World Journal of Pharmaceutical Science and Research

www.wjpsronline.com

Research Article

ISSN: 2583-6579

SJIF Impact Factor: 6.916

Year - 2026

Volume: 5; Issue: 5

Page: 36-42

SIGNIFICANT REDUCTION OF IGG AGGREGATES IN MEDICINAL MONOCLONAL ANTIBODIES

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Article Received: 10 March 2026 || Article Revised: 01 April 2026 || Article Accepted: 21 April 2026

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DOI: https://doi.org/10.5281/zenodo.19910573

How to cite this Article: Barakat M. Shabsoug, Nizar M. Abuharfeil (2026) SIGNIFICANT REDUCTION OF IGG AGGREGATES IN MEDICINAL MONOCLONAL ANTIBODIES. World Journal of Pharmaceutical Science and Research, 5(5), 36-42

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ABSTRACT

Therapeutic monoclonal immune response like all IgG Mab shows self-aggregates. These blends may cause a diminishment in counter acting agent adequacy, motivate a shocking immunological response and hence impact the specification of the item. Its objective is to clear IgG blends as much as could be normal while IgG mix was extended by hatching of the Mab at 50◦C for 30 minutes. Fifty (50) mg/ml of the monoclonal counter acting agent was added to protein A, affinity chromatography. The Eluted IgG test was connected to hydroxyapatite chromatography using 5% polyethylene glycol in 10 mM PBS, pH 7.2. The Eluted IgG test was dissolved in 0.15 M Arg hydrochloride in 10 mM PBS, pH 7.2. Measure end chromatography-HPLC and ELISA performed examine of mixes. The measure of IgG mixes was diminished from 16.3% to 0.1% after protein A chromatography. Development of Arg hydrochloride to hydroxyapatite chromatography moreover decreased the mixes to 0.01 %. This result might be of therapeutic motivating force for the X game plan of mix pure remedial Mab or IVIG in pharmaceutical ventures.

KEYWORDS: Therapeutic Monoclonal Response, Aggregates, Arginine, Hydroxyapatite chromatography.

INTRODUCTION

Medicinal polyclonal intravenous immunoglobulins (IVIG) and Mab (Mabs) are regularly utilized as a part of clinical prescription as compelling medications to treat autoimmune ailments, tumors, viral contaminations and different diseases.[1,2] The noteworthy measures of IgG self-combinations have been found in immunoglobulin (Ig) drugs. Amid the most recent years, serious research of the IgG self-combination was achieved. The Ig combinations were categorized whether they are solvent/insoluble, covalent/noncovalent, reversible/irreversible and native/denatured.[3]

The combination is induced naturally after drawn-out capacity because of a few elements, including acidic pH, higher temperatures, ionic strengths and dilutions.[4,5]

The IgG-IgG oligomers have been found in the pooled IVIG 12-40%, and monoclonal antibody arrangements ranging from 0.1-0.3%. The combination was the consequence of Fab-Fab and additionally, idiotype-anti-idiotype communications.[6,7] Nevertheless, papain monovalent Fab pieces could not frame self IgG-dimers.

Commercial pooled human Ig acquired from a large number of donors are normally utilized for the arrangement of IVIG. In addition, medicinal Mab became one of the fastest developing regions in the pharmaceutical business. Combinations are shaped amid antibody producing forms, including purification, ultrafiltration, pumping, solidifying, defrosting and storing.[8]

These combinations may change the physical properties of the medication readiness by creating high viscosity, which may influence the immunogenicity of the Ig that elicit an ominous immunological inflammatory reaction, cause a lessening in drug effectiveness, and thusly influence the specification of the product. Subsequently, this phenomenon represents a noteworthy issue in the clinical utilization of the antibodies.[9]

A few methods were utilized to expel or to determine the level of combinations in antibody arrangements, including ionic, cationic and hydroxyapatite chromatography amid the purification steps notwithstanding different measures amid drug provision. Hydroxyapatite chromatography is utilized viably for the expulsion of Ig combinations. Hydroxyapatite is a calcium orthophosphate hydroxide in a permeable frame.

The bound Ig proteins are eluted by chloride inclination. IgG monomers are eluted right on time in the first pinnacle, while IgG combinations appear later. A few A.As are utilized as a part of Ig arrangements to diminish the combinations including Arg, lysine, and proline.[10,11] The maximum admissible combination levels for pharmaceutical products are particular criteria. A few arrangements might be stable and safe regardless of specific levels of combinations while for others, little changes in combination level may essentially influence the Ig strength, viability, and security.[12]

Regardless of the different procedures that have been utilized to expel Ig combinations, it is so far unsuccessful to eliminate. In this examination, a mix of Arg and polyethylene glycol in hydroxyapatite chromatography has been utilized in trying to capacity the combinations in the monoclonal antibody arrangements.

MATERIALS AND METHODS

Mab against CD20

Mouse CD20 Monoclonal Antibody was prepared according to known method[13]. Female BALB/c mice (8-12 weeks old) were procured from Jordan university of science and technology. Pristane, 0.5 ml (2, 6, 10, 14 tetramethyl pentadecane, Sigma) was injected intraperitoneally into mice. After 12 days, high cell densities of the prepared clone (1×10^6 cells/0.5 ml PBS) were given intraperitoneally into mice.

The mice were assessed daily for production of ascitic fluid after the injection of hybridoma cells. The abdomen of the mice was completely enlarged about ten days after the injection of cells. Their ascitic fluids were harvested by 19-gauge needle and centrifuged and the related supernatants were collected for characterization. The titer of monoclonal antibody was assessed by ELISA method. The Mab was set up at a convergence of 10 mg/mL.

Protein A chromatography

The Mab protein was loaded on a Protein A column (5 cm x 10 mm), washed with phosphate buffered saline (PBS), pH 7.2 before bound Mab was eluted from the resin using 100 mM glycine (pH 3.5), 100 mM acetic acid (pH 3.0) or 100 mM Arg (pH 3.0). The protein containing fractions were then held at low pH before neutralization with 1 M Tris buffer (pH 8.3). Solution turbidity was observed by absorbance at 410 nm. The protein samples were quantified using absorbance at 280 nm.

Hydroxyapatite (HA) chromatography

A 1 mL column, 20 µm was equilibrated with 10 mM sodium phosphate, 20 mM MES-buffer, pH 6.5, at a linear flow rate of 300 cm/hr (1 mL/min). 100 µL of protein-A purified MAb, or the respective aggregate reference was injected; the column was washed with equilibration buffer, then eluted in a 30 CV linear gradient to 500 mM sodium phosphate, pH 6.5, 5% PEG 5000.The column was equilibrated to 50 mM Tris buffer, pH 8.5. The IgG peaks from the corresponding HA experiments were titrated to pH 8.5, then applied in-line dilution at a 10% sample, 90% equilibration buffer. The column was washed with equilibration buffer and eluted in a 15 CV linear gradient to 50mMTris, 0.5 M sodium chloride, pH 8.5.

Determination of Ig aggregation

Aggregation of Ig was observed by measuring turbidity of the fractions at 410 nm.

SDS-PAGE

To test the homogeneity fractions, vertical electrophoresis was run utilizing 7.5% sodium dodecyl sulphate (SDS), polyacrylamide gel (PAG).

ELISA

The Mab was assessed utilizing ELISA units (eBioscience, Inc, USA).

Size-Exclusion Chromatography

Immunoglobulin monomers, dimers, combinations, and sections were measured by size-exclusion high-performance chromatography (SE-HPLC) on a TSK G3000SW-XL (7.8 mm 30 cm) segment (Tosobiosciences) in a buffer containing 55 mmol/L Na_2HPO_4, 25 mmol/L NaH_2PO_4, 0.4 mol/L NaCl, and 0.1 g/L NaN_3 at a flow rate of 0.7 mL/min.

RESULTS

Two clones of specific hybridoma cells against CD20 were produced B1 and B2. Both were IgG1 and recognized 2 different epitopes according to inhibition ELISA. In ELISA, B2 Mab showed stronger reactivity than B1 so B2 was used throughout the whole study. The ascitic fluid was aspirated from the injected mice and the specific the mab preparation was centrifuged to remove debris followed by filtration at 0.2 µm. The Mab was prepared in a concentration of 10 mg/ml. Then Protein A chromatography and HA chromatography were used. Mab is an acidic protein with an isoelectric purpose of 5.0. At first, the Mab did not bind the hydroxyapatite medium in 10-25 mM Phosphate buffer, pH 7.0 (Fig. 1).

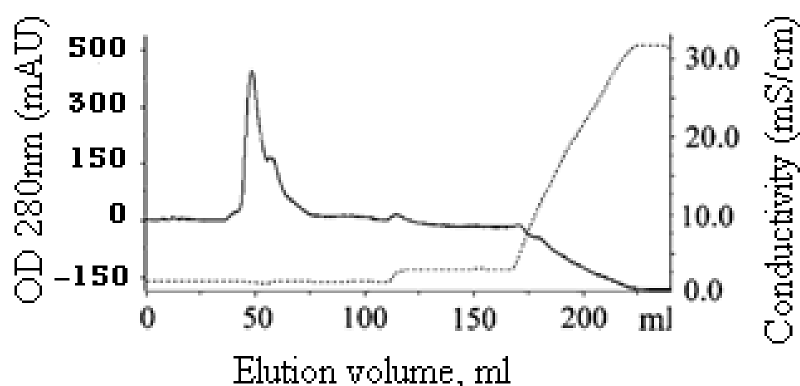

Fig. 1: Hydroxyapatite chromatography. Elution profile of Mab using potassium phosphate buffer only. The protein (4mg) in 25 mM Hepes, pH7.0 was injected into the column with 10 mM phosphate, pH7.0. The absorbance at 280 nm (–) and the conductance of the eluent (mS/cm) (---) were observed continuously. Unbound protein was removed by washing with 60 ml of10 mM phosphate, pH 7.0. Loosely bound protein was removed with a wash of 60 ml of 25 mM phosphate, pH7.0. Finally, an increasing phosphate gradient (25– 400 mM) at pH7.0 was applied to the column over 60 ml. The flow rate was maintained at 2ml/min

Aggregation was observed by measuring turbidity of the fractions at 410 nm.

The addition of 0.1M Arg to the elution buffer of protein A chromatography decreased the Ig aggregate from around 34.4% to 16.3% at low pH value. Moreover, the addition of 5% PEG to the buffer of HA chromatography decreased the Ig aggregates to 0.1%. The sample was dissolved in PBS with 0.1M Arg that decreased the aggregate to around 0.01% (Table 1).

Table 1: Summary of each purification step of the Mab from cell culture.

Purification step	**% of aggregation Before**	**% of aggregation After**
Centrifugation and ultrafiltration	>40	34.3
Protein A chromatography	34.4	16.3
Hydroxyapatite chromatography	16.3	0.1
Arginine in sample buffer	0.1	0.01

SDS-PAGE analysis of the Mab proteins showed no aggregates after protein A chromatography and hydroxyapatite chromatography, and only the heavy 50 kDa band and light chain band 25 kDa were obtained (Fig. 2).

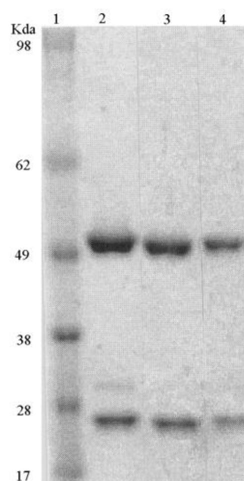

Figure 2: SD-PAGE analysis of monoclonal antibody.

1. MW standard. 2. Mab supernatants after protein A chromatography. 3. Mab after hydroxyapatite chromatography. 4. Mab in buffer with arginine.

DISCUSSION

The presence of contaminants in the Mab products makes the drug of low quality because of the resulting inflammation. Despite the purification strategies utilized as a part of the literature, still, some noteworthy contaminants exist in the Mab products. Utilizing Arg at pH 3.0 followed by hydroxyapatite chromatography, the contaminants were essentially decreased to minimal.

Whereas Arg was found to be a useful excipient during isolation, the addition of His as a stabilizer has an extra benefit due to its buffering capacity with a side chain pKa about pH 6 to be effective in the neutral pH region. Histidine is being utilized as a buffer in numerous lyophilized formulations of medicinal antibodies, for the sample, Herceptin. In an examination, using lyophilized mAb formulations has shown that the best stability was accomplished with the addition of both Arg and His.[14]

In an examination, by Arakawa et al.[15] on the impact of Arg on the solubility of A. As in water, gave some insight to the cooperation between an A.A with a protein. The reaction between Arg and certain amino acids such as Trp, Tyr, His, and Asn showed an enhanced solubility. Addition of Arg to a protein in solution will result in interactions between the A.A and the protein's R-groups by a cation–π interaction, hydrogen bonding, and electrostatic interactions.

Large molecular weight PEG arrangements are effective at lower concentrations, yet low molecular weight PEGs are more favorable. Many PEG polymers with average molecular weights of 1000 or less are approved inactive ingredients in parenteral formulations. Such polymers can be removed from final product by diafiltration or dialysis, while PEG-5000 has an indistinguishable hydrodynamic radius as a globular protein with a molecular weight of 50-100 kD[16] and prevents size-based elimination strategies.

The use of Arg at low pH buffers in protein A chromatography to detach Mab is effective in stabilization and elution. During industrial preparation of Mabs often performed at acidic pH during chromatography. This has a dual effect in both stabilizing and killing of viruses.

Dislodging water surrounding nonpolar buildups is vivaciously ideal and drives combination where hydrophobic interaction is prevailing. At the point when the proteins are close, non-covalent bonds, for sample, hydrogen bonding or ionic interaction can happen between the A.A deposit's R-groups. Most combination occasions are a mix of hydrophobic and non-covalent bonding. At the point when a cosolvent is added to the blend, the relationship between water and protein turns out to be more complicated.[17]

The heat strength of proteins is a mix of non-covalent bonding in the core of the protein, elimination of water from nonpolar buildups and the water in the solvation shell. Cosolvents assume an immediate part in the structure of the solvation shell and in this way the heat strength of the protein.[18] Tell now it is the non-specific collaborations amongst cosolutes and proteins that have been specified. There are large binding samples of a ligand expanding the heat stability of proteins.

Lipid-free HSA in addition to the expansion of a substitute cosolutes likewise increased the protein's T_m, by associating with the binding site and mirroring the lipid, if just feebly. Major proteins have at least one binding site that may assume a part in protein strength and give association site(s) for non-specific stabilizers, for the sample, A.As. The determination of a cosolvent to stabilize a protein's binding site is difficult[15]. In this examination, Mab arrangements could be refined from contaminants to a minimal amount that may diminish the inflammatory impacts of its Medicinal applications.

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