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STANDARDIZATION AND DETERMINATION OF THE SELECTIVITY OF OCTADECYLSILYLSILICA IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Net retention volumes per gram of octadecylsilylsilica have been measured for a large number of mono- and disubstituted benzene derivatives, some monosubstituted hexanes and cyclohexanes and a number of polycyclic aromatic compounds, using *n*-hexane, methylene chloride and a mixture of both solvents as the eluent at 25° and 43.5°.

The retention data are interpreted in terms of the semi-empirical adsorption model, developed by Snyder for bare adsorbents. The effects of adsorbent deactivation, temperature, solute and eluent localization, change of the charge distribution in the solute molecule and adsorption mode of the solute on retention and selectivity are discussed quantitatively.

INTRODUCTION

Chemically modified silicas have been known for many years and have found widespread application in high-performance liquid chromatography (HPLC) and to a smaller extent also in gas chromatography. As the organic layer is chemically bound to the silica, the choice of the chromatographic conditions (temperature and eluent) is not restricted by the stability of a two-phase system. Owing to the loose structure of the bonded phase, the radial mass transfer of solutes is fast in these adsorbents and relatively short analysis times can therefore be achieved. The linear capacity of bonded phases is usually larger than that of bare silicas. As a result of the deactivation by bound organic groups, deactivation by water can be omitted in many instances, which improves the reproducibility of retention data.

Only a few authors have dealt with the retention mechanism in HPLC columns packed with these adsorbents and flushed with apolar or moderately polar eluents. Kirkland[1] examined a polymeric "ether"-bonded phase and concluded that partition is the prime retention mechanism. He suggested that polymeric bonded phases can swell in eluents, if their polarity matches that of the bound layer. Horgan and Little[2] found about equal capacity ratios (k') for equal amounts of chemically bound and conventionally coated Carbowax on silica. Grushka and Kikta[3] and Kikta and Grushka[4] investigated the retention behaviour of some alkyl-bonded phases on silica.

When *n*-hexane is used as the eluent, adsorption of the solute to free silanol groups appears to be important. The decrease in k' with increasing chain length of the alkyl substituent in alkylphenones was ascribed to hindrance of adsorption of the phenones to the silica surface by the bonded groups. Recently, similar retention behaviour on octadecyl silica has been established in gas chromatographic columns[5].

In this paper, the adsorbent properties of octadecylsilylsilica (ODS-silica) is considered in detail. The retention data of a large number of substituted benzenes, hexanes and cyclohexanes and some polycyclic aromatic compounds are interpreted in terms of the semi-empirical adsorption model of Snyder[6]. Actually, this model has been developed to describe adsorption to bare adsorbents, but it is worthwhile examining whether it is also appropriate for characterizing the adsorptive properties of monomeric bonded-phase adsorbents.

THEORETICAL

Snyder[6] assumed that only the first monolayer of eluent is adsorbed at the adsorbent surface and that one type of interaction force plays a dominant role in adsorption. On adsorption of one solute molecule a number of eluent molecules are desorbed, and each of these processes can thus be represented by a single energy term. On polar adsorbents solute adsorption from poorly solvating eluents is mainly controlled by Lewis acid–base interactions[7], whereas London, Debye and Keesom interaction forces are about equal in the eluent bulk and at the adsorbent, and can be neglected. The basic equation of Snyder relates the net retention volume per gram adsorbent (V_N/W) to the electron donor or acceptor strength of the solute and of the eluent with respect to the adsorbent:

$$\log (V_N/W) = \log V_a - \alpha(S^0 - \varepsilon^0 A_s) \tag{1}$$

where V_a is the volume of the adsorbed monolayer of eluent per gram of adsorbent, α is the adsorbent activity ($\alpha = 1$ for a water-free standard adsorbent), S^0 and ε^0 are measures of the Lewis acid or base strength of the solute and the eluent, respectively ($\varepsilon^0 = 0$ for alkanes), and A_s is the surface area of the solute molecule. A_s can be calculated from Van der Waals radii in some instances, but can more appropriately be determined experimentally by ascribing arbitrarily a value of 6 to A_s for benzene. As V_a can be calculated from the BET specific surface area of the adsorbent, assuming a monolayer thickness of 3.5 Å, and S^0 of benzene can be obtained on using *n*-pentane as the eluent, the ε^0 value of any moderately polar eluent can be obtained.

In the following we shall briefly elucidate the origin of the contributions that determine the magnitude of S^0 and A_s. According to Snyder, S^0 is given by the equation:

$$S^0 = \sum_i Q_i^0 - \beta \, f(Q_k^0) \sum_{i \neq k} Q_i^v + q_{ik}^0 \tag{2}$$

The first term of the right-hand side of this equation implies that group (or atom) i of the solute molecule provides a contribution Q_i^0 to S^0. It is clear that this additivity concept cannot be applied to solute groups, the flat adsorption of which is impossible

for steric.reasons. Further, the additivity of Q_i^0 presupposes that the adsorbent surface is energetically homogeneous. However, McDonald[8] pointed out that on a regular silica surface the distance between adjacent silanol groups is about 5 Å, *i.e.*, comparable to the molecular dimensions of the adsorbate. Hence it is impossible for all solute groups to adsorb to active sites. Finally, it is well known that the electron density of a substituent at a phenyl nucleus depends on the electron-withdrawing or -repelling action of other substituents. As S^0 represents a measure of Lewis acid–base interactions, these intramolecular electronic effects will significantly affect its magnitude. These deviations from the additivity rule are accounted for by the two correction terms in eqn. 2.

Suppose that we are dealing with a *m*- or *p*-disubstituted benzene with groups i and k (denoted by i⌀k) and that group k is most strongly adsorbed ($Q_k^0 > Q_\varnothing^0$ and $> Q_i^0$). Then group k will be situated (localized) upon a silanol group. The extent of localization of group k will increase with increasing Q_k^0 and is given by the localization function $f(Q_k^0)$. This means that the –⌀k moiety and the solute ⌀k are adsorbed equally strongly, but that group i in i⌀k is less strongly adsorbed than group i in io. Hence, the correction for solute localization is proportional to $-f(Q_k^0)Q_i^0$. The constant β in eqn. 2 has been set equal to 1 for alumina, but its magnitude should be smaller for silica where the mobility of localized groups is larger than on alumina. This is associated with the fact that the free silanol group can rotate, whereas the alumina sites are partly "buried" aluminium atoms. It is assumed that $f(Q_k^0)$ values obtained on alumina are applicable on silica.

The contribution of intramolecular electronic effects to S^0, represented by the term q_{ik}^0 in eqn. 2, will be explained for substituted anilines. As the adsorption is controlled by Lewis acid–base interaction forces and the amine group has a strong electron-donor ability towards free or reactive silanol groups, an electron-withdrawing substituent will reduce the magnitude of q_{ik}^0. Conversely, q_{ik}^0 increases if the substituent increases the charge density on the amine group. The electron-withdrawing action of substituents can be described by means of Hammett σ constants[9]. These σ constants are defined by means of the dissociation constants of substituted benzoic acids: $\log (K_i/K_{i=H}) = \varrho\sigma_i$, wherein the parameter ϱ is a measure of the susceptibility of the reactive group (here the carboxyl group) for the induced charge shift effected by the substituent i. The magnitude of ϱ depends on the reactive group k, the solvent and, slightly, on temperature. The analogue of the Hammett relationship for the adsorption of a series of solutes which have group k in common ($Q_k^0 > Q_i^0$) from an eluent E is given by the relationship   :

$$q_{ik}^0 = \varrho_k(E)\,\sigma_i \tag{3}$$

ϱ_k will be positive if group k behaves like a Lewis acid and negative if group k behaves like a Lewis base towards the adsorbent sites. Eqn. 3 has been applied by Snyder to pyridines[10] and phenols[11] on alumina and to pyridines on silica[12].

As mentioned above, the A_s value can be estimated from the contributions of the solute groups a_i, which can be calculated from Van der Waals radii (enlarged by 0.5 Å). These data are about equal to experimental A_s data on alumina: A_s(exp., $Al_2O_3) \approx A_s$(calc.) $= \sum_i a_i$(calc.). On silica, however, the a_k values of strongly adsorbed (localized) solute groups appear to be much larger than expected. Snyder[13]

pointed out that the discrepancies between the experimental and calculated A_s values are about proportional to the delocalization function $f(Q_k^0)$:

$$A_s(SiO_2) = \sum_i a_i(\text{exp.}) = \sum_i a_i(\text{calc.}) + \Delta a_k \approx \sum_i a_i(\text{calc.}) + 16f(Q_k^0) \qquad (4)$$

These anomalous A_s values on silica are a result of the adsorbent heterogeneity and the accessibility of the silanol group for solute and solvent localization. The eluent molecules are more strongly adsorbed around strong sites. As group k is preferably localized near free silanol groups[13], eluent molecules have to be desorbed which show a larger effective eluent strength than that of average eluent molecules. The ε^0 values given by Snyder[6] have been determined from retention data of non-localized solutes and therefore these ε^0 values represent an average eluent strength. On that account, the anomalously large $\varepsilon^0 a_k$ value obtained for a localized group k has to be accommodated into the Δa_k term in eqn. 4. The Δa_k values appear to decrease with decreasing water content at the silica surface and become about zero for water-free silica. Apparently, localization is more important at water deactivated silicas. Obviously, the water molecules deactivate the strongly reactive, hydrogen bond-linked silanol groups (which give no localization) first and force the solute molecules to be adsorbed and localized at the isolated free silanol groups. Hence, for adsorbents of the silica type it holds generally that

$$\Delta a_k = \gamma \, \Delta a_k(SiO_2) \qquad (5)$$

where $\Delta a_k(SiO_2)$ represents the increments, holding for silica with 16% water, that have been given by Snyder[6].

From the foregoing it is clear that the characterization of a chemically modified silica requires the evaluation of the parameters $\log V_a$, α, β and γ. The magnitude of all of them may be affected by the presence of the bonded phase. Further, the value of ϱ depends both on the properties of the adsorbent and of the series of solutes investigated. Finally, selective interactions between the solutes and the functional group(s) at the bonded phase should be examined.

The parameters α and $\log V_a$ have been obtained by plotting $\log (V_N/W)$ data of monosubstituted benzenes and chlorobenzenes eluted with n-hexane ($\varepsilon^0 = 0$) against $\sum_i Q_i^0$ values on bare silica, given by Snyder[6].

The parameter γ can be obtained from $\log V_N$ data determined with the eluent n-hexane (H) and some moderately polar eluent E:

$$\{\log[V_N(H)/V_N(E)] - \alpha[q_{ik}^0(H) - q_{ik}^0(E)]\}/\alpha\varepsilon_E^0 = A_s = \sum_i a_i(\text{calc.}) + \gamma\sum_i \Delta a_i(SiO_2) \qquad (6)$$

using ε_E^0, $a_i(\text{calc.})$ and $\Delta a_i(SiO_2)$ values on bare silica given by Snyder[6], and provided that the term $q_{ik}^0(H) - q_{ik}^0(E)$ can be neglected. Eqn. 6 accounts for the localization of m- and p-disubstituted benzenes and is based on an extension of the argument given above for monosubstituted compounds (see eqn. 4).

From eqn. 2, it is evident that β can not be calculated unless ϱ is known. This implies that β and ϱ can be obtained only for series of solutes that have a group k

in common ($Q_k^0 \geqslant Q_i^0$), such as phenols, anilines, pyridines and benzaldehydes. From eqns. 1–3 it follows that the required equation to obtain both β and ϱ is

$$\log[V_N(i\varnothing k)/V_N(\varnothing k)]_E/\alpha - (Q_i^0 - \varepsilon_E^0 a_i) = -\beta f(Q_k^0)Q_i^0 + \varrho_k(E)\sigma_i \qquad (7)$$

A better fit is obtained when a parameter δ is added to the right-hand side of this equation and three-parameter regression analysis is applied.

All relationships given so far are based on the assumption that the solutes examined show flat adsorption at the silica surface beneath the bonded groups. This assumption is reasonable in connection with the rather low surface concentration of the bonded groups, but may be erroneous for large, flat molecules such as polycyclic aromatic compounds. Any restriction of flat adsorption by the presence of the bonded phase can easily be established by comparing experimental S^0 data from the equation

$$S^0 = \frac{1}{\alpha} \log(V_N/WV_a) \qquad (8)$$

holding for the eluent n-hexane, with corresponding data on bare silica. The mode of adsorption of monosubstituted hexanes and cyclohexanes cannot be examined by comparing S^0 values because the contribution of the (cyclo)hexyl increment to S^0 is zero. Hence, the number of adsorbed carbon atoms, m, must be determined from the experimental A_s values of these solutes:

$$A_s = a_i(\text{calc.}) + \gamma \, \Delta a_i(\text{SiO}_2) + m a_{\text{CH}_2} \qquad (9)$$

Comparison of the values of m on the modified and bare silica gives an indication about the restriction of adsorption of the (cyclo)alkyl moiety by the ODS chains.

EXPERIMENTAL

Preparation of octadecylsilylsilica
   A 5-g amount of Merckosorb Si 60 (E. Merck, Darmstadt, G.F.R.) with an average particle size of about 10 μm was refluxed with 2 N hydrochloric acid for 2 h. The activated adsorbent was washed until chloride-free and dried at 150° and 1 mmHg for 12 h in a three-necked flask. The adsorbent was covered with 50 ml of dried and freshly distilled tetrahydrofuran (THF) and the flask was equipped with a cooler, a dropping funnel and an inlet for dry nitrogen to be bubbled through the silica suspension. The reagent [5 g of octadecyltrichlorosilane (Aldrich, Milwaukee, Wisc., U.S.A.) dissolved in 25 ml of THF] was added dropwise while the flask was gently swirled. The reaction mixture was allowed to reflux at 50° for 8 h and was kept at room temperature overnight. The product was rinsed with THF and extracted for 1 h in a Soxhlet apparatus with n-hexane and methanol. Finally, the ODS-silica was dried at 100° and 1 mmHg for 6 h.

Adsorbent characterization
   The specific surface area and the pore-size distribution of the bare silica and the ODS-silica were obtained by the BET technique after Broekhoff and Linsen[14].

The specific surface areas of both adsorbents were identical within experimental error (316 ± 2 and 313 ± 2 m²/g, respectively) but deviated from that stated by the supplier (500 m²/g). The pore volume of the silica (0.79 ml/g) was in accord with that given by the supplier (0.75 ml/g) but was larger than that of ODS-silica (0.60 ml/g). The mean pore diameter was about 100 Å for Merckosorb Si 60 (60 Å according to the supplier) and about 75 Å for the ODS-modified adsorbent.

The elemental analysis of the ODS-silica showed 11.2 wt. % of carbon, which corresponds to a surface coverage of 1.64 μmole/m². From these data it follows that the bonded phase was mainly situated within the pores of the silica particles. The average mutual distance of the ODS chains was about 11 Å.

Chemicals

All solutes (obtained from Fluka, Buchs, Switzerland) had the highest available purity and were used as received. The solvents n-hexane and methylene chloride were supplied by Baker (Delft, The Netherlands) and were dried with molecular sieve 5A before use.

Apparatus and procedure

The home-made apparatus was equipped with an Orlita diaphragm pump (DMP 1515/3), two pulse dampeners in series (Bourdon gauges and capillary resistances), a four-port Valco liquid sample valve with a 3-μl sample loop and a Coleman M55 UV–visible spectrophotometer. The substituted hexanes and cyclohexanes were detected with a Siemens refractive index detector. The column (precision-bore stainless steel, length 25 cm, I.D. 2.1 mm) was surrounded by a water-jacket and thermostated by a circulation pump to within 0.05°. The eluent flow-rate was monitored continuously with a (calibrated) siphon counter (Waters Assoc., Milford, Mass., U.S.A.). The column was packed by the viscosity packing method described by Asshauer and Halász[15]. The slurry (15 wt. % ODS silica in cyclohexanol saturated with water) was degassed and homogenized by ultrasonic treatment and forced into the column by n-hexane at 200 atm. Finally, 200 ml each of n-hexane, absolute methanol and dry methylene chloride were flushed through the column. The weight of ODS-silica in the column was 0.51 g. The HETP of an unretained solute was about 0.3 mm at a linear flow-rate of 1 cm/sec using n-hexane as the eluent.

The solute sample size was about 3 μg. Retention volumes were corrected for eluent hold-up in the column and dead volumes by means of the retention volume of n-pentane. All measurements were made in at least triplicate. The reproducibility of the retention volume was about 10 μl except for strongly adsorbed solutes (for which it was about 1%). The decrease in the precision was probably due to minute traces of water in the eluent. Hence, the activity of the column packing was monitored occasionally by means of the retention data of some polar solutes. Strongly adsorbed solutes showed slightly tailed peaks even with small sample sizes. The influence of isotherm curvature on the net retention volume appeared to be about 5% for α-naphthylamine. It will be shown that these random and systematic experimental errors are small compared with the inaccuracies in the adsorption model to be applied. The following eluents were used: n-hexane (H, $\varepsilon^0 = 0$), n-hexane + methylene chloride (65 : 35) (B, $\varepsilon^0 = 0.22$) and methylene chloride (C, $\varepsilon^0 = 0.32$). The ε^0 value of methylene chloride was as given by Snyder[13], whereas that of the binary eluent was

obtained by interpolation of experimental data of a series of n-pentane–methylene chloride mixtures[13].

RESULTS AND DISCUSSION

Adsorbent activity

Experimental log (V_N/W) data of monosubstituted benzenes and chlorobenzenes are given in Table I. From Q_i^0 values, given in Table II, S^0 values were calculated. A typical plot of log (V_N/W) versus S^0 is presented in Fig. 1.

TABLE I *

LOG (V_N/W) DATA FOR MONOSUBSTITUTED (CHLORO)BENZENES IN n-HEXANE (H, $\varepsilon^0 = 0.0$) AND n-HEXANE–METHYLENE CHLORIDE (65:35) (B, $\varepsilon^0 = 0.22$)

i	No.	∅		Cl∅			∅		Cl∅		
		H, 25°	B, 25°		H, 25°	B, 25°	H, 43.5°	B, 43.5°		H, 43.5°	B, 43.5°
H	1	−0.23 *			−0.36 *		−0.30 *			−0.43 *	
F	2	−0.31 *		m	−0.45 *		−0.44 *		m	−0.46 *	
				p	−0.43 *				p	−0.46 *	
Cl	3	−0.36 *		m	−0.38 *		−0.43 *		m	−0.41 *	
				p	−0.47 *				p	−0.51 *	
Br	4	−0.30 *		m	−0.39 *		−0.39 *		m	−0.44 *	
				p	−0.43 *				p	−0.48 *	
CH₃	5	−0.18 *		m	−0.28 *		−0.27 *		m	−0.30 *	
				p	−0.39 *				p	−0.44 *	
SCH₃	6	0.35	−0.76				0.41	−0.81			
OCH₃	7	0.69	−0.28	m	0.55	−0.60	0.70	−0.37	m	0.39	−0.74
				p	0.52	−0.50			p	0.49	−0.59
NO₂	8	1.05	−0.26	m	0.89	−0.35	0.97	−0.26	m	0.79	−0.53
				p	0.93	−0.43			p	0.83	−0.48
CN	9	1.45	0.16	m	1.30	0.02	1.36	0.03	m	1.20	−0.08
				p	1.36	0.13			p	1.26	−0.01
CHO	10	1.54	0.43	m	1.32	0.20	1.49	0.28	m	1.23	0.09
				p	1.44	0.36			p	1.36	0.23
CO₂CH₃	11	1.66	0.33	p		0.61	1.43	0.27	p		0.45
COCH₃	12		0.72	p	2.05	0.60	1.94	0.59	p	1.79	0.52

* V_N/W data are very small.

As the data for the benzenes and chlorobenzenes can be described with values of α and log V_a which are identical within experimental error, both series were combined:

$$\alpha(25°) = 0.51 \pm 0.01, \quad \alpha(43.5°) = 0.48 \pm 0.01;$$
$$\log V_a = -1.03 \pm 0.03 \text{ at } 25° \text{ and } 43.5°.$$

In Fig. 1 the data points 8 [nitro(chloro)benzenes] and 11 (methylbenzoate) deviate significantly from the line. The average deviations, ΔS^0, are 0.25 ± 0.06 (nitro group) and -0.21 ± 0.18 (ester group). As the data on the other solutes indicate an accuracy of S^0 of 0.03, these figures cast some doubt about the reliability of the Q_i^0 values for the aromatic nitro and ester groups given in Table II. However, neglecting these data points did not significantly influence the magnitude of the α and log V_a values given above.

TABLE II

Q_i^0, $f(Q_i^0)$, σ_i, CALCULATED AND EXPERIMENTAL a_i VALUES OF SUBSTITUENT GROUPS i AT A PHENYL NUCLEUS, AND Q_i^0 AND a_i VALUES OF SUBSTITUENT GROUPS IN AN ALIPHATIC COMPOUND

i	$Q_i^0(\mathscr{Z}-)$ [*]	$F(Q_i^0)$ [**]	σ_i [***]		$a_i(calc.)$ [*]	$a_i(exp.)$ [*]	$Q_i^0(R-)$ [*]	$a_i(calc.)$ [*]	$a_i(exp.)$ [*]
CH$_3$	0.11	0	m	−0.07	0.8	0.8	0.07	1.6	1.6
			p	−0.17					
CH$_2$	0.07	0			0.9	0.9	−0.05	0.9	0.9
CH=	0.25	0			1.0	1.0	(0.25)		
F	−0.15	0	m	0.34	0.4	0.4	1.54	1.2	1.2
			p	0.06					
Cl	−0.20	0	m	0.37	0.7	0.7	1.74	1.5	1.2
			p	0.23					
Br	−0.17	0	m	0.39	1.0	1.0	1.94	1.8	1.8
			p	0.23					
SH	0.67	0	m	0.25	0.9	0.9	1.70	1.8	3.3
			p	0.15					
SCH$_3$	1.29	0.02	m	0.15	1.7	3.2	2.94	2.6	7.4
			p	0.00					
OCH$_3$	1.83	0.13	m	0.12	1.1	4.6	3.61	2.1	9.0
			p	−0.27					
NO$_2$	2.77	0.30	m	0.71	1.3	7.5	5.71	2.3	9.5
			p	0.78					
CN	3.33	0.40	m	0.56	0.6	8.4	5.27	1.5	8.7
			p	0.66					
CHO	3.48	0.42	m	0.36[s]	1.1	8.3	4.97	2.0	9.2
			p	0.43[s]					
CO$_2$CH$_3$	3.45	0.42	m	0.37	2.3	8.1	5.27	3.2	10.5
			p	0.45					
COCH$_3$	4.69	0.45	m	0.38	1.5	9.2	5.27	2.6	9.8
			p	0.50					
OH	4.20	0.45	m	0.12	0.4	7.6	5.60	1.3	8.5
			p	−0.37					
NH$_2$	5.1	0.45	m	−0.16	0.6	8.7	8.0	1.5	8.7
			p	−0.66					
Pyridyl	6.1[ss]	0.45							

[*] Experimental data given by Snyder[6].
[**] Interpolated data from smoothed values[6].
[***] Data given by Ritchie and Sager[16].
[s] Data given by Humffray et al.[17].
[ss] Experimental value given by Snyder[12].

The activity of ODS-silica is small compared with that of bare water-free silicas with the same specific surface area (like Davison Code 62 silica) where $\alpha \approx 0.83$ (ref.6). The large decrease in α indicates that the silane reacts preferably with the strongest sites, i.e., with reactive silanol groups. As a wide-pore silica such as Merckosorb Si 60 has about 8 μmole/m^2 of silanol groups[8] and as only about 5% of these are reactive silanol groups[18], it can be concluded that at a surface concentration of 1.64 μmole/m^2 of ODS groups only free silanol sites are available for adsorption. It is interesting to note that, even at low surface concentrations, adsorbent deactivation by silylation is far more effective than deactivation by adsorption of a monolayer of water.

The decrease in α with increasing temperature is barely significant (dα/d$T \approx$
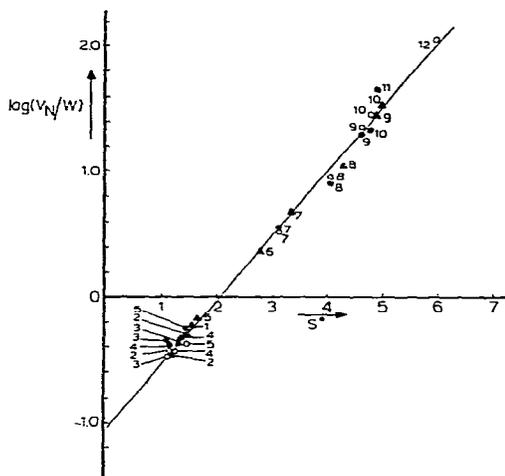
Fig. 1. Experimental log (V_N/W) data for monosubstituted benzenes (▲) and *m*- and *p*-substituted chlorobenzenes (●,○) on ODS-silica *versus* S^0. Eluent: *n*-hexane (25°). Numbering of data points according to Table I.

—16·10^{-4}/°K). Therefore, the standard partial molar entropy of adsorption is small (—0.14 S^0 cal/mole·/°K) and the adsorption process is mainly controlled by enthalpic interaction forces.

The experimental V_a value corresponds approximately to the volume of a monolayer of adsorbed eluent between the ODS bristles.

Eluent localization

For phenols, nitrobenzenes, benzaldehydes, anilines and pyridines it seems to be preferable to estimate β, γ and ϱ simultaneously by applying multiple regression analysis. However, an attempt to do so failed because of the correlation of Δa_i and Q_i^0. Another approach would be to estimate γ and ϱ simultaneously from log (V_N/W) data in two eluents, using the assumption that β is independent of the eluent strength and that γ is independent of the type of substituent group i at the phenyl nucleus. However, the simplest approach is to estimate γ from log (V_N/W) data for monosubstituted benzenes and to assume that γ is independent of the number of substituent groups. The γ values can be calculated straightforwardly by means of eqn. 6, because $q_{ik}^0 = 0$ for these compounds. The results are given in Table III. A variance analysis shows that γ is independent of the temperature and substituent group i. The mean value $\gamma = 0.61 \pm 0.05$ has been used in further calculations. From this large value it can be concluded that the presence of ODS groups on the silica surface promotes the phenomenon of eluent localization, as does water, when adsorbed at a bare silica.

Solute localization

This phenomenon has not previously been examined systematically on silica. Although there is some indication for solute localization on silica[13], its contribution will be smaller than on alumina for reasons given in the theoretical section. Whether β and ϱ depend on the eluent strength has also not been established on silica. We shall examine this point using eqn. 7, assuming that γ is independent of the eluent strength.

TABLE III

γ VALUES FOR MONOSUBSTITUTED BENZENES AND THE RESULTS OF THE VARIANCE ANALYSIS

Statistical procedure for the F-tests:

$$\bar{\gamma} = \sum_{i,T} \gamma_{i,T}/n = 0.61;$$

$$s_{tot.}^2 = \sum_{i,T} (\gamma_{i,T} - \bar{\gamma})^2/(n-1) = 0.0233 \text{ [10 degrees of freedom } (DF)\text{]}.$$

Influence of temperature on γ:

$$s_p^2 = \sum_T DF_T s_{\gamma T}^2/\sum_T DF_T = 0.0131 \quad (DF = 9);$$

$$F_T = s_{tot.}^2(DF = 10)/s_p^2(DF = 9) = 1.78, \text{ i.e., non-significant (90\% level).}$$

Influence of group i on γ:

$$s_p^2 = \sum_i DF_i s_{\gamma i}^2/\sum_i DF_i = 0.0145 \quad (DF = 5);$$

$$F_i = s_{tot.}^2(DF = 10)/s_p^2(DF = 5) = 1.61, \text{ i.e., non-significant (90\% level).}$$

Mean value of $\gamma = 0.61 \pm 0.05$.

Temperature ($^\circ$C)	γ_i						$\bar{\gamma}_T \pm s_{\bar{\gamma}_T}$
	OCH_3	NO_2	CN	CHO	$COOCH_3$	$COCH_3$	
25	0.44	0.71	0.63	0.40	0.62		0.56 ± 0.13
43.5	0.67	0.69	0.77	0.61	0.47	0.69	0.65 ± 0.10
$\bar{\gamma}_i \pm s_{\bar{\gamma}_i}$	0.56 ± 0.16	0.70 ± 0.01	0.70 ± 0.10	0.51 ± 0.15	0.55 ± 0.12	0.69	

Log (V_N/W) data of phenols, nitrobenzenes, benzaldehydes, anilines and pyridines are given in Table IV; chlorobenzenes are not localized.

Preliminary calculation revealed that for any plausible value of β a parameter δ has to be added to the right-hand side of eqn. 7 in order to obtain a good fit of the Hammett plots. The magnitude of δ depends on the solute series and increases with increasing eluent strength. Therefore, δ may be associated with solute–eluent interactions, i.e., secondary eluent effects. As δ is about as large as the standard error of fit of the multiple regression analysis, it is left out of discussion. The β value of the pyridines in the binary eluent has been estimated on the assumption that $\delta = 0$ in view of the limited number of data available.

Further, it appeared that the use of σ constants gives the best description of the intramolecular electronic effects for all solute series examined, except for the phenols where the use of σ^- parameters[16] gives a slightly improved fit.

Finally, p-hydroxybenzaldehyde appeared to show an anomalous adsorption behaviour (to be discussed in the next section) and was excluded from the regression analysis.

The values of β obtained are given in Table V. A comparison of the pooled variance of the β values (s_p^2, 67 degrees of freedom) and the variance between the β values ($s_{tot.}^2$, 9 degrees of freedom) gives $F = s_{tot.}^2/s_p^2 = 1.62$, i.e., not significant at the 90% level (the nitrobenzenes are excluded from these variance analysis because their β values are based on only 1 degree of freedom). Hence β is not significantly dependent of the temperature, group k and the eluent used. Therefore, localization phenomena can be consistently described by parameters (β and γ) that are independent of the eluent strength. An average value of $\beta = 0.51 \pm 0.03$ was obtained. It is noteworthy that the β value of the pyridines (0.58 $\pm$ 0.07) is, within experimental

TABLE IV

LOG(V_N/W) DATA FOR MONOSUBSTITUTED PHENOLS, NITROBENZENES, ANILINES, BENZALDEHYDES AND PYRIDINES IN n-HEXANE (H, $\varepsilon^0 = 0.0$), n-HEXANE–METHYLENE CHLORIDE (65:35) (B, $\varepsilon^0 = 0.22$) AND METHYLENE CHLORIDE (C, $\varepsilon^0 = 0.32$) AT 25 AND 43.5°

i	i-Ø-OH		i-Ø-NO_2				i-Ø-NH_2		i-Ø-CHO				i-C_5H_5N	
	B, 43.5°	C, 25°	H, 25"	H, 43.5°	B, 25°	B, 43.5°	B, 43.5°	C, 25°	H, 25°	H, 43.5°	B, 25°	B, 43.5°	B, 43.5°	C, 25°
H	0.82	0.40	1.05	0.97	−0.26	−0.26	1.20	0.57	1.54	1.49	0.43	0.28	1.89	1.76
m-F		0.79					0.80	0.09						
p-F		0.42					1.22	0.55						
m-Cl	0.78	0.48	0.89	0.79	−0.35	−0.53	0.78	0.06	1.32	1.23	0.20	0.09		
p-Cl	0.83	0.46	0.93	0.83	−0.43	−0.48	1.01	0.27	1.44	1.36	0.36	0.23		0.96
m-Br	0.78	0.49					0.78	0.05						
p-Br	0.84	0.48					0.97	0.21						0.89
m-CH₃	0.75	0.37					1.25	0.61	1.57	1.56	0.60	0.44		1.84
p-CH₃	0.78	0.41					1.29	0.80	2.14	2.10	0.75	0.69	2.07	2.01
m-OCH₃	1.22	0.72					1.66	0.85	2.27	2.20	1.01	0.93		
p-OCH₃	1.32	0.43						1.28	2.40	2.21	0.68	0.63		
m-NO_2	1.45	1.08	1.77	1.66	0.23	0.15	1.15	0.09	2.41	2.30	0.73	0.65		
p-NO_2	1.63	1.38	1.93	1.70	0.24	0.20	1.20	−0.02						
m-CN		1.12					1.56	0.49					1.74	1.18
p-CN	1.77	1.30					1.48	0.28						1.15
m-CHO		1.41											1.99	
p-CHO		1.69												
m-CO_2CH_3		1.22												
p-CO_2CH_3		1.37												
m-COCH₃		1.61						1.33						
p-COCH₃		1.77						1.19						

TABLE V

VALUES OF β, ϱ, δ AND THE STANDARD ERROR OF FIT (s) FOR MONOSUBSTITUTED PHENOLS, NITROBENZENES, BENZALDEHYDES, ANILINES AND PYRIDINES IN n-HEXANE (H), n-HEXANE–METHYLENE CHLORIDE (65:35) (B) AND METHYLENE CHLORIDE (C) AT 25 AND 43.5°

Solute series	Parameter	H, 25°	H, 43.5°	B, 25°	B, 43.5°	C, 25°
Phenols (σ)	$\beta \pm s_\beta$				0.45 ± 0.10	0.50 ± 0.11
Phenols (σ^-)					0.50 ± 0.09	0.57 ± 0.07
Nitrobenzenes		0.92 ± 0.02	0.98 ± 0.00	0.72 ± 0.18	0.46 ± 0.06	
Benzaldehydes		0.58 ± 0.06	0.53 ± 0.08	0.62 ± 0.09	0.45 ± 0.07	
Anilines					0.38 ± 0.09	0.33 ± 0.06
Pyridines					0.63 ± 0.14	0.53 ± 0.09
Phenols (σ)	$\varrho \pm s_\varrho$				0.30 ± 0.17	1.16 ± 0.36
Phenols (σ^-)					0.31 ± 0.13	1.04 ± 0.15
Nitrobenzenes		-0.38 ± 0.03	-0.59 ± 0.00	-0.29 ± 0.20	-0.31 ± 0.06	
Benzaldehydes		-0.40 ± 0.09	-0.62 ± 0.12	-0.84 ± 0.13	-0.88 ± 0.11	
Anilines					-1.82 ± 0.21	-2.60 ± 0.16
Pyridines					-2.37 ± 0.39	-3.39 ± 0.21
Phenols (σ)	$\delta \pm s_\delta$				0.16 ± 0.07	0.21 ± 0.12
Phenols (σ^-)					0.16 ± 0.06	0.27 ± 0.07
Nitrobenzenes		-0.02 ± 0.01	-0.01 ± 0.00	0.14 ± 0.10	-0.09 ± 0.03	
Benzaldehydes		-0.06 ± 0.04	-0.06 ± 0.06	0.26 ± 0.06	0.31 ± 0.05	
Anilines					0.18 ± 0.07	0.27 ± 0.06
Pyridines					zero	0.08 ± 0.06
Phenols (σ)	s				0.17	0.31
Phenols (σ^-)					0.15	0.19
Nitrobenzenes		0.02	0.00	0.14	0.04	
Benzaldehydes		0.08	0.10	0.12	0.09	
Anilines					0.16	0.16
Pyridines					0.10	0.12

error, equal to this average value, although the mutual distance of groups i and k is smaller than in the other series.

Intramolecular electronic effects

The ϱ values obtained are given in Table V. The positive ϱ value obtained for the phenols indicates that the phenolic hydroxyl group accepts electrons on adsorption to silica. Nitrobenzenes, benzaldehydes, anilines and pyridines show negative ϱ values and thus are electron donors towards silica. The absolute value of ϱ_k increases with increasing Q_k^0 of these Lewis bases. The value of ϱ for the nitrobenzenes is uncertain as only four data could be used in the three-parameter regression analysis. The negative ϱ values obtained for the benzaldehydes are striking because, as far as we know, negative ϱ values for acid–base equilibria of benzaldehydes[19] (and acetophenones[19,20]) have been observed only in concentrated sulphuric acid.

The standard deviations of the ϱ values of the phenols decrease when σ^- instead of σ constants are used, contrary to all other series. These σ^- constants are substantially larger than the corresponding σ constants if group i can show mesomeric interactions with group k. It can be expected that mesomeric interactions will be very strong if group k (in a *para*-position) is a proton acceptor and both groups i and k can be adsorbed simultaneously in the vicinity of a silanol group. These requirements

are met in p-hydroxybenzaldehyde. When σ constants are used, the experimental log (V_N/W) value of this solute is about 0.5 larger than predicted. The use of σ^- constants reduces this discrepancy to 0.15. A similar adsorption behaviour is expected for p-hydroxyacetophenone and p-nitrophenol, but has not been observed. The reason might be that the Lewis-base character of the nitro group is too small to stabilize the mesomeric structure, whereas mesomeric interaction in p-hydroxyacetophenone is hampered by the methyl group which forces the carbonyl group out of the plane of the phenyl group on adsorption.

When the influence of the temperature is neglected[21–26], the values of ϱ in Table V show an increase of the susceptibility of group k for substituent effects with an increase in the eluent strength. Obviously, the stability of the (polar) solute–adsorbent complex increases with increasing polarity of the eluent.

Polycyclic aromatic hydrocarbons

The log (V_N/W) data are given in Table VI. The S^0 values at 25 and 43.5° were obtained from eqn. 8. Average values have a precision of about 0.05 and are given in Table VI together with experimental A_s values. The S^0 values are plotted against the number of aromatic carbon atoms in Fig. 2. The data can be described by the equation

$$S^0 = nQ^0_{-C=} - \zeta(n - 6) \tag{11}$$

where the term $\zeta(n - 6)$ accounts for localization. For ODS-silica $\zeta = 0.095 \pm 0.004$ (the data points 3, 5, 6 and 16 are excluded from the calculation of ζ for reasons given

TABLE VI

LOG (V_N/W) DATA FOR SOME POLYCYCLIC AROMATIC COMPOUNDS IN n-HEXANE (H) AND n-HEXANE–METHYLENE CHLORIDE (65:35) (B) AT 25° AND 43.5°, THEIR (MEAN) EXPERIMENTAL S^0 AND A_s VALUES ON ODS-SILICA AND CALCULATED A_s VALUES

Solute	No.	H, 25°	H, 43.5°	B, 25°	$\bar{S}^0$	A_s	A_s (calc.)
Benzene	1	−0.266	−0.304	*	1.50		6.0
Naphthalene	2	0.090	−0.010	*	2.16		8.1
Acenaphthene	3	0.181	0.182	−0.946	2.45	10.0	9.7
Diphenyl	4	0.214	0.224	*	2.53		9.7
Fluorene	5	0.369	0.369	*	2.83		9.7
Bibenzyl	6	0.468	0.452	*	3.02		12.4
Anthracene	7	0.368	0.341	−0.913	2.80	11.4	10.2
Phenanthrene	8	0.386	0.349	−0.913	2.82	11.6	10.2
Pyrene	9	0.445	0.422	−0.921	3.01	12.2	10.7
Fluoranthene	10	0.511	0.486	−0.905	3.08	12.6	10.7
Chrysene	11	0.695	0.660	−0.763	3.45	13.0	12.3
Triphenylene	12	0.704	0.682	−0.666	3.48	12.2	12.3
p-Terphenyl	13	0.664	0.630	*	3.39		13.4
3,4-Benzpyrene	14	0.777	0.735	−0.680	3.61	13.0	12.8
Perylene	15	0.821	0.781	−0.612	3.70	12.8	12.8
Coronene	16	0.957	0.890	−0.459	3.95	12.6	13.8
p,p'-Quaterphenyl	17	1.111	1.069	−0.927	4.29	18.2	17.1

* V_N/W data are very small.
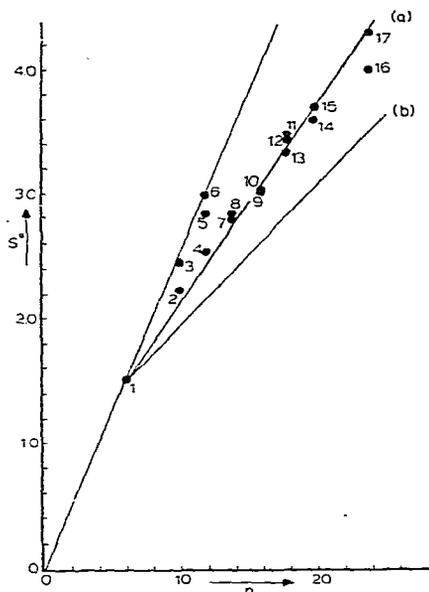
Fig. 2. Experimental S^0 values for some polycyclic aromatic compounds *versus* the number of aromatic carbon atoms (n). Eluent: *n*-hexane. (a) best line through the data points on ODS-silica (the data points 3, 5 and 6 are excluded from the calculation of the regression line), (b) best line through the data points on Davison Code 62 silica[27] (the data points are not given). Numbering of data points according to Table VI.

below), *i.e.*, smaller than the value $\zeta = 0.14$ given by Snyder[27] for a comparable bare silica (Davison Code 62). Hence, the deactivation of the reactive silanol sites by the ODS groups reduces the contribution of localization, probably because of preferential adsorption of the aromatic compounds to the reactive silanol groups of the bare silica[13].

It is noteworthy that the non-planar[28] polyphenyls (data points 4, 13 and 17 in Fig. 2) apparently adsorb flatly like the condensed aromatics. Flat adsorption of condensed and non-condensed aromatics also follows from the experimental A_s data, which appear to be about equal to calculated data (compare columns 7 and 8 in Table VI); A_s(calc.) follows from the equation

$$A_s(\text{calc.}) = 6 + 0.8(h - 6) + 0.25(c - h) \tag{12}$$

where h and c are the number of protons and carbon atoms of the hydrocarbon, respectively[6]. The only exception is coronene, which has a diameter of about 9 Å, *i.e.*, close to the mean mutual distance of the ODS chains (11 Å). Both S^0 and A_s of coronene are significantly smaller than expected. Analogous steric effects have been observed on the adsorption of monosubstituted hexanes to ODS-silica (surface concentration 3.14 μmole/m^2) in gas chromatographic columns[5], and on the adsorption of alkylphenones to nonylsilyl and octadecylsilyl silica in liquid chromatographic columns[3,4].

Acenaphthene, fluorene and bibenzyl show no localization. It is doubtful whether the large S^0 value of bibenzyl can be explained by assuming simultaneous

adsorption of both phenyls on site clusters[6], as this explanation cannot be applied to acenaphthene and fluorene.

Hexyl and cyclohexyl derivatives

The log (V_N/W) data of some monosubstituted (cyclo)hexyl compounds are given in Table VII. The estimates of log V_a and α, deduced from the data obtained with n-hexane as the eluent, are, within experimental error, equal to those derived from the data on substituted benzenes and chlorobenzenes (-1.03 ± 0.02 compared with -1.03 ± 0.03 and 0.46 ± 0.06 compared with 0.51 ± 0.01, respectively).

TABLE VII

LOG(V_N/W) DATA FOR SOME MONOSUBSTITUTED HEXANES AND CYCLOHEXANES IN n-HEXANE (H) AND n-HEXANE–METHYLENE CHLORIDE (65:35) (B) AT 25° AND THE NUMBER OF ADSORBED METHYLENE GROUPS (m)

Substituent	H, 25°	B, 25°	m
Hexanes:			
Cl	−0.23	−1.22	*
Br	−0.24	−1.16	*
SH	−0.23	−0.96	*
CHO	1.24	0.40	1.8
CO$_2$CH$_3$	1.33	0.21	3.2
Cyclohexanes:			
Cl	−0.23	−1.16	*
Br	−0.19	−1.02	*
SH	−0.22	−0.91	*
CN	1.51	0.22	6.8
CO$_2$CH$_3$	1.42	0.30	3.2

* Unreliable results.

The number of adsorbed methylene groups (m) in the binary eluent, calculated with eqn. 9 is given in Table VII. The average values of m are 2.5 ± 1.0 and 5.0 ± 2.5 for the hexanes and cyclohexanes, respectively. Snyder[6] obtained $m = 2.9$ for hexylbenzene on bare silica.

Selectivity

Although the ODS groups do not show any specific interaction with the solutes examined, their presence on the silica influences solute retention. All changes in the V_N/W data caused by the ODS groups are due to the deactivation of the silica and its effect on α, γ and ζ, as discussed in the preceding sections. Whether the magnitude of β and ϱ depends on deactivation could not be established.

In consequence of the large number of factors that influence retention data (even on a rather simple bonded phase such as ODS-silica), the search for specific solute–monomer interactions on polar bonded phases requires a detailed insight into the adsorption mechanism on an alkyl-bonded phase with a comparable surface concentration of monomers. The results obtained for a N-2-cyanoethyl-N-methylaminosilica will be reported in the near future.

CONCLUSIONS

The adsorption equation of Snyder gives a good description of the retention data for the ODS-silica investigated, when the parameters in this equation are adjusted for the deactivation of the silica by the silylation reaction.

The adsorbent activity α decreases considerably (to 0.50) as a result of preferential deactivation of reactive silanol groups. Solute adsorption is mainly controlled by enthalpic interaction forces. The contributions of solute and eluent localization can be described according to Snyder's concept. A parameter $\beta = 0.51$ for the former and a parameter $\gamma = 0.61$ for the latter effect are required in order to obtain an optimal fit to the experimental data.

Intramolecular electronic effects on the adsorption of m- and p-substituted phenols, nitrobenzenes, benzaldehydes, anilines and pyridines to ODS-silica can be described satisfactorily with the Hammett equation. The aldehyde group shows strong mesomeric interaction with the phenolic hydroxyl group. The susceptibility for induced charge shifts caused by substituents increases with increasing proton acceptor strength of the reactive group in the solute molecule and with increasing eluent strength. Phenols are proton donors towards ODS-silica.

All of the results obtained indicate flat adsorption of the solutes to the silica surface beneath the ODS bristles, except those of coronene and substituted (cyclo)-hexanes in the binary eluent, as expected for steric (coronene) or energetic (hexanes) reasons.

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REFERENCES

1 J. J. Kirkland, J. Chromatogr. Sci., 9 (1971) 206.
2 D. F. Horgan, Jr., and J. N. Little, J. Chromatogr. Sci., 10 (1972) 76.
3 E. Grushka and E. J. Kikta, Jr., Anal. Chem., 46 (1974) 1370.
4 E. J. Kikta, Jr., and E. Grushka, Anal. Chem., 48 (1976) 1098.
5 W. E. Hammers, R. H. A. M. Janssen and C. L. de Ligny, J. Chromatogr., in press.
6 L. R. Snyder, Principles of Adsorption Chromatography, Marcel Dekker, New York, 1968.
7 R. S. Drago and B. B. Wayland, J. Amer. Chem. Soc., 87 (1965) 3571.
8 R. S. McDonald, J. Phys. Chem., 62 (1958) 1168.
9 L. P. Hammett, Physical Organic Chemistry, McGraw-Hill, New York, 1940.
10 L. R. Snyder, J. Phys. Chem., 67 (1963) 2344.
11 L. R. Snyder, J. Chromatogr., 16 (1964) 55.
12 L. R. Snyder, Advan. Chromatogr., 4 (1967) 3.
13 L. R. Snyder, J. Chromatogr., 11 (1963) 195; 25 (1966) 264.
14 J. C. P. Broekhoff and B. G. Linsen, in B. G. Linsen (Editor), Physical and Chemical Aspects of Adsorbents and Catalysts, Academic Press, London, 1970, p. 1.
15 J. Asshauer and I. Halász, J. Chromatogr. Sci., 12 (1974) 139.
16 C. D. Ritchie and W. F. Sager, in S. G. Cohen, A. Streitwieser and R. W. Taft (Editors), Progress in Physical Organic Chemistry II, Wiley, New York, 1964, p. 323.
17 A. A. Humffray, J. J. Ryan, J. P. Warren and Y. H. Yung, Chem. Commun., (1965) 610.

18 L. R. Snyder and J. W. Ward, *J. Phys. Chem.*, 70 (1966) 3941.
19 K. Yates and B. F. Scott, *Can. J. Chem.*, 41 (1963) 2320.
20 R. Stewart and K. Yates, *J. Amer. Chem. Soc.*, 80 (1958) 6355.
21 P. D. Bolton and F. M. Hall, *Aust. J. Chem.*, 21 (1968) 939.
22 P. D. Bolton and F. M. Hall, *J. Chem. Soc.*, B, (1969) 259.
23 P. D. Bolton, F. M. Hall and J. Kudrinski, *Aust. J. Chem.*, 21 (1968) 1541.
24 P. D. Bolton, F. M. Hall and I. H. Reece, *J. Chem. Soc.*, B, (1967) 709.
25 P. D. Bolton, C. H. Rochester and B. Rossall, *Trans. Faraday Soc.*, 66 (1970) 1348.
26 I. R. Bellobono and M. A. Monetti, *J. Chem. Soc., Perkin Trans. II*, (1973) 790.
27 L. R. Snyder, *Advan. Anal. Chem. Instrum.*, 3 (1964) 251.
28 L. R. Snyder, *J. Amer. Chem. Soc.*, 67 (1963), 240.