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ADSORPTION BEHAVIOUR OF AROMATIC NITRO COMPOUNDS ON SEPHADEX LH-20

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SUMMARY

By classifying the mechanism of adsorption of solutes to gels into two groups, *viz.*, ionic effects (ion exchange, ion exclusion and ion adsorption) and pure adsorption (hydrogen bonding and π -bonding), and by considering the gel structure as hydrochloric acid, potassium hydroxide, or potassium chloride in methanol are adsorbed on to the gels and the surface of the gel becomes positively charged, the adsorption behaviour of dinitrophenyl derivatives on Sephadex LH-20 in methanol, 0.05 *N* hydrochloric acid–methanol, 0.05 *N* potassium hydroxide–methanol and 0.05 *N* potassium chloride–methanol have been explained. The solutes that have no dissociative groups interact with the gel only by pure adsorption. The solutes that have an acidic group interact with the gel by ion adsorption under the dissociation conditions for the solutes, and those that have a basic group by ion exclusion under both the dissociation and non-dissociation conditions. These behaviours are compared with those in methanol.

Benzene or pyridine added to methanol are assumed to block the interaction sites of gels and hence lower K_{av} values for the solutes are obtained. The presence of two nitro groups and hydroxyl, carboxyl or amino groups on a benzene ring shows the potentiation effect of adsorption to the gel. The dissociative groups favour the ionic effects, which are greater than the sieving effect. A nitro group is assumed to be adsorbed to the gel by hydrogen bonding and this secondary effect is greater than the molecular sieving effect for low-molecular-weight compounds.

INTRODUCTION

The retardation of dinitrophenylated linear monomers and oligomers of polyamides on Sephadex LH-20 was studied under various conditions¹. The values of K_{av} for some solutes were greater than unity and the addition of an inorganic electrolyte to the sample solution caused extreme retardation. In general, when a solute departs from the elution volume predicted on the basis of molecular sieving, *viz.*,

by appearing either earlier or later than expected, some other types of interactions between the solute and gel matrix must occur.

Although the molecular sieving properties of gel materials used in gel chromatography usually predominate, other types of interactions between solutes and gel materials have also been observed when Sephadex was used as the bed material. Gelotte² described some adsorption properties of Sephadex G-25 towards low-molecular-weight substances such as amino acids and alkaloids, and divided these secondary effects into two groups, namely adsorption to the bed materials and superimposed effects. Adsorption is related to the structure of the solutes and superimposed effects depend upon the conditions of the run, *i.e.*, the ionic strength, composition in and pH of the eluent. Kwon³ studied the pH-dependent elution of malonaldehyde on Sephadex G-10 and observed adsorption to the gel matrix below pH 4. Janson⁴ discussed the adsorption behaviour of some substances on Sephadex in different solvents and found that highly charged substances tend to be excluded at an opposite pH range and that the retardation of aromatic amino acids is markedly decreased on the addition of 1 *M* pyridine. Studies of adsorption phenomena on Sephadex have also been made by other workers⁵⁻⁸.

Of the adsorption phenomena, the affinity of dextran gel for aromatic and pseudo-aromatic substances is particularly striking in accordance with the affinity characteristics found for adsorption to cellulose. A planar structure and an extended system of conjugated bonds in the solute favour adsorption⁴. The adsorption of aromatic compounds to dextran gel is presumed to result from the π -electron bonding between them. Janson⁴ stated that in distilled water, the K_d values for acidic aromatic substances are a compromise between the two opposing effects, aromatic adsorption and ion exclusion, and that adsorption increases with increasing salt concentration, although the ion-exclusion effect, which depends on small amounts of fixed carboxyl groups, is eliminated by the addition of small amounts of an electrolyte to the distilled water. He also considered that the increase in aromatic adsorption is caused either by an increase in the number of adsorption sites available or by an increase in the strength of the interaction due to a decrease in the size of the layer of water of hydration which prevents solute-gel interaction.

The interaction of aromatic molecules with Sephadex LH-20 in organic solvents has also been studied⁹. Streuli¹⁰ found that the adsorption values of planar aromatic hydrocarbons on Sephadex LH-20 were linear functions of the resonance energies of these compounds when methanol was used as the eluent. He concluded that the interaction between these aromatic molecules and the gel involved the π -electron cloud, and that Lewis acid-base complexes were formed. Heteroaromatic and polar substituted compounds, such as phenols and anilines, are more strongly adsorbed than predicted from resonance energies, indicating that hydrogen bonding as well as π -bonding participates in the adsorption.

The role of the solvent employed in the solute-gel interaction has also been discussed¹¹. In methanol, π -bonding predominates, but some resonating species are also able to form hydrogen bonds. Dimethylformamide favours sieving, tetrahydrofuran favours hydrogen bonding, and π -bonding is insignificant in both solvents.

The adsorption behaviour of dinitrophenyl derivatives on Sephadex LH-20 in several methanolic eluents has been investigated in this work and the adsorption mechanisms are discussed.

EXPERIMENTAL

The gel used was Sephadex LH-20, which was swollen in the solvent used as the eluent for 24 h prior to being packed in a glass column of 100 cm \times 16 mm I.D. The dry gel weight was 45–46 g and the height of gel bed was 91–93 cm. The eluents used were methanol, 0.05 *N* hydrochloric acid–methanol, 0.05 *N* potassium hydroxide–methanol, 0.05 *N* potassium chloride–methanol, 10% benzene–0.05 *N* hydrochloric acid–methanol, 1% benzene–0.05 *N* hydrochloric acid–methanol, 0.5% dinitrobenzene–methanol and 1 *M* pyridine–methanol. Some aromatic nitro compounds were purchased, and others were prepared in this laboratory. All sample solutions were between 0.05 and 0.1% in concentration and 1-ml portions were applied directly to the top of the gel bed.

The effluent was delivered to a flow cell adapted to a Hitachi Model 124 spectrophotometer equipped with an absorbance recorder. The absorbance of the effluent was recorded continuously. The wavelength at which the peak maximum of a solute was 0.5–0.7 in absorbance (*D*) was selected for each sample. V_0 was determined using Blue Dextran modified as follows: 20 mg of Blue Dextran were dissolved in 1 ml of 10% sodium hydroxide solution and stored overnight. 0.3 ml of dimethyl sulphate was then added, the solution was stirred for 5 h, the sulphuric acid liberated was neutralised with sodium hydroxide solution as soon as possible and the solution was diluted to 10 ml with methanol. V_i was calculated from the values of V_0 , the weight of gel and total gel bed volume (V_t). Other experimental conditions and procedures were as described earlier¹.

RESULTS

The partition coefficients, K_d , of several aromatic nitro compounds and other

TABLE I

K_d VALUES FOR VARIOUS SOLUTES ON SEPHADEX LH-20 IN METHANOL AND METHANOLIC SOLUTIONS OF HYDROCHLORIC ACID, POTASSIUM HYDROXIDE AND POTASSIUM CHLORIDE

Solute	K_d			
	Methanol	0.05 <i>N</i> HCl– methanol	0.05 <i>N</i> KOH– methanol	0.05 <i>N</i> KCl– methanol
Benzene	1.09	1.13	1.10	1.14
Nitrobenzene	1.29	1.32	1.26	1.32
<i>m</i> -Dinitrobenzene	1.36	1.37	1.36	1.36
2,4-Dinitrotoluene	1.33	—	—	—
2,4-Dinitrophenetole	1.36	1.35	1.47	1.38
2,4-Dinitrophenol	1.77	1.59	6.17	5.25
2,4-Dinitrobenzoic acid	1.77	1.57	3.22	2.68
2,4-Dinitroaniline	2.05	2.05	2.05	2.01
2,4-Dinitrophenylaminocaproic acid	1.39	1.41	2.54	2.13
Benzoylaminocaproic acid	0.88	0.86	1.63	1.31
ϵ -Aminocaproic acid	1.11	0.62	1.22	0.65
Benzoic acid	1.25	1.19	2.15	1.84
<i>m</i> -Phenylenediamine	1.60	0.79	1.43	1.46

related aromatic compounds in several eluents are listed in Tables I and II. The K_d values were calculated using equation

$$K_d = (V_e - V_0)/V_i$$

where V_e is the elution volume of the solute, V_0 is the void volume of the gel column, and V_i is the inner volume of the column. In order to discuss the effect of a nitro group and other substituents attached to a benzene ring on the adsorption to the gel some compounds that do not have a nitro substituent were also examined.

TABLE II

K_d VALUES FOR VARIOUS SOLUTES ON SEPHADEX LH-20 IN METHANOLIC SOLUTIONS OF BENZENE, DINITROBENZENE AND PYRIDINE

Solute	K_d			
	0.05 N HCl- 1% benzene- methanol	0.5% Dini- trobenzene- methanol	0.05 HCl- 10% benzene- methanol	1 M pyri- dine-Me- thanol
2,4-Dinitrophenylaminocaproic acid	1.40	1.40	1.16	1.18
2,4-Dinitrophenol	—	1.59	1.48	1.16
2,4-Dinitroaniline	—	1.97	—	1.72
2,4-Dinitrophenetole	—	—	—	1.25
Benzoylaminocaproic acid	0.85	—	—	—

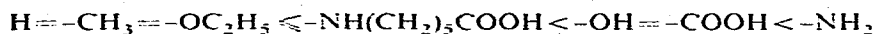
DISCUSSION

In spite of the similar molecular weights of the dinitrophenyl derivatives examined, large differences in K_d values were observed. Besides the substituents on the dinitrophenyl ring, the properties of the eluents also influenced the K_d values.

Elution behaviour of solutes in methanol

The K_d values for all of the solutes examined except benzoylaminocaproic acid are greater than unity, which indicates the participation of adsorptive interactions between the solutes and the gel. The K_d value for benzene is 1.09 and adsorption by π -bonding between the gel and the π -electrons of benzene is considered to occur¹². Upon substituting a nitro group for a hydrogen atom in benzene (nitrobenzene), the K_d value increases to 1.29, and the introduction of a second nitro group (*m*-dinitrobenzene) increases K_d to 1.36. The high electronegativity of the nitro group favours hydrogen bonding between its oxygen atoms and the hydrogen atoms of the gel, and consequently nitrobenzenes utilize hydrogen bonding as well as π -bonding in the adsorption process.

Depending on the type of substituent with which a hydrogen atom of *m*-dinitrobenzene is replaced, the K_d values for 2,4-dinitrophenyl derivatives increase in the following order:



Brook and Munday¹³ found that the adsorption by Sephadex dextran gels of *m*- or *p*-monosubstituted phenols, anilines and benzoic acids can be correlated by the Hammett equation. They also suggested that the benzene derivatives might be adsorbed to the hydroxyether cross-linking groups by hydrogen bonding. In the case of the *m*-dinitrobenzene derivatives, the Hammett equation cannot be correlated with the adsorption behaviour, because the *ortho* effect between the substituent and one nitro group at the *ortho* position of the substituent should be considered.

The K_d values for 2,4-dinitrotoluene and 2,4-dinitrophenetole are similar to that of *m*-dinitrobenzene, as the methyl and ethoxy groups have low electronegativities and hydrogen bonding to the gel is negligible. Carboxyl and hydroxyl groups are more electroattractive and have higher electronegativities. The higher K_d values for 2,4-dinitrobenzoic acid and 2,4-dinitrophenol are due to this higher electronegativity and the partial dissociation of these groups in methanol. The influence of these groups on the K_d values of derivatives is shown in Table III. The increases in K_d caused by the substitution of two nitro groups for hydrogen atoms in the benzene rings of benzoic acid and benzoylaminocaproic acid are identical. This increase is larger than that observed on the substitution of two nitro groups into benzene. The increase in K_d values upon the substitution of a carboxylic acid and two nitro groups into benzene is greater than the sum of the increases observed upon separate substitution of a carboxylic group and two nitro groups into benzene. Both a nitro group and a carboxyl group are electroattractive, and when both two nitro groups and a carboxyl group are introduced the hydrogen bonding is increased due to the potentiation effect.

TABLE III

CHANGES IN K_d VALUES WITH DIFFERENT SUBSTITUENTS USING METHANOL AS ELUENT

Derivative	Parent compound	Substituent	Increase in K_d over that of parent compound
Benzoic acid	Benzene	-COOH	0.16
Nitrobenzene	Benzene	-NO ₂	0.20
<i>m</i> -Dinitrobenzene	Benzene	-NO ₂ (× 2)	0.27
2,4-Dinitrobenzoic acid	Benzene	-COOH, -NO ₂ (× 2)	0.68
2,4-Dinitrobenzoic acid	<i>m</i> -Dinitrobenzene	-COOH	0.41
2,4-Dinitrobenzoic acid	Benzoic acid	-NO ₂ (× 2)	0.52
2,4-Dinitrobenzoylaminocaproic acid	Benzoylaminocaproic acid	-NO ₂ (× 2)	0.51
2,4-Dinitrophenol	<i>m</i> -Dinitrobenzene	-OH	0.41

In methanol, the K_d value for benzoylaminocaproic acid is lower than that of ϵ -aminocaproic acid, suggesting that this compound utilizes the molecular sieving effect more than the adsorption process with π -electrons of the benzene ring and the gel. When two nitro groups are substituted for two hydrogen atoms in the benzene ring of benzoylaminocaproic acid, the K_d value increases considerably owing to the

hydrogen bonding of the nitro groups with the gel. The increase in the K_d value in this instance is identical with that for 2,4-dinitrobenzoic acid.

The influence of amino groups on the adsorption process (*m*-phenylenediamine) is greater than that for nitro groups (*m*-dinitrobenzene).

Elution behaviour of solutes in methanolic solutions of hydrochloric acid, potassium hydroxide or potassium chloride

The changes in K_d values observed upon changing the eluent from methanol alone to 0.05 *N* methanolic solutions of hydrochloric acid, potassium hydroxide and potassium chloride are shown in Table IV. The effects can be classified in six categories:

(a) K_d values for solutes that have no dissociable groups are unchanged (benzene, nitrobenzene and dinitrobenzene).

(b) K_d values for compounds that have an acidic group decrease in the acidic eluent and increase in the alkaline and the neutral eluents (dinitrophenol, dinitrobenzoic acid and benzoic acid).

(c) With dinitrophenylaminocaproic acid and benzoylaminocaproic acid, which have longer chains, the K_d values are unchanged in the acidic eluent although they have an acidic group.

(d) K_d values for ϵ -aminocaproic acid decrease in hydrochloric acid-methanol

TABLE IV

CHANGE IN K_d VALUES ON CHANGING THE ELUENT FROM METHANOL ALONE TO DIFFERENT METHANOLIC SOLUTIONS

Change in K_d	Eluent	0.05 <i>N</i> HCl-methanol	0.05 <i>N</i> KOH-methanol	0.05 <i>N</i> KCl-methanol
Unchanged	Benzene Nitrobenzene <i>m</i> -Dinitrobenzene 2,4-Dinitroaniline 2,4-Dinitrophenetole 2,4-Dinitrophenyl- aminocaproic acid Benzoylaminocaproic acid	Benzene Nitrobenzene <i>m</i> -Dinitrobenzene 2,4-Dinitroaniline	Benzene Nitrobenzene <i>m</i> -Dinitrobenzene 2,4-Dinitrophenetole 2,4-Dinitrophenyl- aminocaproic acid Benzoylaminocaproic acid 2,4-Dinitrophenol 2,4-Dinitrobenzoic acid ϵ -Aminocaproic acid Benzoic acid	Benzene Nitrobenzene <i>m</i> -Dinitrobenzene 2,4-Dinitroaniline 2,4-Dinitrophenetole 2,4-Dinitrophenyl- aminocaproic acid Benzoylaminocaproic acid 2,4-Dinitrophenol 2,4-Dinitrobenzoic acid Benzoic acid
Increased				
Decreased	2,4-Dinitrophenol 2,4-Dinitrobenzoic acid ϵ -Aminocaproic acid Benzoic acid <i>m</i> -Phenylenediamine			ϵ -Aminocaproic acid <i>m</i> -Phenylenediamine

and potassium chloride-methanol and increase in potassium hydroxide-methanol.

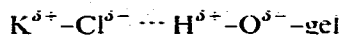
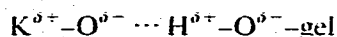
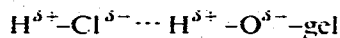
(e) K_d values for dinitroaniline are unchanged in these eluents and those for *m*-phenylenediamine decrease.

(f) K_d values for 2,4-dinitrophenetole increase in the alkaline eluent and are unchanged in other eluents.

The results for the behaviour of acidic aromatic substances in potassium chloride-methanol support Janson's theory⁴, but it is impossible to explain other phenomena with his theory. In the studies by Janson⁴ and Eaker and Porath¹², the probable role of the solvent, which was water when Sephadex G-10 and other unmodified dextran gels were used, was stressed. The apparent inadequacy of Janson's theory in our study is probably due largely to the fact that the solvent and the gel are not water and Sephadex G-10 but methanol and Sephadex LH-20, respectively. In order to explain the reverse elution behaviour when an electrolyte is included in the eluent, the suggestions of Gelotte², Streuli^{10,11} and Brook and Munday¹³ on adsorption behaviour are insufficient. The organophilic Sephadex LH-20 gel might be more polar than the solvent, methanol, in which case polar solutes might be adsorbed through hydrophilic interactions and, by adding an electrolyte to methanol, the polarity of the eluent might increase with respect to the gel phase. However, this hypothesis is still insufficient in order to explain the behaviour of solutes such as dinitrobenzene which do not have any dissociative group and the K_d values of which were unchanged in the four eluents used.

The elution behaviour of the solutes in group (a) indicates that the strengths of hydrogen bonds and π -bonds between the solutes and the gel do not vary when hydrochloric acid, potassium hydroxide or potassium chloride was added to methanol, implying there is neither an increase in the number of adsorption sites of the gel nor a decrease in the size of the layer of hydration which might prevent solute-gel interactions. The elution behaviour of solutes in group (b) in potassium hydroxide-methanol and potassium chloride-methanol can be explained by the change in the solute-gel interactions due to the dissociation or the suppression of dissociation of the acidic groups, but that in potassium chloride-methanol cannot be explained by the dissociation-non-dissociation theory. The elution behaviour of the solutes in potassium chloride-methanol should be the same as that in methanol alone if the increase in K_d values was caused by the dissociation of the acidic groups.

It might be appropriate to consider that the structure of the gel itself might be altered by the addition of hydrochloric acid, potassium hydroxide or potassium chloride to methanol. It may be postulated that hydrochloric acid, potassium hydroxide or potassium chloride in the eluent interacts with the residual hydroxyl group in the gel through hydrogen bonds of the following types:



As a result of these hydrogen bonds, the outside of the gel becomes positively charged in all three instances.

With regard to mechanism, the adsorptive effects can be divided into two classes: (1) the ionic effects involving electrostatic interactions including ion exchange, ion exclusion and ion adsorption; and (2) the pure adsorptive effects involving hydrogen bonding and π -bonding. The data in Table IV might be explained on the basis of the gel-hydrochloric acid (potassium hydroxide or potassium chloride) interactions suggested above, together with the two mechanisms for the adsorption of solutes on to gels.

The solutes that have no dissociable groups are adsorbed to gels only by pure adsorption in the three eluents. The solutes that have an acidic group are inhibited from dissociation in hydrochloric acid-methanol and the ionic effects due to the dissociation are neglected, so that the elution of the solutes occurs earlier than in methanol. These solutes dissociate in potassium hydroxide-methanol or potassium chloride-methanol and the delay in the elution of the solutes due to the ion adsorption between the ion and the gel increases markedly. The solutes that have a basic group are positively charged in hydrochloric acid-methanol and potassium chloride-methanol and exhibit the ion exclusion effect, which persists even in potassium hydroxide-methanol. Several solutes behave similarly in potassium chloride-methanol, hydrochloric acid-methanol and potassium hydroxide-methanol, and in a different manner to that in methanol alone, indicating the occurrence of ion exclusion or ion adsorption between the solutes and the gel. The observation that the K_d values for dinitroaniline are almost identical in three eluents can be explained on the assumption that the ionic effects are absent owing to the formation of a chelate ring by hydrogen bonding between an amino group and the nitro group in the *ortho* position. We assume from the theory mentioned above and the fact that the K_d values for α -aminocaproic acid in hydrochloric acid-methanol and potassium chloride-methanol are similar that the carboxyl group in α -aminocaproic acid does not dissociate in methanol or in potassium chloride-methanol, that the amino group does dissociate, and that the ion exclusion effect occurs in potassium chloride-methanol. The results discussed are summarized in Table V.

Elution behaviour of solutes in methanolic solutions including benzene, dinitrobenzene and pyridine

Hydrogen bonding and bonding by coulombic force are local adsorptions, and the saturation of adsorption by such bonding is determined by the number of interaction sites on the gel surface. The addition of aromatic compounds such as 0.2 *N* sodium salicylate to the eluent in order to saturate the interaction sites in the gel was reported to be rather ineffective⁶. However, dinitrophenyl amino acids are eluted later than the free amino acids in phenol-acetic acid-water (1:1:1) eluent and earlier in 1 *M* pyridine eluent. Further, the interaction of the gel with aromatic compounds was assumed to be completely eliminated¹⁴.

If specific groups in the gel are assumed to act as the adsorption sites for solutes, an appropriate means of preventing these interactions is to block the sites with other compounds. No differences in K_d values were observed on addition of 1%, of benzene or 0.5%, of dinitrobenzene to methanol. However, the effects of the addition of 10%, of benzene or 1 *M* pyridine to methanol might be ascribed to blockage or "saturation" of the adsorption sites of the gel (Table II). Apparently the sites for π -bonding with solutes are blocked in 10%, benzene-methanol and the sites for hy-

drogen bonding in 1 *M* pyridine-methanol. These secondary effects are not completely eliminated by the addition of 10% of benzene or 1 *M* pyridine as the K_d values are still greater than unity.

TABLE V

MAIN SECONDARY EFFECTS CONCERNED IN GEL-SOLUTE INTERACTION FOR ADSORPTION

Abbreviations: P.A. = pure adsorption; I.E. = ionic effects; M.S.E. = molecular sieving effect.

Classification	Solute	Eluant			
		Methanol	HCl-methanol	KOH-methanol	KCl-methanol
a	Benzene Nitrobenzene <i>m</i> -Dinitrobenzene	P.A.	P.A.	P.A.	P.A.
b	2,4-Dinitrophenol 2,4-Dinitrobenzoic acid Benzoic acid	P.A. I.E.	P.A.*	P.A.** I.E.	P.A.** I.E.
c	2,4-Dinitrophenylamino caproic acid Benzoylamino-caproic acid	M.S.E.***	P.A.§	P.A.** I.E.	P.A.** I.E.
d	<i>ε</i> -Aminocaproic acid	I.E.	I.E. §§	I.E. §§§	I.E.
e	<i>m</i> -Phenylenediamine	P.A. I.E.	I.E. §§	P.A. §	I.E. §§
f	2,4-Dinitrophenetole	P.A.	P.A.		P.A.

* Inhibited from dissociation.

** Ion adsorption with positive charge of the gel.

*** Mostly M.S.E.

§ Not dissociated.

§§ An $-NH_2$ group dissociates and ion exclusion with positive charge of the gel.

§§§ A $-COOH$ group dissociates and ion adsorption with positive charge of the gel.

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