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STUDY OF ADSORPTION AND OTHER SIDE-EFFECTS ON GUAR GEL FILTRATION MEDIA AND PREPARATION AND APPLICATIONS OF GUAR GEL ION EXCHANGERS*

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SUMMARY

The elution behaviour of a number of low-molecular-weight substances such as common buffering substances, amino acids, and some aromatic and heterocyclic compounds on guar 5-X30 gel has been investigated. Their gel filtration behaviour was determined by measuring the distribution coefficients. Among inorganic substances, hydroxyl and borate ions were strongly adsorbed on the gel matrix. Among organic compounds, aromatic and heterocyclic substances show a greater tendency than aliphatic substances to be adsorbed. Basic groups in the test substances tend to increase the adsorption, while acidic groups have the opposite effect. A cation exchanger (CM-guar 5-X30) and an anion exchanger (DEAE-guar 5-X30) were synthesized by reaction of guar 5-X30 gel with monochloroacetic acid and 1-chloro-2-diethylaminoethane hydrochloride, respectively, in a strongly alkaline medium. The efficiency of CM-guar 5-X30 and DEAE-guar 5-X30 as packings for ion-exchange chromatography has been demonstrated.

INTRODUCTION

Recently, we described the chromatographic evaluation of cross-linked guar gum gels (guar 5-X30 and 2-X10) as column packings for the gel filtration chromatography of biopolymers in aqueous media[1].

It has been reported[2,3] that with dextran-based gels, many substances show a behaviour that differs widely from what might be expected from their molecular size. Adsorption effects were also observed on cellulose[4] and starch[5,6] matrices. As guar gel is a polysaccharide-based matrix, it was considered necessary to perform similar gel filtration experiments with different substances, *e.g.*, buffering substances, amino acids and some aromatic and heterocyclic compounds, on guar 5-X30 gel, in order to evaluate the efficiency of guar gels in chromatographic separations. The

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synthesis of functional derivatives of guar 5-X30 gel suitable for ion-exchange chromatography has also been carried out and their applications have been studied.

EXPERIMENTAL

Reagents

Guar 5-X30 gel was prepared by the method described earlier[1]. Blue Dextran 2000 (average molecular weight $2 \cdot 10^6$) was obtained from Pharmacia (Uppsala, Sweden). 1-Chloro-2-diethylaminoethane hydrochloride (EGA-Chemie, Steinheim, G.F.R.) and monochloroacetic acid (Sarabhai M. Chemicals, Baroda, India) were used as derivatization reagents. Adenosine 5'-monophosphate (AMP), adenosine 5'-diphosphate (ADP) and adenosine 5'-triphosphate (ATP) were purchased from Centron Research Labs., Bombay, India. 1,3- and 1,4-nitroanilines were obtained from Sisco Research Laboratory, Bombay, India. The amino acids used were BDH, (Poole, Great Britain), E. Merck (Darmstadt, G.F.R.) or Riedel de Haën (Hannover, G.F.R.) chromatographically homogeneous L-isomers. All other organic and inorganic reagents were of analytical grade.

Gel filtration behaviour of substances

Dry guar 5-X30 gel was allowed to swell in 0.05 *M* sodium chloride solution for 30 min and was then freed from fine-grained material by repeated sedimentation and decantation. The gel grains were poured into a glass column under gravity. A circular filter-paper was placed on top of the packed column to protect the surface. After equilibration of the column with distilled water or an electrolyte solution, the substance to be tested was dissolved in 2 ml of water and applied on the column. Elution was then started with the same aqueous solution as was used for equilibration with a hydrostatic pressure of 50 cm. Fractions of 2 ml were collected at a flow-rate of 2 ml/min. Substances to be tested were separately gel filtered through the column, and the yields and distribution constants (K_d values) were determined.

Some gel filtration experiments performed with mixtures of low-molecular-weight substances are described below.

When 25 mg of picric acid alone was filtered through a column of guar 5-X30 gel, an elution profile was obtained as shown in Fig. 1A. However, when 25 mg of picric acid together with 50 mg sodium chloride was gel filtered through the same column, the elution pattern obtained was as shown in Fig. 1B. In the latter instance the zone is not spread out as it is in the former.

When a mixture containing 10 mg of DNP-glycine and 10 mg of sodium chloride was gel filtered through a column of guar 5-X30 gel, the sodium chloride was eluted first and then DNP-glycine. The elution profile is shown in Fig. 2.

Synthesis of ion exchangers

CM-guar 5-X30. In a 1-l three-necked reaction flask, equipped with a 5-cm diameter semi-circular paddle stirrer, were placed 100 ml of benzene. Guar 5-X30 gel (25 g) was then added with vigorous stirring to prevent the formation of lumps. Nitrogen was passed through the mixture for 1 h and the gas inlet tube was then raised above the surface to maintain a nitrogen atmosphere in the reaction vessel. Next, 45 g (1.125 mole) of 50% sodium hydroxide solution were added. The flask was placed in a water-bath at 50° and 28 g (0.29 mole) of monochloroacetic acid were

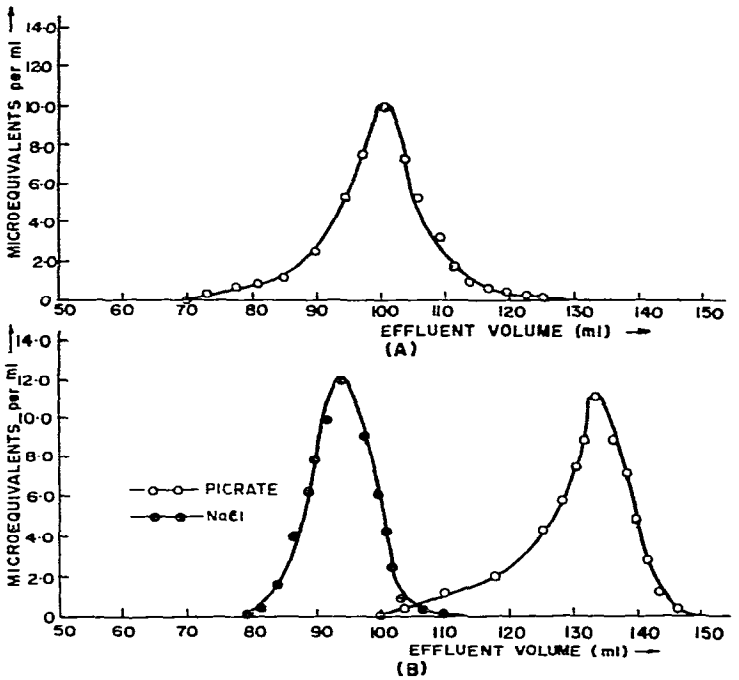

Fig. 1. (A) Elution profile obtained when 50 mg of picric acid was gel filtered through a 40 × 2 cm I.D. column of 100-mesh guar 5-X30 gel with distilled water as eluent. (B) Elution profile obtained when mixture of 50 mg of picric acid and 25 mg of sodium chloride was gel filtered through a 40 × 2 cm I.D. column of 100-mesh guar 5-X30 gel with distilled water as eluent.

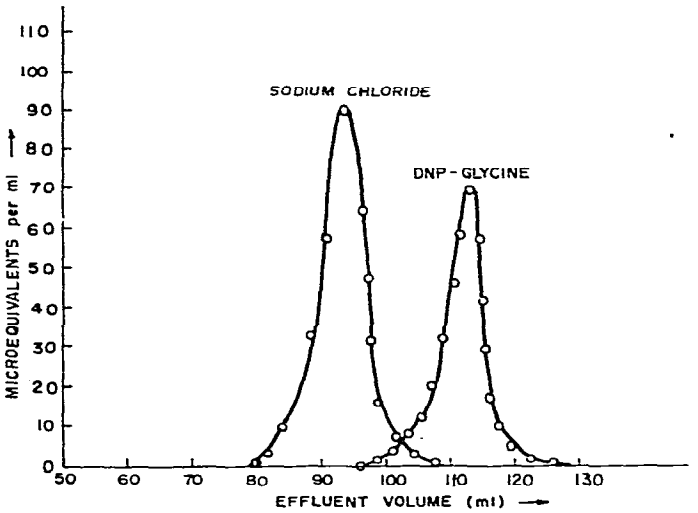

Fig. 2. Elution profile obtained when a mixture of 10 mg of DNP-glycine and 10 mg of sodium chloride was gel filtered through a 40 × 2 cm I.D. column of 100-mesh guar 5-X30 gel with distilled water as eluent.

added in portions, while stirring. The additions were made slowly in order to prevent the temperature from exceeding 55°. The soft, doughy mass was then heated at 40° in a water-bath for 4 h, with stirring. After removal from the water-bath, the reaction flask was cooled in an ice-bath, 200 ml of dilute hydrochloric acid were added in several portions and the solid mass was allowed to settle. After careful decantation of the supernatant liquid, the sediment was washed repeatedly in a similar manner to remove the fines which would not sediment, as well as excess of acid. The sediment was washed with distilled water to ensure complete removal of acid.

The sediment was filtered to remove as much water as possible, then washed with three 200-ml volumes of ethanol, the last washing being made with absolute ethanol. As much as ethanol as possible was drawn off on the filter, and the partially dried product was abraded to a powder. The remaining ethanol was removed by drying *in vacuo* and the carboxylate content of the CM-guar 5-X30 sample so obtained was determined.

DEAE-guar 5-X30. DEAE-guar 5-X30 was prepared by a procedure similar to that used for CM-guar gel, except that 25 g of guar gel 5-X30 were suspended in 100 ml of dioxane, and 45 g (1.125 mole) of 50% sodium hydroxide solution and 51.6 g (0.30 mole) of 1-chloro-2-diethylaminoethane hydrochloride were used.

The soft, doughly mass obtained was heated in a water-bath at 50° for 2 h, with stirring. The reaction flask was cooled in an ice-bath and 200 ml of 1 *M* sodium chloride solution were added in several portions, care being taken to achieve complete mixing. The presence of sodium chloride controlled the swelling and facilitated filtration, which was otherwise difficult under the strongly alkaline conditions required for the removal of coloured side-products from the anion exchangers.

The resulting thick suspension was made strongly acidic by adding sufficient 1 *N* hydrochloric acid, then immediately filtered and washed successively with 200-ml portions of 0.1 *N* sodium hydroxide solution, 0.1 *N* hydrochloric acid and 0.1 *N* sodium hydroxide solution. The cake was tamped down and drained dry to the cracking point between washings but was not rinsed with water. Finally, the cake was suspended in 250 ml of 0.1 *N* sodium hydroxide solution. The supernatant liquid was decanted, and the sediment was washed with water 5–6 times by decantation to remove particles that would not settle. The sediment was filtered to remove as much water as possible, then washed with ethanol and dried as for the preparation of CM-guar 5-X30.

Determination of CM content in CM-guar 5-X30 resin and DEAE content in DEAE-guar 5-X30 resin

Weighed amounts of the CM-guar 5-X30 and DEAE-guar 5-X30 resins were re-swollen separately in 1 *M* sodium chloride solution and the CM and DEAE contents were determined by titration with 0.1 *M* sodium hydroxide solution and 0.1 *M* hydrochloric acid, respectively. From the titration graphs (Fig. 3) the exchange capacity of these resins could be determined.

Cation-exchange chromatography on CM-guar 5-X30

A column (40 × 1 cm I.D.) of CM-guar 5-X30 (cation-exchange capacity 2.5 mequiv./g) was prepared in 2% acetic acid solution. The column was equilibrated

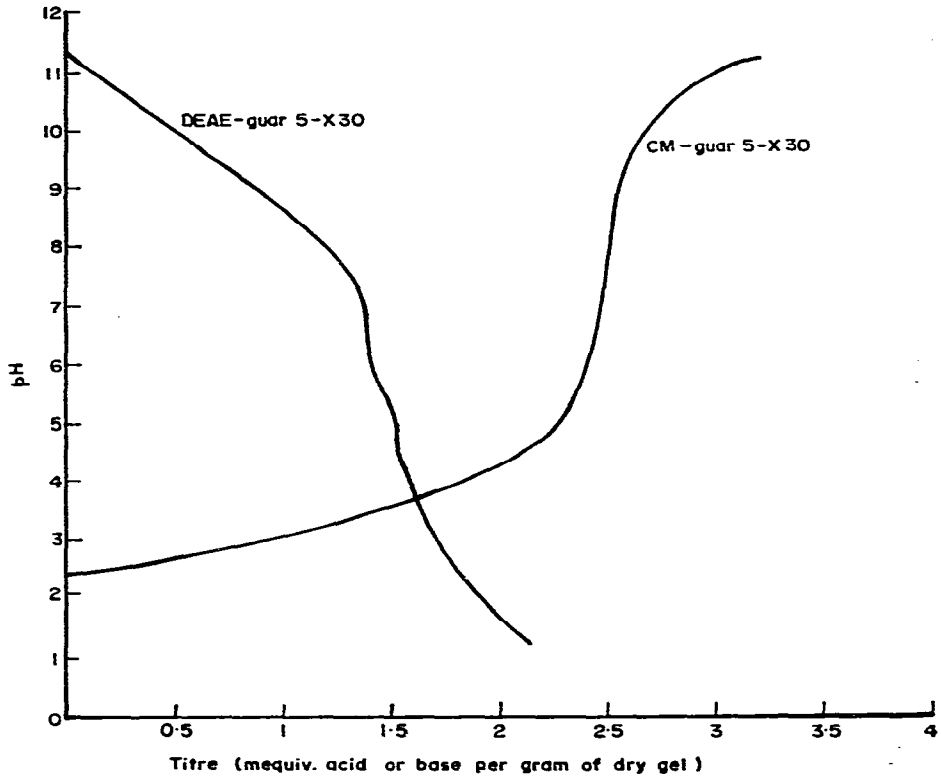

Fig. 3. Titration curves for CM-guar 5-X30 and DEAE-guar 5-X30 gels.

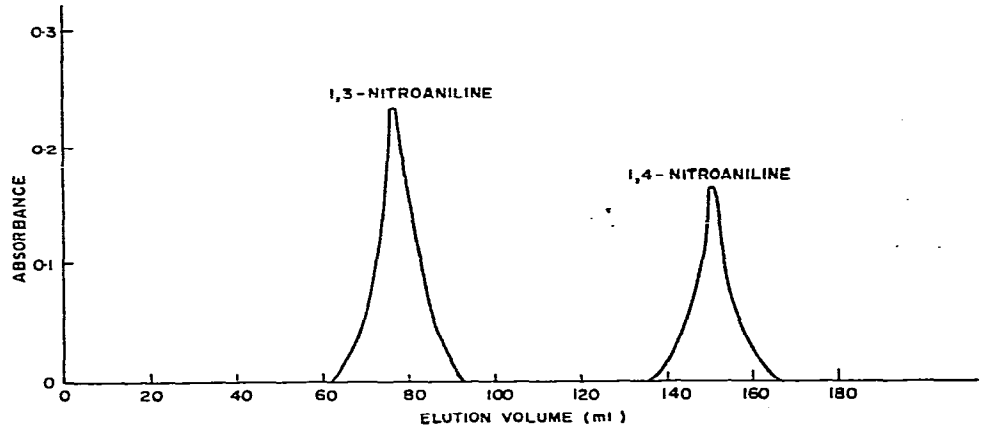

Fig. 4. Separation of 1,3- and 1,4-nitroaniline on a 40 × 1 cm I.D. CM-guar 5-X30 column in 2% acetic acid solution at a flow-rate of 12 ml/h. 1,3- and 1,4-nitroanilines were monitored at 350 and 380 nm, respectively.

with the same solvent. A 1-ml sample of a saturated solution of a mixture of 1,3- and 1,4-nitroanilines in 2% acetic acid solution was applied to the gel bed. The column was eluted with the same solvent at a flow-rate of 12 ml/h, 2-ml fractions being collected. The elution profile is shown in Fig. 4.

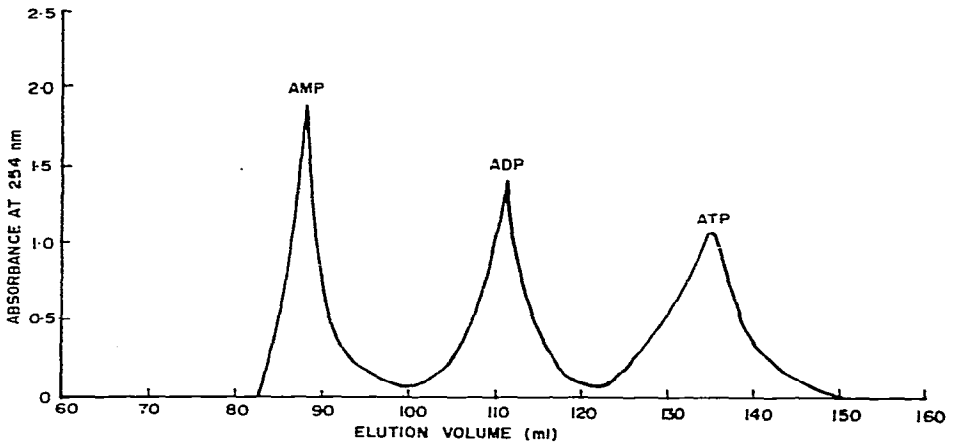

Fig. 5. Separation of a mixture of AMP, ADP and ATP on a 40 × 1.0 cm I.D. DEAE-guar 5-X30 gel column in 0.1 *M* Tris–hydrochloric acid buffer (pH 8.3) at a flow-rate of 10 ml/h.

Anion-exchange chromatography on DEAE-guar 5-X30

DEAE-guar 5-X30 (anion-exchange capacity 1.45 mequiv./g) was equilibrated with 0.1 M Tris–hydrochloric acid buffer (pH 8.3) and packed into a 40 × 1 cm I.D. glass column. The column was then equilibrated with two bed volumes of the same buffer. A 1-ml sample solution containing 0.10% each of AMP, ADP and ATP in the same buffer was applied to the bed. The column was eluted with the same buffer at a flow-rate of 12 ml/h, 2-ml fractions being collected. The elution profile is shown in Fig. 5.

Analysis of column effluents

Acids and bases were determined by titration with dilute sodium hydroxide solution and hydrochloric acid, respectively. Inorganic salts were determined by titration after their passage through an anion or cation exchanger. Amino acids except tryptophan and tyrosine were determined with ninhydrin reagent according to Moore and Stein[7]. Tryptophan, tyrosine and other aromatic substances were determined by measuring their absorption using a spectrophotometer. 1,3- and 1,4-nitroanilines were monitored by measuring the absorbance at 350 and 380 nm, respectively; AMP, ADP and ATP were detected at 254 nm.

RESULTS AND DISCUSSION

Evaluation of distribution coefficients

A substance submitted to gel filtration is characterized by its K_d value, which is calculated from the Wheaton and Baumann[8] absolute distribution coefficient expression:

$$K_d = \frac{V_e - V_0}{V_i}$$

where V_0 is the column void volume as estimated by elution of a totally excluded solute (Blue Dextran 2000), V_i is the inner volume of the column to a a totally in-

cluded solute and V_e is the elution volume of the solute to be tested. A low-molecular-weight solute such as glucose can diffuse freely into and through the grains and has a K_d value of about unity.

In reality, there are some exceptions to these rules. The calculations of K_d values by the above expression give only approximate results. In fact, part of the inner volume, V_i, is water of hydration which is firmly bound to the polysaccharide framework in the gel grains, and is inaccessible to the solute molecules. For an accurate determination of K_d values, the inner volume should be corrected for the water of hydration. In this investigation, V_i was calculated from the water regain (W_r) and the dry weight of the bed material (a):

$$V_i = aW_r$$

No correction for the water of hydration was made. A K_d value of 0.75 therefore indicates non-restricted diffusion in the gel column.

Studies on buffering substances

A number of substances that are commonly used in preparing buffer solutions were tested on the guar 5-X30 gel column. All the substances were eluted quantitatively with distilled water (Table I). Adsorption effects on the bed material were observed with certain substances. This was most pronounced for sodium and potassium hydroxide and for sodium tetraborate, which are known to form complexes with carbohydrates. It is interesting that reversible complex formation with sodium tetraborate[9] due to binding with *cis*-hydroxyl groups of galactose and mannose units of the gels occurs. A similar effect has been observed with pyridine.

TABLE I

ELUTION BEHAVIOUR OF SOME BUFFERING SUBSTANCES GEL FILTERED THROUGH A COLUMN OF 100-MESH GUAR 5-X30 GEL

Eluent: distilled water.

Substance	*Amount (mg)*	K_d	*Yield (%)*
Sodium hydroxide	50	2.10	98.0
Potassium hydroxide	50	2.50	97.0
Ammonium hydroxide	50	0.75	99.5
Sodium hydrogencarbonate	50	0.73	100.5
Sodium carbonate	50	0.74	99.0
Sodium tetraborate	100	1.91	93.0
Sodium acetate	50	0.61	99.5
Sodium citrate	100	0.61	99.0
Sodium phosphate	50	0.66	99.0
Hydrochloric acid	25	0.75	100.0
Formic acid	25	0.80	99.5
Acetic acid	25	0.90	98.5
Citric acid	50	0.90	98.0
Glycine	5	0.75	98.5
Pyridine	5	1.60	98.0

Elution studies on amino acids

Fifteen protein amino acids including glycine (which is listed in Table I) were eluted with distilled water (Table II). Most of the amino acids were eluted with a K_d value of about 0.75, which indicates a free diffusion in the column. The aromatic amino acids exhibit adsorption on the bed material. The basic amino acids are also adsorbed but this adsorption behaviour disappears when the eluent contains sodium chloride electrolyte.

TABLE II

ELUTION BEHAVIOUR OF SOME AMINO ACIDS GEL FILTERED THROUGH A COLUMN OF 100-MESH GUAR 5-X30 GEL

Eluent: distilled water except where indicated otherwise.

Amino acid	*Amount (mg)*	K_d	*Yield (%)*
Alanine	2.0	0.76	99.0
Serine	1.5	0.75	99.5
Leucine	2.5	0.76	98.5
Methionine	5.0	0.75	99.5
Proline	5.0	0.76	99.5
Hydroxyproline	5.0	0.75	100.5
Phenylalanine	2.5	0.85	98.5
Tyrosine	10.0	1.06	98.25
Tryptophan	2.5	1.45	98.0
Aspartic acid	2.5	0.70	99.5
Glutamic acid	2.5	0.72	98.5
Lysine·HCl	2.5	0.85	98.0
Lysine·HCl	2.5*	0.75	98.5
Arginine·HCl	2.5	0.89	99.0
Arginine·HCl	2.5*	0.76	98.5
Histidine·HCl	3.0	0.80	99.0
Histidine·HCl	3.0*	0.74	98.5

* Eluent: 0.05 *M* sodium chloride solution.

Studies on some aromatic compounds

The experiments carried out with some common aromatic substances are summarized in Table III.

Elution behaviour at various pH values

In order to ascertain the effect of pH on the elution profile of the solute, it was considered of interest to study the gel filtration behaviour of sulphanilic acid and tryptophan at various pH values. The results are given in Table IV.

It is seen that the adsorption properties of the test substances vary with pH. It is interesting that the negative sorption of sulphanilic acid becomes more pronounced at higher pH while the adsorption of tryptophan seems to be decreased on increasing the pH.

For the purpose of discussion, we can divide these secondary effects into two categories:

(a) adsorption of solutes on the bed material, which is related to the structure of the test substances and largely independent of the properties of the solvent:

TABLE III

ELUTION BEHAVIOUR OF AROMATIC SUBSTANCES GEL FILTERED THROUGH A COLUMN OF 100-MESH GUAR 5-X30 GEL

Substance	*Amount (mg)*	*Eluent*	K_d	*Yield (%)*
Benzoic acid	5	Distilled water	0.55	99.5
Salicylic acid	5	Distilled water	0.40	98.5
Salicylic acid	5	0.05 *M* NaCl solution	1.40	99.0
Sulphanilic acid	2	Distilled water	0.41	98.0
Sulphanilic acid	2	0.05 *M* NaCl solution	1.0	97.5
Picric acid	1	Distilled water	0.45	95.0
Picric acid	1	0.05 *M* NaCl solution	1.90	98.5
Phenol	2	Distilled water	0.65	98.0
Phenol	2	0.05 *M* NaCl solution	1.5	95.5
Aniline	2	Distilled water	1.45	97.5
Anthranilic acid	5	Distilled water	0.61	99.5

TABLE IV

ELUTION BEHAVIOUR OF SULPHANILIC ACID AND TRYPTOPHAN GEL FILTERED THROUGH A COLUMN OF 100-MESH GUAR 5-X30 GEL AT DIFFERENT pH VALUES

Eluent	*pH*	K_d	
		Sulphanilic acid	*Tryptophan*
0.01 *M* HCl	2.0	0.76	1.95
1.00 *M* acetic acid solution	2.4	0.75	1.90
0.01 *M* ammonia solution	10.6	0.43	0.45
0.01 *M* NaOH solution	12.0	0.45	0.51

(b) charge effect, which depends on the conditions of the column, *i.e.*, ionic strength and pH of the aqueous phases.

Adsorption effects

Among the inorganic substances tested we found that hydroxyl and borate ions are adsorbed on the column. Other inorganic substances may also show adsorption on the gel matrix but to a lesser extent. The adsorption of borate ions has been attributed to the well known complex formation of these ions with the *cis*-diol groups in the gel matrix[9]. Hydroxyl ions are also known to interact with carbohydrate to give the corresponding salt, *e.g.*, sodium cellulose.

Among organic compounds, aromatic and heterocyclic substances have a greater tendency than aliphatic substances to be adsorbed. We also found that basic groups in the test substances increase the adsorption while acidic groups have the opposite effect.

In conclusion, the adsorption effects are not clearly understandable. If they are attributed to the presence of polar (hydroxyl) groups in the gel, then the less pronounced adsorption effect in comparison with cross-linked dextran could be due to the formation of intermolecular hydrogen bonds with *cis*-diol groups.

Charge effect

These effects appeared when some basic substances were subjected to the gel filtration experiments on guar 5-X30 gel and elution was carried with distilled water, but did not occur when the eluent or test solution contained an electrolyte. From Table IV it can be seen that acidic substances show negative sorption when the pH of the eluent is shifted from acidic to basic.

A small number of carboxyl groups present in the gel matrix, introduced during the preparation of the gel, can retard the elution of basic substances owing to salt formation. During elution with salt solutions, these acidic groups are converted into the salts of basic ions and their interactions are eliminated.

In the preparation of CM and DEAE derivatives of guar 5-X30 gel, almost the same conditions were maintained as were used to prepare CM and DEAE derivatives of cellulose[10] and cross-linked dextran[11]. A cation exchanger of capacity approximately 2.5 mequiv./g and an anion exchanger of capacity approximately 1.40 mequiv./g gave nearly ideal titration curves (Fig. 3).

The efficiency of CM-guar 5-X30 as a cation exchanger was demonstrated by the separation of 1,3- and 1,4-nitroanilines in 2% acetic acid solution (Fig. 4). The ability of CM-guar 5-X30 to resolve this mixture is comparable to that of the Enzacryl-CO_2H[12].

DEAE-guar 5-X30 is an effective anion exchanger, as shown by the separation of AMP, ADP and ATP in 0.1 M Tris–hydrochloric acid buffer (Fig. 5). The efficiency of DEAE-guar 5-X30 to resolve this mixture is comparable to that of DEAE derivatives of Enzacryl[12] and cross-linked dextran[13].

Further work on the applications of these ion exchangers is in progress.

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