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Purification of IgG Antibody from Human Serum

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Abstract

In present world monoclonal and polyclonal antibody are widely used in medicine and therapeutics. Hence there is increase in the consumption of plasma derived Immunoglobulin G (IgG) as they are used in the treatment of number of diseases, including neurological diseases and autoimmune conditions. It is necessary to produce IgG derived plasma protein in high quantity to meet the rate of its consumption. Isolation of immunoglobulin in research lab for different application is from cell culture medium, plasma, serum, hybrid cell etc. There are various methods like ammonium sulphate precipitation, affinity chromatography and thiophilic chromatography to purify the desired antibody from human serum sample. Depending upon the biochemical properties of immunoglobulin, different chromatographic techniques are performed to isolate antibody from the source sample. In our present investigation we have purified IgG antibody from human serum sample using protein-An affinity chromatography. After each purification step the biuret test was taken to quantify the protein concentration. The protein a purified sample weighs 5 mg/ml after dialysis was analyzed along with a standard protein marker in polyacrylamide gel electrophoresis. The molecular weight of protein a purified IgG was found in between 20 kda to 90 kda after SDS-PAGE analysis.

Keywords: Human serum; IgG antibody; Ammonium sulphate precipitation;Affinity chromatography; SDS-PAGE

Introduction

Antibodies are belonging to the family of molecules, the immunoglobulin. They constitute the humoral branch of the immune system. The concentration of protein in serum is about 60-80 mg/ml. Among this 55% are albumin protein and 40% are globulin protein. In globulins, 10-20% are immunoglobulin G *i.e.* IgG. Various type of immunoglobulin is found on the surface of lymphocytes. They are also present in exocrine secretions and in intra and extra vascular fluids. Antibodies are the proteins that are primarily produced by B lymphocytes in response to foreign molecules or other agents in the body. The response of our immune system towards foreign antigen is a key mechanism used by a host organism to protect itself against the action of foreign molecules or organisms. B-lymphocytes have specific receptors that can recognize and bind to the antigenic determinants of the antigen and thus stimulates a process of division and differentiation. The lymphoid or plasma cells that predominantly synthesize antibodies.

Immunoglobulin

Immunoglobulins are very specific in nature. They have four polypeptide chains: two identical Heavy (H chain and two identical, non-glycosylated Light (L chains. Each heavy chain carry covalently attached oligosaccharide groups. Heavy chain and a light chain are joined together by disulphide bond. The two identical heavy chains are also joined to each other by disulphide bonds. In the hinge region the disulphide bonds are located in a heavy chain. This region has approximately 12 amino acids that are exposed to enzymatic or chemical cleavage. All four polypeptide chains have some common region *i.e.* Constant (C) and Variable (V) regions. They are found at the carboxyl and amino terminal portions, respectively. A single V region is present in heavy and light chains. In addition light chains also possess a single C region while heavy chains contain three C regions. In both heavy and light chains the V region combine to form two identical antigen binding sites, these parts of the antibody actually bind the antigen.

Immunoglobulin also known as antibody, they are the glycoprotein produced by B cell in response to any antigen. They generally neutralize the antigen and then eliminate it from our body [1]. Their main function includes opsonisation or activation of complementary cascade which generally consist of 64 different proteins in which one protein activate the other protein. There are five different classes or type of immunoglobulin *i.e.* IgG, IgA, IgM, IgE and IgD. Each class of immunoglobulin differs in size, molecular weight, structure and function. Each class of immunoglobulin performs different function due to their difference in heavy chain polypeptide. IgG, IgD, IgE exist as monomer in human serum while IgA when secrete in saliva it is found to be present as a dimer. Beside this, serum IgM class of immunoglobulin exists as pentamer and its monomer are bound to each other by disulphide bound.

Properties of IgG antibody

- **Molecular weight:** 150,000
- **H-chain type (MW):** gamma (53,000)
- **Serum concentration:** 8 to 16 mg/dl
- **Percent of total immunoglobulin:** 75%
- **Glycosylation (by weight):** 3%
- **Distribution:** Intra and extra vascular
- **Function:** Secondary response

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Among the five classes of immunoglobulin IgG is the most abundantly
present antibody in human serum (constitute about 80% of total immu noglobulin in serum). There are 4 subclasses of IgG immunoglobulin *i.e.* IgG1, IgG2, IgG3, IgG4. IgG plays very important function in the human defence mechanism as it opsonize and neutralize the toxin of our body. Beside this, IgG class of immunoglobulin plays an important role in activation of complement system through classical pathway. IgG antibody works against the protenicious antigen and majority of our antigen are protenicious in nature. It is the only class of immunoglobulin which crosses the human placenta. Since it is abundantly present in human serum and has excellent specificity towards the antigen it became the principle antibody that is widely used in immunological research and clinical diagnostics. A delayed response is produced towards the infection by our body through IgG antibody. The longevity of IgG class of antibody in serum makes it useful for most of the passive immunization. In addition in immune deficient people preformed immunoglobulin is given through passive immunization in their body.

Functions of IgG antibody in human immune system

- Activation of complement system.
- Antibody dependent cell mediated cyto-toxicity.
- Neonatal immunity
- Opsinization
- Feedback inhibition of B cells.

Now a day's immunoglobulins are widely used in diagnosis diseases including HIV (ELISA). Immunoglobulin is used in various research areas like molecular medicine, forensic science, biotechnology etc. It is been used in various techniques like western blotting, blood typing and in clinical medicine.

Since immunoglobulin have several important applications, the present investigation was carried out to purify the IgG antibody from human serum sample. Purification ranges from very crude to highly specific methods depending upon the target molecule.

- **Crude:** precipitation of total serum protein.
- **General:** affinity chromatography for purification of particular antibody from the mixture of protein example (IgG) without regard to antigen specificity.
- **Specific:** affinity chromatography for purification of specific antigen sensitive antibody.

There are different purification methods that are used to isolate IgG antibody from human serum

- Ammonium sulphate ($(NH_4)_2SO_4$) fractionation
- Protein-affinity chromatography using protein-A
- Thiophilic affinity chromatography

According to the choice sample, techniques for sample preparation and purification were employed. This is due to the presence of different specific contaminants in the sample and the concentration of required target molecule. However, in most of the cases, affinity chromatography is employed so that in a single step we can minimize the contamination and produces a sample of high purity in a single step (Table 1).

Source of sample	Molecular type	Quantity	Specific contaminant
Human serum	Polyclonal antibody	IgG 8-16 mg/dl	Albumin, transferrin, alpha-2-macroglobulins, haptoglobulin, ceruloplasmin, and other serum protein.
Egg Yolk	Polyclonal antibody	IgY 3-4 mg/dl	Lipids, lipoprotein and vitellin.

Table 1: Different source of antibody sample and their specific contaminant.

Different source of sample has different significant contaminants for example if our source of sample is egg yolk then significant contaminants include lipoprotein, vitellin etc. while if our source of sample is human serum then major significant molecules includes albumin, transferrin and alpha macroglobulin protein. Since the contaminants are different so different purification methods are used in order to get purified target molecule. Apart from the source of sample and its significant contaminants, the concentration of target molecules is also important in the choice of purification technique for example concentration of IgG in human serum sample is present in higher amount *i.e.* 8-16 mg/dl in comparison to other classes of immunoglobulin.

Literature Review

There are various *in vitro* and *in vivo* applications like drug targeting, immunotherapy, environmental analysis, basic research and industrial process etc. in which antibodies are widely used now a days. Generally for the above mention applications, the process of isolation and purification is of great importance. Practically isolation of immunoglobulin in research lab for different application is from cell culture medium, plasma, serum, hybrid cell etc. All the above mention source of antibody contains specific contaminants like lipid, lipoprotein, alpha macro globulins etc.

Munish and his associates had purified IgG from serum sample. For the purification process they had used various methods. These methods were ammonium sulphate fractionation, protein-A affinity chromatography and Thiophilic Affinity Chromatography (TAC). The main aim of their investigation was to select a method that can be used for further studies. Among the three method mention earlier, protein-A affinity chromatography was the best method to get desired molecule in minimum duration of time. This is due to purity of desired molecule and protein concentration. During the affinity chromatography they have estimated the protein concentration at after every purification step [2].

Purified antibodies are of great importance now a day's, there availability is mostly depend upon the purification steps. There are numerous methods to purify antibody but the affinity chromatography is most utilized method that is extensively use. Affinity chromatography involve minimum steps providing simple approach and purify the sample in less duration as compare to other techniques [3-6]. It is very important to remove contaminants as they interfere in the efficient purification process. Centrifugation and filtration are the t

echniques which are generally use to remove particulate matter and hence ensure a clear sample. Through cell debris and other lipoprotein can be removed from the sample. Small contaminants are generally removed by normally transfer the sample to optimum buffer *i.e.* buffer exchange. The buffer exchange purification protocol is done by using dialysis in order to separate small contaminant different type of filtration methods are used like ultra-filtration, microfiltration and cellulose based ultra-filtration [7-9].

Depending upon the biochemical properties of immunoglobulin, different chromatographic techniques are performed to isolate antibody from th e source sample. Biochemical properties include hydrophobic interaction etc. Affinity chromatography is the simple, robust but still most effective technique used for purification of antibody. The principle of affinity chromatography is simply based on the interaction between protein and ligand. The interaction between them is reversible in nature. Hence while performing the experiment ligand forms a covalent bond with the solid support. When sample was passe d through the chromatographic column, antibody get bind to the ligand which is further eluted out in its pure form using denaturing agent [10]. Elution of sample is performed at specific pH in order to isolate stable antibody.

Grodzki and Berenstein had used Ammonium sulphate to precipitate the soluble components of the sample. Precipitation of antibody using ammonium sulphate is called salting out process which is basically depends on the principle of adding Hofmeister or lyotropin series of salt in the solution up to a concentration which results in the competition of ions for the solvent thus resulting in the formation of aggregates which is further precipitate out of solution. Quantity of protein obtained majorly depends upon several factors like time, temperature, pH, and rate of salt addition while the study of Gagnon suggested that crystallization and precipitation can be used to alleviate turbidity [11]. In order to remove the antibody from its sample matrix precipitation is used. Different salts has been used to precipitate antibody from its sample matrix like poly ethylene glycol, octonic acid and ammonium sulphate.

Myhre and Kronvall had investigated that purifying the antibody from human serum sample protein–A is preferred over protein-G as protein-G binds to the contaminants of serum sample like alpha macroglobulin, kininogen along with immunoglobulin which result in reducing the efficiency of purification of immunoglobulin process. Besides this all, protein-G has less binding capacity and stability in comparison to protein-A during elution step. Therefore protein-A is used to purify antibody specifically IgG class from the human serum sample. One of the drawbacks of using this purification technique is that it is restricted to certain classes of immunoglobulin and specific source of sample.

Accordingto Hahn and his associates work done, protein A based resins are most commercially use in affinity chromatography [4-6]. They did a comprehensive study on protein affinity media that are available in today's world. In their investigation they have conclude that the selectivity of the Protein a based media are not suitable for the purification of polyclonal IgG class of antibody. The basis of chromatographic technique is the reversible adsorption of target molecule on the solid support of the column. In various biopharmaceutical industries, affinity chromatography is regularly used to purify the target molecule and to set the purity and quality standards. With the advancement of technology today scientist are focusing to develop more advances in chromatography techniques like invention of biological ligand methods.

In order to separate the antibodies from complex mixture chromatographic and non-chromatographic methods are employed. In order to purify selective antibodies affinity chromatography is use but using this technique for high scale production is not economically cheap. There are several advantages of using protein-A affinity chromatography as protein A has high flow rate, high binding capacity as it bind to Fac, Fab and F(ab)2 region of IgG antibody but sometime it bind to non-specific region of cell protein. It has been observed that tag affinity based purification method is very useful to purify recombinant antibody but it require expensive resins to provide solid support to column.

According to Laemmli, analysis of protein was done. SDS-PAGE is technique used to separate proteins on the basis of their molecular weight. In SDS-PAGE, protein are analysed along with a standard protein marker. Proteins with different molecular weight were found in serum sample ranging from 20 kda to 90 kda. Protein-A affinity chromatography purified IgG showed to bands corresponding to molecular weights 28 and 52 kDa. The molecular weight of proteins 28 and 52 kDa corresponds to light chain (22 kDa) and heavy chain (52 kDa) of IgG class of immunoglobulin molecules [9].

Materials and Methods

Samples used

Samples were used for the purification

- Human serum

Protein quantification

First, quantification of protein was done in the chosen sample (human serum, Egg yolk samples and cow milk sample). In our investigation we have used human serum sample so the total proteins present in the sample was estimated by Biuret method. The estimation of protein was done at after every step of purification to ensure that the proteins are not lost during the purification protocols.

The sample was taken to Biuret test after dialysis, before dialysis as well as eluted sample of affinity chromatography.

Procedure

- Parallel test tubes were taken and reagents were put in them according to the given table.
- Withthe help of distilled water make the volume to 5 ml.
- At 37°C incubate the first test tubes for 10 minutes.
- Take the first test tube, marked it as blank and set the colorimeter at zero at 540 nm.

Measure the optical density of rest of the left test tube using calorimeter.

Sample preparation

- Take 1 ml human serum in a test tube using micro pipette.
- Diluted the 1 ml serum 10 times using distilled water.
- Adjust the pH of diluted serum to 4.0 using 1N HCL.
- Centrifuge the diluted serum at 2800 x g, at 4°C for 40 minutes.
- Added 0.01% charcoal to the supernatant and adjust the pH to 4.0.
- Store it overnight at 4°C.

- Next day centrifuged the charcoal containing diluted serum at 2800 x g, for 40 minutes.
- Filter the supernatant using whatman filter paper.
- Serum sample is prepared and ready to use.

Ammonium sulphate precipitation

Principle: The first step in antibody or protein purification from its natural or raw state is to remove soluble components by forming a solid phase known as 'precipitate'. Therefore through precipitation protein (antibody) is concentrated in the sample. Ammonium sulphate is most commonly used to precipitate the soluble components of the sample. Precipitation of antibody using ammonium sulphate is called salting out process which is basically depends on the principle of adding Hofmeister or lyotropin series of salt in the solution up to a concentration which results in the competition of ions for the solvent thus resulting in the formation of aggregates which is further precipitate out of solution. Quantity of protein obtained majorly depends upon several factors like time, temperature, pH, and rate of salt addition.

Reagent required

- PBS BufferWeighed the salts (NaCl-0.8 g, KCl-0.02 g, Na_2HPO_4-0.144 g, KH_2PO_4-0.024 g) and mix them in 70 ml distilled water. Adjusted the pH of solution to 7.4.Then volume was making up to 100 ml with distilled water.
- Tris HCL-1 MAdjusted the pH to 8.0.
- Saturated $(NH_4)_2SO_4$ Dissolved 12 g in 12 ml water.
- HCL-1 N

Procedure

- Added 1 ml Tris HCL in 10 ml serum sample and adjust pH to 8.0.
- Stirred the serum sample gently.
- Added $(NH_4)_2SO_4$ drop by drop in the serum sample.
- Added ammonium sulphate upto 50% saturation.
- Stirred the serum sample for 1 hour.
- Centrifuge the serum sample containing ammonium sulphate at 2800 x g, 4°C for 20 min.
- Discard the supernatant and again centrifuge the sample at 2800 x g, 4°C for 20 min.
- Collect the pellet and dissolve it in 2 ml PBS.
- Take a dialysis membrane, filled it with above sample and tie it's both end.
- Left the sample for 2 days in dialysis.

After ammonium sulphate precipitation stabilizes protein mixture was obtained which was further purifying by hydrophobic interaction chromatography or affinity chromatography to isolate a specific protein from human serum sample.

Note: It is very important to remove contaminants like undesired protein, cell derbis etc. as they interfare in the efficient purification process. Centrifugation and filtration are the techniques which are generally use to remove particulate matter and hence ensure a clear sample. By this process cell derbis and other lipoprotein can be removed from the sample. Small contaminants are generally removed by normally transfer the sample to optimum buffer *i.e.* buffer exchange. The buffer exchange purification protocol is done by using dialysis.

Purification of IgG by protein-Affinity chromatography

Principle: Affinity chromatography is a technique involves the entrapment process in which the desired molecule is trapped on a solid support. In an affinity chromatography there are two phases:

- Stationary phase
- Mobile phase

The differences in the interaction between these above given phases is the basis of affinity chromatography. The stationary phase is typically a gel matrix which is first loaded into a column. On the other hand mobile phase contain variety of bio-molecule ranging from DNA to protein. The biochemical property of desired molecule is exploited during the purification process. Sometime binding of desired molecule is prevented due to steric interference which inhibits the bonding of desired molecule to the ligand. After the binding of desired molecule to the solid support or Ligand, the stationary phase is removed and eluted out using buffer and dialysis process.

Reagents required

- Resin-Sepharose
- Ligand-Protein A
- PBS (pH-7.4)
- Citric Acid-1 M
- Elution Buffer

Procedure

- Opened the cap of column and poured the Protein-A sepharose resin mixture made in phosphate buffered saline (pH 8) into the column.
- Opened bottom cap of column slowly and allowed the liquid to flow out.
- Filled the column with the resin for around 1 cm in length.
- Ensured that the buffer should always cover the resin.
- To prevent the flow of buffer closed the bottom end of the column
- In order to separate out extra buffer the bottom cap was open.
- Once the buffer has moved into the resin, we applied 200 µl of protein sample to the surface of resin.
- Added 200 µl of PBS in order to remove all unbound protein.
- The IgG will get bound to Protein A at pH 8.
- Added 1000 µl of 0.1 M Citric acid pH-3 and collect eluent in a appendorf tubes.
- The eluent would contain purified IgG.

Qualitative analysis by SDS-PAGE

Principle: SDS-PAGE is technique used to separate proteins on the basis of their molecular weight. During polyacrylamide gel electrophoresis protein are separated through a gel matrix, small protein move faster due to less resistance. Factor like charge of protein and its structure also influence the rate of migration of protein through a gel matrix. In SDS-PAGE, sodium dodecyl sulphate and poly acrylamide is use to reduce the influence of protein structure and charge on the separation of protein so that the separation of protein during SDS-PAGE is only on the basis of length of polypeptide chain.

SDS has strong denaturing effect as it unfold the protein by cleaving its disulphide bonds. Polymerized acrylamide make the matrix suitable for the separation of protein of particular size by forming mesh like structure in the matrix. Migration of protein in a gel depends on the concentration of separating gel. In order to separate small size protein, higher concentration of poly acrylamide is used. After pouring acrylamide solution for making stacking gel, insert the

combs and allow the gel to polymerise. When proteins are migrating through a stacking gel they are highly concentrated but when they are in separating gel their concentration became less due to difference in the rate of migration of glycine ion, chloride ion and protein.

Reagents required

- **12% of separating gel**
- 40% of Acrylamide-Bisacrylamide solution (1.5 ml)
- 1 M Separating Gel Buffer(2 ml)
- 10% SDS (0.5 ml)
- Distilled water-1 ml
- 10% APS-100 µl
- TEMED-10 µl
- **4% stacking gel**
- 40% Acrylamide-Bisacrylamide solution-0.5 ml
- 1 M Stacking Gel Buffer-0.6 ml
- 10% SDS-0.5 ml
- Distilled water-3.4 ml
- 10% APS-100 µlTEMED-10 µl
- **Gel stain solution**
- Coomasic brilliant blue-1 g
- Glacial Acetic Acid-5 ml
- Methanol-50 ml
- Distilled water-45 ml
- **Gel destain solution**
- Glacial Acetic Acid-10 ml
- Methanol-40 ml
- Distilled water-50 ml

Procedure

SDS-PAGE unit preparation:

- Assembled Gel casting unit and set up.
- Checked the Unit for leaks and was dried.
- Inserted the combs between the gel plates and a marking 1 cm below the combs teeth was made and then comb was removed.
- APS and TEMED were added to Separating Gel and immediately gel casting was started until it reached the mark.
- 2.5 ml water was added and for polymerizationgel was kept.
- After polymerization, poured off the water.
- Added APS and TEMED to Stacking Gel and immediately done the gel casting, followed by inserting the comb without introducing air bubble and kept the gel for polymerization.
- After polymerization, marked the wells out on the blank plates.
- Removed the comb slowly and rinsed with water twice.
- Lowered the gel slowly into the cassette.
- Added SDS-PAGE running buffer into the chamber.

Sample preparation, separation and visualization

- Prepared the sample by vortexing 15 µl of protein extract and 5 µl 2x loading buffer.
- Exposed the samples for a few seconds at 100°C.
- 30 µl of samples and protein marker gene (3.4 KD to 204 KD) were loaded into the lanes.
- Gel was Run the gel at 80-100 volts until the bands reached 3/4 length of the gel.
- Between the plates the gel was removed.
- Addition of gel strain in order to cover the gel completely for overnight kept it for overnight at room temperature.
- Decanted the stained Stain and gel destain was added for 3-4 hrs.
- Visualized the bands under white light.

Result and Discussion

The present study was undertaken to isolate and purify Immunoglobulin (IgG) from Human serum sample. The total proteins present in the samples were estimated by Biuret method. The estimation was done at every step to ensure that the proteins are not lost during the purification protocols.

Biuret test was taken with sample of crude serum, ammonium sulphate fractionated sample after dialysis and before dialysis as well as eluted sample of affinity chromatography (Table 2 and Figure 1).

Observation table for standard protein: Stock protein standard 5 mg/ml.

S. No.	Protein (ml)	Conc. of protein (mg)	Distilled water	Biurette reagent		OD at 540 nm
1	0	0	2	3	Keep it for 10 min at 37 °C	0
2	0.1	0.5	1.9	3		0.03
3	0.2	1	1.8	3		0.07
4	0.3	1.5	1.7	3		0.09
5	0.4	2	1.6	3		0.13
6	0.5	2.5	1.5	3		0.15
7	0.6	3	1.4	3		0.19
8	0.7	3.5	1.3	3		0.2
9	0.8	4	1.2	3		0.22
10	0.9	4.5	1.1	3		0.24
11	1	5	1	3		0.26

Table 2: OD of standard protein samples and human serum purification samples.

Formula for calculation of concentration of unknown sample:

$$\frac{\text{OD of Unknown} \times \text{Concentration of known Standard}}{\text{OD of Standard}}$$

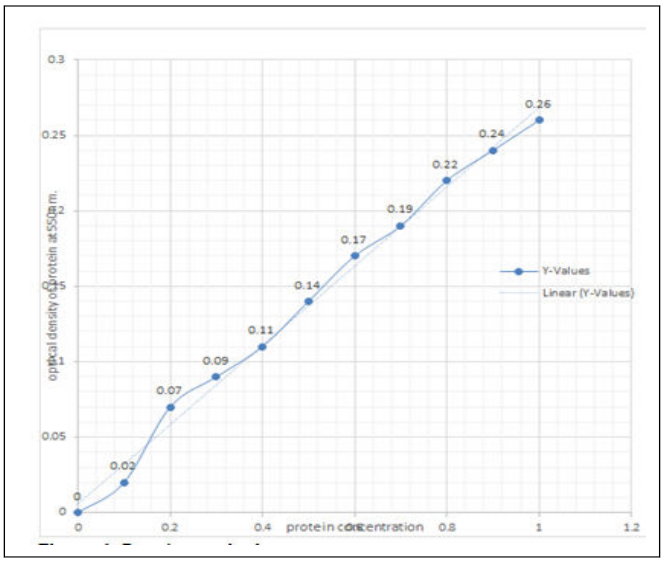

Figure 1: Protein standard curve.

After the preparation of human serum sample, ammonium sulphate precipitation was performed to fractionate IgG from the serum sample. Precipitation was followed by overnight dialysis. Dialysis was done against a phosphate buffer at a specific pH in order to remove ammonium sulphate [11]. Protein content in the serum sample was measured at specific wavelength before and after dialysis at each purification step. Protein concentration in serum sample was found to be 140 mg/ml while protein concentration before and after dialysis was found to be 28.75 mg/ml and 11.87 mg/ml respectively (Table 3 and Figures 2-6).

Protein content in serum sample	Protein content in ammonium sulphate fractionated sample	Protein content in serum sample after dialysis
140 mg/ml	28.75 mg/ml	11.87 mg/ml

Table 3: Protein content in serum sample initially, after ammonium sulphate fractionation and after dialysis.

Figure 2: Human serum sample with settled charcoal after centrifugation for 40 minutes.

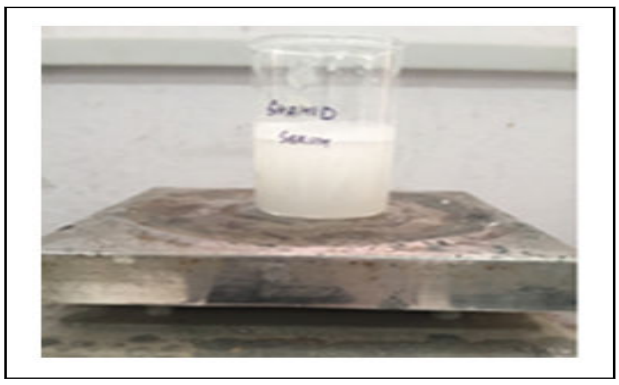

Figure 3: Ammonium sulphate precipitation using magnetic stirrer.

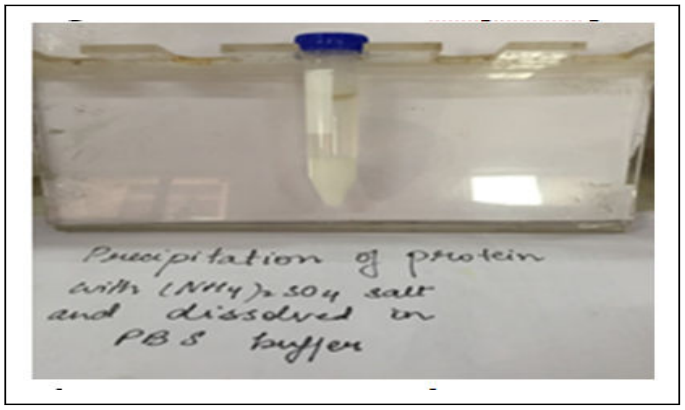

Figure 4: Fractionated serum sample after ammonium sulphate precipitation.

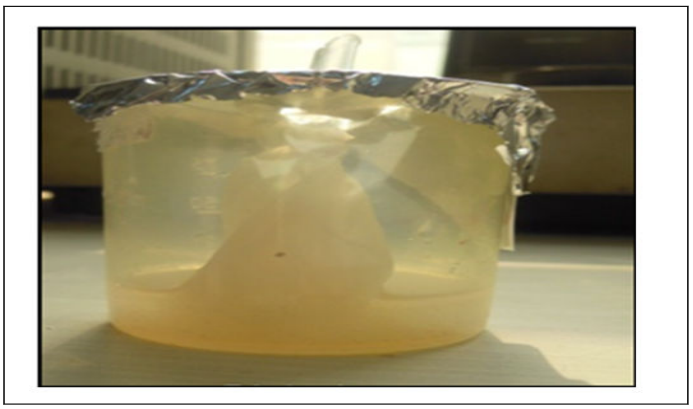

Figure 5: After ammonium sulphate precipitation the fractionated sample is left overnight in PBS for dialysis.

Figure 6: Sample after dialysis as the ammonium sulphate from the fractionated sample is exchange with PBS buffer.

Using protein-A affinity chromatography we have purified IgG antibody specifically from the human serum sample. In this type of chromatography we have used Protein A as it has high affinity towards human immunoglobulin especially IgG antibody. Protein-A was isolated from the cell wall of *staphylococcus auerus*. It forms a covalent reversible bond with the target antibody which was further eluted out in its stable form. Hence it is been used in affinity chromatography to purify monoclonal and polyclonal antibody [12]. The concentration of protein in Protein-A affinity fractionated sample found was 12 mg/ml before dialysis and 5 mg/ml after dialysis (Figure 7). So it is quite clear that there is a reduction in protein content *i.e.* concentration of protein estimated after ammonium sulphate precipitation followed by dialysis is 50% higher than the concentration of protein measured after protein-A affinity chromatography followed by dialysis (Tables 4 and 5).

Fraction number	Absorbance at 540 nm A540
1	0.4
2	1.4
3	1.9
4	1.5
5	0.5

Table 4: Absorbance values for protein-A purified sample.

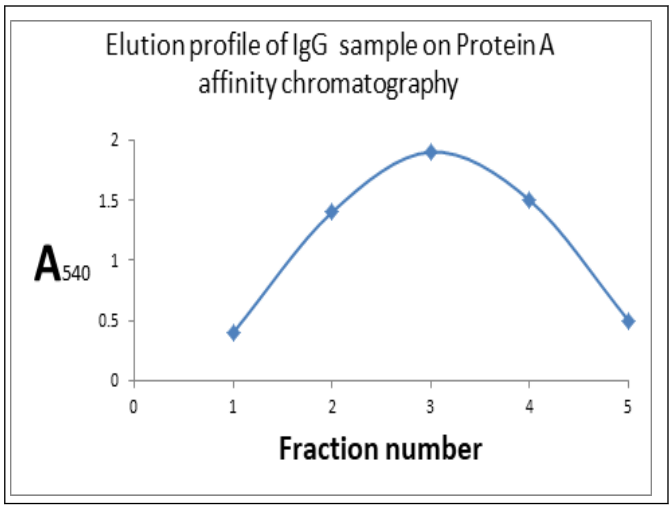

Figure 7: Elution profile of IgG sample on protein affinity chromatography

Initial protein content in human serum sample	Protein content in affinity purified IgG sample	Protein content in affinity purified dialysis sample
140 mg/ml	12 mg/ml	5 mg/ml

Table 5: Protein content in human serum sample initially, after protein-A affinity chromatography and in affinity purified IgG after dialysis.

Protein: A purified IgG sample undergoes dialysis and the protein content in the sample was about 5 mg/ml.

Conclusion

Among all, the choice of purification method would be Protein-A Affinity chromatography because the protein-A purified IgG by this method was fairly pure and has high concentration. Firstly using ammonium sulphate precipitation human serum sample was fractionated as a result the sample became concentrated and can undergo hydrophobic interaction chromatography. After, concentrated human serum sample was dialyzed overnight against phosphate buffer. Dialysis was performed in order to remove ammonium sulphate and hence a homogenized protein mixture is obtained. When we passed the sample through the chromatographic column, antibody get bind to the ligand which was further eluted out in its pure form by using denaturing agent or at pH=3. Elution of sample was performed at specific pH in order to isolate stable antibody. Although the protein concentration was higher in the case of ammonium sulphate precipitation but in case of protein-A affinity chromatography concentration of IgG in protein sample is higher in comparison to other immunoglobulin *i.e.* Protein-A purified IgG sample was undergo dialysis and the protein content in the sample was about 5 mg/ml. Lastly we determined the molecular weight and purity of IgG protein through SDS-PAGE.

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