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ABSTRACT

The properties of sunlight-induced marine photochemical processes were studied through the use of a variety of techniques. Investigations were facilitated through the development of equipment, analytical methods, and photochemical procedures which were designed to avoid many of the problems inherent in such studies in the marine system.

The importance of nitrate and nitrite, transition metals, and organic constituents in photoinducing the reaction of various added labile substrates was considered. Of these the organic constituents were found to exhibit the most significant effect and may be responsible for most of the observed light-initiated reactions in natural seawater.

Organic constituents were also found to generate what was tentatively identified as hydrogen peroxide. The kinetics of its formation and decomposition in seawater were examined.

When natural seawater was irradiated, marked decreases in the physical properties of fluorescence and absorbance were also noted. It is proposed that this may explain some of the observed features of these properties in the ocean.

Methods used to measure light-initiated reactions in seawater were applied to testing its variability with respect to this property. Preliminary results indicate that the photoreactivity of seawater towards a given substrate may vary considerably.

LIST OF ABBREVIATIONS AND SYMBOLS

A	absorbance
D	percent of fluorescence decay (see Section 5.5)
Δ	difference
DOC	dissolved organic carbon
ϵ (a)	molar extinction coefficient (liter/mole cm)
EDTA	ethylenediaminetetraacetic acid
g	grams
h	Planck's constant
HPLC	high pressure liquid chromatography
I	intensity
k	rate constant where subscript indicates order of reaction
λ	wavelength
l	liters
ly	langleys
M	moles or molar
m	meters
min	minutes
ml	milliliters
µg	micrograms
µl	microliters
µM	micromoles (molar)
mM	millimoles (molar)
ν	frequency

ng	nanograms
nM	nanomoles (molar)
NTA	nitrilotriacetate
PAR	photosynthetically active radiation (400-700 nm)
POC	particulate organic carbon
Q	quencher
QSU	quinine standard units (see Section 5.5)
R	color comparison index for epinephrine indicator (see Appendix 2, Section IX)
S °/oo	salinity in parts per thousands (g/kg)
sec	seconds
sen	photosensitizer
ty_2	half-life
TMEM	transition metal enrichment media
VOC	volatile organic carbon
ϕ	quantum yield
yr	years
*	photoexcited state
[]	concentration per litre
X•, R•, etc.	free radicals

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I wish to thank all the members of my committee for their time and patience in this endeavor. I would particularly like to thank my supervisor, Dr. Peter Wangersky, for the encouragement and many enlightening discussions during the course of this work. The members of the student body to whom I owe thanks are many, but those who hold an especially significant place are Dr. Mike McKinnon, Bruce Johnson, and Dr. John Hoff. My wife, Judy, deserves the greatest appreciation of all for it was her understanding and continual support which made the realization of this goal possible.

1. INTRODUCTION

1.1 Abiotic degradation processes

The yearly total input of organic carbon to the oceans from natural and anthropogenic sources has been estimated at 3.6×10^{16} g C (Williams, 1975). This quantity is added to an existing pool which is estimated to be 1×10^{18} g C (based on 0.7 mg C/l). For the oceans to be able to maintain this level, removal and recycling processes must account for a quantity of the organic carbon approaching the yearly input value. Recyling is mainly attributed to biological processes, and these have been extensively studied with the result that microbial degradation is credited with being the major remineralization agent in the oceans. The role of abiotic processes is usually deemed insignificant in comparison to the potential microbial turnover of organic material. However, experimental evidence supporting this observation is extremely limited, simply because few studies of abiotic processes have been conducted.

It is possible to make a case for the significance of abiotic remineralization by balancing the estimated inputs, reservoirs and losses of organic material in the ocean, and then determining the rate of change in the standing DOC pool. Obviously, if the standing DOC load approximates a steady state condition over long periods of time, then the inputs and losses must balance. If microbial activity is the only active remineralization pathway, then it must be 100% efficient; otherwise an accumulation of DOC would result.

Laboratory studies on the decay of plankton (Skopintsev, 1960; Otsuki and Hanya, 1968, 1972 a,b) indicate that 5-10% of the original cellular organic material remains as a soluble organic fraction which is resistant to bacterial decay. By assuming that 5% of the annual input of organic material, which virtually all results from net primary productivity, is inert to microbial degradation, a value of 556 years is obtained for the doubling time (t_2) of the standing DOC (Eq. 1.1).

$$t_2 = \frac{\text{Total DOC}}{\text{5\% of Net Primary Productivity - Loss to the Sediment}} \qquad (1.1)$$

$$= \frac{1 \times 10^{18} \text{ g C}}{1.8 \times 10^{15} \text{ g C/yr} - .1 \times 10^{15} \text{ g C/yr}}$$

$$= 556 \text{ yr.}$$

Since the primary productivity of the oceans has probably been fairly constant for a much longer period than 556 years, the conclusion can be drawn that the oceans should contain far more DOC then they do today unless some other destructive mechanism exists which has not been reproduced successfully in laboratory studies on plankton decay.

Rather than simply to suggest that the biologically inert fraction in these experiments is destroyed in the oceans by purely abiotic mechanisms, it is perhaps more realistic to conclude that for different classes of compounds the recycling will proceed predominantly either biotically or abiotically or more likely through a synergistic interaction involving both processes.

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1.1.1. Thermal processes

Thermodynamically all organic compounds should be unstable in seawater under the prevailing conditions. The kinetics of a particular reaction will determine whether it is oceanographically significant, and the presence of catalysts can dramatically influence reaction rates. Little is known about the kinetics of thermal degradation in seawater, since only a few studies on specific compounds exist. Amino acids have been studied the most thoroughly (Bada, 1971), but because of the slow rates of deamination or decarboxylation, measurements in the laboratory are conducted at temperatures of 100°C or more, and rates are extrapolated back to natural temperatures by using the Arrhenius equation. Using this technique Bada and Miller (1968) determined a half-life for deamination of aspartic acid of 2.8×10^7 years at 0°C or 96,000 years at 25°C. Bada (1971) estimated that the fastest nonbiological degradation of amino acids in via a metal catalyzed oxidation, for which he estimated a half-life of 350 years. This technique was also applied to determining whether a detectable quantity of the bulk DOC in seawater was susceptible to oxidation with a concomitant loss of CO_2 (Bada and Lee, 1976). They found no measurable change in the DOC values of seawater samples from different depths, after heating them at 126°C for 19 days.

It would appear from the evidence so far presented, from relatively few studies, that thermal oxidation pathways are extremely slow. However, a question that comes to mind with regard to employing high temperatures to determine reaction rates in natural water samples is whether

naturally occurring catalysts would survive this treatment or would be destroyed during the initial stages of the reaction.

1.1.2. Photochemical processes

Due to the large input of solar energy into the oceans, photochemical reactions represent at least potentially a significant abiotic process in surface water. Yet, our understanding of such processes, as well as the range of opinions which exist concerning them, is perhaps best exemplified by the following passages.

> Yentsch (1974), from a paper on the decomposition of chlorophyll in seawater: "In the course of these studies, we have concluded that the photo-oxidative effects are probably the more important and it may well be that photo-oxidation is one of the principle mechanisms for the decomposition of organic material in natural waters."

> Horne (1969): "Oxidation may also occur photochemically in the first meter or so of the surface water, and by means of the free oxygen dissolved in seawater in the presence of catalytic surfaces, although the relative importance of such processes does not appear to be known with any degree of clarity."

The slow accumulation of information with regard to such reactions may be due in part to misconceptions about light and the nature of photochemical reactions in general. A tendency to restrict considera-

tion to the immediate vicinity of the surface film might stem from reports in the earlier literature that seawater attenuates ultraviolet radiation very rapidly, and that therefore only surface phenomena are important.

Measurements (Jerlov, 1968) indicate that in clear oceanic water, 5% of the incident light of wavelength 300 nm should reach a depth of 20 m. Even if seawater does attenuate the ultraviolet wavelengths rapidly, as is the case in most coastal waters, there are many photochemical reactions which proceed with high efficiencies at wavelengths well into the visible and even near-infrared regions. Whether such reactions occur in seawater is presently a question without an answer, for there is no composite of experimental evidence on which to formulate such an answer. Most reviews dealing with aspects of marine chemistry either do not cover the subject at all or deal with it in a sentence or paragraph. Nevertheless, a number of papers have appeared which may reveal some characteristics of marine photochemistry.

1.1.2.1. Surface Film

In considering the various processes active in the removal of oil from the sea surface, Pilpel (1968) concluded that oxidation by microorganisms was the most important and might proceed at rates of up to 10 times spontaneous chemical oxidation. Baier (1972), on the other hand, found in experiments conducted in the field and laboratory that bacterial degradation was not fast enough to account for the rapid disappearance of oil films from natural waters. On the basis of results

using internal-reflection infrared spectroscopy, he concluded that the removal of oil films was facilitated by the introduction of O_2 into the organic film (as evidenced by the appearance of ester bands in the infrared spectra) and that this process, combined with bubble breaking at the surface provided the most effective removal mechanism.

Another removal mechanism was discovered by Wheeler (1972) who found that surface films of fatty acids collapsed to form particles on exposure to ultraviolet light. Results indicated that there was an introduction of hydroperoxide groups into the parent fatty acid molecule with resultant polymerization of the products. Instead of polymerization, Timmons (1962) found that the constituents of plankton oil films were converted to smaller and more soluble fragments when exposed to artificial sunlight. Solubilization appears to be a process common to some constituents of crude oil films as well, with low molecular weight acids, sulfoxides and peroxides comprising some of the soluble fraction (Burwood and Speers, 1974; Hansen, 1975; Larson et al., 1977).

An acceleration in the photo-oxidation of films of various fractions of crude oil spread on water was observed when 1-naphthol was added to the films (Klein and Pilpel, 1974). The 1-naphthol apparently acted as a photosensitizer which not only caused an increase in solubilization of the films, but also caused them to spread rather than to contract, as was noted for experiments in which no photosensitzer was added. A case was made for the addition of photosensitizers to oil spills at sea to accelerate their removal by sunlight photo-oxidation.

1.1.2.2. Iodide

The observation that the I/Cl ratios are markedly higher in aerosols above the sea than in sea water itself has been advanced as evidence for sea surface fractionation of these two elements. The photochemical production of I_2 (Eq. 1.2) in seawater (Miyake and Tsunogai, 1963; Merten and Harriss, 1970; Seto and Duce, 1972)

$$2I^- + {}^1/_2\, O_2 + H_2O \xrightarrow{h\nu} I_2 + 2OH^- \qquad (1.2)$$

has been found to occur in the wavelength region of 300-500 nm, and this observation has been advanced to explain the observed enrichment. Direct absorption of light by iodide ion would not appear to be a likely mechanism for this reaction, since the iodide ion absorbs well below the wavelength of 300 nm.

1.1.2.3. Nitrogen nutrients

As a specific topic in the study of the photochemistry of seawater, the nitrogen nutrients (NO_2^-, NO_3^- and NH_3) have received by far the most attention. ZoBell (1933) found that when pyrex flasks containing dilute ammonicial seawater solutions were exposed to sunlight or a mercury arc lamp, a decrease in the NH_3 and increase in NO_2^- and NO_3^- was observed. The same was not found to be true for solutions prepared with distilled water, artificial seawater or autoclaved seawater. The conclusion was that seawater contained labile oxidants or catalysts.

In a similar study (Rakestraw and Hollaender, 1936) using incident

radiation of greater than 220 nm; it was found the NH_3 was converted to NO_2^- in a variety of different seawater samples, but that the same reaction was not observed in either distilled water or in the dark. They also found an efficient photoreduction of nitrate to nitrite.

For a long time after the discovery of the photo-oxidation and photoreduction reactions of the nitrogen nutrients the processes were considered to be insignificant, because it was believed that the wavelength region responsible for the reactions (that below 400 nm) was rapidly attenuated in the sea. With the knowledge that radiation in the region 310 to 365 nm penetrates to at least 20 m (Jerlov, 1951), Hamilton (1964) reinvestigated these reactions using sunlight and 250 nm radiation. Contrary to the earlier findings of ZoBell, he observed no detectable photo-oxidation of ammonia, but did find a significant conversion of nitrate to nitrite. Hamilton proposed that the absence of any NH_3 photo-oxidation in his work might be explained by a lack of the necessary catalyst in the water (from the Tropical Atlantic) used in his experiments. Further evidence for the importance of catalysts in this reaction is taken from the work of Joussot-Dubien and Kadiri (1970), who found that dye-photosensitization of oxygen by visible light in seawater led to the formation of singlet oxygen, which by way of a dark oxidation reaction converted NH_3 to NO_2^-.

Relatively rapid rate estimates (Zafiriou, 1974) were made for the destruction of NO_2^- and NO_3^- in tropical surface waters. It was pointed out, however, that the net effect of the NO_2^- photolysis might be its subsequent recycling through dark reaction pathways to yield

free radicals.

Hung (1970) also observed the photo-oxidation of NH_3 in seawater, but found that both NO_3^- and NO_2^- were products. The disappearance of NH_3 could not be quantitatively explained on the basis of the amount of NO_2^- and NO_3^- formed (Hung, 1972). It was found that 254 nm irradiation of 10 to 60% ammonium acetate in 0 to 3% sodium chloride solution gave some glycine as a product. The formation of amino acids was used to explain the quantitative discrepancy in the amount of ammonia accounted for by considering only NO_2^- and NO_3^- as products. However, no concrete evidence for the formation of amino acids in seawater was found.

1.1.2.4. Xenobiotics

An extensive amount of information on the photochemical degradation of xenobiotics has been compiled (Rosen, 1971; Duursma and Marchand, 1974; Faust, 1975; Crosby, 1976) and only some specific aspects of it will be cited here. In most of these investigations little or no attempt has been made to simulate natural environmental conditions. Very often solvents other than water have been used, and in only a few instances has natural water been employed as a reaction medium. This is justified in most instances by assuming that the reaction of importance proceeds by way of light absorption by the substrate as the initial step. This, of course, limits consideration to only those xenobiotics which absorb at wavelengths greater than 290 nm, when considered in the context of environmental importance. Rosen (1971) points out the importance of carrying out studies under actual environmental conditions

where natural photosensitizers might play an important part in promoting the degradation of xenobiotics.

Miller and Narang (1970) found that DDT was photo-degraded in the presence of aromatic amines at a wavelength of 310 nm. Although this paper is often cited with reference to its environmental significance, it is difficult to assess its importance in view of the fact that the experimental conditions and the photosensitizers used are far from representative of environmental parameters. The importance of natural photosensitizers, such as humic acids, is evident in the photolysis of malathion (Paris et al., 1975), which was found to have a half-life of 990 hours in water containing no humic acid and 15 hours in water containing humic acid. The photolysis of benthiocarb and aldrin in agricultural water (Ross and Crosby, 1973; Ross, 1974) has also be attributed to photosensitization by humic acid.

Apart from the degradation of organic pesticides, the importance of sunlight mediated ractions in the degradation pathways for residual chlorine and its products in seawater has been recognized (Macalady et al., 1977). Sunlight exposure causes a very significant increase in the conversion of these oxidants to bromate ion, which is persistent in natural waters, and has an unknown environmental significance.

1.1.2.5. Metal-organic interactions

Many of the transition metals are found in natural waters as a result of normal geochemical cycles and various anthropogenic inputs. A potential role for these metals in natural water photochemistry is

associated with this ability to form chelates with a wide variety of organic compounds. The resultant chelates are important as a photochemical entity, because they often have absorption bands which are intense, broad, and extend well into the near-ultraviolet or even into the visible portion of the spectrum. Since light in natural waters is restricted to wavelengths greater than 290 nm, an organic compound which is transparent (e.g., amino acids, carboxylic acids, and amines) to this radiation can become susceptible to photolysis by forming a photolabile chelate. A major limitation which restricts the significance of this process is the low concentration of both organic ligands and transition metals. Because of the complex composition of most natural waters, many competing coordination compounds can exist for both metals and ligands. It is therefore essential that a ligand present at a low concentration in these waters must have a large stability constant with some metal ion before a significant concentration of the chelate can exist. This is indeed an oversimplification of the problem, for seawater has a composition made up of over 70 elements present as an unknown number of inorganic dissolved species and solid phases along with a suite of organic compounds, which in the present day inventory account for only 10-34% of the total organic carbon present. One approach used in estimating the importance of different ligands in chelating metal ions has been through the use of computer equilbria modeling (Stumm and Morgan, 1970; Morel and Morgan, 1972; Zirino and Yamamoto, 1972; Morgan and Vuceta, 1976).

Much speculation exists on the presence and importance of natural

chelators in natural waters; but little has been accomplished towards their characterization and even less information exists on their photochemistry. One exception to this has been the studies on acetate; Agahi and Takabatake (1973) found that when aqueous solutions of mercury and acetate were exposed to sunlight, a methyl mercury bond was formed. It a similar study (Jewett et al., 1976) methylmercuric ion and dimethylmercury were found upon irradiation with normal laboratory lighting of aqueous solutions of the reactants at the ppm concentration level. They found a similar result for thallium acetate solutions. Both the mercury and the thallium solutions gave gaseous products which were identified as ethane and CO_2, and also a precipitate which apparently was the elemental form of the metal.

A recent interest in the photolysis of aminopolycarboxylates stems from their rapidly increasing commercial importance. Studies on Cu^{2+} nitrilotriacetate (NTA) (Langford et al., 1973) and Fe^{3+} NTA (Trott et al., 1972) chelates show that both decompose rapidly at radiation wavelengths present in sunlight. The authors suggest that the reaction proceeds by way of a LMCT (ligand to metal charge transfer) transition; based on similar results for Fe^{3+} EDTA (Carey and Langford, 1973) and glycine, they concluded that this may be a general reaction (Eq. 1.3) for aminopolycarboxylate metal complexes.

$$RHNCH_2COOH + \tfrac{1}{2}O_2 \xrightarrow{h\nu} RNH_2 + CO_2 + CH_2O \qquad (1.3)$$

Further studies on the products of Fe^{3+} EDTA (Lockhart and Blakeley, 1975) showed that 8 major products were formed, including glycine.

Natarajan and Endicott (1973) found that of the EDTA chelates with Fe^{3+}, Co^{2+}, Cr^{3+}, Ni^{2+} and Cu^{2+}, only Fe^{3+} and Co^{2+} were photolabile. In a similar study, Lockhart (1976) found that of the EDTA chelates with Na^{+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+} and Hg^{2+}, only Mn^{2+}, Fe^{3+}, and Co^{2+} were photolabile.

1.1.2.6. Other organic compounds

In a few instances, light initiated reactions in seawater have been discovered by testing the stability of substances in light and dark controls, or from the observation that concentration of certain constituents of seawater increase on exposure to light. The latter of these led Wilson et al. (1970) to the conclusion that carbon monoxide (10^{-4}ml $l^{-1}day^{-1}$), ethylene, and propylene (10^{-4}ml $l^{-1}day^{-1}$) were formed by photochemical processes in seawater. Although the concentrations were small, they were significant when compared to the normal concentration of the materials in seawater. The amount produced seemed to be dependent on the concentration of organic material present. The authors do not suggest a mechanism for formation of the observed products, but it is possible that since aldehydes have been identified as a constituent of seawater (Kamata, 1966), their direct photolysis through reactions like 1.4, 1.5 and 1.6 could explain their observations. Unfortunately, the conditions under

$$CH_2O^* \longrightarrow H_2 + CO \qquad (1.4)$$

$$CH_3CH_2CHO^* \longrightarrow CH_2{=}CH_2 + CH_2O \qquad (1.5)$$

$$CH_3CH_2CH_2CHO^* \longrightarrow CH_3CH{=}CH_2 + CH_2O \qquad (1.6)$$

which these reactions and many others have been investigated are so different from seawater that comparisons should be made carefully.

In another study on the chemical instability of purines in seawater and culture media, Antia and Landymore (1974) observed that uric acid and xanthine were degraded by exposure to light of wavelengths greater than 380 nm. On the basis of EDTA inhibition of the uric acid photolysis, trace metals were implicated in the reaction scheme. The addition of EDTA to natural seawater solutions of xanthine, however, caused an increase in the rate of photolysis and this was advanced as evidence for the inhibitory effect of metals on this reaction. In view of the fact that EDTA is also a good reducing agent, it is entirely possible that it is serving as a free radical scavenger and/or a hydrogen or electron source for triplet excited states of photosensitizers. In any case, the influence of EDTA on the photodegradation of xanthine and uric acid does not necessarily implicate trace metals in the reaction. An interesting aspect of this reaction is that neither uric acid or xanthine absorb light above 380 nm. This would imply that the reaction proceeds either as a result of a metal-ligand chelate or it is induced by some other absorbing species.

Carlucci et al., (1969) found that vitamins B_{12}, thiamine, and biotin lost most of their activity over a period of two weeks in sterile seawater solutions which were exposed to sunlight. The destruction of both vitamin B_{12} and thiamine could be explained by direct photolysis, since both absorb at the wavelengths used in the experiment. However, biotin is transparent to the sunlight radiation and, as was the case with

uric acid and xanthine, the initial excitation must originate in a species other than the vitamin molecule.

1.1.2.7. Short-lived reactants

Many photochemical reactions form transient products which often have lifetimes on the order of milli- or microseconds. Because of their short life-times, these products must be studied with special techniques which operate on a time scale fast enough to record their existence. Flash photolysis is such a technique; essentially it amounts to exposing a sample to an extremely high energy flash of light, and immediately measuring the absorbance before the relatively high density of the transient products formed during the flash disappear.

This technique has been used to determine the probable fate of hydroxyl radicals ($OH^{\bullet}$) (Zafiriou, 1974; Zafiriou and True, 1977), which should result from the photolysis of both NO_2^- and NO_3^- at the natural seawater pH of 8.1. Since the $OH^{\bullet}$ is one of the most reactive species known, its lifetime in seawater should be very short. On calculating the pseudo first order rate constants for the reaction of a number of seawater components with the $OH^{\bullet}$, Zafiriou (1974) concluded that it would react almost exclusively with bromide ion, with minor participation from carbonate ion and DOM. The transient absorbance observed during flash photolysis studies of seawater were similar to those expected for a mixture of dihalide ion radicals with no evidence for the bicarbonate radical. Carbonate species, however, were implicated in the decay reactions of the initial free radical transients.

Other evidence for the existence of short-lived species in seawater was given by Swallow (1969), who found that the chlorine anion radical ($Cl_2^{-}\cdot$) and hydrated electrons (e_{aq}^{-}) were formed during the pulse radiolysis of seawater. He proposed that hydrated electrons would be formed under natural conditions by photoionization of aromatic compounds in seawater. He calculated an upper limit for their formation by assuming that all but 14% of the incident light up to 325 nm was absorbed by compounds capable of photoionizing, and that this process was 100% efficient ($\phi = 1$). This gave a maximum production of $\sim 3 \times 10^{12}$ hydrated electrons g^{-1} sec^{-1}, which would in turn be scavenged by O_2 and CO_2 to form the superoxide anion radical (O_2^{-}) and the carbon dioxide anion radical ($CO_2^{-}\cdot$). Zafiriou (1976) points out that if Swallow's calculations are correct, the organic initiators of hydrated electron production would be transformed to free radicals in only 10^3 seconds. He concludes that either these reactions are extremely inefficient with respect to light absorption or quantum yield, or material recycling must occur.

1.2. Conclusion

It should be apparent from the preceding discussion that the photochemistry of seawater may involve many components in a variety of different types of reactions. For many of these reactions the wavelength of the excitation energy is sufficiently long to favor their occurrence throughout the photic region of the water column; they perhaps represent an abiotic process which is competitive with biological recycling processes for some organic materials. However, in view of the experimental conditions used in some of the studies reviewed here, it would be un-

warrented to extrapolate the results directly to the marine system. Yet, even where the natural system has been closely approximated, as in the case of the nitrogen nutrients (ZoBell, 1933; Hamilton, 1964), the results of different workers indicate that seawater may not be a consistent reaction medium.

If the composite of observations concerned with the photochemistry of natural waters is considered as a whole, it is reasonable to conclude that a maze of simultaneous reactions may be occurring of which a few might predominate and set observable trends. Since no systematic study of marine photochemistry has been reported, it has only been possible to consider its role on a conceptual basis. Unfortunately, this has led to a range of opinions varying from complete skepticism to unquestioning belief in the importance of photochemical processes in the ocean.

The following work, therefore, is directed at attacking the question experimentally in an attempt to elucidate the nature of marine photochemical processes. Some of the fundamental questions that have been considered in this work are listed below.

(1) Are any light-initiated changes in either the physical or biological properties of seawater observed?

(2) What is the rate of light-initiated modification of model substances, for which the chemistry is well known, when they are added to seawater under natural conditions or simulated natural conditions?

(3) What products result from light-initiated reactions of either model compounds or natural seawater components?

(4) What are the specific agents of seawater which are responsible for its "photoreactivity"?

(5) What are the general mechanisms of light-initiated reactions in seawater?

(6) Is the "photoreactivity" of seawater variable in time and space?

Conceptually these questions may be tractable, while on an experimental basis some of them may prove to be difficult or even impossible to clearly resolve.

2. ASPECTS OF PHOTOCHEMISTRY

2.1 Introduction

This chapter is included only to clarify some of the terminology used in this thesis, and to provide a convenient list of definitions for terms familiar to photochemists, but unfamiliar to most marine scientists. No attempt has been made to give a comprehensive list of terminology, and the reader is referred to the following texts and articles for further familiarization with the subject:

general photochemistry;

Calvert and Pitts (1966),

Wayne (1970),

Turro (1965), and

Pitts et al. (1963) (vocabulary),

coordination and inorganic photochemistry;

Balzani and Carassiti (1970),

Bucat and Watts (1972) and

Endicott (1976),

photobiology (photodynamic action);

McLaren and Shugar (1964) and

Schenck (1974), and

environmental photochemistry;

Owen (1971) and

Zafiriou (1977).

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Other pertinent references will be given throughout the text, and the literature dealing with relevant aspects of oxyanion photochemistry, transition metal photochemistry, and organic photosensitization is covered in the chapters where those subjects appear.

2.2 Definitions and comments

A. The first law of photochemistry (Grotthuss-Draper Law) states that only radiation which is absorbed by the molecule can be effective in producing a chemical change.

B. The second law of photochemistry (Stark-Einstein Law) states that for the primary process only one quantum of radiation is absorbed by the molecule and that the sum of primary process quantum yields must equal one.

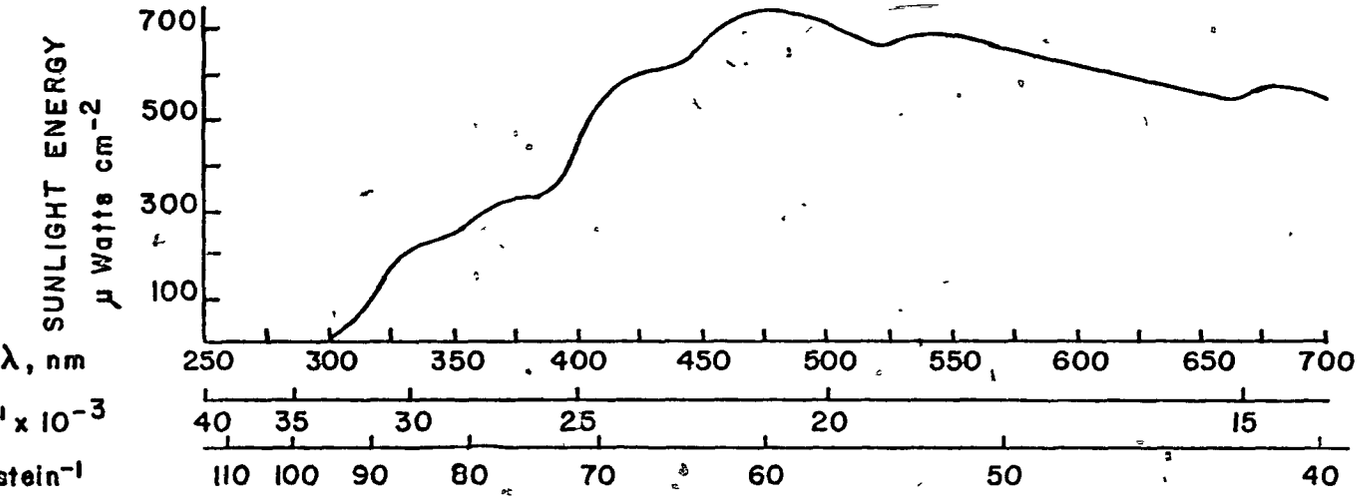
C. An einstein is 6.02×10^{23} photons, where photon refers to a quantum of light energy which is equal to the product of Planck's constant (6.62×10^{-27} erg sec) and the frequency of the radiation (i.e., $q = h\nu = h\,c/\lambda = h\,(3.0 \times 10^{10}\ \text{cm sec}^{-1})/\lambda$ where λ is in angstroms). An einstein at any specific wavelength is then equal to $1.19 \times 10^{16}/\lambda$ ergs or $2.85 \times 10^{8}/\lambda$ calories.

D. The energy of radiation in photochemistry is often expressed in a number of different units including ergs, joules, e V, and calories. Different photochemical reactions have different energy requirements, and they are therefore wavelength dependent with the highest energy reactions occurring at the shortest wavelengths (Figure 2.1).

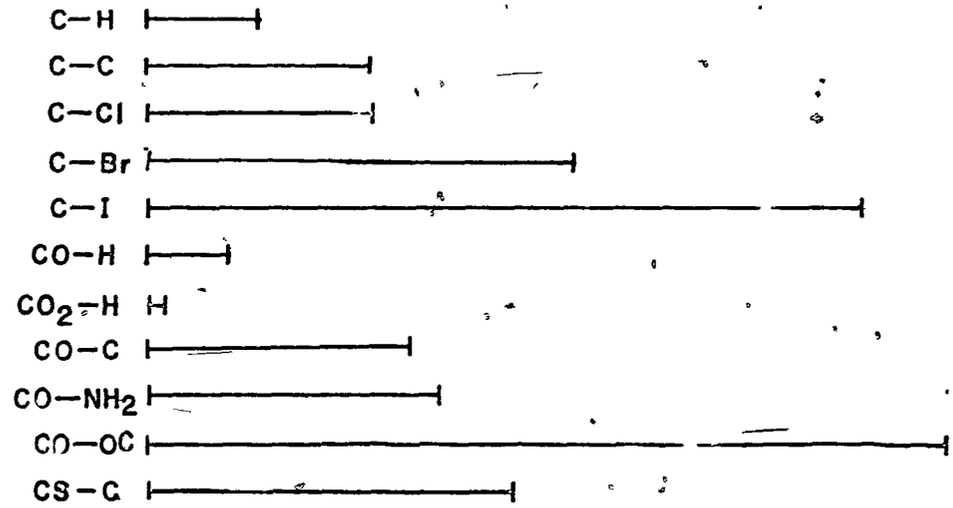
Figure 2.1 The Sea Surface Solar Energy Distribution

and Typical Energies for Bond Dissociation

[1] The bond dissociation energies are for gas phase reactions.



Typical Energies Necessary for Bond Dissociation[1]



E. The quantum yield (ϕ) is a measure of the efficiency of photo-processes, that is

$$\phi_A = \frac{\text{no. of A events occurring}}{\text{no. of photons absorbed}}$$

For primary processes $\Sigma\ \phi_i = 1$, where i represents all of the processes occurring. For chemical processes ϕ has the general definition,

$$\phi = \frac{\text{no. of moles of reactant consumed (or product formed)}}{\text{no. of einsteins absorbed}}$$

Because of secondary reactions ϕ can generally exceed unity and sometimes is as high as 10^4 in chain reactions.

F. Primary processes are those involving the initial act of excitation, and terminate when the excited molcule has undergone reaction or has returned to near its pre-excitation energy level.

G. Secondary processes are actually thermal (dark) reactions of reactive species resulting from the primary process. They are photochemical only in the sense that they are the consequence of light absorption.

H. The singlet excited state (S_n with $n > o$) results when a paired electron is promoted to a higher energy level with retention of electron spin (i.e., paired spins, $\uparrow\downarrow$).

I. The triplet excited state (T_n with $n > o$) results when a paired electron is promoted to a higher energy level with spin inversion occurring (i.e., unpaired spins, $\uparrow\uparrow$). Transitions in which a change of spin occur violate one of the selection rules and are referred

23

to as a forbidden transition. Therefore, $S_0 \rightarrow T_1$ transitions have a low probability of occurring and result in very weak absorption.

J. Photophysical processes are those in which no chemical alteration of the absorber occurs. The Jablonski diagram in Figure 2.2 illustrates some of the possible energy transitions.

K. Primary photochemical processes are those involving a chemical alteration of the excited molecule or an immediate receptor of its excitation energy. The various possible processes are listed in Table 2.1.

L. Photosensitizers are substances which through their own absorption of light can produce a chemical reaction which would not occur in their absence. In this process the photosensitizer serves as the light energy receptor and it can either transfer the energy to a substrate directly (energy transfer process), in which case it undergoes no chemical alteration, or it can interact with the substrate and be chemically changed. Although by definition all reactions originally initiated by the photosensitzer are included, it is of utility to restrict its use to only those processes occurring immediate to the primary act of light absorption. The complexity of environmental systems, however, may preclude any attempt to define distinctly the reaction mechanism, and what would appear to be a photosensitized reaction might instead be the result of a step (a secondary reaction) in the mechanistic sequence that is far removed from the primary light absorption process. To circumvent this problem the all-inclusive terms induced or photoinduced have

Figure 2.2 Jablonski Diagram Showing Absorption and Subsequent Photophysical Modes of Excited State Decay

(1) IC represents internal conversion.

(2) ISC represents intersystem crossing.

(3) VR represents vibrational relaxation process.

(4) S and T represent singlet and triplet state, respectively--with subscripts (i.e., 0, 1, and 2) indicating ground, first, and second excited states, respectively.

(5) Radiative transitions are represented by continuous lines and non-radiative by wavy lines.

(6) Arrows in boxes indicate electron spins in ground and excited states.

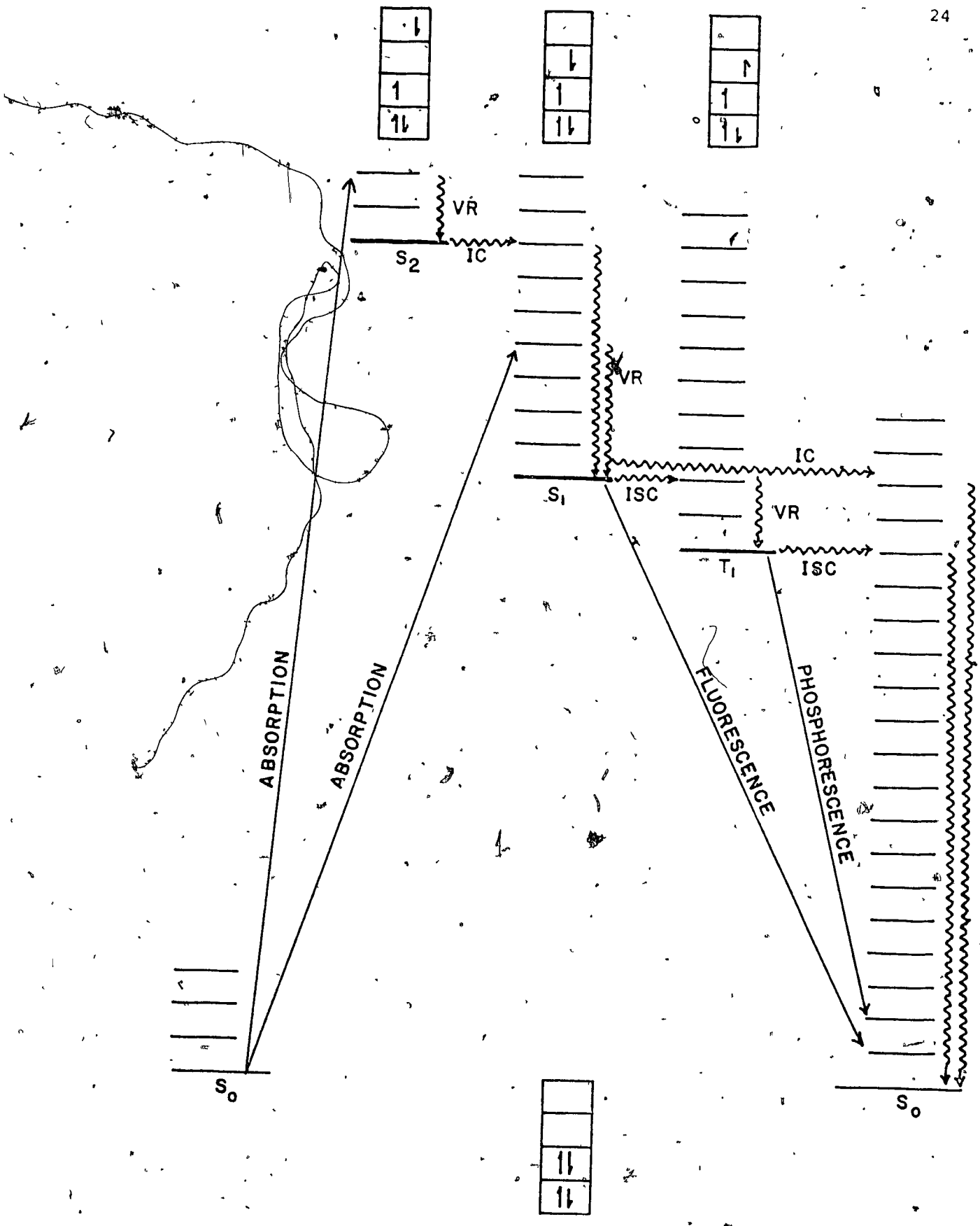
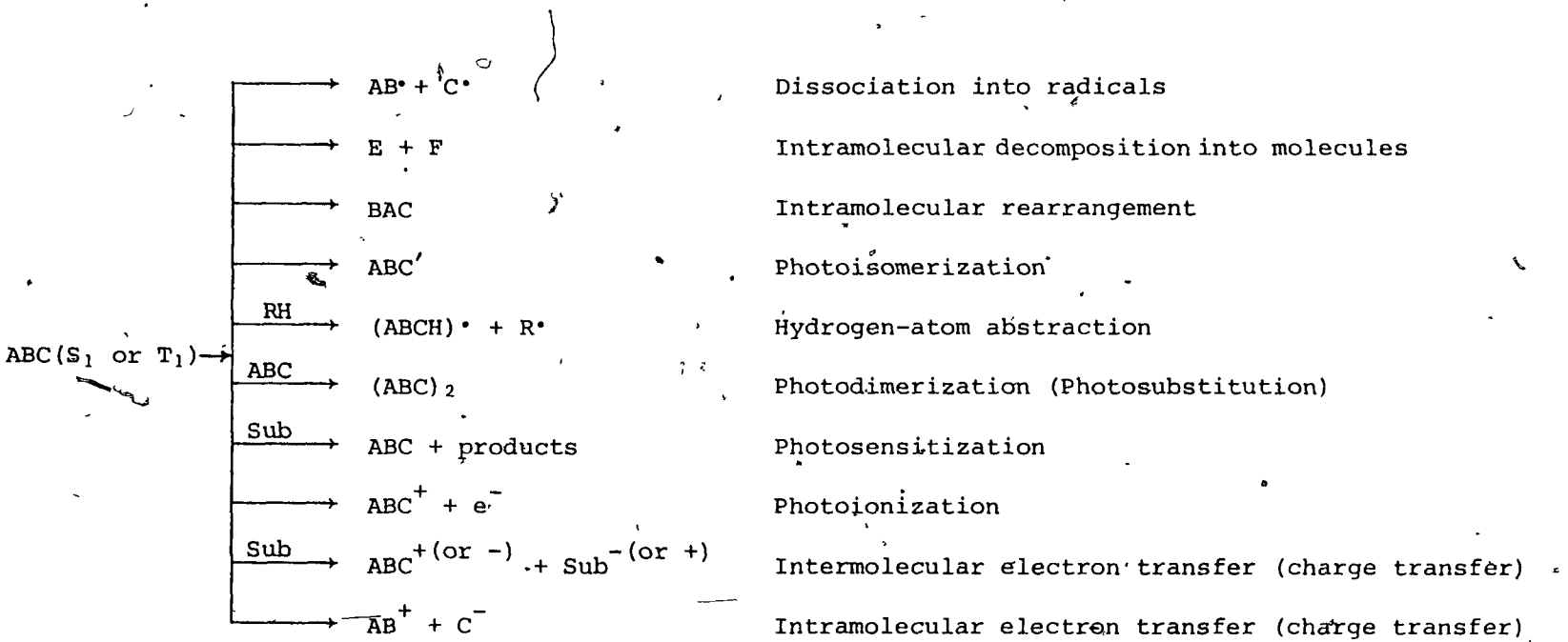

VR
S2
IC
VR
IC
S1
ISC
VR
T1
ISC
ABSORPTION
ABSORPTION
FLUORESCENCE
PHOSPHORESCENCE
S0
S0

Table 2.1 Primary Photochemical Processes[1]

[1] Taken from Calvert and Pitts (1966), p. 367.


ABC(S1 or T1)
AB• + C•
Dissociation into radicals
E + F
Intramolecular decomposition into molecules
BAC
Intramolecular rearrangement
ABC'
Photoisomerization
RH
(ABCH)• + R•
Hydrogen-atom abstraction
ABC
(ABC)2
Photodimerization (Photosubstitution)
Sub
ABC + products
Photosensitization
ABC+ + e−
Photoionization
Sub
ABC+(or −) + Sub−(or +)
Intermolecular electron transfer (charge transfer)
AB+ + C−
Intramolecular electron transfer (charge transfer)

been used to describe those processes which are light initiated but for which the mechanism of the reaction is unknown. This can include metastable products of photolysis that will eventually react or catalyze the reaction of other constituents of the system.

M. Free radicals are species that contain an odd number of electrons. They can be positively or negatively charged or neutral and are often highly reactive. Free radicals are common intermediary products of photochemical reactions and may have an especially important role in secondary reactions in marine photochemistry. One of their most interesting characteristics in this regard is that once initiated they tend to propagate until one of a number of possible termination steps is reached. The common reactions of free radicals follow:

(1) combination,

$$A\cdot + B\cdot \longrightarrow AB$$

$$A\cdot + \cdot O_2\cdot \longrightarrow AO_2\cdot$$

(2) disproportionation,

$$2\,H\text{-}C\text{-}C\cdot \longrightarrow H\text{-}C\text{-}C\text{-}H + \diagup C{=}C \diagdown$$

(3) redox,

$$A\cdot + M^{n+} \longrightarrow A^- + B^{(n+1)+}$$

(4) addition, and

$$A\cdot + \diagup C{=}C \diagdown \longrightarrow A\text{-}\overset{|}{C}\text{-}\overset{|}{C}\text{-}$$

(5) fragmentation.

$$F - C(=O)-O\cdot \longrightarrow R\cdot + CO_2$$

0. Photochemical kinetics, like thermal kinetics, requires a solution to the equation,

$$\text{rate} = -\frac{[dA]}{dt}$$

However, unlike thermal kinetics, the rate of a photochemical reaction can only be accurately defined when the quantum yield and the number of einsteins absorbed (I_A) by the reactant in unit volume and unit time are known. Hence, the rate equation for a photochemical reaction is

$$-\frac{[dA]}{dt} = \phi_A \, I_A.$$

Because of the complications involved in determining ϕ_A and I_A in the seawater system (Section 3.1.6.), the actual reaction rates were not calculated for the studies which follow. Instead relative rates were determined which take into account neither ϕ_A or I_A, but only give the change of concentration of reactant or product with respect to time of exposure to a continuous flux of radiation. No comparison should, therefore, be made between relative rates determined with different light sources.

3. PROBLEMS ASSOCIATED WITH STUDIES IN MARINE PHOTOCHEMISTRY

3.1 Problems unique to photochemical studies in natural waters

In most studies in solution photochemistry, the conditions are selected so that experimental variables are limited to as few as possible in order to obtain the most unambiguous information from the experiment. Operationally the experimentalist sets the conditions which are best suited to his particular problem. However, when the problem is concerned with studying reactions occurring in the natural environment, a comparison of conditions between classical solution photochemistry and marine photochemistry, as indicated in Table 3.1, shows that the two are widely different in many respects. For an experimentally tractable problem, it may be necessary to simplify aspects of the natural system by adopting classical conditions. However, the more closely the study resembles the classical approach the more likely it is to fail in providing an accurate assessment of the real situation. On the other hand, to assume all the prevailing natural conditions as part of the experiment might make the interpretation unrealistically complicated and make any results seemingly dubious. It would seem that the only recourse would be to set conditions somewhere between the two and to base the decision on a careful consideration of the prevailing environmental conditions. By establishing an experimentally realistic goal, the study can become a consideration of only a part of the whole system rather than all of it.

Table 3.1 Comparison of Typical Reactions Conditions

for Classical Solution and Marine Photochemistry

Property	Classical Solution Photochemistry	Marine Photochemistry
wavelength of radiation	usually specified by ε_{max} of studied compound	polychromatic, width dependent on depth and location
solvent	non-aqueous except in coordination photochemistry	seawater
number of reactants	one	number unknown, perhaps many
concentration of reactants and products	high enough to measure conveniently	probably too low to measure easily
phases present	one, homogeneous	heterogeneous
oxygen	usually avoided	always present
competing processes	avoided	possibly thermal, biological, physical and other photochemical
reation rate	significant conversion, μ sec-hours	environmentally significant conversion, hours to years
variability of reaction medium (solvent and reactants)	no	unknown, probably yes

3.1.1. Knowledge of reactants and products

In the classical photochemical approach, a reaction is studied by characterizing the products and following either their rate of appearance or the rate of disappearance of the reactant, for which the initial concentration is known. For a sample of seawater, however, very little is known about the .7 to 2 mg C/l present as organic matter. The situation is somewhat analogous to collecting a sample from a reaction vessel after some unknown reaction time has lapsed, during which some unknown number of reactions were active in altering an unknown number of reactants to give an unknown number of products.

What is known about the identity of the organic fraction in seawater covers only 10 to 34% of the total, depending on whose estimates one uses. The organic compounds listed in Table 3.2 have been identified as constituents of the total "dissolved" organic fraction of seawater, where "dissolved" refers to the organic fraction remaining after filtration through a .45μ filter. The values shown are a composite of different studies on seawater samples, most of which would be of coastal origin. For the sake of comparison it was assumed that the total concentration was 1 mg C/l, with the uncharacterized fraction amounting to 66% of the total. This fraction may be composed of plankton by-products, which, through a type of Maillard reaction, have condensed to form melanoidines (Kalle, 1963). In coastal waters this fraction may contain other principle constituents of terrigenous origin (Prakash, 1971) as well as benthic algal exudates (Sieburth and Jensen, 1969; Khailov, 1963; Craigie and McLachlan, 1964).

Table 3.2 "Dissolved" Organic Components in Seawater

The concentration in μg C l^{-1} are taken from Dawson (1976). The values shown are representative of recent studies which have been presented in the literature.

[1] Based on an approximate average molecular weight for group classification.

[2] Based on typical absorption spectra for the compounds in group classification.

[3] Assumes this fraction has a character similar to soluble fulvic acid fraction from soil with a molecular weight of 1000 of which 50% is carbon.

Component	Concentration $\mu g\ C\ l^{-1}$	Concentration[1] $M\ l^{-1} \times 10^{-7}$	Maximum Absorption wavelength[2], nm
free amino acids	10	.3	< 300
combined amino acids	50	--	< 300
free sugars	20	.3	< 300
combined sugars	200	--	< 300
fatty acids	10	.05	< 300
phenols	2	.02	350
sterols	0.2	.0006	< 300
vitamins	.006	.000002	only biotin < 300
ketones	10	.2	350
aldehydes	5	.1	325
hydrocarbons	5	.03	aliphatic < 300 aromatic > 400
urea	10	.83	< 300
uronic acids	18	.25	< 300
uncharacterized fraction	660	1.3[3]	500-600
total	1000	3.4	--

In general, the absorption spectra of the uncharacterized fraction appears as a broad featureless band extending from the far ultraviolet out at least into the green region. This fraction is probably responsible for most of the absorption at wavelengths greater than 300 nm. For the most part, the remainder of the components are quite transparent to sea level solar radiation wavelengths. There are, however, inorganic components which absorb mainly in the 300-400 nm region; these include NO_2^-, NO_3^- and coordination compounds of many of the transition metals.

3.1.2. Variability of reaction media

In solution photochemistry it is expected that an experiment can be reproduced by using the same set of conditions. Since the solvent can play an important part in determining the course of a reaction, it is of fundamental importance for many reactions to use the same solvent or one with similar characteristics. In marine photochemistry, the solvent is a premixed reaction medium; little is known about the details of its composition, and perhaps what is more important, its variability with respect to time and place of collection. In general the major characteristics of seawater with the same salinity do not vary much from place to place, but the technological difficulties of measuring the minor and non-conservative components leaves the variability of their concentrations open to question. It is certainly reasonable to expect to find differences between different marine environments, such as upwelling, coastal, estaurine, and oceanic areas. The scale of variability could be far smaller if phenomena such as blooms of phyto-

plankton, diurnal migration of zooplankton, schools of fish, phytoplankton patchiness, or fluvial inputs play a significant role in altering seawater composition.

The history of the seawater prior to its use in a reaction might also have a considerable bearing on the observed photochemistry. Previous conditioning of the water by biological, physical or chemical processes will determine its characteristics at the time of collection. Therefore, water exposed to a long period of sunlight radiation might have photochemical characteristics which would be far different than the same water with a previous history of a long period under overcast skies. Temperature and the kinetics of microbial decay might also be important considerations in determining seawater photochemical characteristics.

It is, however, entirely possible that the photochemistry of seawater might be dominated by some overlying feature which gives it approximately the same characteristics everywhere in the oceans.

3.1.3. Concentration of reactants and products

The total molar concentration of organic components in seawater might be in the range of 1-4 μM. However, for most individual compounds the concentrations are usually less than 10^{-7} M. Therefore, analytical techniques with high sensitivity are required, or the substance being determined must be concentrated from large volumes of seawater, this is time consuming and creates serious contamination problems. The latter technique is not suited to studying photochemical reaction solu-

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tions, where the use of small reaction volumes is usually necessary. The alternatives are either to raise the concentration or to try to develop sensitive analytical methods for specific model compounds.

The usual recourse taken by workers studying reactions of natural waters is to raise the concentration of the reactants to levels where changes are easily detected. For zero order reactions (rate dependent only on light intensity) this might be a favorable approach, but for reactions of higher order, especially those where competition between the reactant and some other component (e.g., O_2, trace metals or minor organic components) for a secondary reactive transient exists, the results could be extremely misleading. For instance, in the reaction of the hydrated electron with cystine, the rate is approximately diffusion controled in oxygen-free aqueous solutions. However, if the solution is in equilibrium with air, the reaction would only be important at concentrations of cystine which were in the range of the oxygen concentration. Under natural marine conditions, the concentration of cystine is typically 10^4 to 10^6 times lower than oxygen and other scavengers. Therefore, cystine should not react appreciably by this mechanism under natural seawater conditions.

Very often the significance of environmental photochemical transformation of a substance is determined at concentration from 10^{-4} to 8 molar. Although valuable information may be obtained from such experiments, their relevance is questionable when the results are extrapolated to natural environmental conditions.

3.1.4. Competing processes and reaction rates

The low concentrations of the reactants, the possible high complexity of the light-induced reactions and the possible simultaneous biological, chemical and physical mechanisms for supply, removal or transformation of reactants and products will certainly limit the degree of detailed understanding of the specific photochemical mechanism in the marine environment. Even to eliminate all but the abiotic light-induced reactions could still leave a complex system of competing photochemical processes, the sum of which may alter many of the chemical constituents of seawater. The significance of any one of these processes for a particular component in the natural environment is thus dependent upon the magnitudes of the rates for all of the various chemical, biological and physical transformations involved. The relationship expressed by Eq. 3.1 conceptually provides a simple way of

$$R_S = \frac{\Sigma \text{ Photochemical Rates}}{\Sigma \text{ All Rates}} \qquad (3.1)$$

where R_S = ratio of environmental significance

considering the significance of a photochemical process in the marine environment. A process which is entirely photochemical (where $R_S = 1$) would probably only be possible for the formation of products in which a photochemically unique species is formed. Photochemical transformation of biologically refractory materials might have high R_S values, but compounds which are readily degraded by microbial processes would probably have very low R_S values.

3.1.5. Heterogeneous reactions

Many of the substances which are considered to be part of the dissolved organic fraction of seawater are hydrophobic; hence, they have a strong tendency to absorb on surfaces or coalesce into particles. DOC and POC are associated through a complex equilibrium (Parsons, 1975) in which the displacement is established by the concentration and nature of the organic materials involved and by processes which serve to control the forward and reverse rates (e.g., bubbles, bacteria, inorganic particulates, and chemical condensation reactions). The POC concentration is then highly variable, and dependent both on location and season and may constitute from 2% to 50% of the TOC.

Although the detailed composition of this detrital material is not known, it is certainly composed in part of the remains of dead organisms, and of bacteria that are actively decomposing it. Such particles might represent specific micro-environments within the solution where photochemical processes may proceed with high efficiency. This could be an especially attractive consideration if these particles tend to absorb light as a result of their having incorporated transition metals, portions of photosynthetic apparatus, and condensed polyphenolic polymers, which might be part of the Gelbstoff.

Although the particulate fraction may comprise an important part of the photochemistry of seawater, it also represents a dilemma. If the particulate fraction is removed by filtration or centrifugation its contribution to the photoreactivity of seawater would not be included in experiments. However, if it is retained as part of the sample, the

concomitant bacterial effects must be stopped by some means that does not change the photochemical characterisitcs of the system. The dilemma is further complicated by the lack of knowledge of photochemistry in heterogeneous systems and by the paucity of techniques for studying them. It would seem, then, that a simplification of the natural system is necessary, and that this might best be accomplished by filtration, but, because of the equilibrium between DOC and POC, it can be assumed only that the original POC is removed. Any newly formed POC may have completely different composition and properties.

Aside from the "solid"-liquid phase represented by POC in seawater, it is also necessary to consider the adsorbed organic materials on terrigenous inorganics particles (e.g., clay, silica, and metal oxides), on mineral particles formed in situ by precipitation and at gas-liquid interfaces, as potential photoreactive sites. In many instances, both the photochemical and spectroscopic properties of organic molecules are altered when adsorbed on such surfaces (Nicholls and Leermakers, 1971). Evidence for a variety of photoreactions which take place at metal oxide surfaces (Ritchey and Calvert, 1956; Kuriacose and Markham, 1962; Khenokh and Bogdanova, 1967b; Frank and Bard, 1977) and in surface films at air-water interfaces (Timmons, 1962; Wheeler, 1972; Klein and Pilpel, 1974) have been reported.

3.1.6. Ocean irradiation characteristics

On a sunny day a square meter of the ocean surface may have as much as 1 kilowatt of solar power impinging on it. Approximately 95% of this

enters the water column. Of this about one-half, which consists of the infra-red region, is absorbed by molecules in the upper one meter, and is converted to rotational, translational and vibrational molecular motion. The remainder of the radiation is composed mostly of the visible (700 to 400 nm) and near ultraviolet (400 to 290 nm), wavelenghts and unlike the infrared radiation, it's absorption results in electronic transitions in molecules or ions.

The characteristics of attenuation of the visible and ultraviolet radiation by seawater have been discussed extensively (Holmes, 1957; Duntley, 1963; Jerlov, 1968; Jerlov and Nielsen, 1974). The total attenuation (c) of light in seawater is described by Eq. 3.2 (Jerlov, 1968).

$$c = a_w + a_p + a_y + s_w + s_p \qquad (3.2)$$

where a_w = absorption by water

a_p = absorption by particles

a_y = absorption by organic constituents

s_w = scattering by water

s_p = scattering by particles

Since the absorption and scattering by water itself are constant anywhere in the oceans, these values may be combined ($c_w = a_w + s_w$), and Eq. 3.2 may be rewritten as

$$c - c_w = a_p + a_y + s_p \; . \qquad (3.3)$$

The attenuation properties for the various water masses, shown in

Table 3.3, indicate the large variations that are observed for both the individual components and their summations. Differences are especially marked between regions of low and high productivity or where strong terrestrial influences exist.

The scattering coefficients are of little consequence in the dissipation of light energy, since in Rayleigh scattering the photon collisions are "elastic", and in Raman scattering the photon loses only a small amount of energy to a change in vibrational energy levels of the scattering molecule. Scattering, then, has essentially the net effect of changing the directional character of the light, but only the act of electronic absorption can absorb the photon energy.

Scattering phenomena can, however, exert a strong influence on the net flux of photons across the sea surface. The variation of reflectance on a flat water surface with changing solar elevation is shown in Figure 3.1. If waves are present the reflectance does not change much at high solar elevations, but it is dramatically reduced at lower solar elevations. However, with the presence of white caps and bubbles the albedo might increase to as much as 31% (Fritz, 1951). When the sky is overcast the surface reflectance averages 10%, regardless of solar elevations (Burt, 1953).

The net flux of photons across the sea surface is also a function of the degree of backscattering out of the water, and again is a process which is inversely related to solar elevation. Backscattering is dependent on wavelength-selective molecular scattering (for water this varies as λ^{-4}), which is more important in the blue to ultraviolet

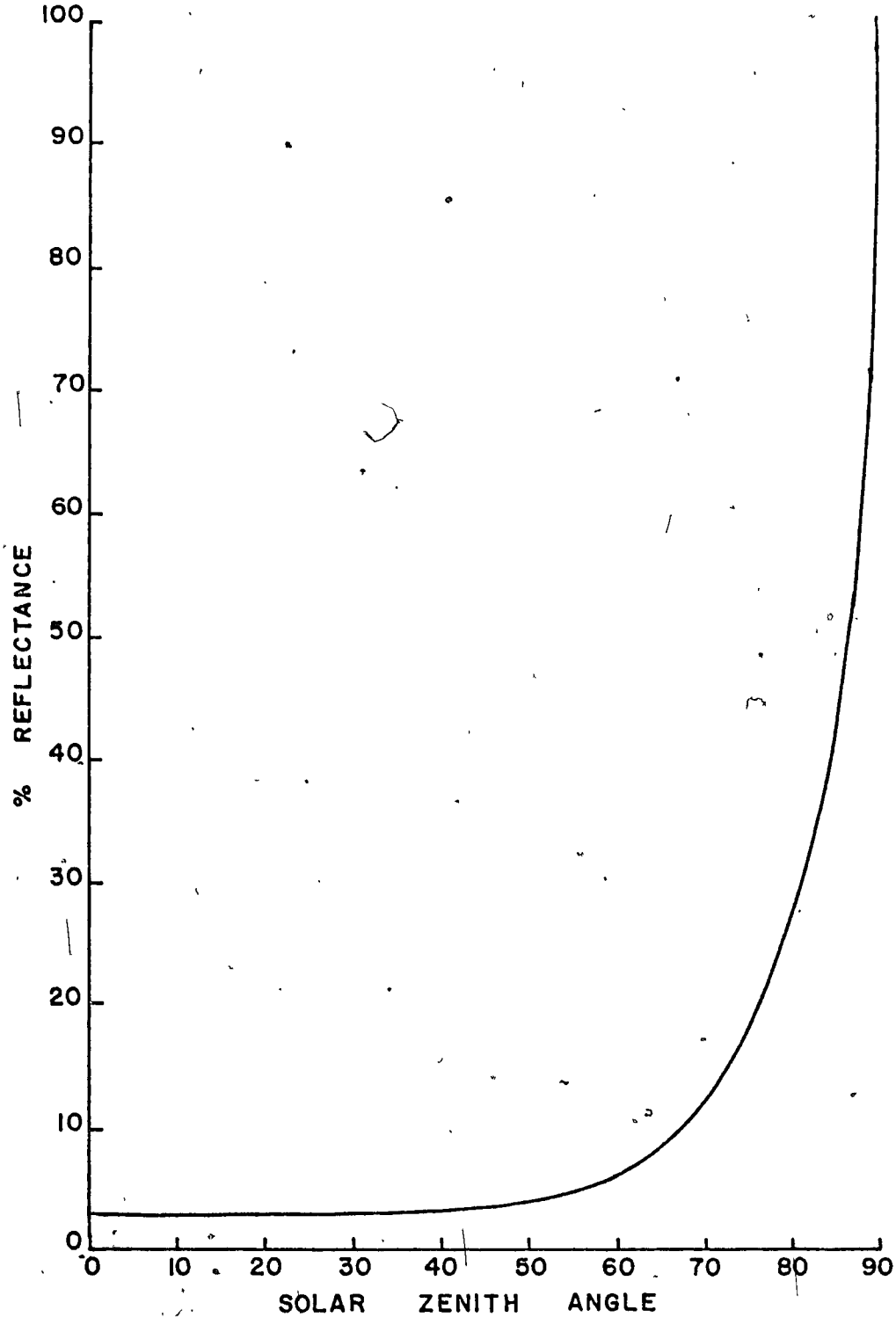
Table 3.3 Regional Attenuation Properties (m^{-1})

Data taken from Jerlov (1968).

Region	Wave-length (nm)	$c-c_w$	s_p	a_p	a_y	a_p+a_y
Sargasso Sea	440	0.05	0.04			0.01
Carribean Sea	665	0.06	0.06	0		0.00
	440	0.09	0.06			0.03
Equator Central Pacific	440	0.09	0.05			0.04
Galapagos	665	0.11	0.07	0.04		0.04
	440	0.24	0.08			0.16
North Atlantic	665				0	
	420				0.03	
North Sea	665				0.01	
	420				0.10	
Bermuda	655	0.10			0	
	380	0.20			0.03	
Kattegat	655	0.23	0.15	0.08	0	0.08
	380	0.54	0.16	0.27	0.11	0.38
South Baltic Sea	655	0.27	0.20	0.07	0	0.07
	380	1.15	0.21	0.28	0.68	0.96
Bothnian Sea	655	0.38	0.28	0.10	0	0.10
	380	1.72	0.31	0.33	1.08	1.41

Figure 3.1 Reflectance of Unpolarized Sun and Sky Radiant Energy from a Horizontal Water Surface

Data is taken from Jerlov (1968).


100
90
80
70
60
50
40
30
20
10
0
% REFLECTANCE
0
10
20
30
40
50
60
70
80
90
SOLAR ZENITH ANGLE

region, and on almost wavelength-independent particle scattering. Therefore, in the clearest ocean waters with the sun at the zenith, 5-7% of blue and violet light is scattered upward (Poole, 1945; Clarke et al., 1970) with little red or orange backscattering. In turbid coastal water, the red and orange will be preferentially backscattered, and the blue to ultraviolet radiation will be attenuated rapidly by absorption.

In general it would appear that under most oceanographic conditions the loss of incident radiation is in the range of 10-20%. The 80-90% remaining in the water column must then be absorbed by the inorganic salts, the water, and the organic materials. The inorganic salts contribute nothing to absorption of seawater from 580-790 nm (Sullivan, 1963), as was demonstrated by comparing distilled water to artificial seawater solutions. Towards shorter wavelengths, a weak steady increase in absorbance is observed in artificial seawater (Lenoble, 1956; Armstrong and Boalch, 1961), but, when compared to natural seawater, the absorbance is considerably less at any given wavelength (Figure 3.2). Gelbstoff is believed to be the agent responsible for this difference in absorbance, and its highly variable concentration with location appears to explain much of the large differences in attenuation, especially in the ultraviolet, which are found for different water masses (Figure 3.3).

The other possible light absorbent in seawater is the water itself. For most purposes in spectroscopy or photochemistry, water is considered to be transparent until the far ultraviolet is reached. However, in oceanic waters where long light pathlengths are possible, water may play an important role in absorbing light energy. Estimating its importance is difficult in view of the fact that its extinction coefficient is not

Figure 3.3 The variation of Photon Flux with Depth in Different Water Types

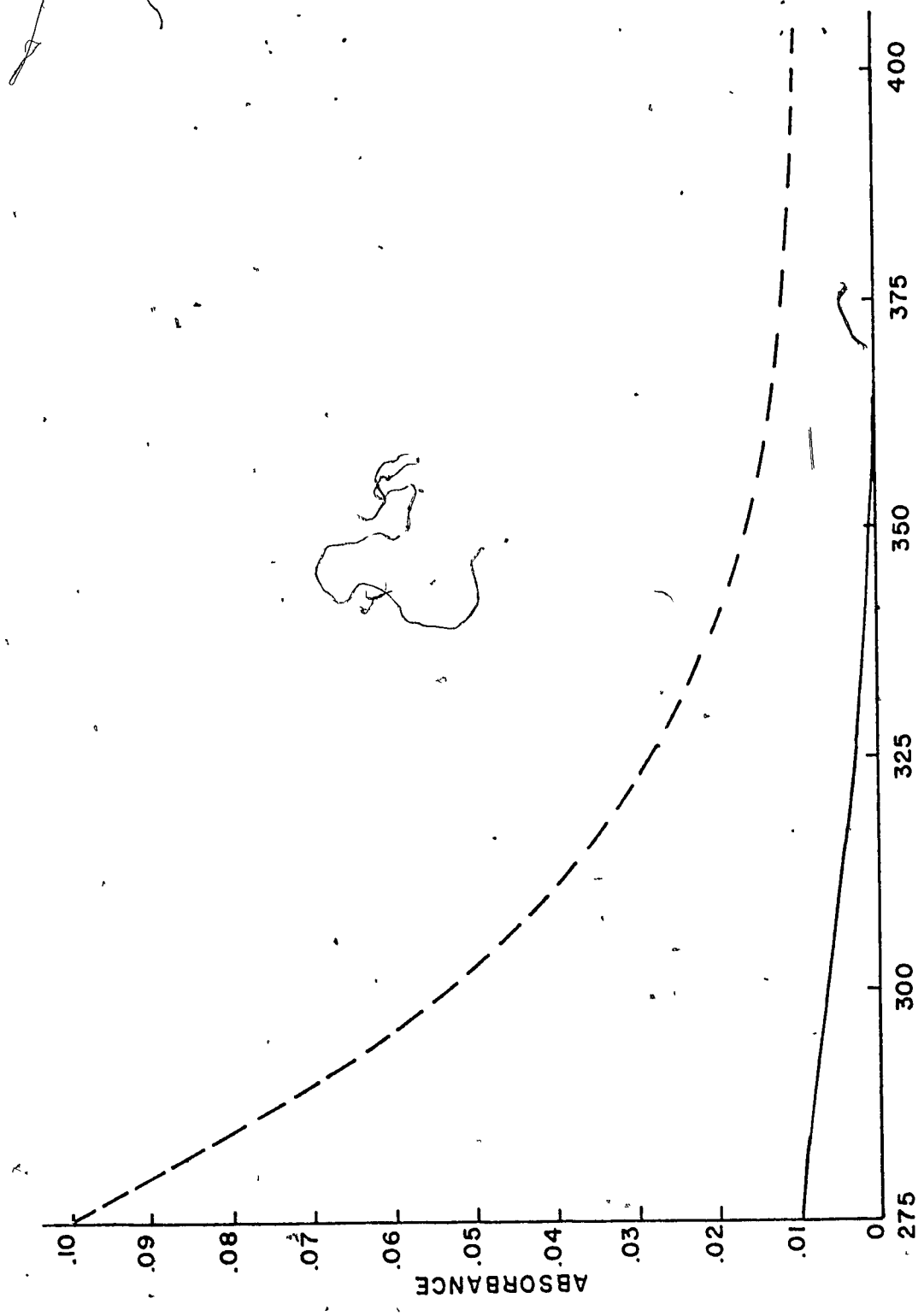
Types I, IA, IB, II, and III represent different oceanic waters and types 1, 3, 5, 7, and 9 represent coastal waters (Jerlov, 1968). Surface light flux measurement was centered at 350 nm with a bandwidth of 10 nm (Pettit, 1932). Light measurement was made at 32°N latitude with sun in zenith.

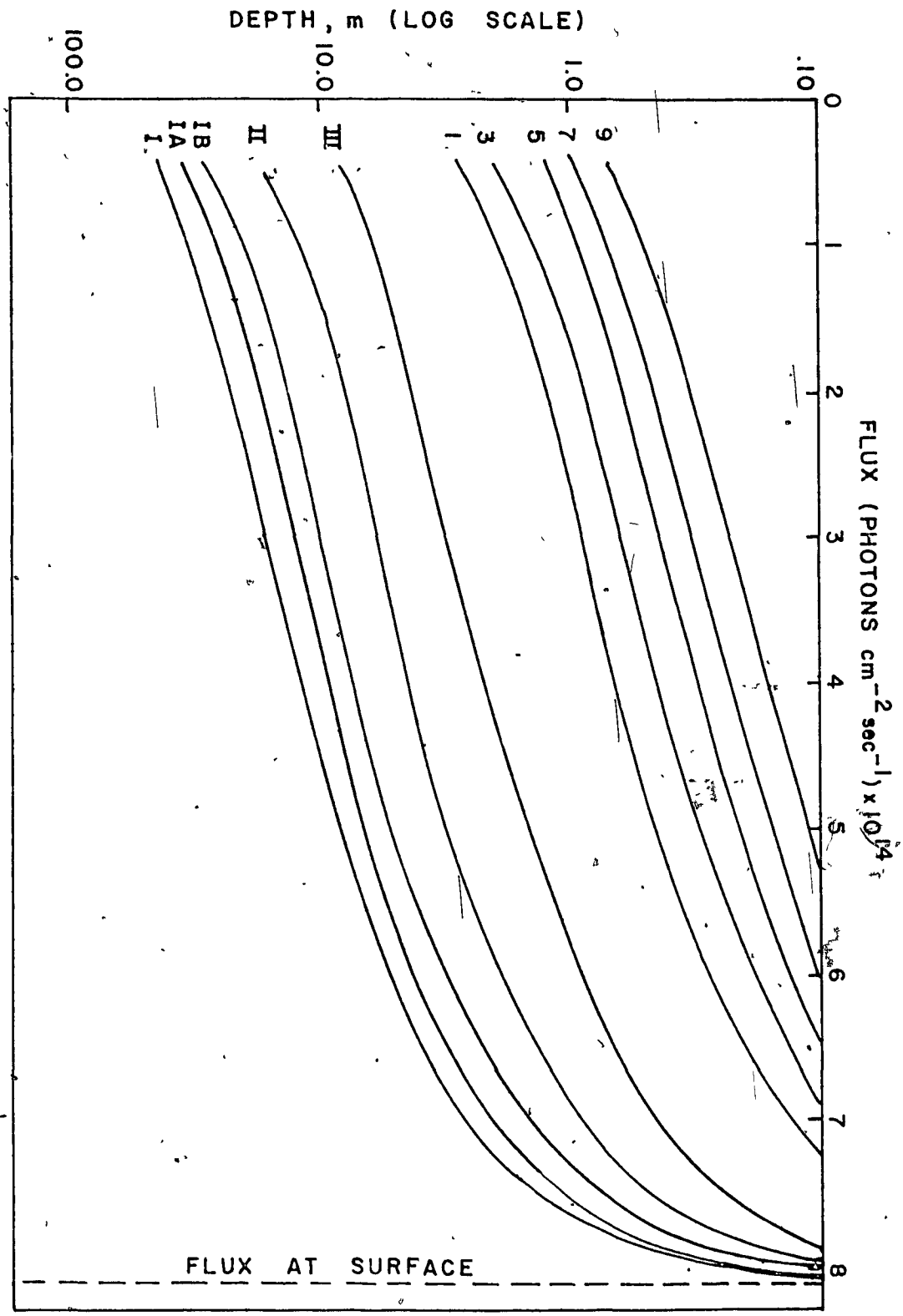
Figure 3.2 Ultraviolet Absorbance of Seawater and Artificial Seawater

Spectra were obtained in 10 cm cells against distilled water. Seawater was collected in July in Halifax Northwest Arm at a depth of 10 m and is approximately 31‰ salinity. The seawater was filtered through a .22 μ filter immediately before running spectra.

— — — seawater

——— artificial seawater


ABSORBANCE
.10
.09
.08
.07
.06
.05
.04
.03
.02
.01
0
275
300
325
350
375
400
WAVELENGTH, nm


DEPTH, m (LOG SCALE)
100.0
10.0
1.0
.10
0
1
2
3
4
5
6
7
8
I
IA
IB
II
III
1
3
5
7
9
FLUX (PHOTONS cm^{-2} sec^{-1}) x 10^{14}
FLUX AT SURFACE

known with any degree of certainty for the region from 200 to 1000 nm (Hale and Querry, 1973) and agreement is even worse for the 300 to 500 nm region.

The fact that many photons are absorbed in the water column in the photochemically accessible region below 700 nm does not mean that all of this energy is devoted to photochemical processes. In fact, most of it is probably converted to thermal energy through photophysical processes. The quantitative importance or significance of a photochemical reaction under environmental conditions is again determined by comparing its rate to those of other processes which give the same end result (Eq. 3.1).

The rate of a photochemical reaction may be determined if the quantum yield and the average number of photons absorbed by the reactant in unit volume and unit time are known. For the simple case of a unimolecular photoreaction (Eq. 3.4) in monochromatic light, the application

$$A + h\nu \longrightarrow \text{Products} \tag{3.4}$$

of the Lambert-Beer Law gives Eq. 3.5.

$$\text{Rate} = -dc/dt = \phi_A I_o (1-10^{-a_A c_A l}) S/V \tag{3.5}$$

where ϕ_A = quantum yield for disappearance of A

I_o = incident intensity

a_A = absorptivity

c_A = concentration of A

l = pathlength

s = surface area of incident light

v = volume of solution irradiated

However, in seawater a number of absorbing species all present at low concentration (with the exception of water) will be competing for polychromatic natural light, which is variable in intensity and wavelength distribution with respect to depth, location, and time of day. Equation 3.6 more closely conforms to the actual environmental situation for determining the rate of reaction 3.4, where the term $(1-10^{-1\Sigma ac})S/V$

$$-dc_A/dt = \frac{S}{V} \sum_{\lambda_1 \to \lambda_2} \phi_A I_0 (1-10^{-1\Sigma ac}) \frac{a_A c_A}{\Sigma ac} \qquad (3.6)$$

represents the total light absorption by the solution, and the ratio $\frac{a_A c_A}{\Sigma ac}$ represents the fraction of light absorbed by the reactant itself.

Recently, a method for calculating environmental direct photolysis rates has appeared in the literature (Zepp and Cline, 1977). In this method the assumption is made that ϕ is not wavelength dependent, and the rate is expressed as the first order equation (Eq. 3.7), where

$$-\frac{d[A]}{dt} = \phi \Sigma k_{a\lambda}[P] \qquad (3.7)$$

$\Sigma k_{a\lambda}$ represents the computer calculated sum of rate constants for all wavelengths of sunlight that are absorbed by the reactant. Even if ϕ is not known, the minimum half-life can be determined (Eq. 3.8) by assuming that ϕ is not likely to exceed unity at the low concentrations of

$$t_{1/2} \leq \frac{0.693}{\Sigma k_{a\lambda}} \qquad (3.8)$$

reactants encountered in natural waters.

The calculation of rates for induced reactions, where the reactant is either sensitized directly or reacts with a secondary intermediate product, becomes far more complicated. The quantum yield for such reactions has the general form of Eq. 3.9.

$$\phi_i = \frac{\text{no. of molecules which undergo reaction}}{\text{no. of photons absorbed by sensitizer}} \qquad (3.9)$$

Because the step of initial photon excitation may be many steps removed from the final process being observed, ϕ_i for that process might be dependent on other extraneous conditions of the reaction system. Even where energy transfer occurs as a first step between the sensitzer and energy acceptor, environmental perturbations may alter ϕ_i. Before this can be tested, however, the sensitizers in seawater must be characterized, quantified, and their absorption spectra carefully determined.

Rates for photochemical reactions are usually measured using a narrow wavelength region. Although the use of this approach in marine photochemistry may have merit in ascertaining specific reaction mechanisms, it should not be considered as a practical solution for determining environmental rates, where the light flux is always polychromatic. Not only can polychromatic radiation affect the overall rate, but it can also affect products and product distributions. As an example of this, consider the photolysis of copper (II) glycinate at 360 nm. The typical products for aminocarboxylates under these conditions are formaldehyde, CO_2, and NH_3. If sea surface sunlight had been used instead, the observed products might also have included CO, H_2, and peroxyformic and

formic acids from the photolysis of formaldehyde in the wavelength region from 290-360 nm.

3.2. Experimental conditions and their control

It is essential in the study of any chemical reaction to control carefully those reaction conditions which may influence reaction results. This is particularly important in marine photochemistry, where the complexity of the reaction system necessitates the control of many variables which may be inherent properties of seawater or extraneous properties resulting from sample handling or experimental design. Unlike the impracticable aspects discussed in section 3.1, the conditions considered in this section are amenable to experimental control or measurement, and the discussion and reaction parameters outlined here served as a basis under which the experimental results in the following sections were obtained.

3.2.1. Contamination

In any work involving the manipulation or analysis of components at microgram or lower concentrations, extreme caution must be exercised to prevent contamination. This is particularly a problem in environmental photochemical studies, where many different contaminants could introduce erroneous results. There are three possible sources of contamination: (1) sampling, sample handling or storage; (2) the reaction vessels; and (3) addition of reagents or buffers. The second source will be discussed in section 3.2.7.

The first source is an inherent part of all oceanographic studies and has created enormous problems, particularly in trace metal analysis. For this reason, with the exception of samples collected in the St. Margaret's Bay program (Section 11), nearly all sampling and sample storage was done with meticulously-cleaned glass containers. In cases where metal concentrations were of particular concern, aged polypropylene containers were used. For the St. Margaret's Bay program, sampling was performed with a Niskin bottle with an external closing mechanism. After collection, the water was removed from the bottle in the minimum possible time. This same sampler was used for all samples collected in that program.

The third source is often justifiably ignored when dealing with chemical reactions where relatively high concentrations of reactants are used, and high to moderate product conversions are obtained. The contamination problem is greatly amplified when dealing with reactants and products at micro- to nanomolar levels. If, for example, the commonly accepted premise that artificial seawater represents the ultimate sample blank is considered on the basis of an analytical comparison (Table 3.4), it is obvious that impurities introduced from high quality salts used to prepare artificial seawater exceed the natural seawater levels for some trace constituents. Even the water itself used to prepare artificial seawater, reagent solutions, or buffer solutions is a potential source of contamination; in the case of pulse radiolysis and flash photolysis studies, it is necessary to take elaborate precautions to purify it.

Table 3.4 Comparison of Some Artificial Seawater Impurities to Their Counterparts in Natural Seawater

Values for artificial seawater were obtained from manufacturer's analytical data for the individual salts used in its preparation. Natural seawater values were obtained from the literature (Brewer, 1975).

Impurity	Molarity in Artificial Seawater	Average Molarity in Natural Seawater	Concentration Ratio ASW/SW
iron	8×10^{-7}	3.5×10^{-8}	23
Manganese	4×10^{-7}	3.6×10^{-9}	111
Chlorate & nitrate	2.4×10^{-6}	variable	50 to .4
iodide	1×10^{-6}	5×10^{-7}	2
other N-compounds	.1 to .2 ppm	---	---

Artificial seawater and waters that were used in experiments were evaluated for purity on the basis of the extent to which a dissolved nonabsorbing substrate underwent photolysis under typical reaction conditions. In Table 3.5, results for different solutions are given which indicate the variability that exists. Three NaCl solutions (A, B and C) all gave widely different results, with C being the most reactive even though it was the best grade (99.999% purity) of the three. Interestingly, the artificial seawater solution D was less reactive than solution A, which contained the salt from which it was made. The lower reactivity in artificial seawater may be indicative of impurities added with other salts used in the formulation, which quench the reaction, but could also be explained by the increased concentration of the glycine-alkaline earth metal complexes formed in artificial seawater.

The high impurity levels of transition metals in all the reagent grade salts used to prepare artificial seawater makes its use as a control reaction blank questionable. The concentration of many of these metals can be significantly reduced by passing the media through highly purified Chelex 100 resin (Davey et al., 1970). Unfortunately, the photoreactivity for different added organic substrates was found to increase when this was tried for both NaCl and artificial seawater solutions (Table 3.5, I, J and K). The increased reactivity was attributed to the formation of NO_2^- and NO_3^-, which were believed to be microbially and photochemically derived from micromolar quantities of organic materials which leached from the Chelex 100 column.

As a result of contamination problems implicit in the use of

Table 3.5 Photoreactivity Test on Various Solutions

Solutions were irradiated in merry-go-round system[4] for 2 hours.

Analytical procedures are described in Chapter 5 and in Appendix 2.

[1] For the 1-^{14}C glycine tests, substrate decomposed only refers to that based on $^{14}CO_2$ yield and is not necessarily quantitative in terms of the amount of glycine reacting.

[2] Super Q water refers to water purified in Millipore system (Appendix 1).

[3] see Appendix 1

[4] see Section 4.2.5.

Solution Desciption	Photoreactivity Test, Substrate and Concentration	Substrate Decomposed[1] $M\ l^{-1}$
A) Super Q Water[2]	1×10^{-7}M 1-^{14}C Glycine	$.52 \pm .03 \times 10^{-10}$
B) Low Organic Water[3]	1×10^{-7}M 1-^{14}C Glycine	$.74 \pm .03 \times 10^{-10}$
C) .68 M NaCl (Fisher Scientific - Lot 744240)	1×10^{-7}M 1-^{14}C Glycine	$1.58 \pm .08 \times 10^{-10}$
D) .68 M NaCl (Fisher Scientific - Lot 705337)	1×10^{-7}M 1-^{14}C Glycine	$1.83 \pm .09 \times 10^{-10}$
E) .68 M NaCl (Spex Ind. - # 1352)	1×10^{-7}M 1-^{14}C Glycine	$2.80 \pm .11 \times 10^{-10}$
F) Artificial Seawater (Using NaCl C)	1×10^{-7}M 1-^{14}C Glycine	$.61 \pm .03 \times 10^{-10}$
G) Super Q Water	5×10^{-6}M Methionine	$.18 \pm .03 \times 10^{-6}$
H) Super Q Water - Distilled off KM_nO_4	5×10^{-6}M Methionine	$.02 \pm .02 \times 10^{-6}$
I) .68 M NaCl	5×10^{-6}M Methionine	$.37 \pm .01 \times 10^{-6}$
J) Solution (I) - Chelex 100 Treated	5×10^{-6}M Methionine	$.63 \pm .01 \times 10^{-6}$
K) Solution (J) - UV Irradiated w/Full Arc	5×10^{-6}M Methionine	$2.49 \pm .05 \times 10^{-6}$

laboratory reagents, their use was limited as much as possible. Where necessity required that they be used on a regular basis (e.g., buffer solution), they were used only at the lowest possible concentration in order to minimize the introduction of impurities that might influence the experimental results.

3.2.2. pH

The surface pH values of the oceans range from 7.6 to 8.4, with a mean near 8.1. The buffer capacity is rather low and is controlled primarily by the carbonate system. The pH can, therefore, be easily altered by changing the dissolved $CO_{2(g)}$ content. Biological processes, physical agitation or the addition of even low concentrations of weakly acidic or basic reagents can alter $[H^+]$ by 2 to 3 orders of magnitude.

Some photochemical reactions in seawater may be highly pH dependent (Figure 6.4). Therefore, in order to avoid ambiguities which might arise from changes in the pH, it was necessary to buffer the solutions. This was done only to the extent that the buffer capacity was just sufficient to maintain the pH during the experiment.

Three buffering systems were used for adjusting and maintaining the pH within ± .05 units (Table 3.6). Small volumes of sterile concentrated buffer solutions were used to minimize dilution and the possibility of bacterial or chemical contamination. The use of the buffer solutions was found not to cause a significant change in the photochemistry of test substances used, at least in the concentrations added to experimental media.

Table 3.6 Buffer Solutions Used to Adjust pH of Photochemical Reaction Media

Buffer	pH of Buffer Solution	Concentration of Buffer M l^{-1}	Used for pH Range	Volume ml l^{-1}
sodium borate + HCl	9.4	.12	8 to 9.5	2.5 to 10
sodium carbonate	11-12	1.0	8 to 8.2	.25 to 1
potassium phosphate + NaOH	8.0	.10	6 to 7.5	.5 to 1

3.2.3. Temperature

Temperature in the surface layer of the ocean varies seasonally and geographically within a range of -1 and 28°C, with a mean of approximately 20°C for the upper 100 m of the ocean between 40°N and 40°S latitude. Rates of some secondary photochemical reactions could vary greatly over this range, but in general the excitation process and most primary processes should not vary significantly over this narrow temperature range.

The primary photophysical process of fluorescence, however, can vary significantly with temperature and, for the sake of accurate comparison of natural fluorescence for different seawater samples, the measurements were always made at 25°C.

Photochemical reactions were conducted at temperatures of 20 ± .1°C for experiments where artificial light sources were used in the laboratory and at 10 ± 2°C for experiments under natural sunlight illumination. These temperatures were chosen primarily because of practical difficulties in maintaining lower temperatures for long periods. Ideally, it would have been better to use the lowest possible operating temperatures to slow the kinetics of competing biological or thermal processes. Bacterial utilization rates of organic substrates, for example, may vary by many orders of magnitude between 0 and 30°C.

There are advantages to be gained in comparing reaction results obtained at different temperatures, when mechanistic characteristics of the reaction are sought. In reaction sequences where the temperature is varied, the O_2 concentrations should be fixed in order to avoid differences due to O_2 solubility (Section 3.2.4.).

3.2.4. Dissolved gases

Of the gases in equilibrium between the atmosphere and seawater, only O_2 and CO_2 are likely to represent potential reactants present at concentrations high enough to make them important. Their solubility in seawater is dependent on both temperature and salinity (Tables 3.7 and 3.8), but in the laboratory the concentration can also vary as a function of the way in which the solution is handled. To avoid complications arising from unpredictable concentration variations, reaction solutions were equilibrated with the atmosphere by rapidly stirring them, for 30-60 minutes, at the reaction temperature before initiating the experiment.

In experiments where sealed reaction vessels were used and no atmospheric source of O_2 was available, possible O_2 consumption was limited by keeping irradiation times short and by using low concentrations of reactants. In the same way, the accumulation of CO_2 and carbonate species were not great enough to affect appreciably the total CO_2 concentration present.

3.2.5. Eliminating biological processes

Marine photochemistry should be studied by reproducing all facets of the natural environment, including the biological processes, which might act to augment or inhibit photochemical reactions or serve as essential steps in overall reaction processes. Since most of the organic materials in seawater originate from the biota, their role is certainly at some point to serve as the major source of reactants for photochemical processes. Photochemistry within any parcel of seawater in the oceans

Table 3.7 Oxygen Solubility in (Moles Liter^{-1}) x 10^{-4} in Seawater as it Varies with Chlorinity (‰) and Temperature

Solubilities are based on an atmosphere of 20.94% O_2 and 100% relative humidity (Carpenter, 1966).

Chlorinity (‰)

Temp. °C	0.0	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0
0.5	4.51	4.40	4.29	4.18	4.07	3.97	3.87	3.78	3.69	3.60	3.52
5.0	3.98	3.89	3.80	3.72	3.63	3.55	3.46	3.38	3.30	3.22	3.15
10.0	3.52	3.44	3.36	3.29	3.21	3.14	3.07	3.00	2.93	2.87	2.81
15.0	3.16	3.09	3.02	2.95	2.88	2.82	2.75	2.69	2.64	2.58	2.53
20.0	2.84	2.78	2.72	2.66	2.61	2.55	2.50	2.45	2.40	2.35	2.30
25.0	2.58	2.53	2.47	2.42	2.37	2.33	2.28	2.24	2.19	2.15	2.11
30.0	2.36	2.31	2.27	2.22	2.18	2.13	2.09	2.05	2.01	1.97	1.94

Table 3.8 Carbon Dioxide Solubility in (Moles Liter^{-1} 760 Torr^{-1}) x 10^{-2} of CO_2 in Pure Water and Seawater.

Values are taken from Murray and Riley (1971).

Chlorinity (‰)

Temp.°C	0.0	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0
0.0	7.709	7.544	7.403	7.271	7.144	7.019	6.894	6.771	6.642	6.515	6.383
5.0	6.372	6.240	6.130	6.019	5.915	5.812	5.708	5.606	5.498	5.392	5.280
10.0	5.347	5.253	5.159	5.068	4.982	4.898	4.812	4.727	4.637	4.548	4.447
15.0	4.545	4.437	4.361	4.285	4.214	4.144	4.073	4.003	3.928	3.853	3.778
20.0	3.887	3.837	3.772	3.708	3.650	3.598	3.537	3.475	3.412	3.350	3.284
25.0	3.379	3.323	3.269	3.214	3.167	3.117	3.069	3.022	2.969	2.926	2.861
30.0	2.979	2.915	2.867	2.820	2.779	2.739	2.700	2.661	2.617	2.574	2.527

could then be largely controlled by the standing concentration and characteristics of materials which are controlled by the kinetics of supply and removal by the biota.

To avoid obscuring the results of photochemical studies, it was necessary to eliminate the effects of the biota over the experimental periods. Some of the common techniques used to accomplish this are autoclaving, chemical sterilization, and filtration. Autoclaving seawater results in forming precipitates of some constituents and probably in a considerable alteration of others. ZoBell (1933) found that autoclaved seawater behaved differently than its precursor as a photochemical reaction media for the oxidation of NH_3.

Use of chemical sterilants, such as sodium azide, mercuric chloride, potassium cyanide, or ethylene oxide can, through their own participation, cause considerable changes in the photochemical characteristics of seawater. This was shown experimentally to be the case for sodium azide, mercuric chloride, and potassium cyanide. Of these, cyanide ion was found to be the most effective bacterial inhibitor at low concentrations, with as little as 7×10^{-5} M l^{-1} completely stopping the microbial utilization of amino acids in seawater. However, even at this low concentration the photoreactivity of glycine was considerably altered (Figure 3.4). Therefore, the use of chemical sterilization of seawater samples was not used.

Perhaps the only means of eliminating the biota from seawater samples without introducing major alterations in the sample's properties is through the use of filtration. This method was used almost exclusively

Figure 3.4 Glycine Photoreactivity in Seawater Containing 25 X TMEM[1] Enrichment

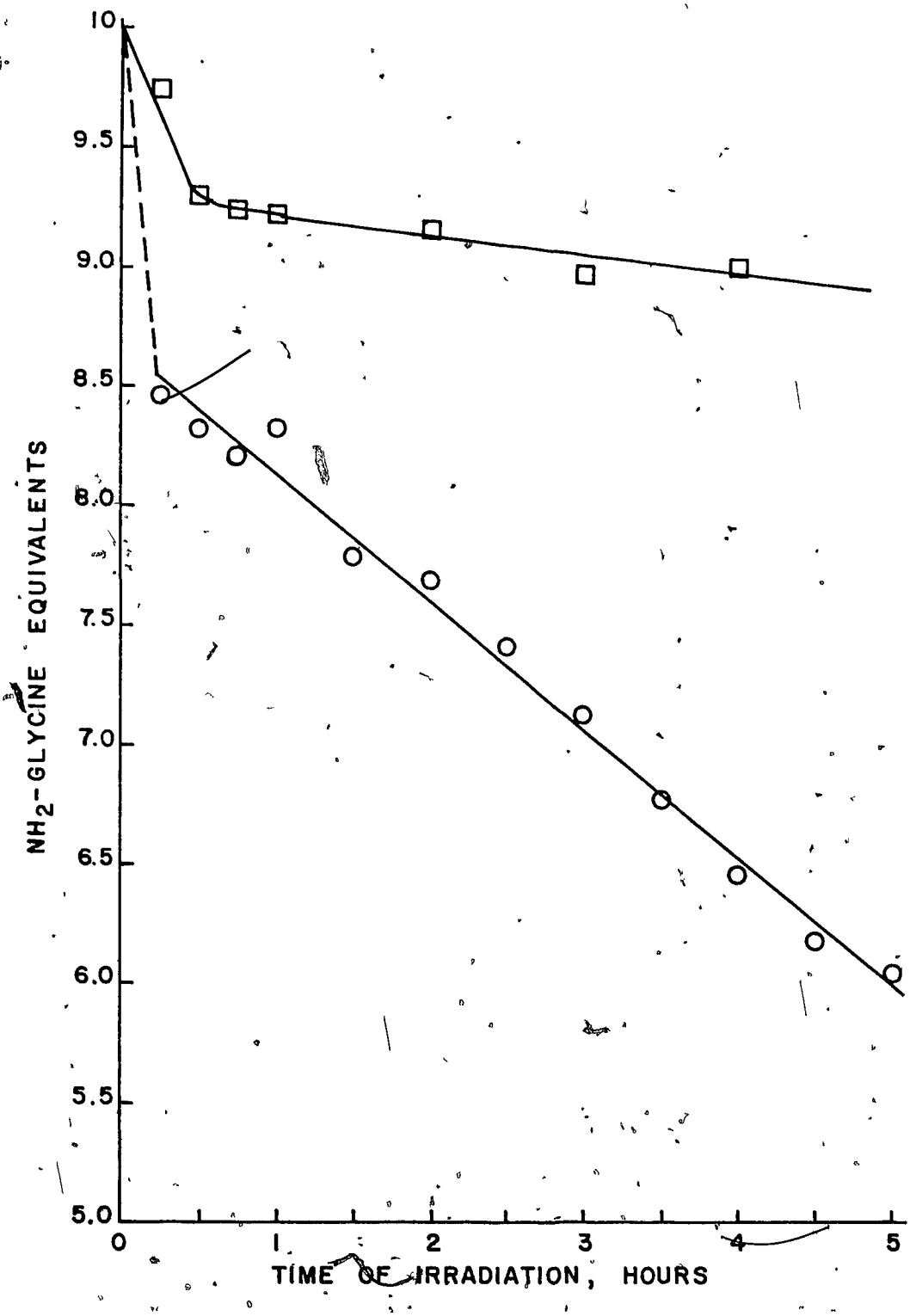
Irradiation was performed in immersion well system (Section 4.2.4.). The Fluram analysis was used to measure the remaining glycine.

○ with 1×10^{-4} M l^{-1} of KCN added as chemical bacterial inhibitor

□ without KCN

[1] see Chapter 8

60


10
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
NH2-GLYCINE EQUIVALENTS
0
1
2
3
4
5
TIME OF IRRADIATION, HOURS

in preparing seawater samples, although it is still fraught with difficulties. Introduction of contaminants from the filter, adsorptive removal of organics by the filter (Quinn and Meyers, 1971), and the removal of possible particulate reaction surfaces were three of the major problem areas connected with this technique. The most unsettling problem however, concerned the observation that for some water samples it was impossible to remove the bacteria by multiple filtrations with .22μ filters.

When microbial contamination did occur, detection was possible by comparing the results from simultaneous light and dark reactions run under otherwise equivalent conditions. Sterile solutions gave neglible dark reactions when compared to light reactions. The extent of light reactions was considered to be the difference between the light and dark reactions. Dark reactions then represent the summation of all dark processes including microbial activity, abiotic reactions, and possibly the activity of cell-free enzymes (Kim and ZoBell, 1974). The source of extensive dark reactions was usually traced to microbial contamination of the sample.

It was found that, under the high light intensities used in the merry-go-round or immersion well systems, microbial activity was completely inhibited (Figure 3.5 and 3.6). In these systems the $^{14}CO_2$ formed from decarboxylation of 1-^{14}C glycine in the irradiated samples was entirely accounted for by photolysis. A corresponding loss of bacteria viability was indicated by the disappearance of ATP during the irradiation, and by post-irradiation inactivity of the bacteria as

Figure 3.5 Light Induced Inhibition of Bacterial Glycine Utilization and Accompanying ATP Loss

Freshly filtered (with 5 μ filter) seawater was buffered to pH 8.1 with borate buffer and enriched with 2.5 ml of sterile SST media (Stein, 1973) per 2000 ml of seawater. A bacterial culture was grown under aerobic conditions at 20°C with normal room lighting. At early stationary phase a few milliliters of this culture media was diluted to 100 ml with fresh seawater of the same salinity and buffer strength. Just before initiating the irradiation 1 μm l^{-1} of 1-^{14}C glycine was introduced.[1] The irradiation was conducted in the merry-go-round system.[1]

Analytical procedures are described in Chapter 5 and in Appendix 2.

—●— $^{14}CO_2$ formed in dark

—■— $^{14}CO_2$ formed in light

—▲— ATP in light

—○— ATP in dark

[1] see Section 4.2.5.

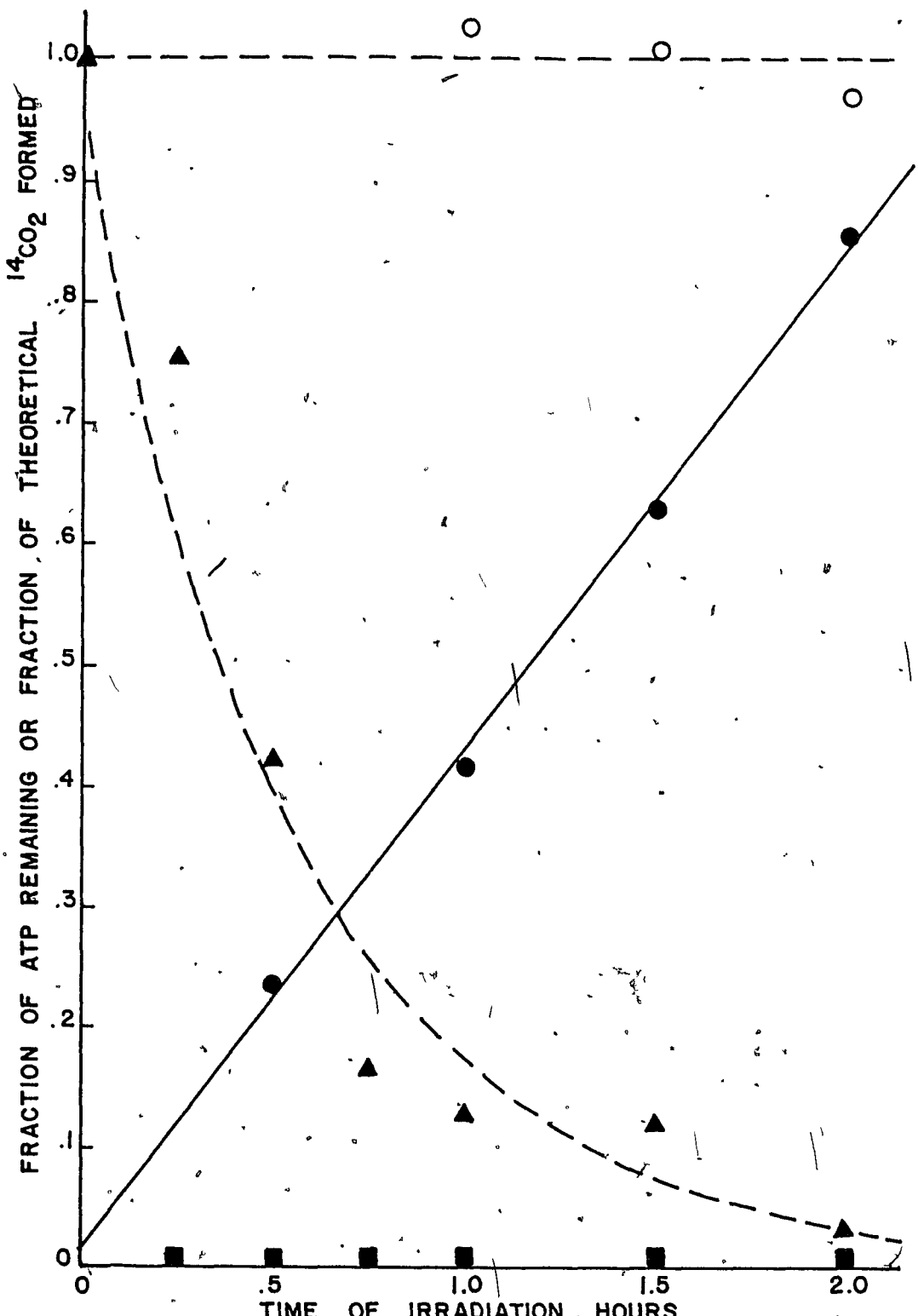

FRACTION OF ATP REMAINING OR FRACTION OF THEORETICAL $^{14}CO_2$ FORMED
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
0
.5
1.0
1.5
2.0
TIME OF IRRADIATION, HOURS

Figure 3.6 Light Induced Inhibition of

Bacterial Glycine Utilization

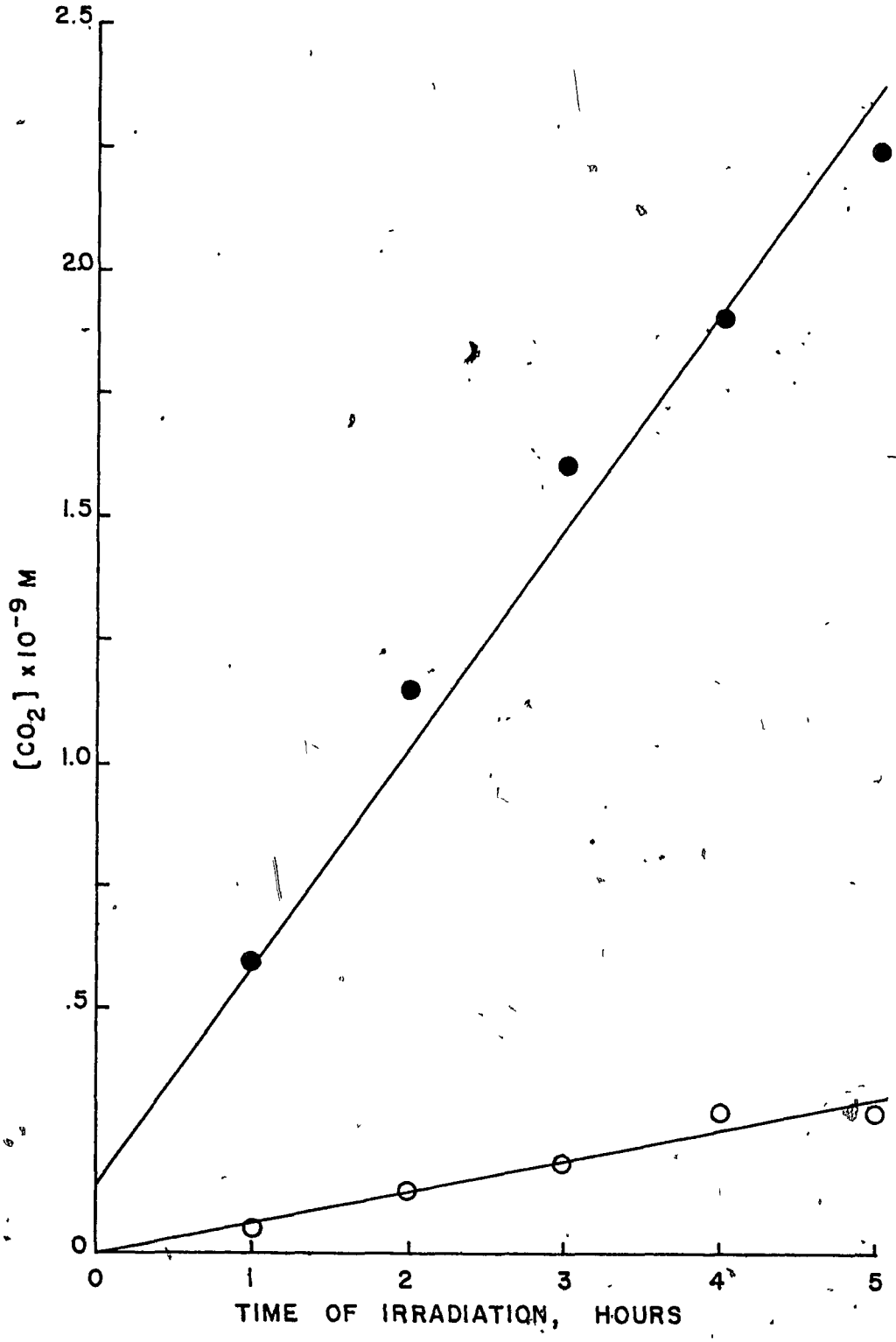
Freshly filtered (with 5 μ filter) seawater was buffered with borate at pH 8.1. The solution was gradually brought to 20°C. The solution was then enriched with 1 x 10^{-7} M l^{-1} of 1-^{14}C glycine and immediately exposed to the radiation in the inner ring of the merry-go-round system.[1]

Analytical procedure is described in Chapter 5 and in Appendix 2.

● dark control

○ irradiated sample

[1] see Section 4.2.5.


2.5
2.0
1.5
1.0
.5
0
$[CO_2] \times 10^{-9}$ M
0
1
2
3
4
5
TIME OF IRRADIATION, HOURS

measured by the rate of labeled substrate utilized.

This inhibition was also observed for sunlight and xenon lamp system reactions (Table 3.9), although the inhibition was not total. This is apparently partially due to the lower energy distribution of these light sources in the 300-400 nm region, where the preponderant amount of the inhibitive effect was observed (Table 3.9).

3.2.6. Wavelength dependence

In order to arrive at any sort of estimate for the rates of photochemical reactions, it is necessary to know what the efficiency of the reaction is at different wavelengths. To determine the quantum yield for a reaction the following basic requirements must be satisfied:

(1) a monochromatic beam of radiation must be used;

(2) a suitable optical train must be employed;

(3) the rate of reactant disappearance or product formation must be measured;

(4) and the number of photons absorbed by the reactant must be determined either with a chemical actinometer or by physical measurement.

The first three requirements can probably be fulfilled for many seawater photochemical reactions, although the environmental relevance of using a monochromatic source should again be questioned (Section 3.16). It is the fourth requirement which deters the use of normal quantum yield measurements for marine photochemical reactions, because it is impossible to determine how much light is actually absorbed by the reactants and

Table 3.9 Wavelength Dependence of Bacterial Utilization of 1-^{14}C Glycine

The same procedure described in Figure 3.5 was used, except that the solutions were irradiated under different conditions for a two hour period.

1 see Chapter 4 for description of equipment

2 samples were irradiated in the outer ring or the merry-go-round system

3 sunlight exposure was made in late May

LIGHT SOURCE[1]	WAVELENGTH REGION NANOMETERS	% THEORETICAL YIELD OF $^{14}CO_2$ FORMED
DARK	--	76.4
HIGH PRESS. Hg[2]	> 290	5.3
HIGH PRESS. Hg	> 310	6.7
HIGH PRESS. Hg	> 400	37.3
HIGH PRESS. Hg	> 500	62.4
XENON	> 300	18.9
SUNLIGHT[3]	> 290	18.8

how much is absorbed by the extraneous compounds in the reaction media. Even the accurate determination of ε is a major porblem in a solution which has only a small light extinction, since light scattering can represent a significant portion of the measured light attenuation.

No attempt was made, therefore, to determine ϕ; instead, a rough estimate of wavelength dependence was used and the extent of a reaction was determined for specified spectral regions (Figure 3.7). These arbitrary divisions give a qualitative approximation as to the depth for which a particular reaction would be important when considered for oceanic or coastal waters with different light attenuation characteristics. From Figure 3.7 it is obvious that, at least for primary photochemical processes occurring in spectral regions A and B, only the upper few centimeters in the most turbid coastal water (Type 9) could be important. In the clearest oceanic water (Type I) this same process could be important to depths greater than 10 m.

The spectral region in which a reaction was occurring was determined using artificial light sources and intensities higher than those naturally observed. To acquire an accurate estimate of the environmental importance of a photochemical process, however, only sunlight or light sources which closely approximate natural light conditions with respect to intensity and energy distribution should be used.

To simulate environmental light conditions in the water column is a complicated task because the variation of incident sunlight intensity is a function of the latitude, water type, depth, weather, season, and time of day. The variable character of natural sunlight in the water

Figure 3.7 Spectral Regions of Radiation Obtained by Using Sharp Cut off Filters[1]

Samples were irradiated in one of the following spectral regions:

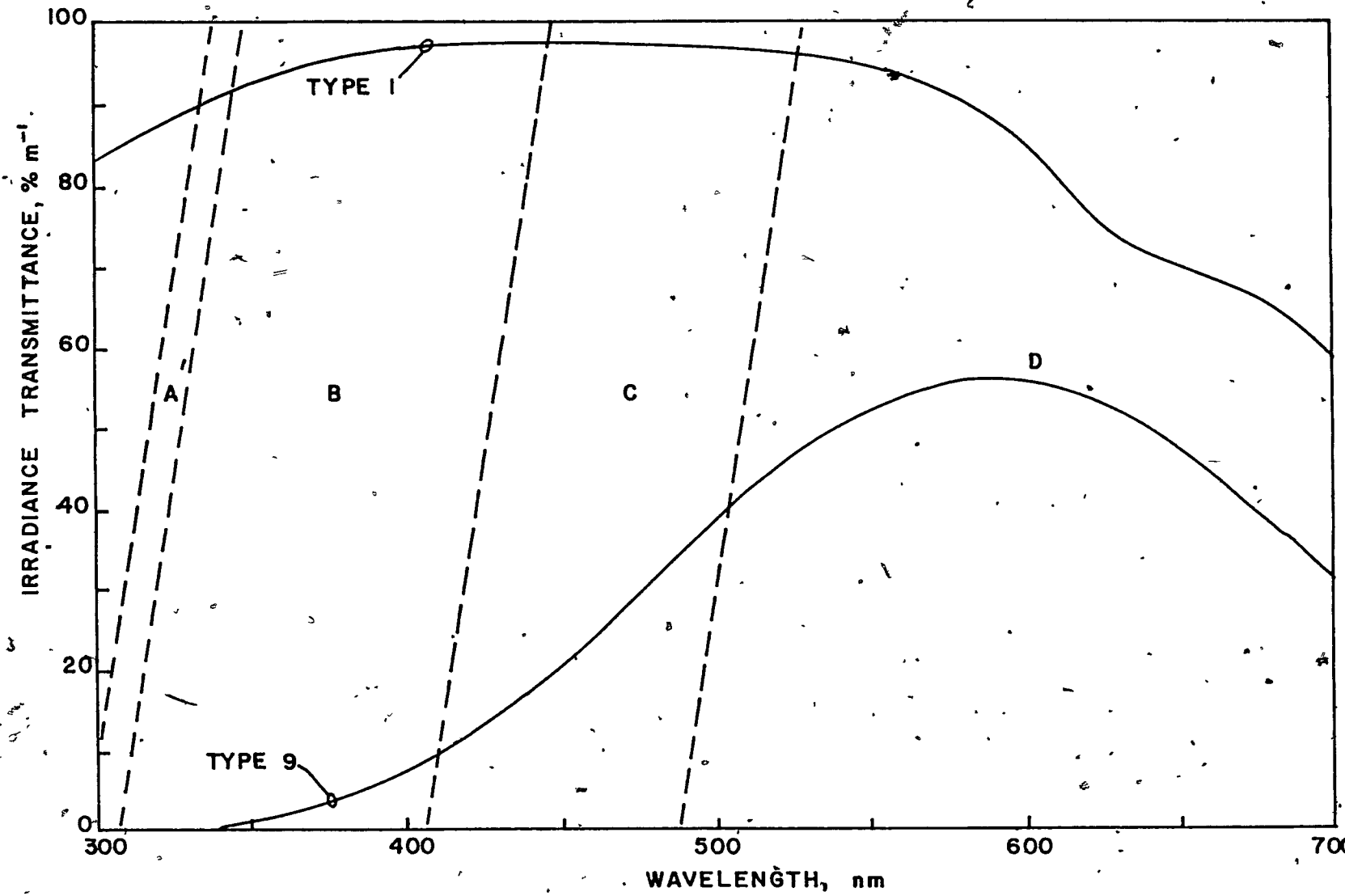
(1) A + B + C + D, > 290 nm;

(2) B + C + D, > 310 nm;

(3) C + D, > 400 nm; and

(4) D > 500 nm

[1] Refer to Appendix 6 for filter combination used.


100
80
60
40
20
0
IRRADIANCE TRANSMITTANCE, % m⁻¹
TYPE 1
TYPE 9
A
B
C
D
300
400
500
600
700
WAVELENGTH, nm

68

column manifests itself as a continual energy redistribution and intensity decrease of the incident radiation with depth.

This complex light regime is porbably best approximated by conducting in situ experiments. However, the inability to reproduce experimental conditions poses a severe limitation on gaining a fundamental knowledge of the underlying principles of marine photochemistry. The mercury arc lamps, which were used for most of the following work, were not intended to provide accurate simulation of natural conditions but were applied to:

(1) measure potential photoreactivity of different seawater components;

(2) examine the mechanistic implications of seawater photoreactivity,

(3) measure rates and yields under carefully controlled conditions,

(4) determine the potential photoreactivity of different seawater samples,

(5) and determine the spectral regions where the reactions are occurring.

To test the validity of these findings with respect to natural environmental conditions, sunlight and a sunlight-simulating xenon source system were used.

3.2.7. Wall effects

Wall reactions are an important aspect of gas phase studies, where high diffusion rates and low concentrations allow a relatively high probability for the collision of excited species with reaction vessel

walls. The surfaces of the reaction vessel can then play a significant role in determining reaction results. Although it is difficult to find similar precedents in solution photochemistry, they probably occur but are marked by the far more significant reactions in the liquid phase. Their importance could be more significant in seawater photochemistry, where low reactant concentrations, almost transparent solutions and small product yields exist. Reaction vessel walls could serve as catalytic surface activated by components of the glass itself or by adsorbed reactants. In either case, reaction yields caused by wall effects should be proportional to the surface area in contact with the solution.

This possibility was tested by increasing the surface area-to-volume ratio and observing the results for the photochemical degradation of methionine and glycine in seawater (Table 3.10). The results indicate that, for the range of sample container sizes used in this work, no appreciable photochemical wall reactions exist.

Seawater characteristics might also be modified in small volume containers by contamination of the solution by substances desorbed from the walls, or by loss of hydrophobic or reactive materials to the walls. This presents a major complication in the analysis of trace metals (Robertson, 1968).

Adsorption of bacteria to container walls of small vessels represents a further problem, in that a considerable acceleration in bacterial activity can occur (Zobell and Andersen, 1936; Heukelian and Heller, 1940; Khaylov and Finenko, 1968). It is essential that both the reaction vessel and the reaction solution be sterile if such problems are to be avoided.

Table 3.10 Test for Wall Reactions in Small Volume Reaction Vessels

The solutions were prepared in sterile 0.7 M NaCl solution. The surface area-to-volume ratio was attained for methionine solutions by using different diameter tubes and for glycine solutions by using thin walled concentric tube inserts. All solutions were irradiated for 2 hours in merry-go-round system.[1]

Analytical procedures are described in Chapter 5 and in Appendix 2.

[1] see Section 4.2.5.

Reaction Vessel Surface Area/Volume Ratio	Substrate and Concentration in M l^{-1}	Substrate Reacting M l^{-1}
3.4	5×10^{-6}, methionine	$9.0 \pm 1.1 \times 10^{-7}$
5.2	5×10^{-6}, methionine	$12.1 \pm 1.0 \times 10^{-7}$
6.9	5×10^{-6}, methionine	$10.0 \pm 0.4 \times 10^{-7}$
10.1	5×10^{-6}, methionine	$9.5 \pm 1.3 \times 10^{-7}$
1.6	1×10^{-7} $1\text{-}^{14}C$ glycine	$5.18 \pm 0.26 \times 10^{-11}$
3.7	1×10^{-7} $1\text{-}^{14}C$ glycine	$4.92 \pm 0.25 \times 10^{-11}$

4. EQUIPMENT AND TECHNIQUES

4.1. Light sources

Choosing the correct light source is of fundamental importance in photochemistry. A number of criteria should be considered when making the selection (Calvert and Pitts, 1966). The source should be selected not only on the basis of spectral distribution, but should also be of sufficient intensity for the purpose in mind. The required intensity is primarily determined by the reaction rate; high intensities are often required where reactions with low quantum yield would otherwise necessitate impractical irradiation periods. This problem could be encountered in marine photochemistry when a process might have a high R_s value and yet be impossible to observe in short-term sunlight exposures because of a slow reaction rate.

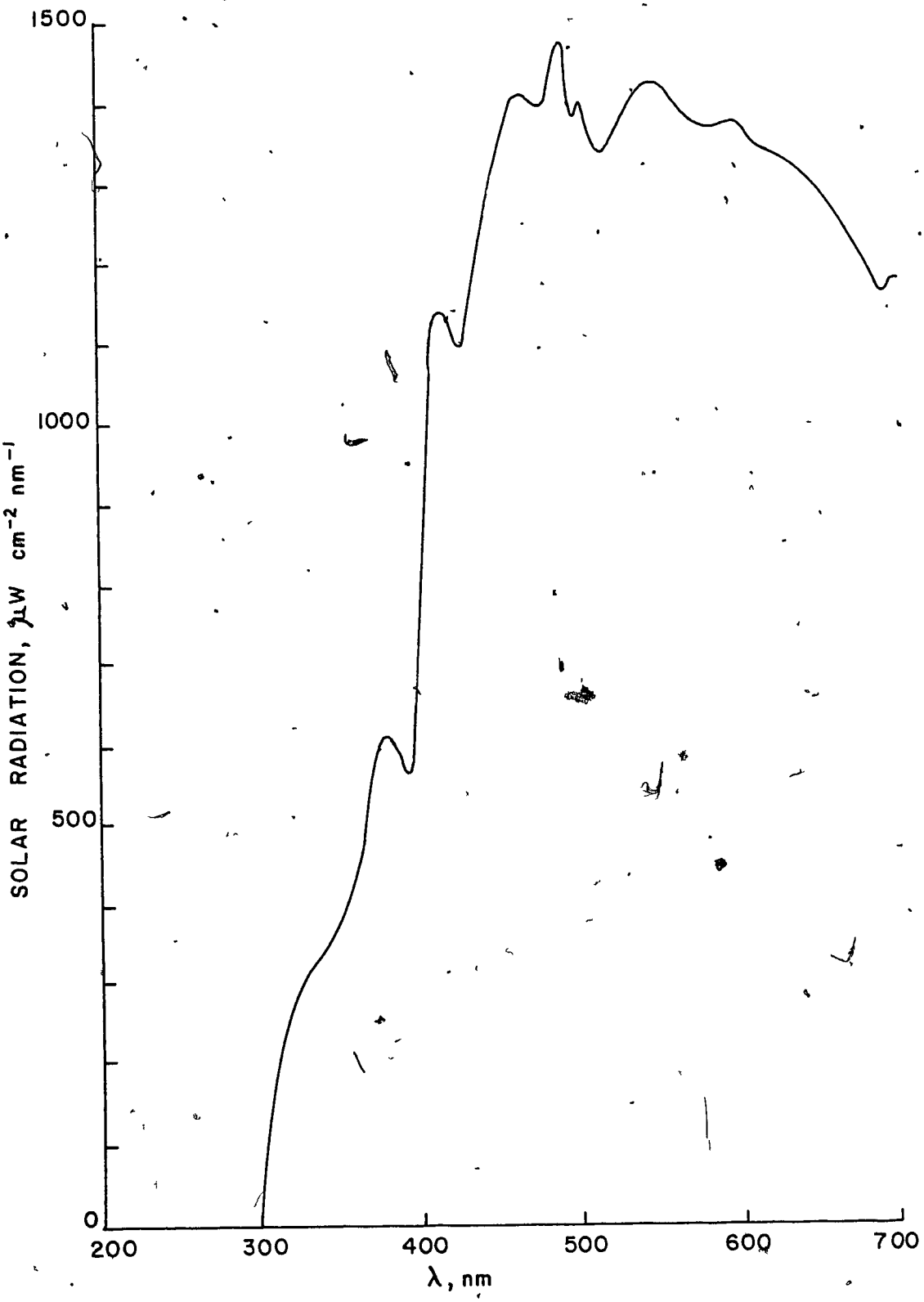
The spectral distribution is also an essential consideration in marine photochemical studies, where radiation from any source should be restricted to wavelengths greater than 290 nm, the lower limit for sunlight at the sea surface.

4.1.1. Sunlight

The solar energy distribution at 41.5° North latitude is shown in Figure 4.1. This approximates the light energy reaching the sea surface at this latitude near noon on a midsummer day. In the visible region (400-700 nm) this distribution does not vary much throughout a day or on a seasonal basis. This is not true for the ultraviolet region,

Figure 4.1 Spectral Distribution of Direct Solar Radiation at Sea Level (adapted from Jerlov, 1976).

Conditions: air mass 1.0, precipitable water 10 mm., ozone 3.5 mm.

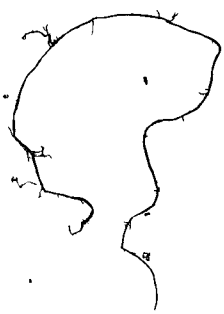

1500
1000
500
0
SOLAR RADIATION, μW cm⁻² nm⁻¹
200
300
400
500
600
700
λ, nm

where large variations occur, especially as the shorter wavelengths are approached (Figures 4.2 and 4.3). This is largely a result of seasonal and daily changes in the zenith angle of the sun, which determines the air mass through which solar radiation must penetrate before reaching the earth's surface.

When using solar radiation as the light source in comparative photochemical experiments, the quantity (flux) and the quality (energy distribution) should be measured. The quantity of radiation is easily determined with the use of a radiometer. However, unless a spectroradiometer is used, the measurements are not indicative of changes in the quality of radiation, especially at the shorter wavelengths. Photochemical reactions with high rates in the ultraviolet region would be highly dependent upon the season, time of day, and weather. Measurement of the total incident visible and ultraviolet radiation is of little value in correcting for such variations, since the ultraviolet represents only a small fraction of the total energy. For comparative studies conducted at different times, variations in the total radiant energy, as measured by the typical solar radiometer, might vary by only a few percent, and yet results for a photoreaction with a high quantum yield in the ultraviolet could conceivably be orders of magnitude apart.

Although it is technically feasible to measure variations in the quality of solar radiation, it may not be warranted because of the expense or the effort involved in making such measurements. Even if such measurements were made, the complexity of the variations in the incident solar radiation and the seawater reaction media might make comparative interpretations difficult.

Figure 4.2 Variation of Total Intensity (Sun and Sky) During the Day for Different Ultraviolet Wavelengths in September (Adapted from Koller, 1965)



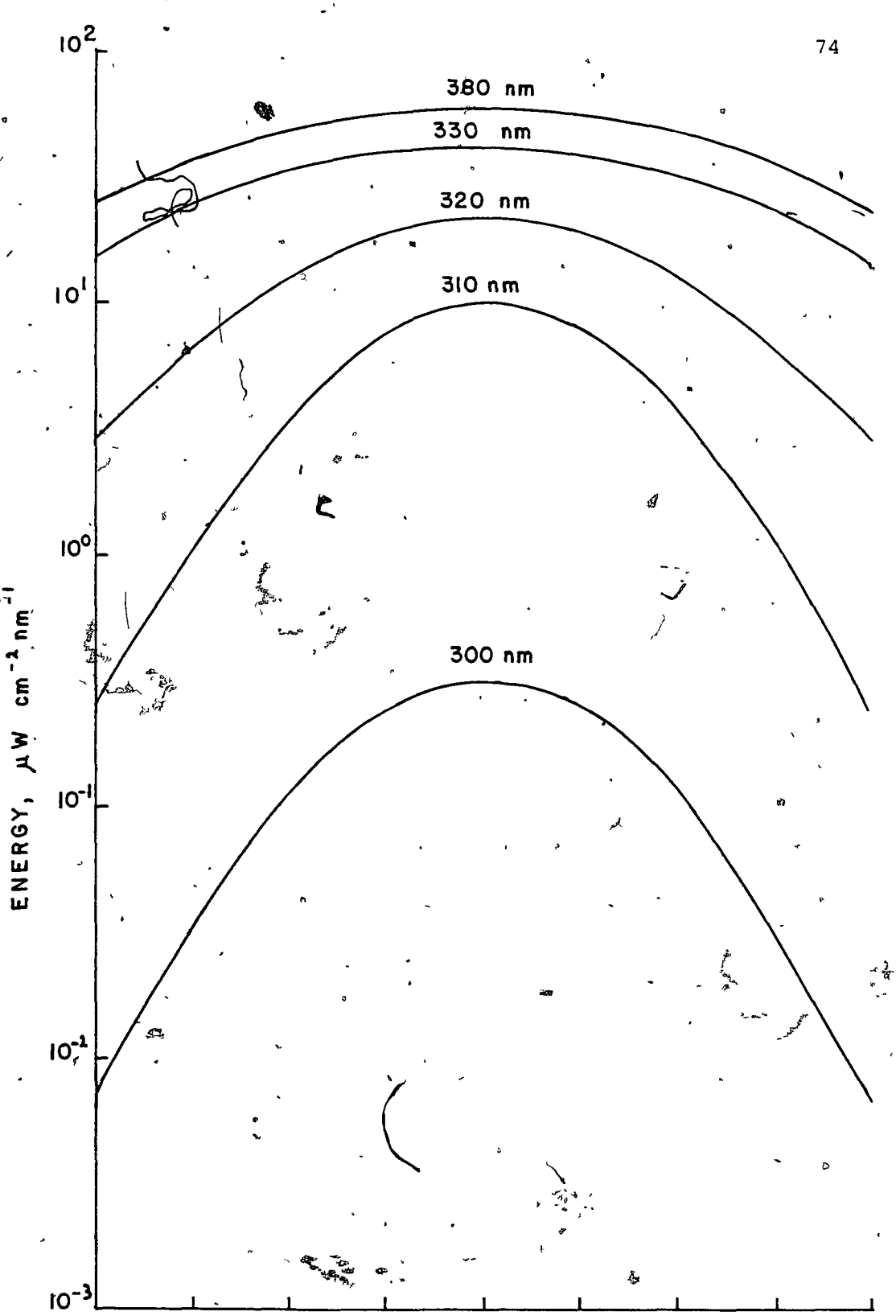
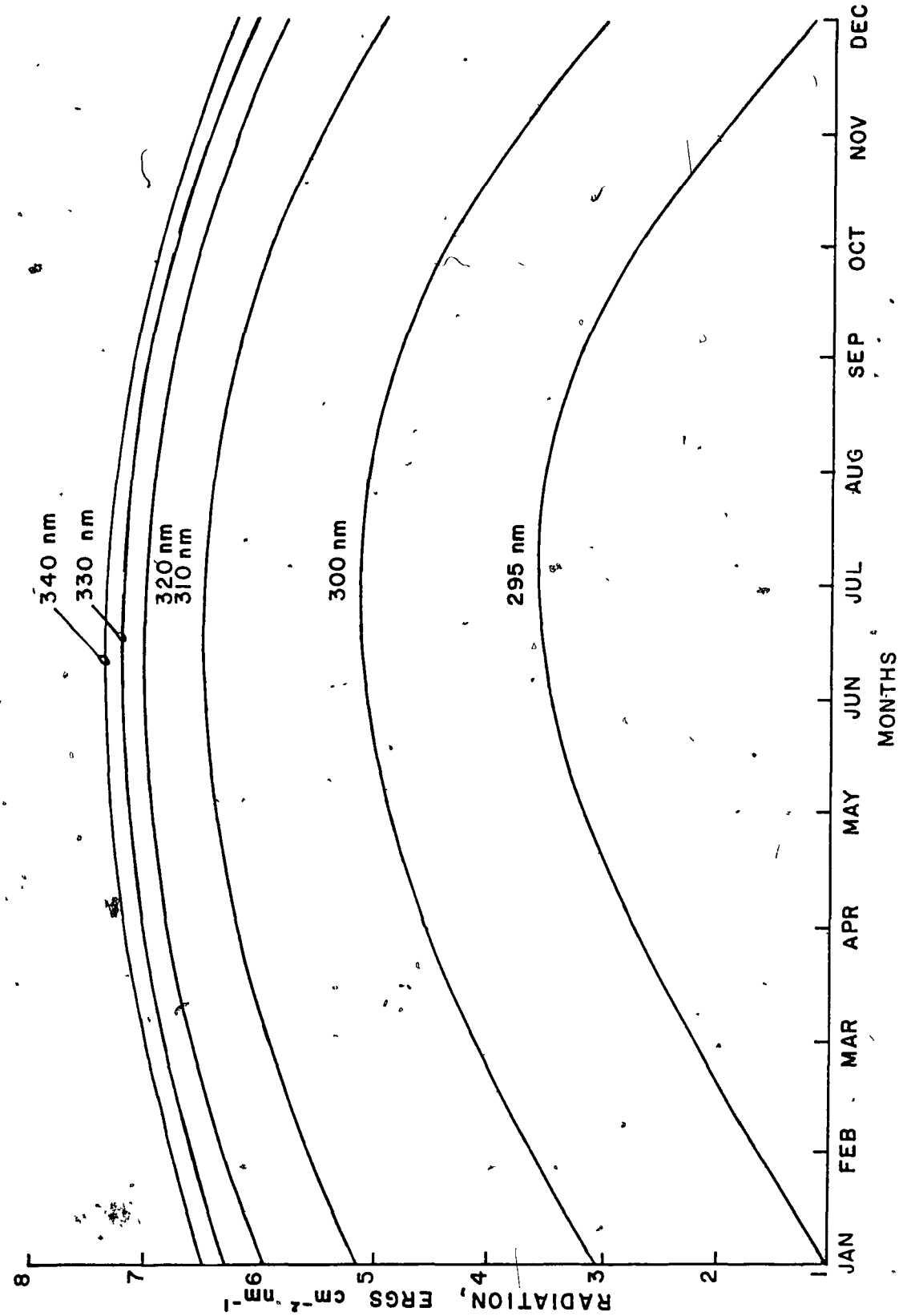

380 nm
330 nm
320 nm
310 nm
300 nm
ENERGY, μW cm⁻² nm⁻¹
10²
10¹
10⁰
10⁻¹
10⁻²
10⁻³
8
9
10
11
12
13
14
15
16
DAYTIME HOUR

Figure 4.3 Monthly Variation in Solar Radiation at 45° Latitude for Various Ultraviolet Wavelengths (Johnson et al., 1976)


340 nm
330 nm
320 nm
310 nm
300 nm
295 nm
RADIATION, ERGS cm⁻² nm⁻¹
8
7
6
5
4
3
2
1
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
MONTHS

75

76.

In view of the difficulties involved in monitoring incident solar radiation accurately, the sun was used as a source to test the validity and to approximate the environmental magnitude of reactions which were otherwise thoroughly investigated using artificial light sources. For this purpose, experiments using natural sunlight were usually conducted only during a period in the middle of the day. This policy provided the least variation in the quality and quantity of the radiation, but also limited the extent to which a reaction proceeded before termination. Unfortunately, clouds and fog in Halifax precluded the success of this policy in over 90% of the sunlight reaction attempts over a period of two years. Even on days in which only 5-20% cloud cover prevailed, which is typical for Halifax, the intensity would fluctuate widely as a passing cloud extinguished most of the direct sun energy component. In the case of intensity dependent reactions, this is a particular problem, and anomalous results could be obtained.

4.1.2. Artificial light sources

Many of the inherent problems of sunlight reactions can be eliminated by using an artificial light source, for which the quantity and quality of radiation are stabilized. The quantity and quality of radiation from an artificial source can, however, vary considerably over long periods of usage. This is especially true for short ultraviolet wavelengths. Most of these changes in output occur during the early stages of lamp usage. The output of the high pressure mercury lamp (Hanovia 679A) used for many of the following experiments decreased by nearly 50% during the first few weeks of regular usage. Within

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several weeks this instability leveled off, and a fairly consistent output was observed after that. Similar changes were noted for all of the arc sources used (Table 4.1). Nevertheless, the normalization of the results from different experiments was possible if the[illegible]e obtained during a relatively short interval during the [illegible]fetime. This assured that changes in intensity for different wavelengths were closely approximated by the total spectrum intensity change. It was possible to compare different experiments in which the radiation was polychromatic by normalizing the results using either a total or a specific bandwidth intensity measurement.

The major problem in using artificial light sources to examine environmental photochemical problems is the difficulty of simulating the solar spectral energy distrubution. The wider the spectral region to be simulated, the more difficult the task becomes. The spectral energy distribution for the high pressure xenon lamp (Table 4.1) gives one of the best approximations for sunlight. Xenon lamps are often used to simulate a solar constant (total solar energy outside of earth's atmosphere). In this work it was employed with a Corning 0-54 filter (Appendix 4) to approximate sea surface sunlight over 1 in^2. The filter transmits only wavelengths greater than 300 nm, but does not sufficiently reduce the near-ultraviolet to make it a close approximation for sunlight in this region. Nevertheless, seawater photochemical reactions run in the xenon reaction system described in Section 4.2.6. gave quite similar results to those observed in sunlight for the same reactions.

Table 4.1 Approximate Percentage of Spectral Energy Distribution in Specific Bands

Values have been calculated from radiated spectral energy distribution for:

sunlight - total sun and sky light at 41.5°N on clear midsummer day (Koller, 1965)

fluorescent - General Electric F40D

sunlamp - General Electric RS-275

high pressure mercury - General Electric XE5000

phosphor coated - General Electric MV 1000/c/BUH

LIGHT SOURCE	WAVELENGTH BANDS IN NANOMETERS									
	290-300	300-310	310-320	320-340	340-360	360-380	380-400	400-500	500-600	600-700
sunlight	.01	.12	.40	1.75	2.39	2.86	3.58	30.40	31.70	26.79
fluorescent	--	.02	.50	.12	.25	1.95	2.20	40.00	40.41	14.55
sunlamp	1.01	2.97	7.69	2.01	1.22	22.44	1.14	21.31	36.33	3.76
high pressure Hg	4.35	8.20	12.52	2.52	1.05	20.50	.79	18.77	29.97	1.33
xenon	1.24	1.36	1.50	3.27	3.67	4.25	4.91	30.57	25.25	23.98
phosphor coated (tower tank light)	--	--	.02	.17	.83	3.03	3.68	21.62	45.58	25.04

Unlike the continuous spectrum of the xenon lamp, the mercury arc lamps have an output which is characterized by strong lines. The GE RS-275 sunlamp is a medium pressure mercury lamp for which the shorter ultraviolet radiation ($\lambda < 280$ nm) are eliminated by passage through an ultraviolet absorbing envelope. In photochemical experiments, this lamp was used in conjunction with a Corning glass 7740 filter to reduce further short wavelength components of the output. Major problems existed in the use of this lamp for photochemical experiments, because of erratic and large intensity fluctuations and short lifetimes. It was used only in a few initial experiments.

The other mercury arc source was a high pressure mercury arc lamp (Hanovia 679A). Output of this lamp differs from the GE RS-275 in that the increased operating pressure causes pressure broadening of the lines, and also an increase in the intensity of the continuous background. The spectral energy distribution, especially for the mercury lines, is still grossly different than for sunlight (Table 4.1). Its use was restricted primarily to diagnostic investigations, for which it was well suited, because of its high intensity and equipment design adaptability. This source was also used in conjunction with filters for the removal of shorter ultraviolet wavelengths. In the immersion well system (section 4.2.4.) it was filtered with a Pyrex 7740 filter sleeve, and in the merry-go-round system (section 4.2.5.) both the filter sleeve and Quickfit reaction tubes served as ultraviolet filters.

The Hanovia 450 watt lamp was found to have excellent stability after a short initial aging period. Variation in output was usually no

more than 2% over a five hour interval. Reaction rates were usually fast enough with this source so that relatively short reaction times provided a reasonable assessment of a reaction. This property was of fundamental importance in minimizing the extent of microbial involvement during a reaction.

4.2. Photoreaction systems

Six different reaction systems were used for irradiation of samples. Since different light sources or different optical geometrics were employed in each system, only qualitative comparisons of results should be made. Some of these systems, and others which will not be discussed here, were used to explore initial ideas or to develop optimal reaction systems for seawater photochemical studies.

Of these, the merry-go-round system was used the most extensively. It afforded the most versatility and, more importantly, it provided a means of simultaneously comparing a number of reactions under nearly identical conditions.

4.2.1. Sunlight

Two procedures were used for sunlight irradiations. The first consisted of filling 500 ml round bottom flasks (Pyrex 7740) with the sample, so that when they were capped with a ground glass stopper only a small volume was occupied by air (enough just to maintain buoyancy). The flasks were carefully sealed by enclosing the stopper and upper portion of the flask neck with a plastic film secured with PVC plastic tape. A lead weight was attached to the neck so that it remained in-

verted when placed in a shallow trough of water. The flask was floated in the trough, which was continually being filled from the bottom with cold seawater (5-10°C). The trough was located on the roof in an area free of shadows. About 50 feet away, the sensor of an Eppley pyranometer (Model 8-48) provided continuous total sun and sky radiation intensity measurements.

In the second procedure, the flasks were replaced with glass stoppered quartz tubes of approximately 34 ml volume. The tubes were supported in a rack inclined at 45°. The support rack was painted a flat black to avoid back reflection through the reaction tubes.[1] The rack was placed on the bottom of the trough and oriented so that the side holding the tubes was facing the sun. Water level in the trough was adjusted to the same level as the liquid level in the tubes. In this way the stoppers were above the water line.

4.2.2. Tower tank experiments

The 35 ml quartz tubes were again used as the reaction containers. They were sealed using the same method described in Section 4.2.1. Two tubes were suspended in the tower tank (for description see Balch et al., 1976) at each depth; one was covered with a black polyurethane opaque coating and was used to determine the extent of dark reactions. The tubes were fastened to a rope, which had a weight on the end, in such a way that the tubes were normal to the rope and the solution-

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[1] Because of internal and external reflection and refraction at interfaces, different shaped containers should give different reaction rates. No attempt was made to evaluate these differences, but they can be significant (Zepp and Cline, 1977).

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containing part of the tube was extended as far as possible from the rope.

Four overhead phosphor coated 1000 watt lamps (GE MV 1000/C/BUH) were suspended from the ceiling over the pool tank; these supplied the total radiation incident on the tubes. The light intensity at the water surface was about 10 to 25% of the midday PAR wavelengths (400-700 nm) in sunlight at the sea surface, but the intensity decayed more rapidly with depth than does sunlight (Table 7.1). This was not due to a significant difference in the attenuation by water, but instead was primarily a function of the distance from the lamps.

4.2.3. Sunlamp system

Initial experiments to test the feasibility of studying seawater photochemistry were performed using a 1 liter water jacketed beaker, which was covered with a 2 mm thick glass plate (Pyrex 7740). A General Electric RS-275 sunlamp was located six inches above the pyrex plate and served as the radiation source for the slowly stirred solution in the beaker. The temperature of the solution was controlled by recirculating water from a constant temperature both through the water jacket of the beaker.

4.2.4. Immersion well system

A standard immersion well reactor using a 450 watt high pressure mercury arc was used for many experiments (Figure 4.4). The reactor was contained in a large enclosure, the walls of which were painted a

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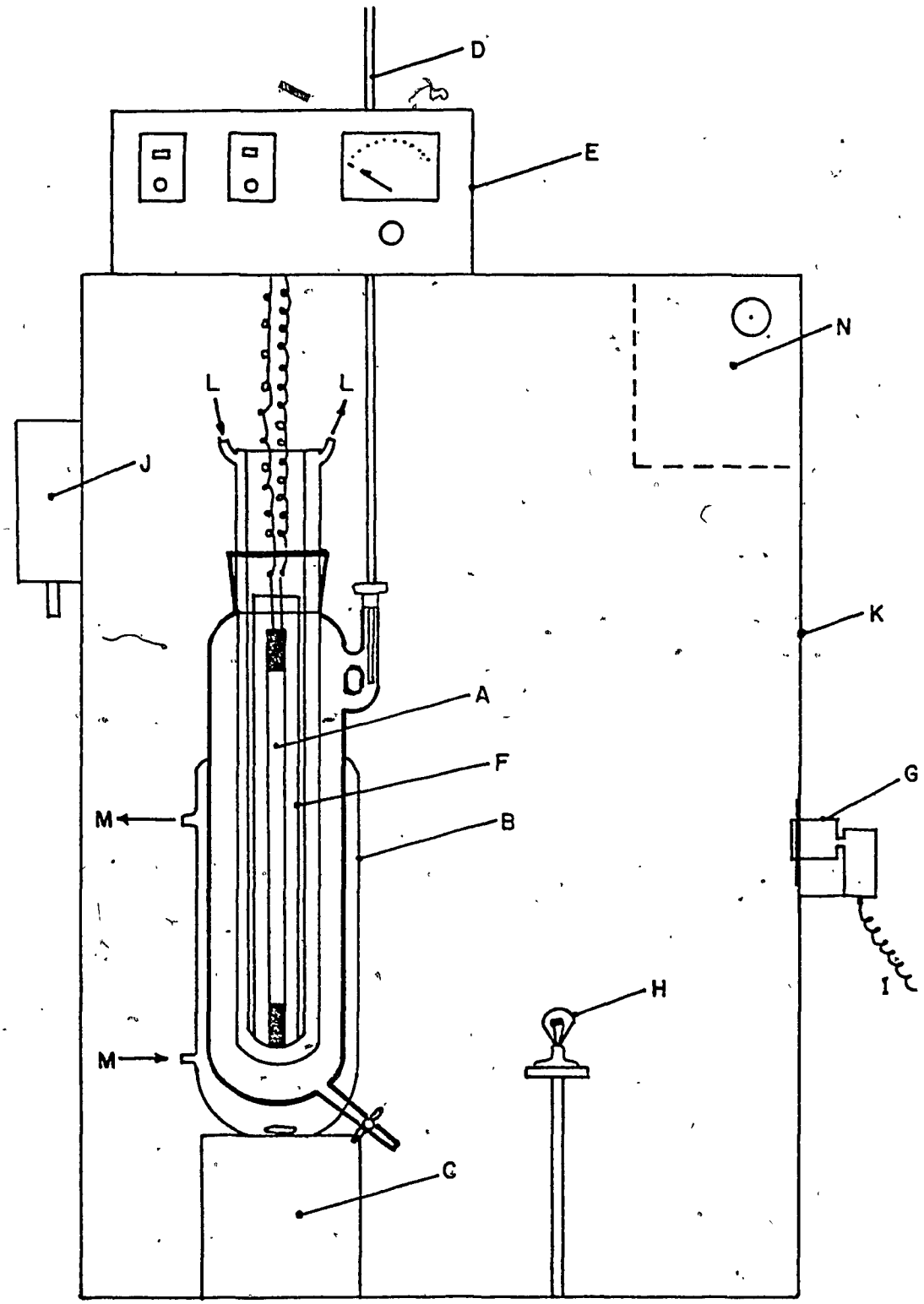

Figure 4.4 Immersion Well Photoreaction System

List of Parts:

A. 450 watt, Hg lamp (Hanovia 679 A).

B. photochemical reaction vessel (from Ace Glass, Catalog # 6523-06)

C. magnetic stirring motor

D. teflon reaction solution or gas inlet tube

E. stablized A.C. power supply for Hg lamp, millimeter, and fine control adjustment for tungsten filament lamp

F. quartz photochemical immersion well (from Ace Glass, Catalog # 6515 A-25) with Pyrex 7740 sleeve filter insert

G. uv sensitive photodiode and operational amplifer (United Detector Technology, UDT-500 uv)

H. tungsten lamp for testing light detection system

I. leads for power source and output to digital voltmeter or strip chart recorder

J. pressure switch (Hanovia 315-60)

K. enclosure with flat black interior

L. cooling water recycle for lamp

M. water recycle for temperature jacket on reaction vessel

N. exhaust fan

flat black to minimize light reflection. The reactor and a UV-sensitive photodiode, which was located on a wall of the enclosure, were mounted so that the light path between them was unobstructed and was a fixed distance. Light intensity, specifically the 365 nm Hg line, was measured throughout reactions and these measurements were used to normalize results of different experiments. Since the intensity was unstable during the first 15 minutes after lamp ignition, the reaction solution was not added to the reactor until after this period. Addition of the reaction solution was made from a point outside of the enclosure by gravity feeding it through an all glass and teflon line. The complete addition of the 600 ml charge of reaction solution required about 20 seconds. Experiments were either continued to the point where the lamp was extinguished, or samples were withdrawn from the outlet at timed intervals during the irradiation.

The filter sleeve (Pyrex 7740) used to reduce the shorter ultraviolet wavelengths from the lamp output initially transmitted a small component of radiation below 290 nm. However, exposure to the intense radiation of lamp soon solarizes Pyrex and considerably reduces its transparency to ultraviolet light (Hanovia, 1964). A much larger infrared component escaped the lamp cooling jacket. Major temperature increases in the reaction solution were avoided by the rapid flow of cooling water from a Laude constant temperature bath through the external sample cooling jacket. Constant stirring of the reaction solution facilitated temperature control.

4.2.5. Merry-go-round system

The merry-go-round photochemical apparatus (Figure 4.5) was patterned after the device described by Moses et al. (1969). The two devices are similar in that a number of samples can be irradiated simultaneously.

A more significant problem resulted from variations in the light transmission characteristics of the various reaction tubes. This was minimized by matching the optical characteristics of the tubes as closely as possible. The matching was checked by comparing the results for the same reaction conducted simultaneously in each reaction tube. For the photolysis of methionine (5×10^{-6} M l^{-1}) in seawater, 8 reaction tubes gave an average concentration of methionine, after photolysis, of 4.004 M l^{-} with a standard deviation of .0164. Analysis was performed by the HPLC determination of dansyl derivatives.

The same test for reproducibility for seawater solutions of 1×10^{-7} M l^{-1} of added 1-^{14}C glycine gave an average of 2.22×10^{-10} M l^{-1} of glycine decarboxylated, with a standard deviation for the 8 reactions of .0951, as determined by $^{14}CO_2$ analysis. The major source of variation usually stemmed from the analysis rather than the photolysis procedure.

The Pyrex 7740 filter sleeve, used to reduce the radiation at wavelengths of less than 300 nm, transmits a small component below 290 nm. Therefore, a secondary filter was necessary to further reduce this radiation. This was accomplished by using Quickfit tubes (29 x 150 mm

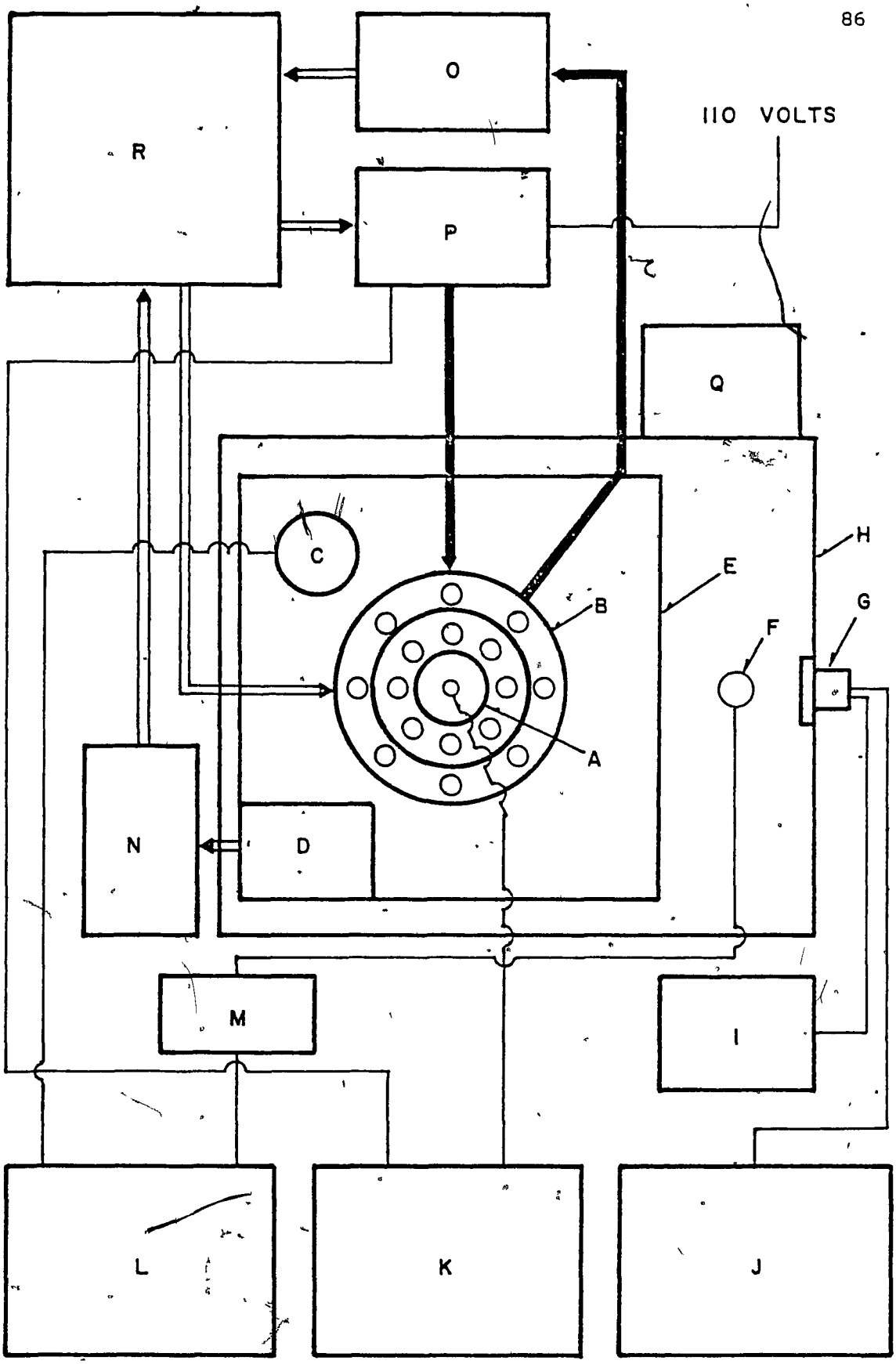
R
O
P
110 VOLTS
Q
C
H
E
B
G
F
A
N
D
M
I
L
K
J

Figure 4.5 Merry-Go-Round Photoreaction System

List of parts:

A. 450 watt, Hg lamp (Hanovia 679A) with pyrex 7740 sleeve filter in quartz immersion well

B. merry-go-round

C. high torque variable speed motor for rotating merry-go-round

D. return pump for bath water

E. temperature controlled bath (± 0.2°C)

F. tungsten lamp for testing light detection system

G. uv sensitive photodiode and operational amplifier (United Detector Technology, UDT-500 uv)

H. enclosure with flat back interior

I. 15 volt power source for light detector

J. fast response recorder with multiple chart speed and multiple range (Linear Inst. Corp., Model 232)

K. stabilized A.C. power supply for Hg. arc lamp

L. filtered D.C. power supply (Electra Products Laboratories, Model D-612T)

M. milliammeter and fine control for adjustment of tungsten filament current

N. heat exchanger

O. heat exchanger

P. pressure switch (Hanovia 315-60)

Q. exhaust and cooling fan

R. Lauda K-2/R temperature controlled bath

Key:

═══ Water for bath temperature control
▬▬▬ Water for lamp cooling
——— electrical connections

Figure 4.6 Xenon Lamp Photoreaction System

List of Parts:

A. power supply - 150 watt (Honovia, Model 28167)

B. lamp housing with F/1.5 optics (Schoeffel LH 150) and equipped with 150 watt xenon lamp (Hanovia 901C-1)

C. filter holders and filters

D. Sample cell holder

E. Sample cell, standard 10 cm. cell with quartz windows and water jacketed for temperature control

F. uv sensitive photodiode and operational amplifier (United Detector Technology, UDT-500 uv)

G. 15 volt power supply for light detector

H. fast response recorder with variable chart speed and variable range (Linear Inst. Corp., Model 232)

I. enclosure with flat black interior

J. temperature controlled water for jacket on sample cell

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with 24/29 standard taper glass stopper), which have a 0% transmission of radiation at and below 290 nm. For further spectral modification of the energy distribution, glass filters were used for the outer ring positions. With the glass filters replaced with opaque covers, these positions could be used for dark controls.

The merry-go-round reactor was immersed in a large temperature controlled water bath. One wall of this bath was made of 1/4 inch thick plate glass, and the other walls and floor were blackened with charcoal impregnated paraffin wax to minimize the reflection of stray light. The bath was situated in the same large enclosure which was used for the immersion wall system (Figure 4.4), so that the light from the outer ring windows of the merry-go-round was projected through the glass wall of the bath and onto a photodiode located on the wall of the enclosure. As the drum of the merry-go-round rotated the light from each window was projected on the photodiode about 12 times each minute. A record of the output from the light detector was made on a fast response strip chart recorder. This output was a representation of the light intensity on the photodiode surface as light from each window flashed across it. The intensity output of the lamp for any spectral region was linearly related to the amplitude of the signal. By using appropriate filter combinations, changes in the total lamp output or output from specific spectral regions could be measured. These measurements provided a means of adjusting reaction results for light intensity changes either during an experiment or between different experiments. After the lamp had attained stability, the fluctuations during an experiment were usually insignificant and applying corrections for them

was unnecessary.

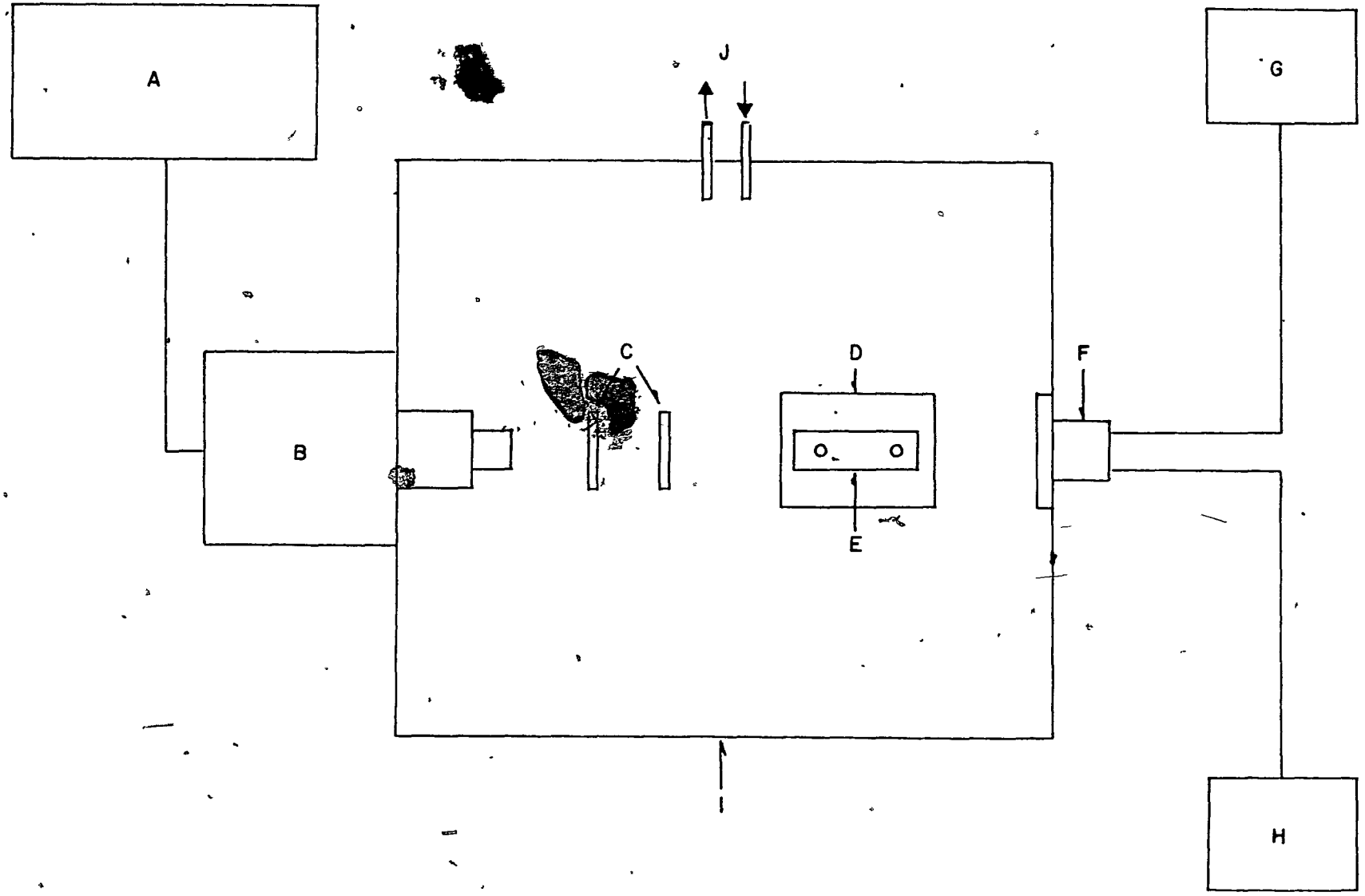
Temperature control in the drum and the external bath were maintained by using 2-heat exchangers and a temperature controlled recirculating bath. Only distilled water was used throughout the system; it was frequently changed to maintain its low light absorption.

Experiments were not started until the bath temperature and intensity of the light source had both stabilized.

4.2.6. Xenon lamp system

The xenon lamp system used in this study is illustrated in Figure 4.6. Irradiation and dark control reaction vessels were both typical 10 cm path length spectrophotometer cells with quartz windows. The entire front window surface was illuminated by the projected beam from the source. Light intensity of the beam was measured with a photodiode located on the wall of the enclosure. The photodiode could be placed so as to measure the incident radiation or the transmitted radiation. For natural seawater samples the low absorptivity changes during irradiation meant that any changes of the intensity measurement were almost entirely due to fluctuations in the source output. Either option provided an accurate estimate of the light intensity.

The sample was not exposed to the light beam until the xenon lamp acquired full stability. Light intensity was measured for the duration of the exposure by recording the light detector output. This value could then be applied to making comparisons between the results of different experiments.


A
J
G
C
D
F
B
E
H

4.3. Light-intensity measurements

4.3.1. Photodiode detector

Basically the same light detection apparatus was used for the immersion well system, the merry-go-round system and the xenon lamp system. The electronic components of this apparatus consisted of a UV-sensitive photodiode and operational amplifier assembly (Appendix 3) and a control and power supply module. According to the manufacturer's specifications for the photodiode, the measurement of light intensity is linear over 12 decades with a maximum deviation in responsivity of .5% over 6 months.

The electrical components for this detector were mounted in a housing which contained a shutter and filter holder for glass or neutral density filters. The shutter provided a means of zeroing the dark voltage signal from the detector on a strip chart recorded or digital voltmeter.

4.3.2. Chemical actinometers

Generally, chemical actinometers are used to determine photon flux over a rather narrow bandwidth region. In this study the broad bandwidth regions of interest require that ϕ for the entire region be approximately the same before an accurate measurement can be made. This requirement is met most closely by $KCr(NH_3)_2(NCS)_4$ (Wagner and Adamson, 1966) for 400 to 600 nm and by the ferrioxalate actinometer (Hatchard and Parker, 1956) for 290 to 400 nm. Even when used in these spectral regions the variation in ϕ is as much as 10%. Therefore, when used over

wide bandwidths these methods can only be expected to give an approximate value for the photon flux in the reaction vessel.

Using these techniques, the photon flux was found to be 9.6 x 10^{-9} einsteins sec^{-1} for the inner ring in the merry-go-round system in the 290 to 650 nm region. For sunlight in the same region the flux was found to be 3.6 x 10^{-9} einsteins sec^{-1}.

4.4. Glassware

To avoid difficulties resulting from adsorbed wall contaminants, the glass reaction vessels were cleaned by placing them in boiling concentrated nitric acid for 30 minutes. The vessels were then thoroughly rinsed with water from a Millipore Super Q system.

Bacterial contamination from the glassware was avoided either by heat sterilization or by washing with 1 molar HCl; glassware was stored containing the HCl solution until it was to be used. For heat sterilization, the glassware was stored in a drying oven at 175°C overnight. The cylindrical tubes used in the merry-go-round system were heat sterilized by storing them in a drying oven inverted with standard taper stoppers in place. As the tubes heated up most of the residual rinse water was forced out by the increasing pressure within the tube. When the nearly dry tubes cooled down, the condensing water vapor created a partial vacuum in the tube. The tubes were stored in this condition until they were to be used.

5. ANALYTICAL METHODS

5.1. Introduction

The low concentrations (often less than micromolar) of most organic and inorganic constituents of seawater means that analytical methods be extremely sensitive, or that concentration of the constituent be preformed prior to the analysis. For organic constituents it is usually necessary to preconcentrate the sample to bring the levels into the detectable concentration range for the analytical method. For amino acid determinations at typical concentration levels found in seawater (10^{-7} to 10^{-9} M l^{-1}), this amounts to concentrating the amino acids contained in from 2 to 23 liters of seawater (Palmork, 1963; Degens et al., 1964; Chau and Riley, 1966; Webb and Wood, 1967; Riley and Segar, 1970; Pocklington, 1970; Clark et al., 1972). The large volumes and the length of time required to perform a single analysis make such methods impractical for use in a diagnostic laboratory study on photochemistry, where the irradiated volume must for practical reasons be small, and the time of an analysis should be short, since many may be required. These requirements were considered as the criteria by which analytical methods to be used in connection with photochemical studies were chosen or designed.

Existing analytical methods for some of the constituents of interest were adequate in terms of sensitivity and the volume of sample required for the analysis. Therefore, standard seawater analytical methods for NO_2^-, NO_3^- and NH_3 (Strickland and Parsons, 1972) were used.

For the NH_3 analysis the difficulties resulting from the exposure of the sample to light during color development of the reagents (Gravitz and Gleye, 1975) was noted and adequate precautions were taken to avoid the problem.

5.2. Amino acid analysis

Factors which led to the choice of the amino acids as model compounds with which to examine seawater photochemical reactivity included an extensive body of information on their photochemical behavior in aqueous solution and on methods of analysis. Although numerous procedures for the analysis of amino acids existed, none of those which had been developed for seawater appeared suitable for use in this study. A survey of existing techniques indicated that some of them, with the appropriate modifications, might fulfill the analytical requirements. One of the major requirements, and perhaps the most limiting, was that the method should work satisfactorily in samples containing the normal concentration of sea salts. A procedure using a gas chromatographic separation of BSTFA derivatives (Gehrke et al., 1969) formed directly in freeze dried salts and a colorimetric method using chloranil-amino acid complexes (Al-Sulimany and Townshend, 1973) both failed to provide satisfactory results, because of incompatibilities with the salt content.

In terms of compatibility with the salt content, efficiency with respect to time of analysis and sensitivity, methods employing fluorescent labeling reagents were the most satisfactory. The major limitation of these methods was the difficulty of reducing the blank, which ulti-

mately set the lower detection limit of the technique. It was for this reason that a promising method using the highly fluorescent o-phthaldehyde derivatives of amino acids (Roth, 1971; Roth and Hampai, 1973; Benson and Hare, 1975) was not used. However, fluorescence detection of amino acids by two other methods did prove to be useful.

5.2.1. Fluram derivatives[1]

Fluram (4-phenylspiro[fuan-2(3H), 1'-phtalan]-3-3'-dione) is a reagent which reacts rapidly with primary amino groups of many compounds to form intensely fluorescent derivatives (Udenfriend et al., 1972). Derivative formation is highly dependent on the solution pH, and for the preparation of amino acid derivatives in seawater the optimum range was found to be from 9.0 to 9.4. The seawater solution was, therefore, buffered before a solution of Fluram in acetone (.08 to .1 mg ml^{-1} of seawater was found to be optimal) was added. The solution was stirred rapidly during the addition; reproducibility of this step was critical in providing good analytical precision.

In samples with greater than 30‰ salinity, a gelatinous precipitate would usually form soon after the addition of Fluram reagent. This did not decrease the extent of derivative formation, but did provide difficulties in the measurement of fluorescence. Relative fluorescence of the derivatives was not appreciably pH sensitive for values greater than 5. It was thus possible to lower the pH of the buffered solution

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[1] A similar method was used by North (1975) for measuring natural levels of primary amines in seawater.

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to dissolve the precipitate.

The excitation and emission wavelengths for the fluorphors of different amines were the same, but the relative intensity of fluorescence varied considerably. The fluorescence of the aspartic acid fluorphor was less than 10% of the phenylalanine derivative, while the NH_3 derivative gave a value which was far less than that observed for aspartic acid. This property of the derivatives makes an accurate assessment of the actual amine concentration impossible where an unknown mixture of compounds containing primary amino groups exists. The concentration of primary amines in a sample was, therefore, calculated in terms of glycine equivalents (the concentration of glycine necessary to produce the same relative fluorescence signal). As the method was used in this work, it provided an estimate of the loss of glycine equivalents from an irradiated sample when compared to a dark control under otherwise identical conditions. In most of these cases a single amino acid was added at a concentration which far exceeded the natural levels of primary amines. Even in this case the method gave an accurate estimate of the extent of decomposition only if no interferring products, such as other primary amines, were formed during the reaction. The dubious nature of results obtained from solutions containing mixtures of amines makes chromatographic separation of the mixture essential to gaining clear understanding of the photochemistry of amino acids in seawater.

5.2.2. Dansyl derivatives

The addition of dansyl chloride (5-dimethylaminonaphthalene sulfonyl

chloride) to basic solutions contiahing primary or secondary amines usually results in the formation of highly fluorescent derivatives. This reaction has been used extensively in biochemistry (Gray, 1967; Seiler, 1970) and has been used for the semi-quantitative detection of amino acids in seawater (Litchfield and Prescott, 1970).

The procedure described in Appendix 2 optimizes the conditions under which the amino acids most effectively compete for a limited amount of dansyl chloride. The dansyl chloride concentration must be far in excess of that needed to label the amino acids, because the sulfonyl chloride is also hydrolyzed. The amount of excess dansyl chloride necessary for a high percentage of amino acid labeling is dependent on the amino acid concentration and also on the second order rate constant for the reaction between the dansyl chloride and the amino acid. Rate constants for different amino acids vary widely; and sufficient time must be alotted for full development with some of the less reactive ones. Several hours were usually needed to give complete labeling of the amino acids used.

The use of this reagent as a direct assay for the amino acid concentration of seawater is of little value, because the sulfonic acid, which is the hydrolysis product of the dansyl chloride, has a strong fluorescence which overlaps the fluorescence maximum for dansyl amino acid derivatives. An essential step in the use of this reagent in seawater analysis is the separation of the sulfonic acid; the procedure outlined in Appendix 2 provides an extract of a mixture of dansyl chloride reaction products which is ready for chromatographic separation.

5.2.3. HPLC separation of amino acid derivatives

The separation of Fluram (Imai et al., 1974) and especially dansyl amino acid derivatives (Seiler, 1970) has been accomplished using the techniques of thin layer chromatography on silica gel or alumina. The availability of high efficiency silica gel and alumina packings for HPLC coupled with a fluorescence detector made the extension of these separation techniques to HPLC possible. The major difficulty which was confronted involved the selection of a suitable solvent system; none of those commonly used for TLC separation of these derivatives were suitable for use in HPLC.

In addition to the usual criteria used for choosing a solvent system for normal phase liquid/solid chromatography, the dielectric constant was also considered. Some solvent systems were found to provide adequate separation, but only a small fluorescence signal was generated. The signal could be greatly increased for either Fluram or dansyl derivatives by using solvent systems which had low dielectric constants (Chen, 1967).

Although the derivatives were separated on a number of different types of column packings, the best results were obtained on small-particle (< 10μ) silica gel columns. Initially, commercially packed columns were used, but their high cost made frequent replacement prohibitive. This replacement was necessary, however, because of a slow deterioration of the packing material during use in the separation of the amino acid derivatives. Instead of using commercially packed columns, the columns were prepared in the laboratory using a neutral

density slurry method (Asshauer and Halász, 1974) and a high pressure packing apparatus similar to those used in other laboratories (Cassidy et al., 1974; Strubert, 1973). The packing apparatus was constructed so that it could be attached directly to the Haskel pump of a DuPont 830 HPLC, which supplied the packing pressure. All HPLC columns used for acquisition of the data in the following sections were packed using this apparatus, with an applied packing pressure of 4000 psi. The columns were all packed with 5μ mean particle size silica gel (Merck, LiChrosorb SI60) equilibrated with the eluting solvent system for several hours prior to the injection of the first samples.

The procedure for preparing the seawater sample for direct injection onto the column was basically the same for either Fluram or dansyl derivatives, and consisted of acidifying the seawater sample and then extracting with ethyl acetate or diethyl ether. Recovery was found to be the lowest for the basic and acidic amino acids and highest for the neutral amino acids. In general, the dansyl derivatives gave better recoveries and were more stable than the Fluram derivatives, especially in the acidic solutions. Because of the low stability and the possible formation of diastereoisomers (Imai, 1974), which appeared in the liquid chromatogram as a pair of peaks for each amino acid, the Fluram derivatives were not used in the HPLC analysis.

Quantification of the separated derivatives was accomplished by integrating the output from the fluorescence detector. The signal was corrected for light absorption in the fluorometer cell of the detector (Eq. 5.1), although this correction was insignificant for the

$$F_C = \frac{1.15A}{10^{-A/4} - 10^{-3A/4}} \qquad (5.1)$$

where F_C = corrected fluorescence signal

F = measured fluorescence signal

A = measured absorbance signal

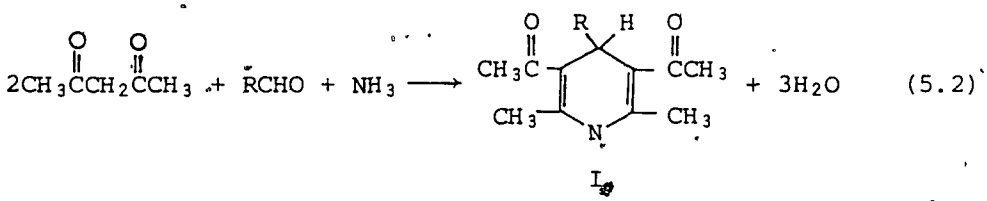
concentration ranges used in most of the experiments. Determination of the actual concentrations was made by reference to an amino acid internal standard (β-alanine, 4-aminobutyric acid or sarcosine), which was added at approximately the same concentration as the reactant amino acid. The analysis was normally used in the range of 1.25×10^{-10} to 2.5×10^{-12} moles, based on the concentration in the injected 10 µl portion of the extract. In this range the method was linear.

A major potential source of error in the HPLC analysis was inherent in the fluorescence detection system, where high performance was only obtained when the lamp output for excitation was stable. Fluctuation in light intensity would result in similar fluctuations in the fluorescence signal, since the two are proportional. The stability of the detector system was tested during operation by comparing the ratios of the amino acid signals to internal standard signal for multiple analyses. This ratio remained the same if the detector was stable throughout an individual analysis. If the ratio varied significantly the analysis was discarded; in sets of sequential triplicate analyses this was seldom observed.

The stability of the detector was also tested by comparing the values of the integral for the internal standard in consecutive analyses. This was possible because of the good reproducibility of the injection volume obtained with the high pressure direct injection valve. With good stability, the integrator output for consecutive triplicate samples was less than 1%, and when variations greatly exceeded this the analytical values were discarded.

5.3. Aldehyde analysis

The Hantzsch reaction (Eq. 5.2) has been used (Belman, 1963) to analyze for low concentrations of formaldehyde in aqueous solutions. The product of this condensation reaction with formaldehyde is 3,5-



(5.2)

I

diacetyl-1, 4-dihydrolutidine (I), which has an intense fluorescence emission at 510 nm.

The procedure described by Belman (1963) was easily adapted to use in seawater solutions and gave a lower detection limit for formaldehyde of approximately 5×10^{-8} M. A linear relationship between the relative intensity of fluorescence and the concentration of formaldehyde was found from 1×10^{-5} to 2×10^{-7} M. Below a concentration of 2×10^{-7} M the standard curve was still well enough behaved to provide a good estimate of the actual concentration.

Although the aldehyde concentration of a sample is calculated from a standard curve for formaldehyde, the method will also measure other aldehydes. The results are, therefore, reported in terms of the formaldehyde equivalents: that is, the concentration of formaldehyde necessary to produce the same relative fluorescence signal.

5.4. Peroxide analysis

An iodometric analytical method for peroxide was developed initially. The procedure consisted of manipulating the sample and reagents under anoxic condition so that low blank values for iodine formation were obtained. Hydrogen peroxide and organic peroxides (Johnson and Siddiqi, 1970) should both be detected with the analysis and their concentration was determined by measuring colorimetrically the amount of I_3^- formed. The method was useful to 5×10^{-7} M l^{-1} of H_2O_2 and was linear in the tested range from 5×10^{-7} to 1×10^{-5} M l^{-1}. Unfortunately, the sensitivity of the method was not great enough to make it useful for photolysis experiments.

Hydrogen peroxide can be quantitatively determined at low concentrations (5×10^{-9} M) by measuring the extent of the peroxidase catalyzed destruction of highly fluorescent scopoletin (6-methoxy-7-hydroxy-1, 2-benzopyrone). This method was first developed to measure H_2O_2 production in radiolysis experiments (Perschke and Broda, 1961) and was later used to measure the natural peroxide levels in seawater (Van Baalen and Marler, 1966). The lack of details concerning the analytical procedure used by VanBaalen and Marler made a detailed study of the method

necessary before it could be used for photolysis experiments. The procedure described in Appendix 2 is based on the results of that study and is designed to optimize the method for its implementation in this work.

VanBaalen and Marler (1966) reported their findings for seawater analysis as H_2O_2 concentration and reported that the addition of catalase caused the complete loss of activity in the peroxidase-scopoletin system, but they provided no other evidence that the oxidant was indeed H_2O_2. This might be an important consideration, in view of the fact that peroxidase can act as a catalyst for other oxidants (Chow et al., 1973) and that catalase accelerates the decomposition of some organic peroxides, although the rates for these processes may be considerably slower than for H_2O_2 (Baldwin, 1957). Based on the rate of destruction of the major oxidant by catalase, the rate of its spontaneous decomposition, and the high mechanistic probability of its formation, it would appear that H_2O_2 is the major oxidant being measured by this procedure in the photolysis experiments (Section 10). It is entirely possible, however, that organic peroxides are formed during the photolysis and that the analysis actual represent a composite concentration value for these and for H_2O_2. For this reason the oxidizing agent measured by this method is referred to in an all-inclusive sense as peroxide.

5.5. Natural seawater fluorescence

Fluorescence spectra of Nova Scotia coastal seawater collected over a period of 1 1/2 years revealed that minor changes did occur in the

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the spectra. However, major excitation (at 380 nm) and emission (at 490 nm) bands were always represented. This feature extended to water collected on the Scotian shelf and was also found to approximate the fluorescence maxima observed for seawater solutions of fulvic acid, benthic algal exudates, and seaward bound river water from Nova Scotian watershed lands. Because of the generality of these excitation and emission maxima they were specified as the wavelengths at which natural seawater fluorescence was measured throughout this work.

The natural fluorescence was measured in accordance with a standard procedure (Appendix 2) for all samples. To normalize the relative intensity values obtained for different samples, they were compared to a fluorescence reference standard. Quinine sulfate was used as the reference standard and a .1 mg/l acid solution was arbitrarily assigned a value of 100 quinine standard units (QSU). The natural fluorescence of a sample was determined from Eq. 5.3. This technique provides an accurate comparison when the fluorescence of the samples being compared

$$F = \frac{I_s}{I_r} \times S \qquad (5.3)$$

where F = natural fluorescence in QSU

I_s = relative fluorescence intensity of sample

I_r = relative fluorescence intensity of standard

S = scale of units: 100 for .1 mg/l quinine sulfate

10 for .01 mg/l quinine sulfate

varies within narrow limits, but widely different values can give erroneous results. This is an especially severe problem in samples

containing high concentration of fluorescent or other light absorbing materials. In such cases the fluorescence should be corrected for excitation and emission light beam attenuation by applying equations which consider the geometry of the fluorometer cell (Duursma, 1974). Such corrections were not necessary for samples from photolysis experiments, because the light absorption at the emission and excitation wavelengths were low.

The extent of the decay of fluorescence in irradiated samples was indicated by determining the change in fluorescence, when compared to a dark control. Results were calculated (Eq. 5.4) in terms of the percentage of decay of fluorescence of the dark control after a blank was subtracted.

$$D = \frac{F_d - F_l}{F_d - F_b} \times 100 \qquad (5.4)$$

where D = % of fluorescence decay

F_d = fluorescence of dark control

F_l = fluorescence of irradiated sample

F_b = fluorescence blank

Seawater that had been exposed to the full spectrum of a 1200 watt high pressure mercury arc for 4 hours was used as the blank. This procedure provided seawater which had a fluorescence intermediate between artificial seawater and low organic water (Appendix 1).

5.6. Iron (II) analysis

To establish whether Fe^{3+} was reduced to Fe^{2+} during photolysis

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experiments it was necessary to determine the rate of Fe^{2+} oxidation in seawater. Its rate of oxidation had been determined in $NaHCO_3$ solutions (Singer and Stumm, 1970); the half-life was found to be .04 minutes at a pH of 8.0. If a half-life comparable to this existed for seawater solutions, the possibility of accumulating enough Fe^{2+} to make its detection possible during a photolysis experiment would be small.

The possible influence of the potential organic and inorganic ligands in seawater on the Fe^{2+} oxidation made the determination of the rates for this reaction necessary. An analytical method for the quantitative measurement of low concentrations of Fe^{2+} in seawater solutions was needed. A colorimetric method with bathophenanthroline has been used for this purpose (Lewin and Chen, 1973), but the large sample volumes and complexity of the procedure made it impractical for its application in this work.

Ferrozine [disodium salt of 3-(2-pyridyl)-5,6-bis(4-phenyl sulfonic acid)-1,2,4-triazine] is a strong and selective chelating agent for Fe^{2+} and it has been used for the quantitative determination of total iron in freshwater (Stookey, 1970; Kundra et al., 1974) and seawater (Lewin and Chen, 1973). The reagent forms a highly light absorbing, stable, and water soluble complex with Fe^{2+} in the pH range of 4 to 9, with little interference from the other ions in seawater. Its use in determining the rate of oxidation of Fe^{2+} or the quantity formed during irradiation was as simple as adding a solution of the reagent to the sample and then measuring the absorbance. The addition of a large excess of the reagent immediately quenched the Fe^{2+} oxidation and minimized the effect of light scattering on attenuation, which was caused by the

formation of colloidal and particulate ferric oxides. Absorbance of the quenched samples varied linearly with Fe^{2+} concentration over the range of .1 to 50 µM (Figure 5.1).

5.7. Collection and measurement of $^{14}CO_2$

The method of collection described by Hobbie and Crawford (1969) was employed with a few minor modifications. These included the use of glass fiber filters in place of paper filters as the absorbent for phenylethylamine, the use of 100 ml dark glass serum bottles in place of flasks, phosphoric acid in place of sulfuric acid and agitation of the solution by stirring rather than shaking.

Counting was done in a Triton X toluene based scintillation fluor. The deviation from the mean for multiple analysis was less than 2% even for samples in which only .1% of the total added substrate activity was converted to $^{14}CO_2$.

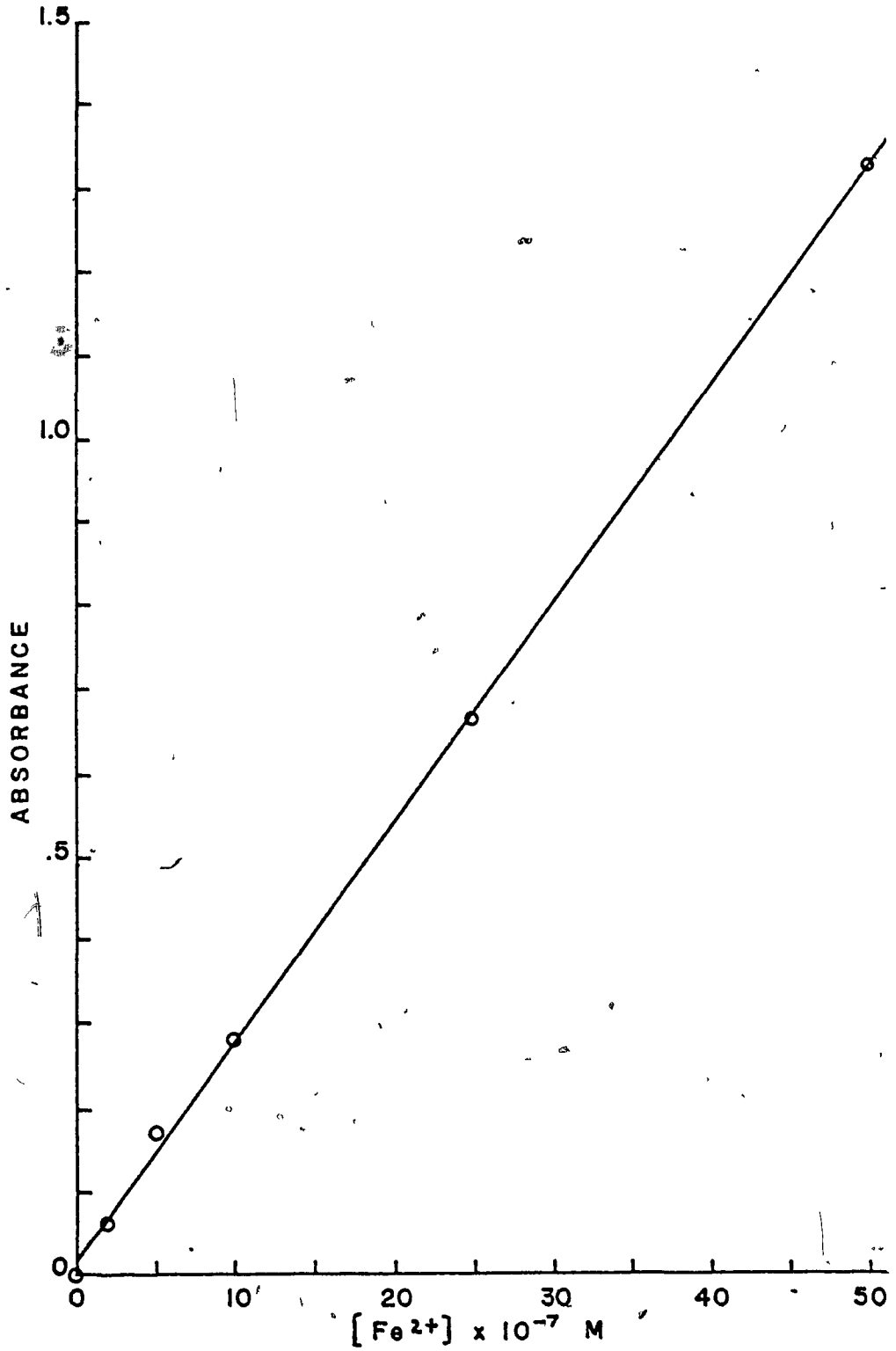
It has been reported (Ragland, 1967) that when phenylethylamine is used as a $^{14}CO_2$ trap, a loss of activity from the cocktail occurs with time. This possibility was tested, but no significant change in counting rate was observed for periods of at least a week. A problem with color development of samples stored at room temperature was observed, but this was remedied by storing samples in a refrigerator at 0-2°C.

5.8. ATP analysis

The analysis of ATP (Holm-Hansen and Booth, 1966; Cheer et al.,

Figure 5.1 Calibration Curve for Fe(II) Analysis in Seawater

see Appendix 2 for analytical procedure


1.5
1.0
.5
0
ABSORBANCE
0
10
20
30
40
50
[Fe2+] x 10-7 M

1974) in bacteria was not sensitive enough for the natural population levels existing in the volumes used in the photoreaction systems. The population levels of bacteria were, therefore, increased by culturing endemic marine bacteria in seawater enriched with SST media (Stein, 1973). Before the enrichment (.125 ml/100 ml of seawater) was made, 1 liter of a freshly collected sample of seawater was filtered through a 5 μ Millipore filter to remove larger organisms.

The bacteria were grown aerobically at room temperature (23°C) under a 12 hour light and 12 hour dark cycle in a 3 l sterile Fernbach flask. The growth phase of the culture was determined by optical density measurements of the culture solution. During the early stationary phase of growth an aliquot of the culture (.5 to 5.0 ml) was diluted to 100 ml with seawater of approximately the same pH, temperature and salinity. This solution was divided in half and the separated solutions were stored under identical conditions, including dim light, for a period of ½ hour. At the end of this period one of the solutions was exposed to irradiation, while other conditions for both solutions were maintained constant. When the irradiation period was terminated both solutions were immediately filtered and analyzed for ATP (Sutcliffe, W. H., Jr., unpublished modification of method of Holm-Hansen and Booth, 1966).

This procedure was normally conducted for a number of different samples simultaneously, so that experimental and culture conditions were as close to the same as possible. In cases where an additional reagent was added, the addition was made prior to the ½ hour holding period.

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For bacteriallogically labile substrates the addition was made just before commencing the irradiation period.

5.9. Ultrafiltration

Seawater was ultrafiltered with an Amicon (Model TC F10) high performance filtration system which was pressurized with purified N_2 (25-50 lbs). Prior to use, the UM-2 Diaflo ultrafiltration membranes were rinsed in distilled water for several hours, with 4 changes of water being made during that time. Buffered seawater (pH 8.0) was filtered through GFC glass fiber filters before introduction into the ultrafiltration apparatus. The water was ultrafiltered at a head pressure of 40-45 lbs; a fairly rapid single pass flow was maintained through the spiral flow channels to minimize concentration polarization.

Ultrafiltered water was run directly into hot nitric acid cleaned glassware and was stored at 0-2°C in the dark until use.

6. INITIAL PHOTOCHEMICAL STUDIES ON SEAWATER REACTIVITY

6.1. Introduction

The apparent lack of information related to marine photochemical studies leaves speculation as the only recourse in predicting what the actual photochemical characteristics of the marine environment are. In fact, in view of the information available it was impossible to decide whether seawater actually possed any unique properties which differentiate it from artificial seawater, salt solutions, or even distilled water in terms of its photochemical reactivity. Even if it did differ, the possible complexity of the system might overshadow any experimental attempts to understand the processes envolved or to measure their magnitudes.

Therefore, the approach taken here is many faceted and is designed as a preliminary excercise in determining qualitatively the nature and magnitudes of the photochemical processes involved, the properties which must be controlled, and the best indicators of photoreactivity which can be measured reliably.

6.2. Change of absorbance

The photochemical oxidation of organic matter in seawater by ultraviolet radiation has been demonstrated (Armstrong et al., 1966). Most of the oxidation occurs at wavelengths of less than 250 nm; oxidation was effectively complete after only an hour exposure to the full spectrum of a 1200 watt mercury arc lamp. All organic compounds absorb to

some degree at wavelengths of less than 250 nm and would probably, with sufficient irradiation time, undergo photochemical destruction under aerobic conditions to give carbon dioxide. This process is further propagated by the addition of hydrogen peroxide, which supposedly serves as an additional source of oxygen. It is probable that hydrogen peroxide also serves as a source of the strong oxidant, hydroxyl radicals, which are produced with high efficiency ($\phi > 1$) at these wavelengths by homolytic fission of the peroxide bond.

At near ultraviolet and visible wavelengths, many natural organic constituents of seawater do not absorb and therefore will not be reactive through primary photochemical processes. However, there is a fraction of the DOC which does absorb throughout the visible and near-ultraviolet region and is therefore at least potentially capable of photoinducing reactions in transparent substances or simple undergoing photochemical change itself. The detailed composition of this light absorbing fraction is not known; it is generally referred to collectively as "Gelbstoff". Because it does absorb light, it is possible to determine qualitatively the extent of its photolability by measuring changes in the absorption spectra of seawater upon irradiation. Seawater containing relatively small amounts of this fraction, such as that in the Eastern Mediterranean or Sargasso Sea, artificial seawater, or seawater in which the organic fraction has been destroyed by intense ultraviolet radiation, all have near-ultraviolet-visible absorption spectra which closely resembles that of distilled water. The measurable light absorption difference between distilled water and seawater for the region from 250 to 600 nm is then almost entirely accounted for by this organic fraction.

To determine whether this fraction is photolabile the absorption spectrum of filtered natural seawater was measured against distilled water at different intervals during its irradiation in the xenon lamp system. No change in the absorption spectrum of the dark control was noted after four days. The irradiated sample underwent major changes in its absorption spectrum during the same period (Table 6.1).

It would appear, then, that the organic chromophores in seawater are labile to solar radiation and that a significant fraction, if not all, would be destroyed by moderate exposure times to sea surface sunlight. The wavelength dependence of this process was not determined, but even if it is restricted to wavelengths which are rapidly attenuated in seawater, the process is efficient enough to alter significantly the transparency of the entire euphotic zone, since all water above the thermocline should have an appreciable residence time near the surface due to vertical mixing. In regions like the Sargasso Sea, where the water is isolated for long periods, the total residence time of water at the surface may be long. In such cases the sunlight induced destruction of "Gelbstoff" could exceed its rate of replenishment, and high transparency would result. This could be the case in Sargasso Sea, where water transparency has been reported to exceed that of distilled water in the blue-violet region (Holmes, 1957).

6.3. Change in TOC or VOC

If there is a significant rate of photo-oxidation in seawater, it is possible that changes in the concentration of organic carbon could serve

Table 6.1 Photochemical Alteration of Seawater Absorbance

λ , nm	INITIAL ABSORBANCE	ABSORBANCE RATIO, LIGHT/DARK				
		6 HOURS	24 HOURS	48 HOURS	72 HOURS	96 HOURS
520	.003	.67	.00	.00	.00	.00
500	.003	.67	.00	.00	.00	.00
480	.004	.50	.50	.00	.00	.00
460	.008	.38	.50	.00	.00	.00
440	.009	.44	.33	.11	.00	.00
420	.010	.90	.70	.30	.20	.20
400	.014	.93	.57	.36	.29	.21
380	.018	.94	.66	.39	.28	.22
360	.026	.96	.77	.58	.58	.50
350	.032	.75	.53	.41	.31	.19
340	.035	.80	.60	.40	.40	.23
330	.043	.74	.60	.40	.30	.23
320	.050	.78	.56	.34	.28	.22
310	.061	.75	.52	.36	.28	.29
300	.075	.76	.56	.39	.31	.25
290	.093	.80	.62	.41	.39	.37
280	.106	.92	.64	.51	.50	.47
270	.129	.89	.74	.59	.58	.54
260	.154	.87	.74	.68	.68	.64
250	.174	.90	.83	.70	.71	.67

as an indicator to the extent of this process. To test this possibility, a series of different experiments were conducted in which natural seawater was irradiated with the sunlamp, the high pressure mercury arc (merry-go-round system), or under natural sunlight. In each case a number of samples was collected at different times during the irradiation and analyzed for VOC and TOC (McKinnon, 1977).

Results of these experiments (Table 6.2), although erratic, do show general trends. The VOC, for instance increases during the early stages of the irradiation and then decays. This is particularly clear in the case of methionine-spiked seawater, where, as evidenced by the odor, a volatile mercaptan or sulfide would seem to be a product of the methionine photolysis. The decrease in the amount of this volatile sulfur compound is indicative of its loss by further reaction to give a non-volatile product(s). Although the methionine concentration as followed by HPLC showed a steady decrease throughout the irradiation, no large net conversion of TOC to VOC was observed.

In those experiments where no organic substrate was added, a general tendency for a decrease in TOC concentration was observed. It would appear that at least some of the natural organic materials in seawater are converted to volatile organic compounds, carbon dioxide, or carbon monoxide. The method of VOC extraction employed should limit the volatile compounds, which could be measured, to low molecular weight hydrocarbons, ketones, sulfides, mercaptans, halocarbons, some amines, and aldehydes other than formaldehyde. The fraction lost from the TOC could very well be carbon dioxide, since the inorganic carbon is lost

Table 6.2 Effect of Various Light Sources on the VOC and TOC Levels in Seawater

Data taken from joint project with McKinnon (1977).

Sample	Light Source	Time of Irradiation (hr)	V.O.C. ($\mu g\ C\ l^{-1}$)	% Difference from the Blank	TOC ($mg\ C\ l^{-1}$)	% Difference from Blank	VOC/TOC (%)	% Δ from Blank
A. Tap Seawater	Sun Lamp	0 (Blank)	25.27		1.03		2.45	
		3.0	37.25	+47	0.96	-6.8	3.88	+56
		14.5	26.51	+ 5	1.06	+3.0	2.50	+ 2
		26.5	30.32	+20	0.93	-9.7	3.26	+33
		0 (Dark)	25.48	+ 1	0.99	-3.9	2.57	+ 5
B. Tap Seawater	Sun Lamp	0 (Blank)	25.45		1.20		2.12	
		1.0	31.67	+24	1.09	-9.2	2.91	+37
		8.0	22.84	-10	1.06	-11.7	2.15	+1.4
		18.0	23.84	- 6	1.10	-8.3	2.17	+2.4
		0 (Dark)	25.63	+ 1	1.17	-2.5	2.19	+3.3
C. Tap Seawater $+0.3\ mg\ C\ l^{-1}$ Methionine	Hg Arc Lamp	0 (Blank)	22.18		1.41		1.57	
		2.0	33.62	+52	1.40	-1.0	2.40	+53
		5.0	29.98	+35	1.41	--	2.12	+35
		10.0	29.19	+32	1.38	-2.0	2.12	+35
		14.0	28.85	+30	1.40	-1.0	2.06	+31
		0 (Dark)	26.29	+19	1.41	0	1.86	+18
D. North West Arm (5 m) (8/6/76)	Natural Sun Light	0 (Blank)	19.16		1.72		1.11	
		1.0	22.23	+16	1.65	-4.0	1.35	+22
		3.5	23.56	+23	1.50	-13.0	1.57	+41
		8.0	17.71	- 8	1.51	-12.0	1.12	+ 1
		0 (Dark)	25.60	+34	1.67	- 3.0	1.53	+38

by acidification of the seawater prior to the TOC analysis. At the pH and pε of air-saturated seawater, carbon dioxide is the inevitable product of all organic compounds, owing to their thermodynamic instability under these conditions, and sunlight might be expected to accelerate this process.

6.4. Reactivity of added organic substrate

Low concentrations of selected organic substrates were added to seawater; the extent of their degradation was determined and used as an indicator of the potential photoreactivity of the sample. Amino acids were used almost exclusively for this purpose for reasons already discussed in Section 5.2.

6.4.1. Reactivity measured as change of glycine equivalents

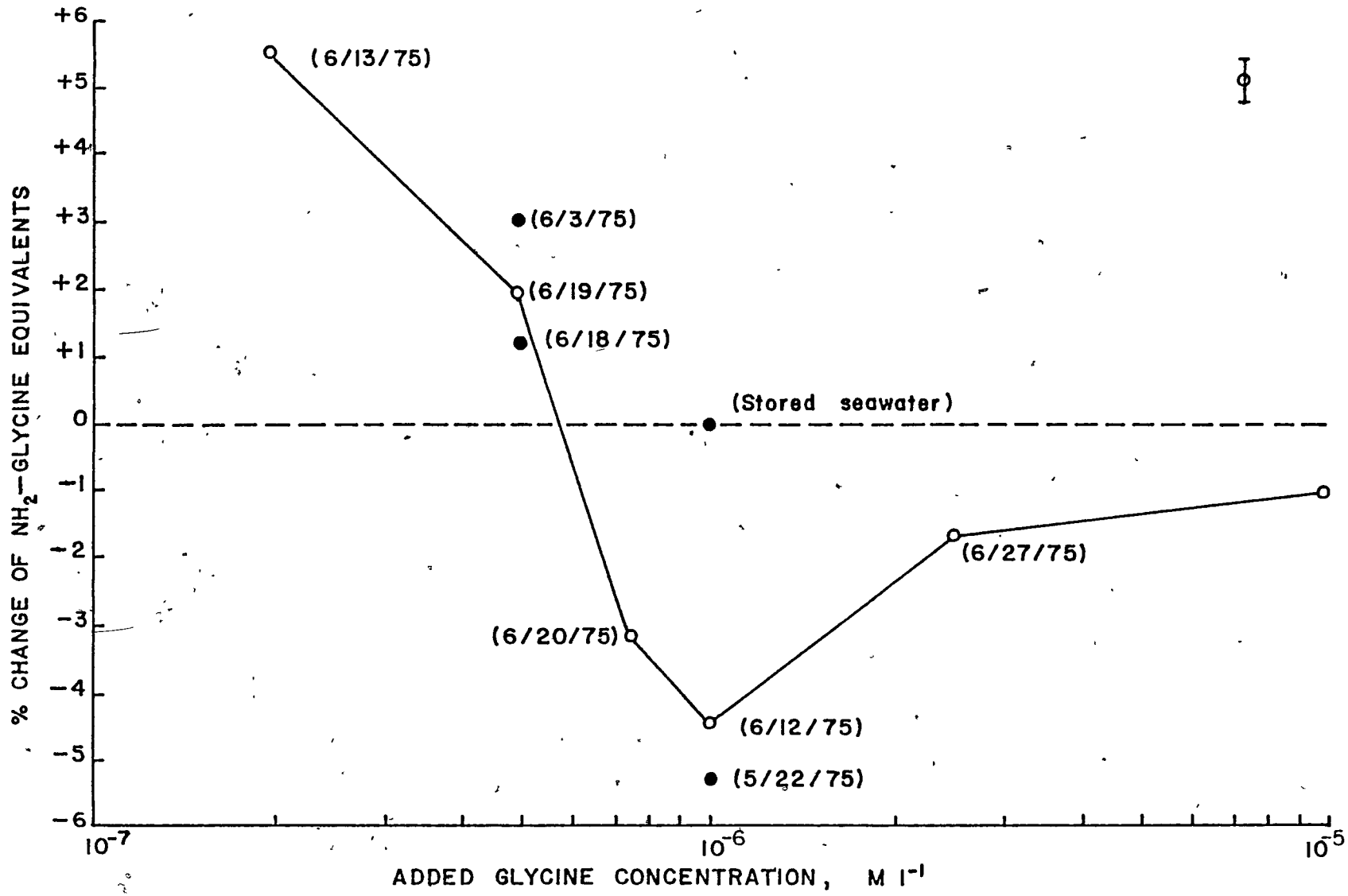
In initial experiments glycine was used as an organic substrate to test the photoreactivity of seawater; experiments were followed by measuring glycine concentration using the Fluram analysis. Solutions were irradiated in the immersion well system. A single reaction generally required a full day to complete. Therefore, an experiment requiring a number of individual reactions might require a week or more to finish. This raised the question of how to maintain a supply of seawater for the reaction media, which would not undergo changes in its properties during the course of the experiment. Unfortunately, even seawater which was filtered at the time of collection and stored in sterile vessels in the dark displayed major changes in glycine photoreactivity with time. Therefore, the procedure of using .22 μ filtered seawater within a few

hours of its collection was adopted. The seawater was collected from the same area (Halifax's Northwest Arm, at 10 m) and was adjusted with Na_2CO_3 to a pH of 8.15 just prior to starting the reaction. Results for both the dark controls and irradiated samples were calculated as the mean of five separate analyses.

In general, the extent of loss of NH_2- glycine equivalents during a 3 hour irradiation was in the range of 1 to 5% for seawater to which 1 to 10 M of glycine had been added. In some cases, however, the NH_2-glycine equivalents actually increased during the irradiation period. This was observed in an experiment in which the substrate concentration was varied, while all other parameters were held the same. The experiment was intended to provide information on the nature of reaction mechanisms for the degradation of glycine in seawater; instead the results (Figure 6.1) reflect the complications which can arise when trying to interpret a conceptually simple experiment, in which all the important parameters were not recognized nor controlled. From this and subsequent experiments it was found that a number of features of the seawater reaction systems had to be adequately controlled or monitored if useful data were to be obtained from the experiment.

One such unrecognized feature was the variability of natural fluorescence of coastal seawater and its rapid decay during irradiation. For the experiment shown in Figure 6.1, a blank control was run for the seawater without glycine present, but to account for the fluorescence decay upon irradiation a second blank is needed for an irradiated sea-

Figure 6.1 Photoreactivity of Glycine in Seawater Measured as Fluram NH_2-Glycine Equivalents


+6
+5
+4
+3
+2
+1
0
−1
−2
−3
−4
−5
−6
% CHANGE OF NH_2−GLYCINE EQUIVALENTS
(6/13/75)
(6/3/75)
(6/19/75)
(6/18/75)
(Stored seawater)
(6/27/75)
(6/20/75)
(6/12/75)
(5/22/75)
10^{-7}
10^{-6}
10^{-5}
ADDED GLYCINE CONCENTRATION, M l^{-1}

water sample containing no glycine. If this blank correction is not made when analyzing for low concentrations of amino acids, the Fluram analysis will give decomposition values which are too high.

The increase in NH_2- glycine equivalents for concentrations of added glycine below 7.5×10^{-7} M must arise either through a more rapid loss of glycine in the dark than in the light samples or through the formation of NH_2-glycine equivalents in the light samples. The later process is possible if the photochemical degradation of other organic constituents, such as compounds containing secondary amino groups (e.g., proline and porphyrins) form compounds containing primary amino groups which form Fluram derivatives. This exemplifies the difficulty in using an analytical method which is nonspecific.

The apparent increase in NH_2-glycine equivalents in the irradiated solution can also be explained by bacterial contamination. Although filtration through .22 μ Millipore filters is an accepted procedure for sterilizing many solutions, including seawater, the method was found to fail on certain samples. In such cases even multiple filtrations failed to remove the bacteria and a viable population remained which could significantly reduce the added labile substrate concentration in the dark controls. In irradiated samples the bacterial activity was completely arrested when the high pressure mercury arc lamp was used as the source. Therefore, if the bacterial utilization of substrate in the dark controls exceeded the combined effect of photochemical loss of glycine and the natural fluorescence decay, then an increase in the observed NH_2- glycine equivalents in the light sample would result.

It is perhaps worthwhile noting that an increase in NH_2-glycine equivalents in irradiated glycine-unspiked seawater was observed during the summer and early fall of 1975, but that no increase was observed during the winter of 1976. This may indicate that the organic chemical composition of the water was different during these two periods.

The question arises as to just how variable are the properties of seawater which control its photochemical characteristics. If they do vary significantly, as the results shown for seawater collected at different dates would attest (Figure 6.1), then all reactions of a single experiment should be conducted simultaneously and should use the same seawater.

6.4.2. Photoreactivity of various amino acids

As measured by analysis with Fluram, glycine was found to decompose slowly in irradiated seawater. In comparison to some amino acids, glycine is relatively unreactive in most photochemical oxidation pathways. To establish what the potential degradation strength of seawater was for various organic compounds, the reactivity of a number of different amino acids was established. This was done by subjecting seawater solutions containing added individual amino acids and prepared with the same seawater, to identical reaction conditions and then determining the extent of degradation. The results (Table 6.3) show that only those amino acids which are readily oxidized react appreciably under the conditions used. This can be seen by comparing the relative rates of the reaction with the hydroxyl radical for these amino acids (Table 6.3).

Table 6.3 Photoreactivity of Amino Acids in Seawater

Seawater of 31‰ salinity was buffered, filtered, and enriched with 5×10^{-6} M l^{-1} of the amino acid. The solutions were irradiated in merry-go-round system for 2 hours at a temperature of 20°C. Analysis was performed immediately on irradiated samples and dark controls using the HPLC dansyl derivative method.

Amino Acid	% Decomposed, LC Analysis	% Decomposed, Fluram Analysis	Rate Constant for Reaction with OH , $M^{-1} sec^{-1}$
Glycine	2	1.2	5×10^7 at pH 8
Alanine	1	4.4	1.2×10^8 at pH 6
Leucine	1	1.8	9.8×10^8 at pH 6
Valine	1	2.4	--- ---
Phenylalanine	3	3.4	3.5×10^9 at pH 6
Tyrosine	26	25.0	--- ---
Tryptophan	92	68.2	8.5×10^9 at pH 6
Proline	1	--	--- ---
Lysine	4	6.0	--- ---
Histidine	--	27.0	3×10^9 at pH 7
Methionine	50	34.8	5.1×10^9 at pH 7

The comparison to the hydroxyl radical rate constants should not be construed to mean that this is the reactive species, for this trend is likely to be similar for other oxidizing radicals and also for reactions which are initiated by photosensitizers (Ray, 1967; Byrom and Turnbull, 1967). The fact that phenylalanine is not appreciably reactive, yet has a rate not greatly different from that of the most reactive amino acids with the OH·, suggests that if an oxidizing radical is involved it is a more selective (a weaker) oxidant than OH·. This is supported by the lack of any observable increase in the reactivity in the series glycine, alanine, leucine, and valine.

Tyrosine, histidine, and especially tryptophan all have weak absorption tails above 290 nm and may therefore be degraded to some extent by direct photolysis initiated by their own light absorption. The photochemical degradation of tryptophan is an interesting case in that above 280 nm it photoionizes to give the tryptophyl radical cation and a hydrated electron (Grossweiner and Usui, 1971). In aerobic aqueous solutions the radical reacts to give N-formylkynurenine, which is an effective photosensitzer at wavelengths above 320 nm (Walrant et al., 1975). No information appears to be available about near-ultraviolet photolysis of tyrosine or histidine. However, both of these amino acids, as well as tryptophan and methionine, are readily oxidized by some photosensitizers (Knowles and Gurnani, 1972). Methionine does not absorb above 290 nm and must therefore be reacting with some photoexcited species or in a secondary process.

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6.5. Reaction products

In determining the actual mechanism(s) or simply the reactive species involved in the case of secondary reactions with amino acids it would be advantageous and maybe even essential to identify and determine yields for the reaction products. Unfortunately, at the micromolar levels of amino acids used, even a quantitative yield of most products could not be analyzed directly, even for those compounds for which a seawater analytical method exists. Although it would be possible to concentrate some compounds with some extraction procedure prior to analysis, this would require large volumes of reaction media.

To make a detailed investigation of reaction products for even simple reactants in the complex seawater matrix may be an excercise in futility, for amino acid photoreactions proceeding with photosensitzers, through metal ligand charge transfer, or secondary free radical oxidation, could all involve transient free radical intermediates which decay to give a complex array of reaction products. Even for a simple amino acid such as glycine, numerous products have been observed (Eq. 6.1) when the substrate initially forms free radicals through interaction of

$$NH_3^+CH_2COO^- \xrightarrow{e^-_{aq},\ OH} NH_3 + CHOCOOH + H_2 + CH_3COOH + CO_2 + HCHO + CH_3NH_2 + HCOOH + \text{minor products} \qquad (6.1)$$

hydrated electrons and OH· formed during x-ray radiolysis of aqueous glycine solutions (Maxwell et al., 1954).

Another complication arises from the stability of the formed products under the reaction conditions. It is entirely possible that products, as a result of their own degradation during the reaction, might never be observed or might provide an inaccurate quantitative measure of the extent of the reaction.

Of the products that are commonly observed in the photolysis of amino carboxylates (e.g., NH_3, CH_2O, and CO_2), only CO_2 could be determined with good precision when the substrate concentration was less than 5×10^{-6} M. Increases in the concentration of each of these products was observed when seawater solutions containing greater than 5×10^{-6}M l^{-1} of glycine were irradiated. Ammonia and CO_2 were also produced in microbially contaminated dark controls, but CH_2O was not observed. For the photochemical reactions, it was usually found that for every mole of glycine destroyed, as determined by the Fluram analysis, slightly more than 1 mole of CH_2O, greater than 1 mole of NH_3, and less than 0.5 mole of CO_2, from the carboxyl group of glycine, was formed. Considerably less than a 1:1 ratio was always observed for the CO_2 produced to glycine reacted ratio. This would suggest that a major part of the products formed result from deamination or dimerization of glycine, and the carboxyl group is incorporated into the products. Since the CO_2 formation was determined by using specifically labeled glycine ($1\text{-}^{14}C$), the results should be reliable. Caution should be used in the interpretation of the results for ammonia and formaldehyde, since analytical interferences in irradiated solutions, the production of these products from other sources, or their destruction during the

reaction may lead to erroneous values. This is particularly evident for NH_3, which was found to be a common product for the seawater photolysis of many amino acids (Table 6.4). For some seawater solutions a net loss of NH_3 was recorded in the irradiated solutions. No extensive investigation was conducted on the reasons for the observed loss, but it might be dependent on the oxidizing characteristics of the seawater, which might in turn be mediated by the concentration of photo-oxidizing agents. These characteristics may be further altered if sufficient concentrations of materials which can serve as scavengers or quenchers of the oxidizing species are present. Results from seawater solutions enriched with transition metals (Cu^{2+}, Ni^{2+}, and Fe^{3+}) and glycine all gave NH_3 yields which were equivalent to or in excess of the amount of glycine destroyed during the irradiation. These metals might serve to deactivate the triplet excited states of photosensitizers or supply a readily reducible substrate to scavenge oxidizing species. The quenching effect of transition metals on the NH_3 oxidation is compatible with the Joussot-Dubien and Kadiri (1970) observation that singlet oxygen (resulting from photosensitization of O_2 by organic compounds in seawater) is the active oxidant, since at least some of these metals are known to quench $^1\Delta$ O_2.

6.6. Dependent seawater properties

Specific properties of seawater such as pH, salinity, oxygen content, or the concentration of certain constituents can have a marked effect on the reactivity of various added substrates. The observed direction and magnitude of the effect which is induced when a property

Table 6.4 Photoreactivity of Amino Acids

in Seawater Determined by Different Analytical Methods

Seawater of 34 ‰ salinity was buffered, filtered, and enriched with 5×10^{-6} M l^{-1} of the amino acid or peptide. The solutions were irradiated in the merry-go-round system for 2 hours while maintained at a temperature of 20°C. The irradiated samples and dark controls were analyzed immediately after the termination of the reaction.

Amino Acid or Peptide	% Decomposed: HPLC Analysis	% Decomposed: Fluram Analysis	% Theoretical NH_3 Formed
Glycine	2.1 ± 0.8	1.2	-3.3
Alanine	0.8 ± 2.0	4.4	-4.3
Valine	0.0 ± 1.2	2.4	4.7
Leucine	0.0 ± 1.2	1.8	4.7
Phenylalanine	2.4 ± 0.7	3.4	-5.9
Tyrosine	26.9 ± 1.2	25.0	8.7
Tryptophan	89.2 ± 1.9	68.0	31.4
Methionine	56.7 ± 0.3	34.8	24.2
Proline	0.8 ± 0.5	--	-2.4
Lysine	3.5 ± 0.3	6.0	3.5
Histidine	---	27.0	11.7
Glycylglycylglycine	---	3.4	6.7

is modified could supply information about photochemical characteristics of the seawater.

6.6.1. Salinity

Seawater was diluted with redistilled Super Q water (Appendix 1) to give solutions of various salinities, each 5×10^{-6} M in added methionine and each buffered to a pH of 8.10. The solutions were irradiated for 2 hours in the merry-go-round system and then analyzed for methionine by the HPLC method.

The reduction of ionic strength by dilution with distilled water is accompanied by a decrease in the concentration of any photoinducing agents which are present in the seawater. The result of this dilution on the reactivity is obvious (Figure 6.2), but the reason for its nonlinear dependence on salinity is not clear. It is possible that this is the result of reaction rate suppression (e.g., reduction of rate for reactants with unlike charges) with increasing ionic strength, but without knowing more about the reactions taking place it is impossible to draw any definite conclusions.

In a similar experiment using the same seawater, in which methionine, tryptophan, and glycine were all present at 5×10^{-6} M, the methionine showed approximately the same reactivity at 35‰ salinity (Figure 6.3) as it did in the experiment in which glycine and tryptophan were not present. It is not possible to determine if glycine is degraded, since it is a reaction product of methionine or tryptophan and is being formed fast enough to show a net increase in concentration. The

Figure 6.2 Effect of Diluting Seawater

with Redistilled Super Q Water

Buffered solutions (pH 8.1) containing 5×10^{-6} M l^{-1} of methionine were irradiated for 2 hours in the merry-go-round system. Analysis on irradiated samples and dark controls was performed using HPLC-dansyl derivative method.

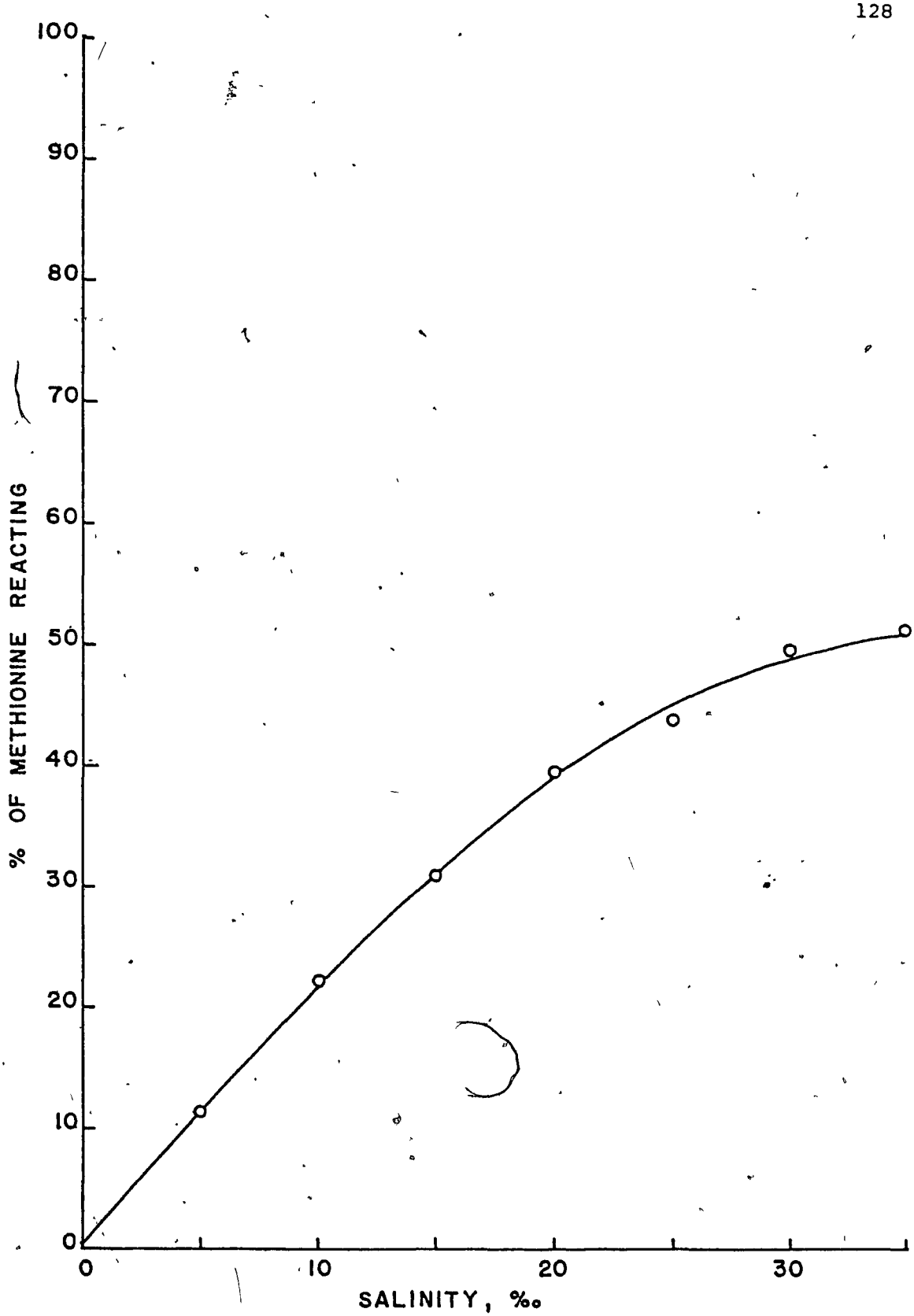
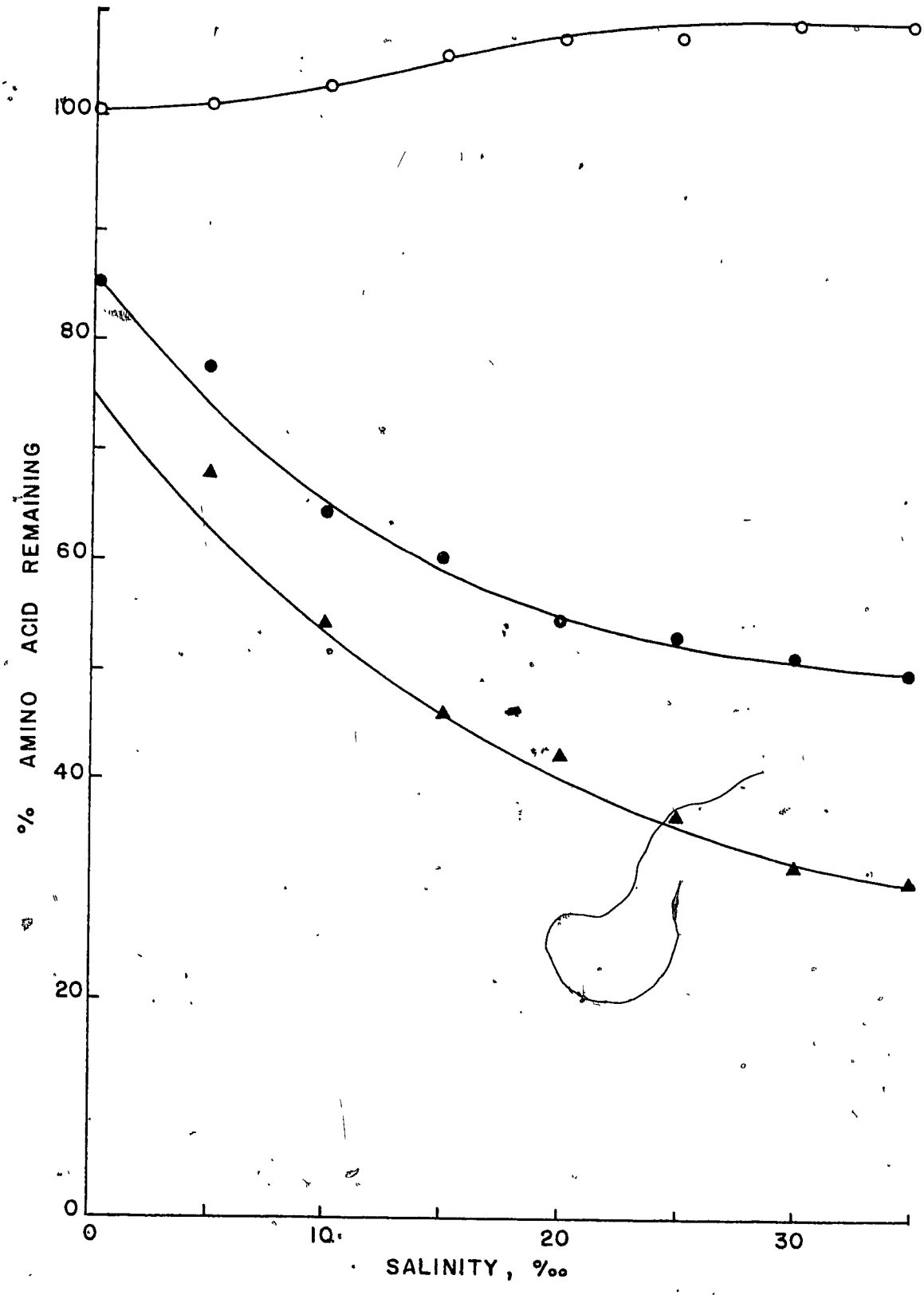

100
90
80
70
60
50
40
30
20
10
0
% OF METHIONINE REACTING
0
10
20
30
SALINITY, ‰

Figure 6.3 Photoreactivity of Glycine, Methionine, and Tryptophan in Seawater Solutions Diluted with Redistilled Super Q Water

Buffered solutions (pH 8.1) containing 5×10^{-6} M l^{-1} each of methionine, glycine, and tryptophan were irradiated for 2 hours in the merry-go-round system. Analysis on irradiated samples and dark controls was performed using HPLC-dansyl derivative method.

- o glycine
- ● methionine
- ▲ tryptophan


100
80
60
40
20
0
% AMINO ACID REMAINING
0
10
20
30
SALINITY, ‰

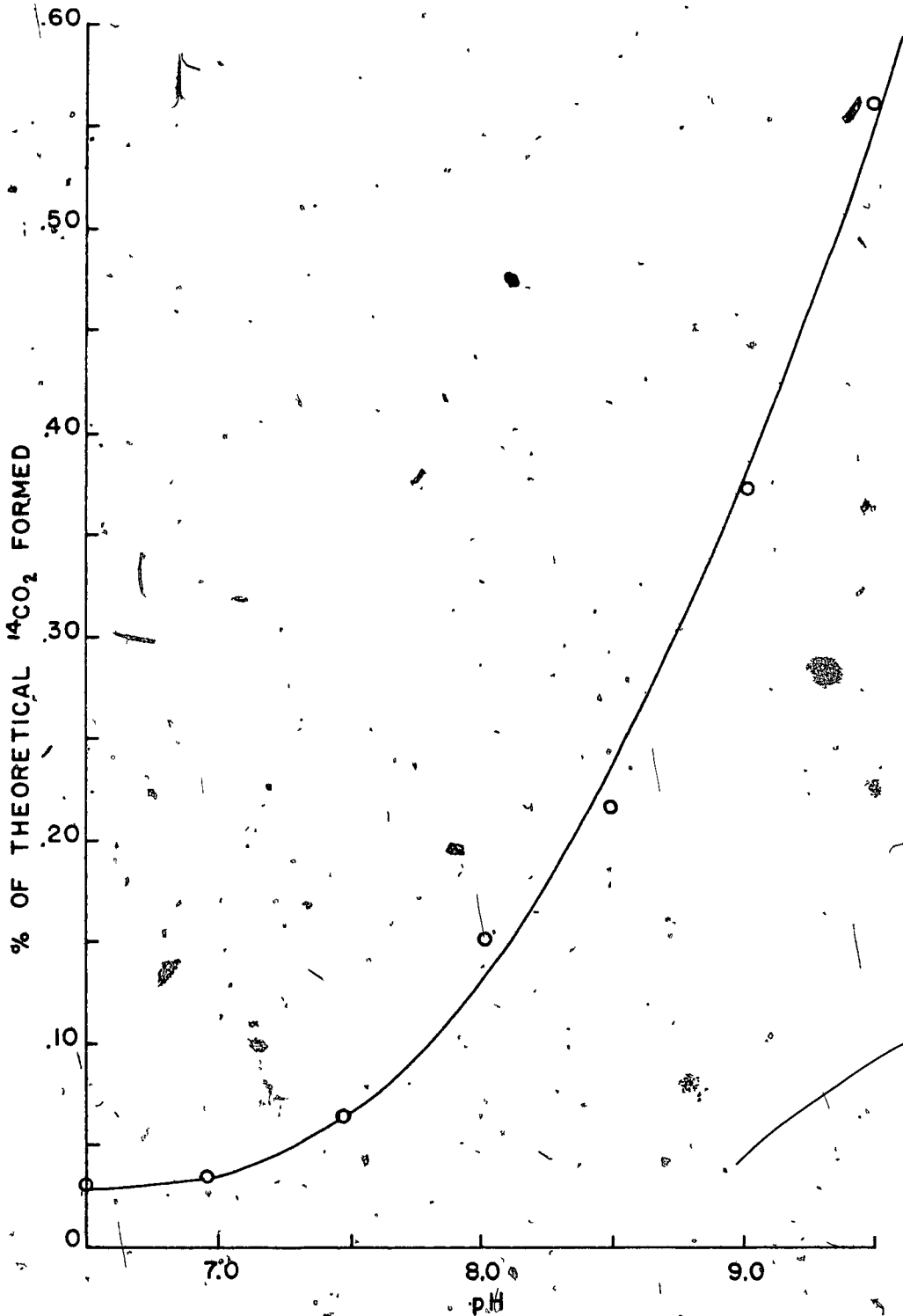
photoinduced destruction of methionine by tryptophan or its photolysis product, N-formylkynurenine, gives an apparent increase in methionine reactivity at low salinity, but not at high salinity (Figure 6.3), suggesting that the reaction might be dependent on ionic strength. Similar results were obtained for the methylene blue photoinduced oxidation of epinephrine (Section 10). Evidence suggests (Section 10.2.4.2.) that the triplet excited state of methylene blue is the oxidant; and the inverse relationship between epinephrine reactivity and salinity may be explained by an increase in quenching of the dye's triplet or singlet excited state with increasing salinity. Fluorescence quenching of organic dyes is certainly influenced by various anions (decreasing efficiency of quenching order: $I^- > Br^- > Cl^- > SO_4^= > NO_3^- > F^-$) and cations through heavy-atom effects which act to decrease lifetimes of excited states. If the triplet excited states of photosensitizers in seawater respond in the same way, then an inverse relationship between the photoreactivity of the reactive substrate and salinity might be expected.

6.6.2. pH

Seawater solutions containing 1×10^{-7} M l^{-1} of 1-^{14}C glycine were adjusted to different pH values with phosphate or borate buffer and then irradiated in the merry-go-round system under the same conditions. The measured $^{14}CO_2$ production showed that a sharp increase in the decarboxylation of the glycine occurred for pH values greater than 7.5 (Figure 6.4). This increase can be attributed to a variety of things, but it is

Figure 6.4 Effect of pH on Glycine Decarboxylation

Seawater was filtered, enriched with 1 x 10^{-7} M l^{-1} of 1-^{14}C glycine (containing .5 μ Ci/50 ml sample), and buffered with phosphate for pH values below 8.0 and borate for 8.0 and above. The solutions were irradiated for 2 hours in the merry-go-round system and then analyzed for $^{14}CO_2$.


.60
.50
.40
.30
.20
.10
0
% OF THEORETICAL $^{14}CO_2$ FORMED
7.0
8.0
9.0
pH

likely that the predominant effect is a result of electron density redistribution in glycine, as its pK of 9.78 is approached. The importance of (+) inductive effects in increasing the reactivity of oxidizing radicals with various organic substrates is well known (Adams et al., 1965) and the reactivity of glycine towards OH• (Scholes et al., 1965) behaves very much the same as the results shown in Figure 6.4 for the same pH range.

The dramatic increase in rate with increasing pH might also indicate that glycine is not predominantly reacting with an anionic species, since the anionic form of glycine becomes increasingly more important at high pH values. This is illustrated by the reaction rate of glycine with hydrated electrons, where the rate decreases with increasing pH (Davies et al., 1965), as a result of the decreasing encounter rate of "like charged" species.

It is impossible to draw any definite conclusions on the reasons for the large pH effect from such an empirical approach. However, the results clearly indicate the importance of controlling pH during photolysis experiments, since the reactivity of glycine in this experiment varied by over 350% in the normal seawater pH range of 7.6 to 8.4.

6.6.3. Constituent effects

Throughout this study the various reactivity tests used all indicated that the test substrate was more reactive in natural seawater than artificial seawater of the same ionic strength. Since the major

constituents and properties, such as pH, O_2 concentration and temperature were the same (within narrow limits), the difference must be attributed to minor constituents which would be expected to vary between the two solutions. The possibility that the observed lower reactivity in artificial seawater was at least in part due to impurities in the salts or water used for their prepration wad discussed earlier (Section 3.2.1.).

Methionine and glycine were the amino acids most frequently used to test the reactivity of seawater. The effect of various substances on the photoreactivity of these amino acids was tested by comparing results from seawater or artificial seawater solutions to the same solutions enriched in these substances (Tables 6.5 and 6.6). Those substances which increased the reactivity for one or both of the amino acids were NO_3^-, NO_2^-, fulvic acid, H_2O_2, riboflavin and transition metals. All of these substances are part of the minor constituents of seawater and all have absorption bands in the near-ultraviolet or visible region. The non-conservative nature of such components means that they should vary in distribution, making seawater a non-uniform reaction media.

The substances which were found to decrease the amino acid reactivity were iodide and oxygen. Both are capable of acting as quenchers of excited states or as scavengers for reactive intermediates. Another scavenger, benzoic acid, commonly used to react with free radical species, was without effect, at least at the concentration em-

Table 6.5 Effect of Various Added Constituents on Methionine Photoreactivity

Solutions were prepared by enriching either artificial seawater (treated with Chelex-100 resin to remove trace metals[1]) or natural seawater with 5×10^{-6} M l^{-1} of methionine and other substances indicated in the Table. Solutions were buffered to a pH of 8.1 and irradiated for 2 hours in the merry-go-round system at a temperature of 20°C. Analysis were performed by HPLC of dansyl derivatives.

[1] It was discovered later that the high reactivity of methionine in this artificial seawater was the result of contamination with NO_2^- and NO_3^- formed by bacterial degradation of organic nitrogen compounds leached from the Chelex-100 during the trace metal removal procedure.

Reaction Media	Substance Added	% of Methionine Lost in 2 Hours of Irradiation
Artificial Seawater[1]	---	12.3
Artificial Seawater	1×10^{-4} M l^{-1} KNO_3	21.4
Artificial Seawater	1×10^{-5} M l^{-1} KNO_2	89.3
Artificial Seawater	5×10^{-6} M l^{-1} KI	10.0
Artificial Seawater	10 X TMEM	11.9
Artificial Seawater	5×10^{-7} M l^{-1} Fulvic Acid	26.2
Freshly Collected Seawater	---	19.4

Table 6.6 Effect of Various Added Substances on the Decarboxylation of Glycine in Seawater

All solutions were prepared with the same freshly collected seawater, which was air-saturated and buffered at a pH of 8.1. Each solution was 1×10^{-7} M in added glycine (containing .5 μ Ci/ 50 ml sample as 1-^{14}C glycine). The solutions were irradiated in merry-go-round system for 2 hours and then analyzed for $^{14}CO_2$.

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Characteristics of Seawater Solution	% of Glycine Decarboxylated
air-saturated	.129
deoxygenated by bubbling with N_2	.396
1×10^{-5} M l^{-1} I^-	.072
1×10^{-5} M l^{-1} Benzoic Acid	.130
1×10^{-5} M l^{-1} IO_3^-	.130
5×10^{-6} M l^{-1} NO_2^-	.488
1×10^{-5} M l^{-1} H_2O_2	.331
5×10^{-7} M l^{-1} Riboflavin	.690
9500 ug l^{-1} Fulvic Acid	.996
10 X TMEM	.267

ployed. The importance of concentration and the nature of the scavenger is better illustrated by comparing the effects of varying concentrations of EDTA and 2-propanol on the reactivity of glycine in seawater (Figure 6.5). EDTA appears to be the more effective scavenger at concentrations above 10^{-5} M, while 2-propanol, although not without effect, shows no sharp changes in the concentration range used. Oxidizing radicals should react with 2-propanol via H-atom abstraction much more rapidly under these conditions than with glycine (e.g., glycine + OH·, $k = 1\text{-}5 \times 10^7\ M^{-1}\ sec^{-1}$; 2-propanol + OH·, $k = 1\text{-}2 \times 10^9\ M^{-1}\ sec^{-1}$). Unfortunately, rate constants for reactions of oxidizing radicals with EDTA were not available, but unless oxidizing radicals in seawater were selective oxidants, there is no reason to believe that EDTA would be than much more reactive than 2-propanol or benzoate ion, which is more reactive than 2-propanol.

It does not appear that a mechanism which protects glycine by simply scavenging oxidizing species in solution explains the results in Figure 6.5. Glycine might instead by reacting in a photosensitized reaction initiated by organic constituents. Glycine is generally considered to be unreactive to dye photosensitization, but increases in CO_2 yield were noted in solutions containing the potential photosensitizers riboflavin and fulvic acid (Table 6.6). In the initial reaction the substrate molecule can react with the triplet excited state of the sensitizer via one of two general mechanisms (Eqs. 6.2 and 6.3).

$$^3\text{Sens} + \text{substrate} \longrightarrow \text{H or } e^- \text{ transfer} \qquad (6.2)$$

$$^3\text{Sens} + \text{substrate} \longrightarrow \text{energy transfer} \qquad (6.3)$$

Figure 6.5 Effect of Increasing Concentrations of EDTA and 2-Propanol on Glycine Photoreactivity in Seawater

The solutions were all prepared using the same freshly collected seawater, which was buffered at pH 8.1 and air saturated. Each solution was 1×10^{-7} M in added glycine (containing .5 μ Ci/50 ml sample as 1-^{14}C glycine). The solutions were irradiated in merry-go-round system for 2 hours and then analyzed for $^{14}CO_2$.

● 2-propanol

O EDTA

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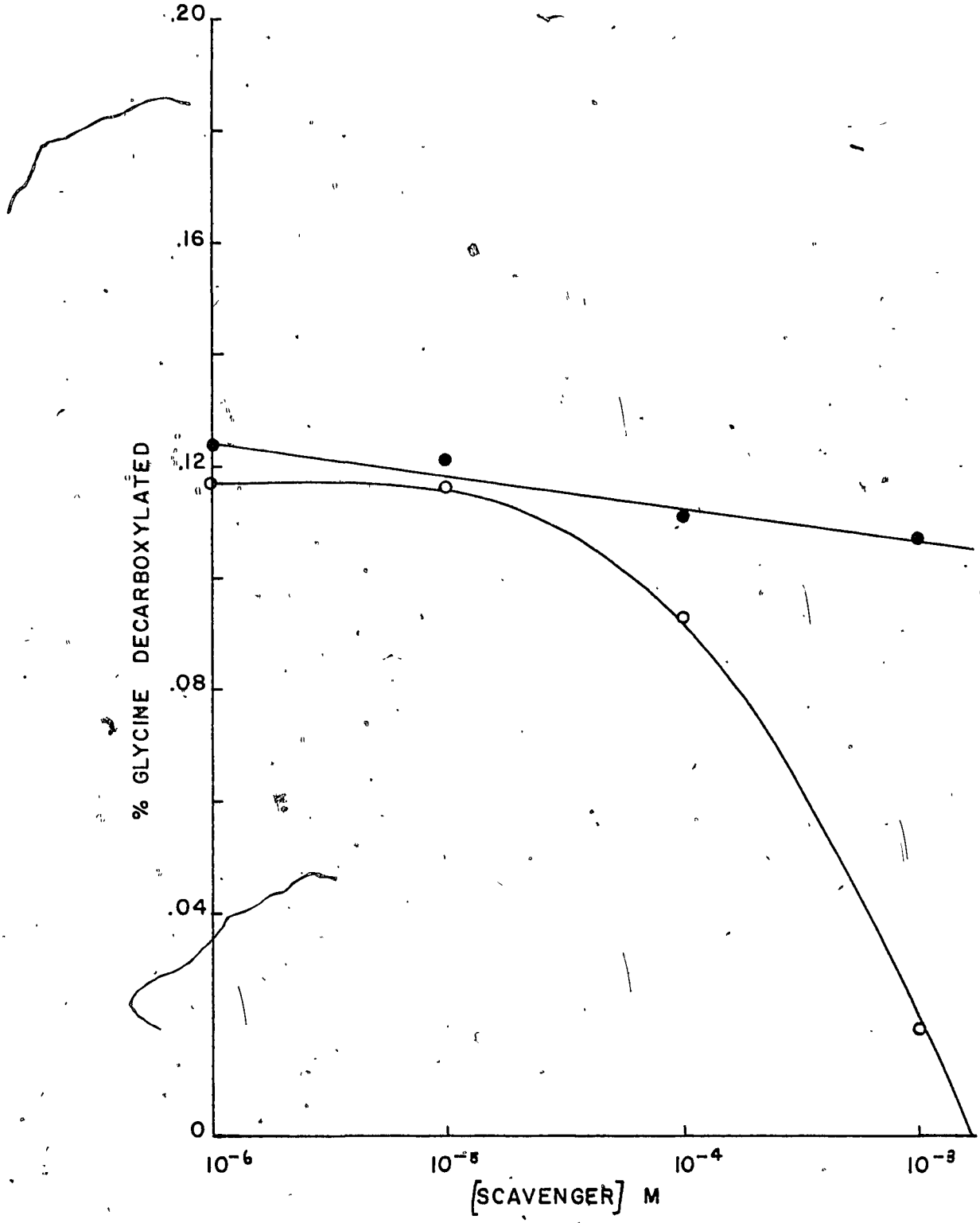
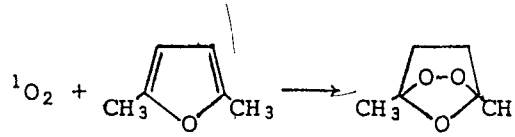

.20
.16
.12
.08
.04
0
% GLYCINE DECARBOXYLATED
10^{-6}
10^{-5}
10^{-4}
10^{-3}
[SCAVENGER] M

Which mechanism predominates will depend on the redox potential of the excited sensitizer and substrate (Berg and Gollmick, 1974), the concentration of sensitizer, substrate, and oxygen, and the reaction rate of substrate with the sensitizer. The rates for energy transfer reactions (Eq. 6.3) will be controlled mainly by the concentration of O_2, since its rate constant, at least for most known sensitizers, falls within the range 1-3 x 10^9 M^{-1} sec^{-1} (Kearns, 1971; Foote, 1976). In surface seawater the concentration of dissolved O_2 is in the range of 2-4 x 10^{-4} M l^{-1}, since this greatly exceeds the normal average total molar concentration of DOC compounds (probably 1-10 μM l^{-1}) which might react with the triplet excited state of natural sensitizers, reactions with O_2 should represent a major process. If the decarboxylation of glycine is due to its reaction with singlet oxygen ($^1O_2$), then the addition of a sufficiently large concentration of a good electron or hydrogen donar would compete with O_2 for the triplet excited state of the sensitizer. This was observed for seawater enriched in I^- (Table 6.6), which is known to undergo photosensitized oxidation in the presence of certain dyes (Kepka and Grossweiner, 1972). It is not clear, however, whether I^- is competing with O_2 in seawater or whether it is acting to quench the excited state of the sensitizers or $^1O_2$, since the later process is also possible at least in aprotic solvents (Rosenthal and Frimer, 1976).

EDTA is a better reducing agent of the excited triplet state of the sensitizer than 2-propanol (Berg and Gollmick, 1974) and should

therefore alter the mechanism of the reaction (Eq. 6.3) when its concentration reaches a level that can compete efficiently for the excited sensitizer or for $^1O_2$. Reactions of $^1O_2$ with EDTA or glycine have not been reported, however, some amines act as quenchers of $^1O_2$ (Kearns, 1971). The usual course of the reaction of readily oxidized substrates (e.g., EDTA, methionine, ascorbic acid, phenol, or allylthiourea) with known dye sensitizers is to form the semireduced dye. In aerobic solutions the semireduced form of the dye will normally reduce O_2 to give the superoxide anion radical (O_2^-), which can disproportionate to give H_2O_2, or undergo oxidation or reduction. One of the observed photolysis products of natural seawater is H_2O_2; its rate of formation can be increased by adding a readily oxidized substrate such as methionine (Section 10.).

Although the evidence suggests that $^1O_2$ could be present in irradiated natural seawater, it is difficult to understand how it can be a significant reactant with all but the most reactive of compounds, since it is quenched rapidly in aqueous solution ($k_D \approx 10^6$ sec^{-1}). Even the reaction with a good $^1O_2$ acceptor like 2,5-dimethylfuran should be insignificant at a concentration of 1×10^{-7} M in aqueous solution (Eq. 6.4). It would appear the net effect of having O_2 present in the reaction



$$k_A^{1} = 1.4 \times 10^8 \ M^{-1}\text{sec}^{-1} \qquad (6.4)$$

$$k_A[A] = 1.4 \times 10 \text{ sec}^{-1} \text{ for } [A] = 1 \times 10^{-7} \text{ M}$$

$$k_D >> k_A[A]$$

---

[1] The rate constant for this reaction was determined in methanol rather than in water. Rate constants for reactions in aqueous solutions are usually much lower.

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solutions is to quench the triplet excited state of the sensitizer. This might explain the increase in glycine decarboxylation in anoxic seawater solutions (Table 6.6).

In considering the quenching of the decarboxylation reaction of glycine by EDTA one further consideration should be taken into account. This is the obvious role of EDTA as a strong chelator; in so doing it will mediate in photoinduced reactions of transition metals, which were also shown to accelerate the reaction (Table 6.6). Unfortunately, this role of EDTA is so familar to marine scientists that its reactive nature and capacity to act in ways other than as a chelator are seldom considered. For instance, the involvement of trace metals in the dark and light degradation of purines in seawater has been based on the observation that EDTA inhibits these reactions (Antia and Landymore, 1974). Considering the effect of EDTA on the reaction of glycine in seawater, the same conclusion could be reached here, but the pronounced effect of other constituents on this reaction makes the interpretation unsustainable.

Determining the relative importance of various constituents on the photoreactivity of seawater is further complicated by the formation of new species during the irradiation. Some of these may play no part in altering the photochemical properties of the reaction system, while others may act to alter the observed kinetics or even the predominant mechanism operating. The formation of peroxides, which accumulate during the irradiation (Section 10.), is an example of such a process.

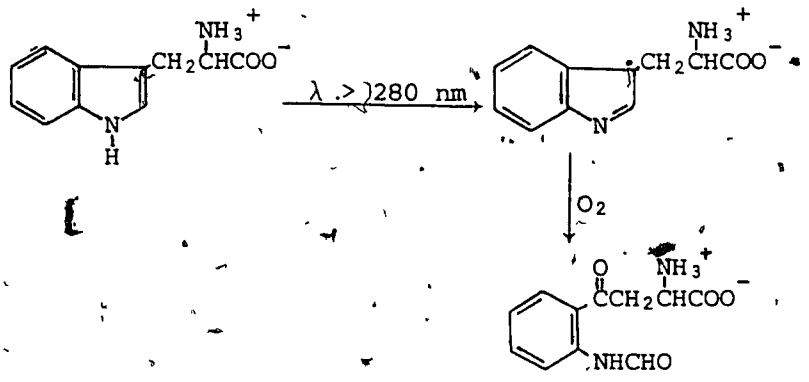
The simultaneous occurrence of O_2^- in solution could then result in an increase in importance of strong oxidizing radicals (Eq. 6.5) (Peters and Foote, 1976) during the course of the reaction and the steady

$$ROOH + O_2^- \longrightarrow RO\cdot + OH^- + O_2 \qquad (6.5)$$

state assumption normally applied to free radical concentrations of solutions irradiated with a continuous radiation source is invalid. Seawater to which H_2O_2 was added at the beginning of the irradiation period gave a higher percentage of decarboxylation of glycine than the same seawater without added H_2O_2 (Table 6.6). For a labile substrate like methionine this effect is far more pronounced.

The complicated nature of seawater photochemistry is exemplified by a study of the photosensitized degradation of methionine by tryptophan, which was investigated to determine the possible importance and characteristics of photosensitized reactions in seawater. The photochemistry of tryptophan has been extensively studied in de-aerated solutions with ESR and flash-photolysis techniques (Baugher and Grossweiner, 1977; Pailthorpe and Nicholls, 1972; Santus and Grossweiner, 1972). The observed transient species are the tryptophyl radical cation, which results from photoejection of an electron from the parent molecule, the 3-tryptophyl radical, and the triplet excited state of tryptophan. Generally, the studies on tryptophan have been conducted with wavelengths of 310 nm or less and only the initially formed transients were considered. If the bandwidth of the incident radiation is broadened to include wavelengths of greater than 320 nm and the reaction duration is

extended, the reaction takes on a new dimension in that N-formylkynurenine is formed (Eq. 6.6), which can function as a photosensitizer in



(6.6)

λ max, 360 nm

the oxidation of tryptophan or other substrates (Walrant et al., 1975). In an aerobic aqueous solution containing methionine and tryptophan, and irradiated with a spectrum of light covering a broad wavelength region (290-400 nm), the reaction probably proceeds as shown in Figure 6.6 (Walrant et al., 1975). Experimental results for such reactions (Table 6.7) display a very similar behavior to those observed for the photolysis of glycine in natural seawater (Table 6.6 and Figure 6.5). Again, O_2 and EDTA act as inhibitors of the reaction, O_2 for the reasons discussed earlier and EDTA probably because it scavenges the 3-tryptophyl radical. EDTA does not protect methionine to the same extent that it does tryptophan, which indicates that methionine is either reacting with the triplet state of tryptophan, O_2^-, $^1\Delta O_2$, e_{aq}^-, $EDTA_{ox}$, or H_2O_2 formed from the disproprotionation of O_2^-. Singlet oxygen should react more rapidly with tryptophan than with methionine, which would not explain why EDTA protects tryptophan to a greater extent than methionine.

Figure 6.6 Mechanism for the Photolysis of Tryptophan at Wavelengths in the Near-Ultraviolet

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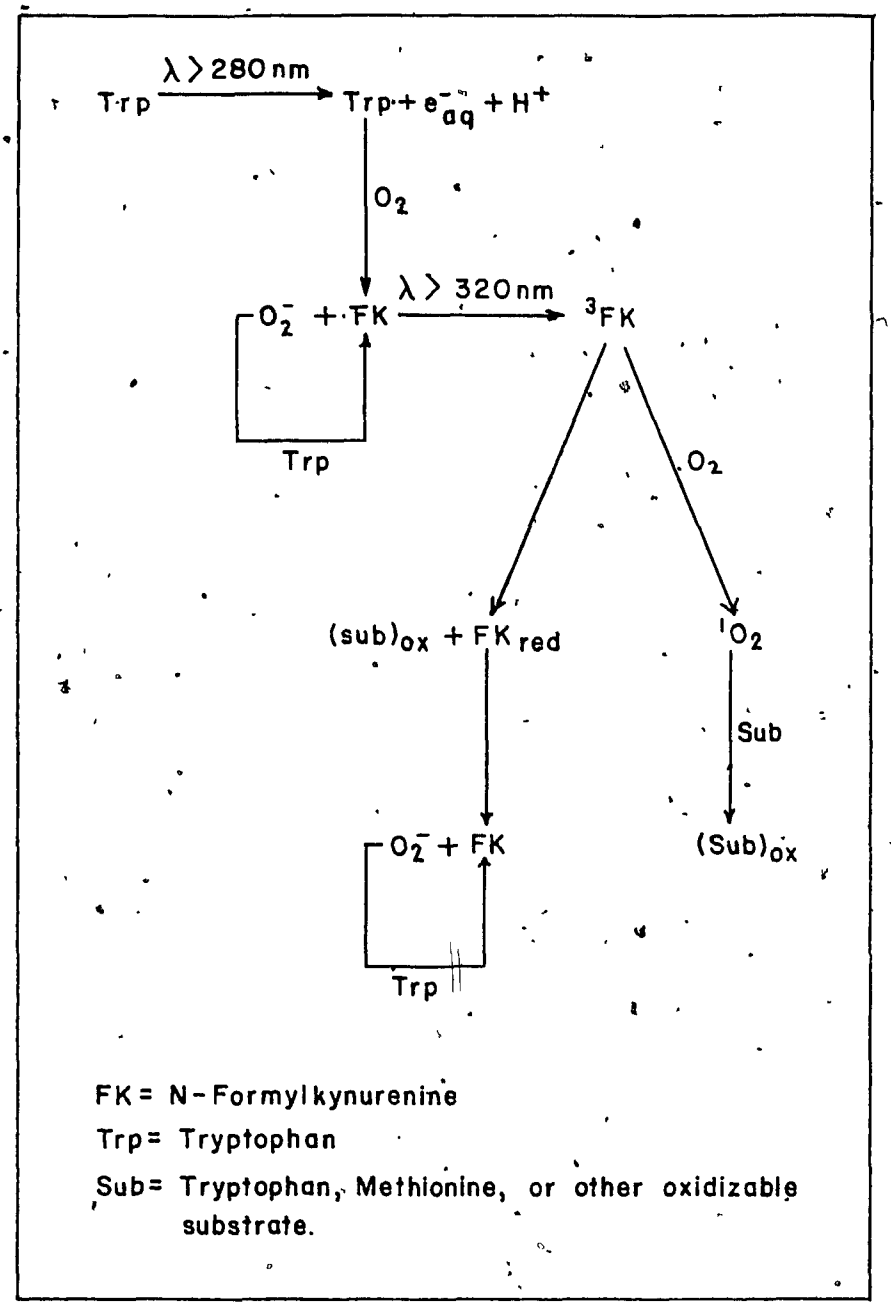
λ > 280 nm
Trp
Trp + e^-_{aq} + H^+
O_2
O_2^- + FK
λ > 320 nm
^{3}FK
Trp
O_2
$(sub)_{ox}$ + FK_{red}
$^1O_2$
Sub
O_2^- + FK
$(Sub)_{ox}$
Trp
FK = N-Formylkynurenine
Trp = Tryptophan
Sub = Tryptophan, Methionine, or other oxidizable substrate.

Table 6.7 Effect of Various Constituents on Tryptophan and Methionine Photoreactivity

The concentration of tryptophan and methionine that was added was always 5×10^{-6} M. All solutions were buffered to pH 8.1 and air-saturated. All irradiations were conducted in the merry-go-round system for 2 hours. Analysis was performed by HPLC of dansyl derivatives.

[1] This seawater was collected at 500 meters in vicinity of Nova Scotian Shelf Break.

Characteristics of the Solution	% of Amino Acid Reacting	
	Methionine	Tryptophan
methionine in Super Q water	2.8	---
methionine and tryptophan in Super Q water	25.0	38.2
methionine and tryptophan in Super Q water, deoxygenated	64.6	95.0
methionine and tryptophan in Super Q water, 1×10^{-3} M l^{-1} EDTA	13.0	6.6
methionine and tryptophan in NaCl solution (I = .684)	29.8	40.8
methionine and tryptophan in 35 ‰ artificial seawater	24.4	38.4
methionine and tryptophan in ∿ 35‰ natural seawater[1]	54.8	80.7
methionine and tryptophan in ∿ 35‰ natural seawater plus 1×10^{-5} M l^{-1} of Cu^{2+}	29.0	17.0

The reaction with e_{aq}^- should be insignificant, because of its much larger rate constant with O_2 (Eqs. 6.7 and 6.8). The triplet state of

$$O_2 + e_{aq}^- \longrightarrow O_2^- \qquad k = \sim 2 \times 10 \ \text{sec}^{-1} \qquad (6.7)$$

$$\text{Met} + e_{aq}^- \longrightarrow \text{Met}_{red} \qquad k = \sim 200 \ \text{sec}^{-1} \qquad (6.8)$$

trytophan is not likely to react with methionine, since the competition for it by O_2 should be much greater. It is difficult to narrow the field of possible reactants any further, but it is interesting to note that the remaining possibilities represent perhaps some of the lowest energy and longest lived species and support the results obtained earlier on the photoreactivity of amino acids in seawater.

In the case of the tryptophan-methionine reactions, there does not appear to be any appreciable salinity or ionic strength effect, as evidenced by comparing the results for reactions run in deionized water, NaCl solution, and artificial seawater. The effect of complimentary reactivity gained in natural seawater is clearly evident from the results shown in Table 6.7. This indicates that the results of the competitive reaction between glycine, tryptophan, and methionine (Figure 6.3) versus salinity cannot be explained in terms of ionic strength dependence. Therefore, the activity coefficients of the reactants cannot be appreciably altered by a change in ionic strength; this implies that the reactants either do not carry different charges or that the net charge of the reactants is the same as the transition state.

7. DECAY OF SEAWATER FLUORESCENCE[1]

7.1. Introduction

Organic components of seawater fluoresce when excited with ultraviolet light (Duursma, 1974). The substances responsible for this fluorescence are introduced to the ocean by rainfall, rivers, organisms, and in situ formation. In coastal waters, a main source of supply of fluorescent substances comes from rivers which drain watershed lands rich in humic substances. This feature of coastal areas has been used as an indicator for water masses (Kalle, 1966; Højerslev, 1971; Zimmerman and Rommets, 1974) and has been considered as a tool which might be used in mixing studies and pollution studies (Kullenberg and ygard, 1971; Duursma, 1974). The use of any property as a tracer requires that the property be relatively conservative within the time scale of the event which is being studied. This has been assumed to be the case for seawater fluorescence; results seem to indicate that at least in the environments studied the property is relatively conservative (Duursma, 1974). This conclusion is derived primarily from the observations that fluorescence is inversely correlated with salinity in regions of fresh water input and that this correlation holds over long distances from the source. Unfortunately, no detailed investigations

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[1] Although the luminescence emission process from seawater has not been established, it is always referred to as fluorescence. It is probable that this is the major process occurring, since visible phosphorescence is not normally observed unless a low collisional deactivation rate of the triplet state exists. Therefore, phosphorescence is unlikely in fluid solutions, like seawater; there are, however, notable exceptions.

on how the characteristics of this fluorescence change with respect to other seawater properties or with time exist. Although the specific characteristics of the fluorescent substances in seawater are unknown, certain intrinsic properties of the fluorescence of organic molecules suggest that the measured fluorescence of seawater solutions all containing the same concentration of natural fluorophors might be dependent on:

1) salinity,
2) temperature,
3) pH,
4) quenchers (e.g., O_2 and other paramagnetic substances, and certain diamagnetic metal complexes),
5) and the irradiation history of the solution.

The last of these properties is particularly interesting with respect to photochemical studies because many organic molecules, which have relatively high quantum yields in photochemical processes, also are highly fluorescent. This is particularly true for known organic photosensitizers; the measurement of natural seawater fluorescence might therefore be indicative of the presence of such substances and even be used to determine their relative stability under environmental conditions.

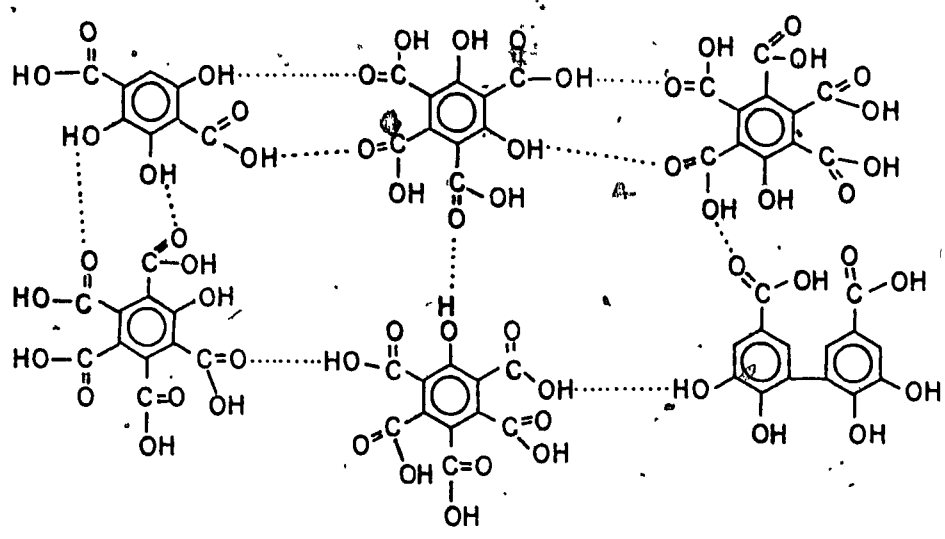
The nature and exact composition of fluorescent substances in seawater is not known, but it is likely that they are a complex mixture of substances derived from different sources (Duursma, 1974) and are therefore subject to considerable variation in their makeup, especially in

regions where large changes in source inputs can occur. This is sometimes apparent from observed excitation and emission wavelength maxima, which are altered as a result of changes in the fluorophor composition from some event, such as a phytoplankton bloom (Traganza, 1969). Generally, the fluorescence spectra for both excitation and emission resemble broad featureless bands with maxima near 370 nm and 490 nm, respectively (Kalle, 1937; Kullenberg and Nygard, 1971). The typical fluorescence spectra of seawater from estuaries, upwelling areas, and the open sea are similar. The difference between them is a matter of intensity (Duursma, 1974). In coastal water, the dominant fluorescent substance appears to be derived from humic materials of terrigenous origin (Figure 7.1), while in the open ocean they are believed to be produced in situ through a condensation reaction of dissociation products from carbohydrates with nitrogenous compounds, such as amino acids (Kalle, 1963). This generally accepted concept has recently been challenged by Karabashev (1976), who prefers to identify the fluorescing substances with the organic matter rather than as a product of its formation.

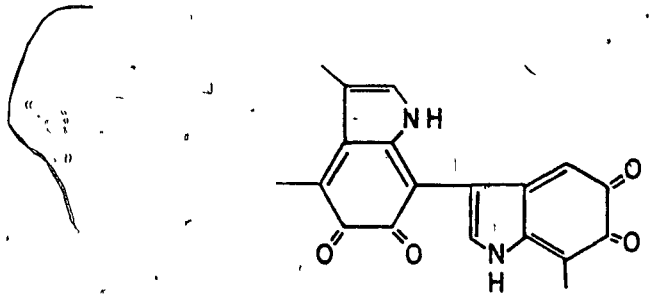
The major product of the proposed in situ reaction is melanin (Figure 7.1); like fulvic acid (Figure 7.1), which is undoubtly a major fluorescence contributor from land, it posseses an extensive chromaphoric system. Both of these materials should act as major absorbers of the light penetrating the ocean's surface, although their photochemical characteristics are unknown. It is entirely possible that they may be conservative with respect to photochemical processes and

Figure 7.1 Proposed Unit Structures for

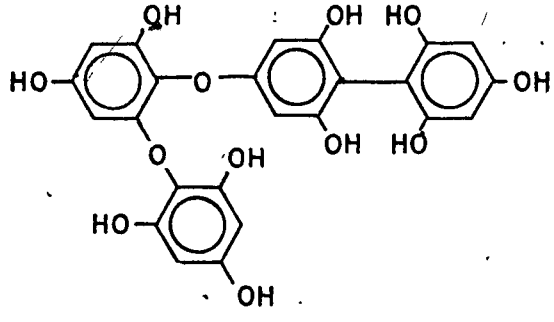
Fulvic Acid, Melanin, and Phloroglucinol-Based Polymer



Fulvic Acid (Schnitzer & Kahn, 1972)



Melanin (Duursma, 1965)



Phloroglucinol-based Polymer (Ragan & Craige, 1976)

that excitation energy is dissipated through purely photophysical processes.

The stability of natural seawater absorbance and fluorescence to light exposure was determined. Further studies were also conducted on fresh water from Nova Scotian streams and on solutions of naturally occurring purified polymers which are representative of some of the natural constituents likely to be present in seawater from the Nova Scotian coastal waters.

7.2 Results and discussion

When a fresh sample of seawater was irradiated in the merry-go-round system, a rapid decay of the natural fluorescence of the sample was observed during the first 10 minutes of the irradiation, with a more gradual decrease thereafter (Figure 7.2). A concomitant decrease in the absorption spectra during this period was proportionally far smaller than the decrease in fluorescence. This indicates that the fraction responsible for fluorescence may be only a small part of that contributing to absorption (see also, Figure 10.10).

In a similar experiment, seawater that had been stored in the dark at room temperature for 4 months prior to the irradiation showed a trend similar to that observed for freshly collected samples (Figure 7.2). Although the initial fluorescence of this water was not measured at the time of collection, its relatively high fluorescence value after standing for 4 months indicates that thermal or microbial decay of the fluorescent materials is small in comparison to its photochemical decay. High microbial and thermal stability of these substances was observed

Figure 7.2 Light Initiated Decay of Natural Seawater Fluorescence

Freshly collected seawater was filtered and irradiated in the merry-go-round system for the times shown. Fluorescence of each sample was determined shortly after the irradiation period.

▲ blank not subtracted

■ artificial seawater blank subtracted

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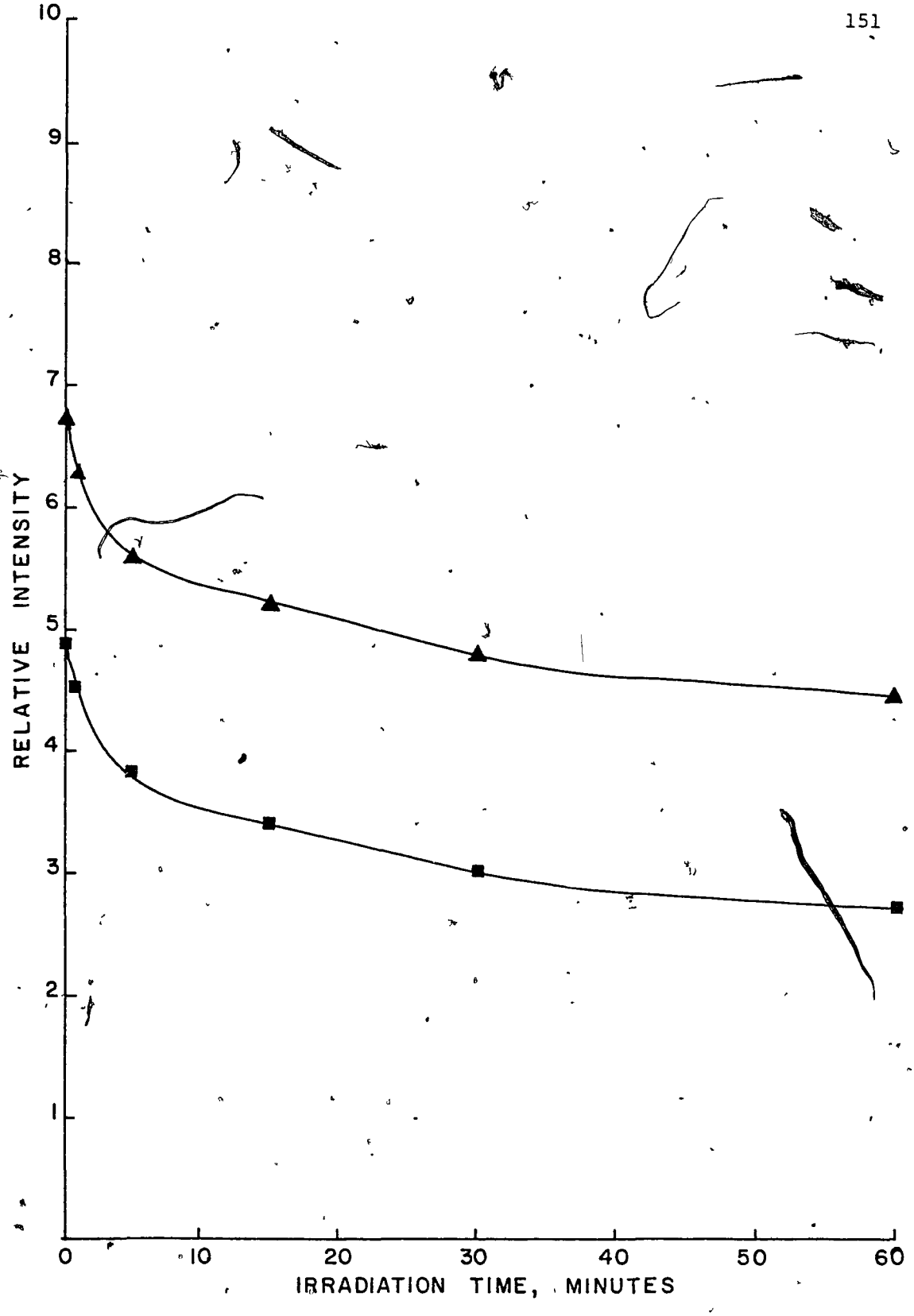
RELATIVE INTENSITY
10
9
8
7
6
5
4
3
2
1
0
10
20
30
40
50
60
IRRADIATION TIME, MINUTES

in other experiments; in fact, an increase in fluorescence was observed on long term storage of some solutions, suggesting that in situ formation of fluorophors was occurring. This possibility is not unlikely in view of the evidence for in situ formation of fluorescent materials in the oceans (Kalle, 1963; Duursma, 1974; Postma et al., 1976). The possibility also exists that this increase is the result of contamination; this has been proposed to explain why the fluorescence of stored Super Q water increases with time (Brown, 1974).

Postma et al. (1976) have also demonstrated that an appreciable decay of fluorescence of Rhine River water occurs in lakes to which the river is a tributary and that the extent of decay is a function of the residence time in the lake. The decay continues as this water mixes into the North Sea. It was suggested that the observed instability of the fluorescence in these waters results from microbial destruction of the land derived "humic" materials. This suggestion is supported by evidence for the microbial decay of fulvic acid in fresh water (Haan, 1972 and 1977). There is, however, no evidence for significant microbial decay in either fresh water or seawater used in this work. Significant utilization of fulvic acid via biological pathways may be very dependent on specific properties of the water; these pathways certainly deserve further consideration.

The light induced decay of fluorescence of land derived fluorophors was examined by exposing seawater solution contianing 10% stream water to artificial light sources (Table 4.1) used to illuminate the 10 m tower tank in the Oceanography Department at Dalhousie University

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(Balch et al., 1976). Solutions were contained in 35 ml sealed quartz tubes, which were suspended in the tank at specific depths (Table 7.1). After an exposure of 48 hours of continuous irradiation, the samples at all depths showed a significant decrease in fluorescence as compared to the dark solutions. On a typical sunny summer day the rate of fluorescence decay would be expected greatly to exceed these values, because of the much higher photon flux, particularly for the spectral region where maximum decay occurs. The tower tank lamps have a significantly lower output of near ultraviolet radiation than does the sun.

A rapid fluorescence decay was observed in water from fresh water streams and in coastal seawater, when irradiated with near-ultraviolet radiation. The same was found for visible radiation, although the decay was far less pronounced (Table 7.2). Therefore, fluorescence should certainly be a non-conservative property in the entire euphotic zone. A lower level of fluorescence near the surface and an increase with depth might be anticipated in regions where the water column is relatively constant with respect to inputs of fluorescent materials and mixing. Depth profiles of natural fluorescence made in such areas often exhibit this feature. Ivanoff (1962) measured fluorescence in the Mediterranean and found low values near the surface, an increase with depth to 75 m, and relatively constant values below that depth. Similar profiles of rapidly decreasing fluorescence with depth in the upper 200 m were observed in the Black Sea (Karabashev, 1970) and the western boundary currents of the tropical Atlantic (Karabashev and Solov'ev, 1974). In the Baltic, an increase in the fluorescence with depth was observed

Table 7.1 Light Induced Decay of Seawater

Fluorescence in Tower Tank Experiment

[1] Light flux was measured only for PAR region (400-700 nm). Coated phosphor lamps were used as the light sources. The flux below 400 nm is proportionally less than for sunlight.

[2] A sea surface sunlight flux of 2000 μ einsteins m^{-2} sec^{-1} was assumed for the PAR region.

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Depth, m	Light Flux[1] μ Einsteins m^{-2} sec^{-1}	% of Typical Solar Flux[2] (Noon at Sea Surface)	% Decrease in Fluorescence
0	288	14.4	53.8
1	160	8.0	43.2
2.5	72.1	3.6	31.6
5.0	24.8	1.2	15.2
7.5	10.5	.53	8.0
10.0	5.04	.25	2.5

Table 7.2 Decay of Natural Fluorescence

in Different Wavelength Regions

The sample was collected at 1 m in St. Margaret's Bay. It was buffered with borate and filtered through a .22 μ Millipore filter. The irradiation time was 2 hours in the outer ring of the merry-go-round system.

[1] see Appendix 5

[2] see Appendix 6

[3] see Section 5.5

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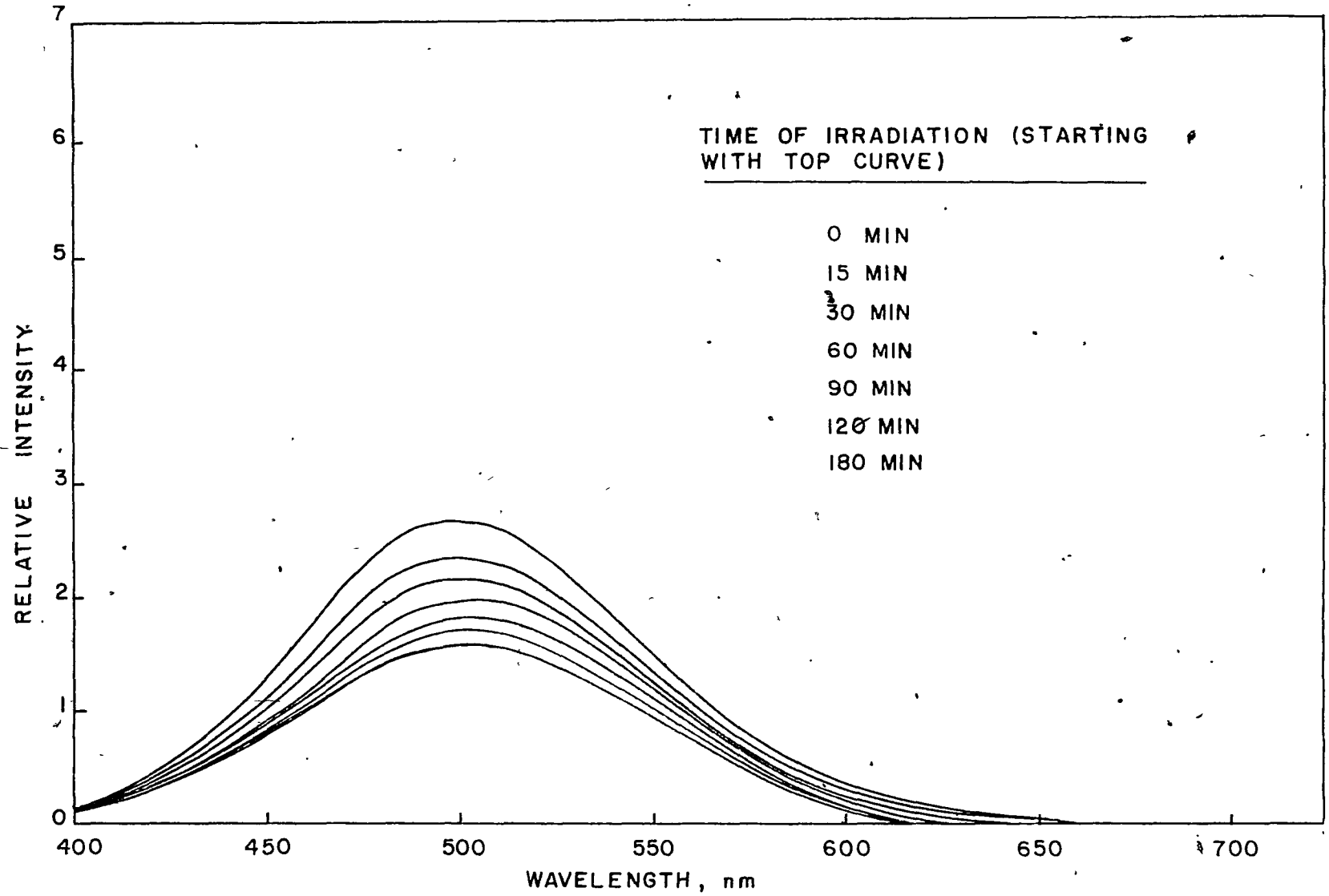
Filter Number[1]	Approximate Spectral Region[2]	Fluorescence Intensity in QSU[3]	% Decay D[3]
Dark	---	7.74	0
CS 7-60	300-400 nm	5.18	43.0
CS 3-70	> 500 nm (D)	6.02	28.9
CS 3-74	> 400 nm (C+D)	5.86	31.5
CS 0-53	> 300 nm (A+B+C+D)	4.75.	50.2

(Højerslev, 1974) which was attributed to an increase in the formation of fluorescent materials as sinking organic matter decomposed. Viewed in another way, the lower fluorescence near the surface might be indicative of an accelerated transformation of natural fluorophors through light induced reactions. This premise, of course, holds only if photolability of natural seawater fluorescence is a property common to all of the regions in question. For over 100 samples taken at various times of the year and from a variety of different locations in Nova Scotian coastal waters, the fluorescence always decreased on irradiation. However, the rate of the decrease varied considerably (see Table 11.2), with no distinct correlation to any of the other measured properties, other than the radiation flux.

If the fluorescence of seawater at any location is the combined contributions of a number of different fluorophors, then the observed response of the sample to light is dependent on the characteristics of the specific fluorophors and upon their concentration. In coastal water, variations in the fluorophor composition are likely to be strongly affected by seasonal and spatial variations in algae and land drainage. The composition of the fluorescent materials of oceanic water impinging on the coast may therefore be dramatically altered. The light induced response of different fluorescent materials can fall into three general categories: (1) fluorescence decreases, (2) fluorescence is stable or rapidly assumes a constant value, or (3) fluorescence increases. Humic substance of fluvial origin appear to fall into category (1) as is shown in Figure 7.3, where the continuous exposure of a sample of stream water to sunlight has caused a diminution in the intensity of emission,

Figure 7.3 Changes in the Fluorescence

Emission Spectra of Stream Water During Exposure to Sunlight


TIME OF IRRADIATION (STARTING WITH TOP CURVE)
0 MIN
15 MIN
30 MIN
60 MIN
90 MIN
120 MIN
180 MIN
RELATIVE INTENSITY
0
1
2
3
4
5
6
7
400
450
500
550
600
650
700
WAVELENGTH, nm

157

but not in its spectral features. When exposed to the same conditions, a sample of seawater collected within three miles of the mouth of the stream also showed a decrease in fluorescence, but differed in that it was more rapid (Figure 7.4) and occurred with a corresponding red shift in the emission maxima from 475 to 490 nm. In Figure 7.4 it appears that this seawater sample has reached a constant value of fluorescence after one hour. With extended irradiation time, however, this fluorescence value would probably continue to diminish, but at a much slower rate, typical of the behavior of seawater fluorescence.

The major component of land derived humic substances is probably fulvic acid, since it is the major soluble organic component of soil leachates (Schnitzer and Desjardins, 1969) and represents as much as 85% of the organic matter in streams rising in swamps (Fotiyev, 1968). The fluorescence emission spectra of a seawater solution of purified fulvic acid[1], of stream water diluted with seawater, and of natural seawater all exhibited similar broad featureless bands which varied in the wavelength of the observed emission maxima (Figure 7.5).

Fluorescence of the purified fulvic acid solution decayed upon irradiation in the same way that seawater solutions of stream water did. Artificial seawater solutions of purified fulvic acid also gave absorption spectra which had the same general broad absorption features of natural seawater (Figure 7.6). Most of the absorption in the natural seawater sample could be accounted for if fulvic acid were on the order

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[1] The purified fulvic acid was supplied by Dr. Donald S. Gamble; it was originally extracted from a Podzol Ph soil from Prince Edward Island, Canada.

Figure 7.4 Decay of Fluorescence with Time in Seawater and Freshwater Exposed to Sunlight

Filtered samples of seawater and freshwater were placed in quartz tubes and exposed to afternoon sunlight on October 24th.

▲ Seawater from vicinity of Halifax Harbour mouth

O Freshwater from Duncan's Cove

[1] The relative intensities have been scaled to 10 and do not represent the quantitative difference between the values for the two curves. The ratio of the actual measured values was 18.4: 1 for the freshwater to seawater samples.

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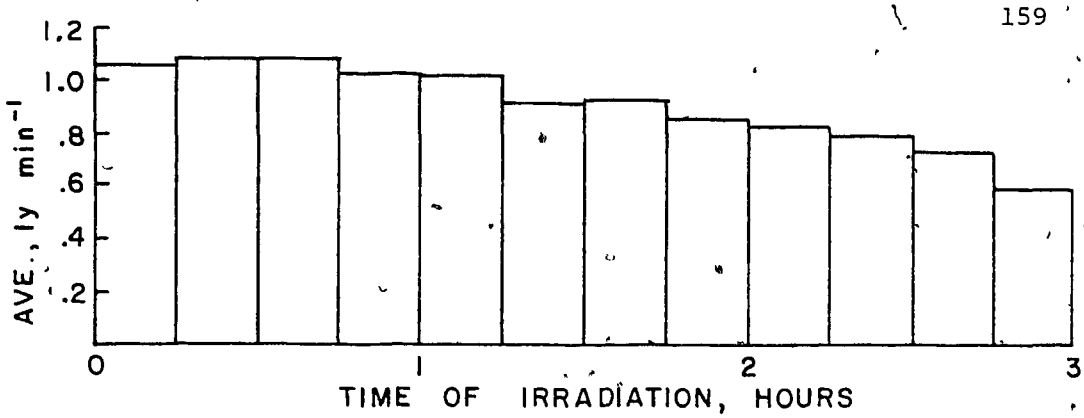

AVE., ly min⁻¹
1.2
1.0
.8
.6
.4
.2
0
1
2
3
TIME OF IRRADIATION, HOURS

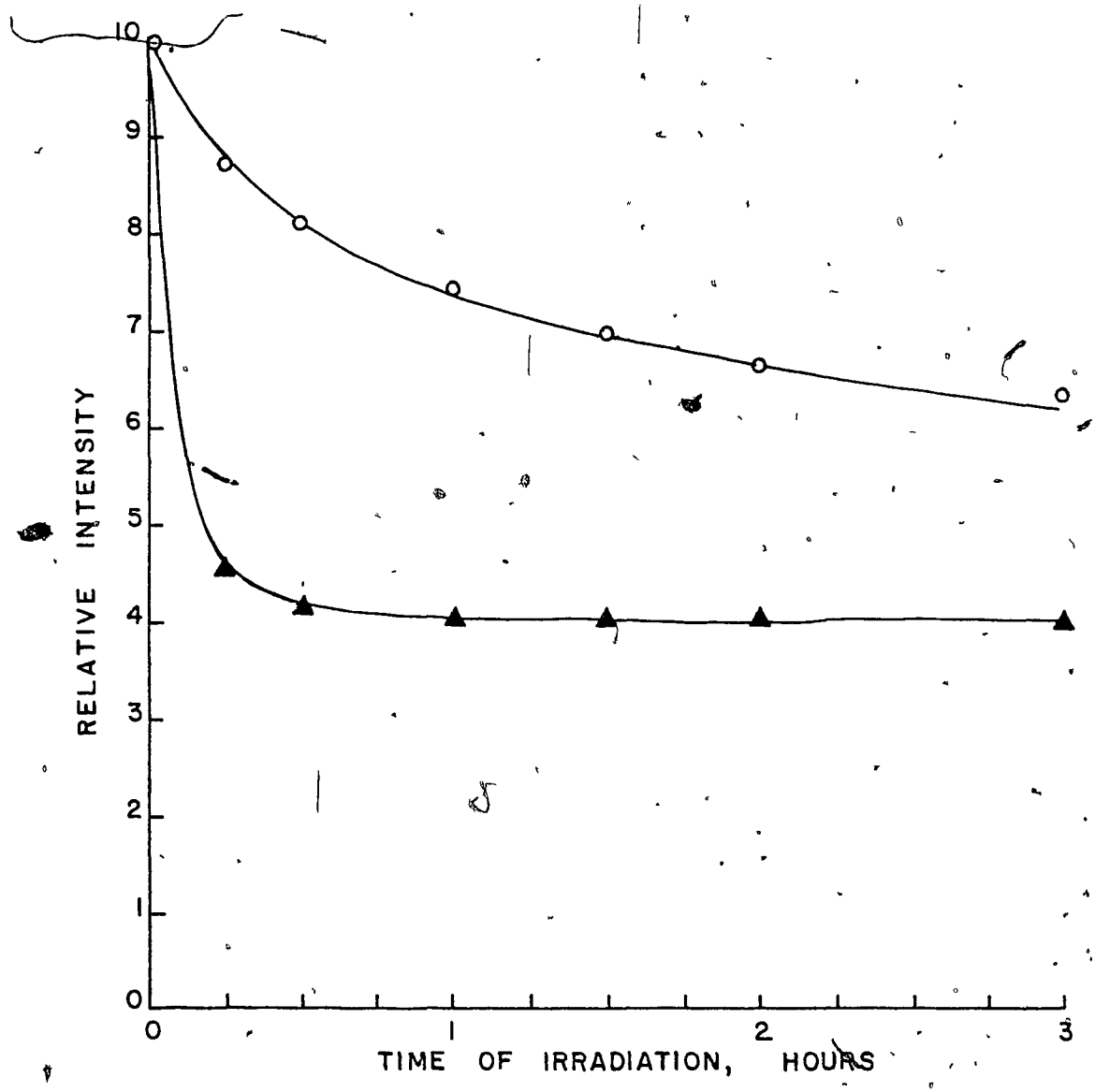

RELATIVE INTENSITY
10
9
8
7
6
5
4
3
2
1
0
0
1
2
3
TIME OF IRRADIATION, HOURS

Figure 7.5 Fluorescence Emission Spectra

for Natural Water Samples and Purified Fulvic Acid Solutions

The solutions were buffered to pH 8.1, with the exception of the stream water which was maintained at its natural level of pH ∿ 4.5. All solutions were filtered immediately before determination of their spectra. An excitation wavelength of 385 nm was used for all samples.

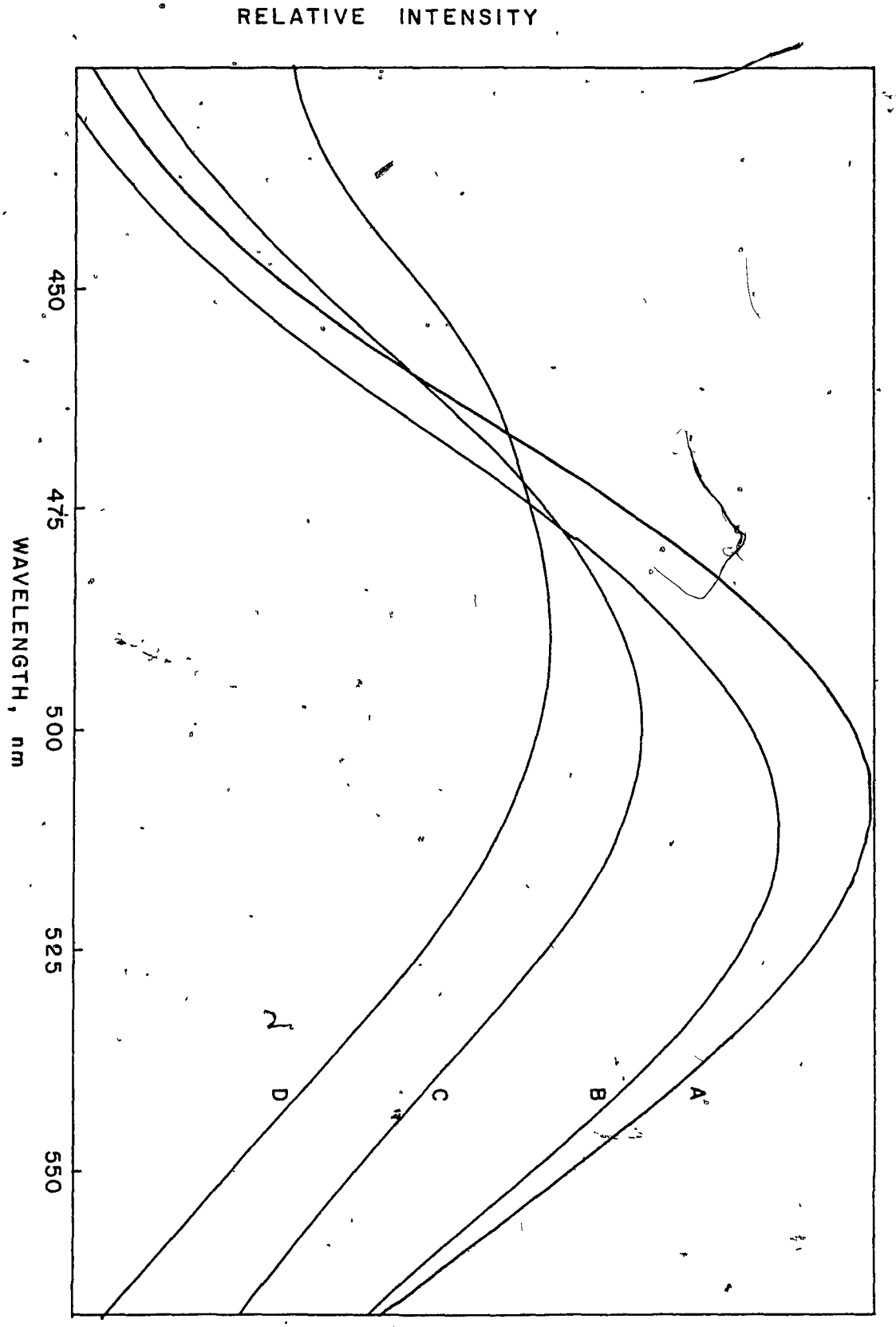
A. 1×10^{-5} M l^{-1} fulvic acid in 31‰ salinity seawater

B. 1×10^{-5} M l^{-1} fulvic acid in redistilled Super Q water

C. stream water

D. seawater

[1] Relative intensity is not scaled alike for each spectra.



160

091

Figure 7.6 Absorption Spectra for Natural Seawater, Artificial Seawater, and Artificial Seawater Solutions Containing Fulvic Acid

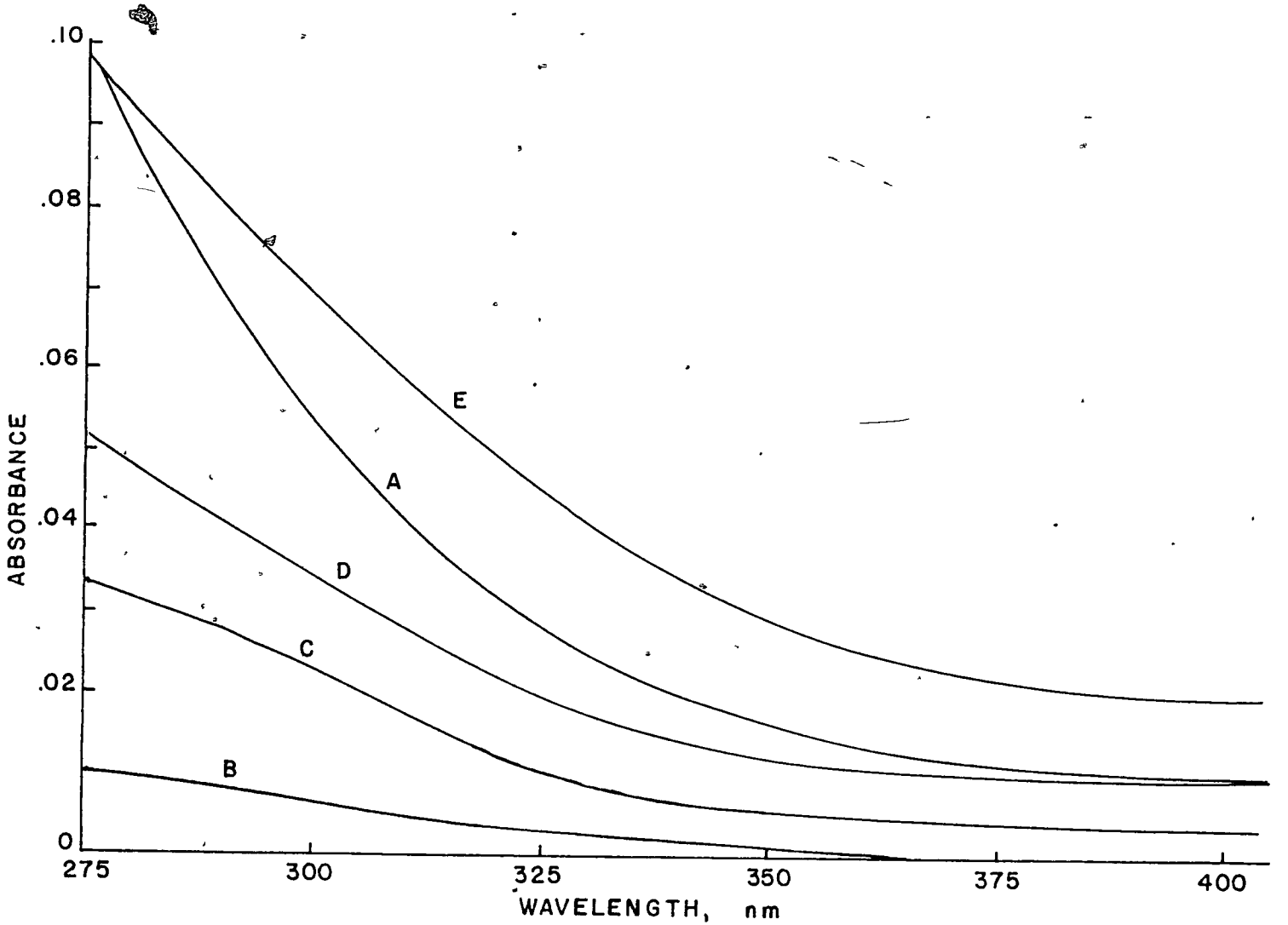
A. natural seawater (31‰ S)

B. artificial seawater (31‰ S)

C. 95 μg l^{-1} of fulvic acid in artificial seawater

D. 190 μg l^{-1} of fulvic acid in artificial seawater

E. 380 μg l^{-1} of fulvic acid in artificial seawater


.10
.08
.06
.04
.02
0
ABSORBANCE
E
A
D
C
B
275
300
325
350
375
400
WAVELENGTH, nm

of 5% (190 μg l^{-1} of fulvic acid) of the TOC. Therefore it would require the addition of only a small increment of organic-rich stream water to cause a significant increase in the light absorption or in the fluorescence of seawater.

Another probable source of seawater fluorescence could be derived from exudates of benthic algae. Ragan (1976a) has isolated and purified water soluble polymeric phenolic compounds from Ascophyllum nodosum and Fucus vesiculosus (brown macrophytes which are abundant along the Nova Scotian coast). Pulsed Fourier transform carbon-13 nuclear magnetic resonance spectra of this polymeric fraction indicate that its structure is similar to the phloroglucinol-based tetramer, 2,2',4,6,6'-pentahydroxy-4'-0-(2-0-(2,4,6-trihydroxyphenyl)-4,6-dihydroxyphenyl) biphenyl (Figure 7.1). Dilute seawater solutions of this substance[1] exhibit a weak fluorescence, which increases if the solutions are irradiated (Figure 7.7). The absorption spectra of these solutions also showed changes after exposure to light (Figure 7.8), which might indicate that an extensive transformation of the polymer is taking place.

Although exudates of bethic algae may not be significant contributors to the budget of fluorescent materials in offshore waters, they could play a significant role in modifying the properties of the coastal regime. The polyphenolic fraction might comprise 15 to 50% of the total organic exudates from brown macrophytes (Sieburth and Jensen, 1969; Sieburth, 1969; Langlois, 1975). The quantity of this material

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[1] A sample of purified polymer was kindly supplied by Dr. Mark Regan from the National Research Council Laboratories, Halifax, Nova Scoita.

Figure 7.7 Fluorescence Excitation and Emission Spectra of Irradiated and Non-Irradiated Seawater Solutions of Phloroglucinol-Based Polymer

The solutions were prepared by dissolving 1.53 mg of the polymer/100 ml of 31‰ l salinity seawater. The solutions were buffered to pH 8.1, stored at 2°C for 24 hours, then filtered and irradiated for 2 hours in the merry-go-round system.

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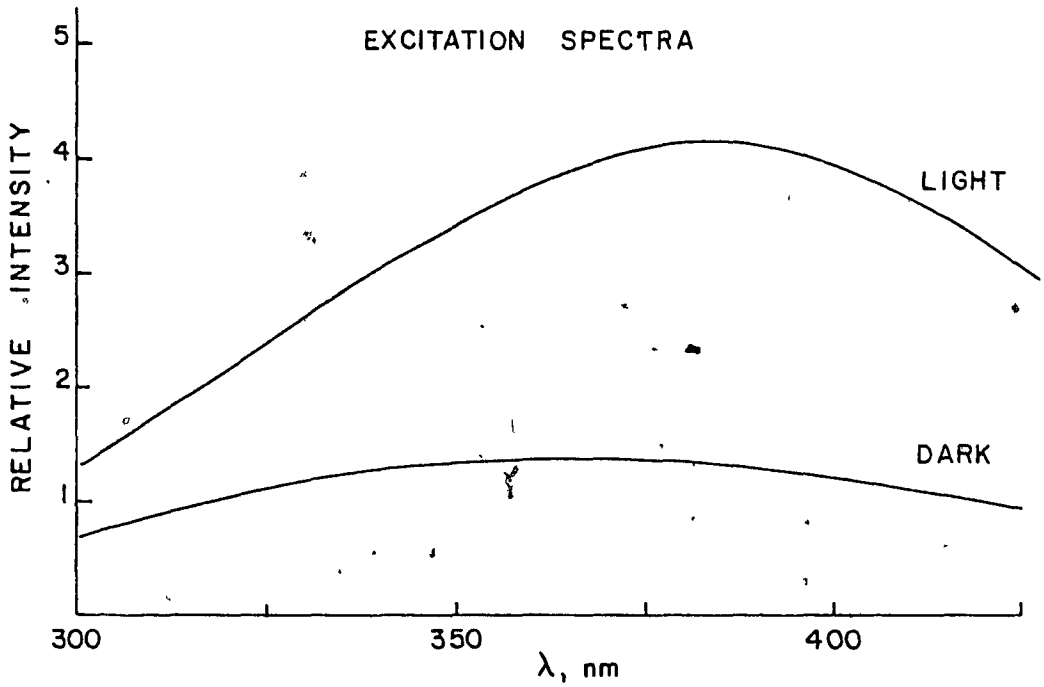

EXCITATION SPECTRA
RELATIVE INTENSITY
5
4
3
2
1
LIGHT
DARK
300
350
400
λ, nm

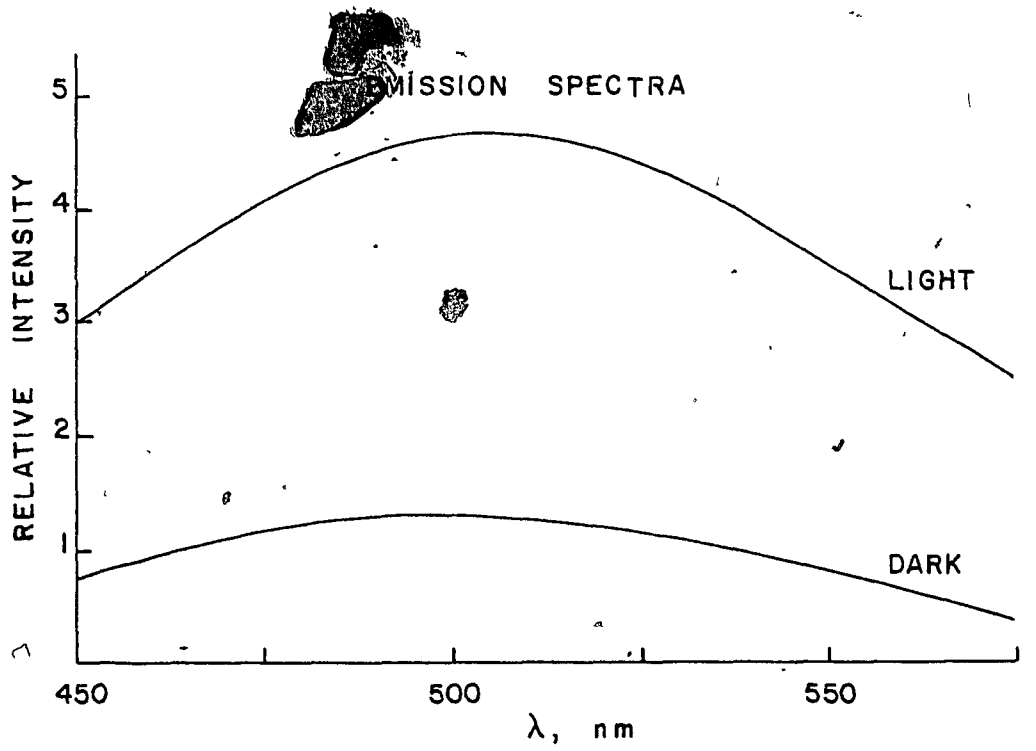
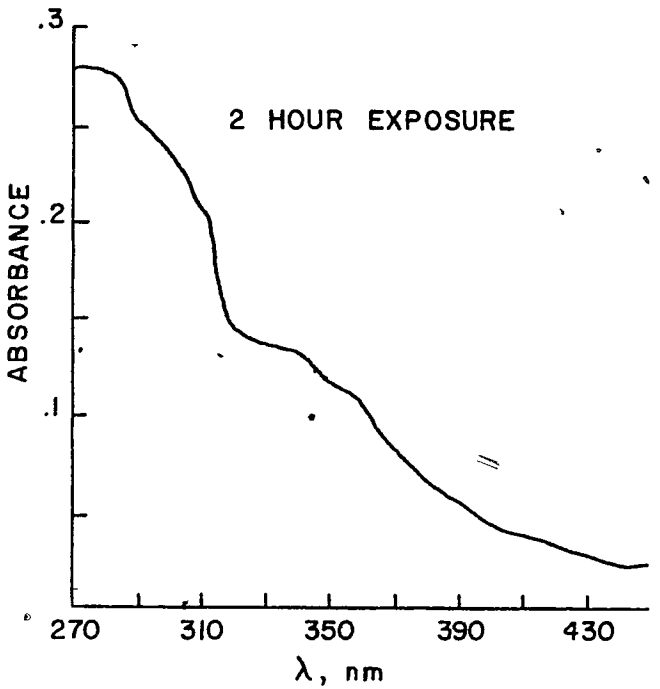
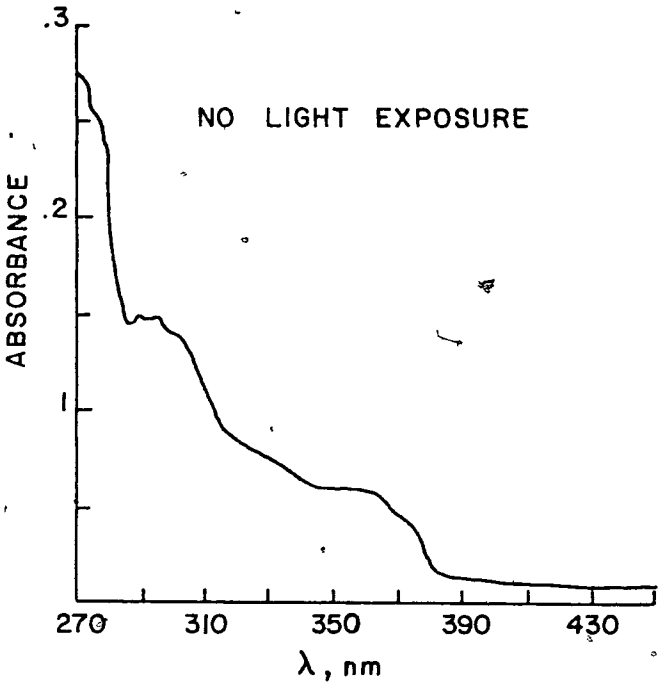

EMISSION SPECTRA
RELATIVE INTENSITY
5
4
3
2
1
LIGHT
DARK
450
500
550
λ, nm

Figure 7.8 Absorption Spectra of Irradiated and Non-Irradiated Seawater Solutions of the Phloroglucinol-Based Polymer

See Figure 7.7 for details of solution preparation. Absorption spectra were determined using a 1 cm pathlength.


2 HOUR EXPOSURE
ABSORBANCE
.3
.2
.1
270
310
350
390
430
λ, nm


NO LIGHT EXPOSURE
ABSORBANCE
.3
.2
1
270
310
350
390
430
λ, nm

165

released may vary diurnally, seasonally, and as the result of local environmental stresses. Although not all of this material is likely to exhibit the properties observed for the polyphenolic extract used here, it is likely, nevertheless, to have a profound effect on the composition of the fluorescent fraction of seawater in regions where macrophytes are abundant.

The observed differences in the light induced change of fluorescence for different seawater samples is shown in Figure 7.10. Samples were collected for 5 different depths at the same station on St. Margaret's Bay (Figure 7.9) over a period of one month, with sampling intervals averaging about 5 days. It was intended that this sampling program be continued over several months to cover the spring pre-bloom, bloom, and post-bloom periods. However, a rash of laboratory equipment failures caused the termination of the program while still in the pre-bloom period and thus limited statistical analysis to a maximum of only six observations for each parameter at each depth. The lines (Figure 7.10) connect the mean values for the data points at each depth and reflect the trends in a specific property with depth. The most obvious feature in each profile is the large range about the mean for the 0 and 5 m depths; the generally lower values of salinity at these depths probably reflect the influence of several small fresh water tributaries to the bay (Figure 7.9). All of the major streams contributing to the bay are deeply colored and can add a significant fraction to seawater fluorescence even when highly diluted. This is perhaps best seen from the inverse relationship between salinity and natural fluorescence (Figure 7.10) and in the correlation between salinity and fluorescence (Figure 7.11). The fact that the three highest values of fluorescence were observed in near

Figure 7.9 Location of St. Margaret's Bay Station

The station (⊕) was located at 63°59' west and 44°35 ' north.

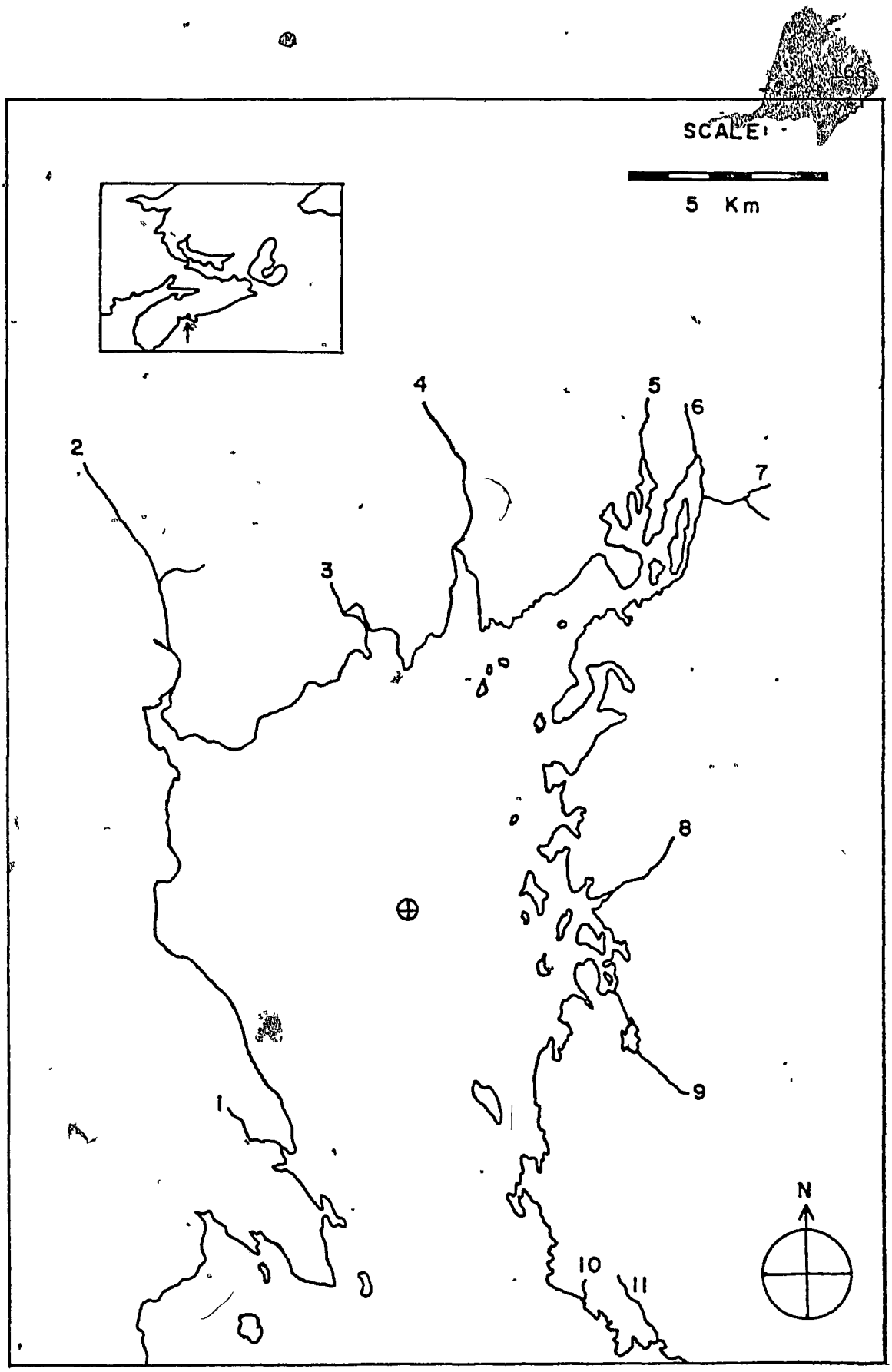

SCALE:
5 Km
1
2
3
4
5
6
7
8
9
10
11
N

Figure 7.10 St. Margaret's Bay Sampling Program: Depth Profiles for Salinity, Fluorescence, Fluorescence Decay, and Chlorophyll a

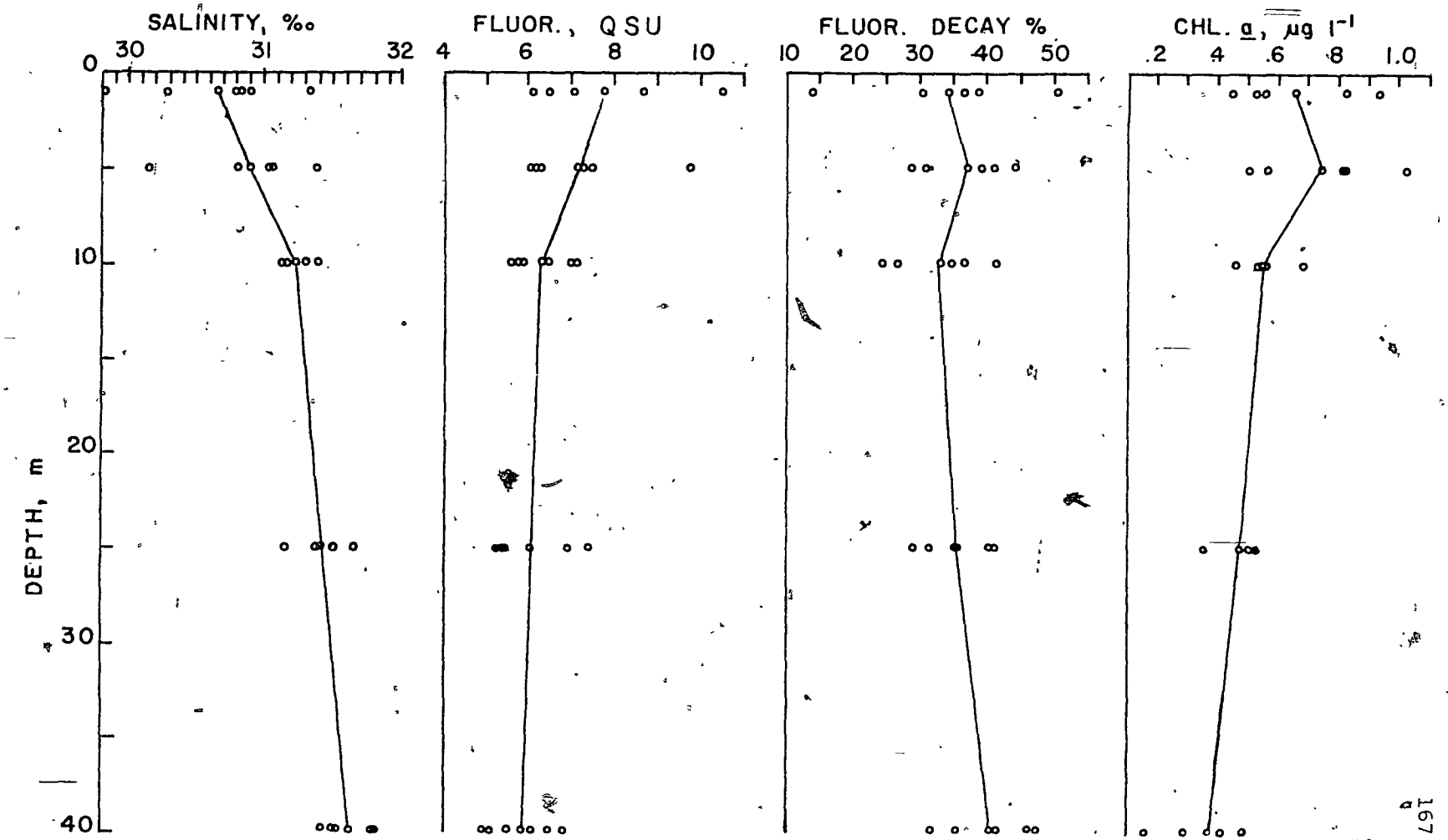
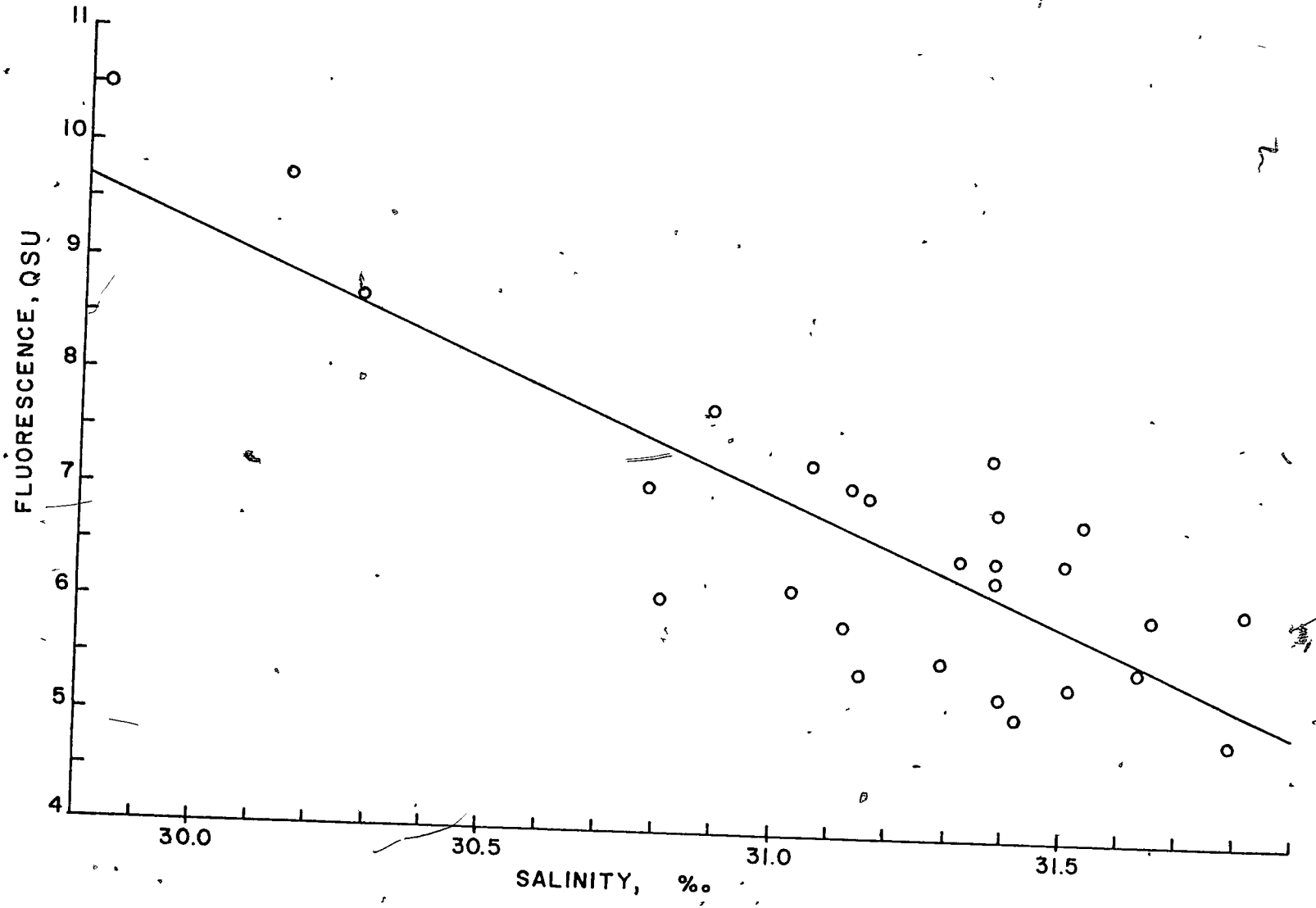

SALINITY, ‰
30
31
32
FLUOR., QSU
4
6
8
10
FLUOR. DECAY %
10
20
30
40
50
CHL. a, µg l⁻¹
.2
.4
.6
.8
1.0
DEPTH, m
0
10
20
30
40

Figure 7.11. Salinity-Fluorescence Correlation

for St. Margaret's Bay Sampling Program



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surface samples shortly after periods of major precipitation indicate the strong influence of fluorophors derived from fresh water on surface water characteristics in the area. This indicates that coastal seawater fluorescence variability can arise from episodic events, as well as from seasonal changes in runoff or soil conditions. The wide spread in values in Figure 7.11 could be the result of the tributaries having different concentrations of fluorescent materials because of differences in their sources or drainage basins.

The strong influence of fresh water sources on the fluorescent properties could mask other sources, which contribute to the fluorescent material budget. An examination of the percent of fluorescence decay (Figure 7.10) for the 1, 5, and 10 m depths indicates that on all but one date a maximum is observed at 5 m. The only exception to this occurred on March 16th, when the surface fluorescence was abnormally high with respect to the 5 m value; this was likely a consequence of the fact that in excess of 45% of the month's total precipitation fell in the 5 days preceding that date. The fluorescence decay maximum at 5 m could be explained by the introduction of another more light labile fluorescent substance at this depth. Since a chlorophyll maximum was observed at 5 m (Figure 7.10) on all sampling dates, it may be that the source was the result of phytoplankton abundance at this depth.

Examination of the data for the 10, 25, and 40 m depths reveals that the maximum in the fluorescence decay always occurs at the 40 m depth. This trend might simply reflect an increasing protection of fluorescent materials from sunlight with increasing depth. However, a

similar trend in particulate and dissolved Mn and Fe is also observed at these depths (Table 11.2). The bay at this station is from 50 to 55 m deep; the increasing trend with depth for these properties might therefore be a result of resuspension from the sediments. This is not to imply that Fe and Mn are a part of the fluorescent materials, but only that a gradient of a fluorescent substance whose source is in the region of the sediments can extend to the 25 to 40 m depths.

It has been assumed throughout the preceding arguments that fluorescence decay is occurring through a zero order reaction, that is, the rate of decay is a function of only light intensity and is independent of the concentration of fluorophors. If, however, the decay is a result of first or higher order reactions, then concentration of fluophors or other reactants becomes of paramount importance and interpretation of data acquired from natural samples, without measuring all significant dependent variabiles, may be impossible.

8. PHOTOREACTIVITY OF GLYCINE IN THE PRESENCE OF TRANSITION METALS

8.1. Introduction

The photochemistry of transition metal coordination compounds is described by an extensive literature which extends back into the last century. At that time it was observed that aqueous solutions of some metals underwent extensive chemical changes when exposed to sunlight. Today the literature includes studies on the photochemistry of nearly all of the transition metals; extensive reviews have appeared on the subject (Adamson, 1969; Balzani and Carassiti, 1970; Endicott, 1970; Bucat and Watts, 1972). Recently there have also been a number of investigations on the environmental photochemical significance of trace metals in natural waters. These have dealt primarily with the sunlight degradation of aminopolycarboxylates (EDTA and NTA) in the presence of Mn^{2+}, Fe^{3+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, and Mg^{2+} (Natarajan and Endicott, 1973; Carey and Langford, 1973; Trott et al., 1972; Langford et al., 1973; Lockhart and Blakeley, 1975; Lockhart, 1976). Other reports include investigation of photoalkylation of Hg^{2+} and Tl^{2+} (Jewett et al., 1975) and the effect of trace metals in seawater and culture media on the photolysis of uric acid and xanthine (Anita and Landymore, 1974).

With respect to the marine environment, there is essentially no information on the photochemical significance of any of the transition metals that are present. Most of them are present at concentrations so

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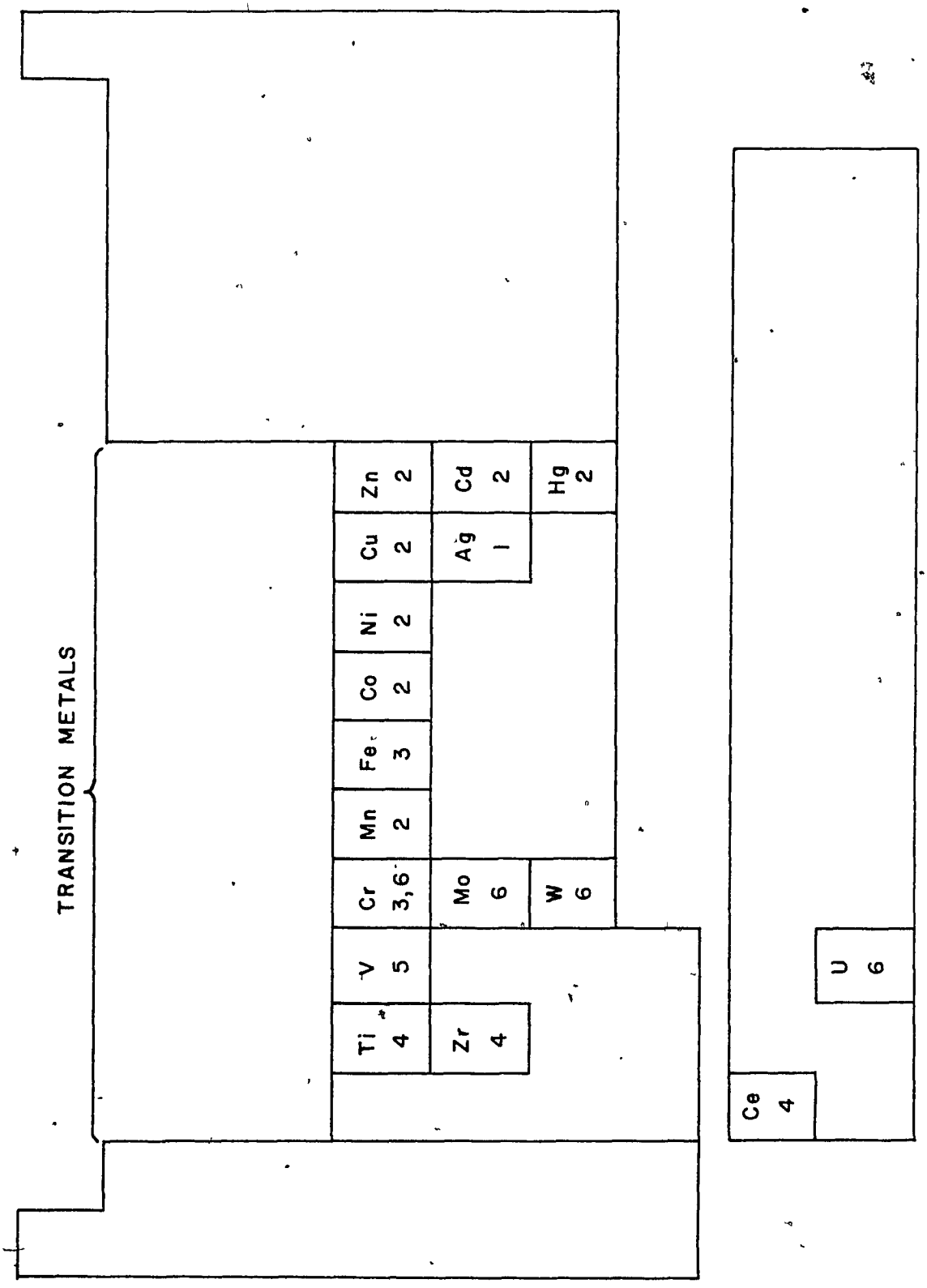
low that they would seemingly be insignificant contributors to the photochemistry of seawater. Also, the predicted activity of many of them is low in seawater and they might exist mainly in a colloidal or particulate fraction rather than in a truly soluble form. Trace metals can, however, induce photochemical reactions when present in solutions as a solid phase. This has been observed for the oxides of Zn (Rubin et al., 1953; Nagarjunan and Calvert, 1964; Frank and Bard, 1977), Cu (Ritchey and Calvert, 1956; Khenokh and Bogdanova, 1967b), Fe (Khenokh and Bogdanova, 1967b; Frank and Bard, 1977) and Ti (Bickley et al., 1973; Frank and Bard, 1977).

Seawater should not be considered a homogeneous reaction medium, especially with respect to organic materials and trace metals. Heterogeneous phases might serve as a site for concentrating reactants that would not otherwise react because of their low concentrations in solution. The low concentrations, however, do not negate the importance of trace metals in inducing reactions in solution, for they act as very efficient catalysts in many systems where they are present at concentrations of less than 0.1 μM. In seawater the sum of the average dissolved concentration for the 17 trace metals as given in Table 8.1 exceeds 0.2 μM. This is, however, no measure of the photochemical importance of these metals in seawater, for each will display different photochemical characteristics. Potentially, the most important photochemical reactions initiated by transition metals in seawater can be grouped into 4 general categories: (1) intramolecular photo-oxidation-reduction (charge transfer), (2) intermolecular photo-oxidation-reduc-

Table 8.1 Potential Photochemically Reactive Transition Metals in Seawater and Their Probable Major Oxidation State

173°


TRANSITION METALS
Zn 2
Cd 2
Hg 2
Cu 2
Ag 1
Ni 2
Co 2
Fe 3
Mn 2
Cr 3,6
Mo 6
W 6
V 5
Ti 4
Zr 4
U 6
Ce 4

tion (charge transfer), (3) photosensitized (energy transfer), and (4) secondary reactions caused by unstable products or metal ions in unstable oxidation states. An individual trace metal can conceivably act through all of these processes; which is more important will be related specifically to the metal ion and organic compound in question, as well as to other properties of the solution. It should be pointed out that other reactions of coordination compounds are possible, such as photosubstitution and photoisomerization, but the net effect of these reactions is usually only reorientation of ligands; therefore these reactions have not been considered here.

Of specific interest in this work are the reactions between trace metals and amino acids, with methionine and glycine being used as representatives of amino acids in seawater. The photoreactivity of methionine induced by transition metals proved to be too low to measure precisely with the analytical methods employed. The sensitive technique of collecting and measuring $^{14}CO_2$ formed from decarboxylation of 1-^{14}C glycine was, therefore, used as the primary means of investigating these reactions.

8.2. Preparation of trace metal solutions

The 18 metal salts shown in Table 8.2 were divided into groups on the basis of compatibility with respect to solubility and redox reactions when combined in solution. Five one liter solutions were then prepared by dissolving the amount of each particular salt in a group (see Table 8.2) in Super Q water. The concentration of individual metals in each

Table 8.2 Composition of TMEM Solutions

[1] At the concentrations used the NO_3^- did not introduce a significant contribution to the observed photoreactivity of seawater solutions. This possibility was examined by replacing the nitrate salts, with the exception of Ag, with other salts (i.e., $FeCl_3 \cdot 6H_2O$ and $(CH_3COO)_2\ UO_2 \cdot 2H_2O$).

[2] Detectable prepcipitates developed in stock solutions 2 and 4 within a week; their shelf-life was considered to be no more than 3 days.

METAL	PROBABLE MAJOR OXIDATION STATE IN SEAWATER	AVERAGE SEAWATER CONCENTRATION $nM\,l^{-1}$	FORMULA	CONCENTRATION IN STOCK SOLUTION, $M\,l^{-1}$	STOCK SOLUTION #
Cr^{6+}	+3, +6	7 (total)	$Cr_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_2O$	3.5×10^{-4}	1
Co	+2	.5	$CoCl_2 \cdot 6H_2O$	5×10^{-5}	1
Cu	+2	15	$CuSO_4 \cdot 5H_2O$	1.5×10^{-3}	1
Mn	+2	5	$MnCl_2 \cdot 4H_2O$	5×10^{-4}	1
Ni	+2	30	$NiSO_4 \cdot 6H_2O$	3×10^{-3}	1
Zn	+2	30	$ZnCl_2$	3×10^{-3}	1
Cr^{3+}	+3, +6	7 (total)	K_2CrO_4	3.5×10^{-4}	2 [2]
Mo	+6	10	$Na_2MoO_4 \cdot 2H_2O$	1×10^{-3}	2
V	+5	50	$NaVO_3$	5×10^{-3}	2
W	+6	.5	$Na_2WO_4 \cdot 2H_2O$	5×10^{-5}	2
Fe	+3	25	$FeNO_3 \cdot 9H_2O$	2.5×10^{-3}	3 [1]
U	+6	15	$UO_2(NO_3)_2 \cdot 6H_2O$	1.5×10^{-3}	3
Ag	+1	3	$AgNO_3$	3×10^{-4}	3
Ce	+4	.1	$(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O$	1×10^{-5}	4 [2]
Ti	+4	20	$K_2TiO(C_2H_4)_2 \cdot 2H_2O$	2×10^{-3}	4
Zr	+4	.3	$ZrOSO_4 \cdot H_2SO_4 \cdot 3H_2O$	3×10^{-5}	4
Cd	+2	1	$Cd(C_2H_4O_2)_2 \cdot 2H_2O$	1×10^{-4}	5
Hg	+2	.2	$HgCl_2$	2×10^{-5}	5

stock solution was such that 10 µl diluted with seawater to one liter would amount to doubling the seawater concentration of those trace metals present in the stock solution. This of course, assumes that the average concentration for each trace metal shown in Table 8.2 is the same as the natural level in the seawater used. Some of the average seawater concentrations used were taken from an early review (Pytkowicz and Kester, 1971) and represent levels which are higher than more recent estimates (Brewer and Spencer, 1975).

Reaction solutions were prepared by adding the required volume of each TMEM (trace metal enrichment media) stock solution to a rapidly stirred seawater solution containing the added amino acid. Sufficient borate buffer solution was added to adjust the pH to 8.1. The total dilution of seawater with reagent and buffer solution amounted to no more than a 0.3% change, even for the highest TMEM enrichments used. Prepared reaction solutions were used immediately after their preparation to reduce possible complications arising from gradual alterations of solution composition through slow kinetic processes.

8.3. Results and discussion

8.3.1. Absorption spectra of trace metal solutions

Many organic compounds which are by themselves photochemically unreactive to near-ultraviolet and visible light can become photochemically active in these regions by formation of chelates with certain transition metals. Absorption bands in the chelate can arise

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from ligand field transitions (localized on the central metal), from internal ligand transition (localized on the organic ligand), or from charge transfer transitions from metal to ligand (MLCT) or from ligand to metal (LMCT). The LMCT transitions are perhaps the most likely to be of importance in leading to photochemical reactions in the natural environment because they are generally or lower energy than those characteristic of other transitions. The very nature of the excited state in LMCT and MLCT transitions (Eqs. 8.1 and 8.2) inclines towards an intramolecular oxidation-reduction

$$[M^{Z+}(L_n)]^{Z+} \xrightarrow[\text{LMCT}]{h\nu} [M^{(Z-1)+}(L_n)^{+}]^{Z+} \qquad (8.1)$$

$$[M^{Z+}(L_n)]^{Z+} \xrightarrow[\text{MLCT}]{h\nu} [M^{(Z+1)+}(L_n)^{-}]^{Z+} \qquad (8.2)$$

process. Whether or not this takes place is dependent on many factors, but the major one is likely to be the stability of the upper and lower oxidation states of the metal and the susceptibility of the ligand to oxidation or reduction. Since most metals in air saturated seawater are already in their highest stable oxidation state, it is unlikely that MLCT transitions will be very important.

Figure 8.1 Electronic Absorption Spectra for Various Transition Metal Seawater Solutions Containing Glycine

Seawater (31‰ salinity) solutions were prepared by first dissolving 1×10^{-2} M l^{-1} of glycine followed by 5×10^{-4} M l^{-1} of the transition metals. The pH of the solutions was adjusted to 8.1 with 0.1N NaOH. After several hours the pH was again checked and readjusted if necessary. The solutions were then filtered directly into a 10 cm pathlength cell and the absorbance spectra was immediately determined against filtered seawater.

A. seawater

B. chromium (III)

C. uranium (VI) as uranyl cation

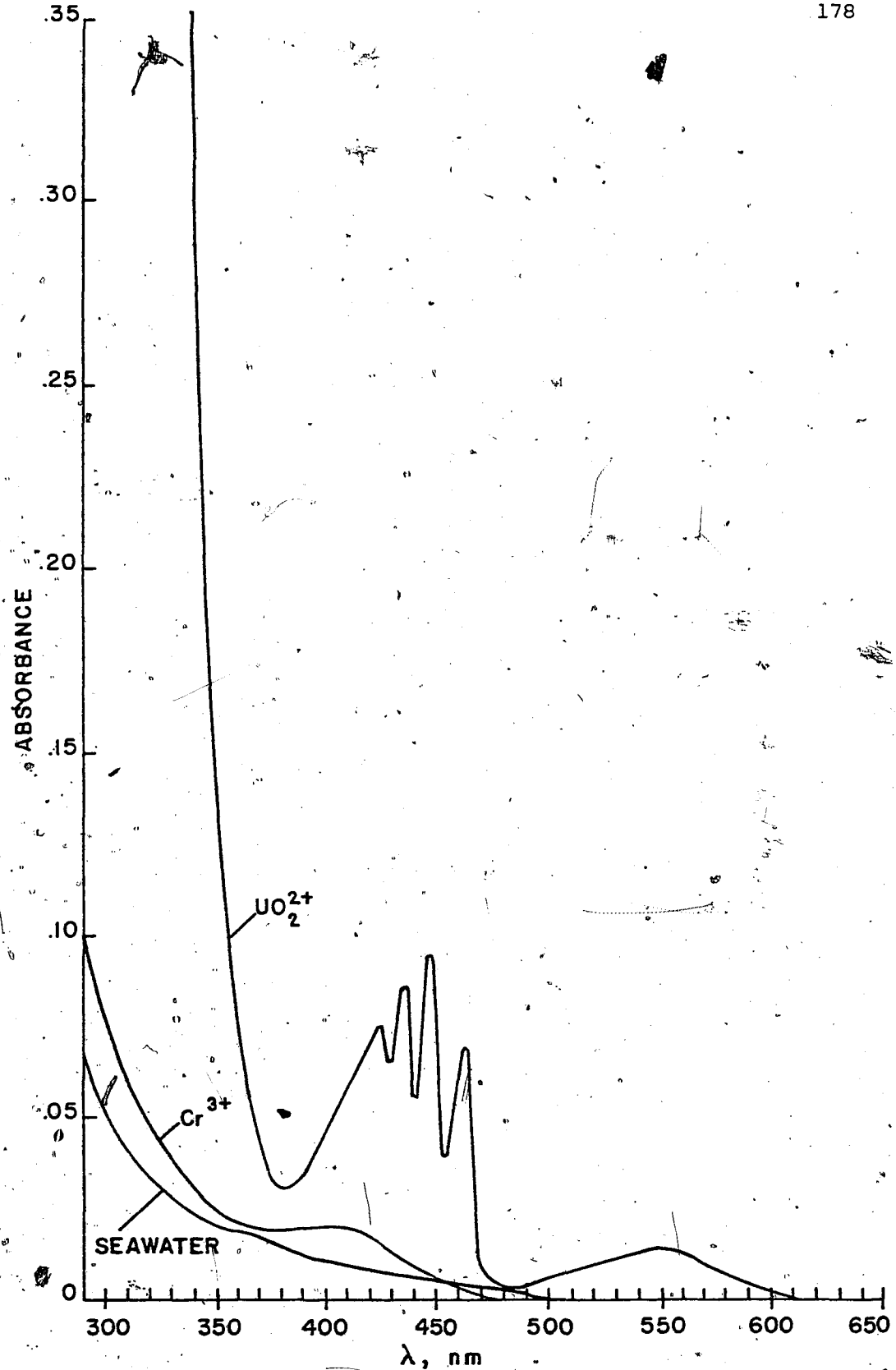

.35
.30
.25
.20
.15
.10
.05
0
ABSORBANCE
UO_2^{2+}
Cr^{3+}
SEAWATER
300
350
400
450
500
550
600
650
λ, nm

Figure 8.1 continued

D. cobalt (II)

E. manganese (II)

F. iron (III)

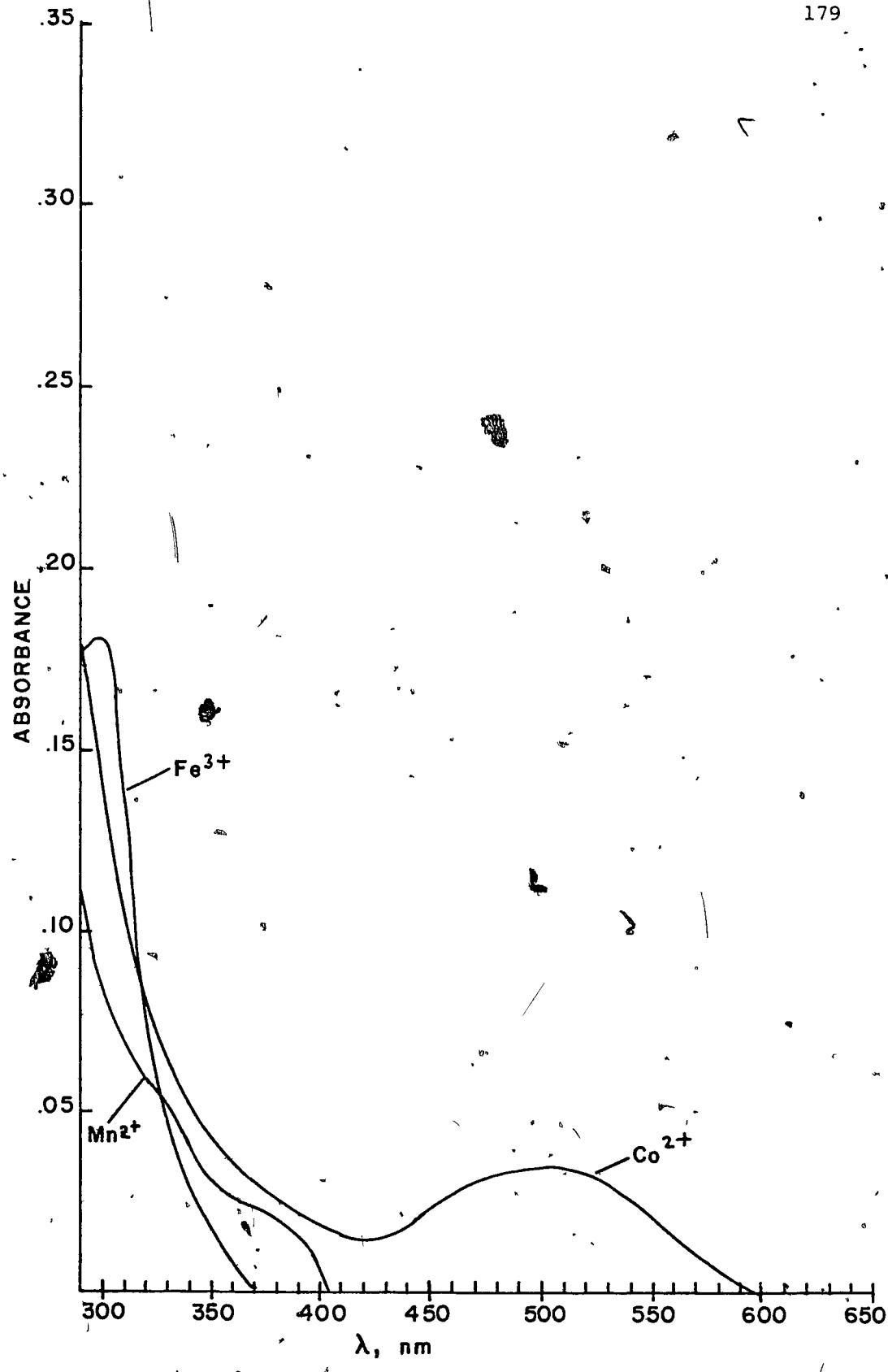

.35
.30
.25
.20
.15
.10
.05
ABSORBANCE
Fe3+
Mn2+
Co2+
300
350
400
450
500
550
600
650
λ, nm

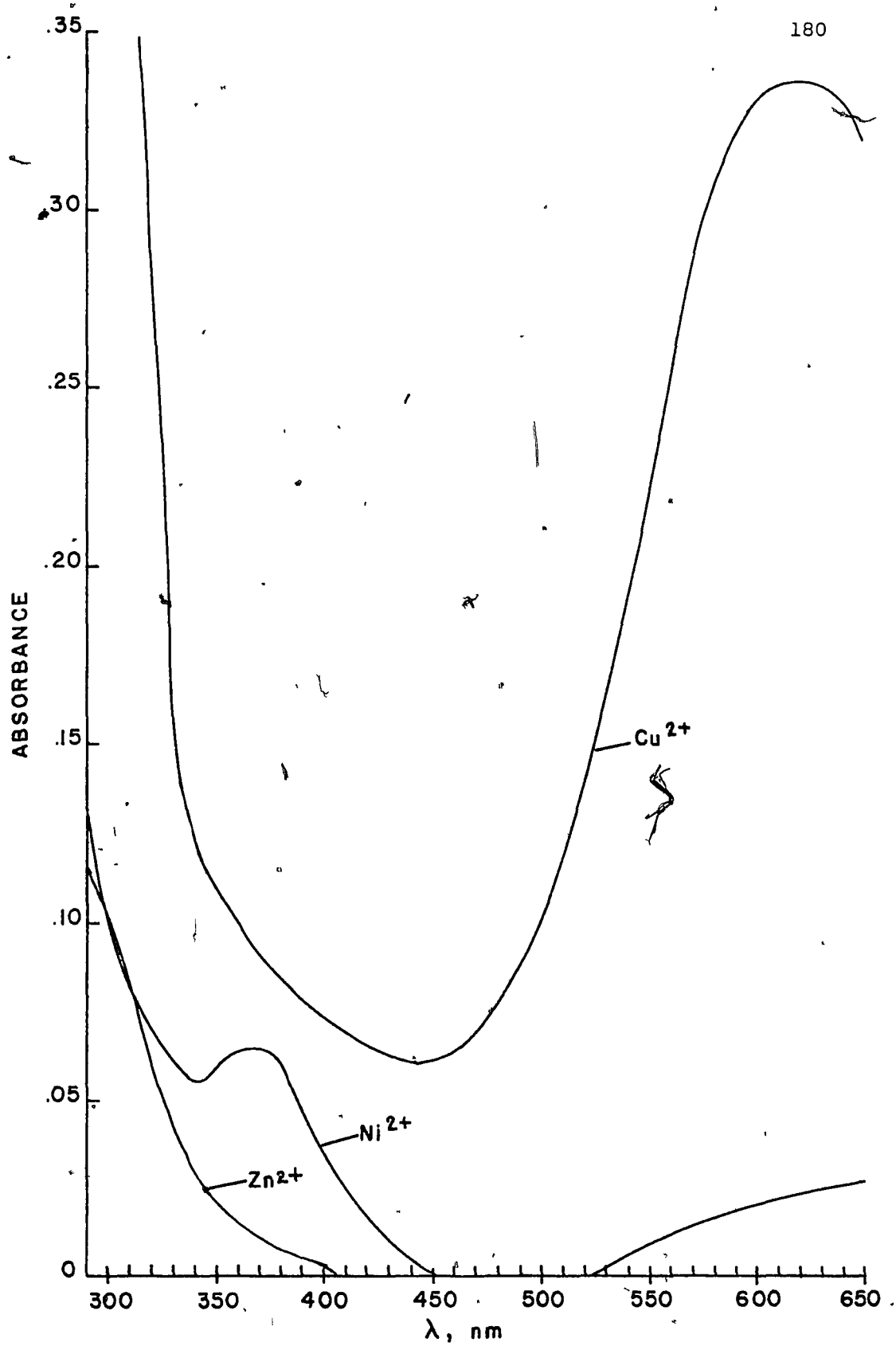
Figure 8.1 continued

G. copper (II)

H. nickel (II)

I. zinc (II)

180


.35
.30
.25
.20
.15
.10
.05
0
ABSORBANCE
Cu 2+
Ni 2+
Zn2+
300
350
400
450
500
550
600
650
λ, nm

Typically, LMCT transitions occur at increasingly longer wavelengths as the metal becomes more oxidizing or the ligand more reducing in character. Complexes having strongly oxidizing central metals or strongly reducing ligands may have charge transfer bands at long wavelengths, indicating that electronic transitions require only a small amount of energy and that the compound might, therefore, be thermally unstable as well. Where a mixed ligand complex exists, as is likely in seawater for many transition metals, the electronic transition will probably still be between the central metal and the most oxidizing or reducing ligand.

The low concentrations of trace metals in seawater make it impossible to determine directly the spectral regions in which their complexes absorb. Absorption spectra for seawater solutions of glycine enriched with individual trace metals (Figure 8.1) give a qualitative idea of the spectral regions in which various trace metals might be photochemically reactive. The high concentration of glycine in these solutions does not insure that the predominant ligand for each metal will by glycine. On the basis of the stability constants of the glycinate complex for most of these metals, this would be the case in distilled water at a lower pH. The absorption bands are not then necessarily the predominant result of glycinate complexes of the metals.

Other electronic absorption transition can result from intermolecular charge transfers between an ion-pair (IPCT, Eq. 8.3) or solvent

(CTTS, Eq. 8.4), and the transition metal complex. Such transitions

$$[ML_n]^{Z+} X^- \xrightarrow[\text{IPCT}]{h\nu} [ML_n]^{(Z-1)+} + X \qquad (8.3)$$

$$[ML_n]^{Z-} + H_2O \xrightarrow[\text{CTTS}]{h\nu} [ML_n]^{(Z-1)-} + e^-_{aq} \qquad (8.4)$$

can generate reactive odd-electron products which might react through secondary reactions with organic substrates or other seawater constituents.

8.3.2. Products of glycine-Cu^{2+} enriched seawater

Photochemical reactions resulting from excitation in charge transfer bands of metal organic-ligand complexes usually result in the formation of free radicals (Endicott, 1970) through homolytic fission of the ligand atom central metal bond. Carbon dioxide, NH_3, aldehydes, and acids are the reported products (Poupko et al., 1973; Khenokh and Bogdanova, 1967a; Neuberg, 1908) of the photolysis of amino acids in the presence of various transition metals. No detailed investigation of the products from such reactions has been made, and it is conceivable that some of the observed products might result from the reactions of free radical intermediates with the amino acid.

Initial investigations on the formation of NH_3 in irradiated seawater solutions of alanine and Cu^{2+} in a 2:1 molar ratio showed that the

percent yield of NH_3 became higher as the concentration of the alanine was decreased from 1×10^{-3} to 1×10^{-5} M. The reason for the increase was not established; it could have been the result of a decreasing solution inner filter effect with decreasing concentration. Another distinct possibility was that the efficiency of the deamination reaction was increasing as the result of some change in the complex or solution characteristics.

The reactions were repeated using 1-^{14}C glycine for concentrations $< 1 \times 10^{-5}$ molar. The reaction products NH_3, formaldehyde, and CO_2 were measured. At concentrations below 1×10^{-5} M the NH_3 continued to show an increase in percent yield with decreasing concentration (Figure 8.2). A similar trend, although far less pronounced, was found for formaldehyde. This might be expected, since formaldehyde is rapidly decomposed in aerobic seawater (Kamata, 1966) and is probably susceptible to photo-oxidation by virtue of its own light absorption in the near ultraviolet. Unlike formaldehyde and NH_3, the percent yield with decreasing concentration of glycine was approximately the same at all concentrations, with perhaps a slight decrease below the micromolar level. Unfortunately, attempts to acquire an accurate product reactant balance failed because of the interference of Cu^{2+} on the Fluram analysis for glycine. Although the results are not quantitatively reliable they do, nevertheless, show an increase in the percent loss of glycine with decreasing concentration.

The analyses for glycine, formaldehyde, and NH_3 lead to the conclusion that the net effect of all reactions in the solution was to

Figure 8.2 Glycine Concentration Dependence

on Product Yields in Copper Photoinduced Reactions in Seawater

The added Cu^{2+} to added glycine ratio in all solutions was 1: 2. Part of the added glycine was present as the 1-^{14}C labeled compound.

All solutions were adjusted to a pH of 8.1 with 0.1N NaOH and then irradiated for 3 hours in the merry-go-round system.

- ● based on Fluram analysis
- ○ based on formaldehyde analysis
- □ based on NH_3 analysis
- ▲ based on $^{14}CO_2$ analysis

FRACTION OF ADDED GLYCINE REACTING
0
.1
.2
.3
.4
.5
.6
10⁻⁷
10⁻⁶
10⁻⁵
10⁻⁴
[GLYCINE], M

184

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increase the efficiency of glycine photolysis. This is contrary to what would be expected if the reactive species in solution was an inner sphere complex containing Cu^{2+} and glycine, since equilibrium models used to compute metal-ligand speciation in natural waters (Morel and Morgan, 1972; Stumm and Morgan 1970; Lerman, 1972) predict that coordination of glycine to Cu^{2+} should decrease as lower concentrations are approached. The behavior of the percent yield of CO_2 is closer to what would be expected, although a more pronounced change with concentration would have been anticipated. The dissimilarity in the percent yield curves for CO_2, formaldehyde, and NH_3 possibly indicate a change in the predominant destructive pathway for glycine, but without further experimental evidence there is no point in discussing this particular possibility further.

It has been assumed that the NH_3 and formaldehyde are arising solely from the destruction of glycine. It is possible, however, that they are products of reactions involving other organic materials in the water. If this is the case, then the preponderance of NH_3 and formaldehyde produced from the other organic materials would increase as the concentration of added glycine in the solutions decreases. This could have been verified by determining the amount of formaldehyde and NH_3 produced in seawater containing only Cu^{2+}, but unfortunately it was not. The measurement of $^{14}CO_2$ avoids such artifacts resulting from organic impurities, but possibly represents only a fraction of total destruction of glycine.

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8.3.3. TMEM experiments

Each transition metal shown in Table 8.1 has the potential to affect the photochemical reactivity of seawater. For any given test used for photoreactivity, a particular transition metal can promote, inhibit, or have no measurable effect. The seawater chemistry of each metal may be the overriding determinant in prescribing the degree to which the metal reacts and the types of reactions in which it will be important. Therefore, metals such as Ti, Zr, W, and Fe, which should exist as highly insoluble oxo and hydroxo compounds, may be photochemically important only in processes involving particulate fractions that contain inorganic species. Their predominant photochemical effect could then involve alterations of surface active materials on the surfaces of particles or the initiation of solution chain reactions through the formation of reactive species, as in the production of hydrated electrons through photoionization. Other metals which are more soluble in seawater (U^{6+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^{1+}, Cd^{2+}, and Hg^{2+}) might react with soluble organic compounds through photoinduced secondary reactions or directly as light sensitive chelates or complexes. The photochemistry of each transition metal ion, therefore, represents a unique case not only in terms of the metal, but also with respect to the organic materials which it will affect.

The main question in the context of this study is to determine what the net combined effect of the 17 trace metals shown in Table 8.1 is upon the photoreactivity of amino acids in natural seawater. This was

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investigated by measuring the amount of $^{14}CO_2$ produced from the decarboxylation of 1-^{14}C glycine in natural seawater, when the transition metal levels were increased in multiples of natural seawater concentration by addition of TMEM. Results indicated that there was a net increase in the decarboxylation of glycine with increasing TMEM concentration (Figure 8.3). The change in going from 1 X, which represents no addition, to 10X is relatively small when compared to the natural (1X) level for the reaction. This implies that the transition metals present at average seawater concentrations can account for only a small fraction of the observed decarboxylation of glycine, and probably other amino acids as well. Whether the added metals are representative of the reactivity of their natural counterparts is open to question. The activities and speciation of the metals could vary considerably between the added and naturally occurring metals. If the primary mechanisms of decarboxylation involve coordination of glycine to metal, than the results probably represent a high estimate of the reaction induced by transition metals.

The effect of specific transition metals on decarboxylation of glycine was also examined. In this case the direction and magnitude of the effect on the photoreactivity of glycine in natural seawater was measured when a 1 x 10^{-6} M addition of an individual metal ion was made to the same seawater (Figure 8.4). The value of one on a relative glycine reactivity scale represents the glycine photoreactivity in seawater without any metal ion addition. Metals showing a significant

Figure 8.3 Effect of TMEM Concentration on Decarboxylation of Glycine in Seawater

Seawater was filtered, enriched with 1×10^{-7} M l^{-1} of d-^{14}C glycine (containing 0.5 μ Ci/50 ml of sample) plus the necessary volumes of TMEM solutions and then buffered to pH 8.1. The solutions were irradiated for 2 hours in the merry-go-round system or in sunlight.

○ merry-go-round system

□ sunlight

188

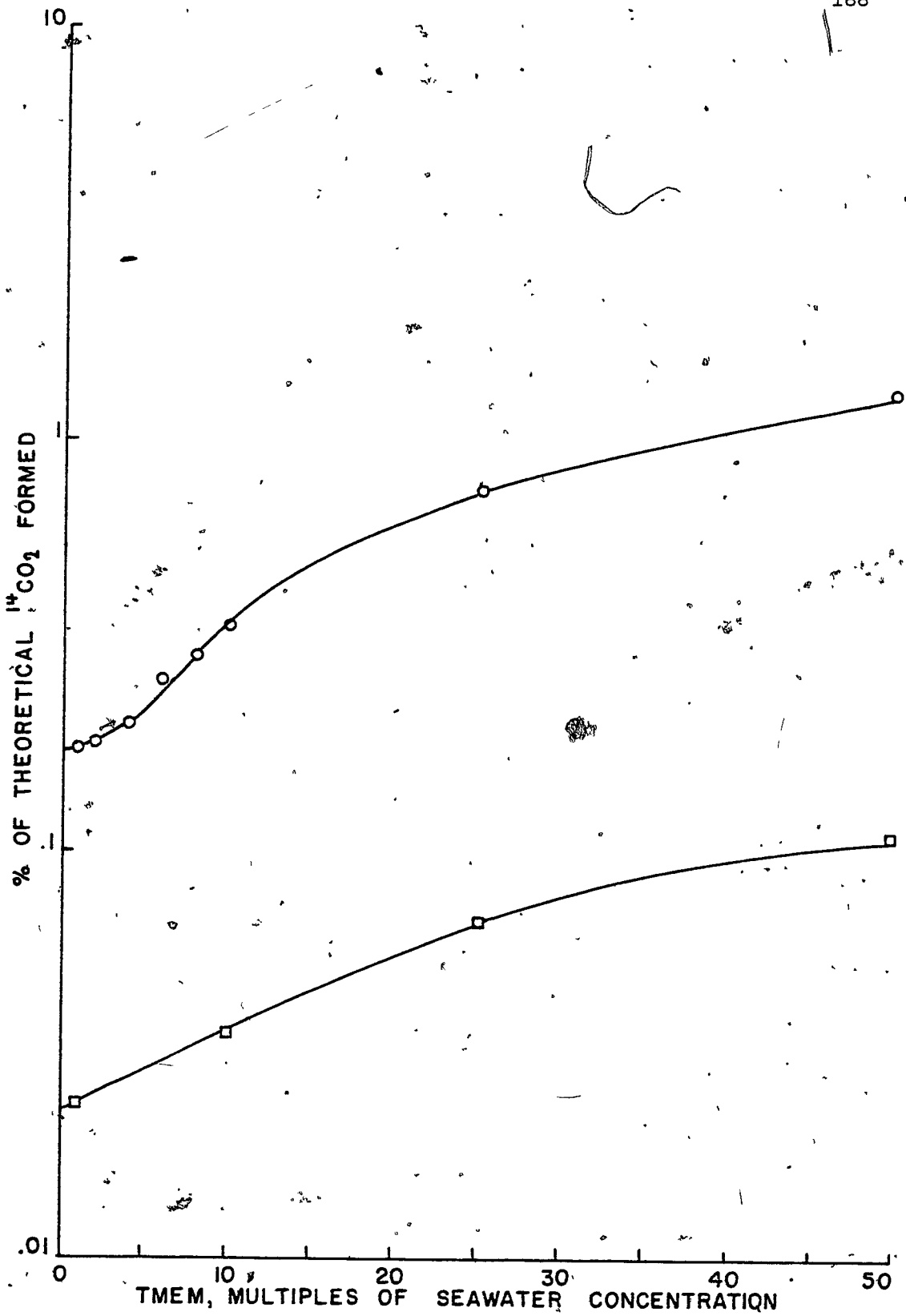
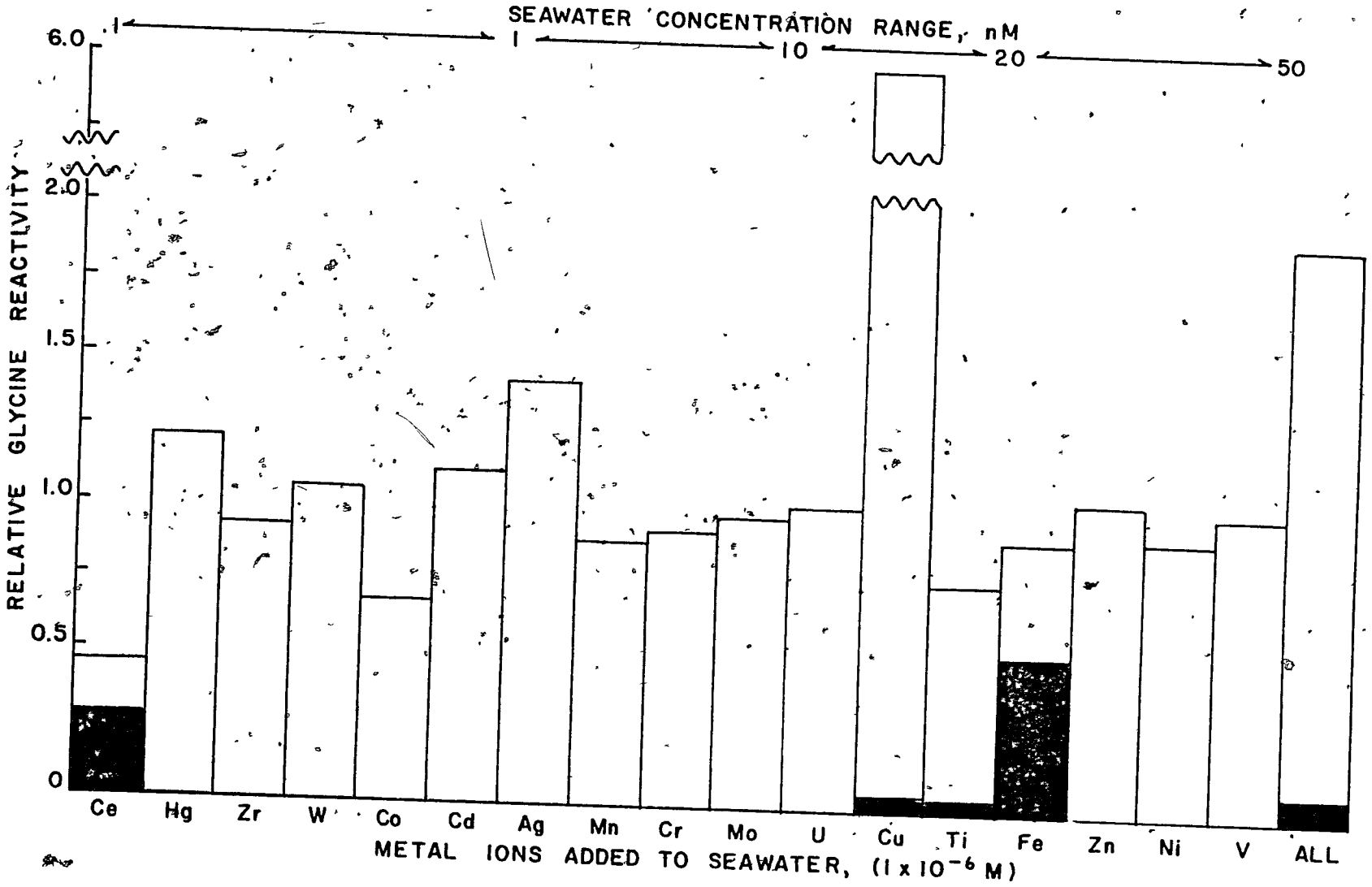

10
1
.1
.01
% OF THEORETICAL $^{14}CO_2$ FORMED
0
10
20
30
40
50
TMEM, MULTIPLES OF SEAWATER CONCENTRATION

Figure 8.4 Effect of Various Transition Metals

on the Photoreactivity of Glycine in Seawater Solutions

Each seawater solution contained 1×10^{-7} M l^{-1} of added 1-^{14}C glycine (containing 0.5 µCi/ 50 ml of sample) and 1×10^{-6} M l^{-1} of the added metal ion(s). The solutions were buffered and then irradiated for 2 hours in the merry-go-round system.

The dark areas represent relative thermal decomposition of glycine.


SEAWATER CONCENTRATION RANGE, nM
1
1
10
20
50
6.0
2.0
1.5
1.0
0.5
0
RELATIVE GLYCINE REACTIVITY
Ce
Hg
Zr
W
Co
Cd
Ag
Mn
Cr
Mo
U
Cu
Ti
Fe
Zn
Ni
V
ALL
METAL IONS ADDED TO SEAWATER, (1 x 10⁻⁶ M)

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reduction in reactivity were Ce^{4+}, Co^{2+}, and Ti^{4+}, while those giving a significant increase were Hg^{2+}, Aq^{1+}, and Cu^{2+}. The importance of direct metal ligand interaction is indicated by the fact that both Hg^{2+} and Cu^{2+} have stability constants with glycine which are much larger than those for the other metal ions. The importance of chelate formation has been observed for Fe^{3+}, Mn^{2+}, and Co^{2+}, which form photosensitive chelates with EDTA in fresh-water up to a pH of at least 8.5. Glycine was not decarboxylated in the presence of these metals, but compared to EDTA it is a weak chelator for them. Caution should be used in generalizing on the photoreactivity of groups of organic compounds, for even though closely related they can exhibit marked differences in photosensitivity which are not reflected by the stability of the chelates that they form with a metal. Aminocarboxylates such as EDTA, glycine, and NTA are all photolabile in the presence of Fe^{3+} (Lockhart, 1976; Carey and Langford, 1973; Natarajan and Endicott, 1973; Trott et al., 1972), but only glycine and NTA react in the presence of Cu^{2+} (Langford et al., 1973) under simulated fresh-water environmental conditions.

Four metal ions (Ce^{4+}, Cu^{2+}, Ti^{4+} and Fe^{3+}) promoted thermal decarboxylation of glycine (Figure 8.4). This was also noted for seawater samples preserved with $HgCl_2$ and stored in the dark at 0-2°C for 24 hours. This suggests that long term storage of seawater samples fixed with $HgCl_2$ should be avoided, since Hg^{2+} is apparently capable of catalyzing the thermal degradation of at least some organic constituents.

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8.3.4. Photoinduced metal oxidation state changes

With the exception of certain confined environments (e.g., particles or the sea surface microlayer), individual transition metals are present in seawater at concentrations in the nanomolar range. In order to be an important agent in the photolysis of organic compounds, they must serve as efficient photocatalysts. Generally, this means involvement in an oxidation-reduction reaction (Eq. 8.5) in which the metal ion is temporarily converted to a higher or lower oxidation state.

$$M^{n+} + L \xrightarrow[k_1]{h\nu} M^{(n-1)+} + L^{1+} \qquad (8.5)$$

$$M^{(n-1)+} + O_2 \xrightarrow[k_2]{h\nu} M^{n+} + O_2^- \qquad (8.6)$$

The rate of return to the photoreactive oxidation state (Eq. 8.6) can be an important consideration in determining its significance as a photocatalyst. Metals for which k_1 is high and $k_1 < k_2$ can function as very efficient catalysts at low concentrations. Such metals can initiate a primary photochemical reaction involving the metal and ligand and a secondary reaction resulting from the rapid re-oxidation of the metal ion. For reactions preceding by way of homolytic fission of metal-ligand bonds and the reduction of O_2 by the reduced metal ion, this amounts to the net production of two free radicals for each primary reaction.

The major effect for metals with $k_2 << k_1$ might be a major shift in the observed equilibrium concentration between different oxidation states of the metal near the ocean surface. Distributions such as this

have been observed for Cr^{3+} and Cr^{6+} (Grimaud and Michard, 1974) and changes in the Fe^{2+} to Fe^{3+} ratio appear to be associated with sunlight exposure in seawater (Lewin and Chen, 1973) and in lake water (McMahon, 1967 and 1969). Lewin and Chen reported finding as much as 25% of the soluble iron in water from Puget Sound present as Fe^{2+}; the re-oxidation of Fe^{2+} had a half-life in excess of 10 hours. In view of the fact that the oxidation of Fe^{2+} in air saturated $NaHCO_3$ solution at pH 8.0 has a half-life of only 2.4 seconds (Singer and Stumm, 1970) the Fe^{2+} form most be stabilized by some component of seawater.

The kinetics of the oxidation of Fe^{2+} in seawater were measured using the Ferrozine method (Section 5.6) to determine whether the rate of oxidation was different than that measured in buffered distilled water by this method. This was indeed the case, for the $t_{1/2}$ for this reaction in air saturated distilled water at 10°C and pH 8.0-8.1 was 10-15 seconds, while in air saturated seawater of 31‰ salinity, at 10°C and pH 8.1 it varied between 2 to 3 minutes for all samples. This is in good agreement with Kester et al. (1975) who found that Sargasso seawater and water from Narragansett Bay had half-lifes at pH 8.0 for this reaction of 3.3 and 5.5 minutes, respectively. The difference in the measured $t_{1/2}$ for the two studies is probably attributable to a marked rate increase with increasing pH in this range.

The rate of oxidation of Fe^{2+} in acid solution is dependent on the anion present and decreases in the following order: OH^{1-}, $P_2O_7^{4-}$, PO_4^{3-}, Cl^{1-}, SO_4^{2-} and ClO_4^{1-} (Stumm and Lee, 1961). The differences in oxidation rates of Fe^{2+} in seawater and distilled water might be

simply related to the predominant anions present in each solution. Therefore, in distilled water, where hydroxo complexes of iron are important, a short half-life would be expected, while in seawater the high concentration of Cl^{1-} and SO_4^{2-} ions intervene by complexing iron and reducing hydrolysis and the oxidation rate of Fe^{2+}. The oxidation rate has also been shown to be very sensitive to low concentrations of organic materials (Theis and Singer, 1973). Different organic compounds studied exhibited a range of effects varying from complete inhibition of the oxidation of Fe^{2+} to an acceleration. Many of the substances which stabilized Fe^{2+} were also capable of reducing added Fe^{2+} even at pH values above 6.

It may be the interaction of Fe^{2+} and Fe^{3+} with the organic fraction in seawater which explain both the slow rate of oxidation of Fe^{2+} observed by Lewin and Chen (1973) and also its mode of formation, which was suggested by them to be the result of photoreduction by organic compounds. To test this possibility, seawater solutions enriched with 10 $\mu m\ l^{-1}$ of Fe^{3+} and 10 $\mu m\ l^{-1}$ of EDTA, NTA, or salicylic acid were irradiated in the merry-go-round system. Upon termination of a 1 hour exposure in the inner ring, Ferrozine reagent was added to the samples and to the dark controls. The differences in color development were then determined spectrophotometrically. The concentrations of Fe^{2+} were not determined, but the relative amounts measured decreased in the order: salicyclic acid (100), EDTA (81), seawater (31), and NTA (0).

9. PHOTOINDUCED REACTIONS OF NO_3^- AND NO_2^-

9.1. Introduction

Most of the inorganic anions of seawater are not subject to photolysis by direct sunlight, because their electronic excitation energies lie well above the maximum photon energy available in sea surface sunlight. This is not true for the oxyanions NO_3^- and NO_2^-, which exhibit weak transitions near 300 and 350 nm, respectively. The resultant primary reactions at these wavelengths could be significant contributors to the pool of secondary reaction products which might react with organic matter in seawater (Zafiriou, 1974).

9.1.1. Photochemistry of NO_3^-

Photolysis studies on aqueous NO_3^- solution led Daniels et al. (1968) to the conclusion that two major primary reactions were occurring (Eqs. 9.1 and 9.2). The formation of oxygen and the pH dependence of

$$NO_3^- \xrightarrow{h\nu} NO_2^- + O \qquad (9.1)$$

$$NO_3^- \xrightarrow{h\nu} NO_2 + O^{\bullet -} \qquad (9.2)$$

the NO_2^- yields were explained in terms of the decomposition of the intermediate peroxynitrate ion (Eq. 9.3), which was formed from the

$$NO_3^- + O \rightleftharpoons [O\text{-}O\text{-}N(O^-)O] \longrightarrow O_2 + NO_2^- \qquad (9.3)$$

reaction of the oxygen atom with NO_3^-. The oxygen atom formed in the

primary process was considered to be in the ground state (^3P), since the excitation energy at 313 nm is only ∿ 91 K cal and is insufficient to form O (^1D). This was also supported by the absence of H_2O_2 in the irradiated solutions, it would probably be the product of $O(^1D)$ with water.

In flash photolysis studies of aerated aqueous solutions of NO_3^- (Barat et al., 1969) a transient absorption was observed at 300 nm. In alkaline solution (pH = 11.3), it decayed over a period of 10 minutes. It was attributed to the peroxynitrite ion ($O-N-O-O^-$). The possibility that its precursor might be $O(^3P)$ or $O^{\bullet -}$ radicals was dismissed, because the addition of $O(^3P)$ scavenger or $OH^\bullet$ scavengers had no affect on the intensity of the transient absorption. They were also unable to find any evidence for the formation of hydrated electrons, which would have been expected if, as other authors had proposed, the NO_3^- absorption was due to a CTTS transition.

The photochemistry of the NO_3^- system would appear to be complex and as yet unsolved. However, the primary reaction leading to the products NO_2 and $OH^\bullet$ (Eq. 9.2) is well documented (Barat et al., 1970; Daniels et al., 1968; Treinin and Hayon, 1970) and would certainly appear to be a potential route for the photochemical interaction of the NO_3^- system with other seawater constituents.

9.1.2. Photochemistry of NO_2^-

Aqueous solutions of NO_2^- undergo no net change when irradiated with ultraviolet light (Holmes, 1926) and as a result the system has received very little attention. Interestingly, a very efficient primary

decomposition (Eq. 9.4) occurs at wavelengths below 300 nm (Treinen and

$$NO_2^- \xrightarrow{h\nu} NO + O^{\bullet -} \qquad (9.4)$$

Hayon, 1970; Treinin, 1970), but the efficiency above 300 nm has not been estimated. Ensuing reactions of the products (Eqs. 9.5-9.9) and the

$$O^{\bullet -} + H_2O \rightleftharpoons OH^{\bullet} + OH^- \qquad (9.5)$$

$$OH^{\bullet} + NO_2^- \longrightarrow NO_2 + OH^- \qquad (9.6)$$

$$O^{\bullet -} + NO_2^- + H_2O \longrightarrow NO_2 + 2OH^- \qquad (9.7)$$

$$NO_2 + NO_2 \rightleftharpoons N_2O_4 \qquad (9.8)$$

$$NO + NO_2 \rightleftharpoons N_2O_3 \qquad (9.9)$$

eventual hydrolysis of the resulting nitrogen oxides, NO_2, N_2O_3, and N_2O_4, lead to a complete re-generation of the NO_2^-. In seawater the formation of these nitrogen oxides is unlikely, for the concentration of NO_2^- is on the order of .1 to .5 μ molar and any $O^{\bullet -}$ ions formed will be removed rapidly through reactions with other seawater constituents. Decomposition pathways of NO and regeneration of NO_2^- are therefore likely to proceed by far different routes in seawater (Eq. 9.10). Nitric oxide

$$2NO + \tfrac{1}{2}O_2 + H_2O \rightleftharpoons 2NO_2^- + 2H^+ \qquad (9.10)$$

might also function as a reactant with other seawater constituents, since it is a free radical and is fairly reactive with some substrates in oxidation and reduction processes. If such reactions do occur a reduc-

tion in NO_2^- concentration might be expected during irradiation.

9.1.3. Chemistry of NO_3^- and NO_2^- photolysis products

The identified products resulting from the primary photochemical reactions of NO_2^- and NO_3^- are the free radicals NO, NO_2, and $O^{\bullet -}$. With a rate constant of 9.6×10^7 sec^{-1} for reaction 9.5, $O^{\bullet -}$ is rapidly protonated in aqueous solutions at a pH near that of seawater. The ensuing reactions of NO and NO_2 are still in question as is their reactivity with organic compounds. However, the chemistry of the $OH^\bullet$ has been extensively studied in flash photolysis and radiolysis experiments, and a large number of rate constants for its reaction with both inorganic and organic compounds are available (Anbar and Neta, 1967; Farhataziz and Ross, 1977). Based on such rate constants, Zafiriou (1974) calculated pseudo-first order rate constants and half-lifes for reactions of various seawater constituents with the $OH^\bullet$. He found that the amount of the $OH^\bullet$ reacting with these constituents decreased in the order, $Br^- >> CO_3^{2-} > HCO_3^- >$ DOM $> NO_3^- > Cl^- > SO_4^{2-}$. What must be considered optimal conditions were used for the DOM fraction, with a concentration of 20 µM and average bimolecular rate constant of 10^9 1 M^{-1} sec^{-1}, yet the $OH^\bullet$ should still react almost exclusively with the bromide ion (Eq. 9.11). Bromine atoms formed in this process

$$OH^\bullet + Br^- \longrightarrow Br^\bullet + OH^- \qquad (9.11)$$

rapidly react with other halide ions to form dihalogen radical anions (Eq. 9.12) (Langmuir and Hayon, 1967; Malone and Endicott, 1972; Zehavi

$$X^{\bullet} + Y^{-} \longrightarrow XY^{\bullet -} \qquad (9.12)$$

and Rabani, 1972; Grossweiner and Matheson, 1957). The stability and rate of formation of XY^{-} will be dependent largely on the oxidizing strength of $X^{\bullet}$ and $Y^{\bullet}$ (Malone and Endicott, 1972). If only the more abundant seawater halide anions (Br^{-} and Cl^{-}) are considered, the $Br^{\bullet}$ should react with Br^{-}, but not Cl^{-}. Also, any $BrCl^{\bullet -}$ that was formed would be expected to decay rapidly (Eq. 9.13), again leading to the formation of $Br_2^{\bullet -}$.

$$BrCl^{\bullet -} + Br^{-} \longrightarrow Br_2^{\bullet -} + Cl^{-} \qquad (9.13)$$

The nature of the transients formed in seawater has been examined through flash photolysis studies (Rao, 1973; Zafiriou, 1974). Although similar excitation conditions were used in each study, the reported identity of the predominant transient was different. Rao reported that $Cl_2^{\bullet -}$ was formed and then disappeared with a second order decay rate ($k_{Cl_2^{\bullet -} + Cl_2^{\bullet -}}$), while Zafiriou identified the transient as a halide anion radical ($BrX^{\bullet -}$) formed through the association of the Br-atom with halide ions in seawater. The possible reasons for this discrepancy have been discussed in detail, with the conclusion that Rao's interpretation is in error (Zafiriou and True, 1977).

Unlike Rao, Zafiriou observed that the transient did not follow second order decay kinetics and was therefore probably reacting with other seawater constituents. The pH dependence of the decay, the appearance of a new weak transient absorption at 600 nm, and a reduction in the decay kinetics of the transient when CO_2 was removed from solu-

tion, strongly implicate the involvement of HCO_3^- in the decay scheme.

In these flash photolysis studies the primary mechanism for halogen atom formation occurs through direct excitation of the halide ion (Eq. 9.14). The hydrated electron formed from this reaction can

$$X^- \xrightarrow[H_2O]{h\nu} X\cdot + e_{aq}^- \qquad (9.14)$$

be manipulated to form OH• by replacing the dissolved air with N_2O, which reacts rapidly with e_{aq}^- to give the precusor to OH• (Eq. 9.15). In

$$e_{aq}^- + N_2O \longrightarrow N_2 + O\cdot^- \qquad (9.15)$$

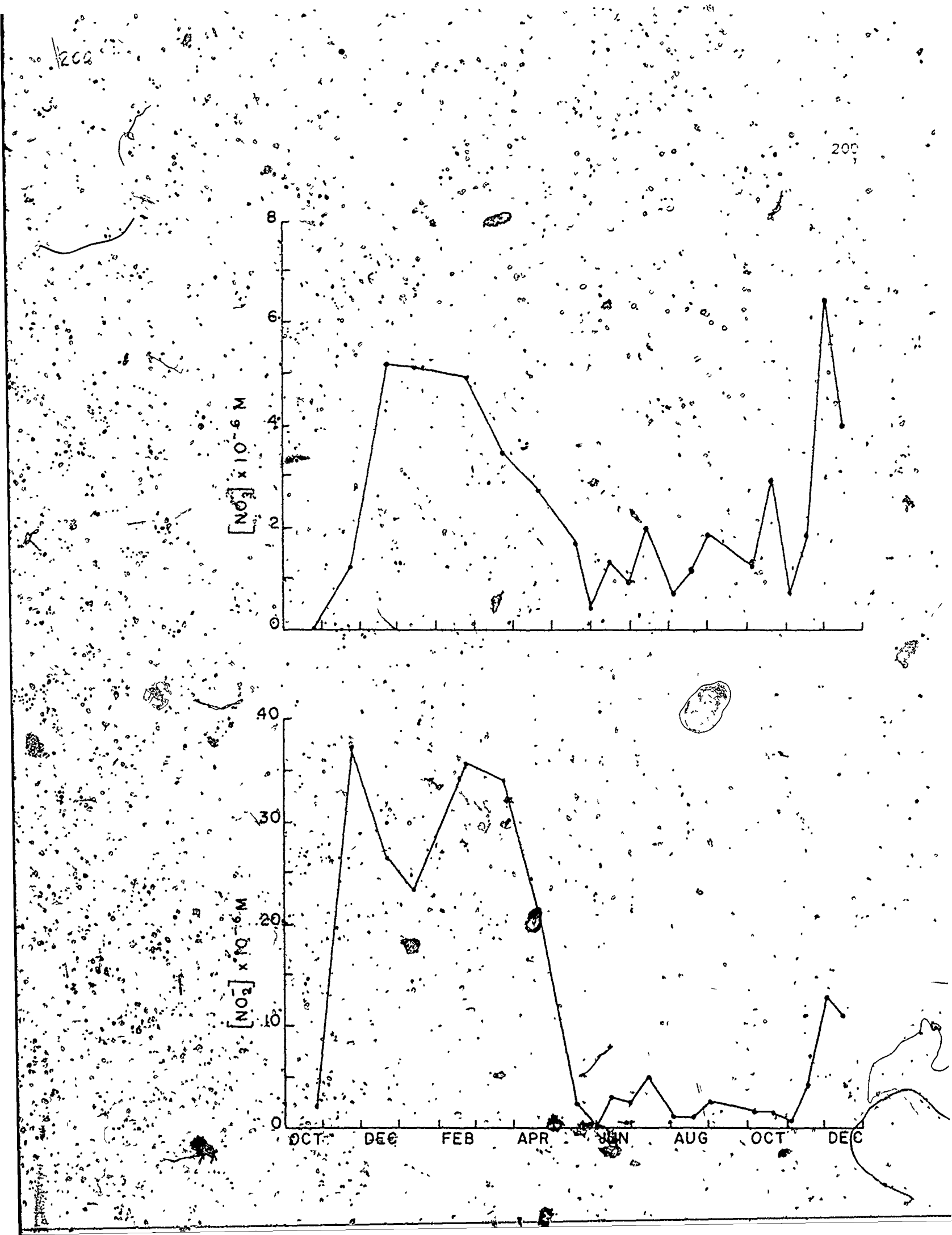
this way the OH•, which is a photolysis product of NO_2^- and NO_3^-, can be studied in terms of its subsequent reactions with seawater constituents. This was applied to a test of the validity of the prediction that the fate of the OH• in seawater was to react with Br^- (Zafiriou, 1974). The prediction was confirmed, for in N_2O saturated seawater a doubling of the transient absorption, ascribed to $BrX\cdot^-$, was observed over that in air saturated seawater.

9.2. Results and discussion

The concentrations of NO_3^- (0-500 $\mu M\ l^{-1}$) and NO_2^- (0-50 $\mu M\ l^{-1}$) have wide ranges in seawater. Their concentrations near the surface tend to be lower and to display high seasonal variability. This variability is expressed in the NO_2^- and NO_3^- data collected for surface water samples from the North West Arm in Halifax (Figure 9.1) and in data from St. Margaret's Bay (Table 11.2).

Figure 9.1: Variation of NO_3^- and NO_2^- Concentration in North West Arm Surface Water[1]

[1] Values are for 1974 and 1975.


200
8
6
4
2
0
$[NO_2^-] \times 10^{-6}$ M
40
30
20
10
0
$[NO_2^-] \times 10^{-6}$ M
OCT
DEC
FEB
APR
JUN
AUG
OCT
DEC

9.2.1. Concentration dependence of NO_2^- and NO_3^- on photoreactivity

At relatively high concentrations, NO_2^- and NO_3^- have been shown to induce photodegradation of methionine and glycine (Section 6.6.3.). These values, however, greatly exceed those measured for North West Arm water or St. Margaret's Bay water. The question remains as to the significance of the photolysis contribution made by these anions at concentrations more typical of normal oceanic and coastal environments. This question was examined by observing the effect on the photoreactivity of methionine by enriching Sargasso seawater (low in NO_2^- and NO_3^-) with increasing increments of either NO_2^- or NO_3^-. The effect for the normal concentration range of these anions in this seawater was found to be small and could make only a minor contribution to the total amount of methionine reacting (Figure 9.2). The same conclusion appears to hold true for the photoinduced decarboxylation of glycine in NO_2^- enriched seawater (Figure 9.3).

The photoinduced degradation of methionine or glycine by NO_2^- must, nevertheless, be an efficient process, for the amount of light absorbed by this anion at these concentrations is small. This apparent high efficiency does not appear to be primarily the result of the near coincidence of the mercury lamp's intense 365 nm line with the near ultraviolet transition of NO_2^-, for midday winter sunlight irradiation of NO_2^- enriched seawater still gave 1/3 of the rate observed in the mercury lamp irradiations.

Figure 9.2 Effect of NO_3^- and NO_2^- Concentration on Methionine Photoreactivity in Seawater

The concentration of added methionine in all solutions was 5×10^{-6} M l^{-1}. The seawater solutions were buffered at pH 8.1. Irradiation was conducted in merry-go-round system for 2 hours at a temperature of 20°C.

▲ NO_3^-

● NO_2^-

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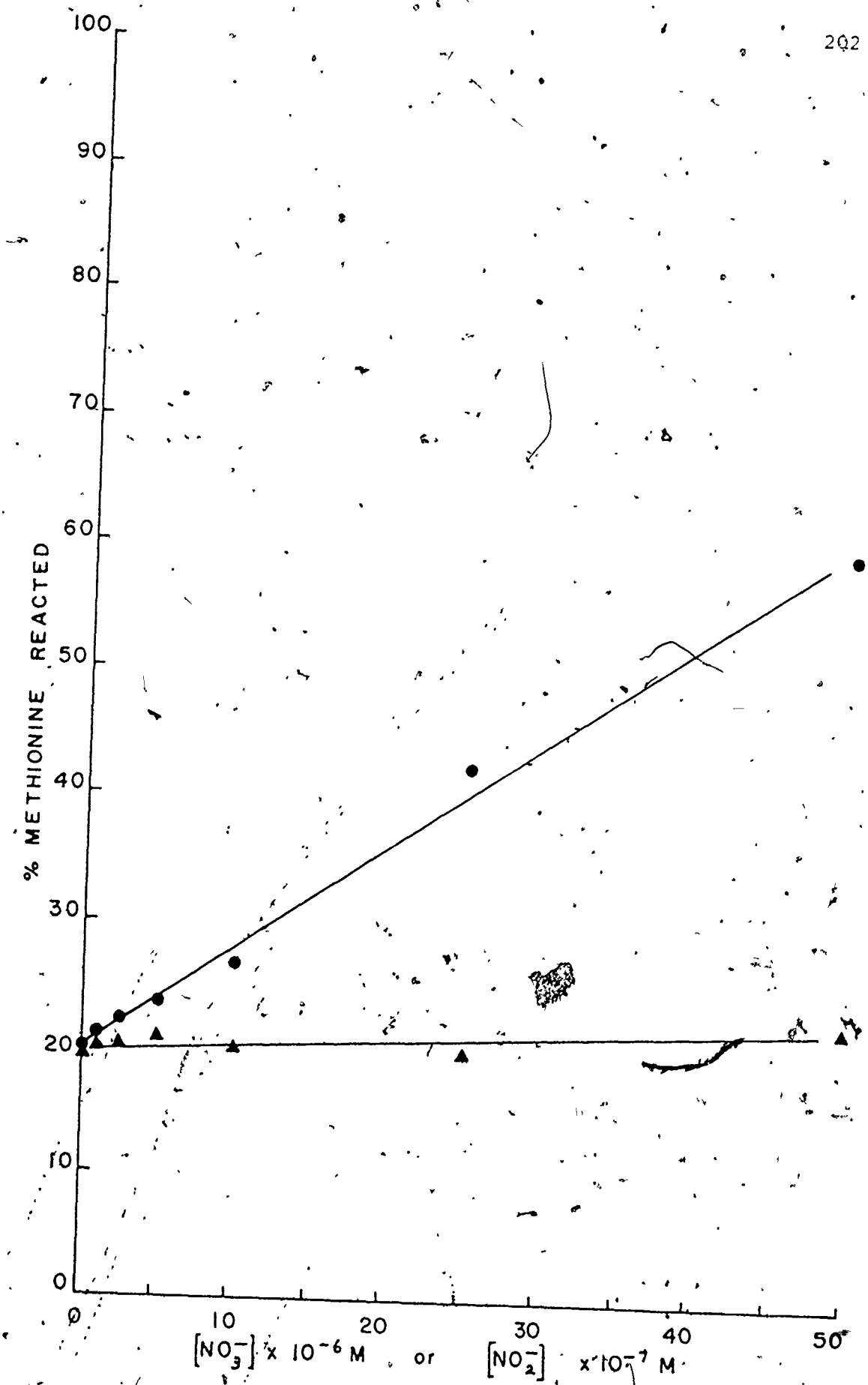
100
90
80
70
60
50
40
30
20
10
0
% METHIONINE REACTED
0
10
20
30
40
50
$[NO_3^-]$ x 10^{-6} M or $[NO_2^-]$ x 10^{-7} M

Figure 9.3 Effect of NO_2^- Concentration on the Photodecarboxylation of Glycine

Seawater was enriched with 1×10^{-7} M l^{-1} of 1-^{14}C glycine (containing .5 μCi/50 ml of sample), NO_2^-, and buffered to pH 8.1. The solutions were irradiated for 2 hours in the inner ring of the merry-go-round system.

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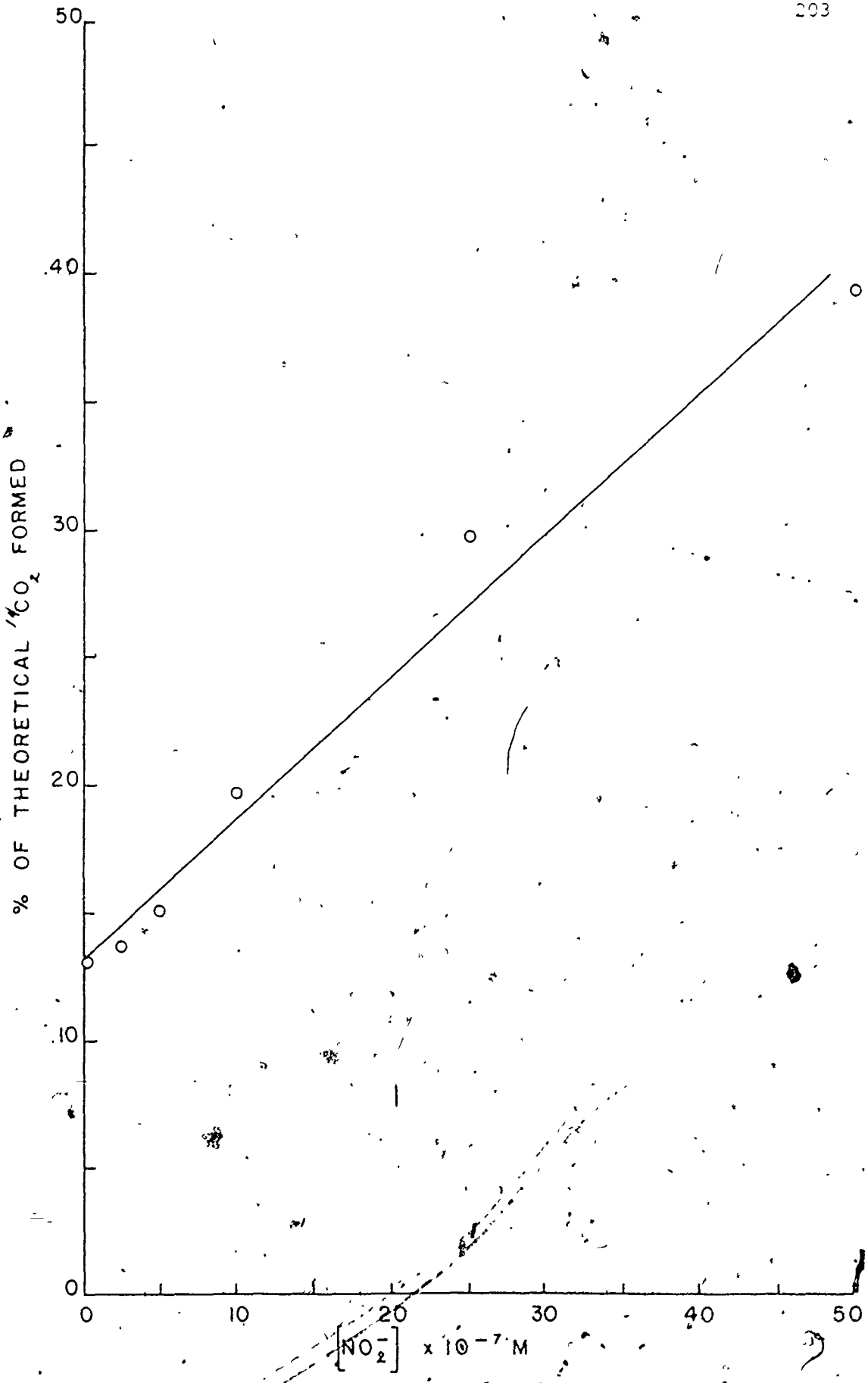

50
40
30
20
10
0
% OF THEORETICAL $^{14}CO_2$ FORMED
0
10
20
30
40
50
$[NO_2^-] \times 10^{-7}$ M

9.2.2. Reactive species in NO_2^- induced photolysis

While a large number of literature values exist for reaction rates of $OH^\cdot$ and e_{aq}^- with organic compounds, little or no information is available on the reactivity of less energetic organic and inorganic free radicals. Nitrite induced photolysis of methionine and glycine might proceed through secondary reactions of such photochemically generated free radicals with these amino acids. It is entirely possible, however, that the predominant free radical formed through NO_2^- photolysis might be unreactive towards amino acids. This possibility was tested by determining the relative rate constants for the disappearance of methionine in NO_2^- solutions of various composition (Table 9.1). The photolysis of NO_2^- in these solutions was used as a source of the $OH^\cdot$ (Eq. 9.4), which should have through secondary reactions with the major reactive anions given exclusively the $OH^\cdot$ in deionized water, $Cl_2^{\cdot -}$ in NaCl solution, $Br_2^{\cdot -}$ in NaBr solution, and $HCO_3^\cdot$ and $CO_3^{\cdot -}$ in $NaHCO_3$ solution. Methionine was found to be relatively reactive in all of these solutions (Table 9.1), with the rate of the reaction decreasing in the order $Br^- > Cl^-$ > artificial seawater > deionized water > HCO_3^-. Based on the oxidizing strength of $Br_2^{\cdot -}$, $Cl_2^{\cdot -}$ and $OH^\cdot$, this order would not have been expected. In deionized water the competing disproportionation reaction of $OH^\cdot$ ($k_{(OH^\cdot + OH^\cdot)} = 5.5 \times 10^9\ M^{-1}sec^{-1}$ at pH 7.0) might reduce the observed rate; however, the concentration of methionine was sufficiently high and its rate with $OH^\cdot$ ($k_{(met + OH^\cdot)} = 5.1 \times 10^9\ M^{-1}sec^{-1}$ at pH 7.0) large enough to rule out this possibility. The competing reaction with NO_2^- ($k_{(NO_2^- + OH^\cdot)} = 7.4 \times 10^9\ M^{-1}sec^{-1}$ at

Table 9.1 Relative Rate Constants for NO_2^- Induced Photolysis of Methionine

Redistilled Super Q water (Appendix 1) was used for all prepared solutions. The pH of the solutions was adjusted with 0.1N NaOH. In the case of the $NaHCO_3$ solution no adjustment was made. The initial concentration of methionine in each solution was 5×10^{-6} M and the relative pseudo first order rate constants were calculated on the basis of concentration determination at $\frac{1}{2}$, 1, $1\frac{1}{2}$, and 2 hours into the reaction. All samples were irradiated simultaneously in the inner ring of the merry-go-round system at constant intensity and constant temperature (20°C).

SOLUTION DESCRIPTION	NO_2^- CONCENTRATION M l^{-1}	RELATIVE PSEUDO FIRST ORDER RATE CONSTANT, sec^{-1}
Deionized Water	5×10^{-6}	$6.2 \pm .4 \times 10^{-5}$
Artificial Seawater (35‰)	5×10^{-6}	$9.1 \pm .5 \times 10^{-5}$
.684 M NaCl	5×10^{-6}	$9.1 \pm .1 \times 10^{-5}$
.684 M NaBr	5×10^{-6}	$1.1 \pm .1 \times 10^{-4}$
.684 M $NaHCO_3$	5×10^{-6}	$3.5 \pm .5 \times 10^{-5}$
Seawater (31‰)	$< 1 \times 10^{-7}$	$2.4 \pm .1 \times 10^{-5}$

pH 7.0) is more likely to be significant, since both NO_2^- and methionine are present initially at the same concentration. This same process is probably reducing the rate in the Cl^- solution, where the rate constant for the reaction between $OH\cdot$ and Cl^- ($k_{(Cl^- + OH\cdot)} = < 10^{-5}\ M^{-1}sec^{-1}$ at pH 7) is sufficiently low for $OH\cdot$ scavenging by NO_2^- to be important.

The similarity of the relative rate constants for the reaction of methionine in artificial seawater, NaCl, and NaBr solutions indicates that the reactive species in seawater may be a dihalogen anion radical. Without further evidence, however, the possibility that the reactant species is something other than $X_2\cdot^-$ cannot be dismissed. For it is possible that disproportionation products of $X_2\cdot^-$ (Eqs. 9.16 and 9.17)

$$X_2\cdot^- + X_2\cdot^- \longrightarrow X_3\cdot^- + X^- \qquad (9.16)$$

$$X_2\cdot^- + X_2\cdot^- \longrightarrow X_2 + 2X^- \qquad (9.17)$$

might have rate constants which are similar to those measured. However, it is unlikely that these products would be formed in significant concentrations at natural levels of NO_2^-.

Although there is no precedent, the possibility also exists that the photolysis of methionine occurs by photosensitization by NO_2^- or through reactions with NO. These possibilities seem unlikely, since large differences in rates were observed for solutions differing only in what would be expected to be ineffectual anionic composition for such reactions. Different quenching rates for the excited state of NO_2^- in these solutions might be postulated, but evidence does not support this,

for Br^- is typically a better quencher of excited states than Cl^- or HCO_3^-.

10. PHOTOINDUCED REACTIONS BY ORGANIC SUBSTANCES

10.1. Introduction

The measured photoreactivity of amino acids in natural seawater is too high to be explained by reactions induced by the combined effect of NO_2^-, NO_3^-, and TMEM alone. Other constituents must, therefore, be contributing to the observed reactivity. Since the organic fraction is responsible for nearly all of the light absorption above 300 nm, it could be a major contributor to the photodegradation of amino acids. The capacity of some specific natural organic constituents to photoinduce the degradation of amino acids in seawater was demonstrated earlier (Section 6.6.3.), where relatively high concentrations of the photosensitzer were used. The determination of the significant concentration levels for photoinduced reactions by these or other organic constituents is not as simple as it was for NO_2^-, NO_3^-, or for transition metals. Most of the organic fraction has not yet been characterized, and for most of these components which have been, little or no information is available on their concentration range and spatial or seasonal variability.

The photochemistry of the organic components of seawater is probably complex. They might behave mechanistically in a number of different ways that depend on their own concentration, the concentration of O_2, and the concentration and characteristics of other constituents of the organic fraction. Photoinduced reactions can generally be categorized into those which are initiated by reactive products of direct

photolysis and those which result from direct or indirect interaction with photosensitizers.

10.1.1. Reactive products from direct photolysis

The most important reactions in this category will probably result from compounds which decompose to form free radical fragments. These can result from homolytic bond fission (Eq. 10.1) or from photoionization (Eq. 10.2). Since the energy of sea surface sunlight has an upper

$$AB \xrightarrow{h\nu} AB^* \longrightarrow A\cdot + B\cdot \qquad (10.1)$$

$$AB \xrightarrow{h\nu} AB^* \longrightarrow AB\cdot^+ + e^- \qquad (10.2)$$

limit of 98 Kcal, only those compounds containing low energy bonds (e.g., -O-O- and -S-S-) should be an important source of radicals resulting from bond fission. Photocleavage in some aromatic compounds, such as bromophenols and phenoxyphenols (Joschek and Miller, 1966), could be important sources of free radicals; both of these types of phenols have been identified in various marine algae (Craigie and Gruening, 1967; Ragan, 1976a, b; Ragan and Craigie, 1976).

For many aromatic compounds, photoionization is a more important process than photocleavage (Grossweiner and Joschek, 1965). The nature of the substituents is a key factor in determining the reaction pathway. Those substituents with negative Hammett σ_p constants (i.e., OH, OR, NH_2, SH, SR, and COO^-) promote the generation of e_{aq}^-, and those with positive σ_p constants (i.e., NR_3^+, NO_2, CN, $COCH_3$, halogens, and CH_2OH) inhibit e^-_{aq} generation (Joschek and Grossweiner, 1966). Some aromatic

heterocyclic compounds also photoionize when irradiated; in fact, indole and some of its derivatives, such as tryptophan, are some of the most efficient at producing hydrated electrons by this process.

The ubiquitous nature of aromatic compounds in the marine environment, especially as porphyrins and polyphenolic materials (Figure 7.1), gives credence to their importance in marine photochemistry. Swallow (1969) calculated a maximum production rate of $\sim 3 \times 10^{15}$ e_{aq}^- $l^{-1} sec^{-1}$ from photoionization of organic compounds at the sea surface at zero solar zenith angle. The calculations were based on the absorption of all incident light up to 325 nm by organic chromophores, which produced e_{aq}^- with a quantum yield of one. The high reactivity of the e_{aq}^- with CO_2 (Eq. 10.3) and O_2 (Eq. 10.4) will mean that it is consumed in these

$$e_{aq}^- + CO_2 \longrightarrow CO_2 \cdot^- \qquad k = 7.7 \times 10^9 \ M^{-1} sec^{-1} \text{ at pH } 7.0 \qquad (10.3)$$

$$e_{aq}^- + O_2 \longrightarrow O_2 \cdot^- \qquad k = 1.88 \times 10^{10} \ M^{-1} sec^{-1} \text{ at pH } 7.0 \qquad (10.4)$$

two reactions. The low concentration of CO_2 at pH values above 8.0 will give a near quantitative conversion of e_{aq}^- to O_2^-. Barring other reactions of O_2^- it should disproportionate to give H_2O_2 (Eq. 10.5).

$$2O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2 \qquad k < 100 \ M^{-1} sec^{-1} \qquad (10.5)$$

Based on Swallow's e_{aq}^- production rate, and assuming their quantitative conversion to H_2O_2 via O_2 reduction, the rate of H_2O_2 formation would be 9×10^{-6} M $l^{-1} hr^{-1}$. This value is unrealistic in view of the high quantum yield for e_{aq}^- generation which was used, and also in terms of the rate of oxidation of organic molecules, since this would require a

net two electron loss per hour from every organic molcule, if the average molar concentration for all organic materials was $\sim 10^{-5}$. However, even if the estimated H_2O_2 rate of production was 10,000 times too high, it could still constitute a significant alteration of the proposed poising of the seawater redox level by the O_2/H_2O_2 couple (Breck, 1974).

10.1.2. Reactions of photosensitizers

Many potential photosensitizing compounds exist in the marine environment. These include phenols, polynuclear aromatic hydrocarbons, quinones, and photosynthetic pigments. Knowledge of their importance in environmental photochemistry is rudimentary, and few investigations have been made in this area. An understanding of the potential role of such compounds in seawater can be derived by applying the concepts of dye photosensitization which have been advanced to explain these processes in biological systems (Spikes and MacKnight, 1970; Spikes and Rizzuto, 1974; Koizumi and Usui, 1974; Foote, 1976). These processes fall into two general categories: Type I, which include H or electron transfer, or Type II, which include all processes where energy transfer is involved. More specifically, these processes involve the interaction of the excited triplet sensitizer molecule with O_2, substrate, or other sensitizer molecules (Figure 10.1). The singlet excited state of the sensitizer is excluded from consideration, because its lifetime is too short to make it a significant reactant in dilute solutions (Eq. 10.6).

$$^1\text{sens} \xrightarrow{Q} \text{sens} \qquad k = \sim 10^8\ M^{-1}\text{sec}^{-1} \tag{10.6}$$

On the other hand, many photosensitizers readily undergo intersystem

Figure 10.1 Primary Reactions of the Triplet

Excited State of Sensitizers and Subsequent Reactions of Products

Definitions of Abbreviations:

sens	sensitizer ground state
^{3}sens	excited triplet state
A	reactive substrate
A*	excited state of substrate
subscript, ox	oxidized form
subscript, red	reduced form
subscript, f-red	fully reduced form

REACTIONS OF SENSITIZERS

Primary Reactions

		Mechanism
(a)	$^3\text{sens} + A \longrightarrow \text{sens} + A^*$	D-Q
(b)	$^3\text{sens} + \text{sens} \longrightarrow \text{sens}_{red} + \text{sens}_{ox}$	D-D
(c)	$^3\text{sens} + A \longrightarrow \text{sens}_{red} + A_{ox}$	D-R
(d)	$^3\text{sens} + A \longrightarrow \text{sens}_{ox} + A_{red}$	D-R
(e)	$^3\text{sens} + O_2 \longrightarrow \text{sens} + {}^1O_2$	D-Q
(f)	$^3\text{sens} + O_2 \longrightarrow \text{sens}_{ox} + O_2^-$	D-O

Subsequent Reactions

(g) $A^* \longrightarrow$ Products

(h) $A^* \longrightarrow A$ + Excitation Energy

(i) $\text{sens}_{ox} + A \longrightarrow \text{sens} + A_{ox}$

(j) $\text{sens}_{red} + A_{ox} \longrightarrow \text{sens} + A$

(k) $\text{sens}_{red} + A \longrightarrow \text{sens}_{f\text{-}red} + A_{ox}$

(l) $\text{sens}_{red} + \text{sens}_{red} \longrightarrow \text{sens}_{f\text{-}red} + \text{sens}$

(m) $\text{sens}_{f\text{-}red} + 2O_2 \longrightarrow \text{sens} + 2O_2^-$

(n) $\text{sens}_{red} + O_2 \longrightarrow \text{sens} + O_2^-$

(o) $A_{red} + O_2 \longrightarrow A + O_2^-$

(p) $O_2^- + AH \longrightarrow HO_2^- + A\cdot$

(q) $O_2^- + A \longrightarrow O_2 + A_{red}$

(r) $^1O_2 + A \longrightarrow$ Products

crossing ($\phi_{ISC} > 0.5$) to the triplet state which has an appreciably longer lifetime than the singlet state (Eq. 10.7).

$$^3\text{sens} \xrightarrow{Q} \text{sens} \qquad k = 10^3 \text{ to } 10^4\ M^{-1}sec^{-1} \qquad (10.7)$$

Whether Type I or Type II mechanisms previal for a specific sensitizer will depend on the properties and concentration of the sensitizer and substrate, and on their rates in the various processes involved (Figure 10.1). In solutions which contain O_2 the Type II D-O mechanisms become very important, for the typical rate constants for the reaction of O_2 with ^{3}sens are in the range of 1 to 3 x 10^9 $M^{-1}sec^{-1}$ (Foote, 1976). For other intermolecular mechanisms to compete successfully for the ^{3}sens in O_2 or air saturated solutions requires that a high substrate or sensitizer concentration be present. If methylene blue is considered representative of a seawater photosensitizer under the conditions given for Figure 10.2, than at micromolar concentration levels none of these amino acids would react to a significant degree by a D-R mechanism. The fact that tryptophan has a rate for this process which probably exceeds that for the major portion of the organic compounds in seawater means that if natural sensitizers are well represented by methylene blue, then the predominant reactions of ^{3}sens are occurring with O_2 and not through D-R, D-Q, or D-D mechanisms.

The significance of the D-R, D-Q, and D-D mechanisms may be greater in heterogeneous reactions and natural macromolecules or molecular aggregates may tend to involve internal H or electron transfer processes analogous to reduction of the excited state triplet quinone in ribo-

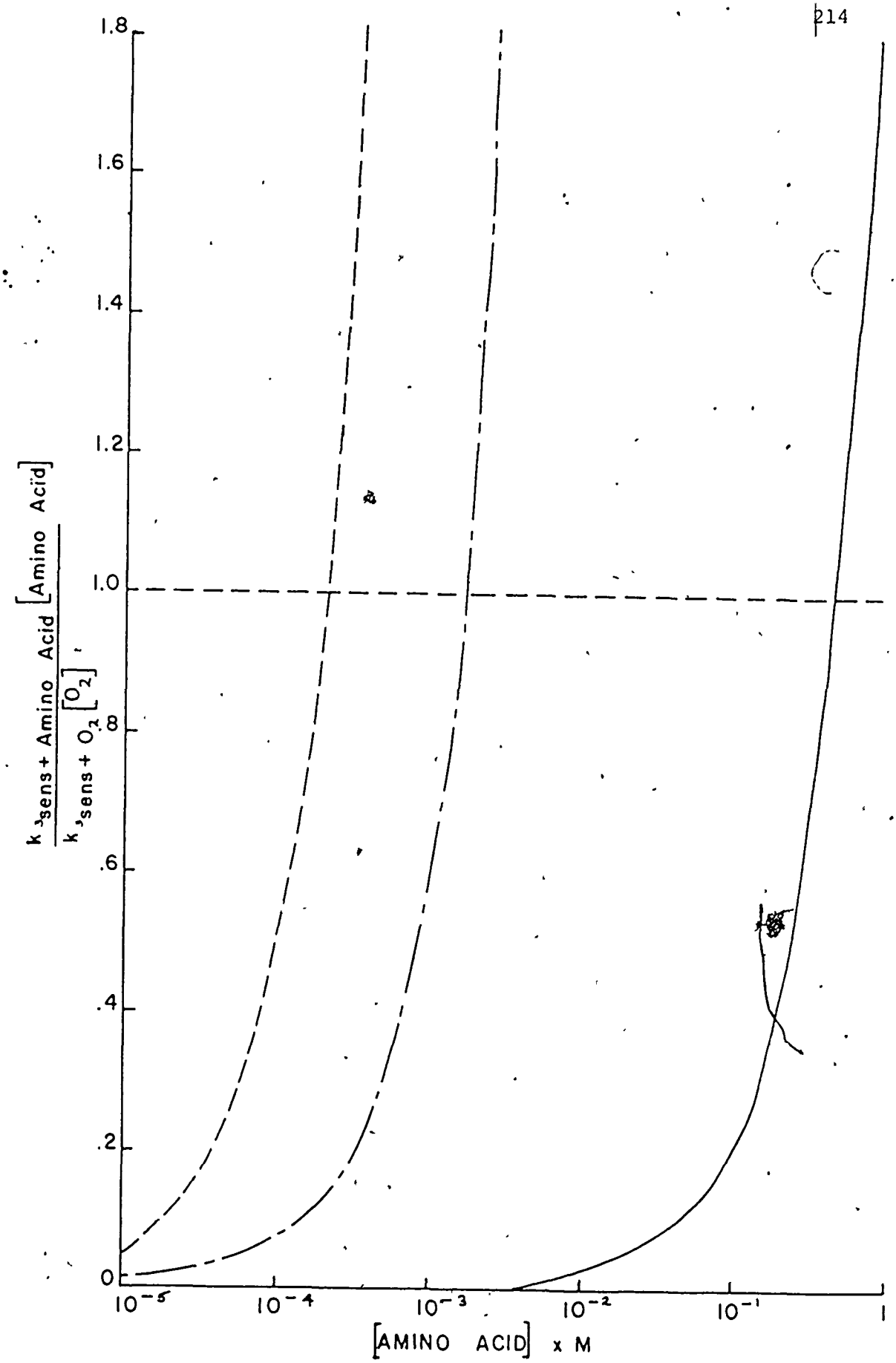
Figure 10.2 Competition Between O_2 and Amino Acids for Triplet State of Methylene Blue

Calculations are for aqueous solutions with oxygen concentration the same as found in air saturated seawater at 20°C and 18‰ chlorinity. Rate constants for amino acid-methylene blue reaction are from Nilsson et al. (1972) and for the O_2-methylene blue reaction a mean value of 2×10^{-9} M^{-1} l^{-1} was assumed (Foote, 1976).

— — Tryptophan

- —— Methionine

——— Histidine


1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
0
$\frac{k'_{sens} + Amino\ Acid\ [Amino\ Acid]}{k'_{sens} + O_2[O_2]}$
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
1
[AMINO ACID] x M

flavin by H-abstraction from the ribityl side chain. It is even conceivable that complexes between sensitizer and substrate or simply physical absorption or adsorption of the substrate by macromolecules, such as the retention of hydrophobic materials by humic acid (Kahn and Schnitzer, 1972), could constitute an isolated reaction environment within seawater.

10.1.3. Reactions of O_2 intermediates

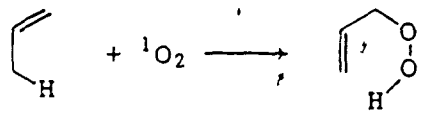
The near universality of reactive oxygen intermediates in aerobic biological and chemical systems has made them a major topic of study in recent years (Kasha and Khan, 1970; Schenck, 1970; Kearns, 1971; Bors et al., 1974; Fridovich, 1976; Foote, 1976). Their generation should also be expected in marine systems through biological, physical, and chemical processes where sufficient energy transfer occurs or where redox phenomena are involved. The reactions of photosensitizers certainly represent a potential source for these reactive intermediates, both through direct generation in D-O processes and as a result of the reactions of O_2 in many of the ensuing processes (Figure 10.1).

If, indeed, $^1O_2$, O_2^-, and peroxides are involved in the photochemistry of seawater, the nature of their probable secondary reactions becomes an important question.

10.1.3.1. Singlet oxygen

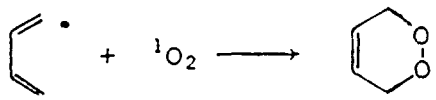
The reactions of $^1O_2$ with organic compounds have been reviewed (Kearns, 1971; Turro, 1974; Foote, 1976). The following six general reaction pathways have been identified and substantiated:

(1) Addition to olefins to give allylic hydroperoxides.



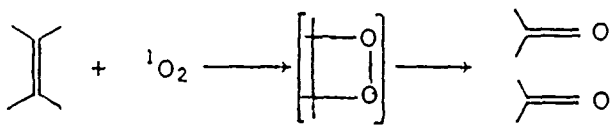
(10.8)

(2) 1, 4-addition to dienes, heterocycles, and certain aromatic compounds to form endoperoxides. These reactions are analogous to Diels-Alder reactions.



(10.9)

(3) 2 + 2 cycloaddition reactions with electron rich olefins, to produce unstable dioxetanes. The dioxetanes are usually unstable and will cleave to give carbonyl compounds.

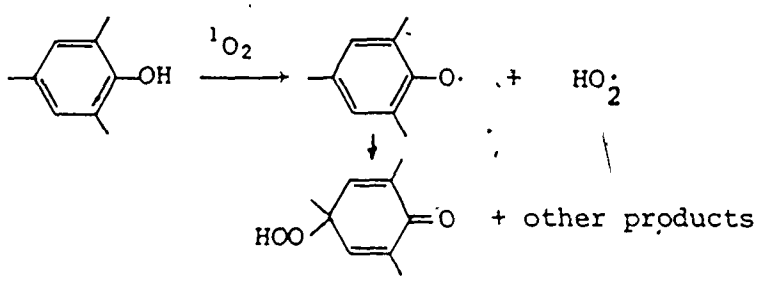


(10.10)

(4) Oxidation of certain heteroatoms such as sulfur, phosphorus, and nitrogen. In reactions of $^1O_2$ with nitrogen, the oxidation of N_2 (Anbar, [illegible]66) and NH_3 (Joussot-Dubien and Kadiri, 1970) have been postulated.

$$2R_2S + {}^1O_2 \longrightarrow 2R_2S{=}O \qquad (10.11)$$

(5) Oxidation of phenols which probably occurs through H abstraction, initially yielding phenoxy and hydroperoxy radicals. Products of phenoxy radical coupling and other decomposition



(10.12)

products are formed.

(6) Physical quenching reactions have been observed for carotenoid pigments. These reactions have rates that are diffusion controlled (2 to 3 x 10^{10} $M^{-1}sec^{-1}$). Azide ion, I^-, O_2^- (Rosenthal, 1975), and sulfides also quench $^1O_2$, at least in some solvents. Certain amines and phenols will preferentially quench $^1O_2$ rather than react chemically with it. Physical quenching has also been observed for the hydrated metal cations of Ni^{2+} and Co^{2+} (Carlsson et al., 1974).

10.1.3.2. Oxygen radicals

In the normal pH range of seawater the hydroperoxy radical ($HO_2\cdot$), which is the conjugate acid of O_2^-, should be unimportant, for its pK_a is 4.8. In comparison to $HO_2\cdot$, the dismutation reaction of O_2^- is slow ($k < 100\ M^{-1}sec^{-1}$) unless catalyzed. Therefore, with a significant rate of generation, low steady state levels of O_2^- can be maintained in irradiated solutions. The appreciably longer lifetime of this species in comparison to $^1O_2$ make it at least potentially a more significant reactant in aqueous solutions, where low substrate concentrations exist. However, comparatively little is known about its reactions with other molecules (Czapski, 1971; Bors et al., 1974; Fridovich, 1976). In general its reactivity will be controlled by its anionic character and

its reduction potential (Meisel and Czapski, 1975). It has the capacity to react as an oxidant or reductant, but in either case it reacts with only those compounds which are easily oxidized or reduced, such as quinones and hydroquinones (Patel and Wilson, 1973; Poupko and Rosenthal; 1973; Rao and Hayon, 1973; Moro-oka and Foote, 1976) and thiols (Barton and Packer, 1970). Unfortunately, many of the reactions in which O_2^- has been reported as the reactant are questionable because of the simultaneous occurrence of $^1O_2$, H_2O_2, ROO•, or RO•.

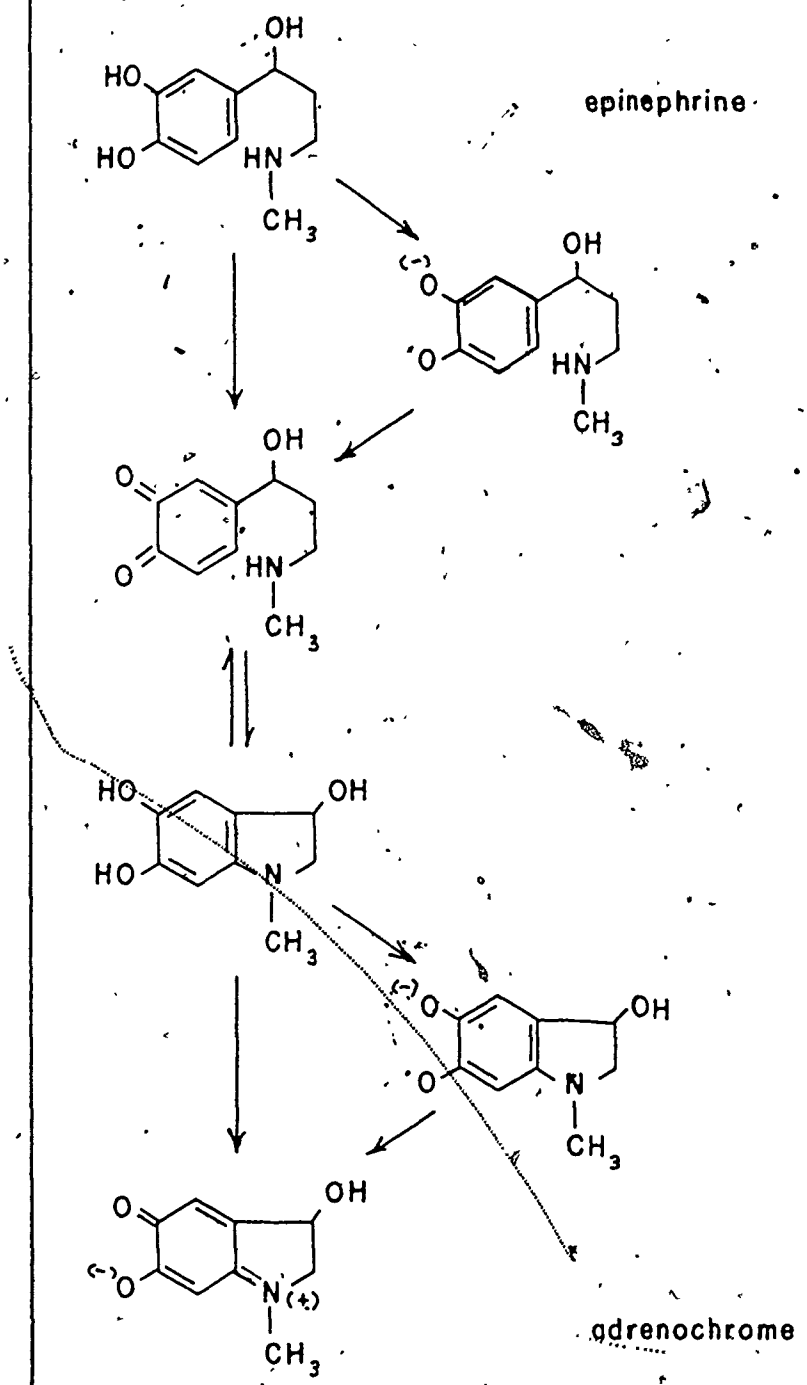
The most common test which has been used to discriminate between O_2^- and other reactive species is the catalyzed dismutation of O_2^- by the enzyme superoxide dismutase. The involvement of O_2^- in a reaction is indicated by a decrease in the reaction rate when the enzyme is added. Some reactions which have been used for this purpose are the oxidation of epinephrine to adrenochrome (Figure 10.3a), the reduction of tetranitromethane to the nitroform anion (Figure 10.3b), the reduction of p-nitroblue tetrazolium chloride (NBT) to the blue formazan derivative (Figure 10.3c), and the reduction of ferricytochrome c to ferrocytochrome c (Figure 10.3d) (Fridovich, 1972; Bors et al., 1974; Fridovich, 1976).

10.1.3.3. Peroxides and peroxy radicals

Hydrogen peroxide, hydroperoxides, and peroxy radicals represent the more stable oxygen intermediate species. Much of the known chemistry of hydroperoxides and peroxy radicals has evolved from studies of autoxidation (Nonhebel and Walton, 1974), and their reactivity in dilute

Figure 10.3 Proposed Reactions of Superoxide Radical Anion with Epinephrine, Tetranitromethane, NBT, and Fe^{3+} Cytochrome c (from Bors *et al.*, 1974)

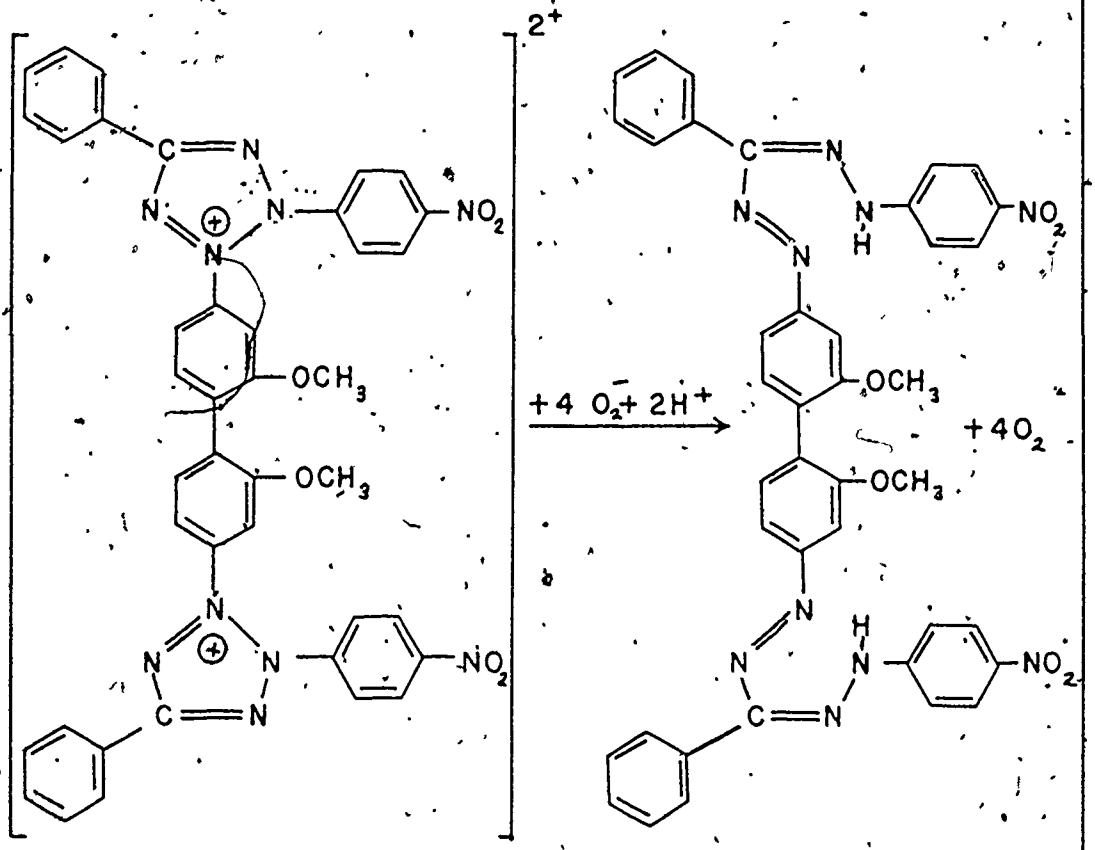
(a) Oxidation of Epinephrine



(b) Reduction of Tetranitromethane

$$O_2^- + C(NO_2)_4 \longrightarrow C(NO_2)_3^- + NO_2 + O_2$$

(c) Reduction of NBT



(d) Reduction of Ferric cytochrome c

$$Fe^{3+}\,Cyt\ c + O_2^{\cdot-} \longrightarrow Fe^{2+}\,Cyt\ c + O_2$$

aqueous solution in the absence of catalysts can only be conjectured as being low. The same holds true for H_2O_2, which should act as a mild oxidant in dilute aqueous solution at a pH of 8.

Peroxides can, however, act as a source of strong oxidants produced as a result of their decomposition by certain pathways. In the presence of catalytic amounts of certain metal ions (Fe^{2+}, Cr^{2+}, Ti^{3+}, Co^{2+}, or Cu^{1+}) peroxides decompose readily to form RO• or OH• radicals (Eq. 10.13).

$$ROOH + M^{n+} \longrightarrow RO\bullet + OH^- + M^{(n+1)+} \qquad (10.13)$$

Decomposition pathways also exist for higher oxidation state metal ions (Fe^{3+}, Co^{3+}, Cu^{2+}, Mn^{3+}, and Ce^{4+}), but in this instance peroxy radicals are generated (Eq. 10.14).

$$ROOH + M^{n+} \longrightarrow ROO\bullet + H^+ + M^{(n-1)+} \qquad (10.14)$$

Hydroxyl and alkoxyl radicals can also be produced in the facile reaction of peroxides with O_2^- (Eq. 10.15) (Peters and Foote, 1976) and through the irradiation of peroxide solutions with near ultraviolet

$$ROOH + O_2^- \longrightarrow RO\bullet + O_2 + OH^- \qquad (10.15)$$

radiation (Eq. 10.16) (Jacob et al., 1977).

$$ROOH \xrightarrow{h\nu} RO\bullet + OH\bullet \qquad (10.16)$$

10.2. Results and discussion

10.2.1. Effective concentration limits for some added sensitizers

The rate of a photosensitizer induced reaction will, of course,

depend to a large extent on the sensitzer concentration and upon its characteristics under the prevailing reaction conditions. A photosensitizer which generates free radical chain reactions may be significant at very low concentrations because of the increased probability of a collision between the substrate molecule and one of the reactive species in the multi-event process. Reaction chains of short duration or single event processes, such as energy transfer to substrate or to O_2, may only be important in homogeneous media if relatively high concentrations of the sensitzer are present. A relative approximation of the importance of specific sensitizers can be estimated by determining the lower limit of the concentration range for which the sensitizer is still inducing a measurable reaction.

For riboflavin induced reactions of methionine, a measurable effect was observed for riboflavin concentrations of less than 5×10^{-9} M (Figure 10.4). At these concentration levels, riboflavin would constitute less than 0.1% of the DOC in seawater if a level of 1.5 mg l^{-1} is assumed, and yet is inducing a significant reaction with methionine present at 1000 times the sensitizer concentration. The capacity of the sensitizer is apparently not irreversibly altered in the reaction, although electronic absorption spectra and fluorescence spectra of the reaction solutions were altered appreciably during the irradiation. This is not surprising, since riboflavin is destroyed rapidly in anerobic or aerobic photolysis to yield a complex product mixture (Song and Metzler, 1967). The yield of products is dependent on the pH, and the major product at pH < 7.0 is lumichrome (I), while in alkaline solution the

Figure 10.4 Concentration Dependence of Riboflavin on Photoinduced Degradation of Methionine

Two solutions were prepared in 35‰ artificial seawater which was buffered with borate buffer to a pH of 8.1:

(a) 5×10^{-6} M l^{-1} in methionine and

(b) 5×10^{-6} M l^{-1} in methionine plus 5×10^{-7} M l^{-1} in riboflavin.

Volumes of the 2 solutions were combined to give the required riboflavin concentrations. The solutions were air saturated and then irradiated for 2 hours in the inner ring of the merry-go-round system or in the outer ring for filtered light reactions.

With the exception of the irradiation period all solution manipulations were performed in the dark or under red light.

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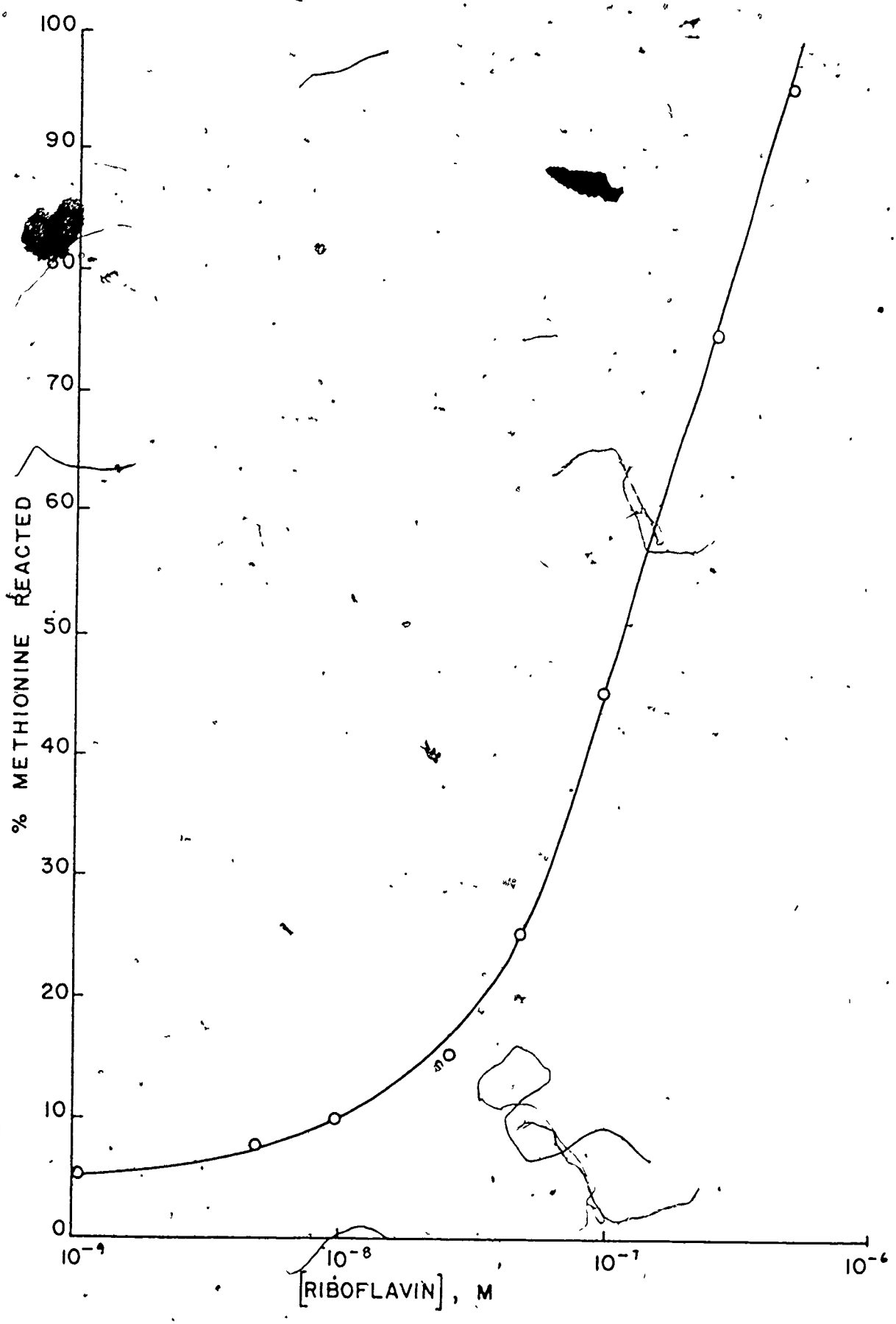
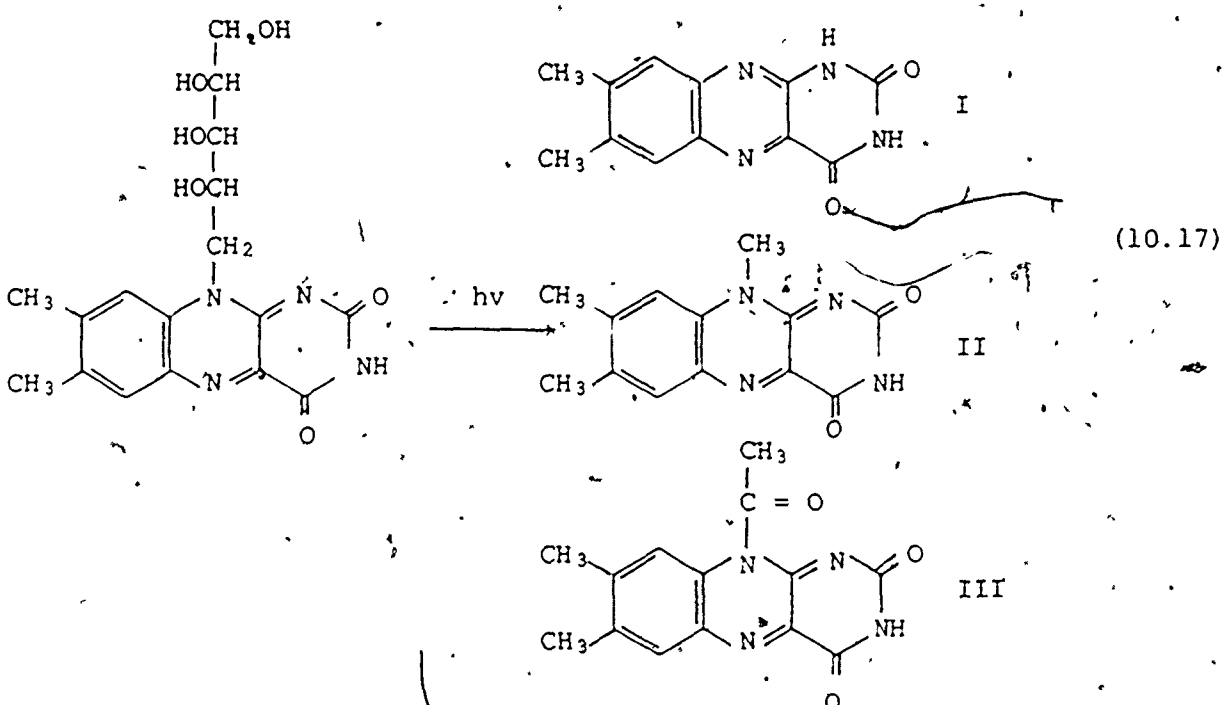

100
90
80
70
60
50
40
30
20
10
0
% METHIONINE REACTED
10^{-9}
10^{-8}
10^{-7}
10^{-6}
[RIBOFLAVIN], M

main product is lumiflavine (II) (Eq. 10.17) (Halwer, 1951). Another



(10.17)

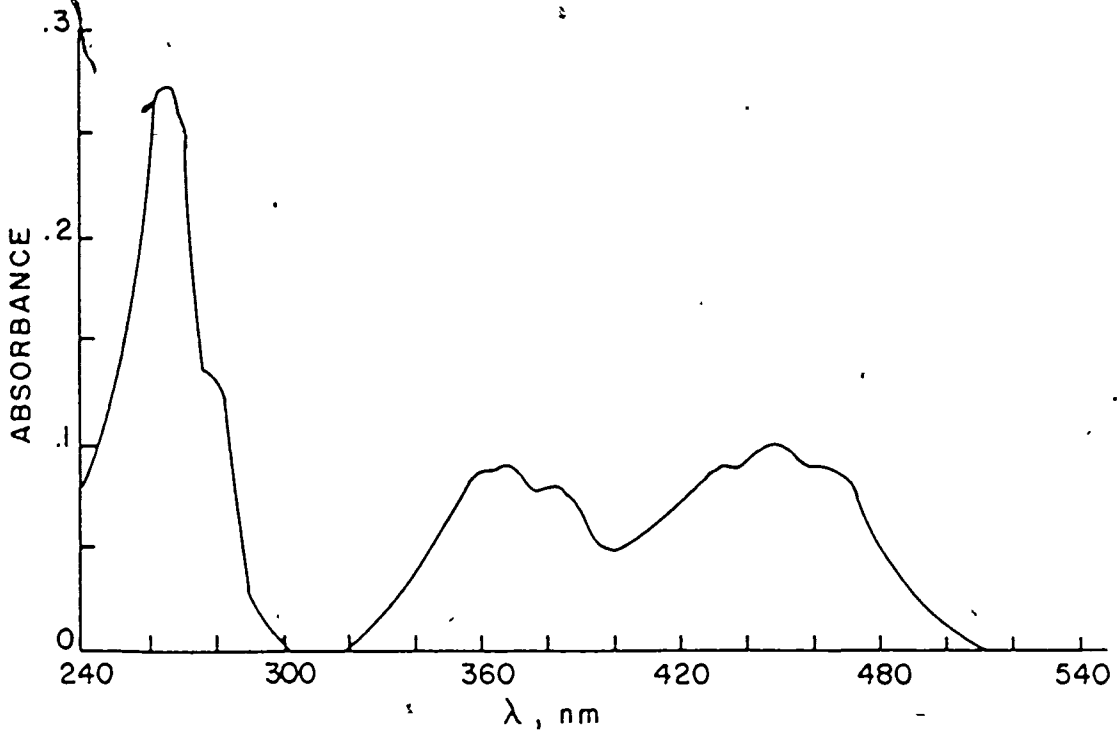
major product, 6-7-dimethyl-9-formylmethyl-isoalloxazine (III), was reported at neutral and acidic pH values, and it, along with lumiflavine, apparently arises from the triplet excited state of riboflavin through intramolecular photoreduction of the isoalloxazine ring by the ribityl side chain (Song and Metzler, 1967; Penzer and Radda, 1967). Neither of these products were identified in seawater riboflavin photolysis. Instead, a nearly quantitative conversion to lumichrome was observed, based on the absorption spectra of the solutions (Figure 10.5), the HPLC analysis and the shift from a green to blue fluorescence maximum, which is characteristic only for lumichrome. The rapid shift of the fluorescence maxima indicated that riboflavin was converted to lumichrome during the earlier minutes of the irradiation. Since it required an hour to reduce the methionine concentration to the values

Figure 10.5 Absorption Spectra for Riboflavin-Methionine Reaction Solution

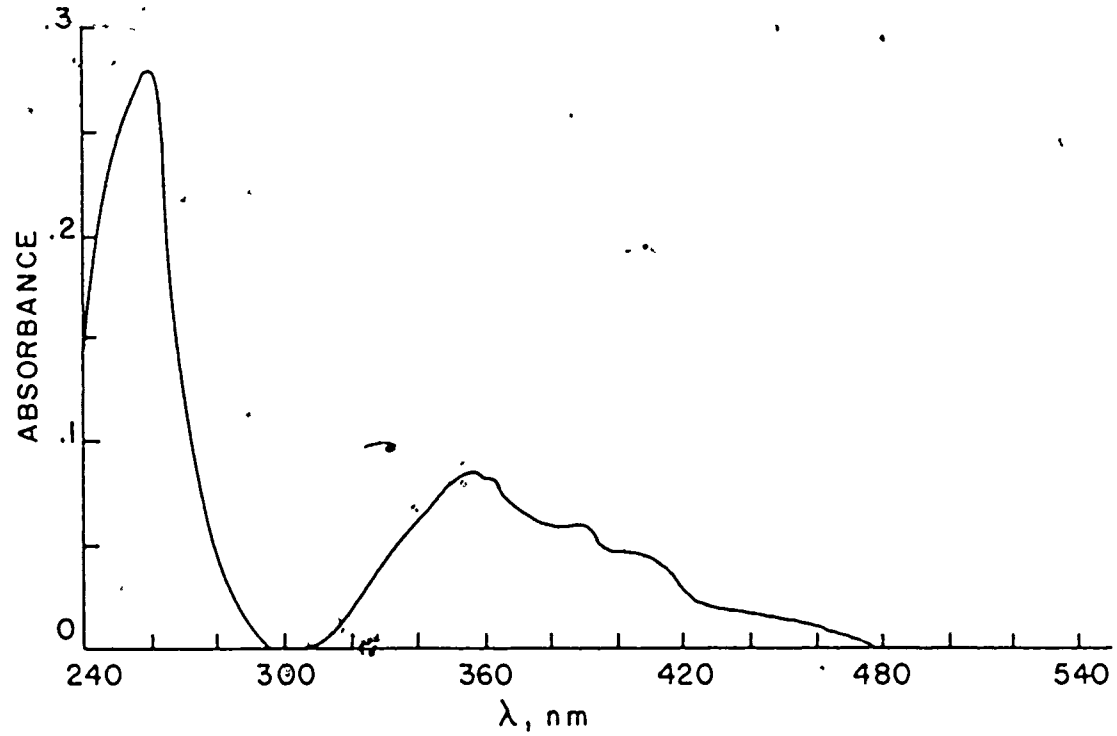
(a) 1×10^{-7} M l^{-1} of riboflavin in dark control (see Figure 10.4)

(b) solution after 2 hour irradiation period for wavelength region > 290 nm.

a.


ABSORBANCE
.3
.2
.1
0
240
300
360
420
480
540
λ, nm

b.


ABSORBANCE
.3
.2
.1
0
240
300
360
420
480
540
λ, nm

shown in Figure 10.4 it implies that lumichrome is probably the major sensitizer of the reaction.

Lumichrome is believed to result from the singlet excited state of riboflavin (Song, 1971), and the absence of lumiflavine or 6,7-dimethyl-9-formylmethyl-isoalloxazine may mean that the triplet state is being efficiently quenched by O_2. This possibility was tested by irradiating a deaerated 1×10^{-7} M riboflavin solution (Figure 10.4), which contained 5×10^{-6} M l^{-1} of methionine. The result was a 2 fold increase in methionine destruction and an approximate 40% decrease in the amount of lumichrome formed, when compared to the same reaction in an air saturated solution. Although the role of O_2 in this reaction is unknown, it is apparent that it does quench the reaction.

Lumichrome could also be formed by photolysis of lumiflavine or 6,7-dimethyl-9-formylmethyl-isoalloxazine. This process is known to be rapid for at least the latter of these 2 compounds (Treadwell et al., 1968). In any case, the end product appears to be the light stable sensitizer lumichrome (Berends and Posthuma, 1962). For wavelengths greater than 290 nm lumichrome appears to be light stable in seawater, since no appreciable change of the absoprtion spectrum (Figure 10.5b) was noted for an extended irradiation period of two hours in the merry-go-round system.

The formation of lumichrome occurred at wavelengths in the near ultraviolet region and in the 400 to 500 nm region, but none was found for wavelengths greater than 500 nm nor was any loss of riboflavin observed. The absorption spectra for solutions irradiated in the > 290 nm and > 400 nm regions were identical, but the destruction of

methionine for the > 400 nm region was only 39% of that observed in the > 290 nm region.

In comparison to the isoalloxazines, information on the mechanisms of photosensitization by natural polyphenolic materials is virtually nonexistent. Their potential importance in mediating reactions in fresh and agricultural water has been demonstrated (Ross and Crosby, 1973; Ross, 1974; Paris et al., 1975). However, the concentration of such substances in seawater is far lower, and their effectiveness as sensitizers may be greatly diminished.

The concentration dependence of two natural polyphenolic substances (fulvic acid and the phloroglucinol-based polymer) was determined for the photoinduced degradation of methionine (Figure 10.6). The relative reaction rate in seawater used to prepare the polymer enriched solutions was .15 $\mu M\ l^{-1} hr^{-1}$. This rate was doubled by approximately 50 $\mu g\ l^{-1}$ addition of the phloroglucinol-based polymer and 240 $\mu g\ l^{-1}$ for fulvic acid. Since the two polymers have an elemental composition which is approximately 50% carbon, the actual increase in terms of μg of C l^{-1} is roughly one half of these values, or less than 10% of the total DOC present in this seawater. In terms of molar concentrations, the observed rate doubling is occurring at 5 to 50 x 10^{-11} M l^{-1} of the phloroglucinol-based polymer and 2.5 x 10^{-7} M l^{-1} for fulvic acid. Molecular weights of 950 for fulvic acid and 10^5 to 10^6 for phloroglucinol-based polymer were assumed.

Although concentrations expressed as molarity or equivalents are more applicable in determining reaction kinetics or characterizing

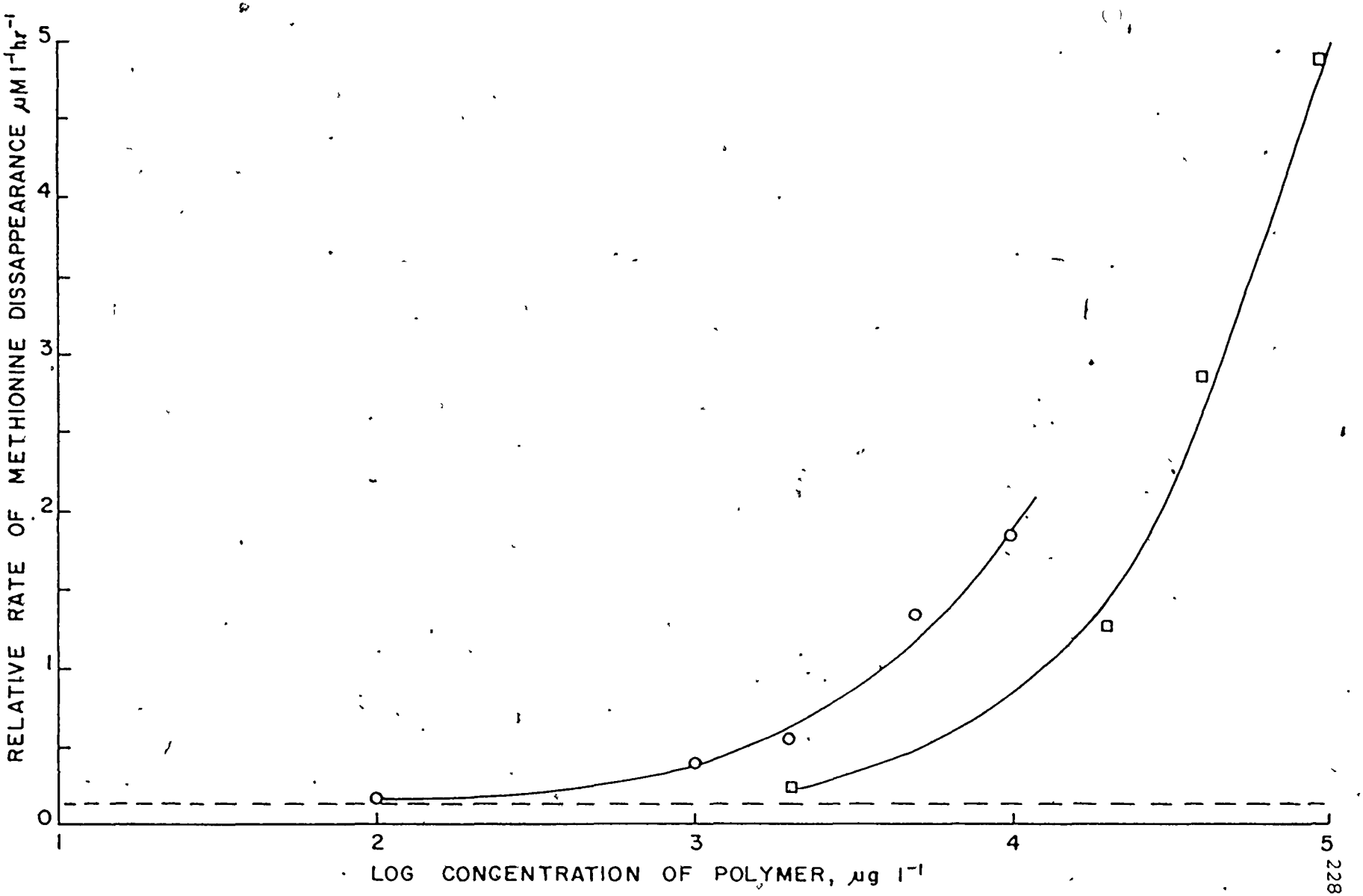
Figure 10.6 Concentration Dependence of Phloroglucinol-Based Polymer and Fulvic Acid on Photoinduced Degradation of Methionine

Solutions were prepared in natural seawater which was buffered with borate and saturated with air. All reaction solutions were initially 5×10^{-6} M in added methionine.

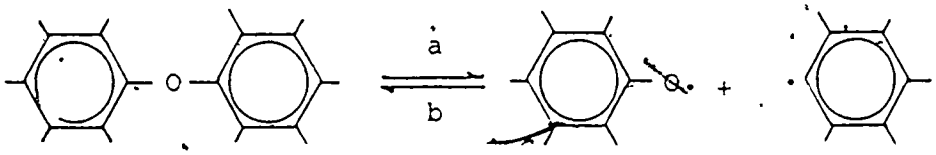
○ phloroglucinol-based polymer

□ fulvic acid

— — — seawater without added sensitizer


RELATIVE RATE OF METHIONINE DISSAPPEARANCE μM l⁻¹hr⁻¹
0
1
2
3
4
5
1
2
3
4
5
LOG CONCENTRATION OF POLYMER, μg l⁻¹

reaction mechanisms, they could be very misleading when applied to these natural polymers. Natural polymers could be subject to considerable alteration of structure through hydrolytic and thermal processes (Ragan and Craigie, 1977) as well as by photochemical processes (Section 7.2). It is reasonable to assume that, like many other phenolic materials, they may polymerize and increase in molecular weight through a process like phenoxy radical coupling (Eq. 10.18b), or dissociate to give lower



(10.18)

molecular weight fragments (Eq. 10.18a). Which trend (i.e., polymerization or dissociation) dominates will be highly dependent, among other things, on the concentration range of the polymer.

10.2.2. Sensitizer induced peroxide formation

Oxygen can be directly reduced by many photosensitizers, or it can react with secondary products to give O_2^-. Either through disproportionation or further reduction, O_2^- yields H_2O_2 a common product in aerobic photosensitization reactions. The occurrence of H_2O_2 in seawater (Van Baalen and Marler, 1966) could mean that it is being generated by similar processes there. However, its production could also be attributed to the metabolic activity of micro-organisms, as exemplified by some blue-green algae (Stevens et al., 1973), or to a host of dark oxidation-reduction reactions which are not light induced. In fact, a low steady state concentration of H_2O_2, resulting from the O_2/H_2O_2 redox couple, has been proposed as the main controlling factor of the

reduction-oxidation properties of seawater (Breck, 1974; Parsons, 1975). The concentration levels measured by Van Baalen and Marler (15 to 200 nM l^{-1}), however, exceed the predicted steady state concentration by several orders of magnitude and indicate that the kinetics of formation must be relatively rapid or that H_2O_2 has a higher stability in seawater than would be expected.

10.2.2.1. Formation and stability of peroxide in prepared seawater solutions

Various materials were compared with respect to their capacity to generate peroxides in seawater (Table 10.1a). Of these, riboflavin and tryptophan have both been shown to form H_2O_2 in aqueous aerobic solutions, when irradiated at wavelengths in the near-ultraviolet region (Massey et al., 1971; McCormick et al., 1976). Photochemical processes involving tryptophan at these wavelenghts can generate H_2O_2 by several routes (Figure 6.6), but the direct interaction of the triplet excited state of tryptophan or N-formylkynurenine with O_2 at the concentrations used here might be more significant than reduction of the sensitizer by substrate with subsequent reduction of O_2. Flash photolysis of aqueous tryptophan solutions revealed that the triplet pathway might be more important (Pailthorpe et al., 1973). The same mechanism may be operating for riboflavin, although in this case intramolecular reduction is possible. However, the rapid transformation of riboflavin to lumichrome, for which intramolecular reduction is not possible, would suggest that a change in the rate of peroxide formation should have been observed shortly after initiating the irradiation. It was not. It is therefore

Table 10.1 Effects of Potential Photosensitizing and Non-Photosensitizing Materials on Apparent Rate of Peroxide Formation

Sample irradiation was conducted in inner ring of merry-go-round system. Peroxide determinations were conducted at 15 minute intervals and the apparent rate was calculated from the best linear fit for these values plotted against time.

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a. EFFECT OF POTENTIAL PHOTOSENSITIZERS ON PEROXIDE FORMATION[5]

Seawater Solutions	Apparent Peroxide Formation Rate $nM \cdot l^{-1}$ hr^{-}
No addition	11.2
95 g l^{-1} Fulvic Acid	19.2
5×10^{-8} M l^{-1} Riboflavin	33.9
5×10^{-6} M l^{-1} Tryptophan	49.6
1×10^{-7} M l^{-1} Isoxanthopterin	13.3
500 µg l^{-1} Phloroglucinol-based polymer	222

b. EFFECT OF NON-PHOTOSENSITIZERS ON PEROXIDE FORMATION

Seawater Solutions	Apparent Peroxide Formation Rate $nM\ l^{-1}\ hr^{-1}$
No addition	11.2
10 X Enrichment of TMEM	4.34
5×10^{-6} M l^{-1} Methionine	18.5
1×10^{-6} M l^{-1} NO_2^-	2.4
1×10^{-6} M l^{-1} NO_2^- + 5×10^{-6} M l^{-1} Methionine	24.0

likely that O_2 is being reduced through a D-O mechanism, and not via the reduced form of the sensitizer (reactions M and N in Figure 10.1). The addition of a readily oxidized substrate can facilitate the formation of O_2^- by increasing the yield of the reduced sensitizer through the D-R mechanism. This might explain the observed increase in the apparent formation rate of peroxide when methionine is added to seawater (Table 10.1b). Reducing agents such as EDTA, methionine (other amino acids), and tetramethylethylenediamine have often been used to increase the D-R pathway and to generate O_2^- in aerobic solutions of various sensitizers, including flavins (Frisell et al., 1959; Massey et al., 1969; Beauchamp and Fridovich, 1971).

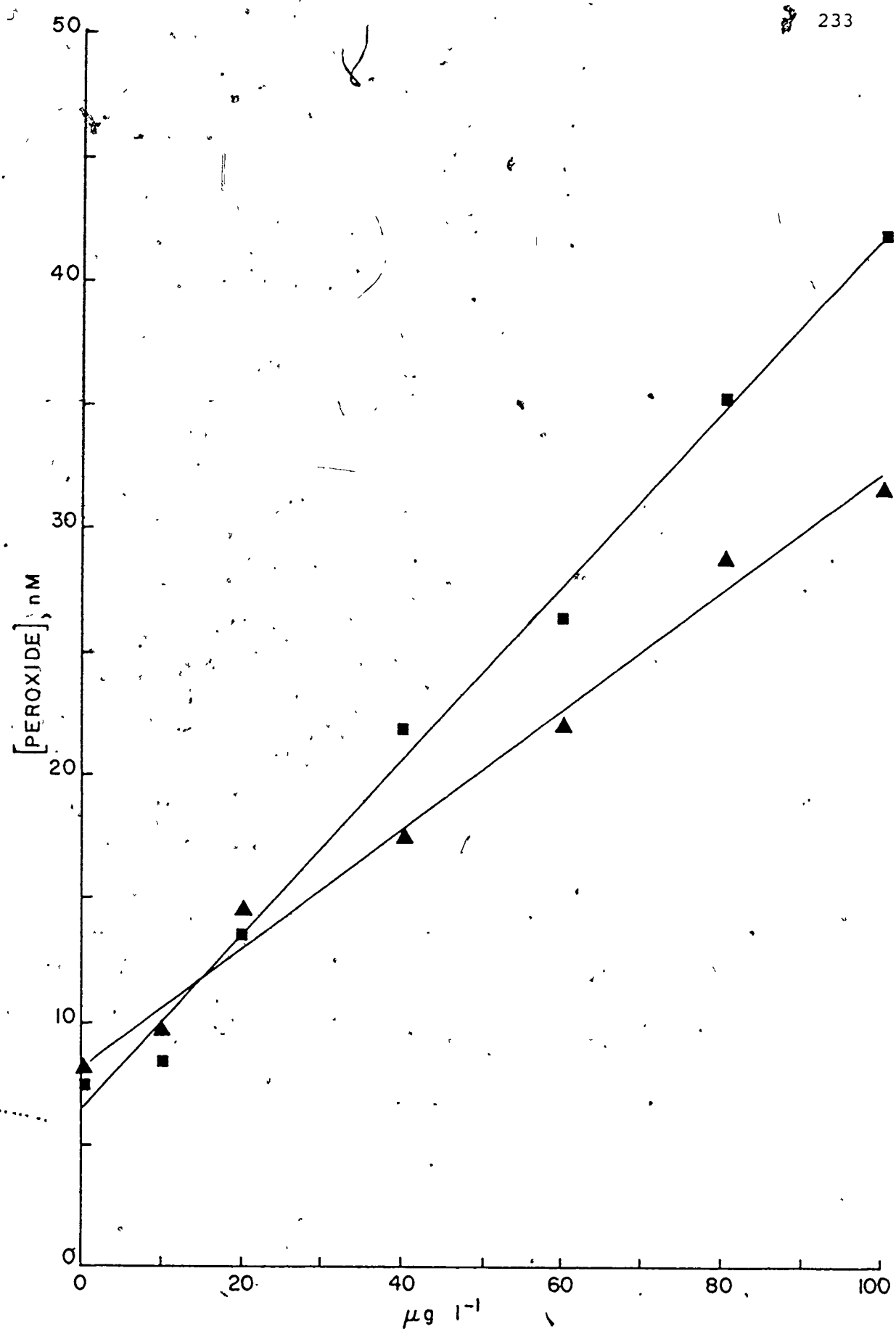
Peroxide formation was also investigated for three other substances: isoxanthopterin (2-amino-4,7-pteridinediol), fulvic acid, and the phloroglucinol-based polymer (Table 10.1a). Only isoxanthopterin failed to give a significant yield of peroxide; this might at least in part be due to its high insolubility in water at a pH of 8.1. The polyphenolic polymers were both found to generate peroxide; the phloroglucinol-based polymer was expecially productive. In fact, for the same concentration of the 2 polymers, there was approximately a 11 fold difference in the amount of peroxide produced (Figure 10.7). Both polymers can cause an appreciable increase in the amount of peroxide accumulated when they are present at low concentrations. Only 12.5 µg of carbon as the phloroglucinol-based polymer and 105 µg of carbon as fulvic acid were necessary to double the accumulated concentration of peroxide in this seawater.

Figure 10.7 Concentration Dependence of Fulvic Acid and Phloroglucinol-Based Polymer on Formation of Peroxide in Seawater

Solutions were prepared in natural seawater which was buffered with borate and saturated with air. The solutions were irradiated in merry-go-round system for 15 minutes and then immediately quenched.

■ fulvic acid solutions are enriched to 10 times $\mu g\ l^{-1}$ values shown on the abscissa

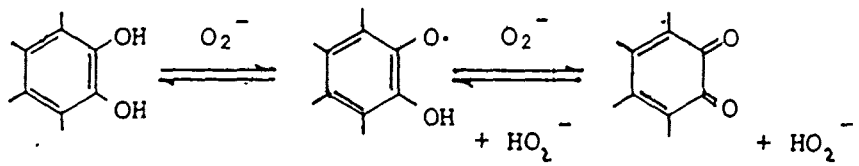
▲ phloroglucinol-based polymer


50
40
30
20
10
0
[PEROXIDE], nM
0
20
40
60
80
100
μg l⁻¹

Measurement of the accumulated peroxide concentration cannot be applied directly to determining its rate of formation. Although peroxides might represent one of the more stable oxidants formed in irradiated solutions, they are probably unstable under the reaction conditions used in these experiments. The observed accumulation rate (k_A, see Eq. 10.19) is actually the summation of the formation rate

$$k_A = k_F - k_L - k_D \tag{10.19}$$

(k_F), the light catalyzed decay rate (k_L), and the dark decay rate (k_D). If this equation is considered only for H_2O_2, and it is assumed that H_2O_2 is entirely formed by disproportionation of O_2^- (eq. 10.5), then k_F is related only to the active photon flux, the concentration of O_2^- producing substances, and their quantum yields, unless other constituents of the solution intervene. Therefore, even if the concentration and characteristics of O_2^- producing substances do not vary among different seawater samples, a change in composition of other constituents could alter k_F. This has already been demonstrated for methionine, and might be an anticipated result for other organic materials (e.g., phenols or quinones, Eq. 10.20) or for various transition metal ions (Eqs. 10.21-10.23), like Cu^{2+} (Klug-Roth and Rabini, 1976) or Fe^{3+} (Barb et al., 1951).



(10.20)

$$Cu^{2+} + O_2^- \longrightarrow Cu^+ + O_2 \tag{10.21}$$

$$Cu^{2+} + O_2^- \xrightarrow{2H^+} Cu^{3+} + H_2O_2 \tag{10.22}$$

$$Fe^{3+} + O_2^- \longrightarrow Fe^{2+} + O_2 \tag{10.23}$$

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The same type of constituent variations could also alter k_D and k_L, for H_2O_2 is unstable in the presence of trace amount of many inorganic and organic materials. On the basis of concentration, the halide ions represent the most notable component group in seawater toward which H_2O_2 is unstable. The reaction with Cl^- yields Cl_2 (Eq. 10.24) and the first order rate constant for the disappearance of

$$H_2O_2 + 2H^+ + 2Cl^- \longrightarrow Cl_2 + 2H_2O \qquad (10.24)$$

H_2O_2 at pH 8.0, 20°C, and in .5 M NaCl is .0082 hr^{-1} (Skopintsev, 1949a). Low concentrations of Cu^{2+} were found to cause a considerable acceleration in the rate, while some organic compounds inhibited the reaction (Skopintsev, 1949b). Under similar conditions, Liebhafsky (1932) investigated the decomposition of H_2O_2 in iodide solutions (Eqs. 10.25 and 10.26) and found a first order rate constant of .69 sec^{-1}

$$H_2O_2 + I^- \longrightarrow IO^- + H_2O \qquad (10.25)$$

$$IO^- + H_2O_2 \longrightarrow I^- + H_2O + O_2 \qquad (10.26)$$

at 25°C for reaction 10.25. Although the reaction has also been studied for bromide solutions (Bray and Livingston, 1923), no information was found for the decomposition near the neutral pH region. However, it is reasonable to anticipate, on the basis of oxidation potentials for the halides, that the decomposition rate would increase in the order $Cl^- < Br^- < I^-$.

Reactions contributing to k_L can result directly from the photolysis of H_2O_2 (Eq. 10.16) of from the reaction of H_2O_2 with secondary

photolysis products such as free radicals (Eq. 10.15) (Hunt and Taube, 1952; Peters and Foote, 1976). Above 300 nm, H_2O_2 photolysis proceeds exclusively to OH• with a primary quantum yield of .5 to .6; the observed non-chain reaction quantum yield is 1.0 to 1.2, as a result of the production of O• (Eq. 10.27), which then reacts with more H_2O_2

$$H_2O_2 \xrightarrow{h\nu} 2OH\bullet \xrightarrow{h\nu} H_2O + O\bullet \qquad (10.27)$$

(Jacob, 1977). Rapid alternative reactions of O• in seawater should lower the quantum yield to less than one. Nevertheless, this high quantum yield should provide a rapid H_2O_2 turnover rate for wavelengths to 370 nm, even though peroxides have a comparitively low molar extinction coefficient above 300 nm. Again, the rate k_L can be dependent on the concentration and nature of minor constituents which scavenge free radicals resulting from the primary photolysis and thereby reduce ϕ to a value which is not less than 0.5.

The importance of some minor constituent variations on k_A for a natural seawater sample are shown in Table 10.1b. It has already been suggested that the role of methionine may be to alter the sensitizer mechanism from D-O to D-R. Another possibility exists, however, if methionine reactions involve free radical intermediates which in the presence of O_2 form peroxides through what amounts to an autoxidation reaction sequence (Eqs. 10.28-10.34). Since radicals (R•) typically

$$\left.\begin{array}{l} \text{Initiator} \longrightarrow X\bullet \qquad (10.28) \\ X\bullet + RH \longrightarrow XH + R\bullet \qquad (10.29) \end{array}\right\} \text{Initiation}$$

$$\left.\begin{array}{l} R\bullet + O_2 \longrightarrow ROO\bullet \qquad (10.30) \\ ROO\bullet + RH \longrightarrow ROOH + R\bullet \qquad (10.31) \end{array}\right\} \text{Propagation}$$

$$ROO\cdot + R\cdot \longrightarrow ROOR \qquad (10.32)$$

$$2R\cdot \longrightarrow RR \qquad (10.33)$$

$$2ROO\cdot \longrightarrow ROOR + O_2 \qquad (10.34)$$

} Termination

have short lifetimes and would be present at very low concentrations, the termination reactions 10.32 and 10.33 should be unimportant, as should reaction 10.34, as long as a sufficient supply of RH is present. Other termination reactions should certainly be active in seawater; however, the predominant product of autoxidation pathways under these conditions should be hydroperoxides. This could explain why NO_2^- inhibits peroxide formation while NO_2^- plus methionine increases its apparent rate of formation (Table 10.1b). In this case NO_2^- acts as the initiator and methionine as the substrate for hydrogen abstraction. When only NO_2^- was added, a considerable reduction in the apparent rate was observed after a short induction period, during which the rate was the same as measured in seawater without any addition. This does not appear to be the result of dark reactions involving H_2O_2 and NO_2^-, for solutions of these two materials undergo no detectable thermal reaction at a pH of 7 to 8 for periods of up to 20 hours (Schwartz and Allen, 1955). The stability of H_2O_2 towards NO_2^- was examined by measuring the decay kinetics of H_2O_2 in seawater with and without a NO_2^- enrichment. Without NO_2^-, k_1 was 1.07×10^{-3} sec^{-1}, and with 5×10^{-6} M of added NO_2^-, k_1 was $.99 \times 10^{-3}$ sec^{-1}; both were determined at 20°C. The difference between the rate constants is within experimental error for the method and cannot be used to explain the observed inhibitory effect of NO_2^- on k_A.

It appears that inhibition is light initiated and may involve a product of NO_2^- photolysis. The resulting inorganic free radicals (e.g., $OH^\cdot$, $Br_2^{\cdot -}$, or $HCO_3^\cdot$) are too short-lived ever to maintain a sufficient steady state concentration to be significant competitors for peroxide or peroxide intermediates. Nitrite photolysis also generates NO and NO_2, which, although not stable, should have appreciably longer lifetimes. If a sufficient steady state concentration of these can be maintained, peroxide or peroxide intermediates could conceivably be removed by reactions such as 10.35 through 10.38. Oxidation of NO by H_2O_2 does not appear to be important, because the

$$2NO + H_2O_2 \longrightarrow 2NO_2^- + 2H^+ \qquad (10.35)$$

$$NO + O_2^- \longrightarrow NO_2 + O^- \qquad (10.36)$$

$$NO_2 + H_2O_2 \longrightarrow 2H^+ + NO_2^- + O_2^- \qquad (10.37)$$

$$NO_2 + O_2^- \longrightarrow NO_2^- + O_2 \qquad (10.38)$$

reaction is very slow even at much higher concentrations (Seddon and Sutton, 1963) than would ever be present in these solutions. Oxidation of NO by the perhydroxyl radical is an important reaction in atmospheric chemistry (Demerjian et al., 1974), but the extent of its reaction with O_2^- (Eq. 10.36) in seawater is unknown. Oxidation of either H_2O_2 or O_2^- by NO_2 is also possible, since NO_2 and its dimer are relatively strong oxidizing agents.

Inhibition of k_A by NO_2^- could be far more complex than the mechanisms involving NO or NO_2 proposed here, but without further information on the chemistry of NO_2^- and its photolysis products in sea-

water, any proposal is only speculative. The same holds true for transition metals, which inhibit peroxide accumulation (Table 10.1b).

It is apparent from the results shown here that the observed k_F in seawater can be controlled by substances other than those responsible for peroxide formation, and that k_A for natural seawater could be determined by a complex array of variables.

10.2.2.2. Formation and stability of peroxides in seawater

The light catalyzed formation of peroxides in freshly collected seawater was demonstrated for numerous samples; all that were tested gave measurable yields of peroxide. Peroxide formation was not found, however, in artificial seawater or deionized water when they were subjected to identical experimental conditions. This indicates that the property is peculiar to seawater and does not arise from an artifact of the experimental procedures.

The reaction(s) inducing peroxide formation were, for the seawater used, restricted to wavelengths of less than 400 nm. This result suggests that major differences in the light source outputs should contribute to major differences in k_A when the same seawater is exposed to different light sources. Although differences were observed, they did not reflect the weighted intensity outputs of the different sources in the near-ultraviolet region. The reasons for this were not determined, but it is likely that k_L is greatly enhanced through primary peroxide photolysis by the strong 313 nm line from the mercury lamp. This increase would be expected because of an increase in the

molar extinction coefficient for peroxides with decreasing wavelength. Therefore, environmentally accurate determinations of k_A are best made using sunlight although some of the difficulties in doing so are reflected by a loss of precision (Figure 10.8a). This probably results in part from inadvertent changes in light intensity and energy distribution, especially for shorter wavelengths (Figure 4.2). Also, the usual problems inherent in field studies manifest themselves even more in an analysis involving unstable reagents, precise aliquot additions, and precise mixing rates to measure an unstable product. In an attempt to eliminate some of the problems, the experiment was conducted in a series of different quartz tubes (Figure 10.8a), with each representing a specific reaction and with the analyses performed in the laboratory within 10 minutes after removal from sunlight. Far better results were obtained when the reaction was conducted in a single quartz vessel with the analyses made near the irradiation site (Figure 10.8b). Part of the difference observed in these two experiments could result from catalytic decomposition of peroxide at container walls. This effect has been observed in homogeneous liquid phase studies on the catalytic decomposition of H_2O_2 (Uri, 1949).

Under controlled laboratory conditions, seawater irradiations with artificial light sources gave far better analytical precision (Figures 10.9a, b). In some cases an initial induction period was observed, after which peroxide levels climbed rapidly. In Figure 10.9b, it is shown that virtually no peroxide accumulation was observed for the first 1/4 hour of the light irradiation. As in the case of added H_2O_2, or

Figure 10.8 Peroxide Accumulation in Seawater

Irradiated with Sunlight

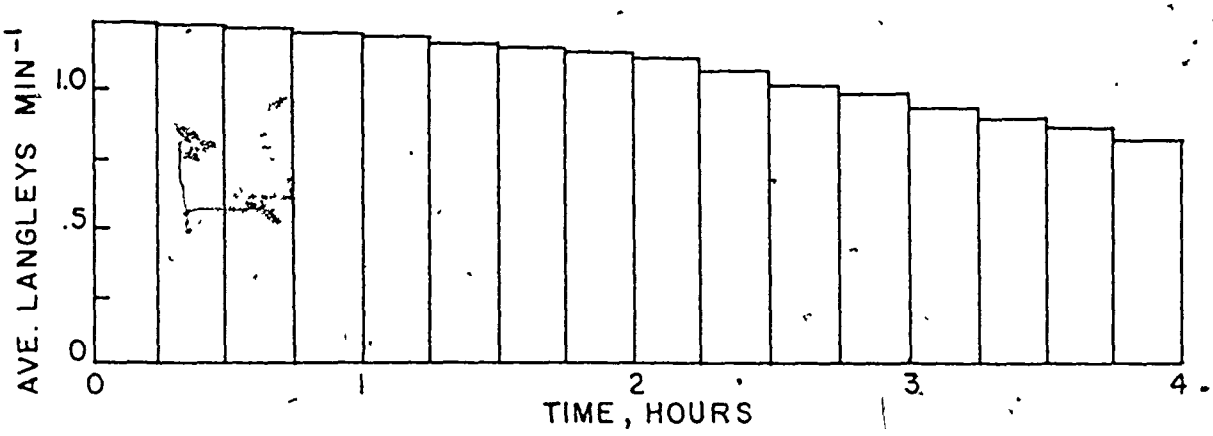
Freshly collected seawater (salinity 31‰) was buffered with borate, filtered, and exposed to sunlight in sealed quartz tube(s) which was immersed in ~ 10°C seawater. Duplicate peroxide analyses were run at the times shown.

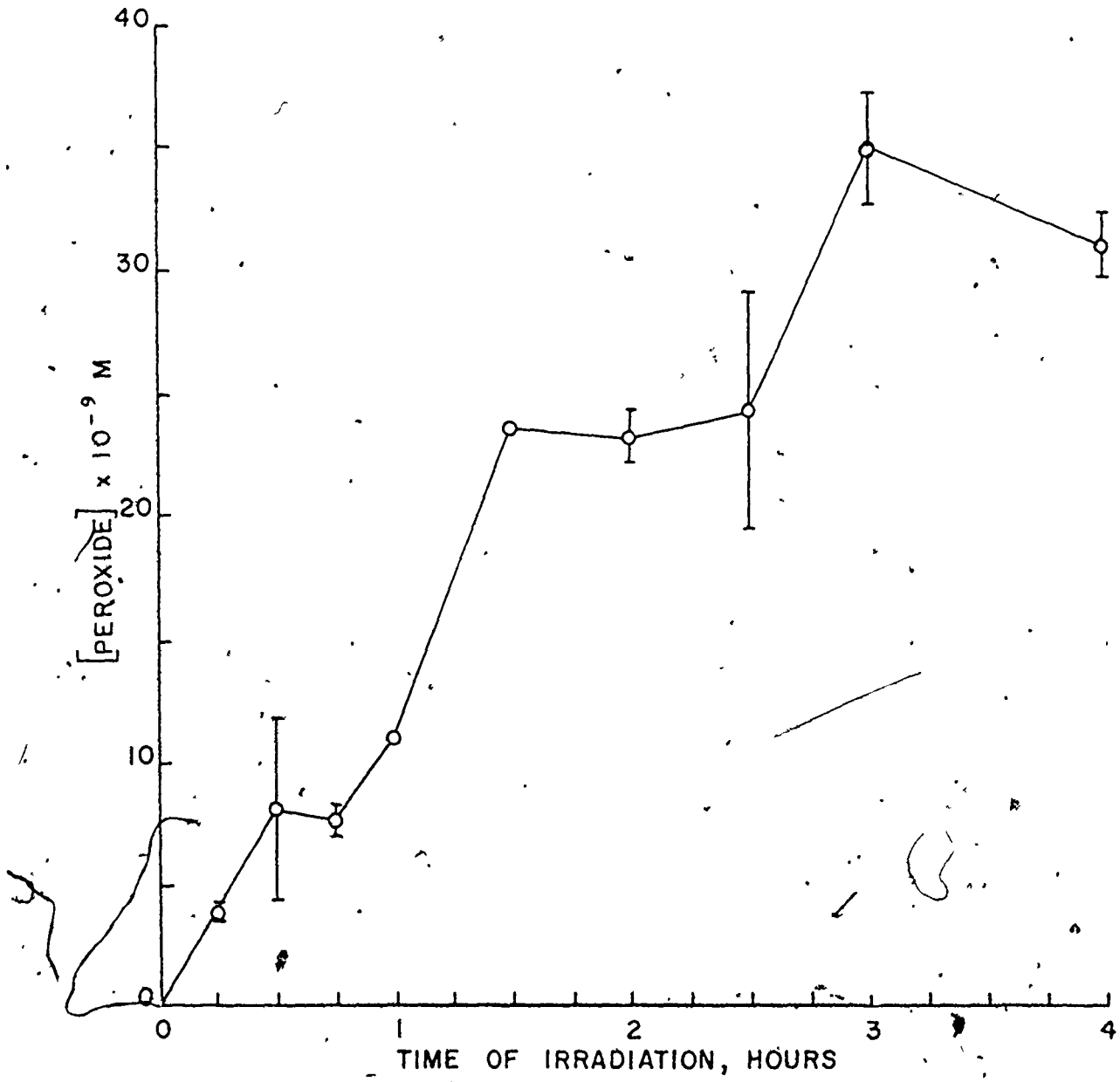
The experiment was performed in late August. Averaged sunlight energy over 1/4 hour periods are shown in the block diagram.

A. multiple quartz tubes

B. single quartz tube

A


AVE. LANGLEYS MIN⁻¹
1.0
.5
0
0
1
2
3
4
TIME, HOURS


40
30
20
10
0
[PEROXIDE] x 10⁻⁹ M
0
1
2
3
4
TIME OF IRRADIATION, HOURS

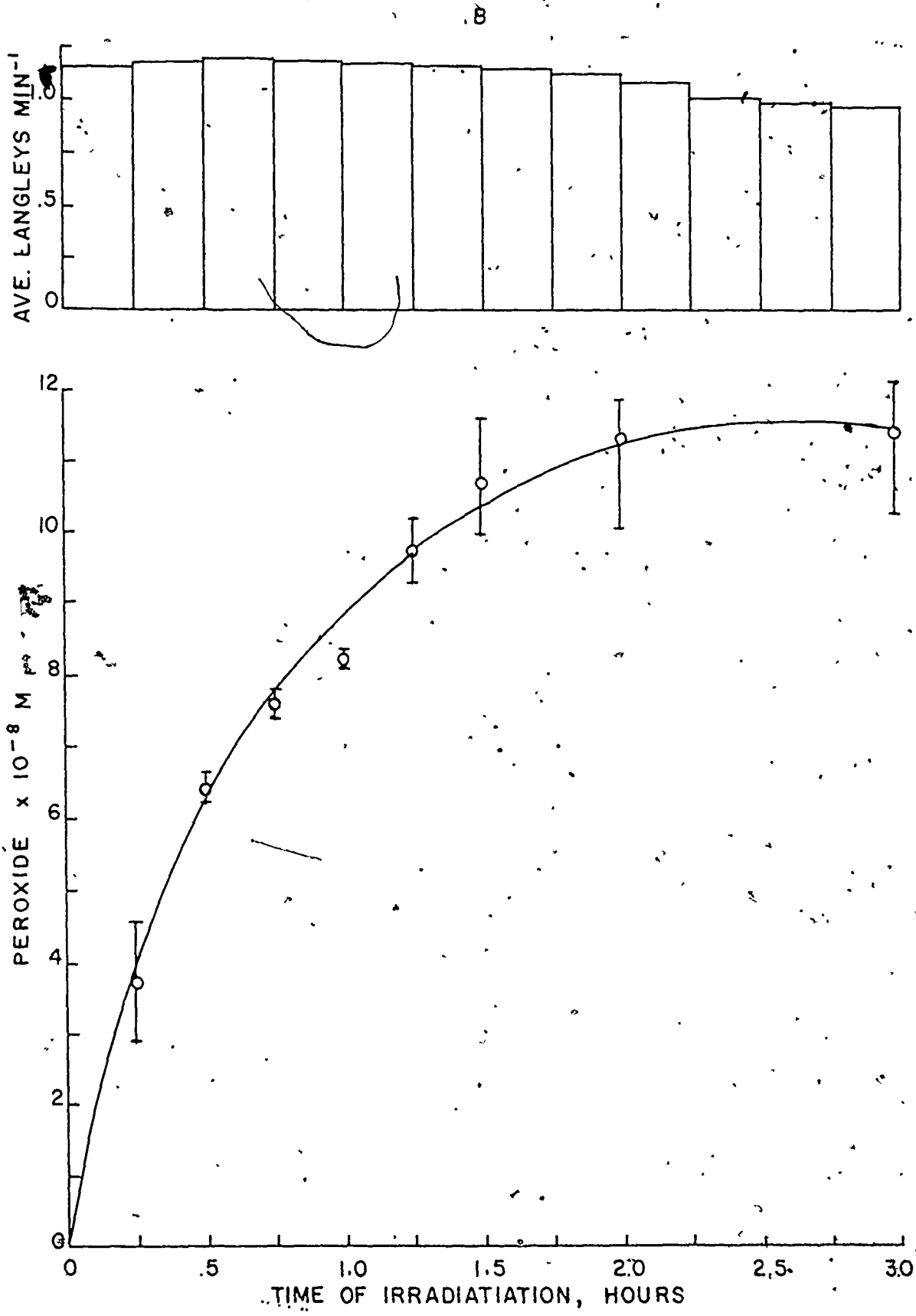

B
AVE. LANGLEYS MIN⁻¹
1.0
.5
0
PEROXIDE x 10⁻⁸ M
12
10
8
6
4
2
0
0
.5
1.0
1.5
2.0
2.5
3.0
TIME OF IRRADIATIATION, HOURS

Figure 10.9 Peroxide Accumulation in Seawater Irradiated with Artificial Light Sources

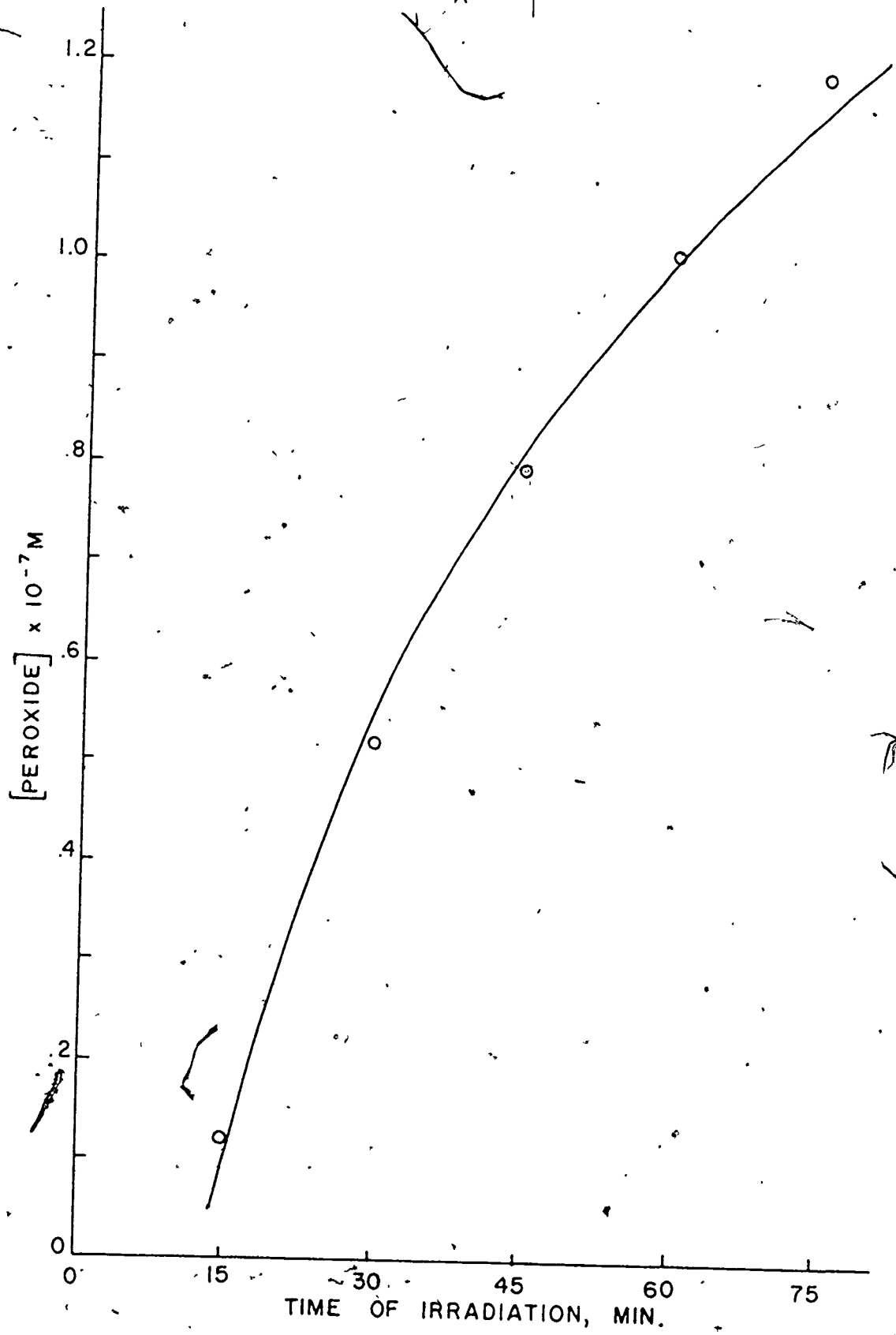
Freshly collected seawater (salinity 31‰) was buffered with borate, filtered, and irradiated at 20°C. Duplicate peroxide determinations were made at the times shown.

A. xenon lamp system (seawater collected in February)

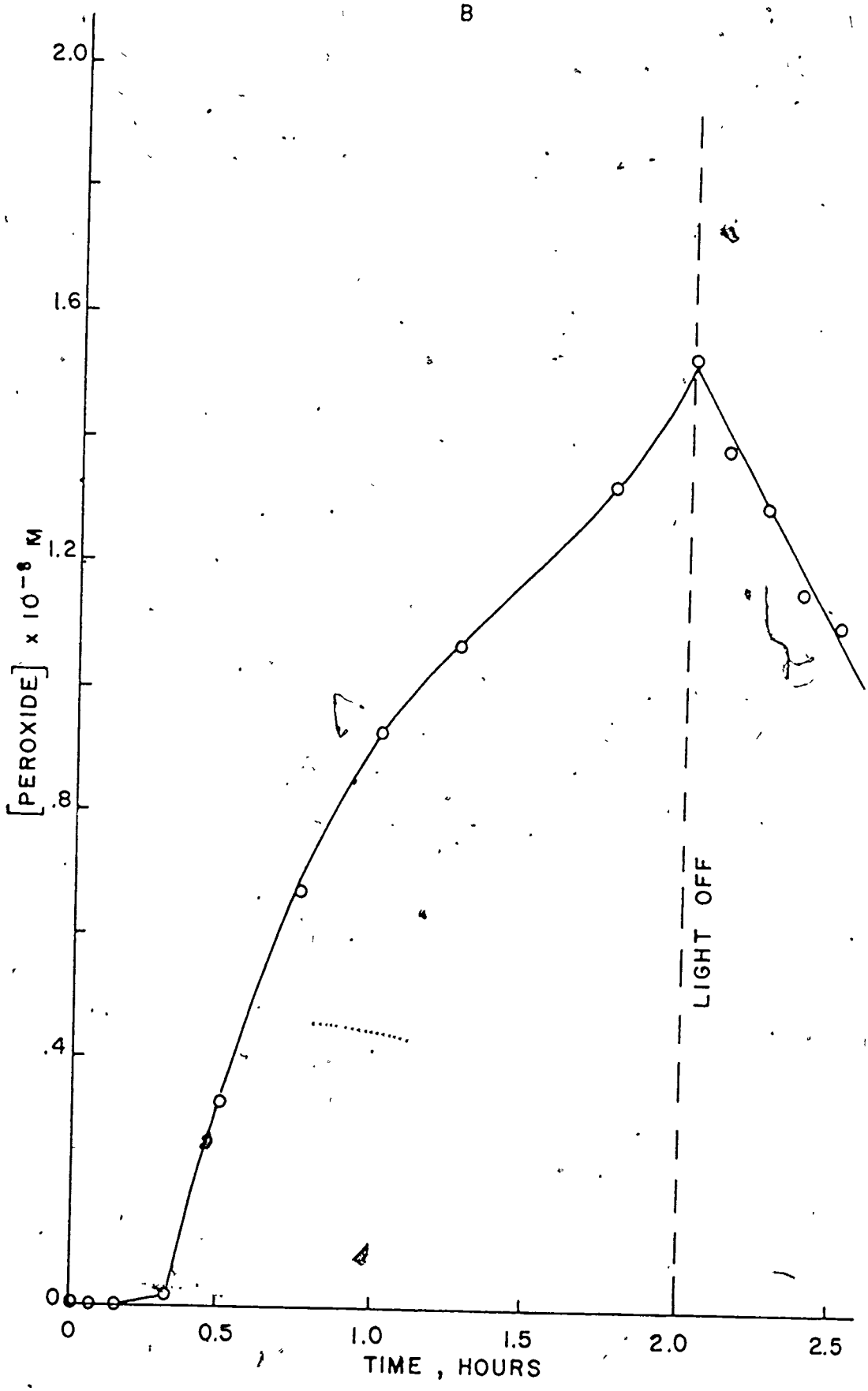
B. merry-go-round system (seawater collected in June)

243

A


1.2
1.0
.8
.6
.4
.2
0
[PEROXIDE] x 10⁻⁷M
0
15
30
45
60
75
TIME OF IRRADIATION, MIN.

B


2.0
1.6
1.2
.8
.4
0
[PEROXIDE] x 10⁻⁸ M
LIGHT OFF
0
0.5
1.0
1.5
2.0
2.5
TIME , HOURS

peroxide formed by added photosensitizers, a significant dark decay rate (k_D) is again observed for peroxides formed during the irradiation of natural seawater (Figure 10.9b). The decay is virtually instantaneous upon the addition of a few micrograms of catalase to seawater containing added H_2O_2 or seawater containing peroxides formed from the substances shown in Table 10.1a. Organic peroxides are either unaffected or are destroyed slowly by catalase (Baldwin, 1957), while the second order rate constant for H_2O_2 decomposition is approximately 10^7 M^{-1} sec^{-1} (Bonnichsen et al., 1947). This suggests that the peroxide which is being measured is H_2O_2, but it does not rule out the possibility that other organic peroxides are present which are not detected in the peroxidase analysis.

10.2.3. Characteristics of photosensitizers in seawater

10.2.3.1. Light stability

An effect of the continuous illumination of seawater was a decrease in the solution absorbance at all wavelengths (Table 6.1). To examine the possibility that there was a concomitant loss of peroxide formation, seawater samples which had been irradiated for different durations were tested by measuring the peroxide accumulation when re-irradiated for a fixed time. The solutions were given 48 hours to re-equilibrate before the second irradiation period was initiated. Peroxide concentrations were compared to solution absorbance (at 310 nm), and fluorescence emission intensity measured (at 490 nm) immediately before the second.

irradiation period (Figure 10.10). These short periods of irradiation reduced the fluorescence and peroxide yield far more than the absorbance, indicating that fluorescence might be used as an indicator for determining the potential of seawater samples to promote certain reactions.

10.2.3.2. Molecular weight range

To estimate the molecular weight of photosensitizing agents, seawater was ultrafiltered using an Amicon UM-2 Diaflo membrane which has a nominal molecular weight cut-off of 1000. The efficiency of the retention of light absorbing components was found to be highly sensitive to the ratio of filtrate flow rate to spiral channel flow rate (Section 5.8); at higher ratios, less retention was observed (Figure 10.11). The reasons for this marked difference are not clear, but it suggests that much of the material involved is not ideally suited to molecular weight fractionations of this type. It does, however, appear that the larger fraction of light absorbing material is of relatively low molecular weight. This is consistent with the observation that the major fraction of humic substances in coastal waters is present in the molecular weight range below 700 (Prakash et al., 1972; Stuermer and Harvey, 1974). Wheeler (1976), however, found that the < 1000 molecular weight fraction never constituted more than 30% of the total visible light absorbing species and averaged about 10% for all areas studied. The difference between Wheeler's results and those presented here may be real or it may be an artifact of the methods or Amicon system used (i.e., stirred verses spiral flow channel). It would seem that results are highly dependent on the conditions chosen. Ogura

Figure 10.10 Light Stability of Fluorescence and Peroxide Forming Entities in Seawater

Freshly collected seawater (February) was buffered with borate, filtered, and irradiated in merry-go-round system for periods of ½, 1, 2, 4, and 7 hours. Fluorescence (Excitation λ 380 nm, Emission λ 490 nm) and absorbance (310 nm in 10 cm cell) measurements were obtained for each solution. After standing at 0°C for 48 hours the samples were re-irradiated for a period of 1 hour in the merry-go-round system and the peroxide concentration was determined for each sample.

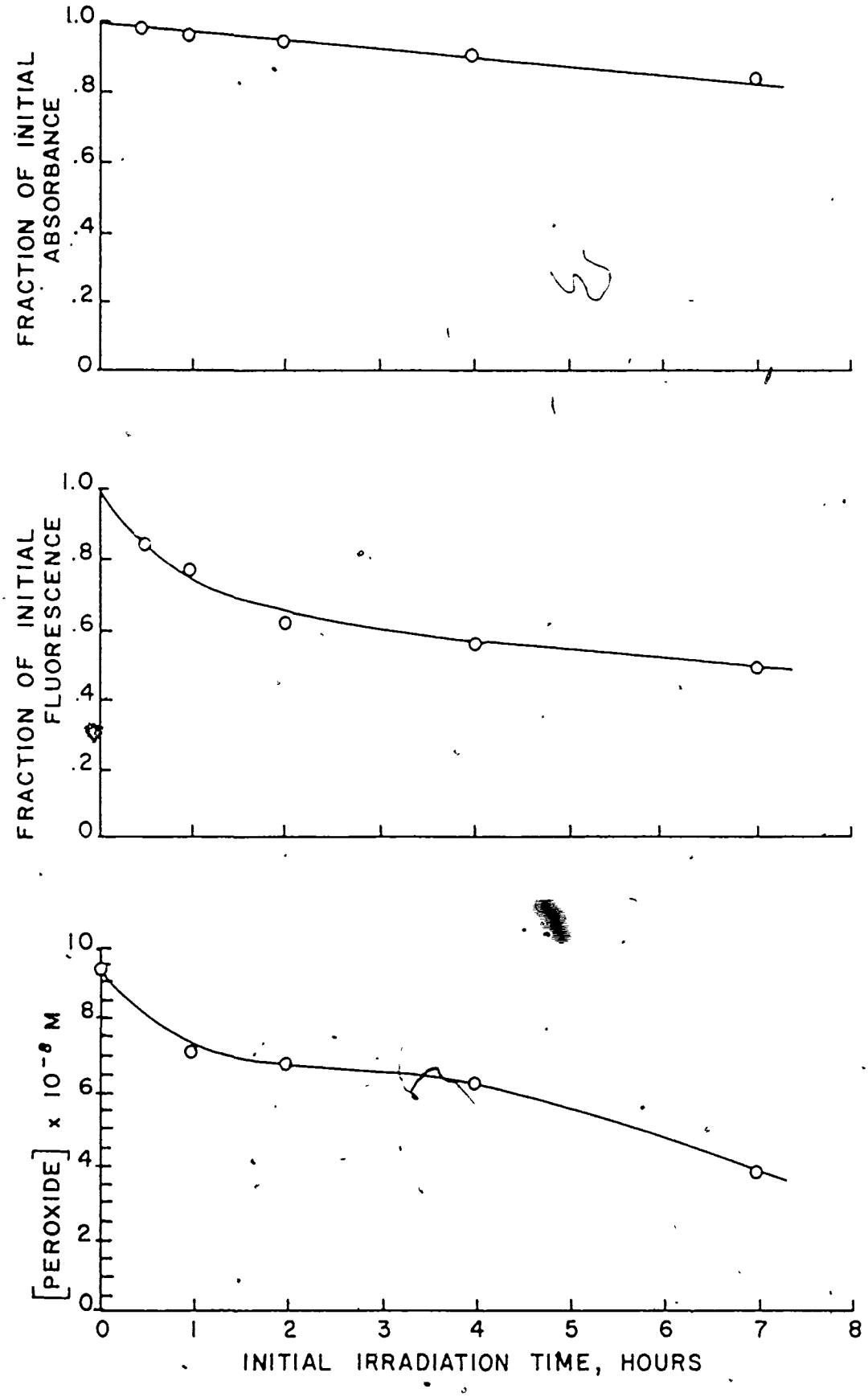

FRACTION OF INITIAL ABSORBANCE
1.0
.8
.6
.4
.2
0
FRACTION OF INITIAL FLUORESCENCE
1.0
.8
.6
.4
.2
0
[PEROXIDE] x 10⁻⁶ M
10
8
6
4
2
0
0
1
2
3
4
5
6
7
8
INITIAL IRRADIATION TIME, HOURS

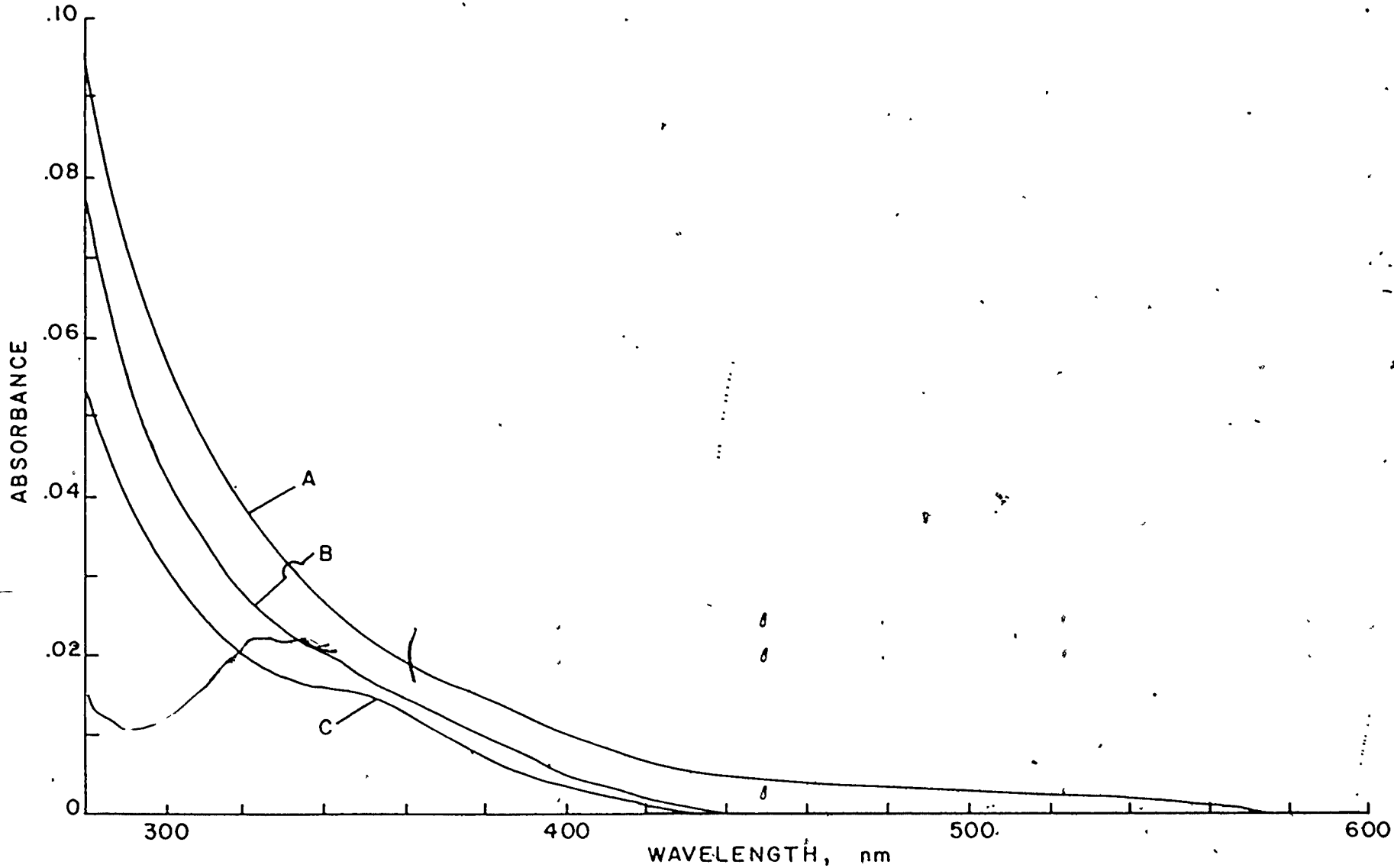
Figure 10.11 Absorption Spectra for GFC Filtered and Ultrafiltered Seawater

All spectra were determined with 10 cm pathlength and against distilled water.

A. GFC filtered only

B. UM-2 filtered with 0.5 ratio of filter flow rate to spiral channel flow rate

C. same as B with flow rate of one


.10
.08
.06
.04
.02
0
ABSORBANCE
A
B
C
300
400
500
600
WAVELENGTH, nm

(1974) points out that fractionation procedures of this kind should always be conducted under the same experimental conditions, because decreased retention will occur for some materials as concentration of organic matter in the concentrate increases. The accuracy of molecular weight determinations by such methods is certainly questionable because calibrations are done with specific compounds which may in no way resemble the molecular dimensions or other properties of natural DOC. Schnitzer (1972) has elaborated on the difficulties of using gel chromatography to measure the molecular weight of humic materials, which give molecular weights that are 2 to 10 times higher than those determined by other methods, and suggests that calibration with well characterized humic fractions would overcome many of these difficulties.

The actual molecular weight cut-off for organic material in seawater may be in question, but fractionation using these filters should, nevertheless, provide a means of semi-selective removal of organic materials without appreciably altering other properties of the solution. With this in mind, samples of seawater were ultrafiltered and then compared to the same seawater without ultrafiltration by measuring specific characteristic photochemical properties of each under identical conditions (Table 10.2). The results show that the photoreactive constituents are removed to a large extent by ultrafiltration, but that an appreciable fraction of what is probably relatively low molecular weight material is not retained by the UM-2 Filter.

10.2.4. Photoreactivity indicators

Besides amino acids, there are many other compounds which can be

Table 10.2 Changes in Some Characteristics of Seawater by Ultrafiltration

[1] This represents the average fractional change of absorbance for wavelengths $\geq$ 300 nm.

[2] Relative rate constants determined over 2 hour irradiation time in merry-go-round system. The added methionine concentration was 5×10^{-6} M l^{-1}.

[3] Relative rate constants determined over 1 hour irradiation in merry-go-round system.

[4] See Section 10.2.4.2.

[5] A, B and C represent same seawater that was used for data in Figure 10.1.

	$\frac{\Delta Abs}{Abs}$ [1]	Fluorescence Intensity	DOC mg C l^{-}	Methionine [2] Reaction, k_1, sec^{-1}	Peroxide [3] Formation, nM l^{-1} hr^{-1}	Epinephrine [4] Indicator, R
GFC Filtered Only, A[5]	0	4.00	.85±.08	2.11±.04x10^{-}	250±4	22.0±0.3
UM2 Ultrafiltered, B[5]	.38	2.46	.72±.05	1.74±.04x10^{-}	211±2	--
UM2 Ultrafiltered, C[5]	.45	1.54	.46±.03	1.06±.04x10^{-}	156±2	16.7±1.5

used as photoreactivity indicators for seawater. Some of these are reactive enough to scavenge quantitatively short-lived radicals like O_2^- when they are generated in radiolysis, photochemical, or dark reactions (Bors et al., 1974). For some of these compounds the reaction rates can be monitored by direct photometric analysis of the reaction solutions. This offers a distinct advantage over the much slower amino acid analysis, but also introduces a new problem in that the absorption spectra for these compounds (Figure 10.12) or their products extends into the near-ultraviolet or visible region. Inner filter effects or reactions initiated by direct photolysis of the indicator reagents, therefore, become a concern. This problem can be alleviated to some extent by using careful controls and limiting radiation wavelengths to certain regions. NBT, for instance, might be useful for wavelengths greater than 400 nm, while epinephrine might be useful at wavelengths greater than 300 nm.

The possible usefulness of such indicating reagents can only be ascertained by thoroughly studying their photochemical behavior in seawater solutions. A cursory look at the characteristics of some potential indicators of seawater photoreactions follows in the next few sections.

10.2.4.1. NBT

Since polychromatic radiation of > 300 nm was used, NBT had limited application because it attenuates radiation of < 400 nm to large extent (Figure 10.12B). Its application in detecting O_2^- resulting

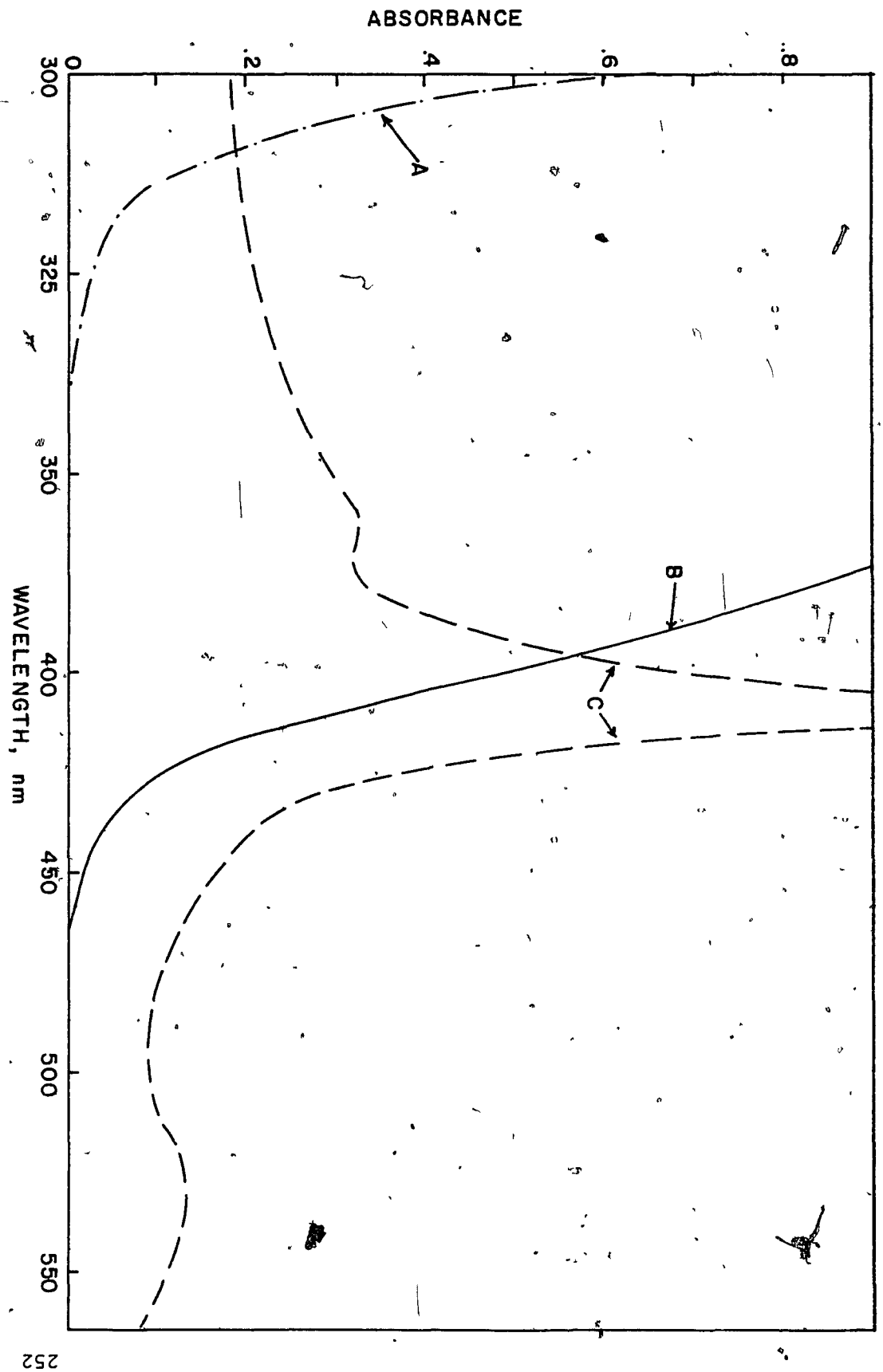
Figure 10.12 Absorption Spectra of Epinephrine, NBT and Fe^{3+} Cytochrome c in Seawater.

All spectra were measured using a 10 cm pathlength. The solutions were all adjusted to pH 8.1 with borate buffer.

A. 1×10^{-4} M l^{-1} of epinephrine

B. 1×10^{-5} M l^{-1} of NBT

C. 1×10^{-6} M l^{-1} of Fe^{+} cytochrome c


ABSORBANCE
0
.2
.4
.6
.8
300
325
350
400
450
500
550
WAVELENGTH, nm
A
B
C

252

253

from reactions at longer wavelengths was tested in 1×10^{-6} M riboflavin seawater solutions containing sufficient methionine to generate O_2^- by the D-R pathway. This reaction required 1×10^{-5} M l^{-1} of NBT to give sufficient color development, indicating that it would probably not serve as a good method for detecting the expected lower levels of O_2^- generated in natural seawater.

Application of this method to natural seawater samples did give positive results, but the yields of blue formazan dye were too low to warrant pursuing the method further.

10.2.4.2. Epinephrine

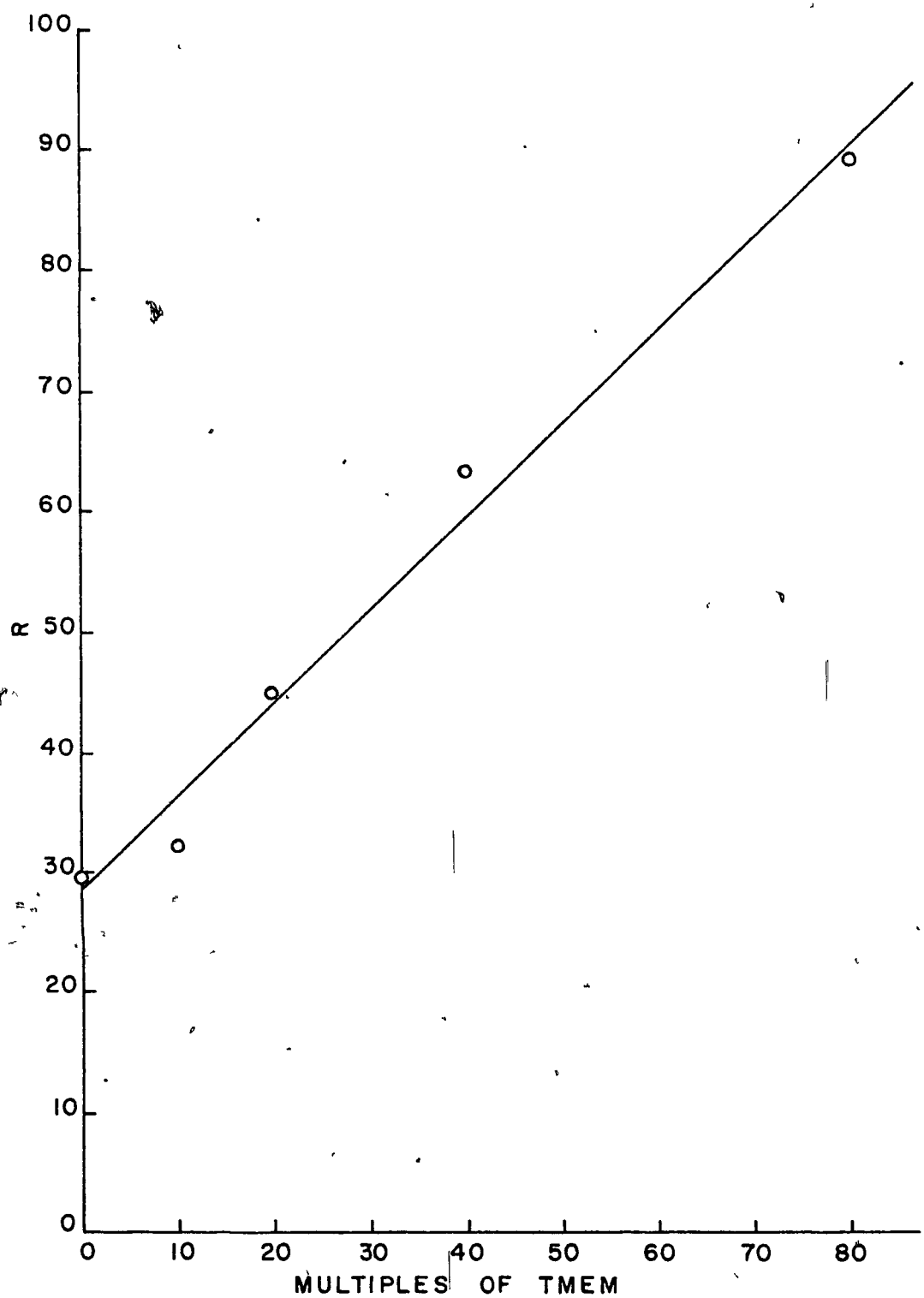
Epinephrine is readily oxidized by weak oxidizing agents to give adrenochrome (Figure 10.3a). This reaction has been used as a sensitive method for detecting O_2^- in aqueous solutions (Misra and Fridovich, 1972) and conceivably could be used to test for the occurrence of this and perhaps other short-lived oxidants in seawater. Interpreting the stoichiometry of such reactions is complicated by an augmentation of rate of autoxidation, which has been explained by the catalytic effect of accumulated adrenochrome (Trautner and Bradley, 1951). Autoxidation can apparently be initiated by trace concentrations of various transition metals, and the result of increasing TMEM concentration is a concomitant increase in the rate of color development in irradiated seawater solutions containing epinephrine (Figure 10.13). This does not constitute a significant problem in seawater, where transition metal concentrations are normally relatively invariant, but in artificial

Figure 10.13 Effect of Transition Metals on

Light Initiated Epinephrine Oxidation in Seawater

The experimental procedure is described in Appendix 2, Section IX. The value R is proportional to solution absorbance at 485 nm and is defined in Appendix 2, Section IX.

254


100
90
80
70
60
50
40
30
20
10
0
R
0
10
20
30
40
50
60
70
80
MULTIPLES OF TMEM

255

seawater solutions the concentration of some transition metals is high enough to be a major interference. For this reason, artificial seawater was not used as a control; instead, Super Q water was used.

Deionized water may still give a control blank which is too large, for the rate of autoxidation is salinity dependent and proceeds about twice as fast at 0‰ salinity as it does at 35‰ salinity (Figure 10.14). In an attempt to overcome the problem of salinity dependence and metal contamination, artificial seawater was passed through purified Chelex 100 resin. This purification step resulted in a 94% decrease in color development (Figure 10.15). Analysis of the Chelex 100 treated artificial seawater revealed that the concentrations of the metals Fe, Mn, Cu, and Zn were reduced by 95% or greater. The decrease in color development, however, could in part be due to impurities introduced in the Chelex 100 treatment. These can include amines, NO_2^-, and NO_3^-; of these NO_2^- was found to be a strong inhibitor of color development in irradiated epinephrine solutions (Figure 10.15).

The difficulties encountered in obtaining an accurate control blank also apply to the medium in which the reference standard was prepared, and again Super Q water was used. Reference standard, here, refers to a specific photosensitizer added at some known concentration to a solution containing the same concentration of epinephrine, and buffered at the same pH as the sample. Both the sample and reference standard solution were irradiated under the same conditions and the ratio of the absorbance change (100 x sample/reference standard) for a set exposure time gave a comparison index which was designated R (see

Figure 10.14 Effect of Salinity on Light Initiated Epinephrine Oxidation Induced by 1×10^{-7} M l^{-1} of Riboflavin

For method and definition of R see Appendix 2, Section IX.

256

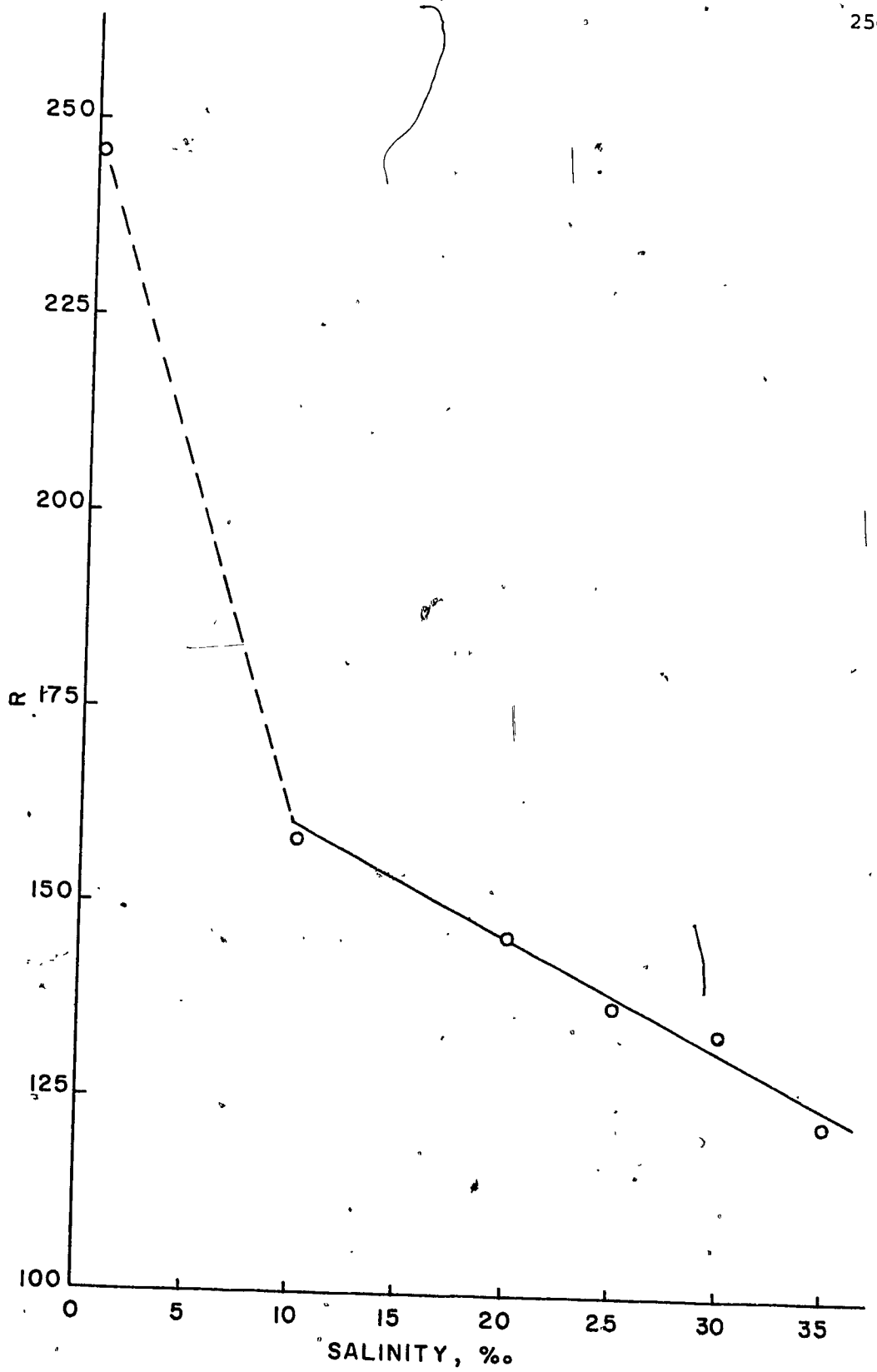


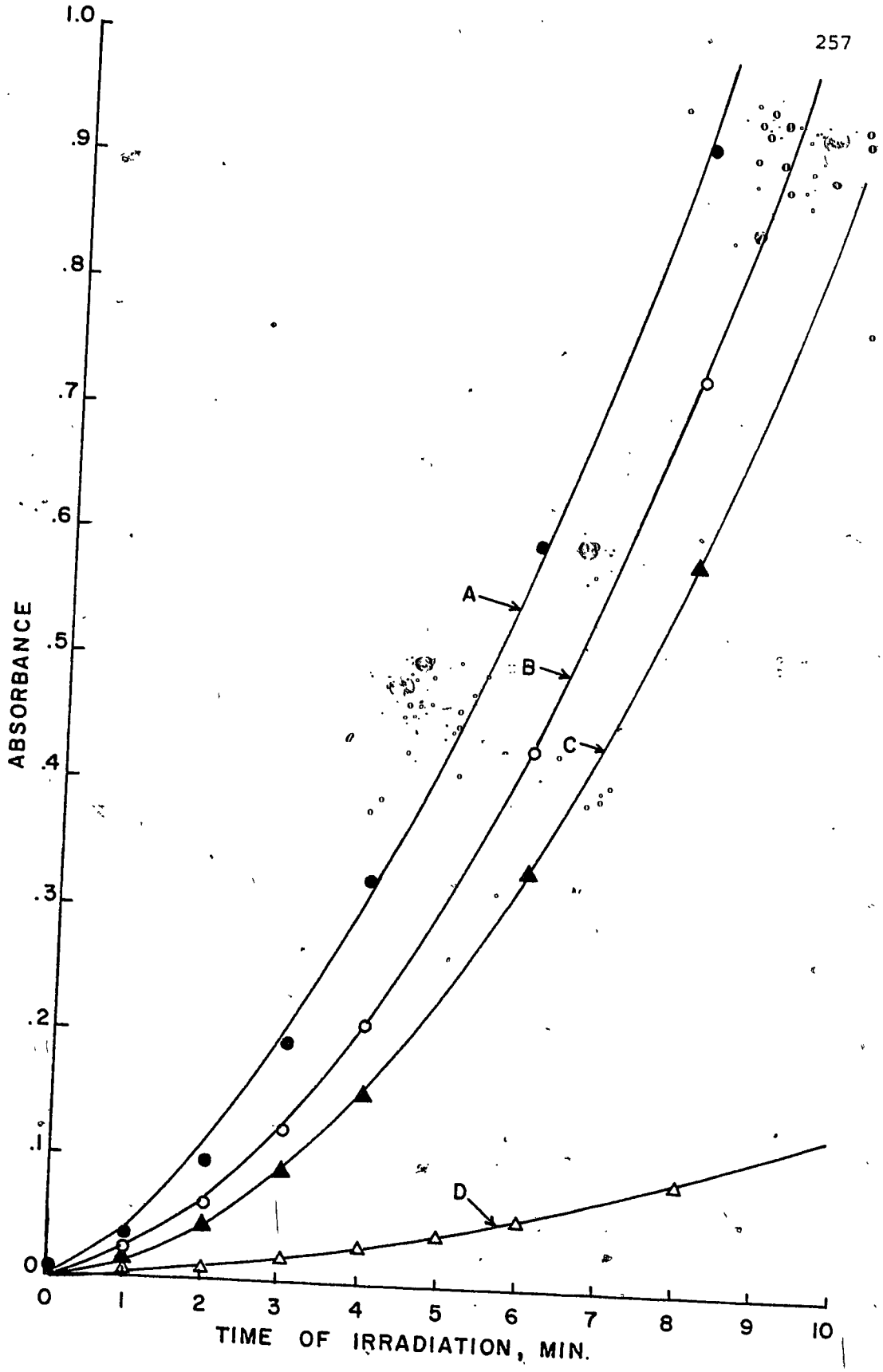
Figure 10.15 Effect of NO_2^- in Seawater and Impurities in Salts Used to Prepare Artificial Seawater on Epinephrine Oxidation

A. seawater

B. seawater with an added 5×10^{-6} M l^{-1} of NO_2^-

C. artificial seawater

D. artificial seawater which was passed through purified Chelex 100 resin


257
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
ABSORBANCE
A
B
C
D
0
1
2
3
4
5
6
7
8
9
10
TIME OF IRRADIATION, MIN.

258

Appendix 2, Section IX). This value should only be used in comparing samples irradiated with the same light source, or sources with the same spectral energy distribution, since it is highly unlikely that the photochemically active spectral region of the reference photosensitizer and seawater will be the same. It is also best to use a reference standard whose absorption maximum is somewhat removed from the absorption maximum for adrenochrome (485 nm); otherwise inner filter effects can be very significant. Methylene blue with absorption maxima at 668 nm and 609 nm was used as the reference standard. The photochemically initiated color development in solutions of epinephrine was linear to at least 10 nM l^{-1} of methylene blue (Figure 10.16).

The photoinduced color development in epinephrine-seawater solutions was common to riboflavin, fulvic acid (Figure 10.17 a,b) and the phloroglucinol-based polymer. It was also observed for seawater diluted with stream water (Figure 10.17c). In all of these solutions the value of R was substantially decreased by adding NO_2^-, N_3^-, or I^-, or by removing dissolved O_2 by sparging with O_2-free N_2 gas. Quenching by N_3^- and I^- and a substantial reduction in R by removing O_2 suggested that $^1O_2$ might be the reactive species. However, exposure of epinephrine to $^1O_2$, generated from the reaction of H_2O_2 and NaOCl (Debey and Douzou, 1970), gave a negligible increase in absorbance. The reaction was also not induced by Br-radicals, since no significant increase in color development was noted for irradiated $Br^- + NO_2^-$ solutions. Nor was the reaction induced by O_2^- or added H_2O_2. The possible involvement of O_2^- was tested by adding superoxide dismutase, which catalyzes

Figure 10.16 Color Development in Irradiated

Epinephrine Solutions Verses Methylene Blue Concentration

259

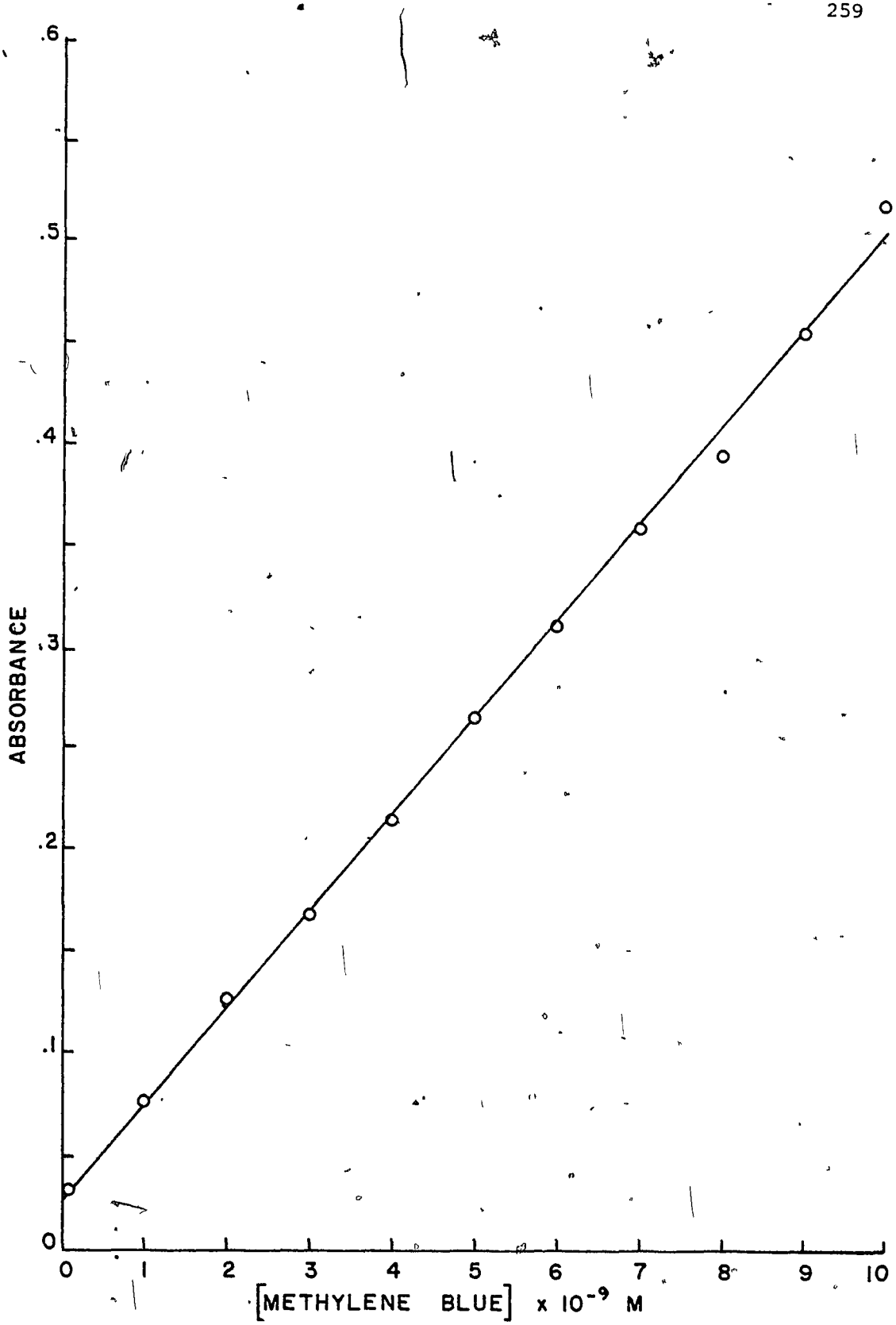


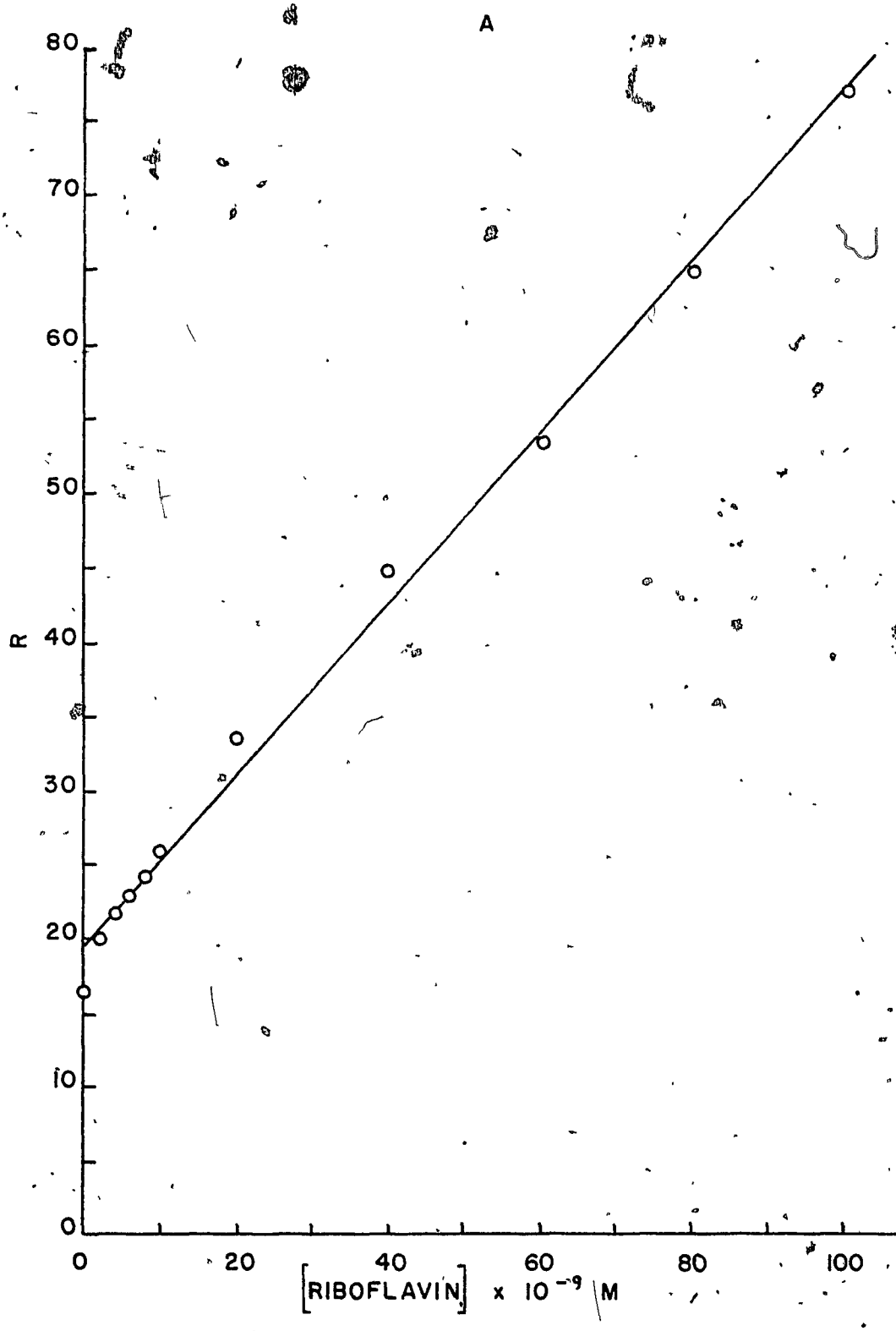
Figure 10.17 Color Development (R) in Irradiated Epinephrine-Seawater Solutions Verses Concentration of Riboflavin, Fulvic Acid, and Stream Water

A. riboflavin

B. fulvic acid (M.W. of 950 was used)

C. stream water from Nova Scotia's South Shore Area

260



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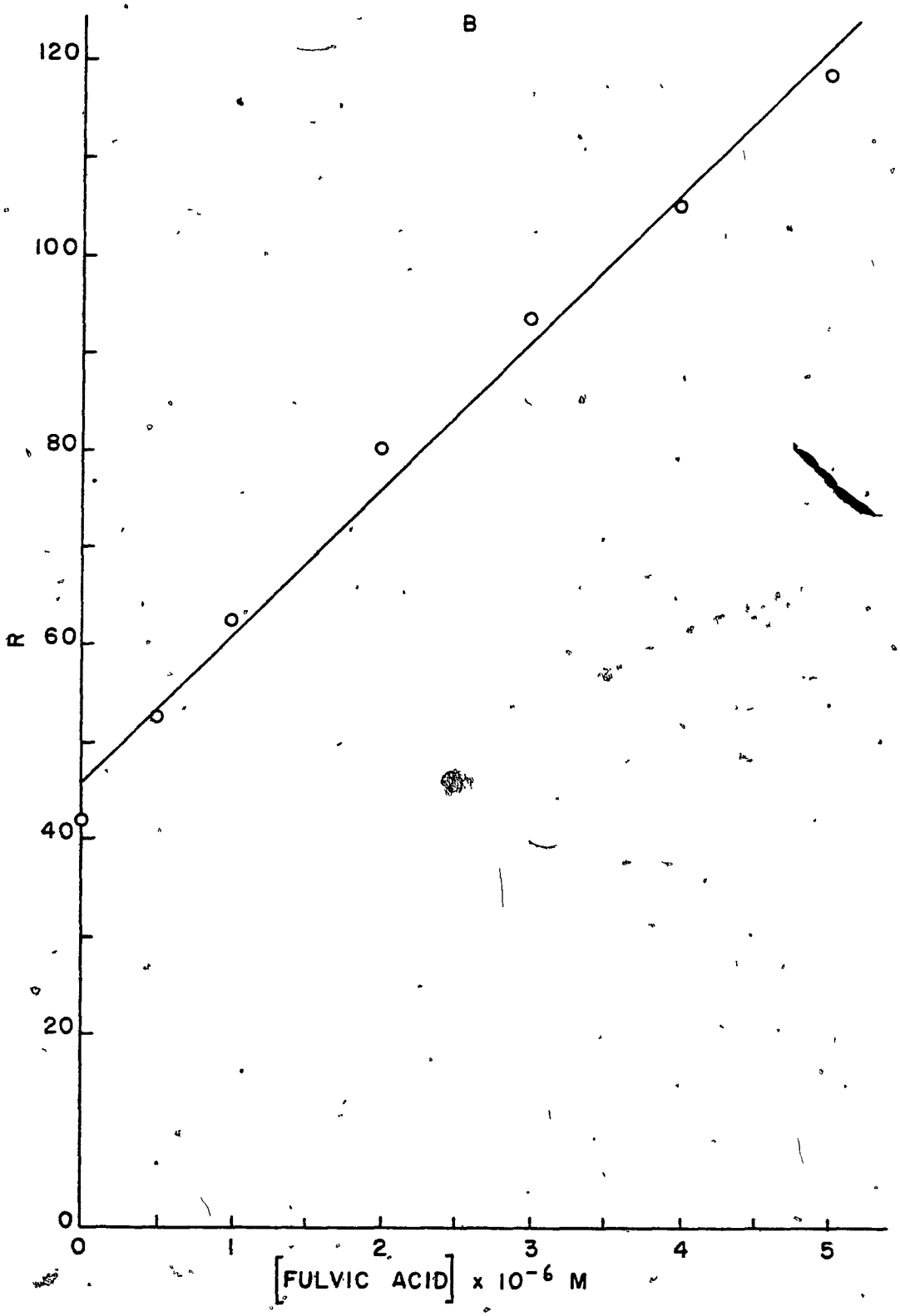
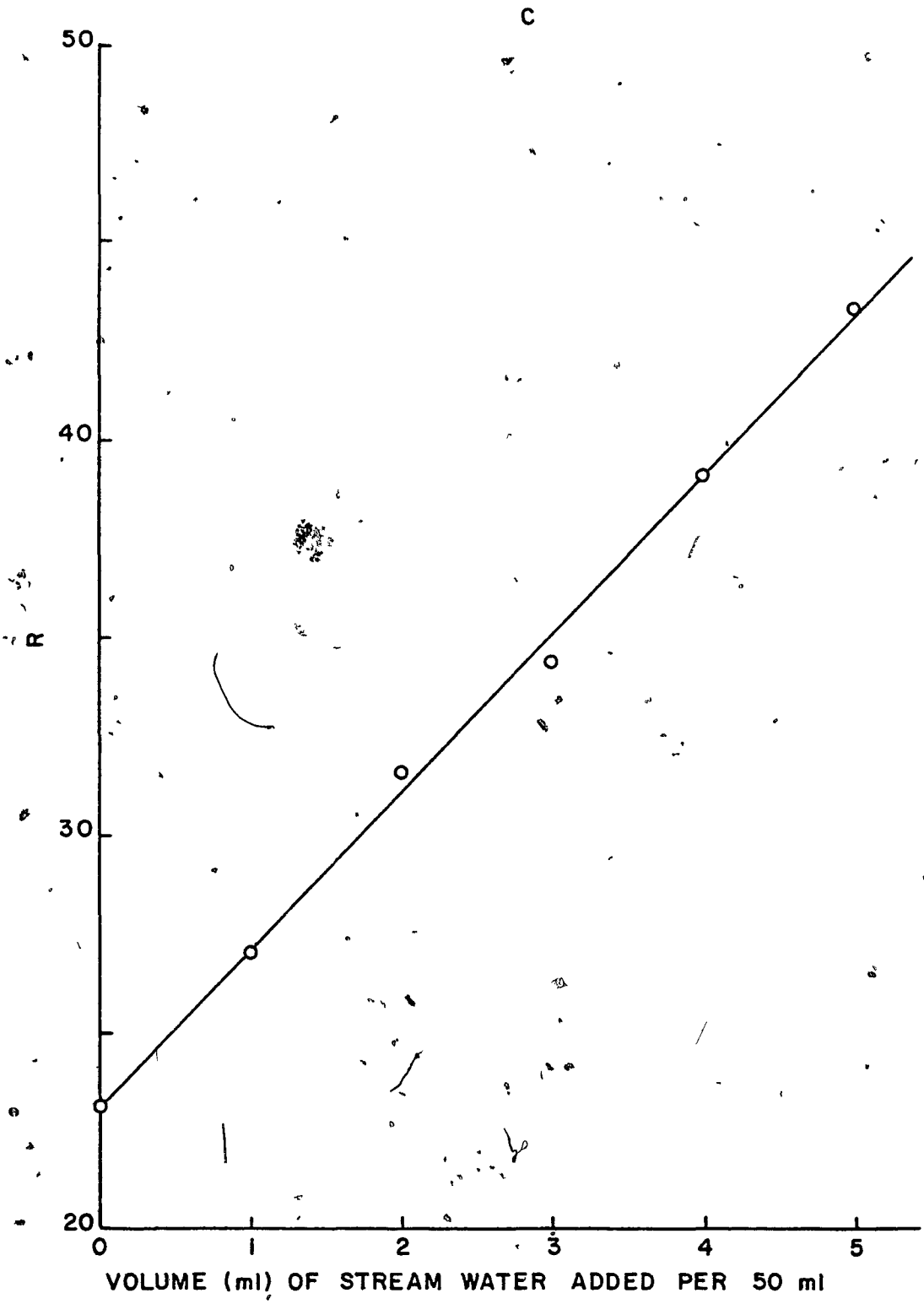


262



the disproportionation of O_2^-. When added at 1000 μg l^{-1}, superoxide dismutase had no effect on the riboflavin-induced reaction with epinephrine. Also, the addition of methionine at 1000 times the concentration of riboflavin (1×10^{-6} M) should have significantly increased R by giving larger yields of O_2^-; instead, the value of R decreased. These observations are in accordance with those of Misra and Fridovich (1972), who found that O_2^- was apparently not involved in the spontaneous autoxidation of epinephrine at pH 8.5 and below, but was at higher pH values. However, co-oxidation of epinephrine by xanthine oxidase (an O_2^- source) at pH 7.8 was inhibited by superoxide dismutase, indicating that O_2^- is a potential reactant with epinephrine at near neutral pH values. Misra and Fridovich (1972) proposed that two reaction pathways exist. One involves O_2^- as the chain-propagating species (Eqs. 10.39-10.42) and the other involves disproportionation

$$RH_4 + O_2^- + H^+ \longrightarrow RH_3\cdot + H_2O_2 \qquad (10.39)$$

$$RH_3\cdot + O_2 \longrightarrow RH_2 + O_2^- + H^+ \qquad (10.40)$$

$$RH_2 + O_2^- + H^+ \longrightarrow RH\cdot + H_2O_2 \qquad (10.41)$$

$$RH\cdot + O_2 \longrightarrow R + O_2^- + H^+ \qquad (10.42)$$

where RH_4 represents epinephrine

R represents adrenochrome

reactions of partially oxidized epinephrine intermediates (Eqs. 10.43-10.46). In seawater solutions containing relatively high concentrations

$$RH_4 + {}^3sens \longrightarrow RH_3\cdot + sens_{red} \qquad (10.43)$$

$$RH_3\cdot + RH_3\cdot \longrightarrow RH_2 + RH_4 \qquad (10.44)$$

264

$$RH_3\cdot + RH_2 \longrightarrow RH\cdot + RH_4 \qquad (10.45)$$

$$RH\cdot + RH\cdot \longrightarrow R + RH_2 \qquad (10.46)$$

of photosensitizers, the levels of epinephrine free radicals may be maintained by the D-R mechanism (Eq. 10.43) at sufficiently high levels for disproportionation reactions to predominate. Epinephrine should be readily oxidized by ^{3}sens, and at 1×10^{-4} M l^{-1} it should compete successfully with dissolved O_2 for the sensitizer. The observed decrease in reaction rate when O_2 was removed might be explained by its role in regenerating the sensitizer (Eq. 10.47), and hence impeding reactions like 10.48 or simply by accelerating sensitizer regeneration,

$$sens_{red} + O_2 \longrightarrow sens + O_2^- \qquad (10.47)$$

$$sens_{red} + RH_3\cdot \longrightarrow sens + RH_4 \qquad (10.48)$$

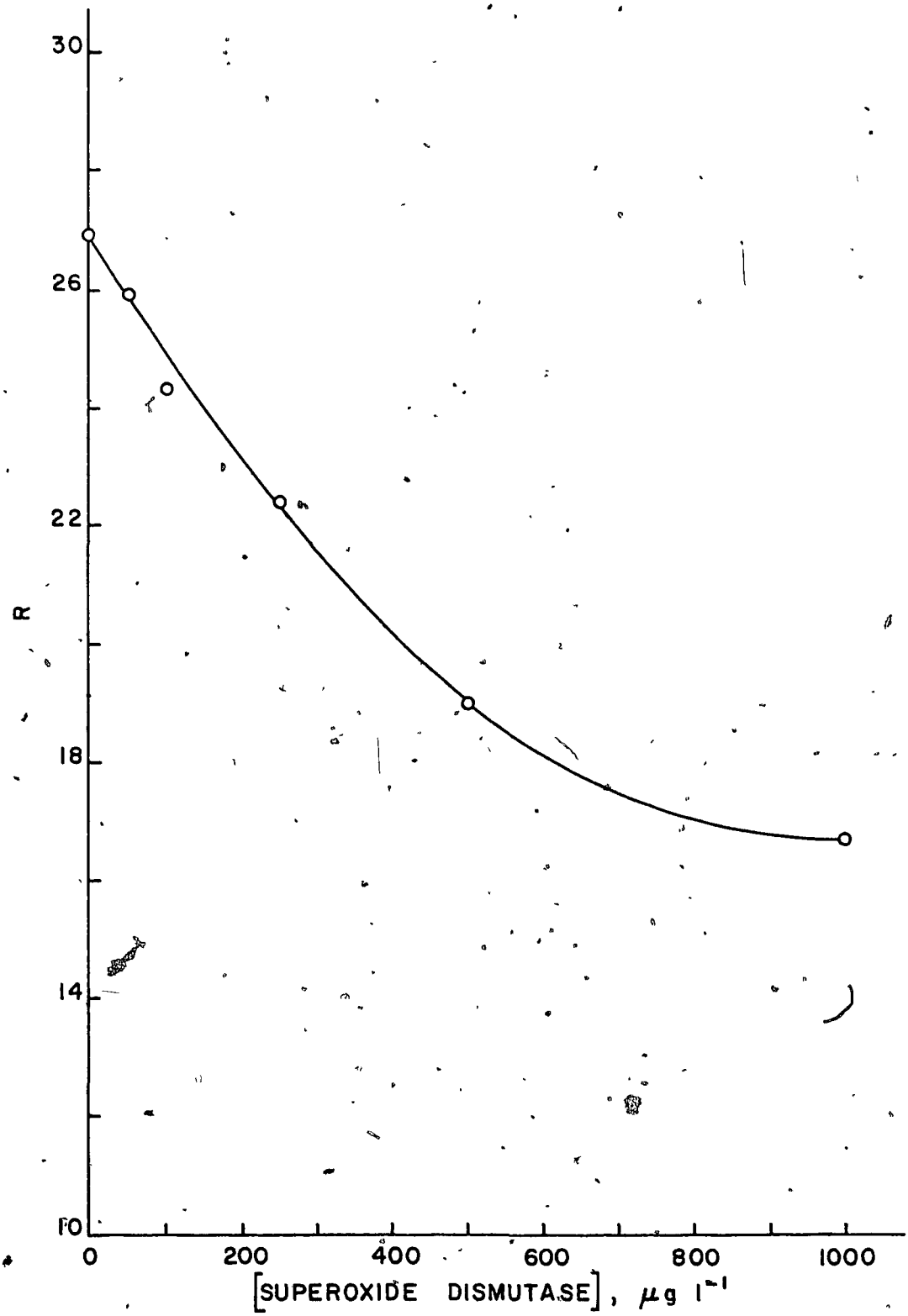
which could be the rate limiting step in the overall reaction.

The use of epinephrine in natural seawater solutions containing no added sensitizers gave R values which were higher than those obtained in artificial seawater or Super Q water. The range for 8 samples, all from the Halifax N.W. Arm or St. Margaret's Bay, was 69.4 to 16.4 with an average of 33.3. For one of these samples, filtered light reactions revealed that 33% of the reaction was occurring in region A, 60% in region B and 7% in region C + D (see Figure 3.7).

As was the case for added sensitizers, N_3^-, NO_2^- and I^- all functioned as inhibitors of color development. However, unlike the results obtained for added sensitizers, superoxide dismutase partially inhibited the reaction (Figure 10.18), and the removal of dissolved O_2 caused an

Figure 10.18 Effect of Superoxide Dismutase on Color Development in Irradiated Seawater Solutions Containing Epinephrine

265


30
26
22
18
14
10
R
0
200
400
600
800
1000
[SUPEROXIDE DISMUTASE], μg l⁻¹

increase, rather than a decrease, in reaction rate. These results indicate that the oxidation of epinephrine in seawater is proceeding by both the ^{3}sens (Eqs. 10.43-10.46) and O_2^- (Eqs. 10.39-10.42) pathways. The increase in reaction rate under anoxic conditions implies that O_2 reduces the efficiency of the sensitizer in oxidizing epinephrine. This could be the result of ^{3}sens quenching by O_2 to produce unreactive $^1O_2$, or consumption of some of the O_2^- in other reactions. Therefore, to facilitate the measurement of ^{3}sens entities in seawater, it might be better to use the epinephrine indicator under anaerobic conditions.

10.2.4.3. Fe^{3+} cytochrome c

Fe^{3+} cytochrome c is readily reduced by O_2^- and by e_{aq}^- (Land and Swallow, 1971) with rate constants of 1.1×10^5 M^{-1} sec^{-1} and 2×10^{10} M^{-1} sec^{-1}, respectively. The e_{aq}^- is also scavenged very rapidly by O_2 ($k \approx 2 \times 10^{10}$ $M^{-1}sec^{-1}$), and in air saturated aqueous solutions, the reduction of Fe^{3+} cytochrome c should occur only with longer-lived reducing species unless sufficiently high concentrations of cytochrome c are used. However, the concentration of cytochrome c that can be used in seawater is limited by solubility. Even when cytochrome c was present at 1 μM l^{-1}, turbidity usually developed in less than 1 hour in seawater of greater than 30‰ salinity. The experiment had to be conducted as quickly as possible after the reagent was mixed with seawater, and even then there was evidence from comparisons with deionized water reaction solutions that the effective concentration was lower than that added.

267

To estimate the light initiated reduction of cytochrome c, a dark control must be substracted from the absorbance of the irradiated sample. This corrects for light scattering due to turbidity increases, and also for a dark reduction of Fe^{3+} cytochrome c, which for polyphenolic materials is quite substantial (Figure 10.19). The dark reduction reactions are not impeded by superoxide dismutase at concentrations of up to 1000 $\mu g\ l^{-1}$, indicating the O_2^- is probably not involved in the reaction.

In irradiated solutions of tryptophan, riboflavin, and fulvic acid, the formation of Fe^{2+} cytochrome c was inhibited by the presence of 500 $\mu g\ l^{-1}$ of superoxide dismutase (Figure 10.20 a,b), thus implying that O_2^- is the active reducing agent. This inhibition was not observed for the phloroglucinol-based polymer (Figure 10.20b), yet this was found to be a copious source of peroxide (Section 10.2.2.1.). If the measured peroxide was indeed H_2O_2, then either it is being formed directly by a two-electron reduction of O_2, which seems unlikely, or the superoxide dismutase is not functioning in its usual capacity in the presence of this polymer. The latter possibility could be due to the enzyme's deactivation by the polymer or to the reduction of Fe^{3+} cyctochrome c via a charge transfer complex with the polymer; in which case, O_2^- would not be involved in the reaction.

The nature of NO_2^- and TMEM inhibition of peroxide formation was also investigated using Fe^{3+} cytochrome c to measure yields of O_2^- during irradiation of solutions containing riboflavin, which was used as the source of the radical, and NO_2^-, methionine; or TMEM (Figure

Figure 10.19 Reduction of Fe^{3+} Cytochrome c

in the Dark by the Phloroglucinol-Based Polymer and Fulvic Acid

- ● 200 µg l^{-1} of the phloroglucinol-based polymer in redistilled Super Q water buffered to pH 8.1
- O 1000 µg l^{-1} of fulvic acid in redistilled Super Q water buffered to pH 8.1

268

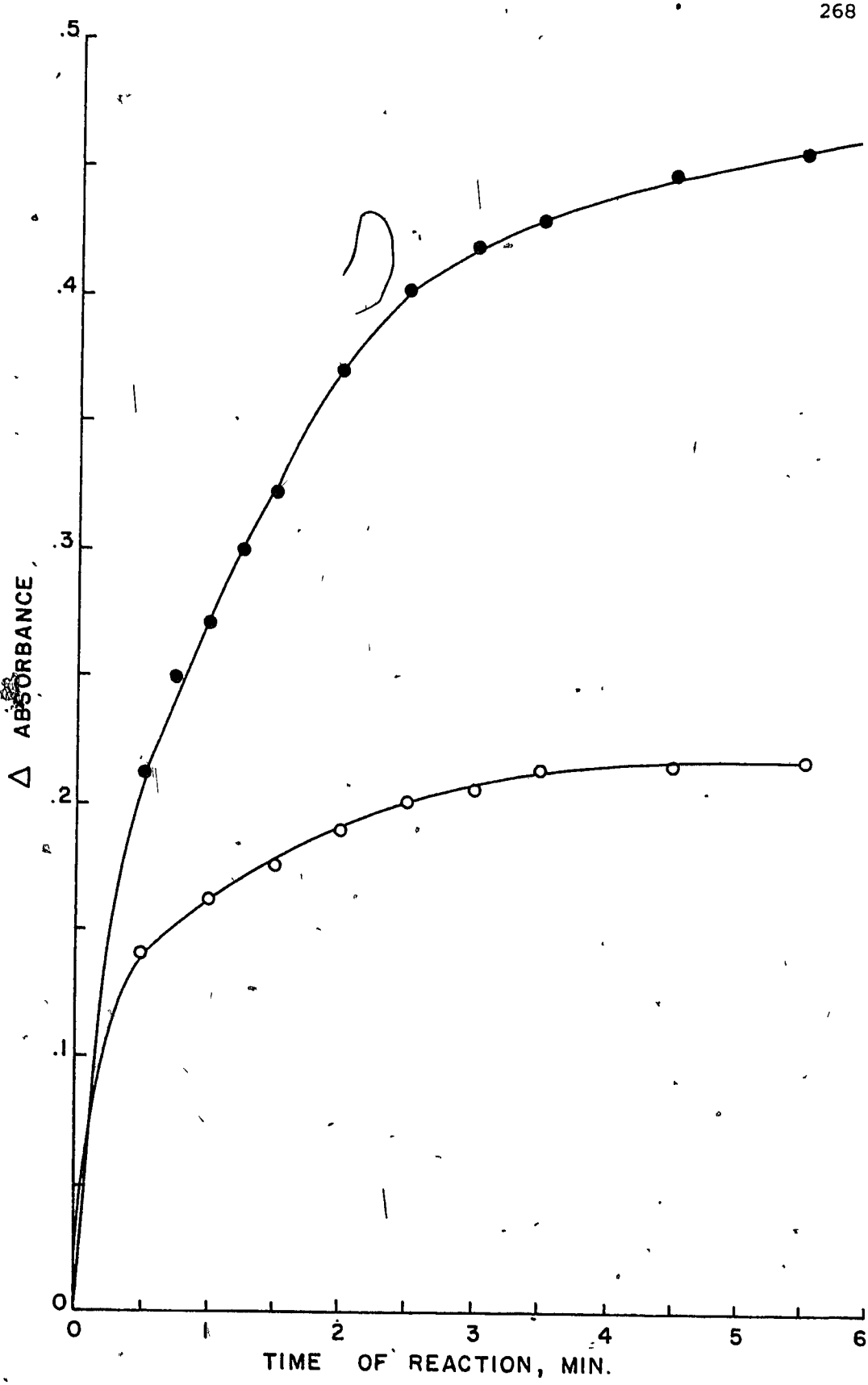


Figure 10.20 Effect of Superoxide Dismutase on Light Initiated Reduction of Fe^{3+} Cytochrome c by Tryptophan, Riboflavin, Phloroglucinol-Based Polymer, and Fulvic Acid

Solutions were prepared in redistilled Super Q water and adjusted to a pH of 8.1. Those solutions to which superoxide dismutase was added contained 500 $\mu g\ l^{-1}$ of the enzyme. All irradiations were conducted in the xenon lamp system.

A. 5×10^{-6} M l^{-1} of tryptophan

B. 1×10^{-7} M l^{-1} of riboflavin

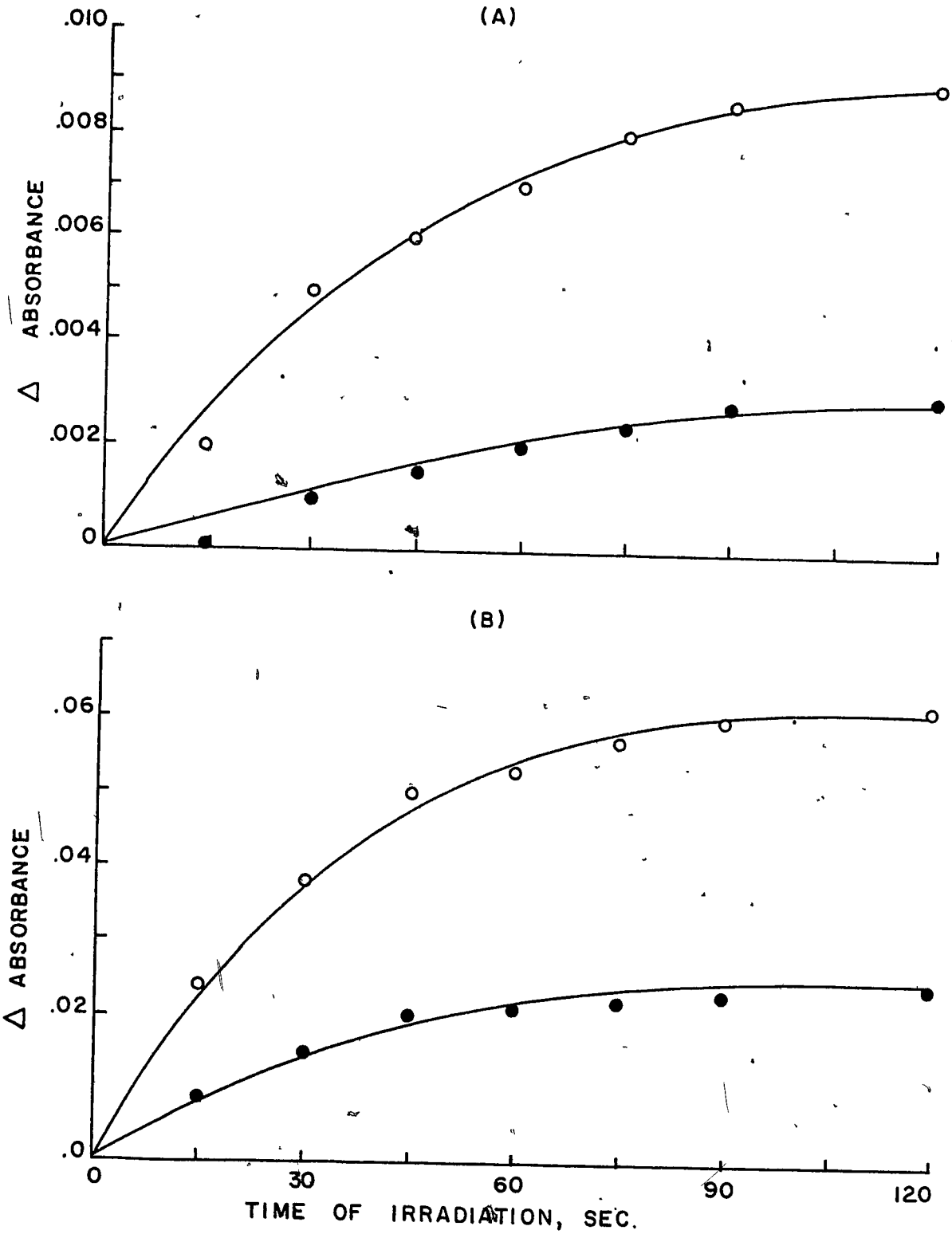
C. 10 $\mu g\ l^{-1}$ of phloroglucinol-based polymer

D. 100 $\mu g\ l^{-1}$ of fulvic acid

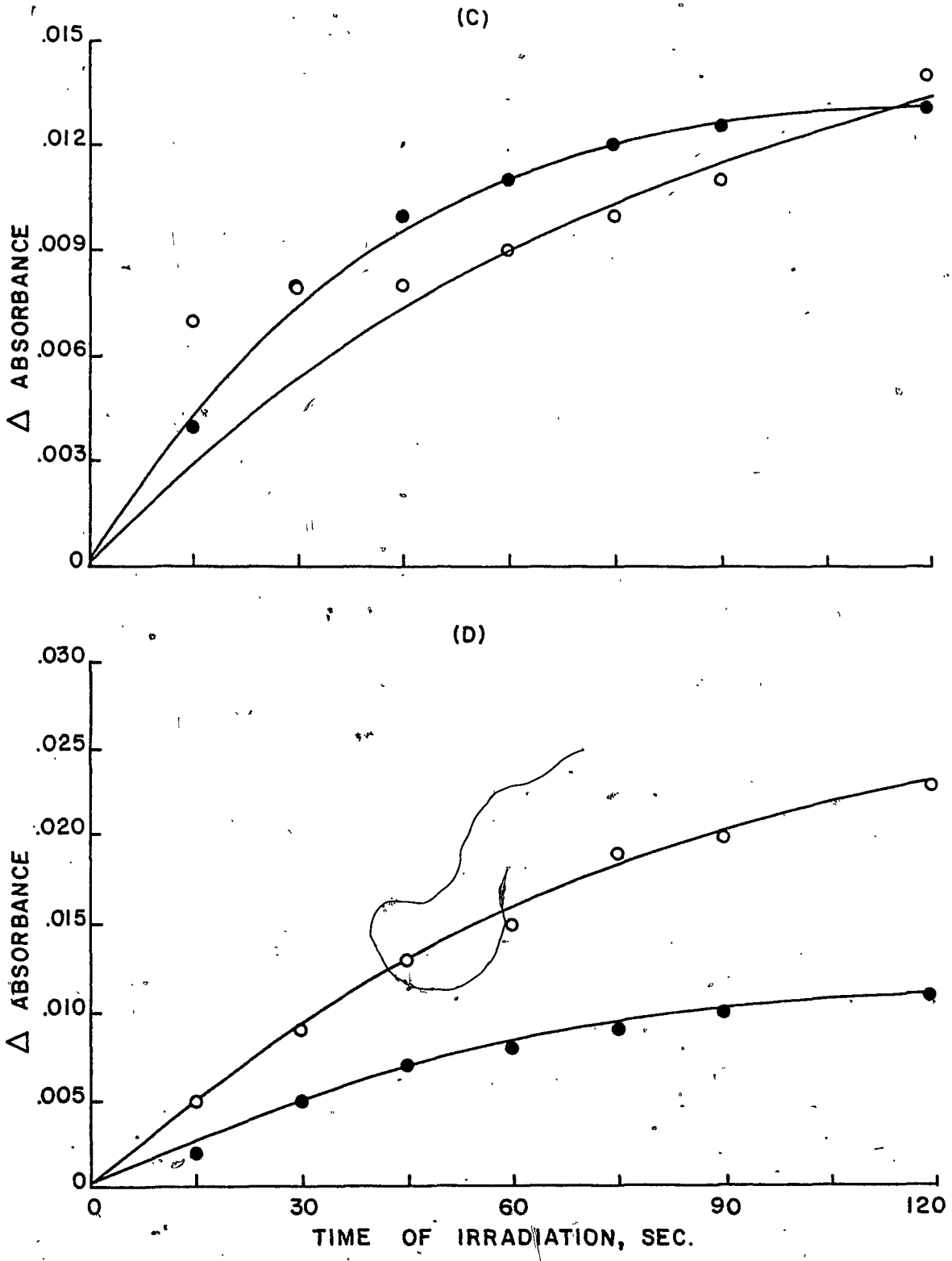
○ without superoxide dismutase

● with superoxide dismutase

269


(A)
.010
.008
.006
.004
.002
0
Δ ABSORBANCE
(B)
.06
.04
.02
.0
0
30
60
90
120
TIME OF IRRADIATION, SEC.

270


(C)
.015
.012
.009
.006
.003
0
Δ ABSORBANCE
(D)
.030
.025
.020
.015
.010
.005
0
Δ ABSORBANCE
0
30
60
90
120
TIME OF IRRADIATION, SEC.

271

10.21). Again, strong inhibition was observed in NO_2^- and in TMEM solutions. The results show that the addition of methionine or methionine plus NO_2^- did not facilitate an increase in O_2^- production. Therefore, the increase in the rate of H_2O_2 formation in their presence would appear to proceed through direct reduction of O_2^- and not via its disproportionation. The O_2^- radical therefore appears to be functioning as an oxidizing agent for reactions where methionine or other easily oxidized organic substrates are present, and probably as a reducing agent for at least some of the transition metal ions present in TMEM.

In seawater, the reduction of Fe^{3+} cytochrome c was observed and the reduction was inhibited by the addition of 500 μg l^{-1} of superoxide dismutase (Figure 10.22). The rate of the reduction was quite rapid in view of the fact that the Xe lamp was used. A rough calculation using ε_{550} of 2.10×10^4 cm^2 mM^{-1} for the reduced minus the oxidized cytochrome c (Massey, 1959) gives a rate of formation for O_2^- of $1\text{-}3 \times 10^{-8}$ M $l^{-1}min^{-1}$. If O_2^- were going completely to H_2O_2 via disproportionation, it would yield $3\text{-}9 \times 10^{-7}$ M $l^{-1}hr^{-1}$ of H_2O_2, which is 3 to 9 times greater than the highest rate of accumulation observed. This calculation is based on an initial rate of O_2^- formation, and might therefore be greatly in error when applied to determining hourly rates. Attempts to quantify results of Fe^{3+} cytochrome c reductions in seawater must also take into account the effective concentration of cytochrome c and the rate of Fe^{3+} cytochrome c oxidation, which occurs through reactions with oxidizing radicals (McCord and Fridovich, 1973).

Figure 10.21 Effect of Methionine, NO_2^-, and TMEM on Fe^{3+} Cytochrome c Reduction Photoinduced by Riboflavin

Solutions were prepared in redistilled Super Q water and adjusted to a pH of 8.1 with borate buffer. Each solution contained 1×10^{-7} M l^{-1} of riboflavin. All solutions were irradiated with the xenon lamp system.

● ———————

○ 5×10^{-6} M l^{-1} of methionine

▲ 5×10^{-6} M l^{-1} of methionine and 5×10^{-6} M l^{-1} of NO_2^-

△ 20 X TMEM

272

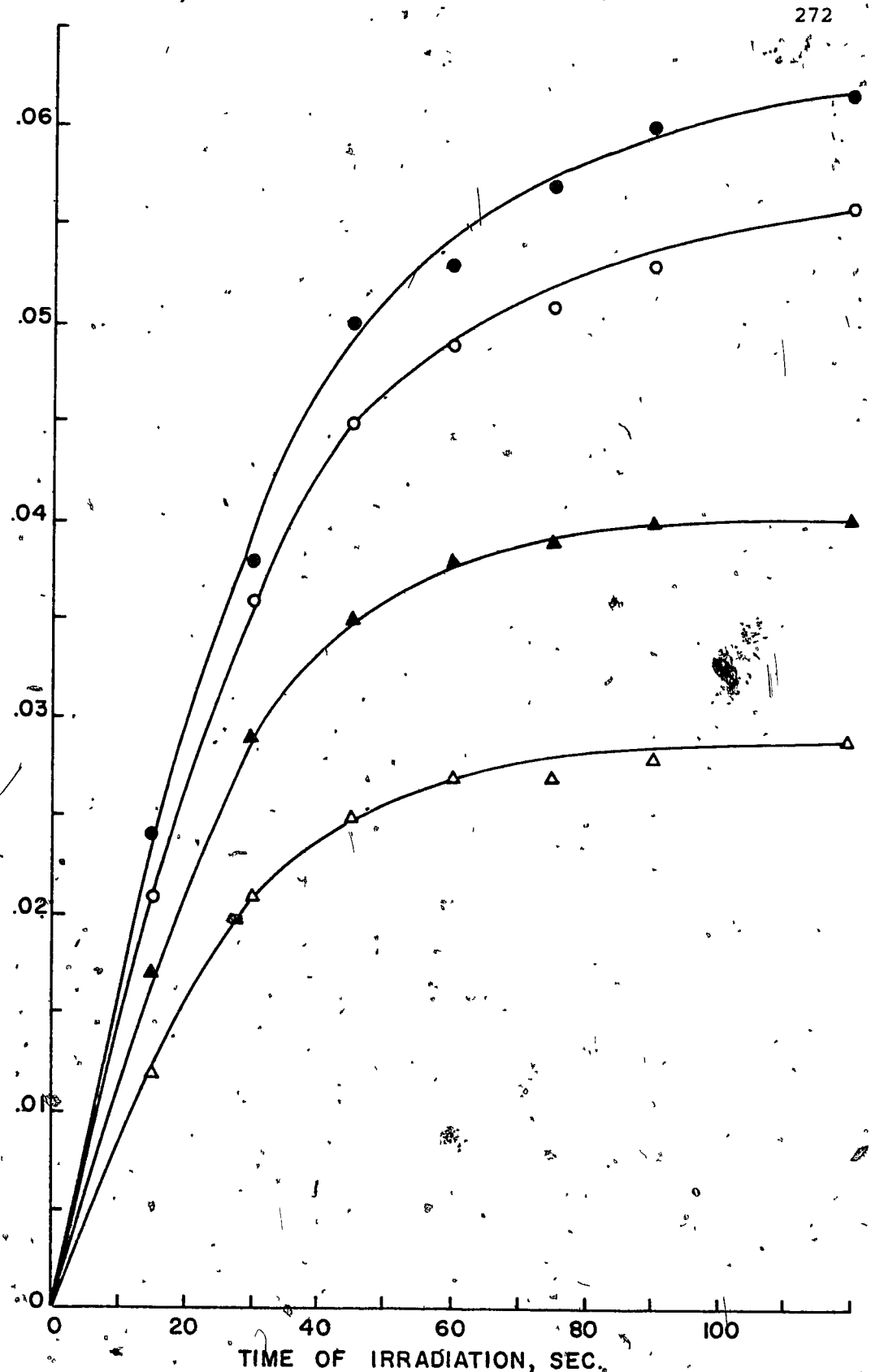

.06
.05
.04
.03
.02
.01
0
Δ ABSORBANCE
0
20
40
60
80
100
TIME OF IRRADIATION, SEC.

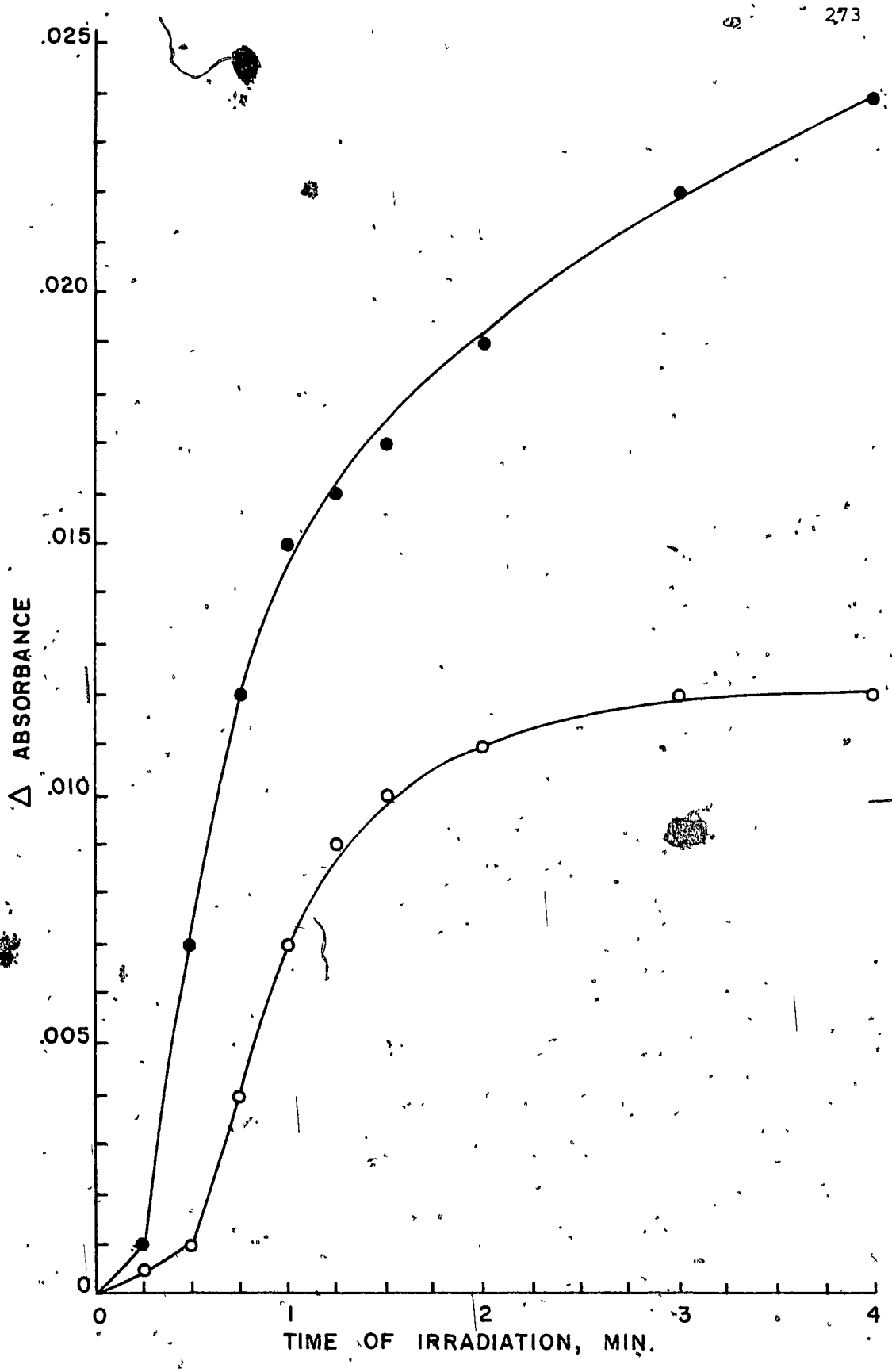
Figure 10.22 Effect of Superoxide Dismutase on Fe^{3+} Cytochrome c Reduction in Seawater

The seawater was buffered to pH 8.1 and filtered. The concentration of superoxide dismutase was 500 μg l^{-1}. Irradiation was conducted in the xenon lamp system.

● without superoxide dismutase

○ with superoxide dismutase

273


.025
.020
.015
.010
.005
0
Δ ABSORBANCE
0
1
2
3
4
TIME OF IRRADIATION, MIN.

11. VARIABILITY OF SEAWATER PHOTOREACTIVITY

11.1. Introduction

Evidence in the preceding several sections indicates that some transition metals, NO_3^-, NO_2^-, and some organic substances can promote the light induced destruction of amino acids. All of these components behave non-conservatively in seawater and might be expected to impart a significant degree of variability to its photochemical characteristics. Inferences about the variability of specific reactions of NO_2^-, NO_3^-, or the transition metals can be made from the abundance of information available on the general distributions and trends of these components in the natural environment. Furthermore, if the photochemistry of seawater were predominantly controlled by certain transition metals, by NO_2^-, by NO_3^-, or by a combination of these components, it would be analytically feasible to measure them. To do the same with the organic fraction is not feasible unless the specific substances involved are known. Unfortunately, the state of the art in marine organic chemistry is still at the point of not having qualitatively identified some 66 to 90% of the dissolved organic matter. For the 10 to 34% of the substances which have been identified and quantified, no clear picture of their variability exists because of the small amount of existing data. The only relatively abundant data on organic materials are the bulk measurements for TOC and DOC; these may bear no discernible relationship to the photochemical character of seawater.

If the specific composition of the organic fraction cannot be determined, then possibly the photochemical characteristics of seawater can be deciphered through the variability of more readily measured general properties. This possibility was explored in a sampling program conducted on St. Margaret's Bay, Nova Scotia. The program was described earlier (Section 7.) with regard to fluorescence measurements. The following is essentially an extension of the same program on the relationship of methionine photoreactivity to other measured properties of the water column.

11.2. Methods

Samples were collected at 1, 5, 10, 25, and 40 meters at the same station, which was located near the center of St. Margaret's Bay (Figure 7.9). A single Niskin bottle with an external closing mechanism was used in sampling. Seawater to be used in various analyses and for photoreactivity tests was transferred immediately after collection to one liter glass bottles. The bottles were sealed, stored on ice in the dark, and returned to the laboratory, where they were immediately filtered through thoroughly pre-washed .22 μ Millipore filters. Analyses and tests were completed as quickly as possible after collection; this usually required no more than 12 hours.

Salinity was determined with a Bissett Berman (Model 6230) salinometer and in situ temperatures with a Beckman (RS-5) thermograph. Data from other research programs, which were run in conjunction with this one, included chlorophyll a, TOC, and dissolved and particulate Fe, Mn, Cu, and Zn.

Irradiation experiments were all conducted in the merry-go-round system with all samples for a particular date run simultaneously. Samples for different dates were corrected for differences in lamp intensity, but the deviations were small. Seawater used in irradiation experiments was buffered to pH 8.1; the added concentration of methionine or 1-^{14}C glycine was 5×10^{-6} M l^{-1} or 1×10^{-7} M l^{-1}, respectively.

11.3. Results and discussion

11.3.1. Natural variability

Data on fluorescence and fluorescence decay were described earlier in Section 7. Figure 7.10 should be referred to for depth profiles on these values, for chlorophyll a, and for salinity. Depth profiles for methionine reactivity, NO_2^-, NO_3^-, and TOC are shown in Figure 11.1; again, the most scatter in values is found at the 1 and 5 meter depths. Interestingly, the variability of the methionine reactivity tends to disappear with increasing depth, with the exception of two wide-spread values which represent the lowest and highest values observed for the month sampling period. To gain any more than a qualitative expression of variability from these profiles of mean values is perhaps not justified in view of the underlying complexity which might control the observed methionine reactivity. Its reactivity may be determined by the summation of effects of a complex array of variables which can generally be categorized as either enhancing or inhibiting. It is difficult to

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[1] Data on dissolved and particulate metals, TOC, and chlorophyll a were obtained from Dr. John Hoff, Dr. Michael McKinnon, and Mr. Kevin Sellner, respectively.

Figure 11.1 St. Margaret's Bay Sampling Program.

Depth Profiles for Methionine Reactivity, TOC, NO_2^-, and NO_3^-

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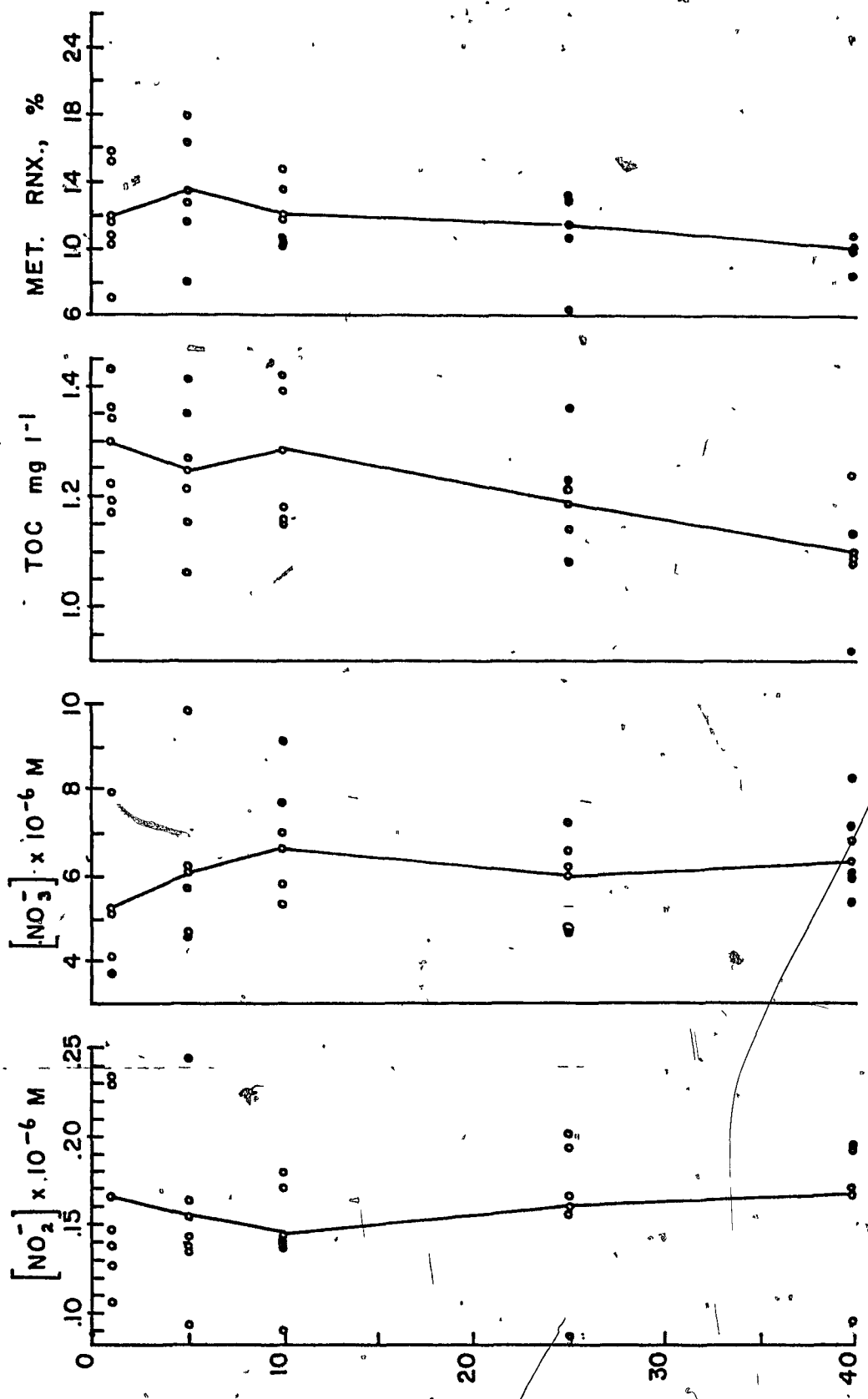
MET. RNX., %
6
10
14
18
24
TOC mg l⁻¹
1.0
1.2
1.4
[NO3⁻] x 10⁻⁶ M
4
6
8
10
[NO2⁻] x 10⁻⁶ M
.10
.15
.20
.25
0
10
20
30
40
DEPTH, m.

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assess how all of these variables, when coupled together, will affect the reactivity. It is, however, possible to speculate on how some of the variables might behave if examined independently.

11.3.1.1. Nitrate and nitrite

The reaction of NO_3^- appears to be very inefficient above 300 nm, and for the concentrations measured it would be expected to contribute little to the reactivity of methionine. Nitrite, even through usually present at a concentration an order of magnitude lower than NO_3^-, should induce the degradation of 0.05 to 0.1 µM of the 5 µM of methionine originally added.

Since the reaction of methionine with NO_2^- probably proceeds through free radical intermediates resulting from NO_2^- photolysis, the reaction should be dependent on the concentration of other organic compounds whose rate of reaction with NO_2^--produced free radicals is comparable to methionine. The low concentrations of methionine and other organic compounds dictates that they will react predominantly with only longer lived radicals of relatively low reactivity. Consequently, only substances which are the most susceptible to free radical attack should be able to affect the measured reactivity of methionine. Sources of variations in the concentration of such substances might arise from the fresh water supply or from biological activity.

Strangely, NO_2^- concentration and methionine reactivity would appear to be inversely related in these natural samples (Figure 11.1). This is difficult to rationalize in view of the NO_2^- induced photolysis of

methionine, unless this process is completely overshawdowed by other, more important processes.

11.3.1.2. Transition metals

Previous experiments on the induced photoreactivity of amino acids by transition metals suggest that their affect on the reactivity of methionine should be insignificant when compared to the photoreactivity observed in natural samples. It is possible, however, that the transition metals might exist in natural seawater in forms which are highly efficient photocatalysts for such reactions, but this seems unlikely, since most transition metals are present at nanomolar concentrations.

In an indirect capacity, however, some of the transition metals could influence methionine reactivity by catalyzing the decomposition of H_2O_2 or by quenching excited states, thereby inducing an effect in the same direction as NO_2^-. Since a number of different transition metals can function in these ways, their combined effects, even though individually present at low concentrations, could be significant. Interestingly, the proposed reaction mechanisms for the metal catalyzed decomposition of peroxides include the formation of strong oxidizing radicals, which engender another sequence of free radical reactions which should contribute to the observed reactivity of methionine.

The likely direction of the transition metals effect is unclear, but there seems to be no obvious consistent correlation between any or all of the measured metals (Fe, Cu, Zn, and Mn; see Table 11.1) and the results for methionine photoreactivity.

Table 11.1 Data from St. Margaret's Bay Sampling Program

The sampling dates are given across the top of the Table.

1 see Section 5.5

	Depth m	3/2	3/9	3/16	3/23	3/26	4/2	4/5
Salinity, ‰	1	30.78	31.32	30.28	29.83	30.89	30.81	30.78
	5	-	31.38	31.03	30.15	31.06	30.80	30.76
	10	31.12	31.38	31.29	31.16	31.13	-	31.27
	25	31.15	31.65	31.51	31.37	31.38	31.39	31.35
	40	31.79	31.81	31.63	31.50	31.53	31.42	31.61
NO_3^-, M l^{-1}	1	-	-	5.1	4.1	3.7	7.9	2.6
	5	5.7	6.2	9.8	4.7	4.6	6.1	4,4
	10	5.3	5.3	9.1	7.7	5.8	7.0	2.6
	25	6.6	6.2	7.2	4.8	4.7	6.2	-
	40	7.2	8.3	6.0	5.4	6.1	6.8	8.0
NO_2^-, µM l^{-1}	1	.137	.146	.105	.229	.126	.233	.090
	5	.35	.142	.092	.138	.163	.243	n.d.
	10	.137	.142	.089	.140	.179	.170	.090
	25	.200	.155	.085	.156	.165	.193	.111
	40	.170	.167	.095	.194	.192	.170	.170
TOC, mg C l^{-1}	1	1.22	1.17	1.34	1.36	1.19	1.43	-
	5	1.21	1.27	1.06	1.15	1.35	1.41	-
	10	1.39	1.39	1.18	1.16	1.15	1.42	-
	25	1.08	1.23	1.36	1.14	1.08	1.21	-
	40	1.08	1.09	.92	1.24	1.10	1.13	-
Chlorophyll a, µg l^{-1}	1	n.d.	.45	.53	.56	.94	.83	.83
	5	n.d.	.51	.57	.82	1.03	>.83	.84
	10	n.d.	.46	.54	.56	.68	.53	1.52
	25	n.d.	.54	.51	.53	.36	.48	2.01
	40	n.d.	.17	.50	.49	.3	.42	.19

	Depth m	3/2	3/9	3/16	3/23	3/26	4/2	4/5
Fluorescence, QSU[1]	1	7.07	6.48	8.69	10.50	7.76	6.09	7.74
	5	7.50	6.30	6.18	9.75	7.29	6.10	6.07
	10	5.88	6.48	5.58	7.02	7.10	5.77	6.36
	25	5.46	6.00	5.38	7.38	6.91	5.28	5.50
	40	4.92	6.08	5.53	6.47	6.82	5.11	5.34
Fluorescence Decay, $\%^{-1}$	1	-	13.9	50.6	38.7	30.4	36.7	60.1
	5	-	28.8	44.3	41.2	31.1	39.4	48.3
	10	-	24.4	41.3	36.6	21.6	34.6	50.7
	25	-	35.3	41.6	40.7	31.5	28.9	51.1
	40	-	35.6	47.7	41.8	32.0	46.3	45.8
Methionine Photoreactivity, %	1	15.2	11.6	7.0	10.2	15.8	10.8	6.4
	5	18.0	16.4	13.4	11.6	8.0	12.8	7.8
	10	11.8	14.7	10.2	10.4	10.6	13.6	10.8
	25	10.6	12.8	6.4	12.8	13.0	11.6	-
	40	10.2	10.8	22.8	10.8	8.6	8.4	3.0
Dissolved Fe, $g\ l^{-1}$	1	.77	.72	1.5	1.3	.68	.76	.76
	5	.86	.67	.83	.96	.46	.70	.69
	10	.57	.72	.54	.45	.44	.59	.42
	25	.38	.49	.48	.40	.38	.44	.44
	40	.34	.60	.66	.47	.46	.54	.58
Dissolved Mn, $g\ l^{-1}$	1	1.5	.99	2.8	4.7	1.8	2.3	2.0
	5	1.8	.89	1.0	3.2	1.2	2.3	2.1
	10	1.1	.88	.79	.91	1.1	2.0	1.0
	25	.44	.51	.38	.65	.48	.83	.77
	40	.46	.85	.47	.68	.75	1.1	1.0

	Depth m	3/2	3/9	3/16	3/23	3/26	4/2	4/5
Dissolved Cu, g l^{-1}	1	.24	.27	.30	.34	.31	.25	.23
	5	.22	.23	.20	.24	.20	.17	.22
	10	.19	.25	.20	.19	.16	.20	.18
	25	.16	.23	.18	.18	.17	.17	.30
	40	.15	.22	.17	.17	.17	.18	.30
Dissolved Zn, g l^{-1}	1	1.1	.65	.97	1.1	2.2	1.3	1.3
	5	.90	.45	.49	.68	1.3	1.6	1.1
	10	.58	.55	.38	.44	1.0	.75	.74
	25	.61	.40	.31	.36	.84	.53	1.9
	40	1.0	.37	.31	.31	.78	.53	2.6

11.3.1.3. Organic photosensitizers

The preceding work implicates some portion of the organic fraction of seawater as the major contributor to amino acid reactivity and to peroxide formation. Since little is known about the composition of this fraction it is impossible to measure it as a distinct component of the photochemical system. An attempt was therefore made to relate methionine reactivity to synoptic measurements, which would hopefully reveal the major or controlling sources of these materials. However, no apparent correlation was found with natural fluorescence, chlorophyll a, or TOC, when the total number of sample values (Table 11.1) for each property was considered. It may be unreasonable to assume that any apparent trends will be observed when testing, as an aggregate, a set of properties which are changing at variable rates and are not homogeneous in time, space, or composition.

The difficulty of describing observations only in terms of specific characteristics of the organic fraction of seawater can be examined in a hypothetical situation where a single organic photosensitizer is mixed uniformly into seawater, and the seawater is then subjected to all of the normal processes of the coastal marine environment. Figure 11.2 depicts the possible alternative light initiated transformation processes which might be acting upon the photosensitizer in the coastal marine environment. For the sake of simplicity, biological and non-light initiated dark reactions have been excluded, although for some organic photosensitizers such processes might be far more significant than light induced transformations.

Figure 11.2 Possible Light Initiated Transformations of a Photosensitizer

Where sens = sensitizer

E = energy

Q = quencher(s)

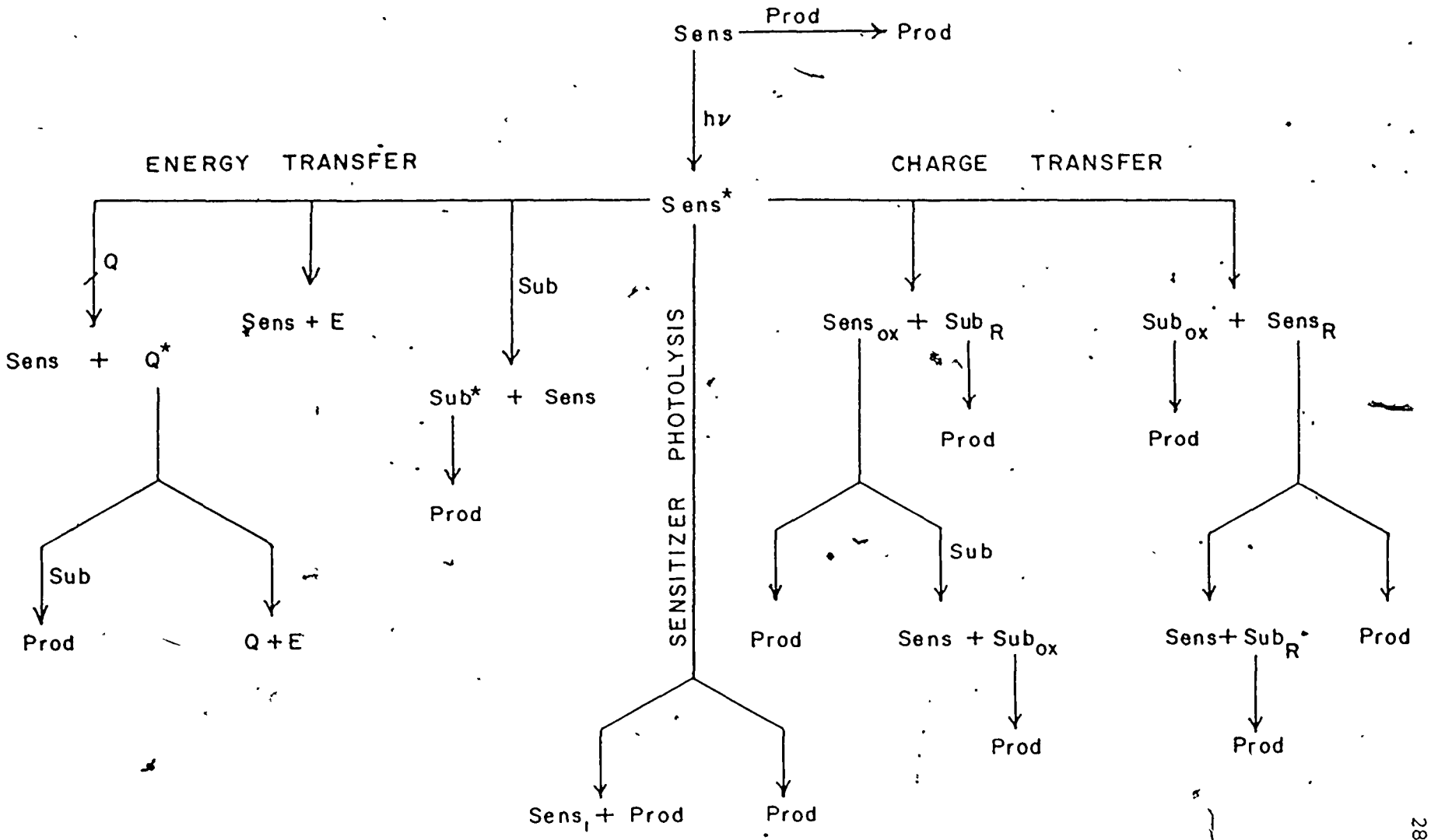
Prod = product(s)

Sub = substrate(s)

$sens_1$ = new sensitizer

subscripts, ox = oxidized form

R = reduced form

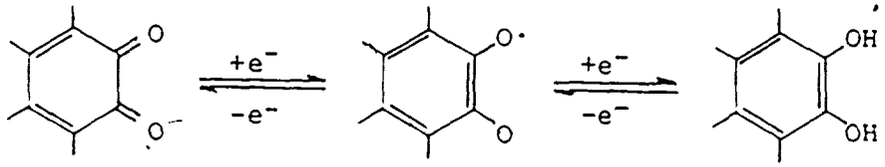

Sens
Prod
Prod
hν
ENERGY TRANSFER
CHARGE TRANSFER
Sens*
Q
Sens + E
Sub
Sens + Q*
Sub* + Sens
Prod
Sub
Prod
Q + E
SENSITIZER PHOTOLYSIS
Sens$_{ox}$ + Sub$_R$
Prod
Sub
Prod
Sens + Sub$_{ox}$
Prod
Sub$_{ox}$ + Sens$_R$
Prod
Sens+ Sub$_R$
Prod
Prod
Sens$_1$ + Prod
Prod

The characteristics of each organic photosensitizer and the distribution of the light energy field in the water column will be major factors involved in determining the photosensitizing properties of any given parcel of water. In some cases the photosensitizers will be destroyed at the surface and will, therefore, exhibit an increasing concentration with depth. In other cases the distribution becomes complicated by transformations of initial sensitizer to another sensitizer (e.g., riboflavin to lumichrome or tryptophan to N-formylkynurenine).

The concentration of a photosensitizer is also subject to its own light induced destruction, stemming directly from its excited state or through interaction with secondary reactive products generated as the result of some photochemical process. Rates for such reactions will be highly dependent on the concentration of the photosensitizer, reactive products, and other reactive substrates which can act as scavengers for the reactive products, thereby protecting the photosensitizer. The concentration of a labile substrate can even determine the principle reaction mechanism operating. For some photosensitizers the predominant product in the presence of a sufficient amount of an oxidizable substrate would be O_2^-, while in its absence it would be $^1O_2$. From arguments given earlier, the formation of $^1O_2$ might amount to the photochemically inconsequential result of quenching the excited state of the photosensitizer. It is possible that some quenching agents, particularly paramagnetic ones, can promote the photochemical activity of the sensitizer by increasing the efficiency of intersystem crossing from

singlet to triplet excited state, thus increasing the total lifetime of the excited states in solution. This in turn can have a marked effect on increasing apparent reaction rates. On the other hand, quenching of both singlet and triplet excited states can occur, O_2 for instance, is about equally efficient at quenching both.

Concentration changes of photosensitizers can also be related to reactions which regenerate them. This can occur rapidly by quenching of excited states, or by processes which involve a photochemical reaction by energy transfer or charge transfer. Regeneration processes could, on the other hand, be much slower, because of control by rate limiting processes.. A possible notable example of this involves natural polyphenolic materials, which can exist in one of three basic forms (Eq. 11.1). If the quinone form is functioning as a photosensitizer



(11.1)

and is reduced in the process, then along with ϕ for this process it's efficiency will be determined by photochemical or thermal regeneration from the reduced semiquinone or hydroquinone forms.

11.3.2. Wavelength dependence

The measurement of seawater photoreactivity with a broad polychromatic source provides little information on the potential importance of the reactions at different depths. Acquisition of this information requires studying reactions at a specific wavelength or in specific wavelength region, and then relating this information to light trans-

mission characteristics of the water mass in question, to gain an estimate of the change of reaction rate with depth.

Rough estimates of reaction rates were made for the disappearance of methionine and for the decarboxylation of glycine for various spectral regions, using the merry-go-round system. Results indicate that the reactions are primarily occurring in the region from 290-500 nm for both amino acids (Table 11.2), and that no measurable change was taking place at wavelengths greater than 500 nm. Reactions in which Corning CS 7-37 and CS 7-60 filters were used revealed that excitation in the spectral region from 330-390 nm accounted for an appreciable fraction of the observed decarboxylation of glycine. Adjusting the CO_2 yields in Table 11.1 for transmittance of the Corning glass filters (Appendix 5) gave a 60-70% yield in this spectral region. It should be pointed out, however, that the yield in this region is probably exaggerated by the strong intensity of the mercury 365 nm line. The results, nevertheless, indicate that a singificant fraction of the reaction is occurring at wavelengths between 350 and 500 nm. Based on typical absorption spectra for coastal water from this area, this means that 1% of the surface reaction rates for specific wavelengths between 350 and 500 nm would be reached at depths of between 4 and 10 meters and for 10% of the surface reaction rates between 1.5 and 5 meters.

Table 11.2 Methionine and 1-^{14}C Glycine as Indicators of Seawater Photoreactivity for Filtered Light Reactions

Freshly collected seawater was buffered to pH 8.1 with borate, filtered (through .22 μ filter), and enriched with either 1-^{14}C glycine or methionine. The solutions were irradiated in inner ring of merry-go-round system.

Concentration of Added Test Substrate	Date of Collection and Depth	Irradiation Wavelength Region and Color Specification Number	Rate of Disappearance of Added Substrate $M\ l^{-1}\ hr^{-1}$
5×10^{-6}M l^{-1} Methionine	March 2, 1976	>300 nm, CS 0-53	1.7×10^{-7}
5×10^{-6}M l^{-1} Methionine	March 2, 1976	>400 nm, CS 3-74	$<0.2 \times 10^{-7}$
5×10^{-6}M l^{-1} Methionine	March 2, 1976	>500 nm, CS 3-70	$<0.2 \times 10^{-7}$
1×10^{-7}M l^{-1} Glycine	April 2, 1976	>300 nm, CS 0-53	2.3×10^{-11}
1×10^{-7}M l^{-1} Glycine	April 2, 1976	>400 nm, CS 3-74	3.1×10^{-12}
1×10^{-7}M l^{-1} Glycine	April 2, 1976	>500 nm, CS 3-70	n.d.
1×10^{-7}M l^{-1} Glycine	April 2, 1976	CS 7-60	8.3×10^{-12}
1×10^{-7}M l^{-1} Glycine	April 5, 1976	>300 nm, CS 0-53	2.9×10^{-11}
1×10^{-7}M l^{-1} Glycine	April 5, 1976	>400 nm, CS 3-74	3.6×10^{-12}
1×10^{-7}M l^{-1} Glycine	April 5, 1976	>500 nm, CS 3-70	n.d.
1×10^{-7}M l^{-1} Glycine	April 5, 1976	CS 7-37	4.8×10^{-12}

12. CONCLUSIONS

In Section 1.2 six fundamental questions were proposed which were each examined during the course of this study, even if only cursorily. The questions and the partial answers which emerged from the preceding experimental work are reiterated here.

(1) Are any light-initiated changes in either physical or biological properties of seawater observed?

A. Seawater exposed to continuous simulated sea surface sunlight irradiation showed a continual decline in absorbance at all wavelengths. An approximation to natural environmental conditions indicated that a 75% reduction in absorbance in coastal seawater would be expected in 2-3 weeks time in an unmixed 10 cm layer at the sea surface. In regions of the oceans where the same water remains in the mixed layer for extended periods and low inputs of absorbers prevail, high water transparency would be expected. (see Sections 6.2. and 10.2.3.1.)

B. Natural seawater fluorescence and river water fluorescence were found to decay on exposure to either sunlight or artificial light sources. A rapid decay over the first few minutes followed by a much slower decline thereafter was usually observed. The decay occurred at wavelengths both less than and greater than 400 nm and should therefore be occurring throughout the euphotic zone (this was borne out in tower tank experiments). The decay is rapid enough so that the usual

vertical profiles of fluorescence should display a rapid increase with depth in the euphotic zone (see Chapter 7 and Section 10.2.3.1.).

(2) What is the rate of light initiated modification of model substances, for which the chemistry is well known, when they are added to seawater under natural conditions or simulated natural conditions?

Amino acids were used for this purpose and in all cases the sunlight-induced degradation greatly exceeded the thermal degradation rate. The rates of degradation of tyrosine, tryptophan, histidine, and methionine greatly exceeded the rates for glycine, alanine, leucine, valine, phenylalanine, proline, and lysine. A case can be made for seawater's predominant nature being a relatively mild reaction medium. (see Section 6.4.2).

(3) What products result from light initiated reactions of either model compounds or natural seawater components?

A. Expected products of amino acid degradation were found (i.e., CO_2, NH_3, and formaldehyde). Attempts to ascertain product-reactant balances yielded incomprehensible data probably because of product instability and analytical artifacts. (see Section 6.5.)

B. A peroxide (probably H_2O_2) was generated by various added organic materials and in natural seawater. Rates of accumulation in natural seawater were found to range from 1×10^{-8} M $l^{-1}hr^{-1}$ to greater than 1×10^{-7} M $l^{-1}hr^{-1}$ for sea surface

sunlight or simulated sunlight conditions. Rapid decay rates were observed for dark reactions and were also postulated to occur by direct photolysis of the peroxide bond. The accumulation rate is then a function of a complex array of variables: the light energy distribution, the concentration of peroxide producing materials, and minor constituents, salinity, and probably temperature. The very fact that the peroxide degrades is an indication that it is involved in seawater redox processes. (see Sections 10.2.2. through 10.2.2.2.)

(4) What are the specific agents of seawater which are responsible for its photoreactivity?

A. The photoreactivity of glycine and methionine was enhanced by NO_3^-, NO_2^-, TMEM, fulvic acid, H_2O_2, riboflavin, and the phloroglucinol-based tetramer. Inhibition was noted for I^-, O_2, EDTA and 2-propanol. (see Section 6.6.3.)

B. Of the transition metals, only Hg^{2+}, Ag^+, and Cu^{2+} promoted a significant increase in photoinduced decarboxylation of glycine, with the order of decreasing effectiveness being $Cu^{2+} >> Ag^+ > Hg^{2+}$. The metal ions Ce^{4+} and Co^{2+} were found to act as inhibitors at μM concentrations. (see Chapter 8)

C. Both NO_2^- and NO_3^- ions were found to photoinduce the degradation of methionine and glycine. Nitrite ion, even though normally present at much lower concentrations, is by far the more important of the two. Relative rate constants in solutions containing different major anions indicate that

the reactive species in NO_2^- photoinduced reactions could be a dihalogen anion radical. (see Chapter 9)

D. The enrichment of seawater with various organic chromophoric materials, indigenous to the marine system, promoted significant increases in the photoreactivity of seawater towards various indicator substrates. Nanomolar and even lower added concentrations of some of these materials induced noticeable changes in seawater photoreactivity. Supporting evidence for the importance of naturally occurring organic materials was obtained through a concomitant reduction of photoreactivity with either in situ destruction of organic chromophores by photolysis or through their removal by ultrafiltration. A reduction in the accumulation rate of peroxide, in the absorbance, and in the fluorescence accompanied the loss of photoreactivity in seawater subjected to these removal methods. (see Chapter 10)

(5) What are the general mechanisms of light-initiated reactions in seawater?

A. The combined evidence from work on NO_2^- and NO_3^-, TMEM, added organic constituents, and natural seawater indicates that it is components of the organic fraction which are mainly responsible for the photoinduced destruction of amino acids and for the formation of peroxide.

B. On theoretical grounds and from experimental evidence, O_2 plays a major role in seawater photoreactions. Although singlet

oxygen ($^1\Delta$ O_2) is likely to be a major product of these interactions, it is unlikely that it will be a major reactant in seawater solutions. Because of its longer lifetime, O_2^- should be a more significant reactant in seawater. Through the use of ferric cytochrome c and superoxide dismutase the occurrence of the O_2^- radical was demonstrated for light-initiated reactions of various added organic semitizers in seawater and in natural seawater itself. The O_2^- radical was not detected in dark redox reactions of natural phenolic materials. (see Section 10.2.4.3.)

C. As a group, free radical reactions may represent the most important general photoinduced process in seawater. For kinetic reasons it is likely that only the least reactive, longest-lived members of free radical chains will be important in modifying organic substrates in solution.

(6) Is the photoreactivity of seawater variable in space or time? Variability for peroxide accumulation rate, methionine reactivity, and glycine reactivity was observed. An attempt to correlate methionine reactivity to other observations obtained in a field program failed to produce any decisive results (see Chapter 11)

It should be emphasized that these conclusions are drawn from experiments conducted using coastal seawater from one region. To extrapolate to water from other regions, especially oceanic, may be premature.

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APPENDICES

APPENDIX 1

METHODS FOR PURIFYING WATER

A. Super-Q Water

Distilled water was passed through a Millipore Super-Q Ultrapure water system with cartridges assembled in the order: prefiltration, carbon (organic absorption), deionization (2), and .22 µm filtration. Purity was such that inorganic ions were below detection limits and the measured organic carbon level averaged less than 0.5 mg l^{-1}. No detectable utilization of added bacterial labile substrate was observed for periods exceeding normal reaction times.

B. Redistilled Super-Q Water

Super-Q water was used to prepare a basic $KMnO_4$ solution. Distillation from this solution gave water which was low in organic materials, gave low blanks in most analytical methods, and exhibited the lowest reactivity towards seawater photochemical indicators. For these reasons it was used for the preparation of reagents and artificial seawater.

C. Low organic water

This water was prepared by passing a mixture of distilled water vapor and O_2 through a quartz column packed with short pieces of quartz tubing; the column was maintained at a temperature of 800°C. The hot effluent vapor from the column was condensed and then redistilled in an all glass system.

D. Seawater for fluorescence blank

Filtered seawater of approximately the same salinity as the samples for which it was to be used was exposed to the full spectrum of radiation from a 1200 watt mercury arc lamp (G.E. UA-11) for 4 hours. The seawater was contained in a 250 ml sealed quartz tube at a distance of 6 inches from the source.

APPENDIX 2

ANALYTICAL PROCEDURES

I. Fluram analysis for amino acids

Reagents:

A. .05 M borate buffer solution, pH 9.4

A one liter solution was prepared by dissolving 16.98 g of sodium borate and .883 g of NaOH in distilled water.

B. Fluram reagent

A 100 ml solution was prepared by dissolving 20 mg of Fluram in high quality acetone. To reduce the reagent blank, the acetone was refluxed over anhydrous cupric sulfate for 3 hours and then distilled through a short packed column in an all glass system.

The prepared reagent solution should not be kept too long, for an increase in the blank occurs with extended storage times.

C. Ethyl acetate

A high quality grade of ethyl acetate was used, which gave low background fluorescence.

Procedure:

Into a 20 ml teflon capped culture tube was pipetted 5 ml of sample. To this was added 1 ml of .05 M borate buffer. The sample was stirred

rapidly with a vortex mixer while 2 ml of the Fluram reagent was added rapidly. The solution was stirred for 15-20 seconds after completing the addition. Following this, .5 ml of .1N HCl was added to clear the turbidity which usually developed during the introduction of Fluram reagent. Fluorescence was measured within 1 hour of derivative preparation with an excitation wavelength of 395 nm and an emission wavelength of 495 nm.

Alternative procedure for higher sensitivity:

The derivative was prepared by the procedure described above; however, in this case the solution was acidified with 1 ml of .2N HCl. Following this, 4 ml of ethyl acetate was added, the tube was sealed with a teflon lined cap and shaken vigorously for 20 seconds. After suspended water had disappeared from the upper organic phase the fluorescence of this phase was measured with an excitation wavelength of 385 nm and emission wavelength of 490 nm. The fluorphor is less stable at lower pH values; therefore, the time elapsed between acidification and the measurement of fluorescence should be as short as possible.

Calibration curve and normalization of fluorescence intensity:

A calibration curve of amino acid concentration versus relative fluorescence intensity was prepared for the concentration range of interest (1×10^{-5} M should probably be considered a maximum upper limit). The fluorescence intensity of each sample was normalized to

the calibration curve by comparing the sample to a standard amino acid solution of approximately the same concentration range. A blank was also determined for the same water sample to which no amino acid had been added. For irradiated seawater samples the blank was determined for seawater which had been subjected to identical conditions. The normalized intensity (I_n) was calculated with the following equation:

$$I_n = \frac{I_c \cdot (I_u - I_b)}{(I_s - I_b)}$$

where I_u = intensity of unknown

I_b = intensity of blank

I_s = intensity of standard

I_c = intensity of the standard as read off the calibration curve

Using the value I_n, the concentration of the unknown was taken from the calibration curve or calculated from its slope.

II. Dansyl derivative preparation

Reagents:

A. Sodium bicarbonate buffer, pH 11

This solution was prepared by combining 650 ml of .05 M $NaHCO_3$ and 295 ml of .1 M NaOH.

B. Dansyl chloride reagent

Dansyl chloride (.1 to .25 g) was dissolved in 100 ml anhydrous spectranalyzed acetone. The concentration of this solution was dependent on the amount of amino acids in the sample. For an amino acid concentration in the range of 1-5 μM, a .15 g dansyl chloride solution was used. The reagent was refrigerated during storage.

C. Internal standard solution

These solutions were prepared by dissolving enough of the amino acid (i.e., β-alanine, 4-amino butyric acid, or sarcosine) in .1 N HCl to give a 5×10^{-4} M solution.

Procedure:

Ten ml of sample and 100 μl of the internal standard solution was pipetted into a 20 ml teflon capped culture tube. To this was added 1 ml of dansyl chloride reagent followed by 1 ml of the buffer solution. This solution was sealed, shaken, and stored in the dark at room temperature for 4 hours. At the end of this period 1 ml of 2 N HCl was added; after a few minutes, 4 ml of ethyl acetate was added and the tube was shaken vigorously for 1 minute. When the phases separated and suspended water disappeared from the upper organic phase, the ethyl acetate extract was ready for direct injection onto to HPLC column.

This extraction procedure gave a linear increase in fluorescence intensity with concentration, for amino acids used in this study, in the range of 5×10^{-8} M l^{-1} to 5×10^{-6} M l^{-1}. For analysis at natural

oceanic concentration levels of amino acids, larger sample volumes, multiple extractions, and concentration of the solvent extracts was necessary.

III. HPLC separation of dansyl derivatives

The solvent extracts containing the dansyl derivatives were injected directly onto a 25 cm silica gel column (Merck LiChrosorb SI 60, 5 μm mean particle size) with a high pressure direct injection valve, containing a 10 μl sample loop. Solvent flow rate (.5 to 2 ml min^{-1}) was maintained with an operating pressure of 2000 psi, with column at room temperature. The eluting solvent was a mixture of methylene chloride, methanol, and acetic acid, which for most neutral amino acids provided a good separation when combined in the volume proportions of 100: 1: 1, respectively. For more polar amino acids, the proportions of methanol and acetic acid relative to methylene chloride were increased. These solvent proportions represent only an approximation of actual conditions necessary for a particular separation, as a result of the column retention properties varying with column age, conditioning and packing reproducibility.

The detection system used was a DuPont 836 fluorescence detector with the excitation source filtered with a Corning CS 7-60 glass filter and emission with a Corning CS 3-72 cut off filter. Typical settings for the concentration range (.1 to 5 μM) normally encountered were: offset 0-3000, range 64, and filtering time constant 0.5 seconds. The output from the detector was integrated with an Infotronics Model

CRS-108, Hewlett Packard 338A, or Hewlett Packard 3370A integrator.

Amino acid concentration in an individual sample was determined by reference to an internal standard amino acid. The amino acid concentration in an irradiated sample (C_{LS}) was calculated by comparison to the dark control sample in accordance with the following equation:

$$C_{LS} = \frac{(A_{LS})(A_{DIS})(C_{DS})}{(A_{DS})(A_{LIS})}$$

where C_{DS} = concentration in dark control

A_{LD} = peak area for light sample

A_{DS} = peak area for dark sample

A_{LIS} = peak area for light internal standard

A_{DIS} = peak area for dark internal standard

IV. Aldehyde analysis

Reagents:

A. Acetate stock solution

This stock solution was prepared by dissolving 154 g (2 moles) of ammonium acetate and 3 ml (∿ .05 moles) of acetic acid in enough Super Q water to make 1 l of solution.

B. .05 M 2,4-pentanedione reagent (Nash, 1953)

This reagent was prepared by dissolving 2 ml of distilled 2,4-pentanedione in 250 ml of the acetate stock solution. The solution was

prepared immediately before the analysis and was kept for no longer than a day.

C. Standard formaldehyde solution

A 2×10^{-2} M solution was prepared by diluting 1.202 g of 37% formaldehyde solution (preserved with 12% methanol) to 500 ml with Super Q water. The concentration was further reduced by diluting 10 ml of this solution with Super Q water to give 1 l of a 2×10^{-4} M solution. Aliquots of this solution were diluted with seawater or artificial seawater to prepare standard solutions of the desired concentration. All solutions were prepared shortly before use.

Procedure:

Five ml of the 2,4-pentadione reagent and 5 ml of sample were combined in a 20 ml glass stoppered test tube. The solutions were thoroughly mixed, and the tubes were immersed in a 37°C water bath to the liquid level inside of tube for a period of 1 hour. After the solutions had cooled to room temperature, their fluorescence was measured at an excitation wavelength of 430 nm and an emission wavelength of 520 nm on an Aminco SPF-125S spectrofluorometer.

A calibration curve was prepared by plotting formaldehyde concentration against relative fluorescence intensity for a series of standard solutions in the concentration range of interest. The fluorescence intensity of each sample was normalized to the calibration curve by comparing the sample to a standard amino acid solution of approximately

the same concentration as the sample. Aside from the common reagent blank, a second blank correction was necessary, because of the change of natural seawater fluorescence during irradiation. The normalized intensity (I_n) was calculated using the following equation:

$$I_n = \frac{I_c \cdot (I_u - I_b + I_{DNF} - I_{LNF})}{(I_s - I_b)}$$

where I_u = intensity of unknown

I_b = intensity of reagent blank (dark control)

I_s = intensity of standard

I_{DNF} = intensity of natural fluorescence for dark control

I_{LNF} = intensity of natural fluorescence for irradiated sample

I_c = intensity of standard as read off calibration curve

Using the value I_n, the concentration of the unknown was taken from the calibration curve or calculated from its slope.

V. Peroxide analysis

Reagents:

A. Scopoletin solutions

A scopoletin stock solution was prepared by dissolving 5 mg of the compound in 500 ml of Super Q water. Gentle heating was necessary to get complete dissolution. From this solution a series of 200 ml solutions were prepared by dilution of the appropriate volumes with Super Q water.

The following series of solutions was found to be useful for H_2O_2 concentration of 5×10^{-9} to greater than 10^{-7} molar. These solutions are

µg Scopoletin/100 µl	Volume stock solution/200 ml
.50	100 ml
.25	50
.10	20
.025	5
.010	2

unstable and should be freshly prepared before use.

B. .05 M Tris buffer, pH 8.0

The buffer solution was prepared by dissolving 3.54 g Trizma (pH 8) in enough Super Q water to make a 500 ml solution.

C. Peroxidase solution

This solution was prepared by dissolving 40 mg of peroxidase in 10 ml of autoclaved Tris buffer (pH 8). The solution was stored at 0°C and was used within 2 days of its preparation.

D. H_2O_2 standard solutions

A 1×10^{-2} M H_2O_2 solution was prepared by diluting 1 ml of a 30% H_2O_2 solution to 1 l with Super Q water. Aliquots of this solutions were diluted with low organic water (Appendix 1, C) to 100 ml to prepare 1×10^{-6} M, 1×10^{-5} M, and 1×10^{-4} M H_2O_2 solutions. The concentration of these solutions were verified by iodometric analysis for H_2O_2.

It is imperative that all glassware used in the preparation of dilute H_2O_2 solutions be meticulously cleaned, and that the water used for dilution be of the highest purity. Even when these precautions were carefully observed, instability of dilute H_2O_2 solutions still created problems. Therefore, the dilute H_2O_2 solutions were freshly prepared for each experiment and further dilution was carried out only seconds before commencement of the analysis.

Procedure:

To analyze for peroxides in photolysis experiments the same seawater was used for the standards, irradiated solutions, and dark controls. The level of peroxide in the seawater was determined by the difference of scopoletin oxidation with and without catalase present.

Five ml of sample, .5 ml of Tris buffer, and 10 µl of scopoletin was added to a 20 ml test tube. The solution was stirred rapidly with a vortex mixer and 50 µl of peroxidase was added quickly. For standards, this procedure was modified by adding an aliquot (5-50 µl) of the 1×10^{-6} M, 1×10^{-4} M, or 1×10^{-4} M peroxide solutions seconds before addition of the peroxidase. The solution fluorescence was measured at an excitation wavelength of 390 nm and an emission wavelength of 490 nm.

The concentration of the scopoletin solution used in a particular analysis is dependent on the concentration of the peroxide. The peroxide concentration range over which the method is useful for a particular scopoletin solution is determined by plotting relative fluorescence intensity against H_2O_2 concentration. The scopoletin solution should

only be used for the concentration range of H_2O_2 for which the calibration curve is linear.

The actual peroxide concentration in irradiated solutions was calculated using the following equation:

$$[\text{Peroxide}] = \frac{I_B(I_L + I_{DNF} - I_{LNF})}{S} - I_B$$

where I_B = intensity of blank for standard

I_L = intensity of irradiated sample

I_D = intensity of dark control

I_{DNF} = intensity of natural fluorescence for dark control

I_{LNF} = intensity of natural fluorescence for irradiated sample

S = slope of calibration curve

VI. Measurement of natural seawater fluorescence

Reagents:

A. Quinine reference standard

A quinine stock solution was prepared by dissolving 100 mg of quinine sulfate in enough .1 M sulfuric acid to make 100 ml of solution. Dilution of 1000 µl, 100 µl, or 10 µl of this solution with .01 M sulfuric acid gave standard reference solutions of 1.0, 0.1, or 0.01 mg l^{-1}, respectively. Super Q water was used in the preparation of these solutions.

B. Seawater fluorescence blank

see Appendix 1, D

C. Borate buffer

The buffer was prepared by dissolving 46.146 g of sodium borate (10 H_2O) in 500 ml of Super Q water, adding 98 ml of 1 N HCl, and diluting to 1 l with Super Q water. When necessary, the pH of the solution was adjusted to 9.4 by titration with small increments of NaOH or HCl solutions. The addition of .5 ml of this buffer to 25-35‰ salinity seawater gave a solution with a pH of 8.1.

Procedure:

Seawater samples were buffered with .5 ml of borate buffer/100 ml, filtered through a .22 μ millipore filter and equilibrated at atmospheric pressure to give an air saturated solution. The fluorescence was measured at an excitation wavelength of 380 nm and an emission wavelength of 490 nm. The quinine reference standard fluorescence was measured immediately before and after each sample determination to detect artifacts introduced by lamp or detector fluctuations. The concentration of standard solution used was determined by the intensity of the sample fluorescence. In general the concentration of the standard solution was such that intensity of sample was always less than, but not greatly different than, the fluorescence intensity of the standard.

VII. Iron (II) Analysis.

Reagents:

A. Borate buffer

The buffer was the same as that used in Appendix 2, VI.

B. .01 M Ferrozine reagent

This reagent was prepared by dissolving .514 g of Ferrozine in enough Super Q water to make 100 ml of solution.

C. Iron (II) standard solutions

Iron (II) stock solutions of 1×10^{-2} M and 1×10^{-3} M were prepared by dissolving ferrous ammonium sulfate in .05 M HCl solution prepared from Super Q water.

Procedure:

Seawater buffered with .5 ml of borate buffer/100 ml was saturated with air by stirring it in a flask open to the atmosphere. The temperature was controlled by immersing the flask in a temperature controlled water bath. An aliquot of the ferrous standard was added and a timer was started. The amount of Fe^{2+} remained at different intervals was determined by rapidly adding a 50 ml aliquot of the sample to a flask containing 1 ml of Ferrozine reagent. The absorbance of this solution was measured at 562 nm in a 10 cm cell against distilled water.

The standards were measured by adding aliquots of the standard Fe^{2+} solution to a flask containing a rapidly stirred solution of .25 ml of borate buffer, 50 ml of water and 1 ml of Ferrozine reagent. Blanks were determined in the same way except, of course, the standard Fe^{2+} solution was omitted.

Iron (II) concentration in samples was determined directly from a calibration curve of absorbance versus Fe^{2+} concentration.

VIII. Measurement of $^{14}CO_2$

Reagents:

A: Glycine, 1-^{14}C solutions

A 1-^{14}C glycine solution with a specific activity of 47.18 mCi mM^{-1} was diluted with .05 N HCl to a concentration of 1 x 10^{-4} M yielding approximately 1.2 x 10^{6} DPM/100 µl. Aliquots of this solution were added to the reaction solutions to provide the final concentration.

B. 1-amino-2-phenylethane (phenethylamine)

The reagent was nearly colorless and gave low CPM values as obtained from supplier. Purification can be achieved by distillation.

C. Scintillation counting solution

A common formulation was used; it consisted of 64 ml of Spectrafluor (a liquid PPO-POPOP scintillator), 500 ml Triton X-100, and 500 ml of toluene (liquid scintillation counting grade).

Procedure:-

Reactions with labeled glycine were carried out in sealed reaction vessels with a small head space volume. To limit loss of $^{14}CO_2$ during transfer of solutions, the pH was raised by adding 50 µl of 1N NaOH/ 50 ml of reaction solution, following the termination of the experiment. The solution was transferred to a 100 ml dark colored serum bottle containing a magnetic stirring bar. The bottle was sealed with a rubber septum to which a plastic cup (Kontes K-882320-0000) had been attached so that it extended down into the bottle and was positioned in the air space above the liquid. The cup held a folded glass fiber filter (Whatman GF/A, 2.4 cm) which had been moistened with .20 ml of 1-amino-2-phenylethane. The solution was acidified by injecting .25 ml of concentrated H_3PO_4 through the septum and then stirred at a moderate rate with the temperature maintained below 25°C. At the end of 1 hour the filters were placed in a glass scintillation vial containing 15 ml of counting solution. The vials were shaken vigorously until the glass filters disintegrated.

Total activity in the solution was determined by adding the same volume of labeled glycine stock solution that was used for the reaction solution to 15 ml of counting solution plus enough Cab-O-sil to form a thixotropic gel.

All samples were counted and converted to DPM by using the channels ratio method of quench correction.

IX. Redox indicators

Reagents:

A. Borate buffer, pH 9.4

The buffer was the same as that used in Appendix 2, VI.

B. Borate buffer, pH 8.1

Borate buffer (pH 9.4) was adjusted to pH 8.1 by titration with 6N HCl.

C. Epinephrine solution

A 1×10^{-4} M solution was prepared by dissolving .916 g of epinephrine in enough .1 N HCl to make 50 ml. The solution was stable for long periods if stored in the dark at a temperature of 0-5°C.

D. Nitro blue tetrazolium (NBT) solution

A 1×10^{-3} M solution was prepared by dissolving 81.8 mg of NBT in enough Super Q water to make a 100 ml solution. The solution was stored in the dark at a temperature of 0-5°C.

E. Ferric Cytochrome c solution

A 1×10^{-4} M solution was prepared by dissolving .124 g of Fe^{3+} cytochrome c in enough .05 M phosphate-NaOH buffer (pH 7.5) to make 100 ml of solution. The solution was stored in the dark at a temperature of 0-5°C.

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F. Methylene blue reference standard solution

This solution was prepared by dissolving .8218 g of methylene blue (91% total dye content) in enough Super Q water to make 200 ml of solution. A ml of this solution was diluted with Super Q water to give 100 ml of a 1 x 10^{-5} M stock solution.

Procedure:

Basically the same experimental procedure was followed for each of the three redox indicators. This procedure consisted of following spectrophotometrically the development of the indicator at some fixed concentration, when the sample containing it was exposed to either a light or dark environment. Comparison was made to a solution prepared with artificial seawater, distilled water, or a reference reaction solution (see Section 10.2.4.).

A. Epinephrine

Seawater samples were prepared by adding .5 ml of borate buffer and 50 µl of epinephrine solution to 50 ml of a sample that was in equilibrium with the atmosphere at the reaction temperature. The sample was irradiated for a period of time which depended on the characteristics of the light source used. Differences in spectral distribution or intensity of different sources considerably altered the time of exposure necessary for sufficient color development of the epinephrine indicator. The inner ring of the merry-go-round system required 4 minutes, the outer ring 8 min, and sunlight or the xenon system 15 to 30 min.

Development of epinephrine in seawater samples was standardized by comparison to a solution of .5 ml of pH 8.1 borate buffer, 50 µl of methylene blue solution, and 50 µl of epinephrine solution in 50 ml of Super Q water. The blank was determined from a solution to which no methylene blue regent had been added. Both the blank and the standard were irradiated for the same period as the sample and all solutions were air saturated.

Color development in the solutions was measured spectrophotometrically at 485 nm in a 10 cm pathlength cell, against distilled water. A relative value of indicator development (R) was then calculated,

$$R = 100 \left(\frac{A_S - A_{BM}}{A_M - A_{BM}}\right)$$

where A_S = absorbance of sample

A_M = absorbance of standard

A_{BM} = absorbance of standard blank

R varies with salinity and therefore only seawater samples varying by no more than a few parts per thousand in salinity should be compared, unless a correction for the salinity difference is made.

NBT

The sample (50 ml) was combined with .5 ml of pH 8.1 borate buffer and .5 ml of NBT solution. The air saturated solution was then irradiated, and the color development due to the formation of Blue

Formazan was measured at 560 nm in 10 cm pathlength cells against distilled water.

Ferric Cytochrome c

The sample (50 ml) was combined with .5 ml of Fe^{3+} cytochrome c solution and .5 ml of borate buffer. The pH 8.1 buffer was used for deionized water and the pH 9.4 buffer for seawater. Formation of the Fe^{2+} cytochrome c was followed by measuring the absorbance at 550 nm in 10 cm pathlength cells against distilled water. The absorption curve for Fe^{2+} cyctochrome c is very sharp and the maximum was determined by scanning.

APPENDIX 3

EQUIPMENT SUPPLIES

Liquid Chromatograph

DuPont 830 with:

- DuPont 835 Multi Wavelength Photometer
- DuPont 836 Fluorescence Detector Option
- Infotronics Model CRS-108, or
- Hewlett Packard 338A, or
- Hewlett Packard 3370A Integrator

Spectrofluorometer

American Instrument Company SPF 125 with:

- Hanovia 901C-1 Xenon Lamp
- IP21 Photomultiplier Tube

Liquid Scintillation Spectrometer

Packard Tri-Carb, Model 3380

Spectrophotometers

- Unicam SP8000
- Cary 14

ATP Photometer Model 2000, JRB Inc.

Salinometer Model 6230, Bissett-Berman

Salinometer-Thermograph, RS-5, Beckman

Photochemical Light Detection System

United Detector Technology, Sant Monica, California.

- Detector/Preamp, UDT500 UV
- Test box, UDT 505 (includes biasing, circuitry, batteries and gain control)

LI-COR, LI-185 Quantum/Radiometer/Photometer (from LAMBDA Instruments Corp., Lincoln, Nebraska)

Detector, LI-192S Underwater Quantum Sensor
Detector, LI-200S Pyranometer Sensor

Eppley Black and White Pyranometer

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APPENDIX 4

CHEMICAL SUPPLIES

Fisher Scientific Company, Montreal, Quebec

Supplied nearly all inorganic salts and most organic chemicals used including solvents for HPLC.

Canlab, Toronto, Ontario

Supplied ferrozine (Cat. # FX279-1).

Eastman Kodak Company, Rochester, N.Y.

Supplied o-phthaldehyde (cat. # 8154),
2-mercaptoethanol (cat. # 4196),
and 2,4-pentanedione (cat. # 1088).

Sigma Chemical Company, St. Louis, Mo.

Supplied amino acids, peptides,
catalase (cat. # C-40),
peroxidase (cat. # P-8250),
superoxide dismutase (cat. # S-5879),
epinephrine (cat. # E-4125),
cytochrome c (cat. # C-2506),
NBT (cat. # N-6876),
TNM (cat. # T-5752),
scopoletin (cat. # S-2500),
and isoxanthopterin (cat. # I8752).

Pierce Chemical Company, Rockford, Ill.

Supplied dansyl chloride (cat. # 217 511).

New England Nuclear, Boston, Mass.

Supplied glycine (1-^{14}C) (NEC-047H)

Amersham/Searle, Arlington Heights, ILL,

Supplied Triton X-100 (# 196145) and,
Spectrafluor PPO-POPOP (# 190650)

APPENDIX 5

TRANSMITTANCE SPECTRA[1] FOR VARIOUS GLASSES

Spectra:

A. Corning filter CS 0-53 (PYREX brand glass), thickness 1.99 mm

B. Corning filter CS 0-54, thickness 2.09 mm

C. glass from Quickfit tube used in merry-go-round system

D. unknown (labeled with Corning glass # 3482, but gives far different spectra), thickness 1 mm

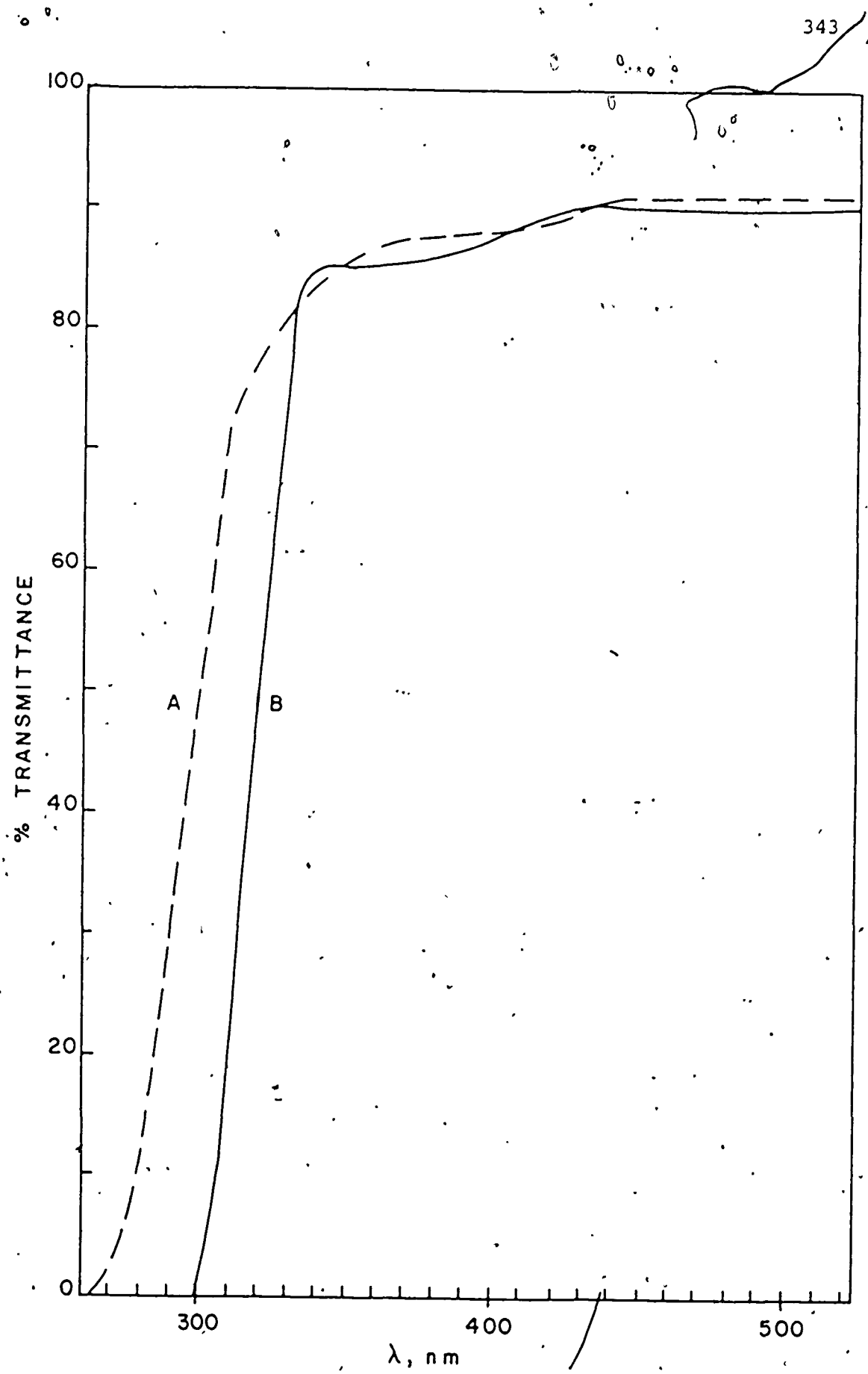
E. Corning filter CS 7-60, thickness 4.60 mm

F. Corning filter CS 7-37, thickness 5.05 mm

G. Corning filter CS 3-74, thickness 2.03 mm

H. Corning filter CS 3-70, thickness 4.50 mm

[1] All spectra were recording using a Cary 14 with reference to air.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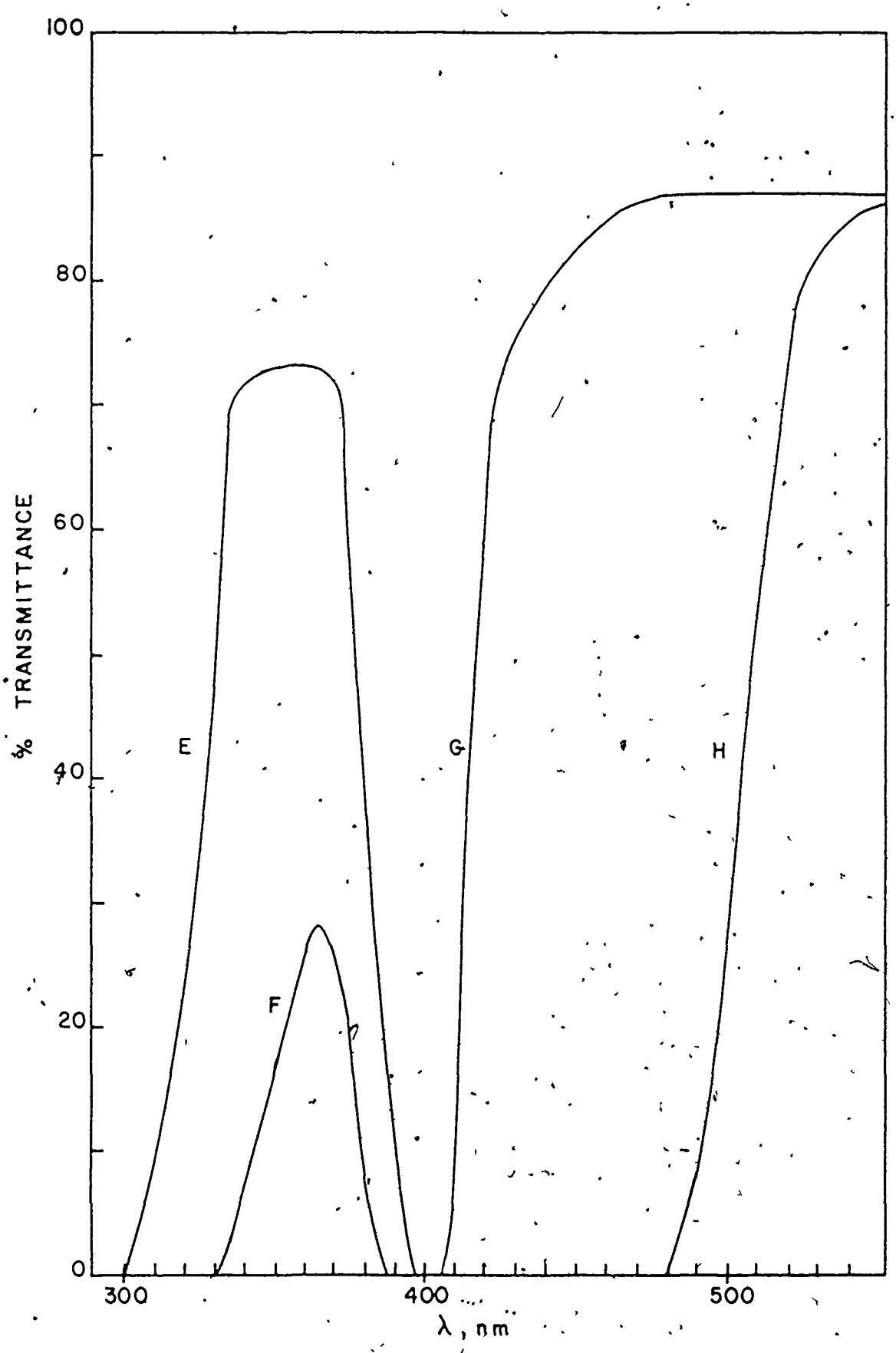
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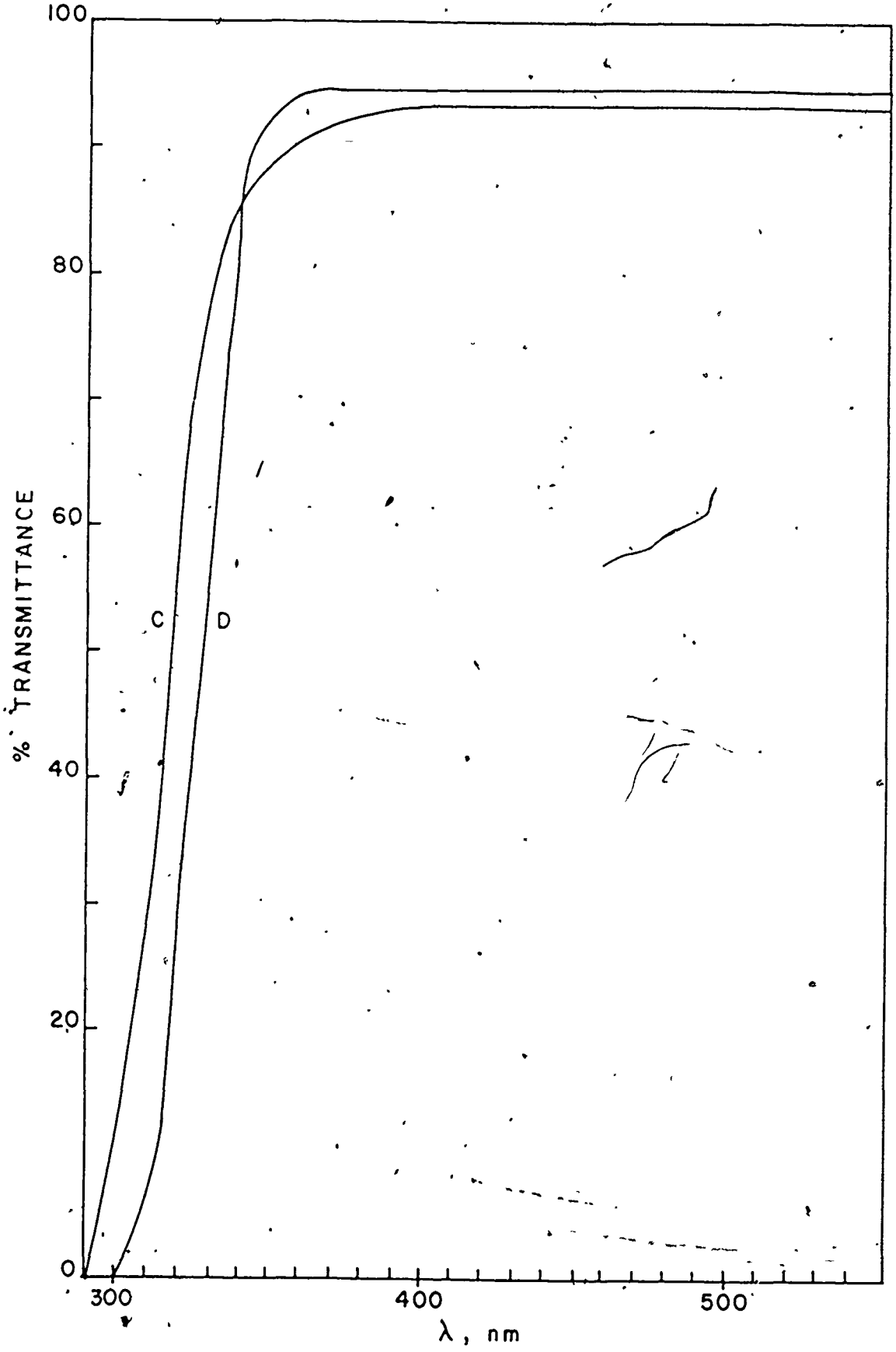
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APPENDIX 6

FILTER COMBINATIONS USED IN IRRADIATION SYSTEMS

System	Spectral Region	Filter[2] 1	Filter[2] 2	Filter[2] 3
sun lamp	290 nm	A	--	--
xenon lamp	300 nm	B	--	--
immersion well	290 nm	A	--	--
merry-go-round				
inner ring	A+B+C+D[1]	A	C	--
outer ring	A+B+C+D[1]	A	A	C
outer ring	B+C+D[1]	A	D	C
outer ring	C+D[1]	A	G	C
outer ring	D[1]	A	H	C
outer ring	300-400 nm	A	E	C
outer ring	330-390 nm	A	F	C

[1] Refer to Fig. 3.7.

[2] Refer to Appendix 5 for filter transmission spectra and color specification number.