Modification of glass capillary inner surface for chromatography

M. CIGÁNEK, K. TESAŘÍK, M. HORKÁ, and K. JANÁK

Institute of Analytical Chemistry, Czechoslovak Academy of Sciences, CS-61142 Brno

Received 15 March 1989

Conditions of glass capillary (50 μm i.d.) inner surface modification by the aqueous solution of ammonia were studied. The resulting silica gel porous layer was modified chemically by methyloctyldichlorosilane. Under given etching conditions (temperature 250—300 °C for 16 h), the 5—10 vol. % aqueous solution of ammonia was optimal. The static way of silylation by 10 vol. % silane solution in toluene for 16 h at temperatures from 250 to 280 °C with the column filled to 50—70 % of its volume is optimal. This method provides a mechanically stable porous layer 1—10 μm thick.

Capillary columns are more often used for analytical purposes these days due to their high efficiency and rapid analysis. Capillary columns of inner diameter less than 100 μm were studied with respect to their use in gas [1—5], supercritical [6—8] and liquid [5, 9—22] chromatography. For capillary columns in high-performance liquid chromatography, the optimal diameter is expressed in units of μm [10]. Larger diameters — providing compromise results — are more suitable, however, for testing new methods of preparation of capillary columns. Low capacity of the columns can be increased by roughening the capillary inner surface and its coating with a stationary phase film of maximal thickness possible. Roughening of the glass capillary inner surface can be done in different ways [3—16, 19, 23—26].

For capillaries of the diameter less than 50 μm, treatment by basic or acidic solutions is suitable. The most suitable seems to be an aqueous solution of ammonia at temperatures above 120 °C when all the reaction components and products are gaseous and can be forced out of the capillary by an inert gas. Such treatment is suitable for all kinds of glass [24].

This work is concentrated on the determination of optimal conditions for preparation of the porous layer on the inner surface of the glass capillary of 50 μm i.d. Its chromatographic properties and mechanical stability were verified.

Experimental

The glass capillaries were prepared from Sial glass (Kavalier, Sázava, Czechoslovakia), only one series of them was prepared from Unihost soft glass (the same manufacturer). The used chemicals were of anal. grade (Lachema, Brno).

The capillaries were drawn on a home-made drawing device [27]. The capillary columns were tested on a Fractovap 210L AC gas chromatograph (C. Erba, Milan) with a flame ionization detector having a carrier gas inlet adjusted to the pressure 0.1—1.2 MPa. Photographs of the capillary inner surface were taken by an Edax lining scanning electron microscope (Philips, Eindhoven, The Netherlands). The capillaries 5 m long, 50 μm i.d., 0.7 mm o.d., were filled up to 70 % of their volume with 3.5—26 vol. % aqueous solution of ammonia. The closed capillaries were then heated in a thermostat at various temperatures ranging from 80 to 350 °C for 16 h. Residual ammonia after the reaction with the capillary inner surface was forced out of the column with nitrogen at the laboratory temperature. Then the capillaries were heated to 250 °C (at the program 50 °C, 5 °C min^{-1}; 250 °C, 80 min) under nitrogen flow. In case of repeated etching, the whole process was carried out again. The inner surface was treated further by silylation with 10 vol. % solution of methyloctyldichlorosilane in toluene, either by the dynamic or by static method. At the dynamic way of silylation, 20 mm^3 of silylation agent was forced through the capillary by compressed nitrogen at the linear velocity 2 cm s^{-1} At the static way, the column was filled up to 30—70 % of its volume with the silylation agent. In both cases, the closed columns were heated at the chosen temperature (180—280 °C) for 16 h. After silylation, the remaining silylation agent was forced out of the column by nitrogen at the laboratory temperature and, under continuous nitrogen flow, the capillaries were heated to 280 °C (at the program 50 °C, 2 °C min^{-1}; 280 °C, 60 min). The pressure of nitrogen used for filling the capillaries with the reaction solutions was adjustable, as needed, in the range 0.1—1.8 MPa. All the solutions used for filling the capillaries were filtered through a glass filter with the pore size 5—15 μm.

Effect of individual steps of the inner surface preparation was followed by studying chromatographic behaviour of particular columns. The used test mixture consisted of benzene, toluene, *p*-xylene, *o*-xylene, and *tert*-butylbenzene. The testing was carried out isothermally in the range of column temperatures 30—140 °C. The temperature of a split injector (split ratio 1 1000) and the detector was 200 °C. The carrier gas was nitrogen with an average linear velocity of about 50 cm s^{-1} Ends of the glass capillary columns were permanently provided with the detector and injector by fused silica capillaries 20 cm long. The columns were connected by microcouples with fused silica capillaries. The quality of the prepared inner surfaces of capillary columns was evaluated by capacity factors, peak symmetry and efficiency of the columns.

Effects of the chemical composition of glass, concentration of the aqueous solution of ammonia, temperature, time and repetition of etching, influence of silylation and stability of the etched layer were investigated.

Results and discussion

Effect of the chemical composition of glass

It is generally known that the activity of the glass capillary and, consequently, also that of the column is caused by the structure of the inner surface with the

Table 1

Effect of the chemical composition of glass and the temperature of etching

Column	Temperature of etching/°C	Capacity factor k			
		Benzene	Toluene	*p*-Xylene	*o*-Xylene
1	Non-etched	0.4	1.1	2.7	2.8
2	Non-etched	0.4	0.7	1.8	2.0
3	200	0.1	0.6	1.3	1.6
4	250	1.0	3.0	5.8	8.0
5	300	1.6	4.9	13.7	16.9
6	350	1.2	3.7	9.0	13.2
7	300	1.4	4.5	13.2	16.3

Column *7* was etched twice at 300 °C for 16 h. Testing temperature of the columns: *1*, *2* — 30 °C; *3* to *7* 50 °C.

centres of Lewis acids and silanol groups. Such chemical composition of the capillary surface has a decisive effect on its chromatographic behaviour.

The capillaries made of soft glass (column *1*) and hard glass (column *2*) were tested without further modification of their inner surface. From the retention values given in Table 1 and from the peak shapes of the tested substances (Fig. 1*a*, *b*) the effect of the composition of glass on the chromatographic behaviour of the column prepared from it is evident. Higher surface activity of column *1* not only increases retention of the solutes in comparison with column *2*, but it also causes solute zones spreading in the column. Further columns were prepared from the Sial hard glass only.

Effect of temperature and ammonia concentration

The capillaries were etched by a concentrated aqueous solution of ammonia at temperatures 200, 250, 300, and 350 °C, for 16 h (columns *3*—*7*, Table 1). Fig. 2 shows photos of the inner surface of the capillaries prepared in such a way taken by the electron microscope. At the temperature 200 °C (Fig. 2*a*), a porous layer about 1 μm thick is formed. Its surface is covered with particles of a spherical shape and of a diameter 0.1—0.2 μm. At the temperature 250 °C (Fig. 2*b*), similar particles are formed on the surface, their diameter is about 0.5 μm, the porous layer thickness varies in the range 1—2 μm. At the temperature 300 °C (Fig. 2*c*) the layer increases to the thickness 8—12 μm and it contains great amount of bubbles of different shape and of the diameter from 1 to 10 μm. Here and there the layer is cracked. At the temperature 350 °C

(Fig. 2*d*), the layer reaches the thickness of 8 μm and contains less bubbles. After repeated etching (Fig. 2*e*), the porous layer thickness does not increase substantially. However, the structure of the layer changes. The number of bubbles in the layer decreases and the dimension of the particles on the surface is about 1 μm.

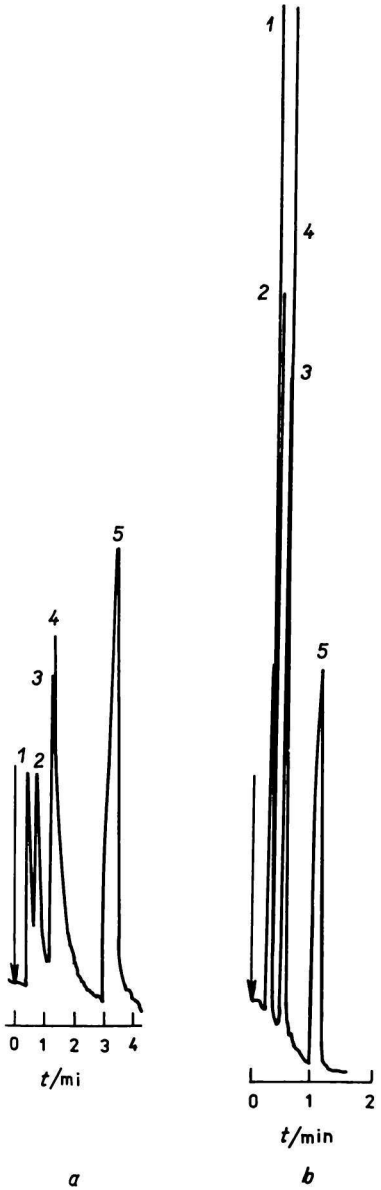
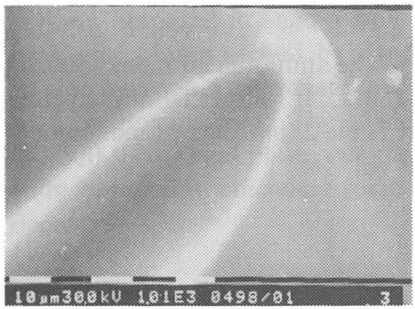
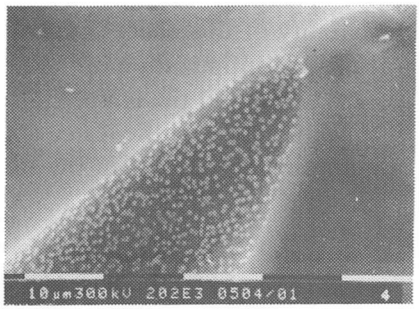
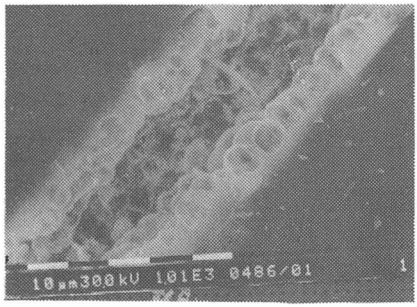


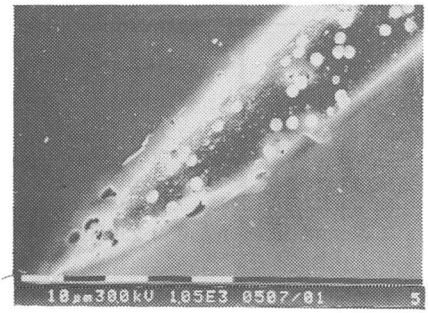
Fig. 1. Effect of the chemical composition of glass: *a*) column from Unihost soft glass; *b*) column from Sial hard glass. Temperature of the columns 30 °C; *1.* benzene, *2.* toluene, *3. p*-xylene, *4. o*-xylene, *5. tert*-butylbenzene.



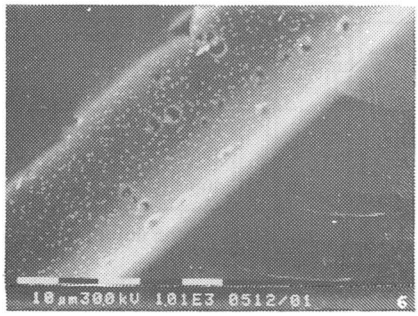


b





d



e

Fig. 2. Effect of the temperature of capillary columns etching by ammonia. *a*) 200 °C; *b*) 250 °C; *c*) 300 °C; *d*) 350 °C; *e*) etched twice at 300 °C.

Also the values of capacity factor of the tested solutes corresponds with visual examination of the photos of the capillary inner surface (Table 1). It follows from the results that with increasing temperature also the thickness of the inner surface etching increases (up to the temperature 300 °C). Under such experimental conditions, the maximal thickness of the layer can be obtained at the temperature about 300 °C. The layer formed at this temperature, however, contains a great number of bubbles and is cracked at some parts. Further treatment of the capillaries prepared in such a way often led to their blocking

with porous layer fragments. In case of the column with a small inner diameter, probability of this effect is great.

Further, optimization of the concentration of the ammonia aqueous solution was carried out at the temperature of etching 250 °C. It follows from the values of capacity factor of the tested solutes given in Table 2 that the optimal content of ammonia solution is in the range of 5—10 vol. %.

Table 2

Effect of the etching by the aqueous solution of ammonia

Column	φ (Ammonia)/vol. %	Capacity factor k			
		Benzene	Toluene	*p*-Xylene	*o*-Xylene
8	3.5	4.7	7.3	11.9	12.6
9	5.0	5.9	9.8	15.6	18.3
10	10.0	5.2	8.9	14.7	15.8
11	15.0	4.1	5.0	8.1	9.0
12	20.0	1.9	2.8	4.9	5.6
13	26.0	0.4	0.6	0.8	0.9

Columns were etched at 250 °C for 16 h, testing temperature 140 °C.

Table 3

Effect of silylation temperature and time

Column	Silylation		Capacity factor k			
	θ/°C	t/h	Benzene	Toluene	*p*-Xylene	*o*-Xylene
14	180	16	1.0	3.0	5.8	8.0
15	280	16	3.4	8.0	21.5	26.0
16	400	2	3.2	8.7	22.7	28.0

For preparation of the columns see Table 2, testing temperature 100 °C.

Effect of the method and temperature of silylation

The dynamic silylation did not sufficiently deactivate the surface of capillaries, which resulted in considerable asymmetry of the tested solute zones. Therefore, the static silylation was used (Table 3). The optimal temperature is 280 °C, the optimal time 16 h. Silylation at high temperatures (persilylation of the surface) destroys the porous layer and blocks the column. It is caused by cracking of the bubbles which form a part of the porous layer.

Fig. 3 presents the chromatogram of the tested mixture on the column etched under optimal conditions and then silylated statically by a 10 % solution of

methyloctyldichlorosilane in toluene. It is evident that the capacity factor of the column prepared in such a way (Table 2, column *9*) increased in comparison with the column etched by concentrated ammonia (Table 1, column *4*). The elution curves are asymmetrical. Their shape shows that the solutes still interact with the porous layer. The efficiency of such columns is in the range from 500 to 1500 theoretical plates per metre which is, in comparison with the theoretically obtainable efficiency for the column with such diameter, an important decrease.

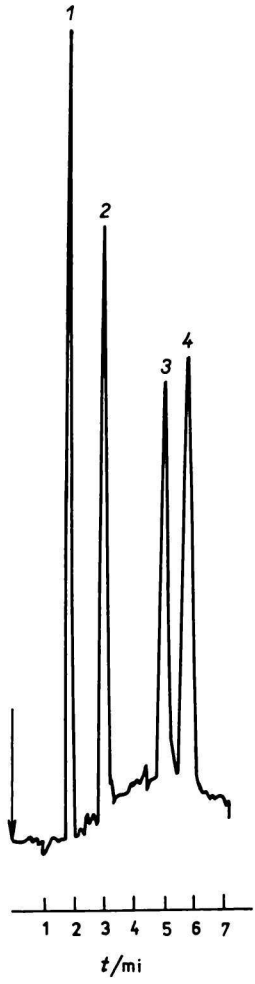


Fig. 3. Chromatogram on the capillary column prepared under optimal conditions, column temperature 140 °C. *1.* Benzene; *2.* toluene; *3. p*-xylene; *4. o*-xylene.

The porous layer stability

An important precondition of applicability of the capillary columns with a porous layer is its surface stability. This demand is very important at columns

Table 4

Effect of the column washing (with its tenfold volume) on the stability of the inner porous layer

Solvent	Capacity factor k			
	Benzene	Toluene	*p*-Xylene	*o*-Xylene
—	7.1	12.0	20.4	23.7
Acetone	6.3	10.1	16.9	19.7
Acetone	5.8	9.4	16.7	19.4
Water	6.1	9.8	16.3	18.8

Testing temperature 100 °C.

with a very small inner diameter which are to be used in liquid chromatography. Therefore, the check of stability of the layer prepared under optimal condition was carried out by washing the capillary with acetone and distilled water. The column was at first washed twice with acetone at the linear velocity about 1 cm s^{-1} (the volume of acetone was always about 10 volumes of the column) and then once with distilled water under the same conditions. Before and after each washing and conditioning of the column, the surface was tested. The results given in Table 4 show that after first washing with acetone, the capacity factors of the test solutes decreased. This is, most probably, caused by washing out of silane which was only adsorbed on the inner surface of the column. Further washing of the column with acetone or water had no substantial effect on retention of the tested substances. Stability of capacity factors shows that under such experimental conditions, the capillary inner surface remains unaffected.

Conclusion

Glass capillary columns of 50 μm i.d. with the silica gel porous layer modified chemically by methyloctyldichlorosilane were prepared. Different thickness and structure of the porous layer can be obtained by the change of ammonia concentration in water, temperature, the time of etching and the way of heating after etching.

The created porous layer proved to be sufficiently mechanically stable in our gas chromatographic tests. Under the given etching conditions (temperature 250—300 °C for 16 h), 5—10 vol. % aqueous solution of ammonia was the best. The static way of silylation by 10 vol. % solution of silane in toluene for 16 h at the temperature 250—280 °C with the column filled to 50—70 % of its volume appears to be optimal.

Acknowledgements. The authors would like to thank Dr. J. Karmazín from the Institute of Physical Metallurgy of the Czechoslovak Academy of Sciences in Brno for the photos of the inner surface of the glass capillary columns.

References

1. Schutjes, C. P. M., Vermeer, E. A., Rijks, J. A., and Cramers, C. A., *J. Chromatogr. 253*, 1 (1982).
2. Kong, R. C., Wooley, C. L., Fields, S. M., and Lee, M. L., *Chromatographia 18*, 362 (1984).
3. Schutjes, C. P. M., Vermeer, E. A., and Cramers, C. A., *J. Chromatogr. 279*, 49 (1983).
4. Aerts, A., Rijks, J. A., Bemgard, A., and Blomberg, L., *J. High Resolut. Chromatogr. Chromatogr. Commun. 9*, 49 (1986).
5. Barbrot, A., Folestad, S., and Larsson, M., *J. High Resolut. Chromatogr. Chromatogr. Commun. 9*, 117 (1986).
6. Peaden, P. A. and Lee, M. L., *J. Chromatogr. 259*, 1 (1983).
7. Lee, E. D. and Henion, J. D., *J. High Resolut. Chromatogr. Chromatogr. Commun. 9*, 172 (1986).
8. Markides, K. E., Fields, S. M., and Lee, M. L., *J. Chromatogr. Sci. 24*, 254 (1986).
9. Kučera, P., *J. Chromatogr. 198*, 93 (1980).
10. Knox, J. H. and Gilbert, M. T., *J. Chromatogr. 186*, 405 (1979).
11. Ishii, D., Tsuda, T., Hibi, K., Takeuchi, T., and Nakanishi, T., *J. High Resolut. Chromatogr. Chromatogr. Commun. 2*, 371 (1979).
12. Krejčí, M., Tesařík, K., and Pajurek, J., *J. Chromatogr. 191*, 17 (1980).
13. Tesařík, K., *J. Chromatogr. 191*, 25 (1981).
14. Tsuda, T., Tsuboi, K., and Nakagawa, G., *J. Chromatogr. 214*, 283 (1981).
15. Tsuda, T. and Nakagawa, G., *J. Chromatogr. 199*, 249 (1980).
16. Tijssen, R., Bleumer, J. P. A., Smit, A. L. C., and van Kreveld, M. E., *J. Chromatogr. 218*, 137 (1981).
17. Krejčí, M., Tesařík, K., Rusek, M., and Pajurek, J., *J. Chromatogr. 218*, 167 (1981).
18. Jorgenson, J. W and Guthrie, E. J., *J. Chromatogr. 255*, 335 (1983).
19. Tsuda, T. and Nakagawa, G., *J. Chromatogr. 268*, 369 (1983).
20. Šlais, K., *J. Chromatogr. Sci. 24*, 321 (1986).
21. Gooijer, C., Baumann, R. A., and Hofstraat, J. W., *J. Chromatogr. Sci. 24*, 361 (1986).
22. Fujimoto, C., Morita, T., and Jinno, K., *Chromatographia 22*, 91 (1986).
23. Traitler, H. and Prévot, A., *J. High Resolut. Chromatogr. Chromatogr. Commun. 4*, 433 (1981).
24. Mohnke, M. and Saffert, W., in *Gas Chromatography*, p. 216. (van Swaay, M., Editor.) Butterworths, London, 1962.
25. Brunner, F. and Cartoni, G. P., *Anal. Chem. 36*, 1522 (1964).
26. Liberti, A., Cartoni, G. P., and Brunner, F., *J. Chromatogr. 12*, 8 (1963).
27. Desty, D. H., Haresnape, J. N., and Whyman, B. H., *Anal. Chem. 32*, 302 (1960).

Translated by H. Kašparovská