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Research paper

Engineered microcrystals for direct surface modification with layer-by-layer technique for optimized dissolution

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Abstract

This investigation relates to a two-step formulation development technique-synthesis of sterically stabilized drug microcrystals followed by direct surface modification by sequential electrostatic adsorption. Stable microcrystals of naproxen were produced by pH-induced reprecipitation in presence of a stabilizer. Sequential layer growth was achieved by the layer-by-layer assembly of biocompatible polyelectrolytes (PEs) and was registered using microelectrophoresis. The coated colloids were characterized using confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). The in vitro controlled release pattern of the drug through the PE diffusion barrier was studied using a diffusion cell assembly at physiological pH of 7.4, both before and after freeze-drying. Thermodynamically stable naproxen microcrystals were obtained by association and had a mean length of 15 μm and a zeta potential of −37.5 mV and were surface modified efficiently using biocompatible polysaccharide/protein-based PEs. Sufficient charge reversal with each layer was evident indicating layer growth with successive deposition cycles. The coating was complete and homogeneous as visualized under CLSM and SEM. The in vitro release study revealed that the stoichiometry of PEs in the complex coating and its molecular architecture played important roles in forming the diffusion barrier, which offered efficient control of the dissolution rate of drug core (up to 50% lower than bare crystal). The release profile fitted zero order release kinetics. This novel formulation technique enables administration of high concentrations of water-insoluble drugs in a stable, tissue compatible form, simultaneously affording sustained release.

Keywords: Naproxen; Layer-by-layer; Sustained release; Injectable; Ophthalmic

1. Introduction

Therapy enhancement via successful delivery of a therapeutic agent is the principal goal of drug delivery research. The most important commercially recognized technologies available for delivery of large quantities of water-insoluble drugs with improved performance characteristics (for e.g. insoluble drug delivery (IDD™) and NanoCrystal®), utilize direct surface modification of a drug micro or nanocrystal via hydrophobic/hydrophilic interactions, enveloping and protecting it from coalescence, simultaneously rendering it less irritating to tissue [1–7]. A novel strategy for direct surface modification of colloidal entities is layer-by-layer surface nanoengineering which utilizes sequential adsorption of oppositely charged PEs forming a complex assembly via electrostatic interactions [8]. The surface properties of coated colloids and capsules can be tuned precisely with respect to tissue compatibility or target recognition by choosing appropriate PEs as coating components and the dissolution rate of the encapsulated substance could be tuned with the number of deposited layers, components used for fabrication of the complex and their concentration, supporting salt concentration and solution pH [9–14]. The layer-by-layer technique has been utilized successfully for direct surface modification of dyes, micronized drug particles, enzyme crystals and DNA-PE complex microparticles for the purpose of achieving controlled release [10,15–18]. However, these investigations have utilized either thermodynamically unstable core particles (due to high-energy input during micronization) or utilized non-biocompatible PEs for multilayer assembly or principally focused the evaluation on per-oral dosage forms.

From a pharmaceutical point of view, a better option would be to synthesize sterically microparticles and subsequently

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subject them to surface modification for controlled release or targeting applications. An efficient tool for achieving the former goal is the synthesis of naturally grown microcrystals via association in presence of stabilizing agents [19,20]. The surface stabilization could be achieved by using any of the known colloidal stabilizers [21–23]. We have chosen Vitamin E tocopheryl polyethylene glycol 1000 succinate-NF grade (TPGS) for its excellent surface stabilization/amphiphilic properties and biocompatibility [24]. We could obtain stable microcrystals for a variety of drugs when they were re-precipitated (by pH change or non-solvent addition) in presence of TPGS (unpublished results).

The motivation behind the present investigation is to exploit the layer-by-layer nanoengineering concept for formulating a tissue-friendly sterile dosage form using re-engineered drug microcrystals with tailored release characteristics: an intramuscularly (i.m.) injectable controlled release system or a controlled release ophthalmic preparation. We have chosen naproxen as a model active pharmaceutical ingredient as it is one of the most potent non-steroidal anti-inflammatory drugs (NSAID) used for its analgesic, anti-inflammatory and anti-pyretic properties for treating rheumatic disorders, mild to moderate pain and acute gout [25–27]. NSAIDs including naproxen have been substituted for steroids in the treatment of ocular inflammation because they have not shown the same propensity to produce side effects in ocular tissues as ophthalmic steroids [28–30]. The envisaged formulation presents the drug microcrystal to the ocular/muscular tissue in a stable form with controlled release properties.

2. Materials

Naproxen, dextran sulfate sodium salt (DS, MW ~ 500 kDa), gelatin (Type A, 120 bloom) and sodium lauryl sulphate (SLS) were purchased from Sigma, Germany. Chitosan (medium molecular weight) and sodium alginate (SA) were purchased from Aldrich, Germany. Poly acrylic acid sodium salt (PAA) (MW ~ 5100) was purchased from Fluka, Germany. TPGS and dialysis membrane (Spectra/Por molecular weight cut-off ~ 6–8000) were kindly provided by M/s Eastmann Chemical BV, Germany and Spectrum Labs, USA, respectively. Rhodamine B isothiocyanate labelled chitosan (chitosan-Rh) was prepared and purified using the principle reported earlier [31]. All the other chemicals and reagents were of analytical grade and were used as procured. Ultra pure water used for all experiments and cleaning steps was obtained from Milli-Q system having a specific resistance greater than 18 mΩ cm.

All the PE solutions were prepared in 0.5 M NaCl solution. The concentrations were 1 mg/ml for DS, SA, gelatin and PAA and 0.5 mg/ml for chitosan. Glacial acetic acid was used to solubilize chitosan and had a concentration of 0.2% (v/v) in final solution.

3. Methods

3.1. Synthesis of microcrystals of naproxen

A 10% (w/v) (approx.) suspension of naproxen was prepared in 1% (w/v) aqueous solution of TPGS. A few microlitres of 1N sodium hydroxide solution were added to obtain a clear solution. The drug was re-precipitated as fine microcrystals by reducing the pH to about 4.5 with 0.1N hydrochloric acid under slow magnetic stirring. The microcrystals were washed repeatedly with water and finally suspended in water to give an approximately 10% (w/v) suspension.

3.2. Layer-by-layer coating with PEs

PE multilayer assembly was accomplished by consecutive adsorption of oppositely charged PEs using the centrifugation protocol as described earlier [8]. In every case, the first layer adsorbed was the positively charged PE. In each experiment, a known volume of approximately 2% (w/v) suspension of the naproxen microcrystal was alternatively incubated with 1 ml of PE solution for 15 min under gentle shaking. Two washing steps with water were carried out in between two adsorption cycles. This process was continued until a desired level of coating was achieved.

The samples were lyophilised using a tabletop assembly (Lyovac GT2, Amsco/Finn-Aqu, Germany) after suspending in a known volume of 5% (w/v) mannitol solution.

3.3. Characterization

The particle size distribution of a suitably diluted sample of the microcrystal suspension was studied with a Malvern Mastersizer 2000 (Malvern Instruments GmbH, Germany) at pH 4.5. The microelectrophoretic mobility of the coated microparticles was measured using a Malvern Zetasizer 4.0 (Malvern Instruments GmbH, Germany) after deposition of every layer. The measurements were carried out with purified water as the flow medium and the mobility was then converted to the ξ-potential using the Smoluchowski relationship. CLSM images were captured with a Leica TCS NT confocal scanning system (Leica, Germany) equipped with a 100 × /1.4–0.7 oil immersion objective. Scanning electron microscopy (SEM) measurements for coated and uncoated microcrystals (an air-dried dilute aqueous suspension on a glass cover slip mounted on the stub) were carried out using a Zeiss DSM 40 instrument (Zeiss, Germany) operating at an accelerating voltage of 15 keV.

3.4. In vitro dissolution study

A known amount of coated drug crystals were placed in the donor compartment (containing 1 ml of phosphate buffer pH 7.4) of a diffusion tube (dimensions: 80 mm [length] × 22 mm [inner diameter]) separated from

the receptor compartment (containing 20 ml of phosphate buffer pH 7.4 and 1% (w/v) of SLS) by a dialysis membrane (MWCO: 6–8000). The diffusion cell assembly was fixed such that the dialysis membrane was always in contact with the medium of the receptor compartment [32,33]. The fluids in both the compartments were stirred slowly using a magnetic set-up. The quantity of drug which had dissolved and diffused into the receptor medium was estimated by UV spectrometry at 229 nm.

4. Results and discussion

4.1. Synthesis of naproxen microcrystals

Naproxen, a propionic acid derivative, is an acidic NSAID, insoluble at acidic pH, but soluble at a pH above 9.5. Microscopic examination of the drug in the native form shows uneven surface and size distribution unsuitable for exploiting the layer-by-layer deposition directly. Recrystallization of the drug from alkaline solution (solubilized as sodium salt) via reduction in the pH results in needle-shaped microcrystals that form sticky aggregates, which are difficult to disperse and handle. However, when recrystallization is carried out in presence of a stabilizer, the resulting microcrystals were rod-shaped, easily redispersible and with appropriate size distribution (for intended application). The microcrystals exhibited characteristic opalescence due to the surface stabilization of the colloidal particles. The phenomenon was also observed when we recrystallized other active agents like ciprofloxacin, acetazolamide, cyclosporine, etc. either by pH change or by non-solvent addition in presence of TPGS. We employed 1% (w/v) of TPGS that reduced the surface tension of the system to 41.02mN/m during the recrystallization step and helped in spontaneous covering of the newly formed hydrophobic surface resulting in lowered total surface energy and enthalpy of the system. Microcrystals had a mean length of 15.8 μm and 90% (by volume) of the particles were below 28 μm with a major proportion of the suspension distributed within the window of 11–20 μm. The method for synthesis was simple and produced thermodynamically stable and galenically acceptable forms of naproxen.

4.2. Layer-by-layer coating of the microcrystals and characterization

The bare microcrystals had a zeta potential of − 37.5 mV to activate subsequent electrostatic self-assembly process upon exposure to PEs. The coating was directly applied to the microcrystals without pre-modification of the crystal surface with positively charged species as the first layer. Upon exposure to oppositely charged macromolecules, there was sufficient surface re-charging corresponding to the charge of the PE employed as evident by the reversal of zeta potential values (Fig. 1). We monitored the layer-by-layer

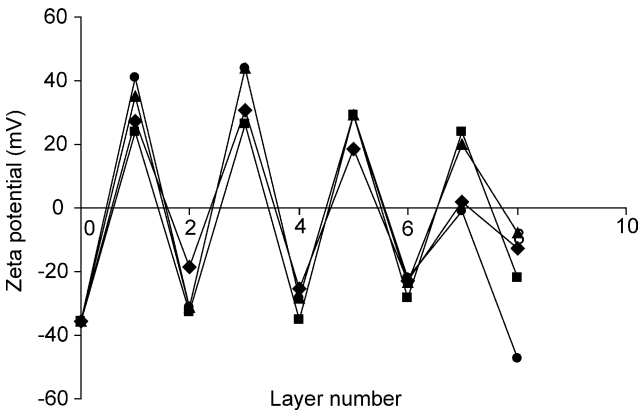

Fig. 1. Electrophoretic mobility of naproxen microcrystals as a function of PE layer number: (◆) SA-gelatin coating, (■) DS-gelatin coating, (●) PAA-chitosan coating (▲) DS-chitosan coating.

deposition process up to a build-up of four adsorption cycles for the PE layer pairs under investigation. The charge reversal obtained represents a change in surface properties with respect to electrical charge (due to charge over-compensation) and hence represent credible evidence for the step-wise growth of PE multilayers.

The coating/washing process was carried out at a pH of about 5.0 ± 0.5 for two reasons: first, to limit dissolution, if any, of the naproxen cores and second, especially when gelatin was used a polycation, because a slightly acidic pH must be maintained to induce maximal ionization of the charged groups for efficient complex formation with the counter PE. The salt concentration was maintained at 0.5 M NaCl to facilitate formation of denser PE multilayers [12]. The suspension was easily dispersible in all cases with gentle shaking and no cake formation was observed upon standing for weeks. The number of multilayers for assuring reduction in pores of the inter-penetrating, non-stratified network of PEs was 14–16. The CLSM image of a typical coated microcrystal is shown in Fig. 2. The multilayer coverage was either visualized by labeled PE as the layer constituent (chitosan with rhodamine label) or by introducing a small quantity of an external dye (rhodamine or fluorescein) that would be adsorbed onto the coating via electrostatic interactions. The coating with PE complex was complete and uniform with no patch-like islets on the surface. There was no aggregation observed both before and after coating—hence the microcrystals were surface-stabilized by virtue of their synthesis method and/or with PE multilayer coating around them. One could obtain empty shells by selectively dissolving the supporting drug microcrystal by an organic solvent (ethanol or acetone) or by raising the pH to above 10 with a drop of NaOH solution. The shells collapsed due to this abrupt loss of the support and could be observed using a fluorescent tag. Hence, the CLSM supports the existence of multilayer coatings on the drug microcrystal.

Macroscopically, the uncoated crystals exhibited marked opalescence. The SEM images of naked and coated

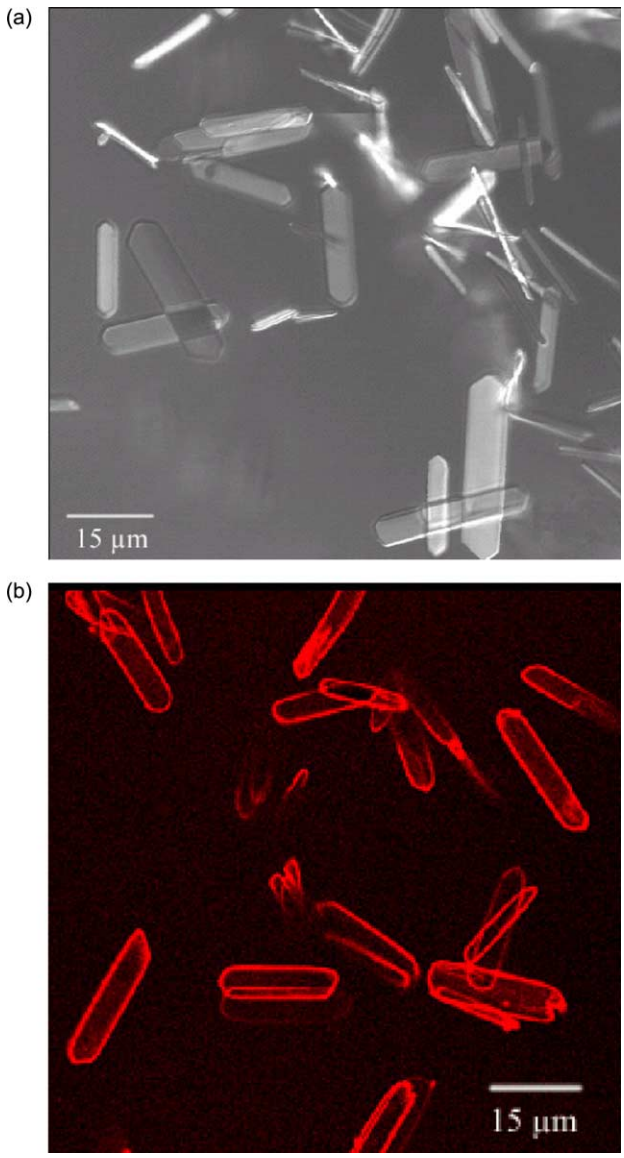

Fig. 2. CLSM images of naproxen microcrystals (a) uncoated, (b) coated with 16 layers of DS-Chitosan (chitosan was tagged with Rhodamine).

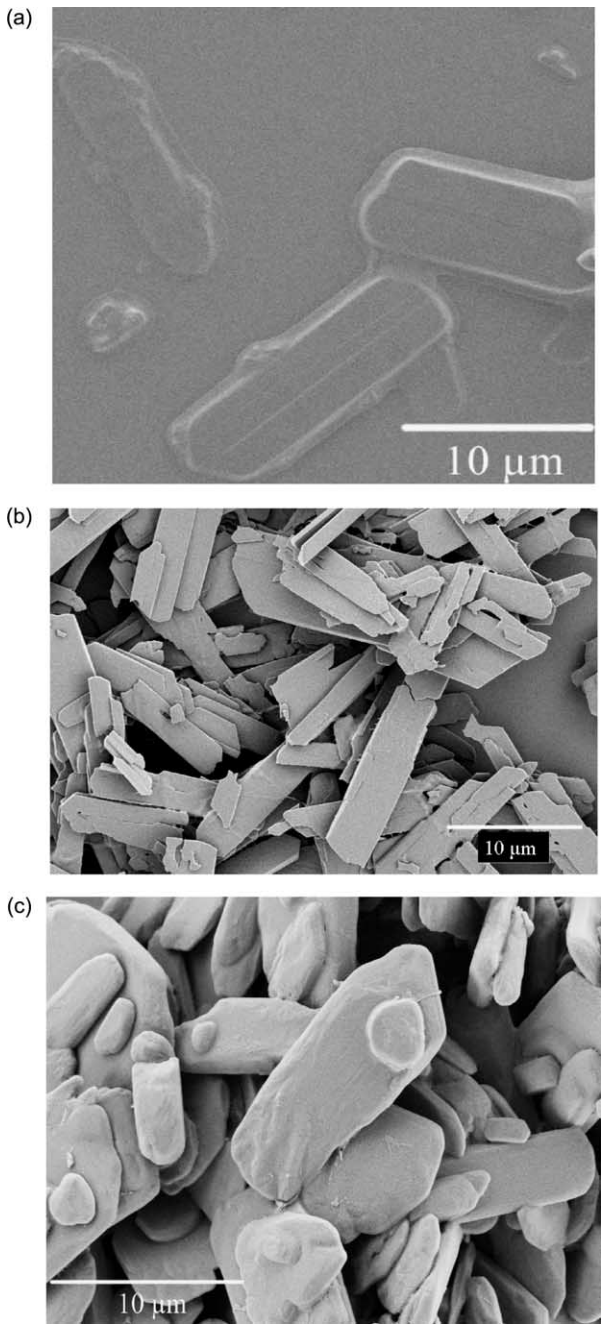

Fig. 3. SEM images of naproxen microcrystals (a) uncoated, (b) coated with eight layers of SA-gelatin A, (c) coated with 14 layers of SA-gelatin A.

microcrystals are shown in Fig. 3. The bare microcrystals had a near-transparent morphology and with increasing number of PE multilayered coatings, an increase in opacity of crystal surface was observed (Fig. 3a–c). The surface of the coated microcrystal was smoothened with PE multilayer deposition and coating was uniform in all cases of PE combinations. The product was an elegant, easily re-dispersible and physically stable formulation.

When stored as an aqueous suspension in distilled water under refrigeration, there was no evidence of aggregation neither macroscopically nor microscopically. However, we carried out lyophilization of the coated samples with mannitol with dual objectives: (1) it would help to assure an adequate shelf-life as the majority of physico-chemical reactions leading to product instability are accelerated when stored in aqueous media, and (2) achieving sterility of the dosage form by a suitable terminal sterilization technique (for e.g. Gamma radiation) would demand the product to be presented in a dry state to minimize the probable degradation. No difference in morphology was observed by SEM examination for the samples re-suspended after lyophilization treatment. We employed the in vitro dissolution profile of the drug microcrystal in a suitable medium as the terminal indicator for projecting any subtle change in the PE coating during processing of the formulation.

4.3. In vitro release profile

We subjected the hydrophilized and improvised drug core to direct surface modification for achieving diffusion-controlled release via PE multilayer membrane. The striking advantage of such a diffusion barrier is the control of rate and selectivity of fluxes of substances across it by simply tuning the local conditions such as pH, ionic strength, etc. [11]. The in vitro dissolution was carried out under simulated conditions that would reflect the actual purpose for which the product is being developed (controlled release i.m. injection or ophthalmic dosage form). The dialysis membrane acted as the semipermeable diffusion barrier that limits the delivery of the drug from the dosage form to the surrounding tissue [34]. To enhance the solubility of naproxen and to maintain continuous sink conditions throughout the study period, 1% (w/v) SLS was used with phosphate buffered saline (pH 7.4) in the receptor compartment (represents systemic component). The dissolution profile of the formulations is shown in Fig. 4. The uncoated microcrystal dissolved completely within 6 h with a $t_{1/2}$ (time required for 50% of the drug payload to be dissolved) of about 185 min. When the microcrystals were covered with a chitosan-polyanion (DS or PAA) pair, no significant difference in $t_{1/2}$ values were observed compared to control and approximately 87% of the encapsulated drug was dissolved in a 6 h period. At the same time, when gelatin A was used in combination with DS or SA, the $t_{1/2}$ values were significantly higher (270 and 360 min, respectively) with upto 70 and 50% of the drug load being dissolved in 6 h.

The limited water solubility of the drug itself at the pH employed resulted in steady-state dissolution of the control formulation (uncoated microcrystal). Mean thickness values for a monolayer obtained by electrostatic adsorption of chitosan or DS or SA or PAA is about 2–4 nm while for gelatin, it is about 16 nm [15,35,36]. This means, for 16 layers of chitosan-polyanion (DS or PAA) pairs, the total thickness of the coating would vary between 32 and 64 nm while for gelatin-polyanion (DS or SA) pairs, the values would be about 126 nm for 14 layers. The major factor controlling the dissolution of the coated microcrystal is the thickness of the PE multilayer covering and the values stated above speak for the observed difference in rates of drug release. In addition, pore-mediated transfer of substances (due to the artifacts of inter-penetrated PE multilayers) accounts for a certain fraction of dissoluted drug [37]. As the number of PE layers is increased, this secondary contribution arising from defects within the network is reduced. However, when the multilayers are exposed to different environmental conditions (with respect to pH, ionic strength etc.), then the PE multilayers undergo molecular rearrangements that may increase or reduce defects [37]. In the present case, this rearrangement factor could be used to explain the differential effects occurring with respect to release pattern when chitosan is replaced by gelatin though layer numbers are reduced in the case of the latter. Gelatin is a weak PE with low charge density; but it is a bulky, high molecular weight protein. This results in formation of a loopy conformation at coating conditions (pH $\sim$ 5.0) leading to adsorption of several monomolecular protein layers resulting in increased layer thickness. This is not the case when chitosan is used with DS or PAA where there is no possibility to form loopy conformation by any of the participating PEs and this may as well give rise to better chances of generation of defects. The imbalance in stoichiometry during assembly of PE multilayers and their subsequent molecular reorganization under the conditions of the diffusion experiments, would lead to stretching or shrinking of the coating. The surface charge on gelatin would preferentially get reduced when switched from condition of assembly (pH 5.0) to conditions of dissolution experiment (pH 7.4) resulting in shrinking of the bulky molecule, thus closing the probable defects and pores. In the case of the chitosan-polyanion pair, the pores may as well remain as such or be closed to a reduced extent than gelatin. This lack of repair in combination with reduced thickness resulted in faster release rates for the chitosan-polyanion pair.

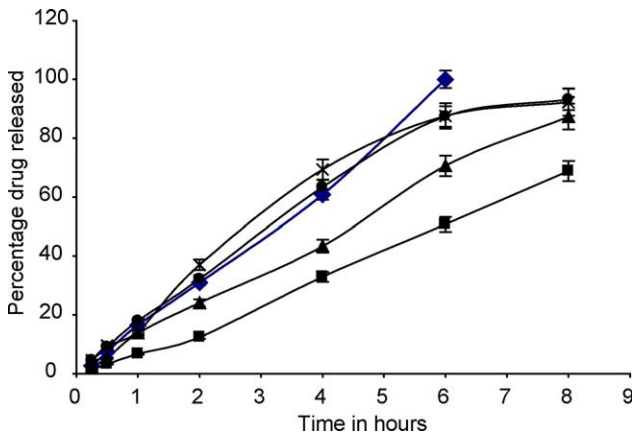

Fig. 4. In vitro dissolution profile of coated and uncoated naproxen microcrystals: (◆) uncoated, (■) SA-gelatin 14 layers, (●) PAA-chitosan 16 layers, (▲) DS-gelatin 14 layers, (x) DS-chitosan 16 layers.

The drug delivery assembly may be classified into a reservoir type controlled release system with a semi-porous rate-controlling membrane. With an increase in the number of PE multilayers, a smoothened topography is observed (by SEM), although the coating remains non-homogeneous chemically (as a result of interpenetrating networks formed upon the layer-by-layer adsorption) and semi-porous in physical terms. Compared to classical diffusion barriers obtained with polymeric materials, the current formulation technique provides a barrier that could be tuned precisely in terms of thickness and composition [12]. As can be seen in the graph (Fig. 4), a constant release profile is maintained with zero order kinetics. We obtain a near-perfect (regression coefficient $R^2 > 0.9$) fitting of the release curves to the zero order equation for all the coated microcrystals, which provides additional corroboration for a diffusion-controlled release mechanism.

One of the factors that should be considered for successful scale-up of these formulations would be sterility.

As PE complexes are vulnerable for thermal decomposition, we intended to use gamma radiation as the means of sterilization. To minimize the probable degradation that may occur during exposure, we deprived the formulation of water by lyophilization. The formulations were resuspended in water and subjected to microscopical analysis (CLSM and SEM) to visualize the physical changes that might have occurred during the freeze-drying process. There was no change observed prior to and after lyophilization treatment. We relied on in vitro dissolution kinetics of the drug microcrystal as the terminal indicator to reflect any subtle changes in physical chemistry of the PE multilayers. The results were encouraging as no significant deviation was observed compared to the in vitro release pattern before lyophilization.

5. Conclusions

In the present study we have shown that direct surface modification of water-insoluble drugs could be used as an effective formulation development tool. This constitutes the first specific application-oriented report on the layer-by-layer nanoengineering technique that employs completely biocompatible excipients (with a GRAS status from USFDA) for direct surface modification of pre-engineered drug microcrystals via a controlled crystallization protocol. The basics of the technology have been reported earlier [15,35,38] and a broad patent for the concept also has been obtained [39,40]. This method of direct surface engineering of the drug accomplishes several desirable properties for parenteral or ophthalmic dosage form: (1) tissue compatibility of preparation and vehicle; (2) controllable particle size; (3) administration of high drug payloads; and (4) stable, elegant and grossly homogenous formulation. One could manipulate the technology depending on the therapeutic demand by suitably tuning the extent of controlled release required which in turn is achieved by choosing the PE pairs, their stoichiometry, assembling conditions (like pH and salt concentration) and number of adsorbed layers. Layer-by-layer nanotechnology as a surface modification process has found practical application in ophthalmology (Focus® Excelens™ contact lens from Ciba vision) and has been explored as a viable tool for surface modification of medical devices for improving the biocompatibility. In coming years we would see increased research towards utilization of the layer-by-layer technology in the micro- and nano-scale for imparting specialized properties for novel drug carrier systems.

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