# Can Supercritical $CO_2$ Dissolve Hydrogen Peroxide? Impregnation Case Study

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## ABSTRACT

In this study, supercritical $CO_2$ is used as an effective solvent to impregnate hydrogen peroxide ($H_2O_2$) into cellulose acetate (CA) non-woven mats. As much as 30 wt% $H_2O_2$ can be impregnated at operating conditions of 25-to-40°C and 1,200-to-1,400 psi. Thermal Gravimetric analysis shows that $H_2O_2$–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, pure $H_2O_2$–$H_2O$ (50:50 w/w) solution evaporates within 20 minutes at this same temperature. Shelf-life studies show that $H_2O_2$–loaded CA mats lose ~50% and ~100% of available $H_2O_2$ in 30 days, when stored at 2-to-8°C and 20-to-23°C, respectively. $H_2O_2$ slowly diffuses and subsequently evaporates from the CA mat at a rate determined by the diffusion of $H_2O_2$ through CA mat. A thin polymer coating minimize the loss of $H_2O_2$, thus increasing the shelf-life of the product. These mats are used as bandages for the continuous delivery of $O_2$ for advanced wound care. *In vitro* studies show that the developed bandages produce $O_2$ in a controlled rate for 24 hours. The findings of this study provide insight into the application of supercritical fluid technology as a viable approach to load $H_2O_2$ into different carriers.

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

## INTRODUCTION

Sub and supercritical $CO_2$ assisted impregnation has been reported as a viable approach for a variety of substances such as drugs [1, 2], flavors [3, 4], dyes [5] etc. The $CO_2$ assisted impregnation has a number of advantages, such as processing without organic solvents and uniform distribution of active substance in the matrix. The impregnation process depends on the partitioning of the active substance between the $CO_2$- 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 [6]. Although 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 [7]. $H_2O_2$ almost instantaneously releases oxygen when contacted with a wound site, due to the catalytic decomposition by an enzyme catalase [8]. 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 [9, 10]. Therefore, adequate supply of oxygen is important for wound healing. Roe et al. showed that topically applied $O_2$ penetrates ~700 μm deep through human skin [11]. The present study demonstrates the effect of impregnation process parameters on the $H_2O_2$ loading and also presents *in vitro* performance of the $H_2O_2$–loaded CA mats such as $H_2O_2$ release, O2 production rate, and shelf-life of the product.

## MATERIALS AND METHODS

### Materials

$H_2O_2$–$H_2O$ solutions, 30:70 and 50:50 wt/wt, are purchased from Sigma Aldrich, USA. Concentrated $H_2O_2$–$H_2O$ solution, 80:20 wt/wt, is obtained by evaporating $H_2O$ from 50:50 wt/wt $H_2O_2$–$H_2O$ solution in vacuum at 25°C. Cellulose acetate (CA) non-woven mats are a donated by Celanese, USA.

### Methods

#### $CO_2$ assisted $H_2O_2$ impregnation

A high-pressure apparatus (Parr instruments, model 5500 with magnetic stirrer drive) is used for $CO_2$ assisted impregnation. CA mats, dried at 100°C for two hours, are loaded into a tea bag that is tied to the impeller shaft. $H_2O_2$–$H_2O$ solution is placed at the bottom of the vessel. The weight ratio of $H_2O_2$–$H_2O$ solution to CA mats is ten. Then the vessel is pressurized with $CO_2$ to 1200 ± 50 psig and temperature is maintained at 25°C using an externally mounted heating band. The system is maintained at these conditions for 60 minutes with vigorous mixing and then slowly depressurized over a period of 15 minutes. $H_2O_2$–$H_2O$ loading is determined by recording the CA mat weight before and after impregnation.

#### Characterization of $H_2O_2$–loaded CA mats

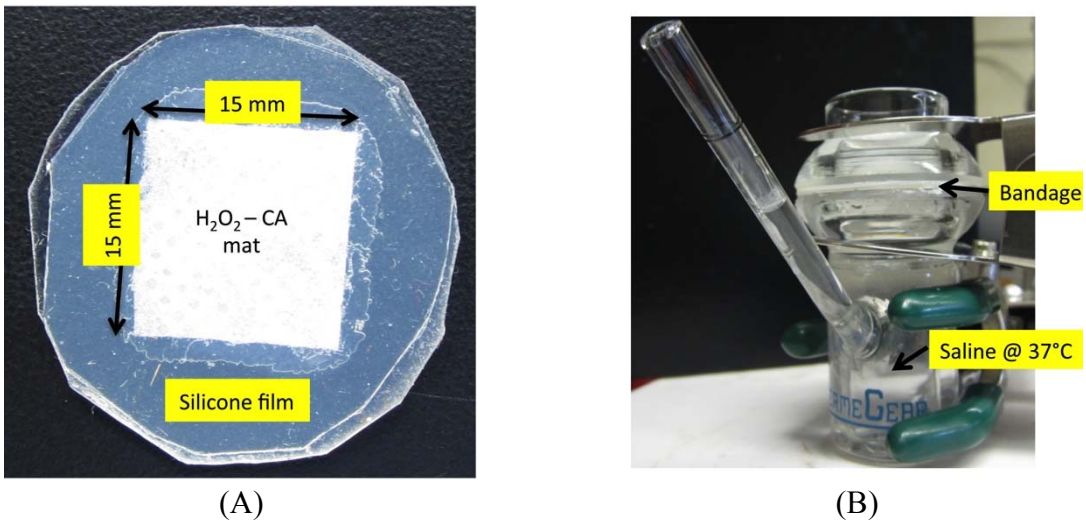
$H_2O_2$ is extracted from $H_2O_2$–loaded CA mats by suspending in 1:10 v/v sulfuric acid–distilled water solution for 15 minutes with moderate stirring. $H_2O_2$ concentration of the solution is determined using permanganate titration (HANNA Instruments, HI 902C, USA). The standard deviation of $H_2O_2$ concentration is ~2% as determined from three repeated titrations of representative $H_2O_2$–loaded CA mats.

The $H_2O_2$–$H_2O$ loading in the CA mats is determined using thermal gravimetric analysis (TGA) (Perkin–Elmer USA, Model Pyris 1 TGA). The furnace is continuously flushed with nitrogen gas at a flow of 3 L/hour. The $H_2O_2$–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.

The morphology of the CA mats is determined using scanning electron microscopy (SEM) (HITACHI SU-70). 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, USA, Model: Desk V TSC) onto the sample before capturing the images.

### *in vitro* $H_2O_2$ release studies

The bandages are made by placing $H_2O_2$–loaded CA mat between medical grade silicone films and Figure 1A shows the digital image of the bandage. A Franz cell is used to determine the *in vitro* $H_2O_2$ release kinetics from $H_2O_2$–loaded CA mats. 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 $H_2O_2$ release using permanganate titration.




(A) (B)

Figure 1. Digital image of the bandage made from $H_2O_2$–loaded CA mats and silicone films (A) and Franz cell experimental set up used for *in vitro* $H_2O_2$ release from bandages (B).

## RESULTS AND DISCUSSION

$CO_2$ assisted impregnation experiments are performed with $H_2O_2$–$H_2O$ (50:50 w/w) solution at a fixed pressure of 1200 psig and temperatures from 25 to 45 °C. The amount of $H_2O_2$–$H_2O$ used in all four experiments is approximately an order of magnitude higher than the saturation solubility of $H_2O_2$–$H_2O$ in $CO_2$. The calculations are based on the pure water solubility in high pressure $CO_2$, since the solubility of $H_2O_2$–$H_2O$ in high pressure $CO_2$ is not available in the literature. $H_2O_2$–$H_2O$–$CO_2$ phase behavior studies are in progress in our laboratories; data will be presented in the conference. Figure 2 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. 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 independent CA mats. The results suggest that lower temperatures are favorable for the $H_2O_2$ loading into CA mats using $CO_2$ process.

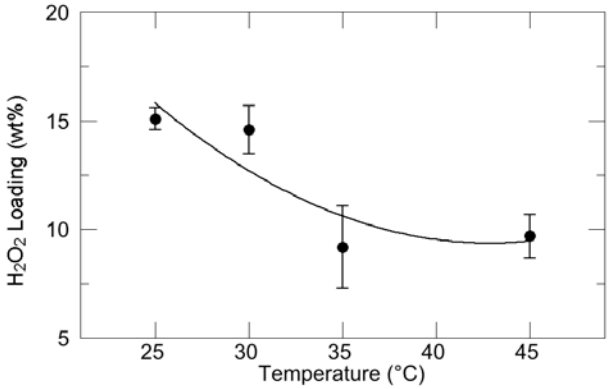



Figure 2. Effect of impregnation temperature on the $H_2O_2$ loading in CA mats at 1200 psig.

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 3, 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 overall conclusion is that $H_2O_2$ loading into CA mats can be tailored by manipulating the $CO_2$ assisted impregnation process parameters.

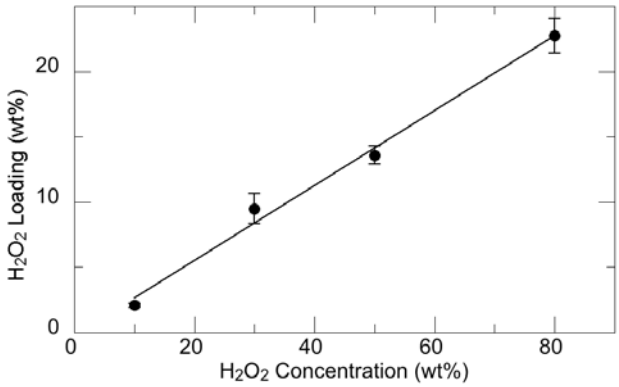
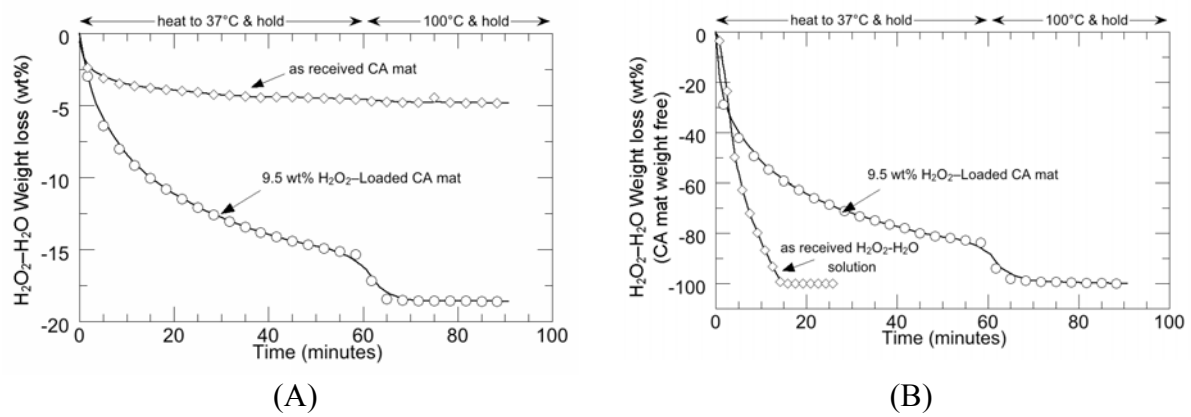



Figure 3. Effect of $H_2O_2$–$H_2O$ solution concentration, used for the $CO_2$ assisted impregnation, on the $H_2O_2$ loading in CA mats at 25°C and 1200 psig.

TGA analysis is used to determine the $H_2O_2$–$H_2O$ loading in the CA mats. Figure 4 shows the typical $H_2O_2$–$H_2O$ weight loss from the 9.5 wt% $H_2O_2$–loaded CA mats. Figure 4A compares the weight loss from the $H_2O_2$–loaded CA mats with that of the as-received CA mats. The results show that CA mats are loaded with ~19 wt% of $H_2O_2$–$H_2O$ whereas as received CA mat has ~5 wt% absorbed moisture or other volatiles. Figure 4B compares the weight loss from the $H_2O_2$–loaded CA mats with that of the as received $H_2O_2$–$H_2O$ (50:50 w/w) solution. The results show that $H_2O_2$–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 $H_2O_2$–$H_2O$ (50:50 w/w) solution evaporates within 15 minutes at this same temperature.




(A) (B)

Figure 4. TGA curves for (A) as received CA mat and 9.5 wt% $H_2O_2$ loaded CA mat (processed at 25°C and 1200 psig) and (B) as received $H_2O_2$–$H_2O$ (50:50 w/w) solution and 9.5 wt% $H_2O_2$ loaded CA mat on CA mat weight free basis.

Figure 5 shows the shelf-life of $H_2O_2$–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 and subsequently evaporates from the CA mats at a rate determined by the diffusion of $H_2O_2$ through CA mats. 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% $H_2O_2$–$H_2O$ solution shelf-life is significantly longer compared to the $H_2O_2$–loaded CA mats, which is due to the presence of $H_2O_2$ stabilizers in the solutions. Further studies are in progress to incorporate stabilizers into the CA mats along with the $H_2O_2$ to determine if the shelf life of the $H_2O_2$–loaded CA mats can be increased.

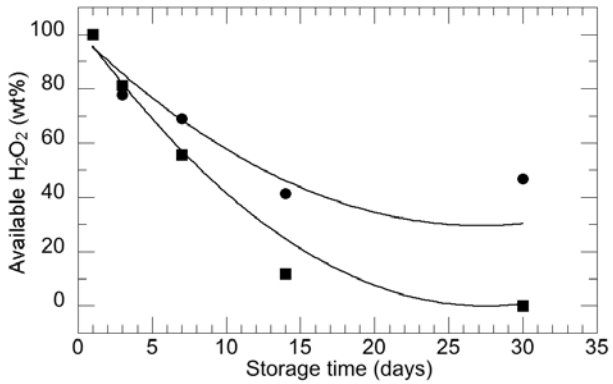
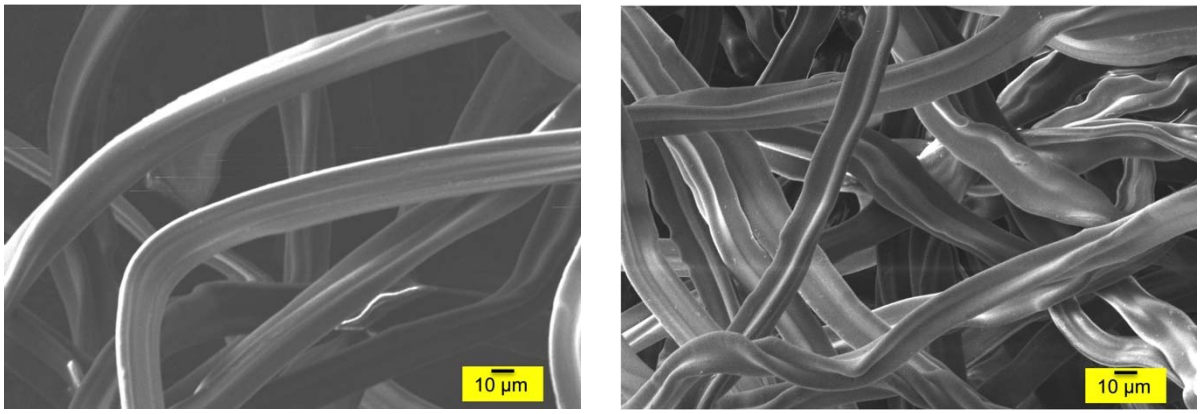



Figure 5. Storage stability of $H_2O_2$–loaded CA mats at 2-to-8°C (●) and 20-to-23°C (■).

Figure 6 shows SEM images of CA mats before and after $H_2O_2$ loading. The morphology of the $H_2O_2$–loaded CA mat (Figure 6B) looks similar to that of the virgin CA mat (Figure 6A), which indicates that the $CO_2$ assisted impregnation process has no effect on the

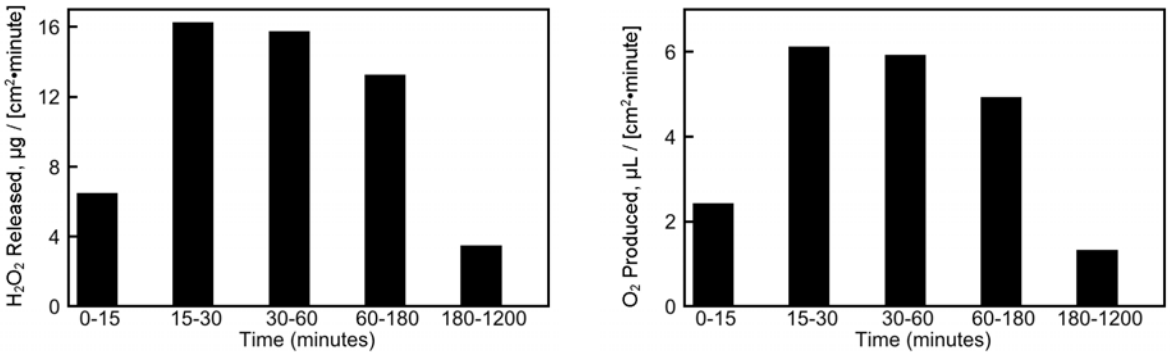
morphology of the mats. Note that CA mats lose their structural integrity if soaked directly in $H_2O_2$–$H_2O$ solutions at $H_2O_2$ concentrations greater than 30 wt%. Hence, $H_2O_2$ impregnation using a $CO_2$ assisted process offers a significant potential for creating novel materials for advanced wound care applications.




(A) (B)

Figure 6. SEM images of CA mats (A) as received and (B) ~22 wt% $H_2O_2$–loaded CA mat.

Figure 7 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 7B 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 ~ 0.25 µL per $cm^2$ of skin. 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.




(A) $H_2O_2$ release (B) $O_2$ Production

Figure 7. *in vitro* $H_2O_2$ release kinetics from the 22 wt% $H_2O_2$–loaded CA mat (15 mm X 15 mm, processed at 25 °C, 1200 psi, and 80:20 w/w $H_2O_2$–$H_2O$ solution used in impregnation) measured in saline at 37°C.

## CONCLUSION

In this study CA mats are successfully impregnated with $H_2O_2$ using sub and supercritical $CO_2$ at mild operating temperatures and pressures. The $H_2O_2$ loading in the CA mats can be tailored between 2-to-25 wt% by manipulating the impregnation process parameters. Maximum $H_2O_2$ loading into CA mats is achieved at temperatures of 25-to-30 °C, which is likely due to the higher partitioning of $H_2O_2$ from $CO_2$–rich phase to CA mats. Shelf-life studies show that storing the $H_2O_2$–loaded CA mats at 2-to-8 °C retains more than 50 wt% of the loaded $H_2O_2$ and thus storing in the refrigerator is preferred over room temperature. The $CO_2$ assisted impregnation process had no effect on the morphology of the CA mats. *in vitro* release studies reveal that sustained $H_2O_2$ release and, thus, $O_2$ production is achieved for 24 hours, using the bandages created from $H_2O_2$–loaded CA mats. We anticipate that these mats can find potential applications for advanced wound care.

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