

333. *The Adsorption of Organic Acid Molecules and their Chromatographic Separation on Ion-exchange Resins.*

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The molecular adsorption of some organic molecules on the strong-base resins Dowex 1 and 2 and Amberlite I.R. 410 have been measured by two indirect methods. Adsorption is even greater on these resins than on the acid exchange materials previously studied.

The interference of adsorption effects with the separation of mixtures of organic electrolytes may be entirely overcome by choice of a suitable solvent mixture. It is shown that typical mixtures of aliphatic and aromatic carboxylic acids can be quantitatively separated by ion-exchange chromatography in 35% dioxan-water as solvent.

THE chromatographic separation of organic acids on ion-exchange materials has been discussed in a number of places.¹ Ideally, a series of acids may be displaced from a basic exchange resin in the order of their dissociation constants, but in practice difficulties, that have been attributed to van der Waals adsorption forces, are so great that, for example, in Partridge's work² on amino-acid mixtures aromatic acids had to be removed before a separation of the simpler acids was attempted.

In the present paper, adsorption data for some organic acid molecules on strong-base exchange resins are recorded, and methods are then given for removing or minimising the molecular-adsorption difficulty in the ion-exchange chromatography of organic materials.

EXPERIMENTAL

Materials.—Amberlite 410 was ground and sieved wet; the 100—120 mesh fraction was used in this work. Dowex 1 and 2 were supplied as micro-beads of 200—250 mesh. The resins were cycled three times with *N*-sodium hydroxide followed by *N*-hydrochloric acid, and the chloride forms were exhaustively washed, dried by suction, left on large filter papers for 14 days, and then stored in stoppered bottles. The moisture content of the air-dried resins was determined by heating separate samples in a vacuum oven, over phosphorus pentoxide, for 24 hr. at 80°. Columns of the resin were always kept under water and protected by soda-lime tubes. When not in actual use they were left in the chloride form.

The sodium hydroxide was standardised with AnalaR potassium hydrogen phthalate previously dried at 120°. A stream of air, free from carbon dioxide, was blown through solutions during titrations and de-ionised (carbon dioxide-free) water was used for dilutions. The acids used were AnalaR where possible.

Capacity Measurements.—The resin column was 10 cm. high and 1.2 cm. in diameter, and contained 5 g. of dry resin chloride. This was converted into the hydroxide form and washed free from alkali. A 0.1*N*-solution of the acid under study was then run down the column at a boundary rate of 2 cm./hr. The narrow outlet tube at the foot of the column had two small, spade-shaped, blacked platinum electrodes fused to the glass, and the resistance of the eluate was measured for each 0.5 ml. until it became constant. The fall of the resistance when acid broke through the column was not entirely sharp, but a plot of Δ (resistance) against Δ (volume) gave a symmetrical maximum which was taken to indicate the retention volume. A similar experiment in which sodium chloride was passed through the column in its chloride form enabled the "dead" volume to be estimated—the small volume of acid solution necessary to replace the solvent in the column at the beginning of each experiment. By subtracting this from the measured retention volumes, corrected values were obtained from which the total amount of each acid retained by the column could be calculated. One further small correction was necessary, as the water in the swollen resin will presumably contain organic acid molecules at virtually the same concentration as the external solution. Dowex 2 in the chloride form absorbs 1.17 g. of water/g. of resin,³ and will therefore retain approximately 0.1 milliequiv. of

¹ See, for instance, Davies, *Biochem. J.*, 1949, **45**, 38; *Research*, 1950, **3**, 447.

² Partridge, *Biochem. J.*, 1949, **44**, 521.

³ Cf. Davies and Owen, preceding paper.

acid/g. from any decinormal solution of a weak acid. A constant deduction of 0.10 milliequiv. has been made from our results, and leads to the values given in Table 1 for the amounts of acid adsorbed by 1 g. of resin in the hydroxide form.

Displacement Measurements.—A suitable quantity of acid was applied in decinormal solution to the top of a 10 cm. regenerated column of Dowex 1. Hydrochloric acid was then run on at the same rate as before. When the boundary reached the bottom of the column, samples were automatically collected, weighed, and titrated with 0.02N-sodium hydroxide in an atmosphere free from carbon dioxide. The last fractions were also analysed for chloride by subsequent titration with 0.02N-silver nitrate. Except for the first and the last fraction the concentration of the eluate was constant, and the flat portion of the concentration curve was made to extend over at least 10–15 ml. in order to fix this equilibrium concentration with accuracy. For the experiments at elevated temperatures jacketed columns were used.

Separation Experiments.—A column containing approximately 50 milliequiv. of resin was used, and for the more complex mixtures this was followed by a second, narrower, column, 6 cm. in height and 0.5 cm. in diameter. A known amount of acid mixture was applied to the top column and displaced with hydrochloric acid as in the earlier experiments. The fractions collected were titrated with sodium hydroxide solution, and the acids present were identified by submitting a drop of each fraction to paper chromatography by Hiscox and Berridge's method.⁴ In overlapping fractions the amount of each acid present was estimated from the relative size of the spots. This method broke down in the separation of phenylacetic acid from benzoic acid, which have almost identical R_F values, and was supplemented by the preparation of S-benzylthiuronium derivatives. The melting points of these, and mixed melting points with pure derivatives, enabled the amount of overlap to be determined.

RESULTS AND DISCUSSION

The results of our capacity measurements are given in Table 1, which shows the amounts of acid, in milliequiv., retained from 0.1N-solution by 1 g. (dry weight) of strong-base resin. Duplicate or triplicate measurements were made for each acid, and did not differ from the mean value shown by more than 0.01 milliequiv., except for phenylacetic acid where the average deviation was 0.06 milliequiv.

TABLE 1.

| Acid | Dowex 1 | Dowex 2 | Amberlite 410 | Acid | Dowex 1 | Dowex 2 | Amberlite 410 |
|-----------------|---------|---------|---------------|-----------------|---------|---------|---------------|
| Hydrochloric... | 3.45 | 3.84 | 3.26 | Butyric..... | 4.50 | 4.23 | 3.91 |
| Formic | 3.92 | 4.24 | 3.80 | Valeric | — | 4.88 | — |
| Acetic | 3.92 | 4.28 | 3.61 | Phenylacetic... | 10.4 | 7.3 | 5.5 |

It may be assumed that hydrochloric acid is retained wholly by salt formation, so that the hydrochloric acid value for each resin represents the exchange capacity of that resin. All the organic acids are retained in greater amounts than this, and the difference is ascribed to molecular adsorption. Figures thus derived for the molecular adsorption from 0.1N-solution are compared in Table 2 with values previously reported⁵ for adsorption on acid resins.

TABLE 2.

| Acid | Dowex 1 | Dowex 2 | Amberlite 410 | Amberlite 100 | Wofatit C |
|--------------------|---------|---------|---------------|---------------|-----------|
| Formic | 0.47 | 0.40 | 0.55 | — | — |
| Acetic | 0.47 | 0.44 | 0.35 | 0.16 | 0.14 |
| Butyric | 1.05 | 0.40 | 0.65 | 0.54 | 0.44 |
| Phenylacetic | 7.0 | 3.5 | 2.3 | 2.0 | 1.7 |

The present measurements were designed primarily to study the effect of molecular adsorption in column separations, and are therefore not strictly comparable with the results of the static experiments recorded in the last two columns of Table 2. Indeed, Davies and Thomas's measurements of the rate of adsorption⁵ make it likely, even after allowance for the smaller particle size of the basic resins, that adsorption equilibrium had not been completely established in our present results. Nevertheless, the two series are in

⁴ Hiscox and Berridge, *Nature*, 1950, **166**, 522.

⁵ Davies and Thomas, *J.*, 1951, 2624.

agreement in showing both the increasing importance of molecular adsorption with rising molecular weight, and the striking effect of an aromatic constituent. Adsorption on the basic resins is always significantly greater than on the acid resins, and this can be attributed to the much greater proportion of carbon in the former. Comparisons of the results for the basic resins amongst themselves must be regarded with some caution, as the Amberlite sample was much coarser than the Dowex resins, and it is possible that adsorption on the former was therefore less complete.

TABLE 3.

| <i>Formic acid</i> | | | | | | |
|------------------------------|---------|--------|--------|--------|--------|--------|
| Concn. of eluting acid | 0.05037 | 0.0750 | 0.1001 | 0.1600 | 0.2002 | 0.3244 |
| Concn. of eluate | 0.0550 | 0.0755 | 0.1220 | 0.1980 | 0.2660 | 0.5080 |
| <i>Acetic acid</i> | | | | | | |
| Concn. of eluting acid | 0.0504 | 0.0750 | 0.1001 | 0.1310 | 0.2002 | 0.3244 |
| Concn. of eluate | 0.0545 | 0.0735 | 0.1100 | 0.1520 | 0.2480 | 0.4880 |
| <i>Propionic acid</i> | | | | | | |
| Concn. of eluting acid | 0.0504 | 0.1000 | 0.2000 | 0.2704 | | |
| Concn. of eluate | 0.0580 | 0.1200 | 0.2960 | 0.4320 | | |

Table 3 contains the results of displacement measurements. It will be seen that when a basic column, to which a little organic acid has been applied, is developed with hydrochloric acid, the displaced acid always emerges at a higher concentration than the eluting acid, and the discrepancy between the two increases with increasing concentration. This is also an effect of molecular adsorption.

The theory of displacement development for non-electrolytes on a normal adsorbent was given by Tiselius.⁶ The substance, or mixture of substances, is forced down a column of adsorbent by a substance with a stronger adsorption affinity than any of the components. This "developer" displaces the other substances which in turn displace each other. If the column is uniform and sufficiently long a steady state is reached in which the components move down the column, at the same rate, in bands arranged in decreasing adsorption affinity. If a volume ΔV is introduced into the column and Δx is the distance moved by a band, $\Delta V/\Delta x_D = \Delta V/\Delta x_1 \dots$

The movement of the front of the developer band by Δx involves a quantity of developer given by :

$$M \cdot \Delta x \cdot a_D + \Delta x \cdot \alpha C_D = \Delta V \cdot C_D$$

where M is the weight of adsorbent per unit length of column, a_D is the amount of developer adsorbed by 1 g. of adsorbent, α is the volume between the particles of adsorbent per unit length of column, and C_D is the concentration of developer. A similar equation applies to each boundary :

$$M \cdot \Delta x \cdot a_1 + \Delta x \cdot \alpha C_1 = \Delta V \cdot C_1, \text{ etc.}$$

and rearrangement of these equations gives

$$(\Delta V/\Delta x - \alpha)/M = a_D/C_D = a_1/C_1, \text{ etc.}$$

The concentration of each component therefore depends upon its specific adsorption.

This theory does not apply to acids or bases moving down exchange columns without modification. For strong electrolytes which are bound on the column by salt formation alone the adsorptive capacity of the resin is simply the exchange capacity and is the same for all, so that the effluent (whether separation is complete or not) will throughout have the same concentration as the developer solution. For a weak electrolyte in the absence of complications from molecular adsorption or hydrolysis, the same considerations will apply if a correction is made for the undissociated molecules absorbed by the swollen resin; the quantity α will retain its original meaning for the completely dissociated developer acid

⁶ Tiselius, *Arkiv Kem. Min. Geol.*, 1943, A, 16, No. 18.

but must be replaced by α' , which allows also for the absorbed molecules, in the equations for a weak-acid boundary. This correction amounts to about 3% for 0.1N-acid of dissociation constant 1×10^{-5} and so is quite inadequate to account for the figures of Table 3, where specific adsorption effects must again be invoked to explain the high and varying eluate concentrations. Under these conditions the equation for the boundary movement of the developer may be written:

$$M\Delta x.\bar{C} + \Delta x.\alpha C_D = \Delta V.C_D, \text{ or } \bar{C}/C_D = (\Delta V/\Delta x - \alpha)/M,$$

where \bar{C} is the exchange capacity per gram of the strong-base resin; but for the weak electrolytes we must write:

$$M\Delta x.\bar{C} + M.\Delta x.a_1 + \Delta x.\alpha' C_1 = \Delta V.C_1, \text{ or } (\bar{C} + a_1)/C_1 = (\Delta V/\Delta x - \alpha')/M,$$

where a_1 is the specific molecular adsorption at concentration C_1 . If we neglect the small difference between α and α' we obtain:

$$(\bar{C} + a_1)/C_1 = \bar{C}/C_D, \text{ or } a_1 = (C_1/C_D - 1)\bar{C}.$$

Davies and Thomas⁵ found that the Freundlich equation, $a = A.C^{1/n}$, fitted their data quite well, and we have therefore plotted our values of $\log a_1$, calculated from the equation above, against $\log C_1$. The points show some scatter, but there is no evidence of curvature in the lines and the values found for the constants are: formic acid, $A = 3.2$, $n = 1.27$ ($a = 0.54$ for $C = 0.1$); acetic acid, $A = 3.6$, $n = 1.04$ ($a = 0.39$ for $C = 0.1$); propionic acid, $A = 3.9$, $n = 1.35$. As shown in parentheses, these results are in fair agreement with the estimates of adsorption made from the capacity measurements (Table 2).

Ion-exchange Chromatography of Acid Mixtures.—Molecular adsorption interferes most seriously with acid separation. We found, for instance, that formic and acetic acids can readily be separated quantitatively on Dowex 1 or Dowex 2 by displacement with hydrochloric acid; but that with a mixture of formic and phenylacetic acids the same procedure led to the displacement of the formic acid (the *stronger* acid), followed by a mixed band containing phenylacetic and hydrochloric acids. We have tested three ways of attempting to overcome this complication of adsorption.

Use of a strongly adsorbed acid as developer. Attempts were made to separate acetic and phenylacetic acids on Dowex 1 and 2 by using a 0.1N-solution of naphthalene-1-sulphonic acid as developer. This is a much stronger acid than the other two and is also likely to be strongly adsorbed by the resin. The results were disappointing; acetic acid emerged in a pure fraction, but it was followed by a mixture of the other two acids. Better results might have been obtained by using slower flow-rates and high concentrations of developer, but as these procedures would have practical disadvantages this method was abandoned.

Use of elevated temperatures. As a rise of temperature would be expected to reduce specific adsorptions, the adsorption of phenylacetic acid from 0.1N-solution was measured at a series of temperatures by the break-through capacity method. The results, in milliequiv./g., are in Table 4.

TABLE 4.

| | | | | | |
|-----------------|------|-----|-----|-----|-----|
| Temp., °c | 20 | 50 | 70 | 80 | 90 |
| Dowex 1 | 10.4 | 7.2 | — | 5.4 | 5.4 |
| Dowex 2 | 7.3 | — | 5.6 | 4.6 | 4.6 |

These results made it appear doubtful whether the improved separations on jacketed columns would compensate for their practical disadvantages, and attention was therefore directed to the third method.

Use of mixed solvents. Changes of solvent, as well as of temperature, may be used to modify the extent of adsorption of a substance. Break-through volumes were therefore measured on Dowex 2 for 0.1N solutions of phenylacetic acid in various dioxan-water mixtures,³ and gave the results shown in Table 5.

Clearly adsorption is strikingly reduced in this solvent, and some typical acid separations with 0.1N-hydrochloric acid as developer were next studied, 35% dioxan (v/v) being used as solvent. Table 6 gives the results. The amounts of each acid applied to, and recovered from, the column are expressed in milliequiv. The order of elution of the acids is that given by reading down Col. 1 of Table 6.

TABLE 5. 0.1N-Phenylacetic acid on Dowex 2.

| | | | | | | | | |
|---------------------------|------|------|------|------|------|------|------|------|
| Dioxan, vol. % | 0 | 10 | 28.9 | 32.5 | 40.0 | 50.0 | 64.4 | 81.5 |
| Capacity (mequiv./g.) ... | 7.34 | 5.40 | 4.38 | 4.06 | 3.76 | 3.75 | 3.77 | 3.76 |

These separations leave little to be desired. So far as can be judged by paper chromatography and the other tests applied the separations were complete, and within the possible error the amount of acid recovered was always the same as that applied. The acids appear in the theoretical order of their dissociation constants and there is a satisfactory separation in this order even of formic and phenylacetic acids, where large adsorption effects lead to a reversal of order in water. It may be noticed, too, that quite small differences in

TABLE 6.

| Acid | Dissociation constant $\times 10^6$ | Applied (mequiv.) | Recovered (mequiv.) | Error, % |
|---|-------------------------------------|-------------------|---------------------|----------|
| <i>Butyric-phenylacetic acid separation</i> | | | | |
| Butyric | 1.51 | 0.968 | 0.974 | + 0.7 |
| Phenylacetic | 4.88 | 0.805 | 0.805 | — |
| <i>Formic-acetic-propionic-phenylacetic acid separation</i> | | | | |
| Propionic | 1.34 | 1.000 | 0.999 | -0.1 |
| Acetic | 1.75 | 1.009 | 1.008 | -0.1 |
| Phenylacetic | 4.88 | 0.804 | 0.804 | — |
| Formic | 17.7 | 1.410 | 1.405 | -0.4 |
| <i>Acetic-phenylacetic-benzoic acid separation</i> | | | | |
| Acetic | 1.75 | 0.953 | 0.949 | -0.4 |
| Phenylacetic | 4.88 | 0.897 | 0.892 | -0.5 |
| Benzoic | 6.30 | 1.060 | 1.058 | -0.2 |

dissociation constant lead to perfect separations so long as complicating factors are eliminated.

This use of appropriate mixed solvents seems likely to be of very general value in the separation of organic mixtures. The beneficial effect of the organic solvent is primarily in the reduction in the amount of solute adsorbed, but will also be exerted in increasing the rate of attainment of adsorption equilibrium, since the exchange of an organic solvent molecule for a solute molecule at an adsorption site will involve a much smaller energy increment than will the desorption of the solute molecule in water. The partition effect will also normally lead to improved separations; in the experiments recorded in Table 6 the effluent concentrations were appreciably greater than decinormal, from which it may be deduced that the partition of the acids between the predominantly aqueous resin phase and the external phase was markedly in favour of the latter.

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