Effect of polyelectrolyte coatings on DEXA release from alginate microspheres

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Introduction

Over the last two decades, the field of controlled drug delivery has been confronted with two major challenges (R. Langer, 2004). The first one has been achieving sustained zero-order release of a therapeutic agent over a prolonged period of time. The second of these challenges is the controlled delivery of a therapeutic molecule or drug in a controlled manner (A. Anal, 2007). One promising approach to this problem is the use of nanoengineered carrier systems utilizing the layer-by-layer (LbL) templating technique. The layer-by-layer (LBL) assembly technique, introduced by Decher in 1991 (Decher, 1991) has come out as a versatile and inexpensive method of constructing polymeric thin films, with control upto nanometer-scale. Films are formed by electrostatic interactions between oppositely charged poly-ion species to create alternating layers (R. Srivastava, 2005) of sequentially adsorbed poly-ions also called "Polyelectrolyte Multilayers (PEMs)". PEMs can be applied on any flat substrate like glass, silicon, colloids (A. Khopade, 2003) and have been utilized in such applications as sensors, electrochromics, and nanomechanical thin films (B. DeGeest, 2006).The acute inflammatory and tissue reactions affect the normal function of numerous implants such as biosensors, pacemakers etc. For example, the accumulation of inflammatory cells and the presence of a fibrous capsule around a glucose sensor reduce the transport of glucose from the capillaries to the sensor thereby reducing its efficacy as a sensor (A. A. Sharkawy, 1997). These problems can be overcomed by several approaches; the one that we are targeting is the delivery of immunomodulating agents such as Dexamethasone (DEXA) or other drugs (anti inflammatory agents like NSAID's) and other tissue response enhancer in the immediate vicinity of the graft or incorporate within the implant to reduce the tissue response against an implant (A. A. Sharkawy, 1998). Thus, the objective of the present work is to obtain a zero-order release profile of dexamethasone over a period of 1 month using nanoengineered alginate hydrogel system that has potential for future application for implantable devices such as glucose biosensor.

Materials and methods

Materials

Alginate (Low viscosity, 2%), Dexamethasone-21-phosphate di-sodium salt (MW-392.5) (DEXA), sodium poly (styrene sulfonate) (PSS, 70 kDa), poly (allylamine hydrochloride) (PAH, 70 kDa), Poly(acrylic acid) (PAA, 45KDa) and Poly (diallyldimethylammonium chloride) (PDDA, 20-35 KDa), 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS) and PBS tablets were purchased from Sigma- Aldrich (India). Sodium azide was purchased from Loba Chemi (Mumbai). Calcium Chloride, barium chloride, magnesium chloride and aluminum chloride and dialysis membrane (10-14 kDa) were purchased from Merck (Mumbai) and Hi Media (Mumbai) respectively. All chemicals were reagent grade and used as received.

Instrumentation

Encapsulation unit Variation J30 (Nisco Engineering AG, Zurich) and syringe pump (Multi-Phaser™, model NE-1000, New Era Pump Systems, NY) have been used for preparing uniform sized alginate microspheres. Nikon YS 100, Zeiss optical microscope with a digital camera and Hitachi S3400 scanning electron microscope were used for microscopic studies. Zeta potential (Electrophoretic mobility) measured using Zetaplus, (Brookhaven Instruments, USA). Helios Alpha UV-Vis spectrophotometer (double beam, Thermo scientific) was used for release studies.

Preparation of alginate microspheres
The microspheres were prepared by using the droplet generator in which a solution of 2% w/v sodium alginate containing defined amount of dexamethasone was filled into the syringe of 60c.c. capacity. The flow rate of the solution was fixed according to the in-built program of the syringe pump. The encapsulation unit works on the principle of aerodynamic force where the sodium alginate solution while passing through the nozzle (diameter = 350μm) breaks up into micron size particles. The flow rate of sodium alginate solution was fixed at 20 ml/hr and the pressure was maintained at 75 mbar. The fine spray of alginate was collected into 250mM calcium chloride solution for gelation under constant stirring (250 rpm) for 20 minutes.

Microspheres image analysis & Surface Morphology
Uncoated and coated microspheres were examined using a Nikon optical microscope at 2.5X, 10X, and 20X magnifications for image analysis and size determination. The shape and surface characteristics of the microspheres were observed by scanning electron microscopy (Hitachi S3400)

Effect of drug loading
The loading and release of small molecules from poly- electrolyte coated microspheres are effectively governed by a number of properties of the system such as the degree of swelling, hydrophilic/hydrophobic balance, pKa (app), charge density, pore size, and the properties of the small molecules such as size, charge, and hydrophilicity. Thus, for the experiment different concentrations of dexamethasone were taken to study the behavior of drug release from the alginate microspheres.

Effect of salt concentration on drug release
To determine the effect of various salts on drug release, different salts like $BaCl_2$, $CaCl_2$, $MgCl_2$, $AlCl_3$ & $ZnSO_4$ were chosen for the study.

LbL coatings of calcium alginate microspheres
The polyelectrolyte used for this experiment were Poly (sodium 4-styrene-sulfonate) (PSS), Poly (acrylic acid) (PAA), Poly-(allylamine hydrochloride) (PAH) and Poly (diallyl dimethyl ammonium chloride) (PDDA). A solution of 2mg/ml concentration of each polyelectrolyte was prepared with 0.25 M $CaCl_2$ with pH adjusted to 7.4. For the coating 3ml of positively charged polyelectrolyte (PAH, PDDA) was added to 1ml of microspheres, kept for 20 min then centrifuged at 500 rpm for 1min and washed with distilled water (twice). In the same manner, a second layer (- vely charge PE's) was deposited on the microspheres. For the crosslinking experiment PAH and PAA were used as pair of polyelectrolytes. EDC was used to crosslink the PAH/PAA multilayers using N-Hydroxysuccinimide (NHS) as a catalyst (R. Srivastava, 2005). The surface charge of the microspheres was measured using the zeta potential analyzer after rinsing and prior to addition of each polyelectrolyte.

Zeta potential (Electrophoretic mobility)
The electrophoretic mobility of the uncoated and LbL coated, cross linked microspheres were measured using Zetaplus (Brookhaven Instruments, USA). The ζ-potential was calculated from the electrophoretic mobility (μ) using the Smoluchowski relation: $\zeta = \mu\eta/\varepsilon$ (where η and ε are the viscosity and permittivity of the solvent, respectively). For this experiment, 50 μl sample solution containing the microspheres was diluted in 2 ml of distilled water and used for analysis (R. Srivastava, 2005).

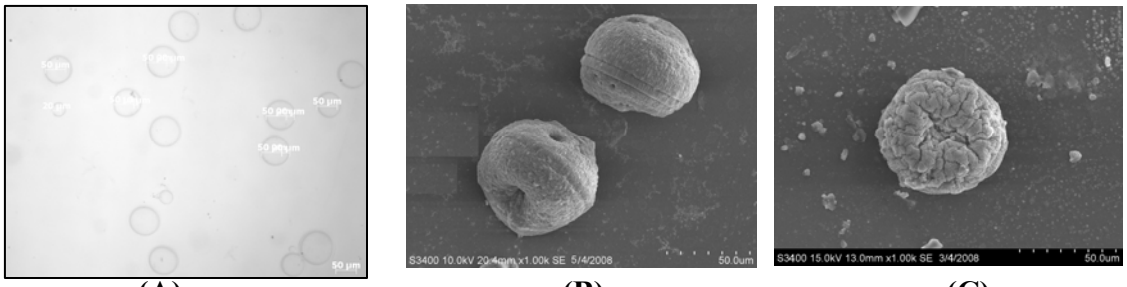
***In- vitro* drug release study**
In-vitro drug release studies were performed using a dialysis membrane (cutoff- 10-14 KDa) in a beaker. Dexamethasone loaded uncoated, coated and cross linked microparticles were put in a beaker containing 100 ml of 0.1M phosphate buffered saline (PBS, pH 7.4) and 0.01%w/v sodium

azide. The samples (in triplicate) were incubated in a 37ºC incubator under sink conditions for the release studies. At preset time intervals, the incubation buffer was collected and replaced with a fresh buffer solution. The amount of released dexamethasone in the collected medium was determined spectrophotometrically at λ_{max} 242 nm. Statistical analysis of the data was performed using analysis of variance (ANOVA: Single factor) with the aid of Microsoft Excel 2003. Difference was considered significant when $p<0.05$.

Results

Microspheres preparation and optimization

For obtaining the uniform sized particles, different conditions were used in terms of alginate concentration, flow rates, pressure, distance between nozzle and collecting plate and $CaCl_2$ concentration. At 2 % w/v alginate at a flow rate of 20 ml/hr with 70mbar pressures at 5 cm distance using 250 mM. $CaCl_2$ concentrations, particles of 60±5 µm were produced as shown in Figure 1 (a)



(A) (B) (C)

Figure.1 (A) Optical microscope images of uncoated alginate microspheres (Size 60 ± 5 µm) (B) SEM images of $(PDDA/PSS)_1$ & (C) $(PAA/PAH)_1$ coated alginate microsphere

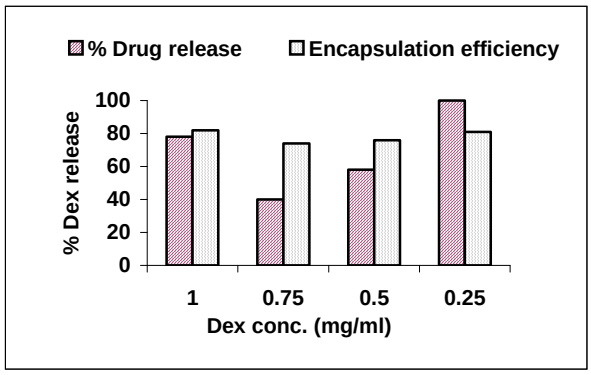


Figure.2 Effect of drug loading on dexamethasone release profile

Effect of drug loading analysis

For the optimization of drug loading, different dexamethasone concentrations were used for release i.e. 0.25, 0.50, 0.75 and 1mg/ml. The 100 % drug was released with 0.25 mg/ml concentration, with very good encapsulation efficiency ≈82%, as shown in figure 2.

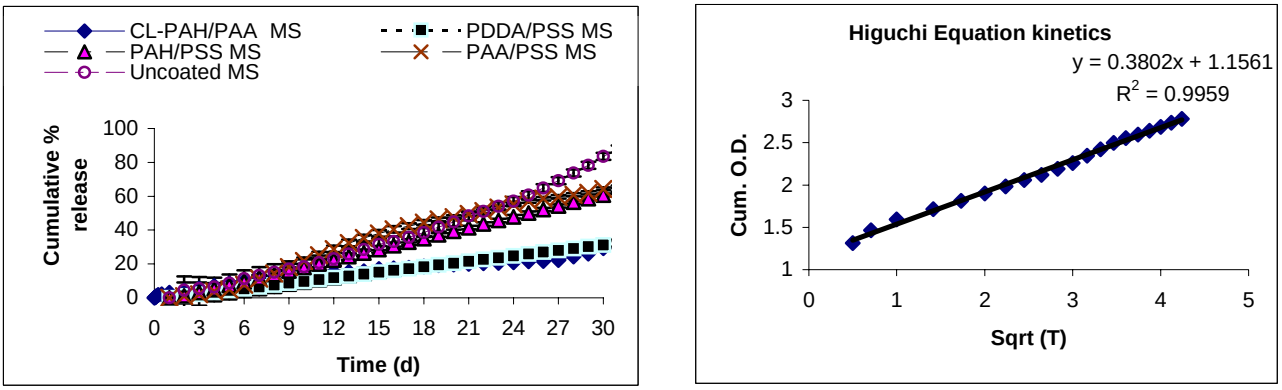
Effect of different salt type on drug release

Different salts were chosen to determine the effect on dexamethasone release behavior, *viz* $BaCl_2$, $CaCl_2$, $MgCl_2$ and $AlCl_3$. In the case of $MgCl_2$ and $AlCl_3$, there was no gelling or clumping of alginate gel. $Bacl_2$ showed good gelling property, but drug entrapment efficiency was very low. With the initial drug loading of 1 mg/ml, 64% was the encapsulation efficiency and only 58% of drug released over a period of 1 month. On the other hand, $CaCl_2$ provides good gelling property alongwith very good drug entrapment efficiency ≈ 80% with the same amount of drug loading and was showing a 78% drug release over a period of one month. Thus, $CaCl_2$ was selected for the further studies.

In- vitro release studies

The *in-vitro* release profile of various polyelectrolytes was performed; results were shown in below figures. The cumulative release of dexamethasone was approximately 100%, 74%, 29%, 39% and 68% for coated, uncoated, PAH/PAA, cross linked PAA/PAH, PAH/PSS and PDAA/PSS microspheres respectively. There was a significant (Student t-test, $P<.05$) difference in the extent of drug release as observed in PAA/PAH coated v/s cross linked PAA/PAH. To study the release

kinetics, data obtained from the *in- vitro* drug release studies were plotted in various kinetic models: zero order, first order (Z. Rahman, 2006) and Higuchi's model (T. Higuchi, 1963). Further, to evaluate the mechanism of drug release data for the first 60% of drug release were plotted in Korsmeyer- Peppas equation (R.W.Korsmeyer, 1983). The corresponding plot (cumulative drug percent release vs Sqrt time) for the equation indicated a good linearity (R^2 =0.9959) and release exponent n = 0.4465 for 1 bilayer system as shown in Figure 4, which appears to indicate the diffusion based release mechanism. Similarly, fits for other curves and models equations can be done. Thus, the application of polyelectrolyte nanofilms on uniform sized alginate microspheres demonstrated a controlled release profile as compared to the uncoated microspheres release profile.



4(a) **4(b)**

Figure.4 (a) Cumulative release profile of Dexamethasone loaded polyelectrolytes (PE's) coated microspheres; 4(b) Higuchi kinetic equation for $(PAA/PAH)_1$ coated MS

Conclusion

The anti inflammatory drug dexamethasone (DEXA) was loaded into microspheres. The maximum loading of DEXA was achieved at pH 7.0 and at a salt concentration of 250 mM. We also found that by manipulating the drug concentration of the release solution, it was possible to control the rate of the release of the drug. Therefore, the continuous release of dexamethasone using LbL coated microspheres would be able to control inflammation around the implant and improve the prolonged existence and detection of glucose sensors *in-vivo*

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