ADSORPTION CHROMATOGRAPHY OF POLAR ORGANIC MOLECULES ON BIOGEL

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SUMMARY

The adsorption properties of phenols, amides, ureas and carboxylic acids on a tightly cross-linked polyamide have been studied. A very good numerical correlation between adsorption and molecular structure of the phenols can be made. Adsorption of amides can be correlated with $-NH_2$ content and unsaturation. Comparisons of these results with results on other gels have been made.

INTRODUCTION

Several recent articles have detailed the adsorption behavior of aromatic compounds on polydextran gels. These have included aqueous[1-3] and non-aqueous[4] studies. Polyacrylamide gels are also known to have adsorptive properties for organic[5] and inorganic materials[6,7].

The present work was undertaken to determine if any rationale exists for the adsorption of organic materials on cross-linked polyacrylamides and to see if adsorption phenomena can be related to molecular structure as it has been on other materials[8,9].

MATERIALS AND METHODS

The chromatographic column was a 0.5 cm I.D., 97-cm glass tube equipped with a stopcock at its outlet. Teflon and metal fittings connected the column to Teflon or Tygon tubing through which the solvent ($M/100$ aqueous sodium chloride) was pumped. A rotary pump (Sigmamotors, Inc.) provided a flow rate of about 1 ml/min. The detection system was a Waters R-4 Differential Refractometer thermostated at 34° and connected to a Sargent Model SRL strip chart recorder.

The polyamide gels used were BioGel P-2 (100–200 mesh) and BioGel P-6 (100–200 mesh). The nominal exclusion limits of these gels are 200–2600 and 1000–5000, respectively. If sieving were the only process taking place, all solutes tested should have had K values of 1.00.

All solutes used were reagent-grade chemicals except the cresols, which were

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practical grade. 0.1 M solutions were used whenever solubility permitted. Otherwise saturated solutions were used.

Samples (50 μl) were injected from a syringe through a septum directly onto the column. At least three elution values were obtained on each compound.

The column was calibrated using a 0.5 % aqueous solution of Blue Dextran to determine the interstitial volume, and acetone and tetrahydrofuran to determine void plus pore volume. These latter two compounds gave identical and reproducible (1 % variation) values. The $V_0 + V_t$ value, as measured by them, was slightly smaller than that for methanol and slightly larger than the same value for dimethyl sulfoxide. Acetone and tetrahydrofuran are regarded as molecules least likely to interact with the gels either through hydrogen, π, or base bonding. They therefore give the best measure of $V_0 + V_t$.

A dilute sodium chloride solution was used as the eluant rather than distilled water as it was noted early in the work that unreproducible elution volumes were obtained for tartaric, citric, and succinic acids if repeated injections of these materials were made using water alone as the eluant. This situation was corrected by the use of $M/100$ NaCl. A similar situation has been noted on Sephadex[10] and has been ascribed to Donnan membrane equilibrium.

RESULTS

K_D values for all compounds run are listed in Tables I and II. These were computed from the standard equation $K_D = (V_e - V_0)/V_t$. The pooled standard deviation for all results is $\pm$ 0.04.

TABLE I

K_D VALUES FOR BENZENE DERIVATIVES ON CROSS-LINKED GELS

Compound	P-2			P-6	G-25[1]
	Exptl.	Calc.	Δ	Exptl.	Exptl.
Benzene	1.69	—			
Phenol	2.20	2.24	0.04	1.43	2.0
Benzoic acid	1.04	0.95	−0.09		
2-Hydroxyphenol	2.81	2.79	−0.02		1.9
3-Hydroxyphenol	2.90	2.79	−0.11		2.65
4-Hydroxyphenol	2.58	2.79	0.21	1.60	2.4
2,3-Dihydroxyphenol	3.41	3.34	−0.07		2.4
3,5-Dihydroxyphenol	3.50	3.34	−0.16		3.15
2-Methylphenol	2.42	2.39	−0.03		2.55
3-Methylphenol	2.44	2.39	−0.05		
2,4-Dimethylphenol	2.48	2.54	0.06		
2,6-Dimethylphenol	2.44	2.54	0.10		
2-Carboxyphenol	1 42	1 50	0 08		2.1
3-Carboxyphenol	1.40	1.50	0.10		1.8
4-Carboxyphenol	1.45	1.50	0.05		1.45
2-Chlorophenol	3.04	3.26	0.22		3.4
3-Chlorophenol	3.40	3.26	−0.14		
4-Chlorophenol	3.18	3.26	0.08	1.77	
2,4-Dichlorophenol	4.48	4.28	−0.20		
2,4,6-Trichlorophenol	4.98	5.30	0.32		
2-Nitrophenol	2.94	3.78	0.84		2.45
3-Nitrophenol	3.74	3.78	0.04		
4-Nitrophenol	3.75	3.78	0.03	1.94	
2,4-Dinitrophenol	2.37	5.32	2.95	1.76	

TABLE II

K_D VALUES FOR WATER-SOLUBLE ORGANIC COMPOUNDS ON POLYAMIDES

Compound	P-2	P-6
Urea	1.37	1.17
Biuret	1.88	1.29
Acetamide	1.06	
N-Methylacetamide	0.94	
N,N-Dimethylacetamide	0.86	0.89
Acrylamide	1.17	
N-tert.-Butylacrylamide	0.97	
N-Vinyl-2-pyrrolidone	1.23	
Hydrochloric acid	1.29	
Trichloroacetic acid	1.17	
Chloroacetic acid	0.96	
Acetic acid	1.08	
Lactic acid	0.79	0.98
Acrylic acid	0.97	
Crotonic acid	1.28	1.00
Oxalic acid	1.04	
Succinic acid	1.22	
Malic acid	1.00	1.03
Tartaric acid	1.08	
Maleic acid	1.26	1.11
Fumaric acid	0.93	0.96
Citric acid	1.19	1.04
Glycine	0.93	
4-Aminobenzoic acid	2.75	
Pyrrole	1.61	
N-Methylpyrrole	1.56	
Pyridine	1.52	
Methanol	1.08	
Dimethyl sulfoxide	0.91	

There is no correlation between K values and molecular weight, pK_a values, or (in the case of the phenols) Hammett constants. All aromatics showed strong adsorption and some of the amides and acids showed some adsorption. Further considerations for each group of compounds is given in the following separate sections.

Phenols

The phenols, of all the compounds chromatographed, show the most pronounced adsorption and an inspection of the data implies this can be correlated with molecular structure. Benzene itself gives a value of 1.69 on P-2 indicating a strong interaction with the gel presumably through the π electrons of the ring. Further substitution of the ring increased adsorption (except for benzoic acid) and was dependent on type and extent of the substitution. Thus phenol has a K greater than that of benzene and all three dihydroxybenzenes have values greater than that of phenol; all these latter values are similar. Trihydroxybenzenes have K values in excess of those for the dihydroxy compounds. If a mixture of all these compounds were chromatographed on P-2 the order of emergence from the column would be benzene, hydroxybenzene, dihydroxybenzenes, trihydroxybenzenes.

The addition of hydroxy groups appears to add a standard increment to the

K value. If the K for benzene is substracted from the K for the hydroxybenzenes and then the values divided by the number of hydroxy groups in the compound an average incremental value of 0.55 ± 0.06 per hydroxygroup is found. A similar type of calculation can be made for all the other phenols tested and incremental values per group found are: $CH_3 + 0.15 \pm 0.05$; $Cl + 1.02 \pm 0.15$; $NO_2 + 1.54 \pm 0.01$; $CO_2H -0.74 \pm 0.07$. The K value for any compounds listed can then be calculated from the equation

$$K_{cpd} = K_{benzene} + \Sigma n \Delta s$$

where Δs are the substituent values listed above. This has been done and calculated values and errors are listed in columns 2 and 3 of Table I.

This type of correlation has been noted by SNYDER[8,9] on alumina and silica gel columns using non-aqueous solvents as eluants. The order he noted for aromatic compounds for increasing adsorption on alumina was $CH_3 < Cl < NO_2 < OH < CO_2H$ and on silica gel $Cl < CH_3 < NO_2 < OH < CO_2H$. The order noted here on P-2 is $CO_2H < CH_3 < OH < Cl < NO_2$. Analyses of OELERT's data[4] obtained for a limited number of phenolic compounds with LH-20 and isopropanol yields the order $CH_3 < CO_2H = OH < NO_2$.

The most glaring differences between experimental and calculated values for the experimental system occur for 2-nitro-, 2,4-dinitro-, and 2,4,6-trichlorophenols. The first compound is known to have interactions between the hydroxyl and the nitro group, hence the substituents cannot interact with the gel independently; 2,4-dinitrophenol gives a very low value and the molecule may be so large it cannot completely diffuse into the gel. The same may also be true for the trichloro compound.

If these three compounds are ignored, agreement between experimental and calculated values is ± 0.09 units. Presumably then the elution value for any phenol, providing intramolecular hydrogen bonding or large size does not interfere, can be predicted by using the K of benzene and the incremental values of the substituents. Incremental values could also be determined for $-Br$, $-I$, $-CN$, $-NR_2$, $-OR$ and other common substituents and these values used to predict K_s for untested compounds.

Amides and ureas

A number of amides and ureas were chromatographed and several generalities on behavior can be made. Adsorption, as in the case of the aromatics, is enhanced by double bonds as shown by acrylamide and the pyrrolidone, by the presence of multiple $-NH_2$ groups as in urea and biuret, and is reduced by the presence of alkyl groups. The N-substituted acetamides show a regular decrease in K values with increasing methylation and do in fact appear to be sieving though they are outside the sieve limits of the gel. Negation of adsorption is also shown by N-tert.-butylacrylamide in relation to acrylamide. Urea and biuret are readily separated in this system.

Although no good assignment of an incremental value can be made because of the scarcity of data it would appear from the urea, biuret and acetamide data that an incremental value for $-NH_2$ is about 0.3–0.4.

Acids

In general the acids showed no pronounced adsorption on BioGel P-2 but differences in behavior do provide some interesting results.

Maleic and fumaric acids are separable because of the differences in K values. This separation is improved if 0.1 M rather than 0.01 M NaCl is used. The K values in this case are fumaric 0.88, maleic 1.34.

Fumaric acid appears to be partially excluded from the gel by its rigid molecular shape while maleic acid, which can conjugate three double bonds and assume a 7-membered ring through intramolecular hydrogen bonding, is strongly adsorbed.

Succinic, citric, and crotonic acids are also strongly adsorbed, but malic, oxalic and acrylic acids are not. At present there does not seem to be the kind of incremental molecular group behavior observed in the phenols.

Increasing carboxyl content does appear to enhance adsorption while an increase in OH content negates this effect (malic, tartaric and lactic acid). Succinic and citric acids are capable of preferred orientations through 7-membered hydrogen-bonded rings which probably increases adsorption. The ring acid 4-aminobenzoic acid is very strongly adsorbed, as might have been expected from phenol behavior.

HARLOW AND MORMAN[11] have chromatographed a number of these acids on Dowex 50W X 12 but no correlation of the behavior of the compounds on P-2 and the ion-exchange resin is apparent. It is interesting to note that on both substrates crotonic acid was more adsorbed than acrylic acid and lactic acid less strongly than acetic acid.

Adsorption of molecules by other gels

A number of compounds were also chromatographed on the polyamide P-6. K values are listed in Tables I and II. A plot of K values on P-2 vs. these values on P-6 indicates a linear relation. A least squares solution for the data yields the equation

$$K \text{ (P-6)} = 0.37K \text{ (P-2)} + 0.63.$$

The pooled standard deviation for fourteen results is $\pm$ 0.08. The largest difference ($+$ 0.25) is found for 2,4-dinitrophenol, implying this compound can more readily diffuse through P-6 than it can through P-2. The data indicate that the degree of adsorption of the molecules is a function of the degree of cross-linking of the gel. The more highly cross-linked gel adsorbs more strongly.

WOOF AND PIERCE'S[1] results on G-25 are also listed in the last column of Table I. A plot of K values from P-2 data vs. those from G-25 data does not immediately suggest a correlation. However, many of the compounds tested are *ortho* substituted and these seem to be the ones that give the largest scatter from a linear result. If more *meta* and *para* substituted aromatics were run it might be possible to show a better correlation of adsorption values on the two gels. A least squares solution of the present data gives the equation

$$K \text{ (P-2)} = 0.56 \ K \text{ (G-25)} + 0.93$$

but the pooled standard deviation is $\pm$ 0.34 (twelve results). If *ortho* substituted compounds are discounted the slope of the line would be closer to 1.

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