CONTROLLED RELEASE OF IMMUNOMODULATING AGENT FROM NANOENGINEERED ALGINATE CARRIERS

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Introduction

Due to the ever increasing amount of biopharmaceuticals, there is a growing need for advanced drug delivery systems (R. Langer, 2004). Over the last two decades, the field of controlled drug delivery has been confronted with two major challenges. 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 controlled manner (A. Anal, 2007). Probable solution to these problems includes the fabrication of a delivery system that releases its drug at a predetermined rate and the development of a delivery system that can respond to changes in the local environment.

A critical problem with implantable glucose sensors is the inflammatory response upon implantation (T. Hickey, 2002). Tissue response against an implant can be reduced by the release of immunomodulating agents (e.g. dexamethasone) or tissue response enhancer in the immediate vicinity of the graft or incorporated within the implant (A. A. Sharkawy, 1998). Therefore, in order to develop a reliable implantable glucose sensor that will remain functional for a long time *in vivo*, it is important to ensure controlled and continuous local delivery of dexamethasone at the implantation site.

Layer by Layer self assembly is the construction of nanometer (nm) scale coatings by the adsorption of alternately charged polyions (N. Pargaonkar, 2005). The attractive feature of this approach is its ability to assemble complex structures from modular components ant integrate them into self assembling constructions for a wide variety of application (R. Srivastava, 2005). Therefore, the goal of this study was to develop a microsphere system that can be used for continuous delivery of dexamethasone. For this purpose, dexamethasone loaded microsphere system has been designed to provide localized dexamethasone delivery at an implant site to suppress the inflammatory response arising out of the implantation procedure.

Materials and methods

Materials

Alginate (Low viscosity, 2%), Dexamethasone 21 phosphate di-sodium salt (MW-392.5), sodium poly (styrene sulfonate) (PSS, 70 kDa), poly (allylamine hydrochloride) (PAH, 70 kDa), PBS tablets were purchased from Sigma- Aldrich (India). Sodium azide was purchased from Loba Chemi (Mumbai). Calcium 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 preparation of alginate microspheres. Nikon YS 100, Zeiss optical microscope with a digital camera and Hitachi S3400 scanning electron microscope were used for image analysis. Model 770 Accusizer (Particle Sizing Systems, CA) used for particle sizing. Zeta potential (Electrophoretic mobility) microspheres was calculated using Zetaplus, (Brookhaven Instruments, USA). Helios Alpha UV-Visible spectrophotometer (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 with dissolved dexamethasone drug was filled into the syringe of 60c.c. capacity. Different flow rate and pressure condition were used for the preparation of uniform sized microspheres. Alginate spray was collected into 250 mM calcium chloride solution for gelation at 250 rpm for 15 minutes.

Microspheres particle size analysis
The mean particle size and size distributions of the microspheres were measured using optical microscopy and Model 770 Accusizer (Particle Sizing Systems, CA).

Microspheres image analysis
Uncoated and coated microspheres were examined using a Nikon optical microscope at 2.5X, 10X, and 20X magnifications for image analysis and size determination.

Surface Morphology
The shape and surface characteristics of the microspheres were observed by scanning electron microscopy (Hitachi S3400)

Layer-by-Layer Self Assembly Technique
For the Layer-by-Layer (LbL) coating of the microspheres, 1ml of PAH (2 mg/ml in 0.25 M $CaCl_2$) was added to 200µl of microspheres and kept for 20 min with intermittent shaking. The particles were then centrifuged at 500rpm for 1min to separate them from the unreacted PAH solution and washed with distilled water. This was followed by addition of 1ml of PSS (2 mg/ml in 0.25 M $CaCl_2$) and kept for 20 min with intermittent shaking. Finally, the microspheres were washed (twice) with distilled water using centrifugation (R. Srivastava, 2005). Similarly, a second bilayer was deposited on the microspheres and finally, images of microspheres obtained using an optical microscope and scanning electron microscope.

Zeta potential (Electrophoretic mobility)
The zeta potential of the uncoated and LbL coated microspheres were measured using Zeta plus (Brookhaven Instruments, USA). For this experiment, 50µl sample solution containing the microspheres was diluted in 2 ml of distilled water and used for analysis (R. Srivastava, 2005).

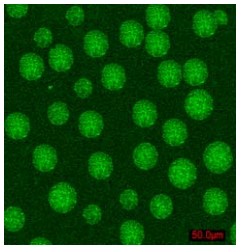
***In- vitro* drug release study**
In-vitro drug release studies were performed using a dialysis membrane in a 250 ml beaker. Dexamethasone loaded microspheres were put in 100 ml of 0.1M PBS (pH 7.4) containing 0.01%w/v sodium azide at 37ºC. At preset time intervals, the buffer sample was collected and replaced with a fresh buffer solution (B.G. De Geest, 2006). The amount of drug released was calculated spectrophotometrically at λ_{max} 242 nm.

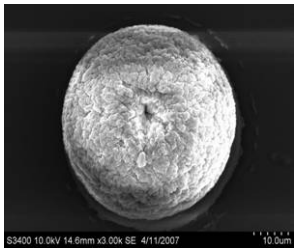
Results

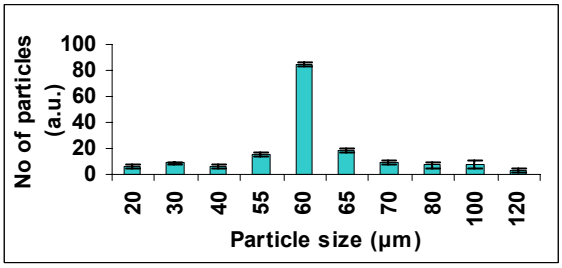
Microspheres preparation and optimization
For obtaining the uniform sized particles, different conditions were used in terms of alginate concentration, flow rates, pressure, distance between nozzle and collecting plate and $CaCl_2$ concentration. At 2 % w/v alginate at a flow rate of 20 ml/hr with 70mbar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

Particle size analysis
The particle size and size distributions of the microspheres were measured using fluorescence microscopy and Model 770 Accusizer (Particle Sizing Systems) and it was observed that maximum

particles were in range of 60±5 µm as demonstrated in Figure 1(b).







1(a) **1(b)**

Figure.1 (a) Fluorescence microscope and SEM images of alginate microspheres (Size 60 ± 5 µm); (b) Particle size determination using DLS

Zeta potential analysis

The results of ζ-potential measurements on LbL coated microspheres shown in Figure 2. The uncoated microspheres had a ζ-potential −25.0 mV ±0.5 which reversed with each coating. The film thickness of the deposited polyelectrolyte nanofilms was calculated by using QCM and was in the region of thickness of 2.1 nm per layer. For charged microspheres electrostatic interactions between the microspheres and the polyelectrolytes are the main driving force for polyelectrolyte adsorption.

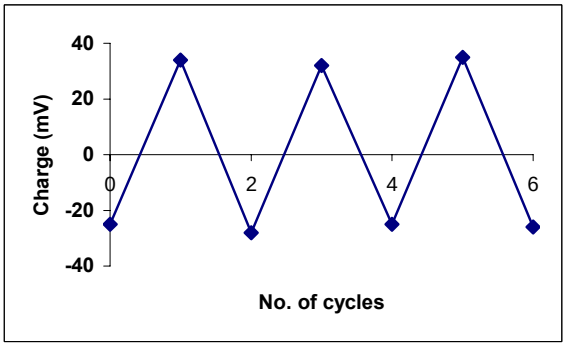


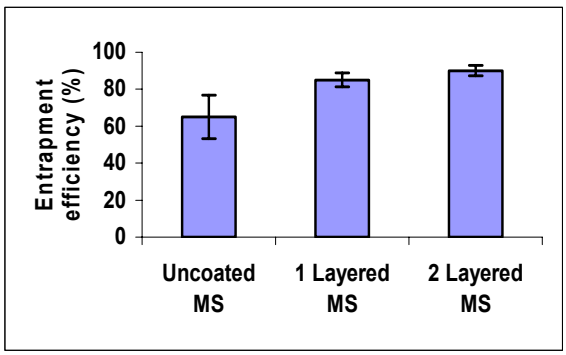
Figure 2. Zeta potential of (PSS/PAH) coated alginate microspheres

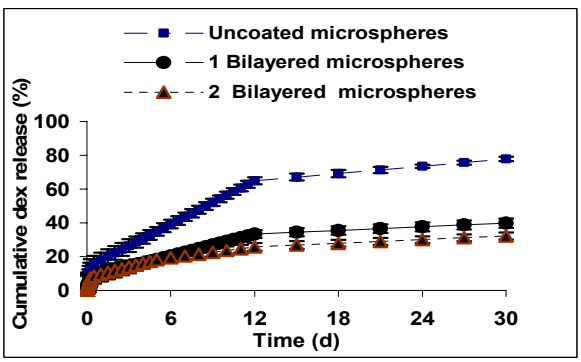
Drug entrapment efficiency

Drug entrapment efficiency was found to be 62%, 74% and 80% for uncoated, $(PSS/PAH)_1$ and $(PSS/PAH)_2$ coated microspheres respectively as shown in Figure 3 (a).

***In- vitro* release studies**

The *in-vitro* release profile of uncoated, one bilayer coated and two bilayer coated alginate microspheres are shown in Figure 3(b). The cumulative release of dexamethasone was approximately 80%, 40% and 30% for uncoated microspheres, one bilayer coated microspheres and two bilayer coated microspheres respectively. There was a significant (Student t-test, P<.05) difference in the extent of drug release as observed in uncoated and coated microspheres. To study the release kinetics, data obtained from *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).

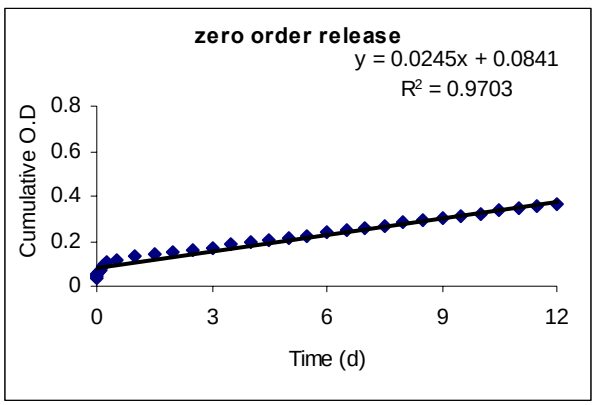


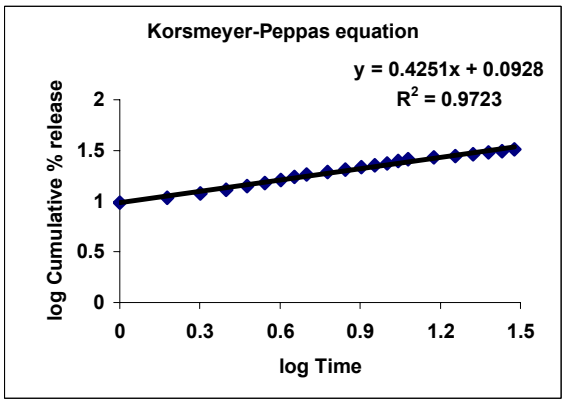


3(a) **3(b)**

Figure 3(a). Drug entrapment efficiency of $(PSS/PAH)_1$, $(PSS/PAH)_2$ coated and uncoated microspheres; 3(b). Comparative release profile of dexamethasone loaded uncoated $(PSS/PAH)_1$ and $(PSS/PAH)_2$ coated alginate microspheres, Mean± SD (n=3)

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 (log cumulative percent drug release vs time) for the equation indicated a good linearity ($r^2 = 0.9723$) and release exponent $n = 0.04215$ for 2 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. Drug kinetics profile for $(PSS/PAH)_2$ coated microsphere fitted to 4(a) zero order kinetic equation; 4(b)Korsmeyer- Peppas equation

Conclusion

The study revealed that uniformly sized dexamethasone-loaded alginate microspheres were prepared using a commercially available droplet generator. The LbL coated microspheres have a high drug loading capacity and also maintained their integrity throughout the release period. 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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