CHARGE-TRANSFER CHROMATOGRAPHY OF AROMATIC HYDROCARBONS ON THIN LAYERS AND COLUMNS*

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Fractional distillation and crystallization, the historic mainstays of the organic chemist, have been supplemented in recent years by an array of chromatographic methods, each of which has extended the powers of the chemist to resolve a mixture into its pure components. There still continue to arise, however, troublesome separation problems unyielding to even these powerful techniques.

In the course of investigations involving the synthesis of certain hydroaromatic compounds from their corresponding polynuclear hydrocarbons by reduction with metals in liquid ammonia, the need arose for a method of analysis to determine the composition of the mixed hydrocarbon product. Although methods for the detection of aromatic substances by chromatography on paper, on acetylated paper[1], on plates covered with acetylated cellulose powder[2], and on thin layers of alumina or silica gel[3] have been reported, we were aware of no similar investigation concerning the analogous partially hydrogenated aromatic molecules. In view of the recognized advantages of thin layers of adsorbents for chromatographic separation in general (*e.g.*, speed, capacity, sharpness of spots), this technique was adopted for a preliminary screening of adsorbents and solvents. Results, however, proved disappointing; hydroaromatic compounds derived from phenanthrene, anthracene, chrysene, and benzanthracene were separated only poorly in the adsorbents, silica gel, alumina and Florisil employing hydrocarbon solvents. R_F values for silica gel, the best of these unsatisfactory adsorbents, are reproduced in Table I.

The wide variation of colors exhibited by the complexes of these hydrocarbons with 2,4,7-trinitrofluorenone (TNF), the reagent employed to spray the chromatograms, suggested a similar variation in the free energy of the charge-transfer complex formation. To test the possibility that adsorbents impregnated with this complexing agent could selectively retard the more strongly complexed components in a mixture, the same hydrocarbons were chromatographed on thin layers of silica gel impregnated uniformly with TNF, with striking results (Table I). The efficacy of the technique has subsequently been confirmed many times with other hydrocarbons and extended to preparative separations on columns and thick layers.

As an addition to the list of empirical methods for the chromatographic separation of a specific class of molecules this technique will undoubtedly prove valuable, but its significance, we believe, is considerably broader. The strength of complex formation is known to be dependent upon the structures of both acceptor and donor

TABLE I

R_F VALUES OF HYDROAROMATICS ON IMPREGNATED SILICA GEL

Solvent: benzene–heptane (1:4).

Hydrocarbon	$R_F \times 100$	
	Silica gel	5% TNF–S.G.*
Phenanthrene**	48	14
Dihydro-	49	18
Tetrahydro-	53	27
Hexahydro-	54	44
Octahydro-	55	54
Chrysene**	35	1
Dihydro-	36	16
Tetrahydro-	36	29
Hexahydro-	38	34
Anthracene**	45	15
Dihydro-	43	37
Tetrahydro-	50	43
Hexahydro-	53	51

* Prepared by method A.
** The reduced isomers are those with hydrogen: (i) at positions 9,10; 1,2,3,4; 1,2,3,4,9,10; and 1,2,3,4,4a,9,10,10a, respectively, for phenanthrene; (ii) at positions 5,6; 5,6,11,12; and 4b,5,6,10b,-11,12, respectively, for chrysene; (iii) at positions 9,10; 1,4,9,10; and 1,4,5,8,9,10, respectively, for anthracene.

molecules. For example, tetracyanoethylene (TCNE) is a π complexing agent which is especially effective for complexing substituted benzenes[4] as compared to naphthalene and higher homologs, while the converse is true of the larger acceptor TNF which forms strong complexes only with aromatic molecules having at least three rings[5]. It follows, in principle at least, that judicious choice of acceptors could provide adsorbents able to preferentially retain any desired donor molecule, either a π-donor (which includes alkenes and alkynes as well as aromatic hydrocarbons), or an n-donor (substances containing atoms with non-bonded electron pairs)[6,7]. Because of the potential usefulness of adsorbents with controllable powers of selective adsorption, we undertook to investigate the properties of acceptor-containing* adsorbents.

CHROMATOGRAPHY ON THIN LAYERS OF IMPREGNATED ADSORBENTS

A note by FRANCK-NEUMANN AND JÖSSANG[8] describes chromatography of several aromatic hydrocarbons on thin layers of silica gel containing trinitrobenzene. During the preparation of this manuscript there came also to our attention a paper by BERG AND LAM[9] relating empirical studies which partially overlap our investigation. Accordingly, we have omitted these aspects in the interest of minimum duplication.

Experimental

Two techniques were developed for the preparation of thin layers of adsorbents impregnated uniformly with an acceptor. Both furnish sharp spots and reproducible results.

* Impregnation with donors is limited by the fact that acceptors are generally bound the more tightly.

In the precoating method (A) a solution of the acceptor was substituted for water in the slurry employed to coat the plates. For example, 5 % TNF plates were produced from 90 ml of a solution of 10 g of TNF in 600 ml of benzene combined with 30 g of silica gel G, followed by activation at 100° for 1 h. In the postcoating, or immersion method (B), regular activated plates were dipped in a solution of the acceptor for a specific time period. Standard conditions employed to obtain the data for TNF and TNB* in Table II were 30 sec in a 2 % solution of the reagent in benzene followed by drying at 60° for 20 min. Although longer immersion time or greater concentration of the solution diminished R_F values below the useful range for our purpose, these simple methods for the variation of acceptor concentration may prove useful for other studies. Similar conditions were employed to prepare plates impregnated with picric acid, chloranil and 9-dicyanomethylene-2,4,7-trinitrofluorenone[10]. However, thin layers of silica gel impregnated with the latter two substances could not be heated without causing visible decomposition, and both proved to be exceptionally sensitive to light. Method B is the method of choice since it is the more rapid and convenient, and in addition it is applicable to the precoated plates and films (*e.g.* Eastman chromatogram sheets) on the market. An alternative method employing an ascending solution of the acceptor is not recommended for general use because of its lesser convenience and the lack of control of acceptor concentration.

Test solutions were prepared in benzene, the concentration of those hydrocarbons with fewer than 4 rings being 0.05 M and that of the higher molecular weight compounds being 0.025 M. Saturated solutions in tetrahydrofuran were employed for those compounds insufficiently soluble in benzene (*e.g.* chrysene, perylene and anthanthrene). Spots were applied with 1 μl pipets. Solvents were chemically pure laboratory reagents. Glass plates 20 × 20 cm and 5 × 20 cm were used, and development was carried out at room temperature by the ascending technique in a jar lined on three sides with filter papers. The distance traveled by the solvent front was 10 cm in all cases. Spots on ordinary plates were detected by spraying with an 0.5 % solution of TNF in acetone, and were viewed in both visible and ultraviolet light. Spraying was unnecessary with the impregnated plates. While colors were often distinctive, subjective interpretation varies considerably, and shades depend on such factors as concentration, impurities, presence of solvent and age.

Results and discussion

In order to demonstrate that our method represents chromatography on a charge-transfer acceptor and not merely chromatography of an undissociated complex formed at the origin, the individual 1:1 complexes of the phenanthrene series (Table I) were prepared and chromatographed on silica gel. In confirmation of the hypothesis, the resulting R_F values were essentially the same as those of the free hydrocarbons spotted on the same plate.

The retarding influence of TNF on the compounds in Table I is apparently felt most strongly by the more fully aromatic substances. Since many of these compounds are new**, quantitative data regarding their relative ease of participation in complex formation are not in the literature. However, qualitatively the magnitude of the decrease in observed R_F value correlates with expectation since complexes of TNF with

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* 1,3,5-Trinitrobenzene.
** Details of their synthesis will be reported separately.

aromatic compounds have been widely studied from organic[11,12], physical[13], and analytical[5,14] viewpoints and it is well established that the strength of complexation is a direct function of the extent of the conjugated system.

Comparison of the R_F values of a large variety of hydrocarbons on silica gel and on the same support impregnated with TNF (Table II) further strengthens the generalization that this acceptor retards elution of aromatic compounds with increasingly strong affinity for molecules with three or more rings. More detailed analysis of the data is aided by comparison of binding constants (B), defined in eqn. 1 (where $\Delta R_F = R_F$ on adsorbent alone $- R_F$ in the presence of complexing agent). This constant which provides a measure of an acceptor's efficiency for diminishing the distance traveled by a substance spotted on the plate is more useful than R_F values for comparison of the relative retardation of different molecules.

$$B = \frac{\Delta R_F \times 100}{R_F \text{ on adsorbent alone}} \tag{1}$$

Increasing values of B for the naphthalene, phenanthrene* and anthracene series are in the order unsubstituted < monomethyl < dimethyl (benzene–heptane as solvent) indicating that the relative strength of absorption is in the same order. This is in agreement with the known enhancement of donor properties of aromatic molecules by methyl substituents[6]. That the position of methyl substitution may override in importance the extent of substitution is evident in the data. Of particular interest are the binding constants of 12-methylbenz(a)anthracene and 1,4-dimethyl-phenanthrene, 89 and 56 respectively, which fall below those of benz(a)anthracene and phenanthrene, 93 and 53 respectively, probably as a consequence of the steric hindrance between a methyl group and a hydrogen atom which distorts these molecules from planarity sufficiently to weaken complex formation. Although the data are insufficient to assess more fully the importance of planarity and the effect of bulky substituents, non-planar molecules (triptycene as well as the two already cited) exhibit comparatively low binding constants suggesting a requirement for a flat non-hindered face for maximum adsorption.

In general, all the molecular structural features (maximum number of condensed aromatic rings, substitution by methyl groups, high degree of unsaturation, planarity) which contribute to the lowering of R_F values, are equally factors known to facilitate complex formation[6]. Tentatively, we conclude that R_F *values of aromatic hydrocarbons on TNF coated plates are inversely dependent upon the presence of structural features which enhance charge-transfer complexation.*

The superior electron-donating ability of methoxyl due to the presence of non-bonding electrons is apparently responsible for the low R_F value of 2-methoxy-naphthalene ($R_F = 0.24$ compared to $R_F = 0.54$ for 2-methylnaphthalene); however this effect is not reflected in the value of B: 13 for the 2-methoxy compound, 26 for the 2-methyl analog. Steric interference with complexation is likely since B for 2-ethyl-naphthalene is only 17 and the spatial requirements of methoxy and ethyl are quite similar. Further investigation is necessary to determine the validity of this explanation and to study the influence of other electron-donating substituents (RO-, NH_2-, HO-, etc.) on the mobility of the parent aromatic molecules.

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* The anomalous R_F value for 1,4-dimethylnaphthalene was rechecked.

TABLE II

BINDING CONSTANTS OF AROMATIC HYDROCARBONS[*] ON IMPREGNATED PLATES

		Benzene–heptane (1:4)			Carbon tetrachloride	
		R_F silica gel	B_{TNF}	B_{TNB}	R_F silica gel	B_{TNF}
1	Biphenyl	0.45	0	9	0.51	—2
2	Naphthalene	0.50	—2	24	0.53	4
3	1-Methyl-	0.50	8	28	0.52	10
4	2-Methyl-	0.54	26	33	0.54	15
5	1,4-Dimethyl-	0.55	35	40	0.54	20
6	2,3-Dimethyl-	0.53	38	40	0.53	17
7	2,6-Dimethyl-	0.53	36	38	0.53	23
8	2-Ethyl-	0.48	17	—	0.59	12
9	2-Methoxy-	0.24	13	33	0.24	8
10	Stilbene	0.40	23	23	0.45	20
11	Fluorene	0.46	24	24	0.46	22
12	Fluoranthene	0.43	88	67	0.45	73
13	Acenaphthylene	0.49	39	51	0.49	29
14	Phenanthrene	0.40	53	43	0 55	49
15	1-Methyl-	0.41	63	56	0.49	55
16	2-Methyl-	0.40	60	53	0.50	54
17	3-Methyl-	0.43	63	60	0.50	50
18	1,4-Dimethyl-	0.41	56	51	0.51	49
19	Anthracene	0.38	66	32	0.58	48
20	2-Methyl-	0.40	75	43	0.50	54
21	9-Methyl-	0.42	86	52	0.52	56
22	9,10-Dimethyl-	0.45	89	93	0.54	72
23	Retene	0.51	63	67	0.59	54
24	Benz(a)anthracene	0.42	93	74	0.49	88
25	1-Methyl	0.39	97	69	0.50	90
26	2-Methyl-	0.40	93	75	0.51	86
27	3-Methyl-	0.40	100	75	0.50	98
28	4-Methyl-	0.39	95	77	0.50	94
29	5-Methyl-	0.39	100	72	0.49	94
30	6-Methyl-	0.39	90	74	0.50	86
31	7-Methyl-	0.37	95	73	0.48	88
32	8-Methyl-	0.40	100	70	0.50	92
33	9-Methyl-	0.38	92	—	0.49	84
34	10-Methyl-	0.38	95	68	0.48	90
35	12-Methyl-	0.38	89	55	0.50	76
36	7,12-Dimethyl-	0.38	95	58	0.50	96
37	3-Methylcholanthrene	0.34	100	79	0.52	100
38	Chrysene	0.42	98	81	0.45	91
39	Triphenylene	0.42	90	86	0.48	90
40	Triptycene	0.28	25	21	0.40	5
41	Pyrene	0.51	84	75	0.57	77
42	3,4-Benzpyrene	0.42	98	90	0.50	100
43	Perylene	0.39	100	90	0.51	98
44	1,2,5,6-Dibenzanthracene	0.30	100	83	0.47	98
45	1,2,3,4-Dibenzanthracene	0.29	100	72	0.48	98
46	2,3,6,7-Dibenzanthracene[**]	—	—	—	—	—
47	1,2,7,8-Dibenzphenanthrene[**]	—	—	—	0.40	100
48	1,2,4,5-Dibenzpyrene	0.28	100	86	0.46	98
49	1,2,3,4-Dibenzpyrene	0.32	100	88	0.46	100
50	3,4,9,10-Dibenzpyrene	0.29	100	86	0.46	100
51	3,4,8,9-Dibenzpyrene	0.31	100	87	0.46	100
52	Anthanthrene	0.38	100	87	0.48	100
53	1,12-Benzperylene	0.36	100	86	0.47	98

[*] The more common compounds were obtained from commercial sources and the remainder were provided through the generosity of Prof. CHARLES HUGGINS from his extensive collection of carcinogens and related compounds.
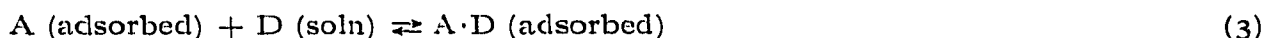
[**] Values uncertain due to extreme insolubility of the compound.

For the smaller acceptor TNB, similar trends are evident in the data in Table II with the difference that B is generally lower, and biphenyl and naphthalene, not retarded by TNF, are bound by TNB. Analogous data for chloranil* are not reported since measurements often furnished erratic results, possibly as a consequence of the sensitivity of these plates which darkened gradually on exposure to light or heat. Despite these problems it is clear that chloranil exhibits diminished preference for higher polycyclic systems compared to TNF or TNB; binding constants for biphenyl, naphthalene, acenaphthylene and phenanthrene were of the same order (approximately 20), with values for certain 4-ring molecules such as the monomethylbenz(a)-anthracenes and 3-methylcholanthrene only moderately higher (25–35).

While variation of solvent may conceivably be employed to alter drastically the binding constant of one compound relative to that of another, this was not observed. Substitution of carbon tetrachloride for benzene–heptane elevated the R_F values and depressed the binding constants on TNF-coated plates generally so that values of B maintained essentially unaltered relationships within the series.

For studies limited to the more soluble hydrocarbons (generally those with fewer than five rings) less polar solvents, such as cyclohexane or isooctane, provide a conveniently broad spread of R_F values.

Quantitative correlation of R_F data with published equilibrium constants for complex formation is hampered by variations in solvents and temperature and by the limited ranges of structural types for which constants have been determined. Attempts to extend existing data for a series of complexes of TNB in chloroform at 25 °[6] via the BENESI–HILDEBRAND procedure[7,15] were frustrated by the low solubility of the higher molecular weight complexes. However, a plot of the available data furnishes an essentially linear relation (Fig. 1). Further study may well disclose agreement less ideal than this appears in view of the fundamental differences between the normal charge-transfer equilibria (eqn. 2), involving dissociation of a complex in solution, and the adsorption equilibria (eqn. 3) presumably operative during chromatographic separations. Various factors difficult to estimate ($e.g.$, altered electron affinity and steric accessibility of the acceptor and the possible importance of relative solubility in the adsorption–desorption process) may be involved.

$$\text{A (soln)} + \text{D (soln)} \rightleftharpoons \text{A·D (soln)} \qquad (2)$$

$$\text{A (adsorbed)} + \text{D (soln)} \rightleftharpoons \text{A·D (adsorbed)} \qquad (3)$$

$$\text{A·D (liq)} \rightleftharpoons \text{A·D (solid)} \qquad (4)$$

Stabilities of crystalline molecular compounds have been correlated directly with melting point by ORCHIN[16]. However, the phase equilibrium (eqn. 4) differs considerably from the adsorption equilibrium (eqn. 3) so that it is hardly surprising to find only a rough correlation between B and the melting points of TNF–hydrocarbon complexes (Fig. 1). Least satisfactory were plots of either the energies for the first π–π* transition of donor hydrocarbons or the charge-transfer transition energies

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* The low solubility of chloranil in benzene and its ready extraction from the plates by the standard solvents (Table II) necessitated the use of tetrahydrofuran–benzene for the preparation of the plates by method B and isooctane for their development.

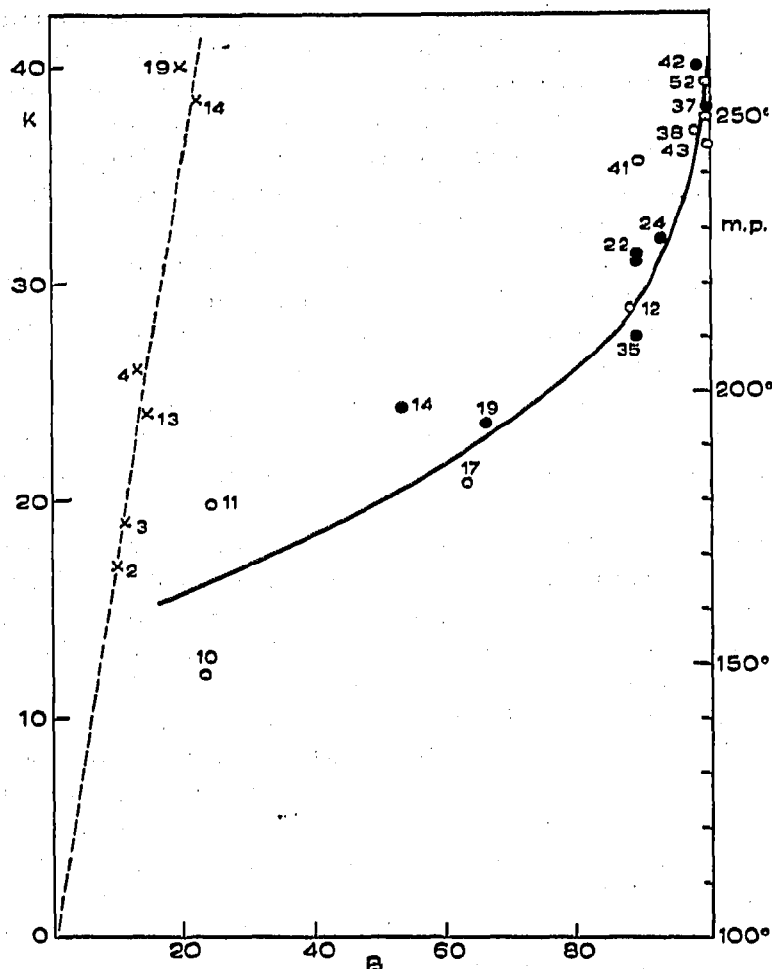($E\pi$) of their TNF complexes[13] vs. B, both of which provided an apparent random scattering of points.

Fig. 1. Relation of binding constants (B) to the equilibrium constants (K) for complex formation with TNB in chloroform at 25° (ref. 6) (— — —), and to the melting points for a series of complexes with TNF (————). (O) reported[12]; (●) found for complexes prepared in our laboratory. Superscript numbers refer to compounds in Table II.

CHROMATOGRAPHY ON THICK LAYERS OF IMPREGNATED SILICA GEL

Search of the literature disclosed no reference to the preparative use of acceptor-impregnated adsorbents.

The convenience of the immersion method for the preparation of thin layers led us to attempt to adapt this technique to thicker layers. Uniform, ridge-free adsorbent layers, 2 mm thick, were prepared by means of the multi-thickness applicator supplied by the Kensington Scientific Co. using a slurry of 60 g silica gel G in 120 ml of water. Plates were air dried at room temperature overnight, and baked in an oven at 110° for 2 h. These plates, after immersion in a 5 % solution of TNB in benzene for as long as 16 h, followed by drying failed to separate adequately hydro-aromatic derivatives of phenanthrene or biphenyl (60–100 mg in chloroform or ben-

zene, applied by streaking or spotting) on development in cyclohexane. Judging by color differences evident in a cross section of the layer, the acceptor had not penetrated much below the surface. Attempts to impregnate similarly prepared plates by an ascending technique with a 5 % solution of TNB in benzene (16 h) provided even less satisfactory results. However, a modification of method A solved the problem. Layers (2 mm) of silica gel G containing TNB were prepared using a slurry of 60 g adsorbent in a solution of 6 g TNB in 120 ml methanol. The plates were dried in air for 2 h and under an infrared lamp for several more hours. Reduced derivatives of phenanthrene and biphenyl, applied as before, were separated into sharp bands by development in cyclohexane. In order to recover the pure compounds, the colored bands were scraped off the plate and transferred to the top of a small column of neutral alumina, activity I; elution with 20–50 % ether in light petroleum ether furnished the hydrocarbons free of TNB.

CHROMATOGRAPHY ON COLUMNS

Although column chromatography utilizing the principle of charge-transfer bonding has some precedent, the method dating from the report by GOLDEWICZ[17] in 1949 that columns of silica gel impregnated with TNB as an "indicator" served for the gross separation of crude hydrocarbon mixtures into saturated, hydroaromatic, aromatic, and resinous fractions, has been little exploited. The only mention in the intervening years is the use of columns of silicic acid impregnated with picric acid or TNF for separation of cycloalkenylnaphthalene, anthracene and phenanthrene reported by KLEMM et al.[18], and a brief note by BUU-HOI AND JACQUIGNON[19] on the use of columns of tetrachlorophthalic anhydride and tetrachlorophthalimide.

The major value of preparative charge-transfer chromatography lies in its ability to resolve mixtures of very closely related molecules. In our hands columns of pure TNF were as ineffective as silica gel or Florisil alone or distillation using a 6 ft. Podbielniak Heliband column for the separation of hydroaromatic compounds in the phenanthrene series; however, a column of silica gel containing 5 % TNF cleanly separated a mixture of tetra-, hexa- and octa-hydrophenanthrene.

The gentle conditions combined with the clean separations possible render the method suitable for isolation of individual products furnished by metal–ammonia reduction of polycyclic aromatic molecules. For example, reduction of 9,10-dihydroanthracene in tetrahydrofuran–ammonia with 2 equivalents of lithium at —33° led to a mixture of 1,4,9,10-tetrahydro- and 1,4,5,8,9,10-hexahydroanthracene in addition to unreacted dihydro compound. Purification by recrystallization was unsatisfactory due to a strong tendency towards co-crystallization. Chromatography of 1 g of the mixture on 400 g of a 10 % TNB–silica gel column separated the mixture which moved down the column on elution with 10–20 % benzene in hexane as individual brightly colored bands, and emerged as colorless solutions.

Treatment of the adsorbent with a solution of the acceptor may take place before or after its introduction into the column. The former procedure has the advantage that a more polar solvent may be employed to dissolve the nitroaromatic compounds which are relatively insoluble in hydroaromatic solvents. For example, activated silica gel (400 g) was treated with a solution of 40 g TNB in 400 ml acetone, and dried first under a heat lamp, and then in a desiccator at oil pump pressure.

Alternatively, a solution of TNB (52 g) in the minimum quantity of warm toluene was poured on the top of a column of silica gel (480 g) in heptane kept warm by means of an external heating tape to prevent crystallization. It was washed in with 200 ml of warm toluene–heptane (1:4), followed by heptane. The heat source was removed, and washing was continued with hexane until no more toluene could be detected in the effluent. The performance of both types of column was comparable. For efficient separations relatively high ratios of acceptor to adsorbent and of impregnated adsorbent to sample to be separated are necessary, as indicated in the example cited.

SUMMARY

    1. Thin layers of silica gel impregnated with acceptors of the charge-transfer type effectively separate a wide variety of aromatic substances, including many of their closely related partially reduced derivatives.

    2. The ratio of the difference between R_F values on acceptor-containing and ordinary plates to the R_F value on the latter $\times$ 100 is termed the binding constant (B). For a series of aromatic hydrocarbons, values of B appear to be directly related to the presence of structural features which contribute to the ability of these compounds to function as charge-transfer donors.

    3. Preparative chromatography using thick layers or columns of impregnated silica gel effectively separates aromatic and hydroaromatic substances of closely related structure.

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