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MEASUREMENT OF THE AVERAGE THICKNESS OF THE FILM OF STATIONARY PHASE IN DYNAMICALLY COATED GLASS CAPILLARY COLUMNS FOR GAS CHROMATOGRAPHY

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SUMMARY

Some procedures for the determination of the average thickness of the film of stationary phase in dynamically coated glass capillary columns were improved and compared. Good agreement was found between the film thickness calculated by use of the specific retention volume of toluene as measured on a packed column, the known β -ratio of statically coated columns and the weight of the stationary phase that can be rinsed out of the column. The measured film thicknesses were also found to be in accordance with those predicted by the Fairbrother-Stubbs equation.

In addition, the influence of the nature of the glass surface on the film of stationary phase is discussed. It is demonstrated that high coating velocities should be avoided when coating smooth surfaces dynamically.

INTRODUCTION

A prerequisite for high efficiency of capillary columns is the presence of the stationary phase as a thin, evenly distributed film along the entire length of the column. The quality of such a film is greatly dependent on the procedure used for its application. Two principal methods are available, static and dynamic. With the static method, the capillary is filled with a dilute solution of the stationary phase and the solvent is evaporated at elevated temperature¹⁻³ or reduced pressure^{4,5-6}. In the dynamic procedure, a plug of coating solution is moved through the capillary, leaving a thin layer of solution on the inner wall⁷; the capillary is then flushed with dry nitrogen for several hours, thus evaporating the solvent of the coating solution. We have concentrated our efforts on the development of the dynamic coating method^{8,9}; recently, however, we have also been successful with the static coating method. Using either of the methods, columns of high efficiency and durability have been obtained with a high degree of reproducibility.

The thickness of the film of stationary phase in the capillary is an important characteristic of the column. An increase in film thickness will result in a decreased column efficiency as expressed by the Golay equation^{1,4b}. Too thin films, on the other

hand, might allow the column wall to play an active role in the partition process which in many instances would result in deformed chromatographic peaks. Several methods have been proposed for the determination of the average film thickness, d_f , in dynamically coated capillary columns: from values of the partition coefficient, K , calculated from measurements of the specific retention volume, V_g , on a packed column⁹⁻¹⁴; from the capacity ratio and β -value of statically coated columns^{8,15}; and from literature values of K or V_g (ref. 16). Further, d_f has been calculated from the difference in volume of the coating solution before and after coating¹⁷⁻¹⁹, and also from the weight of stationary phase that can be rinsed out of the column^{20,21}. The merits of the different methods are disputed. Some workers consider the method of comparison with statically coated columns to be dubious¹², while others regard this method as useful¹⁵. Moreover, the method of weighing the phase rinsed out of the column is sometimes considered to give too low d_f values²², e.g., 0.1 μm as reported by Grob and Grob^{20,21}. Recent work has shown that smooth, compatible surfaces are capable of holding liquid films of thickness up to 0.3–0.2 μm in position^{13,14,23}, which indicates that some higher d_f values previously reported might have been erroneously measured^{8,17}.

It was therefore considered to be of interest to compare the different methods used for the determination of d_f . For this purpose, careful measurements were made of the average film thickness using the different methods for a number of glass capillary columns, both statically and dynamically coated.

EXPERIMENTAL

Capillaries (20 m \times 0.6 mm O.D. \times 0.25 mm I.D.) were drawn on a vertically operated Shimadzu glass-drawing machine using Duran 50 or AR-glass tubing (6 \times 2 mm I.D.) which had been previously cleaned with chromic acid-sulphuric acid, carefully rinsed with water and acetone and dried. Before coating, the capillaries were pre-treated in different ways. Silanization was carried out in the gas phase with hexamethylsilazane-chlorotrimethylsilane (5:1)⁸. Some capillaries were etched with gaseous HF produced by the thermal decomposition of chlorotrifluoroethyl methyl ether²⁴. Etching with HCl was effected according to published methods^{25,26}. Further, a thin, non-extractable layer of Carbowax 20M was applied to the inner surface of some of the HF-etched capillaries using a method described earlier²⁷.

The dynamic coating was executed according to previously described procedures^{8,9,28}. It should be noted that for reproducible results, it is essential that the temperature of the column during the coating should be kept constant by thermostatic control; small changes in temperature will affect the viscosity of the coating solution and the evaporation of the solvent. Further, evaporation of the solvent should be performed using a high flow-rate of dry nitrogen. At slower gas flow-rates, the solvent is removed as liquid plugs and some of the stationary phase is thus transported out of the column⁹; the film thickness will be lower than predicted in such columns.

Statically coated capillaries could be prepared with a high degree of reproducibility when using the methods of Verzele and co-workers^{5,6}. The capillaries were thus filled with a carefully prepared, de-gassed solution of 0.40% (v/v) SF-96 methylsilicone in freshly distilled methylene chloride. One end of the capillary, which had previously been slightly drawn out in a micro-flame, was then blocked with the

aid of silicone gum adhesive (Rhodosil). When the adhesive had hardened, the solvent of the stationary phase solution was evaporated at 25° and a pressure of about 0.05 torr.

Absolute values of the diameter of the capillary at various points along the column were achieved by using a microscope, and the average inner diameter, necessary for the calculation of d_f , was derived from the weights of the column when empty and when filled with mercury.

Prediction of d_f in dynamically coated capillary columns

Some equations for the prediction of d_f in dynamically coated capillary columns have been proposed. Kaiser¹⁸ studied the thickness of the layer of stationary phase as a function of the % (v/v) concentration, c , of the coating solution, the inner diameter of the capillary, d_k , and the velocity of the coating plug, u . The empirical equation

$$d_f = \frac{c}{100 d_k} (0.265u + 0.25) \quad (1)$$

was derived. When investigating wetting phenomena in capillaries in connection with electro-osmotic studies, Fairbrother and Stubbs²⁹ found the relationship

$$d_f = \frac{cr}{200} \left(u \cdot \frac{\eta}{\gamma} \right)^{\frac{1}{2}} \quad (2)$$

to be useful for the calculation of the film thickness, where η and γ are the viscosity and surface tension, respectively, of the coating solution, and r is the column radius. In addition, it has been proposed that eqn. 3 is applicable to the coating of capillary columns³⁰:

$$d_f = \frac{1.34rc}{100} \left(u \cdot \frac{\eta}{\gamma} \right)^{\frac{2}{3}} \quad (3)$$

The values of η and γ required for the calculation are generally not available. They can, however, be obtained by some rapid and simple measurements using an Ubbelohde viscometer and the capillary rise method; such data used in this investigation are presented in Table I.

Measurement of d_f in dynamically coated capillary columns

A number of methods are available for the measurement of the average film thickness in capillary columns. This can be calculated on a basis of the relationship

$$K = \beta k \quad (4)$$

TABLE I

PROPERTIES OF SOLUTIONS OF SILICONE OIL SF-96 IN TOLUENE AT 25°C

Concentration (%, v/v)	Viscosity (η) (10^{-3} kg/m·sec)	Surface tension (γ) (10^{-2} N/m)
10	1.39	2.50
2	0.70	2.45

where K is the partition coefficient, β is the ratio of the total gas volume in the column to the total volume of the stationary phase at the column temperature and k is the capacity ratio $(t_R - t_M)/t_M$, where t_R is the retention time of a test component and t_M is the retention time of an inert component. As K is a constant for a given solvent-solute pair at constant temperature, it can be assumed that for constant experimental conditions¹⁷

$$\beta_1 k_1 \text{ (statically coated)} = \beta_2 k_2 \text{ (dynamically coated)} \quad (5)$$

whence

$$d_f = \frac{r}{2\beta_2} = \frac{rk_2}{2\beta_1 k_1} \quad (6)$$

Values of d_f in dynamically coated capillary columns are thus derived from measurements of k on statically coated columns; the β -values of these columns are readily available: *e.g.*, if a column is coated with a 0.4% (v/v) solution, $\beta = 99.6/0.4$. Moreover, it is possible to determine K from measurements of the specific retention volume, V_g , on a packed column. V_g is related to K by the known equation

$$K = \frac{V_g T_c \rho_l}{273} \quad (7)$$

where T_c is the absolute column temperature and ρ_l is the density of the liquid phase at the column temperature. Eqns. 4 and 6 give

$$d_f = \frac{rk}{2k} \quad (8)$$

and substitution of K gives

$$d_f = \frac{273 rk}{2 V_g T_c \rho_l} \quad (9)$$

The density of SF-96 at 40 °C is 0.954 g/ml. In order to obtain correct V_g values utilizing data from a packed column, careful measurements must be made of the column inlet pressure, column temperature and weight of stationary phase in the packed column³¹. A plot of $\ln V_g$ against $1/T$ for benzene and toluene on SF-96 is shown in Fig. 1. Further, the film thickness can be calculated from the weight of stationary phase that can be rinsed out of a column. For this purpose, the column was rinsed with a low-boiling solvent, *e.g.*, methylene chloride, which was allowed to drip slowly into a small sample cup designed for differential scanning calorimetry (Perkin-Elmer). In order to hasten the evaporation of the solvent, the cup was placed in a gentle stream of cleaned, dust-free air. Rinsing with 5 ml solvent was sufficient for a 20-m

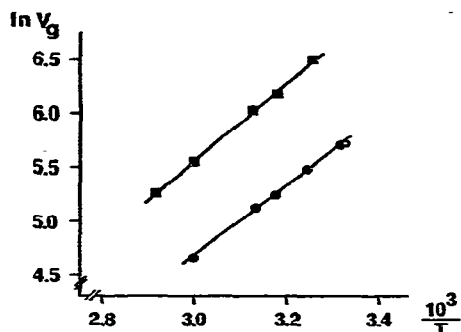


Fig. 1. Graph of $\ln V_g$ against $1/T$ for benzene (●) and toluene (■) on SF-96 silicone oil. V_g is expressed in ml/g. ○, Literature value¹².

column, this procedure taking about 2 h. The cup was then carefully weighed on a Cahn microbalance. Assuming ideal column geometry:

$$d_f = \frac{w}{l d \pi \rho} \quad (10)$$

where w and ρ are the weight and density, respectively, of the stationary phase, l is the column length and d the column diameter. Finally, the use of transparent tubes of known diameter on the inlet and outlet allows the film thickness to be calculated from the difference in volume, V_{diff} , between the original and final coating plug:

$$d_f = \frac{V_{\text{diff}}}{l d \pi} \cdot \frac{c}{100} \quad (11)$$

RESULTS AND DISCUSSION

Eqns. 2 and 3 have been shown to be useful for the prediction of the average film thickness in capillary columns^{8,10-12}. The influence of the support, however, is not taken into consideration in these equations. Novotný and Bartle^{13,14} have demonstrated that on silanized soda-glass, the stable film thickness of SF-96 is limited to 0.2–0.3 μm . However, on HCl-etched soda-glass, *i.e.*, a rougher surface, they were able to prepare stable films of SF-96 with a d_f of up to 1.4 μm (ref. 13). Provided sufficient wetting was achieved and the maximum film thickness that the support would hold was not exceeded, we found that widely differing surfaces acquired similar film thicknesses (Table II). The limited capacity of the surface must also be considered when selecting the coating rate and the concentration of the stationary phase. A high coating rate results in the deposition of a relatively thick layer of phase solution; if the thickness of this layer exceeds that which the surface is capable of holding, the solution film will deteriorate and start to travel along the column as small plugs. This effect is illustrated in Fig. 2, where d_f calculated from eqn. 2 is plotted against d_f measured according to the V_g method for dynamically coated HF- and HCl-etched AR-glass capillary columns. A low-concentration coating solution (2%, v/v, SF-96 in toluene) was used in this test in order to achieve a thin film of

TABLE II
AVERAGE FILM THICKNESS IN DYNAMICALLY COATED DURAN 50 GLASS CAPILLARY COLUMNS, COATED WITH 10% (v/v) SF-96 IN TOLUENE

Column number	Column diameter (mm)	Pre-treatment	Coating rate (mm/sec)	Predicted d_f			Measured d_f			
				Eqn. 1	Eqn. 2	Eqn. 3	Eqn. 6	Eqn. 9	Eqn. 10	Eqn. 11
1	0.262	Silanized	2.0	0.12	0.07	0.041	0.061	0.062	0.07	0.10
2	0.247	HF-etched	3.1	0.13	0.081	0.051	0.074	0.079	0.08	0.04
3	0.247	HF-etched	8.0	0.19	0.13	0.10	0.11	0.12	0.12	0.09
4	0.261	HF-etched	8.0	0.18	0.14	0.10	0.14	0.13	0.13	0.15
5	0.220	HF-etched*	6.9	0.20	0.11	0.08	0.11	0.08	0.11	0.09
6	0.250	HF-etched + Carbowax	10.5	0.21	0.15	0.12	0.13	0.13	0.14	—
7	0.275	HF-etched	11	0.20	0.17	0.13	0.15	0.17	0.16	—
8	0.249	Silanized	17	0.28	0.19	0.16	0.19	0.19	0.19	0.20
9	0.250	Silanized	18	0.29	0.20	0.17	0.21	0.21	0.20	0.15
10	0.241	HF-etched	21	0.34	0.21	0.18	0.19	0.18	0.21	0.20
11	0.262	Silanized	18	0.28	0.21	0.18	0.16	0.16	0.21	0.34
12	0.273	HF-etched	18	0.27	0.22	0.18	0.20	0.20	0.20	0.18
13	0.259	HF-etched	23	0.33	0.23	0.21	0.21	0.21	0.21	0.22
14	0.250	HF-etched + Carbowax	27	0.39	0.24	0.22	0.19	0.19	0.20	—
15	0.251	HF-etched	32	0.44	0.26	0.25	0.25	0.25	0.25	0.34
16	0.248	HF-etched	33	0.45	0.27	0.25	0.26	0.26	0.26	0.18
17	0.248	HF-etched	33	0.45	0.27	0.25	0.27	0.27	0.27	0.24
18	0.248	HF-etched + Carbowax	32	0.44	0.26	0.24	0.25	0.27	0.27	—
19	0.260	HF-etched	33.5	0.44	0.28	0.26	0.27	0.28	0.27	—
20	0.261	HF-etched + Carbowax	47	0.57	0.33	0.33	0.34	0.34	0.33	—
21	0.273	HF-etched	49	0.57	0.36	0.36	0.35	0.33	0.33	—
22	0.239	HF-etched	65	0.82	0.36	0.38	0.37	0.38	0.37	—

* AR-glass.

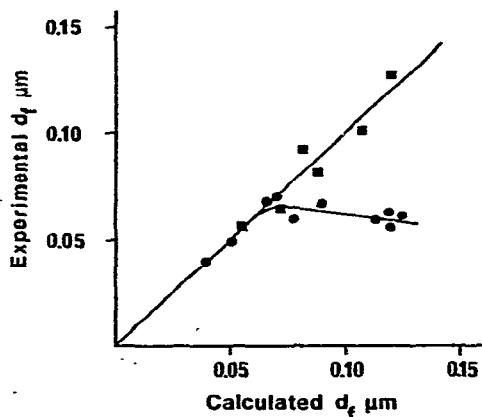


Fig. 2. Graph of experimental film thickness (V_s method) against film thickness predicted from eqn. 2. AR-glass capillaries dynamically coated with 2% (v/v) SF-96 in toluene. ●, Silanized glass columns; ■, HCl-etched glass columns.

stationary phase, well within the limit of what the glass could carry even when using higher coating rates. The strongly etched columns, owing to their greater capacity, showed good agreement between calculated and experimental d_f values, but the smooth capillaries were not coated as predicted at higher rates.

It is therefore evident that too high coating rates should be avoided when applying phase films to smoother surfaces. We recommend that thicker phase films should be prepared by the use of more concentrated stationary phase solutions. Eqn. 2 has been found³² to give a good approximation of film thickness when $R = u\eta/\gamma < 0.09$. On the smooth surface, deviations of measured from predicted d_f values appeared at $R = 0.006$, while on the more etched surface eqn. 2 was found to be valid even up to $R = 0.009$, which was the highest rate tested. Eqn. 3 should be valid for $R \leq 0.003$ (ref. 33); for higher R values too high d_f values are obtained, and eqn. 3 is thus not applicable in this test series.

An advantage of the static coating procedure is that the exact amount of stationary phase in the column can be calculated¹⁷:

$$d_f = \frac{r}{2\beta} \quad (12)$$

This relationship was used for a comparison of the V_g and rinsing methods for determination of d_f . A series of Duran 50 glass capillary columns were thus coated statically, *i.e.*, columns with a known amount of stationary phase were prepared. The results from eleven such columns are given in Table III.

TABLE III

AVERAGE FILM THICKNESS IN SOME 10-m LONG STATICALLY COATED DURAN 50 GLASS CAPILLARY COLUMNS, COATED WITH 0.4% (v/v) SF-96 IN DICHLOROMETHANE

Column number	Column radius (mm)	Column test temp. (°C)	k	V_g (ml/g)	Theoretical d_f (eqn. 12)	Measured d_f	
						Eqn. 9	Eqn. 10
1	0.120	40	2.20	523	0.24	0.23	0.27
2	0.111	40.5	2.40	518	0.22	0.24	0.24
3	0.135	40.5	2.38	518	0.27	0.28	0.27
4	0.103	40.5	2.36	518	0.21	0.21	0.22
5	0.120	41	2.14	503	0.24	0.23	0.25
6	0.115	41	2.22	503	0.23	0.23	0.23
7	0.105	41	2.25	503	0.21	0.21	0.20
8	0.103	41.5	2.17	498	0.21	0.20	0.20
9	0.111	42	2.12	488	0.22	0.22	0.22
10	0.0936	42	2.32	488	0.19	0.20	0.20
11	0.111	42.5	2.13	478	0.22	0.22	0.23

The values of d_f measured according to the two methods are in good agreement with those predicted theoretically (eqn. 12). These methods can therefore be used for accurate determinations of d_f . The values of k obtained from the statically coated columns were then used for the calculation of d_f in dynamically coated capillary columns according to eqn. 6.

The usefulness of the different methods for the measurement of d_f was tested

for capillary columns coated dynamically at different rates. For a given capillary, the four methods tested resulted in similar values of d_f (Table II). However, the method based on the measurement of the decrease in volume of coating solution upon coating occasionally gave divergent results, perhaps owing to some imperfections in the technique. The fact that similar d_f values are achieved in independent ways indicates that the measured values might be regarded as correct. Moreover, the concordant d_f values measured show good agreement with the values predicted from eqns. 2 and 3. The values of R are thus sufficiently low in this test series to allow the application of eqn. 3. For our columns, eqn. 1 predicts too high d_f values, an explanation for which could be that the equation was derived from experiments using another coating solvent, namely *n*-hexane. The ratio of viscosity and surface tension is slightly higher for toluene ($2.1 \cdot 10^{-2}$ sec/m) than for *n*-hexane ($1.7 \cdot 10^{-2}$ sec/m) and the film thickness, by reference to eqn. 2, would therefore be slightly higher when toluene is used as the coating solvent. We therefore consider eqn. 1 to be unsuitable for the prediction of d_f when using our combinations of stationary phase, solvent and column diameter. The influence of column diameter has been thoroughly investigated^{8,10-12}.

It should be noted that the measured and predicted values of d_f are derived as average film thicknesses, and consequently some small variations in the film thickness along the length of the column might occur. Such changes can, by reference to eqns. 2 and 3, be due to variations in the inner diameter. It is thus important to use capillaries that possess the highest possible uniformity of inner diameter for the preparation of high-efficiency capillary columns. Such variations are reported to be less than $\pm 5\%$ (refs. 34 and 35) or $\pm 7.4\%$ (ref. 15). Our experience is that, when carefully operated and utilizing selected pieces of Duran glass tubing, the Shimadzu glass-drawing machine can produce 100-m long capillaries with a tolerance of $\pm 2-3\%$ (Fig. 3). For successful operation, the glass tubing should be adjusted strictly vertical to the furnace; further, it is important to use undamaged feeding rolls. Uneven films might also be due to locally lowered wettability of the glass; the stationary phase might thus form micro-drops³⁶. Adsorption of sample components on the resulting exposed glass surface will result in tailing of the chromatographic peaks. Such an effect has, however, not been observed for the columns tested in this investigation. Another cause of uneven films is that the stationary phase tends to accumulate in cracks and crevices in the surface³⁷; therefore, the highest efficiency is obtained when smooth glass surfaces are used, *e.g.*, unetched Duran 50 glass.

From these results, it can be concluded that the measurement of d_f based on the partition coefficient, K , might be problematical. Values of K and V_g are generally

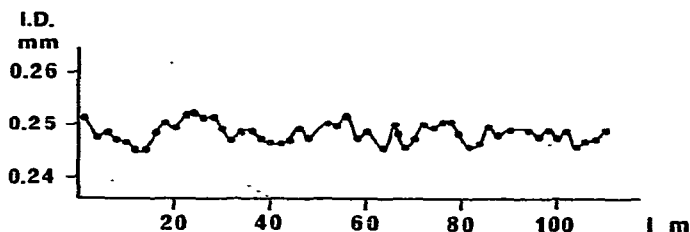


Fig. 3. Uniformity of the inner diameter of a typical piece of Duran 50 glass capillary. Average inner diameter, 0.248 mm.

not available in the literature, and their accurate determination can be difficult. Moreover, the comparison with statically coated capillaries could present difficulties, as some experience is necessary for the proper execution of the static coating method. A further drawback of the methods based on measurement of the capacity ratio is that with thin, non-polar films on polar surfaces, such as Carbowax deactivated glass²⁷, or when sufficient wetting is not attained, the support could influence the retention of the test compound, thus giving too high values of d_f (ref. 27). The most practical solution seems to be to use eqns. 2 or 3 for the prediction of the film thickness and to make occasional checks of the film thickness of typical capillary columns from the weight of stationary phase rinsed out of the column.

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