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THE EFFECT OF SOLVENT CHANGE ON THE SEPARATION PROCESSES OF SEPHADEX LH-20 MODIFIED DEXTRAN

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SUMMARY

Small polar organic molecules are separated from one another on Sephadex LH-20 Modified Dextran either by sieving or by adsorption through π or hydrogen bonds. The dominant mechanism is determined by the solvent employed. Thus dimethylformamide favors sieving, tetrahydrofuran hydrogen bonding, and methanol and acetonitrile π and hydrogen bonding in varying portions.

INTRODUCTION

The adsorption values of planar aromatic hydrocarbons on Sephadex LH-20 Modified Dextran have been shown to be linear functions of the resonance energies of these compounds when methanol is used as the eluant. However, heteroaromatic, and polar substituted compounds such as phenols and anilines, are more strongly adsorbed than predictable from resonance energies, indicating that these compounds utilize hydrogen as well as π bonding in the adsorption process. Calculation indicates that the majority of the adsorption energy is utilized in π bonding¹.

Since methanol and isopropanol² have been the only solvents studied for these generalizations it appeared worthwhile to determine if the phenomena were general or restricted to alcoholic solvents. Accordingly an investigation of several other solvents was made.

EXPERIMENTAL

The apparatus used in these experiments was identical to that previously described¹ except that a Water's R-4 differential refractometer was used as the column monitor in most cases. V_0 was estimated from the elution value of Blue Dextran or polystyrene (mol.wt. 10^6); ($V_0 + V_t$) was equated to the elution volume of acetone. This may not be the best measurement of ($V_0 + V_t$) but it allows a standard base to be used in all calculations. V_0 and ($V_0 + V_t$) did not vary appreciably over 30 day periods. K was calculated from the standard equations.

The gel was swelled in the appropriate solvent for 24 h prior to being packed

in the column. Samples were injected either neat (1-5 μ l) or as 25- μ l samples of a dilute solution. All determinations were made at least in duplicate. V_0 and $V_0 + V_t$ were determined daily.

RESULTS

K values for a variety of aliphatic and aromatic compounds utilizing four dif-

TABLE I

ELUTION VALUES FOR VARIOUS ORGANIC MOLECULES USING SEPHADEX LH-20 AND VARIOUS SOLVENTS

Compound	MeOH	DMF	THF	CH ₃ CN
Hexane	1.00	0.97	0.62	1.19
Acetone	1.00	1.00	1.00	1.00
Methanol	—	1.05	1.80	2.14
Dimethylformamide	1.13	—	1.73	1.45
Tetrahydrofuran	1.22	1.08	—	1.36
Acetonitrile	1.23	0.98	1.15	—
Benzene	1.33	1.00	0.89	1.27
Naphthalene	1.71	0.95	0.85	1.72
Anthracene	2.15	—	—	2.13
Phenanthrene	2.36	0.91	0.75	2.22
Chrysene	3.03	0.86	0.70	2.96
Biphenyl	1.62	0.99	0.71	1.45
Pyridine	1.21	1.15	1.32	2.10
Quinoline	1.63	1.09	1.21	2.54
Pyrazine	—	—	1.23	1.80
Pyrimidine	1.26	1.20	1.36	1.91
Pyrazole	1.28	0.99	2.11	4.13
Imidazole	1.55	1.05	5.97	8.81
Pyrrole	1.61	0.85	—	1.83
Indole	2.08	0.96	1.15	2.50
Carbazole	2.47	0.89	1.01	3.40
1,2,4-Triazole	1.36	1.03	3.34	6.89
Chlorobenzene	1.40	1.05	0.82	1.42
Nitrobenzene	1.50	1.02	0.87	1.27
Benzyl alcohol	1.39	1.02	1.18	2.32
Benzoic acid	1.50	1.03	1.39	2.03
Acetic acid	1.59	0.84	1.24	—
1-Carboxynaphthalene	1.72	1.08	—	—
Phthalic acid	1.36	0.88	1.31	4.35
Isophthalic acid	1.95	—	1.08	—
Phenol	1.65	0.88	1.32	3.34
3-Nitrophenol	1.80	0.85	1.52	5.80
2,3-Dihydroxyphenol	2.14	0.80	1.72	—
1-Naphthol	2.23	—	1.14	5.07
Aniline	1.63	0.90	1.19	1.77
N,N-Dimethylaniline	1.50	0.99	0.74	1.27
sec.-Butylamine	0.95	1.39	2.03	3.44
Morpholine	1.17	1.32	1.77	3.36
1-Naphthylamine	2.18	—	1.01	2.40

ferent solvents are listed in Table I. The solvents were chosen as representative of those often encountered in chromatography. They also show varying characteristics: methanol should be adsorbed to the gel by hydrogen bonds; dimethylformamide (DMF) has both hydrogen bond and π bond characteristics; tetrahydrofuran (THF) should be the least polar solvent and only adsorb to the gel by hydrogen bonds from the gel; acetonitrile has π character in the nitrile bond but is not a particularly good solvating agent.

An inspection of the data indicates that adsorption behavior varies considerably with the solvent. The dependency on sieving, π and/or hydrogen bonding is detailed in the separate discussions below.

DISCUSSION

Adsorption from methanol

The adsorption of the planar aromatic hydrocarbons and the heterocyclics have been previously discussed¹. However the three other solvents used, DMF, THF, and acetonitrile, are all adsorbed from methanol solutions. Hydrogen bonding would seem appropriate for the first two of these, but the gel-acetonitrile interaction is probably through a π bond. The K values, although not large, and thus indicating the relative weakness of the hydrogen bonding process, definitely show adsorption since precision of determinations is ± 0.07 .

Benzyl alcohol is adsorbed indicating a π interaction, the value (1.39) being only slightly greater than that for benzene (1.33) and less than that for phenol (1.65). Multiple OH groups on the benzene ring (2,3-dihydroxyphenol) increase the adsorption of the molecule through additional hydrogen bonds. Acetic acid with approximately the same pK_a as benzoic acid and a much larger constant than that for phenol is adsorbed, but not as strongly as either of the aromatic compounds. The distinctly different values for the isomeric phthalic acids show that separation of isomeric compounds is possible in this system (see also pyrazole and imidazole).

Among the amines the comparatively basic *sec.*-butylamine ($pK_a \sim 10.6$) is not adsorbed, but the weaker base morpholine ($pK_a \sim 8.52$) is, most probably through hydrogen bonding to the ethereal oxygen. All the much weaker aromatic amines are more strongly adsorbed than either of the stronger amines.

Adsorption from DMF

This solvent completely eliminates π interactions between solutes and gel, and only a few compounds show any adsorption, most probably through hydrogen bonds.

Values for the planar aromatic hydrocarbons indicate a slight sieving mechanism; heterocycles show only some weak hydrogen bonding for pyridine and pyrimidine. The pyrrole derivatives cannot compete with DMF for adsorption sites as they could with methanol; neither can acids or phenols.

The two relatively strong bases, *sec.*-butylamine and morpholine, are both adsorbed, more so than the aniline derivatives.

It would appear that sieving is the most predominant process for the gel when DMF is used as the solvent except for hydrogen bonding from the gel to aliphatic amines. One might expect a group separation of aliphatic amines from aromatic amines and aromatic heterocyclics using this system.

Adsorption from THF

This solvent, like DMF, eliminates π bonding (planar hydrocarbon data) but emphasizes hydrogen bonding effects for acids, amines, heterocyclics and phenols.

Methanol and DMF are strongly adsorbed from THF solution by the gel ($K = 1.80$ and 1.73) but acetonitrile is only slightly adsorbed ($K = 1.17$). The result for hexane is difficult to explain unless we assume that acetone is adsorbed from this solvent and consequently all K values are too low by a factor of 1.61 . This seems unlikely in terms of the hydrocarbon data which indicates sieving as was the case with DMF.

All the heterocyclics show some adsorption but it is indicative of hydrogen rather than π bonding. The difference in K values for imidazole and pyrazole is spectacular (3.86 units) showing a very easy separation for these two compounds. The triazole is also very strongly adsorbed.

Benzyl alcohol (1.18) is adsorbed but the chloro- and nitrobenzene are not again indicating the preference for hydrogen bonding.

The principle point of interest for the phenols, aniline, and benzoic acid is that all these compounds are more strongly bound than the corresponding naphthyl compounds. This would appear to be due to the inability of the naphthalene nucleus to penetrate the gel as readily as the smaller benzene ring. This is also shown by indole and carbazole.

The two aliphatic amines are more strongly adsorbed than aniline or the nitrogen heterocycles such as quinoline or pyridine. Primary amines are more strongly adsorbed than secondary or tertiary derivatives.

Adsorption from acetonitrile

This solvent favors π bonding as did methanol and isopropanol. The least squares fit for K vs. resonance energy for the five planar aromatic hydrocarbons tested is:

$$K = 0.0207 (\text{Res. Energy}) + 0.45$$

$s = \pm 0.09$ which is of the same order of magnitude as reproducibility of experimental data.

The equation has nearly the same slope as that for methanol data (0.0207 vs. 0.0199) and the intercepts are similar (0.45 vs. 0.54). Thus neither solvent holds an advantage over the other for separation of the hydrocarbons.

A difference between the solvents is seen, however, when compounds which can hydrogen bond are considered. Hydrogen bonding is of relatively greater importance in acetonitrile solutions than in methanol solutions. Deviations of calculated K values for phenols, anilines, and heterocyclics from experimental K value are 2-5 times as great for acetonitrile data as for methanol data. Since all calculated values are too low the most reasonable explanation seems to be a strong tendency to hydrogen bonds. This is also shown by *sec.*-butylamine, morpholine and acetic acid which all have quite high values.

Again in this solvent as in THF a very large difference is found between the K values for imidazole and pyrazole. Triazole is also very strongly bonded. The behavior is contrasted to that of pyrazine and pyrimidine which are more weakly bonded than pyridine itself.

Methanol, DMF and THF are all adsorbed from this solvent, the greatest adsorption being for methanol. Benzyl alcohol also shows a high value and acetic acid a very high value. Phthalic and isophthalic acid were too insoluble to be determined.

CONCLUSIONS

K values for a variety of aromatic, heterocyclic and aliphatic compounds on Sephadex LH-20 Modified Dextran have been determined for four solvents: methanol, dimethylformamide, tetrahydrofuran, and acetonitrile.

The gel appears to be able to perform separations by at least three mechanisms: sieving, π bonding, and hydrogen bonding. In each solvent a different mechanism prevails.

In DMF sieving is the principle mechanism with a small contribution from hydrogen bonding in a few cases; π bonding is insignificant.

In methanol π bonding is predominant with some resonating species also able to form hydrogen bonds. Sieving does not occur for the molecules studied.

In THF there is some sieving but hydrogen bonding is the principle adsorption mechanism; π bonding is insignificant.

In acetonitrile hydrogen bonding is the principle mechanism but π bonding also occurs. It is less important than in methanol, however.

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