RELATIONSHIP BETWEEN CHROMATOGRAPHIC PERFORMANCE AND SURFACE STRUCTURE OF SILICA MICROSPHERES

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SUMMARY

Spherical silica packing material has been thermally modified at temperatures ranging from 220–950°. The chromatographic performance of the modified columns was tested using several solutes ($k' = 0$–20) with hexane as the eluent. The parameters k', V_r, α, R_s, and Ø have been obtained and correlated with total surface hydroxyl concentration (n_{OH}).

At each corresponding temperature, trimethylchlorosilane was added to the silica, thus effectively blocking the single surface hydroxyl groups. Comparisons of the two sets of results give interesting information concerning the adsorption processes in high-performance liquid chromatography as well as helping with the other important considerations in column performance optimization.

INTRODUCTION

The importance of fully understanding the adsorption processes in liquid–solid chromatography cannot be overstressed. It is, after all, the basis of any predictions that are to be made on chromatographic separations. Therefore, it is obvious that any improvement in the knowledge of adsorption must help the practical chromatographer in the laboratory.

The "assumed" adsorption sites of silica gels, that is the surface hydroxyl groups, have been extensively studied during the past fifteen years, elucidating a surface structure that is now well known[1–3]. IR spectroscopy, chemical modification, and thermogravimetric analysis (TGA) have been extensively used in these studies.

However, a great deal of confusion remains about certain parts of the adsorption process. The most important deficiency of knowledge in this area is the matter of whether the single hydroxyl groups, or the hydrogen-bonded hydroxyl groups, or even the siloxane bridges are the principal adsorption sites in silica gels.

The total surface hydroxyl group concentration is obtained using TGA and heating the silica between two fixed temperatures[1,3–6]. Other workers have obtained the concentrations of the individual surface hydroxyl species[2,5,7,8] using such methods as selective silanization to block a particular hydroxyl species.

This paper sets out to pin-point the active sites involved in the adsorption process on silica gel, from which a greater control of the degree of adsorption, and

hence a better knowledge of separation can be obtained. Another advantage of this data is that surface coverage in bonded phase chromatography can be controlled to a much greater extent, especially as regards deciding which hydroxyl group species should be blocked and which should be coated.

During the course of the investigation, other parameters were noted and are discussed with a view to optimizing the overall column performance.

EXPERIMENTAL

The liquid chromatograph consisted of a "home-made" coil-pump, having a safety valve in the system to avoid any back-pressure to the solvent reservoir. The coil was made of 50-ft. × ¼-in. PTFE tubing and was coiled around two metal supports. The coil was pressurised by a nitrogen cylinder and connected to the injection system by PTFE tubing. A Budenberg gauge (Broadheath, Manchester, Great Britain) in the system gave an accurate pressure drop across the column. A loop injector [Chromatronix (Berkeley, Calif., U.S.A.), Model CSV, 2-μl slug volume] was used and the detector was a variable-wavelength (set at 254 nm) Cecil (Cambridge, Great Britain) Model CE212 UV spectrometer.

The silica gel was from the same 100-g batch of Spherisorb S10W[*]. It was used straight from the manufacturer's container, with no further treatment.

The silica was slurry-packed into a 10.6-cm × 4-mm-I.D. stainless-steel column (with a stainless-steel Swagelok reducing union, fitted with a 2-μm porous stainless-steel frit, at the detector end of the column) using the following method.

1.5 g of Spherisorb were placed in approximately 5 ml of methanol. The slurry was syringed into a pre-column (25 cm × 4 mm I.D.) joined to the column to be used by a straight union and the two columns were topped up with methanol. A stainless-steel coil with 100 ml of methanol was pressurised with nitrogen at 3000 p.s.i. and connected, through an open/shut valve, to the top of the pre-column. The valve was opened and the 100 ml of methanol were run through the column until nitrogen gas was seen coming out of the end of the column. The valve was closed and the pre-column disconnected from the main column. This technique has been shown to be extremely efficient and reproducible and is completed in 15 min[5,9,10].

The top of the column packing was levelled off and a small piece of 5-μm stainless-steel mesh was placed over the top of the column. Another reducing union fitted with a frit was placed over the injector end, and connected to the loop injector.

Hexane was allowed to run through the column until complete equilibration was achieved (approximately 10 min at a flow-rate of 3.0 ml/min). Testing was done using the following solutes: (1) toluene, (2) nitrobenzene, (3) acetophenone, and (4) 2,4-dinitrotoluene. Two runs were made at five different flows, the average values being taken. The five flow-rates were 1.6, 1.8, 2.2, 2.6, and 3.0 ml/min and were taken to obtain average values for the flow-independent parameters that were measured.

On completion of this set of tests, the column was heated with nitrogen flowing through it for 1 h in an oven at 120° to remove all the hexane before the furnace heating. The column was heated with a nitrogen flow again at 220° in a tube furnace for 2

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[*] Spherisorb is made by A.E.R.E., Harwell, Great Britain, and supplied by Phase Separations, Deeside, Great Britain.

h, then cooled. Testing was continued as for the first column ("room temperature"). The column was returned to the 120° oven for 1 h, then heated for 2 h at the next temperature. This process was repeated for the following temperatures: 220°, 340°, 485°, 600°, 705°, and 950°. The reason for the somewhat arbitrary temperatures was that for previous work a furnace was used that was difficult to set at a specific temperature and comparisons were required. The furnace used for the heating was a tube furnace made in the workshop, controlled by a temperature setter (WestGardian) to within ± 5°.

When the complete process was finished, another column was prepared and tested in exactly the same way. The results shown are an average for the two columns.

For the trimethylchlorosilane (TMCS)-treated columns, the following procedure was employed. A 10.6-cm × 4-mm-I.D. stainless-steel column was slurry-packed with 1.5 g of Spherisorb S10W silica. After initial testing with hexane as eluent, 100 ml of 5% TMCS in toluene were passed through the column to give an *in situ* coating to the silica. The column was examined again with hexane eluent and the standard test mixture.

In the case of the TMCS testing, a new column had to be packed for every different temperature. The procedure was to heat the packed column in a nitrogen flow at the required temperature for 2 h, test with hexane, add the TMCS, unpack the column, repack and then proceed to the next temperature.

The silica samples from each column were dried to remove the hexane and stored in a desiccator over phosphorous pentoxide. TGA measurements were obtained for these samples as were small batches of Spherisorb S10W silica heated at the corresponding temperatures for 2 h in a nitrogen atmosphere. The TGA measurements were made on a Stanton Redcroft thermobalance by heating the sample to constant weight at 120°, then to constant weight at 1100°. The weight difference gave the percentage weight loss due to the hydroxyl groups.

RESULTS AND DISCUSSION

At low temperatures the surface of silica possesses two distinctly different types of hydroxyl group, one being the single hydroxyl group, the other being the hydrogen-bonded hydroxyl group. They have quite different properties, for example, in the IR spectroscopy spectra of silica the single hydroxyl group gives a sharp peak at 3780 cm^{-1}, whereas the hydrogen-bonded hydroxyl group gives a broad band from 3200–3650 cm^{-1} (refs. 11 and 12).

These two hydroxyl species are the main factors in adsorption at low temperatures, but the relative importance of each is not known. The reason for the TMCS additions is to block the single hydroxyl groups selectively, leaving the hydrogen-bonded hydroxyl groups available for adsorption. Comparison of the results from the untreated and treated material gives more information about the overall adsorption process.

Heating silica above 500° converts many of the hydroxyl groups to siloxane groups, which may have importance in the adsorption process on silica, *i.e.*,

OH   OH   O
 | | ———→   ╱   ╲
Si — Si   500°   Si — Si
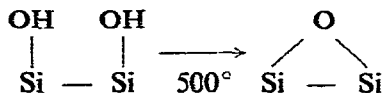
In Fig. 1 the concentration of the total surface hydroxyl groups can be seen to decrease with an increase in temperature. The rate of decrease is large up to 120°, due to the fact that molecular water is being driven off at this temperature. From 120–450°, a slow steady decrease of total surface hydroxyl group concentration occurs, due to the hydrogen-bonded hydroxyl groups being removed[1,4]. Above 500°, the only hydroxyl groups left on the surface are the single hydroxyl groups. They leave the surface at a slower steady rate until at 1100° the surface is completely free of all hydroxyl groups. This result agrees with previous work[1,4,6].
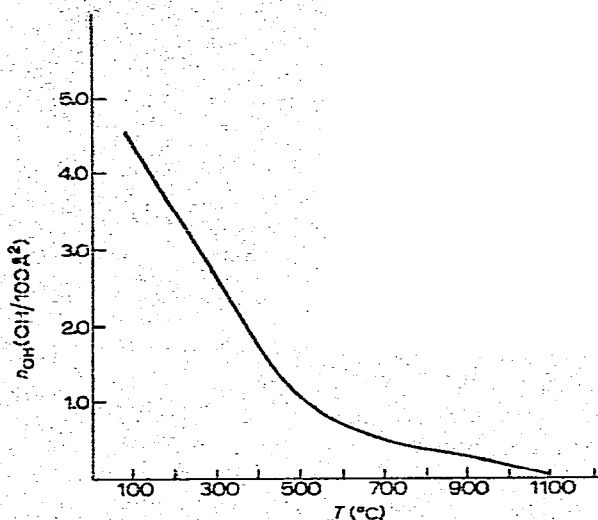

Fig. 1. TGA measurements for Spherisorb S10W silica.

The individual concentrations of the different hydroxyl group species on silica gels have been calculated by several workers[5,7,8,13,14] and are presented here: total n_{OH} = 4.6 OH/100 Å^2, single n_{OH} = 1.2 OH/100 Å^2, and hydrogen-bonded n_{OH} = 3.4 OH/100 Å^2.

How exactly this affects the chromatographic performance of the Spherisorb S10W silica can be seen by comparing Table I, which gives the parameters obtained from the Spherisorb S10W silica chromatograms, and Table II, which gives the parameters from the Spherisorb S10W silica plus TMCS chromatograms.

To clarify the full extent of the temperature changes (i.e., the n_{OH} changes) on the retention characteristics of the silica, the results are presented in Figs. 2–4, showing graphs of the temperature of heating plotted against the various parameters for both the Spherisorb S10W silica and the Spherisorb S10W silica plus TMCS. A few explanatory notes on these parameters are given below:

$$k' \text{ (column capacity ratio)} = \frac{t_r - t_0}{t_0}$$

where t_0 is the retention time for an unretained peak and t_r the retention time for a retained peak r.

TABLE I

MEASURED PARAMETERS FOR SPHERISORB S10W SILICA AT VARIOUS TEMPERATURES

1 = Toluene; 2 = nitrobenzene; 3 = acetophenone; 4 = 2,4-dinitrotoluene.

Parameter	25°				220°				340°			
	1	2	3	4	1	2	3	4	1	2	3	4
k'	0.18	7.54	16.31	21.71	0.48	0.92	1.89	3.16	0.55	1.21	3.7	5.22
V_r	0.17	1.26	2.55	3.35	0.22	0.29	0.42	0.61	0.23	0.32	0.69	0.92
α	4.2		1.33		1.92		1.68		2.19		1.41	
R_s	4.26		1.18		0.81		1.09		1.27		1.05	
Φ	419				417				390.6			

Parameter	485°				600°				705°			
	1	2	3	4	1	2	3	4	1	2	3	4
k'	0.57	1.64	4.17	6.02	0.8	2.57	5.49	7.89	1.02	3.49	7.57	10.84
V_r	0.23	0.39	0.76	1.03	0.26	0.52	0.96	1.31	0.3	0.64	1.23	1.7
α	2.89		1.44		3.21		1.43		3.44		1.43	
R_s	1.64		1.06		2.17		1.18		2.09		1.05	
Φ	399				404				338			

TABLE II

MEASURED PARAMETERS FOR SPHERISORB S10W SILICA PLUS TMCS AT VARIOUS TEMPERATURES

1 = Toluene; 2 = nitrobenzene; 3 = acetophenone; 4 = 2,4-dinitrotoluene.

Parameter	25°				220°				340°				485°			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
k'	0.49	8.9	9.05	9.96	0.42	2.49	2.97	3.56	0.33	0.52	0.67	0.9	0.24	0.24	0.57	0.57
V_r	0.22	1.42	1.44	1.57	0.21	0.51	0.58	0.67	0.2	0.22	0.25	0.28	0.18	0.18	0.22	0.22
α	18.53		1.1		6.06		1.1		1.58		1.33		1.0		1.0	
R_s	3.71		0.36		0		0		0		0		0		0	
Φ	487				505				519				693			

Parameter	600°				705°				950°			
	1	2	3	4	1	2	3	4	1	2	3	4
k'	0.18	0.18	0.18	0.18	0.1	0.1	0.1	0.1	0	0	0	0
V_r	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15
α	1				1				1			
R_s	0				0				0			
Φ	964				1229				1684			

V_r (retention volume, ml) $= t_r \cdot F$
where F is the flow-rate (ml/sec).

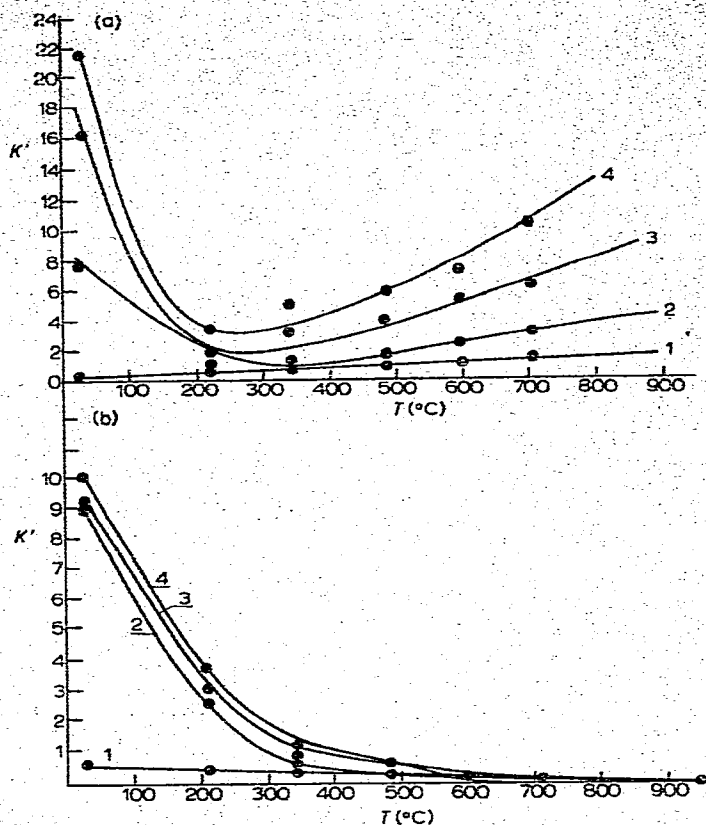α (column selectivity) $= \dfrac{k_1'}{k_2'}$

Fig. 2. k' data for Spherisorb S10W silica (a) without and (b) with TMCS.

where $k_1' = k'$ of solute 1 and $k_2' = k'$ of solute 2.

$$R_s \text{ (resolution between two peaks)} = \frac{1}{4}(N)^{-\frac{1}{2}} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k'}{k' + 1}$$

where N = total number of theoretical plates = $5.54\,(\frac{1}{2}W_r/t_r)^2$, where $\frac{1}{2}W_r$ = width of peak at half height.

$$\Phi \text{ (flow resistance parameter)} = \frac{P\,\delta\,L}{u\,d_p^2}$$

where P = pressure drop across the column (N/m^2), δ = viscosity of solvent (N sec/m^2), u = flow velocity (m/sec), d_p = particle size (μm), and L = column length (m). For this system $\Phi = (P/u) \times 0.02085$ for $L = 10.6$ cm, $\delta = 0.312 \times 10^{-3}$ N sec/m^2, and $d_p = 10\,\mu$m.

Figs. 2 and 3 show that for Spherisorb S10W silica alone, a similar relationship is found for all the different parameters involved in retention, with respect to temperature. The decrease in the value of the retention parameters (k', V_r, α, R_s) up
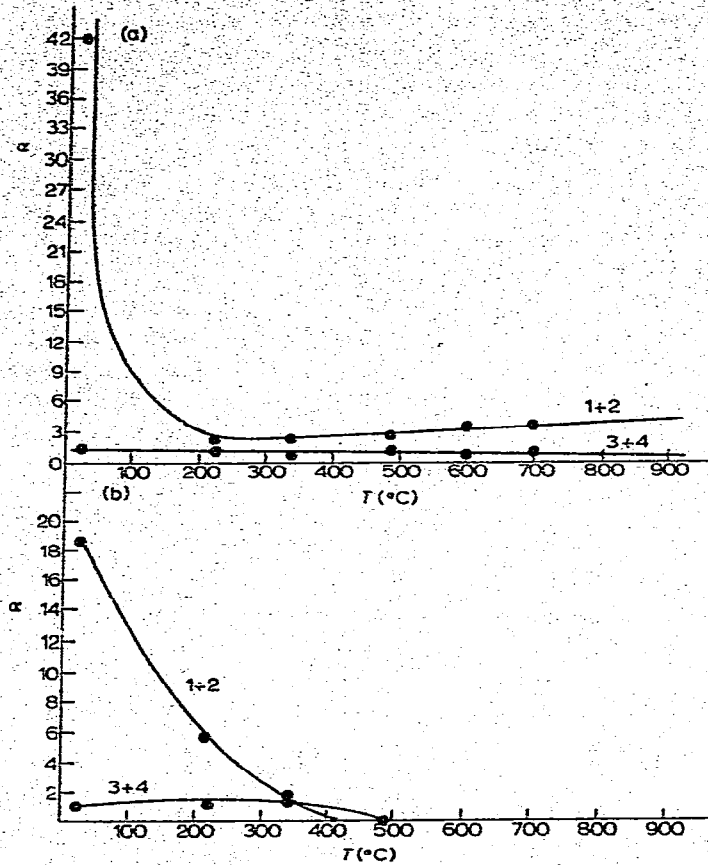
Fig. 3. α data for Spherisorb S10W silica (a) without and (b) with TMCS.
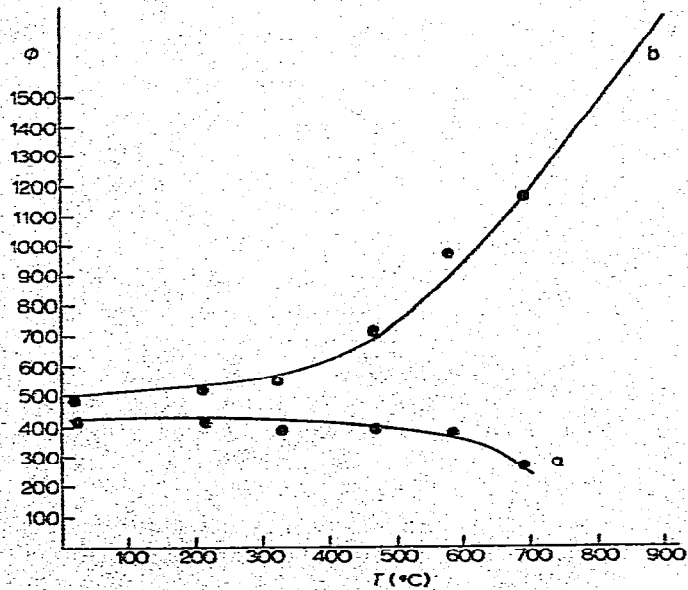

Fig. 4. Φ data for Spherisorb S10W silica (a) without and (b) with TMCS.

to 200° is directly correlated to the initial loss of hydroxyl groups on the silica surface. Above 200° the retention parameters begin to rise gradually, though at a much lower rate of increase than the initial decrease in value. There is nothing noticeably happening at the 450–500° region, which by examination of Fig. 1 would be expected.

A possible explanation of why there is no observed change in the retention characteristics at 450° lies in the postulation of there being more to the simple ideas of adsorption than was at first thought. If the single hydroxyl groups were the only adsorption sites on the silica surface at this temperature, then a reduction in the retention parameters would be expected (due to the fact that the single hydroxyl groups are steadily removed above 500°). But as the graphs in Figs. 2 and 3 show, this is not happening and another adsorption process involving a further active site must be present. It is at these temperatures that the siloxane bridges are formed and adsorption may be occurring through these groups. The active sites would be expected to be more active on these bridges due to their strained structure. Therefore, an increase in adsorption would be expected with larger values for the retention parameters. Combining the expected results of a drop in adsorption caused by the removal of the single hydroxyl groups and an increase in adsorption caused by the formation of the strained siloxane bridges, a steady increase in the retention parameters values would be expected. This is what is observed.

Up to 200° it will be assumed that both the single and the hydrogen-bonded hydroxyl groups play the major part in the adsorption mechanism. This would cause the retention parameters to be large due to the strong adsorption. Once again agreement with results is found. Assuming this, then any removal of the surface hydroxyl groups will cause the retention parameters to decrease. The experimental results bear this out.

Above 200° the hydrogen-bonded hydroxyl groups are being constantly removed but the retention parameters are increasing in value. An explanation of this is that the somewhat sterically hindered single hydroxyl groups with much stronger active centres are taking over in the adsorption process. This would cause the observed increase. The explanation for the process involved above 500° has been mentioned above.

In Fig. 5, a summary of the surface structure of the Spherisorb S10W silica at various temperatures is given, based on the results obtained.

In Fig. 6 the concentration of the surface hydroxyl groups for Spherisorb S10W silica plus TMCS is plotted against temperature. The n_{OH} falls off steadily from 200–500°, indicating the loss of hydrogen-bonded hydroxyl groups. Above 500° the n_{OH} is very close to zero (as it should be, considering the TMCS blocks all the single hydroxyl groups), indicating that the surface is free of hydroxyl groups. In Fig. 7, the surface structure of the Spherisorb S10W silica plus TMCS is shown for various temperatures.

At room temperature TMCS reacts with the silica, effectively blocking all the single hydroxyl groups. Due to the presence of the hydrogen-bonded hydroxyl groups, the adsorption is still quite strong, even though the retention parameters are markedly reduced compared with those of the corresponding untreated silica. Values of k' at 350° are less than 1.0, showing the further removal of the hydrogen-bonded hydroxyl groups. At this stage peaks are coming out together. This is understandable because there are very few hydroxyl groups on the surface, and at 485°, where all the hydroxyl
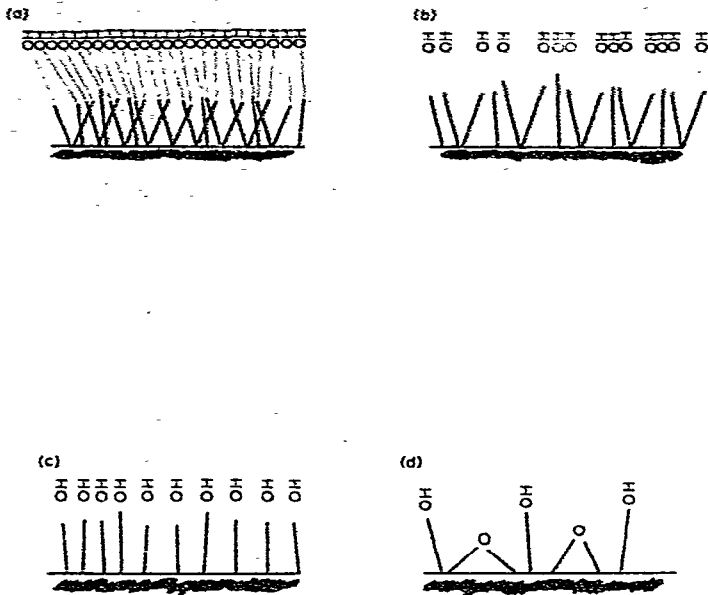
Fig. 5. Surface structure of Spherisorb S10W silica at various temperatures. (a) Room temperature; $k' = 20$. (b) $T = 300°$; $k' = 3$. (c) $T = 450°$; $k' = 5$. (d) $T = 700°$; $k' = 7$.
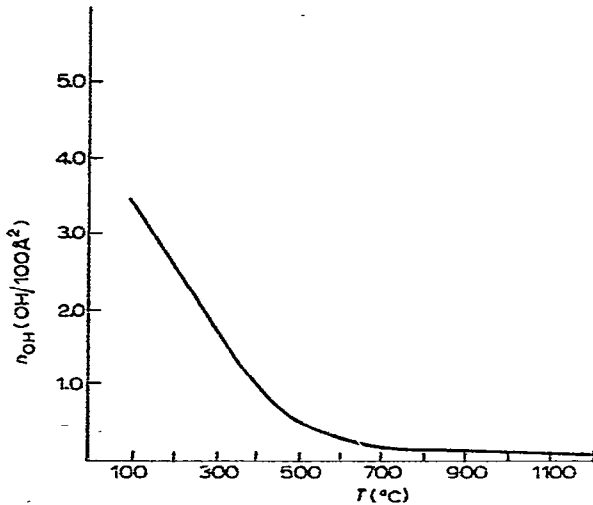

Fig. 6. TGA measurements for Spherisorb S10W silica plus TMCS.

groups have been removed, k' values are almost zero. The remaining adsorption is probably due to the few siloxane bridges that are not sterically blocked.

Fig. 4 shows the relationship between the flow resistance parameter (Φ) and the temperature of heating, for both Spherisorb S10W silica and Spherisorb S10W silica plus TMCS. For the silica alone the value of Φ hardly changes over the whole temperature range until 600°, where it decreases. The flow resistance parameter varies
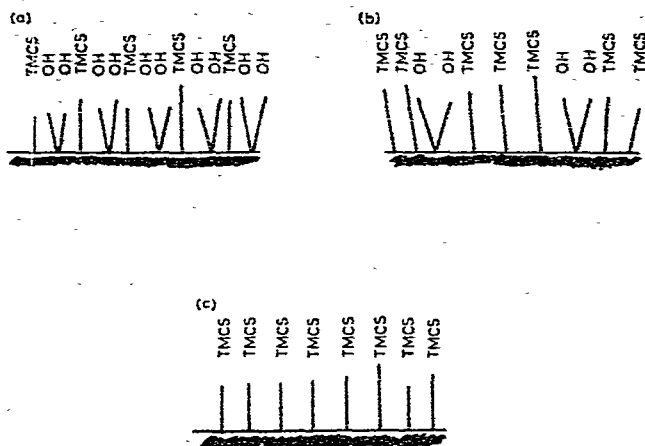
Fig. 7. Surface structure of Spherisorb S10W silica plus TMCS. (a) $T = 200°$; $k' = 4$. (b) $T = 350°$; $k' = 0.9$. (c) $T = 500°$; $k' = 0.5$.

for silicas that have different properties, as well as for varying packing character-istics. Optimum column performance is obtained by having a value of Φ as low as possible. Comparing results obtained for Spherisorb S10W with those obtained for Spherisorb S10W silica plus TMCS gives further details of the column packing struc-ture. The results are similar to those for the untreated silica up to 350°, then the value of Φ suddenly rises rapidly. This would indicate that the nature of the packed bed for the silica alone remains constant until 600°, where some change takes place (perhaps sintering of the small pores), but the deterioration of the silica plus TMCS begins at 350°. An increased percentage of TMCS (*i.e.*, less hydroxyl groups) seems to result in a less permeable column. The reason for this is as yet not clear and future work will involve a close study of Fig. 4.

The values of Φ at low temperatures are similar to those obtained by Knox[15] for slurry-packed spherical silica gel.

CONCLUSION

From the results it seems that both species of hydroxyl groups are important in the adsorption process for 200–500° thermally treated silicas. The k' values for Spherisorb S10W silica and Spherisorb S10W silica plus TMCS indicate that the single hydroxyl groups form stronger adsorption sites than do the hydrogen-bonded hy-droxyl groups. Their concentration is much less than that of the hydrogen-bonded hydroxyl groups (almost one third), but from the graphs of k' in Fig. 2 it can be seen that their contribution to the k' value is over one half. The siloxane bridges become important for silica at a temperature beyond 500° thermally treated even though the silica changes its structure at this temperature and its surface area decreases rapidly[5,16].

The optimum values ($k' = 1.5$–4) for nitrobenzene and acetophenone occur at 300–485° and 200–350°, respectively. Therefore pre-treatment of the Spherisorb S10W silica at a temperature in this region would appear to give optimum results for the solutes tested. Through a better understanding of the complete chromatographic

separation process predictions may become possible. This must be the ultimate aim for any practical chromatographer.

ACKNOWLEDGEMENTS

REFERENCES

1 J. A. Hockey and B. A. Pethica, *Trans. Faraday Soc.*, 58(10) (1962) 2017.
2 L. R. Snyder and J. W. Ward, *J. Phys. Chem.*, 70 (1966) 3941.
3 A. J. Tyler, *Ph.D. Thesis*, U.M.I.S.T., Manchester, 1968.
4 R. P. W. Scott and P. Kucera, *J. Chromatogr. Sci.*, 13 (1975) 337.
5 J. M. Bather and R. A. C. Gray, in preparation.
6 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968, Ch. 7.
7 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968, Appendix II.
8 C. G. Armistead, A. J. Tyler, F. H. Hambleton, S. A. Mitchell and J. A. Hockey, *J. Phys. Chem.*, 73 (1969) 3947.
9 P. A. Bristow, personal communication.
10 D. Ord, personal communication.
11 F. H. Hambleton and J. A. Hockey, *Nature (London)*, 208 (1965) 138.
12 F. H. Hambleton, J. A. Hockey and J. A. G. Taylor, *J. Catal.*, 3 (1971) 20.
13 M. L. Hair and W. Hertl, *J. Phys. Chem.*, 73 (1969) 2372.
14 M. L. Hair and W. Hertl, *J. Phys. Chem.*, 73 (1969) 4269.
15 J. H. Knox, *Chem. Soc. HPLC Summer School*, Sussex University, Brighton, 1975.
16 S. Kapila, W. A. Aue and J. M. Augl, *J. Chromatogr.*, 87 (1973) 35.