

## Chitosan-polyethylene glycol coated cotton membranes for wound dressings

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Membranes have been developed by coating a blend of chitosan (CS) and polyethylene glycol (PEG) of different molecular weights (4000, 10,000 and 20,000) on cotton fabric followed by freeze drying. The influence of PEG molecular weight on the physical characteristics of the membranes has been investigated. The scanning electron microscopy of the coated fabric reveals porous structure. The porosity of the material is found to be 51-70% and the pore size in the range of 75-120 microns. The increase in the PEG content in the blend composition causes destabilization of pores, leading to an increase in the pore size with elongated morphology. There seems to be phase separation between the two components, which is an important factor for the observed behaviour of the porous structure. The air and water permeability of the membranes tends to increase with the increase in PEG molecular weight. The PEG addition to membrane leads to the development of a more flexible matrix depending on the amount of PEG.

**Keywords:** Chitosan, Polyethylene glycol, Membrane, Wound dressing

### 1 Introduction

Biopolymers have generated considerable interest in the field of medical technology since last few decades. These are the materials produced in nature by plants and living organisms, participate in natural biocycle and are eventually degraded and reabsorbed in nature. Owing to the increased environmental awareness, growing public health and environmental regulations, biopolymers such as polysaccharides, cellulose, starch, chitosan and chitin are on the leading front as alternatives to synthetic polymers<sup>1</sup>. Biopolymers have been projected as versatile candidates in the area of wound dressings, sutures, tissue engineering and drug delivery<sup>2-6</sup>. Chitin is one of the most abundant organic materials in nature and in fact chitosan is the partially deacetylated form of chitin. The deacetylated amino groups are responsible for the positive charge of chitosan, which makes it water soluble and reacts readily with a variety of negatively charged materials<sup>7</sup>. Low water sorption ability of chitin yields an insufficient exudates removal from the wound surface. To accelerate the water sorption ability of chitin Tanodekaew *et al.*<sup>8</sup> grafted polyacrylic acid on chitin to obtain a hydrogel

characteristics for wound dressing application. These wound dressings showed excellent cytocompatibility against L929 mouse fibroblasts.

Chitosan (CS) has shown enormous potential as therapeutic material due to its interesting features, such as antimicrobial nature, scar prevention and biocompatibility<sup>9-14</sup>. Wound dressing is one such application where a combination of all such features is reported<sup>15</sup>. Asymmetric chitosan membranes have been prepared by immersion-precipitation phase inversion method for wound dressing application. The dressing consists of a dense skin surface on the top layer supported by a macroporous sponge like sublayer. The tissue compatible asymmetric membrane is impermeable to exogenous microorganism due to its top layer and inherent antimicrobial property of chitosan and show excellent oxygen permeability, controlled evaporative water loss and enhanced fluid drainage ability<sup>16</sup>. Similarly, Mi *et al.*<sup>17</sup> prepared an silver sulfadiazine (AgSD) incorporated asymmetric chitosan membrane with sustained antimicrobial capability by dry/wet phase separation method. This membrane acts as a rate controlling wound dressing to incorporate AgSD and release sulfadiazine and silver ion in a sustained way. The asymmetric chitosan membrane showed prolonged antibacterial activity and decreased potential silver toxicity.

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Recently, nanoparticles are becoming very interesting in the biomedical field<sup>18</sup>. Silver nanocrystalline chitosan (SNC) wound dressing composed of nanosilver and chitosan have been used for treating deep partial thickness wounds. The SNC dressings were found to promote wound healing and combat infection along with the decrease in the risk of silver absorption<sup>19</sup>. In another approach, thermo and pH-sensitive hydrogels were prepared by graft polymerization of NIPAAm onto chitosan by gamma radiation<sup>20</sup>. Yang *et al.*<sup>21</sup> prepared the poly(vinyl alcohol) (PVA)/ chitosan hydrogels for wound dressing application by gamma irradiation combined with freeze-thawing process. It was found that the appropriate amount of chitosan can endow hydrogels with large swelling capacity and mechanical strength and the hydrogels showed good antibacterial activity against *Escherichia coli*.

Chitosan (CS) blends with different polymers such as polyethylene glycols (PEGs) have been reported to offer membranes for wound dressing<sup>22-25</sup>. PEG has exceptional characteristics of protein resistance, low toxicity and immunogenicity along with the biocompatibility. Hence, a combination of CS with PEG would improve the biological characteristics of the blend membranes. PEG is generally blended with CS because of its beneficial effects on biological characteristics of complex membranes. A sub-micrometer porous membrane may also be fabricated by blending CS and PEG<sup>23</sup>. The porous structure is produced by selective dissolution of CS/PEG blend membranes for their multiphase structure. The pore structure, swelling behavior and mechanical properties have been found to be influenced by content and molecular weight of PEG and the degree of crosslinking. This study has further been extended by using two synthetic polymer counterparts *viz.* PVP and PEG for blending with CS for designing microporous membranes<sup>24</sup>. Chen *et al.*<sup>26</sup> prepared trilayer composite wound dressings by plasma activation treatment and subsequently UV-light graft polymerization of N-isopropyl acrylamide (NIPAAm) gel onto nonwoven polypropylene (PP). Chitosan was immobilized onto the surface of PNIPAAm/PP nonwoven composite through crosslinking. Porosity was developed by subsequent freeze drying of the dressing. Wound dressings from electrospun materials potentially offer many advantages over conventional process with its huge surface area and microporous structure. Composite nanofibrous membranes of type 1

collagen, chitosan and polyethylene oxide have been fabricated by electrospinning which were subsequently crosslinked by glutaraldehyde treatment. These membranes were found to promote wound healing and induce cell migration and proliferation<sup>27</sup>.

In our previous study<sup>28</sup>, CS was blended with PEG (molecular weight 20,000) in different ratios and the mixture was coated on cotton fabric followed by the freeze drying to develop porous membranes. It was observed that the morphology as well as the physical structure is significantly influenced by the amount of the PEG content. In order to investigate the influence of the molecular weight of PEG, in the present study, blends of CS and PEG with different molecular weights were prepared and coated on cotton fabric. The physical structure and surface morphology as a function of the PEG molecular weight have been investigated.

## 2 Materials and Methods

### 2.1 Materials

Chitosan (deacetylation, 81.5%, viscosity 325 cp) was received from India Sea Foods, Kerala, India. Polyethyleneglycol of the molecular weights 4000, 10,000 and 20,000 was received from Fluka. Lactic acid and sodium hydroxide were received from Merck, India. Deionised water was used for all the experiments. Cotton fabric (plain weave cloth) was supplied by Century India. Freeze-drier supplied by Heto-Holten, Denmark (-45°C) was used for the cryogenic experiments. Padding mangle machine supplied by R. B. Electronic and Engineering Pvt. Ltd. was used for the coating the fabric.

### 2.2 Preparation of Composite Membranes

The preparation of membranes was carried out as reported earlier<sup>28</sup>. Chitosan was purified by dissolving it in 2% aqueous lactic acid solution under constant stirring for 24 h followed by precipitation under alkaline medium. The precipitated CS was dried under vacuum. The purified CS was dissolved in 2% aqueous lactic acid under constant mechanical stirring under ambient conditions to get 4% CS. The cotton fabric was placed in the CS solution overnight and was subsequently passed under padding mangle at a load of 0.5 kg. The padded cotton fabric was subsequently coated with a squeeze to various thicknesses. The coated membrane was placed in the deep freezer at -70°C for 72 h followed by freeze-drying at -45°C for 8 h and subsequently, was stored

over calcium chloride to avoid any moisturisation of the material.

For CS-PEG blend combinations, PEG of desired molecular weight (4000, 10,000 & 20,000) was added to the CS solution under continuous stirring. The amount of PEG was kept constant as 50% of the CS content in the blended compositions. The coating of this blend solution and the freeze-drying of the resultant matrix was carried out as followed for the CS coated membranes. PEG with molecular weights of 4000, 10,000 and 20,000 have been referred to as PEG-4, PEG-10, PEG-20 and membranes produced by coating CS-PEG blend on cotton have been referred to as CPC membranes in the subsequent text.

### 2.3 Test Methods

#### *Surface Characteristics*

The surface characteristics of the samples were studied using LEO 435 VP (Leica- Oxford Link-Isis), scanning electron microscope (SEM). Thick layer of gold metal is used to provide conduction on the sample. All the samples were pre-dried under vacuum prior to the SEM. Pore size was measured from the micrographs.

#### *Density Measurement*

Density of samples was measured by taking into account the thickness of membranes of specific size and by measuring the weight of the sample. Weight per cubic centimeter was represented as the density of the membranes<sup>28</sup>.

#### *Porosity Measurement*

The porosity of the samples was calculated as per the following relationship<sup>16</sup>:

$$\text{Porosity (\%)} = [(V_m - V_0)/V_m] \times 100 \quad \dots (1)$$

where  $V_m$  and  $V_0$  are the specific volumes of the freeze dried CS composite membrane and the pure chitosan films respectively.

#### *Differential Scanning Calorimetry (DSC)*

DSC study of the samples was carried out on Perkin Elmer DSC-7 system. The thermograms were run in the temperature range 40-200°C under the nitrogen atmosphere and at 10°C/min heating rate. The melting temperature was obtained as the peak of the thermogram. The heat of fusion ( $\Delta H_f$ ) of the sample was obtained from the area under the melting thermograms. Based on the heat of melting of pure

PEG, the amount of PEG in membranes may be obtained as per the following equation:

$$W_{\text{PEG}}^* = \frac{\Delta H_f}{\Delta H_f^*} \quad \dots (2)$$

where  $\Delta H_f$  and  $\Delta H_f^*$  are the heat of fusion values of the membrane sample and pure PEG respectively.  $\Delta H_f^*$  for pure PEG was taken as 180 J/g from DSC thermogram. The PEG content obtained as above needs to be correlated for the fraction of PEG ( $W_{\text{fPEG}}$ ) within the membrane as per the following equation:

$$W_{\text{fPEG}} = W_{\text{PEG}} / [W_{\text{PEG}} + W_{\text{CS}} + W_{\text{COT}}] \quad \dots (3)$$

where  $W_{\text{PEG}}$ ,  $W_{\text{CS}}$ , and  $W_{\text{COT}}$  are the weights of PEG, CS and cotton in membranes respectively.

#### *Thermogravimetric Analysis (TGA)*

TGA study of the samples was carried out on Perkin Elmer TGA-7 system. The thermograms were obtained under nitrogen atmosphere at 10°C/min heating rate in the temperature range 50-600°C.

#### *Bending Length Measurement*

Bending length of the samples was measured using Shirley stiffness tester according to the British Standard 3356:1961. The bending length was determined after conditioning samples under vacuum at 60°C (ref. 28).

#### *Air Permeability Measurement*

Air permeability of the samples was measured on FX-3300 air permeability tester II (TEXTTEST Instruments) as reported earlier<sup>28</sup>. The instrument contains a powerful suction pump drawing air through an interchangeable test head with a circular opening. For the measurement, the test head appropriate for the selected test standard is mounted to the instrument. The specimen (5 cm<sup>2</sup>) is clamped over the test head opening by pressing down the clamping lever, which automatically starts the suction pump. The preselected test pressure (98 Pa) is automatically set and maintained, and after a few seconds the air permeability of the test specimen is digitally displayed in the preselected unit of measure.

#### *Water Permeability Measurement*

Water permeability was measured on Shirley hydrostatic head tester using AATCC test method 127-1977 (ref. 28). The membrane (6 cm × 6 cm) is

subjected on one face to a steadily increasing pressure of water until penetration occurs at three places. The pressure or hydrostatic head at which the water penetrates at three places is taken as a measure of the resistance to the penetration of water through fabric. The specimen size of 6 cm and test area diameter of 5 cm were used for the measurements.

### 3 Results and Discussion

#### 3.1 Membrane Morphology

The investigation using PEG-20 in earlier work<sup>28</sup> has shown that the CS-PEG coated cotton CPC membranes are porous in nature and the porosity ranges between 51% and 70%. However, it is found that the PEG molecular weight has profound influence on the porosity development in these membranes. The influence of PEG molecular weight on the porosity development is shown in Figs 1-3. A systematic evaluation of the porous structure proceeds with the PEG molecular weight and their amount. These observations are similar to that made by Mi *et al.*<sup>16</sup>, where a regular porous structure with a top dense layer was observed. However, in their studies the density of the top layer was controlled by the pre-evaporation of the surface for different time intervals. In the present study, PEG-4, PEG-10 and PEG-20 have been investigated for the structure-property correlation. The data for PEG-20 have been reproduced from our earlier work<sup>28</sup>.

The PEG molecular weight has enormous influence on the density and the porosity of the resultant membranes (Table 1). The porosity for low molecular weight PEG-4 is found to be 58.9% which increases to about 69.5% for identical amount of PEG-20 in the CPC membrane. There is a distinct trend in the partial loss of elongated porous structure and in the formation of the collapsed structure due to the increase in PEG content. As the PEG molecular weight increases, the porous structure tends to be more open with the pore size in the range 75-120 microns. The pores become more elongated and larger in size as observed for the addition of PEG-10 and PEG-20 to the CS matrix (Figs 2 and 3). The porous structure of PEG-20 at 50% content becomes wide open with the appearance of the large pits.

These results may be explained in terms of a cumulative effect of the compatibility and the phase separation of CS and PEG components within the composite matrix. In general, if the compatibility of the two components is better, the phase separation diminishes. It seems that the molecular interaction between CS and PEG-4 is much better as compared to that between CS and high molecular weight PEG-10 and PEG-20. This may be due to the fact that PEG-4 has higher number of terminal hydroxyl groups per unit mass and is expected to interact more intensely due to higher extent of hydrogen bonding with CS

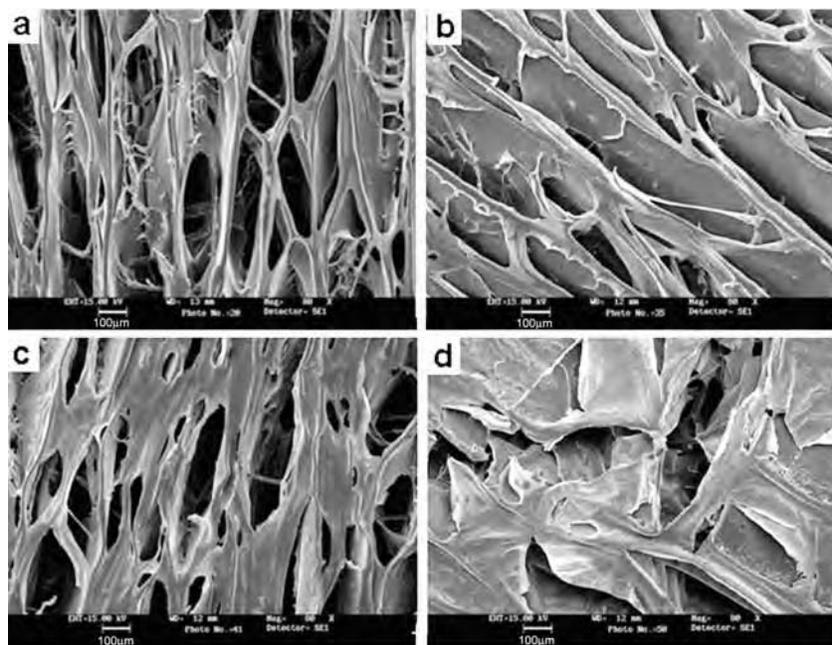


Fig. 1 — SEM images of 1 mm coating on padded cotton fabric (a) CS coated cotton membrane; and CPC membranes with (b) 10% PEG-4, (c) 30% PEG-4 and (d) 50% PEG-4

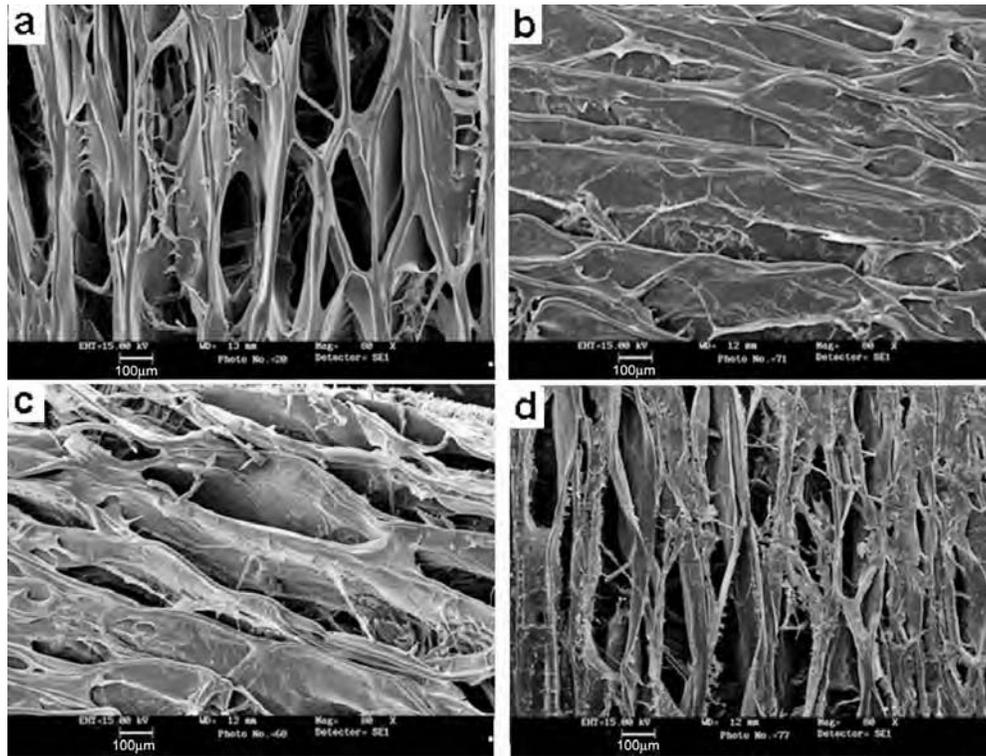


Fig. 2 — SEM images of 1 mm coating on padded cotton fabric (a) CS coated membrane; and CPC membranes with (b) 10% PEG-10, (c) 30% PEG-10 and (d) 50% PEG-10

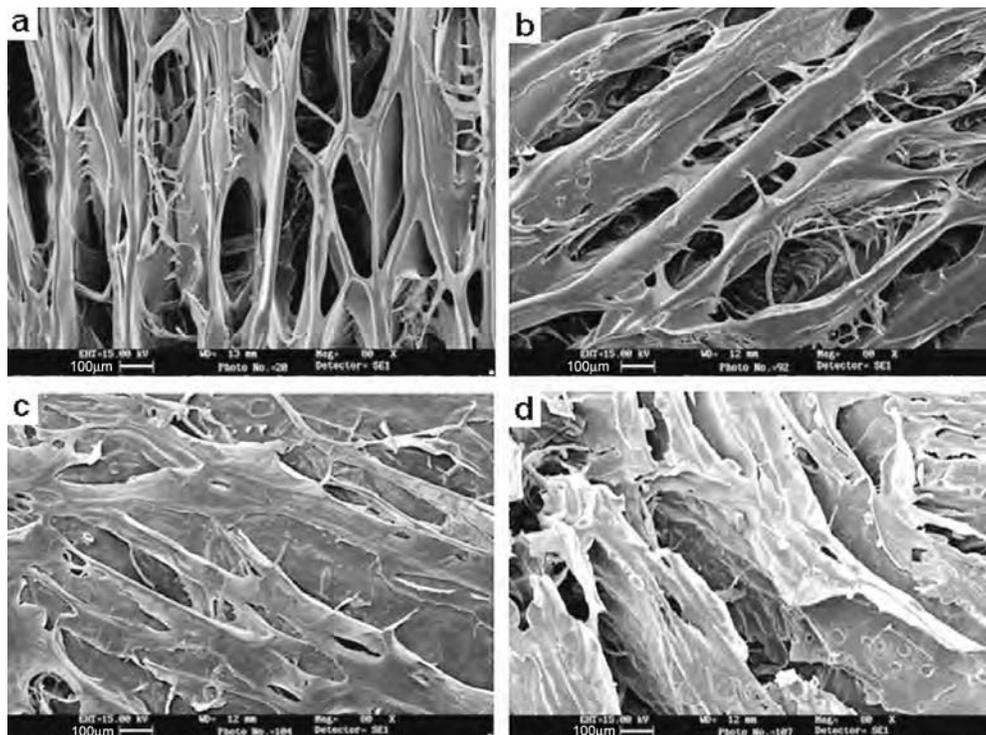


Fig. 3 — SEM images of 1 mm coating on padded cotton fabric (a) CS coated membrane; and CPC membranes with (b) 10% PEG-20, (c) 30% PEG-20 and (d) 50% PEG-20

Table 1—Density and porosity in CPC membranes

Sample	PEG mol. wt.	PEG, %	$V_0$ , cm <sup>3</sup> /g	$V_m$ , cm <sup>3</sup> /g	Density g/cm <sup>3</sup>	Porosity, %
Pure CS film	-	-	1.08	-	0.926	-
CS membrane	-	0	-	2.235	0.447	51.6
CPC membrane	4,000	50	-	2.63	0.380	58.9
CPC membrane	10,000	50	-	2.9	0.345	62.7
CPC membrane	20,000	50	-	3.55	0.282	69.5

$V_0$ —Specific volume of pure chitosan film.

$V_m$ —specific volume of freeze dried CS composite membrane.

molecules. This is the reason that PEG-4 may offer better compatibility with CS and low level of phase separation and hence the pore size is slightly affected.

At higher molecular weight (PEG-20) the hydrogen bonding interaction will be less pronounced which would lead to more isolation of PEG molecules from CS matrix. This is reflected by the collapsing of pores in a regular fashion. Consistent with the observations of Zeng *et al.*<sup>24</sup>, it has been found that the membranes exhibit larger pore size and higher porous structure as the PEG content in CS increases. This has been attributed to the poor interaction of PEG with the CS chains. As a matter of fact, in our studies, the observed diminishing porous morphology in PEG added membranes is the reflection of the limited interaction of these two components in the blended matrix. Based on the interaction of PEG with CS matrix and the generated morphology as well as the porosity, it may be proposed that the PEG-20 is the most appropriate additive for the dressing development.

### 3.2 Membrane Characterization

The DSC thermograms of membranes in lower temperature range (40–80°C) are presented in Fig. 4 and the corresponding data are shown in Table 2. It is observed that the melting of the PEGs with different molecular weights is suppressed as the blended component increases in the CS matrix as compared to the virgin PEG. The decrease in melting point is higher in PEG-4 as compared to that in PEG-20. The melting point decreases from 73°C to 71°C for PEG-20 while it decreases from 69°C to 65°C for PEG-10 and from 63°C to 56°C for PEG-4. These observations suggest that it is the strong hydrogen bonding between PEG-4 (by virtue of high content of terminal hydroxyl groups) and CS molecules which interferes with the crystallization process of PEG during the membrane fabrication step and is reflected in the diminishing melting temperature.

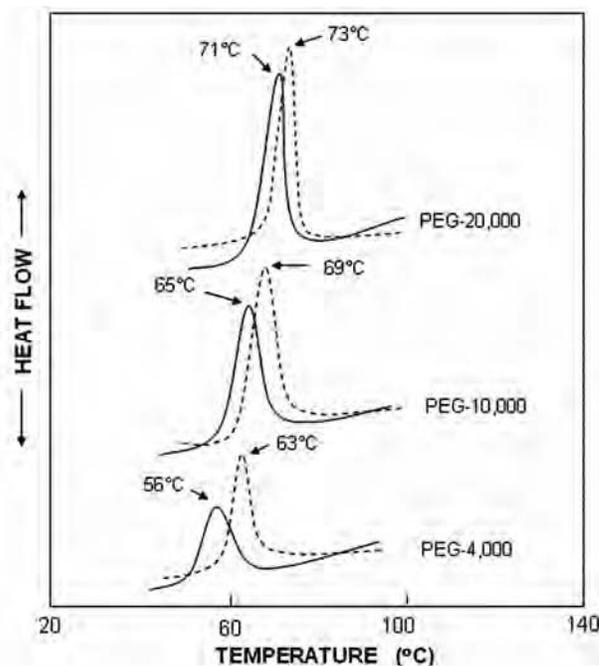


Fig. 4 — DSC thermograms of CPC membranes for different PEG molecular weights (--- virgin PEG and — blended composition)

At higher molecular weight of PEG, the interaction is less effective due to relatively lower content of hydroxyl groups and polyether chain is not much affected during the crystallization process. The melting temperature, as a result, is little affected. The reflection of the strong interaction of PEG-4 with CS is evident from the heat of fusion data (Table 2). The corrected heat of fusion value for PEG-4 in membrane is much lower than that for pure PEG. However, for PEG-10, the difference between corrected and inherent values is less pronounced. This difference is even much less for PEG-20. This is because of the fact that CS-PEG association is stronger in PEG-4 as compared to PEG-20 and does not allow the crystallization of PEG-4 component, as can be seen in free PEG-4. This supports the strong interaction of

Table 2—DSC data of different membranes

Sample	Melting temperature of virgin PEG °C	Melting temperature of blend of membranes, °C	Heat of fusion $\Delta H_f$ , J/g	Corrected heat of fusion, J/g	PEG fraction <sup>a</sup>
CPC membrane (4000 MW)	63	56	5.6	43	0.24
CPC membrane (10000 MW)	69	65	11.8	91	0.51
CPC membrane (20000 MW)	73	71	17.7	136	0.76

<sup>a</sup>Based on the heat of fusion value of 180 J/g for pure PEG<sup>28</sup>.

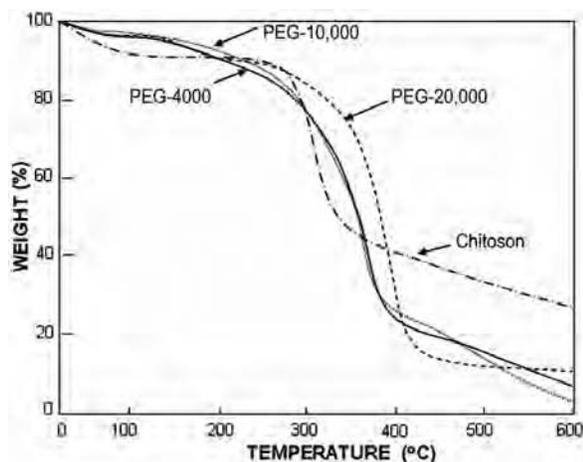


Fig. 5 — Thermogravimetric analysis of CS coated membrane; and CPC membranes with PEG-4, PEG-10 and PEG-20

PEG-4 with CS molecules, which interferes with its crystalline structure. Similar observations have been made by Zeng *et al.*<sup>24</sup> as well.

The TGA thermograms of pure chitosan and composite membranes are shown in Fig. 5. Chitosan shows a typical multi-step degradation pattern. However, the PEG containing composite membranes show better thermal stability as compared to the CS coated membranes. Moreover, PEG-20 shows the most stable matrix as compared to the other blended membranes. This is because of the better thermal stability of PEG-20 molecules which is also reflected by the enhanced thermal stability of membranes.

### 3.3 Membrane Properties

The variation in air permeability of membranes with the PEG molecular weight is presented in Fig. 6 along with the water permeability in the form of pressure to force water to pass through the membrane. The air permeability decreases in CS-PEG composite membrane (PEG-4) as compared to the virgin CS

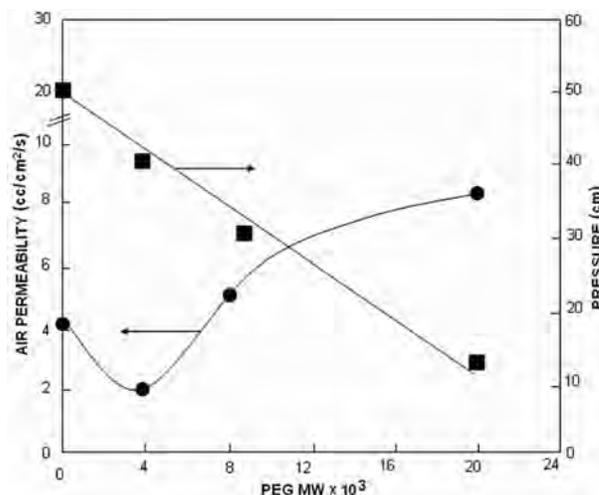


Fig. 6 — Variation in air permeability and water permeability of membranes with the PEG molecular weight in CPC membranes

coated membrane. However, the permeability of membranes increases with the increase in PEG molecular weight. The decrease in permeability of PEG-4 membrane is the indication of considerable changes in the porosity of the membrane after PEG addition. The SEM micrographs have shown that the porosity changes significantly in blended membranes and may lead to the lowering in permeability (Figs 1-3). However, as the molecular weight increases, the compatibility of the PEG component with CS also diminishes and leads to more open and larger porous structure. This, as a result, leads to an increase in the air permeability. The water permeability as a result also increases sharply as evident from the lower pressure needed for the water to pass through. The importance of large pore size in enhancing the permeability has also been demonstrated by Zeng *et al.*<sup>23</sup>.

Since PEG-20 has been evaluated as the appropriate component from the above findings. The bending length characterization has been made for the

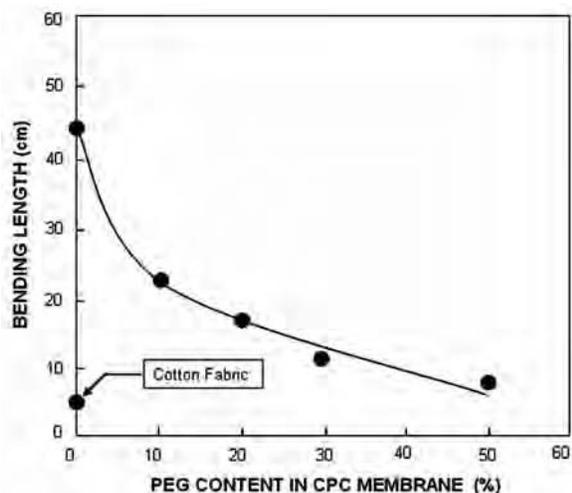


Fig. 7 — Bending length of samples with different PEG-20 contents in CPC membrane

CPC membranes with PEG-20 only. Bending length is a measure of the flexibility in the membranes and has been measured for different amount of PEG-20 in CS-PEG coating (Fig. 7). It is observed that the uncoated cotton fabric has bending length of 4 cm. However, the CS coating leads to almost 11 fold increase in the bending length.

The addition of PEG to CS diminishes the bending length depending upon the PEG content. The higher the PEG content in the CS-PEG blend, the lower is the bending length. These results may be understood from the plasticizing effect of PEG component in the membrane. Chitosan is a rigid molecule, with  $T_g$  of 170°C. The coating of CS introduces high level of rigidity in the cotton fabric. However, once PEG is added to CS the plasticization of the CS chains by PEG molecules takes place and this is reflected in the development of a flexible structure. As the PEG increases, more flexibility is introduced and leads to the lower bending length. The glycols are known for imparting plasticization effect in the hydrogels. This is where, glycerine has been observed to introduce plasticization in CS based hydrogels<sup>29</sup>. Our observations are further supported by the studies of Sparks and Murray<sup>30</sup> on chitosan-gelatin complex in combination with glycerol and sorbitol. The films casted from this combination exhibits plasticizing effect of the glycol.

#### 4 Conclusion

The coating of CS-PEG blend onto the cotton fabric produces membranes with a porous structure

and good permeability. The molecular weight has strong influence over the structural morphology of membranes. PEG with molecular weight of 4000 shows much better interaction with CS as compared to that of higher molecular weight of 20000. This is reflected by the porosity development in the resultant membranes. The PEG-4 containing membranes exhibit 58.9% porosity as compared to 69.5% for PEG with 20000 molecular weight. The low molecular weight PEG-4 forms better hydrogen bonding with CS than PEG-20, and hence shows less phase separation. With PEG-20, the phase separation is more and elongated porous structure is formed. The air and water permeabilities are also influenced in such a way that membrane possesses reasonable values at CS:PEG-20 (50:50) content. The PEG addition is an important criterion for designing a flexible membrane. CS is the rigid molecule with glass transition of 170°C and leads to the rigidity in the coated membrane. The comfort and handling aspects are very important in dressing fabrication to make the interface between the membrane and the tissue smooth. PEG addition to CS is helpful in introducing the flexibility in the coated membranes.

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