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# A new dispersive liquid–liquid microextraction using ionic liquid based microemulsion coupled with cloud point extraction for determination of copper in serum and water samples

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ABSTRACT

A simple and rapid dispersive liquid–liquid microextraction procedure based on ionic liquid assisted microemulsion (IL-μE-DLLME) combined with cloud point extraction has been developed for preconcentration copper ($Cu^{2+}$) in drinking water and serum samples of adolescent female hepatitis C (HCV) patients. In this method a ternary system was developed to form microemulsion (μE) by phase inversion method (PIM), using ionic liquid, 1-butyl-3-methylimidazolium hexafluorophosphate ($[C_4mim][PF_6]$) and nonionic surfactant, TX-100 (as a stabilizer in aqueous media). The Ionic liquid microemulsion (IL-μE) was evaluated through visual assessment, optical light microscope and spectrophotometrically. The $Cu^{2+}$ in real water and aqueous acid digested serum samples were complexed with 8-hydroxyquinoline (oxine) and extracted into IL-μE medium. The phase separation of stable IL-μE was carried out by the micellar cloud point extraction approach. The influence of of different parameters such as pH, oxine concentration, centrifugation time and rate were investigated. At optimized experimental conditions, the limit of detection and enhancement factor were found to be 0.132 μg/L and 70 respectively, with relative standard deviation $< 5\%$. In order to validate the developed method, certified reference materials (SLRS-4 Riverine water) and human serum (Sero-M10181) were analyzed. The resulting data indicated a non-significant difference in obtained and certified values of $Cu^{2+}$. The developed procedure was successfully applied for the preconcentration and determination of trace levels of $Cu^{2+}$ in environmental and biological samples.



## 1. Introduction

Copper ($Cu^{2+}$) is an important essential element and is associated with number of metalloproteins (Vulpe and Packman, 1995; Milne, 1999; Kazi et al., 2010). The major functions of Cu metalloproteins are oxidation–reduction reactions, as Cu-containing enzymes bind and react directly with molecular oxygen. A number of pathological conditions have been attributed to the loss of cupro-enzyme activity (Harris, 1992). The long term high exposure of Cu causes potentially adverse effects to human health. In the presence of cellular reductants, low-molecular-weight $Cu^{2+}$ compounds may play a catalytic role to initiate the free radical reactions. The resulting oxyradicals have the potential to damage cellular lipids, nucleic acids, proteins, and carbohydrates (Kalkan et al., 2002). During infections or inflammatory stress, serum $Cu^{2+}$ concentration increases due to acute-phase activity of interleukin 1 (Meng and Zhang, 2006). The $Cu^{2+}$ is also a hepato-toxic element because its accumulation in fibrotic liver caused by the HCV infection may contribute to hepatic injury (Arain et al., 2014a; Hatano et al., 2000).

Flame atomic absorption spectroscopy (FAAS) has been widely used for the determination of trace quantities of metal ions in biological and environmental samples because it is a relatively simple technique, with high sample throughput and inexpensive equipment (Arain et al., 2014b). However, the direct determination of metals at trace level by FAAS is limited not only by insufficient sensitivity, but also by matrix interference especially in biological samples (Tabrizi, 2007; Duran et al., 2007). Sample preparation plays an important role in analytical process to concentrate and separate the target analytes as well as decrease the interferences

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from the complex matrix samples. A number of sample pretreatment methods have been established for the determination of trace level of copper from different types of samples.

Various preconcentration methods including liquid–liquid extraction (Farajzadeh et al., 2009), coprecipitation (Tuzen and Soylak, 2009), cloud point extraction (Shokrollahi et al., 2008), and solid phase extraction (Bulut et al., 2007) have been proposed for preconcentration of trace elements. Liquid–liquid extraction (LLE) is one of the convenient and simple separation tools used for the metals extraction (Pena-Pereira et al., 2009). These methods, despite of their advantages, suffer from limitations, such as significant chemical additives, solvent losses, complex equipment, large secondary wastes, unsatisfactory enrichment factors and high time consumption, that limit their application (Komjarova and Blust, 2006; Ghaedi et al., 2007; Duran et al., 2008). A new trend in analytical chemistry is miniaturization of preconcentration techniques to reduce the consumption of reagents and decrease waste generation (Asghari et al., 2014)

Recently dispersive liquid liquid microextraction (DLLME) has been attracted much attention due to its simplicity, rapidity, low sample volume, high recovery and enrichment factor (Rezaee et al., 2006; Baharand Zakerian, 2012). But, like other analytical methods, DLLME also has some limitations, the use of highly toxic extractive and dispersive solvents, which in addition to their toxicity can decrease the partition coefficients of analytes in extraction solvents (Farajzadeh et al., 2014; Skrlikova et al. 2011). In this sense, substantial interest has been manifested on the usage of ionic liquids (ILs) as the green solvent to replace the conventional organic solvents in order to extract heavy metal ions and other pollutants (Ho et al., 2013).

These drawbacks of DLLME can be overcome by using them in the form of microemulsion (μE). Ionic liquid based microemulsion-dispersive liquid–liquid microextraction (IL-μE-DLLME), as a new class of extraction media and efficient separation tool, not only overcome the solubility limitations of ionic liquids in a polar solvents but also provide hydrophobic or hydrophilic nano-domains there by expanding their potential in micro-heterogeneous systems use for separation and extraction technique (Gao et al., 2007). ILs are organic salts, non-molecular solvents which exist in the liquid state at room temperature, also termed as room temperature ionic liquids (Shah et al., 2012). A number of extraction methods have been reported in which RTILs have been efficiently employed for the extraction of metal ions (Liu et al., 2005).

The micro-emulsion (μE) is defined as a thermodynamically stable, clear and isotropic dispersion of one liquid into another immiscible liquid, stabilized by a third component, which can be a surfactant (detergent molecules) or a co-surfactant (alcohol or amine molecules (Friberg, 2007; Behera et al., 2007)). Two main methods have been used to prepared μE: Phase titration and Phase inversion methods. Phase inversion method (PIM) depends upon addition of an excess of the dispersed phase or in response to temperature. The PIM method simply involves titrating water into a mixture containing ionic liquid and surfactant, which initially leads to the formation of a water-in-ionic liquid ($H_2O$ /IL) emulsion, then after stirring, it inverts into an ionic liquid-in-water emulsion (IL/$H_2O$) (Shafiq-un-Nabi et al., 2007; Talegaonkar et al., 2008)

The aim of present study was to develop an efficient, rapid and environmental friendly ionic liquid based μE-DLLME method for enrichment of trace levels of $Cu^{2+}$ in biological (serum) and environmental (water) samples. In the developed method the μE consisting of hydrophobic ionic liquid in-water (IL/$H_2O$), was formed by the phase inversion method (PIM). In which water is used as dispersive media to disperse ionic liquid, while surfactant (TX-100) was used to stabilize the μE by reducing the hydrophobic IL/$H_2O$ interfacial tension. The $Cu^{2+}$ preconcentration was mediated by chelation with the 8-hydroxyquinoline (oxine) reagent, followed by extraction with IL-μE. Separation of IL-μE from the aqueous phase could be induced by the miceller cloud point extraction technique. The effect of various experimental parameters on the IL-μE-DLLME were investigated and optimized. The validity of proposed method was checked by analyzing trace levels of $Cu^{2+}$ in certified reference materials (water and serum), drinking water and blood serum samples of adolescent female HCV patients along with the healthy referents.

## 2. Experimental

### 2.1. Chemicals and reagents

Ultrapure water obtained from ELGA lab water system (Bucks, UK), was used throughout the work. Certified standard solution of $Cu^{2+}$ (1000 mg/L) was obtained from the Fluka Kamica (Bush, Switzerland). Working standard solutions were obtained by appropriate dilution of the stock standard solutions before analysis. Concentrated $HNO_3$ (65%) and $H_2O_2$ (30%) were purchased from Merck (Darmstadt, Germany). 1-Butyl-3-methylimidazolium hexafluorophosphate [$C_4$mim][$PF_6$] was purchased from Sigma-Aldrich (Germany). The oxine was obtained from (Merck), prepared by dissolving appropriate amount of reagent in 10 mL ethanol (Merck) and diluting to 100 mL with 0.01 M acetic acid and were kept in refrigerator at 4 °C for one week. The nonionic surfactants Triton X-114 and Triton X-100 were obtained from Sigma (St. Louis, MO, USA). The 0.1 mol/L acetate and phosphate buffer was used to set the pH of the solutions. The pH of the samples and standards were adjusted to the desired pH by the addition of (0.1 mol/L HCI/NaOH) solution in the buffer. All glassware used in the experiments were cleaned with pure water, soaked in 2.0 mol/L of $HNO_3$ and washed with ultrapure water to avoid contamination.

### 2.2. Instrumentation

A Perkin-Elmer Model AAnalyst 700 (Norwalk, CT) flame atomic absorption spectrophotometer was used. The $Cu^{2+}$ hollow cathode lamp was run under the conditions suggested by the manufacturer. A single element hollow cathode lamp was operated at 7.0 mA and spectral bandwidth of 0.7 nm. The analytical wavelength was set at 324.8 nm. The acetylene flow rate and the burner height were adjusted in order to obtain the maximum absorbance signal.

A pH meter (Ecoscan Ion 6, Malaysia) was employed for pH adjustments. Centrifugation was carried out by using Model-1465 centrifuge (speed range 0–6000 rpm, timer 0–60 min, 220/50 Hz, HISTAM-R, Spain). Optical microscopy was used to study the microstructure of selected samples (Hund wetzlar D-35580, medprax, germany) with a 60*0.25 objective lens. Turbidity of all the formulated μEM was analyzed by measuring its absorbance at a wavelength of 600 nm (UV–vis Spectrophotometer Biochrom libraS22, Cambridge, UK).

### 2.3. Sample collection and pretreatment procedure

The blood samples were collected from 90 adolescent girls have hepatitis C (HCV), attending the outpatient clinic and admitted to the hepato-gastroenterology ward at the Civil Hospital in Hyderabad, Pakistan. The HCV patients were tested by anti-HCV Antibodies test (positive RNA test /PCR test). For comparative purpose 75 healthy girls of same age group (12–15 years) as referents (mostly the relatives of patients), were also selected. They all were residents of Hyderabad and different areas of Sindh, Pakistan. At the start of the study, weight, height, blood pressure, and