

Original

Specific separation of polar analytes on a medium polar stationary phase by gas chromatography with carrier gas containing vaporous modifiers

Hiroshi Ito^{1,2*}, Takashi Sugiura³, Hidenori Nagai³, Atsushi Murase¹,
Shuji Kodama⁴, Yoshinori Inoue⁵, Atsushi Yamamoto³, Kazuichi Hayakawa²

¹ Toyota Central R&D Labs., Inc., 41-1, Nagakute, Nagakute-cho, Aichi 480-1192, Japan

² Graduate School of Natural Science and Technology, Kanazawa University, Kakuma-machi, Kanazawa-shi, Ishikawa 920-1192, Japan

³ College of Bioscience and Biotechnology, Chubu University, 1200, Matsumoto-cho, Kasugai-shi, Aichi 487-8501, Japan

⁴ Toyama Institute of Health, 17-1 Nakataikoyama, Imizu-shi, Toyama 939-0363, Japan

⁵ ChromaNik Technologies Inc., 6-3-1, Namiyoke, Minato-ku, Osaka 552-0001, Japan

Received for review December 12, 2006. Accepted December 28, 2006.

ABSTRACT

We have developed a novel generator that constantly introduces a vaporous modifier into the inert carrier gas. The effect of different modifiers on the retention times of analytes using a medium polar stationary phase and the generator was studied. When using formic acid as a modifier, the line of the van't Hoff plot of acetic acid was convexly curved. The inflection point was in the range of 100 to 120 °C, which corresponds to the boiling point of formic acid under pressurized conditions. The inflection point was found to be related to the association of formic acid.

Key words: vaporous modifier, introducer, polar analytes, medium polar stationary phase

Introduction

One of the problems of GC is the use of inert gases such as nitrogen and helium as a carrier gas. The separation in GC is only dependent on an affinity between the stationary phase and an analyte because the affinity of the inert gas may be disregarded. Consequently, the use of GC column with different polarity has been effective in improving the separation of the overlapped peaks.

In the 1970 s, many researchers have examined several organic and inorganic vapors in GC as the carrier gases aiming at diversity of manners in separation and extension of the GC applications.¹⁻⁷ The practical use of GC with modifier seemed to be not far ahead by various investigations in which the method of introducing the modifiers and the hyphenation to many detectors were included. In regard to the steam modifier, specially designed GC

instruments were commercially available which gave the effective results on the analyses of phenols produced in the manufacturing process of the coal liquefaction,⁸ and surfactants and additives in soap.⁹ But GC with steam modifier was deficient in flexibility and simplicity of the hardware and was left behind in the field of separation science that was making rapid progress. Unfortunately, a clear explanation for the separation mechanism in GC with the modifier has not been made. Clarifying the interaction between analytes, modifier and stationary phase during the separation process would be of concerned in the revival of GC with modifier. Therefore, we developed a novel generator of modifier which consists of a simple structure and seems to be applicable for capillary column GC.

In this paper we basically evaluate the prototype generator and

Correspondence: Hiroshi Ito, Organic Analysis Lab., Toyota Central R&D Labs., Inc., 41-1 Nagakute, Nagakute-cho, Aichi 480-1192, Japan
Fax: +81-561-63-6448

E-mail: hiroshi-ito@mosk.tytlabs.co.jp

investigate the elution behaviors of analytes by using various volatile liquids as modifiers. Moreover, we report the fundamental investigation on the behavior of formic acid modifier in the column which indicates the largest change in retention of organic acid analytes.

Experimental

Generator of modifier

A schematic diagram of newly developed generator of modifier is shown in Fig.1. It has a direct ventilation structure and the carrier gas enters into the humidification chamber from gas line saturated with modifier vapor. Quartz or acrylic fiber was used as the absorbent of the modifier and was covered by the punching metal plate. A Teflon® porous membrane film with a pore size of 1.2 μm set at the exit side prevented the contamination of the modifier particle. The generator has a duplex-tube structure and the aqueous solution of 20% ethylene glycol was circulated through the overcoat tube to keep the humidification chamber temperature constant. The amount of vaporous modifier generated was controlled by the dilution rate of the vapor with the carrier gas or by the temperature of the humidification chamber.

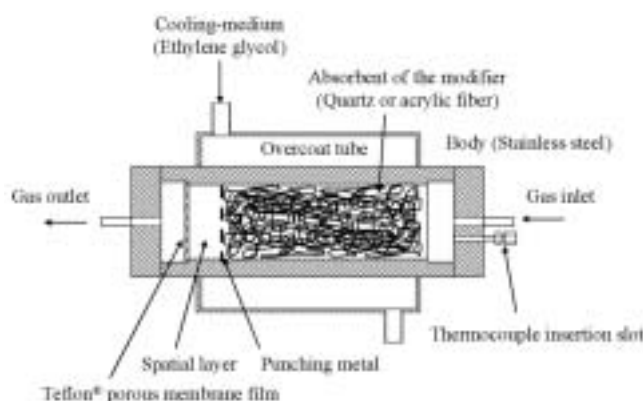


Figure 1. Schematic of the generator

Gas chromatography

The GC instrument used was a GL Sciences (Tokyo, Japan) GC 353 B equipped with a flame ionization detector (FID), which included the prototype generator of modifier. To prevent the condensation of modifier vapor, the carrier gas line between the injector and the generator was heated by a ribbon heater. Operating conditions were as follows: injector temperature, 280 $^{\circ}\text{C}$; detector temperature, 320 $^{\circ}\text{C}$; column temperature, isothermally 80–200 $^{\circ}\text{C}$; carrier gas flow rate, 55.8 mL/min. The calculation of the retention factor, k , was performed by using the column dead volume, V_0 , which was obtained from the signal of a thermal conductivity detector on injecting air.

The column packing material used here was Porapak R (poly

[vinylpyrrolidone–divinylbenzene], 50/80 mesh, Waters, Milford, MA, USA). It was washed with tetrahydrofuran and methanol, and then was heated to dryness in a vacuum prior to packing them into the stainless tubes (1/8 in. o.d. $\times$ 2.17 mm i.d. $\times$ 1 m).

Determination of the modifier concentration in carrier gas

The concentration of formic acid modifier in the carrier gas was determined by ion chromatography (761 Compact IC, Methrom Shibata, Tokyo, Japan) equipped with photometric detector (870–UV, Jasco, Hachioji, Japan). The absorbed solution used here was 3 mM KOH in which the carrier gas was bubbled for 3 min.

Reagents

Straight-chained (C2–C7) fatty acids, their methyl esters, and their 1-amino derivatives were purchased from Wako Pure Chemical (Osaka, Japan). Volumes of 2 μL of working solutions with concentration of 1 mg/mL of each compound were injected into the GC column. Other reagents were purchased from Wako.

Results and discussion

Evaluation of the generator of modifier

There are many methods for generating the vaporous modifiers such as natural or heating vaporization, bubbling, supersonic atomization, nebulization and so on. In this work we chose the method in which the vaporous modifier was vaporized in contact with carrier gas. The generator can be directly placed in the carrier gas line near the injector because its mechanical structure is simple.

Full contact between two phases is important for the constant supply of the vaporous modifier. The acrylic fiber is well-ventilated and exhibits an excellent property for the absorption and desorption of water. To prevent the leak of water drops from Teflon® porous membrane film, a spatial layer between the absorbent and the membrane film in the introducer was necessary. In regard to water supply, we have confirmed that the relative humidity in the carrier gas was constant more than 8 hr after introduction.¹⁰

Next, we monitored the concentration of formic acid in the carrier gas. When using organic compounds as a modifier, quartz fiber which is chemically and physically stable was substituted as an absorbent instead of for the acrylic fiber. The column packed with Porapak R was preconditioned at 120 $^{\circ}\text{C}$. The generator of modifier containing 0.3 g of formic acid was kept at 0 $^{\circ}\text{C}$ with the carrier gas flow of 50 mL He/min. The concentration of formic acid in 10 mL of absorbed solution was 450 ppm after 30 min, but decreased slowly and became constant at 360 ppm 1 hr later. Afterwards, it was approximately constant of about 360 ppm until 6 hr. After the introduction of modifier, the baseline of FID was very stable after 1 hr of introduction until 6 hr and the repeatability of

the GC analysis was satisfactory.

Effect of the modifier on the retention of analytes on Porapak R column

We investigated the effect of steam modifier on the retention of analytes. The non-polar packing material, Gaskropak 54 [poly (styrene/divinylbenzene)], was unstable to steam modifier.¹⁰ We chose the medium polar packing material, Porapak R, as the stationary phase of this study in considering of the application to analytes with wide range of polarities and boiling points.

The retention behavior of acetic acid on the Porapak R column was examined by introducing water, formic acid, lactic acid and propionic acid as modifiers. Figure 2 is van't Hoff plot when the temperature of generator was set at 40 °C except for formic acid of 20 °C. The retention behavior of acetate was not affected by water and was changed slightly by lactate and propionate modifiers. The change in retention time of acetate, however, became larger when using formic acid modifier as the column temperature decreased. In formic acid modifier, van't Hoff plot of acetic acid showed convex curve. The boiling point of formic acid calculated from the pressure at the column entrance (760 + 678 mmHg) is about 124 °C, and thus that in the column is in the region 100 to 124 °C. Curved lines of van't Hoff plot seem to be inflective in this region. It was estimated that formic acid modifier was promoted to self-associate below its boiling point and was adsorbed on a methacrylic acid site of the medium polar stationary phase, and as a result, the decrease of the stationary phase polarity caused the decrease in retention of the analyte.

To elucidate the change of retention behavior by formic acid modifier, the thermal dependence for the retention of formic acid on the Porapak R was examined. A van't Hoff plot of formic acid was curved, and the curved line seemed to be composed by two kinds of straight lines (Fig. 3). The temperature at the intersection of two regression lines was 107 °C, and it agreed with the boiling point of formic acid under pressurized state in the column. This result suggests that the distribution coefficient in the stationary phase was changed at the boiling point of formic acid. Larger the slope of the regression line, higher the content of modifier in the stationary phase depending on the hydrophilic interaction of formic acid. If the polar site of the stationary phase was masked by polar interaction with the modifier, the polarity of the stationary phase might be decreased, as a result, the retention of polar analytes also will rapidly decrease. This hypothesis explains the extreme decrease in retention of acetic acid observed in Fig. 2. On the other hand, the content of formic acid in the stationary phase above boiling point was smaller as shown in Fig 3., and the association characteristics of formic acid will be lost in this range.¹¹

We applied the separation technique with formic acid modifier

to the analysis of high boiling point fatty acids. Generally, fatty acids are prone to give large tailing peaks in the medium polar column. If formic acid introduced successively as a modifier was distributed in the medium polar stationary phase, the modifier can produce in the polar column and the retentions and peak shapes of high boiling point fatty acids change steeply. Figure 4. shows the chromatograms of such compounds with and without formic acid modifier at column temperature of 200 °C. As for the n-valeric acid (b.p.186 °C), the retention time became shorter (4.10 min) with the modifier. Moreover, it showed a clear peak. n-Caproic acid (b.p. 205 °C) and n-heptanoic (b.p. 174 °C) acids, which were strongly adsorbed on the polar stationary phase and not eluted without modifier, could be eluted at 8.08 min and 16.00 min.

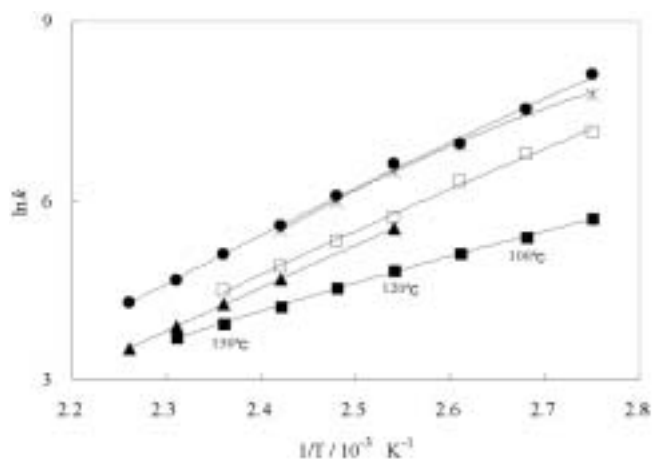


Figure 2. van't Hoff plot of acetic acid with various modifiers
Symbols: ● ; Without modifier,
With modifier: □ ; Lactic acid, ▲ ; Propionic acid,
■ ; Formic acid, * ; H₂O

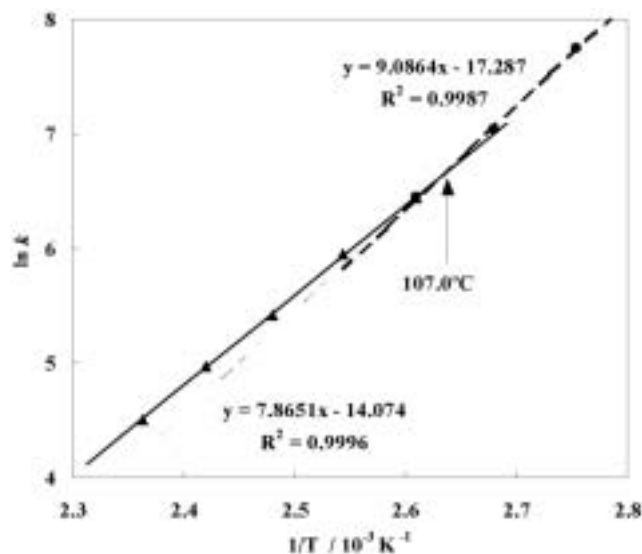


Figure 3. van't Hoff plot for formic acid modifier

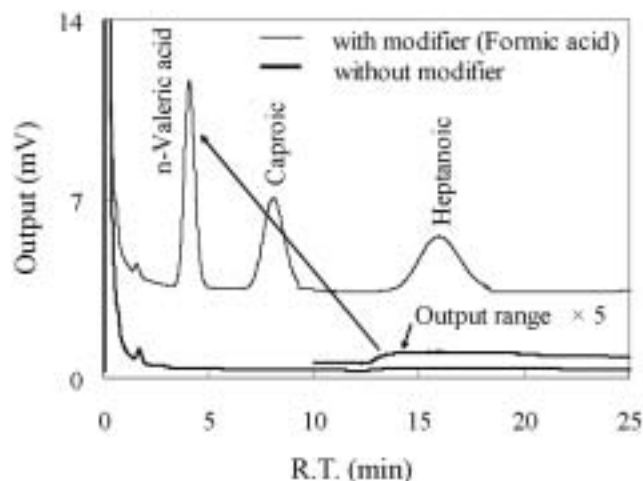


Figure 4. Separation of the high boiling point ingredient by modifier of formic acid

Conclusion

We developed a generator of vaporous modifier into the carrier gas of GC. This equipment was evaluated by applying some modifier compounds to the polar packed column. It was recognized that the elution of volatile acids was fastened by introducing the modifiers. Formic acid modifier changed the elution behavior of analytes in the neighborhood of the boiling point of the modifier. It was presumed that the phase of modifier adsorbed on the packing material changed at this temperature. Further examination of the effect of a modifier to the mobile phase on the retention mechanism will be necessary. However, it is suggested that the present approach might be a significant technique in changing the property of

a stationary phase on GC analysis. However, it was suggested that the presented approach could produce new stationary phases of GC column which were created by using various vaporous modifiers added to the inert carrier gas. Thus, any samples with various polarities might be analyzed by the unique column.

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