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Gas Chromatographic Separation of Isotopic Molecules Using a Cavitand-Impregnated Ionic Liquid Stationary Phase

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Cavitands, which are a class of compounds with deep open-ended cavities, are known to exhibit remarkable molecular recognition ability through host−guest interactions because of their unique structures. It is known that isotopic molecules can be differentiated in the small spaces provided by completely closed capsules in solution. To determine if this subtle molecular recognition ability extends to cavitands, we have developed a new method to facilitate the use of cavitands as stationary phases (SPs) in gas chromatography (GC). These involve a "pseudo heterogeneous two-phase system"; specifically, ionic liquids (ILs) were used as solvents to coat three cavitands with slightly different structures onto GC columns. When cavitand-coated columns were compared with columns coated with only ILs, it was clear that cavitands not only extended the utilization but also substantially improved separation efficiency of the columns. It was found that cavitand-coated columns can effectively separate many different types of isotopic compounds including aromatic hydrocarbons (mixture of chlorobenzene-h_5 and chlorobenzene-d_5, mixture of 1,2-dichlorobenzene-h_4 and 1,2-dichlorobenzene-d_4), alcohols (methanol from its corresponding methanol-d, d_3, d_4), ether, pyridine, and acetonitrile. The results also show that by modifying functional groups of the cavitand, from Et to $C_{11}H_{23}$ or from amide to carboxylate, we can fully retain the molecular recognition ability of the cavitand. The drastic changes in the polarity of the SP from medium polar to nonpolar, or to polar, greatly extends the applicability of these cavitand-coated SPs. Compared to other GC SPs that are known to separate isotopic molecules, these cavitand-coated SPs can separate a relatively wider range of isotopic compounds at relatively lower temperature, with shorter column length and higher efficiency.

We have synthesized a series of cavitands, that is, compounds with deep open-ended cavities and successfully demonstrated that these compounds, because of their unique structures, exhibit remarkable molecular recognition ability through host−guest interactions. Isotopic isomers can be differentiated by fully enclosed capsules,[1−3] but such molecular discrimination has not been observed for other type of host compounds including the widely studied calixarenes and cyclodextrins.[4−11] It is interesting to realize that the molecular recognition ability of calixarenes and cyclodextrins is not limited to solutions but rather extended to other phases as well. For example, both calixarenes and cyclodextrins can be used as stationary phases in gas and liquid chromatography for a variety of different types of separations including chiral separation.[4−10] They can also effectively serve as selective modifiers in capillary electrophoresis.[11] Considering that cavitands, compared to cyclodextrins, cover a larger fraction of a guest's surface, their use as stationary phase (SP) for chromatography could make it possible to perform separations which to date are not possible, for example, separation of isotopic isomers. Unfortunately, in spite of their potential, to date, application of cavitands in separation science is rather limited. It was quite recently that cavitands were found to provide substantial selectivity in the detection of aromatic compounds (benzene, chlorobenzene) by solid phase microextraction.[12] A variety of factors are responsible for the lack of application but the most likely one is due to limited solubility of cavitands which, in effect, makes it difficult to coat or to immobilize them onto a substrate or a column. It may be possible to synthetically modify cavitands to make them more soluble or covalently bind them to a solid

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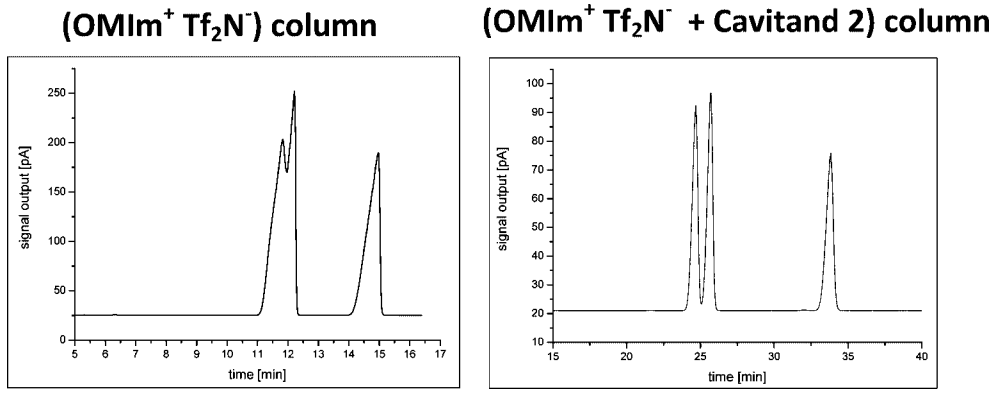
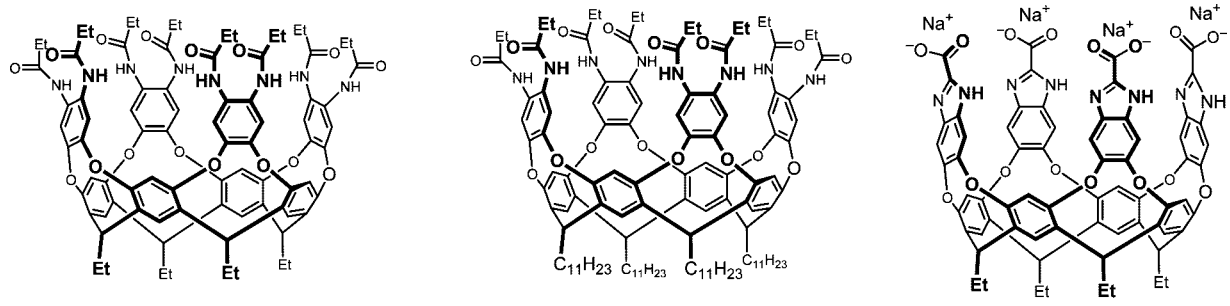
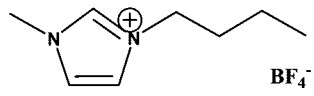


Figure 1. Chromatograms of mixture of *o*-, *m*- and *p*-xylene.

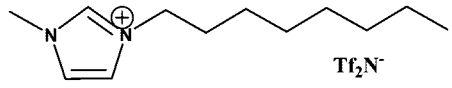
Scheme 1. Structures of Cavitands and ILs



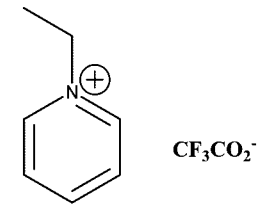
Cavitand 1 **Cavitand 2** **Cavitand 3**



1-butyl-3-methylimidazolium BF_4^-
$[BMIm]^+$ $[BF_4]$-



1-octyl-3-methylimidazolium Tf_2N^-
$[OMIm]^+$ $[Tf_2N]^-$



N-Ethylpyridinium $CF_3CO_2^-$
$[N\text{-}EtPy]^+$ $[CF_3CO_2]$-

support. In fact, in the solid phase microextraction application, the cavitands were covalently bound to sol−gel support. Because complicated and elaborate synthetic schemes are often needed for such immobilization process, they can only be performed by persons with extensive synthetic background and experience. Furthermore, there are always concerns that chemically modified cavitands may not retain the molecular recognition ability. It is therefore, desirable to develop a novel solvent which not only dissolves cavitands but also serves as coating solvent for the gas chromatography column. Room temperature ionic liquids (ILs) with their unique properties may provide the answer for this problem.

ILs are a group of organic salts that are liquid at room temperature. They have unique chemical and physical properties, including being air and moisture stable, a high solubility power, and ability to provide unique solute binding site(s) and virtually no vapor pressure.[13−25] Because of these properties, they can serve as a "green" recyclable alternative to the volatile organic

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Table 1. Resolution (R_s) Values

mixture	[BMIm$^+$ BF_4^-]	[BMIm$^+BF_4^-$] + **cavitand 1**	[OMIm$^+Tf_2N^-$]	[OMIm$^+Tf_2N^-$] + **cavitand 2**	[N-EtPy$^+CF_3COO^-$]	[N-EtPy$^+CF_3COO^-$] + **cavitand 3**
Methanol (D_4-H_4)	0.41 ± 0.04	0.450 ± 0.05				0.34 ± 0.01
Methanol (D_3-H_4)						0.34 ± 0.02
Methanol (D_1-H_4)	0.43 ± 0.01	0.41 ± 0.01				
Methanol (D_4-D_3)	0.42 ± 0.01	0.45 ± 0.02				
p-xylene, *m*-xylene			0.38 ± 0.01	0.80 ± 0.02		
m-xylene, *o*-xylene			2.71 ± 0.01	6.45 ± 0.02		
chlorobenzene (D_5-H_5)	0.19	0.50 ± 0.01				
1,2-dichlorobenzene (D_4-H_4)	0.214	0.42 ± 0.03				
pyridine (D_5-H_5)	0.61	0.76 ± 0.01	0.17	0.43 ± 0.01		0.69 ± 0.03
1,4-dioxane (D_8-H_8)	0.510	0.59 ± 0.01		0.47 ± 0.01		0.43 ± 0.02
acetonitrile (D_3-H_3)	0.23 ± 0.02	0.24 ± 0.01		0.34 ± 0.01		

Table 2. Selectivity (α) Factors

compound	[BMIm$^+$ BF_4^-]	[BMIm$^+BF_4^-$] + **cavitand 1**	[OMIm$^+Tf_2N^-$]	[OMIm$^+Tf_2N^-$] + **cavitand 2**	[N-EtPy$^+CF_3COO^-$]	[N-EtPy$^+CF_3COO^-$] + **cavitand 3**
Methanol (D_4-H_4)	1.04 ± 0.01	1.04 ± 0.01				1.04 ± 0.04
Methanol (D_3-H_4)						1.03 ± 0.01
Methanol (D_1-H_4)	1.038 ± 0.002	1.036 ± 0.001				
Methanol (D_4-D_3)	1.04 ± 0.01	1.04 ± 0.01				
p-xylene, *m*-xylene			1.038 ± 0.003	1.045 ± 0.001		
m-xylene, *o*-xylene			1.26 ± 0.01	1.34 ± 0.01		
chlorobenzene (D_5-H_5)	1.015	1.0058 ± 0.001				
1,2-dichlorobenzene (D_4-H_4)	1.006	1.004 ± 0.003				
pyridine (D_5-H_5)	1.010	1.005 ± 0.005	1.02	1.006 ± 0.001		1.014 ± 0.006
1,4-dioxane (D_8-H_8)	1.006	1.007 ± 0.002		1.006 ± 0.002		1.04 ± 0.02
acetonitrile (D_3-H_3)	1.022 ± 0.007	1.019 ± 0.003		1.024 ± 0.004		

compounds that are traditionally used as industrial solvents. The ILs have, in fact, been successfully used in many applications, including replacing traditional organic solvents in (1) organic and inorganic syntheses, (2) solvent extractions, (3) liquid−liquid extractions, (4) electrochemical reactions, and (5) as a medium for enzymatic reactions. Of particular interest are applications of ILs as stationary phase in GC.[25,26] ILs, because of their low vapor pressure, extremely high boiling point, and high solubility, can, potentially and uniquely, serve as either SP phase or solvent for SP. In fact, it was reported that ILs can be used as SP in GC for the separation of a variety of compounds.[25,26] Recently, we have successfully demonstrated that ILs can be used either by themselves as SP or as solvents to coat fullerenes (C_{60}, amino-fullerene and hydroxy-fullerene) onto GC columns.[27] Furthermore, ILs serve not just as coating solvents but rather synergistically with fullerenes to provide unique properties as stationary phases, namely, dual modal characteristics (i.e., they act as both nonpolar SP as well as polar SP). The polarity of the stationary phase can be adjusted by changing either the type of the ILs and/or by adding either C_{60} (or its amino- or hydroxy derivatives) into the ILs.[27]

The information presented is indeed provocative and clearly demonstrates that it is possible to dissolve cavitands in ILs and to use the cavitand-IL solutions as SP in GC. Such considerations prompted us to initiate this study, which aims to explore these possibilities by initially dissolving cavitands in ILs and coating the GC capillary column with cavitand-IL solutions. Effects of cavitands on gas chromatographic separations will be determined by comparing results obtained with cavitand-IL column to those found with a column coated with only IL. Additionally, results obtained with cavitands with slight difference in structure will be compared to elucidate the mechanism of chromatographic separation, as well as to gain insight into the molecular recognition ability of these unique host compounds as SP in GC.

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EXPERIMENTAL SECTION

Chemicals. 1,2 dichlorobenzene-d_4 (99%), chlorobenzene-d_5 (99%), ethanol-d_5 (98%), methanol-d_1 (99%), methanol-d_3 (99.5%), methanol-d_4 (99.8%), acetonitrile-d_3 (99.8%), pyridine-d_5 (99.5%), dioxane-d_8 (99%) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). Dioxane (99%), *o*-xylene (99%), *m*-xylene (99%) and pyridine (99.5+ %) were supplied by Alfa Aesar (Ward Hill, MA). All other chemicals were obtained form Sigma-Aldrich (Milwaukee, WI) and used as received.

Three ILs used in this study, 1-butyl-3-methylimidazolium tetrafluoroborate (BMIm$^+$ BF_4^-), octyl methyl imidazolium Tf_2N^- (OMIm$^+$ Tf_2N^-), and *N*-ethyl pyridinium trifluoro acetate (N-EtPy$^+$ $CF_3CO_2^-$) were prepared and characterized by ^{1}H NMR and IR as previously reported. The **cavitands 1**, **2**, and **3** (structures are shown in Scheme 1) were prepared using procedures reported previously.[1−3]

Dissolving Cavitands in ILs. As shown in Scheme 1, the three cavitands used in this work have similar structure but because of their polarity have very different solubility, namely, in the scale from lowest polarity to higher polarity is **cavitand 2**, **cavitand 1**, and **cavitand 3**. It is expected that they can be dissolved in ILs which have comparable polarity. As expected, it was found that **cavitand 2** is dissolved in OMIm$^+$ Tf_2N which is a relatively nonpolar IL; **cavitand 3** is soluble in a relatively polar IL such as N-EtPy$^+$ CF_3COO^-, and BMIm$^+$ BF_4^- which has medium polarity can dissolve **cavitand 1**.

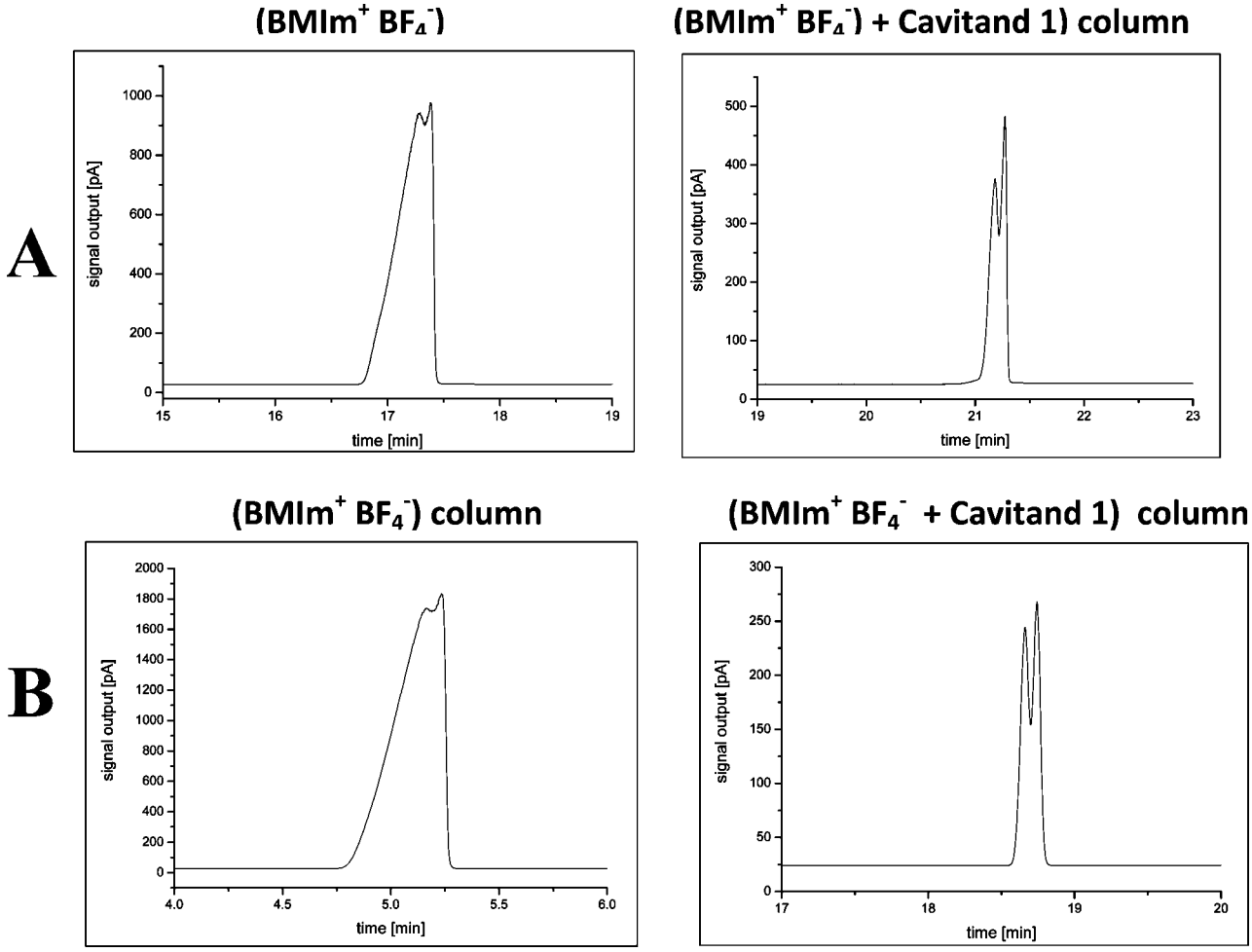

Figure 2. Chromatograms of (A) mixture of 1,2-dichlorobenzene-h_4 and 1,2- dichlorobenzene-d_4 and (B) mixture of chlorobenzene-h_5 and chlorobenzene-d_5.

In general, it was found that 1 mg of cavitand completely dissolved in 0.1 mL of IL in approximately 12 h.

Coating GC capillary columns. IL solutions of cavitands were coated onto GC capillary column (Supelco Corporation) using a static method similar to that previously reported.[27] Essentially, 25 mg of solution of **cavitand 1** in $BMIm^+$ BF_4^- (or **cavitand 2** in $OMIm^+$ Tf_2N^-, or **cavitand 3** in N-$EtPy^+$ CF_3COO^-) was dissolved in 10 mL of dichloromethane and coated onto a 10 m silica capillary tubing (Supelco Corporation) at 40 °C. This same general procedure was used to coat three other 10 m capillary columns with only the IL without any cavitand ($BMIm^+$ BF_4^- or $OMIm^+$ Tf_2N^- or N-$EtPy^+$ CF_3COO^-). Coated columns were flushed with dry nitrogen gas for 60 min, and then conditioned in a GC instrument via use of a temperature gradient (from 30 to 100 at 1 °C/min increment) and held overnight a the upper temperature.

All separations were carried out, in triplicate, on a Hewlett-Packard model 6890 gas chromatograph with nitrogen as a carrier gas, split injection (split ratio of 50:1) and flame ionization detection.

RESULTS AND DISCUSSION

Six 10 m columns (0.25 mm i.d. × 10 m long) were coated with either IL alone or IL plus each of three cavitands (1, 2, or 3) as the stationary phase. They were [$BMIm^+$ BF_4^-], [$BMIm^+$ BF_4^-] + **cavitand 1**, [$OMIm^+$ Tf_2N^-], [$OMIm^+$ Tf_2N^-] + **cavitand 2**, [N-$EtPy^+$ CF_3COO^-] and [N-$EtPy^+$ CF_3COO^-] + **cavitand 3**. The thickness of the coating film was calculated using the following equation[27]

$$d_f = \frac{dc}{400}$$

where d_f is the film thickness in μm, d is the i.d. of the column in μm, and c is the concentration of IL solution in the coating solvent (i.e., dichloromethane) in % (v/v) which in this work was 25 mg of IL in 10 mL of dichloromethane. It was found that all six columns have a similar coating thickness, that is, 0.129 μm, 0.129 μm, 0.116 μm, 0.116 μm, 0.120 and 0.120 μm for [$BMIm^+$ BF_4^-], [$BMIm^+$ BF_4^-] + **cavitand 1**, [$OMIm^+$ Tf_2N^-], [$OMIm^+$ Tf_2N^-] + **cavitand 2**, [N-$EtPy^+$ CF_3COO^-] and [N-$EtPy^+$ CF_3COO^-] + **cavitand 3**, respectively. Theoretical plate numbers of the columns were determined with naphthalene as solute at 100 °C and calculated using $N = 16\,(t_R/W)^2$, where t_R is the retention time of naphthalene and W is the base width of naphthalene peak. All six columns were found to have efficiencies in the range of $N = 21{,}000$ to 28,500 plates. Interestingly, it seems that adding any of the cavitand 1, 2, or 3 into the ILs slightly increases the efficiency of the columns by roughly 2,000 to 4,000 plates. These results clearly indicate that the ILs

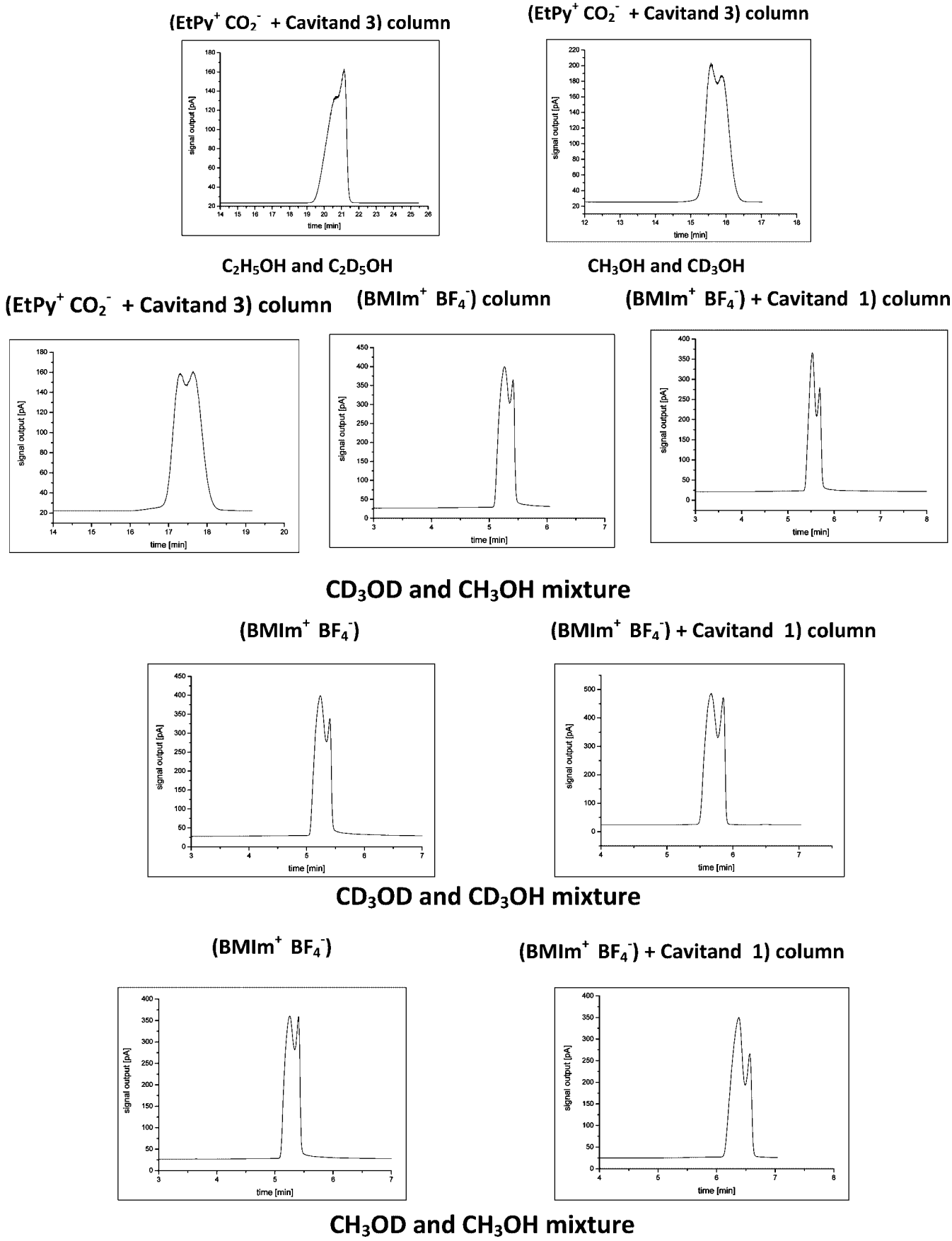

Figure 3. Chromatograms of mixtures of isotopic methanols.

can effectively serve as SPs in GC and that adding cavitands to the ILs further increases the efficiency of the SP.

As described in the introduction, these cavitands were found to exhibit remarkable molecular recognition in solutions;

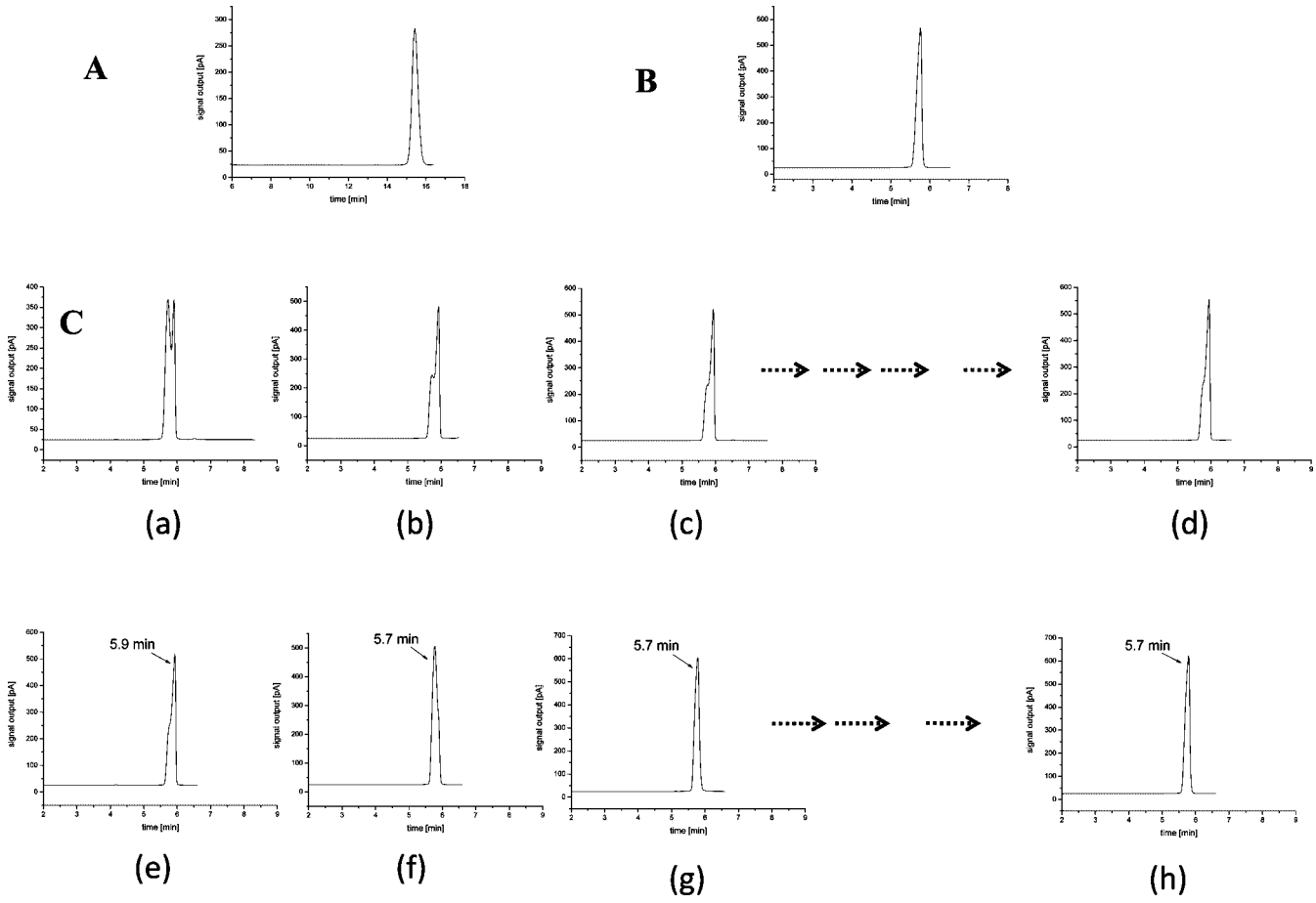

Figure 4. Chromatograms showing deuterium-hydrogen exchange reaction in [$BMIm^+$ BF_4^-] +**cavitand 1** column.

namely, they can distinguish molecules with a very small difference in their structures such as isotopic isomers. To determine if the discrimination ability of these cavitands is effective only in homogeneous liquid phase as we have observed previously or heterogeneously as a liquid stationary phase (SP) toward gaseous solutes as well, we investigated separation efficiencies on these cavitands columns for a variety of solutes which have only very small difference in their structures. These include a mixture of xylene isomers (*o*-, *m*- and *p*-xylene), a mixture of isotopic isomers of either methanol (methanol-h_4, methanol-d_4, methanol-d_3, methanol-d), ethanol (ethanol-h_5 and ethanol-d_5), chlorobenzene (chlorobenzene-h_5 and chlorobenzene-d_5), 1,2-dichlorobenzene (1,2-dichlorobenzene-h_4 and dichlorobenzene-d_4), pyridine (pyridine-h_5 and pyridine-d_5), dioxane (dioxane-h_8 and dioxane-d_8).

Separation of Aromatic Hydrocarbons. It was found that columns coated with either [$BMIm^+$ BF_4^-] or [N-$EtPy^+$ CF_3COO^-] can separate *o*-xylene from *m*- and *p*-xylene but they cannot separate *m*-xylene from *p*-xylene (chromatograms not shown). Adding **cavitand 1** to [$BMIm^+$ BF_4^-] does not provide any improvement, namely, this column can separate *o*-xylene from *m*- and *p*-xylene but cannot separate the latter two isomers; neither does adding **cavitand 3** to [N-$EtPy^+$ CF_3COO^-]. Better separations were found for column coated with either [$OMIm^+$ Tf_2N^-] alone or [$OMIm^+$ Tf_2N^-] with the **cavitand 2** (Figure 1A,B). Three xylene isomers were separated on these columns. However, while the column coated with [$OMIm^+$ Tf_2N^-] alone can separate *m*-xylene from *p*-xylene, they were not baseline separated (Figure 1A). Interestingly, adding **cavitand 2** into this IL column leads to substantial improvement in the separation efficiency: namely, *o*-, *m*-, and *p*-xylene were baseline separated (Figure 1B). While the improved efficiency can be visibly observed in the chromatograms (Figure 1A,B), it is more pronounced when compared in terms of resolution values (R_s) and selectivity factors (α) listed in Tables 1 and 2. As listed, adding **cavitand 2** to [$OMIm^+$ Tf_2N^-] led to improvement in both selectivity factors and resolution values. For example, compared to [$OMIm^+$ Tf_2N^-] column, adding **cavitand 2** to [$OMIm^+$ Tf_2N^-] increases the α value for *p*- and *m*-xylene mixture from 1.038 to 1.045 and from 1.26 to 1.34 for for *m*- and *o*-xylene. Pronounced enhancement, however, can be seen in terms of resolution values. Specifically, compared to column with [$OMIm^+$ Tf_2N^-] alone, adding **cavitand 2** to the IL led to 110% and 138% increase in the resolution for *p*- and *m*-xylene as well as for *m*- and *o*-xylene, (from R_s values of 0.38 and 2.71 (for [$OMIm^+$ Tf_2N^-] column) increase to 0.80 and 6.45, respectively for a column coated with [$OMIm^+$ Tf_2N^-] + **cavitand 2**).

Chromatogram of a mixture of 1,2-dichlorobenzene-h_4 and 1,2-dichlorobenzene-d_4 separated by column coated with [$BMIm^+$ BF_4^-] is shown in the left side of Figure 2A. Compared to other columns coated with other ILs ([$OMIm^+$ Tf_2N^-] and [N-$EtPy^+$ CF_3COO^-] either alone or with added cavitands) this column seems to be able to differentiate 1,2-dichlorobenzene from its corresponding deuterated compound in spite of the fact that the difference between them is very small. However, the

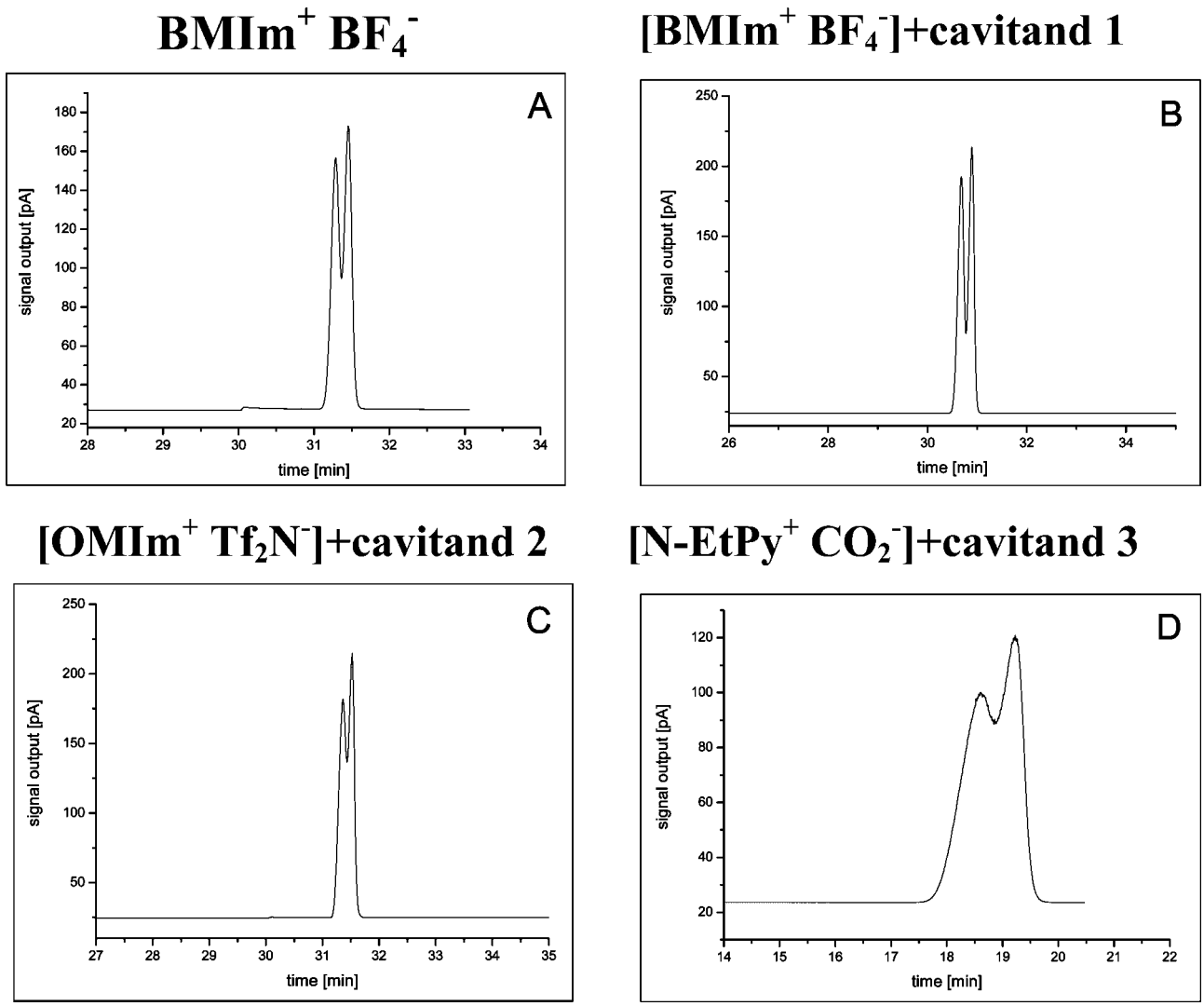

Figure 5. Chromatograms of mixture of dioxane-h_5 and dioxane-d_5.

separation is relatively poor as shown in the chromatogram and in terms of selectivity factor and resolution value (Tables 1 and 2). Isotopic recognition of the column was substantially improved when **cavitand 1** was added to the [$BMIm^+$ BF_4^-] IL. As evident from chromatogram shown in the right side of Figure 2A, the [$BMIm^+$ BF_4^-] + **cavitand 1** column provides substantial improvement in the separation efficiency as seen in the chromatogram and as listed for R_s values in Table 1.

Similar results were also observed for a mixture of chlorobenzene-h_5 and chlorobenzene-d_5, namely, only columns coated with either [$BMIm^+$ BF_4^-] alone or [$BMIm^+$ BF_4^-] + **cavitand 1** can separate them, and that compared to the column coated with [$BMIm^+$ BF_4^-] alone, adding **cavitand 1** substantially improves separation efficiency (see two chromatograms shown in Figure 2B).

Separation of Isotopic Mixtures of Ethanol and Methanol. Figure 3 shows chromatograms of isotopic mixtures of ethanol and methanol. It was found that columns coated with IL alone ([$BMIm^+$ BF_4^-], [$OMIm^+$ Tf_2N^-], and [N-EtPy^+ CF_3COO^-]) cannot separate a mixture of ethanol-h_5 from ethanol-d_5. When **cavitand 3** was added into the [N-EtPy^+ CF_3COO^-] the chromatogram obtained showed a broad peak with a broad shoulder (Figure 3A) indicating that this column can differentiate ethanol-h_5 from ethanol-d_5, but the recognition is not sufficient to lead to effective separation of these two isotopic isomers. (No separation, even for a broadband with broad shoulder, can be obtained by adding **cavitands 1** and **2** into the [$BMIm^+$ BF_4^-] and [$OMIm^+$ Tf_2N^-] ILs, respectively).

Interestingly, the [N-EtPy^+ CF_3COO^-] + **cavitand 3** column was also found to exhibit some separation, but not baseline separated, for a mixture of methanol-h_4 and methanol-d_3 (second chromatogram of Figure 3A) and methanol-h_4 and methanol-d_4 (first chromatogram of Figure 3B). These two methanols mixtures cannot be separated by columns coated with either [$OMIm^+$ Tf_2N^-] or [N-EtPy^+ CF_3COO^-]. Interestingly, the mixture of CD_3OD and CH_3OH can also be separated by columns coated either with [$BMIm^+$ BF_4^-] alone or with [$BMIm^+$ BF_4^-] + **cavitand 1** (Figure 3B second and third chromatograms). Careful inspection of the three chromatograms shown in Figure 3B reveals that while the [N-EtPy^+ CF_3COO^-] + **cavitand 3** column separated the mixture of CD_3OD and CH_3OH into two equal intensity (and area) bands, chromatograms obtained by either [$BMIm^+$ BF_4^-] or [$BMIm^+$ BF_4^-] + **cavitand 1** column contain not two equal bands but rather two

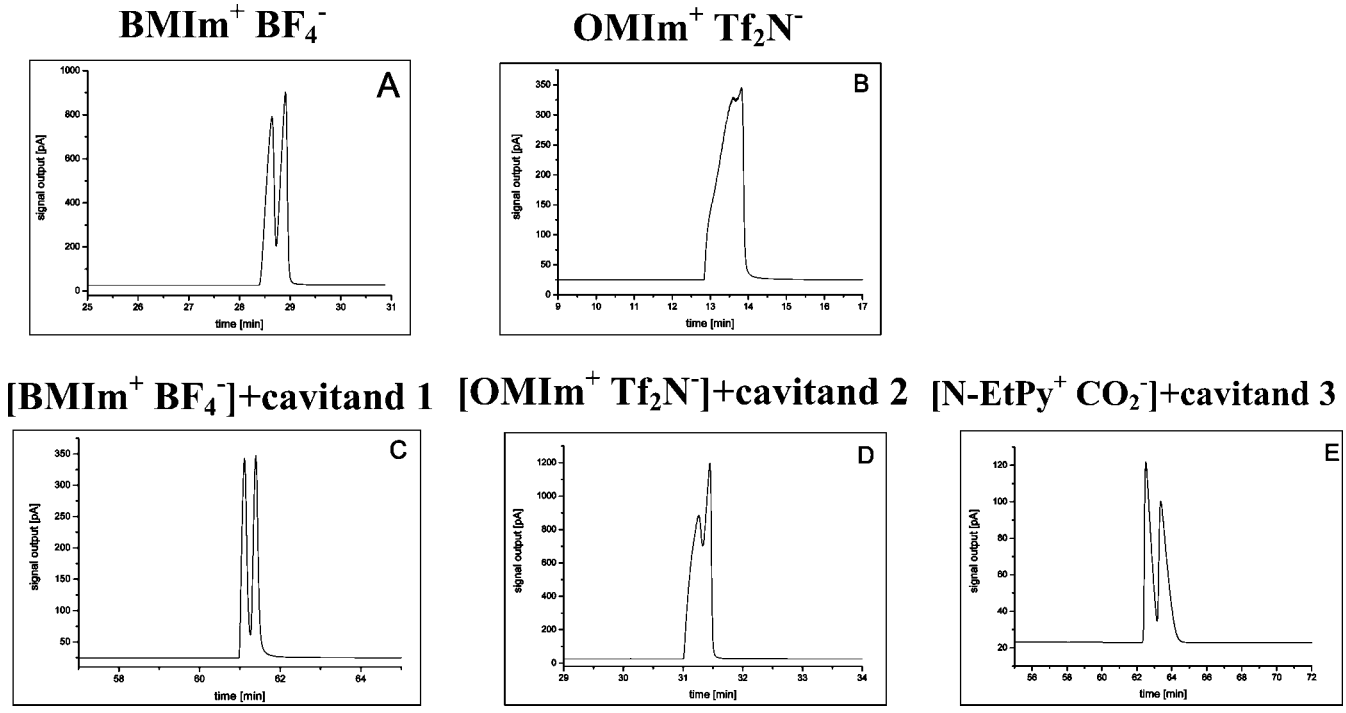

Figure 6. Chromatograms of mixture of pyridine-h_5 and pyridine-d_5.

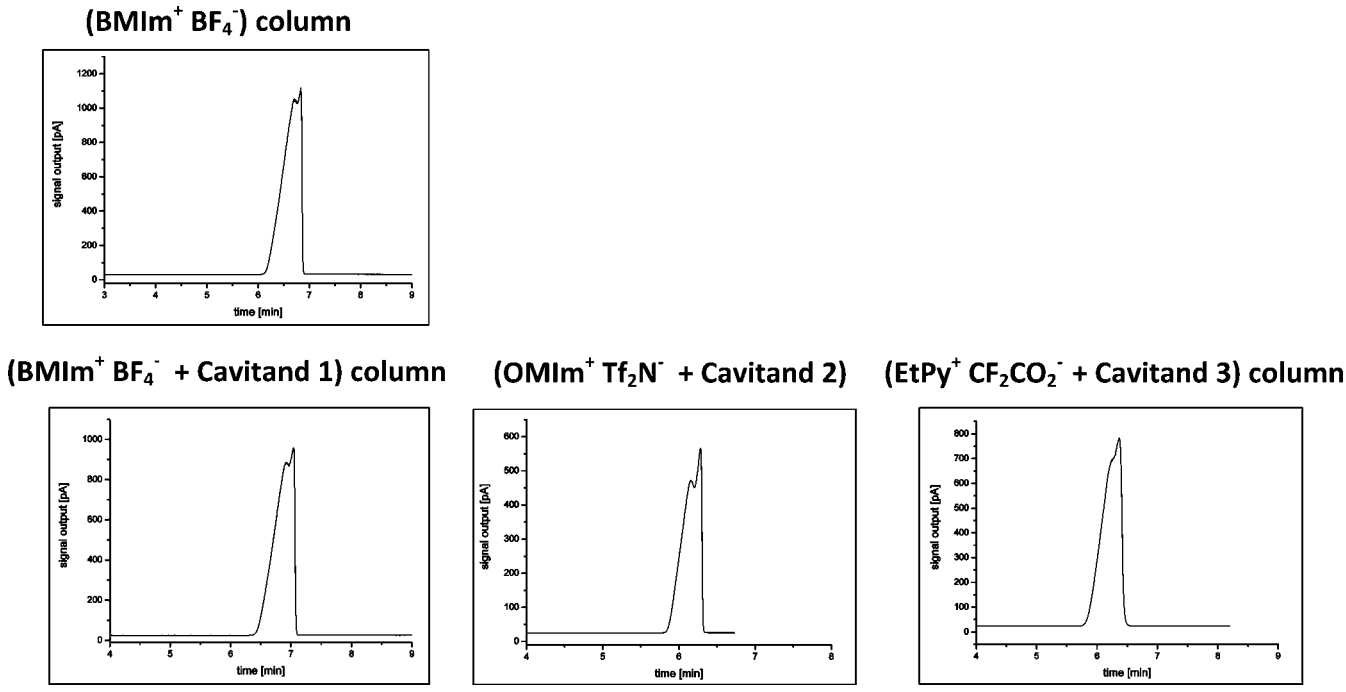

Figure 7. Chromatograms of mixture of acetonitril-h_3 and acetonitrile-d_3.

bands with approximately ~2.5:1 and ~2.2:1 ratio, respectively. In fact, these two columns ([BMIm$^+$ BF$_4^-$] and [BMIm$^+$ BF$_4^-$] + **cavitand 1**) can separate other isotopic mixtures of methanols including mixture of either CD_3OD and CD_3OH, and CH_3OD and CH_3OH (Figure 3C,D) which cannot be separated by other four columns. Again, while rather than two bands with 1:1 ratio as expected, chromatograms obtained by these two columns in all cases contain two bands with approximately ~2.5:1 ratio.

Results obtained seem to indicate that different from other ILs and **cavitand 2** and **3**, in this case for isotopic mixtures of methanol, adding **cavitand 1** into [BMIm$^+$ BF$_4^-$] does not provide any observable difference in the separation in terms of chromatograms, selectivity factors, and resolution values (Tables 1 and 2). Furthermore, it is of particular interest to observe that the two SPs ([BMIm$^+$ BF$_4^-$] or [BMIm$^+$ BF$_4^-$] + **cavitand 1** and [N-EtPy$^+$ CF$_3$COO$^-$] + **cavitand 3**) used in the study have different chromatographic properties, that is, the former can separate only compounds that have a different isotope at the hydroxyl group (CH_3OH and CD_3OD, CH_3OD and CD_3OH or CH_3OD and CH_3OH) while the latter can separate compounds that have a different isotope at the methyl

group and/or hydroxy group (CH_3OH and CD_3OH and CH_3OH and CD_3OD). Furthermore, areas of two bands separated with [$BMIm^+$ BF_4^-] and [$BMIm^+$ BF_4^-] + **cavitand 1** column are in ~2.5:1 ratio, whereas [N-$EtPy^+$ CF_3COO^-] + **cavitand 3** column provides about 1:1 area ratio separation (all mixtures were made in 1:1 v/v ratio).

Additional experiments were then carried out to gain insight into this intriguing ~2:1 ratio of two bands by [$BMIm^+$ BF_4^-] and [$BMIm^+$ BF_4^-] + **cavitand 1** columns. We found that when we injected only CD_3OH into [$BMIm^+$ BF_4^-] + **cavitand 1** column, as expected, there was only one band eluted out with a retention of 5.7 min (Figure 4B). However, when CD_3OD was injected into this column, two overlapped bands eluted out with retention times of 5.7 and 5.9 min, and the area of the first band is about twice that of the second band (Figure 4C(a)). This is rather interesting considering that there were two bands eluted out when only CD_3OD was injected. Furthermore, if CD_3OD was repeatedly injected into the column, the first band decreased concomitantly with the increase of the second band. Figure 5C shows chromatograms obtained for first, second, third up to 11th injection (Figure 4C(a to d)). As shown in Figure 4C(d), for the 11th injection, the chromatogram shows a main band at 5.9 min with a broad shoulder at about 5.7 min. If CD_3OH was then injected into the column, rather than seeing one eluted band as shown in Figure 4B, the chromatogram (Figure 4C(e)) has a single broadband with a retention of 5.9 min and a small shoulder at shorter retention time. If CD_3OH was then repeatedly injected (as shown in Figure 4C(f), 4C(g) and 4C(h) for second, third and sixth injection, respectively), the eluted band increasingly becomes narrower and shifts toward shorter retention time. In fact, the chromatogram obtained with the sixth injection (Figure 4C(h)) is the same as that shown in Figure 4B, namely, it contains a single narrow band with retention time of 5.7 min.

In contrast to the [$BMIm^+$ BF_4^-] + **cavitand 1** column, only one single band was found when either CD_3OH or CD_3OD were injected into [N-$EtPy^+$ CF_3COO^-] + **cavitand 3**. Figure 4A shows the chromatogram obtained when CD_3OD was injected into [N-$EtPy^+$ CF_3COO^-] + **cavitand 3** column.

Taken together, these results seem to suggest that when CD_3OD and other alcohol-OD is injected into the [$BMIm^+$ BF_4^-] + **cavitand 1** (or [$BMIm^+$ BF_4^-] column), the -OD will undergo deuterium-hydrogen exchange with the SP to convert to -OH resulting in a chromatogram with two bands (-OH (first band) and -OD (second band)). Continuous injection of CD_3OD into the column increases deuterium-hydrogen exchange and leads to increasing concentration of deuterium in the column. As a consequence, the first band (-OH band) of the chromatogram decreases while the second -OD band increases. After the 10th injection, there was not much -OH remaining in the SP to offer exchange with -OD of the analyte. As a consequence, only a broad -OD band is seen in the chromatogram.

It is noteworthy to add that because of deuterium-hydrogen exchange between the -OD group of methanol-d_4 and the -OH group of methanol-h_4, a mixture of CD_3OD and CH_3OH should contain four isomers: CD_3OD, CD_3OH, CH_3OH, and CH_3OD. However, as shown in the second and third chromatogram of Figure 3b, instead of four separated bands, only two bands were observed when this mixture was injected into the [$BMIm^+$ BF_4^-] or the [$BMIm^+$ BF_4^-] + **cavitand 1** column. This is because it was found that while [$BMIm^+$ BF_4^-] column and [$BMIm^+$ BF_4^-] + **cavitand 1** column can separate CD_3OD from CD_3OH (Figure 3C) and CH_3OD from CH_3OH (Figure 3D), they cannot separate CD_3OD from CH_3OD, and CD_3OH from CH_3OH (chromatograms not shown). As a consequence, only two bands were observed when a mixture of CD_3OD and CH_3OH was injected into these columns.

On the basis of the structure of the cavitand and IL shown in Scheme 1, it is possible that the deuterium-hydrogen exchange is between the -OD of the alcohol and the -NH of the amide group of the **cavitand 1** and/or the acidic proton of the -CH group between two nitrogens of the imidazolium ring of [$BMIm^+$ BF_4^-] IL. Our assumption on the involvement of the proton of the C−H group between two nitrogens of the imidazolium ring in the deuterium exchange reaction is based on earlier studies[33] which show that the pK_a of this proton is low (22.05 ± 0.03) and comparable to that of the alpha proton of ketone, and the ketone's alpha proton is known to undergo proton-deuterium exchange reaction. More importantly, it was also reported that the proton of this imidazolium ring's C−H group readily undergoes deuterium-hydrogen exchange reaction with a half-life of 4.5 min.[34] However, this deuterium-hydrogen exchange between analyte and SP is seen only with [$BMIm^+$ BF_4^-] and [$BMIm^+$ BF_4^-] + **cavitand 1** columns and not with N-$EtPy^+$ CF_3COO^-] + **cavitand 3**. This is hardly surprising considering that [N-$EtPy^+$ CF_3COO^-] and **cavitand 3** do not have any group available for deuterium-hydrogen exchange. Because of the similarity in the structure of **cavitand 1** and **2**, and of [$BMIm^+$ BF_4^-] and [$OMIm^+$ Tf_2N^-] (Scheme 1), it is expected that **cavitand 2** and ([$OMIm^+$ Tf_2N^-] SP may also be able to provide deuterium-hydrogen exchange for -OD group of alcohols. However, because either [$OMIm^+$ Tf_2N^-] or [$OMIm^+$ Tf_2N^-] + **cavitand 2** columns cannot separate any isotopic mixtures of either ethanol or methanol at all, even if the -OD group undergoes deuterium exchange in these columns, it is not possible to detect as both alcohol-OH and alcohol-OD will elute together as one chromatographic band through this column. It is not expected that [N-$EtPy^+$ CF_3COO^-] or **cavitand 3** are involved in any hydrogen−deuterium exchange reaction because of lack of a suitable proton.

Separation of Isotopic Mixture of Dioxane and Pyridine. Panels A and B of Figure 5 are chromatograms of the isotopic mixture of 1,4-dioxane (dioxane-h_8 and dioxane-d_8) separated on [$BMIm^+$ BF_4^-] and [$BMIm^+$ BF_4^-]+**cavitand 1** column, respectively. It seems from these chromatograms that adding **cavitand 1** into [$BMIm^+$ BF_4^-] does not provide substantial improvement in the separation. However, according to the resolution values listed in Table 1, **cavitand 1** improves resolution of the [$BMIm^+$ BF_4^-] column by 16% (R_s value increased from 0.51 to 0.59 for [$BMIm^+$ BF_4^-] and [$BMIm^+$ BF_4^-] +**cavitand 1** column, respectively). Panels C and D of

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Figure 5 also show separations of this mixture by $OMIm^+$ Tf_2N^-]+**cavitand 2** and [N-$EtPy^+CF_3COO^-$] +**cavitand 3** column, respectively. While the separations by these two columns are not as good as that by [$BMIm^+$ BF_4^-] and [$BMIm^+$ BF_4^-] + **cavitand 1** columns (in terms of R_s values), it is significant because without adding **cavitand 2** or **cavitand 3**, columns coated with only [$OMIm^+$ Tf_2N^-] and [N-$EtPy^+$ CF_3COO^-] IL cannot separate this dioxane mixture at all (chromatograms not shown).

All three cavitands were found to provide significant and positive effects on the separation of isotopic mixtures of pyridine (pyridine-h_5 and pyridine-d_5). As shown in Figure 6A,B, among three columns coated only with ILs, the [$BMIm^+$ BF_4^-] column provides the best relative separation (R_s value is 0.61; Figure 6A), [$OMIm^+$ Tf_2N^-] column offers a very slight possibility (R_s value is 0.17, Figure 6B) while the [N-$EtPy^+$ CF_3COO^-] column cannot separate the mixture at all (chromatogram not shown). Adding either **cavitand 1**, **2**, or **3**, respectively, into these columns substantially improves the separation. The significant and positive effect of cavitands is evident by comparing Figure 6A and 6C (for [$BMIm^+$ BF_4^-] and [$BMIm^+$ BF_4^-] +**cavitand 1** column), 6B and 6D ([$OMIm^+$ Tf_2N^-] and [$OMIm^+$ Tf_2N^-]+**cavitand 2**) column, 6E (for [N-$EtPy^+$ CF_3COO^-] +**cavitand 3** column). In fact, **cavitand 1** and **cavitand 2** were found to increase the resolution value of [$BMIm^+$ BF_4^-] and [$OMIm^+$ Tf_2N^-] column by 24.5% and 153%, respectively (from 0.61 to 0.76 and from 0.17 to 0.43).

Separation of Isotopic Mixture of Acetonitrile. Chromatograms of an isotopic mixture of acetonitrile (CH_3CN and CD_3CN) are shown in Figure 7A–D. Among three columns coated with only ILs, [$OMIm^+$ Tf_2N^-] and [N-$EtPy^+$ CF_3COO^-] column cannot separate the mixture at all. Columns coated with [$BMIm^+$ BF_4^-] provided slight separation with R_s value 0.23 (Table 2, Figure 7A). Addition of **cavitand 2** and **cavitand 3** to [$OMIm^+$ Tf_2N^-] and [N-$EtPy^+$ CF_3COO^-], respectively, resulted in improved separation efficiency (Figure 7C,D). However, adding **cavitand 1** into [$BMIm^+$ BF_4^-] does not seem to provide any enhanced separation, namely, chromatograms 7A and 7B, and the resolution values of 0.23 and 0.24 suggest no significant difference between these two SPs.

CONCLUSIONS

In summary, by simply using ILs as a solvent to coat cavitands onto a GC column we have successfully shown, for the first time, that the remarkable molecular recognition ability of these host compounds is not limited to homogeneous solutions but can be extended to a "pseudo heterogenous two-phase system". The cavitands, which are solubilized in a thin film of IL, can recognize many different types of analytes, not in the solution but rather in the gas phase. It seems that the gaseous analytes repeatedly diffused into the thin film of the cavitand solution (i.e., SP of the GC column) as they eluted through the column. Cavitands exert molecular recognition toward analytes after the latter have diffused into the solution. Since analytes stay in the cavitand solution for only a very short time as they repeatedly diffuse in and out of the solution as they migrate through the column, the molecular recognition ability of the cavitands toward analytes is quite strong and manifested as the analytes migrate through the column. The result were two different retention times for analytes (and hence baseline separation) which have only very minor differences in their structure such as hydrogen(s) versus deuterium(s).

Carefully comparing results obtained with the [$BMIm^+$ BF_4^-] column with the [$BMIm^+$ BF_4^-] + **cavitand 1** column reveals that for all separations, even though chromatographic efficiencies for all separations are relatively similar for both columns, the [$BMIm^+$ BF_4^-] + **cavitand 1** column often provided slightly higher efficiency. These results seem to suggest that **cavitand 1** probably forms inclusion complexes with the imidazolium ring of [$BMIm^+$ BF_4^-]. Such inclusion complex formation would make it difficult to separate the effect on separation provided by the **cavitand 1** from that of [$BMIm^+$ BF_4^-]. In fact, this is not the first time that the imidazolium of [$BMIm^+$ BF_4^-] IL is known to form inclusion complexes with a host compound. In our earlier studies on the interaction between phenol and cyclodextrins (CDs) in [$BMIm^+$ BF_4^-] IL, we found that rather than forming inclusion complexes with CDs, phenol can only adsorb onto the surface of CDs.[35,36] This is because the imidazolium ring of IL forms inclusion complexes with cyclodextrins thereby blocking phenol from entering it.[35,36] Other workers have also found that imidazolium ring can form inclusion complexes with CDs and other host compounds such as calixarenes.[37−40] However, because the open-ended cavitands are structurally deeper compared with that of cyclodextrins and calixarenes, it is expected that even if the cavity of the **cavitand 1** is occupied by the imidazolium ring of $BMIm^+$ the cavitands can still exert their own effect particularly for aromatic compounds because of their deep structural characteristics. That is, it is expected that [$BMIm^+$ BF_4^-] + **cavitand 1** column should have the combined advantages of [$BMIm^+$ BF_4^-] and **cavitand 1**. Those were, in fact, observed here, namely, for the same set of aromatic analytes, [$BMIm^+BF_4^-$] + **cavitand 1** column exhibits relatively better separation efficiency than the [$BMIm^+$ BF_4^-] column. Compared, for example, for the separation of a mixture of chlorobenzene-h_5 and chlorobenzene-d_5 (or a mixture of 1,2-dichlorobenzene-h_4 and 1,2-dichlorobenzene-d_4) the resolution found for the [$BMIm^+BF_4^-$] + **cavitand 1** column was 0.50 which is 2.6 fold better than that by the [$BMIm^+$ BF_4^-] column [or 2 fold better (R_s= 0.42 and 0.21) for the 1,2-dichlorobenzene mixture].

Because of rather large differences in separation efficiencies between the [$OMIm^+$ Tf_2N^-] column and the [$OMIm^+$ Tf_2N^-] + **cavitand 2** column, and between the [N-$EtPy^+$ CF_3COO^-] column and the [N-$EtPy^+$ CF_3COO^-] +**cavitand 3** column, it is not expected that either $OMIm^+$ ring or $EtPy^+$ ring can form inclusion complexes with **cavitand 2** or **cavitand 3**, respectively. This is as expected considering the steric hindrance of the octyl group on the $OMIm^+$ ring (which prevents it from entering cavity of **cavitand 2**) and the highly polar nature of the $EtPy^+$ ring (which makes it difficult to be included in the cavity of **cavitand 3** which is known to be highly hydrophobic).

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The results obtained indicate that by simply modifying the functional group of the cavitand from -Et (**cavitand 1**) to $C_{11}H_{23}$ (**cavitand 2**) or from -amide (**cavitand 1**) to carboxylate (**cavitand 3**), we can fully retain the molecular recognition ability of the cavitand while drastically changing the polarity of the SP from medium polar ([$BMIm^+$ BF_4^-] +**cavitand 1**) to nonpolar ([$OMIm^+$ Tf_2N^-] + **cavitand 2**), or to polar ([N-$EtPy^+$ CF_3COO^-] +**cavitand 3**). This extends the applicability of these cavitands SP to the separation of isotopomers of many different types of compounds ranging from aromatic hydrocarbons, alcohols, to dioxane, pyridine, and acetonitrile. A large number of different compounds were successfully separated with only three cavitands, and since many different cavitands can be synthesized (or modified from currently existing ones) it can be expected that isotopic isomers of many compounds can, in principle, be separated by judiciously selecting a cavitand column. It is true that in some cases baseline separation cannot be achieved. However, it should be remembered that all separations reported in this study were based on the use of columns of only 10 m long. It is, therefore, expected that better separation (baseline separation) can be achieved by increasing the length of the columns and/or use of recycling chromatographic techniques.

Since the columns used in this work were prepared by coating ILs with or without cavitands onto the capillary wall of the columns, there is a concern that the coated SP may leach out. However, we found that retention time and separation efficiency of the columns remain the same even after more than a year and after hundreds of injections. For example, the [$BMIm^+$ BF_4^-] +**cavitand 1** column separated a mixture of pyridine-h_5 and pyridine-d_5 on August 2007 with a retention time (for pyridine-h_5) of 33 min and a resolution R_s of 0.690 ± 0.003. More than 1 year later (on December 2008), it has, within experimental error, the same retention time and resolution for the same mixture (t_r and R_s are 31 min and 0.77 ± 0.06).

This is not the first observation that isotopic molecules can be separated by GC. Several GC capillary columns were reported to provide separation of isotopic molecules.[28−32] For example, using a commercial DB-5HT column (which according to the manufacturer is similar to the DB-5 column used in the reported paper but can operate at relatively higher temperature) which is reported to be able to separate isotopic aromatic molecules, we found that for isotopic compounds such as a mixture of either chlorobenzene-h_5 and chlorobenzene-d_5, or 1,2-dichlorobenzene-h_4 and 1,2-dichlorobenzene-d_4, even at 30 m long, this column has relatively inferior separation efficiency (R_s = 0.38 and 0.16) compared to a 10 m long [$BMIm^+$ BF_4^-] +**cavitand 1** column (R_s = 0.50 and 0.42). More importantly, none of polar isotopic molecules (isotopic alcohols or acetonitrile), which were well separated by the [$BMIm^+$ BF_4^-] +**cavitand 1** column, can be separated by this DB-5T column. In fact, all of reported columns cannot separate many different types of isotopic molecules (aromatic, alcohols, ether, acetonitrile) as reported here by cavitands columns. Rather, they can only separate a certain class of molecules (e.g., either aromatic molecules or aliphatic hydrocarbons) and at relatively higher temperature and longer retention time.[28−32] Furthermore, to provide comparable separation efficiency as those obtained with the cavitands columns, much longer length must be used for these columns (i.e., at least from 30 to 60 m long compared to 10 m of cavitand columns).[28−32]

While the deuterium-hydrogen exchange behavior of the [$BMIm^+$ BF_4^-] + **cavitand 1** column may complicate the chromatographic separation process, it can potentially provides a novel means for simple, inexpensive, and rapid (deuterium) synthesis or enrichment of deuterated compounds which traditionally are either difficult and/or expensive to produce. This can, for instance, be accomplished by initially and repeatedly injecting inexpensive alcohol-OD into the [$BMIm^+$ BF_4^-]+**cavitand 1** columns to completely convert all protons available for deuterium-hydrogen exchange sites in the column to deuterium (this can be monitored as chromatogram changes from two bands eluted to a single band). The desired alcohol-OH is then injected into the column. It is expected that only a few percent of -OH of this compound will be converted to -OD by the column with this injection. However, if the cycle is repeated many times (which can be automated and controlled by a microprocessor), the desired alcohol with high concentration of -OD can be obtained.

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