Liquid Chromatography of Analgesic Drugs on Ion-Exchange Resins

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Ion-exchange resins, cationic and anionic, serve as stationary phases for the liquid chromatography of analgesic drugs and various disubstituted benzenes, using aqueous ethanol as the mobile phase. No ion exchange is involved, but rather the solvent action of the polymer matrix for the organic solutes; however, a certain correlation can be drawn with the acidic or basic strength of the solute. Elution volumes depend strongly on the resin crosslinking and to a smaller extent on the counterion. The counterion affects the sharpness of the bands; ammonium ion gives the sharpest bands in a cation-exchange resin, chloride ion does the same in an anion-exchange resin. Some data are reported for a poly(vinylpyridine) resin.

The first applications of ion-exchange chromatography to the separation of nonionic organic compounds took advantage of complex formation between the organic compounds and inorganic ions held by the exchangers. Thus, sugars were separated on anion-exchange resins containing borate or bisulfite ions. Then it was found that the complexing ions were unnecessary. Sugars could be very effectively separated by chromatography on an anion-exchange resin in its chloride or sulfate form, and by a cation-exchange resin in its sodium or potassium form, using aqueous alcohol eluents (*1–3*). Aliphatic alcohols, esters, ketones, and aromatic hydrocarbons may be separated on columns of anion-exchange and cation-exchange resins, using as eluents aqueous salt solutions or alcohol–water mixtures (*4, 5*). It is evident that ion-exchange resins, particularly those based on polystyrene, can act as solid "solvents" for organic compounds, and particularly compounds containing the benzene ring. The "solvent" action resides in the styrene–divinylbenzene polymer matrix. The ionic groups are important because they solvate and cause the resin to swell, but their chemical nature and the sign of their ionic charge is secondary.

Phenacetin (acetyl-*p*-phenetidine, 4-ethoxyacetanilide), *p*-phenetidine, and acetanilide were separated by "salting-out chromatography" in potassium phosphate solutions on a cation-exchange resin (*6*). Recently a number of analgesic drugs, including caffeine, phenacetin, and acetylsalicylic acid, have been separated by chromatography on pellicular anion-exchange resins using dilute aqueous buffer solutions as eluents (*7–9*). The use of a cation-exchange resin for separating analgesic drugs was also reported (*10*). Analgesic drugs and a number of oxypurines were separated on a copper-loaded chelating resin (*11*). Coordination of the solutes with copper ions played a part in these separations, but the strong retention of phenacetin suggested that the solute–matrix interaction was important too.

Mono- and disubstituted benzenes have been separated on anion-exchange (*12*) and cation-exchange (*13*) resins using water–alcohol eluents. Isomeric butyl alcohols were separated on a potassium-form cation-exchange resin with water as eluent, tertiary butyl alcohol emerging first (*14*). The separation of barbiturates on a pellicular anion-exchange resin was reported (*15*). In none of these separations, except perhaps the last, did ion exchange occur; the dominant mechanism was absorption by the resin polymer matrix.

This investigation describes the use of nonpellicular bead-type and macroporous ion-exchange resins for the chromatography of various disubstituted benzenes and xanthines, using alcohol–water mixtures, electrolyte-free, as eluents.

EXPERIMENTAL

Apparatus. Two liquid chromatography systems were used, both supplied by Chromatronix, Inc., Berkeley, Calif. In one, glass columns 6 mm and 9 mm in diameter and about 40 cm long were used, with a variable-speed pulseless pump, Cheminert Model CMP-2, sample injection valve R6031SV with a 0.5-ml loop, and a Model 200 ultraviolet detector. The other apparatus was the Model 3100 high pressure liquid chromatograph, with stainless steel columns 2 mm in diameter and 50 cm long, an 0.02-ml sample injection loop and ultraviolet detector.

Resins. The cation-exchange resins were all of the sulfonated, cross-linked polystyrene type. The following *gel-type resins* were supplied by Bio-Rad Corp., Richmond, Calif.: AG 50W-X4, AG 50W-X8, AG 50W-X12, each 200–400 mesh; Aminex Q-150 S, 8% crosslinked and 20–35 microns particle diameter; Aminex AG 50W-X4, 20–30 microns particle diameter. The *macroporous resin* Amberlyst-15 was supplied by Rohm and Haas Co., Philadelphia. It was ground and wet-screened through 100 mesh, then washed thoroughly with hydrochloric acid and water.

The anion-exchange resin, AG 1-X8, 200–400 mesh, was of the polystyrene–quaternary ammonium type, supplied by Bio-Rad Corp.

The "PVP" resin was a copolymer of 2-methyl-5-vinylpyridine with 9% of divinylbenzene, below 325 mesh, supplied on an experimental basis by Bio-Rad Corp. The chromatographic behavior of this resin has been described by Freeman (*16, 17*).

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RESULTS

Separations on Cation-Exchange Resins: Effect of Solvent Counterion and Crosslinking. The first experiments were made with a mixture of aspirin, phenacetin and caffeine, using the resin AG 50W-X8, 200–400 mesh. Because of the wide range of particle sizes in this resin the bands were broad, but separation was good, and it was possible to study the effects of solvent and counterion on the elution volumes and band widths. The data are summarized in Tables I and II. The effect of solvent composition is very marked, and the nature of the counterion affects not only the elution volume but also the band width. Ammonium ions give the sharpest bands, and sodium ions the least sharp bands.

This effect is seen in Figure 1. The curves shown here were obtained with a resin of relatively uniform particle size, Aminex Q-150 S (see above). Band spreading is almost certainly determined by the rate of diffusion within the resin, and it appears that the solute molecules diffuse fastest when the counterion is NH_4^+. We have no explanation for this, nor have we been able to correlate the effect with other properties of the ammonium-loaded resin.

Figure 1 shows an effect which we have observed repeatedly, a fluctuation in the absorbance reading immediately following the aspirin peak and, in general, occurring at about 1.5 void volumes. It is caused by a fluctuation in solvent composition which affects the proportion of light energy transmitted by the cell windows. The solvent is a mixture of ethanol and water (1:3 by volume), and if the solvent injected with the sample does not have exactly the same proportion of ethanol, a concentration pulse travels along the column at a rate that depends on the slope of the partition isotherm between the resin and solvent. These partition isotherms have been studied by Ohtaki *et al.* (*18*).

The effect of crosslinking is shown in Table III and Figures 2 and 3. Two series of tests were made. In the first, resins 200–400 mesh, AG-50W, of 4%, 8%, and 12% crosslinking were run in 2-mm × 50-cm columns. In the second series, Aminex resins of controlled particle size were used in columns 2-mm × 150-cm for the 8% crosslinked resin Q-150 S, 2 mm × 100 cm for the 4% crosslinked resin. The 4% crosslinked resin offered more resistance to flow than the 8% resin, which is why we had to use different column lengths and different flow rates.

To compare different column lengths, retention volumes are quoted as multiples of the bulk column volume. The 8% crosslinking gives the highest retention volumes for most compounds but not all. Volumes for 12% crosslinking are much smaller than for 8% or 4% crosslinking. The most likely reason for this behavior is steric exclusion. The 4% resin, taking into account its greater solvent uptake and lower bulk density, is a better absorbent than the 8% resin, and it is seen from Figures 2 and 3 that the relative magnitudes of the retention volumes of different compounds are different for the two resins. The 4% resin gives much better chromatographic separation of the four compounds chosen than does the 8% resin.

A few tests were made with the macroporous cation-exchange resin, Amberlyst-15. The results are shown in Table IV and Figure 4. Considering the fact that no attempt was made to control particle size except for screening through 100 mesh, the sharpness of the peaks is impressive, and suggests what might be achieved if bead-type macroporous resins of closely controlled particle size were available. However, the distribution ratios for different solutes on the macroporous resin did not differ as much among themselves as they did on the gel-type resins. Caffeine and theobromine, for example, could be resolved on the gel-type resins but not on the macroporous resin.

Table I. Effect of Solvent[a]

Counter-ion	Solvent	Peak elution volume: Aspirin	Caffeine	Phenacetin
H^+	Water	44	...	...
	2-Propanol, 33%	12	large	35
	50%	13	large	17
	66%	12	116	...
	2-Propanol, 66% with acetic acid, 5%	...	131	...
NH_4^+	Water	8	...	130
	2-Propanol, 33%	7	11.5	22
	66%	6	12.5	9
	Ethanol, 33%	7	14.5	27
	Ethanol, 25%	7	20	57

[a] Resin, AG 50W-X8. Column size, 6 mm × 45 cm.

Table II. Effect of Counterion[a]

Counter-ion	Caffeine: V,[b] ml	Caffeine: N	Phenacetin: V, ml	Phenacetin: N
NH_4	20.5	68	57	52
K	24	31	58	21
Na	21	26	50	20
Li	31	56	68	34

[a] Resin, AG 50W-X8. Column size, 6 mm × 44 cm. Solvent, 25% ethanol by volume. Flow rate, 24 ml/hr. [b] *V* is the measured peak elution volume in ml, uncorrected for void volume; *N* is the plate number, $(V/\text{standard deviation})^2$.

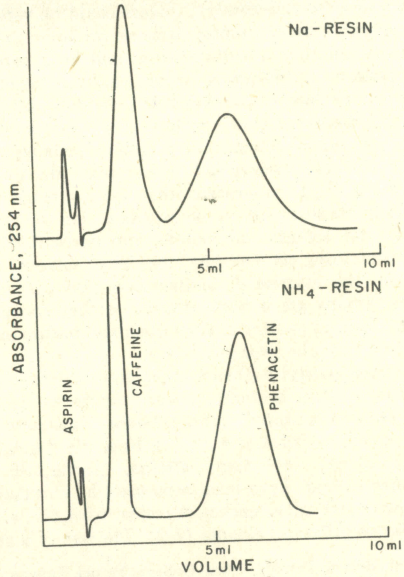

Figure 1. Effect of counterion on resolution. Columns 2 mm × 50 cm; flow rate 12 ml/hr. Quantities introduced: aspirin 4, caffeine 2, phenacetin 1 microgram. Full-scale absorbance 0.32

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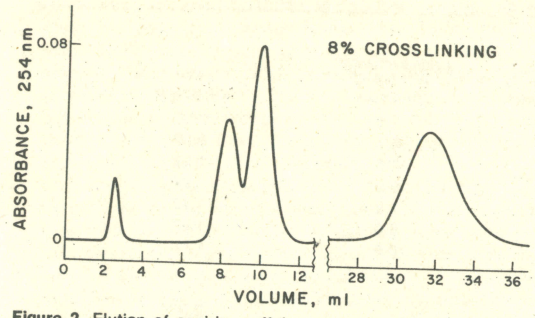

Figure 2. Elution of aspirin, caffeine, acetaminophen (4-hydroxyacetanilide) and salicylamide: quantities, 12, 1.5, 1, and 10 micrograms, respectively. Aminex Q-150 S resin, 2 mm × 150 cm, flow rate 6 ml/hr; solvent, 25% ethanol

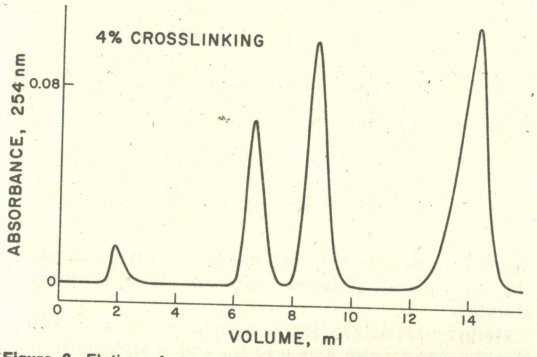

Figure 3. Elution of same mixture as in Figure 2. Aminex AG 50W-X4 resin, 2 mm × 100 cm, flow rate 4 ml/hr; solvent, 25% ethanol

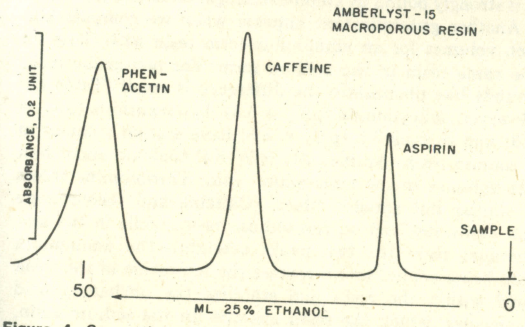

Figure 4. Separation on macroporous resin, ammonium form. Amberlyst-15 resin, 9 mm × 53 cm, flow rate 12 ml/hr; solvent, 25% ethanol. Quantities: aspirin 0.25 mg, caffeine 0.10 mg, phenacetin 0.05 mg. Aspirin peak eluted at 15 ml, one void volume

An example of what can be done with gel-type resins by improved techniques is shown in Figure 5. This is a chromatogram of the drug "Excedrin," with the same constituents as the mixtures shown in Figures 2 and 3, on the same ammonium-form, 4% crosslinked resin used for Figure 3. (In the sodium form, the elution order is changed; the new order is aspirin, salicylamide, caffeine, acetaminophen, with the last two peaks incompletely resolved). This resin gives narrow peaks and excellent resolution of many mixtures but has the drawback that it is soft and

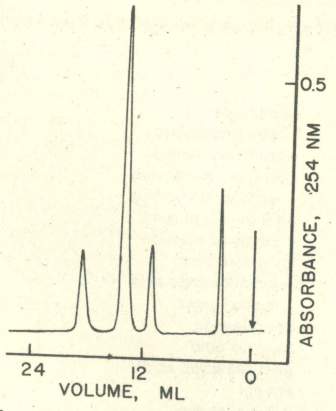

Figure 5. "Excedrin" tablet, 0.035 mg; resin, Aminex AG 50W-X4, 20-30 microns, NH_4 form; column 6 mm × 42 cm, temp. 70°C; eluent, 25% ethanol; flow rate, 12 ml/hr; pressure, 120 lb per square inch. Elution order: aspirin, caffeine, acetaminophen, salicylamide. Aspirin peak shown at double sensitivity

Table III. Effect of Crosslinking[a]

Resin	Compound	Elution volume for crosslinking 4%	8%	12%
AG 50W	2-Ethoxyacetanilide	2.25	2.55	1.2
	4-Ethoxyacetanilide	2.9	3.1	1.4
	Ethyl benzoate	4.85	6.7	3.5
	Acetanilide	2.15	3.76	2.0
Aminex	Aspirin	0.6	0.55	...
	Caffeine	2.0	1.75	...
	4-Hydroxyacetanilide	2.7	2.1	...
	Salicylamide	4.3	6.4	...

[a] Resins, AG 50W-X12 and Aminex. Columns, 2-mm diameter. Solvent, 25% alcohol by volume. Elution volumes are expressed as multiples of bulk column volume. Column lengths were 50 cm for AG-50W, 100 cm for Aminex 4% crosslinked, 150 cm for Aminex 8% crosslinked. Both resins were in ammonium form.

Table IV. Macroporous and Gel-Type Resins

Compound	Elution vol., multiple of bulk column vol. AG 50W-X8	Amberlyst-15
Theobromine	1.3	1.3
Caffeine	1.65	1.2
Acetanilide	3.75	1.85
4-Methylacetanilide	5.75	2.2
4-Methoxyacetanilide	5.1	2.0
4-Ethoxyacetanilide	5.5	1.95
Ethyl benzoate	6.7	3.0

Table V. Anion-Exchange Resin: Counterion Effect[a]

Counterion	Bed depth, cm	Caffeine V	Caffeine N	Phenacetin V	Phenacetin N	2-Methylacetanilide V	2-Methylacetanilide N
Cl	48	17	90	66	60	45	90
Br	44	15	...	74	48	52	58
SO_4	44	...	...	50	22	30	30

[a] Resin, AG1-X8. Column diameter, 6 mm. Solvent, 25% ethanol. Flow rate, 24 ml/hr. Elution volumes in ml; for V and N, see Table II.

Table VI. Elution Volumes in Multiples of Bulk Column Volume

	Resin: Cation exchanger: Q-150S-NH_4 Solvent: 25% Ethanol	Anion exchanger: AG1-X8–Cl 25% Ethanol	PVP 2-Propanol
Acetanilide	2.0	8.1	1.07
2-Methylacetanilide	1.85	3.4	0.92
4-Methylacetanilide	2.9	12.5	1.02
4-Hydroxyacetanilide	2.0	9.9	1.84
4-Methoxyacetanilide	2.7	7.9	1.13
2-Ethoxyacetanilide	2.55	5.0	1.07
4-Ethoxyacetanilide	3.1	10.0	1.00
Ethyl benzoate	6.7	...	...
4-Aminobenzoic acid methyl ester	8.0	...	2.3
Salicylamide	6.4	...	...
Salicylic acid	0.5	...	...
Acetylsalicylic acid	0.65	...	...
Phenol	...	...	2.18
2-Aminophenol	...	...	3.34
3-Aminophenol	...	...	5.20
4-Aminophenol	...	...	2.62
1,3-Dimethylxanthine (theophylline)	1.22	16.0	1.60
3,7-Dimethylxanthine (theobromine)	1.15	1.15	1.9
1,3,7-Trimethylxanthine (caffeine)	1.28	1.22	1.14
Hypoxanthine	1.85	4.2	1.44

collapses under high pressure gradients. It performs better at higher temperatures, where the solvent viscosity is lower, diffusion rates are higher, and the resin is more rigid.

Anion-Exchange and PVP Resins. Only one anion-exchange resin was tested, AG 1-X8. Three counterions were compared, with the results shown in Table V. Chloride is definitely superior in plate number to bromide and sulfate, and the bed expansion is greater with chloride, indicating a more open resin structure which permits faster diffusion. Comparing anion- and cation-exchange resins, the anion-exchange resin gives larger retention volumes for most compounds tested (see Table VI).

The PVP resin was used with 100% isopropyl alcohol as solvent. The recent work of Freeman *et al.* (*17*) shows that this was a bad choice. It is a "levelling solvent" which itself binds strongly with the resin, so that the more weakly bound substrates all elute at around one column volume. The phenols, being acidic, are more strongly bound, and a very good chromatographic separation was achieved for the three isomeric aminophenols.

Comparison of Various Solutes. Table VI presents the result of a large number of tests made in 50-cm columns with the Model 3100 high-pressure liquid chromatograph. The elution volumes are greater, and show greater differences, with the anion-exchange resin than with the cation-exchange resin.

Looking over Table VI, we can make a few generalizations and rationalizations. The most important property that influences the affinity of the solute to the resin appears to be its acid or base strength. Salicylic and acetylsalicylic acids are relatively strong acids (pK about 4) and they are held weakly, if at all, by the cation-exchange resin. They are bound strongly by anion-exchange resins (*7*). Among the four xanthines, theophylline is the most acidic (pK_a = 8.8) and is bound more strongly by the anion-exchange resin than the next most acidic, which is hypoxanthine (pK_a = 8.94). The order of binding of the isomeric aminophenols by PVP is the same as the order of their basic strengths (pK_b: 4.0 meta, 4.55 ortho, 5.55 para) (*19, 20*), with the strongest base being the most strongly absorbed.

Methyl *p*-aminobenzoate, or 4-aminobenzoic acid methyl ester, has a basic amino group and is strongly bound both by the cation-exchange resin and PVP, as one would expect. Salicylamide is strongly bound by the cation-exchange resin, even though it is an acid with pK_a about 8. It is strongly bound by anion-exchange resins also (*7*).

Another generalization appears when we compare elution volumes for an ammonium-form resin with those for the same resin in the sodium form. For non-acidic compounds like phenacetin the difference is small (Table II). However, salicylamide (pK_a = 8.4), hypoxanthine (pK_a = 8.9) and theophyllin (pK_a = 8.8) have elution volumes in a sodium resin (Aminex AG 50W-X4) that are about half the volumes in the ammonium resin. Theobromine shows a similar but smaller effect. (Caffeine and theobromine are easily resolved on the sodium resin, while in the ammonium resin the two peaks overlap.) The ammonium ion, being acidic, would repress the ionization of the weak acid within the resin and stabilize the uncharged acid molecules, which the resin absorbs. In the sodium resin, the weak acids can displace sodium ions and release the salts of the acids, which the resin would not absorb. This effect, if it proves to be general, could help the chromatographer to establish the identity of unknown peaks in mixtures.

Other generalizations can be seen from Table VI, but it is premature to offer rationalizations at this stage.

CONCLUSIONS

We have shown that ion-exchange resins are effective stationary phases for the liquid chromatography of nonionic organic compounds, using electrolyte-free aqueous

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alcohol as eluent. The nature of the counterion affects the elution volumes and has a marked effect on the sharpness of the elution bands. Of the counterions tested, ammonium ion gave the sharpest bands in a cation-exchange resin, while chloride ions gave the sharpest bands in an anion-exchange resin. The degree of crosslinking has a great effect, not only on the elution volumes as a whole, but on the relative volumes found with different solutes. On the whole, the best chromatographic curves were obtained with a resin of 4% crosslinking.

Compared to chromatography on pellicular resins, that on gel-type resins requires more time. This is especially true with the softer resins that deform under high pressure gradients. High flow rates cannot be used with these resins. Gel-type resins have the advantage, however, that they give excellent resolution (Figure 5 indicates theoretical plate heights of 0.2 mm and less) and above all, that they allow elution volumes to be manipulated by changing counterions and crosslinking. For preparative chromatography, the greater capacity of gel-type resins is an obvious advantage.

The use of macroporous resins is promising. When these resins become available in small, uniform particle sizes, they will combine high capacity with good resolution at high flow rates. They are, of course, highly crosslinked by their nature and will not show the range of selectivity orders that is possible with gel-type resins.

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