

Membrane Technology Application for Fractionation Process to Obtain High Quality Glucosamine

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Membrane Technology Application for Fractionation Process to Obtain High Quality Glucosamine

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Abstract

Glucosamine, monosaccharide from chitosan obtained from the chitin deacetylation process, has been used widely in various fields such as nutrition, pharmacy, and cosmetics. Glucosamine can be obtained from the hydrolysis of chitosan. Enzymatic hydrolysis provides the advantage of mild reaction conditions, environmentally friendly, and high yield. But until now, the separation of glucosamine from the chitosan hydrolysis fraction has been an obstacle. Ultrafiltration membranes offer an efficient filtration process because they do not require additional chemicals. The performance of ultrafiltration membranes was analyzed from the fractionation process of chitosan hydrolysis. The PES membranes in 10, 25, and 50 kDa were used to filter hydrolyzed Low Molecular Weight Chitosan (LMWC) in varied concentrations. The experiment was carried out in crossflow membrane module for flat sheet at room temperature in 1 bar. The permeate flux during filtration decreased rapidly at the initial and gradually over time because of fouling and concentration polarization. The more concentrated hydrolyzed LMWC solution resulted higher percentage of rejection up to almost 20% at the same membrane MWCO while higher MWCO resulted lower rejection percentage for the same hydrolyzed LMWC concentration. The FTIR spectrum of the used membranes of all types had absorption bands of glucosamine which proved that the fractionation process occurred. The time retention in HPLC chromatograms of glucosamine produced were similar with standard glucosamine. Thus, ultrafiltration could be applied for hydrolyzed LMWC fractionation process.

Keywords: fractionation; glucosamine; LMWC; MWCO; ultrafiltration

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INTRODUCTION

Glucosamine (2-amino-2-deoxy-D-glucose) is a monosaccharide from chitosan, which is obtained by the chitin deacetylation process. Glucosamine has been used extensively in the fields of nutrition, pharmacy, and cosmetics (Pesek *et al.*, 2016; Dalirfardouei *et al.*, 2016). Glucosamine has been proven effective in reducing osteoarthritis pain (Salazar *et al.*, 2014), skin wound healing (Xavier, 2006), healing of surgical incisions (Xavier, 2006), bone regeneration (Sinha *et al.*, 2011), and so on.

Glucosamine has been produced commercially by complete hydrolysis of chitosan polymers using concentrated HCl (Wu *et al.*, 2011; Sibi *et al.*, 2013). This method is simple and easy, but the use of high concentrations of acid and high temperatures causes environmental pollution, caramel formation, and low yields.

The hydrolysis process using the enzymatic method provides benefits such as mild operating conditions, high yields, and environmental friendliness. Some enzymes that have been proven to hydrolyze chitosan, including the enzyme of papain (Yongchun *et al.*, 2003), cellulase (Xia *et al.*, 2008), pectinase (Abd-Elmohdy, *et al.*, 2010), pepsin (Li *et al.*, 2012a), lipase (Lee *et al.*, 2008), protease (Li *et al.*, 2012b), and α amylase (Rokhati *et al.*, 2013). The disadvantage of enzymatic hydrolysis is the low reaction rate. Glucosamine products from chitosan enzymatic hydrolysis are still mixed with oligo chitosan products (Pan *et al.*, 2011). Therefore, further research is needed to purify glucosamine products.

Ultrafiltration (UF) has been widely used for purification, concentration, and fractionation of macromolecules or fine particle suspensions. The use of UF in the food industry and biotechnology is often associated with macromolecular biopolymers such as polysaccharides and proteins. UF is a promising filtration tool in many applications in various food and beverage industries, including dairy products, fruit and vegetable juices, grapes, sugar and other sweeteners, vegetable oils, and water treatment (Echavarría *et al.*, 2011; Cassano *et al.*, 2010). Ultrafiltration membrane processes have also been proposed for potential applications in more complex systems containing starch (Beolchini *et al.*, 2006; Sakinah *et al.*, 2007).

Parameters used to quantify the efficiency of membrane processes, are flux (J) and solute rejection (R), where flux is defined as

$$J = \frac{V_p}{A \cdot t} \quad (1)$$

Where V_p is volume of permeate, A is surface area of membrane, and t is operating time. While rejection, the fraction of the concentration of solute that does not penetrate the membrane, is formulated as follows (Mulder, 2012)

$$R = 1 - \frac{C_p}{C_f} \times 100\% \quad (2)$$

Where C_p is concentration of permeate and C_f is concentration of feed.

This study aimed to analyze the performance of the ultrafiltration process and to determine whether the fractionation process using ultrafiltration could produce high-quality glucosamine.

MATERIALS AND METHODS

Materials

Low Molecular Weight Chitosan (LMWC) with molecular weight of 50,000-190,000 Da was supplied from Sigma-Aldrich, Germany. Cellulase enzyme from *Aspergillus niger* and β -glucosidase enzyme from almonds were purchased from Sigma-Aldrich, Germany. Tween 80 was supplied from Merck, Germany. Three types of commercial polyethersulfone (PES) membrane of 50 (GR51PP) kg/mol (kDa), 25 (GR60PP) kDa, and 10 (GR81PP) kg/mol (kDa), were obtained from Alfa Laval, Denmark. For the standard glucosamine, the one from Sigma-Aldrich was used.

Chitosan Hydrolysis

LMWC solution of 1% w/v was prepared by dissolving LMWC powder in 0.1 M acetic acid/sodium acetate buffer solution. Then, in chitosan solution 1% w/v (50 ml) was mixed with 4% (w/w chitosan) Tween 80, 0.125 ml cellulase enzyme from *Aspergillus niger*, and 1 ml β -glucosidase enzyme. The mixture was put in shaking incubator at 51°C at a speed of 250 rpm. After hydrolysis, the enzyme was deactivated by heating the solution for 10 minutes in boiling water.

Ultrafiltration of Hydrolysis Product

A crossflow membrane module was used to evaluate flat sheet membrane specimens. Hydrolyzed LMWC solution was filtered using 10 kDa, 25 kDa and 50 kDa ultrafiltration membrane to obtain glucosamine filtrate. The temperature operation was at room temperature and the transmembrane pressure (TMP) was kept constant at 1 bar. Beforehand, the membrane was soaked in distilled water, then the membrane loaded in the unit were precompact by filtering distilled water for 30 minutes in TMP of 3 bar. Flux during the filtration process were measured. The concentrate was recycled back to the reservoir, and the permeate was collected.

Glucosamine filtrate was added with 1 N HCl solution and then concentrated with a rotary evaporator until it reached the concentration of 20%. Concentrate solution was added with ethanol to precipitate glucosamine into glucosamine hydrochloride. Glucosamine hydrochloride crystals were separated from the filtrate using a centrifuge and dried in a vacuum oven at 30°C.

Glucosamine Characterization

In this study, the FTIR spectrum of the hydrolysis product (powder) was recorded with KBr powder (10 mg chitosan powder in 90 mg KBr

powder). The instrument used was the Prestige-21 Shimadzu IR spectrophotometer, in the wavelength range of 500 - 4000 cm^{-1} . The molecular weight distribution of glucosamine products was analyzed using HPLC on the Chromolith® Performance NH_2 column (100 x 4.6 mm) at 30°C. The Cellular Phase is Acetonitrile: Distilled Water (60:40 v / v) with a flow rate of 0.8 ml/minute. The detector used is a refractive index detector (RID) with an injection volume of 20 μl at a time of 30 minutes.

RESULTS AND DISCUSSION

Permeate Flux

In order to study the performance of the filtration process, the variation of permeate flux with time is shown in fig. 1, fig. 2., and fig 3. Hydrolyzed LMWC in varied concentrations, 0.1% w/v, 0.5% w/v, and 1% w/v, were filtered and the flux during the process were measured for 150 minutes.

The permeate flux during filtration decreased rapidly at the first 30 minutes and gradually thereafter. According to Lin *et. al.* (2004), the flux decline during membrane filtration process was caused by fouling phenomena and concentration polarization. Fouling depends on the membrane pore size. It might occur because of partial pore size reduction caused by foulants absorbed on the inner pore walls, pore blockage, or cake and gel layer formation. The absorption of compound could also influence the hydrophilicity of the membrane resulting in flux variation (Mohammad *et. al.*, 2012).

The solute caused concentration polarization phenomenon in hydrolyzed chitosan solution being retained by the membrane while the solvent passed the membrane, the solute would accumulate to form a layer at the membrane interface with a relatively high concentration (Sutskover-Gutman *et. al.*, 2010). The concentrated layer near the membrane was less permeable so the flux declined.

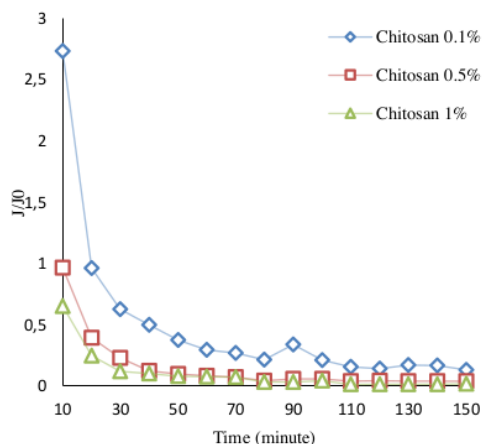


Figure 1. Profile of permeate flux during UF of hydrolyzed chitosan on 10 kDa membrane

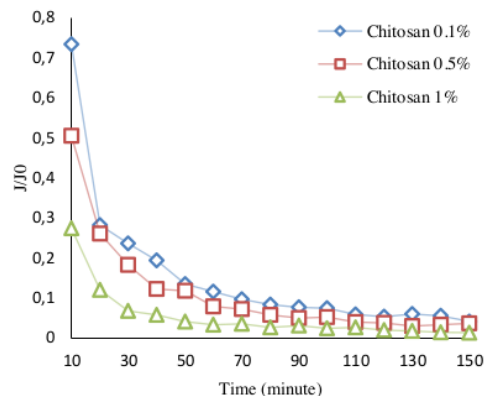


Figure 2. Profile of permeate flux during UF of hydrolyzed chitosan on 25 kDa membrane

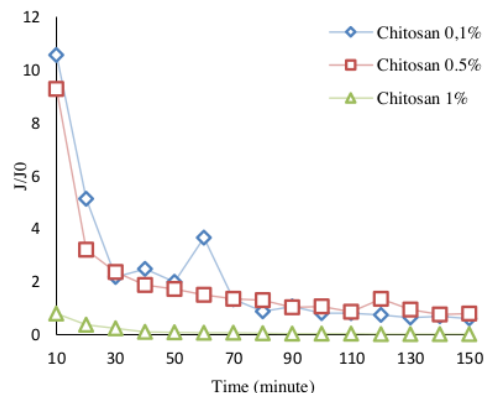


Figure 3. Profile of permeate flux during UF of hydrolyzed chitosan on 50 kDa membrane

Rejection

Membrane selectivity is a measurement of the membrane's ability to hold one material or pass another material. The parameter used to describe selectivity is rejection (R). Therefore, in this study, the rejection of three different concentrations of hydrolyzed LMWC for each membrane's MWCO was measured. The results can be seen in Table 1.

Table 1. Rejection on Hydrolyzed LMWC UF

LMWC Concentration (% w/v)	Rejection (%)		
	50 kDa	25 kDa	10 kDa
1	59.81	81.36	84.57
0.5	50.50	61.56	72.63
0.1	0.99	6.81	12.64

The extent of rejection of solutes by membranes is influenced largely by the pore size or molecular weight cut-off (MWCO) (Zeman and Zydney, 2017). As can be seen from table 1., for each MWCO, the more concentrated hydrolyzed LMWC solution resulted higher percentage of rejection. It can be stated that the

bigger MWCO was, the lesser retained solute of hydrolyzed LMWC was. The used LMWC has the molecular weight of 50-190 kDa, while glucosamine, according to U.S. National Library of Medicine, has the molecular weight of 179,19 Da. This could mean that LMWC which might not had been completely hydrolyzed successfully passed through the membrane. The higher the MWCO of the membrane, the more hydrolyzed LMWC can pass through it, resulted lower rejection percentage.

On the other hand, the higher MWCO resulted lower rejection percentage for the same hydrolyzed LMWC concentration. These results indicated the occurrence of concentration polarization and fouling on

the membrane which was manifested at higher concentrations. These phenomena also showed that fouling significantly influenced membrane rejection behavior (Guo *et al.*, 2012).

Membrane Characterization

Hydrolyzed chitosan was filtered using three different types of ultrafiltration membrane (10 kDa, 25 kDa and 50 kDa) to obtain glucosamine filtrate. The membrane before and after filtration were analyzed using FTIR. The FTIR spectrum shows band to identify the functional group characteristics recorded in mid-infrared region (wavelength of 4000 cm^{-1} to 400 cm^{-1}).

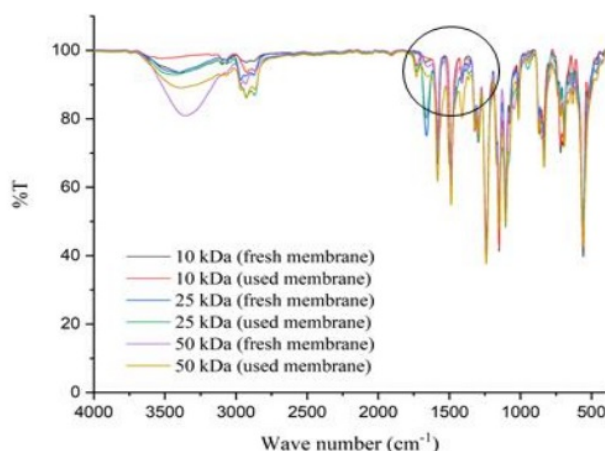


Figure 4. FTIR spectrum of membranes

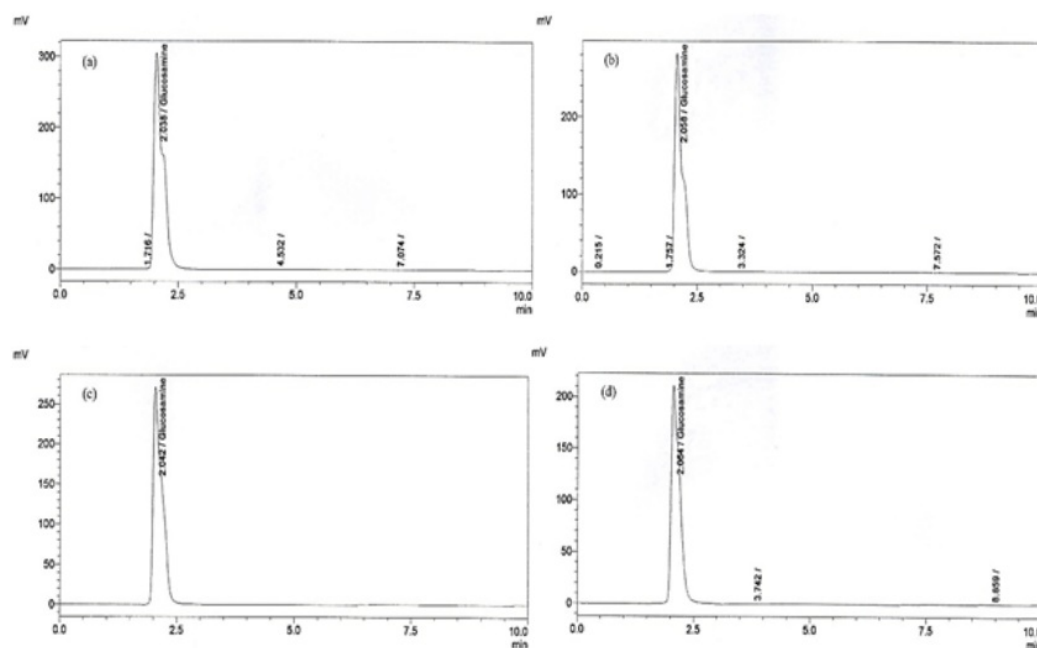


Figure 5. HPLC chromatogram of glucosamine: (a) standard, (b) 10 kDa, (c) 25 kDa, (d) 50 kDa

Specific characteristics of chitosan spectrum are found in the absorption band of amino groups in the wavelength range of 3400 cm^{-1} , 1650 cm^{-1} , and 1560 cm^{-1} . The characteristics of the saccharide polymer structure are in absorption bands with wavelengths of 1377 cm^{-1} (C-H stretching), $1170\text{--}1100\text{ cm}^{-1}$ (stretching C-O-C bridge), and 1033 cm^{-1} (C-O stretching). Meanwhile, according to the research done by Rokhati *et. al.* (2020), glucosamine shows absorption band of O-H stretching at 3359.95 cm^{-1} , N-H bending at 1614.37 cm^{-1} and 1535.29 cm^{-1} , and C-N stretching at 1321.19 cm^{-1} .

Based on fig. 4, N-H bending for secondary amide were present at 1582 cm^{-1} , 1580 cm^{-1} , and 1564 cm^{-1} on 10, 25, and 50 kDa respectively at the used membrane. This proved that fractionation occurred in ultrafiltration process.

In addition, O-H stretching bands were found at 3302 cm^{-1} to 3367 cm^{-1} (Corazzari *et. al.*, 2015). C-N stretching band occurred at 1312 cm^{-1} , 1400 cm^{-1} , also 1322 cm^{-1} on 10, 25, and 50 kDa respectively (Silverstein *et. al.*, 2005). Whereas, the bands around 1650 cm^{-1} corresponded to C=O stretching. C-O stretching around 1088 cm^{-1} to 1094 cm^{-1} on all types of membranes were present (De Queiroz Antonino *et. al.*, 2017).

Molecular weight of chitosan distribution is one of the most important characteristics that affect the function of the polymer (Lin *et. al.*, 2009). The molecular weight distribution of glucosamine was analyzed using HPLC. As can be seen in fig. 5, the eluted peak of standard glucosamine (Sigma-Aldrich) was at 2.038 minutes. The retention time for the glucosamine fractionated using ultrafiltration membrane of 10 kDa, 25 kDa, and 50 kDa respectively were 2.058 minutes, 2.042 minutes, and 2.064 minutes. To sum up, the time retention in HPLC chromatograms of hydrolyzed chitosan products were similar with the retention time of standard glucosamine. Accordingly, the product of fractionation by ultrafiltration process was nearly pure glucosamine.

CONCLUSION

From the study, the permeate flux declined rapidly at initial stage and gradually over time. While more concentrated hydrolyzed LMWC solution resulted higher percentage of rejection at the same membrane MWCO and higher MWCO resulted lower rejection percentage for the same hydrolyzed LMWC concentration. The results of FTIR spectrum showed the presence of chitosan functional group on the membrane surface. The time retention in HPLC chromatograms of glucosamine produced were similar with standard glucosamine.

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