

ANALYSIS OF PREPARATION OF DEXTRAN HYDROGEL MEMBRANES AS A WOUND DRESSING

Avinash Nangia and Cheung T. Hung*

Department of Dermatology, School of Medicine, University of California at
San Francisco, San Francisco, CA 94143-0989, U.S.A.

*Zenith Technology Co. Ltd., 156 Frederick Street, Dunedin, New Zealand

ABSTRACT

Several variables which could affect the polymerization and physico-chemical properties of dextran hydrogel were investigated. The dextran hydrogels were prepared at multiple level of each variable. Water uptake capacity and handling characteristics were employed to evaluate the hydrogels. The effect of each variable on the polymerization of dextran is discussed and an optimum formulation of dextran hydrogel is presented.

INTRODUCTION

An increased understanding of the mechanisms of wound healing process has led to the development of various wound dressings^{1,2}. The purpose of these dressings is

Correspondence:

Avinash Nangia, Ph.D

Department of Dermatology, University of California San Francisco, Surge 110,
San Francisco, CA 94143-0989, U.S.A.

to create a wound environment that will allow the healing to proceed at the maximum possible rate^{3,4}. The essential factors of a dressing to promote wound healing are: high humidity levels between the dressing and wound interface^{5,6}, exudate soaking property, bacterial impermeability and insulation properties⁷.

A semiocclusive hydrocolloid dressing composed of biocompatible dextran, phospholipid and glycerol has recently been formulated in our laboratory⁸. Although it fulfils some of the important requirements, it possesses poor mechanical properties and instability in the presence of large exudate. It is important that an effective wound covering should remain coherent on the wound surface. Stability of a wound dressing is required to provide protection against microbial infection. A wound dressing which does not disintegrate at the wound surface, while maintaining the exudate soaking and easy removal properties of hydrocolloid dressings, will be desirable⁹.

In recent years, considerable interest has been shown in the use of hydrogels in the fabrication of various biomaterials¹⁰. Hydrogels are polymeric networks formed by crosslinking polymer chains. In the presence of water, these networks absorb a significant amount of water to form an elastic gel and yet remain insoluble. They are also biocompatible¹¹ and can be used as sustained drug delivery devices^{12,13}. These properties have prompted several workers to investigate hydrogels as wound dressings¹⁴⁻¹⁸.

This study was designed to identify the variables which are important to produce a dry flexible dextran hydrogel membranes which can be used as an effective wound dressing. Since the physico-chemical properties of a polymeric hydrogel are usually governed by the polymerization conditions^{10,19}, it is the aim of this investigation to evaluate the effect of several potential variables on the nature of the

hydrogel membranes. Since the water uptake capacity of a hydrogel can provide an indication of its porosity, oxygen permeability and mechanical properties²⁰⁻²², each hydrogel membrane prepared was evaluated for its water uptake capacity.

EXPERIMENTAL

Materials

A clinical grade dextran (molecular weight 81,500), two industrial grade dextrans (molecular weight 2,000, 000 and 25,000,000), L- α -phosphatidylcholine from fresh turkey egg yolk and epichlorohydrin were obtained from Sigma Chemicals Co., St. Louis, MO, USA. Sodium lauryl sulphate and sodium hydroxide were purchased from BDH. Polysorbate 85 was procured from ICI. Glycerol BP grade was purchased from Pharmaceutical Sales and Marketing Ltd., New Zealand.

Instruments

Silverson Homogenizer (Silverson Machine Ltd., England) was used to solubilize the phospholipid. Glass rings of 70mm in diameter with unsintered Teflon[®] film or Whatman[®] sealing film securely fastened on one side using a rubber band, were used for casting the hydrogels.

METHODS

Preparation of Dextran Phospholipid Hydrogel Membranes

The hydrogel membranes were prepared as follow: 10 g of dextran (molecular weight 81,500) was dissolved in 30 ml of distilled water. This solution was mixed with 0.3 ml of polysorbate 85, 4 ml of 10 M sodium hydroxide (0.04 moles), 2.8 ml of epichlorohydrin (0.035 moles) and 1.0 g of L- α -phosphatidylcholine solubilized previously in 30 ml of distilled water using 100 mg of sodium lauryl sulphate. The resulting mixture was then homogenized using a Silverson

homogenizer for two minutes and poured into four membrane casting rings. The rings were then maintained at an ambient temperature of $20 \pm 2^\circ\text{C}$ for 12 h till a tack free gel was obtained. The membranes were removed from the rings by unfastening the base film and rinsed with distilled water to remove the residual alkali as well as other unreacted components. The membranes after washing were dried at 80°C .

From this initial method, a number of variables were identified which might affect the physico-chemical properties of the crosslinked dextran membranes. These variables were polymerization temperature, polysorbate 85 concentration, epichlorohydrin (crosslinking agent) concentration and molecular weight of dextran. Table 1 lists the order of the studies and the levels at which each variable was investigated. Each variable was studied sequentially by preparing four batches of the hydrogels for each level listed in Table 1.

Removal of Residual Alkali from the Hydrogel Membranes

The time required for the complete removal of sodium hydroxide from the hydrogel membranes during the washing step was assessed as follows: Gel membranes were prepared according to the procedure outlined above. After polymerization, each membrane was soaked in 250 ml of distilled water for ten minutes with occasional shaking. After ten minutes, the water was decanted and a further 250 ml of fresh distilled water added. This procedure was repeated at intervals of 10, 20, 30, 40, 50, 60, 80, 100 and 120 minutes. 50 ml of the decanted solution removed each time, was titrated against 0.01 M hydrochloric acid using phenolphthalein as the indicator. The cumulative amount of alkali leached from the membrane was then calculated.

TABLE 1
Water Uptake Capacity of Dextran Phospholipid Hydrogel Membranes Prepared
under Different Conditions

| Order of evaluation | Variable | Level | Water uptake capacity (%) ^a (Mean \pm S.D) ^b |
|---------------------|-------------------------------------|-----------------|---|
| First | Temperature (°C) | 20 | 1319 \pm 10 |
| | | 30 | 1516 \pm 28 |
| | | 40 | 1587 \pm 95 |
| | | 50 | 1753 \pm 15 |
| | | 60 | 1875 \pm 35 |
| | | 80 ^c | |
| Second | Polysorbate 85 (ml) | 0.00 | 1206 \pm 32 |
| | | 0.10 | 1248 \pm 44 |
| | | 0.25 | 1282 \pm 65 |
| | | 0.50 | 1328 \pm 14 |
| Third | Epichlorohydrin contents (moles) | 0.01 | - d |
| | | 0.03 | 1324 \pm 14 |
| | | 0.04 | 1308 \pm 24 |
| | | 0.05 | 1274 \pm 40 |
| | | 0.08 | 1196 \pm 36 |
| Fourth | Molecular weight of dextran | 81,500 | 1318 \pm 10 |
| | | 2,000,000 | 1146 \pm 176 |
| | | 25,000,000 | 877 \pm 158 |

a Water uptake capacity = $\frac{\text{Weight of wet gel disc} - \text{Weight of dry gel disc}}{\text{Weight of dry gel disc}} \times 100$

b Mean of four determinations

c Hydrogel membranes were not obtained at this temperature

d No membrane was obtained

Water Uptake Capacity

Circular pieces of the dried membranes were cut with cork borer and measured for initial weight. The discs were allowed to soak in distilled water in a petri dish. After 24 h, the discs were removed, surface dried with Whatman® No.1 filter paper and weighed. The water uptake capacity (%) was calculated by the following equation:

$$\text{Water uptake capacity} = \frac{\text{Weight of wet gel disc} - \text{Weight of dry gel disc}}{\text{Weight of dry gel disc}} \times 100$$

RESULTS AND DISCUSSION

Preparation of Dextran Phospholipid Hydrogel Membranes

The freshly-prepared hydrogel membranes after washing were transparent, stable in water and possessed adequate handling properties. However, after washing and drying at 80°C, the hydrogels shrank to thick glassy membranes and were brittle in nature (see Figure 1). When the dried membranes were rehydrated in water, they swelled and attained the original size and shape but were fragile to handle (see Figure 1).

Dextran is selected as the matrix for the hydrogel because dextran is a nontoxic, nonantigenic polysaccharide. It has previously been demonstrated in our laboratory that phospholipid, when added to a hydrocolloid dressing in the concentration of 10% w/w, can provide optimum occlusiveness at the wound surface^{8,23}. Therefore, a fixed concentration of 10% w/w of phospholipid was incorporated in all the hydrogels. Sodium lauryl sulphate, an ionic surfactant, was used to solubilize the phospholipid in the hydrogels.

Crosslinking between dextran chains and epichlorohydrin, a crosslinking agent, can only take place under alkaline conditions with pH above 12. The amount of

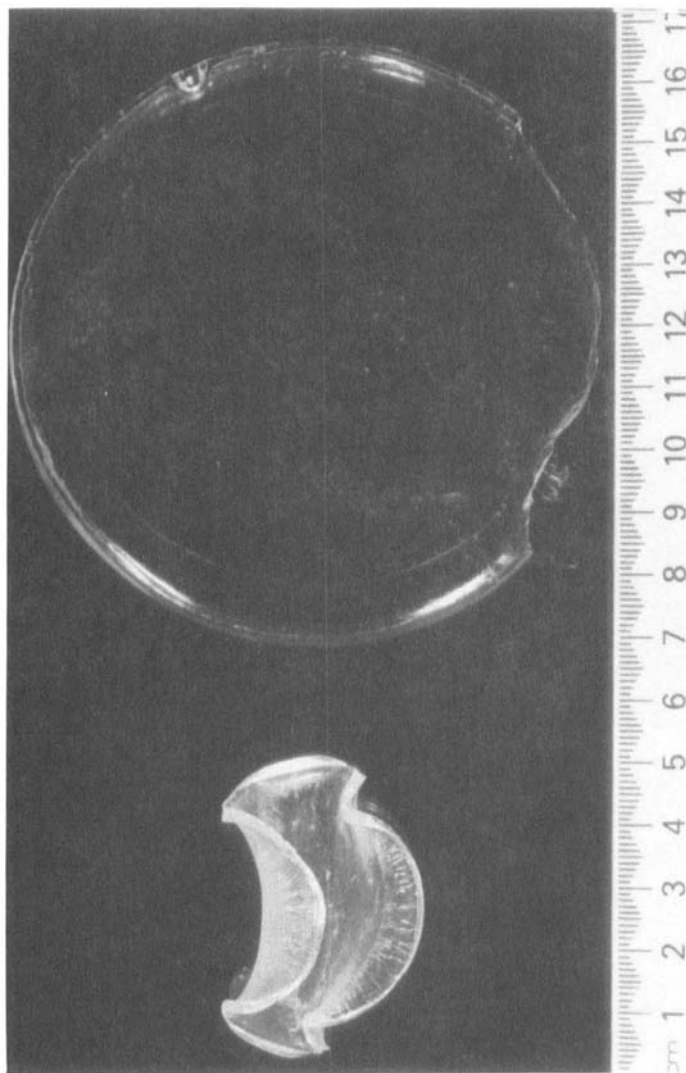


FIGURE 1

Dry and hydrated forms of a dextran hydrogel.

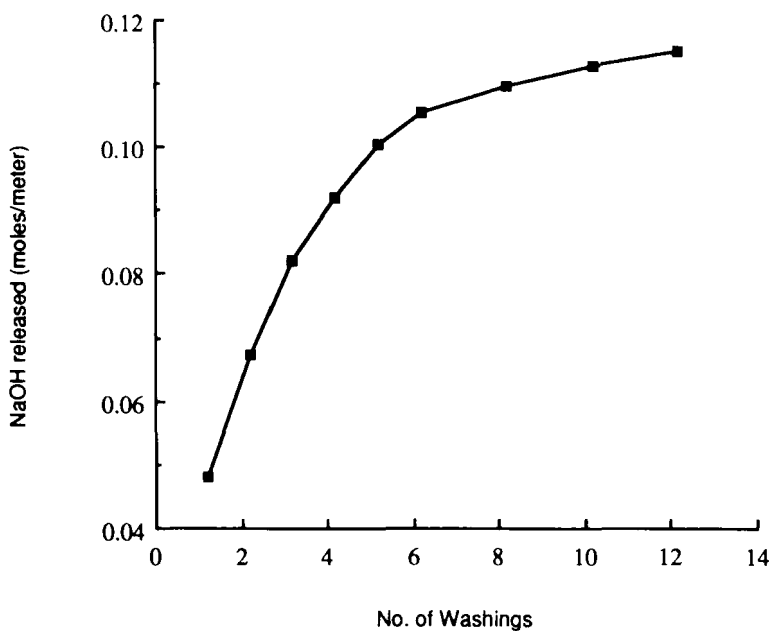


FIGURE 2

Plot showing the release of residual sodium hydroxide from the dextran-phospholipid hydrogel membranes upon repeated washings.

sodium hydroxide required to maintain the pH of the reaction mixture above 12, was found to be 0.04 moles. Therefore, 4 ml of 10 M sodium hydroxide was used in all the studies. Since large quantities of sodium hydroxide will be retained in the hydrogels after polymerization, a washing procedure which ensures its complete removal from the membranes was investigated. Removal of sodium hydroxide is important to prevent its hazardous effects on the wound surface. The amount of residual alkali leached from the hydrogel membranes after repeated washings at different time intervals is shown in Figure 2. Results indicate that leaching of sodium hydroxide was rapid. Most of the alkali can be removed from the membranes after six washings and ten washings essentially removed all the sodium

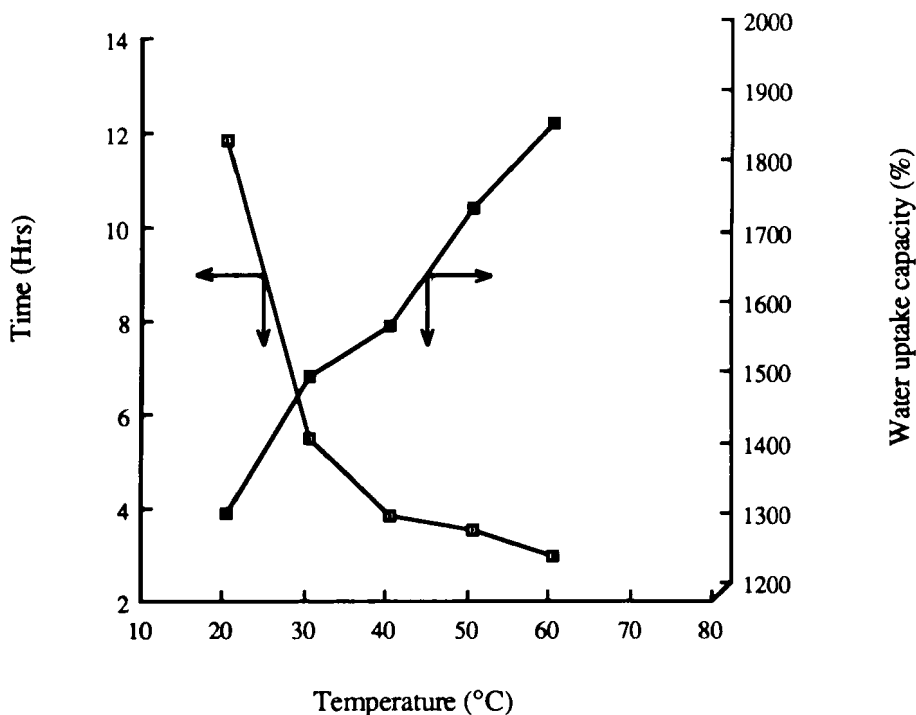


FIGURE 3

Plot showing the effect of temperature on the crosslinking time (□) and water uptake capacity (■) of dextran-phospholipid hydrogel membranes.

hydroxide from the membranes. Ten repeated washings with 250 ml distilled water were then adopted for subsequent studies.

Table 1 summarizes the water uptake capacity for all batches of hydrogels prepared. In this investigation, only those potential factors which can directly influence the physico-chemical properties of dextran hydrogels were studied. Figure 3 shows the effect of various temperatures on the crosslinking time required to obtain coherent hydrogel membranes. At an ambient temperature of 20°C, membranes were cast in 12 h, while at elevated temperatures, setting time was progressively reduced. However, at 80°C, no gel was formed even after 10 h and the colour of

the reaction mixture turned yellow. This was probably caused by the evaporation of epichlorohydrin at higher temperature or due to hydrolysis of dextran in the presence of strong alkali, or to both. The membranes cast at higher temperature had a higher water uptake value than those cast at lower temperature. It may be due to faster crosslinking at elevated temperature resulting in a looser network which is able to entrap larger amounts of water²⁴. Nevertheless, all freshly-prepared hydrogels cast at 30, 40, 50 and 60°C were extremely fragile to handle and were associated with more serious shrinkage upon drying than the hydrogels prepared at room temperature. These results indicate that although higher temperatures favour the formation of hydrogels with higher water absorption capacity, the problem of shrinkage and fragility can not be eliminated. Therefore, crosslinking of dextran at low temperature (20°C) for 12 h was preferred.

Addition of polysorbate 85 to the reaction mixture was found to be essential to avoid phase separation when the crosslinking agent epichlorohydrin content exceeded 0.03 moles. An increase in polysorbate 85 content from zero to 0.5 ml in the reaction mixture, resulted in the formation of slight turbid but uniform membranes. Membranes with various surfactant concentrations were found to have adequate mechanical properties when freshly-prepared but they all suffered from the same shrinkage problem after washing and drying as previously discussed. However, a small increase in water uptake capacity was observed on increasing the surfactant concentration (see Figure 4). In subsequent studies, 0.5 ml of polysorbate 85 was selected because the hydrogel membranes possess the highest water uptake capacity at this level. In addition, this amount dispersed all the levels of epichlorohydrin employed in the next study.

Membranes were successfully formed with various epichlorohydrin content (0.03-0.08 moles) at the selected conditions. However, hydrogels with 0.05 and 0.08

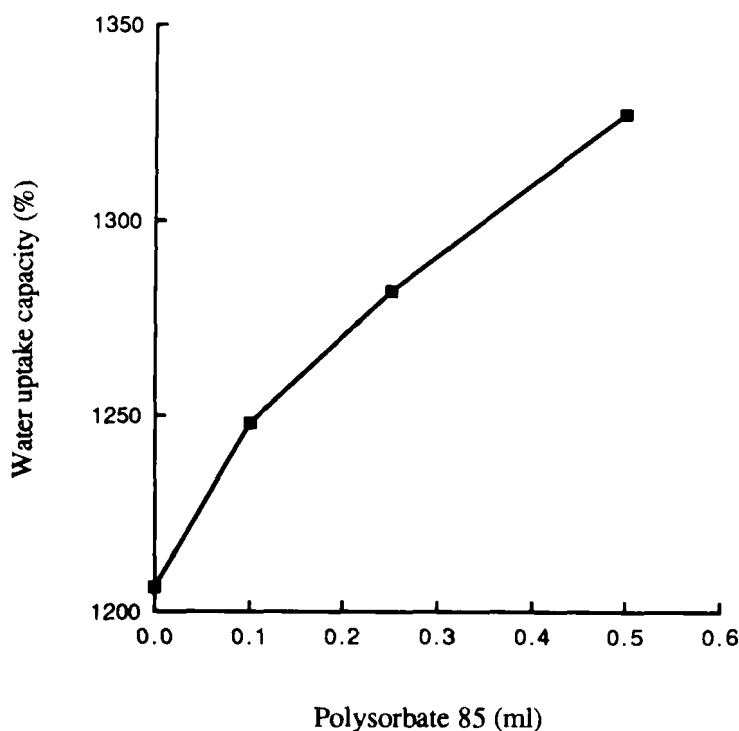


FIGURE 4

Plot showing the effect of polysorbate 85 on the water uptake capacity of dried dextran-phospholipid hydrogel membranes.

mole were extremely brittle even when freshly-prepared. After washing and drying, the hydrogels with low epichlorohydrin content (0.03 and 0.04 moles) shrank to a thick glassy membrane while the hydrogels with higher epichlorohydrin content (0.05 and 0.08 moles) fragmented. A small drop in water-uptake capacity was also observed with increased epichlorohydrin content (see Figure 5). From the above results it seems that the crosslinking density of the dextran hydrogels increases with increasing epichlorohydrin content. It also appears that the shrinkage of the membranes is proportional to the degree of hydrogel crosslinking. Epichlorohydrin content in the region of 0.03-0.04 moles is therefore adequate in

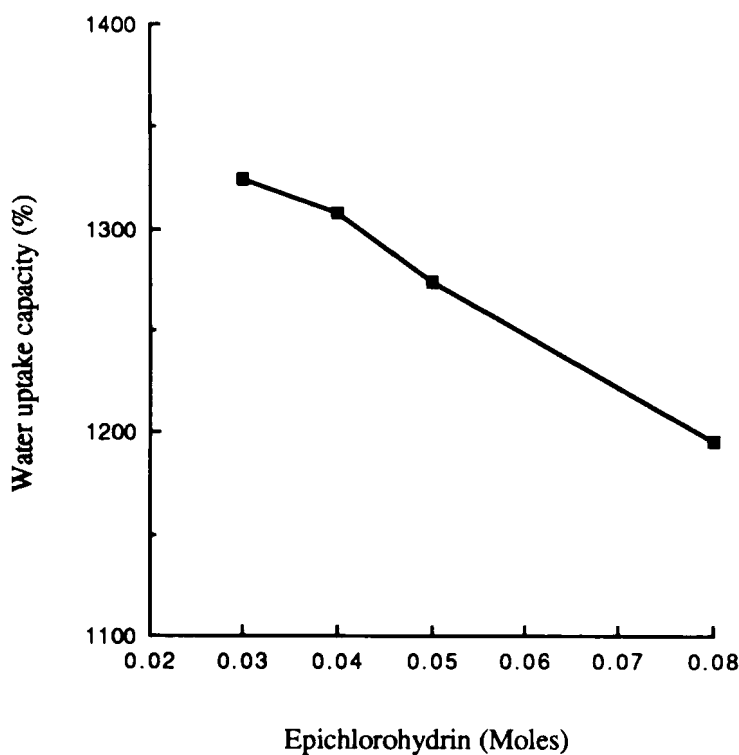


FIGURE 5

Plot showing the water uptake capacity of dried dextran-phospholipid hydrogel membranes as a function of epichlorohydrin content.

crosslinking 10 g of dextran to form water-insoluble membranes. For this reason, the epichlorohydrin content (0.035 moles) initially employed in this investigation was not altered in subsequent experiments.

Membranes were then fabricated using the method outlined previously using different molecular weights of dextran. Freshly-prepared membranes derived from the higher molecular weight dextrans were not as flexible as the membranes produced from the dextran of lower molecular weight (81,500). These membranes experienced the same shrinkage problem as those manufactured earlier. In

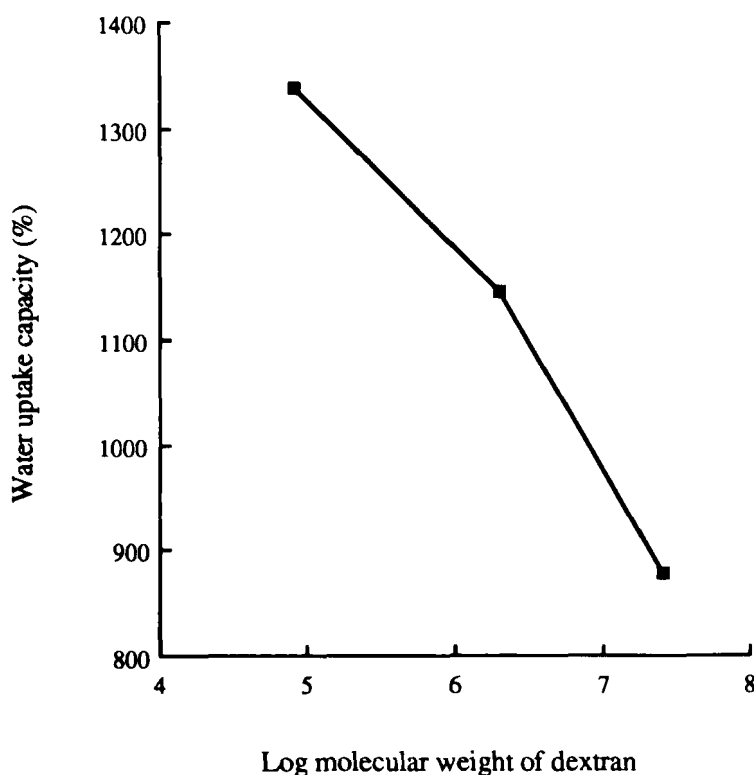


FIGURE 6

Plot showing a relationship between water uptake capacity vs molecular weight of dextran used for casting dextran-phospholipid hydrogel membranes

addition, the water uptake capacity of the membranes was found to decrease as the molecular weight of the dextran increases (see Figure 6). Such an observation also indicates that the crosslinking density of the dextran hydrogels increases with increasing dextran molecular weight. It is possible that, during the crosslinking reaction, dextran of low molecular weight is able to disperse uniformly in the solution so that distance between the interlinking sites are longer and limited, thus reducing the crosslinking density of the membrane. In case of high molecular weights dextrans, the proximity of the interlinking sites due to their longer chain:

allows epichlorohydrin to form more crosslinks, thereby creating a diffusional barrier for the water molecules to enter the network²⁵.

Overall, it appears that the initial formulation used in the synthesis of dextran hydrogel can not be modified to produce dry uniform hydrogel membranes which can be used as a wound dressing. Nevertheless, results obtained indicate that among various polymerization variables involved in the fabrication of dextran hydrogels, temperature and epichlorohydrin content play the most important role. Compared to temperature, epichlorohydrin was found to have only a moderate effect on the physical properties of the hydrogel membranes. Low polymerization temperature and the correct amount of epichlorohydrin are required for the successful formulation of dextran hydrogels.

In an another attempt to modify the shrinkage problem of dextran membranes, the freshly-prepared hydrogels, after washing, were soaked in a 5%v/v glycerol (a hydrophilic plasticizer) aqueous solution for 6 h and then dried at 80°C. The dried membranes still exhibited the same shrinkage problem. However, the dried membranes were flexible in nature.

Crosslinked dextran hydrogel, in the form of dry sterilized beads (Debrisan[®]), has been used extensively as a cleansing agent for moist exudating wounds²⁶. These beads, being hydrophilic, can soak up water upto four times their dry weight²⁷. However, in case of heavy exudating wounds, exudate accumulation remains a problem once the exudate soaking capacity of these beads is saturated. The optimal formulation of dextran hydrogel produced in this study has three times higher water absorption capacity than the Debrisan beads and will be more effective in cleansing the exudating wounds.

Further studies are in progress to form composite dry hydrogel membranes of dextran and other polymeric material to be used as a wound dressing.

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