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Note

Capillary columns with immobilized stationary phases

I. A new simple preparation procedure

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Immobilized coatings in capillary columns offer two advantages. The first has to do with insufficient *wettability*. When a liquid does not permanently spread on a given solid support, bonding the molecules, or at least part of them, to the support surface may solve the problem. The same result may be achieved by cross-linking instead of surface bonding. In many cases of insufficient wettability an ideally uniform liquid film can be deposited for a short time or at low temperature. If cross-linking within the liquid is feasible before the film starts breaking up, the film may be perfectly stabilized. The second advantage is to have a *non-extractable* coating which cannot undergo phase stripping due to large splitless or on-column injection and which permits solvent rinsing to free the column from non-volatile sample by-products or from active breakdown products of the liquid phase.

As far as we know, K. Grob¹, in 1966–1967, was the first to attempt experimentally both bonding to the support surface and producing a cross-linked coating. He achieved bonding of terminal hydroxyl groups and of lithium-organic groups on Si-Cl groups obtained by treating the glass surface with thionyl chloride. Non-extractable coating was produced by *in situ* polymerization of polyolefins such as butadiene with boron trifluoride as a catalyst. Although the basic aims could be attained, the work was discontinued in 1968 because the coating was insufficiently thermostable. The main reason for the failure, as we know today, was the untreated glass surface, the leaching of which we introduced only in 1977².

In 1976 Madani and co-workers³ were the first to re-attack the problem by an entirely new approach. They studied *in situ* synthesis of cross-linked methyl- and phenyl methyl polysiloxanes and developed their work to an impressive level⁴. In addition to realising the two above-mentioned advantages, they were able to tailor a given polarity by preselecting the phenyl:methyl ratio. In 1978 Blomberg and co-workers⁵ entered the field on a similar line and developed their own synthetic and cross-linking techniques⁶.

The column preparation procedures of both groups are relatively sophisticated and laborious. We tried to achieve equivalent results by applying the far simpler methods which have been used industrially for more than twenty years for the pro-

duction of silicone rubber from silicone oils. These methods are comprehensively described in textbooks, for instance in the one by Noll⁷. Our trials based on this literature were almost immediately successful.

SURFACE BONDING *VERSUS* CROSS-LINKING

We have not been able to find a clear-cut definition for the widely used term "bonded phase"^{4,8}. As deduced from its common use, we feel that it emphasizes a covalent bonding between the support surface and the stationary phase, while cross-linking is treated as a secondary aspect or is even suppressed.

We think that instead of theoretically selecting an exact meaning of the term "bonded phase", a proper term should be selected which exactly describes the *experimentally proven* facts involved. From this viewpoint we have first to state that no direct proof has been forwarded so far for the existence of covalent bonds between phase and support, whereas cross-linking within the liquid is an amply proven phenomenon⁷. This statement does not at all lower the merits of the underlying technical progress, since both advantages, film stabilization on an insufficiently wetttable support, and non-extractability, are achieved by cross-linking alone.

The results of our simplified procedure are identical to those of the procedures of Madani and co-workers^{3,4} and Blomberg and co-workers^{5,6}, namely cross-linking, and very probably also bonding to the support. We feel that the practical effects combined with their structural interpretation may properly be described by the term "immobilized phase".

SIMPLIFIED PRODUCTION OF IMMOBILIZED PHASES

Silicone rubber articles are manufactured from liquid silicones by cross-linking induced by organic *peroxides*⁷. It is hard to see why this established and simple technique should not be used to immobilize stationary phases, since, if properly applied, it does not influence the organic coating in ways other than cross-linking and probable bonding to the support surface. According to Noll⁷, the peroxide transforms a methyl group into a radical which then reacts with a methyl group of a neighbouring molecule to form a covalent bond. Corresponding reactions between other groups occur less frequently. The reaction products of most peroxides are volatile or, in case of a side reaction producing a heavier or more polar reaction product, can be extracted from the immobilized phase. The latter case may explain the frequent observation that solvent-extracting a freshly immobilized coating slightly lowers the adsorption activity and the polarity of the column.

Preparation of an immobilized coating simply involves adding a peroxide to the liquid phase before carrying out the regular static or dynamic coating and changing slightly the regular procedure for conditioning the freshly coated column. Instead of programming the temperature to the upper temperature limit of the column, the temperature has to remain for one or a few hours at a level (100–140°C) favouring the cross-linking reaction. Conditioning should then be continued to evaporate the peroxide breakdown products.

The standard peroxide is dibenzoyl peroxide (DBP). Further study may reveal a different peroxide that is even more suitable. A somewhat disadvantageous feature

of DBP is its volatility; possibly it causes some overlapping of the cross-linking reaction with evaporation of the peroxide. Bis(dichlorobenzoyl)peroxide may be advantageous in this respect.

Reasonable immobilization may be obtained by a fairly broad range of peroxide concentrations. Excessive amounts of peroxide produce increased column activity; amounts which are too low result in increased losses of stationary phase upon rinsing. It may be surprising, however, that strongly varied amounts of peroxide hardly influence column characteristics such as polarity, separation efficiency and loading capacity (apparent film thickness). Thus, the density of cross-links does not seem to be a critical factor provided it exceeds a certain minimum.

Immobilization by reaction with a peroxide is, at the present time, limited to silicones of low or very moderate polarity. We have not yet successfully immobilized phases such as OV-17 and OV-225. However, this is not surprising since, for the same reason, these phases are not available as gums.

PRACTICAL PROCEDURE

Column

Immobilization works, as far as cross-linking is concerned, on any kind of support surface. However, maximum advantage in terms of column quality and thermostability is obtained with persilanized glass or fused silica. Persilanization⁹ is carried out the same way as for conventional inert columns.

Phases

All silicones of low polarity (the polarity limit may be represented by the phase OV-61) can be used with the same solvents (*e.g.*, pentane, methylene chloride) and concentration as used without cross-linking. As soon as the peroxide has been added, the solution should be kept dark and cool for a minimum time period.

Peroxide

DBP was used as purchased (*e.g.*, No. 33581 from Fluka, Buchs, Switzerland). We prepared a 2% (w/v) solution in benzene which was kept dark and cool. The concentration of the peroxide in the pure phase was 0.1–1.0% (w/w). An example may eliminate possible misinterpretations. To 10 ml of a 0.2% solution of OV-1 in pentane, 4 μ l of the 2% DBP solution are added with a regular syringe to yield a concentration of 0.4% DBP in pure OV-1.

Conditioning

After regular static or dynamic coating the column is mounted into a gas chromatograph without the outlet to the detector. For 15–20 min a high carrier flow-rate of 2–4 ml/min for a 0.3 mm I.D. capillary is applied. The flow-rate is then reduced to 0.1–0.2 ml/min to prevent premature evaporation of DBP. The temperature is quickly raised to 100°C and is programmed at a rate of 0.2°/min to 130°C. In case the programmer does not offer this low program rate, the temperature is raised manually by keeping it for 1 h each at 100, 110 and 120°C. After maintaining 130°C for at least 1 h, the carrier flow-rate is increased to 2 ml/min, and the temperature is simultaneously set at 200°C for 1 h. The column is cooled, the exit is connected to the

detector, and the first quality test is run, which has to include a capacity ratio or an exact elution temperature in the case of our comprehensive test¹⁰. To check the degree of immobilization, as well as to eliminate possible impurities, the column may be rinsed with two column volumes of methylene chloride and one volume of pentane. The test is repeated. The column may show a slightly reduced adsorption activity. Proper immobilization is demonstrated by a loss of stationary phase of 5% or less, as calculated from the capacity ratio, or by an elution temperature lowered by 1°C at most. Further treatment is as for conventional inert columns.

In case of thickfilm (0.8 μm) columns we have repeatedly observed that prolonged rinsing (twenty column volumes of methylene chloride, overnight) produced a slight apparent increase of film thickness.

To prevent any premature evaporation of DBP we have sealed some columns after short conditioning at room temperature. The reaction at 100–130°C produced surprisingly active columns, however. We suspect that this is due to the water content of the commercially available DBP.

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REFERENCES

- 1 K. Grob, *Helv. Chim. Acta*, 51 (1968) 729.
- 2 K. Grob, G. Grob and K. Grob, Jr., *Chromatographia*, 10 (1977) 185.
- 3 M. Rigaud, P. Chebroux, J. Durand, J. Maclouf and C. Madani, *Tetrahedron Lett.*, 44 (1976) 3935.
- 4 C. Madani and E. M. Chambaz, *J. Amer. Oil Chem. Soc.*, 58 (1981) 63.
- 5 L. Blomberg, J. Buijten, J. Gawdzik and T. Wännman, *Chromatographia*, 11 (1978) 521.
- 6 L. Blomberg, K. Markides and T. Wännman, *J. Chromatogr.*, 203 (1981) 217.
- 7 W. Noll, *Chemie und Technologie der Silicone*, Verlag Chemie, Weinheim, 1960.
- 8 M. L. Lee and B. W. Wright, *J. Chromatogr.*, 184 (1980) 235.
- 9 K. Grob, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 493.
- 10 K. Grob, Jr., G. Grob and K. Grob, *J. Chromatogr.*, 156 (1978) 1.