Hydrogen Peroxide Loaded Cellulose Acetate Mats As Controlled Topical O₂ Delivery Devices

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ABSTRACT

In this study, sub and supercritical CO₂ are used as effective solvents to impregnate hydrogen peroxide (H₂O₂) into cellulose acetate (CA) non-woven mats. As much as 25wt% H₂O₂ can be impregnated at operating conditions of 25-to-45°C and 1,200 psig. Thermal Gravimetric analysis shows that H₂O₂-loaded CA mats lose more than 60% of available H₂O₂ when quickly heated to 37°C and held at this temperature for 60 minutes. However, in comparison, pure H₂O₂-H₂O (50:50 w/w) solution evaporates 100% within 15 minutes at this same temperature. Shelf-life studies show that H₂O₂-loaded CA mats lose ~50% and ~100% of available H₂O₂ in 30 days, when stored at 2-to-8°C and 20-to-23°C, respectively. H₂O₂ slowly diffuses and subsequently

evaporates from the CA mat at a rate determined by the diffusion of H_2O_2 through the CA fibers. We envision applying a thin polymer coating to the CA mat to suppress diffusional losses of H_2O_2 , thus leading to an increased product shelf-life. These mats should find use as bandages for the continuous delivery of O_2 for advanced wound care. *In vitro* studies presented here demonstrate that the developed bandages produce O_2 at a controlled rate for 24 hours.

Keywords: Supercritical CO_2 , Impregnation, Hydrogen Peroxide, Controlled Release, and Wound Healing

1. INTRODUCTION

Sub and supercritical CO_2 assisted impregnation of polymeric materials has been reported as a viable approach for loading a variety of substances such as drugs[1, 2], flavors[3, 4], dyes[5] etc. into the carrier polymer. The CO_2 assisted impregnation process has a number of advantages, such as processing without organic solvents and uniform distribution of active substance in the matrix[3, 6]. The impregnation process depends on the partitioning of the active substance between the CO_2 -rich and polymer-rich phases. Therefore, the relative solubility of the active substance in CO_2 and in the polymer has a significant effect on the amount of active substance loaded into the polymer[7]. Although significant research has been done in the area of CO_2 -assisted impregnation, there are no studies aimed at loading H_2O_2 using this method.

In the present study, cellulose acetate (CA) mats are impregnated with H_2O_2 using CO_2 assisted processing. H_2O_2 is a widely used chemical oxygen producing compound, because it decomposes

into water and oxygen and it carries 47 wt% O_2 per unit mass[8]. H_2O_2 almost instantaneously releases oxygen when contacted with a wound site, due to the catalytic decomposition by an enzyme catalase[9, 10]. Hence, the controlled release of H_2O_2 has the potential to be an effective method for *in situ* sustained oxygen delivery. Oxygen has been used as a therapeutic agent to speed up healing of acute and chronic wounds[11-14]. Therefore, an adequate supply of oxygen is important for wound healing. Roe et al. showed that topically applied O_2 penetrates $\sim 700\ \mu m$ deep through human skin[15], which means that O_2 delivered to a wound site will be readily absorbed. The present study demonstrates the effect of impregnation process parameters on the H_2O_2 loading into the CA fibers that make up the nonwoven mat. H_2O_2 release rate, O_2 production rate, and mat shelf-life data are also presented to demonstrate the efficacy of the *in vitro* performance of the H_2O_2 -loaded CA mats.

2. MATERIALS AND METHODS

2.1. Materials

30 and 50 wt% H_2O_2 in H_2O solutions are purchased from Sigma Aldrich, USA. Concentrated 80 wt% H_2O_2 in H_2O solution is obtained by evaporating H_2O from a 50wt% H_2O_2 - H_2O solution in vacuum at 25°C. Celanese, USA has donated cellulose acetate (CA) non-woven mats.

2.2. Methods

2.2.1. CO_2 -assisted H_2O_2 impregnation

A high-pressure apparatus (Parr instruments, USA) is used for the CO_2 assisted impregnation process. CA mats, dried at 100°C for two hours, are loaded into a tea bag that is tied to the

impeller shaft of the apparatus. Enough $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ solution is placed at the bottom of the vessel of the apparatus to obtain a $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ solution to CA mat ratio often. The vessel is then pressurized with CO_2 to 1200 ± 50 psig and heated to the desired temperature typically maintained between 25 and 40°C . The system is maintained at these conditions for 60 minutes with vigorous mixing and then the vessel is slowly depressurized over a period of 15 minutes. A gravimetric method is used to determine the loading of $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ in the CA mats after the impregnation experiment. Thermogravimetric analysis is used to confirm the gravimetrically determined $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ loading of the CA mats.

2.2.2 $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ solubility in CO_2

In the present study, $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ solubility in CO_2 measurements are performed using a variable-volume view cell as described elsewhere [16, 17]. The details of the variable-volume view cell are provided in the supporting information. The $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ solubility isotherms are determined at temperatures of 25°C to 40°C by observing the dew point pressures. The contents of the cell can be viewed on a video monitor using a camera (Olympus Corporation, Model STC-N63CJ) connected to a borescope (Olympus Corporation, Model F100-024-000-55) placed against a sapphire window secured at one end of the cell. The mixture is isothermally compressed to a single phase, and the pressure is then decreased incrementally until a dew observed on the window. In between each pressure decrease step the contents are stirred vigorously for three minutes, stopped stirring and the system is held constant for another three minutes.

2.3. Characterization of H₂O₂-loaded CA mats

2.3.1. Hydrogen peroxide loading determination

H₂O₂ is extracted from the treated mat by immersing the H₂O₂-loaded CA mats into a 1:10 v/v sulfuric acid–distilled water solution for 15 minutes with moderate stirring. The H₂O₂ concentration of the resultant solution is determined using permanganate titration (HANNA Instruments, HI 902C, USA). The standard deviation of the H₂O₂ concentration is ~2% as determined from three repeated titrations of representative H₂O₂-loaded CA mats.

2.3.2. Thermo gravimetric analysis

The H₂O₂–H₂O loading in a CA mat also is determined using thermal gravimetric analysis (TGA) (Perkin–Elmer, Pyris 1, USA). The furnace is continuously flushed with nitrogen gas at a flow of 3 L/hour. The H₂O₂-loaded CA mats are heated at a rate of 100 °C/minute to 37 °C and held at this temperature for 60 minutes. The mats are then quickly heated to 100 °C at a heating rate of 100 °C/minute and held at this temperature for 30 minutes.

2.3.3. Scanning electron microscopy

Scanning electron microscopy (SEM) (HITACHI SU-70, USA) is used to determine the morphology of the H₂O₂–H₂O loaded CA fibers that make up the nonwoven mats. CA fibers, peeled from a representative mats, are spread on a graphite paste and then a 5 nm platinum coating is applied via spun coat (Denton Vacuum LLC, Desk V TSC, USA) onto the sample before capturing the images.

2.3.4. *in vitro* H₂O₂ release studies

In the present study, the bandages are prepared by placing H₂O₂-loaded CA mat between medical grade silicone films. Figure 1A shows the digital image of the bandage. A Franz cell is used to determine the *in vitro* H₂O₂ release kinetics from the bandages. The cell is filled with 15 mL of saline, 0.9 wt% NaCl in distilled water, and maintained at 37°C. The bandage is mounted as depicted in Figure 1B that mimics the *in vivo* skin attachment. The saline is stirred at 100 rpm and aliquots drawn at a pre-defined time points are assayed for H₂O₂ release using permanganate titration.

Figure 1

3. RESULTS AND DISCUSSION

There is a large amount of solubility and phase behavior data available in literature for the H₂O in CO₂ [18-21]. Figure 2 shows the literature solubility of H₂O in CO₂ and indicates the low solubility of water in CO₂ [19]. At a given temperature the solubility of water in CO₂ increases as pressure increases up to a certain point, after that increasing pressure has no effect on the solubility. There is no literature data available for the H₂O₂-H₂O solubility and phase behavior in CO₂. This study reports the preliminary data on the H₂O₂-H₂O solubility and phase behavior in CO₂, which is important to understand and optimize the CO₂ assisted impregnation process. Figure 3 shows the solubility isotherms for 72:28 w/w H₂O₂-H₂O in CO₂. The H₂O₂-H₂O is more soluble in CO₂ when compared to pure water, which is likely due to the additional hydrogen bonding site of H₂O₂.

Figure 2

Figure 3

Figure 4 shows the phase behavior of $\text{H}_2\text{O}_2\text{--H}_2\text{O--CO}_2$ system at a fixed overall composition, but with varying H_2O_2 concentration in the starting $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ solution. At a given temperature, as the H_2O_2 concentration increases the dew point pressure decreases up to 50 wt%, which is due to the increased intermolecular interactions between H_2O_2 and CO_2 . However, the dew point pressure increases as H_2O_2 concentration increases from 50 to 72 wt% for temperatures below the CO_2 critical temperatures and flatten out above the CO_2 critical temperatures. Further studies are required to elucidate the phase behavior of $\text{H}_2\text{O}_2\text{--H}_2\text{O--CO}_2$ system.

Figure 4

CO_2 assisted impregnation experiments are performed with $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ (50:50 w/w) solution at a fixed pressure of 1200 psig and temperatures from 25 to 45 °C. The amount of $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ used in all the four experiments is approximately an order of magnitude higher than the saturation solubility of $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ in CO_2 . Figure 5 shows the effect of temperature on the H_2O_2 loading in CA mats. As the impregnation temperature increases the H_2O_2 loading in the CA mat decreases. The higher H_2O_2 loading at low temperatures is likely due to the higher partitioning of H_2O_2 from CO_2 -rich phase to the CA mat, which is likely due to strong interactions between the molecular groups on the CA polymers and the H_2O_2 that are favored at low temperatures. However, the H_2O_2 loading in the CA mat is approximately the same at 25-to-30 °C and 35-to- 45 °C. The error bars of the data points are the standard deviation of H_2O_2 loading

in three individual CA mats that are loaded with H_2O_2 in the same experiment. The results suggest that lower temperatures are favorable for the H_2O_2 loading into CA mats using the CO_2 process. Note that even though the solubility of H_2O_2 – H_2O in CO_2 is low (see Figure 3) the CO_2 assisted impregnation can effectively load significant amounts into the CA mats, which signifies the importance of solute-polymer interactions rather than the solubility. These results are consistent with the literature reports where flavors[3] and dyes[22] with low CO_2 solubilities were successfully loaded into fibers/polymers using CO_2 assisted impregnation.

Figure 5

To manipulate the H_2O_2 loading into the CA mats, the impregnation experiments are performed at 25°C and 1200 psig using four different concentrations of H_2O_2 – H_2O solutions. Note that the amount of H_2O_2 – H_2O used in all the four experiments is the same and of sufficient amount to maintain the CO_2 –rich phase saturated during the impregnation process. As shown in Figure 6, the H_2O_2 loading into CA mats increases linearly as H_2O_2 concentration increases in the starting solution used during the impregnation step. The error bars of the data points are the standard deviation of H_2O_2 loading in three individual CA mats that are loaded with H_2O_2 in the same experiment. The results suggest that H_2O_2 loading into CA mats can be tailored by manipulating the CO_2 assisted impregnation process parameters for example temperature and H_2O_2 – H_2O solution concentration.

Figure 6

TGA analysis is used to determine the $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ loading in the CA mats. Figure 7 shows the typical $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ weight loss from the 9.5 wt% $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ -loaded CA mats. Figure 7A compares the weight loss from the $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ -loaded CA mats with that of the as-received CA mats. The results show that CA mats are loaded with ~19 wt% of $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ whereas as received CA mat has ~5 wt% absorbed moisture or other volatiles. Figure 7B compares the weight loss from the $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ -loaded CA mats with that of the as received $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ (50:50 w/w) solution. The results show that $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ -loaded CA mats lose more than 60% of available H_2O_2 when quickly heated to 37°C and held at this temperature for 60 minutes. However, in comparison, 100% of $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ (50:50 w/w) solution evaporates within 15 minutes at this same temperature.

Figure 7

Figure 8 shows the shelf-life of $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ -loaded CA mats determined at 2-to- 8°C and 20-to- 23°C . Approximately 50 wt% of the loaded H_2O_2 is available in the CA mats when stored at 2-to- 8°C for 30 days, whereas the mats stored at 20-to- 23°C loses 100 wt% of loaded H_2O_2 in the same time. H_2O_2 slowly diffuses from the internal space or free volume of the CA fibers and subsequently evaporates from the surface of the CA mats. The rate of loss of available H_2O_2 from the mats is fixed by the diffusion of H_2O_2 through the CA fibers that make up the mat. It is expected that a thin polymer coating will minimize the loss of H_2O_2 , and, hence, increase the shelf-life of the product. Typical over the counter 3 wt% $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ solution shelf-life is significantly longer compared to the $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ -loaded CA mats, which is due to the presence of

H₂O₂ stabilizers in the solutions and due to the cap on the bottle that minimizes vapor losses of H₂O₂. If a thin polymer film is fixed to the outside of the H₂O₂-loaded CA mat, the loss of H₂O₂ from the mat will be suppressed similar to the suppression afforded by the cap on a bottle of 3 wt% H₂O₂-H₂O solution. Further studies are in progress to coat the H₂O₂-loaded CA mats with a low H₂O₂-H₂O permeable polymer, which presumably will improve the shelf-life of the H₂O₂-loaded CA mats.

Figure 8

Figure 9 shows digital and SEM images of virgin, CO₂-treated, and H₂O₂-loaded CA mats. The structural integrity of the both CO₂-treated and H₂O₂-loaded CA mats (Figure 9C and 9E) look similar to that of the virgin CA mat (Figure 9A), which indicates that there is no effect of CO₂ assisted impregnation process on the structural integrity of the CA mats. The morphology of the both CO₂-treated and H₂O₂-loaded CA mats (Figure 9D and 9F) look similar to that of the virgin CA mat (Figure 9B), which indicates that the CO₂ assisted impregnation process has no effect on the morphology of the mats.

Figure 9

Figure 10 shows that CA mats lose their structural integrity if soaked directly in H₂O₂-H₂O solutions at H₂O₂ concentrations greater than 30 wt% (Figure 10C and 10E). Hence direct soaking of CA mats in the H₂O₂-H₂O solutions is not a viable option for loading H₂O₂ into the

mats. Therefore, H_2O_2 impregnation using a CO_2 assisted process offers a significant potential for creating novel materials for advanced wound care applications. However, the morphology of the CA fibers is unaffected due to the direct soaking of the CA mats in the H_2O_2 – H_2O solutions (Figure 10B, 10D, and 10E).

Figure 10

Figure 11 compares the H_2O_2 release rate and corresponding calculated O_2 production rate from the H_2O_2 –loaded CA mats. Both H_2O_2 release and corresponding O_2 production are normalized for a 1.0 cm^2 size CA mat per minute within the time range shown on the abscissa. The O_2 production in Figure 11B is calculated assuming one-half mole of O_2 is created from the decomposition of a mole of H_2O_2 and using the ideal gas equation at 37°C and 1 atm. The total O_2 production from the H_2O_2 –loaded CA mats is an order of magnitude higher than the typical skin O_2 consumption, which is $\sim 0.25 \text{ }\mu\text{L}$ per cm^2 of skin[15]. However, the O_2 production can be tailored by manipulating the amount of H_2O_2 loading into the CA mats. The H_2O_2 –loaded CA mats developed in the present study can potentially be used for topical oxygenation, needed for wound healing, given the available total O_2 production and the sustained O_2 release for long periods of time.

Figure 11

CONCLUSION

In this study CA mats are successfully impregnated with H₂O₂ using sub and supercritical CO₂ at mild operating temperatures and pressures. The H₂O₂ loading in the CA mats can be tailored between 2-to-25 wt% by manipulating the impregnation process parameters. Maximum H₂O₂ loading into CA mats is achieved at temperatures of 25-to-30 °C, which is likely due to the higher partitioning of H₂O₂ from CO₂-rich phase to CA mats. Shelf-life studies show that storing the H₂O₂-loaded CA mats at 2-to-8 °C retains more than 50 wt% of the loaded H₂O₂ and thus storing in the refrigerator is preferred over room temperature. The CO₂ assisted impregnation process had no effect on the morphology of the CA mats. *in vitro* release studies reveal that sustained H₂O₂ release and, thus, O₂ production is achieved for 24 hours, using the bandages created from H₂O₂-loaded CA mats. We anticipate that these mats can find potential applications for advanced wound care.

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Figure1

(A)

(B)

Figure2

Figure3

Figure4

Figure5

Figure6

Figure7A

Figure7B

Figure8

Figure9

(A)

(B)

(C)

(D)

(E)

(F)

Figure10

(A)

(B)

(C)

(D)

(E)

(F)

Figure11

(A) H₂O₂ release

(B) O₂ Production