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CHROMATOGRAPHIC SEPARATION OF PHENOLS USING AN ACRYLIC RESIN

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SUMMARY

A liquid chromatograph is described that uses gas pressure on water to force eluents through the column. A macroporous polyacrylate resin is used in conjunction with methanol–water or basic aqueous eluents for separation of mixtures of phenols. The elution characteristics of twenty-one phenols are described. Several separations of actual mixtures are shown to demonstrate the separation capabilities of this system.

INTRODUCTION

Recently a series of macroporous resins has become available. The surface area of these resins is at least one order of magnitude greater than microporous resins[1,2]. The different structure of the absorbent results in compounds with low dielectric strength adsorbing at much faster rates[1]. The larger pore size allows large molecules to be chromatographed such as α-amylase which has a molecular weight of 100,000[3]. POLLIO AND KUNIN discuss some of the variables influencing the adsorption of protein to macroporous resins[4].

The macroporous XAD series of resins from Rohm and Haas have been used to extract phenols, alkyl sulfonic acid, dyes, steroids, vitamin B-12, and fulvic acid from waste streams[3]. XAD-1 has been used to concentrate organic material from seawater[5]. XAD-2 and XAD-7 have been used to extract and partially separate organic matter from potable water[6]. XAD-2 has been used to extract drugs and metabolic products from urine[7–13]. ZAIKA *et al.* have studied the separations of amino acids, carbohydrates, and nucleotides on XAD-2[14,15].

In this paper a macroporous polyacrylic resin, Rohm and Haas XAD-7, is used for the column chromatographic separation of mixtures of phenols. This resin does not require a stationary liquid phase; hence bleeding is not a problem even at high pressures. It is much lower in cost than the superficially porous supports commonly used in high-speed liquid chromatography. Finally, this macroporous resin is reasonably efficient so that good chromatographic separations can be achieved in a fairly short time.

EXPERIMENTAL

The liquid chromatograph

A schematic diagram of the liquid chromatograph is shown in Fig. 1. Except

where noted, all tubing that carries air or water is 1/4-in. Synflex nylon tubing No. N3-4203 from Globe Machinery and Supply Co., Des Moines, Iowa. This tubing carries a pressure rating of 1500 lbs. The tubing is connected to 1/4-in. pipe fittings with Gyrolock connectors No. 4CM4 from Hoke Inc. All tubing carrying eluent is Teflon® tubing No. To63031 from Chromatronix.

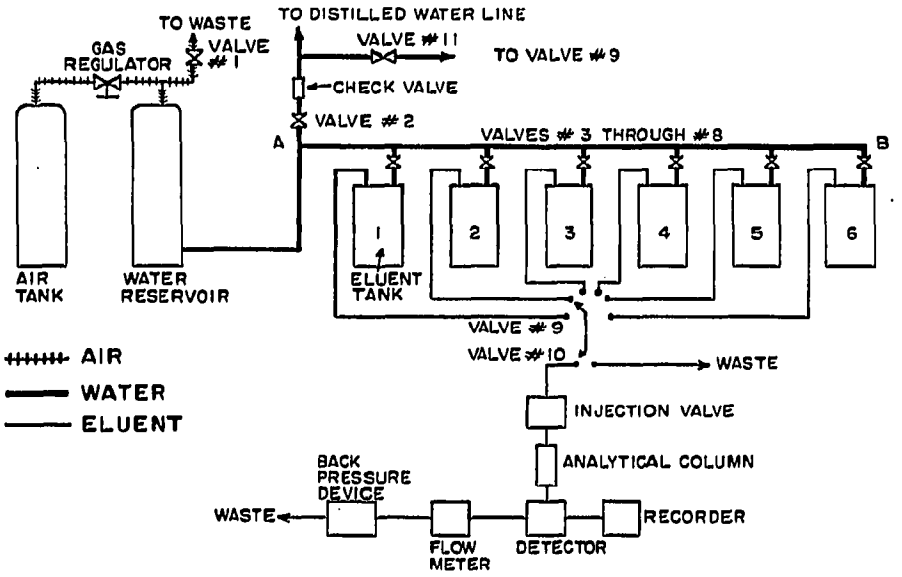


Fig. 1. Schematic diagram of liquid chromatograph.

A helium tank was filled with air at pressures up to 2200 lbs. for use as a source of air pressure. When the liquid chromatograph is operated at pressures greater than 60 lbs., a high-pressure regulator, Matheson Scientific Co. Model No. 580-2 is used. For pressures less than 60 lbs., a low-pressure helium regulator is used. This is connected to the water reservoir with nylon tubing. The water reservoir is an oxygen tank that was modified by drilling a water outlet hole near the bottom tapped for 1/4-in. NPT threads. The fitting at the top was fitted with a connector to go to 1/4-in. NPT threads. To fill the water reservoir, valve No. 1 is opened, releasing air in the reservoir. Simultaneously, valve No. 2, which is connected directly to the distilled water line, is opened. Valves Nos. 1, 2, and 11 are all Hoke valves No. 3212M4B. Valve No. 11 controls the flow of water purging the eluent selection valve, No. 9. The check valve is Hoke No. 6113F4B. The water line going from A to B is made up of 1/4-in. stainless-steel fittings: six tees, one elbow, and six 6-in. nipples. Water inlet valves Nos. 3 through 8 are Hoke valves No. 3222H4B.

A diagram of the eluent tanks is shown in Fig. 2. Each tank is made from 5-in. schedule 80 steel pipe, 12 in. long. In the flange of the tank, eight 3/8-in. holes were drilled 7/16 in. from the edge and equally spaced around the tank. The same diameter holes were drilled in the lid to match. The three holes for air outlet, eluent outlet, and water inlet were drilled 1.75 in. from the edge equally spaced around the lid. The lid was bolted on with 3/8 × 2 1/4 in. bolts and the bolt head was spot-

welded in place. The lid was removed, after which the holes in the lid were enlarged to 7/16 in. The air escape valve is Hoke No. 3222M2B. A 1/4-in. O-ring is placed on the Kel-F® connector (see Fig. 3), which is screwed into the lid so that the O-ring is on the inner side of the lid. Nylon tubing connects to the water inlet using connectors described earlier.

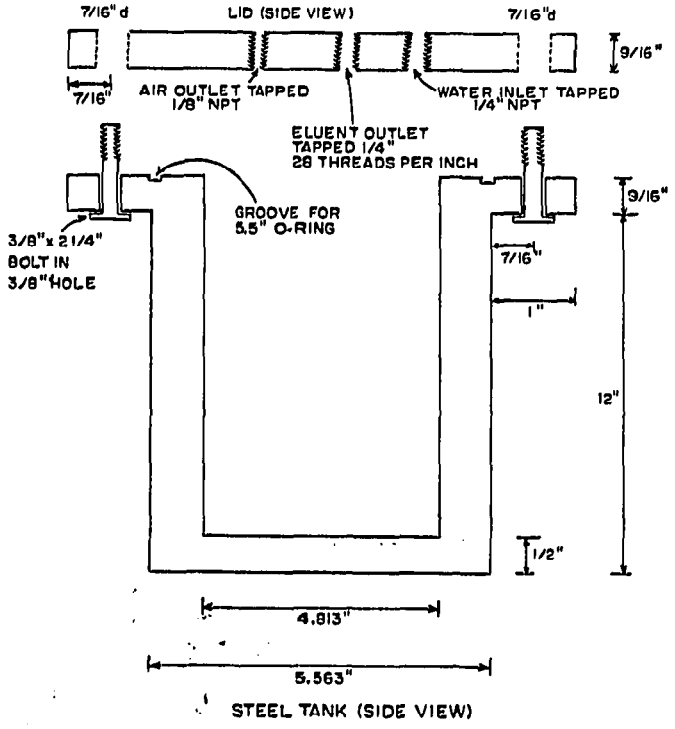


Fig. 2. Diagram of eluent tank.

The eluent goes in a 2.5-l plastic bag, Matheson Scientific No. 12657-10. The liner from the plastic bag cap was removed and a 0.775-in.-diameter hole was drilled in the center. The Teflon® hat (Fig. 4) was put back into the cap replacing the liner. When the cap was screwed back on to the plastic bag, the Teflon® hat was held in place. A short piece of Chromatronix tubing connects the Teflon® hat and the Kel-F® connector. The plastic bag is placed in the steel tank and bolted tight. Teflon® tubing then connects the Kel-F® connector to the eluent selection valve, No. 9, which is a six-way valve, No. R60V6K. Valve No. 10 is No. CAV-3031K and the sample injection valve is No. SV-8031K. All these parts are from Chromatronix. A small sample loop was made and calibrated by filling the loop with 1 *M* HCl, collecting the contents of the loop and titrating this with 0.01 *M* NaOH. The volume of the loop was found to be 38.1 μl.

A Chromatronix Model 200 UV detector was used to monitor the effluent. Roger Gilmont Instruments, Inc. No. 3201 size No. 1 flow meter was used. Adaptors were machined of Kel-F® following the Chromatronix design for the G1-C glass connector to allow attachment of Chromatronix fittings. A 10-ft. length of Chroma-

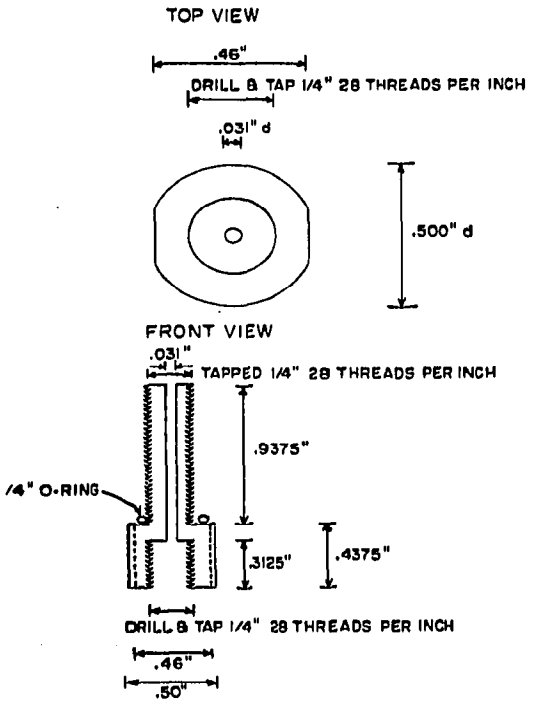


Fig. 3

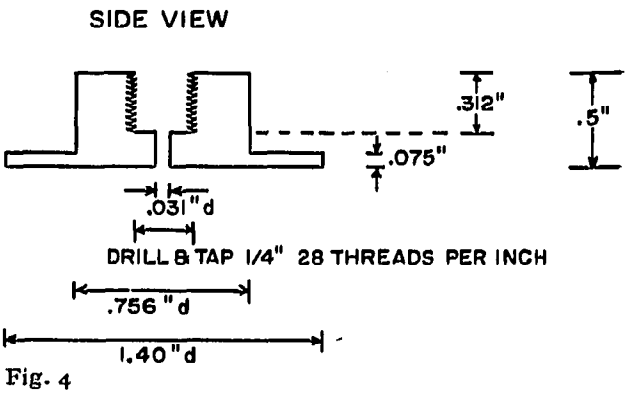


Fig. 4

Fig. 3. Kel-F connector.

Fig. 4. Teflon® hat.

tronix tubing, No. T063012, was used to produce back pressure and prevent gas desolubilization. For a flow-rate of 1.0 ml/min the pressure drop across this tubing is 34 p.s.i. Most of the time this was sufficient. For the rare times when it was not, a Fischer and Porter needle valve, No. 795-609, was used by fusing a Chromatronix No. G1-C glass connector to the needle valve. This allowed the needle valve to be connected to the Teflon® tubing being used.

Operating suggestions

The plastic bags will hold about 2.5 l but it works better if they are filled with no more than 2 l. The bags contain a plasticizer which causes the eluent to become contaminated. Filling the bags with methanol and letting them stand for a week before use eliminates the contamination.

After a plastic bag has been placed in the steel tank and the lid bolted down, all air must be removed from the tank. To remove the air surrounding the plastic bags open the water inlet valve letting water into the eluent tank. Simultaneously open the air release valve at the top of the eluent tank. As soon as water starts to come out the air release valve, close it. To remove any air present initially in the plastic bag, turn the eluent selection valve to that tank while simultaneously turning valve No. 10 to waste position. Leave it in this position until eluent goes through valve No. 10. This eliminates most of the air. Any air remaining will stay there unless the operating pressure is very low (less than 25 to 30 p.s.i.) or the bag is almost empty.

Discussion of design

The construction materials for the liquid chromatograph were chosen for their resistance to corrosion. Only Kel-F®, glass Teflon® and polyethylene contact the liquid so a variety of acids and solvents can be used.

The ultimate source of pressure for the liquid chromatograph is high-pressure air. The air puts pressure on water, which puts pressure on the eluent. This is an indirect system but for reasons of safety it was decided to have liquid rather than gas under pressure in all the parts of the system where this could reasonably be accomplished.

Originally, helium was used as the source of pressure but it was discovered that water vapor seeped into the helium tank. Rather than return the tank to be refilled with helium, which would then be contaminated with water, it was decided to keep the tank and fill it with air. The local fire department refilled the tank whenever necessary with pressures of about 2400 lbs. The maximum pressure that can be placed on the eluent for this liquid chromatograph is 500 lbs., which is the pressure rating of the Chromatronix parts used.

There are several advantages of an air pressure system over a pump. These have been discussed elsewhere[16,17].

Reagents

The adsorbent, XAD-7, a macroporous resin from Rohm and Haas, was ground in a Model 4-E Quaker City mill and sieved dry. The resin that passed through the 325-mesh screen was placed in a 250-ml beaker filled with methanol, stirred, and allowed to settle. Any resin that did not settle to the bottom in 5 min was discarded. This procedure was repeated several times and that which remained was used.

The phenols were obtained from several sources and were the best grade available. They were used without further purification.

The aqueous solutions were either 0.05 *M* NaOH, 0.05 *M* $NaHCO_3$, 0.05 *M* Na_2CO_3 or combinations thereof so as to yield the desired pH.

Choice of flow-rate

The flow-rate used in all cases was 1 ml/min. Using this flow-rate, the height of a theoretical plate for the XAD-7 was 3.5 mm as determined using phenol and 3.8 mm using *o*-chlorophenol.

Distribution coefficients

Distribution coefficients were determined for many phenols by eluting a phenol from a 2.8 mm × 13 cm column at ambient temperature with some combination of water and methanol at 1 ml/min and measuring the retention volume. The volume of solution injected was 38.1 μl. The amount of phenol injected varied from 10 to 50 μg. Using the same column containing the same resin, the interstitial volume, V_0, was determined by eluting a 1% solution of acetone in methanol with 100% methanol and measuring the retention volume of the acetone peak. Acetone was used for the measurement of V_0 because it appeared not to be retained under the conditions of elution. The weight of resin used was measured by waiting until the retention volumes of many phenols were measured, extruding the resin, and weighing it. From these data, the distribution coefficient, D, was determined by using the

formula, $\bar{V} = V_0 + Dg$ where g is the weight of air dried resin in grams and $\bar{V}$ is the retention volume.

RESULTS

Water–methanol system

Distribution coefficients were determined for several phenols between XAD-7 resin and liquid phases containing various combinations of water and methanol. The results are summarized in Figs. 5–7, where log D is plotted as a function of log

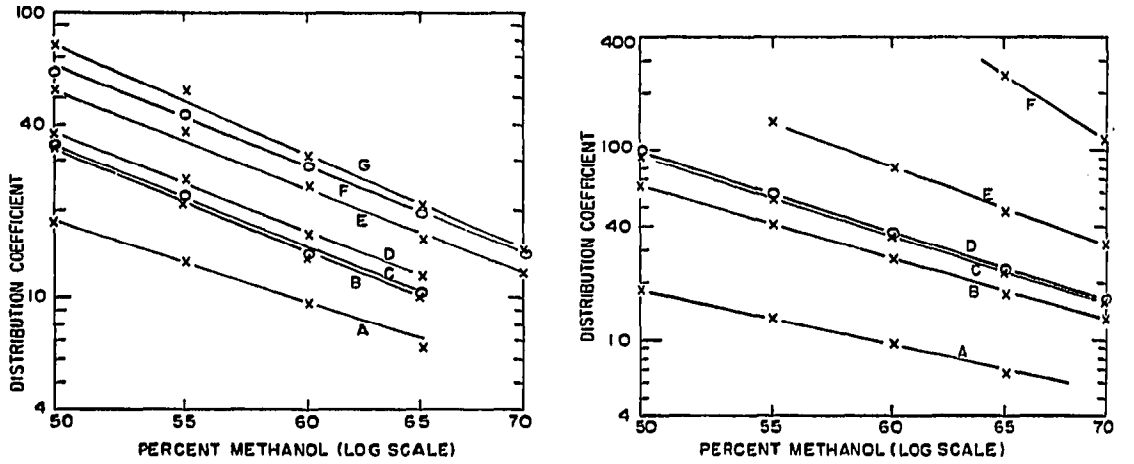


Fig. 5. Distribution coefficients of methyl-substituted phenols on Amberlyst XAD-7 in water–methanol solutions of varying percent. A = Phenol; B = *p*-cresol; C = *m*-cresol; D = *o*-cresol; E = 3,5-dimethylphenol; F = 2,6-dimethylphenol; G = 3,4,5-trimethylphenol.

Fig. 6. Distribution coefficients of bromo-substituted phenols on Amberlyst XAD-7 in water–methanol solutions of varying percent. A = Phenol; B = *o*-bromophenol; C = *p*-bromophenol; D = *m*-bromophenol; E = 2,4-dibromophenol; F = pentabromophenol.

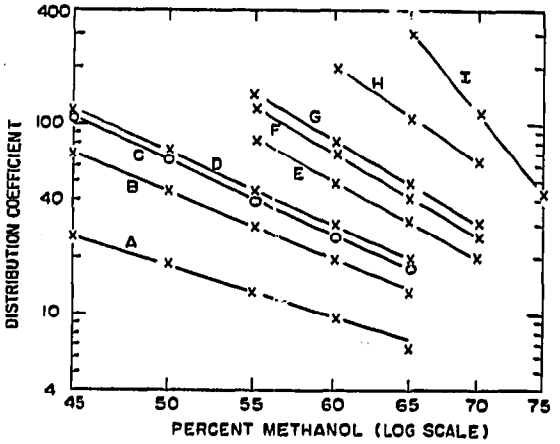


Fig. 7. Distribution coefficients of chloro-substituted phenols on Amberlyst XAD-7 in water–methanol solutions of varying percent. A = Phenol; B = *o*-chlorophenol; C = *p*-chlorophenol; D = *m*-chlorophenol; E = 4,6-dichlorophenol; F = 3,5-dichlorophenol; G = 2,3,6-trichlorophenol; H = 2,3,4,5-tetrachlorophenol; I = pentachlorophenol.

methanol concentration. Since the graphs are linear, each compound can be represented by an equation of the form,

$$\log D = a \log \% \text{ MeOH} + b \quad (1)$$

where a is the slope and b is the intercept. The slope and intercept of all phenols measured are given in Table I.

TABLE I

VALUES OF a AND b FOR EQN. 1 FOR SOME PHENOLS

Compound	$-a$	b
Phenol	3.663	7.479
o-Bromophenol	4.885	10.112
p-Bromophenol	5.253	10.885
m-Bromophenol	5.389	11.146
2,4-Dibromophenol	6.250	13.021
Pentabromophenol	10.749	21.884
o-Chlorophenol	4.558	9.387
p-Chlorophenol	4.992	10.286
m-Chlorophenol	4.939	10.241
2,4-Dichlorophenol	5.899	12.183
3,5-Dichlorophenol	6.398	13.213
2,3,6-Trichlorophenol	6.321	13.150
2,3,4,5-Tetrachlorophenol	7.441	15.528
Pentachlorophenol	13.65	27.23
p-Cresol	4.618	9.366
m-Cresol	4.543	9.245
o-Cresol	3.425	7.365
3,5-Dimethylphenol	4.512	9.405
2,6-Dimethylphenol	4.130	8.809
3,4,5-Trimethylphenol	5.053	10.486
2,4,6-Trimethylphenol	5.016	10.503

Conditions for column chromatographic separation of various phenol mixtures can be predicted from the data in Table I. Using these data it is also possible to derive an equation relating the resolution achieved in separating any two compounds with the percentage of methanol in the eluent. Some of the symbols used in the derivation are illustrated in Fig. 8. The symbols not illustrated are defined below.

$\delta V = \beta/2$

N = number of theoretical plates

V_0 = interstitial volume of the column

a_1, a_2 = value of a for compounds 1 and 2, respectively, as defined in eqn. 1

b_1, b_2 = value of b for compounds 1 and 2, respectively, as defined in eqn. 1

D = distribution coefficient

g = grams of resin used in the column

C = percent methanol in a water–methanol solution

V = retention volume

The usual formula for resolution is

$$R = \frac{2d}{w_1 + w_2} \quad (2)$$

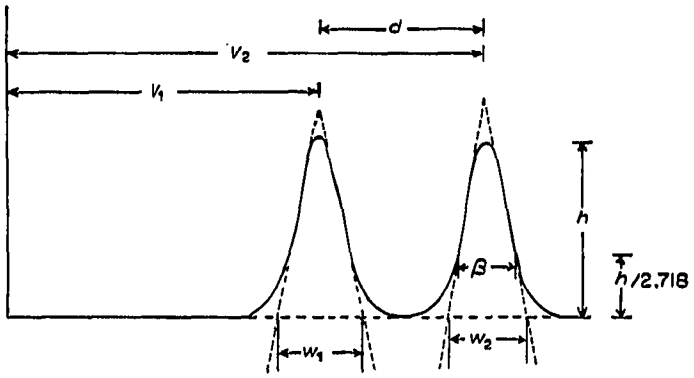


Fig. 8. Typical elution curve illustrating various symbols.

It can be shown that:

$$d = \bar{V}_2 - \bar{V}_1$$
$$w = \beta\sqrt{2}$$
$$\beta = 2(\delta V)$$

Substituting the values of d, w, and β into eqn. 2 gives the result:

$$R = \frac{(\bar{V}_2 - \bar{V}_1)}{(\delta V_1 + \delta V_2)\sqrt{2}} \tag{3}$$

RIEMAN AND WALTON[18] have shown that

$$\delta V = \bar{V}\sqrt{\frac{2}{N}}$$

Substituting this into eqn. 3, gives the result

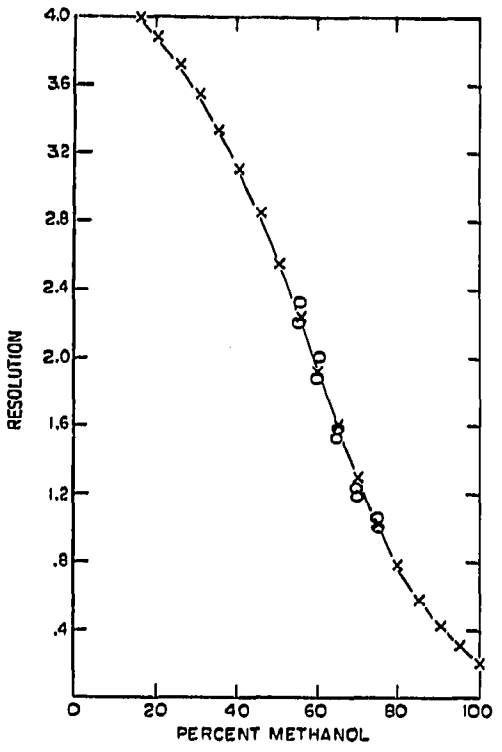
$$R = \frac{(\bar{V}_2 - \bar{V}_1)\sqrt{N}}{2(\bar{V}_1 + \bar{V}_2)} \tag{4}$$

Solving eqn. 1 for $\bar{V}$ and substituting into eqn. 4 yields the following equation

$$R = \frac{g(10^{b_2 + a_2 \log C} - 10^{b_1 + a_1 \log C})\sqrt{N}}{2(2V_0 + g10^{b_2 + a_2 \log C} + g10^{b_1 + a_1 \log C})} \tag{5}$$

The validity of eqn. 5 was proved by eluting a mixture of phenol, *m*-bromophenol, and 2,4-dibromophenol with varying percentages of methanol. The column used was the same length, and contained the same weight resin as was used in determining the distribution coefficients. The resolution was measured between the first two compounds, phenol and *m*-bromophenol, and the last two compounds, *m*-bromophenol and 2,4-dibromophenol. The experimental and theoretical values are shown for each pair of compounds in Figs. 9 and 10.

In using eqn. 5, it is suggested that one decide what is the minimum resolution that is needed. Then find the percentage methanol that the eluent must have to give



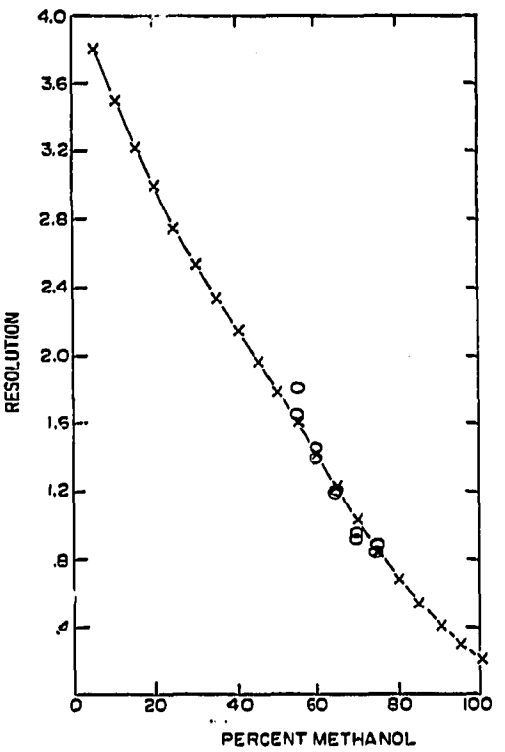


Fig. 9. Resolution of phenol and *m*-bromophenol on Amberlyst XAD-7 in water–methanol solutions of varying percent. ×, Predicted values; ○, experimental values.

Fig. 10. Resolution of *m*-bromophenol and 2,4-dibromophenol on Amberlyst XAD-7 in water–methanol solutions of varying percent. ×, Predicted values; ○, experimental values.

this separation. If a higher percentage of methanol is used for the eluent, the resolution will not be as good as desired. If a lower percentage of methanol is used, the separation will take longer than necessary.

Eqn. 5 will give the resolution between any two consecutive compounds being eluted provided the composition of the eluent remains the same during the entire elution. There are times when it would be advantageous to separate several compounds by gradient or stepwise elution. Eqn. 5 is no help in this situation. However, by looking at the graphs as shown in Figs. 5–7, one can get a clue as to what will work. An illustration of this is the separation of chlorophenols, for which a sample separation is shown in Fig. 11.

Aqueous system

The distribution coefficients were determined for the three mononitrophenols and phenol as a function of pH in completely aqueous solutions. A graph of the results is shown in Fig. 12. In each case for low pH values the distribution coefficient decreases with increasing pH. The explanation for this is that in aqueous solution the distribution coefficient of any phenol in the molecular form must be fairly high.

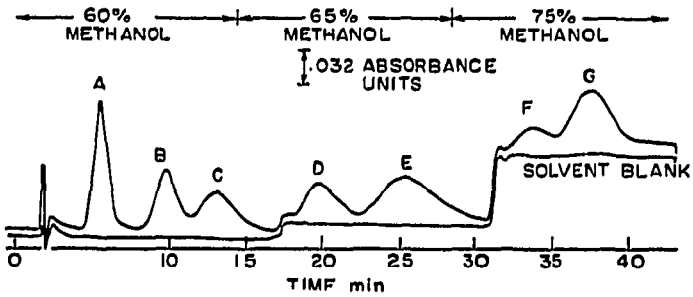


Fig. 11. Separation of chloro-substituted phenols. A = Phenol, 12.6 μg; B = *o*-chlorophenol, 36.6 μg; C = *m*-chlorophenol, 41.4 μg; D = 2,4-dichlorophenol, 85.8 μg; E = 2,3,6-trichlorophenol, 93.8 μg; F = impurity; G = 2,3,4,5-tetrachlorophenol, 29.5 μg. Resin: Amberlyst XAD-7, approx. 350 mesh. Column: 29 cm × 2.8 mm. Flow-rate: 1 ml/min. Volume of sample: 38.1 μl.

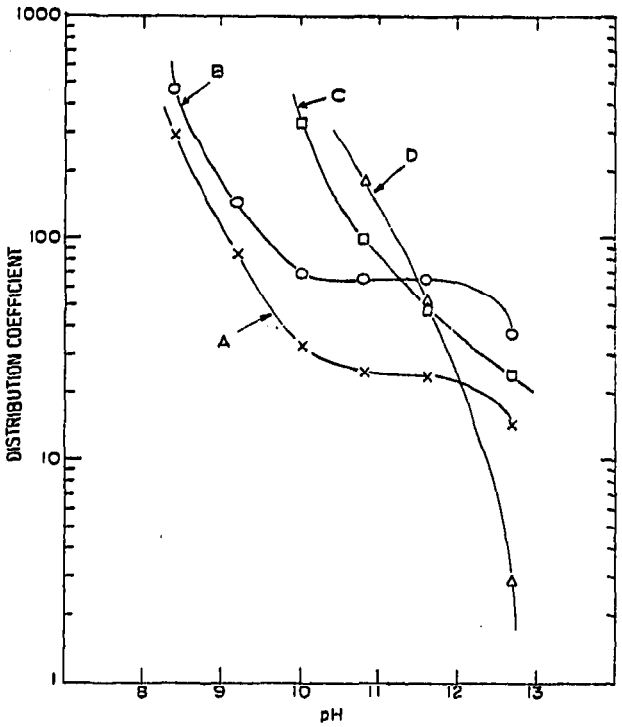


Fig. 12. Distribution coefficient of nitro-substituted phenols on Amberlyst XAD-7 in aqueous solutions. A = *o*-Nitrophenol; B = *p*-nitrophenol; C = *m*-nitrophenol; D = phenol.

The decreasing slope represents the decreasing portion of the phenol in the molecular form.

An examination of Fig. 12 indicates that it should be possible to separate the three mononitrophenols and phenol. Starting with an eluent of pH 10.0, *o*-nitrophenol comes through first followed by *p*-nitrophenol. Changing the pH of the eluent to 12.7, phenol comes through very rapidly followed by *m*-nitrophenol. A chromatogram showing this separation is shown in Fig. 13.

After some experiments had been done with 0.05 *M* sodium hydroxide in 60 % methanol, it was noticed that the resin had been chemically altered so that the separations with aqueous sodium hydroxide could not be repeated. It is believed that the ester group on the resin was partially hydrolyzed. To avoid the possibility of hydrolysis, no eluent should be used that contains mixtures of a strong base and a non-aqueous solvent. In changing from a strongly basic aqueous eluent to a non-aqueous eluent containing methanol, a solvent such as water should be interspersed between the two eluents.

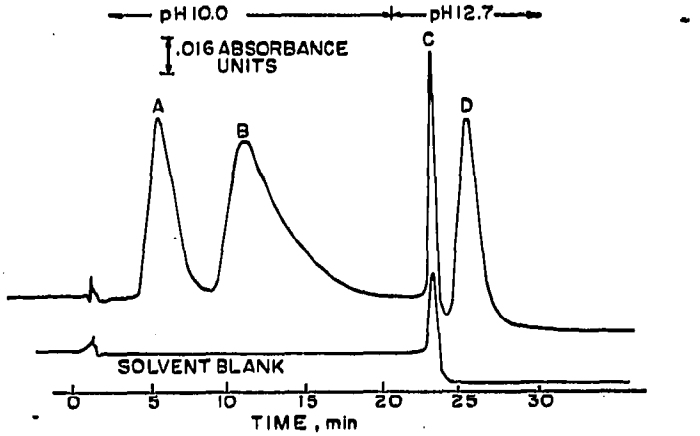


Fig. 13. Separation of nitro-substituted phenols. A = *o*-Nitrophenol, 1.3 μg; B = *p*-nitrophenol, 5.9 μg; C = phenol, 11.2 μg; D = *m*-nitrophenol, 0.95 μg. Column: 2.8 mm × 13 cm. Other conditions were the same as in Fig. 11.

QUANTITATIVE STUDIES

Calibration curve

Varying amounts of phenol were injected on to a 2.8 mm × 13.7 cm column containing XAD-7 and eluted with 65 % methanol. A plot of peak height was found to be linear for 0.3 μg to 200 μg. The peak height for 0.3 μg was about four times the noise level, making the limit of detection about 0.2 μg.

Quantitative study involving no solvent change

A mixture of phenol, *p*-bromophenol, and 2,4-dibromophenol was injected on to a 2.8 mm × 13.7 cm column and eluted with 70 % methanol. The amount of each phenol present was determined by comparing the height of the peak to a calibration curve for each phenol when eluted under identical conditions but without the other phenol present. A typical chromatogram of the separation is shown in Fig. 14. The results are given in Table II.

Quantitative study involving a solvent change

A mixture of phenol, *o*-chlorophenol, and 2,4-dichlorophenol was injected on to a 2.8 mm × 29 cm column and eluted with 60 % methanol for the first 11.25 min followed by 65 % methanol. At min 18.75 the eluent was switched back to 60 %

methanol to equilibrate the column in preparation for the next injection. At min 35, the baseline had leveled off sufficiently to allow the next injection to be made. The amount of any phenol present was determined by comparing the height of a peak to a calibration curve for that phenol when eluted under identical conditions but without the other phenols present.

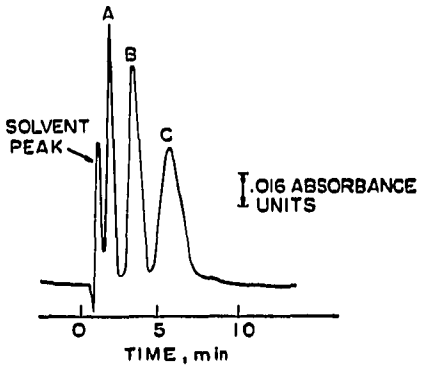


Fig. 14. Separation of bromo-substituted phenols. Sample: A = Phenol, 10.7 μg B = *p*-bromophenol, 35.3 μg; C = 2,4-dibromophenol, 40.2 μg. Eluent: 70 % methanol. Column: 2.8 mm × 13 cm. Other conditions were the same as in Fig. 11.

TABLE II

RESULTS FOR QUANTITATIVE STUDY INVOLVING NO SOLVENT CHANGE

Compound	*μg added*	*μg found*	*Relative error* (%)
Phenol	10.7	10.9	+2.0
p-Bromophenol	35.3	36.0	+1.9
2 4-Dibromophenol	40.2	40.8	+1.4

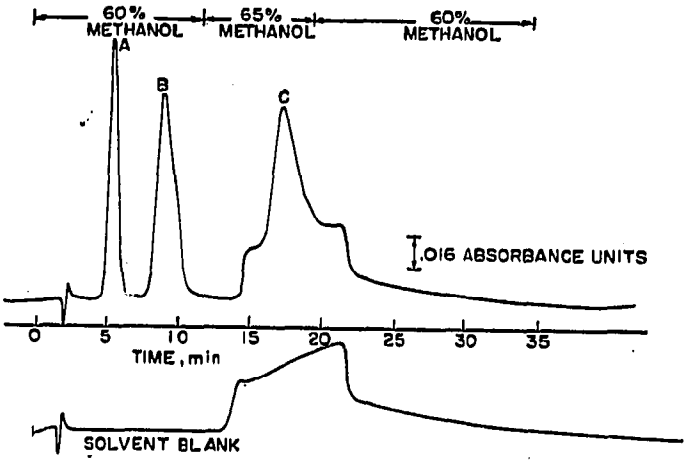


Fig. 15. Separation of chloro-substituted phenols used in quantitative study. A = Phenol, 8 5 μg; B = *o*-chlorophenol, 49.4 μg; C = 2,4-dichlorophenol, 120.5 μg. Column: 2.8 mm × 28 cm. Other conditions were the same as in Fig. 11.

A chromatogram of the mixture is shown in Fig. 15. The results are given in Table III. Normally, the height of the 2,4-dichlorophenol peak would be measured with respect to the baseline as it exists for elution of that compound. However, the baseline here is not well defined so the height was measured with respect to the baseline as established prior to the injection of the sample.

TABLE III

RESULTS FOR QUANTITATIVE STUDY INVOLVING SOLVENT CHANGE

Compound	*μg added*	*μg found*	*Relative error (%)*
Phenol	8.50	8.61	+1.27
o-Chlorophenol	49.4	51.2	+3.58
2,4-Dichlorophenol	120.5	122.2	+1.37

In doing quantitative work, it is important that the sample is not dissolved in a solvent which would be a good eluent for the sample. For example, when phenol is eluted with 65 % methanol on a column of 2.8 × 500 mm, the maximum amount of methanol that can be present in the sample is 55 %. A higher percentage will cause the elution peak to be shorter and broader than it should be.

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