

CHROM. 21 979

## DYESTUFF-COATED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC RESINS FOR THE ION-EXCHANGE AND CHELATING-EXCHANGE SEPARATION OF METAL IONS

P. JONES\*<sup>a</sup> and G. SCHWEDT

*Institute for Inorganic and Analytical Chemistry, Technische Universität Clausthal, Paul-Ernst-Strasse 4, D-3392 Clausthal-Zellerfeld (F.R.G.)*

---

### SUMMARY

Methods are described for the formation of permanent coatings of triphenylmethane-type dyestuffs on high-performance liquid chromatographic-grade neutral polystyrene resins. Bromophenol blue gave an ion-exchange coating with separation properties similar to those of a low-capacity sulphonated resin, although the efficiency was lower. Most of the work was focused on Chrome Azurol S, which produced a chelating-exchange coating. The separation and preconcentration of both divalent and trivalent metal species was studied and found to be strongly influenced by the pH of the eluent, but little affected by the ionic strength. With stepped pH gradients, preconcentration and separation could be achieved using a single column, even in 1 *M* potassium nitrate solution.

---

### INTRODUCTION

Most publications dealing with the determination of trace metals by high-performance liquid chromatography (HPLC) have concentrated on ion-exchange techniques (usually referred to as ion chromatography). Regardless of whether high- or low-capacity substrates are used, retention and separation are normally controlled by polyfunctional carboxylic acids which form relatively weak complexes with the metal ions.

Although ion-exchange separations of metal ions have been used in a number of important applications, as discussed in recent reviews<sup>1,2</sup>, there are still several problems associated with this approach. One particularly important problem is column disturbance due to the ionic strength of the sample solution. Too high an ionic strength can cause a temporary drastic change in column capacity, destroying the separation. As a large number of sample treatment procedures result in highly concentrated solutions, *e.g.*, wet oxidation, further sample handling may be necessary to remove the bulk of the matrix before injection.

---

<sup>a</sup> Permanent address: Department of Environmental Sciences, Plymouth Polytechnic, Plymouth, Devon PL4 8AA, U.K.

An alternative approach to ion exchange is chelating exchange, in which the solid substrate contains chelating groups rather than ion-exchange groups. As the capacity factors of the metal ions depend on the values of the conditional stability constants in the stationary phase, the ionic strength will have much less effect on the chromatography. Therefore, at its simplest, retention can be controlled by varying the pH of the mobile phase containing only a non-complexing acid or salt. Auxiliary complexing agents, such as polyfunctional carboxylic acids, can of course still be used, but competing chelation and pH will then control the capacity factors of the metal ions. Chelating exchange is well known in classical column chromatography, where it has been used principally for matrix isolation. However, an increasing number of publications are appearing that describe metal separations on high-performance liquid chromatographic (HPLC)-grade or near HPLC-grade chelating stationary phases. Nearly all these publications describe substrates with chemically bonded chelating groups made by the workers themselves. Fritz and co-workers studied a variety of chelating functions on resins, as discussed in a book on ion chromatography by Fritz *et al.*<sup>3</sup>, while Faltynski and Jezorek<sup>4</sup> reported the methods of synthesis and chromatographic performance of six bonded chelating functions on silica gel. Risner and Jezorek<sup>5</sup> described a more detailed study of 8-hydroxyquinoline (HQ)-bonded silica gel, with the conclusion that a very lightly loaded column gave the best separations. Chambaz and Haerdi<sup>6</sup> used a similar 8-HQ-bonded silica gel column to study the preconcentration and elution of a range of divalent metals. Iminodiacetic acid (IDA) has also been bonded to HPLC-grade material and a Japanese resin is now commercially available. Toei<sup>7</sup> investigated such a material for the separation and determination of calcium and magnesium in sea water.

Although a number of bonded chelating groups have shown some interesting results, a greater range needs to be investigated in order to ascertain the full potential of chelating-exchange HPLC substrates. One way of achieving this without resorting to lengthy and perhaps difficult syntheses of chemically bonded groupings is to coat a particular substrate with selected compounds. Modifying stationary phases by coating with specific compounds is a well known technique in ion chromatography, but mainly concerns the formation of dynamic ion-exchange coatings using quaternary ammonium- or alkyl sulphonate-based compounds. Few workers have investigated dyestuff coatings on HPLC-grade materials. However, Golombek and Schwedt<sup>8</sup> recently showed that it was possible to achieve excellent high-speed separations of common anions using a dynamic coating of the dyestuff methyl green on a neutral polystyrene-based resin.

We considered that it would be useful to try to extend this idea of dyestuff coatings to produce HPLC-grade cation-exchange and chelating-exchange substrates for the separation of metals. The literature contains many examples of dyestuff coatings, but on resins of large particle size. A recent review<sup>9</sup> shows that most of the publications describe chelating-exchange coatings on anion-exchange resins, with only a relatively small number involving neutral resins. The principal objective of most of this published work was to collect or preconcentrate groups of metal ions from a variety of matrices which, after elution, were then determined by a number of off-line techniques. There appears to be no published work as yet on analytical separations of groups of metal ions on dyestuff-loaded resins of small particle size.

This paper describes a preliminary investigation of the separation character-

istics of HPLC-grade neutral resins for metal cations when coated with organic dyes giving ion-exchange or chelating-exchange properties. Most of the work was focused on the ligand-exchange coatings as these showed the greatest potential for novel separations.

## EXPERIMENTAL

### *Apparatus*

A standard isocratic LC system was used, linked to a post-column reactor as described elsewhere<sup>10</sup> but with the preconcentration column replaced with a sample loop. Both the column pump and the post-column reaction pump were made of stainless steel, Model BT8100 (Biotronic, Maintal, F.R.G.). The column effluent and reagent streams (both kept at  $1 \text{ ml min}^{-1}$ ) were mixed at a simple stainless-steel T-junction and then followed immediately by a reaction coil composed of  $4 \text{ m} \times 0.3 \text{ mm}$  I.D. PTFE tubing tightly wound on a 4-mm diameter glass rod. The detector was a Model BT8200 variable-wavelength UV-VIS spectrophotometer (Biotronic). The injector was a Model 7125 valve fitted with a  $100\text{-}\mu\text{l}$  sample loop (Rheodyne, Cotati, CA, U.S.A.), connected to a stainless-steel column ( $150 \times 4.6 \text{ mm}$  I.D.), filled with Benson BPI-10  $10\text{-}\mu\text{m}$  particle size neutral polystyrene-based resin. This resin was coated with an organic dyestuff as described below. The separations were carried out mostly at room temperature with some at  $60^\circ\text{C}$ , as detailed below.

### *Reagents*

Analytical-reagent grade chemicals and solvents were used unless stated otherwise. All the dyes studied were obtained from Aldrich (Milwaukee, WI, U.S.A.) and the metal standard solutions were prepared from  $1000 \mu\text{g ml}^{-1}$  stock solutions obtained from Merck (Darmstadt, F.R.G.).

### *Elution systems*

A number of elution systems were used, based on acetic acid or lactic acid, or a mixture of both, adjusted to a particular pH with sodium hydroxide solution. For the chelating-exchange work, the ionic strength was adjusted with potassium nitrate. Exact details are given under Results and discussion.

### *Detector systems*

Three post-column reaction reagents were used, depending on the metals under study.

*Calmagite*. This post-column reaction has been described elsewhere<sup>10</sup>, except that the more stable Calmagite replaces Eriochrome Black T. The Calmagite was used to detect the divalent metals, except beryllium. The reagent solution consisted of 0.004% (w/v) Calmagite in *ca.*  $0.3 \text{ M}$  aqueous ammonia. The detector wavelength was set at 610 nm with metal peaks appearing as a decrease in absorbance.

*Pyrocatechol violet (PCV)*. This reagent was used to detect Al, Ga, In,  $\text{Bi}^{\text{III}}$  and  $\text{Fe}^{\text{III}}$ . The post-column reaction solution consisted of 0.004% (w/v) PCV in  $0.5 \text{ M}$  aqueous hexamine adjusted to pH 6.0 with  $2 \text{ M}$  nitric acid. Higher concentrations of hexamine buffer will be required if the pH of the eluent is less than 1.5. The exact concentration of hexamine is not important, provided that the pH of the post-column

reaction is between 5.7 and 6.0. The detector wavelength was set at 580 nm, where metals were detected as an increase in absorbance.

*Chrome Azurol S (CAS)*. This reagent was used to detect Be. It can also be used for Al, Ga and Fe<sup>III</sup> but, unlike PCV, cannot detect In and Bi<sup>III</sup>. (Note: this is the same dye that was used to coat the resin for the chelating-exchange work.) The post-column reagent consisted of a 0.008% solution of CAS in 0.5 M hexamine buffer adjusted to pH 5.6 with nitric acid. For more acidic eluents the same considerations apply as for PCV, taking into account that the optimum pH for the CAS reaction is 5.6. The detector was set at 560 nm, where metal peaks gave an increase in absorbance.

#### *Column coating procedures*

The resin was coated by pumping a 0.2% solution of the dyestuff in methanol-water (20:80), adjusted to pH 3 with acetic acid, through the column until breakthrough occurred, and then the pumping was continued for a further 10 min. The column was washed with deionized water and adjusted to pH 10.5 with ammonia solution until the column effluent was colourless. This non-leaching condition was established using the detector set at the wavelength of maximum absorbance of the dye. To ensure that the column was stripped clean of any adsorbed metals, 20 ml of 0.1 M nitric acid was pumped through, followed immediately by a solution of 0.1 M acetic acid. When the column was left for any length of time, including overnight, it was stored in 0.1 M acetic acid (*ca.* pH 3).

For the results presented in this paper, a coating of bromophenol blue was used for the ion-exchange work and a coating of Chrome Azurol S (CAS) for the chelating exchange study.

## RESULTS AND DISCUSSION

### *Ion-exchange coating*

To obtain a strong-acid cation-exchange surface on the resin requires a coating compound containing sulphonic acid groups. This is not a major problem as many dyestuffs, particularly acid-base indicators, have sulphonic acid groups to make them water soluble. However, to investigate the coating of a large number of dyes on the HPLC-grade resin would be time consuming and costly. Because of this, it was decided to screen a number of dyes on a neutral resin of large particle size packed in glass columns, to obtain some idea of the stability of the coating under a range of conditions. The resin chosen was Amberlite XAD-4 (Rohm and Haas) which, although a macroporous type, subsequently proved to be a useful guide to the behaviour of a dye coating on Benson HPLC resin.

As a result of this XAD-4 screening, methyl orange and bromophenol blue were chosen for a more detailed study on the Benson column. The methyl orange coating was found to "leach" very slowly, subsequently interfering with post-column reaction detection, and so was abandoned in favour of bromophenol blue (a triphenylmethane dye). The bromophenol blue coating was particularly stable (total loading approximately 50 mg) and, once conditioned as detailed under Experimental, gave no observable leaching throughout the study. A check for leaching was carried out several times a day by switching off the post-column reaction pump and monitoring the absor-

bance of the column effluent at the wavelength maximum of the bromophenol blue dye. A 0.1 M lactic acid eluent was chosen to assess the separation performance of the coated column, as this was known to give good results with low-capacity ion-exchange substrates<sup>11</sup>. Fig. 1 shows the separation of Zn, Co and Mg using a Calmagite post-column reactor. Clearly, the column was functioning as an ion exchanger, giving similar retention times to those found on low-capacity chemically bonded phases such as those obtainable commercially. However, the separation efficiency was disappointing, made worse by significant peak tailing. Although the efficiency would undoubtedly improve if 5- $\mu\text{m}$  resins were used, tailing may still be a problem. As 5- $\mu\text{m}$  resins were not available, it was decided that attention should be focused on chelating-exchange coatings.

### Chelating-exchange coating

The same XAD-4 screening procedure as described for ion-exchange coatings was used for this work. Ten chelating compounds were subjected to the screening process as listed in Table I. These were chosen to cover a range of chelating function, dye type and molecular size. Plainly, colour is not an important requirement of the organic compound to form a stable coating, although it is useful, as the depth of coating and the amount of leaching can easily be observed. Nevertheless, two important chelating agents, chromotropic acid and 8-hydroxyquinoline sulphonate, were included, even though they are colourless in the uncomplexed form.

From the results of the screening, Calcon, chromotropic acid, 8-hydroxyquinoline and PAR gave poor, thin coatings, Calmagite, methylthymol blue and xylenol orange gave light coatings and Chrome Azurol S, Chromoxane Cyanine R and pyrogallol red gave fairly deep coatings. Of the last three, Chrome Azurol S was chosen for further study on the Benson column as it appeared to give the deepest coating. In any event, pyrogallol red would not be suitable because it is easily oxidized in alkaline media. It is fortuitous that Chrome Azurol S gave a deep coating as it is stable to oxidation. Also, the chelating function, which is essentially a salicylic acid analogue (Fig. 2), is a weaker complexing group for many metals than the most commonly

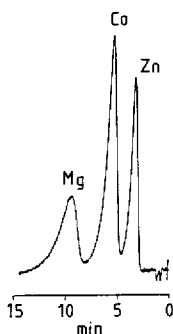


Fig. 1. Ion-exchange separation on a bromophenol blue-coated column. Sample: 100- $\mu\text{l}$  injection of a mixture of cobalt, magnesium and zinc each at a concentration of 10 ppm. Chromatographic conditions: eluent, 0.1 M lactic acid (pH 3.8); detection, post-column reaction with Calmagite solution; wavelength, 610 nm.

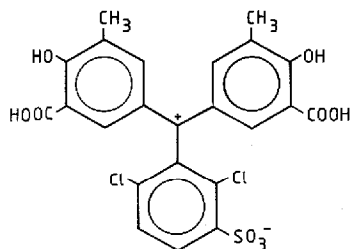


Fig. 2. Structure of Chrome Azurol S.

TABLE I  
LIST OF DYES SCREENED WITH AMBERLITE XAD-4

1. Calcon
2. Calmagite
3. Chrome Azurol S
4. Chromotropic acid<sup>a</sup>
5. Chromoxane Cyanine R
6. 8-Hydroxyquinoline<sup>a</sup>
7. Methylthymol blue
8. PAR
9. Pyrogallol red
10. Xylenol orange

<sup>a</sup> Colourless in uncomplexed form.

investigated iminodiacetic acid or 8-hydroxyquinoline functions. Therefore, it was considered that the separation properties of Chrome Azurol S (CAS) would be an interesting comparison with those of the iminodiacetic acid and 8-hydroxyquinoline bonded substrates. A Benson column was coated with CAS as detailed under Experimental and the results of the investigation are divided into two parts, divalent and trivalent metals. However, beryllium was included with the trivalent metals as it has a similar chemistry to aluminium.

Before any work could be carried out on the chelating properties of CAS, it was important to ensure that ion-exchange effects due to the presence of a sulphonate group (Fig. 2) were eliminated. This was achieved by maintaining a high ionic strength in the eluent. Thus, a concentration of at least 0.2 *M* with respect to potassium nitrate was used for all the chelating-exchange work.

#### Divalent metals

The results of a preliminary study of the separation properties of the CAS-coated column for a number of divalent metals are shown in Fig. 3. Fig. 3A shows a separation under simple pH control and Fig. 3B the separation of the same three

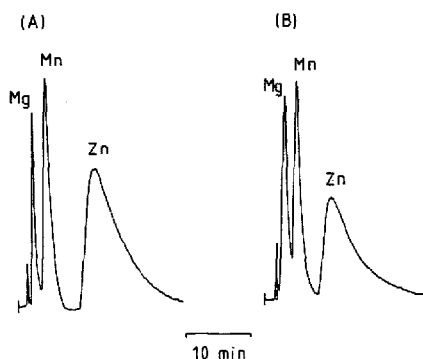


Fig. 3. Isocratic chelating-exchange separation of divalent metal ions on a CAS-coated column. Chromatographic conditions: eluent, (A) 1 *M*  $\text{KNO}_3$  (pH 5.7); (B) 1 *M*  $\text{KNO}_3$  containing 0.05 *M* lactic acid (pH 6.5); detection as in Fig. 1. Sample: (A) 100  $\mu\text{l}$  of a mixture containing 2 ppm of Mg, 5 ppm of Mn and 40 ppm of Zn; (B) 100  $\mu\text{l}$  of a mixture containing 2 ppm of Mg, 4 ppm of Mn and 20 ppm of Zn.

metals with the addition of a complexing carboxylic acid in the eluent. As expected, the elution order was the reverse of that found for an ion-exchange separation. The presence of lactic acid in the eluent speeded up the elution but did not affect the selectivity. As can be seen, the peaks were fairly broad, showing a relatively low efficiency for this column. Nevertheless, sufficient resolution was being achieved to evaluate the CAS coating.

The potential for on-column preconcentration and separation using the same column was investigated with stepped gradients. With an eluent containing 1 *M* potassium nitrate adjusted to pH 8.5, no elution of magnesium, the most weakly held cation, was observed. Even the presence of 0.05 *M* lactic acid in the eluent did not cause elution at this high pH. Injecting a mixture of dipositive metals at pH 8.5 and then stepping the eluent down to a lower pH caused the metals to elute. The peak shapes were much narrower and also better separated than if the chromatography had been carried out isocratically at the lower pH. Although gradients are known to improve performance in this way, the retention times were longer than expected considering the sharp decrease in pH. This could be explained by the fact that the CAS coating was acting as a buffer (four weak acid groups per molecule), resisting the decrease in pH, turning the step into a much more gradual change in gradient. Fig. 4 shows a four metal separation using a two-step gradient with varying sample conditions. Fig. 4A was obtained after a small-volume injection at ppm levels and Fig. 4B was obtained after a large-volume injection at ppb<sup>a</sup> levels. Although quantitative recoveries were not evaluated, this work clearly shows the potential for combined preconcentration and separation on a single column in solutions of high ionic strength.

#### *Trivalent metals and beryllium*

As a class, these metal species needed lower pHs for elution than the divalent metals because of the stronger complex formation with CAS. However, the same elution properties were observed under isocratic conditions, namely, broad peak shapes with a high degree of asymmetry. Table II shows the order of elution expressed as the eluent pH needed to produce a retention time of approximately 5 min. Some of the peak shapes, notably for gallium and bismuth, were very broad but were found to improve (*i.e.*, become sharper) on increasing the temperature of the column. Increasing the column temperature also increased the retention times slightly. Interestingly, increasing the column temperature did not produce sharper peaks for the divalent metals. We considered that this difference in temperature response between the divalent and trivalent metals could be due to the slower kinetics of complex formation usually found with the more highly charged metal cations. Thus, when gallium was involved reasonable separations could only be achieved at high column temperatures. Fig. 5 shows a separation of Al, In and Ga at 60°C.

The effect of auxiliary complexing ligands in the eluent was also studied. It was found that halide ions had a significant, but varying, influence on the retention of these metals, and could provide a useful way of changing the selectivity of the separation. For example, indium and bismuth(III) were markedly affected by the presence of low concentrations of chloride in the eluent, giving significantly shorter reten-

<sup>a</sup> Throughout this article, the American billion (10<sup>9</sup>) is meant.

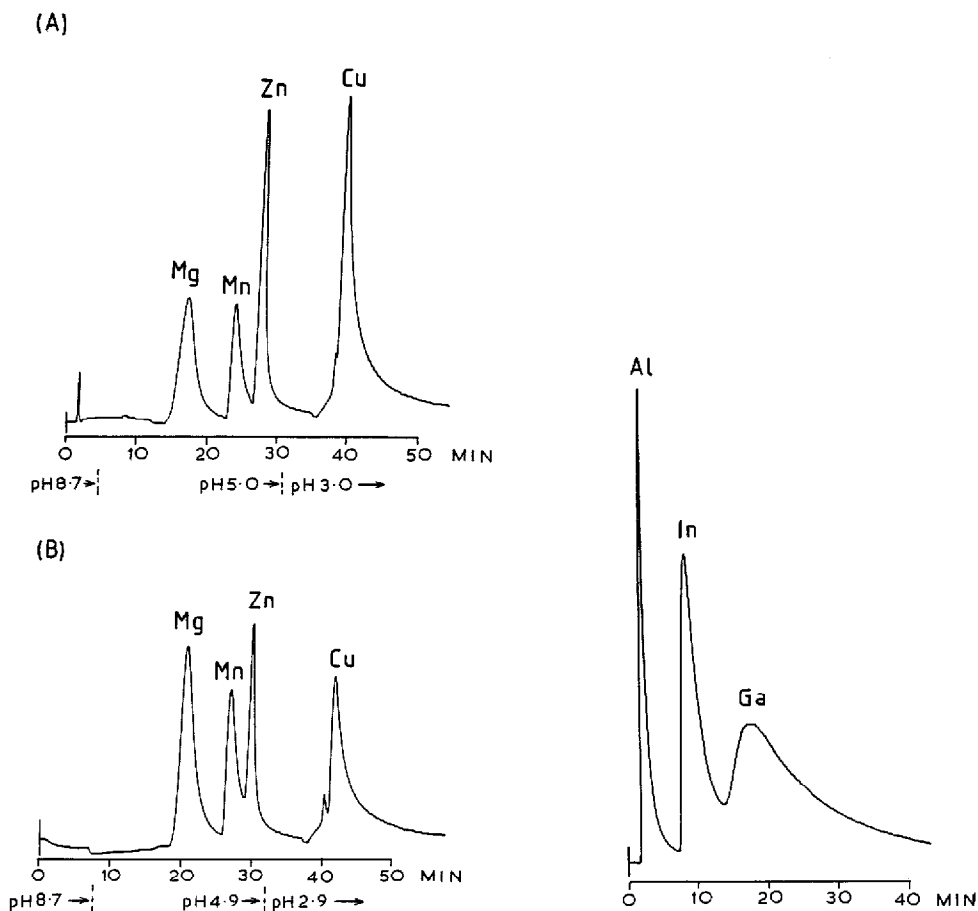


Fig. 4. Chelating-exchange preconcentration and separation of divalent metals using step gradients. Chromatographic conditions: eluent for both (A) and (B), 1 *M*  $\text{KNO}_3$  containing 0.05 *M* lactic acid, adjusted to the appropriate pH for the step gradients as indicated in the diagrams; detection as in Fig. 1. Sample conditions: (A) injection of 100  $\mu\text{l}$  of a mixture containing 5 ppm of Mg, 5 ppm of Mn, 20 ppm of Zn and 20 ppm of Cu; (B) injection of 7 ml of a mixture containing 10 ppb of Mg, 10 ppb of Mn, 20 ppb of Zn and 20 ppb of Cu.

Fig. 5. Chelating-exchange separation of some trivalent metal ions under isocratic conditions. Chromatographic conditions: eluent, 1 *M*  $\text{KNO}_3$  (pH 2.25); column temperature, 60°C; detection, post-column reaction with PCV solution; wavelength, 580 nm. Sample: 100- $\mu\text{l}$  injection of a mixture of 1.5 ppm of Al, 20 ppm of In and 15 ppm of Ga.

tion times, whereas the other elements listed in Table II were unaffected. Fig. 6 shows that the very poor peak for bismuth at room temperature is considerably sharpened by the addition of chloride to the eluent.

The influence of stepped pH gradients after preconcentration was studied, and produced similar results to the work on divalent metals. However, elevated column temperatures and large pH jumps were necessary to obtain reasonably sharp peaks. The preconcentration and elution of aluminium was studied in more detail. Alumi-



TABLE II

pH VALUES OF ELUENT PRODUCING A RETENTION TIME OF CA. 5 min

Element	pH	
	25°C	60°C
Be	3.2	3.0
Al	3.0	2.5
In	2.3	1.9
Ga	2.3	1.7
Fe <sup>III</sup>	1.5	1.5
Bi <sup>III</sup>	1.1	1.1

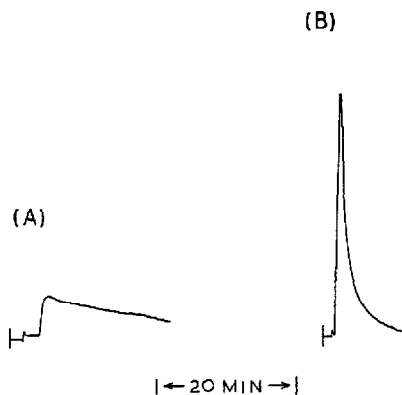


Fig. 6. Effect of chloride on the elution of bismuth(III). Chromatographic conditions: eluent, (A) 0.4 *M* KNO<sub>3</sub> (pH 1.05); (B) as (A) but containing also 0.05 *M* chloride; detection as in Fig. 5. Sample, 100- $\mu$ l injections of 100 ppm of Bi<sup>III</sup>.

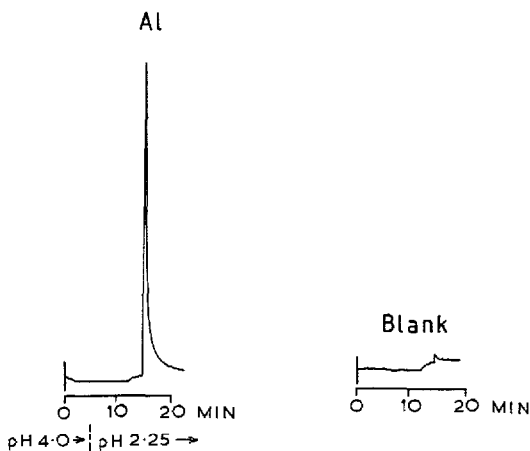


Fig. 7. Preconcentration and elution of aluminium using step gradients. Chromatographic conditions: eluent, 1 *M* KNO<sub>3</sub>, adjusted to the appropriate pH for the step gradient as indicated in the diagram; detection as in Fig. 5. Sample: (left) 5-ml injection of a 50-ppb Al standard at pH 4; (right) blank, 5-ml injection of deionized water.

um was found to be fully retained at pH 4, at which pH all the divalent metals except beryllium and copper were unretained. A step gradient to pH 2.25 then eluted aluminium as a sharp peak (Fig. 7). Under these conditions, indium and gallium gave much longer retention times and iron(III) and bismuth(III) were strongly retained. Using the same conditions as detailed in Fig. 7, an aluminium calibration was attempted in the range 4–16 ppb. A good linear plot was obtained with a correlation coefficient of 0.9999 and a slope of 0.0038 absorbance per ppb.

#### CONCLUSION

The two triphenylmethane dyes chosen gave essentially permanent coatings on the Benson resin with a loading of *ca.* 30 mg per gram. The CAS chelating-exchange coating showed the greatest potential for novel separation possibilities, where the relative insensitivity to ionic strength will be useful for the analysis of samples with high salt concentrations. The ability of the CAS column to handle large-volume injections should also allow preconcentration and separation to be carried out without resorting to the addition of a second column.

#### ACKNOWLEDGEMENTS

P.J. gratefully acknowledges financial support from the Science and Engineering Research Council and the Nuffield Foundation for a 6-month study visit to the Federal Republic of Germany.

#### REFERENCES

- 1 G. Nickless, *J. Chromatogr.*, 313 (1985) 129–159.
- 2 P. Jones, in H. A. MacKenzie and L. E. Smythe (Editors), *Quantitative Analysis of Biological Materials*, Elsevier, Amsterdam, 1988, pp. 77–97.
- 3 J. S. Fritz, D. T. Gjerde and C. Pohlandt, in W. Bertsch, W. G. Jennings and R. E. Kaiser (Editors), *Chromatographic Methods Series, Ion Chromatography*, Hüthig, Heidelberg, 1982, pp. 20–23 and 179–184.
- 4 K. H. Faltynski and J. R. Jezorek, *Chromatographia*, 22 (1986) 5–12.
- 5 C. H. Risner and J. R. Jezorek, *Anal. Chim. Acta*, 186 (1986) 233–245.
- 6 D. Chambaz and W. Haerdi, *J. Chromatogr.*, 482 (1989) in press.
- 7 J. Toei, *Fresenius Z. Anal. Chem.*, 331 (1988) 735–739.
- 8 R. Golombek and G. Schwedt, *J. Chromatogr.*, 452 (1988) 283–294.
- 9 M. L. Marina, V. Gonzalez and A. R. Rodriguez, *Microchem. J.*, 33 (1986) 275–294.
- 10 P. Jones, K. Barron and L. Ebdon, *J. Chromatogr.*, 354 (1986) 407–415.
- 11 P. Jones, K. Barron and L. Ebdon, *Anal. Proc.*, 22 (1985) 373–375.