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GAS CHROMATOGRAPHIC MINI-COLUMNS PACKED WITH VERY FINE LIQUID-MODIFIED PARTICLES OF GRAPHITIZED CARBON BLACK

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SUMMARY

The effect of decreasing the particle size of graphitized carbon black (GCB) from 60 to 20 μm on column efficiency has been investigated. The $\bar{H}_{min.}$ value was found to decrease linearly as the mean particle diameter is decreased. As a result, a plate height of about 0.06 mm, which corresponds to 17,000 plates per metre of column, has been achieved by using 20–25-μm GCB particles. Unlike gas–liquid columns, the optimal mobile phase is practically unaffected by variations in the particle diameter of GCB. Very fine particles of carbon supporting a stationary phase have been evaluated in terms of selectivity, efficiency and column loadibility with varying amounts of liquid phase. For routine use in the analysis of high-boiling, polar compounds, a 21 cm × 0.5 mm I.D. glass column packed with GCB particles with a mean particle diameter of 29 μm, coated with a very thin film of stationary phase and fed with hydrogen as the carrier gas, was found to be the best arrangement in terms of analysis time, efficiency, column loadability and permeability.

Some analytical applications to steroids, oxygenated diterpenes, barbiturates, nitro- and chlorophenols, free fatty acids up to C_{20} and glycols are reported.

INTRODUCTION

Many attempts have been made to obtain highly efficient packed columns by varying parameters that are correlated to the plate height. The interpretation of $\bar{H}/\bar{u}$ data, obtained by varying the column parameters, requires an appropriate choice of the rate equation. A simple plate-height equation for gas–solid packed columns, which has been shown elsewhere[1–3] to give a good reproduction of experimental data over a wide range of carrier gas velocities, is

$$\bar{H} = 2\lambda\bar{d}_p + \frac{2\gamma D_m^0}{u^0} + \frac{\omega\bar{d}_p^2 u^0}{D_m^0} + \frac{2K'}{(K'+1)^2}\bar{t}_d u^0 j \qquad (1)$$

or, in simpler terms,

$$\bar{H} = A + \frac{B}{u^0} + c_g u^0 + c_s u^0 j$$

where $\bar{d}_p$ is the mean particle diameter, D_m^0 is the molecular diffusion coefficient for the mobile phase at the column outlet pressure, K' is the capacity ratio, $\bar{t}_d$ is the mean desorption time of an equilibrium population of sorbed molecules, λ, ω and γ are structural factors related to particle orientation, u^0 is the outlet carrier gas velocity, which is related to the average carrier gas velocity through the expression $\bar{u} = u^0 j$, where j is the James–Martin compressibility factor[4].

Eqn. 1 shows that the plate height is related to the particle diameter and predicts explicity that the column efficiency can be enhanced by reducing the particle size of the column packing.

Plate heights as low as 82 μm (12,000 plates per metre of column) have been obtained by Myers and Giddings[5] by using a 0.5-mm I.D. column filled with 13-μm modified alumina. However, they found that a pressure drop of greater than 150 atm was needed in order to feed a 1-m long column.

Recently, Huber *et al.*[6,7] evaluated the influence of particle size and pressure on the column efficiency. They were able to obtain 10,000 plates per metre of column by making use of 30–35-μm Chromosorb particles coated with 3% (w/w) squalane.

Graphitized carbon black (GCB) modified with various liquid or solid compounds has been shown to be very effective in gas chromatographic analysis. Various papers have dealt with the optimization of the selectivity of this adsorbing material not only by using suitable modifying agents but also by adjusting their surface concentration[8–11].

Very recently, Di Corcia and Giabbai[12] considered the effect of the particle size of GCB on column efficiency. A continuous decrease in the plate height for gas–solid columns packed with partially coated (0.2% PEG 1500) GCB was observed on reducing the mean particle diameter from 185 to 29 μm. As a result, a maximum of 10,000 plates per metre of column was achieved by using $\bar{d}_p = 28$ μm at a carrier gas velocity of 7 cm/sec and with a relatively low pressure drop of 19 atm. It was found also that, unlike gas–liquid columns, GCB packed columns do not exhibit a shift of $\bar{u}_{opt}$ in the direction of smaller velocities as the mean particle diameter is reduced.

In gas chromatography, with few exceptions[13–15] the use of GCB has been confined to the analysis of low and medium boiling compounds by partially coating its surface with modifying agents. This is so because high-boiling compounds have unacceptably long elution times when eluted on only a partially modified carbon surface. On the other hand, it has been shown[9] that retention times can be drastically reduced if the GCB surface is coated with one layer of a modifying liquid.

The purpose of this work was to investigate the possibility of analysing high-boiling compounds by making use of (1) GCB covered with one or more layers of a suitable stationary phase, (2) very short columns of small internal diameter packed with very fine GCB particles and (3) hydrogen as the carrier gas. By using GCB particles with a mean particle diameter of 29 μm packed in a 21-cm column (0.5 mm I.D.) and using hydrogen as the carrier gas, we succeeded in reducing the elution time of a given compound by a factor of about fifteen with respect to a corresponding, conventional column without a loss of efficiency. The gas chromatographic behaviour of GCB covered with one or more layers of PEG 1500 was evaluated in terms of selectivity, capacity ratio, column efficiency and column load. The effect on the column efficiency of decreasing the particle diameter of GCB from 60 to 20 μm is re-

ported. It is noteworthly that by the use of a mean particle diameter of 22.5 μm it has been possible to obtain 17,000 plates per metre of column. The effectiveness of the mini-columns under consideration has been exploited for the elution of steroids, oxygenated diterpenes, free fatty acids up to C_{20}, barbiturates, nitrophenols and chlorophenols up to pentachlorophenol.

EXPERIMENTAL

Carbopack C, which is an example of GCB, of 80–100 mesh was supplied by Supelco (Bellefonte, Pa., U.S.A.). It was ground and sieved to obtain the appropriate particle size ranges.

Column packings were prepared by a procedure reported elsewhere[16]. For analytical applications, three column packings were used:

(1) Carbopack C + 1% Quadrol;
(2) hydrogen-treated Carbopack C + 1% PEG 20M;
(3) Carbopack C + 0.3% trimesic acid + 0.6% PEG 1500.

Hydrogen-treated Carbopack C was kindly supplied by Dr. F. Bruner, who treated GCB following a procedure described previously[17]. Trimesic acid was dissolved in methanol together with PEG 1500 and the column was left overnight at 150° under a gas flow. Under these conditions, a partial esterification reaction should occur between the acidic compound and the terminal hydroxyl groups of PEG 1500. This modification can account for the anomalously high thermal stability which the column packing shows even at 270°.

Glass columns 25 cm long were used and a 21 cm × 0.5 mm I.D. section was filled with the packing material. The first 4 cm on the inlet side, having an I.D. of 2 mm, were left empty to be used as an expansion chamber for injected samples.

The packing procedure is very critical and is carried out by gently vibrating with the aid of a vibrator making about 500 rev/min. If this operation is carried out correctly, the weight of Carbopack C packed into a 21 cm × 0.5 mm I.D. column is 0.047–0.049 g. Columns filled with larger amounts of carbon exhibit a considerable decrease in permeability and efficiency.

Another critical operation is the introduction of the carrier gas into mini-columns packed with very fine (25–33 μm) GCB particles. Even in the first few moments, the pressure of the carrier gas at the column inlet must be increased very slowly. This precaution is very important and, if the operation is not carried out correctly, the column shows an anomalously low permeability and some void spaces may occur in the material filling the column. In order to feed the column gradually with the carrier gas we inserted between the gas cylinder and the column inlet a 1.5 m × 1 mm I.D. stainless-steel tube filled with 20–25-μm GCB particles. In this way, any pressure increase operated with the regulator of the gas cylinder is very finely regulated and controlled by this restriction.

A common gas chromatographic apparatus was used (Model GI, Carlo Erba, Milan, Italy), equipped with a flame-ionization detector. In order to be able to work with pressure drops of the carrier gas higher than 5 atm, the line of the carrier gas was connected directly to the column inlet. Also, when hydrogen was used as the carrier gas, a 3:2 mixture of nitrogen and hydrogen was used to feed the detector.

RESULTS AND DISCUSSION

A number of glass columns (21 cm × 0.5 mm I.D.) were prepared using particle sizes of Carbopack C in the ranges 61–45, 45–33, 33–25 and 25–20 μm. For each particle size range, the relative amount of PEG 1500 deposited on the carbon surface was varied from 0.6 to 4%, which corresponds to approximately one and eight layers of stationary phase, respectively. For each column, the average theoretical plate height was determined as a function of the average mobile phase velocity by using methyl hexyl ketone as the eluent at 55°.

In Fig. 1 $\bar{H}_{min.}$ values for each $\bar{H}/\bar{u}$ curve are plotted *versus* $\bar{d}_p$ for Carbopack C modified with 0.6% and 4% PEG 1500.

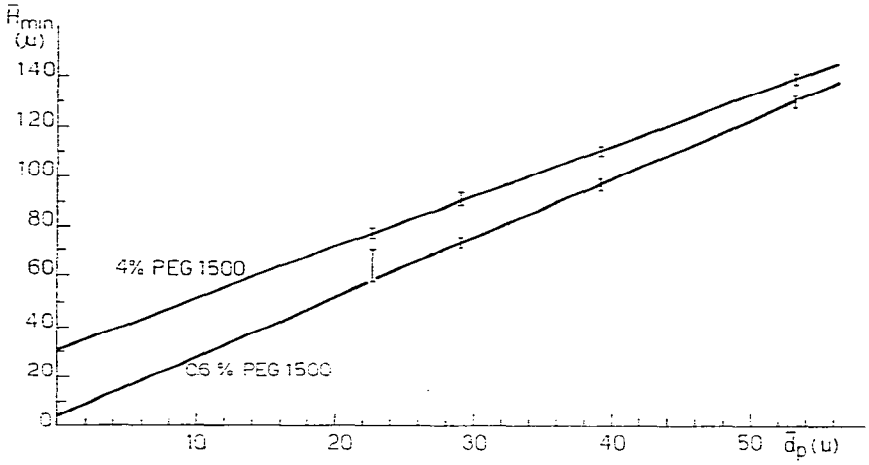


Fig. 1. $\bar{H}_{min.}$ *versus* d_p curves for columns packed with Carbopack C + PEG 1500. Column, 21 cm × 0.5 mm I.D.; carrier gas, nitrogen.

From these curves it can be seen that in the range of particle diameters considered there is a gradual, linear decrease of $\bar{H}_{min.}$ on reducing $\bar{d}_p$, regardless of the amount of stationary phase deposited on the carbon surface. It is noteworthy that by using a mean particle diameter of GCB of 29 μm coated with one layer of PEG 1500 we succeeded in obtaining, with good reproducibility, a plate height of 73 μm, which corresponds to more than 13,500 plates/m. This means that working with a 21-cm long column packed with Carbopack C modified with a monolayer of a liquid phase it is possible to obtain an efficiency of about 2850 plates. Another interesting result is that a pressure drop of carrier gas of only about 4.8 atm is needed to feed the column considered above.

It can be seen that several attempts were made to evaluate the $\bar{H}/\bar{u}$ curve for a column packed with Carbopack C particles with a mean diameter of 22.5 μm and coated with one layer of PEG 1500. Only in one instance did we succeed in obtaining 17,000 plates per metre of column at the optimal carrier gas velocity, as would be expected from the $\bar{H}_{min.}/\bar{d}_p$ curve. This difficulty in duplicating the $\bar{H}_{min.}$ value can be accounted for by the tendency of the fine GCB particles to stick together, probably owing to the presence of electrostatic charges on their surface. As a consequence,

this effect might cause some difficulties in obtaining a homogeneous, close packing of GCB particles in the gas chromatographic column. As one would expect, the tendency for sticking together became more and more marked on decreasing both the mean particle diameter and the amount of modifying agent deposited on the carbon particles. In fact, no difficulties were encountered in using 20–25-μm GCB particles coated with 4% PEG 1500, which corresponds roughly to eight layers of liquid on the carbon surface. Also, in very recent work[12] on the use of fine GCB particles coated with only 0.2% PEG 1500, it was found that non-linear decrease in $\bar{H}_{\text{min.}}$, on reducing $\bar{d}_p$, was found by using $\bar{d}_p$ values of less than 50 μm.

With GCB particles coated with a monolayer of PEG 1500 molecules, it is interesting to discuss the behaviour of the $\bar{H}_{\text{min.}}/\bar{d}_p$ curve. The linear decrease of $\bar{H}_{\text{min.}}$ as $\bar{d}_p$ is decreased can be explained by taking into account eqn. 1, putting $u_0 = (u_0)_{\text{opt}}$ and assuming $c_g \gg c_s j$, as seems to occur in gas-adsorption chromatography. Then, we have

$$H_{\text{min.}} = (2\lambda + \sqrt{2\gamma\omega})\,\bar{d}_p$$

In addition to a linear decrease in $H_{\text{min.}}$ on reducing $\bar{d}_p$, the approximate equation above also predicts that the plate height tends to zero as the particle diameter is reduced. In other words, under the approximation made, this expression predicts that an enormously high number of plates can be achieved by using GCB particle diameters in the submicron region. In the range of particle diameters evaluated, this hypothesis appears to be supported by our experimental results.

In Fig. 2, for a given mean particle diameter of Carbopack C, $\bar{H}_{\text{min.}}$ values are plotted as a function of the loading of the liquid phase using both nitrogen and hydrogen as the carrier gas. A steady decrease in column efficiency occurs on passing from one layer (0.6%, w/w) to about four layers (2%, w/w) of PEG 1500. Also, the increase in the plate height is reduced on passing from four to eight layers of stationary phase (4%, w/w). This effect can be explained qualitatively by considering that the increase in the number of layers of the modifying agent deposited on GCB

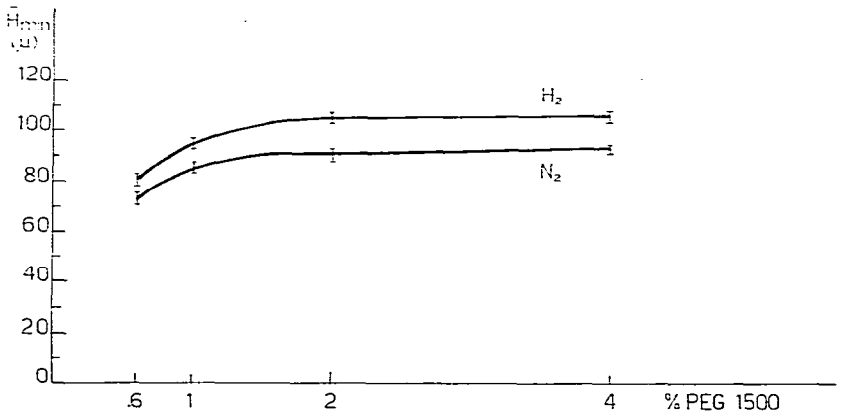


Fig. 2. Effect on $\bar{H}_{\text{min.}}$ of variation of the liquid loading on GCB particles (25–33 μm) and of variation of the carrier gas. Column, 21 cm × 0.5 mm I.D.

gradually modifies the chromatographic process of elution, which changes from adsorption on a monolayer to solution into the liquid phase. This modification can cause a gradual increase in the contribution to the plate height of the mass-transfer effect, relative to the stationary phase. It should be pointed out that this term is generally considered not to affect the band spreading in a gas-adsorption, dynamic process.

The effect of using hydrogen as carrier gas on the optimal column efficiency was evaluated. It can be seen that the $\bar{H}_{min.}$ *versus* percentage PEG 1500 data indicate a constant decrease in efficiency when hydrogen is used in place of nitrogen. This loss is not higher that 10%. On the other hand, we found hydrogen to be useful for analytical purposes, as in this instance the maximum column efficiency is reached at carrier gas velocities about three times higher than by using nitrogen.

Column loadability in some instances can be a limiting factor in the use of micropacked columns with internal diameters comparable to those of capillary columns. Table I gives, for a well retained compound (3-methyl-2-butanol), the maximum amount of sample injected that is still eluted as a symmetrical peak at 55° in a 0.5-mm I.D. column packed with Carbopack C (61–45 μm) modified with various percentages of PEG 1500. The loadability of these columns is also compared with that of routinely used 5% PEG 1500-coated Chromosorb W (100–120 mesh) packed in a conventional 1.5-mm I.D. column. It can be seen that, even when the Carbopack C surface is shielded by only one layer of PEG 1500, the column shows a sufficiently large loadability, which does not create difficulties if a flame-ionization detector is used. As expected, the column loadability increases as the liquid phase is increased and it is noteworthy that a micropacked column filled with Carbopack C modified with 4% PEG 1500 has a loadability only about three times lower than that of a conventional, gas–liquid packed column.

TABLE I

MAXIMUM AMOUNT OF SAMPLE (3-METHYL-2-BUTANOL) THAT CAN BE INJECTED AND STILL BE ELUTED AS A SYMMETRICAL PEAK

PEG 1500(%)	*Amount of sample (μg)*
0.6	0.1
1.0	0.4
2.0	0.8
4.0	2.0
Chromosorb W + 5% PEG 1500	7.5

The very short micropacked columns were also evaluated in terms of the separation power for a given mixture as the amount of liquid phase is varied. The selectivity of these chromatographic materials was also compared with both a commonly used, partially coated (0.2% PEG 1500) Carbopack C column and with the conventional gas–liquid column mentioned above. In Table II, relative retention times at 55° for C_5 alcohols and butanol are reported. We chose such a complex mixture in order to evaluate the feasibility of using the columns considered to separate various kinds of geometrical isomers. With respect to GCB modified with 0.2% PEG 1500, a large variation in relative retention times occurs where a more or less closely packed

TABLE II

RELATIVE RETENTION TIMES FOR C_5 ALCOHOLS

Alcohol	*PEG 1500 (%)*					*Chromosorb W + 5% PEG 1500*
	0.2	*0.6*	*1.0*	*2.0*	*4.0*	
Butanol	466	680	826	903	998	1192
2-Methyl-2-butanol	438	523	501	485	477	471
3-Methyl-2-butanol	628	747	773	779	775	836
3-Pentanol	778	828	835	843	852	875
2-Pentanol	1000	1000	1000	1000	1000	1000
Cyclopentanol	894	1357	1790	1930	2800	3450
2-Methyl-1-butanol	1327	1453	1585	1653	1698	1880
3-Methyl-1-butanol	1577	1616	1743	1785	1802	1930
Pentanol	2296	2006	2108	2215	2320	2590

monolayer of modifying agent is present on the GCB surface. This effect can be accounted for by considering that in the former instance adsorption occurs on the solid medium while in the latter eluate molecules are adsorbed on the PEG 1500 monolayer. As the number of layers is increased, more or less large modifications in the chromatographic process still occur and GCB coated with increasing amounts of stationary phase tends to behave like the corresponding gas–liquid column. Variations in the separation factors that occur when the GCB surface is coated with an increasing number of layers is probably due to the fact that the chromatographic process changes from adsorption on the outer layer of liquid film to solution into the bulk liquid.

From our results, a partially shielded GCB surface appears to have the highest selectivity.

Columns packed with GCB supporting a thin film of stationary phase generally have a higher separation power than a gas–liquid column. Moreover, it appears that the selectivity of GCB coated with thin films of liquid phase can be changed by changing the liquid to solid ratio.

Considering the permeability of the mini-columns studied, Table III gives some pressure drops just needed to obtain a linear carrier gas velocity of about 9 cm/sec, which corresponds to about the optimal value, with nitrogen as the carrier gas on a column packed to a length of 21 cm with GCB particles of different size ranges coated with 1% PEG 1500. No substantial difference in the column permeability was found on varying the percentage of liquid phase.

TABLE III

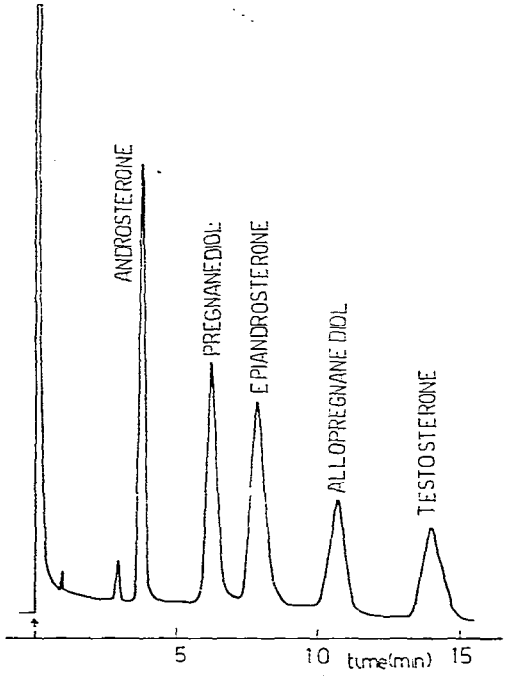
PRESSURE DROPS REQUIRED TO OBTAIN A LINEAR CARRIER GAS VELOCITY OF 9 cm/sec

Particle size range (μm)	*Pressure drop (atm)*
61–45	1.4
45–33	2.6
33–25	4.8
25–20	8.0

To give experimental evidence of the usefulness in chromatographic analysis of mini-columns packed with very fine GCB particles coated with very thin films of stationary phase, some interesting analytical applications concerning the elution of polar, high-boiling compounds were studied. In all instances, the elutions were performed by using Carbopack C of particle size 25–33 μm packed in 21 cm $\times$ 0.5 mm I.D. glass columns with hydrogen as the carrier gas. Under these conditions, the columns had an efficiency of about 2400 plates at a carrier gas velocity of about 21 cm/sec.

Fig. 3 shows a chromatogram for a steroid mixture obtained at 245° on hydrogen-treated Carbopack C coated with 1% PEG 20M. Testosterone is eluted with a retention time of about 15 min. It should pointed out that on a conventional, 1-m column with nitrogen as the carrier gas testosterone could be eluted after as long as about 4 h. It has been previously reported[18] that the treatment of GCB with hydrogen at 1000° has the effect of eliminating residual, acidic active centres. For the analysis of steroids, hydrogen-treated Carbopack C was found to be useful as untreated Carbopack C caused partial decomposition of testosterone and to some extent also of pregnanediol.

By using the same column packing, a rapid separation of some oxygenated diterpenes was performed, as shown in Fig. 4. Also in this instance, untreated Carbopack C caused the complete decomposition of sclareole, which is eluted as the first peak.



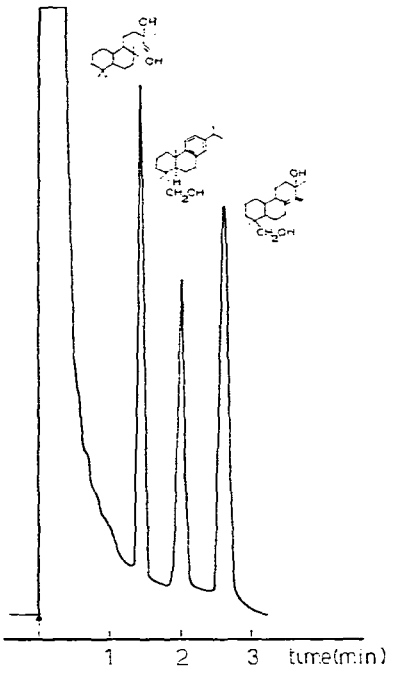
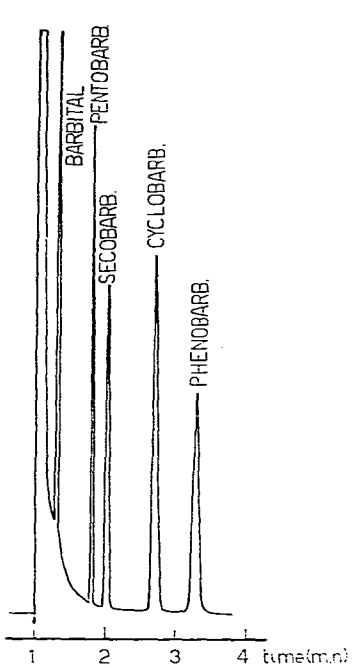


Fig. 3. Chromatogram showing the elution of some steroids. Column, 21 cm $\times$ 0.5 mm I.D.; packing, hydrogen-treated Carbopack C (33–25 μm) + 1% PEG 20M; carrier gas, hydrogen; dead time, 0.9 sec; temperature, 245°.

Fig. 4. Chromatogram showing the elution of some oxygenated diterpenes. Temperature, 235°; other conditions as in Fig. 3.



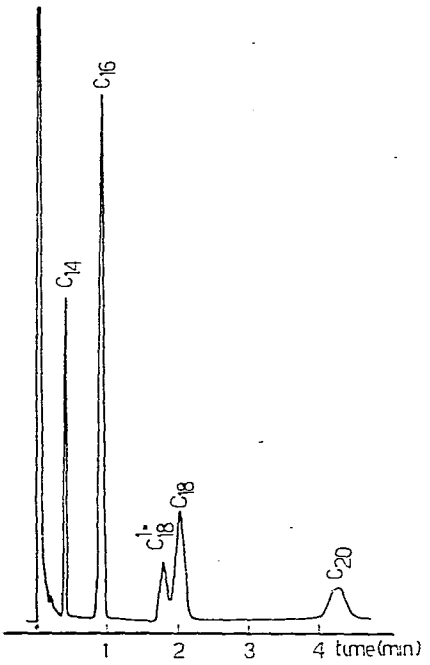


Fig. 5. Chromatogram showing the elution of some barbiturates. Column, 21 cm × 0.5 mm I.D.; packing, Carbopack C (33–25 μm) + 0.3% trimesic acid + 0.6% PEG 1500; carrier gas, hydrogen; dead time, 1.0 sec; temperature, 235°.

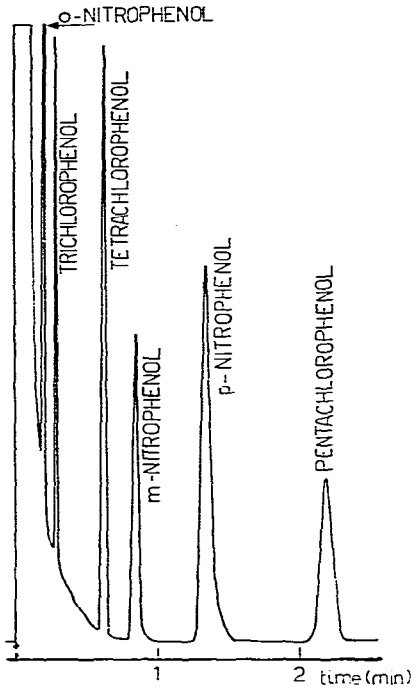
Fig. 6. Chromatogram showing the elution of some C_{14}–C_{20} free fatty acids. Conditions as in Fig. 5.

Fig. 5 shows a chromatogram for a barbiturate mixture eluted at 235° on Carbopack C modified with 0.3% trimesic acid +0.6% PEG 1500. It is interesting that phenobarbital, which is the most retained of the commonly encountered barbiturates, is eluted on this column within about 3.5 min at 235°. Moreover, at this column temperature, no bleeding of the stationary phase occurred as this column packing is stable up to 270°.

With the same packing material and the same column temperature, a mixture of high-boiling free fatty acids up to C_{20} was eluted with a retention time of about 4 min, as shown in Fig. 6. It should be pointed out that with a conventional column packed with GCB modified with FFAP, oleic acid could be eluted with a retention time of about 110 min[13].

It is known that when eluted on classical gas–liquid columns, polychlorophenols and nitrophenols have large retention times because of their high polarity. As shown in Fig. 7, by the use of a mini-column packed with suitably modified Carbopack C, we were able to elute pentachlorophenol with a symmetrical peak within 130 sec. Also, *p*-nitrophenol was eluted within 80 sec as an only slightly tailed peak. This retention time is very low compared with the 42 min needed to elute the same compound on a conventional modified GCB column[14].

When the thermal stability of the modifying agent is a critical factor, mini-columns fed with hydrogen can be a great aid. Quadrol, when deposited on Carbopack C, is very effective for the linear elution of glycols. At 150°, using 1% Quadrol-modified Carbopack C, glycerol is eluted in 30 min on a conventional packed column



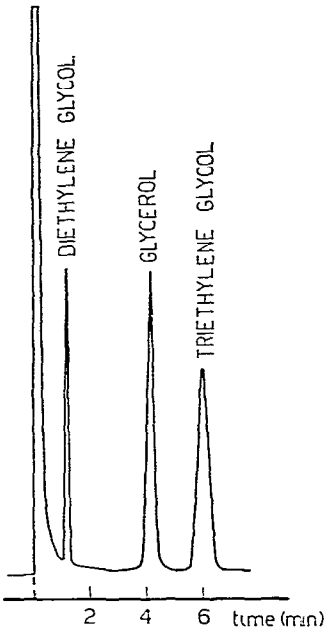


Fig. 7. Chromatogram showing the elution of some chloro- and nitrophenols. Temperature, 210°; other conditions as in Fig. 5.

Fig. 8. Chromatogram showing the elution of some glycols. Column, 21 cm × 0.5 mm I.D.; packing, Carbopack C (33–25 μm) + 1% Quadrol; carrier gas, hydrogen; dead time, 0.9 sec; temperature, 125°.

(80–100 mesh in a 1.1-m column with nitrogen as the carrier gas). At this column temperature, however, the packing under consideration has a short life. On the other hand, by using 25–33-μm GCB packed in a 21-cm length of column, glycerol could be eluted within 4 min at 125°, as shown in Fig. 8. At this temperature, the characteristics of the packing material remained unaltered over a long period.

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