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Guanidinium ionic liquid-based surfactants as low cytotoxic extractants: Analytical performance in an *in-situ* dispersive liquid–liquid microextraction method for determining personal care products

Idaira Pacheco-Fernández, Verónica Pino*, Juan H. Ayala, Ana M. Afonso

Departamento de Química, Unidad Departamental de Química Analítica, Universidad de La Laguna (ULL), La Laguna, Tenerife, 38206, Spain

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ABSTRACT

The IL-based surfactant octylguanidinium chloride (C_8Gu-Cl) was designed and synthetized with the purpose of obtaining a less harmful surfactant: containing guanidinium as core cation and a relatively short alkyl chain. Its interfacial and aggregation behavior was evaluated through conductivity and fluorescence measurements, presenting a critical micelle concentration value of 42.5 and 44.6 mmol L^{-1}, respectively. Cytotoxicity studies were carried out with C_8Gu-Cl and other IL-based and conventional surfactants, specifically the analogue 1-octyl-3-methylimidazolium chloride (C_8MIm-Cl), and other imidazolium- (C_{16}MIm-Br) and pyridinium- (C_{16}Py-Cl) based surfactants, together with the conventional cationic CTAB and the conventional anionic SDS. From these studies, C_8Gu-Cl was the only one to achieve the classification of low cytotoxicity. An *in situ* dispersive liquid–liquid microextraction (DLLME) method based on transforming the water-soluble C_8Gu-Cl IL-based surfactant into a water-insoluble IL microdroplet via a simple metathesis reaction was then selected as the extraction/preconcentration method for a group of 6 personal care products (PCPs) present in cosmetic samples. The method was carried out in combination with high-performance liquid chromatography (HPLC) and diode array detection (DAD). The method was properly optimized, requiring the use of only 30 μL of C_8Gu-Cl for 10 mL of aqueous sample with a NaCl content of 8% (w/v) to adjust the ionic strength and pH value of 5. The metathesis reaction required the addition of the anion exchange reagent (bis[(trifluoromethyl)sulfonyl]imide – 1:1 molar ratio), followed by vortex and centrifugation, and dilution of the final microdroplet up to 60 μL with acetonitrile before the injection in the HPLC-DAD system. The optimum *in situ* DLLME-HPLC-DAD method takes ~10 min for the extraction step and ~22 min for the chromatographic separation, with analytical features of low detection limits: down to 0.4 μg L^{-1}; high reproducibility: with RSD values lower than 10% (intra-day) and 16% (inter-day) for a spiked level of 15 μg L^{-1}; and an average enrichment factor of 89. The requirement of low volumes (30 μL) of a low cytotoxic IL-based surfactant allows the method to be considered less harmful than other common analytical microextraction approaches.

1. Introduction

Ionic liquids (ILs) constitute a well-known group of organic salts with melting points below 100 °C [1]. These non-conventional solvents present a unique set of properties, such as high chemical and thermal stability, negligible vapor pressure at room temperature, and ease of synthesis. Several of their properties, including solubility, viscosity, and solvation ability with a variety of compounds, can be tuned by modifying their chemical composition [2].

There is an impressive number of applications of ILs as promising alternatives to toxic conventional organic solvents, given their adequate solvation ability and their claimed non-toxicity, because they do not generate volatile organic compounds to atmosphere [3–5]. Nevertheless, diverse studies have stated the toxicity of several ILs [6–11]. In this sense, it results quite important to design appropriately the IL structure with improved physico-chemical

* Corresponding author.
E-mail addresses: ipacheco@ull.edu.es (I. Pacheco-Fernández), veropino@ull.edu.es (V. Pino), jayala@ull.edu.es (J.H. Ayala), aafonso@ull.edu.es (A.M. Afonso).

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properties and safer toxicological profiles. In general, literature studies agree that the cation moiety plays an important role in the toxicity of the resulting IL, being morpholinium [9], guanidinium [6,11], piperazinium [11], and choline [6] cations, those providing lower toxicity compared to the commonly used imidazolium, pyridinium, pyrrolidinium, ammonium, and phosphonium-based ILs. An increase in the side chain length attached to the IL cation can also lead to a significant increase in the IL toxicity [6,7,10,11], whereas the functionalization of the side chain attached to the IL cation (particularly incorporating ether groups) may reduce the toxicity of the resulting IL [7,8]. Despite the knowledge of proper IL design to produce less toxic ILs, the wide majority of analytical applications using ILs as extraction solvents utilize imidazolium-based ILs [3,12].

IL-based surfactants constitute a type of ILs derivatives capable of forming micellar aggregates when dissolved in water at concentrations above the critical micelle concentration (CMC) [13–16]. This new type of cationic surfactants can be monocationic or multicationic [17], and present lower CMC values than conventional surfactants with similar structures [15], permitting in this way to take advantage of their micellar properties in extraction methods with much lower amounts of reagents [13].

Applications of IL-based surfactants as solvents in sample preparation methods initially involved the extraction of organic compounds from aqueous samples with the aid of microwaves [18–21] or ultrasounds [22–25]. IL-based surfactants have also been used in micro solid-phase extraction (μ-SPE) methods linked to solid supports forming hemimicelles and admicelles [26,27]. In these extraction schemes, lower amounts of IL-based surfactants are required since this type of aggregates can form at concentrations below the CMC. In several cases, the method is further improved by using magnetic nanoparticles as solid support for these hemimicelles/admicelles, allowing the use of magnetic-based microextraction approaches [28–30].

More recently, our group has proposed the inclusion of a preconcentration strategy for IL-based surfactants [31] through the application of the *in-situ* dispersive liquid–liquid microextraction (DLLME) technique [32,33]. In this method, a water-soluble IL-based surfactant is used as extractant solvent. Then, a metathesis reaction is accomplished, and the water-soluble IL is transformed into a microdroplet of water-insoluble IL by controlling the appropriate ratio of IL-based surfactant/anion exchange reagent. The formed microdroplet contains the preconcentrated analytes [3,31]. Thus, the IL-based surfactant 1-hexadecyl-3-butylimidazolium bromide ($C_{16}C_4$Im-Br) has been used successfully in this integrated extraction-preconcentration mode for the determination of polycyclic aromatic hydrocarbons (PAHs) in toasted cereals [31], and for determining copper (II) in water samples [34]. 1-hexadecyl-3-methylimidazolium bromide (C_{16}MIm-Br) has also been used as optimum extractant solvent in a microwave-assisted extraction procedure followed by an *in-situ* DLLME preconcentration step to determine PAHs, alkylphenols and butylparaben from sediments [35].

The focus of the current study is to utilize for the first time a guanidinium-based IL as extraction solvent, with the purpose of proposing this new generation of ILs as solvents of low cytotoxicity. The IL-based surfactant octylguanidinium chloride (C_8Gu-Cl) is synthetized, its micellar behavior is evaluated, and its cytotoxicity is investigated in comparison with several conventional surfactants and a group of imidazolium and pyridinium IL-based surfactants. Once proper cytotoxicity is shown for C_8Gu-Cl (particularly compared with ILs and conventional surfactants), the *in-situ* DLLME application is carried out for a group of personal care products (PCPs), including parabens and benzophenones, from cosmetic samples.

2. Experimental

2.1. Chemicals, reagents and materials

The analytes studied in this work included a group of personal care products: methylparaben (MePa, 99.5%), ethylparaben (EtPa, 99.5%), propylparaben (PPa, 99.5%), isopropylparaben (iPPa, 98%), benzophenone (BP, 99.5%), and benzophenone-3 (BP3, 99.5%). MePa, EtPa, and PPa were purchased from Dr. Ehrenstorfer GmbH (Augsburg,Germany); BP and BP3 were supplied by Sigma-Aldrich (Steinheim, Germany); and iPPa was acquired from Alfa Aesar (Karlsrube, Germany). Individual standard solutions (1000–3000 mg L^{-1}) were prepared in acetonitrile supplied by VWR International Eurolab (Barcelona, Spain). Intermediate standard solutions containing all analytes at concentrations of 5 mg L^{-1}, 20 mg L^{-1} and 100 mg L^{-1} (in acetonitrile) were prepared. All solutions were stored protected from light at 4 °C. Working solutions in ultrapure water (10 mL) were prepared by dilution of the intermediate solutions at concentration levels ranging from 5 to 500 μg L^{-1}.

Octylamine (99.5%) and 1H-pyrazole-1-carboxamidine hydrochloride (99%), obtained from Sigma-Aldrich, were used to synthetize the IL-based surfactant. Lithium bis[(trifluoromethyl)sulfonyl]imide (Li-NTf_2), used as anion exchange reagent during metathesis, was also supplied by Sigma-Aldrich.

The cytotoxicity produced by the tested surfactants was evaluated against the J774.1 murine macrophage cell line (ATCC TIB-67, American Type Culture Collection LG Promochem, Spain). A cytotoxicity detection kit (lactate dehydrogenase; Roche Applied Science) was used according to the manufacturer's recommendations.

The conventional nonionic surfactant Triton X-100, the conventional cationic surfactant cetyltrimethylammonium bromide (CTAB), the conventional anionic surfactant sodium dodecyl sulfate (SDS), the imidazolium-based surfactant 1-octyl-3-methylimidazolium chloride (C_8MIm-Cl), and the pyridinium-based surfactant hexadecylpyridinium chloride (C_{16}Py-Cl), were supplied by Sigma-Aldrich. The imidazolium-based surfactant C_{16}MIm-Br was synthetized and characterized in our laboratory according to a previous work [36].

Ultrapure water (18.2 mΩ cm) was obtained from a Milli-Q gradient A10 system (Millipore, Bedford, MA, USA). Pyrene (>97.0%), acetic acid (99%), hydrochloric acid (36.5–38%, v/v), sodium hydroxide (>99%), and HPLC-grade acetonitrile, were purchased from Sigma-Aldrich. Absolute ethanol was acquired to Panreac (Barcelona, Spain), and sodium acetate trihydrate (99.5%) was acquired to Scharlau (Barcelona, Spain).

Three commercial facial tonics were analyzed. They were purchased in a local store. Facial tonic −1 was commercially labelled as paraben-free. MePa was tagged in facial tonic −2 and facial tonic −3, whereas PPa was also tagged in facial tonic −2.

The *in-situ* DLLME procedure and the conductivity measurements of the IL-based surfactant solutions were performed in 15 mL Glass PIREX® centrifuge tubes. A 25 μL syringe supplied by Hamilton (Reno, Nevada, USA) was employed to handle the microdroplet obtained in the *in-situ* DLLME method.

2.2. Instrumentation and equipment

A NMR spectrometer AVANCE™ (500 MHz) from Bruker (Massachusetts, USA) and a LCT Premier time of flight (TOF) Mass Spectrometer with electrospray ionization (ESI) from Water Micromass (Singapore) were used for the characterization of the IL-based surfactant.

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