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THE INTERACTION OF PHENOLS, ANILINES, AND BENZOIC ACIDS WITH SEPHADEX GELS

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SUMMARY

The adsorption by Sephadex dextran gels of monosubstituted phenols, anilines, and benzoic acids can be correlated by the HAMMETT equation. The interaction of these benzene derivatives with the gel therefore operates through the hydroxyl, amino, and carboxylic groups respectively. It is suggested that the benzene derivatives are adsorbed onto the hydroxyether cross-linking by hydrogen bonds. Halogen-substituted benzene derivatives are exceptions as they are more strongly adsorbed than the HAMMETT equation predicts. There are indications that the halogen-substituted derivatives are adsorbed by the dextran chains.

INTRODUCTION

Phenols are strongly adsorbed by columns of Sephadex* dextran gels at the hydroxyether groups which cross-link the dextran chains¹. The phenols interact with the gel through the phenolic hydroxyl group², since the distribution coefficients (K_D) of a series of monosubstituted phenols can be correlated by the HAMMETT linear free-energy relationship³. An exception to this rule is the halogen-substituted phenols. These interact with the gel more strongly than would be predicted from the HAMMETT equation.

HAMMETT found that for most *meta*- and *para*- substituted benzene derivatives, a plot of the logarithm of equilibrium constant (K) for one reaction against $\log (K)'$ for a second reaction is linear, as shown in eqn. 1.

$$\log \frac{K}{K^0} = \rho \log \frac{(K)'}{(K^0)'} \quad (1)$$

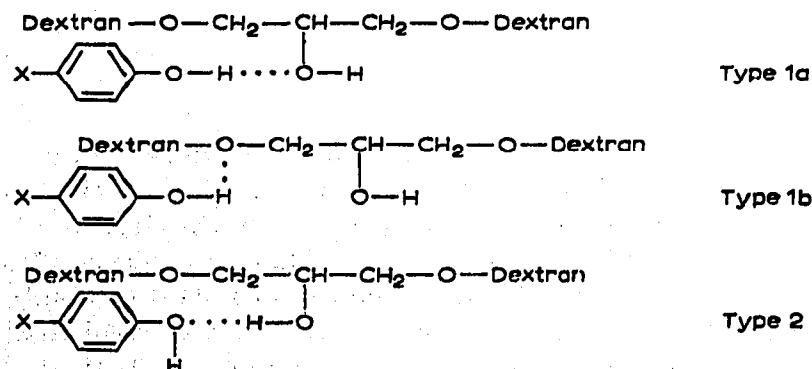
where K^0 refers to the unsubstituted benzene derivative, and ρ (reaction constant) is a proportionality constant. The ionisation of substituted benzoic acids was chosen

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

as a standard and $\log ((K)'/(K^0)')$ was defined as the substituent constant σ . Eqn. 1 becomes

$$\log \frac{K}{K^0} = \rho\sigma \quad (2)$$

A plot of $\log K_D/K_D^0$ against σ for a series of substituted phenols was linear². This indicates that the interaction with the gel depends on the transmission of the electronic effects of the substituents through the aromatic ring to the hydroxyl group, and hence that the hydroxyl group must be the reaction centre. It was suggested that the phenols interact with the gel by hydrogen bonds of one of the following types.



Sephadex gels also strongly adsorb substituted anilines and benzoic acids⁴. If their distribution coefficients can be correlated by the HAMMETT equation, the arguments given above for phenols should also apply to anilines and benzoic acids. Values of K_D for substituted anilines and benzoic acids on Sephadex G-10 were therefore found by measuring their elution volumes (V_e) from a column of the gel. The K_D values were used to test the validity of the HAMMETT equation under these conditions.

In addition, K_D values of substituted phenols, anilines, and benzoic acids were measured on columns of methylated Sephadex (LH-20). From these values, it was possible to decide whether the solutes interacted with the gel by a type 1 or type 2 process, since the methylated gel cannot act as a hydrogen donor.

MATERIALS AND METHODS

The column (diameter 1.5 cm) was packed with Sephadex gel to a height of 11.0 cm as described by DETERMANN⁵. Samples, 0.5 ml of eluent containing 0.5 to 1.0 mg of solute and 0.1 mg of Blue Dextran 2000 as internal standard, were introduced onto the column with a hypodermic syringe.

The eluents were acetate buffer (pH 4.0) for phenols, acetate buffer (pH 2.2) for benzoic acids, and 0.1 *N* sodium hydroxide solution for anilines.

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

Values of K_D were calculated⁵ from eqn. 3

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

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

V_0 was determined as the elution volume of Blue Dextran 2000 (Pharmacia). V_t was calculated as the product of the water regain (manufacturer's value) and the dry weight of the gel. V_e was obtained from the chart. Elution volumes were reproducible to ± 0.5 ml and the solutes were eluted as symmetrical peaks.

RESULTS AND DISCUSSION

Values of K_D for a series of *meta*- and *para*-substituted phenols², anilines and benzoic acids on Sephadex G-10 are shown in Table I. All the compounds were strongly adsorbed by the gel since their values⁶ of K_D are greater than 1.0.

TABLE I

K_D VALUES FOR MONOSUBSTITUTED PHENOLS, ANILINES, AND BENZOIC ACIDS ON SEPHADEX G-10

No.	Substituent	K_D value		
		Aniline	Phenol ²	Benzoic acid
1	H	4.6	7.2	10.7
2	<i>m</i> -CH ₃	5.8	10.2	16.1
3	<i>p</i> -CH ₃	5.6	10.1	14.7
4	<i>p</i> -C ₂ H ₅	—	12.6	—
5	<i>p</i> -(CH ₃) ₃ C	—	—	32.3
6	<i>m</i> -OH	—	12.7	21.7
7	<i>p</i> -OH	—	8.8	23.1
8	<i>m</i> -CH ₃ O	6.2	—	15.5
9	<i>p</i> -CH ₃ O	4.6	8.0	15.3
10	<i>p</i> -C ₂ H ₅ O	4.8	—	16.1
11	<i>m</i> -NH ₂	3.5	—	—
12	<i>p</i> -NH ₂	2.6	—	—
13	<i>p</i> -CH ₃ CONH	2.9	—	—
14	<i>m</i> -NO ₂	15.4	16.2	11.1
15	<i>p</i> -NO ₂	26.3	16.9	10.5
16	<i>m</i> -CHO	—	7.8	—
17	<i>p</i> -CHO	—	7.7	7.7
18	<i>m</i> -COOH	—	—	21.1
19	<i>p</i> -COOH	—	16.0	20.1
20	<i>p</i> -CN	—	—	—
21	<i>m</i> -CH ₃ CO	—	—	—
22	<i>p</i> -CH ₃ CO	8.3	—	—
23	<i>m</i> -F	7.9	11.0	10.3
24	<i>p</i> -F	5.7	9.3	9.5
25	<i>m</i> -Cl	19.1	25.6	23.6
26	<i>p</i> -Cl	16.7	22.8	20.4
27	<i>m</i> -Br	27.5	36.1	36.3
28	<i>p</i> -Br	23.8	32.5	30.9
29	<i>m</i> -I	45.1	—	—
30	<i>p</i> -I	42.6	57.8	55.1

The buffers were chosen so that the solutes were present only in their non-ionised states, to avoid complications from ion-exclusion⁶. For similar reasons, those substituents which give ions in the buffer are not included in Table I.

Table II gives values of K_D for substituted phenols, anilines and benzoic acids on Sephadex LH-20 (methylated). Here again, all compounds tested are strongly adsorbed by the gel. In all cases, K_D values for Sephadex G-10 are greater than for LH-20 since there is more cross-linking in G-10

HAMMETT plots for monosubstituted anilines and benzoic acids on Sephadex G-10 and for monosubstituted phenols, anilines and benzoic acids on Sephadex LH-20 are shown in Figs. 1-5 respectively. K_D^0 refers to the unsubstituted benzene derivative. Values of σ were taken from the review by JAFFÉ⁷.

In all cases, a reasonable correlation exists for substituents other than the halogens. This is similar to results for phenols² on Sephadex G-10. Hence the interaction of anilines with the gel must be through the amino group and benzoic acids through the carboxyl group.

The slopes of the correlation lines, which give the reaction constant ρ , are shown in Table III. The slopes were calculated by a least-squares treatment.

TABLE II

 K_D VALUES FOR MONOSUBSTITUTED PHENOLS, ANILINES, AND BENZOIC ACIDS ON SEPHADEX LH-20

No.	Substituent	K_D value		
		Aniline	Phenol	Benzoic acid
1	H	2.7	5.9	7.5
2	<i>m</i> -CH ₃	3.4	7.0	11.2
3	<i>p</i> -CH ₃	3.4	6.5	10.6
4	<i>p</i> -C ₂ H ₅	—	9.5	—
5	<i>p</i> -(CH ₃) ₃ C	—	14.6	25.0
6	<i>m</i> -OH	—	8.3	13.3
7	<i>p</i> -OH	—	5.9	13.7
8	<i>m</i> -CH ₃ O	3.8	—	10.7
9	<i>p</i> -CH ₃ O	3.1	6.8	11.0
10	<i>p</i> -C ₂ H ₅ O	3.5	—	13.5
11	<i>m</i> -NH ₂	2.1	—	—
12	<i>p</i> -NH ₂	2.0	—	—
13	<i>p</i> -CH ₃ CONH	2.3	4.4	10.8
14	<i>m</i> -NO ₂	6.7	9.7	6.8
15	<i>p</i> -NO ₂	10.7	9.7	6.1
16	<i>m</i> -CHO	—	6.2	—
17	<i>p</i> -CHO	—	7.1	5.9
18	<i>m</i> -COOH	—	8.2	14.9
19	<i>p</i> -COOH	—	10.6	14.6
20	<i>p</i> -CN	—	—	5.4
21	<i>m</i> -CH ₃ CO	3.8	—	—
22	<i>p</i> -CH ₃ CO	5.3	—	—
23	<i>m</i> -F	4.4	7.5	7.5
24	<i>p</i> -F	4.0	6.6	6.9
25	<i>m</i> -Cl	8.4	15.6	15.3
26	<i>p</i> -Cl	7.9	14.9	12.9
27	<i>m</i> -Br	10.8	21.7	22.2
28	<i>p</i> -Br	11.3	20.2	19.1
29	<i>m</i> -I	16.7	—	34.4
30	<i>p</i> -I	16.4	30.5	38.0

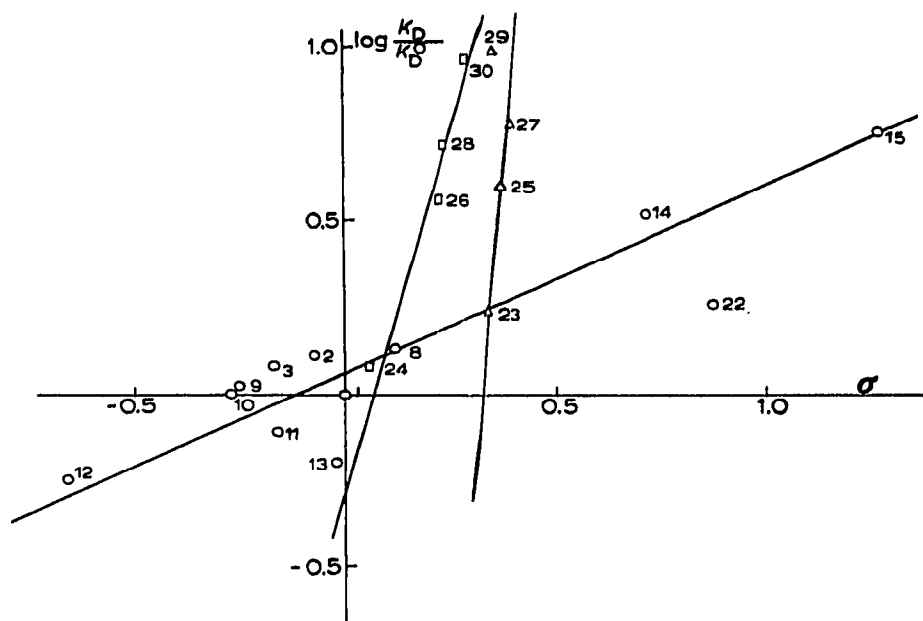


Fig. 1. HAMMETT plot for substituted anilines on Sephadex G-10. \odot , non-halogenated anilines; \triangle , *m*-halogen substituted anilines; \square , *p*-halogen substituted anilines.

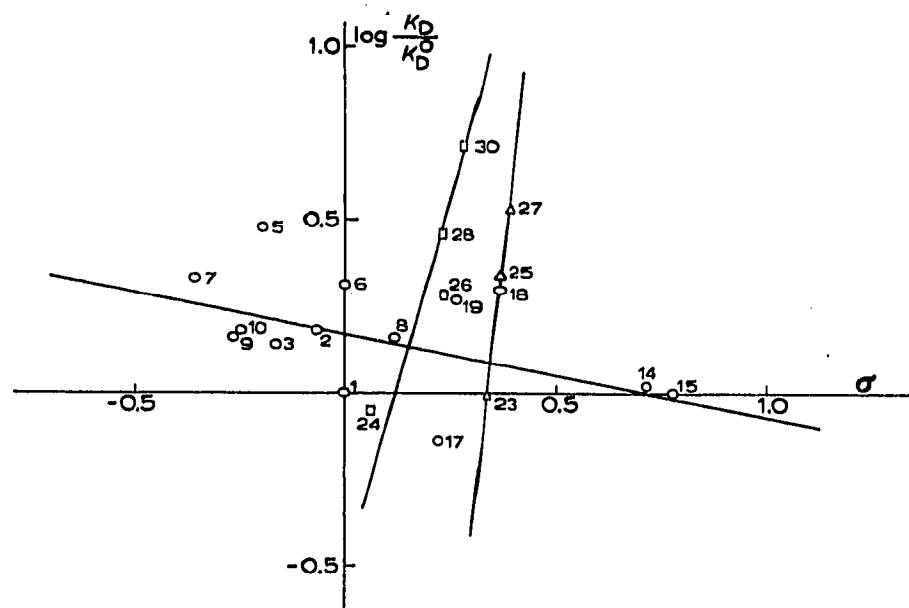


Fig. 2. HAMMETT plot for substituted benzoic acids on Sephadex G-10. \odot , non-halogenated benzoic acids; \triangle , *m*-halogen substituted benzoic acids; \square , *p*-halogen substituted benzoic acids.

In the case of anilines and benzoic acids, values of ρ are the same, within experimental error, for Sephadex G-10 as for Sephadex LH-20. This implies that the position on the gel at which interaction takes place is unchanged by methylation, which is evidence for bonding of type I. On the other hand, the ρ value for phenols on Sephadex G-10 is larger than on Sephadex LH-20, which suggests some difference in the types of interaction.

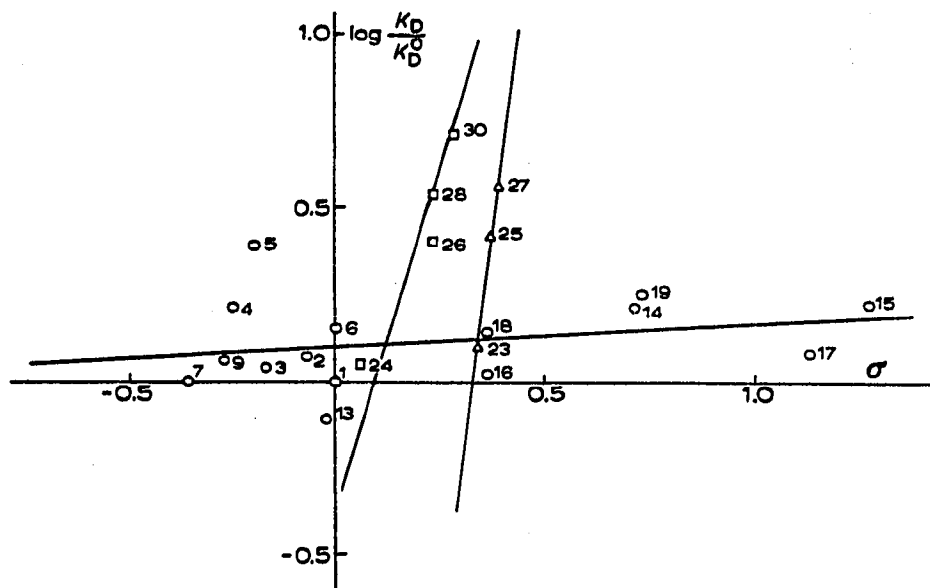


Fig. 3. HAMMETT plot for substituted phenols on Sephadex LH-20. \odot , non-halogenated phenols; Δ , *m*-halogen substituted phenols; \square , *p*-halogen substituted phenols.

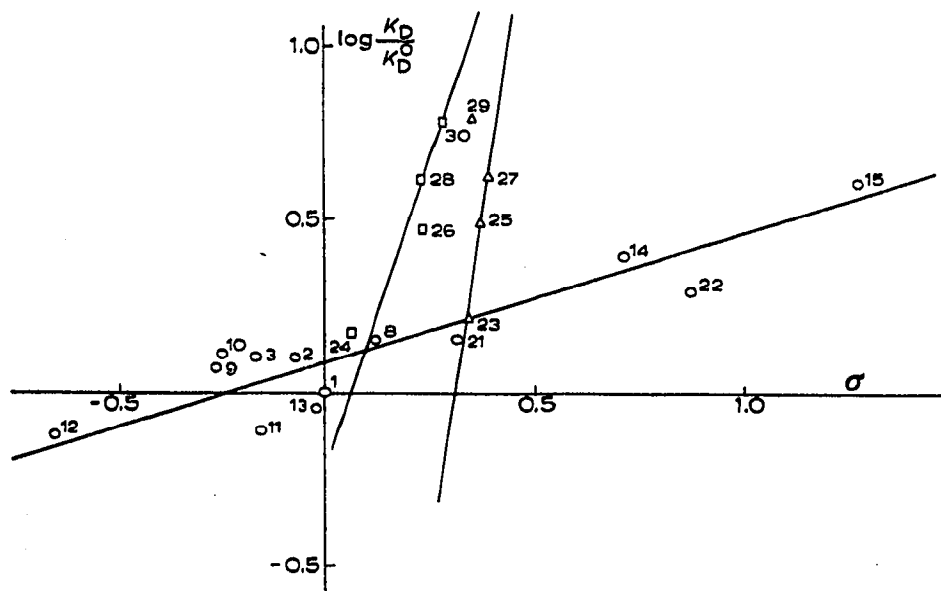


Fig. 4. HAMMETT plot for substituted anilines on Sephadex LH-20. \odot , non-halogenated anilines; Δ , *m*-halogen substituted anilines; \square , *p*-halogen substituted analines.

Eqn. 2 predicts that the line of the HAMMETT plots should pass through the origin. However, all the lines cross the y axis above the origin. One explanation of this phenomenon is that the substituents themselves interact slightly with the gel, increasing the adsorption of the substituted, relative to the unsubstituted, derivative. The scatter of some of the points may also be due to the same cause.

The halogen-substituted benzene derivatives behave similarly to halogenated

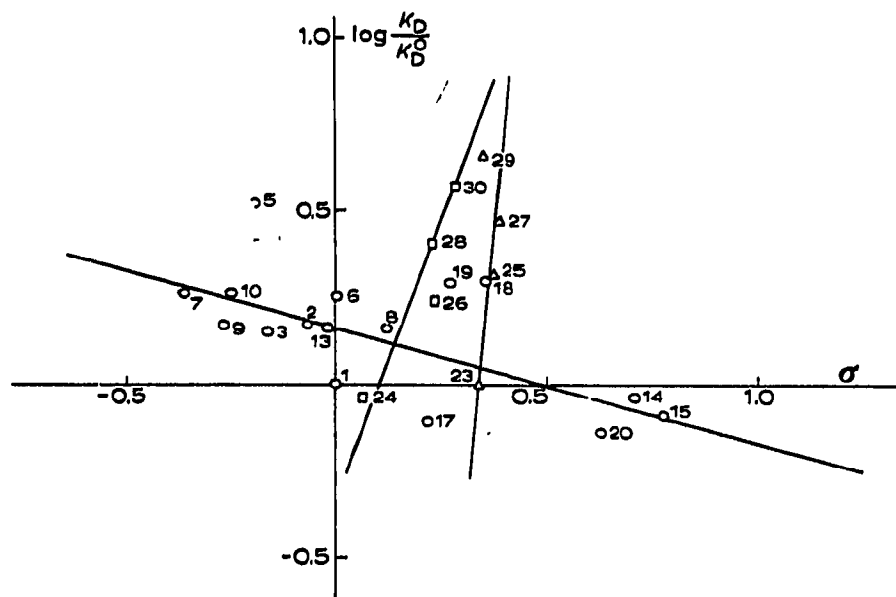


Fig. 5. HAMMETT plot for substituted benzoic acids on Sephadex LH-20. \odot , non-halogenated benzoic acids; \triangle , *m*-halogen substituted benzoic acids; \square , *p*-halogen substituted benzoic acids.

TABLE III

REACTION CONSTANTS ρ FOR MONOSUBSTITUTED PHENOLS, ANILINES AND BENZOIC ACIDS

	<i>Sephadex G-10</i>	<i>Sephadex LH-20</i>
Phenols	0.10	0.06
Anilines	0.47	0.34
Benzoic acids	-0.23	-0.32

phenols² on Sephadex G-10. They are adsorbed much more strongly than would be predicted from the HAMMETT equation. As can be seen from Figs. 1 to 5, the *meta*-substituted halogen derivatives lie on one straight line and the *para*-substituted derivatives on a second. The slopes of these lines are given in Tables IV and V.

The large difference in the slopes of the HAMMETT plots for halogenated and non-halogenated benzene derivatives suggests that the types of interaction with the gel are not the same. It seems probable that the halogenated benzene derivatives bond to the gel through the halogen atom rather than the side-chain. It is unlikely

TABLE IV

REACTION CONSTANTS ρ FOR *meta*-HALOGEN-SUBSTITUTED PHENOLS, ANILINES, AND BENZOIC ACIDS

	<i>Sephadex G-10</i>	<i>Sephadex LH-20</i>
Phenols	10	10
Anilines	14	8
Benzoic acids	12	12

TABLE V

REACTION CONSTANTS ρ FOR *para*-HALOGEN-SUBSTITUTED PHENOLS, ANILINES, AND BENZOIC ACIDS

	<i>Sephadex G-10</i>	<i>Sephadex LH-20</i>
Phenols	3.1	3.9
Anilines	4.2	3.6
Benzoic acids	4.4	3.2

that hydrogen-bonding is involved since, if it were, the K_D values of the halogenated derivatives would be in the order $F > Cl > Br > I$. In fact, the values are in the reverse order.

It is unlikely that the halogen atoms interact with the hydroxyether cross-links of the gel. More probably, the halogen-substituted derivatives are adsorbed by the dextran chains since halogen molecules and halogen compounds are known to interact with starch⁸ and dextran⁹.

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