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## MONOMERIC AND POLYMERIC DERIVATIZATION IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC MATERIALS

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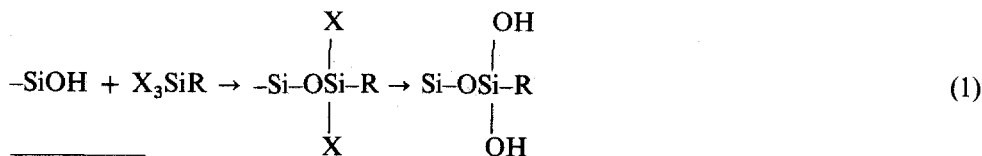
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### SUMMARY

Data and arguments are presented showing that the clear distinction between so-called "monomeric" and "polymeric" derivatised silica gel phases is not warranted. Our conclusion is that most "polymeric" phases are in fact "monomeric".

Monomeric high-performance liquid chromatographic (HPLC) stationary phases are derivatized silica gels obtained with silanes having only one reactive function, *e.g.*, chlorodimethyloctylsilane. After bonding, all further reaction is impossible and only one alkyl group can be bonded per silanol function available in the starting material. Polymeric HPLC stationary phases are obtained with silanes having more than one reactive functional group, *e.g.*, octadecyltrichlorosilane or aminopropyl-triethoxysilane\*. After bonding via a first reactive function, there are other reactive functions left in the silane moiety, even after forming a siloxane bridge between two neighbouring silanol groups and two reactive silane functions of one silane reagent molecule. These reactive functions can hydrolyse with traces of water in the medium and the newly formed silanol group can bond another silane molecule. This leads to polymeric derivatization.

The above summarizes the reasoning developed in the recent literature. Often it is then suggested or ascertained that monomeric derivatization is to be preferred because superior results are obtained. These tendencies in HPLC are clearly presented in Majors' review<sup>1</sup>. The important difference between the two kinds of phases is that as a result of multi-functional silane bonding, more silanol functions appear in the end product than were originally present on the silica gel matrix. Unreacted functional groups indeed hydrolyse during washing or handling the reversed phase in non-anhydrous conditions. This is formulated as follows:



\* The terms "monomeric or polymeric" derivatization are generally used in the literature. A case could be made for using the terms "monolayer or multilayer" instead, as pointed out by the reviewer of this paper. We prefer, however, to retain the currently used nomenclature even if it is not perfect.

where X is a chlorine or alkoxy function. Two silanol groups are present where originally there was only one. With a relatively dense loading of the silica gel surface and with large R groups, the silanol groups are relatively effectively shielded. Such phases are, however, more polar than those in which two methyl groups are bonded to the silane reagent. The polar interference of these silanol groups, it is stated, leads to disadvantageous properties of polymeric phases, inducing tailing and low efficiency. "End capping" or trimethylsilylation of the still accessible silanol functions can remove this difficulty. This needs extra effort, however, and the overall result is not expected to be as reproducible or reliable as with monomeric derivatization. A disadvantage of monomeric derivatization is that the lower degree of bonding leads to phases that are more liable to dissolution by polar solvents in reversed-phase HPLC. Polymeric phases would be more stable. The above is only a very short summary and

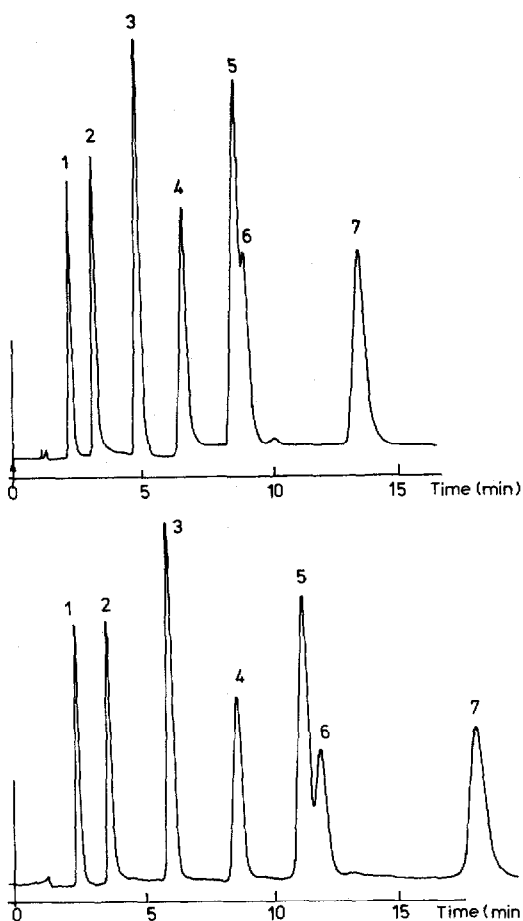


Fig. 1. Chromatogram obtained on a Varian 5020 instrument with a  $25 \times 0.46$  cm I.D. column packed with  $10\text{-}\mu\text{m}$  RSiL octadecylated with chlorodimethyloctadecylsilane to 20% bonded material. Polarity mixture sample: 1 = benzyl alcohol; 2 = acetophenone; 3 = anisole; 4 = benzyl chloride; 5 = toluene; 6 = 1-nitronaphthalene; 7 = naphthalene. Upper trace, without end capping; lower trace, same chromatographic conditions but after trimethylsilylation of the phase.

the interested reader is referred to Majors' review<sup>1</sup> and to a paper by Berendsen and De Galan<sup>2</sup> for more details and for further references on the subject.

According to the above views, a derivatized phase fully loaded with dimethyl-octadecyl chains would have no accessible residual silanol functions. End capping would therefore be unnecessary and should not in fact affect the chromatographic performance of such a reversed-phase silica gel. This is not so, as is shown in Fig. 1.

After end capping, the retention time is substantially increased (although there is no measurable increase in the percentage of bonded material) and the peak shape and efficiency are slightly better. The situation regarding "monomeric" and "polymeric" phases is therefore not clear. Even "monomeric" phases are better end capped.

As polymerization of polyfunctional silanes is induced by traces of water, we examined the influence of added water. Some results are given in Table I.

The derivatizations mentioned in Table I were carried out on 5- $\mu$ m ROSiL (Nos. 1-7) and on 10- $\mu$ m RSiL (Nos. 8-15), which are spherical and irregular shaped silica gels from Alltech (Arlington Heights, IL, U.S.A.). The dried gel was heated in dry xylene at about 80-90°C and the desired water amount was added. After equilibration a four-fold excess of silane was added and the mixture was heated for 4 h. The derivatized silica gel was washed thoroughly with xylene, acetone and methanol before drying. The percentage loading was determined by thermogravimetric analysis (TGA) as the weight loss between 200 and 900°C. The blank value between these temperatures for underivatized material was deduced.

The degree of loading increases on adding water to the reaction medium. It is surprising, however, that this also occurs with the monofunctional silane phases.

TABLE I

EFFECT OF THE PERCENTAGE OF WATER ADDED TO THE DERIVATIZING REACTION MEDIUM ON THE PERCENTAGE OF ORGANIC MATERIAL BONDED ON SILICA GEL BY REACTION WITH MONO- AND TRIFUNCTIONAL SILANES

No.	Silane	Water added (%)	Bonded organic material determined by TGA (%)
1	$\text{Cl}_3\text{SiC}_8\text{H}_{17}$	0	11.6
2		1.5	15.5
3		3.0	18.5
4	$\text{ClMe}_2\text{SiC}_8\text{H}_{17}$	0	12.0
5		1.5	15.0
6		3.0	15.6
7		7.0	18.5
8	$\text{Cl}_3\text{SiC}_{18}\text{H}_{37}$	0	10.5
9		0.2	11.0
10		0.5	12.0
11		1.0	13.9
12		1.5	16.0
13		2.0	17.0
14		5.0	25.6
15		10.0	30.0

With trichlorooctadecylsilane, a loading above 20% leads to materials that have rubbery characteristics. This can actually be felt and seen by compressing the phase with a spatula. Not surprisingly, this leads to columns with increased back-pressure. According to Berendsen *et al.*<sup>3</sup> the limiting monofunctional covering for a 8 nm average pore diameter silica gel and for silanes with longer alkyl chains is *ca.* 3.0  $\mu\text{equiv./m}^2$ . For 10- $\mu\text{m}$  RSiL with a surface area of *ca.* 300  $\text{m}^2/\text{g}$  and an average pore diameter of 8 nm, 1 g of the material can take up 900  $\mu\text{equiv.}$  of octadecyltrichlorosilane. This would lead to a weight increase of *ca.* 280 mg, assuming the extra chlorine atoms (two for monofunctional bonding, only one for bifunctional bonding) are replaced by hydroxyl groups. On pyrolysis in TGA analysis, all carbon and hydrogen presumably is lost and the two hydroxyl groups would eliminate water. Only 242 mg would be lost by 1280 mg of the phase, or about 20%. All loadings above this level must be (partially) polymeric in character.

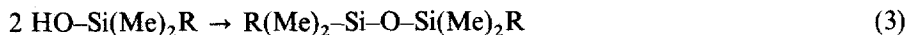
It is obvious that calculations such as those above are easy but that the conclusions cannot be accepted too rigorously. The difficulties and pitfalls in this respect have been discussed at length by De Galan and co-workers<sup>2,3</sup>. The assumption that silicon is not eliminated from derivatized phases by TGA is also questionable. In some instances we have found silicon-containing volatile compounds by combined pyrolysis-gas chromatography-mass spectrometry. Still, we believe that the general line of the above reasoning is correct.

Erhard and Kováts<sup>4</sup> gave some slightly higher experimental figures than De Galan and co-workers for the highest possible coverage of silica gel surfaces: 4.1  $\mu\text{mol/m}^2$  for decyldimethyl chains and 4.74  $\mu\text{mol/m}^2$  for trimethylsilyl groups. It is obvious that coverage should be expressed in this way and not as a percentage of bonded material. At the highest possible coverage this percentage is therefore determined by the specific surface area of the silica gel (full hydration and a normal pore size distribution are presumed). A 10% bonded octadecylsilica gel can be highly or only partially loaded according to the specific surface area. Very different chromatographic results can be expected for both materials. In Fig. 1, the 20% coverage is very high for the silica gel used and for monomeric substitution. However, end capping has a marked effect.

With a monofunctional silane the derivatizing reaction is simple to formulate:



Adding water to such a reacting mixture would presumably lead to hydrolysis of the Cl-Si bond and to the formation of a hexaalkyldisiloxane:



Reaction 3 removes silane reagent from bonding possibilities. Instead of a smaller amount of bonded silane, adding water rather increases the number of bonded chains. In our opinion, this shows that the following reaction is easier than reaction 1 or 2:



This does not mean that reactions 1 and 2 do not occur; even in rigorously dry

conditions silane is bonded extensively by silica gel. The less reactive, better "hidden" silanol functions probably react, however, via path 4 and for this some water has to be present in the medium. We think that this shows that the silane is first adsorbed on the silica gel and reacts from this state. Further, even with a large percentage of water in the medium (3% is more than equivalent to the amount of silane used), the degree of loading is virtually the same for the trifunctional and monofunctional silanes, even under polymerization-promoting conditions (added water). When water is added to the derivatization reaction medium with trifunctional silanes, oligomers such as dimers or trimers must be formed. These still bear reactive functions and compete for bonding with the original silane reagent. With up to 3% of added water no oligomers seem to bond, otherwise the TGA % would be higher. We explain this by assuming that the concentration of these oligomers is low and by the greater steric hindrance of these larger molecules. Multi-step addition of more than one polyfunctional silane to a single silanol function in the matrix also apparently does not occur with only 3% of water added. Otherwise, again, the "polymeric" phase would lead to a decidedly greater TGA % than that of the "monomeric" phase. To reach more than 20% bonding or, in other words, to obtain polymeric phases with octadecyl chains on the silica gel we used, a large excess of water is necessary. These are not the conditions for reversed-phase synthesis as generally described in the literature. The coverage of derivatized silica gels should be expressed in  $\mu\text{mole}/\text{m}^2$ ; as long as this does not exceed 3–3.5  $\mu\text{mole}/\text{m}^2$  no polymeric bonding is present, even when the silane reagent



Fig. 2. Chromatogram obtained with a Varian LC 5020 instrument with  $25 \times 0.46$  cm I.D. columns packed with spherical 5- $\mu\text{m}$  ROSiL derivatized with octyldimethylchlorosilane (above) and octyltrichlorosilane (below). Both phases end capped. Chromatographic conditions and sample as in Fig. 1.

was trifunctional. Some available commercial phases approach this level of coverage, but none exceeds it. We believe, therefore, that many phases obtained with trifunctional silanes and therefore with supposedly polymeric character are in fact as "monomeric" as phases obtained from monofunctional silanes. There is a difference in polarity, though, between phases obtained with mono- and trifunctional silanes (two trimethylsilyloxy groups against two methyl groups). There is a difference in selectivity, but the performance of "monomeric" and "end capped polymeric" phases is virtually the same, as shown in Fig. 2.

#### ACKNOWLEDGEMENTS

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#### REFERENCES

- 1 R. Majors, *J. Chromatogr. Sci.*, 18 (1980) 488.
- 2 G. Berendsen and L. de Galan, *J. Liquid Chromatogr.*, 1 (1978) 561.
- 3 G. Berendsen, K. Pikaart and L. de Galan, *J. Liquid Chromatogr.*, 3 (1980) 1437.
- 4 J. F. Erard and E. Kováts, *Anal. Chem.*, 54 (1982) 193.