

Determination of Inorganic Anions by High-performance Liquid Chromatography Using a Micellar Mobile Phase

Frank G. P. Mullins and (the late) Gordon F. Kirkbright

Department of Instrumentation and Analytical Science, University of Manchester Institute of Science and Technology, P.O. Box 88, Manchester, M60 1QD, UK

High-performance liquid chromatography using micellar hexadecyltrimethylammonium chloride as the mobile phase is shown to provide a versatile and efficient technique for the separation of iodate, nitrite, bromide, nitrate and iodide. The mechanism of distribution of the inorganic anions using an aqueous micellar mobile phase with a hydrophobic column packing is investigated. Anion partitioning between the micelles and the mobile phase and between the conditioned stationary phase and the mobile phase both contribute to the retention of the inorganic anions. The influence of the concentrations of hexadecyltrimethylammonium chloride, sodium chloride and acetonitrile on the retention mechanism is also investigated.

The application of micellar chromatography to the analysis of anions is demonstrated via the determination of nitrate and nitrite in domestic water samples.

Keywords: *Micellar chromatography; inorganic anions; high-performance liquid chromatography*

Following the development of ion chromatography by Small *et al.*¹ considerable interest has been shown in the determination of inorganic anions by high-performance liquid chromatography (HPLC). In the procedure employed by Small *et al.* a column packed with a proprietary anion-exchange resin was incorporated into commercial instrumentation. Skelly² reported the HPLC separation of inorganic anions using an eluent containing an octylamine salt. Iskandarani and Pietrzyk³ and Molnar *et al.*⁴ demonstrated the determination of anions by using tetrabutylammonium salts on a styrene-divinylbenzene resin and a bonded stationary phase, respectively. Cassidy and Elchuk^{5,6} reported the analysis of inorganic anions using tetrabutyl- and tetramethylammonium salts on a stationary phase coated with cetylpyridinium chloride. Reeve⁷ used a buffered aqueous eluent containing hexadecyltrimethylammonium bromide (CTAB) to separate inorganic anions on a cyano-bonded normal-phase column. De Kleijn⁸ used hexadecyltrimethylammonium chloride (CTACl) in order to obtain the separation of inorganic anions using the same conditions as those employed by Reeve.⁷

This paper reports the separation of iodate, nitrite, bromide, nitrate and iodide using CTACl above its critical micellar concentration. The elution behaviour of the inorganic anions is discussed in terms of a micellar multi-equilibrium theory. The control of mobile phase parameters, such as solvent composition and ionic strength, permits the quantitative separation and detection of the anions at concentrations in the $\mu\text{g dm}^{-3}$ (p.p.b.) range.

Experimental

Apparatus

A Waters Associates M6000A pump equipped with a Rheodyne 7125 valve injector (20 or 200- μl loop) connected to a Waters Lambda Max variable-wavelength UV detector was used in conjunction with a 250×5 mm i.d. $5 \mu\text{m}$ particle size octadecylsilica column (Spherisorb ODS) (HPLC Technology). Retention times and peak areas were measured using a recording integrator (Hewlett-Packard, Model 3390A).

Reagents

Inorganic salts. Sodium nitrite, sodium nitrate, sodium iodide, ammonium bromide and potassium dihydrogen ortho-

phosphate were of AnalaR grade (BDH Chemicals Ltd., Poole). Potassium iodate was of analytical-reagent grade (General Pharmaceuticals Co. Ltd., Sudbury).

Disodium hydrogen orthophosphate. Analytical-reagent grade material was obtained from Fisons Scientific Apparatus, Loughborough.

Hexadecyltrimethylammonium chloride. Supplied by Fluorochem Ltd., Glossop, and recrystallised twice from acetone-chloroform.

Acetonitrile. HPLC-grade acetonitrile was supplied by Rathburn Chemicals, Peebleshire.

De-ionised water. Water distilled from glass and further de-ionised by using a Waters I de-ioniser (Gelman Sciences) was employed throughout this work.

Procedure

Mobile phases were prepared by adding the appropriate amount of surfactant to water and filtering through a $0.22\text{-}\mu\text{m}$ filter (Duropore). The column was conditioned by passage of the mobile phase for 90 min at a flow-rate of 1.5 ml min^{-1} in order to yield reproducible retention times. Stock solutions of the test solutes were prepared in water and diluted to the appropriate working concentrations with water. These concentrations were $100 \mu\text{g ml}^{-1}$ (100 p.p.m.) for each anion. Efficiencies of up to 30 000 plates m^{-1} were obtained when using these micellar mobile phases.

Results and Discussion

Charged surfactants have been widely used as mobile phase modifiers to improve the partitioning characteristics of charged solutes in reversed-phase liquid chromatography. Various studies have focused on identifying the interactions that occur in the presence of the counter ions and several retention mechanisms have been proposed. The first assumes an ion-exchange mechanism,⁹ while the second assumes ion-pair formation in the mobile phase prior to its adsorption on to the non-polar stationary phase^{10,11}; a third model is that proposed by Bidlingmeyer *et al.*,¹² referred to as the "ion-interaction" model. This does not require ion-pair formation in either phase and is not based on ion exchange. In this model

the hydrophobic surfactant is assumed to adsorb on to the stationary phase as a primary layer, while the counter ions occupy the secondary layer, thus establishing an electrical double layer. As the mobile phase also contains the hydrophobic salt, a dynamic equilibrium is established between the double layer, the hydrophobic salt and the solute.

A micellar mobile phase differs from a conventional ion pairing mobile phase in two important respects. Firstly, micellar solutions can be regarded as microscopically heterogeneous, being composed of the micellar aggregate and the bulk surrounding medium; an ion pairing mobile phase can be considered as homogeneous. Secondly, the concentration of surfactant is above its critical micellar concentration (CMC), *i.e.*, the concentration above which micelle formation becomes appreciable. The concentration of hexadecyltrimethylammonium chloride used in this study was above the CMC values in all experiments. (CMC for hexadecyltrimethylammonium chloride is 9.9×10^{-4} M). The chromatographic properties of aqueous solutions of surfactants above their CMCs have been studied by other workers.^{13,14} Kirkbright and Mullins¹⁵ reported separation of organic anions (sodium dithiocarbamates) when using CTAB above its CMC. Terweij-Groen *et al.*¹⁶ discussed the distribution mechanism of ionisable acids using CTAB above its CMC and proposed a dynamic ion-exchange model to explain the results obtained.

The effect of CTACl concentration in the mobile phase was investigated by measuring the capacity ratios for several inorganic anions on a column initially loaded by elution with water containing 1.3×10^{-1} M CTACl and then subsequently eluted with water alone. Very strong retention of the inorganic anions occurred when no CTACl was present in the eluent. As shown in Fig. 1, retention decreases when CTACl is present in the eluent.

When strongly hydrophobic cationic surfactants are present in the mobile phase the hydrophobic surface of the stationary phase becomes saturated with respect to the adsorption of the surfactant. This confers an ion-exchange capability on the stationary phase. Cassidy and Elchuk^{5,6} reported use of cetylpyridinium chloride to coat the stationary phase "permanently," but used tetrabutyl- and tetramethylammonium

salts in the mobile phase. Their equilibration procedure also employed the use of acetonitrile in the initial conditioning step, thus increasing the over-all cost of the analysis. Knox¹⁷ proposed that retention was a linear function of the charge density on the surface of the stationary phase. Hung and Taylor¹⁸ reported adsorption isotherms for CTAB that indicate a high loading of the stationary phase at 0% organic modifier. Therefore, the strong retention of anions on a saturated stationary phase can be attributed to strong anion interaction with the very high charge density on the surface.

The decrease in retention of the anions as the concentration of CTACl is increased (Fig. 1) can be attributed to anion interaction with micelles in the mobile phase. The retention time of the selected anions can be reduced by increasing the concentration of the micellar reagent [Fig. 2 (a), (b)]. If a more rapid analysis is required, organic modifiers such as methanol or acetonitrile can be used but are not necessary. Partitioning of aromatic acids between the aqueous solution and the micellar phase was reported by Sepulveda.¹⁹ Anion association with a cationic micelle has been shown to occur in the electric double layer on the micelle surface.²⁰

The retention of the anions on a loaded octadecyl-bonded silica column in the presence of CTACl micellar mobile phase follows the order

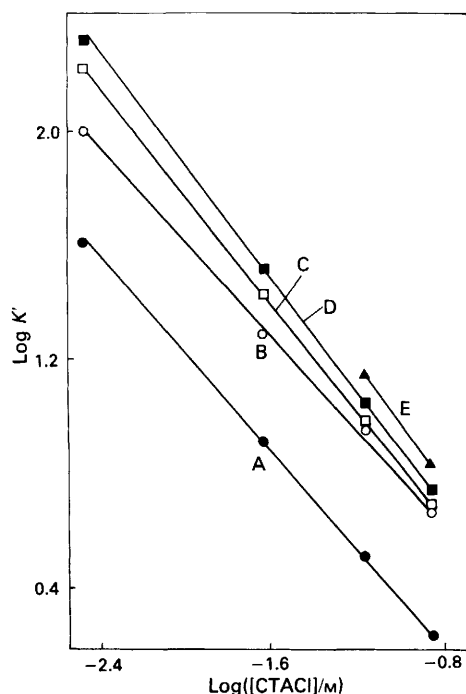
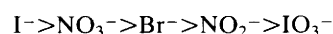


Fig. 1. Variation of log(capacity ratio) with log[hexadecyltrimethylammonium chloride] for A, iodate; B, nitrite; C, bromide; D, nitrate; and E, iodide

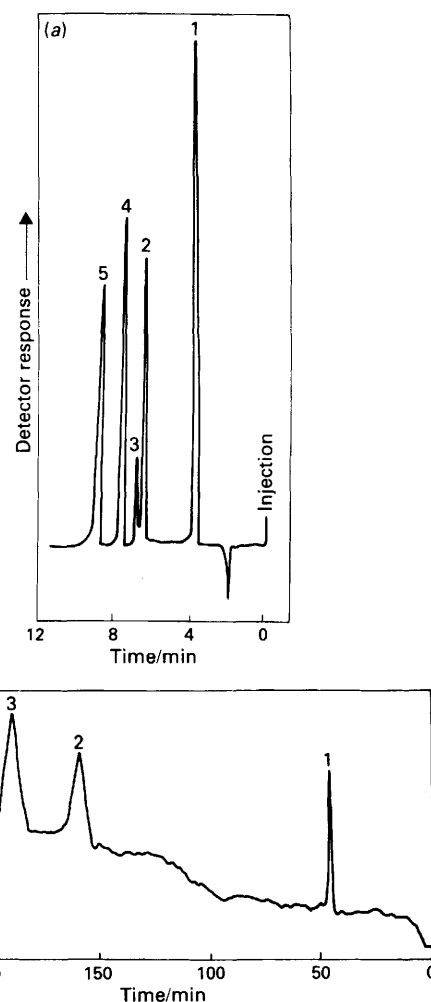


Fig. 2. Micellar chromatograms of anions. 1, Iodate; 2, nitrite; 3, bromide; 4, nitrate. Conditions: packing, C_{18} -bonded silica; column, 250×5 mm i.d., $5 \mu\text{m}$ particle size; detection, UV at 205 nm; flow-rate, 1.5 ml min^{-1} ; pressure drop, 1600 lb in^{-2} ; and sample concentration, $100 \mu\text{g ml}^{-1}$ with $20\text{-}\mu\text{l}$ sample loop. Solvent: (a) 1.36×10^{-1} M CTACl; (b) 5×10^{-4} M CTACl in water

This order is similar to the anion selectivity order found on a typical strongly basic anion exchanger. Fig. 2 illustrates a separation of five inorganic anions with a 1.36×10^{-1} M CTACl micellar mobile phase. No buffer salts or organic modifier were used in order to accomplish this separation.

Armstrong and Nome²¹ reported the derivation of the equation

$$\frac{V_s}{V_e - V_m} = \left[\frac{(K_{mw} - 1)}{K_{sw}} \right] C_m + \frac{1}{K_{sw}} \quad \dots (1)$$

to describe the partitioning of a solute between water and the stationary phase K_{sw} , between water and the micelles K_{mw} and between the micelles and the stationary phase K_{sm} . The equilibrium theory proposed by Armstrong and Nome²¹ is used to quantify the reduction in retention with surfactant concentration. From the retention data obtained the ratio $V_s/(V_e - V_m)$ was calculated for each solute, where V_s is the volume of stationary phase, V_m the volume of the mobile phase in the column and V_e the elution volume of the solute. V_m was taken to be the void volume and V_s the total column volume minus V_m . This ratio was plotted *versus* the concentration of surfactant in micelles (total surfactant concentration minus CMC) and the results shown in Fig. 3 were used to obtain the intercept, where the micelle concentration is zero. For each solute, K_{sw} and K_{mw} were calculated from the slope and intercept using linear regression (Table 1). A single comparison can be made with the data reported by Bartet *et al.*,²³ who obtained a value of 1.1 for the exchange constant of $\text{NO}_3^-/\text{Br}^-$; the value obtained in this work was 1.03 (see Table 1).

The presence of non-aqueous phases can cause changes in both partial molar volume and the apparent relative molecular mass of a micelle. Guveli *et al.*²⁴ reported studies on the addition of aliphatic alcohols to micellar solutions. The effect of variation of the acetonitrile concentration in the mobile

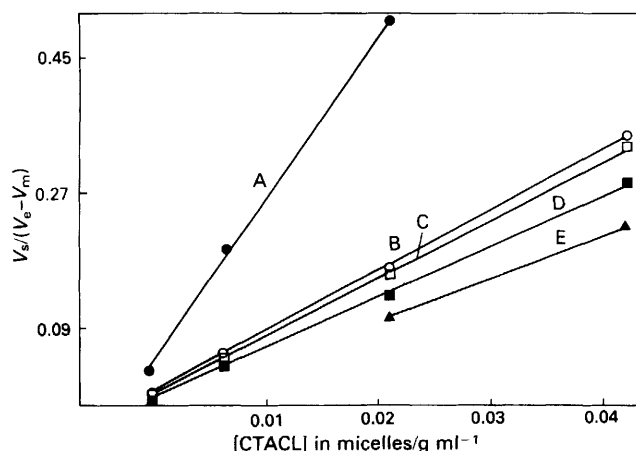


Fig. 3. Variation of $V_s/(V_e - V_m)$ with concentration of hexadecyltrimethylammonium chloride (in micelles in g ml^{-1}). A, Iodate; B, nitrite; C, bromide; D, nitrate; and E, iodide

Table 1. Calculated equilibrium constants of a CTACl micellar system for nitrate, bromide and nitrite

| Ion | K_{sw} | $K_{mw}^{*†}$ | K_{ms} |
|-----------------|----------|---------------|----------|
| NO_2^- | 282.9 | 2397 | 0.118 |
| Br^- | 351 | 2842 | 0.124 |
| NO_3^- | 411.9 | 2929 | 0.141 |

* In order to determine K_{mw} values the partial specific volumes (\bar{v}) must be known. For CTACl, $\bar{v} = 0.977 \text{ ml g}^{-1}$ as determined by Armstrong and Stine.²² The K_{mw} values are given per surfactant molecule.

† $k^{\text{NO}_3^-/\text{Br}^-} = K^{\text{NO}_3^-/\text{Cl}^-} / K^{\text{Br}^-/\text{Cl}^-}$
where k = exchange constant.

phase is illustrated in Fig. 4. A decrease in the capacity ratio is observed with a corresponding increase in organic modifier (acetonitrile) concentration.

In Fig. 5 the variation of the reciprocal of the capacity ratio (k^{-1}) *versus* that of the square root of the ionic strength of the medium ($\mu^{-1/2}$) is shown; as the sodium chloride concentration is increased, the capacity ratio is seen to decrease. Iskandarani and Pietrzyk²⁵ have reported similar results and postulated that if a linear relationship exists between k^{-1} and $\mu^{-1/2}$, the retention of the solute anion in the presence of the surfactant on a bonded phase results from a complex set of equilibria

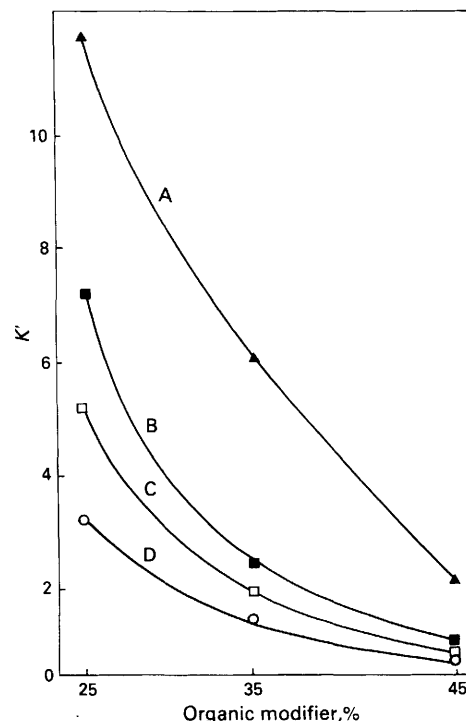


Fig. 4. Variation of capacity ratio (k') with concentration of organic modifier (acetonitrile). A, Iodide; B, nitrate; C, bromide; and D, nitrite

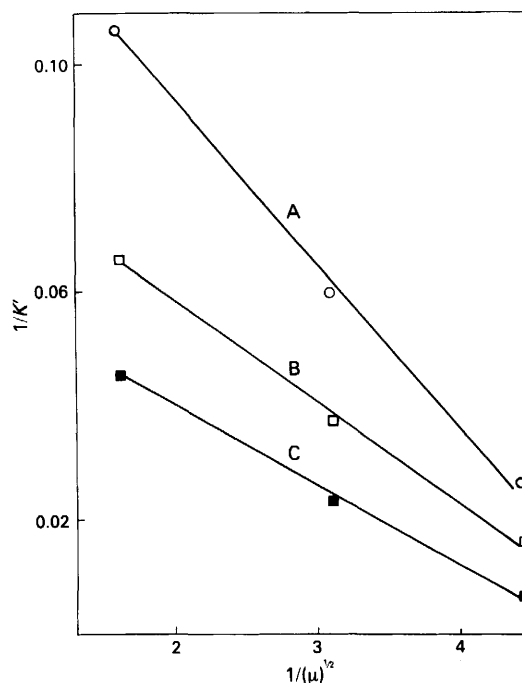


Fig. 5. Variation of the inverse of capacity ratio with the inverse of square root of ionic strength (μ). A, Nitrite; B, bromide; and C, nitrate

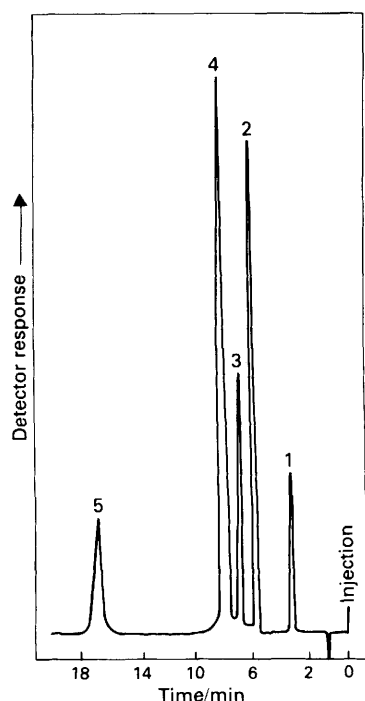


Fig. 6. Micellar chromatogram of anions. 1, Iodate; 2, nitrite; 3, bromide; 4, nitrate; and 5, iodide. Conditions: packing, C_{18} -bonded silica (Spherisorb ODS); column, 250×5 mm i.d., $5 \mu\text{m}$ particle size; solvent, 1×10^{-2} M CTACl buffered to pH 6.8, (10 mM phosphate), 35 + 75 V/V acetonitrile - water; detector, UV, at 205 nm; flow-rate, 1.5 ml min^{-1} ; pressure drop, 2300 lb in^{-2} ; and sample concentration, $100 \mu\text{g ml}^{-1}$ and $20\text{-}\mu\text{l}$ sample loop

taking place within a double layer at the bonded phase surface. Cantwell and Puon²⁶ have derived a quantitative explanation in terms of the Stern - Guoy - Chapman (SGC) theory of electrical double layer to account for the linear relationship between the reciprocal of the square root of the ionic strength and the reciprocal of the capacity ratio. The linearity observed in Fig. 5 may indicate the establishment of dynamic equilibria between the inorganic anion and the electrical double layer on the bonded phase and the electrical double layer on the micelle surface.

Fig. 6 illustrates a separation of inorganic anions using a 0.018 M phosphate-buffered acetonitrile - water (35 + 65 V/V) mobile phase containing 1×10^{-2} M CTACl.

The above results show that conventional HPLC can be used with UV detection for the determination of inorganic anions, namely IO_3^- , NO_2^- , Br^- , NO_3^- and I^- . One of the attractive features of this procedure is the ability to control retention by control of the concentration of aqueous micellar hexadecyltrimethylammonium chloride rather than by the use of organic modifiers.

Linearity and Sensitivity

For nitrate, nitrite, bromide and iodide the calibration graphs obtained were linear for injected masses between $100 \mu\text{g}$ and 20 ng ; correlation coefficients of $0.969\text{--}0.990$ ($n = 4$) were obtained and good repeatability (r.s.d. approximately 0.05) was observed. The limits of detection for the anions investigated were nitrite (4 ng), bromide (5 ng), nitrate (3 ng) and iodide (20 ng). A sample of domestic water was analysed by using this procedure. This analysis indicated nitrate to be present at $10 \mu\text{g ml}^{-1}$, and nitrite at 100 ng ml^{-1} (Fig. 7).

It is thus clear that micellar chromatography can provide an inexpensive and convenient means of separation and detection of inorganic anions of environmental significance, i.e., nitrate, nitrite, bromide, iodide and iodate. The limitation in

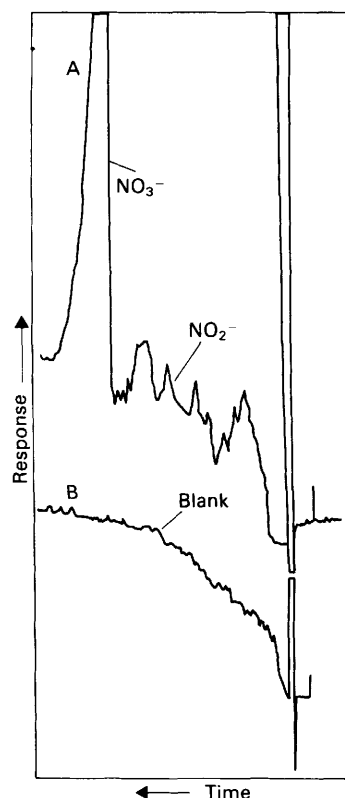


Fig. 7. Micellar chromatogram of (A) domestic tap water and (B) blank conditions: packing, C_{18} -bonded silica (Spherisorb ODS); column, 250×5 mm i.d., $5 \mu\text{m}$ particle size; solvent, 1×10^{-2} M CTACl, 25 + 75 V/V acetonitrile - water; detection, UV at 205 nm; flow-rate, 1.5 ml min^{-1} ; and pressure drop, 1500 lb in^{-2} ; $200\text{-}\mu\text{l}$ sample loop

wavelength response of the detection system employed in this work at present has prevented examination of the use of a micellar mobile phase for the separation and determination of other species, such as chloride and sulphate. Alternative methods of detection are currently under investigation.

We acknowledge Thames Water Authority for full support of this work and partial funding.

References

- Small, H., Stevens, T. S., and Bauman, W. C., *Anal. Chem.*, 1975, **47**, 1801.
- Skelly, N. E., *Anal. Chem.*, 1982, **54**, 712.
- Iskandarani, Z., and Pietrzyk, D. J., *Anal. Chem.*, 1982, **54**, 2427.
- Molnar, I., Knauer, H., and Wilk, D., *J. Chromatogr.*, 1980, **201**, 225.
- Cassidy, R. M., and Elchuk, S., *J. Chromatogr.*, 1983, **262**, 311.
- Cassidy, R. M., and Elchuk, S., *J. Chromatogr. Sci.*, 1983, **21**, 454.
- Reeve, R. W., *J. Chromatogr.*, 1979, **177**, 393.
- de Kleijn, J. P., *Analyst*, 1982, **107**, 223.
- Van de Venne, J. M., Hendrikx, J. L. H. M., and Deelder, R. S., *J. Chromatogr.*, 1978, **167**, 1.
- Wittmer, D. P., Nuessle, N. O., and Haney, W. G., *Anal. Chem.*, 1975, **47**, 1422.
- Horvath, C., Melander, W., and Molnar, J., *J. Chromatogr.*, 1976, **125**, 129.
- Bidlingmeyer, B. A., Deming, S. N., Price, W. P., Sachok, B., and Petrusek, M., *J. Chromatogr.*, 1979, **186**, 419.
- Weinberger, R., Yarmchuk, P., and Cline Love, L. J., *Anal. Chem.*, 1982, **54**, 1552.
- Yarmchuk, P., Weinberger, R., Hirsch, R. F., and Cline Love, L. J., *Anal. Chem.*, 1982, **54**, 2233.
- Kirkbright, G. F., and Mullins, F. G. P., *Analyst*, 1984, **109**, 491.

16. Terweij-Groen, C. P., Heemstra, S., and Kraak, J. C., *J. Chromatogr.*, 1978, **161**, 69.
17. Knox, J. H., and Hartwick, R. A., *J. Chromatogr.*, 1981, **204**, 3.
18. Hung, C. T., and Taylor, R. B., *J. Chromatogr.*, 1981, **209**, 175.
19. Sepulveda, L., *J. Colloid Interface Sci.*, 1974, **46**, 372.
20. Stigter, D., *J. Phys. Chem.*, 1964, **68**, 3603.
21. Armstrong, D. W., and Nome, F., *Anal. Chem.*, 1981, **53**, 1662.
22. Armstrong, D. W., and Stine, G. Y., *J. Am. Chem. Soc.*, 1983, **105**, 2962.
23. Bartet, D., Gamboa, C., and Sepulveda, L., *J. Phys. Chem.*, 1980, **84**, 272.
24. Guveli, D. E., Kayes, J. B., and Davis, S. S., *J. Colloid. Interface Sci.*, 1981, **82**, 307.
25. Iskandarani, Z., and Pietrzyk, D. J., *Anal. Chem.*, 1982, **54**, 1065.
26. Cantwell, F. F., and Puon, S., *Anal. Chem.*, 1979, **52**, 623.

Paper A4/67

Received February 13th, 1984

Accepted March 22nd, 1984