

CHROM. 4071

## The interaction of organic acids and bases with Sephadex gels

Columns of Sephadex\* gels are usually used to fractionate substances according to their molecular dimensions<sup>1</sup>. However, these gels possess other properties which can also be used for certain separations. Firstly, Sephadex gels, particularly those which are highly cross-linked, reversibly adsorb molecules with  $\pi$ -electron systems<sup>2</sup>, provided that their molecular sizes are small enough to permit diffusion into the gel-matrix. Secondly, organic ions are usually partially excluded from Sephadex gels<sup>2</sup>. It has been pointed out that the separation of the geometrical isomers, maleic and fumaric acids on columns of Sephadex G-10 in a buffer of pH 5.0 depends on a combination of ion-exclusion and gel-adsorption<sup>3</sup>.

It was argued that in buffers of high pH, the acids would be ionised and partially excluded from columns of the gel. This would lead to a low value of  $K_D$  for the acid, where  $K_D$  is the distribution coefficient of the acid between the internal and external aqueous phases of the gel<sup>2</sup>. On the other hand, in buffers of low pH, the acids would be unionised and tend to be adsorbed by the gel either by  $\pi$ -electron interaction or by hydrogen-bonding of the acid hydroxyl groups to the gel matrix, giving a high value of  $K_D$ . Hence, the dissociation constant of the acid will determine whether the acid is adsorbed by or excluded from the gel in a buffer of a given pH. It should therefore be possible to separate any two organic acids, provided that they have different dissociation constants, by suitable choice of pH of the buffer.

The shape of a plot of  $K_D$  values of an acid against pH of the buffer should be as shown in Fig. 1. At any pH lower than A, the acid is unionised, and at any pH

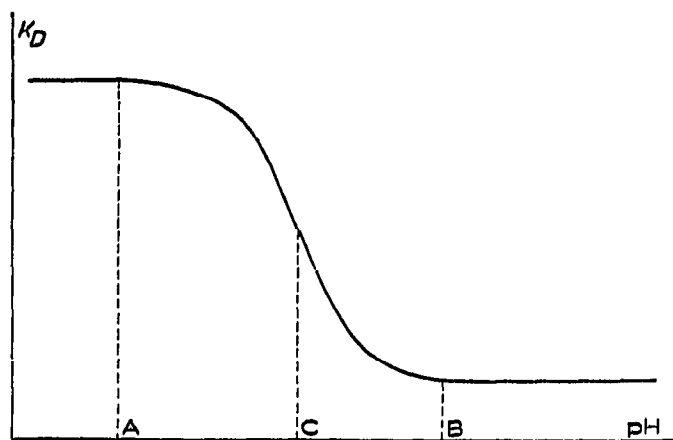


Fig. 1. The effect of pH on the  $K_D$  value of an organic acid.

greater than B, it is fully ionised. The dissociation constant of an acid HA is given approximately by:

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

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

By taking the logarithm of each side of the equation, we have:

$$pK_a = \text{pH} - \text{Log} \frac{[\text{A}^-]}{[\text{HA}]}$$

At the point C in Fig. 1,  $[\text{HA}] = [\text{A}^-]$  and hence  $pK_a = \text{pH}$ , *i.e.* the pH at point C is numerically equal to the  $pK_a$  of the acid.

It should be possible to apply a similar argument to organic bases. A plot of  $K_D$  values against buffer pH for bases should have low  $K_D$  values in buffers of low pH, since the bases will be ionised, and high  $K_D$  values in buffers of high pH.

The distribution coefficients of a number of organic acids and bases on columns of Sephadex G-10 were therefore measured at different values of pH.  $K_D$  values were plotted against pH for each substance and the shape of the curve compared with Fig. 1. Values of  $pK_a$  were read off from the plots and compared with literature values.

#### *Materials and methods*

The column, diameter 1.5 cm, was packed<sup>4</sup> with Sephadex G-10 gel to a height of 8.0 cm. A circle of filter paper was placed on the top of the column to protect the surface. The effluent from the column was continuously monitored with an L.K.B. Uvicord I flow analyser, which measured U.V. absorbance at 254 nm, connected to a Leeds and Northrup "Speedomax H" strip-chart recorder. Samples, 0.5 ml of buffer containing sufficient solute to give a reasonable absorbance, were introduced onto the column with a hypodermic syringe. The solutes were eluted with McIlvain buffers of 0.1 *M* ionic strength over the pH range 2-8 and Sörensen-Walburn buffers (0.1 *M*) over the range 8-13.

Values of  $K_D$  were calculated using the following equation<sup>2</sup>:

$$K_D = \frac{V_e - V_0}{V_t}$$

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.  $V_e$  was measured with the recorder chart and measuring cylinder.  $V_0$ , determined as the elution volume of Blue Dextran 2000 (Pharmacia) was 4.8 ml.  $V_t$ , calculated as the product of the water regain (manufacturers value) and the dry weight of the gel, was 5.5 ml.

#### *Results and discussion*

Plots of  $K_D$  values against pH for several phenylacetic acids are shown in Fig. 2. The curves have the predicted shape. The complete curve for benzoic acid cannot be investigated as Sephadex gels cannot be used in solutions more acid than about pH 2. The values of  $pK_a$  derived from the plots in Fig. 2 are shown in Table I. Literature values for the dissociation constant of phenylisopropylhydroxyacetic acid and phenylisopropylacetic acid were unavailable. These were therefore measured by the method described by ALBERT AND SERJEANT<sup>5</sup>. Agreement between values of  $pK_a$  from the curves and from the literature<sup>5</sup> is satisfactory.

$K_D/\text{pH}$  plots for benzoic and *m*-toluic acids are shown in Fig. 3. A plot for phenol is shown in Fig. 4. Here, again, the shape of the curves and the values of  $pK_a$  which they give (Table I) are satisfactory. Plots for aniline, *N*-methylaniline,

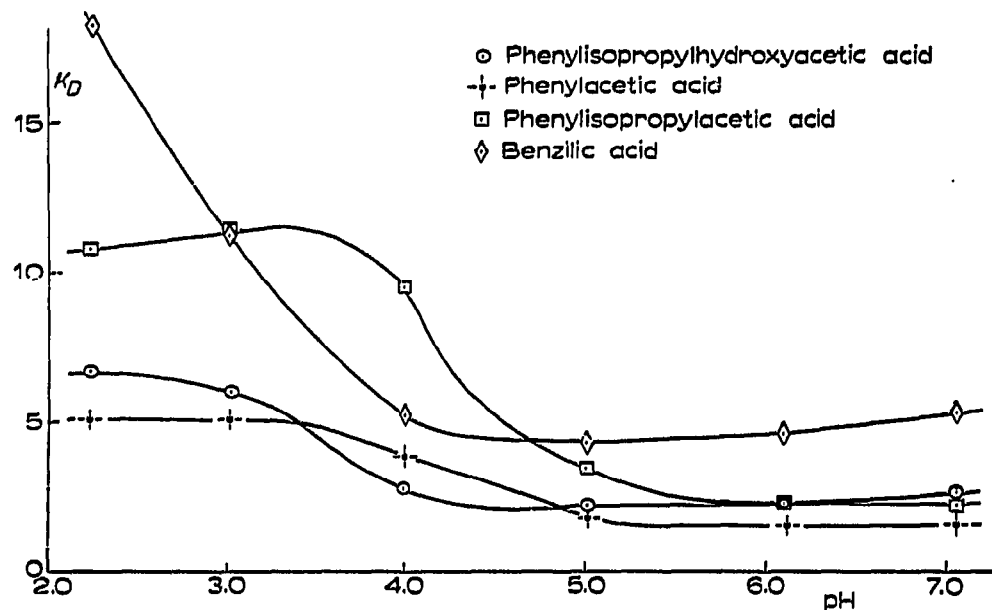


Fig. 2. The effect of pH on the  $K_D$  value of phenylacetic acids.

TABLE I

A COMPARISON OF DISSOCIATION CONSTANTS WITH LITERATURE VALUES

	$pK_a$	
	Estimated from $K_D/pH$ plots	Literature
Phenylacetic acid	4.3	4.31
Phenylisopropylacetic acid	4.2	4.07 <sup>a</sup>
Phenylisopropylhydroxyacetic acid	3.6	3.53 <sup>a</sup>
Benzoic acid	4.2	4.18
<i>m</i> -Toluic acid	4.3	4.27
Phenol	10.0	9.98
Aniline	4.5	4.58
<i>N</i> -Methylaniline	4.9	4.85
<i>N,N</i> -Dimethylaniline	5.0	5.06
<i>p</i> -Aminobenzoic acid	4.9	4.85

<sup>a</sup> Determined by the method of ALBERT AND SERJEANT<sup>5</sup>.

and *N,N*-dimethylaniline, as examples of organic bases, are shown in Fig. 5. The shapes of the curves are less symmetrical than those of acids, but the values of  $pK_a$  derived from them (assuming that the  $pK_a$  is numerically equal to the pH at which the slope of the curve is greatest) are in agreement with literature values (Table I).

A plot for *p*-aminobenzoic acid is shown in Fig. 6. At high pH, the  $K_D$  value is low due to exclusion of the carboxylic acid anion. As the pH is reduced,  $K_D$  increases as the anion is converted to the unionised acid. At still lower pH, the amine group is protonated and consequently  $K_D$  is reduced. The  $pK_a$  corresponding to the addition of a proton to the carboxylic acid anion is in agreement with the literature

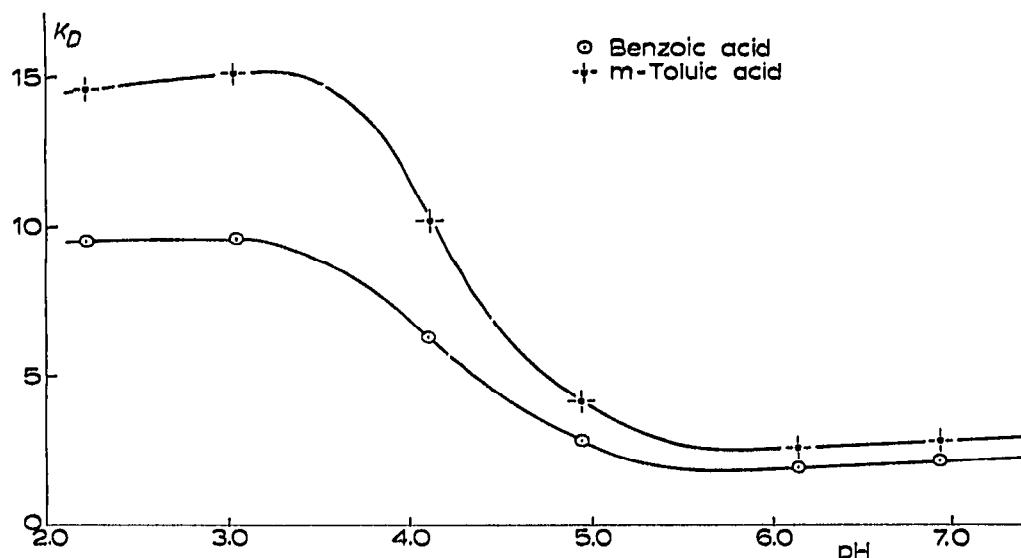


Fig. 3. The effect of pH on the  $K_D$  value of benzoic and *m*-toluic acids.

value. The  $pK_a$  corresponding to protonation of the amine group cannot be estimated from the graph as the shape of the curve cannot be measured below pH 2.

If a substance is adsorbed by the gel, it will have a  $K_D$  value greater than 1. All the examples of organic acids and bases (when unionised) are strongly adsorbed by the gel. This may be due either to  $\pi$ -electron interaction, as the acids and bases used in this investigation contain phenyl rings, or to hydrogen bonding.

A combination of ion-exclusion and gel-adsorption by columns of Sephadex G-10 gels has been shown to account for the shape of curves obtained by plotting  $K_D$  values against pH for several organic acids and bases. Reasonably accurate dissociation constants could be estimated from the curves. Sephadex G-10 gels should

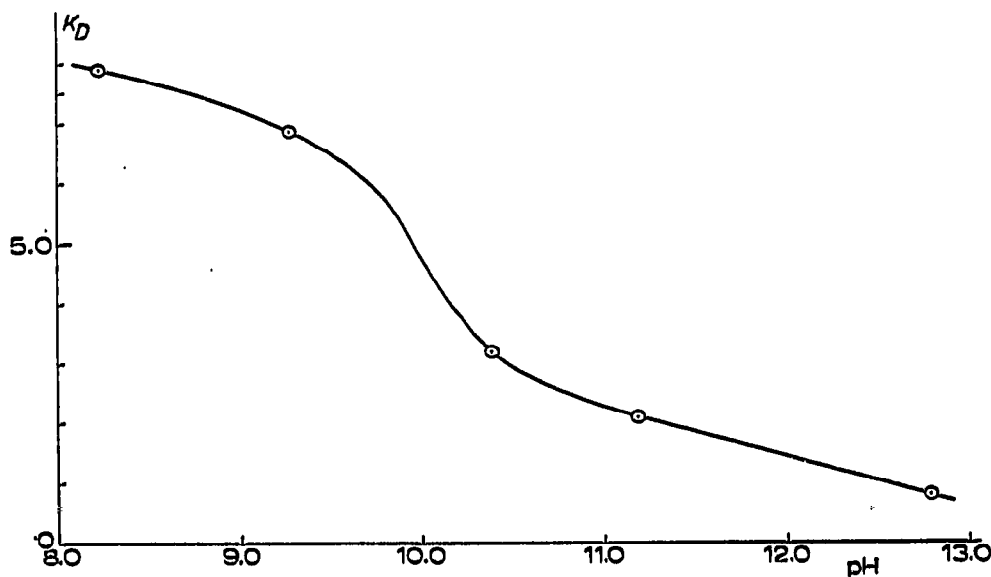


Fig. 4. The effect of pH on the  $K_D$  value of phenol.

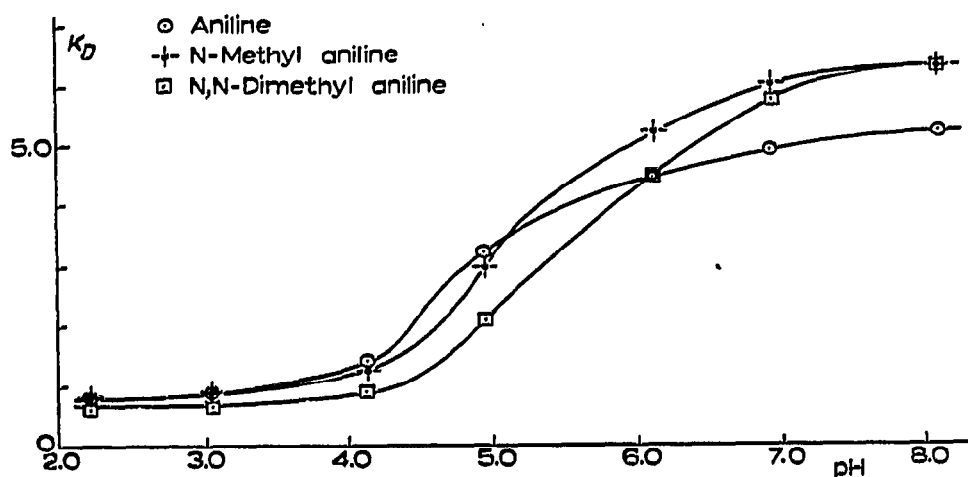


Fig. 5. The effect of pH on the  $K_D$  value of aniline derivatives.

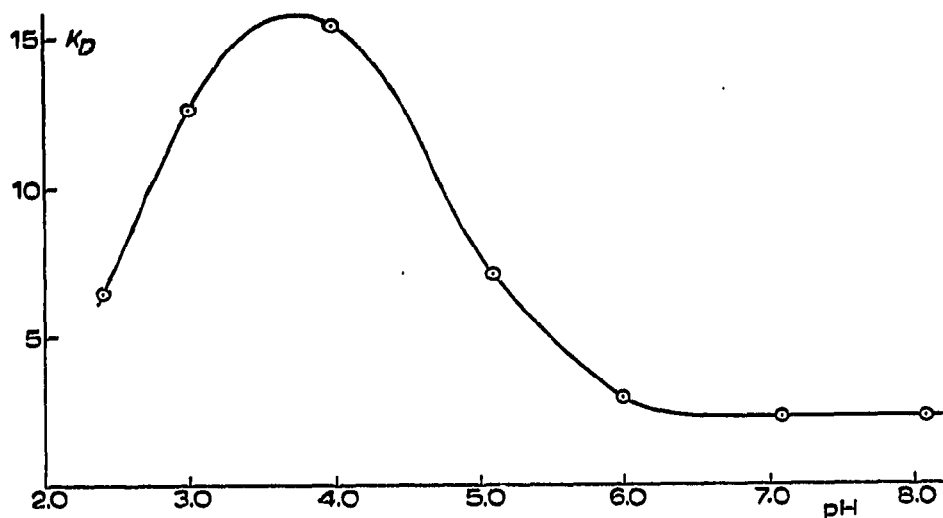


Fig. 6. The effect of pH on the  $K_D$  value of *p*-aminobenzoic acid.

therefore be useful for separating mixtures of acids and bases with different dissociation constants.

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