Pharmaceutical Research, Vol. 22, No. 5, May 2005 (© 2005)
DOI: 10.1007/s11095-005-2600-0

Research Paper

Controlled Release of Dexamethasone from Microcapsules Produced by Polyelectrolyte Layer-by-Layer Nanoassembly

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Received November 20, 2004; accepted January 28, 2005

Purpose. In an effort to expand the application of core-shell structures fabricated by electrostatic layer-by-layer (LbL) self-assembling for drug delivery, this study reports the controlled release of dexamethasone from microcrystals encapsulated with a polyelectrolyte shell.
Methods. The LbL self-assembly process was used to produce dexamethasone particles encapsulated with up to five double layers formed by alternating the adsorption of positively charged poly(dimethyldiallyl ammonium chloride), negatively charged sodium poly(styrenesulfonate) and depending on the pH positively or negatively charged gelatin A or B onto the surface of the negatively charged dexamethasone particles. The nano-thin shells were characterized by quartz crystal microbalance measurements, microelectrophoresis, microcalorimetry, confocal microscopy, and scanning electron microscopy. *In vitro* release of dexamethasone from the microcapsules suspended in water or carboxymethylcellulose gels were measured using vertical Franz-type diffusion cells.
Results. Sonication of a suspension of negatively charged dexamethasone microcrystals in a solution of PDDA not only reduced aggregation but also reduced the size of the sub-micrometer particles. Assembly of multiple polyelectrolyte layers around these monodispersed cores produced a polyelectrolyte multilayer shell around the drug microcrystals that allowed for controlled release depending on the composition and the number of layers.
Conclusions. Direct surface modification of dexamethasone microcrystals via the LbL process produced monodispersed suspensions with diffusion-controlled sustained drug release via the polyelectrolyte multilayer shell.

KEY WORDS: controlled release; dexamethasone; layer-by-layer; microcapsules; polyelectrolytes.

INTRODUCTION

Recently, a layer-by-layer (LbL) self-assembly for preparing ultra-thin films based on the electrostatic attraction (1–5) between oppositely charged polyions began to receive attention for its application in drug delivery (6–11). In this method, the spontaneous sequential adsorption of dissolved anionic and cationic polyelectrolytes, depending on the charge of the substrate, leads to the formation of ordered multilayer assemblies on the solid substrate surface (2,12–15). The reversal of surface charge after each immersion is the crucial factor for a stepwise growth of the multilayer films by this electrostatic LbL self-assembling process (16). Unlike liposomes, polyelectrolyte multilayer microcapsules are tough and homogenous (6). Templates such as organic and inorganic colloid particles can be coated and the templates then decomposed to produce stable capsules with defined size, shape, and shell thickness (17). In addition, the LbL process can be completed on almost any substrate. However, the substrate particles must have a change in the solution in which the coating is performed and the substrate must not dissolve in the aqueous coating solution (1,6–8).

Qui *et al.* (6) and Ai *et al.* (7) demonstrated that this process can be used to encapsulate drug microcrystals with stable nanoshells. In these studies, it was shown that the release rate of the drugs depended on the solubility and the size of the microcrystals, the number of layers and thickness of the shell, and the type of polyion used in the LbL assembling process. For the poorly water soluble drug furosemide, it was shown that gelatin/polyelectrolyte bilayers was a substantial improvement over shells constructed from poly(allylamine) or chitosan and reduced drug release up to 300 times when compared to uncoated drug microcrystals (7). Antipov *et al.* (14,16) concluded that these polyelectrolyte shells assembled around a core consisting of a low molecular weight compound provide barrier properties for release under conditions where the core dissolves and the composition of the shell is optimized.

Based on these studies, the LbL encapsulation process might solve many problems associated with drug formulation, release, and delivery (17–19). In an effort to expand the application of core-shell structures fabricated by this electrostatic LbL technique for drug delivery, this study also reports the controlled release of dexamethasone from nano/microcrystals encapsulated with a polyelectrolyte shell. Dexa-

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methasone is a glucocorticoid that is clinically used for its anti-inflammatory and immunosuppressive effects and was chosen as a model drug because of its potential use in specialized delivery systems in treating peritumoral edema associated with brain tumors and for treating and preventing retinal diseases. A number of side effects, such as hypertension, hydroelectrolytic disorders, hyperglycemia, peptic ulcers, and glucosuria, which restricts the use of dexamethasone in conventional prolonged delivery systems, could be addressed by an alternative delivery system (20). In addition, the advantageous properties of gelatin were further explored; the use of sonication in polyelectrolyte solution to produce monodispersed microcapsules is shown; and the use of heat of adsorption measurements to follow the LbL assembling process is reported.

MATERIALS AND METHODS

Materials

Micronized dexamethasone USP (MW: 392.47) was obtained from Spectrum Chemicals (Gardena, CA, USA). Cationic poly(dimethyldiallyl ammonium chloride) (PDDA; MW 400,000; Sigma Aldrich, St. Louis, MO, USA), anionic sodium poly(styrenesulfonate) (PSS; MW 70,000), and the charged polypeptides, gelatin type A (acid pretreated or porcine gelatin; Bloom strength 300, MW 50,000~100,000) and gelatin type B (alkali processed or bovine gelatin; Bloom strength 225, MW 50,000~100,000), were obtained from Sigma Aldrich. The solvents and dispersion mediums used in this study were 10 mM phosphate buffer pH 5.8 (PBS; Sigma Aldrich), purified deionized water, and 0.2 M PBS buffer 7.2 (21). Fluorescein-5-isothiocyanate (FITC; Sigma Aldrich) was used for labeling both uncoated and encapsulated dexamethasone microcrystals.

Layer-by-Layer Assembly on Dexamethasone Particles

The presence of a charged surface is a prerequisite for successful layer-by-layer assembly. A dexamethasone suspension was prepared in PBS buffer pH 5.8 with a concentration of 2 mg/ml and the charge measured. Dexamethasone is hydrophobic and insoluble in water. Based on the negative charge on the suspended drug particles, 5–10 mg of micronized dexamethasone was suspended in 5 ml of PDDA solution (2 mg/ml in PBS buffer pH 5.8). The isoelectric point of PDDA is 12 and therefore has a net positive charge at pH 5.8 (3). The solution was then sonicated for 15 min in the presence of PDDA. The suspension was transferred to 1.5 ml centrifuge tubes and centrifuged at 10 000 RPM for 5 min (model 5804R, Eppendorf, Westbury, NY, USA). The separated drug particles were washed three times with PBS buffer. This completed the first layer of polymer on the drug particles. Zeta potential measurements indicated reversal to positive charge due to PPDA masking the negative drug particle surface charge. The particles were then resuspended in 5 ml of PSS solution (2 mg/ml at pH 5.8), stirred for 20 min to ensure coating, centrifuged, and then washed three times. At this pH, PSS carries a net negative charge because the isoelectric point of PSS is below 1.0. This completes the assembly of the first layer. Where gelatin was used in the assembling process, it was dissolved in the PBS pH 5.8 at a concentration of 2 mg/ml. Gelatin A has a very weak positive charge at pH 5.8 because the isoelectric point of gelatin A is ~7–9 while gelatin B has a net negative charge because the isoelectric point is ~4–5. Depending on the charge, gelatin was layered with either PDDA or PSS. This process was repeated for the assembly of multiple layers around the dexamethasone particles by this LbL assembling process. There was approximately 20–30% loss of material during the entire procedure. The composition of the layers studied were dexamethasone core/$(PDDA/PSS)_4$/PDDA; dexamethasone core/$(PDDA/\text{gelatin A})_4$/PDDA, dexamethasone core/$(PDDA/\text{gelatin B})_4$/PDDA, and dexamethasone core/PDDA/$(PSS/\text{gelatin A})_4$/$(PSS/PDDA)_1$.

Zeta Potential Measurements

To ensure the reversal of charge after each polyion coating, the zeta-potential of the suspended particles were measured and the reported results represents the mean of 10 measurements determined with a Zeta-plus photon correlation spectroscopy and microelectrophoresis instrument (Brookhaven Instruments, Holtsville, NY, USA).

Particle Size and Morphology

Dexamethasone powdered samples were suspended in purified/filtered water containing the cationic polyelectrolyte PDDA (2 mg/ml) or anionic PSS (2 mg/ml) and sonicated from 0.5 to 8 min (model 1510, 40 KHz; Branson Ultrasonics, Danbury, CT, USA). Samples from these suspensions were added to a small volume stirred cell to obtain a desired obscuration, and then the geometric particle size was measured by Malvern laser light scattering (Malvern Mastersizer X; Malvern, UK) using a 100-mm Fourier transform lens. As an estimate for the size of the PDDA-coated dexamethasone particles filtered through a 1.2-μm filter, the intensity weighted mean diameter determined by photon correlation spectroscopy (PCS; Malvern Zetasizer 4, He-Ne-laser λ 633 nm) at 25°C under an angle of 90 degrees was measured. All samples were diluted with demineralized particle-free water to an adequate scattering intensity prior to the measurement.

A Philips XL 30 scanning electron microscope (Philips, Eindhoven, The Netherlands) was used to obtain photomicrographs of the sonicated dexamethasone micronized particles. Samples were mounted on a metal stub with an adhesive and coated under vacuum with carbon (Emscope TB500 sputter-coater; Emscope Laboratories, Ashford, UK) before being coated with a thin gold-palladium film (Eiko Engineering Ion Coater IB-2; EIKO Engineering, Ibaraki, Japan). Confocal laser scanning microscopy (model DMI RE2; Leica, Allendale, NJ, USA) and fluorescent spectrometry (Photon Technology International, Lawrenceville, NJ, USA) were used to analyze optical properties and structures of encapsulated dexamethasone particles. For fluorescence, the particles were resuspended in deionized water and 1 μl of FITC added; FITC attaches to PDDA. The fabricated particles were incubated overnight with FITC.

Quartz Crystal Microbalance Study of the Layer-by-Layer Assembly

Prior to polyion multilayer formation on the dexamethasone microcrystals, the coating procedure was elaborated on

gold electrode resonators of 9-MHz quartz crystal microbalance (QCM; USI-Systems Inc., Kyoto, Japan). The resonators were immersed in a polyion solutions for 15 min, removed, and dried. The added mass and the coating thickness (ΔL) can be calculated from the frequency shift (ΔF), according to the Sauerbrey equation and using a special scaling (7). For the instrument used in this study, the calibration was ΔL (nm) = 0.017 ΔF (Hz). These optimized assembly conditions were applied to the LbL shell assembly on microcrystals.

Microcalorimetric Measurements

The heat involved in the formation of the layers at 25°C was measured with a micro differential scanning calorimeter (Micro DSC III; Setaram, Caluire, France) in isothermal mode using 1-ml batch mixing vessels. The suspended drug core particles were placed in the bottom of the mixing vessel while the polyelectrolyte solution was added to the top reservoir. Once the instrument was equilibrated at 25°C, the plunger was pushed down allowing the electrolyte solution to come in contact with the drug suspension. The heat measured, once corrected for the heat involved in mixing and the addition of solvent without electrolyte, represents the heat involved in the attachment of the polyelectrolyte molecules to the core particle surface.

In Vitro Release

In this study, the release of dexamethasone from suspensions and gels (1% carboxymethylcellulose aqueous gel) was measured using vertical Franz-type diffusion cells (Permegear Inc., Bethlehem, PA, USA). The system used in this study consisted of six cells each with a polyethylene sample ring with a 1-cm-diameter hole at the center, the same size as the opening in the vertical receptor cells, which is placed on top of the 0.2-μm cellulose acetate membranes (Osmonics Inc., Minetonka, MN, USA) and then filled with the suspension or semisolid. The membrane with the sample was placed on top of the vertical receptor cell and clamped tightly into place. The receptor cells were filled with the dissolution medium (phosphate buffer pH 7.2), and a small magnetic stirrer placed in each cell was used for mixing. Samples were removed from the receptor cell at predetermined times, filtered, and suitably diluted. The amount of dexamethasone in solution was determined by measuring the ultraviolet absorbance at 239 nm with a Multispec-1510 spectrophotometer (Shimadzu, Kyoto, Japan). The UV-method was calibrated and complied with generally accepted specifications for linearity and precision.

RESULTS

Dexamethasone is a white, odorless, crystalline powder ($C_{22}H_{29}FO_5$, MW = 392.47). The commercially available product is micronized (total particles <20 μm, ≥99%; total particles <10 μm, ≥75%). The drug is stable in air and practically insoluble in aqueous media (20,21). Scanning electron photomicrographs (Fig. 1) of the micronized powder shows the aggregation of the small drug particles. Sonication for 10 min of an aqueous suspension (0.5% w/v) reduced the extent

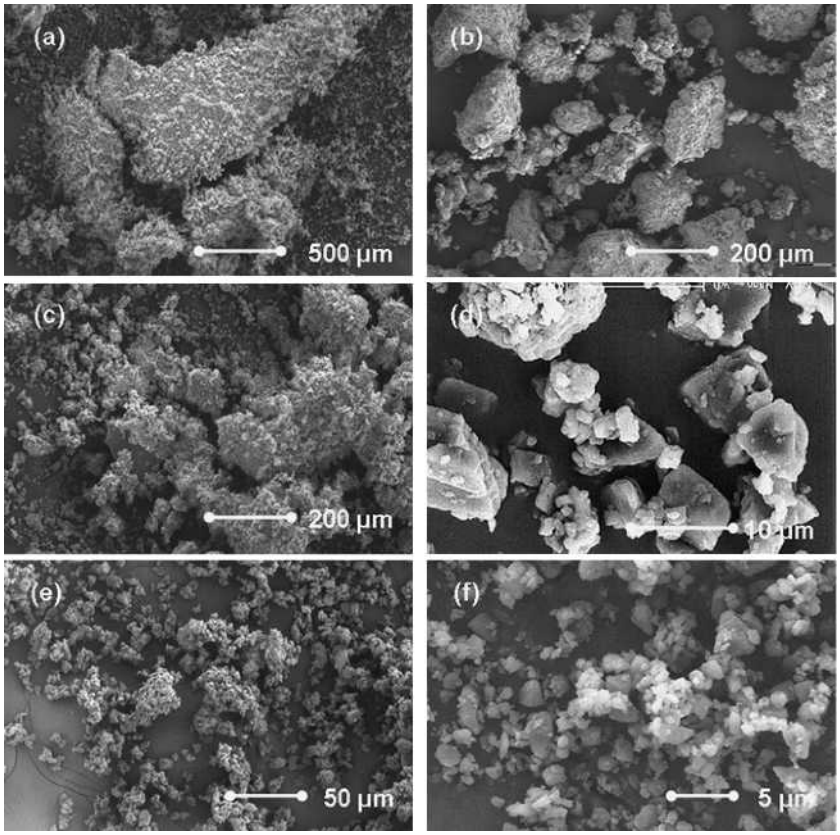

Fig. 1. SEM photomicrographs of (a) micronized dexamethasone powder and powder recovered from (b) an aqueous suspension sonicated for 10 min; (c) an aqueous suspension containing 2 mg/ml PSS sonicated for 10 min; (d) close-up of sample c; (e) an aqueous suspension containing 2 mg/ml PDDA sonicated for 10 min; (f) close-up of sample e.

of aggregation, but the powder was still very cohesive. When a similar suspension of the drug was prepared in a 2 mg/ml solution of the anionic electrolyte PSS and sonicated for 10 min, aggregation was significantly reduced, and close inspection of the particles (Fig. 1d) show larger individual particles, 10–20 μm, coated by smaller particles. Sonication of a suspension prepared in a 2 mg/ml solution of the cationic polyelectrolyte PDDA not only reduced aggregation but also reduced the size of the individual particles (Fig. 1f). In this sample, a large number of fine particles, <1 μm, was observed.

Comparison of the particle size (27.93 μm) of the powder dispersed in deionized water in the sample cell of the particle analysis instrument with a magnetic stirring bar to the sonicated sample (Fig. 2) measured under the same conditions showed that the mean volume particle size of the micronized drug powder was 11.23 μm with a normal unimodal particle size distribution. The mean volume particle size of the powder sonicated in the PSS solution, 9.37 μm, was not different from that of the suspension sonicated in water. Sonication in a PDDA solution, 2 mg/ml, reduced the mean size of the particles to 2.54 μm. UV analysis of the filtered solutions after sonication confirmed that the drug did not dissolve in the suspension mediums. Figure 3a shows the effect of an increase in sonication time on the mean volume particle size of the dexamethasone powder in the PDDA solution. The mean size decreased according to a first-order decay process (r^2 = 0.993), and a maximum decrease in the particle size was reached after 8 min. Sonication up to 30 min did not lead to a further decrease in particle size.

Because microscopic evaluation showed that the suspension in a PDDA solution prepared by sonication led to a significant reduction in the size of the dexamethasone particles as shown in Fig. 1f; this suspension was filtered and the particle size distribution of the filtered solution measured. The PCS measured size distribution of the dexamethasone suspension in 2 mg/ml PDDA solution sonicated for 10 min and filtered through a 1.2-μm filter is shown in Fig. 3b. The number mean size of the particles was 150 nm, and the mean volume diameter was 420 nm. The filtered sub-micrometer particles were polydispersed with a wide size distribution (distribution and SEM picture shown in Fig. 3b).

Figure 4 shows confocal photomicrographs of the micronized dexamethasone particles sonicated in the absence and presence of FTIC-labeled PDDA. Without the addition of PDDA, the cohesive, small dexamethasone particles adhere to each other to form large aggregates. When sonicated in a solution containing PDDA, the particles are coated with the PDDA, and the coated particles do not aggregate as shown in Fig. 4b. The small particles are uniformly dispersed throughout the medium. Computer generated 3D images (Fig. 4) of the coated particles shows the fluorescence intensity of the labeled polymer shell, which indicated the thickness of the polymer shell around the individual dexamethasone core crystals. Figure 5 shows a single dexamethasone particle with a diameter of ~2.5 μm coated with multiple layers of PDDA and gelatin. In solution, the addition of the polyelectrolyte layers almost doubled the diameter of the particle.

Surface electrical potential (zeta potential) for the dexamethasone nano/microcrystals at each stage of the layering process are shown in Fig. 6. The uncoated drug is negatively

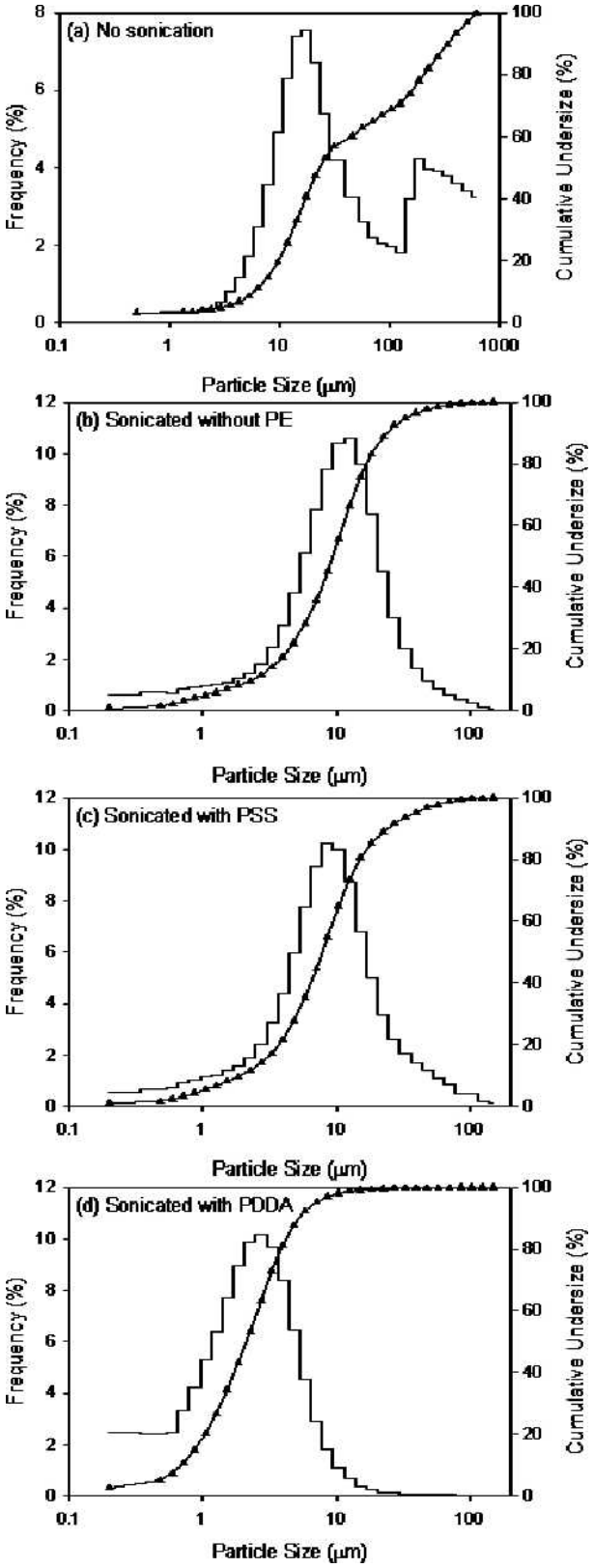

Fig. 2. Particle size distribution profiles for micronized dexamethasone measured in deionized purified water with and without polyelectrolytes or sonication: (a) Drug powder without sonication; (b) drug powder sonicated without PDDA for 10 min; (c) drug powder sonicated with PSS for 10 min; (d) drug powder sonicated with PDDA for 10 min.

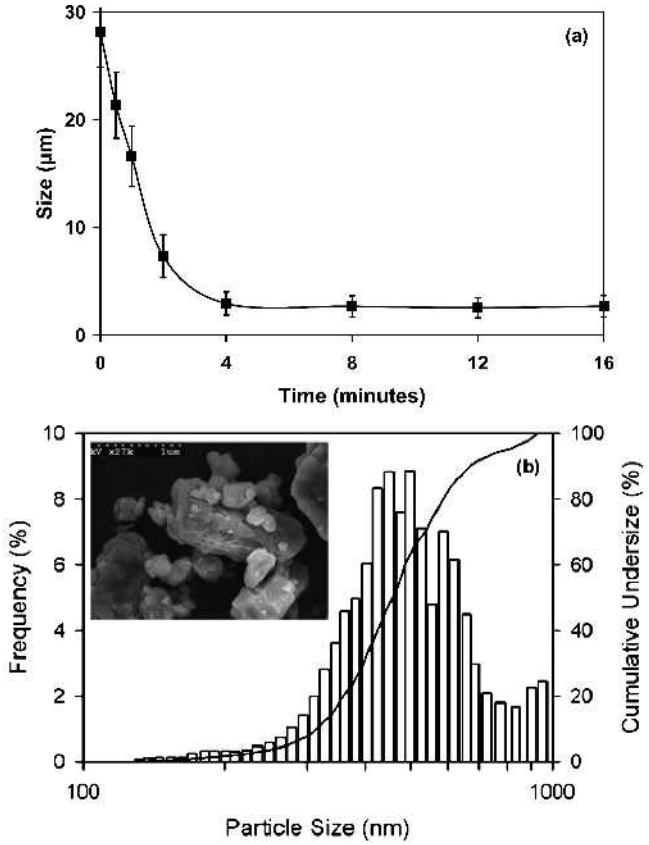

Fig. 3. (a) The change in mean volume particle size of dexamethasone when sonicated in a solution of the cationic polyelectrolyte PDDA. (b) Particle size distribution (mean size ~420 nm) for the PDDA-coated particles prepared by sonication and filtered through a 1.2 μm membrane filter. This represents the size distribution of the nanosized particles produced by the sonication process. Insert is a SEM picture of the nanosize particles; the bar represents 1 μm. Mean ± SD, n = 5.

charged with ζ-potential –32 mV when suspended in PBS buffer. The first PDDA layer reversed the charge to +30 mV. The first PSS coating changed the charge to –50 mV. The reversal in change followed the same trend with additional layers and the magnitude of the change remained constant up to 3 complete bilayers (Fig. 6). The surface ζ-potential of the fourth PSS layer declined from –50 mV at the first to –30 mV at the fourth layer. Probably, at some areas PSS (MW = 70 000) did not completely overcome the more bulky PDDA layer (MW = 400 000). This decline could limit total thickness of the shell after 4 bilayers of PDDA/PSS. When PDDA(+) was altered with the polyampholyte gelatin, the gelatin layers tended to have smaller ζ-potentials, averaging –15 mV for gelatin A and –22 mV for gelatin B. When gelatin A, predominantly positively charged at pH 5.8, was layered with PSS(–) the average zeta potential of the layers was +10 mV. The reversal in change and magnitude of the change remained constant with additional layers up to 4 bilayers of PDDA/gelatins or gelatin A/PSS (Fig. 6).

The alternating surface charge of coated drug crystals is strong evidence that the layer-by-layer assembly of the oppositely charged components was successful. In this study, PDDA was always added as the final layer (Fig. 6) because sonication studies (Figs. 1 and 2) showed that when the dexamethasone particles were coated with this polyelectrolyte, it reduced aggregation of the dexamethasone suspensions and ensured the production of monodispersed microcapsules. In addition, the results demonstrated that the amphoteric polyelectrolyte gelatin A with theoretically a net positive charge at pH 5.8 can have local charge imbalances allowing it to be layered with the strongly positively charged PDDA or the negatively charged PSS.

Heat of adsorption measurements (Fig. 7) was also used to monitor the LbL assembly. The data in Fig. 7 shows that the interaction between the negatively charged dexamethasone and the positively charged PDDA was an endothermic process that required 21.4 mJ. PSS interacted with the PDDA layered over the dexamethasone crystals by an exothermic process that produces 5.5 mJ heat. The heat of adsorption was more than the 3.1 mJ produced when the solution of PSS was added to a solution of PDDA. Additional layers of PDDA/PSS layered onto the first bilayer produced consistent endothermic (PDDA, 2.7 mJ) and exothermic (PSS, 2.6 mJ) heats of adsorption. When PSS was replaced with gelatin A or gelatin B, the magnitude of the heat of adsorption involved in the interaction of the positively charged PDAA with the negatively charged polyelectrolyte was reduced to 1.5 mJ for gelatin A and 1.9 mJ for gelatin B when compared to 5.5 mJ when PSS was layered onto PDDA (both endothermic processes). The heats of adsorption were different for the two gelatins, and the heat of adsorption involved in the layering process was higher than the heat of mixing with PDDA. When the gelatin and PSS solutions was mixed, the heat produced by the addition of gelatin A was higher, 0.9 mJ, than gelatin B, 0.4 mJ. This demonstrated that the positively charged gelatin A more strongly interacted with PSS than gelatin B, which is negatively charged at pH 5.8. However, alternating PSS with gelatin A at pH 5.8 during the assembling process produced such small heat changes that it was not possible to measure it accurately with the microcalorimeter. Still constant and repeatable changes in the heat of adsorption measurements were also strong evidence of the layering process.

Because it is difficult to measure the thickness of the polyion capsule, QCM measurements were used to estimate shell thickness (7,10,16). The average frequency for the first PDDA layer was 147 Hz, which corresponded to a thickness (ΔL) of ~2–3 nm. Similarly, the thickness of the other LbL assemblies used in this study was calculated and the results are listed in Table I. The PDDA/PSS layers were the thinnest with the PDDA/gelatin layers on average being at least 4 times as thick as this layer. The PDDA/gelatin layer assembled with gelatin B was about 1.7 times as thick as gelatin B layers. As reported earlier, PSS/gelatin A layers was the thickest at 50–60 nm (7). The measured shell thickness that was seen under the confocal microscope for the hydrated nano-shells (Figs. 4 and 5) indicated that the gelatin layers swelled significantly when suspended in water. In addition, previous studies have found that layer thickness estimated from QCM measurements is half of the thickness on microtemplates such as drug crystals (7,10,16). The QCM data listed in Table I also confirmed the successful assembling of the polyion shells around the negatively charged dexamethasone crystals.

Release profiles of dexamethasone in PBS buffer pH 7.2 are shown in Figs. 8a and 8b. A solution of dexamethasone in

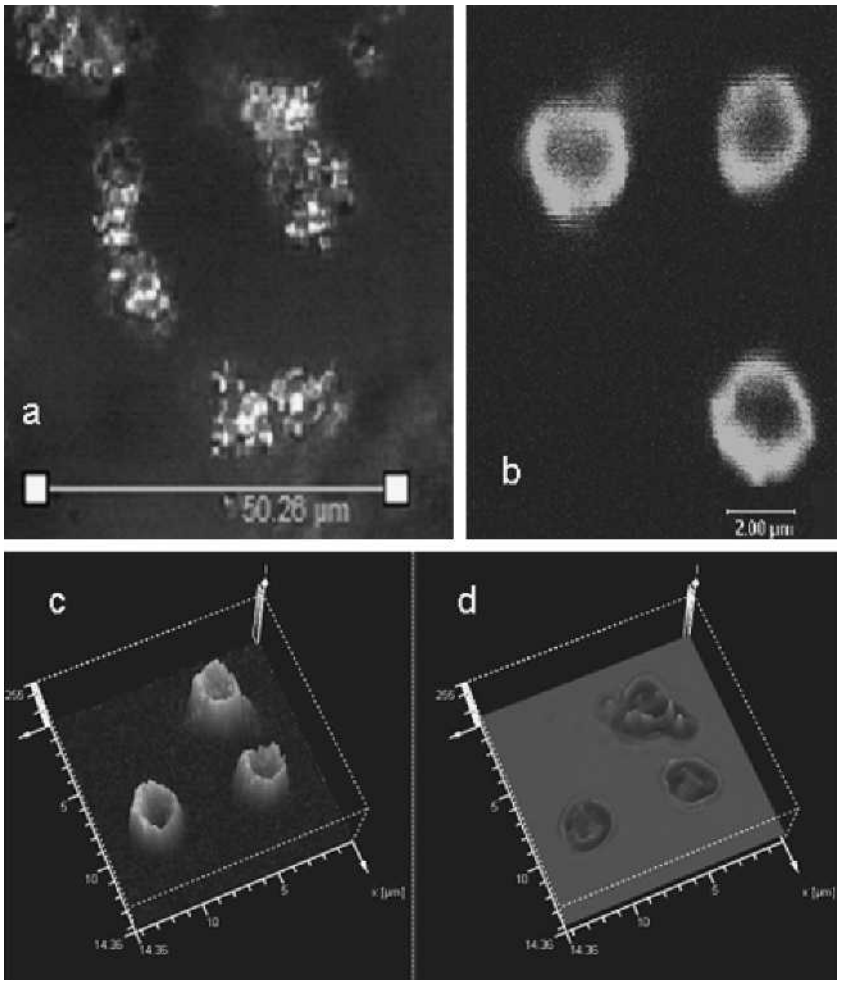

Fig. 4. Confocal photomicrographs of (a) aggregated dexamethasone particles sonicated without polyelectrolyte, (b) deaggregated particles coated with FTIC labeled PDDA; (c) and (d) are fluorescent and bright field mode computer-generated 3D images of dexamethasone particles layered with PDDA labeled with FITC showing the fluorescence intensity of the labeled polyelectrolyte shell around individual dexamethasone core crystals.

an ethanol water mixture (1:1 v/v) almost instantaneously diffused throughout the receptor cell (>90% dissolved in 2 min). Dexamethasone release from an aqueous suspension sonicated in the absence of PDDA was much slower than a suspension prepared in a PDDA solution. When the suspension in the PDDA solution was filtered through a 1.2-μm filter, the dissolution of the nanosized particles was slightly faster than that of the unfiltered sample. LbL coating of the dexamethasone particles with $(PDDA/PSS)_4$/PDDA slowed down the release compared to the PDDA sonicated particles, but the release from these particles were very similar to the suspension prepared without PDDA (Fig. 8a).

LbL assembling with gelatin of $(PDDA/gelatin\ A)_4$/PDDA, $(PDDA/gelatin\ B)_4$/PDDA or PDDA/$(PSS/gelatin\ A)_4$/$(PSS/PDDA)_1$ significantly decreased dexamethasone release from the suspended microcapsules. Sustained drug release from the microcapsules reached 80% for PDDA/gelatin A, 60% for PDDA/gelatin B, and 60% for gelatin A/PSS coated particles after 2 h (Fig. 8). The release profiles were best described by a model that represents systems where drug diffusion occurs through a polymeric structure or network, $M_t/M_\infty = kt^n$ ($r^2 > 0.990$), where M_t/M_∞ is the fractional release of the drug, t is the release time, k is a constant incorporating structural and geometric characteristics of the controlled release device, and n is the release constant, indicative of the mechanism of drug release (22,23). Matching amphoteric gelatin A and B according to their charge at a specific pH with the correct polyelectrolyte slowed down the release of the drug more than when predominantly positively gelatin A was layered with positively charged PDDA (Fig. 8).

In Fig. 9, the release from gels containing 0.1% w/v of the dexamethasone microcrystals or microcapsules suspended in 1% sodium carboxymethylcellulose gels are shown. The release was measured using vertical Franz-type diffusion cells with 1-cm-diameter opening. The rates of release from the gels were in the same order as the release from the suspensions as shown in Fig. 8: PDDA coated particles (0.163 μg cm^{-2} min^{-1}) > $(PDDA/PSS)_4$ coated particles (0.076 μg cm^{-2} min^{-1}) ≥ uncoated particles (0.071 μg cm^{-2} min^{-1}) > PDDA/$gelatin\ A)_4$ coated particles (0.043 μg cm^{-2} min^{-1}) > $(PDDA/gelatin\ B)_4$ coated particles (0.033 μg cm^{-2} min^{-1}).

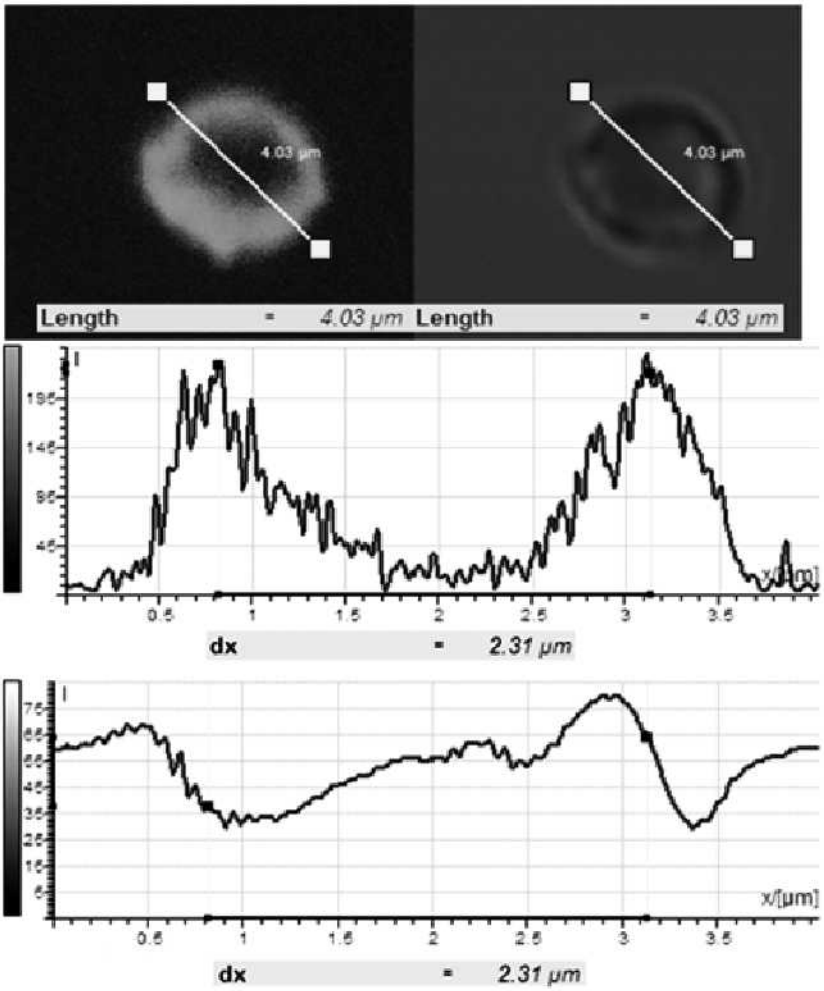

Fig. 5. Confocal photomicrograph of a dexamethasone particle where the bright field image (right) indicates the presence of the core and the characteristic peripheral fluorescent ring (left) indicates the shell. For this microparticle, the fluorescence intensity peak to peak distance measured show the diameter of the particle is 2.31 µm.

DISCUSSION

This study reports the successful liquid dispersion of cohesive sub-micronized dexamethasone crystals by electrostatic coating with a positively charged polyelectrolyte, PDDA, and the subsequent self-assembling of multiple layers of polyions around the crystals to produce controlled release microcapsules in a two phase process as shown in Fig. 10. The effective adsorption of the first layer of PDDA was evidenced by the microelectrophoresis measurement of the reversal in charge and the enhanced dispersability of the dexamethasone particles because of the colloidal stabilization produced by the positively charged PDDA coating. Stabilizing of the practically monodispersed particles could also reduce the Ostwald maturation of the colloidal particles of the scarcely soluble dexamethasone. The shell formed around the microcrystals also preserved the integrity and the shape of the original crystals.

Continued assembling of multiple polyelectrolyte layers around the cores produced an electrostatic polyelectrolyte multilayer shell around the drug microcrystals. The shell thickness and diameter of the microcapsules assembled

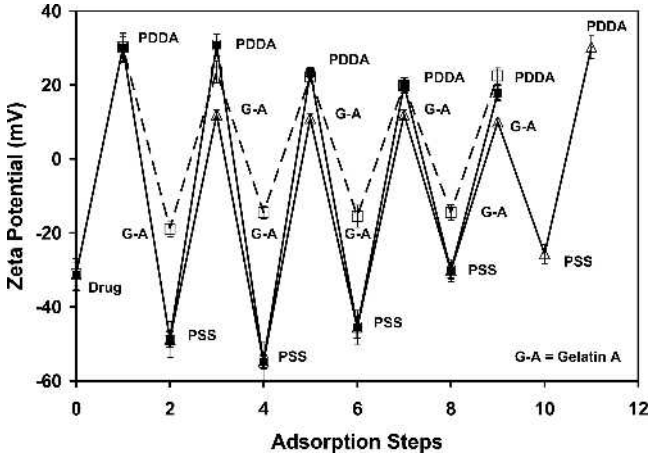

Fig. 6. Changes in the zeta potential of the coated dexamethasone particles as a function of the number of adsorption steps for capsule composition of dexamethasone core/$(PDDA/PSS)_4$/PDDA (solid line and solid squares), dexamethasone core/$(PDDA/gelatin\ A)_4$/PDDA (broken line and open squares), and dexamethasone core/PDDA/$(PSS/gelatin\ A)_4$/$(PSS/PDDA)_1$ (solid line and open triangles).

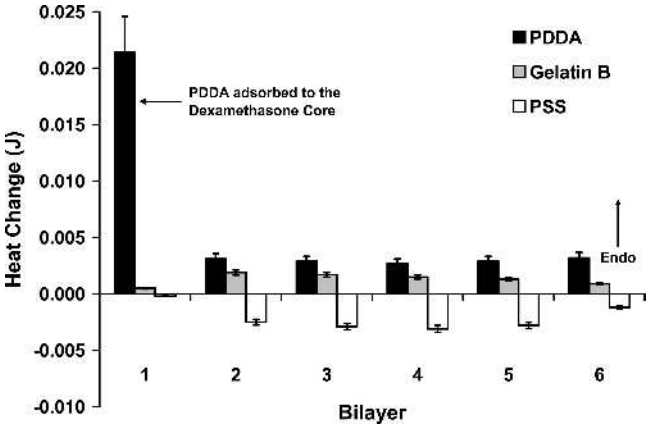

Fig. 7. Changes in the heat of adsorption for the assembling of the polyelectrolyte bilayers. For each bilayer, the heat of adsorption for both the positive (PDDA) and negative ions (gelatin B and PSS) are shown. Mean ± SD, n = 4.

around the microcrystals can be varied with the precision of a few nanometers by varying the combinations of polyelectrolytes used in the assembling process. The encapsulation efficiency of this method was very high. Typically the polymer: drug ratio of 10:1 is used in conventional polymer-based drug delivery systems. In the LbL shell approach studied here, the shell thickness of the capsule range from 25 to 60 nm, which is much smaller than the core drug crystal dimensions and gives a polymer shell to drug core thickness ratio of about 1:50.

Microelectrophoresis measurements showed that the binding of PDDA to the negatively charged dexamethasone caused a large reversal in charge. The reversal in change for the binding of PSS to PDDA was similar; but the binding of gelatin A or B to PSS or PDDA caused smaller changes in charge. Gelatin is a bulky large molecular weight protein that is a weak polyampholyte with a low charge density. In other studies, type A gelatin was used in the LbL assembling process where the pH was adjusted to an acidic pH, which is below the isoelectric point and the molecule is predominantly positively charge (7,8). Results reported here show that when a solution of type A gelatin is adjusted to a pH that is acidic but above the isoelectric point, it can still be assembled with a positively charged polyion PDDA. Thus the variation in cationic, anionic, and the nonionic characteristics of gelatin A and B can be used to promote binding with the drug core or

Table I. Film Thicknesses for Layer-by-Layer Assembly on Dexamethasone Core Surface Calculated from Frequency Shifts Measured with Quartz Crystal Microbalance [ΔL (nm) = 0.017 ΔF (Hz)]

Layer	Thickness (nm)
PDDA	2–3
PDDA/PSS	4–6
PDDA/gelatin A	6–8
PDDA/gelatin B	11–12
PSS/gelatin A	10–15
$(PDDA/PSS)_4$	10–14
$(PDDA/gelatin\ A)_4$	25–35
$(PDDA/gelatin\ B)_4$	45–55
$(PSS/gelatin\ A)_4$	50–60

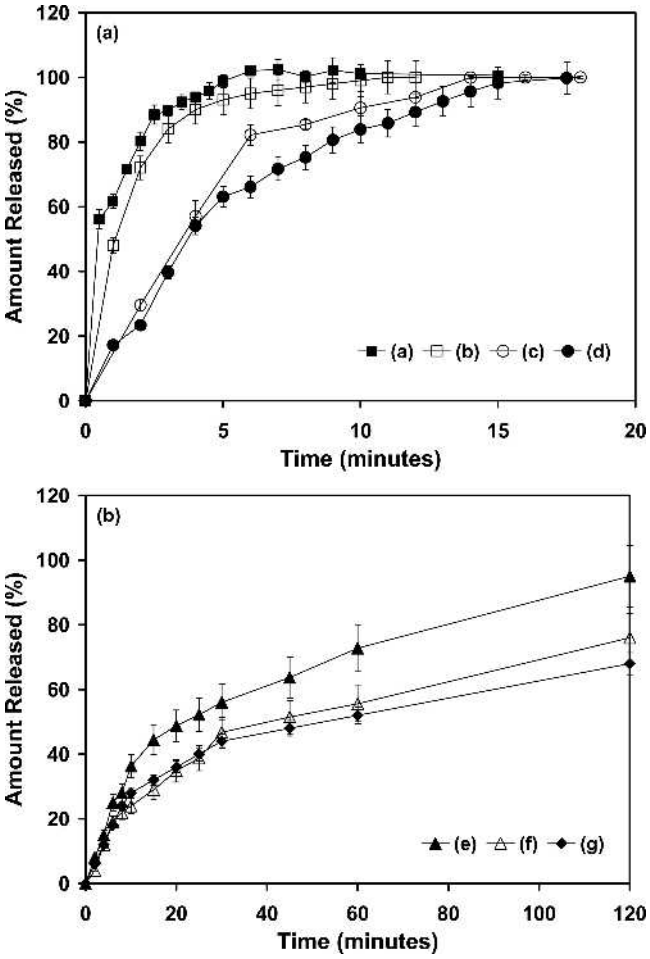

Fig. 8. Release profiles of dexamethasone particles in PBS buffer pH 7.2: (a) coated with 1 layer PDDA sonicated and filtered (<1.2 μm); (b) coated with 1 layer PDDA sonicated and unfiltered; (c) dexamethasone core/$(PDDA/PSS)_4$/PDDA; (d) particles sonicated without PDDA; (e) dexamethasone core/$(PDDA/gelatin\ A)_4$/PDDA; (f) dexamethasone core/$(PDDA/gelatin\ B)_4$/PDDA; (g) dexamethasone core/PDDA/$(PSS/gelatin\ A)_4$/$(PSS/PDDA)_1$. Mean ± SD, n = 6.

other polyions. The PSS or PDDA/gelatin layers were thicker than PDDA/PSS layers because gelatin has a loopy conformation at coating conditions leading to adsorption of several monomolecular layers with increased layer thickness (8). This is not the case with PDDA/PSS, which give rise to a thinner more porous coating (7). The thickness of the gelatin layers also depended on the type and pH of assembling because at pH 5.8, gelatin A formed thicker layers with negatively charged PSS and gelatin B formed thicker layers with positively charged PDDA.

Heat of adsorption measurements show that the binding of PDDA to the negatively charged dexamethasone microcrystals and to gelatin is an endothermic process and that the heat produced by this initial coating with PDDA is larger than for the assembling of subsequent layers. The binding of PDDA with the dexamethasone core and between PDDA and gelatin A is an entropically driven process such as that known to govern interactions between polyelectrolytes and proteins or between nucleic acids and proteins (24,25), whereas the interaction between the two polyions PSS and PDDA is a mildly exothermic interaction (26,27). The inter-

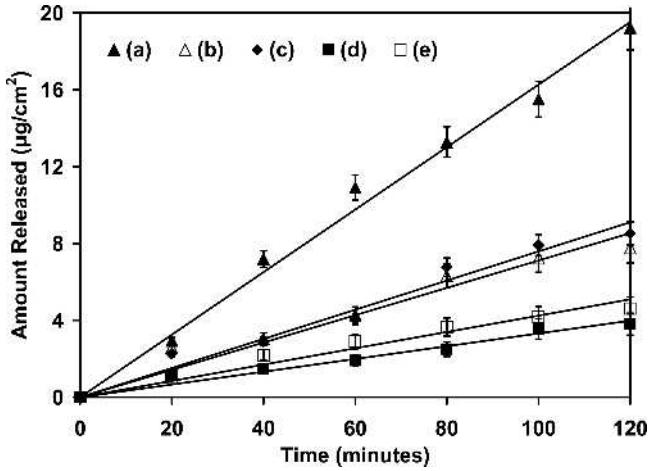

Fig. 9. Release of dexamethasone from the microcrystals and microcapsules suspended in a 1% w/w carboxymethylcellulose gel for microcrystals: (a) coated with 1 layer PDDA; (b) dexamethasone core/$(PDDA/PSS)_4$/PDDA; (c) sonicated without PDDA; (d) dexamethasone core/$(PDDA/gelatin\ B)_4$/PDDA; (e) dexamethasone core/PDDA/$(PSS/gelatin\ A)_4/(PSS/PDDA)_1$. Mean ± SD, n = 6.

action between PDDA and the drug cores and gelatin is associated with a loss of chain conformational entropy of the polyion combined with a release of counterions from the protein and the polyelectrolyte during the formation of charge/charge interactions between the two macromolecules and between the core and the charged polymer (25). The loss of configurational entropy of the polymer chains is compensated for by a gain in entropy associated with the release of the counterions producing a positive enthalpy. For the binding between the strongly charged PSS and PDDA, direct Coulombic interactions between the positive and negative charges present on the interacting macromolecules leads to binding and the formation of the polyion layers (26,27).

The drug delivery assembly reported here for dexamethasone may be classified as a reservoir type controlled system with a semi-permeable rate controlling membrane. As can be seen from Figs. 8 and 9 constant release profiles were maintained with almost zero-order kinetics (23). It provides additional prove for a diffusion-controlled release mechanism. The release of the dexamethasone from the drug involves two processes: a) bulk solution diffuse into the capsules to dissolve the drug crystals and b) the dissolved drug molecules diffuse out of the capsules. The dissolution of the crystal cores proceeds from the surface toward the crystal center and small crystals dissolved faster than larger crystals. Microscopic evaluation (Figs. 4 and 5) of the shells around the crystals revealed swelling of the gelatin shelled during the dissolution process. Such swelling is probably caused by osmotic pressure inside the shells accompanying the dissolution of the core material.

Because uncoated dexamethasone crystals dissolve faster than the encapsulated crystals, it is expected that at the early stages the dexamethasone concentration in the capsules are high, close to saturation. This will remain saturated with drug till the crystals within each shell in exhausted (16). The high concentration of the dexamethasone inside the shells caused by the dissolution of the core can result in a large concentration gradient across the capsule wall and as a consequence cause a high osmotic pressure inside the capsule, therefore the diffusion of dexamethasone through the capsule wall is accelerated. During this time, the permeability of the capsule wall controls the release rate. Structural differences between the PDDA/gelatin A layer *vs.* the PSS/gelatin A and PDDA/gelatin B multilayers determined the rate of this diffusion process. For example, dissolution data suggest that the diffusion through the single PDDA layer is five times faster than through the PDDA/gelatin B multilayers. In addition, the surface charge on gelatin B would be reduced when switched from conditions of assembly pH 5.8 to conditions of diffusion pH 7.2, which means the bulky gelatin molecule would shrink thus closing probable defects and pores, but simultaneously the multilayers will also swell. Swelling will take place evenly in all directions depending on the adhesion of the layer to a

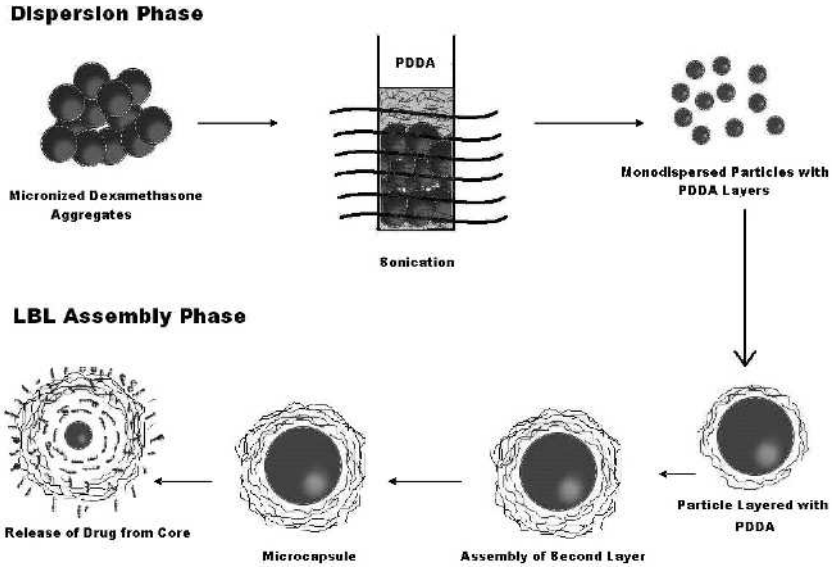

Fig. 10. Illustration of the monodispersion of the micronized dexamethasone drug particles by electrostatic layer-by-layer encapsulation followed by the subsequent controlled release of the drug through the insoluble porous shell.

rigid support. This constrains the swelling to the direction perpendicular to the support, which increases the thickness of the shell. However, as more drug dissolves, it is conceivable that the increase in hydrostatic pressure driven by the difference in osmotic pressure creates a tension in the wall, which may widen existing or create new pores thus increasing drug release. A combination of these processes then produces the controlled release as seen in Figs. 8 and 9.

CONCLUSIONS

The results of this study suggest that the combination of sonication and LbL coating can be used to produce monodispersed suspensions of dexamethasone microcrystals and that direct surface modification via the LbL process produced diffusion-controlled drug release via the polyelectrolyte multilayer nano-thick shell. In addition, for the first time, the simultaneous micronization of crystalline drug particles and polyion coating during sonication of the drug particles in an oppositely charged polyion solution is demonstrated. Although the optimum LbL microcapsules prepared in this study obtained 100% release within 2 h compared to up to 8 h reported for PLGA microspheres (28) and from 4 to 35 days for insoluble PLGA scaffolds (29), the capsules prepared in this study were significantly smaller, ~0.5–5 μm vs. ~15–100 μm. This self-assembling system is also an efficient controlled drug release delivery system because for the LbL microencapsulated poorly soluble dexamethasone, both the initial burst and the incomplete unloading of the encapsulated drug is reduced. Also, these nanothin shells occupy a small portion (~1% w/w) of the whole capsule. Therefore, drug loading of polyelectrolyte microcapsules is very efficient. The small microcapsules (0.5–5 μm) can be used for injection for either fast or slow release of the drug. Suspensions made from the very small monodispersed particles can also reduce irritation associated with the administration of suspensions in the eye. Future studies will look at changes in the gelatin layers during binding and upon exposure to heat and cross-linking/film hardening agents. The use of microcalorimetry to characterize the LbL process will also be further studied.

ACKNOWLEDGMENTS

This work is supported by NSF no. 0210298 "Nanoengineered Shells" and Louisiana Board of Regents 2002/05-RDA19 grants. Any opinions, findings, conclusions, or recommendations expressed in this material are those of the authors and do not necessary reflect the view of these funding agencies.

REFERENCES

1. G. Decher. Fuzzy nanoassemblies: toward layered polymeric multicomposites. *Science* **227**:1232–1237 (1997).
2. Y. M. Lvov, G. Decher, and H. Möhwald. Assembly, structural characterization and thermal behaviour of layer-by-layer deposited ultrathin films. *Langmuir* **9**:481–486 (1993).
3. Y. M. Lvov, K. Ariga, I. Ichinose, and T. Kunitake. Assembly of multicomponent protein films by means of electrostatic layer-by-layer adsorption. *J. Am. Chem. Soc.* **117**:6117–6123 (1995).
4. J. Mendelsohn, C. Barrett, V. Chan, A. Pal, A. Mayes, and M. Rubner. Fabrication of microporous thin films from polyelectrolyte multilayers. *Langmuir* **16**:5017–5023 (2000).
5. B. Tieke, F. van Ackern, L. Krasemann, and A. Toutianoush. Ultrathin self-assembled polyelectrolyte multilayer membranes. *Eur. Phys. J. E* **5**:29–39 (2001).
6. X. Qui, S. Leporatti, E. Donath, and H. Möhwald. Studies on the drug release properties of polysaccharide multilayers encapsulated ibuprofen microcrystals. *Langmuir* **17**:5375–5380 (2001).
7. H. Ai, S. A. Jones, M. M. de Villiers, and Y. M. Lvov. Nanoencapsulation of furosemide microcrystals for controlled drug release. *J. Control. Rel.* **86**:59–68 (2003).
8. D. B. Sheney and G. B. Sukhorukov. Engineered microcrystals for direct surface modification with layer-by-layer technique for optimized dissolution. *Eur. J. Phar. Biopharm* **58**:521–527 (2004).
9. Y. M. Lvov, A. Antipov, A. Mamedov, A Möhwald, and G. Sukhorukov. Urease encapsulation in nanoorganized microshells. *Nano Lett.* **1**:125–128 (2001).
10. Y. M. Lvov and F. Caruso. Biocolloids with ordered urease multilayer shells as enzymatic reactors. *Anal. Chem.* **73**:4212–4217 (2001).
11. G. Ibarz, L. Dahne, E. Donath, and H. Möhwald. Smart micro- and nanocontainers for storage, transport, and release. *Adv. Mater.* **13**:1324–1327 (2001).
12. I. Suzuki, T. Ishizaki, H. Ihoue, and J. Anzai. Modification of polyelectrolyte layered assembly using an active ester of azobenzene carboxylate. *Macromolecules* **35**:6470–6474 (2002).
13. G. B. Sukhorukov, A. A. Antipov, A. Voigt, E. Donath, and H. Möhwald. pH-controlled macromolecule encapsulation in and release from polyelectrolyte multilayer capsules. *Macromol. Rapid Commun.* **22**:44–46 (2000).
14. A. A. Antipov, G. B. Sukhorukov, S. Leporatti, I. L. Radtchenko, E. Donath, and H. Möhwald. Polyelectrolyte multilayer capsule permeability control. *Coll. Surf. A: Physicochem. Eng. Aspects* **198–200**:535–541 (2002).
15. Z. Sui, D. Salloum, and J. B. Schlenoff. Effect of molecular weight on the construction of polyelectrolyte multilayers: stripping versus sticking. *Langmuir* **19**:2491–2495 (2003).
16. A. A. Antipov, G. B. Sukhorukov, E. Danoth, and H. Möhwald. Sustained release properties of polyelectrolyte multilayer capsules. *J. Phys. Chem. B* **105**:2281–2284 (2001).
17. G. B. Sukhorukov, E. Danoth, E. Moya, S. Susha, A. Voigt, A. Hartmann, and H. Möhwald. Microencapsulation by means of step-wise adsorption of polyelectrolytes. *J. Microencapsul.* **17**: 177–185 (2000).
18. O. P. Tiourina and G. B. Sukhorukov. Multilayer alginate/protamine micronized capsules: encapsulation of α-chymotrypsin and controlled release study. *Int. J. Pharm.* **242**:155–161 (2002).
19. G. B. Sukhorukov, E. Donath, S. Davis, H. Lichtenfeld, F. Caruso, V. I. Popov, and H. Möhwald. Step-wise polyelectrolyte assembly on particle surfaces-a novel approach to colloid design. *Polym. Adv. Technol.* **9**:759 (1998).
20. Martindale. *The Extra Pharmacopoeia*, 30th ed. The Pharmaceutical Press, London, 1993.
21. *The United States Pharmacopeia XXVI and National Formulary 19*. The United States Pharmacopeial Convention, Bethesda, MD, USA, 2000.
22. N. A. Peppas. Analysis of Fickian and non-Fickian drug release from polymers. *Pharm. Acta Helv.* **60**:110–111 (1985).
23. R. W. Baker. *Controlled Release of Biologically Active Agents*, John Wiley & Sons, New York (1987).
24. M. T. Record, M. L. Lohman, and P. De Haseth. Ion effects on ligand-nucleic acid interactions. *J. Mol. Biol.* **107**:145–158 (1976).
25. V. Ball, M. Winterhalter, P. Schwinte, P. Lavalle, J. C. Vogel, and P. Schaaf. Complexation mechanism of bovine serum albumin and polly(allylamine hydrochloride). *J. Phys. Chem. B* **106**:2357–2364 (2002).
26. M. Bezan, M. Malavasic, and G. Vesnaver. Surfactant binding to oppositely charged polyions. *Berichte der Bunsen-Gesellschaft* **100**:1054–1058 (1996).
27. C. Wang and K. C. Tam. Interaction between polyelectrolyte and oppositely charged surfactant: effect of charge density. *J. Phys. Chem. B* **108**:8976–8982 (2004).
28. H. Eroglu, H. S. Kas, L. Oner, O. F. Turkoglu, N. Akalan, M. F. Sargon, and N. Ozer. The in-vitro and in-vivo characterization of PLGA: L-PLA microspehere containing dexamethasone sodium phosphate. *J. Microencap.* **15**:603–612 (2001).
29. H. Kim, H. W. Kim, and H. Suh. Sustained release of ascorbate-2-phosphate and dexamethasone from porous PLGA scaffolds for bone tissue engineering using mesenchymal stem cells. *Biomaterials* **24**:4671–4679 (2003).