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Influence of storage temperature and moisture on the performance of microsphere/hydrogel composites

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ABSTRACT

The current study involved investigation of the effect of storage temperature and moisture on the performance of poly(lactide-co-glycolide) (PLGA) microsphere/poly(vinyl-alcohol) (PVA) hydrogel composites. Physical aging occurred in composites stored at 25 °C due to structural relaxation. The glass transition temperature (Tg) and enthalpy of relaxation of the composites increased leading to a slower cumulative % release. The Tg of composites incubated at 40 °C, 75% RH decreased significantly due to the plasticization effect of absorbed water, whereas no change was observed in the Tg of microspheres alone; indicating that the hydrogel component enhanced water absorption. PLGA degradation occurred leading to significantly faster dexamethasone release following incubation at 40 °C, 75% RH for 1 month. No significant change was observed in the *in vitro* release profiles of composites after 6 months storage at 25 °C, 60% RH, however, release was accelerated following 12 months storage. Accordingly, exposure of the composites to ambient temperature/moisture during storage, shipping or handling may cause physical aging, plasticization, and degradation and hence, their performance may be affected. The extent to which the performance of the composite is affected by storage temperature and moisture is a net effect of physical aging and moisture induced plasticization/hydrolytic degradation.

1. Introduction

Hydrogel/microsphere composite formulations have been investigated as a new type of drug delivery system as these formulations possess the advantages offered by both components. The microspheres provide sustained and controlled drug release, whereas the hydrogels significantly improve the biocompatibility of the system. These composite formulations have been utilized to delivery both small and large molecules locally as well as systematically (Lee and Lee, 2009; Lee and Tan, 2009; Chen and Liu, 2012; Shin and Lee, 2013). They have also been investigated as coating materials for implantable biosensors to enhance their *in vivo* functionality (Patil and Papadmitrakopoulos, 2007; Bhardwaj and Sura, 2010; Wang and Papadimitrakopoulos, 2013). Poly (lactic-co-glycolic) acid (PLGA) polymers are the most commonly used polymers to prepare microsphere formulations as their safety has been well documented and they are capable of providing various drug release profiles. As for the hydrogel component, various materials have been investigated, such as poly (vinyl-alcohol) (PVA), and alginate (Patil and Papadmitrakopoulos, 2007; Lee and Tan, 2009; Bhardwaj and Sura, 2010; Wang and Papadimitrakopoulos, 2013). Despite the fact that many studies have been conducted on the biological, physical and chemical properties of PLGA microsphere/hydrogel composite formulations, limited information has been reported on the effect of storage temperature and moisture on the performance of these polymeric composite formulations.

In general, PLGA microspheres are mostly prepared *via* the solvent evaporation/extraction method. In this method, the polymer is dissolved in an organic solvent followed by evaporation or extraction of the organic solvent into an external aqueous phase resulting in solidification of the polymer particles. PLGA microspheres produced by solvent removal (solvent quenching) is analogous to thermal quenching of polymers from the molten state, which results in polymeric matrices with higher structural energy and lower density compared to the equilibrium state. The glass transition temperature (Tg) of various PLGA polymers used for microsphere formulations is usually in the range of 40–60 °C. Accordingly, the microsphere components are in the glassy state at the temperatures encountered during processing, and storage and tend to relax toward a lower equilibrium energy state over time. This polymer chain relaxation is known as physical aging or structure relaxation. Physical aging is characterized by an endothermic peak during thermal scanning, which is also referred to as the 'overshoot' or 'overheating' peak (Rouse and Mohamed, 2007; Rawat and

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Burgess, 2011). The area of this peak (the enthalpy of relaxation) indicates how the storage conditions have affected the material. Physical aging leads to increased polymer stability *via* a reduction in the free volume fraction and thereby an increase in density, which may decrease water penetration (Liggins and Burt, 2004; Rouse and Mohamed, 2007; Allison, 2008; Rawat and Burgess, 2011). These changes in density and free volume will affect drug release profiles. In addition to potential physical aging during storage at temperatures below the Tg of the composites, the presence of moisture may induce chemical degradation of PLGA microspheres. Although PLGA polymers are insoluble in water, they degrade *via* hydrolytic attack of their ester bonds (Zolnik and Burgess, 2007). Accordingly, dispersion of microspheres inside a hydrogel matrix will significantly enhance water absorption and hence microsphere degradation.

In the present work, changes in the Tg, enthalpy of relaxation and molecular weight associated with storage of PLGA microspheres/PVA hydrogel composite systems were determined under various temperature and humidity conditions. The effect of storage temperature and moisture on the *in vitro* release profile of dexamethasone-loaded PLGA microspheres/PVA hydrogel composites was also investigated. This work was performed with the objective of obtaining an understanding of the influence of storage temperature and moisture on the performance of PLGA microsphere/hydrogel combination systems as well as to select appropriate storage conditions for these composite formulations.

2. Materials and methods

2.1. Materials

Dexamethasone, poly(vinyl alcohol) (PVA, Mw 30–70 kDa), sodium chloride (ACS grade) and sodium azide were purchased from Sigma–Aldrich (St. Louis, MO). PVA (99% hydrolyzed, Mw 133 kDa) was purchased from Polysciences, Inc. (Warrington, PA). PLGA Resomer® RG503H (inherent viscosity 0.32–0.44 dl/g) and PLGA 503H was a gift from Boehringer-Ingelheim. Methylene chloride, sodium mono-hydrogen phosphate (ACS grade), acetonitrile (ACN, HPLC grade), dimethyl sulfoxide (DMSO, ACS grade) and tetrahydrofuran (THF, HPLC grade) were purchased from Fisher Scientific (Pittsburghm, PA). Disodium hydrogen phosphate (ACS grade) was purchased from VWR International. NanopureTM quality water (Barnstead, Dubuque, IA) was used for all studies.

2.2. Methods

2.2.1. Preparation of PLGA microsphere/PVA hydrogel composite films

2.2.1.1. Preparation of PVA solutions. PVA (99% hydrolyzed, Mw 133 kDa) aqueous solution (5%, w/v) was prepared *via* mixing polymer powder (1 g) with distilled water (20 ml). The mixture was heated to 80 °C for approximately 45 min, while slowly stirring. When the entire polymer was dissolved and the mixture become clear, the solution was cooled back to room temperature and kept overnight to remove all the air bubbles as well as allow the dissolved polymer to reach equilibrium.

2.2.1.2. Preparation of PLGA microspheres. Dexamethasone loaded PLGA microspheres were prepared using an oil-in-water emulsion solvent extraction/evaporation technique as described previously (Hickey and Kreutzer, 2002; Zolnik and Burgess, 2008; Rawat and Burgess, 2010). Briefly, 2 g PLGA were dissolved in 8 ml methylene chloride and 200 mg of dexamethasone were then dispersed in this solution. This organic phase was emulsified in 40 ml of a 1% (w/w) PVA (average Mw 30–70 kDa) solution and homogenized at 10,000 rpm for 1.5 min using a Power Gen 700D Homogenizer (Fisher Scientific, Pittsburg, Pennsylvania). The resultant emulsion was poured into 500 ml of a 0.1% (w/w) PVA (average Mw 30–70 kDa) solution and stirred under vacuum to achieve rapid evaporation of methylene chloride. The hardened microspheres were washed three times with de-ionized water and collected *via* filtration (0.45 μm). The prepared microspheres were kept under vacuum overnight and later stored at 4 °C until further use.

2.2.1.3. Preparation of PLGA microsphere/PVA hydrogel composite films. The PLGA microspheres/PVA hydrogel composite films were prepared by dispersing PLGA microspheres into PVA hydrogel (5%, w/v) solution, then this suspension was filled into a pre-made mold (15 mm × 30 mm × 2 mm) and subjected to three freeze–thaw cycles of 2 h freezing at −20 °C followed by 1 h thawing at 25 °C.

2.2.2. In vitro stability investigation of PLGA microsphere/PVA hydrogel composites

2.2.2.1. Storage conditions. The investigated storage conditions were: (1) 40 °C and ambient humidity; (2) 25 °C and ambient humidity; (3) 40 °**C** and 75 ± 2% relative humidity (RH); (4) 25 °C and 75 ± 2% RH; (5) 25 °C and 60 ± 2% RH; and (6) and under refrigeration and freezing conditions, 4 and −20 °C, respectively.

2.2.2.2. Dexamethasone storage stability. Dexamethasone storage stability was determined through high performance liquid chromatography (HPLC) analysis. 5 mg of the PLGA/PVA composites were first dissolved in 1 ml DMSO followed by dilution using THF to 10 ml. The dexamethasone concentration was determined using HPLC. A Perkin Elmer HPLC system (series 200) with a UV absorbance detector (Perkin Elmer, Shelton, CT) set at 240 nm was used. The mobile phase consisted of acetonitrile/water/phosphoric acid (30/69.5/0.5, v/v/v). A Perkin Elmer C18 (4.6 mm × 15 mm) analytical column was used with the flow rate set at 1 ml/min. The chromatographs were analyzed using a PeakSimple™ Chromatography System (SRI instruments, Torrance, CA).

2.2.2.3. Gel permeation chromatography. The molecular weight of the PLGA polymer was determined by gel permeation chromatography (GPC; Waters) with an evaporative light scattering detector (ELSD). The mobile phase was THF with a flow rate of 2 ml/min at 40 °C. 5 mg of the composites were dissolved in 1 ml DMSO, then diluted to 10 ml using THF and filtered through 0.45 μm filters for GPC analysis. The data collection and analysis were performed using Waters Millenium software. Polystyrene standards (2000, 900, 824, 400, 200, 110, 43, 18.80, 17.60, 6.93, 2.61, 0.98 kDa) were used for calibration and weight average molecular weights (Mw) were calculated.

2.2.2.4. Equilibrium swelling. The equilibrium water content (H) was experimentally determined using Eq. (1), where W_s is the equilibrium swollen weight of PLGA/PVA composite films, W_d is the dry weight of the films and $W_{d(\mathrm{PVA})}$ is the dry weight of PVA present in the films:

$$H = \frac{(W_s - W_d)}{W_{d(PVA)}} \times 100\% \quad (1)$$

The PLGA/PVA composite films were dried in a vacuum desiccator at room temperature till virtually no change in weight was observed. To measure W_s, the swollen films were removed from the PBS buffer solution (pH 7.4, 0.1 M, 37 ± 0.5 °C) and gently wiped with a lint-free tissue prior to weighing.

2.2.2.5. Thermal analysis. A temperature-modulated differential scanning calorimetry (TM-DSC) was used to determine the glass transition temperature (Tg) and enthalpy of relaxation of the microspheres (TA instrument Q100 differential scanning calorimeter,

Table 1
Microsphere drug loading, and PLGA molecular weight of composites stored at −20, 4, and 25 °C.

Temperature		−20 °C	4 °C	25 °C
Parameter/time	Day 0	12 months	12 months	12 months
PLGA MW (kDa)	26.09 ± 0.38	25.91 ± 0.89	25.71 ± 1.09	24.51 ± 1.23
Dexamethasone loading (%, w/w)	7.43 ± 0.24	7.48 ± 0.10	7.52 ± 0.38	7.46 ± 0.27

New Castle, DE). The samples were heated at a rate of 2 °C/min from −20 °C to 200 °C at a modulating oscillatory frequency of 0.4 °C/min. The thermograms were used to determine the glass transition temperature (Tg) and the enthalpy of relaxation (peak area) of the microspheres using Universal Analysis software (TA Instruments). Specifically, the Tg is determined by the reversing heat flow that describes a kinetic event, whereas the relaxation endotherm is represented by the non-reversing heat flow.

2.2.2.6. In vitro release. *In vitro* release tests of dexamethasone loaded PLGA microsphere/PVA hydrogel composites were determined as described previously (Zolnik and Leary, 2006; Zolnik and Burgess, 2007). Briefly, the composites were immersed in 250 ml Pyrex® glass bottles containing 200 ml of 0.1 M phosphate-buffered saline (PBS) (pH 7.4) and incubated at 37 °C under constant agitation (100 rpm). Sink conditions were maintained. The weight of each composite was approximately 47 mg. At pre-determined time points, one ml samples were taken and replaced with fresh PBS. The concentration of dexamethasone in each sample was determined using HPLC as described above. Cumulative percent release at a given time point was calculated as: cumulative percent release = (amount released at sampling time/total amount released) × 100. The values are reported as mean ± standard deviation ($n = 3$).

3. Results

3.1. Effect of storage temperature on the performance of the PLGA/PVA composites

Since the PLGA microspheres are in a rubbery state above their Tg (approximately 42 °C), storage temperatures below the Tg were chosen: refrigeration conditions (4 °C, normal storage conditions); ambient conditions (25 °C); and −20 °C was used as a control (polymer chains have no or minimal mobility at approximately 50 °C below Tg).

The initial molecular weight (Mw) of the PLGA, as determined by GPC, was approximately 26 kDa. As shown in Table 1, there was no significant change in PLGA molecular weight or dexamethasone loading (therefore, no dexamethasone degradation) for the PLGA microspheres after storage at −20, 4, 25 °C over a 12-month period. Since the PLGA microspheres were well dispersed inside the PVA hydrogel matrix, no particle aggregation occurred.

The Tg of the PLGA microsphere/PVA hydrogel composite was approximately 40 °C one day after preparation. The observed Tg of the composite was the Tg of the PLGA microspheres. The PVA in the composites was partially crystalline with a melting point at 220 °C and partially amorphous with a Tg at approximately 80 °C (Sakurada, 1985). However, the amount of PVA present in the composite was too low to be detected *via* the DSC thermal scan. No significant change was observed in the Tg of the PLGA/PVA composites after incubation at -20 and 4 °C for up to 12 months in comparison to the initial values. However, the Tg of the composites gradually increased from 39.28 °C (day 0) to 50.23 °C after 30 days storage at 25 °C as shown in Fig. 1.

The enthalpy of relaxation of the PLGA/PVA composites, when stored at 25 °C, was determined *via* a non-reversing heat flow plot

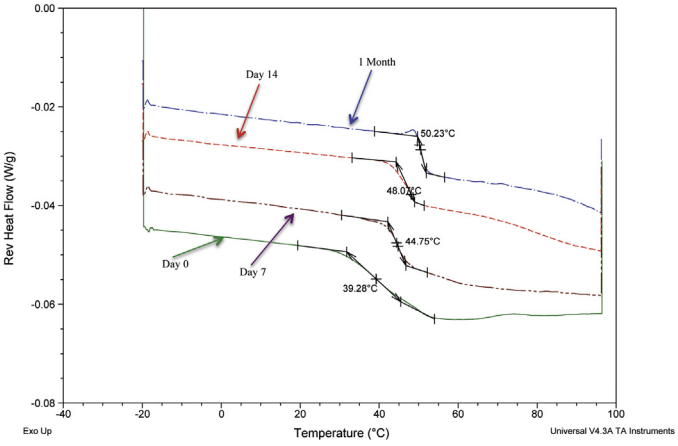

Fig. 1. DSC thermograms showing increase in glass transition temperature with time following incubation of dexamethasone-loaded PLGA microsphere/PVA hydrogel composites at 25 °C.

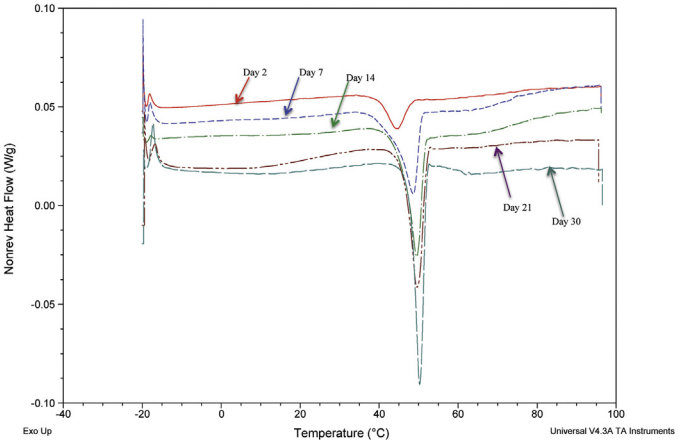

Fig. 2. DSC thermograms showing increase in enthalpy of relaxation with time following incubation of dexamethasone-loaded PLGA microsphere/PVA hydrogel composites at 25 °C.

as shown in Fig. 2. The enthalpy of relaxation increased rapidly during the first 7 days when stored at 25 °C followed by a slower increase from day 8 to day 30. The enthalpy of relaxation increased by approximately 7 J/g after 1-month storage at 25 °C. However, there was no significant difference in the enthalpy of relaxation after 1 month storage at either −20 or 4 °C (Fig. 3).

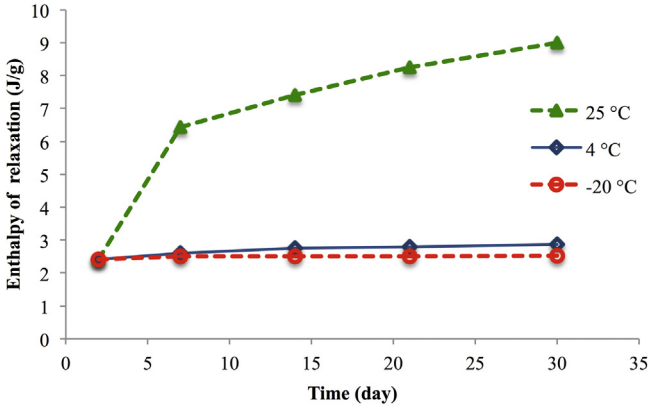

Fig. 3. Enthalpy relaxation of the PLGA microsphere/PVA hydrogel composites stored at −20, 4 and 25 °C.

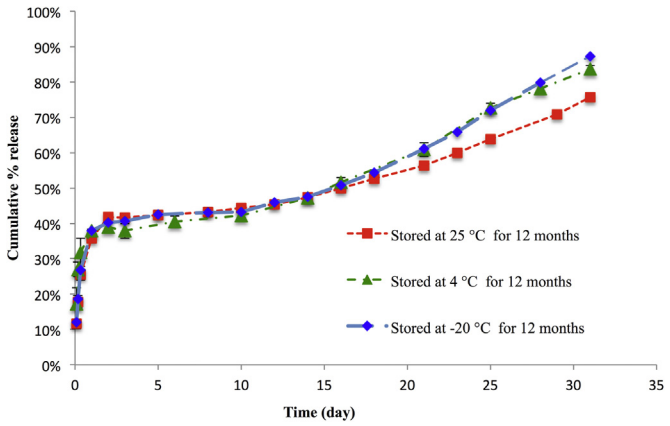

Fig. 4. *In vitro* release profiles of dexamethasone-loaded PLGA microsphere/PVA hydrogel composites: (1) incubated at −20 °C for 12 months; (2) incubated at 4 °C for 12 months and (3) incubated at 25 °C for 12 months.

Fig. 4 shows the *in vitro* release profiles of: (1) composites stored at −20 °C for 12 months (control); (2) composites stored at 4 °C for 12 months, and (3) composites stored at 25 °C for 12 months. A typical triphasic release profile was observed with an initial burst release (first 48 h) of approximately 45%, followed by a lag phase of approximately 10 days, and then a secondary zero order release phase. As shown in Fig. 4, the dexamethasone release profiles of the composites stored at 4 °C overlapped with those stored at −20 °C. The *in vitro* release profiles of the composites stored at 25 °C for 12 months were also triphasic. The burst release and lag phases were the same as those stored at −20 and 4 °C, however, the release rate of the secondary zero order phase was less and there was a decrease in the cumulative percent release of approximately 8% by day 31.

The equilibrium swelling properties of the PVA hydrogels (with/without PLGA microspheres) after storage at −20, 4 and 25 °C for 12 months are shown in Fig. 5. It was observed during the swelling studies that the PVA hydrogels (with/without PLGA microspheres) reached equilibrium swelling approximately one hour following incubation in the buffer solution. The amount of dexamethasone released during this period was negligible. Therefore, the presence of dexamethasone did not affect the results of the swelling studies. The percentage equilibrium swelling of the

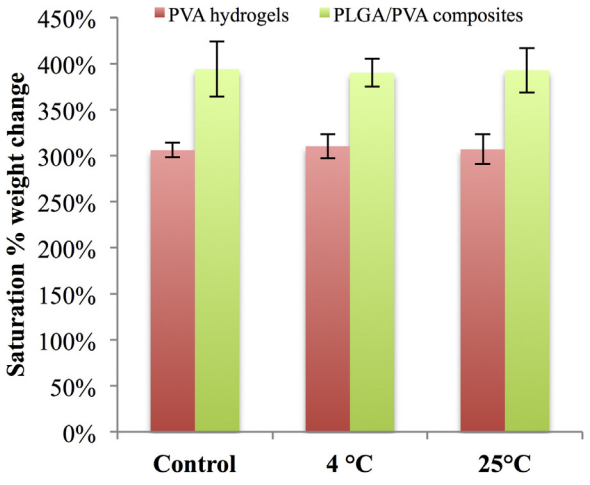

Fig. 5. Saturation % weight change of PVA hydrogels with and without dexamethasone-PLGA microspheres immersed in PBS buffer (pH 7.4) at 37 °C after 12 months storage at -20 (control), 4, and 25 °C.

Table 2
The glass transition temperatures of the PLGA microspheres and the PLGA/PVA composites following 1 week storage at: (1) 40 °C, 75% RH; and (2) 25 °C, 75% RH.

Storage condition	Tg of PLGA microspheres	Tg of PLGA/PVA composites
Control (−20 °C)	42 °C	40 °C
40 °C and 75% RH	43 °C	33 °C
25 °C and 75% RH	48 °C	45 °C

PVA hydrogels with/without PLGA microspheres was approximately 390% and 305%, respectively. No significant change was observed post incubation at −20, 4, 25 °C for 12 months.

3.2. Effect of storage humidity on the thermal properties of the composites

To determine the effect of moisture on the thermal properties of the PLGA microspheres and the composites, samples were incubated at 25 °C and 75% RH and 40 °C and 75% RH. The PLGA microspheres were used as a control to determine the effect of the presence of PVA hydrogel on water absorption upon exposure to moisture. Samples were stored for only one week to avoid potential interference from moisture induced hydrolytic degradation of the PLGA microspheres. As shown in Table 2, the Tg of the PLGA microspheres incubated at 40 °C and 75% RH remained almost unchanged (approximately 43 °C), whereas the Tg of the composites decreased from approximately 40 °C to approximately 33 °C. On the other hand, the Tg of the microspheres incubated at 25 °C and 75% RH increased from approximately 42 °C to approximately 49 °C and the Tg of the composites increased from approximately 40–45 °C.

3.3. Accelerated stability study

The PLGA microspheres and PLGA/PVA composites were incubated at 40 °C and 75 ± 2% RH for 1 month to investigate accelerated stability. No dexamethasone degradation was observed. However, PLGA degradation occurred after 1-month storage at 40 °C and 75% RH. The Mw of the PLGA decreased from approximately 26 kDa to approximately 6 kDa. The *in vitro* release profiles of the PLGA/PVA composites after 1-month storage are shown in Fig. 6. The dexamethasone release rate was significantly enhanced compared to the control group (samples stored at −20 °C). Instead of the typical triphasic release profile, a biphasic release profile was observed. More than 90% of the dexamethasone was released in 3 days.

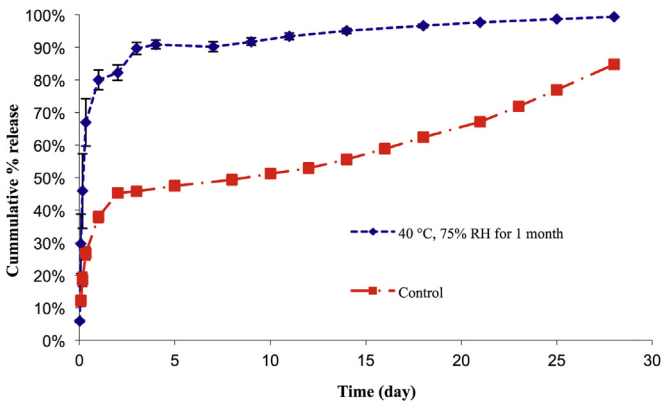

Fig. 6. *In vitro* release profiles of dexamethasone-loaded PLGA microsphere/PVA hydrogel composites: (1) control; and (2) composites incubated at 40 °C and 75% RH for 1 month.

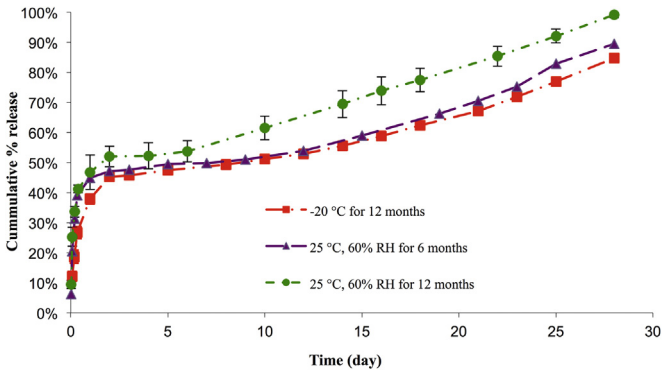

Fig. 7. *In vitro* release profiles of dexamethasone-loaded PLGA microsphere/PVA hydrogel composites: (1) control; (2) composites incubated at 25 °C, 60% RH for 6 months, and (3) composites incubated at 25 °C, 60% RH for 12 months.

3.4. Long-term stability study

The PLGA/PVA composites were incubated at 25 °C and 60 ± 2% RH for 6 and 12 months to determine their long-term stability. No dexamethasone degradation was observed up to 12 months storage under this condition. As illustrated in Fig. 7, the samples incubated at 25 °C and 60% ± 2% RH for 6 months exhibited a similar *in vitro* release pattern to the control group (samples stored at −20 °C). However, the dexamethasone release was accelerated after 12-month storage under this condition. The initial burst release in the first 24 h increased by approximately 7% and the lag time decreased from approximately 10 days to 5 days.

4. Discussion

The lack of change in PLGA Mw after incubation at −20, 4 and 25 °C for 12 months indicates that PLGA microspheres are not susceptible to degradation at ambient temperature below their Tg over a 12 month period (Table 1). Comparing the initial Tg values of the PLGA/PVA composites (approximately 40 °C) to the Tg of the PLGA microspheres (approximately 42 °C) indicates that the presence of the PVA hydrogel matrix has no significant effect on the thermal properties of the microspheres. The observed increase in the Tg and enthalpy of relaxation of the composites stored at 25 °C for 12 months (Figs. 1 and 2) was a result of physical aging. Physical aging at temperatures below the Tg is a result of secondary ß relaxations in polymer chains that arise from localized vibrational motion and reorientation of small side chains (Allison, 2008). Large-scale translational and rotational motions of the polymer backbones are hindered in the glassy state (Liu and Bhandari, 2006).

The change in the rate of structural relaxation at 25 °C (faster increase in enthalpy of relaxation in the first week followed by a slower rate of increase (Fig. 3)) is attributed to the fact that the rate of structural relaxation is a function of free volume of the polymeric matrix (Allison, 2008). The more free volume, the further the system is away from the equilibrium state, which leads to faster rates of structural relaxation. The free volume decreases with time as the polymeric matrix shifts toward the equilibrium state, which in turn results in a reduced structural relaxation rate (Liu and Bhandari, 2006). In addition, the rate of structural relaxation also depends on the polymer chain mobility, which is closely related to the incubation temperature (Zhang and Boucher, 2013). Therefore, the low PLGA chain mobility at 4 and −20 °C (approximately 38 and 62 °C below the microsphere Tg, respectively) may be responsible for the lack of any significant change in the enthalpy of relaxation following 12 months incubation at these temperatures (Fig. 3).

The observed slower secondary zero order release rate of composites after storage at 25 °C for 12 months may be due to: (1) aggregation of the PLGA microspheres; (2) change in the properties of the PVA hydrogel; (3) change in the properties of the PLGA microspheres; and (4) a combination of these three factors. Since the microspheres were well dispersed and immobilized inside the PVA hydrogel matrix, no particle aggregation occurred during storage. The lack of change in the swelling properties of the PVA hydrogels (with and without the microspheres) indicated that the PVA hydrogels remained stable. Accordingly, it is speculated that physical aging of the microspheres is responsible for the observed slower release rate. As discussed above, physical aging reduces the free volume of the PLGA matrix with time, which in turn results in a denser polymer matrix and slower water penetration rate. As a result, the PLGA degradation rate and the dexamethasone diffusion are reduced leading to the observed slower release rate.

It is known that water molecules absorbed into an amorphous polymeric matrix plasticize the system, leading to increase in polymer chain mobility and decrease in the Tg of the polymer (Hancock and Zografi, 1994). This could explain the observed significant decrease in the Tg of the composites upon incubation at 40 °C and 75% RH for one week (Table 2). No such change occurred in the Tg of the PLGA microspheres probably due to their relative hydrophobicity, which would require a much longer time to absorb a similar amount water as the composites (Table 2). This indicates that the presence of the hydrogel significantly enhances water absorption. In addition, the lack of change in the Tg of the microspheres could be a result of physical aging and water plasticization effects canceling each other out, since these two factors have the opposite effect on the Tg. The observed increase in the Tg of the microspheres and the composites (at 25 °C and 75% RH for one week) indicates that the effect of physical aging is greater compared to the effect of water plasticization under these conditions. Physical aging would decrease the impact of plasticization from water molecules through reducing water penetration as discussed above. Comparison of the change in the Tg of the composites incubated at 25 °C and 75% RH to the change in the Tg of those incubated at 40 °C and 75% RH reveals that water absorption is strongly dependent on storage temperature.

In addition to the plasticization effect, the presence of moisture also induces hydrolytic degradation of PLGA. This is apparent in the drastically accelerated dexamethasone release rate and the significant decrease in the Mw of PLGA microspheres following storage at 40 °C and 75% RH for 1 month. Since the Tg of the composites stored under these conditions quickly decreased to a value below 40 °C (Table 2), the microspheres were in the rubbery state making them more susceptible to hydrolytic degradation. In addition, it is known that PLGA degradation is auto catalyzed by its acidic degradation products. Accordingly, once degradation starts, the reaction rate would increase with time due to the gradual build up of local acidity. The results of the long-term stability study (25 °C and 60% RH) indicated that no significant PLGA degradation occurred up to 6 months (Fig. 7). This observation is a result of the net effect of the storage temperature and the humidity. The rate of physical aging decreases with time as the system shifts toward the equilibrium state, whereas the rate of the moisture induced hydrolytic degradation increases with time. Accordingly, the presence of moisture is the dominant factor in the later stage of this long-term stability study.

5. Conclusions

The shelf-time stability of PLGA microsphere/PVA hydrogel composites is reported for the first time. An important finding of this work is that the moisture content of the hydrogel negatively impacts the PLGA microsphere stability by increasing the hydrolytic

degradation of the polymer. In addition, the *in vitro* release profiles of the composites following storage is affected by both the moisture content/PLGA microsphere degradation and the physical aging of the PLGA polymer. For example, at 25 °C and 60% relative humidity, the physical aging played a dominate role during the first 6 months leading to no apparent change in the release profiles, whereas the moisture content/PLGA microsphere degradation was the dominate factor during the second 6 months resulting in an accelerated release rate. Under the same humidity conditions, moisture absorption strongly depends on the storage temperature. For example, the Tg of the composites significantly decreased after 1 week storage at 40 °C and 75% RH as a result of water plasticization, whereas the Tg of the composites increased following 1 week storage at 25 °C and 75% RH as a net effect of physical aging and moisture content. Based on the results of the current work, storage temperatures of 4 °C or below may be considered appropriate for microsphere/hydrogel composite formulations. However, the storage stability test conditions and results may vary depending upon the properties of the microsphere and hydrogel components of the composites.

Acknowledgements

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