THIN-LAYER SOLUBILIZATION CHROMATOGRAPHY

I. PHENOLS

JOSEPH SHERMA AND LYAL V. S. HOOD*
Department of Chemistry, Lafayette College, Easton, Pa. (U.S.A.)
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INTRODUCTION

Solubilization chromatography is a method for separating organic nonelectrolytes having a small solubility in water. It is a type of partition chromatography which employs ion-exchange resin as the stationary phase and aqueous solutions of organic compounds as the mobile phase. With this technique, a series of phenols[1] (among other compounds[2,3]) have been separated by elution with acetic acid through a column of strongly-acidic cation-exchange resin. Mixtures of phenols were later separated by development with acetic acid on a sheet of filter paper loaded with the same type of resin (paper solubilization chromatography[4]). This report demonstrates the combination of solubilization chromatography and thin-layer chromatography and its application to phenol separations.

Phenols of various kinds have been chromatographed on thin-layer plates made from a wide range of sorbents, for example silica gel[5], polyamides[6], and unbound aluminum oxide[7]. Thin layers of ion-exchange resin have apparently not been employed. In fact, only one report of the use of ion-exchange resins in thin-layer chromatography has come to our attention. BERGER *et al.*[8] separated three radioactive halides by development with molar sodium nitrate and three organic dyes by development with a 4:4:1 mixture of acetic acid, methanol and acetone. To prepare their layers, however, they used equal parts of cellulose powder containing 5 % plaster and 200–400 mesh anion- or cation-exchange resin. In the present work, our layers are composed only of resin and binder.

EXPERIMENTAL

Apparatus and reagents

"Chromatofilm" thin-layer chromatography apparatus, manufactured and supplied by Research Specialties Co., Richmond, Calif., was used throughout this work.

The ion-exchange resins employed were the strongly-acidic cation-exchange resin, Dowex 50W-X8, 200–400 mesh (Lot No. 4673-30; Dow Chemical Co., Midland, Mich.), and the strongly-basic anion-exchange resin, Dowex 1-X4, 200–400 mesh (Lot No. 4697-27). The Dowex 50 was used as supplied except for some experiments in which the ammonium form of the resin was employed. Conversion from the hy-

* Present address: Department of Chemistry, University of Florida, Gainesville, Fla. (U.S.A.).

drogen form was accomplished by passing a saturated solution of ammonium chloride through a column of the resin until the pH of the effluent and influent were identical. The resin was then washed with distilled water. The Dowex 1 was cleaned and converted to the chloride form by successively passing 150 ml of 1.5 *M* sodium hydroxide, 150 ml of distilled water, 150 ml of 1.5 *M* hydrochloric acid, and a final 150 ml of distilled water through a column of resin.

All chemicals used were of the best grade commercially available. Developing solutions were prepared by proper dilution of reagent-grade acetic acid and methanol. Individual test solutions of the phenols were prepared by dissolving 0.20 g of catechol, resorcinol, phenol, *o*-cresol, *m*-cresol or *p*-cresol in distilled water (or the minimum necessary amount of methanol) and diluting to 25 ml with additional water. The first three phenols could be completely dissolved in water, but the cresols could not. A mixture solution of the six phenols was prepared by the combination of individual solutions containing 1.2 g of each phenol in 25 ml of water including a minimum amount of methanol if necessary.

Preparation of layers

A slurry of ion-exchange resin was prepared by a modification of the method used by SMITH AND FOELL[9] for silica gel. One gram of soluble starch and 30.0 ml of distilled water were added to 25.0 g of dry hydrogen-form Dowex 50 resin in a 250-ml erlenmeyer flask. The flask was heated with occasional stirring on a steam bath for 20 min at 75–80°. The flask was allowed to cool and more water, usually less than 10 ml, was added to give a slurry of the proper consistency. The consistency of this mixture is very important: it should be thin enough to allow five plates to be coated but thick enough so that the coating does not run after application to the plates. With the Dowex 50 in the ammonium form and the Dowex 1, the slurry was prepared using wet resin from the column, and less water (10–20 ml) was added before heating with the starch.

Glass plates, 8 in. × 8 in., were soaked overnight in "cleaning solution", rinsed with distilled water, and dried. Five of these plates were positioned on the aligning tray. The variable-thickness spreader was adjusted, using gauges supplied with it, to give a layer of 0.3 mm thickness. It was found that thicker layers tended to crack more readily and that thinner layers were difficult to apply evenly. The slurry was mixed well and poured into the spreader, which was then drawn over the plates at a slow, steady rate. The plates were allowed to stand in place for 15 min and were then stored in a storage rack until used.

In the beginning, attempts were made to dry the plates in an oven after the standing period (at 60–70°, or by gradually increasing the temperature from 25° to 70°), but this usually resulted in cracked layers. Calcium sulfate was tried in place of starch as the binder, but this also usually resulted in cracked layers being formed. The use of calcium sulfate is not desirable anyway because of the possibility of ion exchange occurring.

After some practice and experience, it was possible to prepare good layers of resin almost every try by use of the procedure described above.

Spotting techniques

Initial zones of the test solutions were applied to the plates using Peerless

wood applicator sticks (Diamond Match Co., New York 17, N.Y.). Compared with micropipettes, it was easier in this way to apply a detectable amount of solute to a smaller area without disturbing the layer. Three dabs of test solution were used for each spot; this corresponded to about 5 μl. The plates were consistently developed in the direction opposite to which the spreader was moved in the preparation of the layers. The solutes were spotted on an origin line 18.0 cm from the top of the plate, and the solvent was allowed to rise to within 3.0 cm of the top. The distance of development was therefore 15.0 cm in all cases. A line was drawn across the layer with a grease pencil to mark this position 3.0 cm from the top. No line was drawn at the origin, but the plastic template which had lateral markings was used as a guide in placing the initial solute spots. Up to seven initial zones, one inch apart, were placed on one plate. The spots were air dried for 15 min after application.

Whenever mixtures were chromatographed, individual reference zones of each constituent in the mixture were placed on the same plate. Mixtures which were studied included the one containing all six phenols described above, as well as all possible binary mixtures of the six phenols. These two-component mixtures were prepared on the plate by successive application of individual sample solutions to the same area of the plate, with drying in between.

Development

The developing chamber (Model-200D) was lined with Whatman No. 1 filter paper. Three hundred ml of developing solvent were poured down the sides of the chamber to completely saturate the paper. The chamber was covered for 20 min to allow equilibration of the atmosphere inside. One or two plates were placed on the A-frame in a near-vertical position with the origins at the bottom. The plates were put into the chamber as quickly as possible so that it was uncovered for a minimum amount of time. The solvent was allowed to rise until it reached the line drawn across the top of the plates. For irregular solvent fronts, the solvent was permitted to rise until the average of the front was at the line. The plates were then removed and air-dried in a horizontal position for 15–20 min, depending on the solvent.

In some cases, unidirectional multiple development was employed. After the first development, the plate was removed, air-dried, and redeveloped. Up to three consecutive developments were tried.

Detection

The dried plates were sprayed with a diazotized benzidine solution. This was prepared by mixing 20.0 ml of a stock benzidine solution (1 l of solution containing 5.0 g benzidine and 14.0 ml conc. HCl) with 20.0 ml fresh 10 % sodium nitrite solution at 0° with constant stirring. This reagent was stable for only 2–3 h. Some of the spots appeared at once, others after several hours. Consequently, the plates were allowed to stand overnight before evaluation.

During preliminary work other reagents were tried, but none proved as successful for detecting all of the phenols as the one above. These included diazotized sulfanilic acid, sodium molybdate, acidic potassium permanganate, ferric chloride plus potassium ferricyanide, and *p*-nitroaniline followed by sodium carbonate. The usual vigorous, corrosive sprays suggested for thin-layer chromatography attacked the organic layer and were useless.

RESULTS AND DISCUSSION

R_F values were computed by dividing the distance moved by each phenol (measured to the front edge of the zone) by the distance moved by the solvent front. For multiple chromatography, the R_F values were determined from the total distances the solute and solvent moved in the several developments.

Solubilization chromatography on cation-exchange resin layers

(*1*) *Hydrogen-form resin.* In the hope of being able to compare the results obtained with those from earlier work in columns[1] and on paper[4], the first chromatographic system tried included Dowex 50 in the hydrogen form as the stationary phase and aqueous solutions of acetic acid as the mobile phase. The acetic acid caused severe cracking of the layers and very irregular solvent fronts. Therefore, we switched to aqueous solutions of methanol (which had been used in earlier column studies of ketones[2]) and found the resultant chromatograms were much easier to spray and evaluate.

Table I shows the R_F values obtained with methanol. Times for development ranged from 1.0–1.5 h. At lower methanol concentrations the spots were generally less than 2.0 cm in length. At higher methanol concentrations, catechol and resorcinol

TABLE I

R_F VALUES ON DOWEX 50W-X8 (H^+) LAYERS

Phenol	*R_F values*									
	Concentration of methanol (M)									
	0.0	*1.0*		*2.0*	*3.0*	*4.0*	*5.0*	*6.0*	*7.0*	
Catechol (orange)	0.41	0.41	0.47	0.45	0.51	0.58	0.60	0.59	0.46	0.66
Resorcinol (yellow)	0.43	0.43	0.47	0.46	0.58	0.58	0.59	0.66	0.66	0.71
Phenol (purple)	0.27	0.29	0.30	0.30	0.31	0.32	0.30	0.39	0.37	0.41
o-Cresol (light blue)	0.15	0.23	0.25	0.24	0.22	0.23	0.26	0.28	0.39	0.39
p-Cresol (yellow)	0.15	0.20	0.20	0.21	0.21	0.21	0.24	0.29	0.26	0.35
m-Cresol (purple)	0.19	0.21	0.23	0.23	0.22	0.24	0.31	0.32	0.33	0.30

gave zones up to 3.0 cm in length. The colors of the zones after spraying with diazotized benzidine are shown in parentheses. Duplicate runs are shown for 1.0 and 7.0 *M* methanol to indicate the degree of reproducibility to be expected. In general, reproducibility was best for lower concentrations of methanol.

The results shown in Table I for starch-bound thin layers of cation-exchange resin are generally comparable to those obtained on columns of resin and resin-papers. In columns[1], the distribution coefficient (*C* value) for a given phenol decreased as the molarity of eluting solvent increased. This indicated a decrease in affinity for the stationary resin phase. On cation-exchange paper[4], R_F values for a given phenol increased with an increase in concentration of the developing solvent, again indicating a decrease in affinity for the resin phase. Table I shows a general increase in R_F value for each phenol as the molarity of methanol increases. This trend was expected

because as the molarity of organic constituent in the mobile phase increases, it can better compete with the resin phase for the solute molecules. The R_F values in Table I rise in a parallel manner for each of the phenols. In column solubilization chromatography[1], plots of log C *vs.* molarity of eluent were a series of more or less parallel straight lines, as were plots of R_F *vs.* molarity of developer in paper solubilization chromatography[4]. As a consequence, a separation of a mixture of phenols which was possible with any one concentration of methanol could not be enhanced by changing solvents, as was true for the column solubilization chromatography of most compounds studied[1–3].

Another interesting comparison among the data for phenols on columns of resin, resin-papers and on thin layers of resin is their affinity for the stationary phase when the solvent was water. Focusing on just four phenols, the order of increasing affinity for the resin-paper as measured by decreasing R_F values was *o*-cresol > phenol > resorcinol > *p*-cresol[4]. On thin layers of resin, the R_F sequence was resorcinol > phenol > *o*-cresol = *p*-cresol. Only two of these were studied on columns of resin[1], and *o*-cresol had much more affinity than phenol. Considering the many different forces which can contribute to the retention of a phenol by the various stationary phases, there is no reason to believe that the migration sequences should be the same. Much more work is needed to clarify the results presented above, but they seem to indicate that interactions between the phenols and the cellulose and/or binder of the resin-paper may be much more important than any interactions with the starch, and that starch-bound layers of resin may function in a manner analogous to a column of resin alone.

With the results of Table I, separations of binary mixtures of the phenols were planned and performed as follows: by development with water, phenol and *p*-cresol were separated by 0.80 cm and phenol and *m*-cresol were separated by 0.30 cm (distances given are between the leading edge of the slower-moving zone and the trailing edge of the faster-moving zone; all separations were performed at least three times and the distances given are the average for all the replicates; in every run, separation was achieved every time); by development with 3.0 M methanol, phenol and *o*-cresol were separated by 0.50 cm and *m*-cresol and resorcinol were separated by 1.7 cm; by development with 4.0 M methanol, catechol and *o*-cresol were separated by 1.3 cm, catechol and *p*-cresol were separated by 1.6 cm and catechol and *m*-cresol were separated by 1.2 cm; by development with 5.0 M methanol, catechol and phenol were separated by 1.8 cm; by development with 6.0 M methanol, *o*-cresol and resorcinol were separated by 3.2 cm and *p*-cresol and resorcinol were separated by 3.0 cm; and by development with 7.0 M methanol, phenol and resorcinol were separated by 2.3 cm. Besides these, the six-component test solution was subjected to double development with 4.0 M methanol. Three zones resulted: a blue-purple zone containing the cresols (R_F 0.21) was separated by 1.2 cm from the purple phenol zone (R_F 0.29) which was separated by 1.0 cm from an orange zone (R_F 0.39) containing resorcinol plus catechol.

Attempts to separate the three cresols from each other by single development with concentrations of methanol greater than 7.0 M or by multiple development at various solvent concentrations all failed. Likewise, conditions within the present system could not be found by which catechol could be consistently separated from resorcinol.

(2) *Ammonium-form resin.* Table II shows the results of developing the phenols with methanol on Dowex 50 in the ammonium form. Development times ranged from 55 to 70 min. Spot sizes varied from less than 1.0 cm to almost 4.0 cm in length,

TABLE II

R_F VALUES ON DOWEX 50W-X8 (NH_4^+) LAYERS

Phenol	*R_F values*				
	Concentration of methanol (M)				
	0.0	*2.0*	*4.0*	*6.0*	*8.0*
Catechol (brown)	0.24	0.24	0.29	0.32	0.34
Resorcinol (red)	0.25	0.27	0.29	0.43	0.43
Phenol (yellow)	0.21	0.20	0.25	0.32	0.34
o-Cresol (yellow)	0.15	0.15	0.16	0.21	0.29
p-Cresol (yellow)	0.13	0.14	0.19	0.22	0.27
m-Cresol (yellow)	0.15	0.15	0.19	0.27	0.27

resorcinol and catechol being the most diffuse. Note that the colors of the developed zones after spraying with diazotized benzidine were different on the different resin form.

Similar to the results on the hydrogen-form resin, the R_F values increased with increasing molarity of methanol in a roughly parallel manner for each phenol. Again, the solubilizing effect of the methanol is readily apparent. The order of affinity of the phenols for the ammonium-form resin was the same as for the hydrogen-form (resorcinol < catechol < phenol < cresols), but the actual R_F values were substantially lower. This greater affinity for the ammonium-form layer compared with the hydrogen-form layer is just opposite to the results obtained in column studies of the effect of the counter ion of Dowex 50 when various ketones were eluted with water[10]. No explanation can be offered for these differences, unless the starch is involved.

This system exhibited much less selectivity toward the phenols. No separations could be performed with any molarity of methanol as the developer.

Solubilization chromatography on anion-exchange resin layers

Table III shows the results of developing the phenols with methanol on layers of Dowex 1-X4 in the chloride form. Times for development ranged from 25 to 75 min, being much faster with high solvent concentrations. The developed zones were generally less than 2.0 cm in length, becoming only slightly more diffuse with increasing concentration of methanol. R_F values for all concentrations of methanol between 0.0 and 6.0 *M* were 0.10 or less.

The much greater affinity of the phenols for the resin phase as evidenced by the very low R_F values throughout Table III was expected. Dowex 1 is more organic in nature (more carbon atoms per functional group) than Dowex 50, and one of the most important interactions[11] between the phenols and the stationary phase was undoubtedly the London dispersion forces involving the hydrocarbon parts of the resin.

It is interesting that the order of the R_F values on the anion-exchange resin tends to reverse trends established on the cation-exchange resin. At high concen-

TABLE III

R_F VALUES ON DOWEX 1-X4 (Cl^-) LAYERS

Phenol	*R_F values*					
	Concentration of methanol (M)					
	0.0	*6.0*	*7.0*	*12*	*19*	*22*
Catechol (yellow)	0.09	0.10	0.10	0.17	0.27	0.31
Resorcinol (red-brown)	0.07	0.08	0.08	0.13	0.22	0.25
Phenol (orange)	0.09	0.11	0.12	0.21	0.37	0.37
o-Cresol (orange)	0.05	0.08	0.09	0.16	0.37	0.45
p-Cresol (orange)	0.06	0.08	0.08	0.17	0.39	0.45
m-Cresol (orange)	0.07	0.08	0.09	0.17	0.38	0.40

trations of methanol, the cresols exhibited the least affinity for the anion exchanger, having R_F values about 1.5 times as large as resorcinol and catechol, which always had the highest R_F values on the cation exchanger.

Only with very high concentrations of methanol did the phenols migrate significantly. The system was selective enough, however, to allow various separations to be performed. For example, with 19 *M* methanol (75 %), resorcinol was separated from every phenol except catechol by at least 0.70 cm, and catechol was separated by 0.70 cm from *p*-cresol. Triple development with 22 *M* methanol (90 %) separated catechol (R_F 0.12) by at least 0.50 cm from every phenol except resorcinol. Even catechol and resorcinol, which showed little differential migration in the other systems, were substantially separated. Further development was impossible because the layers cracked after the third solvent pass.

Salting-out chromatography on cation-exchange resin layers

Salting-out chromatography[12] is a method by which water-soluble nonelectrolytes have been separated by elution through ion-exchange resins with aqueous salt solutions as eluents. We decided to develop the phenols by thin-layer salting-out chromatography in a system including Dowex 50 in the ammonium form as the stationary phase and aqueous solutions of ammonium sulfate as the mobile phase.

TABLE IV

R_F VALUES ON DOWEX 50W-X8 (NH_4^+) LAYERS

Phenol	*R_F values*		
	Concentration of ammonium sulfate (M)		
	0.0	*1.0*	*3.8*
Catechol	0.24	0.13	0.070
Resorcinol	0.25	0.15	0.090
Phenol	0.21	0.11	0.050
o-Cresol	0.15	0.070	0.030
p-Cresol	0.13	0.060	0.020
m-Cresol	0.15	0.070	0.030

The results for the individual phenols are shown in Table IV. Times for development ranged from 50 to 105 min. The developed zones were 0.60 to 2.5 cm in length, being more compact as the salt concentration increased. The colors of the sprayed zones were the same as for the ammonium-form layers developed with methanol. At high salt concentrations, the solvent front was unusually irregular.

These preliminary experiments indicate that salting-out chromatography on thin layers of ion-exchange resin is a feasible procedure worthy of future detailed study. We did not expect to be able to separate the phenols with it because their R_F values when developed with water (R_{F0}) were too low. The R_F values did decrease as expected with increasing salt concentration but apparently not selectively. It should be possible to find selective systems for producing separations of nonvolatile water-soluble solutes with high R_{F0} values. The salt eluents would tend to decrease and separate the R_F values if the results were comparable to column salting-out chromatography.

COMMENTS

The purpose of this research was to present a new separation procedure combining thin-layer chromatography with partition chromatography on ion-exchange resins, and to compare the results of this procedure with results from similar types of chromatography reported earlier. A limited number of phenols were chosen for study, but the method would undoubtedly be applicable to virtually any uncombined phenols or derivatives which could be detected successfully. In fact, additional phenols were examined, although not in detail, and showed evidence of useful migration in several of the systems. These included pyrogallic acid, phloroglucinol, *o*-nitrophenol, *o*-phenylphenol and *p*-hydroxybenzaldehyde. Further work is now in progress to extend this separation technique to other classes of compounds and to study other experimental variables in the process.

As in all differential migration methods, the behavior of individual solutes is subject to considerable variation, such as in R_F and the size of individual zones. This is especially true in thin-layer chromatography because the preparation of nearly identical layers from day to day is a genuine art. The reproducibility of R_F values shown in Table I (which was typical but not the best we could have shown) is quite good except for two or three values. It was generally possible to reproduce values to $\pm$ 0.03 R_F units throughout this work.

The phenols were undoubtedly very slightly ionized during their migrations. It is probable, however, that the actual ion exchange between the stationary phase and these ions was small enough to be neglected compared with other solute–resin interactions, and that the procedure was essentially a separation of nonelectrolytes by a partition process.

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SUMMARY

The R_F values of a series of six phenols have been determined using thin-layer chromatographic techniques on layers of ion-exchange resin. These data have been used to predict and perform the separation of various mixtures of these phenols by development with aqueous solutions of methanol. The procedure is called thin-layer solubilization chromatography, and it is compared to solubilization chromatography in columns and on paper.

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