Original Paper

Ultralow-Temperature HPLC Using Low-Molecular-Weight Hydrocarbons as Mobile Phases

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Abstract

In ultralow-temperature HPLC, analyte retention is often enhanced, inhibiting elution. To solve this problem, we have investigated the use of low-molecular-weight hydrocarbons, methane and ethane, as the mobile phase in a monolithic ODS column. Analyte retention was successfully reduced by the use of these mobile phases, and elution of mono- and di-chloromethane and *n*-octane, which were not eluted in our previous work using a liquid nitrogen based mobile phase, was achieved. The analysis of octane structural isomers revealed that, in cryogenic HPLC, the retention of branched octanes was significantly reduced compared to the retention of *n*-octane, *i.e.*, the retention factor of *iso*-octane (2,2,4-trimethylpentane) was almost negligible. The retention factors of branched octanes were distributed between those of *n*-pentane and *n*-heptane in HPLC at -176°C , whereas, in gas chromatography at 50°C , these values were between those of *n*-heptane and *n*-octane.

Keywords: Ultralow-temperature HPLC; Octane structural isomers; Retention factor; Monolithic column; Low molecular weight hydrocarbons

1. Introduction

Temperature is one of the most critical parameters affecting physical and chemical properties of materials. Up to the present date, various methods to measure the wide variety of properties over broad temperature range, from ultralow to ultrahigh temperatures, have been developed. For the analysis of organic compounds at ultralow-temperatures, photo spectroscopy and mass spectroscopy have been employed in general. In infrared spectroscopy, measurements at or near the liquid nitrogen temperature (-196°C) have become common, and instruments for such measurements are commercially available. Moreover, supersonic jet spectrometry makes possible the measurement of optical spectra at ultralow-temperatures lower than a few tens K [1,2]. Kinetics at ultralow-temperature ($<25\text{ K}$) have been investigated using laser-induced fluorescence spectroscopy [3]. In time-of-flight secondary ion mass spectrometry (TOF-SIMS), equipment for low temperature measurements

($< -100^{\circ}\text{C}$) is commercially available. Interestingly, electrochemical reactions in the liquid phase at near liquid nitrogen temperature have been reported [4]. In the field of separation analysis, gas chromatography (GC) at cryogenic temperatures has been reported [5–8]. However, analyte for cryogenic GC is limited to permanent gases because strong adsorption prevents the elution of other compounds from the column.

Chromatography is not only useful for the separation of mixtures, it can also be used to study interactions between the stationary phase and compounds [9–11]. The development of chromatography under cryogenic conditions will allow to investigate interactions between compounds at ultralow-temperatures, and will contribute to understanding the chemistry at cryogenic conditions. Results of low-temperature HPLC down to -65°C have been reported, including analysis of thermally labile compounds [12–14], preparative separation of unstable compounds [15,16], selectivity enhancement in enantiomer

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separation [17,18], and the use of water ice as the stationary phase [19], in which organic solvents and their mixtures were used as non-freezing mobile phases. The use of liquid carbon dioxide as an alternative mobile phase for low-temperature HPLC has also been reported [20–23]. However, freezing makes these mobile phases unsuitable for cryogenic HPLC at near liquid nitrogen temperatures.

Recently, we have developed HPLC at near liquid nitrogen temperatures using pure liquid nitrogen as a mobile phase, and have achieved separation of methane, ethane, and propane, which were not eluted in cryogenic GC because of strong adsorption [24]. At ultralow-temperatures, the interaction between the analytes and the stationary phase was significantly enhanced. The addition of a low-molecular-weight alkane or alkene to the liquid nitrogen mobile phase was effective for promoting analyte elution, or reducing the interaction between the analyte and the stationary phase [24,25]. However, this type of mobile phase is not useful for eluting polar and high molecular weight compounds, where the interaction with the stationary phase is significantly high. The simplest solution to the problem of eluting highly retained-analytes, or analytes that interact strongly with the stationary phase, is to use a mobile phase of high elution power. As described above, low-molecular-weight alkanes and alkenes have the potential to enhance analyte elution compared to liquid nitrogen. Therefore, in this study, we have investigated ultralow-temperature HPLC using low-molecular-weight hydrocarbons as the mobile phase. The unique retention behavior of octane structural isomers in cryogenic HPLC is also reported.

2. Experimental

2.1. Apparatus and procedures

The apparatus used for cryogenic HPLC consisted basically of the same arrangement as that used in our previous work [25]: a gas cylinder, an injector (sample loop, 0.5 μ L, Model 7520, Rheodyne, Berkeley, CA, USA), a column (Chemcobond 5-ODS-H 1.0 $\times$ 150 mm (d.p. 5 μ m), Chemco, Osaka, Japan or MonoCap C18 Fast-flow 0.2 $\times$ 150 mm, GL Sciences, Tokyo, Japan), an ultralow-temperature bath, a ribbon heater for controlling the column temperature, a splitter with a resistance tube, and a quadrupole mass spectrometer (MS) equipped with an electron ionization source (GC-MS QP5050, Shimadzu, Kyoto, Japan).

The column was refrigerated by immersion in a liquid nitrogen bath and the column temperature was maintained at -176 to -177 $^{\circ}$ C by the ribbon heater surrounding the column. The mobile phase was supplied at a constant pressure of 1.0 to 2.0 MPa. The pressure at the outlet end of the column was maintained above 0.7 MPa by a resistance tube, to ensure that the mobile phase in the column

remained liquid. The MS was used in selected-ion monitoring (SIM) mode. All analytes were injected in their gaseous state using the valve injector at approximately 0.5 and 0.1 MPa for packed and monolithic columns, respectively. When vaporization of the analytes was insufficient, the analytes were heated by hot water. Argon was used for t_0 measurement. The elution time of each analyte was defined in the same manner as described in our previous study [24,25]. When the peak top time was not affected by the loading amount, this value was used as the elution time. However, when peak top time was reduced by the increase in loading amount, the peak end time (independent of loading amount) was used as the elution time (marked with an arrow in the figures). This reduction in peak top time will be caused by over loading of analytes.

GC measurement as a reference was performed using the cryogenic HPLC apparatus at room temperature, or a gas chromatograph equipped with a flame ionization detector (GC-17A, Shimadzu) with the ODS column.

2.2. Chemicals

Methane (>99.999%), a mixture of nitrogen and methane (N_2/CH_4 , 50/50 (v/v)), ethane (>99.7%), and ethylene (>99.9%) were purchased from Taiyo Nippon Sanso (Tokyo, Japan) and used as mobile phases. The analytes, dichloromethane, *n*-hexane, *n*-heptane, *n*-octane, 2-methylheptane, 4-methylheptane, 2,4-dimethylhexane, 3-methylheptane, 2,2,4-trimethylpentane (*iso*-octane), and 3-ethyl-3-methylpentane, were purchased from Wako Pure Chemical Industries (Tokyo, Japan), except for chloromethane (Sumitomo Seika Chemicals, Osaka, Japan). The standard gases of propane (99.5%) and propylene (99.5%) were purchased from GL Sciences.

3. Results and discussion

3.1. Elution power of liquid alkane mobile phase

In the previous research, it was found that retention of alkenes, based on interaction with π electrons, is significantly enhanced at liquid nitrogen temperatures compared to that of alkanes [25]. Propylene was therefore used as a probe analyte for evaluating the elution power of pure liquid methane and ethane. Since the melting points of methane and ethane are -182.5 and -183° C, respectively, at 0.1 MPa, a column temperature of approximately -176° C was employed for the study.

In our previous study at -196° C, propylene was not eluted from the packed ODS column with liquid N_2/CH_4 (50/50, v/v) as the mobile phase, and addition of 4% ethylene was necessary for its elution [25]. Figure 1A shows the relationship between ethylene content added to the N_2/CH_4 mobile phase and the retention factor, k , of propylene at approximately -176° C. The propylene retention factor for the N_2/CH_4 mobile phase without

Fig. 1. Difference in elution power for propylene in ultralow-temperature HPLC at approximately -176°C . (A) Effect of C_2H_4 content added to the N_2/CH_4 mixture. Estimated retention factor for propylene was approximately 4.9 without C_2H_4 addition. The chromatogram for propylene and argon (t_0) with mobile phases of (B) liquid methane and (C) liquid ethane. Column: packed ODS column (d.p. $5\ \mu\text{m}$). Flow: constant pressure mode at 1.0 MPa. Detection: SIM mode at $m/z = 42$ and 20 for propylene and argon, respectively.

ethylene was estimated *ca.* 4.9. The chromatograms for propylene at approximately -176°C are shown in Figs. 1B and 1C, in which pure methane and ethane, respectively,

Fig. 2. Analysis of propane and propylene using (A) packed and (B) monolithic ODS columns in ultralow-temperature HPLC at approximately -176°C . Mobile phase: liquid methane. Flow: constant pressure mode at (A) 1.0 MPa and (B) 2.0 MPa. Detection: SIM mode at $m/z = 44$, 42 and 20 for propane, propylene and argon, respectively.

were used as the mobile phase. Since the viscosity of liquid ethane was higher than that of liquid methane, the elution of argon (t_0 maker) was delayed with the liquid ethane mobile phase. Note that our system employed constant pressure mode for supplying a mobile phase to the column. In Fig. 1B, the fragment of propylene was also observed with a SIM monitoring at $m/z = 20$ for argon.

The retention factors of propylene using pure liquid methane and ethane were 1.3 and 0.0043, respectively. In Fig. 1C, the separation of argon and propylene was not observed due to the significantly small retention of propylene. Therefore, it was evident that the use of low-molecular-weight hydrocarbons is effective in suppressing the strong adsorption of analytes on the stationary phase in ultralow-temperature HPLC. With ethane as the mobile phase, the retention of propylene was close to zero, and the elution power of liquid ethane was approximately 300 times higher than that of liquid methane in this case.

In cryogenic HPLC, reduction in retention is essential for the analysis of highly retained analytes, such as polar

Fig. 3. Analysis of mono-/di-chloromethane using (A) liquid methane and (B) liquid ethane mobile phases in ultralow-temperature HPLC at -177°C . Column: monolithic ODS column. Flow: constant pressure mode at (A) 2.0 MPa and (B) 1.5 MPa. Detection: SIM mode at $m/z = 50, 49$ and 20 for monochloromethane, dichloromethane and argon, respectively.

compounds or compounds with a relatively large molecular weight. In general, the retention factor is affected by the column phase ratio, *i.e.*, $k = K(V_s/V_m)$, where K , V_s , and V_m are the equilibrium constant, volume of stationary phase, and volume of mobile phase, respectively. Therefore, a decrease in the phase ratio, $\phi = V_s/V_m$, can reduce analyte retention, and the use of a monolithic column was effective for this purpose, because the permeability of a monolithic column is higher than that of a packed column [26–28].

As shown in Fig. 2, the retention factors of propane in packed and monolithic ODS columns were 0.48 and 0.18, respectively, with a pure liquid methane mobile phase at approximately -176°C . That is, for propane, the retention in a monolithic column is approximately 1/2.7 times that in a packed column. The use of a permeable monolithic column was effective in enhancing analyte elution. In our previous study, it was found that the retention of alkanes in an ODS column was higher than that in a bare-silica column. Therefore, the amount of ODS functional groups, or carbon content, in the packed column would be higher than in the monolithic column.

Interestingly, for propylene, the retention factor in the monolithic column ($k = 1.3$) was the same as that in the packed column ($k = 1.3$), as shown in Fig. 2. This difference in behavior suggests that residual silanol groups, which significantly interact with π electrons [24], are more abundant in the monolithic column than in the packed column.

3.2. Analysis of chloromethane and dichloromethane

When chloromethane was analyzed in the packed ODS column with pure methane mobile phase at -177°C , no elution was observed. The analysis of chloromethane using the monolithic ODS column, in which successful elution was observed, is shown in Fig. 3A. However, the retention factor was significantly large ($k = 58$), and elution of dichloromethane was not observed. With a pure ethane mobile phase, the retention factor of chloromethane decreased to 4.7, as shown in Fig. 3B. For chloride compounds, the elution power of liquid ethane as the mobile phase was higher than that of liquid methane, similar to the case of propylene. However, the values of $\ln(k_{\text{inCH}_4}/k_{\text{inC}_2\text{H}_6})$ for propylene and chloromethane were 5.7 and 2.5, respectively. With the liquid ethane mobile phase, the elution of alkenes was more sensitive than that of chloride compounds. With the pure ethane mobile phase, dichloromethane was also eluted from the monolithic ODS column.

As noted above, the retention factor of propylene was almost constant in both the packed and monolithic columns owing to the residual silanol groups. The successful elution of chloromethane in the monolithic column, that is, the reduction in chloromethane retention, indicated that chloromethane interacted mainly with the ODS functional groups but not with silanol groups under the cryogenic conditions.

As shown in Fig. 3, elution time of chloromethane and dichloromethane were determined at the peak end time. This behavior indicated the overloading of analytes. In our previous study, we proposed the combined retention mechanism for ultralow-temperature HPLC, *i.e.*, adsorption exchange and pseudo-partition [25]. Since the overloading behavior was observed for highly retained analytes, it was estimated that the large retention would be provided by the adsorption exchange mode. The overloading behavior shown in Fig. 3B indicated that (1) the use of liquid ethane mobile phase was not effective to suppress the adsorption of mono- and di-chloromethane and (2) the slow desorption rate will result in the overloading behavior. As shown in Fig. 3B, magnitude of overloading of dichloromethane was superior to that of chloromethane. The desorption rate of dichloromethane would be significantly smaller than that of chloromethane.

3.3. Analysis of octane structural isomers

The use of liquid methane and ethane as mobile phases was effective in suppressing the retention of propylene and chloromethane. When the carbon number in an alkane increased, the retention factor of the alkane also increased. Therefore, with liquid nitrogen as the mobile phase, elution of relatively large alkanes, such as octane, was impossible. However, the use of liquid ethane as the mobile phase resulted in successful elution of *n*-hexane, *n*-heptane, and *n*-octane in the monolithic ODS column at -176°C , as shown in Fig. 4A. However, *n*-decane was not eluted under

Fig. 4. Analysis of alkanes in (A, B) ultralow-temperature HPLC at -176°C and (C) GC at 22°C . Analytes: (A) linear alkanes (C6–C8) and (B, C) *n*- and *iso*-octanes. Column: monolithic ODS column. Detection: SIM mode (the m/z value for each compound is indicated in the figure).

these conditions.

Figure 4B shows the chromatograms for *n*-octane and *iso*-octane (2,2,4-trimethylpentane). As shown in this figure, although *n*-octane showed moderate retention ($k = 6.9$), the retention of *iso*-octane was almost negligible. In GC at 22°C with He mobile phase, the retention factors of *n*- and *iso*-octanes were 314 and 94, respectively, as shown in Fig. 4C. That is, the retention of *iso*-octane was significantly suppressed in HPLC at -176°C compared with that in GC at 22°C .

Figure 5A shows the relationship between the carbon atom number and $\ln k$ value for alkanes in cryogenic HPLC. As shown here, the $\ln k$ value for linear alkanes was almost proportional to the carbon number (C5 to C8). This behavior is general in chromatography, and the same behavior was also observed in GC at 50°C (Fig. 5B). In both cryogenic HPLC and GC at 50°C , the retention factors of branched octanes were smaller than that of *n*-octane. However, the magnitude of the reduction in retention is significant in cryogenic HPLC. In GC measurements, the retention factors of branched octanes fell between the retention factors of *n*-heptane and *n*-octane. In contrast, the

Fig. 5. Difference in retention behavior for branched octane isomers between (A) ultralow temperature HPLC at -176°C and (B) GC at 50°C . Column: monolithic ODS column.

retentions of branched octanes were distributed between those of *n*-pentane and *n*-heptane in cryogenic HPLC. It is thus clear that selectivity for structural isomers is enhanced in cryogenic HPLC.

4. Conclusions

The use of a liquid hydrocarbon as the mobile phase, instead of one based on liquid nitrogen, was effective in reducing analyte retention in ultralow-temperature HPLC, and the elution power of ethane was higher than that of methane. In addition, the use of a highly permeable monolithic column reduced retention. The combination of a low-molecular-weight hydrocarbon as the mobile phase with a monolithic ODS column achieved elution of the highly retained analytes mono- and di-chloromethane and octanes. In the analysis of octane structural isomers, considerable enhancement in selectivity was observed, the retention factors for branched octanes in cryogenic HPLC being significantly reduced. However, detailed study is necessary to clarify the mechanism of the significant reduction in retention, and this will contribute to the investigation of the interactions under cryogenic conditions.

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