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COMPARISON OF METHODS FOR THE DEACTIVATION OF GLASS OPEN-TUBULAR COLUMNS WITH PEG 20M

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SUMMARY

A new deactivation method for glass capillary columns is described. Vapours of PEG 20M are bled from a short pre-column on to the capillary column and act as the deactivating agent. Bare columns, as well as coated columns, can be deactivated and eventually be re-deactivated after deterioration.

Columns deactivated with this new method compare favourably with columns deactivated by other methods involving PEG 20M, with respect to adsorption and catalytic activity. The influence of the deactivating agent on the polarity of SE-30 coated columns is small.

PEG 20M deactivated roughened surfaces are amenable to subsequent coating with polar phases. We have concluded that the gas-phase deactivation method has decisive advantages over other methods involving PEG 20M.

INTRODUCTION

Glass capillary columns have found wide acceptance for the analysis of complex biochemical and environmental samples. The catalytic and adsorptive activity of the column wall, however, still presents a major problem in many applications.

Current methods of surface deactivation are open to criticism, notably for analyses above 200° and for trace analyses at the picogram level. The use of columns with a relatively thick film of stationary phase[1] in order to mask rather than eliminate column wall effects, is a workable alternative but the separation times on these columns are relatively long.

The need for well-deactivated columns of high phase-ratio (500) is obvious

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and in recent years a vast body of literature on column-wall deactivation has become available.

In a different context Aue *et al.*[2] described the formation of a film of chemically bonded PEG 20M on diatomaceous earth supports and in a subsequent paper[3] they showed that these surfaces were highly inert. Cronin[4], Schomburg *et al.*[5], Blomberg[6] and Grob and Grob[7] applied variants of the method to glass capillary columns and obtained efficient deactivation. In all of these variants the PEG 20M was applied as a solution in dichloromethane.

Recently, we described the deactivation of glass open-tubular columns with PEG 20M via the gas-phase[8], as based on the work of Ives and Giuffrida[9] on packed columns. We showed that vapours of PEG 20M bled from a short pre-column on to a glass open-tubular column were very effective in deactivation.

In this paper we have presented a more detailed description of the possibilities and limitations of this gas-phase method compared with other methods involving PEG 20M.

EXPERIMENTAL

Gas-phase deactivation procedure

A glass tube (8 × 0.25 in. O.D., 2 mm I.D.), packed over a length of 3 in. with 5% PEG 20M on Chromosorb W AW, was inserted in the hot zone of the injection port of a gas chromatograph. In the oven compartment the exit end of this precolumn was tapered to about 1.2 mm to match the outer diameter of the capillary column to be deactivated. The connection was made with shrinkable PTFE tubing. The temperature of the pre-column was *ca.* 5–10° higher than that of the capillary column. The exact temperatures were not critical and in most experiments were *ca.* 260 and 250°, respectively. The PEG 20M was allowed to bleed through the capillary column overnight, at a carrier gas flow-rate of *ca.* 3 ml/min.

Preparation of apolar columns

A series of five thin-film coated SE-30 columns was prepared, differing with respect to the deactivation method. All columns were 15 m × 0.4 mm I.D., Duran 50 glass, and coated according to Bouche and Verzele[10] with 0.2 μm of SE-30. After coating, the columns were conditioned by temperature programming at 1°/min to 235° and were kept at that temperature overnight.

For deactivation, we used our gas-phase method, applied to the bare column wall (No. 1) or to the coated and conditioned column (No. 2), the PEG 20M treatment according to Grob and Grob[7] but deleting the step involving Emulphor (No. 3) and the PEG 20M treatment of Blomberg[6] (No. 4). Column No. 5 was not deactivated. A sixth column was coated with a film of 0.5-μm SE-30 instead of 0.2 μm and was not deactivated (*c.f.* Table II).

Preparation of polar columns

A paired series of polar phase-coated columns was prepared using different methods for the stabilization of the stationary phase film. Stabilization by surface roughening was obtained by growing $BaCO_3$ particles on the column wall according to Grob *et al.*[11]: by the deposition of NaCl particles from a NaCl sol, as

described by Franken *et al.*[12] after some procedural modifications; or by the formation of whiskers according to Schieke *et al.*[13,14]. Stabilization by the incorporation of colloidal silica into the stationary phase (SPOT columns[15]) was achieved using Silanox 101 (ref. 16) or Cab-O-Sil (ref. 15) as the stabilizing agent. In this latter method the surface active and deactivating agent benzyltriphenylphosphoniumchloride was omitted in order to investigate the deactivating effect of the PEG 20M bleeding products only.

Prior to coating, but after surface roughening or after deposition of the colloidal silica, one column of each pair was deactivated via the gas-phase; the non-deactivated column served as a comparison.

Evaluation of columns

As a measure of the adsorption properties of variously-deactivated columns we compared the peak width of 2-hydroxybenzaldehyde (2-HB) with that of *n*-undecane (*n*-C_{11}). The peak width of 2-HB is strongly influenced by the adsorptivity of the column wall in contrast to the peak width of *n*-C_{11}.

At 190° oven temperature 0.5 μl each of *n*-C_{11} and 2-HB were injected separately using a carefully deactivated all-glass splitter, split ratio 1:1000. The carrier gas was helium at a flow-rate of 10 cm/s. The "deactivation effect", *DE*, was calculated as a percentage:

$$DE = \left(\frac{W_{0.1\ n\text{-}C_{11}}}{W_{0.1\ 2\text{-HB}}}\right) \cdot f_D \cdot 100\% \tag{1}$$

in which $W_{0.1}$ is the peak width at 10% of the peak height of the compound indicated and f_D is a correction factor required to correct *DE* to 100% in the absence of adsorption. From the definition of HETP, it follows that

$$f_D = \sqrt{\frac{H_{2\text{-HB}}}{H_{n\text{-}C_{11}}}} \tag{2}$$

H is given by the Golay equation for $k = 0$:

$$H = \frac{2D}{u} + \frac{1}{24} \cdot \frac{r^2 u}{D} \tag{3}$$

in which u denotes the mean linear velocity of the carrier gas, r the column radius and D the diffusion coefficient of the injected compound. Diffusion coefficients for 2-HB and *n*-C_{11} were obtained from Fuller *et al.*[17]. Substitution in eqn. 2 then yields a correction factor $f_D = 1.192$.

As a measure of the catalytic activity of coated columns we used the reaction rate constant (k_d) of endrin decomposition at 217° oven temperature. Endrin is known to be very sensitive to active sites on the column wall[18]. The decomposition obeys a first order reaction and accordingly we write

$$a_{t_R} = a_0 \exp(-k_d t_R) \tag{4}$$

where a is the amount of endrin present and the subscripts t_R and 0 denote time. Least-squares fitting of eqn. 4 to the experimental data for the endrin peak area *versus* retention time, *i.e.*, at various carrier gas velocities, yields k_d.

Finally, the influence of the polar PEG 20M deactivating agent on the polarity of non-polar SE-30 columns was evaluated in terms of the retention of β-BHC (1.2.3.4.5.6-hexachlorocyclohexane, β-isomer) relative to HCB (hexachlorobenzene) at 217°. The retention of β-BHC is greatly increased by polar impurities in the stationary phase while the retention of HCB is not. Experiments were carried out after one week and again after 10 weeks of continuous use at 220°.

RESULTS AND DISCUSSION

Non-coated columns

Optimal experimental conditions for the gas-phase deactivation process were investigated on the basis of the *DE* values of the variously-treated columns. As illustrated in Fig. 1 the *DE*-value is a function of time and in the example given approaches 100% after about 8 h. No set of conditions could be found in which the necessary deactivation time was substantially less than 8 h. Hence, for practical reasons we standardized the deactivation time to 16 h (overnight). Using an overnight treatment, variation of the pre-column temperature between 230 and 280° did not influence the effect of the gas-phase deactivation. A pre-column temperature of less than 230° however did not yield a well-deactivated column within 16 h. In all experiments the capillary column temperature was 5–10° less than that of the precolumn.

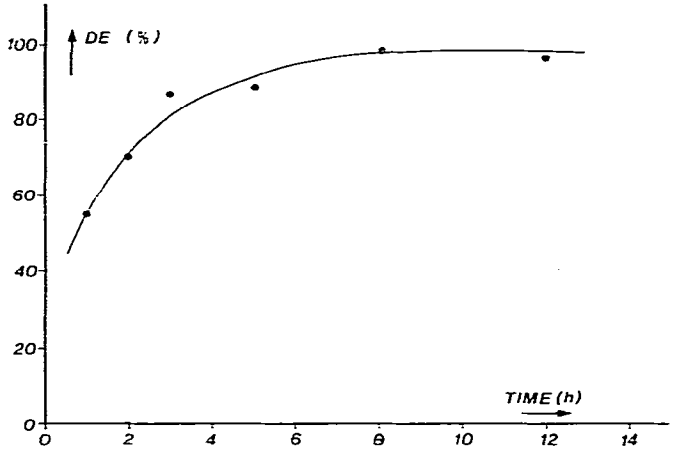

Fig. 1. *DE* values *versus* deactivation time, measured for a 50 m × 0.25 mm I.D. Duran 50 glass capillary column. Column temperature, 250°; pre-column temperature, 260°; carrier gas, helium; flow-rate, 1.5 ml/min.

It was established that each 10-m portion of a 50 m × 0.25 mm column yielded the same *DE*-value (within experimental error) at each stage of the deactivation process. We therefore conclude that the process of deactivation takes place uniformly over the entire column length at a rate determined by slow-reaction kinetics rather than by a limited availability of the PEG 20M bleeding products. This is consistent with the

observation that the effect of the gas-phase deactivation process is not influenced by column length, column diameter or carrier gas flow-rate.

We may conclude that a major advantage of the gas-phase deactivation procedure is that the results are independent of the experimental conditions over a wide range. The column lengths investigated ranged from 10 to 130 m, pre-column temperature from 230 to 280°, column diameter from 0.2 to 0.6 mm and the carrier gas flow-rate from 1 to 15 ml/min. The deactivation time was between 16 and 72 h. Rinsing with dichloromethane or methanol after deactivation, which is an essential step in Blomberg's method to remove the excess of PEG 20M degradation products, did not influence the *DE* value of gas-phase deactivated columns. Apparently, these degradation products are absent after gas-phase treatment.

An unexplained observation was that a packing of 5% PEG 20M on Chromosorb W AW loses its effectiveness as a packing for the pre-column after about 6 months storage. Therefore the packing is freshly prepared every 3 months.

The adsorption properties of columns deactivated according to Grob and Grob[7] and Blomberg[6] were compared with columns deactivated via the gas-phase on the basis of the *DE* values, *c.f.* Table I. Both the gas-phase and the Blomberg method yield well-deactivated columns. However, neither the Blomberg column nor the Grob column withstands heat treatment at 250° for 16 h, whereas the gas-phase deactivated column remains almost stable.

In practice, Blomberg-type columns are indeed satisfactory for temperatures up to 200° but deteriorate within a few days at higher temperatures.

TABLE I

DE VALUES OF NON-COATED DEACTIVATED COLUMNS BEFORE AND AFTER HEAT TREATMENT AT 250°

Deactivation method	*DE*	
	Before heat treatment	*After heat treatment*
Gas-phase[8]	94	91
Blomberg[6]	91	75
Grob and Grob[7]*	23	
	48**	0

* The step involving Emulphor or Triton was omitted.
** After two deactivations.

Apolar columns

The influence of different PEG 20M deactivation methods on the polarity and catalytic activity of the SE-30 columns 1–6 (*c.f.* Experimental) is summarized in Table II. All four methods do influence the polarity of the column but the polarity changes during ageing are only pronounced with column No. 3. Both gas-phase methods and the Blomberg method yield columns with a polarity closely resembling that of the pure SE-30 liquid stationary phase. The interpretation of these data is facilitated by comparison with relative retention data for other stationary phases: 1.57 for the low-polar OV-17, 2.18 for the medium-polar OV-210 and 5.86 for the polar OV-225.

The catalytic activity of these columns towards picogram amounts of pesticides, including endrin, is illustrated in Fig. 2. Endrin does not elute from columns 4 and

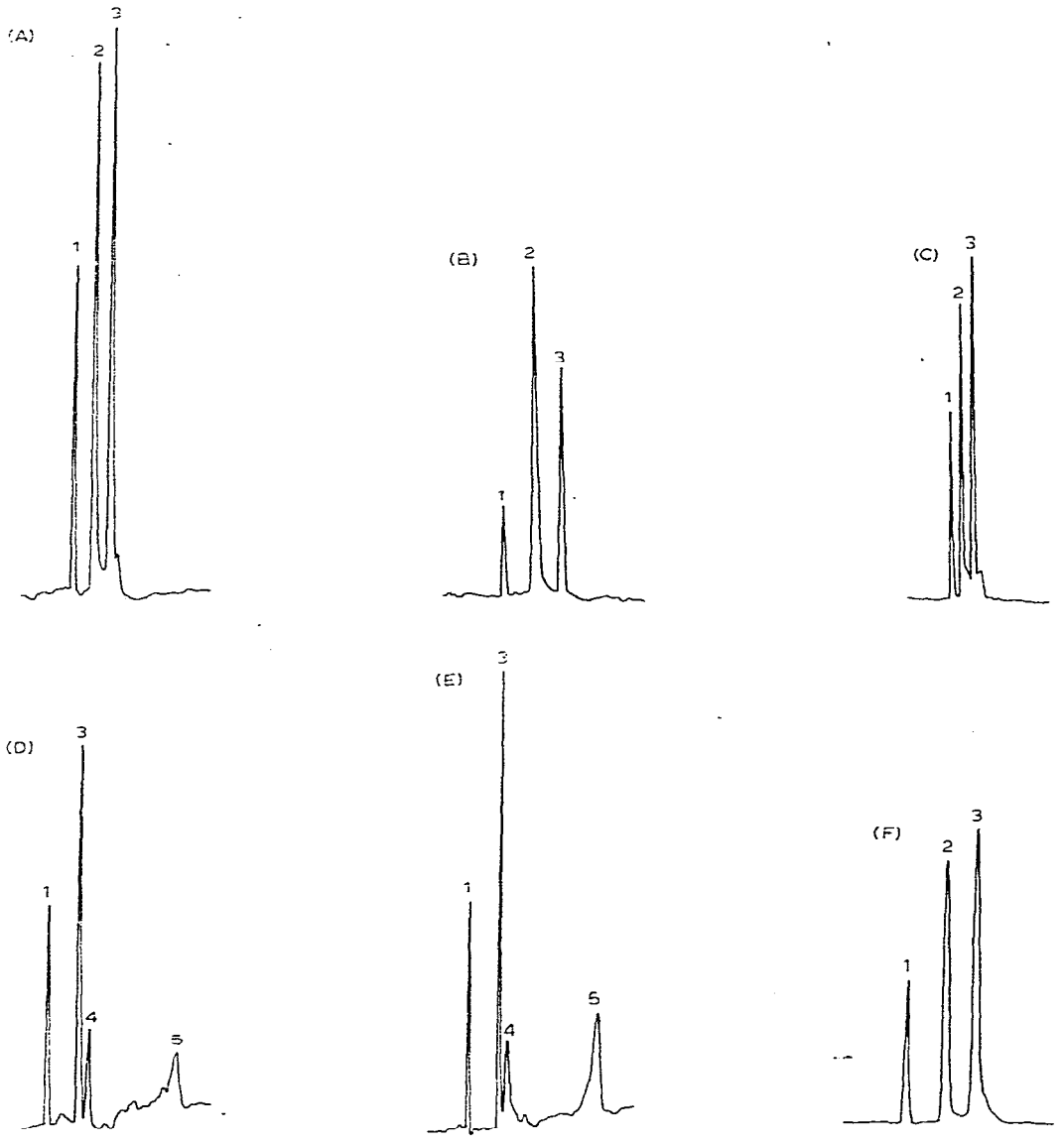

Fig. 2. Chromatograms from 1 μl of a test mixture containing 1.5 pg of dieldrin and 7.5 pg each of endrin and *p,p'*-DDD on (A) column 1, (B) column 2, (C) column 3, (D) column 4, (E) column 5 and (F) column 6. For column numbers, see text. Peaks: 1 = dieldrin; 2 = endrin; 3 = *p,p'*-DDD; 4 = endrin decomposition product 1; 5 = endrin decomposition product 2. Oven temperature, 217°; detector (ECD) temperature, 300°; carrier gas, Ar–CH_4 (95:5); pre-pressure 0.2 atm; purge gas, Ar–CH_4 (95:5); flow-rate 25 ml/min. Injection device, ref. 19.

5, due to total catalytic breakdown. A numerical evaluation of catalytic wall activity in columns 1–3 and 6 is given in Table II.

Deactivation via the gas-phase *after* coating appears to be even more effective than prior to coating. This provides possibilities for re-deactivating coated columns

TABLE II

POLARITY AND ACTIVITY TEST ON SE-30 COLUMNS

α = Retention of β-BHC relative to HBC at 217°, *c.f.* Experimental.

Column No.	*Deactivation*	α_1*	α_{10}**	$k_d \cdot 10^{-4}$***
1	Gas-phase[8]	0.983	0.967	4.6
2	"Pepping-up"	1	0.977	0.9
3	Grob and Grob[7]	1.170	1	10.2
4	Blomberg[6]	1	0.972	***
5	No deactivation	0.956	0.956	***
6	No deactivation ($d_f = 0.5\ \mu$m)	0.956	0.956	1.6

* After 1 week at 220°.
** After 10 weeks at 220°.
*** No endrin response.

("pepping-up") after deterioration due to prolonged use, provided that the stationary phase is stable up to at least 220°.

The results with column No. 6 confirm the effectiveness of a thick film of the stationary phase itself as a deactivating agent[1].

Polar columns

Table III summarizes the results obtained with pairs of deactivated and non-deactivated polar columns. In all cases the gas-phase deactivated column shows a significantly lower catalytic activity, *i.e.*, a lower k_d value, than the non-deactivated one. This is even true for the PEG 20M coated columns 11 and 12. Apparently, the PEG 20M derived deactivating agent is of a quite different nature than the bulk of the PEG 20M.

The effect of film thickness on catalytic activity is evident from a comparison of the k_d value of column 7 with that of 9 and of column 8 with that of 10, respectively.

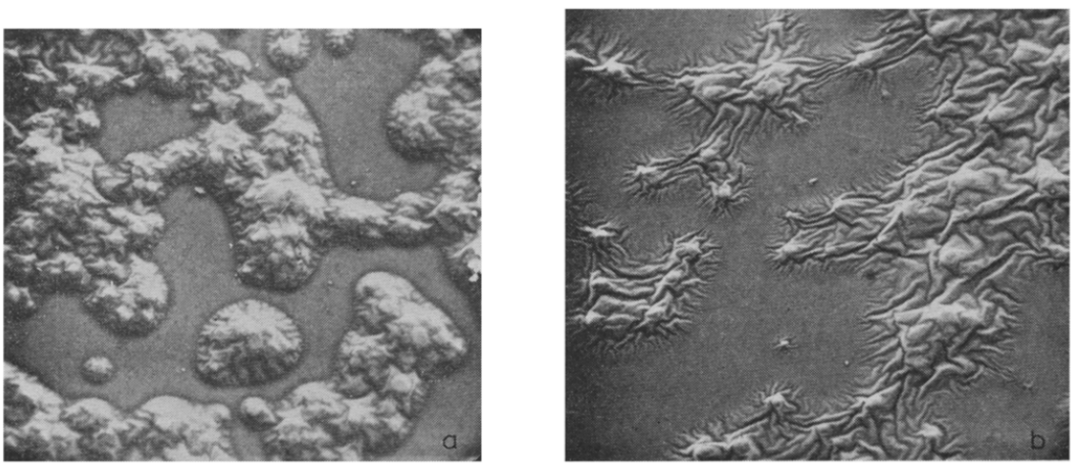

Fig. 3. Scanning electron microscope (SEM) pictures (20 × 20 μm) of (a) non-deactivated and (b) deactivated NaCl/OV-17 columns (columns 13 and 14, respectively). Scanning electron micrographs were taken using a "Stereoscan" (Cambridge Instruments, Cambridge, Great Britain).

TABLE III

RESULTS OBTAINED WITH POLAR COLUMNS

Column No.	*Coating solution*	*Roughening*	*Deacti-vation*	*Length (m)*	*I.D. (mm)*	*Column temperature (°C)*	*k*	*N/m**	*CE (%)*	*Carrier gas*	*$k_a \cdot 10^{-4}$ at 217° (sec^{-1})*	*Coating method*
7	12% (w/v) OV-17 in *n*-hexane	$BaCO_3$	No	20	0.3	190	11.5	2120	57	He	42.9	Dynamic mercury plug method***
8	As column 7	$BaCO_3$	Yes	20	0.3	190	6.4	2312	60	He	5.1	
9	40% (w/v) OV-17 in hexane	$BaCO_3$	No	18.5	0.3	220	11.3	2478	67	He	10.9	
10	As column 9	$BaCO_3$	Yes	20	0.3	220	8.6	2524	67	He	2.4	
11	12% (w/v) PEG 20 M in CH_2Cl_2	$BaCO_3$	No	20	0.3	190	8.4	2562	68	He	1.1	
12	As column 11	$BaCO_3$	Yes	20	0.3	190	10.5	2613	70	He	0.4	
13	40% (w/v) OV-17 in toluene	NaCl sol	No	26	0.4	225	15.0	2768	98	N_2	4.4	
14	As column 13	NaCl sol	Yes	26	0.4	225	13.1	2483	94	N_2	1.0	
15	0.2% (w/v) SP 2401 in acetone	Silanox	No	13	0.4	220	—	830	30	He	No endrin response	Static method
16	As column 15	Silanox	Yes	13	0.4	220	11.3	1881	68	He	2.0	
17	0.3% (w/v) OV-17 in acetone	Cab-O-Sil	Yes	15.5	0.5	220	19.9	1647	76	He	43.2	
18	As column 17	Cab-O-Sil	Yes**	—	—	—	—	—	—	—	6.7	

* Number of theoretical plates, *N*, per metre, calculated for the methyl ester of docosane-1-carboxylic acid.

** Column 17 after "pepping up".

*** Ref. 20 after procedural modifications.

From a comparison of column 7 with 11 it appears that the shielding effect of PEG 20M is much more pronounced than that of OV-17.

The influence of the polar interjacent layer on the wettability of the glass surface is illustrated in Fig. 3a and b, in the form of scanning electron micrographs (SEM) of columns 13 and 14, respectively. On the non-deactivated surface of column 13 the OV-17 liquid phase pools around the sodium chloride crystals and shows a finite contact angle. Consequently, part of the surface is not covered with stationary

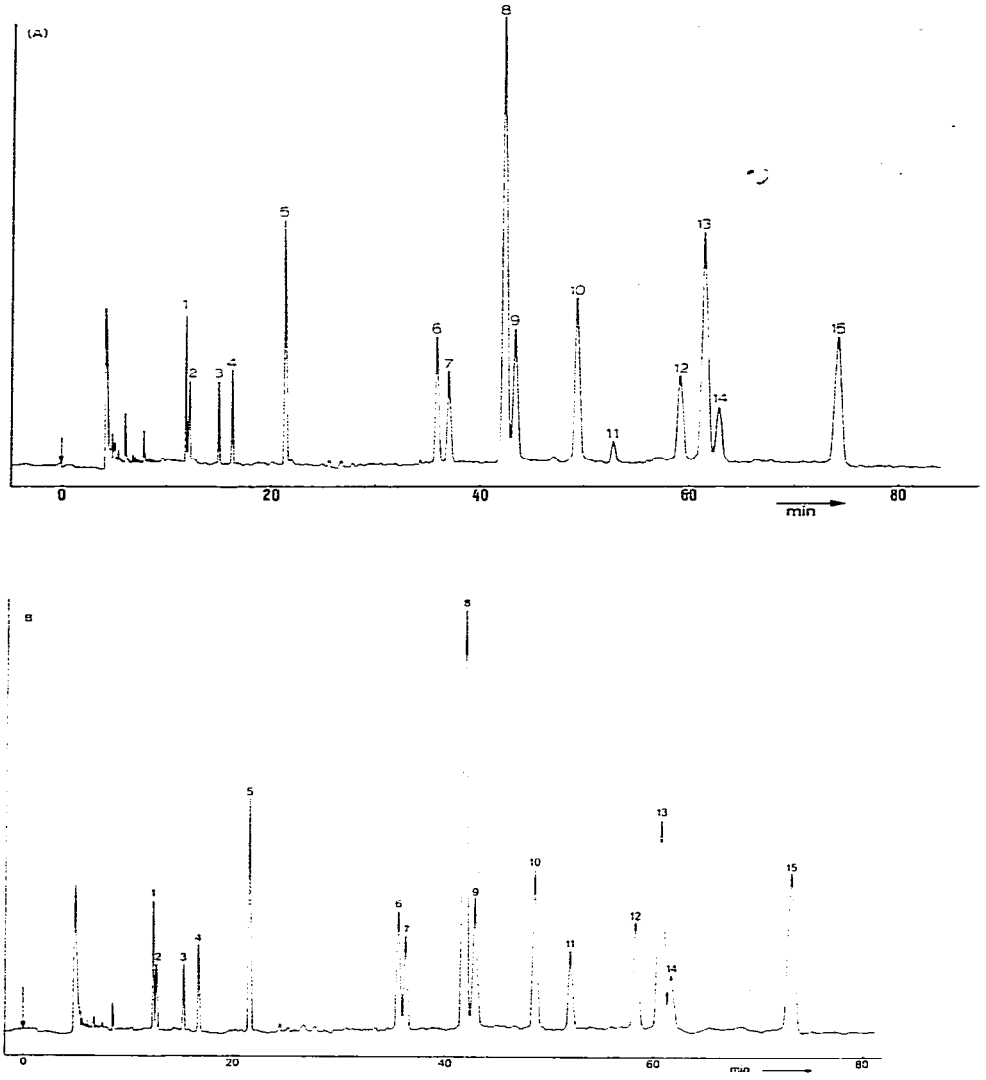

Fig. 4. Separation of a mixture of chlorinated pesticides on (A) column 13 and (B) column 14. For column numbers, see text. Peaks: 1 = HCB; 2 = α-BHC, 3 = γ-BHC; 4 = β-BHC; 5 = aldrin; 6 = *o,p'*-DDE; 7 = α-endosulfan; 8 = *p,p'*-DDE; 9 = dieldrin; 10 = *o,p'*-DDD; 11 = endrin; 12 = *o,p'*-DDT; 13 = *p,p'*-DDD; 14 = β-endosulfan; 15 = *p,p'*-DDT. Oven temperature, 217°; carrier gas, nitrogen; pre-pressure, 0.2 atm; injection device, ref. 19.

liquid (Fig. 3a), in contrast to the situation on a gas-phase deactivated surface (Fig. 3b) where no finite contact angle is observed. These different wettabilities, however, are not reflected in the coating efficiencies, (CE; ref. 21), because the diameter of these open spots is negligible compared with the column diameter.

The exception is the pair of columns containing Silanox 101 (columns 15 and 16). The silanized apolar surface of Silanox 101 is not wetted by the medium-polar SP 2401 stationary phase and consequently no stable dispersion of Silanox 101 and SP 2401 is obtained[15]. This results in a low coating efficiency, an excessive bleeding rate and a continuous decrease in the capacity ratio. After deactivation however, the polar intermediate layer of the deactivation agent improves the wettability of the Silanox 101 surface and a stable column of reasonable CE is obtained.

Fig. 4 shows the analysis of a 15-compound test mixture of pesticides on columns 13 and 14. The non-deactivated column (No. 13) gives a weak endrin response. Note that the catalytic activity is also reflected in the reduced peak areas of *p,p'*-DDT and *o,p'*-DDT.

The gas-phase deactivation was also applied to highly active surfaces such as Cab-O-Sil and whiskers. For the whisker columns no endrin response was obtained, even after repeated re-deactivation of the coated column. An OV-17 column, containing Cab-O-Sil, deactivated prior to coating, still was rather active (column 17). The catalytic activity however was substantially decreased by deactivation after coating (column 18).

CONCLUSION

Treatment of glass capillary columns with PEG 20M via the gas-phase as described, offers a simple and effective method for reducing both the catalytic and the adsorptive activity of the column wall. The surfaces obtained are compatible for subsequent coating with polar stationary phases. The deactivating layer withstands continuous use at 220° and intermittent use up to at least 250°. The method can be applied both to coated and non-coated columns.

This enables a re-deactivation of columns if adsorption or catalytic activity develop during prolonged use. A slight increase of the polarity of non-polar columns must be accepted. Whisker columns cannot be deactivated by this method.

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