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Ionic Liquids as Advantageous Solvents for Headspace Gas Chromatography of Compounds with Low Vapor Pressure

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The potential of ionic liquids as solvents for headspace gas chromatography was investigated. Three compounds with boiling points above 200 °C were selected to demonstrate the feasibility of the concept described. 2-Ethylhexanoic acid, formamide, and tri-*n*-butylamine as examples of acidic, neutral, and basic analytes were dissolved in acidic 1-*n*-butyl-3-methylimidazolium hydrogen sulfate (1), neutral 1-*n*-butyl-2,3-dimethylimidazolium dicyanamide (2), and 2 containing 1,8-diazabicyclo[5.4.0]undec-7-ene to adjust basic conditions. All analytes could be determined with limits of detection and limits of quantification in the low-ppm concentration range.

Ionic liquids (ILs) are salts[1,2] or mixtures[3] of salts that melt below room temperature. They represent an important class of innovative solvents. Their relevance is reflected by continuous emergence of new applications, mainly in the field of homogeneous catalysis.[4] In technical applications, they serve as superior electrolytes for electric devices.[5] Some utilization has been also reported for various analytical purposes, e.g., for matrix-assisted laser desorption/ionization mass spectroscopy (MALDI-MS),[6] for capillary electrophoresis (CE),[7−12] as stationary phases for gas chromatography (GC),[13−15] for liquid-phase microextractions,[16,17] and as modifying additives for eluents in liquid chromatography.[18,19]

Analysis of process-related residual reactants and solvents in pharmaceuticals, as regulated by the official authorities, plays a tremendous role in quality assurance and quality control. The permanently demanded improvement of analytical performance in chemical, clinical, and pharmaceutical laboratories relies on either simplified sample preparation or thorough chemometric automation. For example, multivariate analysis and classification of the respective pharmaceuticals and intermediates using near-infrared reflectance spectroscopy are powerful tools for analytical automation[20] but, in general, simplifying analytical sample preparation bears the highest potential of improving sample throughput and time savings in an easy manner.

Headspace gas chromatography (HSGC) is a widely used analytical technique for the determination of volatile substances in solids and liquids.[21−23] However, for compounds with a low vapor pressure, the sensitivity in HSGC is rather limited. The increase of the vapor pressure by increasing the thermostating temperature is restricted by excessive pressure buildup in the vial.

A contemporary approach to overcome this problem is the use of ILs as versatile solvents, since these low-melting organic salts are easily synthesized or commercially available, respectively.[4] They have ideal physicochemical properties for optimized headspace conditions, as they are chemically inert, fairly good solvents for organic substances with moderate to high polarity. They can be tailored as application-specific systems with remarkably high

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stability[24,25] at temperatures even above 350 °C and exhibit negligibly low vapor pressures.[26] The relevance of these solvents is reflected in a recent patent application.[27] The intention of this work was to evaluate their obvious potential and to demonstrate the superiority of this concept for challenging analytical problems in order to stimulate a broader use of ILs by the community of analytical scientists.

EXPERIMENTAL SECTION

Reagents and Standards. Formamide (FA) [75−12−7], bp 210 °C, vapor pressure 1.87 Pa (0.014 Torr, 20 °C);[28] racemic 2-ethylhexanoic acid (EHA) [149−57−5], bp 228 °C, vapor pressure 4 Pa (20 °C);[29] tri-*n*-butylamine (TBA) [102−82−9], bp 216 °C, vapor pressure 25.6 Pa (0.192 Torr, 25 °C);[30] and 1,8-diazabicyclo[5.4.0]undec-7-ene [6674−22−2] (DBU) were purchased from Aldrich. Ionic liquids 1-*n*-butyl-3-methylimidazolium hydrogen sulfate (**1**), 1-*n*-butyl-2,3-dimethylimidazolium dicyanamide (**2**), and 1-*n*-butyl-2,3-dimethylimidazolium hydrogen sulfate (**3**) were synthesized according to published procedures.[31−33] The precursor 1-*n*-butyl-2,3-dimethylimidazolium chloride exhibited polymorphism, and X-ray structures of the two different types of crystals are described (Figure S-1). Polymorphism in the crystalline state of imidazolium salts has been reported previously.[34,35] A detailed description of the improved synthesis and analytical characterization is given in the Supporting Information.

Apparatus. Two detection systems were used in this study: a mass selective detector for FA and a flame ionization detector for EHA and TBA.

System A. The analysis of FA was performed on a poly(ethylene glycol) fused-silica capillary column (J&W Scientific; length 60 m, inner diameter 0.255 mm, film thickness 0.25 μm). A CE Instruments gas chromatograph GC 8000 Top (Thermo Electron) equipped with a Fisons mass selective detector MD 800 (Thermo Electron) was used. The carrier gas was helium (99.999% purity) with a constant flow rate of 1 mL/min and a split ratio of 1:40. The temperatures of the capillary column and the injection port were set to 200 °C. The mass selective detector used electron impact (EI^+, 750 V) ionization and was operated in the single ion monitoring (SIM) mode, monitoring only the base peak of the formamide mass spectrum (45 m/z). The temperature of the column interface into the detector was 180 °C, and the temperature of the ion source was 150 °C. For headspace sampling, a CTC autosampler CombiPal was used (CTC Analytics AG), equipped with a 2.5-mL airtight syringe (Hamilton). The syringe temperature was set at 150 °C, fill speed at 0.5 mL/s, injection speed at 0.5 mL/s, pullup delay at 500 ms, preinject delay and postinject delay at 500 ms, respectively, without needle flush. A 2.0-mL aliquot of every sample solution containing 200 mg of matrix was filled into a 10-mL headspace vial (Chromacol), and the vials were hermetically sealed. The headspace samples containing penicillin G benzathine spiked with 100 ppm FA (based on the product) were incubated at 180 °C for 15 min with shaking. The agitator speed was set at 500 rpm. The injection volume was 1.0 mL (Figure 1a).

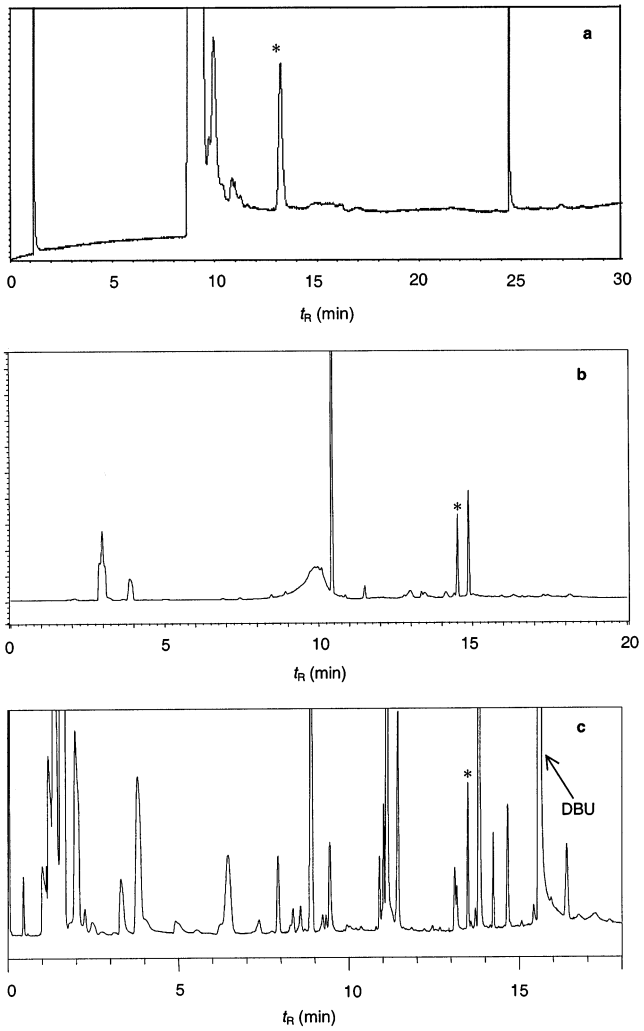

Figure 1. Chromatograms of production samples dissolved in ILs applying high temperature thermostating HSGC: (a) penicillin G benzathine spiked with 100 ppm formamide (*) in $BMMI^+$ dicyanamide, thermostated at 180 °C, 15 min; (b) ampicillin sodium containing 0.4% 2-ethylhexanoic acid (*) in BMI^+ hydrogensulfate, thermostated at 150 °C, 15 min; (c) potassium clavulanate spiked with 100 ppm tri-*n*-butylamine (*) in $BMMI^+$ dicyanamide/DBU, thermostated at 150 °C, 15 min.

System B. The analyses of EHA und TBA were performed on a dimethylpolysiloxane-phase fused-silica capillary column (Agilent; length 30 m, inner diameter 0.53 mm, film thickness 2.65 μm). An Agilent gas chromatograph HP6890 (Agilent) equipped with a flame ionization detector (FID) was used. The carrier gas was helium (99.999% purity) with a constant flow rate of 4 mL/min and a split ratio of 1:40. The temperatures of the capillary

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Table 1. Validation Results

analyte	detection mode	slope (area ppm^{-1})	intercept (area)	correlation coefficient r	repeatability (RSD, $n = 6$) (%)[a]	mean recovery (%)	LD (ppm)	LQ (ppm)
2-ethylhexanoic acid	FID	88.4	400.3	0.9994	9.6	95	22	65
tri-*n*-butylamine	FID	0.0480	0.06019	0.9999	2.0	93	8	24
formamide	SIM	155905	1.220×10^6	0.9998	10.9	101	13	39

[a] At $c = 100$ ppm.

column and the injection port were set to 250 °C, the temperature of the FID was set to 250 °C. For headspace sampling, a Perkin-Elmer headspace sampler HS40 was used (Perkin-Elmer). The temperature of the transfer line was kept at 180 °C; the injection pressure was set at 15 psi for 0.15 min. To minimize degradation products, the incubation temperature has been optimized to 150 °C for ampicillin sodium containing 0.4% EHA (Figure 1b) and for potassium clavulanate spiked with 100 ppm TBA based on the product (Figure 1c). A 2.0-mL aliquot of every sample solution containing 200 mg of matrix was filled into a 20-mL headspace vial (Perkin-Elmer); the vials were hermetically sealed and incubated for 15 min with shaking. The injection volume was 0.6 mL.

RESULTS AND DISCUSSION

Ionic Liquids. The ILs finally used in this study were 1-*n*-butyl-3-methylimidazolium hydrogen sulfate (**1**) and 1-*n*-butyl-2,3-dimethylimidazolium dicyanamide (**2**), respectively. The choice of the anions such as hydrogen sulfate as a Bronstedt acid or dicyanamide as a coordinating anion was governed not only by the nature of the particular solutes but also by the affordability of these room-temperature ionic liquids. The hydrogen sulfate, especially, is very inexpensive. These salts can be dried at elevated temperatures to virtually anhydrous solvation media, thus preventing hydrolysis of any reactive analytes. The choice of the 2-methylimidazolium cation in **2** was intentional, as carbene formation could be avoided upon addition of strongly basic additives. Furthermore, 1-*n*-butyl-2,3-dimethylimidazolium hydrogen sulfate (**3**) was also used and found to be equally suitable. Controlled temperatures as high as 180 °C could be used without any problems.

Analytes. As representative examples, three analytes with boiling points above 200 °C were selected to demonstrate the feasability of the HSGC approach described: EHA, FA, and TBA as acidic, neutral, and basic compounds. For EHA and TBA, FID was used as detection mode, whereas for FA, which exhibits very low FID response, mass selective detection with SIM was used. To the best of our knowledge, no successful application of HSGC for the direct determination without derivatization or extraction of EHA and FA has been published so far. The quantification of FA by static HSGC at low temperatures reported in the literature failed due to the lack of sensitivity.[36] Only the quantification of TBA with a vapor pressure significantly higher than the two other compounds has been described.[37]

Matrixes. To demonstrate the routine applicability, three authentic production samples, spiked with the respective analyte used in the drug synthesis, were chosen: (a) FA in benzylpenicillin benzathine [1538−09−6], which is practically insoluble in aqueous systems, freely dissolved in 1-*n*-butyl-2,3-dimethylimidazolium dicyanamide ($BMMI^+$) dicyanamide as a neutral solvent, (b) EHA in ampicillin sodium [69−52−3] dissolved in 1-*n*-butyl-3-methylimidazolium (BMI^+) hydrogen sulfate as an acidic solvent, and (c) TBA in potassium clavulanate [61177−45−5] dissolved in $BMMI^+$ dicyanamide, containing an excess of DBU to generate basic conditions.

Validation. To estimate the sensitivity of the applied system, a limit test was determined without optimization of all instrumental parameters for the three representative compounds dissolved in pure ILs. A validation according to the current guideline of the International Conference on Harmonization (ICH/Q2B) was performed.[38] Selectivity was assessed by identification using GC/MS for FA and by standard addition for TBA and EHA, respectively. An incubation time of 15 min was sufficient in all experiments to ensure reproducible results. Blank runs have been applied to check a possible carryover of analytes. The limit of detection (LD) and the limit of quantification (LQ) were calculated from the slope s and residual standard deviation σ of a linear regression of the signal areas obtained at six concentration levels (5, 10, 50, 100, 250, and 500 ppm based on the whole sample solution) by the following equations, $LD = 3.3\ \sigma/s$, $LQ = 10\ \sigma/s$. Recovery was calculated as found/expected value, and the observed value was calculated from the calibration function. The results, including repeatability at the 100 ppm level, are summarized in Table 1. Representative chromatograms are shown in Figure 1. Finally, we tested the use of a smaller volume of IL (0.5 mL) spiked at the 100 ppm level under otherwise identical conditions and found the response to be comparable.

Since the compounds selected are solvents or reagents frequently used in the pharmaceutical industry for the production of active drug substances and since static HSGC is an official method of the European and U.S. Pharmacopoeias, the enhanced sensitivity of the analytical system would be of general importance in routine quality control; the official limits of EHA and FA are 0.8% and 220 ppm, respectively; for TBA, the limit of 0.2% specified as aliphatic amines in potassium clavulanate is applicable.[39] Although the official limit for EHA is relatively high compared to the sensitivity of the system presented, this compound still is a

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representative example for fatty acids, which are of great importance at low concentrations in many biological matrixes.

CONCLUSIONS

We have shown that ILs are well suited for trace analysis of high-boiling, low vapor pressure residual solvents in pharmaceutical drug products. It was demonstrated that, by proper choice of the IL, challenging analytical problems involving neutral, acidic, and basic analytes can be successfully solved.

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SUPPORTING INFORMATION AVAILABLE

Optimized synthesis and characterization of the ionic liquids **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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