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Research paper

Diffusional characteristics of freeze/thawed poly(vinyl alcohol) hydrogels: Applications to protein controlled release from multilaminate devices

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Abstract

The incorporation of a model protein, bovine serum albumin (BSA), into poly(vinyl alcohol) (PVA) hydrogel films during the freezing and thawing process and its subsequent release behavior were investigated. The effect of the number of freezing and thawing cycles as well as the stability of BSA were examined. BSA release profiles were not significantly different from gels prepared after 3 or 5 cycles. However, the rate and overall amount of PVA dissolution were considerably higher for gels prepared after 3 cycles. These observations were then applied to the development of novel, freeze/thawed PVA laminates. Laminates containing gel layers prepared after 3 or 5 cycles were successfully prepared with good stability over a 6 month swelling period. These structures, containing distinct layers of very specific properties, could be used to achieve zero-order release behavior.

Keywords: Poly(vinyl alcohol); Freezing/thawing process; Bovine serum albumin; Laminate

1. Introduction

Poly(vinyl alcohol) (PVA) hydrogels prepared using freezing and thawing techniques have great potential for biomedical and drug delivery applications [1,2]. In addition to desirable mechanical and swelling properties, these materials exhibit a size exclusion phenomenon due to the presence of crystalline regions that render the network insoluble [3]. For example, the solute diffusion coefficient has been related to the crystalline volume fraction [3] through a linear approximation that was developed from an analysis of the permeation of theophylline and FITC-dextran through such semicrystalline PVA gels. Controlled drug delivery from PVA microparticles prepared by such freezing and thawing techniques has also been examined previously [4]. The preparation techniques involved the use of an aqueous solution of PVA that was dispersed in corn oil with sodium lauryl sulfate as the surfactant. Challenges faced during such a process included the proper agitation of the particles particularly after the oil was partially frozen. The PVA microparticles were found to be capable of initially fast release rates of a model protein, bovine serum albumin (BSA), followed by a period of constant release over a longer time of 7 days.

The incorporation and release of other drugs and proteins have also been investigated. The release of oxprenolol, theophylline, and various growth factors from freeze/thawed PVA gels has been examined [5–11]. Oxprenolol or theophylline release was affected by the number of freezing/thawing cycles [5]. These results demonstrated how drug release could be optimized for certain mucoadhesive controlled release applications by controlling freezing and thawing conditions. Mucoadhesive PVA gels were further examined for use in epidermal bioadhesive systems for the controlled release of epidermal growth factor or ketanserin to accelerate wound healing [7].

For certain applications, it would be desirable to incorporate proteins or drugs into thin films of PVA gels prepared by freezing and thawing techniques. Ideally, an ease of processing would be involved with the incorporation of such solutes during the freezing and thawing process. However, there are certain challenges that may occur with the incorporation and release of a drug or protein when considering such freeze/thawed PVA gels for a variety of applications.

In this work, the incorporation of a model protein, BSA, into PVA hydrogel films during the freezing and thawing process and the subsequent release behavior were investi-

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gated. The focus of this work was to address the effect of the number of freezing and thawing cycles as well as the stability of BSA during preparation techniques. In addition, several other issues were addressed including the effect of the conditions of the environmental media on the overall gel behavior. In particular, the effect of complete release media changes as opposed to only partial changes was investigated to determine if swelling and diffusion characteristics were altered in any way. Such information was important in developing experimental techniques and analyzing results in terms of different possible applications. It was necessary to consider such aspects of the behavior of freeze/thawed PVA films prepared in the presence of a model protein in order to apply the information to the development of novel PVA laminates.

2. Novel freeze/thawed PVA laminate hydrogels

Extensive analysis of the preparation of PVA gels by freezing and thawing techniques has shown that several parameters significantly alter the overall structure, morphology, and stability of the resulting materials. In particular, the number of freezing and thawing cycles, concentration of aqueous solution, and molecular weight of PVA can be manipulated to control properties such as the overall water content, mechanical strength, adhesive characteristics, and diffusive properties. An interesting approach to developing such gels for various biomedical and pharmaceutical applications involves looking at more than one material with optimal characteristics. Rather, by utilizing the properties of different freeze/thawed gels, one may design a layered structure of such gels with overall optimal characteristics for a certain application.

The idea of adding various layers of drug-loaded polymer films in a way as to prepare a multilayered structure containing high drug concentration in the center and low concentration in the other layers was first proposed by Lee [12]. The associated mathematical analysis showed that drug release from such devices could be done under zero-order conditions. Further design of such systems has been investigated by Lu et al. [13] in order to achieve an overall constant rate of drug or protein release over time. Laminated polymer matrix systems were investigated to designed and modeled as drug delivery devices capable of constant release rates. Photopolymerization techniques were used to prepare such diffusion controlled devices with initial drug concentration profiles that were spatially nonuniform. Additionally, the diffusivity of drugs was examined as another variable that could affect overall release kinetics. Obviously, when implementing such ideas, considerable difficulties may be encountered in trying to obtain the appropriate variation of properties throughout the gel.

In this work, we are taking advantage of the ease of processing associated with freeze/thawed gels as well as the ability to manipulate parameters to optimize certain properties. In preparing novel freeze/thawed PVA laminates, fresh PVA solution is added to a previously formed gel and then subjected to additional freeze and thawing cycles to create a layered structure. Each structure contains varying properties due to the exposure of each layer to a different number of freezing and thawing cycles. In addition, each layer could be prepared using different concentrations of PVA or even different PVA molecular weights. In preparing such laminates, other factors may also be considered such as the thickness of each layer or even the concentration of a drug, protein, or solute in each layer. It is apparent that there are almost boundless opportunities in designing such laminates due to the number of parameters that can be varied to impact the resulting behavior of the gels.

3. Experimental

3.1. Incorporation of model drug

Aqueous solutions of PVA were prepared by dissolving PVA (Elvanol® HV, E.I. duPont de Nemours, Wilmington, DE, $\overline{M}_n = 64\,000$, polydispersity index = 2.02, degree of hydrolysis = 99.0%) in deionized water for 6 h at 90°C. Bovine serum albumin (Fraction V Powder, Sigma Chemical Co., St. Louis, MO) was dissolved in a small amount of water at 25°C and then added to the cooled PVA solution to obtain a final solution of 15 wt.% PVA and 0.5 wt.% BSA. The solutions were cast between glass microscope slides with 0.7-mm thick spacers. The samples were then exposed to 3 or 5 cycles of freezing for 8 h at −20°C and thawing for 4 h at 25°C. Control PVA gels were also prepared in the same manner without the addition of BSA.

3.2. Release from freeze/thawed PVA

Release studies were conducted with thin disks of 12 mm diameter using a USP II apparatus in deionized water at 37°C. The release medium was analyzed for both BSA released and PVA dissolved. The UV absorbance was measured at 292 nm to determine the concentration of BSA. The dissolution of PVA was also determined by complexing a 5 ml sample of aqueous PVA with 2.5 ml of a 0.65 M boric acid solution and 0.3 ml of a 0.05 M I_2/0.15 M KI solution and then diluting to 10 ml with deionized water at 25°C. The absorbance of visible light at 671 nm was then measured to determine the concentration of complexed PVA in solution.

The effect of complete water changes versus partial water changes during release experiments was also investigated to determine if frequent environmental changes were necessary to maintain uniform release characteristics. Water changes were implemented by completely replacing the environmental swelling media with fresh water at 8 different time intervals during a 48-h period. Partial water changes were examined by replacing the swelling media at only 3 different time intervals during the same 48-h period. Addi-

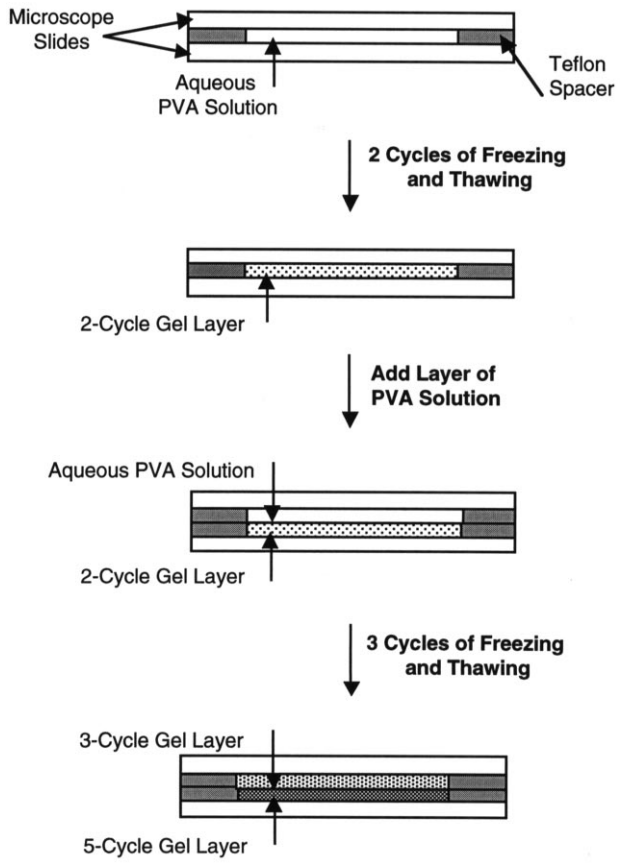

Fig. 1. Preparation of laminates of freeze/thawed PVA hydrogels.

tionally, PVA release was analyzed both from samples first allowed to thaw before analysis, and from samples that were analyzed during the thawing period (immediately after the last period of freezing).

3.3. Preparation of PVA laminate hydrogels

A feasibility study was carried out to prepare freeze/thawed PVA laminates by freezing and thawing processes. The preparation of laminates containing two PVA layers, one prepared with 3 cycles of freezing and thawing, and another with 5 cycles of freezing and thawing, is depicted in Fig. 1. A 15 wt.% aqueous PVA solution of $\overline{M}_n = 64\,000$ was placed between two microscope slides with 0.7-mm thick Teflon spacers and exposed to two cycles of 8 h freezing at −20°C and 4 h thawing at 25°C. At the beginning of the second thawing period, the top microscope slide was removed and additional aqueous PVA solution was poured on top of the already existing gel prepared with two cycles. An additional set of 0.7 mm thick spacers was added and the microscope slide placed back on top. The sample was then allowed to remain at room temperature for the remaining 4 h of the second thawing cycle to allow for the interpenetration of polymer chains between the layers. The sample was then exposed to an additional three cycles of freezing (8 h) and thawing (4 h).

4. Results and discussion

4.1. Incorporation of BSA drug

BSA was incorporated into the physical crosslinked structure of the gel during the freezing and thawing process. Good gelation of the PVA solution was observed in preparing thin films. Therefore, the presence of this intermediate size protein (BSA, molecular weight = 66 000, radius of gyration = 35.9 Å [14]) did not prevent the folding and alignment of PVA chains that was necessary for the formation of crystalline regions that served as physical crosslinks within the network [3]. Of course, there are likely some limitations associated with the preparation of such gels in the presence of proteins or drugs. It is quite possible that there is a certain range in PVA molecular weight as well as initial BSA and PVA concentrations in which such gels are successfully prepared. However, a 15 wt.% aqueous solution of PVA of $\overline{M}_n = 64\,000$ resulted in gels that were successfully loaded with 0.5 wt.% BSA. It must be noted, that this technique is not 'universal,' as the stability of each protein employed must be checked.

The UV spectrum was monitored throughout the process with a UV/Vis spectrometer (Lambda 10 model, Perkin Elmer, Norwalk, CT), indicating that the protein remained stable upon being exposed to repeated freezing and thawing cycles.

4.2. Release from freeze/thawed PVA

In analyzing the results of PVA dissolution, the PVA loss was determined as the weight percent of the total gel. Therefore, the results were normalized by the weight of each original gel that contained both water and PVA. All gels had similar initial weights before being placed in water. PVA gels exposed to frequent water changes and infrequent water changes demonstrated similar dissolution behavior in terms of total PVA dissolution as well as the rate of dissolution as shown in Fig. 2. Gels exposed to only three complete water changes during a 48-h time period showed no signifi-

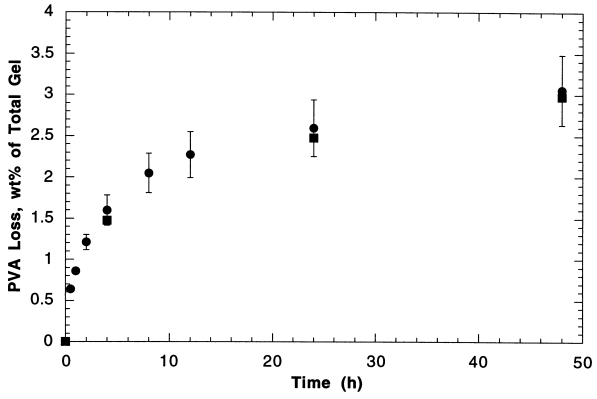

Fig. 2. PVA loss in water at 37°C of 3-cycle PVA samples with frequent (●) and infrequent (■) water changes.

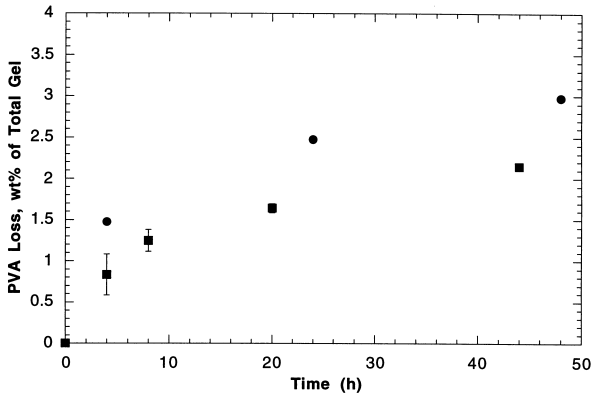

Fig. 3. PVA loss in water at 37°C of 3-cycle PVA samples from an initially frozen (●) and an initially thawed (■) state.

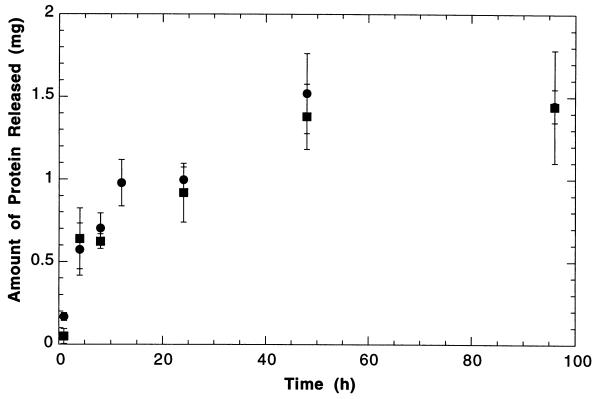

Fig. 4. BSA release in water at 37°C from PVA samples with three (●) and five (■) cycles of 8 h freezing and 4 h thawing.

cant effects from the lack of frequent replenishment of the swelling media. In a Fickian diffusion model, the diffusive driving force is a concentration differential across a surface. Therefore, these results indicated that the concentration of PVA in the wash solution was not high enough to impede the further dissolution of PVA from the gel.

The frequency of swelling medium changes did not alter the diffusional characteristics of freeze/thawed PVA gels. Another important issue was the initial state of the PVA gel, with respect to time allowed for thawing at room temperature, when placed in the swelling medium. In order to examine this effect, we studied the PVA dissolution behavior of gels that were placed in water immediately after the third cycle of freezing and gels that were allowed to thaw for 4 h before being placed in water. These results are shown in Fig. 3 where the PVA loss in terms of wt.% of the total gel is plotted as a function of time. There is an overall effect of the initial gel conditions on the rate and overall amount of PVA loss. For example, a lower amount of PVA was released from the gels that were completely thawed for 4 h prior to swelling in water. These results indicate that further crosslinking likely occurred during the thawing process as the PVA chains were allowed to further rearrange and crystallize. With the increase in crystallinity and associated physical crosslinking, lower PVA dissolution occurred in water. Therefore, it is very important to maintain consistencies when analyzing the PVA gels after the preparation techniques. Any significant differences in the time allowed for thawing can result in an overall significantly different structure.

PVA gels were further analyzed in terms of protein release from gels of varying cycles of freezing and thawing. The release profiles of BSA from gels exposed to 3 and 5 cycles of freezing and thawing are shown in Fig. 4. For both materials, a large part of the BSA was released during the first 48 h with a slightly faster rate of diffusion from the 3-cycle gels. Approximately 1.5 mg of BSA was released from both the 3- and 5-cycle gels over a time period of 2 days. The rate of protein release appears to be relatively constant between 4 and 48 h. During the first 4 h the rate was considerably higher as approximately 0.5–0.7 mg of BSA was released. The diffusion also seemed to be quite sensitive to changes that occurred in the physical structure of the material during the first 24 h. During this time, the system exhibits significant rearrangement due to loss in crystallinity and PVA chain dissolution.

Not all PVA chains participated in the formation of stable crystallites during the freezing and thawing process. The fractional dissolution of PVA was monitored as shown in Fig. 5. It is apparent that more PVA diffused out of the samples exposed to only three cycles of freezing and thawing. Essentially, as the number of freezing and thawing cycles was increased, more PVA chains participated in the formation of stable crystals as shown before [3,4]. Although there was a significant difference in PVA dissolution, the release of BSA was not significantly different between the samples prepared with three or five cycles. This was an interesting result particularly when considering such materials for release applications. By increasing the number of freezing and thawing cycles, less PVA dissolution occurred.

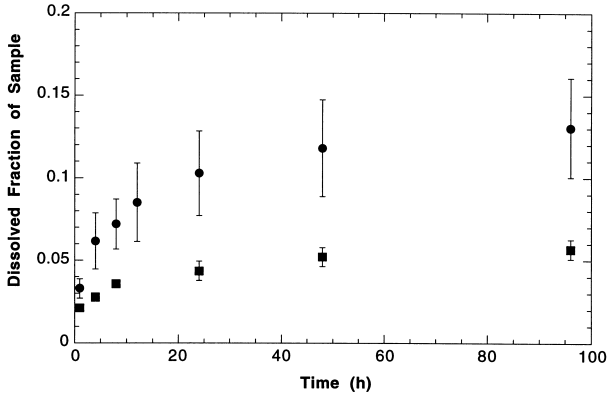

Fig. 5. Fractional PVA dissolution in water at 37°C of PVA samples prepared in the presence of BSA with three (●) and five (■) cycles of 8 h freezing and 4 h thawing.

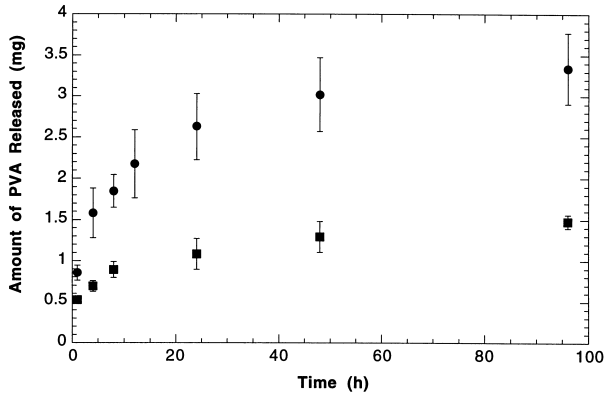

Fig. 6. Weight of PVA dissolved in water at 37°C from samples prepared in the presence of BSA with three (●) and five (■) cycles of 8 h freezing and 4 h thawing.

This was desirable from the viewpoint of achieving a minimum level of leachable polymer from a particular device. An increase in the freezing and thawing cycles did not significantly alter the protein release profile; however, it did serve to enhance the stability in terms of lowering PVA dissolution. The overall amount of PVA released in terms of total weight is shown as a function time in Fig. 6.

The ability to incorporate a protein into the structure of the gel speaks to the possibility of using this system for drug delivery either alone or during other applications. There are obvious differences observed between gels exposed to varying preparation conditions. By more drastically varying the number of freezing and thawing cycles, we expect to increase the differences in diffusive characteristics as well. By taking advantage of some of the differences in the overall properties of PVA gels that are prepared with varying preparation conditions, it is possible to develop novel materials using benign manufacturing processes.

4.3. PVA laminate hydrogels

The multilaminate PVA systems prepared in this work contained a 5-cycle gel layer and a 3-cycle gel layer (as shown in Fig. 1). The layers adhered well to each other even upon being placed in water. Thin disks of 12 mm diameter were placed in deionized water at 37°C and monitored over time. Even after 6 months in water, the overall structure was intact with the layers adhering well. Interdiffusion of the PVA chains occurred allowing for the adhesion between layers. This adhesion allowed for the successful preparation of freeze/thawed PVA laminates.

Laminates of freeze/thawed PVA hydrogels were successfully prepared to contain layers of varying cycles of freezing and thawing. Such materials appear to remain stable in terms of retaining its original layered structure upon swelling in water at 37°C for 6 months. This feasibility study has provided insight into developing a novel group of laminate hydrogels for a wide variety of pharmaceutical applications.

5. Conclusions

This work investigated the incorporation of a model protein into thin films of freeze/thawed PVA gels. BSA was successfully incorporated into the gel structure. The ability of PVA to crystallize in the presence of the intermediate molecular weight protein was not hindered. Although the BSA release profile was not significantly different from 3- and 5-cycle gels, the rate and overall amount of PVA dissolution was considerably higher for 3-cycle gels.

Acknowledgements

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