Original Article

# Purification of Human Serum Albumin by Ion Exchange Chromatography

Nima Padashi[1], Mehdi Arjmand[2], Samira Rajaei[3], Ali Dabbagh[4]

## Abstract

**Background:** Albumin, one of the most important plasma proteins, has a difficult process of synthesis and production. We compared two different methods for albumin purification: Carboxymethyl cellulose (CM cellulose) resin exchange and Diethylaminoethyl cellulose (DEAE cellulose) resin exchange in order to determine which resin could be more beneficial.

**Materials and Methods:** two ion exchange resins were used DEAE cellulose resin and CM cellulose resin. All resins were recruited according to the standard preparation protocol. The final results were analyzed using SDS-PAGE technique.

**Results**: in DEAE Cellulose resin, nearly more than 75% of the purified protein was albumin; while, in CM cellulose resin, more than 90% was albumin.

**Conclusion:** albumin purification using CM cellulose resin is much more efficacious compared to DEAE cellulose resin. Though significant laboratory findings were demonstrated in this study, clinical studies are needed to confirm clinical outcomes.

**Keywords:** human serum albumin; carboxymethyl cellulose; diethylaminoethyl cellulose; ion exchange chromatography

**Please cite this article as:** Padashi N, Arjmand M, Rajaei S, Dabbagh A. Purification of Human Serum Albumin by Ion Exchange Chromatography. J Cell Mol Anesth. 2016;1(4):158-62.

1. Department of Chemical Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. South Tehran Branch, Islamic Azad University, Tehran, Iran
3. Department of Immunology, Tehran University of Medical Sciences, Tehran, Iran
4. Anesthesiology Research Center, Department of Cardiac Anesthesiology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

**Corresponding Author:**
Ali Dabbagh, MD; Professor, Fellowship in Cardiac Anesthesiology, Anesthesiology Research Center, Cardiac Anesthesiology Department, Shahid Beheshti University of Medical Sciences, Tehran, Iran; Email: alidabbagh@yahoo.com; alidabbagh@sbmu.ac.ir
Received: August 19, 2016
Accepted: September 14, 2016

## Introduction

Albumin is a one of the most important plasma proteins with essential roles in the perioperative period and in patients undergoing anesthesia or in the critical care setting; a number of well documented roles are considered for albumin including:

- Carrier of proteins, hormones, vitamins, drugs (including anesthetic drugs)
- Volume expander with oncotic properties
- Prevention of interstitial fluid accumulation due to its oncotic properties(1-4)

However, synthesis and production of albumin is not an easy task; it takes time and needs sophisticated methods; often, mandating great monetary fund (1, 5, 6). A number of methods have been proposed to refine albumin from vital fluids, including ion exchange chromatography. Ion exchange chromatography has a very potent property to differentiate proteins with even one amino acid difference (7).

In this study, we compared two different methods for albumin purification: Carboxymethyl cellulose (CM cellulose) resin exchange and Diethylaminoethyl cellulose (DEAE cellulose) resin exchange. The results were compared using SDS

PAGE to determine the degree of albumin purity and to assess which resin could be more beneficial (8-13).

# Methods

Two ion exchange resins were used in this study: Diethylaminoethyl cellulose (DEAE cellulose) resin with CAS Number: 9013-34-7; particle size of 60-130 μm; PubChem Substance ID 24894457; source: Sigma-Aldrich Chemie GmbH, Germany (8-10) and Sodium Carboxymethyl cellulose (CM cellulose) resin with CAS Number: 9004-32-4; average molecular weight of ~90,000; MDL number: MFCD00081472; source: Sigma-Aldrich, Saint Louis, MO, USA (8-10). All resins were prepared and recruited according to the standard preparation protocol provided by the manufacturer.

**Buffer preparation**

Since the pH of buffer should be 1 unit different from the product and also, the pH of serum human albumin is in the range of 4.8-5.6; so, the pH of DEAE cellulose resin should be above 6.6 and the pH of CM cellulose resin should be below 3.8. It has been demonstrated that the CM cellulose resin forms insoluble complexes with serum albumin with a maximum precipitation at pH 4-5 (9, 11).

For preparation of solutions, the following steps were used:

- For DEAE cellulose bis-tris buffer or bis-tris propane buffer with 20 molar concentration and $Cl^-$ as its counter-ion are considered the best buffers; however, we used tris buffer with 20 molar concentration in pH of 7.6 and $Cl^-$ as its counter-ion
- For CM cellulose resin, the best buffer is lactic acid or formic acid buffer with 50 molar concentration in pH of 3.6 and $Na^+$ as its counter-ion; we used this preparation with formic acid

**Determination of counterion concentration**

For DEAE cellulose, the concentration of counterion is 0.05 to 0.25 molar. So, five different concentrations (0.05, 0.1, 0.15, 0.2 and 0.25 molar) of tris buffers were created. Then, 0.5 milliliter of each of these 5 buffers was added to 0.5 milliliter of DEAE cellulose. Afterwards, 0.5 milliliter of plasma is added and the resulting mixture was stirred by shaker to create a suspension. The resulting suspension was centrifuged for 5 minutes with 13000 rpm. Then, the extracted fluid was assessed for its protein content using SDS-page to find out the exact concentration of protein in which human serum albumin (HSA) was attached to the resin; i.e. the starting concentration of counterion; then, to find out the concentration in which HSA was detached from the resin; i.e. the concentration for counterion washout.

For CM cellulose the concentration of counterion is 0.05 to 0.2 molar. The same process of suspension production and washout which was described above for DEAE cellulose was done for CM cellulose by using 50mM formic acid for producing 0.05, 0.1, 0.15 and 0.2 molar concentrations.

For DEAE cellulose, starting buffer concentration was 0.1 molar Chloride ($Cl^-$) solution in order to disappear HAS. However, for CM cellulose buffer, starting buffer concentration was 0.05 molar Sodium ($Na^+$) solution in order to disappear HAS.

**Technique of albumin purification**

First, the resin was washed with 3 fold of the primary buffer volume. Each vial of resin contained 0.5ml of resin; so, at first, 0.5ml of tris buffer with 0.1M $Cl^-$concentration was added to resin and mixed with 2500 rpm shaker to create a suspension; then, the yielding suspension was centrifuged for 5 minutes with 1300 rpm to achieve the resin through the final sediment. Again, the supernatant was removed and the above centrifugation process was repeated.

After resin was prepared, the sample was added in the following process:

- 0.5ml of the start buffer was added to the resin
- 0.5ml of plasma was added to this mixture
- The combination was mixed with shaker for 1 minutes with 2500 rpm
- The final suspension was centrifuged for 5 minutes with 1300 rpm
- The yielding supernatant was procured
- 0.4ml of this solution was obtained and the remaining was wasted
- 0.5 of the start buffer was added to the above 0.4 ml solution and mixed with shaker for 5 minutes with 2500 rpm
- The latter suspension was centrifuged with 1300 rpm for 5 minutes and the supernatant was obtained
- 0.4ml of this last solution was mixed with 0.5 ml of the start buffer inside vial number 1 and the

remaining fluid was wasted

- This latter solution was mixed with shaker for 1 minutes with 2500 rpm and then, centrifuged with 1300 rpm for 5 minutes
- 0.4 ml of the solution in the latter stage was added to 0.5 ml of washing buffer and again mixed with shaker for 1 minutes with 2500 rpm; then, was centrifuged with 1300 rpm for 5 minutes
- 0.4 ml of the above solution was spilled to vial number 2
- The above process of shaking and centrifugation was repeated twice

Now it was the turn for separation of all resin-attached proteins through the following order:

- 0.5ml of 1M tris buffer was added to resin and was mixed with shaker for 1 minutes with 2500 rpm; then, centrifuged with 1300 rpm for 5 minutes
- 0.4ml of the supernatant was spilled to vial number 3 and the remnant was wasted
- The above process was repeated twice
- the same steps were done for CM cellulose resin with its own buffers

The results were finally analyzed with SDS-PAGE technique, using 60 volts current for 3 hours.

## Results

As seen in figures 1 and 2, in all tests performed with the start buffer, the extract of the first column contained large amounts of albumin and other proteins (the large blue bands). Higher amounts of albumin could be contributed to older versions of resins and also, its low capacity (Figures 1 and 2).

As seen in Figure 1, the three lanes which were related to the extraction phase were from DEAE Cellulose resin; however, other proteins were attenuated significantly, denoting that albumin contributed to more than 70% of all proteins; in other words, the purification process was successful. In the 3rd stage, which was resin retrieval phase, nearly all resin attached proteins were washed out and nearly more than 75% of the purified proteins were albumin.

On the other hand, as seen in Figure 2, in the three lanes related to protein extraction from CM cellulose resin, nearly all the other proteins other than albumin were washed out from the bands; meaning that albumin comprised more than 80% of the protein

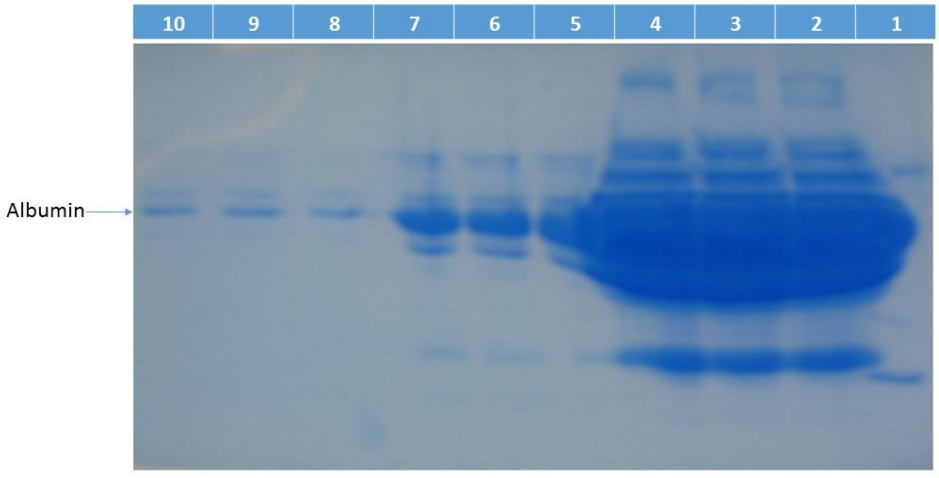


**Fig. 1.** Results of SDS page analysis of samples on DEAE Cellulose resin(The lanes are numbered from right to left)

- Lane 1 is representative of protein marker.
- Lanes 2, 3 and 4 are representatives of extracted proteins from first, second and third stages of adding samples to the columns
- Lanes 5, 6 and 7 are representatives of washed proteins from first, second and third stages of washing the columns
- Lane 8, 9 and 10 are representatives of extracted proteins from first, second and third stages of protein retrieval of the columns.

contents; in other words, albumin purification was done successfully. In the 3rd phase, i.e. resin retrieval phase, all resin bound proteins were washed out. More than 90% of the CM Cellulose-bound proteins were albumin

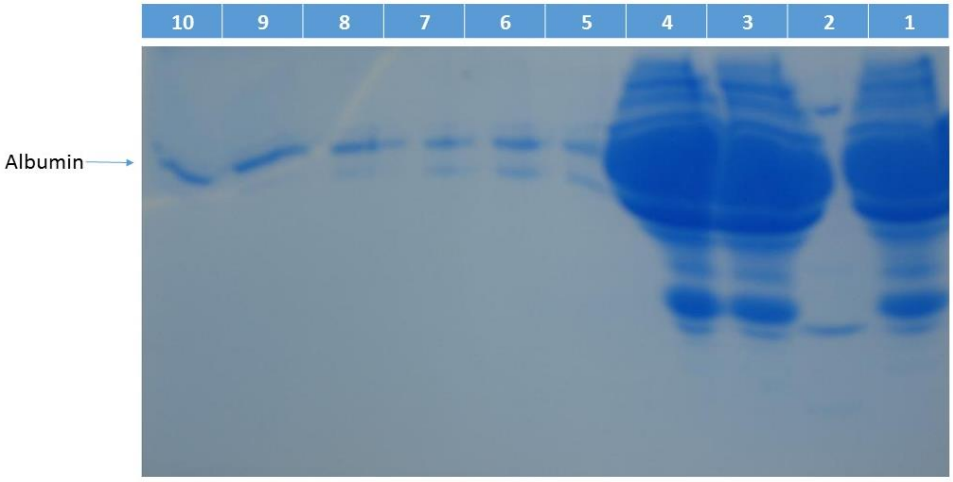


**Fig. 2.** Results of SDS page analysis of samples on CM Cellulose resin (The lanes are numbered from right to left)

- Lane 2 is representative of protein marker.
- Lanes 1, 3 and 4 are representatives of extracted proteins from first, second and third stages of adding samples to the columns
- Lanes 5, 6 and 7 are representatives of washed proteins from first, second and third stages of washing the columns
- Lane 8, 9 and 10 are representatives of extracted proteins from first, second and third stages of protein retrieval of the columns.

# Discussion

The results of our study demonstrated the improved efficacy of albumin purification using Sodium Carboxymethyl cellulose (CM cellulose) resin compared to Diethylaminoethyl cellulose (DEAE cellulose) resin.

DEAE and CM cellulose, are two exchangers which can be used for protein separation. DEAE cellulose is an anion exchanger while carboxymethyl cellulose is a cation exchanger (8-10). In one study, it was found that "DEAE ion-exchange and Protein G affinity columns in tandem" are useful methods with fast and reproducible results for separation and purification of proteins, especially albumin (12); however, in our study, CM cellulose resin yielded better than DEAE cellulose resin.

One of the most probable explanations for the results of this research could be related to the size of pores in these two different kinds of resins and also the isoelectric point of HSA (14, 15). The pore size of CM cellulose resin could be different from 150-180 µm to 250-350 µm which is higher than DEAE cellulose resin's pore size (60-130 µm). As the PI of HSA is 4.8, in the pH of 3.8 which is related to CM cellulose resin, the human albumin serum has positive charge. In this pH other impurities are deleted better from the albumin which resulted in high purity of HSA. The role of buffer and pH in ion exchange chromatography is more important regarding pore size and in this case, if we had bis-tris or bis-tris propane buffer the DEAE Cellulose could yield in a better purifying effect.

This study though had significant laboratory findings and vivid protocols, could be completed with clinical studies in order to confirm clinical outcomes of the current research.

## Conclusion

This study demonstrated that albumin purification using CM cellulose resin is much more efficacious compared to DEAE cellulose resin.

## Acknowledgment

The authors would like to acknowledege the kind efforts os Anesthesiology Resaech Center, SBMU, Tehran, Iran and also, the kind help of personnel of Department of Chemical Engineering, Science and Research Branch, and, South Tehran Branch, Islamic Azad University, Tehran, Iran

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

## References

1. He XM, Carter DC. Atomic structure and chemistry of human serum albumin. Nature. 1992;358(6383):209-15.
2. Boppana R, Kulkarni RV, Mutalik SS, Setty CM, Sa B. Interpenetrating network hydrogel beads of carboxymethylcellulose and egg albumin for controlled release of lipid lowering drug. Journal of microencapsulation. 2010;27(4):337-44.
3. Roberts I, Blackhall K, Alderson P, Bunn F, Schierhout G. Human albumin solution for resuscitation and volume expansion in critically ill patients. Cochrane Database Syst Rev. 2011(11):Cd001208.
4. Nishimura A, Tabuchi Y, Kikuchi M, Masuda R, Goto K, Iijima T. The Amount of Fluid Given During Surgery That Leaks Into the Interstitium Correlates With Infused Fluid Volume and Varies Widely Between Patients. Anesth Analg. 2016.
5. Sugio S, Kashima A, Mochizuki S, Noda M, Kobayashi K. Crystal structure of human serum albumin at 2.5 A resolution. Protein engineering. 1999;12(6):439-46.
6. Haefliger DN, Moskaitis JE, Schoenberg DR, Wahli W. Amphibian albumins as members of the albumin, alpha-fetoprotein, vitamin D-binding protein multigene family. Journal of molecular evolution. 1989;29(4):344-54.
7. Jakus V, Hrnciarova M, Carsky J, Krahulec B, Rietbrock N. Inhibition of nonenzymatic protein glycation and lipid peroxidation by drugs with antioxidant activity. Life sciences. 1999;65(18-19):1991-3.
8. Salama A, El-Sakhawy M, Kamel S. Carboxymethyl cellulose based hybrid material for sustained release of protein drugs. International journal of biological macromolecules. 2016.
9. Hoang B, Ernsting MJ, Roy A, Murakami M, Undzys E, Li SD. Docetaxel-carboxymethylcellulose nanoparticles target cells via a SPARC and albumin dependent mechanism. Biomaterials. 2015;59:66-76.
10. Chen X, Liu L, Jiang C. Charge-reversal nanoparticles: novel targeted drug delivery carriers. Acta pharmaceutica Sinica B. 2016;6(4):261-7.
11. Zadow JG, Hill RD. The precipitation of proteins by carboxymethyl cellulose. The Journal of dairy research. 1975;42(2):267-75.
12. Qi Y, Yan Z, Huang J. Chromatography on DEAE ion-exchange and Protein G affinity columns in tandem for the separation and purification of proteins. Journal of biochemical and biophysical methods. 2001;49(1-3):263-73.
13. Desjardins PR, Rabkin SW, Jacobs HK. DEAE-cellulose chromatography of creatine kinase isoenzymes--effect of pH and serum. Clinical biochemistry. 1979;12(3):77-82.
14. Barbosa LR, Ortore MG, Spinozzi F, Mariani P, Bernstorff S, Itri R. The importance of protein-protein interactions on the pH-induced conformational changes of bovine serum albumin: a small-angle X-ray scattering study. Biophys J. 2010;98(1):147-57.
15. Lee SY, Bang S, Kim S, Jo SY, Kim BC, Hwang Y, et al. Synthesis and in vitro characterizations of porous carboxymethyl cellulose-poly(ethylene oxide) hydrogel film. Biomaterials research. 2015;19:12.