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ACID-WASHED GRAPHITIZED CARBON BLACK AND ITS APPLICATION IN THE SEPARATION OF SOME ACIDS

ANTONIO DI CORCIA*, ARNALDO LIBERTI and CLAUDIO SEVERINI
Istituto di Chimica Analitica, Università di Roma, 00185 Rome (Italy)

SUMMARY

Acid-washed graphitized carbon black modified with a suitable liquid phase has been exploited for the analysis of mixtures of biological interest. Phenobarbital can be accurately determined at the 6 ppm level. 3-Hydroxybutyric acid can be determined at the physiological level by direct injection of undeproteinized serum.

INTRODUCTION

Although the first paper on gas chromatography (GC), by James and Martin[13] in 1951, dealt with the quantitative analysis of free fatty acies, and in spite of the large amount of subsequent work on the analysis of acids, there are still some unresolved or difficult problems: for example, trace analysis of acidic pollutants in water, fatty acids in fermentation broth, hydroxyacids in blood and barbiturates at the ppm level in biological materials.

In chromatography of solutions containing acidic compounds, the column is usually deactivated either with an acid (usually H_3PO_4) or with a liquid phase itself containing an acidic functional group. Generally, these columns have several limitations: thermal instability of H_3PO_4 at temperatures higher than 190°C; partial decomposition of the stationary phase by H_3PO_4 at temperatures higher than 190°C; anomalous interactions between H_3PO_4 or the acidic group of the liquid phase and non-acidic compounds, such as alcohols; strong interactions between the acidic liquid phase and acidic, polar eluates which results in long analysis times and poor selectivity for acidic compounds having similar molecular structure.

In recent years, graphitized carbon blacks (Carbopack) deactivated with H_3PO_4 (ref. 1), trimesic acid[2] or coated with FFAP[3,4] have been used for elution of acids. These chromatographic systems showed the same limitations as those mentioned above.

In our laboratory, we observed that if one part of Carbopack is immersed in 25 parts of water, the suspension continuously stirred for 24 h and the supernatant liquid then separated by filtration and placed in contact with the glass electrode of a pH electrometer, a reading is obtained corresponding to a pH of 10.5. It has also been noted[5] that washing the surface of graphitized carbon balcks (CGBs) with an acidic

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solution is effective in eliminating basic adsorption sites. Furthermore, after this chemical treatment, the pH of a suspension of Carbopack in water decreased to 6. Also, symmetrical peaks for acids were obtained after elution on even the uncoated surface of Carbopack.

In this work, the ability of acid-washed (AW) Carbopack to give symmetrical peaks for acids without the use of a deactivating acidic agent has been exploited in the analysis of some acid-containing solutions. In particular, by modifying the surface of Carbopack C with Apiezon and PEG 20M, a mixture of barbiturates was separated. Calibration curves for phenobarbital showed no chemisorption of this compound at the 5 ppm level. Moreover, by modifying the surface of Carbopack B with PEG 20M, we were able to detect about 30 ppm of 3-hydroxybutyric acid in blood by direct injection of undeproteinized plasma.

EXPERIMENTAL

Carbopack B and C, which are examples of graphitized carbon blacks, were kindly supplied by Supelco (Bellefonte, PA, U.S.A.) The procedure of washing Carbopacks with an acidic solution in order to obtain a carbon surface free from basic centres has been reported previously[5].

Coating of the Carbopack C surface was carried out in the usual way[6]. Both Apiezon and PEG 20M were deposited on Carbopack from methylene chloride: 0.2% Apiezon + 0.35% PEG 20M on Carbopack C AW was conditioned for 12 h at 250°C; 4% PEG 20M on Carbopack B AW was conditioned for the same time at 240°C.

A Model Gl gas chromatograph (Carlo Erba, Milan, Italy) equipped with a flame ionization detector was used. The nozzle of the detector was made of quartz. Tailed peaks for hydroxyacids were noted when the chromatographic apparatus was equipped with a stainless-steel nozzle.

RESULTS AND DISCUSSION

By using a 2-m column packed with Carbopack B AW + 5% PEG 20M, perfectly symmetrical peaks and baseline separations were obtained for elution of an aqueous mixture containing C_2–C_7 fatty acids, C_1–C_5 alcohols, ethyl acetate and acetaldehyde at 20 ppm. This kind of analysis is of biological interest as these substances may be produced by bacterial fermentation. It should also be noted that no chromatographic system is suitable for simultaneous elution of acids and alcohols. If the column is deactivated in order to obtain linear elution of acids, alcohols are eluted as tailed peaks due to anomalous interactions between eluates and H_3PO_4.

Because of the widespread use of barbiturates, therapeutic monitoring and drug overdose analysis is an important task in clinical laboratories. Several relevant gas–liquid chromatographic procedures have been described previously[7–11], although the plethora of methods only serves to illustrate the lack of general method to meet all needs. Many of the procedures reported give good results in the analysis of drug overdoses, where the blood barbiturate concentration is high, but fail in therapeutic monitoring, where the concentration levels in biological fluids are low. This is due to partial irreversible adsorption of barbiturates on the columns.

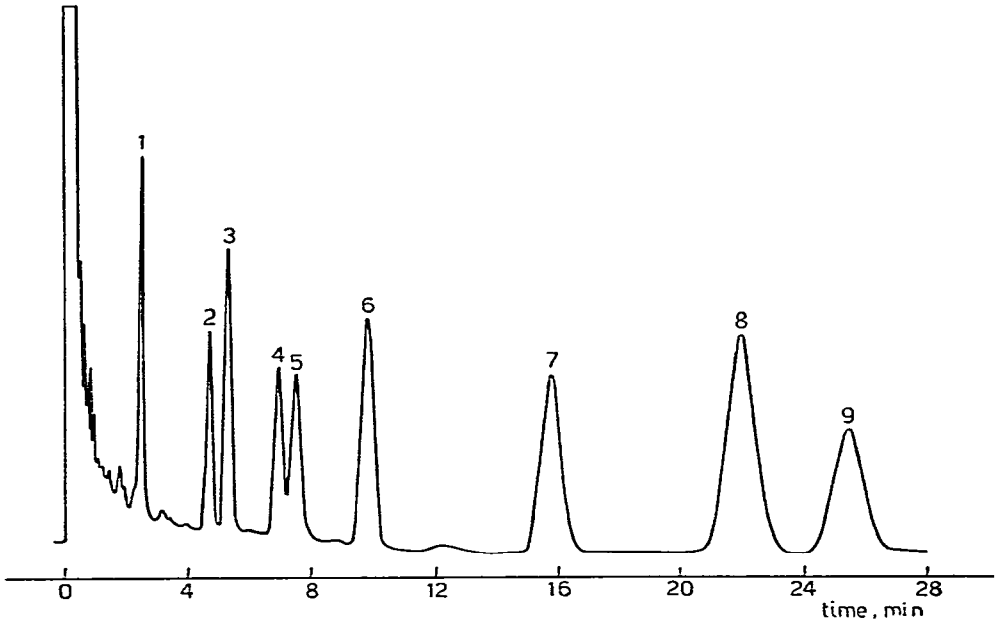

Fig. 1. Chromatogram of a barbiturate mixture. Packing: Carbopack C AW + 0.35% PEG 20M + 0.2% Apiezon. Column: 1 m × 2 mm. Carrier gas: hydrogen. Injected amount: 1 μl of a solution containing 80–100 ppm of each component. Dead time: 5.8 sec. Temperature: 235°C. Peaks: 1 = barbital; 2 = diallylbarbital; 3 = allylisobarbital; 4 = amobarbital; 5 = pentobarbital; 6 = secobarbital; 7 = cyclohexenylbarbital; 8 = phenobarbital; 9 = cycloheptylbarbital.

Fig. 1 shows the elution of a mixture of the most frequently encountered drugs on Carbopack C AW suitably modified with 0.35% PEG 20M and 0.2% Apiezon.

Chemisorption of barbiturates does not occur on the acid-washed surface of graphitized carbon blacks. To substantiate this and to determine the limit of detection of phenobarbital, quantitative measurements of this compound were made. A paraffin was chosen as internal standard since partial chemisorption of a given compound can be recognized by injecting it in variable amounts together with an inert eluate and measuring the response factor at any given concentration. Standard solutions at concentrations lower than 166 ppm were prepared by diluting the starting solution containing phenobarbital and the reference compound. Results are reported in Table I. It appears that the quantitative determination of phenobarbital at the ppm level can be made with an error not higher than 6%.

The value of gas chromatography in the diagnosis of, and studies on, human diseases is now well recognized. A number of metabolic disorders are characterized by increased concentrations of organic acids, in biological fluids and blood and urine samples are routinely examined by chromatographic methods in several hospital laboratories.

In many cases, the organic acids of biological interest are present in blood at the ppm level and are very polar in nature, such as the hydroxyacids. Consequently, the quantitative elution of these compounds presents serious problems due to chemisorption. Other sources of errors accrue from deproteinization, removal of charged

TABLE I

RESPONSE FACTOR FOR PHENOBARBITAL *VS.* HEXADECANE AT VARIOUS CONCENTRATIONS

Concentration (ppm, w/w)	*Response factor*	*S*
166	0.79	0.032
55	0.80	0.031
18	0.80	0.028
6	0.78	0.043

compounds and derivatization of organic acids. Serious difficulties are encountered in derivative preparation due to the inhibition caused by residual amounts of water.

It has been reported[12] that the concentration of 3-hydroxybutyric acid in blood can increase from about 20 ppm (physiological level) to about 60 ppm for patients affected by diabetes. The monitoring of this organic acid can be of great aid in the early diagnosis of this disease. Fig. 2 shows the elution of undeproteinized serum of a patient with a glycemia value equal to 2.2. A well-defined peak for 60 ng of 3-hydroxybutyric acid is observed. This elution was accomplished by the use of Carbopack B AW coated with 5% PEG 20M. The column was used over a long period of time, and accumulation in the injection port of proteins contained in serum did not affect the chromatographic profile.

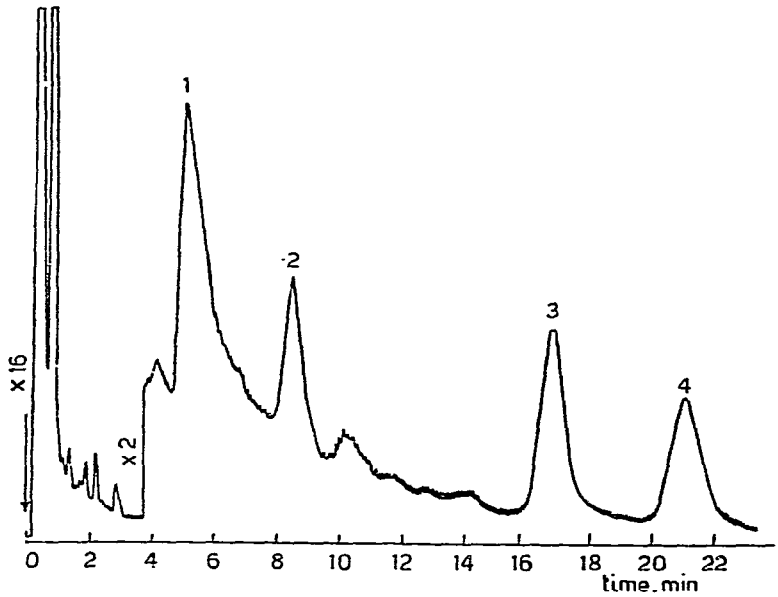

Fig. 2. Chromatogram of 3-hydroxybutyric acid from undeproteinized serum. Packing material: Carbopack B AW + 4% PEG 20M. Column: 1 m × 3 mm. Carrier gas: nitrogen. Injected amount: 2 μl of serum. Dead time: 12 sec. Temperature: 195°C. Peaks: 1 = lactic acid and artifact from acetoacetic acid; 2 = 3-hydroxybutyric acid; 3 = enanthic acid (internal standard), 10 ppm; 4 = unknown.

The same procedure was followed for 3-hydroxybutyric acid. Enanthic acid was chosen as internal standard. The results obtained showed no chemisorption of the hydroxyacid and a well-defined peak for 7 ppm of this acid in water. At this level of concentration, the error of quantitative measurement is $\leqslant 10\%$.

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