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Aqueous two-phase based on ionic liquid liquid–liquid microextraction for simultaneous determination of five synthetic food colourants in different food samples by high-performance liquid chromatography

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ABSTRACT

A rapid and effective method of aqueous two-phase systems based on ionic liquid microextraction for the simultaneous determination of five synthetic food colourants (tartrazine, sunset yellow, amaranth, ponceau 4R and brilliant blue) in food samples was established. High-performance liquid chromatography coupled with an ultraviolet detector of variable wavelength was used for the determinations. 1-alkyl-3-methylimidazolium bromide was selected as the extraction reagent. The extraction efficiency of the five colourants in the proposed system is influenced by the types of salts, concentrations of salt and [C_nMIM]Br, as well as the extracting time. Under the optimal conditions, the extraction efficiencies for these five colourants were above 95%. The phase behaviours of aqueous two-phase system and extraction mechanism were investigated by UV–vis spectroscopy. This method was applied to the analysis of the five colourants in real food samples with the detection limit of 0.051–0.074 ng/mL. Good spiked recoveries from 93.2% to 98.9% were obtained.

1. Introduction

Synthetic or natural food colourants are usually added to foodstuffs to make them more visually attractive to consumers and to restore their original appearance when it has been lost during production processes (Kucharska & Grabka, 2010). Studies demonstrate that the chronic consumption of synthetic colourants seems to predispose to neurotoxicity in young and adult animals and some synthetic food colors could be reduced by azoreductase enzymes in intestinal bacteria and in liver cells with the release of aromatic amines to the organism (Hildenbrand, Schmahl, Wodarz, Kimmel, & Dartsch, 1999; Nagaraja & Desiraju, 1993). Considering both the potential effects on human health and the need for knowledge of the components of food, detection of synthetic dyes in different food samples is an important issue to deal with. Various analytical techniques, included spectrophotometric methods (Turak & Ozgur, 2013), voltametric methods (Mo et al., 2010), differential pulse polarography (Chanlon, Joly-Pottuz, Chatelut, Vittori, & Cretier, 2005), thin layer chromatography (Oka et al., 1994), high-performance liquid chromatography (HPLC) and so on (Minioti, Sakellariou, & Thomaidis, 2007; Pereira Alves, Brum, Branco de Andrade, & Pereira Netto, 2008), have been developed to facilitate the simultaneous determination of various synthetic food colourants. Preconcentration method was usually used combined with these techniques to minimise potential interferences from diversified components present in food samples and concentrate the analyte from a low content of food sample (El-Shahawi, Hamza, Al-Sibaai, Bashammakh, & Al-Saidi, 2013; Pourreza & Ghomi, 2011; Shen, Zhang, Prinyawiwatkul, & Xu, 2014).

Aqueous two-phase system (ATPS) is a very promising technology for separation and pretreatment (Tang et al., 2014; Jiang, Lu, Tan, Liang, & Cui, 2014). Compared with the traditional organic solvent extraction and solid phase extraction, ATPS is considered to be environmentally friendly due to the bulk of both phases consist of water and no use of volatile organic solvent in the whole process. Since a new type of ATPS consisting of ionic liquid (IL) and salts were reported in 2003 by Rogers and co-workers for the first time, the IL–salt ATPS (IL–ATPS) has been applied to the separation and preconcentration of organic compounds, inorganic compound and biomolecules in different matrices (He, Li, Liu, Li, & Liu, 2005;

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Passos et al., 2013; Tan, Li, & Xu, 2012; Yang et al., 2014). The IL–salt ATPS (IL–ATPS) has many advantages, such as low viscosity, little emulsion formation, no need of using volatile organic solvent, quick phase separation, high extraction efficiency, and gentle biocompatible environment.

In this paper, an IL–ATPS based on hydrophilic ionic liquid, 1-alkyl-3-methylimidazolium bromide ([C_nMIM][Br]), and salt was established to extract and determine the commonest five synthetic food colourants, tartrazine (Ta), sunset yellow (SY), amaranth (Am), ponceau 4R (Pon) and brilliant blue (BB), in different food samples with HPLC. The phase equilibrium of IL–ATPS and the factors on the extraction of colourants, such as the types of salts and IL, concentrations of salt and [C_nMIM][Br], as well as the extracting time and centrifugation time were discussed in detail. The [C_4MIM]Br-salt ATPS was not easy to emulsify and [C_4MIM]Br was diffluent in water and methanol (HPLC mobile phase). Colourants could be extracted into IL phase and analysed by HPLC with direct sampling after ATPS extraction. Under the optimum conditions, the proposed method was applied to preconcentration colourants in real food samples. In addition, the mechanism of IL–ATPS formation was discussed, and the extraction mechanism of the IL–ATPS was investigated by UV–vis spectroscopy.

2. Experimental

2.1. Apparatus

An Agilent 1260 HPLC system (Agilent, USA), equipped with a 1260 infinity quaternary pump, and a 1260 infinity variable wavelength detector (190–600 nm), was used for colourants determination. Chromatographic separation was achieved on a Eclipse plus-C_{18} column (4.6 mm × 150 mm × 3.5 μm) (Agilent, USA). All spectra measurements were carried out by using a model UV-2501 spectrophotometer (Shimadzu, Japan); all pH values were measured by a PHS-25B pH-metre (Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China) and the phase separation was assisted with a TG16-WS centrifuge (Hunan Xiangyi Centifuge Instrument Co., Ltd., Chang sha, China).

2.2. Standard solutions and reagents

The standard stock solutions of the colourants, tartrazine (Ta; C.I. Food yellow 4; 0.5 mg/mL), amaranth (Am; C.I. Food red 9; 0.5 mg/mL), sunset yellow (SY; C.I. Food yellow 3; 0.5 mg/mL), ponceau 4R (E124) (Pon; C.I. Food red 7; 0.5 mg/mL), brilliant blue (BB; C.I. Food blue 2; 0.5 mg/mL) were obtained from the National Research Center for Certified Reference Materials (Beijing, China).The mixed standard solutions containing all colourants at 20.0 μg/mL was prepared by mixing and dilution of appropriate aliquots from standard stock solution of each substance. Working solutions were prepared by appropriate dilutions of the mixed standard solutions with water.

HPLC-grade methanol was purchased from Merck KGaA Co., Ltd. (Germany). 1-butyl-3-methylimidazolium bromide ([C_4MIM][Br]), 1-hexyl-3-methylimidazolium bromide ([C_6MIM][Br]), 1-octyl-3-methylimidazolium bromide ([C_8MIM][Br]) and 1-decyl-3-methylimidazolium bromide ([C_{10}MIM][Br]) were obtained from Shanghai Cheng Jie Chemical Co., Ltd. (Shanghai, China). 0.3 g/mL [C_nMIM]Br aqueous solution was prepared by distilled water. $K_2HPO_4{\cdot}3H_2O$ was used as the salt for the aqueous two-phase systems. Milli-Q water (Millipore, Bedford, MA, USA) was used throughout the study. Britton–Robinson (B–R) buffer solutions were prepared by mixing the mixed acid (composed of 0.04 mol L^{-1} H_3PO4, HAc and H_3BO_3) with 0.2 mol L^{-1} NaOH in proportion. Unless otherwise specified, all other reagents were analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.3. Preparation of phase diagram

Phase diagrams were determined by the means of cloud-point method (Li, He, Liu, Li, & Liu, 2005). A certain of [C_nMIM]Br was put into a 10.0 mL centrifugal tube. The tested salt solution was added dropwise to the test tube until turbidity. Whereafter, a two-phase system was achieved and water was added dropwise to the test tube to obtain a clear one-phase system. More salt solution was added again to afford a two-phase system. The composition of this mixture was noted and so on. The bimodal curve was applied to characterise the phase diagram (Vollhardt & Fainerman, 2010). In the region above the bimodal curve, the system is divided into two phases; in the region below the bimodal curve, the system is of a homogeneous phase.

2.4. Preparation of the sample solution

All samples, including soft drink, sugar-based, instant powdered drink and gelatin-based confectionery, were obtained from a local market. Appropriate amounts (0.1–5 g) of the samples were dissolved in 15 mL of water. The carbonated drinks were degassed by ultrasonication for 5 min to remove the carbon dioxide. A warming process (50 °C, 30 min) was used for the complete dissolution of the sugar-based, instant powdered drink and gelatin-based confectionery. These solutions were centrifuged and the upper solutions were filtered through 0.45 μm micro-pore filter membrane. The filtrate was transferred to volumetric flask of 25.0 mL and completed to the mark with distilled water and prepared for IL–ATPS extraction.

2.5. Extraction procedure

0.5 mL of 0.3 g/mL [C_4MIM]Br, 0.50 mL B–R buffer solution and a certain of the mixed standard solution or the sample solution were placed in a 10.0 mL centrifugal tube. The mixture was diluted to 5.0 mL with distilled water and then 5.5 g $K_2HPO_4{\cdot}3H_2O$ was added. A turbid solution was easily formed after gentle blending. The tube centrifuged for 4 min at 3000 rpm to ensure a complete phase separation. The upper aqueous phase was removed with a syringe, and the volume of the IL phase collected was almost 120 μL. Then 20.0 μL of [C_4MIM]Br phase was injected into HPLC for quantification.

2.6. Phase behaviour of IL-ATPS

The extraction efficiency depends on the structure of analyte and its affinity towards the extractant (described as partitioning coefficient), phase ratio and the number of extraction in the liquid–liquid extraction system. Among these factors, partitioning coefficient K is the most important physical and chemical parameter for the study of phase behaviour. Higher separation efficiency can be achieved when a greater partitioning coefficient K and a lower phase ratio R were selected (Wuhan University, 1995). In this paper, quantitative extraction of the five colourants was accomplished with a one-step extraction by using IL–ATPS. So the distribution behaviours of these colourants between IL phase and salt phase were characterised by the extraction efficiency (E), partition coefficient (K) and phase ratio (R).

The parameters E, K and R were defined as follows:

$$E = \frac{C_{IL}V_{IL}}{C_{aq}V_{aq} + C_{IL}V_{IL}} \times 100\% \quad (1)$$