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Dextran-Coated Magnetic Supports Modified with a Biomimetic Ligand for IgG Purification

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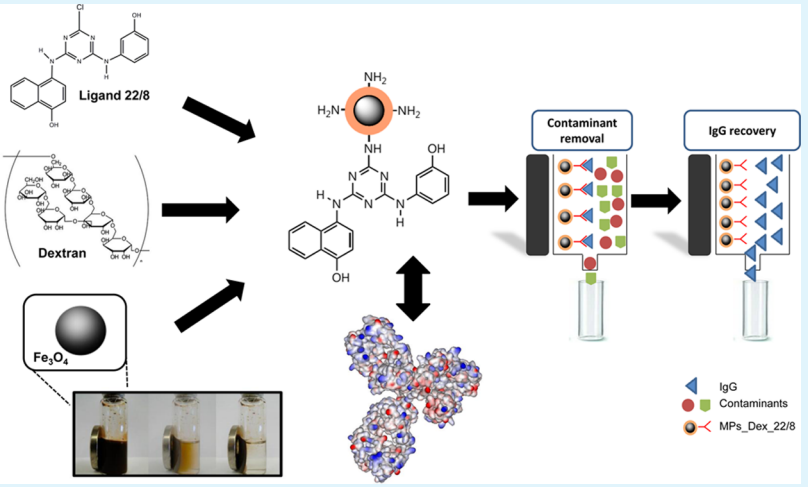

ABSTRACT: Dextran-coated iron oxide magnetic particles modified with ligand 22/8, a protein A mimetic ligand, were prepared and assessed for IgG purification. Dextran was chosen as the agent to modify the surface of magnetic particles by presenting a negligible level of nonspecific adsorption. For the functionalization of the particles with the affinity ligand toward antibodies, three methods have been explored. The optimum coupling method yielded a theoretical maximum capacity for human IgG calculated as 568 ± 33 mg/g and a binding affinity constant of 7.7×10^4 M^{-1}. Regeneration, recycle and reuse of particles was also highly successful for five cycles with minor loss of capacity. Moreover, this support presented specificity and effectiveness for IgG adsorption and elution at pH 11 directly from crude extracts with a final purity of 95% in the eluted fraction.

KEYWORDS: *magnetic particles, dextran, immobilization, synthetic affinity ligand, IgG purification*

1. INTRODUCTION

Full antibodies and engineered antibody formats can be designed to bind to a diversity of antigens with high specificity, and further conjugated with other therapeutics for increased efficiency.[1] For the in vivo administration of antibodies, demanding production and purification processes are required in order to avoid contaminations and produce safe, pure, and consistent products. Simultaneously, industries have the challenge to reduce total manufacturing costs. Downstream processing can account for 50−80% of the total production costs; therefore, there is the need to design purification strategies that will target high purity and product yield as well as cost minimization.[2,3]

Affinity-based methodologies are widely employed on traditional antibody purification processes, and are based on the selective recognition between the antibody molecule and a complementary ligand immobilized in a solid matrix, commonly agarose or derivatives.[3] Nonspecific interactions are reduced with increased yield and contaminants can be eliminated in a single step. The affinity ligands mostly used to capture antibodies are biospecific ligands which are natural immunoglobulin binding ligands (protein A, protein L).[4,56] However, these ligands are costly, labile, and can leach under certain conditions. An alternative and promising choice is the use of synthetic affinity ligands mimicking the biological receptors.[7−9] Although presenting lower binding constants, the purity obtained with the biomimetic ligands is still high with the advantages of being inexpensive, scalable to produce, durable and extraordinarily stable under harsh conditions.[3] A good example of biomimetic ligands toward antibodies is ligand 22/8, a protein A mimetic.[10] In addition, the support for ligand attachment is also a key step for binding the target molecule. The immobilization of ligands on agarose beads has been extensively studied on literature.[3,7] However, packed bed chromatography and bed expanded systems present some limitations, namely clogging and diffusion limitations.[3,11]

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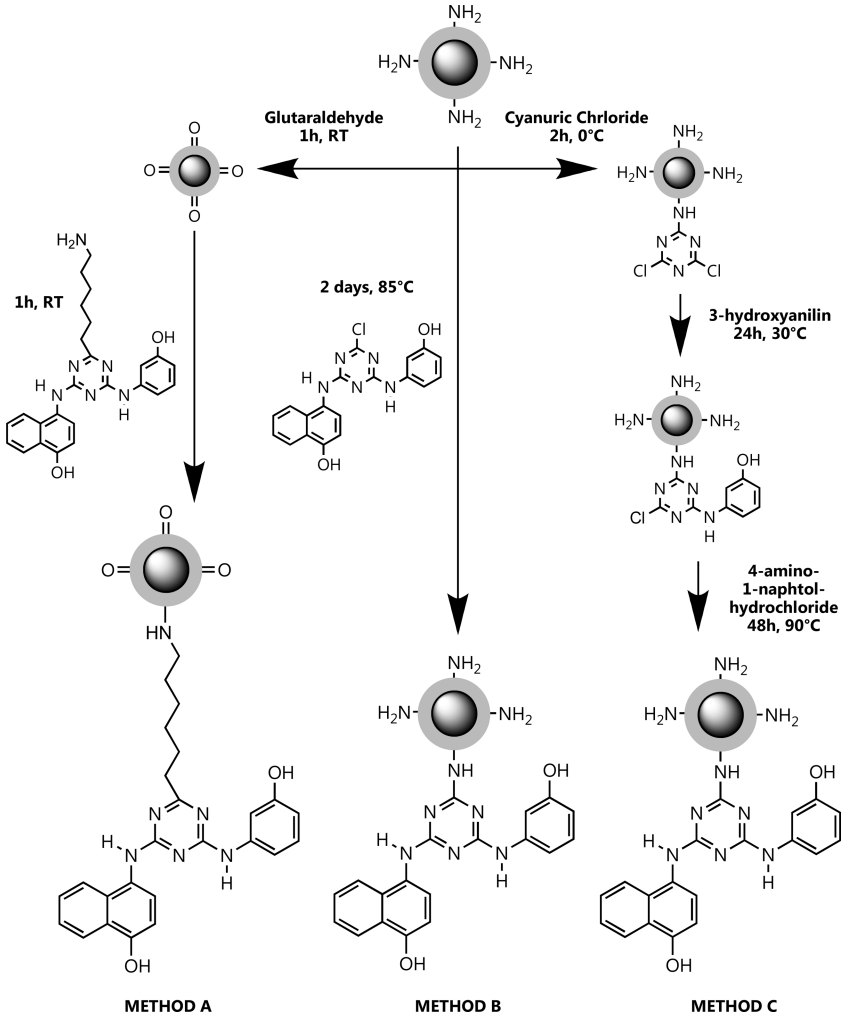

Figure 1. Schematic representation of the synthetic affinity ligand 22/8 Immobilized onto MPs coated with dextran by three different methods: method A, the ligand 22/8 was used in solution phase with a six carbon spacer; method B, the ligand 22/8 was also used in solution phase but without spacer; and method C, the ligand 22/8 was directly synthesized onto the support (ChemDraw 11).

Iron oxide magnetic particles (MPs) appear as a challenging and a suitable choice for bioseparation applications because this support can contribute to cost reduction and process integration.[2,3] MPs present attractive features such as superparamagnetism, which greatly facilitates manipulation, recovery, and reutilization, particularly in high-gradient magnetic separation devices.[12,13] Other advantageous characteristics of MPs concern the small size of the particles providing a high surface area to volume and minimum diffusion limitations.[14,15] MPs present low colloidal stability because of the highly active surface and high surface area to volume ratio, which increases the particles agglomeration. Both phenomena have impact on the size, shape, and stability of the particles. In solution, the impact of these might bring some disadvantages in the applicability of these supports.[15,16] The coating of MPs appears as an essential strategy for particle stabilization, and different coating agents can be applied. MPs coating with polymers, particularly biopolymers such as polysaccharides, attracted attention of researchers as these are known to increase biocompatibility, chemical functionality, and colloidal stability of different materials. In addition, biopolymers are renewable, nontoxic and biodegradable which make them an environmental and sustainable choice.[15] Some of the polysaccharides most used for covering MPs, include agarose,[17] chitosan,[18] starch,[19] dextran,[20] and gum Arabic.[21,22] Dextran, a neutral polysaccharide produced by lactic acid bacteria, is a conventional polymer used for coating MPs. MPs coated with dextran (MPs_Dex) are mostly used in biomedical applications for resonance magnetic imaging and there are already preparations available in the market.[15] These supports were also explored for bioseparation and biosensing applications.[23,24] In the bioseparation field, dextran-coated MPs have already been applied for the separation of proteins,[25,26] cells,[27] organelles,[28] and for isolation of target bacteria by immunomagnetic particles,[29] through the exploitation of the natural interactions between sugars and biological receptors.

This work focused on the preparation of a new magnetic support, based on iron oxide magnetic particles coated with dextran for bioseparation processes, taking into account the characteristics of iron oxide magnetic particles coated with gum Arabic (MPs_GA) previously studied.[21] The novelty of this work relies on the combination of a low cost and inert polymer with a robust synthetic ligand mimicking protein A for the purification of IgG from purified and unpurified mixtures.

2. EXPERIMENTAL PROCEDURE

Materials. (3-Aminopropyl)triethoxysilane (APTES) 98%, 3-hydroxyanilin 98%, 4-amino-1-naphtol hydrochloride 90%, cyanuric

chloride 99% were acquired from Aldrich (Sintra, Portugal). Sodium hydroxide 99% was purchased from Panreac (Cascais, Portugal). Albumin from bovine serum, dextran from *Leuconostoc mesenteroides*, glutaric dialdehyde 50 wt % sol in water, gum arabic from *acacia tree*, iron(III) chloride hexahydrate 98%, iron(II) chloride tetrahydrate 99%, and N,N-dimethylformamide 99% were acquired from Sigma (Sintra, Portugal). Anthrone 97%, sodium bicarbonate 98%, and sulfuric acid 98% were from Sigma−Aldrich (Sintra, Portugal). Human normal immunoglobulin (Gammanorm) was purchased from Octapharma (Lisboa, Portugal). Protein quantification assay used was bichinchoninic acid (BCA) kit from Sigma. For SDS-PAGE gels, the reagents used were 30% acrylamide/bis solution 37.5:1, sodium dodecyl sulfate solution 10% purchased from BIO-RAD. Ammonium persulphate 98% (PSA), N,N,N,N-tetramethylethylenediamine 99% (TEMED), and bromphenol blue sodium salt were acquired from Roth (BetaLab, Queluz, Portugal). Glycerol 99% purchased from Sigma−Aldrich (Sintra, Portugal). SDS micropellets 99% (sodium dodecyl sulfate), tris base 99.9% ultrapure for molecular biology, and glycine 99% ultrapure for molecular biology were purchased from NZYTech (Lisboa, Portugal). 2-Mercaptoethanol 99% purchased from Aldrich (Sintra, Portugal). Hydrochloric acid 37% (concentrated) was acquired from Panreac (Cascais, Portugal). To stain polyacrylamide gels , we used the Silver Stain Plus kit from BIO-RAD (Amadora, Portugal). LMW-SDS Marker Kit (18.5 kDa −96 KDa) was from NZYTech (Lisboa, Portugal).

Methods. *Synthesis, Amination, Stability Study, and Characterization of Bare and Dextran-Coated MPs.* Bare MPs and dextran-coated MPs were synthesized by the coprecipitation of $FeCl_3$ and FeC_2 salts, using a Fe^{2+}/Fe^{3+} molar ratio of 0.5, through the addition of a base under an inert atmosphere, following the Massart method.[30] The syntheses were performed at room temperature for the bare MPs and at 60 °C for the dextran-coated MPs (MPs_Dex). For the MPs_Dex, 2.0 g of a 50 mg/mL aqueous solution of the biopolymer was added dropwise immediately after the addition of the iron solution. The synthesized MPs were washed several times with distilled water using a magnet for separation. To quantify the yield of biopolymer coating MPs, we analyzed the amount of biopolymer in the washes after synthesis by the anthrone method.[31] MPs were then aminated by using 3-aminopropyltriethoxy silane (APTES),[21] yielding amination densities of 214 ± 44 μmoL NH_2/g MPs. Finally, to evaluate the storage stability at 4 °C and the stability of the supports on amination, we analyzed all the washes performed in the intermediate steps by the anthrone method to determine the quantity of biopolymer released. All samples were characterized by Fourier transform infrared (FTIR) spectroscopy on a Perkin-Elmer Spectrum BX instrument. Samples were prepared by grounding and mixing with KBr in a proportion of 1:100. The magnetization of the magnetic particles in solution were characterized by using a vibrating sample magnetometer (VSM) (DSM 880 VSM) at INESC-MN facilities (Lisbon, Portugal). The samples were prepared in milli-Q water with a concentration of 6.1 mg/mL and were used 30 μL of each sample in a vertical quartz rod. Transmission electron microscopy (TEM) was utilized for the characterization of particle morphology and estimation of the size of the magnetic core. The dried particle samples were prepared by evaporating dilute suspensions on a carbon-coated film and TEM performed in an Analytical TEM Hitachi 8100 with Rontec standard EDS detector and digital image acquisition. For all supports the physical properties (hydrodynamic diameters and zeta potential) were determined by Dynamic light scattering (DLS), using a Zetasizer Nano ZS from Malvern. For these analyses, samples with a final concentration of 0.05 mg/mL in milli-Q water were prepared.

Immobilization of the Biomimetic Ligand 22/8 onto Dextran-Coated MPs. For the immobilization of the biomimetic ligand 22/8 onto MPs_Dex, three different methods were tested (Figure 1). In method A, the ligand 22/8 has a six carbon space arm and was previously synthesized in solution phase and purified[7] by Dr. Abid Hussain from our group. For the immobilization procedure, the aminated particles (10 mg/mL) were washed five times with distilled water and resuspended in a solution of glutaraldehyde with a final concentration of 5% (v/v). The suspensions were sonicated for 10 min and subsequently incubated for 1 h at room temperature with constant shaking. Afterward, the particles were washed five times with milli-Q water. The support was then incubated in a 1:1 stoichiometry (taking into account the number of amines available) with the ligand 22/8 previously dissolved in DMF:H_2O (50:50) and centrifuged for 5 min at 13000 rpm to make sure the insoluble part was discarded. The incubation proceeded for 1 h at room temperature at 300 rpm in an orbital shaker. Finally, to block the remaining functional groups, we washed modified supports five times with distilled water and were incubated 1 h at room temperature with constant shaking in the presence of a solution of 100 mMol/L glycine in distilled water.

For method B, the ligand 22/8 was synthesized in solution phase[32] and kindly provided by Telma Barroso from our group. For this immobilization procedure the aminated MPs were incubated with 5 mol equiv (taking into account the number of amines available) of the ligand 22/8 dissolved in DMF:H_2O (1:12) and with 1 equivalent of sodium bicarbonate. Incubation occurred for 2 days at 85 °C with constant shaking. In methods A and B, final washes were collected in order to quantify the amount of ligand bound to the particles (by measurement of absorbance at 280 nm). However, it was not possible to quantify the exact amount of ligand bound because of the extremely low solubility of the ligand.

Finally, in method C, ligand 22/8 was synthesized directly on the particles. The aminated support was resuspended in 50% (v/v) acetone/water and reacted with 5 mol equiv (according to the amount of amines available) of Cyanuric chloride, dissolved in acetone, during 2 h at 0 °C at 300 rpm. In the end of this reaction, the MPs were washed one time with acetone, one time with 50% (v/v) acetone/water and finally five times with water. The first nucleophilic substitution on triazine ring was then performed by adding 2 equivalents (relative to the amount of amines) of 3-hydroxyanilin in water. This reaction proceeded for 24 h with stirring at 30 °C and after the reaction the particles were washed five times with water. Finally, for the second nucleophilic substitution, 5 mol equiv of 4-amino-1-naphtol- hydrochloride, in the presence of 5 equiv. of sodium hydroxide, dissolved in 50% (v/v) DMF/water, were added to the reaction and left to incubate for 48 h with stirring at 90 °C.

After every procedure in methods A, B, and C, the particles were washed sequentially with 50% (v/v) DMF/water, water, and finally resuspended in water for storage.[21]

Assessment of Human IgG and Bovine Serum Albumin Binding to Affinity Magnetic Supports. The MPs_Dex modified with affinity ligand 22/8 (250 μL at 6.0 mg/mL) were tested with a pure solution of human IgG (hIgG), and with a pure solution of Bovine Serum Albumin (BSA). The particles suspensions were washed with regeneration buffer (0.1 M NaOH in 30% (v/v) isopropanol), followed by deionized water to neutralize the pH. These cycles of washes were repeated two times. Then, particles were equilibrated with binding buffer (50 mM phosphate, pH 8). After preparation of the supports, 250 μL of a hIgG or BSA solution in binding buffer (1 mg/mL) was added to the particles and incubated for 15 min at room temperature with constant stirring, after which the supernatants were separated by magnetic separation and removed. Particles were then washed five times using binding buffer (250 μL) following the same methodology. Bound protein was then eluted with a 50 mM Glycine−NaOH, pH 11 buffer. Reuse of the modified supports were repeated five times for the binding of hIgG, where after each cycle of adsorption and elution the supports were regenerated two times using regeneration buffer followed by deionized water to neutralize the pH. All samples were analyzed by BCA assay (microplate reader assay), in order to quantify the amount of protein bound to and eluted from the supports.[21] Nonmodified particles (MPs and MPs_Dex) were tested at the same time and in the same conditions. To assess biopolymer and iron leaching, we incubated the magnetic supports separately with binding, elution, and regeneration buffers, and the supernatants recovered by magnetic separation. The amount of biopolymer and iron in the supernatants were quantified by the anthrone[31] and magnetite[33] methods, respectively. Adsorption isotherms of hIgG on the magnetic supports were estimated by partition equilibrium experiments. Solutions of hIgG (0−18 mg/mL;

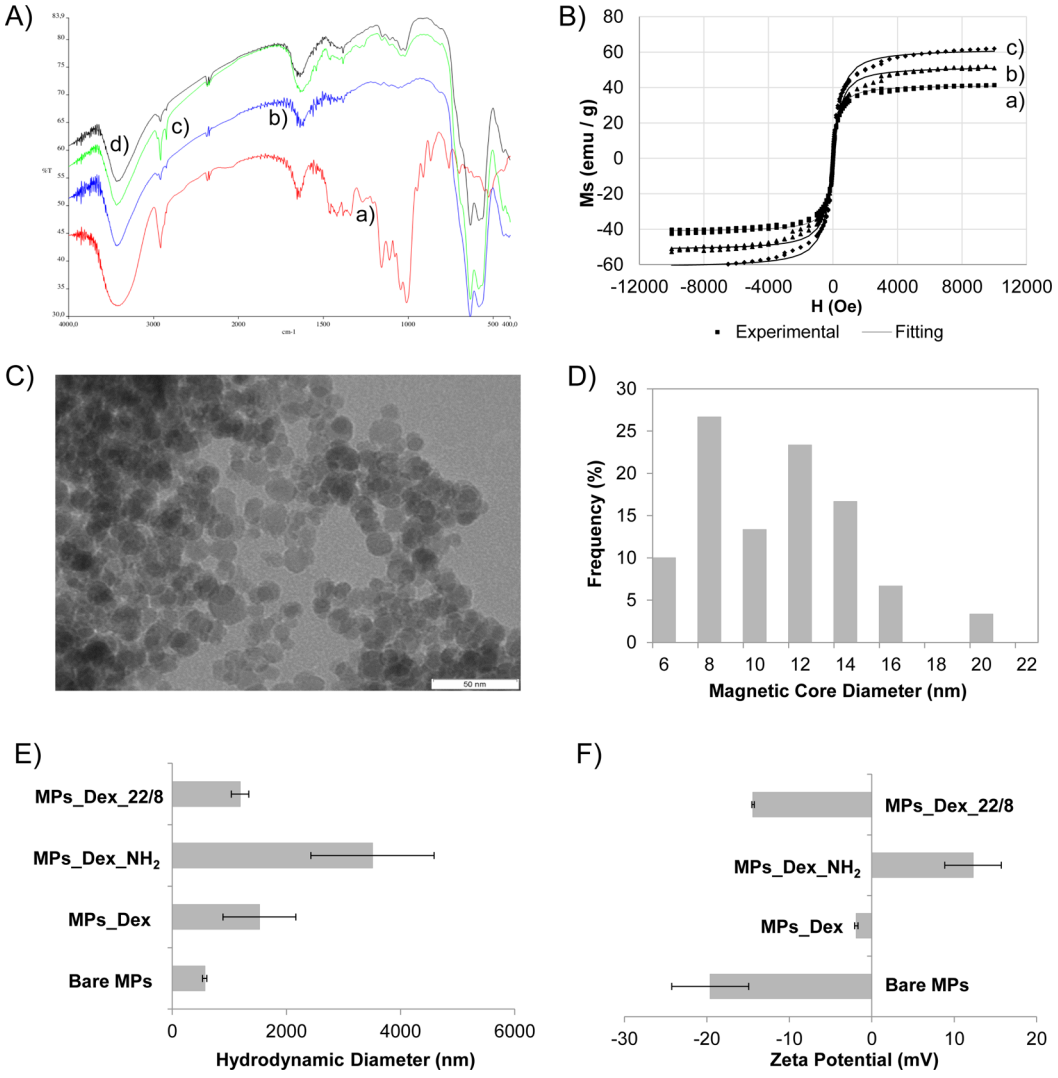

Figure 2. (A) Magnetic particle characterization by FTIR spectra for dextran (curve a), bare MPs (curve b), dextran-coated MPs (curve c), and MPs_Dex functionalized with 22/8 (curve d). (B) VSM curves for bare MPs (curve a), dextran-coated MPs (curve b), and MPs_Dex functionalized with 22/8 (curve c). (C) TEM image of dextran-coated MPs. (D) Grain size distribution from TEM. (E) Hydrodynamic diameter. (F) Zeta potential (n = 2).

250 μL) in phosphate buffer (50 mM, pH 8) were incubated with 250 μL at 6.1 mg/mL of MPs_Dex functionalized with ligand 22/8 by method C, as previously described in literature.[21]

Assessment of Monoclonal Antibody Magnetic Purification from Crude Extracts. The functionalized (MPs_Dex_22/8 by Method C) and nonfunctionalized supports (MPs_Dex) (500 μL with 54 mg/mL) were washed sequentially with regeneration and binding buffers, as described above, and then incubated for 15 min at 4 °C with 500 μL of a CHO cell culture supernatant. The solution in which the particles were suspended was removed by magnetic separation, and then MPs were washed five times with binding buffer (500 μL). After washing, MPs were divided in two equal portions and protein recovery was tested for two elution buffers: (i) 50 mM glycine−HCl, pH 3 and (ii) 50 mM glycine−NaOH, pH 11. All collected samples (loading, flowthrough, and elutions) were analyzed by SDS-PAGE 12.5% Acrylamide/Bisacrylamide in denaturing conditions and stained with Silver Staining kit (BioRad). A BCA assay was also performed in order to quantify the amount of total protein in each of the samples collected.

3. RESULTS AND DISCUSSION

Preparation and Characterization of Affinity Magnetic Supports. Magnetic supports were prepared by the chemical coprecipitation of iron salts and coated with dextran, a neutral polysaccharide well-known as a coating agent. Upon MPs coating, dextran presented high stability toward storage and modification with amino-silanes, as no biopolymer was released over a period of 160 days and during the amination step. The prepared magnetic particles were then characterized by FTIR, VSM, TEM and DLS. The analysis of FTIR spectra (Figure 2A) confirmed the presence of dextran on the surface of the particles. The characteristic dextran peaks at 1427 cm^{-1}, due to C−H bond bending, and around 1000 cm^{-1}, due to the stretching vibration of the alcoholic hydroxyl (C−OH), were visible in the spectra of coated MPs. The characterization by TEM revealed the existence of spherical magnetic cores (Figure 2C) with an average diameter of 12 nm (Figure 2D) and a size distribution between 8−12 nm, as observed previously by Batalha and co-workers.[21] The spherical magnetic cores tend to form agglomerates, more pronounced upon dextran coating, as assessed by an increase on the hydrodynamic diameter (Figure 2E) of MPs_Dex. This phenomenon has already been observed in other works and might be attributed to the noncovalent interactions between the coating biopolymers and neighbor

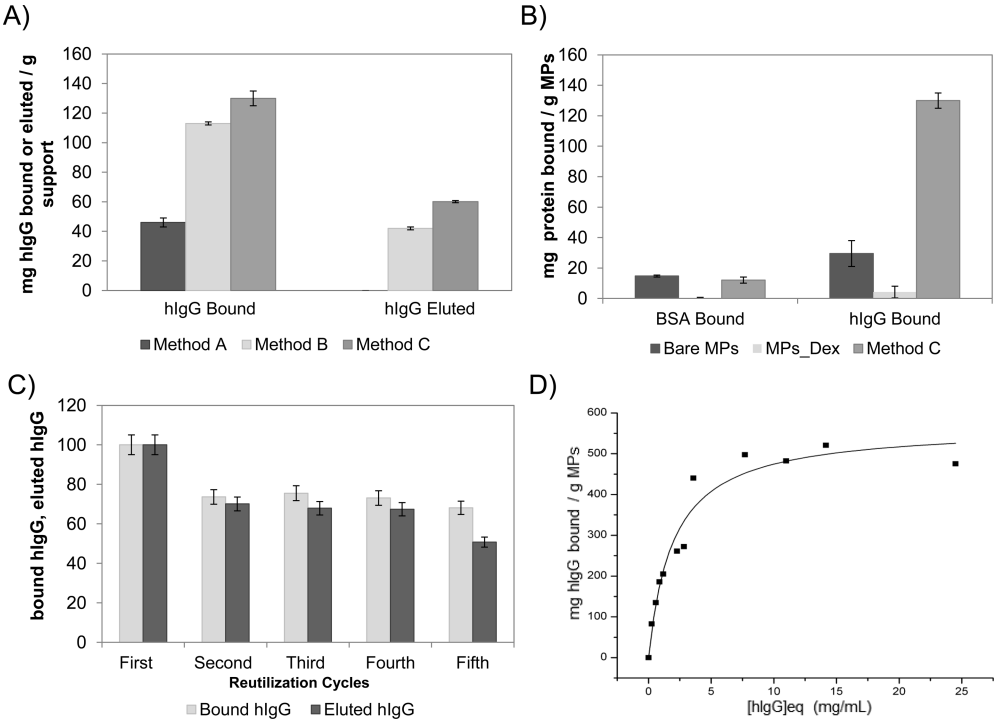

Figure 3. (A) Binding and elution of hIgG to MPs_Dex modified with ligand 22/8 (n = 2); (B) binding of BSA and hIgG to MPs_Dex modified with ligand 22/8 through Method C (n = 2); (C) reutilization of MPs_Dex modified with ligand 22/8 through Method C for binding and elution of hIgG (n = 2); and (D) binding of hIgG at the surface of MPs_Dex modified with ligand 22/8 by Method C. Representation of q (the amount of bound hIgG in equilibrium per mass of solid support) as function of C_{eq} (the concentration of hIgG in equilibrium). Experimental data were fitted with the expression $q = (Q_{ma}x \times C_{eq})/(K_d + C_{eq})$ for the Langmuir isotherm (OriginLab 6.1 software), where Q_{max} corresponds to the maximum concentration of the matrix sites available to the partitioning solute (which can also be defined as the binding capacity of the adsorbent), and K_d is the dissociation constant (n = 2).

Table 1. Comparison of Binding Isotherm of Human IgG to Immobilized Protein A and Ligand 22/8 onto Different Supports and to Ligand 22/8 Immobilized on MPs_Dex through Method C

support	K^a (M^{-1})	Q_{max} (mg of hIgG adsorbed/g of support)
protein A on agarose	3.7×10^5	17
commercial protein A on MPs	3.3×10^5	109
ligand 22/8 on agarose	1.4×10^5	152
ligand 22/8 on cellulose membrane	3.0×10^5	630
Ligand 22/8 on MPs_Ga	1.5×10^5	344
ligand 22/8 on MPs_Dex	7.7×10^5	568

particles.[21,34] The hydrodynamic diameter for MPs_Dex agglomerates decreases slightly upon modification with ligand 22/8, since this functionalization can create steric restrictions, alteration of surface charge and increased hydrophobicity.[21] Through zeta potential analysis (Figure 2F), the presence of the dextran was confirmed as well as the modification of the surface of the particles with ligand 22/8. When coated with dextran, the particles presented a zeta potential of −1.88 mV, because of the neutral charge of the biopolymer, which is corroborated with the values determine by Xu and co-workers.[35] After chemical modification of MPs_Dex with ligand 22/8, the zeta potential of the supports became more negative. These changes in the zeta potential show a surface charge rearrangement due to the presence of new functionalization groups.[21]

Finally, through VSM analysis, it was possible to ascertain the magnetic properties of the supports. The curves represented in Figure 2B show reversibility and symmetry which represents a typical no hysteresis curve characteristic of the superparamagnetic behavior of the particles synthesized. In terms of saturation magnetization, the values obtained were 41.5 emu/g for bare MPs (0.9955), 52.0 emu/g for MPs_Dex (0.9946), and 62.0 emu/g for MPs_Dex modified with ligand 22/8 (0.9933). The saturation magnetization value obtained for the bare MPs is consistent with the values referenced in the literature.[36]

Affinity Magnetic Separation of Antibodies. Our group has previously shown the suitability of gum Arabic as a coating agent to produce magnetic supports modified with the affinity ligand 22/8 for antibody separation. However, the charged nature of gum Arabic can interfere with the adsorption of biocomponents and increase nonspecific interactions. The inertness of MPs_Dex magnetic supports for binding hIgG has been assessed and compared with bare agarose, the traditional support for chromatography, bare MPs and gum Arabic coated MPs. Agarose presented the lowest nonspecific interactions (0 mg/g hIgG bound to unmodified agarose), followed by MPs_Dex (4 ± 4 mg of hIgG per gram of dried MPs), MPs coated with gum arabic (28 ± 3 mg of hIgG per gram of dried MP), and bare MPs (60 ± 2 mg if hIgG per gram of dried MP).[21] MPs_Dex presented seven times less capacity for binding to hIgG, when compared with gum Arabic coated MPs.[21] The differences in the chemical composition of the biopolymers can explain the different reactivity they impair to the magnetic supports. Nonetheless, coating MPs with

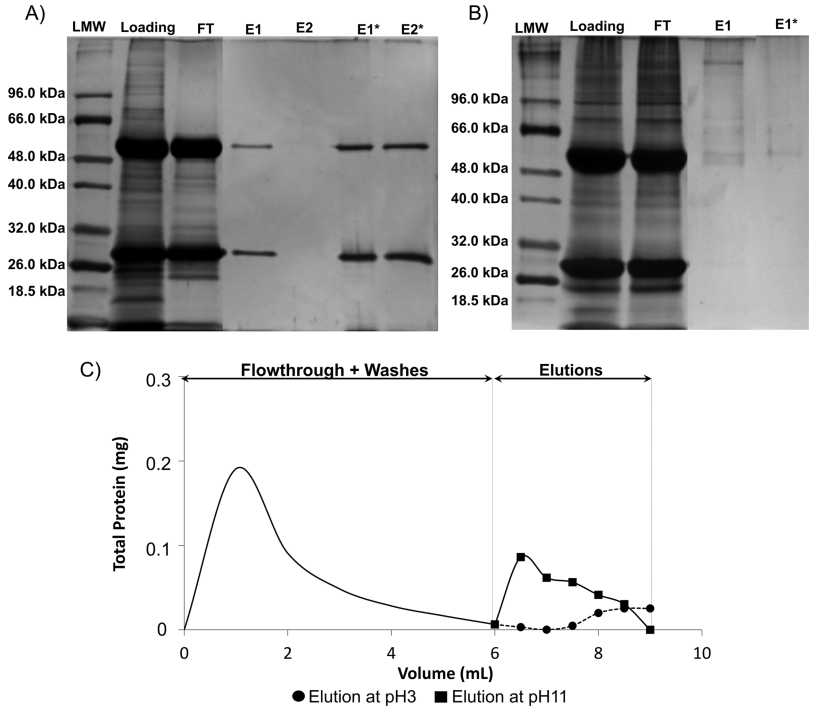

Figure 4. SDS-PAGE gel (12.5%) in denaturation conditions to verify (A) binding capacity of MPs_Dex_22/8 for IgG from a crude extract and purity of fractions, (B) inertness of MPs_Dex for IgG. LMW (low molecular weight); loading (sample of the crude extract incubated with the adsorbent); FT (flowthrough); E1 (first elution with 50 mM glycine − HCl, pH 3); E1* (first elution with 50 mM glycine − HCl, pH 11), and (C) washes and elution profiles for IgG onto MPs_Dex_22/8. The squared and circled points represent the elution profiles at pH 11 and 3, respectively.

biopolymers is likely to create a net of porous structures that leaves reactive iron oxide partly exposed to create interactions and might have some contribution in the nonspecific adsorption of each support. MPs_Dex particles were further on explored for hIgG purification from pure solutions, through the conjugation of a synthetic affinity ligand mimicking protein A, named as ligand 22/8. Three different methods for the covalent attachment of the synthetic ligand onto MPs have been tested (Figure 1). In method A, ligand 22/8 was synthesized in solution-phase with a six carbon spacer. In method B, ligand 22/8 was also synthesized in solution-phase but without a six carbon spacer. Finally, for method C, ligand 22/8 was synthesized directly on the solid support. In method A, there is the need to use a strong cross-linker (glutaraldehyde) which can also react with amine groups from neighboring particles, therefore reducing the free aldehyde groups available to react with the amine groups from the ligand. In addition, the solubility of the ligand is very poor. Method B is performed at high temperature (80−90 °C), at which the less reactive chloride of the ligand is substituted. Consequently, the quantity of ligand that is immobilized on the support may be compromised. In the case of method C, this is a multistep reaction where the coupling of the triazine ring is done at 0 °C through the most reactive chloride, and therefore less likely to result in low reaction yields. Previous works have also shown that immobilization of very insoluble triazine ligands through direct synthesis on the solid support yields best results for protein adsorption.[9]

By analyzing the quantity of hIgG bound and eluted from the supports (Figure 3A), method A revealed to be the less suitable method followed by method B. Method C seems to be the best method to immobilize ligand 22/8 and to produce affinity magnetic supports toward IgG. To assess the recovery of protein, we studied the elution buffer 50 mM glycine−NaOH, pH 11, because of iron leaching at acidic pH, previously observed.[21] In method A it was not possible to quantify eluted protein. In method B, it was possible to elute 42 ± 1 mg of hIgG eluted/g of MPs which corresponds to 37% of the bound protein, whereas for method C, 46% of bound protein was eluted. As a result of these studies, MPs_Dex with ligand 22/8 immobilized by method C (MPs_Dex_22/8) appear as the most promising magnetic supports with a binding capacity of 130 ± 5 mg of hIgG/g of MPs and a elution capacity of 60.1 ± 0.7 mg of hIgG/g of MPs, and further studies were performed.

MPs_Dex_22/8 were tested for binding to a model contaminant protein, bovine serum albumin (BSA), for which the support should not present affinity. The magnetic support bound 12 ± 2 mg of BSA/g of MP, a 10-fold lower value when compared to the quantity of hIgG bound (130 ± 5 mg of hIgG bound/g of MP) (Figure 3B). The regeneration and reuse capacity of the particles was also studied. As shown in Figure 3C particles retain about 70% of the initial protein binding and elution capacity until the fifth stage of recycling. The pH resistance of the support was evaluated in order to assess the release of iron and dextran and therefore infer on eventual ligand leaching, which is covalently bound to the polymer. The total amount of dextran released after using five times the support, was 0.0007% of the total amount of dextran initially adsorbed, and during the first and second cycle of reutilization there was no dextran release. In terms of magnetite release, we observed that after five cycles of reutilization the support lost 0.39% of the initial magnetite which corresponded to 19 ng of iron. In the first cycle of reutilization there was a leaching of 0.09 mg/L Fe (corresponding to 0.0006% of initial iron) during the elution step, that is comparable with the results of Batalha and co-workers.[21] These observations, together with the

retention of protein attached to the support after elution and regeneration, can account for the loss of capacity of the support throughout the reutilization cycles.

The adsorption isotherm of human IgG on the magnetic support MPs_Dex_22/8 (Figure 3D) was fitted in a Langmuir type isotherm and compared with data available in the literature (Table 1). The commercially available protein A modified MPs show experimental adsorption of 109 mg hIgG adsorbed/g MPs.[37] Through the fitting of the adsorption curve of hIgG, an affinity constant of 7.7×10^4 M^{-1} (K_a) and a theoretical maximum capacity of 568 ± 33 mg hIgG adsorbed/g MPs (Q_{max}) were obtained with a correlation factor of 0.95. The affinity constant value is in the same order of magnitude to the Protein A and ligand 22/8 immobilized on different supports. The Q_{max} value for MPs_Dex_22/8 is nearly two times higher than the same ligand immobilized on MPs_GA,[21] four times higher than the same ligand immobilized on agarose and thirty times higher than the natural Protein A immobilized on agarose.[7] Only the cellulose membrane revealed a higher binding capacity, which was not compensated by the low recovering capacity shown by this support.[32]

The magnetic support MPs_Dex_22/8 was finally employed in the small-scale purification of an IgG monoclonal antibody directly from CHO cell culture supernatants (Figure 4) without any initial step to remove impurities. The recovery of pure IgG was visible at pH 3 and pH 11, but in larger yields for the latter. From 56% of total protein bound to the support, there was a recovery of 5 and 16% of total protein at pH 3 and 11, respectively (Figure 4C). Through analysis of the SDS-PAGE gel by densitometry analysis with software Image J, it was estimated that the loading sample contains about 60% of IgG (in terms of total protein present) and that the purified IgG presents 95% purity. The inertness of the MPs_Dex particles was also assessed (Figure 4B) with the crude samples, showing the absence of protein bound to or eluted from the support.

4. CONCLUSION

Iron oxide magnetic particles with a dextran coating are a promising support for the magnetic separation of biomolecules, because of the ease of preparation and chemical modification, low cost, reduced nonspecific adsorption, and high stability. In particular, the covalent attachment of a synthetic affinity ligand mimicking protein A turned these particles viable for the one-step recovery of IgG. Our results show that the direct synthesis of the ligand on the magnetic support yielded the best antibody-capturing properties. In addition, this support MPs_Dex_22/8 also showed low nonspecific adsorption in the presence of BSA and no major loss of capacity after five cycles of protein purification. Moreover the estimated values for affinity constant for ligand 22/8 were comparable with those found for protein A and ligand 22/8 immobilized on different adsorbents, but with the advantage of presenting considerable higher maximum capacity for antibody adsorption. When contacting the magnetic adsorbent with mammalian cell culture supernatants rich in IgG, the MPs_Dex_22/8 supports were able to purify IgG when eluting at pH11 with a purity of 95%.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

MPs, iron oxide magnetic particles; MPs_Dex, iron oxide magnetic particles coated with dextran; MPs_GA, iron oxide magnetic particles coated with gum Arabic; MPs_Dex_22/8, iron oxide magnetic particles coated with dextran modified with ligand 22/8; hIgG, human IgG; BSA, bovine serum albumin

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