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CHROMATOGRAPHIC DETECTORS:

NOVEL OPERATIONAL MODES,

CHARACTERISTICS AND CORRELATIONS

by

Hameraj Singh

A thesis submitted in partial fulfilment of the requirements for the degree of Ph.D in chemistry at Dalhousie University, Halifax, Nova Scotia.

October, 1994.

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ABSTRACT

Novel operational modes, fundamental characteristics and correlations of three common chromatographic detectors: the electron capture detector (ECD), the flame photometric detector (FPD), and the flame ionisation detector (FID) are reported.

Conventional ECDs are driven by unipolar polarisation sources. Following the discovery that a.c. constant-frequency polarisation yields better sensitivities and comparable linear ranges, studies of other bipolar polarisation regimes were conducted. The results indicate that it is possible to operate bipolar constant-current ECDs with response by a decrease of frequency as compared to conventional systems that respond by an increase of pulser frequency. In addition, the bipolar pulsed (constant frequency) ECD is very sensitive and produces a calibration curve that is entirely linear, from the 5 attomoles/second α-hexachloro-cyclohexane detection limit to the limit established by the extent of the standing current.

Filters are commonly employed to reduce detector noise, but the nature of chromatographic noise has never been fully investigated. The effect of three types of filters (two digital and one analog) on the noise from the ECD, FPD and FID, as well as the nature of the noise were therefore investigated. The results show that chromatographic noise is mainly white noise (of a fundamental nature, reducing with filter time constant in square-root fashion and giving a constant noise "power" spectrum) that is converted into low frequency noise by the filter, thus the nature and sophistication of the filter are not very important. These results were supported by simulations, and equations were developed for the calculation of fundamental detector noise.

Detector response ratios are widely employed, especially for probing peak identity and purity. Since integration is a common procedure in chromatography, an integral response ratio algorithm for automatic response ratio determination was developed. The integral method proved superior to the existing slope ratio method; and seems promising, particularly for sequential detection where the shape and retention time of peaks may vary from detector to detector.
ABBREVIATIONS AND SYMBOLS USED

a.c. alternating current

α-HCCH α-hexachloro-cyclohexane

AVG non-weighted moving average filter

β' high energy electrons produced by radioactive decay of $^{63}$Ni

d.c. direct current

ECD electron capture detector

FID flame ionisation detector

FIR finite impulse response filter

FPD flame photometric detector

GC gas chromatograph

MDA minimum detectable amount, at S/N = 2.

$N_{pp}$ peak to peak deviations of baseline noise

PMT photomultiplier tube

RC resistor-capacitor

RMS, $\sigma$ root mean square, standard deviation

RR response ratio

RRC response ratio chromatogram

RSD relative standard deviation

SNR signal to noise ratio

VCO voltage controlled oscillator
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Permission is hereby granted to Dr. Walter A. Aue (thesis supervisor) to use any or all of this material in whatever way he deems appropriate.

Hameraj Singh
CHAPTER 1

Introduction

1 The Electron Capture Detector

1.1 Preamble

Gas chromatography is a common technique for the separation and quantitative analysis of the components in a complex mixture, and electron capture detection is one of several selective techniques available for the analysis of the effluent from a gas chromatographic column. The ultra-sensitive and highly selective electron capture detector (ECD) was invented and further perfected by Lovelock (1-3).

The high sensitivity and selectivity of this detector for specific classes of chemicals (halides and halide derivatives, as well as nitro, sulphur and organometallic compounds), and its relative insensitivity towards hydrocarbons have made it indispensable in analytical laboratories. Typical applications are the determination of: pesticide residues in food, soil, water and animal tissues; atmospheric pollutants, for example, CFC's (chlorofluorocarbons) and PCB's (poly-chlorinated biphenyls), dibenzodioxins and dibenzofurans; and anti-knock agents in fuel.

The ECD is an ionisation type detector utilizing a radioactive material (usually $^{63}$Ni or $^3$H) as the ionization source. However, non-radioactive ECDs have been described (4-5). Contrary to early belief, the ECD is a destructive detector - for strong electron
capturers (6). The ECD functions by relying on the ability of some substances to capture free electrons. These thermal (or secondary) electrons are derived from the ionisation of the carrier gas by the primary electrons resulting from radioactive decay of the foil. An electric field is normally applied across the electrodes of the detector to monitor the current (due to free electron collection), and response.

Unlike most other ionization detectors, the response of the ECD is based on the concentration of the components in the carrier gas, and its response is the result of a decrease in standing current. Unfortunately, there are a wide variety of operating variables for the ECD. The standing current, sensitivity, selectivity, and linear range of the ECD are affected by both the gas chromatographic and detector conditions. Among these are:

1. Polarization mode (d.c., a.c., pulse), and voltage,
2. Width, amplitude, and frequency of pulses (pulsed mode),
3. Detector temperature,
4. Type, flow rate and grade of the carrier and scavenger gases,
5. Stationary phase of gas chromatographic column (column bleed),
6. Column temperature,
7. Conditioning time of the detector,
8. Sample residues ("memory effect"),
9. Type of radioactive source,
10. Geometry of the detector,
11. Pressure within the detector, and
12. Contamination by atmospheric oxygen.

The optimization of these variables is difficult, if not impossible, since each electron capturing substance would require individual optimization, and several variables are interdependent. Hence, reported results are often valid only for a specific type of detector and set of operating conditions.

1.2 Electron Capture Cell Configurations and Operational Modes of Polarisation and Response.

1.2.1 Cell Configurations.

There are four basic configurations for the electron capture detector. These are: parallel plate, coaxial, asymmetric, and two chamber design (7-9).

The parallel plate and some variations of the coaxial design have symmetric electrical fields. The other designs result in a non-symmetric field. Available ECDs invariably conform to one (or a variation) of these designs. Because of the extreme sensitivity of the ECD to contaminants, the two chamber design of Aue and Siu (9) is interesting since its foil is purged by pure carrier gas and cannot be contaminated by the effluent from the chromatographic column.

1.2.2 Polarisation Modes

In principle, the ECD is based upon the absorption of free electrons by electron capturing substances. The radioactive foil (β- emitter) used in these systems is responsible for the production of free electrons via ionization of the carrier gas. The electrons are
collected at the anode and result in the 'standing current' of the detector. The presence of an electron capturing substance in the detector results in a decrease of collectable electrons and hence a reduction of standing current. This reduction - after some electronic processing - produces a peak in the chromatogram.

There are several techniques used in the sampling of electrons within the ECD. These are: conventional "d.c." mode (constant voltage); conventional "pulsed mode" (constant frequency); conventional "constant current" mode (variable frequency); d.c. "constant current" mode (variable voltage); and a.c. - constant frequency mode. Brief descriptions of each of these techniques follow, and for quick reference they are summarized in Table 1.1.

1.2.2.1 Conventional Constant Voltage - "d.c." mode

In this mode, a constant negative potential is applied to the cathode (radioactive foil); or a constant positive potential to the anode (collecting electrode). This potential causes the drift of electrons to the anode. An increase in current is observed with increasing potential until a plateau is reached when all the free electrons are collected. For analytical purposes, the detector is normally operated at a voltage which corresponds to about 85 % (commonly referred to as the 'knee') of the maximum current plateau.

An intrinsic problem with detectors operated in this mode is the existence of a net positive space charge close to the cathode. This occurs because, under the applied potential, electrons are sampled at a much higher rate, i.e. the drift speeds of (unimpeded) electrons are some 3 to 4 orders of magnitude faster than those of the much heavier
cations and anions. The presence of the d.c. space charge has been blamed repeatedly for ambiguous responses (7-8).

1.2.2.2 Conventional "pulsed mode" - Constant Frequency

This technique was introduced by Lovelock as a refinement of the d.c. mode (10). In essence, electrons are sampled by application of short, strong pulses, whose width, amplitude and frequency are preselected.

Under such a pulsing regime, the electron concentration within the ECD is never constant, but varies in a saw-tooth fashion. During the application of the pulse, the electrons move to the collecting electrode and their concentration within the cell rapidly decreases. In the interval, when the pulse is off, the electron concentration again builds up (due to continuous irradiation of the carrier gas by the radioactive source). It will reach a constant value (plateau, steady state), provided the interval between the pulses is long enough. If the pulses are much shorter than the interval (e.g. 1 μs vs 1 ms), virtually all of the capture reaction is supposed to occur under "field free" conditions (7). Compared with its predecessor (the constant-voltage d.c. method), improved sensitivity and linear range have been obtained by this technique (8).

1.2.2.3 Conventional "Constant-Current" mode - Unipolar Pulsing

In the operational mode described above, a chosen set of pulse parameters are kept constant and response is directly measured as a reduction in current, as effected by the analyte. In a variation of that technique, the detector current is kept constant by
electronically varying the pulse period (more correctly, the pulse repetition time, i.e. the operating frequency of the pulser). A change in this frequency indicates the presence of an electron capturing species. This conventional "constant-current" mode was introduced by Maggs, Joynes, Davies, and Lovelock (11) and still remains the preferential one primarily because of its wide linear range (approximately four orders of magnitude).

In principle, the detector current is compared with a reference current and a constant ratio of the two is maintained via a feedback loop that controls the frequency of the excitation pulses. The analyte entering the ECD causes a reduction of current by capturing electrons. The frequency of the pulses commensurately increases to enhance the collection of electrons and thus keeps the detector current at its appropriate level. This change in frequency, plotted versus time, constitutes the chromatogram.

1.2.2.4 "d.c." - Constant-Current mode

Here, constant-current operation is achieved by varying the polarizing d.c. voltage via a current limiting device (12) or a variable power supply with associated feedback circuitry (13). Analytes passing through the detector cause a reduction of standing current. The change in voltage necessary to re-establish the preset current level is monitored in time to give the chromatogram.

The method does offer an improvement of linearity as compared to the d.c. constant voltage mode. In fact, the linear range and detection limit (four orders of magnitude and 0.1 pg of aldrin) are comparable to the conventional "constant-current" (variable frequency) mode. This goes to show that constant current operation is able to
produce longer linear ranges than the other techniques.

The reason, simply put, is that to maintain a constant current, a certain number of free electrons have to be collected; the remaining electrons being available for capture. Therefore, as the amount of analyte increases, a lower percentage of analyte molecules is being converted to anions (the rate of anion production being about constant), and the system can never run out of some (however few) electrons to capture. Without the "constant current" restraint, a large amount of analyte would simply exhaust the available supply of electrons.

1.2.2.5 "a.c." - Constant Frequency mode

Sampling of electrons within the ECD using bipolar techniques is still in its infancy. The technique, which was developed in this laboratory, initially used a variable auto-transformer ("Variac") to control the amplitude of regular household current - 60 Hz sine waves (14). More complex, commercially available function generators and a 'home made' programmable pulser were later used to define the basic features of the a.c.-ECD regime, using various waveforms (15, 16). However, the rectangular waveform seems to have prevailed as the mode of choice because it produced more definitive current profiles and it also allowed easy comparisons with the corresponding unipolar excitation modes.

Compared to the unipolar current profiles, the a.c. profile displays a descent and hence an additional response maximum at high frequencies. It has been suggested that the position of the low frequency ascent and response maximum, (which is similar to the one seen in the unipolar modes) is primarily determined by the cation-electron
recombination rate and that the high frequency descent and response maximum is primarily determined by the onset of electron oscillation, as mediated by a migrating negative space charge. The sensitivities and linear ranges of bipolar operation (at the two response maxima) are comparable with those obtained from typical unipolar variable-current modes (15).

Table 1.1: ECD Polarisation modes

<table>
<thead>
<tr>
<th>Polarisation mode</th>
<th>Condition</th>
<th>Readout</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional d.c. (unipolar)</td>
<td>Constant voltage</td>
<td>Current</td>
</tr>
<tr>
<td>Conventional pulsed (unipolar)</td>
<td>Constant frequency</td>
<td>Current</td>
</tr>
<tr>
<td>Conventional pulsed (unipolar)</td>
<td>Constant current</td>
<td>Frequency</td>
</tr>
<tr>
<td>d.c. (unipolar)</td>
<td>Constant current</td>
<td>Voltage</td>
</tr>
<tr>
<td>a.c. (bipolar)</td>
<td>Constant frequency</td>
<td>d.c. current</td>
</tr>
</tbody>
</table>

1.3 Basic Processes within the ECD.

A high steady-state concentration of electrons within the ECD can be achieved by the action of ionizing radiation on carrier (and/or purge) gas molecules, when the rates of ion pair formation and ion pair recombination are equal. The capture of free electrons and hence reduction of the 'standing current' constitutes the ECD response. The following
section gives a very brief outline of these processes within the ECD, at least as far as they are currently understood.

1.3.1 Primary Radiation Sources.

Generally, a radioactive source (commonly $^{63}$Ni) is used for the generation of ion pairs within the ECD. However, non-radioactive ECDs do exist (8). Apart from $^{63}$Ni, tritium has been widely used. Tritium sources are of high specific activity, yielding high sensitivities associated with a large standing current. Unfortunately, the maximum temperature limit for tritium foils (225°C for Ti$^3$H and 325°C for Sc$^3$H) is low and enhances the susceptibility of tritium detectors to contamination. $^{63}$Ni can be safely used up to 400°C, (and probably higher). Other suitable radiation sources and their characteristics have been described (17).

1.3.2 Production of Thermal Electrons.

Beta rays (high energy electrons) from the decay of a radio-isotope, react with molecules of the carrier gas to produce large numbers of ion pairs. Through collisions with carrier gas molecules, the secondary electrons thermalize (slow down to ambient kinetic energy levels). [$\beta^-$ particles released from the radioactive decay of $^{63}$Ni have a maximum energy distribution of 67 keV; and approximately 36 eV are consumed in forming a nitrogen ion pair. Thus, many ion pairs are formed from one $\beta^-$ particle, i.e. the $\beta^-$ particles lose their energy in steps during collision with nitrogen (or other carrier/purge gases) until their energy is less than that necessary for the formation of ion
pairs. The energetic secondary electrons produced (~ 18 keV) are thermalized (to ca. 0.07 eV, at 300 °C) through collision with carrier gas molecules (8).]

\[ \beta^- + N_2 \rightarrow N_2^+ + e^- + \beta^- \text{ (weaker)} \]

Some of the cations may recombine with electrons. The nature of the cations varies, i.e. "N$_2^{++}$" in the equation above represents a series of ions derived from N$_2^+$ by clustering, such as N$_2^+$(H$_2$O)$_n$, N$_4^+$, etc. The rate of production of thermal electrons is essentially constant and independent of the presence of the typically minute amounts of electron capturers. However, it is subject to the random nature of radioactive decay and any change in the nature or density of the carrier gas (8). The width of the ionization zone depends on both the nature and density of the carrier gas, and on the type of radioisotope. Early estimates varied widely. The experimentally determined range suggests that 50 % of the ion pairs are generated within 3.8 mm (in nitrogen at ambient temperature and pressure) of a $^{63}$Ni foil (18-19).

Thermal electrons may be lost on conductive surfaces, or by recombination; they may also be captured, or collected by the anode through the application of a constant or intermittent potential usually applied to the cathode (the radioactive foil). The rate at which electrons are collected determines the baseline current of the detector under a prescribed set of analytical conditions.

1.3.3 Electron Attachment.

When a molecule capable of capturing electrons enters the ECD, it may react either associatively or dissociatively to produce a negative ion. Other mechanisms,
generally regarded as insignificant, have also been described (20). In associative capture, a stable molecular ion is formed:

\[ e^- + AB \rightleftharpoons AB^- + \text{energy} \]

but in the dissociative capture reaction an unstable molecular ion results which spontaneously disintegrates into a free radical and an anion:

\[ e^- + AB \rightarrow AB^- \rightarrow A^0 + B^- \pm \text{energy} \]

where \( AB \) = electron capturing molecule,

\( AB^- \) = negative molecular ion, and

\( A^0 \) and \( B^- \) = dissociation products.

In the non-dissociative capture reaction, the stable molecular ion exists at a lower energy level compared to the parent molecule. This energy difference, which is the electron affinity of the electron capturer, is exhausted by either inelastic collisions with other molecules or emission of radiation (17). For the dissociative reaction, an activation energy is required to split the parent molecule. Energy equilibrium is preserved by the relative kinetic energy of the reactants, both before and after collision; by the energy released through the formation of the negative ion; and by the energy released or acquired during the dissociation of the molecule.

The overall effect of either mechanism is the formation of (heavy) anions and a corresponding reduction of (light) electrons from the plasma. A reduction of current is thus observed. Details of the electron attachment processes have been published (8,17).
1.4 The "Classical" Mechanism.

This model, also referred to as the stirred-reactor model, was developed for the constant frequency, unipolar pulse mode of ECD operation. It may be noted that the d.c. constant-voltage mode was regarded as obsolete mainly because of its short linear range, and the presence of space charges. In addition, the pulse mode allowed an easy kinetic treatment, once certain simplifying assumptions had been made.

The assumptions and postulates of this model (valid only for conditions of a long pulse period, approximately one milli-second; and a short pulse width, about 0.5 micro-second) are that the electrons reach thermal equilibrium and the plasma is homogenous, during the pulse-free period (i.e. the period during which no potential is applied to the cell); that at certain levels of voltage the detector current is independent of the applied potential (typically above 50 volts); that the detector current is due to the collection of 'all' electrons when the pulse is applied; that anions are relatively immobile and hence do not contribute to the current; that cations are much more abundant than electrons; that the neutralization rate of cations and anions is several orders of magnitude greater than the recombination rate of cations and electrons (the typical estimate being $10^5$ to $10^8$ times greater); and that the response is dependent upon anion neutralization.

Detailed reviews of the theory and operation of the ECD have been published (7,8). This classical mechanism was later modified by Grimsrud and co-workers (21,22) who considered electrostatic forces between the ions and thus included this concept into their hypothesis. In addition to the model described earlier, the following postulations were added: that cations migrate during the pulse free interval and may be lost to
grounded surfaces or collected by the anode, with the baseline current thus reflecting the net charge of cations and electrons collected, while anions are not collected; and that bipolar regions with fuzzy borders exist within the ECD - an inner region consisting mainly of electrons surrounded by a sheath of cations.

Later work with this detector yielded results that could not be reconciled with the classical mechanism (such as hypercoulometric behaviour, i.e. the experimental fact that one analyte molecule appears to capture more than one electron). In addition, mechanistic studies carried out by API-MS (Atmospheric Pressure Ionization Mass Spectrometry) suggested that recombination and neutralization rates were, in fact, of comparable magnitude (23). These among other observations led to the evolution of the alternative space charge mechanism of ECD operation.

1.5 The Space Charge Mechanism.

The process of ECD operation via a space charge mechanism was proposed for a two chamber d.c. ECD by Aue and co-workers (24-26). In essence, it does not equate the initial electron capture reaction with observed response, but postulates that response is essentially caused by a migrating negative space charge of (analyte derived) anions that impedes the collection of electrons.

Compared to the classical mechanism, the following aspects differ sharply from the 'stirred reactor' theory. In the space charge mechanism, the ECD is considered to be made up of two characteristic regions, and anions migrate to the anode where they may be collected. The observed magnitude of response is due to a faster cation-electron
recombination rate (associated with the slow moving anion space charge), and is not only dependent on the chemical reactions within the cell but also on physical parameters such as temperature, pressure, and the physical size and shape of the detector.

In addition to providing a satisfactory explanation of the striking effect that the impedance of the detector is much greater - and its response much, much smaller - in the "reversed-field" mode (as compared to the "regular-field" mode where the radioactive foil is always the cathode, and the "collecting" electrode always the anode); the space-charge mechanism is consistent with the observation of hypercoulometric behaviour; and is also compatible with the (experimentally realized) concept of a non-radioactive ECD whose gas-phase chemistry does not include cations. None of these effects are readily accounted for by the classical mechanism. However, experimental observations have yielded evidence that more than one mechanism may be operative under certain ECD conditions (27); and the observation of hypercoulometry in commercially available, conventional low-volume ECDs suggest that a space charge mechanism may be operative even when "clean" unipolar pulse regimes are employed (28, 29).

2 The Flame Photometric Detector

The Flame Photometric Detector (FPD) has been used to selectively detect gas chromatographic effluents for nearly 3 decades. Since its inception in 1966 (30), the detector's ruggedness, sensitivity and selectivity has propagated its use not only for gas, liquid and supercritical fluid chromatography, but also for other separation techniques such as capillary electrophoresis (31).
The detector response is based on the emission from excited molecular species in a hydrogen-rich flame, using air or oxygen as the oxidant. Although its initial use was mainly propagated for monitoring sulphur and phosphorus compounds, for example in pesticide residue analysis, the detector is now known to respond to a large number of other elements (32).

The original FPD design used a back focussing mirror to enhance light throughput, to a single photomultiplier tube (PMT). However, one of the earliest modifications (33) replaced the mirror with a second interference filter and PMT, thus allowing simultaneous monitoring of sulphur and phosphorus compounds. Apart from producing dual-channel chromatograms from a single injection, response ratio analysis was done for identification of species containing both phosphorus and sulphur.

The FPD has managed to survive in the wake of stiff competition from other selective, sensitive and linear responding (to sulphur) detectors for example the mass spectrometer, mainly due to the exorbitant cost of the latter. However, despite its shortcomings, it is still widely used, particularly for monitoring sulphur compounds in the environment (34); in the petroleum industry (35) and a variety of organometallic compounds (36).

Other recent developments in the FPD include the pulsed flame photometric detector (37), which uses a combustible gas flow which cannot sustain a continuous flame. The pulses of the emitted light (controlled by pulses to an igniter) are amplified by a lock-in amplifier. This seems to offer better sensitivity and selectivity as compared to the regular FPD. Another chemiluminscent detector is the Reactive Flow Detector.
(RFD) developed by our group (38). The RFD behaves similarly to the FPD with the notable exception that it produces quenching free response and the flame that supports the flow can be used as a regular Flame Ionisation Detector (FID). An RFD/FID prototype has been connected to the output of an ECD. This set-up allows the sequential detection of a single analyte by three disparate detectors, whose response ratios may then be utilized for analyte identification, among other things.

3 The Flame Ionisation Detector

The FID has been around for over 3 decades and is the most widely used gas chromatographic detector (39). The FID responds to organic compounds which ionise in a hydrogen/air (oxygen) flame. Molecules that do not ionize readily, for example CO$_2$, N$_2$, CS$_2$, etc. do not give any significant response. However, since the detector responds to most organics, it is classified as a general detector as opposed to the previously described selective ECD and FPD.

Since the hydrogen/air flame is relatively cool, response is due to chemiionisation as compared to thermal ionisation in hotter flames. The energy for chemiionisation supposedly arises from free radical recombination (40) and response to carbon compounds occur by the reaction:

$$\text{CH} + \text{O} \rightarrow \text{CHO}^+ + \text{e}^-$$

The electrons are collected by an appropriately biased electric field and the resulting electron current can be observed, usually via an electrometer on a strip-chart recorder.
4 Objectives of this work

4.1 The Electron Capture Detector

Within an ECD, sampling of electrons is the one process that has received the greatest amount of attention. Conventional sampling techniques employed a negatively biased constant voltage (d.c.) source or unipolar pulsed source, connected to the radioactive foil, driving the electrons to the "collecting electrode" (anode) across the plasma. Of course, focusing on the electrons is the most logical choice, since it is electrons that react with analyte molecules and that the analyst wants to collect. However, it has recently been shown that an alternating current polarization source performs just about as well as its unipolar counterpart, even though in an a.c. driven ECD there is an equal and opposite half wave in the "reversed-field" direction to each "regular-field" half wave, i.e. the foil is half of the time negatively polarised and half of the time positively polarised. Under the homogeneous conditions postulated by the 'classical' theory this would lead to zero current and zero response since the same number of opposite charges would be transported to each electrode and the conventional d.c. electrometer - which is opaque to a.c. - would integrate these to zero.

This study was initiated to evaluate the merits and demerits of practical bipolar pulsing regimes, both in the two chamber ("space charge") and the single chamber ("stirred reactor") design ECDs. At the same time, this work would be expected to contribute to a better understanding of the mechanisms operating within these detectors.

The proposed experimental work includes the following tasks:

(a). Develop a "constant-current" type ECD based upon a.c. polarisation of the detector;
(b). Investigate the effects (both on baseline current and response) of separating (by a field-free interval) the bipolar pulses of an a.c. regime, including any space charge polarization and relaxation effects;

(c). Develop a bipolar pulsed (not continuous a.c.) polarization system that operates at constant frequency; and

(d). Develop a bipolar pulsed (not continuous a.c.) constant-current system for ECD excitation.

4.2 Characteristics and Correlations

Filters are commonly employed to decrease chromatographic noise and hence improve the signal to noise ratio (SNR). Filters are however normally evaluated on white noise, i.e. noise of an infinite number of frequencies, and the performance of digital filters is often superior to analog filters. But do filters behave the same way on chromatographic noise? In other words, is chromatographic noise white noise? To answer this question, three common filters (normally used in chromatography) - one analog and two digital (a simple moving window and a weighted moving average) filters - will be tested for their effects on the noise from three common chromatographic detectors: the ECD, FPD and FID. In addition, an attempt will be made to quantify the minimum noise from these detectors - both experimentally and theoretically.

Response ratios are widely used in chromatography to evaluate peak purity and identity. Ratios can be done manually or automatically. Computer generated slope ratios have been initiated and widely used by our group on dual channel data from a FPD.
However, integration is a common procedure in chromatography, and peak area determination is less likely to be affected by shifts in shape and retention time. Therefore automatic determination of response ratios using peak areas should also be more precise. Using different chromatographies, this study will compare an integral based algorithm with a slope ratio based algorithm with the aim of determining their use in specific circumstances.
CHAPTER 2

Experimental

This section gives a concise description of the general experimental equipment, chemicals, and techniques. Where more specialized equipment is used, it will be described in the appropriate section(s).

2.1 Instrumentation.

2.1.1 Tracor 550 Gas Chromatograph.

This commercially available instrument included the conventional $^{63}$Ni dual chamber Tracor ECD (Figure 2.1). Carrier gas (pre-purified nitrogen, Linde) was further refined by sequential passage through a hydrocarbon trap (Chromatographic Specialties Inc., model HT 200-2, Brockville, Ontario, Canada); a glass moisture trap (Chromatographic Specialties Inc.,); and finally through a Supelco (Supelco Inc., Bellafonte, PA, U.S.A.) carrier-gas purifier unit (capable of removing traces of oxygen and water), and then split into two separately controlled streams - one used as purge and the other as column carrier gas. The chromatographic column was a 1 m, 2 mm i.d. pyrex tubing, packed with 5% OV-101 on Chromosorb W, AW (100-120) mesh.

For analyses involving α-HCCH, a column flow of 35 mL/min and a purge flow of 50 mL/min was used, with injector temperature at 250 °C, column temperature at 150 °C, transfer line temperature at 270 °C, and detector temperature at 300 °C. The analyte
was introduced with a Hamilton 1 µL or 10 µL syringe, employing the "hot empty needle" technique, when appropriate.

The general instrument performance was periodically monitored in the (historically oldest, instrumentally simplest and diagnostically most revealing) d.c. mode by using the d.c. mode power source originally supplied with the chromatograph. The Tracor dual-

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**Figure 2.1:** Cross-sectional view of Tracor Dual Chamber ECD. Adapted from: "Operation and Service Manual - Nickel-63 High Temperature Electron Capture Detector." Tracor Inc. Texas, 1972.
chamber $^{63}$Ni ECD was polarized in most experiments by 'lab built' modules whose performance and parameters were monitored by a Tektronix 2215A 60 MHz Oscilloscope (Tektronix Inc., OR, U.S.A.) and/or a JDR Instruments (San Jose, CA, U.S.A.) DFC 100 Multifunction counter. The ECD output signal was monitored by a Linear Instruments strip chart recorder and/or a Fluke 75 multimeter, via the Tracor's own electrometer. A "home-made" variable frequency RC filter was sometimes used (generally at 1 s cut-off), for noise reduction purposes.

2.1.2 Shimadzu GC-8A Gas Chromatograph

The ECD on this instrument is of the coaxial type (Figure 2.2). The coaxial construction results in a more symmetric field ("homogenous" ion-pair distribution) and hence lower impedance to electron collection, as compared to the Tracor ECD which has an asymmetric field with non-homogenous ion-pair distribution.

Pre-purified Nitrogen (Linde) was screened by a hydrocarbon trap (Chromatographic Specialities Inc., model HT 200-2) in sequence with a high capacity moisture trap (Chromatographic Specialities Inc.) before it was fed to the chromatograph via a Supelco carrier-gas purifier unit. The chromatographic column was a 50 cm, 2 mm i.d. pyrex tubing, packed with 5% OV-101 on Chromosorb W, AW (100-120) mesh.

Analytical conditions were: injector/detector temperature: 260 °C; column temperature: 160 °C; carrier and purge gas flow: 30 mL/min. Sample introduction was achieved by use of a Hamilton 1 µL or 10 µL syringe, when appropriate.

For a.c. constant frequency experiments, the detector was polarized by a
Figure 2.2: Cross-sectional view of Shimadzu ECD.
Wavetek 5 MHz XCG/Sweep Generator, Model 183 (Wavetek, San Diego, California) and for pulsed - constant frequency and constant current experiments, by the programmable pulser described in the following section.

Instrument performance was monitored in the d.c. mode using a simple "lab-made" electrometer, which was also used for all experiments. An oscilloscope, multifunction counter, and multimeter (all described in section 2.1.1) were used to monitor various parameters, when desired. Chromatograms were obtained using a Fisher Recordall Series 5000 strip chart recorder.

2.1.3 Programmable Bipolar Pulse Generator.

This is a customised unit designed and built in the Department's Electronic Shop by Brian Millier. It is microprocessor-based, and capable of generating sequences of up to three individual square wave pulses within a single repetition sequence. The sequence repetition time as well as the delay time and pulse width of each pulse is programmable. Periods as long as one second are attainable; and pulse delay(s) and width(s) are programmable to a resolution of 600 nanoseconds. All timing parameters are programmed using a terminal or a computer connected to the pulser through an RS-232 serial data link. The polarity and amplitude of the individual pulses, as well as the d.c. offset are controlled by potentiometers on the unit. Maximum attainable pulse amplitude is 125 volts in either direction, with a slew rate of 150 V/μs. The circuit design does not allow for overlapping pulses. The unit may optionally be triggered by an external pulse generator, in which case the sequence repetition time is controlled by the external
Figure 2.3: Schematic of programmable bipolar pulse generator. Courtesy of B. Millier, Electronics Shop, Department of Chemistry, Dalhousie University.
generator. However, the pulse width, delay, polarity and amplitude could only be changed if the experiment was interrupted, i.e. these parameters cannot be varied (as would the frequency) in say, a feedback circuit. Figure 2.3 shows a schematic block diagram of the pulser and detailed circuit diagrams are given in Appendix 1.

2.1.4. Shimadzu GC-4BMPF Gas Chromatograph

This chromatograph is equipped with both a dual channel FPD and an FID. The analyte flow to the detector was via a 100 x 0.3 cm i.d. glass packed column (5% OV-101 on Chromosorb W, 100/120 mesh). Nitrogen (pre-purified, Linde) was further refined by sequential passage through hydrocarbon and moisture traps (both from Chromatographic Specialties Inc.), before being purged through the chromatographic column at 25 mL/min. The air (extra dry, Linde) and hydrogen (pre-purified, Linde) flows necessary for the flame(s) were also pretreated by their own set of sequential hydrocarbon and moisture traps.

General conditions were, injector temperature: 250 °C, detector temperature: 280 °C, and a column temperature that varied with the analysis. Further details on operating conditions will be given in the appropriate sections of results and discussion, when deemed necessary.

Detector signals were monitored via the instrument's own electrometer(s) on a Fisher Recordall Series 5000 dual pen strip-chart recorder, or the data were collected by a 386 IBM compatible PC with 80287 math co-processor, VGA display adaptor and multi sync monitor. The data were collected at a frequency of 10 Hz by the CHROM program.
(41) or by dedicated software and hardware of the 10 channel multiplexing FPD (42).

2.1.5 Series RFD/FID Detectors

This unit is designed as an attachment to the Shimadzu ECD described earlier. A schematic of the RFD/FID prototype is shown in Figure 2.4. When connected, it allows sequential detection of any analyte by the ECD, RFD and FID. The ECD signal was monitored in the constant-current mode (as supplied), while the RFD and FID signals were monitored by a Tracor electrometer unit modified to work as a 'stand alone' electrometer and polariser.

2.2 Chemicals

The main analyte used in the ECD study was the strong electron capturer $\alpha$-hexachloro-cyclohexane, commonly referred to as $\alpha$-HCCH. This was supplied by ICN Labs. Inc. (Irvine, CA, U.S.A.) and was used without further purification.

Working standards were prepared by sequential dilution of a stock solution. The solvent used was hexane (Caledon Laboratories Ltd., Georgetown, ON, Canada), 'distilled in glass', 'non-UV'. High sensitivity work required that the solvent be distilled from calcium hydride under a stream of nitrogen, and used immediately thereafter (43).
Figure 2.4: Schematic of the RFD/FID
CHAPTER 3

Constant-Current Electron Capture Detection

with Response by Decrease of Frequency

3.1 Introduction.

As originally invented by Lovelock, the first commercial electron capture detectors were driven by direct-current sources. Because of their then low sensitivities, short linear ranges and various problems poorly understood but commonly attributed to space charges, the d.c. mode gave way to unipolar pulsed methods of operation, to the extent that the "constant-current" (variable frequency) technique has become the standard offering of manufacturers of GC-ECD systems. Pulsing regimes have generally yielded better sensitivities as well as an extended linear range. Unipolar "constant-current" (frequency-modulated) operation usually produces a linear range of about four orders of magnitude. However, the Tracor ECD used in this study (and some other ECD's not specially designed for "constant-current" operation) realises only three orders of magnitude linearity (18). This has been attributed to the two-chamber ('space charge') design of this detector as compared to the single chamber ('stirred reactor') configuration of many other commercial models, such as the Shimadzu ECD also used in this study.

Constant-current operation in the unipolar mode - both d.c. (13) and pulse (11) driven, has clearly generated an extended linear range. Later, electron capture detectors
have been shown to perform equally well with bipolar (a.c.) as with conventional unipolar polarization sources in non "constant-current" configuration (14-16).

The pioneering paper describing a.c electron capture detection suggests an interesting possibility. Operating an ECD under a "constant-current" constraint that responds to analyte by a decrease (as compared to the conventional increase) of frequency (15). That it would, is not immediately perceptible. Under constant-voltage d.c. or unipolar pulse constant-frequency polarization, analyte entering the ECD captures free electrons and effects a decrease of cell current. This drop has been compensated for by an increase of the d.c. voltage (12, 13) or an increase of the pulse frequency, i.e. the conventional constant-current mode of operation (11). These modes utilize feedback circuitry to keep the ECD current constant and have been very successful in improving the linear range of the system. The typical value is about four orders compared to the two orders (at maximum) under non-constant current conditions. Thus, it would seem obvious that an ECD used with feedback circuitry has to respond by an increase of the polarizing source, for d.c. polarization an increase of voltage, and for unipolar pulses an increase of frequency.

However, initial work with a.c. polarization suggests that the electron concentration within the ECD is directly proportional to the pulser frequency (at high frequencies), as compared to the low frequency region (and the conventional unipolar pulse mode) where the electron concentration varies inversely with the frequency of the applied pulses (15).

The current study was designed to verify whether or not an ECD operating under
a "constant-current" constraint could indeed produce linear response by decrease of frequency. Additionally, a.c. constant frequency profiles varied with the individual detector, but the overall patterns were the same. The two detectors used in this study vary immensely in geometry, construction and volume, thus corresponding to the two extremes. The Tracor two chamber construction results in a heterogenous plasma distribution while the Shimadzu coaxial design presents a much more homogenous ion-pair distribution within the cell. Thus it is interesting to compare the performance of the two detectors under an a.c. constant-current regime.

3.2 Experimental.

In unipolar constant-current ECDs, either voltage or frequency could be used as the recorded variable. However, theoretical arguments suggest that the use of voltage is not a viable option for the bipolar a.c. constant-current ECD.

Electronic circuitry was designed that in essence compares the ECD current (after conversion to a voltage) with a predetermined reference voltage (indicative of the desired ECD operating current), and maintains the former at the same level by a feedback loop that regulates the frequency of pulses applied to the detector. The two detectors necessitated different electronic configuration for a.c constant-current operation. Figure 3.1 show the block diagrams of the systems used for the Tracor and Shimadzu ECDs respectively. A detailed schematic of the pulser unit is included in Appendix 1. This unit was designed and built by Brian Millier of the Electronics Shop, Department of Chemistry, Dalhousie University.
Figure 3.1: Block diagrams of the a.c. constant-current systems used for the Tracor ECD (above) and Shimadzu ECD (below). I/V = current to voltage converter, VCO = voltage controlled oscillator, F/F = Flip Flop.
In its experimental configuration, the electrometer (for the Tracor system the electrometer supplied with the original instrument and for the Shimadzu system the lab-made electrometer) serves as the current to voltage converter. This voltage is further amplified (by a factor of 10) through an inverting (or by operator's choice, non-inverting) operational amplifier and then summed with a voltage from the reference potentiometer, by a summing operational amplifier circuit with a gain of 200. The polarity of the voltage resulting from the detector (i.e. after the first operational amplifier) is opposite to that of the reference potentiometer; thus the setting of the reference potentiometer in effect determines the quiescent current of the detector, since it controls the frequency of the VCO (voltage controlled oscillator) which is fed by the output of the summing amplifier. A sample of the voltage being fed to the VCO is sent to the recorder to monitor VCO frequency. This gives the chromatogram (plot of frequency vs. time). The VCO has a highly linear voltage to frequency transfer ratio, allowing this form of frequency monitoring to be valid. At the higher extremes of its range the VCO does not produce a square wave with a precise 50% duty cycle, therefore a simple flip-flop follows the VCO to give a 'perfect' waveform. A potentiometer to precisely control the d.c. offset voltage (thus converting the +15 V unipolar pulses to ±7.5 V a.c. pulses), and a buffer amplifier (amplification factor of 2) complete the circuit.

Analytes passing through the ECD cause a reduction of current (as is usually the case). At high frequencies the baseline current is re-established by a decrease of the operating frequency, as compared to an increase of frequency in the conventional unipolar "constant-current" systems. The first operational amplifier of the circuit serves either in
an inverting or non-inverting configuration to allow operation at high or low frequencies, respectively; and the reference voltage could be set to either positive or negative values, thus allowing the appropriate base frequency and response mode.

3.3 Results and Discussion.

Current-frequency and response-frequency profiles generated by alternating current polarization of ECDs have been reported, but for the purposes of this study these core experiments were repeated under similar conditions to allow adequate comparison (some of the ECDs and instrumentation described in the pioneering paper on a.c. electron capture detection (15) were not available here).

For ease of reference, Figure 3.2 show current/frequency profiles by a.c. polarization of the Tracor and Shimadzu ECDs. While the overall pattern (initial rise with frequency to a plateau, then a decline as the frequency becomes higher) is the same; the behaviour of each detector is different at the low and high frequency regions. The low frequency region correlates well with the corresponding unipolar counterpart, and with the cation-electron recombination rate (15, 44). The difference in this region is dependent on detector construction. In the Tracor two chamber design (essentially an electron generating chamber connected by a narrow electrically insulating channel to the electron collecting chamber) electrons, once pushed over to the collecting chamber, if not collected have little chance of recombining with cations. Thus, at low frequencies, the forward biased half-wave acts similar to a d.c. constant voltage drive, i.e. at 10 Hz in Figure 3.2, the Tracor detector shows a current of about 1 nA. However, in the "stirred
Figure 3.2: a.c. current/frequency profiles of the Tracor ECD: +/- 5 V, and of the Shimadzu ECD: +/- 2 V; rectangular wave. Tracor ECD polarized by programmable pulser, and Shimadzu ECD polarized by Wavetek function generator.
reactor" design of the Shimadzu ECD; cation-electron recombination is dominant at low frequencies, hence the current is very low (re: the 10 to 100 Hz region of Figure 3.2). As frequency increases and the reverse biased half-wave becomes shorter than the time for cation-electron recombination (ca. 0.5 ms) essentially all the \( \beta' \) generated electrons are collected with the forward biased half-wave; and the current increases to a plateau level. This occurs at about \( 10^3 \) to \( 10^4 \) Hz for the Tracor detector and at about \( 10^3 \) to \( 10^5 \) Hz for the Shimadzu ECD.

At still higher frequencies, the plateau comes to an end and current decreases with increasing frequency. This decline has been associated with the phenomenon of electron oscillation - the pulses become too short to transport the electron out of the plasma to the collecting electrode. Electron oscillation becomes dominant at very high frequencies and reduces the current to zero. The frequency at which this happens is different for the two detectors - the shorter the inter-electrode distance, the higher the frequency (15). Figure 3.2 shows that the drop from the plateau occurs at frequencies of about an order of magnitude lower (\( > 10^4 \) Hz) in the Tracor ECD (of longer inter-electrode distance) as compared to the Shimadzu ECD (\( > 10^5 \) Hz).

For any individual detector, the frequency at which oscillation occurs could be shifted approximately an order of magnitude to higher frequencies. That is an extension of the "plateau" region shown in the current-frequency profile of Figure 3.2. This change in optimum oscillation frequency could be brought about simply by using an argon - 5% methane mixture as carrier gas, instead of nitrogen. The mobility of electrons in Ar - 5% \( \text{CH}_4 \) is about an order of magnitude higher compared to electron mobility in nitrogen.
Therefore, the current decline due to electron oscillation shifts to correspondingly higher frequencies. However, the rise in current at low frequencies (100 - 1000 Hz region) is independent of electron mobility and remains unaffected, since it is contingent on the cation-electron recombination rate (16). Speculatively, a wider plateau region would result in a longer linear range (of the calibration curve) for an a.c. constant-current system.

With unipolar constant current systems, either voltage or frequency could be used as the recorded variable. However, the same cannot be said for an ECD operating under constant current conditions when utilizing an a.c. polarizing source. Figure 3.3 shows the current-voltage profiles for the Shimadzu ECD under d.c. and a.c. constant-frequency polarization.

Under a d.c. drive, the current rises with voltage and then reaches a "steady state" - further increase of voltage does not increase the current. This was used as the basis of a d.c. constant-current drive for electron capture detectors (12,13). In that study, at d.c. voltages as high as 1000 V the current plateau was not affected.

With a.c. polarization, the current rises with amplitude until all electrons present during the regular field are collected. It reaches a maximum and then declines with further increase of amplitude (Figure 3.3). This occurs because the reverse field current keeps increasing, thus decreasing the overall current. [The d.c. electrometer used with the Shimadzu ECD has a time constant of about 1 s, hence only the 'net' current - sum of electrons and positives collected - is observed].

The contribution of the reverse-field current to the overall current is dependent
Figure 3.3: Current-voltage profile by d.c. - constant voltage and a.c. - constant frequency excitation. Shimadzu ECD.
upon the detector geometry and the operating frequency of the polarizing source. For the "stirred-reactor" configuration of the Shimadzu ECD (Figure 2.2) and the high a.c. polarizing frequency (200 KHz); the contribution of the reverse field current is very significant (Figure 3.3). This suggests that pulser frequency is the only reasonable monitoring parameter for an ECD operating under a constant-current constraint with a.c. polarization.

Conventional unipolar frequency-modulated, "constant-current" systems operate on the correlation that exists between current and frequency; i.e. the frequency is used as the indicating parameter. The profile for such a system correlates in time with the low-frequency region of the a.c. profile in Figure 3.2. In addition, response correlation between the two polarization modes has been reported (45).

For the conventional system, pioneered by Maggs, Joynes, Davies, and Lovelock (11); and on the low frequency end of the a.c. profile (Figure 3.2), the ECD current (I) increases with frequency (f), i.e.

\[ I \propto f \]  \hspace{1cm} (3.1)

Thus, the electron concentration \([e^-]\) in the cell decreases with increasing frequency, or

\[ [e^-] \propto 1/f \]  \hspace{1cm} (3.2)

The analogous assumption for a.c. constant current electron capture detection at high frequencies - clearly obvious from Figure 3.2, it would be

\[ [e^-] \propto f \]  \hspace{1cm} (3.3)

Analyte entering the ECD captures free electrons and reduces the current. In the
conventional case the decline in current is counteracted by an increase of pulser frequency (Equation 3.2). However, in the novel mode described herein, the reduction in current is compensated for by a decrease of the VCO frequency (Equation 3.3).

A typical current-frequency profile could be run under "clean" (analyte free), and "doped" (analyte present) conditions. Such a profile is shown for the Tracor ECD in Figure 3.4, with various amounts of α-HCCH injected. This was done with a bipolar square wave input of constant amplitude throughout, and constant frequency for each data point.

At constant frequency, detector response is expressed by the vertical distance (i.e. the difference in current, solid line in Figure 3.4) between the analyte-free, and the analyte-containing lines. However, under a constant-current constraint, detector response would be represented by the horizontal distance (i.e. the difference in frequency, dotted line) between the "clean" and "doped" curves of Figure 3.4.

With frequency-modulated "constant-current" systems, the upper end of the linear range is imposed by the upper end of the available pulsing frequency range, or by a breakdown of the proportionality between current and frequency. For conventional constant-current systems, the latter holds true for low detector operating currents, and the former for high detector currents. In practice, low detector currents are used (less than 50% of the detector's maximum current), since at higher operating current the noise significantly lowers the sensitivity and the linear range is thus shortened at its lower end (i.e. the detection limit). Experiments carried out on both detectors suggest that the same holds true for an ECD operating under an a.c. constant-current constraint.
Figure 3.4: Current-frequency profiles of a.c. ECD under various amounts of α-HCCH injected. Tracor ECD.
Figure 3.5: Calibration curves for α-HCCH on the Tracor ECD under various polarization modes using α-HCCH as the analyte. The response axes for the three plots were deliberately offset to place the curves on the same graph.
Table 3.1: Performance of Tracor ECD under various Polarization Regimes.

<table>
<thead>
<tr>
<th>Polarization mode</th>
<th>MDA(^a) (10^{-18}) mole/s</th>
<th>Linearity(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d.c. - constant voltage (-18 V)</td>
<td>140 ± 23</td>
<td>1.8 ± 0.05</td>
</tr>
<tr>
<td>a.c. - constant frequency</td>
<td>34 ± 3</td>
<td>2.0 ± 0.02</td>
</tr>
<tr>
<td>(±20 V, 70 KHz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.c. - constant current (0.5 nA)</td>
<td>30 ± 3</td>
<td>2.5 ± 0.05</td>
</tr>
</tbody>
</table>

Table 3.2: Performance of Shimadzu ECD under various Polarization Regimes.

<table>
<thead>
<tr>
<th>Polarization mode</th>
<th>MDA(^a) (10^{-18}) mole/s</th>
<th>Linearity(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d.c. - constant voltage (-0.2 V)</td>
<td>100 ± 11</td>
<td>2.0 ± 0.04</td>
</tr>
<tr>
<td>a.c. - constant frequency</td>
<td>34 ± 0.3</td>
<td>3.0 ± 0.02</td>
</tr>
<tr>
<td>(±2 V, 300 KHz)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.c. - constant current (0.5 nA)</td>
<td>170 ± 27</td>
<td>1.2 ± 0.02</td>
</tr>
</tbody>
</table>

\(^a\) Minimum detectable amount

\(^b\) Orders of magnitude
Under a.c. constant-frequency conditions, ECD response increases with amplitude up to the maximum value tested: ±70 V (16). The pulsing unit used for this experiment gave an a.c. amplitude of ±15 V, thus low response may be expected. In a conventional system, the lower the response the better is the linear range and it is expected that an a.c. constant-current system would behave in a similar fashion.

Conventional (unipolar) "constant-current" operation has always provided a detector with extended linearity, and it was expected that the a similar result would be obtained in this experiment. Figure 3.5 shows calibration curves obtained under d.c. - constant voltage, a.c. - constant frequency, and a.c. - constant current modes of operation of the Tracor ECD; and Tables 3.1 and 3.2 summarises the performance data of the two detectors under the three different polarization modes.

The data suggest that for the "heterogenous" Tracor ECD, the bipolar a.c. constant current mode is superior to any of the other modes tested. However, the same cannot be said for the more "homogenous" Shimadzu ECD.

Having proven that it is possible to drive an ECD with alternating current under a constant-current constraint, it may be reasonable to speculate how this high frequency system works, as compared to the low frequency conventional system. How do these two differently responding systems keep the current constant?

In the low frequency region, the constant-current drive - whether d.c., unipolar pulsed, or bipolar (a.c. or pulsed) - works against the cation-electron recombination rate (44). And in the high frequency region, the constant-current regime - a.c. or bipolar pulsed types only - cycles so fast that it becomes less and less efficient at electron
collection with increasing frequencies; that is electron oscillation as opposed to electron collection becomes dominant (15).

In a constant-frequency system, such as that of Figure 3.2, the area of most efficient electron collection is represented by the plateau region. Since electron collection is high, the cation-electron recombination rate is low and so is the free electron concentration. On both sides of the plateau, the recombination rate as well as the concentration of free electrons is large - especially so on the high frequency side (15, 44).

The electron capture reaction is first order in electrons (response is proportional to analyte concentration). In addition, electrons that are captured by analyte molecules form heavy anions. The heavier and slower anions increase the cell impedance, thus the cation concentration increases and so does their recombination with free electron (24). A constant-current system will therefore have to work to keep electrons away from both the electron capture and the recombination reactions by collecting with greater efficiency, that is by moving in frequency toward the plateau region of the current profile. Therefore, for the low frequency regime, a increase and for the high frequency regime, a decrease in frequency results.
CHAPTER 4

A Perfectly Linear, High-Sensitivity Electron Capture Detector

4.1 Introduction

Initially ECDs were polarized by constant voltage (d.c.) sources and later by pulsing regimes. The move from continuous to pulsed excitation did reveal much of what is currently known about these detectors. Even though d.c. sources may have been unconsciously discriminated against in the early days, pulsing regimes still remain the polarization mode of choice for commercial instrument manufacturers. Most chromatographers believe that pulsed sources do allow the capture reaction to occur under ‘field free’ conditions, and that enhanced response, hence enhanced sensitivity, results. They also believe that unipolar pulsing allows the linear range to span a greater percentage of the baseline current.

With the advent of alternating current sources, and the associated theory that at high frequencies the electrons oscillate as a result of the bipolar nature of the polarisation regime, interesting questions arise. What would happen if the bipolar pulses of an a.c. regime were separated? That is if ‘field-free’ intervals were inserted between pulses of alternating polarity. In effect, instead of forcing electrons to oscillate continuously, they would be allowed to relax. What would the current and response profiles then look like? Would there be enhanced capture? And enhanced response? Would the linearity of the system be affected? And what other benefits or detriments would be associated with the
use of different bipolar pulse sequences? This series of experiments was designed with the aim of finding answers to these and similar questions.

4.2 Experimental

The Tracor gas chromatograph and conditions are as described in Section 2.1.1. The ECD excitation source was the programmable pulser described in Section 2.1.3. The pulser output was always connected to the radioactive foil of the ECD, thus a "negative" pulse imposed a "regular" field and a "positive" pulse a "reversed" field across the detector cell. Further details of experimental conditions and parameters will be given when called for in the discussion.

4.3 Predictions, Results and Discussion.

4.3.1 The Current Profile.

The pioneering paper describing a.c. electron capture detection (15) suggests that due to the alternating bipolar nature of the polarization regime, electron oscillation (as opposed to collection) takes place at high frequencies. This oscillation of electrons has the effect of 'storing' (increasing the residence time of) electrons in the upper (foil) chamber of the Tracor two-chamber detector, causing enhanced cation-electron recombination and a reduced current.

At low frequencies, the current is low because the regular-field is on only 50% of the time, and the reversed-field half-cycle produces a small positive current (the d.c. electrometer used only sees the net current), but at high frequencies the pulse widths
become too small and changes in polarity too quick to effectively 'push' all electrons over to the collecting electrode; thus a reduced current (and, at very high frequencies, no current) is observed. Somewhere in between (at medium frequencies) a current plateau exists. These observations suggest that at high frequencies, should the positive and negative portions of the a.c. regime be separated, a higher current level may be attained since electron oscillation could be interrupted (oscillation being defined as movement in one direction followed immediately by movement in the opposite direction). However, the positive portion still remains and the current level is not expected to reach the maximum d.c. limit (at maximum d.c. current, all electrons are collected) or even the corresponding unipolar negative pulse level, but be close to (and above) the a.c. plateau level.

Experimentally, using rectangular a.c. waveforms with a 12 μs cycle, the two pulses (positive and negative portions of the a.c. waveform) were incrementally separated and the current levels were monitored. These were compared to current levels from a normal a.c. regime of the same amplitude (± 75 V), and a unipolar pulse train. Each of the regular-field pulses used in this study (which for 6 μs exert -75 V) would, by the tenets of the classical theory of ECD operation be strong enough to clear the cell of electrons (7-8, 21-23). The results are presented in Figure 4.1.

The results clearly show that as the positive and negative portions of the a.c. waveform are separated (following the profiles from right to left), the current level rises. This rise continues and stabilises to a plateau level when the two pulses are approximately 15 μs apart. A similar study, but toward a different end, produced comparable results (16).
Figure 4.1: A comparison of the current profiles produced with a.c. (○), bipolar (□) and unipolar pulse (△) square wave sequences, in the Tracor ECD. a.c. excitation and bipolar pulsing: ± 75 V, bipolar pulse widths = 6 μs with incremental separation of pulses such that the distance between any two pulses is the same; unipolar negative pulse: -75 V, width = 6 μs. Maximum d.c. current: 2.88 nA.
The current remains at this level up to an interval of about 60 μs between the pulses. At low frequencies, the decline in the observed current was comparable for polarisation by either the unipolar negative pulse train or the bipolar pulse train, suggesting that the observed current is dependent on the cation-electron recombination rate. But why these similarities and differences between the profiles?

At low frequencies (ca. 0.1 - 5 KHz), it is the two periodically pulsing regimes whose profiles join, that is the positive pulse of the bipolar pulse sequence is ineffective in this region. This is reasonable since the reversed-field current is only a minute fraction of the regular-field current (15), and the system has sufficient time to relax between pulses [ca. ≥ 0.5 ms - the time required for cation-electron recombination (44)]. At very low frequencies, both profiles must drop to zero, since the distance between the pulses is so large as to make the pulses ineffective.

For the a.c. profile however, the current does not, and cannot, drop to zero (for this particular detector design, cf. Fig. 3.2). A strong 75 V is continuously being imposed in one or the other direction. As the frequency becomes lower and lower, i.e. the "d.c." portions of opposing polarity becomes longer and longer, the measurements will show correspondingly alternating regular- and reversed-field d.c. currents, if the pulse width is significantly longer than the time constant of the measuring circuit. However, when the pulse width is shorter (as is the case for ≥ 100 Hz in Figure 4.1) then the net d.c. current is seen - it approaches a level that is slightly lower than half the conventional d.c. current at -75 V (15).

At medium frequencies, the bipolar regimes reach slightly different current
plateaus. The left side rise to the plateau is governed by the cation-electron recombination rate (44). The two pulsing regimes reach higher plateaus than the a.c. regime - a consequence of the heavier a.c. regime's higher reversed field current and non-relaxation.

At high frequencies, it is the a.c. and the bipolar pulsed profiles that join and jointly drop to zero. However, the unipolar pulsed profile continues on a plateau level corresponding to the -75 V d.c. current. This is as expected, since as the frequency becomes higher the unipolar pulses more and more become like d.c. However, the bipolar pulses are starting to approach each other closely (at 83 KHz they would theoretically merge) and begin to resemble an a.c. regime. The a.c. pulses become too short in that region to drive electrons to the collecting electrode (15), that is electrons oscillate at high frequencies until they recombine with cations or collide with some conductive structure (in an analyte free system). The two profiles join and start to drop from the plateau at about 67 KHz, a frequency that is dependent on the pulse amplitude (15).

One way of investigating the current profile, would be to start with a negative pulse train of reasonable amplitude, width, and (high) frequency; and superimpose a positive pulse train of equal amplitude and frequency, whose position is slowly shifted relative to the negative pulse train. This would define the effect the positive pulse has upon the current as it moves away from a negative pulse and approaches the following one. In effect, we would be able to see whether the presence of a positive pulse striking before the negative pulse would have the same effect as after it. (Of course, one could
as well start with a positive pulse train and superimpose a negative one).

However, before this is done, it would be appropriate to determine what pulse width should be used to maximize free electron collection - particularly in light of the fact that little knowledge exists about the behaviour of bipolar ECD systems at high frequencies. Figure 4.2 presents the current profiles obtained from a unique application of the bipolar programmable pulse generator. With the pulse sequence repeating every 200 µs, and a positive pulse at 1 µs and a negative pulse at 100 µs delay time in the sequence, the third pulse of negative polarity was shifted along to various positions to cover the repetition cycle, and hence to double the strength of the fixed negative pulse (when coming close to its 100 µs delay time). These results clearly indicate that a negative pulse width of 2 µs is insufficient to collect all the free electrons; when the two negative pulses add up to a combined width of 4 µs, the current still rises. However, a 5 µs pulse does effectively collect 'all' free electrons (i.e. increasing the width to 10 µs does not further increase the current).

The current profiles of Figure 4.2 do suggest that bipolar pulses do produce other effects on the charge polarization within the ECD. The stationary part of the pulse sequence resembles bipolar pulsing at 5 KHz - positive and negative pulses strike evenly every 100 µs; and the 'mobile' negative pulse strikes in the available intervals.

The "mobile" negative pulse ("2") is moved from a position closely following the stationary positive pulse ("1") to a position closely preceding, then closely succeeding the central negative pulse ("3"), to a position closely approaching the starting positive pulse ("1") of the next sequence, that is the "mobile" negative pulse strikes just before and after
Figure 4.2: Current profiles with various pulse widths. Tracer ECD. Polarization source: programmable pulser. 3 pulses: pulse 1: +100 V (fixed), delay = 1 μs; pulse 2: -100 V (mobile), delay (D) = variable; pulse 3: -100 V (fixed), delay = 100 μs. Sequence repeats every 200 μs. Pulse widths = 2 μs (□) or 5 μs (○).
both positive and negative stationary pulses. The effect of these four close encounters are more clearly seen from the 2 μs profile of Figure 4.2.

These four cases are typical of a bipolar pulsed system, and for the 2 μs profile, they represent a system in which both recombination and collection of ionic species account for significant fractions of the available charge, i.e., the pulses are too weak to clear the cell of electrons. In each of the four close encounter cases, the fairly constant current level is being severely disturbed. This disturbance occurs on the average, at about 8 μs - a time that is consistent with the mobility of free electrons (46). And the disturbance appears to be consistent, regardless of pulse polarity and precedence - it does not matter whether the pulses are of the same or opposite polarity, or which pulse leads and which follows.

In the central region of Figure 4.2 where the 2 μs negative pulses get close to each other, we can expect them to act like one 4 μs pulse; and they seem to do so by producing, as expected, a higher current. But why is the mobile pulse when not close to its centrally located companion, less efficient at electron collection?

In the region where the two negative pulses are spaced far from one another (about 10 to 80 μs and 120 to 190 μs), the time span is enough to allow the system to relax. The only explanation that could be envisioned is a process in which some of the electrons, having been pushed by the first negative pulse into the detector channel leading to the anode - but not far enough through to be immediately collected - are both pulled back (by the positive space charge they left behind) and pushed back (by their own negative space charge) before the second negative pulse strikes. However, if the second
negative pulse should strike immediately after the first, the electrons cannot be pushed/pulled back, but are rather given another push forward to the anode for collection. Since collection is irreversible, closely spaced negative pulses (of no relaxation interval) collect a larger current than more widely spaced collection pulses.

The results show that when pulses of the same polarity approach each other, an increase of current occurs; and if the closely approaching pulses are of opposite polarity a drop in current is observed. But is the latter effect reasonable? It may make sense if the positive pulse precedes the negative (collecting) pulse, but at the right side of Figure 4.2 it doesn't. When the negative pulse precedes the positive pulse the current should not decrease since electron collection is final. Why would a positive pulse (and more so a weak one, not capable of producing a significant increase of reverse-field current) following a collecting pulse effect a decrease of current? The following paragraphs examine this question in more detail.

The interesting effect is an increase and decrease of current that occur only upon close encounter of the pulses (at high frequencies). This may be related to the fact that a decrease of current from the plateau level with increasing frequency (speculatively attributed to the onset of "electron oscillation") was observed in the Tracor ECD to begin at about 4 KHz, when excited by an alternating potential of amplitude +/- 10.6 V, sine wave (15). Figure 4.2 suggest that the effect would be discernable at a sequence repetition frequency of 10 KHz.

Therefore, using the bipolar programmable pulser with different (+/-) pulse amplitudes for each data set, and a pulse width of 5 μs at a frequency of 10 KHz, the
position of the positive ("disturbing") pulse was varied with respect to the stationary negative ("collecting") pulse, and the resulting current profile(s) determined. These results are presented in Figures 4.3 and 4.4. In addition, the current level in the absence of the positive pulse (negative pulses only) is indicated in each plot by a horizontal line.

As expected, at a high pulse amplitude (± 100 V) the current level is clearly lower than that produced by the unipolar negative pulse train, i.e. the presence of the strong positive pulse effects the collection of a (small) number of cations and the d.c. electrometer sees only the 'net' charge collected. However, it would seem that at low voltages (± 20 V, ± 50 V), the positive half of the pulse train has the effect of reducing the effective cation-electron recombination rate, thus allowing more electrons to be carried over by the ensuing negative pulse to the collecting electrode. This, paradoxically, results in a current level higher than that caused by the negative pulses only. It is possible (though mere speculation) that the positive pulse somehow disturbs the plasma distribution, thus reducing cation-electron recombination so that more electrons are collected with the subsequent negative (collection) pulse. The variation of observed current with pulse amplitude is shown in Figure 4.5.

The second order cation-electron recombination reaction is highly concentration sensitive. This reaction in conjunction with the activity of the radioactive foil determines the highest possible concentration of ion-pairs in a clean detector (29, 44). In addition, the distribution of ion-pairs in the ECD is heterogenous over the dimensions of the detector cell, due to the roughly exponential distribution of ion-pairs generated by the $^6$Ni betas, i.e. a relatively thin layer of fairly dense plasma lines the radioactive foil (16, 18).
Therefore any process that disturbs the charge distribution in the detector will affect the recombination rate, and allow overall higher charged-particle concentrations to exist.

This is exactly what the positive pulse does: it pulls electrons toward and pushes cations away from, the radioactive foil. In contrast, the negative pulse does the opposite and effects electron collection. The resulting increase in the overall electron concentration allows a larger current to be later collected. But why is there a voltage dependence?

In Figures 4.3 to 4.5, at low voltages the current is low. Therefore, few charged particles are collected, hence they must recombine and possibly recombination proceeds close to its maximum rate. In contrast, at high voltages the current is close to its maximum value, i.e. most charged particles are collected, and very few recombine (a low recombination rate).

Therefore, at a very low voltage setting the effect of the disturbing pulse on the overall plasma distribution must be small, since the rate of ion-pair generation is far higher than the rate of ion-pair collection. However, the disturbing pulse affects the collection process much more strongly. Electrons that are not collected when the negative pulse is on have a high chance of recombining with the greater number of wider distributed cations. When the time is too short for relaxation to occur, the number of collected electrons (and the current) severely drops. The effect is so strong that the bipolar current remains below the unipolar current even in the middle of the plot.

At the very high voltage setting the effect of the disturbing pulse on the ion-pair distribution could be strong and that should decrease the recombination rate. However,
Figure 4.3: The effect on the ECD standing current produced by varying the position of the positive pulse in a bipolar two pulse regime. Pulse amplitudes: ±10 V, ±20 V, repetition time: 100 μs. Pulse widths: 5 μs. The horizontal line indicates the corresponding unipolar current level. D = delay time.
Figure 4.4: The effect on the ECD standing current produced by varying the position of the positive pulse in a bipolar two pulse regime. Pulse amplitudes: ±50 V, ±100 V, repetition time: 100 μs. Pulse widths: 5 μs. The horizontal line indicates the corresponding unipolar current level. D = delay time.
Figure 4.5: Variation of maximum bipolar current as percentage of unipolar current, with respect to pulse amplitude. Tractor ECD. Polarisation source: programmable pulser.
ion-pair collection is high, hence the recombination rate must be low, thus the effect has to be small. Therefore the bipolar current remains close to the unipolar current, but below it since the positive pulse collects a small reverse current which is summed with the electron current by the slow electrometer.

At intermediate voltage settings, the system is not governed by either a high recombination rate, nor by a high collection rate; thus the number of collected ion-pairs should be comparable to the number of recombined ion-pairs. This gives the positive pulse the chance to disturb the charged particle distribution enough to suppress the recombination rate and a higher current is collected from the now higher charged-particle concentration.

These results also indicate that as oscillation is interrupted and the system presumably relaxes between pulses, the current increases to a plateau level, that is as the bipolar pulses are separated, the current rises and levels off when the pulses are approximately 15 μs apart (re: Figures 4.3 and 4.4). In common with the behaviour of a.c. sources, this plateau becomes more prominent as the amplitude of the pulses is increased. But what happens in the relaxation interval? In other words, why does the increase to and decrease from the plateau occur?

The profiles of Figure 4.3 and 4.4 indicate that the current drops dramatically when the positive pulse closely leads or follows the negative pulse and especially at low voltages, the current drops to zero. In mechanistic terms, the effect has to be different for each configuration. The "disturbing" positive pulse preceding the "collecting" negative pulse is expected to produce a decrease in current, but why should a positive
pulse following a negative pulse produce a decrease in current, especially so since electron collection is final?

The only reasonable speculation for the current drop on the left side of Figures 4.3 and 4.4 (where the negative pulse leads the positive pulse) is that the electrons were transported from the foil chamber through the central channel into the electron collecting chamber, but they did not actually reach the anode during the negative pulse. If no positive pulses were to follow, some of the electrons would migrate to the collecting electrode, being driven there by their own negative space charge. Some might also return through the channel to the foil chamber, being pushed there by their own space charge, and being pulled there by the net positive space charge of the plasma.

However, if the positive pulse should hit before the system has had time to relax, most of the electrons (that were pushed by the preceding negative pulse) would be driven back to the foil chamber, where they either recombine with cations or again pushed over to the collecting chamber (by the subsequent negative pulse). However, this assumed scenario will work only if the pulses are so weak that they are just able to push electrons out of the plasma and into the channel and at low voltages, the experimental results bear this out. At high voltages, the subsequent positive pulse cannot reverse the electron flow since the pulses are strong enough to drive the electrons all the way to the anode where they are collected.

The right side decrease in current from the plateau level in Figures 4.3 and 4.4 is expected. The ECD behaves like a rectifier diode - it allows the current to flow much more easily in the regular-field direction than in the reversed-field direction (24).
Therefore, the positive pulse would only polarize the charge distribution in the foil chamber because it is too weak to push larger numbers of cations into the collection chamber and electrons into the foil. Thus, if the subsequent negative pulse strikes before the system has had time to relax (≤ 15 μs), it would need extra strength to overcome the polarisation and effect electron collection. Since both pulses are of equal (but opposite) amplitude, the effect is expected to be less dependent on voltage; an expectation that is confirmed by the experimental results.

As a matter of completeness, it was interesting to observe what effect the variation of positive pulse strength would have on the current. With this in mind, the width and the amplitude of the positive pulse were varied (each separately) while the parameters of the negative pulse were kept constant. Figure 4.6 presents the current profile when both the positive and negative pulse amplitudes are equal, but the width of the positive pulse is varied. It should be noted that although these experiments were done at a frequency of 10 kHz - a region where electron oscillation is expected to take place under a.c. conditions, any two pulses were separated by a minimum of 24 μs, which should be enough to allow the system to relax (the effect of electron oscillation is not present or is minimal). These results suggest that there exists an optimum positive pulse width for producing the maximum current output. The position of this maximum varies with the amplitude of the pulses. At low amplitudes it takes a wider positive pulse to give maximum current output; while at high amplitudes the width of the positive pulse required to optimize current output is smaller. That is the system depends - though not linearly - on the "effective voltage" (pulse amplitude and width). A similar
Figure 4.6: Effect on the standing current caused by variation of the positive pulse width, in a bipolar two pulse regime. Repetition time: 100 μs, pulse 1 (negative): delay = 1 μs, width = 5 μs. Pulse 2 (positive): delay = 30 μs. Pulse amplitudes: (○) +/- 50 V, (△) +/- 75 V, (□) +/- 100 V.
Figure 4.7: Effect on the standing current caused by variation of the positive pulse amplitude, in a bipolar two pulse regime. Repetition time: 100 μs. Pulse widths: 5 μs. Pulse 1 (negative) delay = 1 μs, pulse 2 (positive) delay = 50 μs. Pulse 2 amplitude = -50 V (v), -75 V (□), and -100 V (O).
behaviour was observed with the use of unipolar pulse sequences (18).

According to the classical theory of ECD operation, there are two major processes occurring within the detector that decrease the number of charged particles. These are cation-electron recombination and cation-anion neutralization. The latter requires the presence of an analyte (electron-capturer), and since these experiments were done in 'pure' nitrogen gas, no analyte-derived anions were present. This then leaves as the only explanation the recombination process. However, even though cation-electron recombination is expected to be the dominant factor, it should be noted that the presence of the reversed-field pulse will result in the collection of (a small number of) cations at the collecting electrode, and of (some) electrons at the foil; with the collected 'net' charge being reported as the current.

It would seem that at lower voltages, as the width of the positive pulse is increased, it slows the recombination reaction by separating cations from electrons with the latter then being collected by the ensuing negative pulse. The positive pulse may even reduce (to a small extent) the amount of cations in the upper (foil) chamber of the detector, giving electrons less of a chance to recombine with the fewer cations present. As the positive pulse width increases it increases the positive ("reversed-field") current, and thereby reduces the overall current as seen by the d.c. electrometer.

Figure 4.7 shows the current profile produced when the amplitude of the positive pulse is varied with respect to a constant negative pulse amplitude, with both pulses being of the same width. Similar reasoning to that used above, suggests that at a low negative pulse amplitude there is a noteworthy increase in the current level. However, since the
pulse width is small, increasing the positive pulse amplitude does not cause a significant drop in the standing current.

In a sense, these experiments confirm the fact that the presence of a positive pulse, paradoxically, can increase the detector's standing current. It rules out any suspicion that the effect seen in the earlier curves may have been an electronic artefact.

4.3.2 The Response Profile

What is responsible for response within the ECD? As far as the primary process is concerned, the obvious answer is: the capture of electrons by analyte molecules. But what happens then? Two conflicting answers have been given to this question. The "Classical Model" relies on neutralization of analyte-derived anions as the reaction responsible for response. However, one of its major drawbacks is that it cannot explain hypercoulometry (the apparent capture of more than one electron by an analyte molecule). The other explanation relies on the "Space Charge Model", which assumes the slow migration to, and possible collection at, the anode of analyte-derived anions, a process that creates a negative space charge that, in turn, impedes electron migration and thus enhances cation-electron recombination; the consequent reduction in current is seen as response. This Space Charge mechanism is compatible with the phenomenon of hypercoulometry. There is no clear-cut evidence to suggest that either one of these mechanisms alone is entirely responsible for response. Both may take place simultaneously; to what extent is unknown (18, 28).

If the current profiles presented in the previous section may be used as a guide,
then it is fairly obvious that, as the positive pulse is moved away from the negative pulse (from left to right), more electrons are left over as a result of reduced cation-electron recombination. These electrons essentially remain in the upper foil chamber of the detector until they are collected by the next negative pulse. How would this affect response? The presence of the positive pulse effects 'storage' of electrons, hence when the analyte enters the detector, enhanced capture is expected to take place. This, of course, may result in a larger response which may even be hypercoulometric. A recent publication suggested that all response in commercially available, conventional ECDs is hypercoulometric, if based not on the moles of analyte but on the moles of anions formed from it (28). However, at low currents, even though the capture rate may be high, the response may not be seen because the current is not available to translate the "capture" into "response". Therefore, the prediction is that the response would increase as the positive pulse is moved away from the negative pulse. Of course, as the positive pulse closely approaches the ensuing negative pulse, and the current decreases steeply, the response would also drop.

The experimentally derived results are presented in Figures 4.8 and 4.9. The response profiles, superimposed on the corresponding current profiles, turn out as predicted, i.e. an increase in response with the movement of the positive pulse away from the negative pulse. In addition, at higher voltages, two response maxima corresponding to the inflection points of the current profile are observed. The magnitude of the response maxima varies for obvious reasons: the higher response maxima occurs where many more electrons and hence a higher current is available to translate electron capture into response.
Figure 4.8: (*) Response profile produced by the bipolar two pulse sequence, superimposed on the corresponding current profile (o). Pulse amplitude: +/- 20 V, repetition time: 100 μs, pulse width: 5 μs. Analyte: 5 pg α-HCCH. D = Delay time.
Figure 4.9: (○) Response profile produced by the bipolar two pulse sequence, superimposed on the corresponding current profile (○). Pulse amplitude: +/- 50 V, repetition time: 100 μs, pulse width: 5 μs. Analyte: 5 pg α-HCCH. D = Delay time.
Constant-voltage d.c. excitation of an ECD produces a response/voltage profile that shows a maximum corresponding to the 'knee' of the current/voltage curve. However, a.c. excitation suggests that the response increases with the voltage (16). As to what this excitation mode would produce as the voltage of the pulses is increased, can only be ascertained by experiment. Figure 4.10 presents the response/voltage profile superimposed on the current/voltage profile at a frequency characteristic of the higher response maximum. Interestingly enough, this picture is very similar to a current/voltage plot from conventional d.c. polarisation. The voltage is much higher in the bipolar pulse plot, but then that voltage is only "on" for a short fraction of the time. At high voltages, most of the electrons are collected hence unavailable for capture and response is low, and at low voltages the baseline current is very low, the cell current (analyte present) is lower, and the difference between the two (the response) must also be low. It should be noted that at these conditions the electron capture rate is high, but the observed response does not reflect that. Somewhere between these extremes must lie a maximum - at about the 'knee' of the current/voltage profile. This is not necessarily different from the results of the a.c. study - the maximum a.c. amplitude used was ± 70 V, which was probably not enough to effect a decrease in response.

To answer another question frequently asked of ECD performance: is the response coulometric or hypercoulometric? If the behaviour of the higher (right side) maximum under bipolar pulsing conditions does indeed resemble d.c. (and therefore a typical 'space-charge' mechanism) response should turn out to be hypercoulometric. The right side axis of Figure 4.10 represents the response profile in terms of the number of electrons captured.
per molecule of $\alpha$-HCCH. The result clearly shows that the response is hypercoulometric even at low voltages. At maximum, it suggests the apparent 'capture' of close to four electrons per molecule of analyte. Presumably, like its d.c. counterpart, the position of the response maximum should shift to higher voltages with an increase in the amount of the analyte, and the hypercoulometric ratio should increase with pressure.

4.3.3 Sensitivity and Linear Range

In practice, the performance of any quantitative chromatographic system is judged by its sensitivity and linearity. How does the system described herein perform? At its best, with $\alpha$-HCCH as the analyte, a minimum detectable amount of $5 \times 10^{-18} \text{ mol/s}$ and a linear range of approximately three orders of magnitude was obtained (Figure 4.11). This is with the positive pulse ending 3 μs before the negative pulse strikes (or a positive pulse delay of 93 μs in a 100 μs pulse period). More importantly, however, is the observation that the linearity ends because the detector standing current has been completely exhausted (see chromatograms in Figure 4.12). This is most unusual. Conventional ECD calibration curves (non constant-current) become non-linear when response exceeds around, say, 10 - 30% of the standing current. Linearity right up to 100% of the standing current appears incompatible with the kinetics of a second-order capture reaction. To date, this is the only case in which the linear range of an ECD system extends all the way to zero standing current. In other words, this polarisation mode produces a totally linear ECD response. Since the ECD responds by a reduction of standing current, it should be obvious that, if the total current is completely "consumed" (speculatively: completely
Figure 4.10: Effect of pulse amplitude (at constant frequency) on the current and response, at the right side (+/-) response maximum. Analyte: 5 pg α-HCCH. Bipolar pulsing: repetition time = 100 µs, pulse widths = 5 µs. Pulse 1 (negative) delay = 1 µs; pulse 2 (positive) delay = 93 µs. (○) current, (+) response.
Figure 4.11: Calibration curve of α-HCCH produced at a positive pulse delay of 93 μs.

Bipolar pulsing: repetition time = 100 μs. Pulse 1 (negative): delay = 1 μs, width = 5 μs.

Pulse 2 (positive): delay = 93 μs, width = 5 μs. Pulse amplitude: ±75 V.
Figure 4.12: Chromatograms depicting the total consumption of 'free' electrons. ECD excitation conditions as for Figure 4.11. α-HCCH = 5 pg, 20 pg, 50 pg, 100 pg, (s) = solvent peak. Note that the extra peaks in the right side chromatograms are presumably oxidation products of the solvent, hexane (43).
impeded by the negative space charge) the linear range can extend no further.

These results suggest that if the standing current could be increased, for instance by using a tritium containing foil (whose ionisation rate is much larger than that of nickel-63), then the linear range of the system could be further increased (in terms of higher loadings of analyte). It should be noted however, that the detection limit of the system may be affected since a higher activity foil produces a larger noise level (and the detection limit is quoted at a signal to noise ratio of 2).

However, another practical alternative is to use an argon-methane mixture as carrier gas (5% methane in argon being a commonly used ECD carrier gas). This approach has previously been utilized in this laboratory with a.c. polarization of the Tracor ECD, yielding a maximum of 4.5 nA current using the argon-methane mixture as compared to only 3.2 nA with nitrogen (16). Speculatively, use of the argon-methane mixture may result in a linear range in excess of the three orders of magnitude realized in this study.

How does this extraordinary effect of a perfectly linear calibration curve occur? The obvious but non-committal answer is that it is caused by the presence of the positive pulse. Other than a.c. excitation done by our group, there is one other publication relating performance to bipolar pulsing of ECD's. Simon and Wells (47), for their bipolar constant-current ECD, used a pulse regime consisting of an initial extraction pulse (negative portion), immediately followed by a reverse-biased (positive) pulse. (Note: this is opposite of our better mode). They suggested that the positive pulse "repairs" the inhomogeneity within the plasma caused by the collecting pulse, and minimizes the
collection of anions. And, in common with this study, linearity improved (but not to the exhaustion of the standing current).

The fact that the observed response is hypercoulometric (Figure 4.10) suggests a major contribution from the space charge mechanism. The Varian detector used in Simon and Wells' study is of completely different design than that used in ours. The former can be described more or less as a 'stirred reactor', but the Tracor ECD (used in this study) is of a two chamber construction and has often been used to model non-homogenous ECD kinetics.

The one thing that is very obvious is that the presence of the positive pulse does improve the linearity. In the Varian detector, the positive pulse immediately follows the negative one; improved linearity was observed but the response became non-linear at high analyte concentrations. On the other hand, the Tracor detector was excited with the positive pulse striking 3 μs before the collecting pulse, and the calibration curve was completely linear. This does suggest that the position of the positive pulse influences the linearity of the system - a suggestion that is easily demonstrated by experiment. Using the programmable bipolar pulser, the linear range was determined when the positive pulse followed the negative pulse by 1 μs (the left response maximum), and 44 μs (in the current plateau region) of the response profiles as shown in Figures 4.8 and 4.9.

The linear ranges of the calibration curves suggest that, as the reverse biased pulse is shifted from one collecting pulse toward the next, the linearity only improves drastically when the reverse biased pulse strikes close to the subsequent collecting pulse. More precisely, at a fixed frequency of 10 kHz, with the positive pulse at delay times of
7, 50, and 93 μs; the linearity was 61, 58, and 100 % respectively, of the baseline currents.

Exactly how this effect occurs is unknown. The basic electron-capture reaction may be represented as:

$$e^- + A \rightarrow A^-$$

where A represents the analyte molecule. The rate of reaction is given by:

$$\text{Rate} = k [e^-][A]$$

where k is the capture constant for the analyte. The results imply a first order reaction, which suggest that for varying analyte concentrations, the rate and hence response, is directly proportional to the analyte concentration (and the system is linear) as long as the electron concentration is not significantly depleted. Experience has shown this level to vary with the excitation mode of the detector. For the Tracor ECD used in this study, the determined values (for 10 % deviation from linearity) are about 34 % by d.c. excitation, and approximately 47 % (of baseline values) for the unipolar pulsed mode. However, even if we were to consider the linearity as a percentage of the maximum d.c. current (2.88 nA), our bipolar regime allows a linear range extension of 2 times that of the corresponding unipolar polarisation regime (Table 4.1).

A 100 % linearity could mean that either the free electron concentration is unimportant (an unlikely situation) or that the presence of the reverse biased pulse does truly increase the concentration (residence time) of electrons within the detector. Electron "storage" would also be congruous with the observed excellent sensitivity.
Table 4.1: Variation of linearity of Tracor ECD with polarisation mode.

<table>
<thead>
<tr>
<th>Polarisation mode</th>
<th>Linearity as % of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>maximum d.c. current</td>
</tr>
<tr>
<td>direct current</td>
<td>28</td>
</tr>
<tr>
<td>pulsed unipolar</td>
<td>34</td>
</tr>
<tr>
<td>pulsed bipolar</td>
<td>(68)**</td>
</tr>
</tbody>
</table>

* All constant voltage, constant frequency

** Baseline current limited
CHAPTER 5

A Study of the Relaxation Phenomenon in a "Stirred Reactor" Design

Electron Capture Detector

5.1 Introduction

Bipolar polarization of an ECD with a non-homogenous ion-pair distribution resulted in a high sensitivity detector that also produced a perfectly linear calibration curve (Chapter 4). The non-relaxed a.c. constant frequency polarized detector performed better than (and sometimes comparable to) its unipolar counterpart. However, a.c. constant frequency polarization of the Shimadzu ECD (of nearly "homogenous" ion-pair distribution) at high frequency resulted in a much more sensitive detector with a longer linear range of the calibration curve: 34 attomole s\(^{-1}\) and 3 orders of magnitude for a.c. constant frequency polarization (at 300 KHz, ±2 V) as compared to 100 attomole s\(^{-1}\) and about 2 orders of magnitude linearity for d.c. polarization (Table 3.2.)

The basic processes occurring within ECD's are the same, regardless of their geometry. However, the shape of the detector affects the kinetics of these processes. In the Tracor ECD (Figure 2.1), the electron collecting chamber can be safely assumed to be relatively free of cations. Cations being larger and slower compared with electrons have little chance of making it through the narrow channel into the electron collecting chamber unless, of course, they are being "pushed" or "pulled" by an appropriate field.
gradient and even then the contribution is small. A similar situation, cannot be assumed for the Shimadzu ECD (Figure 2.2.) where the design and geometry allows the electron-generating and electron-collecting processes to occur within the same chamber.

This study was designed to evaluate the effects of the bipolar relaxation phenomenon within a "stirred-reactor" design ECD. More specifically: would the Shimadzu ECD behave similarly to the non-homogenous Tracor ECD? And, if not: what mechanistic processes within a homogenous type ECD are responsible?

5.2 Experimental

The operating conditions of the Shimadzu gas chromatograph are as described in Section 2.1.2. The ECD was polarized by 9 V batteries (connected in series when necessary), and the programmable pulser described in Section 2.1.3. Further details of experimental conditions and parameters will be given when appropriate, in the Results and Discussion section.

5.3 Results and Discussion

5.3.1. Detector Homogeneity

Electron capture detectors can be classified into two major categories. Cell configurations that give "non-homogenous" and "close to homogenous" ion-pair distributions are frequently described in the literature (7, 8). The parallel plate and "centre pin" coaxial designs are the ones that give a more homogenous plasma distribution. The Shimadzu ECD used for this study is of the latter type. Computer
simulation of the possible mechanism(s) within ECDs are generally based on homogenous ion-pair distribution. Such theories are replete in the literature and the cell is often referred to as a "stirred reactor", although obviously no physical stirring takes place.

If indeed the ion-pair distribution was homogenous, then under a d.c. drive, the same current-voltage and response-voltage profiles would be obtained whether the foil was negatively polarized (electrons collected) or positively polarized (positive ions collected), i.e. the regular or reversed field modes respectively. However, even in a symmetrical cell construction, this is obviously not the case. The impedance of an ECD to ion collection is two-fold - physical and chemical. In fact, an ECD operates much like a diode (or rectifier); that is it allows the current to flow more easily in one direction than in the other. It is this behaviour that allowed the ECD to function adequately with an a.c. polarizing source using a d.c. amplifier, without any additional circuitry (15).

Figure 5.1 shows the current-voltage and response-voltage plots for the Shimadzu coaxial "centre pin" ECD operated in the regular and reversed field d.c. modes. It is clear from these profiles that even if the plasma is homogenous, the detector is not electrically isotropic, i.e. electrons are more easily collected in the regular field mode compared to cations in the reverse field mode. In a "clean" (analyte free) ECD, under a given electrical field strength, the velocity of unimpeded electrons is about 3 to 4 orders of magnitude faster compared to cations. Generally, it is the "residence time" of cations (and in the presence of analyte, anions) that determines the current. However, other factors are important. The rate of electron generation by interaction of β radiation with molecules of the carrier and/or purge gas (and if analyte is present, the electron capture
Figure 5.1: Current-voltage and response-voltage profiles of the Shimadzu ECD when operated in the "regular" and "reversed-field" d.c. modes. Filled symbols: current, open symbols: response to 5 pg α-HCCH.
rate of the particular analyte), the cation-electron recombination rate (and in the presence of analyte, the cation-anion neutralization rate), and space charges that control current flow within the detector. These "chemical" parameters are responsible for the electrical anisotropy of the Shimadzu ECD.

In a non-homogenous detector, such as the Tracor ECD, electrical anisotropy is brought about primarily by asymmetric detector geometry. In fact, asymmetric electrode placement within ECD's is very common. The main impetus favouring the asymmetric geometry is that the more difficult it is to collect electrons, the better the chance of capture and hence the higher the response. However, the space charge theory has suggested that a higher response is possible without greater capture, a suggestion that has been demonstrated by experiment (48).

In a d.c. drive, for the regular field direction, electrons are driven to the collecting electrode and cations to the foil; in the reverse field mode the observed current is due to cation collection, while electrons are lost by cation-electron recombination and by collection on the foil. For the pulse mode, the space charge is different as compared to that created by a continuous d.c. field. In fact for a coaxial centre pin ECD, operating in the pulse mode the maximum d.c. current is never achieved. Grimsrud and Warden (49) estimated that in the pulse mode, up to 25% of maximum d.c. current (due to electron collection) is not seen, the reason being that the electron current is reduced due to migration of positive ions to the collecting electrode and only the 'net' current is observed. However, like the d.c. mode response in the regular field pulse mode is much much greater as compared to the reverse field mode.
5.3.2. The Current Profile

Current-frequency profiles by a.c. polarization of the Tracor and Shimadzu ECDs are shown in Figure 3.2. These profiles, as well as comparison of the respective d.c. profiles suggest that the "impedance to electron collection" of the Shimadzu ECD is about an order of magnitude lower compared to the Tracor detector. For the Tracor system, the optimum relaxation conditions were observed at a pulse repetition time of 100 μs, which would suggest that, if present, a similar effect would be observed in the Shimadzu ECD at frequencies close to 100 KHz.

Figure 5.2 presents the current profile obtained by polarizing the Shimadzu ECD with bipolar pulses of varying (+/-) amplitudes, at a pulse repetition time of 12 μs, using pulse widths of 1 μs. The current level obtained with positive pulses only is compared with that obtained by varying the position of reverse-biased (negative) pulse, in the bipolar two pulse sequence.

Similar to the Tracor system (Figure 4.3 and 4.4) the current rises dramatically and becomes higher than that due to the collecting pulses only (at the same frequency), when the reverse biased pulse approaches the subsequent collecting pulse. The effect is more dramatic at low voltages, as is also the case for the non-homogenous Tracor ECD. However, when the reverse bias pulse immediately follows the collecting pulse, the observed current decreases below that due to the collecting pulse sequence. This effect was absent in the two chamber Tracor detector.

The profiles were done under "clean" pulse conditions, i.e. no analyte present. Hence, the dominant processes are thermal electron generation and cation-electron
Figure 5.2: The effect on the ECD standing current produced by varying the position of the reverse bias pulse in a bipolar two pulse regime. Pulse width: 1 μs, repetition time: 12 μs. The horizontal lines represent the corresponding unipolar current levels.
recombination:

Ionization: \[ M + \beta^+ \longrightarrow M^+ + e^- + \beta^- \text{ (weaker)} \] (5.1)

Recombination: \[ e^- + M^+ \longrightarrow M \] (5.2)

where \( e^- \) = electrons or any anion and \( M^+ \) = any cation

Electron loss by reaction with electron capturing species of the carrier gas, by diffusion to the walls and by ventilation with the carrier gas flow, are generally low and thus considered insignificant. Also, cations could be produced by ionization of species other than carrier gas molecules: such as stationary phase bleed, carrier gas impurities, etc.

In the Tracor detector, it was suggested that electrons once pushed through the channel and into the collection chamber have little chance of recombining with cations and are essentially collected. However, in the Shimadzu ECD, a reverse bias pulse immediately after the collecting pulse essentially "pushes" the electrons into the positive space charge zone (close to the foil) and hence increases cation-electron recombination and the observed current drops. It should be noted that the current was monitored via an electrometer with a 1 s time constant - thus only the 'net' current was observed. In addition, a reverse bias pulse would collect positives, which as discussed earlier could reduce the maximum observable current by as much as 25%. But, the major effect has to be on the cation-electron recombination reaction. If it was not, the bipolar current profile would be similar to the unipolar current profile, but somewhat lower due to positive ion collection.
With some delay (about 2 μs) between the reverse bias pulse following the collecting pulse, the effect reaches a maximum; and an electrical double layer once again develops (cations close to the foil and electrons further away). As would be expected the negative pulse would push the electrons away from the collecting electrode, but now, the "pushed" electrons encounter electrostatic repulsion from the electrons of the "double layer". Recombination is thus much reduced and the free electrons are collected by the next positive pulse. The current increases to a maximum when the reverse bias pulse is about 1.5 μs before the collecting pulse when the current begins to drop. The positive and negative pulses are so close together that electrons oscillate with the field, and are not collected.

5.3.3. The Response Profile

For unipolar pulse (constant frequency) operation, ECD response shifts with the effective voltage, i.e. response is dependent on pulse amplitude and width (V*t). This is generally the case - for the d.c. constant voltage mode, response is very dependent upon the voltage. Thus, for a bipolar system response could be expected to vary with voltage, but their exact interdependence could only be ascertained by experiment.

Similar to the Tracor detector, a bipolar pulsed "stirred reactor" (Shimadzu) ECD behaves in a similar way to d.c. polarization (cf. Figures 4.10 & 5.3). Such a behaviour is indicative of a space charge phenomenon. It should be noted that the space charge theory is different for heterogenous and homogenous ECDs (24, 29).

For a "non-homogenous" detector, a slow moving space charge of anions (formed
Figure 5.3: Current-voltage and response-voltage profiles for the bipolar pulsed Shimadzu ECD. Response to 5 pg α-HCCH. Pulse repetition time: 12 μs, pulse widths: 1 μs, negative pulse delay: 4 μs.
by interaction of electrons with molecules of the analyte) impedes by electrostatic repulsion, electron collection and increases cation-electron recombination. The scenario is different in a "homogenous" ECD. Cation-anion neutralization is much slower compared to cation-electron recombination. This slower neutralization effectively increases the cation concentration within the detector (since electrical neutrality must prevail). This increased cation concentration results in increased cation-electron recombination. In both systems the increased recombination results in the observed response being higher than the coulometric limit. It should be noted that a higher response does not necessarily mean greater reaction (electron capture by analyte), since reaction and response have been separately monitored within an ECD; and reaction can be of the same extent while response could be higher (26).

Much more important to this study is the effect voltage has on response in a bipolar system as the position of the negative pulse is varied with respect to the positive pulse. Figures 5.4 and 5.5 show the variation in response with voltage as the reverse bias pulse is moved to different positions in the repetition cycle of the forward bias pulse. It is clearly obvious from these profiles that while the response at the edge (reverse-bias pulse just before the forward bias pulse) is independent of voltage, the central response maximum is highly dependent upon the voltage and the position of the negative pulse. The best response being observed at ± 2.5 V, and a reverse bias pulse delay of 6 µs in a 12 µs bipolar pulse period.

Interestingly, a two point simplex optimization procedure using ± volts and negative pulse delay as variables [the pulse period of 12 µs and pulse width of 1 µs was
**Figure 5.4:** Response profile (V) produced by the bipolar two pulse sequence, superimposed on the corresponding current profile (●). Pulse amplitude: ± 2 V (above), ± 3 V (below); repetition time: 12 μs, pulse width: 1 μs. Analyte: 5 pg α-HCCH.
Figure 5.5: Response profile (o) produced by the bipolar two pulse sequence, superimposed on the corresponding current profile (●). Pulse amplitude: ± 2.5 V, repetition time: 12 μs, pulse width: 1 μs. Analyte: 5 pg α-HCCH.
showed that maximum response could be obtained at ± 2.3 V at a reverse bias pulse delay of 6.0 - 6.4 μs, that is close to that presented in Figure 5.5. However, if the simplex procedure is expanded to include the pulse period as an additional variable, then the response maximum is located at a negative pulse delay of 16 - 18 μs, a pulse period of 20 - 22 μs, and ± 2.2 V. A response profile done at 20 μs pulse period, ± 2.2 V with the variable being the negative pulse delay showed that the response profile essentially followed the current profile.

From a mechanistic point of view, it would be interesting to see whether the relative response varies with the type of electron capturer. With this in mind, the response of pentachloroethane, nitrobenzene, and tetrabromoethane [Supplier: Organic Mini-Stockroom (Model 0-1000A) of Chem-Service, West Chester, PA.], as well as two unknown peaks which showed up in the chromatogram were compared at different positions of the negative pulse. For this experiment ± 2.3 V pulses, with the sequence repeating every 12 μs were used. Although an arbitrary amount of analyte was tested in the mixture, it was ensured that the concentration present was within the linear range, simply by injecting multiple amounts and checking whether the response varied in proportion to the injection volume. Table 5.1 presents the response with respect to nitrobenzene, the central peak in the 5 peak chromatogram. Interestingly, the response of the different electron capturers is about the same regardless of the position of the negative pulse. This does suggest that it is one major mechanism that controls the response, in the ECD, regardless of the nature of the electron capturer, i.e. associative, dissociative, etc.
Table 5.1: Response vs. position of reverse bias pulse in a bipolar two pulse sequence.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Negative pulse delay (µs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.8</td>
</tr>
<tr>
<td>Pentachloroethane</td>
<td>1.5 ± 0.0</td>
</tr>
<tr>
<td>Unknown #1</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>1.0</td>
</tr>
<tr>
<td>Unknown #2</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Tetrabromoethane</td>
<td>2.4 ± 0.1</td>
</tr>
</tbody>
</table>

Hyercoulemetry, the phenomenon that suggests that an ECD consumes more electrons than moles of analytes supplied, has been observed before within asymmetric design ECDs. The hypercoulometry ratio varies with the type and operating conditions of the detector; and does not allow the ECD to be used with a great degree of precision as a gas phase coulometer, i.e. without using calibration standards. However, hypercoulometry is useful in that the higher the ratio the greater the sensitivity of the detector.

The more asymmetric a detector, the longer it takes for electrons to be collected. In the presence of analyte, the chance of electron capture would be higher and could lead to a large hypercoulometric ratio. However, in a symmetric ECD, one would not expect
hypercoulometric behaviour. The largest hypercoulometric ratio has been found in a pressurized, d.c. driven, asymmetric ECD. There, at 5 atm pressure, one molecule of analyte seemed to be able to capture up to 50 electrons (48). On the basis of electrostatic repulsion alone, such a capture is impossible. Hypercoulometry was therefore explained on the basis of an increased cation-electron recombination rate, in the presence of anions.

Hypercoulometry was observed in a unipolar pulse driven, axially offset concentric ECD (28); and in a bipolar asymmetric ECD (Chapter 4, Figure 4.10). The right side axis of Figure 5.5 shows that hypercoulometry is present in a bipolar pulsed "stirred reactor" type ECD, and under these conditions one molecule of $\alpha$-HCCH seems capable of capturing three electrons.

Table 5.2 gives the peak to peak noise level, SNR, MDA, the hypercoulometric ratio, and the linearity as the position of the negative pulse is varied with respect to the collecting pulse, when the pulse sequence repeats every 12 µs.

The data show that within a bipolar two pulse sequence, when the position of the positive pulse is fixed and that of the negative pulse is varied, the minimum detectable amount and SNR improve as the reverse bias pulse occurs close to (but just before) the collecting pulse. However, it would seem that the presence and position of the reverse-bias pulse affects the noise within the detector. The minimum noise level is achieved when the negative pulse is just before the collecting pulse and the worst noise level is seen when the negative pulse closely follows the collecting pulse.

It is very unlikely that the reverse-bias pulse could affect the noise due to the random nature of radioactive decay. Therefore, it is more likely that the noise is due to
Table 5.2: Performance characteristics of the Shimadzu ECD when polarized with a bipolar two pulse sequence; where the position of the reverse bias pulse is varied with respect to the positive pulse. Pulse width: 1 μs, pulse amplitude: ± 2.3 V, pulse sequence repeats every 12 μs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0</th>
<th>1.3</th>
<th>6.2</th>
<th>8.5</th>
<th>10.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noise (pA)</td>
<td>3.0 ± 0.2</td>
<td>3.3 ± 0.3</td>
<td>2.2 ± 0.2</td>
<td>2.7 ± 0.1</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>$^1$SNR</td>
<td>2110 ± 76</td>
<td>1389 ± 58</td>
<td>2399 ± 117</td>
<td>3806 ± 94</td>
<td>7562 ± 184</td>
</tr>
<tr>
<td>$^2$MDA</td>
<td>93 ± 5</td>
<td>141 ± 13</td>
<td>82 ± 7</td>
<td>51 ± 2</td>
<td>46 ± 4</td>
</tr>
<tr>
<td>$^3$HCR</td>
<td>1.0</td>
<td>0.7</td>
<td>0.8</td>
<td>1.6</td>
<td>1.2</td>
</tr>
<tr>
<td>$^4$Linear range</td>
<td>1.2</td>
<td>1.0</td>
<td>1.6</td>
<td>1.1</td>
<td>1.3</td>
</tr>
</tbody>
</table>

*: Positive pulses only
$^1$: Signal to Noise Ratio (based on peak height analysis)
$^2$: Minimum Detectable Amount (attomole/sec)
$^3$: HyperCoulometric Ratio
$^4$: Linear Range (orders of magnitude)

ion (in particular positive ion) collection. This would seem to corroborate with the observed current profile where a larger current was seen when the reverse bias pulse was close to, and before the collecting pulse. Also, for the optimized regular field mode d.c. operation, the noise level was half that of the optimized reversed field d.c. mode. In fact,
at their respective response maximum, the peak to peak noise levels were 0.6 pA and 1.2 pA respectively, for the regular- and reverse-field modes of d.c. operation. Thus, it would seem that the collection of ions, as opposed to electron collection, influences the observed noise level.

As the reverse bias pulse moves across its range (from just after the collecting pulse to just before it), the sensitivity varies but not in a similar pattern. The position of the negative pulse influences the amount of electrons apparently captured per molecule of analyte ($\alpha$-HCCH). This is quite easily seen from the data in Table 5.2. The best hypercoulometric ratios are observed at 8.5 and 10.8 $\mu$s. At shorter delay times, the amount of electrons apparently captured per molecule of analyte is less than that when the positive pulses only are present.
CHAPTER 6

A Bipolar Pulsed (Constant Frequency) Electron Capture Detector

6.1 Introduction.

Current-frequency profiles generated by a single unipolar pulse of fixed amplitude and width, and by using an alternating voltage source, show considerable difference at low frequencies. In addition, the profile for an a.c. source showed a decline from the plateau level at high frequencies. The low frequency end difference was due to the large pulse width of the forward biased half-wave (which is comparable to the normal field d.c. mode); while the high frequency decline has been attributed to electron oscillation.

However, for the Tracor two-chamber detector the bipolar frequency profiles generated by alternating pulses of the same width and amplitude are similar to the unipolar pulse profile at low frequencies: 10 Hz to 10 KHz (Figure 4.1). This similarity was unexpected since a reversed bias pulse supposedly hampers electron collection, and may assist with cation collection and should therefore effect a reduction of current.

The relaxation phenomena observed within the same detector suggest that for a bipolar system response is very much dependent upon whether the reverse bias pulse occurs before or after the collecting pulse. This series of experiments was designed to undertake a more comprehensive study of bipolar pulsed ECDs, both in the two-chamber Tracor detector and in the single chamber Shimadzu ECD.
6.2 Experimental

The gas chromatographs and operating conditions are described in Sections 2.1.1 and 2.1.2. The ECD polarization source was the bipolar programmable pulser described in Section 2.1.3. Further details of experimental conditions and parameters will be given in the Results and Discussion section.

6.3 Results and Discussion

6.3.1 The Tracor Detector

The following four waveforms were used in this study:

These waveforms were generated by the bipolar programmable pulser and fed directly to the ECD. Detector current was monitored via the electrometer supplied with the Tracor gas chromatograph.

Waveform I is a single unipolar pulse of 5 μs width and -50 V amplitude; waveform II consists of a 5 μs pulse of -50 V amplitude followed by a 20 μs delay time and a +50 V, 5 μs pulse; waveform III is the opposite of waveform II: a 5 μs pulse of +50 V amplitude followed by a 20 μs delay, then a -50 V, 5 μs pulse; and waveform IV
was similar to waveform III, with the exception that the pulses were separated by 2 μs.

For the Tracor detector a negative pulse is usually referred to as the forward-bias pulse, since it allows electron collection, and the positive one the reverse-bias pulse. The detector was polarised with these pulses over a range of frequencies from 100 Hz to 20 KHz, the maximum allowable frequency for bipolar waveforms type II and III, and to 70 KHz for waveform type IV.

Figure 6.1 shows the current-frequency and response-frequency profiles generated for the four waveforms, respectively. Over the frequency range examined, the current and response profiles for three waveforms (types I-III) are similar, that is there seems to be no observable difference in the profiles even though the excitation waveforms are different.

However, when the positive pulse occurs before the collecting pulse, the current profile is a trace higher, especially at high frequencies. Having ruled out experimental variation of the data as the source for the difference; and referring to the relaxation phenomenon described in Chapter 4, it seems possible that the current should be higher. This is because for that particular waveform (type III) cation-electron recombination is much reduced. This effect is obviously frequency dependent - as is the case for the unipolar pulse profile. However, a bipolar pulse profile with the positive pulse before the negative pulse has a much more significant effect on the recombination of ion pairs within the ECD. If this is true, then if the reverse bias pulse strikes much closer to and before the collecting pulse; say 2 μs (waveform type IV) as compared to 20 μs for the previous profiles, then the current profile should be different. In fact, if cation-electron
Figure 6.1: Current-frequency and response-frequency profiles for the Tracor ECD when polarised by waveform types I-IV. Analyte: 5 pg α-HCCH.
recombination is indeed reduced under 'non-relaxed' conditions then there should be many more 'free' electrons which should be much easier to capture. Hence, one would expect the response profile to be much higher when compared to the unipolar profile, or to the bipolar sequence which has the two pulses 20 μs apart.

The experimental current-frequency and response-frequency profiles for waveform type IV is also shown in Figure 6.1. The current profile suggests that the electron collecting efficiency of this waveform is not as good as its unipolar counterpart or the bipolar relaxed waveforms (types II and III). However, as expected the response profile is very much higher in the 'non-relaxed' state.

Much more important to this study was to determine whether the bipolar pulse waveforms (compared to the unipolar pulse waveform), when used as ECD polarisation sources, would affect the linearity of the constant-frequency system, and hopefully translate any increase to a constant-current system. The linearity of the first three waveforms was tested with the pulse sequence repeating every 300 μs (or a base frequency of 3.3 KHz). The relevant data are presented in Table 6.1.

At the same base frequency, the baseline current and noise levels are about the same regardless of the type of polarisation waveform. The lower limit for noise in an electron capture detector is generally dictated by the random fluctuation of radioactive decay. However, absolute response is less for waveform type II, as compared to the other two waveforms for which the responses are about the same. The larger response for waveforms type I and III results in better minimum detectable amounts (at a signal to noise ratio of 2).
Table 6.1: Performance characteristics of the Tracor ECD when polarised by unipolar and bipolar waveforms at a constant frequency of 3.3 KHz.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Waveform type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Baseline current, I₀ (pA)</td>
<td>916</td>
</tr>
<tr>
<td>Noise (pA)</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>¹² Sensitivity (pA/pg)</td>
<td>16.5±0.5</td>
</tr>
<tr>
<td>¹² MDA (10⁻¹⁸ mol s⁻¹)</td>
<td>49±3</td>
</tr>
<tr>
<td>Linearity (% of I₀)</td>
<td>48</td>
</tr>
</tbody>
</table>

¹ Analysis based on peak height
² Analyte: α-HCCH
³ MDA: Minimum Detectable Amount

The major effect of the various pulsing regimes seems to be in the extent to which the standing current could be sampled by an electron capturing analyte before the system exhibits non-linearity. With the reverse bias pulse 20 μs after the collecting pulse, the system is only linear to 36% of the baseline current, as compared to 48% and 78% respectively, for the unipolar pulse profile and the bipolar pulse profile where the forward bias pulse is 20 μs after the reverse bias pulse.
From the data presented in Chapter 4, it would be expected that the effect produced when a reverse bias pulse occurs before a collecting pulse is dependent upon the separation of the pulses, as well as the frequency (obviously, pulse width and amplitude would also play a role, but a smaller one when considered in the present context). At higher frequencies and closer distances between the positive and negative pulses, the effect reaches its maximum at 10 KHz with the reverse bias pulse being separated by 3 μs from (and before) the collecting pulse; that is 100% linearity.

6.3.2 The Shimadzu Detector

The design of this system is unlike that of the Tracor detector in that detector polarisation and current monitoring is achieved through the same electrode. This results in a positive pulse being the collecting pulse and the negative pulse being the reverse-bias pulse as compared to the Tracor system where a negative pulse was the collecting pulse and the positive pulse was the reverse-bias pulse. However, the general pattern of the pulses remained the same, although of different width, amplitude and separation distance between the bipolar pulses. The following three waveforms were used for this study:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+15 V</td>
<td>0 V</td>
<td>-15 V</td>
</tr>
</tbody>
</table>
These waveforms were generated by the bipolar programmable pulser and fed to the Shimadzu ECD via a 1:1, 6 mH torroidal transformer. Waveform A is a single unipolar pulse of +15 V amplitude and 1 μs width. Waveform B consists of a 1 μs, -15 V pulse that strikes 2 μs before the collecting pulse (of 1 μs width and +15 V amplitude). Waveform C is the opposite of waveform B in that the collecting pulse strikes 2 μs before the reverse bias pulse. The detector was polarised over a wide range of frequencies between 100 Hz and about 100 KHz.

Figures 6.2 to 6.4 show the current-frequency and response-frequency profiles for the three waveforms. These profiles show that while the current-frequency profiles for waveform types A and C are similar, their response profiles are slightly different. The response maxima for waveform C extends over a wider range of frequencies. However, both the current-frequency and response-frequency profiles for waveform B are different when compared to the other two.

Chapter 5 describes the relaxation phenomenon within the Shimadzu ECD, and the data suggest that relaxation occurs with about 2 μs separation between the reverse-bias and forward-bias pulses. Similar to the Tracor detector, the major effect is seen at high frequencies. Also comparable in that regard is that a reverse-bias pulse striking before a collecting pulse significantly decreases cation-electron recombination, and thus there are more 'free' electrons available. When the current-frequency profiles due to waveforms A and B are compared, it is observed that the profile due to waveform B shift towards commensurately lower frequencies. Since more 'free' electrons are available due to decreased cation-electron recombination, this behaviour is not unexpected. However,
Figure 6.2: Current-frequency and response-frequency profiles for the Shimadzu ECD when polarised by a unipolar waveform, consisting of a 1 μs, +15 V pulse (waveform type A). Response to 5 pg α-HCCH.
Figure 6.3: Current-frequency and response-frequency profiles for the Shimadzu ECD when polarised by a bipolar waveform, initially of a 1 μs, -15 V pulse followed by a 2 μs delay time then a 1 μs, +15 V pulse (waveform type B). Response to 5 pg α-HCCH.
Figure 6.4: Current-frequency and response-frequency profiles for the Shimadzu ECD when polarised by a bipolar waveform, initially of a 1 μs, +15 V pulse followed by a 2 μs delay time then a 1 μs, -15 V pulse (waveform type C). Response to 5 pg α-HCCH.
when the reverse-bias pulse strikes after the collecting pulse, there is no significant
difference in the current-frequency profiles except at high frequencies, where the pulse
period is short. The difference there is due to the effect the reverse-bias pulse has on the
following sequence, more specifically, the next collecting pulse.

When the reverse-bias pulse follows the collecting pulse, it does not matter if there
is a separation or not, since the work of the collecting pulse is already done. In addition,
as suggested above, at high frequencies an effect is due to the presence of the reverse-bias
pulse at some (but close) distance to the collecting pulse. Figure 6.5 compares the
current-frequency profiles due to a reverse-bias pulse, a reverse-bias pulse followed
immediately by a forward-bias pulse, and a forward-bias pulse followed immediately by
a reverse-bias pulse.

The behaviour of the reverse-bias pulse was elaborated upon by Simon and Wells
(47). A reverse-bias pulse immediately following a collecting pulse makes little
difference to the current profile when compared to that generated by the collecting pulse
only. And a collecting pulse immediately after a reverse-bias pulse allows significant
collection of electrons. This can be explained on the basis of electron mobility versus
cation mobility, electrons being 3-4 orders faster compared to cations are more easily
collected. Therefore, the small reverse-bias pulse before the collecting pulse changes the
cation-electron recombination rate and hence the electron current, thus the current-
frequency profile shift towards higher frequencies. The cations being larger, heavier, and
slower are little affected, but a significant portion may be collected. It was suggested that
up to 25% of the electron current may not be seen due to cation collection (49). The
Figure 6.5: Current-frequency profiles for the Shimadzu ECD when polarised by unipolar and bipolar waveforms. Pulse widths: 1 µs. For the bipolar waveform, the opposite polarity pulses follow one another immediately.
currents are integrated and summed out due to the 'slow' time constant of the electrometer.

For the Tracor two-chamber detector, the presence of a reverse-bias pulse before a collecting pulse led to the system being completely linear. That surprising result prompted further questions: Is the effect truly an ECD one? Is it dependent upon detector geometry? These among other questions prompted an investigation into the effect of bipolar pulsing waveforms on a single chamber "stirred reactor" type ECD.

Table 6.2 presents the relevant data, at the response maxima for each of the three waveform types. Similar to the Tracor detector, a reverse-bias pulse, close to and before the collecting pulse, does improve the sensitivity and linearity of the Shimadzu detector, when operated in the bipolar pulsed (constant frequency) mode.
Table 6.2: Performance characteristics of the Shimadzu ECD when polarised by unipolar and bipolar waveforms.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Waveform type</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Baseline frequency (KHz)</td>
<td>12.5</td>
<td>4.5</td>
<td>20.1</td>
<td></td>
</tr>
<tr>
<td>Noise (pA)</td>
<td>1.1</td>
<td>0.86</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>$^{1,2}$Sensitivity (pA/pg)</td>
<td>$12.8 \pm 1.1$</td>
<td>$29.4 \pm .59$</td>
<td>$12.7 \pm 0.42$</td>
<td></td>
</tr>
<tr>
<td>$^{1,2,3}$MDA ($10^{-18}$ mol s$^{-1}$)</td>
<td>$68 \pm 6$</td>
<td>$20 \pm 4$</td>
<td>$68 \pm 3$</td>
<td></td>
</tr>
<tr>
<td>Linearity (% of $I_{oc}$)</td>
<td>15</td>
<td>49</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

1 Analysis based on peak height

2 Analyte: α-HCCH

3 MDA: Minimum Detectable Amount
CHAPTER 7

Bipolar Pulsed Constant-Current Electron Capture Detection

7.1 Introduction.

Constant-current electron capture detectors, utilizing the frequency of the polarising source as the monitoring parameter are widely available from gas chromatograph manufacturers. Such circuits generally utilize a single unipolar pulse of appropriate polarity, to give an electrical field gradient favouring electron collection. The pulses are generally of short width (≤ 1 μs) and high amplitude (≥ 50 V). This is reasonable since it is the best (in terms of sensitivity and linearity, 0.1 pg detection limit and 4 orders of magnitude linearity for lindane, a strong electron capturer) and safest available, to date.

The use of bipolar pulsing regimes has shown that it is possible to increase the extent to which the standing current of the constant-frequency system can be sampled, even to the maximum of 100%. Previous experiments revealed that detector geometry plays a very important role: For the "stirred-reactor" type Shimadzu ECD, it was possible to sample up to 49% of the baseline current, before the system exhibited non-linearity; and for the "non-homogenous" Tracor ECD, the system could be sampled up to the maximum and was therefore entirely linear.

Constant-current systems, whether in the unipolar pulsed, d.c., or a.c. mode have generally increased the linear range of ECDs. One main impetus for this work therefore,
was to determine whether a bipolar pulsed constant-current ECD is better as compared to its unipolar counterpart, or if there are other limiting parameters. Additionally, as may be seen from Figure 4.1 (cf. Chapter 3), a bipolar pulsed ECD should give response by a decrease of frequency, as compared to the conventional unipolar systems that respond with an increase of pulser frequency. It was hoped that a bipolar pulsed constant-current system would prove superior to the other constant-current systems.

7.2 Experimental

The basic principle of the operational feedback circuitry was similar to that described in Section 3.2. However, some of the individual components were different. In particular, the VFC 32 (voltage to frequency converter) chip used in the present circuit is linear over 6 orders of magnitude, as compared to 3 orders linearity for the chip used in the \textit{a.c. constant-current circuit}. As was the case for the \textit{a.c. constant-current study}, the experimental configuration of the systems was different for the Tracor two-chamber (two electrode) detector, and the Shimadzu single-chamber (one electrode) detector. Here the constant current circuit has an additional component; the output of the VCO triggers the bipolar programmable pulser, which sends the desired polarising waveform to the ECD.

7.3 Results and Discussion.

A comparison of the current-frequency profiles generated by unipolar, \textit{a.c.}, and bipolar pulsing of the Tracor ECD (Figure 4.1) shows that at low frequencies (ca. 100 Hz
to 10 KHz), the unipolar and bipolar current-frequency profiles are very similar. This low frequency region has been correlated with the cation-electron recombination rate, and it is expected that the same would hold true for the bipolar waveform; since the forward-bias pulse is the dominant one. In fact, said argument was proposed for the low frequency end of the a.c. regime and was supported by experimental work and theoretical simulation of the detector (44). At high frequencies (ca. 60 to 90 KHz), the bipolar and a.c. profiles are strikingly similar, that is electron oscillation seems to be the dominant mechanism controlling the current at these frequencies. At medium frequencies (ca. 10 to 60 KHz), the bipolar plateau is higher than the wider a.c. plateau. The difference in the current levels has been attributed to the bipolar relaxation phenomenon described in Chapter 4.

The similarity of the unipolar and bipolar pulsing waveforms at the low frequency end suggests that constant-current operation would be possible in the bipolar pulsed mode, giving response by an increase of frequency. Several different bipolar waveforms were used in this study; in particular, a bipolar 'relaxed' waveform, a bipolar 'semi-relaxed' waveform, a 'non-relaxed' bipolar waveform, and an alternating waveform where a forward-bias pulse of fixed amplitude and width is alternated with a reverse-bias pulse of similar amplitude and width, such that at any frequency the distances between the forward-bias and reverse-bias pulses are the same. The performance of these waveforms were evaluated against a single unipolar pulse in both the Tracor and Shimadzu ECDs.

The similarity of the a.c. and bipolar pulses at the high frequency end suggests
Figure 7.1: Current-frequency profiles at the low frequency end for the Tracor ECD when polarised with bipolar pulses. Analyte: α-HCCH.
Figure 7.2: Current-frequency profiles at the high frequency end for the Tracor ECD when polarised with bipolar pulses. Analyte: α-HCCH.
that like the a.c. constant-current study (Chapter 3), a bipolar alternating waveform would
give response by a decrease of the pulser (baseline) frequency. Response by a decrease
of frequency was successful for the a.c. study in both detectors.

Current-frequency profiles done under "clean" (analyte-free) and "doped"
conditions generally outline how a constant-current system may respond. Such profiles
are shown for the Tracor detector at both low and high frequencies in Figures 7.1 and 7.2,
respectively. The bipolar waveform of equally spaced forward and reverse-bias pulses
of ±100 V, 5 μs width, was generated by the bipolar programmable pulser operating in
the alternating mode. The analyte, α-HCCH, was injected with a Hamilton 1 μL syringe.
Detector current was evaluated from the recorder response, by peak height analysis.

In the constant-frequency mode, regardless of frequency, response is indicated by
the vertical difference between the "clean" and "doped" curves (the solid line) in Figures
7.1 and 7.2. However, for the constant-current, frequency-modulated modes of operation,
detector response is given by the horizontal difference between the "clean" and "doped"
curves (the dotted line) in Figures 7.1 and 7.2. For low frequencies (Fig. 7.1), the
constant-current response is indicated by an increase of the pulser frequency; and at high
frequencies (Fig. 7.2), the detector response is given by a decrease of the baseline
frequency.

The profiles of Figure 7.2 are quite unique, in that they suggest that the use of
high detector reference currents is favoured for a linear response by decrease of
frequency. This is unlike the case for the unipolar or low frequency bipolar end, where
low detector currents are favoured. The profiles suggest that for bipolar constant-current
operation at the high frequency end, detector operating currents of about 1.0 to 1.5 nA are favoured, for a linear response. Below about 1.0 nA, an exponential response may be obtained. In fact, these suggestions were confirmed by experiment. A calibration curve done at 0.7 nA showed that response is indeed non-linear; while response was linear at 1.0 and 1.5 nA reference currents. At 1.0 nA, the minimum detectable amount was 0.2 pg of α-HCCH, and response was linear over 1.9 orders of magnitude.

The bipolar constant-current experiments were done with four basic waveforms and the results were compared to that of the unipolar waveform. For the Tracor ECD, the bipolar waveforms were:

Waveform I consists of a 5 μs, -100 V pulse followed immediately by a reverse-bias pulse of the same width and amplitude. This waveform may sometimes be referred to as the "non-relaxed" waveform, since there is no separation distance between the two pulses of opposite polarity. Waveform II, termed the "semi-relaxed" waveform has a 3 μs separation distance between the pulses of opposite polarity; and waveform III, the "relaxed" waveform, has 15 μs separation distance between the forward and reverse-bias pulses. Waveforms I, II, and III are fixed bipolar units that are utilized in the feedback
Waveform IV is the bipolar alternating waveform for which at any given frequency, the distance between two consecutive forward-bias (or reverse-bias) pulses contains a reverse-bias (or forward-bias) pulse that is equidistant from its two neighbouring pulses. These waveforms were evaluated both in the configuration shown (collecting pulse precedes the reverse-bias pulse), and in the opposite configuration where the collecting pulse follows the reverse-bias pulse. However, for the latter configuration, waveforms I, II and III all gave exponential calibration curves; but a linear range experiment done with 22 μs separation distance between the reverse-bias and collecting pulses showed that response becomes linear. This is consistent with the observations of the relaxation phenomenon described in Chapter 4.

A single unipolar pulse of -100 V, 5 μs width and the bipolar alternating waveform (type 4) gave similar result: 0.1 pg detection limit and 4 orders of magnitude linearity with α-HCCH as the analyte, thus suggesting that for the bipolar alternating waveform, the reverse-bias pulse does not get close enough to the forward-bias pulse to induce any noticeable effect. When the reverse-bias pulse immediately follows the forward-bias pulse (waveform type I), response improves but the noise also increases thereby yielding a worse detection limit and a shorter linear range when compared to the unipolar pulses only. However, when a separation distance is introduced between the collecting and reverse-bias pulses (waveform types II and III); the response is still high but the noise decreases thus improving the minimum detectable amount to 6 fg/s of α-HCCH. With 15 μs separation distance, the upper frequency limit of the pulsing waveform was achieved within the linear range, thus resulting in another perfectly linear
calibration curve. Figure 7.3 shows the calibration curve done with waveform type III and the insert shows a chromatogram of 120 fg of $\alpha$-HCCH, i.e. close to the detection limit.

For the Shimadzu detector, bipolar constant-current operation at the low frequency end showed that, similar to the Tracor detector, a bipolar waveform gave higher response as compared to its unipolar counterpart. Also similar was that a reverse-bias pulse immediately before a collecting pulse significantly increased the noise: 0.33 ± 0.05 pA as compared to 0.10 ± 0.02 pA, for a 1 μs, $+15$ V pulse and a 1 μs, $-15$ V pulse, respectively. For a fixed bipolar waveform with the reverse-bias pulse before the collecting pulse, the performance varied with the separation distance between the two pulses, within the fixed pulsing unit. Table 7.1 summarises the data obtained at a reference current of 0.5 nA.
Figure 7.3: Calibration curve of α-HCCH done under constant-current conditions (0.5 nA) with waveform type III. Tracer ECD. Insert: Chromatogram of 120 fg of α-HCCH.
Table 7.1: Performance of the Shimadzu ECD in the constant-current mode, with a bipolar pulsing unit consisting of a reverse-bias pulse followed by a collecting pulse, but with various separation distances. Pulse amplitude: ± 15 V. Pulse width: 1 µs.

<table>
<thead>
<tr>
<th>SD(µs)</th>
<th>Parameter</th>
<th>²S/N</th>
<th>³MDA</th>
<th>⁴Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>²S/N</td>
<td>6.07</td>
<td>0.3</td>
<td>2.8</td>
</tr>
<tr>
<td>0.1</td>
<td>²S/N</td>
<td>13.97</td>
<td>0.1</td>
<td>2.5</td>
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<tr>
<td>0.2</td>
<td>²S/N</td>
<td>15.08</td>
<td>0.1</td>
<td>3.0</td>
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<tr>
<td>0.5</td>
<td>²S/N</td>
<td>13.15</td>
<td>0.1</td>
<td>3.0</td>
</tr>
<tr>
<td>1</td>
<td>²S/N</td>
<td>6.95</td>
<td>0.3</td>
<td>3.0</td>
</tr>
<tr>
<td>2</td>
<td>²S/N</td>
<td>5.93</td>
<td>0.3</td>
<td>3.0</td>
</tr>
<tr>
<td>³ALT</td>
<td>³MDA</td>
<td>5.32</td>
<td>0.4</td>
<td>2.7</td>
</tr>
<tr>
<td>⁶UNI</td>
<td>⁴Range</td>
<td>3.89</td>
<td>0.5</td>
<td>2.7</td>
</tr>
</tbody>
</table>

¹SD: Separation Distance between the pulses
²S/N: Signal to Noise Ratio
³MDA: Minimum Detectable Amount of α-HCCH (pg)
⁴Range: Linearity in orders of magnitude
⁵ALT: Alternating waveform
⁶UNI: Unipolar pulse only
CHAPTER 8

Noise and Filters

8.1 Introduction

In chromatographic (and generally analytical) detection systems, the applicability of a particular detector is judged on its minimum detectable amount (MDA), its dynamic range - preferably the linear dynamic range, and its precision. The precision and the detection limit (hence the dynamic range) are related to the quantity of noise present in the system. Therefore information on the noise characteristics of a detector can be used to optimize its performance.

Chromatographic noise is defined as the full amplitude of the baseline fluctuations. It includes all random variations of the detector signal whose frequency is of the order of one or more cycles per minute, that is short-term drift and spikes included, but long-term drift excluded (50).

The amount of noise present in a system is important since it defines the MDA. The MDA\textsubscript{chrom} (a strictly chromatographic definition) is defined as the amount or flow of a sample that gives a detector signal of two times the noise level (50):

\[
MDA_{\text{chrom}} = 2 \times \frac{N_{\text{g/mol}}}{S} \times A \text{ (g or mol)}
\]
\[ MDA_{chrom} = 2 \times \frac{N_{p-p}}{S} \times \frac{A}{w_{60.7}} \text{ (g/s or mol/s)}; \]

where \( S \) = the signal (peak height),
\( A \) = the amount injected (in g or mol),
\( w_{60.7} \) = the width of the peak (in seconds) at 60.7\% of its height (equal in a Gaussian peak to 2\( \sigma_p \)), and
\( N_{p-p} \) = the peak-to-peak fluctuation of the baseline, i.e. baseline noise.

However, the chromatographic literature presents detection limits obtained by different techniques. Another common definition gives the MDA as the amount or flow of sample that produces a signal of three times the noise, where noise is measured as the root-mean-square (RMS) noise, or the standard deviation (\( \sigma \)) of the baseline fluctuations. This definition is an earlier IUPAC recommendation and is of spectroscopic origin and will therefore be referred to as \( MDA_{spec} \):

\[ MDA_{spec} = 3 \times \frac{RMS}{S} \times A \text{ (g or mol)} \]

or

\[ MDA_{spec} = 3 \times \frac{RMS}{S} \times \frac{A}{w_{60.7}} \text{ (g/s or mol/s)}; \]

(Note: The first number in each equation, i.e. \( p = 2, 3, \text{ etc.} \), is the level-of-confidence or probability-related parameter that - for purposes other than this study - can be adjusted to suit analytical objectives.)

Obviously, each of the equations above simply relates the detection limit to the
commonly used signal to noise ratio (SNR); with noise being whatever the analyst wants it to be:

\[ MDA = p \times \frac{A}{SNR} \text{ (g or mol)} \]

or

\[ MDA = \frac{p}{2\sigma_p} \times \frac{A}{SNR} \text{ (g/s or mol/s)} \]

The only other common way of documenting chromatographic detection limits is pictorial. It displays the recorder trace of a signal small enough, a baseline long enough, and noise large enough, to allow evaluation by the reader.

With several approaches being used to document detection limits, how does the analyst correlate one set of measurements with the other to obtain a reasonable comparison? Apart from the confidence level parameter of the two numerical assessments (S/N = 2, 3) the only other difference is the quantification of noise; whether it is measured as \( N_{pp} \) or calculated as RMS. Do these two assessments correlate? And can one set of measurements accurately predict the other?

Under ideal circumstances of short-term noise and normal (Gaussian) distribution, a reasonable correlation between the theoretically time-dependent \( N_{pp} \) and the theoretically time-independent RMS of baseline fluctuations equates \( N_{pp} \) with five times the RMS noise. That is because for a Gaussian distribution, 5*RMS includes more than 99% (99.4%) of the measurements. But is chromatographic noise always Gaussian?

Filters are widely used to reduce noise and thus improve the SNR. An
improvement in SNR generally leads to a better detection limit. Filters are normally evaluated on white noise, i.e. noise containing all frequencies; and the performance of digital filters is often superior to analog filters in that digital filters can clip 'interfering' frequencies close to those of interest without adversely affecting them. But is the filter type of importance to chromatography?

To answer these and related questions, this study is designed to evaluate the effect of three types of filters (one analog and two digital) on noise from three common chromatographic detectors: the ECD, the FPD, and the FID; and also to determine the noise characteristics of these detectors.

8.2 Experimental

The ECD data were obtained from the Tracor and Shimadzu detectors described in Chapter 2. These were both operated in the d.c. mode. The FID data were from the Shimadzu GC-4BMPF chromatograph and from the FID of the sequential unit described earlier. The FPD data came from the Shimadzu 2-channel detector described in Section 2.1.4. Data for some \( N_{p-p} \) evaluations were measured on a strip-chart recorder, and computer acquired data were \textit{via} a lab-made interface using the CHROM-8 program (41). In addition, some of the FPD data were acquired by a specialised unit equipped with a 600 rpm wheel carrying a semicircular variable interference filter, supported by hardware and software for operation as a ten-channel flame photometric detector with wavelength as the third dimension ["3-D FPD"] (42). The wheel spun at 600 rpm, i.e., at an acquisition time of 5 ms for each 100 ms data point.
All raw data flows were routed through conventional electrometers of time constants: RC = 0.1, 0.22 and 1 s, with the exception of those coming from the 3-D FPD. The photomultiplier output of the latter was processed by a high-speed amplifier and split into ten 5-ms segments per revolution of the wheel; the data were then assigned to one of three ranges of decadic sensitivity, summed by a gated integrator, converted into digital form, and forwarded to a computer for storage and display. In the 3-D FPD software, two types of low-pass digital filters were available for smoothing operations on the ten-channel data: a non-weighted moving average ("AVG") with operator-defined window width; and a "Hamming window" weighted moving average - a conventional finite-impulse-response ("FIR") filter with operator-defined, fully variable cut-off frequency and the choice of 32, 64 or 128 taps (42).

The conventional ("2-D") FPD, the ECD and the FID (the latter two via a lab-built preamplifier) were monitored on the same Shimadzu GC-4BMPF electrometer, which fed an interface and thence a two-channel computer program named "CHROM-8" (41). This program contains, among other routines, the FIR filter algorithm. It also provides a routine that sorts baseline fluctuations according to the magnitude of their deviation from the mean and feeds the resulting array of (raw or smoothed) baseline data to the Dalhousie University Chemistry Department undergraduate network program offering least-squares fitting of Gaussian and other types of curves. The enforced Gaussian fit yields the nominal standard deviation (hereafter called σ\text{fit}), and its graph allows superimposition of the arrayed data for visual inspection. (Note that data files can be imported, a pair at a time, from the newer ten-channel 3-D FPD program into the older
two-channel CHROM-8 program - but not vice versa.)

In addition to the smoothing and evaluating functions that are confined to the computer, the raw or treated data flow can be returned from the CHROM-8 program - or from the 3-D FPD program through the CHROM-8 program - to the analog domain; and it can, via a simple, lab-made three-pole RC filter with various time constants, be recorded on a chart. The blueprint of the analog filter's conventional circuit is given in Appendix 1; it will be referred to as "RC". Raw or smoothed data can also be forwarded directly to a laser printer. And obviously, the electrometer output can remain completely in the analog domain, i.e. it can provide in the conventional manner - usually via the RC filter - confirmatory baselines or chromatograms on the strip-chart recorder.

The CHROM-8's "SIGMA" lab-developed routine (51) employs very heavy digital filtering of the baseline noise (with an operator-adjustable time constant) in order to establish a smooth "zero line", from which the real baseline fluctuations are measured. The algorithm then uses the conventional formula for calculating the RMS deviation:

\[
\text{RMS} = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n}} = \sigma
\]

[Note: The definitions use the value, not the +/- range of the standard deviation.]

The 3-D FPD "MDA" program (42) uses a second-order least-squares fit of the baseline (from 50 points selected by the computer at equal intervals on those stretches of the chromatogram that the operator designates as "baseline"). The deviations \(x_i\) are then measured from this smooth line. They are used directly (recourse to the mean \(x_i\) having become superfluous):
The "MDA" program proceeds to compute numerical values for the two common types of detection limits. For measurement of the standard deviation, the computer measures $2 \sigma_p$ as the width of the peak, in seconds, at 60.7% of its height. To determine the "chromatographic" $S/N_{pp} = 2$ detection limit on the screen, the operator uses cursors to define the signal - $S$ (the peak height), and the peak-to-peak baseline noise - $N_{pp}$. The latter is available in two versions, depending on whether or not the operator's pattern recognition and judgement is taken into account.

The first version is "objective" and "inclusive" (i.e. it is independent of the operator and is representative of all signal excursions, thus including the sharp and strong fluctuations that appear to extend beyond the normal distribution of noise and are hence called "spikes" or "outliers"). The $N_{pp}$ noise thus measured is referred to as "$N_{all}$". The second version is "subjective" and "exclusive" (i.e. it allows the operator to define the core of the noise, thereby excluding what appear to be spikes). The $N_{pp}$ noise thus measured is referred to as "$N_{core}$". Obviously, $N_{all} > N_{core}$ in experimental situations; in simulations (which start with Gaussian distributions) $N_{pp} = N_{all}$. All simulations were carried out using SigmaPlot's (Jandel Scientific, San Rafael, CA, USA) Gaussian random number generator, non-weighted moving averaging and operator defined boxcar averaging.

For this study, $n$ equals $10^3$ to $10^4$ data points (about 2 to 17 minutes worth of chromatographic data acquisition time).
8.3 Results and Discussion

8.3.1 Effect of Filters on Noise

In chromatography and other sectors of analytical chemistry the amount of noise is of utmost importance. This is because it defines the detection limit of a particular system. Once the signal is optimised, noise is normally further reduced by filtering, thereby offering an improvement in the SNR and the detection limit. In gas chromatography, for a typical packed column the peak width (at half height) may be, say 10 s. This is equivalent of a signal of 0.1 Hz, a relatively low frequency. Filters act by frequency discrimination, thus it is typical to use low-pass filters - filters that block high frequencies and allow low frequencies to get through. Normally, the frequency discrimination (generally referred to as a band or window) is such that the filter acts as close as possible to the analyte peak without adversely affecting it. Usually a 5 to 10 % reduction in the peak height (of the analyte peak) is acceptable.

Filters are of several different types. There are analog filters which can be subdivided into passive or active filters - the latter type generally makes use of an operational amplifier and thus requires a source of power, hence the term "active". A simple passive analog filter consists of a resistor and a capacitor, while the more complicated active variety may consist of a series of resistor-capacitor (RC) network depending on the desired bandpass. These active filters are designated as 3 pole, 6 pole etc., depending on the number of RC networks the signal traverses. The higher the number of poles, the sharper the bandpass, that is the filter can operate closer to the signal without significantly cropping it.
Digital filters, on the other hand, are normally associated with computers. The signal is normally collected at twice the frequency of the electrometer-amplifier circuit, in order to prevent aliasing. For example, our programs samples at 0.1 s intervals, therefore a 0.2 s RC filter precedes (or is built into) the interface.

The simplest digital filters are of the boxcar and the ensemble averaging type. In the boxcar filter, the desired number of data points (the boxcar length) are averaged to give one new data point, i.e. the filtered output is shorter than the original data set by a factor of the boxcar length. For example, if 1000 data points are collected and processed by a four point ensemble averaging filter, the output has 250 data points.

A simple non-weighted moving average filter, like ensemble averaging, uses a specified window width (of data points), which for symmetry is normally available in odd-numbered window widths. In this type of data filtering, the window moves sequentially from data point to data point and at the specified window width, takes the average of the window. The resulting filtered data points are therefore about the same length as that in the unfiltered set, except that the compromised end pieces of half the window width must be discarded. For example, for a window width of three and 1000 data points, the filtered output contains 998 data points. The first and last points are deleted since the algorithm did not have a "real" data point to work with. A weighted moving average algorithm on the other hand, applies weighting coefficients to the data points while carrying out the smoothing process, the points nearest the center of the filter being given the greatest weight. [Note: to 'filter', generally implies an irreversible, and of necessity fast, reduction of noise on data during the acquisition phase; to 'smooth',
Figure 8.1: Unfiltered and smoothed chromatograms. Upper figure: unfiltered chromatogram with solvent and analyte peaks. Lower figure: The same data file smoothed by the FIR algorithm.
suggests a slower, and algorithmically more complex, reduction of noise on already acquired and safely stored data. Thus, the same algorithm, if fast enough, can serve as either a filter or smoother. In this study, a simple three pole RC filter is used primarily as a filter, but occasionally as a smoother. The fast non-weighted moving average filter functions as either a filter or a smoother; and the slower FIR algorithm is available as a smoother only.]

The fact that filtering improves the SNR is undisputed. Figure 8.1 shows an example. The upper figure shows a typical unfiltered chromatogram (really slightly filtered by the unavoidable RC constant of the acquisition system), obtained via the fast acquisition system of the 3-D FPD. The noise is such that no peaks are clearly discernable. The lower figure shows the identical data file now smoothed by the FIR algorithm to about 10% peak height reduction. The solvent and analyte peaks are now clearly visible and a detection limit can be calculated.

Figure 8.1 clearly shows that filtering of a noisy chromatogram is useful. But is the extent of filtering important? And would filtering affect the $N_{pp}$ noise and the RMS noise to the same or different extents? In other words, is the $N_{pp}/$RMS ratio constant with the extent of filtering? Or does it vary?

8.3.2 Effect of Filters on the $N_{pp}$/RMS Ratio

Different types of filters and smoothing algorithms tend to produce a similar effect on the noise (52, 53). However, their effects on a peak are different. The upper 10% of the peak is its most vulnerable part since the signal there undergoes, say a 170° change
of direction. Thus, even though the different filters do adequate noise jobs, they do clip peaks to different extents. Intuitively, if noise were treated as a succession of small, sharp peaks, then as the extent of data smoothing increases, the sharp tips of these peaks will become round tops. As this happens, the peak-to-peak noise will decrease rapidly, but the root-mean-square noise will decrease slowly. The ratio of the $N_{pp}/\text{RMS}$ noise will therefore decrease with the extent of filtering.

The intuitive, predicted shape shift effect on the $N_{pp}/\text{RMS}$ ratio is presented in Figure 8.2. For simplicity, the triangular, sinusoidal, and hemispherical waveforms are restricted to the same single frequency and amplitude. The $N_{pp}$ values are all the same. However, the RMS values and hence the $N_{pp}/\text{RMS}$ ratio, vary by a factor of two. Generally, the spikier the excursions of original noise from the 'true' baseline (i.e., the larger their height to width ratio) and the less they are filtered, the higher will be the $N_{pp}/\text{RMS}$ ratio. On the other hand, the rounder the original noise features and the more they are filtered, the lower will be the $N_{pp}/\text{RMS}$ ratio. Furthermore, the more severely a particular type of filter clips the baseline deviations (noise), the more it will depress the $N_{pp}/\text{RMS}$ ratio.

Figure 8.3 shows the experimental version of the theoretical arguments presented above. It shows 3-D FPD baseline noise measured as $N_{ull}$, $N_{core}$, and RMS as it decreases with increasing filtering by the AVG filter. Clearly the two main types of noise measurements do depend to different degrees on the extent of filtering. Thus their ratios also depend on the extent of filtering: The value of $N_{ull}/\text{RMS}$ changes from about 9 at 0.1 s, to 5.7 at 1 s, to an extrapolated 3.6 at 10 s; therefore the conversion factor between the
two types of detection limits, \( \text{MDA}_{\text{chron}} \) and \( \text{MDA}_{\text{spect}} \), must vary accordingly. This trend is evident in all of the tested detector/filter combinations, and will be discussed later.

### 8.3.3 Noise "Distribution"

The literature presumes that noise is Gaussian, that is normally distributed. But if this were the case, there would exist a clear (though sampling-time dependent) statistical correlation between \( N_{\text{all}} \) and RMS (54). However, our measurements show that this is not always the case, therefore, the distribution cannot always be normal. To check

\[
\begin{align*}
N_{p-p} & & \text{RMS} & & \frac{N_{p-p}}{\text{RMS}} \\
\uparrow & & & & 4.8 \\
\downarrow & & & & 3.5 \\
\uparrow & & & & 2.8 \\
\downarrow & & & & 2.4
\end{align*}
\]

\[\text{Figure 8.2: Geometric figures treated as noise. The triangular, sinusoidal, and hemispherical waveforms are of a single frequency and amplitude.}\]
Figure 8.3: Variation of noise with the extent of filtering by the AVG filter. Noise: 15 min baseline from segment # 2 (ca. 430 to 460 nm) of the 3-D FPD. \( \text{H}_2 \): 50 mL/min, air: 40 mL/min, R374 PMT @ -950 V.
the distribution, this study employs a Gaussian fit program and the enforced $\sigma_{\text{fit}}$ measurement at 60.7% of the peak height, is included among the probes of this study.

Figure 8.4 shows an example of an unsmoothed (though, of course, filtered by the 0.22-second time constant of the electrometer) FID baseline. Two spikes appear prominently on the chart-recorded baseline (see insert), and these episodes last long enough to enter the distribution as several data points. Thus the standard deviation measured by RMS is larger than that measured by $\sigma_{\text{fit}}$. Which measurement should then be considered, RMS or $\sigma_{\text{fit}}$? And on a chart recorded baseline, how should the peak-to-peak noise be measured? $N_{\text{all}}$ is about four times as large as $N_{\text{core}}$ in Figure 8.4, and the $N_{\text{all}}/\sigma_{\text{fit}}$ ratio is 30 - a very high and unreasonable value. This does suggest that it may be better to use the subjective $N_{\text{core}}$ rather than the objective $N_{\text{all}}$ measurement.

Generally, most detectors are not as spiky as the FID of Figure 8.4. Normal distributions are often approached. As an example, the top graph of Figure 8.5 shows the noise from 22 minutes of regular FPD operation. Surrounding the solid line of the enforced Gaussian fit are two dashed lines, drawn to demarcate the square-root band, i.e. $+/- [(\text{number of occurrences})^{1/2}]$. Asymmetric distributions not attributable to spikes are also possible. The bottom graph of Figure 8.5 shows an example; the solid line again marking the enforced fit, the dashed line indicating the presumed "true" distribution. It is not known what caused these asymmetries; and therefore the possibility that they represent mere artifacts of data treatment, particularly when coming from the 3-D FPD acquisition system and the FIR filter operating with a long time constant on a perhaps oversmoothed baseline, cannot be excluded. What matters in the current context,
however, is that such asymmetries can and do occur. However, as will be shown later, in most cases $\sigma_{\text{fit}}$ and RMS agree well thereby suggesting that chromatographic noise is mainly Gaussian.

8.3.4 Noise Frequency

It may be argued that deviations from the Gaussian shape could be due to noise processes of different duration and distribution, which would come sequentially to the fore as smoothing became increasingly effective. That would suggest the presence of weaker

![Noisy FID, No Filter](image)

**Figure 8.4**: Unfiltered FID baseline with spikes. Insert: conventional recorder trace.
Figure 8.5: Examples of noise distributions. Upper graph: Gaussian, unfiltered 2-D FPD noise. Enforced Gaussian fit (solid line) and deviation as [# of occurrences]^{1/2} (dashed lines). Lower graph: Asymmetric, heavily filtered 3-D FPD noise with enforced Gaussian fit (solid line) and presumed true distribution (dashed line).
and slower (initial) noise components - with time constants within and perhaps beyond the filter range - which would become visible only as the stronger and faster components were being progressively quelled by the filter. If present, these different noise components would show their signatures at different frequencies in a noise power spectrum.

A typical FPD baseline (see Figure 8.1) does suggest (at least visually) the presence of fast and slow noise components. A baseline may have noise components from different sources, for example: column bleed, dust particles, flame flicker, photon shot noise, and electronic noise from the measurement system. Early FPD designs utilised mirrors and lenses. The use of these optical components however did not lead to any significant improvement in the signal to noise ratio (30, 55). This suggested that the noise in early FPD's was dominated by flame flicker, not photon shot noise.

Lured by this, one of the early experiments of our group attempted background subtraction using continuous, electrometer-acquired two-channel data from a conventional FPD. Contrary to expectations, the noise did not decrease but rather increased by a factor of about $2^{1/2}$. A similar attempt at background subtraction using the multiplexed data from a single PMT and electrometer system, with 5 ms acquisition time per channel (42) also produced an increase of about 1.4. If there were noise components slower than the 5-ms segment acquisition time of the 3-D FPD system, they should appear in two or more wavelength segments (provided they do so optically) - and they could therefore be annulled or at least significantly decreased by the subtraction of two suitably chosen and scaled segments from one another. Thus, for these FPD cases, it appears that the
relatively slow noise which appears, especially in the heavily filtered baselines does not originate as slow noise in the detector (e.g. as flame or flow flicker); but must owe its existence to white noise being converted into low frequency noise. And why not?

If the noise of an FPD is due to the quantum nature of light ("photon shot noise") then it must be white. However, chromatographic detection systems are far too slow to display the profiles of these fast primary events. In a typical case, the time constant of the electrometer may be 0.1 s and the full-scale response of the strip-chart recorder 1 s. In addition, analog or digital filters with selectable time constants in the 1 to 10 s range are often used.

8.3.5 Other Detectors and Filters

So far, the discussion has focused on a representative sample of the observed effects of filters on noise. This section presents a wider cross-section of the results. Table 8.1 presents a selection of four noise sources, all treated by the same filter. This selection includes the conventional electron capture and flame ionization detectors, a conventional photomultiplier tube's dark current and the flame photometric detector in the intermittently sampled 3-D version. Other systems behave similarly, some of these are covered in various later graphs emphasizing different aspects of filters and noise.

Table 8.1 is designed to allow a moderately detailed, numerical comparison of noisy baselines from individual sources, and the results of their smoothing by the same FIR algorithm. The numbers represent noise or noise-ratio measurements by the various definitions described earlier, and may be compared only with due caution. They carry,
Table 8.1: FIR smoothing of noise from different sources

<table>
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<tr>
<th>FIR cut-off(^\text{f})</th>
<th>Noise (arbitrary units)</th>
<th>N(_{\text{all}})</th>
<th>N(_{\text{core}})</th>
<th>RMS</th>
<th>(\sigma_{\text{fit}})</th>
<th>RMS</th>
<th>(\sigma_{\text{fit}})</th>
<th>RMS</th>
<th>(\sigma_{\text{fit}})</th>
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<td>(^{63})Ni-ECD, DC mode, 5 min</td>
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<td>533</td>
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<td>5.1</td>
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</table>

* in Hz, nominal
at best, two significant digits, since a larger number of significant digits may not yield more information. In fact, numerical detection limits are, by definition, one significant digit numbers. One book entitled "Detection in Analytical Chemistry" states: "One finds, for example, that at least 13 replicates are necessary to obtain s within 50% of the true σ [90% confidence interval]" (56). The important data in terms of detection limits are, of course, the ratios as given in the last four columns. (The first four columns of plain noise measurements have been included to provide the complete picture. The data, coming as they do from different detectors, conditions, amplifiers, attenuations, etc., are of necessity given in arbitrary units and are hence comparable only within a particular set, not across the Table.) Here the primary focus will be on the \( \frac{N_{all}}{RMS} \) and \( \frac{N_{all}}{\sigma_{fl}} \) ratios since \( N_{all} \) represents the objective and most commonly used noise measurement; and also because the \( N_{all} \) results span wider ranges, thus revealing clearer trends.

To start with, Table 8.1 suggests that different noise sources do produce different types of noise. The highest and the lowest ratios in the "non-filtered" mode are about a factor of two apart, with the FID being the highest. The assumption of different types of noise is also supported by different distributions. In a perfectly Gaussian distribution, RMS and \( \sigma_{fl} \) would have to be the same, and so would have to be the ratios \( \frac{N_{all}}{RMS} \) and \( \frac{N_{all}}{\sigma_{fl}} \). Examination of Table 8.1 shows, however, that the two measurements differ to a small but significant extent. Now, RMS is larger in the FID (as expected from Figure 8.4) and the 3-D FPD; while \( \sigma_{fl} \) is larger in the ECD and the covered photomultiplier tube. These relationships apply (with minor exceptions) throughout the monitored smoothing ranges. The differences between RMS and \( \sigma_{fl} \) are, however,
relatively small: they hardly show up on a summary log/log plot.

Perhaps the most striking trend apparent in Table 8.1 is that all the ratios decline (again with minor exceptions) as the extent of FIR smoothing increases. This is in agreement with the relationships obtained with the AVG filter as portrayed in Figure 8.3. Figure 8.3 is, however, somewhat limited in its range: the AVG filter is restricted to a window width of 50 data points, i.e. 5 seconds (42). (This is because the AVG filter is also used in the acquisition mode, in which case it has to calculate averages that keep up with the 3-D FPD data flow.) In contrast, the time constant of the FIR smoother is unrestricted. Figure 8.6 therefore presents a close-up look at two ratios in two different detectors (conventional FPD on top and FID on the bottom), over a range that extends to longer time constants and includes more data points. The suggested dependence is illustrated by solid least-square linear regression lines. Given the kind of subject and the type of plot, the data points are expected to show considerable scatter. Note also that these baselines - in contrast to those of Figure 8.3 - were obtained from an electrometer of RC = 0.22 s, it would therefore have made little sense to measure data points below that time constant.

Figure 8.6 confirms that the $N_{\text{all}}/\text{RMS}$ and $N_{\text{all}}/\sigma_{\text{fit}}$ ratios decrease as the influence of the smoothing algorithm increases. The $N_{\text{core}}/\text{RMS}$ and $N_{\text{core}}/\sigma_{\text{fit}}$ ratios are not shown here; they display similar but less dramatic behaviour. They are also closer to values typically expected for a rule-of-thumb $N_{p-p}/\sigma$ ratio (compare Table 8.1). So far the systems behave as expected, that is, a decrease of the ratio with the extent of filtering. However, only numerical, not visual behaviour has been considered - and the interrelated
Figure 8.6: Variation of FPD and FID noise ratios with FIR smoothing. The lines are linear least squares regression fits. Upper graph: 12 minutes of conventional FPD noise. Unfiltered ratios are 9.7 and 8.8. Lower graph: 10 minutes FID noise. Non-filtered ratios are 16 and 13.
topics of noise, filters and detection limits may contain significant perceptual components. Better expressed: "Anyone who tries to analyse a time series without plotting it first is asking for trouble" (57). One potential troublespot in this study is the \( N_{\text{core}} \) definition. As suggested earlier this may be a better value to use, but there is no better way of defining where to draw that line than by plotting an example. Figure 8.7 shows about 15 minutes of noise from the 3-D FPD, smoothed with the AVG filter set at window widths of 0.1 (i.e. "unfiltered") to 5 seconds. The traces show how the subjective \( N_{\text{core}} \) definition (on the right) ties in with the objective \( N_{\text{all}} \) measurement (on the left).

It would have been possible to replace the analyst-assessed limits of \( N_{\text{core}} \) by a computer-calculated test for outliers thereby changing \( N_{\text{core}} \) into a seemingly 'objective' measurement. However, that was not deemed necessary since there is no binding set of rules for determining detection limits. Therefore, in the context of this exploratory study, it would make little sense to introduce outlier tests for despiking baselines, thus the common sense and pattern recognition of the analyst was allowed to prevail. Underlying this illustration of objective vs. subjective evaluation is not only the analyst's assessment of what can be called an outlier or spike; it is also, and perhaps more importantly, the analyst's perception of which noise features do and which do not perturb the recognition of a superimposed analyte peak, i.e. of which do and which do not influence the practical detection limit.

Beyond defining \( N_{\text{core}} \) and providing a visual record of a sample datafile, Figure 8.7 offers still further material for discussion. The traces, which are derived from integrated 5-ms segments of the 3-D FPD signal sampled at 100 ms intervals, and are
Figure 8.7: Experimental 3-D FPD noise smoothed by the AVG filter.
reproduced by a laser printer giving relatively high resolution (compared to, say, conventional traces from a strip-chart recorder that might take a second or so to traverse full scale with thick pen and wide deadband). After about one minute into the top trace of Figure 8.7 two strong, positive excursions are present. These persist in two more traces below, then subside. In contrast, the strongest positive excursion of the bottom trace occurs at about four-and-a-quarter minutes. Its presence can be followed up to the top trace, but just barely. The obvious question is: Why do different traces emphasize different episodes?

The measurement of $N_{\text{ttlI}}$ is restricted to the two excursions of highest positive and negative amplitude. Filters, on the other hand, take a broader view and simply redistribute net total energy. Consequently, the discrete energy of a strong but lone excursion, which may appear with dominant amplitude under slight smoothing, might lose that dominance under major smoothing to the combined energy of a few - not quite as strong but closely positioned and equidirectional, hence mutually reinforcing - excursions. Such an interpretation implies that original noise is very fast. This conclusion is supported by the earlier-described failure to eliminate what appeared to be slow noise from the 2-D (i.e. the continuous monitoring via two PMT's) and the 3-D (i.e. the intermittent monitoring via one PMT) flame photometric chromatograms, as well as by the later-shown simulations and resulting square-root relationships.

Yet, this only means that original slow noise is not evident in the baselines considered here; in many other cases of chromatography and spectroscopy it may well be (e.g. as flicker noise). The temporal effect becomes even visually apparent in the bottom
three traces, where noise features not only decrease in amplitude but also merge and broaden in time.

However, Figure 8.7 can be taken beyond questions of visual perception. Its raw data happen to be the same as those of the earlier Figure 8.3 - and, combined, these two figures raise several interesting and, more importantly, easily answered questions. In Figure 8.3, the relationship of noise to window width (filter time constant) appears linear. Furthermore - although surprisingly more so for $N_{\text{all}}$ and $N_{\text{core}}$ than for RMS - the slope is close to one half, suggesting a classical square-root dependence based on the randomness of noise. If all initial noise were indeed fast and normally distributed, would that translate into an amplitudinal square-root relationship of noise with the filter's time constant? Would both $N_{\text{all}}$ and RMS follow it - i.e. would both perhaps become parallel, and would the $N_{\text{all}}$/RMS ratio therefore become constant? Such parallelism, if it were to occur would offer support - of a theoretical if not of a practical nature - for proposing a single rule-of-thumb factor that could be generally used to interconvert the two types of measurements. For an answer to these and related questions, the effect of filters on noise from different detectors and noise simulations are relied upon.

So far, the experimental curves used mainly FPD noise. What about FID and ECD noise? Figure 8.8 show these, plus a PMT dark current noise - all as taken from the same, conventional electrometer and filtered by the same, common FIR filter. (The "unfiltered" noise levels are arbitrarily included in Figure 8.8 at the RC time constant of the electrometer, i.e. at 0.22s.)

Figure 8.8 shows some expected and some unexpected features. The $N_{\text{all}}$/RMS
ratios decrease with the filter time constant as expected. Again as expected, there are differences between the different detectors. What seems unexpected, however, is that all (least-squares second-order regression) curves are clearly non-linear; and that their slopes vary from about zero to about one. Are the FID and the ECD, as well as the PMT dark current, fundamentally different from the FPD that produced the close-to-square-root slope of Figure 8.3?

![Figure 8.8: Effect of FIR filter on FID, ECD, and PMT noise.](image)
The answer is no, and part of that answer can be given by an experiment that uses the same FPD flame sampled by the two different acquisition systems. The "regular FPD" noise, i.e. noise that came through the same electrometer (and the same FIR filter) as the three noise records of Figure 8.8 show the same general trend. It does make sense that the time constant of the electrometer [which is different in nature and larger in value than the time constant of the fast 3-D FPD acquisition system (42)] should reduce the slope to essentially zero because in that region the noise is already filtered, i.e. the expected FIR filter action is preempted.

It is more difficult to account for the steeper slopes of Figures 8.8, and for these speculation is resorted to. Several possibilities come to mind, such as the presence of peculiar noise components in that temporal region, the preferred diminution of excessive spikes by the filter, etc. The most reasonable explanation, however, may be the nature of the two filters that sequentially act on the data: the RC circuit of the electrometer and the weighted-average window of the FIR filter. The amplitudinal transfer functions of these two filters are obviously quite different (58); not to mention the likely phase shift induced by the RC circuit. The double pass by disparate filters may well have produced the observed effect. While interesting in terms of practical performance, and supportive in terms of the changing \( N_{all}/\text{RMS} \) value, the curvatures must be considered to be of uncertain origin and hence unsuitable for predicting or extrapolating noise behaviour.
8.3.6 Simulated Noise

Random noise was simulated using SigmaPlot's Gaussian random number generator and smoothed by boxcar and non-weighted moving average algorithms. This simulation used a preformed Gaussian distribution of noise amplitudes - despite the fact that most, if not all, of the random elements in initial noise are in the time domain. What is presumed random and dominant in initial noise are, of course, the temporal intervals: between betas being emitted in the ECD, between PMT electrons being ejected by photons in the FPD, between charged particles being formed and collected in the FID. Seen through larger filter windows, short intervals between these primary events appear as positive, long intervals as negative amplitudinal excursions. Therefore, even though 'real' noise occurs in the time domain, the data collection and processing system displays their randomness in the amplitude domain.

For noise calculations, the initial FID events can be assumed to have the same amplitude, i.e. that of a unit charge; and the PMT electron avalanches can be assumed to be of similar strength (at least those avalanches set off by photons from the FPD flame). In contrast, the ECD's ion-pair yield of individual beta rays varies - in a predictable, inherent manner due to the concomitant loss of neutrinos; in a much less predictable, condition-dependent manner due to the particulars of isotope plating, cell dimensions, foil contamination and beta backscattering from nearby surfaces (18).

A plot of the effect of the non-weighted moving-average filter on a simulated random noise input of a single frequency is shown on the left side of Figure 8.9. The boxcar traces look similar, but are computed only to a boxcar length of 64 initial data
points. Both simulated types of baseline look very much like the real one shown in Figure 8.7; except that, there, some spikes are clearly present and the window can open to only 50 data points. The earlier discussed waxing and waning of particular noise episodes across adjacent windows can be observed in the simulated traces of Figure 8.9 as well, thus supporting the adopted interpretation.

The numerical $N_{pp}$ ($= N_{null}$) and RMS noise variation with window width and boxcar length is shown in Figure 8.10. Least-squares linear regression lines are drawn through the points which are averages of five replicates from different random inputs. As expected, the two filters behave alike and it is the RMS line that now shows the square-root slope. The $N_{pp}$/RMS ratio declines, over three orders of time variation, from about 8 to about 5. Compared to the experimental Figure 8.3, this decline is somewhat less steep. There should also exist a slight dependence of the $N_{pp}$ value on the number of data points, particularly if the number were much smaller (54) since the probability of drawing the largest, sparsest numbers increases with the number of draws, at least in the case of a perfect (i.e. smooth and limitless) Gaussian distribution.

Far more important in the present context, however, is the fact that the "theoretical" $N_{pp}$/RMS ratio of initially Gaussian noise is filter-width dependent, just as is the experimental ratio. In other words, in the absence of additional information about a particular system, the analyst cannot correlate its noise measurements - $N_{pp}$ and RMS, and, more importantly, its corresponding detection limits $MDA_{chrom}$ and $MDA_{spec}$, by simply assuming the initial noise to be Gaussian. Even if that assumption is correct, the conclusion to which it leads is not. Note also that this particular simulation assumes
Figure 8.9: Simulated single- and multi-frequency Gaussian noise smoothed by the AVG filter.
Figure 8.10: Variation of $N_{pp}$ and RMS values of simulated single frequency noise as smoothed by the AVG filter. Deviations are calculated from 5 independent data sets.
initial random noise of only a single, high frequency - there are no other noise components present that could complicate the picture. The term "frequency" refers here to the hypothetical input frequency, i.e. the reciprocal of the temporal interval between initial random data points being introduced into the simulation.

It is also possible to introduce random noise at several different acquisition frequencies, and then smooth the compounded initial noise. For algorithmic convenience, noise of different frequencies is produced here from separate sets of random numbers treated by the AVG filter and multiplied by the square root of its window width to equalize amplitudes. These constituent noise files, and their sum, are thus close to amplitudinally random in nature. Although the results of filtering the summed noise are easy to predict, they are still instructive to plot and are presented as the right side traces of Figure 8.9. The comparison of the left and right sides of Figure 8.9 shows noisy baselines of a different character.

Figure 8.11 shows the $N_{pp}$ and RMS curves as derived from five independent replicates of the whole procedure described above. The procedure thus simulates - for the left side of the plot - the response of aboriginal multi-frequency noise to filters of comparable window width mimicking, for instance, the behaviour of a chromatographic baseline that contains random source noise with initial frequencies in the 10 to 0.3 Hz range, and is being filtered with time constants in the corresponding 0.1 to 100 second range.

The results of filtering initial multi-frequency noise can be compared to the results of filtering initial single-frequency noise (Figure 8.10). It is obvious why Figure 8.11
displays a flatter section (very roughly of slope 1/4 in the region where noise was introduced, as compared to the following, steeper region roughly of slope 1/2). The narrow window widths can reduce only the faster, not the slower noise components. Once the window width exceeds the time constants (the reciprocals of the introduction frequencies) of all five initial noise components, the curve assumes its familiar

![Graph showing variation of $N_{p-p}$ and RMS values of simulated multi frequency noise as smoothed by the AVG filter. Deviations are calculated from 5 independent data sets.](image)

Figure 8.11: Variation of $N_{p-p}$ and RMS values of simulated multi frequency noise as smoothed by the AVG filter. Deviations are calculated from 5 independent data sets.
square-root slope. That, as in Figure 8.10, is then merely the wake, i.e. the inherent residual, of the initial perturbations. Regardless of slope, however, the $N_{pp}/\text{RMS}$ ratio keeps decreasing throughout the whole time range, similar to the decrease for experimental noise shown in Figure 8.8.

8.3.7 Noise, Filters and Detection Limits

From the results presented so far, it is obvious that no single or single-series of measurements can adequately establish a correlation between peak-to-peak and standard-deviation based measurements for general use. Nor can theoretical relationships be confidently employed as long as the noise distribution has not been experimentally established. The best that can be done under the circumstances is to estimate its range of behaviour over as many typical cases and conditions as possible.

This has been done in Figure 8.12. Ten early experimental series are represented, comprising five types of noise sources, three types of filters, and three types of noise definitions (the fourth, $\sigma_{\text{fit}}$, would have merely bloated this particular representation). The overall variation of the $N_{pp}/\text{RMS}$ ratio covers the range from about 2 to 10 in this self-explanatory bar graph. The bars denote the largest and the smallest ratio measured in each experimental set; in general, large ratios are derived from little-filtered, small ratios from much-filtered runs. The top axis shows the ratio itself; the bottom axis the factor this implies for converting one of the two common detection limits to the other, i.e. $\text{MDA}_{\text{chron}}$ at $S/N_{pp} = 2$ to $\text{MDA}_{\text{spec}}$ at $S/\text{RMS} = 3$.

Figure 8.12 reinforces earlier conclusions: There is no simple and clear connection
Figure 8.12: Comparison of $N_{p/p}$/RMS and $\text{MDA}_{\text{chrom}}/\text{MDA}_{\text{spec}}$ ranges for different detector and filter combinations. '2', '4': segment # in 3-D FPD; '(2)': segment # 2 monitored through stationary wheel and electrometer; 't': total of 10 segments; 'PMT': dark current of Hamamatsu R-374 PMT.
between the $N_{pp}$ and RMS measurements - and what connection there is depends to a large extent on conditions. Thus - if both types of detection limits must be reported - it is up to the analyst to measure both under the prevailing lab conditions (rather than to use an unreliable conversion factor). Obviously, however, that is a time-consuming process. Thus there is still some practical value to be found in narrowing down conditions and thereby tightening correlations, to the point where a rough conversion factor may be made to serve the less exacting cases.

The difference between the "spikes included" $N_{all}$/RMS and the "spikes excluded" $N_{core}$/RMS values in Figure 8.12 is substantial; as expected it is mainly due to unsmoothed (really lightly smoothed) noise specimen; their highest values are 10 and 6.8, respectively. With heavy smoothing, the two ratios move closer together (also compare Table 8.1). Thus the range of values is usually wider in the "spikes included" sets. These circumstances would suggest that, for a reasonably accurate correlation of $N_{pp}$ and RMS-based detection limits, the subjective "spikes excluded" measurement might prove the better choice if true spikes are present and the smoothing is but light. If spikes are absent and the smoothing is heavy, the objective "spikes included" treatment may be preferred.

On heavily smoothed datasets from the three conventional detectors FID, ECD and FPD, the $N_{all}$/RMS ratio usually falls between 5 and 6, remarkably close to the statistical value of 5. That implies a range of 3.3 to 4 for the $MDA_{brm}$/MDA$_{spec}$ ratio. The $N_{core}$/RMS ratios are smaller, but not by much. (These numbers depend, of course, on the permissible extent of smoothing, in other words on the width of the analyte peak. As will
be shown later, the longest time constants used for Figure 8.12 would severely oversmooth fast chromatograms.)

The measurements on the 3-D FPD may be of less general importance. It appears that the very low light level and/or the unconventional data acquisition method do influence the observed distribution of noise: the "spikes excluded" ratios seem disproportionately lower. The effect is, however, relatively small, and of concern only for this particular detector configuration. It does not influence the following comparisons (which, because of the ease of instrumental and algorithmic accessibility, and the clarity of illustration, were done on a 3-D FPD datafile). If the ranges shown in Figure 8.12 are symptomatic, the nature of the filter/smooother - whether FIR, AVG or RC - does not seem to play a major role, and possibly other conventional filter types would display similarly undistinguished behaviour.

The general "guidelines" suggested above for estimating noise, noise ratios and detection-limit conversion factors, do go as far as the data of Figure 8.12 will allow. The primary task is, however, to detect peaks - and for that reason the real-life example of a 3-D FPD detection-limit file is shown. The same datafile is treated with three different filters, to the extent reasonable or possible. The original datafile is displayed in Figure 8.1, and a 5 minute portion is chosen for Figure 8.13. As the noise is being shrunken and flattened by the filters, and the analyte peak slowly emerges, the question of how far to take the process arises. A first choice may be the maximum signal/noise ratio ($\text{SNR}_{\text{max}}$), at least for straightforward qualitative detection. If, however, the filtered datafile is also to be used for quantitation, the argument could be made that the peak should not be
unduly broadened (resulting in a loss of resolution) by the smoothing process. Therefore a detection-limit assessment at the particular extent of filtering that reduces the peak height by an arbitrary but still (generally) tolerable ten percent (SNR\textsubscript{10%}) is included. As it turns out, all three filters are capable of reaching that threshold.

Figure 8.14 shows graphically how the filter affects the analyte peak height, and what SNR results. Note that noise is measured here as \( N_{\text{core}} \); similar plots can be generated for noise measured as \( N_{\text{all}} \), RMS or \( \sigma_{\text{in}} \). From their respective SNR values, the corresponding detection limits can be easily calculated (see Experimental). The double plot of Figure 8.14 is simple and instructive. In order to emphasize the controlling role of the analyte peak - whose width defines the extent of smoothing possible or permissible - the curves are normalized such that the 10% peak-height reduction appears at the same position; and that the three lines are superimposed on one another. This necessitates the use of three formally different abscissae.

There is, however, no real contradiction between the three different scales. Time constants of different types of filters cannot be directly compared. Filter action depends on the nature as well as on the sophistication of the particular filter mechanism or algorithm; and the transfer functions of the three filters, operating here on not-quite-Gaussian noise, can at best be roughly estimated. The most glaring difference, i.e. the approximate factor of 1.7 between the time constants of the low-pass RC filter on one hand, and the AVG moving window on the other, is indeed expected (58, 60).

All three curves in the top graph - a semi-logarithmic plot of peak height reduction vs. filter time constant - appear to be linear. When the top graph is compared with the
Figure 8.13: Five minutes of 3-D FPD chromatogram as smoothed by FIR, AVG and RC filters. Left: Raw data with arrow marking the position of the analyte peak. Middle: Data smoothed to SNR_{10\%} level. Right: Data smoothed to SNR_{max}.
Figure 8.14: Plots of reduction in peak height and SNR vs. time constants of FIR, AVG and RC filters.
bottom graph, it turns out that the SNR increases until the peak height has been reduced by roughly one half! To filter that far may or may not endanger accuracy, depending on the analytical objectives.

As expected, the fast but unweighted AVG filter performs worse than the slow but weighted FIR filter (running on its maximum of 128 taps). In terms of using either for trace analysis, however, the difference is minor. The AVG and RC filters do not overtly reach their $\text{SNR}_{\text{max}}$ level, but must be close to doing so. The three-pole RC filter is a very simple representative of its kind. Considerably sharper roll-offs can be achieved with units of higher order, say 5- or 6-pole filters. However, the main advantage of the higher-order filters may only be that they can work a bit closer to the analyte peak without seriously cropping it - a small advantage if no initial noise components are present in that frequency region. It is not surprising that the three disparate filters differ little in chromatographic performance. Filters, particularly complex and expensive ones, are designed to cut on or off within the smallest time constant increment, i.e. they are expected to offer the sharpest roll-off. Thus they are often judged by how well they can suppress sine waves of one frequency versus another (59). Yet most of the slow noise seen in this study appears to be just the square-root carry-over of fast noise; meaning that it represents the minimum contribution of transformed aboriginal noise that, because of its random and uncorrelated nature, cannot be circumvented, subtracted or, for that matter, further suppressed by any algorithm. Though it can, of course, be reduced - square-root fashion - by an increase of filter time constant or window width. In contrast, if the initial noise were to contain additional, sizeable amounts of truly slow components - components
close but not equal to the dispersion of the analyte peak - then the higher-order filters may, on a relative basis, lead to larger improvements in the detection limit.

The typical improvement in SNR, hence in detection limits, to be expected, given that most of the initial noise is white, and that the analyte peak height is not seriously diminished by the filter, can be easily estimated. The noise should be reduced by a factor of approximately \((t_F/t_A)^{1/2}\), where \(t_F\) and \(t_A\) are the (effective, comparable) time constants of filter and amplifier. As a typical order-of-magnitude example, the chromatographically permissible time constant of an RC filter may be 1 second, and the RC constant of the electrometer circuit that feeds it 0.1 second. The approximate decrease in noise that can then be obtained from the filter is \(10^{1/2}\), i.e. about threefold - and the approximate increase in SNR should be the same (provided the peak-height has not been significantly compromised). This estimate agrees with practical experience, in which it was found that filters improve detection limits for conventional detector channels by factors of, typically, three to four.

On the other hand, fast acquisition systems, like that of the 3D-FPD, allow more noise to pass. Hence they will also allow more noise to be removed over the wider permissible range of the smoothing process: such systems will appear to offer larger improvement factors. For instance, the maximum SNR improvement shown in Figure 8.14 is close to five. It is similarly obvious that excessively spiky baselines lead to apparently larger improvement factors (for the same time-constant ratio of filtered to unfiltered data), particularly if noise is measured as \(N_{\text{all}}\).

The data of Figure 8.14 raises the question of how far the smoothing process
should actually be driven and also shows that it is possible to derive clean data, as those on the right side of Figure 8.13, from the likes of those on the left side of Figure 8.13; and also suggests that the action of the three disparate filters could hardly be visually distinguished.

Visually, the noise on the middle of Figure 8.13 still appears faster than, hence qualitatively different from, the analyte peak. However, on the right side traces, this difference has essentially vanished and noise could now be perceived as a sequence of smaller peaks, particularly if this were a temperature-programmed run. In other words, the analyte peak in the middle set of Figure 8.13 is distinguished from noise by size and width, while that on the right side is distinguished by size only. So, how far to filter? For qualitative work, maybe just enough to distinguish the peak from the noise. But for quantitative work, the peak height should not be unduly truncated. At maximum, the SNR$_{10\%}$ level should not be exceeded.

### 8.3.8 Fundamental Noise

Fundamental noise is the inherent, unavoidable part of the short-term baseline fluctuations that are normally referred to as noise. The simplest unavoidable noise contribution is the one based on the atomic structure of matter. Detector reactions, as all chemical (or nuclear) events, are random at the molecular level. This implies that the standard deviation of their rate (and hence the noise of the baseline if no other source of variation is present) can be described as a square-root function of the time interval over which the process is being observed. The emission of beta particles by the radioactive
foil of the ECD, the generation of photons by chemiluminescence in the FPD, and the formation of ion pairs by chemiionization in the FID, certainly qualify as such primary, random events. How does this fundamental noise compare with the experimentally measured one?

8.3.8.1 Fundamental ECD Noise

The chemistry of the ECD reactions leading to the detector's response involve beta decay and ion-pair generation, followed by the capture of electrons by analyte molecules. The noise-determining step is the one with the lowest rate of discrete events. Since one beta ray forms many ion pairs on its travel through the gas phase, i.e. since there are many more collected electrons than beta rays - the minimal noise characteristics should depend primarily on the number of (effective) radioactive decays.

However, there exists a problem unique to beta decay. Betas are not monochromatic; they roughly conform to an exponential distribution in energy. A further complication is their backscattering from internal detector surfaces, particularly those of high atomic weight (16, 18). The exponential distribution of the ion pair yields of individual betas must distort and broaden the initially Gaussian distribution of their counts (the intervals between sequential decays that translate into noise amplitudes). Relatively short intervals between betas of relatively high energy must lead to the largest positive excursions of the baseline; relatively long intervals between betas of relatively low energy to the largest negative ones. On the other hand, the ECD may have a built-in damping system in its chemistry and physics. For instance, the second-order ion pair
recombination, and other reactions, are much faster in regions or during episodes of high ionic density. The ECD thus represents a system far more complex than that of the FPD, in which (effective) photons produce electron avalanches of approximately the same magnitude; or that of the FID, in which unit charges are produced and collected.

A cursory simulation suggests that the RMS value of Gaussian noise may increase by a factor of about 1.6 if randomly chosen Gaussian distribution values are multiplied by randomly chosen exponential distribution values within limits reasonable for a real-life $^{63}\text{Ni}$ system (18). Of the total specified foil activity (15 mCi), fewer than half of the betas will have the correct direction for reaching the gas phase (neglecting isotopic purity as well as coating thickness, contamination, and curvature of the foil). Therefore, an arbitrary assumption that one half of all betas are "effective" in generating gaseous ion pairs, is made.

The foil in the Shimadzu ECD is relatively new, but the one in the Tracor detector is about two decades old and its originally specified activity has apparently dropped from 15 to 11.9 mCi. Of the drop, about 2 mCi is due to age (decay); the rest is possibly due to analytical contamination. The apparent value of 11.9 mCi is derived from the ratio of maximal d.c. currents then vs. now, i.e. 3.0 vs. 2.37 nA. In addition, the Tracor ECD is a two-chamber unit designed for d.c. and constant-frequency operation, while the Shimadzu detector is a one-chamber unit designed for constant-current operation: the two thus offer a wide-ranging comparison.

For the measurement, both ECD's are used in d.c. mode at their SNR-optimized potential; i.e. the way they would serve in conventional d.c.-ECD analysis. That means,
however, that not all possible current is withdrawn (if it were, no electrons would remain for capture and the detector would not respond to analyte). However, the electrons that are not withdrawn (34% in the Shimadzu, 38% in the Tracor) do kinetically and electrostatically influence the sampled system.

From the foil activity, it is easy to estimate the number of electrons generated per second. The square root of this number, as is well known from counting statistics, represents the standard deviation of the random, minimal noise. It can be translated back to current and, after conversion from RMS to $N_{pp}$, can be compared to the experimentally measured noise. It is possible to measure RMS noise directly but, for ease of experiment and chromatographic relevance (as discussed earlier), $N_{pp}$ is preferred.

There are also a number of assumptions or approximations hidden in this estimate. However, they should not call into question a one-significant-digit result. The easiest-overlooked of these may be the time constant or transfer function of the filter (or electrometer). Given the noise-equivalent-bandpass characteristics of integrating vs. low-pass RC circuits (58), and given the previous discussion in comparing non-weighted moving-window averaging with 3-pole RC smoothing (Figure 8.14), the effective integration time can be assumed to roughly equal 1.7 times the nominal time constant of the RC filter.

The Shimadzu ECD has a 15 mCi $^{63}$Ni cylindrical foil; it is operated at 0.20 V d.c., resulting in a 1.97 nA (of a maximum 3.0 nA) baseline current, and it makes use of a lab-made power supply and electrometer of RC = 1.0 s (i.e. of an estimated 1.7 s integration time). Under these conditions, the $(N_{pp}/RMS)_{1.7n}$ ratio is close to 5.5. The
"effective" disintegrations (d) per second, i.e. the number of betas likely to reach the gas phase are:

\[ 15 \times 10^{-3} \text{ Ci} \times 3.7 \times 10^{10} \text{ d/(s Ci)} \times 0.5 = 2.8 \times 10^8 \text{ d/s} \]

These betas can produce a maximum current of 3 nA, thus

\[ \frac{3 \times 10^{-9} A}{1.6 \times 10^{-19} \text{ As/e}^-} = 1.9 \times 10^{10} \text{ e}^-/s, \]

suggesting that, on average, 68 electrons are produced by each beta. For an integration time of 1.7 s, the RMS noise, adjusted for the exponential ion-pair yield of betas, is:

\[ N_{\text{RMS}} = (2.8 \times 10^8 \text{ d/s} \times 1.7 \text{ s})^{1/2} \times 1.6 = 3.5 \times 10^4 \text{ d}, \]

which, when converted to current, pared to the baseline of 1.97 nA, and finally changed to N\text{p}p noise, gives

\[ N_{\text{p}p} = \frac{3.5 \times 10^4 d}{1.7 s} \times \frac{68 e^-}{d} \times \frac{1.6 \times 10^{-19} \text{ As/e}^-}{e^-} \times \frac{1.97}{3.0} \times 5.5 \]

Therefore, \( N_{\text{p}p} = 8 \times 10^{-13} \text{ A} \)

This compares well with the experimental result of 0.6 pA.

The analogous calculation for the Tracor ECD uses an estimated foil activity of 11.9 mCi and an estimated integration time of 0.17 s for the Tracor electrometer's 0.1 s RC time constant. The \((N_{\text{p}p}/\text{RMS})_{0.17s}\) conversion factor is 6. The detector is operated at -18 V at a baseline current of 1.48 nA. Hence the RMS noise equals:

\[ (11.9 \times 10^{-3} \text{ Ci} \times 3.7 \times 10^{10} \text{ d/(s Ci)} \times 0.5 \times 0.17 \text{ s})^{1/2} \times 1.6 \]

\[ N_{\text{RMS}} = 9.8 \times 10^3 \text{ d}, \text{ and} \]
\[ N_{p-p} = \frac{9.8 \times 10^3 d}{0.17 s} \times \frac{68 e^{-} d}{d} \times \frac{1.6 \times 10^{-19} As}{e^{-}} \times \frac{1.48}{2.37} \times 6.0 \]

\[ N_{p-p} = 2 \times 10^{-12} \text{ A} \]

Again, this compares reasonably with the experimental result of 4.5 pA peak-to-peak noise.

No doubt experiments and estimates could both be improved, for example by measuring the foil beta count directly. Still these two experiments suggest - in unison and with sufficient cogency - that (clean) ECD noise is predominantly caused by a fundamental process, and that this process is the decay of the radioisotope.

For ease of calculation, the derived equation for calculating the RMS noise for the ECD can be condensed to

\[ RMS_{ECD} = I \times f_e \times \sqrt{\frac{1}{A_{eff} \times Ci \times t}} \]

where,

I = current in amperes (A),

\( f_e = 1.6 \), a factor that describes the widening of the random emission noise by the exponential beta energy distribution,

\( A_{eff} \) = effective activity of the ECD foil in Ci (assumed to be 0.5 x specified activity),

\( Ci \) = number of disintegrations per second of one Curie (3.7 x 10^{10}), and

\( t \) = integration time of the measurement (= 1.7 x RC of electrometer).
8.3.8.2 Fundamental FPD Noise

One of the simplest experiments for characterizing FPD noise is to decrease the light throughput from a (constant) flame, while measuring both the PMT current and the corresponding noise level right down to the dark current. The experiment can be extended to the high-input side by increasing the emissivity of the flame, i.e. by making it larger and hotter. This also tests whether the flame starts to produce flicker noise at particular flow conditions.

Figure 8.15 shows the result of this experiment. (It would have been interesting to produce for the ECD a graph similar to the one shown in Figure 8.15 for the FPD. However, that would have meant varying the random input itself, for instance by inserting a cylinder that would cover a variable fraction of the radioactive foil. For obvious reasons, that was not done.) About half of the points were obtained by intercepting a variable fraction of the constant FPD light beam with the opaque edge of a variable interference filter wheel that was mounted on one side of the detector; the other half were obtained by observing the light beam through a conventional, filterless FPD channel (mounted on the other side) and then adding larger and larger amounts of air to the flame. All data points still fall on one line in the log-log plot of Figure 8.15; and, as determined by least-squares linear regression, the line has a slope very close to one half. This indicates, as expected, a shot noise limited process.

It does not, however, identify photon shot noise (really: the temporal randomness in the sequence of luminescent flame events) as the main source. There are several other sources that could have been responsible for the observed square-root relationship (58-60).
Fortunately, an estimate can help to clarify the case.

From the current and gain of the PMT it is easy to estimate the number of photons that strike the photocathode during a particular time interval and eject an electron. The square root of this number represents the standard deviation of the random, minimal noise. It can be translated back to current and, after conversion from RMS to $N_{pp}$, can be compared to the experimentally measured noise of Figure 8.15.

The photon yield for the data points on the left side of Figure 8.15 is about 20%, the typical value for the Hamamatsu R-374 photomultiplier tube (61) receiving light from the leading edge of the variable interference filter (ca. 400 nm). The photon yield for the data points on the right side of Figure 8.15 is an average that could be determined from the background spectrum and the sensitivity profile of the PMT; it drops strongly toward the red. The question is, however, whether the photon yield should be included in the noise calculation. While at least five photons strike the photocathode for each ejected electron, only photoelectrically effective ones count and need to be counted. Non-effective photons might as well have struck photoelectrically inert parts the arrangement. The noise-determining step in any multistep process is obviously the one with the smallest rate of discrete events (here: the generation of electrons from the photocathode). The decision to exclude the photon yield from the calculation is also supported by the fact that Figure 8.15 shows no detectable discontinuity in the shift-over from mono- to poly-chromatic photons.

The gain of the R-374 PMT at -700 V (the voltage used in the experiment) is estimated as $2.0 \times 10^4$. The estimate is based on the specified gain at -1000V ($=5.3 \times 10^5$
(61)) and measurement of the gain ratio between -1000 and -700V (=26). At one nanoampere of baseline current (which is a fairly typical value for operation of the FPD, and allows the much lower dark current to be neglected),

\[
1.0 \times 10^{-9} A \times \frac{0.37 \text{ s}}{1.6 \times 10^{-19} \text{ As/e}^{-}} \times \frac{1 \text{ hv}}{2 \times 10^{4} \text{ e}^{-}} = 1.2 \times 10^{5} \text{ hv},
\]

hence the RMS noise of photoelectrically effective photons (hv) is \((1.2 \times 10^{5})^{1/2} = 3.5 \times 10^{2}\) hv (for an integration time of 0.37s). Changing back into current and converting RMS to \(N_{pp}\) noise - with the conversion factor \((N_{pp}/\text{RMS})_{0.37s} = 8\) derived earlier - yields \(N_{pp}\):

\[
3.5 \times 10^{2} \text{ hv} \times \frac{2 \times 10^{4} \text{ e}^{-}}{1 \text{ hv}} \times \frac{1.6 \times 10^{-19} \text{ As/e}^{-}}{0.37 \text{ s}} \times 8 = 2 \times 10^{-11} \text{ A}
\]

Figure 8.15: Peak-to-peak noise vs. current in a Hamamatsu R-374 photomultiplier tube at -700V; with different light input from a typical FPD flame (see text for details).
Again, the estimated noise compares well with the measured noise of $2.4 \times 10^{-11}$ A. The accuracy and credibility of both experiment and estimate could obviously be improved, e.g. by using photon counting, boxcar averaging, monochromatic photons (just in case), direct RMS evaluation, etc. However, even the present comparison suggests very strongly that the randomness of luminescent events, i.e. the counting statistic of arriving photons, generates the major part of the noise. In other words, this typical FPD produces noise that is random and close to the fundamental minimum.

These detailed calculations can be summarised in a more user friendly version as:

$$RMS_{FPD} = \sqrt{\frac{I \times e \times g}{t}}$$

where,

$I = $ PMT current in A,

$e = $ electronic charge ($= 1.6 \times 10^{-19}$ As), and

$g = $ PMT gain at the voltage of the experiment, and

$t = $ effective time constant of the RC circuit.

8.3.8.3 Fundamental FID Noise

A pure hydrogen/air flame contains very few ions; the FID baseline current is therefore very low. Jentzsch and Otte mention a value of "$\leq 0.75 \times 10^{-11}$ A for an empty capillary replacing the column [and] obviously dependent on the cleanliness of the gases and the apparatus" (62). For "spectroscopic" types of flames, Alkemade et al. summarize: "The ionization found is often due to metallic or organic impurities. In pure $H_2$ flames
some residual ionization close to thermal equilibrium may be found, involving NO⁺ and 
H₃O⁺ ions. The latter ion is formed by the recombination reaction H + H + OH -> H₃O⁺  
+ e⁻" (63).

In chromatographic practice, it is almost impossible to exclude some contamination 
by carbon and silicon compounds. However, the system may still behave in a 
fundamental, random manner. Consequently, rather than maintain a minimalist approach 
and attempt to design and test the cleanest FID possible, a just reasonably clean FID (in 
this case part of the three-detector ECD-RFD-FJD combination) was used. The FID was 
deliberately doped with small amounts of typical contaminants: either directly by adding 
different levels of methane, or indirectly by adding different levels of bleed from a 
Carbowax-20M column, to the hydrogen stream (the latter arrangement bypasses and 
thereby spares the Shimadzu ECD from possible contamination). This may simulate a 
high baseline; or it may simulate a - continuously introduced - analyte. Figure 8.16 
shows the interesting result of this experiment. Increasing the rate of ionization over a 
decade from that of the "pure" hydrogen/air flame results, indeed, in a square-root 
increase of noise, thereby suggesting a random process. At still higher background levels 
- i.e. higher than a tenfold increase and thus perhaps beyond typical operating conditions 
of the FID - the noise increases proportionally with the current.

In a spectroscopic system, the latter type of noise is referred to as "multiplicative"  
and normally attributed to "analyte flicker" (64). In spectroscopic systems it is indeed 
common to observe a change-over from additive to multiplicative noise - e.g. from photon 
shot noise to analyte fluctuation noise - as the signal level increases. A similar
change-over appears to occur in the chemiionization system of the FID.

The experiment used the Tracor electrometer of RC = 0.10 s time constant, i.e. of an assumed 0.17 s integration time. The \((N_{pp}/\text{RMS})_{0.17s}\) ratio equals approximately 6 in this FID. The baseline current in the absence of deliberately added column bleed or constantly bled-in analyte is \(8.6 \times 10^{-12}\) A; the p-p noise at this point (the lowest data point of Figure 8.16) is \(2 \times 10^{-14}\) A. (This compares favourably with the noise level mentioned by Jentzsch and Otte, i.e. \(\leq +/- 5 \times 10^{-14}\) A (62); however, this comparison must remain highly approximate in the absence of any information on time constants.)

The number of ion pairs generated within the integration interval is then

\[
8.6 \times 10^{-12} A \times \frac{0.17 s}{1.6 \times 10^{-19} \text{As}/e^-} = 9.1 \times 10^6 e^-
\]

and \(N_{pp}\) equals

\[
\sqrt{9.1 \times 10^6 \times \frac{1.6 \times 10^{-19} \text{As}/e^-}{0.17 s} \times 6} = 2 \times 10^{-14} A
\]

The estimated magnitude of noise that originates from the randomness of ion-generating events thus compares well with the value measured in a "clean" FID flame. Although - as in the ECD and FPD cases - experimental accuracy could no doubt be improved, the data are good enough to allow us to presume that most if not all of the FID's baseline noise is fundamental in character; i.e. that it is due to the atomic nature of matter and that, consequently, it is determined by, and cannot be reduced below, counting statistics.

Once again, the calculations for the RMS FID noise can be summarised into a simple formula:
\[ \text{RMS}_{\text{FID}} = \sqrt{\frac{I \times e}{t}} \]

where,

- \( I \) = baseline current in A,
- \( e \) = electronic charge, and
- \( t \) = time constant of the electrometer.

**Figure 8.16:** Peak-to-peak noise vs. current in a flame ionization detector; with different levels of methane, or Carbowax-20M-derived bleed, added to the hydrogen flow.
The fact that this statement applies not only to the FID but also to the ECD and the FPD, i.e. to each of the three detectors tested, is welcome. For one, it highlights a fundamental property that these three (and perhaps more) otherwise disparate chromatographic detectors have in common. Also, that fundamental property defines the theoretically minimal noise level: a value which may allow the chromatographer to assess how well an actual detector is performing, or how clean it is, or how (or whether) the fluctuations of its baseline could be further reduced.
CHAPTER 9

Automatic Response Ratios from an Integrative Algorithm

9.1 Introduction

Response ratios (RR's) from dual- or multiple-channel detectors or sensors are commonly used as indicators of chemical or physical properties and they are frequently determined on chromatographically separated analytes. They can support a variety of analytical tasks, for example: the assessment of peak purity (65), the subtraction of matrix components (41) or interfering peaks (65), the production of element-specific chromatograms (32, 66), and the determination of physicochemical constants (67, 68). RR values are determined manually or automatically and can be plotted as "response ratio chromatograms" [RRC's] (65).

In studies involving the dual- or multi-channel (42) flame photometric detector (FPD), our group determined RR's based on slope ratios (i.e. by comparing the first differentials) of the two luminescence outputs. The use of slopes served the purpose well (32, 42, 65, 66), but it was suggested that determining RR's as area ratios (i.e. by comparing the integrals) might prove advantageous (65). Integration is a common procedure in chromatography (69); and it seemed likely that area ratios should be less vulnerable to shifts in phase, increases in noise, and - particularly in the case of sequential detection - discrepancies in retention time and incongruities in peak shape.

For these reasons an integrative approach to RR determination was pursued and
its results compared to those of the earlier developed differential approaches (65). As a test system the dual-channel FPD (Section 2.1.4) that supported the prior study, was used and its two channels were set to monitor two strong bands of the most common FPD analyte, sulphur. These two bands of the blue $S_2$ emission [$\nu = 0 \rightarrow 9$ at 394 and $0 \rightarrow 10$ at 405 nm of the system $B^3\Sigma_u^- \rightarrow X^3\Sigma_g^+$ (70)] - are known to produce fairly constant response ratios, even when peaks exceed the quadratic range or are quenched by co-eluting hydrocarbons (71). To test the algorithms to their limits, the analyte was injected in variable & low, variable & high, and constant & high and very high amounts; and the chromatograms, obtained from two different detector conditions, were used both with and without heavy digital filtering.

9.2 Experimental

Several series of multiple di(tert-butyl) disulphide injections into the Shimadzu GC-4BMPF gas chromatograph with dual-channel FPD were stored in computer memory via a lab-developed interface and acquisition program (41). The injections were done at variable & low (about 0.5 to 2 ng), at variable & high (about 5 to 20 ng) and, later, at constant high and very high (20 and 100 ng) levels of analyte. The variable injected amounts and volumes were selected such that the different sulphur peaks spanned the available screen range, and that the different volumes of solvent (acetone) produced tails of different prominence, i.e. of variable quenching ability. The low-analyte files deliberately included peaks that were close to the detection limit and hence encrusted by strong noise. Figure 9.1 shows an example.
Figure 9.1: Typical "unfiltered" chromatogram of "low & variable" analyte injections from ca. 0.5 to 2.0 ng of di(tert-butyl)disulphide in acetone. The numbers marking the sulphur peaks are the same as those used in Tables 9.1 and 9.2. Channel 1 (405 nm); H₂: 200 mL/min, air: 50 mL/min.
Both high-analyte and low-analyte files were examined "unfiltered" and "heavily filtered". The latter procedure used the FIR filter with Hamming window and 32, 64 or 128 taps, whose cut-off frequency was adjusted to approach the maximum signal/noise ratio. Separate sets of individually "optimized" parameters (slope thresholds for start/stop commands, etc.) were used for differential and integral RR determinations.

The above variations were carried out at a set of general flow conditions that would typically be used for a larger number of FPD-active elements including sulphur. To guard against concentration-dependent spectral changes, further data files were generated by using conditions whose gas flows were optimized for the S\textsubscript{2} luminescence, and by repeatedly injecting the same amounts of solvent and analyte. Figure 9.2 offers a simplified graphic comparison of the present integrative with the earlier differential approach: the latter averages the weighted absolute-slope ratios (the steeper the slope the heavier the weight) for the whole-peak mode; or the weighted positive-slope ratios (up the peak, first "half") and negative-slope ratios (down the peak, second "half"), for the split-peak mode (65). Note from Figure 9.2 that the split-peak mode excludes a center slice containing the peak apex: slope ratios from the center, where both slopes go through zero, can under adverse conditions produce erroneously large or small numbers, particularly if the two peaks reach their maximum amplitude at a slightly different time. No such apex effect occurs in the integral mode; in fact, there the apex region represents the most important part of the measurement.

Accordingly, the new integral RR algorithm was written for the whole-peak mode only. Peak start and stop criteria were similar to those of the differential system (65).
Figure 9.2: Schematic representation of three different ways to evaluate chromatographic peaks (one peak only shown) for determining response ratios based on integral (area) or differential (slope) measurements. A = amplitude, S = slope, w = weighted.
Also as in the differential system, the objective was to cover not all but just the diagnostic parts of the peak. What mattered was not the most accurate determination of the peak areas themselves, but the most precise determination of their ratio. The often unreliable base of the peaks (the lowest 5 to 10 percent of peak height) was therefore routinely excluded by suitable cut-on/cut-off thresholds.

Briefly, the integrative algorithm searched for "valid" peaks (in a procedure similar to the one used by the differential algorithms), then integrated them and determined their ratio. To be accepted as "valid", a peak had to meet all of the following criteria:

1. The peak had to be located within lower and upper "clipping" levels, as defined by the operator with the help of cursors superimposed on the chromatograms. The lower clipping level allowed the operator to exclude noise or solvent-caused baseline dips; the upper clipping level allowed the exclusion of peaks that exceeded the range of the original data acquisition system (and were of concern only if one or both of the chromatograms had been previously reduced in amplitude).

2. For sole purpose of integration, the peak was assumed to "start" when its slope exceeded a "minimum slope" threshold, set by the operator in percent of full scale (= percent of screen height) per minute. The peak was assumed to "stop" when the slope (multiplied by -1 for the descent) dropped below the same slope threshold. (Note: The algorithm was designed to process roughly symmetric peaks. If strongly asymmetric ones should be encountered on a regular basis, the operator may prefer to define separate and different slope thresholds for ascent and descent.) The necessary threshold values could be easily estimated on the screen by imagining tangents drawn through the desired start
and stop points of some conveniently located peak.

The slope was calculated from the amplitude difference between two data points, with the distance (i.e. the time) between the data points set by the operator. For instance, a setting of "1" meant carrying out the slope determination over two adjacent data points or 0.1 second, "5" meant 0.5 second, etc. This was done to reduce the effect of short-term noise (if present). Slope values were calculated for every data point. Obtaining meaningful slope values thus depended on choosing the appropriate width of the 'moving window' in which the slope appeared. (Note: If the signal had already been smoothed before the slope threshold criterion was applied, this choice mattered but little.)

3. In order to be accepted by the algorithm as a valid peak "start", a specified, uninterrupted number of slope values had to exceed the set "minimum slope" value. For instance, if the operator answered "10" to the prompt requesting the "number of consecutive data points", at least 10 slope values in a row (one second's worth of chromatographic time) all had to fall above the slope threshold. The setting thus defined how long a peak-commencing upward (or a peak-terminating downward) slope had to last in order to trigger peak start and stop commands. Again, which "number of consecutive data points" to set was easily estimated on the screen from the width of the most slender peak.

With all prompts answered, i.e. all thresholds defined, the program searched for "valid" slopes throughout the chromatogram. It declared PEAKSTART and PEAKEND times if, and only if, all of the above criteria had been met for a particular peak. The algorithm (CHROM-8) to achieve this was included in a collection of in-house
chromatographic routines called "CHROM". It analyzed the first-channel signal to define PEAKSTART and PEAKEND, then imposed these times on the second channel. This worked well if the two channels, as in this study, were fairly similar.

Note that in analytical methodology, the two channels could, for instance, originate from FPD wavelength regions spaced far apart, or they could even originate from different detectors (in parallel or in sequence). In addition, the sample could contain several elements or functional groups with different response characteristics. Consequently, if the two channels were to yield peaks grossly different in amplitude and perhaps symmetry, it would be reasonable to include both channels in the peak diagnostics. To achieve this with minimal effort, an alternative algorithm (CHROM-9) was assembled. At its heart was a routine that took the average of the two channels, performed the diagnostics on the averaged peaks, and then imposed the derived PEAKSTART and PEAKEND times back onto the two constituent (original) channels, there to serve the subsequent computation of area-based response ratios. In addition, a subroutine "CHROMTS" ("time-shift") allowed one chromatogram to be temporally shifted until it best matched its twin in elution behaviour. The latter feature was developed with sequential (as opposed to synchronous) detector channels in mind, for example to be used with the sequential detectors described in Figure 2.4. For the current simultaneous signals from adjacent S₂ bands, however, the integrative CHROM-9 algorithm was unnecessary and the TS option inappropriate. Also, whenever double-checked on the data files of this study, CHROM-9 produced similar results as CHROM-8.
The signal (amplitude, chromatographic response) at PEAKSTART time was defined as the average inside a 0.3 second window (a third of the sum of three successive data points, with the PEAKSTART datum in the middle). A similar smoothing procedure was used for the signal at PEAKEND time. A horizontal baseline (really: a truncation line) was then defined by calculating the arithmetic mean of the smoothed PEAKSTART and PEAKEND signals. The "peak area" (for purpose of calculating area ratios) was obtained by summing all 0.1-second datapoint amplitudes above that line, from PEAKSTART to PEAKEND.

The algorithm then calculated the area ratio of corresponding peaks in the two channels. (Any magnification factors previously applied to one or both chromatograms were automatically factored into this response-ratio calculation.) The numerical RR values, together with the PEAKSTART and PEAKEND times (to a tenth of a second), were forwarded to a matrix printer, in tabular form.

Meanwhile, the RR values appeared on the screen in the form of a (logarithmic) RRC, superimposed on the two constituent chromatograms. With the help of the CHROM-9 program, all three could be individually magnified or reduced in amplitude. The screen images could also be vertically offset and, if so desired, forwarded singly or in combination to a printer. Figure 9.3 shows an example of a coarse "screen dump", as routinely obtained from a matrix printer.
Figure 9.3: Response-ratio chromatogram with its constituent (405 and 394 nm) chromatograms. Scaled for easier viewing; response ratios and wavelengths manually inserted. Screen-dumped on 8-point matrix printer (hence the stepped appearance). The caption shown on top, "A:ints1a", is the automatically printed file title.
9.3 Results and Discussion

There is little doubt that dual-channel RR algorithms perform well on strong peaks towering above straight and smooth baselines; just as conventional integrator algorithms do (69). The obvious question, however, is how well they cope with the more difficult types of terrain in which smaller peaks - overlapping one another, perhaps - rise from an undulating baseline overgrown with noise. In such cases, conventional integrators have been shown to produce errors (in estimating single-peak areas) of up to 40% (72).

The test protocol therefore offered chromscapes of considerable challenge: by including non-filtered data (i.e. data filtered only by the RC circuit of the electrometer); by approaching the detection limit; by letting peaks ride on strong or weak solvent tails (and thus be strongly or weakly quenched); and by injecting variable amounts of both analyte and solvent.

Table 9.1 shows an example of such a worst-case scenario: it lists the RR values for the sulphur peaks numbered in the Figure 9.1 chromatogram. Table 9.1 lists in addition the means, together with the relative standard deviation (+/-% RSD) values, both for a single peak determined by all four algorithms (row), and for all peaks determined by a single algorithm (column). As expected for a worst-case scenario, the "precision" of the measurements is unacceptably large - with the important exception, however, of the 7.2% RSD value for the integral (area-ratio) mode. In contrast, the differential (slope-ratio) mode produces not only very large relative deviations; it also produces highly inaccurate means.

This is as expected, since summed differential ratios should be much more
Table 9.1: Response Ratios from a Non-Filtered, Low and Variable Level Chromatogram

<table>
<thead>
<tr>
<th>Peak</th>
<th>Area Ratios</th>
<th>Slope Ratios</th>
<th>Avg(%RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Whole Peak</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Split Peak</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>First Half²</td>
<td>Second Half²</td>
</tr>
<tr>
<td>1</td>
<td>0.851</td>
<td>2.305</td>
<td>1.319</td>
</tr>
<tr>
<td>2</td>
<td>0.716</td>
<td>1.639</td>
<td>1.141</td>
</tr>
<tr>
<td>3</td>
<td>0.786</td>
<td>1.689</td>
<td>1.129</td>
</tr>
<tr>
<td>4</td>
<td>0.731</td>
<td>1.416</td>
<td>1.350</td>
</tr>
<tr>
<td>5</td>
<td>0.762</td>
<td>1.448</td>
<td>1.095</td>
</tr>
<tr>
<td>6</td>
<td>0.734</td>
<td>1.344</td>
<td>1.260</td>
</tr>
<tr>
<td>7*</td>
<td>0.779</td>
<td>0.809</td>
<td>0.736</td>
</tr>
<tr>
<td>8</td>
<td>0.639</td>
<td>0.798</td>
<td>0.880</td>
</tr>
<tr>
<td>9</td>
<td>0.729</td>
<td>1.613</td>
<td>1.622</td>
</tr>
<tr>
<td>10</td>
<td>0.721</td>
<td>1.171</td>
<td>1.376</td>
</tr>
<tr>
<td>11</td>
<td>0.786</td>
<td>1.984</td>
<td>1.763</td>
</tr>
<tr>
<td></td>
<td>0.749 (7.21)</td>
<td>1.474 (30.8)</td>
<td>1.243 (23.9)</td>
</tr>
</tbody>
</table>

* Off-scale peak

1 Conditions: H₂ 200 ml/min, Air 50 ml/min. Time constant of electrometer. RC = 0.22s. Injections vary from 0.5 to 2.0 ng di(tert-butyl) disulphide in acetone.

2 The "Half" is actually less than 50% since the centre slice that contains the peak apex is excised by the split-peak algorithm - see Figure 9.2.
### Table 9.2: Response Ratios from a Filtered, Low and Variable Level Chromatogram

<table>
<thead>
<tr>
<th>Peak</th>
<th>Area Ratios</th>
<th>Slope Ratios</th>
<th>AVG(%RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Whole Peak</td>
<td>Split Peak</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>First Half</td>
</tr>
<tr>
<td>1*</td>
<td>0.808</td>
<td>0.904</td>
<td>0.885</td>
</tr>
<tr>
<td>2</td>
<td>0.756</td>
<td>0.760</td>
<td>0.770</td>
</tr>
<tr>
<td>3</td>
<td>0.787</td>
<td>0.784</td>
<td>0.771</td>
</tr>
<tr>
<td>4</td>
<td>0.767</td>
<td>0.786</td>
<td>0.761</td>
</tr>
<tr>
<td>5</td>
<td>0.789</td>
<td>0.788</td>
<td>0.770</td>
</tr>
<tr>
<td>6</td>
<td>0.730</td>
<td>0.723</td>
<td>0.714</td>
</tr>
<tr>
<td>7**</td>
<td>0.799</td>
<td>0.837</td>
<td>0.831</td>
</tr>
<tr>
<td>8</td>
<td>0.712</td>
<td>0.715</td>
<td>0.708</td>
</tr>
<tr>
<td>9</td>
<td>0.748</td>
<td>0.776</td>
<td>0.770</td>
</tr>
<tr>
<td>10</td>
<td>0.716</td>
<td>0.722</td>
<td>0.722</td>
</tr>
<tr>
<td>11*</td>
<td>0.764</td>
<td>0.810</td>
<td>0.786</td>
</tr>
<tr>
<td></td>
<td>0.761(4.3)</td>
<td>0.782(7.1)</td>
<td>0.772(6.7)</td>
</tr>
</tbody>
</table>

* Values obtained in zoom mode to avoid screen resolution problems

** Off-scale peak in non-filtered Chromatogram.

1 Conditions: as in Table 9.1, except FIR cut-off frequency of 0.2 Hz.

susceptible to severe variations in slope, i.e. to noise obscuring the algorithmically important parts of the peak. The numbers of Table 9.1, measured under deliberately marginal conditions, clearly indicate that integral measurements (of truncated peaks) provide preciser and more accurate data than differential measurements.

Again as expected, the situation is much improved by heavily filtering the
chromatograms before measuring the RR values. Table 9.2 shows the results. All RSD values are now acceptable. (On a poor data file such as this, a relative standard deviation of less than +/- 10 % is considered to represent a reasonable criterion of acceptability). The means are very close to one another, and are certainly close to the "integral" mean of Table 9.1. It is interesting to note that the four algorithms now differ less among each other when evaluating the same peak (most values are in the 1 to 2 % RSD range, with the median at 1.7%), than peaks differ among each other when evaluated by the same algorithm (these values all fall into the 4 to 7% RSD range).

As expected, the precision improves, when larger amounts of analyte are used. Figure 9.4 shows the "non-filtered" first-channel chromatogram of this series. It should be mentioned that the better RR values are not due to a diminution of quenching: the sulphur peaks are still quenched by the same percentage as in Figure 9.1. This is not immediately obvious from the picture - the solvent appears to tail very little at the higher attenuation - but percent quenching depends only on the concentration of the quencher, not on the concentration of the analyte (73). The quencher, i.e. the solvent and/or its fragmentation products, has not reached the limit of its quenching power - it has reached the limit of its own luminescence. The fact that the linear range of (probably CH and CC) luminescence has indeed been exceeded, can be seen from the very similar peak heights resulting from very different volumes of solvent.)

Table 9.3 contains the RR values for the chromatographic data file shown in Figure 9.4, i.e. for the typical example of a "non-filtered", high-analyte level run. The individual integral and differential algorithms now produce essentially the same RSD
Figure 9.4: Typical "unfiltered" chromatogram of "high & variable" analyte injections from ca. 5 to 20 ng of di(tert-butyl) disulphide in acetone. The numbers marking the sulphur peaks are the same as those in Table 9.3. Channel 1 (405 nm); H₂: 200 mL/min, air: 50 mL/min.
Table 9.3: Response Ratios from a Non-Filtered, High and Variable Level Chromatogram

<table>
<thead>
<tr>
<th>Peak</th>
<th>Area Ratios</th>
<th>Slope Ratios</th>
<th>Avg. (%RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole Peak</td>
<td>Split Peak</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>First Half</td>
<td>Second Half</td>
</tr>
<tr>
<td>1</td>
<td>0.795</td>
<td>0.801</td>
<td>0.828</td>
</tr>
<tr>
<td>2</td>
<td>0.795</td>
<td>0.819</td>
<td>0.809</td>
</tr>
<tr>
<td>3</td>
<td>0.801</td>
<td>0.797</td>
<td>0.809</td>
</tr>
<tr>
<td>4</td>
<td>0.803</td>
<td>0.805</td>
<td>0.811</td>
</tr>
<tr>
<td>5</td>
<td>0.811</td>
<td>0.806</td>
<td>0.808</td>
</tr>
<tr>
<td>6</td>
<td>0.816</td>
<td>0.819</td>
<td>0.826</td>
</tr>
<tr>
<td>7</td>
<td>0.822</td>
<td>0.825</td>
<td>0.827</td>
</tr>
<tr>
<td>8</td>
<td>0.821</td>
<td>0.821</td>
<td>0.821</td>
</tr>
<tr>
<td>9</td>
<td>0.835</td>
<td>0.833</td>
<td>0.830</td>
</tr>
<tr>
<td>10</td>
<td>0.834</td>
<td>0.856</td>
<td>0.874</td>
</tr>
<tr>
<td>11*</td>
<td>0.888</td>
<td>0.855</td>
<td>0.833</td>
</tr>
<tr>
<td>12</td>
<td>0.839</td>
<td>0.834</td>
<td>0.833</td>
</tr>
<tr>
<td>13</td>
<td>0.834</td>
<td>0.832</td>
<td>0.838</td>
</tr>
<tr>
<td>14</td>
<td>0.828</td>
<td>0.851</td>
<td>0.836</td>
</tr>
<tr>
<td>15**</td>
<td>0.779</td>
<td>0.880</td>
<td>0.890</td>
</tr>
<tr>
<td>16</td>
<td>0.843</td>
<td>0.846</td>
<td>0.847</td>
</tr>
<tr>
<td>17**</td>
<td>0.843</td>
<td>0.872</td>
<td>0.870</td>
</tr>
</tbody>
</table>

|       | 0.823(3.1)  | 0.832(3.0)  | 0.833(3.0) | 0.840(2.8) |

*   Off-scale peak.
**  Values obtained in zoom mode to avoid screen resolution problems.

1  Conditions: as in Table 9.1 except injections from 5 to 20 ng di(tert.-butyl) disulphide in acetone.
(close to 3%) for all peaks combined. When the action of the four algorithms is compared for the same peak, the RSD values are generally lower (mostly between 0.5 and 3 %, median 1.1% RSD).

The same chromatogram as given in Figure 9.4, but now heavily filtered, produces data hardly different from those of Table 9.3, at least as far as the mean response ratios are concerned. Also, the RSD values for each of the four algorithms, working separately on all peaks, are again similar (close to 2.7%). However, the RSD values for the four algorithms working on the same peak now fall mostly into the 0.1 to 0.3 % range (median 0.23%): a significant improvement when compared to the "non-filtered" data.

It thus appears that all tested integral and differential algorithms concur within relatively narrow margins of error (certainly below 1% RSD) when measuring the same, good-quality peak. As supported by this concurrence - and the low probability of all four algorithms having the same bias - all four algorithms are considered to be essentially accurate. The variability of the RR values themselves thus seems to be due not to algorithmic but to chromatographic, i.e. spectral differences. This should come as no surprise in light of the differently sized and differently quenched peaks. Although the S₂ bands predominate, smaller contributions of other excited sulphur species (71, 74) may still be present - not to mention carbon-based emissions and/or background luminescences. Chromatographic response ratios, in addition to their many other roles in analysis, can thus serve as exquisitely sensitive probes of spectrochemical change.

The relatively strong RR variation of differently sized and quenched peaks suggested that the algorithms' potential involvement in the comparatively large +/- 3%
RSD spread be investigated. To obtain data files that excluded, as far as convenient, any spectral variation, the detector conditions were optimized for the S\textsubscript{2} emission (earlier conditions had been those that afforded good overall response to a rather wide variety of elements, including sulphur). Also, the same analyte amount and the same injection volume were used throughout each experimental run.

Table 9.4 shows the result from one set of heavily filtered chromatograms, as obtained from very high, constant levels of analyte, just bordering the upper end of the linear range. The obvious difference to the earlier, variable-analyte runs is the much lower RSD value for the same algorithm working on all 16 peaks: it is of similar magnitude as the RSD value for all four algorithms working on the same peak. A lower (but still constant) analyte level gave the same result, i.e. the RSD values for heavily filtered chromatograms were close to 0.5\% for the integral and the two split-peak differential modes.

The values for the whole-peak differential mode were, however, clearly worse (in the 1 to 1.5\% range). Speculatively, this is a consequence of working close to the upper linear-range limit. The likely effect to consider here is the behaviour of the peak apex. It is the peak apex that approaches or exceeds the linear range; and it is the peak apex where, as suggested earlier, slope ratio measurements are likely to show the greatest error. Accordingly, the apex-free split-peak values of the differential approach agree well with the (whole-peak) values of the algorithmically quite different integral approach; but they disagree with the values of the otherwise similar whole-peak differential approach - both in mean and in \% RSD (0.734, 0.736 and 0.737 vs. 0.746; and 0.42, 0.41 and 0.38 vs. \textit{...})
Table 9.4: Response Ratios from a Filtered, Very High and Constant Level Chromatogram

<table>
<thead>
<tr>
<th>Peak</th>
<th>Area Ratios</th>
<th>Slope Ratios</th>
<th>Avg(%RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Whole Peak</td>
<td>Split Peak</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>First Half</td>
</tr>
<tr>
<td>1</td>
<td>0.739</td>
<td>0.745</td>
<td>0.736</td>
</tr>
<tr>
<td>2</td>
<td>0.733</td>
<td>0.739</td>
<td>0.733</td>
</tr>
<tr>
<td>3</td>
<td>0.733</td>
<td>0.753</td>
<td>0.733</td>
</tr>
<tr>
<td>4</td>
<td>0.733</td>
<td>0.746</td>
<td>0.734</td>
</tr>
<tr>
<td>5</td>
<td>0.731</td>
<td>0.739</td>
<td>0.731</td>
</tr>
<tr>
<td>6</td>
<td>0.731</td>
<td>0.745</td>
<td>0.732</td>
</tr>
<tr>
<td>7</td>
<td>0.733</td>
<td>0.744</td>
<td>0.734</td>
</tr>
<tr>
<td>8</td>
<td>0.741</td>
<td>0.748</td>
<td>0.743</td>
</tr>
<tr>
<td>9</td>
<td>0.738</td>
<td>0.749</td>
<td>0.739</td>
</tr>
<tr>
<td>10</td>
<td>0.737</td>
<td>0.743</td>
<td>0.738</td>
</tr>
<tr>
<td>11</td>
<td>0.737</td>
<td>0.777</td>
<td>0.738</td>
</tr>
<tr>
<td>12</td>
<td>0.735</td>
<td>0.743</td>
<td>0.736</td>
</tr>
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<td>0.733</td>
<td>0.740</td>
<td>0.736</td>
</tr>
<tr>
<td>14</td>
<td>0.731</td>
<td>0.742</td>
<td>0.734</td>
</tr>
<tr>
<td>15</td>
<td>0.735</td>
<td>0.746</td>
<td>0.736</td>
</tr>
<tr>
<td>16</td>
<td>0.732</td>
<td>0.741</td>
<td>0.736</td>
</tr>
<tr>
<td></td>
<td>0.734(0.419)</td>
<td>0.746(1.209)</td>
<td>0.736(0.406)</td>
</tr>
</tbody>
</table>

1 Conditions: H<sub>2</sub> 50 ml/min, Air 40 ml/min.

FIR cut-off frequency 0.2 Hz. Injections of 100 ng di(tert -butyl) disulphide in acetone.
1.2; respectively). Since the apex-burdened whole-peak differential mode is here the odd algorithm out, its forced inclusion in the group of four brings about an RSD value higher than expected.

In other words, if the spectral conditions are fairly constant, three algorithms will produce essentially the same response ratio - with both types of RSD values being around 0.5%. In contrast, the fourth (the whole-peak differential mode) is a bit off and its RSD a bit worse (typically in the 1 to 1.5% range). However, even this poorer precision should still be satisfactory for most analytical purposes.

9.4 Conclusion

To conclude - and at the same time to include summaries of some other directly comparable datasets, Table 9.5 lists the RSD values for three typical chromatographic files being exposed to all four algorithmic treatments. (Other files done for this study follow the same trends.) Several conclusions and expectations can be made from these files:

1. The algorithms do perform satisfactorily over the whole concentration range - even peaks close to the detection limit (Figure 9.1) can be successfully evaluated.

2. In the particular case of S₂ - and likely of some other FPD emitters as well (cf. ref. 71) - care has to be taken that the spectral characteristics of the emitter do indeed remain constant throughout the concentration/quenching ranges of analysis. Under reasonable circumstances, the algorithms are sensitive enough to pick up changes in spectrum smaller than 1% - a change that would, for example, not be normally recognized in conventional
Table 9.5: Summary of Relative Standard Deviations (%) of Dual-Channel FPD response ratios as determined by different algorithms on chromatograms varying in detector conditions, analyte levels, and time-constant settings.

<table>
<thead>
<tr>
<th>No. of peaks</th>
<th>Analyte Injection Level$^1$</th>
<th>Chromatogram filtered$^2$</th>
<th>Area Ratios</th>
<th>Slope Ratios</th>
<th>Median of Single Peak RSD's$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Low, variable</td>
<td>No</td>
<td>7.2</td>
<td>31</td>
<td>24</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>Yes</td>
<td>4.3</td>
<td>7.1</td>
<td>6.7</td>
</tr>
<tr>
<td>17</td>
<td>High, variable</td>
<td>No</td>
<td>3.1</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>Yes</td>
<td>2.6</td>
<td>2.7</td>
<td>2.6</td>
</tr>
<tr>
<td>16</td>
<td>Very high, constant</td>
<td>No</td>
<td>0.42</td>
<td>3.0</td>
<td>1.3</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>Yes</td>
<td>0.42</td>
<td>1.2</td>
<td>0.41</td>
</tr>
</tbody>
</table>

1 Typical levels of di(tert-butyl) disulphide in acetone:
   Low and variable: 0.5 to 2.0 ng;
   High and variable: 5 to 20 ng, both at $H_2 = 200$, and Air = 50 mL/min;
   Very high and constant: 100 ng, $H_2 = 50$, Air = 40 mL/min.

2 "No" represents an RC time constant of 0.22 s.
   "Yes" represents an FIR filter with filter taps and cut-off frequency set to give peak height reduction in the 50 to 60 % range (close to maximum SNR).

3 Median of same-peak RSD values as determined by the 4 algorithms (see utmost right columns in Tables 9.1 to 9.4).

Typical levels of di(tert-butyl) disulphide in acetone: (Hence, such algorithms may find use in spectrochemically motivated work.)
3. In this study, heavy filtering invariably improved the precision of RR data. Although the dependence of RSD values on the time constant of the filter has not been investigated in any detail, it is obvious that dual-channel chromatograms should be routinely filtered before the response ratios of their peaks are determined.

4. In general, the split-peak values - although having less than half of the peak to operate on - are as precise, or preciser than, the whole-peak values in the differential mode. This is speculatively attributed to the - slope-ratio-wise - less precise apex section being excised by the split-peak algorithm.

5. On noisy chromatograms, the integral (area ratio) approach performed better than the differential (slope ratio) approach.

6. The integral algorithm is at present available in whole-peak mode only. However, the addition of a split-peak integral mode - if desired for checks of peak purity, peak overlap, etc. - should require only the minor software adjustment of bisecting the peak at the apex. Similarly, operator-selected (i.e. cursor-defined) peak slices could be evaluated by the integrative (as opposed to the already existing differential) approach.

7. General principles suggest that - in comparison with response ratios determined by the differential (slope) method - response ratios determined by the integral (area) method should be less susceptible to those detrimental effects of shifts in retention time and/or concentration profile that arise from monitoring two sequential detectors. They should also be less susceptible to electronic phase shifts that can occur even when two channels of the same detector are monitored by their own electrometers.
APPENDIX 1

Circuit Diagrams

Figure A.1: Schematic of Programmable Pulser Logic Board
Figure A.2: Schematic of Programmable Pulser Amplifier Board.
Figure A.3: Schematic of a.c. Constant-Current Feedback Circuit.
Figure A.4: Schematic of Three Pole Variable Frequency RC Filter.
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