CHROM. 11,597

Note

Optimization of reaction conditions for the preparation of chemically bonded supports

I. The role of the diluent (or solvent)

C. J. LITTLE, A. D. DALE and J. A. WHATLEY

Physical Methods Department, Roche Products Limited, 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)

(Received November 6th, 1978)

Recent studies concerning the chemical bonding of stationary phases to silica have led to the development of stable supports suitable for use in high-performance, reversed-phase liquid chromatography[1-3]. The need to improve the efficiency and selectivity of these supports requires the definition of optimal procedures for their manufacture, such as the nature of the silane used, diluent, diluent temperature and the sequence of silanization reactions which should include the "capping" of the unreacted silanol sites.

Choice of solvent, or better "diluent", as the reaction is heterogeneous, is an important parameter, the choice of which is very confusing in the literature. It would appear that the most popular solvents are toluene[4,5] and xylene[6,7]. Other references cite the use of petroleum fractions[8,9], *n*-hexane[10], isooctane[11], benzene[12,13], dioxan[14,15], tetrahydrofuran[16] and even water[17]. We suspect that water does not result in a bonded support, but produces a precipitate of hydrolysed silane on the surface of the silica.

This paper reports the results of a systematic study of the role of the diluent in the preparation of chemically bonded supports.

EXPERIMENTAL

The apparatus was as described previously[4].

Preparation of supports

All bonding experiments were performed under identical conditions[4]. Octadecyltrichlorosilane (0.5 g) was dissolved in the solvent (50 ml). When methanol is used as the solvent, this procedure ensures that the trichlorosilane is converted into the dimethoxysilane prior to heterogeneous reaction with the silica, thus avoiding com-

petitive reactions of the chlorosilane between the solid surface and the diluent. To this slurry, Partisil 10 (5 g) was added and the reaction was allowed to proceed for 24 h at ambient temperature. The excess of reagent was then washed from the silica by five decantations with the diluent. The bonded supports were dried overnight at 60°, slurried in isopropanol[18] and packed into identical columns (10 × 0.42 cm I.D. Apollo Tubing) using methanol as the packing eluent. All columns were packed at 7500 p.s.i.g. for 30 min.

The carbon contents of the supports were measured by combustion micro-analysis. The evaluation of the supports has been described in detail elsewhere[4].

RESULTS AND DISCUSSION

The experiments were designed to establish the preferred diluents for chemical bonding. Solvents covering a wide range of polarity were used. Pyridine was included as a basic solvent in order to take up the liberated hydrogen chloride and so enhance the reaction velocity. Based on evidence that the silica surface has a maximal silanol group formation at pH $\approx$ 3 (ref. 19), we included acetic acid as a bonding solvent in the hope that all of the potential active sites would become available for bonding.

A summary of the effects of the bonding solvent is given in Table I, which lists all of the solvents investigated in approximate order of effectiveness. We tried to correlate this trend with the physical properties of the solvents but the only ones that seem to be relevant are ε^0 and the solubility of water in the solvent. There appears to be a sharp distinction between the water-miscible and water-immiscible solvents, the former giving rise to the poorest bonded supports. Ethyl acetate, with an intermediate

TABLE I

COMPARISON OF SOLVENTS USED IN THE CHEMICAL BONDING OF SILICA SUP-PORTS

Bonding solvent	ε^0	Solubility of H_2O (%)	Resolution (R)[***] Hydrocarbons: $R_{1,3}$	Ketones: $R_{5,7}$	Nitroimidazoles[4]: $R_{8,9}$
Carbon tetrachloride	0.18	0.05	8.3	5.7	3.9
Dichloromethane	0.42	2	7.8	5.5	3.7
(Chloroform)[*]	0.40	0.5	(5.7)	(2.9)	(2.2)
Hexane	0	0	5.3	3.2	2.5
Diethyl ether	0.38	1.2	5.8	3.6	2.3
Toluene	0.29	0.05	5.8	3.7	1.5
Ethyl acetate	0.58	3.3	3.1	—	1.4
Acetic acid	Large	S[§]	2.6	SR	1.5
Dioxan	0.56	S	SR	1.9	1.4
Methanol	0.95	S	SR	1.7	2.0
Acetone	0.56	S	SR	NR	SR
Acetonitrile[**]	0.65	S	SR	NR	SR
Nitromethane[**]	0.64	10	NR	NR	SR
Pyridine[**]	0.71	S	NR	NR	SR

[*] Chloroform stabilized with ethanol which probably accounts for these anomalous results.
[**] Indicates a flocculent product which settles with difficulty.
[***] SR = slight resolution, insufficient for calculation: NR = no resolution.
[§] S = miscible in all proportions.

water solubility, appears to be the link between the two classes of solvents. The halogenated hydrocarbons appear to be the best of the water-immiscible solvents, carbon tetrachloride being optimal. Examples of the chromatography of this optimally bonded silica are given in Fig. 1. Acetonitrile, nitromethane and pyridine produce the worst bonded supports. The product is flocculent and settles with difficulty. Columns packed with these supports result in a high pressure drop between the top and bottom of the column. This evidence is in accord with the formation of a polymeric film over the surface of the silica.
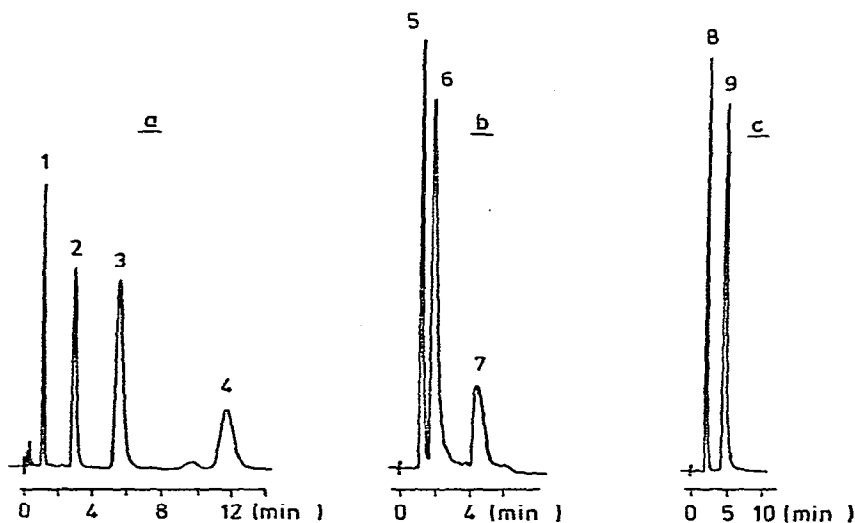

Fig. 1. High-performance liquid chromatography of (a) hydrocarbons, (b) ketones and (c) nitroimidazoles on octadecylsilica, bonded in carbon tetrachloride. Peaks: 1 = benzene; 2 = naphthalene; 3 = diphenyl; 4 = anthracene; 5 = acetone; 6 = diethyl ketone; 7 = 4-heptanone; 8 = misonidazole metabolite Ro 05-9963; 9 = misonidazole Ro 07-0582 (see ref. 19).

Further evidence that the nature of the bonding solvent can play a vital role in defining the bonded surface, is shown in Fig. 2. The percentage of carbon on the bonded silica is plotted against reaction time. The reaction in toluene is shown to be virtually complete after 10–15 min at room temperature. When methanol is used, thus converting the trichlorosilane into a trimethoxysilane, the reaction is incomplete after 24 h at room temperature. It will be demonstrated in a subsequent paper[20] that this reaction never achieves the same level of bonding as is achieved in toluene. Presumably there are sites on the silica surface that differ in reactivity and the activation energy of the trimethoxysilane system is insufficient to allow bonding to take place. This evidence throws into doubt the usefulness of a wide range of commercially available silane esters for use in the chemical bonding of silica.

In conclusion, we wish to emphasize the importance of using the optimum solvent for chemically bonding supports for high-performance liquid chromatography. It would appear that the halogenated hydrocarbon solvents are to be favoured. The differences between these solvents are not dramatic but the best resolution and column efficiencies appear to be generated by the use of carbon tetrachloride as the
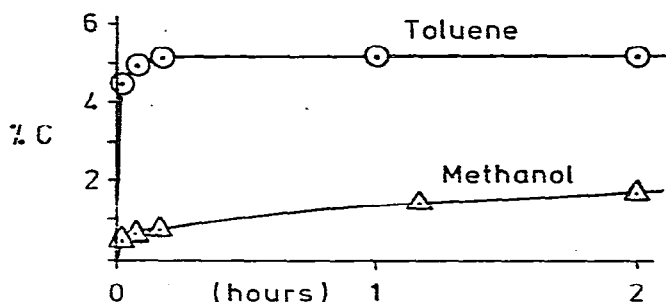
Fig. 2. Variation of carbon coverage of the bonded silica with reaction time.

bonding solvent. In this solvent, the silica remains as a slurry for most of the reaction time. This factor may be related to the efficacy of the solvent for bonding. However, it poses problems in the decantation stage and we have adopted the procedure of diluting with dichloromethane (1:2), which results in a more rapid sedimentation.

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