Research Article

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PLGA based drug delivery systems (DDS) for the sustained release of insulin: insight into the protein/polyester interactions and the insulin release behavior

Verónica Lassalle[a]* and María Luján Ferreira[b]

Abstract

BACKGROUND: Drug delivery systems (DDS) were designed using insulin as model drug and poly (lactic–co-glycolic) copolymers (PLGA) as polymeric matrix. The carriers were synthesized by direct self-assembly of the insulin and the polyester under mild conditions.

RESULTS: The kind and level of association between the protein and the polymer were studied using computational methods (combined MM2/PM3) and spectroscopic tools (Fourier transform infrared (FTIR), energy dispersive X-ray (EDX) and X-ray fluorescence spectroscopy (XFS)). The effect of the number average molecular weight (M_n) of the copolymer on the association efficiency (AE) drug–polymer as well as on the release profile has been explored. Mathematical models were used to predict the insulin release kinetic and mechanism.

CONCLUSIONS: Satisfactory protein/PLGA association efficiencies (between 77 and 99%) were registered depending on the M_n of the PLGA. Hydrophobic and hydrophilic interactions were detected between the protein and the polymeric network by computational analysis. *In vitro* release studies demonstrated that copolyesters of about 8600 and 1500 Da were suitable for the gradual release of insulin while PLGA oligomers of average molecular weight between 700 and 800 Da were unsuitable as DDS. The insulin release kinetics fits well with the Korsmeyer model, following the anomalous transport mechanism.

Supporting information may be found in the online version of this article.

Keywords: protein delivery; biomaterials; insulin; PLGA; release mechanism

INTRODUCTION

During the past two decades formulations that control the rate and period of drug delivery (e.g. time-release medications) and target specific areas of the body have become increasingly common and complex. Different systems based on biodegradable polymers have been explored for these purposes. Special attention was placed in homo and copolymers derived from lactic acid (i.e. D and L-poly(lactic acid), poly (lactic-co-glycolic acid, etc).[1,2] Several devices, such as microspheres, nanoparticles, hydrogels, lipid-based systems, *in situ* prepared parenteral drug delivery systems and complexes have been investigated to entrap and release a great number of drugs.[3,4] Nanoencapsulation techniques often rely on formation of emulsions with subsequent solvent evaporation. A high-energy input is necessary to obtain nanoemulsions and hence nanoparticles. Under those conditions, protein stability is often compromised through temperature, pH, and shear stress at interfacial surfaces with high amounts of organic solvents.[5] The surfactants and co-solvents can be toxic at high doses and may be limited in their daily and per-dose uptake levels.

In situ forming depots or other systems, based on complexation and assembly of proteins with functionalized polymers, were designed to overcome some drawbacks of conventional formulations. These advantages include reduction of manufacturing costs and complexity and minimization of the use of harmful additives.[6]

However, articles in the open literature concerning these kinds of delivery systems are still limited compared with the vast amount of information on nano and microparticles.[7] For instance, Dai *et al*. have investigated the formation of insulin and lauryl sulphate complexes using the hydrophobic ion-paring technique. They have characterized comprehensively the physiological and biological properties of the complex in a solid state and have evaluated their bioactivity through *in vivo* experiments.[8] Earlier, Slanger *et al*. reported the formation of diastereoisomer complexes between D-PLA (homo and block copolymers) and L-insulin as

* *Correspondence to: Verónica Lassalle, INQUISUR, Depto de Química, Avda Alem 1253,8000, Bahía Blanca,Prov. Bs As R Argentina. E-mail: veronica.lassalle@uns.edu.ar; vlassalle@plapiqui.edu.ar*

a *INQUISUR, Depto de Química, Avda Alem 1253,8000, Bahía Blanca,Prov. Bs As R Argentina*

b *PLAPIQUI-UNS-CONICET-Camino La Carrindanga Km 7, CC 717, 8000 Bahía Blanca,Prov. Buenos Aires, R. Argentina*

representative of poly(L-amino acids). They described mainly the preparation and the *in vitro* release of the protein from the stereocomplex microparticles.[9] Kissel *et al*. have studied the *in situ* formation of parenteral drug delivery systems after intramuscular or subcutaneous injection. These authors used insulin as model protein and amphiphilic polyesters composed of PLGA chains grafted onto an amine-substituted poly(vinyl alcohol) backbone. They hypothesized that by contact with isotonic fluids aqueous suspensions of positively charged particles could offer the possibility to form a semi-solid depot at the injection site due to ion-mediated particle aggregation.[10] Bodmeier *et al*. reported several articles on the design and application of *in situ* forming depots as drug delivery systems.[11,12] In one of these they investigated the influence of poly(lactide-co-glycolide) (PLGA) type (molecular weight and end-group functionality) on the leuprolide release from *in situ* forming microparticle (ISM) systems. ISM systems are based on an emulsion of the PLGA solution dispersed in an oil phase. The polymer droplets solidify after contact with aqueous fluids and form microparticles *in situ*. They found that 6-month controlled release leuprolide ISM could be obtained by blending poly(lactides) (PLA) with different molecular weights.[13]

Here, a drug delivery system (DDS) prepared by simple self-assembly of insulin on poly(lactic-co-glycolic) copolymers has been prepared. The main goal of this work is to gain insight into the kind and nature of the interactions between the protein and the polymeric networks in the DDS formation. To this purpose, FTIR, SEM/EDX and a combined MM2 minimization/PM3 minimization, have been utilized. To the best of the authors knowledge, there is scarce (if any) information in the open literature concerning the use of computational tools to explain the nature of the interactions medicaments/polymer in DDS. Another aim of this contribution was to evaluate the performance of the fabricated DDS in terms of the *in vitro* release of insulin, analyzing the feasible release mechanisms. In general, for the release description, theoretical approaches have focused on mechanistic modelling of the release kinetics. These models distinguished pure Fickian diffusion either under the assumption of sink conditions inside the diffusion layer or under the assumption of saturation and matrix erosion.[14,15] Here, mathematical models were further applied to analyze the kinetics and determine the mechanism for the insulin release.

MATERIALS AND METHODS

Materials

Amorphous PLGA copolymers with M_n ranged between 1500 and 8600 Da, and oligomers of M_n between 700 and 800 Da were utilized. The copolyesters were synthesized in our labs from D/L lactic acid 85% (Sintorgan S.A, Argentina) and glycolic acid (from Fluka Chemika (Switzerland) through enzymatic polymerization. The protocol for PLGA polymerization as well as the characterization of the copolymers has been published in previous works.[13] Porcine neutral insulin was supplied by Betasint U-40 (Beta Laboratories, Argentina) as an aqueous commercial solution. Analytical grade solvents provided by Dorwill (Argentina, SA) were used in all the described procedures. The buffer solution of pH 7 (disodium hydrogen phosphate) was provided by Merck. The 0.1 mol L^{-1} solution of phosphate buffer saline (PBS, pH = 7.4) was prepared from 137 mmol L^{-1} NaCl, 2.7 mmol L^{-1} KCl, 4.3 mmol L^{-1} Na_2HPO_4 and 1.4 mmol L^{-1} KH_2PO_4.

Preparation of DDS

Insulin/PLGA DDS were prepared using 25–30 mg of PLGA (dissolved in 3 mL of CH_2Cl_2) and 1.44 mg of insulin (1 mL of aqueous insulin commercial solution) pre-treated with 1 mL of ethanol under stirring for 30 min to avoid protein aggregation. The PLGA/insulin weight ratio was kept almost constant in all experiments at 17 : 1. Both solutions (organic and aqueous/ethanolic) were contacted by adding the insulin to the polymeric solution using a syringe. 0.5 mL of buffer pH = 7 was further added to maintain neutral media during the procedure. Formation of complexes was allowed for 24 h at 37 °C under stirring, and was evidenced by the formation of a cloudy emulsion. Then, precipitation was performed in 10 mL of bidistilled water and the CH_2Cl_2 was eliminated by evaporation. The solid was recovered by filtration and washed several times with bidistilled water.

Determination of the association efficiency (AE)

The amount of insulin incorporated to the polymeric moieties was quantified in terms of the AE. It was calculated indirectly from the entrapment supernatant using UV/visible spectroscopy. The AE was defined by the following expression:

$$AE(\%) = \frac{total\ insulin\ amount - free\ insulin\ amount}{total\ insulin\ amount} \times 100$$

where the *total insulin amount* is the insulin initially fed to the system (mg) and the *free insulin amount* (mg) is the non-associated insulin in the supernatant. The last value was measured by UV/visible at 275 nm, using a calibration curve relating absorbance (*A*) and concentration of insulin (mg mL).[17]

Characterization of DDS

FTIR spectroscopy was employed to analyze the structure of the protein and PLGA after entrapment. A Nicolet (USA) FTIR 520 spectrometer was used for recording transmission spectra in the range 4000–400 cm^{-1}. The DDS spectra were obtained by casting a CH_2Cl_2 solution on a KBr window, so the assay was performed after solvent evaporation.

A UV/visible spectrophotometer (Shimadzu 160 Japan, equipped with a computer assisted system for data acquisition) was used to quantify the entrapped and released insulin. To do so a calibration curve relating absorbance (*A*) and protein concentration (mg mL^{-1}) was constructed. Detailed information about the construction of calibrations curves is available elsewhere.[17]

Scanning electronic microscopy (SEM, JEOL 35 CF 1983, Tokio Japan) from CRIBABB (Bahía Blanca, Argentina) was used to examine the morphology of the carriers. SEM/EDX and X-ray fluorescence spectroscopy were used for qualitative determination of protein on DDS.

The MM2 program in the Chem3D Ultra (from Cambridge Soft, versions 5.0 and 11.0 for graphic presentation) was employed. A short model of PLGA was used and different aminoacids were placed initially 3 Å away from the PLGA model. The interaction was modeled to take place by the lateral residue of the aminoacids of the insulin. After this step another minimization step was performed using the PM3 facility in the same Chem3D Ultra. No solvent effect was analyzed, considering the interaction to be in the gas phase.

In vitro release of insulin

The protocol for the insulin release from PLGA-based DDS was optimized in previous work.[14] Briefly, about 15–25 mg of DDS was

incubated in 3 mL of 0.1 mol L^{-1} PBS solution (at pH7) at 37 °C in a water bath under continuous stirring. The incubation media (PBS) and temperature reproduces the physiological environment.[18]

Two replicates tubes were employed for each condition and the vials were sampled at various release times. The release assay was designed for a period of 24 h. Released protein was measured in the supernatant, withdrawing 250 µL of supernatant at different intervals of time and diluted to 3 mL with bidistilled water. The samples were centrifuged at 6000 rpm for 40 min and analyzed by UV/visible spectroscopy. A calibration curve of absorbance (*A*) against concentration of released protein (expressed as mg mL^{-1} solution of supernatant) was employed.[17]

Release data modeling

The following mathematical models were fitted to the raw release data to predict the *in vitro* release kinetic:

(1) The zero-order rate describes systems in which drug release is independent of its concentration:[19]

$$C = K_0\, t \tag{1}$$

K_0 is the zero-order rate constant expressed in units of concentration/time and t is the time.

(2) The first-order kinetic implies that release rate is concentration dependent:[20]

$$\log\, c = \log\, c_\theta - \frac{kt}{2.303} \tag{2}$$

where, C_0 is the initial concentration of drug and k is the first-order constant.

(3) The Higuchi model describes the release of drugs from an insoluble matrix as a square root of time dependent process based on Fickian diffusion:

$$Q = Kt^{1/2} \tag{3}$$

K is a constant reflecting the design variables of the system.[11]

(4) The Hixson–Crowell model describes the release from systems in which there is a change in surface area and diameter of particles or any carrier:[21]

$$Q_0{}^{1/3} - Q_t^{1/3} = K_{HC}t \tag{4}$$

Q_t is the amount of drug released in time t, Q_0 is the initial amount of the drug and K_{HC} is the rate constant of the Hixson–Crowell rate equation.

(5) The Korsmeyer model[22,23] refers to a semi-empirical equation based on a power law expression to describe the drug release from swelling controlled systems:

$$\frac{M_t}{M_\infty} = Kt^n \tag{5}$$

where M_t/M_∞ is the fraction of drug released at time t, K is the rate constant and n is the release exponent. The n value is used to characterize different release mechanisms.[24,25]

The goodness of fit was compared on the basis of the correlation coefficients r^2 and the kinetic constant $\boldsymbol{k}$. Models with best fit are those having highest (closest to 1) correlation coefficients.

RESULTS AND DISCUSSION

Characterization of PLGA/insulin delivery systems

The incorporation of the protein in the polymeric network was verified by FTIR spectroscopy. The spectrum of the pure insulin was compared with the corresponding insulin/PLGA physical mixture (in a ratio insulin/PLGA = 1/17) and with the DDS (Fig. 1). A significant difference between physical mixture and DDS spectra is observed. The spectrum of the physical mixture shows an additive effect of insulin and PLGA, in which characteristic peaks of the protein are clearly distinguished at 1720, 1660 and 1590 cm^{-1}. However in the spectrum of the prepared carriers these bands appear shifted to 1735 and 1665 cm^{-1}, while the signal at 1590 cm^{-1} is not detected.

The shift of the carboxylic stretching peak (1720 cm^{-1}) may be ascribed to the protein interactions with the polymer chains.[26] A different behavior was observed for the peak recorded at 1660 cm^{-1}, which was shifted to 1665 cm^{-1}. Such a small change may be reasonably assigned to a disturbance of the insulin intramolecular interactions owing to modification of the molecular environment. The presence of the polyester chains provides the possibility of depletion of the existing intramolecular interactions by predominant intermolecular interactions (e.g. insulin/PLGA).[27]

Qualitative determination of insulin on polyester chains was further assayed by SEM/EDX and X- ray fluorescence spectroscopy and the results obtained agree with those from FTIR and SEM-EDX.

It is important to highlight that, according to FTIR and UV/visible analysis undertaken in previous work, the stability of the insulin was not affected during the DDS formation procedure.[17]

Morphological examination of the DDS by SEM reveals that almost spherical nano scale aggregates were formed. Micrographies of raw PLGA and PLGA/insulin carriers are compared in Fig. 2(a) and (b), respectively. The co-polyester prepared by enzymatic synthesis shows a typical amorphous and uniform polymeric surface.

The carriers are formed by spherical nanoscale particles highly aggregated. Aggregates of non-uniform sizes have been observed. Although the morphology was not examined in detail, according to SEM assays the average size was estimated between 300 and 800 nm.

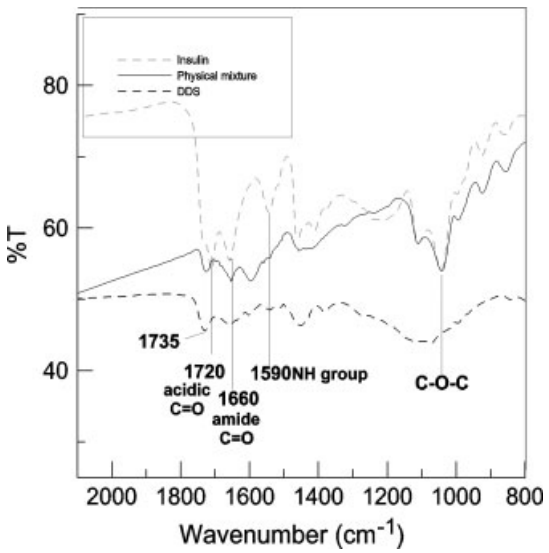

Figure 1. FTIR spectra of pure insulin, PLGA/insulin physical mixture and DDS.

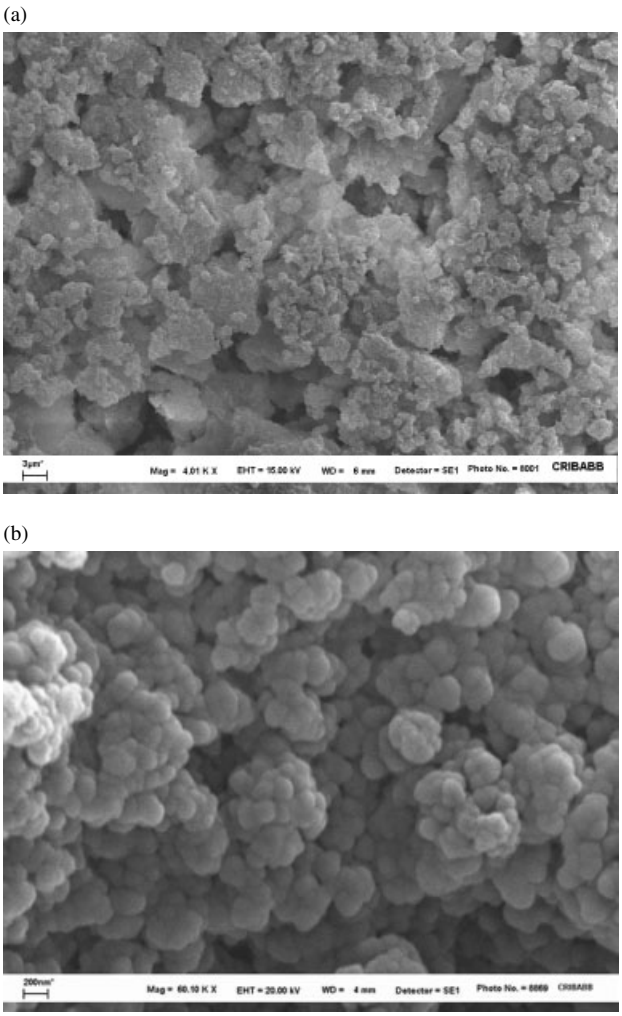

Figure 2. SEM micrographies of (a) raw PLGA and (b) insulin/PLGA DDS.

Association efficiency between insulin and PLGA

The AE was the parameter chosen to measure the amount of drug loaded in the polymeric matrix. The dependence of AE on PLGA M_n is listed in Table 1 in terms of AE%. Satisfactory association levels, roughly 100%, were reached using PLGA with M_n of 1500 and 8600 Da, respectively. Similar AE values were reported in the open literature.[11,28] For instance, Slanger *et al*. formulated PLA/insulin stereocomplexes for the controlled release of insulin. The authors reported yields ranging between 80 and 85% depending on the method and the solvent employed for precipitation.[9]

On the other hand, an important decrease in the AE was observed when PLGA oligomer of low molecular weight (M_n = 753) was used.

This decrease could be explained in terms of the insulin/polyester interactions, as will be described in the following sections. However, partial degradation of the PLGA oligomer could also explain the lower AE value obtained in that case.

Investigation on the insulin/PLGA interactions

Insulin's empirical formula is $C_{254}H_{377}N_{65}O_{75}S_6$ with a molecular weight of 5734 Da. It has two polypeptide chains linked by two disulfide bonds.[29]

To gain insight into the interactions of PLGA/insulin during DDS formation, a combined MM2 minimization/PM3 minimization was performed in tandem. The goal was the calculation of the ΔH°_f for conformations where the lateral chains of the main AA of insulin (in chains A and B) were in contact with a short model of Polylactic-co-glycolic (PLGA). The structure of the co-polyester is illustrated in Fig. 3. The randomness of such a structure is based on previous studies of the enzymatic synthesis and characterization of PLGA copolymers.[16]

Table 1. Association efficiency AE (%), as a function of the number average molecular weight of the used PLGA

Mn of PLGA (Da)	AE (%)[a,b]
753	72 ± 4.9
1548	99 ± 3.5
8600	97 ± 1.8

[a] Calculated from UV/visible analysis. Amount of insulin in the feed = 1.440 mg.
[b] As defined in the Experimental section
The data presented in the table are the result of two entrapment tests.

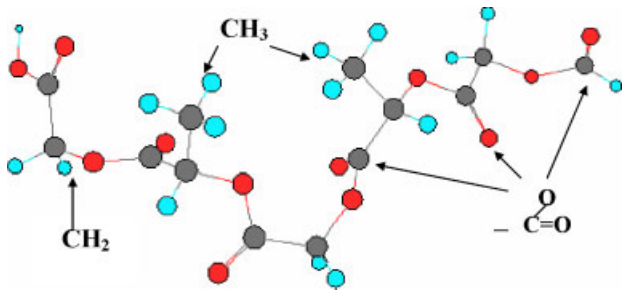

Figure 3. Short model of random PLGA.

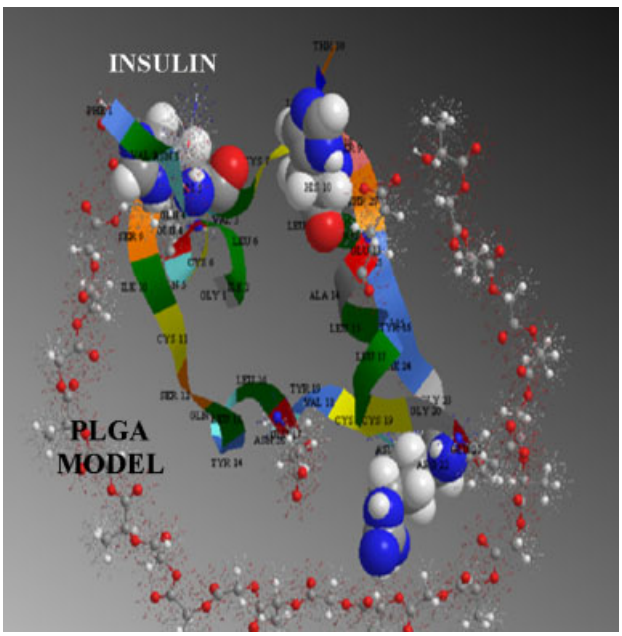

Figure 4. Interaction of the insulin chains A and B with a PLGA model of near 1500 Da. In this figure the histidine are shown in space filling, the glutamate in ball and stick.

The lateral groups of different amino acids were contacted with this short model of PLGA. Figure 4 illustrates the contact between glutamate (AA from insulin) and the short model of PLGA.

The interaction between lateral residue of AA from insulin and the short model of PLGA was considered for the MM2/PM3 study. For the selected conformation amino acid–short model of PLGA, the PM3 output is the standard formation enthalpy (ΔH°_f). The minimization procedure implies performing sequential changes in the structure in such a way that a local minimum (or a global minimum) may be found, using a comparison with a gradient norm until a cut value is found.

Therefore it is possible to calculate the reaction enthalpy (ΔH°_r). This value is obtained with the amino acid and the PLGA short model infinitely far away each other and the amino acid adsorbed on the PLGA by the lateral chain side. This is very difficult to study experimentally because the pure AA would interact through the amide or acid moiety and much less through the side chain. When proteins like insulin are analyzed in their interactions with polymers, the main contact protein–polymer is from the lateral residues of the protein with the specific interaction groups of the polymer.

Table 2 presents the results for ΔH°_r for the different amino acids following interactions between lateral residue of AA from insulin and the short model of PLGA.

It is evident that strong insulin/PLGA interactions take place in the case of arginine, glutamate, histidine, serine, tyrosine, leucine and asparagine. Specially privileged are the interactions with hydrophilic AA, i.e. histidine, glutamate and arginine. In spite of this, the number of interactions with hydrophobic AA (i.e. with leucine, serine, aspargine and phenylalanine) is higher due to the fact that insulin contains a larger number of such amino acids in its structure. Therefore hydrophobic interactions appear to be responsible for the entrapment of insulin in PLGA-based DDS. The available literature supports these results, in reporting hydrophobical as the main force between protein and polymeric substrates in DDS.[30,31] For instance, Simon *et al*. have prepared self-assembling nanocomplexes from insulin and water-soluble branched polyesters. They investigated the effect of the incorporation of hydrophobic moieties (polylactic acid) on the hydrophilic polymeric backbone. These authors found that using a simple mixing procedure and short incubation times, it was possible to increase complexation capacity of the higher grafted polyesters. The reason for this behavior was assigned to the more pronounced hydrophobic interactions with the non-polar residues of insulin.[32] The presence of hydrophobic interaction does not make the interaction weak *per se*. Hydrophobic interactions may be very strong.

Table 2. ΔH°_{int} (gas phase) for the interaction between the short model of random PLGA (Fig. 3) and different main aminoacids present in chains A and B of insulin. Short model PLGA $\Delta H^\circ_{f\ PLGA}$[b)] = 491.9 Kcal/mol

AA	$\Delta H^\circ_{f\ AA}$[a)]	$\Delta H^\circ_{f\ AA\text{-}PLGA}$[c)]	ΔH_{int}[d)]
Alanine	−100.13	−594	−1,97
Arginine	51,9	−459	−19
Asparagine	−132,8	−632	−7,3
Cysteine	−88,5	−583,9	−3,5
Phenylalanine	−71,1	−568,9	−5,9
Glutamate	−215	−727,1	−20,2
Glutamine	−144,7	−637,5	−0,9
Histidine	78,25	−431,2	−17,6
Leucine	−107,8	−606,5	−7,2
Serine	−137,8	−638	−8,3
Tyrosine	−116	−615	−7,1

[a] $\Delta H^\circ_{f\ AA}$ Standard formation enthalpy of individual *AA*.
[b] Standard formation enthalpy of Short Model of PLGA.
[c] $\Delta H^\circ_{f\ AA\text{-}PLGA}$ Standard formation enthalpy found after the minimization of the *AA-PLGA* conformational interaction.
[d] $\Delta H^\circ_{int} = \Delta H^\circ_{f\ AA-PLGA} - (\Delta H^\circ_{f\ AA} + \Delta H^\circ_{f\ PLGA})$ Standard enthalpy change for the interaction *AA-PLGA*.

The effect of the length of the polymeric matrix on the feasibility to interact with the protein has been evaluated. Figure 5(a), (b) and (c) shows PLGA models of Mn 700, 1500 and 3000 Da, respectively, in contact with insulin chains (A and B). The points of contact between the insulin and the polyester increase with the PLGA molecular weight, becoming optimal, in a bidimensional model, with PLGA of about 1500 Da. The length of the chain in the oligomer is not enough to establish adequate contact with the whole protein molecule. As a consequence it is difficult for the insulin to remain linked to the oligomeric networks. This fact partially justifies the lower AE value obtained for PLGA oligomer in comparison with those values registered for the higher molecular weight PLGA. In the case of the 1500 Da, the interaction is optimum because the length of the PLGA matches with the 'border' bidimensional length of the insulin. Employing higher molecular weight copolymers, for example with Mn of 8600 Da, this kind of contact is not improved. There are several portions of the PLGA that remain away from close interaction with insulin because of interactions with other portions of the same PLGA molecule (intramolecular interactions) or potential interactions with other PLGA molecules.

Computational studies considering these kinds of interactions are scarce in the open literature, to the best of the author's knowledge. Even when authors are aware of the importance of the solvent as significant dielectric media in terms of ionic interactions, the PLGA structure has covalent bonds and mainly ester groups and methylene groups (non-polar), with some of them able to generate H-bonding and hydrophobic interactions with proteins. There is also evidence indicating that although the identities and sizes of residues aligned on a given fold vary greatly, the polarities are strongly preserved.[30,33] The interaction and placement of hydrophobic residues seems to be a more critical determinant of protein structure than the role of polar residues and local sequence-dependent interactions.[31,34] The investigation by Huang *et al*. presents a very interesting and clear explanation of protein hydrophobic interactions.[35] From their computational approach and experimental results, they concluded that the classic arrangement of hydrophobic and polar residue achieves much more than might have been expected by theorists or experimentalists, such as the selection of a target fold topology from a large number of alternatives. Considering the available information, the molecular modeling is sufficiently theoretically and experimentally based – even considering its simplicity – to validate the results presented within this study.

In vitro release studies

Figure 6 shows the insulin released, expressed as percentage of cumulative insulin released (with respect to the initial amount of insulin added), as a function of the incubation time (24 h) for polyesters of different average molecular weight. Comparing the plots in Fig. 6, a marked burst effect is noted in the case of

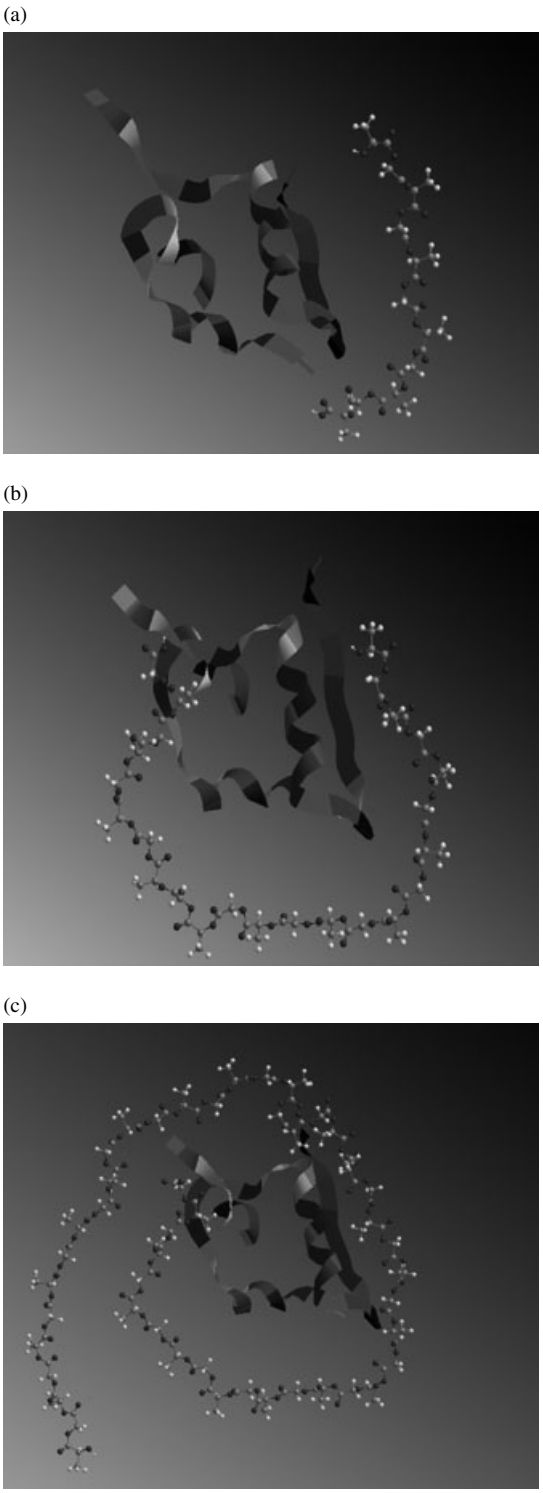

Figure 5. Interaction of the insulin chains A and B with PLGA of different Mn: (a) 750, (b) 1500 and (c) 8600Da.

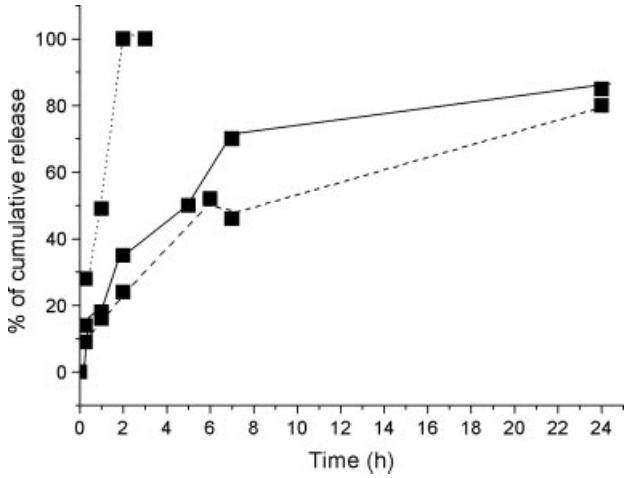

Figure 6. Release profiles, expressed as percentage of cumulative insulin released, as a function of the incubation time (24 h) for polyesters of different average molecular weight. PLGA 8600 Da (———); PLGA1500 Da(- - - -); PLGA753 Da(· · · ·). ± S.D. of the percentage cumulative release data was in the range: ±4.25 and 6.30 using PLGA 8600Da; ±3.42 and 4.80 using PLGA 1500 Da and ±4.35 and 6.75 using 753 Da.

the polyester of 753 Da, since 49% of the total insulin in DDS was released during the first hour of incubation. As expected, complete release (100% cumulative release) occurred quickly within the 5th hour of test. The lack of control over the release of the entrapped insulin could be due to two main causes. First, due to the rapid hydration and differential degradation of the PLGA oligomer. The extent of hydrolytic degradation depends on many physico-chemical characteristics of the polymer including hydrophilic/hydrophobicity, amorphous/crystallinity, molecular weight, and additives. Considering the characteristics of the oligomer (amorphous and with Mn = 753 Da) and based on the release evidences, it was proposed that heterogeneous degradation occur under the release conditions.[36,37] Second, the solubility of PLGA oligomers with average molecular weight lower than 800 Da in PBS solution at pH = 7.4 has been reported in the open literature.[38] Therefore both effects contribute to the failure of PLGA oligomers as systems for the controlled release of insulin under the studied conditions.

For DDS prepared with PLGA of 1500 and 8600 Da the burst effect is less evident. Both PLGA show a percentage cumulative release of about 16–18% during the first hour of the test. From the second hour of incubation and later, a considerable difference is evidenced between the formulations prepared from these different PLGAs. The DDS obtained from PLGA with higher Mn (Mn = 8600 Da) shows a faster increase of protein in solution (35% cumulative release) during the second hour of test. The carrier prepared from PLGA with lower Mn (Mn = 1500 Da) has better control in the release since almost 24% cumulative release was registered at the same incubation time. A similar trend is observed until the end of the test (24 h). The amount of insulin released from DDS prepared with 1500 Da PLGA leads to lower levels of protein in solution at different intervals of time compared with the formulation obtained from higher Mn PLGA. The final cumulative release after 24 h, reached 80 and 85% for PLGA of 1500 and 8600 Da, respectively. Although the two magnitudes are comparable, it is clear from Fig. 6, that the control of release was more effective using the lower molecular weight copolyester. These findings agree with results derived from the computational analysis with regard to the level of protein/PLGA interaction as a function of the polymer Mn (see Fig. 5). The burst effect is frequently reported in the published literature on insulin-drug delivery devices, sometimes with higher percentages than those registered in this work.[39] It is important to highlight that besides the protein/PLGA interactions, the morphology (shape, size and

size distribution) of the DDS have an impact on the release profile, specially on the magnitude of the burst effect.[40] Since this aspect was not well-explored in the present contribution, it will be studied and discussed in complementary future work.

Release profiles similar to those included in Fig. 6 have been observed for insulin and other proteins released from different devices by different authors.[41] For instance, insulin nano-aggregates encapsulated in water-soluble chitosan and poly (α, β,-l-malic acid) were prepared by Fan *et al*. These authors found that insulin release from polymeric multilayers was pH dependent. Besides the released insulin, in this case the protein should be unbound with the polyelectrolytes. They observed a slow release in the PBS solution at pH 7.4 after a rapid initial release as the burst effect.[42] Cui and co-workers also found incomplete release of insulin from PLGA copolymers of different composition and average molecular weight. The authors designed the *in vitro* assays for 24 h and reported that almost 60–70% of insulin was released at the end of the test.[7] They observed that an increase in the molecular weight of PLGA 50/50 led to a reduction in both the initial (burst effect) and final release over 24 h. Cui *et al*. explained the observed release behaviour by the retarded effect of the polymer meshwork generated by the longer chains in the co-polymers with higher molecular weight.[7]

Kinetics and mechanism for *in vitro* release

To optimize and predict the delivery of insulin from the prepared DDS a fundamental understanding of the mechanisms controlling its release is necessary. The applied mathematical models described in the Experimental section, have been selected on the basis of published articles regarding DDS using different polymeric matrixes.[24,25,43,44]

The experimental release data were fitted to Equations (1) to (5) by plotting the following:

- cumulative percentage drug release vs time (zero-order kinetic model);
- log cumulative percentage drug remaining vs time (first-order kinetic model);
- cumulative percentage drug release vs square root of time (Higuchi model);
- log cumulative percentage drug release vs log time (Korsmeyer model);
- cube root of percentage remaining drug vs time (Hixson–Crowell cube root law).

The results of these fittings are given in Table 3, expressed in terms of the correlation factor (r^2) and the constant (k) as a function of the PLGA employed, i.e. Mn = 1500 and 8600 Da.

Obviously, the insulin release mechanism is known to be complex. The intention is not to provide a model for the overall process. The goal is to use simple approaches to release modeling that may allow simple characterization and comparison of different insulin release systems.

Table 3 shows that the best model was the Korsmeyer for any of the polyesters used. This result indicates that the release of insulin is a log (time) dependent process.

From the data in Table 3, it is clear that the values of r^2 corresponding to the PLGA with Mn = 8600 Da shows a greater deviation from 1, than the r^2 values of 1500 Da PLGA. This may be related to complex interaction between the polymeric moieties and the protein. Considering the higher length chain of PLGA 8600 Da, it is possible that desorption–resorption of the insulin molecules occurs during the incubation procedure on the same PLGA molecule or on different ones. It was hypothesized that the increase in viscosity of the media (in combination with the different swelling of the polymeric matrix) has an influence on the release mechanism.[45]

Table 3. Results of kinetic model fitting (release rate constants k and correlation coefficients, r^2) for DDS prepared from different Mn PLGA

	PLGA 1500[a]		PLGA8600[a]	
Mathematical model	**k**	**r^2**	**K**	**r^2**
Zero order	3.026(h^{-1})	0.8338	2.770(h^{-1})	0.7360
First order	0.0622(h^{-1})	0.9672	0.0714(h^{-1})	0.8965
Higuchi	16.462($h^{-1/2}$)	0.9485	17.317($h^{-1/2}$)	0.8961
Hixson–Crowell	0.072($h^{-1/3}$)	0.9397	0.0788($h^{-1/3}$)	0.8457
Korsmeyer	17.673(h^{-n})	0.9702	23.121(h^{-n})	0.9146

[a] 1500 and 8600 are the average molecular weight of the PLGA in Da.

Table 4. Calculated exponent *n* and mechanism of release

Drug release mechanism	Exponent n[(a)]	References
Fickian diffusion	0.43	16
Anomalous transport	0.85 > n > 0.43	16a
Case II transport	0.85	16a
Anomalous transport	0.5046	This work PLGA1500[(b)]
Anomalous transport	0.4516	This work PLGA 8600[(b)]

[a] values of ***n*** considering spherical carriers.
[b] 1500 and 8600 refer to the Mn of the used PLGA.

Although use of the Korsmeyer model requires detailed statical analysis, the calculated exponent n gives an indication of the release mechanism.[46] It is worth noting that the n value depends on the shape of the used device.[43,44] Therefore it was assumed, based on SEM characterization, that PLGA/insulin carriers were spherical in shape to perform the n calculation. The values obtained are included in Table 4 as a function of the PLGA.

The n data reveal that the best approximation for insulin kinetics and release mechanism is anomalous transport independent of the Mn of the PLGA used as matrix. The anomalous transport kinetic is a process partially controlled by viscoelastic relaxation of the matrix during water penetration. It is intermediate between Fickian and Case II transport. This last process is associated with stresses and transition-states in hydrophilic glassy polymers, which swell in water or biological fluids. This term also includes polymer disentanglement and erosion.[23,24]

It is important to highlight that the n exponent further depends on the size distribution of the carriers. An in-depth study of morphology of the prepared DDS was not the goal of this contribution. Therefore it was assumed that the DDS were spherical in shape based on SEM evidence but no correction was performed regarding the possibility of the formation of aggregates of different sizes. Consequently, the mechanism for insulin release from PLGA-based carriers should be considered as an approximation and more detailed studies are currently required for more precise mechanistical information.[47,48] However, the practical use of this simple modelling is clear.

CONCLUDING REMARKS

Insulin/PLGA delivery systems were prepared using a relatively simple and low cost procedure. MM2/PM3 analysis suggested that hydrophobic protein/polyester interactions were mainly responsible for the DDS formation and a preferential interaction with PLGA of 1500 Da was found compared with copolyesters of 8600 and 753 Da.

The *in vitro* release assays revealed that the Mn of the copolymers greatly affect the release profile. 1500 Da PLGAs was the most efficient in terms of the interaction with insulin and the computational results allowed us to propose a potential reason for this experimental finding.

According to mathematical models the insulin release kinetics correlates with the Korsmeyer model. As an approximation, anomalous transport was found to be the most probable mechanism for protein release from PLGA DDS.

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Supporting information

Supporting information may be found in the online version of this article.

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