Analytical Methods

Development and optimization of a naphthoic acid-based ionic liquid as a "non-organic solvent microextraction" for the determination of tetracycline antibiotics in milk and chicken eggs

Jiajia Gao [a], Hui Wang [a,1], Jingang Qu [a], Huili Wang [b], Xuedong Wang [a,*]

[a] Key Laboratory of Watershed Sciences and Health of Zhejiang Province, Wenzhou Medical University, Wenzhou 325035, China
[b] College of Life Sciences, Wenzhou Medical University, Wenzhou 325035, China

ABSTRACT

In traditional ionic liquids (ILs)-based microextraction, ILs are often used as extraction and dispersive solvents; however, their functional effects are not fully utilized. Herein, we developed a novel ionic liquid 1-butyl-3-methylimidazolium naphthoic acid salt ($[C_4MIM][NPA]$) with strong acidity. It was used as a mixed dispersive solvent with conventional $[C_2MIM][BF_4]$ in "functionalized ionic liquid-based non-organic solvent microextraction (FIL-NOSM)" for determination of tetracycline antibiotics (TCs) in milk and eggs. Utilization of $[C_4MIM][NPA]$ in FIL-NOSM method increased extraction recoveries (ERs) of TCs by more than 20% and eliminated the pH adjustment step because of its strong acidity. Under optimized conditions based on central composite design, the ERs of four TCs were 94.1–102.1%, and the limits of detection were 0.08–1.12 µg kg^{-1} in milk and egg samples. This proposed method provides high extraction efficiency, less pretreatment time and requires non-organic solvents for determination of trace TC concentrations in complex animal-based food matrices.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Tetracycline antibiotics (TCs) are broad spectrum agents, exhibiting high activity against infections caused by Gram-positive and Gram-negative bacteria, as well as chlamydia, mycoplasmas, rickettsiae and protozoan parasites (Nikolaidou, Samanidou, & Papadoyannis, 2009). Eight types of TCs are now commercially available, of which oxytetracycline (OTC), tetracycline (TC), chlorotetracycline (CTC) and doxycycline (DC) are commonly administered to food-producing animals in China (Liu et al., 2009). In recent years, the abundant and in some cases improper use of TCs has resulted in the presence of TC residues in edible animal foods, which may be toxic and dangerous for human health (Jing et al., 2010; Sun, He, He, Zhang, & Chen, 2009). Additionally, when consumed for long periods, low doses of TCs in foods can lead to drug-resistant bacteria in humans (Ahmadi, Shahbazi, & Karami, 2015). Accordingly, the maximum residue limits of TCs have been established by EU, FAO/WHO and FDA as 0.1 mg kg^{-1} in milk and 0.2 mg kg^{-1} in egg samples. Due to the potentially adverse effects of TCs in food, it is critical to develop reliable ana-lytical methods for routine monitoring of TCs in foods that are rapid, precise, economical in cost and time, and harmless to the environment (Lu, Zhang, Guo, Zhang, & Sun, 2015). Currently, TCs are determined by HPLC with different detection modes, such as ultraviolet, fluorescence and mass spectrometry (Freitas, Porto, Vieira, & Siqueira, 2010; Jia, Xiao, Hu, Asami, & Kunikane, 2009; Wang, Yang, Zhang, Mo, & Lu, 2008; Yu, Tao, Chen, Wang, & Yuan, 2011). Before HPLC analysis, extraction and preconcentration procedures are very important for the determination of TCs at low levels. Liquid-liquid extraction (LLE) (Zhao, Zhang, & Gan, 2004) and solid-phase extraction (SPE) (Jin et al., 2010; Pailler, Krein, Pfister, Hoffmann, & Guignard, 2009) have been extensively applied to the pretreatment of TCs in liquid food samples. Additionally, dispersive liquid-liquid microextraction (DLLME), in-tube solid-phase microextraction (SPME), hollow fiber based liquid phase microextraction (HF-LPME) and dispersive micro solid-phase extraction have been developed for determination of TCs (Hu, Pan, Hu, Huo, & Li, 2008; Noorfatimah et al., 2015; Shariati, Yamini, & Esrafili, 2009; Zhang, Zhang, & Jiao, 2015). Except for DLLME, most of these methods are characterized as instrument-based techniques and still require time-consuming extraction steps. There are several prominent advantages for traditional DLLME, such as microliter-level volumes required for extraction and dispersive solvents and short extraction times. However,

---

* Corresponding author.
  E-mail address: zjuwxd@163.com (X. Wang).
¹ Co-first author.

an obvious disadvantage of traditional DLLME is the need for organic solvents as the extraction and dispersive solvents. Consequently, ionic liquid-based microextraction techniques are becoming more widespread in recent years (Dong et al., 2013; Rajabi, Asemipour, Barfi, Jamali, & Behzad, 2014).

Ionic liquids (ILs), consisting of organic cations and inorganic or organic anions with melting points at or below 100 °C, have been widely applied as "green solvents" to improve extraction and enrichment performance as compared to the traditional use of organic solvents. Currently, ILs are widely used in microextraction procedures as dispersive or extraction solvents according to their different solubilities. Several previous studies have shown that the alkylimidazolium hexafluorophosphate-based IL, ([C$_n$MIM] PF$_6$), is an appropriate extraction solvent in DLLME procedures (Sun, Chen, & Shi, 2014). The literature indicates that three kinds of [C$_n$MIM]PF$_6$-based DLLME are widely used. The first is based on traditional DLLME and involves a small amount of organic solvent that functions as the disperser. The extraction process is initiated by the formation of a cloudy solution, and the analyte enriched IL phase is then sedimented by centrifugation (Carro, Gonzalez, & Lorenzo, 2013; You, Wang, Liu, & Shi, 2013; Zhang, Gao, Peng, Li, & Zhou, 2011). The second approach is *in situ* IL-based DLLME involving an *in situ* metathesis reaction. A hydrophilic IL, such as [C$_n$MIM]Cl or [C$_n$MIM]BF$_4$, is dissolved in the aqueous phase to promote interaction between the IL and analytes. An ion-exchange reagent, such as NH$_4$PF$_6$ or LiNTf$_2$, is then introduced to perform the *in situ* metathesis reaction (Yao & Anderson, 2009). A turbid solution with fine IL microdroplets is formed and produces a hydrophobic IL. A significant advantage of this method is that the metathesis reaction and extraction are accomplished in one step making it rapid and amendable to high-throughput analysis. More recently, a "whole ILs-DLLME" was introduced, in which the hydrophilic ionic liquid [C$_n$MIM]BF$_4$ or [C$_n$MIM]Cl was used as the disperser to aid in the dispersion of the hydrophobic ionic liquid [C$_n$MIM]PF$_6$ extractor into the aqueous phase (Gao, Yang, Yu, Liu, & Zhang, 2012; Sun et al., 2014). Importantly, no organic solvent is utilized in this IL-based DLLME procedure making this method more environmentally friendly.

Because the chemical and physical properties of ILs can be easily adjusted by suitable selection of cation and anion species, the structural adjustability is an important advantage of ILs and functionalized ILs are easily obtained (Sun et al., 2014). Although hydrophobic and hydrophilic ILs are used as extraction and dispersive solvents, respectively, in the "whole ILs-DLLME", the functional effects of ILs are not utilized as part of the microextraction procedure. In this investigation, we introduced 1-naphthoic acid into the imidazolium ring to synthesize a novel ionic liquid 1-butyl-3-methylimidazolium naphthoic acid salt ([C$_4$MIM][NPA]). In microextraction procedures, the mixture of two ILs, including [C$_n$MIM]BF$_4$ and [C$_4$MIM][NPA], was selected as the dispersive solvent. Addition of this novel ionic liquid provided two prominent benefits: (1) it promoted a non-polar environment due to its lower solubility than [C$_n$MIM]BF$_4$ in aqueous solution, increased volume of the sedimented phase resulting from [C$_n$MIM]+, and thus enhanced extraction recoveries for TCs; and (2) because [C$_4$MIM] [NPA] is strongly acidic in aqueous solution, it acts as a pH modifier. Thus, with the addition of the novel ionic liquids, the pH of the solution will be simultaneously changed eliminating the need for an additional pH adjustment step.

Based on the considerations discussed above, this study aimed to develop a novel ionic liquid and to further use its beneficial functional effects to enhance ILs-based microextraction. The goal of the novel ionic liquid was to improve extraction efficiency of TCs and to simplify and expedite the extraction procedure by eliminating the pH adjustment step. As no organic solvents are used in this newly developed microextraction procedure, we designated

this method as "functionalized ionic liquid-based non-organic solvent microextraction (FIL-NOSM)". The optimized FIL-NOSM method achieved higher extraction efficiencies for simultaneous determination of four TCs based on a simple, rapid and environmental friendly procedure. Optimization of the major operational parameters was conducted using a response surface method (RSM) based on central composite design (CCD). The optimized method was compared with other common methods to evaluate its advantages and feasibility for determining trace levels of TCs in animal-derived foods. To the best of our knowledge, this is the first application of the FIL-NOSM method, based on [C$_4$MIM] [NPA], for the pretreatment of trace-level TCs in milk and egg samples.

2. Materials and methods

2.1. Chemicals and reagents

Certified reference standards (purities >98%), including TC, OTC, CTC and DC, were obtained from Sigma-Aldrich (St. Louis, MO, USA). Chromatographic-grade methanol and acetonitrile were purchased from Merck Co., Ltd. (Darmstadt, Germany). Ionic liquids of 1-butyl-3-methylimidazolium hexafluorophosphate ([C$_4$MIM][PF$_6$]), 1-hexyl-3-methyl-imidazolium hexafluorophosphate ([C$_6$MIM][PF$_6$]), 1-octyl-3-methylimidazolium hexafluorophosphate ([C$_8$MIM][PF$_6$]), 1-ethyl-3-methylimidazolium tetrafluoroborate ([C$_2$MIM][BF$_4$]) and ammonium hexafluorophosphate (NH$_4$PF$_6$) were purchased from Shanghai Chengjie Chemical Co., Ltd. (Shanghai, China). The novel ionic liquid, 1-butyl-3-methylimidazolium naphthoic acid salt ([C$_4$MIM][NPA]), was synthesized by our group as described below. Other chemicals were of analytical grade and purchased from Tianjin Chemical Reagent Co., Ltd. (Tianjin, China). Ultrapure water (18.2 MΩ cm, 25 °C) from a Millipore Milli-Q system (Bedford, MA, USA) was used for preparation of the mobile phase and sample solutions.

2.2. Instrumentation

An Agilent 1260 HPLC system equipped with a tunable UV detector was used for tetracycline quantification. A Zorbax Eclipse SB-C$_{18}$ column (5 μm, 4.6 mm × 250 mm) was utilized for separation of analytes from a 20 μL sample loop injection. The mobile phase consisted of a 0.01 mol L^{-1} Na$_2$HPO$_4$ aqueous solution (pH 2.5) and acetonitrile (20:80, V/V) at a constant flow rate of 0.8 mL min^{-1}. The column temperature was kept at 25 °C, and the detection wavelength was set at 355 nm. Ultrasound mixing was carried out by a model SB-5200D ultrasound cleaner (Ningbo Scientific Biology Corp., Ningbo, China). The phase separation was performed with a model TDL-50C centrifuge (Anting Instrument Factory, Shanghai, China). A 310P-01N pH meter (ThermoFish Scientific, Waltham, MA, USA) was applied for pH measurement. A XH-D vortex mixer (Shanghai Zhengqiao Scientific Instrument Corp., Shanghai, China) was used for mixing solutions.

2.3. Collection and preparation of samples

Milk and chicken egg samples were purchased from Wenzhou Baixin Supermarket (Wenzhou, China). Aliquots of milk (3 mL, pH = 6.81) were mixed with 200 μL of 20% acetic acid aqueous solution in a 10 mL centrifuge tube, followed by the addition of 2.8 mL water. The sample was ultrasonically shaken for 1 min and stored in a refrigerator at 4 °C for 15 min. Finally, each sample was centrifuged for 15 min at 3000 rpm and filtered with a 0.22 μm PTFE membrane filter. After the above procedures, the pH of pretreated milk samples was changed to 5.37.