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Protein Separation using Microfiltration under Electric Field

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***Abstract*— Protein separations have been carried out using a variety of techniques like microfiltration, ultrafiltration, membrane chromatography, high performance tangential flow filtration. Membrane based methods have been the basis for all types of separation and purification of proteins and other biotechnologically important products. Here we have designed a unique method for the separation of proteins under the influence of an electric field. We hypothesize that the application of an electric field will utilize the charges present on the proteins thus separating them which in turn reduce the time required for their separation and the flux resistance. With the use of electric field, the separation of proteins enhances. Towards this aim we have designed a unique separation apparatus which will separate proteins in a continuous manner, varying the electric field. For this investigation, polyvinylidene fluoride (PVDF) membrane with 0.2 and 0.5 micrometer pore size diameters will be used and proteins will be separated. This apparatus can be used for separation of proteins from whey by microfiltration under influence of electrical field. Effect of various parameters such as voltage, membrane pore size, feed composition and flow rate was observed with and without electric field. It was observed that the concentration of proteins was increased in the presence of electric field and it increased with the increase in voltage**.

Keywords-Whey; Electric Field; Voltage; Microfiltration; Pore Size

I. INTRODUCTION

The purification of protein has been widely used in electrodialysis, ultrafiltration and microfiltration when comparing studies on the use of conventional membrane for different separation [1,2]. The newly developed techniques superimpose additional forces such as pressure hydraulic force. Recently dynamic filtration has represented a further possibility for reducing the surface layer on the membrane of the rotating disc filtration [1-3]. However, the principal disadvantage of this technique is that it cannot be separated in high concentrations of protein.

A newly developed type of electric force – an electro microfiltration has been used in this study for the separation of protein from whey. Effect of parameters such as voltage, membrane pore size, feed flow rate and composition of feed has been studied. Microfiltration was chosen to increase the permeate flux.

Whey Protein is a mixture of globular proteins isolated from whey, the liquid material created as a by-product of cheese production. Whey protein is commonly marketed and ingested as a dietary supplement, and various health claims have been attributed to it in the alternative medical community [4]. Whey is leftover when milk is coagulated during the process of cheese production, and contains everything that is soluble from milk after pH is dropped to 4.6 during the coagulation process [5]. Whey can be denatured by heat . High heat (such as sustained temperatures above 72 associated with the pasteurization process) denatures whey protein. Denaturing the whey protein triggers hydrophobic interactions with other proteins and the formation of a protein gel [6]. The protein in cow's milk is 20% whey protein and 80% casein protein [7] whereas the protein in human milk is 60% casein protein and 40%whey protein [8]. The protein fraction in whey constitute approximately 10% of the total dry solids in whey. This protein is typically a mixture of beta-lactoglobulin (65%), alpha-lactalbulin (25%), bovine serum albumin (8%), and rest immunoglobulins [9]. Laboratory experiments have suggested that whey protein and its components might reduce the risk of cancer in animals, suggesting an avenue for future medical research [10]. The use of whey proteins as a source of amino acids and its effect on reducing the risk of diseases such as heart disease, cancer and diabetes has been the focus of ongoing research [11]. Whey is an abundant source of branched chain amino acids (BCAA's) [12] which are used to stimulate protein synthesis [13].

II. BACKGROUND

A. The purification of proteins was first studied in membrane process under the influence of an electric field by Young G. Park (2005). An example is presented of the membrane process showing how filtration time was reduced by the use

of electric field. Trans membrane pressure was reduced by 20% as electric field was increased. The concentration of proteins in the membrane process in the presence of electric field was reduced by over 300% in comparison with the membrane process without electric field. Hydraulic electro filtration provided another substitute to cross flow filtration for the purification proteins [14].

B. Electrically enhanced cross filtration for the separation of lactoferrin from whey protein mixture was done by Guillaume Brisson, Michel Britten, Yves Pouliot (2007). The effect of applying an external electric field during lactoferrin(LF) and whey protein solution microfiltration was studied. The impact of electric field strength and polarity on the permeation flux and protein separation were investigated. The influence of LF iron saturation was also assessed. The electrically enhanced microfiltration (EMF) were performed on a purpose built flat sheet module and operated in a full recirculation mode at low trans membrane pressure(0.7×105Pa) and feed velocity (0.05m/s). The results showed than application of an electric field had an important impact on protein transmission.[15]

III. EXPERIMENTAL STUDY

The details of the experimental investigations are as follows:

A. *Experimental Setup*

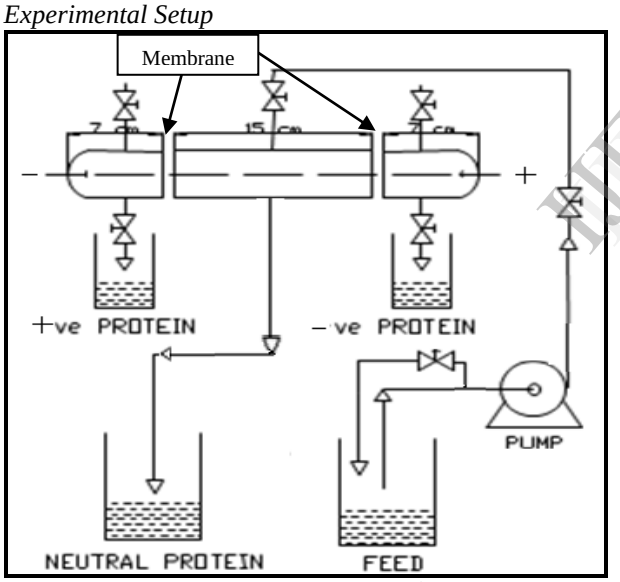

Figure1: Schematic Drawing of Setup

B. *Equipment Specification*

- Inner diameter: 47mm
- Outer diameter: 60mm
- Total length: 29 cm (7cm+15 cm+7cm)

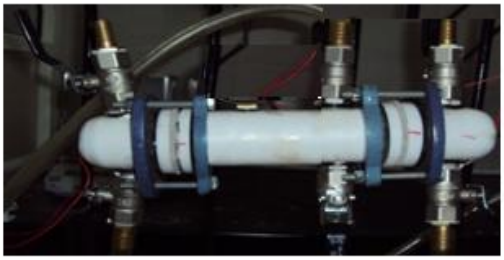

Figure2: Close View of Equipment

C. *Experimental Requirement*

Table1: Experimental Requirement

Requirement	Specification
Pump	0.33 HP 1440 RPM 130V / 50hz 0.8 A Continuous
Membrane	Material:polyvinylidene fluoride (PVDF) Microfiltration. (0.2 µm&0.5 µm, 47 mm diameter)
Method of analysis	Ultraviolet Specrophotometer, BSA
Piping	Moc: PET

D. *Experimental Procedure*

We used Whey as our raw material, which was under consideration for the separation of its constituent protein. We employed Cross Microfiltration under electric field for the separation. Experimental setup was assembled as shown in figure 1. The membranes used were materials provided by Millipore (USA). They had pore sizes of 0.2 and 0.5 µm with a 47-mm diameter-dimension. Membranes were prepared from a polyvinylidenefluoride (PVDF) using the phase inversion technique; and they were hydrophilic and hydrophobic in nature. Two flat Copper plates having area of $1cm^2$ were installed in either side of lobes as electrodes dipped in a buffer solution as shown in schematic diagram. The experiments were carried out using a conventional MF plant as shown in figure 1. The apparatus was made of Teflon for the prevention of electrical conductivity. All the experiments were carried out at room temperature.

While studying the separation of proteins under electric field we considered following four parameters and observed the effect of variation in their values on protein separation. Those parameters are: 1] Voltage 2] Membrane pore size 3] Composition of feed 4] Flow rate of feed. We maintained electric condition by employing 0 V, 10 V, 20V. We had membranes of pore size 0.2 and 0.5 µm. We performed experiments with the feed concentration of whey: water as

1:2 and 1:4. We used two set of flow rates which are 0.1354 l/s and 0.054 l/s. We analysed the effect of each parameter by varying that parameter with above mentioned values while keeping all other parameters constant.

Fresh whey was collected from nearby Katraj dairy, which was stored in cold storage at -10 ^{o}C. Consistent quality of raw material was ensured. This feed material of composition (1:2) was passed with the help of pipe and pump arrangement having specification mentioned above through equipment set up as discussed in figure no. 1. Flow rate was maintained at 0.1354 l/s. First electric voltage of 0 V was applied, i.e. no electric field inside the equipment was maintained and the separation was analysed. Three fractions of product were collected as shown in schematic diagram, as Left Hand Side (LHS)-fraction collected from cathode side, middle fraction and Right Hand Side (RHS)-fraction collected from anode side of equipment and neutral fraction from centre of the equipment. And then quantitative analysis for each fraction was performed using UV Spectrophotometer. Then voltage was increased to 10V and 20V and the same procedure was repeated.

Method of Analysis

- 1 mg/ml solution of BSA standard solution was prepared.
- Then 0,10,20,30…100 microliters(µl) of Bovien Serum Albumin (BSA) standard solution of 1 mg/ml solution were taken up and made up each sample solution up to 0.2 ml.
- 2 ml of Bradford reagent was added in each sample solution.
- The absorbance for each sample was estimated with the help of UV spectrophotometer within the stipulated residence time (200 sec.).
- The absorbance results were compared with the standard BSA curve and concentration of proteins present in the given sample were found out.

  And thus data obtained from analysis was expressed in tabulated form.

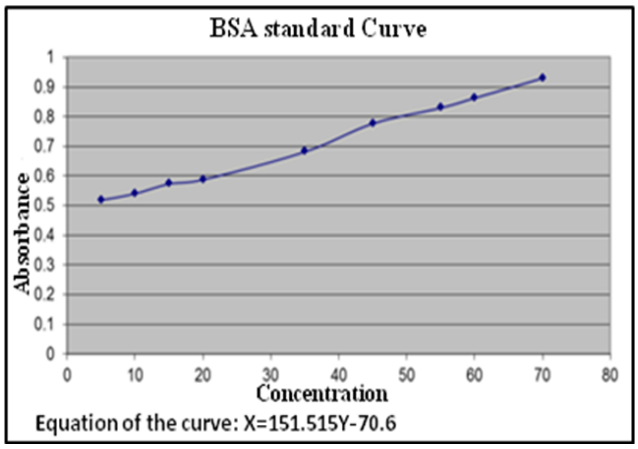

Figure3:Standardisation Curve

IV. RESULTS AND DISCUSSION

A. *Effect of Voltage*

The experiments were performed by varying voltage applied as 0 V, 10 V, 20 V and keeping membrane size, flow rate and composition of feed constant. The quantitative analysis of fractions collected on either side of equipment was carried out and plotted in terms of concentration on Y- axis vs voltage values occupying X-axis entries.

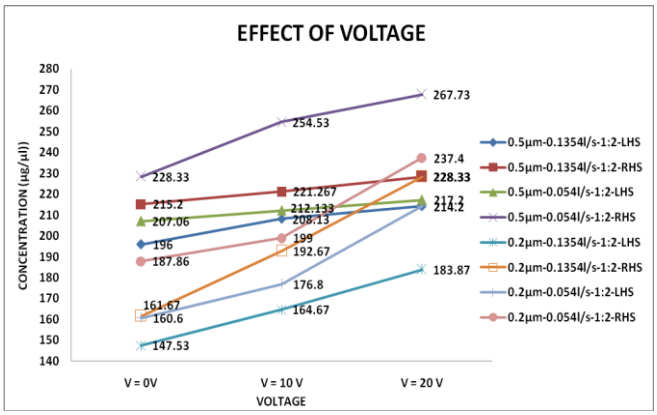

Figure4: Effect of Voltage

As mentioned elsewhere, two electrodes were used for separation. As expected Proteins were more attracted towards anode predominantly. Our experiment also supports the same. Presence of electric field facilitates the separation of proteins, which has been observed in our experimental studies.

As the applied voltage increases, the concentration of proteins in anode side fractions i.e., Right hand side product increases.

B. *Effect of Flow Rate*

The experiments were performed by varying flow rate as 0.1354 l/s and 0.054 l/s and keeping membrane size, composition of feed and voltage applied constant. The quantitative analysis of fractions collected on either side of equipment was carried out and plotted in terms of concentration on Y- axis vs flow rate occupying X-axis entries.

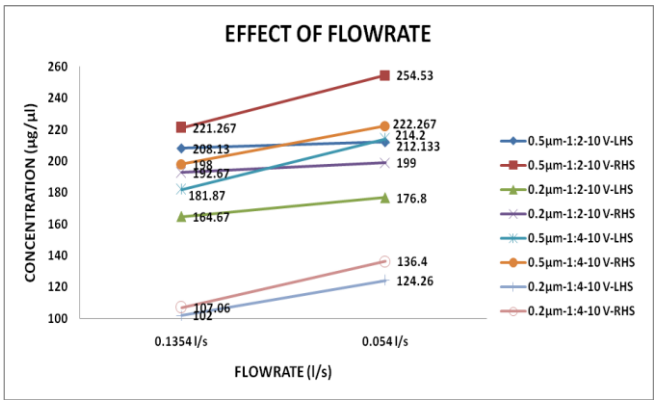

Figure5: Effect of FlowRate

Flow rate determines residence time of feed material inside the equipment, during which electric field is applied. As the flow rate decreases, residence time of feed solution inside the equipment increases and thus the time of exposure of feed solution to the electric field increases which results in increase in the concentration of proteins in anode side fractions i.e., Right hand side product increases. And adverse effect has been observed for higher values of flow rate.

C. Effect of Membrane Size

The experiments were performed by varying membrane size as 0.2 µm and 0.5 µm and keeping flow rate, composition of flow and voltage applied constant. The quantitative analysis of fractions collected on either side of equipment was carried out and plotted in terms of concentration on Y- axis vs membrane size occupying X-axis entries.

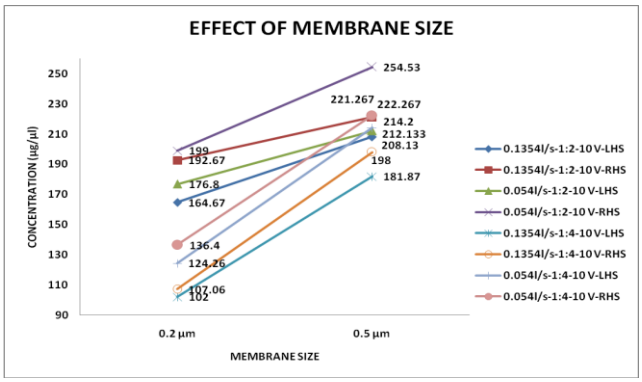

Figure6: Effect of Membrane Pore Size

As the membrane pore size increases, more amounts of proteins are observed in both the fractions.

D. Effect of Composition of Feed

The experiments were performed by varying composition of feed as 1:2 and 1:4 (whey:water) and keeping membrane size, flow rate and voltage applied constant. The quantitative analysis of fractions collected on either side of equipment was carried out and plotted in terms of concentration on Y-axis vs composition of feed occupying X-axis entries.

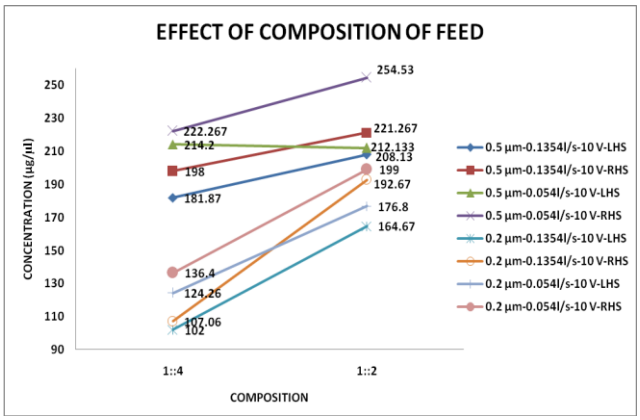

Figure7: Effect of Composition of Feed

On diluting the feed solution, the concentration of proteins decreases.

V. CONCLUSION

The separation of proteins increases by the application of electric field as compared to without electric field. It is directly proportional to the applied electric field. The introduction of electric field indicates more concentration of proteins in the Right Hand Side (RHS) of the apparatus as compared to the Left Hand Side (LHS). This can be attributed to the fact that the electrode present on the RHS of the apparatus is having positive charge (anode) and since most of the proteins are negatively charged, they get attracted towards it and hence we observe large concentration. The concentration of Proteins increases on increasing the pore size of the membrane and less dilution of feed.

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REFERENCES

[1] C. Serra, M. Wiesner and J. Laine, Chem. Eng, Sci.,72 (1999) 1.
[2] U. Holeschovsky and C. Cooney, AIChE J., 37(1991) 1219.
[3] Z. Lararova and W. Serro, Sep. Sci. Technol., 37 (2002) 515.
[4]. Marshall, K (2004). "Therapeutic applications of whey protein". Alternative Medicine Review 9 (2): 136–156. PMID 15253675.
[5.] Spurlock, D. "Isolation and Identification of Casein From Milk Course Notes". Retrieved 27 June 2014.
[6] Foegeding, EA; Davis, JP; Doucet, D; McGuffey, MK (2002). "Advances in modifying and understanding whey protein functionality". Trends in Food Science & Technology 13 (5): 151–9. doi:10.1016/S0924-2244(02)00111-5
[7] Jay R. Hoffman and Michael J. Falvo (2004). "Protein - Which is best?". Journal of Sports Science and Medicine (3): 118–130.
[8] Luhovyy BL, Akhavan T, Anderson GH (2007). "Whey proteins in the regulation of food intake and satiety". Journal of the American college of Nutrition 26 (6): 704S–712S. doi:10.1080/07315724.2007.10719651. PMID 18187437
[9] Haug A, Høstmark AT, Harstad OM, A; Høstmark, AT; Harstad, OM (25 September 2007)."Bovine milk in human nutrition – a review". Lipids Health Dis 6: 25. doi:10.1186/1476-511X-6-25. PMC 2039733 PMID 17894873.
[10] P.W. Parodi (2007). "A Role for Milk Proteins and their Peptides in Cancer Prevention".Current Pharmaceutical Design 13 (8): 813–828. ISSN 1873-4286
[11] Krissansen GW (December 2007). "Emerging health properties of whey proteins and their clinical implications". J Am Coll Nutr 26 (6): 713S–23S.doi:10.1080/07315724.2007.10719652. PMID 18187438
[12] Rieu I, Balage M, Sornet C, et al. (April 2007). "Increased availability of leucine with leucine-rich whey proteins improves postprandial muscle protein synthesis in aging rats".Nutrition 23 (4): 323–31. doi:10.1016/j.nut.2006.12.013. PMID 17367997
[13] Kimball Scott; Jefferson, LS (2006). "Signaling Pathways and Molecular Mechanisms through which Branched-Chain Amino Acids Mediate Translational Control of Protein Synthesis". The Journal of Nutrition 136 (1): 227S–31S. PMID 16365087
[14] Effect of an electric field during purification of protein using microfiltration -Young G. Park- Desalination 191 (2006) 404–410
[15] Electrically-enhanced crossflow microfiltration for separation of lactoferrin from whey protein mixtures- Guillaume Brisson, Michel Britten, Yves Pouliot- Journal of Membrane Science 297 (2007) 206–216