THE INFLUENCE OF PVA.cl.CITRIC ACID/CHITOSAN MEMBRANE HYDROPHILICITY ON THE TRANSPORT OF CREATININE AND UREA

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ABSTRACT

The influence of cross-linking and membrane hydrophilicity on the transport rate had been studied using a membrane prepared from a mixture of chitosan/PVA cross-linked citric acid (PVA.cl.CA) for creatinine and urea transport. The optimum mole ratio of PVA:citric acid as well as the best composition of chitosan:PVA.cl.CA were determined using creatinine transport study. Using the optimum compositions, further study was done using different thickness of the membrane in transporting creatinine, urea and a mixture of 3 species (creatinine, urea and vitamin B12). Membrane characterization was done using FT-IR spectrophotometer, water absorption test, TG/DTG and SEM. The results showed that the optimum composition PVA:citric acid was obtained to be 90:1, having % WU of 113.74% and creatinine transport percentage of 18.16%. Meanwhile, the optimum composition of chitosan:PVA.cl.CA was found at 4:6 ratio having % WU and % transport of 136.67% and 24.26%, respectively. The optimum transport capacity was found for membrane thickness of 50 µm with WU% at 139.61% and the percent transport of creatinine and urea each was 38.93% and 60.36%. The presence vitamin B12 in the solution is proved to disturb the transport of creatinine and urea through the membrane. Finally, hydrophilicity seemed to give substantial contribution in the transport process as well as the mechanical strength of the membrane.

Keywords: chitosan; PVA; transport; cross-linking; hydrophilicity

INTRODUCTION

Hemodialysis is a biomedical method used to replace kidney function in the removal of metabolic waste substances from the body. The principle of hemodialysis is based on the diffusion of the toxic compound uremic from the body to the dialysate solution through a semi-permeable membrane as a response to the differences in concentration or pressure [1]. Membrane is a selective and semipermeable thin layer sited between two phases, a source phase...
containing entrained components, and an acceptor phase containing the components that can pass through the membrane. The transport through the membrane is achieved due to the gradient concentration in both phases. The permeability nature of the membrane means that the membrane can block certain species that are larger than the membrane pore size, and pass other species with smaller sizes. Selective properties of these membranes can be used in the separation process. To be used for hemodialysis, membranes should (1) have high permeability, which means that they can transport metabolites as quick as possible and (2) be hemocompatible or not rejected by the blood [2].

Chitosan is a polycationic biopolymer derived from chitin and is hydrophobic by nature. Chitosan is not soluble in water, but the repeatability of amine groups in its structure enable it to be dissolved in dilute acids. In acidic condition, the amine groups can easily be protonated and become positively charged. Researchers have been using chitosan for hemodialysis membranes due to its ability to form a thin film. However, the positively charged surface of chitosan will cause the disturbance of plasma proteins circulation, which in turn will cause protein adsorption on the surface of the membrane. Hence, it is necessary to modify the surface of chitosan prior to using it as a hemodialysis membrane [3-4].

One of the strategies that can be employed to increase the uniformity of pore size distribution as well as biocompatibility of the membrane is homogeneous blending, by which the equivalent ratio of hydrophobic and hydrophilic groups of the materials can be arranged. An appropriate ratio of hydrophilic and hydrophobic groups on the membrane is necessary to maintain the mechanical properties and biocompatibility of the surface. The ability of hydrophilic group to be connected to water molecules and the hydrophobic group to be connected the surface of the material will reduce the surface tension arising from the contact of a solid material with a solution of system [5]

One of synthetic polymers that can be used for blending with chitosan is PVA (polyvinyl alcohol). PVA is a hydrophilic, elastic, non-toxic, no carcinogenic and biodegradable polymer [6]. The combination between PVA and chitosan can be used as a basic material to prepare hemodialysis membrane [7]. The combination of the two materials aims to neutralize the positive charge of the chitosan as well as increase the hydrophilicity and mechanical properties of the membrane. Improving the biocompatibility of chitosan on blood by grafting PVA on the skeletal structure of chitosan followed by mixing resulted chitosan-PVA composite with PVA [8]. The process was able to produced more biocompatible membranes, as indicated by the reduction of protein adsorption on the membrane surface as well as a higher mechanical strength of the membrane. When used alone, pure PVA exhibited high level of swelling when converted into a membrane, making it difficult to be used as a membrane without any modification. The high level of swelling might cause a leakage during transport processes through the membrane, thus, it is necessary to modify the material through crosslinking with more selective groups [6]. In addition, crosslinking reaction will also increase membrane rigidity [9] which is more favorable in a membrane. A mixture of PVA/chitosan crosslinked with glutaraldehyde and glyoxal as crosslinkers, demonstrated that the mixture was able to decrease membrane permeability for vitamin B12 [10]. The studied of crosslinking reaction of PVA with maleic acid, and obtained that the level of swelling of PVA declines with increasing maleic acid content as crosslinker agent [9].

Our research is aimed to improve the transport ability of PVA, using crosslinking reaction of citric acid with PVA. The OH-group located along the open chain of PVA made this material easy to modify by substituting the hydroxyl group with other groups according to particular purposes [7] in this case for hemodialysis membranes. The crosslinking reaction of PVA with citric acid is done in order to incorporate the reactive -COOH groups of citric acid to the backbone chain of PVA. The substitution is expected to increase the active sites on the surface of PVA membrane, through which creatinine and urea can be transported via hydrogen bonds formation.

Next, to improve the mechanical properties as well decrease the hydrophilicity of PVA crosslinked with citric acid, the composite was mixed with the more hydrophobic chitosan. The appropriate ratio of the hydrophilic groups and the hydrophobic groups is expected help produce a membrane with high mechanical strength and flexibility. In this study, the relationship between the hydrophilicity of the membrane with the ability to transport urea and creatinine was also studied.

**EXPERIMENTAL SECTION**

**Materials**

Materials used in this study are chitosan, poly(vinyl alcohol) (PVA), citric acid, urea, creatinine, vitamin B12, potassium mono-hydrogen phosphate (K2HPO4), phosphoric acid solution (H3PO4), acetic acid solution (CH3COOH), 1 M sodium hydroxide (NaOH) solution, 3 M hydrochloric acid (HCl) solution, picric acid, 4-dimetilaminobenzaldehyda (DAB), ethanol (C2H5OH) 96% and filter paper. All chemicals were purchased from Merck and of analytical grade.
Table 1. Composition of PVA crosslinked citric acid

<table>
<thead>
<tr>
<th>Type of materials</th>
<th>PVA (g)</th>
<th>Citric acid (g)</th>
<th>Mol PVA</th>
<th>Mol citric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA1</td>
<td>0.375</td>
<td>0.0235</td>
<td>70</td>
<td>1</td>
</tr>
<tr>
<td>CA2</td>
<td>0.375</td>
<td>0.0182</td>
<td>90</td>
<td>1</td>
</tr>
<tr>
<td>CA3</td>
<td>0.375</td>
<td>0.0136</td>
<td>120</td>
<td>1</td>
</tr>
<tr>
<td>CA4</td>
<td>0.375</td>
<td>0.0109</td>
<td>150</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2. Amounts of PVA.cl.CA and Chitosan for membranes prepared with different proportion volume ratio

<table>
<thead>
<tr>
<th>Type of membrane</th>
<th>PVA.cl.CA</th>
<th>Chitosan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.79 x 10^-3 M (mL)</td>
<td>0.09375 M (mL)</td>
</tr>
<tr>
<td>CA2-CS3</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>CA2-CS4</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>CA2-CS5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>CA2-CS6</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>CA2-CS7</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>CA2-CS8</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

Instrumentation

The membrane instrument used for the transport consisted of two cylindrical chambers glass clamped together with the membrane held between them. The effective volume of each chamber was 50 mL and the effective diameter of the membrane was 2.5 cm. The phycochemical characterization of the done using FTIR spectrometry. A thermogravimetric analyzer (Perkin Elmer) was used to measure the thermal weight loss of the membrane at the temperature range of 30–900 °C with heating rate of 5 °C/min under nitrogen stream. Membrane surface was characterized using scanning electron microscopy (SEM) Shimadzu U8000. Prior to analysis, samples were coated with platinum using Magnetron sputter coating equipment Instruments (Shimadzu). The Analytical balance, UV-spectrophotometer (772-Spectrophotometer) and pH meter (HM-30R) were used for measurements of sample.

Procedure

Synthesis of PVA crosslinked with citric acid (PVA.cl.CA)

PVA.cl.CA with four different compositions was prepared by first dissolving 0.375 g of PVA powder (g) in 20 mL distilled water. Afterwards, different amounts of citric acid were added with constant stirring for 2 h at 80 °C to reach the desired mole ratio (see Table 1). While stirring, 10 mL of 5% acid solution was also added. The resulted materials were then analyzed using FTIR for functional groups analysis.

Preparation of PVA.cl.CA membrane

The membrane was prepared by mixing a constant amount of chitosan (5 mL) with PVA.cl.CA obtained in 2.2. The mixture was stirred for 20 h and dried in an oven at 60 °C. Afterwards, 1M NaOH was added to help remove the membrane from Petri dish. The membrane was then washed with distilled water until neutral, after which it was air dried. Later, it was used for creatinine transport experiment. The optimum mole ratio of PVA and citric acid is obtained from the membrane that exhibits the highest % creatinine transport.

Determination of the appropriate ratio of chitosan and PVA.cl.CA

The determination of appropriate ratio of hydrophilic group (PVA.cl.CA):hydrophobic (chitosan) was conducted using the optimum ratio of PVA and citric acid obtained in 2.3. Membranes were prepared using the composition in Table 2. The membranes generated were used to transport creatinine. The optimum ratio of chitosan:PVA.cl.CA is the membrane exhibiting the highest % creatinine transport.

Preparation of chitosan and PVA.cl.CA blend membrane thickness variation

Using the optimum composition from 2.4, membranes with different thickness were prepared using a composition shown in Table 3. Three different thicknesses were used and each is coded as T200, T100 and T50, respectively.

Swelling Test

Dried membranes with different compositions as prepared using method 2.2–2.4 were weighed, then soaked in phosphate buffer for 6 h. The membranes were taken out every 1 h interval and weighed. The membrane swelling percentage was calculated using the following formula:

\[
\text{Swelling} \% = \left( \frac{w_w - w_d}{w_d} \right) \times 100
\]

where \( w_w \) and \( w_d \) are the weight of hydrated and dried membranes, respectively.

Transport Test

Transport test was performed using a transport device with the membrane sited in the middle of device. The source phase contained 50 mL either creatinine 15 ppm or urea 500 ppm standard solutions in phosphate buffer, while the acceptor phase only contained 50 mL the phosphate buffer solution. The
transport tests were carried out for 6 h, with samples from acceptor phase taken out every 1 h for analysis using UV-visible spectrophotometer.

**Study of membrane transport capacity for urea, creatinine and vitamin B_{12}**

Transport process experiments were done by using the following metabolites: urea 500 ppm, creatinine 15 ppm, and vitamin B_{12} 20 ppm in 50 mL dissolved phosphate buffer solution in the source phase and 50 mL phosphate buffer only in the acceptor phase. Each compartment is equipped with magnetic stirrer. Solute permeability in passing through the membrane was determined by analyzing the concentrations of each solute in the acceptor phase with a UV-Vis spectrophotometer at 0–6 h intervals. For the purpose of analysis, urea was initially complexed with 4-dimetilaminbenzaldehida (4-DAB) in acidic condition, and the absorbance was measured at 425 nm. Creatinine was measured at 486 nm picric acid-NaOH as complexing agents. The pink vitamin B_{12} solution was measured at 361 nm without using any complexing agent.

**Membrane thickness analysis**

Dried membranes were soaked with 10 mL of phosphate buffer solution to allow it to inflate in proportion to the size of the membrane used in the transport process. Afterwards, the membranes were measured for their thickness on three different spots to obtain the average thickness of the membrane.

**Characterizations**

The membranes were characterized using different methods. Membrane surface was characterized using scanning electron microscopy (SEM) Shimadzu U8000. Prior to analysis, samples were coated with platinum using Magnetron sputter coating equipment Instruments (Shimadzu). Meanwhile, the analysis of the membrane pore size distribution was done using Surface Area Analyzer. A thermogravimetric analyzer (Perkin Elmer) was used to measure the thermal weight loss of the membrane at the temperature range of 30–900 °C with heating rate of 5 °C/min under nitrogen stream. The weight losses at different stages were recorded and analyzed. The tensile strength of the membranes was measured using a Shimadzu testing machine AG-I-250 KN, using membrane size of 1 cm x 10 cm.

**RESULT AND DISCUSSION**

**Synthesis and Characterization of PVA Crosslinked with Citric Acid (PVA.cl.CA)**

Cross-linking reaction was done using acid catalyst to facilitate the proton transfer process to the citric acid molecules, and was carried was carried out at 80 °C to accelerate the process. The mechanism of cross-linking reaction of PVA with citric acid can be explained through a two-stage reaction: 1) citric acid molecule is protonated due to acidic condition from acetic acid (Scheme 1); 2) –OH groups from PVA is then bound to the protonated citric acid (Scheme 2). The first stage, protonation of citric acid -COOH group in by proton from acetic acid, will happen very fast. Protonation may occur on both the -COOH groups located at the end of citric acid molecule. Oxygen atom attached to the carbonyl C-atom is electron-rich and therefore can easily accept proton. As a result, C atom of the carbonyl group will have a positive partial charge and can be easily attacked by electron pair from hydroxyl group of PVA, as shown in the second reaction stage. In this stage, the crosslinking structure of PVA - citric acid is formed. The crosslinking reaction is called intramolecular type reaction, since the crosslinking reaction occurs in the same PVA chain. The second stage of the reaction is the slow step reaction, and is therefore the rate determining step of the reaction.
Although the structure of citric acid crosslinked PVA is described as intramolecular crosslinking, it is also possible for intermolecular crosslinking to occur. Such structure is formed when two chains of PVA is crosslinked with citric acid, as can be seen in Fig. 1.

**FT-IR Spectra Analysis**

FT-IR was used to characterize the functional groups of PVA, citric acid as well as PVA.cl.CA, and the IR spectra can be seen in Fig. 2. PVA spectra showed a broad band at 3400 cm$^{-1}$, indicating the presence of O-H groups, while an absorption band at 1110 cm$^{-1}$ can be attributed to the vibration of C-O in the vinyl acetate groups. Citric acid spectra showed a wide absorption band in the region 2900 to 3400 cm$^{-1}$, which can be attributed to stretching vibration of O-H from of the carboxylic group. The band ranged from 1689 to 1759 cm$^{-1}$ is typical absorption band of carbonyl (C=O) of carboxylic group. The spectra of PVA.cl.CA showed a broader band at 3400 cm$^{-1}$. The higher intensity of this band might be attributed to the presence of O-H, both from the carboxylic group of citric acid, and -OH groups of PVA. The absorption at 1087 and 1234 cm$^{-1}$ which were absent in both PVA and citric acid spectra, might be ascribed to C-O ester groups, indicating the crosslinking between citric acid and PVA chains, as seen in Fig. 2. Meanwhile, absorption band at 1658 cm$^{-1}$ in PVA.cl.CA spectra indicated the decrease of carbonyl groups after crosslinking, as exhibited by the smaller intensity of the absorption band.

**TG/DTG Analysis of the Membranes**

Thermograms from TGA and DTG analysis of PVA and PVA.cl.CA are shown in Fig. 3. Thermogravimetry was conducted at temperatures between 30–900 °C at a heating rate of 5 °C min$^{-1}$. As can be seen in Fig. 3(A), there is a slight weight loss (approximately 5%) between 30–150 °C, which can be associated to the evaporation of water due to hygroscopic hydroxyl groups. The second decline occurred at temperatures between 225–325 °C, associated with heat-related degradation of PVA molecules. Meanwhile, form Fig. 3(B), it can be seen that there is a weight loss at the temperature between 30-250 °C (16.32%), which is much larger than that of PVA, indicating a much heavier mass of water associated with hydroxyl group. The second weight loss occurred between 250–500 °C, which can also be attributed to heat-related degradation of the molecule. Both data show that the citric acid crosslinked PVA has a higher thermal stability than pure PVA, as indicated from the higher temperature range for the weight loss associated both the release of water as well as and heat-related degradation processes.

**Determination of Optimum Membrane**

The first step, determination of the best ratio for the cross linking reaction between citric acid with PVA was carried out using creatinine permeation through the membranes which were prepared with different compositions of starting materials, as shown in Table 1. Creatinine permeation was selected for this experiment due to the higher molecular weight (113 g/mol), which higher than that of urea (60 g/mol). Since larger molecular weight is equivalent to larger molecular size,
It can be assumed that if the membrane is able to perform permeation of creatinine, the membrane will also be able to be used for the permeation of urea. From Fig. 4, it can be seen that the highest creatinine permeation was obtained for CA2 membrane (18.16%). Thus the best mole ratio of citric acid to PVA occurs at a ratio of 1:90.

After getting mole ratio between PVA with citric acid, the next step is setting the balance ratio of hydrophilic to hydrophobic group forces the material through the mixing between PVA.cl.CA with chitosan. The right blend of hydrophilic groups with hydrophobic membrane will result in a certain hydrophilicity values with optimum performance. The range of values appropriate hydrophilicity, membrane can work optimally in the transport process. Hydrophilicity of the membrane plays a role in determining the permeation rates of the membrane permeate. When hydrophilicity achieves the right composition, the membrane expands according to the optimal water content capacity. Water content contained in the membrane transport process is a means to permeate through the membrane. The determination of the optimum equivalent ratio of chitosan with citric acid crosslinked PVA was carried out by using the membranes for creatinine transport and for swelling percentage. As can be seen from Table 2, the membranes were prepared with different mole ratio of the starting materials. The results are given in Fig. 5.

From Fig. 5, it can be seen that the longer the transport time, the higher the transport percentage. Furthermore, the transport percentage tends to increase with increasing amount of PVA.cl.CA in the membrane. And can be seen from Table 4, that the greater the content PVA.cl.CA in blended membranes will increase the ability swelling of membrane. In other words, the increased content of PVA.cl.CA in membrane continued to increase the hydrophilicity of the membrane and it is also correlated with an increase in creatinine transport capability. This might be due to the fact that the addition of PVA to chitosan membranes is able to improve the membrane hydrophilicity. It can also be seen from Fig. 5 that the membrane with the most optimum composition is membrane CA2CS4 (PVA.cl.CA:Chitosan of to 6:4), as indicated by the highest percentage of transport percentage of the membrane.

Experimental results also indicate that for all chitosan-PVA membranes, the swelling percentages are higher than that of pure chitosan membrane. Thus, it can be argued that PVA.cl.CA is more hydrophilic than chitosan [11]. It was also found that best proportion ratio of hydrophilic and hydrophobic (hydrophilicity membrane) plays an important role in the transport process [11-13]. This result is consistent with the results in the study of chitosan/poly(ethylene oxide) (PEO) membrane. The increase in membrane

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**Table 3.** Composition of membrane for different thickness

<table>
<thead>
<tr>
<th>Type of membrane</th>
<th>Chitosan (mL)</th>
<th>PVA.cl.CA (mL)</th>
<th>Acetic acid (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;50&lt;/sub&gt;</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>T&lt;sub&gt;100&lt;/sub&gt;</td>
<td>4</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>T&lt;sub&gt;200&lt;/sub&gt;</td>
<td>8</td>
<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 4.** Swelling percentage and creatinine permeated percentage for CA2 membrane with different proportion volume of PVA.cl.CA:Chitosan

<table>
<thead>
<tr>
<th>Type of membrane</th>
<th>% Swelling</th>
<th>% Creatinine permeated</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA2-CS8</td>
<td>116.85</td>
<td>19.81</td>
</tr>
<tr>
<td>CA2-CS7</td>
<td>127.07</td>
<td>22.52</td>
</tr>
<tr>
<td>CA2-CS6</td>
<td>12731</td>
<td>22.84</td>
</tr>
<tr>
<td>CA2-CS4</td>
<td>136.67</td>
<td>24.26</td>
</tr>
<tr>
<td>CA2-CS3</td>
<td>142.11</td>
<td>23.02</td>
</tr>
</tbody>
</table>

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Fig 4. The relationship between % transport vs. contact time for PVA–citric acid mixture with different mol ratio

Fig 5. Creatinine permeated percentage for membranes with different mole ratio of chitosan:PVA.cl.CA, measured at different transport time (2, 4, and 6 h)
Table 5. Swelling percentage for CA2-CS4 membrane with different thickness

<table>
<thead>
<tr>
<th>Membrane type</th>
<th>Thickness (μm)</th>
<th>% Swelling</th>
<th>% Creatinine permeated</th>
<th>% Urea permeated</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{50}$</td>
<td>40-50</td>
<td>139.61</td>
<td>38.93</td>
<td>60.36</td>
</tr>
<tr>
<td>$T_{100}$</td>
<td>110-120</td>
<td>132.71</td>
<td>26.18</td>
<td>52.03</td>
</tr>
<tr>
<td>$T_{200}$</td>
<td>180-230</td>
<td>114.78</td>
<td>12.31</td>
<td>26.80</td>
</tr>
</tbody>
</table>

The effects of vitamin B$_{12}$ on the transport of creatinine and urea

The effects of vitamin B$_{12}$ on creatinine and urea transport were studied by combining the three species in the source phase. The transport of the three species mixture was done for 6 h using a membrane $T_{50}$, and the results can be seen in Fig. 7.

Vitamin B$_{12}$ is one of the molecules present in blood together with creatinine and urea. Having a molecular weight of 1355 g/mol, the molecular size of vitamin B$_{12}$ is so large that it hinders the transport processes of creatinine and urea. The transport percentages of creatinine and urea transport for the three species mixture are lower than when urea and
creatinine were transported individually. The transport percentage of creatinine decreased from 38.93% to 33.52%, while transport percentage of urea decreased from 60.36% to 40.06%. When mixed together, the much large molecular size of vitamin B$_{12}$ seems to have a significant influence on the transport percentage of the other two metabolites. In addition, Fig. 8 also shows that there did not seem to be any transport of vitamin B$_{12}$ through the membrane, as pointed out by the almost zero absorbance of vitamin B$_{12}$ at 360 nm during UV-Vis analysis. It seems that the molecular size of vitamin B$_{12}$ is too large to pass through the membrane pores. Additionally, the presence of vitamin B$_{12}$ in the source phase also reduces the percentage of creatinine and urea transport. With very large molecular size, vitamin B$_{12}$ might hinder creatinine and urea to enter the membrane pores [13-14]. The competition of the three metabolites to approach the membrane surface may also cause additional collision between the molecules, so that it is difficult for creatinine and urea to enter the membrane pores which lead to so longer transport time. The presence of vitamin B$_{12}$ that covers the pores might also be one of the reasons for the decline in the transport percentage for both creatinine and urea, as the molecules were able to block urea and creatinine from passing through the membranes. This result is supported with the SEM image shown in Fig. 8.

**CONCLUSION**

The crosslinking reaction of PVA with citric acid is done in order to incorporate the reactive -COOH groups of citric acid to the backbone chain of PVA. The substitution is expected to increase the active sites on the surface of PVA membrane, through which creatinine and urea can be transported via hydrogen bonds formation. From all data pertaining to the transport of both creatinine and urea, it can be seen that there is a linear relationship between hydrophilicity and the transport capacity of the membranes. It was also found that best proportion ratio of hydrophilic and hydrophobic (hydrophilicity membrane) plays an important role in the transport process. The appropriate proportion of hydrophobic and hydrophilic groups allows the membrane to have higher transport capacity. The presence of vitamin B$_{12}$ in the source phase also reduces the percentage of creatinine and urea transport.

**SEM images**

SEM images of PVA.cl.CA/chitosan membrane before and after the transport of urea, creatinine as well as the three metabolites (urea, creatinine and vitamin B$_{12}$) are shown in Fig. 8. It can be seen from Fig. 8 that prior to use, the membranes look smooth and porous. However, after being used for the transport of 3 species, the membrane surface became more rugged and compact, with small flocks covering the pores. While it is not clear in this research whether the flocks on the surface are vitamin B$_{12}$ adsorbed on the membrane surface, it is evidence that the changes of the membrane surface considerably decreased the transport percentage of both urea and creatinine.
REFERENCES