Sequential-injection Analysis

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by

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Synopsis

Process Analytical Science (PAS) is a rapidly developing sub-discipline of Analytical Science. Tried and tested analytical principles are applied in modified instrumental architectures that enable the real-time monitoring of key process constituents. Process Analyzers are becoming vital and valuable components of sophisticated distributed control strategies. Their acceptance and usefulness is resulting in ever increasing demands on the process analysis researcher. Chemical sensors, first believed to be the ultimate solution for the process controller, have not enjoyed the wide spread application initially predicted. Their long term reliability has not materialized in all but a few cases. An intermediate or alternative approach is required which will incorporate the conceptual simplicity and size of sensors and the predictable and controlled environment of well established flow-based sample

manipulation procedures such as flow-injection analysis (FIA) and the various branches of chromatography.

This study describes the development of such a technique which has been called Sequential-injection Analysis (SIA). The theoretical basis on which the technique is founded is outlined together with the progression of thinking which lead to its conceptualisation. Its successful implementation depends entirely on microprocessor controlled flow programming. The development of a device control and data acquisition package was mandatory and is described. The study then focuses on establishing the operational parameters affecting the design of a SIA manifold.

Having established the manifold design principles, SIA is evaluated as an approach to sample manipulation. The wet chemical unit operation of trace enrichment is applied to SIA. Although the use of SIA for many traditional FIA applications is envisaged, the use of SIA principles with chemical sensors as the means of detection is seen as the ultimate application of this flow-based analytical technique. Some would be so bold as to claim that it is possibly the basis of successful implementation of chemical sensors. Its usefulness and advantages over FIA in such an application is demonstrated. The hardware requirements for the future optimum development of this approach to process analysis as well as some future areas of work conclude the study.

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Samevatting

Die wetenskap van prosesanalise is 'n snelontwikkelende subdissipline van die Analitiese Wetenskap. Beproefde analitiese beginsels word aangewend in gewysigde instrumentasie wat die monitering van hoof prosesbestanddele in reële tyd moontlik maak. Prosesanaliseerders word steeds belangriker en waardevoller komponente in gesofistikeerde verspreide-beheer strategieë. Hulle aanvaarding en nuttigheid bring eskalerende eise mee vir die prosesanalitiesenavorser. Chemiese sensors wat eers beskou is as die eindoplossing vir prosesbeheer, geniet nie die wydverspreide toepassing wat oorspronklik voorspel is nie omdat hulle langtermyn betroubaarheid net in 'n paar gevalle bewys is. 'n Tussentydse of alternatiewe benadering, wat die konseptuele eenvoud en grootte van die sensors kombineer met die voorspelbare en beheerde milieu van goed gevestigde vloeigebaseerde monsterhanteringsprosedures, soos vloei-inspuitanalise (VIA) en chromatografie, word benodig.

Hierdie studie beskryf die ontwikkeling van so 'n tegniek wat sekwensiële-inspuitanalise (SIA) genoem word. Die teoretiese basis waarop die tegniek berus, word geskets saam met die vooruitgang in denke wat gelei het tot sy totstandkoming. Die suksesvolle implementering van die tegniek maak volkome staat op vloeibeheer deur midel van 'n mikroverwerker. Die ontwikkeling van 'n apparaatbeheer- en dataverkrygingsprogram was daarom noodsaaklik en word beskryf. Daarna fokus die studie op die bepaling van bedryfsparameters wat die ontwerp van 'n SIA vloeisisteem beïnvloed.

Nadat die ontwerpbeginsels vir die vloeisisteem vasgestel is, word SIA geëvalueer as 'n benadering tot monster-manipulasie. Spoorverryking as eenheidsbewerking word aangewend ten opsigte van SIA. Al word die gebruik van SIA beoog vir sommige tradisionele VIA aanwendings, word die gebruik van SIA beginsels met chemiese sensors as metingswyse beskou as die uiteindelike aanwending van hierdie vloei-gebaseerde analitiese tegniek. Sommige sal hulle verstout om te sê dat dit moontlik die basis vir die suksesvolle implementering van chemiese sensors is. Die nut en voordele bo VIA in so 'n aanwending word aangetoon. Die studie word afgesluit met die hardeware behoeftes vir die toekomstige optimum ontwikkeling van hierdie benadering tot prosesanalise, en 'n aantal toekomstige studievelde.

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Exodus 31:3 ..... and I have filled him with the Spirit of God, with skill, ability and knowledge in all kinds of crafts-- What do we have that has not come from almighty God?

A work such as this is never the result of a single individuals efforts. Numerous people, by their encouragement, tolerance, motivating, and cooperation contribute beyond what they imagine. This work would not have been completed without the constant encouragement and interest by my wider family, Bible study group, friends, and colleagues.

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Sharon, Andrew, Stephen, and Nicole, I have done this as much for YOU as for me!

Here I stand on the edge of an ocean of truth. I have picked up a few grains of sand, but the whole ocean lies beyond me, unknown.

– Isaac Newton

Chapter 1

Evolution of Sequential-Injection Analysis

1.1 Flow-based Analysis for Process Analysis

Process Analysis is a branch of Analytical Science, established in the 1950s in the petrochemical industry, which has enjoyed considerable interest and growth in recent years. This has manifested itself in several areas. Notably:

- In 1982 the Centre for Process Analysis (CPAC) was established as a joint industry / university venture with start up funding from the National Science Foundation (NSF). The aim of this venture was to stimulate research and technology transfer between academia and industry in the field of Process Analytical Chemistry
- In 1992, the journal Process Control and Quality was established to provide an outlet for technical papers, review articles, and case studies in the field of Process Analysis.
- Clevett, a renowned Process Analysis consultant and editor of the above mentioned journal, published a book entitled "Process Analyzer Technology" (John Wiley and Sons, New York, 1986) which is fast becoming a reference book for this emerging field.

- In 1994, the third international symposium on Process Analysis, Anatech will be held in Europe. The preceding two symposia were held in Europe (1990) and the United States (1992). In each case, delegates form all over the world listened to papers from authors originating on all five continents.
- Major corporations have established research programmes focused specifically at the development of technology in this field.
- Several international instrument manufacturers have established programmes to develop instrumentation for the field.
- Several small groups focused on the research, development, and technology transfer in this field have begun to appear in most industrialized countries.
- In South Africa, we have noticed an increased awareness in industry of the benefits of Process Analysis. Instrument suppliers are adding Process Analyzers to their product range.
- At Mintek, the decision was taken in 1992 to establish a Process Analytical Science research group. The aims of this group are to develop technology and instrumentation for Process Analysis in the metallurgical and associated chemical industries both locally and internationally.

From this we can see that Process Analysis is grounded on a strong research base. This base stretches across disciplines involving various analytical techniques, associated fields of engineering, and computational science.

One technique that has enjoyed attention in many of the avenues mentioned above is the field of flow-based analysis. At CPAC, the Flow-injection Analysis (FIA) group is

highly successful and has, through a stimulating visiting researcher programme, had an impact on groups right around the world. In fact it was during a year long visit by the author to CPAC at the University of Washington that Sequential-injection Analysis (SIA) had its origin. The evolution of this technique from its roots in FIA is an interesting discussion and serves to illustrate the relationship between SIA and other flow-based analysis techniques. It will become clear from this discussion that FIA and SIA share many theoretical elements, instrumentation, and applications. It will also show what additional avenues and benefits have been realized by adopting this novel approach to flow-based analysis.

This discussion will begin by examining the origin, theory, and instrumentation of FIA. As this is developed, common elements which affect SIA will be highlighted. The discussion will then turn to gaining an understanding of the implementation of flow-based analysis in process analysis. This discussion will highlight the need for certain refinements to the then existing flow-based methods of analysis, that eventually led to the development of SIA. Having defined SIA, some of the major areas of research requiring attention in this new field of endeavour will be identified. These areas form the basis of this study.

1.2 PRINCIPLES OF FLOW-BASED ANALYSIS

1.2.1 *Birth of Flow-injection Analysis*

FIA may be defined as an automated sample manipulation method which relies on the injection of a well defined volume of sample solution into an unsegmented flowing stream with subsequent detection of an analyte in a suitable flow through detector. The term, Flow-injection Analysis, was coined by Ruzicka and Hansen[1] in 1975 and built on a steadily emerging realization that the previous requirement for homogenous mixing of the sample and reagent in the flow system and equilibrium conditions were indeed not true requirements.

A thorough review of the early history has been published by Stewart[2]. In this publication, the author makes the point that "when a new technique is introduced to the analytical chemistry community, it is usually the case that the process is the sum of many individual concepts developed by different investigators." This has indeed been true of FIA, in fact the technique continues to grow and develop with new aspects being added all the time. Of course, the danger of such development is that confusion can result particularly in the area of nomenclature, as different workers apply their own terminology. Some have even criticized the very term *Flow-injection Analysis*. However, the widespread use of the term will make the task of an IUPAC group investigating nomenclature in flow-based techniques a difficult one, particularly if they

aim to make radical changes to the conventions which have become accepted through wide spread use.

Some debate also follows when variations of an existing technique are proposed and then given a new name. One example of this is the approach which has been termed *reversed Flow-injection Analysis (rFIA)* where the reagent is injected into a continuously flowing sample stream. Is this a new technique or simply a flavour of the existing technique? Arguments for both sides are equally convincing. It is the opinion of this author that heated debate on these subjects is counter productive and diverts attention from the natural growth and development of the field of study. Rather this energy should be channelled into proclaiming and investigating the benefits of the latest development.

This investigation reports on an interesting and potentially powerful development in the field of flow-based analysis. No space will be allocated to debating whether this development represents a fundamental advancement of the field to the point of justifying a new name or whether it is simply a variation on the existing technology. Rather it will be taken as a *fait accompli* and the potential that this new approach opens and the possibility for further development of the field that it unleashes will be highlighted. As further work is conducted in the field, the advantages that the new approach offers will determine whether the new terminology remains or disappears.

1.2.2 *Non-equilibrium conditions*

Practising analytical chemists must master a wide range of sample handling procedures. Aside from the mechanical task of weighing, these procedures include pipetting, mixing, decanting, separating, concentrating, diluting, and other volumetric functions. Despite the mundane nature of many of these operations, robotic sample handling has largely been limited to manipulations in a highly repetitive environment or where safety considerations preclude the presence of human operators.

Despite rapid and widespread advancement in the detectors used to measure components in prepared samples, until the mid 70s, few advancements had taken place in the field of automated sample handling. Sophisticated detectors and analytical instrumentation equipped with auto samplers were still dependant on the prowess (and speed) of the analyst responsible for sample preparation.

In wet chemical sample manipulation (see Figure 1a), flowing systems were seen to offer a means of simulating a conveyor belt of beakers to which various manipulation actions were applied (see Figure 1b), e.g. an aliquot of a required reagent was added. Samples in the simulated conveyor belt, were separated by air bubbles thereby ensuring the integrity of each sample (see Figure 1c). This mode of operation prevailed from 1966 when Skeggs[3] defined the concept of segmented-flow analysis. Essentially this approach still depended on homogenous mixing of the sample with the reagent – a

concept which is engrained in wet chemists throughout their training as a the basis for reproducible measurement.

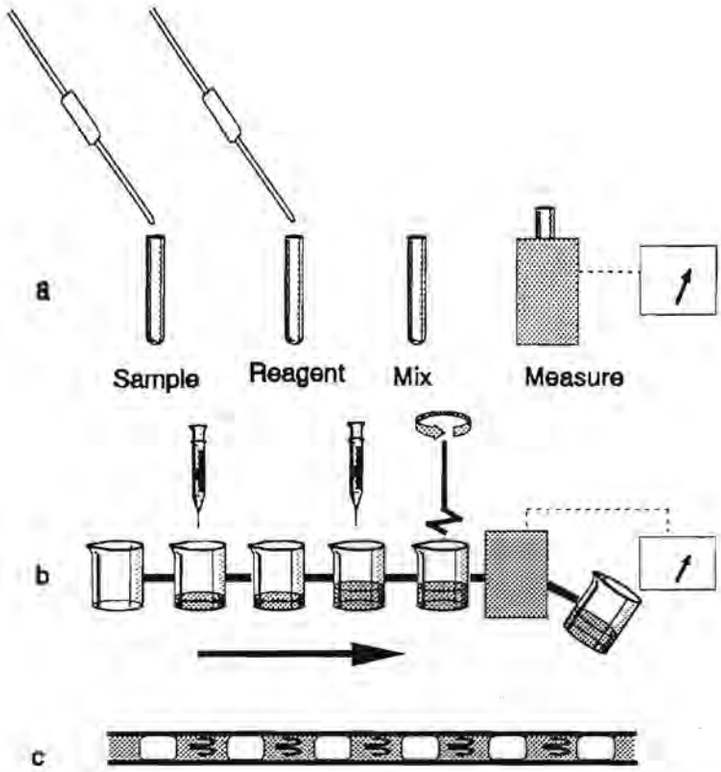

Figure 1: Development of automated sample manipulation procedures in wet chemistry. a. manual methods, b. conveyor belt, c. air segmented continuous flow methods

In contrast, FIA does not impose this limitation, in fact after injecting a well-defined sample into a continuously flowing stream and allowing the sample to be propelled down a flow conduit, a continuum of ratios between sample and reagent is achieved (Figure 2). Reproducible measurement is obtained because the geometry, flow rate, and timing of the system are kept constant.

This means that sub-stoichiometric conditions, or incomplete reactions, or other non-equilibrium conditions can prevail without compromising the reproducibility of the measurement. The implications of this have played a significant role in the wide

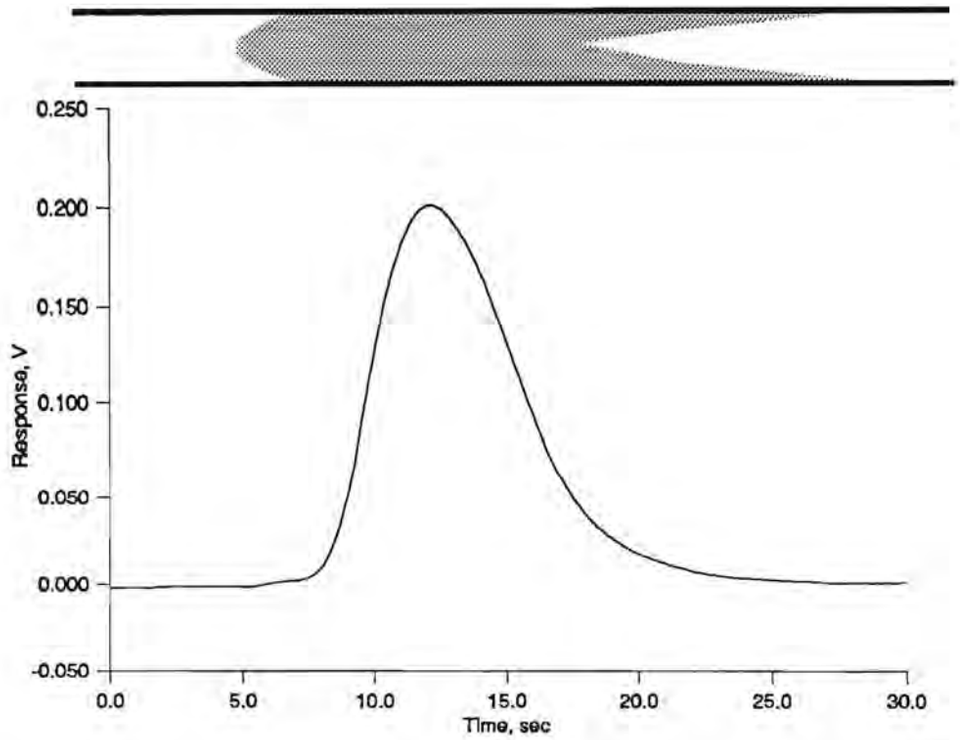

Figure 2: Dispersion of the sample zone into the carrier stream as it is propelled towards the detector under conditions of laminar flow. A continuum of ratios of sample to carrier concentration is achieved.

acceptance of FIA as a valuable tool in many areas of analysis. For example, measurements requiring lengthy colour developing times could be automated and speeded up because the requirement for complete colour development was removed. It also meant that unstable transient species could also be used in the detection process because these were reproducibly produced in the well-defined dispersion-controlled environs of a flow-injection manifold.

1.2.3 *Dispersion*

Reproducible dispersion is the basis for analysis by flow-injection methods. From the early days of FIA, dispersion was recognized as being fundamental to the optimum design and understanding of this approach to sample manipulation. Initially chemical engineering hydraulic models were employed as predictive estimators of the flow-injection response curve. These models are limited to the description of dispersion in the manifold (resultant from laminar flow) and do not consider the contribution from the injection valve, mixing and connection components, and detector flow cell. Furthermore, theoretical studies have been undertaken for systems where there is no chemical reaction and those where chemical reactions take place. DeLon Hull and co-workers[4] recently reviewed a wide spectrum of models that have been applied to the dispersion phenomenon in flow-injection systems and concluded that despite extensive efforts in the development of models, a uniformly acceptable understanding or description of dispersion is still not available.

Of course, while it was necessary for the theory to follow, the utility of FIA was not limited by the absence of a uniform theoretical description of the process and still today, an empirical approach is often adopted. In fact the technique was born out of a empirical approach despite certain errors in early thinking. For example, turbulent flow was initially assumed to govern mixing in flow manifolds. These incorrect assumptions were rectified as the theory was developed. Hence the development of a sound theoretical foundation is fundamental to the wide acceptance and universal application

of key principles of operation. Also, a good understanding of some of the underlying principles, albeit in a fragmented approach, provides a basis for sound manifold design. A more important rationale for expending effort in the study of the theoretical principles behind a particular technique is that such studies often point the way to possible advances in the field.

This was in fact what led to the development of SIA. Conclusions drawn from the Random walk model suggested a course of action that eventually gave birth to SIA. This is discussed in detail in paragraph 1.5.2.

Ruzicka and Hansen[5] defined the conceptually simple and practically useful dispersion coefficient, $D = C^0/C$, where C^0 is the concentration of the sample material before the dispersion process begins and C is the concentration of the sample after the dispersion process in the element of fluid that yields the analytical readout (refer to Figure 2). The special case, D^{max} where $C = C^{max}$, the maximum of the recorded curve, was also defined. Subscripts indicating whether the dispersion coefficient refers to sample or reagent are also frequently used. It is important to remember that D considers only the physical process of dispersion and not the ensuing chemical reactions. The use of this simple relationship has dominated experimental design and the development of the technique. Even when FIA advanced to the utilization of double injection modes which generated well defined stacks of samples and reagents, this parameter was used despite the more important concept of zone penetration which only emerged during the present study.

For convenience, sample dispersion has been defined as *limited* (D = 1-3), *medium* (D = 3-10), and *large* (D >10). Flow-injection systems designed accordingly have been used to achieve a number of different sample manipulation unit operations. Zagatto *et al*[6] pointed out that in confluence systems, these parameters should be re-evaluated.

Zagatto *et al*[6] proposed a generalized parameter, volumetric fraction, to describe the composition of specific element of fluid after a dispersion process. They defined volumetric fraction $X_{s,t,k}$, where s is the solution considered, t is a temporal coordinate, and k is a spatial coordinate. X without subscripts refers to the volumetric fraction of the sample at the time corresponding to peak maximum at the detector. This parameter offers the possibility of evaluating more complex manifolds.

Although Ruzicka and Hansen always recognized the limitations of their approach to theoretical modelling with respect to the influence of chemical reactions[7], it was not until 1981 that the kinetic implications of a chemical reaction were included in theoretical models by Pardue and Fields[8,9], and Painton and Mottola[10,11].

Betteridge *et al.*[12] took a different approach by developing a model which operates on individual molecules. The so-called Random walk model was chosen because it is well suited to investigating sample size, chemical kinetics, and the combination of reaction rate and physical dispersion. This model successfully predicted many observations made with real flow-injection systems. For example, peak height decreases exponentially with time when no chemical reaction is occurring, and high flow rates

produced rapid mixing and high longitudinal dispersion. Most important for this investigation into SIA though, the Random walk model suggested that mixing, the fundamental requirement for FIA, could take place with no nett displacement of the sample; i.e. it is possible to have D > 1 without traversing a manifold of length, *L*. This important concept is discussed in more detail in paragraph 1.5.2 and is the foundation on which SIA is built.

1.2.4 *Manifold Design Criteria*

Having identified that dispersion was an important criteria in the design of flow-injection manifolds, workers set about defining rules and guidelines for the design of effective manifolds. These criteria have been summarized in a series of rules by Ruzicka and Hansen[5]. These rules govern the effect of sample volume, reactor length, flow rate, and reactor geometry on dispersion. They also address means of increasing sampling frequency while maximizing sensitivity and other measurement phenomenon, e.g. FIA titration.

While a good understanding of these principles is helpful in the design of effective flow-injection manifolds, many practitioners prefer to rely on a straight forward empirical approach. In this regard, FIA is quite forgiving and a system which has not been optimized can yield absolutely satisfactory results. There are nevertheless, dangers associated with such an approach. Again this illustrates the tremendous power of a

reproducible sample handling procedure. A less than optimized sequence, if carried out in a reproducible fashion can (and often does) yield reliable results.

1.3 HARDWARE

One of the factors which ensured FIA's initial widespread acceptance has also hampered its long term development. Components needed to put together a flow-injection manifold can be inexpensive and are commonly found in most laboratories. To assemble a flow-injection manifold, a minimum configuration would include

- some means of propelling the sample - (gravity feed should not be excluded),
- a means of introducing the sample - early systems though not ideal made use of a hypodermic syringe and a length of silicone tubing to act as a septum,
- a flow through detector - a simple LED-based photometer can be assembled in most electronic workshops, and
- a recording device - an inexpensive laboratory recorder is the obvious choice.

While such an arrangement would deliver a crude system with small chance of application in a routine fashion, it illustrates the point that FIA has low capital requirements. Even if one were to go for a more sophisticated system making use of a good peristaltic pump, electrically actuated injection valve, simple detector, and microprocessor control, the individual components would cost approximately US$6 000 (R20 000).

There are a few instrument manufacturers who supply good commercial laboratory analyzers, one or two who cater for the process environment and then only for specific applications, and one with a specific application who offers a portable field analyzer for environmental monitoring.

It is estimated that in the USA, there are about 50 flow-injection analyzers in the process environment. All of these are home built systems. Instrument suppliers are reticent to get involved in this area. The following reasons for this are given:

- Providing the equipment is not enough, it is necessary to also supply methodology. Individual users have specific requirements and low volume applications cost money to develop and support. Industry is generally not prepared to pay the price necessary to cover these costs.
- Components are readily available either off the shelf or borrowed from chromatographers. Many laboratories have opted for the development of their own systems. Time and again this has proved to be a costly exercise as an important and often overlooked aspect of reliable systems is the software and hardware necessary for reliable and versatile data acquisition and device control.
- Process analyzers are strongly dependent on custom made sampling systems.

While early investigations of the technique can happily be undertaken with a minimum outlay of capital, a sound approach is to quickly identify a reputable vendor of complete systems. This frees the laboratory to concentrate efforts on the development of suitable chemistries of measurement and leave the instrument design and software development

to instrument vendors. Such vendors can afford to offer a versatile and thoroughly debugged system which is configurable to meet individual requirements.

Also in the early development of SIA, hardware (specifically the pump) had a negative impact on progress. While the sinusoidal flow syringe pump was suitable for demonstrating the principle, the sinusoidal flow pattern complicated operation of the analyzer. Several investigators reverted to the use of peristaltic pumps. Considering that SIA was developed specifically (though not exclusively) with the process environment in mind and peristaltic pumps are not ideally suited to the process environment, this is a step backwards rather than an advancement. The specification for ideal SIA instrumentation is set out in paragraph 1.6.5.

1.4 Flow-based Analysis for Process Analysis

1.4.1 *Automated Wet Chemical Analysis*

Automation in the laboratory environment received a considerable boost with the introduction of FIA by Ruzicka and Hansen in 1975[1]. This approach to sample manipulation gained world-wide popularity in both industrialized and developing countries. Instrumentation costs were small compared to many other methodologies, and the assembly of flow-injection manifolds from components borrowed from chromatographers or machined by in-house machine shops meant that even laboratories with a limited budget could participate.

It was the wet chemical laboratory which benefitted most from the power and versatility of FIA. The classical literature was scoured for suitable wet-chemical procedures that could be implemented in flow-injection manifolds. Separation, trace enrichment, dilution, and many other typical unit operations carried out in a wet chemical laboratory were adapted for use in flow-injection manifolds. Colorimetric, electrochemical, and other detectors were equipped with suitable flow cells and incorporated into manifolds. Many classical methods of analysis, which over the years had been replaced by instrumental techniques such as atomic-absorption spectrophotometry, obtained a new lease on life. Also, methods which had been discarded because it was difficult to ensure reproducible handling of a sample or which made use of unstable reagents that could be prepared *in situ,* could again be explored in the controlled environment of a flow-injection manifold. Electrochemists, beginning to feel the impact of sensitive instrumental techniques, found new avenues opening to them as flow-injection provided them with novel options in the manipulation of samples prior to electrochemical detection.

FIA was not just a curiosity for the researcher. Rather there is a growing trend to replace the tedium of manual sample manipulation in service laboratories with flow-injection analyzers fed by autosamplers. A dramatic new approach to wet-chemical analysis is emerging[47].

Part of the reason for its growing popularity is evident when examining the steps required to develop a flow-injection method (Figure 2). When confronted with a

particular measurement request, the *modes operandi* for the flow-injection method developer is as follows:

- Determine the measurement parameters, e.g. analytical range, required precision, etc.
- Establish whether an existing wet chemical method is suitable.

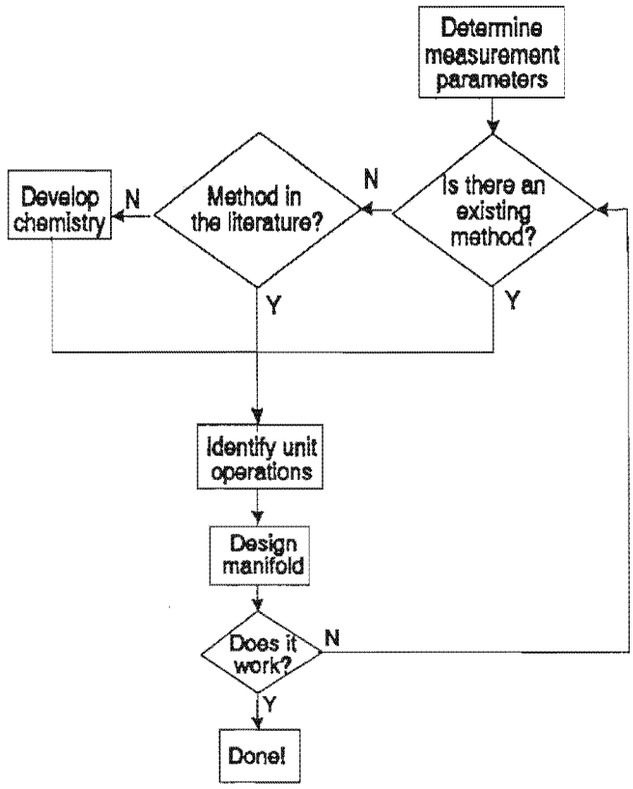

Figure 3: Application development flow diagram

- If not, search the literature for a suitable measurement chemistry.
- If none, can be found develop a chemistry from scratch.
- Identify the unit operation which together form the method. In this regard, a proven battery of flow-injection unit operations, and flow-injection components such as dialysis units, phase separators, flow-through stirrers, etc. is of great assistance.
- Design an FIA manifold that will achieve the desired sample manipulation.
- Test the methodology in the flow-injection manifold. Pay special attention to the optimization of manifold parameters and reagent concentrations, and interferences.

A closer look at this sequence of events reveals two important considerations.

(1) An established "tool box" of unit operations and flow-injection components is invaluable to the method developer.

(2) Established and proven chemistries of measurement, where available, can be used. This cuts down development time significantly and also contributes a high degree of confidence to the final methodology.

These considerations proved equally important when FIA began to make its way into the process environment. Equipped with suitable robust instrumentation and proven chemistries of measurement, process analysts could keep development time to a minimum and efficiently demonstrate the versatility of FIA in the process environment.

As is the case in their laboratory counter parts, flow-injection process analyzers are often in-house developed systems. There are presently numerous flow-injection process analyzers in daily use in the United States and Europe. The first systems in South Africa began field tests last year and the first full commercial installations were commissioned early in 1994. All indications are that the use of this approach will continue to increase in the future particularly as reliable commercial systems come onto the market. Frequently, end-users consider reliability and robustness as prime criteria when selecting instrumentation. In this regard flow-based analyzers have much to offer.

1.4.2 *Advantages*

Flow-based process analyzers provide several excellent features that are absent in many continuous analyzers and sensors[5,13]. Of note are the advantages in the area of automated calibration, self-diagnosis, and self-cleaning:

- Calibrants are subjected to the same treatment as the samples.
- The baseline provides an immediate indication of a drifting detector or fouling of the system.
- Because a small sample bolus is injected into a continuously flowing stream, the analyzer is constantly flushed with a clean solution.
- The ability to make use of highly reactive, oxidizable, light-sensitive, or otherwise unstable reagents which can be generated *in situ* sometimes makes this approach the only possible solution to the monitoring of a particular process stream.
- There are several additional advantages such as a high sampling frequency, good reproducibility, and computer-compatible hardware which ensures that process analyzers are conveniently incorporated into existing or planned distributed process control systems.

With such advantages, it did not take long for these flow-injection analyzers to make their way into the plant environment. Many successful installations followed[14-17].

1.4.3 *Disadvantages*

Nevertheless, there is always the need for improvement. Despite the above mentioned successes in areas as varied as the monitoring of fermentation processes, sea water, and redox scrubbers, there still exist some barriers to the extensive wide scale deployment of flow-injection process analyzers. These may be summarized as follows:

- Usually a new manifold must be developed for each application. Manifolds are often complex and prone to blocking.
- Most flow-injection methods are suitable for the determination of single elements. Few multi-element detectors are presently used.
- In spite of the fact that FIA uses pump speed typically of the order of 1 $cm^3.min^{-1}$, this still translates into about 10 dm^3 of reagent per stream per week. This can prove to be excessive particularly when expensive reagents are being used wastefully essentially as carrier solution.
- There is a lack of robust commercial instrumentation, system devices, and manifold components.

Of course these barriers do not apply to all applications and flow-injection process analyzers will continue to enjoy wide application.

While sensors continue to enjoy much research funding it would appear that the their wide scale use is not eminent. Experts in the field point to early in the 21st century as being the time when we can expect to begin to enjoy the fruits of research into the field of sensors[21]. This is a long time to wait and presently, the overwhelming conclusion of

process analysts is that most chemical sensors can not be relied upon without some level of sample preconditioning or manipulation. Many sensor researchers would agree. Chemical sensors are simply not robust enough yet. Furthermore the resources required for their development make all but the highest volume applications quite beyond economic viability.

An assessment of these barriers and a broad consideration of process monitoring requirements indicated the need for a more versatile flow-based sample manipulation technique. The development of such a technique and its application is the subject of this study.

1.4.4 *Instrumentation*

The last barrier mentioned above, that of inadequate commercial instrumentation, will not be addressed in this study. Instead, some of the minimum specifications for future commercial systems will be highlighted, with specific reference to the process environment. It is hoped that this will hasten their development and thereby ensure the future wider utilization of flow-based process analyzers.

A characteristic of FIA since its inception has been the use of home-built systems. While commercial systems have been available, these have often focused on a particular application. This goes contrary to one of FIA's major benefits - its usefulness for a host of different measurements. Also, fittings and components are frequently borrowed

from the chromatographer despite significant difference in operating conditions, notably pressure.

While the absence of versatile commercial systems can usually be tolerated in the laboratory, in the process environment it has severely limited the wide spread application of FIA as a process analyzer technique. Only organizations with large research departments and budgets can afford to develop their own in-house flow-injection process analyzers.

Instrument suppliers seeking to address this need should heed the following guiding specifications.

1.4.4.1 Sampling system

Generally, sampling systems are developed to meet the requirements of the particular process being monitored. The following desirable options should be provided for:

- The sample may have to be pumped out of the process.
- The sample may have to pass through a polishing filter.
- For high value or toxic sample there should be a provision to return unused sample to the process.
- The ability to monitor multiple streams should be incorporated.
- In some cases it may be necessary to carry out some form of sample conditioning, e.g. cooling.

- Maintenance requirements should be kept to a minimum and should be conveniently undertaken.
- Some form of self diagnosis should be considered.
- High dissolved solids and particulate material must be handled in an unattended fashion for extended periods of time.

1.4.4.2 Calibration

Most detectors used in flow-based analysis require regular calibration. The mechanical system used should be as simple as possible, probably dependant on a multi-position selection valve. Of greater importance, the calibration algorithms and strategies require careful thought and attention. Some options which should be handled by the controlling software include the ability to:

- Intersperse calibration measurements with sample measurements.
- Average replicate measurements after sensible outlier rejection.
- Select the number of calibrants.
- Apply one of several curve fitting algorithms and even multi-variable calibration techniques. This option will become more important as there is a move towards multi-element techniques based on detector arrays.
- Periodically check the validity of the calibration.
- Waive calibration if the previous calibration is still valid.
- Include statistical measures to establish the goodness of fit of the calibration curve.

1.4.4.3 Pump

The means of propelling streams is one of the weakest components in present flow-based process analyzers. One of two options are generally applied. Either chromatographic reciprocating pumps or peristaltic pumps are used. Both of these have their limitations. One commercial system makes use of a pressurized reagent container to propel the carrier stream. Included in the specifications for the ideal pump are the following:

- robust - able to withstand continual use for extended periods of time with little or no scheduled maintenance,
- all wetted parts to be able to withstand corrosive solutions and organic solvents.
- not prone to blockage,
- be easily controlled using TTL or switch controls (forward, reverse, and stop),
- device actions should be rapid and without significant inertia,
- flow rates in the range 0.5 to 15 $cm^3.min^{-1}$,
- constant flow rates over extended periods,
- smooth, reproducible, and pulseless flow,
- pressures of up to 700 kPa,
- small and compact in size,
- multiple pumping channels (± 4 channels per pump),
- self priming,
- low power consumption, and
- have the option of inherent safety.

A pump which satisfies all of these criteria is still to be developed. It is unlikely that all requirements will be satisfied in a single pump. rather it is expected that a range of pumps will cover the various desired features.

1.4.4.4 Valves

Various valves are required in flow-injection analyzers. These range from simple two way valves, to multi-position selection valves (3 to 10 ports), and multiport (usually 6, 8 or 10) injection valves. These valves should have the following characteristics:

- robust - able to withstand continual use for extended periods of time with little or no scheduled maintenance,
- small and compact in size,
- be able to withstand pressures of up to 700 kPa,
- be easily controlled using TTL or switch controls,
- ports should be of the same diameter as the manifold tubing,
- flow channels should not be torturous,
- all wetted parts should be resistant to corrosive solutions and organic solvents,
- switching should be rapid and reproducible,
- low power consumption, and
- have the option of inherent safety.

Valves which satisfy most of these criteria are already available.

1.4.4.5 Detectors

There is a dangerous tendency to simply move reliable laboratory detectors into the process environment. This practice often fails. A superior approach is to develop a whole new range of detectors which are based on proven analytical concepts used in laboratory analyzers. These detectors should be designed with process conditions in mind with a minimum number of vulnerable parts, and requiring negligible scheduled maintenance. Because these detectors can be configured for a specific analyte, it is often possible to simplify their design. One of the most important components in any flow-injection detector is the flow-cell. Time spent in ensuring a sound design is well spent. In this regard, strong guiding principles are:

- minimize dead volume,
- simplify flow paths,
- avoid bubble traps, and
- ensure convenient maintenance schedule.

To date most detectors are single channel devices. This is seen as a short coming in present analyzer designs. Diode array spectrophotometers (uv, vis, and ir regions), and electrode arrays for electrochemical detectors promise to be important detectors in future multi-component analyzers. Robust commercial systems, specifically diode array detectors, are beginning to make their way into the market.

1.4.4.6 Device Control and Data Acquisition

In the design of a device control and data acquisition system, the main consideration should be versatility. The ability to easily swop devices and detectors and programme the device events in a convenient fashion is of paramount importance.

The rate of change of the detector response is such that a data acquisition rate of 10 sec^{-1} per channel is more than adequate. Some redundancy to allow for statistical smoothing of the data is desirable, though not essential. There is a need to allow for different input voltages from the detector. A reliable amplifier and facility for offset adjustment is quite adequate.

Devices may require either transistor-transistor-logic (TTL) or switch control and both options must be available. Analyzer diagnostic systems often rely on digital inputs. These requirements have been adequately addressed in the FlowTEK™ software package which will be discussed in more detail in Chapter 2.

1.4.4.7 Data output

While most process control systems have sophisticated calculation capabilities, it is most advantageous to carry out all calculations at the process analyzer itself and only release final concentration data (for samples and where required, calibrants) to the process control system. Either a 4 to 20 mA signal or ASCII data compliant with one

of the serial communications protocols is regarded as a minimum requirement. Convenient access to raw signals at the analyzer facilitates debugging during commissioning and analyzer breakdowns.

1.4.4.8 Housing

Some process analyzers are installed in specially constructed analyzer shelters and then the demands on the analyzer housing are fairly lenient. Where this is not the case, protection from the elements and plant environment is required. Generally, an IP55 coded housing is sufficient. In the choice of the analyzer housing material, careful cognisance of the plant environment is required. This is particularly important in corrosive environments. In explosive environments (Div 1 Class 1) where inherently safe instrumentation is required, it may be necessary to purge the analyzer housing and equip the door of the housing with a cut off switch that powers the analyzer down when the housing is opened.

1.4.5 *Future of FIA in Process Analysis*

While this study focuses on SIA, it is worth considering the future of FIA in the process environment because it highlights important considerations for the future of SIA as well. Weaknesses and opportunities in this area could impact on SIA. These ideas have been developed through various discussions both with members of industry and with fellow researchers and colleagues in the United States of America

It is most likely that as the merits of FIA become more widely known, this mode of plant monitoring will gain rapid acceptance. Where instrument suppliers offer robust and generic instrumentation, plant laboratories will often be in a position to suggest a suitable measurement chemistry or even develop the methodology themselves. This is a most desirable options and represents an efficient means of meeting process monitoring requirements. This development presents an interesting challenge for the analytical chemist. In many other areas of development in analytical science, instrumentation plays a dominant role and a good understanding of physics and computer science is necessary. As FIA finds wider acceptance there will be a growing need to apply sound chemistry in the development of innovative measurement solutions.

It can be expected that there will be a growing interest and effort in the direction of the development of multi-element methodology. This will necessitate studies using detector arrays such as diode array spectrophotometers, electrode arrays, and multi-dimensional sensors. Chemometric data handling procedures will form vital components of such systems. The need for carefully designed chemistries of measurement will inevitably become more important as selectivity is derived from both the detector, the data handling, and the chemistry of measurement.

Miniaturization is a logical development in the evolution of flow-based process analyzers. Not only will this assist in reducing reagent consumption, but smaller devices will advance the progression towards chemical sensors. Also FIA is seen as a

useful tool for environmental monitoring. In this area there is a growing tendency to move the monitoring device to the sample source or make use of portable systems.

As more chemical sensors are developed and come onto the market, it can be expected that these will be used in FIA manifolds in the beginning. To date, many sensors, particular in the field of biochemistry, have relied on FIA to present the sample to the transducer. This will be discussed in more detail in a subsequent chapter. Some would argue that FIA will provide the launch point for the extensive use of chemical sensors in distributed process monitoring systems. In fact, it is not inconceivable that flow-based analyzers as we know them today, or some variation of them, will form the basis of true in-line process chemical sensors in the future.

1.5 Evolution of a new Flow-Based Analysis Technique

We have noted successful applications of FIA in a number of different applications, and for the foreseeable future, we can expect flow-injection process analyzers to proliferate in modern chemical, biochemical, metallurgical, and environmental applications. The use of chemical analyzers in the process control strategy represents a significant shift in thinking for many process control engineers. For the most part, process control systems are based on physical measurements such as flow-rate, pressure, electrical resistance, etc. While this has resulted in processes which are operated under statistical control, verification of the process performance can only really be achieved by chemical analysis, usually in a remote plant laboratory. This approach is seen as unacceptable in

the design of quality management systems for the production process, e.g. ISO 9000. In such systems, the emphasis is on quality assurance during the process rather than after-the-fact. Process analysis brings the process controller a step closer to ensuring excellent control of the plant and real time quality assurance. At this stage, lengthy development times, the cost of these analyzers, and their maintenance requirements mean that only a few critical streams are monitored.

What process control engineers really want is a whole battery of chemical sensors in a fully distributed system. A high degree of redundancy would ensure reliability and facilities for cross validation. Calls for such capabilities have resulted in considerable research activity in the area of sensor design. Yet researchers will clearly have to come up with novel ways of addressing several severe recurring problems. A case in point is the fibre optic sensor, where quoted disadvantages[19] include long response times, limited long-term stability, limited dynamic range, and the absence of automated verification of the sensor response.

It is worth noting that at present sensors are operated in a mode which strives for steady state response. In some cases this is appropriate and successful in sensors that measure physical phenomenon such as temperature. However, where fragile sensing elements are used that are prone to decomposition or change, a different approach is required. Two principles evident in living organisms apply.

(1) The first makes use of a high degree of redundancy, where the sensing elements are constantly being renewed and old and failing elements are decommissioned on an ongoing basis to be replaced by new sensing elements.

(2) The second operates on an impulse-response basis where two distinct states can be identified for the sensor. The sensing element is subjected to some or other stimulus - it receives an impulse. The sensor in turn generates a response which is transmitted via the nervous system to the brain where it is interpreted. The response is not a continuous signal. Rather it is modulated allowing the sensing surface to re-establish itself between each impulse-response couple. In this way, longevity, reliability, and repeatability is ensured.

Focusing on this approach in Nature and applying it to process analysis, the taste bud provides an excellent model for the next generation of automated wet-chemical analyzer. Process solution (saliva), which has solubilized the analyte (flavour), washes over an array of minute sensors (taste buds). A central processor (the brain) receives and combines data from various process analyzers (senses such as taste, smell, sight, and touch), and through a process of pattern recognition, identifies a taste. The saliva continues to flush the sensors in preparation for the next analysis. During this time, the taste sensors are also calibrated using the saliva as calibrant.

This model suggests an exciting route for the development of future generations of process analyzers. Unlike its predecessor, continuous flow analysis, FIA (and SIA) are impulse-response measurement types and represent the first step in the progression to

the model suggested above. Between injections (impulses), the baseline and chemical environment is returned to a known state. The output from the detector (response) is recorded for transmission to some interpretative device. In FIA, the period between impulses is used simply to re-establish the baseline by flushing away the previous reaction products.

Using a chemical sensor as the detection device in an flow-based manifold now opens up some interesting options. Between impulses, the sensing surface can be renewed. This principle was demonstrated in the development of flow-optrodes[20,21]. Considering these devices, and Nature's example in the taste bud, it became apparent that a further development of the flow-injection concept was in order and could lead to a more advantageous use of sensors in flow-based analyzers. A different approach to solution sequencing was required.

1.5.1 *Flow Programming*

The development of FIA from its inception is based on the introduction of a well defined sample zone into a continuously flowing carrier stream. During the passage to the detector, the sample, being subjected to various physical and chemical interactions, is converted into a detectable species. The success of this technique is totally dependant on a reliable and well designed solvent delivery system.

A review of the FIA literature[5,22-24] soon reveals that an overwhelming majority of applications favour the use of a constant flow rate. Indeed, the ease with which such an environment is obtained when using a simple peristaltic pump, has contributed significantly to the wide acceptance of FIA. There are however several recognized disadvantages of FIA *per se*[25]:

(1) It is wasteful. Reagents are pumped continuously irrespective of whether they are being used or not. Although this action also serves to flush the flow manifold and detector between measurements, where the reagent is costly, this is not a desirable arrangement.

(2) A requirement for increased reaction time implies the use of longer reaction coils. Longer reaction coils result in increased dispersion which may be undesirable.

(3) The chemical kinetics are concealed within the physical process of dispersion. While most chemistries are extremely rapid and so kinetic studies are not practical, kinetic differences in slower reactions can be employed to add a measure of selectivity to otherwise unspecific methods[26]. Constant flow conditions preclude this approach.

(4) It is difficult to maintain a constant flow rate for extended periods of time. Differences in temperature, solution viscosity, and elasticity of pump tubing result in variations in the flow rate.

Wider use of computerized device control has allowed for the development of sequencing techniques which can collectively be termed flow programming. Broadly speaking, two concepts apply, *viz*, stopped-flow FIA[27,28] and reversed or oscillating

flow[29,30]. These two techniques address the above mentioned disadvantages in most instances and raise questions around the requirement for monotonous flow.

It is apparent that the only reason why this mode of thinking has become entrenched is due to the dominance of linear, preferably constant, and forward flow in the theory of FIA. The convenience of being able to inject the sample into the continuously flowing carrier stream at any time has had a strong influence on the development of FIA and its supporting theory.

Rios *et al*[31] and Toei[32] were the first to deviate from this approach. They demonstrated in experiments where the flow rate was varied (positive or negative ramp), that as long as the injection is synchronized with the start of the flow change cycle, reproducible results can be obtained. This observation holds important implications for addressing some of the limitations experienced with systems based on monotonous flow.

1.5.2 *The Random Walk Model*

A theoretical description of the zone dispersion process in the analyzer tubing is a central issue in flow-based techniques. As dispersion is a random process, the random walk model can be used to describe it. Einstein used the random walk model to explain Brownian motion[33]. He showed that a group of molecules taking a series of random steps will finally reach a Gausian distribution around the origin, the spread being determined by the number of steps and the mean size of each step. For a molecule

experiencing laminar flow, the total displacement per step can be considered a combination of the laminar flow and the random walk.

It was Giddings[34] who laid the foundation for providing a theoretical basis for the dispersion process long before flow-injection was even conceptualized, when he applied the random walk model to describe the dynamics of chromatographic processes. Betteridge and co-workers[12,35] built on this to use this model to describe zone dispersion in FIA, albeit for conditions of constant linear forward flow. They pointed out that because this model deals with individual molecules and not assemblages, it is easy to simulate the effects of sample size, chemical kinetics, and the competing effects of the rate of chemical reaction and physical dispersion. Furthermore, conceptually it is easier than theories based on a series of imaginary tanks[36]. In a series of simulations they simulated the dispersion process both in the presence and absence of a chemical reaction and showed the agreement with experimental observations.

Each molecule in their simulated sample plug is represented by x,y, and z co-ordinates constrained within the boundaries of the hypothetical tube in such a fashion as to give a uniform number of molecules per unit volume. The molecules were moved so as to simulate both random dispersion (Δd) and longitudinal transport by laminar flow (Δf). Thus for each cycle, $(x_i, y_i, z_i)_{new} = (x_i, y_i, z_i)_{old} + \Delta d + \Delta f$. Without flow, random dispersion causes the initial rectangular injection to become Gausian. In the presence of laminar flow, the resultant sample peak becomes skewed Gausian as is frequently observed in flow-injection profiles. The real value of the random walk model is that it

leads to the conclusion that efficient mixing of the reacting components can be achieved without actually travelling any nett distance: efficient mixing can be achieved simply by oscillating the injected zone back and forth.

Although Betteridge and co-workers confined themselves to conditions of constant linear forward flow, this is not a requirement for the random walk model - the two terms in the equation above are independent. Thus it became evident that these same principles would hold for a system where linear flow programming was applied. The contribution from the longitudinal transport (Δf) would simply be altered to reflect the changing flow conditions defined in the flow programme. The dispersion contribution (Δd) would remain the same.

In classical FIA the need to travel the full length of the channel is only necessary because the sample zone has to be transported from the injector past a mixing point, through a detector, and then to waste. Use of a flow programme, rather than constant monotonous flow, requires synchronization of sample zone injection with the start of each flow cycle. A system configuration was required which will allow sample zone injection, reagent addition, mixing, measurement, and ejection of the reacted mixture by a combination of forward and reversed flow steps.

While linear flow programming traditionally refers only to the flow pattern employed, i.e., the rate and direction of flow, the inclusion of a mechanism of selecting different streams to be subject to the flow programme may also be added. (Of course, if the

composition of the different streams altered the diffusion constant, this would have an effect on the Δd term.)

The group at the University of Washington[25] used these ideas for the basis of an extension to FIA which was called Sequential-injection Analysis (SIA)[37].

1.5.3 *Sensor Injection*

SIA provides a robust methodology for performing automated wet-chemical analysis. Reagents, samples, and wash solution are selected sequentially using a selection valve and are drawn into a reaction coil. The reaction products are then expelled through the flow cell of a suitable detector giving rise in the process to a measurable signal. Although the new methodology falls short of the standard of our optimized and extremely versatile taste buds, it takes us one step closer to this example of an idealised process analyzer. The major deviation of a sequential-injection analyzer from the model from Nature can be found in the need for a selection valve. The selection valve provides the system with the capability to sequence reagents, samples, and calibrants. In the taste bud this is not necessary as the selectivity of the taste bud is built into the array of sparingly selective sensors that together provide the required level of selectivity through a process of pattern recognition. In SIA, selectivity comes primarily from the chemistry employed. Future work should address the development of sensors that incorporate the chemistry, and therefore have built-in selectivity. The use of solid interfaces and membranes in this regard will prove invaluable.

It is not unreasonable to envisage an instrumental arrangement that presents process solutions, after suitable physical modification, to an array of sparingly selective sensors. The responses from these sensors would then be manipulated to yield selective information on critical plant constituents. Such an approach offers some hope for the wide scale utilization of chemical sensors as it offers a means of overcoming existing limitations in the areas of calibration, reagent renewal, sensor lifetime, and sensor diagnostics.

1.5.4 *Instrumental Layout*

The instrumentation used in SIA is quite similar to that used by FIA and includes a pump, selection valve, reaction coils, and a detector equipped with a flow-through cell. Components are linked with narrow-bore teflon tubing and one of several connector types. Several arrangements of these components have been used. Initially the detector was placed between the pump and selection valve (see Figure 4). Later this arrangement was altered to place the detector in one of the arms of the selection valve. The reason for this will be discussed in a subsequent chapter. Also some workers have used a peristaltic pump or micro volume piston pump called a Tecuria pump (Figure 4c). The advantages and implications of this approach will also be discussed in a subsequent chapter.

Each measurement starts by drawing wash solution into the manifold. This solution flushes the reaction products from the manifold at the end of the experiment. The

selection valve is then advanced and successive aliquots of sample and reagent solution are drawn into the manifold. In the manifold the resultant stack of reagents and sample are transported (with oscillation or stopped flow, if required) through a reaction coil to the detector by reversing the flow of the pump. As the detectable species passes through the detector flow cell, the signal is registered and can then be related to concentration using normal calibration procedures.

1.6 Research Requirements for Sequential-Injection Analysis

Once the initial concept of SIA had been demonstrated[37], several areas of research became apparent and form the body of this study.

1.6.1 *Device Control and Data Acquisition*

The effective and reliable use of flow programming implies the requirement for a highly reproducible means of generating the flow programme. While early FIA analyzers were often manually operated and data acquisition and display was typically achieved by means of a chart recorder, this is clearly not adequate in SIA. SIA relies implicitly on flow programming for the determination of volumes of reagents and samples. Where a non-linear flow rate profile is used the need to synchronize the start of an experiment with a specific instant in the flow cycle is of paramount importance.

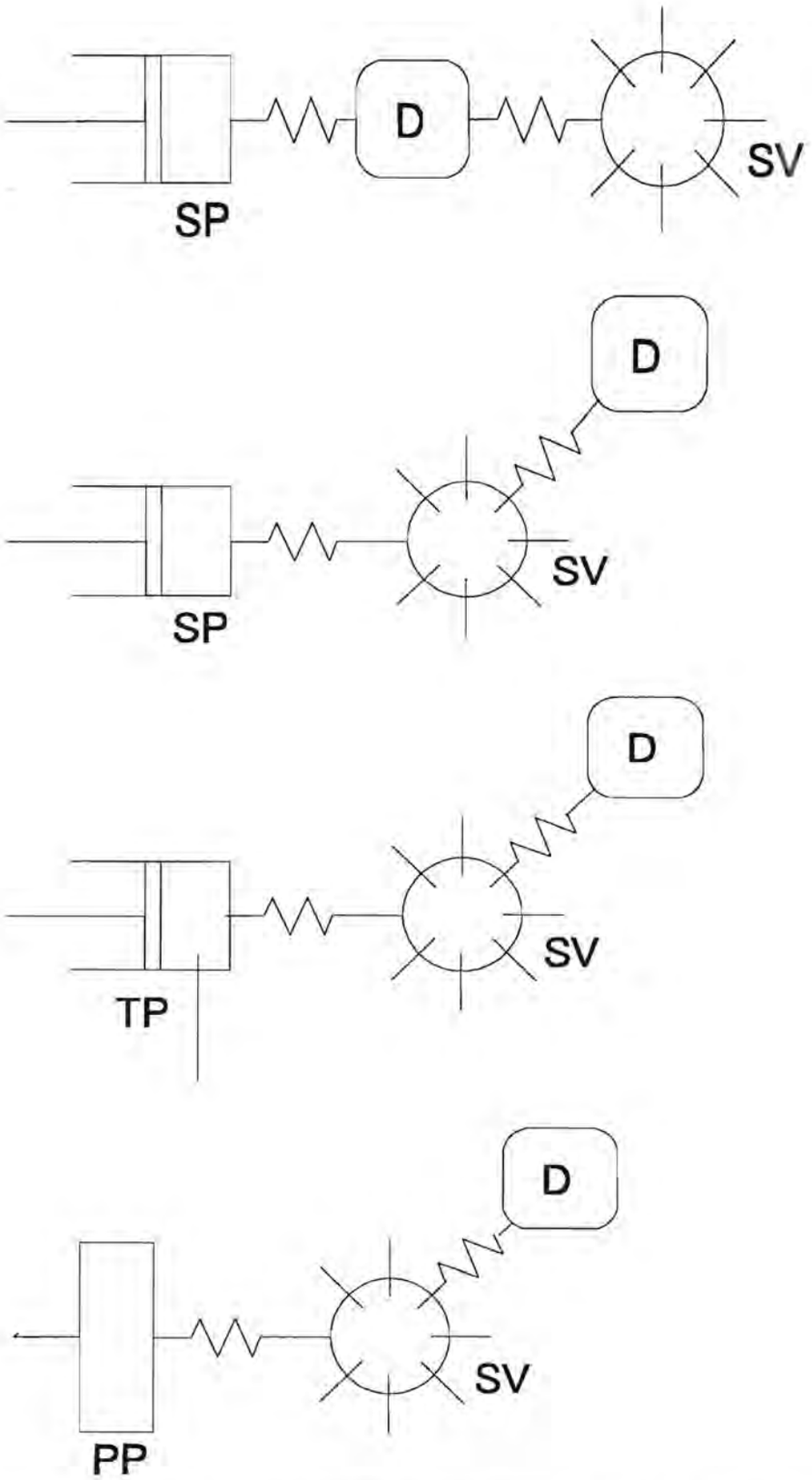

Figure 4: Some variations in initial manifold arrangements. SP - Syringe Pump, TP - Tecuria Pump, PP - Peristaltic pump, D - Detector, SV - Selection Valve

Volumes in SIA are frequently determined by the time that a particular stream is selected or on the number of strokes a pump executes. Control of such parameters is best achieved under micro processor control. Also in the research environment, the ability to alter the sequencing order and relative volumes is most important and should be executed conveniently.

Data acquisition from one or more detectors and subsequent manipulation of the data is also best achieved electronically. Subsequent examination of the data and the execution of various diagnostic procedures requires data to be in an electronic format, (typically as an ASCII file).

These specifications were used as a starting point for the design of a PC-based device control and data acquisition package called FlowTEK™.

1.6.2 *Manifold Design Principles*

Although meaningful results were obtained using the initial manifold design, that manifold was clearly in no ways optimized. Workers at the University of Washington and Mintek investigated basic manifold design principles. Parameters that were investigated included flow reversal, sample and reagent volumes, and factors that had a direct bearing on the degree of zone penetration. Whereas dispersion proved to be a key parameter in the description of flow-injection manifolds, zone penetration is a more important parameter in SIA. It is interesting to note that many of the principles

established in this study are directly applicable in flow-injection manifolds that make use of double injection techniques to achieve a stack of well defined sample and reagent zones.

This investigation also began to highlight some of the important considerations for suitable devices such as pumps, selection valves, and flow-through detector cells.

1.6.3 *Application to Measurement Problems*

Having examined the principles of good manifold design, the developed theories must be tested with some real applications. Trace enrichment and separation using sorbent extraction was selected as interesting typical sample manipulations that an SIA system would have to tackle. The suitability of SIA for this application is demonstrated.

1.6.4 *Sensor Injection*

SIA will doubtless find application in certain specific areas, particularly in the field of process analysis. In fact already, Shell Development Company makes use of SIA to monitor pH. Its true application though will probably be as a front end to sensors. To date sensors have been notoriously unreliable. Already in the biochemical field, analysts are requiring sensors to be part of flow-injection manifolds particularly when operated in the process environment. SIA offers a more versatile sample manipulation

strategy for chemical sensors than does FIA. This is demonstrated by investigating a cyanide selective sensor and its operation in an SIA manifold.

1.6.5 *Design Criteria for Instrumentation*

As is the case for FIA, SIA instrumentation is generally borrowed from chromatographers or built from scratch in precision machine shops. One company in the United States advertises itself as being a supplier of components for FIA and SIA. This represents a significant step forward for the technique and heralds the wide acceptance of the technology by a broad spectrum of users. Nevertheless, catalogues are still thin in this area and the following specification are an attempt to set out some important requirements for future commercial components and SIA systems. The similarity to specifications for similar devices for flow-injection process analyzers is quite evident.

1.6.5.1 Pump

- The flow rate must be highly reproducible and reliable. Continuous maintenance free operation for periods of weeks is necessary for process applications.
- Remote control via TTL or switch contacts should enable immediate stop, start, forward, and reverse pump actions. Pump inertia should be negligible. The ability to control the pump speed via an analog input is seen as a desirable though

not essential option. In this regard, the option of having either a high or normal pump speed may be adequate.

- Flow rates of between 0.5 and 10 $cm^3.min^{-1}$ are seen as being optimal.
- Pump pulsing should be kept to a minimum.
- All wetted parts resistant to a broad range of solvents and acids. It is unlikely that a single material would be able to satisfy all requirements for inertness and therefore two or three options should be available. While this is an important criteria, it is not as critical as for FIA as by far the majority of time, only the wash solution will be in contact with pump components.
- The pump should not be adversely affected if it runs dry. It should also be self priming.
- Connection to typical 0.5 mm, 0.8 mm, and 1.5 mm i.d. tubing should be by means of standard fittings
- Power requirements should be low enabling the pump to be incorporated in portable systems. It would be desirable to offer a 12 V version.
- An intrinsically safe option would be required for certain applications.
- Physical size should be kept as small as possible.

1.6.5.2 Selection Valve

- Continuous maintenance free operation for periods of weeks is necessary for process applications.
- Flow paths should have a minimal effect on dispersion.

- Various flow path options should be available, including dead stop, flow through individual, flow through to common.
- All wetted parts resistant to a broad range of solvents and acids. It is unlikely that a single material would be able to satisfy all requirements for inertness and therefore two or three options should be available.
- Valve ports should match the dimensions of the flow manifold and should not include torturous paths.
- Remote control via TTL or switch contacts should enable random selection of ports using 4 bit digital control. The ability to sequentially step through ports should also be available. Some means of feed back indicating the present valve position is required.
- Power requirements should be low enabling the valve to be incorporated in portable systems. It would be desirable to offer a 12 V version.
- Back pressures of up to 700 kPa must be accommodated. For some applications 2000 kPa would be desirable.
- Connection to typical 0.5 mm, 0.8 mm, and 1.5 mm i.d. tubing should be by means of standard fittings.
- An intrinsically safe option would be required for certain applications.
- Physical size should be kept as small as possible.

1.6.5.3 Detectors

The same criteria as for FIA hold (see paragraph 1.4.4.5).

1.7 REFERENCES

1. J. Ruzicka and E.H. Hansen, *Anal. Chim. Acta*, **78**, (1975), 145.
2. K.K. Stewart, *Talanta*, **28**, (1981), 789.
3. L.T. Skeggs, *Anal. Chem.*, **38**, (1966), 31A.
4. R. DeLon Hull, R.E. Malick, and J.G. Dorsey, *Anal. Chim. Acta*, **267**, (1992), 1.
5. J. Ruzicka and E.H. Hansen, Flow-injection analysis, 2 nd ed., Wiley Interscience, New York, (1988).
6. E.A.G. Zagatto, B.F. Reis, and H. Bergamin F., *Anal. Chim. Acta*, **226**, (1989), 129.
7. J. Ruzicka, and E.H. Hansen, *Anal. Chim. Acta*, **99**, (1978), 37.
8. H.L. Pardue, and B. Fields, *Anal. Chim. Acta*, **124**, (1981), 39.
9. H.L. Pardue, and B. Fields, *Anal. Chim. Acta*, **124**, (1981), 65.
10. C.C. Painton, and H.A. Mottola, *Anal. Chem.*, **53**, (1981), 1715.
11. C.C. Painton, and H.A. Mottola, *Anal. Chim. Acta*, **154**, (1983), 1.
12. D. Betteridge, C.Z. Marczewski, and A.P. Wade, *Anal. Chim. Acta*, **165**, (1984), 227.
13. G.D. Christian and J. Ruzicka, *Chemical Engineering*, (Mar 1988), 57.
14. K.S. Johnson, and R.L. Petty, *Anal. Chem.*, **54**, 1982, 1185
15. C.P.. Parker *et al.*, *Biotechnol. Lab.*, **4** (2), (1986), 23.
16. M.J. Whitaker, *J. Am. Lab.*, (Mar 1986), 154.
17. G.D. Marshall, M.J.C. Taylor, S.J.S.Williams, and D.E. Barnes, *Process Control and Quality*, **2**, (1992), 249.

18. T. Seiyama, Chemical Sensor Technology, vol 1, Elsevier Press, Amsterdam, 1988.

19. J.O.W. Norris, *Analyst*, **114**, (1989), 1359.

20. R.J. Berman, and L.W. Burgess, *Proc. Int. Soc. Opt. Eng.*, **1172**, (1988), 301

21. R.E. Dessy, *Anal. Chem.*, **61**, (1989), 1079A.

22. M. Valcarcel, and M.D. Luque de Castro, Flow-injection Analysis - Principles and applications, Ellis Horwood, Chichester, 1987.

23. J. Moller, Flow-injection Analysis, Springer Verlag, Berlin/Heidelberg, 1988.

24. J.L. Burgnera, Flow-injection Atomic Spectroscopy, Marcel Dekker, New York, 1989.

25. J. Ruzicka, G.D. Marshall, and G.D. Christian, *Anal. Chem.* **62**, (1990), 1861.

26. N. Lacy, Optosensing for signal enhancement and multi component parts per billion determinations in flow-injection analysis, Ph.D. thesis, University of Washington, Seattle, WA, 1990.

27. J. Ruzicka, and E.H. Hansen, *Anal. Chim. Acta*, **99**, (1978), 37.

28. J. Hungerford, G.D. Christian, J.Ruzicka, and J. Giddings, *Anal. Chem.*, **57**, (1985), 1974.

29. S. Olsen, J. Ruzicka, and E.H. Hansen, *Anal. Chim. Acta*, **136**, (1982), 101.

30. J. Ruz, A. Rios, M.D. Luque de Castro, and M. Valcarcel, *Talanta*, **33**, (1988), 199.

31. A. Rios, M.D. Luque de Castro, M. Valcarcel, *Talanta*, **32**, (1985), 845.

32. J. Toei, *Talanta*, **35**, (1988), 425.

33. A. Einstein, *Ann. Phys.*, **17**, (1905), 549.

34. J.C. Giddings, Dynamics of chromatography, Marcel Dekker, New York, 1965.

35. C.D. Crowe, H. Levin, D. Betteridge, A.P. Wade, *Anal. Chim. Acta*, **194**, (1987), 49.

36. O. Levenspiel, Chemical Reaction Engineering, 2 nd ed., Wiley, New York, 1972.

37. J. Ruzicka, and G.D. Marshall, *Anal. Chim. Acta*, **237**, (1990), 329.

Chapter 2

Data Acquisition and Device Control

2.1 INTRODUCTION

Numerous papers have been devoted to the use of microprocessor-controlled flow-injection analysis (FIA)[1]. Different aspects of the control of FIA systems have been described. Most authors agree that computer control and data acquisition leads to superior results and improved use of this versatile method of sample manipulation. In the author's laboratories, there was a need for a software package that would satisfy the requirements of several different groups of people, *viz.* research scientists, service analysts, and plant analysts. The possibility of using one package both in the laboratory environment for research, method development, and service analysis, and in the plant environment for process analysis has not been adequately addressed in commercially available packages. Such an approach demands a modular design and adaptable user-defined set-up options. Integration of the hardware and software must be such as to afford maximum flexibility and user-selectable configuration. This level of flexibility is often absent in commercial systems where the requirement for simplicity places constraints on flexibility.

The researcher demands a system by which simple and flexible configuration of the manifold, single measurements under various conditions and comparison of the resultant peak profiles, the use of a variety of devices and detectors, repeated performance testing, and convenient system documentation are all easily achieved. The service analyst, on the other hand, needs a reliable data acquisition system with the facility to load and save standard methods of analysis. The ability to control an auto sampler is also important. The process analyst, in turn, requires a system that can repeat a measurement at a predetermined frequency, with the added feature of being able to specify a sequence of measurement methods or sub procedures, e.g., calibration, followed by several measurements, and then a wash sequence. (This approach to process analysis will be discussed in more detail below.) The facility of allowing certain basic instrumental diagnostic tests should not be excluded for the process analyzer.

One of the attributes of FIA is that it is reasonably simple and inexpensive to purchase or to build components and assemble these into an FIA manifold This has lead to the wide use of in-house systems. These systems can be operated manually with detector output being directed to a chart recorder. This approach is even used in primitive service laboratories. However, the additional advantages of manpower saving and improved quality of data can be achieved through the coupling of the FIA system to a computer for automated device control and data acquisition.

In the study of sequential-injection analysis $(SIA)^2$ it quickly became apparent that micro-processor control was mandatory. In this novel technique by which the familiar manual laboratory processes of mixing various reagents and carrying them to a suitable detector is automated in a flow system, precise control of a sequence of events ensures reproducible and accurate results. Although this technique can be implemented in the laboratory, its primary application will likely be in the plant environment. In this environment, the ability to manipulate the data and turn it into useful process monitoring or control data should also be incorporated in the device control and data acquisition package. Also, the use of SIA in sensor-based systems will greatly enhance the usefulness of these sensors. Research into the technique and its various applications as well as method development will still take place in the analytical laboratory. Our requirement for a versatile package was therefore extended to include computer control and data acquisition for the related technique of SIA both for the process environment and in the research laboratory. The need for a package that would satisfy all of these requirements prompted the development of this software package.

2.2 INSTRUMENTAL DESIGN

A definition of the terminology used in this chapter and the program is given in the glossary at the end of the chapter.

2.2.1 *Computer*

This program was written for an IBM PC (or compatible) with an enhanced graphics adaptor (EGA) or visual graphics array (VGA) screen. A minimum of 256 kilobytes random access memory (RAM) is required. Although the program can be run on a computer equipped with a 360 kilobyte floppy disk, this places a limit on the number of data files that can be saved and has certain speed implications. The program has been written for the DOS environment but will run in a DOS shell of the Windows environment.

2.2.2 *Interface board*

A general-purpose analog and digital input/output interface board (PC-30B from Eagle Electric, Cape Town, South Africa) was used to interface the computer to the analytical instrumentation. Subsequently the facility to interface other boards, notably the ADA 2200 (Real Time Devices), was also added. Support for other interface boards is simplified by the modularity of the program structure which ensures that all interface-specific commands are kept together. The minimum specifications of this board are set out in Table I.

In the planning of this program's architecture, particular attention was given to maintaining modularity and including user-definable flexibility. These two requirements were not allowed to compromise requirements for a simple and user-friendly interface.

Facilities are provided for the connection of up to eight user-definable devices (e.g., pumps, valves, on/off switches) to digital output points. Four digital input points are configured to enable a measure of instrument diagnostics (e.g., to test whether the reagent reservoir is empty) or other diagnostic signals to the software. Four analog input ports allow for a maximum of four detector or other analog devices to be sampled by the program. Figure 5a illustrates the relationship between the computer and analytical apparatus for FIA. The relationship between the computer and analytical apparatus for SIA is given in Figure 5b.

Table I

Minimum specifications for FlowTEK *interface board*

(satisfied by the PC-30B and ADA 2200 interface boards)

Analog input channels	4 single ended (12 unused by the program)
	12-bit resolution
	Input range (0 to 10 V)
	Input impedance (>100 kΩ/100 p)
	Acquisition rate ($3x10^4$ sec^{-1} variable)
Digital I/O	24 in 3 ports programmable as Input or Output
	TTL compatible
Required power	100 mA at ± 5V
PC connection	Uses a fully bussed full length 8-bit slot of an
	80x86 computer

2.2.3 *Distribution board*

A distribution board was built which allows for easy connection of the devices and detectors to the interface board. A variable gain amplifier circuit was included on this distribution board to enable connection to analog devices with an output range and offset different to that available on the interface board. (This variable gain amplifier allows signals as low as 0 to 10 mV to be amplified 1000 times and an offset of ±12 V

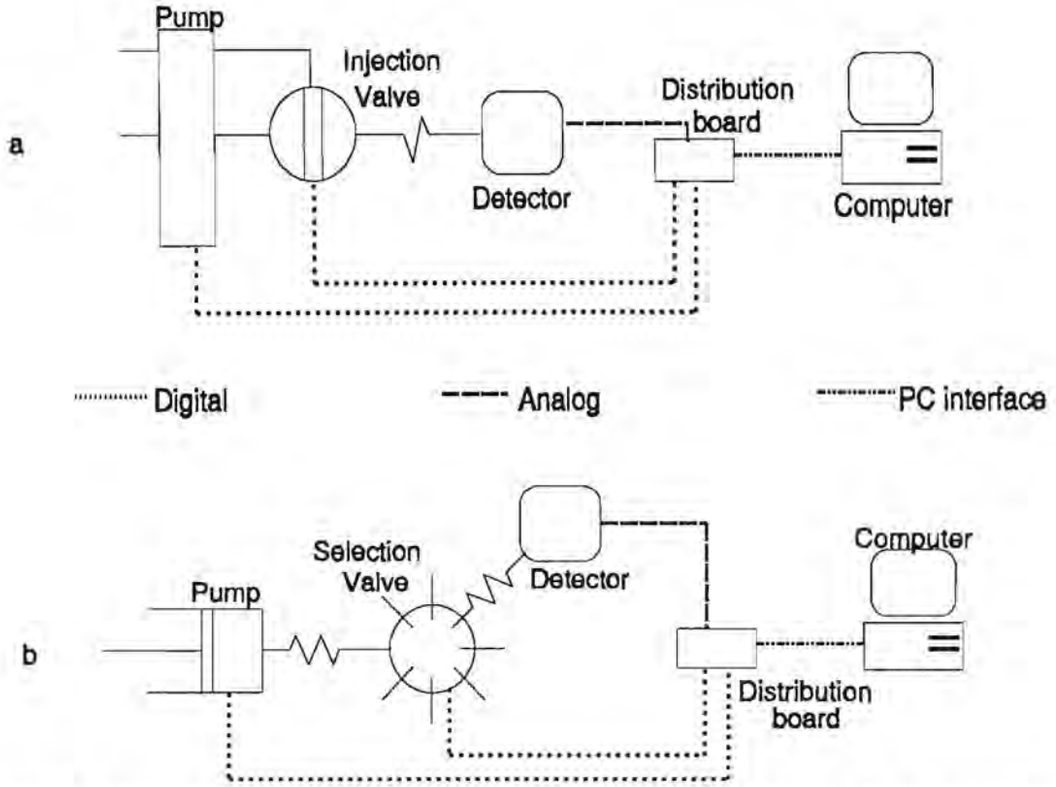

Figure 5: Manifolds and their relationship to computer hardware. a. Flow-injection manifold. b. Sequential-injection manifold.

to be eliminated.) Incorporated in the amplifier circuitry are three levels of electronic filtering with time constants of 0.01, 0.1, and 1 second. Equipped with this electronic signal smoothing facility, it was not necessary to include any signal smoothing routines in the software. These of course can be applied to the raw data using a third party package.

Circuitry was added to the digital I/O lines to enable both TTL and switch control of digital input and output signals.

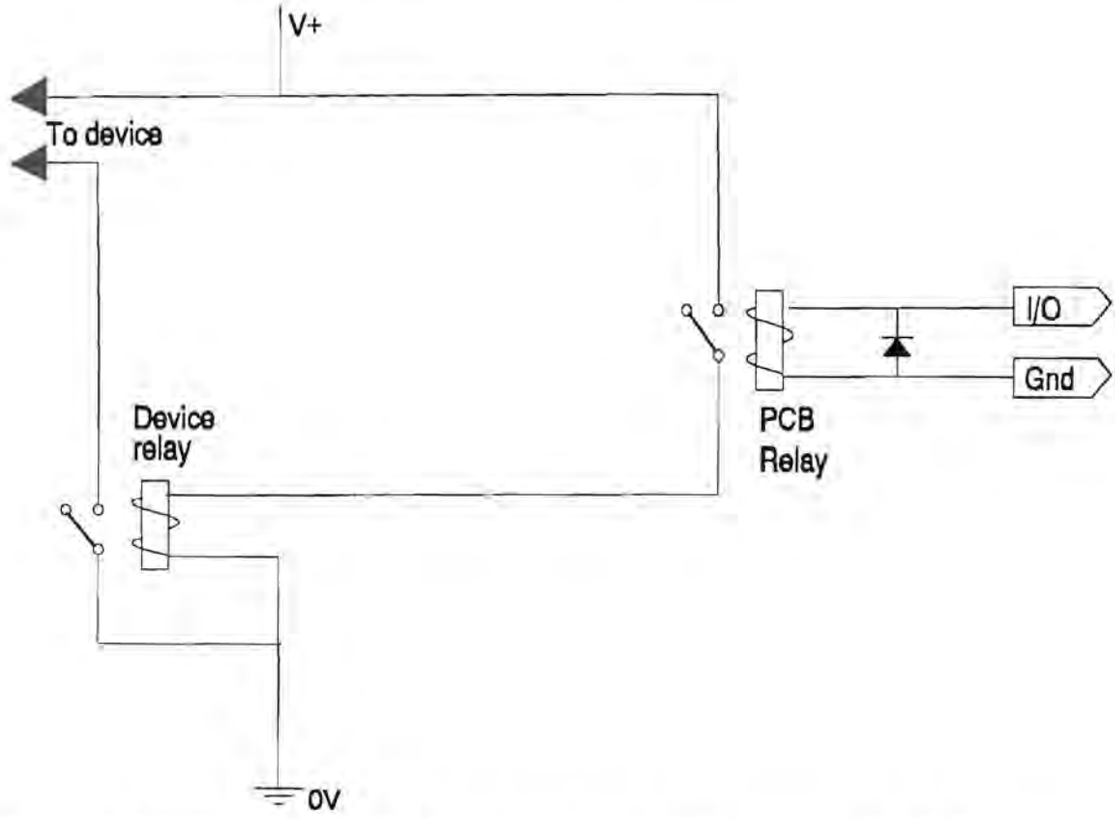

Figure 6: External relay for devices without built-in TTL control. The PCB relay is equipped with a coil of 5V and 500 Ω.

Some of the features described by Clark *et al*[3] in a similar program were implemented in this program. The main differences in the user interface between this package and that of Clark *et al* are in the Methods development module. Features which greatly simplify the definition of methods, particularly for SIA, have been included. Of particular note is the ability to "insert time" between events. This facility is required because in SIA experiments, volume of reagents and sample aspirated is determined by time[2,4]. The data handling facilities of this package have also been enhanced, and more user-defined options are provided.

The modularity of the program was achieved by the independent development and debugging of 20 source files. The source files were compiled and linked using the

Turbo C++ Project Make facility (Borland Corp. Scotts Valley, CA, USA). Figure 8 gives the dendrogram of the program. Each level in the program is activated through menu choices. Menu options are chosen by hitting the key corresponding to the first letter of the menu choice. The main menu is shown in Table II. Each option can have sub-menus below it. Progress back towards the main menu is achieved by hitting Esc or Q for Quit.

The configuration selected by the user is saved to a configuration file on exiting the program. This configuration file is read at the start-up of the program, thereby ensuring the maintenance of the experimental environment from one session to the next. Of course, changes to the configuration are easily achieved through the various menu options. The current state of all user-selected parameters may be displayed on the screen, or as a hard copy on the printer, with the Note Pad menu choice. The parameters that are saved in the configuration file are listed in Table III. At start-up, the last used method file and calibration set up parameters are also read thus ensuring continuity from one experimental session to the next..

Table II

Main Menu Options

Menu option	Description
Once	Executes a single measurement
Repeated	Executes a procedure resulting in several measurements
Calibrate	Performs a least squares regression using either an exponential, rectangular hyperbola, or first, second, or third order model on one of the peak parameters (peak height, area, time, or width at a specific height)
History	Displays one of the peak parameters for up to one hundred measurements
Method	Defines an experiment method allowing various device events to be programmed, e.g., pump off after x seconds, inject at y seconds, detector off, etc.
File	Allows various file name selections and manipulations such as delete, directory and a shell to the disk operating system
Setup	Provides for basic user choices such as mode of auto zero operation, integration limits, the number of detector, and screen display options
Notepad	Lists the present settings for all user-selected options
Quit	Exit from the program

Table III

User-selected configuration parameters and program defaults

Parameter	Default
Path	c:\flowtek\data
Reduced data file	default.red
Method file	default.met
Calibration file	default.cal
Profile file root	default
Save all profiles	Yes
Main procedure file	None
Detector display mode	Paged
Re-scale y axis	Yes
Initial y axis range (arbitrary units)	-0.005 to 1.0
Number of detectors	1
For each detector:	
Minimum integration limit (sec)	0
Maximum integration limit (sec)	60
Height at which width is measured (arbitrary units)	0.1
Time at which peak height is measured (sec)	At peak maximum
Auto zero mode	Every measurement
Time at which zero response is measured (sec)	0
Auto zero offset	0

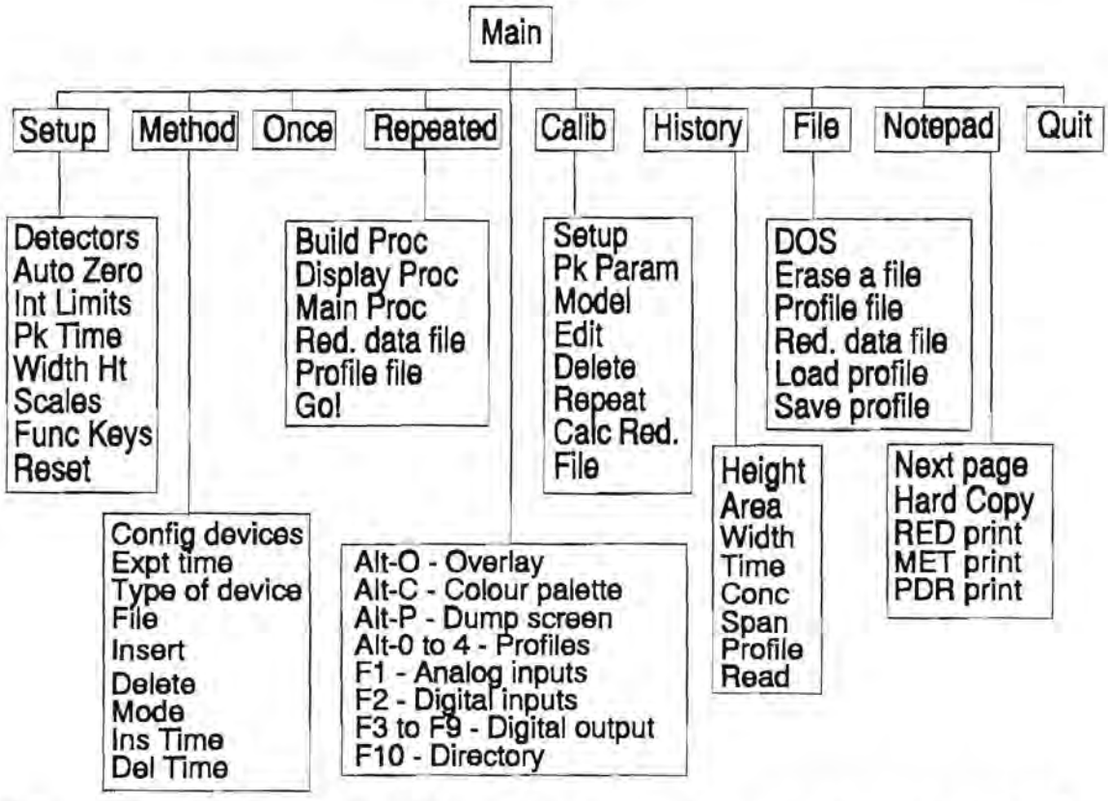

Figure 7: Dendrogram of FlowTEK program showing the various menu options and program functions.

Figure 8 represents an image of the main screen. The top line is reserved for the menu options. The second line is for user input when prompted, and messages from the program. The rest of the screen is divided into four boxes, three of which are always visible. As data are collected, they are plotted in real time as a peak profile in the profile box. The present value of the response and time is also given in a box which appears during each measurement. The scale on the time and response axes can be selected to best display the peak profile. On completion of the measurement, the peak parameter box is updated. This peak parameter box provides useful information which is not easily obtained from a chart recorder plot, *viz.* peak height ,peak area, peak width at a particular height, peak time, and concentration. A line diagram in the device box depicts the state of each of the manifold devices during the experiment.

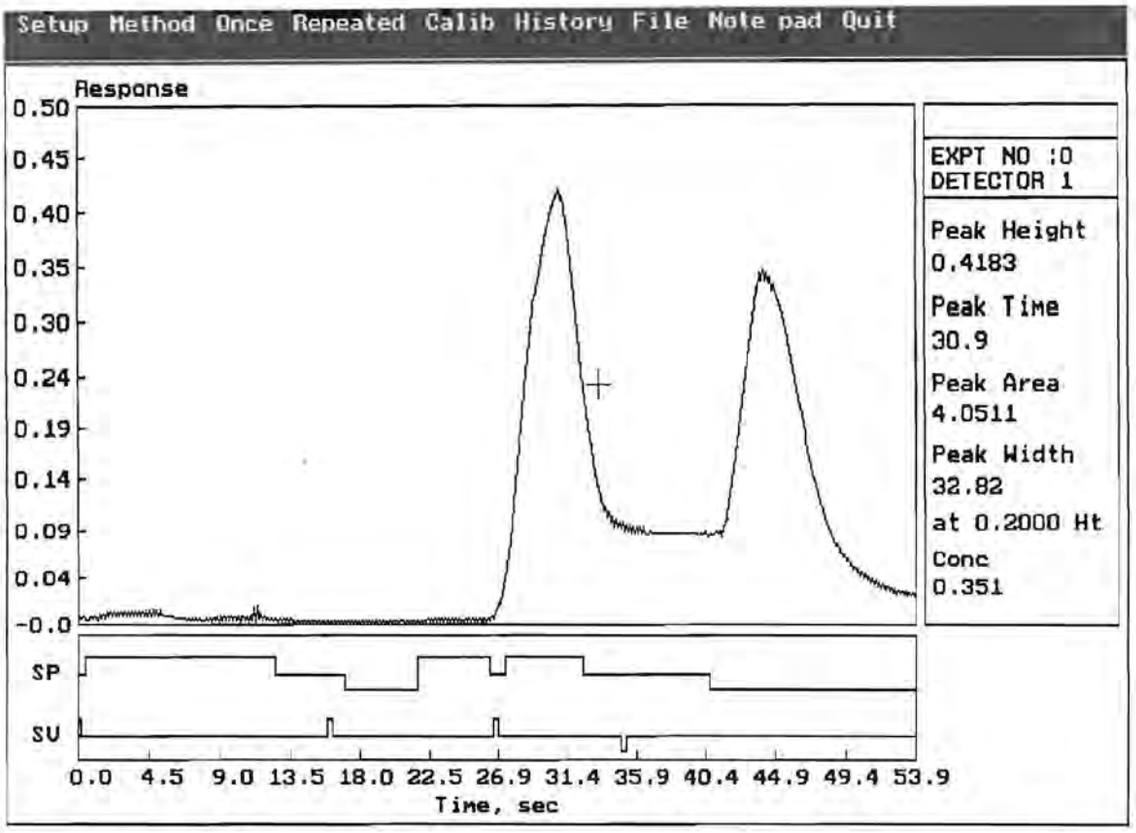

Figure 8: Typical flow-injection profile as depicted on the Main menu screen. Note the device events are depicted schematically in the device display box.

The scaled time and response co-ordinates of a cross-hair cursor are given in the fourth box, which becomes visible if the arrow-key-driven cross-hair cursor is activated between measurements. This facility is useful to pinpoint the actual response at a given time, or the exact time of a particular response phenomenon.

A peak overlay facility is activated with a hot key. This facility enables subsequent profiles to be overlaid in different colours instead of clearing the screen between each measurement. This option has proved most useful in the research environment where peak shape provides additional diagnostic information. In fact, it is our experience that

the amount of diagnostic information provided by this approach is far superior to that obtained from a chart recorder.

The peak parameters (height, area, width, time, and concentration) together with the date and time of the measurement, the profile file name, and method name are stored in one data file called the 'Reduced Data' file (see Table IV for an example). The entire profile, together with the program parameters used in the experiment, as well as just the profile data can be stored for each measurement. The file containing just the profile data is called the abridged profile file and is useful when importing profile data into third party packages for further data manipulation of the response data. These data are stored as the original analog-to-digital raw data. Numbers are therefore integers in the range 0 to 4095. Experiment profiles may be recalled and displayed for comparison in up to four pop-up windows on the main screen. Alternatively they may be overlaid in the main response profile box.

Methods are stored in method files. The definition and design of methods are discussed in more detail below. Procedures are stored in procedure files. (A procedure is a list containing other procedures, methods and wait periods.) Procedures provide a powerful facility for the control of experiments. The use of Procedures is described in more detail below.

Table IV

Typical FlowTEK *Reduced data file*

Date	Time	Peak Height	Peak Time	Peak Area	Peak Width	Width Height	Conc	Profile File	Method File
1994-04-14	16:48:12	2.5513	36.26	17.0731	5.69	1.00	0.0000	DEFAULT1.1	SIACN1.MET
1994-04-14	16:49:10	2.5073	36.15	21.0239	5.81	1.00	0.0000	DEFAULT1.2	SIACN1.MET
1994-04-14	16:50:08	2.4658	35.93	22.5973	5.71	1.00	0.0000	DEFAULT1.3	SIACN1.MET
1994-04-14	16:51:06	2.8906	35.60	24.7214	6.37	1.00	0.0000	DEFAULT1.4	SIACN2.MET
1994-04-14	16:52:03	2.7856	35.93	24.2471	6.22	1.00	0.0000	DEFAULT1.5	SIACN2.MET
1994-04-14	16:53:01	2.7173	36.48	22.9830	6.40	1.00	0.0000	DEFAULT1.6	SIACN2.MET
1994-04-14	16:53:59	2.9272	35.82	27.2263	7.74	1.00	0.0000	DEFAULT1.7	SIACN3.MET
1994-04-14	16:54:57	2.9053	35.82	25.7511	7.04	1.00	0.0000	DEFAULT1.8	SIACN3.MET
1994-04-14	16:55:53	2.8223	36.81	25.2674	7.42	1.00	0.0000	DEFAULT1.9	SIACN3.MET

All data files are stored in American standard code for information interchange (ASCII) format. This feature is particularly important for the reduced data file and peak profile file, since it facilitates the importing of the data from these files into any editor, statistical package, or spreadsheet package. A macro-driven spreadsheet for Quattro Pro has been written that imports the data from the reduced data file and places each number or label in its respective cell in the spreadsheet. Graphing and statistical macros are envisaged for this spreadsheet. This mode of operation can also be used to produce user-defined reports.

2.4 Method Design and Development

A method in this program consists of a list of device events and the time (in seconds), after the start of an experiment, that the event should occur. In addition, information such as the duration of the measurement, time after the start of an experiment that data

collection should begin, and various set-up options such as the integration limits and auto-zero mode also form part of the method. Up to eight devices may be defined in each method. Each device can assume one of up to three digital states (e.g., for a pump the three states of forward, off, and reverse can be defined). Analog control of devices (e.g., programable control of pump speed) has not been included. For devices which require more than three states, a single device can be spread over more digital output points.

When the method option is chosen from the main menu, a new screen is obtained which provides a full screen representation of the line diagram in the device box on the main screen. In addition, another box is provided which lists the actual event time and actions for each device. On this page, the devices used are defined and the duration of the measurement is selected. A cross-hair cursor is used to insert and delete events. Alternatively, the absolute time and device choice may be entered from the keyboard. For example, in response to prompts, the user would respond with the characters in bold print, at time **0** set device **1**, the pump, **F**orward and device **2**, the injection valve, to the **L**oad position. The "Insert" and "Delete" keys are used to insert or delete time between events. This option is useful during method development in the optimization of the method. It also proved to be essential in SIA where time determines volumes of selected reagents and samples. Method files can be saved and retrieved for later use or inclusion in a procedure.

2.5 DATA ACQUISITION AND INSTRUMENT CONTROL

On execution of a measurement, a loop is entered in the program that consists of three tests. This loop is executed at maximum speed until the time set for the measurement has passed.

The first test establishes whether a device event should occur. If so, the necessary value is sent to the relevant digital output port to execute the event. The status of the port before an event is maintained by the software to ensure that an event on one device does not change the present status of the other devices linked to that port. The required output values and the time at which they are to occur are calculated and stored in an array when the method file is loaded.

The second test in the data acquisition and device control loop monitors the keyboard to establish whether the "Esc" key has been pressed. The "Esc" key aborts the measurement and returns control to the main menu. All other input from the keyboard is ignored.

The third test determines whether 0.1 second has elapsed since the last collected data point. If so, the next data point is collected for each detector. If not, the data collection routine is skipped. Each data point is not simply a single reading from the analog-to-digital (A/D) converter. The PC-30B interface board is equipped with a 12-bit monolithic A/D converter. The board contains logic which allows any sequence of

the 16 channels to be sampled. (A maximum of four channels are allowed in this program.) Use is made of the direct memory access (DMA) mode of data collection. In this mode, the selected channels (usually only one) are sampled at the maximum data acquisition rate (3×10^4 sec^{-1}) until sixteen values have been obtained for each selected channel. These digital values are stored directly in the computers memory at an address passed to the data acquisition sub-routine. The maximum elapsed time in the collection of this data when four channels are used is 2.13×10^{-3} sec. For practical purposes, this data is collected instantaneously. On completion of the direct memory storage of the acquired data, the data for each channel are sorted and the two highest and two lowest values are discarded. It is assumed that these may be outliers. The response for each channel is then calculated as the average of the remaining values.

In this way, a data point for each of the detectors is obtained every 0.1 second. However, that data point represents the average of the 12 middle values from 16 values acquired at the maximum acquisition rate. A user-selected mathematical function is then applied to the response from the detector (e.g., transmission data can be converted to absorbance) and the resultant point is plotted on the screen. The Response-Time box is also updated every 0.5 seconds.

At the end of measurement, a calculation routine is executed. In this routine, the user's choice for auto-zero is implemented. By auto-zero, we mean that a zero offset is subtracted from every data point. The value subtracted is obtained at a user-specified time during the measurement (usually at the start of the experiment). The user also

chooses whether this auto-zero option is to be implemented and if so, whether a new offset is obtained for every measurement or just at the start of the procedure. Having implemented the desired auto-zero calculation, the peak height, time of peak maximum, peak area, peak width at a specified height, and concentration are calculated.

For peak height measurement, two options exist. Either the maximum height of the peak is given, or the height at a specified time after the start of the measurement is determined. This enables the implementation of the so-called electronic dilution method[5]. The peak time is then set at either the time at which the peak maximum occurred or the chosen time after the experiment starts. Peak area is determined by Simpson's rule (reviewed in ref. 6). Peak width at a specified height is a useful parameter in FIA titration[1]. If certain manifold design rules have been observed[1], concentration of the sample (C_s) is related to peak width (Δt) according to the following proportionality:

$$\Delta t \propto \log_{10} C_s$$

If a calibration calculation has been performed or a calibration file has been loaded, the concentration of the analyte in the sample can be determined using the calculated regression coefficients and the chosen peak parameter. If no calibration has been carried out, the regression coefficients are taken to be zero, i.e. a concentration of zero is reported.

2.6 CONTROL BY PROCEDURES

In the Repeated mode of operation, a Main Procedure file is defined and executed. The main procedure consists of a combination of other procedures, methods, and a WAIT operation which allows a pause (of up to 8 hours) between measurements or until a digital input is received at a particular digital input point. This option is of particular use in the process environment where the maximum analysis throughput may not be required or an external event may signal the need for a measurement. On initiating a procedure, an algorithm that makes use of a stack structure is executed until the end of the main procedure is encountered. This structure allows nested procedures. (Recursive calls of a single procedure will however result in a stack overflow.)

The facility of being able to define and execute a procedure is an extremely useful one. It allows the analyst to define the sequence of events in an analysis scheme. This means that it is no longer necessary for the same method to be executed repeatedly. However, by design of a suitable procedure, various useful options may be included. For example, standards may be analyzed in duplicate in a calibration procedure. This could be followed by a measurement procedure which analyses each sample in triplicate (samples could, of course, be drawn from one of several sources), and after every 10 samples, a wash method could be executed. The main procedure could be concluded with a repeat of the calibration procedure (to test calibration validity) and a short method at the end to flush the tubing with wash solution and switch off the various devices. The procedure can be aborted at any time by using the "Esc" key. Countless

variations on this theme can be developed. The use of procedures opens the option of leaving the analyzer unattended in the process environment or, together with an autosampler, for overnight laboratory use. It also provides the researcher with the ability to acquire data without necessarily being in attendance. This means that the researcher can busy himself in a more productive fashion.

The reduced data file, containing the peak parameter values for each measurement in the procedure, also contains the method file name used to generate the data. This, together with the time of analysis, allows for adequate sample identification. The reduced data file also keeps a record of the profile file name. The full peak profile for each measurement can be saved. This allows the user to investigate unexpected results at the end of a procedure by looking at the peak profiles.

2.7 CALIBRATION

Several user-selected options have been included in the calibration routine.

- The data used for the calibration can be selected from any reduced data file. The only prerequisite is that the data form a contiguous block in the reduced data file. Alternatively, the user may type in calibration data from the keyboard.
- Up to five standards may be used.
- Each standard can be analyzed once, in duplicate, or in triplicate. Where more than one reading is taken for a particular concentration, the spread of readings

are depicted on the calibration graph. The regression calculation uses the arithmetic mean of the readings.

- The regression can be carried out on any of the peak parameters.
- A least-squares regression using either a linear, quadratic, cubic, exponential, or rectangular hyperbola model is performed on the calibration data. Another option allows for all five models to be applied. The model which gives the best correlation coefficient (r^2) is then adopted.

The regression coefficients and calculated correlation coefficient are displayed together with all the calibration data. The calibration curve is also displayed for visual inspection and evaluation of the regression model applied to the data.

Two methods of editing the data, besides the manual entry of data from the keyboard, are presented. In the first case, a simple deletion of any data point is allowed (provided that there are sufficient data for the selected model). Deleted points are marked as deleted for future reference. In the second case, any measurement may be repeated, and the new data value replaces the old.

2.8 DIAGNOSTICS USING THE HISTORY OPTION

A facility is provided to display graphically, as points or bars, any one of the peak parameters. A maximum of 100 measurements from a reduced data file can be displayed. A cross-hair cursor allows for any measurement to be selected and all the

peak parameters for that measurement to be displayed in a peak parameter box. The profile, if it were saved for the experiment, may also be conveniently recalled and viewed in a pop-up window.

This menu option provides a pseudo chart recorder type of display, but has the added advantage of allowing any of the peak parameters to be displayed. The display of the profile allows a closer *post mortem* examination of the profile of a measurement of particular interest.

2.9 System Performance

The program has been tested in both flow-injection and sequential-injection applications where it was used for automated data acquisition and device control. The repeatable control of the devices improved the precision and accuracy of the data. In one application, the program was used to synchronize pump events in a dilution method described by Clark *et al*[7]. The precision attained improved by about an order of magnitude when electronic control was used.

2.9.1 *Study of fundamental parameters*

The software went beyond enabling researchers to duplicate, in a more convenient fashion, what they were already doing manually.

- Of course, the tedium of a simple manual task such as injecting a sample slug into a flowing stream was removed from the user and left to the computer.
- More importantly though, the software opened up areas of endeavour and research not previously possible. For example, the technique of flow reversal, where the sample is oscillated back and forth in the reaction tube before being dispelled to the detector, can only be investigated with the aid of computer control.
- Of greater consequence for the present investigation, precise computer control is the foundation on which SIA is built. Volumes are determined by accurate control of pumping times. Complex selection valve programmes are routinely and repeatably handled by the software without any intervention by the researcher.
- Having the flow-injection or sequential-injection profile stored on electronic media makes the manipulation of the data using third party packages both feasible and most enlightening.
- Research and method development requires making continual changes to the method employed. The convenient graphical user interface proves invaluable in this regard. In particular for SIA, the facility of being able to insert and delete time between device events and thereby change the respective volumes, was found to be particularly useful. Investigations into the effect of sample volume, for example, are directly affected by this option.

2.9.2 *Method development*

The investigation of the suitability of various chemistries for the analysis of a particular sample or the development of a new method of analysis from sound principles of chemistry, is conveniently handled by the package whether the chosen platform is flow-injection or sequential-injection analysis.

- Methods (as used in FlowTEK - see the glossary for the definition) are conveniently constructed and optimized using the Method menu option.
- Interference studies are simplified with the flexible profile recall facilities and trend monitoring using the History menu option.
- Various calibration models can be investigated and compared.
- Analytical figures of merit such as precision, working range, sample throughput, and detection limits are derived from data stored on electronic media in the reduced data file.
- Once established, a hard copy of Methods, and other key configuration parameters can be produced for inclusion in the investigators note book.

2.9.3 *Flow-based analysis as a diagnostic research tool*

Some workers have used flow-based analysis to track the progress of a particular experiment. A good example of this was the investigation of various aspects of supported liquid membranes (SLMs) using flow-based analysis. Barnes[8] made extensive use of this software to optimize the composition of the membrane, study the

formation and degradation of an SLM, and explore transport mechanisms, as well as parameters such a membrane geometry. Again the availability of key data in electronic format and the ability to leave the experiment unattended to gather data over extended periods proved most valuable.

2.9.4 *Service analysis*

While the software conveniently handles the acquisition of data in a service laboratory environment, manipulation of the data and report writing is best handled by a third party package such as a spread sheet package. Nevertheless, the repeatability of computer control improves the quality of analytical results by removing the influence of poor duplication. Also analyst errors are effectively eliminated.

2.10 FUTURE DEVELOPMENTS

2.10.1 Windows™ *platform*

With proliferation of the Windows operating environment, it is inevitable that users of the software will require the porting of the software to the Windows platform. In such an upgrade, specific attention will be given to exploiting the power of the graphical user interface to simplify the configuration, utilization, and understanding of flow-based analysis. Data manipulation and report writing tools, already available in the Windows environment, must be tapped to enhance the power of the package in these areas.

Options should be developed with both the researcher, routine laboratory analyst, and process analyst in mind.

2.10.2 *Sequential-injection analysis*

Research in SIA in the future is likely to centre around three areas:

1) Application development.
2) Operational simplification and manifold miniaturization.
3) The development of sample manipulation capabilities for sensors.

Software which streamlines research in these areas will contribute significantly to the development and general acceptance of SIA. As pumps are developed and improved, a comparison of time-control of SIA experiments with volume-control will necessitate the addition of a module to the method routine. This routine should also allow the random selection of selection valve ports. (At present valve ports are accessed sequentially.)

2.10.3 *System configuration*

Flow-based analysis techniques frequently require the user to configure the manifold for a particular measurement. Some instrument suppliers have dealt with this by providing encoded manifold cartridges which are simply slotted into a chassis and automatically configure the software for the required measurement. While this level of automated system configuration is appropriate in many environments, it does remove a level of flexibility (which has always been regarded as a positive characteristic of flow-based

analysis) from the analyst. A software package which allows intuitive configuration of the manifold and even provides expert comments on manifold design would provide the best of both worlds.

2.10.4 *Multi-array detectors*

A major field of endeavour in research into flow-based techniques in the coming years will be centred on the use of multi-array detectors such as diode array spectrophotometers and arrays of sparingly selective electrodes. Future software will have to allow the acquisition and manipulation of multi-channel detector output.

2.10.5 *Process monitoring and control*

The incorporation of a third construct in Procedures called a Directive will enhance the versatility of procedures, particularly in the process environment. These directives are instructions to the software or hardware to perform user-specified options at particular points in a procedure or method. Such options could conceivably include various calibration options, diagnostic facilities, alarms and warnings, and some of the set up options which, once they have been selected in the present package, are fixed for the entire duration of the experiment .

Planning for a package with these capabilities is under way.

2.11 REFERENCES

1. J. Ruzicka, and E.H. Hansen, "Flow-injection Analysis", 2nd ed., Wiley Interscience, New York, (1988).
2. J. Ruzicka, and G.D, Marshall, *Anal. Chim. Acta*, **237**, (1990), 329.
3. G.D. Clark, G.D. Christian, J. Ruzicka, G.F. Anderson, and J.A. van Zee, *Anal. Instr.*, **18**(1), (1989), 1 .
4. J.Ruzicka, G.D. Marshall, and G.D. Christian, *Anal. Chem.*, **62**, (1990), 1861.
5. S. Olsen, J. Ruzicka, and E.H. Hansen, *Anal. Chim. Acta*, **136**, (1982), 113.
6. M.A. Sharaf, D.L. Illman, and B.R. Kowalski, "Chemometrics", Wiley-Interscience, New York, (1986).
7. G.D. Clark, J. Ruzicka, and G.D. Christian, *Anal. Chem.*, **61**(15), (1989), 1773.
8. D.E. Barnes, Supported liquid membranes, Ph.D. Thesis, University of Pretoria, (1993).

2.12 GLOSSARY OF TERMINOLOGY

Table V

Terminology used in FlowTEK

Detector	e.g., uv/vis spectrophotometer, ion-selective electrode, atomic-absorption spectrophotometer, etc.
Device	e.g., pump, injection valve, switch, auto sampler, etc.
Method	Series of device events, event times, and instrument set-up options which together result in a single measurement.
Peak parameters	Peak height, area, width at a certain height, time of peak maximum, and concentration
Reduced data	Date and time of a measurement, peak parameters, method file name, and profile file name
Procedure	A combination of methods, other procedures, and WAIT steps
Profile	Plot of detector response versus time for the duration of a measurement
Configuration	A set of user-selected options
Auto zero	The process of subtracting a constant from all data points. The constant is measured at some point during the experiment (usually at the beginning) and corresponds to the detector response in the absence of analyte
Event	A change in the status of a device

Chapter 3

Factors affecting zone penetration

3.1 INTRODUCTION

There is a tendency by many users of flow-based techniques to apply empirical techniques in the choice of operating conditions and design of manifolds. This is unfortunate, as much excellent work has been done on establishing the rules[1] which govern important parameters such as dispersion. A thorough understanding of these rules provides a sound platform on which method development can be built. Even workers who use optimization techniques such as the simplex optimization technique benefit by an understanding of the guiding principles as it enables a more intelligent setting of boundary conditions.

While FIA is forgiving enough to allow an unstructured approach, SIA requires a more stringent approach. Most of the early papers[2-4] dealing with SIA set out important principles which affect manifold dimensions and design. While a good understanding of the principles that affect FIA proves useful in understanding SIA, some findings were totally unexpected and provided some important guidelines which could be re-applied to certain variations of FIA.

3.2 DISPERSION COEFFICIENT OR ZONE PENETRATION

Karlberg[5] has called flow-injection analysis "the art of controlling sample dispersion in a narrow tube". A complete definition for flow-injection analysis[6] appeared in 1978 and was based on a combination of sample injection, reproducible timing, and controlled dispersion. As knowledge of the dispersion process increased, the concept of flow-injection analysis was further refined. In the ensuing years several models have been used to gain greater understanding of the dispersion process. Many of these have been discussed in Ruzicka and Hansen's monograph on FIA[1]. Initially, workers thought that flow in the manifold conduits was of a turbulent nature. This was subsequently found to be incorrect and workers realized that laminar flow and radial mixing were two strong governing influences in determining the dispersed sample profile. As the sample bolus is transported down the conduit, diffusion and convection are the two forces which exert these influences.

Under laminar flow conditions (found to pertain by calculation of the Reynold's number, Re < 2100), the fluid velocity profile is found to acquire a parabolic centro-symmetric distribution; the fluid velocity at the centre of the conduit is twice as fast as the mean flow velocity. At the walls of the conduit, the velocity tends to zero. The effect of this is that convection, resultant from the concentration gradient between the sample and the surrounding carrier stream, has a larger area over which to operate. The combination of these two influences (and several other mechanical influences) result in dispersion of the sample zone into the surrounding solution. This is required

for chemical reactions to take place between the sample and the reagent. While little can be done to influence convection (it is a function of concentration, distance travelled, and the radial distance from the tube axis), secondary mechanical measures can be introduced to enhance mixing in the radial direction. These include:

- the geometric disorientation of the flow path by coiling , knitting, or meandering the tube,
- the use of single bead string reactors, or
- the use of flow through mixing chambers.

Frequently, the full implications of dispersion in this dynamic arrangement operating under conditions of laminar flow, has suffered by the gross simplifications applied. One of the most serious of these may be attributed to the fact that detectors generally provide a radially integrated signal - a homogenized representation of the physical and chemical processes at work. Nothing could be further from the truth. The true impact and implications of the non-homogenized concentration profile, though often mentioned, has not always received its due attention. A simplistic understanding of dispersion theory as experienced through the eye of a detector such as a spectrophotometer, can result in one considering a uniform distribution of a particular species in a particular cross section of the flow conduit. This simplistic understanding can flaw an understanding of flow-based systems. This is particularly true as one considers single-line, double-injection, and sequential-injection techniques.

While dispersion has proved to be an important parameter in FIA, it is becoming increasingly apparent that for SIA, it is perhaps not the most suitable parameter. In fact some would argue that it is not even the most appropriate building block of FIA theory particularly where one or other chemical reaction is occurring. It is interesting to note though, that dispersion continues to dominate the theory of FIA. This can be ascribed to its conceptual simplicity as a guiding principle and its wide use from the outset of the technique.

For SIA, a different, though not unrelated parameter, was found to be more useful. For a technique which operates on a stack of well defined sample and reagent zones, the concept of zone penetration was identified independently by workers at the University of Washington[4] and Mintek[7] as being of fundamental importance. The importance of zone penetration can be ascribed to the fact that this influence has a dramatic impact on the surface area over which a concentration gradient exists and therefore over which axial mixing takes place. For a chemical reaction to take place, elements of sample fluid must come into intimate contact with elements of reagent fluid. For manifolds where no reaction takes place, zone penetration and band broadening must be kept to a minimum.

With the emphasis on zone penetration, radial influences should not be overlooked as it is these that ensure contact between the reacting components. In most cases, this is best achieved by mechanical means, but then only once one zone has significantly penetrated the next.

It is interesting to note that zone penetration is just as useful for certain variations of FIA, notably double injection FIA. With the insight gained in the principles governing the optimization of a SIA manifold as detailed in this chapter, some benefit may be gained by revisiting FIA manifolds which utilize a double-injection approach.

3.3 MANIFOLD DESIGN

Early work in the field of SIA[2] soon showed that a single manifold was sufficient, irrespective of the chemistry to be employed. This manifold, once optimized, could be 'cast in stone'. The variability from one method of analysis to the next is introduced through microprocessor sequencing of reagents and control of the pump, and in some cases, by changing the detector. Changing the detector could be as simple as selecting a new wavelength for a photometer, but could also include exchanging an optical detector for an electrochemical one.

Researchers in the laboratory of Ruzicka and Christian[2-4] and in Mintek's Process Analytical Science group[7] have given attention to the rules governing the design of an SIA manifold. These rules must be applied to achieve the desired mixing regimen.

3.4 INSTRUMENTAL SET-UP

Figure 9 depicts a typical sequential-injection manifold. The manifold consists of three main components plumbed with narrow-bore (0.5 to 1.5 mm i.d.) tubing. The syringe

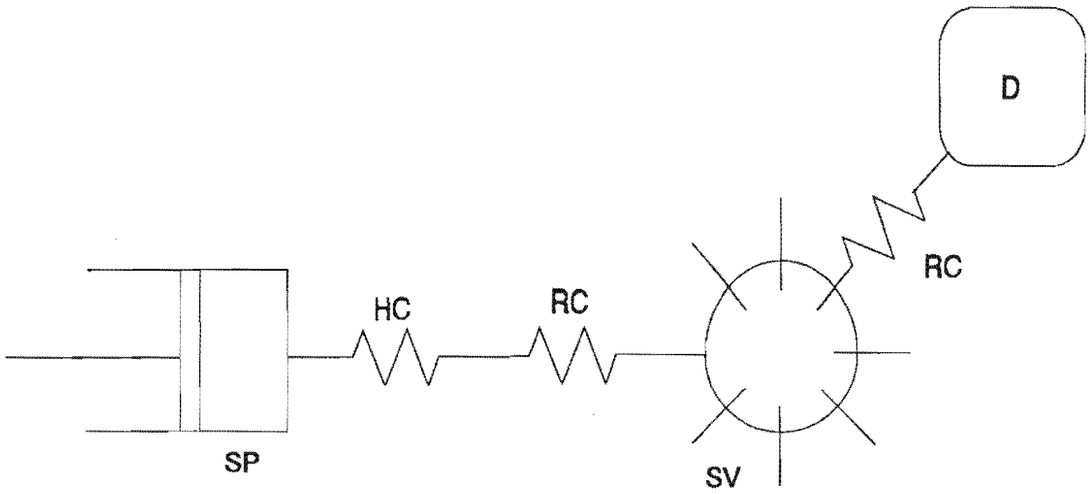

Figure 9: Sequential-injection manifold. P - pump, HC - Holding coil, RC - Reaction coil, SV - selection valve, D - detector.

pump draws sample and reagent solutions sequentially into the first reaction coil (RC) and holding coil (HC). These streams are selected by the multi-port selection valve. As this stack of sample and reagent solutions are propelled through the manifold, they penetrate one another. The pump propels the resulting merged zones through the second reaction coil (RC) and to the flow cell of a suitable detector where a signal is registered. The syringe barrel acts as a reservoir of wash solution. Reactions take place in the tubing of the reaction coils.

The detector can be placed either between the valve and the syringe, or downstream from the selection valve. In the first published work[2], the detector was placed between the valve and syringe. In this position a double peak results. The first peak is

registered when the detectable species passes through the detector on its way to the syringe. The second results when these same products, now somewhat more dispersed, are expelled through the detector and valve to waste. An exception to this case, as was demonstrated in the first publication, occurs when the chemistry is contained within the detector, i.e. in so-called sensor-injection.

Subsequent experimentation showed that a more desirable position for the detector is downstream from the valve in the waste line. In this position, the familiar FIA response profile results. Also, and more importantly, the reaction products are moving under positive pressure through the detector. In the former position, the first peak is obtained under negative pressure. The chance of bubbles passing through the detector under these conditions is far greater than in the latter position. One consideration for the second position is that for half the experiment, the solution in the flow cell is stationary, while the wash, reagent, and sample solutions are drawn up. Usually this has no effect on the detector.

Attention has to be given to avoid sample carry-over from one measurement to the next. The carry-over results from the sample solution in the short length of tubing between the sampling point or reservoir and the selection valve. Several measures to overcome this problem have been proposed[3] including the following:

- A portion of the wash solution is expelled via the sample line before a fresh sample is drawn up. This method can be used if there is a large well mixed reservoir of sample solution.

- A large enough sample volume can be drawn up so that the contaminated leading aliquot does not come into contact with the reagent during zone penetration of the reagent zone.
- The contaminated sample zone can be expelled through an auxiliary waste line.

The pump[8] used initially for SIA has provided a sinusoidal flow pattern. This is not a prerequisite for SIA. All that is required is a reproducible flow pattern. Further work is required to optimize the pump system, and to reduce the analysis time.

In fact syringe type pumps are not even the only pumps which can be used for SIA. Peristaltic pumps can also be used, provided that exact control with respect to stop, start, forward, and reverse, is possible. An interesting option is presented in the use of a new pump design as supplied by Tecuria. This design makes use of a novel piston action that propels the stream and obviates the need for check valves. The advantage of this design is that by connecting the inlet of the pump to the wash reservoir, it is not necessary to first draw up wash solution. The pump dispenses minute (3 mm^3) aliquots of fluid at such a rapid rate that apparent constant flow is experienced. This is further enhanced by inclusion of a pulse damper between the pump and holding coil. This pump can be driven by a stepper motor which further adds to its precision, specifically with regard to stopping and starting.

3.5 MANIFOLD DIMENSIONS AND GEOMETRY

Early work in the development of SIA soon established some guiding principles in the choice of syringe and reactor volumes[3]. When the Alitea sinusoidal-flow pump is used, only a segment of a full pump rotation is used. The volume of the stroke of the piston has been determined previously[8] and may also be determined empirically. The maximum volume for a particular syringe is given by $V = 2R\pi r^2$, where R is the radius of the cam and r is the radius of the syringe. Ruzicka and Gübeli[3] pointed out that once the stroke volume has been fixed, the combined reagent and sample volumes are fixed, as the stroke volume should be at least four times the combined volume of the sample and reagent. The holding coil should be large enough to prevent the sample or reagents from entering the syringe barrel. The reaction coils should not exceed one-third the volume of the wash solution, thereby ensuring that they are adequately flushed during every experiment. With these guidelines, the only parameters still required before a manifold can be constructed are the diameter of the tubing and the geometry of the reaction coils. Less stringent boundary conditions pertain when a flow through pump is used.

Once the manifold has been assembled, the next stage in the development of a method is the choice of pump and selection-valve events that will provide the required sample manipulation. Several factors must be considered.

3.5.1 *Flow-reversal*

The principle and feasibility of using flow-reversal to enhance zone penetration in SIA has been demonstrated before[2]. Gübeli *et al*[4] investigated this further, and concluded that the first flow-reversal, and its length are most effective in providing mutual zone penetration. Furthermore, they stated that multiple flow-reversals would probably be used only for difficult solution-handling tasks, such as the mixing of zones of very different viscosities.

This is best understood by considering a schematic representation of the dispersion profiles of a sample zone just before the first zone reversal (Figure 10b). It can be seen that the element of sample fluid with the highest concentration is well placed just prior to the first reversal to penetrate the trailing zone (Figure 10c) in a similar fashion to the leading edge during uptake.

3.5.2 *Flow rate*

The use of a sinusoidal flow rate, once a method has been developed, has no adverse effect. It should however be borne in mind during method development. Equations describing the flow rate at each stage in an experiment have been given[8]. Flow rate is, of course, proportional to the pump speed, and the range of flow rates employed can be varied by changing the pump speed. The effect of pump speed on zone penetration and sensitivity is discussed below.

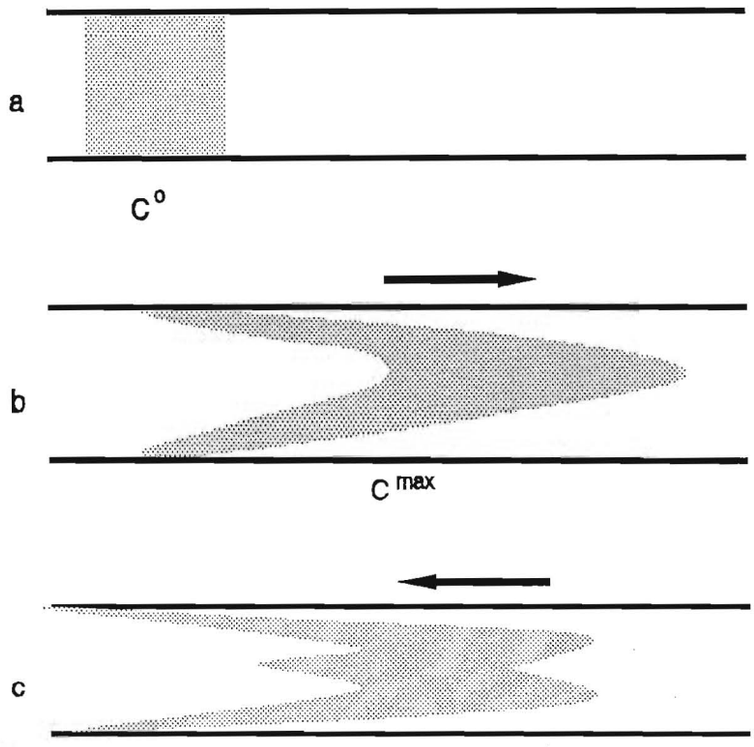

Figure 10: Dispersion of the a) sample plug due to laminar flow just b) prior to, and c) after zone reversal.

3.5.3 *Sample and reagent volumes*

Gübeli *et al*[4] have conducted an in-depth study on the effect of sample and reagent volume on zone penetration and sensitivity. Their conclusions have been summarized in three rules:

- "Changing the injected sample volume is an effective way to change the sensitivity of the measurement. Dilution of overly concentrated injected sample material is best achieved by reducing the injected sample volume."
- "Injecting at least twice as large a reagent zone volume as the sample zone volume, while keeping the volume of the sample zone less than or equal to 0.5 $S_{½}$, allows the optimum conditions for single reagent based chemistries to be

met." ($S_{½}$ is defined as the sample volume that yields a dispersion of 2 in the manifold).

- "Two reagent chemistries can be accommodated provided that the sample volume is kept below the $S_{½}$ value, the sample zone is surrounded by the reagent zones, and the concentration of the injected reagents are sufficiently high" to prevent sub-stoichiometric mixtures.

3.6 EXPERIMENTAL

3.6.1 *Instrumental*

The sequential-injection system depicted in Figure 9 was constructed using an Alitea dual-piston sinusoidal-flow syringe pump (Alitea USA, Medina, WA), a 10-port electrically-actuated selection valve (Model ECSD10P, Valco Instruments, Houston, TX), and a Milton Roy Spectronic Mini 20 spectrophotometer (Opto Labor, South Africa). Data acquisition and device control were achieved using a PC30-B interface board (Eagle Electric, Cape Town, South Africa) and an in-house assembled distribution board. The FlowTEK[9] software package for computer-aided flow-analysis was used throughout for device control and data acquisition.

3.6.2 *Reagents*

Analytical-reagent-grade reagents were used unless otherwise stated. Deionized water was used in the preparation of all solutions.

Bromothymol blue dye solution and sodium tetraborate carrier solution were prepared in the usual way[1]. In these studies, a system where no chemical reaction was involved was specifically chosen to keep a study of the zone penetration phenomenon as simple as possible. The obtained concentration profiles can be used to draw conclusions in systems where chemical reactions will take place.

3.6.3 *Experimental procedure*

A blue dye was used to carry out a study of the effect of different parameters on zone penetration and precision. Two measurements were carried out for each variation of a parameter. In the first measurement, the dye was selected first, and a buffer solution second. In the second measurement, a buffer was sucked up first and the dye was sucked up next. The response of the detector were monitored at 620 nm. The responses from the two experiments were overlaid, and the area of the zone of overlap was calculated from the response data. Precision calculations were carried out on 10 repetitions of the procedure.

3.7 RESULTS AND DISCUSSION

Unlike FIA where, in most cases, the sample bolus is surrounded by reagent, in SIA the sample and reagent zones are stacked in the manifold conduit. As these zones move through the manifold, mutual dispersion takes place and the zones penetrate one another. The degree of penetration can be measured in various ways. At the University of Washington, researchers borrowed parameters from peak-resolution theory developed by chromatographers to obtain a measure of the zone penetration[4]. This approach yields useful results, but is difficult to determine automatically. Furthermore, when large sample or reagent volumes are used, low overlap figures are obtained. (This, of course, demonstrates the uneconomical use of reagent and/or sample solution, but gives a false impression of the degree of zone penetration.)

In the present work, we chose to integrate the area of overlap and use that as a measure of zone penetration. Given the response data, this figure can be obtained automatically. Of course, it too suffers from certain limitations. It does not indicate how sensitive the measurement will be, as it does not take into account the concentration of the sample and reagent. It would not, for example, predict whether aspirating the sample first is preferable to aspirating the reagent first. It only measures the degree of zone penetration and not the ratio of reaction components in a given element of fluid. This approach is useful for the comparison of systems where changes in dispersion and the rate of dispersion are the only parameters to be considered.

The reproducibility of penetration plays a major role in the attainable precision of measurement. Precision is measured by determining the relative standard deviation (s_r) of 10 measurements at a given concentration. It is surprising that such good precision is attained in SIA systems, since reaction takes place at an interface with steep concentration gradients. In experimental work to date, good precision has been obtained if peak height is measured. The area and peak width measurements, however, give poorer precision.

In the present study, precision and zone penetration were used to evaluate the effect of three parameters, viz. tubing diameter, reaction tube geometry, and pump speed.

3.7.1 *Effect of tube diameter*

Several factors should be borne in mind when considering the optimum tube diameter. These include the resultant back-pressure in a length of tubing, the vulnerability to blockages, and the degree of radial dispersion attainable.

Smaller diameter tubing gives rise to higher back-pressures. When the pressure in the reverse stroke drops below the partial pressure of the dissolved gasses, bubbles form in the tubing, which results in poor reproducibility of the flow pattern and can lead to spurious signals from the detector. The back-pressure is also related to pump speed, and so for smaller diameter tubing, a reduced pump speed may be necessary. When the pump is forced to work under conditions of high back-pressure, it starts to labour, and

poor precision results. Table VI shows the marginally poorer precision obtained when the narrow-bore tubing is used.

Table VI

Effect of tubing diameter on zone penetration and precision

Tube inner diameter, mm	1.5	0.8	0.5
Zone penetration	0.197	0.335	0.363
s_r , peak 1	0.007	0.013	0.022
s_r , peak 2	0.011	0.017	0.021

The narrower the tubing, the more vulnerable it is to blockage. With suitable choice of the wash solution and adequate sample filtering, this problem can be minimized.

The ratio of inside tubing surface area to inner diameter decreases as the diameter increases. Therefore, the frictional effect of the tubing walls decreases with increasing tube diameter. This helps to reduce axial dispersion, and narrow peaks are obtained (Figure 11). Table VI shows the negative effect this has on the attainable zone penetration. (Note that, for the tubing used in sequential-injection manifolds, laminar flow is maintained.)

The tubing leading to the selection valve for reagent lines should be as large as is practically possible, in order to minimise back-pressure. The diameter and length of the

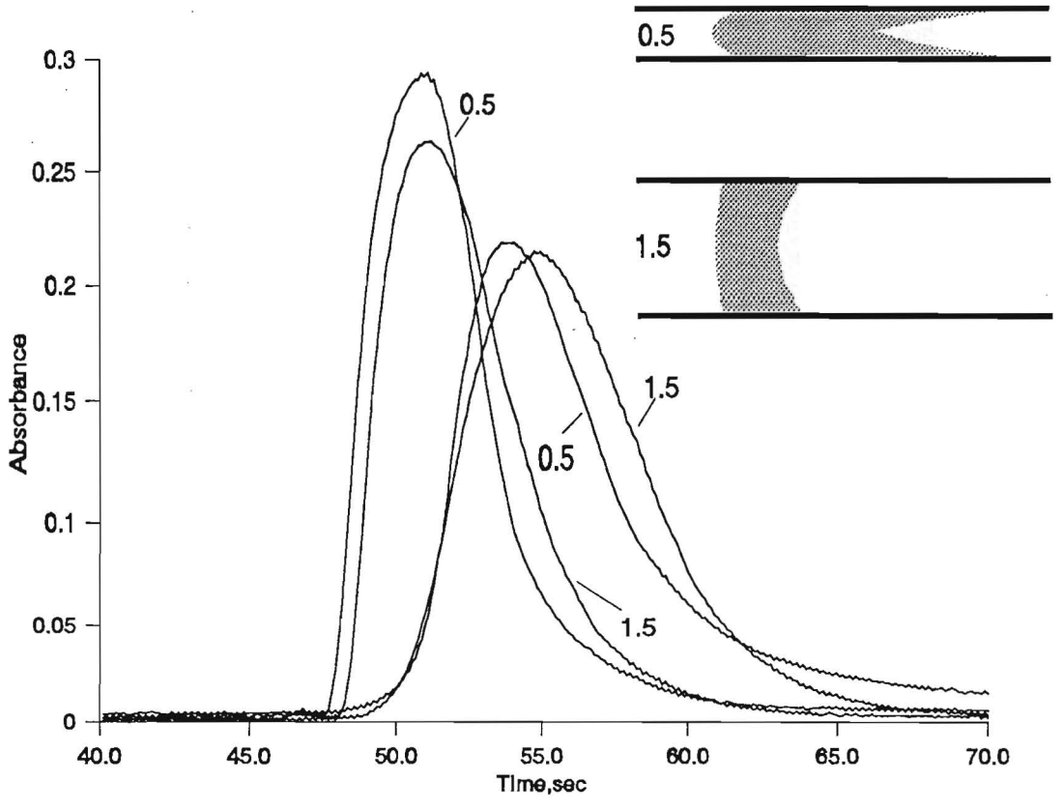

Figure 11: Effect of tube diameter on zone penetration

sample line tube should be kept to a minimum to reduce problems associated with carry-over. The diameter of the holding coil (see Figure 9) can also be larger (it is simply an extension of the syringe barrel), provided that the reaction products do not penetrate this coil.

3.7.2 *Effect of reaction tubing geometry*

Various reactors have been described in the literature on FIA manifolds[1]. Where the reactor consists of a length of tubing, various geometries have been proposed. Three were evaluated to establish the effect of reactor geometry on zone penetration and precision (see Table VII).

Table VII

Effect of reactor geometry on zone penetration and precision

Reactor geometry	Straight	Coiled	Knitted
Zone penetration	0.223	0.241	0.273
s_r, peak 1	0.024	0.014	0.023
s_r, peak 2	0.013	0.026	0.017

As may be expected from what has been observed in FIA, the straight tube results in greater axial dispersion. A coiled reactor gives less axial dispersion, and a knitted reactor gives the least axial dispersion of the three evaluated. Figure 12 shows the peak profiles for a straight reactor and a knitted reactor. Whereas with FIA it was desirable to minimize axial dispersion to minimize dilution, in SIA axial dispersion promotes zone penetration and therefore straight reactors are more desirable. Workers who use double-injection in FIA should take note of these results as they usually also seek to maximize zone penetration. The reactor geometry did not have a marked effect on precision.

Of course once zone penetration has taken place, it is desirable to promote radial mixing in order for reaction to take place. For this reason, a short length of knitted coil just prior to detection is usually incorporated in the manifold.

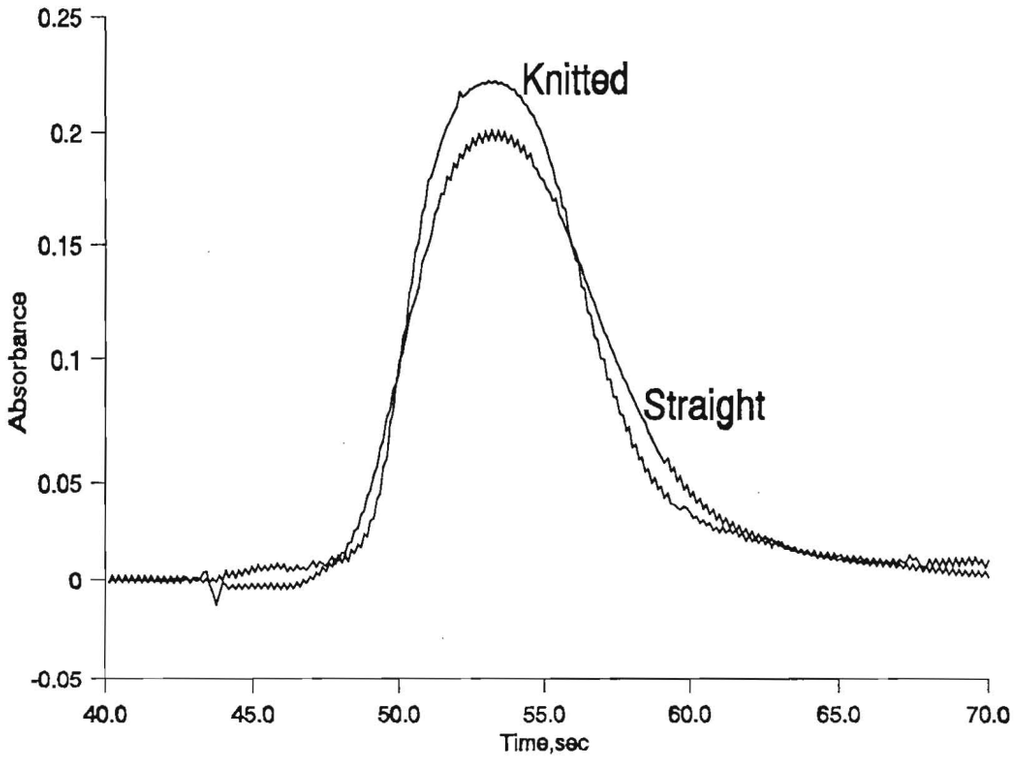

Figure 12: Effect of reactor geometry on zone penetration.

3.7.3 *Effect of pump speed*

In the consideration of the pump speed, a measurement cycle may be split into two periods. The first is the period during which wash solution is drawn up. The second is the period in which the sample and reagent zones are drawn up, one or more flow-reversals are carried out, and the detectable species are expelled through the flow cell of the detector. The first period lengthens the reaction time, and constitutes a disadvantage of SIA when syringe pumps are used. There is no reason why this should not be carried out at a higher speed, provided that the pump will allow this. Future improvements to the pump and controlling software should allow the loading of the

wash solution at accelerated speeds without causing cavitation. Flow through pumps in SIA e.g. peristaltic pumps, would eliminate this step.

We have already noted that the flow rate will change continuously when a sinusoidal flow pump is used. However, the range of flow rates can be altered by changing the pump speed. Figure 13 shows the wide range of flow rates encountered at a fixed pump speed. Theory would suggest that the volume drawn up can be kept constant by adjusting the times for sucking to compensate for the altered pump speed employed. In practice this is not the case. If a simple inverse ratio of time to pump speed is used, reduced volumes are obtained for higher pump speeds. This is probably due to the imperfect flow dynamics of the pump, i.e., start up and stopping are not instantaneous. For this reason, sampling times were not altered and the volumes used at slower pump speeds were smaller than at higher pump speeds. Previous work[4] has shown that greater zone penetration occurs when smaller volumes are used. This was indeed observed in the present investigation (see Figure 14) where the zone penetration at a pump speed setting of 10 was found to be 0.339 while that for a pump setting of 30 was found to be 0.200. Despite the smaller individual peaks for the slower pump speed, the intersection of the two peaks was almost exactly the same due to the greater mutual penetration. SIA would therefore appear to favour slower pump speeds.

Of even greater importance in the consideration of pump speed is the effect of pump speed on back-pressure. At excessively high pump speeds, the back-pressure becomes too high. A balance must be obtained between pump speed, speed of analysis, and

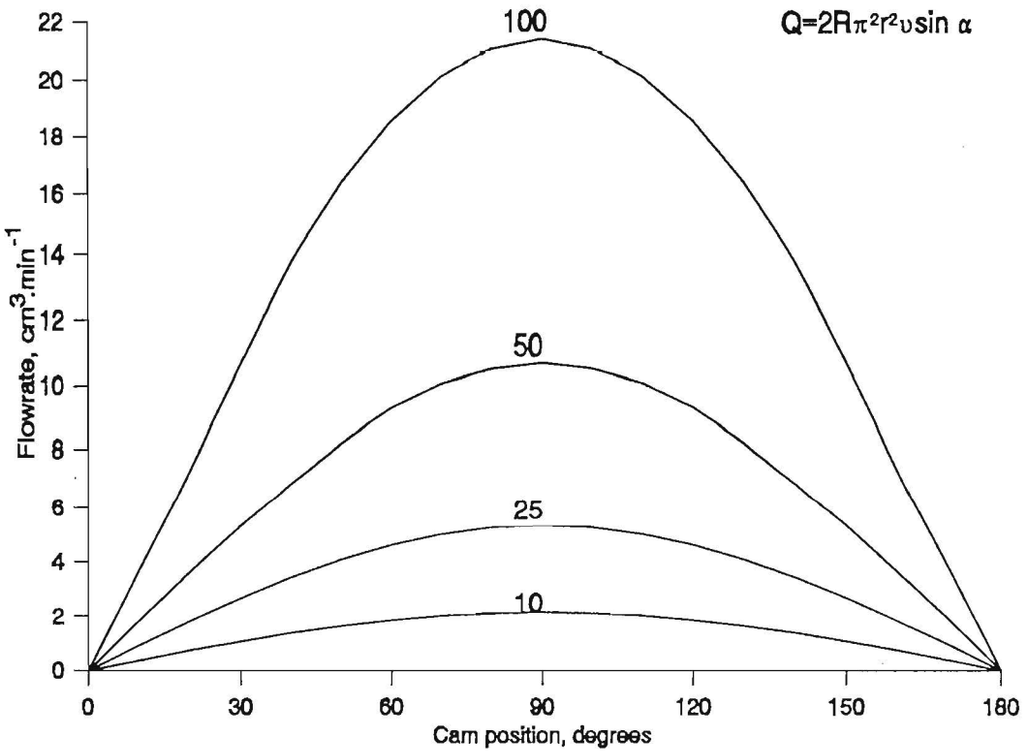

Figure 13: The flow rate at different pump speeds. (The area under the curve between particular cam positions gives the theoretical volume.)

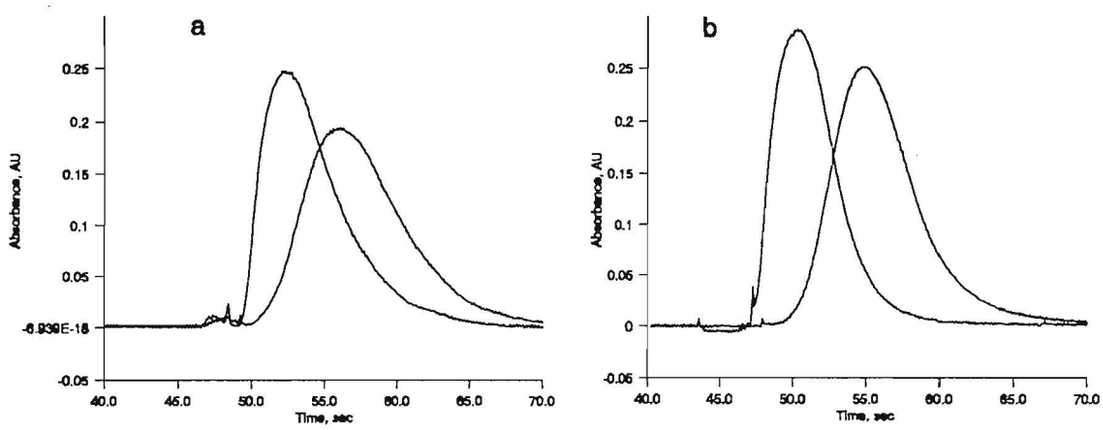

Figure 14: Effect of pump speed on zone penetration. Pump setting of a) 10 and b) 30.

reagent consumption. Empirical studies when using narrow-bore tubing (0.5 mm to 1.5 mm i.d.) have indicated that a pump speed setting (on the Alitea pump) of between

10 and 30 gives good pump performance with a typical measurement cycles of 60 seconds. Zone penetration studies suggest the preferential use of slower pump speeds.

The effect of pump speed on zone penetration if a constant-flow syringe pump or flow-through pump is used will be less complicated and more meaningful.

3.7.4 *Order of injection*

In double-injection FIA, the order in which the sample and reagent zones are introduced has a minimal effect, as the volume of the injection loops is usually much less than the system volume. The second zone therefore has a similar distance to travel as the first zone. In SIA, where at least one flow-reversal takes place, and zone volumes are of the same order as reactor volumes, this is not the case. The dispersion of the first zone introduced is greater than that of the second. This can be seen by the difference in peak heights in the figures depicting peak profiles. One must therefore decide which zone to introduce first. Clearly, the kind of application will dictate the order chosen. The following must be considered. When sensitivity is important, the reagent, at a sufficiently high concentration to ensure an excess, should be introduced first and allowed to penetrate the sample zone. The sample zone will experience less dispersion. If buffering of the sample by the wash solution is required, the order must be reversed. If solubility considerations prevent the reagent concentration from being increased, sandwiching of the sample between two reagent zones is an option to be considered.

3.8 CONCLUSIONS

Further steps have been taken towards defining the parameters that affect the design of the manifold for SIA. Decreasing the tube diameter results in increased back-pressure and prevents the miniaturization of flow conduits. When a tube diameter of 0.8 mm or 1.5 mm is used instead of 0.5 mm, improved precision is attained without an excessive decrease in zone penetration.

Unlike FIA, where knitted reactors are preferred, in SIA straight reactors allow greater zone penetration through axial dispersion. The same principle will hold for FIA when double injection is used as a means of introducing fixed volumes of sample and reagent in a stacked-zone configuration analogous to SIA. The optimum arrangement for chemical reaction to occur is obtained by enhancing mixing of the penetrated zone just prior to detection using one or other mechanical means, e.g. a short length of knitted tubing.

Pump speed, when a sinusoidal flow pump is used, is a complicated parameter to evaluate. This is particularly so when the effect of pump speed on zone penetration is investigated. When a constant-flow pump is used, the evaluation of this parameter is more meaningful. For sinusoidal flow, the effect of pump speed on the back- pressure can be empirically monitored and optimized. Faster pump speeds are desirable when analysis times are to be minimized. Slower pump speeds are preferred when maximum zone penetration is required.

The longer path length and therefore greater dispersion observed in the first zone selected must be considered in the design of an analysis procedure. Optimum use of the two dispersion patterns will ensure sensitive and reproducible measurements.

SIA has reached the point where a fixed manifold can be designed. Dimensions for the optimized system used in this investigation are given in Figure 15. The device sequence for a simple binary system is given in Table VIII.

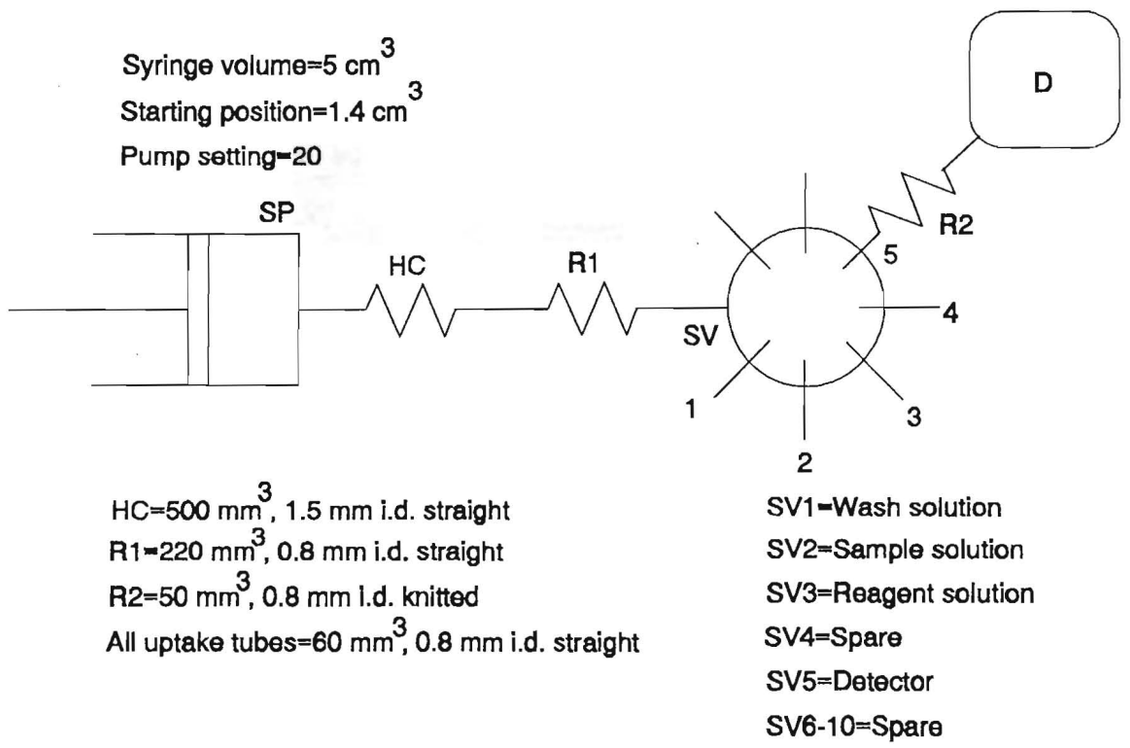

Figure 15: Manifold dimensions for optimized SIA manifold using a sinusoidal flow syringe pump.

Further developments will concentrate on using the versatile controlling software to manipulate sample and reagents in novel ways to achieve desired sample handling procedures. This will enable the development of a generic process analyzer with the

single option of exchangeable detectors. Soon researchers will look at this simple manifold, and seek ways to miniaturize and simplify it. The areas that will come under the spot light are the stream propulsion system and the stream selection system.

Table VIII

Optimum device sequence for a binary system

Time, sec	Pump	Valve	Description
0	Off	Wash	Pump off, select wash solution
2.5	Reverse		Draw up wash solution into syringe
27.5	Off	Sample	Pump stop
29		Sample	Select sample stream
30	Reverse		Draw up sample solutions
35	Off		Pump stop
35.5		Reagent	Select reagent stream
36	Reverse		Draw up reagent solution
41	Off		Pump stop
41.5		Detector	Select detector line
42	Forward		Pump stack of zones to detector
70	Reverse	Home	Return pump and valve to starting position

3.9 REFERENCES

1. J. Ruzicka and E.H. Hansen, Flow-injection analysis, 2nd ed., Wiley Interscience, New York, (1988).
2. J. Ruzicka and G.D. Marshall, *Anal. Chim. Acta*, **237**,(1990), 329.
3. J. Ruzicka and T. Gübeli, *Anal. Chem.*, **63**, (1991), 1680.
4. T. Gübeli, G.D. Christian and J. Ruzicka, *Anal.Chem.*, **63**, (1991), 2407.
5. B. Karlberg, *Anal. Chim. Acta,* **180**, (1986), 16.
6. J. Ruzicka and E.H. Hansen, *Anal. Chim. Acta*, **78**, (1978), 37.
7. G.D. Marshall and J.F. van Staden, *Proc. Control and Quality*, **3**, (1992), 251.
8. J. Ruzicka, G.D. Marshall and G.D. Christian, *Anal. Chem.*, **62**, (1990), 1861.
9. G.D. Marshall and J.F. van Staden, *Anal. Instr.*, **20**(1), (1992), 79.

Chapter 4

Application to Trace Enrichment

4.1 INTRODUCTION

In the application of many instrumental techniques, the need for separation and trace enrichment procedures often arises. Specifically, the effect of interferents in a sample solution may be overcome by separating the interferent and the analyte. Also, preconcentrating the analyte provides a way to extend the useful dynamic range of measurement techniques. Manual procedures to achieve this have been in use for many years and are widely practised. With the constant drive to automate, flow-based techniques have emerged which provide convenient and precise methods of separation and trace enrichment. Such in-line matrix removal and preconcentration techniques using specifically FIA, have been well researched and are in routine use[1-6].

In many cases in advocating preconcentration, flow-analysis researchers have tended to slavishly adhere to the principles of chromatography despite the fact that other options exist. This is not surprising as the similarity between flow-based analysis and modern chromatography is obvious. Separation and trace enrichment techniques cover a wide spectrum of sample manipulations and include automated solvent extraction[7], supported liquid membranes (SLMs)[8], sorbent extraction[9], and various forms of chromatography.

In the case of the latter, either ion exchange resins[1-4] or chelating agents covalently bound on a solid support[5] or the novel method of flow-injection donnan analysis[6] have been employed.

In most cases, methods which employ flow-based sample manipulation techniques have been element specific and limited to a particular suite of sample types. In considering sorbent extraction as a means of achieving separation and trace enrichment, Ruzicka and Arndal[10] introduced the concept of an open flow-injection system. In so doing, they demonstrated an approach to sorbent extraction where a variety of selective and not-so-selective complexing reagents may be used. The chemical interaction between the analyte complex and column material is reversible thereby enabling easy stripping of the analyte simply by changing the polarity of the carrier stream. Unlike chromatography which takes advantage of small changes in the affinity of various compounds to attain selectivity, sorbent extraction adopts an "all or nothing" approach. A compound is either all sorbed or there is no interaction with the sorbent. The selectivity of the analysis lies either in the detector or conceivably in a multi-component analysis of the obtained data from an array of sparingly selective detectors. This approach presents the opportunity of using a single manifold for various sample types and analytes by simply adjusting the detector and possibly some of the reagents. It also points to the existing solvent extraction know-how to guide the design of specific separations.

In this study we will concentrate on sorbent extraction and establish the usefulness of SIA as a means of executing the required sample manipulation. By approaching sorbent extraction as solvent extraction using a solid organic phase, a clearer picture of the guiding principles is obtained. This approach was muted as a possibility by Malamas *et al* [5] Berge and Going[11] studied a suitable chelating exchanger *viz.* 8-quinolinol-5-sulfonic acid. Lacy[9] demonstrated how FIA could be utilized for both separation and trace enrichment and obtained additional sensitivity by moving the separation step right into the flow cell.

In chromatography, differences in the affinity of various compounds for the chromatographic material are exploited. In sorbent extraction, the complexing reagent and support bed can be viewed as an immobilized organic phase. Conditions are chosen which strongly favour the partitioning of a particular compound onto the sorbent while unwanted compounds are allowed to pass unrestrained. The sorbed compound is then quantitatively stripped from the support by simply changing the polarity of the carrier stream. This means that the bonds between the analyte and complexing reagent are left intact.

Although various supports such as macro reticular resins or porous glass beads have been used, the use of hydrophobic polymeric supports has several advantages[12]:

- They are stable over the entire pH range,
- they exhibit excellent adsorption characteristics,

- they are uniform in size, and
- they are mechanically robust.

Hydrophobic polymeric supports are commonly used in guard columns for chromatographic systems. Because of the high capacity of this material, (particle size *ca* 35μm and a pore size of 8 nm), micro columns can be used.

In understanding sorbent extraction systems, an understanding of some of the basic principles which govern solvent extraction proves most useful.

4.2 Comparison to Solvent Extraction

The principles of solvent extraction was first quantitatively described by Koltoff and Sandell[13] and subsequently, theoretically by Irvine and Williams[14]. Since then the subject has been thoroughly studied and presented in several monographs[15,16]. Equilibrium concepts are especially crucial in predicting separation behaviour and efficacy. Two important classes of equilibria can be identified:

- Mechanical which defines the spatial distribution of macro bodies.
- Molecular which defines the spatial distribution of molecules and small assemblages of molecules.

Mechanical equilibrium falls outside the scope of the present investigation and deals primarily with motion that results in macroscopic systems as they seek to achieve a minimum potential energy, e.g. a boulder rolling down a hill.

Giddings[17] in his excellent monograph on separation science points out that molecular equilibrium, unlike mechanical equilibrium, is complicated by entropy. Entropy being a measure of randomness, reflects the tendency of molecules to scatter, e.g. Brownian motion. This scattering process is not the only force working on molecules. As we considered the random walk model for describing SIA, we saw, for example, that the overall flow pattern imposed on a manifold when a solution is pumped had a major impact on the final position of a molecule at a particular point in time.

In separation science, there are additional intermolecular forces at work. The magnitude of these forces is dependant on the properties of the two molecules involved. One force which is particularly strong (and always exploited when carrying out solvent extractions) is that which is dominated by hydrogen bonds. Any given water molecule (or other hydrogen bonded molecule) surrounds itself with a shell of other water molecules; the attraction between them resulting from hydrogen bonds. Any molecule or ion dissolving in the water molecule must disrupt this shell. This is only permissable if the intruding species has strong linkages with the water. An intruding non-polar molecule can only offer weaker linkages with the water molecules and so the water molecules tend to expel the intruder resulting in the so called hydrophobic effect. The expelled non-polar molecules associate with other non-polar molecules to form a separate phase. From this we see that solubility (and the partitioning phenomenon) are governed by intermolecular forces influenced strongly by polarity. To make any use of this observation, some scale of solubility is required. Several have been suggested.

The best known was described by Hildebrand[18]. The solubility parameter of a substance is defined as follows:

$$\delta = \sqrt{\frac{\Delta E_v}{V}}$$

where ΔE_v is the energy necessary to vaporize the molecules in volume, V. δ values correlate strongly with polarity and are given in ref 17 p. 29.

Although, a complex interaction has been shown to exists between the two phases of a binary solvent extraction system and the analyte of interest, it is permissible to view solvent extraction in much simpler terms. This qualitative simplification is permissible if it guides one towards a useful rule of thumb: "The solubility of the metal salt of interest in the two phases may be used as a first approximation of the distribution of the metal salt between the two phases." This means that a table δ values can be used to predict partitioning and aid in the selection of suitable phases to maximize selectivity. To achieve separation, the solubility of the analyte in one of the phases is maximized while that of the unwanted matrix is minimized. For example, to promote the solubility of a particular metal salt in the organic phase, the coordinated water molecules of the hydrated metal ion are replaced by an organic molecule which typically has an anionic group ($-OH^-$, $-SH^-$) and an uncharged basic group (=N-, =O).

In a solvent extraction experiment, the complexing reagent is dissolved in one of the phases. On complexation with the analyte, the complex partitions itself preferentially

into one of the phases because of increased solubility in that phase. This principle may be applied to sorbent extraction if we envisage a system where the organic complexing reagent is immobilized on a suitable hydrophobic support. The sorbent, formed in this way and assembled into a suitable geometry can be placed in a flow manifold. The sorbent then represents the organic phase. The aqueous phase is represented by the aqueous carrier stream. The sample injected into such a manifold passes over the sorbent making intimate contact with the immobilized extractant. These collisions along with their resulting turbulence promotes the transport of the analyte across the phase boundary between the phases. The metal of interest partitions between the aqueous carrier phase and the stationary organic phase according to the distribution coefficient of the system. For this to be successful the distribution coefficient should be high (>100). To strip the analyte from the sorbent, the carrier is changed to favour the analyte in the mobile phase. This is achieved simply by changing the polarity of the mobile phase.

4.3 EXPERIMENTAL

The aim of this suite of experiments was to demonstrate the versatility of SIA for a typical sample manipulation application, *viz.* sorbent extraction.

4.3.1 *Apparatus*

The manifolds depicted in Figure 18 to Figure 20 were used in this study. They were assembled using the following components:- An Alitea C4-V pump (Alitea USA, Medina, WA), an ECSD10P Valco electrically actuated 10 port selection valve (Valco, Houston, TX), and a Spectraphysics UV2000 scanning UV-Vis spectrophotometer (Anatech Instruments, Randburg). The flow cell of this detector has a path length of 6 mm and an i.d. of 2 mm. FlowTEK, the software package described in chapter 2, was used for device control and data acquisition.

Teflon tubing with an i.d. of 0.5 mm was used in the manifold. The sorbent support was loaded into a small glass column with an i.d. of 1.5 mm and a length of 8 mm (see Figure 16). After positioning a small wad of glass wool in the one end of the column, the column was wet-packed by carefully drawing a slurry of methanol and the sorbent into the column with a syringe. When the column was full, glass wool was packed into the other end as well. The tiny glass column was then placed into a perspex holder. Standard Upchurch flangeless fittings were used to secure the column in place. These fittings are ideally suited because it is possible to allow the teflon tubing to protrude about 1 mm beyond the end of the fitting. This short length of tubing is inserted into the glass column thereby ensuring effective connection. Furthermore, the small ferule acts as an effective seal when the connector is firmly tightened up against the glass column. The internal volume of the columns was about 14 mm^3.

The polymeric support was obtained from a small disposable syringe pre-column (Dionex) with the brand name of On-guard-RP. It is a macroporous divinylbenzene with a pore size of *ca* 8 nm and a specific surface area of *ca* 300 $m^2.g^{-1}$.

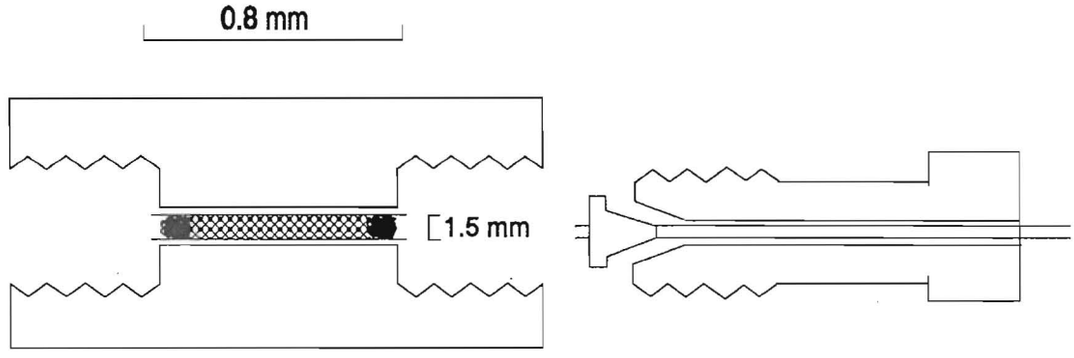

Figure 16: Miniature column used for sorbent extraction. The polymeric support is held in place by two small wads of glass wool. Two flangeless fittings seal the column in the perspex holder.

4.3.2 *Reagents*

All reagents were of analytical reagent grade and deionized water was used throughout. Standard solutions of Cu^{2+} were prepared by serial dilution of a standard copper sulphate stock solution.

Sodium diethyldithiocarbamate (NaDDTC) is a white crystalline powder that is readily soluble in water and ethanol. Numerous elements form complexes with NaDDTC. These complexes are fairly insoluble in water but are readily soluble in organic solvents. For this reason, this complexing reagent is frequently used for both gravimetric and photometric determinations. Masking agents and appropriate reaction

conditions are selected to obtain selectivity[12]. Copper forms a brown complex with NaDDTC which can be stabilized in aqueous solutions by the addition of a suitable organic colloid (e.g. gum arabic, pectin). It is, however, desirable to extract the coloured complex into a suitable organic solvent. The complex forms in the pH range 4 - 11 and gives an absorption maximum at 448 nm (see Figure 17) when the complex is formed in a 1:20 MeOH:H_2O mixture.

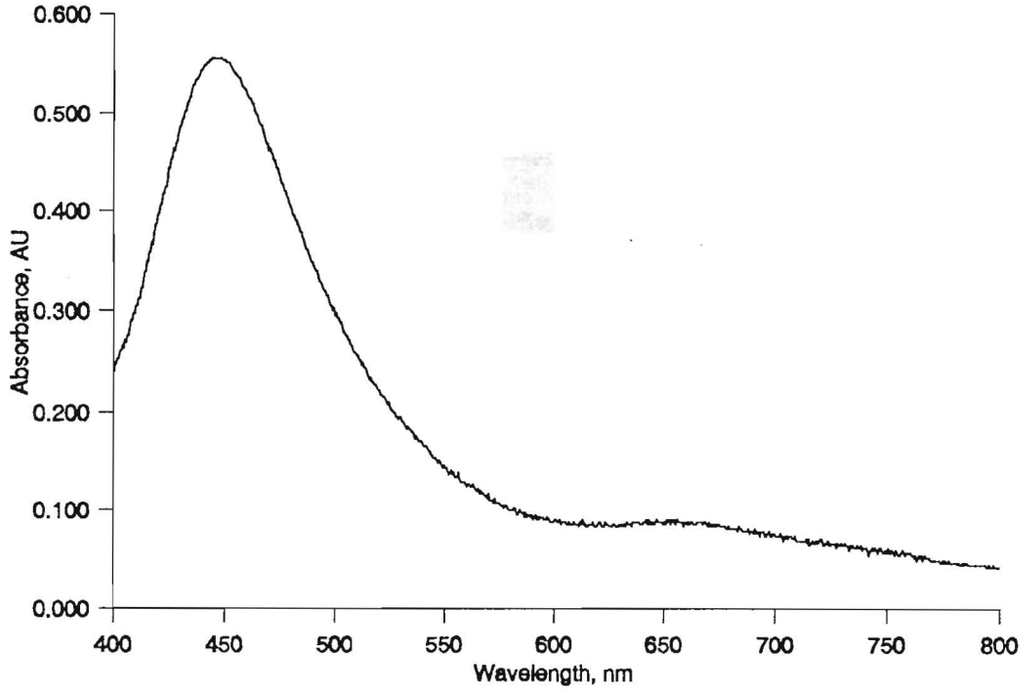

Figure 17: Spectrum of $Cu(DDTC)_2$ complex. Cu^{2+} concentration 5 $mg.dm^{-3}$ in 1:20 MeOH:H_2O mixture, pH=4. Peak maximum - 448 nm.

A 1 $g.dm^{-3}$ NaDDTC solution was prepared by dissolving 0.1 g of the trihydrate salt in 100 cm^3 of water.

4.3.3 *Procedure*

To achieve the aim of this suite of experiments, several manifolds were evaluated and their particular attributes were studied. The response profiles were analyzed to ascertain what exactly was happening in the manifold. The effect of different experimental conditions on the attainable performance was investigated.

The first manifold (see Figure 18) is particularly aimed at systems which make use of aggressive organic solvents. This manifold is however, only necessary when a peristaltic pump or other pump susceptible to damage by aggressive organic solvents is used. Peristaltic pump tubing is not normally suited to aggressive organic solvents. When a pump with suitable wettable components that are resistant to organic solvents is used, one of the other manifolds can be applied. Care must be taken when using this manifold that sufficient organic solvent is used to completely flush the complex from the sorbent bed. Also the holding coil must be large enough to ensure that the organic solvent does not come into contact with vulnerable pump components.

In this manifold, first the flush solution is drawn up into the holding coil, then the sample solution, and finally the complexing reagent. When this stack of sample and reagent zones is pumped over the sorbent bed, the complexing reagent first attaches itself to the hydrophobic sorbent material. As the sample passes through the sorbent bed, the copper is extracted and immobilized on the sorbent. No sooner is it immobilized on the sorbent but the organic strip solution arrives and displaces the

complex from the sorbent and flushes it to the detector for measurement. In this way, the sorbent is left in the required state for the next measurement. Having established that the manifold did in fact work as expected, this manifold was not pursued any further.

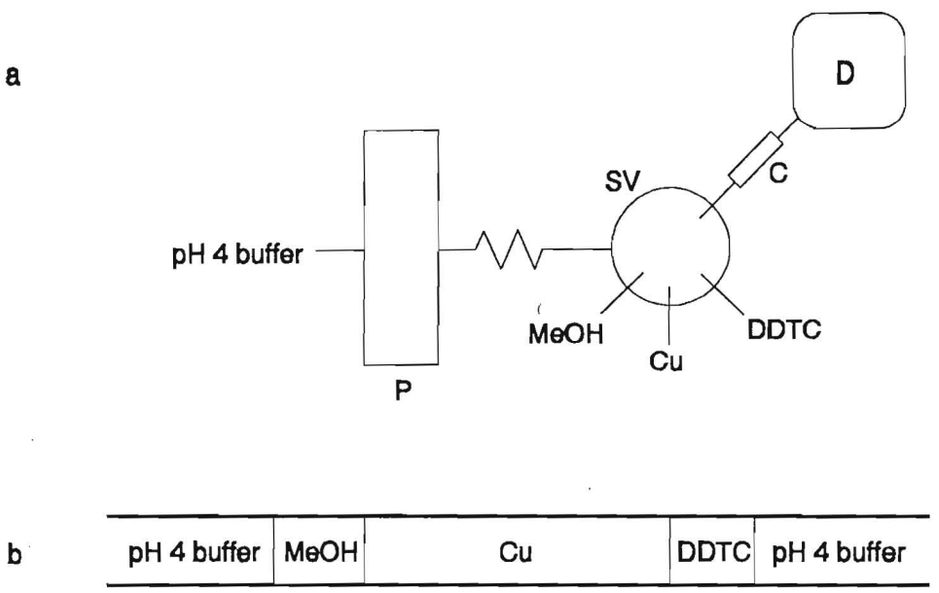

Figure 18: a) SIA manifold for sorbent extraction when using an aggressive stripping solution, P - pump, SV - selection valve, D - detector, C - column. b) Sequence of reagents in the SIA reaction coil (see text for details)

In the second manifold, an attempt was made to keep the complexing reagent and sample separate until they meet on the sorbent. To do this, the manifold set out in Figure 19 was assembled. Once again, while investigating this manifold, the tremendous value of FlowTEK was clearly illustrated. In addition to the excellent device control attributes of the package and accurate timing, the ease with which

volumes are changed by adjusting the relevant times in the method made the investigation of this manifold feasible.

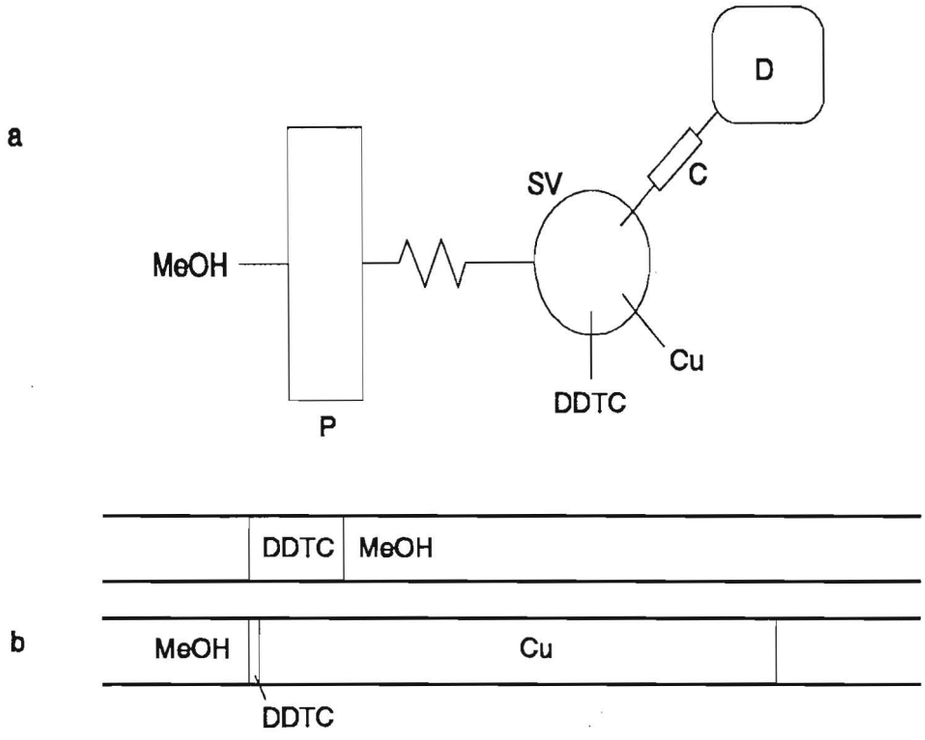

Figure 19: a) SIA manifold for sorbent extraction with pre-sample column loading, P - pump, SV - selection valve, D - detector, C - column. b) Sequence of reagents in the SIA reaction coil (referred to as Manifold A in the text).

In this manifold, the organic strip solution is moved to the carrier. Of course, this has a deleterious effect on the pump tubing. However, by using a suitable solvent resistant tubing and methanol as the solvent, we were able to use this arrangement. A further advantage of moving the solvent to the carrier is that it is always pumped under positive pressure in the system. In the manifold depicted in Figure 18, bubbles tended to form as the solvent with its low partial pressure was sucked into the manifold. These bubbles gave rise to spurious signals in the detector.

In the manifold designed for pre-sample column loading, a small portion of organic reagent is drawn up into the coil and then immediately spewed out to the sorbent extraction column. After this, the sample is drawn up into the coil and then pumped to the column. Once again, the complexing reagent extracts the copper from the passing solution. The methanol, now the carrier stream, flushes the complex from the column and carries it to the detector. The weaknesses of this manifold will be discussed under results.

The third manifold (Figure 20) proved to be the best and also has the simplest device event list (see Table IX). In this manifold, the methanol is again placed in the carrier stream. First, the sample solution is drawn into the manifold. The selection valve is then advanced and a small portion of complexing reagent is drawn up into the coil. Finally, the stack of zones is pumped to the column. In the now familiar sequence, the complexing reagent is adsorbed onto the sorbent. When the sample passes, the copper is extracted, and the sample matrix is pumped (via the detector) to waste. When the methanol reaches the column, it displaces the complex, and carries it to the flow cell of the detector.

In these experiments, the extraction chemistry takes place on the surface of the column. In fact, it is possible to visually observe a discolouration of the column as the sample passes through it. For this reason, tube lengths between the selection valve and the column, and between the column and the detector were kept to an absolute minimum. The holding coil is made long enough to contain the sample. As zone penetration is

undesirable, knotting the holding coil and even using larger diameter tubing is permissable and even desirable.

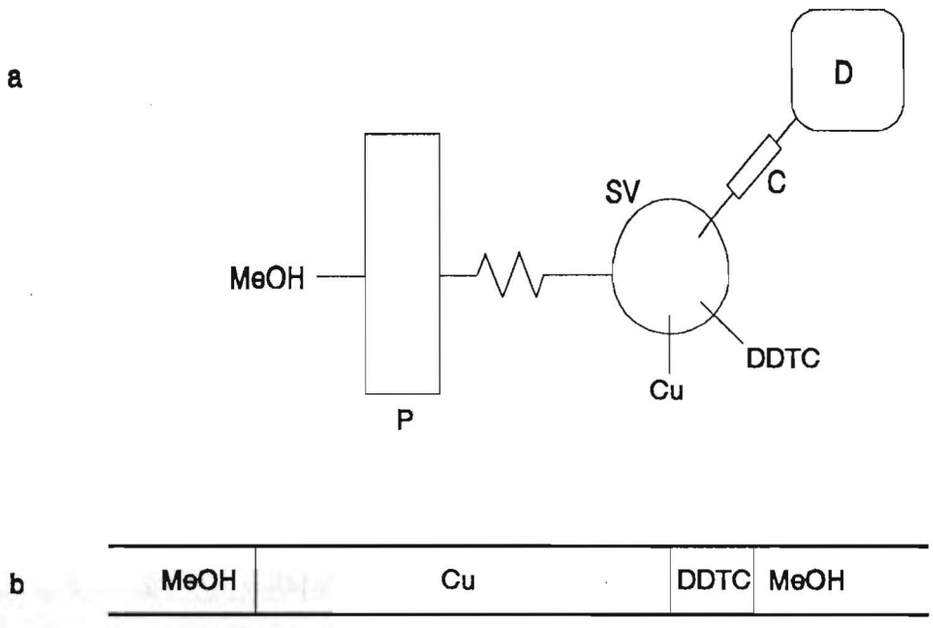

Figure 20: a) Optimized SIA manifold for sorbent extraction, P - pump, SV - selection valve, D - detector, C - column. b) Sequence of reagents in the SIA reaction coil (referred to as Manifold B in the text).

Table IX

Device events for sorbent extraction experiments

Manifold A (Figure 19)		Manifold B (Figure 20)	
Time, sec	Event*	Time, sec	Event*
0	SV - DDTC	0	SV - Cu
5	P - Reverse	5	P - Reverse
6.8	P - Off	15	P - Off
			SV - DDTC
7	SV - Detector	18	P - Reverse
10	P - Forward	19.8	P - Off
			SV - Detector
12	P - Off	23	P - Forward
	SV - Sample		
15	P - Reverse	50	P - Off
25	P - Off		
	SV - Detector		
28	P - Forward		
60	P - Off		

* P refers to pump and SV refers to selection valve

4.4 RESULTS AND DISCUSSION

The manifold described in Figure 19 was assembled and the method described in Table IX was entered into FlowTEK. When the method was executed, the response profile given in Figure 21a was obtained. Clearly, this is not a conventional response profile and it is worth pausing to explain the observed response profile. It is necessary to refer to the manifold and more specifically, the diagrammatic representation of the zone stack that pertains and is given in Figure 19. The peaks emerge in the order i, ii, and then iii.

When the complexing reagent is pumped down the column channel, the length of tubing between the column, through the selection valve and even into the holding coil contains the complexing reagent. The residual amount left in the holding coil as a result of dispersion is reduced by pumping out to the column slightly more than what was drawn up (2 seconds versus 1.8 seconds). When the sample is loaded, it carries with it on its way to the pump, a small volume of the complexing reagent on the leading edge of the sample zone. As the pump is reversed, the trailing edge becomes the leading edge as the sample is directed down the column channel. The residual amount of complexing reagent in the tube between the valve and the column reacts with the new leading sample edge. Because of dispersion, there is a small amount of methanol present and this ensures that most of this leading edge passes through the column resulting in peak i. The profile between i and ii is what the detector sees while the sample zone passes through the column and detector. Note during this time, the complexing reagent now

immobilized on the column is extracting the copper from the sample zone. A small amount of the complex is not held by the column and passes through. Peak ii is the original leading edge that is now at the end of the sample zone again mixed with some methanol (and more dispersed than the front sample - complexing agent interface). It also passes unretained through the column. Finally, the main methanol zone arrives at the column and washes the sorbed complex off the column and through the detector (peak iii). While the precision of peak ii was poor, both peaks i and iii are sensitive to concentration and yield reproducible results which can be used to quantify the amount of copper in the sample. Peak i is typically about one quarter of the size of peak iii and can be used to extend the dynamic range of the method.

An experiment was then carried out to seek to simplify this profile. To do that, it would be necessary to eliminate the complexing reagent from the holding coil at the start of the sample uptake step as well as in the length of tubing between the selection valve and the column. This can be achieved by increasing the time spent dispensing the loaded complexing reagent relative to the time spent drawing it up. Bearing in mind that the full internal volume of the column is 14 mm^3, it was decided to reduce the time of uptake and keep the dispensing time constant (2 seconds or 33 mm^3) while still ensuring that there was sufficient complexing reagent to coat the sorbent.

The results of that experiment are given in Figure 22. The x-axis represents the volume of complexing reagent that is drawn up into the manifold. Because the time to dispense this volume was kept constant (2 seconds), effectively more and more of the wash

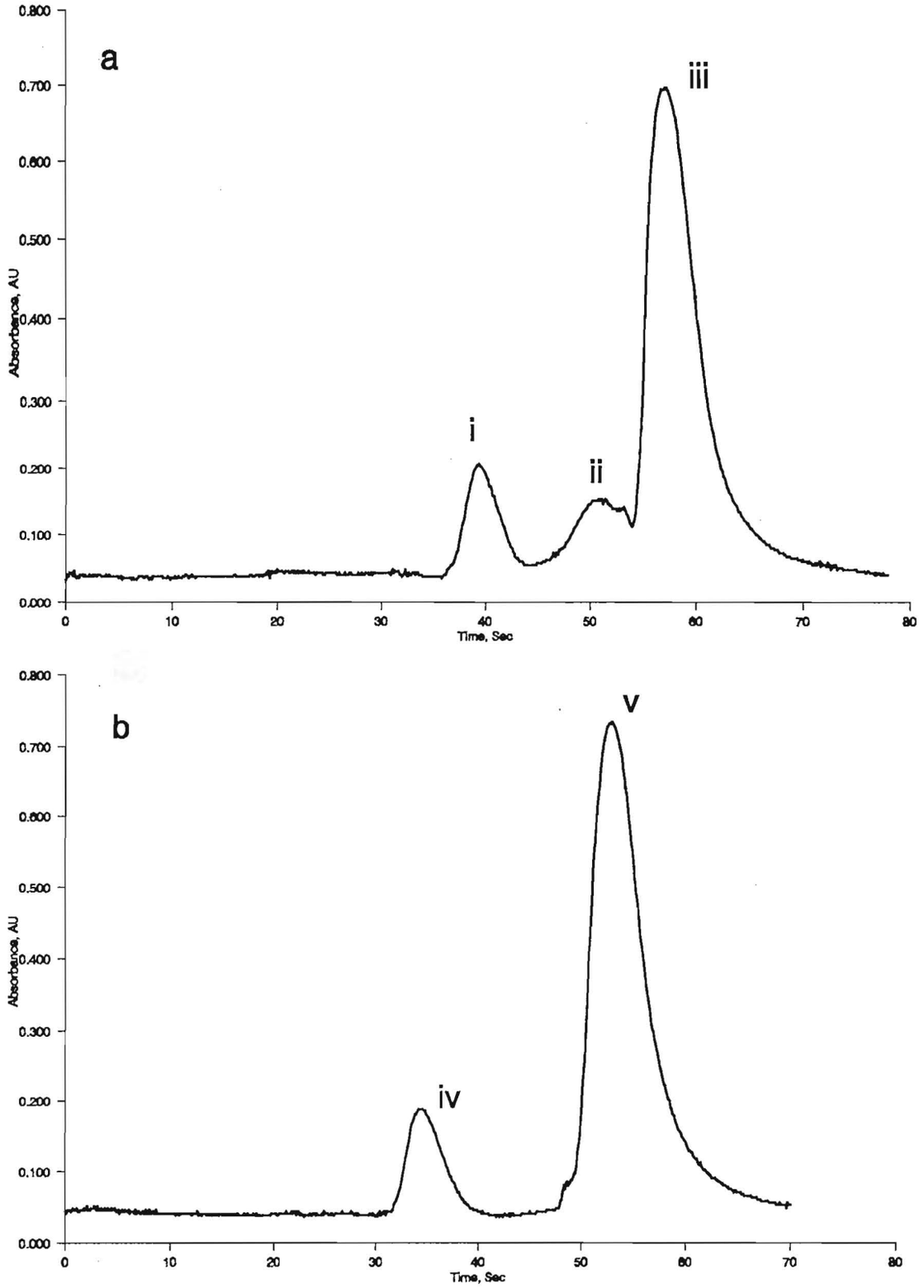

Figure 21: a) Response profile for the enrichment of $Cu(DDTC)_2$ using Manifold A. b) Response profile for the enrichment of $Cu(DDTC)_2$ using Manifold B.

solution was being dispensed to the column channel before loading the sample. As more and more wash solution is sent to the column, the peak height decreases. Lest the reader conclude that this reduction is rather due to insufficient complexing reagent, an experiment was repeated where the volume of reagent dispensed to the column was fixed at 20 mm^3 but now instead of dispensing some of the merged zone, the time spent dispensing (1.2 seconds) was made equal to the time spent drawing up the reagent . The attained peak height was restored to about 1.4 AU as compared to *ca* 0.75 AU obtained with a 2 second dispensing time. This proves that when too much flush solution is sent to the column channel before sample loading, it effectively washes some of the complexing reagent from the column before complexation can take place. For all subsequent experiments, the method given in Table IX was used, i.e. load complexing reagent for 1.8 seconds and dispense it for 2 seconds.

Next the effect of sample volume was determined for two levels of copper (refer to Figure 23). In the first case, a 5 $mg.dm^{-3}$ copper sample solution was used. It soon became evident that while for the smaller volumes (< 200 mm^3), a fairly linear relationship between sample volume and response is obtained, when absorbance values got above 1.0 AU, severe curvature of the curve was obtained. The sample concentration was halved and the experiment was repeated. Here the linear region extended further to *ca* 300 mm^3. At higher levels, curvature was again observed. This curvature may be more as a result of some of the complex being flushed from the column by the large sample zone than normal curvature of spectrophotometric responses at high absorbance values.

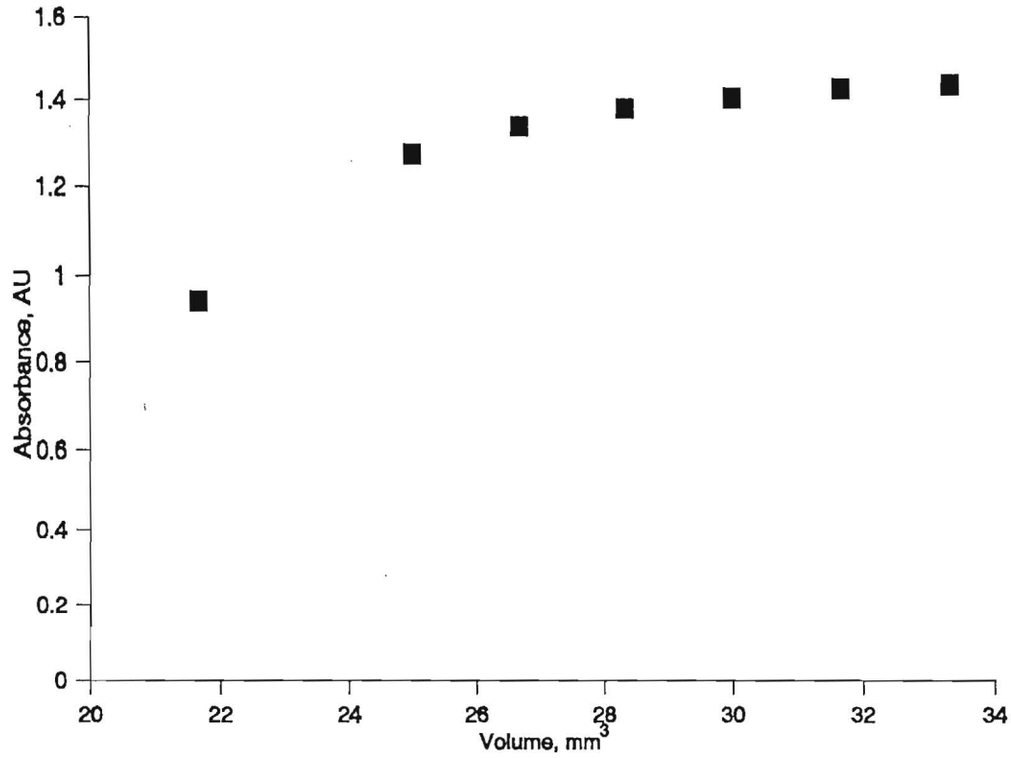

Figure 22: Effect of changing the relative ratio between complexing reagent drawn up and that dispensed to the pump. In each case, 33 mm^3 was dispensed, different volumes were aspirated.

There is a way of eliminating peak ii, *viz.* by adopting the manifold given in Figure 20 and the method given in Table IX. This manifold gives rise to the profile depicted in Figure 21b where two instead of three peaks are observed. Peak iv results from the complex formed at the interface between the trailing edge of the sample (when moving towards the pump) and the complexing reagent. The trough between iv and v corresponds to the sample zone passing through the detector; its copper having been retained on the column by the complexing reagent. Peak v is the peak resultant from the methanol stripping the copper complex from the column. As for the previous manifold, both peaks can be use to quantify the copper present in the sample.

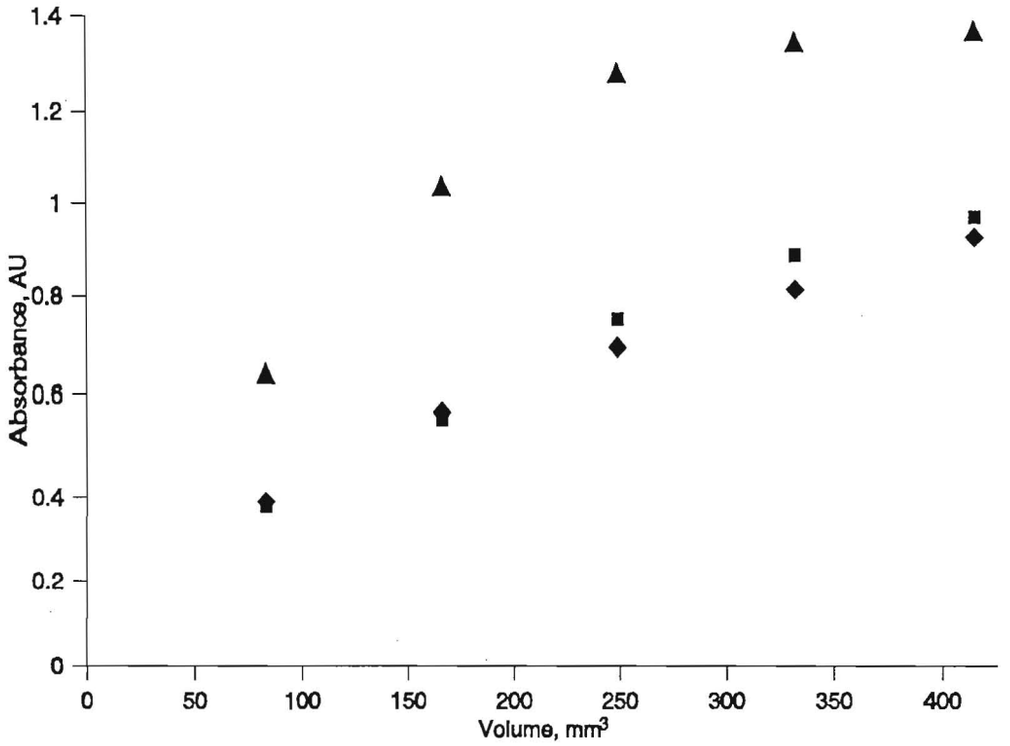

Figure 23: Effect of sample volume on response. ♦ - 2.5 mg.dm^{-3} Cu, Manifold A, ■ - 2.5 mg.dm^{-3} Cu, Manifold B, ▲ - 5.0 mg.dm^{-3} Cu, Manifold A.

Using this manifold, the effect of sample volume on response was determined. The results are plotted as a ■ in Figure 23. The trend was similar though marginally higher than the earlier manifold. This is not unexpected as that portion of the complex represented by peak ii has now effectively been incorporated into peak v.

Having settled on this manifold, analytical figures of merit were determined. A calibration was carried out. The graph of the curve is given by the straight line (r^2= 0.9963): $Resp = 0.164\ [Cu^{2+}] + 0.171$.

The unusually high intercept on the y-axis can be attributed to the different refractive indices of methanol and water. This was confirmed by running a blank. The reproducibility of the method was determined by calculating the relative standard

deviation (s_r) for 10 replicates of a 2.5 mg.dm^{-3} copper solution. It was found to be 0.013. The sample throughput when a 150 mm^3 sample is used is about 60 h^{-1}. When the volume is increased to 420 mm^3, the throughput is reduced to 40 h^{-1}. Without optimizing the system for trace enrichment, an enrichment factor of 2.5 is achieved when a sample volume of 420 mm^3 is used with Manifold B.

In this experimental work, no attempt was made to prove the developed methodology suitable for a particular sample as that was not the intent of the experiment. Rather the concept of sorbent extraction has been demonstrated to be applicable, and even convenient, using SIA. The final manifold is far simpler than some of those reported for FIA[9 p 140]. Furthermore, having been shown to work for Cu and DDTC, there is no reason why SIA should not work for other systems employing other complexing reagents such as PAR, PAN, dithizone, diphenylcarbazone, etc.

4.5 REFERENCES

1. S. Olsen, L.C.R. Pessenda, J. Ruzicka, and E.H. Hansen, *Analyst*, **108**, (1983), 905.
2. Z. Fang, S. Xu, and S. Zhang, *Anal. Chim. Acta*, **164**, (1984), 41.
3. S.D. Hartenstein, J. Ruzicka, and G.D. Christian, *Anal. Chem.*, **57**, (1985), 21.
4. Z. Fang, J. Ruzicka, and E.H. Hansen, *Anal. Chim. Acta*, **164**, (1984), 23.
5. F. Malamas, M.Bengtsson, and G.Johansson, *Anal. Chim. Acta*, **160**, (1984), 1.
6. J.A. Koropchak, and L. Allan, *Anal.Chem,* **61**, (1989), 1410.

7. B. Karlberg and S. Thalander, *Anal. Chim. Acta, 98*, (1978), 1.

8. D.E. Barnes, Supported liquid membranes, Ph.D. Thesis, University of Pretoria, Pretoria, (1993).

9. N. Lacy, Optosensing for signal enhancement and multi-component parts per billion determination in flow-injection analysis, Ph.D. Thesis, University of Washington, Seattle, WA, (1990).

10. J. Ruzicka, and A. Arndal, *Anal. Chim. Acta*, **216**, (1989), 243.

11. D.G. Berge and J.E. Going, *Anal. Chim. Acta*, **123**, (1981), 19.

12. Anon. "Interaction® separations, #2, May 1989, Interaction Chemicals, Inc. Ragina Patel Mountain View, CA.

13. Koltoff and Sandell, *J. Am. Chem. Soc.* **63**, (1941), 190.

14. H. Irvine, and R.J.P. Williams, *J. Chem. Soc.* **71**(1949), 1841.

15. Stary, J. "Solvent extraction of metal chelates", Pergamon press, (1964).

16. G.H. Morrison and H. Freiser "Solvent extraction in analytical chemistry" John Wiley and Sons, New York, (1957).

17. J.C. Giddings, "Unified separation science", John Wiley and sons, New York, (1991).

18. J.H. Hildebrand, and R.L Scott, "The solubility of non-electrolytes", 3rd ed., Dover, New York, (1964).

19. J. Fries, and H. Getrost, "Organic reagents for trace analysis", E. Merck, Darmstadt, (1977).

Chapter 5

Sensor Injection

5.1 What is a Sensor?

The term "sensor" is one which invokes a myriad of images, not the least of which have their origins in science fiction. The term is not always used where it is strictly appropriate, but rather to leverage some advantage from these images. Frequently, it simply implies small and compact. Ideally, it should be characterized by unattended operation. Though the ideal of unattended operation is constantly strived for, it is seldom achieved. Rather than debate the validity of any particular definition, we will adopt a pragmatic approach and adopt a definition which will give some clarity to this investigation.

We will distinguish between physical and chemical sensors[1]. A further characterization will distinguish between the measurement of physical attributes and chemical concentration. Then a matrix can be used to characterize all sensors (see Table X) on the basis of what the sensor measures and the principle it employs to carry out the measurement. Physical sensors rely on some physical phenomenon to produce a response which can be related to a physical or chemical parameter. Chemical sensors

on the other hand, depend on a chemical reaction to generate a signal that can be related to a physical or chemical parameter.

Table X

Characterization of sensors

Physical Sensors	Chemical Sensors
Physical parameters e.g. Thermometer	Physical parameters e.g. Chemisorption humidity sensor[1] p66
Physical Sensors Chemical composition e.g. Evanescent wave chemical sensor[1] p241	Chemical Sensors Chemical composition e.g. Oxidase-based glucose sensor[1] p210

Physical sensors are well established and widely used in many areas. Many present plant control systems are strongly, and even exclusively, dependant on physical sensors, e.g. temperature, flow rate, and pressure. We will focus our attention on sensors which yield information on chemical composition and, having identified the category of physical sensors, will pay no further attention to it.

There is no consensus on a precise definition for chemical sensors (as apposed to a detectors). Therefore, in this investigation, a sensor will be defined as a detector where the chemistry of measurement is contained within the sensing unit. For example,

under this definition, a photometer is a detector, an optrode where the chromogenic reagent is immobilized on a suitable sensing device, would be termed a sensor. This definition does not warrant further discussion and simply serves to give the reader a term of reference when considering the use of flow-based analysis as a sample presentation tool for sensors. Principles established in this investigation will, in the main, be applicable to all sensors which satisfy this definition and are used for the monitoring of liquid streams.

Despite some classical examples of most reliable chemical sensors such as the glass electrode for pH measurement (developed in 1930), chemical sensors have frequently been plagued with untold failures. Flow-based analysis, notably SIA, could improve this situation in the future.

5.2 PRESENT STATE OF THE ART

Before we look to the future though, a brief tour of the history of chemical sensor development is appropriate. Seiyama[1], notes that Jonson developed one of the first sensors (based on catalytic combustion) in 1923. The next significant advancement occurred when Wagner developed the theory of electromotive force (emf) of a solid electrolyte cell in 1957. This opened the door for the proliferation of electrochemical sensors of various shapes, designs, and electrochemical principles. These include ion selective electrodes, oxide semiconductor type gas sensors, and ion-selective field-effect-transistors (ISFETS). In the ensuing years, electrochemical sensors dominated

the field almost exclusively until superior optical fibres and supporting hardware came out of the communication industry.

Early chemical sensors drew on the sensitivity, and selectivity of electrochemical reactions. Such reactions were directly compatible with the emerging fields of micro electronics and the first chemically-sensitive field-effect-transistors (CHEMFETS) were first described more than two decades ago. Since then many papers and a few reviews[2,3] have followed. Many of the early problems have been addressed by moving as many of the manufacturing steps to the wafer level as possible. Some problem still exist and not all manufacturing steps can be carried out at wafer level. The Pacific Northwest Laboratory in the USA has made some significant steps in manufacturing reliable ISFETS. In fact in a plenary paper at the Signals and Sensors Symposium held in Dublin in 1992, Domansky *et al*[4] described how they are able to make multiple sensing ISFETS which are stable for up to 2 months. These workers believe that CHEMFETS are now ready for commercialization and blame the lack of commercially available CHEMFETS on inadequate marketing rather than technical problems. The solution is available, all that is required, according to these researchers, is a suitable problem. In contrast to criticisms pertaining to the life time of sensors, these workers report certain pH sensitive devices which have a life time of years, membrane ISFETS last a couple of months, but enzyme FETS still seldom last more than a few days.

While electrochemistry characterized many early developments and continues to enjoy research funding, there is a recent interest in the utilization of optical methods for

sensors[5]. Surface acoustic waves (SAW) and sensors which make use of optical fibres are the subject of numerous studies. A recent review[6] highlights the advantages and disadvantages of optical chemical sensors. These are summarized in Table XI.

Table XI

Strengths and weaknesses of optical chemical sensors

Advantages	Disadvantages
• Electronically passive	• Interference from ambient light
• Immune to electromagnetic interference	• Photodegradation or leaching of the optically active component
• Easily miniaturized	• Frequent recalibration required
• Corrosion resistant	• Limited dynamic range
• Suited to chemical (organic and inorganic) and biological analytes	• Slow response because sample and detector are in different phases
• Suited to remote sensing	• Limited life time
• Intrinsically safe	
• Small in size	

The advantages have proved to be so attractive that the disadvantages have all been overcome to a greater or lesser extent. Furthermore, all of the disadvantages can be addressed if some form of sample manipulation is employed. At a recent meeting

addressing the use of chemical sensors in the field of biotechnology[7], there was strong agreement on the fact that (bio)sensors can really only be used in practice if they are coupled with a suitable sample presentation system. This is particularly so if some of the problems related to stability, life-time, and convenient calibration are to be addressed.

Recent advances in micromachining, and electronics have been widely employed by researchers and continue to produce more reliable sensors. Advances in the life sciences (e.g. biotechnology) as well as new materials are also making significant contributions to the field of sensors. However, even though much research funding has been allocated to the development and study of chemical sensors, it may convincingly be argued that overall, sensors are not yet economically viable or practically implementable. Nevertheless, the potential rewards are massive, and so research funding continues to be allocated to this broad field of endeavour.

Optical fibres and associated optical chemical sensors have induced somewhat of a renaissance in optical methods. Interest covers the full spectrum of optical techniques; absorption, luminescence (including phosphorescence and chemiluminescence), and reflectance. Dramatic advance in performance and quality of light sources (lasers and light emitting diodes), optical fibres, amplifiers, and photodetectors have promoted rapid progress. These robust solid state components have enabled the development of solid state measuring equipment such as photometers based on a LED[8] and a versatile, robust,

and miniature fluorometer. When the chemistry of measurement is immobilized in these detectors, novel and powerful sensors result.

Work continues in making various sensors more reliable and robust. Chemometricians research the use of neural networks for pattern recognition[9] using the output from arrays of sensors. Materials scientists investigate new materials for use both as substrates and chemically active components. Eventually all of these elements must be incorporated to yield a true sensor array with all the attributes of our own olfactory system.

Until this is achieved, the coupling of sensors to appropriate sample manipulation systems clearly represents a workable intermediate and provides a means of employing optical and electrochemical sensors in the short to medium term. In so doing, a better understanding of the principles of application will be gained and may open the door to widespread acceptance of these powerful little devices.

The editorial board of Chemical Sensor Technology, vol 1[1] is of the opinion that fundamental research being carried out at present will really only reach commercialization in the next century. This will not prevent utilization of available sensors in current applications. Flow-based sample presentation systems could provide the required intermediate successes.

5.3 The use of Sensors for Process monitoring

As the value of process inventories increases with the scale of new plants and the cost of reagents and the value of products, there is a growing requirement to maintain strict statistical control of plant conditions through out the production process. The need for this control has been further expanded by the need for continuous environmental monitoring.

Present control systems generally seek to maintain a particular set point for critical process components. Control strategies of the future can be expected to become adaptive so that upset conditions or variations in the feed materials or required products are dynamically controlled during production. Obviously under such conditions it will be unacceptable to wait for results to come back from a remote laboratory. Even present control systems are dependant on rapid and frequent results. Also, monitoring only physical parameters such as temperature, flow rate, and pressures, while most important, is inadequate.

This problem is being addressed through the development and implementation of process analyzers. Many progressive chemical and pharmaceutical companies have sophisticated and well developed distributed process analyzer systems as well as the supporting research, and maintenance facilities to ensure their continued development. A frequent criticisms levelled at present process analyzers is that they are somewhat unreliable and too maintenance intensive. Unattended operation, as is experienced when

using certain physical sensors, is constantly held up as the standard to aspire to. Future chemical sensors stand a chance of reaching these lofty ideals.

Earlier the weaknesses of optical sensors were enumerated. Some of these may be expanded to describe the limitations of sensors in general:

- short life times - this may be addressed by making use of convenient disposable devices. Also if costs can be contained, a high degree of redundancy can be built into a sensor array and thereby enhance life time.
- inaccurate - because present sensors are often not stable, regular recalibration is needed. It is difficult if not impossible to calibrate sensors *in situ* without some form of sample (and calibrant) presentation system. Sensors are often poisoned by harsh process concomittants.
- poor reproducibility - this is also related to the lack of stability in present sensors and a wide variety of operating conditions.
- poor selectivity - as data processing power is increased and the concept of an array of sparingly selective electrodes is expanded, this problem may be resolved.
- response time of sensors to changing conditions is bedeviled by the need to achieve equilibrium across phase boundaries.

Before sensors are accepted on any significant scale, these problems will have to be adequately addressed. While for the long term, fundamental research is being directed at these shortcomings, in the short term, sample presentations schemes based on flow-analysis can go a long way to minimizing these problems.

5.4 SENSORS AND FLOW-BASED ANALYSIS

Flow-based sample manipulation systems (such as FIA and SIA) are well suited to acting as a versatile sample presentation system. All that is required is for the sensor to be incorporated in a suitable flow cell. This approach offers several powerful options when applying sensors for process monitoring and control:

- Frequent calibration is conveniently achieved by simply presenting the sensor with appropriate calibration standards.
- The sensing surface can be rejuvenated by periodically (or continuously) flowing a suitable reagent solution through the flow cell containing the sensor.
- The integrity of the sensor can be tested by monitoring a baseline and periodically sequencing a check sample.
- Because the sensor is exposed to the harsh sample for a fraction of the time and then only to a small volume, sensor life time can be expected to improve.
- The sample is presented to the detector in a controlled and reproducible manner thus improving the precision of measurement.
- Reproducible sample handling procedures obviate the need for equilibrium conditions.

Experiments were carried out to demonstrate the ability of SIA to behave as an efficient sample presentation system for sensors. A potentiometric sensor developed at Mintek for the determination of cyanide[10] is used as typical sensor. Principles demonstrated for this sensor can be applied to other sensors as well. In particular because of their

present short stable life times, it is expected that many biochemical sensors will benefit most from this approach to sensor utilization.

5.5 EXPERIMENTAL

5.5.1 *Carrier stream*

The carrier stream, which has been described before[10], acts as an ionic strength buffer and manifold cleaning solution.

Calibration solutions were prepared freshly on a weekly basis and are stored in dark coloured bottles to minimize photodegradation of the cyanide. A range of calibrants were generated by serial dilution of a stock solution prepared by dissolving sodium cyanide in a 0.1 $mol.dm^{-3}$ caustic solution.

5.5.2 *Instrumentation*

The SIA manifold used in this investigation is given in Figure 24a. An Alitea C-4V peristaltic pump (Alitea USA, Medina, WA) is coupled to a Valco ECSD10-P multiposition selection valve (Valco, Houston, TX) using teflon tubing with an i.d. of 0.8 mm. Figure 24b represents the equivalent FIA manifold and illustrates the relative simplicity of SIA manifolds and strong relationship between FIA and SIA.

The cyanide sensor comprises an ion selective electrode of the first type and a single junction Thalamid-type reference electrode. This reference electrode was selected because of its independence to cyanide concentration. No significance can be attributed to the absolute voltages given in the diagrams because of the dynamic nature of the measurement process and the signal handling electronics employed in conjunction with the electrode assembly.

The electrodes are mounted in an optimized flow cell (see Figure 25) to provide excellent sensitivity and minimal dead volume. The FlowTEK package described in chapter 2 was used for device control and data acquisition. A typical method flow programme is given in Table XII. (The pump was halted while the valve position was advanced.)

Table XII

Method flow programme for cyanide determination

Time, sec	Pump	Valve Port
0	Off	1 (Home position)
2	Reverse	1, 2, 3, or 4*
5	Off	5
7	Forward	5
45	Off	

* The different ports were fed by bottles containing the calibration solutions. In the simulated plant experiment, Port 1 was fed by the simulated plant vessel.

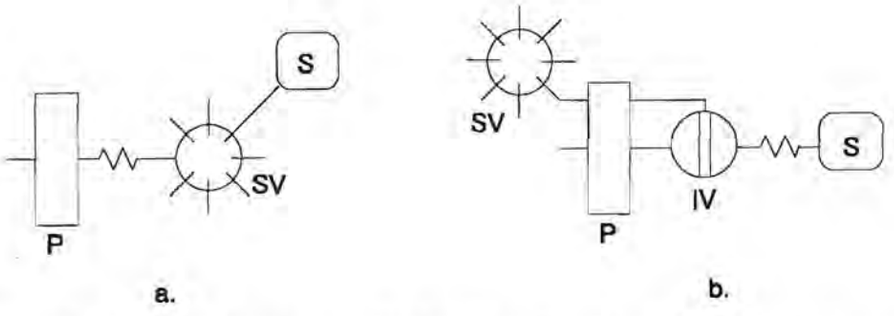

Figure 24: a. Sequential-injection manifold and b. equivalent flow-injection manifold for the determination of cyanide, P - pump, SV - selection valve, IV - injection valve, S - cyanide sensor in flow cell

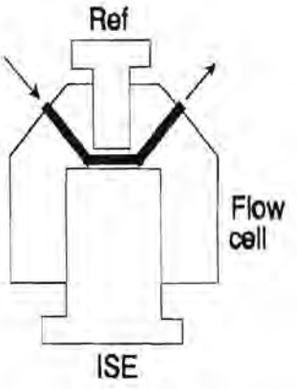

Figure 25: Cyanide ion selective electrode (ISE) mounted opposite a reference (Ref) electrode in a flow cell. Arrows indicate the flow path.

5.5.3 *Experimental Procedure*

The purpose of this suite of experiments is to demonstrate the power of SIA to enhance the utilization of sensors. This was achieved by carrying out the following experiments:

- adapt the existing flow-injection methodology used for the determination of cyanide to a sequential-injection manifold.
- investigate the impact of various manifold variables on sensitivity and precision.
- optimize the operational conditions.
- determine the analytical figures of merit which pertain.
- apply the sequential-injection analyzer to a simulated plant situation to demonstrate its usefulness for plant monitoring and control.
- consider SIA as a useful tool for sensor testing.

5.6 RESULTS AND DISCUSSION

5.6.1 *From FIA to SIA*

Considerable work has been carried out in the laboratory at Mintek to develop the method for the determination of cyanide using potentiometric detection[10]. This work has gone as far as to be tested and applied in the laboratory, on the pilot plant, and even in the process environment. In the latter, it has been used to control the addition of cyanide in a gold extraction process. The method has been found to be reliable and yields an accurate and precise result. It was for this application that the sensor used in this investigation was developed.

The process of migrating the methodology from the FIA platform to the SIA platform was a trivial one. The optimum sample size determined below corresponds to the optimum sample loop size. Flow-rates are directly comparable. Reaction coil lengths can also be transferred directly. The carrier stream was also adopted without changes.

5.6.2 *System Optimization*

The peristaltic pump was found to provide a more stable flow than the sinusoidal flow pump used for the studies described in chapter 3. This is clearly illustrated by comparing the precision attainable in a SIA manifold using the sinusoidal flow pump to that obtained when a peristaltic pump is used. A 120 $mg.dm^{-3}$ cyanide solution was

analyzed 20 times in two different manifolds that had been optimized. Firstly, the manifold was assembled with a sinusoidal flow syringe pump. Secondly, the syringe pump was replaced with a peristaltic pump. Appropriate changes were made to the flow programme. As far as possible, other operating conditions were matched. When the sinusoidal-flow pump was used, the relative standard deviation (s_r) was 0.032. The relative standard deviation (s_r), for the system which uses a peristaltic pump, was found to be 0.019. In addition, the fact that the peristaltic pump is a flow through pump and therefore does not first need to be loaded with wash solution also makes operation more convenient, and less time consuming. Consequently, the peristaltic pump was used as the stream propulsion device for the rest of the investigation.

Having selected the linear flow peristaltic pump, the impact of pump speed was investigated. Although there is an increase in sensitivity for pump settings greater than 300, under such conditions, precision suffered and carrier stream consumption was increased. The poorer precision may be attributed to the increased inertia of the pump and elasticity in the manifold which is accentuated at higher pump speeds. Any variability in starting and stopping the pump translates into discrepancies in the overall flow programme and therefore the sample volume. It is also likely (although not a pre-requisite) that at slower pump speeds, it is possible to get closer to equilibrium conditions. For all subsequent experiments, a pump speed of 300 which translates into a flow rate of 1 $cm^3.min^{-1}$ was used.

In FIA, sample volume is determined by the size of the sample loop on the injection valve (refer Figure 24b). In SIA, sample volume is determined by the duration of pumping with the sample port selected in the selection valve. Table XIII gives the times used, the calculated sample volume with a flow rate of 1.0 cm^3. min^{-1}, and the measured response from the sensor.

Table XIII

Sample Times and resultant Volumes

Sample Time, sec	Volume, mm^3	Response, V
1.0	16.7	1.01
2.0	33.3	1.62
3.0	50.0	1.69
4.0	66.7	1.74
5.0	83.3	1.86
10.0	166.7	2.03

The profiles (for sample volumes corresponding to 1, 3, and 10 sec) obtained under similar conditions are given in Figure 26. There is, as may be expected, an increase in peak height from small to large sample volumes. It is also interesting to take note of the peak shape for each sample. For sample volumes larger than 50 mm^3 (3 sec), the peak looses its familiar shape. This observation is easily explained. Given the

manifold used (notably the short reaction coil between the selection valve and the flow cell), for larger sample volumes, there is a region in the middle of the sample bolus where carrier solution has not penetrated. The concentration of the sample in these regions is therefore the same as in the original sample. Normally we would expect the signal to plateau under these conditions. The continued increase in signal despite no change in concentration can be attributed to the electrode slowly achieving an equilibrium state.

[As an aside, it is important to note that particularly when measuring plant solutions of varying ionic strength this situation is undesirable as the ionic strength buffer used as the carrier does not impact on that portion of the sample that is giving rise to the measured signal.]

As is the case for FIA, equilibrium conditions are not a requirement for SIA. The only requirement is a reproducible environment. From Table XIII it is clear that there is not much benefit gained from increasing the sample volume beyond 50 mm^3. Also, at high levels, it takes longer to re-establish the baseline (see Figure 26). A slight decrease in repeatability was also observed for larger sample volumes.

The excessive signal tailing observed in response profiles for the cyanide sensor is a function of the sensor response characteristics. Once the ions in the selective membrane have been disturbed by the passage of cyanide through the flow cell, it takes time for them to re-orientate themselves to conditions devoid of cyanide.

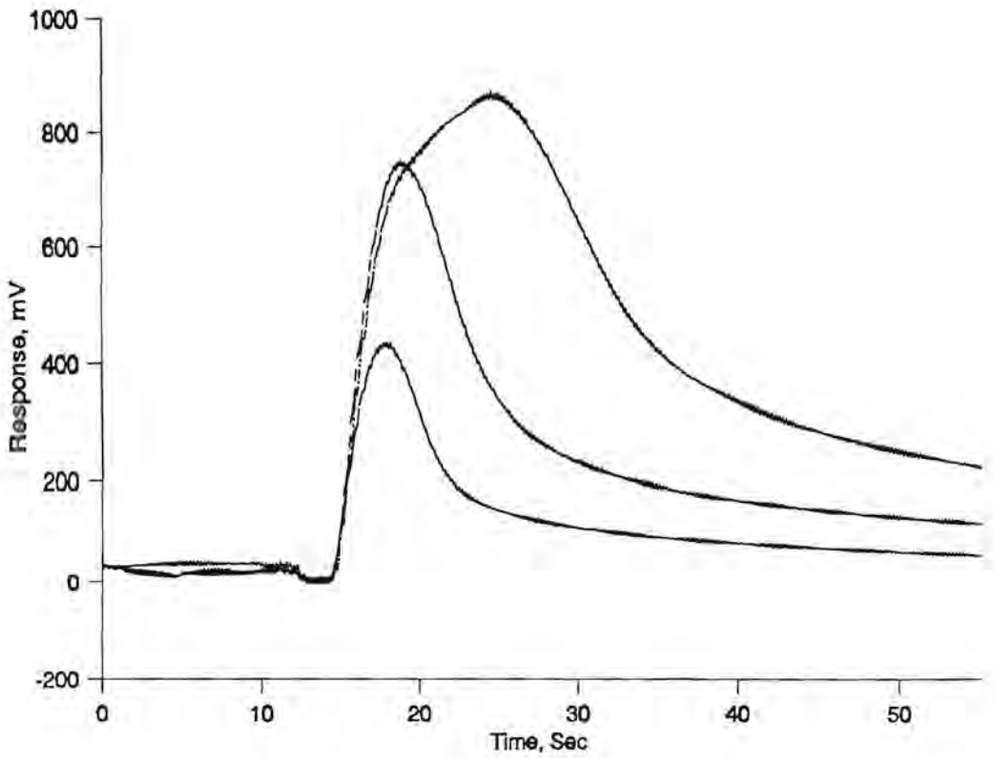

Figure 26: Response profiles for different volumes of sample. Volumes as per Table XIII corresponding to sample times of 1, 3, and 10 seconds.

It has been suggested that this tailing can be minimized by adding a small amount of cyanide to the carrier stream[11]. Experiments were carried out to ascertain the impact of doping the carrier stream with small amounts of cyanide. The comparison of the response profile for a solution containing 196 $mg.dm^{-3}$ of cyanide injected into a carrier without cyanide and one containing 10 $mg.dm^{-3}$ of cyanide is given in Figure 27. [The baseline of the two profiles were overlaid to assist comparison of the two profiles.] The cyanide in the carrier did not simply raise the baseline to achieve the desired reduction in tailing. Rather, the only effect that it had was to reduce the attainable sensitivity. A similar tail profile was achieved with a notable reduction in attainable sensitivity. This experiment brings into question the practice of doping the carrier stream with cyanide to reduce tailing. The only advantage of this practice would be to

yield a stable baseline. In SIA, even this advantage falls away because a shoulder on the leading edge of the profile develops when there is cyanide in the carrier stream. [The higher shoulder in the diagram is for the lower peak obtained with cyanide in the carrier. In some experiments, particularly when the solution in contact with the sensor had been stationary for a day or more, this shoulder was more pronounced]. This shoulder can be attributed to the fact that the solution in the flow cell is stationary while the sample is loaded and thus a temporary equilibrium is established during the sample loading period. This equilibrium is disturbed the moment flow commences through the flow cell. The disturbance of this equilibrium conditions gives rise to a shoulder at the start of the next profile. This temporary equilibrium is independent of flow when there is no cyanide present in the carrier stream.

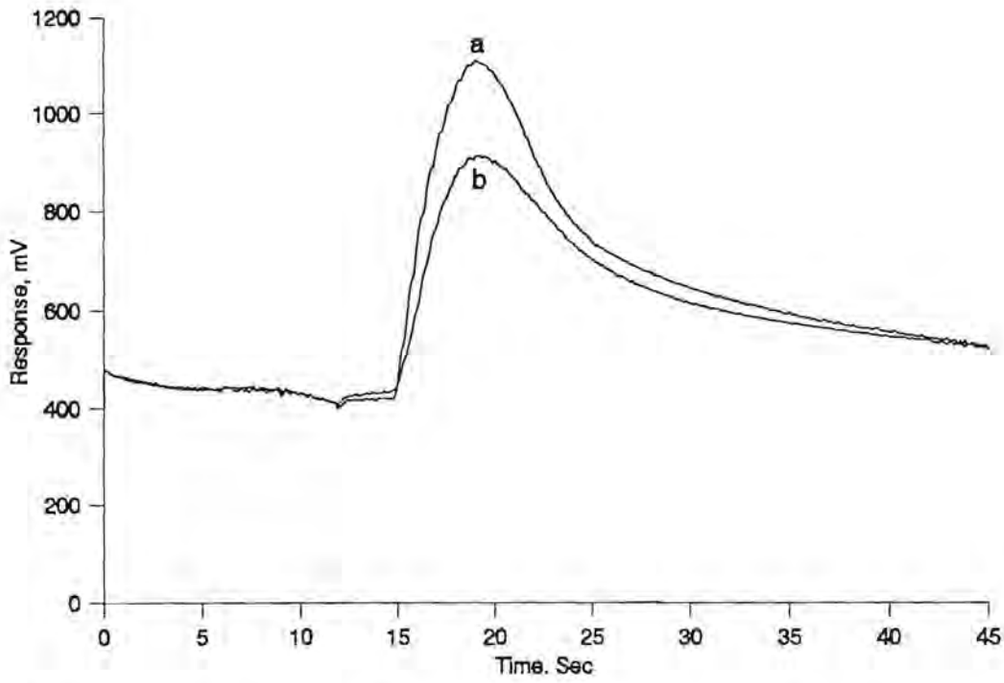

Figure 27: Influence of cyanide in the carrier stream. a) no cyanide b) 10 mg.dm^{-3} cyanide in the carrier stream.

5.6.3 *Analytical Figures of Merit*

Once an optimized system had been obtained, analytical figures of merit were determined. Figure 28 provides a trace of response profiles obtained when three standards were measured in triplicate and the second standard was measured 10 times. The precision of the peak height measurements is very good; the relative standard deviation (s_r) was determined for 10 measurements of a cyanide solution (192 mg.dm^{-3}) and found to be 0.016. Area measurements were less precise though still good enough for most purposes (s_r = 0.030). (No advantage could be gained by using peak area measurements). When peak height response was plotted against the natural logarithm of the concentration, a straight line was obtained (r^2 = 0.9997), with the following equation: $\text{Resp} = 1.620\ ln\ [CN^-] + 3.341.$

A sample throughput of 80 samples per hour is attainable.

5.6.4 *Use of SIA for sensor testing*

The concept of using flow-injection analysis as a diagnostic technique for the development and testing of sensors was proposed by Yerian *et al*[12]. These workers investigated the development and testing of an immobilized urease sensor. They pointed out that many papers on sensors seldom provide critical information on sensor performance, e.g. speed of response, reproducibility, sensitivity, selectivity, and lifetime. They then went on to demonstrate the power of flow-based analysis (specifically FIA) for monitoring sensor performance during the development phase and also once the

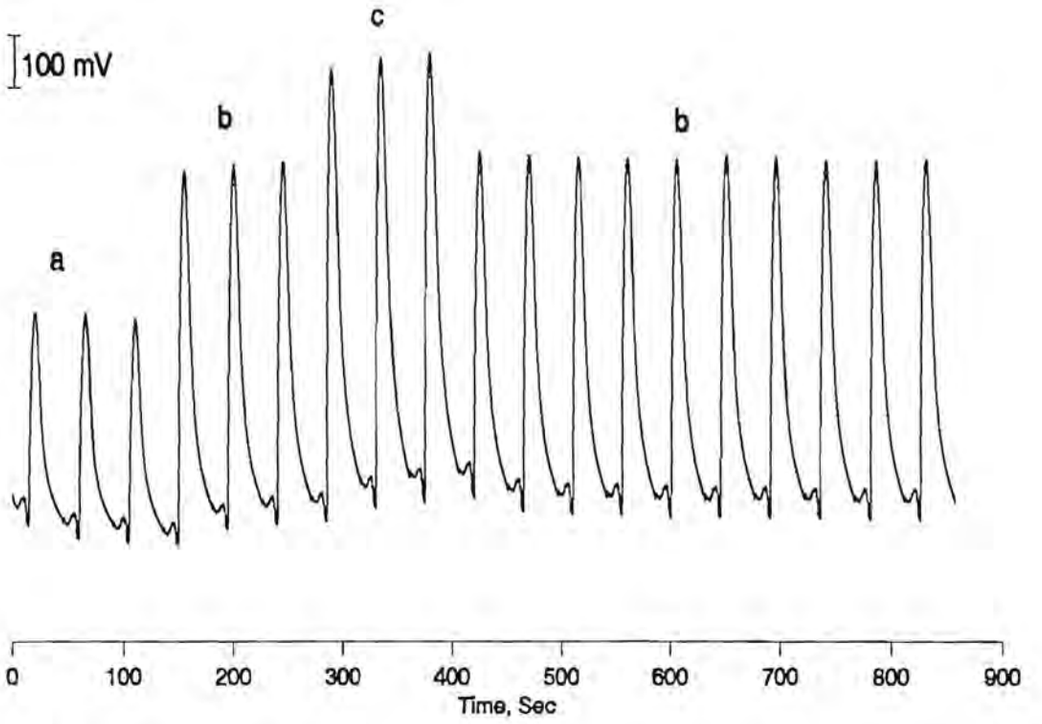

Figure 28: Response profiles obtained for the replicate injection of cyanide solutions with the following concentrations a) 96 $mg.dm^{-3}$, b) 192 $mg.dm^{-3}$, and c) 288 $mg.dm^{-3}$

sensor design had been finalized. With their proposed manifold, they were able to test one sensor at a time.

While these workers demonstrated the feasibility of flow-based analysis for the testing of sensors, they did not address the question of testing large numbers of sensors. In most cases, sensors are mass produced. Nevertheless, each sensor must be individually tested as failure rates of 30 per cent are not uncommon. SIA provides a convenient platform for a test rig. Consider the manifold depicted in Figure 29. A test solution is repeatedly drawn up via one of the ports of a multiposition selection valve. The test solution is dispensed sequentially to each of the sensor flow cells. The resultant response profiles are compared to predefined minimum standards, and faulty sensors are

quickly and conveniently identified. Using any of the convenient commercial connectors, or even a simple tubing sleave, flow cells are easily changed to allow rapid testing of large batches. Also, tests such as the comparison of different types of sensors, or sensor life time studies, as well as a host of other tests can conveniently be carried out using this test manifold.

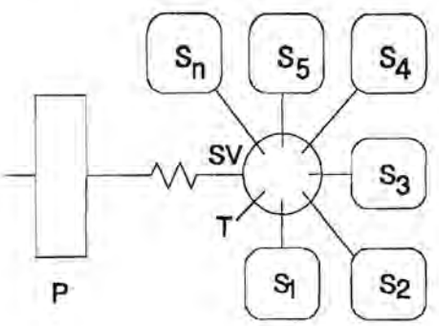

Figure 29: Sensor testing manifold. P - Pump, SV - Multi-position selection valve, T - Test solution, S_1 to S_n - Sensors to be tested.

5.6.5 *Process Monitoring*

The main area of application for sensors will be in the process environment. Process environments as different as hospital wards and chemical plants are envisaged. It is therefore important to demonstrate, at least in concept, that this mode of operation is feasible. To do this, a plant situation was simulated in the laboratory. A vessel was filled with a solution containing cyanide. The sequential-injection analyzer was programmed to sample and monitor the cyanide concentration in the simulated process vessel. Several random adjustments were made to the cyanide in the process vessel to simulate changing plant conditions. In particular after 620 measurements a spike of

concentrated (2.4 $g.dm^{-3}$) cyanide solution was added to the simulated process vessel and after 1480 measurements, the process solution was diluted with water. Every four hours the analyzer was automatically recalibrated.

The output from this experiment is given in Figure 30. The analyzer was able to track major and minor changes in cyanide concentration in the simulated process vessel. While this simple test certainly does not demonstrate the long term application of SIA in a process environment - a test period of at least a few months would be required for that - it does demonstrate the concept of process monitoring using sensors with a sequential-injection front end. The slope of calibration curves throughout the test period was constant. The intercept did however, vary. The effect of temperature on the reference potential and changing pumping characteristics is believed to be the reason for this. This is not a problem provided that the analyzer is calibrated frequently. The sequential-injection front end makes calibration a trivial exercise. The analyzer was found to run conveniently and was characterized by consistent performance, low sample consumption (± 1.2 dm^3 per day), and accurate analysis.

5.7 FUTURE WORK

The present sequential-injection hardware has been adapted from flow-injection equipment. As was the case for FIA in the early days, this has hampered the development and widespread acceptance of SIA. The extent of this incompatibility is particularly evident when comparing the dimensions of components used for sample

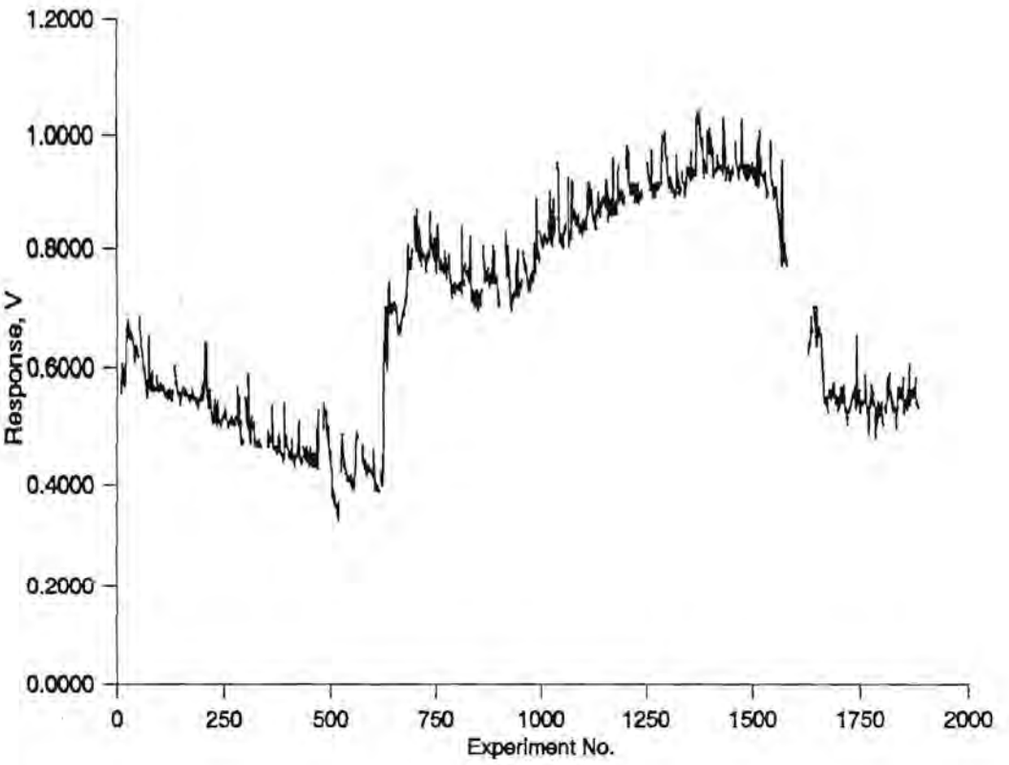

Figure 30: Cyanide monitoring using a sequential-injection analyzer in a simulated process environment.

presentation to the dimensions of typical sensors. Differences span three order of magnitude. Clearly there is much room for innovative research. Novel propulsion and stream selection methods will have to be developed. Miniaturized pumps and valves have been described though more as research curiosities than devices ready for commercialization. The same micro machining techniques used in the production of sensors could be used to prepare sample presentation front ends. Once integrated into compact packages, such devices will have tremendous potential as portable devices or *in-vitro* measurement systems.

5.8 REFERENCES

1. T. Seiyama, Chemical Sensor Technology, Vol. 1. Elsevier, Amsterdam, (1988).
2. L.A.R. Pioda, V. Stankova, and W. Simon. *Anal. Lett.*, **2**, (1969), 665.
3. W.E. Morf, The principles of ion-selective electrodes and of membrane transport, Elsevier, Amsterdam, (1981), 266.
4. K. Domansky, J. Janata, M. Jasowicz, and D. Petelenz, *Analyst*, **118**, (1993), 335.
5. J.N. Zemel, J.V. der Spiegel, T. Fare, and J.C. Young, Fundamentals and Applications of Chemical sensors, The American Chemical Society, (1986).
6. R. Narayanaswamy, *Analysts*, **118**, (1993), 317.
7. J. Ruzicka, Private communication, (1992).
8. G.D. Marshall, M.J.C. Taylor, D.E. Barnes, and S.J.S. Williams, *Process Control and Quality*, **2**, (1992), 249.
9. M.Hartnett, D. Diamond, P.G. Barker, *Analyst*, **118**, (1993), 347.
10. M.J.C. Taylor, D.E. Barnes, G.D. Marshall, D.R. Groot, S.J.S. Williams, *Process Control and Quality*, **3**, (1992), 173.
11. C. Pohlandt-Watson, *Mintek report M275*, Mintek, Randburg, South Africa, (1986).
12. T.D. Yerian, G.D. Christian, and J.Ruzicka, *Anal. Chim. Acta*, **204**, (1988), 7.

Chapter 6

Summary

6.1 AUTOMATED DATA ACQUISITION AND DEVICE CONTROL

FIA has made significant advances since its definition in 1975. Many of these advances can be attributed to the sheer ingenuity of investigators. In recent years though, that ingenuity has been able to draw on the power of micro processor-base device control and data acquisition packages. In commercial software systems, the emphasis has been on striving for greater degrees of automation. To this end, present commercial systems are configured automatically when a particular manifold cartridge is inserted into the instrument. Of course such convenience carries a hefty price tag and are severely limiting to the researcher. The researcher requires the same powerful device control and data acquisition facilities but with the flexibility to make frequent changes to device events and conveniently acquire and compare results from one experiment to the next.

With the invention of SIA, it soon became obvious that accurate computer control of device events was mandatory. Volume is determined by time or pump cycles rather than physical injection loops, as is the case for FIA. It was therefore vitally important to ensure repeatable control of devices. Also, early indications were that computer-

aided device control and data acquisition could open up new fields of endeavour for all flow-based research, (eg. stopped-flow experiments could only be investigated meaningfully when the timing of devices could be accurately controlled).

These considerations prompted the development of a software package aimed specifically at the researcher. The guiding principles applied in the development of this package were as follows:

- It should provide useful diagnostic information to permit speedy method development and convenient evaluation of research findings.
- It should be flexible and allow a high degree of user-configurability.
- The device control method development option should be convenient and intuitive.
- Data should be stored in a format that would allow data manipulation in third party packages.

These objective have been realized in a user-friendly package called FlowTEK™. Use of the program for existing FIA applications has resulted in superior data. The package generates and stores information-rich response profiles and peak parameters (peak height, peak area, peak time, and peak width at a particular height). In the research environment, it has led to more productive use of researcher time. The tedious and often repetitive operation of the experiment is handled by the computer, thus freeing the researcher for more stimulating and productive activity.

In the present investigation, the package was used extensively, specifically to:

- acquire important data for studying the effect of various parameters on analyzer performance,
- investigate the suitability of SIA for trace enrichment and separation,
- explore the potential of sensor injection methodologies, and
- control all device events necessary to carry out these experiments.

In the research environment, the package was found to go beyond merely acting as a convenient tool, to even highlighting new areas of endeavour: peak shape is an under utilized source of information in flow-based analysis.

6.2 SYSTEM CONFIGURATION

The initial work carried out on SIA demonstrated its potential. Those early manifolds were far from optimized and yet they still pointed to the viability of the technique. To gain wide acceptance though, a clearer understanding of the controlling influences that impact on important parameters such as sensitivity and repeatability was required. The present study built on the excellent work being carried out at the University of Washington (UW) in the laboratories of Ruzicka and Christian. Important guide lines were established to assist in the design of SIA manifolds. Of course, these guidelines must be interpreted in the light of the application at hand; it may not always be desirable to maximize sensitivity.

Factors that were considered at the UW were:

- the interdependence of various tube volumes, particularly when a syringe pump is used,
- sample and reagent volumes, and
- the effect of flow-reversal as a means of ensuring zone penetration and good mixing between sample and reagents.

In the present investigation, attention turned to manifold design principles, *viz.*:

- the effect of tube diameter,
- the effect of reactor geometry,
- the implications of varying pump speed, and
- the order of sample and reagent selection.

An interesting consideration that comes from this study is that double-injection FIA has a lot in common with SIA. Both begin with a stack of well defined sample and reagent zones which must penetrate one another in order to ensure that the desired chemical reactions take place. This suggests that principles established for SIA are directly applicable to double-injection FIA.

At the conclusion of this study it was possible to specify the optimum manifold configuration to achieve good precision and maximum mutual penetration of the various zones.

6.3 SORBENT EXTRACTION USING SIA

Separation science had its origin at the very instant of creation. Even primitive man practised the art in the isolation of dies. Analytical techniques which now fall under the umbrella of separation science number more than 30. It therefore seems appropriate to ensure that SIA is capable of carrying out separations. Sorbent extraction was selected because of the versatility it offers and the large repository of known chemistries that it can draw on.

Various manifold configurations and flow programmes were evaluated. A manifold which provides excellent results was assembled. Good calibration curves were attained at various levels of enrichment. The repeatability of the system was demonstrated. Other performance criteria all point to a viable approach to trace enrichment.

A key attribute of the final manifold is its simplicity. This is an important consideration for applications in the process environment. Furthermore, the proposed method satisfies all of the requirements set for sorbent extraction in FIA, *viz.*:

- rapid and quantitative partition,
- rapid and complete elution, and
- an extensive selection of off-the-shelf complexing reagents.

Swopping from the aqueous sample solution to the organic stripping solution is easily achieved by simply selecting a different port in the selection valve. Whereas for

manual solvent extraction, manipulation of the organic phase is troublesome frequently requiring the handling of emulsions, in sorbent extraction using SIA, these manipulations are carried out by the apparatus under computer control. In principle the attainable level of enrichment should exceed that of manual methods because of the small volume of organic phase that is required. Proper choice of the stripping solvent ensures that the enriched analyte is stripped into a small volume of organic solvent. In classical solvent extraction, the ratio of organic to aqueous phase is physically limited to that which can be reliably handled in the separation funnel.

Having demonstrated the feasibility and usefulness of SIA for trace enrichment and separation using sorbent extraction, the way is open to apply it to particular applications. Reference to existing chemistries of separation will also reduce the development cycle. In the process environment, the potential of being able to carry out complex sample manipulations, such as trace enrichment, in an automated fashion using SIA, is most exciting.

6.4 SENSOR INJECTION

There is a strong long-term drive to provide plant personnel with reliable devices that can provide critical information on the chemical composition of feed materials, process streams, and effluent streams. The need for robustness, and low maintenance requirements without detracting from the demand for accuracy and precision continues to point to an array of sparingly selective sensors scattered in a distributed fashion

through out a process. This is now attainable with sensors that measure physical phenomenon but not yet realistically attainable for chemical sensors. For this to be realized, particularly in the short term, some form of sample manipulation is required.

SIA has proved to be equal to the task. When the sensor is incorporated into a suitable flow cell, the following benefits accrue:

- recalibration is conveniently and easily accommodated,
- *in situ* rejuvenation of the sensor is feasible,
- the sensor is only exposed to the potentially harsh sample solution for a short time, for the rest of the time, the sensor is exposed to wash solution, and
- various pre-measurement manipulations can be carried out in the manifold.

If we accept the definition of a chemical sensor as being a transducer where the chemistry of measurement is incorporated in the detection device, we can envisage sensor arrays incorporated in sequential-injection manifolds. Such devices will provide a level of redundancy that can be used to improve robustness and reliability. Such a system brings us closer to a low maintenance and versatile means of monitoring key process components.

6.5 WILL SIA REPLACE FIA?

It is unlikely that SIA in its present form will replace or supersede FIA. Few sample manipulation techniques match FIA in flexibility. The only requirement for successful

implementation in FIA is a repeatable (though not constant) flow pattern. This has resulted in the enthusiastic acceptance of FIA in service laboratories and has revitalized interest in classical wet chemistry.

In SIA careful planning and method design is required. Attention must be given to ensuring that zones are contained within the reaction coil and that device events are carefully synchronized. This requires closer attention during the method development phase. Nevertheless, once a method has been developed, SIA tirelessly and slavishly repeats the device sequence which generates the desired analytical results.

SIA does have several key advantages over other flow-based techniques:

- Manifolds are simple and robust, typically comprising a single line manifold with three devices: a pump, selection valve, and detector.
- The selection valve doubles as an injection valve and sample stream selector thus reducing the number of devices required.
- Carrier and reagent usage is kept to an absolute minimum.
- Widely differing applications all use the same flow manifold. In this investigation, there was no material difference in the optimized manifold used to study the impact of instrumental parameters, sorbent extraction using SIA, and sensor injection. At most there was a detector change or inclusion of the sorbent extraction cartridge.

6.6 WHAT HAS SIA TAUGHT US?

In this investigation we have discovered that SIA can be applied to complex sample manipulation procedures such as trace enrichment, and that the usefulness of sensors is increased by incorporating them in a sequential-injection manifold. We have also developed a versatile device control and data acquisition package suited to research into flow-based methods of analysis such as FIA and SIA. Though most necessary and most valuable, these are not the most important finding of this investigation.

To date, flow-based analytical methodologies have been based on the physical determination of volumes. The injection valve with its sample loop is fundamental to FIA and all fields of instrumental chromatography. Even in segmented flow analyzers packets of sample and reagent are defined by the mechanical and physical introduction of bubbles.

In SIA, control is shifted from the mechanical to the temporal. Given a repeatable flow programme, volumes are determined by time. This means that we can do away with an injection valve or bubble introduction mechanism. While it is true that at present we must use a selection valve to sequence sample zones, this is only the beginning. In future this requirement will fall away as innovative stream selection options emerge. Looking to Nature we can expect membranes to play an important role in facilitating selection. Also it can be expected that electrical charge either coupled to membranes or in isolation will provide a means of selection. This suggests some exciting possibilities

for miniaturization and the combination of the sample manipulation manifold and the detector into a single unit. This is only conceivable because SIA has taught us that no nett flow is necessary for mixing to take place.

In the initial papers on SIA, the random walk model was used as a theoretical basis for SIA. It was this model that taught us that no nett flow is required for mixing (between sample and reagent) to take place, specifically at the molecular level. Flow is only useful for transporting components from one point in the manifold to the next. We were reminded that mixing at the molecular level takes place under the influence of entropy which is a random process. Mixing is fundamental to flow-based sample manipulation procedures.

The volumes employed in present flow-based systems are surely only appropriate because of the scale of manifold components., notable the tubing diameter and dimensions of detector flow cells. With these dimensions, the influence of longitudinal transport by laminar flow dominates the mixing process. As dimensions are reduced, the influence of the dispersion process will increase and the mixing of zones will largely come under the influence of entropy.

These observations suggest a research direction that will lead to the development of integrated flow-based sensors that have been miniaturized to the extent where manipulation close to the molecular level is attained but which exploit the advantages offered by flow-based sample manipulation.

Appendix A

Publications and Presentations

J.Ruzicka, G.D. Christian, and G.D. Marshall, Poster, CPAC sponsors meeting, Seattle, WA, Nov 1989

G.D. Marshall and J.F. van Staden, Anatech '92, Atlanta, Georgia, Apr 1992.

G.D. Marshall and J.F. van Staden, 3rd IUPAC symposium on Analytical Chemistry in the exploration, mining, and processing of materials, Sandton, South Africa, Aug 1992.

G.D. Marshall and J.F. van Staden, FIA Workshop, Sandton, South Africa, Feb 1994.

G.D. Marshall and J.F. van Staden, Analytica '94, Cape Town, Dec 1994.

J. Ruzicka, G.D. Marshall and G.D. Christian, *Anal. Chem.*, **62**, (1990), 1861.

J. Ruzicka and G.D. Marshall, *Anal. Chim. Acta*, **237**,(1990), 329.

G.D. Marshall and J.F. van Staden, *Anal. Instr.*, **20**(1), (1992), 79.

G.D. Marshall and J.F. van Staden, *Proc. Control and Quality*, **3**, (1992), 251.

G.D. Marshall and J.F. van Staden, *Submitted to Anal. Chim. Acta,* (1994).

G.D. Marshall, FlowTEK™ *1.1 Reference Manual,* Mintek, Randburg, (1992), 104pp.