

Chapter 3

Factors affecting zone penetration

3.1 INTRODUCTION

There is a tendency by many users of flow-based techniques to apply empirical techniques in the choice of operating conditions and design of manifolds. This is unfortunate, as much excellent work has been done on establishing the rules¹ which govern important parameters such as dispersion. A thorough understanding of these rules provides a sound platform on which method development can be built. Even workers who use optimization techniques such as the simplex optimization technique benefit by an understanding of the guiding principles as it enables a more intelligent setting of boundary conditions.

While FIA is forgiving enough to allow an unstructured approach, SIA requires a more stringent approach. Most of the early papers²⁻⁴ dealing with SIA set out important principles which affect manifold dimensions and design. While a good understanding of the principles that affect FIA proves useful in understanding SIA, some findings were totally unexpected and provided some important guidelines which could be re-applied to certain variations of FIA.

3.2 DISPERSION COEFFICIENT OR ZONE PENETRATION

Karlberg⁵ has called flow-injection analysis "the art of controlling sample dispersion in a narrow tube". A complete definition for flow-injection analysis⁶ appeared in 1978 and was based on a combination of sample injection, reproducible timing, and controlled dispersion. As knowledge of the dispersion process increased, the concept of flow-injection analysis was further refined. In the ensuing years several models have been used to gain greater understanding of the dispersion process. Many of these have been discussed in Ruzicka and Hansen's monograph on FIA¹. Initially, workers thought that flow in the manifold conduits was of a turbulent nature. This was subsequently found to be incorrect and workers realized that laminar flow and radial mixing were two strong governing influences in determining the dispersed sample profile. As the sample bolus is transported down the conduit, diffusion and convection are the two forces which exert these influences.

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

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

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

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

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

For SIA, a different, though not unrelated parameter, was found to be more useful. For a technique which operates on a stack of well defined sample and reagent zones, the concept of zone penetration was identified independently by workers at the University of Washington⁴ and Mintek⁷ as being of fundamental importance. The importance of zone penetration can be ascribed to the fact that this influence has a dramatic impact on the surface area over which a concentration gradient exists and therefore over which axial mixing takes place. For a chemical reaction to take place, elements of sample fluid must come into intimate contact with elements of reagent fluid. For manifolds where no reaction takes place, zone penetration and band broadening must be kept to a minimum.

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

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

3.3 MANIFOLD DESIGN

Early work in the field of SIA² soon showed that a single manifold was sufficient, irrespective of the chemistry to be employed. This manifold, once optimized, could be 'cast in stone'. The variability from one method of analysis to the next is introduced through microprocessor sequencing of reagents and control of the pump, and in some cases, by changing the detector. Changing the detector could be as simple as selecting a new wavelength for a photometer, but could also include exchanging an optical detector for an electrochemical one.

Researchers in the laboratory of Ruzicka and Christian²⁻⁴ and in Mintek's Process Analytical Science group⁷ have given attention to the rules governing the design of an SIA manifold. These rules must be applied to achieve the desired mixing regimen.

3.4 INSTRUMENTAL SET-UP

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

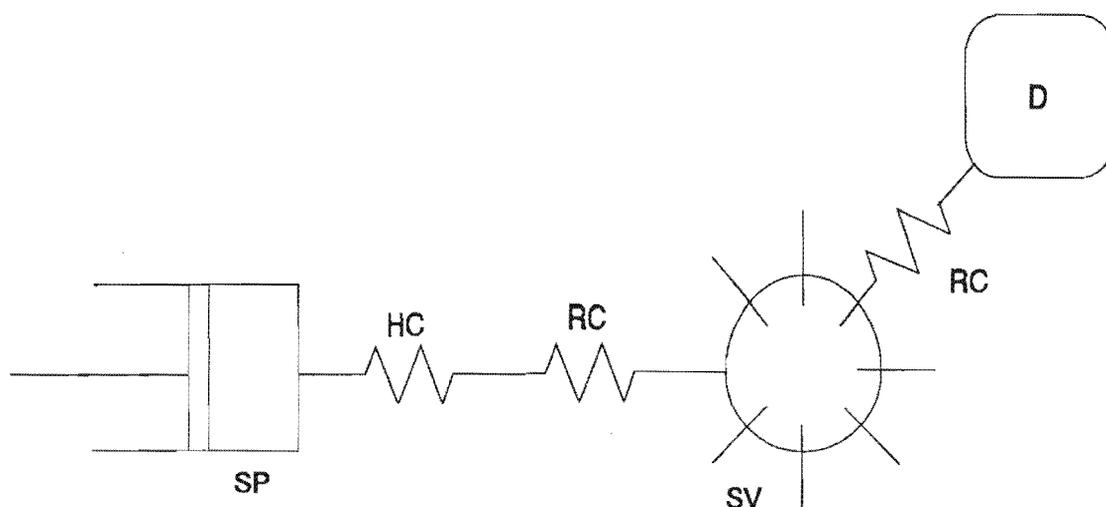


Figure 9: Sequential-injection manifold. P - pump, HC - Holding coil, RC - Reaction coil, SV - selection valve, D - detector.

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

The detector can be placed either between the valve and the syringe, or downstream from the selection valve. In the first published work², the detector was placed between the valve and syringe. In this position a double peak results. The first peak is

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

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

Attention has to be given to avoid sample carry-over from one measurement to the next. The carry-over results from the sample solution in the short length of tubing between the sampling point or reservoir and the selection valve. Several measures to overcome this problem have been proposed³ including the following:

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

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

The pump⁸ used initially for SIA has provided a sinusoidal flow pattern. This is not a prerequisite for SIA. All that is required is a reproducible flow pattern. Further work is required to optimize the pump system, and to reduce the analysis time.

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

3.5 MANIFOLD DIMENSIONS AND GEOMETRY

Early work in the development of SIA soon established some guiding principles in the choice of syringe and reactor volumes³. When the Alitea sinusoidal-flow pump is used, only a segment of a full pump rotation is used. The volume of the stroke of the piston has been determined previously⁸ and may also be determined empirically. The maximum volume for a particular syringe is given by $V = 2R\pi r^2$, where R is the radius of the cam and r is the radius of the syringe. Ruzicka and Gübeli³ pointed out that once the stroke volume has been fixed, the combined reagent and sample volumes are fixed, as the stroke volume should be at least four times the combined volume of the sample and reagent. The holding coil should be large enough to prevent the sample or reagents from entering the syringe barrel. The reaction coils should not exceed one-third the volume of the wash solution, thereby ensuring that they are adequately flushed during every experiment. With these guidelines, the only parameters still required before a manifold can be constructed are the diameter of the tubing and the geometry of the reaction coils. Less stringent boundary conditions pertain when a flow through pump is used.

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

3.5.1 *Flow-reversal*

The principle and feasibility of using flow-reversal to enhance zone penetration in SIA has been demonstrated before². Gübeli *et al*⁴ investigated this further, and concluded that the first flow-reversal, and its length are most effective in providing mutual zone penetration. Furthermore, they stated that multiple flow-reversals would probably be used only for difficult solution-handling tasks, such as the mixing of zones of very different viscosities.

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

3.5.2 *Flow rate*

The use of a sinusoidal flow rate, once a method has been developed, has no adverse effect. It should however be borne in mind during method development. Equations describing the flow rate at each stage in an experiment have been given⁸. Flow rate is, of course, proportional to the pump speed, and the range of flow rates employed can be varied by changing the pump speed. The effect of pump speed on zone penetration and sensitivity is discussed below.

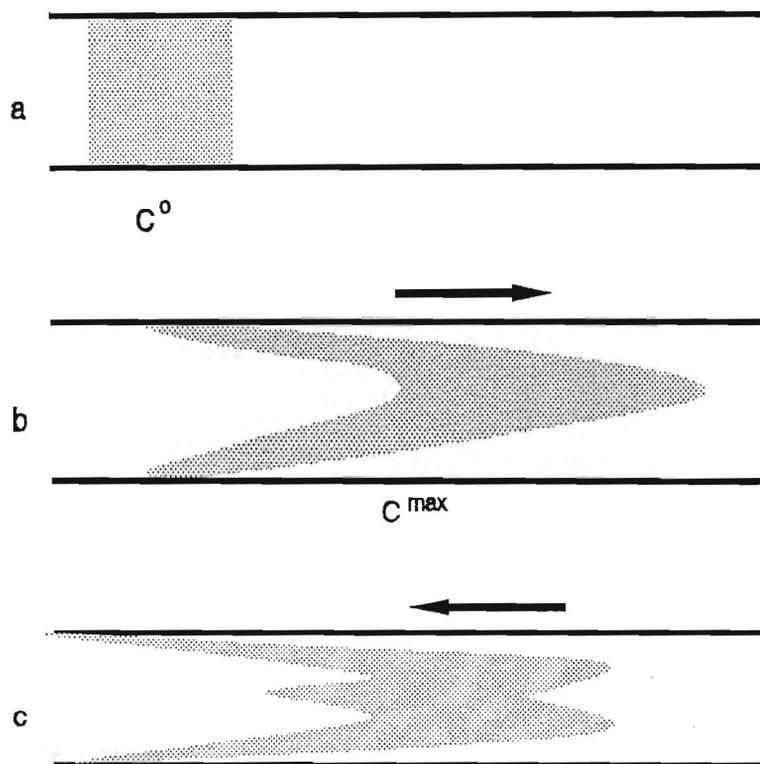


Figure 10: Dispersion of the a) sample plug due to laminar flow just b) prior to, and c) after zone reversal.

3.5.3 Sample and reagent volumes

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

- "Changing the injected sample volume is an effective way to change the sensitivity of the measurement. Dilution of overly concentrated injected sample material is best achieved by reducing the injected sample volume."
- "Injecting at least twice as large a reagent zone volume as the sample zone volume, while keeping the volume of the sample zone less than or equal to $0.5 S_{1/2}$, allows the optimum conditions for single reagent based chemistries to be

met." ($S_{1/2}$ is defined as the sample volume that yields a dispersion of 2 in the manifold).

- "Two reagent chemistries can be accommodated provided that the sample volume is kept below the $S_{1/2}$ value, the sample zone is surrounded by the reagent zones, and the concentration of the injected reagents are sufficiently high" to prevent sub-stoichiometric mixtures.

3.6 EXPERIMENTAL

3.6.1 *Instrumental*

The sequential-injection system depicted in Figure 9 was constructed using an Alitea dual-piston sinusoidal-flow syringe pump (Alitea USA, Medina, WA), a 10-port electrically-actuated selection valve (Model ECSD10P, Valco Instruments, Houston, TX), and a Milton Roy Spectronic Mini 20 spectrophotometer (Opto Labor, South Africa). Data acquisition and device control were achieved using a PC30-B interface board (Eagle Electric, Cape Town, South Africa) and an in-house assembled distribution board. The FlowTEK⁹ software package for computer-aided flow-analysis was used throughout for device control and data acquisition.

3.6.2 *Reagents*

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

Bromothymol blue dye solution and sodium tetraborate carrier solution were prepared in the usual way¹. In these studies, a system where no chemical reaction was involved was specifically chosen to keep a study of the zone penetration phenomenon as simple as possible. The obtained concentration profiles can be used to draw conclusions in systems where chemical reactions will take place.

3.6.3 *Experimental procedure*

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

3.7 RESULTS AND DISCUSSION

Unlike FIA where, in most cases, the sample bolus is surrounded by reagent, in SIA the sample and reagent zones are stacked in the manifold conduit. As these zones move through the manifold, mutual dispersion takes place and the zones penetrate one another. The degree of penetration can be measured in various ways. At the University of Washington, researchers borrowed parameters from peak-resolution theory developed by chromatographers to obtain a measure of the zone penetration⁴. This approach yields useful results, but is difficult to determine automatically. Furthermore, when large sample or reagent volumes are used, low overlap figures are obtained. (This, of course, demonstrates the uneconomical use of reagent and/or sample solution, but gives a false impression of the degree of zone penetration.)

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

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

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

3.7.1 *Effect of tube diameter*

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

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

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

Table VI

Effect of tubing diameter on zone penetration and precision

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

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

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

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

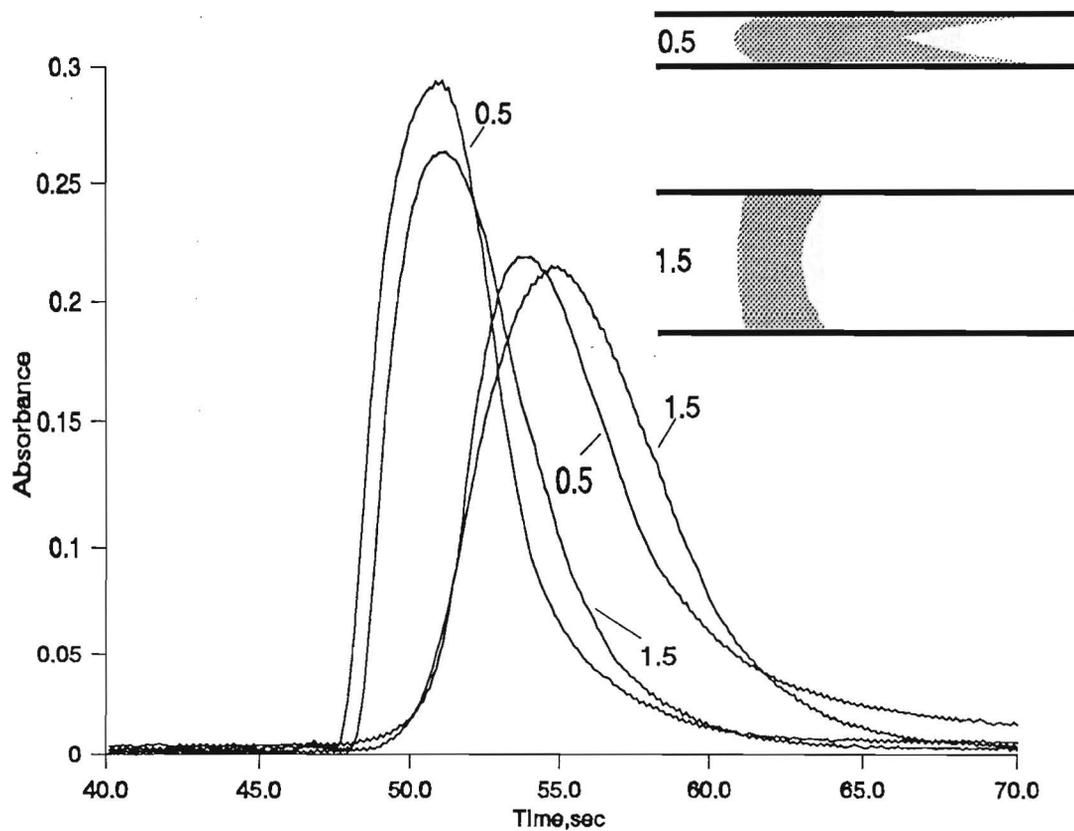


Figure 11: Effect of tube diameter on zone penetration

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

3.7.2 *Effect of reaction tubing geometry*

Various reactors have been described in the literature on FIA manifolds¹. Where the reactor consists of a length of tubing, various geometries have been proposed. Three were evaluated to establish the effect of reactor geometry on zone penetration and precision (see Table VII).

Table VII

Effect of reactor geometry on zone penetration and precision

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

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

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

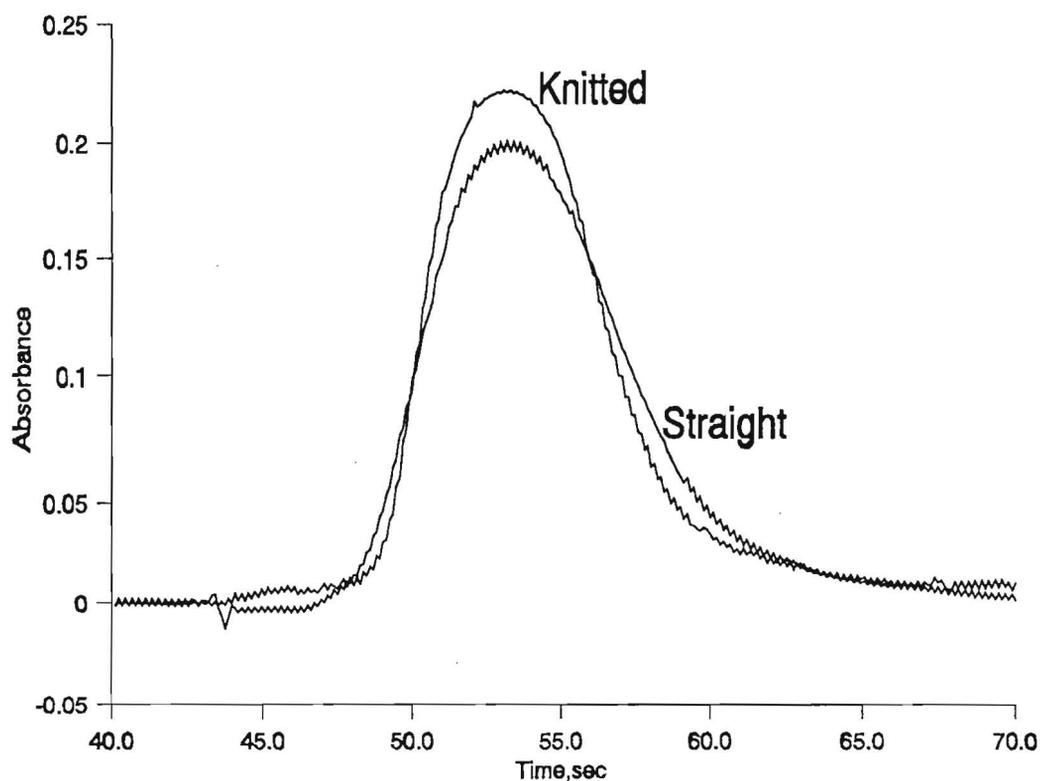


Figure 12: Effect of reactor geometry on zone penetration.

3.7.3 *Effect of pump speed*

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

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

We have already noted that the flow rate will change continuously when a sinusoidal flow pump is used. However, the range of flow rates can be altered by changing the pump speed. Figure 13 shows the wide range of flow rates encountered at a fixed pump speed. Theory would suggest that the volume drawn up can be kept constant by adjusting the times for sucking to compensate for the altered pump speed employed. In practice this is not the case. If a simple inverse ratio of time to pump speed is used, reduced volumes are obtained for higher pump speeds. This is probably due to the imperfect flow dynamics of the pump, i.e., start up and stopping are not instantaneous. For this reason, sampling times were not altered and the volumes used at slower pump speeds were smaller than at higher pump speeds. Previous work⁴ has shown that greater zone penetration occurs when smaller volumes are used. This was indeed observed in the present investigation (see Figure 14) where the zone penetration at a pump speed setting of 10 was found to be 0.339 while that for a pump setting of 30 was found to be 0.200. Despite the smaller individual peaks for the slower pump speed, the intersection of the two peaks was almost exactly the same due to the greater mutual penetration. SIA would therefore appear to favour slower pump speeds.

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

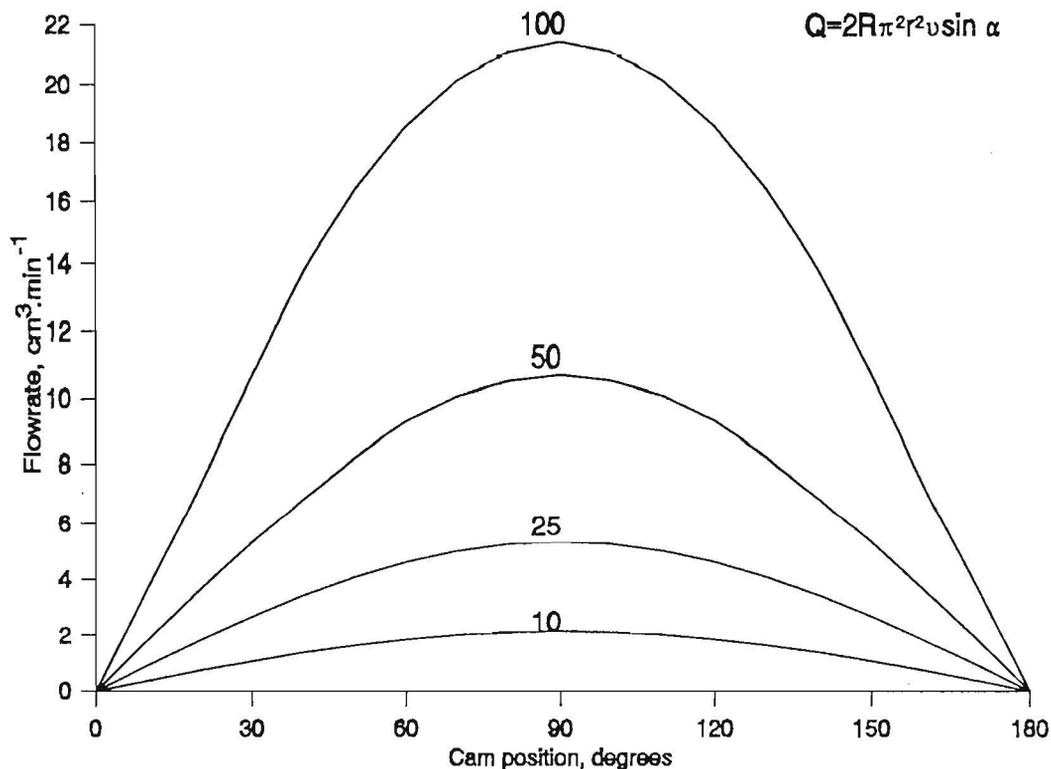


Figure 13: The flow rate at different pump speeds. (The area under the curve between particular cam positions gives the theoretical volume.)

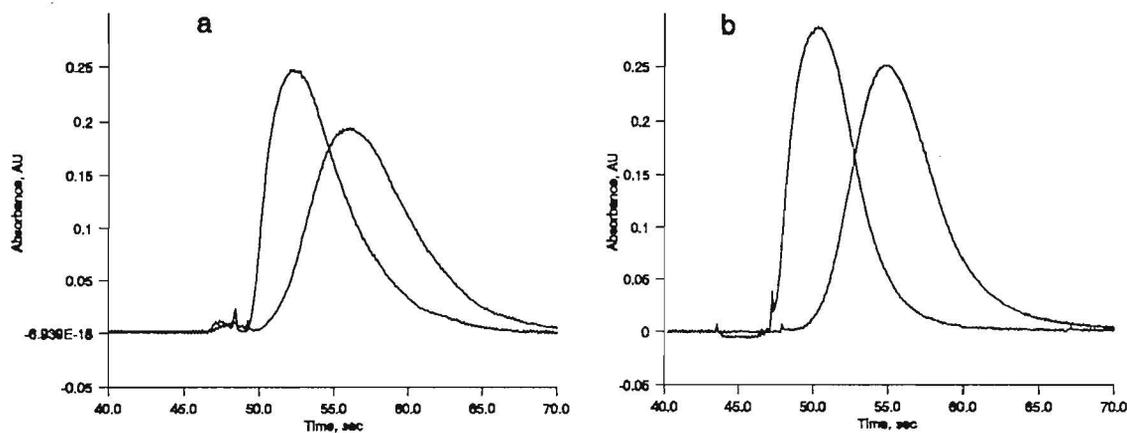


Figure 14: Effect of pump speed on zone penetration. Pump setting of a) 10 and b) 30.

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

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

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

3.7.4 *Order of injection*

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

3.8 CONCLUSIONS

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

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

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

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

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

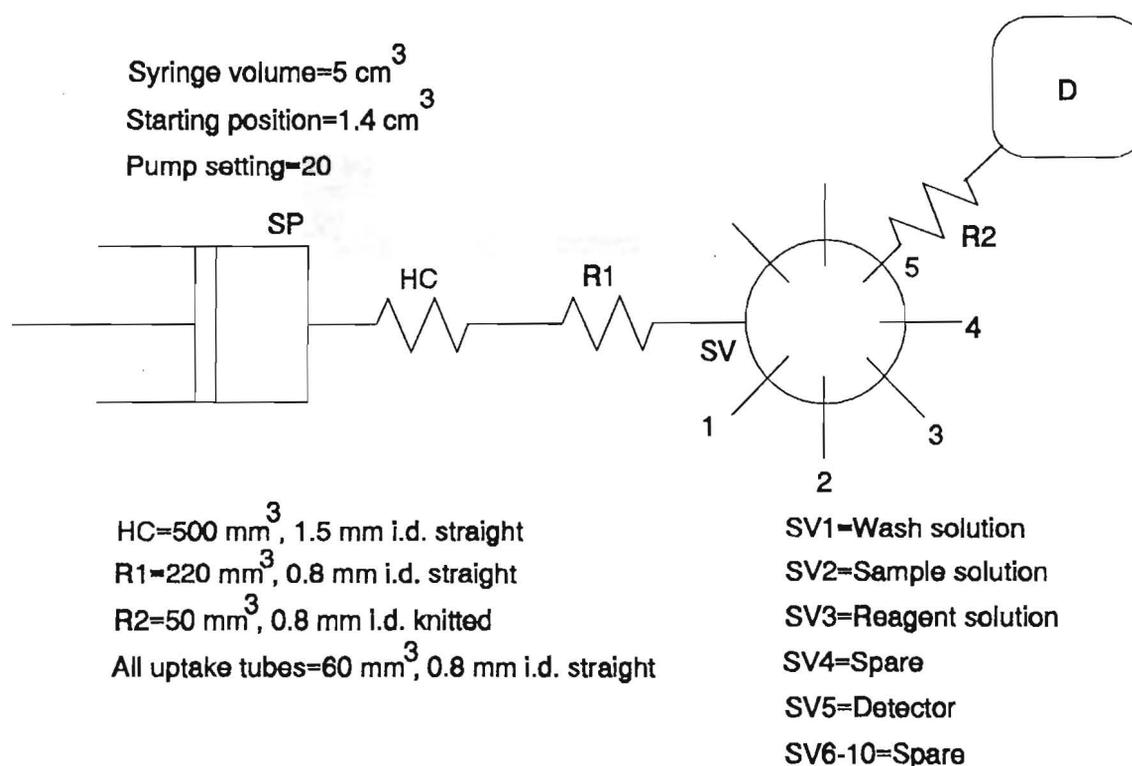


Figure 15: Manifold dimensions for optimized SIA manifold using a sinusoidal flow syringe pump.

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

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

Table VIII

Optimum device sequence for a binary system

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

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