Journal of Chromatography, 592 (1992) 101–108
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2393

Application of a pellicular anion-exchange resin to the separation of inorganic and organic anions by single-column anion exchange

Richard C. Ludwig

Supelco Inc., Supelco Park, Bellefonte, PA 16823-0048 (USA)

ABSTRACT

Applications are shown of a pellicular anion-exchange resin, prepared by hydrophobically coating a quaternized latex on a mono-disperse, non-porous resin. The low capacity material is ideal for single-column ion chromatography with both conductivity and indirect ultraviolet detection. The pellicular nature of the resin allows rapid isocratic and gradient separations. The resin shows excellent efficiency and is stable under high pressures and over a wide pH range. Stability of the coated resin to pressure, solvents and buffers is discussed.

The separation of common inorganic anions, including fluoride, phosphate, chloride, nitrite, bromide, nitrate and sulfate, can be performed at levels below 1 ppm by direct injection. A large number of eluent ions and pH values can be used to optimize the selectivity. Organic acids can be separated rapidly. A rapid separation (11 min) of the common 5'-nucleotide phosphates was performed on the 15-cm column. In addition, a high-efficiency separation of the nucleotides and co-enzymes was performed on a 25-cm column. The elution order of bases was different when performing the separation with acidic and basic eluents.

INTRODUCTION

High-performance ion-exchange chromatography, or ion chromatography, was introduced in 1975 with the advent of suppressed ion chromatography [1]. This was followed by the development of low-capacity ion exchangers, which allowed single-column ion chromatography, in 1979 [2]. The dramatic increase in performance of both techniques has been due to the use of small, rigid particles with easily accessible exchange groups. Porous silicas were used, as functionalization was easily confined to the surface, resulting in rapid diffusion kinetics [3]. Additionally, reversed-phase silicas have been coated with a number of ion-pair reagents or ionic surfactants to create dynamic ion exchangers of variable capacity [4–6]. Pellicular resins have been prepared by agglomerating anion-exchanging microparticles onto the surface of lightly sulfonated supports [7].

In order to increase the speed of analysis for large biomolecules, non-porous supports with very small particle size have been used [8,9]. The small particle size is necessary to obtain sufficient surface area and capacity.

The material described in this report is based on the hydrophobic binding of a submicron anion-exchange latex on mono-disperse, non-porous polystyrene resin as first described by Warth *et al.* [10]. The present material uses a partially cross-linked 5-μm mono-disperse, non-porous resin as the support. The resulting product is a resin-based pellicular anion exchanger of low capacity. As such it is suitable for single-column ion chromatography and provides rapid efficient separations of a number of compound classes.

Initial work on this project concerned characterization and optimization of the coating process. It was found that both salt concentration and the amount of acrylate latex affect the final capacity of the column. In addition it was found that the coated columns were stable to packing and flushing with methanol.

A number of applications have been demon-

0021-9673/92/$05.00

strated on this material. While inorganic anions are the most common use of ion chromatography, organic acids, sulfonic acids and nucleotides have also be separated with this material.

EXPERIMENTAL☆

Column materials

The strong-base anion-exchange latex suspension consisted of a 10% suspension of 0.1 μm functionalised methylmethacrylate. The suspension is commercially available as BPA-1000 (Supelco, Bellefonte, PA, USA). The latex had a surface area of 55 m^2/g, an exchange capacity of 3.1 mequiv./g, and operated from pH 2 to 14.

The support resin was non-porous 5 μm polystyrene containing 3% divinylbenzene.

The materials were slurry packed in stainless-steel columns, 15 and 25 cm × 4.6 mm I.D., and retained with 2 μm stainless steel-frits.

Coating procedure

Loading versus amount of latex. The specified amount of latex suspension was added to 2.25 g of 5 μm resin wetted with methanol. The mixture was diluted to 15 ml with 0.05 *M* NaCl and sonicated for 25 min to allow equilibration.

Loading versus salt concentration. A 2-ml volume of latex suspension was added to 2.25 g of resin wetted with methanol. The mixture was diluted to 15 ml with the specified concentration of NaCl and sonicated for 25 min to allow equilibration.

Column capacity. The actual column capacity was measured by loading the column with 25 ml of 0.5 *M* $NaNO_3$. The column was then rinsed with 25 ml of deionised water and the nitrate was displaced using 25 ml of 0.005 *M* Na_2SO_4. The nitrate was then quantitated by UV absorption at 230 nm.

Column evaluation

The mobile phases consisted of 0.025 *M* KH_2PO_4 (pH 4.5) and the application conditions were as described in the figure legends. The flow-rate was 1 ml/min, unless otherwise stated and the apparatus comprised a Spectra-Physics 8100 pump, a Rheodyne 7125, Valco C6U or Wisp 712 injector and a Kratos 757 UV detector at 254 nm.

☆ *Patent note:* Preparation of both the coated material and the mono-disperse support are covered by USA Patent Applications.

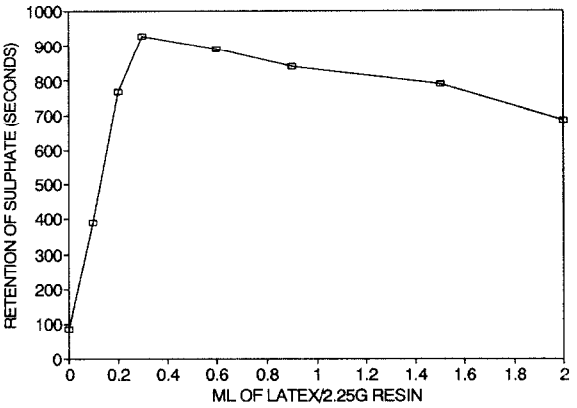

Fig. 1. Retention *versus* amount of latex. The procedure is described in the Experimental section.

RESULTS AND DISCUSSION

Coating mechanism

The submicron latex particles are held in place by hydrophobic binding to the polystyrene–divinylbenzene support. Figs. 1 and 2 illustrate the variation in capacity achieved by varying the amount of latex available for coating and the NaCl concentration. Effective capacity was determined by measuring the retention of sulfate ion under constant chromatographic conditions. Fig. 1 shows a rapid increase to the maximum, followed by a much

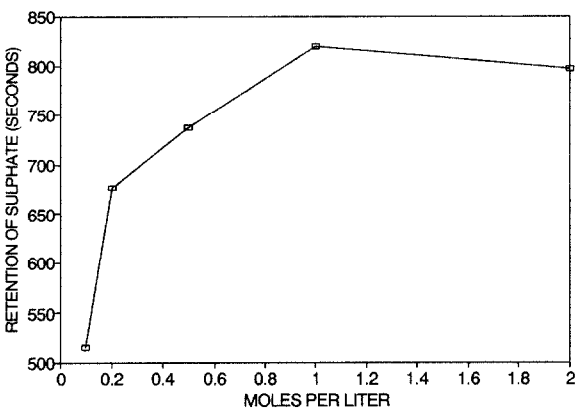

Fig. 2. Retention *versus* sodium chloride concentration. The procedure is described in the Experimental section.

TABLE I

RETENTION OF ANIONS

Conditions: column, 15 cm × 4.6 mm I.D. Sal-PS; buffer, 1 m*M* phthalate pH 6.5; flow-rate, 1 ml/min; sample, 20 µl of 100 ppm of each anion; detection, indirect UV at 265 nm.

Column	Retention time (s)						
	F^-	$H_2PO_4^-$	Cl^-	NO_2^-	Br^-	NO_3^-	SO_4^{2-}
3336 (preflush)	81	111	150	189	323	397	788
3336 (postflush)	83	111	149	188	316	387	789
3333 (preflush)	80	113	143	178	304	370	710
3333 (postflush)	81	114	137	170	283	348	672
3485 (Initial)	84	105	122	145	217	261	515
3485 (after 500 injections)	86	105	134	148	219	261	511

slower decrease with additional latex. The decrease may be due to dilution of the resin–salt mixture and increasing concentration of the suspending agents. Increasing the NaCl concentration, shown in Fig. 2, increases the capacity up to a maximum at about 1 *M* NaCl. The binding appears to be mainly governed by hydrophobic interaction of the two resins.

Stability evaluation

Column No. 3336 was flushed with methanol at 30°C. Evaluation after re-equilibration with the phthalate mobile phase indicated that the column exhibited little loss of retention (Table I). The most significant changes were for bromide and nitrate. Tailing, initially present, was significantly reduced and retention was slightly lower. Column No. 3333 was flushed with methanol overnight at 50°C. Chloride, nitrate and sulfate showed 4–5% reduction in retention and the peak shapes of bromide and nitrate were improved.

Column No. 3485 withstood over 500 injections without loss of performance.

Coating reproducibility

Twenty-six columns were prepared using a standardized coating and packing procedure and two lots of the polystyrene support. The capacity of these columns was evaluated using a 0.025 *M* potassium phosphate mobile phase and guanosine 5′-monophosphate as the test probe. The packing and evaluation were carried out over several days using several lots of mobile phase. No external temperature control was used. A control chart of the capacity factor is shown in Fig. 3. Control limits calculated using the moving range predict that greater than 95% of the columns should have capacity factors between 5.0 and 6.6. Average efficiency of the 15-cm columns was 6703 plates, with a standard deviation of 1296. The capacity as measured using nitrate adsorption/desorption was 0.3 mequiv. per 15 cm × 4.6 mm I.D. column.

Application

Common inorganic anions. Common inorganic anions, including fluoride, phosphate, chloride, ni-

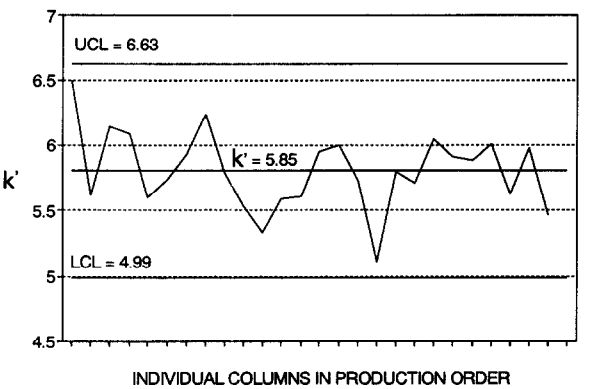

Fig. 3. Control chart of capacity factor (*k'*). Conditions: column, 15 cm × 4.6 mm I.D. Sal-PS; buffer, 0.025 *M* KH_2PO_4; flow-rate, 1 ml/min; sample, 10 µl of uracil (0.7 µg/ml) and adenosine, uridine and guanosine 5′-monophosphates (4 µg/ml each); detection, UV at 254 nm, at ambient temperature. Data shown are for guanosine 5′-monophosphate.

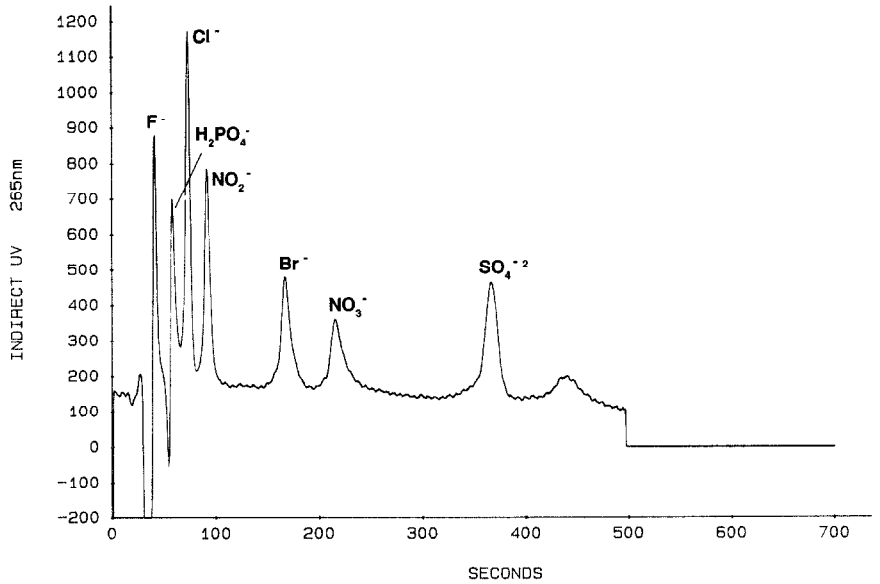

Fig. 4. Chromatogram of common inorganic anions. Conditions: Column, 15 cm × 4.6 mm I.D. Sal-PS; buffer, 1 m*M* phthalate pH 5.0; flow-rate, 2 ml/min; sample, 100 μl of F^- (0.76 ppm), $H_2PO_4^-$ (3.88 ppm), Cl^- (1.4 ppm), NO_2^- (1.84 ppm), Br^- (3.2 ppm), NO_3^- (2.48 ppm) and SO_4^{2-} (3.84 ppm); detection, indirect UV at 265 nm, 0.016 a.u.f.s., at ambient temperature.

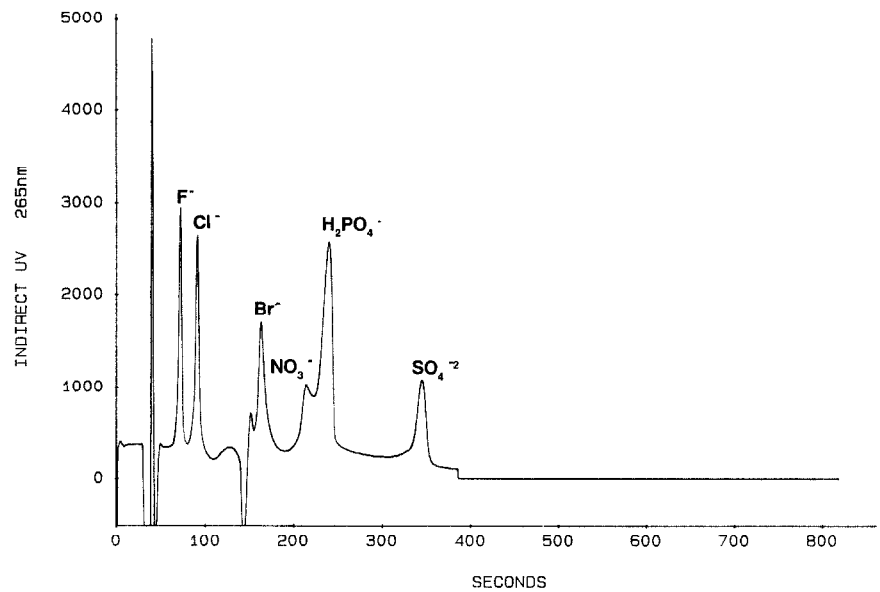

Fig. 5. Chromatogram of common inorganic anions. Conditions: column, 15 cm × 4.6 mm I.D. Sal-PS; buffer, 1 m*M* *p*-hydroxybenzoate pH 8.6; flow-rate, 1 ml/min; sample, 20 μl of F^-, Cl^-, Br^-, NO_3^-, $H_2PO_4^-$ and SO_4^{2-}; detection, indirect UV at 265 nm, 0.04 a.u.f.s., at ambient temperature.

trite, bromide, nitrate and sulfate, were determined in a wide variety of samples at widely varying levels. Fig. 4 shows that these anions can be determined quite rapidly at levels approaching 100 μg/l with direct injection in the single-column mode on the pellicular material.

Fig. 5 shows the use of an alternative mobile phase at a pH of 8.5. The higher pH increases the retention of fluoride and carbonate and shifts phosphate towards the end of the chromatogram. Such operation at basic pH is not possible with silica-based materials.

Organic acids. Figs. 6–8 show separations of various organic acids. Formic and acetic acids, important in the power industry, can be separated using various eluents. Concentrated free lactic acid forms intermolecular esterification products which can be separated from the monomeric acid. These impurities were not present in a freshly dissolved sample of the lithium salt. Selectivity on the pellicular material is not as high as on materials specifically designed for organic acid analysis in juices and wines.

Sulfonic acids. Fig. 9 shows the rapid separation of short-chain sulfonic acids, using the conditions similar to those used for inorganic anions. Retention of the sulfonic acids can be varied quite easily by

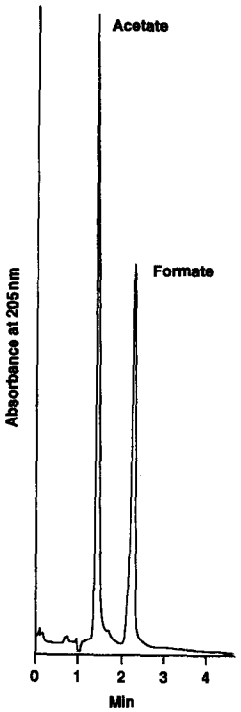

Fig. 6. Chromatogram of organic acids. Conditions: column, 15 cm × 4.6 mm I.D. Sal-PS; buffer, 0.02 *M* KH_2PO_4; flow-rate, 1 ml/min; sample, 90 μl of acetate (172 ppm) and formate (133 ppm); detection, UV at 205 nm, 0.016 a.u.f.s., at ambient temperature.

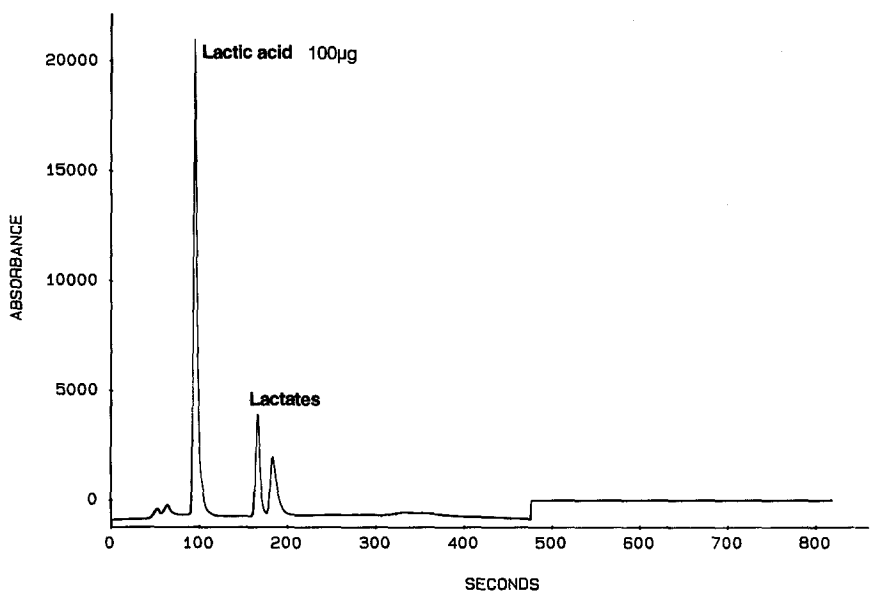

Fig. 7. Chromatogram of lactic acid. Conditions: column, 25 cm × 4.6 mm I.D. Sal-PS; buffer, 0.025 *M* KH_2PO_4; flow-rate, 1 ml/min; sample, 10 μl of 10 mg/ml lactic acid; detection, UV at 205 nm, 0.016 a.u.f.s., at ambient temperature.

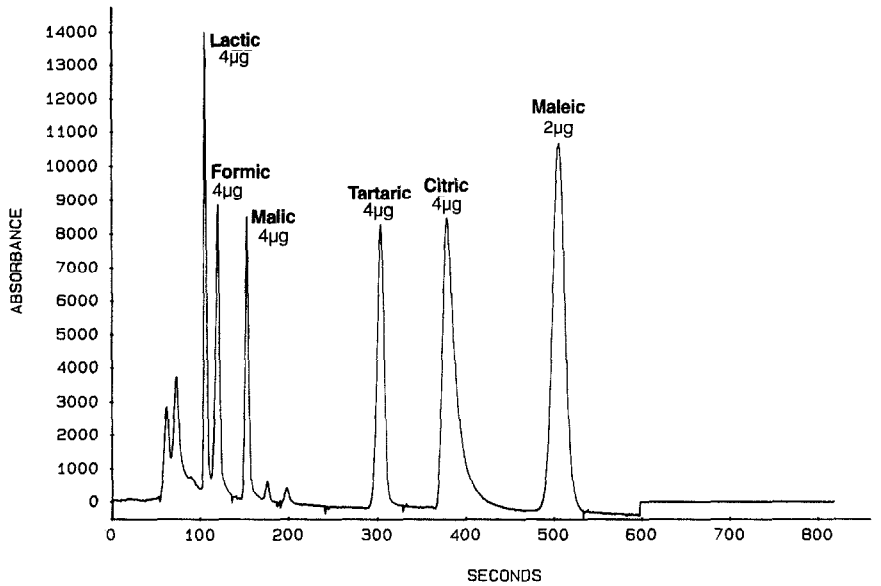

Fig. 8. Chromatogram of organic acids. Conditions: column, 25 cm × 4.6 mm I.D. Sal-PS; buffer, 0.025 *M* KH_2PO_4; flow-rate, 1 ml/min; sample, 10 µl of lactic, formic, malic, tartaric and citric acids (0.4 mg/ml each) and maleic acid (0.2 mg/ml); detection, UV at 205 nm, 0.016 a.u.f.s., at ambient temperature.

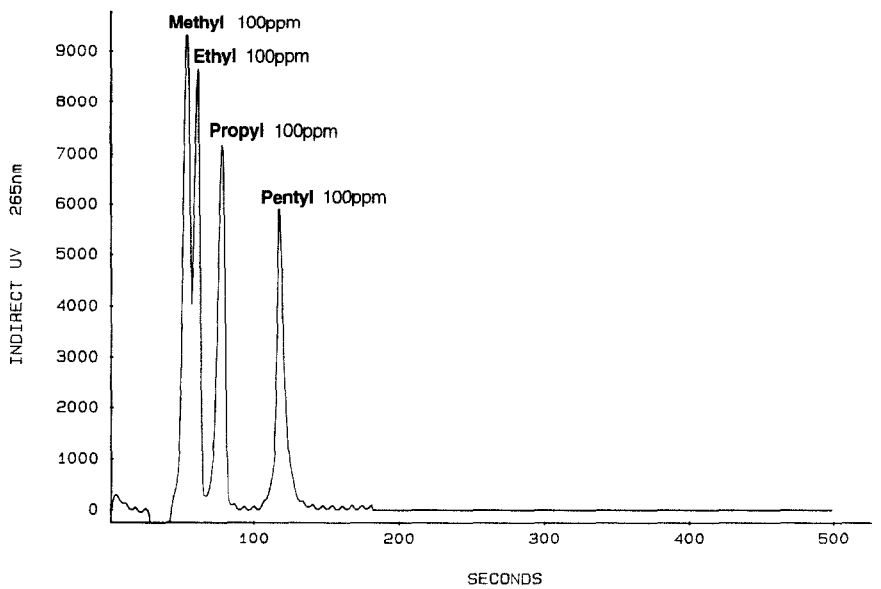

Fig. 9. Chromatogram of sulfonic acids. Conditions: column, 15 cm × 4.6 mm I.D. Sal-PS; buffer, 1 m*M* phthalate pH 6.0; sample, 20 µl of methyl-, ethyl-, propyl- and pentylsulfonic acids (100 ppm each); detection, indirect UV at 265 nm, 0.04 a.u.f.s., at ambient temperature.

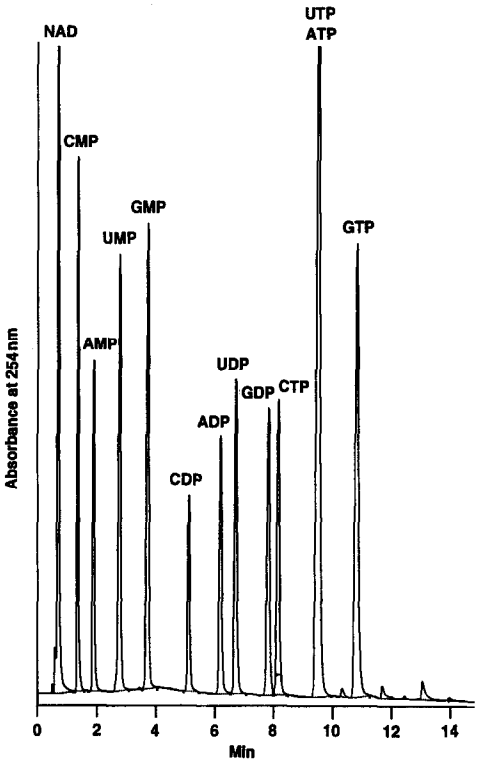

Fig. 10. Chromatogram of nucleotides. Conditions: column, 15 cm × 4.6 mm I.D. Sal-PS; buffer, 0.01 *M* Tris chloride pH 8.5 with a sodium chloride gradient from 0.05 to 0.3 *M* in 15 min; flow-rate, 2 ml/min; sample, 100 µl of 30–50 nmol of each nucleotide; detection, UV at 254 nm, 0.04 a.u.f.s., at ambient temperature.

altering the strength of the mobile phase. However, as the aliphatic chain length increases, tailing becomes more evident. Due to the absence of organic modifier, the aliphatic portion may be partitioning into or hydrophobically binding to the resin support.

Nucleotides. Complex nucleotide mixtures are often separated by ion-exchange or ion-pair chromatography. Two mobile phase systems were developed for the separation of nucleotides on the pellicular material. Fig. 10 shows a rapid separation of twelve commonly occurring nucleotides in less than 10 min, using an NaCl gradient in a Tris–acetate buffer of pH 8.5. The Tris buffer eliminates the baseline rise associated with phosphate or citrate buffers. To improve resolution and allow the separation of additional nucleotides and co-enzymes normally found in biological extracts, a 25 cm × 4.6 mm I.D. column was used (Fig. 11).

CONCLUSIONS

The process of coating a quaternized latex on a mono-disperse, non-porous resin can be varied to provide columns with different capacity and retention. The coating process can be standardized to provide reproducible retention.

Hydrophobically coated anion-exchange columns are stable in the presence of methanol and

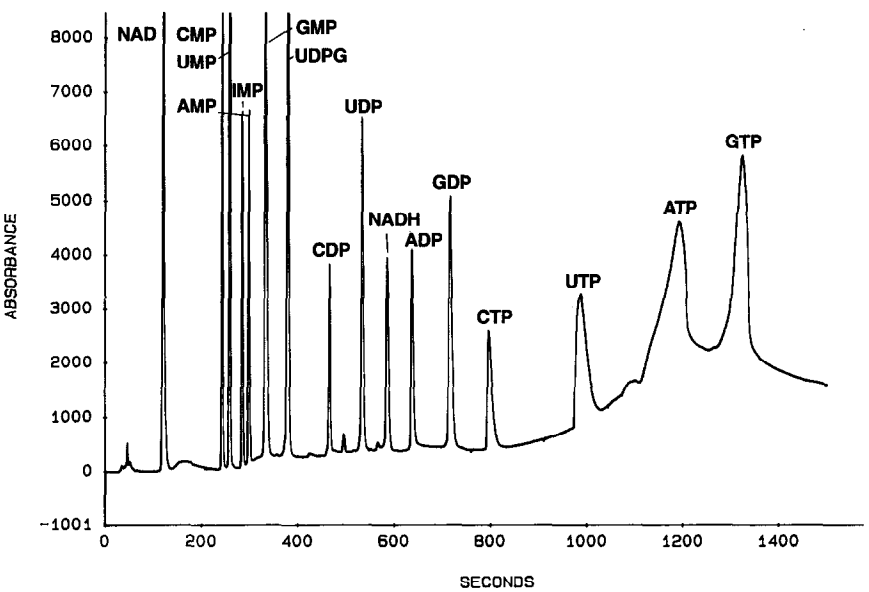

Fig. 11. Chromatogram of nucleotides. Conditions: column, 25 cm × 4.6 mm I.D. Sal-PS; buffer, 0.005 *M* KH_2PO_4 pH 5.6 to 0.5 *M* KH_2PO_4 pH 4.5 gradient in 20 min; flow-rate, 2 ml/min; sample, 100 µl of 30–50 nmol of each nucleotide; detection, UV at 254 nm, 0.04 a.u.f.s., at ambient temperature.

buffers.

Inorganic anions can be analyzed at levels as low as 100 μg/l by direct injection on this packing material.

Organic acids of various types can be analyzed. Selectivity for the organic acids commonly analyzed in wine is not as great as when specialty ion exclusion-type columns are used.

REFERENCES

1 H. Small, T. Stevens and W. Bauman, *Anal. Chem.*, 47 (1975) 1801.
2 D. Gjerde, J. Fritz and G. Schmuckler, *J. Chromatogr.*, 186 (1979) 509.
3 R. Nieman and R. Clark, *J. Chromatogr.*, 317 (1984) 271.
4 C. Knight, R. Cassidy, B. Recoskie and L. Green, *Anal. Chem.*, 56 (1984) 474.
5 R. Cassidy and S. Elchuck, *J. Chromatogr.*, 262 (1983) 311.
6 D. DuVal and J. Fritz, *J. Chromatogr.*, 295 (1984) 89.
7 H. Small and T. Stevens, *U.S. Pat.*, 4 101 460 (1978).
8 Y. Kato, T. Kitamura, A. Mitsui and T. Hashimoto, *J. Chromatogr.*, 398 (1987) 327.
9 M. Rounds and F. Regnier, *J. Chromatogr.*, 443 (1988) 773.
10 L. Warth, J. Fritz and J. Naples, *J. Chromatogr.*, 462 (1989) 165.