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High pressure liquid chromatography of phenols and metal ions

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by

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CHROMATOGRAPHIC SEPARATION OF PHENOLS USING AN ACRYLIC RESIN

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 adsorbent 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 acids, dyes, steroids, vitamin B-12, and fulvic acid from waste streams (3). XAD-1 has been used to concentrate organic material from sea water (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 (7-11). Zaika has studied the separations of amino acids, carbohydrates, and nucleotides on XAD-2 (12,13). Button has used XAD-8 to reduce the color of Kraft paper bleach plant waste from 1500 ppm to 200 ppm in a single operation (14). The XAD-8 decolorized 50 bed volumes before needing to be regenerated.

At the present time very few applications have been made

of the XAD resins as column packing for high pressure liquid chromatography. Grieser and Pietrzyk have used XAD-2 for the separation of phenols (15). So far, no use has been made of XAD-7 for high pressure liquid chromatography.

In this thesis a macroporous polyacrylic resin, Amberlite XAD-7 from Rohm and Haas, 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.

The Liquid Chromatograph

Instrumental design

A schematic diagram of the liquid chromatograph is shown in Figure 1. Except where noted, all tubing that carries air or water is 1/4" Synflex nylon tubing No. N3-4203 from Globe Machinery and Supply Co., Des Moines. This tubing carries a pressure rating of 1500 lbs. The tubing is connected to other parts with Gyrolock connectors No. 4CM4 from Hoke Inc. All tubing carrying eluent is Teflon tubing No. T063031 which is connected to other parts with tube end fittings No. TEF-107. All of these parts are available from Chromatronix Inc.

A helium tank was filled with air at pressures up to 2200

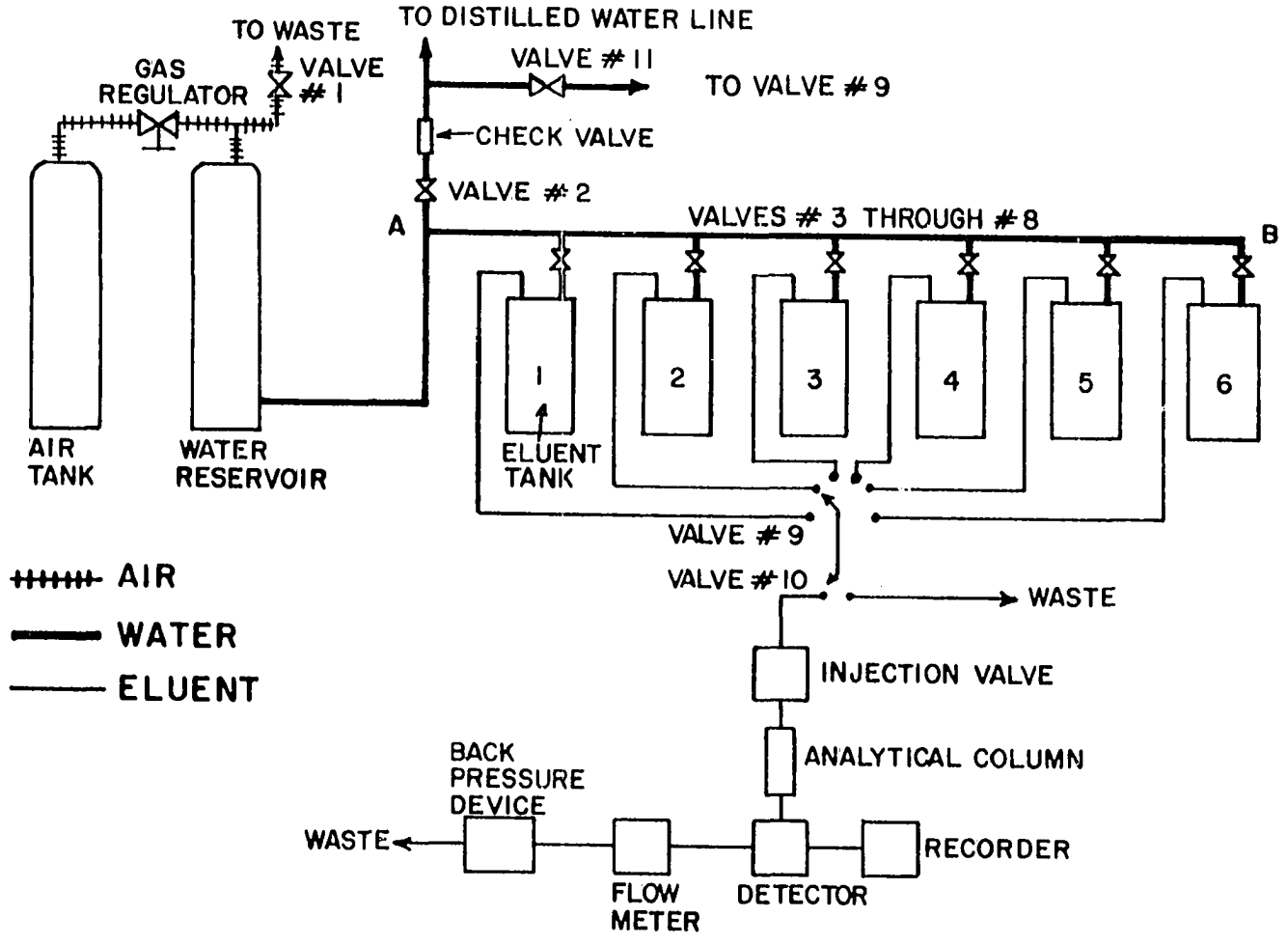


Fig. 1. Schematic diagram of liquid chromatograph

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. No. 580-2, 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" NPT threads. The fitting at the top was fitted with a connector to go to 1/4" NPT threads. To refill the water reservoir, open valve No. 1 releasing air in the reservoir. Simultaneously, open valve No. 2 which is connected directly to the distilled water line. Valves No. 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" stainless steel fittings: six tees, one elbox, and six 6" nipples. Water inlet valves, Nos. 3 through 8 are Hoke valves No. 3222H4B.

A diagram of the eluent tanks is shown in Figure 2. Each tank is made from 5" Schedule 80 steel pipe, 12" long. In the flange of the tank, eight 3/8" holes were drilled 7/16" 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" from the edge equally spaced around the lid. The lid was bolted on with 3/8 x 2 1/4" bolts and the bolt head was spot welded in place. The lid was removed after which the holes

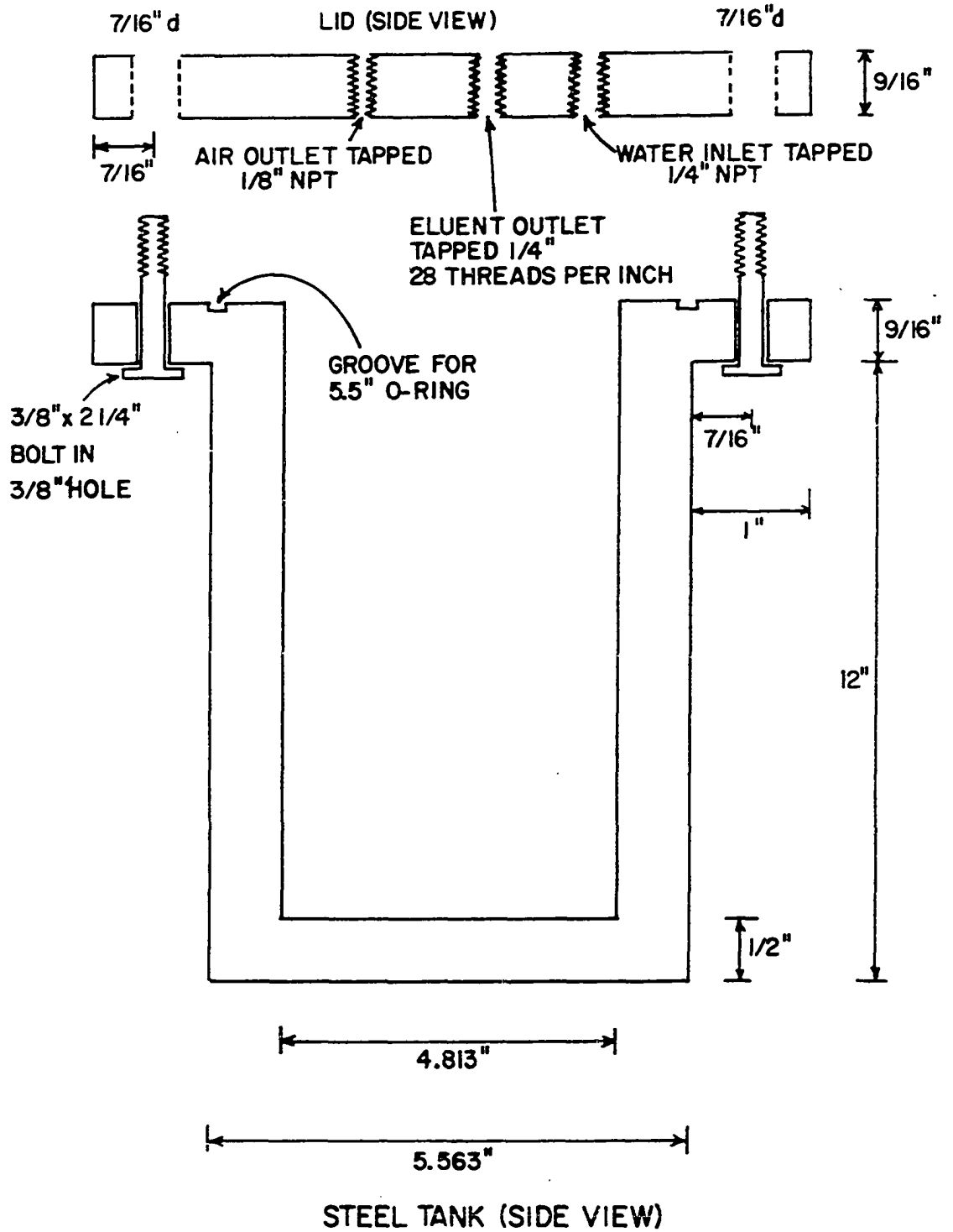


Fig. 2. Diagram of eluent tank

were enlarged to 7/16". O-rings between the lid and the tank were 5 1/2" I.D. 1/8" thick, 70 to 80 durometer hard purchased from Tech Syn Corporation. These o-rings are harder than ordinary o-rings. Probably ordinary o-rings will work. The air escape valve, Hoke #3222M2B, is screwed into the air outlet hole on the outer side of the lid. A 1/4" o-ring is placed on the Kel-F connector (see Figure 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.

The eluent goes in a 2.5 liter plastic bag, Matheson Scientific Co. #12657-10. The bag is apparently coated with some sort of plasticizer which it was found necessary to remove. Filling the bag with methanol and letting it stand for a week was found to be sufficient. The liner from the plastic bag cap was removed and a .775" diameter hole was drilled. The Teflon hat (see Figure 4) was put back into the cap replacing the liner. When the cap was screwed back onto 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. Chromatronix tubing then connects the Kel-F connector to the eluent selection valve, #9, which is a six-way valve, Chromatronix R60V6K. Valve #10 is Chromatronix #CAV-3031K. The sample injection valve is Chromatronix #SV-8031K. A small sample loop was made and calibrated by filling the

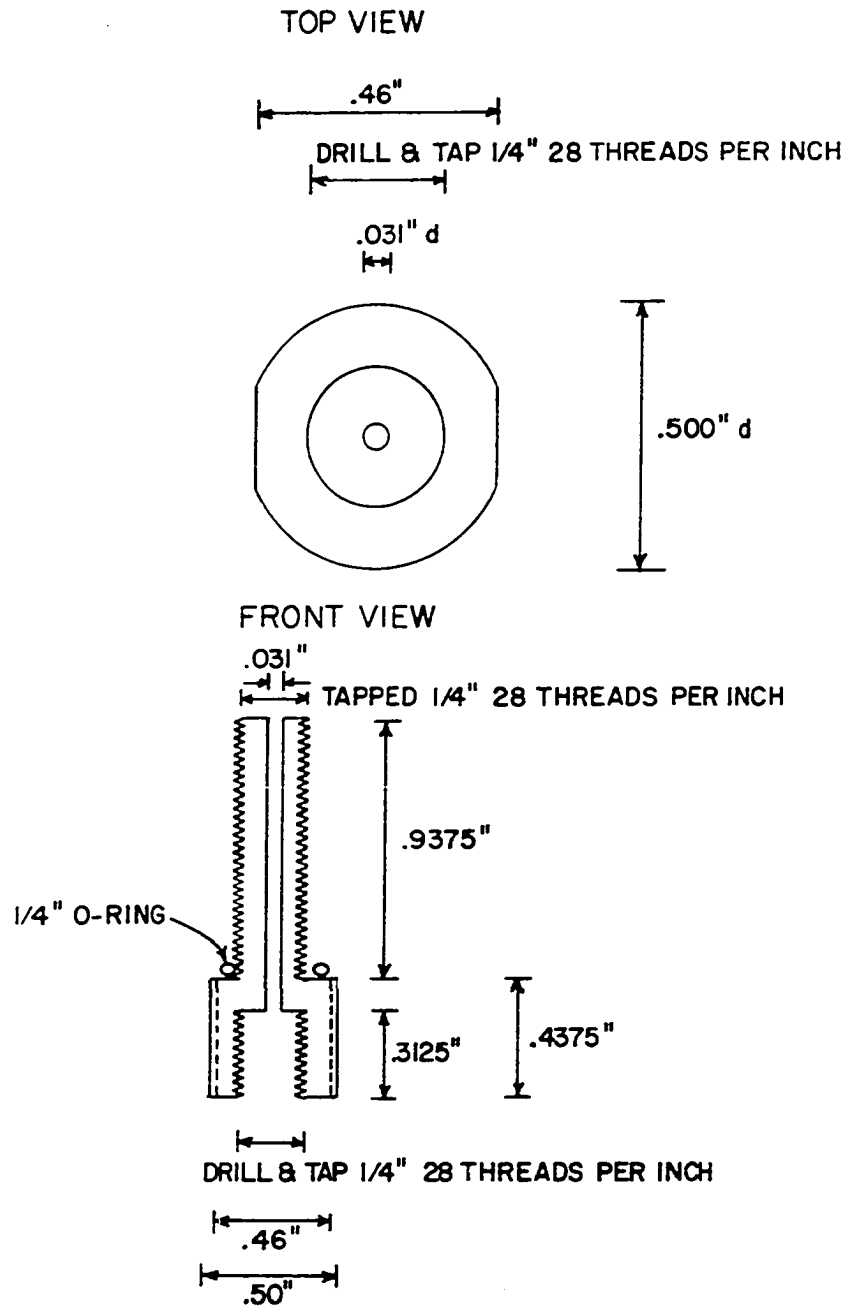


Fig. 3. Kel-F connector

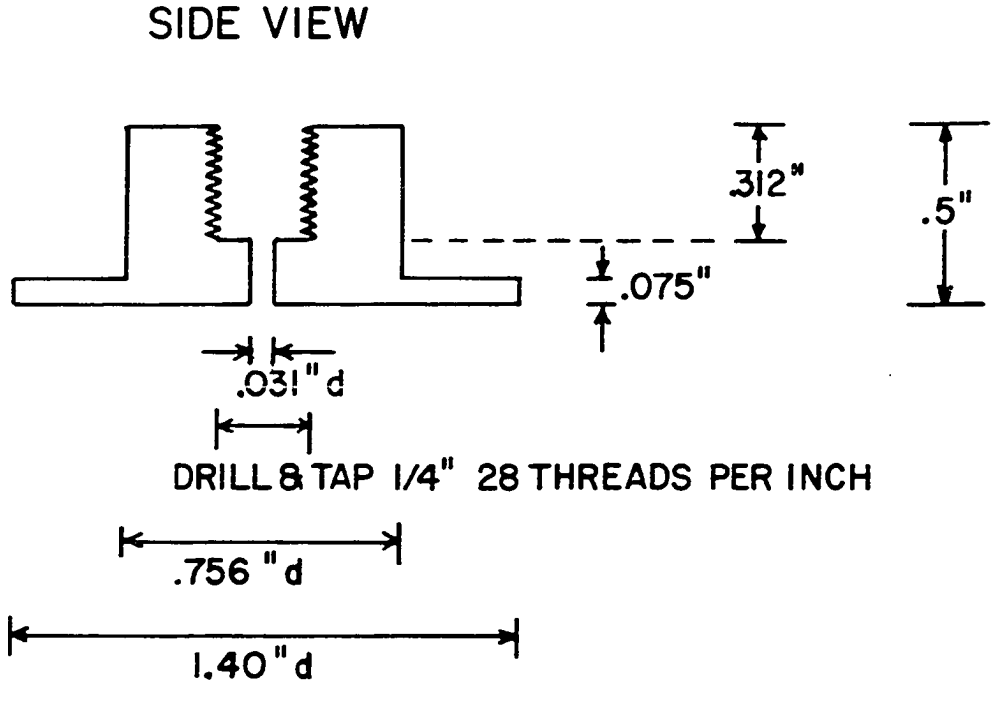


Fig. 4. Teflon hat

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 microliters.

A Chromatronix model 200 UV detector is used to monitor the effluent. This has a 253.7 nm mercury line source and a 1 cm light path, 8 microliter dead volume flow through cell. It is a double beam differential photometer with a photomultiplier which can be attenuated from 0.01 to 5.12 absorbance units with a linear dynamic range from .0001 to 3.0 absorbance units.

Roger Gilmont Instruments, Inc. catalog number 3201 size number 1 flow meter is used. Adaptors were machined of Kel-F following the Chromatronix design for the G1-C glass connector to allow attachment of Chromatronix fittings.

A ten foot length of Chromatronix tubing, catalog number T063012, was used to produce back pressure and prevent gas desolubilization. For a flow rate of 1.0 ml/minute the pressure drop across this tubing is 34 psi. 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 number G1C glass connector to the needle valve. This allowed the needle valve to be connected to the Teflon tubing being used.

Operating suggestions

When loading a tank with eluent, a couple suggestions are in order. First, the plastic bags will hold about 2.5 liters

but it works best if the bags are filled no fuller than two liters. Secondly, 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 but inside the eluent tank, 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, number 9, to that tank while simultaneously turning valve number 10 to waste position. Leave it in this position until eluent goes through valve number 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 psi) or the bag is almost empty.

The procedure for removing the eluent bag from an eluent tank is to shut off the water inlet valve, hook a short piece of Tygon tubing to the air release valve leading to a waste receptacle and open the air release valve. As soon as water stops flowing out the air release valve, the nylon tubing can be disconnected at the tank lid and nuts holding the lid on removed.

Discussion of pumping systems

When using a piston type pump the liquid is pushed through the column in pulses. Pulses are undesirable because they produce more noise in the detector and also decrease the effi-

ciency of the column.

One way of reducing the amount of pulsation is by using damping devices in the form of a coil of tubing (16). The eluent goes from the pump, through the coil and then into the injection port. Pulsing is decreased by the flexing of the coil.

Another way to decrease pulsing is by using a feed back device (17,18). Two types have been described. In both cases when the pulse reaches its maximum, solvent is allowed to escape and return back to the reservoir. When the pulse is less than the average some of the trapped solvent is under greater pressure than the liquid coming from the pump and so the trapped solvent is pushed into the tubing leading to the column. In one case this is accomplished by the use of a side arm containing gas under pressure (18). In the other case this is accomplished by means of a steel diaphram (17).

For low to intermidiary pressures, another way of eliminating pulses is available and that is by eliminating the pump and going to a pressure system. For low pressures it is permissible to have helium or some other inert gas put pressure directly on the liquid.

One way of doing this has been described by Seymour, Sickafoose, and Fritz (19). The eluent was placed in polyethylene bottles which were encased in steel jackets. Helium pressure up to 90 lbs could be imposed on the surface of the eluent to push it through the column. This method has the

advantage of being inert in any acid.

Buist and Higgins describe a simple pressure system that will maintain the pressure within 0.05 lbs of the desired pressure (20). It has a maximum pressure rating, however, of 2 lbs. Another pressure system used eluent reservoirs made of long coils of 1/8" o.d. nylon or Teflon tubing (21). Since the surface area in contact with the pressurizing gas is very small compared to the volume of eluent in the tubing, greater pressures could be used without much gas dissolving in the liquid. Chromatronix Inc. has developed a commercial liquid chromatograph utilizing this same idea.

One other way of utilizing pressure systems for intermediate pressures is by the use of an impermeable barrier between the gas and the eluent. Bonnelycke uses a steel bellows as the reservoir for the eluent which is contained in a tank made of Plexiglass (22). The pressure limit for this system is 200 lbs but if the tank had been made of stronger materials, higher pressures would have been possible.

Huber and Van Urk-Schoen have built a pressure system where the eluent goes in a Teflon bellows (23). High pressure nitrogen puts pressure on one tank of oil which compresses the bellows in a second tank. With this system, it is possible to use acids as eluent under high pressure. The eluent is added to the bellows through a hole in the top of the tank and apparently removed the same way. It appears that rinsing out all of one eluent to prevent contamination before changing to another

eluent would be a little difficult.

Jentoft and Gouw built a high pressure system where air under pressure pushes mercury from one tank to another tank (24). The eluent floats on top of the mercury in the second tank and is pushed out of the tank as mercury comes in. Here again, when changing eluents it appears that rinsing out all the first eluent to prevent cross contamination would be a little difficult.

Young and Maggs have described a pressure system where the eluent goes in a polyethylene wash bottle which is placed in a brass pressure vessel (25). In this case, the bottle appears to be easily removed for replenishing or changing eluents. Acids could be used for eluent with this system.

Discussion of liquid chromatograph described in this thesis

Construction materials for this 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 under pressure in all the parts 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 than can be placed on the eluent for this liquid chromatograph is 500 lbs which is the pressure rating of the Chromatronix parts used.

The pressure system described in this thesis is essentially the same as that which Young and Maggs have described (25). It has the advantage in that it is less susceptible to explosions if a leak or a break should develop. It has the disadvantage in that if any hole or loose connection develops inside the tank containing the eluent reservoir, water can get mixed with the eluent without the operator realizing this has happened.

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 five minutes 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

Experimental

A methanol-water solution containing 450 ppm phenol and 1,447 ppm o-chlorophenol was injected onto a 0.28x13 cm column containing 350 mesh XAD-7 and eluted with 45% methanol-55% water at varying flow rates. The volume of solution injected was 56.5 microliters. The retention volume, $\bar{v}$, and width of each peak was measured and using these values, the height of a theoretical plate was calculated. The formula used was $H = (L/16)(w/\bar{v})^2$ where H = height of a theoretical plate, L = length of the column and w = width of the peak as illustrated in Figure 8.

Results

For a flow rate of 1.6 ml/min, the HETP is 6 mm. For flow rates greater than 1.6 ml/min, the HETP increases rapidly as the flow rate increases. For lower flow rates, the HETP decreases as the flow rate decreases but does not decrease sufficiently to justify going to much lower flow rates.

One other factor influencing the choice of flow rate was that for most operations a flow rate greater than 1.25 ml/min required the use of high pressure regulator at its lower range.

A flow rate less than 1.25 ml/min required the use of a low pressure regulator at its higher range. Since the latter is more precise, it was decided to use a flow rate less than 1.25 ml/min. The flow rate chosen was 1.0 ml/min which gives a linear flow rate of 41 cm/min assuming a void volume of 40% (9). The HETP at this flow rate is 3.5 mm.

Distribution Coefficients

Experimental

Batch distribution coefficients Phenol and p-bromophenol were dissolved in various solvents at concentrations ranging from 200 to 250 ppm. To each solution was added approximately 0.5 gram of XAD-7. The solutions were shaken for one hour on a Burrell Wrist Action Shaker. The final concentration of solution was determined by gas chromatographing one microliter of solution on a Beckman GC4 gas chromatograph. The height of the resulting peak was compared to a calibration curve. Operating parameters were as follows:

Liquid Phase: 10% Dexil 300 GC

Solid Support: Chromosorb W DMCS

Column Dimensions: 4 ft x 0.25 in. o.d.

Detector: FID

Helium Flow: 100 ml/min

Temperatures:

Injection Port: 230°

Detector: 250°

Column: 100 to 150°

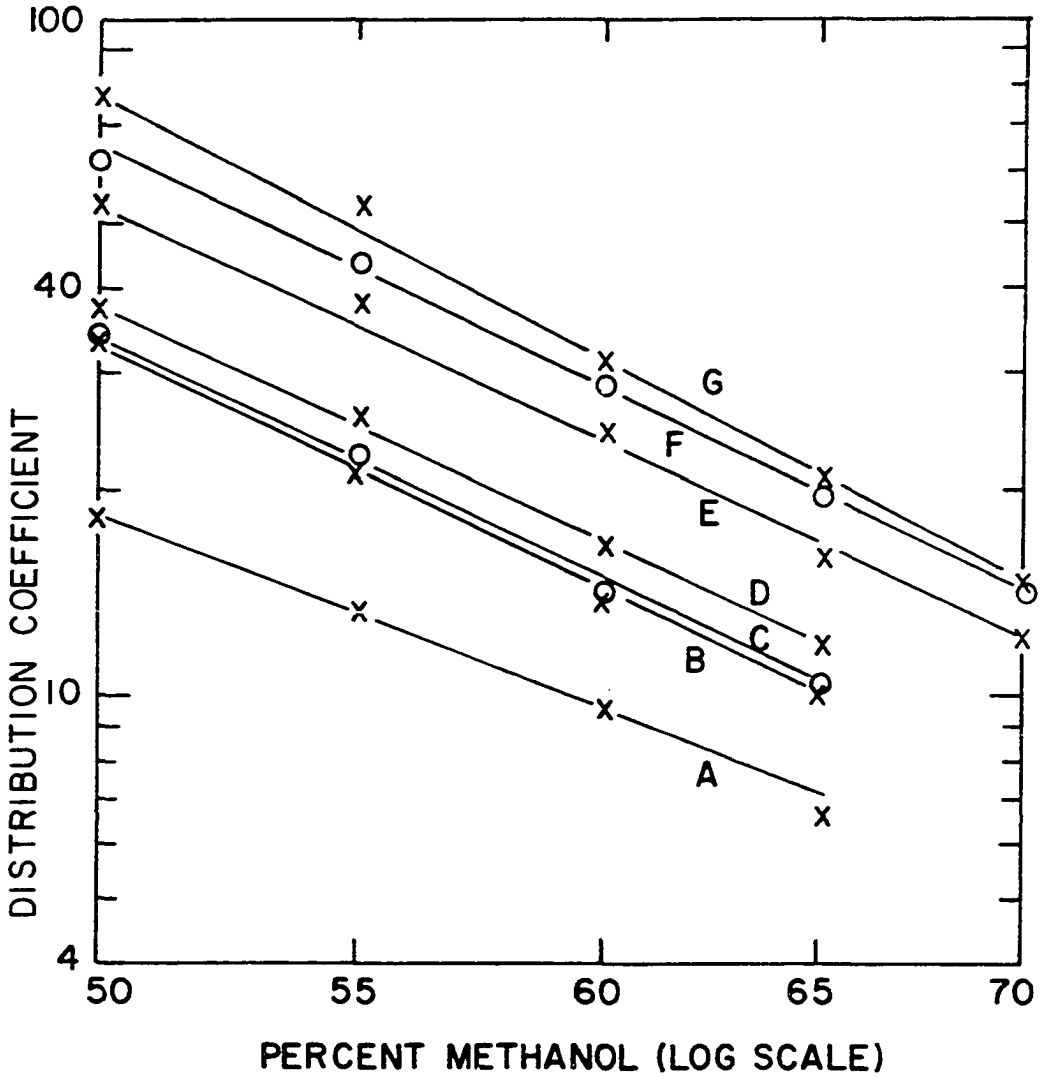
Column distribution coefficients Distribution coefficients, D, were determined by eluting a compound from a 2.8 mm x 13 cm column with some combination of water and methanol at 1 ml/min and measuring the retention volume, $\bar{v}$. The volume of solution injected was 38.1 µl. Using the same column containing 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 these measurements because it appeared not to be retained under the conditions of elution. The weight of the resin used was measured by waiting until all distribution coefficients were measured, extruding the resin into a beaker, allowing it to air dry, and weighing it. From these data, the distribution coefficient was determined by using the formula, $\bar{v} = v_0 + Dg$ where g is the weight of air dried resin in grams.

Results

Water-methanol system The distribution coefficients of several phenols between XAD-7 and varying combinations of water and methanol were determined by column methods. The resulting values of D were graphed on log graph paper as a function of percent methanol. These graphs are shown in Figures 5 through 7. Since each graph is linear, a compound can be represented by an equation of the form

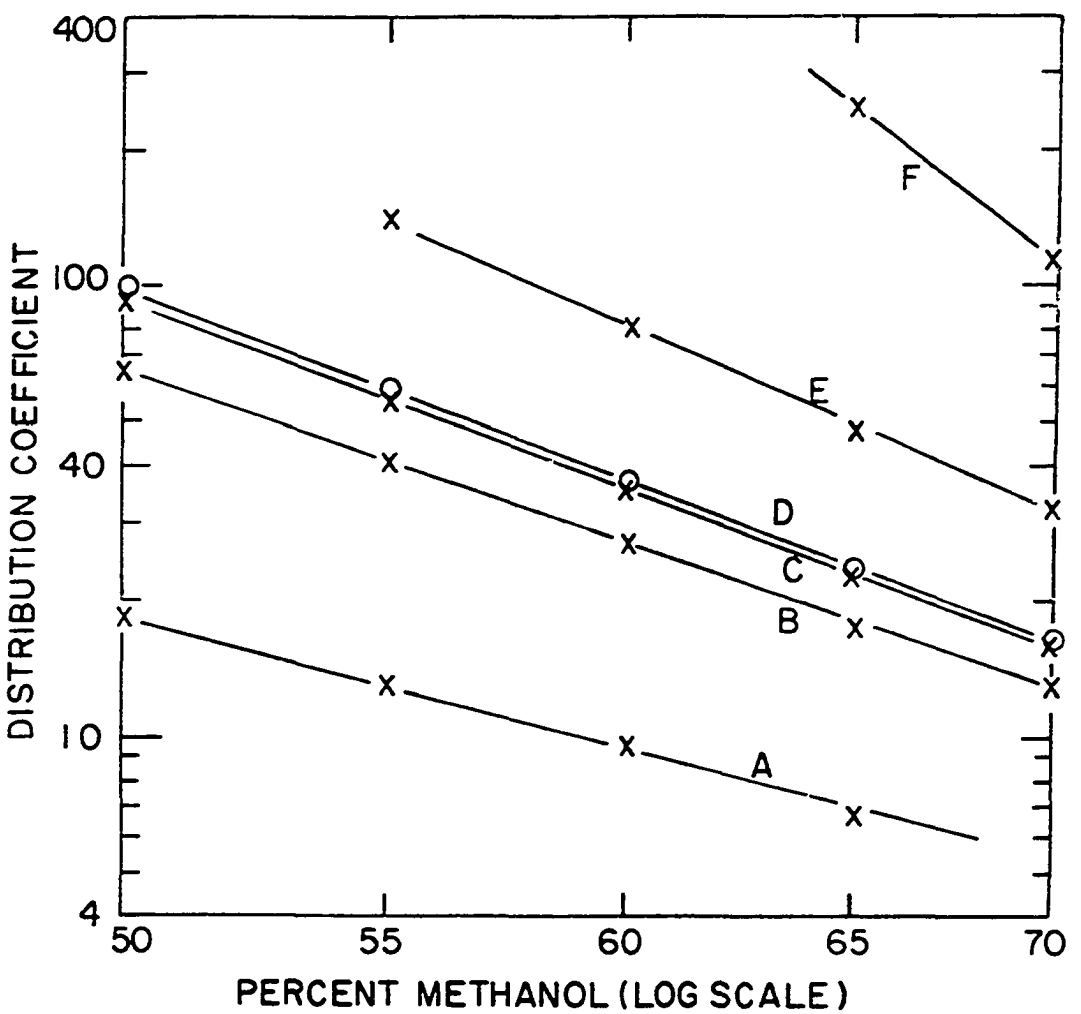
$$\log D = b + a \log C \qquad (1)$$

where a is the slope of the line, b is the intercept, and C is



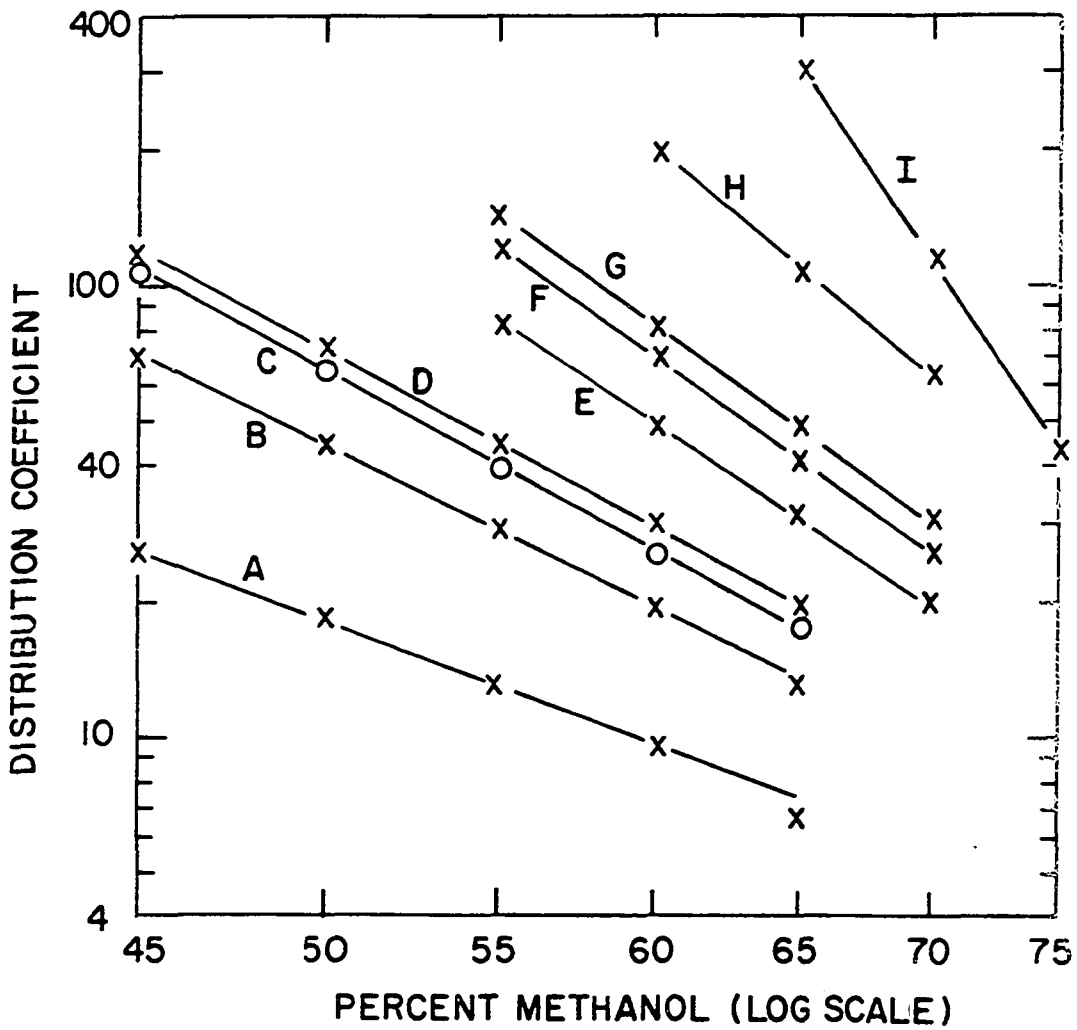
A, phenol; B, p-cresol; C, m-cresol; D, o-cresol; E, 3,4-dimethylphenol; F, 2,6-dimethylphenol; G, 3,4,5-trimethylphenol

Fig. 5. Distribution coefficients of methyl substituted phenols on Amberlite XAD-7 in water-methanol solutions of varying percent



A, phenol; B, o-bromophenol; C, p-bromophenol; D, m-bromophenol, E, 2,4-dibromphnol; F, pentabromophenol

Fig. 6. Distribution coefficients of bromo substituted phenols on Amberlite XAD-7 in water-methanol solutions of varying percent



A, phenol; B, o-chlorophenol; C, p-chlorophenol; D, m-chlorophenol; E, 2,4-dichlorophenol; F, 3,5-dichlorophenol; G, 2,3, 6-trichlorophenol; H, 2,3,4,5-tetrachlorophenol; I, pentachlorophenol

Fig. 7. Distribution coefficients of chloro substituted phenols on Amberlite XAD-7 in water-methanol solutions of varying percent

the percent methanol in the water-methanol mixture. The slope and intercept of all phenols were measured and are given in Table 1.

Table 1. Values of a and b for Equation 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

Using these data it is possible to derive an equation that predicts the resolution that will be achieved in separating compound #1 from compound #2 using a given percent methanol as the eluent. Some of the symbols used in the derivation are illustrated in Figure 8 showing a typical chromatogram of the separation of two compounds. Other symbols not illustrated are defined below.

- N number of theoretical plates
- v_o interstitial volume of the column
- a_1, a_2 value of a for compound 1 and compound 2 respectively as defined in Equation 1
- b_1, b_2 value of b for compound 1 and compound 2 respectively as defined in Equation 1
- D distribution coefficient
- C percent methanol in a water-methanol mixture used as the eluent
- g grams of resin used in the column

The usual formula for resolution is

$$R = \frac{2d}{w_1 + w_2} \tag{2}$$

It can be shown that

$$d = \bar{v}_2 - \bar{v}_1 \tag{3}$$

Substituting Equation 3 into Equation 2, the result is

$$R = \frac{2(\bar{v}_2 - \bar{v}_1)}{w_1 + w_2} \tag{4}$$

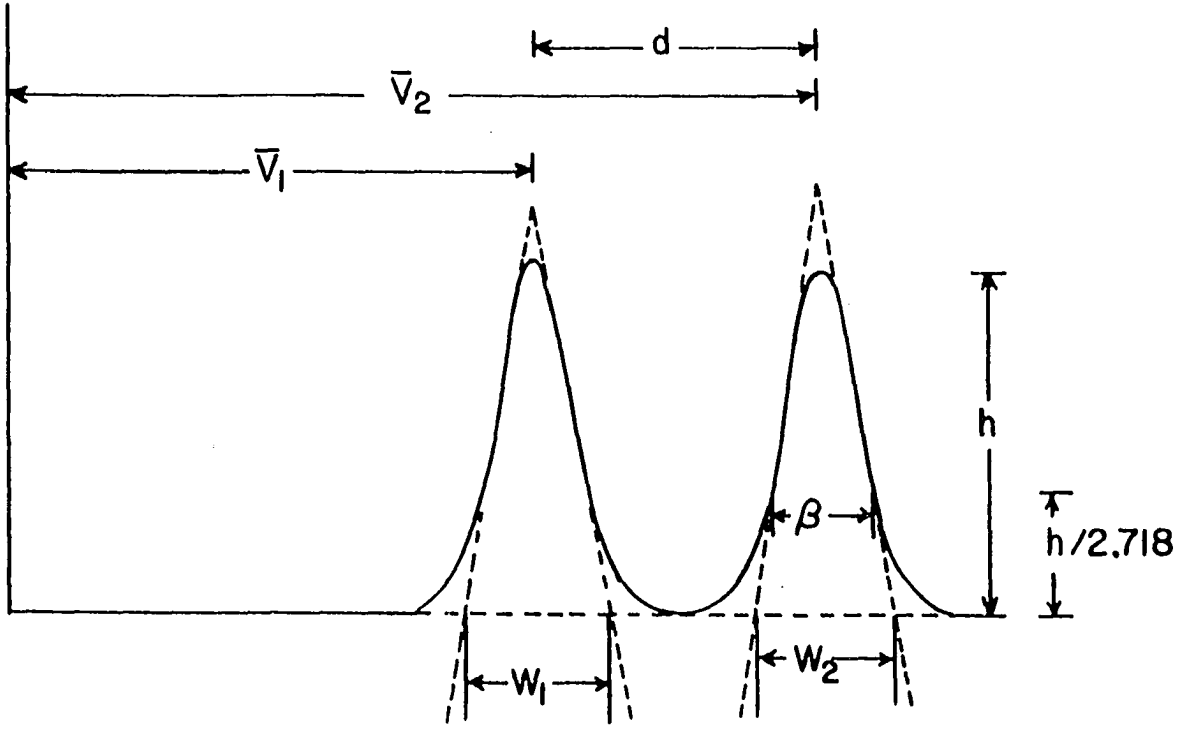


Fig. 8. Typical elution curve illustrating various symbols

The definition of theoretical plates is

$$N = 16(\bar{v}/w)^2 \tag{5}$$

Solving this equation for w and substituting into Equation 4 gives the result

$$R = \frac{\sqrt{N}}{2}\left(\frac{\bar{v}_2-\bar{v}_1}{\bar{v}_2+\bar{v}_1}\right) \tag{6}$$

From the definition of distribution coefficient, the following equation can be derived:

$$\bar{v} = v_o + Dg \tag{7}$$

where g stands for the number of grams of resin. Let $\bar{v}_2$ be the elution volume of compound two, having distribution coefficient D_2 and substituting Equation 7 into Equation 6, gives the result

$$R = \frac{g\sqrt{N}}{2}\left(\frac{D_2-D_1}{2v_o+D_2g+D_1g}\right) \tag{8}$$

Solving Equation 1 for D and substituting into Equation 8 yields the result

$$R = \frac{g\sqrt{N}}{2}\left[\frac{10^{b_2+a_2 \log C} - 10^{b_1+a_1 \log C}}{2v_o + g\,10^{b_2+a_2 \log C} + g\,10^{b_1+a_1 \log C}}\right] \tag{9}$$

A computer program written in Fortran IV language that will compute the value of R is shown in Figure 9. In using this program, the first card must have the following information in the order listed:

1) Number of theoretical plates.
2) V_o for the column being used.

```
  2    READ,AN,VO,G,A1,B1
       RT=SQRT(AN)
 10    READ,A2,B2
       IF(A2.EQ.0)GO TO 80
       PRINT15,A1,B1,A2,B2
 15    FORMAT('2','A1=',F8.4,3X,'B1=',F8.4,3X,
      X'A2=,',F8.4,3X,'B2=',F8.4)
       PRINT20
 20    FORMAT('0PERCENT METHANOL'2X'RESOLUTION')
       C=5.0
 25    CC=ALOG10(C)
       AA2=(10**(B2+A2*CC))*G
       AA1=(10**(B1+A1*CC))*G
       S=(RT/2.)*(AA2-AA1)/(2.*VO+AA2+AA1))
       PRINT65,C,S
 65    FORMAT('B',F8.1,F18.6)
       C=C+5.0
       IF(C.LE.100)GO TO 25
       A1=A2
       B1=B2
 70    GO TO 10
 80    STOP
       END
$ENTRY
70.70,0.846,.1429
-3.618,7.437
-5.363,11.106
-6.259,13.039
0.00,0.00
```

Fig. 9. Computer program to solve Equation 9

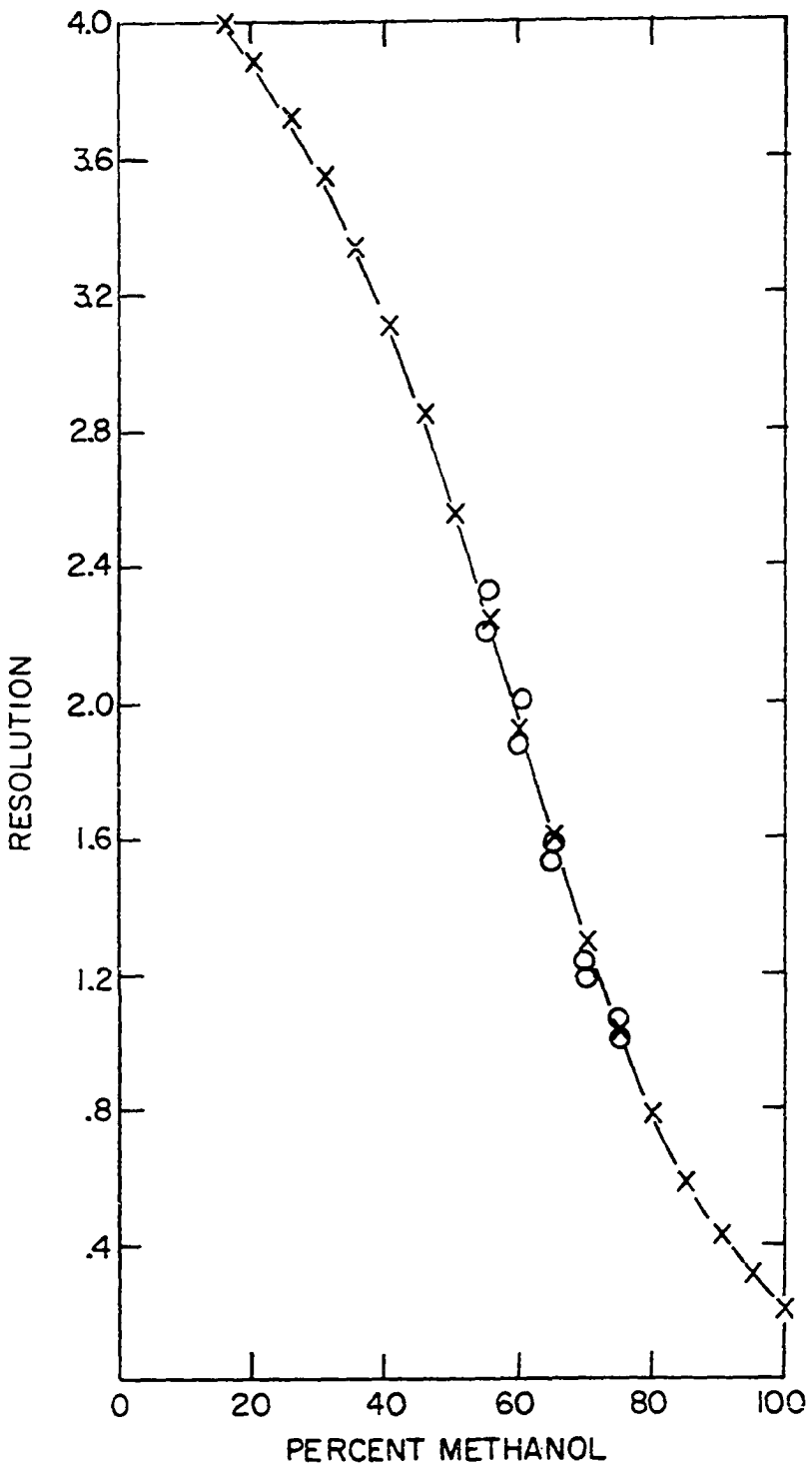
3) Grams of resin contained in the column.

Each succeeding card must contain the value a and b in that order for one compound. The last data card must contain the number zero. The program will then compute the resolution between adjacent compounds from 0% to 100% methanol in increments of 5%.

The validity of Equation 9 was proved by eluting a mixture of phenol, m-bromophenol, and 2,4-dibromophenol with varying percentages of methanol. The resolution was measured between the first two compounds, m-bromophenol and phenol, and the last two compounds, m-bromophenol and 2,4-dibromophenol. The experimental and theoretical values are shown for each pair of compounds in Figures 10 and 11. The test for Equation 9 was done with a column that was the same length and had the same weight of resin as the column that was used to measure the distribution coefficients of the phenols. No attempt was made to see if the formula was valid for columns of different lengths or columns containing different amounts of resin.

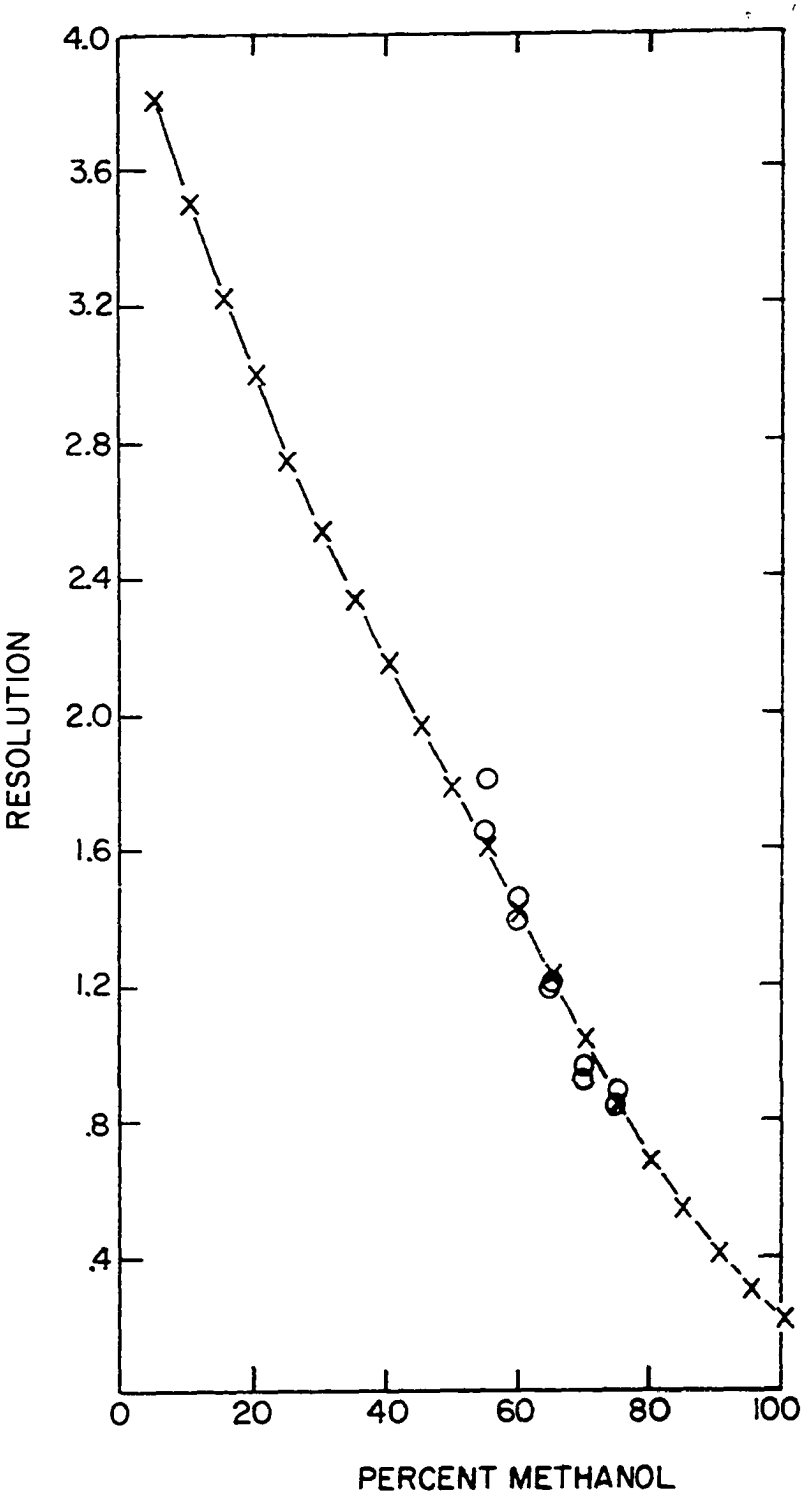
In using the formula it is suggested that one decide what is the minimum resolution that is needed. Then find the percent methanol that the eluent must have to give this separation. If a higher percentage of methanol is used for the eluent, the resolution will not be as good as desired. If a lower percentage of methanol is used, the separation will take longer than necessary.

Equation 9 will give the resolution between any two con-



XXX predicted values, 000 experimental values

Fig. 10. Resolution of phenol and m-bromophenol on Amberlite XAD-7 in water-methanol solutions of varying percent



XXX predicted values, 000 experimental values

Fig. 11. Resolution of m-bromophenol and 2,4-dibromophenol on Amberlite XAD-7 in water-methanol solutions of varying percent

secutive compounds being eluted provided the composition of the eluent remains the same for the time that those compounds are being eluted. There are times when it would be advantageous to separate several compounds starting with an eluent of one composition and gradually changing the composition until the last compound is eluted. Equation 9 is no help in this situation. However, by looking at the graphs as shown in Figures 5 through 7, one can get a clue as to what will work. An illustration of this is the separation of chlorophenols as shown in Figure 12. Other separations in which the eluent remains the same composition throughout the elution are shown in Figures 13 and 14. These separations could have been predicted to work by using Equation 9.

Aqueous system Distribution coefficients were determined for the three mono nitro phenols and phenol as a function of pH in completely aqueous buffer solutions. A graph of the results is shown in Figure 15. 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. The decreasing slope represents the decreasing portion of the phenol in the molecular form. The reason why the distribution coefficients of p-nitrophenol and o-nitrophenol level off at high pH values and the other two phenols do not, is uncertain but may be due to differences in ionic strength of the solutions used. In spite of the fact

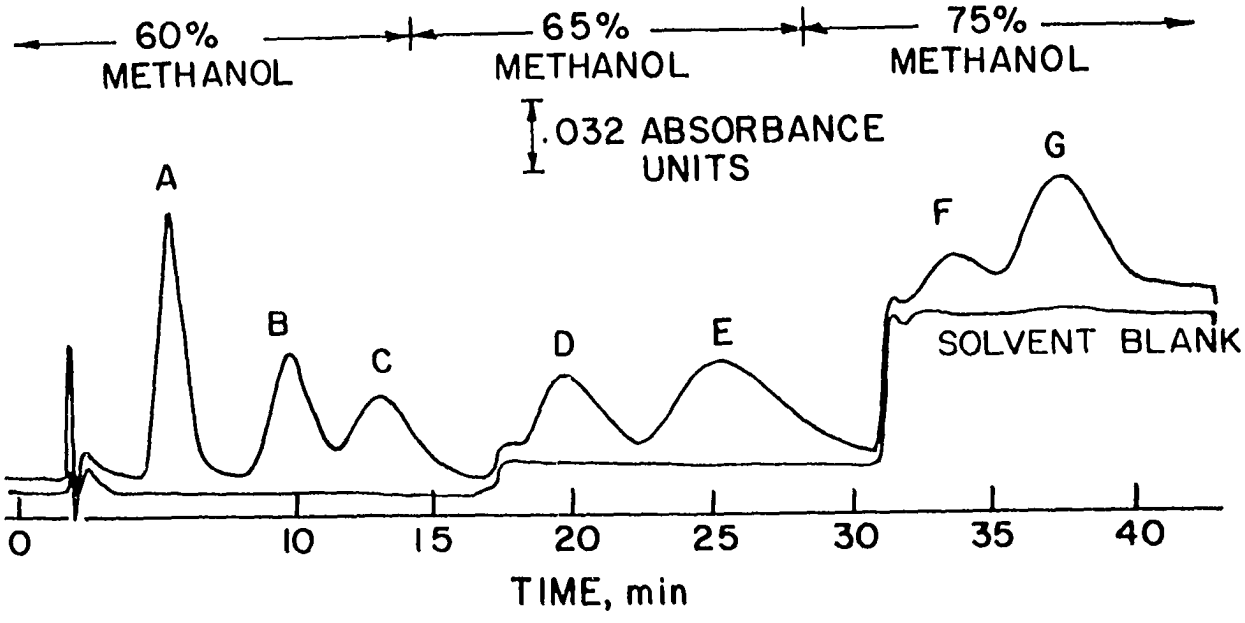


Fig. 12. Separation of chloro substituted phenols

Sample: 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: Amberlite XAD-7, approx. 350 mesh; Column: 29 cm x 2.8 mm; Flow rate: 1 ml/min; Volume of sample: 38.1 µl

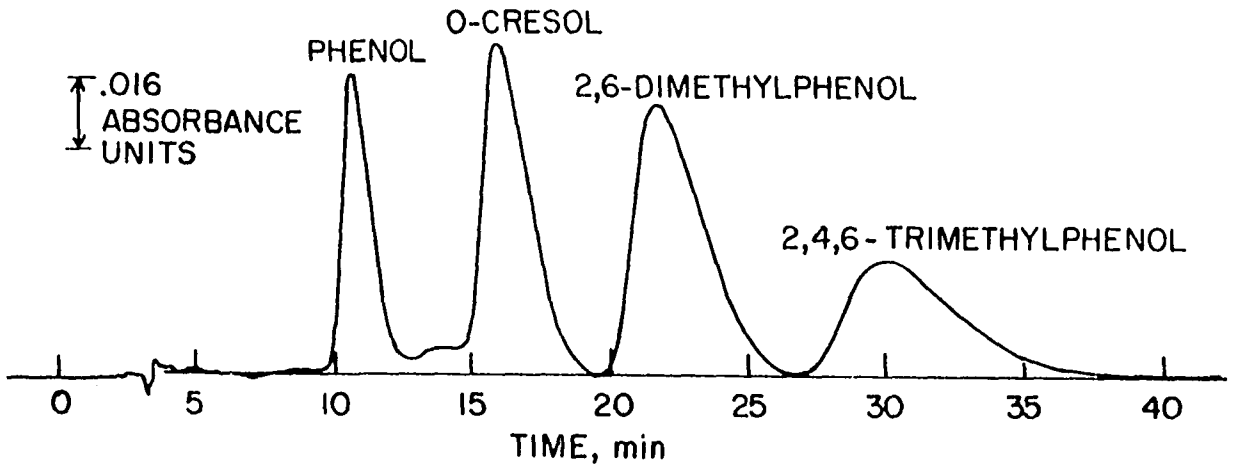


Fig. 13. Separation of methyl substituted phenols

Sample: phenol, 16 µg; o-cresol, 37 µg; 2,6-dimethylphenol, 61 µg; 2,4,6-trimethylphenol, 75 µg;
Column, 50 cm x 2.8 mm; Eluent, 55% methanol; Flow rate, 1 ml/min;
Volume of sample, 38.1 µl

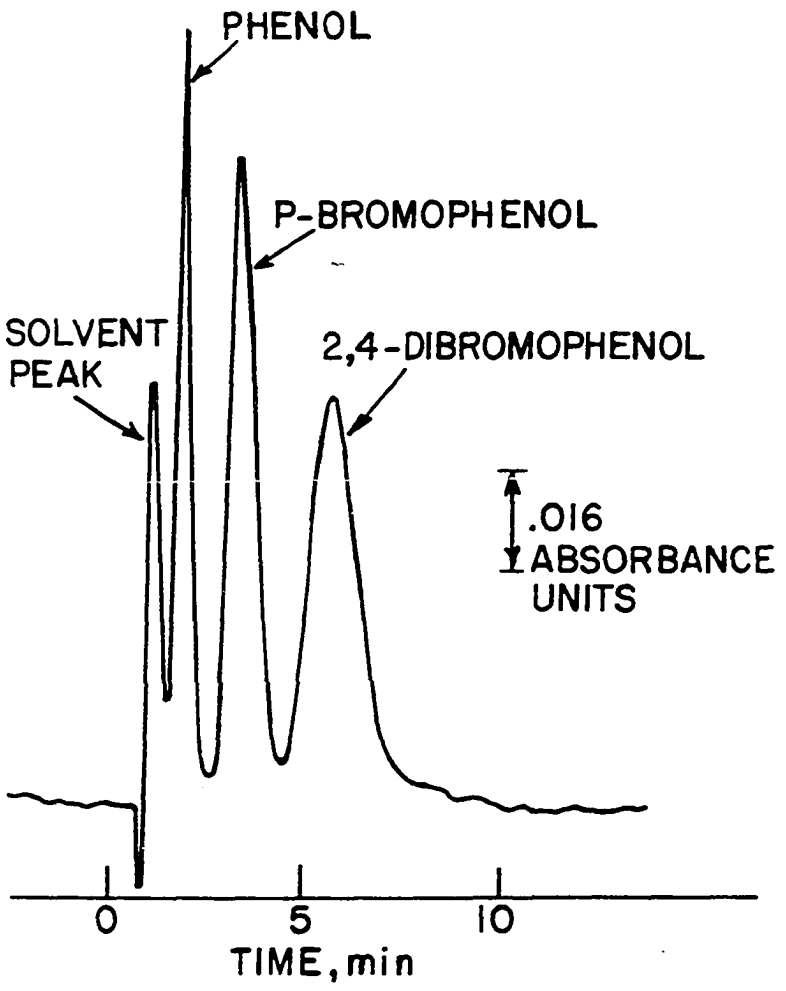


Fig. 14. Separation of bromo substituted phenols

Sample: phenol, 10.7 µg; p-bromophenol, 35 µg; 2,4-dibromophenol, 40 µg;
Eluent, 70% methanol; Column, 2.8 mm x 13 cm; Flow rate, 1 ml/min; Sample volume, 38.1 µl

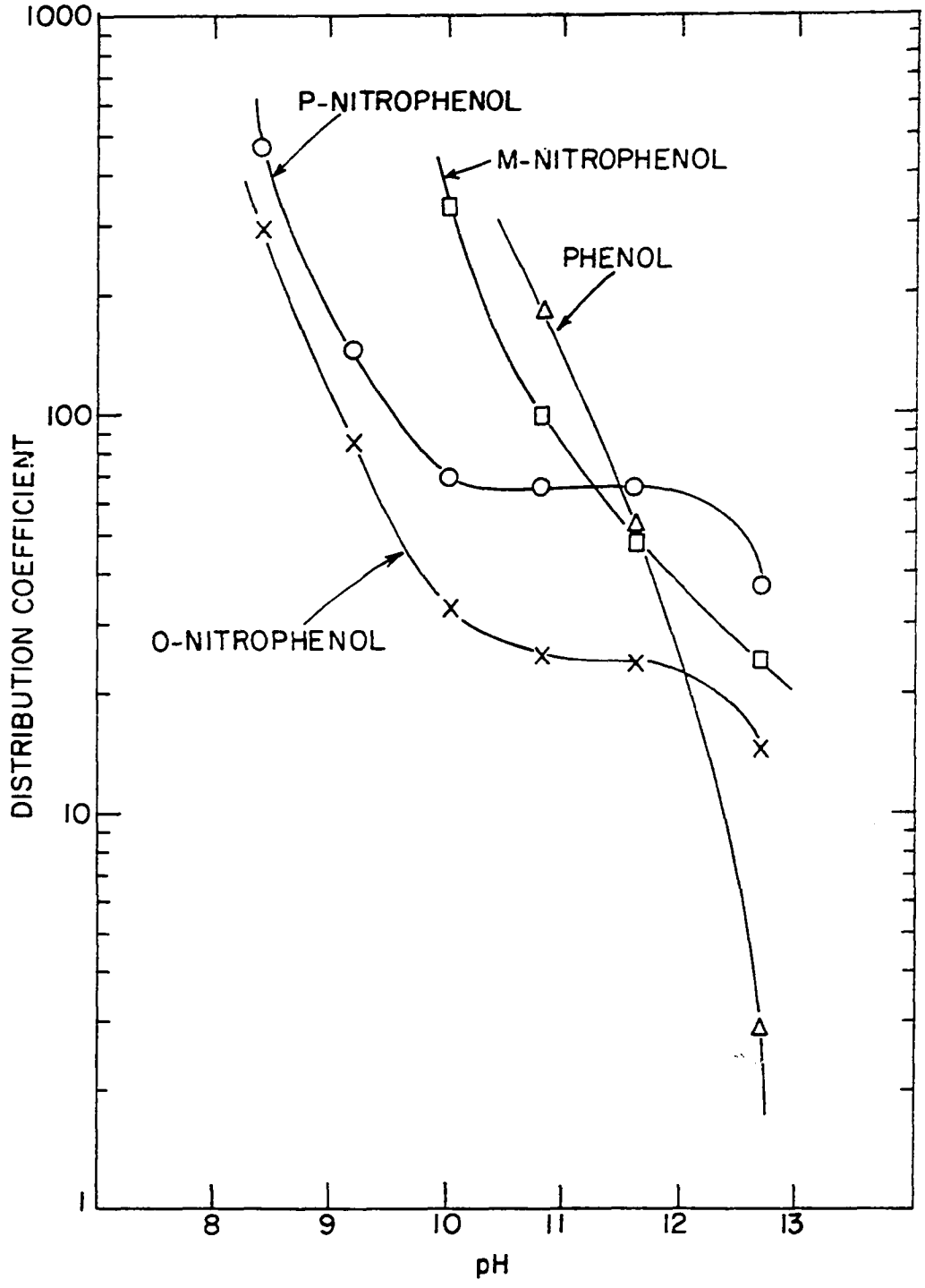


Fig. 15. Distribution coefficient of nitro substituted phenols on Amberlite XAD-7 in aqueous solutions

that the total molarity of the buffer for all solutions was the same (0.05 M), the ionic strength varies. The ionic strength of the pH 12.7 solution is especially different. The retention of organic compounds on XAD-2 has been shown to be greatly affected by ionic strength (26,27). The effect is probably also present for XAD-7.

An examination of Figure 15 would indicate that it should be possible to separate all four compounds. Starting with an eluent of pH 10.0, o-nitrophenol should come through first followed by p-nitrophenol. If the eluent is changed to pH 12.7, phenol should come through very rapidly followed by m-nitrophenol. A chromatogram showing such a separation is shown in Figure 16.

Using basic aqueous solutions, it is possible to separate the ortho, meta, and para isomers of the chloro phenols and bromo phenols. Chromatograms of these separations are shown in Figures 17 and 18.

Precaution

The solvent 0.05 M NaOH-60% methanol was used as an eluent with the column at temperatures of 50°C for two or three days. In elutions which followed this it was found that the $\bar{v}$ was much less than it should be. Also, separations which had worked before this solvent was used did not work after it had been used. When this resin was discarded and replaced with fresh resin the problem no longer existed. It is believed that the ester functional group on the resin hydrolyzed in the

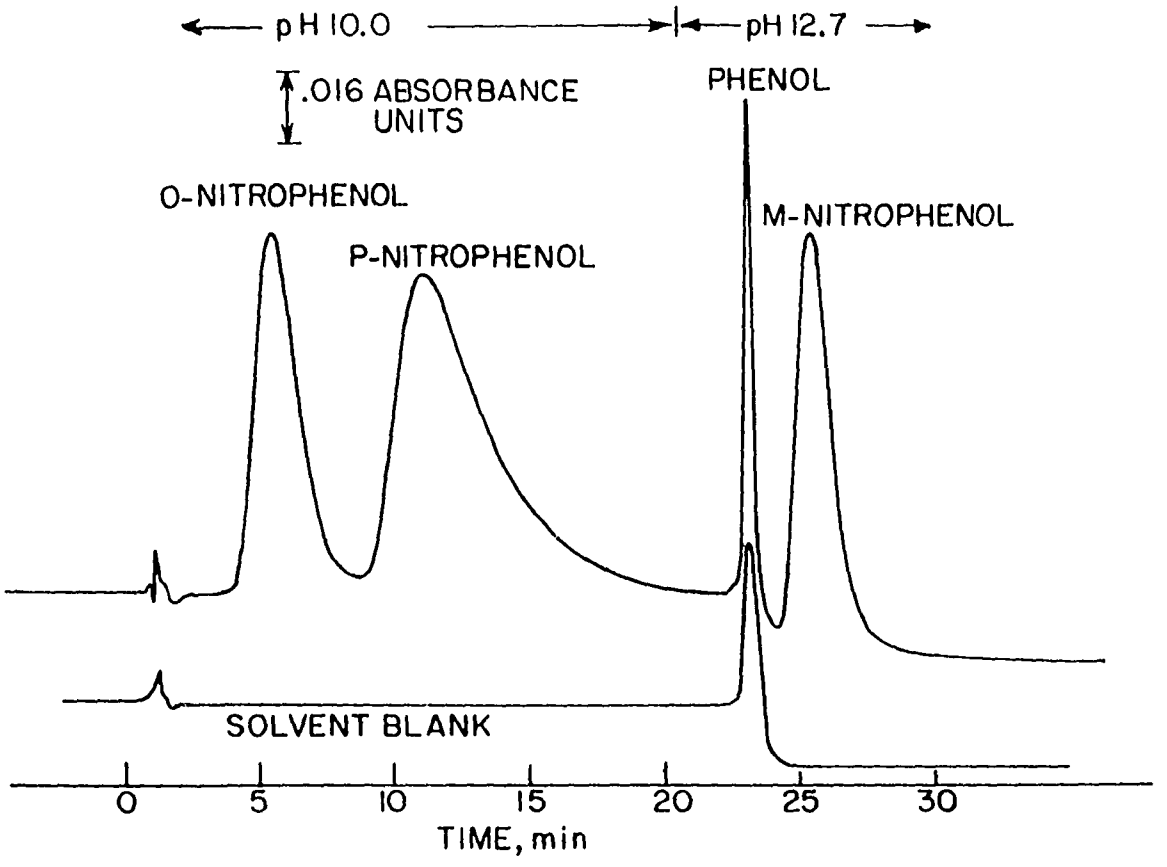


Fig. 16. Separation of nitro substituted phenols

Sample: o-nitrophenol, 1.3 µg; p-nitrophenol, 5.9 µg; phenol, 11.2 µg; m-nitrophenol, 0.95 µg
Column, 2.8 mm x 13 cm; Flow rate, 1.0 ml/min; Sample volume 38.1 µl; Resin, Amberlite XAD-7, approx. 350 mesh

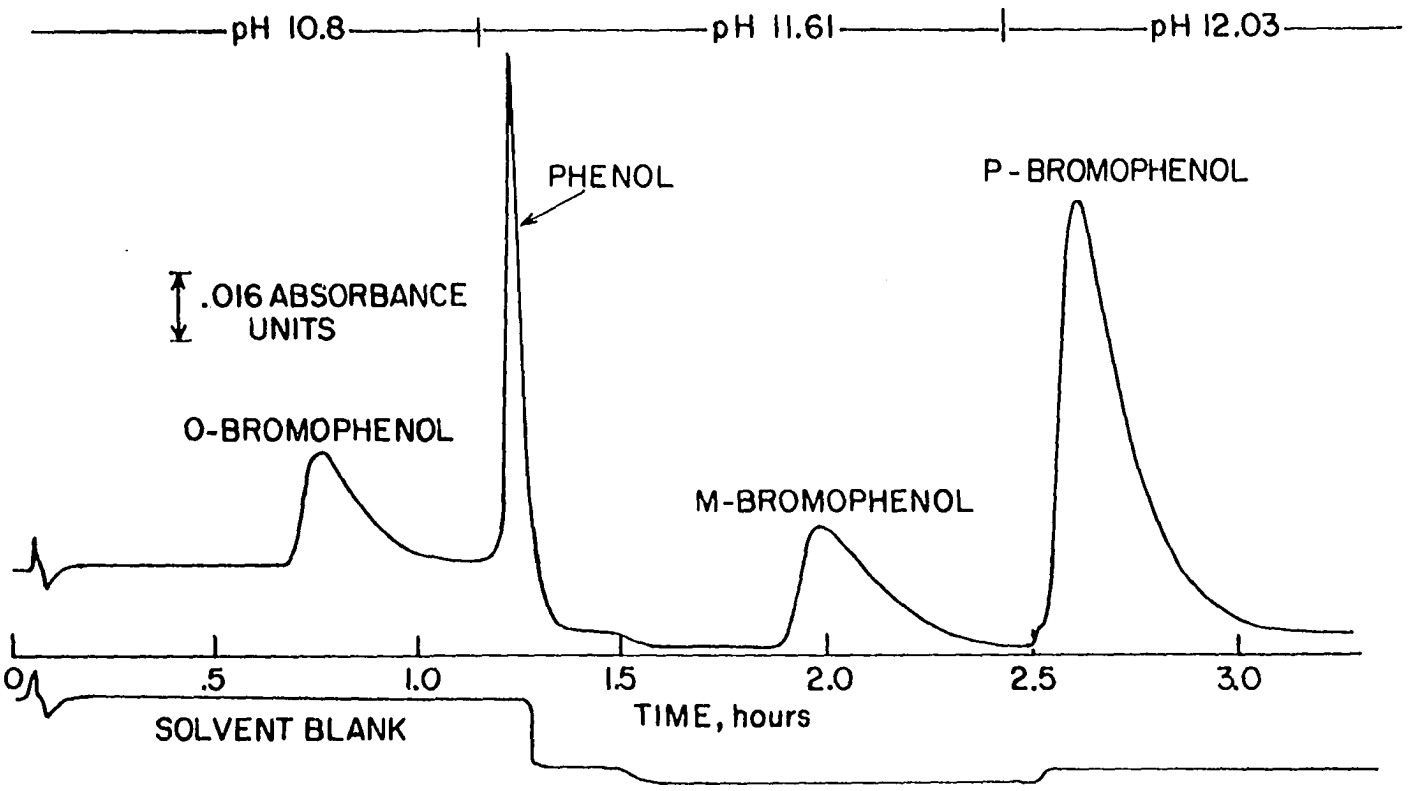


Fig. 17. Separation of bromo substituted phenols

Sample: o-bromophenol, 16 µg; phenol, 14 µg; m-bromophenol, 26 µg; p-bromophenol, 22 µg
Column: 2.8 mm x 50 cm; Other conditions same as Fig. 16

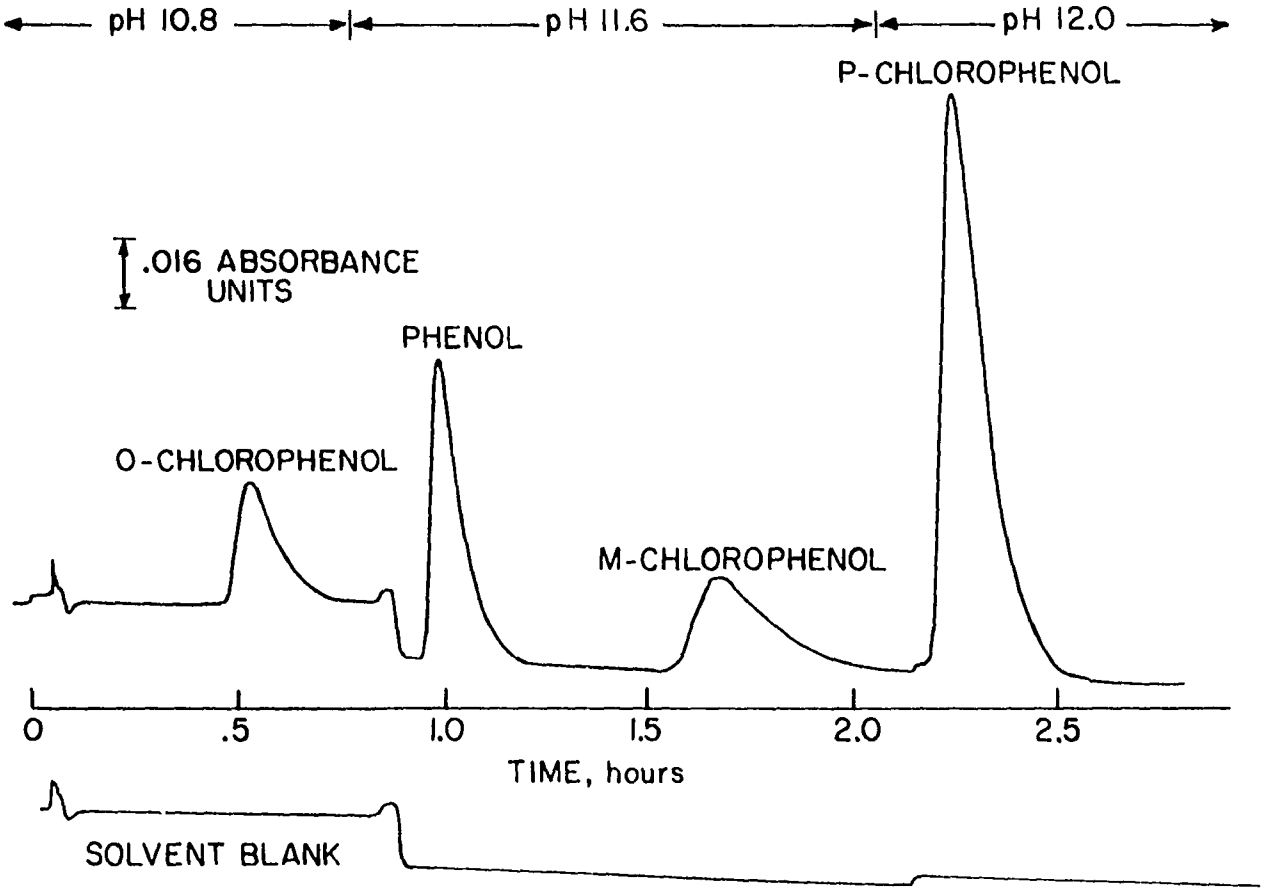


Fig. 18. Separation of chloro substituted phenols

Sample: o-chlorophenol, 16 µg; phenol, 14 µg; m-chlorophenol, 15 µg; p-chlorophenol, 17 µg
Column: 2.8 mm x 50 cm; Other conditions same as Fig. 16

presence of methanol and sodium hydroxide.

Until further work is done to determine what conditions will cause hydrolysis and what will not, it is suggested that the following precautions be taken:

1) Do not use any eluent that contains mixtures of strong base and nonaqueous solvents.

2) In changing from an aqueous strongly basic eluent to a nonaqueous solvent such as methanol prevent the two eluents from overlapping by interspersing a solvent such as water between them. The volume required is often about 6 to 8 bed volumes based on the point where the base line levels off or else where it goes from one level to another.

Quantitative Studies

Calibration curve

Procedure Phenol (0.3 g) was dissolved in methanol and diluted to 100 ml with water, making sure that the amount of methanol was less than 25%. This stock solution was diluted with water to give solutions with varying concentrations of phenol. Each solution was injected onto a 2.8 mm x 13.7 cm column of XAD-7, 350 mesh, and eluted with 65% methanol-35% water. The volume of sample injected in each case was 38.1 microliters.

Results A plot of peak height versus micrograms of sample was found to be linear for 0.3 microgram to 300 micrograms (see Figure 19). The peak height for 0.3 microgram was

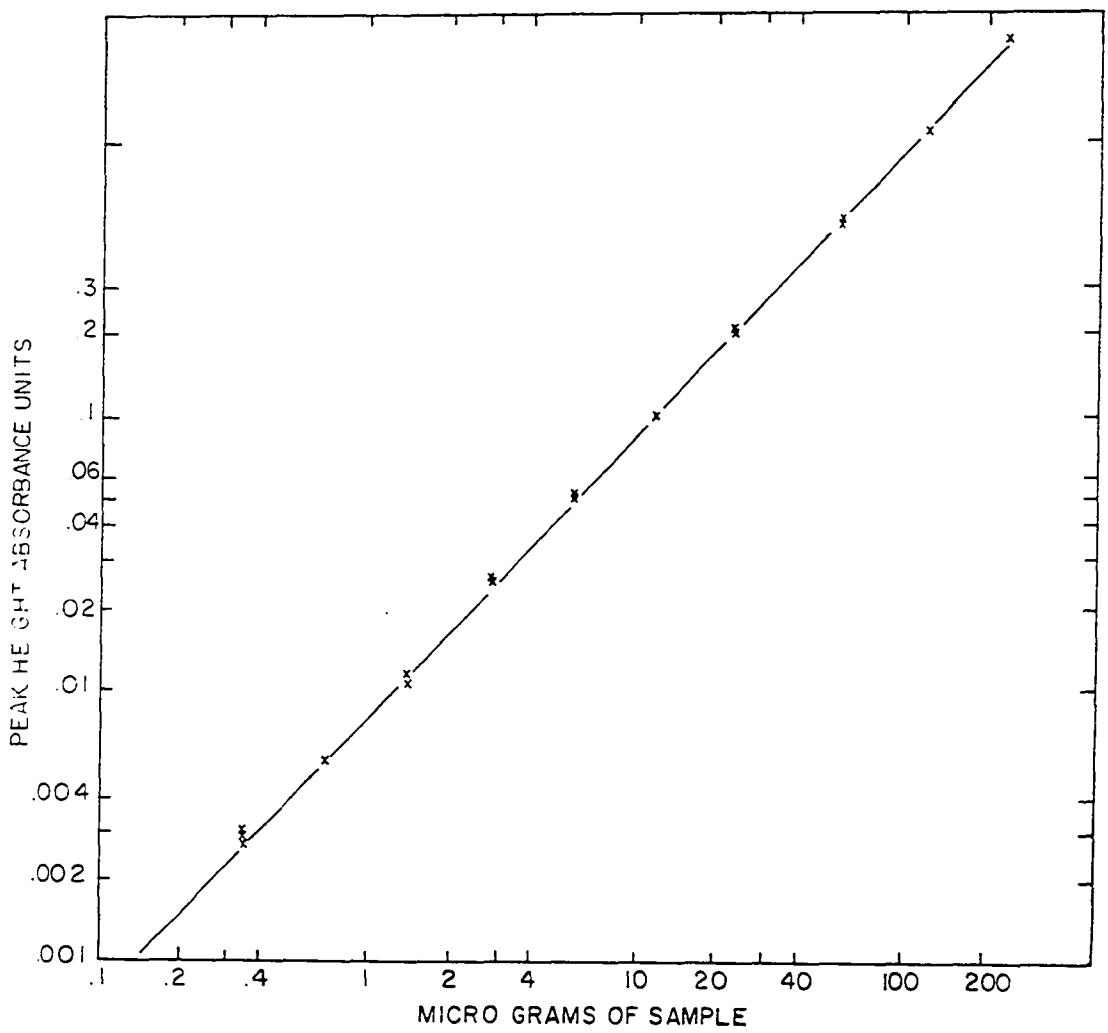


Fig. 19. Calibration curve for phenol

Conditions: Volume of sample, 38.1 µl; Flow rate, 1.0 ml/min;
Eluent, 65% methanol-35% water;
Resin, Amberlite XAD-7, 350 mesh;
Column, 2.8 mm x 13.7 cm

about four times the noise level making the limit of detection about 0.2 microgram.

Quantitative study involving no solvent change

Procedure Phenol, p-bromophenol, and 2,4-dibromophenol were dissolved in water and methanol making sure that the percentage of methanol was less than 25%. 38.1 microliters of this solution was injected onto a 2.8 mm x 13.2 cm column of XAD-7, 350 mesh, and eluted with 70% methanol-30% water. The amount of 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 phenols present.

Results A chromatogram of the mixture is shown in Figure 14. The results are given in Table 2.

Table 2. 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%

Quantitative study involving a solvent change

Procedure A mixture of phenol, o-chlorophenol, and 2,4-dichlorophenol was dissolved in a combination of water and methanol, making sure that the percentage methanol was less than 30%. 38.1 microliters of this solution was injected onto

a 2.8 mm x 29 cm column of XAD-7, 350 mesh, and eluted with 60% methanol for the first 11.25 minutes followed by 65% methanol. At minute 18.75 the eluent was switched back to 60% methanol to equilibrate the column in preparation for the next injection. At minute 35, the base line 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.

Results and discussion A chromatogram of the mixture is shown in Figure 20. The results are given in Table 3.

Table 3. 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%

Normally, the height of the 2,4-dichlorophenol peak would be measured with respect to the baseline as it exists at the $\bar{v}$ for that compound. However, the location of the baseline for this $\bar{v}$ is not well defined so the height was measured with respect to the baseline as established prior to the injection of the sample. In spite of this, the resulting calibration curve was linear but did not pass through the origin. After

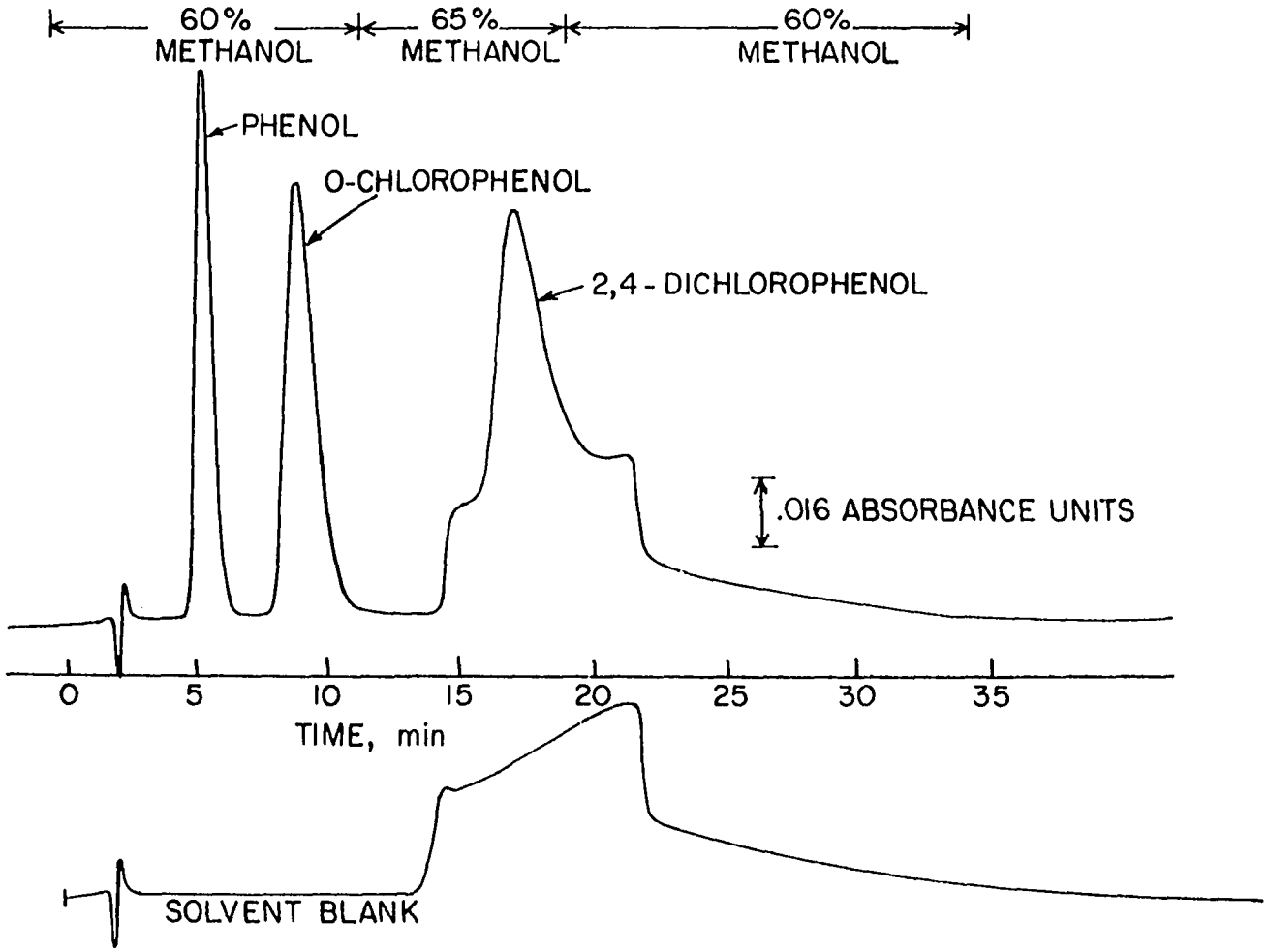


Fig. 20. Separation of chloro substituted phenols used in quantitative study
Sample: phenol, 8.5 µg; o-chlorophenol, 49.4 µg; 2,4-dichlorophenol, 120.5 µg
Column: 2.8 mm x 28 cm; Other conditions same as Fig. 12

changing from 60% methanol to 65% methanol, the baseline jumps due to the higher absorbance of the second solvent. The baseline continues to rise slowly and if one did not change back to 60% methanol, would do so for thirteen minutes after which it would level off. The gradual increase in base line is believed due to the adsorbance of methanol onto XAD-7. When changing from 60% to 65% methanol, the column gradually picks up more methanol until equilibrium is reached. The opposite effect occurs in changing from 65% to 60% methanol.

Precaution

In doing quantitative work, it is important that the sample not be dissolved in a solvent which would be a good eluent for the sample. When the sample is injected, it hits the column in a little band. If the sample solvent is a good eluent, it will cause the sample to be eluted faster while the sample solvent is passing over it. This causes the sample to be spread out on the column resulting in shorter peak height. In the case of phenol being eluted with 65% methanol on the thirteen centimeter column, the maximum percent methanol in which the sample could be dissolved was 25%. Using a 50 cm column, the maximum was 55%. (See Figure 21.) Figure 22 shows a chromatogram of a peak that has been broadened due to the sample being dissolved in a high percentage of methanol. In Figure 22A the sample was dissolved in 2% methanol-98% water. In Figure 22B the sample was dissolved in 100% methanol.

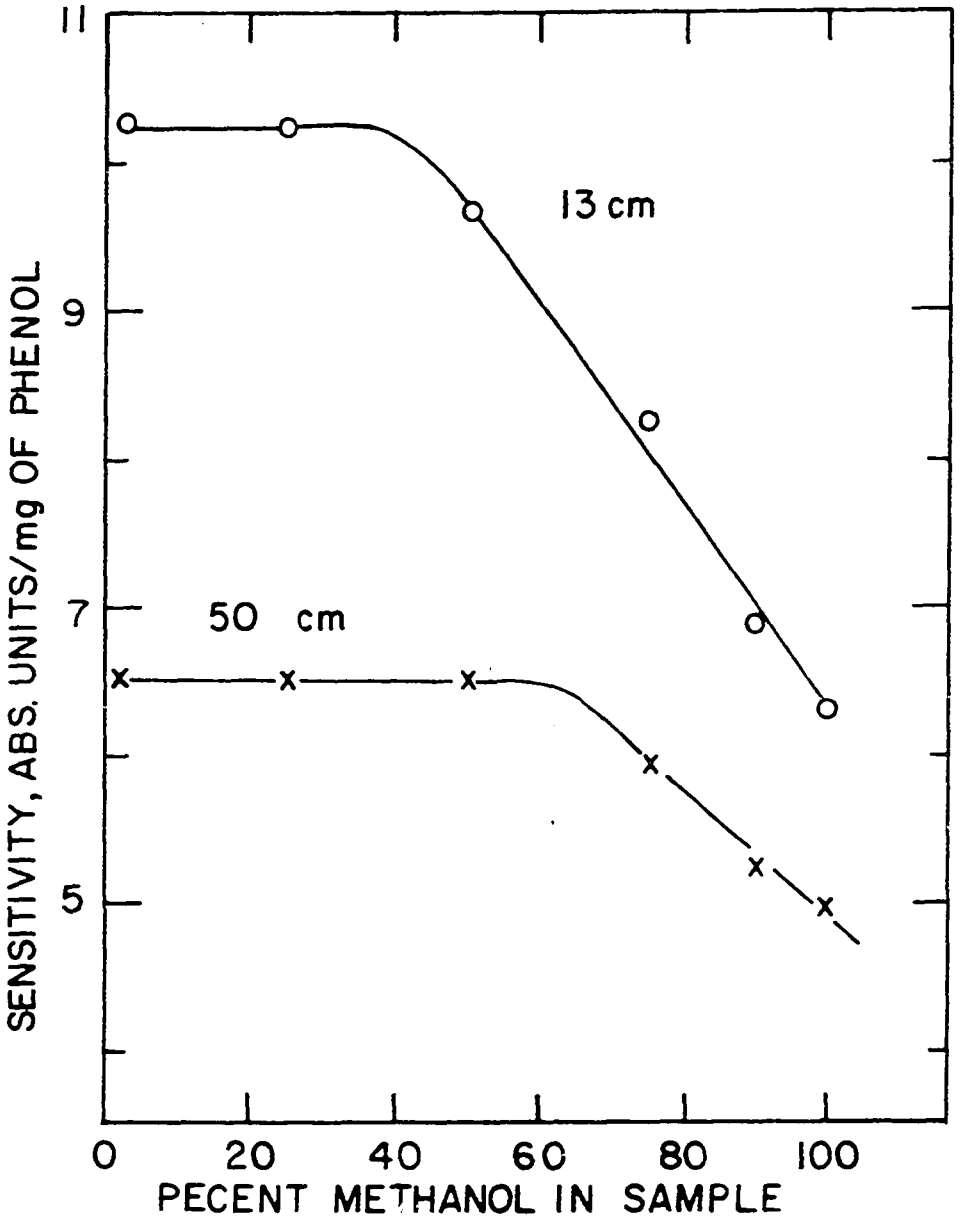


Fig. 21. Sensitivity of phenol (height of peak in absorbance units per mg of phenol) vs. percent methanol in sample solution for a 2.8 mm x 13 cm column and a 2.8 mm x 50 cm column

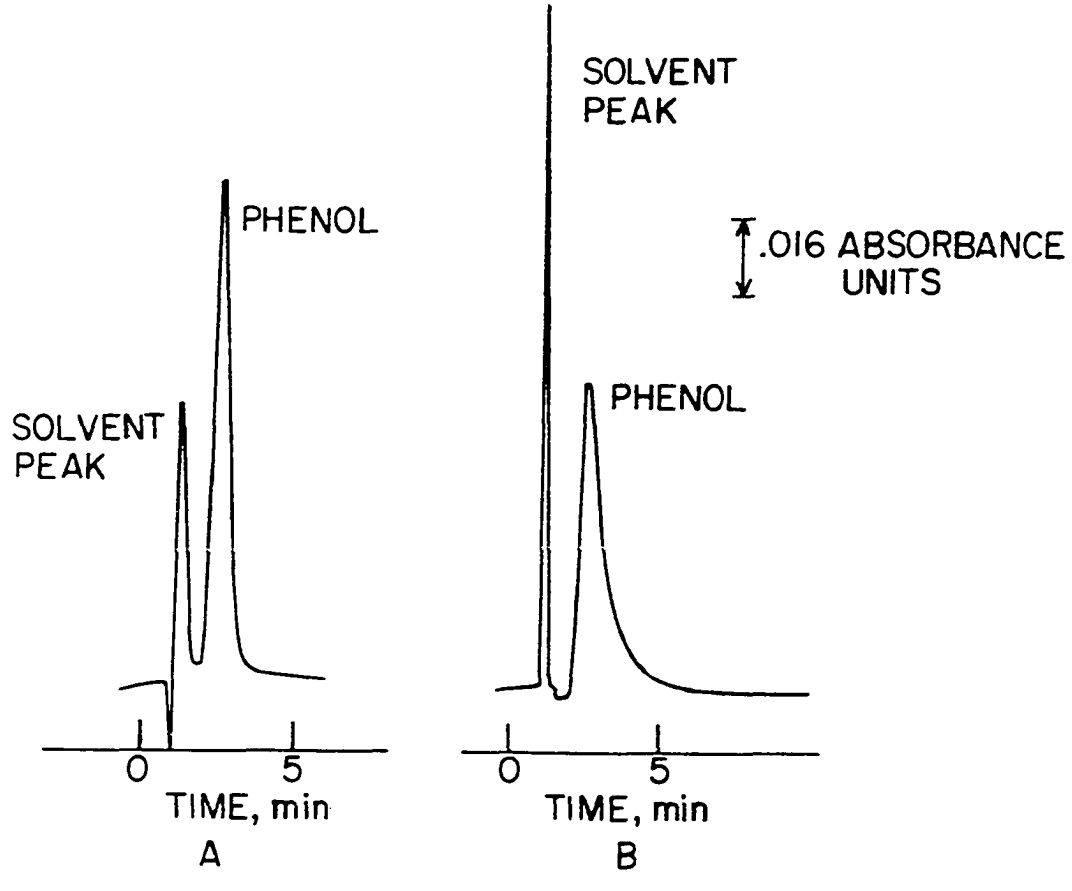


Fig. 22. A. Elution of 10.7 µg phenol dissolved in 2% methanol-98% water
B. Elution of 10.7 µg phenol dissolved in 100% methanol; Column, 2.8 mm x 13.2 cm; Eluent, 65% methanol; Other conditions same as in Fig. 12

EVALUATION OF XAD-7

Adsorption chromatography is today an important technique for the separation and analysis of organic mixtures. Most advances in the theory and practice of the method have come from workers primarily concerned with achieving a particular separation, rather than contributing to the theory of the method (28). Someone who has contributed extensively to the theory is L. R. Snyder. Using his theory, it should be possible to predict the behavior of a large number of organic compounds on the adsorbents: silica, alumina, Magnesia, and Florisil. However, in recent years, many new adsorbents have been placed on the market. It would be useful to find out if the general principles that Snyder developed apply to these adsorbents also.

One new adsorbent is Amberlite XAD-7 produced by Rohm & Haas. Many uses of this adsorbent have been described (3,29) but no one has ever made an attempt to show whether the adsorbent obeys the theory as proposed by Snyder. That is the goal of this part of the thesis.

Principles of Adsorption[1]

The relative adsorption of a sample on an adsorbent, D,[2] depends on two quantities, 1) the relative volumes of adsorbed

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[1]Symbols used in this section are listed in the appendix along with their definition.

[2]D, distribution coefficient, is sometimes also given the symbol K' or R^o.

and nonadsorbed phases and 2) the net energy of adsorption (30). Consider the first effect. A thermodynamic equilibrium constant, K_{th}, is defined such that

$$K_{th} = N_{xa}/N_x \tag{10}$$

where N_{xa} is the mole fraction of sample in the adsorbed phase and N_x is the mole fraction of sample in the nonadsorbed phase. In elution chromatography, the number of moles of sample in the adsorbed phase, n_{xa}, is usually small compared to the number of moles of solvent in the adsorbed phase, n_{sa}. Likewise, the number of moles of sample in the nonadsorbed phase, n_x, is small compared to the number of moles of solvent in the nonadsorbed phase, n_s. Thus, there exists the approximate relationship

$$N_{xa} = n_{xa}/n_{sa} \tag{11}$$

and

$$N_x = n_x/n_s \tag{12}$$

This results in the equation

$$K_{th} = n_{xa}n_s/n_x n_{sa} \tag{13}$$

Since D has been defined as

$$D = C_{xa}/C_x \tag{14}$$

where C_{xa} is the concentration of sample adsorbed in moles/gram of resin and C_x is the concentration of sample not adsorbed in moles per ml of solvent, D can be defined as follows:

$$D = \left(\frac{n_{xa}}{g}\right)/\left(\frac{n_x}{v}\right) \tag{15}$$

Combining Equations 13 and 15 results in the equation

$$D = \frac{K_{th} n_{sa} V}{n_s g} \tag{16}$$

Let V_a be the volume in ml of adsorbed solvent per gram of resin which equals $n_{sa}V/n_s g$. Substituting this value of V_a into Equation 16, the result is

$$D = K_{th} V_a \tag{17}$$

Thus D equals the ratio of sample in the adsorbed phase to that in the nonadsorbed phase times the volume of solvent adsorbed.

To calculate V_a, assume the adsorbed solvent exists as a monolayer. This monolayer is usually about 35 Å thick (31,32) so that

$$V_a = (3.5 \times 10^{-4})(\text{surface area in } m^2/g) \tag{18}$$

This gives an answer with units of ml.

The second factor on which the adsorption of a sample depends is the net energy of adsorption. To take this into consideration let the equilibrium constant K_{th} be related to standard free energy in the usual way.

$$\log K_{th} = -\Delta G_a^\circ / 2.3RT \tag{19}$$

where R is the gas constant, T is the absolute temperature and ΔG_a° is the free energy of adsorption. To simplify the derivation, let $-\Delta G_a^\circ/2.3RT$ be ΔE. Substitute this into Equation 17 and the result is

$$\log D = \log V_a + \Delta E \tag{20}$$

In liquid chromatography, a competition exists between

the sample molecule X and solvent molecule S

$$X_l + mS_a \rightleftarrows X_a + mS_l \tag{21}$$

This can be thought of in the following way. A sample molecule from the liquid phase will displace m solvent molecules on the adsorbent. The net equation is

$$\Delta E = E_{xa} + mE_{sl} - E_{xl} - mE_{sa} \tag{22}$$

The terms E_{xa}, E_{sl}, E_{xl}, and E_{sa} refer to the dimensionless (partial molal) energies of the sample, x, and solvent, s, molecules in the adsorbed, a, or liquid, l, phases. For most adsorption systems, E_{sl} and E_{xl} are much less important than the terms E_{sa} and E_{xa} (33). Therefore, to a close approximation,

$$\Delta E = E_{xa} - E_{sa} \tag{23}$$

Equation 20 can now be written as

$$\log D = \log V_a + E_{xa} - mE_{sa} \tag{24}$$

The adsorption energy of the sample for the adsorbent, E_{xa}, can be thought of as depending on the adsorbent and on the sample. The same is true for E_{sa} so that these can be written:

$$E_{xa} = f(x)f(A_i) \tag{25}$$

$$E_{sa} = f(s)f(A_i) \tag{26}$$

Assuming $f(A_i)$ is the same for the sample and the solvent, it shall be called α so that it is now possible to write Equation 24 as

$$\log D = \log V_a + \alpha f(x) - \alpha m f(s) \tag{27}$$

The term f(x) represents the energy of adsorption of the sample for the adsorbent. This energy will be called S^o. If A_s is the area of the adsorbed sample molecule and A_e is the area of the adsorbed solvent molecule, then

$$m = A_s/A_e \tag{28}$$

If ε is defined as the energy required for each solvent molecule to adsorb per unit area, then

$$\varepsilon = f(s)/A_e \tag{29}$$

Solving for the term mf(s), the result is εA_s. Substituting these terms into Equation 27, the result is Snyder's equation

$$\log D = \log V_a + \alpha(S^o - A_s\varepsilon) \tag{30}$$

For any one adsorbent, α is usually one unless the adsorbent has been modified in some way such as by the addition of water. In all the work described in this thesis, it is assumed the characteristics of the resin remain the same so alpha will always be equal to one.

Discussion of Snyder's Equation

Elutropic strength

The parameter in Snyder's equation which takes into consideration the effect of the solvent is elutropic strength, ε. For small values of elutropic strength, the solvent has very little energy of adsorption. Thus, the sample molecule is much more likely to stay adsorbed resulting in large values of D. For large values of elutropic strength, the solvent has

enough energy to displace any sample that is adsorbed to the adsorbent. Thus, the sample comes right through the column and D values are low. One of the weakest solvents known is pentane which is arbitrarily given the value of zero. To calculate the solvent strength of some other solvent, ε_2, let the D value for benzene using this solvent be D_2. For pentane let $\varepsilon = \varepsilon_1$ and the D value for benzene using the solvent pentane be D_1.

$$\log D_2 = \log V_a + \alpha(S^o - \varepsilon_2 A_s) \tag{31}$$

$$\log D_1 = \log V_a + \alpha(S^o - \varepsilon_1 A_s) \tag{32}$$

Subtracting Equation 32 from Equation 31, the result is

$$\log D_2/D_1 = \alpha A_s(\varepsilon_1 - \varepsilon_2) \tag{33}$$

For an adsorbent of standard activity, α is one. For convenience, Snyder has decided to let A_s for benzene be 6. This leaves the value of ε_2 as the only unknown so its value can now be determined.

Molecular area, A_s

Once values of elutropic strength are known, it is possible to graph log D as a function of ε. According to Snyder's equation, the slope should equal $-A_s$ and the intercept equals $\log V_a + S^o$. This allows A_s to be calculated experimentally.

It is possible to predict A_s if it is assumed that A_s equals the sum of the contributions of each group that makes up the molecule, a_i.

$$A_s = \sum_i^i a_i \tag{34}$$

Values of a_i have been calculated for alumina (34), silica (34), Florisil (35), and Magnesia (36). As previously stated, the A_s value for benzene is 6. Since the area of the benzene molecule is 50 $\mathring{A}^2$ it can be stated that there exists a conversion factor: 1 $A_s \approx 8.5$ $\mathring{A}^2$.

Sample adsorption energy, S^o

The experimental calculation of S^o values can be determined from the graph of log D as a function of ε. As was pointed out previously, the intercept of the graph equals log $V_a + S^o$. If the surface area of a resin is known, values of S^o can be calculated.

Assuming that the sample adsorption energy, S^o, of a compound is equal to the sample adsorption energy of the substituents that make up the molecule, Q_i^o, it is possible to predict S^o. The equation used is

$$S^o = \sum^{i} Q_i^o \qquad (35)$$

Values of Q_i^o have been compiled for alumina and silica (37).

In order for the adsorption energy of any group i for the adsorbent to remain constant, three conditions must be met.

1) The adsorbent surface lying beneath each group of the sample molecule must be essentially constant.

2) The electronic structure of group i must not change; i.e. it must remain independent of the remainder of the sample molecule.

3) The relative orientation of i with respect to the adsorbent

surface must not change.

Any failure of the experimental S^o value to equal the predicted value is probably due to one of the above three reasons.

Comparison of XAD-7 to Other Resins

In order to compare XAD-7 to other resins, it was necessary to compare percent methanol to elutropic strength. In order to accomplish this, the batch distribution coefficients of two phenols were determined for several solvents. The results are given in Tables 4 and 5. If one graphs log D as a function of elutropic strength, the result is a straight line. On the basis of this graph an equation can be written for phenol which is

$$\log D = -4.65\varepsilon + 2.74 \tag{36}$$

From Table 1, the equation for phenol in water-methanol solvents is

$$\log D = 7.48 - 3.66 \log C \tag{37}$$

Equating the two expressions for log D and solving for log C yields the following result:

$$\log C = 1.28\varepsilon + 1.3 \tag{38}$$

Doing the same for p-bromophenol yields the result

$$\log C = 1.38\varepsilon + 1.38 \tag{39}$$

Since Equations 38 and 39 are essentially the same, it was decided to establish the hypothesis that there exists one and only one equation relating percent methanol and elutropic strength, that equation being

Table 4. Distribution coefficients of phenol as a function of elutropic strength

Solvent	ε	D
Acetone	0.56	1.56
Isopropyl ether	0.28	13.5
Ethylether	0.38	8.6
Carbontetrachloride	0.18	130
Pentane	0	375

Table 5. Distribution coefficients of p-bromophenol as a function of elutropic strength

Solvent	ε	D
Carbon tetrachloride	0.18	210
Xylene	0.26	29
Benzene	0.32	8.5
Chloroform	0.40	10.0

$$\log C = 1.3\varepsilon + 1.3 \quad (40)$$

If Equation 40 is true, it is possible to describe the distribution coefficient of any phenol as a function of elutropic strength. An example is shown for 2,4-dibromophenol. From Table 1, one can write the equation

$$\log D = 13.021 - 6.25 \log C \quad (41)$$

Combining Equations 40 and 41 gives the result

$$\log D = 13.021 - 6.25(1.3\varepsilon + 1.3) \quad (42)$$

$$\log D = 4.9 - 8.12\varepsilon \quad (43)$$

This equation is similar to Snyder's equation (Equation 30) and will allow the calculation of S^o and A_s for 2,4-dibromophenol. The surface area of XAD-7 is 450 sq meters/gram (3). Using the Equation 18, the value of $\log V_a$ is -0.803. Since the characteristics of XAD-7 have not been modified in any way, the value of α is one. Using these values for α and $\log V_a$, the resulting values of A_s and S^o have been worked out for all the phenols chromatographed as described earlier and are included in Table 7.

As previously discussed, it is possible to predict values of S^o using the equation

$$S^o = \Sigma Q^o. \quad (44)$$

For example, S^o for p-bromophenol can be represented by the equation

$$S^o = Q^o_{phenol} + Q^o_{Br}. \quad (45)$$

S^o for phenol can be represented by

$$S^{o} = Q^{o}_{phenol} \qquad (46)$$

From Equation 46, $Q^{o}_{phenol} = 3.52$. Using this value in Equation 45, $Q^{o}_{Br} = 1.34$. By this technique, Q^{o}_{i} values were calculated for each substituent on the basis of the meta and para isomers. The average of these values was taken as the Q^{o}_{i} value for that substituent. The results are tabulated in Table 6 along with Q^{o}_{i} values for alumina and silica for comparison.

Table 6. Values of Q^{o}_{i}, group contributions to adsorption energy

Substituent	XAD-7 Q^{o}_{i}	XAD-7 Q^{o}_{ortho}	Alumina Q^{o}_{i}	Silica Q^{o}_{i}
-Br	1.38	-0.34	0.33	-0.17
-Cl	1.09	-0.35	0.20	-0.20
$-CH_3$	0.64	-0.44	0.06	0.11
$-NO_2$	0.80	-0.20	2.75	2.77
 -OH	3.52		9.26	5.70

As stated earlier, Equation 44 is valid only if the electronic structure of a substituent does not change as its position on the molecule changes. Since the electronic structure of an ortho substituted isomer is usually different than that of either the meta or para substituted isomer, the energy of adsorption should also be different. This difference in energy is taken into account by modifying Equation 44 to

$$S = \sum_{i} Q_i^o + Q_{ortho}^o \qquad (47)$$

where Q_{ortho}^o shall be interpreted to mean the additional adsorption energy of any group in an ortho position. Using Equation 47, values of Q_{ortho}^o have been calculated and are included in Table 6. To illustrate the use of Equation 47, suppose one desires the S^o value of 2,4-dibromophenol. The following values are summed.

Q_{phenol}^o	3.52
$2Q_{Br}^o$ @ 1.38 =	2.76
$Q_{Br,ortho}^o$	-.34
Total	5.94

Therefore, the S^o value for 2,4-dibromophenol is predicted to be 5.94. The experimental value was 5.70. Predicted values of S^o along with experimental values are shown in Table 7.

Just as values for Q_i^o were calculated, group contributions to molecular area (a_i) can be calculated using the comparable formula

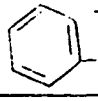
$$A_s = \sum_{i} a_i + a_{ortho} \qquad (48)$$

This was done with the resulting values summarized in Table 8. From this the value for molecular area of a phenol can be predicted. This has been done with the results shown in Table 7 along with the experimental values for molecular area.

Table 7. Predicted and experimental values of adsorption energy, S^o, and molecular area, A_s, for several phenols

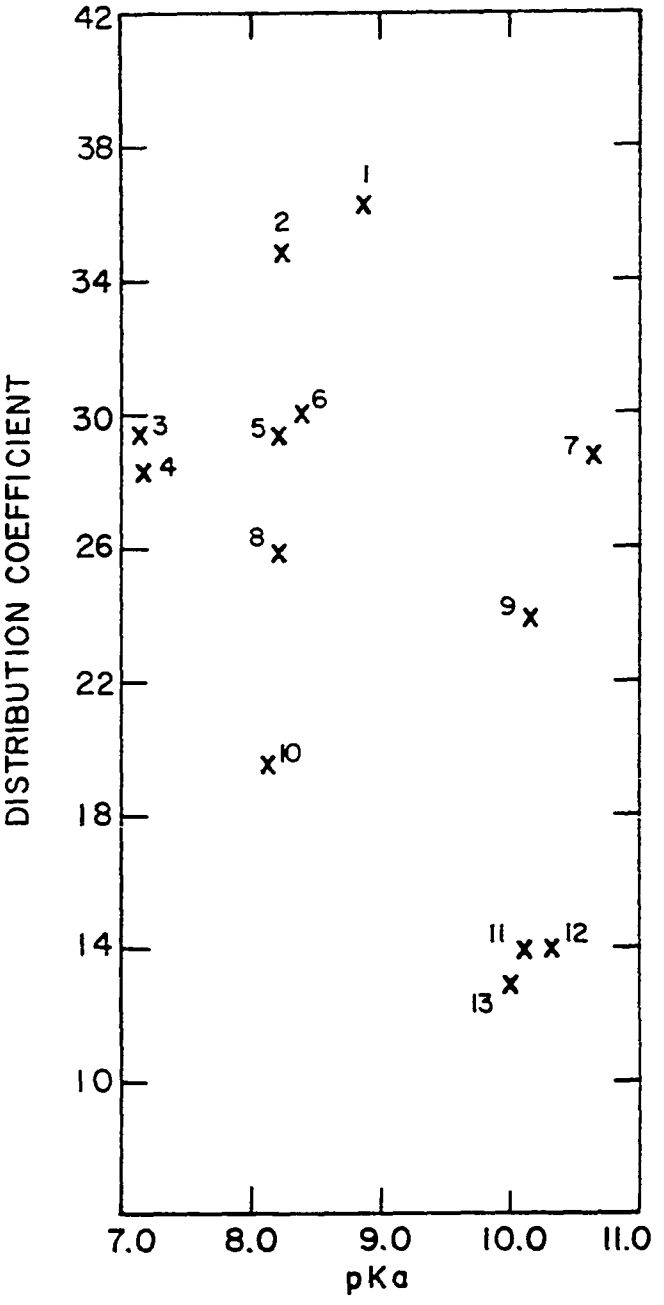
Compound	Experimental S^o	Predicted S^o	Experimental A_s	Predicted A_s
o-Bromophenol	4.56	4.56	6.35	6.35
p-Bromophenol	4.86	4.90	6.82	6.92
m-Bromophenol	4.94	4.90	7.01	6.92
2,4-Dibromophenol	5.70	5.94	8.12	8.51
Pentabromophenol	8.72	9.74	13.97	14.42
o-Chlorophenol	4.26	4.26	5.93	5.93
p-Chlorophenol	4.60	4.61	6.49	6.45
m-Chlorophenol	4.62	4.61	6.42	6.45
2,4-Dichlorophenol	5.32	5.35	7.67	7.62
3,5-Dichlorophenol	5.70	5.70	8.32	8.14
2,3,6-Trichlorophenol	5.74	6.09	8.22	8.79
2,3,4,5-Tetrachlorophenol	6.66	7.53	9.67	11.00
p-Cresol	4.17	4.16	6.00	5.96
m-Cresol	4.14	4.16	5.91	5.96
o-Cresol	3.72	3.72	4.45	4.45
3,5-Dimethylphenol	4.34	4.80	5.87	7.16
2,6-Dimethylphenol	4.24	3.92	5.37	5.65
3,4,5-Trimethylphenol	4.72	5.44	6.57	8.36
2,4,6-Trimethylphenol	4.79	4.56	6.52	5.34
o-Nitrophenol	4.13	4.13	5.11	5.11
m-Nitrophenol	4.33	4.32	5.59	5.59
p-Nitrophenol	4.32	4.32	5.59	5.59
phenol	3.52	-	4.76	-

Table 8. Values of a_i, group contributions to molecular area

Substituent	XAD-7 a_i	XAD-7 $a_{i,ortho}$	Alumina a_i	Silica a_i
-Br	2.16	-0.57	0.5	1.0
-Cl	1.69	-0.52	1.6	0.7
$-CH_3$	1.20	-1.51	0.8	0.8
$-NO_2$	0.83	-0.48	2.5	7.5
 -OH	4.76			13.6

A comment

Whereas most compounds lie in a flat configuration with respect to the adsorbent, phenols will sometimes lie in a vertical configuration when a strong eluent is being used (38). That is, the OH substituent will be adsorbed to the resin and the remaining portion of the molecule will be as far removed as possible. When this is the case, the adsorption of any phenol depends not on the size of the molecule or on the total adsorption energy of all substituents but on the acidity of the phenol (38). If, in the experimental work done for this thesis, the phenols have been adsorbed in a vertical configuration, the conclusions made in the previous pages are incorrect. In order to defend these conclusions a graph relating the distribution coefficient of many phenols for XAD-7 in 60% methanol-40% water as a function of the pKa of that phenol (39) is shown in Figure 23. On the basis of this graph, it is contended that there is no relationship between distribution coefficient and pKa. Therefore, it is probably the case that the phenols lie in a



1, m-bromophenol; 2, p-bromophenol; 3, p-nitrophenol; 4, o-nitrophenol; 5, m-chlorophenol; 6, m-nitrophenol; 7, 2,6-dimethylphenol; 8, p-chlorophenol; 9, 3,5-dimethylphenol; 10, o-chlorophenol; 11, m-cresol; 12, p-cresol; 13, phenol

Fig. 23. Distribution coefficients of several phenols on XAD-7 in 60% methanol as a function of the pKa of the phenol

flat configuration and the conclusions drawn in the previous pages are correct.

It seems logical that the configuration would be flat in this case. The adsorbents on which the observation was made that phenols lie in a vertical configuration are polar over the entire surface. XAD-7 is not. Beside each ester functional group of XAD-7 is a long hydrocarbon chain. Thus, it would be easy for the hydroxyl portion of a phenol to be attracted to the ester functional group of the adsorbent. At the same time the remainder of the phenol molecule being less polar would be attracted to the hydrocarbon portion of the adsorbent. The result being a flat configuration.

DETERMINATION OF BISMUTH BY FORCED FLOW LIQUID CHROMATOGRAPHY

Until about six months ago, detectors for liquid chromatography were capable of monitoring the effluent from a liquid chromatograph at only one to five discrete wavelengths. Some detectors have only one wavelength available which is usually 254 nm. Other detectors have two wavelengths available which usually include the additional wavelength of 280 nm. A few detectors have other wavelengths available. Within the past six months, two companies have introduced UV detectors capable of monitoring column effluent at any wavelength. Varian Aerograph has accomplished this by taking a spectrophotometer already available and equipping it with a flow through cell. Schoeffel Instrument Company has built a detector that contains a monochromator. Until detectors of this type become widely available it will be important to consider the absorbance of the desired species under the conditions in which it is eluted at the wavelengths available on most detectors.

Bismuth is an example of one such species. It can be eluted from a cation exchanger with 0.5 M HBr, conditions under which no other metal ion is simultaneously eluted. The bromo complex of bismuth in 0.5 M HBr has an absorptivity of 37,000 at 254 nm. Another acid that has been used up to the present time in the ion exchange separation and determination of metal ions is hydrochloric acid (40). The chloro complex of bismuth has an absorptivity of 47,000 when dissolved in 6 M HCl (41).

However, the 47,000 absorptivity of the chloro complex occurs at 225 nm. The absorptivity at either 254 nm or 280 nm is at most 1/10th that at 225 nm. But this absorptivity is valid only if the concentration of HCl used to elute the bismuth is 6 M. For lower concentrations of acid, the absorptivity of a metallic species is often less (42,43). In using a cation exchanger, the approximate molarity of HCl to elute the bismuth is about 0.5 M (44). Thus the actual absorptivity of the bismuth chloro complex at either 254 nm or 280 nm may be less than 1/10th of 47,000 as previously suggested. So, unless one has a detector capable of monitoring column effluent at 225 nm, hydrobromic acid is the better acid to use in a cation exchange method for determining bismuth.

Some work has already been done using hydrobromic acid in the separation and determination of different metals using a cation exchanger. Fritz and Garralda used hydrobromic acid with a cation exchanger to separate mercury, bismuth, and cadmium from each other and other metals (45). Bismuth was determined by evaporating the hydrobromic acid and titrating the bismuth with EDTA. Strelow and Boshoff used hydrobromic acid with the cation exchanger AG 50W-X4 to separate thorium from rare earths, zirconium, barium, and other metals (46). Most other metals are eluted first with 5.5 M HBr. Thorium is then eluted with 5 M HNO_3. Nelson and Michelson have published distribution coefficients for most metals using hydrobromic acid with the cation exchanger, Dowex 50-X4 (47). Potential

separations as indicated by these studies were illustrated. Dybczynski and Maleszewska used Dowex 50 and 6 M HBr-0.0035 M Br_2 to retain gold while eluting platinum, palladium, iridium, and rhenium (48). Gold was then eluted with acetylacetone.

There are already methods available for determining bismuth that make use of the high absorptivity of the bromo complex. Nielsch and Boltz have developed a method for bismuth in which enough hydrobromic acid is added to the sample to make the solution 1.75 M (49). The absorbance is measured at 375 nm. Beer's Law is obeyed for concentrations of bismuth in the range of 1 to 15 μg/ml. Stolyarova has proposed a method for bismuth in which the sample is dissolved in 1 M KBr plus HCl (50). Beer's Law is obeyed for concentrations of bismuth from .2 to 6 μg/ml.

One colorimetric method that is very selective was developed by Lau, Droll, and Lott using Ammonium 1-pyrrolidinedithiocarbamate (51). With this method, lead(II), silver(I), tin(IV), mercury(II), thorium(IV), aluminum(III), iron(III), and many others do not interfere when present at 40 fold excess. Three NBS standards were measured for bismuth with acceptable results. The only metals that interfere are thallium(III) and platinum(IV). The interference of thallium is eliminated by reducing thallium(III) to thallium(I). The interference of platinum(IV) is circumvented by measuring at a higher wavelength. The procedure is a little tedious requiring a double extraction and adjustment of pH to 10.5.

Cheng, Bray, and Melsted have developed a method for bismuth using diethyldithiocarbamate (52). Cadmium, mercury, copper, and silver do not interfere in 10 fold excess. A lead base bearing metal, NBS standard, was analyzed for bismuth. Based on this analysis, a 1,000 fold excess of lead, 110 fold excess of antimony, and 55 fold excess of tin do not interfere. The limit of detection for the method was 1 ppm.

The advantages of the method described in this thesis are that it is very selective, reasonably fast, has a sensitivity better than 12 ppb, and allows the sample to be dissolved in solutions which are much more acidic than is required for most methods.

Equipment

The liquid chromatograph

The liquid chromatograph used was the same as that used for part one of this thesis. A few modifications were made, however.

In the eluent line between valve #9 and #10, Figure 1, was placed an on-off valve, Chromatronix #CAV2031K. This was used to turn off the flow of eluent through the column. Previously, this was done by turning the six-way selector valve midway between two openings. This works but is not recommended.

Another change was made in preparation for the time when the liquid chromatograph would be equipped with a gradient elution device. This was the installation of a size #1 flow

meter, Roger Gilmont #F7160, in the water line after the water reservoir and before valve #3. The manufacturer reports in the description of the flow meter that, "glass under pressure is always subject to danger, hence the need for protective tubes, the following safe working pressures are recommended: Size #1, 500 lb etc." When the flow meter was installed it was found that pressures greater than 20 pounds caused the flow meter to leak. For this reason, the following modifications were made: The hole in the retainer nut was enlarged slightly at the bottom so it would push down on the o-ring just underneath it. The little threaded cap on the capillary valve that holds the adapter o-ring tight against the valve block was remade of stainless steel. A 1/4" o-ring in the capillary valve assembly was replaced with a similar o-ring made of Vyton.

Justification for placing the flow meter in the water line was based on the assumption that the flow rate of water passing through the water line was equal to the flow rate of the eluent passing through the column. Therefore, only one calibration curve would be necessary to determine the flow rate of any eluent passing through the column.

Another change in the liquid chromatograph was to replace the Teflon hats with pieces constructed of polypropylene. The Teflon hats occasionally decomposed to the extent that water passed through them and into the eluent bag. After changing from Teflon to polypropylene, no problems with decomposition have been encountered. Teflon has been described as having

the property of not being attacked by acids, bases, or organic solvents (53). For this reason it was immediately suspected that either the machinist or supplier made a mistake and actually made the hats out of some other plastic such as nylon. Both the supplier and the machinist have given their assurance that there was no mistake. At least three Teflon hats were remade with the same result. There are different grades of Teflon. Perhaps this is an explanation of the problem.

Column

Preliminary work on this project was done using a Chromatronix column LC-6M-13. This column is supplied by Chromatronix with the dimensions 6.3 mm x 13 cm. For the work on this project it was shortened to 6 cm. After about a month of use on this project, the non-Teflon plastic parts on the column decomposed. The column was replaced with a Kel-F rod, 1/4" x 6" long. A 2.6 mm hole was drilled the length of the rod. Each end was threaded on the outside to yield threads 1/4", 28 threads per inch. This allowed each end of the column to be connected to a Chromatronix tube end fitting by means of a Chromatronix coupling. In the outlet end of the column was placed a small wad of glass wool. The 0.8 mm diameter of the tubing that follows the column is smaller than the diameter of the column and keeps the glass wool in place. This worked at all pressures used which included pressures as high as 400 lbs. Taking the glass wool into consideration the dimensions of the column were 2.6 mm x 13 cm. This column holds 0.30 g of A-15

resin.

The column was packed by connecting the top of the column to a 1/8" x 6" stainless steel pipe by means of a Kel-F Reducer. (See Figure 24) To the other end of the pipe was attached a 1/8" coupling to which can be attached a brass bushing from Chromatronix #107A54. To the bottom of the column was attached a short length of Chromatronix tubing to keep the resin from coming out. Slurry the resin in a suitable solvent and by means of a pipette, transfer the resin to the pipe. Screw the bushing onto the top of the pipe. Disconnect the tubing on the liquid chromatograph that goes to the injection port and connect it to the brass bushing. Turn on the liquid chromatograph which will force the resin into the column. Turn off the liquid chromatograph and if the pipe was completely filled with solvent, the column can be disconnected without any of the resin in the pipe coming out. To remove the resin from the pipe, turn on the liquid chromatograph again and any resin remaining in the pipe will be forced out.

Experimental

Reagents

The hydrobromic acid used in this study was Baker Analyzed Reagent which is contaminated with a little bromine. In order to decrease the intensity of the solvent blank, the bromine was removed by passing the HBr through a column of Amberlite XAD-7 from Rohm and Haas. As long as the concentration of hydro-

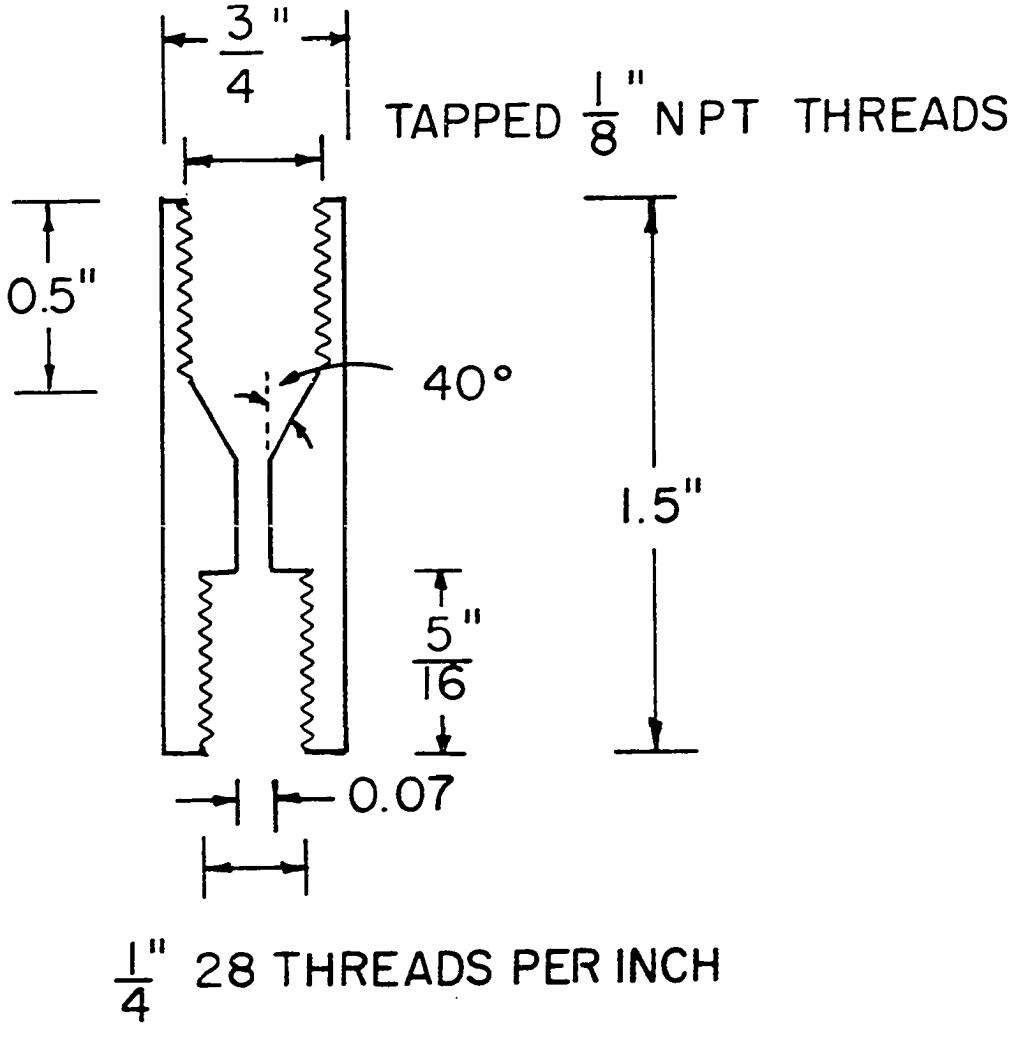


Fig. 24. Kel-F reducer

bromic acid is greater than about 4 or 5 molar, the bromine is retained by the XAD-7. In this case, the hydrobromic acid was passed through the column as the concentrated reagent just as it was supplied. In order to elute the bromine from the column, pass water through it and the bromine is rapidly eluted.

Resins

The resins used in this study were Dowex 50W-X8 and AG MP-50 which were supplied by Bio Rad and Amberlyst 15, Amberlite 200, Amberlite XE-284, Amberlyst XN-1005 and Amberlite XAD-7 which were supplied by Rohm and Haas. The AG MP-50 was supplied as 100 to 200 mesh beads. The Dowex 50W-X8 was supplied as 200 to 400 mesh. Both these resins were used without further treatment. The resins supplied by Rohm & Haas were ground, sieved, and the fines decanted. The mesh size used was 250 to 325 mesh. Decanting fines was done by placing a few grams of resin in a 250 ml beaker along with methanol. After stirring, the resin was allowed to settle and any resin which had not settled to the bottom in two or three minutes was poured out along with most of the methanol.

In the remainder of this thesis, Amberlyst 15 is abbreviated as A-15.

Sample preparation

Solutions for interference study All solutions except antimony and tin used for the interference study were prepared by dissolving about 10 m moles of the chloride or nitrate of the desired metal in 100 ml of either 1 M or 0.1 M HNO_3. Ali-

quots of these solutions were mixed with an aliquot of a similarly prepared bismuth solution and diluted to volume. The final concentration of solvent for each interferent study is given in Table 11.

A 0.001 M antimony(III) solution was prepared by dissolving 1.2175 g of antimony metal in 20 to 30 ml of concentrated sulfuric acid. It was evaporated to dryness on a hot plate set at low heat and heat lamp shining on it from above. This takes about two days. The residue was dissolved in 100 ml of 2 M HCl while stirring with a glass stirring rod. One ml of this solution was taken and mixed with 10 ml of 1 M HCl after which it was diluted to 100 ml. Aliquots of this solution were then mixed with bismuth solution.

A 0.001 M antimony(V) solution was prepared by dissolving 1.2175 g of antimony metal in about 10 to 20 ml of aqua regia. This was evaporated to dryness on a hot plate and the residue dissolved in 25 ml of 6 M HCl which was then diluted to 100 ml. One ml of this solution was diluted to 100 ml with water.

A tin(IV) solution was prepared by dissolving tin in aqua regia and evaporating to dryness. This was dissolved in 100 ml of 2 M HCl which was further diluted with water.

NBS 53b About 150 mg of the alloy was dissolved in 5 ml of concentrated HBr and six drops of concentrated nitric acid from a Pasteur pipet. The solution was then evaporated to dryness using a heat lamp. The residue was dissolved in 20 ml of 2 M HBr and diluted to 100 ml with water.

In using this procedure, sometimes a precipitate formed and sometimes it did not. When a precipitate did form, it was allowed to settle and solution to be injected was obtained by decanting some of the clear solution on top. Since antimony (III) was present it was necessary to wait 3 minutes longer before changing eluents from 0.5 M HBr to 0.05 M HBr.

Standard bismuth solution Standard bismuth was prepared by taking 0.1 gram of bismuth metal and dissolving it in 10 ml of 6 M HNO_3 and diluting this to 200 ml with water. Five ml of this was diluted to 500 ml with 1 M HNO_3. This was diluted by factors of 0.1, 0.15, 0.2, and 0.25 to yield solutions from which a calibration curve could be made. After each injection of an unknown, an injection was made of the standard that had a peak height closest to that of the unknown.

Distribution coefficients

Distribution coefficients for any compound were determined by measuring the retention volume and using Equation 7. The parameter, v_o was determined by injecting 0.1 M HNO_3 onto the column and measuring its retention volume.

Spectrophotometric Detection

To determine the suitability of the UV detector for monitoring bismuth the UV spectrum was obtained. The sample was prepared by dissolving bismuth chloride in concentrated HBr and evaporating to dryness under a heat lamp. After being repeated, the sample was dissolved in 0.5 M HBr and the spec-

trum of this solution obtained. Nelson and Michelson have discussed the effectiveness of this procedure in replacing any chloride existing with bromide (47). The resulting spectrum is shown in Figure 25. The wavelength of maximum absorbance is at 256 nm with an absorptivity of 37,000. The absorptivity at the wavelength which the detector operated at (254 nm) is essentially the same.

Choice of Eluents and Cation Exchanger

Based on the work of Fritz and Garralda, there are three metals that have distribution coefficients on the cation exchanger Dowex 50W-X8 in hydrobromic acid that are about the same as bismuth (45). Those are antimony(III), cadmium(II), and tin(IV). In order to get a more complete picture of the behavior of these metals, distribution coefficients for each metal were determined in several concentrations of HBr (see Figure 26). Based on these results, it was decided that cadmium was the most likely metal to interfere. Therefore, in choosing the resin and eluting solvent, one condition would be that bismuth and cadmium must be resolved.

The higher the concentration of HBr, the faster the bismuth is eluted but at the same time the resolution of bismuth and cadmium is decreased. Therefore, for each cation exchanger, the approximate maximum concentration of HBr that would resolve bismuth from cadmium was determined. Once the maximum concentration of HBr was determined, the time required to elute

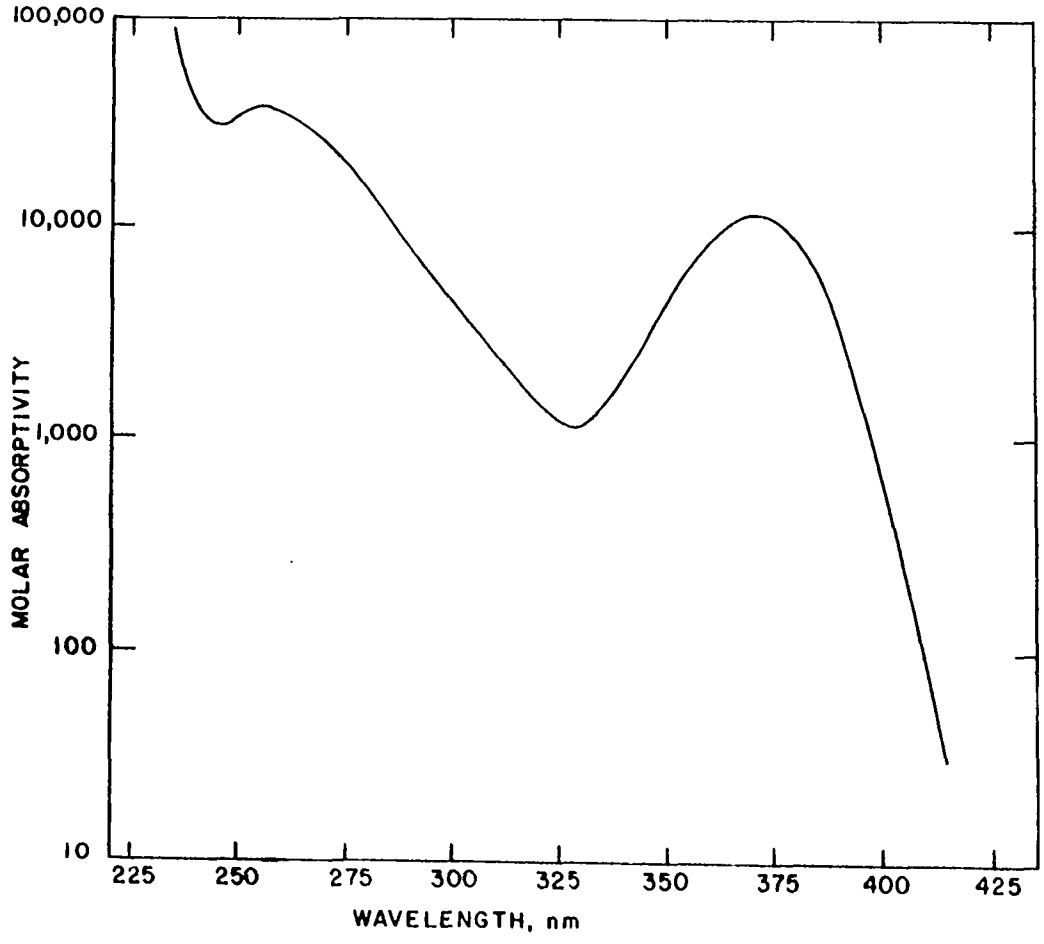


Fig. 25. U.V. spectrum of bismuth(III) in 0.5 M HBr

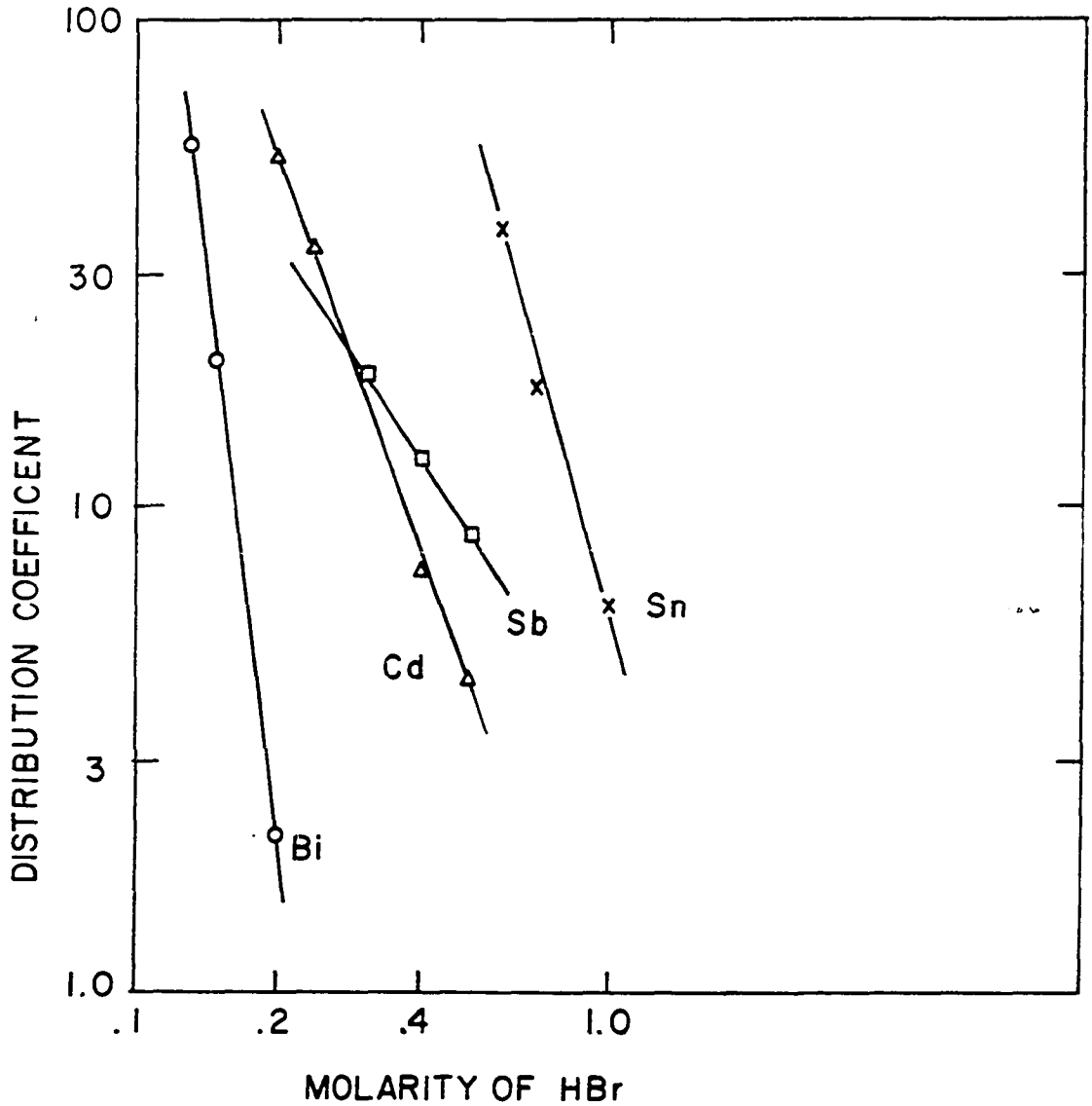


Fig. 26. Distribution coefficients for bismuth(III), cadmium (II), antimony(III), and tin(IV) on Dowex 50W-X8

bismuth was measured. Results are given in Table 9. Based on these results, A-15 was definitely better than the other resins.

Table 9. Comparison of various cation exchangers for the elution of bismuth

Resin	Conc. of HBr to resolve Bi from Cd (Molarity)	Time required to completely elute Bi (Minutes)
Amberlite XE-284	<0.35	>5
Dowex 50W-X8	0.35	3
Amberlite 200	0.35	5
Ag MP-50	0.25	6
Amberlyst 15	0.50	0.2
Amberlyst XN-1005	<0.35	>4

Since for A-15, 0.5 M HBr was the maximum concentration of HBr that would resolve bismuth from cadmium, it was chosen as the eluent to elute bismuth. Bismuth will adsorb to A-15 in just about any dilute acid. One possible acid is 0.05 M HBr which was chosen for the sorbing eluent.

On the basis of the results so far, a tentative procedure was established. A sample containing bismuth was dissolved in 0.1 M HNO_3. While 0.05 M HBr was passing through the column containing A-15 resin, 214 µl of the bismuth solution was injected. At 1.5 minutes, the eluent was switched to 0.5 M HBr which elutes the bismuth. At 2.5 minutes, the eluent was changed back to 0.05 M HBr to equilibrate the column in preparation for the next injection. At 3.5 minutes, the next injec-

tion was made. A typical chromatogram is shown in Figure 27.

Since on Dowex 50W-X8, antimony and tin have distribution coefficients somewhat similar to bismuth, it was thought desirable to determine their distribution coefficients on A-15 to make sure that they would not interfere. The results are shown in Figure 28 along with the distribution coefficients of bismuth and cadmium. They indicate that antimony and tin should not interfere and also that it should be possible to separate bismuth, cadmium, and antimony using 0.5 M HBr. If a solution containing mercury, bismuth, cadmium, and antimony in 0.5 M H_2SO_4 is injected onto a column of A-15 while 0.05 M H_2SO_4 is passing through, all four metals are retained. Switch to 0.05 M HBr and mercury is easily eluted. Switch to 0.5 M HBr and bismuth, cadmium, and antimony are separately eluted. A chromatogram of the separation is shown in Figure 29.

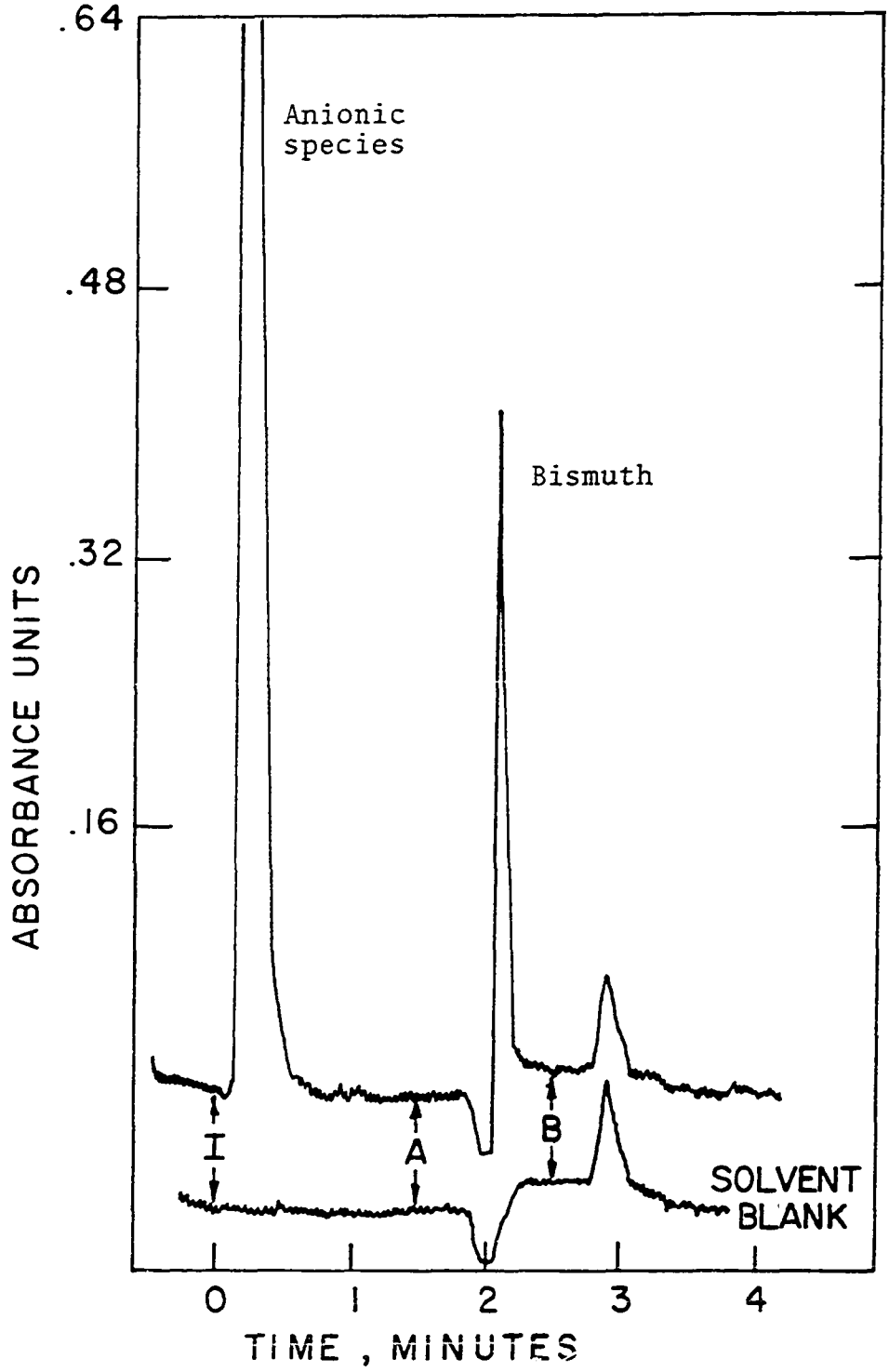
Acceptable Sample Solvents

The effect of varying the concentration of several acids used to introduce the sample was studied. Figures 30 and 31 illustrate the results. Table 10 summarizes the acceptable range of concentration for the acids studied. The lower limit is believed to be determined by the formation of basic salts, e.g., basic nitrate ($Bi(OH)_2NO_3$), basic sulfate ($(BiO)_2SO_4$, and oxychloride (BiOCl). Apparently, these species have a higher absorptivity than the bismuth bromide complex accounting for the increase in peak height observed at lower concentrations.

Fig. 27. Typical chromatogram of bismuth analysis

Conditions: Sample size, 214 µl; Sample, 4.78×10^{-6} M bismuth(III) in 0.1 M HNO_3; Flow rate, 3.2 ml/min; Column, 2.6 mm x 13 cm; Resin, Amberlite A-15, 250 to 325 mesh

I, Inject sample; A, Change eluents from 0.05 M HBr to 0.5 M HBr; B, Change eluents to 0.05 M HBr


.64
.48
.32
.16
ABSORBANCE UNITS
Anionic
species
Bismuth
I
A
B
SOLVENT
BLANK
0
1
2
3
4
TIME , MINUTES

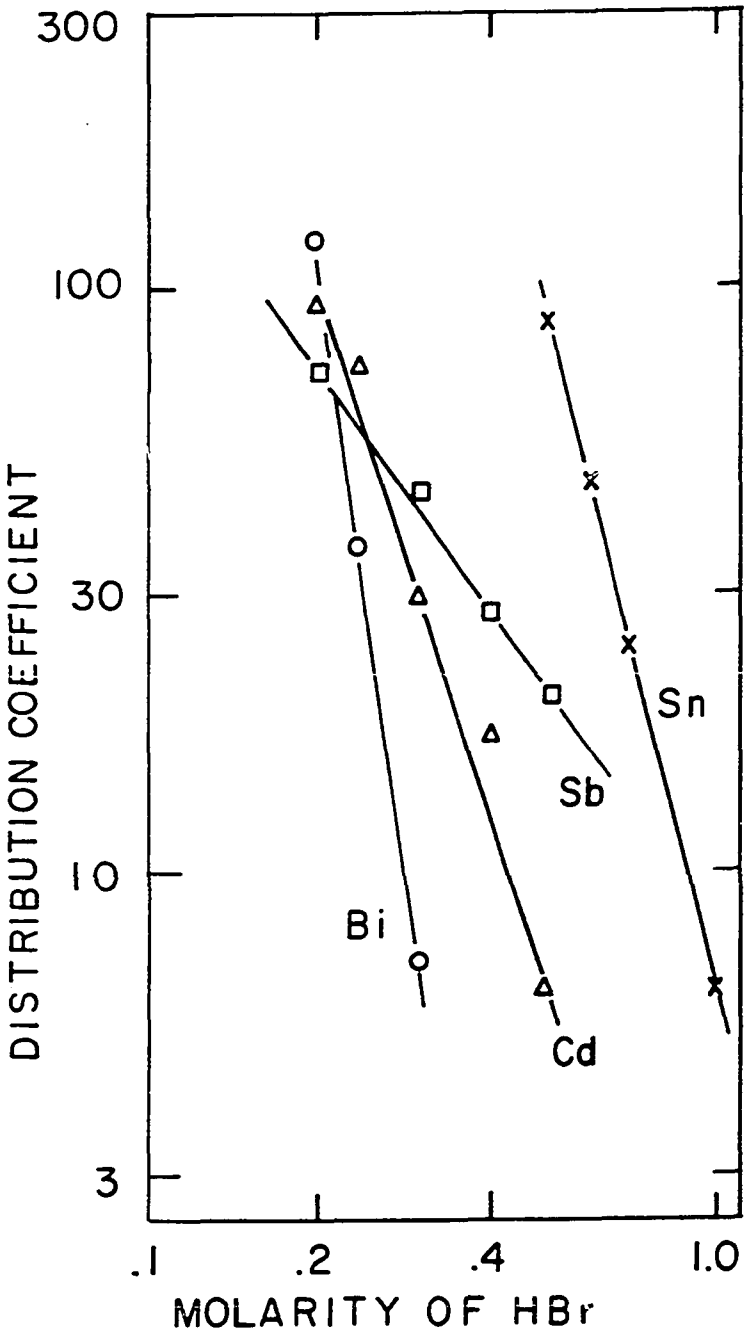
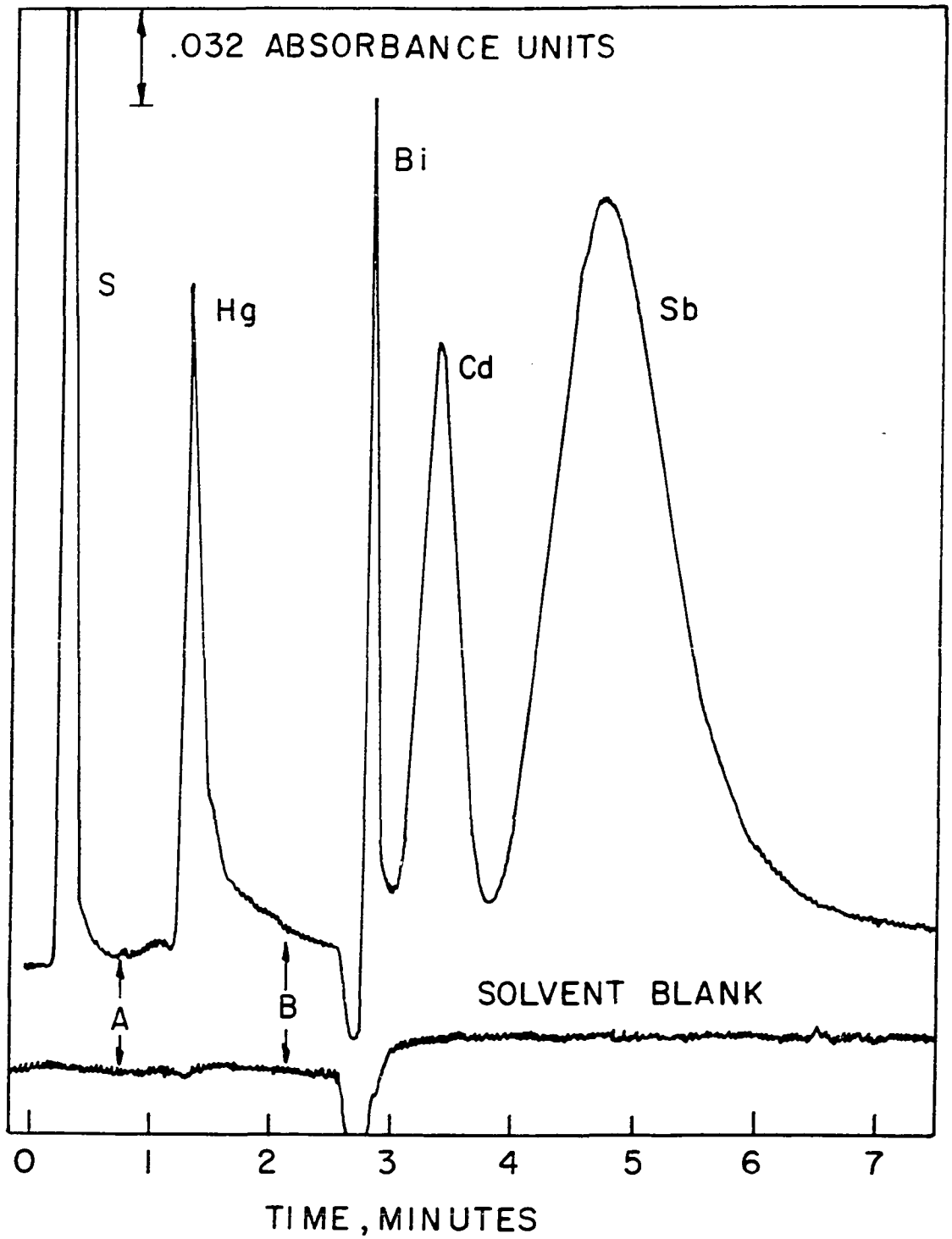


Fig. 28. Distribution coefficients for bismuth(III), cadmium (II), antimony(III), and tin(IV) on Amberlyst 15

Fig. 29. Separation of 6.5 µg Hg(II), 0.17 µg Bi(III), 600 µg Cd(II), and 23 µg Sb(III) in 0.5 M H_2SO_4

Sample loop: 214 1; Column: 2.6 mm x 13 cm; Resin: Amberlyst 15, 250 to 325 mesh; Flow rate: 2.7 ml/min

Initial eluent: 0.05 M H_2SO_4; A, Change eluents to 0.05 M HBr; B, Change eluents to 0.5 M HBr; S, Solvent peak


.032 ABSORBANCE UNITS
S
Hg
Bi
Cd
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SOLVENT BLANK
0
1
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TIME, MINUTES

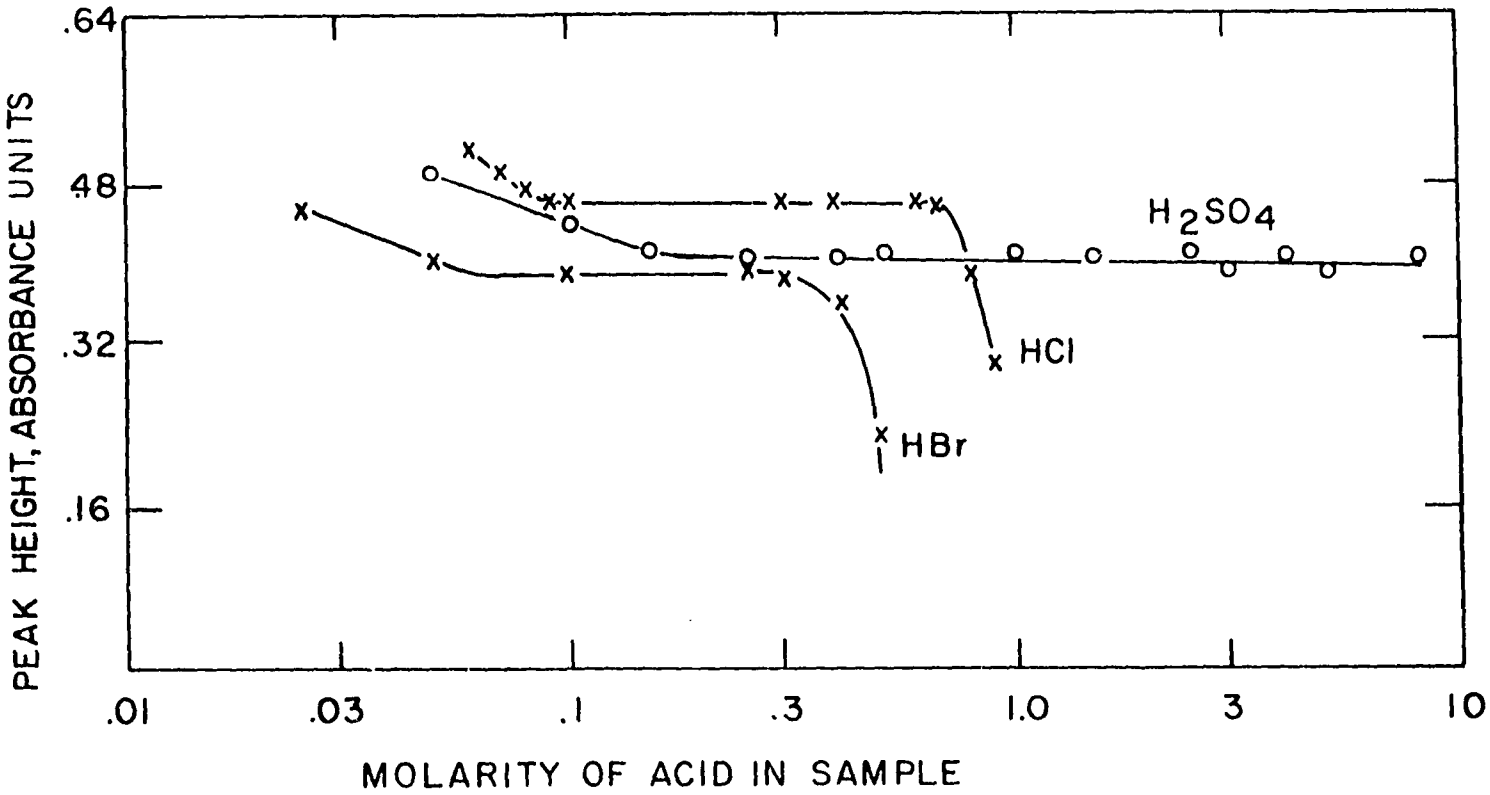


Fig. 30. Peak height of bismuth vs. concentration of acid in sample

Amount of bismuth injected each time: HCl, 0.247 µg; HBr, 0.211 µg; H_2SO_4, 0.216 µg; Sample loop, 214 µl; Flow rate of 0.05 M HBr, 3.2 ml/min

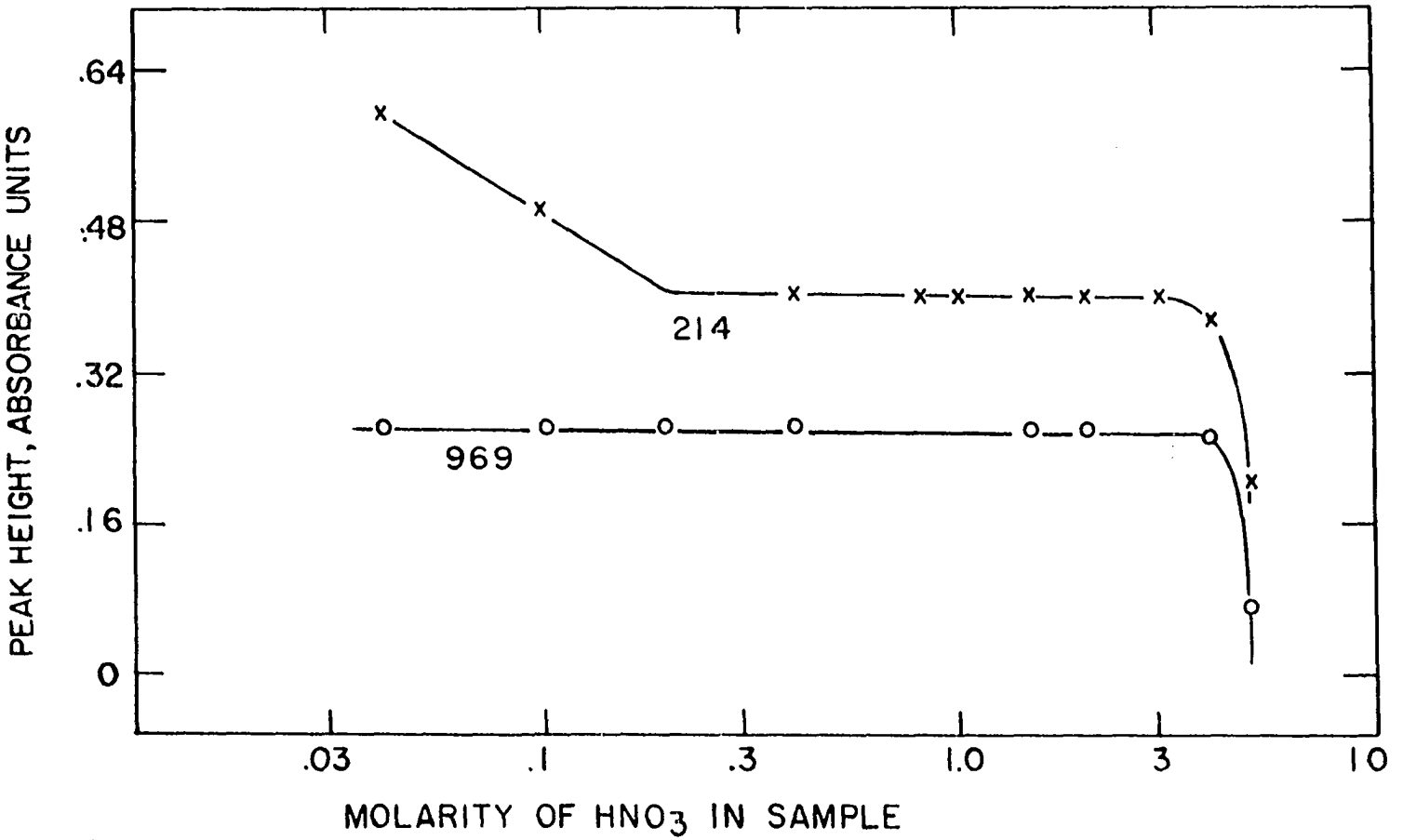


Fig. 31. Peak height of bismuth vs. concentration of nitric acid in sample Amount of bismuth injected each time: 214 µl sample loop, 0.214 µg; 969 µl sample loop, 0.031 µg

Table 10. Acids and the concentration range of each that can be used as a solvent for the bismuth sample

Acid	Sample loop size, µl	Lower limit (Molarity)	Upper limit (Molarity)
HBr	214	0.07	0.2
HCl	214	0.1	0.7
H_2SO_4	214	0.15	8
HNO_3	214	0.25	3
HNO_3	969	0.05	4

For higher concentrations of bismuth and low concentrations of acid the solubility of these species is exceeded and in these cases, the peak height decreases. The upper limit is believed to be due to the ability of the solvent in which the sample is dissolved to elute the bismuth. For large sample loops, a large amount of sample solvent will pass over the bismuth that first enters the column. If this sample solvent is a good eluent for bismuth, the bismuth will be smeared out on the column or possibly eluted off the column. When the eluent is changed to 0.5 M HBr, the result will be a shorter peak. This explanation appears to be valid in the case of the sample solvents HCl, HBr, and HNO_3. For sulfuric acid, according to Strelow et al. (54) any concentration greater than 1.5 to 2 M is a good eluent for bismuth and thus one would expect the peak height of bismuth to drop sharply at approximately this concentration. As shown in Figure 30, this does not happen.

Choice of Initial Eluent

When the bismuth is first injected, it is essential that the eluent passing through the column retain the bismuth until all other species which are easily eluted are eluted. It was assumed that the ideal initial eluent would be one that retained bismuth better than any other species present in solution. If this was the case, bismuth would be held nearest to the top of the column. It should be in the same position that it would be in if there were no other metal ions present in the sample and thus the presence of other metal ions would have no effect on the sorption and later elution of bismuth. A solvent that meets these criteria is 0.05 M H_2SO_4 (54).

Using 0.05 M H_2SO_4 and determining the peak height of bismuth as a function of the concentration of HNO_3 in the sample, it was found that the results are not independent of the concentration of HNO_3 in the sample. If 0.05 M HBr is used as the initial eluent the results are independent as shown in Figure 31. Based on this observation, the assumption that the ideal initial eluent is the one that sorbs bismuth better than any other species present, appears to be incorrect.

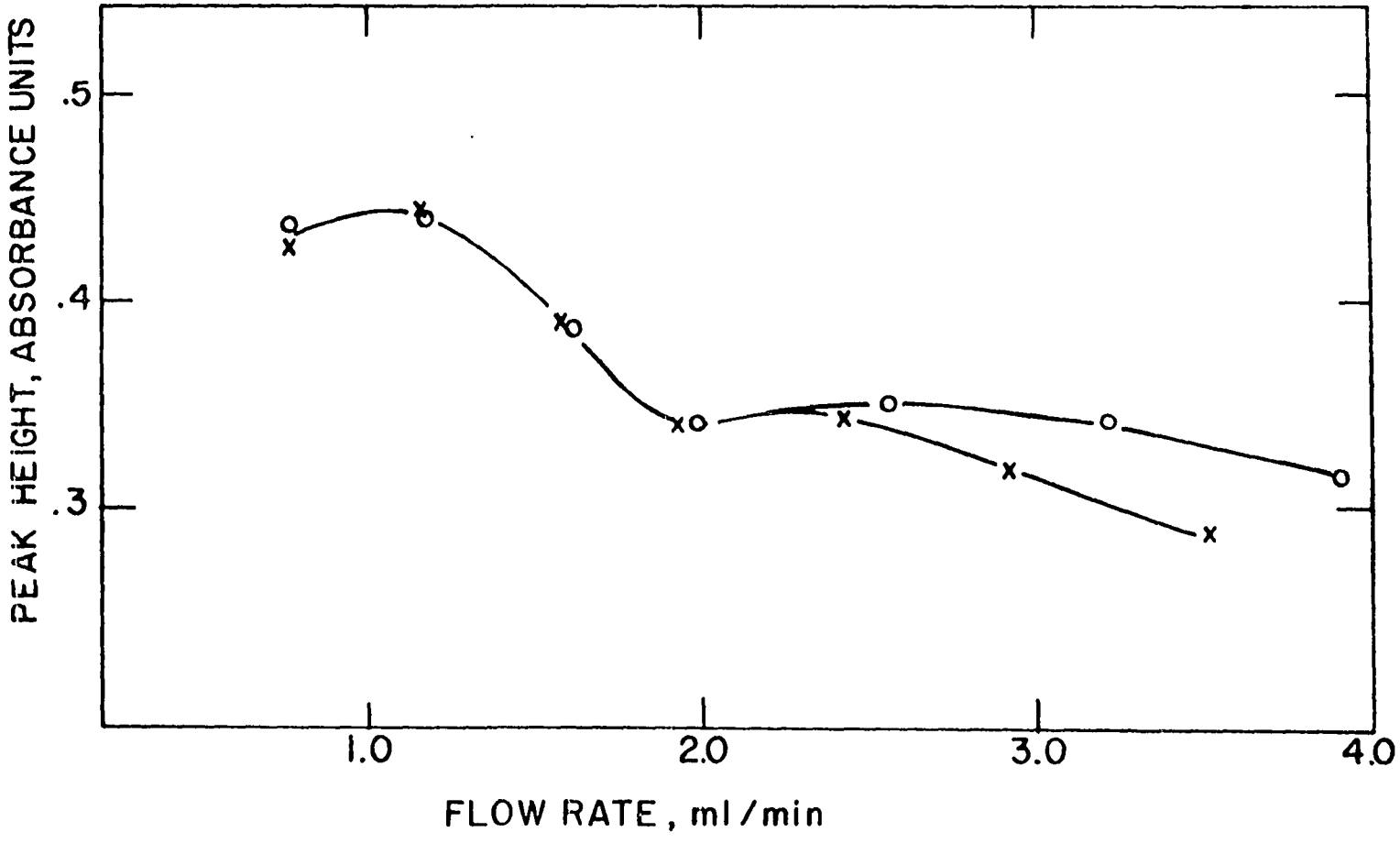
Flow Rate

A study was made of the bismuth peak height as a function of flow rate by injecting a 214 µl sample of 0.82 ppm bismuth in 0.3 M H_2SO_4 onto the column. The initial eluent was 0.05 M HBr which was changed to 0.5 M HBr at 3.0 ml. At 7.5 ml, the

eluent was switched back to 0.05 M HBr and the next injection was made when the base line became level. The flow rate was measured while using the eluent, 0.05 M HBr and having the injection port in load position.

The results are shown in Figure 32. In this case as the flow rate was increased from 1.2 ml/min to 2 ml/min, the peak height decreased rapidly. For higher flow rates, the change in peak height was much less. In a study done earlier using Dowex 50W-X8 instead of A-15 and 0.25 M HBr instead of 0.5 M HBr, a similar result was obtained in this region (see Figure 33). With the A-15 resin, there appears to be a maximum peak height at 1.2 ml/min, whereas with Dowex 50W-X8, no such maximum occurs. Any explanation for this is surely speculative but one possibility is based on greater vertical diffusion of the bismuth throughout the column at slower flow rates.

A similar study by Seymour et al. has been done on the elution of iron from an anion exchanger (19). Qualitatively, the results in that study resemble the results shown in Figure 32 with the flow rate of 2 ml/min to 4.5 ml/min in that study corresponding to 1.2 ml/min to 2 ml/min in Figure 32. Since the diameter of the columns used were different one would expect a better comparison based on linear flow rate. Taking the same range of flow rates but converting to linear flow rate, one finds that from 16 cm/min to 32 cm/min in the study by Seymour et al. corresponds to 50 cm/min to 80 cm/min on Figure 32.



XX: 1st set of measurements; OO: 2nd set of measurements

Fig. 32. Peak height of bismuth on A-15 resin vs. flow rate of 0.05 M HBr

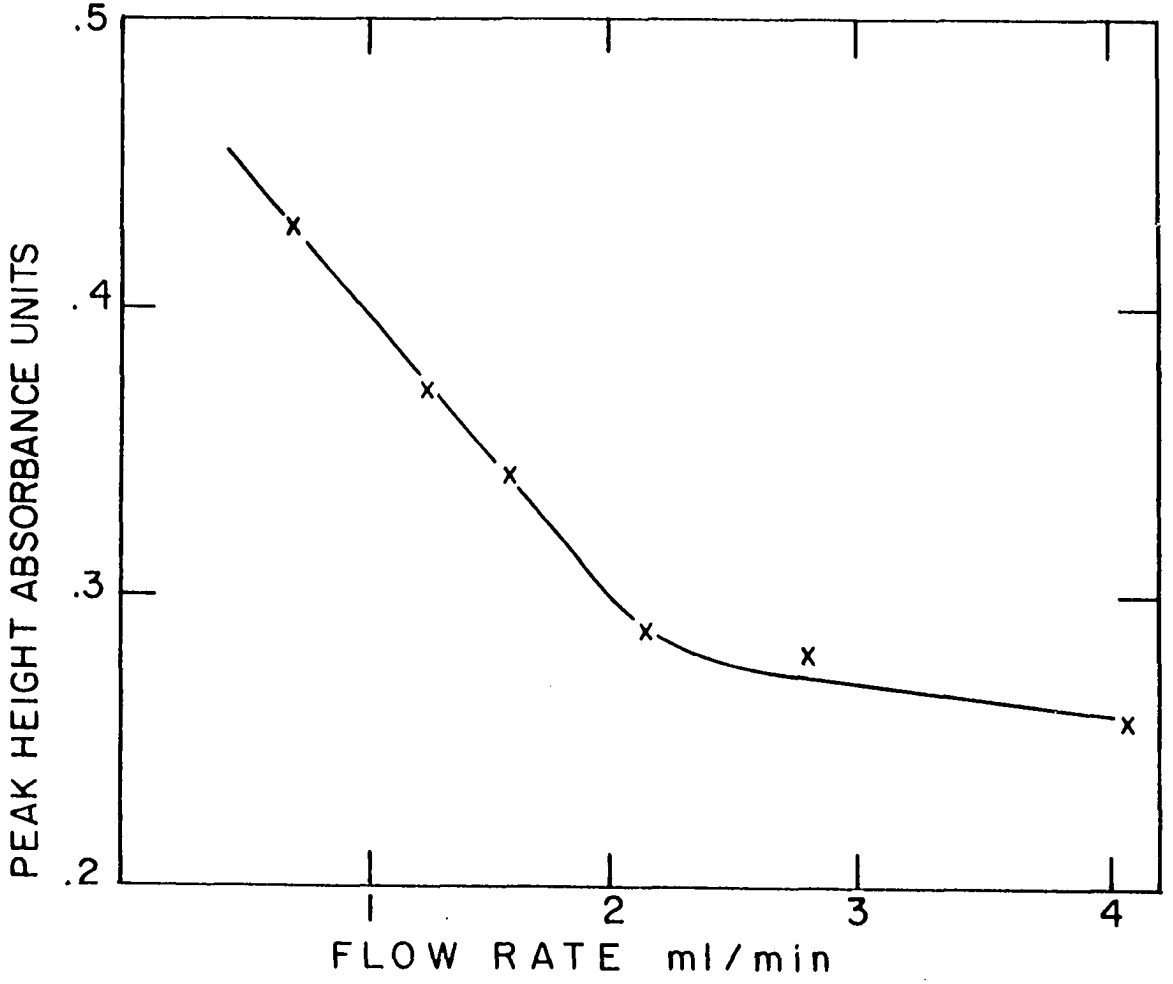


Fig. 33. Peak height of bismuth on Dowex 50W-X8 resin vs. flow rate of 0.05 M HBr

One other observation can be made in the study of peak height vs. flow rate by Seymour et al. For low values of flow rate, the graph is beginning to level off as if a maximum was being approached. If a maximum did exist, it would correspond to the maximum observed in this study.

When the experiment using A-15 was carried out the peak height was measured at each flow rate starting at the high flow rates and proceeding to lower flow rates. When a complete set of measurements had been obtained, the flow of eluent was stopped and the results were calculated and graphed. After obtaining the results, they differed from that obtained using Dowex 50 (Figure 33) so much that the experiment was repeated. The results from the second run are shown as dots with circles around them. For the most part, the results from the two runs are identical. At flow rates greater than 2.3 ml/min, they are not identical. Perhaps this points out an answer to a problem that has been observed in other situations when a flow rate of 3.2 ml/min was used. A sample containing bismuth was injected onto the column and eluted. Following this, other samples were chromatographed. Then a sample of the same bismuth solution as injected originally was reinjected and eluted. The peak height of the original sample and the last sample should reproduce but often they do not. This difficulty has been surmounted by following each unknown with a standard. Perhaps the difficulty would not exist if flow rates of 2.3 ml/min or less had been used.

The flow rate of 3.2 ml/min used in the bismuth project was chosen on the basis of results obtained with Dowex 50W-X8. After switching to A-15, it was assumed 3.2 ml/min was still an acceptable flow rate.

Procedure

The sample containing bismuth was dissolved in either HNO_3, H_2SO_4, HCl, or HBr having a concentration within the limits described in Table 10. The pressure on the liquid chromatograph was adjusted so as to yield a flow rate of 3.2 ml/min when the injection valve was set in "inject" position with 0.05 M HBr flowing through the column. The valve was returned to the "load" position so that the sample could be loaded and injected. At 1.5 minutes the eluent was changed to 0.5 M HBr. During the first 1.5 minutes any anionic species present in the sample are eluted. In many cases, this was more than adequate time but it was used only to ensure that conditions would be the same in the analysis of many different kinds of samples. At 2.5 minutes, all the bismuth was eluted and so the eluent was switched back to 0.05 M HBr. At 3.5 minutes the column is sufficiently equilibrated with the 0.05 M HBr that the next sample could be injected. The injection port may be returned to load position any time after 2.5 minutes.

Both the recorded peak height and area of the peak are directly proportional to the concentration of bismuth in the sample. When area was measured it was determined over a time span beginning when the chromatogram trace went through zero

going up and continued for the next 0.3 minutes. A typical chromatogram is shown in Figure 28.

Using this procedure most metal ions are left on the column. The metal ions build up on the column occupying exchange sites at the top effectively making a shorter column. To counteract the build-up of metal ions on the column it was found necessary to alternate injections of unknown samples with a standard having a concentration between 50% and 200% of that of the unknown. An indication of the build-up of metal ions on the column is the increase in the peak height of the standard. When the peak height of the standard had increased by about 10 to 20%, the metal ions that had accumulated were stripped off with 4 M HCl. At the conclusion of a series of samples, any metal ions present on the column were stripped off with 4 M HCl leaving the column saturates with this acid until the next time samples had to be analyzed.

Each time a series of samples were analyzed, it was often the case that the first two or three trials did not reproduce. Therefore, the procedure was repeated two or more times at the beginning of a series to be certain the peak height reproduced.

Special cases If any one of the elements, antimony (III), mercury, cadmium, or molybdenum(VI) were present the procedure had to be modified. Cadmium and antimony(III) are eluted immediately after bismuth and so it was necessary to wait for them to be eluted before switching back to the 0.05 M HBr. For cadmium, the delay was one-half minute. For antimony

(III), the delay was 3 minutes. Both molybdenum(VI) and mercury are eluted with the 0.05 M HBr. If molybdenum(VI) was present in 1,000 fold excess, the change to 0.5 M HBr was delayed 1.5 minutes. For a 100 fold excess, the change was delayed 1.0 minute. Any time the change to 0.5 M HBr was delayed, the change to 0.05 M HBr and the next injection were delayed by the same length of time.

If mercury was present in 1,000 fold excess, the elapsed time for 0.05 M HBr and 0.5 M HBr to pass through the column was increased in both cases. The switch from 0.05 M HBr to 0.5 M HBr was made at 4.0 minutes and the switch back to 0.05 M HBr was made at 7.5 minutes. The next injection could be made at 8.5 minutes. Mercury came through very quickly in the presence of 0.05 M HBr but tailed badly. Evidently when the change to 0.5 M HBr was made, some mercury which was still on the column was eluted giving a high result for the bismuth. The mercury that was being eluted with the 0.5 M HBr also tailed necessitating a longer elution time for 0.5 M HBr.

When using lower attenuation levels on the detector such as was necessary to analyze the solution containing 12 ppb bismuth, it took longer for the base line to level off after each injection. As a result, the change of eluents was delayed until the base line leveled off.

Calibration Curve

A calibration curve relating peak height to concentration is shown in Figure 34. By the use of three different sample loops it is possible to use the method for a wide range of concentrations. The lower limit obtained using the 969 µl sample loop was 12 ppb which gives a signal about three times the noise level. Since the signal depends on the absolute amount of bismuth in the sample loop rather than the concentration, using larger sample loops should make possible the analysis of even lower concentrations.

At low concentrations there is a slight curvature in the calibration curve but otherwise the curve is linear. A calibration curve relating the area under the peak as a function of concentration can also be prepared. At concentrations less than 0.1 ppm the blank and the unknown had areas which were large in comparison to their difference. For this reason use of area for determining concentration of bismuth is not recommended.

For the interference studies, a separate calibration curve relating both area and height to concentration was prepared from 0.3 to 1 ppm bismuth. For both area and height, the curve was linear and extended through zero.

Interference Study

Bismuth was determined in the presence of a 1,000 fold molar excess of many different ions. The results of this study

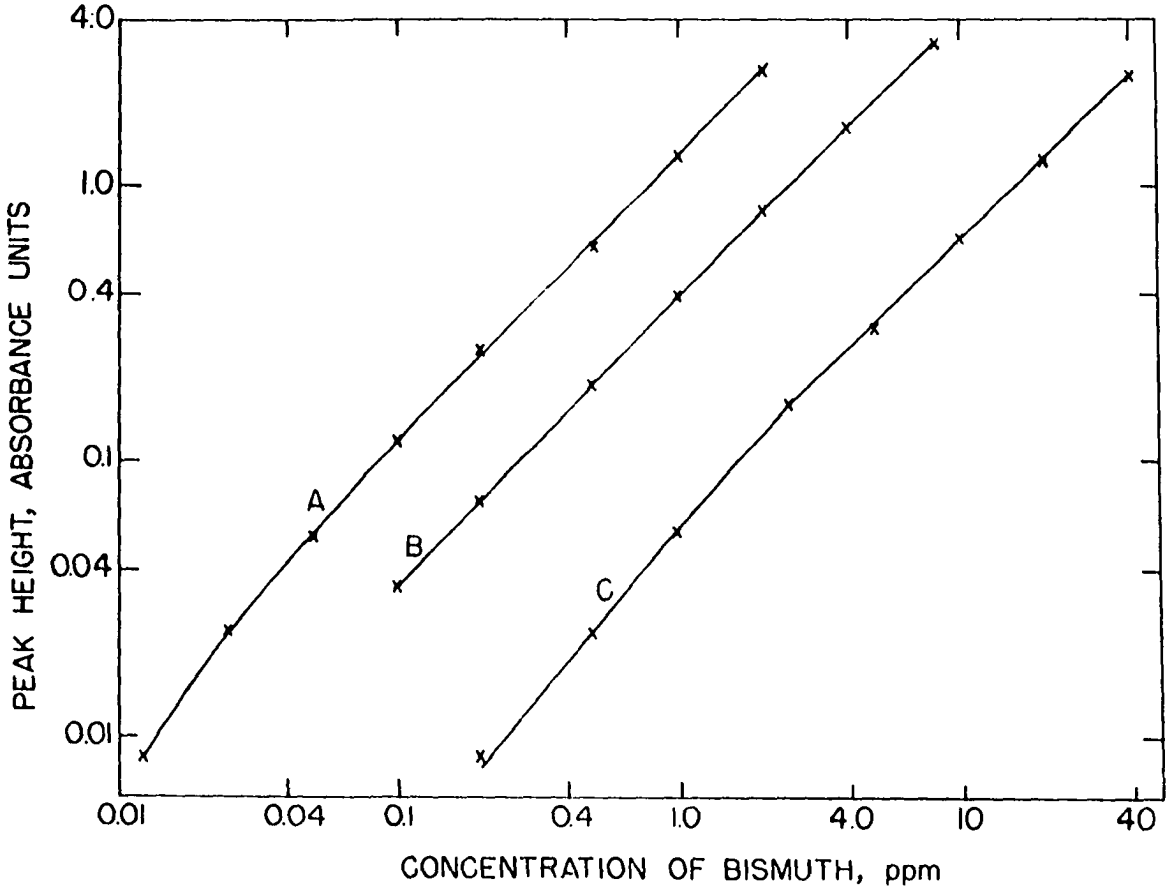


Fig. 34. Calibration curve for bismuth using different size sample loops. Sample loop sizes: A, 969 µl; B, 214 µl; C, 38.1 µl

are given in Table 11. In case the error turned out to be greater than 3%, the interference study was repeated for that particular interferent at a lower excess. In practically all cases, the relative error obtained by using peak height was less than the relative error obtained using peak area. The relative standard deviation of repeated trials, each one obtained by comparing to the standard that immediately followed it was 1.6%.

The NBS standard 53b (lead base bearing metal) with certificate analysis as shown in Table 12 was analyzed as described earlier. The resulting percentage of bismuth present was found to be 0.0752% with the relative standard deviation of 1.36 pph for four trials. The certificate analysis is .075%.

The only element that seriously interfered in the determination of bismuth was tin. When a 1,000 fold excess was present the value for bismuth was only 22% of what it should have been. In order to decrease the relative error to less than 3% the excess of tin had to be less than 13 fold if area is used to determine concentration and less than 5 fold excess if height is used to determine concentration. It is believed that the tin on the column acts as an inorganic ion exchanger and retains some of the bismuth. Substantiation of this is based on the fact that when one attempts to elute tin(IV) with 0.5 M HBr, it tails badly. One could account for the tailing if one assumed that the tin partially hydrolyzes. If it does hydrolyze, the tin exists in a form that is the same as that used in inorganic

Table 11. Analysis of bismuth in the presence of other metals

Interferent	Conc of Bi^{+3} M x 10^6	Molar ratio inter/Bi^{+3}	Solvent	Rel error, % (area)	Rel error, % (height)
Mo(VI)	4.78	100	0.1 M HNO_3	+0.8	+2.14
Mo(VI)	4.78	1,000	0.095 M HNO_3	+9.15	+5.40
Mn(II)	4.78	1,000	0.14 M HNO_3	-2.32	-1.51
Zn(II)	4.78	1,000	0.1 M HNO_3	+0.30	-1.33
Cr(III)	4.78	100	0.1 M HNO_3	+1.35	+1.57
Cr(III)	4.78	1,000	0.1 M HNO_3	-5.25	-4.75
Mg(II)	4.78	1,000	0.1 M HNO_3	+1.72	+0.60
Hg(II)	4.78	10	0.1 M HNO_3	+8.33	+1.46
Hg(II)	4.78	100	0.1 M HNO_3	+25.54	+9.88
Hg(II)	4.78	1,000	0.14 M HNO_3	+29.68	+8.65
Pb(II)	4.78	100	0.1 M HNO_3	+1.62	+0.83
Pb(II)	4.78	1,000	0.1 M HNO_3	+5.18	+3.55
Sb(IV)	2.76	1,000	0.17 M HCl	+2.27	+1.37
Sb(III)	1.10	1,000	0.24 M HCl	-15.48	-3.13
Sb(III)	2.76	400	0.24 M HCl	-0.58	-0.51
Tartrate	4.78	100	0.1 M HNO_3	+2.19	+2.22
Tartrate	3.82	1,000	0.1 M HNO_3	-5.16	-3.70
K(I)	3.82	1,000	0.1 M HNO_3	-2.02	-2.65
Cu(II)	3.82	1,000	0.1 M HNO_3	-1.86	-1.06
Co(II)	3.82	1,000	0.1 M HNO_3	+1.50	+0.03
Cd(II)	3.82	1,000	0.1 M HNO_3	-1.64	-0.70

Table 11. (Continued)

Interferent	Conc of Bi^{+3} M x 10^6	Molar ratio inter/Bi^{+3}	Solvent	Rel error, % (area)	Rel error, % (height)
Fe(II)	3.82	1,000	0.13 M HNO_3	-1.91	-0.74
Fe(III)	4.78	100	0.10 M HNO_3	+0.63	-0.57
Fe(III)	4.78	1,000	0.14 M HNO_3	-4.99	-7.47
Ba(II)	3.82	100	0.13 M HNO_3	-4.14	-2.73
Ba(II)	4.78	1,000	0.26 M HNO_3	-15.6	-23.4
Al(III)	4.78	1,000	0.12 M HNO_3	-0.94	-0.83
Ca(II)	4.78	1,000	0.1 M HNO_3	+0.97	-0.64
Sn(IV)	2.76	2	0.13 M HCl	-3.0	+1.10
Sn(IV)	2.76	10	0.13 M HCl	-2.1	-5.82
Sn(IV)	2.76	25	0.13 M HCl	-5.6	-8.01

Table 12. National Bureau of Standards Certificate of Analysis for sample NBS 53b

lead	84.35%	arsenic	0.042%
antimony	10.28%	nickel	0.006%
tin	5.06%	silver	0.003%
copper	0.209%	iron	0.002%
bismuth	0.075%	aluminum	0.0007%

ion exchangers. Donaldson and Fuller have studied the cation exchange properties of hydrous SnO_2 and find that for bivalent metal transition ions, the selectivity series closely parallels the order of equilibrium constants for the reaction (55)

$$M^{2+} + H_2O \rightleftharpoons MOH + H^+$$

Bismuth has a hydrolysis constant of 12.42 (56), so if one assumes that it has the same properties as the ions that Donaldson and Fuller studied, it would be expected to be retained by SnO_2. Another experiment that supports the explanation just proposed is based on the effect of tin on the elution of antimony. A mixture containing the three ions, cadmium, bismuth, and antimony(III) was injected onto a column of A-15 while 0.05 M HBr was passing through the column and then eluted with 0.5 M HBr. The result was three peaks, one for each element injected. Then, tin was injected onto the column with most of the tin being eluted with 0.7 M HBr. Following this, cadmium, bismuth, and antimony were again injected onto the column and eluted with 0.5 M HBr. This time the antimony peak was missing. When the resin was replaced with fresh resin

and the same solution reinjected, the antimony peak was then present. Here again, it is believed that the tin sorbs the antimony. No hydrolysis constants for antimony(III) were found in the literature but the fact that it has a great tendency to hydrolyze would indicate a high hydrolysis constant (57). Thus, with a high hydrolysis constant, one would expect antimony to be sorbed by the tin still left on the column.

Antimony has another peculiar behavior under the conditions used to determine bismuth. If 2.4 micromoles of antimony(III) is injected onto the column previously described, it appears the column is overloaded in that the front edge of the elution peak is pushed forward enough to overlap with the bismuth peak. The back edge of the antimony elution peak is not affected. If the amount of antimony(III) injected is reduced to .5 micromoles the elution peak is normal and looks just like the antimony peak shown in Figure 29. The 2.4 micromoles represents only about 0.6% of the capacity of the column which is usually not enough to overload the column. When antimony was checked as an interferent for bismuth it was possible to determine bismuth in the presence of 1,000 fold excess of antimony(III) provided the amount of antimony present was less than 0.5 micromoles.

Suggestions for Future Work

When one injects a sample onto a column, the ideal situation is that the species one wants to measure is strongly

sorbed and every other species passes right through the column. When this is the case, no matter how much else is present, the species to be measured will not be affected. Once everything else has passed through the column, the species to be determined can be eluted and the peak height measured.

In the method just discussed, the only species that goes right through the column at the time of injection is mercury. Everything else is sorbed right along with the bismuth and so this is not an ideal procedure. What appears to be an obvious procedure that meets the criteria of an ideal method is to use an anion exchanger. At fairly low concentrations of hydrobromic acid, bismuth forms an anionic species, whereas no other metal except mercury does. So if injected onto an anion exchanger while dilute HBr is passing through, the bismuth should sorb while everything else passes through. If the concentration of HBr is decreased enough, the bismuth should change to a cationic species and be eluted. This has been tried and unfortunately, bismuth is not eluted. Another way of eluting bismuth from an anion exchanger that works for hydrochloric acid is to use an eluent composed of a mixture of hydrochloric acid and perchloric acid (41). This was tried using varying combinations of hydrobromic acid and perchloric acid. No combination was found that would successfully elute bismuth.

It is believed that an anion exchanger similar to strong base anion exchangers currently available but with a lower capacity should be a solution to the problem just stated. Low

capacity cation exchangers have been developed and it has been shown that the distribution coefficient of different species is lower than on a similar cation exchanger with higher capacity (58). If this characteristic holds true for anion exchangers as well, it should make it possible to elute the bismuth bromo complex from an anion exchanger. If this is the case, it should be possible to develop a method for bismuth coming closer to meeting the characteristics of an ideal ion exchange method than the method discussed in this thesis.

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APPENDIX: SYMBOLS USED

a	Slope of the graph when log D is graphed as a function of log C
a_1	Value of a for compound #1
A_c	Area of adsorbed solvent molecule (see Equation 19)
a_i	The contribution to A_s of individual substituents of a sample $A_s = \sum_i a_i$
A_1	Area of the adsorbed solvent molecule
A_s	Area of the adsorbed sample molecule
b	Intercept of the graph when log D is graphed as a function of log C
b_1	Value of b for compound #2
C	The concentration in percent of methanol used to elute a compound from the column. The remaining percent is water.
C_x	Concentration of sample not adsorbed in moles/liter of solvent
C_{xa}	Concentration of adsorbed sample in moles/gram of resin
d	Difference in $\bar{v}$'s of two chromatographic peaks
D	Distribution coefficient of a compound. $D = C_{xa}/C_x$
D_1	Value of distribution coefficient for compound #1
ΔE	Net sample adsorption energy $E = -\Delta G^\circ/2.3RT$
E_{sa}	Dimensionless free energy of the adsorbed solvent molecule
E_{s1}	Dimensionless free energy of the nonadsorbed solvent molecule
E_{xa}	Dimensionless free energy of the adsorbed sample molecule
E_{x1}	Dimensionless free energy of the nonadsorbed sample molecule

$f(A_i)$ The portion of E_{sa} or E_{xa} due to the resin

$f(S)$ The portion of E_{sa} due to the solvent. See Equation 17.

$f(x)$ The portion of E_{xa} due to the sample. See Equation 16.

g Grams of resin contained in the column

ΔG_a° Standard free energy of adsorption of the sample

h Maximum height of a chromatographic peak

H Height of a theoretical plate. $H = L/N$

i A substituent of any compound such as Br in p-bromophenol

K_{th} Thermodynamic equilibrium constant, $K_{th} = N_{xa}/N_x$

L Length of column

m Number of solvent molecules having an area equivalent to the area of one sample molecule. $m = A_s/A_e$

N Number of theoretical plates. $N = 16(\bar{v}/w)^2$

n_s Moles of solvent not adsorbed

n_{sa} Moles of adsorbed solvent

n_x Moles of sample not adsorbed

n_{xa} Moles of adsorbed sample

N_x Mole fraction of sample not adsorbed. $N_x = n_x/n_s$

N_{xa} Mole fraction of adsorbed sample. $N_{xa} = n_{xa}/n_{sa}$

Q_i^o The contribution to S^o of individual substituents of a sample. $S^o = \sum^i Q_i^o$

R Resolution of two peaks. $R = 2d/(w_1+w_2)$

R Universal gas constant

S^o Same as $f(x)$

S_a Solvent molecule adsorbed to the resin

S_i Solvent molecule not adsorbed