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PREPARATION AND CHARACTERIZATION OF CHEMICALLY BONDED THIN-LAYER CHROMATOGRAPHIC PLATES

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SUMMARY

Although the concept of reversed-phase thin-layer chromatography (TLC) is not new, the preparation and detailed characterization of chemically modified TLC plates have not been previously reported. In this study, detailed evaluations of various organically modified TLC plates have been carried out. Methyl, ethyl, hexyl, dodecyl, and octadecyl alkyltrichlorosilanes have been used to form bonded layers of varying behavior. The nature of these layers has been examined using several types of test compounds in conjunction with both conventional iodine staining techniques and radioactive tracer techniques. By use of these techniques the interactions of a number of different types of test molecules with the bonded polymeric films have been studied. From these studies, dodecyltrichlorosilane has been found to give chromatographically preferred coatings, along with a reversal in compound elution order. These results compare closely to results obtained on octadecyl reversed-phase high-pressure liquid chromatographic columns.

INTRODUCTION

Although the concept of reversed-phase thin-layer chromatography (TLC) is not new, the preparation and detailed characterization of chemically modified TLC plates have not been fully examined. Previously, several authors[1–6] have reported on the use of plates impregnated with non-polar stationary phases. In these cases, solvent systems for this type of chromatography are often saturated prior to use with the same material used to impregnate the plate.

The advantage of bonded coatings over physically coated phases is well known for high-pressure liquid chromatographic (HPLC) use[7,8]. Similar advantages exist for TLC use. In addition, since the bonded phases are not removed by solvents, an unidentified compound may be scraped from the plate, extracted with an appropriate solvent and analyzed by ancillary techniques without interference from the bonded layer. This advantage may not necessarily exist when physically coated plates are used.

Another potentially useful application of bonded phase plates arises from their analogous behavior with chemically bonded reversed-phase HPLC columns. In

this respect, an initial separation may be designed using reversed-phase TLC and adapted to reversed-phase HPLC with minimal effort.

In this study, organically modified TLC plates have been prepared from varying chainlength alkyltrichlorosilane monomers. The chromatographic behavior of these bonded phase plates has been examined by both conventional iodine staining and by radioactive tracer techniques.

EXPERIMENTAL

Analtech 250-μm silica gel G plates (Analtech, Newark, Del., U.S.A.) were used in all experiments. Prior to modification, each plate was dried at 110° for at least 2 h. All modifications were carried out in a glass reaction chamber (Fig. 1) which was also dried for 2 h at 110° before use. Two 5 × 20 cm plates were placed back to back in the reaction chamber and the chamber sealed while being maintained at 110°. The chamber was removed from the oven and a drying tube attached to exclude moisture.

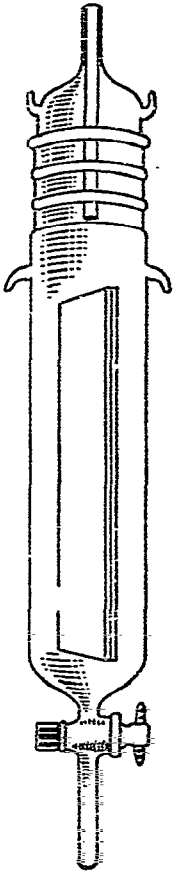

Fig. 1. Reaction vessel for preparing chemically bonded TLC plates.

After having removed the chamber and plates from the oven, the reactions were carried out using the following procedure: 500 ml of a 1% solution of a particular alkyltrichlorosilane monomer in dry toluene were slowly drawn (*i.e.* at 100 ml/min) into the reaction chamber and the chamber sealed. The solution was allowed to react for 10 min and drained from the chamber. The chamber and plates were subsequently rinsed twice with 500-ml portions of dry toluene. The plates were then rinsed with 500 ml of water-saturated toluene (15 min), removed from the chamber and dried at 110° for a minimum of 2 h.

Reagents

The toluene used was analytical reagent grade obtained from Mallinckrodt (St. Louis, Mo., U.S.A.) which was allowed to stand over activated molecular sieve (Union Carbide, New York, U.S.A.) for at least 4 days prior to use. Acetonitrile (distilled in glass) was obtained from Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.). Methyltrichlorosilane, ethyltrichlorosilane, and octadecyltrichlorosilane were obtained from Aldrich (Milwaukee, Wisc., U.S.A.). Hexyltrichlorosilane, and dodecyltrichlorosilane were obtained from K & K Labs. (Plainview, N.Y., U.S.A.) and PCR (Gainesville, Fla., U.S.A.), respectively. All silane monomers were used in the condition received. The labeled [7-^{14}C]-*o*-hydroxybenzoic acid was obtained from New England Nuclear (Boston, Mass., U.S.A.).

Detection

All compound detection, except for the labeled *o*-hydroxybenzoic acid was made using iodine vapor staining. Radioactive *in situ* counting experiments were carried out on a Varian Berthold Model LB2723 radio scanner.

RESULTS AND DISCUSSION

Chemically modified TLC plates were prepared by reacting various alkyltrichlorosilanes with precoated silica gel plates. After modification, these plates were examined using a test series of compounds which are listed in Table I. Retention behavior, spot spreading, development time, and plate reproducibility were studied.

Shown in Table I are tabulated retention data for each test compound on the various alkyltrichlorosilane-modified plates. These data were obtained with acetonitrile–0.01 *M* KH_2PO_4 solution (40:60). All R_F data are mean values obtained from 3–5 replicate plates for a particular alkyltrichlorosilane modification. R_F values for each test compound were reduced as a function of increasing hydrocarbon chainlength for the bonded phase, with the greatest relative reduction for the non-polar compounds. Also, as the bonded hydrocarbon chainlength was increased, increased separation between the test compounds was observed. The greatest selectivity for the test solutes occurred on the dodecyl- and octadecyl-modified plates. These results are consistent with recently reported HPLC data on similar bonded phases[9]. In that work, columns were prepared from ethyltrichlorosilane, hexyltrichlorosilane, dodecyltrichlorosilane and octadecyltrichlorosilane. Increased compound retention and selectivity were noted with the longer chainlength (dodecyl and octadecyl) bonded phases.

Summarized in Table II are calculated values of adjusted spot widths, w, relative spot broadening, s [where $s = (w/R_F) \times 100$], and overall plate development

TABLE I

VARIATION IN COMPOUND RETENTION AS A FUNCTION OF BONDED HYDROCARBON CHAINLENGTH FOR MODIFIED TLC PLATES

Solvent system, acetonitrile–0.01 *M* KH_2PO_4 (40:60). All R_F data are mean values obtained from 3 to 5 replicate plates per alkyltrichlorosilane modification.

Compound	*R_F values for various modified TLC plates*				
	Methyl	*Ethyl*	*Hexyl*	*Dodecyl*	*Octadecyl*
p-Hydroxybenzoic acid	0.90 ± 0.035	0.87 ± 0.014	0.81 ± 0.006	0.82 ± 0.021	0.79 ± 0.015
Methyl *p*-hydroxybenzoate	0.89 ± 0.0	0.84 ± 0.007	0.76 ± 0.015	0.71 ± 0.047	0.62 ± 0.059
Ethyl *p*-hydroxybenzoate	0.89 ± 0.021	0.84 ± 0.028	0.75 ± 0.015	0.68 ± 0.047	0.58 ± 0.068
Propyl *p*-hydroxybenzoate	0.88 ± 0.028	0.83 ± 0.035	0.71 ± 0.012	0.61 ± 0.026	0.51 ± 0.072

TABLE II

VARIATION IN EFFICIENCY AS A FUNCTION OF BONDED HYDROCARBON CHAINLENGTH FOR VARIOUS ALKYLTRICHLOROSILANE MODIFIED TLC PLATES

Solvent system, acetonitrile–0.01 *M* KH_2PO_4 (40:60). All data are mean values obtained from 3 to 5 replicate plates per alkyltrichlorosilane modification. w = Relative spot width; $s = (w/R_F) \times 100$.

Compound	*Various alkyltrichlorosilane plates*									
	Methyl		*Ethyl*		*Hexyl*		*Dodecyl*		*Octadecyl*	
	w	*s*	*w*	*s*	*w*	*s*	*w*	*s*	*w*	*s*
p-Hydroxybenzoic acid	0.15	16.7	0.13	14.9	0.12	14.8	0.12	14.6	0.16	20.3
Methyl *p*-hydroxybenzoate	0.15	16.9	0.10	11.8	0.15	19.7	0.12	16.7	0.14	22.6
Ethyl *p*-hydroxybenzoate	0.15	16.9	0.13	15.5	0.13	17.3	0.10	14.7	0.14	24.1
Propyl *p*-hydroxybenzoate	0.15	18.2	0.12	14.5	0.12	16.9	0.12	19.7	0.12	23.5
Run times	60–75 min		60–75 min		75–90 min		90–105 min		120–150 min	

times. The plates which produced chromatographically preferable spot shapes were those modified with either dodecyl or octadecyltrichlorosilane. The shorter chain-length coatings, methyl and ethyl, produced poorly-formed irregular spots. Although the octadecyl-modified plates produced well-formed spots, the relative widths of these spots were increased compared to spots obtained with similar compounds on the dodecyl plates. These results are in good agreement with efficiency data reported for similar HPLC studies[9] with the exception of the increased spot broadening in the case of the octadecyl plates. These differences are reconcilable in terms of long plate development time and poor solvent flow on the octadecyl-modified plates. These were not factors in the reported HPLC experiments.

The effect of hydrocarbon chainlength on chromatographic performance was further investigated in terms of spot distribution. Shown in Fig. 2 are *in situ* radioactive scans of [7-^{14}C]-*o*-hydroxybenzoic acid obtained on the various alkyltrichlorosilane-modified plates. These data are in close agreement with the test mixture data obtained by conventional iodine staining techniques as previously discussed.

Based on the above results, dodecyltrichlorosilane-modified plates were found to be chromatographically preferable since they provided the best overall performance in terms of compound retention and spot integrity. Also, the dodecyl plates had reasonable development times of approximately 75–90 min at ambient temperature.

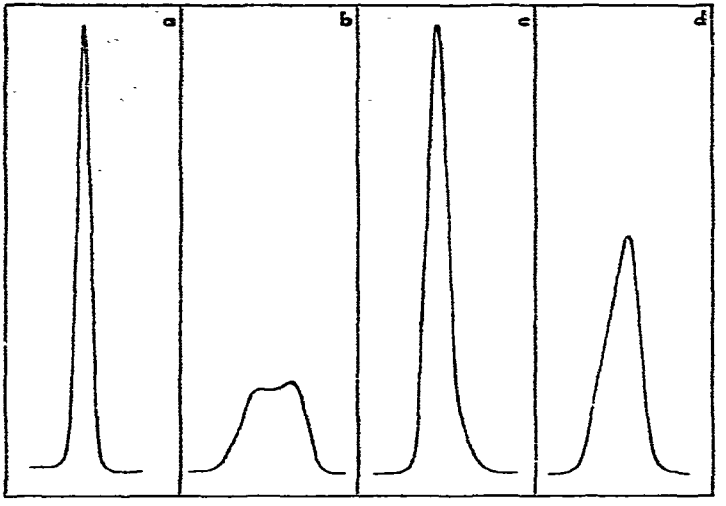

Fig. 2. Effect of bonded hydrocarbon chainlength on spot distribution. (a) Unmodified silica G plate, (b) methyl-modified silica G plate, (c) dodecyl-modified silica G plate, (d) octadecyl-modified silica G plate. Solvent systems: (a) acetic acid–chloroform (5:95), (b–d) acetonitrile–0.01 *M* KH_2PO_4 (40:60). Test solute: *o*-hydroxybenzoic acid.

Correlation studies

Additional dodecyl-modified plates were prepared and evaluated using several different test mixtures. Correlation studies between separations obtained on C_{12} reversed-phase TLC plates and C_{18} reversed-phase HPLC columns were made. The C_{18} columns were prepared by an *in situ* method using 200 ml of dry toluene to establish controlled pre-reaction conditions. The complete details of preparation have been previously described[10,11].

Summarized in Tables III–VI are the results of these correlation studies. All TLC R_F and HPLC k' data are mean values obtained from at least triplicate determinations. Accompanying representative chromatograms of these data appear in

TABLE III

COMPARISON OF TLC AND HPLC REVERSED-PHASE SEPARATIONS FOR VARIOUS HOMOLOGS OF *p*-HYDROXYBENZOIC ACID ESTERS

Mobile phase, acetonitrile–0.01 *M* KH_2PO_4 (40:60). TLC: dodecyltrichlorosilane-modified plates; HPLC: octadecyltrichlorosilane-modified packing. Figures in parentheses indicate elution order.

Compound	*Retention data*	
	TLC (R_F)	*HPLC (k')*
p-Hydroxybenzoic acid	0.82 (1)	0.37 (1)
Methyl *p*-hydroxybenzoate	0.72 (2)	1.25 (2)
Ethyl *p*-hydroxybenzoate	0.68 (3)	1.75 (3)
Propyl *p*-hydroxybenzoate	0.61 (4)	2.54 (4)
Butyl *p*-hydroxybenzoate	0.56 (5)	3.84 (5)

TABLE IV

COMPARISON OF TLC AND HPLC REVERSED-PHASE SEPARATIONS FOR SELECTED AROMATIC ALCOHOLS

Mobile phase, acetonitrile–0.01 *M* $(NH_4)_2CO_3$ (40:60). TLC: dodecyltrichlorosilane-modified plates; HPLC: octadecyltrichlorosilane-modified packing. Figures in parentheses indicate elution order.

Compound	*Retention data*	
	TLC (R_F)	*HPLC (k')*
Phenol	0.59 (1)	1.11 (1)
p-Cresol	0.55 (2)	1.49 (2 and 3)
m-Cresol	0.54 (3)	1.49 (2 and 3)
o-Cresol	0.51 (4)	1.57 (4)

TABLE V

COMPARISON OF TLC AND HPLC REVERSED-PHASE SEPARATIONS FOR VARIOUS AROMATIC AMINES

Mobile phase, acetonitrile–0.01 *M* $(NH_4)_2CO_3$ (40:60). TLC: dodecyltrichlorosilane-modified plates; HPLC: octadecyltrichlorosilane-modified packing. Figures in parentheses indicate elution order.

Compound	*Retention data*	
	TLC (R_F)	*HPLC (k')*
Aniline	0.73 (1)	1.32 (1)
p-Toluidine	0.70 (2)	1.96 (2)
3-Chloroaniline	0.69 (3)	2.28 (3)
o-Tolidine	0.60 (4)	2.50 (4)

TABLE VI

COMPARISON OF TLC AND HPLC REVERSED-PHASE SEPARATIONS FOR VARIOUS AROMATIC AMINES

Mobile phase, acetonitrile–0.01 *M* $(NH_4)_2CO_3$ (30:70). TLC: dodecyltrichlorosilane-modified plates; HPLC: octadecyltrichlorosilane-modified packing. Figures in parentheses indicate elution order.

Compound	*Retention data*	
	TLC (R_F)	*HPLC (k')*
Aniline	0.60 (1)	1.90 (1)
p-Toluidine	0.52 (2)	3.23 (2)
3-Chloroaniline	0.41 (3)	4.16 (3)
o-Tolidine	0.32 (4)	5.71 (4)

Figs. 3a–d. In each case, good agreement between TLC and HPLC results was observed. In all comparative studies, the same mobile phase and solvent system compositions were used to carry out both HPLC and TLC separations. In those cases where good resolution of the test mixture components was obtained using the dodecyl-modified plates, concurrently good separations were observed using the octadecyl column as shown in Figs. 3a and 3d. When little or no separation between the various test compounds was observed by TLC, accompanying poor or no separation was obtained by HPLC as shown in Fig. 3b. Also by changing the TLC solvent system

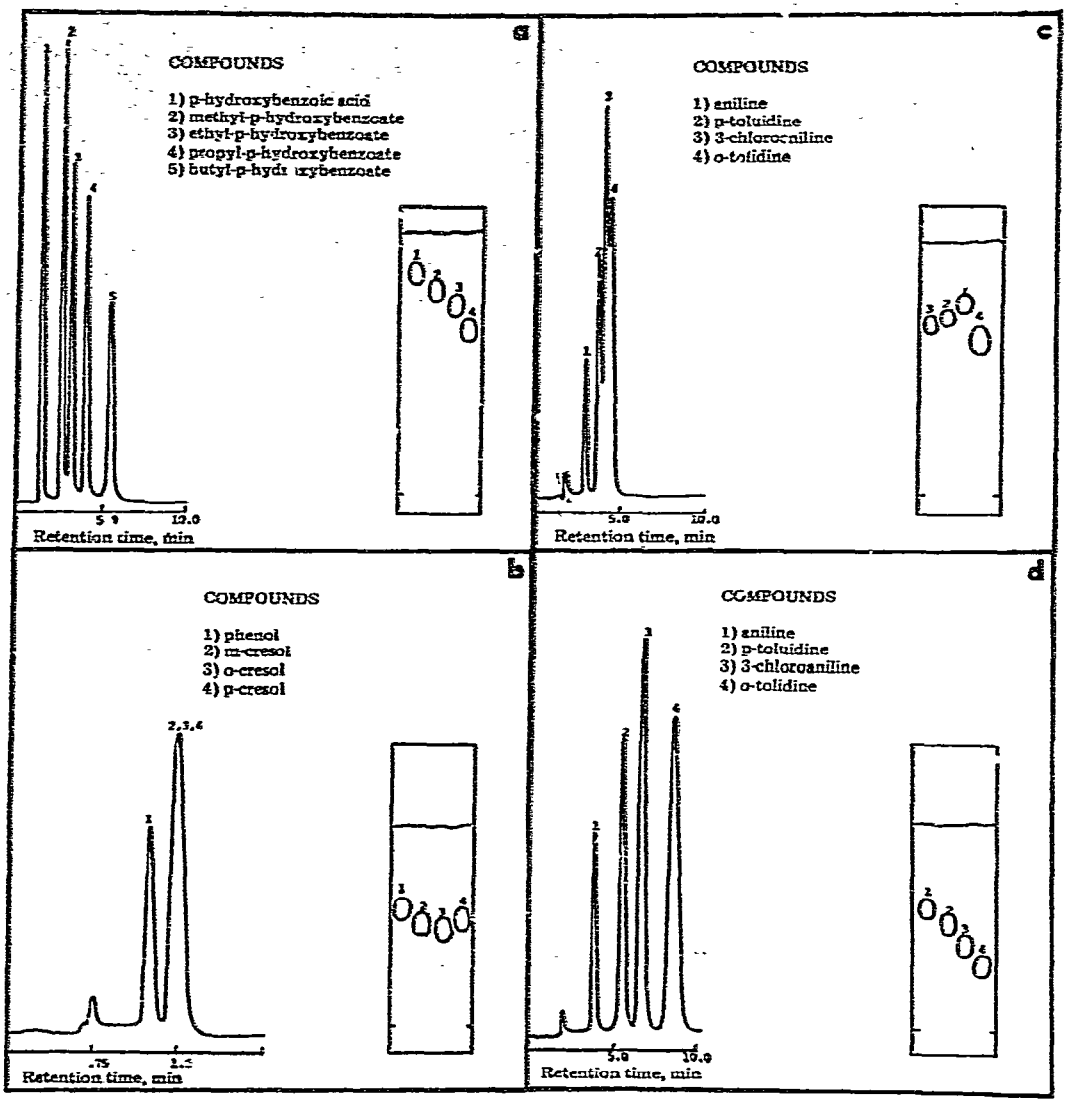

Fig. 3. Comparison between reversed-phase TLC plates (C_{12}) and similar reversed-phase HPLC packing (C_{18}). HPLC conditions: column, C_{18} on micro-silica (average particle diameter $\approx 10\,\mu$); mobile phase, (a) acetonitrile–0.01 *M* KH_2PO_4 (40:60), (b) and (c) acetonitrile–0.01 *M* $(NH_4)_2CO_3$ (40:60), (d) acetonitrile–0.01 *M* $(NH_4)_2CO_3$ (30:70). TLC conditions: plates, C_{12} on silica gel G; solvent system, same as listed above for HPLC.

composition resolution could be either enhanced or decreased; similar changes in the HPLC mobile phase composition resulted in improved or reduced compound separation as shown by comparing Figs. 3c and 3d.

In each comparative study, good agreement between column and TLC data was obtained; changes in compound type (*i.e.* acid or base) and mobile phase compositions resulted in concurrent changes in separation by both techniques. These

data make the use of chemically modified reversed-phase TLC plates an attractive means of initially investigating and designing a reversed-phase separation prior to the use of HPLC. Thus, one can quickly determine if all compounds will be eluted from the reversed-phase LC column and if a separation is easy or difficult by reversed-phase TLC without tying up HPLC equipment. In addition, the hydrophobic nature of the bonded layer offers added advantages and alternatives to the synthetic chemist who desires further analysis on very polar compounds which elute at very low R_F, are unresolved on regular silica gel plates, or are chemically labile under strongly acidic or basic adsorption TLC conditions.

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