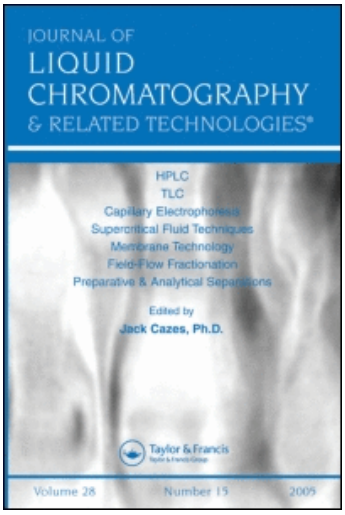

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THE USE OF AN α-CYCLODEXTRIN MOBILE PHASE IN THE THIN-LAYER CHROMATOGRAPHIC SEPARATION OF ORTHO, META, AND PARA SUBSTITUTED PHENOLS

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ABSTRACT

An aqueous solution of α-cyclodextrin (cyclohexaamylose) is demonstrated to be a very effective mobile phase in thin-layer chromatographic separations. The chromatographic behaviors of twenty-six substituted phenolic and naphtholic compounds using polyamide thin-layer stationary sheets are described. The R_f values were found to be dependent upon both the structural features of the phenolic compounds and the concentration of α-cyclodextrin in the mobile phase. A possible mechanism that accounts for the observed chromatographic behavior is presented. The advantages and disadvantages of the aqueous α-cyclodextrin mobile phase over the traditional pure or mixed organic solvent systems typically employed are discussed.

INTRODUCTION

Phenolic compounds are prevalent in a wide variety of chemical processes. They are extensively used as intermediates in industrial applications (such as in the manufacture of different dyes, herbicides, pesticides, plastics, drugs, etc.) and are present in the waste water effluents from coal distilling and coking plants. Trace levels (< 1 ppm) of many substituted

phenols are toxic to aquatic and mammalian life and have an adverse effect on both the odor and taste of water (1-3). Due to these serious, detrimental effects on water quality, the separation and identification of substituted phenols continue to be a subject of particular interest and significance.

Various chromatographic techniques have been utilized (4,5) to separate and determine phenols, but most of these suffer from one or more drawbacks. One popular and simple technique is thin-layer chromatography (TLC) (6,7). Table 1 summarizes many of the TLC systems that have been developed for the separation of phenols (8-32). As can be seen, most require organic solvent mobile phases and/or specially prepared stationary phases. The problem with many of these mobile phases stems from the environmental and safety hazards that they pose (i.e. their toxicity, flammability, and the added expense of providing for a safe working area). Additionally, one is severely limited in the variables that can be adjusted when attempting to obtain an optimum separation (such as a change in the pH, ionic strength, buffer components, or addition of water or other highly polar species).

Recently, the novel use of aqueous macromolecular solutions as the mobile phase has been proposed in an attempt to overcome many of these difficulties (33-37). Thus far, two such general systems have been detailed. Armstrong has described the use of micellar systems in various TLC or HPLC separations (33-35) while we have jointly reported the first use of aqueous cyclodextrins in the TLC separation of substituted isomeric benzoic acids (36). In these systems, dubbed "pseudophase liquid chromatography" (37), the desired component in a mixture does not partition directly to the bulk solvent in the mobile phase, but instead binds to the discrete macromolecular species present in the solution (i.e. the micelle or cyclodextrin). Numerous physical organic studies (38) have shown that cyclodextrins are capable of forming inclusion

TABLE 1

Summary of Published TLC Systems for the Separation of Phenols

Stationary Phase	Mobile Phase	Compounds Separated
Silica gel, ref 8-10	benzene:HAc (7:3 or 98:2) or benzene:MeOH (9:1)	> 20 phenols
Silica gel, ref 11-13	benzene:acetone (7:3 to 9:1)	dichloro-, chloro-, and aminophenols
Silica gel, ref 14	$CHCl_3$:acetone (1:1)	hydroxybenzoic acids, phenol
Silica gel, ref 15-17	benzene or cyclohexane: $CHCl_3$:Et_3N (5:4:1); or CH_2Cl_2	alkylphenols, chlorocresols
Silica gel, ref 18	CH_2Cl_2:cyclohexane (5:1) or cyclohexane:cyclohexanone (3:1)	phenols (as their 4-acetyl-2-nitrophenyl derivatives)
Cellulose, ref 19	benzene:HAc:HOH (125:72:3)	various phenols
Al_2O_3, ref 20,21	MeOH, EtOH, acetone, or benzene:heptane (3:1)	naphthol, phenol, or hindered phenols
Al_2O_3, ref 22	benzene:$C_4H_8O_2$ (2:1)	cresols, xylenols
Silufol, ref 23	benzene:acetone (9:1)	cresols, dihydroxybenzenes
Crystalline aluminum hydroxide, ref 24	2 % EtOH in benzene	nitrophenols
Polyamide, ref 25,26	benzene:MeOH:HAc (45:8:4)	various phenols
Polyamide impregnated silica gel, ref 27, 29	EtOH:cyclohexane (1:1) or benzene:MeOH:HAc (45:8:4)	polyhydric phenols, polyphenols
Cellulose or starch impregnated polyamide, ref 28	benzene:MeOH:HAc (80:13:7)	polyhydric phenols
Silica gel + $AgNO_3$, ref 23	benzene:acetone (9:1)	cresols, dihydroxybenzenes
Silica gel + 4,4'-diazido-2,2'-stilbenedisulfonic acid or + anilinium chloride, ref 30-32	benzene:acetone (9:1) or benzene:EtAc (3:1 or 9:1)	various phenols such as aminophenols, cresols, xylenols, etc.

complexes with a whole host of aromatic molecules. More importantly, differences in the binding of ortho, meta, and para isomers were observed. In this work, we take advantage of the selective binding of substituted phenolic compounds to α-cyclodextrin (α-CD) and demonstrate the TLC separation of a series of phenols and naphthols via use of an aqueous α-CD mobile phase and polyamide stationary phase.

EXPERIMENTAL

Materials. The phenolic standards employed were: 2,4,6-trichlorophenol, 2,6-dimethylphenol, p-phenylphenol, o-phenylphenol, p-hydroxybenzoic acid, p-hydroxybenzaldehyde, o-hydroxybenzoic acid, p-bromophenol, p-chlorophenol, and p-methoxyphenol (Eastman Kodak Company); phenol and resorcinol (Mallinckrodt Chemical Works); p-nitrophenol, o-nitrophenol, p-tert-butylphenol, and 8-amino-2-naphthol (Matheson, Coleman & Bell); 1-naphthol, 2-naphthol, catechol, hydroquinone, and picric acid (2,4,6-trinitrophenol) (Fisher Scientific Company); and o-aminophenol, m-aminophenol, p-aminophenol, m-nitrophenol, and p-iodophenol (Aldrich Chemical Company). All of these compounds are the best available reagent grade materials and were used as received except for the amino-derivatives which were recrystallized prior to use. α-Cyclodextrin was purchased from Sigma Chemical Company and used without further purification. The Polygram polyamide-6 UV_{254} thin-layer sheets were obtained from Brinkmann Instruments, Inc. Distilled, deionized water was used in the preparation of the α-CD stock solutions. Spectral grade acetone, ether, or chloroform (Fisher Scientific Company) was used to dissolve the standard phenolic substances employed in this study.

METHODS. Aqueous solutions of varying α-CD concentration (0.00, 0.025, 0.05, 0.075, 0.10, and 0.11 M) were prepared and utilized as the mobile phase in the subsequent TLC experiments.

In all cases, 20 x 20 cm polyamide TLC sheets were employed as the stationary phase. The standard aromatic hydroxy-containing compounds were prepared by dissolving 1 - 10 mg of the desired compound in about 10 ml of the spectral grade solvent. Typically, about 0.1 - 5 μl of these standard solutions were then spotted on the polyamide sheets with a microsyringe and, after drying, the sheets were developed with the appropriate mobile phase. All ascending TLC were run in air tight rectangular chambers (Analtech) which were lined with solvent-soaked saturation pads (Analtech) in order to ensure saturation of the ambient atmosphere in the chamber with the volatile components of the mobile phase. The temperature for all runs was 25.0° C and the development time ranged from about one hour (if [α-CD] = 0.00, 0.01, or 0.025 M) to a maximum of about two and one-half hours (if [α-CD] = 0.11 M).

A few compounds (such as the nitrophenols) were easily detected as yellow spots, directly or after exposure to concentrated ammonia fumes. Most of the other compounds were detected as fluorescence-quenched dark spots against a bright fluorescing background either before or after exposure to ammonia fumes (from a beaker of concentrated ammonium hydroxide) when illuminated at either 254 or 366 nm with a model UVSL-25 Mineralight handlamp. A few compounds were detected by their own fluorescence (8-amino-2-naphthol, hydroxybenzoic acids). Some substances (such as p-methoxyphenol, phenol, p-tert-butylphenol, and 2,6-dimethylphenol) were difficult to visualize using the mentioned detection methods. Hence, a new spot test was developed whereby they could be easily detected. The chromatogram was soaked or sloshed with an aqueous 0.07 - 0.10% potassium permanganate solution and the compound detected as a yellow-brown spot against the pinkish-purple background. The spots must be marked fairly quickly since the entire chromatogram will even-

tually turn yellow. Tracings of typical chromatograms are shown in Figure 1. The reported R_f values were determined from such chromatograms and represent the average of at least five identical runs which were in good agreement unless otherwise noted.

RESULTS

Aqueous solutions of α-CD proved to be a very effective and selective mobile phase in the TLC separation of a wide variety of phenols and naphthols (many of which are positional isomers) on polyamide thin-layer sheets (42). The results are summarized in Table 2 which lists the R_f values as a function of the α-CD concentration in the mobile phase. Except as noted in Table 2, the compounds all moved as distinct spots and the R_f values for the pure standards were found to be essentially the same as those determined for the same compounds when present as part of a mixture (refer to the chromatograms in Figure 1).

With the exception of the aminophenols, and to a lesser extent, phenol and the hydroxyphenols, no appreciable separation of the compounds was achieved if a distilled water mobile phase was used (i.e. all R_f values were 0.10 or less). However, if an aqueous α-CD mobile phase was employed, the separation of many isomeric compounds (such as the nitrophenols, phenylphenols, naphthols) as well as other groups of compounds (such as p-Y-phenols, Y = Cl, Br, I, OH, NO_2) became possible (refer to Table 2). Typically, for any given isomeric family of ortho, meta, and para substituted phenols, the R_f value for the para isomer is larger than that for the ortho isomer, with the meta isomer having an intermediate value. These results all reflect the relative ability of these compounds to bind to the α-CD (see Discussion Section).

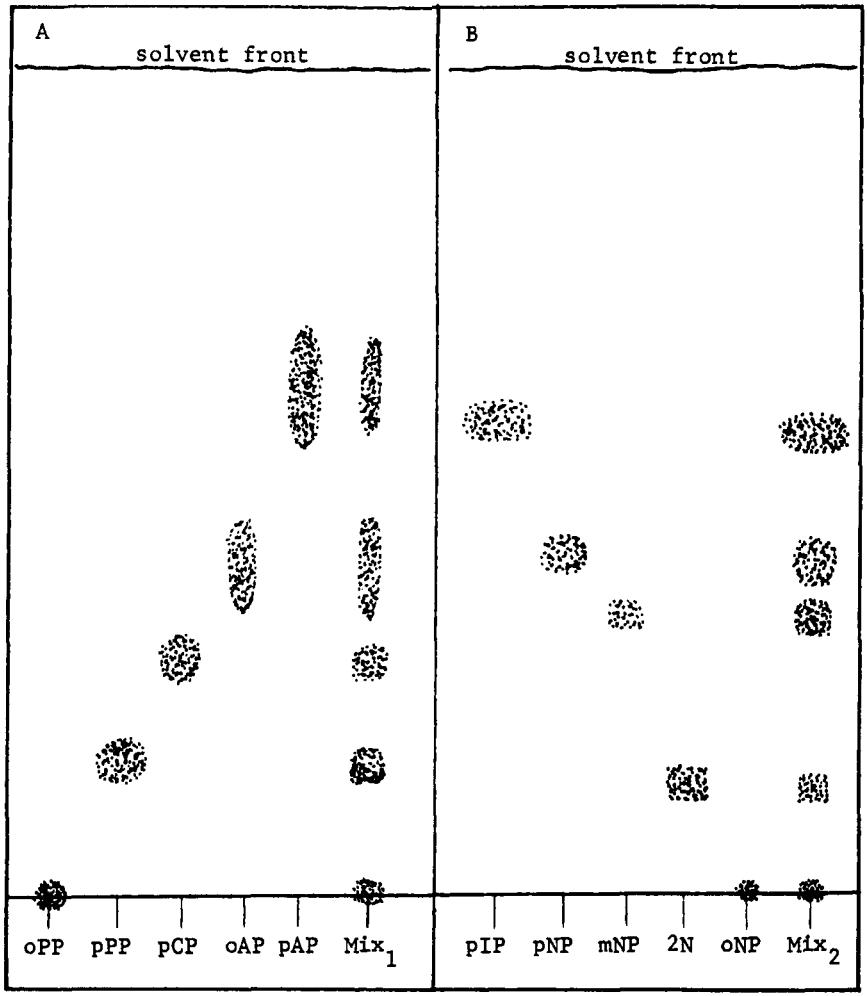


FIGURE 1

Tracing of typical polyamide thin-layer chromatograms developed with 0.10 M α-CD at 25° C. The compounds separated on chromatogram A were: o-phenylphenol (oPP), p-phenylphenol (pPP), p-chlorophenol (pCP), o-aminophenol (oAP), p-aminophenol (pAP), and a mixture of these five (Mix_1); and on chromatogram B: p-iodophenol (pIP), p-nitrophenol (pNP), m-nitrophenol (mNP), 2-naphthol (2N), o-nitrophenol (oNP), and a mixture of these five compounds (Mix_2).

The R_f values of many compounds were found to be dependent on the concentration of the α-CD used. In general, for those compounds which bind to α-CD, the R_f values increased as the α-CD concentration increased, whereas those that don't bind exhibited essentially constant R_f values (see Table 2). Figure 2A shows how changes in the α-CD concentration affect the R_f values for five compounds (their behavior is typical of all the rest). In all cases, the change in R_f appears to be a curvilinear function of the α-CD concentration and tends to approach a maximum value. Plots such as the one shown in Figure 2A can help one select the appropriate α-CD concentration so that optimum separation of the desired components in a mixture will result. It is important to note that the order of R_f values can sometimes change depending upon the α-CD concentration. For instance, the R_f value for p-hydroxybenzaldehyde is larger than that for p-iodophenol at 0.025 M α-CD while the reverse is true at 0.10 M α-CD. Usually, the best separations are achieved at the higher concentrations of α-CD. However, the upper limit of the α-CD concentration that can be used in the aqueous mobile phase is set by its solubility in water (~0.15 M at 25° C) (38).

The strength of binding between α-CD and a phenol can be quantitatively expressed in terms of a formation equilibrium constant. These values are given, where available, (in terms of log K) in Table 2. As can be seen, the isomer in any given family of compounds (such as o-, m-, and p-nitrophenol for instance) with the larger formation equilibrium constant (log K) has the larger ΔR_f value (Table 2). When an attempt was made to correlate the ΔR_f value for <u>different</u> families of phenols with their formation constants, the same general trend was seen, but there were some notable exceptions (refer to Figure 2B). For example, o-hydroxybenzoic acid had the

TABLE 2

R_f Values of Substituted Phenolic Compounds on Polyamide TLC Sheets Using Aqueous Solutions of α-CD as the Mobile Phase[a]

Phenolic Compound	R_f Values at Indicated [α-CD]				log K[b]
	0.00M	0.025M	0.05M	0.10M	
o-Nitrophenol	0.00	0.00	0.00	0.00	0.90[c]
m-Nitrophenol	0.04	0.13	0.22	0.34	1.73[c]
p-Nitrophenol	0.03	0.13	0.25	0.39	2.53[c]
o-Phenylphenol	0.00	0.00	0.01	0.01	----
p-Phenylphenol	0.02	0.06	0.08	0.16	----
o-Hydroxybenzoic acid	0.17(s)	0.18(s)	0.18(s)	0.18(s)	1.80[d]
p-Hydroxybenzoic acid	0.11	0.27	0.43	0.64	3.30[d]
p-Hydroxybenzaldehyde	0.12	0.22	0.29	0.43	----
p-Methoxyphenol	0.15	0.22(t)*	0.28(t)*	0.45(t)*	----
p-Chlorophenol	0.03	0.10	0.20	0.29*	----
p-Bromophenol	0.01	0.12	0.24	0.40	----
p-Iodophenol	0.02	0.18	0.34	0.56	----
o-Aminophenol	0.35(s)	0.31(s)	0.36(s)	0.39(s)	----
m-Aminophenol	0.39	0.40	0.42	0.40	----
p-Aminophenol	0.55(s)	0.57(s)	0.64(s)	0.61(s)	----
1-Naphthol	0.01	----	0.02	0.02	----
2-Naphthol	0.00	0.02	0.06	0.14	1.51[e]
8-Amino-2-naphthol	0.03	----	0.04	0.04	----
2,4,6-Trinitrophenol	0.00	----	0.02	0.02	----
2,4,6-Trichlorophenol	0.00	0.05	0.11	0.20	----
Phenol	0.17	0.19(s)*	0.21(s)*	0.25(s)*	1.28[e]
p-tert-butylphenol	0.01	----	0.18(s)*	0.41(s)*	1.92[e]
2,6-Dimethylphenol	0.07(d)	----	0.08(d)	0.13(d)	----
Catechol	0.13	0.14	0.22*	0.20*	----
Resorcinol	0.11	0.18	0.15	0.14	----
Hydroquinone	0.27	0.29	0.24	0.25	----

[a] R_f values represent the average of at least six determinations that were within ± 0.01 unless otherwise indicated

[b] Log K represents the log of the formation binding constant for the indicated phenol with α-CD.

[c] Taken from reference 39.

[d] Taken from reference 40.

[e] Taken from reference 41.

The (t) represents a slight tailing of the spot, (s) indicates streaking, and (d) refers to a diffuse spot. An asterisk (*) indicates that the R_f values were only within ± 0.04 of each other.

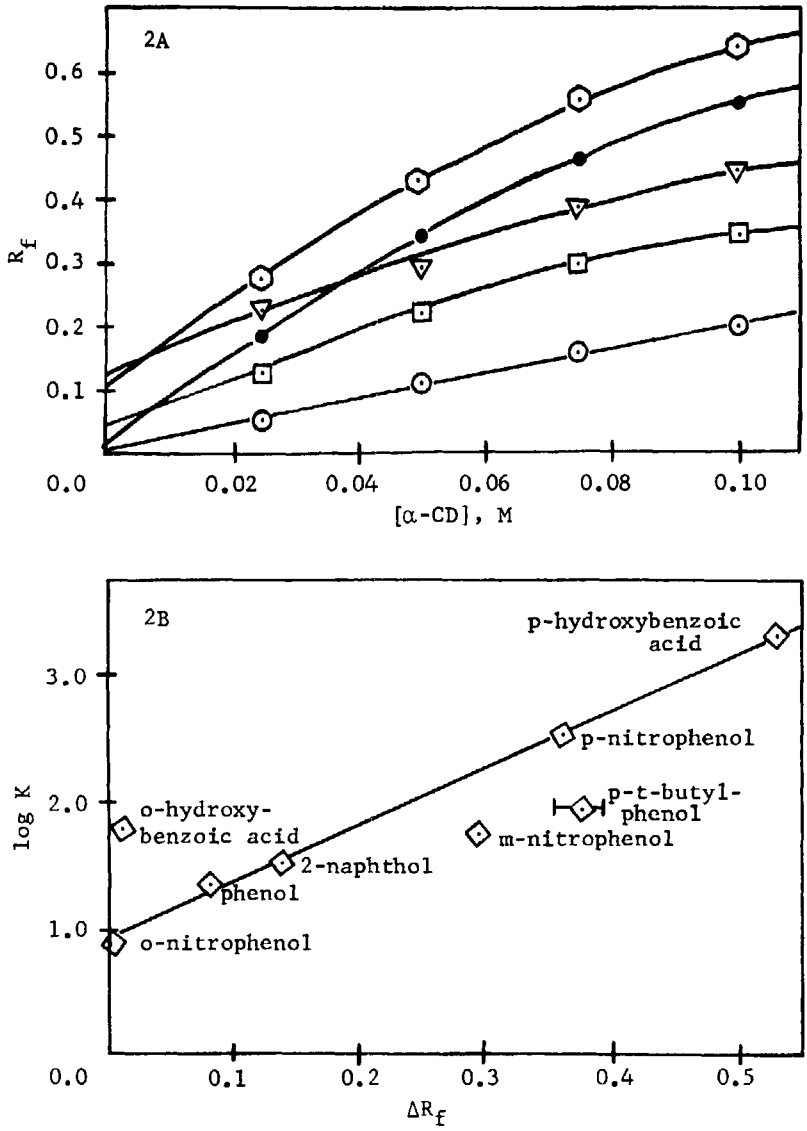


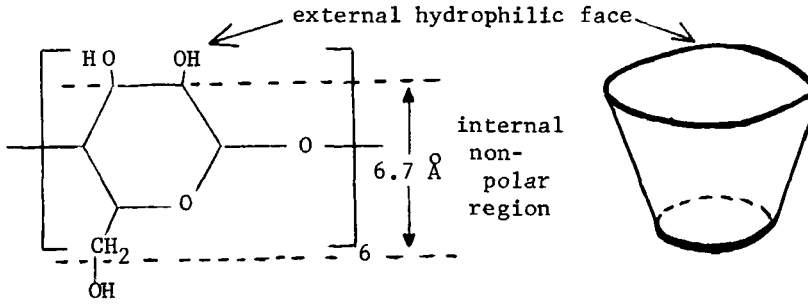
FIGURE 2

2A shows the plot of R_f values vs. α-CD concentration for 2,4,6-trichlorophenol (⊙), m-nitrophenol (⊡), p-hydroxybenzaldehyde (▽), p-iodophenol (●), and p-hydroxybenzoic acid (○). 2B shows a plot of log K (the formation equilibrium constant for the binding of the indicated phenol to α-CD) vs. ΔR_f (the difference between the R_f value in 0.11 M α-CD and that in distilled water) for eight different phenolic compounds.

larger K, yet lower R_f value compared to phenol. Part of the reason for this observed poor correlation stems no doubt from the fact that the binding constants were determined in very acidic media (38), whereas our R_f values were determined in a neutral, aqueous α-CD medium. Clearly, more data is required before any meaningful conclusions can be made regarding such quantitative correlations. However, they can be empirically used to help one predict the expected chromatographic behavior in separations involving the α-CD mobile phase.

DISCUSSION

In order to discuss the results, it is necessary to first briefly describe some of the pertinent properties of α-CD and the factors that govern its ability to bind phenols. α-CD is a cyclic oligosaccharide consisting of six α-(1,4)-linked D-glucopyranose units that has an internal hydrophobic cavity and two external hydrophilic faces (Structure I). Its



STRUCTURE I

structure can be represented by a "V" shaped hollow truncated cone whose central void is 4.7 - 5.2 Å in diameter and has a depth of 6.7 Å. The six primary hydroxyl groups all lie on

the rim of the cavity on the side with the smaller circumference while the twelve secondary hydroxyls lie on the opposite, more open face (38,41,43).

α-CD can interact with a variety of aromatic compounds in a dynamic equilibrium fashion to form 1:1 complexes in which the aromatic molecule is either partially or fully included in the cavity. The most important parameter in determining whether such complexation can occur is the geometry and size of the guest molecule in relation to the dimensions of the α-CD. If the guest is too large, it will not fit into the cavity and hence not bind, whereas if it is too small, it will easily pass in and out of the cavity with little or no appreciable binding. For any series of the same isomeric family, the degree of binding can be qualitatively correlated to the "fit" of the substrate in the cavity (38,41).

The forces responsible for the binding have been variously ascribed to: (1) Van der Waals interactions, (2) hydrophobic interactions with concurrent release of high energy water molecules from the α-CD cavity and relief of strain energy in the α-CD ring system upon complex formation, and (3) hydrogen bonding between the appropriate moiety of the guest (the phenolic hydroxyl group) and the secondary hydroxyl groups on the rim of the cavity (38-41, 43-48). A combination of the latter two forces appears to dominate in the binding of phenols to α-CD (39,44).

With this aspect of cyclodextrin chemistry in mind, it becomes possible to speculate on the possible mechanism of separation of phenols on polyamide using an α-CD mobile phase. Adsorption of phenols on a polyamide stationary phase is caused by hydrogen bond formation with the polyamide carbonyl groups. The mechanism of chromatographic separation then is dependent on the breaking of the phenolic/polyamide hydrogen

bond as a result of solvation of the hydrophobic part of the phenol with the nonaqueous solvent and/or solvation of the phenolic group with an aqueous or polar solvent (49,50). When a phenol complexes with α-CD, the binding forces involved (supra vide) are such that both of these "solvation" requirements are at least partially fulfilled. Hence the separation mechanism with the aqueous α-CD mobile phase appears to be essentially the same as that observed for conventional systems, except that with the α-CD phase, geometry and size determine the degree of binding (and hence, separation) while solvent polarity dictates the separation observed for the more traditional mobile phases. This ability of α-CD to selectively bind molecules according to their size and geometry and hence simultaneously "solvate" both the hydrophobic and hydrophilic portions of the phenolic compounds is unique and cannot be duplicated by any pure or mixed solvent system. In order to predict the degree of separation of phenolic compounds on polyamide using an α-CD mobile phase, one needs merely to determine or estimate their relative binding ability to the α-CD.

By referring to the characteristics of α-CD just outlined and several literature reports concerning their interaction with phenols (38-48), several generalizations can be drawn that will quantitatively help explain the observed chromatographic behaviors of the phenols studied in this work and will serve as useful guides in predicting the behavior of others. They are as follows:

(1) In the absence of steric factors, complex formation usually involves insertion, into the cavity, of the more hydrophobic substituent or portion of the molecule that is most effective at "filling the hydrophobic cavity," with approach and penetration being from the more open (and

accessible) secondary hydroxyl side of the cavity. The types of substituents on the phenolic compound capable of penetrating in this fashion include the $-NO_2$, -COOH, -X (halides such as I, Br) (47,48), and alkyl or phenyl groups (38,41,43). Even though a series of phenols may contain just these substituents, differences in their binding are observed due to differences in the substituents' hydrophobicity as well as in their ability to fill the cavity and to influence--via inductive and resonance effects--the hydrogen bonding ability of the phenolic hydroxyl group toward the secondary hydroxyl groups on the α-CD. The differences in R_f values observed for the series of p-Y-substituted phenols (Y = phenyl, NO_2, COOH, Br, I, etc.) in this study are most probably due to this reason. This means that it is possible to separate various p-substituted phenols from each other as is demonstrated in this work.

(2) Certain substituents, namely the phenolic hydroxyl (-OH) and amino ($-NH_2$) groups, apparently cannot penetrate the α-CD cavity due to unfavorable solvation and/or dipole orientation factors (44). The fact that the ortho, meta, and para isomers of aminophenol and hydroxyphenol (catechol, resorcinol, hydroquinone) exhibited no significant changes in their R_f values when chromatographed with the α-CD mobile phase is most likely a consequence of this factor. These compounds contain only the $-NH_2$ and/or -OH groups, neither of which are capable of penetrating the α-CD cavity. Hence, little--if any--binding occurs, and the R_f values are all similar to those observed when using only distilled water as the mobile phase. This suggests that isomers of phenols containing only these groups will not show any enhanced separation in the presence of α-CD.

(3) Provided that there is a group present that can penetrate the α-CD cavity, the order of binding ability is para > meta >

ortho. This is explicable in terms of the para isomer's better ability to fit and effectively fill the cavity (38, 43). Additionally, the phenolic hydroxyl group of para isomers can, upon binding, effectively interact (via hydrogen bond formation) with the α-CD's secondary hydroxyl groups. This leads to their greater stability (39). Although the meta isomers can penetrate the cavity about as well as the para isomers, their phenolic hydroxyl group will be buried in the cavity where it cannot effectively interact via hydrogen bond formation with the α-CD's hydroxyl groups. The strength of their binding is thus greatly diminished compared to the para isomer (39). In comparison with the para or meta isomers, the binding of the ortho isomer is generally very meager due mostly to steric hindrance. Additionally, the binding ability of some ortho isomers (i.e. those containing a substituent capable of hydrogen bonding, such as the $-NO_2$ group in nitro-phenol) can be further reduced due to intramolecular hydrogen bonding between the phenolic hydroxyl group and the other substituent present (38,39). Since the degree of binding to α-CD dictates the degree of separation on polyamide, one would expect the following order of R_f values for any given ortho, meta, and para isomeric family ($R_{f\ para} > R_{f\ meta} > R_{f\ ortho}$). Qualitatively,the results of this study are in accord with this expectation (refer to R_f values for o,p-phenyl-, o,p-carboxylic acid- and o,m,p-nitrophenols in Table 2). The use of an α-CD phase is thus particularly effective in the TLC separation of o-, m-, and p- substituted isomers of a particular compound.

(4) Although little data is available, some more highly substituted phenols have been reported to bind to α-CD (38, 41,44). These results seem to indicate that some binding is possible for compounds possessing a suitable substituent at

the 4 position (supra vide) and non-hydrogen bonding groups at the 2 and/or 6 positions. Substituents at the 3 and/or 5 position inhibit complexation with α-CD due to steric hindrance (38,39,44) while hydrogen bonding groups at the 2 and/or 6 position greatly decrease the binding capability due to intramolecular hydrogen bond formation. An α-CD mobile phase should then be capable of separating at least some di- and tri-substituted isomeric phenols (for instance, the separation of 2,4-di- and 2,4,6-trisubstituted phenols from their other corresponding 3,4-di-, 4,5-di-, 2,3,4-tri-, and 3,6,5-trisubstituted isomers should be feasible in many instances). In this work, we show that it was possible to separate 2,4,6-trichlorophenol (TCP) from 2,4,6-trinitrophenol (TNP) (Table 2). Presumably, this separation is due to the fact that the two ortho nitro groups of TNP form intramolecular hydrogen bonds with the phenolic hydroxyl group which diminishes its binding ability (compared to that for TCP).

Although the four generalizations just outlined seem to be able to adequately describe the chromatographic behaviors exhibited by the phenols studied in this work, their general validity as well as applicability to other systems must await further study and verification.

CONCLUSION

An α-CD mobile phase has been shown to be particularly effective in the TLC separation of ortho, meta, and para substituted phenols as well as a series of different para substituted phenols. Additionally, some tri-substituted phenols and naphthols were separated on polyamide. The results of this study and a previous one concerning the separation of benzoic acids (36) tend to suggest that the use of an aqueous α-CD mobile

phase is general and applicable to a wide variety of substituted aromatics.

Table 3 compares some previously reported TLC methods for the phenols used in this study with the present method. As can be seen, the use of the α-CD mobile phase affords as good as or much better separations for the indicated phenols than do the traditional methods. The use of α-CD eliminates the need for the organic solvents (along with their assorted problems, supra vide) as well as the need for prior derivatization of the phenols (refer to the second last entry in Table 3). No single traditional solvent system appears to be as effective as α-CD in separating the wide variety of phenols studied in this work.

In addition to the obvious advantages just mentioned, the use of an aqueous α-CD mobile phase will allow for easier adjustment of the solution conditions (pH, ionic strength, etc.) when attempting to improve separations. α-CD has been reported to enhance the absorption and fluorescence intensity of many aromatic compounds (38, 41,43). Hence, in the separation and subsequent detection of such compounds, the use of an α-CD mobile phase should lower the detection limits and increase the sensitivity of the TLC method. We are currently pursuing this possible application.

The main disadvantage in using the α-CD mobile phase is that no separation is possible for those compounds that do not bind to α-CD. For instance, in the present work, the separation of some ortho isomers (o-nitrophenol and o-phenylphenol) from 1-naphthol and 2,4,6-trinitrophenol would not be possible with α-CD. For these substances, the conventional mobile phase systems would have to be used in order to obtain the desired separations. Alternatively, one could use aqueous solutions of some larger cyclodextrins (β-CD or γ-CD) and obtain separation since they can bind some substituted phenols as well as larger aromatic molecules.

TABLE 3

Comparison of Method with some Previously Published TLC Systems for the Separation of Phenolic Substances

TLC System Employed	R_f Values for Indicated Phenols[a]								
	P	pMP	oNP	mNP	pNP	1N	2N	pCP	pBP
Silica gel; cyclohexane:EtAc (4:1); ref 7	.53	.39	.32	.40	.47	---	---	---	---
Polyamide; benzene; ref 50	.16	---	---	---	.02	.14	.12	.13	.13
Polyamide; EtAc; ref 50	.75	---	---	---	.51	.12	.10	.74	.75
Polyamide; MEK:cyclohexane (3:2); ref 49	.62	.60	---	---	.38	.56	.53	.49	---
Polyamide; acetone:cyclohexane (65:35); ref 49	.62	.66	.79	---	.45	.61	.56	---	---
Polyamide; diethyl ether:cyclohexane (3:2); ref 51	.58	.49	---	---	---	.52	.42	---	---
Polyamide; $CHCl_3$:cyclohexane (3:2); ref 51	.08	.10	---	---	---	.10	.08	.08	---
Silica gel; either CH_2Cl_2:cyclohexane (5:1) or cyclohexane:cyclohexanone (3:1); (determined as the 4-acetyl-2-nitrophenyl-derivatives); ref 18	.82 .62	--- .50	.45 .34	.50 .49	.53 .55	.73 .58	.65 .69	--- ---	--- ---
Polyamide; 0.10 M aqueous α-CD; this work	.25	.45	.00	.34	.39	.02	.14	.29	.40

[a]Abbreviations for phenols: phenol (P), p-methoxyphenol (pMP), o-nitrophenol (oNP), m-nitrophenol (mNP), p-nitrophenol (pNP), 1-naphthol (1N), 2-naphthol (2N), p-chlorophenol (pCP), and p-bromophenol (pBP).

The use of these cyclodextrins in TLC or HPLC separations will be the topic of a subsequent publication.

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