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ENZACRYL® GEL PACKINGS

INORGANIC SALT COMPATIBILITY, pH STABILITY AND ION-EXCHANGE DERIVATIVES

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SUMMARY

The elution behaviour of simple inorganic salts on Enzacryl® gel K1 was found to depend on both radius of the hydrated anion and electrostatic interaction. An approximately linear, inverse relationship was found to exist between the distribution coefficient, K_{ds}, measured under conditions of static equilibrium, and the charge transfer to solvent absorption maximum, $\nu_{max.}$, for the anions studied. Transition metal cations were strongly adsorbed but could be eluted with sodium chloride solution. At 25°, Enzacryl gel was shown to be stable to 1 M HCl and the pendant acylmorpholino residues were only slowly hydrolysed by 1 M NaOH. Hydrolysis of Enzacryl gel K2 with 1 M NaOH at 100° gave a cation exchanger, Enzacryl-CO_2H. An anion exchanger Enzacryl-DEAE was obtained by reaction of Enzacryl gel K2 with N,N-diethylethylene diamine in boiling ethylene glycol. The efficacy of Enzacryl-CO_2H and Enzacryl-DEAE as packings for ion-exchange chromatography has been demonstrated.

INTRODUCTION

Recently, we have described the chromatographic evaluation of bead polymerized, cross-linked poly(acryloylmorpholine) networks (Enzacryl® gels K1 and K2) as column packings for gel permeation chromatography (GPC) in water[1] and polar organic solvents[2].

Enzacryl gel is a unique matrix for aqueous GPC in that the linear poly(acryloylmorpholine) chains carry few labile protons. Consequently, the elution volume of the solvent corresponds to that of ^2H_2O or ^3H_2O. This facilitates rapid and accurate determination of Wheaton and Baumann[3] absolute distribution coefficients.

In the present paper we report on some other aspects of aqueous chromatography with Enzacryl gel. These include the elution behaviour of inorganic salts, the stability of the matrix to extremes of pH and the synthesis of functional derivatives suitable for ion-exchange chromatography.

EXPERIMENTAL

Commercial materials

Enzacryl gels K1 and K2 (60–210 μm), respective molecular weight ($\bar{M}_n$) exclusion limits of approx. 10^4 and $5 \cdot 10^4$ for polyethylene glycols in water, were obtained from Koch-Light (Colnbrook, Great Britain). Blue Dextran 2000, average molecular weight ($\bar{M}_w$) of approx. $2 \cdot 10^6$ was purchased from Pharmacia (London, Great Britain).

Analytical-grade inorganic salts, N,N-diethylethylenediamine, adenosine 5'-monophosphate (AMP), adenosine 5'-diphosphate (ADP), adenosine 5'-triphosphate (ATP), 3-nitroaniline and 4-nitroaniline were obtained from the usual suppliers.

GPC of inorganic salts

Dry Enzacryl gel K1 was dispersed in distilled water and allowed to swell for 24 h to attain equilibrium. The mixture was then degassed, stirred into a slurry and poured into 45 × 2.5 cm glass GPC columns. Packing was completed under a flow-rate of 15 ml/h·cm².

For gel chromatography, columns were connected to the proportionating pump of a BTL Chromofrac fraction collector and eluted at ambient temperature. Sample solutions (1.0 ml) were applied directly through the solvent inlet line. Effluents were collected in 1.0-ml fractions at a flow-rate of 2.0 ml/h·cm². Usually, the concentration of each solute in the applied samples was that which gave 0.2 M anions.

The chromatographic behaviour of sodium chloride was investigated at concentrations of 0.01, 0.02, 0.05, 0.1, 0.2, 1.0, 2.0 and 5.0 M.

In the case of transition metal ions, sorptive interaction necessitated elution with 2 M sodium chloride solution.

Evaluation of distribution coefficients

For each column the void volume, V_0, was determined by measuring the elution volume of Blue Dextran 2000. The elution volume, V_g, of a standard solute, glucose, was determined concurrently. Subsequently, the Wheaton and Baumann absolute distribution coefficient, K_d, for each non-adsorbed salt with elution volume V_e was calculated from the equation:

$$K_d = K_d' \cdot \frac{V_e - V_0}{V_g - V_0} \tag{1}$$

For a given salt V_e was taken as the volume of solvent displaced from the column before the emergence of the peak maximum; K_d' is the absolute distribution coefficient for glucose (0.73)[1].

In the case of each sodium salt, a "static distribution coefficient", K_{ds}, was calculated from equilibrium swelling measurements[4]. A 4-ml aliquot of a 0.2 M solution of each salt was added to 1.0-g samples of Enzacryl gel K1. Swelling was allowed to proceed to equilibrium over 48 h after which Na⁻ concentrations in the supernatants were determined in order to calculate the change in salt concentration. The value of K_{ds} was calculated from the equation:

$$K_{ds} = \frac{v_p - v_i}{v_p} \tag{2}$$

where v_p and v_i are the respective volumes within the gel inaccessible to a totally excluded, polymeric solute and the salt. It was assumed that v_p corresponded to the volume of solvent imbibed by the beads on swelling to equilibrium. The parameter v_i was calculated using the expression:

$$v_i = \frac{v(c_2 - c_1)}{W_g c_2} \tag{3}$$

where W_g is the weight of dry Enzacryl gel taken for swelling, v is the total volume of solution, c_1 the initial salt concentration and c_2 the final salt concentration in the solution after swelling to equilibrium.

Stability to extremes of pH

Samples of dry Enzacryl gel K2 were allowed to swell to equilibrium in 1 M hydrochloric acid and 1 M sodium hydroxide and the resulting xerogels incubated at 25° in suspension in these reagents over 9 days. Aliquots of each xerogel suspension were withdrawn after 0, 2, 4 and 9 days. The xerogels recovered were washed exhaustively with distilled water, shrunk by equilibration with ethanol, rinsed with diethyl ether and dried under reduced pressure. Weighed quantities of the recovered beads were re-swelled in 2 M sodium chloride and titrated with 0.1 M sodium hydroxide.

Enzacryl-CO₂H

The alkaline degradation of Enzacryl gel K2 described above was repeated at 100°. Aliquots of the reaction mixture were withdrawn after 8, 24, 48 and 72 h and the reaction stopped immediately by pouring into a mixture of excess 1 M hydrochloric acid and crushed ice. Each sample of hydrolysed xerogel was washed to neutrality with distilled water, treated with ethanol and diethyl ether as described above, dried under reduced pressure and the carboxylate content of the samples of Enzacryl-CO₂H so obtained estimated.

Enzacryl-DEAE

Dry Enzacryl gel K2 was suspended in an excess of a mixture of dry ethylene glycol and dry N,N-diethylethylene diamine (5:1, v/v) and stirred magnetically while boiling under reflux at 175°. Aliquots of the reaction mixture were withdrawn after 8, 24, 48 and 72 h, poured on to crushed ice and washed to neutrality with distilled water. The samples of hydrated gel so obtained were equilibrated with ethanol, rinsed with ether and dried under reduced pressure. Weighed amounts of the Enzacryl-DEAE samples were re-swelled in 2 M sodium chloride and the diethylamino group content estimated by titration with 0.1 M hydrochloric acid.

Cation-exchange chromatography on Enzacryl-CO₂H

Enzacryl-CO₂H (cation-exchange capacity 0.78 mequiv./g) was equilibrated with aqueous acetic acid (2%, v/v), stirred into a slurry and packed into a 1.0 × 45 cm glass column. A 1-ml sample of a saturated solution of a mixture of 1,3- and 1,4-nitroanilines in aqueous acetic acid (2%, v/v) was applied to the gel bed. The column was eluted with the same solvent at a flow-rate of 10 ml/h collecting 2-ml fractions.

Anion-exchange chromatography on Enzacryl-DEAE

Enzacryl-DEAE (anion-exchange capacity 0.74 mequiv./g) was equilibrated with 0.1 M Tris–HCl buffer (pH 8.3) and packed into a 1.0 × 45 cm glass column. A 1.5-ml sample of a 0.33 % solution of a mixture of AMP, ADP and ATP in equal proportions by weight in the same buffer was applied to the gel bed. The column was eluted with more Tris–HCl buffer at a flow-rate of 14 ml/h collecting 2-ml fractions.

Analysis of column effluents

Inorganic anions were determined by an adaption of the fluorescence quenching techniques described by Brooks and Davis[5]. In general, 0.5-ml aliquots of column effluent were mixed with 3.0 ml of the appropriate fluorescent reagent directly in a 1-cm path-length cell of a Perkin-Elmer MPF 2A fluorescence spectrophotometer. A $5 \cdot 10^{-2}$ M solution of quinine sulphate in $5 \cdot 10^{-2}$ M H_2SO_4 was used as fluorescent reagent for Cl^-, I^- and NCS^-. This solution was diluted ten-fold with $5 \cdot 10^{-2}$ M H_2SO_4 to estimate Br^-. The excitation wavelength, $\lambda_{ex.}$, was 390 nm and the emission wavelength, $\lambda_{em.}$, 450 nm. In the case of SO_4^{2-} and NO_3^- the fluorescent reagent was a 10^{-4} M solution of fluorenone; $\lambda_{ex.}$ and $\lambda_{em.}$ were 300 and 340 nm, in 50 % (v/v) ethanol, respectively.

Transition metal cations were determined by atomic absorption spectroscopy using a Hilger and Watts Atomspek spectrophotometer. Na^- concentration was determined with an EEL flame spectrophotometer.

Most organic solutes were monitored spectrophotometrically. Thus 1,3- and 1,4-nitroaniline were monitored by measuring absorbance at 350 nm and 380 nm, respectively; AMP, ADP and ATP were detected at 254 nm and Blue Dextran at 260 nm.

RESULTS AND DISCUSSION

Chromatographic behaviour of simple inorganic salts on Enzacryl gel is typified by the elution profiles for sodium chloride at various sample concentrations (Fig.
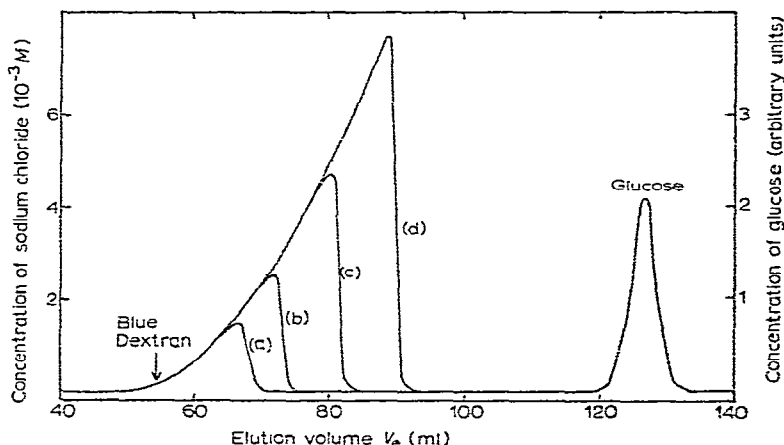

Fig. 1. Elution profile for NaCl on Enzacryl gel K1 in water at a sample concentration of (a) 0.01 M, (b) 0.02 M, (c) 0.05 M and (d) 0.1 M.

TABLE I

K_d VALUES FOR NaCl CHROMATOGRAPHED ON ENZACRYL GEL K1

Column dimensions, 2.5 × 45 cm; sample volumes applied, 1 ml.

Sample conc. (M)	K_d	Sample conc. (M)	K_d
0.01	0.11	0.5	0.58
0.02	0.17	1.0	0.69
0.05	0.25	2.0	0.79
0.1	0.33	5.0	0.82
0.2	0.42		

1). The chromatograms all exhibit a "leading tail" followed by a sharp decrease in concentration immediately after the peak maximum is reached. In each case appearance volume corresponds to the column void volume and the leading edges of the chromatograms are superimposable. The obvious concentration dependence of the peak maximum distribution coefficient K_d (Table I and Fig. 2) is indicative of salt exclusion by a mechanism other than simple molecular sieving except at very high solute concentrations. Similar results have been obtained by Neddermeyer and Rogers[6] for GPC of inorganic salts on Sephadex G-10 and G-25. Since these column packings were found to contain small amounts of carboxylate ion, the "leading tail" was ascribed to a Donnan salt exclusion effect.

A number of metal chlorides and inorganic sodium salts were eluted on Enzacryl gel K1 at a sample ion concentration of 0.2 M ions and the K_d values recorded (Tables II and III). The similarity of the K_d values of the chlorides of sodium, potassium, lithium, magnesium and hydrogen suggests independence of the salt exclusion mechanism of the nature of the cation. It follows, therefore, that the wide variation in K_d of the inorganic sodium salts is a consequence of the exclusion mechanism being anion dependent. As Enzacryl gel K1 is a practically neutral carrier, it is unlikely that ion exclusion results solely from the Donnan effect. Non-ideal permeation
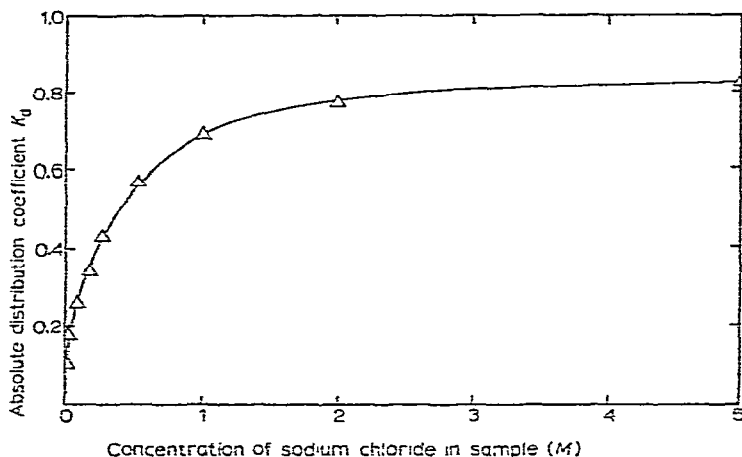

Fig. 2. Variation in K_d with NaCl concentration on Enzacryl gel K1 in water.

TABLE II

K_d VALUES OF VARIOUS CHLORIDES CHROMATOGRAPHED ON ENZACRYL GEL K1

Column dimensions, 2.5 × 45 cm; sample volumes applied, 1 ml.

Salt	K_d	Salt	K_d
HCl	0.45	KCl	0.44
LiCl	0.45	$MgCl_2$	0.42
NaCl	0.43	$CuCl_2$	adsorbed

TABLE III

K_d VALUES OF SODIUM SALTS CHROMATOGRAPHED ON ENZACRYL GEL K1

Column dimensions, 2.5 × 45 cm; sample volumes applied, 1 ml; Na^+ concentration, 0.2 M.

Salt	K_d	Salt	K_d
NaCl	0.43	NaSCN	1.05
NaBr	0.56	$NaNO_3$	0.73
NaI	0.91⁻	Na_2SO_4	0.34

behaviour of inorganic solutes may be regarded as one aspect of the broader phenomenon of "additional exclusion". This may be envisaged as adverse micropartition of solutes between regions within the individual gel beads where the constrained solution concentration of poly(acryloylmorpholine) chains is high and regions consisting of relatively pure solvent[2].

As a result of its concentration dependence, the distribution coefficient of a given ionic solute diminishes on traversing an Enzacryl gel column owing to the continuous dilution effect. Consequently it is preferable to compare distribution coeffi-

TABLE IV

K_d VALUES OF SODIUM SALTS ON ENZACRYL GEL K1, CTTS ν_{max} VALUES AND HYDRATED ION RADII

Anion	K_d	CTTS $\nu_{max.} \times 10^3$ (cm^{-1})*	Hydrated anion radius r_H (nm)**
Cl^-	0.69	54.5	0.332
Br^-	0.94	50.3	0.330
I^-	1.69	44.2	0.331
SCN^-	1.34	45.0	—
NO_2^-	0.88	52.0	—
NO_3^-	0.88	—	0.335
HSO_4^-	0.58	60.0	—
SO_4^{2-}	0.50	57.1	0.379
SO_3^{2-}	0.58	44.0	—
$S_2O_3^{2-}$	0.49	46.5	—
$H_2PO_4^-$	0.48	59.0	—
HPO_4^{2-}	0.65	56.0	—
CO_3^{2-}	0.34	—	0.394
$[Fe(CN)_6]^{4-}$	0.59	—	0.422

* From data given in ref. 11.
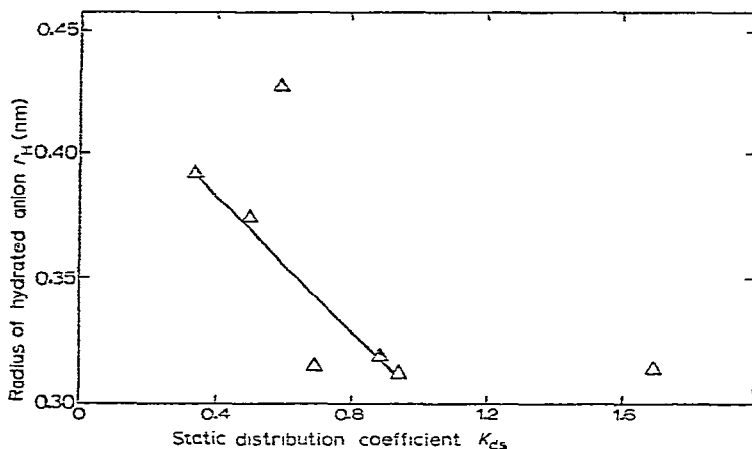** From data given in ref. 10.

Fig. 3. Relationship between r_H and K_{ds} for anions of simple sodium salts on chromatography on Enzacryl gel K1 in water at 0.2 M sample anion concentration.

cient, K_{ds}, obtained under defined "static" equilibrium conditions (Table IV). It is well documented[7-9] that the overall partition of a salt between the gel and external solution is governed principally by hydrated ion radii and electrostatic interaction. Consequently, it was no surprise to find only poor correlation (Fig. 3) between K_{ds} and the hydrated anion radius, r_H, as given by Nightingale[10]. We have found that a better correlation exists between K_{ds} and charge transfer to solution (CTTS) wavelength maxima, v_{max}. (Table IV and Fig. 4). It should be noted that both r_H and v_{max}. are determined for anions at infinite dilution, whereas K_{ds} is measured at 0.2 M salt concentration. Experiments carried out at much greater dilution would have been less rewarding because the anions are almost totally excluded at high dilution.

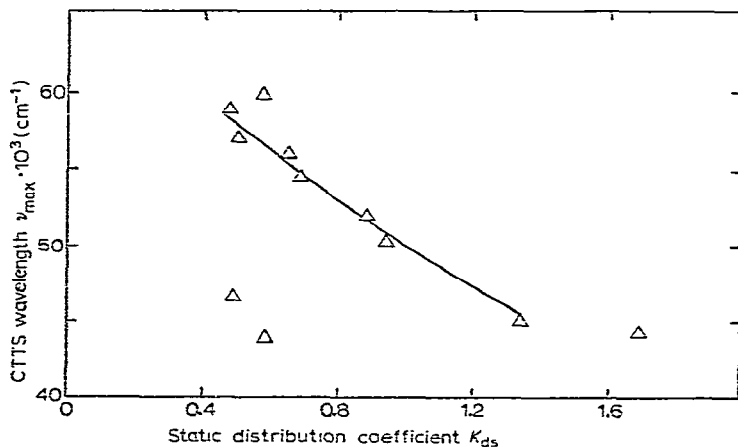The wavelength of the CTTS absorption band of an anion in solution is govern-

Fig. 4. Relationship between v_{max}. and K_{ds} for anions of simple sodium salts on chromatography on Enzacryl gel K1 in water at 0.2 M sample anion concentration.

TABLE V

ELUTION VOLUMES, V_e, FOR TRANSITION METAL CHLORIDES CHROMATOGRAPHED ON ENZACRYL GEL K1 IN 2 M NaCl

Column dimensions, 2.5 × 45 cm; sample volumes applied, 1 ml; Cl⁻ concentration 0.2 M.

Salt	V_e (ml)	Salt	V_e (ml)
CrCl₃	162	CoCl₂	130
MnCl₂	133	NiCl₂	141
FeCl₂	132	CuCl₂	246

ed by both the charge and size of the hydrated anion[11]. A solvated anion will absorb radiation to form a radical and a solvated electron.

$$X^-_{aq} \xrightarrow{\ h\nu\ } (X\cdot + e^-)_{aq}$$
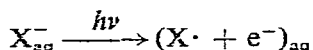
The electron is bound within a volume defined by the environment of the ion. CTTS spectra, therefore, are well suited to the studying the properties of hydrated ions in solution. The absorption wavelength $\nu_{max.}$, of the ion in solution is related to the charge and size of the anion by

$$h\nu_{max.} = IP - \Delta H^0_s(X\cdot) + \frac{17.8\ e^2}{R_d}$$

where h is Planck's constant, IP the ionisation potential, ΔH^0_s (X·) the enthalpy of solvation of the radical, e the electronic charge and R_d the radius parameter characterising the ionic cavity. This is the distance between the ion centre and the near-inside edge of the solvent shell surrounding the ion, and approximates to values predicted from an analysis of partial molar ionic volumes[11].

It is apparent from Fig. 4 that sorptive retardation of SCN⁻ and I⁻ is taking place. Only SO_3^{2-} and $S_2O_3^{2-}$ fail to fit the $\nu_{max.}$ versus $K_{d\cdot}$ relationship. This may be
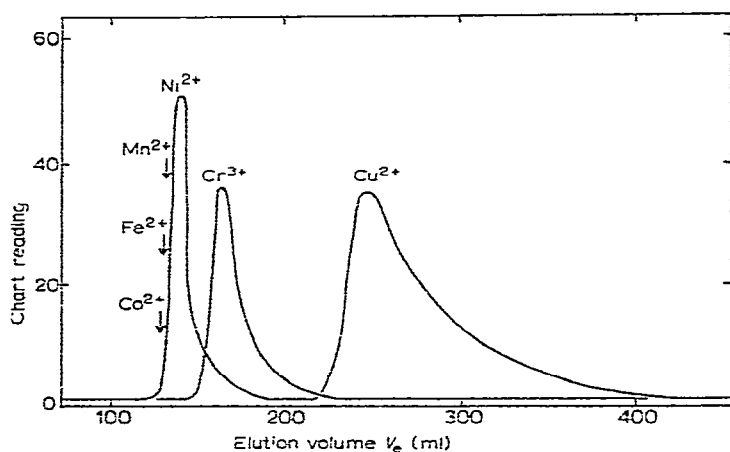

Fig. 5. Elution profile for some transition metal chlorides on Enzacryl gel K1 in 2 M aqueous NaC at a sample concentration of 0.2 M.

TABLE VI

HYDROLYSIS OF ENZACRYL GEL K2 BY 1 M NaOH AT 25°

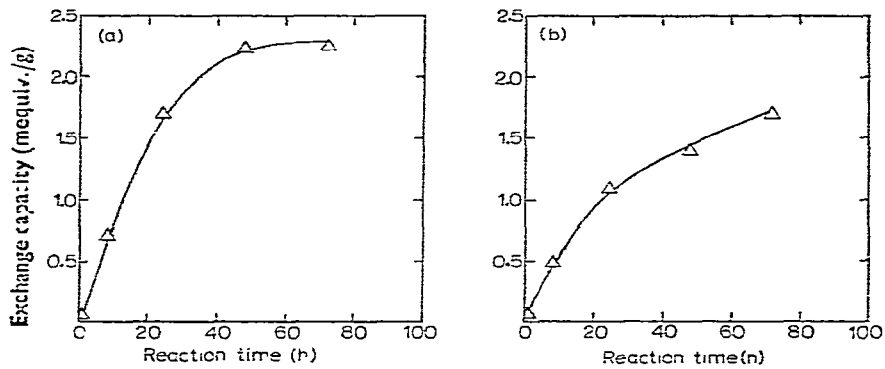| Time (days)	Capacity (mequiv./g)
0	0.00
2	0.03
4	0.11
9	0.21

Fig. 6. (a), Preparation of Enzacryl-CO_2H by hydrolysis of Enzacryl gel K2 with 1 M NaOH at 100°. (b), Preparation of Enzacryl-DEAE by reaction of Enzacryl gel K2 with N,N-diethylethylene diamine in boiling ethylene glycol.
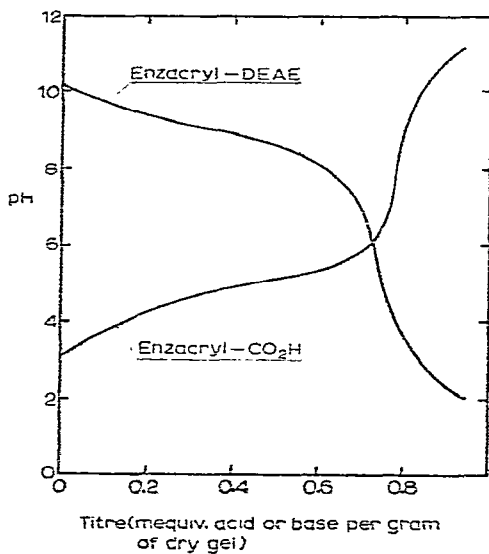

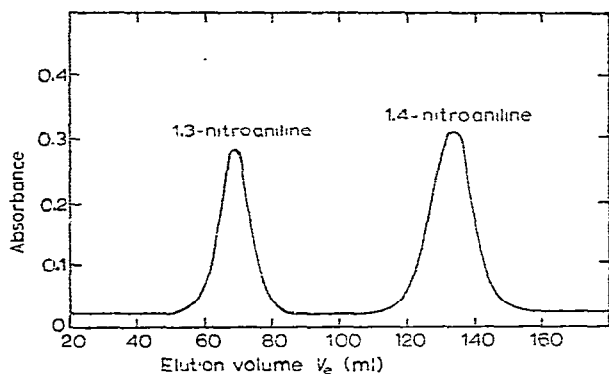Fig. 7. Titration curves for Enzacryl-CO_2H and Enzacryl-DEAE.

Fig. 8. Chromatography of a mixture 1,3- and 1,4-nitroaniline on Enzacryl-CO_2H in 2% aqueous acetic acid.

due to an incorrect assignment of ν_{max}, or some other process taking place such as intramolecular transitions.

It is interesting to note that transition metal ions are strongly adsorbed on Enzacryl gel. This may be a consequence of weak complexing with the oxygen lone pairs of the morpholine residues. The ions may, however, be eluted with dilute sodium chloride solution (Table V and Fig. 5). In this medium the eluted transition metals may not all be in the form of simple hydrated species. Transition metal ions are also strongly adsorbed on cross-linked dextran[12-14].

The possibility of a small number of ionic sites on the Enzacryl gel matrix led us to investigate its stability to extremes of pH. The matrix is little affected by 1 M HCl at 25° as no hydrolysis is detected after 9 days. However, with 1 M NaOH at 25° slow hydrolysis occurred with the formation of carboxylate groups (Table VI). The stability of Enzacryl gel to extremes of pH compares favourably to other hydrophilic GPC supports, for example, cross-linked polyacrylamide[15].
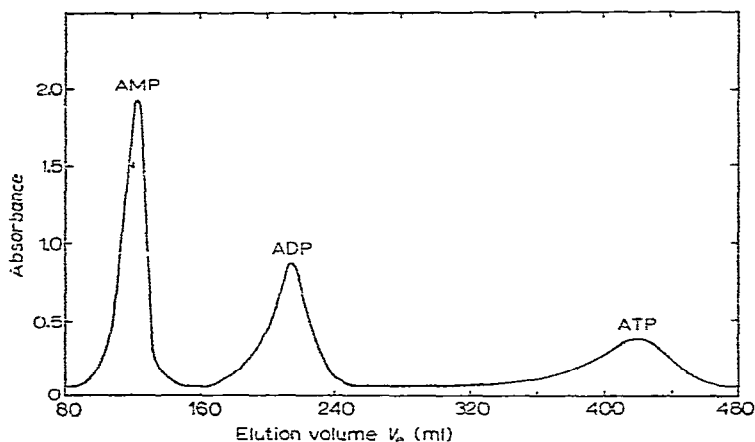

Fig. 9. Chromatography of a mixture of AMP, ADP and ATP on Enzacryl-DEAE in 0.1 M Tris–HCl (pH 8.3).

Controlled alkaline hydrolysis of cross-linked polyacrylamide has been used to prepare cation-exchange derivatives[15] and the same technique was used on Enzacryl gel K2 in the present studies (Fig. 6a). Anion-exchange derivatives of Enzacryl gel K2 were prepared by reaction with N,N-diethylethylene diamine in refluxing ethylene glycol (Fig. 6b). Cation and anion exchangers of approximately 0.75 mequiv./g capacity, Enzacryl-CO_2H and Enzacryl-DEAE, respectively, gave near ideal titration curves (Figs. 7a and b).

The efficacy of Enzacryl-CO_2H as a cation exchanger was demonstrated by the separation of 1,3- and 1,4-nitroanilines in 2% acetic acid (Fig. 8). Enzacryl-DEAE was shown to behave as an anion exchanger by the separation of AMP, ADP and ATP in 0.1 M Tris–HCl buffer (Fig. 9). The ability of Enzacryl-DEAE to resolve this mixture is comparable to that of the DEAE derivative of cross-linked dextran[16].

In view of the applicability of cross-linked poly(acryloylmorpholines) in non-aqueous GPC the possibility of utilising Enzacryl-CO_2H and Enzacryl-DEAE in mixed and non-aqueous solvents should not be overlooked.

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