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Nanoencapsulation of Insulin Using Blends of Biodegradable Polymers and *In Vitro* Controlled Release of Insulin

Vida Rahmani[1], Kayghobad Shams[1]* and Hamidreza Rahmani[2]

[1]*Department of Chemical Engineering, Isfahan University of Technology, Isfahan 84156-83111, Iran*
[2]*Department of Animal Science, Isfahan University of Technology, Isfahan 84156-83111, Iran*

Abstract

The purpose of this study was achieving an optimum formulation with low initial burst and steady-state release of insulin from the nanoparticles prepared with different blends of poly(lactide-co-glycolide) (PLGA), polylactic acid (PLA), poly(ε-caprolactone) (PCL) and Eudragit® RS100 polymers. Insulin was encapsulated in different blends of these polymers using W/O/W double emulsion technique. Methylene chloride was used as the organic solvent and poly(vinyl alcohol) was used as the stabilizer in the external aqueous phase. The prepared nanoparticles revealed high encapsulation efficiencies (average 81.0%), showing that different compositions of the polymers did not have much effect on encapsulation efficiencies and loading capacities. AFM analyze showed a minimum particle size of 300 nm and maximum particle size of 900 nm. The *in vitro* release profiles indicated that the PLGA/PLA: 45%/55% blend was the optimum mixture, which its insulin release profile had the minimum initial burst, followed by a smooth and uniform drug release. The *in vitro* release profiles were modeled with the Higuchi and Diffusion model and were in better agreement with the diffusion model. In conclusion, the appropriate blend of these polymers might be interesting for drug encapsulation and its release pattern for further researches.

Keywords: Nanoencapsulation; Controlled release; Initial release; Insulin; Poly(lactide-co-glycolide); Polylactic acid; Poly(ε-caprolactone), Eudragit® RS100

Introduction

Insulin is a peptide hormone consisting of two peptide chains, which are connected by two disulphide bridges [1]. This hormone is produced by the β cells in pancreas and has a main role in controlling blood glucose level by facilitating its uptake in the organism cells, especially muscle and adipose tissues [2]. Insulin has a key role in the treatment of diabetes mellitus, which is growing into epidemic proportions worldwide [3]. Diabetes mellitus is caused due to a disorder of blood glucose regulation. In type I diabetes, or insulin-dependent diabetes mellitus, the pancreatic β cells are destroyed by an autoimmune-mediated destruction. Type II diabetes, or non-insulin-dependent diabetes mellitus, is caused by disorder of both insulin resistance and secretion [4].

For the treatment of type I diabetes and many patients with type II diabetes, a constant basal insulin supply is needed to mimic a near-normal physiological insulin secretion pattern [5,6,7]. Because insulin has a short biological half-life, in addition to mealtime treatment, injection of one or more doses of intermediate- or long-acting insulin is necessary to satisfy the patient's basal requirement of insulin [6,8]. This mode of administration has many disadvantages, such as physiological stress, pain, inconvenience, cost, risks, infection, inability to handle insulin and the localized deposition of insulin, leading to local hypertrophy and fat deposition at the injection sites [8], particularly in infants and kids. In recent years insulin delivery by non-invasive routes has gained significant attention, including oral, ocular, nasal, buccal, rectal, pulmonary, and transdermal drug delivery systems [5,9]. Oral route is clearly the most convenient and desired alternate that offers the maximum advantage in patient compliance [9,10]. However insulin undergoes rapid degradation by the gastrointestinal enzymes [11], and after oral administration less than 0.5% of the initial dose is absorbed [2,12]. Therefore, there has been great interest in developing an insulin formulation that could provide a controlled release profile of the drug for longer periods of time [13]. It is proved that encapsulation of proteins protects them from gastric pH and enzymatic attack, resulting in the release of the entrapped molecule in a controlled fashion [14].

For the maximum protection of the drug, the integrity of the encapsulating material should be maintained until permeation through the intestine wall. This can be best achieved by the use of polymers, which must be biodegradable and biocompatible. Poly(lactide-co-glycolide) or PLGA, is an approved biodegradable polymer that degrades to toxicologically acceptable lactic and glycolic acid [13] and has been widely used in drug delivery systems and insulin encapsulation [6,15-19]. Polylactic acid or PLA as another bioabsorbable polymer with no toxicity has also been used for insulin encapsulation [20,21]. Poly(ε-caprolactone) or (PCL), also a biodegradable polymer, and Eudragit® RS100, a nonbiodegradable but biocompatible polymer, also have had applications in insulin encapsulation, but only with a 50/50 ratio [2,22].

The W/O/W double emulsion method is the most common method used for the encapsulation of protein drugs, due to its relevant simple process and no need to expensive instruments [23]. In this method, the aqueous drug solution is dispersed in an organic solution containing dissolved polymers, to form the primary W/O emulsion. Then, this primary emulsion is dispersed in a larger volume of water containing a surfactant/an emulsifier and the second W/O/W emulsion is formed. With the removal of the organic solvent, solid micro/nano particles are formed [23]. Drug release from micro/nano particles can be divided into an initial burst release phase followed by a slow continuous release phase. The initial burst release is usually defined as the amount of drug

***Corresponding author:** Kayghobad Shams, Department of Chemical Engineering, Isfahan University of Technology, Isfahan 84156-83111, Iran, Tel: 98 311 391 5610; E-mail: k_shams@cc.iut.ac.ir

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released in the first 24 hours [24]. In insulin-loaded particles, the initial burst release, as a critical process, causes hypoglycemia and must be seriously controlled.

There have been many challenges on controlling the initial burst release of insulin from the micro/nano particles. De Rosa et al. tried achieving slow release of insulin from microspheres prepared by adding different non-ionic surfactants to the primary emulsion [15]. Takenaga et al. reduced the initial burst release by using hydrophilic additives such as glycerin, ethanol and distilled water throughout the preparation procedure [6]. Choi and Kim used zinc-complexed insulin and reported no initial burst and a constant release rate [7]. Hinds et al. used a combination of two PEGylation and microencapsulation technologies and obtained negligible burst release [18]. Martins et al. improved insulin release from the microspheres by reinforcing the alginate matrix with chitosan and/or dextran sulphate [14]. Han et al. produced insulin-loaded nanoparticles by the isoelectric point deposition method and reduced the burst release [19]. Zhang et al. [25] fabricated a novel pH-responsive oral protein drug delivery made of starch nanoparticles (SNPs) as backbone and poly(l-glutamic acid) (PGA) as graft chains by click reaction, and studied it's in vitro insulin release. Mortazavian et al. [26] studied statistical optimization and in vitro characterization of insulin nanoparticles containing thiolatd N-diethyl methyl chitosan (DEMC-Cys) and N-dimethyl ethyl chitosan (DMEC-Cys) conjugates. Zabihi et al. [27] prepared nanoparticles of insulin/hydroxy-propyl-methylcellulose (HPMC)–poly-lactic-co-glycolic acid (PLGA) by a modified supercritical CO_2 anti-solvent technique. They could prepare uniform particles were with the smallest particle size of 35 nm, the maximum product yield of 88%, and the highest insulin loading of 55.2%. Andreani et al. [28] developed and characterized silica nanoparticles (SiNP) coated with hydrophilic polymers as mucoadhesive carriers for oral administration of insulin. These efforts were materialized mostly by using additives or complicated preparation methods. In the present work, we have tried to control the initial burst release of insulin from the nanoparticles without the use of any extra additives or complex methods. Insulin was encapsulated using various blends of PLGA, PLA, PCL and Eudragit® RS100. Regarding the different physicochemical and molecular properties of these polymers, it is postulated that an optimum combination of them in encapsulation, because of structural and molecular weight differences would lead to a coating and a mass transfer resistance that could reduce the initial burst release and release insulin in a more steady and controlled manner. Therefore, the objective of this study was investigating the effect of different blends of PLGA, PLA, PCL and Eudragit® RS100 on insulin encapsulation efficiency, its initial burst and in vitro release profile. In addition, the in vitro release profiles of insulin were modeled with Higuchi and diffusion models and respectively the Higuchi dissolution constants and the Diffusion coefficients for insulin in the different polymeric blends were calculated.

Materials and Methods

Materials

Regular human insulin (100 IU/ml) was obtained from Exir Pharmaceutical Company (Tehran, Iran). Poly(DL-lactide-co-glycolide) (average molecular weight 5,000-15,000, lactide/glycolide ratio 50:50), polylactic acid (average molecular weight 60,000), poly(ε-caprolactone) (average molecular weight 70,000-90,000) and poly(vinyl alcohol) (average molecular weight 31,000-50,000, 87-89% hydrolyzed) were purchased from Aldrich. Eudragit® RS100 was a gift from Akbarieh Company (Tehran, Iran). Methylene chloride was purchased from Merck. HPLC grade acetonitrile was obtained from Acros Organics. All other chemicals were of analytical grade.

Methods

Polymeric formulations: The combinations of the candidate coating polymers (PLGA, PLA, PCL, and Eudragit® RS100) for encapsulation of insulin were grouped in three sets. Because of the existing promising and independent reports on use of PLGA and PLA in insulin encapsulation [6,15-21], blends of PLGA and PLA were studied first. Blends of PCL and Eudragit® RS100, which their 50/50% combination was previously used for insulin encapsulation [2,22] were also studied in another set of experiments. Thus five various combinations of PLGA and PLA with 0%, 25%, 50%, 75% and 100% PLGA and five other combinations of Eudragit® RS100 and PCL with 0%, 25%, 50%, 75% and 100% Eudragit® RS100 were used for insulin encapsulation, as shown in Table 1. After the preparation of the encapsulated insulin nanoparticles and studying the *in vitro* release profile of insulin from the formulations, the most promising formulation, with the more steady insulin release, was determined. To fine-tune the formulation and to achieve a more precise optimum formulation, the range of compositions was divided in 5% increments, again used for insulin encapsulation, and the optimum formulations were determined. In the third set of the experiments, the two polymers not used in the optimum formulations, were combined with the optimum formulations in different percentages. Then, the *in vitro* release profiles of these formulations were studied. Finally, insulin release from compositions with equal percentages of three and four polymers in the formulations, as shown in Table 2, were investigated following the same procedures.

Preparation of nanoparticles: Insulin-loaded polymeric nanoparticles were prepared by the multiple emulsion technique, using methylene chloride as the organic solvent and poly(vinyl alcohol) or PVA as the emulsifying agent. The multiple emulsion technique described by Damgé et al. and Hoffart et al. was used with minor adjustments for the preparation of nanoparticles [22,29]. Briefly, using a caped test tube, 1 ml of an aqueous solution of insulin (100 IU/ml) was emulsified in 10 ml methylene chloride, containing 250 mg polymers of various formulations, by sonification for 30 s at 60 W. The resulting

Formulation No.	PLGA (%)	PLA (%)	Eudragit® RS100 (%)	PCL (%)
1	0	100	0	0
2	25	75	0	0
3	50	50	0	0
4	75	25	0	0
5	100	0	0	0
6	0	0	0	100
7	0	0	25	75
8	0	0	50	50
9	0	0	75	25
10	0	0	100	0

Table 1: Polymeric formulations used in the first stage of the experiments.

Formulation No.	PLGA (%)	PLA (%)	Eudragit® RS100 (%)	PCL (%)
11	33	33	33	0
12	33	33	0	33
13	33	0	33	33
14	0	33	33	33
15	25	25	25	25

Table 2: Polymeric formulations used in the last stage of the experiments.

water-in-oil emulsion was poured into another test tube containing 40 ml of PVA aqueous solution (0.1%) and again was sonicated for 1 min at 60 W, resulting to the formation of the second water-in-oil-in-water emulsion. After evaporation of methylene chloride, the nanoparticles were isolated by centrifugation for 40 min at 11,000×g (BHG Hemle, model Z369, Germany). The supernatant phase containing the free insulin (not entrapped within the polymeric encapsulation) was saved for analyzes. The nanoparticles were washed two times with deionized water and lyophilized (at -40°C and 10 mmHg for 6 hours, Ogawa Seiki, model OSK 2139, Japan) and stored at -20°C until use.

Characterization of nanoparticles: The mean diameter of nanoparticles was determined using Atomic Force Microscopy (AFM, Bruker, Germany). The operation mode for AFM was static mode. The Image Plus 2.9 software was used for image analysis. The amount of free insulin was determined by high performance liquid chromatography (HPLC), using a Jasco Series 900 instrument, consisting of a Jasco model 980 HPLC pump and UV-975 detector. The reverse-phase column (C18, μBondapak®, average particle size 10 μm, length 300 mm) was kept at room temperature. The mobile phase consisted of 60 volume of 1 mmol sodium sulphate and 0.2% triethylamine in water, pH 3.2 adjusted by phosphoric acid, and 40 volume of acetonitrile, as described by Rajan et al. [30]. The solution was filtered through a 0.45 μm membrane. The eluent was monitored with a flow rate of 1 ml/min and a UV detector set at 214 nm [30].

The encapsulation efficiency and loading capacity were calculated by Equations 1 and 2, respectively [8]:

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{Amount of insulin Used} - \text{Weight of Free Insulin}}{\text{Amount of Insulin Used}} \times 100\% \quad (1)$$

$$\text{Loading Capacity (\%)} = \frac{\text{Weight of insulin Used} - \text{Weight of Free Insulin}}{\text{Weight of Dried Nanoparticles}} \times 100\% \quad (2)$$

***In vitro* experiments:** The in vitro release of insulin from various formulations was evaluated using phosphate-buffered saline (PBS, pH=7.4). 5 mg of each formulation was suspended in 2 ml of buffer and incubated longitudinally in a water bath at 37°C and 100 strikes/min. At appropriate times (5 hours, 1, 2, 3, 4, 5, 6, 7 days) 500 μl of supernatant was collected after centrifugation for 15 min at 6900×g and was replaced by 500 μl fresh buffer. The insulin content of the samples was determined using HPLC.

***In vitro* release modeling:** The in vitro release profiles of insulin were modeled using the celebrated Higuchi and Diffusion models. The regression was carried out using MATLAB R2008a curve fitting toolbox.

Higuchi model: The Higuchi model is the first example of a mathematical model for describing drug diffusion in a matrix system. Equation 2 gives the model expression.

$$Q=A[D(2C-C_s)\,C_s t]^{1/2} \quad (2)$$

Where, Q is the amount of drug released in time t per unit area A, C is the initial concentration of drug, C_s is the drug solubility in the matrix media, and D is the diffusivity of the drug molecules in the matrix substance [31].

The simplified model of Equation 2 is:

$$Q=K_H \times t^{1/2} \quad (3)$$

Where, K_H is the Higuchi dissolution constant [31]. Equation 3 was used to determine the Higuchi release rate constants for various formulations.

Diffusion model: The diffusion model is based on Fick's second law of diffusion. For a radial unsteady-state molecular diffusion in a sphere with constant diffusivity, Flick's second law is expressed as:

$$\frac{\partial C}{\partial t} = D\left(\frac{\partial^2 C}{\partial r^2} + \frac{2}{r}\frac{\partial C}{\partial r}\right) \quad (4)$$

Where, C is the drug concentration, t is time, D is the diffusion coefficient and r is the radius. It is assumed that the drug distribution in the polymeric matrix is uniform; hence the initial condition follows Equation 5.

$$t=0\ C=C_{initial}\ 0< r <R \quad (5)$$

Where, $C_{initial}$ is the initial concentration of the drug in the sphere matrix and R is the radius of the sphere.

The first boundary condition is given by:

$$t>0 \quad \frac{\partial C}{\partial r} = 0 \text{ at } r = 0 \quad (6)$$

For the second boundary condition, it is assumed that the perfect sink condition is established. This assumption is reasonable, since the perfect sink condition is achieved when the concentration of the released drug is always less than 10 percent of its saturation solubility. The solubility of insulin in PBS at pH 7.4 is approximately 7 mg/ml [32], making the 10 percent of the saturation solubility about 0.7 mg/ml. The amount of insulin used in the experiments was 1 ml, which contained 100 IU insulin. With assuming an encapsulation efficiency of 100%, 100 IU insulin would be encapsulated in 250 mg of polymer. Thus, the amount of insulin encapsulated in 5 mg polymer would be 2 IU. Each IU of insulin is equal to 1/22 mg insulin, so the maximum concentration of insulin in 2 ml PBS would be 2/22 mg insulin, in other words 1/22 mg/ml, which is much lower than 0.7 mg/ml. Thus, the perfect sink condition is obtained.

The boundary condition for sink condition is:

$$T > 0\ C = C_\infty \quad \text{at } r = R \quad (7)$$

Where, C_∞ is the drug concentration on the surface in equilibrium with the surroundings. The solution for Equation 4 with the initial and boundary conditions given by Equations 5, 6 and 7 is [33]:

$$\frac{M_s}{M} = 1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty} \exp\left(-\frac{n^2\pi^2}{R^2} Dt\right) \quad (8)$$

Where, M_t represents the cumulative amount of insulin released at time t and M is the cumulative amount of insulin released after a long time. If protein release was leveled off below 100%, the experimentally determined plateau value was considered as 100% reference value for protein diffusion [34].

Results and Discussion

Characterization of nanoparticles

The AFM results of the insulin-loaded polymeric nanoparticles showed a minimum particle size of 300 nm and maximum particle size of 900 nm. Three samples of the AFM images are shown in Figure 1. The prepared nanoparticles showed high encapsulation efficiency, with an average of 81.0%. As shown in Table 3, there was a maximum 5.4% difference in the encapsulation efficiencies between different formulations. The results indicated that different compositions of the polymers did not have much effect on the encapsulation efficiencies and also loading capacities.

In vitro release of insulin

In what follows, the Data represent the mean obtained from two

separate experiments. Figure 2 shows the in vitro release profile of insulin for formulations 1-5 in PBS at 37°C and pH 7.4 for 7 days. Formulation 1 (0% PLGA-100% PLA) released 27.1% of its insulin content after 1 day incubation. Insulin release from formulations 4 (75% PLGA) and 5 (100% PLGA) were the most rapid, releasing respectively 65.4% and 67.3% of their insulin content just in the first day. The percent release of formulations 2 (25% PLGA) and 3 (50% PLGA) after 1 day of incubation was lower than formulation 1, 22.6% and 19.5%, respectively. Figure 3 shows the percent of insulin released in the first day of incubation in PBS at 37°C for formulations 6-10. Formulation 8 (50% Eudragit® RS100) had the minimum initial burst release, releasing 29.1% of its insulin content after 1 day. Formulations 7 (25% Eudragit® RS100) and 6 (0% Eudragit® RS100-100% PCL) also released low amounts of their insulin content after 1 day, 32.6% and 36.0%, respectively. Formulations 9 (75% Eudragit® RS100) and 10 (100% Eudragit® RS100) showed higher amounts of insulin release in the first day, 48.2% and 54.9%, respectively. Generally with the increase of the Eudragit® RS100 percentage in the formulation, the initial burst release was increased. This was attributed to the lower molecular weight of Eudragit® RS100 compared to PCL. It is speculated that as the molecular weight of polymer decreases, the polymeric coating exhibits less resistance to mass transfer, and the diffusion of insulin takes place easier across the polymeric film, hence the initial burst release is increased. As shown in Figures 2 and 3 the slowest release was related to the formulation with 50% PLGA. Therefore, the range between 50% PLGA and 75% PLGA, and also 25% PLGA and 50% PLGA were selected and experiments with an accuracy of 5% were carried out in this range. Table 4 shows the polymeric formulations used in the second stage of the experiments. Figure 4 shows the *in vitro* release profiles of insulin for the PLGA/PLA formulations containing 25% to 50% PLGA (the formulations containing less than half PLGA). Formulations 19 (45% PLGA) and 18 (40% PLGA) have slightly less insulin release in the first day of incubation compared to formulation 3, 16.3% and 16.4%, respectively. While the insulin release from formulations 17 (35% PLGA) and 16 (30% PLGA) in the first day of incubation was more than formulation 3, 21.1% and 21.6%, respectively.

As shown in Figure 5, all these formulations have released more insulin compared to formulation 3 (50% PLGA). Formulation 20 (55% PLGA) released 41.0% of its insulin content in the first day of incubation. Insulin release from formulation 21 (60% PLGA) and 22 (65% PLGA) was 54.2% and 61.4%, respectively, after 1 day of incubation. The percent release of insulin from formulation 23 (70% PLGA) after 1 day was 62.3%.

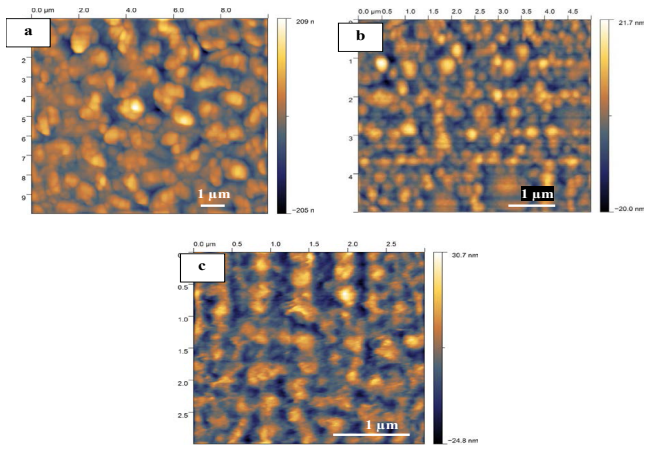

Figure 1: AFM images of encapsulated insulin nanoparticles. a) PLA nanoparticles b) 45% PLGA-55% PLA nanoparticles c) 20% PLGA/PLA (45/55)-80% PCL.

Polymeric Formulation	Encapsulation	Loading Capacity (%)
100% PLA	81.8 ± 1.2	1.49 ± 0.022
100% PLGA	82.6 ± 0.7	1.50 ± 0.013
100% PCL	81.8 ± 0.6	1.49 ± 0.011
100% Eudragit® RS100	80.5 ± 0.8	1.47 ± 0.015
25% PLGA-75% PLA	78.7 ± 2.1	1.43 ± 0.038
30% PLGA-70% PLA	81.6 ± 1.4	1.49 ± 0.025
35% PLGA-65% PLA	81.5 ± 0.9	1.48 ± 0.016
40% PLGA-60% PLA	80.6 ± 0.9	1.47 ± 0.016
45% PLGA-55% PLA	80.1 ± 1.7	1.46 ± 0.031
50% PLGA-50% PLA	80.6 ± 1.0	1.47 ± 0.018
55% PLGA-45% PLA	80.6 ± 0.8	1.47 ± 0.015
60% PLGA-40% PLA	81.7 ± 2.2	1.49 ± 0.040
65% PLGA-35% PLA	80.8 ± 0.4	1.47 ± 0.007
offd% PLGA-30% PLA	80.8 ± 0.5	1.47 ± 0.009
75% PLGA-25% PLA	81.7 ± 1.5	1.49 ± 0.027
25% PCL-75% Eudragit® RS100	80.2 ± 2.1	1.46 ± 0.038
50% PCL-50% Eudragit® RS100	79.6 ± 0.6	1.45 ± 0.011
75% PCL-25% Eudragit® RS100	81.4 ± 0.7	1.48 ± 0.013
80% PLGA/PLA (45/55)-20% PCL	81.9 ± 0.5	1.49 ± 0.009
60% PLGA/PLA (45/55)-40% PCL	82.4 ± 1.3	1.50 ± 0.024
40% PLGA/PLA (45/55)-60% PCL	81.6 ± 1.7	1.49 ± 0.031
20% PLGA/PLA (45/55)-80% PCL	79.3 ± 1.4	1.44 ± 0.025
80% PLGA/PLA (45/55)-20% Eudragit® RS100	82.4 ± 1.4	1.50 ± 0.025
60% PLGA/PLA (45/55)-40% Eudragit® RS100	81.1 ± 0.8	1.48 ± 0.015
40% PLGA/PLA (45/55)-60% Eudragit® RS100	81.6 ± 1.8	1.49 ± 0.033
20% PLGA/PLA (45/55)-80% Eudragit® RS100	81.8 ± 0.9	1.49 ± 0.016
33% PLGA-33% PLA-33% Eudragit® RS100	77.2 ± 2.5	1.41 ± 0.046
33% PLGA-33% PLA-33% PCL	79.4 ± 1.9	1.45 ± 0.035
33% PLGA-33% Eudragit® RS100-33% PCL	82.1 ± 0.3	1.49 ± 0.005
33% PLA-33% Eudragit® RS100-33% PCL	81.5 ± 0.7	1.48 ± 0.013
25% PLGA-25% PLA-25% Eudragit® RS100-25% PCL	82.0 ± 1.0	1.49 ± 0.018

Table 3: Encapsulation efficiencies and loading capacity for polymeric formulations.

Figure 6 illustrates the percent of insulin released from all PLGA/PLA formulations after 1 day of incubation in PBS at 37°C and pH 7.4. The results show that the blend of PLGA/PLA with 45/55 weight percent ratio is an optimum blend, which the release profile of insulin has the minimum initial burst release followed by a smooth and uniform drug release. It is observed that as the PLGA percent in the formulation increases from 0 to 50%, the initial insulin release is nearly the same. But with more increase in the PLGA percent of the formulation, the amount of insulin released in the first 24 hours is significantly increased.

Because of their different physicochemical and structural properties, it is speculated that PLGA and PLA form nanocapsules with a two layer

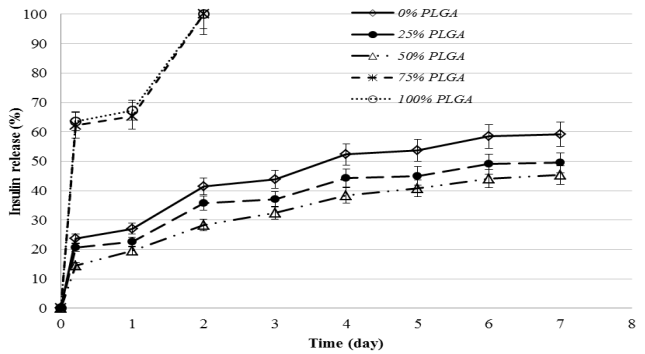

Figure 2: The *in vitro* release profile of insulin from formulations 1-5 in PBS at 37 °C and pH 7.4 for 7 days.

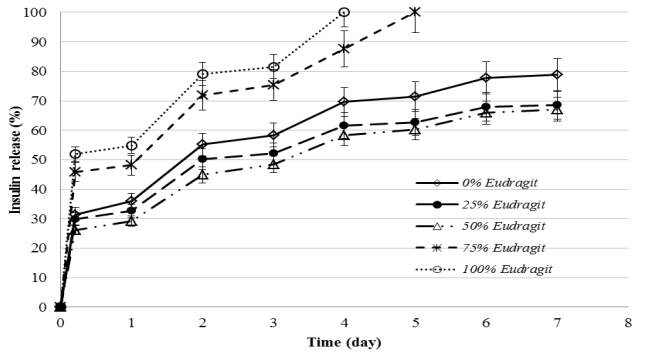

Figure 3: Percent of insulin released from the formulations 6-10 after 1 day of incubation in PBS at 37°C and pH 7.4.

Formulation No.	PLGA (%)	PLA (%)
16	30	70
17	35	65
18	40	60
19	45	55
20	55	45
21	60	40
22	65	35
23	70	30

Table 4: Polymeric formulations used in the second stage of the experiments.

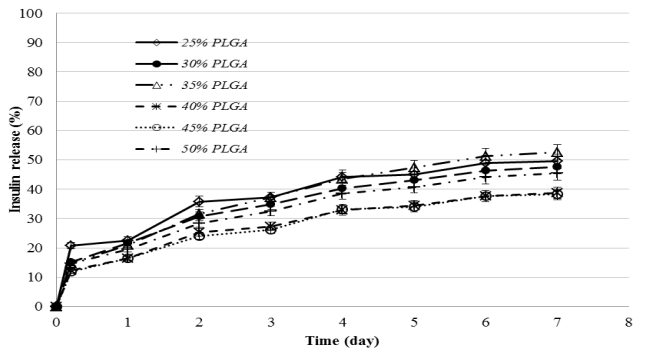

Figure 4: The *in vitro* release profile of insulin from formulations 2, 3, 16-19 in PBS at 37°C and pH 7.4 for 7 days.

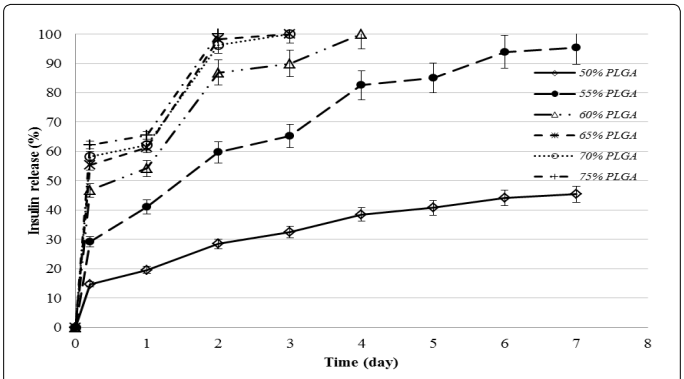

Figure 5: The *in vitro* release profile of insulin from formulations 3, 4, 10-13 in PBS at 37°C and pH 7.4 for 7 days.

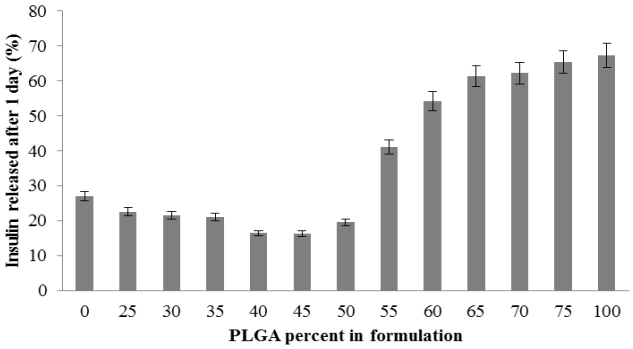

Figure 6: Percent of insulin released from all PLGA/PLA formulations after 1 day of incubation in PBS at 37°C and pH 7.4.

structure. PLGA forms the inner-layer wall facing the aqueous solution of insulin because of its hydrophilicity, while hydrophobic PLA forms the outer-layer wall preventing the leakage of insulin to the surface of the nanoparticles [35]. Protein drugs usually penetrate through the pores or channels formed in the nanoparticles. In the beginning, water enters the surface pores and dissolves the protein for release [31]. In this study, it appears that the outer hydrophobic PLA layer prevents water from diffusing into the polymer layer and generally with the increase of the PLA percentage in the formulation, the initial burst release is decreased. Thus, nanoparticles containing more than 50% PLGA in their formulation released insulin rapidly compared to the formulations having more hydrophobic PLA in their combination.

Another important factor determining the amount of drug release is the glass transition temperatures (T_g) of the polymers. PLA has a glass transition temperature of around 55°C, while the glass transition temperature of PLGA is about 40°C. With incubating the nanoparticles in a water bath at the temperature of 37°C, which is close to PLGA's glass transition temperature, the nanoparticles having more PLGA in their formulation transfer from the glassy to the rubbery state and release insulin faster. Therefore, the rapid release of insulin from the formulations containing more PLGA than PLA, is attributed to the low glass transition temperature and the more hydrophilicity of PLGA compared to PLA. With using the PLGA/PLA: 45/55 blend, the initial burst release was controlled successfully and insulin was released in a steady-state and uniform manner. For investigating the effect of adding PCL and Eudragit® RS100 to the optimum blend, formulations as shown in Table 5 were prepared and used for insulin encapsulation. Figure 7 shows the in vitro release profile of insulin for the optimum formulation and formulations 24-31. As shown in Figure 7, with the

addition of PCL and Eudragit® RS100 to the optimum formulation, the initial burst release was increased. This was attributed to the smaller size of their nanoparticles, as shown in Figure 1. With the decrease in the particles diameter, the area/volume ratio increases and the area for insulin diffusion increases. This leads to higher amounts of initial burst release.

In the final stage of the experiments, insulin release from compositions with same the percentage of three and four polymers in the blends was studied. The results are shown in Figure 8. In general, the amount of insulin released from blends with three and four polymers was more than the optimum PLGA/PLA: 45/55 blend. This may be attributed to the increase in polymer branches and attainment of a less dense and less compact coating, which in turn results in a less mass transfer resistance and a higher diffusion of insulin through the polymeric coating.

In vitro release modeling

Higuchi model: The results and the correlation coefficient values for fitting the in vitro release profiles to the Higuchi model are shown in Table 6. The results listed in Table 6 showed the goodness of the fit for most cases and indicated that the experimental data were in good agreement with the Higuchi model, with an average correlation coefficient of 0.887.

Diffusion model: With fitting the experimental data to the diffusion model Fick's diffusion coefficient for insulin in different blends of the polymeric formulations used was evaluated. The results and the correlation coefficients are shown in Table 7. Table 7 shows that the experimental data in all cases are in good agreement with the diffusion model, with an average correlation coefficient of 0.941. The comparison of Tables 6 and 7 reveals that the experimental data is in superior agreement with diffusion model.

Formulation No.	PLGA/PLA: 45/55 (%)	PCL (%)	Eudragit® RS100 (%)
24	80	20	0
25	60	40	0
26	40	60	0
27	20	80	0
28	80	0	20
29	60	0	40
30	40	0	60
31	20	0	80

Table 5: Polymeric formulations used in the third stage of the experiments.

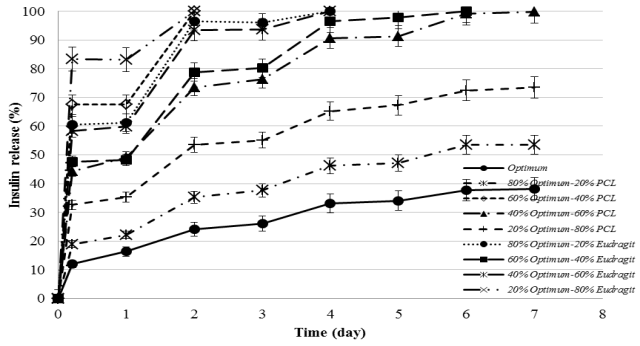

Figure 7: The *in vitro* release profile of insulin from formulations 3, 24-31 in PBS at 37°C and pH 7.4 for 7 days.

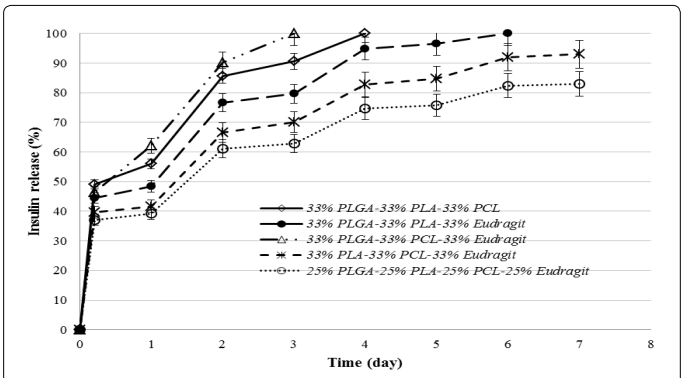

Figure 8: The *in vitro* release profile of insulin from formulations 11-15 in PBS at 37°C and pH 7.4 for 7 days.

Polymeric Formulation	K_H	R^2
100% PLA	24.71	0.958
100% PLGA	74.11	0.818
100% PCL	32.87	0.918
100% Eudragit® RS100	52.08	0.850
25% PLGA-75% PLA	20.82	0.951
30% PLGA-70% PLA	19.44	0.963
35% PLGA-65% PLA	21.08	0.984
40% PLGA-60% PLA	15.70	0.964
45% PLGA-55% PLA	15.49	0.969
50% PLGA-50% PLA	18.37	0.968
55% PLGA-45% PLA	38.64	0.971
60% PLGA-40% PLA	54.30	0.903
65% PLGA-35% PLA	64.24	0.869
70% PLGA-30% PLA	64.17	0.845
75% PLGA-25% PLA	73.32	0.826
25% PCL-75% Eudragit® RS100	46.04	0.893
50% PCL-50% Eudragit® RS100	27.62	0.933
75% PCL-25% Eudragit® RS100	29.01	0.885
80% PLGA/PLA (45/55)-20% PCL	21.84	0.952
60% PLGA/PLA (45/55)-40% PCL	74.73	0.774
40% PLGA/PLA (45/55)-60% PCL	42.47	0.876
20% PLGA/PLA (45/55)-80% PCL	30.99	0.881
80% PLGA/PLA (45/55)-20% Eudragit® RS100	57.98	0.776
60% PLGA/PLA (45/55)-40% Eudragit® RS100	46.07	0.868
40% PLGA/PLA (45/55)-60% Eudragit® RS100	56.92	0.796
20% PLGA/PLA (45/55)-80% Eudragit® RS100	81.87	0.611
33% PLGA-33% PLA-33% Eudragit® RS100	54.53	0.890
33% PLGA-33% PLA-33% PCL	45.56	0.892
33% PLGA-33% Eudragit® RS100-33% PCL	61.85	0.938
33% PLA-33% Eudragit® RS100-33% PCL	39.05	0.900
25% PLGA-25% PLA-25% Eudragit® RS100-25% PCL	35.17	0.878

Table 6: Higuchi dissolution constants and the correlation coefficients for various formulations.

Conclusion

In this study encapsulated insulin nanoparticles were prepared by the W/O/W multiple emulsion technique using various blends of PLGA, PLA, PCL and Eudragit® RS100. The AFM results of the insulin-loaded polymeric nanoparticles showed a minimum particle size of 300 nm and maximum particle size of 900 nm. The polymer ratios did not have much effect on the encapsulation efficiency and all formulations had approximately the same encapsulation efficiency

Polymeric formulation	D/r^2 $\times 10^7$ (1/s)	D_{min} [a] $\times 10^{16}$ (cm^2/s)	D_{max} [b] $\times 10^{16}$ (cm^2/s)	R^2
100% PLA	4.38	0.98	8.88	0.964
100% PLGA	15.51	3.49	31.41	0.898
100% PCL	4.37	0.98	8.84	0.951
100% Eudragit® RS100	4.80	1.08	9.73	0.943
25% PLGA-75% PLA	4.56	1.03	9.25	0.958
30% PLGA-70% PLA	3.86	0.87	7.81	0.979
35% PLGA-65% PLA	3.50	0.77	7.08	0.971
40% PLGA-60% PLA	3.76	0.85	7.62	0.973
45% PLGA-55% PLA	3.70	0.83	7.50	0.967
50% PLGA-50% PLA	3.76	0.84	7.61	0.974
55% PLGA-45% PLA	3.66	0.82	7.41	0.969
60% PLGA-40% PLA	7.55	1.70	15.29	0.952
65% PLGA-35% PLA	11.64	2.62	23.58	0.929
70% PLGA-30% PLA	12.06	2.71	24.42	0.922
75% PLGA-25% PLA	14.35	3.23	29.06	0.893
25% PCL-75% Eudragit® RS100	4.77	1.07	9.66	0.948
50% PCL-50% Eudragit® RS100	4.05	0.91	8.20	0.948
75% PCL-25% Eudragit® RS100	4.53	1.02	9.17	0.946
80% PLGA/PLA (45/55)-20% PCL	3.82	0.86	7.73	0.943
60% PLGA/PLA (45/55)-40% PCL	17.26	3.88	34.95	0.876
40% PLGA/PLA (45/55)-60% PCL	4.94	1.11	10.00	0.945
20% PLGA/PLA (45/55)-80% PCL	4.71	1.06	9.54	0.942
80% PLGA/PLA (45/55)-20% Eudragit® RS100	11.93	2.68	24.16	0.918
60% PLGA/PLA (45/55)-40% Eudragit® RS100	5.82	1.31	11.78	0.931
40% PLGA/PLA (45/55)-60% Eudragit® RS100	10.58	2.38	21.42	0.921
20% PLGA/PLA (45/55)-80% Eudragit® RS100	71.75	16.14	145.29	0.953
33% PLGA-33% PLA-33% Eudragit® RS100	5.49	1.24	11.13	0.947
33% PLGA-33% PLA-33% PCL	4.79	1.08	9.70	0.922
33% PLGA-33% Eudragit® RS100-33% PCL	6.62	1.49	13.41	0.912
33% PLA-33% Eudragit® RS100-33% PCL	7.82	1.76	15.84	0.948
25% PLGA-25% PLA-25% Eudragit® RS100-25% PCL	4.80	1.08	9.71	0.939

[a]Calculated based on $Diameter_{min}$=300 nm

[b]Calculated based on $Diameter_{max}$=900 nm

Table 7: Diffusion coefficients and the correlation coefficient values for different formulations.

values, with the average 81.0% and maximum difference of 5.4%. For the formulations containing PLGA and PLA, it was observed that with the increase of the PLGA percent in the formulation from 0 to 50%, the initial insulin release remained nearly unchanged. But with more increase in the PLGA percent of the formulation, the amount of insulin released in the first day was significantly increased, presumably because of the low glass transition temperature and the more hydrophilicity of PLGA compared to PLA. The formulation with PLGA/PLA: 45/55 had the minimum burst release, releasing only 16.3% of the encapsulated insulin in the first 24 hours, followed by a smooth and uniform drug release in the next days. For blends containing PCL and Eudragit® RS100, insulin release was increased with the increase of Eudragit® RS100 percentage in the formulation. This was attributed to the lower molecular weight of Eudragit® RS100 compared to PCL, which made insulin diffusion across the polymeric film more rapid. In general, the amount of insulin released from blends of three and four polymers was more than the optimum PLGA/PLA: 45/55 blend. This is was attributed to the increase in polymer branches and formation of a less resistive coating due to the use of more branched polymers, and also the reduction of nanoparticles' sizes, which results in higher diffusion of insulin across the polymeric coatings. In conclusion the optimum blend of these polymers might be interesting for drug encapsulation and its release pattern for further researches.

References

1. Moslemi P, Najafabadi AR, Tajerzadeh H (2003) A rapid and sensitive method for simultaneous determination of insulin and A21-desamido insulin by high-performance liquid chromatography. J Pharm Biomed Anal 33: 45-51.

2. Damgé C, Socha M, Ubrich N, Maincent P (2010) Poly(epsilon-caprolactone)/ eudragit nanoparticles for oral delivery of aspart-insulin in the treatment of diabetes. J Pharm Sci 99: 879-889.

3. Elsayed A, Remawi MA, Qinna N, Farouk A, Badwan A (2009) Formulation and characterization of an oily-based system for oral delivery of insulin. Eur J Pharm Biopharm 73: 269-279.

4. Ravaine V, Ancla C, Catargi B (2008) Chemically controlled closed-loop insulin delivery. J Control Release 132: 2-11.

5. Khafagy el-S, Morishita M, Onuki Y, Takayama K (2007) Current challenges in non-invasive insulin delivery systems: a comparative review. Adv Drug Deliv Rev 59: 1521-1546.

6. Takenaga M, Yamaguchi Y, Kitagawa A, Ogawa Y, Mizushima Y, et al. (2002) A novel sustained-release formulation of insulin with dramatic reduction in initial rapid release. J Control Release 79: 81-91.

7. Choi S, Kim SW (2003) Controlled release of insulin from injectable biodegradable triblock copolymer depot in ZDF rats. Pharm Res 20: 2008-2010.

8. Wang SL, Xie SY, Zhu LY, Wang FH, Zhou WZD (2009) Effects of poly (lactic-co-glycolic acid) as a co-emulsifier on the preparation and hypoglycaemic activity of insulin-loaded solid lipid nanoparticles. IET Nanobiotechnol 3: 103-108.

9. Jain D, Panda AK, Majumdar DK (2005) Eudragit S100 entrapped insulin microspheres for oral delivery. AAPS PharmSciTech 6: E100-107.

10. Carino GP, Mathiowitz E (1999) Oral insulin delivery. Adv Drug Deliv Rev 35: 249-257.

11. Aboubakar M, Puisieux F, Couvreur P, Deyme M, Vauthier C (1999) Study of the mechanism of insulin encapsulation in poly(isobutylcyanoacrylate) nanocapsules obtained by interfacial polymerization. J Biomed Mater Res 47: 568-576.

12. Trotta M, Cavalli R, Carlotti ME, Battaglia L, Debernardi F (2005) Solid lipid micro-particles carrying insulin formed by solvent-in-water emulsion-diffusion technique. Int J Pharm 288: 281-288.

13. Hamishehkar H, Emami J, Rouholamini NA, Gilani K, Minaiyan M, et al. (2009) The effect of formulation variables on the characteristics of insulin-loaded poly(lactic-co-glycolic acid) microspheres prepared by a single phase oil in oil solvent evaporation method. Colloids Surf B Biointerfaces 74: 340-349.

14. Martins S, Sarmento B, Souto EB, Ferreira DC (2007) Insulin-loaded alginate microspheres for oral delivery - Effect of polysaccharide reinforcement on physicochemical properties and release profile. Carbohydr Polym 69: 725-731.

15. Rosa GD, Iommelli R, La Rotonda MI, Miro A, Quaglia F (2000) Influence of the co-encapsulation of different non-ionic surfactants on the properties of PLGA insulin-loaded microspheres. J Control Release 69: 283-295.

16. Yamaguchi Y, Takenaga M, Kitagawa A, Ogawa Y, Mizushima Y, et al. (2002) Insulin-loaded biodegradable PLGA microcapsules: initial burst release controlled by hydrophilic additives. J Control Release 81: 235-249.

17. Kang F, Singh J (2005) Preparation, in vitro release, in vivo absorption and biocompatibility studies of insulin-loaded microspheres in rabbits. AAPS PharmSciTech 6: E487-494.

18. Hinds KD, Campbell KM, Holland KM, Lewis DH, Piché CA, et al. (2005) PEGylated insulin in PLGA microparticles. In vivo and in vitro analysis. J Control Release 104: 447-460.

19. Han Y, Tian H, He P, Chen X, Jing X (2009) Insulin nanoparticle preparation and encapsulation into poly(lactic-co-glycolic acid) microspheres by using an anhydrous system. Int J Pharm 378: 159-166.

20. Ma J, Zeng P, Ye C, Wang Y, Fan Y (2001) An improved interfacial coacervation technique to fabricate biodegradable nanocapsules of an aqueous peptide solution from polylactide and its block copolymers with poly(ethylene glycol). Colloid Polym Sci 279: 387-392.

21. Ibrahim MA, Ismail A, Fetouh MI, Göpferich A (2005) Stability of insulin during the erosion of poly(lactic acid) and poly(lactic-co-glycolic acid) microspheres. J Control Release 106: 241-252.

22. Damgé C, Maincent P, Ubrich N (2007) Oral delivery of insulin associated to polymeric nanoparticles in diabetic rats. J Control Release 117: 163-170.

23. Ye M, Kim S, Park K (2010) Issues in long-term protein delivery using biodegradable microparticles. J Control Release 146: 241-260.

24. Luan X, Skupin M, Siepmann J, Bodmeier R (2006) Key parameters affecting the initial release (burst) and encapsulation efficiency of peptide-containing poly(lactide-co-glycolide) microparticles. Int J Pharm 324: 168-175.

25. Zhe Zhang, Hongling Shan, Li Chen, Chaoliang He, Xiuli Zhuang, et al. (2013) Synthesis of pH-responsive starch nanoparticles grafted poly (L-glutamic acid) for insulin controlled release. European Polymer Journal 49: 2082-2091

26. Mortazavian E, Amini M, Dorkoosh FA, Amini H, Khoshayand MR, et al. (2014) Preparation, Design for Optimization and in Vitro Evaluation of Insulin Nanoparticles Integrating Thiolated Chitosan Derivatives. Journal of Drug Delivery Science and Technology 24: 40-49

27. Zabihi F, Yang M, Leng Y, Zhao Y (2015) PLGA–HPMC nanoparticles prepared by a modified supercritical anti-solvent technique for the controlled release of insulin. The Journal of Supercritical Fluids 99: 15-22

28. Tatiana A, Leonardo M, Esteban NL, Ana LR. de Souza, Charlene P, et al. (2015) Effect of mucoadhesive polymers on the in vitro performance of insulin-loaded silica nanoparticles: Interactions with mucin and biomembrane models. European Journal of Pharmaceutics and Biopharmaceutics 93: 118-126

29. Hoffart V, Ubrich N, Simonin C, Babak V, Vigneron V, et al. (2002) Low molecular weight heparin-loaded polymeric nanoparticles: formulation, characterization, and release characteristics. Drug Dev Ind Pharm 28: 1091-1099.

30. Rajan DS, Gowda KV, Mandal U, Ganesan M, Bose A, et al. (2006) Development of RP-HPLC for analysis of human insulin. Indian J Pharm Sci 68: 662-665.

31. Dash S, Murthy PN, Nath L, Chowdhury P (2010) Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm 67: 217-223.

32. Lakhiari H, Muller D (2004) Insulin adsorption on coated silica based supports grafted with N-acetylglucosamine by liquid affinity chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 808: 35-41.

33. Wong HS, Wang JJ, Wang CH (2001) In vitro sustained release of human immunoglobulin G from biodegradable microspheres. Ind Eng Chem Res 40: 933-948.

34. Van Tomme SR, De Geest BG, Braeckmans K, De Smedt SC, Siepmann F, et al. (2005) Mobility of model proteins in hydrogels composed of oppositely charged dextran microspheres studied by protein release and fluorescence recovery after photobleaching. J Control Release 110: 67-78.

35. Liu R, Huang SS, Wan YH, Ma GH, Su ZG (2006) Preparation of insulin-loaded PLA/PLGA microcapsules by a novel membrane emulsification method and its release in vitro. Colloids Surf B Biointerfaces 51: 30-38.