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Magnetic aqueous two phase fishing: A hybrid process technology for antibody purification

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ABSTRACT

The potential to combine aqueous two-phase extraction (ATPE) with magnetic separation was here investigated with the aim of developing a selective non-chromatographic method for the purification of antibodies from cell culture supernatants. Aqueous two-phase systems (ATPS) composed of polyethylene glycol (PEG) and dextran were supplemented with several surface modified magnetic particles (MPs) at distinct salt concentrations. The partition of pure human IgG in the upper and lower phases as well as the amount adsorbed at the MPs surface was investigated, indicating that MPs coated with dextran and gum Arabic established the lowest amount of non-specific interactions. The binding capacity of gum arabic coated particles modified with aminophenyl boronic acid (GA-APBA-MP) was were found to be excellent in combination with the ATPS system, yielding high yields of antibody recovery (92%) and purity (98%) from cell culture supernatants. The presence of MPs in the ATPS was found to speed up phase separation (from 40 to 25 min), to consume a lower amount of MPs (half of the amount needed in magnetic fishing) and to increase the yield and purity of a mAb purified from a cell culture supernatant, when compared with ATPE or magnetic fishing processes alone.

1. Introduction

The challenging promise of new biotechnology derived drugs for the treatment of several diseases such as cancer and auto-immune disorders is being mostly fulfilled by monoclonal antibodies (mAbs) [1,2]. The traditional methods of mAb purification are too expensive and complex to scale up to a level sufficient to deal with the increasing product demand [2]. The chromatography-based technologies now available for extraction and purification of antibodies at industrial scale are reaching a point close to their maximum capacity [3]. Hence novel, low cost and easy to scale up non-chromatographic methods for the downstream processing of antibodies are receiving special attention from the academic and industrial communities [4]. Among these, aqueous two phase extraction (ATPE) and magnetic fishing represent interesting alternatives to chromatographic methods worth exploiting. Aqueous two phase systems (ATPSs) form when solutions of two incompatible polymers or one polymer and a salt, are mixed together above certain concentrations [5]. The partition of substances between two aqueous phases is influenced by complex phenomena which involve van der Waals forces [6], hydrogen bonds, charge interactions, hydrophobic interactions and steric effects [7]. Still, ATPE represents a promising alternative for antibody purification with tests already performed in industrial settings [11]. Magnetic particles (MPs) modified with ligands typically employed in chromatographic media (e.g. ion-exchange, affinity) constitute highly selective adsorbents for target molecules [8]. In addition, the magnetic responsive nature of these particles allows their selective manipulation and separation in the presence of other suspended solids with promising applications in process integration. Magnetic separation has been tested at small-scale for IgG capture from cell culture supernatants, and provided similar yields and purity when compared to chromatography columns typically employed [14]. However, the lack of large scale magnetic separators and the high cost of commercially available MPs are the major challenges faced by this technology [9]. The objective of this work was to assess the possibility of combining ATPE and magnetic fishing in a unique new hybrid extraction process. ATPSs based on PEG and dextrans were supplemented with MPs possessing different surface functionalities and tailor-made for mAb purification. The basic core of MPs (iron oxide) was firstly coated with

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polymers, as Gum Arabic, and then modified with aminophenyl boronic acid (APBA) an affinity ligand that has already shown feasibility to capture mAbs directly from cell culture supernatants [10–12]. The hybrid process where gum Arabic MPs coated with APBA were supplemented to PEG/dextran ATPSs also showed high recovery of IgG with high purity, while speeding up the phase separation process and facilitating the recycling of polymers.

2. Materials and methods

2.1. Chemicals

Sodium silicate ($Na_2SiO_3 \cdot 5H_2O$), amino phenyl boronic acid (APBA), tris(hydroxymethyl)amino methane, dextran from *Leuconostoc mesenteroides*, HEPES, glutaraldehyde, tetraethoxy silane (TEOS), gum arabic, anthrone, sodium hydroxide, 3-glycidyloxypropyl trimethoxysilane (GLYMO), ferric sulfate hydrate $[Fe_2(SO_4)_3] \cdot H_2O$, ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$), (3-aminopropyl) triethoxysilane (APTES), and alizarin red were purchased from Sigma–Aldrich. Poly(ethylene glycol) with molecular weight 3350 and 8000 were purchased from Sigma (St. Louis, MO, USA). Poly(ethylene glycol) with molecular weight 1000, 3500, 6000 and 10,000 were purchased from Fluka (Buchs, Switzerland). Dextran with an average molecular weight of 500,000 was purchased from Fluka (Buchs, Switzerland). The reagents used for SDS-PAGE gels were, ammonium persulphate (APS), *N*,*N*,*N*,*N*-tetramethylethylenediamine (TEMED), 30% acrylamide/bisacrylamide-solution, SDS micropellets (sodium dodecyl sulphate) and silver stain plus kit were purchased from BIO-RAD.

2.2. Biologicals

Polyclonal human immunoglobulin G (IgG) for therapeutic administration (Gammanorm) was purchased from Octapharma, as a 165 g/l solution containing 95% of IgG. A Chinese Hamster Ovary (CHO) cell supernatant containing monoclonal human IgG directed against interleukin-8 was produced in house (details of mAb production can be found in SI).

2.3. Magnetic aqueous two phase extraction studies

Magnetic particle synthesis, surface modification and characterization was carried out as described in [12], and full details can be found in Supplementary Information. Aqueous two-phase systems were prepared by weighting the corresponding stock solutions of PEG and dextran polymers along with salt in order to achieve the desired final composition of each system. Pure IgG extraction studies were performed by adding 1 ml of 1 g/l IgG stock solution dissolved in PBS buffer. In IgG extraction studies from CHO cell culture (1.16 g/l IgG), the supernatant loading ranged from 1 to 1.5 ml. All systems were prepared in 15 ml graded test tubes to a total final weight of 5 g by adding water (Milli-Q), PEG of molecular weight 3350 Da of 40% stock solution (final 5% w/w), dextran 500,000 Da of 20% stock solution (final 8% w/w), MP at a final concentration of 0.02% (w/w) and NaCl in the concentration range of 100–500 mM. IgG extraction studies were carried out by thoroughly mixing each system components in a vortex shaker for 15 min, followed by phase separation at room temperature. After phase separation, the test tubes were held on a magnetic separator and samples of each of the phases along with magnetic particles were collected. The particle washing was carried out for five times, the first one with Milli-Q water and then four consecutive washes with 20 mM HEPES buffer at pH 8.5 (0.5 ml volume each wash). Elution of adsorbed IgG was then triggered using 1.5 M Tris–HCl buffer at pH 8.5 (5 elution fractions with 0.5 ml volume each). Total protein content was quantified using the Bradford method. The amount of IgG in both bottom and top phases and bound to MPs were quantified on a porous protein-A affinity column from Applied Biosystems connected to an Äkta purifier system from GE-Healthcare (Uppsala, Sweden). The adsorption buffer was composed by 50 mM phosphate buffer and 150 mM NaCl, at pH 7.4, whereas elution was carried out using 12 mM HCl and 150 mM NaCl. IgG concentration was determined from a calibration curve obtained using Gammanorm IgG as a standard. The purity of protein preparations on top and bottom phases was evaluated by SDS-PAGE. The respective gels were prepared according to a standardized protocol. The low molecular weight marker and the samples for running gels were prepared by adding 10 μl of each sample and 5 μl of sample buffer followed by boiling for 2 min. The gel ran for 80 min at 150 V and 250 mA. For detection of the protein bands, the gel was stained with silver stain coloration kit (BioRad).

For the study of the best incubation period between GA-APBA-MPs and a pure human IgG solution, the same protocol has described above was followed with the exception that IgG extraction studies were carried out by thoroughly mixing each system components in a vortex shaker for 10, 15, 20, 25 or 30 min, followed by phase separation at room temperature. In order to assess the recycling properties of the GA-APBA-MPs, up to five cycles of magnetic ATPSs were carried out with the same particles by regenerating them after each round through a washing with regeneration buffer (0.1 M NaOH, 30% (v/v) isopropanol).

3. Results and discussion

3.1. Selection of polymer-coated magnetic particle suitable for ATPSs

Magnetic particles (MPs) are commonly coated with biological or synthetic polymers with the aim of (i) isolating the magnetic core against harsh conditions, (ii) improving biocompatibility, (iii) introducing functional groups for further chemical modification, and (iv) increasing colloidal stability. The polymers selected in this work for the coating of MPs were dextran-500000, gum arabic, carboxymethyl dextran, PEG-3500, EOPO, jeffamine-M-2070, polyacrylic acid sodium salt-15000, UCON-2000 and UCON-3520. This selection was based on commonly employed polymers in ATPSs for biomolecules purification, as well as on polymers previously selected for the coating of MPs applied in antibody magnetic fishing [13]. In order to develop a hybrid process combining MPs and ATPE for antibody purification, the first step was the selection of the most suitable polymer for the coating of MPs [14]. For this purpose, MPs coated with the selected natural and synthetic polymers with distinct properties, were prepared, and then supplemented to PEG/dextran ATPSs, followed by investigation of IgG partitioning in the upper and lower phases (Fig. 1 in SI) as well as binding to particles (Fig. 1) at different salt concentrations (100, 200, 300, 400 and 500 mM NaCl). In general it was observed that an increase in salt concentration decreased the IgG concentration in the lower dextran phase with simultaneous rise of IgG concentration in the upper PEG-rich phase, particularly for salt concentrations greater than 200 mM (Fig. 1 in SI). The rise of salt concentration also decreases non-specific ionic interactions and enhances hydrophobic interactions which promote the transfer of IgG between phases [15]. On the other hand, the amount of IgG adsorbed onto polymer coated MPs (which usually partition to the bottom phase) tend to increase with the increase in salt concentration (Fig. 1). This effect is particularly evident for EOPO, PEG, jeffamine and polyacrylic acid coated MPs. Particles coated with UCON and carboxymethyl dextran yielded the highest amounts of human