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THE INTERACTION OF PHENOLS WITH SEPHADEX GELS

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SUMMARY

The adsorption by Sephadex dextran gels of a series of monosubstituted phenols can be correlated by the Hammett equation both in acid and alkaline solutions. This suggests that the interaction of a phenol with the gel operates through the hydroxyl group. Halogen-substituted phenols, however, are exceptions as they are more strongly adsorbed than would be predicted from the Hammett equation.

INTRODUCTION

Columns of Sephadex* dextran gels, in addition to fractionating mixtures according to molecular size, adsorb aromatic and heterocyclic compounds from aqueous solution[1], provided that the molecules are small enough to diffuse into the gel matrix. This adsorption effect, which is particularly marked in the more tightly cross-linked gels, can frequently be used to separate mixtures of similar molecular weights. For example, Sephadex gels interact strongly with phenols[2–4]. This behaviour of phenols raises two questions. Firstly, with which part of the gel structure does the phenol interact, and, secondly, how does the phenol bond to the gel.

The first question has been elegantly answered by DETERMANN AND WALTER[5], who have shown that the source of adsorption is the hydroxy-ether groups which cross-link the dextran chains. The second question has not so far been satisfactorily answered. The bonding of any aromatic compound to Sephadex is usually ascribed to a rather vague interaction of π-electrons with the gel matrix. However, WOOF AND PIERCE[6] have noted that the adsorption of a substituted phenol depends on whether the substituent donates or withdraws electrons from the aromatic ring. This suggests that the hydroxyl group takes part in the interaction with the gel. Further support for this hypothesis would be provided if the adsorption of a series of monosubstituted phenols by Sephadex gels was shown to obey a linear free energy relationship of the Hammett type[7].

* Sephadex gels are cross-linked polysaccharides manufactured by Pharmacia, Uppsala, Sweden.

For *meta*- and *para*-substituted benzene derivatives, a plot of the logarithm of the equilibrium constant K for one reaction against log K' for a second reaction is usually linear. Hammett formalised this linear free energy relationship in eqn. 1

$$\log \frac{K}{K_0} = \varrho \log \frac{K'}{K_0'} \quad (1)$$

where K_0 refers to the unsubstituted benzene derivative, and ϱ is a proportionality constant. The ionisation of substituted benzoic acids (K') was chosen as a standard, and log K'/K_0' was defined as the Hammett substituent constant σ. Hence, eqn. 1 becomes

$$\log \frac{K}{K_0} = \varrho \sigma \quad (2)$$

The proportionality constant ϱ (reaction constant) depends on the sensitivity of the reaction centre (hydroxyl group in the case of phenols) to changes of electronic environment made by changing the substituent.

If the adsorption of a series of substituted phenols in aqueous solution by a Sephadex gel can be correlated by the Hammett equation, then the phenols must interact with the gel partially or wholly through the hydroxyl group, since the electronic effects of the substituents will be transmitted to it through the aromatic ring.

The equilibrium constants (K_D) for the interaction of phenols with Sephadex G10 (highly cross-linked) were therefore found by measuring their elution volumes (V_e) from a column of the gel. These values of K_D were then used to test whether the Hammett equation could be applied to this system.

MATERIALS AND METHODS

Column preparation and operation

The column, of diameter 1.5 cm, was packed with Sephadex G10 gel to a height of 12.5 cm as described by DETERMANN[8]. A circle of filter paper was placed on the top of the column to protect the surface. Samples, 0.5 ml of buffer containing between 0.5 and 1.0 mg of the phenol, were introduced onto the column with a hypodermic syringe. The same column was used throughout the whole series of experiments.

The column eluent was aqueous acetate buffer of pH 4.0 and ionic strength 0.1 M or aqueous 0.1 N sodium hydroxide. These eluent solutions neutralised the slight ion-exchange properties that the gel exhibits when distilled water is used[1].

The effluent from the column was continuously monitored with an L.K.B. Uvicord I flow analyser which measures ultraviolet absorbance at 254 nm. The flow analyser was connected to a Leeds and Northrup "Speedomax H" strip-chart recorder.

Values of K_D were calculated in the usual way[1] using eqn. 3

$$K_D = \frac{V_e - V_0}{V_i} \quad (3)$$

where V_e is the elution volume of the phenol, V_0 the void volume of the column, and V_i the internal aqueous volume of the gel.

V_e was measured with the recorder chart and measuring cylinder. V_0, determined as the elution volume of Blue Dextran 2000 (Pharmacia), was 7.5 ml. V_i,

calculated by multiplying the water regain (manufacturer's value) by the dry weight of the gel, was 8.6 ml. Elution volumes were reproducible to ± 0.5 ml and the phenols were eluted as symmetrical peaks.

TABLE I

K_D VALUES FOR MONOSUBSTITUTED PHENOLS

No.	*Phenol*	*K_D value*	
		pH 4.0 acetate buffer	*0.1 N sodium hydroxide*
1	Phenol	7.2	0.58
2	*m*-Cresol	10.2	0.72
3	*p*-Cresol	10.1	0.76
4	*p*-Ethylphenol	12.6	0.93
5	*p*-Hydroxybenzoic acid	16.0	—
6	*m*-Hydroxybenzaldehyde	7.8	1.05
7	*p*-Hydroxybenzaldehyde	7.7	1.42
8	*p*-Methoxyphenol	8.0	0.60
9	Resorcinol	12.7	—
10	Quinol	8.8	—
11	*m*-Nitrophenol	16.2	2.96
12	*p*-Nitrophenol	16.9	5.41
13	*m*-Fluorophenol	11.0	1.10
14	*p*-Fluorophenol	9.3	0.99
15	*m*-Chlorophenol	25.6	2.38
16	*p*-Chlorophenol	22.8	2.85
17	*m*-Bromophenol	36.1	3.20
18	*p*-Bromophenol	32.5	3.90
19	*p*-Iodophenol	57.8	6.00
20	*m*-Aminophenol	—	0.49
21	*p*-Aminophenol	—	0.43

RESULTS AND DISCUSSION

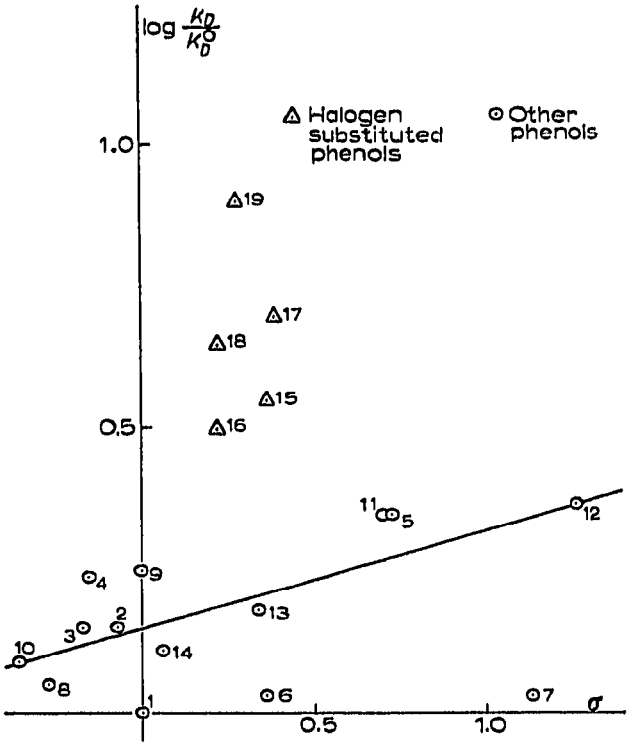
Values of K_D for a series of *meta*- and *para*-substituted phenols in pH 4.0 aqueous acetate buffer and 0.1 *N* sodium hydroxide solution are given in Table I. Acetate buffer of pH 4.0 was chosen as one of the eluents because, under these conditions, all the phenols exist in the unionised form. On the other hand, in 0.1 *N* sodium hydroxide solution, the phenols are fully ionised.

A K_D value greater than 1.0 indicates that a substance is adsorbed by Sephadex[1]. Table I shows that in pH 4.0 buffer, all the substituted phenols are strongly adsorbed. In 0.1 *N* sodium hydroxide, the adsorption effect appears to be much smaller. This is probably due to ion exclusion. It has been shown[1] that some ions are partially excluded from Sephadex gels. As the series of phenols in 0.1 *N* sodium hydroxide exist as anions, the K_D values are likely to be a combination of ion exclusion and adsorption effects. K_D values for *p*-hydroxybenzoic acid, resorcinol and quinol were not measured in 0.1 *N* sodium hydroxide because of the added complication of the ionisation of the substituent groups. For similar reasons, values for *m*- and *p*-aminophenols in pH 4.0 buffer were not included in Table I.

Fig. 1 is a plot of log K_D/K_D° against Hammett substituent constant for mono-

substituted phenols in pH 4.0 buffer. $K_D°$ refers to unsubstituted phenol. Values of σ were taken from the review by JAFFÉ[9].

For substituents other than the halogens, the correlation is reasonably good, particularly as no account was taken of effects likely to interfere with the phenol–gel bonding, such as solvent–phenol and solvent–gel interactions. The reaction constant ϱ, obtained by calculating the slope of the best straight line, is 0.104 with a standard deviation of 0.064.



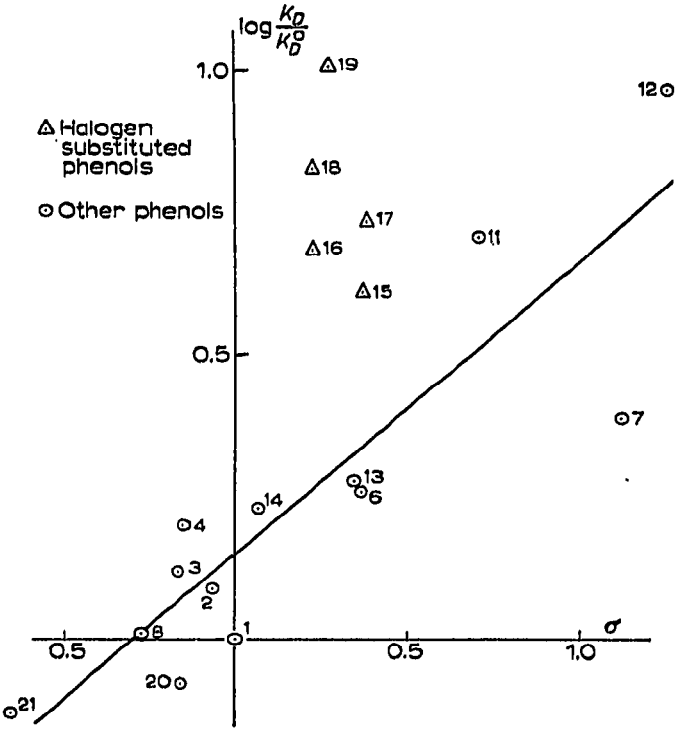


Fig. 1. Hammett plot for a series of substituted phenols in pH 4.0 acetate buffer.

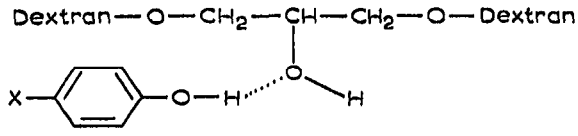
Fig. 2. Hammett plot for a series of substituted phenols in 0.1 N sodium hydroxide solution.

The phenols containing halogen substituents, with the exception of the fluorides, are considerably more strongly bound to the gel than would be predicted from a Hammett correlation. A possible explanation is that the interaction of these compounds is due to the halogen rather than the hydroxyl group.

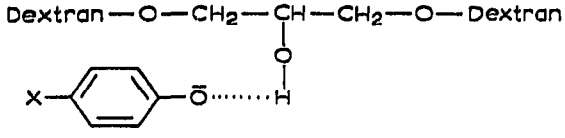
The Hammett plot for the series of phenols in 0.1 N sodium hydroxide solution is shown in Fig. 2. The correlation is again reasonably good, giving a ϱ value of 0.49 with standard deviation 0.08. Here, also, the phenols with halogens as substituent are more strongly adsorbed than expected.

Except in the case of halogen-substituted phenols, the adsorption of monosubstituted phenols by Sephadex gels, in both pH 4.0 acetate buffer and 0.1 N sodium hydroxide, can be correlated by the Hammett equation. Hence, it seems probable that the bonding of the phenols to the gel takes place through the hydroxyl group. However, the reaction constant ϱ for the two eluents is different, suggesting that the bonding in pH 4.0 buffer is somewhat different from that in 0.1 N sodium hydroxide.

This could be explained by hydrogen bonding to the gel at two different positions on the phenol, *i.e.* in pH 4.0 buffer



and in 0.1 *N* sodium hydroxide



REFERENCES

1 B. GELOTTE, *J. Chromatog.*, 3 (1960) 330.
2 C. G. NORDSTRÖM, *Acta Chem. Scand.*, 21 (1967) 2885.
3 J. A. DEMETRIOU, F. M. MACIAS R., M. J. MCARTHUR AND J. M. BEATTIE, *J. Chromatog.*, 34 (1968) 342.
4 T. C. SOMERS, *Nature*, 209 (1966) 368.
5 H. DETERMANN AND I. WALTER, *Nature*, 219 (1968) 604.
6 J. B. WOOF AND J. S. PIERCE, *J. Chromatog.*, 28 (1967) 94.
7 L. P. HAMMETT, *Physical Organic Chemistry*, McGraw-Hill, New York, 1940.
8 H. DETERMANN, *Gel Chromatography*, Springer-Verlag, New York, 1968, p. 42.
9 H. H. JAFFÉ, *Chem. Rev.*, 53 (1953) 191.