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A NEW METHOD TO PREPARE CHITOSAN MEMBRANE AS A BIOMEDICAL MATERIAL*

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Abstract This paper reports a new method to prepare chitosan membrane which could be used as a biomedical material. Addition of a fixation agent composed of alcohol, glycerol and potassium hydroxide can accelerate the sol-gel transformation process and hence shorten the preparation period. The present method takes about 6 h to get a flexible membrane with fine appearance. The physical and biological properties of the membrane were also investigated and compared with the membrane prepared by conventional method.

Keywords Chitosan, Membrane, Sol-gel transformation, Fixation agent

INTRODUCTION

Chitosan is a biodegradable natural polymeric material having good physical and biological properties. It was reported that chitosan could enhance coagulation, suppress fibroblast growth, and improve interstitial cell growth[1-6]. Chitosan could be used to prepare controlled-release medicine membranes[7], artificial skin[8], and anti-adhesion membranes for post-operative applications[9], *etc.*

However, few chitosan membranes have been used in clinical practice. One of the important reasons is that the preparation by conventional methods requires a long time, and the prepared membrane is not good enough in its strength and flexibility. The scope of this study is to find an effective method to prepare chitosan membranes with good physical and biological properties.

EXPERIMENTAL

Materials

Chitosan (100 mesh, 90% deacetylated, M_v = 1.75 × 10^4) was a product of Qingdao Haihui Biological Engineering Co. Ltd. Potassium hydroxide, anhydrous alcohol, glycerol and acetic acid were chemically pure. Two kinds of fixation agent were prepared: fixation agent A was a mixture of anhydrous alcohol and glycerol, and fixation agent B was a mixture of anhydrous alcohol, glycerol and potassium hydroxide.

Procedure

A definite amount of powdered chitosan was dissolved in 1.5% acetic acid at 30℃ to form a 1%–2% solution. 5% of glycerol was then added as a plasticizer. The bubble-free solution was cast in a mold and immersed in the fixation agent. After fixation, the membrane was dried in an oven at desired temperature, then washed with distilled water, and finally oven-dried. The membrane so obtained had a thickness of ca.10 μm.

Characterization

The appearance of the membrane was characterized by its color, transparency and surface smoothness. The

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hardness of the membrane was measured at 20℃ with a Shore D hardness tester. Tensile testing was performed with a LDS-500 tensile instrument at 20℃ and a cross-head speed of 50 mm/min. Gravimetric analysis was used to measure the water content of the membrane and its ability to absorb water.

RESULTS AND DISCUSSIONS

Factors Affecting the Preparation and the Appearance of Membranes

Conventionally, the membrane is produced via evaporating solvent directly from chitosan solution. Normally it takes 100 h for a complete process to produce a membrane. For improving the efficiency, we chose a sol-gel process and selected anhydrous alcohol as the fixation agent. This compound is miscible with acetic acid and easy to evaporate. When it was added to chitosan/acetic acid solution, it can extract acetic acid from the solution and evaporate rapidly. Table 1 shows that the time for fixation decreases with increasing concentration of the fixation agent.

Table 1. Relationship between the concentration of fixation agent and fixation time

Concentration of alcohol (%)	Fixation time (h)	Appearance of the membrane
0 (pure water)	> 36	Not fixed
60	> 36	Not fixed
85	30	Gel on surface
90	24	White gel
95	18	White gel
100	12	White gel

The temperature during fixation also affects the fixation time and the properties of the membrane. Results in Table 2 reveal that the fixation time decreases with increasing temperature. It is favorable for saving time, but when the temperature is higher than 70℃, the surface of the membrane becomes rough, and some bubbles were encapsulated in the bulk of the membrane. This is caused by the rapid increase of the viscosity of the solution during the evaporation process. When the temperature is even higher, the membrane becomes yellow, owing to oxidation of the material.

Table 2. Influence of fixation temperature on the fixation time and the appearance of the membrane

Fixation temperature (℃)	Fixation time (h)	Appearance of wet membrane	Appearance of dry membrane
55	3.0	White gel	Colorless, transparent, smooth
65	2.5	White gel	Colorless, transparent, smooth
70	2.0	White gel, a few bubbles on the edge	Colorless, transparent, smooth
75	1.5	White gel, bubble on the edge	Light yellow, transparent, little rough
80	1.0	Light yellow, bubble in the bulk	Light yellow, transparent, little rough bubble in bulk

Influence of Glycerol Content on the Properties of Membranes

Pure chitosan membrane is not suitable for use as a biomedical material (such as anti-adhesion material after surgical operation) because of its brittleness. We select glycerol as the plasticizer because it has the functions of keeping wet, lubrication, and plasticizing. It is non-toxic and harmless to the human body. However, during the fixation process, glycerol will partially dissolve in alcohol, hence decrease its plasticizing efficiency. To avoid the loss of the plasticizer, a certain amount of glycerol was added to alcohol as a component of the fixation agent. Furthermore, addition of potassium hydroxide to the fixation agent B neutralizes the membrane so as to avoid the inflammation near the wound, which is caused by the acidity of the membrane.

From the results of tensile testing and hardness measurement, it is found that the flexibility of the membrane improves with increasing glycerol content, as shown in Figs. 1–3. Elongation at break reaches a maximum at 8% glycerol content. Although the tensile strength decreases accordingly, it is not important for the use.

Furthermore, addition of glycerol will improve the ability of wet keeping of the membrane, which is also

helpful in its medical performance. As revealed in Fig. 4, the water content and the ability to absorb water increase continuously with the increase of glycerol content.

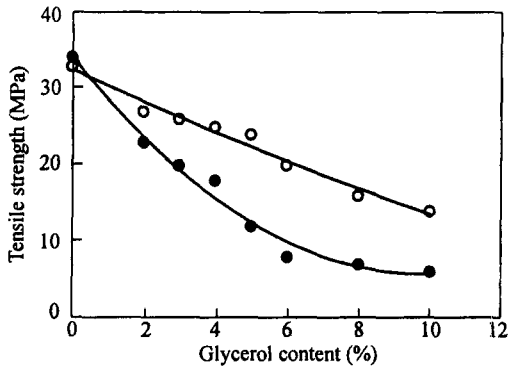

Fig. 1 Influence of glycerol content on the tensile strength of the membrane prepared with fixation agent A (○) and B (●)

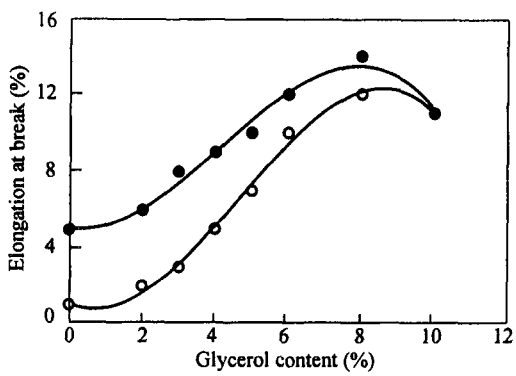

Fig. 2 Influence of glycerol content on the elongation at break of the membrane prepared with fixation agent A (○) and B (●)

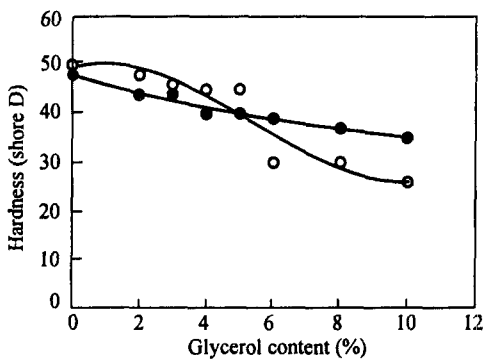

Fig. 3 Influence of glycerol content on the hardness of the membrane prepared with fixation agent A (○) and B (●)

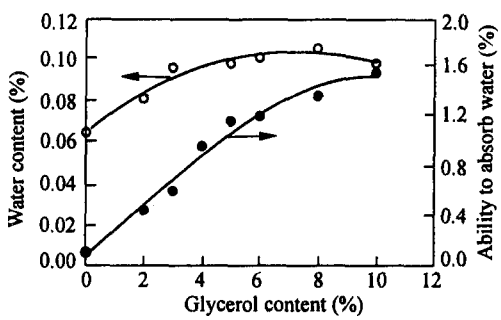

Fig. 4 Influence of glycerol content on the water content (○) and ability to absorb water (●) of the membrane prepared with fixation agent B

Comparison of the Present Method with Conventional Methods

There are several methods to prepare chitosan membranes. For comparing the advantage and disadvantage of our method with conventional methods, three membranes were prepared via different methods, as shown in the following procedures.

0.5 g powdered chitosan was dissolved in 20 mL 1.5% acetic acid at 30℃. 1.6 mL 5% (*V/V*) glycerol solution was then added and stirred. The bubble-free solution was cast in three molds for further use.

Method A (conventional method): chitosan solution was dried at room temperature for 4 days to get a transparent membrane.

Method B (modified conventional method): chitosan solution was dried at room temperature for 4 days. Subsequently, it was neutralized with a dilute potassium hydroxide solution and washed with distilled water. Then it was oven-dried at 40℃ for 1 h to get a semi-transparent membrane with slightly white color.

Method C (present method): chitosan solution was immersed in a fixation agent composed of 140 mL anhydrous alcohol and 11.5 mL glycerol at 70℃. After 2 h, the membrane was washed with distilled water. Then it was oven-dried at 60℃ for 3 h to get a transparent membrane.

Figure 5 shows the stress-strain curves of the chitosan membranes prepared via different methods. The conventional method (A) can provide a membrane having balanced strength and flexibility. However, it normally takes 100 h for a complete process to produce a membrane. Method B produces a hard and brittle membrane that

has no practicability. The present method (C) takes less time (about 6 h for the complete process) and gives a flexible membrane with fine appearance. Although its strength is relatively low it is suitable for use as an anti-adhesion membrane.

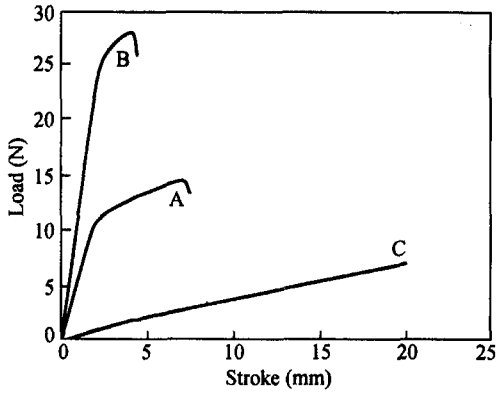

Fig. 5 Stress-strain curves of the chitosan membranes prepared via different methods

Evaluation of the membrane *in vivo* reveals that the membrane prepared via conventional method (A) will induce inflammation near the wound where the membrane was used as anti-adhesion material after a surgical operation, which is caused by the acidity of the membrane. In comparison, during the fixation step of method C, most of the acetic acid in solution was extracted by the fixation agent and evaporated. The pH of the membrane equals 6–6.5 after fixation and drying. Then it was washed with distilled water to give a neutral membrane.

CONCLUSION

Chitosan membrane that could be used as a biomedical material was prepared via a sol-gel transformation method. The fixation agent composed of alcohol, glycerol and potassium hydroxide can accelerate the sol-gel transformation process and hence shorten the preparation period. The conventional method normally takes 100 h for a complete process to produce a chitosan membrane, whereas the present method takes less time (about 6 h for the complete process) and furnishes a neutral membrane with fine appearance. Addition of glycerol can not only improve the flaxibility of the membrane but also improve the ability of wet keeping.

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