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COLUMN CHROMATOGRAPHY ON POLYSTYRENE RESIN USING AQUEOUS SYSTEMS

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SUMMARY

Chromatography on the polystyrene resin Amberlite XAD-2, using water or dilute aqueous solutions as eluants, provides useful separations of biologically important compounds of low molecular weight. Adsorption effects govern separations. Compounds with aromatic or non-polar groups are retarded on the resin, while ionic or polar compounds pass through the column with little or no retention. The importance of pH is discussed. A nucleoside may be separated from the corresponding nucleotide or free base, the order of elution being nucleotide–base–nucleoside. Pyrimidine nucleosides may be separated from the strongly adsorbed purine nucleosides and amino acids may be separated into groups. Protonation of a molecule resulted in a smaller elution volume.

INTRODUCTION

Chromatography on cross-linked dextran (Sephadex*) and cross-linked polyacrylamide (Bio-Gel) has been utilized extensively for the separation of a large variety of organic compounds. Relatively little information is available about chromatography on cross-linked polystyrene resins which do not contain ion-exchange groups, particularly when using aqueous eluants.

The principle of gel filtration developed by PORATH AND FLODIN[1] was applied by VAUGHAN[2] to a non-aqueous system to fractionate polystyrene on columns of polystyrene–divinylbenzene resin with benzene as eluant. Lipid mixtures have also been separated on polystyrene resins with benzene[3]. CORTIS-JONES[4] separated a number of organic compounds of low molecular weight on polystyrene resins with benzene or other organic solvents as eluants. Macroreticular polystyrene gels have been developed by MOORE[5] for the determination of molecular weight distribution of polymers in non-aqueous media.

Few attempts have been made to use polystyrene resins for chromatography in

* Mention of commercial names does not imply endorsement by the U.S. Department of Agriculture.

aqueous media. CORTIS-JONES[4] reported that substances as widely different in molecular weight as egg albumin and glucose could not be separated on polystyrene resin with aqueous eluants. However, WHEATON AND BAUMAN[6] found that non-ionic organic compounds of low molecular weight could be separated by elution with water through columns of cross-linked polystyrene ion-exchange resins such as Dowex 50.

Gel filtration effects were observed during chromatography with water as eluant of oligosaccharides[7,8] and polyhydric alcohols[9] on Dowex 50 cation-exchange resin. HOUGH *et al.*[10] eluted raffinose, sucrose, and glucose, in that order, with water from an anion-exchange resin Permutit De-Acidite, but observed no separation on Permutit polystyrene beads.

Separation of monosaccharides analogous to partition chromatography was observed on cation-exchange resins with water[8,11] and on anion-exchange resin with aqueous ethanol[12] as eluant. Anomeric glycosides were resolved with water on Dowex 1 X2 [OH^-] although free sugars were not eluted from the column[13].

Salting-out chromatography[14] was useful in separating alcohols, ethers, amines, aldehydes, and ketones. Non-ionic compounds were eluted from columns of cation- or anion-exchange resins with electrolyte solutions in order of their increasing hydrophobic character. Ionic and non-ionic materials were separated on ion-exchange resins by ion exclusion[15].

Recently new resins, polystyrene cross-linked with divinylbenzene, have become available under the name of Amberlite XAD. These stable non-ionic resins are in the form of beads each of which is an agglomeration of a large number of microspheres. This structure imparts to the resin macroreticular porosity, uniform pore size distribution, and high surface area. These resins are designed to remove water-soluble organic materials from aqueous solution by adsorption. Van der Waals forces are considered to be involved in the adsorption mechanism[16]. The hydrophobic portion of the solute molecule is selectively adsorbed on the polystyrene surface of the bead while the hydrophilic portion is oriented toward the aqueous phase.

RILEY AND TAYLOR[17] employed Amberlite XAD-1 polystyrene resin for concentration of trace organic compounds such as surfactants, insecticides, and dyes in sea water. We have reported previously the use of Amberlite XAD-2, a higher surface area analog of Amberlite XAD-1, for separation of water-soluble meat flavor precursors[18] and for removal of excess picric acid used to deproteinize tissue extracts[19]. In the present study we report on the separation of a number of compounds of low molecular weight found in tissue extracts on columns of Amberlite XAD-2 resin with aqueous solutions as eluants.

EXPERIMENTAL

Polystyrene resin, Amberlite XAD-2 (20–50 mesh, Rohm and Haas Co.), was washed extensively with methylene chloride–methanol (1:3), methanol, and finally water to remove UV absorbing impurities. A slurry of resin in water was poured into glass columns fitted with sintered glass supports to give resin beds of 1.5 × 43 cm, 1.6 × 40 cm, 1.5 × 84 cm, and 2.9 × 34.5 cm. Elution was generally carried out with water under gravity flow. When 0.001 *N* HCl or 0.001 *N* NaCl was used as eluant, the column was equilibrated with the eluant prior to sample application.

Purine and pyrimidine derivatives as well as amino acids were obtained from

Sigma Chemical Co., St. Louis, Mo.; creatinine from Fisher Scientific Co., Fair Lawn, N. J.; glucose from J. T. Baker Chemical Co., Phillipsburg, N. J.

Solutions of standard compounds (0.5–10 mg) were prepared in water with the addition of hydrochloric acid or sodium hydroxide when necessary to effect solution.

The column effluent was monitored by means of a Gilford Model 2000 multiple absorbance recording spectrophotometer to detect UV absorbing compounds. The wavelength was set at the absorbance maximum for each compound. Fractions of 2.5–4.5 ml were collected with a Beckman Model 132 fraction collector at a flow rate of 1–2 ml/min.

Amino acids were estimated by the ninhydrin procedure of COCKING AND YEMM[20] and identified by thin-layer chromatography. Glucose was estimated by the anthrone procedure of TOENNIES AND KOLB[21].

RESULTS AND DISCUSSION

Our experiments showed that useful separations may be obtained on columns of polystyrene resin using water and dilute aqueous solutions as the eluting media. Tables I–IV list the elution volumes of standard compounds on Amberlite XAD-2 columns.

It has been shown[19] that amino acids can be separated into two groups on Amberlite XAD-2. Thus, leucine, isoleucine, phenylalanine, and tyrosine were separated from the other amino acids found in meat extract. Table I shows the elution volumes of a number of amino acids from a 1.6 × 40 cm column of Amberlite XAD-2. Most amino acids elute from the column early, while those possessing large alkyl chains (leucine) or aromatic groups (tyrosine) are retarded. TLC of the eluted fractions showed that the two groups of amino acids were almost completely separated.

TABLE I

ELUTION VOLUMES (ml) OF STANDARD COMPOUNDS FROM AN AMBERLITE XAD-2 1.6 × 40 cm COLUMN

Compound	*Sample medium*[a]*/eluant*		
	0.4 N HCl/ water	*0.001 N NaCl/ 0.001 N NaCl*	*0.4 N HCl/ 0.001 N NaCl*
Glycine	51	55	45
Glutamic acid	51		48
Histidine · HCl	51		45
Leucine	81		75
Tyrosine	81		75
Lysine · HCl		55	
Taurine		55	
Methionine		76	
Inosine		200	
Hypoxanthine			72
Glucose		55	

[a] Solutions (1 ml) of 3–6 mg of each compound were applied to the column.

TABLE II

ELUTION VOLUMES (ml) OF STANDARD COMPOUNDS FROM AN AMBERLITE XAD-2 1.5 × 43 cm COLUMN

Eluant: water; sample medium: HCl, pH 1.

Compound	*Elution volume*
Glycine	60
Glucose	72
Inosinic acid Hypoxanthine	96[a]
Leucine	108
Inosine	232
Guanosine	236

[a] One peak was recorded at 247 mμ for samples containing a mixture of inosinic acid and hypoxanthine. Inosinic acid was eluted on the leading edge of the peak.

Elution of amino acids with water or with 0.001 *N* sodium chloride gave similar separations. In mixtures of inosine, hypoxanthine and amino acids, inosine was completely separated while hypoxanthine was eluted together with the second group of amino acids.

Previously CLEAVER AND CASSIDY[22] showed that adsorption of amino acids on polystyrene-based ion exchangers was independent of true ion exchange. According to MOORE AND STEIN[23] the polystyrene matrix of the ion exchanger plays an important role in the resolution of amino acids. Thus, glycine, alanine, valine, and leucine, amino acids of identical charge, are separated on Dowex 50 as a result of greater affinity of the resin for longer aliphatic side chains.

TABLE III

ELUTION VOLUMES (ml) OF STANDARD COMPOUNDS FROM AN AMBERLITE XAD-2 1.5 × 84 cm COLUMN

Eluant: water.

Compound	*Sample medium*		
	HCl[a]	*NaOH*	*Water*
Glucose	110		
Glycine	108	108[b,c]	107
Isoleucine	156		
Uracil	163	164[c]	
Uridine	235	227[c]	
Inosine		408[b]	421
Adenosine			~490[e]
Hypoxanthine Uric acid		180[b,d]	188

[a] Sample solution, pH 1.
[b] Sample solution, pH 13.
[c] Sample solution, pH 8.
[d] One peak was recorded at 260 mμ for a mixture of hypoxanthine and uric acid, with uric acid being eluted on the leading edge.
[e] Very diffuse peak.

The order of elution of nucleic acid derivatives from XAD-2 resin (nucleotide–base–nucleoside) was different from that observed on elution from Sephadex[24] or Bio Gel[25] (nucleotide–nucleoside–base). Ionization rather than molecular weight apparently influences the separation on polystyrene.

On a small column of resin, 1.5 × 43 cm (Table II), inosine was separated from hypoxanthine, but inosinic acid was not resolved from hypoxanthine. On a column 2.9 × 34.5 cm, containing a larger amount of resin, similar sample load gave well separated peaks for inosinic acid, hypoxanthine, and inosine.

Purine and pyrimidine derivatives may also be resolved on Amberlite XAD-2 (Table III). Pyrimidines tend to be eluted somewhat sooner than purines, *e.g.* uracil ahead of hypoxanthine, and uridine ahead of inosine. This trend was observed on Sephadex[24,26] and Bio-Gel[25,27] columns.

CORTIS-JONES[4] has reported that with organic solvents as eluants the presence of hydroxyl groups tends to cause a molecule to be eluted earlier from a polystyrene resin column than a molecule of higher molecular weight but having a fewer number of hydroxyl groups.

In the present work, using aqueous systems, hydroxyl groups were found to affect the elution of a molecule from the resin. Glucose was eluted from columns of Amberlite XAD-2 without noticeable retardation. Also, uric acid, containing three hydroxyl groups, was eluted ahead of hypoxanthine, containing one hydroxyl group. Uric acid was not resolved from hypoxanthine on a 1.5 × 84 cm column but quite well resolved on a 2.9 × 34.5 cm column.

The role of pH was found to be very important in the chromatography of ionizable compounds on polystyrene resin in aqueous media. Not only was the pH of the eluant important in determining the elution volumes of compounds, but also the pH of the sample solution applied to the column. When creatinine in 0.05 *N* HCl was applied to a polystyrene column and eluted with water it emerged from the column as a sharp peak at 153 ml. Similarly, on chromatography of creatinine dissolved in 0.001 *N* HCl and eluted with the same solvent, a sharp peak at 170 ml was obtained. However, when a solution of creatinine in water was applied to the column and eluted with water, the creatinine emerged as a somewhat broad peak at 294 ml. In acidic solution creatinine (p*K* 4.8) exists as a cation and is not significantly adsorbed by the resin. In neutral solution creatinine is in the form of uncharged free base and is strongly adsorbed by the resin since it is potentially an aromatic system (imidazole).

KWON[28] has reported similar results in the case of malonaldehyde chromatographed on Sephadex G-10 where the elution volume was dependent on the pH of the eluant. In acidic solvents a chelate structure was considered to be present for malonaldehyde, which, owing to its pseudo-aromatic nature, was more strongly adsorbed to the column material than the linear molecule in neutral or basic solutions.

From the consideration of their p*K* values[29] it is evident that inosine, guanosine, and adenosine are still unionized in 0.001 *N* HCl and are strongly adsorbed on Amberlite XAD-2. The elution volumes of these compounds are quite large and the peaks very broad. Apparently purines in the free base form tend to be strongly adsorbed on polystyrene resin.

According to SWEETMAN AND NYHAN[26] protonation decreases adsorption. The elution volume of adenine (p*K* 4.2) from Sephadex G-10 was decreased when the

TABLE IV

ELUTION VOLUMES (ml) OF STANDARD COMPOUNDS FROM AN AMBERLITE XAD-2 2.9 × 34.5 cm COLUMN

Compound	*Eluant/sample medium*				
	Water/ water	*Water/ acid*	*Water/ base*[a]	*0.001 N HCl/ 0.001 N HCl*	*0.1 N HCl/ 0.1 N HCl*
Glucose	135	145[b]			
Glycine		137[c]	136	140	
Histidine	142	140[c]			
Leucine	211				
5′-Inosinic acid	137	169[b]		191	
Hypoxanthine		248[b]	243	256	
Tyrosine		248[b]			
Creatinine	294	153[c]		170	
Uric acid			127[d]		
5′-Adenylic acid				192	
Adenine				204	
Cytosine				148	
Cytidine				181	
Guanine				199	
Uridine				316	
Inosine	579	580[c]		568	453
Guanosine				657	351
Adenosine				735	327

[a] Sample in NaOH solution, pH 10.
[b] Sample solution in 0.1 *N* HCl.
[c] Sample solution in 0.05 *N* HCl.
[d] The column was washed with water after chromatography of a sodium hydroxide-containing sample. An initial run of uric acid following chromatography of an acid-containing sample yielded an elution volume of 173 ml.

eluant was changed from pH 7 to pH 5, while no effect was observed on the elution volumes of hypoxanthine, xanthine, and guanine (p*K* 2.0, 0.8 and 3.3, respectively). This reasoning can be applied to the present experiment. When inosine, guanosine, and adenosine were eluted from Amberlite XAD-2 with 0.1 *N* HCl, markedly smaller elution volumes were obtained together with sharpening of the peaks as compared to elution with water or 0.001 *N* HCl using the same column (Table IV).

Inosinic acid also exhibited differences in elution volumes depending on whether the sample was dissolved in water or in dilute acid. For inosinic acid dissolved in water the elution volume (Table IV) was 137 ml, while for inosinic acid dissolved in 0.1 *N* HCl the elution volume was 169 ml. A larger elution volume (191 ml) was observed for inosinic acid when elution was carried out with 0.001 *N* HCl. In acid solution the unionized form of the molecule may predominate over the charged species which are not adsorbed by the resin.

In the case of uracil and uridine the elution volumes were unchanged for samples of pH 1 or pH 8. Elution volumes of glycine and hypoxanthine were also independent of sample pH as well as of the eluting medium.

Anomalous behavior of uric acid during chromatography on Amberlite XAD-2 was noted. Sodium hydroxide was used to dissolve samples containing uric acid. In

the first chromatographic run uric acid was eluted with water at 173 ml, after glycine (139 ml). In a subsequent run performed directly after the initial one uric acid was eluted from the column at 125 ml, ahead of glycine, the elution volume of which was unaffected (140 ml). It was also noted that the effluent was not basic as expected. When a column of Amberlite XAD-2 was treated with 0.5 bed volumes of 1 *N* sodium hydroxide and subsequently washed with water, the residual sodium hydroxide was difficult to remove. No satisfactory explanation for these observations can be offered at this time, although it appears that sodium hydroxide is adsorbed by the polystyrene resin. It has been reported[24] that hydroxyl ions are adsorbed to Sephadex.

As had been noted previously in the case of gel filtration[30], the peaks that elute from the column early are quite sharp, but those that elute late become progressively more diffuse due to strong interaction with the resin phase. A similar trend was noted with Amberlite XAD-2. For example, a compound not retained on the resin—glucose (6.64 mg)—could be eluted in a 70-ml-wide band, while a compound showing strong adsorption—inosine (2.27 mg)—was eluted in a 270-ml-wide band from a 1.5 × 43 cm column of XAD-2 with 0.001 *N* HCl or water as eluant.

The results indicate that useful separations of water-soluble organic compounds may be achieved on polystyrene resin by taking advantage of adsorption effects. Since chromatography may be carried out with water or very dilute electrolyte solutions, the use of polystyrene resin may be more advantageous than other resins where solutions of high ionic strength are necessary to elute compounds from the column.

Amberlite XAD-2 may be conveniently employed for fractionation of nucleic acid derivatives, particularly for resolution of base–nucleoside pairs, and for separation of purine nucleosides from pyrimidine nucleosides. This resin may also be useful for desalting of water-soluble aromatic compounds.[18] Further experiments with aqueous eluants of various pH values will undoubtedly result in other useful separations on columns of Amberlite XAD-2.

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