Journal of Chromatography, 153 (1978) 381–389

CHROM. 10,815

SCREEN FOR THE EVALUATION OF CHEMICALLY BONDED SUPPORTS USED IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

C. J. LITTLE and A. D. DALE

Physical Methods Department, Roche Products Ltd., P.O. Box 8, Welwyn Garden City, Herts. (Great Britain)

and

M. B. EVANS

Applied Chemistry Department, School of Natural Sciences, Hatfield Polytechnic, Hatfield, Herts. (Great Britain)

(First received August 8th, 1977; revised manuscript received December 7th, 1977)

SUMMARY

A screen is proposed for the evaluation of chemically bonded supports; for use in reversed-phase high-performance liquid chromatography. Application of the screen to a range of test mixtures has revealed that, in general, octadecyl-bonded silica is the most useful.

INTRODUCTION

A chemically bonded support is the normally accepted terminology for describing the chemical linking of organic moieties on to chromatographic adsorbents such as alumina and silica gel. The advantages of chemically bonded supports in routine analysis stem from their long-term stability as well as compatibility with gradient elution techniques. Although widely used, the logic behind the choice of a bonded support for a particular separation has become very vague.

Conventionally, a stationary phase was chosen after partition experiments had indicated the desired selectivity. Such experiments are difficult to perform with bonded phases and in any case, the effect of the monolayer so formed is significantly less than the multilayer film associated with the adsorbed stationary phase. Consequently, comparisons between adsorbed and bonded phases are probably meaningless. Consistent with this viewpoint, difficulties have been encountered when reversed-phase chromatography has been used in an attempt to determine dynamic partition coefficients[1–3].

Applications of chemically bonded supports for reversed-phase chromatography are legion[4–11]. Many use octadecyl-bonded silica. Recently, a vast range of silanes has been made generally available thus prompting an escalation of the number of bonded silicas evaluated and made commercially available. It is interesting to note

that the majority of applications advocating the use of such bonded materials employ adsorption chromatography with non-aqueous solvent systems[4–7,12–15] and no real effort, apart from the investigation of chain length of the bonded alkyl groups[16,17], appears to have been made to see if a better separation could have been achieved on more readily available supports. It was to this end that a column screen was instigated.

EXPERIMENTAL

Apparatus

Modular equipment was used comprising: constametric I pump (LDC); variable-wavelength UV detector (Cecil Instruments, Model CE 212); syringe loading injection valve (Rheodyne, Model 7120) and a Servoscribe I.S. recorder (Smiths Industries).

Reagents

Silanes were purchased from Petrarch Systems (Magnus Scientific, Sandbach, Cheshire, Great Britain) and Aldrich (Milwaukee, Wisc., U.S.A.).

Preparation of bonded supports

In all cases, the following procedure was used. Silica (10 g) was slurried in toluene (100 ml) to which the appropriate silane was subsequently added (1 g). With occasional stirring, the reaction was allowed to proceed at ambient temperature for approximately 24 h. The supernatant solvent was subsequently decanted off. Toluene (100 ml) was added and the bonded silica was again dispersed and allowed to settle. The supernatant was decanted-off. This procedure was repeated several times using methanol as solvent (usually 3–5 washes) until no residual silane was observed in the supernatant. Finally, the methanol dispersion was filtered, washed with methanol and dried at 40°.

Column packing

The procedure used has been described previously[18]. All columns were packed by the same procedure and under the same conditions (*i.e.* 7500 p.s.i. for 30 min). Extreme care was taken to ensure that all the end fittings, column tubing (Apollo, from Accles and Pollock, Warley, Worcestershire, Great Britain), dimensions and support material (Partisil 5) were the same for each column, thus reducing the possibility of ascribing peculiar chromatography to factors other than chemical modifications of the support.

Chromatography

All chromatography was performed under isocratic conditions using a solvent composition found to be suitable for the application but not necessarily optimal. Test mixtures used and the corresponding eluent composition are given in Table I.

The fundamental chromatographic parameter chosen to characterise the chemically modified supports is that of resolution R, determined from the expression

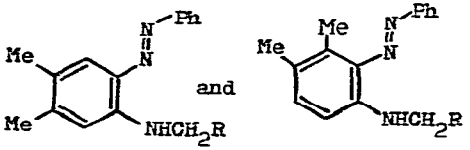
$$R = \frac{2\Delta t}{W_1 + W_2}$$

TABLE I

COMPOSITION OF TEST MIXTURES AND THEIR CORRESPONDING ELUENTS

Me = methyl, Ph = phenyl.

Test mixture		*Methanol*	*Water*
PAH	benzene, naphthalene, diphenyl and anthracene	60	40
Phenol-1	phenol, *p*-cresol and 2,4-xylenol	20	80
Phenol-2	phenol, *p*-cresol, *p*-bromophenol, *p*-iodophenol	20	80
Mefruside	see Fig. 2	20	80
2-NI	see Fig. 5	10	90
Ketone	see Fig. 1	40	60
Sugar	arabinose, xylose, fructose and sucrose	90*	10
Azo compounds	*n*-(I) and *iso*-(II) azo dyes	90	10



where R = (HOCH$_2$–CHOH–CHOH–CHOH)–

* Acetonitrile.

where Δt is the difference in retention time of the solutes and W_1 and W_2 are the peak widths at the base of the peak respectively. Unlike the commonly used capacitance factor (k^1) we feel that R is of more practical use as well as being easier to comprehend.

The column screen

The column screen involves the evaluation of a wide range of bonded supports for each separation under investigation.

Test mixtures were chosen to represent relatively simple molecules of similar functional group together with more complex mixtures of drugs and their metabolites. The latter are included in order to justify the relevance of the screen to real applications.

An evaluation of the screen must take into account the following parameters: alkyl chain length; steric effects due to bulky groups; basicity; acidity; polarity of the bonded phase.

The use of the screen should be aimed at providing the following information: the best column for a given separation; trends which can be used to probe the underlying mechanism of reversed-phase liquid chromatography; and a limited number of bonded supports suitable for use in a more restricted screen.

Furthermore, application of the screen would be expected to restrict the proliferation of publications describing novel bonded phases.

Application of the screen

The proposed screen has been applied to at least 20 bonded supports (the number is being increased continuously) based upon various alkyl, aryl, acidic, polar and unsaturated functional groups. Identification of the bonded supports mentioned in Table III is given in Table II.

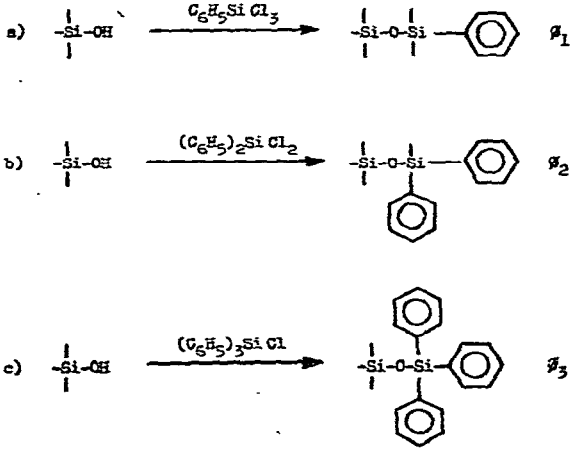
TABLE II

IDENTIFICATION OF SOME BONDED SUPPORTS LISTED IN TABLE III

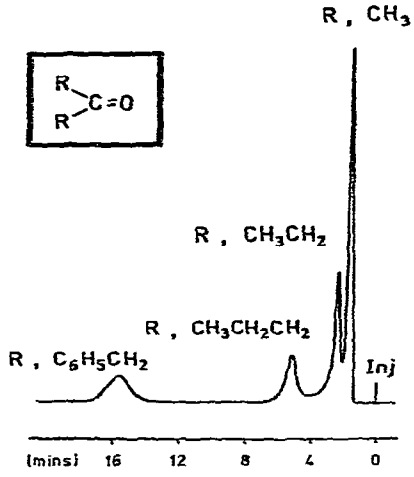
Column packing		*Identification*
Support	*Modification*	
Partisil-5	Hexyl	C_6
	Nonyl	C_9
	Tetradecyl	C_{14}
	Octadecyl	C_{18}
	Monophenyl	φ_1
	Diphenyl	φ_2
	Triphenyl	φ_3
	Diphenyl, sulphonic acid	φ_2/SCX
	Diphenyl, sulphonamide	φ_2/S-NH_2
	Amino propyl	NH_2
	Propyl ethylenediamine	en
	Propionitrile	CN
	Methacrylate	MA
	Allyl	All
	Glycidoxypropyl	GLY
	Octadecyl, sulphonic acid	C_{18}/SCX
	n-Butyl germanium	BuGe
Alox-T, 5μ	Octadecyl	Al-T/C_{18}

Alkyl bonded supports. Three different separations (illustrated by Figs. 1, 2 and 3) have been used to demonstrate the relationship between resolution R and alkyl chain length C_n. In each case, R was found to increase linearly with C_n (Fig. 4). These examples show zero resolution on silica alone ($n = 0$). Of the examples studied so far only the nitroimidazole separation gives a positive resolution on silica alone. It is conceivable that the degree to which a group of compounds is affected by changes in C_n could be utilised to improve some complex separations.

Steric effects due to bulky groups. Three phenyl-bonded supports were made according to the scheme



Reaction scheme for chemical bonding with mono-, di- and tri-phenyl silanes.



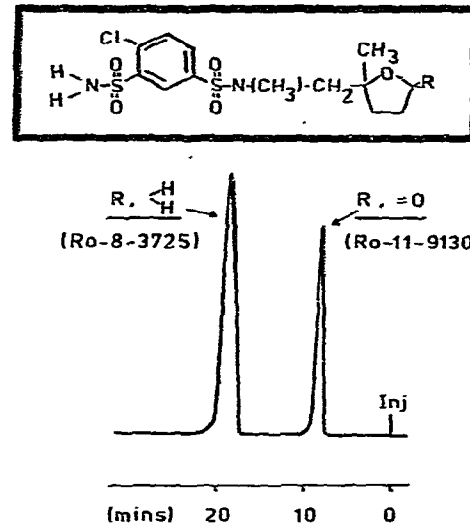
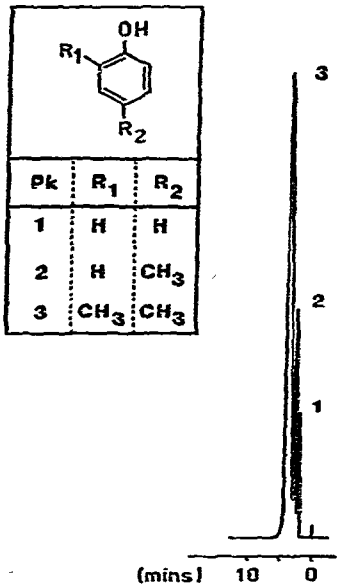


Fig. 1. Ketone separation on octadecyl Partisil (5 μm). Eluent, methanol–water (4:6); flow-rate, 1 ml/min; detection, UV (254 nm).

Fig. 2. Sulphonamide separation on octadecyl Partisil (5 μm). Eluent, methanol–water (2:8); flow-rate, 1.5 ml/min; detection, UV (248 nm).

As the bulkiness of the bonded phenyl groups increases from $\varphi_0 \rightarrow \varphi_1 \rightarrow \varphi_2 \rightarrow \varphi_3$ (where $\varphi_0 = C_0$ = unbonded silica) steric exclusion of the solute from the support surface can be expected to increase far more dramatically than that due to increasing chain length of similar carbon number. This provides a tool for investigating the elution mechanism of reversed-phase chromatography.



Pk	R_1	R_2
1	H	H
2	H	CH_3
3	CH_3	CH_3

Fig. 3. Phenol-1 separation on octadecyl Partisil (5 μm). Eluent, methanol–water (2:8); flow-rate, 0.8 ml/min; detection, UV (275 nm).

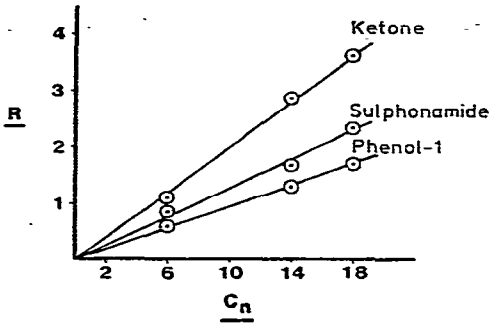


Fig. 4. Relationship between resolution R and alkyl chain length C_n.

If liquid partition was the dominating mechanism an increase in retention and resolution would be expected for increasing alkyl and aryl coverage of the silica surface. On the other hand, if adsorption was the dominating mechanism an increase in support coverage would be expected to decrease retention and resolution. At first sight, the separation of the 2-nitroimidazole radiosensitiser [Ro-07-0582] from its metabolite [Ro-11-9130] (Figs. 5 and 6) would fall into the latter category. If, however, the resolution data is superimposed on the corresponding resolution data for the alkyl coverage (Fig. 7) in such a manner that phenyl corresponds to hexyl, diphenyl corresponds to dodecyl and triphenyl corresponds to octadecyl, a more complex situation is observed. Going from phenyl to triphenyl the general decrease in retention and resolution upholds the adsorption as the dominating mechanism but the increase in retention and resolution in going from silica to phenyl can be attri-

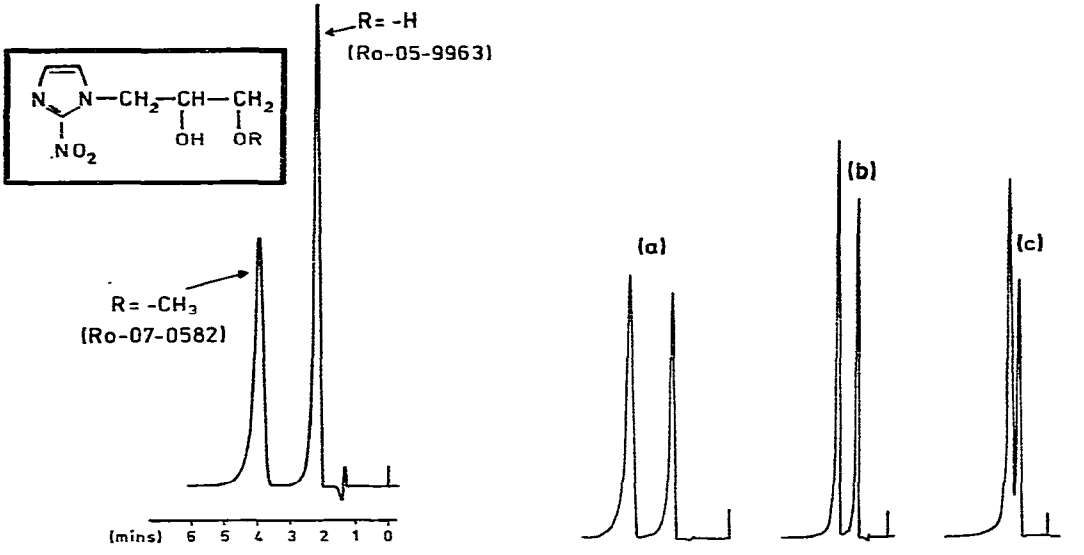
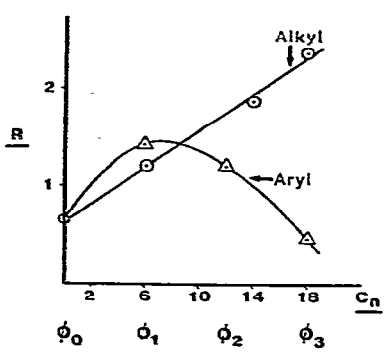


Fig. 5. Nitroimidazole separation on octadecyl Partisil (5 μm). Eluent, methanol–water (1:9); flow-rate, 1 ml/min; detection, UV (324 nm).

Fig. 6. Nitroimidazole separation on mono- (a), di- (b) and tri- (c) phenyl-bonded silicas.



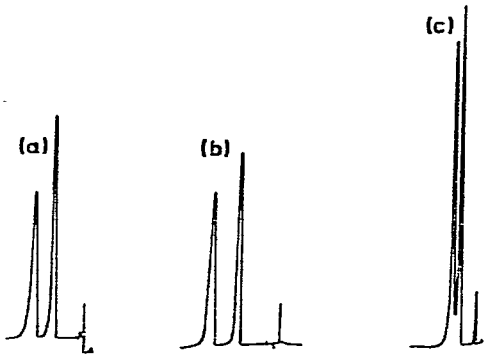


Fig. 7. Comparison between the effects of carbon number C_n and phenyl carbon number φ_n on resolution for the nitroimidazole separation.

Fig. 8. Sulphonamide separation on mono- (a), di- (b) and tri- (c) phenyl-bonded silicas.

buted either to partition effects or to adsorption modified by hindrance to the desorption process. The problem is magnified by the continued increase in retention and resolution by increasing alkyl coverage. Perhaps the increase in carbon number merely increases the hindrance to the desorption process. A similar result[18] is obtained with the separation of the sulphonamide, Mefruside [Ro-8-3725] from its metabolite [Ro-11-9130] (Fig. 8) except that the maximum retention and resolution occurs nearer to diphenyl (Fig. 9).

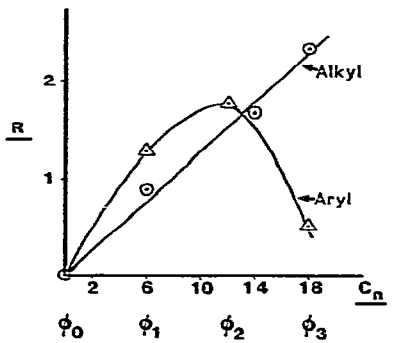


Fig. 9. Comparison between the effects of carbon number C_n and phenyl carbon number φ_n on resolution for the sulphonamide separation.

Acidic and basic bonded supports. At this stage it is worth referring to a summary of the screen to date (Table III) which attempts to classify the performance of the bonded supports investigated for each application. Not every bonded support is listed, but those that are have been chosen as being representative or illustrative of a point of particular interest. Most of the bonded supports investigated fall into the zero–moderate category.

In general, the acidic and basic bonded supports gave poor resolution. The only application giving significant separation with these supports was that of the nitroimidazoles. However, this separation gave a similar separation on unbonded

TABLE III

SUMMARY OF COLUMN SCREEN

Performance	*PAH*	*Phenol-1*	*Phenol-2*	*Mefruside*	*2-NI*	*Ketone*	*Sugar*	*Azo compounds*
Zero	CN	NH_2	$\varphi_{(1-3)}$	CN	NH_2	CN	C_{14}	C_{18}
Poor	C_{18} | SCX	C_6	CN	C_6	BuGe	$\varphi_{(1-3)}$	CN	—
Mod.	(Al-T) | C_{18}	C_{14}	All	C_{14}	CN	MA	BuGe	—
Good	C_{14}	MA	C_{14}	φ_2	φ_1	C_{14}	NH_2	C_{18} | SCX
Best	C_{18}	(Al-T) | C_{18}	C_{18}	C_{18}	C_{18}	C_{18}	en	SCX

silica. Consequently, one can conclude that the bonded groups have made very little difference to the separation.

Exceptions relating to these bonded supports occur in the applications where ion-exchange or ion-pair formation can be invoked (*i.e.* sugars and azo compounds). An interesting trend to emerge from the sugar screen is the improvement in resolution as the number of bonded amino groups is increased. This work will be reported in greater detail in a future publication[19].

Polar bonded supports. The general remarks referring to acidic and basic bonded supports also apply to polar supports such as nitrile and methacrylate. The major exception to these comments refers to the resolution of the phenol-1 mixture where methacrylate produces a good resolution. However, the resolution offers no advantage over the octadecyl-bonded supports of either silica or alumina. To date no application has been found for this group of supports which cannot be better performed by other supports.

Miscellaneous. Apart from the octadecyl Alox T support, which is comparable to octadecyl Partisil for the phenol-1 separation, the only noteworthy result is that of *n*-butyl germanium in the sugar separation. This application serves to emphasize the importance of screening several supports prior to submitting a new application for publication. This is the only potential application we have found for *n*-butyl germanium so far. If we had published on the basis that it was superior to octadecyl Partisil a misleading situation would have arisen through not taking into account the even better resolution of aminopropyl Partisil and ethylenediamine-bonded Partisil.

CONCLUSIONS

Apart from applications involving ion-exchange or ion-pair mechanisms, octadecyl-bonded supports appear to be the most useful for reversed-phase chromatography.

Although inconclusive at this stage, there is some evidence that the predominant role of the bonded phase in reversed-phase chromatography is to modify the adsorption effects of the support. Almost certainly there is more than one mechanism acting and it is hoped that more conclusive evidence will be forthcoming.

Already there is sufficient evidence to propose a restricted column screen. It would appear that silica, octadecyl silica, aminopropyl silica, ethylenediamine-bonded silica and a strong cation-exchange-bonded silica should suffice to give the maximum of information for the minimum of effort.

REFERENCES

1 A. Pryde, *J. Chromatogr. Sci.*, 12 (1974) 486.
2 E. Grushka (Editor), *Bonded Stationary Phases in Chromatography*, Ann Arbour, Michigan, 1974.
3 J. F. K. Huber, E. T. Alderlieste, H. Harren and H. Poppe, *Anal. Chem.*, 45 (1973) 1337.
4 J. M. McCall, *J. Med. Chem.*, 18 (1975) 549.
5 H. Poppe, *Anal. Chem.*, 47 (1975) 1483.
6 J. A. Schmit, R. A. Henry, R. E. Williams and J. F. Dieckman, *J. Chromatogr. Sci.*, 9 (1971) 645.
7 R. V. Vivilecchia, R. L. Cotter, R. J. Limpert, N. Z. Thimot and J. N. Little, *J. Chromatogr.*, 99 (1974) 407.
8 B. B. Wheals and R. N. Smith, *J. Chromatogr.*, 105 (1975) 396.
9 N. E. Hoffman and J. C. Liao, *Anal. Chem.*, 48 (1976) 1104.
10 D. M. Takahashi, *J. Chromatogr.*, 131 (1977) 147.
11 M. J. Fasco, L. J. Piper and L. S. Kaminsky, *J. Chromatogr.*, 131 (1977) 365.
12 M. Novotný, S. L. Bektesh and K. Grohmann, *J. Chromatogr.*, 83 (1973) 25.
13 K. Hostettmann and H. M. McNair, *J. Chromatogr.*, 116 (1976) 201.
14 B. B. Wheals, *J. Chromatogr.*, 107 (1975) 402.
15 R. S. Ward and A. Pelter, *J. Chromatogr. Sci.*, 12 (1974) 570.
16 K. Karch, I. Sebastian and I. Halász, *J. Chromatogr.*, 122 (1976) 3.
17 K. Karch, I. Sebestian, I. Halász and H. Engelhardt, *J. Chromatogr.*, 122 (1976) 171.
18 C. J. Little, A. D. Dale, D. A. Ord and T. R. Marten, *Anal. Chem.*, 49 (1977) 1311.
19 C. J. Little, A. D. Dale and M. B. Evans, in preparation.