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Gas Chromatographic Analysis Using Grafted Chromosorb G by Thionyl Chloride And Grignard Reagent

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The surface of a chromosorb G (NAW) support (45-60 mesh, S = 0.5 m^2/g) was grafted by two steps, the first one was chlorination of the silica surface using thionyl chloride ($SOCl_2$) and the second by reacting Grignard reagent with chlorophenyl magnesium. A hybrid surface structure (organic/inorganic) of the chromosorb had an extra characteristics of hydrophobicity. The variation of surface grafted support was realized by plotting the relationship between variation of the logarithm of retention volume Vs and the reverse of absolute temperature log Vs = f(1/T). Distinguish the new support with a good stability at high temperature degrees which enable its application in gas chromatography. Essential oils' mixtures (α-pinene, β-pinene, cineole, fenchone, borneol and anethole) and some fatty acid (C8, C10, C12, C14 and C16) in standard (GLC-30) and in laurel oil extracted from wild fruit and cultivated laurius nobilis were determined using the modified chromosorb G by gas chromatographic analysis. The analytical results were characterized by high precision, accuracy and reproducibility.

Key Words: Chromosorb G, Chlorination, Essential oils, Laurel oil, Grignard reagent.

INTRODUCTION

In chromatography the separation is affected by the nature of stationary phase, so the popular use of GLC technique causes a vast variation of stationary liquid phases. The solute may be separated at similar temperature degrees if the reciprocal effects between the stationary phase and the solute were different[1-13].

Many stationary phases have the general structure shown in Fig. 1. A stationary phase of polydimethyl siloxane, in which all the -R groups are methyl groups ($-CH_3$), is non-polar and often makes first choice for a new separation. The order of elution when using polydimethyl siloxane usually follows the boiling points of the solutes, with lower boiling solutes eluting first. Replacing some of the methyl groups with other subsistents increases the stationary phase's polarity, providing greater selectivity. Thus, in 50 % methyl-50 % phenyl polysiloxane, 50 % of the -R groups are phenyl groups ($-C_6H_5$), producing a slightly polar stationary phase. Increasing polarity is provided by substituting trifluoropropyl ($-C_3H_6CF_3$) and cyanopropyl ($-C_3H_6CN$) functional groups or using a stationary phase based on polyethylene glycol[1-17].

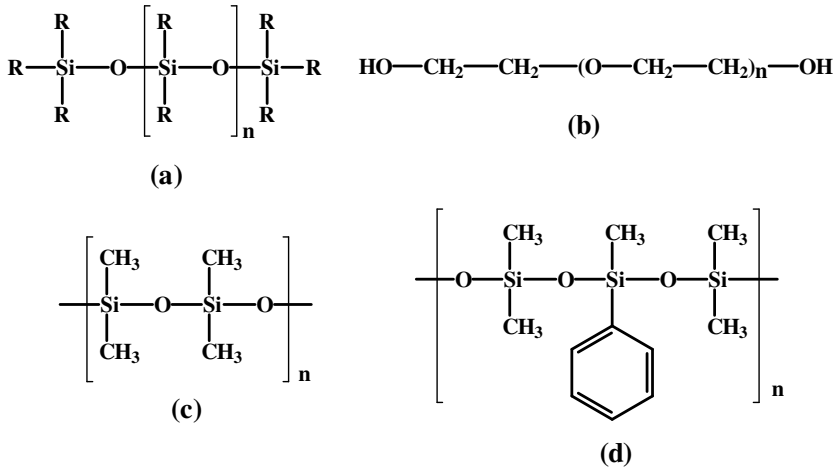


Fig. 1. Many stationary phases: (a) polydialkyl siloxane, (b) polyethylene glycol, (c) Dimethyl polysiloxane, (d) 95 % methyl-5 % phenyl polysiloxane

Diatomaceous supports: Two types of supports are made from diatomite. One is pink and derived from firebrick and the other is white and derived from filter aid. Diatomite itself is a diatomaceous earth. Diatomite is composed of diatom skeletons or single-celled algae that have accumulated in very large beds in numerous parts of the world. The skeletons consist of a hydrated microamorphorous silica with some minor impurities (metallic oxides)[4-6,12].

Modification of surface of chromatographic support: In GLC, the solutes separation depends on its distribution between the carrer gas and the liquid stationary phase that cover the solid phase to prevent of solute molecules adsorption on the uncovered solid phase surfaces that cause differences in retention times for similar molecules. This is caused by virtue of existence of active sites as silanole groups attached forcedly with the solute by hydrogenic bonds, therefore tailing of the peak. The deactivation of the surface support takes place by making it inert by a proper method as a chemical grafting or superficial condensation of the polymer to produce a new form differs from the surface structure of the uncovered support[1-17].

Preparation of organomagnesium compounds (Grignard reagents): Grignard reagents are prepared from organic halides by reaction with magnesium.

$$\underset{\text{Organic halide}}{RX} + \underset{\text{Magnesium}}{Mg} \longrightarrow \underset{\text{Organomagnesium halide}}{RMgX}$$

Anhydrous diethyl ether is the customary solvent used when preparing organomagnesium compounds. Sometimes the reaction does not begin readily, but once started, it is exothermic and maintains the temperature of the reaction mixture at the boiling point of diethyl ether (35 °C). The halide reactivity order is I > Br > Cl > F and alkyl halides are more reactive[13,14].

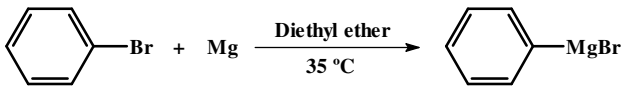
EXPERIMENTAL

The chromatograms were obtained by using a GC-9A gas chromatograph equipped with a flame ionization detector (FID) and chromatopac C-R3A printer (Shimadzu Co.), (2 m × 2 mm) stainless steel column, 1 μL syringe (Hamilton Co.), chromosorb G (NAW) 45-60 mesh for gas chromatography-Merck, special reactor for grafting. All solvents and chemicals used GR grade.

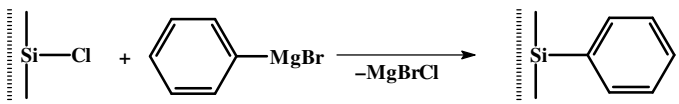
Chemical grafting: Chromosorb granules were dried (45-60 mesh) at 105 °C for 2 h then the grafting was made through two stages:

Chlorination of the chromosorb G (NAW): Chlorination of the chromosorb G (NAW) was made by using of thionyl chloride by placing the chromosorb support in a stainless steel column equipped with a special injector within the grafting oven by passing nitrogen gas throughout the treatment period. The temperature was elevated to 120 °C for 2 h then thionyl chloride injected for several times by 1 mL/30 min to ensure the replacement of superficial silanol groups by chloride groups at 80 °C for 3 h then the temperature was raised to 120 °C for extra 3 h. A binding between the silanol group and the chlorination material was made according this reaction:

Treatment with Grignard reagent: After preparing Grignard reagent from the bromobenzene an activated magnesium metal in a rate of mol to mol in presence of the ether as an activator proceeds according to the reaction:



Grignard reagent was transferred into a round bottom flask equipped by reflux condenser and the chlorinated chromosorb G was added in the presence of nitrogen gas. The temperature was elevated to 60 °C for 3 h, then the solvent was vapourized by using a rotary evaporator, then the dried support washed by the diethyl ether, by ethyl alcohol and finally with distilled water to get the chemically modified chromosorb G (by phenyl groups) according to this reaction:



RESULTS AND DISCUSSION

The modified process leads to the formation a new bonding structure of Cromosorb G is discerning through two ways:

Plotting the relation between variation of the logarithm of retention volume and the reverse of absolute temperature log Vs = f(1/T): The modification superficial structure of grafted chromosorb G was studied by "inverse gas chromatographic method" in the range 40-100 ºC and column dimension (2 m × 2 mm) using dichloromethane, benzene and *n*-pentane as auxiliary solutes. The plotted relationship log Vs = f(1/T) shown that, a decreasing of volume retention was noticed with grafted chromosorb G by condensation phenyl group compared to free chromosorb G (Fig. 2). Fig. 2 shows that ΔVs between grafted and free chromosorb G decreases with decrease of auxiliary solute polarity. By conclusion of the three auxiliary solutes approve the modification of the superficial structure of the chromosorb G support surface.

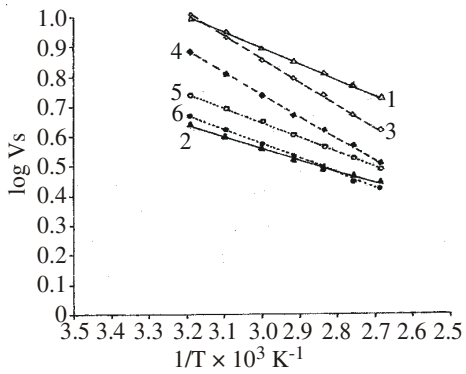


Fig. 2. Variation of log Vs = f(1/T) on (2 m × 2 mm) packed columns using free chromosorb G (1,3,5) and grafted chromosorb G (2,4,6): 1,2-*n*-pentan, 3,4-benzene, 5,6-dichloromethane

Hydrophobicity: For the estimation of the changes in the hydrophobicity after modification, we compared dispersibility of the free and grafted chromosorb G in soluble mixture water/benzene. As shown in Fig. 3, the free chromosorb G disperses in the water layer only. Due to the presence of the hydrophobic alkyl groups on the external surface of grafted chromosorb G and the hydropholicity of the rest at the surface, the grafted chromosorb G was found in organic phase at the benzene-water boundary.

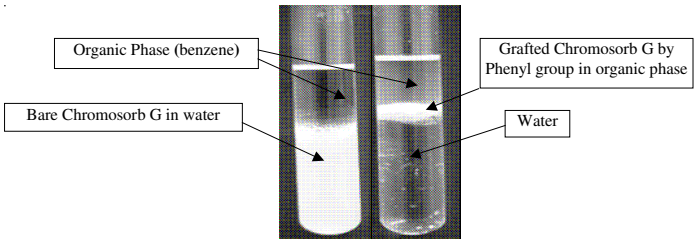


Fig. 3. Free chromosorb G (left) and grafted chromosorb G by phenyl group in organic phase (right) dispersed in water/benzene (organic phase) system

Applications

Identification of essential oils: The essential oils' mixtures, which are using in pharmaceutical industries as gastric antiseptics[18] was determined using grafted chromosorb G. The most important ones of them are as follows: α-pinene, β-pinene, cineole, fenchone, borneol, anethole.

The chromatographic conditions for analysis are as the following: analytical stainless steel column (2 m × 2 mm) packed with modified chromosorb G by phenyl group, programmed column temperature between 35-125 ºC, with increasing temperature rate 6 ºC/min, detector was flame ionization, flow rate of N_2 carrier gas 40 mL/min, the injection volume was 0.2 µL and injected port temperature 175 ºC. A mixture of the six essential oils prepared in a concentration of 0.2 g/5 mL for each one by using the chloroform as solvent (Fig. 4). Fig. 4 shows that the separation was completed with a high precision and reproducibility.

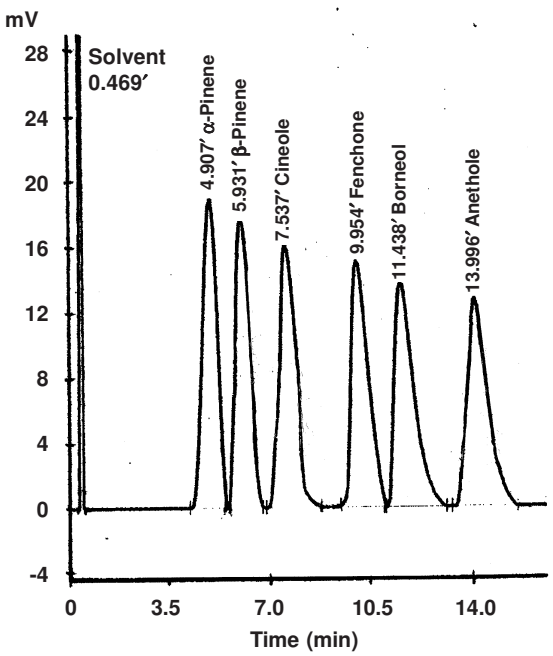


Fig. 4. Chromatogram for some essential oils (column 2 m × 2 mm packed with modified chromosorb G, programmed temperature 35-125 ºC with increasing rate 6 ºC/min, flow rate of N_2 40 mL/min, the injection volume 0.2 µL and injected port temperature 175 ºC)

Comparison of the rate of lauric acid in laurel oil extracted from wild fruit and cultivated laurius nobilis using the modified chromosorb G: The fatty acid as methyl esters of a standard mixture containing five components: methyl caprylate, methyl caprate, methyl laurate, methyl myristate, methyl palmitate by a rate of 20 %

from each (GLC-30 standard mixtures acc. USP-27) was identified (Fig. 5). These esters were well identified at the determination conditions: analytical stainless steel column (2 m × 2 mm) packed with modified chromosorb G by organic group, programmed column temperature between 90-235 ºC, with increasing temperature rate 6 ºC/min, detector was a flame ionization, flow rate of N_2 carrier gas 40 mL/min, the injection volume was 1 µL and injected temperature was 250 ºC.

The laurel oil extracted from wild fruit after its esterification as a form of methyl ester (according of the British Pharmacopaedia 2007)[19] and determined the quantity of some fatty acids of laurel oil ester at the same conditions was studied (Fig. 6). It was found that, the mean rate of lauric acid was 33.52 % (RSD % = 3.1, n = 5).

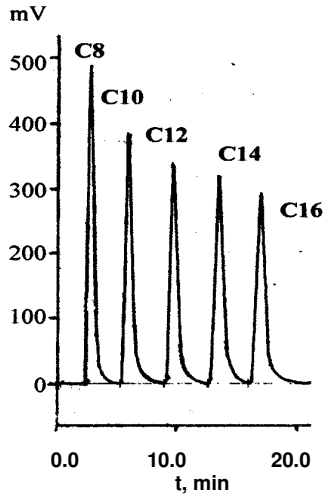


Fig. 5. Chromatogram of standard mixture of fatty acids (C8, C10, C12, C14 and C16) as methyl esters (GLC-30) (column 2 m × 2 mm packed with modified chromosorb G, programmed temperature 90-235 ºC with increasing rate 6 ºC/min, flow rate of N_2 40 mL/min, the injection volume 0.2 µL and injected port temperature 250 ºC)

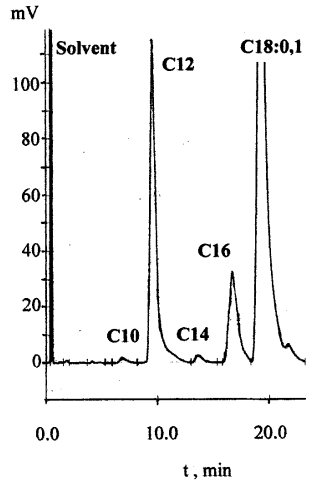


Fig. 6. Chromatogram of laurel oil extracted from wild fruit of laurius nobilis (column 2 m × 2 mm packed with modified chromosorb G, programmed temperature 90-235 ºC with increasing rate 6 ºC/min, flow rate of N_2 40 mL/min, the injection volume 0.2 µL and injected port temperature 250 ºC)

The extracted laurel oil from cultivated fruit laurius nobilis after its esterification as methyl ester and determined the quantity of fatty acids at the same conditions was determined (Fig. 7). It was found that, the mean rate of lauric acid was 12.24 % (RSD % = 3.4, n = 5). The results indicate that the laurel oil extracted from the fruits of wild laurel trees of the mountains was contained of lauric acid 2.74 times more than laurel oil extracted from the fruits of cultivated laurel trees. These results were almost identical with results using gas chromatography capillary columns; where the rate of lauric acid was 33.74 and 12.06 % in laurel oil extracted from wild fruit and cultivated laurius nobilis, respectively[20].

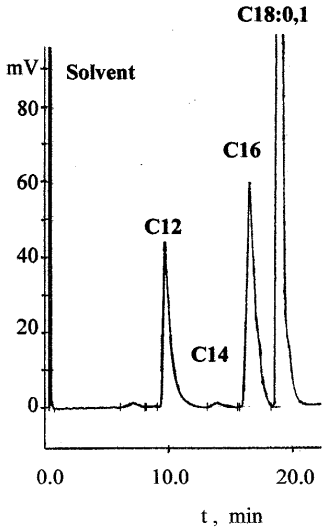


Fig. 7. Chromatogram of extracted laurel oil from fruit of cultivated laurius nobilis (column 2 m × 2 mm packed with modified chromosorb G, programmed temperature 90-235 ºC with increasing rate 6 ºC/min, flow rate of N_2 40 mL/min, the injection volume 0.2 µL and injected port temperature 250 ºC)

Conclusion

The surface of a chromosorb G support was grafted by two steps, the first one was a chlorination of the silica surface using thionyl chloride and the second by reacting Grignard reagent with chlorophenyl magnesium. Distinguish the new support with a good stability at high temperature degrees which enable its application in gas chromatography. Essential oils' mixtures (α-pinene, β-pinene, cineole, fenchone, borneol and anethole) and some fatty acid (C8, C10, C12, C14 and C16) in extracted laurel oil from wild fruit and cultivated laurius nobilis using the modified chromosorb G by gas chromatographic analysis were determined. The analytical results were characterized by high precision, accuracy and reproducibility.

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