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SEPARATION OF UNDERIVATISED BARBITURATES BY CAPILLARY COLUMN GAS CHROMATOGRAPHY

PREPARATION OF MEDIUM POLAR POLYMETHYLSILOXANE COLUMNS TO OPTIMIZE SELECTIVITY

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SUMMARY

Methods for the preparation of immobilized non-polar polymethylsiloxane capillary columns are described, for the analysis of underivatized barbiturates. The columns produced can resolve most of the common underivatized barbiturates, but lack the selectivity for certain difficult-to-separate barbiturates.

The preparation of immobilised medium polar capillary columns in which compounds having the fundamental structure of the barbiturates incorporated into the stationary phases is described. This method offers a way of extending the selectivity of stationary phases enabling difficult separations to be achieved. It should be possible to extend this process to any groups of related, and difficult-to-separate mixtures.

INTRODUCTION

The widespread misuse of barbiturates[1] has made it necessary for forensic science laboratories to provide analytical services which specifically identify all common barbiturates. One aid to this task is the use of capillary column gas chromatography (GC). Many capillary systems have been described[2–7] which are capable of resolving certain barbiturate mixtures but no single column seems to possess adequate resolving power for all such compounds. In fact it is common practice to use a combination of two capillary columns[8,9], one containing non-polar and the other polar phases for the separation of barbiturate mixtures.

Because of their high polarity and low volatility, it is necessary that any capillary GC system for barbiturates should satisfy the four criteria of thermal stability, high efficiency with good deactivation and selectivity. These four qualities are seldom found in one single column especially selectivity. Although progress has been made in the preparation of well deactivated non-polar columns by means of persilylation[10,11], these stationary phases usually lack the selectivity for certain difficult-to-separate barbiturate pairs. We would agree with Verzele[12] that, for capillary GC, the

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limits of efficiency have been reached and that the accent of column research should be directed towards higher selectivity through a wider choice of stationary phases. Numerous methods for the preparation of columns coated with medium polar or polar phases have been described[13–18] but many of them have serious drawbacks. Berendsen *et al.*[19] have shown that greater selectivity in high-performance liquid chromatography (HPLC) can be achieved by choosing a liquid phase which possesses some of the chemical features of the materials being separated, in their case fluorosilicone polymers for the analysis of fluorocarbons. By incorporating the barbiturate structure into immobilised stationary phases in capillary column gas chromatography we have found that selectivity to certain difficult-to-separate barbiturates can be improved.

The work presented falls into two sections. The synthesis of monomers containing the barbiturate moiety and the *in situ* incorporation of these, by peroxide treatment, into an SE54 stationary phase to form a three-dimensional co-polymeric phase.

EXPERIMENTAL

Characterisation of barbiturate monomers

Unless otherwise stated, NMR spectra were recorded in deuterated chloroform solution with a Perkin-Elmer R32 NMR spectrometer, and are given in parts per million (δ) downfield from an internal tetramethylsilane (TMS) standard. The abbreviations s, d, t, q and m refer to singlet, doublet, triplet, quartet and multiplet respectively.

Infrared (IR) spectra were recorded on a Perkin-Elmer 157 prism spectrophotometer. The abbreviations s, m and w refer to strong, medium and weak respectively. Mass spectra were recorded on a M.S.S. mass spectrometer.

Synthesis of 5-(2-vinyl dimethyl siloxy propyl)-5-(1-methylbutyl)-barbituric acid (I)

Conversion of 5-allyl-5-(1-methylbutyl)barbituric acid into 5-(2-hydroxypropyl)-5-(1-methylbutyl)barbituric acid according to Maynert and Washburn[20]. Concentrated sulphuric acid (25 ml) was added to 5-allyl-5-(1-methylbutyl)barbituric acid (5 g) in a round bottomed flask equipped with a magnetic stirrer bar for 12 min at room temperature. The yellowish brown solution was poured into ice-water (150 ml). The white precipitate formed was filtered, washed with water (50 ml) and dried in an oven at 100°C. The product was recrystallised twice from ethanol to give colourless crystals of 5-(2-hydroxypropyl)-5-(1-methylbutyl)barbituric acid (4.2 g, 78%), m.p. 204–206°C (lit.[20], 215–216°C).

IR(Nujol): 3350 cm^{-1} (m, O–H), 3050 cm^{-1} (m, N–H), 1700 cm^{-1} (s, C=O).

NMR[$(C^2H_3)_2CO$]: δ 9.90 (s, 2H, N–H), δ 3.80 (m, 2H, CH_2–C–C=O).

$C_{12}H_{20}N_2O_4$ requires: C = 56.25, H = 7.81, N = 10.93; found: C = 56.54, H = 8.09, N = 11.12.

Conversion of 5-(2-hydroxypropyl)-5-(1-methylbutyl)-barbituric acid into 5-(2-vinyldimethylsiloxypropyl)-5-(1-methylbutyl)barbituric acid (I). To a solution of 5-(2-hydroxypropyl)-5-(1-methylbutyl)barbituric acid (2 g) in dimethyl formamide (distilled, 6 ml) in a flask equipped with stirrer and reflux condenser, was added imidazole (3 g, 6 equiv.), moisture being kept out by means of a Drierite-filled tube.

Vinyldimethylethoxysilane (2 ml, 1.5 equiv., Petrarch system, Bristol, PA, U.S.A.) was added at room temperature. The reaction was stirred overnight at 80°C to give the desired product after purification by column chromatography (silica gel 60; diethyl ether–chloroform (1:4, v/v), R_F 0.35). The product was recrystallised with light petroleum (b.p. = 40–60°C) to give colourless crystals, m.p. 138–40°C.

IR(Nujol): 3100 cm^{-1} (m, N–H), 1680 cm^{-1} (s, C=O), 1260 cm^{-1} [w, Si–$(CH_3)_2$], 800 cm^{-1} [m, Si–$(CH_3)_2$].

NMR (C^2HCl_3 with no TMS added): δ 0.15 (s, 6H, Si–CH_3), δ 3.85 (m, 1H, CH–O–Si), δ 5.50–6.10 (m, 3H, CH=CH_2), δ 8.50 (s, 2H, N–H).

Diagnostically important fragmentation in mass spectrum, *m/e* 325 (M − 15, 100 relative intensity), 313 (M − 27, 561), 238 (M − 102, 10), 223 (M − 117, 68), 211 (M − 129, 16).

Synthesis of 1,3-dimethyl-5-allyl-5-(1-methylbutyl)-barbituric acid (II)

To a mixture of 5-allyl-5-(1-methylbutyl)barbituric acid (10 g) in aqueous sodium hydroxide (4 g in 40 ml water, 2.5 equiv.), was added dimethyl sulphate (20 ml, *ca.* 5 equiv.).

The reaction was exothermic and completed in about 30 min. Thin-layer chromatography (TLC) on silica gel showed complete conversion into product after 30 min [R_F 0.49, diethylether–chloroform (1:4, v/v)]. Water (50 ml) was added to the reaction mixture, and the product was extracted into diethyl ether (3 × 100 ml), washed with water (30 ml), dried (anhydrous sodium sulphate) and filtered. After removal of solvent, a crude product was obtained in the form of a pale-yellow oil. Purification by column chromatography [silica gel 60; diethylether–chloroform (1:4, v/v)] yielded a colourless oil (8 g).

IR (film): 1660 cm^{-1} (s, C=O), 920 cm^{-1} (s, =C–H).

NMR (C^2HCl_3): δ 2.70 (d, 2H, CH_2–C=C), δ 3.25 (s, 6H, N–CH_3), 4.90–5.80 (m, 3H, CH=CH_2).

$C_{14}H_{22}N_2O_3$ requires: C = 63.15, H = 8.27, N = 10.53; found: C = 63.57, H = 8.47, N = 10.40.

Synthesis of 1,3-diallyl-5-ethyl-5-isoamylbarbituric acid (III)

To a mixture of 5-ethyl-5-isoamylbarbituric acid (2 g) and a catalytic amount of fine copper powder in aqueous sodium hydroxide (0.8 g in 8 ml water, 2.2 equiv.), was added allyl bromide (4 ml, 5 equiv.). The reaction was exothermic and the colour of the reaction mixture changed from colourless to milky. TLC on silica gel showed complete conversion into product in about 5 h [R_F 0.65, acetone–chloroform (3:7, v/v)].

Water (20 ml) was added to the reaction mixture and the product was extracted into diethyl ether (3 × 100 ml), washed with sodium hydroxide (1 *M*, 50 ml), water (50 ml), dried (anhydrous sodium sulphate) and filtered. After removal of solvent, a crude product was obtained in the form of a pale-yellow oil. After purification by column chromatography [silica gel 60; acetone–chloroform (3:7, v/v)], a colourless oil (1.46 g, 62%) was obtained.

IR (film): 3050 cm^{-1} (m, =C–H stretch), 1680 cm^{-1} (s, C=O), 1000 and 940 cm^{-1} (s, =C–H bend).

NMR (C^2HCl_3): δ 4.50 (d, 4H, N–CH_2), δ 5.00–5.50 (m, 4H, CH_2 = C), δ 5.50–6.20 (m, 2H, CH=C).

$C_{17}H_{26}N_2O_3$ requires: C = 66.66, H = 8.50, N = 9.15; found: C = 66.32, H = 8.84, N = 9.15.

Preparation of non-polar immobilised polymethylsiloxane columns

Cleaning and drawing of glass tubing. Glass capillaries (0.2–0.4 mm I.D. and *ca.* 0.8 mm O.D.) were drawn from Pyrex glass tubes (3.5 mm I.D. and 6.0 mm O.D.) on a Carlo Erba GCDM 60 glass-drawing machine. Before drawing, the glass tubes were cleaned with chromic acid for 1 h, washed with water, acetone, and finally dried with a stream of dry nitrogen.

Leaching. The capillary column was filled with 20% hydrochloric acid (Aristar grade, BDH) by means of a water pump.

The column was then removed from the hydrochloric acid solution and air was allowed to be drawn into the column for 8% of the column length. The full end was sealed and the other end sealed under vacuum. The column was placed in an oven at 180°C for 13 h.

Rinsing and dehydration. Both ends of the leached column were opened and the hydrochloric acid inside was displaced by one volume of 2% hydrochloric acid (pH 3) employing a water pump. This solution was, in turn, displaced by half a volume of methanol (Analar grade, BDH). The rate of rinsing being not more than 2 cm sec^{-1}. The methanol was flushed out and the column was placed in an oven at 250°C for 2 h. This dehydration step was accomplished under a slow carrier gas flow of nitrogen.

Deactivation by persilylation. Persilylation was performed as described below with diphenyltetramethyldisilazane (DPTMDS) although in some experiments, octamethylcyclotetrasiloxane (D_4) and hexamethyldisilazane (HMDS) were used instead. All silylating agents were obtained from Fluka (Buchs, Switzerland).

By means of a syringe, the silylating agent was sucked into the column until it filled 10% of its volume.

This plug was then moved through the column by nitrogen pressure, at a rate of 0.5 cm sec^{-1} for DPTMDS (1 cm sec^{-1} for D_4 and 2 cm sec^{-1} for HMDS) and the nitrogen flow stopped immediately after the plug had left the column. Both ends of the column were then vacuum sealed (oil pump), and the column placed in an oven, the temperature of which was programmed from 50°C to 400°C at 5°C/min and held at 400°C for 16 h. The column was allowed to cool slowly in the closed oven for 1 h and one end of the persilylated column then opened under methylene chloride (Analar grade, BDH). The percentage of the column length filled with methylene chloride was a rough estimate of the degree of persilylation[21]. Filling to about 70% was generally achieved. Finally the capillary was rinsed with 5 ml of methylene chloride and dried in an oven at 150°C for 2 h. This dehydration step was accomplished under a slow carrier gas flow of nitrogen.

Coating. Solutions for static coating were prepared using pentane (Analar grade, BDH) as solvent for SE-54 (Phasesep). When the stationary phase had dissolved, 0.1–1%, w/w, of dicumyl peroxide (DCUP) (2% in toluene, BDH) was added and the resulting coating solution degassed for 1 min (ultrasonic bath). The column was completely filled with coating solution from a syringe under pressure.

A further 10% of this solution continued to be passed through the column before the pressure was discontinued. One end of the column containing no liquid

phase was drawn out into a fine capillary and the solution pressurised into the vacant volume. The fine end of the column was then sealed with molten wax. About 30 min was allowed for the wax to solidify and a vacuum (oil pump) was applied to the other end, evaporation of the solvent being assisted by immersing the column in a water-bath at room temperature. Care must be taken not to allow the wax in the sealed end to touch the water in the waterbath. Evaporation was terminated when only 1–2 coils at the sealed end were still filled with solvent. This normally took about 13 h for a 23-m column.

Immobilisation. After static coating, the column was flushed with nitrogen for 1 h and cured according to the method of Blomberg *et al.*[22]. For this the capillary was sealed under vacuum and then placed in an oven at 140°C for 30 min. In some experiments, columns were cured according to the method of Grob *et al.*[23], in which the vacuum sealed capillaries were kept for 1 h at 160°C and then 1 h at 180°C. After curing, the oven was rapidly cooled and the columns flushed with dry nitrogen at room temperature. The columns were then conditioned at 250°C for 1 h and their performance tested with the Grob test mixture[24,25]. Finally the columns were washed slowly with 5 ml of methylene chloride over a period of 5–6 h, dried in a stream of nitrogen and conditioned overnight at 300°C before re-testing.

Preparation of selective medium polar polymethylsiloxane columns

Cleaning of the glass tubing, leaching, rinsing, dehydration and deactivation by persilylation were carried out as described for the non-polar columns.

A solution for static coating was prepared by first dissolving SE-54 in pentane and then adding from 5 to 50% (w/w) of the unsaturated barbiturate monomers (I, II or III). Finally, 0.5–10% (w/w) DCUP (2% in toluene) was added. The ratio of barbiturate monomer to that of DCUP was varied in order to find the optimal amount for each of the barbiturate monomers. After static coating as described above, the column was flushed with dry nitrogen for 1 h and cured according to the method of Blomberg *et al.*[22] detailed above. The oven was cooled rapidly and the column flushed with dry nitrogen at room temperature. The ends of the columns were straightened under a flow of nitrogen using a Carlo Erba GESM 102-20 automatic electrical end-straightening machine. The column was conditioned at 250°C for 1 h and tests run. Finally the column was washed with 5 ml methylene chloride, dried in a stream of nitrogen and re-conditioned overnight at 300°C before re-testing.

Gas chromatography

Capillary GC was performed with a standard Hewlett-Packard Model 5710A gas chromatograph modified for capillary GC analysis and equipped with a flame ionisation detector. The carrier gas was nitrogen with injector and detector temperatures of 300°C.

RESULTS AND DISCUSSION

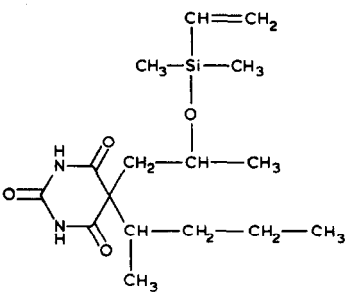
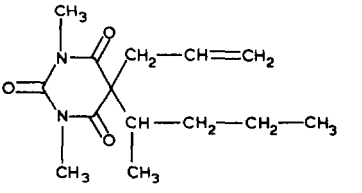
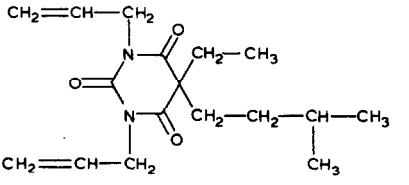
The effect of incorporating the vinyl silicone derived barbiturate (I) (Table I) into the non-polar SE54 stationary phase was dependent upon the concentration of the monomer and the time of curing. As might be anticipated, the higher the concentration of the monomer the greater the polarity of the resultant phase. This can

readily be seen in chromatograms for a test mixture of standard barbiturates on two columns, one containing 2% and one with 10% by weight of barbiturate monomer (I) incorporated, as compared with that of the same mixture on a reference SE-54 column (Fig. 1). A further observation was that the activity was considerably affected by the curing time at 140°C being much greater as the time of curing increased. Also, the activity of the column became more apparent as the concentration of the monomer incorporated increased, as shown by increased tailing behaviour (Fig. 1B and C) from 2 to 10% incorporation. Clearly the performance of these columns was no improvement on the reference SE-54 column (Fig. 1A) especially for brallobarbital, which failed to elute, and cyclobarbital and phenobarbital which could not be resolved on the column employing 10% incorporation of the barbiturate. One explanation for the performance of these columns may be the incorporation of the barbiturate into the polymer as the free acid form although it might have been anticipated that this was beneficial in the same way that incorporation of formic acid vapour into the carrier gas affects chromatographic responses[26–28].

In order to test whether the "active" hydrogens on the nitrogen of the barbiturate were giving rise to this effect a series of columns were prepared in which the hydrogens were replaced by methyl groups. It was also felt that little advantage was

TABLE I

STRUCTURE OF BARBITURATE MONOMERS SYNTHESIZED

No.	*Barbiturate monomers*	*Structure*
I	5-(2-Vinyldimethylsiloxypropyl)-5-(1-methylbutyl)barbituric acid	
II	1,3-Dimethyl-5-allyl-5-(1-methylbutyl)barbituric acid	
III	1,3-Diallyl-5-ethyl-5-isoamyl-barbituric acid	

being gained from the use of a silicone derivative and this was replaced by the more easily synthesised 5-allyl barbiturate (II) (Table I). Columns having barbiturate monomer (II) at concentrations from 5 to 50% by weight were prepared. These columns were slightly acidic in nature and showed greater selectivity and better performance than the previously made columns and greater selectivity than the reference SE54 column. This is illustrated by the resolution of amylobarbital and nealbarbital (peaks 5 and 6 respectively in Fig. 2). The selectivity, as expected, was much greater for the higher level of incorporation of monomer (50%) compared with the lower (8%) level of incorporation. Furthermore, nealbarbital has the larger retention time of the two barbiturates. This would be expected since the structure of the incorporated barbiturate bears a stronger resemblance to nealbarbital than it does to amylobarbital and a stronger interaction between the former with the incorporated barbiturate seems to have been established.

The problem of activities still seems to be present, giving rise to some tailing of the chromatographed species. One reason for this may be the presence of small amounts of the peroxide and perhaps breakdown products still present in the stationary phase matrix.

The possibility of retained material should be reduced if less peroxide is used. Also, while some selectivity has been introduced by the incorporation of the monomer (II), these columns were still unable to resolve a mixture of butabarbital, buto-

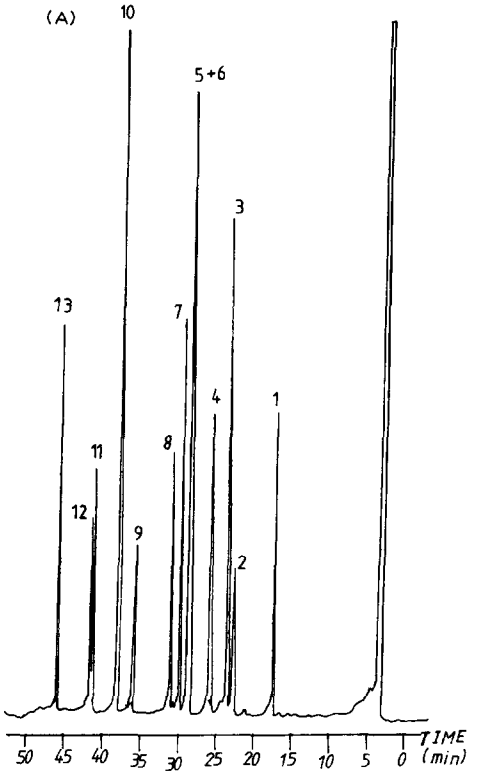


Fig. 1.

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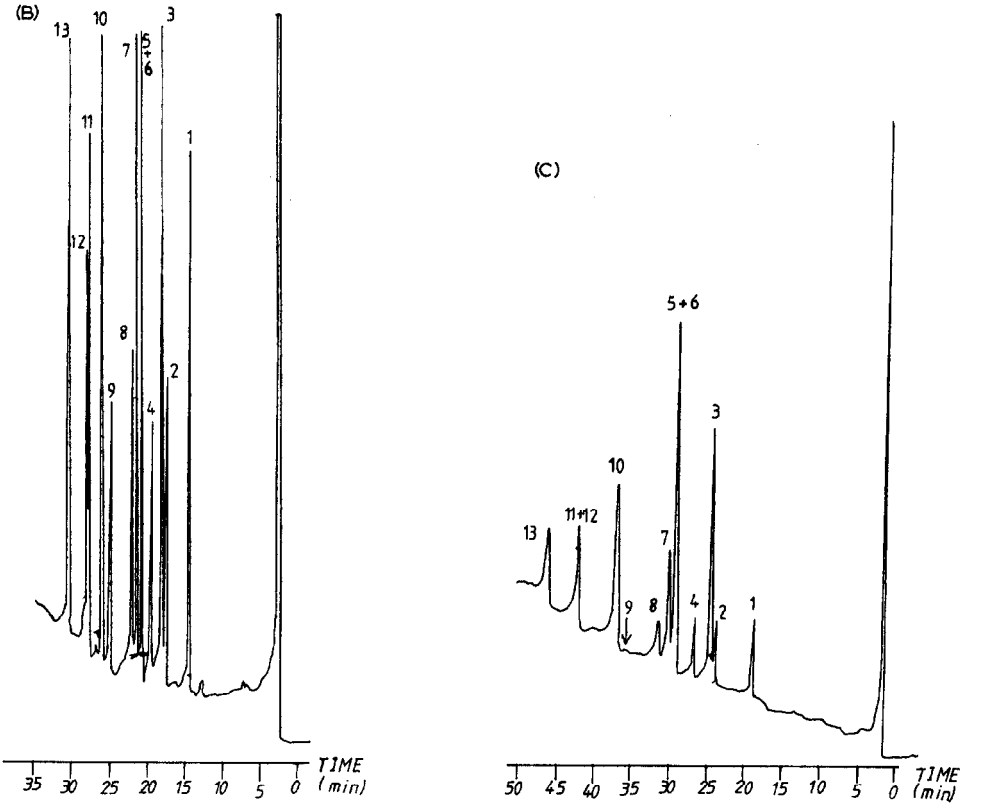

Fig. 1. Gas chromatograms of a standard barbiturate mixture. (A) Reference SE-54 column; 20 m × 0.29 mm I.D., DPTMDS persilylated. Chromatographed at 120°C for 2 min, programmed at 2°C/min to 220°C and held at the upper temperature for 32 min. (B) SE-54 column with 2% by weight of the barbiturate monomer (I) incorporated; 19 m × 0.29 mm I.D., DPTMDS persilylated. Chromatographed at 120°C for 2 min, programmed at 4°C/min to 250°C. (C) SE-54 column with 10% by weight of the barbiturate monomer (I) incorporated; 18 m × 0.23 mm I.D., DPTMDS persilylated. Chromatographed as in A. Peaks: 1 = barbital; 2 = allobarbital; 3 = aprobarbital; 4 = butalbarbital; 5 = amylobarbital; 6 = nealbarbital; 7 = pentobarbital; 8 = vinbarbital; 9 = brallobarbital; 10 = methylphenylbarbital; 11 = phenobarbital; 12 = cyclobarbital; 13 = heptabarbital.

barbital and butalbital although partial resolution of butabarbital from butobarbital and butalbital was attained (Fig. 3). One reason for this failure to resolve this mixture may have been that the site of attachment of the incorporated barbiturate (II) via the 5-position may be sterically hindered preventing ready access to the chromatographed species. Furthermore, most barbiturates differ from each other by variations in the pattern of substitutions at the 5-positions hence it might be expected that greater selectivity would be introduced if the barbiturates were attached to the polymer at some point removed from the 5-position leaving the 5 substituents free to interact with the chromatographed species. For this reason the N,N'-diallyl derivative of amylobarbital (III) was synthesised and incorporated (10–50%) into an SE-54 column.

A comparison with a reference SE-54 column in which 1% of DCUP was used

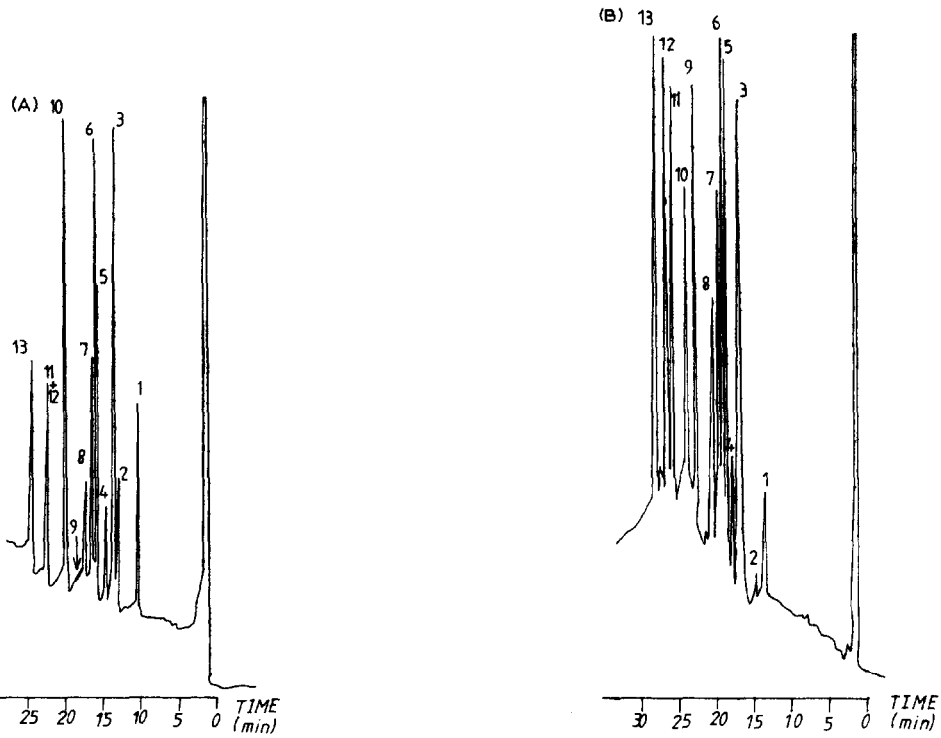

Fig. 2. Gas chromatograms of a standard barbiturate mixture. (A) SE-54 column with 8% by weight of the barbiturate monomer (II) incorporated; 13 m × 0.23 mm I.D., DPTMDS persilylated. Chromatographed at 120°C for 2 min, programmed at 4°C/min to 220°C and held at the upper temperature for 5 min. (B) SE-54 column with 50% by weight of the barbiturate monomer (II) incorporated; 18 m × 0.23 mm I.D., D_4 persilylated. Chromatographed as in A. Peak assignments as in Fig. 1.

as curing agent, with an SE-54 column incorporating 30% by weight of barbiturate monomer (III) and using 3% DCUP as curing agent, best illustrates the results obtained (Fig. 4). In each case waste products were removed by washing the cured columns, which were of the same column dimensions, with 5 ml of methylene chloride. If the barbiturate monomer (III) had not been incorporated into the SE-54 then it would have been expected that amylobarbital and nealbarbital would have similar retention times on the new to that of the reference column. Clearly this is not so because these two barbiturates elute at a larger retention time (Kovats Indices 2020 and 2060 for amylobarbital and nealbarbital respectively) and higher temperature (*ca.* 220°C) on the new than on the reference column (*ca.* 150°C, Kovats Index 2000). Furthermore, selectivity has been introduced into the new column as illustrated by the almost baseline separation of the two compounds albeit with some tailing (Fig. 4B). However, one surprise was that amylobarbitone, which most closely resembles the incorporated barbiturate monomer (III), eluted before nealbarbital implying that the latter compound shows greater reactivity towards the incorporated compound. Explanations for this may lie in the incomplete incorporation of the N-N'-diallyl amylobarbital (III) into the SE54 resulting in the presence of at least one intact N-allyl group.

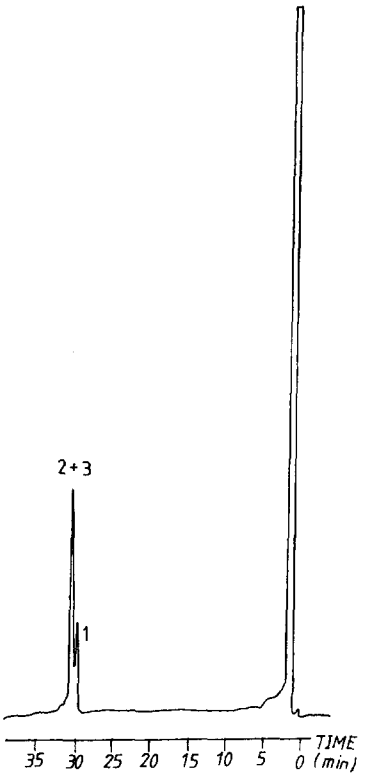

Fig. 3. Gas chromatogram of butabarbital (1), butobarbital (2) and butalbital (3) on an SE-54 column with 50% by weight of the barbiturate monomer (II) incorporated; 18 m × 0.23 mm I.D., D_4 persilylated. Chromatographed at 125°C for 4 min, programmed at 2°C/min to 170°C and held at the upper temperature for 8 min.

The difference in selectivity between the reference and the new column is also demonstrated by the chromatographic characteristics of butabarbital, butobarbital and butabital. On the reference SE-54 column, butabarbital can be partially resolved from butobarbital (Fig. 5A) but butobarbital and butalbital cannot be separated (Fig. 5B). However, the modified column can partially resolve these latter pair (Fig. 5C).

Another difficult-to-separate pair of barbiturates, brallobarbital and hexobarbital can also be resolved on the barbiturate (III) incorporated column with baseline separation (Fig. 6B); the reference SE-54 column is unable to resolve these two compounds (Fig. 6A).

The general performance of the N,N′-diallyl amylobarbital (III) incorporated SE-54 immobilised column when compared with a reference SE-54 immobilised column as indicated by the Grob test mixtures shows that the former column is acidic whereas the reference column is slightly basic in character, furthermore, the elution sequence changed, with 1-octanol eluting after undecane on the new column compared with the reference SE-54 column for Grob test mixture I and 2,6-dimethylphenol, 2,6-dimethylaniline and 2-ethylhexanoic acid eluting after dodecane for the

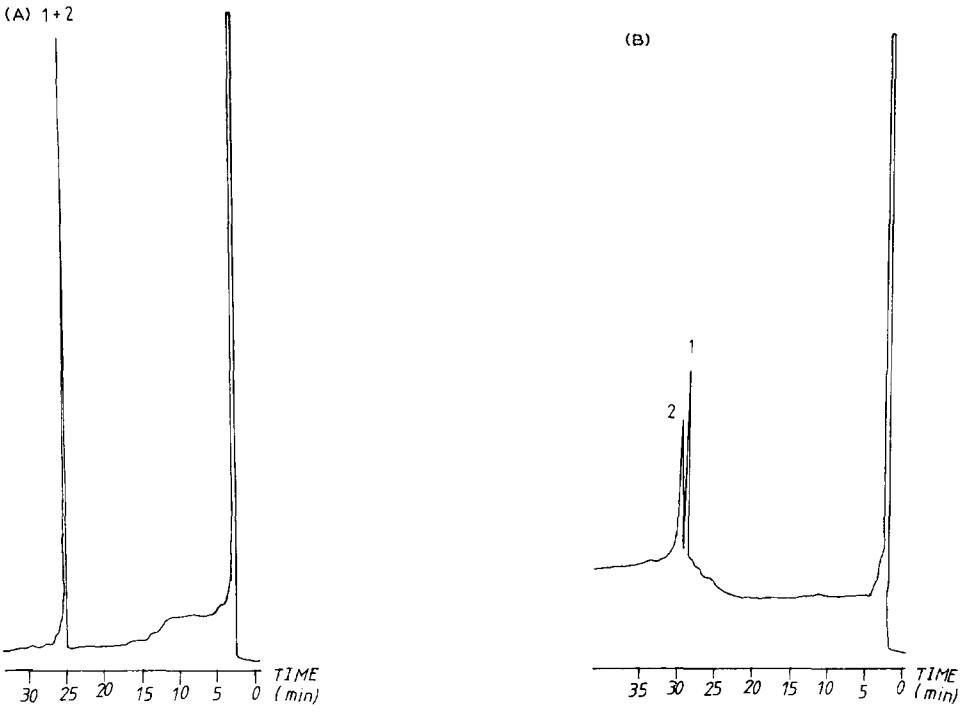

Fig. 4. Gas chromatograms of amylobarbital (1) and nealbarbital (2). (A) SE-54 column with 1% by weight DCUP; 23 m × 0.23 mm I.D., DPTMDS persilylated. Chromatographed at 120°C for 2 min, programmed at 4°C/min to 150°C and held at the upper temperature for 20 min. (B) SE-54 column with 30% by weight of the barbiturate monomer (III) incorporated and 3% by weight DCUP; 23 m × 0.23 mm I.D., DPTMDS persilylated. Chromatographed as in A except the upper temperature was 220°C.

Grob mixture II. This increase in retention of the polar components of the Grob mixtures on polar phases is, of course, to be expected.

CONCLUSIONS

The preparation of immobilised medium polar capillary columns in which compounds having the fundamental structure of the groups of compounds to be separated incorporated into the stationary phases offers a way of extending the selectivity of stationary phases thereby enabling difficult separations to be achieved. It should be possible to extend this process described for barbiturates to any groups of related, and difficult-to-resolve mixtures.

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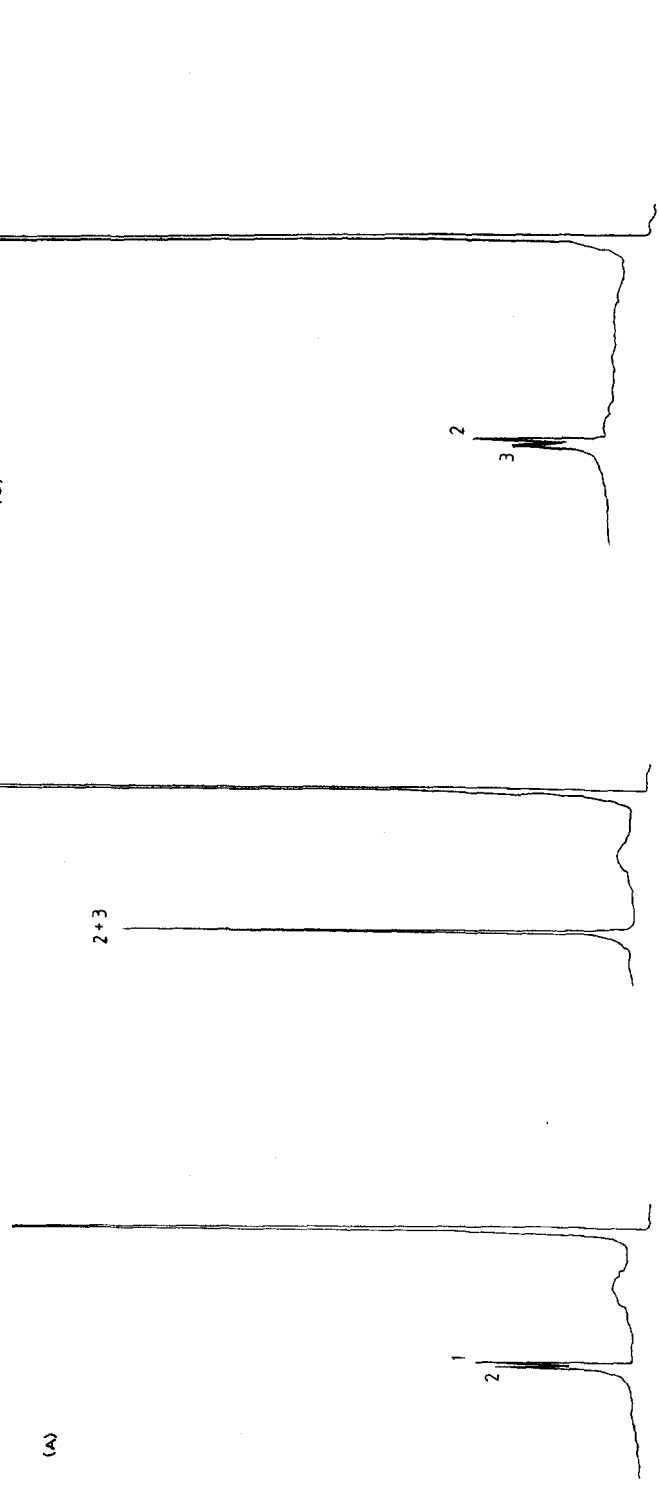

Fig. 5. Gas chromatograms of butabarbital (1) butobarbital (2) and butalbital (3). (A) SE-54 column with 1% by weight DCUP; 23 m × 0.23 mm I.D., DPTMDS persilylated; separation of butabarbital from butobarbital chromatographed at 125°C for 4 min, programmed at 4°C/min to 150°C and upper temperature held for 15 min. (B) Column as in A; butobarbital and butalbital were unresolved under optimal conditions. Chromatographed as in A. (C) SE-54 column with 30% by weight of the barbiturate monomer (III) incorporated and 3% by weight DCUP; 23 m × 0.23 mm I.D., DPTMDS persilylated; separation of butobarbital and butalbital. Chromatographed as in A except the upper temperature was 210°C.

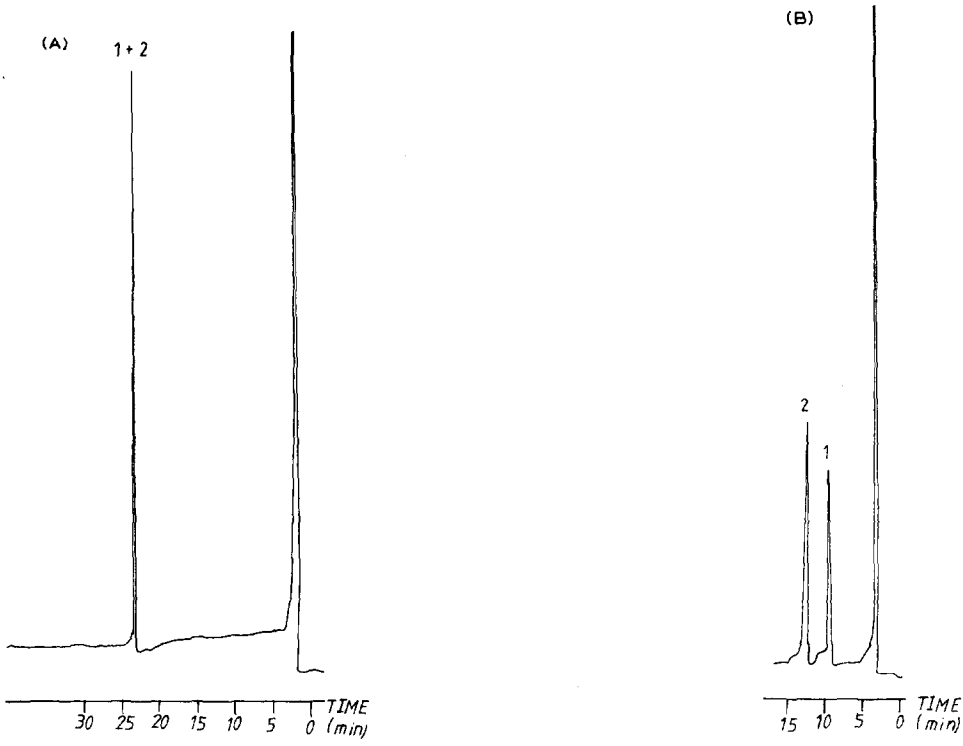

Fig. 6. Gas chromatograms of hexobarbital (1) and brallobarbital (2). (A) SE-54 column with 1% by weight DCUP; 23 m × 0.23 mm I.D., DPTMDS persilylated; hexobarbital and brallobarbital were unresolved under optimal conditions. Chromatographed at 120°C for 2 min, programmed at 4°C/min to 180°C and upper temperature for 10 min. (B) SE-54 column with 30% by weight of the barbiturate monomer (III) incorporated and 3% by weight of DCUP; 23 m × 0.23 mm I.D., DPTMDS persilylated. The two compounds were resolved. Chromatographed at 210°C for 4 min, programmed at 4°C/min to 240°C and held at upper temperature for 10 min.

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