Establishment of Modified Chitosan Membrane For Wound Dressing Applications

Khin Saw Oo*, Yee Yee Than** Zaw Naing Naing***

* Associate Professor, Department of Chemistry, Kyaing Tong University
** Associate Professor, Department of Chemistry, Kyaing Tong University
*** Lecturer, Department of Chemistry, D.S.A

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Abstract: In this research work, wound dressing materials were prepared as composite membranes from chitosan, sodium alginate and calcium chloride by autoclaving method. The important parameters for wound dressing materials are tensile strength, elongation at break, tear strength, antibacterial activities and wound healing activity. Moreover biodegradability of composite membrane is one of the important parameters for reduction environmental pollution. This research work concerns with the preparation, characterization and application of biodegradable modified chitosan composite membranes (MCM) for wound dressing materials. Physicomechanical properties of MCM were also studied. According to these properties, optimum ratio was chosen. The characterization was also carried out by FT IR, SEM, XRD and TG-DTA analyses. Swelling nature of that membrane was carried out in various temperatures and pH. The biodegradability of prepared MCM was studied by soil burial method. In vitro antibacterial activity of prepared MCM-1 was investigated using agar disc diffusion method. The prepared MCM-1 was tested for the antibacterial activity against (a) *Staphylococcus aureus* (b) *Pseudomonas aeruginosa*. In vivo the healing activity of the prepared membrane was utilized by using wistar rats. In the view of results were achieved the prepared modified chitosan composite membrane have the potentially to be as a useful wound dressing material in some medical fields.

Keywords: Biodegradable modified chitosan membrane (MCM), antibacterial activity, soil burial, wound dressing.

I. Introduction

This research work is mainly on preparation and characterization of modified chitosan membrane for wound dressing applications. Composites are materials made from two or more constituent materials with significantly different physical or chemical properties, that when combined, produce a material with characteristics different from the individual components. This individual components remain separate and distinct within the finished structure. In the present work, chitosan was chosen because of its biodegradability and unique properties. Sodium alginate was chosen because of its novel hemostatic and antibacterial properties. Calcium has numerous physical and chemical characteristics which enable to be used in a wide range of applications. According to the objectives of this research, chitosan calcium alginate membranes prepared by using chitosan, sodium alginate and calcium chloride. These membranes were in safe hands after suitable characterization, determination and necessary analyses. The prepared membranes were used in medical field for multi-purposes.

II. Experimental

In preparation of modified chitosan membrane, the following materials were used: chitosan, sodium alginate, calcium chloride, acetic acid and glycerine. The procedure for membrane was shown in the following flow chart and the prepared
hydrogel was shown in Figure 1. The commercial chitosan were identified by moisture and ash contents. The prepared modified chitosan membranes (MCM) were characterized by analytical instruments and physicomechanical properties.

Figure 1. The flow chart of preparation of modified chitosan membrane
III. Results and Discussion

The degree of swelling and modified chitosan membrane was shown in Figure 2. The physicomechanical properties of prepared modified chitosan membranes (MCM) were determined for the purpose of optimization of the desired products in Table 1. From the results of these properties, modified chitosan membranes (MCM-1) sample was chosen as optimum condition from the other samples (i.e., MCM 2 to 9) for the desired products.

Table 1. Physicomechanical Measurement of Modified Chitosan Membranes

<table>
<thead>
<tr>
<th>No</th>
<th>Test</th>
<th>ZNN-1</th>
<th>ZNN-2</th>
<th>ZNN-3</th>
<th>ZNN-4</th>
<th>ZNN-5</th>
<th>ZNN-6</th>
<th>ZNN-7</th>
<th>ZNN-8</th>
<th>ZNN-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thickness (mm)</td>
<td>0.10</td>
<td>0.14</td>
<td>0.15</td>
<td>0.11</td>
<td>0.15</td>
<td>0.09</td>
<td>0.20</td>
<td>0.11</td>
<td>0.15</td>
</tr>
<tr>
<td>2</td>
<td>Tensile Strength (MPa)</td>
<td>5.7</td>
<td>1.7</td>
<td>3.7</td>
<td>1.3</td>
<td>3.4</td>
<td>3.7</td>
<td>1.8</td>
<td>4.8</td>
<td>0.12</td>
</tr>
<tr>
<td>3</td>
<td>Elongation at Break (%)</td>
<td>15</td>
<td>2</td>
<td>16</td>
<td>1</td>
<td>10</td>
<td>8</td>
<td>2</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Tear Strength (kJ/m)</td>
<td>16.7</td>
<td>7.8</td>
<td>12.5</td>
<td>4.6</td>
<td>6.7</td>
<td>13.3</td>
<td>3.9</td>
<td>10.0</td>
<td>4.6</td>
</tr>
</tbody>
</table>

* The name of the sample (1, 2, 3,...) means the amount of sodium alginate (ml).

The prepared membrane (MCM-1) was characterized by FT IR, SEM, XRD and TG-DTA analyses were shown in Figures (3 - 7). From the results of FT IR analysis the functional groups of chitosan and MCM could be confirmed in Table 2. According to SEM micrographs similar pattern of pores are distributed on the surface of (MCM). The MCM-1 shows the sponge like nature and cluster form. This indicates that this membrane has good sorption properties. With respect to the XRD analysis of the MCM-1, it can be assumed that the semi-crystallize nature which support to normal sorption character for wound dressing.
Figure 3. FT IR spectrum of modified chitosan membrane

Figure 4. FT IR spectrum of chitosan

Table 2. FT-IR Spectral Assignment for Chitosan and Modified Chitosan Membranes

<table>
<thead>
<tr>
<th>Wavenumber, cm⁻¹</th>
<th>Observed</th>
<th>From References</th>
<th>Characteristic Vibration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chitosan</td>
</tr>
<tr>
<td>3290</td>
<td>3248</td>
<td>3650-3200</td>
<td>νOH</td>
</tr>
<tr>
<td>2873</td>
<td>2926</td>
<td>3300-2700</td>
<td>νCH</td>
</tr>
<tr>
<td>1616</td>
<td>1608</td>
<td>1680-1580</td>
<td>νC=O</td>
</tr>
<tr>
<td>1417</td>
<td>1411</td>
<td>1450-1365</td>
<td>δCH</td>
</tr>
<tr>
<td>1259</td>
<td>1149-1020</td>
<td>1300-800</td>
<td>νC=O</td>
</tr>
</tbody>
</table>
From the results of TG-DTA analysis, thermal stability of MCM-1 was found to be stored at room temperature in Table 3.
Figure 7. TG-DTA analysis of modified chitosan membrane

Table 3. Thermal Degradation Analysis Data of MCM

<table>
<thead>
<tr>
<th>Temperature Range (°C)</th>
<th>Weight Loss (%)</th>
<th>Peak’s Temperature (°C)</th>
<th>Nature of Peak</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-130</td>
<td>17.85</td>
<td>-</td>
<td>Endothermic</td>
<td>Removal of moisture and loss of volatile materials from the membrane.</td>
</tr>
<tr>
<td>130-440</td>
<td>51.25</td>
<td>-</td>
<td>Exothermic</td>
<td>Loss of hydroxyl group and depolymerization.</td>
</tr>
<tr>
<td>440-585</td>
<td>8.90</td>
<td>-</td>
<td>Exothermic</td>
<td>Complete combustion and residual weight is trace elements of Na and Ca.</td>
</tr>
</tbody>
</table>

From the purpose of antibacterial activities test of MCM-1, the *vitro* study was carried out by agar disc diffusion method (Figures 8 - 10). *In vivo* study, the MCM-1 was shown in Figures 11 and 12 on *S. aureus* and Figures 13 and 14 on *P. aeruginosa*. 

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Figure 8. Antibacterial activity of MCM on *Staphylococcus aureus*.
(a) Acetic Acid (control)
(b) MCM
(c) Amoxycillin (Standard)

Figure 9. Antibacterial activity of MCM on *Pseudomonas*.
(a) Acetic Acid (control)
(b) MCM
(c) Ciprofloxacin (Standard)

Figure 10. Antibacterial activity of control, MCM and antibiotic.
Figure 11. Effect of MCM dressing on open wound (S. aureus)

Figure 12. Effect of MCM dressing on open wound (S. Aureus)
In this work, biodegradation of MCM-1 was tested by soil burial test. It shows this process of MCM for 2 months (Figure 15). The prepared modified chitosan membrane (MCM) was shown in (Figure 16).
In this research work, chitosan, sodium alginate and calcium chloride were mixed in various ratios of the following: (1) 1 g of chitosan in 110 mL of 2% acetic acid, (2) (1 mL - 9 mL) of 2% sodium alginate solution and (3) (1 mL - 9 mL) of 2% calcium chloride solution. Totally nine types of modified chitosan membranes (MCM) were prepared as the desired products.

The physicomechanical properties (such as tensile strength, percent elongation at break, tear strength) of prepared MCM were determined for the purpose of optimization of the desired products. Based on the results, MCM-1 sample was chosen as optimum condition from the other samples (i.e., MCM 1 to 9) for the desired products.

Figure 15. The physical appearances of MCM

Figure 16. Prepared modified chitosan membrane (MCM)

IV. Conclusion

In this research work, chitosan, sodium alginate and calcium chloride were mixed in various ratios of the following: (1) 1 g of chitosan in 110 mL of 2% acetic acid, (2) (1 mL - 9 mL) of 2% sodium alginate solution and (3) (1 mL - 9 mL) of 2% calcium chloride solution. Totally nine types of modified chitosan membranes (MCM) were prepared as the desired products.

The physicomechanical properties (such as tensile strength, percent elongation at break, tear strength) of prepared MCM were determined for the purpose of optimization of the desired products. Based on the results, MCM-1 sample was chosen as optimum condition from the other samples (i.e., MCM 1 to 9) for the desired products.
From the results of FT IR analysis, the functional groups could be confirmed. According to SEM micrographs show sponge like nature and cluster form. This indicates that membrane has good sorption properties. With respect to the XRD analysis of MCM-1, it can be assumed that semi-crystalline nature which supports to normal sorption character for wound dressing. From the results of TG-DTA analysis, thermal stability of MCM-1 was found to be stored at room temperature. According to the determination of swelling MCM-1, the swelling ratio was found to be not only the highest at acidic medium but also the lowest at alkaline medium.

For the purpose of antibacterial activities test of MCM-1 the \textit{vitro} study was carried out by agar disc diffusion method. In \textit{vivo} study, MCM-1 shows more than 98 % of reduction in wound area after 12 days on \textit{P.aeruginosa} and 14 days on \textit{S.aureus}. In this work, biodegradation of MCM was tested by soil burial test. It can be seen clearly significant deformation of MCM.

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First author-Dr Khin Saw Oo Associate Professor, Kyaing Tong University  
e mail address- ook6692@gmail.com  

Second author-Dr Yee Yee Than, Associate Professor, Kyaing Tong University  
e mail address- yeethan52@gmail.com  

Third author- Zaw Naing Naing, Lecturer, DSA, E mail address-47demon47@gmail.com  

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