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Note

Optimization of reaction conditions for the preparation of chemically bonded supports

II. Bonding sequence (including "capping") and reaction temperature

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In Part I¹, we considered the effect of changing the nature of the solvent used during the chemical bonding of supports for use in high-performance liquid chromatography (HPLC).

An additional parameter that must be considered during the bonding process is the degree of coverage of the silica surface. Peak asymmetry has long been attributed to unreacted silanol sites². The problem is complicated by the generation of silanol groups from the hydrolysis of unreacted chlorosilane moieties. This significantly reduces the hydrophobicity of the support³ and is of particular importance when trichlorosilanes are used for bonding, as a certain percentage of these chlorosilane groups do not react with the silica surface.

Attempts to eliminate the unreacted silanol sites have included the use of monochlorosilanes^{2,3} and the "capping" of unreacted sites with trimethylchlorosilane^{4,5}, the smallest monochlorosilane available. Other silanes have been used, such as dimethyldichlorosilane^{3,6}.

We have extended these studies in order to define the optimal procedure for producing reversed-phase bonded supports for HPLC.

EXPERIMENTAL

The apparatus and reagents⁷ and column packing^{7,8} were as previously described. The evaluation of the supports has been described in detail elsewhere⁷.

General methods of bonding

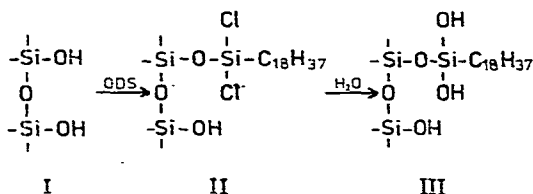
For each of the categories (a) to (c) the following conditions were employed. Partisil-10 (5 g) was added to octadecyl trichlorosilane (0.5 g) previously dissolved in toluene (50 ml). The reaction was allowed to proceed for 24 h at ambient temper-

ature. For subsequent resilanization, trimethylchlorosilane (0.5 g) was added to the heterogeneous mixture and the reaction allowed to proceed for a further 24 h. For category (d), a higher percentage of trichlorosilane (1 g per 5 g of Partisil 10) was used. For category (e), the reaction mixture was refluxed for 2 h to ensure completeness of reaction.

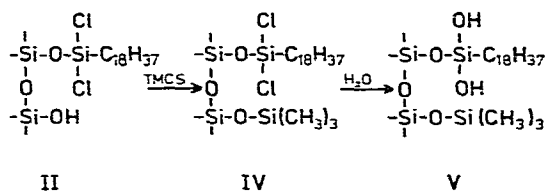
RESULTS AND DISCUSSION

Three main categories of bonding can be defined (Fig. 1).

(a) Conventional bonding with a Trichlorosilane



(b) Unreacted silanol groups "CAPPED"



(c) Unreacted & hydrolysed groups "CAPPED"

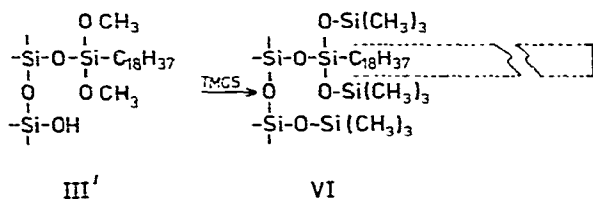


Fig. 1. Schemes for chemical bonding.

(a) Conventional bonding with a trichlorosilane. This process involves the reaction of a trichlorosilane with the silica surface. After washing the excess of reagent from the silica by multiple decantations with the bonding solvent, the structure designated II is obtained. When used in an aqueous reversed-phase mode, residual chlorosilane groups are hydrolysed to III. This structure features in the majority of published work to date.

(b) "Capping" the unreacted silanol groups. This process is an extension of the conventional bonding procedure. After the primary silanization with, for example, octadecyltrichlorosilane (ODS), trimethylchlorosilane (TMCS) is added to the reaction mixture. Being a more compact, smaller molecule than ODS, TMCS can react

with the residual surface silanol groups, particularly those deep within the support particle, which were previously unavailable to the ODS owing to steric factors. After reaction has taken place, all excess of reagent was washed from the support. When used in an aqueous reversed-phase mode, residual chlorosilane groups are hydrolysed to V.

(c) "Capping" of unreacted and hydrolysed silanol groups. Both processes described so far do not account for the hydrolysis of the unreacted chlorosilane groups. Roumeliotis and Unger³ approached this problem by synthesizing their own monochlorosilanes. We feel that this procedure is unnecessary and that the alternative hydrolysis or alcoholysis of the chlorosilane followed by resilanization with TMCS is preferable. In practice, this procedure is straightforward. After the primary-silanization, unreacted ODS is washed from the support using the bonding solvent. Methanol is then added in order to convert the chlorosilane II into the methoxy derivative (III)¹, (Fig. 1). The support is washed free of methanol using the bonding solvent and TCMS is added. After washing the residual silane from the support, the final product VI is obtained.

Two further categories can be identified, which arise from efforts to reduce unwanted adsorption effects still further: (d) conventional bonding as in (a), but with a higher concentration of reagent (1 g per 5 g of silica); and (e), as for (d) but the reaction mixture was refluxed for 2 h to ensure completeness of reaction.

A comparison of each category of bonding was carried out using a polyaromatic hydrocarbon test mixture. It is obvious from Fig. 2 that the resolution of the hydrocarbons increases as the residual silanol groups are decreased. Heavier loading

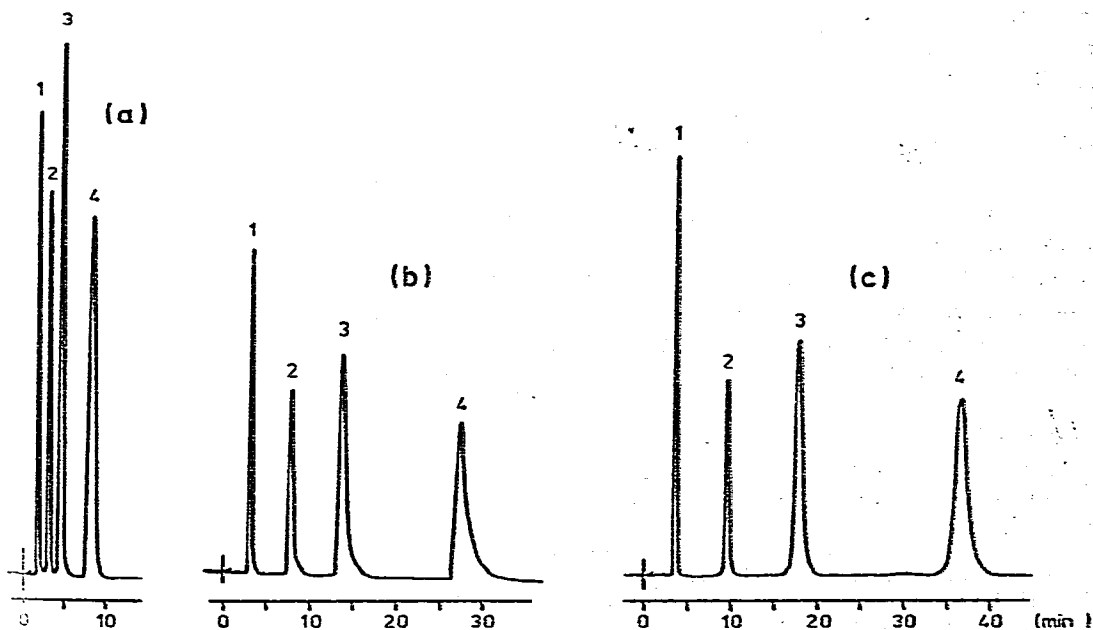


Fig. 2. Chromatograms illustrating the difference between (a) normal bonded silica, (b) bonded and "capped" silica and (c) bonded, hydrolysed and "capped" silica. Column: 10 × 0.42 cm I.D., packing as in text). Eluent: methanol-water (6:4), flow-rate 1 ml/min. Detection: UV at 254 nm. Peaks: 1 = benzene; 2 = naphthalene; 3 = diphenyl; 4 = anthracene.

of the bonded support only partially improves the chromatography, with resolution similar to (b). There is less tailing than for (b) but the efficiency is significantly less. This confirms the results of previous work⁹.

No advantage is gained by refluxing the reaction mixture.

Further, this indicates that for maximal resolution and efficiency, methanolysis followed by "capping" is to be preferred. The same trends occur for other test mixtures such as ketones and nitroimidazoles⁷. They also occur when the chain length of the bonded alkyl group is increased from C₁₈ to C₂₂ (ref. 10). In both instances the major difference is between (a) and (b). Although the difference in resolution between (b) and (c) is less significant, the improvement in peak symmetry makes (c) the support of choice.

It is relevant, at this stage, to consider the general practice of refluxing in order to bond the silica. We have already shown¹ that, at room temperature, the reaction is virtually complete after 10–15 min reaction time. An extension of this work to include refluxing after 24 h at room temperature (Fig. 3) indicates no advantage in the practice. Confirmation of this result is achieved by comparison of (d) and (e).

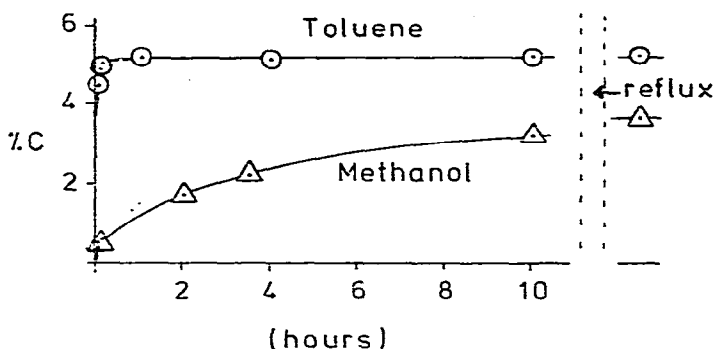


Fig. 3. Variation in carbon content of the silica support with reaction time and temperature.

In conclusion, we advocate that the optimal bonding procedure for the preparation of reversed-phase supports is method (c), which involves alcoholysis of the primary silanization followed by resilanization with TMCS. Our standard practice now is to combine this result with our previous work on solvent optimization. We now use carbon tetrachloride as the bonding solvent¹. More details of this improved methodology will be reported later.

REFERENCES

- 1 C. J. Little, A. D. Dale, J. A. Whatley and M. B. Evans, *J. Chromatogr.*, 171 (1979) 431.
- 2 E. Grushka (Editor), *Bonded Stationary Phases in Chromatography*, Ann Arbor Sci. Publ., Ann Arbor, Mich., 1974.
- 3 P. Roumeliotis and K. Unger, *J. Chromatogr.*, 149 (1978) 211.
- 4 D. C. Hunt, P. J. Wild and N. T. Crosby, *J. Chromatogr.*, 130 (1977) 320.
- 5 J. H. Knox and A. Pryde, *J. Chromatogr.*, 112 (1975) 171.
- 6 R. K. Gilpin and M. F. Burke, *Anal. Chem.*, 45 (1973) 1383.
- 7 C. J. Little, A. D. Dale and M. B. Evans, *J. Chromatogr.*, 153 (1978) 381.
- 8 C. J. Little, A. D. Dale, D. A. Ord and T. R. Marten, *Anal. Chem.*, 49 (1977) 1311.
- 9 E. J. Kikta and E. Grushka, *Anal. Chem.*, 48 (1976) 1098.
- 10 C. J. Little, A. D. Dale and M. B. Evans, *J. Chromatogr.*, 153 (1978) 543.