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The effect of the ionic liquid cation on the DNA stability and purification using aqueous biphasic systems

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PURPOSE OF THE ABSTRACT
The research on nucleic acids has led to important biological discoveries placing them as pivotal therapeutic agents or targets, namely in cancer, cardiovascular, neurological and infectious diseases [1]. From basic to applied research, highly pure and intact nucleic acids are always required. Nevertheless, designing isolation and purification protocols while ensuring the biological stability and integrity of nucleic acids is a challenging task due to their easy instability. Furthermore, conventional extraction procedures of nucleic acids employing volatile organic solvents (VOCs) have serious concerns associated to their toxicity [2]. Therefore, the finding of cost-effective and more sustainable methodologies able to selectively extract and purify nucleic acids, while keeping their stability and integrity, is a top priority for their employment in therapeutic applications.
Aqueous biphasic systems (ABS) can be seen as more sustainable liquid-liquid systems since they avoid the use of volatile and hazardous VOCs [3]. As a major component of these systems, water provides a mild environment where biological activity and native structure of labile molecules can be preserved [4]. ABS can be thus used to develop more biocompatible separation and/or purification platforms. Recently, novel and alternative ABS composed of deep eutectic solvents (DES) were explored to selectively separate DNA from proteins in a single-step [5]. Despite a few number of reports are found on the development of alternative and greener purification processes [5,6], the evaluation of the nucleic acids stability and long-term preservation is still a missing part. Moreover, the use of DES is more restricted in terms of the molecular structures of the respective constituents and their integrity as phase-forming components in ABS can be compromised [7]. In this context, ABS composed of ionic liquids (ILs) allow a larger range of phase-forming components to be explored, while providing higher and more selective extraction efficiencies for a wide range of biocompounds [3].
The aim of this work is to demonstrate the applicability of IL-based ABS to preserve and purify DNA from biological matrices. A screening of several ILs ([N111(2OH)]Cl, [N111(2OH)]Br, [N4444]Br, [P4444]Br, and [C1C2im]Br) was initially carried out at different weight fractions (5, 15, and 30 wt%) in aqueous buffered solutions in order to evaluate the effect of IL cation on the structural stability of DNA. According to the results obtained, the structural stability of DNA in IL aqueous buffered solutions is pH dependent, where the increase of the IL concentration and consequent decrease of pH leads to an unfavorable DNA stabilization capability (Fig. 1). Additionally, a selective DNA partitioning between the two aqueous phases in ABS composed of ILs and polymers was found, which is dependent on the pH (Fig. 1). In this way, ABS are here proposed to be used in more biocompatible purification platforms of nucleic acids; these can be further used in back-extraction procedures for the recovery of DNA and its further therapeutic applications by changes in the pH and phase-forming components nature.

FIGURES

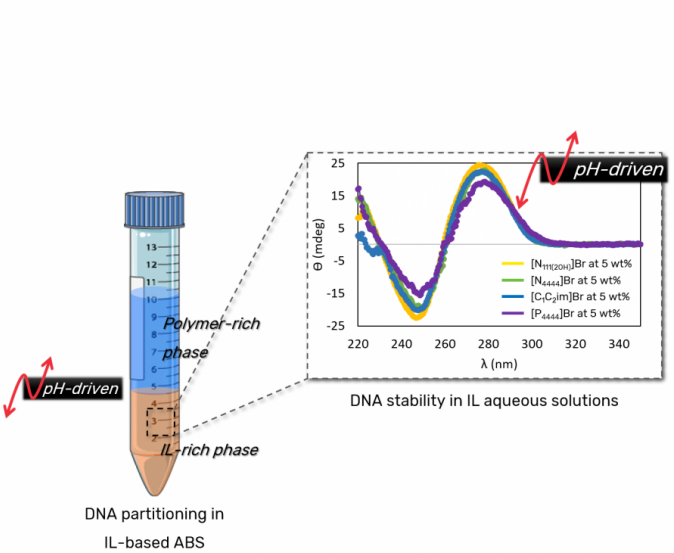

FIGURE 1

Illustration on the use of IL-based ABS as pH-driven purification systems of nucleic acids.

DNA - deoxyribonucleic acid;

IL - ionic liquid;

ABS - aqueous biphasic system.

FIGURE 2

KEYWORDS

Nucleic Acid Stability | Purification | Ionic Liquids | Aqueous Biphasic System

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