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Rajendar R. Mallepally
Virginia Commonwealth University, rrmallepally@vcu.edu

Chance C. Parrish
Virginia Commonwealth University, parrishcc2@vcu.edu

Mark A. McHugh
Virginia Commonwealth University, mmchugh@vcu.edu

Kevin R. Ward
University of Michigan - Ann Arbor

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Hydrogen peroxide filled poly(methyl methacrylate) microcapsules: potential oxygen delivery materials

Rajendar R. Mallepally,*1 Chance C. Parrish1, Mark A. McHugh1 and Kevin R. Ward2,3

1 Department of Chemical and Life Science Engineering, Virginia Commonwealth University, Richmond, VA 23284, USA
2 Department of Emergency Medicine, University of Michigan, Ann Arbor, MI 48109, USA
3 Michigan Center for Integrative Research in Critical Care, University of Michigan, Ann Arbor, MI 48109, USA

Corresponding author: rrmallepally@vcu.edu

Abstract

This paper describes the synthesis of H2O2–H2O filled poly(methyl methacrylate) (PMMA) microcapsules as potential candidates for controlled O2 delivery. The microcapsules are prepared by a water-in-oil solvent emulsion and evaporation method. The results of this study describe the effect of process parameters on the characteristics of the microcapsules and on their in vitro performance. The size of the microcapsules, as determined from scanning electron microscopy, ranges from ~5 to 30 µm and the size distribution is narrow. The microcapsules exhibit an internal morphology with entrapped H2O2–H2O droplets randomly distributed in the PMMA continuous phase. In vitro release studies of 4.5 wt% H2O2 loaded microcapsules show that ~70% of the H2O2 releases in 24 hours. This corresponds to a total O2 production of ~12 cc per gram of dry microcapsules. Shelf-life studies show that the microcapsules retain ~84 wt% of the initially loaded H2O2 after nine months storage at 2-to-8 °C, which is an attractive feature for clinical applications.

Keywords: Microcapsules, Controlled release, H2O2 encapsulation, O2 delivery, and topical oxygenation

1. Introduction

Oxygen is essential for cellular metabolism and organ functioning and the lack of sufficient oxygen at the cellular level causes severe damage to cells and tissues (Tandara and Mustoe, 2004). Thus, sustained delivery of oxygen using chemical oxygen producing materials has significant importance in advanced health care situations, such as wound healing (Rodriguez et al., 2008; Sen, 2009) and tissue engineering (Harrison et al., 2007). H2O2 is a widely investigated chemical oxygen producing compound, since it decomposes into water and oxygen and it carries 47 wt% O2 per unit mass. In general, the H2O2 decomposition reaction rate is slow, however many catalysts accelerate this reaction rate, one among them is catalase which is in abundant amounts in blood. H2O2 almost instantaneously decomposes when contacted with catalase. Hence, the controlled release of H2O2 has the potential to be an effective method for sustained oxygen delivery in the presence of catalase. The most effective way to control the delivery of O2 is to encapsulate H2O2 within a biocompatible polymer matrix. Figure 1 shows a schematic diagram of the controlled oxygen production via this chemical oxygen delivery strategy. In this envisioned application, H2O2-loaded polymeric microcapsules are administered into the colon and H2O2 diffuses out of the microcapsules at a fixed rate and contacts catalase, which generates O2. Although the encapsulation of a drug in a polymer matrix provides an effective method to
control drug delivery, encapsulating aqueous solutions in a hydrocarbon-rich polymer presents major engineering challenges (Atkin et al., 2004) addressed in this present study.

Figure 1. Schematic diagram of controlled oxygen production via a chemical oxygen generation strategy.

A solvent emulsion and evaporation technique is a versatile encapsulation method that has been used to encapsulate a variety of compounds in a different polymeric carriers (Li et al., 2008; O'Donnell and McGinity, 1997; Rosca et al., 2004). The basic principle of an emulsion-based encapsulation process is to create an oil/water or water/oil emulsion, depending on the type of compound to be encapsulated, followed by solvent evaporation from the emulsion droplets, which results in the formation of microspheres or microcapsules. Although research has been reported on the preparation of oil-filled polymer microcapsules by various techniques (Berkland et al., 2007; Dowding et al., 2004, 2005; Yow and Routh, 2006), there is limited research on the preparation of water-filled polymer microcapsules (Atkin et al., 2004; Bean et al., 2012). A few research groups have reported on the encapsulation of H\textsubscript{2}O\textsubscript{2} in polymers (Ng et al., 2010; Stefanescu et al., 2011), however the microcapsules synthesized in these studies have very small amounts of encapsulated H\textsubscript{2}O\textsubscript{2} and quantitative H\textsubscript{2}O\textsubscript{2} release data are not presented in these studies.

In the present study, H\textsubscript{2}O\textsubscript{2}–H\textsubscript{2}O filled poly(methyl methacrylate) (PMMA) microcapsules are prepared by a solvent emulsion and evaporation method. PMMA is used because of its biocompatibility and non-toxicity and is also an FDA approved polymer for many biomedical applications, for example bone cements and dermal fillers (Lemperle et al., 1998; Nollenberger and Albers, 2013; Vollrath et al., 2012). In this study no attempt is made to prove the biocompatibility of the PMMA. Figure 2 shows a schematic diagram of the microencapsulation process where an emulsion is created and the subsequent evaporation of solvent from the droplets results in the formation of microcapsules. For this process to be effective the two primary constituents, H\textsubscript{2}O\textsubscript{2}–H\textsubscript{2}O solution and PMMA, must dissolve in a mutual, highly volatile solvent and the resultant single-phase solution must be immiscible with the continuous oil phase used in the evaporation step. Acetone is chosen as the mutual solvent following the previous work of Atkin et al. (Atkin et al., 2004). The acetone-rich phase consists of PMMA, H\textsubscript{2}O\textsubscript{2}–H\textsubscript{2}O solution, acetonitrile, and acetone at weight ratios of 1:1:1:22, respectively. In this instance acetonitrile serves two purposes as it minimizes both microcapsules agglomeration and decomposition of H\textsubscript{2}O\textsubscript{2} (Kim, 1979). Once the acetone-rich phase is emulsified into an oil phase containing a surfactant, both acetone and acetonitrile evaporate from the emulsion droplets causing the PMMA to vitrify and subsequently trap H\textsubscript{2}O\textsubscript{2}–H\textsubscript{2}O solution in the PMMA matrix. This study reports the preparation and performance characteristics of H\textsubscript{2}O\textsubscript{2}-loaded poly(methyl methacrylate) (PMMA) microcapsules as effective controlled oxygen producing materials. The effect of different process parameters are systematically investigated to demonstrate the impact
of these parameters on the characteristics and performance of the microcapsules. Data are presented on the \( \text{H}_2\text{O}_2 \) loading, in vitro \( \text{H}_2\text{O}_2 \) release rate, \( \text{O}_2 \) production rate, and shelf-life of the PMMA microcapsules synthesized in this study.

![Microcapsules preparation process](image)

Figure 2. Schematic representation of microcapsules preparation process used in the present study.

2. Materials and Methods
2.1 Materials
PMMA with weight average molecular weights (\( M_w \)) of 15,000, 96,000, 350,000, and 996,000 g/mol) are purchased from Sigma Aldrich, USA and used as received. Hydrogen peroxide (50 wt\%) in water solution is purchased from Sigma Aldrich, USA and is concentrated to 80 wt\% by evaporating water in vacuum. Acetone, acetonitrile, hexane, hexadecane, and dichloromethane are purchased from Fischer Scientific, USA and used as received. Croda Inc. USA generously donated the dipolyhydroxystearate (DPHS) surfactant used in these studies and Span 80, the other surfactant investigated in these studies, is purchased from Sigma Aldrich, USA.

2.2. Microcapsules preparation
The \( \text{H}_2\text{O}_2-\text{H}_2\text{O} \) solution with 80:20 (w/w) ratio is used in the entire study, except in section 3.3 where microcapsules are prepared using 50:50 (w/w) \( \text{H}_2\text{O}_2-\text{H}_2\text{O} \) solution. Briefly, PMMA (4 wt\%) and \( \text{H}_2\text{O}_2-\text{H}_2\text{O} \) solution (4 wt\%) are dissolved in a mixture of (22:1 w/w) acetone and acetonitrile. This single-phase solution is emulsified (high shear homogenizer, IKA USA, model T25 Ultra-Turrax) into mineral oil containing a surfactant. The ratio of acetone-rich phase to oil-rich phase is 1:4 w/w. The acetone and acetonitrile are then evaporated by continuously stirring the emulsion for \(~18\) hours. The resultant microcapsules are separated from the mineral oil-rich phase by centrifuging (Fischer Scientific, USA, model: AccuSpin™ 400) at 3000 rpm for 15 minutes and the recovered microcapsules are washed twice with hexane. The microcapsules are dried at room temperature in air and are stored at 2-to-8 °C until use.

2.3. Microcapsules characterization
The morphology of the microcapsules is determined using scanning electron microscopy (SEM) (HITACHI SU-70). Dry microcapsules are spread on a sticky carbon tape glued to a one-inch aluminum SEM stub and then a 10 nm platinum coating is applied via spun coat (Denton Vacuum, LLC, USA, Model: Desk V TSC) onto the sample.
The $\text{H}_2\text{O}_2$–$\text{H}_2\text{O}$ loading in the microcapsules 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 microcapsules are heated at a rate of 10 °C/minute to 120 °C and held at this temperature for two hours. The microcapsules are then quickly heated to 200 °C at a heating rate of 100 °C/minute and held at this temperature for 30 minutes.

$\text{H}_2\text{O}_2$ loading in the microcapsules is determined using permanganate titration (Automatic potentiometric titration system, HANNA Instruments, HI 902C, USA). Approximately 50 mg of microcapsules are dissolved in dichloromethane and distilled water is then added to the solution to extract the $\text{H}_2\text{O}_2$. The recovered aqueous phase is assayed for $\text{H}_2\text{O}_2$ concentration. The standard deviation of $\text{H}_2\text{O}_2$ concentration is ~2% as determined from three repeated titrations of representative $\text{H}_2\text{O}_2$ solutions.

2.4. In vitro $\text{H}_2\text{O}_2$ release studies
Six filter paper bags, each loaded with ~50 mg of microcapsules, are immersed in saline solution, at 37°C, located in separate 20 mL screw-capped glass bottles. A bench top incubator, set at 120 rpm and 37°C, is used to continuously shake the bottles. At a specific time a filter bag is removed from a given bottle and the amount of peroxide in the saline solution is determined by permanganate titration as mentioned in section 2.3.

3. Results and Discussion
3.1. Effect of continuous phase
Hexadecane and mineral oil are selected to study the effect of the continuous phase on the characteristics of the microcapsules since an acetone-rich phase is immiscible with both oils. Microcapsules are prepared using DPHS–mineral oil and using DPHS–hexadecane as the continuous phases, keeping all other parameters constant. In both cases the concentration of DPHS in the oil phase is 3 wt% based on the oil-phase weight. Figure 3 shows the SEM images of the microcapsules prepared from both continuous phases. Although the maximum microcapsule size is ~10 µm in both cases, the microcapsules created in mineral oil have a narrow size distribution that ranges from 5 to 10 µm (Figure 3a), whereas the microcapsules created in hexadecane have a broad size distribution that ranges from 1 to 10 µm (Figure 3b). In the emulsification process the droplet formation step determines the size and size distribution of the resulting microcapsules (Lam et al., 1996; Maa and Hsu, 1996). Droplets of acetone-rich phase emulsified in a continuous oil phase undergo a disruptive stress, that causes droplet break-up, and a cohesive stress, that stabilizes the droplet. The disruptive stress depends on the flow regime or fluid motion and the cohesive stress depends on the interfacial tension. A stable droplet size results from the balance between disruptive and cohesive stresses (Mu et al., 2005). The interfacial tension is expected to be equal for mineral oil or hexadecane as the continuous phase, since both are hydrocarbons with an approximate surface tension of 28 mN/m (Jones and Wedeven, 1971; Rolo et al., 2002). Hence, the disruptive stresses play a significant role in fixing droplet size. Disruptive stresses depend on the flow regime that is fixed by the Reynolds number ($Re$), which quantifies the importance of inertial forces relative to viscous forces. $Re$ numbers are calculated using equation (1)
\[ Re = \frac{\rho_c N D^2}{\mu_c} \]  

where \( \rho_c \) and \( \mu_c \) are the density and viscosity of the continuous phase, respectively, \( N \) is the homogenization speed, and \( D \) is the impeller diameter. In a batch stirred tank reactor an \( Re \) number less than 10,000 indicates laminar flow and a number greater than 10,000 indicates turbulent flow. Values of the \( Re \) numbers listed in Table 1 show that the emulsification process is in the laminar regime when mineral oil is used and the turbulent regime when hexadecane is used. In both cases ~4 wt% H\(_2\)O\(_2\) is loaded into the microcapsules, which indicates that the continuous phase has no significant effect on H\(_2\)O\(_2\) loading. Turbulent mixing occurs when hexadecane is used and a broad microcapsules size distribution is obtained due to the fluctuations inherent with this type of mixing. Laminar mixing occurs when mineral oil is used and a narrow size distribution of microcapsules is obtained. Mineral oil is used as the continuous phase in all further microcapsule preparations reported in this study.

<table>
<thead>
<tr>
<th></th>
<th>Viscosity (mPa*s)</th>
<th>Density (kg/m(^3))</th>
<th>( Re^c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral oil</td>
<td>15.0(^a)</td>
<td>890(^a)</td>
<td>6127</td>
</tr>
<tr>
<td>Hexadecane</td>
<td>3.0(^b)</td>
<td>770(^b)</td>
<td>26238</td>
</tr>
</tbody>
</table>

\(^a\) taken from ref. (Mu et al., 2005), \(^b\) Dortmund data bank, \(^c\) calculated at \( N = 10,000 \) rpm and \( D = 0.025 \) m using equation 1.

Figure 3. Scanning electron microscope images of H\(_2\)O\(_2\) loaded PMMA microcaps prepared using mineral oil (a) and hexadecane (b) as continuous phases.

3.2. Effect of surfactant on the emulsion stability
The surfactant plays a significant role in the stability of the emulsion (Tadros, 2006). Two separate acetone-in-mineral oil emulsions are created using DPHS (3 wt%) and Span 80 (9 wt%). Note that it is not possible to create a stable emulsion with a concentration below 9 wt% Span 80. Figure 4a shows the images of the two emulsions after one day and 15 days of preparation. The emulsion created using DPHS remains stable as compared to the emulsion created using
Span 80. Figure 4b shows a schematic diagram of the orientation of the two surfactants at the oil/water interface. DPHS has two hydrophobic alkane tails and a bulky ethylene oxide hydrophilic head, which makes DPHS a strong emulsion stabilizer compared to Span 80, which has only one hydrophobic alkane tail and a small hydrophilic head (Tadros, 2006). DPHS is used as the surfactant in all further microcapsule preparations reported in this study.

![Figure 4b](image)

Figure 4. Emulsion stability using DHPS and Span 80 as the surfactants (a) A refers to the mineral oil rich phase and B refers to the acetone rich phase and (b) surfactant orientation at the oil-water interface (Anonymous, 2011).

3.3. Effect of PMMA molecular weight
The effect of PMMA molecular weight (M_w) on the characteristics of the microcapsules is investigated using four different M_w polymers under identical processing conditions. All the four batches of microcapsules are prepared using 50:50 (w/w) H_2O_2–H_2O solution. Table 2 lists the characteristic microcapsules size and H_2O_2 loading from these experiments and Figure 5 shows the SEM images of the microcapsules. As the M_w of PMMA increases from 15,000 g/mol to 996,000 g/mol, the maximum microcapsule size increases from 10 µm to 40 µm and it is evident that the size distribution also increases. As the M_w of PMMA increases the viscosity of the acetone-rich phase is expected to increase (Bueche, 1955; Kulicke and Kniewske, 1984) resulting in large viscous stresses that resist droplet break-up and consequently result in larger-size microcapsules. Yang et al. report similar results where microparticle size increases as the molecular weight of the polymer increases (Yang et al., 2001). As noted, the microcapsule size distribution is significantly narrower when lower molecular weight PMMS is used. All four batches of microcapsules are made at 10,000 rpm homogenization speed and the induced shear stresses at this speed are likely only sufficient to create homogeneous droplets when the lowest M_w PMMA is used.

![Figure 5](image)

**Table 2. Influence of PMMA molecular weight on the microcaps characteristics.**

<table>
<thead>
<tr>
<th>PMMA Molecular Weight (g/mol)</th>
<th>Microcapsule size (µm)</th>
<th>H_2O_2 loading (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15,000</td>
<td>2–10</td>
<td>2.4</td>
</tr>
<tr>
<td>96,700</td>
<td>5–15</td>
<td>4.0</td>
</tr>
<tr>
<td>350,000</td>
<td>5–25</td>
<td>4.0</td>
</tr>
<tr>
<td>996,000</td>
<td>5–40</td>
<td>7.0</td>
</tr>
</tbody>
</table>

*a* as observed in SEM images; *b* determined from permanganate titration
The data in Table 2 also show that H$_2$O$_2$ loading in the microcapsules increases as the M$_w$ of PMMA increases. The higher loading is likely due to the slower diffusion of H$_2$O$_2$ molecules through the acetone-rich phase containing high M$_w$ PMMA. These results are consistent with literature studies reporting that drug loading increases as the molecular weight of the polymer increases (Li et al., 2008). Hence, the M$_w$ of PMMA can be used as a controlling factor to tailor microcapsule size and H$_2$O$_2$ loading.

3.4. Effect of homogenization speed and homogenization time

SEM images in Figure 6 show the change in microcapsules size when the homogenization speed is increased from 10,000 to 24,000 rpm and the homogenization time is increased from 5 to 15 minutes. Three batches of microcapsules are prepared using 15,000 g/mol M$_w$ of PMMA and different homogenization speeds and homogenization times. When homogenizing at 24,000 rpm the emulsion temperature rises from ~25 to ~50 °C, due to the high mechanical energy input into the system. As a result of this temperature increase, acetone and acetonitrile evaporate quickly from the emulsion droplets, which leads to faster formation of microcapsules. Therefore, to avoid any process-induced differences to the microcapsules during homogenization, the temperature of the mineral oil-rich phase is maintained at ~25°C, the same temperature used when
homogenizing at 10,000 rpm. Figure 6a and 6b show the maximum microcapsules size is ~10 µm and size distribution are very similar when homogenizing at 10,000 and 24,000 rpm. At a fixed homogenization speed of 24,000 rpm, increasing the homogenization time from 5 minutes to 15 minutes (Figure 6b and 6c) has no significant effect on the size and size distribution of the microcapsules. However, Figure 6c shows broken microcapsules are obtained likely due to the rupture of hardened microcapsules at this elevated rpm and the longer homogenization time. For all three conditions, the H₂O₂ loading is 4.3-to-4.5 wt%, indicating that the H₂O₂ loading is not affected by these process parameters. These results show that low rpm rates and relatively short processing times are sufficient to create reasonably monodisperse H₂O₂-loaded PMMA microcapsules. The average microcapsule size obtained in this study is an order of magnitude smaller than the average size of H₂O₂–loaded poly(lactic-co-glycolic acid) (PLGA) microspheres reported by Ng et al (Ng et al., 2010), where the size ranges from 25 to 250 µm.

![SEM images of H₂O₂–H₂O loaded PMMA microcapsules at different processing conditions and with 15,000 g/mol PMMA.](image)

3.5. Microcapsules internal morphology

Figure 7a shows an example of the external morphology of typical H₂O₂–H₂O loaded PMMA microcapsules obtained in this study. The image in Figure 7b reveals an internal morphology with 1-2 µm diameter H₂O₂–H₂O droplets randomly distributed in the PMMA "continuous phase". Since the PMMA concentration in the acetone-rich phase is near the saturation limit, PMMA precipitates rapidly as acetone starts to evaporate from the emulsion droplets. Randomly
dispersed H₂O₂–H₂O droplets do not have sufficient time to coalesce and form a single core given the high rigidity of vitrified PMMA, which has glass transition temperature is ~105°C (Atkin et al., 2004). In a typical batch production of microcapsules, it is not unexpected that a few microcapsules are formed in which randomly dispersed H₂O₂–H₂O droplets coalesce into a single H₂O₂–H₂O core surrounded by a PMMA shell as seen in Figure 7c. However, this single core-shell microcapsule morphology is expected to be in the minority relative to the multi-H₂O₂–H₂O droplet morphology shown in Figure 7b.

![Figure 7](image-url)

**Figure 7.** SEM images of H₂O₂–H₂O loaded PMMA microcapsules synthesized in this study; (a) typical microcapsule close view, (b) inside view of microcapsules with multiple H₂O₂–H₂O cores, and (c) a microcapsule with a single, H₂O₂–H₂O core.

### 3.6. Thermal analysis of microcapsules

When TGA analysis is used to determine the amount of H₂O₂–H₂O loading in the microcapsules it is important to distinguish H₂O₂–H₂O from H₂O₂ loading, where H₂O₂ loading is determined using permanganate titration (see section 2.3). Figure 8 shows a typical H₂O₂–H₂O weight loss curve for microcapsules loaded with 4.5 wt% of H₂O₂. Figure 8a compares the H₂O₂–H₂O weight loss from microcapsules with that of as-received PMMA showing that ~1.4 wt% of absorbed moisture or other volatiles are present in the as-received PMMA. The microcapsules are loaded with 8.4 wt% of H₂O₂–H₂O, which is obtained by subtracting the weight loss of as-received PMMA from the total weight loss of microcapsules. Note that PMMA thermally...
degrades at temperatures higher than 200 °C (Kandare et al., 2006) and therefore, the weight loss up to 120 °C is only considered as H$_2$O$_2$–H$_2$O loss.

Figure 8b compares the H$_2$O$_2$–H$_2$O weight loss from microcapsules with that of as-received H$_2$O$_2$–H$_2$O (50:50 w/w) solution. H$_2$O$_2$–H$_2$O solution evaporates within 10 minutes even before the sample reaches 120 °C, whereas the microcapsules only lose ~20% of available H$_2$O$_2$–H$_2$O over the same time period. This observation reinforces the conclusion that H$_2$O$_2$–H$_2$O solution is predominantly encapsulated within the PMMA matrix, in agreement with the SEM micrographs shown in Figure 7b. Once the temperature reaches 120 °C, which is slightly above the PMMA glass transition temperature of ~105 °C, PMMA exhibits rubbery behavior and H$_2$O$_2$–H$_2$O more rapidly diffuses from the polymer. In 30 minutes the microcapsules lose ~85% of available H$_2$O$_2$–H$_2$O and the remaining 15% loss occurs in 90 minutes as the sample is held at 120 °C. The slow diffusion of H$_2$O$_2$ and H$_2$O is likely due to hydrogen bonding between each of these compounds and the carbonyl (–C=O) groups of PMMA (Chen et al., 2005; Panarin et al., 2001). Further evidence of the hydrogen bonding character of H$_2$O$_2$ is detailed in literature reports of H$_2$O$_2$ forming strong hydrogen bonds with the oxygen of a siloxane, an Si-O-Si group (Żegliński et al., 2006), and with the carbonyl oxygen of polyvinylpyrrolidone (Panarin et al., 2001).

Figure 8. Weight loss from H$_2$O$_2$–H$_2$O loaded PMMA microcapsules and H$_2$O$_2$–H$_2$O (50:50 w/w) solution; (a) total weight basis, (○) 4.5 wt% H$_2$O$_2$ loading and (◇) as received PMMA which shows moisture loss, (b) PMMA-free basis, (○) 4.5 wt% H$_2$O$_2$ loading and (◇) H$_2$O$_2$–H$_2$O (50:50 w/w) solution. The heating rate is 10 °C per minute up to 120°C.

3.7. Shelf-life of H$_2$O$_2$–H$_2$O loaded PMMA microcapsules
The loss of H$_2$O$_2$ from the microcapsules occurs either by decomposition or diffusion to the microcapsule surface followed by evaporation. H$_2$O$_2$ is more stable at low temperatures (Żegliński et al., 2007), therefore, the microcapsules are stored at 2- to-8 °C and the shelf-life is determined by measuring H$_2$O$_2$ content for periods of up to nine months. Figure 9 shows the H$_2$O$_2$ loss on a PMMA-free basis during a nine month storage period. The results show that ~84% of loaded H$_2$O$_2$ is available after nine months of storage. The long shelf-life of these microcapsules is an attractive feature for clinical applications.
Figure 9. Available H$_2$O$_2$ in the 4.5 wt% H$_2$O$_2$–loaded PMMA microcapsules when stored at 2- to-8°C.

3.8 *in vitro* H$_2$O$_2$ release studies

Figure 10 compares the H$_2$O$_2$ release rate and corresponding calculated O$_2$ production rate from the H$_2$O$_2$–H$_2$O loaded PMMA microcapsules synthesized at 10,000 and 24,000 rpm using 15,000 g/mol PMMA. The H$_2$O$_2$ release profile in Figure 10a is biphasic with an initial fast release rate up to 60 minutes followed by a slower release rate from 60 minutes to 24 hours. In the first 60 minutes the H$_2$O$_2$ release follows zero-order kinetics with no initial burst, which indicates that H$_2$O$_2$ is encapsulated in the PMMA matrix and the release is a diffusion controlled process. The internal morphology of a microcapsules (Figure 7b) indicates that there is a distribution of diffusion path lengths, which results in a "time averaged" delivery of H$_2$O$_2$ to the solution surrounding the microcapsules. In 24 hours, ~70% of available H$_2$O$_2$ releases from the microcapsules. The O$_2$ production in Figure 10b is calculated assuming one-half mole of O$_2$ is created from the decomposition of H$_2$O$_2$ and using the ideal gas equation at 37 °C and 1 atm. The data in Figure 10b show that ~12 cc of cumulative O$_2$ is produced over a 24 hour period from a gram of dry microcapsules, regardless of the homogenization speed used to create the microcapsules. Although not shown here, the release of H$_2$O$_2$ and, hence, the production of O$_2$ are essentially identical when the homogenization time is increased from five to 15 minutes at both 10,000 and 24,000 rpm homogenization speeds. Hence, either increasing the homogenization speed or homogenization time does not have a significant effect on the size of the microcapsules, the H$_2$O$_2$ release rate, or the O$_2$ production rate.

H$_2$O$_2$–loaded PMMA microcapsules synthesized in the present study have two advantages as compared to the previously reported H$_2$O$_2$–loaded PLGA microspheres (Ng et al., 2010). The total amount of O$_2$ produced is one to two orders of magnitude higher than the O$_2$ production reported by Ng et al. (Ng et al., 2010). The microcapsules of present study produce sustained O$_2$ for 24 hours whereas the microspheres produced by Ng et al. (Ng et al., 2010) last for 4 hours. The microcapsules in the present study are candidate materials for topical oxygenation, needed for wound healing, given the available O$_2$ and its release for long periods of time. Note that the *in vitro* cytotoxicity and *in vivo* performance of the H$_2$O$_2$–loaded PMMA microcapsules are not reported in this study. However, there are literature studies reporting the application of biomaterials loaded with peroxide containing compounds which, in most cases, utilize a catalyst to convert the peroxide to oxygen. These studies show that the H$_2$O$_2$ containing materials when used in combination with the peroxide decomposition catalyst are biocompatible.
and non-toxic to cells and tissues (Camci-Unal et al., 2013; Harrison et al., 2007; Li et al., 2012; Oh et al., 2009; Wang et al., 2010).

Figure 10. Performance of H$_2$O$_2$–H$_2$O loaded PMMA microcapsules, at 37 °C, synthesized using different homogenization speeds (▲) 10,000 rpm and (●) 24,000 rpm, both with 15,000 g/mol PMMA and a homogenization time of five minutes.

Figure 11 compares the H$_2$O$_2$ release rate and corresponding O$_2$ production rate from the 4.5 wt% H$_2$O$_2$–loaded PMMA microcapsules after one day and after six months of storage at 2-to-8 °C. After six months of storage, the rate of H$_2$O$_2$ release in the first 60 minutes is equivalent to that observed with freshly prepared microcapsules indicating storage time has no effect on the H$_2$O$_2$ release in this initial time period. However, the 24 hour, cumulative release of H$_2$O$_2$ is decreased by 10% after six months of storage. During storage, H$_2$O$_2$ slowly diffuses through the PMMA continuous phase and, as a result, more hydrogen bonds can be formed between H$_2$O$_2$ and the carbonyl groups of PMMA. The decrease in cumulative H$_2$O$_2$ release is likely due to the increase in number of H$_2$O$_2$–PMMA hydrogen bonds. Figure 11b shows the total O$_2$ production is decreased by ~20% after six months storage due to the combined effect of an ~11% storage loss of H$_2$O$_2$ and a 10% lower cumulative release.

Figure 11. Effect of storage time on the H$_2$O$_2$ release rate at 37 °C from the H$_2$O$_2$–H$_2$O loaded PMMA microcapsules after one day (●) and after six months (▲) of storage at 2-to-8°C.
4. Conclusions
We have successfully synthesized H$_2$O$_2$–loaded PMMA microcapsules. The microcapsules size and size distribution are independent of homogenization speed and time for a given molecular weight PMMA. Microcapsules size and H$_2$O$_2$ loading increases as the molecular weight of the PMMA increases. Controlled production of O$_2$ is achievable with H$_2$O$_2$–loaded PMMA microcapsules that can be used for systemic and topical oxygenation for a period of 24 hours. A gram of dry microcapsules produces a total of 10-to-12 cc of O$_2$ in 24 hours. This O$_2$ delivery rate is approximately one to two orders of magnitude higher than the typical skin O$_2$ consumption (Roe et al., 2010). The H$_2$O$_2$–loaded PMMA microcapsules also have potential applications as anti-microbial agents. Animal studies are in progress to assess the in vivo performance of these microcapsules and those results will be published elsewhere.

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