Immobilized 1,3-Dialkylimidazolium Salts as New Interface in HPLC Separation

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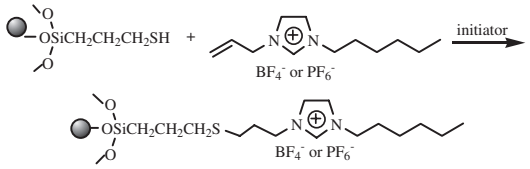
Silica particle chemically modified with ionic liquid is used for the first time as the stationary phase in HPLC for the separation of alkaloids following a dual mechanism.

The increasing interests in ionic liquids (ILs) largely stem from their unusual combination of properties such as environmentally benignity, nonvolatility, nonflammability.[1] So, ILs have found wide applications in various fields, e.g., as alternative solvents in routine extraction procedures,[1,2] electrolytes in electrochemistry,[3] solvents in chemical reaction,[4] reaction media in homogeneous catalysis,[5] and as lubricants.[6] In the analytical science, coated IL films are employed as the stationary phase in gas chromatography (GC)[7] and also in capillary electrophoresis (CE).[8] Recently, they are used as the background electrolytes[9] and especially as additives in mobile phase in HPLC.[10] Surface immobilized IL has been used for heterogeneous catalysis.[11] However, use of surface-immobilized IL for HPLC separation has not been reported. Here we present the investigation of utilizing IL modified stationary phase as the new separation interface in HPLC.

1-Allyl-3-hexylimidazoliums ([AHIm]) combined with BF_4^- or PF_6^- counterion were prepared using the well-established method.[12] The products reacted with 3-mercaptopropyltrimethoxysilane (MPS) modified silica via the radical chain transfer addition reaction (Scheme 1) between allyl and thiol groups in the presence of azodiisobutyronitrile (AIBN).[13] Formation of chemically immobilized layer of IL fragment on silica surface was characterized by the Raman spectrometry. The obvious Raman shift at 2578 cm^{-1} in MPS modified silica was attributed to the stretching vibration of S–H (Figure 1). After reacting with vinyl ionic liquid, its intensity decreased dramatically. The absorption bands at 3107 cm^{-1} and 3164 cm^{-1} were ascribed to the unsaturated C–H in imidazolium rings. Together with the enhanced intensity of saturated C–H between 3000 and 2800 cm^{-1}, we concluded that the IL was successfully grafted onto silica surface. The surface analysis indicated 0.58 mmol·g^{-1} of the ILs were grafted and the coupling yield of [AHIM] salts with MPS-based silica was about 50%. IL-modified silica was packed into 150 × 4.6 mm i.d. stainless steel column by slurry-packing procedure for HPLC investigation, respectively.

Alkaloids including ephedrines (1, norephedrine; 2, ephedrine and 3, pseudoephedrine, a typical pair of diastereoisomeric compounds; 4, methylephedrine) and tropane alkaloids (5, anisodamine; 6, scopolamine; 7, atropine) were chosen as the probe analytes. Ephedrines are the forbidden drugs in Olympic games and tropane alkaloids are widely used as anti-cholinergic drugs. Ephedrines are well retained on both IL-based columns with high resolution and selectivity (Figure 2a). It is worth noting that only 1% of organic solvent is added in mobile phase for an effective separation. While on C_{18} (Chromatorex C_{18}, 5 μm) or C_8 column (Zorbax Eclipse XDB-C_8, 5 μm), ephedrines exhibit very poor resolution under the similar conditions (Table 1). Comparatively, on IL-based columns the desired capacity factor (k'), superior selectivity and separation efficiency were achieved. Whereas, poor peak shapes and Rs were observed on the quaternary ammonium salts column (Table 1, SAE column). This confirms the speciality and importance of the IL-modified silica. Similarly, alkaloids **5**, **6**, and **7** are well separated on both IL-based columns (Figure 2b). The selectivity and the efficiency



Scheme 1. Preparation of ionic liquid-modified stationary phase.

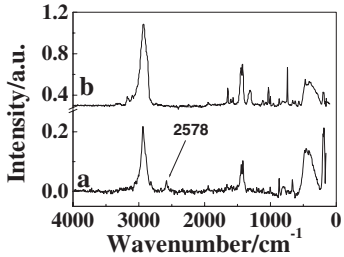


Figure 1. Raman spectra of a: MPS-modified silica and b: ionic liquid-modified silica.

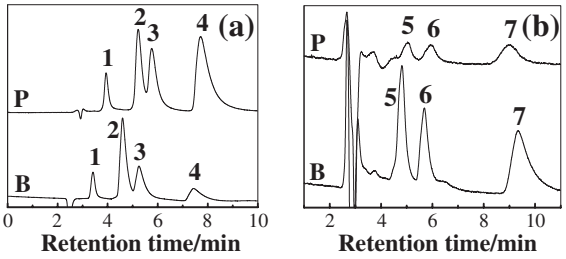


Figure 2. Typical chromatograms of (a) ephedrines and (b) tropane alkaloids on ionic liquid-modified silica. B: on [AHIm]BF_4-based column; (a) mobile phase, 1% methanol in 0.05 mol·L^{-1} KH_2PO_4 at pH 3.0; flowrate, 0.7 mL·min^{-1}; (b) methanol-0.05 mol·L^{-1} NaH_2PO_4 (10/90); flowrate, 0.6 mL·min^{-1}. P: on [AHIm]PF_6-based column; (a) 0.02 mol·L^{-1} KH_2PO_4; (b) methanol-0.02 mol·L^{-1} KH_2PO_4 (10/90); both flowrates are 0.6 mL·min^{-1}. Both the columns are 150 × 4.6 mm i.d.. The data were detected at 210 nm with an UV–vis detector.

of IL-based column have nearly no difference after repeated use, which means the good stability of it.

The pH value in mobile phase plays an important role in the separation. At lower pH, the analytes are eluted in shorter time with better peak shape, but they are retained longer at high pH. The reason is that at lower pH these basic solutes are liable to accept protons, which makes them less hydrophobic. Therefore a reversed phase mechanism may be involved in the separation. Additionally, the increase of buffer concentration could decrease the retention time by reducing the interaction between residue silanols and basic solutes.

It is seen from Figure 2 that the elution order of ephedrines on IL-based columns are the same as that on C_{18} or C_8 column (k' value, showed in Table 1), indicating a similar separation mechanism. Ephedrines also have similar k' values on IL-based columns and on C_8 column, which is ascribed to the similar alkyl chain length (i.e., C_6 in ionic liquid, and C_8) and so they have nearly the same hydrophobicity. Though alkaloid **2** and **3** have the same chemical structures, **3** forms stable intramolecular hydrogen bond more easily than **2**, which makes it more hydrophobic and retain longer on the column than the latter. This evidence also verifies that the separation on IL-based silica follows a reversed phase mechanism that compounds with larger polarity (hydrophilicity) will be eluted sooner. The elution order of phloroglucinol < hydroquinone < phenol or trimesic acid < *p*-phthalic acid < benzoic acid on the IL-based columns also confirms a reversed phase mechanism. It is concluded that long substituted alkyl chains and the hydrophobic counter ions make ionic liquid-modified silica the reversed phase stationary phases.

Alkaloid **5** is eluted before **6** on IL modified columns while on C_{18} or C_8 column, it displays an inverse order (see k' value, Table 1). It is well known that alkaloid **6** has higher hydrophilic property than **5**, which leads to a smaller retention time of **6** on C_{18} or C_8 column. However, when adding sodium dodecyl sulfate (SDS) as the ion pair agent to the mobile phase, **5** and **6** are eluted in the same order on C_{18} column as that on IL-based column.[14] Though alkaloid **6** has less basicity than **5** because of the steric hindrance effect, the oxonium salt formed at lower pH on the epoxy group of alkaloid **6** could form ion pair with the pairing ion, resulting in its stronger retention on C_{18} column. The same situation can be expected on IL-based column that at lower pH the formation of ion pair between oxonium salt of alkaloid **6** and the BF_4^- or PF_6^- anion confined on silica leads to a stronger retention of **6**. So ion-pairing interaction plays an important role in the separation on these new packings. In addition, it has been proved that ionic liquids possess ion pair characteristic and can form ion-pairing complex with analytes in electroanalysis.[15]

In a word, this kind of IL-modified stationary phases display characteristics not only of reversed phase chromatography but also ion-pair chromatography. The analytes can be effectively separated on IL-based stationary phase without addition of ion pair agent[16] or triethylamine[17] in mobile phase as that on C_{18} column.

In summary, chemically immobilized IL on silica surface is utilized as a new interface for the HPLC separation of alkaloids. Effective separation is ascribed to both the hydrophobicity and the ionic property of IL-modified stationary phases. Considering the large family of ionic liquids with tunable substituted alkyl chains and counter anions, IL-based stationary phase has great variety and can be investigated further in HPLC. Moreover, the letter presents a new addition to the versatile applications of IL.

Table 1. Comparison of k' (capacity factor) and Rs (resolution) on varied columns

Comp.	[AHIm]BF_4		[AHIm]PF_6		C_{18}		C_8		SAE	
	[a1]k'	Rs	[b1]k'	Rs	[c1]k'	Rs	[d1]k'	Rs	[e1]k'	Rs
1	0.46		0.48		6.5		0.46		0.46	
2	1.0	3.2	0.97	3.8	13	5.1	0.95	3.6	0.51	0.4
3	1.2	1.4	1.2	1.3	17	2.6	1.3	2.8	0.61	0.5
4	2.2	2.9	1.9	2.6	18	0.9	1.3	0.6	0.81	0.9
	a2		b2		c2		d2		e2	
5	0.86		0.92		4.0		0.76		0.54	
6	1.2	1.6	1.3	1.3	2.9	2.4	0.45	2.8	0.55	0.2
7	2.6	3.8	2.4	3.0	7.6	4.6	1.7	5.2	0.72	2.0

a1: mobile phase, 1% methanol in 0.02 mol·L^{-1} KH_2PO_4; a2, b2, e2: methanol-0.02 mol·L^{-1} NaH_2PO_4 (10/90); b1: 0.03 mol·L^{-1} KH_2PO_4; c1: water at pH 3.0 adjusted with H_3PO_4; c2: 20% methanol in 0.02 mol·L^{-1} KH_2PO_4, d1: 0.02 mol·L^{-1} KH_2PO_4; d2: 10% methanol in 0.02 mol·L^{-1} KH_2PO_4; e1: 0.01 mol·L^{-1} NaH_2PO_4.

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