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Influence of high inlet pressure gas chromatography (HIPGC) on analytes retention time

Influência da elevada pressão de entrada em cromatografia gasosa (HIPGC) no tempo de retenção

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Received: April 17, 2018
Accepted: May 11, 2018

Abbreviations: high inlet pressure gas chromatography (HIPGC); retention time (tR); flame ionization detector (FID); reduced plate height (h); linear mobile phase velocity (vopt).

Resumo
A cromatografia gasosa com injeção sob alta pressão é uma ferramenta analítica que oferece diversas vantagens, como alta eficiência, reduzido tempo de análise e fácil migração de compostos com alta massa molecular. Neste trabalho foi realizado um estudo básico sobre o desempenho cromatográfico de colunas capilares compactadas (comprimento de 25 cm) utilizando um cromatógrafo a gás modificado. Este sistema, desenvolvido em nosso laboratório, consistiu em um novo dispositivo conectado ao injetor visando a aplicação de cromatografia gasosa em dois modos diferentes: baixa e alta pressão de entrada (até 2000 psi). A pressão de entrada foi variada entre 500 e 2000 psi e a influência desta transição de pressão na eficiência cromatográfica foi avaliada, sendo comparada com outras abordagens para GC. Os resultados obtidos aplicando este instrumento, baseado em um cromatógrafo a gás comercial modificado, mostram que com o aumento da pressão de entrada é possível reduzir o tempo de análise, bem como aumentar a intensidade do sinal analítico. Além disso, este trabalho mostra o uso de colunas capilares empacotadas com fase estacionária de HPLC como uma abordagem alternativa para GC sem programação de temperatura.

Palavras-chave: cromatografia gasosa de alta pressão de entrada; coluna capilar empacotada; cromatografia gasosa.

Abstract
High inlet pressure gas chromatography (HIPGC) is an analytical tool that offers several advantages including high efficiency, fast analysis time, and easier elution of high molecular mass compounds. In this paper a basic study regarding the chromatographic performance of packed capillary columns (length to 25 cm) utilizing a modified commercial GC instrument was carried out. This lab-made GC system consisted in a new device connected to the GC injector aiming the application of gas chromatography in two different modes: low inlet pressure and high inlet pressure (c.a. 2000 psi). The inlet pressure was varied between 500 - 2000 psi and the influence of this pressure transition in the chromatographic efficiency was evaluated being compared with other GC approaches. The obtained results applying this modified GC instrument shows that increasing the inlet pressure is possible to reduce the analysis time as well as improve the analytes signal. In addition, this work shows the use of packed capillary columns packed with standard HPLC stationary phases as an alternative approach to GC analysis without requiring temperature programming.

Keywords: high pressure inlet gas chromatography; packed capillary column; gas chromatography.
1. Introduction

Over the past few years there has been a growing demand for high-throughput separations in numerous fields (e.g., environmental, food, clinical or biological analysis), where either the time response delivery must be reduced, or the productivity enhanced (or in the ideal case both), considering the large number of analyzed samples and diversity of compounds. In order to achieve these goals robust analytical techniques such as liquid chromatography and gas chromatography has been utilized[1–4]. Although gas chromatography (GC) is in some cases faster, and provides higher separation efficiency, having better properties for combination with a wide range of sensitive and selective detectors, it also has some limitations such the requirement that the molecules to be analyzed must be thermally stable and sufficiently volatile. Liquid chromatography (LC) on the other hand provides better selectivity, a much wider application range and has proved to be more rugged than GC[2,3,5–7]. Once GC and LC are complementary techniques, it is logical that the development of an ideal separation technique would combine the advantages coming from each one of these techniques. This would include the high efficiency of open tubular GC columns and the selectivity of LC packing materials, enabling the analysis of a wide range of compounds. A good example of this ideal technique stands out as high inlet pressure gas chromatography (HIPGC)[8–11].

The main difficulties in employing HIPGC are related to the instrumentation, since high pressure conditions requires appropriate connections to succeed in working at such high pressure range, while the injection system must be suitable to transport the intact sample at different pressures[13]. Although some research laboratories have developed instruments for HIPGC, a large number of instrumental drawbacks did not allow these research groups to build robust instrumentation in order to evaluate the technique further[9,14].

Along the last decades several instruments have been developed in our laboratory based upon modification of standard commercial instruments to operate under supercritical fluid chromatography, unified chromatography and other chromatographic modes[15–21]. In all this instrument, when using the mobile phase at high-pressures the injection was carried out via HPLC valves. Although the results have been satisfactory, when using gas as mobile phase the HPLC valves are unsuitable to maintain a reasonable seal system.

Employing the experience obtained in the development of different instrumentation, accessories and laboratory devices, we sought a simple, versatile and inexpensive way to develop a HIPGC instrumental system. This paper discusses the development and application of an instrumental HIPGC system by simple modification of commercial GC equipment through a new device connected to the instrument injector to be used at column inlet pressures up to 2000 psi.

2. Materials and Methods

2.1. HIPGC instrumentation

A standard gas chromatographic system from Shimadzu (Kyoto, Japan), model GC 17A, was modified to perform as a HIPGC analytical system. The instrumental apparatus consists of an external high-pressure nitrogen supply source; a sample introduction system (standard split/splitless injector), an oven, a column, and a flame ionization detector.
(FID) (Figure 1). A total of 5 different instruments were developed until a satisfactory experimental performance was achieved. The system described in this paper corresponds to the fifth developed modification. A schematic drawing of this system containing all main parts is depicted in Figure 2.

**Figure 1.** Overview of the HIPGC system developed: A) gas supplier; B) device designed and built to control all gases through the system and C) modified GC system.

**Figure 2.** Main components of the HIPGC system employed in this work: 1) Manometer, 2) Micrometer valve, 3) T4 connection, 4) Three-way valve, 5) Gas filter (mobile phase), 6) Trap (purge flow), 7) Trap (split flow), 8) Buffer, 9) Syringe holder, 10) Modified injector device; 11) Injection port - liner used: split liner; 12) Stainless steel union (Valco); 13) Capillary tube (restrictor); 14) Packaged capillary column; 15) FID detector and 16) Gas chromatograph.
The high-pressure nitrogen system consists of two standard gas cylinders used to deliver the nitrogen gas to a third empty cylinder, responsible for boosting the nitrogen mobile phase at pressures up to 2500 psi. This latter cylinder was connected to a high-pressure regulator used to control the inlet pressure before a micrometric stainless-steel valve via 1/16” (HOKE series 1600) used to control the nitrogen (mobile phase) flow. Figure 3 shows a photo detailing the developed nitrogen pressurization system. A carrier gas was used to assist the injection process (split/splitless) being this system controlled by a back-pressure regulator consisting in a three-way solenoid valve usually employed to control the mobile phase flow rate. In the case when splitless injection mode is selected this solenoid valve is maintained closed.

The injector was also modified, and new connections were built aiming to achieve a proper septum position adjustment avoiding the deleterious backpressure effect over the injection syringe and prolonging the septum life. In this case, a pre-column (fused silica tubing) had one end attached to the injector with the other end coupled to a 1/19 union (Valco) that is connected to the packed capillary analytical column. The other end of the analytical column was connected to a capillary (fused silica tubing) restrictor placed into the FID detector set to 300 °C.

2.2. Reagents, solutions and materials

Ibuprofen analytical standard was acquired from Sigma (Steinheim, Germany). Biodiesel prepared from soya bean oil were obtained from Cargill (Mairinque, Brazil). C9-C12 analytical standards were from Sigma (Steinheim, Germany) while the solvents pentane and hexane were from Mallinckrodt. Standard stock solutions of C9-C12 were prepared at 1000 µg L⁻¹ in hexane. An intermediate standard solution was prepared by the dilution of stock solutions with hexane. Working standard solutions were prepared daily by the dilution of the intermediate standard solution in hexane. All tubing employed in the flow control system was 1/16” o.d., made from stainless steel. The data acquisition system consisted of standard PC (Personal Computer) fitted with standard Shimadzu software used for gas chromatography.

2.3. Preparation of the analytical columns

To produce the capillary analytical columns, a fused silica tubing of 0.25 mm of internal diameter were used. These capillaries were cut aiming several column lengths. At one end of the tube was introduced a glass fiber filter to serve as column frit. A long tube (0.50 mm i.d. vs 10 cm) was impregnated with an epoxy resin in order to fix the frit being this capillary introduced 5 cm
into the analytical column while other remaining 5 cm were fixed in other tube (0.25 mm i.d. vs 30 cm). This column structure was filled by the stationary phase being this same procedure necessary to seal the other column end after the packing procedure. Considering the reduced diameter applied in this work the columns was packed employing a slurry packing process. In this methodology a HPLC pump (LC-20AD from Shimadzu) was coupled to a stainless-steel tube connected to another stainless-steel reservoir containing the stationary phase (C18) suspension (Figure 4). This reservoir was connected to the capillary tube structure previously prepared. Afterwards, the packaging process starts with the system being purged using methanol as solvent in such a way that it pushes the particle suspension into the capillary column. After the capillary tube totally filled, the system must be turned off and the column will be taken only after complete depressurization system for finishing the packing step. Figure 4 displays a general view of the packing system utilized.

3. Results and Discussion

The instrumental system developed for HIPGC consists of a GC commercial instrument, a pressure system, manometer, micrometric valve, connection T, three-way valve, gas filter (mobile phase), trap (purge flow), trap (split stream), injector device modified, liner (SPME), capillary fused silica as a restrictor, packed capillary column, FID detector, as illustrated on Figure 2. The pressure system developed consists of two cylinders of N₂ gas connected to a third cylinder (capacity of 3 L) through a T connection and two return valves, which the springs from the valves were replaced by stronger springs to withstand the pressure range of 1-2000 psi.

Figure 4. Simplified scheme of the packaging system with suspended particles utilized for the preparation of capillary columns: (a) gas cylinder, (b) packaging solvent reservoir, (c) pneumatic amplifier pump, (d) valves, (e) T-connection, (f) suspended particulate reservoir, (g) and (h) restrictor (fused silica tube). Modified from Rezemini, Andrea Lucia. “Study about the packaging process and evaluation of columns for capillary liquid chromatography”. Master’s dissertation in Analytical Chemistry, Institute of Chemistry of São Carlos, University of São Paulo, São Carlos, 1997.
avoiding gas return. The purpose of this pressure system configuration was to increase the gas pressure instead of using a booster (a more expensive device) while assuring the use of higher pressures for a longer time and reducing the mobile phase flow rate ($N_2$). As the inlet pressure on the HIPGC system is higher than that achieve in standard GC, the GC injector device is not feasible to be employed as such. In the absence of commercial availability of injection devices that support pressure in the HIPGC range, changes on the GC injector were required. The modifications were developed to increase the withstand of higher pressures avoiding possible leakage and septum deformation.

Figure 5 shows the main mechanical modifications developed at the injector connections to minimize the effect of backpressure under the syringe. The built device consists of (a) a piece designed for septum entrapment avoiding the movement of the septum and its deformation (b) a needle guide was embedded in the piece what eliminates possible leaks and (c) this device was made in stainless steel for guarantee resistance mechanical. The diameter and height of PTFE plug at the tip of the gastight syringe was modified and optimized to avoid deformation and possible leak (S1- supplementary supporting). The HIPGC instrumental system and modification on syringe plug allowed the injection of liquid sample by syringe. The mobile phase flow rate was adjusted by using proper restrictors. Although there are a variety of restrictor types such as fused silica, stainless steel, Pyrex glass, in this instrumental system a fused silica restrictor was used. The restrictor dimensions were optimized according to length of column utilized.

In order to validate the HIPGC system, a packed column (250 µm x 20 cm x 3 µm, C18) has been used. The column was connected to the injector using $N_2$ as carrier gas for separation of a mixture containing saturated hydrocarbons (C9-C12). The restrictor was fitted using an open capillary (L = 1.5 m, 50 µm I.D.) to obtain a flow rate ~ 2.5 mL/min. The oven temperature was set at 30 °C and FID temperature at 280 °C. Relative Standard Deviation (RSD) of retention times and peak areas have been measured with C9 ($n = 4$) and the values obtained (< 0.2% and < 2%, respectively) confirmed the suitable setup of the instrument for HIPGC pressure in the range 1-2000 bar.

Figure 6 depicts the van Deemter plots for the developed system and columns, showing the reduced plate height (h) against linear mobile phase velocity.
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(v_{opt}) for octane at 90 °C with an inlet pressure ranging from 500 to 1500 psi for two different capillary columns: Figure A – 250 µm x 12 cm length packed particles C18 3.0 µm (column A); and Figure B – 250 µm x 20 cm length packed with particles C18 3.0 µm (column B). These results are showing an inversely proportional dependence between h and inlet pressure while \( v_{opt} \) are shifted to faster flow rates, for both columns. The slight difference on the efficiencies obtained for column A and B, under the pressure range (500 - 1500 psi) is expected once the total efficiency increases proportionally to the column length. Therefore, column B reported lower levels of h being consequently considered more efficient.

Despite this correlation, it must be highlighted that the longer is the packed capillary column a higher flow resistance should be expected requiring higher column inlet pressure levels to maintain the chromatographic performance as well as more robust instrumentation in order to avoid leaks\([22]\). Increasing the temperature reduces analysis time and column efficiency, but the maximum operating temperature at the developed system was 90 °C. The theoretical plates per meter (N/m) obtained for column A and B were 70,000 and 73,000, respectively. These results were slightly higher than that previously observed for packed capillary column (21.5 cm x 250 µm i.d. x 5 µm ODS-2) that was 50,000 suggesting that the particle diameter (3 µm in our case against 5 µm in the previously reported one) directly affect the column efficiency\([9]\).

Figure 7 shows the relative retention time (t_R) for octane by HIPGC. Operating conditions: column 250 µm x 20 cm length packed with particles C18 3.0 µm; nitrogen at 2000 psi, column temperature 90 °C (isothermal) and FID 280 °C.
From Figure 7, it is observed that the best results were achieved at higher inlet pressure conditions, similar of SFC (1200 - 2000 psi), suggesting that these conditions allowed improvements in terms of gas solvating properties as well as can reduce the retention time. In addition, the results illustrate a trend to retention time increasing with the loss of mobile phase density or decrease of pressure inlet (1000 - 500 psi) hence at low levels of pressure, the octane $t_R$ slightly increased. Considering this fact, at pressure levels lower than 1000 psi it was observed that the retention time changes dramatically reinforcing that the octane is dependent on pressure inlet column. Measurements were also made for other hydrocarbons ($C_9$-$C_{12}$) being the trends in the retention time profiles similar. Figure 8 (A and B) shows a typical chromatogram of a mixture of these compounds at pressure inlet of 2000 and 1500 psi at 90 °C, respectively. The separation of hydrocarbons on packed capillary column confirms that the approach of using columns shorter than open tubular columns, small particles, reduced inner diameter and high-pressure inlet, decrease the analysis time becoming the analytical method faster with satisfactory levels of chromatographic efficiency.

Figure 9 illustrate a comparison between a representative chromatogram of biodiesel (B100) (Figure 8A) obtained by this developed HIPGC system and another chromatogram obtained by a GC-FID common analysis (Figure 8B). It can be seen a large difference between the obtained retention time for biodiesel and triglycerides.
HPIC isothermal analysis between 6.0 – 6.5 minutes while traditional GC analysis reporting a retention time from 22 to 25 minutes with temperature ramp. In such cases were just a triacylglycerides profile is adequate; a much shorter analysis time can be obtained to generate the required data. This fact confirms that at high-pressure conditions the analysis can be faster and perform without high temperature conditions.

Bartle et al. [9] showed the separation of several hydrocarbons on packed capillary column (21.5 cm x 250 µm i.d. x 5 µm ODS-2) at 120 bar, column temperature at 40 °C, ramping at 5 °C to 220 °C with subsequent detection by FID at 400 °C; in this work the retention time for octane was about 20 minutes. Comparing this work to the results prior discussed, the present paper presents the results obtained by using a small column (20 cm x 250 µm i.d. x 3.0 µm C18) without temperature programming, at pressure inlet of 2000 psi, as well as reports an octane retention time of 3.0 minutes which is about 6 times shorter than previously reported under similar conditions[9]. This fact shows an important role of inlet pressure in the t_R of the compounds reinforcing the applicability of HIPGC as an alternative for GC common analysis mainly in cases when faster analysis is required.

4. Concluding Remarks

The modified instrument used to HIPGC analysis showed experimental results reinforcing that by increasing the injector inlet pressure a reduction on the analysis time is obtained. This proposed approach was limited according to the instrumental design to an inlet pressure of 2000 psi being this insufficient for the analysis of much higher molecular compounds at temperatures up to 90 °C. The results showed that utilizing a traditional GC instrument and packed capillary columns with HPLC type stationary phase in HIPGC renders satisfactory chromatographic efficiency and faster analysis time when compared to traditional GC.

Acknowledgement

The authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, FAPESP (Grants 2017/02147-0, 2015/15462-5 and 2014/07347-9) and to CNPq (307293/2014-9) for the financial support provided.

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