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ORIGINAL RESEARCH

Negative effect and removal of trace amounts of 1,3-dialkylimidazolium ionic liquids in samples from biorefineries

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Abstract Ionic liquids (ILs), based on 1,3-dialkylimidazolium cations, are frequently used solvent components or auxiliaries for various types of biomass in biorefinery approaches. Unless washing and sample preparation have been carried out very carefully, analytical samples often contain residual traces of such ionic liquids. These residues can compromise the quality of physicochemical analyses, as was demonstrated for monosaccharide analysis after hydrolysis by gas chromatography, high-performance thin-layer chromatography, or ion chromatography (IC), and even damage analytical equipment, such as gas chromatographic capillaries or IC electrodes. We suggest a simple procedure—short stirring with solid elemental sulfur adsorbed on alumina as the scavenger—as a pretreatment of the analytical samples to remove interfering imidazolium IL traces. The chemistry underlying this pretreatment is the conversion of the 1,3-dialkylimidazolium cation to the corresponding, water-insoluble, neutral, volatile 1,3-dialkylimidazole-2-thiones. Given the negative effect of imidazolium IL impurities, the minor extension of the sample preparation by one short additional step appears to be a small price to pay for an unperturbed and instrument-safe analysis.

Anna F. Lehrhofer and Yuko Yoneda have contributed equally.

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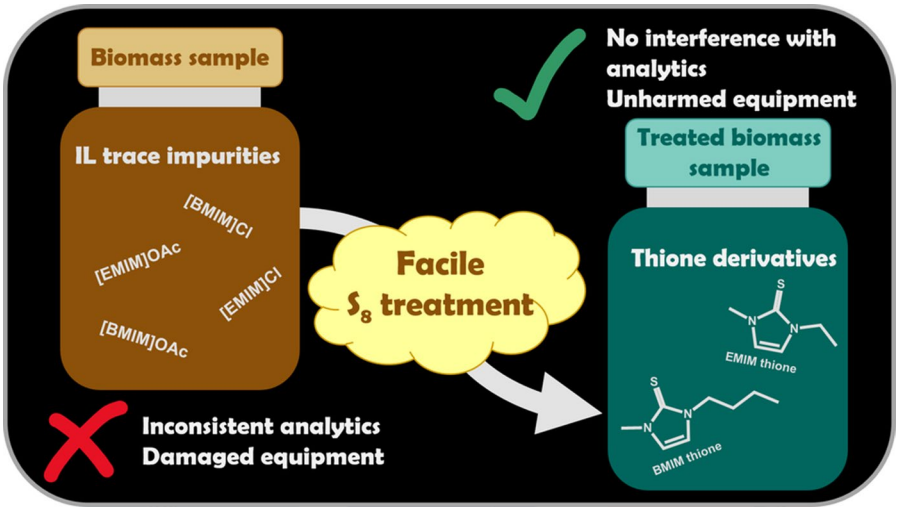
Graphical abstract



Keywords Biorefinery · Biorefinery analytics · Cellulose · Gas chromatography (GC) · High-performance thin-layer chromatography (HPTLC) · Ion chromatography (IC) · Ionic liquid · Monosaccharide analysis · NMR spectroscopy

Introduction

1-Butyl-3-methylimidazolium (BMIm) and 1-ethyl-3-methylimidazolium (EMIm), both as either acetate or chloride, were the first ionic liquids (ILs) to be reported to dissolve cellulose (Swatloski et al. 2002), and in biorefinery research are thus often referred to as "first-generation" ILs. After the first hype about ionic liquids being able to directly dissolve wood and other biomass and about potential applications in biomass pulping and separation, the views on ionic liquids have now become more realistic and objective. There seem to be unsurmountable general problems with recycling and byproduct accumulation when it comes to biomass fractionation or wood pulping by ILs—and the often-praised advantages of the ILs, superior solubilization power and negligible vapor pressure, turned into distinct disadvantages when it came to IL recycling and purification. Further, the claim of chemical inertness, which has frequently been proposed as one of the ILs´ main assets, was no longer valid when it was demonstrated that imidazolium ILs react with the reducing ends of cellulose and contained carbonyl groups (Ebner et al. 2008; Liebert et al. 2009) or that the IL anions, in particular the acetates, cause trace acetylation of the biomass treated with the IL (Köhler et al. 2007; Zweckmair et al. 2015). Natural aging, especially thermal stress, causes byproduct formation in imidazolium ionic liquids, mainly imidazole and *N*-methylimidazole, which can be seen as almost ubiquitous trace impurities in IL samples that are not meticulously purified (Liebner et al. 2010; Wendler et al. 2012).

Consequently, advancement of ILs proceeded very fast: the next generations of ILs, which were distillable, more inert towards biomass constituents or phase-separable, were able to tackle the initial drawbacks more efficiently (King et al. 2011; Holding et al. 2014; Vijayaraghavan and Macfarlane 2014; Ahmad et al. 2016; Kakko et al. 2017; Brandt-Talbot et al. 2017; Song et al. 2018; Achinivu et al. 2014). Still, the first-generation imidazolium ionic liquids have seen a revival: not in biomass fraction or wood pulping, but for instance in fiber spinning—where the short contact times and the pure starting celluloses minimize side reactions and purification issues (Zhang et al. 2019 and 2020; Hettegger et al. 2022) —or in biomass pretreatments to increase accessibility and improve saccharification and enzymatic digestibility (Mora-Pale et al. 2011; Tadesse and Luque 2011; Zhang et al. 2014;

Elgharbawy et al. 2016; Tu and Hallett 2019; Azizan et al. 2020; Ehaz and Sohail 2020; Amini et al. 2021; Quesada-Salas et al. 2022; for recent reviews see: Haldar and Purkait 2021; Lin et al. 2022; Roy and Chandawat 2023). Some of these approaches are still at the lab scale, but all of them require analytical monitoring.

In many biorefinery scenarios, analysis of the monosaccharide profile is a key analytical approach to characterize carbohydrate biomass, fermentation liquids, cellulosic pulps, or cellulosic fibers. Commonly used methods are acidic hydrolysis or methanolysis of the accessible polysaccharides, which have been recently compared with their pros and cons being evaluated in detail (Becker et al. 2021). Over the last decade, the analytical techniques for monosaccharide quantification have been thoroughly optimized and reviewed in the literature. Three main approaches are used today: ion chromatography (IC) (McGregor et al. 2017; Grünewald et al. 2021), gas chromatography (GC) after derivatization (Becker et al. 2013a, 2013b) and high-performance thin-layer chromatography (HPTLC) (Böhmdorfer et al. 2016; Zweckmair et al. 2016; Oberlerchner et al. 2018).

In the present study, we briefly demonstrate the negative impact of trace imidazolium-based ionic liquids on each of these three analytical techniques and communicate a simple and general method for removing these traces prior to analysis to diminish further interference with the analytical process and instrumentation. The common ionic liquids 1-ethyl-3-methylimidazolium as acetate (EMIm-OAc, **1a**) and chloride (EMIm-Cl, **1b**) as well as 1-butyl-3-methylimidazolium, also as acetate (BMIm-OAc, **2a**) and chloride (BMIm-Cl, **2b**) were studied.

Materials and methods

General

All chemicals were purchased from Sigma-Aldrich (Schnelldorf, Germany) and of the highest purity available. Solvents of the highest available purity (HPLC grade) were used for all extractions and workup procedures. Deionized water was used throughout. HPTLC was performed using Merck silica gel 60 F_{254} pre-coated plates, 20 × 10 cm.

GC/MS analysis

GC/MS analysis was carried out on an Agilent 7890A gas chromatograph with an Agilent 5975C triple-axis mass selective detector (MSD) and pressurized splitter (25 kPa constant pressure) for simultaneous detection by MS and FID. The capillary, an Agilent VF-5ht Ultimetal (30 m × 0.25 mm i.d., 0.1 µm film thickness), was operated at constant flow (2.5 mL/min) with helium as carrier gas. The sample (1 µL) was injected with an automated liquid sampler into a temperature-programmed multi-mode inlet that was operated under the following conditions: 15:1 split, 65 °C (0.1 min), then 500 °/min to 380 °C (5 min). The oven temperature was set to 60 °C (5 min), then 10 °C/min to 380 °C. The MS was operated in scan mode, 29–2050 *m/z*, EI mode, 70 eV ionization energy, ion source temperature: 230 °C, quadrupole temperature: 150 °C, transfer line: 280 °C. The entire system was controlled by Agilent Masshunter B.7.02.

IC-PAD analysis

Analysis was carried out on an ICS3000 ion chromatography system (Thermo-Dionex, USA) with a DP gradient pump, AS-1 autosampler, and an ED-3000 electrochemical detector. A Dionex™ CarboPac™ PA20 column (3 × 150 mm) with a PA20 CarboPac™ guard column (3 × 30 mm) was used. Injection volume 3 mL, flow rate 0.30 mL min^{-1} at 20 °C, system temperature 20 °C. Linear-gradient elution: 0–15 min with 2 mM NaOH, 15–22 min with 10 mM NaOH + 0.5 M sodium formate, 22–24 min with 200 mM NaOH, 24–30 min with 2 mM NaOH. Electrochemical detection was done with an Au working electrode and an Ag/AgCl reference electrode with 0.5 s potential sweeps (potentials: E1 for detection, E2-E4 for cleaning; E1 = 0.10 V, 0–0.4 s; E2 = –2.00 V, 0.41–0.42 s; E3 = 0.60 V, 0.43 s; E4 = –0.10 V, 0.44–0.50 s). Data processing was done with Chromeleon 2.0 software.

HPTLC analysis

Samples (2 µL, 1 µL for the thiones) were sprayed as bands at 50 nL/s with an Automatic TLC Sampler (ATS 4, Camag, Muttenz, Switzerland) equipped with a 25 µL syringe. After each deposition, the syringe was rinsed with neat acetone. Two different

elution protocols were used, one for the separation of monosaccharides, the other to demonstrate the separation of the treated ionic liquids. For the analysis of monosaccharides, elution was performed with 10 mL of *n*-butanol/*iso*-propanol/acetic acid/boric acid (6/14/1/3, v/v/v/v) in an Automated Developing chamber 2 (Camag) to a distance of 60 mm. Images were taken with the TLC Visualizer (Camag) with UV light at 366 nm (fluorescence mode) after derivatization with aniline. For the separation of treated ionic liquids, elution was performed with ethyl acetate/hexane (3/1, v/v) in a twin trough chamber to a distance of 60 mm. Images were taken with the TLC Visualizer (Camag) with UV light at 254 nm (fluorescence quenching mode).

All instruments were controlled with VisionCats 2.5 software or later (Camag).

NMR spectroscopy

Solution-state NMR spectra were recorded using a Bruker Avance II 400 spectrometer equipped with a cryogenically-cooled broadband observing (BBO) 5 mm probe-head (CryoProbe™ Prodigy, N_2-cooled). The NMR experiments were performed with z-gradients at RT at resonance frequencies of 400.13 MHz for ^{1}H, 100.61 MHz for ^{13}C, and 40.54 MHz for ^{15}N using standard Bruker pulse programs. Chemical shifts are given in parts per million (ppm) and were referenced to the respective solvent as internal reference (DMSO-d_6; 2.50 ppm for ^{1}H, 39.52 ppm for ^{13}C). ^{1}H NMR spectra were recorded with 32k complex data points and apodized using a Gaussian window function (lb = –0.30 Hz and gb = 0.30 Hz) prior to *Fourier* transformation. ^{13}C NMR *J*-modulated spectra using WALTZ16 ^{1}H decoupling (Bruker pulse program "*jmod*") were recorded with 65k complex data points. S/N was enhanced utilizing an exponential window function (lb = 1.0 Hz) before *Fourier* transformation. ^{1}H-^{1}H COSY using gradient pulse for selection (Bruker pulse program *"cosygpqf*") was used to determine homonuclear shift correlation and acquired with 2048 × 256 data points. The multiplicity-edited HSQC experiment (Bruker pulse program "*hsqcedetgpsp.3*") was performed using adiabatic pulses for inversion of ^{13}C and GARP-sequence for broadband ^{13}C-decoupling, optimized for ^{1}J(CH) = 145 Hz and acquired with 1024 × 256 data points. For determination of long-range ^{1}H-^{13}C couplings, an HMBC experiment using gradient pulses and a low-pass filter without decoupling (Bruker pulse program "*hmbcgplpndqf*") was performed and acquired with 1024 × 256 data points. To determine ^{1}H-^{15}N couplings, ^{1}H-^{15}N HMBC experiments (Bruker pulse program "*hmbcgplpndqf*") were performed acquiring 1048 × 128 data points; ^{15}N shifts were extracted from the obtained 2D spectra. All NMR data was acquired and processed using Bruker TopSpin 4.3.0 and/or 3.2.7 software.

Compound identification

Detailed analytical data of compounds **3** and **4** can be found in the supporting information (NMR spectra, IR spectra, QTOF MS data, microanalysis).

Preparation of an analytical sample of 1-ethyl-3-methyl-1,3-dihydro-2*H*-imidazole-2-thione (**3**)

Vacuum-dried 1-ethyl-3-methylimidazolium chloride (**1b**, 1.01 g, 6.89 mmol, 1.0 equiv.), elemental sulfur (0.234 g, 7.30 mmol, 1.06 equiv.), and K_2CO_3 (1.14 g, 8.26 mmol, 1.2 equiv.) were mixed, flushed with Ar and refluxed in anhydrous MeOH (10 mL) for 3 h. The yellow suspension was cooled to RT, the solvent was removed *in vacuo* and deionized H_2O (10 mL) was added. The suspension was briefly heated to reflux to fully dissolve impurities, cooled to RT, acidified with 1 M HCl (10 mL), and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with 2 × 20 mL of 1 M HCl and 20 mL of brine, dried over $MgSO_4$, and concentrated *in vacuo*. The product was obtained as a yellow oil (0.678 g; 69%). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 7.15 (d, 1H, ^{3}J = 2.4, 5-CH), 7.12 (d, 1H, ^{3}J = 2.4, 4-CH), 3.95 (q, 2H, ^{3}J = 7.3, N-CH_2), 3.45 (s, 3H, N-CH_3), 1.21 (t, 3H, J = 7.3, CH_3) ppm. ^{13}C NMR (100.61 MHz, DMSO-d_6): δ 160.9 (C = S), 118.3 (4-CH), 116.5 (5-CH), 41.9 (N-CH_2), 34.3 (N-CH_3), 14.1 ppm (CH_3), see Fig. 1. ATR-FTIR: ν = 3157, 3131, 3187, 2959, 2931, 2874, 1567, 1458, 1413, 1398, 1357, 1328, 1264, 1222, 1166, 1088, 954, 735, 709, 672 cm^{-1}. HRMS (LC ESI-QToF-MS): *m/z* = 143.0636 (calcd. 143.0643 for $C_6H_{11}N_2S$, $[M+H]^+$). Microanalysis for $C_6H_{10}N_2S$: calcd. C 50.67, H 7.09, N 19.70, S 22.55; found C 50.72, H 6.99, N 19.82, S 22.46.

Preparation of an analytical sample of 1-butyl-3-methyl-1,3-dihydro-2*H*-imidazole-2-thione (**4**)

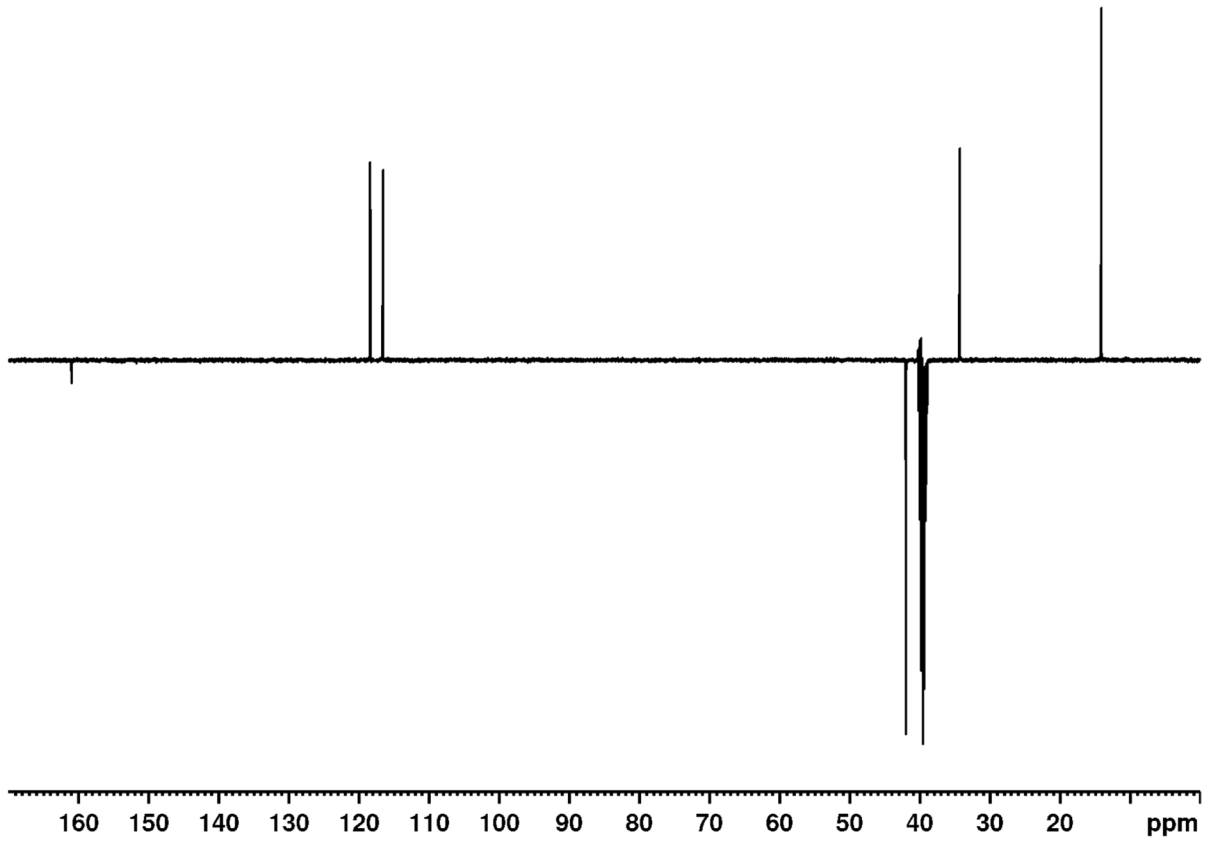


Fig. 1 ^{13}C NMR spectrum of 1-ethyl-3-methyl-1,3-dihydro-2*H*-imidazole-2-thione (**3**)

Vacuum-dried 1-butyl-3-methylimidazolium chloride (**2a**, 1.00 g, 5.72 mmol, 1.0 equiv.), elemental sulfur (0.191 g, 5.95 mmol, 1.04 equiv.), and K_2CO_3 (0.999 g, 6.87 mmol, 1.2 equiv.) were mixed, flushed with Ar and refluxed in anhydrous MeOH (9 mL) for 3 h. The yellow suspension was cooled to RT, the solvent was removed *in vacuo* and deionized water (10 mL) was added. The biphasic suspension was briefly heated to refluxing temperature to fully dissolve impurities, cooled to RT, and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with 2 × 20 mL of 1 M HCl and 20 mL of brine, dried over $MgSO_4$, and concentrated *in vacuo*. The product was obtained as a yellow oil (0.799 g; 82%). ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 7.13 (d, 1H, ^3J = 2.4, 5-CH), 7.11 (d, 1H, ^3J = 2.4, 4-CH), 3.92 (q, 2H, ^3J = 7.4, N-CH_2), 3.45 (s, 3H, N-CH_3), 1.64 (m, 2H, N-CH_2-CH_2), 1.26 (m, 2H, CH_2-CH_3), 0.89 ppm (t, 3H, ^3J = 7.3, CH_3). ^{13}C NMR (100.61 MHz, DMSO-d_6): δ 161.3 (C = S), 118.2 (4-CH), 117.1 (5-CH), 46.6 (N-CH_2), 34.4 (N-CH_3), 30.4 (N-CH_2-CH_2), 19.2 (CH_2-CH_3), 13.6 ppm, see Fig. 2. ATR-FTIR: ν = 3157, 3131, 3187, 2959, 2931, 2874, 1567, 1458, 1413, 1398, 1357, 1328, 1264, 1222, 1166, 1088, 954, 794, 735, 709, 672 cm^{-1}. HRMS (LC ESI-QToF-MS): *m/z* = 171.0941 (calcd. 171.0956 for $C_8H_{15}N_2S$, $[M+H]^+$). Microanalysis for $C_8H_{14}N_2S$: calcd. C 56.43, H 8.29, N 16.45, S 18.83; found C 56.51, H 8.14, N 16.54, S 18.85.

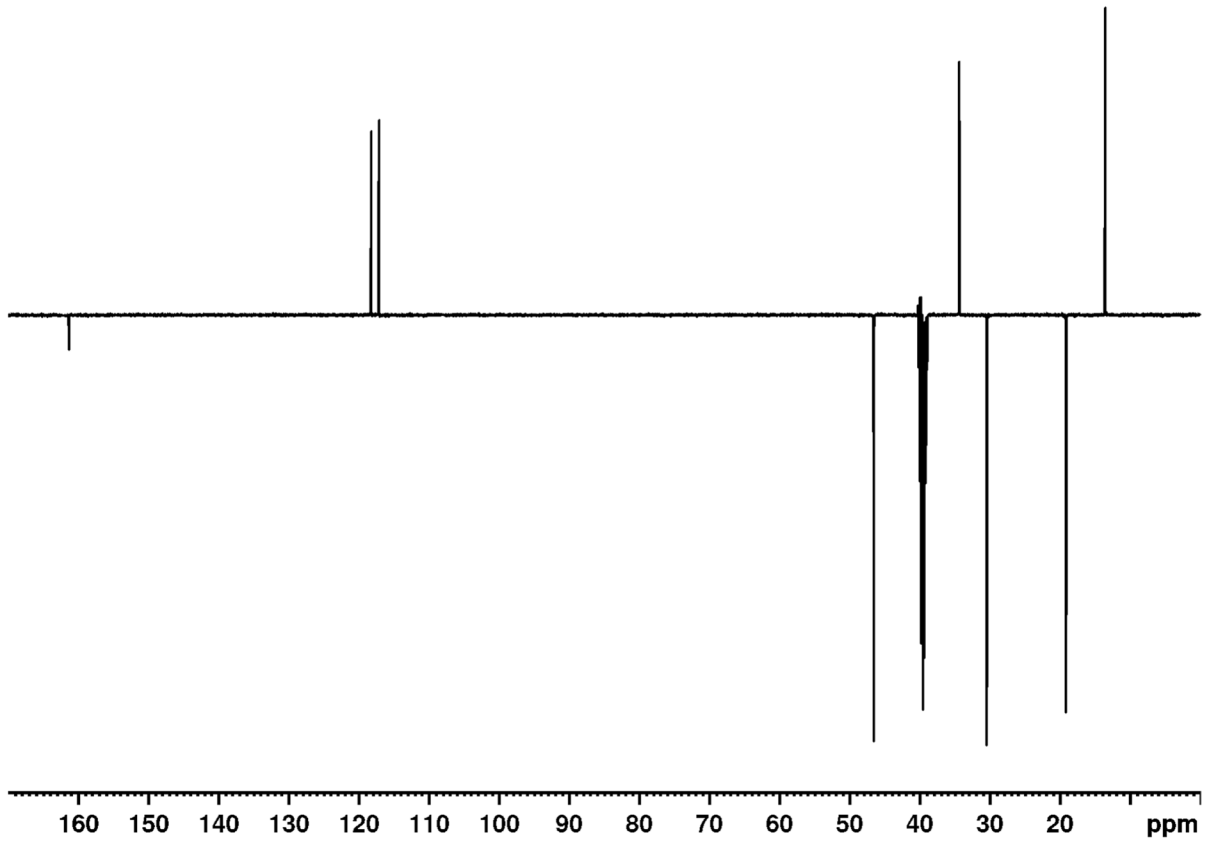


Fig. 2 ^{13}C NMR spectrum of 1-butyl-3-methyl-1,3-dihydro-2*H*-imidazole-2-thione (**4**)

Protocol for removal of 1,3-dialkylimidazolium traces from analytical samples by conversion into the corresponding 2-thiones.

Preparation of sulfur@alumina:

Sulfur (0.5 g, *p.a.*) was dissolved in toluene (200 mL, freshly distilled), and alumina (5.00 g, Brockmann grade 1) was added. The slurry was stirred for 5 min and then evaporated to dryness. The resulting sulfur@alumina (10 wt%) was obtained as an off-white, free-flowing powder, which was stored in an airtight, dark flask at room temperature. Note: the toluene must be freshly distilled to remove stabilizers which otherwise could distort the HPTLC or GC chromatograms. For later use in aqueous media, alumina Brockmann grade 5 was used instead of grade 1.

Removal of IL traces:

To the analyte solution (for GC or HPTLC measurement) 5 mg of a sulfur@alumina (10 wt%, from Brockmann grade 1 alumina, 10 mg per 5 mL of solution) was added and the solution was gently shaken or slowly stirred for 5 min, allowed standing for 5 min to let the solids settle, filtered through a 0.45 µm syringe filter, and then used directly for analysis. In the case of aqueous solutions for IC analysis, sulfur@alumina prepared from Brockmann grade 5 was used and the solution was filtered through Whatman filter paper, with the protocol otherwise unchanged.

Results

Since the pretreatment of biomass with ionic liquids is very common in biorefinery approaches, and the subsequent trace-free removal of the ionic liquids is rather demanding—if not entirely impossible on a larger scale—the presence of trace ILs in ionic liquids is a very prevalent phenomenon. Although ILs are versatile solvents known for several unique properties, such as low volatility, high thermal stability, and tunable physicochemical characteristics, the presence of trace amounts of ILs in analytical samples from biorefineries can have significantly negative effects on both analytical methodology and analytical equipment. In the following, we have selected the three analytical techniques—ion chromatography (IC), gas chromatography (GC), and high-performance thin-layer chromatography (HPTLC)—which are commonly used to analyze mono- and oligosaccharides in very different samples and matrices from biorefineries. Irrespective of the individual analytical case, traces of 1,3-dialkylimidazolium ionic liquids can generally interfere with the measurements, either by influencing the hardware or by falsifying the method´s analytical quality parameters. While adulterated results for analytical samples might still be tolerable on a case-by-case basis, permanent negative impacts on analytical hardware are generally unacceptable.

The potential negative effects of IL impurities in analytical samples include more IL-specific impacts, such as the contamination of analytical instruments (*cf.* the GC example below), which is aggravated by the low volatility and general stability of the ILs, and the interference with chromatographic separation along with suppression of analyte signals (*cf.* the HPTLC and IC cases below). Apart from those, there might be general impurity effects, such as modification of the sample matrix with regard to polarity, which possibly become interesting for MALDI-MS analyses, memory effects, of which the retention of the non-volatile ILs in GC capillaries is a good example, and corrosion of analytical instrumentation in the case of ILs with chloride anions (for example **1b**, **2b**).

Understanding and addressing the negative impacts of IL traces in analytical samples is crucial to ensure the reliability and accuracy of analytical results in biorefinery research. Although IL contamination has scarcely been recognized as a problem to date, the results presented below suggest that many of the analytical results obtained so far—with either of the three discussed methods IC (HPAEC-PAD), HPTLC or GC, working on IL-contaminated samples for mono-/oligosaccharide determination—have been more or less erroneous. There was therefore an urgent need to consider the potential interference of ILs in the chosen analytical methods and to develop strategies to eliminate or at least mitigate these effects.

Ion chromatography (IC)

Ion chromatography (IC), mostly used with pulsed amperometric detection (PAD), is a standard technique in the analysis of carbohydrates and related substances. It has the advantage of a direct analysis of mono- and oligosaccharides without the need for derivatization (as required in gas chromatography) at high sensitivity (ppm). In biorefinery scenarios, the separation and quantitation of fermentable sugars in hydrolysates or residual sugars in fermentation broths is a permanent analytical task. In pulp analysis, the monosaccharide composition of the hemicelluloses is a frequent question, addressed by hydrolysis/methanolysis followed by IC analysis. In standard IC-PAD, carbohydrates are separated in an NaOH eluant at a pH of 10 or above and analyzed with high sensitivity by oxidation at the anode, usually an Au electrode. The chromatographic separation, under identical measurement conditions, usually remains stable and highly reproducible. In PAD, a rapid sequence of electric potential changes, the “pulse”, is applied which is usually repeated every 0.1 to 0.5 s. The current at the actual measuring potential, at which the analyte is oxidized, is recorded as output. The measuring pulse is preceded and followed by oxidative and reductive cleaning pulses, which aim at regenerating the gold working electrode and ensuring stable and reproducible detection conditions. In standard carbohydrate analysis by IC, this approach keeps the electrode clean and the measurement highly reproducible. The standard deviation of a repeated glucose determination (1 mM in water, pH 10) can be as low as 0.05% or even less.

Figure 3 shows the measured monosaccharide concentration of an aqueous solution containing 10 mM methyl β-D-glucopyranoside and 5 mM methyl β-D-galactopyranoside. The values remained constant when the measurement was repeated 20 times. By

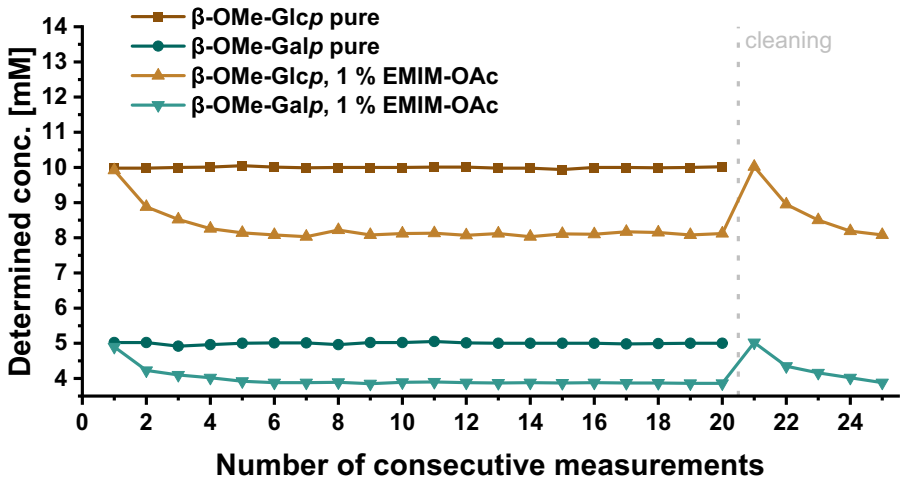


Fig. 3 Consecutive IC-PAD measurements (1 – 20) of an aqueous solution containing 10 mM methyl β-D-glucopyranoside and 5 mM methyl β-D-galactopyranoside (β-OMe-Glc*p* pure/β-OMe-Gal*p* pure), demonstrating the constancy of the output value. Consecutive IC-PAD measurements (1–25) of the same aqueous solution containing additionally 1 wt% (rel. to the sum of the two monosaccharide derivatives) EMIm-OAc (β-OMe-Glc*p*, 1% EMIm-OAc / β-OMe-Gal*p*, 1% EMIm-OAc). Between runs 20 and 21, the electrode was washed with hot DMSO (100 °C) and wiped with a polypropylene wipe

contrast, when the solution contained 1 wt% EMIm-OAc (**1a**, rel. to the sum of the two monosaccharides), the signal decreased over six measurements and remained rather constant afterward at about 80% of the starting value. An increase in the IL content caused a faster decrease but with similar end values (Fig. 4). Evidently, the IL traces contaminated the working electrode and decreased its effectivity so that seemingly smaller carbohydrate concentrations were reported. When the IL-free carbohydrate mixture was measured after the series of IL-containing samples, the concentration values remained lower: the electrode contamination effect was obviously long-lasting or even permanent. The values did not increase over 20 more measurements. Washing of the electrode in hot (100 °C) DMSO and/or polishing with a soft polypropylene wipe fully returned the initial capacity of the electrode and removed the contamination. It is obvious that these cleaning processes—at least the mechanical one—damage the gold layer of the electrode and are detrimental to the working electrode in the long term. Clearly, it would be advisable to avoid

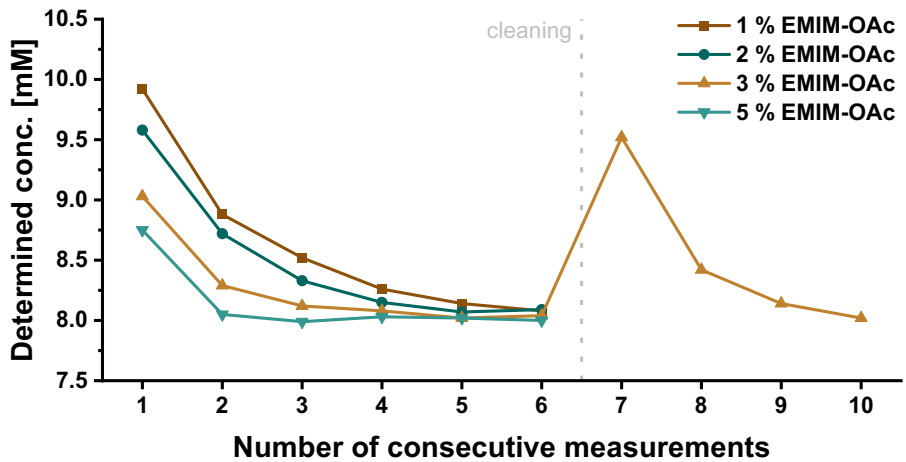


Fig. 4 Consecutive IC-PAD measurements (1–6) of an aqueous solution containing 10 mM methyl β-D-glucopyranoside and different concentrations of EMIm-OAc (wt%, rel. to the monosaccharide derivative). For the analyte with 3 wt% EMIm-OAc, the effect of electrode cleaning (see Fig. 3) is shown (between runs 6 and 7)

such tenacious contaminations of the electrode from the very beginning.

The fact that the electrode response decreases and then remains constant during subsequent measurements is indicative of a passivation process which covers the electrode by a very thin, probably even monomolecular layer, which still allows the electrode reactions to proceed, but at a lower rate, as reagents and electrons must pass through the additional layer. The layer also seems to protect the underlying electrode from further passivation, otherwise, the electrode response would continuously decrease and not remain constant at the lower level, as observed in our experiments. Concluding from our recent work on 1,3-dialkylimidazolium ion chemistry in connection with beta-irradiation (Jusner et al. 2023, Lehrhofer et al. 2024), we assume that the imidazolium-derived *N*-heterocyclic carbene (Jahnke and Hahn 2017), which is responsible for the observed chemical processes during electron beam treatment in ILs, is also generated at the electrode surface and covers the electrode tightly until removed mechanically or by suitable solvents. It is known that such carbenes form exceptionally stable coordination complexes/addition compounds with transition metals—among them Au—a chemistry that has been exploited for transition metal-based anticancer agents, nanoparticles, or glycoside synthesis (Zhang et al. 2024; Teles 2015; Porchia et al. 2018; Hosoya et al. 2014; John and Ghosh 2010; Yokota et al. 2008). Similar complex formation occurs between *N*-heterocyclic carbene and Au surfaces, such as on the material of the IC working electrode (de Souza et al. 2019; Lv et al. 2018; Mechelke et al. 2017). If this is indeed true, the observed passivation would not only impact the measurement but very slowly consume the electrode´s Au layer and thus permanently damage it.

The effects of the presence of traces of IL have not been investigated for IC conditions other than HPAEC-PAD (e.g. for anion or cation determinations), so no statements can be made about the effects of traces of IL under different IC conditions and modes.

High-performance thin-layer chromatography (HPTLC)

HPTLC allows easy and fast quantification of monosaccharides, also in complex matrices, such as those obtained in biorefinery approaches, fermentation broths, or hydrolysis liquors. While IC, GC, or HPLC have the risk of capillary or column clogging, HPTLC does not suffer from such restrictions. Constituents that are not completely dissolved or immobile in the mobile phase remain at the starting point of the TLC plate and do not interfere with the later separation along the stationary phase. In an HPTLC setup with sampling, plotting, development, and quantitation (UV/VIS scanning) being done in an automated way, the analytical figures of merit are well comparable to HPLC for biorefinery applications. Figure 5 shows the separation of D-glucose and D-galactose in the presence of different amounts of the ionic liquid EMIm-OAc. While the R_f values were not influenced by the presence of the IL, the signal intensities of the spots were evidently reduced, with higher IL concentrations having a more pronounced suppressive effect. While the concentration of both monosaccharides in the solution was in fact always the same, the presence of IL (1%, 0.5%, 0.2%) falsified the result when the spots were read out by the automatic scanner so that the reported monosaccharide concentrations were much lower than the expected values.

The presence of IL was clearly not compatible with reliable quantification of the monosaccharide content by HPTLC. The effect was similar for the monosaccharides and oligosaccharides tested. Interestingly, onic and uronic acid detection seems to be more severely affected, so that in these cases sometimes a complete “disappearance” of the analyte occurs in the presence of the IL (not shown). This effect might be a consequence of the ionic (ion pair) interaction of the onate/uronate anion with the imidazolium cation, which is stronger than the rather weak IL-analyte interaction in the case of neutral monosaccharides.

Gas chromatography (GC)

The negligibly small vapor pressure of 1,3-dialkylimidazolium-based ILs turns into a significant drawback if these ionic liquids enter a gas chromatography system as a trace impurity of an analyte. While solvent and conventional, volatile analyte components will enter the capillary as gases and will be separated during the transport along the stationary phase, the IL will mostly remain in the inlet and liner and contaminate them, and this contamination will increase in the case of multiple injections. Small amounts of the IL

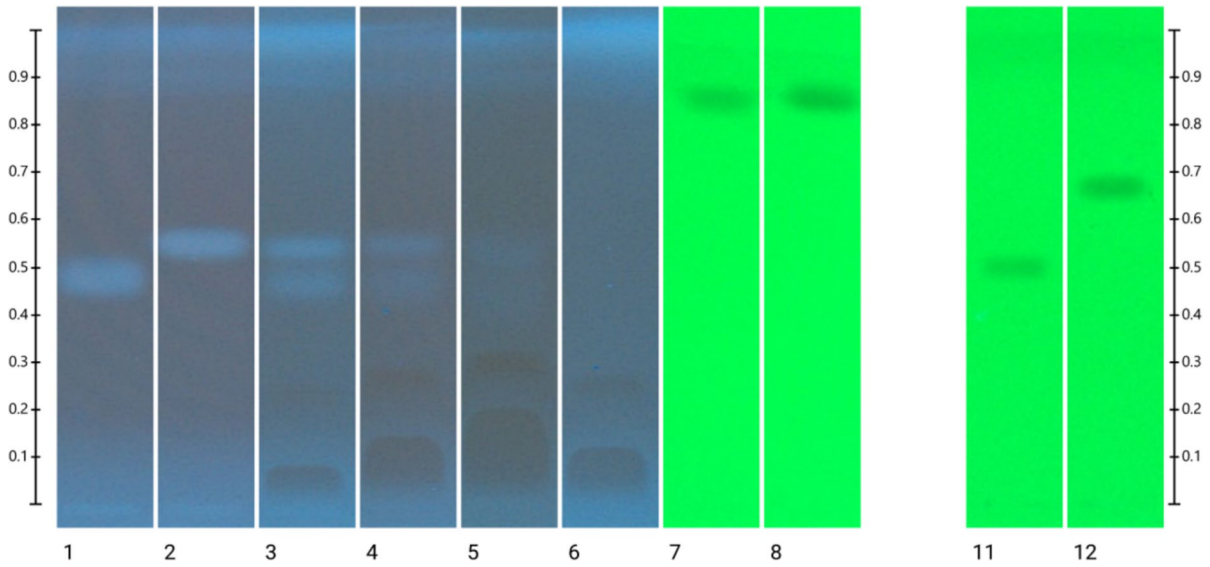


Fig. 5 HPTLC chromatograms of a model separation of equimolar mixtures of D-glucose and D-galactose. Track 1: 0.03 mg mL^{-1} D-glucose only; track 2: 0.03 mg mL^{-1} D-galactose only; Tracks 3–5: 0.03 mg mL^{-1} of each sugar, with 3) 0.2%, 4) 0.5% 5) 1% of EMIm-OAc (**1a**); track 6: EMIm-OAc (**1a**) only; track 7 + 11: EMIm-thione (**3**); track 8 + 12: BMIm-thione (**4**). Tracks 1–5: separation conditions for monosaccharides; tracks 7–8 and 11–12: separation conditions for treated ionic liquids

will be dragged along with the carrier gas in the form of neutral ion pairs, enter the capillary, slowly travel deeper into it, and may eventually reach the detector. This causes a gradual increase of the background in the case of MS detection, with "classical" symptoms of capillary bleeding and contamination (see Fig. 6a). Since it is almost impossible to flush out the IL once it entered the capillary, the only remedies are to shorten the capillary mechanically from its liner side, trying to remove at least the most heavily IL-contaminated part, and to perform a thorough cleaning of the inlet and replacing the contaminated liner (Fig. 6b), or eventually using a new capillary. It is obvious that IL traces in analytical GC samples – which can even impair the analytical hardware in the manner described – are a highly undesirable occurrence that should be avoided as far as possible.

Removing 1,3-dialkylimidazolium IL traces from analytical samples

Given the negative effects that 1,3-dialkylimidazolium IL traces in analytes can have on different analytical methods, it seemed obvious that a protocol to neutralize such impurities and render them harmless would be most welcome. We started with the requirement that up to 5 wt% of IL (relative to the sum mass of the analyte) should be removed, although the actual content in most samples would probably be one (to two) orders of magnitude lower. Further, a possible additional treatment should evidently interfere as little as possible with the analyte and the analytical techniques, of which we focused on IC, HPTLC and GC (vide supra). Adsorption and ion exchange techniques, although well-suited to remove the IL, would be generally unsuitable as it cannot be guaranteed that ionic components in the analyte, *e.g.*, frequent analyte components, such as onic and uronic acids, carboxylic acids, and hydroxy-acids, would not be retained as well.

After quite extensive and unfortunately rather unsuccessful trials to remove the IL traces by adsorption and separation techniques, we resorted to a chemical modification approach. Ideally, this would require a selective modification of the IL that cancels out its disadvantageous analytical properties, but without compromising the chemical integrity of any other component of the analyte and solvent. Considering the complexity of analytes in the field of biorefinery

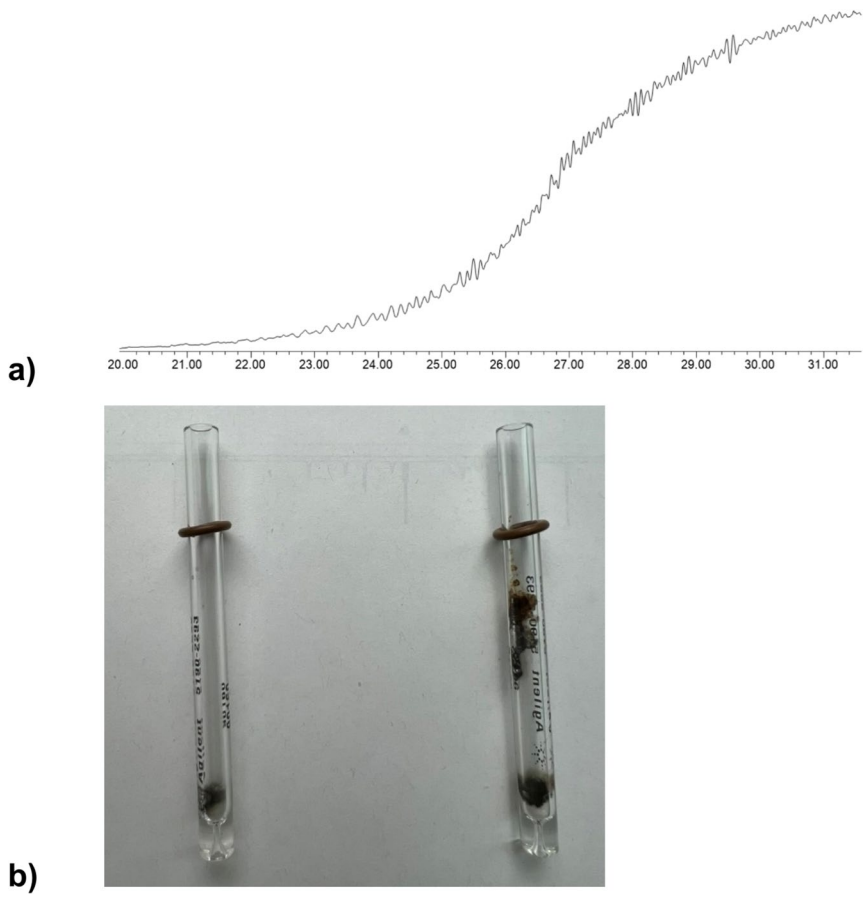
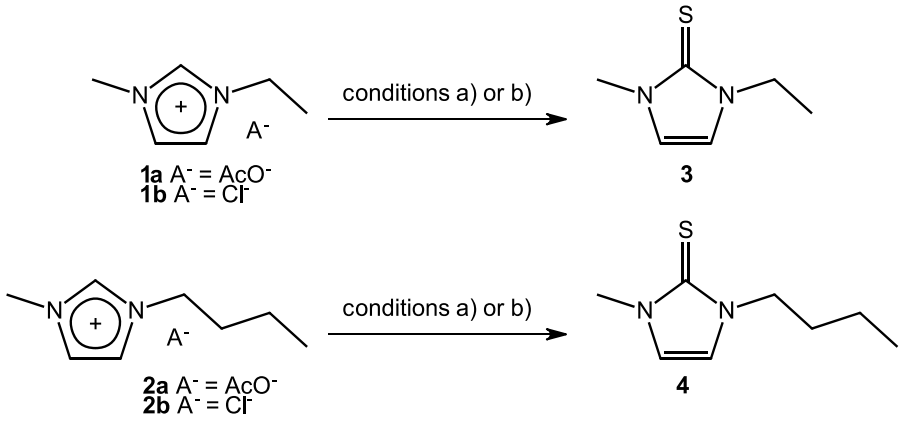


Fig. 6 **a** Flushing a GC capillary (DB-5ms) after previous sugar analysis of samples containing trace IL impurities (0.1 wt% EMIm-OAc), section of chromatographic trace, t > 20 min. The increased background noise and "bleeding" remained constant and did not decrease for more than 4 h. **b** Mildly contaminated (left) and heavily contaminated GC liners which require thorough cleaning or exchange

analytics, this seemed rather utopian until we were reminded – admittedly serendipitously in conjunction with studies on beta-irradiation studies of ILs (Jusner et al. 2023; Henniges et al. 2012) and residual chromophores in cellulosics (Rosenau et al. 2007)—of the very facile formation of the carbene derived



Scheme 1 Conversion of 1,3-dialkylimidazolium cations from ionic liquids into the corresponding 2-thiones by stirring with elemental sulfur. Conditions **a** preparative synthesis of an authentic sample according to a modified literature protocol (Laus et al. 2013), conditions **b** protocol for removing IL traces from analytical samples

from 1,3-dialkylimidazolium cations and its neat follow-up reaction with elemental sulfur to the corresponding thiones (Laus et al. 2013), see Scheme 1.

The reaction with S_8 converts 1,3-dialkylimidazolium cations on a preparative scale in nearly quantitative yields into the corresponding 2-thiones when stoichiometrically equivalent amounts of co-reactants are used. If the co-reacting sulfur is offered in a large excess, as in the present case where only IL traces are present, the conversion is complete within a few minutes, and the yields are practically quantitative. The reaction is largely independent of the reaction medium: it proceeds equally well in aprotic media, in protic media such as alcohols or pyridine, and also in aqueous solvent mixtures (tested up to 65 vol% of water in acetone or ethanol). The resulting thiones are neutral heterocyclic compounds: upon reaction, the 1,3-dialkylimidazolium moiety loses its cationic and aromatic character.

In contrast to their 1,3-dialkylimidazolium precursors, the thiones behave quite "nicely" in the analyses: in IC, the thiones are redox-neutral and thus "blinded out", becoming invisible under the usual conditions of electrochemical carbohydrate detection, without having any negative influence on the other (carbohydrate) analytes. Most notably, there is no negative effect on the stability of the electrode response as in the case of the IL traces, so the IL conversion into the thiones combined with measurement on a freshly conditioned working electrode provides consistent and constant quantification (Fig. 7). In TLC, the thiones show clear spots when their concentration is high enough (Fig. 8). In low concentrations, such as those of the IL traces in the analytes, the thiones are scarcely TLC-detectable, and their formation reliably cancels out the negative quantification effects of the IL traces on the carbohydrates. In GC analysis, the thiones are readily volatilized and transported through the capillary without residues and interferences (Fig. 9), giving clear and well-distinguishable peaks.

The reagent used to prepare the thiones, elemental sulfur that is mainly present as the cyclic octamer S_8, has very low general reactivity under the conditions of sampling and analysis, so that a reaction with components of the analyte other than the IL is rather unusual, if not highly unlikely. Optimization trials have established that the sulfur is best applied as precipitate on neutral alumina (sulfur@alumina, 5 wt%, a mixture of basic and acidic alumina) which is simply prepared by adding the alumina to a solution of sulfur

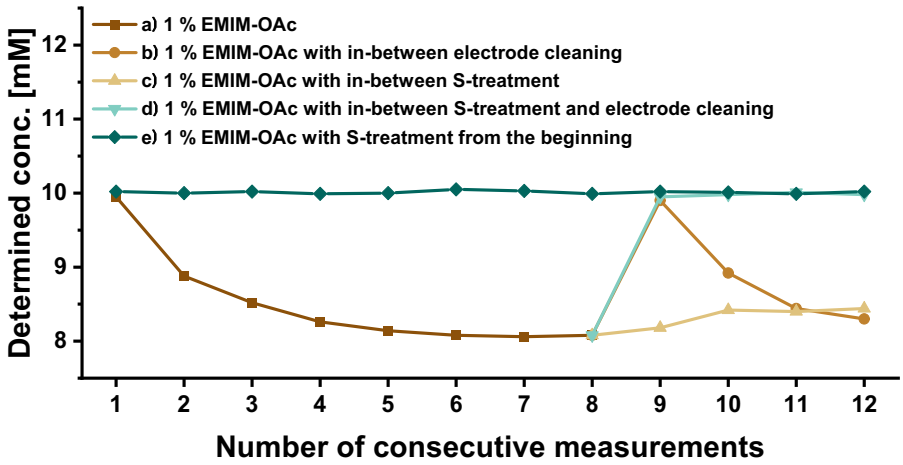


Fig. 7 Consecutive IC-PAD measurements (1–6) of an aqueous solution containing 10 mM methyl β-D-glucopyranoside and 1 wt% EMIm-OAc (rel. to the monosaccharide derivative). **a** direct measurement on a freshly cleaned electrode (1–7); **b** direct measurement on a freshly cleaned electrode (1–7) followed by another electrode cleaning step and further measurement (8–12); **c** direct measurement on a freshly cleaned electrode (1–7) followed by sample treatment with elemental sulfur and further measurement (8–12) without another electrode cleaning step; **d** direct measurement on a freshly cleaned electrode (1–7) followed by sample treatment with elemental sulfur, another electrode cleaning step and further measurement (8–12); **e** sample treatment with elemental sulfur before measurement on a freshly cleaned electrode. For the S_8-treatment, see the protocol in the Materials and Methods section. Note that the purification protocol gives improvements only when used from the beginning on a clean, non-contaminated electrode, or after an in-between electrode cleaning step

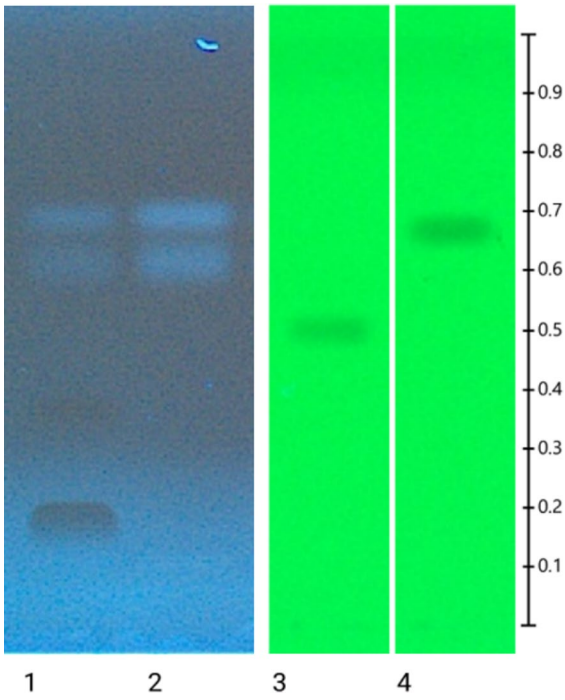


Fig. 8 HPTLC chromatograms of a model separation of equimolar mixtures of D-glucose and D-galactose containing 0.5% of EMIm-OAc (eluant: methanol/water, v/v = 6/4). Track 1: direct measurement, track 2: measurement after IL removal through conversion into the corresponding thione by S_8-treatment (separation conditions for monosaccharides). Tracks 3 and 4: HPTLC of thiones derived from EMIm and BMIm (separation conditions for thiones / treated samples)

in toluene and evaporating the solution to dryness. The alumina has two functions: first, the basic centers aid the formation of the corresponding *N*-heterocyclic carbene (Gurau et al. 2011), which then immediately reacts with the sulfur, and second, the formed thiones are obviously adsorbed to a large extent to the alumina surface where they just had been formed so that the concentration of thiones in the analysis solution is thus significantly reduced. It should be noted that alumina cannot be replaced by silica, Celite®, or other carriers because the alkaline centers are a prerequisite for substantial carbene formation and thus for the thione formation to proceed. Also, neutral alumina cannot be replaced by basic alumina (in an attempt to further expedite IL conversion), as this alumina variant would adsorb acidic components in the analyte, such as organic acids, which is not the case with neutral alumina. We took water-free alumina (Brockmann grade 1) for preparation of the solid sulfur@alumina to be used in combination with all non-aqueous solvents, and Brockmann grade 5 alumina (non-hygroscopic) for use in aqueous media and binary water-containing solvents. The solubility of sulfur in other very apolar media, such as alkanes, cycloalkanes, benzene or toluene, is also noticeable but is much higher for sulfur than for sulfur@alumina. Although the derivatization reaction itself proceeds readily and the release of sulfur into the solvent would be minimal, these apolar solvents should be replaced by polar or polar protic solvents before subsequent analysis to avoid unnecessary uptake

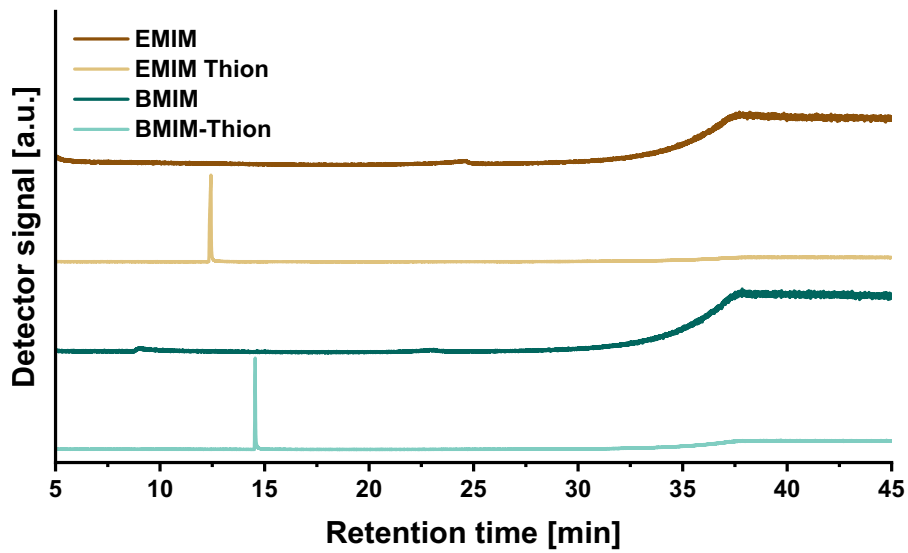


Fig. 9 GC chromatographic traces of the thiones and their parent ILs. From top to bottom: EMIm-OAc (**1a**), 1-ethyl-3-methyl-1,3-dihydro-2*H*-imidazole-2-thione (**3**), BMIm-OAc (**2a**), 1-butyl-3-methyl-1,3-dihydro-2*H*-imidazole-2-thione (**4**). For the behavior of the ILs in GC, *cf.* also Fig. 6a

of sulfur into the analytical systems. After solvent screening, we can recommend all common solvents, such as ethyl acetate, THF, dioxane, dichloromethane, pyridine, chloroform, methanol, ethanol, 2-propanol or their mixtures—which are commonly used for GC and HPTLC detection—for the IL-removal protocol and subsequent analysis.

Conclusions

Traces of 1,3-dialkylimidazolium ionic liquids impair the accuracy of analyses by GC, IC and HPTLC—the negative influence of the IL traces on these analytical methods for monosaccharide determination is reported here for the first time. Even more detrimental, the IL traces can permanently compromise the integrity of IC detector electrodes and GC capillaries and deteriorate the quality of the measurements. In the case of ion chromatography, it can be assumed that the formation of the 1,3-dialkylimidazolium-derived carbene and its strong interaction with the electrode material (Lv et al. 2018) is the reason for the loss of sensitivity. In gas chromatography, the missing (or extremely low) volatility of the ILs and subsequent thermal degradation cause the undesired capillary effects and the loading of the capillary/liner with contaminants that are hard to remove. The reason for the negative influence on the detectability of carbohydrates (and carbohydrate derivatives) in HPTLC can only be speculated at present. Although it is known that 1,3-dialkylimidazolium ionic liquids react with the reducing ends of aldoses of cellulose (Ebner et al. 2008), this reaction cannot take place in the case of the methyl aldopyranoside compounds used, and the concentration of IL would also be far too low to explain the observations of the apparent "disappearance" of the analytes.

To avoid such problems, an easy remedy is introduced: suspending elemental sulfur—a roughly tenfold molar excess—in the solution of the analyte for 10 min under stirring. This will convert the 1,3-dialkylimidazolium ions into the corresponding 1,3-dialkyl-imidazole-2-thiones, which have two favorable properties: their adsorbates at alumina exhibit very low extractability into most apolar, aprotic and protic solvents and—if they should dissolve nevertheless—have no negative effect on IC, HPTL, or GC separation. If freshly precipitated sulfur on neutral alumina is used, the stirring time can in most cases be reduced to 5 min and the formed thione is reliably absorbed onto the solid phase in standard GC and HPTLC solvents. Even in rather polar aprotic solvents, such as DMSO or DMF (which would be rather non-standard solvents for GC measurements anyway), only thione traces are dissolved and the main part remains bound to the alumina matrix.

The method presented also works with other 1-alkyl-3-methylimidazolium ILs, which are reliably converted into their corresponding 2-thione derivatives. 1-Alkyl-3-methylimidazolium ILs with additional 2-substituent do not react because the C2-carbene intermediate required for thione formation cannot be formed.

If hydrolysates or similar samples from biomass treatments involving ILs are to be analyzed by GC, we generally recommend sulfur pretreatment before injection. The same applies to HPTLC or IC analysis, to eliminate measurement errors resulting from IL impurities contained. The additional step in the analytical protocol can easily be integrated into sample preparation (addition, stirring, and filtration), does not constitute a significant extension of analysis time, and does not involve any hazardous chemicals or tedious techniques. We thus hope that after a necessary "familiarization phase", the additional step will become a matter-of-course protocol in the respective analysis approaches for biomass hydrolysates in which the presence of IL traces cannot be ruled out with certainty.

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Authors' contributions All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was written by TR. All authors commented on previous versions and read and approved the final manuscript.

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Availability of data and material No datasets were generated or analysed during the current study. Data available from the authors upon request.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication All authors agreed to the publication in the submitted form.

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