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EFFECT OF REPEATED CROSS-LINKING OF SE-54 STATIONARY PHASE FILM ON THE CHROMATOGRAPHIC PROPERTIES OF CAPILLARY COLUMNS

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SUMMARY

The effects of repeated cross-linking of SE-54 stationary phase with azo-*tert.*-butane on the degree of stationary phase film immobilization and on the chromatographic properties were studied. The optimum numbers of repeated immobilizations in the columns for gas and liquid chromatography were determined. Excessive repetition of immobilization adversely affected the chromatographic properties. The contribution of chemical bonding to the degree of stationary phase immobilization was studied with capillaries silylated with octamethylcyclotetrasiloxane.

INTRODUCTION

The quality of capillary columns in gas chromatography has been increased markedly by stationary phase film immobilization[1]. Perfect immobilization is obtained by the cross-linking of stationary phase chains and their chemical bonding to the capillary surface [2]. One well established method of silicone stationary phase immobilization consists in cross-linking the polysiloxane chains by reactions of free radicals. These originate from methyl and vinyl groups in the stationary phase by the action of radical reaction initiators[1]. In the same way, bonds between the stationary phase and the silylated capillary surface are also formed, as Grob and Grob[3] have shown for silylation with divinyltetramethyldisilazane. For a silylated surface carrying only methyl groups, however, they supposed a substantially lower occurrence of bonding with the stationary phase[2].

As radical reaction initiators, most often organic peroxides are used, and also dialkylazo compounds, especially azo-*tert.*-butane. The application of azo-*tert.*-butane has certain advantages over organic peroxides; its main decomposition products are nitrogen, isobutane and isobutene, which do not react with the polysiloxane chain, do not oxidize oxidizable phases[4], do not change the stationary phase polarity and do not increase the column activity[5]. Optimum conditions for cross-linking non-polar and medium-polarity silicone phases by means of azo-*tert.*-butane have been established[5,6]. Owing to its volatility, azo-*tert.*-butane can be diffused to the stationary phase film in the form of vapour. This procedure permits multiple repetition of cross-linking with the same column.

After the first application of azo-*tert.*-butane the SE-54 stationary phase extractability is only about 5%, nevertheless some workers have recommended carrying out cross-linking several times[7,8]. According to the literature[7,8], however, the stationary phase extractability after three or five cross-linkings does not change from that of the same phase cross-linked only once (5%). Apart from stating that the phase remains gummy, the separation properties of the columns were not described.

It is still unclear if further cross-bonds are formed during the repeated cross-linking or whether the possibilities of cross-linking are exhausted after the first cross-linking of the stationary phase film.

The aim of this work was to establish the effect of repeated cross-linking of SE-54 stationary phase film on its extractability and on the efficiency and capacity of columns coated with this material. The effect of silylation of the capillary surface with octamethylcyclotetrasiloxane on the stationary phase immobilization was also studied.

EXPERIMENTAL

Materials and chemicals

Glass tubes from Simax Glas (Kavalier Sázava, Czechoslovakia), octamethyl-cyclotetrasiloxane (VCHZ Synthesia, Kolín, Czechoslovakia), SE-54 silicone stationary phase (W. Günter, Düsseldorf, F.R.G.), azo-*tert.*-butane (Ventron, Karlsruhe, F.R.G.), *n*-butane (Kaučuk Kralupy nad Vltavou, Czechoslovakia) were used. Other chemicals of analytical-reagent grade were supplied by Lachema (Brno, Czechoslovakia).

Apparatus

The device for drawing the glass capillaries and the device for filling and coating the capillaries with a solution of the stationary phase in a liquefied gas were designed in the Institute of Analytical Chemistry (Czechoslovak Academy of Sciences, Brno, Czechoslovakia). The capillaries and capillary columns were tested and thermostated in a Fractovap Model 2300 AC gas chromatograph with a flame ionization detector (Carlo Erba, Milan, Italy).

Procedures

Capillary surface treatment. Prior to coating, some capillaries were leached with 20% hydrochloric acid at 170°C[9] and silylated with octamethylcyclotetrasiloxane[10] (D_4) for 2 h at 420°C.

Capillary coating and column testing. Capillaries *ca.* 15 m × 0.25 mm I.D. were filled with a 0.4% (w/v) solution of the SE-54 stationary phase in liquefied butane[11]. The capillaries were coated statically without using a vacuum[11]. Immediately after coating, they were blown with nitrogen and conditioned at a low nitrogen flow-rate by heating from 40 to 230°C at 1°C/min. The columns were tested for their separation efficiency and the capacity ratio of the solutes with a mixture of C_7–C_{11} *n*-alkanes at 80°C and a mixture naphthalene and methyl nonanoate at 110°C. For reasons of economy of time, the average linear velocity of the carrier gas was adjusted to 25 cm/s.

Immobilization. The column was blown with a stream of nitrogen saturated at 25°C with azo-*tert.*-butane vapour for 1 h per 10 m of column length at a flow-rate of

2 μl/s. A sealed column was heated in a thermostat from 40 to 220°C at 10°C/min and then left at 220°C for 1 h. After immobilization, the column was always conditioned under a flow of nitrogen by heating from 60 to 240°C at 1°C/min and then for 1 h at 240°C. After testing, the column was subjected to another cycle of immobilization, conditioning and testing. After a certain number of cycles, it was washed with chloroform of volume 1 ml per 1 m of column length at a flow-rate of 1.4 μl/s and tested again.

Resistance of stationary phase film to solvents. The column was washed with 10 ml of the chosen solvent, filled with this solvent and left for 48 h. The solvent was then ejected and the column was washed with 4 ml of dichloromethane. Conditioning was carried out under a flow of nitrogen with heating from 60 to 240°C at 3°C/min and then for 15 min at 240°C. After testing with the mixture of *n*-alkanes, the column was subjected to the action of another solvent.

The extent of extractability of the stationary phase by a solvent (z) is most often interpreted as the degree of stationary phase film cross-linking. This term, however, does not relate specifically to stationary phase extractability because the immobilization includes not only cross-linking but also bonding of the stationary phase chains to the capillary surface. A more suitable term for characterizing the extent of immobilization seems to be the degree of non-extractability or the degree of immobilization, designated z', which is used in this work. The degree of stationary phase film immobilization in the treated columns was calculated as the ratio of the capacity ratio of undecane after washing the column to that of the same solute determined after the last immobilization, expressed as a percentage. The changes in the column efficiency (number of theoretical plates per metre of column) and the capacity ratio of the selected solutes during immobilization were expressed as the relative efficiency (N_{rel}) and the relative capacity ratio of the solute (k_{rel}). These represent the percentage of the column efficiency and the capacity ratio of the solute prior to first immobilization.

Unless stated otherwise, all the results given are mean values from at least three columns under the same experimental conditions.

RESULTS AND DISCUSSION

Dependence of the degree of immobilization on its repetition

The repeated immobilization of SE-54 stationary phase film initiated by azo-*tert*.-butane was studied on silylated columns and on the columns with the capillary surface untreated.

As can be seen from Fig. 1, the degree of immobilization increases with increasing number of immobilizations. The possibilities of cross-linking (the existence and steric accessibility of methyl and vinyl groups, their compatible configuration in adjacent chains) are therefore not exhausted after the first immobilization. It is evident from comparison of the two curves in Fig. 1 that the cross-linking continues even with multiple repetition of immobilization (up to 10 times), even though the stationary phase film in the silylated columns is fully immobilized. Wright *et al.*[5] observed a slight decrease in the polarity of polar silicone stationary phases after their immobilization with azo-*tert*.-butane and connected this effect with fixation of the non-polar azo-*tert*.-butane decomposition products on the stationary phase film. It can be
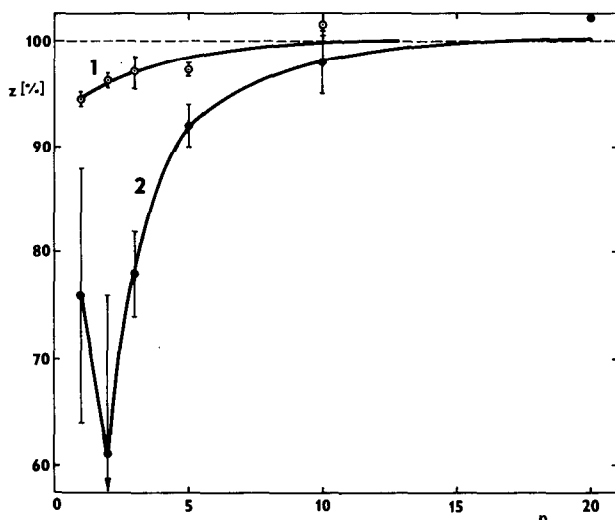
Fig. 1. Dependence of the degree of immobilization, z' (%), of SE-54 stationary phase film ($d_f = 0.25 \, \mu$m) on repeated immobilization with azo-*tert.*-butane ($n =$ number of repeated immobilizations). 1, Columns with the surface modified by leaching and silylation; 2, columns with the surface untreated.

assumed that on repeated immobilization, this fixation effect may become a source of other cross-links.

For the practical application of the capillary columns, it is necessary to use the optimum cross-linking to prevent any marked decrease in mass transfer through the stationary phase film. The optimum degree of immobilization of the stationary phase film is 95% for the columns used in gas chromatography. Denser cross-linking together with chemical bonding is more advisable for the columns used in liquid and supercritical fluid chromatography. It follows from Fig. 1 that one immobilization is sufficient to immobilize the stationary phase film with azo-*tert.*-butane when preparing the capillary columns for gas chromatography. Resistance of the immobi-

TABLE I

EFFECT OF STATIC ACTION OF THE SOLVENTS ON THE CHROMATOGRAPHIC PROPER-
TIES OF COLUMNS (0.25 × 15 mm I.D.) WITH REPEATEDLY IMMOBILIZED STATIONARY
PHASE SE-54 ($d_f = 0.25 \, \mu$m)

The degree of immobilization (z') and the number of theoretical plates per metre of column length were calculated from the characteristics of the n-undecane peak.

No. of immobilizations	Initial state		Water		Methanol		Acetonitrile	
	z' (%)	n/m	z' (%)	n/m	z' (%)	n/m	z' (%)	n/m
1	93.8	3400	92.5	3200	82.3	2800	80.9	3000
2	95.9	3450	95.9	3350	86.5	3050	87.8	3200
3	95.5	3000	95.5	3200	91.0	3050	92.5	2800
5	97.1	3000	96.3	2600	91.1	2800	86.8	2600

lized film to the long-term action of solvents was verified statically with water, methanol and acetonitrile as components suitable for the preparation of mobile phases for reversed-phase liquid chromatography. The results are given in Table I. Of the solvents used, water hardly affects the chromatographic properties of the columns and methanol and acetonitrile cause further extraction of the stationary phase. For the column immobilized three times, the decrease in the degree of stationary phase immobilization is negligible. The column immobilized five times showed tailing after extraction with acetonitrile and its capacity decreased.

The course of curve 2 in Fig. 1 is interesting from the theoretical point of view. The dispersions in the degree of immobilizations assessed after the first, as well as after the second immobilization are fairly large, and show a considerable effect of the conditions during saturation of the stationary phase film with azo-*tert.*-butane (*e.g.*, variation of temperature and nitrogen flow-rate, and also the effect of the film homogeneity) and an effect of the time sequence of heating and further conditioning of the columns. Still, it can be observed systematically that the degree of stationary phase film immobilization is lower after the second than after the first immobilization. The number of columns tested was increased to six (immobilized once) or seven (immobilized twice). To verify the effect, one of the columns was broken into two halves after the first immobilization and both halves were tested. Then, one was washed with a solvent and the other was immobilized a second time. The degree of immobilization was lower again ($z'_1 = 65\%$, $z'_2 = 53\%$).

It is assumed that the decrease in the degree of immobilization in this instance is illusory and is only a consequence of the decrease in the capacity ratio of the solutes after washing the column with the solvent. The decrease in capacity ratio, however, cannot be caused by the loss of the stationary phase in the column, but is probably due to the decreased interaction of the solutes with the stationary phase after its washing. This interpretation is also confirmed by the result of a test in which the column, after its first immobilization, was washed with the solvent and, after testing, immobilized again. The result was very close to that in the preceding test ($z'_1 = 65\%$, $z'_2 = 50\%$). A third immobilization forms a system so rigid that washing with the solvent hardly affects the stationary phase film. For the silylized columns, the dependence of the degree of immobilization on its repetition has a monotonous course; the chemical bonding of the stationary phase film to the surface limits the changes in its arrangement.

Silylation of the capillary surface with D_4 increased the stationary phase immobilization markedly (see Fig. 1). The degree of the stationary phase film immobilization after the first immobilization was 18% higher with the silylated columns. Comparison with data in the literature[3] shows that the immobilization is affected in the same way by chemical binding of the stationary phase to the surface silylated with an agent containing vinyl groups (divinyltetramethyldisilazane) as with an agent containing methyl groups (D_4). From the point of view of surface wettability and suitable acid–base properties, the latter agent is preferred for silylation of the capillary before its coating with the SE-54 stationary phase.

Effect of repeated immobilization on chromatographic properties of the columns

A large number of cross-links in the stationary phase film may be the cause of the lower diffusive permeability of the stationary phase film and, consequently, of the

decrease in interactions of the solute with the functional groups of the stationary phase. This is manifested, on the one hand, by a decrease in the column efficiency and, on the other, by a change in its capacity. It can be also affected by the decomposition products of the initiator fixed on the stationary phase film.

The dependence of the relative efficiency of the capillary columns determined for undecane on the number of immobilizations is shown in Fig. 2. Whereas the first immobilization does not affect the efficiency of the capillary columns, further immobilizations decrease it. The decrease in efficiency is less marked with the columns with a silylated surface (see curves 1 and 3). When comparing these efficiencies with the efficiency of a column prepared in the same way but with the stationary phase film non-saturated with azo-*tert.*-butane, the reason for the decrease in efficiency can be attributed to the stationary phase film cross-linking. The dependences of the relative efficiencies of the columns determined after washing the columns with the solvent on repeated immobilization show similar curses (curves 2 and 4). The efficiency of most of the silylated columns increased slightly after washing, whereas the efficiency of the columns with an untreated surface decreased. The dependences of the column efficiency on repeated immobilization expressed for naphthalene and methyl non-anoate acid had similar courses, as shown in Table II. After multiple repetition of immobilization, the efficiency of the columns with a silylated surface decreased to *ca.* 80% of the original value. To retain the original column efficiency, however, only one or a maximum of two immobilizations are permissible.

The effect of repeated stationary phase film immobilization on the capacity ratio of undecane is shown in Fig. 3. Whereas the relative capacity ratio on the
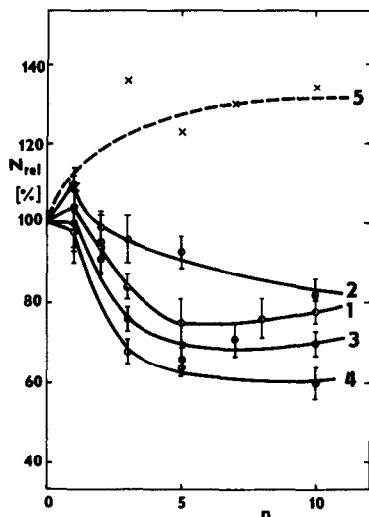

Fig. 2. Effect of repeated immobilization of SE-54 stationary phase film on the relative efficiency of the capillary columns (n = number of repeated immobilizations). 1, 2, Columns with the surface modified by leaching and silylation; 3, 4 columns with the surface untreated; 1, 3, columns tested for efficiency prior to their washing with the solvent; 2, 4, column efficiency after washing the columns with the solvent; 5, column with the untreated surface heated and conditioned in the same way as for immobilization but without azo-*tert.*-butane vapour in the column.

TABLE II

EFFECT OF REPEATED IMMOBILIZATION ON THE COLUMN EFFICIENCY (NUMBER OF PLATES PER METRE)

Column 1: 13 m × 0.25 mm I.D., silylated, immobilized with azo-*tert.*-butane. Column 2: 12 m × 0.25 mm I.D., surface untreated, immobilization only simulated without azo-*tert.*-butane.

Column No.	Solute	Initial state	No. of immobilizations				After washing
			1	5	8	10	
1	Decane	3450	3500	3050	2450	3000	2950
	Naphthalene	3450	3400	3150	2750	2650	2800
	Methyl nonanoate	3650	3550	3150	3000	3200	3200
2	Decane	2500	2650	2750	3150	3050	–
	Naphthalene	3200	3450	3050	3350	3400	–
	Methyl nonanoate	2850	3100	3350	3300	3350	–

non-immobilized comparative column practically does not change (curve 1), a marked decrease occurs on the columns with silylated surfaces after the third immobilization and on the columns with untreated surfaces even after the first immobilization. After 5–7 immobilizations the capacity ratio of undecane on both types of the columns stabilized at 86–88% of the original capacity ratio, which correlates well with levelling of the degree of immobilization on both types of columns. The decrease in the capacity ratio cannot be caused by the presence of azo-*tert.*-butane decomposition products in the stationary phase film because, owing to their non-polar character, they should slightly increase the capacity ratio of undecane. The decrease in the capacity ratio of naphthalene and methyl nonanoate acid was of a similar character but less marked. It
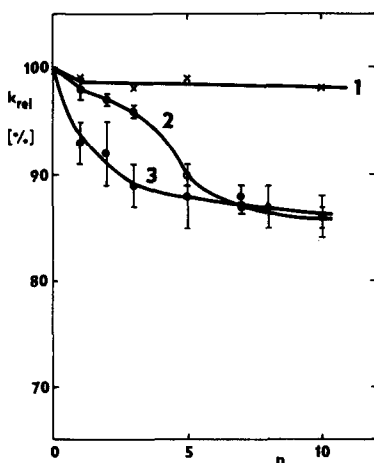

Fig. 3. Change in the capacity ratio of undecane during repeated immobilization of SE-54 stationary phase film (k_{rel} = relative capacity factor of undecane; n = number of repeated immobilizations). 1, Column with the untreated surface subjected to simulated immobilization (see 5 in Fig. 2); 2, columns with the surface modified by leaching and silylation; 3, columns with the surface untreated.

can therefore be assumed that similarly to the decrease in the column efficiency, the decrease in the capacity ratios is also caused by a decrease in interactions of the solutes with the stationary phase.

CONCLUSION

The repeated immobilization of an SE-54 stationary phase film can increase the degree of immobilization up to 100%. Multiple immobilization of the stationary phase film, however, is not desirable from the point of view of the chromatographic properties of the columns. The columns show the optimal separation properties after the first immobilization, and after further immobilizations their efficiency and capacity decrease. It is sufficient to immobilize the capillary columns in the described way once for gas chromatography and twice or at most three times for liquid chromatography.

Silylation of the surface with D_4 increased the degree of immobilization of the stationary phase film markedly, which shows that the stationary phase film immobilization is due to both cross-linking and chemical bonding to the capillary surface.

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