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A novel aqueous biphasic system formed by deep eutectic solvent and ionic liquid for DNA partitioning

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ABSTRACT

In this work, aqueous biphasic systems (ABSs) composed of ionic liquids (ILs) and deep eutectic solvents (DESs) were developed and utilized to efficiently extract DNA for the first time. Four kinds of ILs/DESs were mainly constituted by betaine/carboxylic acid and betaine/carbohydrates respectively. While another DES ([TBAB][PPG400]) was formed by polypropylene glycol 400 (PPG 400) and tetrabutylammonium bromide (TBAB). The phase-formation ability of the studied ABSs was evaluated by using [TBAB][PPG400]/(ILs/DESs) and [TBAB][PPG400]/inorganic salts. The results revealed that the phase forming ability of ABSs involved with the size of anion alky chain of ILs, the viscosity, the density and the hydrophilicity of DESs, ionic radius and ionic valence of inorganic salts. Then the system comprising [TBAB][PPG400]/IL was selected to ascertain the optimum extraction conditions for the extraction of DNA by the influence factor experiments. Meanwhile the maximum extraction efficiency could be attained 99.60%. Mixed sample experiments were implemented to separate DNA/cytochrome C (Cyt-c) and DNA/bovine hemoglobin (BHb), where the DNA mainly partitioned to IL-rich bottom phase. It turned out that the relationship between the isoelectric point of analytes and the pH of the system played an important role in the separation process. The result also showed that the studied system can be applied to selectively separate mixtures of nucleic acids and proteins in a single-step. Moreover, the developed system was successfully applied to the extraction of DNA from bovine whole blood with satisfactory result. Finally, the extraction mechanisms associated with the separation process were explored by FT-IR spectra, circular dichroism spectra (CD), dynamic light scattering (DLS), transmission electron microscopy (TEM). Overall, the novel systems have been proven to be a remarkable performance in the separation of DNA, which is expected to be widely used and provide further possibilities in separation fields.

1. Introduction

With the environment pollution pricking up and the increasing attention of the whole world, the bearing original ideal “green chemistry” has emerged along with many new challenges to chemistry workers. The researches on developing benign solvents have become a huge concern in academia, for which the use of ionic liquids (ILs) has been under the spotlight. ILs are organic salts with a melting point of below 100 °C. As a class of green solvents, they present many excellent properties, such as high chemical stability, non-flammability, negligible volatility and high ionic conductivity [1,2]. Based on these remarkable advantages, ILs have been widely used as an alternative reaction media to replace conventional organic solvents. Recently, deep eutectic solvents (DESs) have appeared as a new type of solvent. DESs are eutectic mixtures composed of hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs). Owing to hydrogen bond interactions, DESs display a melting point lower than the pure components [3,4]. In addition, most of DESs can be synthesized by simple methods (i.e. heating and mechanical stirring) without further purification steps [5]. As an analogue of ILs, DESs share many attractive characteristics with regular ILs while being much cheaper and environmentally friendlier [6–8]. For these reasons, there has been a remarkably increase in the use of ILs and DESs as green solvents. Specially, aqueous biphasic systems (ABSs) based on ILs and DESs have been extensively proposed and employed in the separation of target compounds [9–12].

Aqueous biphasic system is a liquid-liquid separation technique and formed by two immiscible aqueous-rich phases, e.g. two polymers, two salts or polymer and salt above a certain critical concentration [13,14].

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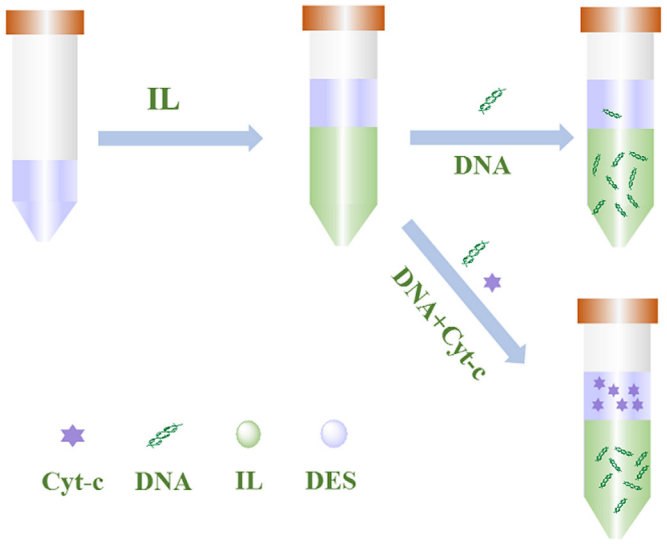
Its advantages over conventional liquid-liquid extraction systems have been well marked, including environment-friendly, low cost, capable of continuous operation, ease of scaling-up, etc [15–17]. Above all, as a major component of two phases, water provides a mild environment where biomolecules can preserve the biological activities and native structure. Therefore, ABSs are recognized as a cleaner and more biocompatible platform to separate and purify several biocompounds [18–20]. Up to now, ABSs based on ILs and DESs to extract biomolecules have achieved substantial progress. Xu et al. have investigated the extraction process of proteins by chloride-based DES-ABS [9]. Li et al. obtained high extraction efficiency for proteins using a series of green betaine-based DESs as phase composition of ABSs [10]. A novel ABS combined PEG-based DES with quaternary ammonium salts were introduced to efficiently partition RNA [11]. Nevertheless, most of these works concentrated mainly on forming ABS by ILs or DESs with common inorganic salts, and there were seldom successful attempts in the separation of complex mixtures in a single-step. It is only more recently that a few researches come up. For instance, Neves et al. investigated liquid-liquid systems formed by two ILs (cholinium bis(trifluoromethylsulfonyl)imide ([Chol][NTf_2]) combined with six [P_{66614}]-based ILs) to find out the factors ruling their immiscibility domains and ions exchange [21]. Silva et al. developed the ABS comprised of cholinium ILs and Pluronic L-35 and discussed their ability to selectively separate naringin and rutin [22].

The high purity nucleic acid has essential applied value in the field of life science. The impurity (e.g. proteins and polysaccharides) always disturbs the purification of nucleic acid [23]. DNA molecules are known as the genetic information carriers. Conventional extraction procedures of DNA with organic solvents create a series of problems ascribed to their toxicity [24,25]. Therefore, it is crucial to search for safer and more effective DNA purification methods. In this respect, ILs and DESs as green solvents provide a promising alternative. According to the research of Mondal and colleagues, DNA was soluble and kept the long-term structural and chemical stability in bio-based DESs [26]. Also, Hua Zhao has demonstrated that the major interaction between ionic solvents and DNA is the electrostatic attraction, while DNA molecules maintained a double helical structure in most ionic solvent systems, especially in aqueous solutions of ILs and DESs [27].

Aiming at developing more performant novel systems to extend the category of ABSs, novel ABSs composed of DESs and ILs were proposed in this work. Herein, betaine-based ILs/DESs refer to betaine/carboxylic acid and betaine/carbohydrates respectively, while another DES composed of polypropylene glycol 400 (PPG400) and tetrabutylammonium bromide (TBAB). To the best of our knowledge, there are no reports in the literature referring to the ABSs formed by ILs and DESs. As a special type of salts, ILs are herein used to prepare ABS with DESs for the first time without any common salts. Whereafter, the partition behavior of the developed ABSs was investigated by selecting DNA as the target analyte. The concentration of DNA was determined by a UV–vis spectrophotometer at 260 nm. The phase-formation ability of the studied systems are compared with ABSs formed by [TBAB][PPG400]/DESs/H_2O and [TBAB][PPG400]/inorganic salts/H_2O separately. The studied ABSs also allow the exploration of some factors during the extraction process so as to determine the optimal conditions of the extraction. Moreover, the studied system was used to selectively separate mixtures of DNA and proteins in a single-step. Finally, the mechanisms of the extraction process were discussed by FT-IR spectra, circular dichroism spectra, dynamic light scattering and transmission electron microscopy. A graphical abstract of extraction process has been provided as indicated in Scheme 1.



Scheme 1. DES/IL-based ABS for the extraction of DNA and selective separation of DNA and Cyt-c.

2. Experimental

2.1. Reagents and instrumentation

Formic acid (purity ≥ 88%), acetic acid (purity ≥ 99.5%), propionic acid (purity ≥ 99%), n-butyric acid (purity ≥ 99%), D-(+)-glucose (purity > 99%), D-sorbitol (purity > 99%), Na_2CO_3 (purity ≥ 99.8%), NaH_2PO_4 (purity ≥ 99.0%), K_2HPO_4 (purity ≥ 98%) and NaCl (purity ≥ 99.5%) were acquired in Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). $C_6H_5Na_3O_7{\cdot}3H_2O$ (purity ≥ 99.0%) was supplied by Tianjin Fengchuan Chemical Reagent Technologies Co., Ltd (Tianjin, China). Sucrose was purchased from Xilong Chemical Co., Ltd. Betaine (purity 99%), tetrabutylammonium bromide (purity ≥ 99%), xylitol (purity 99%), bovine hemoglobin (BHb) and DNA sodium salt from salmon testes were provided by Shanghai Source Biological Technology Co., Ltd (Shanghai, China). Cytochrome C (Cyt-c) and polypropylene glycol with a molecular weight 400 g (PPG400) were gained from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). Bovine whole blood sample was obtained from Jiaozuo biological technology Co. Ltd. (Jiaozuo, China). All reagents utilized were analytical grade and needed no further purification. All solutions in this work were prepared using ultra pure water (18.25 MΩ cm, 25 °C).

Materials were dried in a DZF-6051 vacuum drying oven (Shanghai, China) and weighed by a FA1104B electronic balance (Shanghai, China). Deep eutectic solvents and ionic liquids were synthesized in a DF-101S heat collection-constant temperature type magnetic stirrer (Henan, China), then characterized by an INOVA-400 proton nuclear magnetic resonance spectra (^{1}H NMR) (Varian, USA) and an FT-IR spectrometer (PerkinElmer, USA). A QYC200 incubator shaker (Shanghai, China) was used to blend the aqueous biphasic systems. A TGL-16C high-speed centrifuge (Shanghai, China) was utilized to accelerate the phase separation. The concentration of DNA solution was obtained by a UV2450 spectrophotometer (SHIMADZU, Japan). A MOS-500 circular dichroism (CD) spectrometer (Bio-Logic, France) was applied to assess the structure and stability of DNA. Aggregation phenomena was explored by a Zetasizer Nano-ZS90 dynamic light scattering (DLS, Malvern, Britain). The microstructures and binding characteristics of samples were observed in a JEM-3010 transmission electron microscope (TEM, JEOL, Japan).

2.2. Synthesis and characterization of DESs and ILs

For the preparation of DESs, both of the HBDs (PPG 400, D-(+)-glucose, sucrose, D-sorbitol and xylitol) and HBAs (TBAB and betaine) species were simply mixed together to synthesize five kinds of DESs at proper molar ratios with magnetic agitation at 80.0 °C, until a clear and homogeneous liquid formed. And a known amount of water was added when it is difficult to produce a clear DES. The different