Poly(d,l-Lactide-Co-Glycolide) Encapsulated Poly(Vinyl Alcohol) Hydrogel as a Drug Delivery System

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Purpose. The efficiency of encapsulation of water-soluble drugs in biodegradable polymer is often low and occasionally these microcapsules are associated with high burst effect. The primary objective of this study is to develop a novel microencapsulation technique with high efficiency of encapsulation and low burst effect.

Method. Pentamidine was used as a model drug in this study. Pentamidine/polyvinyl alcohol (PVA) hydrogel was prepared by freeze-thaw technique. Pentamidine loaded hydrogel was later microencapsulated in poly(lactide-co-glycolide) (PLGA) using solvent evaporation technique. The microcapsules were evaluated for the efficiency of encapsulation, particle size, surface morphology, thermal characteristic, and drug release.

Results. Scanning Electron Microscope (SEM) studies revealed that the microcapsules were porous. The microcapsules were uniform in size and shape with the median size of the microcapsules ranging between 27 and 94 μm. The samples containing 10% PLGA showed nearly three times increase in drug loading (18–53%) by increasing the hydrogel content from 0–6%. The overall drug release from the microencapsulated hydrogel, containing 3% and 6% PVA, respectively, was significantly lower than the control batches.

Conclusions. The use of a crosslinked hydrogel such as PVA can significantly increase the drug loading of highly water-soluble drugs. In addition, incorporation of the PVA hydrogel significantly reduced the burst effect and overall dissolution of pentamidine.

KEY WORDS: Poly(lactide-co-glycolide); PLGA; microencapsulation; hydrogel; efficiency of encapsulation; burst effect.

INTRODUCTION

Microencapsulation of small particles in envelopes of polymeric, waxy, or other protective shell materials has become a well-established technology for coating and isolating substances. Sustained release microcapsules using synthetic biodegradable polymers have been developed for numerous therapeutic agents (1). One of the advantages of these formulations is that no follow-up surgical removal is required once the drug supply is depleted (1). The most widely investigated polymers are the aliphatic polyesters based on lactic acid and glycolic acid (PLGA).

Several methods have been developed for the microencapsulation of a wide variety of drugs using PLGA (2–6). However, the double water-in-oil-in-water (w/o/w) emulsion method has been widely accepted for this purpose (7–10). These microcapsules are generally smooth, spherical and provide sustained drug release. However, the release rate is greatly influenced by the physicochemical characteristics of the drug, the polymer, the organic solvent used in the preparation, and the emulsification process (11). Moreover, the efficiency of encapsulation of water-soluble drugs in PLGA is often low (7,12) and several attempts have, therefore, been made to increase the efficiency of encapsulation by changing processing parameters such as inner aqueous phase/polymer phase volume ratio and the polymer phase viscosity (13–15). Furthermore, these microcapsules, are often associated with high initial drug release, (i.e., burst effect) (7,12).

The primary drawback of burst effect is that it is unpredictable and thus undesirable (16). Moreover, even when the burst effect is desirable (e.g., for the wound treatment), the amount of burst cannot be significantly controlled (17). During the last few years, attempts have, therefore, been made to avoid burst effect. Several advanced technologies to avoid burst effect include surface extraction of the drug prior to *in vivo* usage (18,19), using double walled microspheres with layers made of different inert or erodible polymers (20), modifying the surfaces of the drug-loaded microcapsules via an outer layer polymer coating (21,22), and heterogeneously structured composite (23–25). Unfortunately, many of these methods involve additional costly steps, which also result in reduced efficiency of encapsulation or the introduction of additional materials.

The primary objective of this study is to develop a novel microencapsulation technique for the development of controlled release formulation of water-soluble drugs with high efficiency of encapsulation and low burst effect. Pentamidine is used in this study as a model drug because of its high water solubility.

MATERIALS AND METHODS

Chemicals

The copolymer poly(d,l-lactide-co-glycolide), PLGA 50:50 (RG 506; inherent viscosity 0.8; Mw 100,000) was obtained from Boehringer Ingelheim (Germany). The surfactant, L-α phosphatidylcholine was obtained from Avanti Polar-lipids, Inc. (Birmingham, AL, USA). Pentamidine, polyvinyl alcohol (PVA) (98–99% hydrolyzed; average molecular weight 70,000-100,000; viscosity of a 4% aqueous solution at 20°C 11–14 cps), chloroform, and dichloromethane, were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Experimental Design

The experiments were designed to study (i) the effect of PVA concentrations and (ii) the effect of PLGA concentrations. The effect of PVA concentrations was studied at three levels, 0, 3, and 6% w/v. The effect of PLGA concentrations was also studied at three levels, 5, 10, and 15% w/v. Pentamidine was used as a model drug in this study. Similar to PLGA, PVA is also biocompatible and extensively used in the preparation of controlled release formulations (26,27) including parenterals (28).

Preparation of Microencapsulated Hydrogel

Pentamidine/polyvinyl alcohol (PVA) hydrogel was prepared by freeze-thaw technique (29,30). In short, an aqueous

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solution was prepared by dissolving a specific amount of pentamidine and PVA (as listed in Table I) in 500 μl deionized boiled water. The solution was frozen by cooling at –20°C for 16 h. The solution was then allowed to thaw at room temperature for 8 h. This procedure constituted one full cycle. Based on the results of previous experiment (30) the samples were subjected to four freeze-thaw cycles. The result of the freeze-thaw process was the formation of a rigid hydrogel.

Pentamidine-loaded hydrogel was later microencapsulated in poly(lactide-co-glycolide) (PLGA) using the solvent evaporation technique (12). In short, the hydrogel was emulsified with 5 ml of dichloromethane containing PLGA (as listed in Table I). The polymer solution was previously mixed with 500 μl of lipophilic surfactant L-α-phosphatidylcholine in chloroform (10 mg/ml). The emulsification was carried out by sonication at room temperature (at output 4, 50 W) for 40 s (ultrasonic probe, Sonic & Materials Inc., CT). The resulting emulsion was further emulsified in 1 ml of an aqueous solution of PVA (1%) by vortexing for 25 s and then diluted in 100 ml of PVA aqueous solution (0.3%). The system was stirred magnetically (at 500 rpm) for 4 h at room temperature to allow complete evaporation of the solvent. The control batches were prepared by dissolving a specific amount of pentamidine (as listed in Table I) in 500 μl deionized water and emulsified in 5-ml dichloromethane as described above. The processing conditions used for these batches were exactly same as the test batches.

Thermal Analysis

Differential scanning calorimetry (TA DSC 2920, New Castle, DE) of pentamidine, PLGA, crystalline PVA; crosslinked PVA hydrogel with pentamidine, and microencapsulated hydrogel was performed to characterize their physical state. About 5 mg of a sample was weighed, crimped into an aluminum pan and analyzed at a scanning rate of 3°C/minute. The glass transition temperature (Tg) was calculated using TA universal analysis software by extrapolating the linear portion of the thermograms above and below the glass transition point and determining the midpoint.

Particle Size and Morphology

Surface morphology and appearance of microcapsules were examined by scanning electron microscope (SEM), Hitachi S570 (Tokyo, Japan). Samples for SEM were mounted on metal stubs and coated with gold to a thickness of 200–500 Angstrom. Particle size distribution was determined by a Coulter LS130 analyzer (Beckman Coulter Inc. Fullerton CA). For each sample a background run of deionized water was performed. A sample of microcapsules (2 mg) was added to the deionized water in a micro sample cell and counting was performed for 120 s. After subtraction of the background, the particle size distribution calculation was performed. The size distribution was expressed in volume%.

Determination of Pentamidine Content

For each formulation, a 20-mg sample was dissolved in 1 ml of dichloromethane. To the solution was added 10.5 ml of methanol followed by ultra centrifugation (35,000 rpm at 15°C) to completely separate the precipitated copolymer. The efficiency of extraction and recovery of pentamidine was measured independently with five different samples. The efficiency of extraction was at least 98%. The amount of drug in each sample was determined using a high performance liquid chromatography (HPLC).

HPLC Analysis

The chromatographic system used in this study consisted of a Waters Model 600 programmable solvent delivery module, Waters Model 717plus auto sampler, and a Waters Model 996 photodiode array detector (Waters, Milford, MA, USA). The chromatography was performed using a μbondapack C-18 column (Waters 10 μm, 3.9 × 300 mm); the mobile phase consisted of 18% acetonitrile, 2% methanol, 0.2 M ammonium acetate and 0.5% triethylamine; a flow rate of 1.5 ml/min. The identity of the eluting peaks was verified using a diode array detector. The concentration of pentamidine in each sample was determined by intrapolating the peak height to the pentamidine standard curve. Each experiment was performed in triplicate.

In Vitro Dissolution Studies

For each formulation, a 40 mg sample was placed in a 10 ml tube and incubated in 5 ml of pH 7.4 phosphate buffer saline (PBS) with constant shaking (20 rpm) at 37°C. Samples (600 μl) were collected at scheduled times using a filter pipette and centrifuged for 10 min at 10,000 rpm. Particles were all sedimented at the bottom of the dissolution tube and cau-

Table I. Description of Batch Formula

Batch	PLGA		PVA		Amount of pentamidine (mg)
	Amount (mg)	Concentration in DCM (%)	Amount (mg)	Concentration (%)	
A (Control)	400	5	0	0	40
B	400	5	60	3	40
C	400	5	120	6	40
D (Control)	800	10	0	0	80
E	800	10	60	3	80
F	800	10	120	6	80
G (Control)	1200	15	0	0	120
H	1200	15	60	3	120
I	1200	15	120	6	120

PLGA: poly (lactide-co-glycolide); PVA: polyvinyl alcohol; DCM: dichloromethane.

tion was taken not to disturb the sediment. As a result, no significant amounts of particles were removed during the sampling. The samples were analyzed for drug content using HPLC method. Fresh PBS solution was added to the incubated sample (600 μl) to maintain sink conditions. At the end of the dissolution experiment, the samples were freeze dried and the amount of drug remaining in the samples were analyzed following the digestion of the microcapsules with dichloromethane and extraction with methanol as described earlier. Dissolution studies of each formulation were performed in triplicate.

Mass Loss Study

In an effort to monitor the mass loss or degradation of the microencapsulated hydrogel, the batch F, (i.e., 10% PLGA and 6% PVA) was selected. Twenty-one samples of 10 mg each were weighed individually and each sample was placed in a 10-ml tube and incubated in 5 ml of pH 7.4 phosphate buffer saline (PBS) with constant shaking (20 rpm) at 37°C. At each of the preset sampling time, three samples were filtered through Millipore filter (0.2 μ) and washed with 15 ml deionized water. Each of the filtrated samples was weighed accurately after drying.

Statistical Analysis

The efficiency of encapsulation of pentamidine and the amount of drug released from the different formulations of microcapsules during the *in vitro* study was compared using SAS software package. A p value of <0.05 was considered as evidence of a significant difference.

RESULTS AND DISCUSSION

Nine different formulations of microcapsules were prepared to study the effect of PVA and PLGA concentrations. Both concentrations (3 and 6%) of PVA used in this study produced stable rigid hydrogel. However, microencapsulation of the 6% PVA/pentamidine samples was relatively more difficult than the 3% PVA hydrogel. This was especially true for the high viscous samples (15% PLGA). Processing of these samples resulted in the formation of relatively larger agglomerated particles.

Thermal Analysis

DSC thermograms in Fig. 1 show that the endotherm (at 190°C), characteristic for melting of pentamidine, was absent in the microcapsules as well as in the pentamidine-loaded hydrogel. This could be attributed either to the absence of pentamidine or to the formation of a solution of pentamidine in PVA or to the formation of an amorphous polymorph of pentamidine within the hydrogel due to the freeze-thaw process. Because the system consisted of pentamidine and PVA without possible loss of pentamidine, the absence of the melting endotherms of pentamidine could not be attributed to the absence of pentamidine but rather to the existence of pentamidine in the dissolved state in PVA or formation of an amorphous polymorph. The lowering in the melting temperature of PVA in the pentamidine-loaded hydrogel (peak at 213.1°C) compared to the crosslinked PVA sample without pentamidine (peak at 229.6) and broadening of the peak in the hydrogel further confirmed this conclusion. The melting temperature of PVA in the microcapsules peaked at 222.5°C. The inconsistency in the lowering of the melting peaks of PVA was an indication that it was not the result of an interaction between the drug and PVA but was due to the presence of drug in the polymer. In fact, pentamidine acted as an impurity in PVA, therefore causing the melting endotherm of PVA to become broader and to occur at a lower temperature than PVA alone. During microencapsulation and/or hydrogel formation, the polymer was loaded with pentamidine. At the end of the process, when solvent was removed pentamidine could either crystallize out or remain dissolved in the polymer

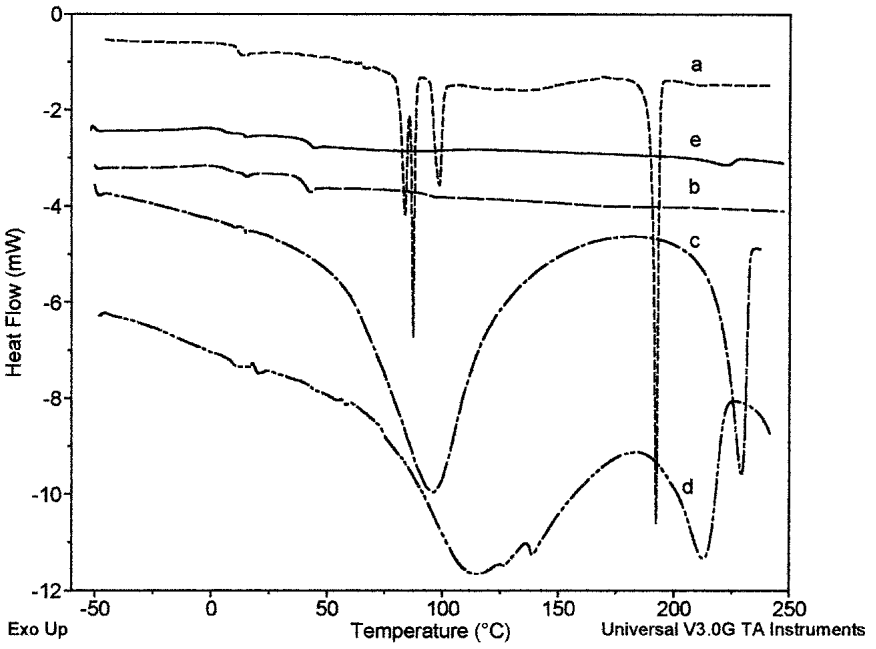

Fig. 1. DSC thermograms of: (a) pentamidine; (b) PLGA; (c) PVA hydrogel; (d) PVA/pentamidine hydrogel; (e) microencapsulated hydrogel.

or transform to an amorphous polymorph. In the former case, where drug crystallizes out, two endotherms would be seen in the DSC trace: one for the melting of the drug and another for the melting of the polymer. These would correspond to the melting endotherms of each of the drug and the polymer alone. In the latter cases, where the drug dissolves in the polymer or transform to an amorphous polymorph, the endotherm for the melting of the drug would be absent and the melting endotherm for the polymer would shift to a lower temperature. One of these two latter situations may have occurred in our study. There was no significant change in the glass transition temperature of PLGA in the microcapsules compared to the pure PLGA (50°C). It can therefore be concluded that pentamidine in the microcapsules was not in the crystalline state and also there was no interaction between PLGA and the pentamidine-loaded PVA hydrogel.

Particle Size and Morphology

Figure 2 shows the SEM pictures of the microcapsules: (a) control microcapsules (Batch D) and (b) microcapsules containing hydrogel (Batch F). Only one SEM picture from each group is included because all batches within the same group show similar surface morphologies. The visual differences among the different batches, within the same group, were similar to the differences within the same batch. The microcapsules of the control group were all spherical in shape with a smooth surface. In contrast, the surface of the microcapsules containing hydrogel was relatively porous. But these microcapsules were also spherical in shape. The presence of pores on the surface of these microcapsules was, possibly, due to the presence of rigid hydrogel. In traditional microencapsulation using solvent evaporation technique (w/o/w), the polymers precipitate on the surface of the liquid emulsion followed by evaporation and/or extraction of the organic solvent. In the present technique, the PLGA solution precipitated on the hydrogel particles and produced a thin coating due to evaporation of dichloromethane. One would expect a smooth surface when the process of solvent evaporation is slow. The presence of pores indicates a rapid evaporation of dichloromethane. These microcapsules were also analyzed for size distribution and the results are listed in Table II. The microcapsules did appear relatively uniform in size. In general, the microencapsulated hydrogel had significantly higher ($p < 0.05$) median particle size than those samples without the hydrogel. Irrespective of the amount of PLGA, an increase in the amount of PVA (3-6%) also increased the median particle size. However, a comparison among these three groups of the microcapsules (A-C, D-F, and G-I) showed that although the third group (G-I) contained the higher amount of PLGA (15%), the median particle size of these batches were significantly smaller ($p < 0.05$) than the second group (D-F; PLGA 10%). This contradictory observation was may be due to the high viscosity of the 15% PLGA solution. Because of the high viscosity, these batches were difficult to process and there were a significant number of large polymer lumps, which had to discard during the washing process. The loss of these polymer lumps may have influenced the median particle size of these batches and reduced the observed value for the same. The median particle size, based on volume% of the microcapsules, from different batches was between 27 and 94 μm. The particles were all smaller than 145 μm.

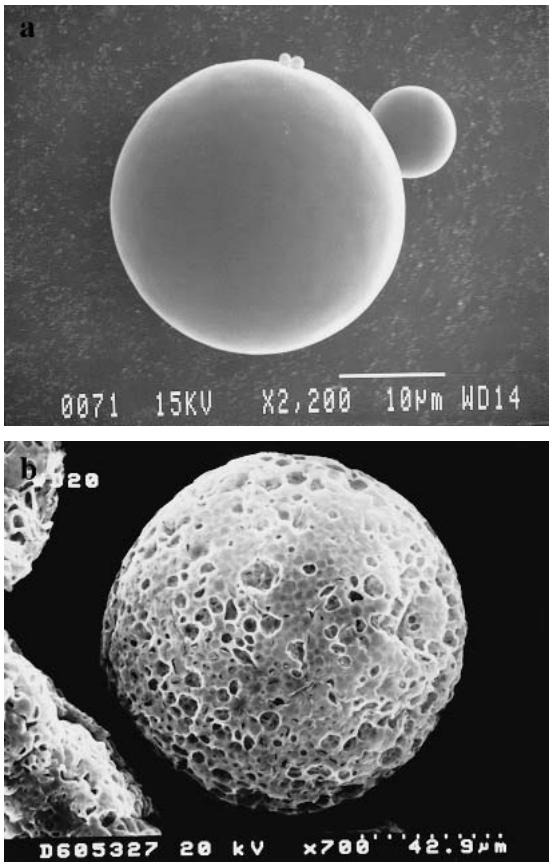

Fig. 2. Typical SEM photographs of microcapsules. (a) Control microcapsules (Batch D); (b) Microencapsulated hydrogel (Batch F).

Efficiency of Encapsulation

Efficiency of encapsulation of pentamidine was determined by measuring the total amount of pentamidine present in each 20 mg sample (i.e., core loading experimental), and comparing this value with the expected amount of pentamidine in each of the samples based on the drug loading during the preparation, (i.e., core loading theoretical) (Fig. 3). The efficiency of encapsulation of all microencapsulated hydrogel was significantly higher than the control batches. The efficiency of encapsulation increased significantly ($p < 0.05$) from approximately 23–31%, when the hydrogel concentration was increased from 3–6% and the PLGA concentration was maintained at 5%. When the PLGA concentration was maintained at 10%, the efficiency of encapsulation was also increased

Table II. Particle Size Distribution Based on Volume %

Batch	Median size (μm)	80% Confidence (μm)
A	27	11–87
B	45	20–85
C	54	25–93
D	48	32–83
E	80	45–139
F	94	65–144
G	43	27–62
H	52	25–77
I	63	38–108

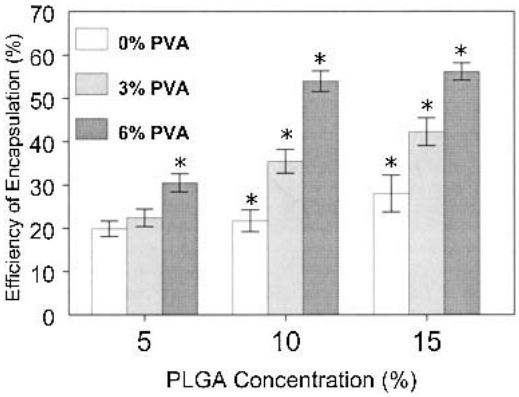

Fig. 3. Effect of PLGA and PVA concentrations on the efficiency of encapsulation of the microcapsules. *Significant Difference, $p < 0.05$.

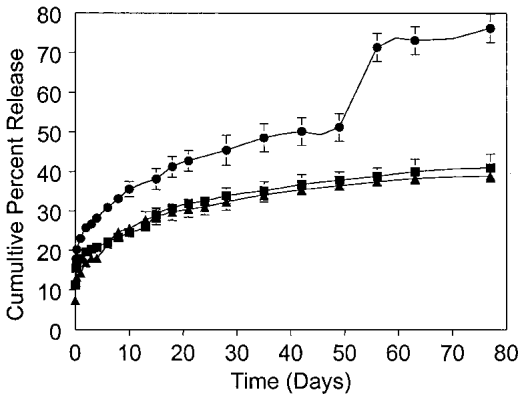

Fig. 5. Dissolution profiles of microcapsules prepared with 10% PLGA. Batch D (●); Batch E (■); Batch F (▲).

significantly ($p < 0.05$) from approximately 22–36% and 54%, due to the incorporation of 3% and 6% PVA, respectively. Similar results were also observed, when the PLGA concentration was maintained at 15%, the efficiency of encapsulations of all three batches were significantly different ($p < 0.05$). These batches showed approximately 2-fold increase in encapsulation when the PVA concentration was increased to 6%.

In vitro Drug Release

Figures 4, 5, and 6 show the dissolution profiles of the microcapsules. All microencapsulated hydrogel batches showed significantly lower initial "burst effect" as well as an overall slow dissolution of pentamidine throughout the studies. All three batches of the control microcapsules (A, D, and G) showed significantly different ($p < 0.05$) drug release comparing with the respective microencapsulated hydrogel batches. Moreover, these three control batches also showed significantly different drug release when compared with each other ($p < 0.05$), but there was no rank-order correlation existing among these three controls; the initial drug release from batch D is higher than batch A. Because the control batch D contained higher amount of PLGA, one would expect relatively lower drug release comparing with the control batch A. This contradictory observation may be explained by considering the solubility of pentamidine in water. Pentamidine is soluble in water and the solubility at room temperature is 100 mg per 1 ml. The first set of the microcapsules (Batches A–C) contained 40 mg pentamidine; as a result the total amount of the drug was dissolved within the 500 μl of deionized water. In contrast, the second set of the microcapsules (Batches D–F) contained 80 mg of pentamidine in 500 μl of deionized water. As a result, there was some excess amount of pentamidine that was not in the dissolved state. These suspended drug particles were trapped within the surface of the batch D and released within the first 2 days of the dissolution and resulted in a significantly higher ($p < 0.05$) drug release during this period. However, once these drug particles were released, drug release from this batch (D) followed a rank-order correlation, (i.e., an increase amount of PLGA reduced the dissolution profiles). Although, the third control batch G also contains a significant amount of suspended drug particles, drug release from this batch showed relatively lower drug release. This slow release may be due to the presence of an excess amount of PLGA in this batch, which ultimately increased the coating thickness and reduced the overall drug release, as expected. A comparison of the overall dissolution profiles of these three control batches (A, D, and G) also showed that "batch A" showed a second "burst effect" between 2 and 3 days, during which the drug release abruptly increased from 23–34%, whereas "batch D" showed the "second burst" effect between 49 and 56 days, but "batch G" did not show any such "burst effect". The appearance of initial "burst effect" during the dissolution of microcapsules is mainly due to the presence of drug on or near the

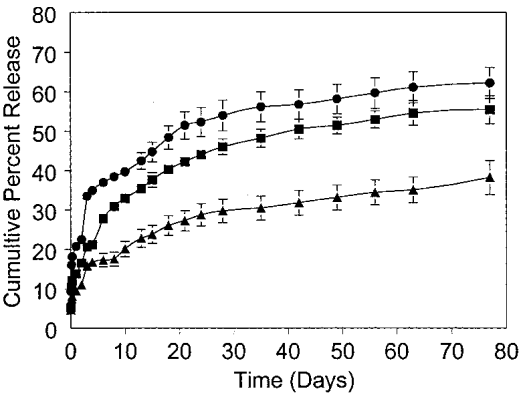

Fig. 4. Dissolution profiles of microcapsules prepared with 5% PLGA. Batch A (●); Batch B (■); Batch C (▲).

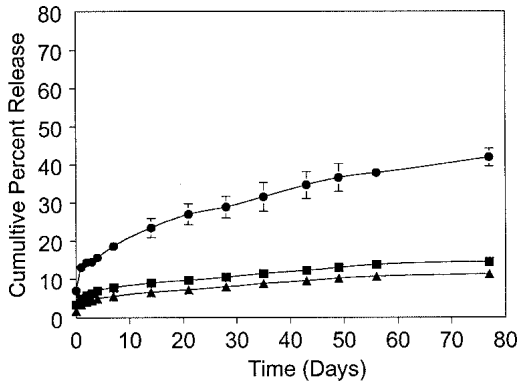

Fig. 6. Dissolution profiles of microcapsules prepared with 15% PLGA. Batch G (●); Batch H (■); Batch I (▲).

surface of the microcapsules, however, the appearance of the second "burst effect" is mainly due to the erosion and/or degradation of the matrix. So, excess amounts of PLGA (as in batches D and G) also delayed or eliminated the appearance of the second "burst effect".

When the concentration of PLGA was maintained at 5%, the drug release within the first 24 h decreased significantly ($p < 0.05$) from approximately 21–14% due to the presence of 3% PVA. This trend continued when the PVA concentration was increased further. The initial release decreased to approximately 10% when the concentration of PVA was 6%. Similar results were also observed when the concentration of PLGA was maintained at 10%, (i.e., an increase in the amount of PVA) significantly reduced ($p < 0.05$) the initial drug release when compared with the control microcapsules.

When the concentration of PLGA was maintained at 15%, the overall drug release from all three batches within this set reduced drastically. The initial drug released within the first 24 h from the control batch (G) was significantly higher ($p < 0.05$) than the two test batches (H and I). The initial drug release from the control batch was approximately 13% and the drug release from the microencapsulated hydrogel was between 3 and 5%, respectively, during the same period. The overall drug release from these microencapsulated hydrogel (Batches E and F) was extremely low due to the synergistic effect of high amount of PLGA and PVA hydrogel. The overall slow release from these microencapsulated hydrogel was either due to degradation of pentamidine within the matrix or the entrapment of pentamidine within the hydrogel matrix. The later was the situation in the present studies, because the total amount of unreleased pentamidine was determined within the matrix at the end of the dissolution experiments. In traditional PLGA microcapsules, drug release, toward the end of the dissolution studies, is governed primarily by the erosion and/or degradation of the PLGA and shows high drug release. In the present studies, the hydrogel maintained its matrix even at the end of 77 days and drug release occurred only through the diffusion and dissolution of pentamidine. SEM picture was taken at the end of the dissolution study after freeze-drying. The presence of hydrogel matrix was evident in this picture (picture not included).

Mass Loss Study

Mass loss during a specified sampling period was determined by measuring the weight loss (%) using the following equation:

$$\text{Weight loss (\%)} = [(\text{initial weight} - \text{residual weight})/\text{initial weight}] \times 100.$$

The initial mass loss during the first 7 days was 16% and the mass loss continued steadily up to 42 days followed by a rapid mass loss between 42 and 56 days. The weight of the samples decreased from 69–50% of the initial weight during this period. This observation was consistent with the SEM picture taken at the end of the dissolution study (picture not included).

CONCLUSIONS

The microencapsulated hydrogel prepared in this study was porous and spherical in shape. Irrespective of the concentration of PVA hydrogel, the use of a crosslinked hydrogel can significantly increase the drug loading of highly water-soluble drugs. Thermal analysis showed that pentamidine was not present within the hydrogel in the crystalline form. As a result, incorporation of the PVA hydrogel significantly reduced the burst effect and overall dissolution of pentamidine. The technique used was successful to prepare small microcapsules. This technique can be used to prepare long acting formulations of various bioactive compounds. The conditions used in this technique were very mild and would be appropriate for drugs with various physicochemical characteristics. Controlled release systems suitable for long-term delivery of drugs can be prepared by using microencapsulated hydrogel with optimum concentration of PVA hydrogel and PLGA.

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