TOWARDS DEVELOPMENT OF OPTIMAL

SEQUENTIAL INJECTION ANALYSIS METHODS

by

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We accept this thesis as conforming

to the required standard

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Department of **INTERDISCIPLINARY STUDIES**

The University of British Columbia
Vancouver, Canada

Date **OCT 16, 1985**
ABSTRACT

Currently, several research groups in the pulp and paper industry are actively pursuing the development of improved detection strategies for priority pollutants. A new technique in analytical chemistry called sequential injection analysis, may be able to provide a robust, inexpensive, automated method for detection of resin acids (known fish toxins) with appropriate use of immunochemical sensing. Most new analytical techniques, however, require fundamental studies in order to understand and optimize the physical processes that occur during the analysis. Towards this end, a dual-channel sequential injection analyzer has been designed and used for fundamental studies of dispersion. In an attempt to simplify the development of sequential injection methods, a unique graphical user interface with a virtual manifold has been proposed and implemented for control of the analyzer. The software is able to automatically and systematically manipulate over 20 instrumental parameters in search of optimal operating conditions; all information is recorded in a comprehensive database for rapid recall and display.

The first dataset to be collected on the analyzer includes over 6,800 experimental dispersion profiles that were created by injection of a tracer dye. The effects of injection volume, flow rate, and manifold geometry were examined and quantified using peak moments. The random-walk model was shown to hold
for sequential injection peak profiles which undergo multiple flow reversals of varying length. Optimization of the mutual penetration between two sequentially injected zones was investigated using several new descriptors for zone penetration, sensitivity, throughput and reagent economy. When the combined conditions of maximum zone penetration and sensitivity were considered, the optimal sample and reagent injection volumes were shown to be independent of manifold length and flow rate.

To gain further insight into the sequential injection technique, a computer simulation based on the random-walk model was proposed and implemented. A unique injection procedure was demonstrated, which simulates the sequential loading of multiple zones, in addition to the flow reversal process. Simulated dispersion profiles agree well with experimental dispersion profiles created under laminar flow conditions. Visualization of the theoretical concentration profiles which occur during injection and flow reversal allowed prediction of improved sensitivity at the point of zero net fluid movement.
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GLOSSARY

12-bit \( 2^{12} = 4096 \)

2D Two dimensions

3D Three dimensions

A Amperage

ADC Analog to digital converter

ADC units Digital values from 0 to 4095 produced by analog to digital conversion of 0 to 10 volts

\( A_0 \) Area of the zone overlap (ADC units • s)

\( A_S \) Area of the sample zone (ADC units • s)

BI Binary Input

BO Binary Output

C Concentration (Molar)

\( C^0 \) Initial concentration (Molar)

\( C^{\text{max}} \) Maximum concentration (Molar)

\( C^0_r \) Initial concentration of reagent (Molar)

\( C^0_s \) Initial concentration of sample (Molar)

\( C^{\text{max}}_{\text{OL}} \) Maximum height of the overlap (Molar)

CPU Central Processing Unit

D Dispersion number (dimensionless); \( D = C^0/C \)

d Distance from the valve to the detector (cm) used in the simulation

d_t Tube diameter (m)

\( \Delta d \) Equivalent length of the detection zone (cm) used in the simulation

DAC Digital to analog converter

DACA Data Acquisition and Control Adapter, manufactured by IBM

\( D_{\text{axial}} \) Axial diffusion coefficient (cm² s⁻¹)

DIP Switch Dual In-line Pole switch
DLL Dynamic Link Library; a collection or library of computer code used for programming in the Windows environment

$D_m$ Molecular diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$)

$D_R$ Dispersion number of reagent zone (dimensionless);
$D_R = \frac{C_R^0}{C_R}$

$D_{\text{radial}}$ Radial diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$)

$D_S$ Dispersion number of sample zone (dimensionless);
$D_S = \frac{C_S^0}{C_S}$

$E$ Peak excess (dimensionless)

FIDO Flow Injection Development and Optimization system

FIA Flow Injection Analysis

Form A window or frame on the computer screen (or display)

GUI Graphical User Interface

HP Hewlett-Packard

HPIB Hewlett-Packard Interface Bus

$\Delta l$ Length of the injection zone (cm) used in the simulation

i.d. Internal diameter of a tube (cm)

IBM International Business Machines, Inc.

$l_D$ Isodispersion point (s); the point in time when an adjacent sample and reagent zone have mutually penetrated each other to the same degree

IEEE-488 A general purpose interface bus; a standard for transmitting data and control commands between electronic equipment

$k_{1,2,3,4}$ Weighting coefficients for the composite optimization parameter

$k_T$ Sum of $k_1$ to $k_4$

$l$ Length of one step or flow reversal

$L_D$ Length of tube from the valve to the detector (cm)

$L_P$ Length of tube from the valve to the pump (cm)

$M$ Molar (moles $\text{L}^{-1}$)

$M_0$ Zeroth peak moment (ADC units $\cdot$ s)

$M_1$ First peak moment or centroid (s)

$M_2$ Second central peak moment or variance ($\text{s}^2$)

$M_n$ Higher-order central peak moments, where $n > 2$
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>MB</td>
<td>Megabyte</td>
</tr>
<tr>
<td>MDI</td>
<td>Multiple document interface; serves as a “container” form for all other subordinate forms of a Windows application</td>
</tr>
<tr>
<td>n</td>
<td>Number of steps or flow reversals</td>
</tr>
<tr>
<td>ODBC</td>
<td>Open database connectivity (a standard database protocol introduced by Microsoft to allow sharing of data recorded in different formats)</td>
</tr>
<tr>
<td>P</td>
<td>Zone penetration parameter (dimensionless) or exponent used in laminar flow convection equation</td>
</tr>
<tr>
<td>PC</td>
<td>Personal Computer</td>
</tr>
<tr>
<td>pH</td>
<td>The negative log of the hydronium ion activity</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million; $1 \text{ mg L}^{-1}$</td>
</tr>
<tr>
<td>ppb</td>
<td>Parts per billion; $1 \mu\text{ g L}^{-1}$</td>
</tr>
<tr>
<td>PTFE</td>
<td>Polytetrafluoroethylene (chemically inert fluoropolymer used for manifold tubing)</td>
</tr>
<tr>
<td>PVC</td>
<td>Polyvinylchloride (used for peristaltic pump tubing)</td>
</tr>
<tr>
<td>$\Delta q$</td>
<td>Length of a molecular step in the axial direction due to convective flow (cm)</td>
</tr>
<tr>
<td>Q</td>
<td>Flow rate ($\text{mL min}^{-1}$)</td>
</tr>
<tr>
<td>$Q_{\text{min}}$</td>
<td>Minimum flow rate ($\text{mL min}^{-1}$)</td>
</tr>
<tr>
<td>$Q_{\text{max}}$</td>
<td>Maximum flow rate ($\text{mL min}^{-1}$)</td>
</tr>
<tr>
<td>r</td>
<td>Radius (cm)</td>
</tr>
<tr>
<td>$r_0$</td>
<td>Tube radius (cm)</td>
</tr>
<tr>
<td>rad</td>
<td>Radians</td>
</tr>
<tr>
<td>RAM</td>
<td>Random Access Memory</td>
</tr>
<tr>
<td>Re</td>
<td>Reynolds number (dimensionless)</td>
</tr>
<tr>
<td>$R_E$</td>
<td>Optimization parameter for reagent economy (dimensionless)</td>
</tr>
<tr>
<td>$md$</td>
<td>A random number uniformly distributed between 0 and 1</td>
</tr>
<tr>
<td>ROM</td>
<td>Read Only Memory</td>
</tr>
<tr>
<td>$R_{\text{OPT}}$</td>
<td>Composite optimization parameter (dimensionless)</td>
</tr>
<tr>
<td>$R_P$</td>
<td>Optimization parameter for sample zone penetration (dimensionless)</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>$R_s$</td>
<td>Optimization parameter for sensitivity (dimensionless)</td>
</tr>
<tr>
<td>RS-232</td>
<td>The standard asynchronous communications adapter (serial port, COM1 – COM4) on most personal computers</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative standard deviation (%)</td>
</tr>
<tr>
<td>$R_T$</td>
<td>Optimization parameter for system throughput (dimensionless)</td>
</tr>
<tr>
<td>S</td>
<td>Peak skew (dimensionless)</td>
</tr>
<tr>
<td>SIA</td>
<td>Sequential Injection Analysis</td>
</tr>
<tr>
<td>SSE</td>
<td>Sum of the squares of the errors</td>
</tr>
<tr>
<td>$T$</td>
<td>Temperature (degrees Celsius)</td>
</tr>
<tr>
<td>$t$</td>
<td>Time (s)</td>
</tr>
<tr>
<td>$t_b$</td>
<td>Time for the detector to return to baseline (s)</td>
</tr>
<tr>
<td>$t_{b_{\text{max}}}$</td>
<td>Maximum time for the detector to return to baseline (s)</td>
</tr>
<tr>
<td>$t_i$</td>
<td>Time of the $i$th signal (s)</td>
</tr>
<tr>
<td>$U$</td>
<td>Linear flow velocity (cm s$^{-1}$)</td>
</tr>
<tr>
<td>$\bar{U}$</td>
<td>Average linear flow velocity (cm s$^{-1}$)</td>
</tr>
<tr>
<td>UV-visible</td>
<td>Ultraviolet-visible range of the electromagnetic spectrum (ca. 200 to 800 nm)</td>
</tr>
<tr>
<td>V</td>
<td>Voltage</td>
</tr>
<tr>
<td>$V_D$</td>
<td>Volume of the tube from the valve to the detector ($\mu$L)</td>
</tr>
<tr>
<td>VGA</td>
<td>Video Graphics Association (which standardizes video electronics)</td>
</tr>
<tr>
<td>$V_R$</td>
<td>Reagent volume ($\mu$L)</td>
</tr>
<tr>
<td>$V_S$</td>
<td>Sample volume ($\mu$L)</td>
</tr>
<tr>
<td>$W_O$</td>
<td>Baseline width of sample and reagent zone overlap (s)</td>
</tr>
<tr>
<td>$W_R$</td>
<td>Baseline width of reagent zone (s)</td>
</tr>
<tr>
<td>$W_{RE}$</td>
<td>Baseline width of reagent excess (s)</td>
</tr>
<tr>
<td>$W_S$</td>
<td>Baseline width of sample zone (s)</td>
</tr>
<tr>
<td>$x$</td>
<td>x-dimension (cm)</td>
</tr>
<tr>
<td>$y$</td>
<td>y-dimension (cm)</td>
</tr>
<tr>
<td>$y_i$</td>
<td>The $i$th signal (ADC units)</td>
</tr>
<tr>
<td>$z$</td>
<td>z-dimension (cm)</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Radius of the pump head (cm)</td>
</tr>
</tbody>
</table>
Δ  Delta or difference

\( \lambda_{\text{max}} \)  Wavelength of maximum absorption (nm)

\( \mu \)  Fluid dynamic viscosity (kg m\(^{-1}\) s\(^{-1}\))

\( \rho \)  Fluid density (kg m\(^{-3}\))

\( \sigma \)  Population standard deviation (s)

\( \sigma^2 \)  Variance or second central moment of the peak (s\(^2\))

\( \omega \)  Pump head frequency (radians s\(^{-1}\))

\( \zeta \)  Laminar flow factor (dimensionless)

\( \propto \)  Indicates proportionality

\( \infty \)  Infinity
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1. Introduction

"If, therefore, anyone wishes to search out the truth of things in serious earnest, he ought not to select one special science; for all the sciences are conjoined with each other and interdependent."

René Descartes

1.1 Overview

Recent years have brought considerable change to the pulp and paper industry. Increased social pressures for better environmental protection have created novel opportunities for the development of analytical methods that are capable of detecting priority pollutants [1]. The need to detect these chemicals at low concentrations in a complex sample matrix created the impetus for development of new techniques which provide both selective and sensitive determination of individual analytes. As a result, information gathered by these new chemical sensing methods can be used to improve process efficiency by providing greater control over manufacturing processes.
An ideal instrument would operate in both at-line and on-line modes [2, 3]. At-line operation involves a human technician presenting samples to the analyzer as they arrive from various sample points in the plant. The technician is thus in a position to regularly check on the operation of the analyzer and can rapidly change from one analytical chemistry to another as needs arise. On-line use can be as a permanent installation for routine process analytical measurements, or for short-term in-situ analysis for process optimization and diagnostics. In the on-line mode, an analyzer would be dedicated to running particular predetermined analyses on samples which are transported directly to it from the main plant reaction vessels, pipes, or effluent streams.

A successful form of continuous flow analyzer, flow injection analysis [4, 5], has found its way into routine industrial monitoring applications in the petrochemical, nuclear, water quality, pharmaceutical, biotechnology, agricultural, and (to a limited extent) pulp and paper industries. However, there are a few recognized drawbacks that limit the robustness of flow injection commercial analyzers in the industrial environment. These include the long-term reproducibility of flow rates using peristaltic pumps, and the need for a different manifold for each chemistry. By far the most significant factor is the rubber or PVC pump tubing used for fluid propulsion. Peristaltic pumps operate on the principle of a series of moving rollers that pinch a flexible pump tube against an adjustable tension device. As the rollers move in one direction they trap and move small segments of fluid in the tube much like the peristaltic action of swallowing. The tubing eventually wears out (over a period of 2-3 weeks of continuous
use) which leads to flow-rate drift and poor flow-rate calibration, necessitating replacement and recalibration of the tubes on a regular basis. As well, the ability to vary the chemical conditions within the analyzer (e.g., volume of injection, degree of mixing, selection of reagent) to allow detection of different analytes is somewhat restricted; usually, an entirely new manifold needs to be designed and optimized. Recently, however, a new technique has been described [6], which overcomes many of these inherent difficulties. This new technique is called sequential injection analysis, and will form the focus of this thesis.

1.1.1 Detection Strategies

Most "real" chemical sensing involves detection of specific analytes or analyte classes in a complex (natural) matrix. Selective determination of the large number of known organic compounds is, without doubt, the new and most challenging analytical frontier. Characterization of relatively pure individual organic compounds is achieved by infrared spectrometry, mass spectrometry, and magnetic resonance techniques [7, 8]. Commonly, analysis of mixtures of organics (or selected organics present in mixtures) can require complicated, expensive, "hyphenated techniques" such as gas chromatography - mass spectrometry (GC-MS), and can only be done by highly trained personnel in better equipped analytical laboratories. However, specially designed selective molecular sensing technologies based on methods of spectrophotometric analysis [9, 10] can often fulfill appropriate fully quantitative and semiquantitative
screening functions at a fraction of the cost and in a form that far more analytical chemists can use.

1.1.2 Resin Acid Detection

Resin acids are liberated during the pulping process of coniferous wood and are toxic to fish at the 1-2 mg L\(^{-1}\) level [11]. On-site detection of resin acids in pulp mill process streams and effluent is difficult to achieve. Methods of analysis for their detection have relied on sophisticated instruments such as gas chromatography [12-20], high-performance liquid chromatography [21-24], or gas chromatography – mass spectrometry [25-27]. These techniques require extensive analytical procedures including preliminary isolation of the resin acids by either liquid or solid-phase extraction and / or derivatization. Often, laboratory results aren't available for days – which may be long after a toxicity breakthrough occurs at the mill. Therefore, several research groups in Canada have focused on development of simple, rapid, quantitative, and inexpensive methods for determining concentrations of resin acids in pulp mill process streams and effluent discharge.

UV-visible colorimetric absorption techniques using chemically selective reagents are widely used and accepted in analytical laboratories. They require inexpensive equipment, and analyses of this nature can usually be performed by non-specialists. Recently, two simpler methods for detection of resin acids have been developed using spectrophotometric techniques [28-29]. In the work by Kester et al.
[29], a conventional flow injection regime proved to be a somewhat satisfactory means of providing an automated, quantitative, safer method of analysis that requires a minimal volume (less than two millilitres per sample) of the corrosive and possibly carcinogenic reagents that are currently used for resin acid detection in some British Columbia pulp mills. In order to improve the analysis further still, a glass flow-cell which incorporates extraction, reaction, and detection was conceived, constructed, and implemented with success. Unfortunately the detection level reached by this system is at the 20 ppm level in the concentrated extract, therefore necessitating a 10- to 100-fold preconcentration step before analysis. This work, however, illustrated the potential for automation of complex chemical assays in the pulp and paper industry using much simpler instruments and less expensive methods of detection.

It was obvious at this stage, that reagents or chemistries which provided much greater sensitivity and selectivity were needed in order to provide the industry with a method capable of detecting resin acids in their real sample matrix at the sub-ppm level. Immunochemical sensing [30-31] was thus considered as a potentially powerful way to meet these criteria, while still working within the realm of simple spectrophotometric detection. The reaction conditions for such methods are necessarily milder and easier to use. As well, immunochemical analysis is easily adaptable to continuous-flow methods such as flow injection analysis [32-35], or better still, the new technique of sequential injection analysis.
1.1.3 Immunochemical Sensing

In the most general sense, an immunoassay is a technique for measuring the presence of a substance using an immunological reaction [36-37]. Although the individual methods used for such measurements vary considerably, all involve reaction of a specific antibody (reagent) with a specific antigen (analyte). Detection can be made by colorimetry, fluorescence, chemiluminescence, radiology, or by electrochemical sensor. Advantage of the inherently high degree of specificity can be taken when it is necessary to analyze a single analyte "lost" in a complex natural matrix. This is especially important for organic compounds which are difficult to detect using conventional methodologies. When developing an immunochemical assay, the largest obstacle to overcome is finding an antibody that specifically and selectively binds to the antigen of interest. Fortunately, activity in this area of research has increased greatly over the past ten years and development and determination of polyclonal and monoclonal antibodies has become more common in modern biotechnology laboratories. A limited quantity of polyclonal antibodies for resin acids is now available at the Forest Products Biotechnology group in the Faculty of Forestry, Department of Wood Science, at the University of British Columbia. Attempts to obtain a monoclonal antibody cell line for resin acids continues to be one of their research goals. The original scope of this thesis included combining the new technique of sequential injection analysis with antibodies which are specific for one particular resin acid, or at least one class of resin acids. However, as anticipated, the length of time necessary for collaborating researchers to obtain and prepare quantities of suitable
monoclonal antibodies was such that a monoclonal cell line was not available at the time when decisions concerning the direction of this work had to be made. As well, the amount of work which proved necessary to design, build, characterize, and optimize a suitable analyzer was more than could be imagined at the time. Therefore, this project was narrowed to include only the development, characterization, and optimization of the sequential injection system, including a computer model for studying the sequential injection technique. Application of monoclonal antibodies for resin acids to the sequential injection analysis system (described in this thesis) must be left to future researchers when the antibodies become available.

1.2 Principles of Flow Injection Analysis

Flow injection analysis is a versatile, continuous flow, sample preparation and delivery methodology, which was first introduced in 1975 [38]. Since then, it has seen rapid and extensive development [4-5] with over 5000 research papers published to date. The rapid growth of flow injection techniques is due to its very wide range of applications as a means for sample transport and sample preparation for analyte determination. Flow injection can automatically and reproducibly mix samples and reagents, and then deliver the reacted and dispersed species to a wide range of detectors which accept a microlitre-scale flowing stream, such as atomic absorption spectrophotometers, inductively coupled plasmas, UV-visible spectrophotometers, ion selective electrodes, and so on. The performance of a flow injection system is greatly enhanced by addition of a microcomputer for control, data acquisition, and near real-
time data processing. Indeed, there are types of experiments that would not be feasible without computer control. The automated nature of and reproducible timing of flow injection analysis often increases sample throughput and precision, allows fast kinetic chemistries to be examined, and provides an ideal analyzer for continuous monitoring of process streams, effluent discharge, and both natural and potable waters [2-3].

A simple flow injection manifold is shown in Figure 1-1. The sample stream is channeled through a sample loop (typically 25 to 200 μL) mounted on the injection valve. Injection is performed by turning this valve causing the carrier / reagent stream to flow through the sample loop, flushing the sample towards the reaction coil. The flow lines are usually inert polytetrafluoroethylene (PTFE) or stainless steel tubing (typically 0.5 - 1.5 mm i.d.). Low pressure peristaltic pumps with rubber or poly(vinyl chloride) (PVC) pump tubing are most often used for stream propulsion, and usually flow rates for the carrier and / or reagents range from 0.1 to 5.0 mL min⁻¹. If necessary, additional reagents can be merged into the carrier / reagent stream before the continuously dispersing sample zone passes through the reaction / dispersion coil towards the flow-through detector.
Figure 1-1. A general flow injection manifold showing flow lines, peristaltic pump(s), injection valve, reaction coil, and detector.

Use of coiled reaction tubes, single-bead string reactors, and knotted reactors enhance mixing of the sample with the reagent(s). This phenomenon has been attributed to the increased radial mixing caused by secondary flow [4-5]. Finally, the sample reaches a detector where the signal is measured as a transient response. Since the originally homogeneous sample zone has dispersed into the carrier / reagent stream, a concentration profile is formed as shown in Figure 1-2. This is the peak shape that is recorded at the detector. Analyte concentration is proportional to peak height, area, or width. The concentration, C, can be measured at any point along the concentration profile. The dispersion number, D, is measured as C₀ / C, and thus takes on a value of greater than 1.

Dispersion within the manifold occurs by two principle processes, diffusion and convection. Variables such as reaction coil length, tube diameter, flow rate, temperature, and sample size affect the degree of dispersion. These parameters can
be effectively optimized to produce the most desirable result (e.g., maximum sensitivity, maximum reproducibility, maximum sample throughput, etc.).

![Figure 1-2. The concentration profile formed after dispersion of an initially square injection plug.](image)

### 1.3 Principles of Sequential Injection Analysis

Sequential injection analysis [6] is a very recent analytical development which is highly suited for process control and remote environmental monitoring. It provides a convenient way to develop, test and implement chemistries which allow sensitive, selective detection of chosen analytes. The total hardware required for a simple single-channel sequential injection instrument shown in Figure 1-3 is typically only a single selector valve, a single high-precision pump (of either syringe or peristaltic design), a detector which is able to accept a flowing stream, and (usually) a computer to control the timing and synchronization of all units to the necessary high precision. Despite this
simplicity, sequential injection systems can fulfill many of the liquid handling functions that could otherwise be done only by a laboratory robot or by a human technician.

Figure 1-3. A simple sequential injection analyzer showing the bi-directional syringe or peristaltic pump, reaction coil, and the multiport selection valve.

Sequential injection analysis offers significant advantages for on-process analytical chemistry, laboratory-based analysis and automated research studies. The advantages of such systems over conventional flow injection analysis include:

- a simpler manifold; usually only one pump and one valve are necessary
- the ability to incorporate diverse chemical analyses in the same manifold without reconfiguration
- long-term stability of flow rates when syringe pumps are being used; no need for recalibration of flow rates
• the ability to vary the injected volume of sample and reagent(s) dynamically via computer

• the ability to obtain optimal mixing conditions while minimizing sample and reagent use

Two major disadvantages already noted by others are:

• the requirement for relatively sophisticated operating software

• the reduction in throughput (relative to flow injection analysis) due to an aspiration cycle necessary to load the sample or reagent(s), and to fill the syringe with wash (if a syringe pump is used)

In sequential injection analysis, the computer-controlled valve and pump are programmed to select appropriate volumes of various reagents, samples, standards and buffers, mix them for a well defined time in a highly reproducible manner, detect the product(s) formed (using the same forms of sensors as available for flow injection analysis), and automatically clean out the instrument in preparation for the next sample. Spectrophotometric, electrochemical, thermal, chemiluminescence, fluorescence, atomic emission, photoacoustic, mass spectrometric and other forms of detection are possible. Typical method precision is as for flow injection systems, and relative standard deviation of 1% or less for replicate analyses is usually achievable.
The simplest analytical method for mixing a sample with a reagent zone to form a detectable product using the sequential injection technique is as follows:

1. The multiposition valve is turned to the line containing wash or carrier solution (position 1 in Figure 1-4) and a volume sufficient to flush out the entire reaction / dispersion tube and the detector flow line is aspirated into the manifold towards the pump. The volume aspirated should be at least 4-5 times the volume of the manifold which needs to be flushed with wash. If a bi-directional peristaltic pump is used, this step is usually not necessary.

2. The pump is paused briefly (usually 1 s) to prevent an undesired pressure surge while the multiposition valve turns to the sample line (2). The desired sample volume is aspirated for the length of time necessary at the current flow rate (e.g., 2.4 s at 2.0 mL min\(^{-1}\) will inject a 40 μL volume).

3. The pump is again paused briefly while the multiposition valve turns to the reagent line (3). A sufficient volume of reagent is aspirated and mutual dispersion of the sample and reagent zones begins immediately, allowing chemical reaction to occur. This step is not necessary if a chemical reaction is not required to effectively detect the analyte of interest (e.g., detection of chloride using a chloride-specific electrode).
Figure 1-4. Sequential loading of sample and reagent zones; product formation occurs as sample and reagent mutually penetrate each other.

4. The flow is then stopped, the multiposition valve is switched to the detection line (position 4 in Figure 1-5) and the flow is reversed to propel the sample / product / reagent zone towards the detector for measurement.

5. If increased mixing is necessary, the flow can be periodically reversed back and forth to enhance the dispersion process occurring between the sample and reagent zones, and therefore, increase the amount of sample reacted. If longer reaction time is necessary, the flow can be stopped while the sample / reagent interface is either in the reaction / dispersion tube or in the flow-through detector. Stopping the flow while the sample / reagent interface is in the detector allows kinetic measurements to be made on the reaction rate.

6. A sufficient volume of wash effectively rinses the manifold flow lines including the detector cell in preparation for the next analysis.
If necessary, the detector can be calibrated using a standard solution (shown at position 5) by the single-standard calibration technique for sequential injection analysis recently reported in the literature [39].

Figure 1-5. After the flow is reversed sample and reagent continue to mix and react as the product zone approaches the flow-through detector.

It should be noted that the above procedure assumes that the sample line is climatized to the current sample solution. If this is not the case (e.g., the sample line is placed in a new sample solution), then sufficient volume of the sample solution should be aspirated and expelled to waste in order to ensure that sample carryover does not occur.

It is easy to see why this technique approximates manual wet-chemical methods of analysis. It also provides improved protection for the analyst from noxious reagents and prevents contamination of samples by the analyst. A large number of conceivable reaction chemistries can be handled in this way. The simple yet robust design of the
instrument allows the analytical measurements to be made (i) in the place where the
analytical need exists, (ii) by the person who needs the analytical data, (iii) within a
reasonable amount of time, and (iv) with minimal maintenance / down time. The
bi-directional syringe pump is far better suited to remote, long-term unattended
operation than the peristaltic pumps commonly found in flow injection analysis. Thus,
there is a growing interest in this technology from researchers who need to do
unattended, automated process control and environmental monitoring.

1.4 REVIEW OF SEQUENTIAL INJECTION ANALYSIS LITERATURE

When this project was started in January of 1992, only three papers on
sequential injection analysis had appeared in the literature. Since then, at least 17
additional research papers which discuss the principles of or the application of the
sequential injection technique have been published. What follows is a brief review of
the majority of the known publications as they appeared in the literature since 1990 in
chronological order.

The paper entitled “Sequential injection: a new concept for chemical sensors,
process analysis and laboratory assays” by Ruzicka and Marshall appeared in
Analytica Chimica Acta in 1990 [6]. This paper introduced the concept of sequential
injection which they say arose from consideration of the random-walk model (it was the
work of Betteridge, Marczewski and Wade that introduced this to flow injection [40]). In
1991, Ruzicka and Gübeli reported the application of stopped-flow sequential injection
analysis to an assay of traces of a proteolytic enzyme using fluorescence detection [41]. They obtained highly reproducible results and a detection limit of 7.2 ng mL\(^{-1}\) of the pure active enzyme. Gübeli et al. also reported the first fundamental study of sequential injection analysis using a sinusoidal flow pump [42]. They considered optimization of zone penetration between two and three sequentially injected zones, and demonstrated the analysis of chloride and phosphate by this method. Several guidelines for method development were presented, however, they used a sinusoidal flow pump (discussed in Chapter 2) at one flow rate using one manifold dimension. The work done in this thesis uses their work as a starting point for further investigation of optimal operating conditions for the sequential injection technique.

In 1992, Ruzicka published a review of flow-injection, stopped-flow, and sequential injection methodologies [43] where he emphasized the need for a greater understanding of the effect of stopping and reversing the flow in the sequential injection process. Christian and Ruzicka discussed methods of exploiting the stopped-flow injection method for chemical assays [44] and pointed out the additional degree of information (kinetic) that can be obtained this way. Pollema et al. [45] reported the first immunochemical sensing method with the use of a sequential injection analyzer. They used the method to investigate short-time antibody binding by immobilizing the antibodies on 4.5 µm diameter magnetic beads. The magnetic beads could be aspirated by the syringe pump into the 1.0 mm i.d. reaction coil and held in place with an electromagnet under computer control while reagents were passed through them. The spent beads could then be released by the magnet and flushed to waste. They
achieved a detection limit of 155 ng mL\(^{-1}\) (ppb) using fluorescence detection with a sampling frequency of 30 samples h\(^{-1}\). It is a method along these lines that would be most suitable for detecting low concentrations of resin acids in mill streams and is the ultimate goal of this line of research.

Baron \textit{et al.} [39], published a method which uses the sequential injection technique which enables detector calibration using only one standard as well as dilution of high sample concentrations. Chunget \textit{et al.} [46], outlined a sequential injection methodology for fermentation monitoring. Marshall and van Staden [47] investigated the parameters affecting zone penetration in sequential injection analysis. They considered the effect of tube diameter (0.5, 0.8, and 1.5 mm i.d.), reaction tube geometry (knitted, coiled, and straight), and sinusoidal pump speed (maximum amplitude of 3.2 to 6.4 mL min\(^{-1}\)) on the degree of zone penetration as calculated by the area of overlap between two adjacent injected zones (this is discussed more in Chapter 4). Their results indicated that greater zone penetration occurred when one uses smaller tube diameters, but that this was obtained at the expense of precision. Straight tubes were also shown to give improved zone penetration over coiled or knitted tubes. The work in this thesis will also expand on these results by considering the effect of straight and coiled tubes using multiple linear flow velocities.

In 1993, Shu \textit{et al.} [48], published a method for monitoring D-lactic acid in pork by immobilized D-lactate dehydrogenase, and Lukkari \textit{et al.} [49], published a method for determination of total ammonium-nitrogen and free ammonia in a fermentation
medium, both using sequential injection analysis. Guzman et al. [50] demonstrated the ability of the sequential injection technique to handle a complex chemical analysis (fluorometric assay of factor thirteen) which involved six different solution zones, two different chemical reactions, appropriate dilutions, and the acquisition of conventional quantitative peak data and kinetic information. Guzman and Compton [51] then compared the analysis of factor thirteen by the sequential injection method to the same assay performed by a Zymark Benchmate robot. Ivaska and Ruzicka [52] found that by appropriate selection of pump tube and pump head rotation frequency, sufficient injection reproducibility could be achieved using a peristaltic pump; it was previously assumed that a syringe pump was necessary to achieve a high degree of reproducibility of injection volume. Pollema and Ruzicka [53] characterized planar concentration gradients for cell-perfusion studies using a sequential injection system.

More recently, a review of sequential injection analysis and its future possibilities has been published [54] as well as a review of sequential injection analysis for electrochemical measurements and process analysis [55]. Shu et al. [56], again reported a method for monitoring D-lactic acid, this time during a fermentation process. Cladera et al. [57], reported the design of a sequential injection system which uses an ordinary automatic titration burette actuated by a stepper motor for stream propulsion. They report that they can achieve similar precision of fluid movement by using this device as can be achieved using a peristaltic pump. Finally, the first known article on the use of sequential injection analysis for process monitoring in the pulp and paper industry in Finland was reported [58]. The analysis is based on the formation of a
coloured complex between Ca\textsuperscript{2+} and o-cresolphthalein complexone for the determination of calcium in white water of a paper machine. Their working range of 5-500 mg L\textsuperscript{-1} of Ca\textsuperscript{2+} was achieved with a sample volume of 30 µL. The method was tested with off-line samples and in an on-line application at a paper mill for periods of 6 hours (every 10 minutes) over a period of several days. They found that the method worked well at monitoring the fluctuation of calcium concentration in real time. During the on-line testing, a continuously flowing side stream of the fiber suspension from the head box of the paper machine led to a continuously operating (6000 rpm) process centrifuge (Westfalia). The centrifuge occasionally introduced small air-bubbles into the sample stream, and therefore improvement in the sampling system was suggested. This recent paper further illustrates the possibility of this line of research in the pulp and paper industry.

1.5 Scope of the Thesis

At the start of this thesis, there were no commercially available systems for performing sequential injection analysis, even though pumps, valves and suitable flow-through detectors had been available for quite some time. It was apparent from the literature that the major obstacle to overcome when designing sequential injection methods was the writing of sophisticated control software for development of analytical methods. Therefore, a major portion of this thesis is devoted to the design and development of a new sequential injection analysis system (Chapter 2) which is capable of not only performing a sequential injection method, but also automatically
exploring system variables. In addition, programming of new methods is simplified by a unique graphical user interface developed particularly for this purpose.

Instrumentation-oriented research requires a significant time investment for the student to become familiar with programming and electronics. The advancement in science obtained through proving some new theory can only be attained once the complicated apparatus is available. This impediment comes at a time when the ability to do research is increasing in momentum exponentially, society’s body of knowledge in different disciplines is increasing at the same rate, there are still academic boundaries which deter interdisciplinary studies, and researchers grounded in different disciplines will have very different skill sets. In order to help transfer this new technology (sequential injection analysis) from one discipline to another (from analytical chemistry to pulp and paper, and biotechnology), an extremely simplified interface between the end-user and the instrument was necessary.

An analysis performed by the sequential injection method is governed by two fundamental processes. The first, which occurs in almost all flow analyses, is physical dispersion of the injected sample into a suitable carrier stream due to convective flow and molecular diffusion. The second process is chemical reaction, which may or may not be necessary in order to detect the analyte. An analysis which relies solely on the first process (dispersion) would be the determination of chloride with a flow-through ion-selective electrode. An assay which includes the second process (dispersion and chemical reaction), would be the analysis of chloride by its reaction with a reagent
(such as mercury (II) thiocyanate and iron (III) nitrate) during transport to a
spectrophotometric detector. In the first case, the dispersion process is acting alone,
while in the second case dispersion and chemical reaction occur simultaneously. In
either case, it is dispersion which is the primary physical phenomenon which governs
the chemical concentration gradient produced within the tube, and as such, has the
most significant influence on the output of the analysis. It is also evident from the
literature that this area of research still needs considerable attention.

Chapter 3 will focus on an investigation of dispersion profiles (without chemical
reaction) produced by the sequential injection analysis system described in Chapter 2.
The automated optimization software will be used for this comprehensive empirical
investigation and its performance evaluated. The dispersion profiles will be subjected
to numerical analysis by moments, in order to investigate the effects of varying the
system operating conditions. The number of flow reversals, and length of the flow
reversal step will be investigated through examination of the peak variance to confirm
the applicability of the random-walk model to sequential injection analysis.

Chapter 4 introduces new parameters which aim to quantify the degree of zone
penetration between two adjacent injection zones. The effect of linear flow rate,
sample and reagent injection volumes, and manifold dimensions on mutual penetration
of two sequentially injected zones will also be investigated. Study of this area of
sequential injection analysis should form the fundamental focus of research in the next
few years.
In Chapter 5, the random-walk model is introduced by outlining previous work, and discussing its relevance in the present work. The basic premise of the random walk model relies on is the continuous tracking of the three-dimensional position of individual molecules as they progress through the manifold under a defined set of conditions. In each iteration of the model, every molecule is allowed a three-dimensional diffusional step of random length, in addition to an axial movement due to convective flow according to a laminar flow profile. This model will likely be the only feasible numerical solution for the sequential injection technique for quite some time. A novel simulation of the injection procedure, developed in this work to accommodate the sequential stacking of zones in the reaction coil will be illustrated. Numerical simulations done with this model will be compared in detail to experimental dispersion profiles obtained in Chapter 3. Investigation of several model parameters will be done in an attempt to improve the agreement between experimental and predicted results. Parameters tuned include sample size, degree of laminar flow, effective diffusion coefficient, and laminar flow profile.

In summary, the overall scope of this thesis includes:

- development of the hardware, software, and computer interface of a new sequential injection analyzer using a graphical user interface
• characterization of the analyzer using a tracer dye to create dispersion profiles which are subjected to moment analysis and recorded as retrievable records in a comprehensive database

• investigation and optimization of the parameters affecting zone penetration for reagent-based assays under typical analyzer conditions

• development of a model to theoretically explain the dispersion process found in the sequential injection technique

Upon completion of this thesis, a sophisticated analyzer will be developed and ready for use in investigating automated resin acid detection in the pulp and paper industry with the use of polyclonal or monoclonal antibodies. As well, a comprehensive database of the system response including nearly 7,000 dispersion profiles obtained during this present work will be available for instantaneous recall. A greater understanding of the optimal operating conditions of this type of analyzer will also be obtained. Finally, a model which can be used to study the molecular distribution of the molecules within the flow manifold will be available for theoretical research in this area.
1.6 References


2. Sequential Injection Analysis System

"The software is the instrument."
National Instruments slogan, ca. 1992

2.1 INTRODUCTION

Research into the development of flow injection methods has been primarily dominated by empirical control over flow rate and experimental timing. This is mainly a result of the complex, interdependent nature of the system variables. Computerization of control of this process has been done by several researchers in order to achieve improvements in precision, reproducibility, automation, and throughput. In a sequential injection method, however, an even greater flexibility of solution handling is achieved. Since there is no physically separate injection loop (present in flow injection analysis), both sample and reagent volumes can be manipulated through the control software. This has a profound impact on a researcher's ability to create variation in the concentration gradients within the reaction coil. Thus, software-driven, empirical optimization techniques will have an even greater overall influence in finding optimal conditions of a sequential injection method than ever before. This chapter will describe
the hardware and software of a dual-channel sequential injection analysis system, designed and constructed to automatically perform empirical optimization experiments. A virtual manifold is proposed and implemented as a Windows-based graphical user interface for sequential injection analysis.

2.1.1 Brief History of Computer-Controlled FI A Systems

In most cases, an analytical flow system can be conceived as two essential elements, namely, the physical hardware which manipulates the fluids, and the computer software which manipulates the physical hardware. When flow injection systems were first being assembled in the mid to late nineteen seventies, the hardware requirements were relatively simple. An analytical system comprised of a simple injection valve (electrically actuated or manual) which provided injection of a discrete sample volume in line with a reaction coil connected to a flow-through sensor. Fluid drive was usually performed by a peristaltic pump lent from a previous generation of hardware called air-segmented flow analysis, or by a constant-head flow device. Typically, only a chart recorder was necessary to capture the transient output signal at the detector. Measurement of peak height from a stable baseline was used to quantify the analysis. Obviously, simple systems of this nature had their limitations with respect to efficient control and optimization of the analysis at hand.

In the early nineteen eighties, researchers and analytical manufacturers began to realize the benefits of controlling the analytical hardware with personal computers
running specialized software. This step into the next generation of hardware systems was made possible by the computer revolution which started about this time, resulting in personal computers becoming more viable as a research tool in the laboratory and the industrial sector. Stewart et al. [1] gave the first report of computer control over a flow injection system. Subsequent software programs to control flow injection systems [2-7], however, rarely did more than turn the pump(s) on or off, turn an injection valve at a pre-selected time, and digitize the detector response for peak storage. However, replacement of the chart recorder with computerized data acquisition techniques allowed research into peak shape to become much more accessible. By this time, peak width and peak area, as well as a multitude of other peak descriptors based on the digitization of the response profile could be used for quantitation, and allow scrutiny of the analytical method. Much more could be determined from an analysis than just chemical concentration at one point in time (as in a peak height measurement).

Instruments of this nature became increasingly common throughout the nineteen eighties with several researchers exploring more complex computer-aided optimization techniques [8-9]. For example, optimization techniques which are based on a factorial design or simplex optimization [10-12] have been investigated extensively [13-17]. The ultimate goal of this line of research is to get the control software to either (i) map out the effects (via factorial design) of the most significant parameters on the given analysis, or (ii) intelligently search an n-dimensional parameter space for optimal operating conditions. Self-tuning flow injection systems would be the natural consequence of this direction of investigation. Simplex optimization is preferred in
situations where there are too many variables to adequately examine individually. For example, to factorially investigate ten values of each of five parameters would require $10^5 = 100,000$ experiments to be tried. At a fast pace of 100 experiments per hour, it would take over 40 days of non-stop experimentation (assuming constancy of calibration throughout). Thus, the focus of research had turned towards more efficient methods of optimization which intelligently explore an $n$-dimensional space.

Optimization-oriented software for flow injection analysis of this nature has the following basic characteristics. The software comprises of an initial set-up routine which defines flow rate boundaries for a given number of pumps (perhaps as many as 5 to 7). The software then chooses flow rates for each pump so as to effect different chemical conditions within the manifold (concentration ratios, residence time, etc.) and begins recording the detector response. Variation in the flow rates is usually done by either a complete factorial design or by more efficient optimization techniques such as the composite modified simplex [12-13]. Thus, the majority of the previous work has been on modification of flow rates of several pumps for an individual one-step injection procedure (even though the optimization algorithms used are quite capable of optimizing procedural situations).

2.1.2 Computer Control Over Sequential Injection Systems

Optimization of a sequential injection method is an order of magnitude more complex than its flow injection predecessor since the researcher must deal with a multi-
step or procedural process. This creates an additional dimension of information to be optimized in the analysis. In the system developed as part of this work, as many as twenty-one instrumental parameters are tunable in any step of the analytical method. Furthermore, these parameters to be optimized not only include flow rate, but also valve positions, sample volume, reagent volume(s), flow reversals, delay timing, and so on. Hence, greater overall control over the chemical conditions is only achievable through this technique after a substantial investment in more sophisticated control software has been made [20, 27].

A unique approach, with the use of a Graphical User Interface (GUI) to create a virtual manifold on the computer screen is proposed and implemented in this work in order to simplify the complexity of (i) designing the analytical procedure, and (ii) mapping of the effects of the tunable parameters. In this first step towards computerized optimization of SIA techniques, more efficient mathematical tools such as simplex optimization will not be considered. Instead, what is desired is a system with the ability to independently map out the effects of all system parameters completely, thereby providing a more comprehensive, informative dataset. A general overview of the interfaced hardware, and the most significant capabilities of the software interface that is proposed and implemented in this work will now be the focus of discussion.
2.2 Sequential Injection Analysis Hardware

The underlying concept behind the principle of sequential injection analysis is hardware simplicity. For industrial monitoring and process control, the undesirable characteristics of flow injection systems (such as multiple pumps, multiple valves, and multiple hardware configurations) need to be eliminated if robust, low-maintenance analyzers are to gain widespread industrial acceptance.

Although only one pump, one multi-position valve, and one detector are necessary to perform a given analysis, the system developed here, for automated SIA response-surface mapping is, in actuality, a dual-channel system. This increase in interfaced hardware enhances the flexibility and "open-endedness" of the instrument as a research tool. More elaborate experimentation can be performed on this instrument than on one which is limited to one pump and one valve. The final methods developed on this instrument would likely be implemented on much simpler hardware systems consisting of little more than one pump, one valve, and one detector.

2.2.1 Computers and Interfaces

The SIA control computer is a 33 MHz, 80486 DX Intel-based, IBM-compatible, personal computer. It has a 1 MB super VGA video card, 128k cache, and 8 MB of RAM. There are two physical hard drives on the system totaling 235 MB of storage space. By today's standards, this system is now limited by the video card, the CPU,
and the cache. Upgrading any of these would improve the speed at which the system is able to redraw its graphical interface and interact with the user. However, this was considered an advanced system at the time it was bought (1991).

The SIA control computer uses two multi-purpose IBM Data Acquisition and Control Adapter (DACA) boards (obtained from Mendelson Electronics; Dayton, OH, USA) as shown in Figure 2-1. These each feature sixteen binary inputs, sixteen binary outputs, four channels of 12-bit analog to digital conversion (ADC), and two channels of 12-bit digital to analog conversion (DAC). The pin assignments for each of these boards are shown in Table 2-1 and Table 2-2. Board #1 is used to control the two six-position valves, the six mini-valves (only five were used), two accessories (e.g., stir motors), and send a reference or acquisition request to the diode array spectrophotometer control computer. It is also used for monitoring the uni-directional valve position, the bi-directional valve position, and up to four analog detectors. Board #2 is used for control over the ten-position valve, and the two pumps (speed and direction). It is also used for monitoring the syringe microswitches (indicating full or empty syringes), the current position of the ten-position valve, and for tracking the position of the two syringes through their linear potentiometers connected to analog input channels 1 and 2. The input and output position codes for the ten-position valves are created by the appropriate combination of the numbers 1, 2, 4, 8, and 10, corresponding to BO 1, 2, 3, 4, and 5, and BI 8, 9, 10, 11, and 12, respectively.
One additional IBM DACA board was interfaced to a Hewlett-Packard Diode Array Spectrophotometer (HP8452) control computer (an Intel 80286 CPU, with 1 MB of RAM). This DACA board allows the SIA control computer to send a reference request signal (via BO14 on DACA #1 and BI4 on DACA #3) and an acquisition request signal (via BO15 on DACA #1 and BI5 on DACA #3) to the diode array control computer which runs a DOS-based program written in-house in Microsoft Professional BASIC version 7.00 by Ivan H. Brock. This software continuously polls DACA board #3 for a reference request or an acquisition request and sends the appropriate instruction to the diode array detector through an IEEE-488 interface. The detected signals for up to two wavelengths are sent back to the SIA control computer through BNC cables connecting analog output 0 and 1 on DACA #3 and analog input 0 and 1 on DACA #1, respectively.
This hardware configuration allows the diode array computer to be easily interfaced to the SIA control computer as a detector through the existing interface boards, without specialized Windows drivers and minimal software overhead on the control computer. In addition, other detection sources can be quickly connected to these same channel inputs (analog input 0 and 1 on DACA #1) when the diode array detector is not in use. This also allows the diode array and the SIA system to be used at the same time for separate analytical purposes with minimal hardware manipulation.
Table 2-1. Pin-out used for IBM Digital Acquisition and Control Adapter (DACA) board #1.

<table>
<thead>
<tr>
<th>IBM DACA Board #1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Binary Output</strong></td>
</tr>
<tr>
<td>00 Valve Toggle</td>
</tr>
<tr>
<td>01 Forward/Reverse for Bi-directional Valve</td>
</tr>
<tr>
<td>02 Mini-Valve 1</td>
</tr>
<tr>
<td>03 Mini-Valve 2</td>
</tr>
<tr>
<td>04 Mini-Valve 3</td>
</tr>
<tr>
<td>05 Mini-Valve 4</td>
</tr>
<tr>
<td>06 Mini-Valve 5</td>
</tr>
<tr>
<td>07 Mini-Valve 6</td>
</tr>
<tr>
<td><strong>Binary Output</strong></td>
</tr>
<tr>
<td>08 Valve Address:</td>
</tr>
<tr>
<td>09 Address of 6 is the Uni-directional Valve</td>
</tr>
<tr>
<td>10 Address of 7 is the Bi-directional Valve</td>
</tr>
<tr>
<td>11 Unused</td>
</tr>
<tr>
<td>12 Accessory 1</td>
</tr>
<tr>
<td>13 Accessory 2</td>
</tr>
<tr>
<td>14 HP Reference Request Line</td>
</tr>
<tr>
<td>15 HP Acquisition Request Line</td>
</tr>
<tr>
<td><strong>Digital to Analog Output</strong></td>
</tr>
<tr>
<td>0 Unused</td>
</tr>
<tr>
<td>1 Unused</td>
</tr>
<tr>
<td></td>
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<td></td>
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</tbody>
</table>
Table 2-2. Pin-out used for IBM Digital Acquisition and Control Adapter (DACA) board #2.

<table>
<thead>
<tr>
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<th>Binary Input</th>
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<tbody>
<tr>
<td>00</td>
<td>00</td>
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<tr>
<td>01</td>
<td>01</td>
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<td>02</td>
<td>02</td>
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<td>13</td>
<td>13</td>
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<tr>
<td>14</td>
<td>14</td>
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<tr>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Digital to Analog</td>
<td>Analog to Digital</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
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<td>2</td>
<td>2</td>
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<td>3</td>
<td>3</td>
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<tr>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

- **Binary Output**: Decimal values corresponding to specific functions.
- **Binary Input**: Decimal values corresponding to specific functions.
- **Analog Output**: Analog values corresponding to specific functions.
- **Analog Input**: Analog values corresponding to specific functions.

- **00**: Syringe 1 Microswitch - Emptied
- **01**: Syringe 2 Microswitch - Filled
- **02**: Syringe 1 Microswitch - Emptied
- **03**: Syringe 2 Microswitch - Filled
- **04**: Unused
- **05**: Unused
- **06**: Unused
- **07**: Unused
- **08**: 1 Input Position
- **09**: 2 Code for
- **10**: 4 Ten-Position
- **11**: 8 Valve
- **12**: 10
- **13**: Unused
- **14**: Unused
- **15**: Unused
- **0**: Pump 1 Speed Output
- **1**: Pump 2 Speed Output
- **2**: Syringe 2 Position Input
- **3**: Unused
2.2.2 Valves

2.2.2.1 Valve Types

Three multi-position valves have been interfaced through the software. Two are six-position, pneumatically-actuated rotary valves (Rheodyne, Inc.) and the third is a ten-position, electronically-actuated valve (Valco Instruments Co. Inc., Houston, TX, USA). Both of the six-position valves were constructed after an original design by Wentzell et al [10]. The controller circuit boards were modified for this work in order to include feedback of valve position to the computer. The circuit diagram for the controllers used for the six-position valves is shown in Figure 2-2.

The second six-position valve was modified still further to enhance its functionality. An additional pneumatically-actuated directional switch was added to allow switching of the rotational direction of the valve. The software was modified to incorporate this feature and includes a routine to choose the direction of rotation “intelligently,” taking the shortest route to the next position. The schematic for the additional circuitry for the second, more advanced six-position valve is shown in Figure 2-3. The bi-directional, pneumatic ratchet mechanism throws a directional lever (lower right) which toggles between clockwise and counter-clockwise positions. This valve is typically used preferentially over the non-reversing valve due to its advanced functionality.
Figure 2-2. Valve circuitry constructed to control each of the six-position valves at addresses 6 (this diagram) and 7 (without inverter on BO 8 line) with valve position feedback loop; taken after reference 10 with several modifications.
A third multi-position valve was interfaced through the DACA board as well. Manufactured by Valco Inc., this ten-position valve is self-contained and is electrically actuated (versus pneumatically), thus requiring no compressed air line. Its greater reliability offsets the greater speed of the pneumatically actuated six-position valves; speed is now less important since the system pauses for 1.0 s at each position in its rotation in order to maintain consistent timing.

In addition, up to six, small 12 V solenoid valves can be interfaced through the software. In this case, two combination “mini-valves” were used, one with two outlets, and the other with three outlets, for a total of five solenoid valves (i.e., future addition of
one more solenoid valve is possible). Six binary outputs on the DACA board control the six valves (high = open, low = closed), through a reed-relay control interface which provides the valve with either 0 or 12 V at approximately 800 mA. The circuits were optically isolated as shown in Figure 2-4 in order to minimize electrical noise between the solenoid valves and the rest of the interfaced circuitry. Although this circuit is repeated six times in an interface box, only five of these valves (one two-way and one three-way) are interfaced to the DACA board through binary outputs 2 through 7 on board #1.

![Electrical schematic for optically isolated solenoid mini-valves.](image)

**Figure 2-4.** Electrical schematic for optically isolated solenoid mini-valves.

### 2.2.2.2 Feedback Control

After extended periods of continuous operation, the potential exists for a solenoid valve to misfire. Overheating of the solenoids might cause the valve to stick and miss a valve movement. If this is done only once in a long-term optimization, the reagent or sample could potentially be diluted with a large quantity of wash, for example, thus diminishing the validity of all future runs. This problem is not as
apparent in flow injection systems in which contamination of the sample or reagents
could only occur if a pump flow reverses inappropriately. Safety checks are built into
the software for the multi-position valves which are hard-wired with a feed-back loop
allowing the computer to check if the valve has arrived in the correct position before
executing a pump movement. At this point the software can stop further execution of
the experimental runs, and signal the user to provide maintenance for the
malfuntioning valve. However, while this solution works well for all three multi-position
valves, it is difficult to implement on the small solenoid mini-valves since it is difficult
(but perhaps not impossible) to physically determine whether or not they have opened
after application of the appropriate voltage. Appropriate inclusion of either an
inductance coil on the solenoid of the mini-valve, or a miniature flow meter near the
mini-valve would potentially enable the software to sense whether or not the valve has
fired at the appropriate time.

2.2.3 Pumps

2.2.3.1 Syringe Pumps

It has been argued [26, 28] that the important characteristic of a liquid propulsion
system is repeatability, and not necessarily linearity. Reproducibility of flow rate is
especially critical for sequential injection analysis where it is the reproducibility of the
flow rate which determines the reproducibility of the sample and reagent injection
volume. To this end, a sinusoidal syringe pump was manufactured and distributed by
Alitea for these systems in an attempt to obtain the most *repeatable* flow rate possible. The reciprocating cam-mechanism of one of these pumps is such that the syringe moves with the y-component of a circular motion. This produces a flow rate that follows a sine function with a frequency equal to the frequency of the rotating pump head according to

\[ y = a \sin(\omega t) \]

where \( y \) is the syringe position, \( a \) is the radius of the pump head (cm), \( \omega \) is the pump head frequency (rad s\(^{-1}\)), and \( t \) is the time (s) since the syringe was empty.

These sinusoidal pumps were the first devices on the market to address the needs of the new sequential injection analysis technique. Several other pumps which achieve the necessary precision offering linear flow are now being manufactured. The newer pumps operate with stepper motors and screw drives, which were initially avoided due to their presumed imprecision.

A great deal of work was done in this project in an attempt to improve the general characteristics of the Alitea sinusoidal flow syringe pumps. Initially, the possibility of (1) linearizing the sinusoidal flow, and (2) operating the pump by tracking its absolute movement instead of by timing was investigated. It was thought that this could be done with a continuous loop feedback control. A 10 cm linear potentiometer was mounted on the side of the syringe pump so that the potentiometer could track the
position of the syringe head. This method of measuring the syringe position was accurate to ca. 10 \( \mu \)L for the following reasons. The linear potentiometer returned a potential difference from 0 to 10 volts depending on the position of the syringe. This was converted by the DACA board into a signal with 12-bit precision, but since the potentiometer was approximately twice as long as one full syringe stroke of 5.0 mL, the precision of measurement was reduced by one half:

Equation 2-2

\[
\frac{1 \text{ stroke}}{\frac{1}{2} \times 2^{12} \text{ ADC units}} \times \frac{5000 \ \mu \text{L}}{1 \text{ stroke}} = \frac{2.4 \ \mu \text{L}}{\text{ADC Unit}}
\]

Still greater imprecision is created since the software loop cycled only fast enough to read the potentiometer ADC value at a resolution of about 3 to 4 units corresponding to about 7.2 to 9.6 \( \mu \)L. This is not precise enough for accurately dispensing solutions which are sometimes as low as 10 to 20 \( \mu \)L. This uncertainty can be reduced by changing the size of the syringe to 1 mL (a factor of 5) and by changing the size of the linear potentiometer (a factor of 2), but a smaller syringe size limits the flexibility for optimization purposes (less volume can be pumped per stroke). Electronically scaling the output of the linear potentiometer to provide a better match for the analog input channel would also conceivably improve the precision by a factor of two.

The pump control unit sends a voltage signal to the pump motor which has been damped by capacitors. This creates a significant hysteresis effect in the pump circuitry, thereby causing a delay in the change of pump motor speed. Figure 2-5 shows the
voltage sent to the control unit as a step function over time (0V to 10V to 0V) and the voltage received by the pump motor (after signal processing in the control unit). As shown, the hysteresis effect is significant, indicating recharge times as high as 10 seconds. This eliminates the possibility of instantaneous flow rate change during or between sequential injection steps. The same effect will occur for the peristaltic driven pumps manufactured by Alitea, since the same pump motor and electronics are used.

![Figure 2-5. Plot of voltage sent to the control unit versus voltage received at the pump motor as a function of time.](image)

From the foregoing, it was concluded that the control software must allow the pumps sufficient time to power up. An instantaneous request for a change of flow rate between SIA method steps would produce inaccuracies in fluid movement as the pump adjusted to the new speed over a finite length of time. Additionally, the pumps were
subsequently operated based on time and not absolute displacement (although the latter would still be possible with the inclusion of a more sophisticated tracking device). No physical changes were made to the Alitea pumps other than mounting a linear potentiostat on the outside of the housing to track the absolute position of the syringe.

### 2.2.3.2 Peristaltic Pumps

The peristaltic pump is by far the most common pump used for continuous flow methods of analysis. While this type of pump dominated flow injection systems worldwide, it was initially thought that it would be unable to provide the necessary flow rate precision required for hydrodynamic injection by SIA. Today, however, the peristaltic pump is commonly used in sequential injection systems too; they are capable of producing the necessary precision if the pump head frequency is reasonable compared to the aspirated volume [28]. On this system, the minimum pump speed allowed is one-quarter of the maximum speed for the pump, at which the relative standard deviation of replicate peaks is generally not compromised.

Peristaltic pumps require periodic recalibration in order to minimize the effects of flow rate drift due to tube wear; this is especially true if long-term optimization programs are to be run. The software automatically recalibrates the pump after a user-specified number of runs have been made (see the "Priming and Calibration" section later in this chapter). The length of pumping time necessary to achieve a precise calibration was investigated. Table 2-3 shows data from the automated calibration of the peristaltic
pump (at a relatively low flow rate) with the use of 0.76 mm i.d. pump tubing. The pump was calibrated by pumping carrier solution (e.g., 1.0 M KCl) through the reaction coil, through a six-position or ten-position valve, and then into a plastic bottle resting on a digital balance (accurate to less than a milligram). The software determines the mass of the container on the balance (through an RS-232 interface port) before and after running the pump at a user-specified rate for a user-specified length of time. Correction is made for the specific gravity of the solution used for calibration. In the case of this calibration experiment, the pump was operated at 50% of full speed, which corresponds to sending it a 12-bit digital signal of \((50\% \times 2^{12} - 1) = 2047\). The calculated minimum flow rate, \(Q\) (mL min\(^{-1}\)) is the projected flow rate at 25% of the maximum pump speed. This lower limit was chosen in order to ensure a relatively high pump head frequency is obtained for the desired flow rate (i.e., less pulsation and greater precision) and will be discussed in more detail in Chapter 3.
Table 2-3. Peristaltic pump calibration data indicating a lower standard deviation when longer calibration times are used.

<table>
<thead>
<tr>
<th>Calibration Time (s)</th>
<th>DAC / Q</th>
<th>Rep 1</th>
<th>Rep 2</th>
<th>Rep 3</th>
<th>Rep 4</th>
<th>Rep 5</th>
<th>Average</th>
<th>Relative Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAC / Q</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAC-units</td>
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<td></td>
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<td></td>
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<tr>
<td>mL min^-1</td>
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<td>mL min^-1</td>
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<tr>
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<tr>
<td>Q_min</td>
<td></td>
<td>300</td>
<td>0.36</td>
<td>0.36</td>
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<td>Q_max</td>
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<td>300</td>
<td>1.46</td>
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<td>1.45</td>
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<td>1.47</td>
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The results in Table 2-3 indicate that little improvement in flow rate calibration precision is achieved after 120 seconds. Improved relative standard deviation would be expected at lower calibration times for tubing with higher flow rates due to the relative increase in mass difference. For the majority of this work, 120 seconds is the default setting for calibration time.

2.2.4 Detectors

Virtually any type of detector which outputs an analog signal can be interfaced to the SIA software provided that a sampling frequency of 10 Hz is sufficient. The software is able to acquire input from two analog channels of either 0 to 10 V or -5 to +5 V (set by DIP switches on the acquisition card).

For some studies, a simple log-amp photometric detector incorporating a quartz halogen light source with interference filters and axial geometry flow cell was used and is described elsewhere [24]. The majority of the studies made use of a diode array spectrophotometer (HP8452, Hewlett-Packard, Palo Alto, CA, USA) capable of 2 nm resolution from 190 to 820 nm, with a single wavelength acquisition rate of 10 Hz. Simultaneous monitoring of up to six wavelengths on the spectrophotometer is possible, however, only two wavelengths were transferred to the SIA control computer through the DACA interface (see Figure 2-1). All data were acquired by the SIA control computer at 10 Hz.
2.3 Sequential Injection Analysis Software

It was realized from the outset that there is an inherent need for more sophisticated software in order to perform sequential injection analysis with any degree of competence [20]. It was hoped that this would not be a significant deterrent in the development and widespread acceptance of this technique. The issue of sophistication advances further if we desire the software to be able to empirically optimize a given sequential injection procedure. This portion of the project addresses the issue of software sophistication while making the human-instrument interface easier to use and more easily understandable than any of its predecessors.

2.3.1 Programming Graphical User Interfaces

In the late nineteen eighties and early nineteen nineties a major software revolution occurred for IBM-compatible personal computers (IBM-PC). This transition from DOS-based software to more graphically oriented Windows-based software occurred relatively quickly and with it came the necessary tools to program sophisticated graphical user interfaces. Microsoft Visual Basic and Microsoft Visual C++ or QuickC have been the most popular programming software packages available. While Microsoft Visual Basic (using standard Basic language conventions) is faster and easier to program [21, 23], the software written in the C-language is lower level, thus making the final application ultimately run faster. The Microsoft Visual Basic language was chosen in order to speed up application development, and any deficiencies in
operating speed of the finished product can be compensated for by use of high-end computer hardware which very recently became inexpensive.

In traditional BASIC programming, it is the program which controls execution of the subroutines; this is known as procedural programming. In Visual Basic, the software is considered to be event-driven, in that the user decides on the events which occur. The software presents a list of choices to the user through a graphical representation of text-boxes, buttons, graphics, and so-on, and waits for the user input. The user then decides which event is necessary to run now and activates it by either keystrokes or mouse clicks. When an event occurs (such as a mouse click) the software then executes an event procedure, a list of instructions to carry out upon acknowledgment of a specific event. It is for this reason that the user has more control over the application which leads to greater control over the hardware which is interfaced to the computer.

By moving to an event-driven programming language such as Microsoft Visual Basic, programming of the instrument by the end-user is simplified considerably. With a Graphical User Interface (GUI), virtual instruments [22] can be created and manipulated on a computer screen with mouse movements. As well, the operator is able to see the status of the hardware from a concise diagram of it on the computer screen, and the operator is able to choose and change physical settings on the instrument simply by pointing to the appropriate pump, valve, detector, or accessory on the computer screen. This is the most significant step towards simplification of a
complex programming situation, and through this substantial simplification of the interface, it is hoped that the ultimate user will be able to better focus their attention and intellect on the chemical analysis method they are trying to develop, rather than programming of the software.

2.3.2 Flow Injection Development and Optimization System

Regardless of how open-ended and flexible a hardware/software system is designed to be from the outset, it inevitably has its experimental limitations. Advanced flow injection systems used for laboratory research purposes such as the Flow Injection Development and Optimization (FIDO) system [10, 18] are no exception. The FIDO system consisted of up to nine pumps, four valves, photometric and electrochemical detectors, and sophisticated software routines for control. The software (written in Microsoft QuickBASIC version 4.0) was designed to automatically develop and optimize FIA methods by either or both of simplex optimization techniques and response-surface mapping.

The FIDO software is a cornerstone to optimization software for flow injection systems and is therefore, the starting point for development of the current sequential injection software primarily due to the similarity in hardware and interfacing circuitry. Some of the lower level FIDO control routines have been copied and then modified for current use. The objective was to develop a finished software product which removed
the complexity of the programming environment from the user, but allowed extreme
flexibility in flow programming for a very wide range of chemical analyses.

2.4 **Graphical User Interface (GUI) for SIA**

The objective of this portion of the project was to create a software environment
which overcame many of the limitations of previous flow optimization software and met
the following requirements:

- *Flexible:* the user should be able to instruct the software to perform a variety
  of different functions, experiments, optimizations, pertaining to research into
  SIA methods development. As such, the software is well suited to a research
  and development environment.

- *Graphical:* the set-up of complex methods, and optimization of system
  parameters should be simplified with a comprehensive graphical user
  interface.

- *Modular:* portions of the code should be able to be added or removed
  quickly and easily to modify the functionality of the instrument (e.g., adding
  new types of pumps or valves).
• **Efficient:** experiments, manifolds, and optimizations should be easy to modify or reuse.

• **Database access:** the large amount of data created should be stored in an expandable, comprehensive database for future reference.

2.4.1 Program Overview

The software programs and files with their respective versions and authors or manufacturers used for this system are listed in Table 2-4. All of the interface was written in Microsoft Visual Basic Professional Version 3.0. A dynamic link library (DLL) file, "VBIO.DLL", written in-house in Microsoft QuickC for Windows, facilitated input and output functions for the IBM DACA boards. Microsoft Visual Basic provides custom controls that are used to access the database which is administered by Microsoft Access. However, a program (available from Microsoft Bulletin Board) called "COMLAYER.EXE" must be run which installs a "Compatibility Layer" between version 3.0 of Microsoft Visual Basic and version 2.0 of Microsoft Access. Without this program, Microsoft Visual Basic is only compatible with version 1.1 of Microsoft Access. Microsoft Query is used to search for and transfer data from Microsoft Access to Microsoft Excel for further processing. Microsoft Excel is used for plotting two-dimensional graphs and three-dimensional response-surface maps for visualization of the data.
Table 2-4. Software programs or files used to implement sequential injection analysis software.

<table>
<thead>
<tr>
<th>Program / File</th>
<th>Latest Version Used</th>
<th>Manufacturer / Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsoft Visual Basic for Windows, Professional</td>
<td>3.0</td>
<td>Microsoft Corporation Redmond, WA, USA</td>
</tr>
<tr>
<td>Microsoft Access</td>
<td>2.0</td>
<td>Microsoft Corporation Redmond, WA, USA</td>
</tr>
<tr>
<td>Microsoft Excel</td>
<td>5.0a</td>
<td>Microsoft Corporation Redmond, WA, USA</td>
</tr>
<tr>
<td>Microsoft Query</td>
<td>1.0</td>
<td>Microsoft Corporation Redmond, WA, USA</td>
</tr>
<tr>
<td>Central Point Icon Editor</td>
<td>2.0</td>
<td>Central Point Software Beaverton, OR, USA</td>
</tr>
<tr>
<td>VBIO.DLL</td>
<td>1.0</td>
<td>Ivan Brock, Graduate Student UBC, Vancouver, BC, Canada</td>
</tr>
<tr>
<td>COMLAYER.EXE</td>
<td>1.0</td>
<td>Microsoft Corporation Redmond, WA, USA</td>
</tr>
</tbody>
</table>

Figure 2-6 shows an outline of the sequential injection analysis software system. There is a parent Multiple Document Interface (MDI) form (top box in diagram) which serves as a container for all other forms†. The other boxes in the diagram which are connected with plain lines are the other most significant forms of the system. The lines with double-headed arrows indicate storage and retrieval of data from the indicated files or tables in the database. Manifold, calibration, bottle content and peak data files are stored as ASCII text files where the extension "xxx" represents a number from 001 to 999. Method and icon files are stored as binary files.

† The term “form” refers to a window or frame on the computer screen (or display) that is customized as the interface of the application. Input boxes, graphics, and pictures are included on a form to give the form a specific functionality.
Figure 2-6. Overview of SIA software showing the most significant forms and how the data are stored and retrieved from disk.

2.4.2 Parent Multiple Document Interface

Figure 2-7 shows the main Multiple Document Interface (MDI) form. This serves as the outermost shell within which all other forms are contained. The only menu for the system appears at the top with a buttonbar for all of the primary forms. Any forms which are active but minimized appear as icons at the bottom of this main form. Scroll controls are available for the method step number and the number of replicates can be set. The form can be sized appropriately, as well as minimized to the desktop to allow the user to perform other functions on the computer such as file management, data management, or report writing while the SIA system is not in use.
2.4.3 The Virtual Manifold

The most significant form of the SIA software system is the "Manifold" form. In addition to allowing the user to program all of the devices interfaced to the computer, it shows a real-time graphical status of the entire system. On this form, shown in Figure 2-8, the syringe positions and the valve positions change in concert with the actual device (as a result of feedback loops at the interface level). Since "a picture is worth a thousand words", the user is better able to visualize the status of the system (e.g., the position of the pumps, the connections made with the multi-position valves, etc.) from a form of this nature rather than a form full of words and numbers. This is especially important when the possible combinations of valve positions increases substantially. For example, while a mere 216 combinations of valve positions are possible with the
manifold shown in Figure 2-8, the entire system is capable of 2160 unique valve position combinations. This would be difficult to comprehend if it were not for a graphical user interface of this nature which allows the user to trace out the tubing connections made by the valves at any given time. As well, the user can change these connections and instrumental settings by simply pointing to a new valve position, for instance, and clicking the mouse. If necessary, this form can be sized to only include one pump and one valve, or other valve types (e.g., the ten-position valve) can be added. Tubes, detectors, and accessories can be dropped into place from the icon form to build the virtual instrument on the screen.

Figure 2-8. Sequential injection manifold form showing controls for two pumps, graphics for two syringes, two six-position valves, a three-position mini-valve (on top of left syringe), a two-position mini-valve (on top of right syringe), and controls for acquisition, delays, and a stir cell.
2.4.4 Viewing, Editing, and Using Icons

The icon viewer form presents the "building blocks" of a manifold. The virtual manifold is constructed from icons placed on a grid. The user can scroll through directories of different tube shapes (elbows, T-pieces, etc.) and accessories before selecting the appropriate fitting to be picked up and dropped into place on the manifold form. If a piece is required that does not yet exist, the user can double-click on a similar icon (if there is one), which activates an icon editor program (Central Point Icon Editor, Central Point Software, Beaverton, OR, USA). The user can then edit the existing icon (or start with a blank screen) into the desired piece, name the new icon, and save it to disk for future use. The new icon will be displayed in the icon viewer form and can be used in any subsequent virtual manifold.

Figure 2-9. Icon viewer form which is used to scroll through various tubes and accessories which are used to build a virtual manifold on the screen.
2.4.5 Designing a Manifold

Designing a manifold is a relatively straight-forward process. However, the user must decide on the most efficient use of the manifold form for two reasons. First, due to limitations in system resources inherent to Microsoft Windows, the grid has a maximum size of 17 icons across and 8 icons down (136 in total). Second, at this time, the pump and valve positions are fixed, thus limiting the number of connections or accessories that can be shown around these devices. Future versions of this software will likely include more flexibility in their positioning.

Once the physical hardware on the bench is in place, the user then constructs the virtual manifold on the screen, keeping in mind the above limitations. Figure 2-10 shows an example of a completed manifold used in the determination of phosphate. Labels with arrows have been added for explanation purposes only and do not appear on the manifold normally. Reagent 1 (R₁) is ammonium heptamolybdate, Sample (S) is a phosphate sample, and Reagent 2 (R₂) is ascorbic acid. A syringe pump is represented by the tall black rectangle which slowly fills with blue as the syringe fills with solution. The syringe is currently in its fill mode (arrow below syringe box is pointing down), and connected through the three-position mini-valve to the reaction coil, which is connected to an H₂O bottle connected to the six-position valve. In the example shown, the pump has been instructed to aspirate (draw solution into itself) at 2 mL min⁻¹ for 30 seconds. This is illustrated by the arrow below the syringe window which is pointing down. The detector is represented by a light source and light sensor,
the waste container is represented by a jerry can, and the sample and reagent containers are represented by brown glass bottles.

![Diagram of a virtual manifold for the determination of phosphate.](image_url)

Figure 2-10. A virtual manifold for the determination of phosphate.

When bottle icons are dropped onto the manifold, the software prompts the user for a bottle number which is used to keep track of the bottle contents. On a separate form called bottle contents, the reagent and sample names can be recorded for each numbered bottle. Thus, when the user clicks on a bottle on the virtual manifold, the contents of the bottle are displayed in the title bar.

The pump, detector, stir cell, and delay time settings can all be adjusted by means of their horizontal scroll bars. The valve positions can be adjusted by clicking on the line that the user would like the valve to move to. For instance, by clicking on
the line to the right of the detector, the virtual manifold will immediately redraw itself showing the new connection of the reaction coil connected through to the detector. At the same instant, the real valve on the bench moves to the position which connects it to the detector.

Thus, all of the pumps, valves, and accessories which comprise the analyzer can be quickly and easily manipulated from the virtual manifold. Once drawn, the manifold can be recorded to disk as an ASCII "*.man" manifold file for future retrieval.

2.4.6 Recording a Method

For method recording purposes, the method editor form (Figure 2-11) is used. When a given step of the method is set up on the virtual manifold, the numerical values for all of the settings can be transferred onto the method editor form by pushing the "Get" button on the lower right corner. A comment which describes the current step (e.g., "Aspirate reagent one for 10 seconds at 2 mL min⁻¹") can be recorded in the text box appearing above the control buttons. If desired, the current method step can now be manipulated numerically through this form, instead of graphically through the virtual manifold.

As many steps as necessary can be added to a given method simply by pressing the "Insert" and "Delete" buttons. The "Get" and "Send" buttons transfer the settings from and to the virtual manifold. The resulting multi-step method can be stepped
through by pressing the up or down step number arrow on the parent MDI form (Figure 2-7, top left corner). After the user has ensured that the method will execute as desired (by watching the instrument step through the procedure), the method can be saved to disk for future retrieval as a binary "*.met" method file.

Figure 2-11. Method editor form showing settings for two pumps, two miniature solenoid valves (mini-valve one and two), two six-position valves (multi-valve one and two), one ten-position valve (multi-valve three), as well as time settings for stirring, acquisition, and delay.

A method for the colorimetric determination of phosphate is shown in Figure 2-12 as an example. Each manifold configuration for each of the five steps of the method are shown in sequence. The user would see only one manifold which would automatically change as the user executed the method. The syringe initially loads a large volume of distilled water (30.0 seconds at 2.000 mL min⁻¹) from the bottle connected directly to the mini-valve at the end of the syringe (Figure 2-12a). The mini-valve is then switched to the reaction coil which is connected to the six-position valve
(Figure 2-12b). The six-position valve is simultaneously connected to the ammonium heptamolybdate bottle. A small quantity of this solution is aspirated (3.0 seconds at 2.000 mL min\(^{-1}\)), before the six-position valve is switched to the phosphate sample. The same volume of phosphate is aspirated (Figure 2-12c), as is the second reagent, ascorbic acid (Figure 2-12d). Finally (Figure 2-12e), the pump direction is reversed (arrow below the syringe), and the six-position valve is connected to the flow-through detector. The syringe is operated for a maximum of 60.0 seconds (although it will automatically stop when empty), and the detector is monitored for the same length of time. The solution in the reaction coil passes through the detector and into the waste container (jerry can).

In this example, the sinusoidal syringe pump is used, and therefore the flow rate is non-uniform. The 2.000 mL min\(^{-1}\) reading on the manifold refers to the flow rate encountered at maximum amplitude. The flow rate decreases at each end of the syringe stroke but since only 60 degrees of pump rotation are used (versus 180), the flow rate only decreases by ca. 14% from this value. For this reason, the top scroll bar in the pump one frame (indicating volume) is not used. If a linear flow pump was used (i.e., peristaltic) the scroll bar would indicate the volume corresponding to 30.0 seconds at 2.000 mL min\(^{-1}\) (i.e., 0.500 mL).
Figure 2-12a-e. A method for the determination of phosphate is shown as an example. In (a), the syringe draws in water from the bottle to its left for thirty seconds. Then, the syringe is connected to the reaction coil through the mini-valve and a sequence of (b) ammonium helptamolybdate, (c) phosphate sample, and (d) ascorbic acid are sequentially aspirated for three seconds each into the reaction coil. Finally, in (e), the six position valve is connected to the detector channel and the solution is expelled through the flow cell to waste while acquiring the detector signal for sixty seconds.
2.4.7 Manifold Priming and Pump Calibration

In FIA, the pump(s) generally need to be run uni-directionally for a length of time long enough to flush any air bubbles out of the manifold. In SIA, however, the priming procedure is more intricate. To prime a multi-position valve such as the ten-position, or one of the six-position valves, the priming and calibration form is used as shown in Figure 2-13. The valve number (1, 2, or 3) is selected and the user decides whether to either (i) load on one line and empty on many, or (ii) load many lines and empty on one. The first case would be desirable if the user wanted to take wash or air into the reaction coil through one line and use it to flush out several other lines when shutting down the system. The second case would be desirable if the user wanted to prime several different lines with several different solutions by loading them each individually into the reaction coil and then expelling them to a waste line.

The selection of valve lines to use for loading and emptying can be accommodated by the form in the following way. The line numbers contained within the "Load" frame on the form allow only one line to be marked at a given time. If the user clicks on line four for example, line one would be deactivated and line four would be marked for loading. In the "Empty" frame, any number of valve lines can be marked (or "checked") for emptying on, and the pump will aspirate on the load line and empty on each empty line sequentially. If the user wishes to perform option (ii) above, clicking on the "Switch" button, will reverse the load and empty frames allowing loading on many lines and emptying on only one. The volume aspirated in each priming cycle is
determined by the "Prime Time" box which indicates the length of time in seconds that the pump should aspirate at the maximum priming speed available (set elsewhere). Multiple priming cycles on the same line can be done (by setting the number of replicates) if the line to be primed is long relative to the reaction coil line; this ensures that the line being primed is completely flushed with its respective solution without fear of contamination of the pump.

Figure 2-13. Priming and calibration form showing controls for specific valve and pump to prime and calibrate.

If a syringe pump is used, then in addition to priming the valve, the entire syringe must be flushed and climatized to the wash solution with all air bubbles removed. This is often difficult to do when the syringe is directly connected to a reaction coil that is of comparable volume to the syringe itself. It was decided, therefore, to connect the mini-
valves (with two or three exit ports) directly to the end of the syringes for most applications. This allows faster priming of wash solution which can be aspirated from a short line going directly into a wash bottle, thereby bypassing the reaction coil completely.

The calibration portion of the form is used for calibrating the peristaltic pumps. The calibration speed is chosen at half of full speed \( (\frac{1}{2} \times 2^{12}) - 1 = 2047 \) for at least 120 seconds as shown earlier (Table 2-3). The valve line box indicates the line which is connected to a digital scale such that the pump expels fluid into a container sitting on the scale platform. The software reads the digital scale to obtain an initial weight, the pump operates for the specified length of time, and the software then calculates the flow rate (at half speed) by mass difference. Different densities in wash solutions can be accommodated by the value in the specific gravity box. The minimum flow rate box is calculated based on one-quarter of full pump speed in order to minimize pulsing and to avoid any non-linearities and non-zero intercepts in the pump speed calibration which occur at low frequencies. The user can then specify the number of runs between calibrations, as appropriate, in order to adjust for any flow rate drift occurring over extended lengths of operation; this is particularly useful for long-term optimizations which are sensitive to flow rate.
2.4.8 Summary Form

An example record is shown with the experimental summary form in Figure 2-14. All of the displayed data are stored in an expandable database. The current record number, 7275, is the 68th peak of an optimization. This is the second replicate of an optimization using the method "Fe_P2_10.met" combined with variable record number 31, in which variable 1 is 9.6 seconds and variable 2 is 2.4 seconds. The peak data, stored as an ASCII file under the name shown, is displayed in the bottom right window which can toggle as a text comment editor (for the file "Fe_D1.txt") for the experiment.

![Experiment Summary Form](image)

Figure 2-14. Experimental summary form which displays all of the important experimental data for each run.
2.4.9 Automated Optimization

Another objective of this work was to simplify the setup procedure for investigation of any of the sequential injection variables for optimization and method development. To set up an optimization, the following procedure is used:

1. Load or design the manifold and the method to be optimized.

2. Scroll to the *instruction step* of the method which is to be optimized.

3. Switch to the method editor form.

4. Double-click on the *parameter* in the current step which is to optimized (e.g., pump one time, valve two position, etc.)

5. The variables form, Figure 2-15, will appear which lists all of the related information about the parameter to be optimized (top box).

6. The user can then enter the boundaries for that parameter in the current instruction step.
Figure 2-15. Variable record form showing record number 31 which involves two variables.

The form in Figure 2-15 shows the parameters for a two variable optimization. The two variables shown are the length of time for pump one to operate when instruction number one and two (out of three) are executed. In successive runs, during instruction number one, pump one will operate for 2.4 to 14.4 seconds in 2.4 second increments (six levels total), while during instruction number two, pump one will operate for 0 to 14.4 seconds in 2.4 second increments (seven levels). This amounts to 42 individual experiments to be mapped. This form can expand to the right to accommodate as many as five variables, or variables can be individually deleted.
Once the variable is set up, it is recorded in the experimental database as a record (variable record number 31 in the example shown) which can be later retrieved with either the same method or used on a different method. This eliminates redundancy in record keeping if the same parameters need to be optimized in several different methods. A realistic maximum of five variables has been set in order to simplify programming.

After the variables have been defined, the user must set the number of replicates of each method to run (on the MDI parent form, Figure 2-7), and switch to the experimental summary form (Figure 2-14). By pressing "New" on the experimental summary form, the system moves to the end of the experimental database and creates a new blank record. The user then optionally enters the common data that will appear in each record, such as number of variables, concentration units, acquisition frequency, data units, operator, $C^0$ value, variable record number, and all of the path and file information for recording results. Then, by pressing the "Fill" button, new records are created, one for each experimental run, which contain the relevant information for performing all of the experiments. For example, if variable one had six steps, variable two had seven steps, and three replicates of each method were desired, the total number of new records created would be $6 \times 7 \times 3 = 126$. In this way, three replicates of each of the seven levels of variable two would be recorded for each of the six levels of variable one. As the records increase, the variable values are incremented, the peak number is incremented, the replicate number is incremented, and the extension of the peak data file name is incremented numerically to match the peak number of the
database (peak number 68 is shown in Figure 2-14). At this point, the database has been primed with the relevant information to run each experiment and record the results, such as the date, time, peak height, peak area, baseline, time to peak maximum, peak moments, and the entire peak data file which contains the digitized detector response.

To run the experimental records, the user must simply point the system to the starting record number, specify which record to stop on, and request that the system begin experiments. The system then reads each experimental instruction from the database, performs the method, records the results, and moves on to the next record much like a ticker-tape machine. By taking this organizational approach to record keeping, individual runs of an optimization experiment can be re-run at a later time, if necessary, to correct problems that are encountered such as air-bubbles or running out of reagent. This was impossible in the previous FIDO software for example, where the entire optimization had to be restarted from the beginning.

2.4.10 Data Analysis and Representation

There are several efficient Windows-based software programs now available for manipulation of data which are recorded in database format. These highly integrated software packages simplify processing of the data in the native database (e.g., Microsoft Access Version 2.0) or after transferring to a spreadsheet (e.g., Microsoft Excel Version 5.0a). Transferring the data to the spreadsheet is done via a “Query”
which can be performed on the database files (using Microsoft Query Version 1.0) in order to efficiently extract only the necessary data for further numeric processing or for graphical representation (including three dimensional surfaces). With all of these integrated tools commercially available, it was deemed unnecessary to incorporate any advanced data analysis or graphical analysis subroutines into the sequential injection software.

2.5 Conclusions

A new design for a sequential injection analysis optimization system has been proposed and implemented with a Windows-based graphical user interface. The unique virtual manifold used for system control and programming makes a significant step towards improved control over these new systems. This development of virtual instrument control was deemed necessary from the outset due to the complexity of optimizing a sequential injection method. As anticipated, the interface has performed well, and over 10,000 experimental runs have been recorded to date, with no notable problems.

The design and implementation of this hardware/software system is the first step in the body of this work. The advantages of such a system will now be exploited in the rest of this thesis. In Chapter 3, the results obtained by using this analysis system to map out the effects of the most significant parameters affecting dispersion will be discussed. In Chapter 4, the dispersion profiles described in Chapter 3 will be used to
explore the concept of zone penetration in a sequential injection analysis. The data reported in Chapter 3 will also be used for comparison with the random-walk model for sequential injection analysis proposed in Chapter 5.
2.6 References


3. Experimental Design and System Characterization

"When the only tool you have is a hammer, every problem begins to resemble a nail."

Abraham Maslow

3.1 INTRODUCTION

The first comprehensive set of experimental data produced on the sequential injection analysis system that is described in Chapter 2 encompasses an empirical investigation of the instrumental parameters thought to be most influential on peak shape [1-10]. These parameters include the injection volume, flow rate (i.e., linear flow), valve-to-detector distance, and manifold configuration (straight or coiled). The influence of the flow reversal [3-8] is also investigated by considering its effect on peak shape for several reversal lengths (I) and for multiple reversals (n). Typical values of the above parameters for sequential injection analysis are investigated with the use of the automated optimization routines of the analyzer by injection of a tracer dye. In this way, the influence of dispersion (without chemical reaction) and the effect of the system parameters on the peak shape are investigated and stored in a database. Information
from this dataset is used to (i) ensure the analyzer is operating as anticipated, (ii) demonstrate the ability of the analyzer to automatically and independently investigate several operational parameters, (ii) characterize the system response, (iii) understand the factors influencing peak shape to a greater degree, (iv) optimize zone penetration (Chapter 4), and (v) provide a comparison with peak profiles produced with the random-walk model (Chapter 5).

3.1.1 Peak Descriptors

Peak descriptors can be used to numerically evaluate the differences in peak shape caused by variation of the instrumental parameters. Besides the usual peak shape descriptors of peak height, peak area, and time to maximum peak height, statistical moments can be used to evaluate a digitized peak profile [13-20]. Moments have been used for peak shape evaluation in non-segmented flow systems on several occasions, and have been used to an even greater extent for chromatographic peak shape analysis [13-14]. The zeroth and first statistical moments can be calculated as

\[ M_0 = \int_{t=0}^{\infty} y(t) dt \]

\[ M_1 = \frac{\int_{t=0}^{\infty} t y(t) dt}{M_0} \]
where $M_0$ and $M_1$ are the zeroth and first ordinary moment, respectively, and $y_i(t)$ is the value of the function at time $t$. The second and all higher central moments can be calculated once the zeroth and first ordinary moments are known according to

$$M_n = \frac{\int_{I=0}^{\infty} (t_i - M_1)y_i(t)dt}{M_0}, \quad n \geq 2.$$  

In general, all odd higher moments characterize peak asymmetry while even higher moments characterize peak broadness. It should be noted that moments higher than $M_3$ and $M_4$ become increasingly sensitive to both noise and inaccuracies in determination of the peak truncation points.

In this work, the summation method [15] is used for determining the statistical moments by adding small vertical slices of the peak over the pre-determined integration limits according to

$$M_0 = \sum_{all \ i} y_i(\Delta t)$$  

$$M_1 = \frac{\sum t_i y_i}{M_0}$$  

$$M_n = \frac{\sum (t_i - M_1)^n y_i}{M_0}, \quad n \geq 2$$
where $\Delta t$ is the data acquisition period (0.1 s in this work), $y_i$ is the detector response in the $i$th interval, and $t_i$ is the time in seconds of the $i$th interval. The value of $i$ includes all data points between the beginning and the end of the peak profile. The method used for calculation of peak start and stop points is discussed below.

The derived functions of skew (measure of asymmetry relative to a Gaussian curve) and excess (measure of "flatness" relative to a Gaussian curve) can be calculated according to

Equation 3-7

$$S = \frac{M_3}{M_2^{3/2}}$$

and

Equation 3-8

$$E = \frac{M_4}{M_2^2} - 3$$

where $M_2$, $M_3$, and $M_4$ are the second, third, and fourth moments respectively. A full Gaussian curve has skew and excess values of 0. For reference, one half of a Gaussian curve, bisected vertically, has a skew of 0.995 (Figure 3-1) while that of a right triangle has a value of 0.566 [18]. Similarly, one half of a Gaussian curve, bisected horizontally based on equal area (lower half) has an excess of 1.377 while that of a square profile has an excess of -1.200.
Figure 3-1. Half Gaussian peak profiles (shaded area) bisected (a) vertically and (b) horizontally.

It is imperative that the zeroth and first ordinary moments be calculated accurately because of the dependence of the higher moments on these values. The zeroth and first moments express the peak area and peak centroid respectively. These moments are calculated using the data points which fall within the peak truncation limits, where the peak starting point is taken as time zero. Others have considered the effects of peak truncation on moment calculations [18-20], and have stressed the importance of obtaining reliable integration limits, especially for the higher order moments which puts a disproportionately higher weight on data which is furthest from the first statistical moment. In a digitized strip of data, peak start and stop points can be determined either from the change in slope of the data set or from when the signal exceeds some threshold value such as 10%, 1%, or 0.1% of the peak height or maximum scale reading. In this work, it was found from manual inspection that the most reliable results were obtained from peaks which were considered by using a combination of these two techniques. In other words, the software most accurately predicted the start and stop position of the peak profile (in all cases which were visually...
examined) by using the following method. The time \( t_i \) in the dataset was taken as the peak *starting* point if it satisfied the following criteria:

1. The value \( t_i \) must occur before peak maximum.

2. The slope of the signal calculated by subtracting \([(y_{i-1} + y_i) / 2]\) from \([(y_{i+1} + y_{i+2}) / 2]\) and dividing by 0.2 s, must be *more than* 0.7 ADC units per second. The value \( y_i \) is the 12-bit analog-to-digital signal at time \( t_i \), and 0.7 ADC units is the standard deviation of the baseline signal for the current system using the diode-array spectrophotometer, expressed in analog-to-digital conversion units.

3. The difference between \([(y_{i+1} + y_{i+2}) / 2]\) and the average baseline signal \((n = 40)\) must be greater than 0.2% of the full-scale reading.

Similarly, the time \( t_i \) in the dataset was taken as the peak *ending* point if it satisfied the following criteria:

1. The value \( t_i \) must occur after peak maximum.

2. The slope of the signal calculated by subtracting \([(y_{i-1} + y_i) / 2]\) from \([(y_{i+1} + y_{i+2}) / 2]\) and dividing by 0.2 s, must be *less than* 0.7 ADC units per second. The value \( y_i \) is the 12-bit analog-to-digital signal at time \( t_i \), and 0.7 ADC
units is the standard deviation for the current system using the diode-array spectrophotometer, expressed in analog-to-digital conversion units.

3. The difference between \( \frac{[(y_i + y_i) / 2]}{2} \) and the average baseline signal \( (n = 40) \) must be less than 0.1% of the full-scale reading.

In this way, the steeper slope found at the beginning of the peak and the more gradual return to baseline at the end of the peak are more readily determined since they are considered under asymmetrical conditions. Several hundred peaks created under various conditions were manually inspected to ensure the accuracy of the peak truncation points as calculated by the above criteria. Excellent agreement was found in all cases although the recording of an air bubble passing through the flow cell produces unpredictable results. In addition, each of three replicate peaks created under identical conditions had computer-determined peak starting points which agreed within ±0.1 s of the average value and peak ending points within ±1.0 s.

It is expected for this system, that the zeroth moment (peak area) will be stable (i.e., replicate injection volumes should be precise), linearly proportional to injection volume (i.e., the zeroth moment is a measure of the number of injected molecules), inversely proportional to flow rate (i.e., the average length of time an individual molecule spends in the detector and contributes to the peak area decreases as the flow rate increases), and independent of manifold geometry (i.e., the total number of molecules passing through the detector is independent of the path traveled to get
there). As well, it is expected that the first moment (peak centroid) will be stable (i.e., the flow rate will consistently cause the centroid of the peak to arrive at the detector at the same time for replicate analyses), linearly proportional to injection volume (i.e., the length of time necessary for the centroid to arrive at the detector increases with the length of time necessary to inject the sample), linearly proportional to manifold length (i.e., increasing the manifold length requires that all of the molecules, on average, travel a further distance which takes a greater amount of time), and inversely proportional to flow rate (i.e., the velocity at which the peak centroid travels from the injection valve to the detector increases with flow rate and therefore the arrival time is proportionally decreased). Finally, it is expected that the second moment (variance) will increase exponentially with injection volume (i.e., the variance of a dispersed distribution profile increases exponentially as the width of the original distribution profile is increased), increase proportionally with manifold length (i.e., according to the random-walk model), and be inversely proportional to the flow rate (i.e., the peak area and, therefore, the width or variance of a dispersion profile decreases as the flow rate increase since the molecules are passing through the detection zone at a faster rate).

Confirmation of these assumptions will be investigated in this chapter in order to validate the utility of statistical moments as peak descriptors for quantifying dispersion profiles created by sequential injection analysis. This will also aid in quantifying the most significant parameters effecting peak shape created by this system. The possibility of using the higher order moments in representations such as skew and
excess for studying the effect of the flow reversal process on the dispersion profile will also be investigated.

3.1.2 Multiple Flow Reversals

The concept of multiple flow reversals for increasing dispersion without increasing the manifold dimensions has been introduced recently [4-5]. Using a flow injection manifold and methodology, it has been shown that the degree of dispersion, as measured by the dispersion coefficient, is linearly related to the length of the flow reversal, and by a factor of a square root with respect to the number of reversals [3]. This supports the applicability of the random-walk model (originally used by Giddings to explain the separation process in chromatography [21]) to "flow pulsing" in flow injection analysis [3]. The model (in its simplest form) is given by the equation

Equation 3-9

\[ \sigma^2 = nl^2 \]

or

Equation 3-10

\[ \sigma = n^{1/2}l \]

where \( l \) is the length of a given step, \( n \) is the number of steps taken, and \( \sigma \) is population standard deviation, which represents a relative spread or dispersion of the objects. In chromatography the objects are the molecules being separated, while in flow injection analysis the objects are the molecules dispersing within the manifold. Assuming that
the concentration gradient produced during the sequential injection process is negligible, it is expected that a similar relationship should hold between the variance ($M_2$ or $\sigma^2$) of a peak profile produced by the sequential injection method, the number of reversals ($n$) and length of the reversals ($l$). This will also be investigated in this chapter.

3.2 EXPERIMENTAL

3.2.1 Reagents

Dispersion experiments were done using a carrier stream of 1.0 M KCl. The non-reactive dye used to study the dispersion process was 1.5 mM K$_3$Fe(CN)$_6$ made up in 1.0 M KCl. All solutions were made up in MilliQ de-ionized water and sonicated for approximately 5 minutes to remove as much dissolved gases as possible (to reduce bubble formation during the reduced-pressure aspiration cycle). Any loss in volume during the sonication process was made up with the addition of more water. It was found that bubble formation within the manifold (caused by the reduced pressure during aspiration of solutions) could be eliminated to a large extent by raising all solution containers above the analyzer by ca. 100 cm. The constant head produced in this way was sufficient to minimize the deleterious effect of air bubbles on the dispersion profile.

A maximum absorbance of this dye in the visible range was found at $\lambda_{\text{max}} = 416$ nm. The molecular diffusion coefficient is taken as $7.6 \times 10^{-6}$ cm$^2$/s$^{-1}$ [22-23].
The linearity of the detector response within the range of concentrations investigated
on the system was determined by preparing 11 solutions ranging from 0.0 to 1.5 mM
K₃Fe(CN)₆ and measuring their absorbance by using the same flow cell as that used for
the dispersion studies. In order to determine the stability of the reagent for long-term
unattended optimization experiments, these same solutions (stored in screw-top glass
vials) were re-measured after a period of 1, 5, and 12 days.

3.2.2 Sequential Injection Manifold

A diagram of the sequential injection manifold employed for the dispersion
experiments is shown in Figure 3-2. The peristaltic pump was used instead of the
syringe pumps in order to obtain linear flow throughout the region of study. All manifold
tubing (except pump tubing) is flexible 0.84 mm internal diameter
polytetrafluoroethylene (PTFE) tubing (Cole Parmer, Chicago, Illinois, P/N 6417-31).
The distance from the valve to the pump tubing (Lᵥ) is large enough (200 cm) to
prevent any dye solution from reaching the pump tubing during any injection cycle. The
tube which connects the pump to the valve (Lᵥ) is connected to the central connector
on the 10-position valve (by flanging at the valve head). The flow cell is connected to
the valve through one of the ten selectable valve lines (position 4). Tubes connected
at valve position 1 and 3 (by flanging at the valve head) were 1.0 m long and lead to
the wash solution (1.0 M KCl), while position 2 leads to the dye solution (1.5 mM
K₃Fe(CN)₆). The tubing between the pump and the valve (Lᵥ) and the tubing between
the valve and the detector ($L_d$) was kept as straight as possible or coiled in around a 1.0 cm or 6.5 cm cylinder to produce different degrees of secondary flow.

A previously reported system [5] employed a larger diameter "holding coil" (50 cm x 1.32 mm i.d. = 684 µL) nearest the pump which is connected to a second diameter coil (100 cm x 1.02 mm i.d. = 817 µL) nearest the 10-position valve. The tube connecting the valve and the detector had yet another dimension (115 cm x 0.5 mm i.d. = 226 µL). Implementation of these three dimensions serves to (i) increase the holding volume of the system (so that large volumes can be injected without reaching the pump), (ii) reduce the internal friction during the aspiration cycle (which will reduce bubble formation), and (iii) keep tubing as narrow as possible in the most critical areas. Other researchers [7-8] found a deterioration in system precision due to the gas bubbles which form under the reduced pressure when 0.5 mm tubing was used. The internal diameter of 0.84 mm for the PTFE tubing used in the current studies is chosen as a compromise between conditions (i) to (iii) above, while satisfying the necessity of using the same diameter tube throughout the system for consistency.
3.2.3 Flow Cell

The standard quartz flow cell (Hellma, Fisher Scientific, Vancouver, B.C.) used in all experiments had a 1.0 mm i.d. by 1.0 cm light path, equivalent to 7.85 μL volume. The tube connecting the valve and the detector ($L_D$) was connected by a flange at the valve head. The other end of the tube was forcibly inserted into the quartz flow cell all the way up to the light path where the flow path makes a 90-degree bend, and then sealed in place with epoxy resin. The dimension $L_P$ of 15, 50 and 100 cm incorporates the length of the 0.84 mm PTFE tubing from the light path of the flow cell back to the valve connection of the tube which connects to the pump (i.e., it includes the dead-volume of the valve head which has an i.d. of ca. 0.8 mm. A 0.84 mm i.d. PTFE tube
was also sealed with epoxy in the exiting line of the flow cell and led to a waste container.

3.2.4 Peristaltic Pump Tubing

Two separate Alitea pumps (either syringe or peristaltic) are controllable by the software system described in Chapter 2. Although the two syringe pumps have the advantage of pulseless flow, the complications introduced by the sinusoidal flow pattern is undesirable for the current investigation. Thus, peristaltic pumps were used under conditions which minimized pulsing (described further in Chapter 5) and a flow rate calibration was performed every 20 injections to correct for drift.

Tygon® pump tubing (Cole-Parmer, Chicago, Illinois) was used in all studies. Flow rates of 0.5 and 1.0 mL min⁻¹ were achieved by using 0.64 mm i.d. tubing, 1.0 and 2.0 mL min⁻¹ by using 1.30 mm i.d. tubing, and 4.0 and 6.0 mL min⁻¹ by using 1.65 mm i.d. tubing. The appropriate selection of pump tubing diameter maintained sufficient precision in most cases for the injection volumes of 40 through 240 µL created by the peristaltic pump. A series of experiments were performed to determine the reproducibility of the injection volume when using the peristaltic pump. The pump was operated for the length of time necessary to inject the desired volume at the current flow rate while the manifold tubing was suspended in a waste container seated on a digital balance. By mass difference, the actual volume of solution moving through the
tube could be determined and the relative standard deviation of the injected volume was calculated.

3.2.5 Experimental Basis Set

The results of the current study are recorded in a comprehensive database (serving as a future reference) and are used for the zone overlap optimization done in Chapter 4, and for comparison with the simulated peaks created by the random-walk model discussed in Chapter 5. Any cross-section of experimental data can be immediately “queried” from the database using search filters and displayed graphically for analysis or comparison with other data. This investigation also serves as an initial exercise for the newly developed automated sequential injection analysis system described in Chapter 2.

The experimental parameters investigated in this study are shown in Table 3-1. Three different manifold lengths ($L_D$) were used, and at each length, three configurations were investigated for the two segments of tube $L_P$ and $L_D$ (straight or coiled at 1.0 cm or 6.5 cm). In all cases, the tubing from the valve to the pump ($L_P$) was 200 cm. This produced 9 (3 x 3) variations altogether. At each of these nine variations, a variable amount of dye was injected (40 to 240 µL in 40 µL increments) before injecting a variable amount (0 to 240 µL in 40 µL increments) of “spacer” (wash solution of 1.0 M KCl). This created 42 (6 x 7) additional variations which were each measured at six different flow rates (0.5, 1.0, 2.0, 3.0, 4.0, and 6.0 mL min$^{-1}$) producing
252 (6 x 6 x 7) unique experimental conditions. These 252 experimental conditions were performed at each of the 9 manifold configurations to produce 2,268 (9 x 252) experimental conditions, each performed in triplicate, thereby resulting in 6,804 (3 x 2 268) individual experiments to be logged on the analyzer and recorded in the database.

The choice of injection volumes and flow rates results in a dataset which covers the majority of conceivable operating conditions for an analysis performed by the sequential injection method. The minimum volume of 40 µL was chosen since injection volumes of any less than 40 µL were prone to irreproducibility (see Results and Discussion). At 1.0 mL min⁻¹ the volume of 40 µL corresponds to a valve head rotation of 442 degrees, which is slightly more than one revolution. Ivaska also found that reproducibility in the injection volume diminishes if less than one pump head revolution is used [9]. Increments of 40 µL for the injected volume and the spacer volume produced regular spacing intervals for response surface mapping and data analysis. The flow rates were chosen such that the injection volumes of 40 µL (which are based on the time for injection) could be achieved by using even multiples of 0.1 s, thereby reducing any round-off errors which might arise otherwise. The upper limit of 240 µL was chosen because in the extreme case of two 240 µL volumes being stacked sequentially, the maximum linear distance traveled from the valve towards the pump would be ca. 87 cm under plug flow conditions, or 174 cm under laminar flow conditions for which the linear axial velocity at the tube center is twice the average linear velocity. This approaches the total length of tubing between the pump and the valve.
Further increase in this length (to allow larger injection volumes) would only increase the friction and result in a pressure drop within the manifold during the aspiration cycle (thereby enhancing bubble formation).

Previous work in this area [5] specified injection volumes in multiples of $S_{x}$ values. The $S_{x}$ value for a system is the injection volume necessary to produce a dispersed peak profile which has a height equivalent to one-half of $C^0$. This is a convenient point of reference since all flow systems will have their own unique value of $S_{x}$ for reference [1]. However, since $S_{x}$ varies with manifold dimension, flow rate, and the flow reversal distance, there would be a much greater variation in injection volumes necessary in this study if multiples of $S_{x}$ were used in each case, in addition to increasing the complications of cross-comparison within the multi-dimensional dataset created. However, data which indicate the $S_{x}$ value at each flow rate and manifold dimension (without a spacer injection) will be shown for reference.

The configuration column in Table 3-1 indicates that the two segments of tube, $L_p$ and $L_d$, were either straight or coiled around a cylinder of 6.5 cm or 1.0 cm diameter. The manifold tubing was kept as straight as possible in the straight tube configuration. In all cases, unavoidable bends in the tubing occur at the injection valve and the detector (Figure 3-2), and may contribute to secondary flow. Coiled tubes were used to enhance secondary flow and reduce dispersion. Although this does not represent an exhaustive investigation, several combinations of straight and coiled tubes were used in order to investigate the effect of secondary flow in sequential injection analysis. The
tube \( L_D \) is listed as straight in all configurations with a manifold length of 15 cm because the tube is too short to coil.

Table 3-1. Experimental parameters (each run in triplicate) for the flow rate, sample zone volume, reagent zone volume, manifold length or volume (from valve to detector), and manifold configuration (coiled or straight).

<table>
<thead>
<tr>
<th>Manifold Length / ( L_D ) Volume (( L_D, V_D ))</th>
<th>( L_P / L_D ) Configuration</th>
<th>Flow Rate (mL min(^{-1}))</th>
<th>Injection Volume (( \mu L ))</th>
<th>Spacer Volume (( \mu L ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.0 cm / straight / straight</td>
<td>0.5</td>
<td>40</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>83 ( \mu L )</td>
<td>6.5 cm / straight</td>
<td>1.0</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>1.0 cm / straight</td>
<td>2.0</td>
<td>120</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>160</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>200</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>240</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>50.0 cm / straight / straight</td>
<td>0.5</td>
<td>40</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>277 ( \mu L )</td>
<td>straight / 1.0 cm</td>
<td>1.0</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>1.0 cm / 1.0 cm</td>
<td>2.0</td>
<td>120</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>160</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>200</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>240</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>100.0 cm / straight / straight</td>
<td>0.5</td>
<td>40</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>554 ( \mu L )</td>
<td>straight / 1.0 cm</td>
<td>1.0</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>1.0 cm / 1.0 cm</td>
<td>2.0</td>
<td>120</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>160</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>200</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>240</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Number of Combinations</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

3.2.6 Analytical Procedure

The following analytical procedure was used in all cases except in the multiple flow reversal investigation:
1. The 10-position valve was set to the first position (thus connecting the valve to the dye supply tube) and 40, 80, 120, 160, 200, or 240 μL of dye was aspirated at a flow rate of 0.5, 1.0, 2.0, 3.0, 4.0, or 6.0 mL min⁻¹.

2. The flow was stopped for 1.0 s as the valve turned to the next position (thus connecting the valve to a tube containing wash solution, 1.0 M KCl) and 0, 40, 80, 120, 160, 200, or 240 μL were aspirated at the same flow rate as that used in step 1.

3. The flow was stopped for 1.0 s as the valve turned to the next position (thus connecting the valve to the flow cell tube).

4. The flow was then reversed and the solution was driven at the same flow rate through the flow cell and into the waste container. The flow would continue for a pre-determined length of time which was sufficient to wash all traces of dye solution from the system. During this time, the signal from the diode-array spectrophotometer was acquired (at 416 nm) by the control software and subsequently stored to disk as an ASCII file.

5. In all experiments the flow rate was automatically calibrated with the digital balance after every 20 injections using a 120 s calibration period.

The following procedure was used in the multiple flow reversal investigation:
1. The 10-position valve was set to the first position (thus connecting the valve to the dye supply tube) and 120 μL (3.6 s) of dye was aspirated at 2.0 mL min\(^{-1}\).

2. The flow was stopped for 1.0 s while the 10-position valve was switched to the second position to allow 640 μL (19.2 s) of 1.0 M KCl to be aspirated in order to move the sample plug further into the manifold away from the valve.

3. The flow was again stopped for 1.0 s while the valve moved to the detection line at the next position. The detection line consists of a 50 cm segment of tube coiled around a 1.0 cm diameter cylinder which connects the valve to the detector, and a 100 cm segment of straight tube connecting the detector to the waste container.

4. The flow was then restarted in the forward direction (towards the valve) for a total displacement of either 80, 160, 240 or 320 μL (2.4, 4.8, 7.2, or 9.6 s, respectively). The same carrier solution (1.0 M KCl) was present in the detection line.

5. The flow was then instantaneously changed to the opposite direction (towards the pump) until the same volume of fluid was displaced as in step 4. This constitutes one "flow reversal step" \((n = 1)\), which was performed from 1 to 8 times. Note that the total displacement (160, 320, 480 or 640 μL) of one flow reversal step is twice the volume displaced in one direction of the step.
6. After all reversals had taken place, the flow was stopped for 1.0 s, restarted towards the detector and the resulting peak profile was recorded. The flow towards the detector was of sufficient volume (6.0 mL) to ensure all traces of the dye were flushed through the detection line to waste, thereby eliminating any carryover. An injection profile which did not include the flow reversals in steps 4 and 5 was also included in the dataset for reference. Reported results are an average of three replicates performed for each combination of flow reversal length (I) and number of reversals (n).

3.3 RESULTS AND DISCUSSION

At the present time, no specific dispersion theories have been suggested to explain the dispersion profile created by the sequential injection process [11]. In the limited amount of research done in this field, dispersion theories developed for flow injection systems (assuming a rectangular block input function and unidirectional flow) have been assumed to be valid for use in sequential injection analysis as a first approximation [5-7]. Two major differences between these two injection methods (which are likely to influence the dispersion profile) are (i) the asymmetric concentration gradient created during injection of the sequential injection zone (shown in Chapter 5) and (ii) the influence of the flow reversal where the concentration profile inverts itself one or more times. However, flow injection theories on their own are still far from complete and are often limited by boundary conditions and an inability to incorporate non-uniformity in the experimental apparatus [1-2]. The degree of the discrepancies
created by (i) and (ii) above have not yet been quantified and, as such, any attempt to relate the peak profiles created by a sequential injection system to flow injection theory should be done with caution. The work of Reijn et al. [12] who considered the effects of timed and delta input functions on the flow injection response curve might be a good starting point. However, until further work can be done in the theoretical research area of sequential injection analysis, researchers are limited to describing the characteristics of the analyzer by empirical experimentation with limited theory for guidance [11].

3.3.1 Absorbance of Dye

When performing long-term optimization experiments using a tracer dye, it is initially necessary to (i) determine the most suitable wavelength for detection, (ii) ensure that the detector response is linear within the working range, and (iii) ascertain that the absorbance of the dye solution remains stable over long periods of time. A sufficient volume of 1.5 mM K$_3$Fe(CN)$_6$ was pumped through the flow cell (to ensure saturation) before stopping the flow and scanning a full UV-visible spectrum (using 1.0 M KCl as a reference solution). Figure 3-3 indicates an acceptable wavelength of maximum absorption at $\lambda_{\text{max}} = 416$ nm, which was used in all dispersion experiments. The highest point of this peak should produce the most stable reading and would correspond to $C^0$ for the system under study since it represents the absorbance of the undiluted dye in the flow cell.
Figure 3-3. Absorbance spectrum of a 1.5 mM solution of K₃Fe(CN)₆ made up in 1.0 M KCl. In all dispersion experiments the dye is monitored at λ<sub>max</sub> = 416 nm.

The linearity of the response at subsequent dilution from the C<sup>0</sup> value was next ascertained by preparing 11 solutions ranging in concentration from 0.0 to 1.5 mM K₃Fe(CN)₆ and measuring them in the same way as C<sup>0</sup>. The absorbance of these solutions at 416 nm was also measured after 1, 5, and 12 days. Figure 3-4 shows the linearity and stability of the dilutions over the 12 day period. Therefore, the absorbance of the dye solution produces a linear response from the detector and shows no signs of deterioration with time.
3.3.2 Reproducibility of the Injection Volume

The injection volume is determined by the flow rate and the length of time the pump is operated. Therefore, the uncertainty in the injection volume is a function of the uncertainty in the starting and stopping position of the rotating pump head [9]. This amount of error is negligible in comparison to the variable positioning of the 8 rollers located around the pump head. Discrete elements of fluid are pinched between the rollers as the pump head rotates, and the volume of one of these elements can be calculated if the pump head frequency is known. For example, for a 40 µL injection volume dispensed at a flow rate of 1.0 mL min⁻¹ by a pump which has been calibrated using 0.64 mm i.d. pump tubing, the total rotation of the pump head is 442 degrees. At
45 degrees per roller, this corresponds to 9.82 rollers, each delivering 4.07 μL of fluid on average. The volume of this element of fluid varies with the diameter of the pump tubing, and therefore larger diameter tubing should be avoided since it will generally have a larger volume of fluid moved by each roller. Larger diameter tubes cannot be ruled out, however, since they are required to effect higher flow rates due to the upper limit of the rotational frequency of the pump. Because it is difficult to ensure that the roller heads line up from one injection to the next, a small amount of uncertainty would be expected in the actual injected volume (as measured by mass difference), and further, the discrepancy in volume would be expected to exhibit periodicity over several injections. Figure 3-5 shows the relative standard deviation (RSD) of 5 replicate injections of 40, 80, and 120 μL at 6 different flow rates. In general, the RSD decreases with increasing injection volume, although the average magnitude of the standard deviation for the entire dataset ranged from a low of 0.18 μL to a high of 1.30 μL and an average of 0.87 μL. It is clear that, for the pump currently being used, injection volumes of less than 40 μL would have too much relative uncertainty to produce reliable results. However, since the uncertainty at 40 μL is a little suspect, an average of triplicate measurements is made in almost all data presented in this work, thereby reducing the systematic noise of the dataset.
Figure 3-5. Relative standard deviation of 40, 80, and 120 µL injection volume (n = 5) as function of flow rate.

As previously described, the periodicity of the fluid movement due to the alignment of the pump head rollers was investigated by connecting the pump, through the valve, to a digital balance and recording the amount of fluid moved by mass difference. In Figure 3-6 and Figure 3-7 the periodicity of the actual injection volume is shown for the movement of 40 and 120 µL of fluid, respectively. The 40 µL injection appears to repeat itself after 6 injections while the 120 µL injection repeats after only 3. This is attributed to the alignment of the roller head on sequential injections. All injections were performed immediately one after another and no other pump head movement occurred between each 40 or 120 µL movement. We can conclude that the pump head does indeed affect the reproducibility of the injection volume, and furthermore, that this reproducibility is periodic. It is interesting to note that if
reproducible alignment of the peristaltic pump rollers on subsequent injections could be achieved, then the reproducibility of the injection volume could be improved (i.e., compare every sixth injection in Figure 3-6 starting at injection number 2). It should be noted that when subsequent sequential injection operations are performed, additional randomization in the pump position will occur at each step, such that the overall precision of the analysis is likely to be better than the series of subsequent injections as shown here. As well, the irreproducibility of injection volume will not result in an irreproducibility of equal magnitude in the detector response to product concentration, especially if the product is formed at the interface of two adjacent zones of high volume.

Figure 3-6. Injection volume as measured by mass difference for a 40 µL injection at 1.0 mL min⁻¹.
### 3.3.3 Calculation of $S_N$ Value

As the injected volume of dye is increased, its height asymptotically approaches the value of $C^0$ as shown in Figure 3-8. The injection volume necessary to reach $C/C^0 = 1/2$ or $D = 2$ is referred to as $S_N$. It is generally assumed that for flow injection peaks the increase in peak height below $S_N$ is approximately linear [1, 5]. The value of $S_N$ is a function of the dimension and geometry of the flow channel [1]. Because of the asymptotic increase above $S_N$, it is generally believed that any increase in injection volume beyond $S_N$ is done at the expense of sample consumption and decrease in throughput, with minimal improvement in sensitivity but some improvement in precision.
This assumes that this same relation holds true for the sequential injection technique [5, 8]. A discrepancy exists for SIA, however, in that although the volume from the injection valve to the detector is constant, by increasing the sample volume ($V_s$), the average distance traveled by the sample zone into the flow channel towards the pump is increased, thereby increasing the overall effective manifold volume. More importantly, the concentration gradient of the injected sample zone cannot be considered to be a rectangular block input function as will be demonstrated using the random-walk model in Chapter 5. However, these simplifying assumptions do not cause too much concern in calculation of $S_{kn}$, which can still be determined in the usual way.

![Figure 3-8. Peak profile as a function of injection volume at 2.0 mL min$^{-1}$ and 15 cm valve-to-detector distance; profiles from injections of 40, 80, 120, 160, 200, and 240 μL are shown (smallest to largest, respectively).](image)
For reference, the values of peak height ($C_{\text{max}} / C^0$) for the experimental conditions under consideration are shown in Figure 3-9. For each plot, the value of $S_{x}$ can be found by finding the injection volume which produces a response (peak height) of 0.5. These data demonstrate the dependence of $S_{x}$ on the reactor volume (governed by the length of the tube from the valve to the detector), flow rate, and manifold configuration. In general, the trend is similar for straight and coiled configurations at each manifold length. As expected, manifolds with the shortest valve-to-detector distance, $L_D$, approach the maximum peak height at the lowest injection volumes and therefore have the lowest $S_{x}$ values.
Figure 3-9. Effect of flow rate, valve-to-detector (L_D) distance, and manifold geometry on maximum peak height as a function of injection volume.
According to flow injection theory, a plot of $-\log(1 - \frac{C^\text{max}}{C^0})$ versus injection volume should produce a linear response. This has been shown to hold true for the sequential injection technique [5] although the results of the current work show that this relationship breaks down at higher flow rates (Figure 3-10). As the flow rate is increased, it becomes increasingly difficult to achieve a response with a peak height of one-half $C^0$. This should be borne in mind when optimizing injection volumes when working at higher flow rates.

![Figure 3-10. The effect of the injection volume on the peak height for the 100 cm coiled manifold.](image)

### 3.3.4 Zeroth Moment

As predicted by theory, and shown in Figure 3-11, the zeroth moment (peak area) is independent of the manifold geometry, linearly proportional to injection volume, and inversely proportional to the flow rate. The linearity and stability of these results is important since all of the higher order moments use the zeroth moment for
normalization. As well, inspection of the precision of the data indicates that the peak truncation method employed produces consistent results. The units for the zeroth moment (peak area) are (analog-to-digital-conversion-units x seconds) expressed as ADC • s.

Figure 3-11. Effect of flow rate on the zeroth moment (peak area) as a function of injection volume for different manifold geometries.
3.3.5 First Moment

As expected, the first moment, which signifies the peak centroid or center of mass, is linearly dependent on injection volume, inversely proportional to flow rate, and dependent on manifold geometry (Figure 3-12). The linearity and stability of this value is also significant since all higher order moments are calculated relative to this value. Straight tubes produce peaks which have a higher first moment due to the decrease in radial mixing which causes more molecules to lag behind along the walls of the tube. Hence, similar to flow injection analysis, throughput can be increased by increasing radial mixing. This will have to be balanced, however, with the need for greater dispersion that promotes penetration of adjacent zones (see Chapter 4). It should also be noted that choosing a flow rate of less than 2.0 mL min$^{-1}$ with the manifold dimensions shown here increases the first moment substantially, as does increasing the manifold length.
Figure 3-12. Effect of flow rate on the first moment (peak centroid) as a function of injection volume for different manifold geometries.

3.3.6 Second Moment

The second moment (variance) of the peak profile is expected to be inversely related to the flow rate, exponentially related to injection volume, and dependent on manifold geometry. Figure 3-13 shows the second moment plotted on a logarithmic scale against injection volume at all six flow rates. These data indicate that the second moment does increase with increasing injection volume and decreasing flow rate. This
is to be expected since larger injection volumes will have an increased peak width and reduction of the flow rate increases the length of time the injected zone takes to pass through the detector. Increasing the manifold length increases the second moment and reduces the influence of injection volume, since the ratio of sample volume to manifold volume is decreased. At both manifold lengths, coiling reduces the second moment by increasing the degree of radial mixing relative to axial dispersion. This is also in agreement with current flow injection theory.

Figure 3-13. Effect of flow rate on the second moment (peak variance) as a function of injection volume for different manifold geometries.
3.3.7 Skew

Skew is a measure of the degree of peak asymmetry. It is useful to quantify this for sequential injection peaks since a greater degree of peak asymmetry is anticipated due to the asymmetrical concentration gradient of the initial injection plug. The data in Figure 3-14 indicate that the peak profiles range in skew from almost symmetrical at 0.5 mL min$^{-1}$ with a long coiled manifold, to highly skewed peaks created at 4.0 to 6.0 mL min$^{-1}$ with a short straight manifold. The degree of skew appears to be independent of injection volume except in the case of the 15 cm coiled manifold where skew decreases with increasing injection volume. Since the distance between the valve and the detector is so short in this case, the data indicate that the concentration distribution found at the detector is influenced to a large degree by the mixing occurring during the injection process. Thus, an increase in radial mixing relative to axial mixing has the overall effect of reducing peak skew. The skew is also reduced when the distance between the valve and the detector is increased, in agreement with the tanks-in-series model, which asserts that the peak symmetry approaches a Gaussian distribution as the number of mixing stages (tanks) increases [1].
Figure 3-14. Effect of injection volume and flow rate on skew of peak profile at different manifold geometries.

3.3.8 Excess

The degree of excess is a measure of the "flatness" of a peak profile relative to a Gaussian distribution which has an excess of 0. Taller, more peaked profiles will have a negative value for excess while flatter more dispersed profiles will have a positive value. The degree of excess follows a trend very similar to skew under all conditions (Figure 3-15). Decreasing the flow rate or increasing the manifold length are both
effective ways of decreasing the excess since both conditions allow more time for radial mixing, thereby creating taller, more "peaked" profiles. In addition, the degree of radial mixing caused by coiled tubes also reduces the excess at both manifold lengths (Figure 3-15b and d). It is noteworthy that relatively consistent near-zero values for excess are achieved in the case of the 100 cm coiled manifold, which implies that the distribution profile has achieved a Gaussian shape and reached a degree of dispersion which is relatively uninfluenced by flow rate.

Figure 3-15. Effect of injection volume and flow rate on excess of peak profile at different manifold geometries.
It is clear from the foregoing survey of peak descriptors calculated on the sequential injection peak profiles created by the analyzer, that a comprehensive range of peak shapes has been created. In all of the above cases, only the dispersion profiles resulting from the injection of a given sample volume without further flow reversal have been considered. With such a system, however, it is of course possible (and usually necessary for reagent-based analyses) to change the injection valve to another position and continue to reverse the flow while a second zone is injected. In such a situation, the first zone undergoes further dispersion since it must travel farther into the manifold towards the pump before the flow is reversed towards the detector. This creates even greater diversity in the dispersion profile since the distance traveled by the first zone towards the pump before reversing introduces yet another variable. Still greater variability can be achieved by reversing the direction of flow several times at variable amplitudes. Multiple flow reversals will be discussed in the next section.

3.3.9 Multiple Flow Reversals

At the outset, it was important to design and implement a sequential injection system that would be able to map out the effects of several variables of different types with equivalent dexterity. Indeed, this has been achieved with the sequential injection system described in Chapter 2 which, unlike its predecessor FIDO, can investigate parameters such as delay time, valve position, volume of fluid moved, pump speed, stop-flow time, number of flow reversals, and amplitude of flow reversals. Although the
investigation of each of these parameters provides insight into the optimal operating conditions of such an analyzer, it is the last two which are demonstrated now because of their significance in the original conception of the sequential injection technique [4, 5, 7].

The effect of one or more flow reversals was investigated for the injection of 120 μL of dye using a flow rate of 2.0 mL min⁻¹. For the system under consideration, 120 μL of dye produced a peak height which ranged from a maximum detector response of $C_{\text{max}}/C^0 = 0.184$ (with zero flow reversal steps) to a minimum of $C_{\text{max}}/C^0 = 0.132$ (with eight flow reversals steps). The two parameters investigated were (i) the step length of a flow reversal ($l$) and (ii) the number of flow reversals ($n$). The step length of a flow reversal ($l$) is normalized by subtracting the variance of the peak profile created with one flow reversal. Figure 3-16 shows that a linear relationship exists between the change in peak variance relative to that for one flow reversal $\Delta(\sigma^2)$ and the number of flow reversals ($n$). This is in agreement with the basic premise of the random-walk model given in Equation 3-9. A linear relation between the change in the square root of the variance relative to one reversal length ($\Delta\sigma$) and the flow reversal length ($l$) given in Equation 3-10 is also shown to hold in Figure 3-17. This is in agreement with results by Marshall [3] who found a linear relationship by performing a similar experiment using a flow injection system (square plug injection), where peak height was used as a relative measure of dispersion instead of peak variance. Hence, the general relation $\sigma^2 \propto nl^2$ is shown to hold for the
sequential injection technique, and forms the basis of the applicability of the random-walk model for simulation purposes as will be discussed in Chapter 5.

Figure 3-16. Effect of the number of flow reversals ($n$) and the flow reversal length ($I$) on the variance of a 120 µL injection at 2.0 mL min$^{-1}$. 
Figure 3-17. Effect of the reversal length (normalized) on the square root of the variance for 2 through 8 reversals.

The implications of the above agreement are significant for sequential injection analysis in that it is the length of the flow reversal which is critical in determining the degree of variance (dispersion) of the peak profile. That is, increasing the length of a reversal step is far more effective than increasing the number of reversals between two sequentially stacked zones in the sequential injection system. It must be kept in mind, however, that increasing the flow reversal step length necessitates a longer distance between the pump and the valve which can have negative effects on the system volume and the measurement precision due to increased pressures.
In addition to the second moment, the skew and excess of the peak profiles were considered under conditions of multiple flow reversals. The skew of the peak profile would be expected to approach 0 (a symmetrical true Gaussian) in comparison to the previous results shown due to the increased manifold dimensions. The peak skew in all cases was reduced to 0.5. There is no discernible trend in the data caused by different reversal lengths since the variation in skew at this level is within the noise level of the measurement. The peak excess shown in Figure 3-18, however, does indicate a general increase for longer reversal lengths and at greater numbers of reversals. As well, one reversal length of 160 μL (2 x 80 μL) actually has the effect of reducing the excess slightly, thereby creating a narrower profile than one without reversal. This effect decreases as the ratio of the reversal length relative to the zone length increases (i.e., at higher reversal lengths).
Figure 3-18. Effect of the number of reversals and the reversal length on peak excess.

### 3.4 Conclusions

Several conclusions can be drawn from this work which describes a comprehensive dataset of sequential injection dispersion profiles created on the analyzer described in Chapter 2. The K₃Fe(CN)₆ solution used for all experiments is linear within this experimental working range and stable for at least 12 days. The reproducibility of the injection volume is periodic and dependent on the alignment of the pump roller head. In general, the injection volume delivered by the peristaltic pump is within ca. 2.0 µL of the target value. For this reason, smaller injection volumes than 40 µL were not included in the dataset, and all injections were performed in triplicate.
thereby allowing averaging of the results. The value of $S_x$ is shown to be dependent on system flow rate and manifold geometry. As well, the linear relationship between $-\log(1 - \frac{C_{max}}{C^0})$ and injection volume is shown to break down at higher flow rates for the sequential injection method.

The usefulness of peak moments as descriptors for sequential injection peak profiles is considered for the first time. The peak moments are shown to be an effective means of discriminating between various peak shapes for the sequential injection method. The trends in the moment calculations are as anticipated and the stability of these descriptors is only compromised in the third and fourth moments at higher flow rates, as evidenced by noise in the skew and excess data. These descriptors should find much greater use in peak shape analysis of sequential injection peak profiles due to the increased variability of peak shape produced by this new technique.

Finally, an investigation of the influence of multiple flow reversals indicates agreement between sequential injection analysis and the random-walk model. This signifies that the model can be used at the molecular level to predict dispersion profiles created by the sequential injection technique (assuming a one-phase liquid with homogeneous flow), and will be discussed further in Chapter 5.
3.5 REFERENCES


4. Optimization of Zone Overlap in Sequential Injection Analysis

"It is not always easy even for scientists to tell whether their theories are made valid because they accord with reality, or because they accord with other scientists and funding agencies."

Benjamin Woolley

4.1 INTRODUCTION

In order to perform most reagent-based assays, the sequential injection technique relies on the simultaneous merging of two or more zones of sample or reagent(s) stacked in the manifold tubing. Therefore, the study of the mutual penetration of two adjacent zones should form the focus of fundamental research in this field. Only by understanding the factors which influence this zone penetration under typical sequential injection conditions, can we have any hope of adequately optimizing the sequential injection process. Obviously, the ability to optimize a process by understanding it, has its advantages over an empirical optimization or a "black box" approach. However, there will undoubtedly be a limit to our understanding when basic
assumptions, physical laws, and formulae will no longer be able to explain the complex behaviour that real sequential injection systems exhibit; all too often, there exists too many interacting variables and non-uniformities in the system to be adequately addressed simultaneously. When this limit is reached, chemometric techniques such as simplex optimization will likely be employed in an attempt to “blindly” search for optimal operating conditions which produce the most desired output from the analyzer.

Since the sequential injection technique is such a new method of analysis, a better fundamental understanding of the underlying factors affecting mutual penetration of the sample and reagent zones needs to be pursued at this time. Very little work has been done in this field so far [3-9]. Previously, theoreticians working in the field of flow injection analysis have usually concerned themselves with studying the dispersion of an entire injected sample volume (which was habitually defined by the physical geometry of the manifold) under a continuous, unidirectional flow regime [1-2]. Such convenient computer control over the volume of the injected zone(s) was rarely considered before the introduction of sequential injection analysis, and was therefore not normally within the scope of a theoretical study; neither was the concept of intermittent pump movement during sample or reagent introduction, especially in conjunction with the flow reversal process. The introduction of these new factors on the analytical process results in a severe increase in the complication of our understanding of the resultant dispersion profile. All of these new factors (variable injection volumes, intermittent pump movement, and flow reversal) need to be addressed in order to come to a satisfactory explanation of the system output.
The first paper considered to be a fundamental investigation of the merging process which occurs in sequential injection analysis was written by Gübeli et al. and appeared in *Analytical Chemistry* in 1991 [3]. They introduced the concept of *zone overlap* as the key parameter which must be controlled in order to produce a meaningful readout for reagent based chemistries. Chemical reactions can only occur within elements of fluid in the region of zone overlap between mutually dispersed sample and reagent zones. It is the relative concentration of the sample and reagent within this zone (and reaction time) which determine the extent of the chemical reaction and, therefore, the characteristics of the product concentration profile. Gübeli et al. [3], investigated the concept of zone overlap by overlaying peak profiles created by separate injection of sample and reagent volumes as shown in Figure 4-1. Note that the reagent zone appears first since it is closest to the detector upon flow reversal, and that the sample zone is more dispersed since it has travelled a further distance than the reagent zone. The isodispersion point \((I_d)\) represents the point of mutual zone penetration. It is generally understood that the most efficient method of penetrating the sample zone with reagent is by aspirating the sample zone first and then the reagent zone. If necessary, the reader can replace any reference to the “sample” and “reagent” zone in this work with the “first-loaded” and “second-loaded” zone, respectively.
Figure 4-1. Overlap of sample and reagent zones are shown by overlaying profiles which were created on separate injections (each 240 µL).

Gübeli et al. [3] used a zone penetration parameter, $P$, to facilitate comparison of profiles produced with different injection volumes of sample and reagent, which was defined as

\[
P = \frac{2W_0}{W_s + W_R}
\]

where $W_0$ is the baseline width of the sample and reagent peak overlap, $W_s$ is the baseline width of the sample zone, and $W_R$ is the baseline width of the reagent peak.\(^\dagger\)

\(^\dagger\) This interpretation assumes a misprint in the original publication (Analytical Chemistry, 63 (1991), page 2408, Equation 1) which defines $P$ as $P = 2W_0 (W_s + W_R)$. In order to obtain a range of $[0, 1]$ for $P$ as the text states, a division sign must be placed after $W_0$. 
This parameter relies on the peak width at the baseline intersection of tangents drawn at the peak inflection points to determine the degree of zone penetration (Figure 4-1). A value of 0 would be expected for no overlap while a value of 1 would be expected for complete (time-domain) overlap. Marshall and van Staden [9] used the overlapping peak area instead of width for calculating the degree of zone penetration, $P$. This parameter, however, does not discriminate between the sample and reagent zones for optimization purposes and does not take into account the sensitivity of the analysis.

For example, it would be expected that a value of $P = 1$ (indicating complete overlap) is more likely to be obtained at (i) lower injection volumes and (ii) greater axial dispersion. Both of these conditions reduce the radial concentration of sample and reagent in the tube at any point in time, thereby reducing the product peak height, and therefore, the sensitivity of the analysis. Moreover, optimization of a sequential injection procedure may include finding conditions which maximize the throughput, reagent economy or reproducibility. Often, these optimal operating conditions can only be obtained by a compromise of at least one of the other desirable characteristics of the analysis.

In light of the foregoing, several additional optimization parameters will be introduced here in order to consider which operating conditions produce an "optimal" analysis by the sequential injection method. In this chapter, the results from a systematic investigation of factors known to affect dispersion (such as injection volume, flow rate, and manifold geometry) during the sequential injection procedure will be discussed [3, 9].
In order to study the degree of zone penetration occurring under varying analytical conditions, the sample and reagent zones were recorded separately, and then overlayed one on top of the other. The experimental peak profiles obtained in the dataset described in Chapter 3 will be used. Injection of three or more zones will be governed (in general) by the same principles outlined for two zones.

4.1.1 Zone Overlap Descriptors

Optimal operating conditions for a given sequential injection method will vary depending on several competing factors. Optimal conditions should include a combination of maximum zone penetration, minimum sample and reagent waste, maximum sensitivity, and maximum sample throughput. Maximizing or minimizing any one of these conditions inevitably results in compromising one or more of the other conditions. For example, an increase in zone penetration (as defined by P) is expected at higher dispersion numbers \(D = C^o / C^{max}\) thereby lowering the sensitivity by a substantial decrease in peak height. Increased sensitivity can usually be achieved by increasing the sample or reagent volume but this improvement is limited as \(D_s\) (the sample zone dispersion) decreases from 2 to 1, and is usually at the expense of wasted reagent. As well, using greater sample and reagent volumes increases the length of time for the previous run to be washed out of the system, thereby decreasing sample throughput. Obviously, optimal operating conditions are going to have to be considered for each specific application and compromises will have to be made.
Each of the optimized conditions mentioned above is governed primarily by (i) the volume of the injected sample and reagent zones, (ii) the flow rate, and (iii) the manifold geometry (straight tubes versus coiled, with variable valve-to-detector distances). The tube diameter would also be expected to play a role in optimization studies [9]. However, as was mentioned in Chapter 3, only one tube diameter (0.84 mm) is considered here. The purpose of this work is to investigate the effect each of these factors has on creating various optimized conditions (i.e., maximum penetration, maximum sensitivity, minimum reagent waste, and maximum throughput). A ratio, which describes each optimization parameter on a scale of 0 to 1 (from least to most optimal), has been calculated so as to understand how each is influenced by injection volumes, flow rate, and manifold geometry.

### 4.1.1.1 Zone Penetration

In this work, the degree of sample zone penetration is calculated as

Equation 4-2

\[ R_p = \frac{A_o}{A_s} \]

where \( A_s \) is the area of the sample zone and \( A_o \) is the portion of \( A_s \) subjected to zone overlap. A value for \( R_p \) near 0 indicates minimal sample overlap by the reagent zone while a value near 1 indicates the most complete sample zone overlap. This ratio, however, does not consider the extent to which the reagent zone is penetrated by the sample zone since it is usually beneficial to have the reagent volume in excess of the
sample volume. A high value for $R_p$ produces a situation which is much like conventional flow injection analysis where reagent is present in all regions of the sample zone. It is in this situation that the most reproducible and meaningful detector response will be found. A low reagent to sample ratio, however, would limit $R_p$ to the maximum area of the reagent which would be roughly equivalent the maximum volume of the overlap. The resulting ratio would be somewhat less than 1 indicating that there is a portion of the sample zone which is not penetrated by reagent. It should be kept in mind, however, that high $R_p$ values would likely be found at high reagent-to-sample volume ratios, a condition that has the potential to waste reagent. Thus, reagent economy must also be considered for optimization purposes.

4.1.1.2 Sensitivity

If the height of the product peak is used to quantify the amount of analyte present in the sample, then the sensitivity of an analysis depends on finding the maximum of this value. The maximum of the product peak will normally occur in the element of fluid which contains the highest overall concentration of sample and reagent (usually the isodispersion point, Figure 4-2). If the sample zone is saturated with reagent due to a high reagent-to-sample volume ratio or if $C_R^0 >> C_s^0$, then the maximum product peak height will be shifted towards the maximum of the sample zone where the analyte has the greatest concentration. In either case, a descriptor is calculated by
considering the highest point of the zone overlap (which may be the height of the sample zone) according to

Equation 4-3

\[ R_s = \frac{2C_{DL}^{\text{max}}}{C^0} \]

which will approach 0 for a very dispersed sample and reagent profile (i.e., high \( D_s \) and \( D_R \)), and approach 1 for a sample and reagent zone which have undergone minimal dispersion. In practice, the height of the overlap on the \( C/C^0 \) scale is usually less than 0.5 (indicating a dispersion value \( D \) greater than 2 for either \( l_D \) or the maximum of the sample zone) and therefore, the factor of 2 is included in order to scale the \( R_s \) ratio over the range \([0, 1]\). This descriptor must be applied with caution since the actual maximum of the product peak will have a greater dependence on the initial reagent-to-sample concentration ratio.

4.1.1.3 Reagent Economy

When reagents are limited or expensive, as is the case in many biotechnology applications, it may be necessary to consider conditions which minimize the degree of reagent waste. Reagent is wasted when there exists periods of time when only reagent is present in the detector, and therefore, no product can be formed or detected. In this study, this effect is estimated by

Equation 4-4

\[ R_E = \left( 1 - \frac{W_{RE}}{W_R} \right) \]
where $W_{RE}$ is the baseline width (measured in seconds) from the start of the reagent peak profile to the start of the sample peak profile and $W_R$ is the baseline width (measured in seconds) of the reagent zone as shown in Figure 4-2. The baseline width is measured from the baseline intersection of tangents drawn at the peak inflection points (as is often done in chromatography). This method of calculating the peak width de-emphasizes the effect of the low concentration of sample or reagent that exists in the leading or tailing edge of the peak profile and improves the reproducibility of the measurement. The ratio $R_E$ is expected to increase from 0 to 1 as the degree of reagent excess decreases. Higher values of $R_E$ are expected for lower reagent-to-sample volume ratios, however, this condition reduces sensitivity and zone penetration, and therefore, a compromise must be sought. Although this ratio is most applicable when the zones are of similar magnitude (i.e., $C_R^0 \approx C_s^0$), this ratio will still reach a high value even when the reagent peak profile is much taller than the sample, and is therefore a more useful optimizing parameter for determining excess reagent than the equivalent peak area ratio. As well, the degree of sample waste is inherently taken into consideration when optimizing the zone penetration parameter ($R_P$) which only reaches a value of 1 when the sample zone is completely penetrated by the reagent.
Figure 4-2. Overlap of sample and reagent zone showing the degree of reagent excess ($W_{RE}$) relative to the reagent width ($W_R$). The isodispersion point ($I_D$) represents the point of mutual zone penetration.

### 4.1.1.4 Throughput

Some applications of sequential injection analysis such as those found in high-volume analytical laboratories, may require a high sample throughput in order to maximize profit margins. For this dataset, throughput is estimated by considering the relative length of time for the peak to return to baseline at the detector after the sample and reagent zones have passed. The largest value in the dataset is used for normalizing the rest of the experiments considered according to
where $t_a$ is the time for the detector response to return to baseline and $t^{\text{max}}_a$ is the maximum length of time for the detector response to return to baseline for the series of experiments under consideration. In this way, the value for $R_r$ will range from 0 to 1 as the return time to the baseline decreases. In this work, the start time is considered to be at the onset of detector recording (which occurs after the sample and reagent zones are loaded), even though the throughput is affected to some degree by the length of time taken to initially load the zones into the injection line. However, since the time taken to load the zones is proportional to the length of time for detector recording, and the descriptor is normalized, the calculated result by inclusion of the zone-loading time would be approximately the same as the one used here.

4.1.1.5 Composite Function

The four conditions of maximum (i) sample zone penetration, (ii) sensitivity, (iii) reagent economy, and (iv) throughput can usually only be optimized at the sacrifice of one or more of the other conditions. For example, sensitivity may be improved at the expense of reagent economy or sample throughput. For any particular application, however, it may not be necessary to optimize all of the above conditions simultaneously. That is, if the sensitivity of an analysis is more important than reagent economy or throughput, optimal operating conditions might favour higher sample and
reagent volumes without concern for processing time. Or, for long-term unattended field-screening purposes where it is known that the monitored analyte has a relatively high concentration, reagent economy might be improved at the expense of sensitivity.

In order to investigate optimal conditions in several analytical situations, a weighted composite function of the above optimization parameters is investigated. This function is tunable for the desired influence of each parameter by appropriate weighting of the coefficients. This function is calculated as

Equation 4-6

\[ R_{\text{OPT}} = \frac{1}{k_T} \left[ k_1 R_p + k_2 R_s + k_3 R_E + k_4 R_T \right] \]

where the first term is a measure of sample zone penetration (Equation 4-2), the second term is a measure of sensitivity (Equation 4-3), the third term is a measure of reagent economy (Equation 4-4), and the fourth term is a measure of throughput (Equation 4-5). The relative weighting (on a scale of 0 to 1) for each of the coefficients, \( k_1, k_2, k_3, \) and \( k_4 \), is set according to the relative importance of the respective term to the analytical requirements. The value \( k_T \) is the sum of \( k_1 \) through \( k_4 \), thus maintaining a scale of 0 to 1 for the \( R_{\text{OPT}} \) value. Investigation of the \( R_{\text{OPT}} \) value with different weightings for profiles created over the full range of physical parameters (such as injection volumes, flow rate, and manifold geometry) will provide a better understanding of the influence each parameter has on the analyzer output.
4.2 Experimental

The experimental dataset described in Chapter 3, which was obtained using the sequential injection system described in Chapter 2, is evaluated here. The overlap of two peaks are considered by overlaying separate injection profiles for the sample and reagent zone. Peaks in the dataset which are considered to be reagent zones are ones in which 40, 80, 120, 160, 200, or 240 μL of dye is injected with 0 μL of spacer (1.0 M KCl) following it. Peaks which are considered to be sample zones are ones in which 40, 80, 120, 160, 200, or 240 μL of dye in 1.0 M KCl is injected with 40, 80, 120, 160, 200, or 240 μL of spacer following it (corresponding to the space taken by the reagent zone). It is assumed that the first injected zone is the sample zone because this produces an overlap situation which increases the likelihood of the sample zone being completely penetrated by reagent (as will become evident in the Results and Discussion).

4.3 Results and Discussion

Before quantifying optimal operating conditions based on the peak descriptors previously discussed, it is first necessary to consider several extreme examples of zone overlap which occur in the dataset. The first situation to consider is the shortest possible distance from the valve to the detector (15 cm) shown in Figure 4-3 at 2.0 mL min⁻¹ and Figure 4-4 at 0.5 mL min⁻¹. The four extreme possibilities for the sample and reagent injection volumes (V_s and V_r, respectively) are (a) V_r = V_s = 40 μL,
(b) $V_R = V_s = 240 \mu L$, (c) $V_R = 40 \mu L$, $V_s = 240 \mu L$, and (d) $V_R = 240 \mu L$, $V_s = 40 \mu L$

where $V_R$ is the reagent volume, and $V_s$ is the sample volume. Through inspection of these figures, it is possible to create a variety of peak dispersion profiles by changing the order of injection in addition to the injection volume. For example, injection of a 40 $\mu L$ zone changes shape considerably depending on whether it was injected before or after a 240 $\mu L$ zone (Figure 4-3d sample zone and Figure 4-3c reagent zone, respectively). These two figures also illustrate the reason for injecting the sample zone prior to the reagent zone since the overlap situation in Figure 4-3d will produce the most reproducible, meaningful detector response, similar in nature to flow injection analysis. In this situation, the entire sample zone is saturated with reagent and the product peak height will occur near the sample zone peak maximum. If the sensitivity of the analysis needs improvement, the sample zone volume can be increased such as in Figure 4-3b, although a limit to the height of the sample zone (or the overlap zone), is quickly reached. In situations of high sample volume (Figure 4-3b and c), the degree of penetration reaches a limit, and the tailing end of the sample peak becomes starved for reagent. In these cases, the product peak maximum is found closer to the isodispersion point between the two zones where reagent and sample concentration are at a maximum. Finally, in situations of low sample and reagent volume (Figure 4-3a), sample and reagent are conserved, and the throughput is high (ca. 180 samples hr$^{-1}$), but these benefits come at the expense of sensitivity and reproducibility.

The peak profiles are quite similar in Figure 4-4 where the flow rate has been reduced to 0.5 mL min$^{-1}$. The most notable change is the time scale which is three
times larger than that in Figure 4-3, thereby decreasing the throughput by a similar factor. In this case, decreasing the flow rate has almost no advantage since there is only a minor improvement in reproducibility and no improvement in the height of the isodispersion point.
Figure 4-3. Overlapped sample and reagent zones created by injecting either low volumes (40 μL) or high volumes (240 μL) of the dye, at 2.0 mL min⁻¹ with a valve-to-detector distance of 15 cm. In (a) $V_R = V_S = 40 \mu$L, (b) $V_R = V_S = 240 \mu$L, (c) $V_R = 40 \mu$L, $V_S = 240 \mu$L, and (d) $V_R = 240 \mu$L, $V_S = 40 \mu$L.

Figure 4-4. Overlapped sample and reagent zones created by injecting either low volumes (40 μL) or high volumes (240 μL) of the dye, at 0.5 mL min⁻¹ with a valve-to-detector distance of 15 cm. In (a) $V_R = V_S = 40 \mu$L, (b) $V_R = V_S = 240 \mu$L, (c) $V_R = 40 \mu$L, $V_S = 240 \mu$L, and (d) $V_R = 240 \mu$L, $V_S = 40 \mu$L.
Increasing the distance from the valve to the detector to 100 cm has a significant influence on the peak shape, as shown in Figure 4-5 and Figure 4-6. In all cases, the peak centroid and dispersion have increased. There is a more notable difference between high and low flow rates (Figure 4-5 and Figure 4-6, respectively) at this greater manifold length. For example, the same degree of zone penetration is evident in Figure 4-5a and Figure 4-6a, yet the heights of the zones in Figure 4-6a indicate improved sensitivity (i.e., they are approximately twice as high) and much greater symmetry is evident (this of course comes at a cost of a much lower throughput).

From the foregoing, it is clear that optimal operating conditions are arrived at through a dynamic balance between injection volume, flow rate, and manifold dimension. Further variation in peak shape and zone penetration can be achieved by modifying the shape of the manifold (i.e., straight tubes versus coiled), however only manifolds with the tubes wound around a cylinder of 1.0 cm diameter will be considered here since this situation is most commonly performed in practice. In general, it is the situation shown in (d) in each of these figures that is the most useful, especially when incorporating maximized sample zone height and throughput, with minimized reagent waste.
Figure 4-5. Overlapped sample and reagent zones created by injecting either low volumes (40 μL) or high volumes (240 μL) of the dye, at 2.0 mL min\(^{-1}\) with a valve-to-detector distance of 100 cm. In (a) \(V_R = V_S = 40 \, \mu L\), (b) \(V_R = V_S = 240 \, \mu L\), (c) \(V_R = 40 \, \mu L, V_S = 240 \, \mu L\), and (d) \(V_R = 240 \, \mu L, V_S = 40 \, \mu L\).

Figure 4-6. Overlapped sample and reagent zones created by injecting either low volumes (40 μL) or high volumes (240 μL) of the dye, at 0.5 mL min\(^{-1}\) with a valve-to-detector distance of 100 cm. In (a) \(V_R = V_S = 40 \, \mu L\), (b) \(V_R = V_S = 240 \, \mu L\), (c) \(V_R = 40 \, \mu L, V_S = 240 \, \mu L\), and (d) \(V_R = 240 \, \mu L, V_S = 40 \, \mu L\).
It is true that efficient zone penetration can also be obtained by "sandwiching" the sample zone between two reagent zones [3]. Although this is not hard to achieve in practice, the zone overlap dynamics of the second and third zones are going to follow similar trends as the first and second zones. Therefore, two-zone penetration is the focus of the current study, while consideration of the mutual penetration of three (or more) zones using similar descriptors and analytical conditions is left for future investigation.

4.3.1 Zone Overlap Descriptors

The $R_F$, $R_S$, $R_E$, and $R_T$ values will first be considered independently so as to examine their influence on the system response. Then, combinations of the functions will be considered for specific applications by appropriate weighting of the composite function. Realistically, an adequate understanding of the simultaneous combination of only two, or possibly three, of these optimization factors will be possible.

4.3.2 Analysis of Response Surface Maps

The data here are presented in the form of response surface maps [10-11] where the volume of the reagent and sample zones form the x and y axes and the overlap descriptor constitutes the z (or response) axis. Each shade of grey on the response surface indicates a 10% change on the response axis. As well, the reader should pay close attention to the x and y axes which may change direction (40 µL to
240 \mu L or 240 \mu L to 40 \mu L) in order to provide the best perspective on the response surface. The 3-D view and orientation of the surface will not be varied, and the sample volume will always appear on the left axis and the reagent volume on the right axis.

4.3.2.1 Zone Penetration

The first and most important descriptor in sequential injection analysis is zone pentration. This has been noted in the work of Gübeli et al. [3] who used a slightly different descriptor defined in Equation 4-1. The data shown in Figure 4-7, however, indicate the degree of sample zone penetration as defined by Equation 4-2. Shown are individual response surface maps for 15, 50, and 100 cm manifolds at 0.5 and 4.0 mL min\(^{-1}\). In all cases maximum penetration of the sample zone occurs at the lowest volume of sample and highest volume of reagent. This is an obvious result but as the sample volume is decreased, so is the sensitivity of the analysis. If the sensitivity of the analysis is not of concern, then it is recommended that the smallest sample volume that can be reproducibly injected be used, while a minimum of twice as much reagent be used (consider the leftmost corner of the response surface map where the increase in response as \( V_R \) increase from 40 to 80 \mu L is significant). In this way, the sample zone will be almost completely penetrated (\( R_p > 0.9 \)) and as long as \( C_R^0 \gg C_s^0 \), the product concentration profile will have the characteristics of a flow injection response profile with the maximum height near the sample zone. Increasing
the reagent volume to greater than twice the sample zone provides no further improvement (consider all points where $V_s = 40 \mu$L).

There appears to be no advantage to increasing the manifold length beyond 15 cm since the surface maps (a), (c), and (e) all appear similar in shape, as do (b), (d), and (f). The greatest amount of zone penetration occurs in (f) where a plateau is reached at $V_s < 120 \mu$L and $V_R > 120 \mu$L. This occurs because higher flow rates over longer distances cause the bolus shape to extend axially and penetrate the next zone to a greater degree. It is also important to note that increasing the reagent volume is less effective at increasing zone penetration than decreasing the sample volume (i.e., the slope of the map is steeper from right to left than from front to back).

It must be remembered that this parameter only considers the area of the sample peak which is overlapped by reagent and does not take into account the height of the overlapped zone or the sample peak. Thus, if sensitivity of the analysis is important, then the information provided by this parameter must by considered in conjunction with the sensitivity parameter which is discussed next.
Figure 4-7. Response surface maps of sample zone penetration ($R_p$) as defined by Equation 4-2.
4.3.2.2 Sensitivity

When optimizing a reagent-based chemistry, it is usually necessary to search for conditions which produce the most sensitive analysis. In flow injection analysis, this is typically done by increasing the sample injection volume and ensuring that the system dispersion is kept to a minimum. A limit is reached, however, as $D_s$ approaches 1, or when the reagent on either side of the sample is unable to completely penetrate the center of the sample zone (thereby creating a “double peak” due to a lack of reagent).

For sequential injection analysis, it is expected that the sensitivity of the analysis will increase with increasing injection volumes of sample and reagent (assuming a stoichiometric concentration ratio between the sample and reagent). Figure 4-8 shows the sensitivity of the analysis as measured by the maximum height of the overlap (Equation 4-3) which is initially the isodispersion point, and then shifts to the height of the sample or reagent zone as the ratio $V_R : V_S$ or $V_S : V_R$, respectively, becomes large. These surfaces indicate that lower flow rates (at all manifold dimensions) produce the most sensitive analysis. This is to be expected since the slope of the interface between the two zones (which has a significant influence on the height of the isodispersion point) is expected to be much greater due to a decrease in parabolic extension of one zone into the next. The surfaces also indicate that a maximum (plateau) is reached at lower volumes for shorter manifold lengths, although the same height (degree of sensitivity) is attainable for all manifold lengths (e.g., a value of about 0.95 is reached for $R_s$ in all cases for 0.5 mL min$^{-1}$).
One final important feature is found within these response surface maps. As mentioned previously, the measure of sensitivity increases first with the isodispersion point, and then reaches a maximum defined by the height of the sample or reagent zone (usually whichever is the lesser of the two injected volumes). By considering the injection of a constant volume of sample with an increasing amount of reagent, it would be expected that the sensitivity would increase with the reagent volume (if the reagent concentration is limiting). It is also expected that the sensitivity will reach a maximum after which the sensitivity will decrease with the decreasing peak height of the sample zone. The peak height of the sample zone decreases due to the proportionally longer distance that the sample zone must travel as the reagent zone is increased. This relationship is evident in the data by considering Figure 4-8a along the line where $V_s = 80$ μL and $V_R$ ranges from 40 to 240 μL. The sensitivity first increases with reagent volume and then decreases. By close inspection, the same trend is evident on other surfaces as well. It is also important to note that this same decrease after maximum sensitivity does not occur when considering lines of constant (low) reagent volume (e.g., Figure 4-8a where $V_R = 80$ μL). This leads to an important point that this particular measure of sensitivity of the analysis must be used with caution in that since $C_R^0 \gg C_s^0$, the upper limit reached at high $V_s$ with $V_R = 80$ μL is actually somewhat pessimistic. It is for this reason, that for optimization purposes, this parameter should be considered in conjunction with the zone penetration parameter which improves upon saturation of the sample zone with reagent. This will be done later in this chapter.
Figure 4-8. Response surface maps of sensitivity ($R_s$) as defined by Equation 4-3.
One of the significant advantages of performing a reagent-based chemistry by the sequential injection method is the ability to control the volume of reagent used in the analysis and, therefore, significantly reduce the amount of reagent waste. Although increasing the reagent volume increases the degree of sample zone penetration, this too will reach a maximum after which reagent will begin to be wasted. The degree of reagent economy is quantified here by the parameter $R_E$ as defined in Equation 4-4 and shown in Figure 4-9. Higher values of $R_E$ indicate a reduction in time where the detector contains reagent but no sample. The response is generally flat in nature, indicating little dependence on sample and reagent volumes. The largest difference in $R_E$ (i.e., $0.65 < R_E < 0.95$) is present in (e) and thus reagent economy should be taken into greater consideration when developing methods with low flow rates and long manifolds. This again indicates that zone penetration is less effective at low flow rates, thereby decreasing the degree to which the reagent zone is penetrated by the sample zone. In general, this data show the greatest dependence on the volume of the reagent zone with the least reagent wasted when the least amount is used. This obvious result needs to be balanced with the degree of zone penetration and sensitivity which are lowest at low reagent zone volumes. Therefore a combination of these parameters will be considered later in this chapter.
Figure 4-9. Response surface maps of reagent economy ($R_E$) as defined by 4-4.
4.3.2.4 Throughput

In high-volume analytical laboratories, one of the most important criteria upon which one judges an analytical system is its sample throughput rate. The throughput of a system will determine the number of samples that can be processed per unit time, thereby decreasing the cost per analysis. In this work, the throughput of the system is measured by the length of time for the detector signal to return to baseline after both sample and reagent zones have passed. The value of $R_T$ as defined by Equation 4-5 is normalized by the longest time for the signal to return to baseline for the entire dataset. Figure 4-10 shows the influence of sample and reagent volume on this parameter, however, data for 2.0 mL min$^{-1}$ have replaced the 4.0 mL min$^{-1}$ data since this parameter is effectively higher than 0.9 for all flow rates higher than 2.0 mL min$^{-1}$. As expected, $R_T$ decreases slightly with increasing manifold length and significantly decreasing with flow rate. However, it is at lower flow rates and longer manifold lengths that the greatest zone penetration and most sensitive analyses are found. Thus, any zone penetration or sensitivity advantage that a lower flow rate or longer manifold length presents is diminished when this function is taken into consideration.
Figure 4-10. Response surface maps of throughput \( R_T \) as defined by Equation 4-5.
4.3.2.5 Composite Function

Several combinations of these four optimization parameters will now be considered and the trade-offs between various optimal conditions will be contemplated. With appropriate weighting of \( k_1, k_2, k_3, \) and \( k_4 \), the composite function defined in Equation 4-6 will allow visualization of optimum operating conditions which exist as compromises between two or more factors.

4.3.2.5.1 Maximum Sample Zone Penetration and Sensitivity

The first, and most significant combination to consider is conditions which produce the greatest sample zone penetration \( (R_p) \) with the greatest sensitivity \( (R_s) \). This is equivalent to combining the surface maps in Figure 4-7 and Figure 4-8, and essentially illustrates the trade-off between increasing the sample volume to obtain better sensitivity with a reduction in zone penetration as a consequence. These maps are shown in Figure 4-11 where \( k_1 = k_2 = 1, \) and \( k_3 = k_4 = 0 \). The shape of these surfaces is similar in all cases. As anticipated, the maximum response “plateau” is found where \( V_R \) is greater than \( V_S \), and is triangular in shape. Surprisingly, the lowest value of sample and reagent which creates optimum zone penetration and sensitivity is the same for every manifold length and (volumetric) flow rate. In every case, a value of \( V_S = 80 \) \( \mu \text{L} \) and \( V_R = 120 \) \( \mu \text{L} \) reaches the highest plateau, and injection of volumes larger than this do not improve the composite function by a significant amount. These volumes, which do not seem to be related to the \( S_N \) value for each system (compare with \( S_N \) graphs in Chapter 3), are more likely a function of tube diameter which effects
the length to area ratio of the injected zones. These optimal values would likely be proportionally lower (with $V_R = 1.5 V_S$) at a smaller tube diameter, however, additional data would be needed to confirm this. Marshall and van Staden [9] showed that reduction in tube diameter results in greater zone penetration (as measured by Equation 4-1 using area instead of baseline width). For reference, the $S_{x}$ value for the conditions shown in each surface map is approximately (a) 60 µL, (b) 60 µL, (c) 120 µL, (d) 140 µL, (e) 150 µL, and (f) > 240 µL.

It is evident that any decrease in sensitivity is counter-balanced with a nearly equal increase in zone penetration, thereby maintaining the level of optimum $R_{opt}$. Taking this into consideration, there is little advantage to increasing the distance between the valve and the detector more than the minimum necessary (usually about 15 cm) unless the degree of zone penetration is ultimately more important than sensitivity. Even if this is true, an increase in zone penetration can be effected by aspirating the two zones further (towards the pump) before reversing the flow towards the detector, or by multiple flow reversals [3]. The extreme situation to consider here is to have the detector right at the valve (e.g., by using a fiber-optic cable). It will be shown theoretically in Chapter 5 that the greatest sensitivity is achieved by monitoring the product concentration at the valve.
4.3.2.5.2 Maximum Sample Zone Penetration and Reagent Economy

If the sensitivity of the analysis is sufficient for the samples requiring measurement, then it would be necessary to consider conditions which produce maximum zone penetration with minimum reagent consumption. This is shown in Figure 4-12, again by using the composite function with appropriate weighting of the coefficients. In general, reducing the sample volume as much as possible (while maintaining reproducible injection volume) and ensuring that the reagent volume is at least twice the sample volume produces the most optimal analysis for this application. Only slight improvement is found by lowering the flow rate or increasing the manifold length. This composite function is dominated by the zone penetration surface which is more dramatic than the reagent economy surface, and is primarily influenced by the choice in sample volume.
Figure 4-11. Response surface maps for the composite function, $R_{OPT}$, where $k_1 = k_2 = 1$, and $k_3 = k_4 = 0$ according to Equation 4-6.
Figure 4-12. Response surface maps for the composite function, \( R_{OPT} \), where \( k_1 = k_2 = 1 \), and \( k_2 = k_4 = 0 \) according to Equation 4-6.
4.3.2.5.3 Maximum Sample Zone Penetration, Sensitivity, and Throughput

After having considered conditions which produce optimal zone penetration with the greatest sensitivity, it is now interesting to combine these results with conditions which produce the highest throughput. Optimization of this situation would produce the most reliable, sensitive, and fastest analysis possible. Figure 4-13 indicates that the same optimal volumes of \( V_S = 80 \, \mu L \) and \( V_R = 120 \, \mu L \) are still maintained, however the composite function (which now includes throughput) is significantly increased by an increase in flow rate. Increasing the flow rate to 6.0 mL min\(^{-1}\) only serves to increase the irreproducibility in the analysis owing to an increased likelihood of creating air bubbles, decreased precision of injection volume, and a shortened length of time during which a reliable measurement can be made.

4.3.2.5.4 Maximum Sample Zone Penetration, Sensitivity, Reagent Economy, and Throughput

Finally, if reagent economy is included in the composite function, the response surfaces shown in Figure 4-14 are generally the same as those in Figure 4-13. Although the surfaces become less sensitive to local variations because of the combination of all four optimization parameters, the general relationship of high reagent to sample volume ratio is still apparent. As well, improvement due to increased flow rate at all manifold lengths is evident at 4.0 mL min\(^{-1}\) due to a substantial improvement in throughput. The influence of reagent excess \((R_E)\) is noticeable at low flow rates (0.5 mL min\(^{-1}\)) and high \( V_R \) where the optimal response begins to decrease slightly. As
well, there is a slight shift in optimum at 0.5 mL min$^{-1}$ to a slightly lower reagent injection volume, although the same general shape to the "plateau" applies.
Figure 4-13. Response surface maps for the composite function, $R_{OPT}$, where $k_1 = k_2 = k_4 = 1$, and $k_3 = 0$ according to Equation 4-6.
Figure 4-14. Response surface maps for the composite function, $R_{OPT}$, where $k_1 = k_2 = k_3 = k_4 = 1$ according to Equation 4-6.
4.4 CONCLUSIONS

The optimization of the zone overlap of a single sample and reagent zone is considered under conditions of variable injection volume, linear flow rate, and manifold dimension. Initially, several extreme sets of conditions are shown and their relevance to optimal sequential injection operating conditions are discussed. Several new descriptors are presented for optimizing the zone overlap under various analytical requirements. These descriptors are evaluated in terms of the information and guidance that they can provide when designing a new method. All four optimization parameters are shown as response surface maps on their own, and several are combined using a composite function. In general, the combination of optimization parameters for sample zone penetration ($R_P$) and sensitivity ($R_S$) are the most significant when deciding upon optimal conditions. In almost all cases, the minimum injection volumes for $V_S$ and $V_R$ which provide maximum penetration and sensitivity, are independent of the flow rate and the manifold length.

The results here can also be used to gain a better understanding of conditions which will produce optimal overlap of more than two zones. Optimal conditions for the first and second injected zone should be similar for the second and third zone, and so on. It has already been shown [3] that by increasing the number of zones stacked in the manifold, multiple-reagent chemistries can be employed by sandwiching the sample zone between two different reagent zones. Consideration of this situation using these
new descriptors should constitute a separate study, in addition to validating the results of this chapter with real chemistries.
4.5 REFERENCES


5. Random-Walk Model for Sequential Injection Analysis

"A good simulation, be it a religious myth or scientific theory, gives us a sense of mastery over experience. To represent something symbolically, as we do when we speak or write, is somehow to capture it, thus making it one's own. But with this appropriation comes the realization that we have denied the immediacy of reality and that in creating a substitute we have but spun another thread in the web of our grand illusion."

Heinz R. Pagels

5.1 INTRODUCTION

The random-walk model of Betteridge et al. [1] gave practitioners a powerful conceptual basis for understanding flow injection analysis. The model involved simulation of multiple discrete "molecules" within the flow manifold. In each $\Delta t$, each molecule took a diffusion step and then was moved a distance along the tube due to flow rate and laminar flow. Users could simulate ten parameters including sample size, reaction rate, molecular diffusion coefficient, temperature and flow rate; as such, it was the first realistic attempt at modeling the complex interactions of molecular diffusion,
convection and reaction kinetics. The speed of laboratory microcomputers at that time limited the number of molecules simulated to ca. 1500, and so only semiquantitative predictions of peak height versus time were possible. Despite this, simulations compared against experimental results were found to be of practical use [2]. It was recognized at the time that a 100-fold increase in computational speed was necessary if complex problems (e.g., pH gradients, solvent extraction) were to be adequately addressed. Recently, Wentzell and co-workers revisited the random-walk model [3] for flow injection analysis. By lifting some earlier limitations on computational speed they obtained accurate predictions of peak shape and duration using up to 1,000,000 molecules (piece-wise) per simulated run.

The advent of sequential injection analysis in 1990 brought with it an experimental requirement for far more stringent control of flow and timing. In early FIA systems flow was continuous and unidirectional, and a microcomputer (if present at all) served solely as a recording device - now the microcomputer has become an essential part of the analyzer. The sequential injection analyzer built for this work has as many as 21 parameters under computer control, any of which can change in each of the 3 to perhaps as many as 15 sequential steps that comprise a method. Designs for future sequential injection analyses can be expected to produce more complex peak shapes than FIA because of the mutual partial overlap of multiple zones (reagents, sample and spacers) occurring in conjunction with at least one flow reversal operation. It would be extremely difficult to create a continuous model that dealt with this complex injection
and dispersion procedure. This leaves discrete-time simulation as the only real viable solution to modeling these interactions.

Keeping in mind the recent further advances in computational speed of the desktop computer, it is proposed in this work that the sequential steps of SIA operation should be amenable to random-walk modeling. Towards this end, this chapter will report a novel simulation technique which is able to simulate the physical sequential injection procedure, as well as incorporate the flow reversal and dispersion throughout the entire manifold. This model can thus be used to gain a better understanding of physical dispersion within the manifold and to predict optimum zone penetration and timing for real sequential injection methods.

Definable parameters in this model include flow rate (sinusoidal or linear flow), number of zones injected, internal tube diameter, temperature, valve-to-detector distance, detector volume, number of flow reversals, and length of each flow reversal. In sinusoidal flow, the pump period, pump start angle, valve switching time, and the syringe and cam radius are all user-specified. The model can track up to four sequentially stacked zones of reagent or sample. The diffusion constant, relative concentration, and zone length (specified by time) can be specified for each injected zone. Dispersion profiles produced with this model will be shown in comparison with experimental peaks under similar operating conditions.
5.2 Theory

The random-walk model, originally proposed in 1905 by Einstein [4] to explain Brownian motion, has been used to simulate many physical systems which incorporate stochastic events [5-7]. Two similar areas of study which have made use of this model for simulating manifold conditions are chromatography [8-9] and flow injection analysis [1-2]. In both cases, the model was shown to correspond well with experimental results.

The basic premise that the flow injection simulations relied on is the tracking of the three-dimensional position of individual molecules as they progress through the manifold under a defined set of conditions. Each molecule tracked is allowed a three-dimensional diffusional step of random length, in addition to an axial movement due to convective flow according to a laminar flow profile. The justification for the diffusional step is as follows. The relative spread of the sample (originally injected as a square "plug") is given by the probability $\langle y^2 \rangle$ of finding a molecule at a distance $y$ from the origin [6] according to

Equation 5-1

$$
\langle y^2 \rangle = \frac{1}{2\sqrt{\pi D_m t}} \int_{-\infty}^{\infty} y^2 \exp\left(-\frac{y^2}{4D_m t}\right) dy
$$
where $D_m$ is the molecular diffusion constant for the process and $t$ is the length of time per step. If a large number of molecules take a large number of steps, $n$, of average length, $l$, at $v$ steps per unit time, then the relative spread is given [6] by

Equation 5-2

$$\langle y^2 \rangle = 2D_mt = n l^2 = vtl^2.$$  

In Chapter 3, it was shown that this relationship holds for the sequential injection technique by considering the second moment of the peak profile as a relative measure of dispersion. With appropriate statistical treatment, it can be shown [6] that the average movement of a given step of $\Delta t$ in any one dimension is $\Delta l \approx \sqrt{2D_m\Delta t}$. Correcting for viscosity $\eta$ (mPa·s) of an aqueous solution (and therefore temperature, °C) via the relation [15]

Equation 5-3

$$\log\left(\frac{\eta_{20}}{\eta_T}\right) = \frac{[137023(T - 20) + 8.36 \times 10^{-4}(T - 20)^2]}{(109 + T)}$$

leads to the corrected step length of

Equation 5-4

$$\Delta l = \frac{\sqrt{2D_m\Delta t}}{(\eta_T/\eta_{20})}$$

in any one dimension. The actual distance moved in $\Delta t$ seconds for a given dimension is given by $\Delta x = \pm rnd(2\Delta l)$ since $\Delta l$ is the average step length and $rnd$ is a random number uniformly distributed in the range [0, 1], where the sign of the movement is
taken as random with equal likelihood of either outcome. This distance is more easily computed in the simulation by the equivalent expression

\[ \Delta x = \Delta y = \Delta z = 4\Delta l \text{md} - 2\Delta l. \]

A new coordinate for each dimension is calculated from the old coordinate for the \( \text{ith} \) molecule in this way, via

\[ x_{i(\text{new})} = x_{i(\text{old})} + \Delta x \]
\[ y_{i(\text{new})} = y_{i(\text{old})} + \Delta y \]
\[ z_{i(\text{new})} = z_{i(\text{old})} + \Delta z + \Delta q \]

where \( \Delta q \) is due to the axial convective movement due to laminar flow at the tube radius, which is determined by

\[ r_i = \sqrt{x_{i(\text{new})}^2 + y_{i(\text{new})}^2}. \]

If \( r_i > r_0 \) then the new radius would lie outside of the tube boundaries. In this simulation, as in the original flow injection simulation [1], this is corrected by assuming a bounce boundary, where the new coordinate \((x_{i(\text{new})}, y_{i(\text{new})})\) is set equal to the old coordinate \((x_{i(\text{old})}, y_{i(\text{old})})\) and the new axial velocity is set equal to half of the old axial velocity (i.e., \( \Delta q_{\text{used}} = 0.5\Delta q \)).
More recently, Wentzel et al. [3] made several modifications to the model in order to determine the effect of step size and wall approximations on the model. The mean molecular step size, \( \Delta l = \sqrt{2D_m \Delta t} \), will influence the validity of the algorithm because of approximations made in calculating axial flow rate and in wall interactions. The easiest method of reducing the step size (\( \Delta l \)), and therefore improve the simulation results, would be to decrease the time interval between iterations, \( \Delta t = 1/N \), by increasing \( N \), the number of iterations per second. This modification, however, comes at the cost of increased computation. The original work by Betteridge et al. [1] used \( N = 1 \), throughout, while Wentzel et al. [3] show peak profiles for \( N = 1, 5, 10, \) and \( 100 \), which asymptotically approach the limiting profile as \( N \) gets larger, and \( \Delta t \) gets smaller. The conclusion reached by Wentzell was that the mean step size should be no more that 5 to 10% of the tube radius. In the work presented here, the value of \( N \) can be calculated by first solving the mean molecular step size for \( \Delta t \)

\[
\Delta t = \frac{\Delta l^2}{2D_m}.
\]

By using \( \Delta l = 0.002 \) cm, corresponding to 5% of the tube radius, and \( D_m = 7.6 \times 10^{-8} \) cm\(^2\) s\(^{-1}\) for the experimental dye used, a result of 0.26 s for \( \Delta t \), corresponding to \( N = 3.8 \) is obtained. In this work, \( N = 10 \) was used (unless otherwise noted) which provides a sufficiently small step size according to Wentzell's recommendations.
Two other compensating factors were also attempted by Wentzell. The first modification was to add the average axial velocity between points \((x_{i(old)}, y_{i(old)})\) and \((x_{i(new)}, y_{i(new)})\) instead of the axial velocity at point \((x_{i(new)}, y_{i(new)})\) only. The second was to calculate the actual position of the molecule after the "wall bounce" experienced. However, they found it computationally simpler to reflect the molecule back along its original trajectory, rather than correctly bounce it off the wall. This is a valid approximation as long as the flow profile is radially symmetrical. Their results showed that inclusion of these two compensating factors produced the same results as when the step size was sufficiently small. Thus, these compensating factors are generally only necessary for small \(N\), and have not been considered here.

Assuming parabolic laminar flow conditions hold, the flow velocity (cm s\(^{-1}\)) at any given radius, \(r\), from the center of the tube can be expressed as

**Equation 5-11**

\[
U_r = U_{\text{max}} \left( 1 - \frac{r^2}{r_0^2} \right)
\]

where \(U_{\text{max}} = 2\bar{U}\) (according to Taylor [10]), \(\bar{U}\) is the average flow velocity (cm s\(^{-1}\)) and \(r_0\) is the radius of the tube (cm). Thus, \(\Delta q\) can be calculated by

**Equation 5-12**

\[
\Delta q = \Delta t U_{\text{max}} \left( 1 - \frac{r^P}{r_0^P} \right)
\]
where $P = 2$ for regular laminar flow according to Taylor [10]. Modification of the flow profile has been done [3] by adjusting the power factor used in Equation 5-12, and by modifying the asymmetry of the flow profile. In the former case, improved fit between simulated and experimental dispersion profiles was realized in some situations when $P$ was adjusted to values other than 2 (i.e., 1, 3 and 4 were also tried by Wentzell). The effect of this factor will be investigated in this work as well, using a corrected value of $U_{\text{max}} = \bar{U}(P+2)/P$. Wentzell found that modification of the flow profile asymmetry was not able to compensate for the experimental profiles obtained with secondary flow (i.e., when tubes were coiled) as anticipated [3], and thus, is not considered in this model.

A typical sequential injection procedure requires that the flow be started, stopped, and paused, several times, in addition to changing direction, and that the dispersing sample must pass through non-ideal valve and detector geometry. Ideal laminar flow requires a finite amount of time to set up and is reduced by such imperfections in the manifold geometry. This is sometimes referred to as "developing flow" and it typically takes 60 to 70 pipe diameters before laminar flow is fully developed. This corresponds to 6 to 7 centimeters of tube length or 47 - 55 μL of injection volume before we can expect stable laminar flow (assuming an approximate tube diameter of 0.1 cm). Consequently, the likelihood of the experimental flow profile exhibiting ideal laminar characteristics is reduced, especially for injection volumes or fluid movements less than 40 μL.
Hence, in order to approximate this non-ideal behaviour, a new parameter, $\zeta$, which adds an average-flow term to the flow profile, was proposed in this work. This parameter is used to adjust the axial flow equation according to

\begin{equation}
U_r = \zeta U_{\text{max}} \left(1 - \frac{r^2}{r_0^2}\right) + (1 - \zeta) \overline{U}
\end{equation}

where $0 \leq \zeta \leq 1$, $U_{\text{max}} = 2\overline{U}$, $\overline{U}$ is the average flow velocity (cm s$^{-1}$) and $r_0$ is the radius of the tube (cm). In this way, the non-ideal flow conditions are approximated by adding the average flow velocity term on the right. In the limiting cases when $\zeta = 1$ the flow is purely laminar, and when $\zeta = 0$ the flow is purely plug flow.

### 5.3 Injection Procedure

Previously, flow injection systems were simulated with the use of the random-walk by randomly placing a large number of molecules within a defined hypothetical "sample loop" with the center of the sample zone taken as the origin of the coordinates. Then, for each molecule, a random diffusion step was added, as well as the convective step due to a parabolic flow rate for each iteration of the simulation. The simulation proceeded as the molecules were propelled and diffused towards the detector. Peak profiles were then created by either (i) integration of the molecules in each of several zones that the manifold tube had been divided into, or (ii) integration of the molecules passing through a discrete detector zone as time passes. The former technique allows
visualization of the dispersion process as it occurs, while the latter creates peak profiles which are comparable with those formed with an experimental detector.

The injection procedure for the sequential injection technique is much more complex, and thus, the difficulty in using this type of model lies in devising an appropriate injection procedure that conforms as closely as possible to reality. It was thought that instantaneous injection of a square plug in the same manner as the simulated flow injection process would be gravely over-simplifying the situation, especially for long injection times. A simulated injection procedure which incorporates connection of a multi-position valve to several positions with sequential stacking of sample and reagent zones into the manifold was needed. To this end, the following injection procedure is proposed and has been implemented in this model:

1. The model begins by simulating the valve movement to the first position for the length of time specified by the user (usually 1.0 s, corresponding to 10 iterations when $\Delta t = 0.1$ s). Since there are no molecules injected at this point, this step only serves to increment the model timer from the “start” of the analysis.

2. A predetermined number of molecules are positioned randomly in the tube of radius, $r_0$, with axial boundaries of $0 \leq z \leq \Delta i$ according to Figure 5-1. The number of molecules (typically 2500) and the length (cm) of $\Delta i$ (typically 2.0 cm) are both user-defined.
Figure 5-1. Tube variables used in the sequential injection simulation; the tube is described by $x$, $y$, and $z$ Cartesian coordinates, with the multi-position valve interface defined at $z = 0$.

3. The flow is reversed (towards the negative $z$-direction) for one iteration of $\Delta t$ (typically 0.1 s). A random diffusional step of length according to Equation 5-5 is added to each dimension. Then, the new $x$- and $y$-coordinates are used to determine the convective step size which is added in the (axial) $z$-direction. If sinusoidal flow is requested, the current flow rate is determined from the current pump head angle, which is subsequently updated for each $\Delta t$.

4. A fraction of the (2500) molecules will have stepped far enough in the $z$-dimension such that their new $z$-coordinate is negative (i.e., they have moved from the supply line, through the valve and into the injection line). These molecules are transferred to a new array in memory and are considered to be "injected." The molecules that did not step far enough in the negative $z$-direction (i.e., they are still in the supply line) are discarded.
5. Steps 2, 3, and 4 are repeated for the length of time of injection of this zone. For example, to inject 3.0 s of this sample or reagent, with $\Delta t = 0.1$ s would require 30 iterations of steps 2 through 4.

6. After the first zone is injected into the injection line, the valve movement must again be simulated. This is done by setting the flow rate to zero (as it is in reality while the valve moves), for the length of time of the valve movement. During this time, random diffusional movement of the injected molecules is still allowed to occur as it would in reality.

7. If there are further zones to inject (up to four sequentially stacked zones are possible in this model), then the above process (steps 2 through 6) is repeated with the molecules of the four (maximum) injected zones being “tagged” as molecule type 1 through 4 in their respective array in memory.

8. If multiple flow reversals are requested, then the molecules are tracked while the flow rate is alternated from positive to negative for the user-specified length of time.

9. Finally, the flow is set to positive (towards the detector) and the molecules are moved back through the valve and into the line connecting the valve and the detector. Each of the four molecule types with coordinates such that $d \leq z_i \leq d + \Delta d$ according to Figure 5-2 are integrated separately, and displayed
graphically in real time as overlapping peaks on the simulation detector. The distance \( d \) is determined by the length of tubing from the valve to the detector, and \( \Delta d \) is calculated such that the volume of the detection zone is equivalent to the volume of the experimental detector assuming a constant tube radius. The simulation is stopped after the user-defined detection time has expired or after five seconds of post-peak baseline have been tabulated, whichever comes first.

Figure 5-2. Simulation parameters for the detection line, and the detection zone.

10. The total number of molecules simulated in each zone is recorded in the database (where all of the other simulation information for each run is stored as individual records), and the peak profile is recorded as a text file to disk for future retrieval. If replicate runs are desired (in order to increase the number of simulated molecules and thus reduce stochastic noise), the simulator repeats steps 1 through 9. The new number of simulated molecules in each zone is added to the previous values, and the new peak profiles are overlaid on top of the old ones.
5.3.1 Theoretical Considerations of the Injection Procedure

5.3.1.1 Molecular Concentration

There are several issues to consider regarding this injection procedure. The concentration of molecules placed in the region $\Delta i$ (determined by the number of molecules placed in the zone and the length of the injection zone, $\Delta i$) should be great enough to provide a smooth simulation, without overfilling the maximum array size of 32,767 molecules for a given injected zone. For example, if 5000 molecules are placed in the injection zone, and of these, approximately 2000 molecules move through the valve on each iteration of 0.1 s, then any more than 16 iterations (equivalent to 1.6 s of injection time) would exceed the maximum array size. If it is overfilled on the first injection, the simulation automatically cuts the number of injected molecules in half (thereby reducing the molecular concentration by a factor of 2) and doubles the number of replicates to run. In this way, the same total number of molecules are simulated, over twice as many runs.

5.3.1.2 Axial Step

The distance $\Delta i$ should be great enough such that, at the prescribed flow rate and iteration time, no molecules are able to make an axial step greater than this distance. This prevents the situation where gaps might occur in the molecular spacing within the manifold, especially nearest the center of the tube where the molecules are
making the largest axial steps due to laminar flow. If these molecular gaps occurred, "sawtooth" peak profiles might be produced at the detector as the concentration of molecules fluctuates. This situation was prevented by ensuring that \( \Delta i \) exceeds the distance moved by a molecule in one iteration at twice the average linear flow velocity, including the maximum axial diffusional step possible.

### 5.3.1.3 Supply Line

The purpose of step 4 in the injection procedure is to simulate the supply of fresh sample or reagent at full concentration at the beginning of each iteration. If the supply line was not recharged after each step, the concentration of molecules within it would soon become parabolically distorted with the center of the tube (nearest the \( +z \) end of the injection zone) depleting of molecules first. More importantly, by only simulating say 2 cm of supply line instead of the full length of 20 to 100 cm, a large number of calculations are eliminated since less molecules need to be simulated. This makes the approximation, however, that the flow rate step is sufficiently great to minimize the effect of molecules drifting "backwards" from the injection tube into the supply line. This unlikely situation would only occur near the wall boundaries where \( U \) approaches zero but the diffusional step in the \( z \)-direction can still be large enough for an "injected" molecule to move back into the supply line.
5.3.1.4 Detection Zone

The length of the detection zone is determined from the user-specified volume of the detector, using the same internal diameter tubing as the manifold. Since the physical flow-cell and manifold tubing used in the sequential injection analysis system have an internal diameter of 1.0 mm and 0.84 mm respectively, $Δd$ (in Figure 5-2) can be extended to maintain constant detection volume as an approximation. This means that although the length of the integration zone for the experimental flow-cell and the simulation are not the same, their total volumes are.

5.3.1.5 Determination of Simulated $C^o$

The question arises as to what value to use for $C^o$ so that the simulated peak profiles can be compared on the same vertical scale as experimental peaks. Having an accurate simulated value for $C^o$ would easily facilitate comparison without the need for normalizing peak profiles by peak area as had been done in previous work [3]. As well, knowing $C^o$ would allow the simulation to predict actual peak height instead of just relative peak height. In order to determine $C^o$, the concentration of molecules for an undispersed sample must be known, that is, the number of molecules found within the detection zone if the pure sample was flowing through it. Intuitively one would like to use the molecular concentration found in the sample line injection region $Δi$, although this would not be truly representative of the actual injected sample since these molecules are replaced at every iteration. Instead, $C^o$ was calculated by dividing the
total number of molecules that actually become part of the simulated sample plug (and are not discarded in step 4 of the injection procedure) by the injection volume. This gives the molecular concentration at the point of injection even though by the end of the injection period, molecules have dispersed throughout a volume greater than the injection volume. Filling the detection zone with this molecular concentration and integrating the molecules provides a value for $C^o$ which can be used to normalize the entire simulated peak profile for comparison with experimental profiles. It will be shown that peak areas calculated on this basis are in excellent agreement with experimental peak areas under all conditions.

5.4 Experimental Peaks

In flow injection analysis, others have successfully developed specialized apparatus which creates conditions which are very close to perfect laminar flow [14]. Physically, this is not difficult to do when working with a sample zone of specific volume, and unidirectional, continuous flow. The sequential injection technique, however, involves repeatedly starting and stopping the flow, in addition to flow reversal of the injected zone(s) back through a possibly imperfect valve channel before reaching the detector. Therefore, it is thought that construction of an apparatus capable of producing perfectly laminar flow under these conditions would be extremely difficult, if not impossible. However, it is possible to minimize any physical perturbations in the manifold which would reduce the laminar flow characteristics of the experimental flow
profile. Towards this end, the following steps have been taken to minimize such perturbations:

1. The manifold tubing was kept as straight as possible in order to minimize secondary flow.

2. The pulsing from the peristaltic pump was reduced as much as possible by appropriately adjusting the tube tension, coating the pump tube with silicone oil, and keeping the distance between the pump and the supply lines or waste lines as long as possible (since friction in the tube can dampen the pulsing).

3. There is a 180° bend at the injection valve which is unavoidable due to the nature of the mechanism. The flow channel in the valve plate has similar dimensions to the manifold tubing and does not have any dead volume.

4. An axial flow-cell with a very small total volume (7.85 μL) was used which has an internal diameter similar to the manifold tubing (1.00 mm and 0.84 mm, respectively). The manifold tubing from the valve was inserted into the flow-cell all the way up to the detection zone (at a 90° angle) where the flow enters the light path. Thus, there are no unions in the manifold tubing between the valve and the detection zone.
Experimental peak profiles from the tracer dye, 1.5 mM K₃Fe(CN)₆, made up in 1.0 M KCl, dispersing in a stream of 1.0 M KCl, are used for comparison to the model. An injection volume of 80 µL, injected at a flow rate of 2.0 mL min⁻¹, with a valve to detector distance of 15.0 cm is used as a standard peak profile for the majority of the comparisons. These particular conditions produce convection-dominated peaks. Thus, peaks which are influenced to a larger extent by diffusion (e.g., low flow rates and longer valve-to-detector distances) were also compared to ensure the validity of the model. Although a valve-to-detector distance of 15 cm may seem relatively short, it must be remembered that due to the flow reversal, the first injected molecules of an 80 µL zone will travel over 30 cm into the manifold, and then back 30 cm to the valve, before traversing the 15 cm distance to the detector, thus traveling a total of 75 cm.

5.5 INTERFACE

The simulation software is written in Microsoft Visual Basic Version 3.0 in order to speed up development time and provide a graphical user interface. As well, data for each simulation are stored in a database written in Microsoft Access Version 2.0. This approach allows ease of access and provides compatibility with Microsoft Excel Version 5.0a spreadsheets and graphing functions for comparison with experimental data. Microsoft Query 1.00 was used to search and extract data from the database, for import into Microsoft Excel. Bitmaps of the screen were captured using Central Point Screen Capture 2.0. A maximum of four simulated profiles for each simulation are stored as a commas delimited text file.
5.5.1 Multiple Document Interface Form

The Flow Simulation form (Figure 5-3) is the multiple document interface (MDI) form which serves as a container for all other subordinate forms. The simulation status box on this form is continuously updated and indicates the current part of the analysis being simulated. Also shown are the database scroll arrows and current record number (bottom left corner), record start and stop numbers, the current replicate, completed replicates and total replicates. The update button records any changes the user makes on any of the forms in the database, the run button starts the simulation, and the pause button stops the calculations until it is pushed again.

Since each simulation constitutes one record, the record start and record stop boxes indicate which records should be run after pushing the run button. The user can specify the number of replicates to run in the bottom right box which are shown as current replicate, completed replicates, and total number of replicates. Simulating with a greater number of molecules increases the smoothness of the calculated profile. By performing the simulation in multiple replicates, the number of molecules held in memory at any one time is greatly reduced, thereby freeing memory for other applications. As well, a given replicate will take less time to calculate, which reduces the probability of interrupting the simulation in the middle of a run if the user wants to shut down the simulator (an action which would otherwise result in data loss).
Figure 5-3. The Flow Simulation form (shown) serves as a container (multiple document interface, MDI) for all other forms.

### 5.5.2 Simulation Profile Form

In the example simulation shown in Figure 5-4 of the Simulation Profile form, Dye A and Dye B are stacked sequentially for 1.2 seconds each into the manifold while the flow is to the left. Then, the flow direction is reversed and fluid is pushed towards the detector (to the right) for 15 seconds as indicated in the time row under “Detector.” The “Total Time” shows the simulation to be currently at 16.60 seconds from the start of the injection process (i.e., including the loading time). The total number of molecules that constitute each zone are listed for each of Dye A and Dye B, as well as the total number of molecules expected at the detector. The diffusion constant is listed for each dye as 0.000005 cm² s⁻¹. The left peak profile shown corresponds to Dye B while the right peak profile corresponds to Dye A due to the fact that the Dye B molecules were loaded into the injection tube last and, upon flow reversal, would reach the detector first. As expected, even though both zones are of the same size, the peak profile for
Dye A is lower than Dye B since it has traveled a greater distance into the injection tube before flow reversal.

Figure 5-4. The Simulation Profile form shows the manifold tube with four central injection zones and the simulated peak profiles.
5.5.3 Physical Settings Form

The tube diameter and distance to the detector are included on the physical settings form, shown in Figure 5-5. The injection length corresponds to $\Delta i$ and the detector volume is used to calculate $\Delta d$ according to the given tube diameter. The value for the number of molecules injected corresponds to the number of molecules randomly placed in the zone $\Delta i$ in step 2 of the simulation. The date and time stamps are set upon completion of the last replicate for a given simulation.

![Physical Settings Form](image)

Figure 5-5. The Physical Settings form includes simulation data for manifold dimensions (tube diameter, valve-to-detector distance, injection length, and detector volume), physical parameters (number of molecules injected and temperature), as well as a time and date stamp.
5.5.4 Flow Settings Form

The Flow Settings form (Figure 5-6) indicates the flow pattern and the sinusoidal flow parameters. Either linear or sinusoidal flow can be selected. If a linear flow is selected, then the flow rate is based on the value entered in the flow rate box (in this case 2 mL min⁻¹). If a sinusoidal flow is selected, the value in the flow rate box is not used, and the flow rate during any Δt is calculated based on the entries in the sinusoidal flow parameters section. If the pump is only operated over a certain angular range (so as to minimize the flow rate difference) then the starting and stopping angles can be entered. In the example shown in Figure 5-6, with the start and stop angles at 60 and 120 degrees respectively, the flow rate varies only by 14% of the maximum rate. As well, if a sinusoidal flow rate is used, it is important to specify the length of time the pump has reversed on the wash line before stacking the sample or reagent zones. That is, if the pump head starts at 60 degrees and the flow is reversed for 20 seconds to load wash solution into the manifold before stacking the first zone, the pump head will be at a new angle when it starts the injection sequence. Since the flow rate during any Δt varies with the pump head angle, a different volume of sample or reagent would be injected in the same specified length of loading time. The initial length of time for loading of the wash solution is specified in the very leftmost segment of the injection tube (labeled “Wash”) shown in Figure 5-4.
Figure 5-6. The Flow Settings form shows flow pattern settings and sinusoidal flow parameters.
5.5.5 Time Settings Form

The Time Settings form shown in Figure 5-7 is used to specify the timing of the simulation. The valve move time is the length of time the flow stops between valve positions while diffusion is still allowed to occur. The time per iteration corresponds to $\Delta t$ which equals $1/n$. The time per reversal is the length of time to reverse the flow in one direction before reversing it again to its original direction.

Figure 5-7. The Time Settings form is used to specify the length of time to pause for one valve movement, the length of time for each iteration, and the length of time for each reversal.
5.5.6 Model Settings Form

The Model Settings form is used to specify the power factor $P$ used in determining the axial flow profile as discussed previously. The laminar factor corresponds to $\zeta$ which determines the fraction of the $z$-dimensional step that is influenced by laminar flow versus plug flow. Alpha factors 1, 2 and 3 are additional parameters that can be accessed by the simulation calculations for testing purposes.

![Model Settings Form](image)

Figure 5-8. The Model Settings form is used to enter up to five additional numerical parameters in the model calculations.
5.6 Results and Discussion

The design and construction of this model which simulates the physical processes occurring within the manifold as realistically as possible was accomplished without any noteworthy difficulties. In the following discussion, the ability of the model to accurately predict peak profiles that are generated on the analyzer under straight-tube conditions will be examined. It should be understood that the experimental peak profiles, although not ideal due to hardware limitations, represent flow conditions which are primarily laminar, and where secondary flow and turbulence are minimized. The influence of the molecular diffusion coefficient, detector volume, injection volume, valve-to-detector distance, flow rate, and laminar flow profile on the model will be considered for comparison to the experimental peak profiles. Validation of the simulation results with data from other researchers is not yet possible since the sequential injection technique is so new. Other fundamental studies showing dispersion profiles created under primarily laminar flow conditions for the sequential injection technique have not been published.

5.6.1 Investigation of Random-Walk Model Parameters

The ability of the model to accurately predict dispersion profiles produced on an actual sequential injection analyzer lies, in part, in the accuracy of the parameters used in the simulation. The effect that each parameter produces on the generated profile are
examined here. A procedure of injecting 80 μL of tracer dye into a stream flowing at 2.0 mL min⁻¹ through a 0.84 mm internal diameter tube with a valve-to-detector distance of 15 cm will be assumed as the standard profile unless otherwise noted. For all experiments, the tracer dye, 1.5 mM K₃Fe(CN)₆, made up in 1.0 M KCl, is injected into a stream of 1.0 M KCl.

5.6.1.1 Effect of Diffusion Coefficient

The molecular diffusion coefficient directly affects the average random step length and should therefore have a significant influence on the simulation output. The literature [3, 11] suggests that under the current experimental conditions the molecular diffusion coefficient for the tracer dye should be $7.6 \times 10^{-6}$ cm² s⁻¹. This nominal value is used as a basis for the majority of the simulations in this work although multiplication factors of this number have been used to calculate simulations for comparison to ensure its validity. The significant effect which the diffusion coefficient has on the peak profile is demonstrated in Figure 5-9, where the diffusion coefficient has been modified over a range of three orders of magnitude. However, it should be noted that typical values of the diffusion coefficient for similar aqueous solutions are within one to two orders of magnitude of the nominal value for the dye used here. Increasing the molecular diffusion increases radial mass transfer, thereby reducing the effect that the convective movement has on any given molecule. That is, on average, molecules moving slowly along the walls of the tube will have a greater ability to step into the
faster moving laminae nearer the tube center. This reduces the tailing and produces a more symmetric, less dispersed profile (e.g., Figure 5-9f).

It should be noted that for the dimensions being considered, the effect of increasing the diffusion coefficient has a much more significant effect on the peak profiles shown in Figure 5-9 due to increased radial mass transfer relative to axial mass transfer. This is to say, an increase in the diffusion coefficient by two orders of magnitude actually reduces the overall peak dispersion in the axial dimension due to a more significant reduction in the effect of the laminar flow profile. Modification of this factor selectively (e.g., $D_{\text{radial}}$ versus $D_{\text{axial}}$) may provide improved agreement between the model and more symmetric experimental profiles which have been influenced by (i) secondary flow conditions (such as with coiled tubes) or (ii) greater manifold lengths.

To facilitate a simple comparison between the simulated and experimental peak profiles, (i) the sum of the squares of the errors (SSE) between the simulated and experimental peak profiles, and (ii) the area of the simulated profile relative to the experimental profile, will be considered. Figure 5-10 shows these two parameters as a function of the diffusion coefficient multiplication factor, and both indicate the best general agreement occurs near $1.0 \ D_m$. In Figure 5-10, the peak area reaches a maximum and then reduces again towards 1 at high values of $D_m$ due to an overall reduction of slower molecules which linger near the walls of the tube causing an increase in the overall peak area by spending more time in the detector. The SSE increases dramatically at this point (i.e., $100 \ D_m$) because of the disagreement in
vertical height at every point in the dispersion profile and not because of a disagreement in peak area (cf. Figure 5-9f).
Figure 5-9. Effect of the molecular diffusion coefficient ($D_m$) on simulated peaks (thick lines) relative to experimental peaks (thin lines); the multiplication factor used relative to $7.6 \times 10^{-6}$ cm$^2$ s$^{-1}$ is shown for each profile.
Figure 5-10. Effect of molecular diffusion coefficient multiplication factor on (i) the sum of the squares of the errors (SSE) between experimental and simulated peaks, and (ii) the simulated peak area relative to the experimental peak area.

5.6.1.2 Effect of Simulated Flow-cell Volume and Tube Diameter

Obviously the detector volume will have a significant influence on the resulting peak profile since it affects the axial range of molecules to be integrated. A larger volume would be expected to produce peaks which rise more slowly and take longer to return to baseline. The experimental flow-cell has an optical path length of 1.0 cm and an internal diameter of 1.0 mm, giving a calculated cell volume of 7.85 μL. Figure 5-11 shows the sum of the squares of the errors between the experimental and simulated
peak profiles using different simulated detector volumes. Adjustment of the simulated
detector volume near this value indicates that this volume is indeed optimal.

![Graph showing the effect of varying the simulated flow-cell volume on the sum of the squares of the errors (SSE).](image)

**Figure 5-11.** Effect of varying the simulated flow-cell volume on the sum of the squares of the errors (SSE).

The internal diameter of the experimental manifold tubing was nominally 0.8 mm. However, the average diameter was determined empirically to be 0.840 mm by mass difference using distilled, de-ionized water and a 6.00 m length of tube. Modification of the internal diameter of the simulated tube near the nominal and experimental values generated profiles which were compared to the experimental peak. The SSE between the experimental peak and the simulated peaks at different internal diameters, shown in Figure 5-12 indicates an optimal internal diameter which is closer to the measured value of 0.84 mm.
Figure 5-12. Effect of modifying the internal diameter of the simulation tube on the sum of the squares of the errors (SSE) between the simulated and experimental profiles and the simulated peak area relative to the experimental peak area.

5.6.1.3 Effect of Simulated Injection Time

Another factor which may influence the fit between the simulated and experimental peak profiles is the accuracy of the injection volume. If a volume slightly greater than or slightly less than 80 μL was injected into the experimental manifold (due to instrumental error) the recorded peak would be larger than or smaller than the expected profile. Since the volumetric flow rate of the pump is calibrated periodically by mass difference, any discrepancy between the specified and the actual flow rate of the system is not expected to be of much concern. However, slight inaccuracy in the experimental injection volume may arise from an inertial lag in flow rate as the flow rate
changes from 0 to 2 mL min\(^{-1}\) at the start of the injection period, and from 2 to 0 mL min\(^{-1}\) at the end of the injection period.

Figure 5-13 shows the SSE and relative peak area by varying the simulated volume from 60 to 100 µL in comparison to the 80 µL experimental peak. As expected, the simulated peak area increases linearly with the injection volume and indicates an optimum very near 80 µL, as does the SSE. Since there is relatively good agreement between peak areas, adjustment of the simulated or experimental injection volume is deemed unnecessary. This may not be the case, however, at shorter injection times and higher flow rates where the inertial lag may have a greater effect.

![Graph showing SSE and Relative Peak Area vs. Simulated Volume](image)

Figure 5-13. Effect of injection volume on simulated peak areas relative to an 80 µL experimental peak.
5.6.1.4 Effect of Laminar Flow Profile

The effect of the laminar flow profile on the simulated peak shape was also investigated. Through comparison of the simulated and experimental peak profiles, it was clear that better agreement could be made if the tailing portion of the simulated profiles (due to laminar flow) could be reduced, thereby shifting the molecules towards the peak centroid. Two methods of modifying the flow pattern have been attempted in this work. The first involves modifying the power of the exponent in Equation 5-12, which produces a change in the axial cross-section of the laminar flow profile according to Figure 5-14. As the power, $P$, increases, the profile becomes broader and the molecular velocity along the tube walls increases while the molecular velocity in the center of the tube decreases. A second flow pattern modification was proposed by introducing a new factor, $\xi$, into the convective movement terms of the simulation. This factor reduces the degree of the laminar flow profile by replacing it with an average-flow component and will be discussed in section 5.6.3.
First, in order to be convinced that the flow regime was primarily laminar for the experimental profiles obtained, and that a significant amount of secondary flow (or turbulence) was not created, a comparison between three peaks was made. Two peaks are from an 80 µL injection volume flowing at 2.0 mL min\(^{-1}\), with a valve-to-detector distance of 100 cm. The longer manifold length was used here so as to increase the influence of laminar flow and diffusion. One peak profile was obtained with the tubing (before and after the injection valve) wound in a 1 cm diameter coil (to enhance secondary flow) while the other was kept as straight as possible. The third peak was simulated with the model which assumes a perfectly laminar flow throughout. It is clear from Figure 5-15 that the simulated peak is significantly more similar to the
experimental profile obtained with straight tubing than the one with coiled tubing. Thus, we can be reasonably certain that the peak profiles obtained with the experimental apparatus using straight tubing are primarily influenced by laminar flow, and that the laminar flow simulated is more perfect than that experienced by the experimental plug. It is likely the assumptions made by the model (such as zero developing flow time, and perfectly cylindrical manifold geometry) that are limiting the fit between the experimental and simulated peak.

Figure 5-15. Comparison of simulated and experimental profiles for an 80 µL injection at 2.0 mL min\(^{-1}\) with a valve-to-detector distance of 100 cm.

Furthermore, the Reynolds number, Re, can also be used to predict whether the flow will be laminar or turbulent in the experimental system [16]. The Reynolds number
is simply a ratio of the inertia forces to viscous forces, and, flow is laminar when the viscous forces dominate. The Reynolds number is defined by

\[ \text{Equation 5-14} \quad \text{Re} = \frac{\rho Ud_i}{\mu} \]

where \( \rho \) is the fluid density, \( U \) is the linear fluid velocity, \( d_i \) is the tube diameter, and \( \mu \) is the dynamic viscosity of the fluid. Since the Reynolds number is unitless, any consistent system of units can be used. The flow is consider laminar for \( \text{Re} < 2000 \), unstable due to the onset of turbulence for \( 2000 < \text{Re} < 4000 \), and turbulent for \( \text{Re} > 4000 \). By considering an "order of magnitude" calculation, we can take the fluid density to be 1000 kg m\(^{-3}\), the linear velocity to be 0.1 m s\(^{-1}\) (which corresponds to ca. 3 mL min\(^{-1}\)), the diameter of the pipe to be 0.001 m, and the dynamic viscosity of the fluid to be 0.001 kg m\(^{-1}\) s\(^{-1}\) (which is the value for water at ca. 21° C). The resulting Reynolds number from this calculation is 100, which is well below the upper boundary of 2000 for laminar flow.

The shape of the laminar flow profile can now be investigated by modifying the power, \( P \), in Equation 5-12. Wentzell et al. [3] found for their flow injection work, that although there was no change in the peak position, the peaks became significantly narrower and taller as \( P \) increased. They indicated that modification of \( P \) improved agreement between experimental and theoretical results in some instances, which (they suggest) means that the nature of the flow profile changes with experimental conditions. Figure 5-16 shows the effect of the power factor on the simulations created
with the SIA model. The general shape change is in agreement with Wentzell et al., with the best overall fit occurring for $P = 2$. These data suggest that an increase in the power factor does have the ability to shift the tailing molecules towards the peak centroid, which improves agreement in the peak tail. However, this is at the expense of a significant lack of agreement near the peak maximum. The SSE between the simulated peaks (using different values of $P$) and the experimental peak is shown in Figure 5-17, as well as the relative peak areas. These data also indicate that the best agreement occurs for $P$ near 2. However, the possibility of the optimal value for $P$ changing with experimental conditions still exists.

Figure 5-16. Effect of power factor on simulated peak profiles (thick line) relative to experimental profile (thin line).
Figure 5-17. Effect of power factor, $P$, on (i) the sum of the squares of the errors (SSE) between simulated and experimental peak profiles, and (ii) the simulated peak area relative to the experimental peak area.

5.6.2 General Agreement of the Random-Walk Model for SIA

The ability of the model to generate accurate peak profiles under typical analytical conditions will now be considered. If the model is able to provide us with a reasonably accurate peak profile for the dispersion of a dye in a carrier stream, it will ultimately be useful for studying and optimizing the various parameters affecting peak profiles in general. The general premise of the model ($\sigma^2 \propto n/l^2$) was shown to hold for an experimental peak produced by the sequential injection technique in Chapter 3. Peak profiles created with different manifold lengths, at different flow rates, using different injection volumes will now be compared to experimental profiles created under
similar conditions. For all simulation profiles shown in this section, the detector volume is 7.85 μL, the manifold tube internal diameter is 0.84 mm, the power factor (P) is 2, the diffusion coefficient is $7.6 \times 10^{-6}$ cm² s⁻¹, and the number of iterations per second is 10. Unless otherwise specified, the injection volume is 80 μL, the flow rate is 2.0 mL min⁻¹, and the valve-to-detector distance is 15 cm.

5.6.2.1 Valve-to-Detector Distance

Figure 5-18 shows the predicted peak profiles by the random-walk model in relation to the experimental profiles. The flow rate was 2.0 mL min⁻¹ and the injection volume was 80 μL. Favorable agreement is found in all three cases. The simulation peaks start and stop at approximately the same time as the experimental peaks. In general, similar discrepancies in shape are found at each length; the simulated peak drops too quickly after peak maximum before crossing over the experimental peak in the tail section. Since peak height and peak area are the most commonly quoted peak descriptors in flow analysis they are shown here for reference (Figure 5-19). There is good agreement between the peak height and area for the simulated and experimental profiles. In general, however, the simulated peak profiles have heights and area which are slightly lower than the experimental ones (except at 100 cm where the simulated peak height exceeds the experimental slightly, possible due to noise).
Figure 5-18. Comparison of simulated and experimental peak profiles (thick lines and thin lines, respectively) for valve-to-detector distances of (a) 15 cm, (b) 50 cm, and (c) 100 cm.
Figure 5-19. Peak height and peak area as a function of valve-to-detector distance for simulated and experimental peak profiles.

5.6.2.2 Flow Rate

The effect of the simulated flow rate, shown in Figure 5-20, again indicates good general agreement between simulated and experimental peak shape. The injection volume was 80 \( \mu \text{L} \) and the valve-to-detector distance was 15 cm. It is interesting to note the improved agreement for 0.5 and 1.0 mL \( \text{min}^{-1} \) during the last one-third of the peak profile. This would indicate that laminar flow conditions near the tube walls are maintained to a greater extent for the experimental profiles at lower flow rates. This effect may be due partly to a lesser degree of hysteresis when starting and stopping the flow. Disagreement in the tailing (Figure 5-20) and the peak height (Figure 5-21) increases with flow rate. This is thought to be due primarily to a difference in laminar
flow profile. In general, the model is less sensitive to peak height than are the experimental profiles. The model accurately predicts the inverse dependence of the peak area on flow rate; there is excellent agreement at 1.0, 2.0, and 4.0 mL min\(^{-1}\), although the cause of the discrepancy at 0.5 mL min\(^{-1}\) is unclear.

Figure 5-20. Comparison of simulated and experimental peak profiles (thick lines and thin lines respectively) for an 80 µL injection at (a) 0.5, (b) 1.0, (c) 2.0, and (d) 4.0 mL min\(^{-1}\).
Figure 5-21. Peak height and peak area as a function of flow rate for simulated and experimental peak profiles.

5.6.2.3 Injection Volume

By far the largest discrepancy between the simulated and experimental peak profiles arises from variation of the injection volume. The predicted peak height is significantly lower than the experimental, as shown in Figure 5-22 and Figure 5-23. However, the peak areas agree relatively well, with only a modest decrease in the simulated value at higher injection volumes. This indicates a lack of agreement between the distribution of molecules within the manifold tube and not in the total number injected. Again, improved agreement would be achieved if the tailing molecules were shifted towards peak maximum as is clearly evident in Figure 5-22d.
Figure 5-22. Comparison of simulated and experimental peak profiles (thick lines and thin lines respectively) as a function of injection volume; the flow rate was 2.0 mL min\(^{-1}\) and the valve-to-detector distance was 15 cm.
Figure 5-23. Peak height and peak area as a function of injection volume for simulated and experimental peak profiles; the flow rate was 2.0 mL min\(^{-1}\) and the valve-to-detector distance was 15 cm.

### 5.6.3 Laminar Flow Factor

It became evident that improved agreement between the simulated and experimental flow profiles would be attained if the tailing molecules of the simulation (near the tube walls) could be brought forward to nearer the peak maximum. An attempt to achieve this through modification of the convective movement terms of the simulation is presented here. The addition of \(\zeta\) and the average-flow term to Equation 5-13 was thought to produce an overall reduction in the laminar flow characteristic of the generated profile.
As expected, the anticipated transformation of the simulated peak profile from one that is almost a square plug (Figure 5-24a, \( \zeta = 0.00 \)) with minimal dispersion, to the asymmetric peak profile created by a purely laminar flow profile (Figure 5-24f, \( \zeta = 1.00 \)) occurs for \( 0.00 \leq \zeta \leq 100 \). In order to maintain consistency with previous investigations, the peaks shown were created using an injection volume of 80 \( \mu \)L, a flow rate of 2.0 mL min\(^{-1}\), and a valve-to-detector distance of 15 cm. However, further investigation of the laminar flow factor using 40, 160, or 240 \( \mu \)L (where the agreement between simulated and experimental peaks is less apparent), would be warranted to investigate any possible improvement at these injection volumes. By close examination of the tailing between 5 and 10 seconds in Figure 5-24e and Figure 5-24f, it is clear that an optimal value for \( \zeta \) lies between these two values. As predicted, the tailing molecules shown in Figure 5-24f have indeed been moved off of the walls and towards the peak maximum. Further reduction of \( \zeta \) below 0.80 causes too much of a shift, decreasing the agreement substantially. In Figure 5-25, the SSE and relative peak area are considered as a function of \( \zeta \). Both indicate an optimum value between 0.80 and 1.00. In Figure 5-26, the simulated peak profiles for \( 0.80 \leq \zeta \leq 1.00 \) are shown (relative to the experimental profile). Close examination of Figure 5-26a and Figure 5-26f reveals that improvement in the tailing portion of the peak profile can indeed be made by fine-tuning this factor. Figure 5-27 indicates the optimal value for \( \zeta \) to be near 0.88 according to the SSE, and 0.94 according to the relative peak areas (although if 0.88 were used, the simulated peak would be no more than 1% larger than the experimental peak).
Figure 5-24. Effect of laminar flow factor, $\zeta$, on simulated peak profile (thick line) relative to experimental peak profile (thin line) for $0.00 \leq \zeta \leq 1.00$. The injection volume was 80 µL, the flow rate was 2.0 mL min$^{-1}$, and the valve-to detector distance was 15 cm.
Figure 5-25. Effect of $\zeta$ on (i) the sum of the squares of the errors (SSE) between the simulated and experimental peaks, and (ii) the simulated peak area relative to the experimental peak as shown in Figure 5-24.
Figure 5-26. Effect of laminar flow parameter, $\zeta$, on simulated peak profile for $0.80 \leq \zeta \leq 1.00$. The injection volume was 80 $\mu$L, the flow rate was 2.0 mL min$^{-1}$, and the valve-to-detector distance was 15 cm.
Figure 5-27. Effect of $\zeta$ on (i) the sum of the squares of the errors (SSE) between the simulated and experimental peaks, and (ii) the simulated peak area relative to the experimental peak as shown in Figure 5-26.

To consider the effect $\zeta$ has on the flow profile at longer tube lengths, similar profiles were compared for a valve-to-detector distance of 100 cm, as shown in Figure 5-28. The flow rate was 2.0 mL min$^{-1}$ and the injection volume was 80 $\mu$L. There is much greater agreement near the peak maximum (between 10 and 20 seconds) for $\zeta = 0.80$ (Figure 5-28b) than for $\zeta = 1.00$ (Figure 5-28f), however, there are now too many molecules between 20 and 30 seconds, and not enough between 35 and 50 seconds. An optimal $\zeta$ value again resides near 0.85, according to the SSE shown in Figure 5-29, the relative peak areas for all simulated values is too low by as much as 4%.
Figure 5-28. Effect of $\zeta$ on the simulated peak profiles (thick line) relative to the experimental peak profile (thin line). The injection volume was 80 $\mu$L, the flow rate was 2.0 mL min$^{-1}$, and the valve-to-detector distance was 100 cm.
Figure 5-29. Effect of $\zeta$ on (i) the sum of the squares of the errors (SSE) between the simulated and experimental peaks, and (ii) the simulated peak area relative to the experimental peak as shown in Figure 5-28.

Although it may be concluded that the optimal $\zeta$ value is not significantly influenced by manifold length, it is much more dependent on the injection volume. Figure 5-30 shows the simulated results at higher injection volumes (160 and 240 $\mu$L) for three values of $\zeta$ (0.60, 0.80, and 1.00). For the 160 $\mu$L injection, an optimal value near 0.80 (or possibly a little higher) is indicated by an excellent improvement in peak tail agreement. The contributions of plug-flow likely come from starting and stopping the flow periodically, and manifold non-ideality. Lack of agreement near the peak maximum is still not understood or accounted for, although the overall peak height agreement is improved at $\zeta = 0.80$ as well. There is significant improvement in the agreement between the tailing portion of the peak (as well as peak height) for $\zeta = 0.80$.
relative to $\zeta = 1.00$ and $\zeta = 0.60$. At an even larger injection volume of 240 µL (Figure 5-30d-f), the best agreement again appears to be near $\zeta = 0.80$. This series of figures (Figure 5-30d-f) clearly demonstrates the ability to improve the peak shape agreement by reduction of the laminar flow profile, especially in the tailing region of the peak.

Figure 5-30. Simulated and experimental peak profiles (thick lines and thin lines respectively) for 160 and 240 µL injection volumes at 2.0 mL min$^{-1}$ with a valve-to-detector distance of 15 cm.
All of the profiles shown thus far have used a flow rate of 2.0 mL min\(^{-1}\). Since laminar flow conditions should be easier to attain at lower flow rates, the optimal value for \(\zeta\) would be expected to be inversely proportional to flow rate. Thus, flow profiles at 0.5 mL min\(^{-1}\) would be expected to have an optimal \(\zeta\) value closer to 1.00, while flow profiles at 4.0 mL min\(^{-1}\) would be expected to have an optimal \(\zeta\) value of less than 0.80. Figure 5-31 shows that this is indeed true. At 0.5 mL min\(^{-1}\), the peak tail agrees best at \(\zeta = 1.00\), and is already "overshooting" at \(\zeta = 0.80\). On the other hand, close inspection of the profiles generated at 4.0 mL min\(^{-1}\) indicates that an optimal \(\zeta\) value lies somewhere between 0.80 and 0.60 (where it begins to "overshoot" as well).
Figure 5-31. Effect of $\zeta$ on simulated peak profiles (thick line) at 0.5 mL min$^{-1}$ and 4.0 mL min$^{-1}$ relative to experimental profiles (thin line) with an injection volume of 80 $\mu$L and a valve-to-detector distance of 15 cm.

Thus, it has been shown that improved agreement between the simulated and experimental peak profiles can be accomplished by inclusion of an average-flow term (moderated by $\zeta$) in the convection equation of the simulation. This is significant for
two reasons. First, it tells us that the experimental peak profiles have not been created under perfectly laminar conditions; if they were, they should exhibit much greater tailing. Second, by empirically determining the effect of the system parameters on $\zeta$ (which is primarily influenced by flow rate), one could conceivably correct the simulation to match the experimental profiles in the majority of cases. This would allow the researcher to use the simulation as an accurate optimization tool for predicting experimental peak profiles under various conditions. However, it should be kept in mind that laminar flow conditions which are free from the influence of secondary flow are rarely found in practice. Hence, further work should first focus on incorporating secondary flow into the convective equation of the model.

In the present state, the model can be used to accurately predict dispersion profiles under flow conditions which are primarily dominated by laminar flow. This allows us to study the predicted output profiles for optimization purposes, and allows us to study the theoretical molecular distribution within the manifold tubing at any point in time. This will be the topic of the next section of this chapter.

5.6.4 Cross-Sectional Molecular Distribution

A significant advantage of developing an iterative model lies in the ability to examine the progress of the simulated analysis at any point in time. This allows the researcher to come to a greater understanding of the analytical procedure since the predicted movement of the molecules within the manifold under varying analytical
conditions can be visualized. To facilitate this, the molecules within the tube can be plotted after each iteration (by the simulation software) in a frame which represents the actual manifold dimensions. This picture of the molecular cross-section can be recorded to disk as a bitmap file for subsequent examination.

The sequential injection of two 80 µL zones at 2.0 mL min⁻¹ with a valve-to-detector distance of 15 cm is shown in Figure 5-33, Figure 5-34, and Figure 5-35. Each frame represents a cross-section of the manifold tube with a vertical scale of 0.84 mm (y-direction) and a horizontal scale of 58 cm (z-direction). The depth of the frame (x-direction) only includes molecules which are less than 10% of the tube diameter from the center of the tube (see Figure 5-32). In this way, the two dimensional cross-section represents an axial slice (or "slab") through the tube without bias from the different thicknesses of the tube as seen from the side. The vertical line closest to the center of the frame is the multi-position valve interface, and the two vertical lines near the right represent the 7.85 µL detection zone. Molecules within the detection zone are integrated to produce a detector response. The distance between the valve and the left end of the tube is 40 cm, the distance between the valve and the right end of the tube is 18 cm, and the distance between the valve and the left edge of the detection zone is 15 cm.

† There are several utility programs available that operate in the Windows environment and allow sequential viewing of bitmap files with a time delay between each frame. Playing back the bitmapped files which are recorded during the simulation, produces an animation of the spatial distribution of the molecules as the analysis proceeds.
Figure 5-32. Molecules found within the shaded “slab” are used in the cross-sectional molecular distribution plots.

The simulation begins with the multi-position valve connected to the line containing the molecules to be injected (although the detector outline is still shown). The simulation allows the analyzer 1.00 s to turn to this position, and therefore, the first molecules are seen moving into the tube towards the left at 1.10 s. The parabolic flow profile is immediately evident in the first step the molecules take, since, as an approximation, the model assumes the flow rate and laminar flow profile start instantaneously. By dividing the tube into 1 cm segments and integrating the number of molecules in each cylinder created, an axial dispersion profile of the molecules can be shown for each iteration (thin line on top of the molecular distribution). The injection of the first zone continues for a total of 2.40 s with an iteration period of 0.10 s. Although a frame was recorded for each simulation iteration, several frames have been left out in these figures for brevity.

By 3.40 s the injection period is complete and the flow is stopped instantaneously (also an approximation). It is interesting to note that very few
molecules are predicted to be near the walls of the tube throughout the zone, and thus wall interaction calculations in the model would play a relatively minor role at this point. The zone of molecules is now over 30 cm in length, which is over 15 cm longer than an 80 μL zone would be under plug flow conditions. From 3.40 s to 4.40 s the flow remains stopped as the injection valve moves to the next position (the detector line). During this time, the molecules are allowed to take their diffusion steps without convective steps. Although the injected zone does not appreciably lengthen during this time, it does appear to be slightly more radially diffused at 4.40 s (e.g., examine molecular concentrations at the center of the tube nearest the valve). As such, it is important to include this pause with molecular diffusion in the model.

From left to right, the concentration gradient is shown to increase quickly at first, and then more gradually for the majority of the injected zone, until it reaches a value near $C^0$ at the valve interface. A key point can now be made based on the molecular concentration gradient across the injected zone. Simplification of the model, by making the assumption that this zone has a constant concentration throughout, and can therefore be injected as a "square plug" (as in flow injection or chromatography models) would be oversimplifying the situation too much since the concentration gradient throughout the zone is significant. By the use of this unique injection procedure, it is now possible to visualize the true concentration gradient in the simulated tube.

At 4.50 s, the first molecules of the second zone to be injected have stepped through the multi-position valve. The concentration profile must now be considered
since, in black and white, it is difficult (if not impossible) to see the difference between the molecules of each zone (a colour animation of the two different molecular zones provides a much greater contrast). By 6.80 s the second zone is completely injected, and, not surprisingly, it has a similar concentration profile as the first zone at 4.40 s. The tailing (right-most) edge of the first zone now has a concentration gradient which is the inverse of its leading (left-most) edge. With this model, the nature of these predicted axial concentration profiles can be shown for the first time. The overlap of these profiles is the most likely point of reaction, and would constitute the product concentration profile. Although the overlap appears to be skewed to the left at 6.80 s, it will be shown in the next section that the product concentration across the zone is actually quite symmetric when radial molecular distribution is taken into account.

By 7.80 s the molecules have been allowed their diffusional steps (without convection) for one second as the valve moves to the detection line. At 7.90 s the flow is reversed and the molecules begin to move back through the valve, towards the detection zone. The initially abrupt concentration profile at the valve interface now begins to disperse. By 9.00 s, 40 \( \mu \)L of the second zone have moved back through the valve and the concentration gradient across this zone is approximately symmetrical. At this point, molecules of this zone are just entering the detector. By consideration of the concentration profile appearing in the detector over the next four frames, one can understand why the detector response increases so quickly and "washes away" so slowly, creating a very skewed peak profile.
At 10.30 s, the isodispersion point is reached at the valve, where the mutual overlap of the concentration profiles is quite symmetric. This point would actually be anticipated at 10.20 s which is exactly 2.40 s (equivalent to 80 µL) after the flow was reversed. At this point in the analysis we have, in essence, an 80 µL zone positioned on either side of the injection valve, with each zone partially dispersed into the other. The concentration of each zone at this interface is approximately 0.5 C°. This demonstrates unequivocally that mutual dispersion between two injected zones in a narrow bore tube is possible with zero net movement of fluid, according to the random-walk model [12-13]. This was one of the advantages presented in favour of the sequential injection method since zero net movement of fluid means sample and reagent consumption are minimized.

As the flow continues to the right, the overlapped zone becomes more skewed and the isodispersion point reaches the detector at 11.80 s. After this point, the concentration of the zone on the right slowly decreases while the concentration of the zone on the left increases slightly until its peak maximum is reached in the detector. Finally, the concentration of both zones slowly diminishes to zero, with the molecules along the walls of the tubes lingering the most; in fact, some molecules are still seen near the walls as far as 20 cm to the left of the valve, even at 20.00 s.
Figure 5-33. Cross-sectional molecular distribution during injection of an 80 μL sample zone through the valve (central vertical line).
Figure 5-34. Cross-sectional molecular distribution during injection of an 80 µL sample zone through the valve (central vertical line).
Figure 5-35. Cross-sectional molecular distribution during injection of an 80 μL sample zone through the valve (central vertical line).
5.6.5 Cross-Sectional Zone Penetration

The mutual overlap of two zones does not necessarily mean that radial mixing in the area of overlap is complete or homogeneous. Again, this can be shown with the random-walk model as a function of time. The simulation tube was divided axially into 1.0 mm segments to produce several hundred individual cylinders. Each cylinder was further divided into 50 concentric cylinders of equal volume. During each iteration of the simulation, a point is made on the simulation tube for each cylinder with at least one molecule from each of the two zones. A point is plotted at the corresponding axial position of the cylinder (z-position) and the positive and negative y-position for that cylinder, thus creating a distribution profile which is symmetric about the tube axis. In this way, the actual zone-penetration can be shown as a cross-section in a plane parallel to the axial center of the tube without bias due to radial positioning. The penetrated zones thus shown, indicate the most probable location of chemical reaction and therefore product formation. The concentration of these penetrated zones can be integrated over the axial distance of the tube (at 1.0 cm intervals as before), thus showing the predicted concentration of the product zone at any point in time.

The axial and radial zone penetration for the two 80 μL zones examined in the previous section, are shown in Figure 5-36, Figure 5-37, and Figure 5-38 as a function of time. Each frame represents a 60 cm long tube with an internal diameter of 0.84 mm and a valve-to-detector distance of 15 cm. The simulation starts by pausing for 1.0 s, injecting the first zone for 2.4 s, pausing for another 1.0 s, before beginning injection of
the second zone at 4.40 s. At 4.50 s the second zone has made its first step into the injection tube, and subsequently the zone penetration initially appears as a very thin region at the interface between the two zones. As the injection proceeds, the zone penetration increases in thickness, and distorts parabolically down the length of the tube according to the laminar flow profile. The concentration profile of the penetrated zones increases in width proportionally, and increases in height approximately equally over the entire zone. This indicates that the height of the overlapping area at say 7.80 s in the previous simulation (Figure 5-34) is not indicative of the product concentration. That is, if the entire tube is taken into consideration at any instant in time, the isodispersion point would not be expected to have a greater concentration of product (using equivalent sample and reagent concentrations) due to the radial separation of the two zones. A detector response, however, is still likely to maximize near the isodispersion point since the product concentration will increase with time (as the two zones pass through the detector) and then minimize when the sample or reagent concentration in the flow-cell approaches zero.

At 7.80 s the flow is reversed and the molecules are propelled towards the detector. Inversion of the radial distribution of the penetrated zones can be seen from 8.00 s to 11.00 s in Figure 5-37. At 10.20 s the simulation is again at the point of zero net fluid movement and the peak height of the concentration profile of penetrated zones is predicted to be maximum at the valve interface. This indicates that the optimal location for detection of product is at the valve. Future designs of multi-position valves which incorporate a sensor (e.g., a fiber-optic cable) within the valve mechanism might
see improved sensitivity at reduced sample and reagent consumption. This is in experimental agreement with the decreased injection volume necessary to achieve maximum sensitivity when using the shortest manifold lengths (Chapter 4). If a longer reaction time is necessary, the flow can be stopped while the product maximum is situated in the detection zone. Progression of the zone penetration towards the detector causes the concentration to diminish such that a maximum of approximately one-half of the height reached at the valve interface is realized at the detector.
Figure 5-36. Axial cross-section of zone-penetration between two 80 µL zones injected sequentially at 2.0 mL min⁻¹.
Figure 5-37. Axial cross-section of zone-penetration between two 80 µL zones injected sequentially at 2.0 mL min⁻¹.
Figure 5-38. Axial cross-section of zone-penetration between two 80 µL zones injected sequentially at 2.0 mL min⁻¹.
By monitoring the zone penetration concentration profile as a function of time, it can be shown that the peak height of this profile increases slowly and approximately linearly as the second zone is loaded. Then, the rate of increase in peak height is increased as the flow is reversed, with a maximum height being reached at the point of zero net fluid movement. Figure 5-39 shows this effect as a function of time. After peak maximum is reached, the maximum concentration of the penetrated zones quickly decays at an exponential rate. Thus, it may be concluded that the random-walk model predicts a significantly narrower and taller concentration profile of penetrated zones when the displacement is at zero net fluid movement. Greatest sensitivity would, therefore, likely be achieved at this manifold position. Similar results would be expected if three stacked zones were considered, except that the point of zero net fluid movement occurs when the middle zone is centered at the valve interface.
Figure 5-39. Relative peak maximum for zone penetration as a function of time, as predicted by the simulation.

5.7 CONCLUSIONS

A numerical simulation based on the random-walk model has been proposed and developed for the sequential injection process. This is the first time this analytical process has been modeled. A unique injection procedure is incorporated into the model which simulates the sequential stacking of up to four zones in the manifold tubing. There is generally good agreement between simulated and experimental peak profiles created under laminar flow conditions. The model is able to successfully predict peak profiles created at a variety of flow rates, injection volumes, and valve-to-
detector distances typical of sequential injection analysis. It was found that inclusion of an average-flow term (the magnitude of which is controlled by ζ) in the convective movement equation of the model improves the correlation (especially in the peak tail) between the simulated and experimental peak profiles. As expected, the degree of laminar flow (ζ) achieved in the experimental profiles is shown to be inversely proportional to the flow rate.

The model has been used to improve our fundamental understanding of the processes occurring during the injection process and flow reversal. The molecular concentration gradients of two zones as a function of time have been shown, and used to prove (theoretically) that mixing can indeed occur with no net fluid movement. As well, the cross-sectional distribution of zone penetration has been examined and used to determine that the theoretical maximum penetration increases upon flow reversal, reaching its highest point at the valve interface. Further use and modification of this model will provide new insight into the sequential injection method.

Further improvements in the agreement of the model with experimental peak profiles might be attained if the approximation of instantaneous flow rate change at the start and stop of an injection period was refined. As well, the model may be improved by accounting for the true valve and detector geometry. An attempt could also be made to improve the laminar flow characteristics of the experimental peak profiles, which have been shown to be somewhat less than perfect probably due to (i) the valve and detector geometry, (ii) the pulsing caused by the peristaltic pump, or (iii) the repeated
starting and stopping of the flow. Further investigation could be done with the current model using tracer dyes with different diffusion coefficients, and the effect of temperature could be investigated.

The next major obstacle to overcome with this model is to account for secondary flow characteristics found when the manifold tubes are coiled. This would enable the researcher to develop optimal experimental methods with the model which can be implemented in practice. As a final step towards reality, incorporation of the effects of chemical concentration gradients and reaction kinetics should be attempted. It is assumed that the computational power of the personal computer will continue to increase in future years enabling the inclusion of these calculations without a significant delay in generation of simulated peak profiles.
5.8 REFERENCES


6. Conclusions and Further Work

"The measure of our intellectual capacity is the capacity to feel less and less satisfied with our answers to better and better problems."

C. W. Churchman

6.1 SEQUENTIAL INJECTION ANALYSIS SYSTEM

For the first time, a significant step towards simplification of method development for sequential injection analysis has been made. The virtual manifold presented in Chapter 2, combined with the greater hardware simplicity of a sequential injection system has proven to be a synergistic combination. Not only can new chemistries be incorporated without physically reconfiguring the manifold (one of the design ideals of sequential injection analysis), but with the new interface, almost all system variables can be automatically explored with a "few clicks of the mouse." This removes the complexity of the software interface from the hands of the researcher who can then focus greater attention on developing new methods or exploring new reaction kinetics or chemistries. Increased utilization of graphical user interfaces which simplify complex
software control over mechanical systems will become more and more apparent as this science evolves. In essence, it is a graphical model on a computer screen that has been established in order to make this field more understandable, and more useful, to researchers across the disciplines.

6.2 Experimental Design and System Characterization

Never before has the control over injection volume and peak shape been so evident in a flow system. This control results in peak shapes that vary widely, and by direct manipulation of the method, virtually any degree of dispersion can be achieved within the same physical manifold. The results from a comprehensive empirical investigation of peak shape is presented in Chapter 3 in an attempt to gain new insight into the sequential injection technique. The study took advantage of the ability of the analyzer to quickly and independently perform a large number of experiments (over 6,000). All of the data were stored within an efficient database for rapid recall and display. Peak moments are found to be useful for characterizing sequential injection peak shape. The reproducibility and periodicity of the injection volume when using a peristaltic pump is demonstrated. In addition, the random-walk model is shown to hold for the sequential injection process, thus validating its use, in Chapter 5 for simulation purposes.
6.3 Optimization of Dispersion in Sequential Injection Analysis

In Chapter 4, the concept of zone penetration was further explored with the use of the analyzer created in Chapter 2 and the dataset tabulated in Chapter 3. The major factors influencing the mutual penetration of two adjacent zones are injection volume, flow rate, and valve-to-detector distance. In this study, only coiled tubes are taken into consideration since this is usually the format that the manifold takes on in practice; straight tubes greater than one metre in length are awkward to work with on a real analyzer. Four parameters which allow a relative measure of the degree of sample zone penetration, sensitivity, reagent economy, and throughput of the analysis were introduced, and were considered under conditions of varying analytical requirements. A composite function was used which enabled the search for optimum conditions using two or more of the four "essential" optimization parameters. It was found that the sample zone penetration and the sensitivity parameters are the most important when designing a method. When only these two parameters were considered, the optimal volumes of sample and reagent to inject were found to be independent of valve-to-detector distance and flow rate (assuming fast reaction conditions). Additionally, it was found that increasing the distance between the valve and the detector beyond the minimum necessary to physically connect them provides no significant advantage for zone penetration, and only decreases the sensitivity. Longer manifold lengths can be simulated by further aspiration of the injected zones towards the pump before sending the mixture to the detector for measurement (or via multiple flow reversals). However, this requires extension of the distance between the pump and the valve to ensure that
the injected zones never reach the pump. Finally, a study should be done to confirm the utility of these optimization descriptors with a real, suitable chemistry.

6.4 RANDOM-WALK MODEL FOR SEQUENTIAL INJECTION ANALYSIS

The random-walk model has provided the first simulation tool for studying the sequential injection technique. The model was shown to correlate well with sequential injection peak profiles which are created under laminar flow conditions, and possible areas of further refinement of the model were discussed. The addition of an average-flow term in the convective step was able to correct for the discrepancy in the tailing portion of the peaks. Other discrepancies in the correlation between the simulated and experimental profiles may be attributed to “developing flow” when the laminar flow profile is created at the beginning of fluid movement. The model also allowed visualization of the (theoretical) cross-sectional concentration gradients which occur during the sequential injection process. This model should have a significant impact on studying heterogenous chemistries such as the use of magnetic beads in biotechnology applications since the model works at the physical / particle level. Reaction kinetics and variation in manifold geometry could be implemented with little difficulty. It is for these reasons that the random-walk model will likely be the most useful aid in studying sequential injection analysis for quite some time.