Effect of Adsorbed Metal Ions and Buffer Nature on IgG Separation from Human Plasma by Column Chromatography Using an Ion Exchange Resin, Amberlite IRC-718

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ABSTRACT: Fractionation of human plasma on ion exchanger resin was performed on Amberlite IRC-718 saturated with metal ions. Depletion of human immunoglobulin G was carried out by column chromatography using Tris-HCl, pH 7 at different concentrations. Results showed that, when Cu^{+2} and Ni^{+2} were adsorbed on the resin, one or two fractions of purified IgG were obtained, respectively. Whereas Fe^{+2} and Zn^{+2}, both retain IgG and serum albumin or serum albumin alone. Furthermore, the Ni^{+2}-resin retention of serum proteins is too strong that the use of 700 m*M*Tris-HCl cannot liberate any other proteins than nonadsorbed serum albumin. In conclusion, this investigation demonstrates that immobilized metal ion affinity chromatography with Cu^{2+}, Ni^{2+}, and Fe^{2+} immobilized on Amberlite IRC-718 has the potential to be developed as part of a process to purify IgG out of untreated human plasma as acceptable adsorption and elution levels of IgG could be achieved. J Appl Polym Sci 115: 324–329, 2010

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INTRODUCTION

Serum proteins are a reflection of ongoing physiological or pathological events.[1] They, may often, serve as indicators of diseases and are rich sources for biomarker discovery, and play a central role in clinical diagnosis. However, the large dynamic range of proteins in serum makes the analysis very challenging because high abundant proteins (tens to approximately 2 mg/mL) including albumin, immunoglobulins, antitrypsin, haptoglobin, and transferrin tend to mask those of lower abundance.[2] The high abundance of albumin and immunoglobulins (about 80% of total serum proteins) is a major problem in proteomic studies, which use serum, plasma, cerebrospinal fluid, or synovial fluid samples.[3] Depletion of abundant serum proteins will help in the discovery and detection of less abundant proteins that may prove to be informative disease markers.[4] In addition, the depletion of IgG in human serum is employed successfully for the treatment of immune disorders including systemic lupus erythematosus, rheumatoid arthritis, myasthenia gravis, alloimmunization, and cancer.[5–9] The depletion of IgG is commonly achieved by protein A/G affinity adsorbents, which binds to the Fc region of the IgG,[10] however, specific antibodies can also be used. Human immunoglobulin G is an important plasma protein with many applications in therapeutics, immunodiagnosis, and immunochromatography. These applications generally require highly pure IgG.[11]

The high specificity of the bioligands provides excellent selectivity. However, in spite of their high selectivity, protein A/G or antibody carrying adsorbents also have some drawbacks, which are worth considering: (i) the cost of the biomolecules tends to be very high[12] (ii) these bioligands are difficult to immobilize in the proper orientation (iii) antibodies may leak from the matrix and such contamination cannot, of course, be tolerated in clinical applications.[13]

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Recently, immobilized metal ion affinity chromatography (IMAC) has shown great potential in the purification of proteins and peptides,[14] and several types of IMAC columns have been applied.[15,16] The ligands (metal chelates) in IMAC are low cost and have high stability, capacity, and selectivity. It is a versatile technique as the same ligand can be used for the purification of different proteins, and the same chelating resin can be used to chelate different metal ions.[17–19] Proteins retention on IMAC supports is affected by a wide range of factors, such as, surrounding chemical environment, nature of chelating groups, and the specific metal ion.[19]

Synthetic chelating ion exchange resins are receiving considerable attention due to their application in different areas, such as, the removal of heavy metals, heterogeneous catalysis, solid electrolytes, ion exchange membrane, ion selective electrode, and purification of industrial waste.[20] Resins with iminodiacetic acid (IDA) functional group such as Chelex 100, Amberlite IRC-718 (formerly IRC-718), Purolite S930, and Lewatit TP 207 were mainly applied due to their high selectivity and low manufacturing cost.[21] The IDA group could provide electron pairs for chelation; it forms stable coordinate covalent bonds with divalent metal ions. For example, the resins Chelex 100 and Amberlite IRC-718 have been used to treat the waste effluent discharged from printed circuit board manufacturing, which contains Cu^{2+}, Ni^{2+}, Co^{2+}, and Cd^{2+}.[22]

One of the few commercial chelating ion exchange resins available is Amberlite IRC-718; its chelating ability is attributed to iminodiacetic groups.[23] This acidic chelating resin has a high affinity and selectivity for heavy metal cations; this is achieved by an iminoacetic acid functionality chemically bonded to macroreticular resin matrix.[24] The macroreticular structure of Amberlite IRC-718 provides a number of advantages over traditional gel resins; it is highly resistant to osmotic shock and has improved kinetics of ion exchange.[24] Amberlite IRC-718 was used for several purposes such as extraction of heavy metals (Cu^{2+}, Ni^{2+}, Fe^{2+}…) from solutions and for ion exchange chromatography.[25]

In an earlier publication, we have reported on the sorption properties of Amberlite IRC-718 toward the divalent metal ions Fe^{2+}, Cu^{2+}, Zn^{2+}, and Ni^{2+}, which are present at trace levels in natural aquatic systems. In view of the wide interest in the purification of human IgG, and due to reported successful applications of Amberlite IRC-718 in the sorption of several metal ions and in IgG purification, the aim of this work is to study the influence of the adsorbed metal ion on Amberlite-718 and the composition of the buffer on the adsorption and desorption of human IgG onto the resin with different immobilized metal ions in a search for the ideal conditions for the purification of human IgG.

EXPERIMENTAL

Reagents

Unless otherwise indicated, all chemicals were obtained from commercial sources and were used as received; the ion exchange resin containing iminodiacetate groups, Amberlite IRC-718, obtained as a sodium salt was purchased from Rohm and Haas company (USA); bulk density 750 g/L, swelling (%) 30, total exchange capacity 1.35 mequiv/mL (Fernandez et al., 2005), particle size of 16–50 mesh was used without further purification. The following metal ion salts were purchased from Fluka (Buchs, Switzerland) and were also used as received without further purification: Cu(II) acetate, Ni(II) acetate, Zn(II) acetate, and Fe(II) chloride.

Sorption of the metal ions on the polymer

Sorption of the metal ions on the polymer was studied by the batch equilibrium technique as previously described.[20] Duplicate experiments involving 0.100 g of dry, 16–50 mesh size, resin samples were suspended in 25 mL of sodium acetate–acetic acid buffer adjusted to pH 7 with continuous shaking and left for 2 h to equilibrate; this buffer system has been used in several earlier publications.[20] To this mixture, 25 mL of metal ion solution containing a total of 15 mg metal ion were added. After being shaken for a definite period of time at 25°C, the mixture was filtered and the amount of metal ion remaining in the filtrate was determined by atomic absorption spectrometry, using standard solutions for calibration. The amounts of metal ions, Fe(II), Cu(II), Zn(II), and Ni(II) loaded on the resin were 298, 172, 138, and 124 mg/g of resin, respectively. The experimental conditions do not cause any leakage of the four metal ions used and no precipitation of metal ions was observed.

Depletion of human immunoglobulin G (IgG)

Blood samples from healthy donors were collected on anticoagulant (129 m*M* sodium citrate). The samples were centrifuged for 5 min at 3000 rpm with Sigma 3K30 C centrifuge at 4°C and the supernatant was used or stored at −20°C until use without further treatment. The column (1 × 10 cm) packed with the metal ions saturated resin was equilibrated with Tris/HCl 25 m*M*, pH 7 (adsorption and equilibration buffer). One milliliter of human plasma was diluted (1 : 5) with the equilibration buffer and loaded into the column (bed volume of 5 mL). After

TABLE I
Mass Balance for Chromatographs of Human Plasma Eluated with Tris-HCl, pH 7

Metal		Cu^{2+}		Ni^{2+}		Fe^{2+}		Zn^{2+}	
Protein recovery		mg[a]	%[b]	mg[a]	%[b]	mg[a]	%[b]	mg[a]	%[b]
Injection	(Initial quantity)	21.15	100	21.15	100	21.15	100	21.15	100
Washing	25 m*M*	16.13	90.45	14.51	82.79	15.05	85.34	7.25	43.73
Elution	100 m*M*	0.46	2.17	0.91	4.30	0.38	1.79	0.92	4.37
	300 m*M*	1.02	4.82	1.17	5.53	1.31	6.19	0.67	3.16
	500 m*M*	0.27	1.28	0.31	1.46	0.11	0.52	0.12	0.56
	700 m*M*	0.23	1.08	0.03	0.14	0.19	0.89	0.01	0.04
Total		17.11	80.89	16.93	80.04	17.04	80.56	8.97	42.41

[a] Mass calculated from protein concentration determined by Bradford[21] method.
[b] Percentage relative to injected protein mass.

plasma injection, the column was washed with equilibration buffer until proteins were not detected in the column outstream by absorption at 280 nm. Adsorbed proteins were subsequently eluted with discontinuous step gradient of Tris/HCl, pH 7 at several concentrations. Regeneration of the column was achieved by washing the column with 0.5 *M* NaOH. Proteins adsorption–desorption experiments were repeated 10 times by using the same beads, which were cleaned with 50 m*M* NaOH solution and then re-equilibrated with the adsorption medium after each desorption step; no significant differences in the results were noticed. All chromatographic procedures were carried out at ambient temperature at a flow rate of 0.5 mL/min. Total proteins were estimated using the method of Bradford[26] modified by Macart and Gerbaut.[27] Purity and protein contents of different peaks obtained were checked using polyacrylamide gel electrophoresis 7.5% in presence of sodium dodecyl sulphate (SDS) as described by Laemmli.[28]

RESULTS AND DISCUSSION

Initial fractionation of human plasma at the flow rate of 0.5 mL/min resulted in the chromatographs corresponding to each metal ion. The adsorption and washing were carried out in 25 m*M* Tris-HCl buffer of pH 7.0 and elution was achieved with buffers at the same pH but with different concentrations of Tris (100–700 m*M*). The eluates obtained were controlled for their purity using SDS-PAGE.

Protein recovery

Protein quantities were determined, in the initial solution and pooled peaks obtained from chromatographic fractions, using the Bradford method.[26] Except for Zn^{2+}, which gave the least recovery of liberated proteins of 42.4%, all other metal ions gave about 80% of injected proteins as shown in Table I. Similar results have been obtained by Vançan et al.[29] who employed Cu^{2+}, Fe^{2+}, Ni^{2+}, and Co^{2+} immobilized on IDA-Sepharose.

Purification of IgG from human plasma

Results of human plasma fractionation on Cu(II)-resin are displayed in Figure 1(A). The first peak obtained in the adsorption/washing buffer contains all proteins of the plasma. The elution with 100 m*M* Tris-HCl buffer liberates two major proteins corresponding to IgG and serum albumin (lane 2) as compared to native human plasma (lane S) and purified IgG (Sigma) (lane Ig). On the other hand, elution with 300 m*M* Tris-HCl buffer yielded purified IgG (lanes 3), whereas elution with 500 and 700 m*M* Tris-HCl afforded IgG contaminated with higher molecular weight proteins (lane 4, 5).

Results of human plasma fractionation on Ni(II)-resin are shown in Figure 1(B). The first peak obtained in the adsorption/washing buffer contains most proteins of the plasma. Elution with 100 and 300 m*M* Tris buffer liberates purified IgG (lanes 2, 3) as compared to purified IgG (Sigma). The peak obtained with 500 and 700 m*M* Tris-HCl buffer contained proteins with higher molecular weights (lanes 4, 5).

Human plasma fractionation on Fe(II)-resin is given in Figure 2(A). The first peak in the chromatogram obtained in adsorption/washing buffer contains all proteins of the plasma. The elution with 100 m*M* Tris-HCl buffer, however, liberates two major proteins corresponding to IgG and essentially serum albumin (lane 2) as compared to native human plasma (lane S) and purified IgG (Sigma) (lane Ig). The peak obtained with 300 m*M* Tris-HCl buffer contained mainly albumin (lanes 3), whereas elution with 700 m*M* Tris-HCl afforded one protein with lower molecular weight is observed (lane 4).

Results of human plasma fractionation on Zn(II)-loaded resin are shown in Figure 2(B). Peak 1 obtained in the adsorption/washing buffer contains serum albumin (lane 1). No apparent proteins were

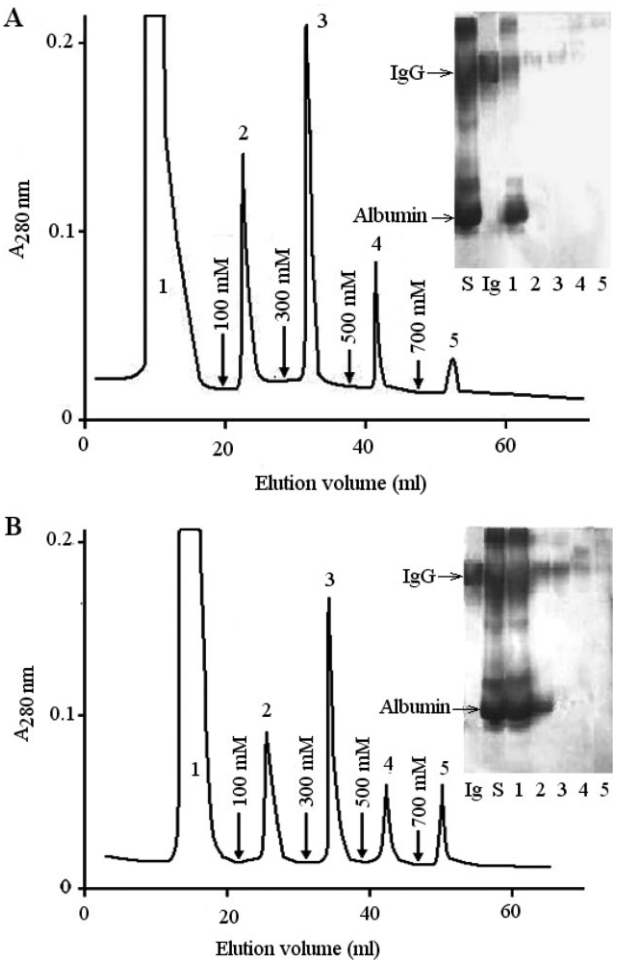

Figure 1 Low flow rate chromatography of human plasma on Amberlite IRC-718-Cu^{2+} (A) and Ni^{2+} (B). A sample of 1.7 mL of human plasma was diluted to 1/5 in 25 m*M* Tris/HCl pH 7 buffer and then applied onto the resin in 5 mL column prepared as described in the materials and methods section. The column was washed with the buffer (peak 1) followed by increasing concentrations of Tris/HCl; 100 m*M* (peak 2), 300 m*M* (peak 3), 500 m*M* (peak 4), and 700 m*M* (peak 5). All peaks were run on SDS-PAGE on 7.5% polyacrylamide gel in nonreducing conditions and stained with Coomassie Brillant Blue (CBB) according to Laemmli.[28] Arabic numerals correspond to peak numbers from the chromatograph. Lane Ig: Human IgG (Sigma), S: human plasma.

observed in the next peaks; this could be due to the fact that concentration of Tris (700 m*M*) is not sufficient to desorb any protein. The pooled fractions were then concentrated using polyethylene glycol and the results obtained were the same.

From these observations, it is clear that IgG from human plasma has been adsorbed onto the resin loaded with different immobilized metal ions. Therefore, one of the objectives of this investigation was to study the adsorption of human IgG onto the resin with different immobilized metal ions in a search for an ideal ligand, to which IgG binding would be sufficiently strong to capture the protein selectively, but not too strong that could hinder application (elution could then only be possible with EDTA as verified by Sidenius et al.[30] for selenoprotein P. Results of this investigation reveal that Cu^{2+} and Ni^{2+} are more efficient in the adsorption and desorption of IgG, followed by Fe^{2+}, which retains IgG and serum albumin where they elute together with 100 m*M* Tris-HCl. When Zn^{2+} was employed, most proteins were retained and IgG was not liberated even with Tris-HCl of upto 700 m*M*. In the case of Fe^{2+}, IgG was widely contaminated with albumin. The results obtained in this study show that IMAC with Cu^{2+},

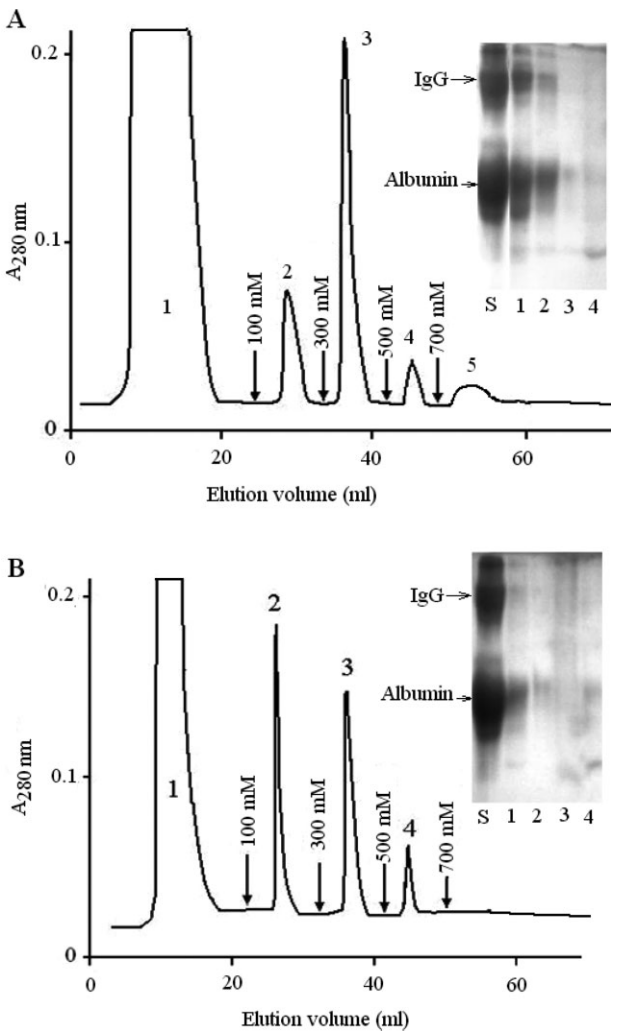

Figure 2 Effect of buffer system on adsorption and elution of human IgG chromatography on Resin-Fe^{2+} (A) and Zn^{2+} (B). Buffer composition is Tris/HCl pH 7; 100, 300, 500, and 700 m*M* to obtain peaks 2, 3, 4, and 5, respectively. Peaks were controlled for their purity with SDS-PAGE on 7.5% polyacrylamide gel in nonreducing conditions and stained with Coomassie Brillant Blue (CBB) according to Laemmli.[28] Arabic numerals correspond to peak numbers from the chromatograph. Lane Ig: Human IgG (Sigma), S: human plasma.

Ni^{2+}, and Fe^{2+} immobilized on Amberlite IRC-718 has the potential to be developed as part of a process to purify IgG out of untreated human plasma, as acceptable adsorption and elution levels of IgG could be achieved. Furthermore, Cu^{2+} and Ni^{2+} have good selectivity to human IgG probably due to the coordination of these ions to the resin and some sites present on the IgG molecule. In contrast, it seems that Zn^{2+} ions have stong affinity toward IgG as Tris/HCl 700 m*M* cannot eluate it. Experiments with human plasma also indicated that IgG can be efficiently separated from plasma proteins using Tris as eluent at biological pH. Similar results have been obtained by Vancan et al.[29] using Cu^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+} immobilized on IDA-Agarose as chromatographic gel and imidazole as eluent buffer. Regeneration of the resin with 0.5 *M* NaOH did not affect its ability to bind proteins; this has also been reported by Karatas et al.[31] and Yavuz et al.[32] who showed that the use of NaOH at this concentration did not affect the ability of the resins to bind IgG. In conclusion, these resins are recyclable and therefore can be employed for several times in IgG purification.

Although, the experiments were not designed to identify the site of interaction between the IgG (and other plasma proteins) molecule and the immobilized metal ion, this site could probably be present in the Fc region of the antibody. Hale and Bleider[33] identified the Fc region of the humanized IgG monoclonal antibody molecule as the region, where the binding site to immobilized metal ions is located. Therefore, human IgG should also bind to the Fc region as the humanized antibody has a murine Fab region fused to a human Fc region. This is a desired situation, as the human IgG to be produced by purification through immobilized IMAC would have sites for complex formation with antigens not involved in the adsorption and elution step and therefore, would certainly be preserved. Thus, immobilized IMAC is not only one of the most popular techniques for protein purification, but it is also a very efficient method for studying protein structure in terms of His residue accessibility.[34,35] As postulated by Sulkowski,[36–38] the affinity of proteins for chelated Cu^{+2} requires at least one accessible His residue. When proteins are retained on chelated Ni^{+2}, they have more than one His residue and the adsorption on chelated Zn^{+2} and Co^{+2} signify a cluster of His residues accessible for coordination.[39]

CONCLUSION

In this investigation, the influence of the nature of metal ions adsorbed on Amberlite IRC-718, a chelating resin containing IDA as ligand attached to the copolymer of styrene and divinyl benzene of macroporous matrix structure resin, and Tris-HCl buffer systems on the purification of human IgG was evaluated and assessed. When the resins were loaded with Cu^{+2} and Ni^{+2}, one or two fractions of purified IgG were obtained, respectively. Whereas, $Fe^{+2}$and Zn^{+2}, both retain IgG and serum albumin or serum albumin alone. Furthermore, the Ni^{+2}–resin retention of plasma proteins is too strong that the use of 700 m*M* Tris-HCl cannot liberate any other proteins than nonadsorbed serum albumin. Results obtained from this investigation revealed that IMAC with Cu^{2+}, Ni^{2+}, and Fe^{2+} immobilized on Amberlite IRC-718 has the potential to be developed as part of a process to purify IgG out of untreated human plasma as acceptable adsorption and elution levels of IgG could be achieved.

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