RESEARCH ARTICLE

The performance of bovine serum albumin filtration by using polyethersulfone-Tetronic 304 blend Ultrafiltration Membrane [version 1; peer review: 2 approved with reservations]

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Abstract

Background: Membrane technology has been widely applied for protein purification. In applications for protein separation, a membrane with stable filtration performance is necessary. In this work, two types of hollow fiber membranes with different characteristic were used to study the filtration profile of bovine serum albumin.

Methods: A single piece of hollow fiber module was used for ultrafiltration testing using UF0 and UFT304 membranes. Flux and rejection of BSA solution were collected based on a pressure-driven inside filtration model.

Results: Ultrafiltration experiments showed that the flux of UFT304 membrane was higher than that of UF0 membrane in all applied pressure condition. Solute rejection was achieved up to 90% for ultrafiltration of BSA solution by using UF0 membrane obtained on the operating pressure of 0.5 atm.

Conclusion: In general, UFT304 membranes has better ultrafiltration performance for BSA separation than UF0 membranes. The UFT304 membrane has a more stable flux for up to two hours of filtration.

Keywords

albumin, solute rejection, polyethersulfone, ultrafiltration

This article is included in the **Mathematical, Physical, and Computational Sciences** collection.

Open Peer Review

Approval Status

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version 2 (revision) 07 Nov 2019	↑	view ↑
version 1 24 Jun 2019	? view	? view

1. **Lisendra Marbelia**, Gadjah Mada University, Yogyakarta, Indonesia

2. **Alagumalai Nagendran** , Alagappa Government Arts College, Karaikudi, India

Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: Razi F: Writing – Original Draft Preparation; Mulyati S: Writing – Original Draft Preparation; Arahman N: Conceptualization, Formal Analysis, Investigation, Methodology

Competing interests: No competing interests were disclosed.

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Introduction

Albumin proteins are one of the most important substances in the human body. Albumin maintains the balance of vascular fluid, forms new cell tissue, and functions acts as an antibody¹. Albumin can help to reduce the risk of stroke and coronary heart disease². Continuous experimentation is performed to establish the best method for isolation of protein from egg white to get the best quality albumin. The most popular technique is precipitation with Na_2SO_4 or $(\text{NH}_4)_2\text{SO}_4$ at 25°C and pH 4-7. The protein-purifying method used in the precipitation technique can result in a large albumin yield, but the protein product contains the residue of precipitate materials, namely ammonium sulfate or sodium sulfate, which can potentially harmful³. The low purity and loss of albumin during the purification process has led researchers to search for alternative methods to address these short-comings.

Rapid development in the field of biotechnology has allowed for the separation of proteins with safer and more efficient methods, namely purification using membrane technology (ultrafiltration, UF). This method has been reported to successfully achieve protein fractionation through UF with several advantages compared with the conventional separation using biology/chemistry methods⁴⁻⁶. The main advantage being generating a product with high purity level and low toxicity⁷.

Despite these advantages, ultrafiltration technology also has some drawbacks. In many cases, protein molecules are easily absorbed on to the surface of hydrophobic membranes, such as polyethersulfone (PES), resulting in fouling. As a consequence, membrane flux decreases drastically along with the operation time. Modification of membrane surface traits is one solution to minimize the fouling of the membrane surface^{8,9}.

In this research, the performance of two type hollow fiber membranes for BSA separation was investigated, one made of PES/N-Methyl-2-pyrrolidone (NMP) and PES/MNP/Tetronic 304 system. Tetronic 304 is a non-ionic surfactant with two bonds of polyethylene oxide (PEO) and two bonds of polypropylene oxide (PPO) to ethylene diamine group. This surfactant potentially improves hydrophilic traits of the PES membrane due to its PEO group. This study aimed to investigate and establish the profile of BSA solution filtration using a standard PES membrane and a membrane modified with Tetronic 304 surfactant for permeability and rejection parameters.

Methods

We used two types of hollow fibre membrane made of polyethersulfone (PES, E6020P: BASF Co. Ludwigshafen, Germany; FN. 218189-009)/N-metilpirrolidon (Merck Germany, 606-021-00-7) (labeled as UF0), and PES/NMP/Tetronic 304 (BASF, Germany) system (labeled as UFT304). UF0 membrane has inner and outer diameters of 699 and 776 μm , respectively. UFT304 membrane has an inner diameter of 705 μm and the outer diameter of 796 μm . The hydrophilicity characteristics based on water contact angle data are 74.0° and 62.0° for UF0 and UFT304 membrane, respectively. Protein model was bovine serum albumin (BSA) Cohn fraction V, pH 7 (WAKO Pure Chemical Industries, Japan; 017-23294).

Characterization of hollow fibre membrane

Membrane characterization was performed to establish the morphology of all membrane types using scanning electron microscopy (SEM, Hitachi Co, S-800, Japan) with voltage acceleration at 20 kV, with a convergence angle of -30°, beam current x. 6.09 mm; y.5.79 mm, and diameter of the beam of 0.006 nm. Before the scanning electron microscopy (SEM) test, the membrane was soaked in liquid nitrogen for around five minutes, after the membrane turned rigid. This treatment was performed to prevent any change in the membrane structure when placing the sample on the SEM plate. The membrane was dried in freeze-drier overnight. One piece of the membrane was taken randomly and mounted on the sampling tubs (Hitachi, 10-004139-1, stubs Ø25 x 16mm x M4, 2 x 90°) wrapped with carbon tape, and then coated with osmium coater (Neoc-STB, Meiwafoysis Co., Ltd., Japan) sputtering. The sample was placed on the SEM panel for the picture-capturing process at 100 and 3000 magnification for whole and enlarged cross-section, respectively. Captured images were processed using *Irfan View* v.3.45

The designing of ultrafiltration module

The ultrafiltration module was designed using a single membrane module as previously described¹⁰. Ultrafiltration module consisted of a peristaltic pump (Watson Marlow, SciQ 323), a circuit of hose for feed flow, two pressure gauges (WIKA 213.53), valve, and hollow fibre membrane installed in the circuit. It was also equipped with feed, permeate, and retentate container.

Albumin solution-separating process

BSA solution was made at a concentration of 500 ppm at constant pH of 4.5. The filtration of BSA solution was conducted by flowing the feed solution to the membrane using a peristaltic pump (Watson Marlow, SciQ 323). The permeate has recorded a result of filtration, and the retentate was returned to the feed tank. The permeate was collected every 10-minute filtration, and its weight was measured by an auto digital balance (Shimadzu, Japan; ATY224). The received data of permeate weight were then converted in a volume unit. Flux and permeability of albumin solution were measured with Equation 1) and Equation 2)¹¹. The filtration process is carried out at several operating pressures, which are 0, 5; 1.00; and 1.5 Atm.

$$\text{Flux} = \frac{V}{A \cdot t} \quad (1)$$

$$\text{Permeability} = \frac{V}{A \cdot t \cdot P} \quad (2)$$

V is a permeate volume, A is the membrane surface area, t is filtration time, and P is operational pressure. BSA concentration in permeate and retentate was then analyzed using a spectrophotometer. Rejection capability of the membrane can be determined using the equations as follows¹¹:

$$\text{Rejection} = \frac{C_F - C_P}{C_F} \times 100\% \quad (3)$$

C_F and C_P are protein concentration in sample and permeate analyzed by a spectrophotometer UV-Vis (Shimadzu UV-1800) at a wavelength of 278.0.

Result

Membrane structure

SEM images of the two membrane are shown in [Figure 1](#) (see underlying data¹²). The morphological structure of membrane of the inner and outer surface were recorded with SEM at 3000x magnification as shown in [Figure 1b](#) and [Figure 1c](#). Differences in pore structure and macrovoid pattern are clearly visible. The UFT304 membrane had larger pores with greater quantity of longer macrovoids. This likely effected ultrafiltration process of BSA protein as discussed below. [Figure 1b](#) and [Figure 1c](#) also shows a large sponge structure in the middle of UF0 membrane which is absent in the UFT304 membrane. The increase in number and size of macrovoid was caused by the addition of polymeric surfactant. During the solidification process of the membrane in the coagulation batch, part of the additive leached out of the polymer system. Therefore, a large macrovoid structure was formed.

Flux and rejection of BSA

One important parameter for the membrane performance is its capability to filter solutions, referred to as flux or permeability. Another important parameter is the capability of membrane to remove components or particles from the solution, commonly known as solute rejection. In this study the ultrafiltration capability of the membrane was tested using a single membrane of hollow fibre (single module) designed with pressure driven inside (PDI) flow. The filtration profile of the albumin protein solution using UF0 and UFT304 membranes at various operational pressures is shown in [Figure 2a](#) (underlying data¹³). The flux of UFT304 membrane at the beginning of this experiment with an operating pressure of 0.3 atm was 63.60 L/m².h.

Flux increased significantly with the increase of the operating pressure which reached 161.00 L/m².hour at a pressure of 1.5 atm. The flux of UF0 membrane at the starting point (operating pressure 0.3 atm) was much lower, 32.0 L/m².h. At the highest operating pressure (1.5 atm), the flux of UF0 membrane was about 53.0 L/m².h.

The difference of flux between the two membranes is likely due to the differences in pore size. Tetronic 304 is a polymeric additive which acts as a pore-forming media. This additive is usually called the membrane-modifying agent (MMA). The increase of membrane pore size with blending MMA into the polymer solution is one of the easiest methods to improve membrane performance fabricated by the phase inversion method¹⁴.

The rejection capability of albumin solution of the two membranes is shown in [Figure 2b](#) (underlying data¹⁵). Albumin rejection by UF0 membrane >90% was achieved at 0.5 atm. Increasing the operating pressure resulted more albumin particles slipping into the permeate section. For all of the tested operating pressures the rejection of albumin solution by the two membranes remained above 80%.

Filtration stability

Filtration stability is the capability of a membrane to filter solute per time unit and operating pressure. This test corresponds to the durability of the membrane (lifetime). The result of stability test of the membrane on BSA filtration using UF0 and UFT304 membranes at 0.5 atm are shown in [Figure 3](#) (underlying data¹⁶).

Figure 1. Membrane morphology observed by scanning electron microscopy (SEM).

Figure 2. The ultrafiltration performance of membrane in term of flux (a), and solute rejection (b).

Figure 3. Filtration stability of UF0 and UFT304 membrane.

The permeability of the UFT304 membrane at the initial operating pressure of 0.3 atm was $90.75 \text{ L/m}^2\cdot\text{h}\cdot\text{atm}$. Meanwhile, the permeability of UF0 membrane at 0.3 atm much lower, $14.38 \text{ L/m}^2\cdot\text{h}\cdot\text{atm}$. The performance of filtration in these two membrane types was closely related to the morphological structure of the membrane as shown in Figure 1. Compared with the UF0 membrane, the UFT304 membrane had a larger pore structure with a higher pore density. The structure of finger-like macrovoid was also longer, and there was no sponge structure in the middle of membrane section. This difference of pore structure likely resulted in the UFT304 membrane's higher filtration capability.

Along with longer filtration time, the permeability of these two membrane types decreased due to the fouling in membrane

pore surface. However, the permeability of UFT304 membrane was still at $5.60 \text{ L/m}^2\cdot\text{h}\cdot\text{atm}$ after 120 minutes of filtration. In contrast the permeability of the UF0 membrane which was almost 0. The filtration profile of UFT304 membrane is more stable than UF0 membrane. The stability of filtration for UFT304 membrane was closely related to the hydrophilicity property of this membrane. UFT304 membrane was more hydrophilic with water contact angle of 62.0° , compared with UF0 membrane with water contact angle of 74.0° . The hydrophilic membrane has the capability to hold the fouling better than hydrophobic membrane¹⁷.

The capability to reduce fouling in this hydrophilic membrane is related to the interaction force between membrane surface and solution particle (protein). The content of polyethylene oxide

(PEO) chain in the Tetronic additive becomes a block (steric repulsion) for protein molecules, preventing them reaching the membrane surface¹⁸. Thus, the protein molecule unlikely to attach the membrane surface. Therefore, the fouling process can be minimized.

Conclusion

The application of ultrafiltration membrane technology to separate albumin protein was conducted with two types of hollow fibre membranes, namely UF0 and UFT304. Based on the results of SEM analysis, UFT304 membrane has more finger shape structures than UF0 membrane. The results of the ultrafiltration test showed that the UFT304 membrane has better performance in terms of the stability of the flux. The rejection of the BSA solution was higher than 80% for both membranes.

Data availability

Underlying data

Figshare: Supplementary data 1. <https://doi.org/10.6084/m9.figshare.8109335.v1>¹³

This project contains the following underlying data:

1. Membrane flux.csv (Raw data for membrane flux underlying Figure 2a)

Figshare: Supplementary data 2. <https://doi.org/10.6084/m9.figshare.8109332.v3>¹⁵

This project contains the following underlying data:

2. Membrane Rejection.csv (Raw data for solute rejection underlying Figure 2b)

Figshare: Supplementary data 3. <https://doi.org/10.6084/m9.figshare.8109338.v2>¹⁶

This project contains the following underlying data:

3. Water permeability of BSA solution.csv (Raw data for BSA filtration profile underlying Figure 3)

Figshare: SEM images. <https://doi.org/10.6084/m9.figshare.8215862.v2>¹²

This project contains the following underlying data:

- SEM images.pdf (Raw SEM images)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Grant information

The author(s) declared that no grants were involved in supporting this work.

Acknowledgement

We express our gratitude to Syiah Kuala University for the funding to conduct this research.

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Reviewer Report 01 November 2019

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Alagumalai Nagendran

Polymeric Materials Research Lab, PG & Research Department of Chemistry, Alagappa Government Arts College, Karaikudi, India

F Razi et al. reported the performance of BSA filtration by PES-Tetronic 304 UF membranes. Authors have reported enough characterization and comprehensive discussion. It may be indexed and authors should consider the following issues:

1. Commercial membranes used made it less attractive. If we synthesize, it can optimize the performance characteristics much better. For example: BSA Rejection reported in this study is 80%. If we fabricate, still higher rejection can be achieved
2. The role of Tetronic 304 in the modified membrane may be clearly explained in discussion section. Is it ok for long term operation?

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Ultrafiltration Membranes, Proton Exchange Membranes

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 02 Nov 2019

Nasrul Arahman, Syiah Kuala University, Banda Aceh, Indonesia

1. Commercial membranes used made it less attractive. If we synthesize, it can optimize the performance characteristics much better. For example: BSA Rejection reported in this study is 80%. If we fabricate, still higher rejection can be achieved

Response: Thank You. The statement raised by the reviewer is correct that the synthesized membrane can optimize the characteristic of membrane and its performance on filtration

1. The role of Tetronic 304 in the modified membrane may be clearly explained in discussion section. Is it ok for long term operation?

Response: thank you for the valuable comments. In this study we examined for 2 hours filtration. More examination for long term operation is planned in our future study.

Competing Interests: We declare that there is no competing interest

Reviewer Report 06 August 2019

<https://doi.org/10.5256/f1000research.20527.r51291>

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Lisendra Marbelia

Department of Chemical Engineering, Gadjah Mada University, Yogyakarta, Indonesia

This article tries to compare the performance of two membranes type for BSA filtration. The two membranes are PES based membranes, UF0 and UFT304. Both membranes are PES/NMP based membranes, in which UFT304 is modified with Tetronic 304.

From the characterization in this study, it was seen that UFT304 is more hydrophilic (lower contact angle) and also more porous (fingerlike structure in SEM). Because of these properties, filtration results showed that UFT 304 has higher flux (fig 2.a) and also more sustained (fig 3) for a longer BSA filtration test, but with a slightly lower solute rejection (fig 2.b).

These above-mentioned finding and conclusions are obtained, shown and well explained in this article.

Some suggestions and revision which should be applied to improve the quality of the paper are as follows:

1. In the abstract, results part...It is written that UFT304 is better since it has higher flux. But for the rejection it is only mentioned for the UF0 membranes. Better mention for the UFT304.
2. In the introduction, last paragraph about the Tetronic modified PES membranes... please add reference here.
3. In the method, more detailed information should be added on how the data were recorded. First, for the filtration with several pressure, how long was the filtration? Is it 10 minutes? Please write it accordingly. Also, related to the filtration experiments, it is not mentioned about the replications (in supplementary, it is shown that there are data replications). The authors should add this information in the methodology. How many replications and how the average data was calculated. Furthermore, the authors should add error bar to the graphs (2a, 2b and 3).
4. In the result, when discussing flux and rejection of BSA, it is written that the differences between the two membranes is due to differences in pore size. The authors should differentiate between pore size and porosity. Also in the discussion, it is mentioned pore density...It would be more appropriate to refer to this as porosity (finger like structure seen on SEM), otherwise it might be confused with pore size and density on the surface which are not observed in this study.

Typos:

- Introduction, last paragraph - NMP instead of MNP
- Method, first paragraph - N-Methyl-2-pyrrolidone instead of N-metilpirrolidon

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Membrane Technology, Wastewater Treatment, Bioprocess Engineering

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 02 Nov 2019

Nasrul Arahman, Syiah Kuala University, Banda Aceh, Indonesia

Comment 1

In the abstract, results part...It is written that UFT304 is better since it has higher flux. But for the rejection it is only mentioned for the UF0 membranes. Better mention for the UFT304.

Response

Thank You for comments. We revise the sentence as follow: Solute rejection reaches 90 and 88% for ultrafiltration of BSA solution on the operating pressure of 0.5 atm using UF0 and UFT304 membranes, respectively

Comment 2.

In the introduction, last paragraph about the Tetronic modified PES membranes... please add reference here.

Response

Thank You for your suggestion. We putted a reference in last paragraph of introduction about the Tetronic modified PES membranes

Comment 3.

In the method, more detailed information should be added on how the data were recorded. first for the filtration with several pressure, how long was the filtration? Is it 10 minutes? Please write it accordingly. Also, related to the filtration experiments, it is not mentioned about the replications (in supplementary, it is shown that there are data replications). The authors should add this information in the methodology. How many replications and how the average data was calculated. Furthermore, the authors should add error bar to the graphs (2a, 2b and 3).

Response: Thank You for reviewer suggestion. We have added the information in methods part. We have revised the graphs in Fig. (2a, 2b, 3) by including error bar.

Comment 4.

In the result, when discussing flux and rejection of BSA, it is written that the differences between the two membranes is due to differences in pore size. The authors should differentiate between pore size and porosity. Also in the discussion, it is mentioned pore density. It would be more appropriate to refer to this as porosity (finger like structure seen on SEM),

otherwise it might be confused with pore size and density on the surface which are not observed in this study.

Response: The point raised by the reviewer is correct. We have revised our explanation as porosity.

Comment 5.

Typos:

Introduction, last paragraph - NMP instead of MNP

Method, first paragraph - N-Methyl-2-pyrrolidone instead of N-metilpirrolidon

Response: Thank You for the correction. We have revised the typo

Competing Interests: We declare that there is no competing interest

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