Chapter 4

Application to Trace Enrichment

4.1 INTRODUCTION

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

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

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

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

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

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

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

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

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

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

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

4.2 Comparison to Solvent Extraction

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

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

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

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

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

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

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

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

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

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

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

4.3 EXPERIMENTAL

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

4.3.1 *Apparatus*

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

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

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

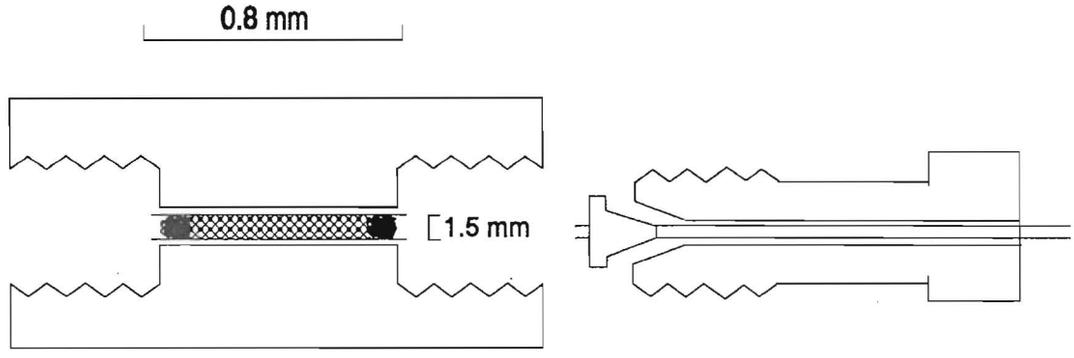

Figure 16: Miniature column used for sorbent extraction. The polymeric support is held in place by two small wads of glass wool. Two flangeless fittings seal the column in the perspex holder.

4.3.2 *Reagents*

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

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

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

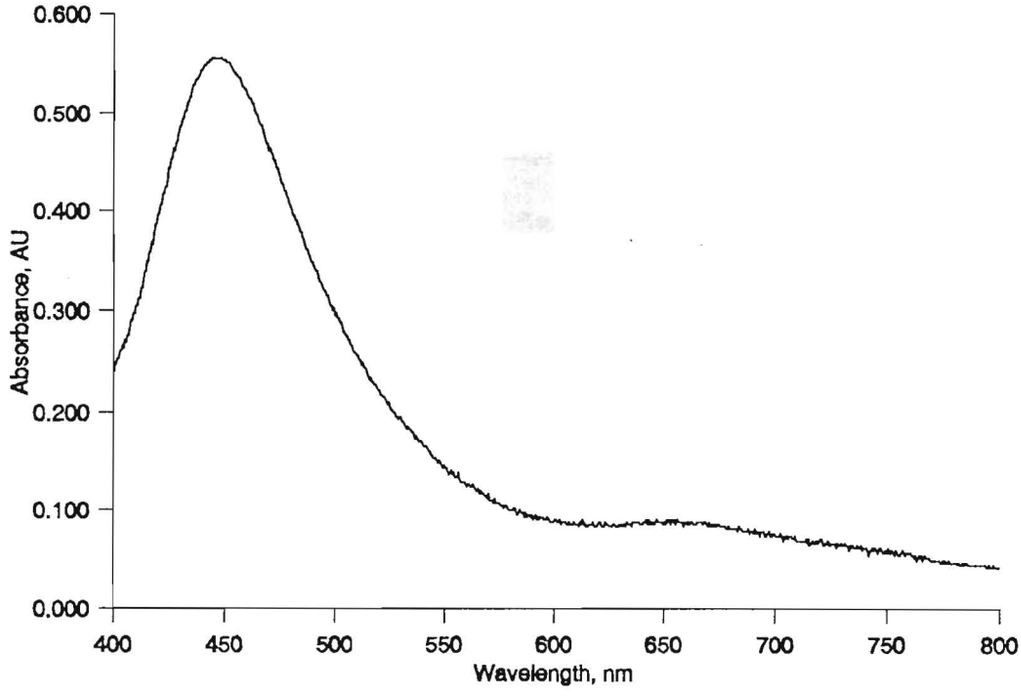

Figure 17: Spectrum of $Cu(DDTC)_2$ complex. Cu^{2+} concentration 5 $mg.dm^{-3}$ in 1:20 MeOH:H_2O mixture, pH=4. Peak maximum - 448 nm.

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

4.3.3 *Procedure*

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

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

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

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

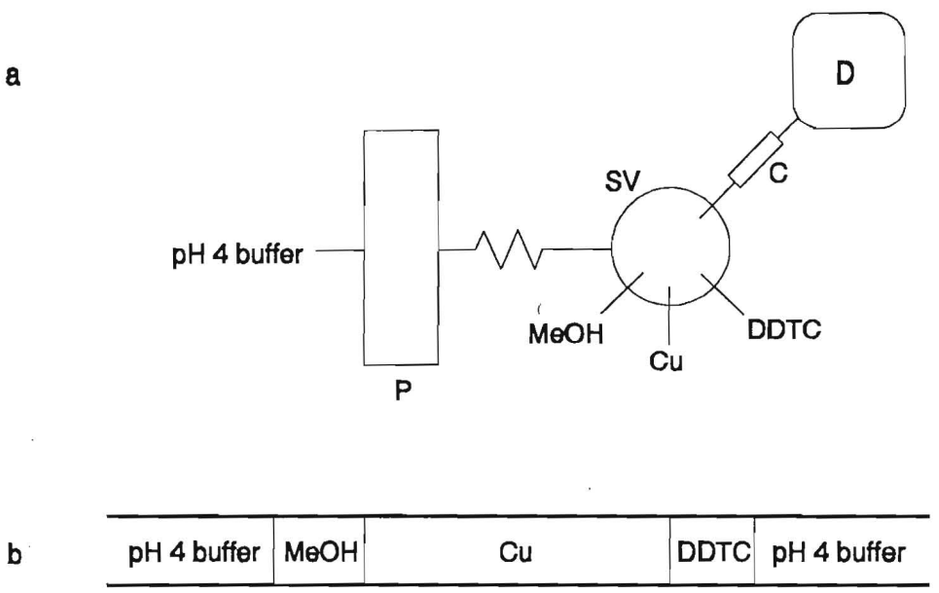

Figure 18: a) SIA manifold for sorbent extraction when using an aggressive stripping solution, P - pump, SV - selection valve, D - detector, C - column. b) Sequence of reagents in the SIA reaction coil (see text for details)

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

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

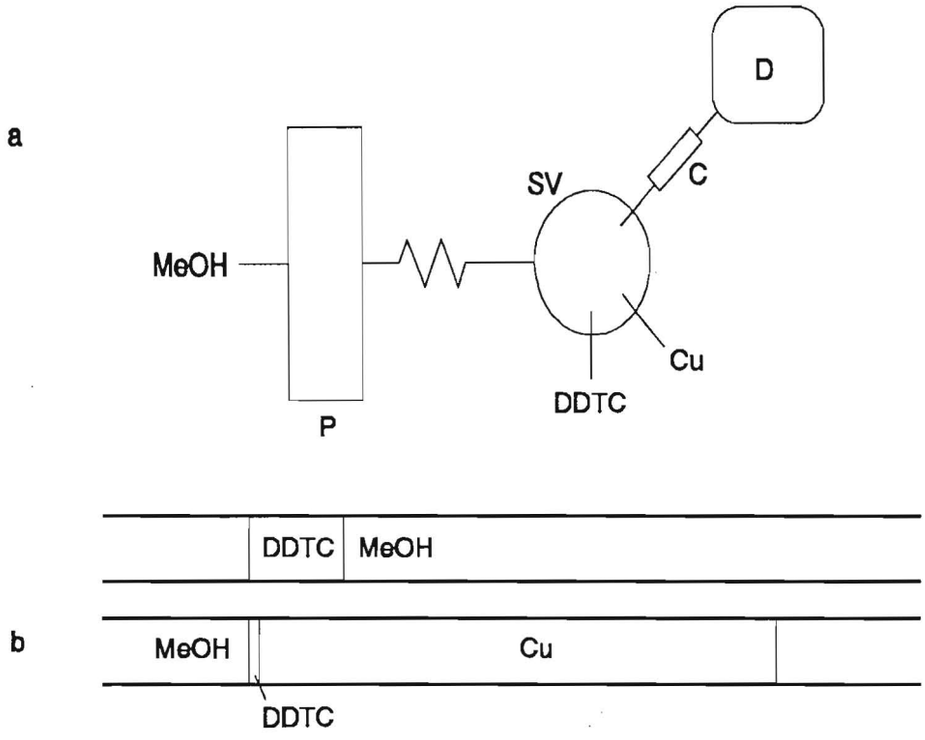

Figure 19: a) SIA manifold for sorbent extraction with pre-sample column loading, P - pump, SV - selection valve, D - detector, C - column. b) Sequence of reagents in the SIA reaction coil (referred to as Manifold A in the text).

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

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

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

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

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

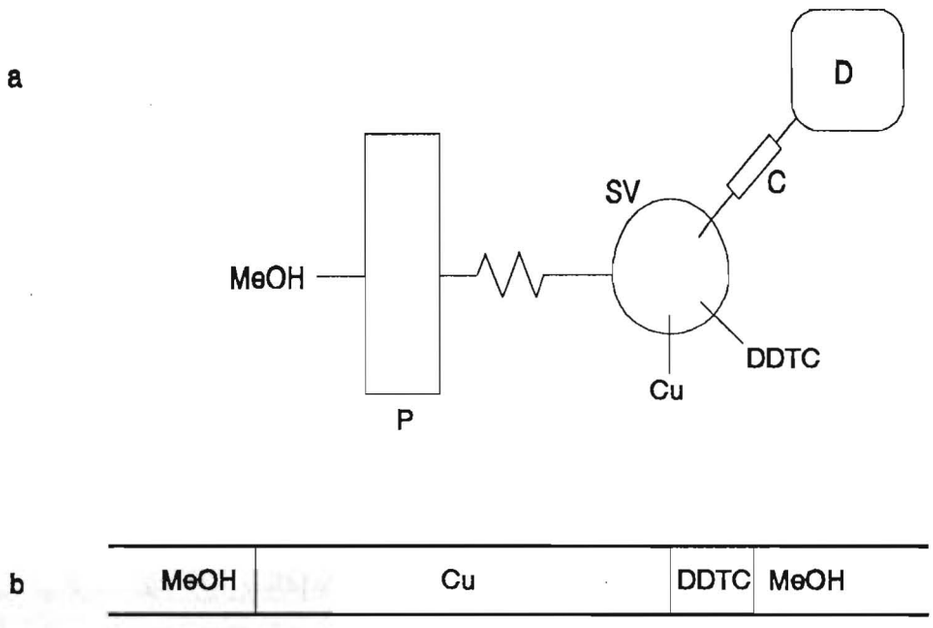

Figure 20: a) Optimized SIA manifold for sorbent extraction, P - pump, SV - selection valve, D - detector, C - column. b) Sequence of reagents in the SIA reaction coil (referred to as Manifold B in the text).

Table IX

Device events for sorbent extraction experiments

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

* P refers to pump and SV refers to selection valve

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4.4 RESULTS AND DISCUSSION

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

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

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

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

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

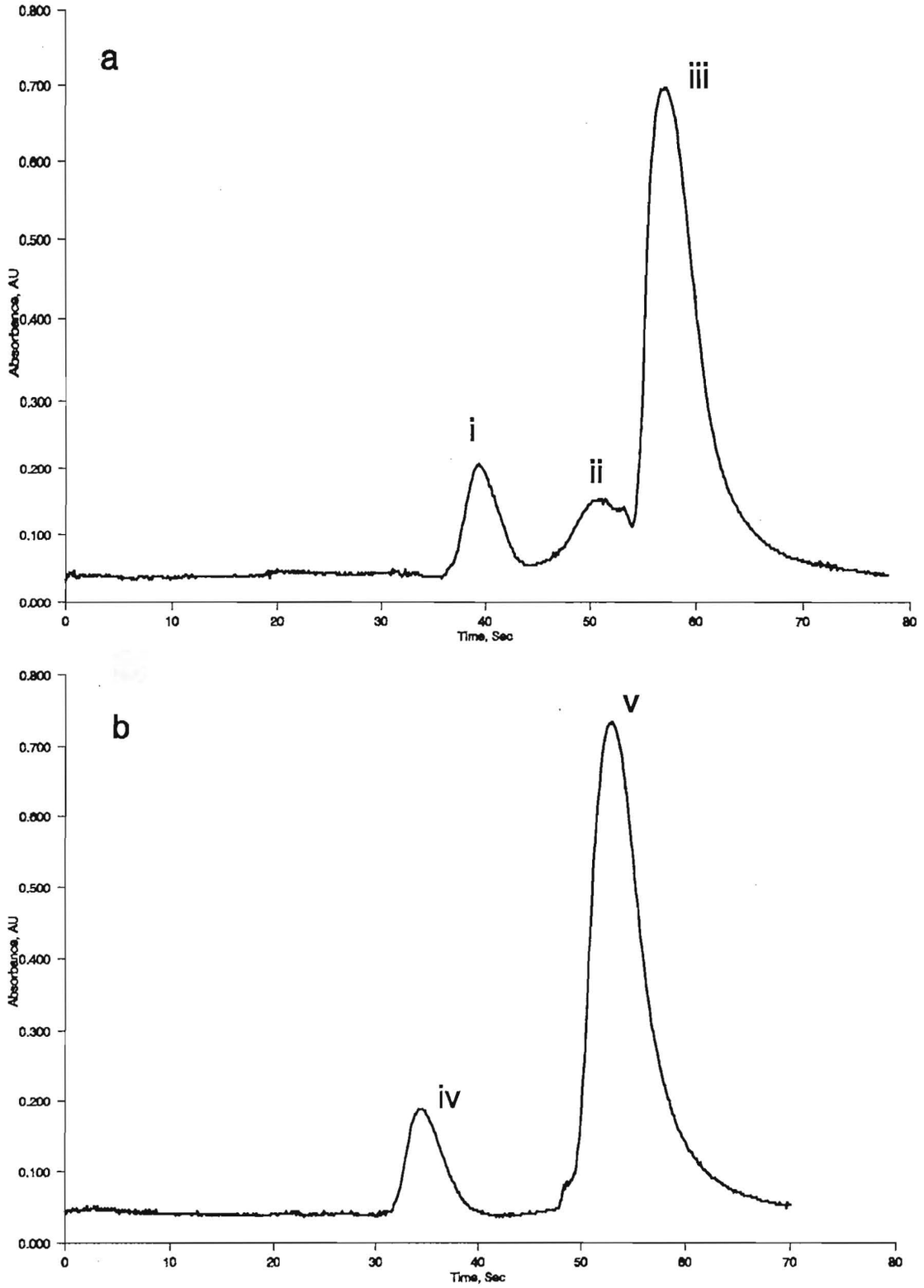

Figure 21: a) Response profile for the enrichment of $Cu(DDTC)_2$ using Manifold A. b) Response profile for the enrichment of $Cu(DDTC)_2$ using Manifold B.

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

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

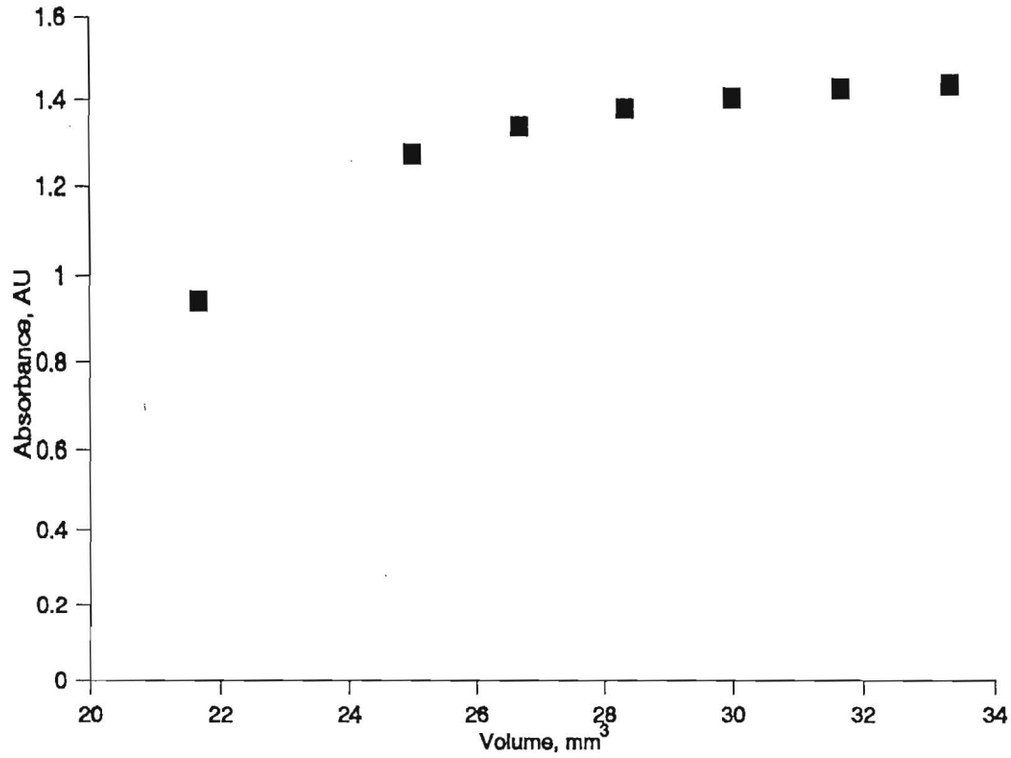

Figure 22: Effect of changing the relative ratio between complexing reagent drawn up and that dispensed to the pump. In each case, 33 mm^3 was dispensed, different volumes were aspirated.

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

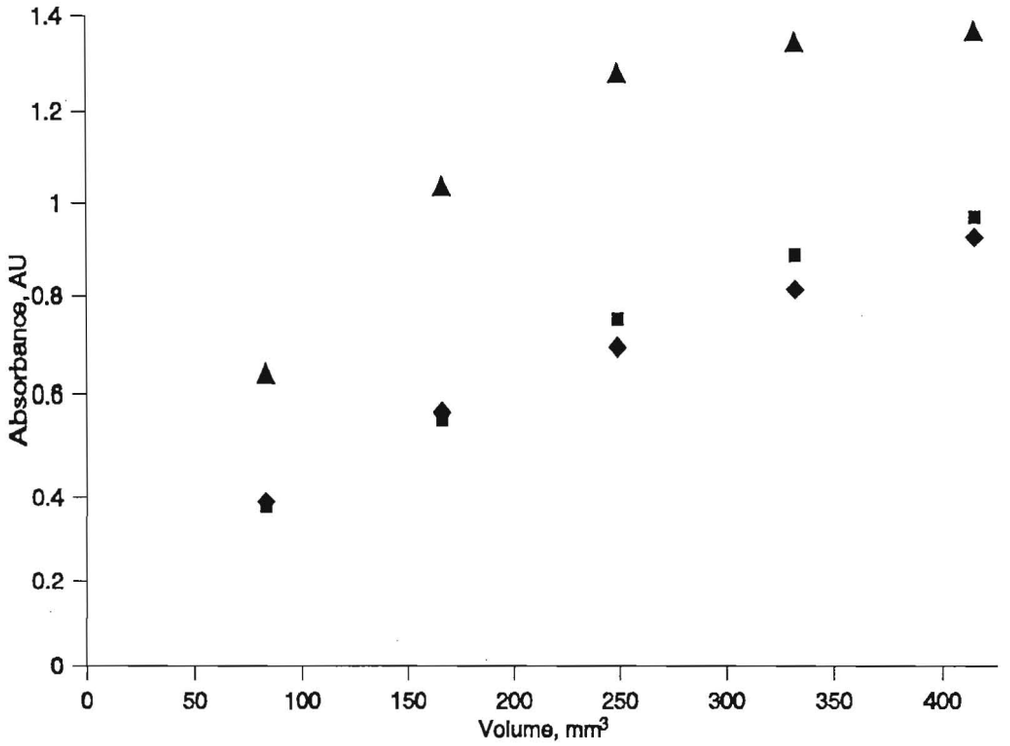

Figure 23: Effect of sample volume on response. ♦ - 2.5 mg.dm^{-3} Cu, Manifold A, ■ - 2.5 mg.dm^{-3} Cu, Manifold B, ▲ - 5.0 mg.dm^{-3} Cu, Manifold A.

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

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

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

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

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

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