Preparation of Hydrogels by Redox Initiation Via Free Radical Polymerisation for Biomedical use as Wound Dressings

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Abstract

Preparation of hydrogels for biomedical use as wound dressings was studied. A partially hydrated hydrogel of sodium 2-acrylamido-2-methylpropanesulfonate (Na-AMPS) was prepared by redox initiation via free radical polymerisation in aqueous solution. The results showed that the hydrogel sheets had good coherency. The equilibrium water absorption (EWC) of hydrogel sheet was 98%, reaching to equilibrium within 30 min and the water molecule evaporated from the hydrogel up to 240 min at water retention of 22%. The WVT rate of Na-AMPS hydrogel was about 85 g/m^2.hr which was in the range of commercial products. The oxygen permeability of the hydrogel sheet was about 300-400 x 10^{-11} cc.mm./cm^2.s.cmHg. Finally, it was found that this hydrogel was non-toxic that suitable for use in biomedical application.

Keywords: Redox initiation, Hydrogel, Wound dressings

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1. Introduction

The development of synthetic hydrogels can be traced back to the pioneer studies of Wichterle and Lim [1] who employed 2-hydroxyethyl methacrylate (HEMA) as a hydrophilic monomer. The lightly crosslinked polymer of this material was used as a soft contact lens, to a more limited extent, as a general biomedical polymer. In recent year, much research has been considered on synthetic hydrogels for biomedical applications, such as contact lenses, skin adhesive, drug delivery especially for wound dressings where the hydrogel was used as the skin cover to protect the inner tissues, retaining body fluids, keep moist environment and regulating body heat [2-5]. Sodium 2-acrylamido-2-methylpropane sulfonate (Na-AMPS) was converted from AMPS-H^+ which is interesting molecular design monomer by its hydrophilicity, thermal stability, stability over a broad pH range, resistance to hydrolysis, ionic character and it can dissolve in water to avoid the organic solvent which toxic to the cell. Previous works [6-9], how to prepare AMPS acid hydrogels including Na-AMPS hydrogels by different methods and studied the properties of the hydrogel, not focusing on the properties requirement of wound dressings. The aim of this present work is to design and characterization of synthetic hydrogels of Na-AMPS using redox initiation via free radical polymerisation for biomedical use as wound dressings. At present, Thailand continues to import wound dressing commercial products at great expense. This present research work is therefore aimed at developing a capability to manufacture these products in Thailand with the potential application on wound dressings.

2. Experimental Procedure

2.1 Preparation of Na-AMPS Solution

The water-soluble monomer used in this work was 2-acrylamido-2-methylpropanesulfonic acid (AMPS-H^+) [10], it was commercial product (Aldrich, assay 99%) and was used without further purification. An aqueous solution of Na-AMPS was prepared by dissolving AMPS acid in distilled water (40% w/v), cooling in an ice-bath, and then neutralizing to pH 7 with sodium hydroxide solution.

2.2 Hydrogel Synthesis

At room temperature, potassium persulfate ($K_2S_2O_8$) as the free radical initiator at a concentration of 0.5% mol (relative to the monomer) and potassium metabisulfite ($K_2S_2O_5$) and ferrous sulphate ($FeSO_4$) as coinitiators (reductants) were add into the 40% w/v Na-AMPS solution [11].

$$S_2O_8^{2-} + Fe^{2+} \rightarrow SO_4^{2-} + SO_4^{-}\bullet + Fe^{3+}$$

$$S_2O_8^{2-} + S_2O_5^{2-} \rightarrow SO_4^{2-} + SO_4^{-}\bullet + S_2O_5^{-}\bullet$$

The aqueous solutions were each poured into a vertical sheet-forming mould consisting of two parallel plates covered with Teflon® sheets as release liners. Spacers were used to control the sheet thickness.

2.3 Analysis of the Hydrogel Sheets

2.3.1 Water Transport Properties

After synthesis, hydrogel is partially hydrated hydrogel, then equilibrate it in air at room temperature (EWC_{air}), after that each hydrogel sheet was immersed in distilled water at room temperature (37 ºC) and its increase in weight with time was followed. The water content (WC) was calculated using the following expression in equation (1).

$$WC\ (wt\ \%) = [(W_w - W_d)/W_w] \times 100\ \% \quad (1)$$

where W_d and W_w are the dry and wet weights of the hydrogel respectively.

When the equilibrium water content (EWC) had been reached, the hydrogel was removed from the distilled water and re-equilibrated in air at room temperature to constant weight. The water retention (WR) of the hydrogel was similarly plotted as a function of time.

The water vapour transmission rate (WVTR) was measured using the ASTM E96-93 (1990) Water Cup Method in equation (2).

$$WVTR = (G/t) / A \quad (2)$$

where

WVTR = water vapour transmission rate ($g/m^{2.}.hr$)

G = weight loss (g)

t = time (hrs)

A = test area (cup mouth area) = $2.83 \times 10^{-3}\ m^2$

G/t = slope of the straight line graph (g/hr)

For each WVTR determination, the hydrogel was first equilibrated in air to its EWC_{air} and a circular-shaped sample (diameter = 5 cm, thickness = 0.12 cm) cut and placed in position on the water cup. The edges of the sample were sealed with paraffin wax. The cup contained 20 ml of distilled water which maintained a saturated water vapour-filled space below the sample.

The complete water cup assembly was then weighed accurately and placed in an incubator at 37±1°C and relative humidity 55-60%. Weight measurements were made every 30 min for 8 hrs.

2.3.2 Oxygen Permeability

The oxygen permeability (Dk) was obtained using a 210T Permiometer. Hydrogel samples were cut to size using a size 7 cork borer then measurement the thickness (0.10 ± 0.02 mm) using a micrometer. Each sample was placed over an electrode with a lens tissue saturated in 0.1 M potassium chloride (KCl) as an electrode bridge in between the sample and the electrode. A column was then placed over the sample, and a constant stream of N_2 gas passed through the sample until a steady current was obtained. This steady current was then recorded as i_0. In all cases, i_0 in this study was found to be zero, signifying that there was no O_2 gas in the system before measurement. Then, O_2 gas was introduced into the system and new steady current reading obtained recorded as i. The oxygen permeability (Dk) was calculated using equation (3).

$$Dk = i\ (\mu A) \times L\ (mm) \times (6 \times 10^{-11}) \quad (3)$$

Where

L = thickness (mm)

i = current obtained for O_2-passing through the hydrogel

2.3.3 Cytotoxicity and SEM studies

In this experiment, the Na-AMPS hydrogel sheet samples were studied using cytotoxicity testing with L929 cells (mouse fibroblasts) by direct contact method. High-density polyethylene (HDPE) and natural rubber containing carbon black were used as negative and positive controls, respectively.

The substances used were:

(1) Mouse fibroblasts, ECACC No. 85011425, cell concentration = 6×10^4 cells/disc

(2) Dulbecco's Modified Eagle's Medium (DMEM) with 10% (v/v) fetal bovine serum, L-glutamine, penicillin (100 units/ml) and streptomycin (100 µg/ml) (Gibco ™, Paisley, UK) for fibroblast culture

(3) Non-toxic dental wax with 1 mm diameter and 15 mm length

(4) Phosphate buffer saline (PBS) pH 7.4

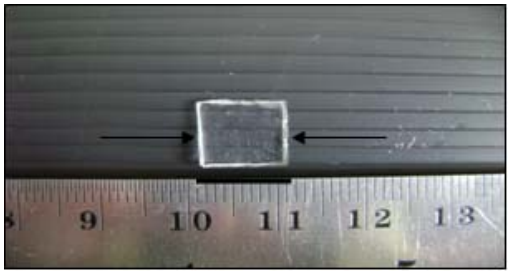
(5) 0.01% v/v neutral red in phosphate buffer saline

Hydrogel test specimens were cut into 1 x 1 cm in size and saturated with growth medium. The samples were placed in the middle of a 35 mm dish and fixed with non-toxic dental wax. L929 cells were then seeded into the dish at a density of $6x10^4$cells/dish and incubated for 48 hrs at 37°C. Cell morphology and the toxic zone were evaluated by inverted phase contrast light microscopy after the 48 hrs exposure to the cells. The cells were stained with 0.01% neutral red in phosphate buffer saline (PBS) for membrane integrity. Finally, the samples were gold sputtered under vacuum and examined microscopically using a Jeol JSM-5410 Scanning Electron Microscope to observe the cellular morphology and behaviour of the L929 cells on the samples. Each sample was tested in triplicate and the tests repeated twice.

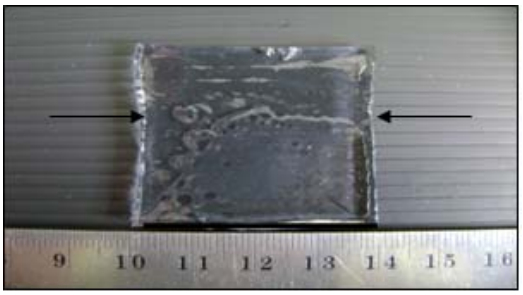
3. Results and Discussion

3.1 Water Transport Properties

The study of water adsorption, in going from EWC_{air} to EWC_{water}, an illustration of how much the hydrogel sheets swelled due to water absorption is shown in Fig. 1. The swollen sheet had approximately 4-fold increases in length and width and 3-fold increase in thickness.



(a) Hydrogel sheet before swelling (1 x 1 x 0.12 cm), Time (t = 0 min), water content = 22%



(b) Hydrogel sheet after swelling (4 x 4 x 0.35 cm), Time (t = 30 min), Water content = 98%

Fig. 1 40% Na-AMPS hydrogel with 1% mol EGDM using redox initiation

Water Absorption

The hydrogel sheet was immersed in distilled water at room temperature (37 ºC). The hydrogel sheet could absorb water molecules into its structure very quickly in the initial time then slowly absorption rate until reaching the equilibrium at water contents (EWC) of 98% as shown in Fig. 2. The water absorption occurred by the different osmotic pressures between hydrogel and the water. In the initial time, the water molecule can go into the hydrogel structure very quickly because the large difference of osmotic pressure. Besides, the polymer molecule colud expand extremely. Until the osmotic pressure nearly equal, hydrogel has more water in its structure then it can absorb water very slowly then the initial time. Finally, the water molecule stop to go into the hydrogel, because of the osmotic pressure was balanced, this reaching the equilibrium water content within 30 min.

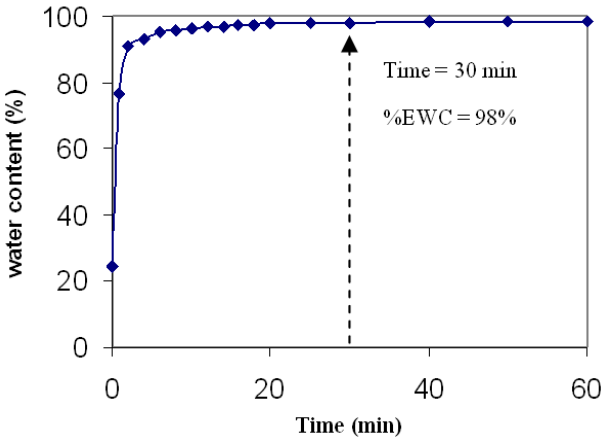


Fig. 2 Water absorption - time profiles for the hydrogel sheets when immersed in distilled water at 37°C.

Water Retention

Fig. 3 shows, Water retention - time profiles for the hydrogel sheets. The results suggested that the water molecule evaporated from the hydrogel up to 240 min. At the initial stage, the water content in the hydrogel decrease with time in a slight and progressive manner until reaching 240 min. After that the water content remained stable and water retention was calculated to be 22%.

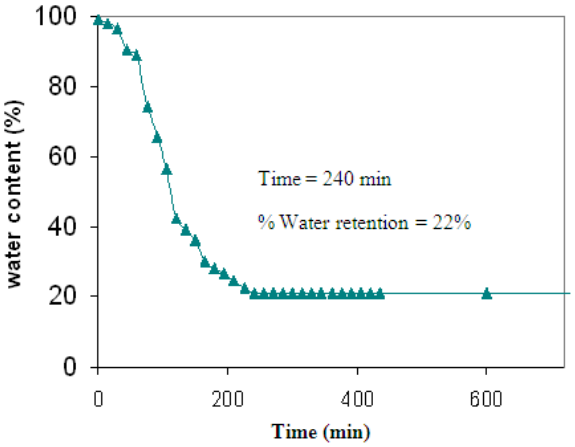


Fig. 3 Water retention - time profiles for hydrogel sheets when left in air at room temperature (continued from Fig. 2)

Water Vapour Transmission Rate

The water vapour transmission (WVT)-time profiles are shown in Fig. 4. From the linear plots, a WVT rates of 85 g/m^2.hr, which, for medical purposes, the WVT rates is deemed to be a suitable rate for the controlled evaporative water loss from a second-degree burn based on the WVT rate ranges of the commercial products as given in Table 1 . These confirm that the hydrogels could effectively decrease evaporative water loss to a significant extent and thereafter control it at a constant rate.

However, in the case of a hydrogel for use as a wound dressing, its ability to release water by evaporation is just as important as its ability to absorb water so that the overall balance of water transport can maintain a moist environment at the wound surface.

Table 1, shows that the WVT rate of Na-AMPS hydrogel is about 85 g/m^2. hr. The WVT rate of Na-AMPS hydrogel from this work is in the range of commercial wound dressings. A higher value of WVT rate may affect a faster drying of the wound. However, for an ideal WVT rate for wound dressing, the value must not be too high because it will cause a dry condition in the wound area. On the other hand, if the WVT rate value is too low, then it will make the wet condition of the wound also accumulation of exudates which may cause the deceleration of healing process and the inflection from bacterial.

Table 1 The value of WVTR of some commercialized wound dressings [12]

Wound dressing Types	WVTR (g/m^2.hr)
Biabrone	154
Metalline	53
Op site	33
Omiderm	208
Na-AMPS (our work)	85

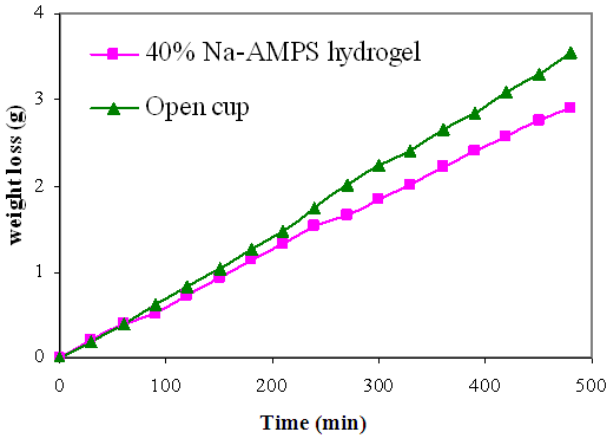


Fig. 4 Water vapour transmission - time profiles for the hydrogel sheets at 37°C and 55-60% relative humidity.

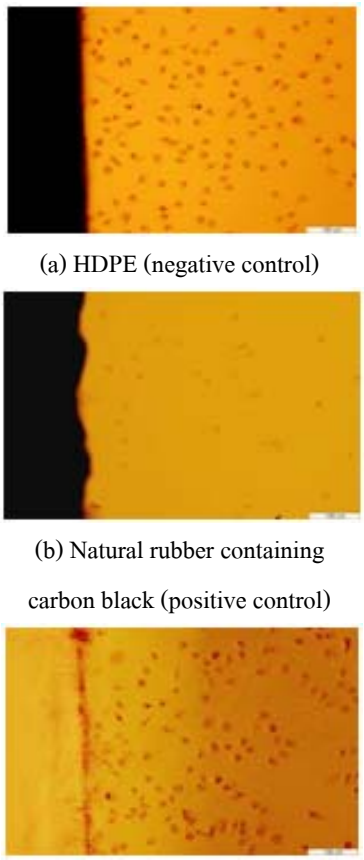
3.2 Oxygen Permeability

It was found that the Dk of the Na-AMPS hydrogel sheets were about 300-400 x 10^{-11} cc.mm./cm^2.s.cm Hg, this being significantly higher than other hydrogel polymers, such as, polyHEMA whose Dk is 145 x 10^{-11} cc.mm./cm^2.s.cm Hg [13]. From the point of view of oxygen permeability, the high Dk values of the Na-AMPS hydrogels are advantageous for their uses as wound dressings since they would readily allow oxygen exchange to and from the wound surface. This oxygen exchange allows the wound to "breathe" which, in turn, aids the wound healing process.

3.3 Cytotoxicity

Cytotoxicity is basically defined as the quality of being toxic to cells. The cytotoxicity testing is a necessary part of the overall testing procedure for any material that is to be used in a biomedical application which involves direct contact with living tissue.

The SEM results in Fig. 5 show that the hydrogel sheet is clearly non-toxic since they exhibit similar cell responses to the HDPE negative control. This similarity extended to both cell viability (number) and morphology (size and shape).



(a) HDPE (negative control)

(b) Natural rubber containing carbon black (positive control)

(c) 40% Na-AMPS hydrogel

Fig. 5 Scanning electron micrographs (magnification x 200) showing the L929 cells after cytotoxicity testing for 48 hrs at 37°C.

4. Conclusions

The hydrogel had high water absorption of 98% reaching to the equilibrium within 30 min. The water molecules evaporated from the hydrogel at water retention was 22%, up to 240 min. The WVT rate was found to be 85 g/m^2.hr, which was the range of commercial wound dressings. The oxygen permeability of the hydrogel sheet was about 300-400 x 10^{-11} cc.mm./cm^2.s.cmHg. From the cytotoxicity test, it was found that this hydrogel was non-toxic that suitable for use in biocompatible process.

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