

GRAFT COPOLYMERS AS SUBSTRATES IN COLUMN EXTRACTIONS

II. CHARACTERISTICS OF HYDROPHILIC-ORGANOPHILIC COLUMNS IN CHROMATOGRAPHIC SEPARATIONS

ROBERT F. HORNBECK

Lawrence Radiation Laboratory, University of California, Livermore, Calif. (U.S.A.)

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INTRODUCTION

The utilization of copolymers consisting of organophilic side chains grafted to hydrophilic polymers as column supports in extraction chromatography has been reported in a previous paper¹. The operating characteristics of such columns can be studied by the techniques developed for ion exchange resins, to which they are analogous in many ways. This paper gives the results of such a study, indicating both the similarities and the differences between extraction chromatography columns and ion exchange columns.

EXPERIMENTAL

Reagents and equipment

Reagents and equipment in general were the same as reported in the previous paper except that columns of various lengths and diameters were used. The grafts of polystyrene to cellulose were furnished by Dr. A. J. RESTAINO, and the Carbowax-styrene grafts and surface-grafted Kel-F were prepared by the author.

The lanthanide/HDEHP system

The lanthanide elements are extracted from dilute acid solution by di-(2-ethylhexyl) orthophosphoric acid, symbolized as HDEHP. For suitably pure HDEHP, a plot of the log of the distribution coefficient *versus* the atomic number gives a straight line relationship for the lanthanides². Y³⁺ also falls in this group with an apparent atomic number of 67.6. The rare earth elements were reported to be separated on columns containing adsorbed HDEHP: microgram quantities were separated on a column with a Celite substrate³ and milligram quantities on a cellulose/HDEHP column⁴. Consequently, it was decided to study the characteristics of the graft copolymer columns using the rare earth/HDEHP system.

By analogy with liquid-liquid extraction of the trivalent lanthanides, the reaction between a lanthanide ion and HDEHP adsorbed on a column support can be written as



Here HDEHP is written in the dimeric form in accordance with current convention. As reported by PEPPARD *et al.*⁵, the above equation is valid if the formal ratio of metal ion to complexing agent is small, *i.e.*, much less than 1:6; at higher ratios, mixed complexes may be formed. It has been postulated that as the maximum ratio of 1:3 is approached, a three-dimensional polynuclear species is formed that has limited solubility in toluene. Gelatinous precipitates have been observed to appear in liquid-liquid extractions under these conditions; extraction columns have greatly diminished flow rates on approaching saturation but revert to normal flow rates as elution of the adsorbed metal ion proceeds. This phenomenon was observed in experiments designed to measure column capacity (described in the next section).

The equilibrium constant for the above reaction is given by

$$K = \frac{(M[H(DEHP)_2]_3)_{org}}{[H^{3+}]_{aq}} \cdot \frac{[H^+]^3_{aq}}{([HDEHP]_2)_{org}^3} \quad (2)$$

If D is the distribution ratio (defined as the concentration of the metal in the organic phase divided by the concentration of the metal in the aqueous phase) and Q is the effective concentration of the HDEHP on the resin, expressed in millimoles per milliliter of swollen resin, one obtains:

$$D = Q^3 \frac{K}{[H^+]^3} \quad (3)$$

For a given concentration of HDEHP on an extraction resin

$$\log D = -3 \log [H^+] + \text{constant} \quad (4)$$

Partition measurements were made in the following manner: tracer quantities of ¹⁵², ¹⁵⁴Eu were shaken for several hours in centrifuge cones containing dilute HCl at various concentrations and styrene grafted cellulose impregnated with a fixed amount of HDEHP. The resin was removed by centrifuging and measured portions of resin

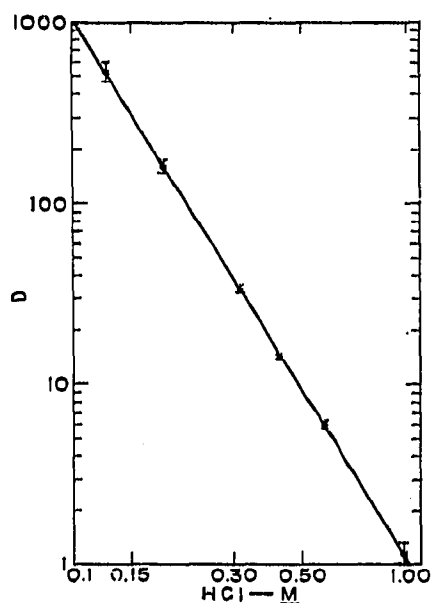


Fig. 1. Distribution ratio (D) for Eu *versus* HCl concentration.

and aqueous supernate were counted in a fixed geometry to determine the relative amount of activity in each. The activity in the resin was corrected for the activity in the adsorbed aqueous solution on the resin. The distribution ratio was obtained by dividing the c.p.m./ml of swollen resin by the c.p.m./ml of aqueous solution. The exact HCl concentration at equilibrium was determined by titrating aliquots of the aqueous solution with a standardized base. A plot of the log of the distribution ratio *versus* the log of the acid concentration is given in Fig. 1. The vertical bars represent the extremes of multiple determinations, the circles the mean values. The straight line was drawn as the best fit through the circles and has a slope of -2.96 .

Elution characteristics of the column

The elution characteristics of a column containing adsorbed HDEHP can be related to the partition measurements by modifying the plate equilibrium theory developed for ion exchange resins by MARTIN AND SYNGE⁶ and extended by BEUKENKAMP *et al.*⁷ An expression for describing the elution of lanthanides from such a column can be derived using the following symbols:

- V = the volume of eluate that has passed through the column
- V_R = the retention volume of the solute (the volume of eluate to maximum metal ion concentration)
- D = the distribution ratio in a plate
- V_i = the effective interstitial volume of the column
- Q = the effective concentration of the HDEHP on the column in millimoles per milliliter
- ϕ = the volume of eluate that has passed through the maximum metal ion concentration, *i.e.*, $\phi = V - V_i$
- ϕ^* = the volume of eluate that has passed through the maximum metal ion concentration at the point when $V = V_R$.

As in the case of an ion exchange column, for elution with a solution of constant concentration of hydrogen ion we have

$$V_R = DV_i + V_i \quad (5)$$

When $V = V_R$, $\phi = \phi^*$ and

$$\phi^* = V_R - V_i = DV_i \quad (6)$$

Assuming equilibrium concentrations in a given plate on the column and substituting for D from eqn. (3),

$$\phi^* = \frac{Q^3K}{[H^+]^3} V_i \quad (7)$$

To obtain calculated values of ϕ^* , Q^3K is calculated from the partitioning measurements described in the previous section, and V_i is determined by measuring the total volume of aqueous solution on the column. At slow flow rates, approaching equilibrium conditions, it is not possible to distinguish between interstitial aqueous solution and adsorbed aqueous solution; therefore, under these conditions the effective interstitial volume of the column is the same as the total aqueous volume.

Effective interstitial volume, V_t , was measured by two methods. In the first method, a weighed portion of polymer containing a known amount of adsorbed HDEHP was soaked in excess aqueous solution and packed into a tared glass column. The packed column, from which excess liquid had been removed, was weighed, and the weight of aqueous solution was found by difference; the volume of solution on the column was obtained by dividing the weight by the density.

The second method arose out of a procedure developed to measure the capacity of the column. A solution containing a known concentration of europium with added $^{152}, ^{154}\text{Eu}$ tracer was passed through the column until the concentrations in the input and effluent solutions were the same. The column was washed with 0.05 M HNO_3 until no measurable europium could be found in the effluent. The total amount of Eu in the washings was determined by tracer assay. Virtually none of the complexed Eu was removed from the column under these conditions; hence, the total weight of Eu in the washings divided by the concentration in the original solution gave the volume of aqueous solution on the column. If care was taken to insure equilibration of the europium solution with the column and to attain complete saturation of the column capacity, results from this method agreed within the limit of experimental error with interstitial volumes determined by the first method (*i.e.*, within $\sim 5\%$).

The column was subsequently washed with 1 M HCl until the remainder of the europium was removed. A simple measurement of the amount of Eu in the eluate gave the total capacity of the column. A formal ratio of 1 to 3 (Eu^{3+} to HDEHP) was found.

The observed values of ϕ^* were obtained in the following manner. A column of known V_t was washed with several bed volumes of 0.05 M HNO_3 solution. When the wash solution had dropped to the layer of glass powder above the resin bed, the column stopped. Lanthanide tracer in 1–2 ml of 0.05 M HNO_3 was added to the column, the solution was allowed to run through, and the column was washed several times with a few milliliters of the HNO_3 solution. When the last of the wash solution had run out, the column was placed over a fraction collector equipped with a drop counter. Hydrochloric acid solution of the appropriate concentration was added to the top of the column and the collection of effluent was begun. The operation of the column was checked by collecting the first few milliliters one drop at a time and measuring the volume to the appearance of chloride ion. If the volume so collected was significantly (10–15%) less than V_t , the column was assumed to be channelling and was repacked.

Effluent fractions were collected in 1–3 ml portions, depending on the length of run anticipated, and counted; the counts per minute were plotted against effluent volume. The value V_R was found by taking the volume at peak maximum, and ϕ^* was obtained by subtracting V_t from V_R .

Examples of calculated and observed ϕ^* values for the elution of Eu are given in Table I.

One major difference between extraction columns and those made from ion exchange resins is that in the former the concentration of complexing agent, hence the capacity, can be varied at will at the time the column is prepared (up to the limits of the adsorption capacity of the resin). Additional confirmation of the validity of eqn. (7) was obtained by examining the relationship between concentration of adsorbed HDEHP and the retention volume. Several columns were prepared in which the concentration of HDEHP was varied over the range of 0.25 to 0.9 mmoles/ml of bed

TABLE I

CALCULATED AND OBSERVED ϕ^* VALUES IN NON-GRADIENT ELUTIONS OF Eu

Column designation ^a	V_t^b	Q^3K	Eluate HCl, (N)	ϕ^* Calc.	ϕ^* Obs.
A-1	1.27	0.549	0.425	9.1	8.9, 9.2, 9.0 (9.0) ^c
A-2	1.09	0.549	0.392	10.0	9.7, 10.1 (9.9)
A-3	1.15	0.549	0.392	10.5	10.6, 10.3 (10.5)
B-1	1.04	0.584	0.370	12.0	12.3
B-2	1.21	0.584	0.370	14.0	14.0
C	1.39	1.021	0.48	12.9	12.8
D	1.15	1.84	0.56	12.0	11.7, 12.5 (12.1)

^a Columns were 20–22 cm long with cross-sectional areas in the range of 0.08 to 0.09 cm². Flow rates were 3 to 5 ml/h.

^b Column supports were cellulose-g-polystyrene that contained 60–70% aqueous fraction. HDEHP concentrations were in the range of 0.33 to 0.49 mmoles/ml.

^c Numbers in parentheses are mean values.

volume. Europium tracer was adsorbed and eluted in the standard manner, and the ϕ^* values were determined. From eqn. (7), one obtains

$$Q(K)^{1/3} = \left(\frac{\phi^*}{V_t}\right)^{1/3} [H^+] \quad (8)$$

When $(\phi^*/V_t)^{1/3}[H^+]$ was plotted against the monomeric HDEHP concentration on the resin, the curve approached a straight line that extrapolated to the origin (Fig. 2), indicating that eqn. (8) is valid up to a concentration of approximately 0.5 mmoles/ml. Deviations at the higher concentrations indicate that the effective concentration is less than that calculated in making up the column.

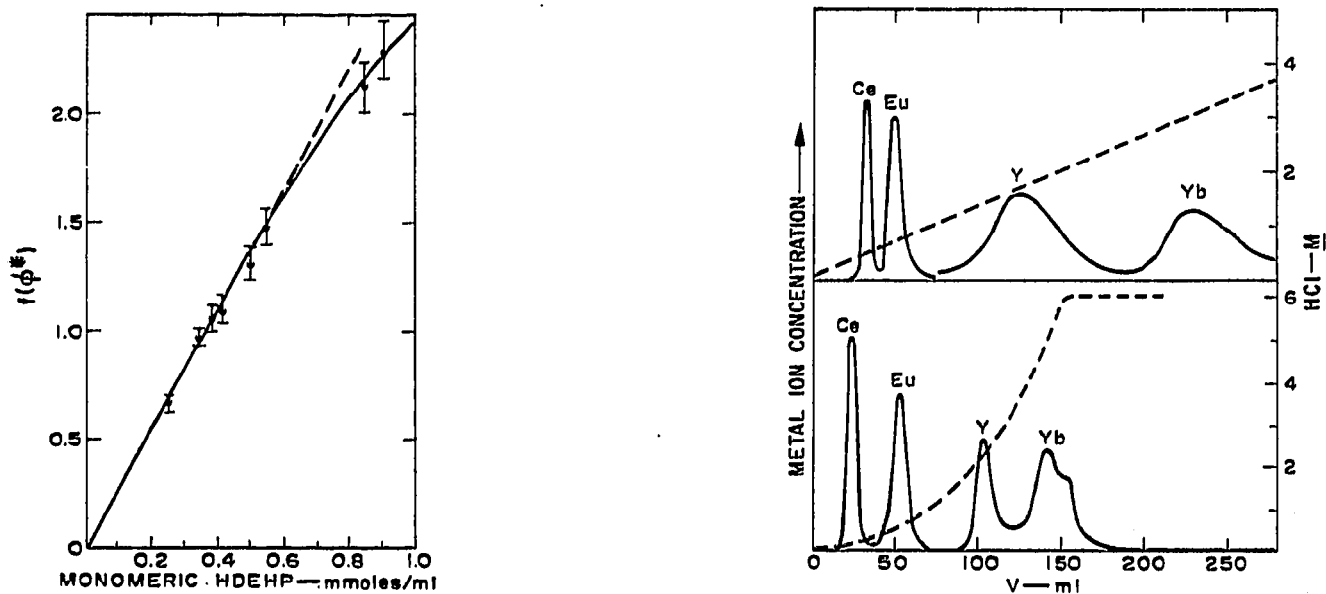


Fig. 2. Values of $(\phi^*/V_t)^{1/3}[H^+]$ versus HDEHP concentration.

Fig. 3. Comparison of linear (upper) and exponential (lower) gradients in the elution of selected lanthanides. Solid curves, relative concentrations of metal ion in eluate; dashed curves, HCl concentrations of eluate. In the curves for Y and Yb, the ordinate values have been multiplied by 5.

Elution chromatography

Separation and elution of the lanthanide elements and yttrium on an HDEHP column is conveniently accomplished with a gradient elution technique with dilute HCl acid as the eluent³. During the run, the concentration of HCl in the eluting solution is increased slowly from 0.1 *M* to approximately 4 *M*. Techniques are available for producing gradients of various shapes⁸. Two preliminary runs were made with a few selected tracers, one with a linear gradient and a second with an exponential gradient (Fig. 3). The exponential gradient distributes the peaks more uniformly and therefore is preferable, although in either case resolution is poor, especially for the heavier elements. More will be said in the next section about the problem of resolution.

The retention volumes of the lanthanides in a gradient elution can be calculated by an integration procedure, modifying eqn. (7) to allow for the changing concentration of hydrogen ion. By the same reasoning employed by SCHWAB *et al.*⁹, one obtains the following expression:

$$\int_0^{\phi^*} f^3(\phi) d\phi = Q^3KV_t \quad (9)$$

where $f(\phi)$ is a term for hydrogen ion concentration as a function of the volume of effluent. The integral can be evaluated numerically by cubing the hydrogen ion concentration of each fraction, multiplying by the volume of the fraction and summing the values over the volume collected. The ϕ^* values for the elutions in Fig. 3 were determined in this manner and are listed in Table II under the column "Summation".

TABLE II
CALCULATED AND OBSERVED ϕ^* VALUES FOR GRADIENT ELUTIONS

Gradient	Element	Calculated ϕ^*		ϕ^* Observed
		Summation	Integration	
Linear	Ce	33.0	— ^a	33.5
	Eu	50	46.6	49.5
	Y	120	120	124
	Yb	217	219	227
Exponential	Ce	19.3	— ^a	23.8
	Eu	48	49	54
	Y	99	101	103
	Yb	137	144	142

^a Acid concentration in the eluate prior to elution of the Ce peak deviated markedly from that calculated from the derived expression for $[H^+]$ as a function of volume; hence ϕ^* values for Ce could not be calculated from these expressions. The deviations occurred principally as the result of mechanical problems at the start of the run in establishing a smooth flow of liquid from the reservoir of high concentration acid to the feed reservoir that provided the input solution to the column.

If a suitable mathematical expression can be derived for the rate of change of hydrogen ion concentration as a function of volume, an integration can be performed and the resulting equation can be used to calculate retention volumes directly. For

example, the hydrogen ion concentration of the eluate in the case of the linear gradient elution in Fig. 3 can be expressed by

$$[\text{H}^+] = f(\phi) = 1.364 \times 10^{-2} \phi \quad (10)$$

and

$$f^3(\phi) = 2.538 \times 10^{-6} \phi^3 \quad (11)$$

Substituting in eqn. (9) and integrating, one obtains

$$6.34 \times 10^{-5} (\phi^*)^4 = Q^3 K V_t \quad (12)$$

Similarly one can obtain for the exponential gradient elution

$$9.23 \times 10^{-2} (e^{0.072\phi^*} + 1) = Q^3 K V_t \quad (13)$$

The ϕ^* values listed in the column under "Integration" in Table II were calculated using eqns. (12) and (13) respectively.

The observed values of ϕ^* were obtained exactly as before.

Resolution of the column

From study of the operating characteristics of columns made from graft copolymers, it quickly became obvious that the resolution of peaks in chromatographic separations on columns of the same length and capacity is dependent principally on the particle size of the polymeric support and on the rate of flow. However, as the particle size is decreased, the resistance to flow increases until impractically slow rates result (*e.g.*, less than 1 ml/h).

The resolving power of a column of given length and capacity was measured by adsorbing and eluting europium tracer in the usual way, as described in the section on Elution characteristics of the column. After the elution curve was plotted and V_R was determined, the data were normalized to standard peak height and retention volume. Peak shapes obtained under various conditions could thus be compared directly by superimposing them as shown in Fig. 4.

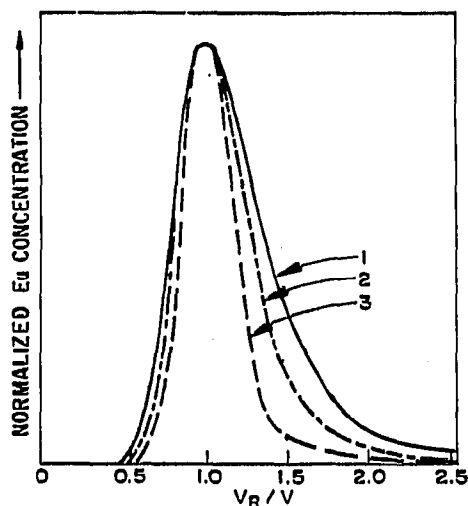


Fig. 4. Elution peaks of Eu tracer on various columns. Curve 1 = cellulose-g-polystyrene, -200 mesh, 0.33 mmoles HDEHP per ml; curve 2 = Carbowax-g-polystyrene, -325 mesh, 0.84 mmoles HDEHP per ml; curve 3 = 1:1 HDEHP-chloroform adsorbed on 110-115 mesh Kel-F powder. All columns were 20 cm long. Flow rate was 30 cm/h for curve 1 and 5 cm/h for curves 2 and 3. Concentrations are normalized to standard peak height.

To ascertain the cause of the broad band widths observed on elution, the following experiment was devised. A sample of tracer europium was adsorbed on a 20-cm column and washed with 10 bed volumes of 0.05 *M* HNO₃. Successive small portions of the resin a few millimeters deep were removed and counted. The experiment was then repeated on a column eluted with 0.5 *M* HCl until a portion of the activity appeared in the eluate. The data are plotted in Fig. 5. It is obvious that band-widening occurs at the elution or the desorption step and not during adsorption or washing.

To test this conclusion further, a kinetic experiment was performed in which the tracer solution was stirred rapidly with a portion of resin for a given period of time and centrifuged quickly. The radioactivities of each resin and its aqueous portion were determined by counting; they were then recombined and the process repeated until equilibrium was attained. A plot of percent adsorbed *versus* time of mixing (Fig. 6) shows a markedly faster rate of approach to equilibrium for the adsorption than for desorption. It also shows that the rates of adsorption as well as desorption are faster for small particle size than for large particle size.

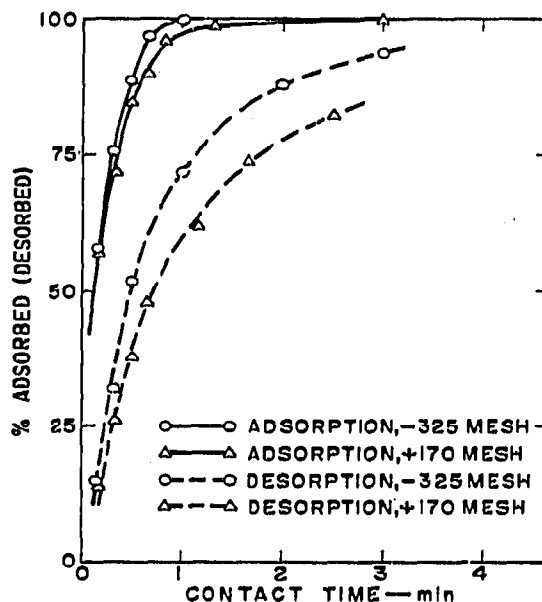
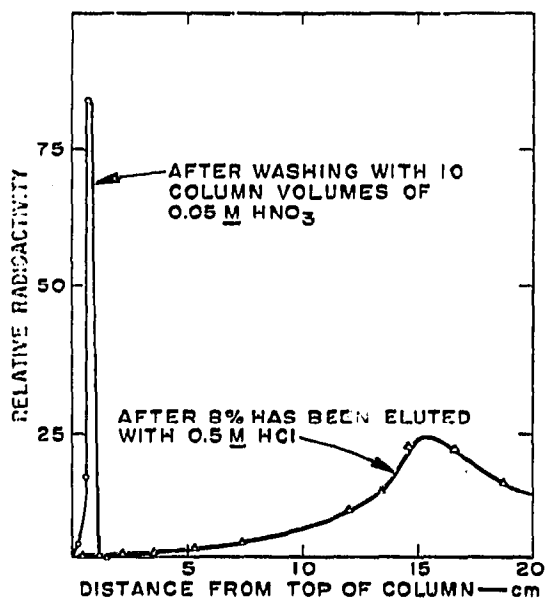


Fig. 5. Distribution of tracer Eu on a graft copolymer column at two stages in the elution process.

Fig. 6. Kinetics of adsorption-desorption. The ordinate (% adsorbed) is the measured concentration of metal ion on the resin, divided by the concentration at equilibrium, multiplied by 100.

The band-widening can be described in terms of ionic processes as follows. Adsorption can occur quickly as europium ions meet the surface of the particle and are complexed by the HDEHP molecules located there. Concentrations at tracer levels do not saturate the surface sites and adsorption occurs easily. In time, the europium migrates to the interior of the particle until it is spread uniformly throughout the polymeric matrix. Desorption occurs more slowly since those ions that found their way to the interior of the particle must now diffuse to the surface while the process of elution is going on, *i.e.*, as europium ions are depleted from the outer layers of the particle. Macro amounts of metal ion would be expected to exhibit additional band-widening, since surface sites are more likely to be saturated during adsorption,

increasing the probability that an ion would move on to the next "plate" before equilibrium was attained (Fig. 7 illustrates this effect).

An obvious approach to the band-width problem is to limit adsorption to the surfaces of particles. A new type of column support was therefore developed by grafting styrene to Kel-F powder in the presence of dissolved Carbowax. The Kel-F does not absorb the solvent mixture and grafting occurs principally at the surface. The product can adsorb both the organic complexing agent and the aqueous solution, but only on the surface of the particle rather than throughout the bulk of the polymer as in the previous grafts. Under these conditions, adsorbed metal ions are not free to diffuse to the interior of the resin particle but are constrained to remain in the surface film, which consists of the swollen polystyrene-poly(ethylene glycol) matrix. By grafting to finely screened Kel-F powder (e.g., 250–325 mesh), one obtains a product that compares favorably in HDEHP capacity with the simple Carbowax-styrene graft, approaching 1 mmole/ml of bed volume. In addition, flow rates are far higher, so that a column under gravity flow must be controlled with a stopcock to keep it from running too fast. Typical elution curves, at a constant acid concentration, for tracer and macro amounts of europium are given in Fig. 7. Note that the band width for tracer Eu is almost identical with that of Curve 3 in Fig. 4 although the lineal flow rate is six times as great.

Gradient elution of a sample of mixed selected carriers (5 mg of each) on a 50 cm \times 0.71 cm² column at an average flow rate of 10 ml/h is illustrated in Fig. 8. The column material was a 12% surface graft of styrene-Carbowax on -325 mesh Kel-F powder and was loaded with 0.86 mmoles of HDEHP per ml of column volume. Arrows indicate the retention volume calculated from eqn. (9) by numerical integration of the term $\Delta V[H^+]^3$. The value of Q^3K for Eu was determined by non-gradient elution of Eu from the column with 0.75 M HCl and the values for the other

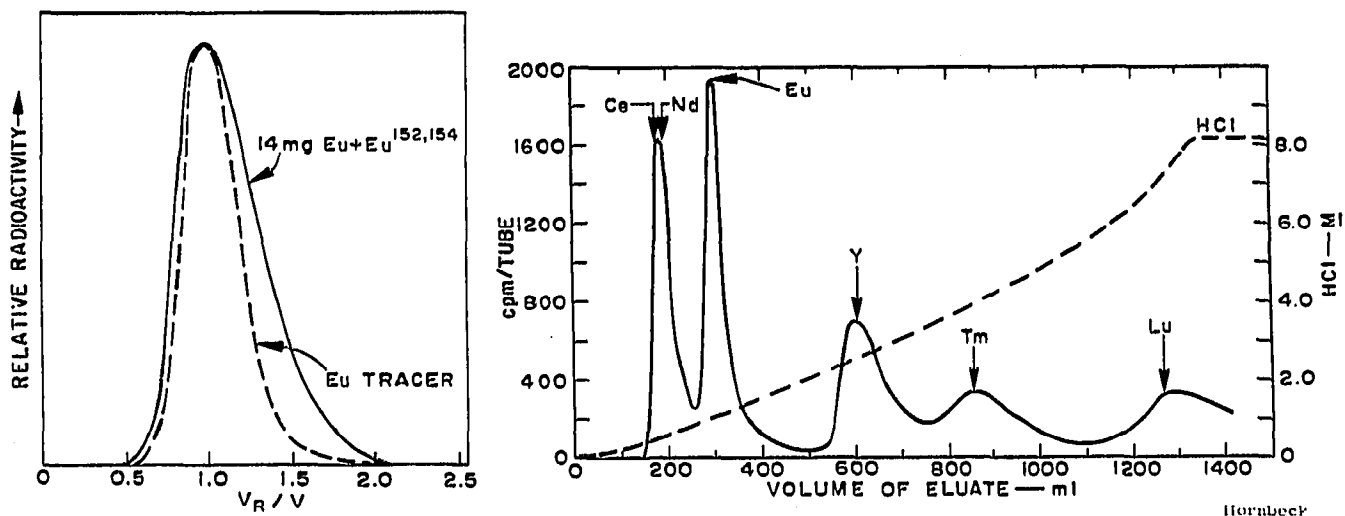


Fig. 7. Elution curves for Eu on a surface-grafted Kel-F column. Column length, 20 cm; lineal flow rate, 30 cm/h; substrate, 7% Carbowax-polystyrene on -200 mesh Kel-F; HDEHP, 0.5 mmoles per ml. The curve for the tracer is virtually congruent with curve 3 in Fig. 4.

Fig. 8. Gradient elution of selected lanthanides on a surface-grafted Kel-F column. Solid curve: elution of lanthanides; dashed curve: HCl concentration, (right ordinate) as determined by titration with standardized base solution.

lanthanides were obtained from distribution ratios for liquid-liquid extraction. The elution curve of Fig. 8 shows that separation of the lanthanides is still poor. It appears that the slow kinetics of the desorption process on a graft copolymer column are such as to limit its usefulness for chromatographic separations of this kind.

DISCUSSION AND CONCLUSIONS

The nature of the environment of the adsorbed HDEHP is an intriguing problem, which, although it goes beyond the scope of this investigation, nevertheless intrudes itself upon our attempts to understand and predict column behavior. Considering the Carbowax-styrene grafts, which appear to be homogeneous in composition, the three most likely models of adsorption are as follows: (1) The adsorbed complexing agent is uniformly distributed throughout the polymer as in a solid solution, (2) it is predominately adsorbed on the organophilic (*i.e.*, polystyrene) side chains as a molecular sheath, or (3) it is present as tiny globules trapped within the polymeric matrix. It is possible that the actual condition is best represented by a mixture of the above models depending on the total loading of the HDEHP on the resin. However, some evidence suggests that the first of these models is to be preferred. Microscopic examination of graft copolymers before and after adsorption indicates uniform penetration throughout the particles, both for an adsorbed aqueous solution and for an organic solution of an organophilic dye. A few experiments were performed in which the complexing agent was diluted with an inert, nonvolatile substance (mineral oil) and adsorbed on the resin in the usual manner. Measurements of the elution of tracer activity from columns made from these substrates demonstrate that the effective concentration of HDEHP is still a function of the mass concentration per unit volume of swollen column material. That is, the added diluent served merely to increase the overall volume of the polymeric matrix rather than to dilute the HDEHP on a mass-to-mass basis. Thus, it may be that aqueous and organic phases are intimately mixed at the molecular level throughout the matrix. For the surface-grafted Kel-F support, this would be true only for the layer of solvent-swollen graft on the surface of the Kel-F particle. Considerably more work needs to be done on this aspect of the problem before we can adequately understand the molecular and ionic processes occurring on the extraction columns.

By analogy with other chromatographic separations, one would expect to improve resolution by increasing the operating temperature. A few trial runs on columns at elevated temperatures gave decreased retention volumes of the adsorbed species but no significant reduction in band-width. The columns are extremely difficult to operate at elevated temperature because of the great propensity of the column material to form and hold air bubbles. Although the ordinary precautions were observed (*i.e.*, careful degassing of column material and solutions, pre-heating of the eluent and passage through glass wool, etc.), long runs inevitably were terminated by bubble formation or column separation. Nevertheless, sufficient data were obtained to conclude that resolution was not improved by the elevated temperature.

Since the uncrosslinked graft of styrene to Carbowax is soluble in toluene, Kel-F powder was coated with such a solution and the solvent was evaporated. The result was a surface-coated (as opposed to surface-grafted) particle that was similarly capable of adsorbing HDEHP and of maintaining water wettability. A column made

from this substrate was inferior in performance to the surface-grafted Kel-F, principally because of the very long tailing of an elution peak. This could possibly be minimized by limiting the thickness of the copolymer coating. However, since the capacity for holding HDEHP was already low compared to the surface-grafted material, it was not felt worthwhile to pursue this approach.

It can be stated that the general characteristics of extraction columns made from graft copolymer substrates can be described by expressions similar to those developed for ion exchange resins at slow flow rates where equilibrium conditions are closely approached. The quantitative description of elution peaks at faster flow rates requires a greater understanding of the reaction mechanisms and diffusion processes within the solvent-swollen polymeric matrix. Empirical methods have established guide lines for varying substrate properties to achieve the proper balance between capacity and flow rate for optimum resolution by the column.

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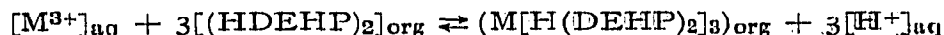
SUMMARY

The operating characteristics of extraction columns made from graft copolymers containing an adsorbed complexing agent are described by expressions analogous to those developed for ion exchange resins. Several kinds of graft copolymers are compared, and guidelines are developed for optimizing column resolution.

The trivalent lanthanides are known to be eluted in order of increasing atomic number by gradient elution with hydrochloric acid on a column containing adsorbed di-(2-ethylhexyl) orthophosphoric acid (HDEHP). Adaptation of the plate equilibrium theory enables one to calculate ϕ^* , the volume of effluent to maximum metal ion concentration, from the expression

$$\int_0^{\phi^*} f^3(\phi) d\phi = Q^3 V_i K$$

where $f(\phi)$ is a term for the hydrogen ion concentration as a function of the volume of effluent, Q is a variable related to the concentration of the HDEHP, V_i is the effective interstitial volume of the column, and K is the equilibrium constant for the exchange reaction



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