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IMMOBILIZATION OF POLYETHYLENE GLYCOL IN CAPILLARY COLUMNS FOR GAS CHROMATOGRAPHY

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SUMMARY

Two methods are described for the immobilization of polyethylene glycol. Immobilization was achieved by reaction with methyl(vinyl)cyclopentasiloxane (V_5) and dicumyl peroxide or ethylene oxide. Fused-silica capillaries coated with V_5-immobilized polyethylene glycol 40M could be used over a wide temperature interval, 30–300°C. Further, immobilization makes feasible the use of thick films. The utility of this column type is demonstrated by several examples. Especially notable is the excellent performance achieved with water solutions and also free acids.

INTRODUCTION

Carbowax 20M is a stationary phase that is frequently used in capillary gas chromatography (GC). It has been of special value in connection with fused-silica columns, since it was one of the first polar phases that could be successfully deposited on fused-silica surfaces[1]. The usefulness of Carbowax 20M is however restricted by several factors. First, the upper temperature limit is relatively low, 220–240°C; and the lower temperature limit is only 60°C. Secondly, the phase may easily be decomposed, *e.g.*, by trace amounts of oxygen and water. Thirdly, degradation may be catalyzed by metal ions which occur in relatively high concentrations in some polyethylene glycols (PEGs)[2]. Fourthly, Carbowax 20M contains a relatively high percentage of low-molecular-weight material[3,4]. In order to achieve improved high-temperature stability of PEG phases, such a phase of high molecular weight, Superox-4, was prepared by Verzele and co-workers[5,6]. Drawbacks of this phase are its low solubility and the high viscosity of its solutions, which makes column preparation difficult. A new type having lower molecular weight, Superox 20M, has recently been described[7]. A particularly fruitful approach to stabilization of PEG stationary phases was taken by De Nijs and De Zeeuw[8–10]. By *in situ* immobilization of PEGs, they obtained columns that were coated with a non-extractable and water-compatible layer of stationary phase. Further, the high efficiencies obtained upon static coating were preserved by the polymerization *in situ*, and the columns could be used for temperature-programmed work up to 240°C. No details of the preparation of such

columns were given however. Columns coated with "bonded" PEG can now be purchased from several companies, e.g., CP^{tm} Wax 57CB from Chrompack (Middelburg, The Netherlands), and BP 20 from SGE (Ringwood, Victoria, Australia). Further, a method for immobilization of PEG stationary phases by reaction with γ-glycidoxypropyltrimethoxysilane was recently presented by Traitler and Kolarovic[11].

Immobilization of PEGs can be achieved in several ways. In this work, two methods will be described. A copolymer of a high-molecular-weight PEG and vinylsiloxane was thus prepared in the presence of dicumyl peroxide in situ. In the other method, PEG-coated capillaries were treated with ethylene oxide.

EXPERIMENTAL

Two different methods were used for immobilization of PEGs. In the first method, fused-silica capillaries (Hewlett-Packard, Avondale, PA, U.S.A.) or leached AR-glass capillaries[12] were deactivated with Carbowax 20M at 280°C as described earlier[13,14]. The capillaries were then statically coated with a solution of polyethylene glycol CP^{TM} Wax 40M (Chrompack), methyl(vinyl)cyclopentasiloxanes (V$_5$) and dicumyl peroxide (DCUP) (E. Merck, Darmstadt, F.R.G.) in methylene chloride. The contents of V$_5$ and DCUP were 4 and 2% respectively of the PEG amount. After coating, the capillaries were flushed with dry nitrogen for 1 h at room temperature. Some columns were also prepared using the dynamic coating method, with toluene as solvent, and the proportions of PEG, V$_5$ and DCUP were as above. In some experiments, octamethylcyclotetrasiloxane (D$_4$) (Ohio Valley Specialty, Marietta, OH, U.S.A.) was used instead of V$_5$.

After placing the coated capillary in a gas chromatograph, the carrier gas (hydrogen) was adjusted to 0.1 ml/min and the GC oven temperature was programmed at 5°C/min from 40 to 150°C, the final temperature being maintained for 30 min. The carrier gas flow was then increased to about 1 ml/min and the oven programmed at 5°C/min to 200°C, then isothermal for 1 h, after which the column was ready for a preliminary test. Finally, the column was rinsed with 10 ml of methylene chloride. Equally good results were achieved when a static curing method was used. In that method the coated capillary was vacuum sealed and heated at a rate of 5°C/min from 40 to 175°C, then isothermal for 3 min and finally cooled.

V$_5$ was synthesised by acidic hydrolysis of methyl(vinyl)dichlorosilane (Petrarch, Bristol, PA, U.S.A.) in water[15]. After washing until neutral and separating from the water phase, the siloxane was dried over calcium sulphate. By fractionated distillation at 10 mm Hg, the different cyclics were separated.

The second method for immobilization was performed on AR-glass capillaries that were leached with hydrochloric acid solution according to Grob et al.[12]. Such capillaries were thus coated statically with a solution of PEG 40M in methylene chloride. After coating, the capillaries were flushed with nitrogen as described above and then conditioned for 2 h at 150°C before a preliminary test. The capillaries were then filled with ethylene oxide (AGA, Lidingö, Sweden) and both ends were sealed. They were then heated at 5°/min to 280°C, and the final temperature was maintained for 3 h. Cooling was carried out slowly and finally, the columns were rinsed during 20 h with 10 ml of methylene chloride.

TABLE I

CHARACTERISTICS OF SOME CAPILLARY COLUMNS COATED WITH POLYETHYLENE GLYCOL

FS = Fused silica; HETP = height equivalent to a theoretical plate.

Column No.	Glass type	Curing method	Coating efficiency	HETP for naph- thalene at 90°C (mm)	% decrease in $k_{naphth.}$ after extraction	Kováts retention index at 90°C	
						Octanol	Naphthalene
1	FS	V_5	78	0.39	10	1533	1666
2	FS	D_4	45	0.68	50	1523	1650
3	AR	V_5	76	0.32	20	1547	1651
4	AR	Ethylene oxide	61	0.38	33	1559	1682
5	AR	not cured	50	0.44		1500	1617
6	FS	V_5 only			≈ 96		
7	FS	DCUP only			≈ 97		
8	FS	Heating only			≈ 97		

RESULTS

It may be of interest to test copolymers of ethylene glycol and silicones as stationary phases. Such a phase, OV-330, is available from Ohio Valley Specialty, but its viscosity is too low to allow good coatings of the capillaries, and in addition,
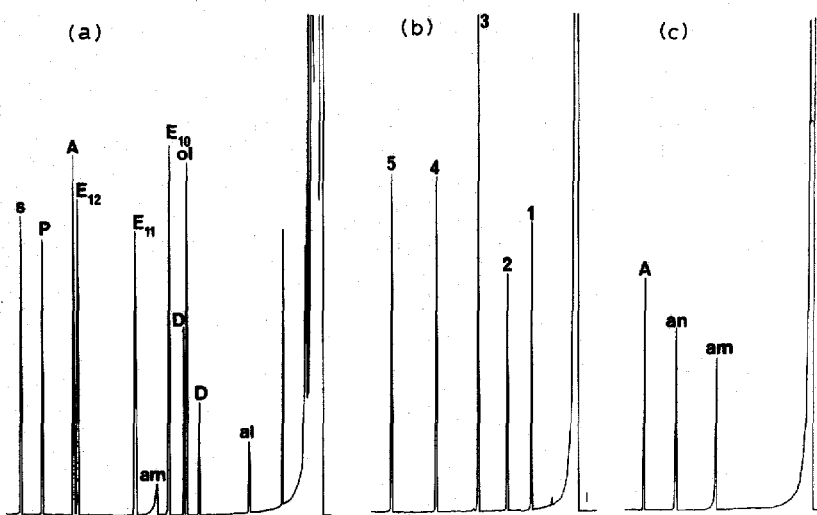

Fig. 1. Gas chromatograms (flame ionization detection, FID) of some test mixtures on a 14.5-m fused-silica capillary column coated with immobilized Carbowax V_5. Chromatogram a: Grob test mixture, injection at 60°C, programmed at 5°C/min. Peaks: al = nonanal; D = 2,3-butanediol; ol = octanol; E_{10}, E_{11}, E_{12} = C_{10}, C_{11}, C_{12} acid methyl ester respectively; am = dicyclohexylamine; A = 2,6-dimethylaniline; P = 2,6-dimethylphenol; s = 2-hexanoic acid. Chromatogram b: free acid test mixture, injection at 80°C, programmed at 5°C/min. Peaks: 1 = acetic acid; 2 = propionic acid; 3 = butyric acid; 4 = valeric acid; 5 = caproic acid. Chromatogram c: amine test mixture, injection at 70°C, programmed at 5°/min. Peaks: am = dicyclohexylamine; an = aniline; A = 2,6-dimethylaniline. Each peak corresponds to ca. 1 ng of substrance.

it could not be cross-linked. Another interesting approach would be block copolymers of polydimethylsiloxane and polyethylene oxide[16]. In the work presented here, however, the purpose of siloxane addition was to make immobilization possible.

Access to polar columns is of importance in GC. The advantages of selectivity will, however, be fully utilized only on high-performance columns, *i.e.,* columns that show high efficiency, good deactivation and high temperature stability. In this work, deactivation was achieved by treatment with Carbowax 20M[17]; further, such a treatment also creates a surface that possesses the wettability necessary for the formation of even films of carbowax[18]. The purpose of the immobilization is to achieve a high degree of physical and chemical stability of the stationary phase film.

Both of the two procedures used for immobilization were effective, the capacity ratio for naphthalene thus being reduced by only 10–30% after rinsing the cured columns with methylene chloride, Table I. Immobilization with D_4 was, however, less efficient. Fused-silica capillary columns prepared by the V_5 method showed somewhat acidic properties, and were thus excellent for free acids, but straight-chain amines could not be eluted (Fig. 1). The usefulness of fused-silica Carbowax coated columns for the separation of free acids was also noted by Schomburg *et al.*[19].

The mechanism of the immobilization of PEG–V_5–DCUP is not yet fully understood, but the low degree of immobilization that was achieved with D_4, however, indicates the importance of the vinyl groups for the curing reaction. Sandra *et al.*[20] reported that a polyol stationary phase, RSL-310, can be immobilized by heat-curing with peroxides. *In situ* heating of PEG 40M–DCUP, however, only resulted in a very thin non-extractable layer of stationary phase, Table I. Similar results were obtained upon heating of PEG 40M–V_5 or PEG 40M only, Table I; efficient immobilization was achieved only when PEG 40M, V_5 and DCUP were heated together. Polyethylene glycol 40M was chosen for two reasons. Firstly, it is a purified product with a low content of residual metal ions. Secondly, it was considered that the relatively high molecular weight would make less significant the effect of chain scission during the reaction. Such a chain scission may be caused by the action of the peroxide. Further, it was found that columns that were cured with a PEG of high average molecular weight but containing a relatively large amount of low-molecular-weight
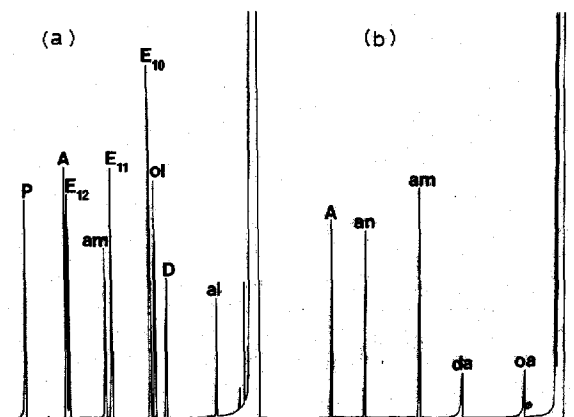

Fig. 2. Gas chromatograms (FID) of some test mixtures on a 10-m AR-glass capillary column coated with Carbowax immobilized with ethylene oxide. Chromatogram a: Grob test mixture, conditions and peaks as in Fig. 1. Chromatogram b: amine test mixture, injection at 50°C, programmed at 5°C/min, peaks as in Fig. 1; oa = octylamine; da = decylamine.
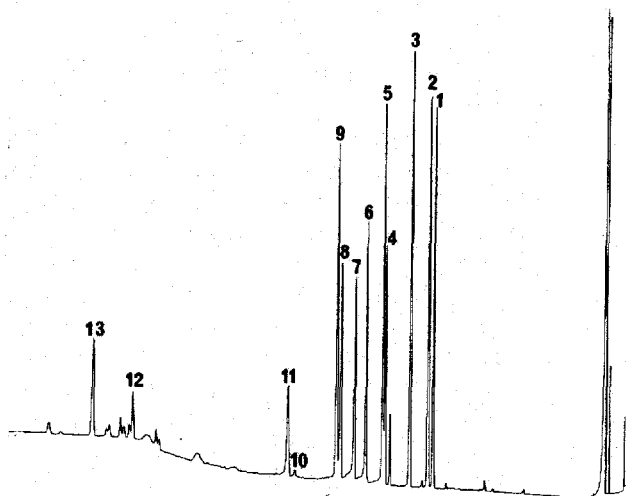
Fig. 3. Gas chromatogram (FID) of a tricyclic antidepressant test mixture on a 10-m AR-glass capillary column coated with Carbowax immobilized with ethylene oxide, $d_f = 0.30$ μm. Split injection at 80°C, rapidly programmed to 200°C, then programmed at 3°/min to 250°C. Peaks: 1 = trimipramine; 2 = amitriptyline; 3 = imipramine; 4 = nortriptyline; 5 = desmethylnortriptyline; 6 = desipramine; 7 = protriptyline; 8 = desmethyldoxepine; 9 = chlomipramine; 10 = desmethylchlomipramine; 11 = maprotiline; 12 = opipramol; 13 = lofepramine. Each peak corresponds to *ca.* 1 ng of substance.
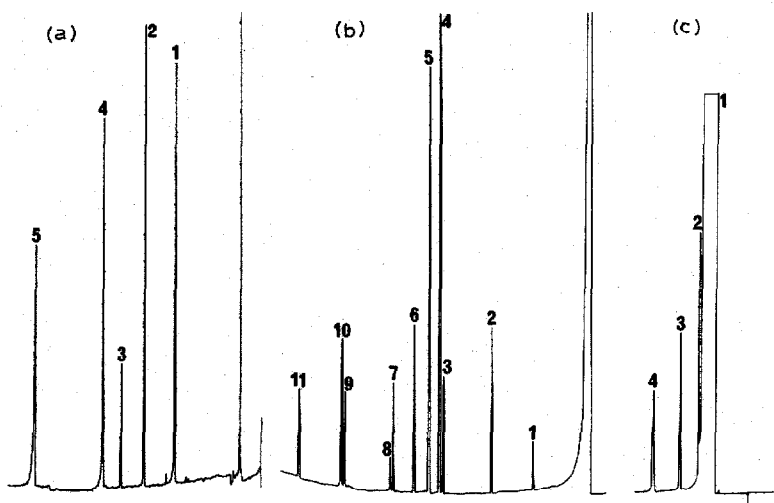

Fig. 4. Gas chromatograms (FID) of some test solutions on fused-silica capillaries coated with immobilized Carbowax–V$_5$. Chromatogram a: water solution of alcohols on a 10-m column, $d_f = 0.4$ μm. Split injection at 100°C, programmed at 10°C/min to 240°C. Peaks: 1 = ethylene glycol; 2 = decanol; 3 = undecanol; 4 = diethylene glycol; 5 = glycerol. Peaks 1, 2, 4, *ca.* 0.5 ng and peak 5, *ca.* 1 ng. Chromatogram b: fatty acid methyl ester test mixture on a 20-m column, $d_f = 0.1$ μm. Split injection at 150°C, programmed at 7°C/min. Peaks: 1 = 14:0; 2 = 16:0; 3 = 18:0; 4 = 18:1; 5 = 18:2; 6 = 18:3; 7 = 20:0; 8 = 20:1; 9 = 22:0; 10 = 22:1; 11 = 24:0. Chromatogram c: alcohol test solution on a 20-m fused-silica capillary column coated with immobilized Carbowax–V$_5$, $d_f = 0.1$ μm. Isothermal at 27°C, H$_2$ flow-rate 22 cm/sec. Split injection. Peaks: 1 = ethanol; 2 = 1-propanol; 3 = 1-butanol; 4 = 3-methyl-l-butanol. Each peak corresponds to *ca.* 3 ng.

material, *i.e.*, Carbowax 20M, showed severe adsorption of hydrocarbons. The use of high concentrations of peroxide, 5%, for curing of PEG 40M also led to columns that showed hydrocarbon adsorption. After curing, the columns were rinsed with

solvent, 10 ml methylene chloride, in order to remove short chain fragments and reaction by-products. Only one rinse was necessary, since a second rinsing did not change capacity factors (k).

Our second method for immobilization is based on the manufacturing process for PEGs. The method works perfectly on leached soda-glass, but not on fused silica. A reason for this difference may be that alkali metal ions in the soda-glass surface catalyze the reaction. Such a catalytic effect was also observed by Traitler and Kolarovic[11], when modifying with γ-glycidoxypropyltrimethoxysilane. Since the PEG 40M is hydroxyl-terminated, it may be speculated that an immobilization in this case is achieved by chain extension. Curing takes place at 280°C, by which is achieved the special advantage that deactivation and immobilization are affected in one step. Thus, in this case, no Carbowax pre-treatment is necessary. The good deactivation is demonstrated in Fig. 2. This type of AR-glass column is useful for the separation of bases; the separation of an amine test mixture is demonstrated in Fig. 2. In addition, the utility of this type of columns is shown for the separation of underivatized antidepressants, Fig. 3.

Column polarity, as reflected by the Kováts retention indices of octanol and naphthalene at 90°C, was slightly changed by immobilization (Table I). Further, the different methods for immobilization lead to somewhat different polarities. Coating
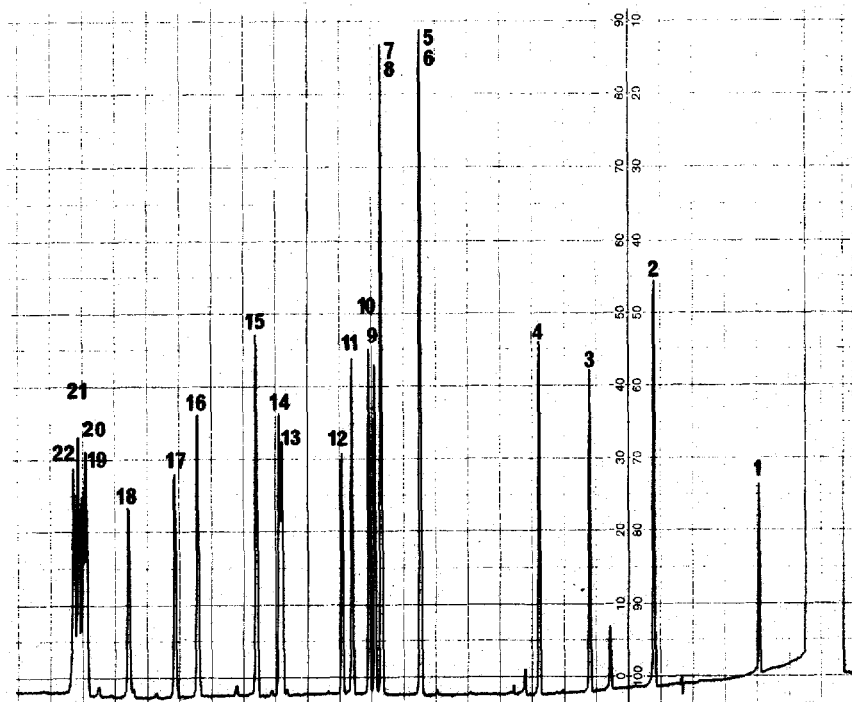

Fig. 5. Gas chromatogram (FID) of a polyaromatic hydrocarbon standard test mixture on the fused-silica column used in Fig. 4c. Splitless injection at 70°C, isothermal for 2 min and then programmed at 3°/min. Peaks: 1 = naphthalene; 2 = biphenyl; 3 = acenaphthylene; 4 = fluorene; 5 = anthracene; 6 = phenanthrene; 7 = 2-methylanthracene; 8 = 2-methylphenanthrene; 9 = 1-methylanthracene; 10 = 1-methylphenanthrene; 11 = 9-methylphenanthrene; 12 = phenylnaphthalene; 13 = fluoranthene; 14 = 9,10-dimethylanthracene; 15 = pyrene; 16 = 1-methylpyrene; 17 = dihydrotetracene; 18 = benzo[c]phenanthrene; 19 = benz[a]anthracene; 20 = cyclopenta[c,d]pyrene; 21 = chrysene; 22 = triphenylene.

efficiency was good, 60–70% (Table I). For thick stationary phase films, we obtained the highest efficiency when using the dynamic coating method; for thin films, the static coating method gave the best results.

Immobilized phases were suitable for separation of water solutions (Fig. 4a). The temperature should then be above 80°C, otherwise the water will be incompletely eluted from the column. Water thus remaining in the column may alter the retention properties, as recently described by Grob and Habich[21]. The retention times of alcohols will be thereby increased and the peaks will become broader. Such uneluted water can however easily be removed by a short conditioning at temperatures above 110°C. Separations of aqueous solutions on Carbowax coated fused-silica columns were also demonstrated by Schomburg and co-workers[22].

A further virtue of the modified PEG, is that it can , when occurring as thin films in the column, be used at lower temperatures than corresponding non-cured phases. This is demonstrated by the separation of an ethanol solution of some alcohols, Fig. 4c. Moreover, immobilization stabilizes thick films of stationary phase, which is of course a convenient way to increase the retention of low boiling solutes. Thick-film columns (film thickness, $d_f > 1$ μm) should however not be used below 60°C.

The separation of a fatty acid methyl ester standard test solution is shown in Fig. 4b; and of a standard mixture of some polyaromatic hydrocarbons in Fig. 5. It
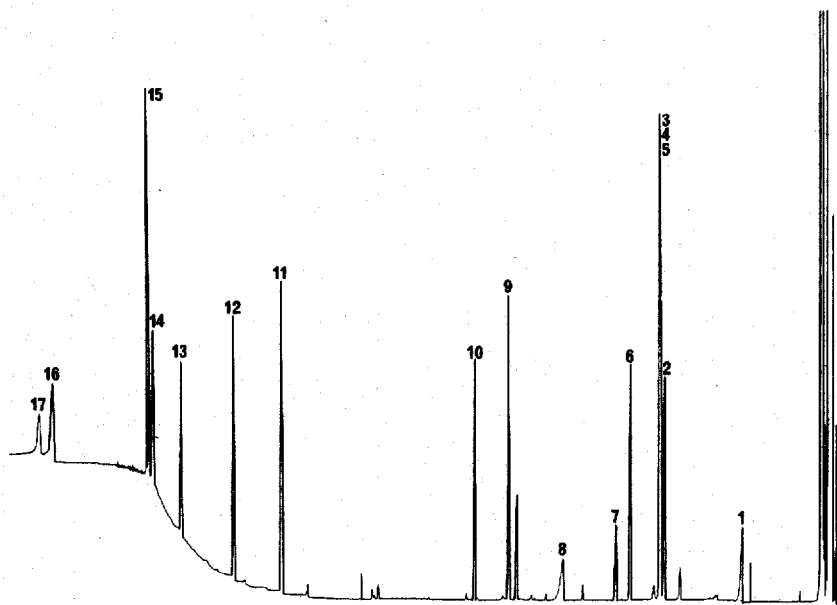

Fig. 6. Gas chromatogram (FID) of a drug test mixture on a 10-m fused-silica capillary column coated with immobilized Carbowax-V$_5$, $d_f = 1$ μm. Split injection at 70°C, isothermal for 3 min, then programmed at 5°C/min to 275°C. Peaks: 1 = propylhexedrine; 2 = amphetamine; 3 = methylamphetamine; 4 = phentermine; 5 = ethylamphetamine; 6 = propylamphetamine; 7 = amphepramone; 8 = ephedrine; 9 = fendimetraline; 10 = phenmetrazine; 11 = methylphenidate; 12 = benzocaine; 13 = phenacetine; 14 = metaqualone; 15 = procaine; 16 = ethylmorphine; 17 = codeine. Each peak corresponds to ca. 1 ng of substance.

may be noted that the pair chrysene–triphenylene is separated on modified PEG which is in agreement with our earlier findings[14].

Ethylene oxide curing leads to an improvement in high temperature stability. The maximum temperature of programmed runs may thus be around 260°C for such columns, cf., Fig. 3. A more enhanced temperature stability was achieved on immobilization with V_5. Such columns can be programmed to 300°C. For separation of a drug test mixture, Fig. 6, the temperature was programmed to 275°C.

In conclusion, the exact mechanism for the PEG immobilization is not known, i.e., whether surface bonding takes place or chain extension or some other type of reaction. The modification, however, results in the PEG properties that considerably increase the versatility of this type of stationary phase, and that was the object of the work presented here.

ACKNOWLEDGEMENTS

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