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Difficulties of protein encapsulation into the biodegradable polymers; a way to overcome the problems

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INTRODUCTION AND OBJECTIVES

Considerable advances in biotechnology have resulted in the discovery of a large number of therapeutic and antigenic proteins. However, their unique properties, such as high molecular weight, easy degradation, instability and low bioavailability, make traditional dosage forms not proper to deliver them. Biodegradable polymeric microspheres are ideal vehicles for controlled delivery applications of drugs, peptides and proteins. Amongst them, poly (lactic-co-glycolic acid) (PLGA) has generated enormous interest due to their favorable properties and also has been approved by FDA for drug delivery. A major obstacle to the development of these devices is the need to retain the structure and biological activity of encapsulated proteins during manufacturing process. Further, due to the high cost, limited availability and tedious isolation of these macromolecules, the encapsulation technique must ensure high efficiency of entrapment. Therefore, high loading and low burst release in uniform microspheres is also of great interest in developing controlled-release systems of therapeutic proteins. Over the past decade, various technologies including, phase-separation (coacervation), spray-drying and different methods of solvent evaporation have been proposed for the preparation of PLGA microcapsules. In this review, we would like to share our own exploration and experience regarding novel oil in oil encapsulation method employed to prepare PLGA insulin-loaded microspheres which allowed us to improve the encapsulation efficiency of the protein, to minimize the burst release and to maintain the structural integrity and conformation of the drug. The review will also discuss different conventional and novel techniques of preparing vaccine, antigen, peptide and protein loaded PLGA microspheres and shed the light on the multiple issues particularly stability aspects scientists are confronting during formulation processes.

MATERIALS AND METHODS

Materials

PLGA (LA to GA was 50:50) was supplied by Boehringer Ingelheim, Germany.

Microsphere preparation and characterization

Insulin-loaded microcapsules were produced by a single phase o/o solvent evaporation method. The protein solution was incorporated in acetonitrile PLGA solution at V/V ratio of (1: 5). This solution is then dispersed into 30 ml mineral oil in the presence of 3% Span 80 and stirred for 2 hours at 5000 rpm with an impeller type stirrer to ensure complete evaporation of acetonitrile. Microspheres were collected by centrifugation at 20,000 rpm for 30 min at 10^{o}C and washed four times with n-hexane to complete remove of mineral oil. Particles were filtered, dried, and stored under refrigeration in a desiccator until used.

The characteristics of the microcapsules were determined by various methods: the surface morphology and size of microparticles by atomic force microscopy and scanning electron microscopy, insulin crystalinity and drug-polymer interactions by XRD and DSC, chemical integrity and aggregation of insulin using HPLC and SDS-PAGE, the protein secondary structure by far ultraviolet-circular dichroism, the antigenicity activity of insulin with ELISA techniques.

RESULTS AND DISCUSSION

The PLGA microparticles were spherical and discrete (Fig.1; SEM image) with smooth surfaces (Fig.2; AFM image). This could be attributed to the lack of internal aqueous phase during the o/o technique as compared to honey comb structure of microparticles fabricated by w/o/w method.

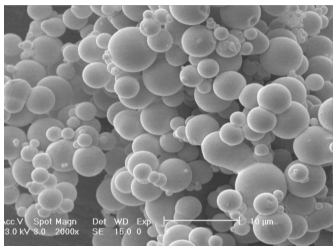


Figure 1: SEM image

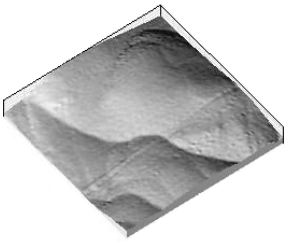
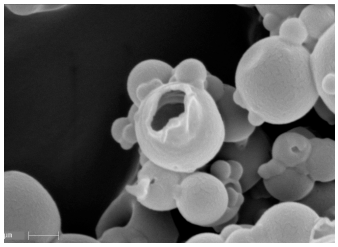


Figure 2: AFM image

The SEM image of microparticles during release study revealed that the microparticle's surface is covered with a thin layer (Fig.3). The encapsulation efficiency of insulin in the PLGA microspheres prepared in this study was 51.18 ± 6.85 (n=3). Improved protein loading was achieved through application of an external oil phase to avoid hydrophilic protein (insulin) leakage**.** Although 52% encapsulation efficiency may not be high enough, but in comparison with many other similar studies involved protein-loaded PLGA microspheres (Aguiar 2004; Porjazoska 2004; yeh 2004) this is virtually a high value. In most of these reports, w/o/w technique has been employed to prepare protein-loaded PLGA microparticles and low encapsulation efficiency has been attributed to drug leakage to aqueous external phase. Fig. 4 illustrates the cumulative release of insulin from PLGA microspheres. More than 90% of insulin was released from PLGA microspheres during 3 days, while only about 24% released as the burst effect.



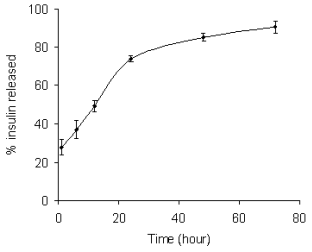


Figure 3. SEM of release **Figure 4. Released insulin**

Burst release is a mostly unwanted event in microspherical drug delivery systems. 24% release might be generally a substantial burst release; however, considerable burst release of proteins from microspheres, particularly in the low size range, is frequently reported in the literatures (Cui 2007; Ibrahim 2005).

Encapsulated and released insulin was dispersed in crystalline state in the polymer matrix as confirmed by DSC (Fig. 5) and XRD (Fig.6).

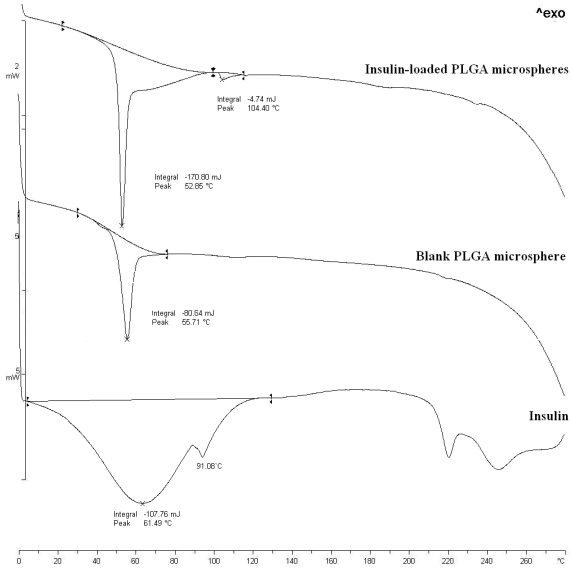


Figure 5. DSC thermograms

Crystalline proteins are usually less prone to chemical degradation than the amorphous form (Lai 1999). Direct interaction between protein molecules is relatively weak in most crystals, thus minimizing the loss of biological activity.

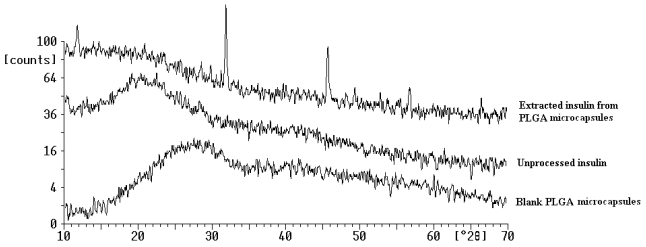
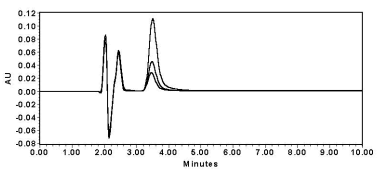


Figure 6. XRD patterns

Chemical integrity and lack of aggregation of insulin were proved using HPLC (Fig. 7) and SDS-PAGE (Fig. 8), respectively.



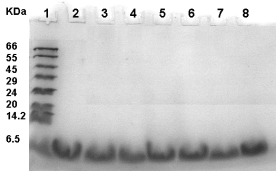


Figure 7. HPLC analysis **Figure 8. SDS-PAGE**

The protein secondary structure preservation was checked by far ultraviolet-circular dichroism (Fig. 8).

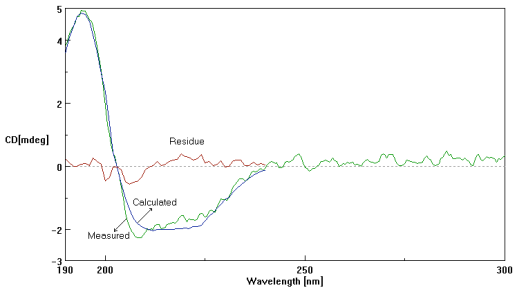


Figure 8. CD analysis

ELISA results indicated that the antibody binding was unchanged after encapsulation (84% as relative to protein content determined by HPLC; n = 2). These results confirmed that the receptor binding epitopes on insulin were maintained after encapsulation.

CONCLUSIONS

Ease of manufacturing under mild preparation conditions, high level of drug entrapment, desirable release pattern with relatively low initial burst effect and an ability to preserve protein structure are the advantages which are offered by the developed protein encapsulation method.

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