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Design of bio-based ionic liquids for the purification of IgG antibodies

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PURPOSE OF THE ABSTRACT

Recent advances on the production and use of antibodies as therapeutic drugs and in research/diagnostic purposes led to their recognition as value-added proteins. Within the biopharmaceuticals class, immunoglobulin G (IgG) is the most widely used type of antibodies in a variety of biomedical applications [1]. Due to the continuous increase on the use of IgG in various high-end applications, there is thus a boost in demand for high quality/high purity IgG, not only for therapeutic applications but also for applications in cutting-edge diagnosis and research. Still, antibodies are currently highly expensive, which has been preventing their widespread applications, mainly due to the lack of cost-effective purification techniques. Processes for the purification of IgG typically require various steps, viz. clarification, concentration and polishing by chromatographic techniques (such as ion exchange, gel permeation and affinity chromatography) [2-3]. However, when envisaging the antibodies widespread use as promising biopharmaceuticals, there is a crucial demand for developing cost-effective and sustainable techniques to obtain high quality/low cost IgG.

In this work, aqueous biphasic systems (ABS) formed by bio-based ionic liquids (ILs) and biocompatible polymers (polypropyleneglycol, PPG 400) were investigated to purify IgG from rabbit serum samples. The bio-based ILs investigated are composed of ions derived from natural sources, namely constituted by the cholinium cation and anions derived from plants natural acids. The novel ILs have been designed, synthesized, characterized and used in the creation of ABS as liquid-liquid extraction/separation techniques. The respective ternary phase diagrams were determined at 25ºC to infer on mixture compositions required to form aqueous systems of two phases, further applied in the extraction of pure immunoglobullin G (IgG) to identify the most promising bio-based ILs, and finally employed in the purification of IgG from complex and real matrices of rabbit serum. Remarkably, the complete extraction of IgG to the IL-rich phase was achieved in a single-step. With pure IgG a recovery yield of 100% was obtained, while with rabbit serum this value slightly decreases to ca. 85%. However, a 58% enhancement in the IgG purity was achieved when compared with its purity in serum samples. The stability of IgG before and after extraction was also evaluated by size exclusion high-performance liquid chromatography (SE-HPLC), Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Fourier Transform Infrared Spectroscopy (FTIR). In most ABS formed by bio-based ILs, IgG retained its native structure, without degradation or denaturation effects, supporting therefore their potential as remarkable platforms for the

purification of high-cost biopharmaceuticals [4].

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FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

BIOPHARMACEUTICALS | PURIFICATION TECHNIQUES | AQUEOUS BIPHASIC SYSTEMS | IONIC LIQUIDS

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