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STUDIES ON SAMPLE PRECONCENTRATION IN ION CHROMATOGRAPHY

I. DESIGN OF AN AUTOMATED, SINGLE PUMP PRECONCENTRATION SYSTEM WITH DIRECT UV ABSORBANCE DETECTION

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SUMMARY

An automated sample preconcentration system for ion chromatography is described which uses a microprocessor-controlled pump coupled to a low-pressure switching valve and two high-pressure switching valves. The microprocessor in the pump is used to actuate these valves in a timed sequence which permits precise loading of a selected volume of sample onto an ion-exchange pre-column, which is then eluted through an ion-exchange analytical column. The system offers a high level of flexibility in selection of sample loading and elution parameters and can be programmed for unattended operation. Evaluation of the system was performed using mixtures of nitrite, bromide and nitrate in the concentration range 0–100 ppb, with detection by UV absorbance at 205 nm. The relative standard deviation of ten replicate analyses was approximately 1% for concentrations of 5–100 ppb, with slightly higher values being recorded for solutions of 1 ppb concentration. Using this system, detection limits of 0.05, 0.07 and 0.03 ppb for nitrite, bromide and nitrate, respectively, were recorded for a 15-ml sample volume, and cross-contamination between samples was less than 0.01%.

INTRODUCTION

The use of high-performance liquid chromatography (HPLC) for the determination of inorganic anions has received a great deal of attention over recent years and numerous separation and detection methods have now been developed[1]. Close examination of these ion chromatographic methods suggests that whilst separation of the inorganic anions is quite satisfactory, detection of trace levels of anions can be difficult. Indeed, commonly employed detection methods such as conductivity[2,3], direct UV absorbance[4], electrochemical[5] and indirect or "vacancy" methods[6] all have roughly equivalent detection limits in the range 100–500 ppb*[1]. For this reason,

* Throughout this article, the American billion (10^9) is meant.

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ion chromatographic techniques give best results when applied to samples with ionic concentrations greater than 1 ppm. Conventional ion chromatography is therefore of limited utility in ultra trace applications where the anions of interest are present in the concentration range 1–100 ppb.

Some adaptations have been suggested to enable very dilute samples to be reliably analysed and these have included new detector designs[7–9], the use of large injection volumes[10,11] and sample preconcentration techniques[12]. The most widely applicable method is sample preconcentration, which commonly involves the use of a small ion-exchange pre-column mounted before the analytical separation column[12]. Relatively large sample volumes are passed through the pre-column (generally referred to as a "concentrator" column), after which the trapped sample anions are eluted onto the analytical column. In this method, the sample is applied to the concentrator column using a large volume syringe or more usually, with a pump. In both cases, another pump is required to deliver the eluent.

In this paper we describe a fully automated trace enrichment system which uses a single microprocessor-controlled pump. The system design allows for flexibility in selecting the volume of sample to be concentrated, precise control of the sample volume and flow-rate used, and provides the additional benefit of unattended operation. This apparatus is ideally suited to non-suppressed ion chromatography and its features are illustrated using direct UV absorbance detection.

EXPERIMENTAL

Instrumentation

The liquid chromatograph used consisted of a Waters Assoc. (Milford, MA, U.S.A.) Model M590 pump and events unit, Model M481 variable-wavelength UV detector, together with a solvent select valve, two pneumatic column switching valves and a Model M730 data module. The separator column used was a Waters IC Pak A (50 × 4.6 mm I.D.) methacrylate-based anion exchanger (10 μm, 30 μequiv/ml) and the concentrator column was a custom made Waters Assoc. Guard Pak (5.0 × 6.0 mm I.D.) packed with methacrylate anion exchanger (25 μm, 15 μequiv/ml). The concentrator column was housed in a Waters Assoc. Guard Pak pre-column module.

Reagents

All water used was doubly distilled and passed through a Millipore (Bedford, MA, U.S.A.) Milli Q water purification system. Standard solutions (1000 ppm) of nitrate, nitrite and bromide were prepared by dissolving appropriate amounts of analytical grade sodium salts in pure water. These solutions were diluted daily to give the trace solutions required.

The eluent used was 6.0 m*M* sodium methanesulphonate at pH 8.0, prepared by adjusting the pH of a weighed amount of methanesulphonic acid (Tokyo Kasei Kogyo, Tokyo, Japan) with sodium hydroxide, followed by dilution to volume. The eluent was freshly prepared every two days and was filtered through a 0.45-μm filter and degassed in an ultrasonic bath before use.

Procedure

The pump microprocessor was programmed to actuate the switching valves in a timed sequence, the details of which are described under Results and Discussion (Table I and Figs. 2–4). Further chromatographic parameters are listed in the caption to Fig. 5.

RESULTS AND DISCUSSION

System design

A conventional sample preconcentration system consists of an eluent pump, concentrator column, analytical column and sample pump (or large volume syringe), interconnected with a six-port high-pressure switching valve. The lay-out and operation of such a system is illustrated in Fig. 1, which shows that the sample is loaded (Fig. 1a) onto the column in the opposite direction to that in which it is eluted (Fig. 1b). This technique, which is sometimes referred to as "backflushing", is used to minimise band broadening in the concentrator column through compression of the sample ions into a compact band in the initial stages of elution from the concentrator column.

In such systems, it is common to employ a relatively inexpensive pump either to deliver directly a known volume of sample to the concentrator column, or alternatively to displace sample from a large volume injection loop. The former method offers the advantage of flexibility in selection of the sample volume but has the disadvantage of poor reproducibility unless a high-precision pump is used. The reverse is true of the loop injection method, which has high precision but reduced flexibility. In addition, neither approach readily permits either preconditioning of the concentrator column with a different eluent in order to enhance retention of solute ions during the sample loading step, or rapid re-equilibration of the pre-column with eluent following sample loading.

In view of these deficiencies, we have investigated the design of a preconcentration system offering flexibility, reproducibility and automation, based on the use of a single, high-precision pump for delivery of both eluent and sample. Moreover,

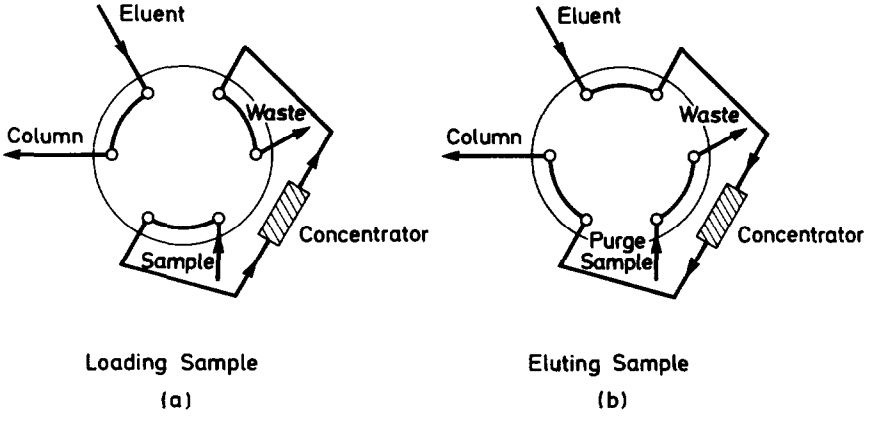

Fig. 1. Conventional sample preconcentration apparatus using a six-port high-pressure valve and two pumps. The flow-paths used for loading (a) and elution (b) of the sample are shown.

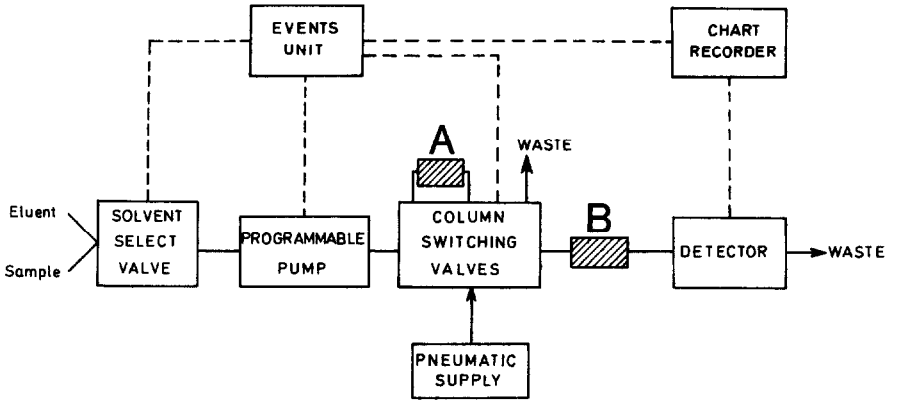

Fig. 2. Schematic representation of an automated, single pump sample preconcentration system using a microprocessor controlled pump, a low-pressure switching valve and two high-pressure switching valves. A = Concentrator column; B = analytical column.

the system was required to have the additional features of backflush elution and negligible cross-contamination between successive samples. Studies relating to on-line preconcentration in reversed-phase HPLC[13,14] suggested that two six-port switching valves (or alternatively one ten-port switching valve) would provide the required interconnections necessary to achieve the above aims.

Fig. 2 shows a schematic representation of the system developed, which used a microprocessor-controlled pump coupled to two high-pressure six-port column

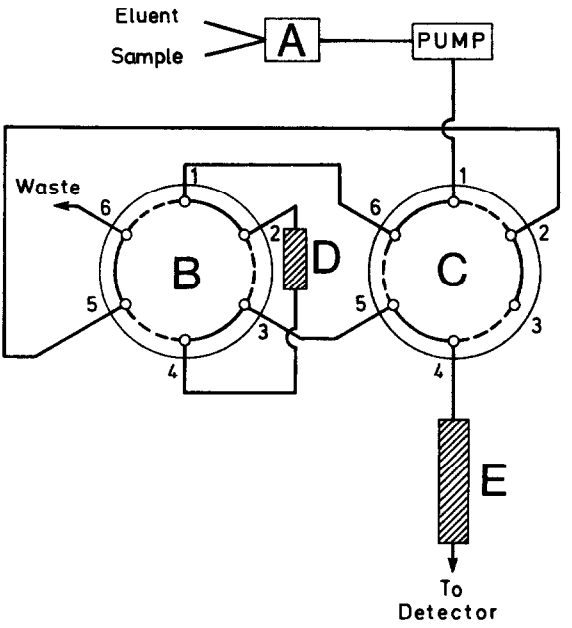

Fig. 3. Details of interconnections used for the preconcentration system A = Solvent select valve; B,C = six-port high-pressure switching valves; D = concentrator column; E = analytical column.

switching valves and a low-pressure solvent select valve. The high-pressure valves were pneumatically operated and the low-pressure valve was electrically operated, with all valves being actuated by electronic pulses from the pump via the events unit. Details of the interconnections used for the column switching valves are illustrated in Fig. 3.

System operation

The basic steps involved in preconcentration and elution of an aqueous sample are illustrated in Fig. 4, with the microprocessor in the pump being programmed to actuate the switching valves in the required sequence. In Fig. 4a, the two column switching valves (labelled B and C) have determined the flow-path and the solvent select valve was set to draw eluent, which was pumped through the concentrator column and thence through the analytical column. This configuration was the "analysis mode" and was the normal mode required for equilibration of the concentrator and analytical columns with a new eluent, or for analysis of a preconcentrated sample.

When a sample was to be loaded onto the concentrator column, the valves were switched to the positions shown in Fig. 4b, which was the "sample flush mode". The purpose of this step was to flush the pump inlet line and connecting tubing with sample. In the system used, 20 ml of sample was used for this flushing step, which was carried out at a high flow-rate in order to reduce the time required. At the completion of this step, the concentrator column was inserted into the flow stream (Fig. 4c) and the system entered the "sample load mode", in which the pump delivered a known volume of sample to the concentrator column, at a precise flow-rate. This step was clearly the most important with regard to precision of replicate analyses.

The next stage was the "eluent flush mode", which is depicted in Fig. 4d. Here the pump inlet line and the connecting tubing were flushed with eluent, again at a high flow-rate. The final step in the process was to return to the analysis mode (Fig. 4a) in which both the concentrator and analytical columns were eluted, causing the preconcentrated sample ions to be backflushed onto the analytical column and hence separated. At the commencement of the analysis mode, the microprocessor in the pump was used to activate the data module, which automatically switched off at the conclusion of the analysis.

Table I provides a summary of the basic steps performed during the operation of the preconcentration system and also indicates the typical duration and flow-rate used for each step. It should be emphasised that the sequence shown in Table II contains only the essential steps and in practice, more complex sequences were employed. For example, Table I suggests that the analytical column was inserted into an eluent stream flowing at 1.2 ml/min (step 9), which would have produced a pressure shock greatly detrimental to the methacrylate packing material. Instead of this, the eluent flow was ceased before the analytical column was switched into line and the flow-rate was then progressively increased using additional steps in the sequence.

The sequence described in Table I offered a high degree of flexibility in the variation and refinement of the preconcentration procedure and was also suitable for unattended operation using the microprocessor clock to initiate and conclude the process. The volume of sample loaded was varied by altering either the flow-rate or

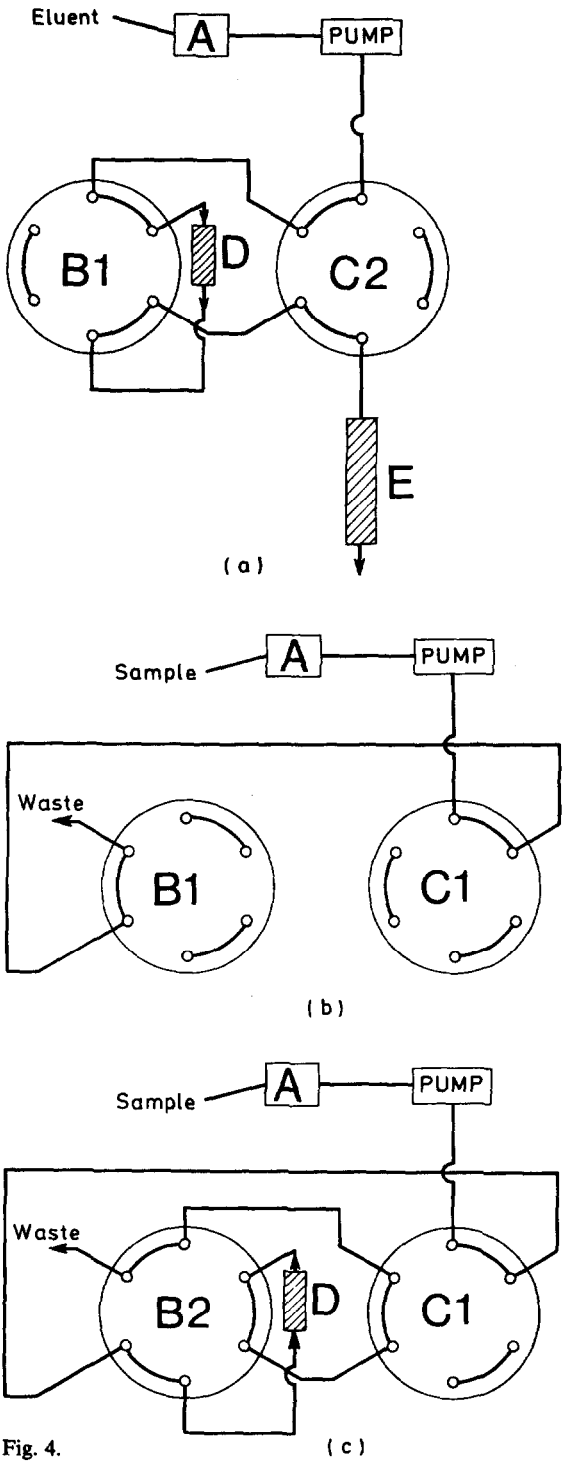
Eluent
A
PUMP
B1
D
C2
E
(a)
Sample
A
PUMP
Waste
B1
C1
(b)
Sample
A
PUMP
Waste
B2
D
C1
(c)

Fig. 4.

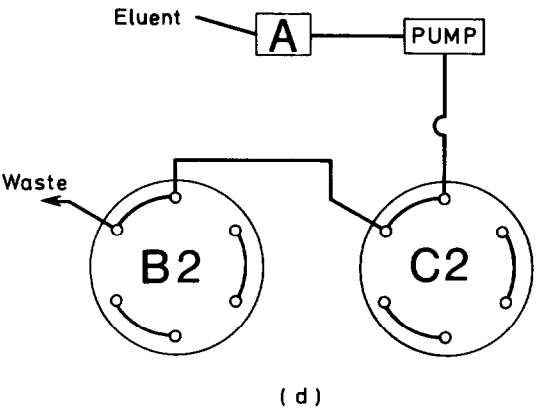

Fig. 4. Flow-paths used at various stages of sample preconcentration and elution. See text for discussion. (a) Analysis mode; (b) sample flush mode; (c) sample load mode; (d) eluent flush mode. A–E as in Fig. 3.

duration of step 5; in practice, we have found it most convenient to vary flow-rate within the range 0.01–3.0 ml/min, using a constant sample loading time of 5 min. Preconditioning of the concentrator column with a weak eluent in order to enhance retention of sample anions during the loading step was possible by incorporating additional flushing and equilibration steps using the flow-paths given in Figs. 4b and c, respectively, with the weak eluent used as an additional input to the solvent select valve. This approach can be necessary to ensure quantitative retention of weakly bound anions such as fluoride and silicate. Similarly, rapid re-equilibration of the concentrator column with eluent at the commencement of the analysis mode was also possible with this system and was achieved by passing a small volume of concentrated eluent through the concentrator column immediately prior to introduction of the normal eluent. When required, this step was performed using the flow-paths given in Figs. 4a and d, with the solvent select valve directing the concentrated eluent to

TABLE I

BASIC STEPS FOR THE PRECONCENTRATION AND ANALYSIS OF A SAMPLE

The flow-rates and durations of the individual steps are representative of those used in routine operation of the preconcentrations system. The flow-paths indicated in the table refer to those given in Fig. 4.

Step	*Time (min)*	*Solvent*	*Flow-path*	*Flow-rate (ml/min)*
1	0	Eluent	a	1.2
2	0.50	Sample	b	1.2
3	0.55	Sample	b	20.0
4	1.55	Sample	b	1.0
5	1.70	Sample	c	1.0
6	6.70	Eluent	d	1.0
7	6.85	Eluent	d	20.0
8	7.85	Eluent	d	1.2
9	8.00	Eluent	a	1.2
10	20.0	Eluent	a	0

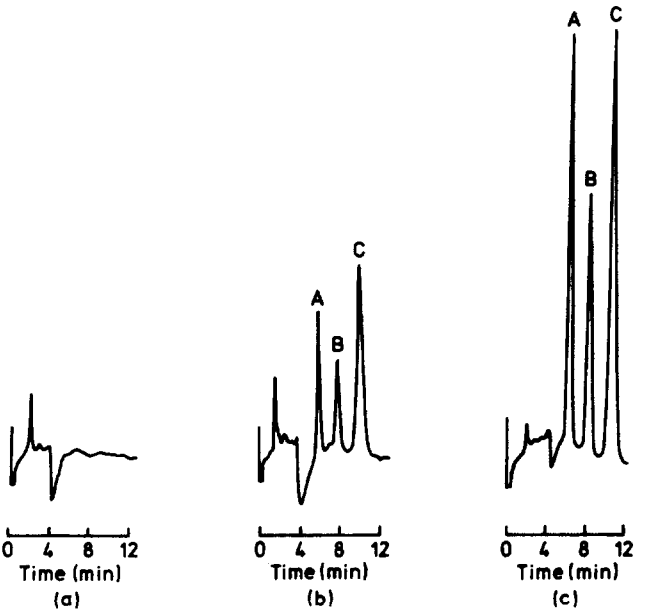

Fig. 5. Chromatograms obtained for trace mixtures of nitrite (A), bromide (B) and nitrate (C), using the preconcentration system with direct UV absorbance detection. (a) Blank; (b) 1.3 ppb of each anion; (c) 20 ppb of each anion. Conditions: analytical column, Waters Assoc. IC Pak A 50 × 4.6 mm I.D. methacrylate anion exchanger; concentrator column, Waters Assoc. Guard Pak containing methacrylate anion exchanger; eluent, 6.0 m*M* methanesulphonate at pH 8.0; flow-rate, 1.2 ml/min. Detection: UV absorbance at 205 nm. Detector sensitivity: 0.02 a.u.f.s. (a, b); 0.05 a.u.f.s. (c). Sample volume: (a) 15 ml; (b) 15 ml; (c) 5 ml.

the pump. Indeed, other similar refinements were possible since the solvent select valve could select from as many as four input solutions.

System evaluation

In order to evaluate the preconcentration system, samples containing nitrite, bromide and nitrate at trace levels were analysed using methanesulphonate as eluent, with direct UV absorbance detection at 205 nm. This eluent has previously been shown to be useful for ion-exchange separation of these inorganic anions with low-wavelength UV detection[15,16]. Fig. 5 shows chromatograms obtained at concentrations of 1.3 ppb (b) and 20 ppb (c) of each ion, together with a blank run (a). Note that both the sample volume and detector sensitivity in Fig. 5c were reduced in comparison to those used in Figs. 5a and b. A calibration plot for the concentration range 0–20 ppb of the three anions is given in Fig. 6 for a 15-ml sample volume. Detection limits for nitrite, bromide and nitrate, calculated from Figs. 5 and 6 were 0.05, 0.07 and 0.03 ppb, respectively, for a sample volume of 15 ml.

The precision of the preconcentration system was determined using ten replicate analyses of solutions of the three anions at various concentrations. The results are given in Table II which shows that relative standard deviations (R.S.D.) of measured peak heights were approximately 1% for concentrations of ions ranging from 5–100 ppb. At a concentration level of 1 ppb, a slight increase in R.S.D. was observed.

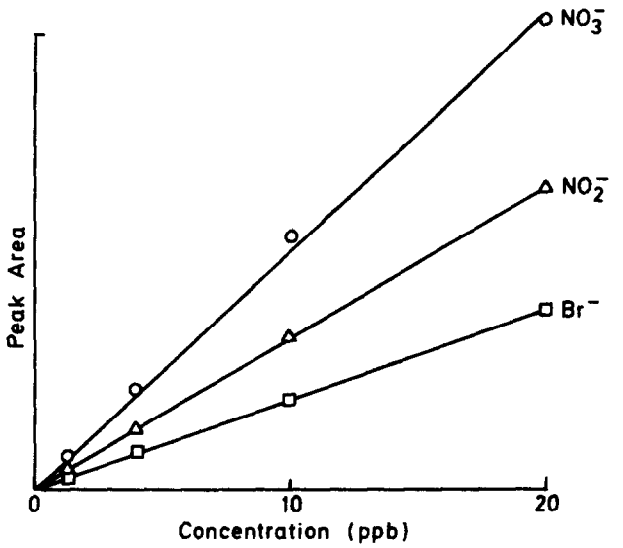

Fig. 6. Calibration plot for nitrite, bromide and nitrate obtained using the chromatographic conditions described in Fig. 5. A sample volume of 15 ml was used for each concentration tested.

Retention times were reproducible to within 1% for replicate analyses, however a slight increase in retention time (up to 3.8%) was observed between the lowest and highest sample concentrations. This increase can be attributed to the use of larger sample volumes at the lower concentrations, which results in some movement in the band of sample ions along the concentrator column, in the direction of sample loading. This in turn results in a slight increase in retention when these ions are back-flushed from the concentrator column. Cross-contamination of samples was measured by alternatively analysing a 100 ppb standard solution and a blank, and was found to be less than 0.01%.

TABLE II

PRECISION DATA FOR TEN REPLICATE ANALYSES OF MIXTURES OF NITRITE, BROMIDE AND NITRATE AT VARIOUS CONCENTRATION LEVELS

The experimental conditions used are given in Fig. 5.

Concentration (ppb)	*Volume concentrated (ml)*	*Detector sensitivity (a.u.f.s.)*	*Peak-height precision (% R.S.D.)*		
			Nitrite	*Bromide*	*Nitrate*
100	5	0.10	1.34	0.59	0.68
20	10	0.05	0.97	1.46	0.75
5	15	0.02	1.10	1.04	1.18
1	15	0.01	2.95	1.85	2.64

CONCLUSIONS

In this paper, we have described a single pump, automated sample preconcentration system suitable for non-suppressed ion chromatography. This system offers a high level of flexibility in selection of sample loading and elution parameters and also permits the inclusion of additional steps for preconditioning or rapid re-equilibration of the concentrator column, when these steps are necessary. Very precise results were achieved in the analysis of ultra trace solutions of nitrite, bromide and nitrate.

This system is currently being applied to conductivity and indirect UV absorbance detection methods and will be used for a systematic study of the experimental factors governing the sample preconcentration process.

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REFERENCES

1 P. R. Haddad and A. L. Heckenberg, *J. Chromatogr.*, 300 (1984) 357.
2 J. A. Glatz and J. E. Girard, *J. Chromatogr. Sci.*, 20 (1982) 266.
3 D. T. Gjerde, J. S. Fritz and G. Schmuckler, *J. Chromatogr.*, 186 (1979) 509.
4 R. J. Williams, *Anal. Chem.*, 55 (1983) 851.
5 J. E. Girard, *Anal. Chem.*, 51 (1979) 836.
6 P. R. Haddad and A. L. Heckenberg, *J. Chromatogr.*, 252 (1982) 177.
7 J. M. Keller, *Anal. Chem.*, 53 (1981) 344.
8 E. Pungor, F. Pal and K. Toth, *Anal. Chem.*, 55 (1983) 1728.
9 J. F. Alder, P. R. Fielden and A. J. Clark, *Anal. Chem.*, 56 (1984) 985.
10 A. E. Buchholz, C. I. Verplough and J. L. Smith, *J. Chromatogr. Sci.*, 20 (1982) 499.
11 A. L. Heckenberg and P. R. Haddad, *J. Chromatogr.*, 299 (1984) 301.
12 R. A. Wetzel, C. L. Anderson, H. Schleicher and G. D. Crook, *Anal. Chem.*, 51 (1979) 1532.
13 R. W. Frei and U. A. Th. Brinkman, *Trends Anal. Chem.*, 1 (1981) 45.
14 C. E. Werkhoven-Goewie, U. A. Th. Brinkman and R. W. Frei, *Anal. Chem.*, 53 (1981) 2071.
15 J. P. Ivey, *J. Chromatogr.*, 267 (1983) 218.
16 P. E. Jackson, P. R. Haddad and S. Dilli, *J. Chromatogr.*, 295 (1984) 471.