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Formulation of Insulin-Loaded Polymeric Nanoparticles Using Response Surface Methodology

D. Attivi
Laboratoire de Pharmacie Galénique et Biopharmacie, Faculté de Pharmacie, Nancy Cedex, France

P. Wehrle
Laboratoire de Pharmacotechnie, Faculté de Pharmacie, Illkirch Cedex, France

N. Ubrich
Laboratoire de Pharmacie Galénique et Biopharmacie, Faculté de Pharmacie, Nancy Cedex, France

C. Damge
Centre Européen d'Etude du Diabéte, Faculté de Médecine, Strasbourgh, France

M. Hoffman and P. Maincent
Laboratoire de Pharmacie Galénique et Biopharmacie, Faculté de Pharmacie, Nancy Cedex, France

ABSTRACT The objective of this work was to formulate new oral insulin-loaded nanoparticules using the response surface methodology. The insulin nanoparticles were prepared by a water-in-oil-in-water emulsification and evaporation method. The polymers used for the encapsulation were blends of biodegradable poly-epsilon-caprolactone (PCL) and of positively-charged, nonbiodegradable polymer (Eudragis RS®). A central composite design has been built to investigate the effects of three controlled variables: ratio of polymers (PCL/RS ratio), volume, and pH of the aqueous solution of polyvinyl alcohol. The nanoparticles were characterized by measuring the amount of entrapped insulin, the particle size, the polydispersity of the obtained particles, the zeta potential, and the amount of insulin released after 7 hours. A second-order model was evaluated by multiple regression and was statistically tested for each of the studied controlled variable. The obtained polynomials proved efficient to localize an optimal operating area highlighted by the use of three-dimensional response surfaces and their corresponding isoresponse curves. An interesting formulation given by the models was selected, prepared, and evaluated. The corresponding quantity of entrapped insulin was 25 IU per 100 mg of polymer, and the particle size was 350 nm with a polydispersity of 0.21. The quantity of released insulin was 4.8 IU per 100 mg of polymer after 7 hours and the zeta potential was +44 mV. All these collected values were in perfect accordance with values estimated by the models. Finally, the results suggested that PCL/RS 50/50 nanoparticles might represent a promising formulation for oral delivery of insulin.

KEYWORDS W/O/W emulsion, Nanoparticles, PCL, Eudragit RS, Insulin, Response surface methodology, Experimental design

INTRODUCTION

With the advances in biotechnology, a wide variety of peptides and proteins can now be produced and will represent most of the new drugs brought onto the market in this century. However, despite the rapid progress in large-scale manufacturing of these therapeutic molecules,

Address correspondence to P. Wehrle, Laboratoire de Pharmacotechnie, Faculté de Pharmacie, 74 Route du Rhin, BP 24, Illkirch Cedex 67401, France; E-mail: wehrle@aspirine.u-strasgb.fr

convenient and effective oral delivery of these drugs remains a major challenge. Indeed, their large molecular size, their lack of permeability through the gastrointestinal tract, their short plasma half-life, their biodegradation by enzymes, and the importance of maintaining a correct conformation all limit their biological activity after oral administration. Throughout the world, considerable research effort has been devoted to developing appropriate, painless delivery systems to replace injectable insulin dosage forms. An oral insulin dosage form would be preferred by diabetic patients over the currently available parenteral formulations. Indeed, the oral route is the most physiological and the most easily accepted by patients. The use of absorption enhancers (Nishihata et al., 1981) and protease inhibitors (Fujii et al., 1985) have improved the oral bioavailability of insulin. When insulin was included within a multiple emulsion, small amounts of biologically active insulin were absorbed after oral administration to rats (Silva-Cunha et al., 1998). Some polymeric dosage forms have also attracted interest. Indeed, insulin-loaded poly(alkyl cyanoacrylate) nanospheres decreased fasting glycemia after oral administration in diabetic rats (Damge et al., 1988). Poly(isobutyl cyanoacrylate) nanocapsules dispersed in a biocompatible microemulsion also facilitated the absorption of insulin following intragastric administration to diabetic rats (Watnasirichaikul et al., 2002). Furthermore, poly(isobuty cyanoacrylate) nanospheres combined with sodium cholate significantly reduced the mean blood glucose level after oral administration to diabetic rats (Radwan, 2001).

Recently, polymeric nanoparticles prepared by a water-in-oil-in-water emulsification method from a mixture of a biodegradable polyester and a polycationic polymer (Hoffart et al., 2002) promoted the oral absorption in rabbits of heparin, which is not usually bioavailable by the oral route (Jiao et al., 2002). In order to test the potential of this new particulate system with insulin, an attempt to optimize the formulation containing insulin was made, by determining how various factors influenced the physicochemical properties of the nanoparticles prepared. Among these properties are particle size, which may control the in vivo absorption of nanoparticles and the drug release kinetics; and particle zeta potential, which influences physical stability and possible mucoadhesion but also drug entrapment efficiency and drug release. The factors studied were 1) the ratio PCL/Eudragit RS, since the biodegradable PCL and the polycationic polymer have already been identified as an interesting association (Jiao et al., 2002); 2) the volume; and 3) the pH of the external polyvinylic alcohol aqueous solution used during the preparation of nanoparticles. Response surface methodology using a central composite design was chosen because it allows the determination of the influence of these combined factors on nanoparticle properties with a minimum of experiments. In particular, it was possible to estimate second-order polynomial equations with a view to selecting the most appropriate formulation. The analysis of the models was helped by the use of three-dimensional response surfaces, and their corresponding isoresponse curves were used to select formulations that have physicochemical properties expected to produce the desired effect in vivo. The collected information was also used to increase understanding of the behavior of the system under study.

The nanoparticle formulations corresponding to the experimental design runs were then evaluated according to different criteria affecting the quality of nanoparticles: quantity of entrapped insulin, particle size, polydispersity, quantity of insulin released after 7 hours, and zeta potential. The scientific literature shows that these are the main properties that determine the physical stability as well as the biopharmaceutical properties of the nanoparticles (Box & Draper, 1987; Lewis et al., 1999).

MATERIALS AND METHODS

Materials

Poly(epsiloncaprolactone) (PCL, MW=42,000 Da), a biodegradable polymer, was purchased from Aldrich (Steinheim, Germany). Eudragit RS® (RS), an acrylic polycationic, nonbiodegradable polymer (MW=150,000 Da), was a gift from Röhm Pharma GmbH (Darmstadt, Germany). Human insulin (Actrapid®) was generously offered by Novo Nordisk. Polyvinyl alcohol (PVA, MW=30,000 Da, 88% hydrolysis), used as a surfactant in the second emulsion, was obtained from Sigma (St Louis, MO). The software

TABLE 1 Central Composite Design (Coded Levels)

Batch #	PCL/RS (X_1)	Volume of PVA (X_2)	pH (X_3)
1	−1.000	−1.000	−1.000
2	+1.000	−1.000	−1.000
3	−1.000	+1.000	−1.000
4	+1.000	+1.000	−1.000
5	−1.000	−1.000	+1.000
6	+1.000	−1.000	+1.000
7	−1.000	+1.000	+1.000
8	+1.000	+1.0000	+1.000
9	−1.682 (−α)	0.000	0.000
10	+1.682 (+α)	0.000	0.000
11	0.000	−1.682(−α)	0.000
12	0.000	+1.682(+α)	0.000
13	0.000	0.000	−1.682(−α)
14	0.000	0.000	+1.682(+α)
15	0.000	0.000	0.000
16	0.000	0.000	0.000
17	0.000	0.000	0.000
18	0.000	0.000	0.000
19	0.000	0.000	0.000
20	0.000	0.000	0.000
21	0.000	0.000	0.000
22	0.000	0.000	0.000
23	0.000	0.000	0.000

Nemrod® LPRAI (Marseille le Merlan, France) was used for generating the experimental design, modeling the response surface, and calculating the statistical evaluation.

Preparation of Nanoparticles

The preparation of nanoparticles was carried out by the multiple emulsion (w/o/w) technique previously described by Hoffart et al. (2002). Briefly, 1 mL of an aqueous insulin solution (100 IU) was first emulsified in methylene chloride (10 mL) containing the polymers (0.25 g) by sonication for 30 secoonds at 60 W. The resulting water-in-oil emulsion was thereafter added to various volumes of polyvinyl alcohol (PVA) aqueous solution (0.1%) and sonicated again for 1 minute, resulting in the formation of the second water-in-oil-in-water emulsion. After evaporation of methylene chloride, the polymers precipitated and the nanoparticles were isolated by centrifugation. The nanoparticles were washed three times with deionized water before freeze-drying.

Experimental Design

A Central Composite Design was used to investigate the effect of three factors (PCL/RS ratio as X_1, volume of the PVA aqueous solution as X_2, and pH of the aqueous PVA solution as X_3) on the response variables [size (Y_1) and polydispersity (Y_4) of nanoparticles, zeta potential (Y_3), amount of insulin released in vitro (Y_2), and encapsulation efficiency (Y_5)]. This experimental design allows estimating a second-order polynomial design with only 2^K+2^K+n central experiments with k the number of controlled variables or factors. For three variables, the design is built with eight experiments at the corners of the cubic experimental field, six axial points (one at the center of each cube face) at a distance±alpha to the center of the cube, and nine center points. The number of central points and the distance alpha are calculated in order to meet orthogonality and rotatability criteria, which confer the greatest efficiency to this type of design. Orthogonality implies that the estimations of the regression coefficients of the polynomial (in direct relation to the factors effects)

TABLE 2 Composition of the 23 Batches and Collected Results

Batch #	PCL/RS ratio	Volume of PVA (mL)	pH	Size (nm)	Polydispersity index	Zeta potential (mV)	Entrapped insulin (IU/100 mg)	Released insulin (IU/100 mg)
1	1.9	34.0	3.9	338	0.48	33	26.00	5.46
2	7.2	34.0	3.9	370	0.50	30	27.00	3.38
3	1.9	45.9	3.9	355	0.20	33	25.00	3.20
4	7.2	45.9	3.9	374	0.40	30	25.00	2.75
5	1.9	34.0	6.5	414	0.65	34	26.00	4.25
6	7.2	34.0	6.5	659	1.00	42	26.00	2.50
7	1.9	45.9	6.5	395	0.50	33	25.00	4.25
8	7.2	45.9	6.5	538	1.00	47	25.00	2.50
9	0.1	40.0	5.2	365	0.30	49	25.00	6.00
10	9.0	40.0	5.2	357	0.30	48	28.00	1.68
11	4.5	30.0	5.2	343	0.20	46	33.00	3.80
12	4.5	50.0	5.2	368	0.47	44	26.00	2.50
13	4.5	40.0	3.0	356	0.32	38	24.00	1.80
14	4.5	40.0	7.4	648	1.00	37	24.00	1.92
15	4.5	40.0	5.2	352	0.30	46	27.00	2.10
16	4.5	40.0	5.2	334	0.12	44	27.00	2.17
17	4.5	40.0	5.2	362	0.20	47	27.00	2.00
18	4.5	40.0	5.2	355	0.30	43	27.00	1.50
19	4.5	40.0	5.2	345	0.20	45	27.00	2.00
20	4.5	40.0	5.2	339	0.20	46	27.00	1.90
21	4.5	34.0	3.9	360	0.30	45	27.00	2.00
22	4.5	34.0	3.9	356	0.20	44	27.00	1.80
23	4.5	45.9	3.9	337	0.20	38	26.00	2.50

are independent of each other, giving therefore an unbiased value of the associated effect. The quality of prediction of the response within the experimental domain depends on rotatability. Once this property is respected, the variance of prediction of the response only depends on the distance alpha from the center and not on the direction in which one moves from the center. Therefore, no direction is favored above another (Box & Draper, 1987; Lewis et al., 1999; Wehrie et al. 1996).

The limits of variation of X_1, X_2, and X_3 are 0.1–9, 30–50 mL, and 3–7.4, respectively. The limits of the experimental domain for the volume of the aqueous PVA solutions were based on previous results obtained with low molecular weight heparin (Hoffart et al., 2002). pH was selected to around ±2 pH units around the isoelectric point of insulin defined as 5.4. The ratio of polymers was selected in order to cover a wide range of possibilities.

The runs of experimental design and the composition of the 23 batches are shown in Tables 1 and 2.

This statistical design provides a second-order polynomial describing linear and quadratic effect, as well as the interactions of each of the studied factors on the considered response variable. The general model corresponds to the following equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3$$

Determination of Response Variables

Insulin Encapsulation Efficiency

The amount of insulin entrapped within polymeric nanoparticles was determined by radioimmunoassay (RIA) (Insulin-CT kit, Cis Bio International, Gif sur Yvette, France). The drug entrapment efficiency was expressed as the quantity of insulin (IU) entrapped per 100 mg of polymer.

TABLE 3 **a. Models and Statistical Evaluations of Nanoparticle Size. b. Models and Statistical Evaluations of Nanoparticle Size Polydispersity. c. Models and Statistical Evaluations of Nanoparticle Zeta Potential. d. Models and Statistical Evaluations of Entrapped Insulin. e. Models and Statistical Evaluations of Released Insulin**

Particle size (nm)

Model term	Regression coefficient	Level of significance
b_0	348.4	[a]
b_1	31.2	[a]
b_2	−5.6	7.6%
b_3	77.6	[a]
b_{11}	8.6	[c]
b_{22}	6.6	[c]
b_{33}	58.4	[a]
b_{12}	−14.4	[b]
b_{13}	42.1	[a]
b_{23}	−20.1	[a]
ANOVA	F=187.34	[a]
R^2	0.912	

Polydispersity

Model term	Regression coefficient	Level of significance
b_0	0.221	[a]
b_1	0.078	[b]
b_2	−0.006	76.0%
b_3	0.199	[a]
b_{11}	0.057	[c]
b_{22}	0.070	[b]
b_{33}	0.184	[a]
b_{12}	0.041	11.7%
b_{13}	0.079	[c]
b_{23}	0.029	25.6%
ANOVA	F=34.78	[a]
R^2	0.855	

Zeta potential (mV)

Model term	Regression coefficient	Level of significance
b_0	44.4	[a]
b_1	1.0	[c]
b_2	0.0	91.1%
b_3	2.1	[a]
b_{11}	−0.2	53.3%
b_{22}	−1.5	[b]
b_{33}	−4.1	[a]
b_{12}	0.8	14.1%
b_{13}	3.5	[a]
b_{23}	0.5	30.6%
ANOVA	F=31.09	[a]
R^2	0.574	

Continued

TABLE 3 a. Models and Statistical Evaluations of Nanoparticle Size. b. Models and Statistical Evaluations of Nanoparticle Size Polydispersity. c. Models and Statistical Evaluations of Nanoparticle Zeta Potential. d. Models and Statistical Evaluations of Entrapped Insulin. e. Models and Statistical Evaluations of Released Insulin (Continued)

Quantity of entrapped insulin (IU/100 mg)

Model term	Regression coefficient	Level of significance
b_0	26.911	[a]
b_1	0.443	12.8%
b_2	−1.228	[a]
b_3	−0.073	79.0%
b_{11}	−0.351	19.0%
b_{22}	0.710	[c]
b_{33}	−1.235	[a]
b_{12}	−0.125	73.2%
b_{13}	−0.125	73.2%
b_{23}	0.125	73.2%
ANOVA	F=6.25	[b]
R^2	0.812	

Quantity of insulin released after 7 h (IU/100 mg)

Model term	Regression coefficient	Level of significance
b_0	1.985	[a]
b_1	−0.974	[a]
b_2	−0.372	[a]
b_3	−0.080	19.9%
b_{11}	0.761	[a]
b_{22}	0.517	[a]
b_{33}	0.061	28.0%
b_{12}	0.204	[c]
b_{13}	−0.121	14.1%
b_{23}	0.361	[b]
ANOVA	F=76.37	[a]
R^2	0.912	

Level of significance—P value: [a]P<0.001 (0.1%); [b]P<0.01 (1%); [c]P<0.05 (5%); P>0.05 (5%): not significant.

Size, Polydispersity, and Surface Potential Analysis

The nanoparticles were analyzed for their size distribution, polydispersity, and their surface potential using a Zetasizer® II (Malvern Instruments, UK).

In Vitro Release Experiments

Freeze-dried nanoparticles (100 mg) were suspended in 20 mL of saline phosphate buffer (PBS, 0.011M, NaCl 0.15M, pH 7.4) in a flask containing Tween® 80 (0.1%) and incubated into a water bath at 37°C under gentle magnetic stirring (150 rpm). At various time intervals, the colloidal suspension was centrifuged for 30 min at 40,000 g. The supernatant was removed and assayed for insulin according to the RIA method described in the commercial kit.

RESULTS AND DISCUSSION

All the tested parameters allowed the nanoparticles to be manufactured correctly, although major differences appeared in their physico-chemical characteristics. In order to facilitate this report reading, results

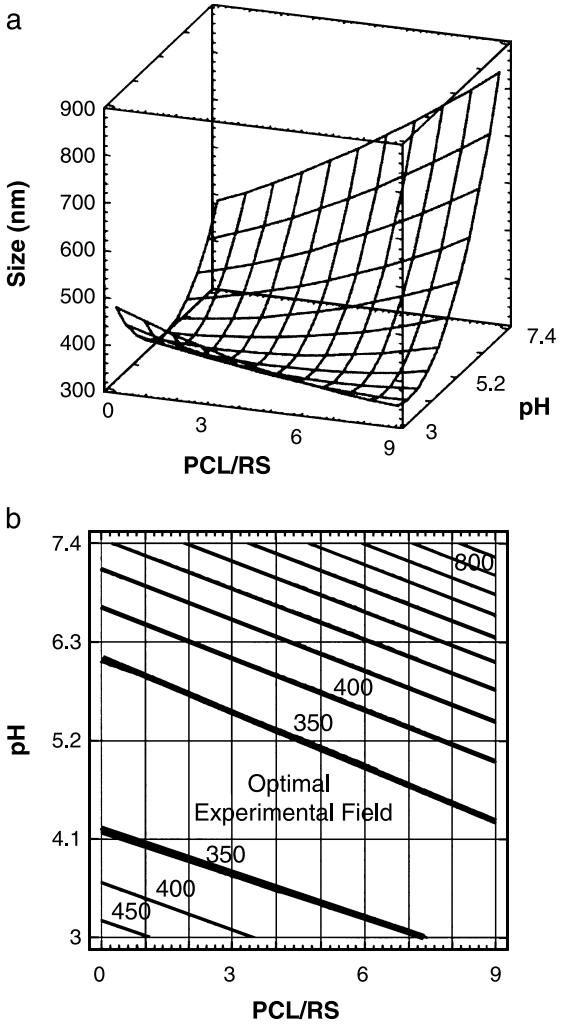

FIGURE 1 **Three (1a) and Two-dimensional (1b) Plots for Particle Size as a Function of the Formulation Variables: PCL/RS Ratio and pH of PVA Solution. (PVA Volume = 40 mL).**

will be presented according to each specific parameter, i.e., particle size, size polydispersity, nanoparticle zeta potential, encapsulation efficiency, and insulin release.

Particle Size

The estimated model and statistical validation are reported in Table 3a. This model was found to generate the highest F value [analysis of variance (ANOVA)] with a coefficient (R^2) of 0.912. The effects of PCL/RS ratio and pH of PVA solution are highly significant in this model, whereas volume of PVA solution plays a minor role. Thus, a variation of PVA volume from 30 mL to 50 mL does not alter the particle size, because the volume of the PVA solution is always large enough to allow a similar precipitation rate of the polymer during solvent evaporation. On the contrary, an increase of the pH of the PVA aqueous solution increases the size considerably: based on its isoelectric point, insulin is negatively charged at pH 7.4. Thus, insulin can interact with Eudragit and consequently decrease the electrostatic repulsions between insulin nanoparticles, which could favor loose agglomeration of nanoparticles and explain the increase in size. When the ratio PCL/RS decreases, the particle size also decreases. This is not surprising, since we have shown previously that Eudragit RS behaves like a surfactant allowing the production of very "small" nanoparticles (Chernycheva et al., 2003). A significant interaction between these two factors is also highlighted: for the lowest pH (pH=3), varying the polymer ratio has no great effect on the particle size (450–350 nm); whereas at the highest pH (pH=7.4), an increase of the size (500–800 nm) is observed (Figs. 1a and 1b). For a physiological pH of 7.4 and an intermediate ratio PCL/RS of 4.5, the particle size is around 650 nm. An

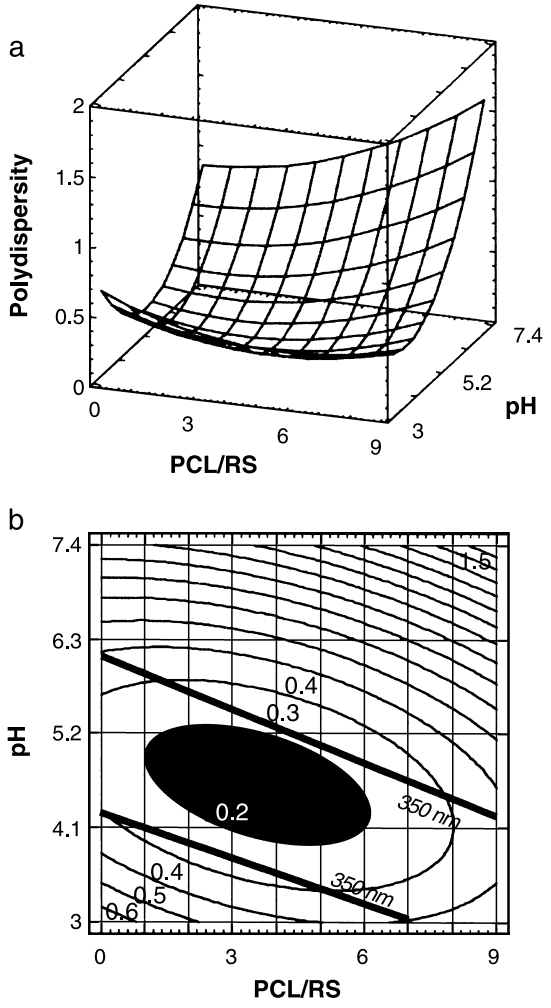

FIGURE 2 **Three (2a) and Two-dimensional (2b) Plots for Polydispersity of Size as a Function of the Formulation Variables: PCL/RS Ratio and pH of PVA Solution. (PVA Volume = 40 mL).**

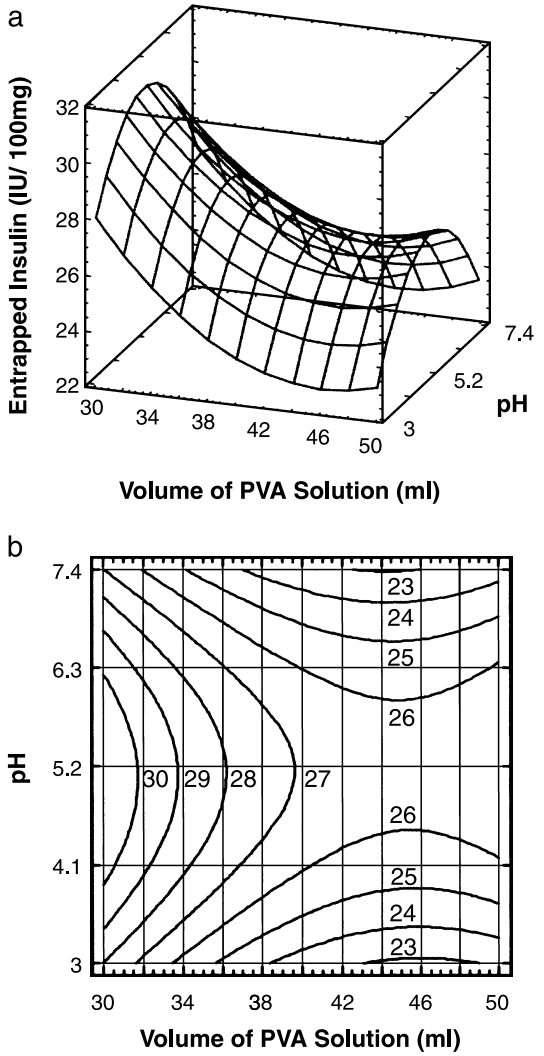

FIGURE 3 **Three (3a) and Two-dimensional (3b) Plots for Quantity of Encapsulated Insulin as a Function of the Formulation Variables: Volume and pH of PVA Solution. (PCL/RS ratio = 4.5).**

optimal area, drawn between two thick lines on Fig. 1b, highlights the operating conditions giving a stable suspension with the smallest particle size.

Size Polydispersity

After nanoparticle formation, the size population frequently follows a multimodal distribution. The polydispersity index is used to describe the diameter spread in a sample of particles. When this index is close to 1, the size range becomes wide. It is therefore better to obtain a value close to 0. The estimated model for polydispersity is valid (Table 3b). The coefficient of correlation R^2 is 0.855 and the model gives a p-value <0.01 (ANOVA). The polydispersity variations go overall in the same direction as the particle size. Increasing both pH and PCL/RS ratio leads to an increase of the polydispersity index. By superimposing the optimal area for the particle size and the best operating conditions allowing the polydispersity index reduction, it is possible to localize a black optimal zone in which size and polydispersity are respectively smaller than 350 mm and less than 0.2 (Fig. 2a and b).

Nanoparticle Zeta Potential

The ANOVA test validates the second-order model (Table 3c), but the low correlation coefficient ($R^2=0.574$) shows poor predictability of the model due to lack of fit. Nevertheless, Student's-t-test applied to each of the regression coefficients leads to identification of significant linear and quadratic effects of the pH and, to a lesser extent, a quite significant effect of the ratio PCL/RS.

The volume of PVA does ot contribute to the model. In general, the zeta potential remains strongly positive. The PCL unloaded nanoparticles have a zeta potential of around -2 mV. On one hand, encapsulated insulin does not modify this value, but on the other hand, Eudragit RS, even in very small quantities, imposes its value of zeta potential. This observation suggests that, when the objective is to reach a positive zeta potential, it is useless to add large amounts of Eudragit RS.

The nanoparticle zeta potential will play an important role in the interactions with mucin glycoprotein, i.e., mucus of the gastrointestinal tract. Indeed, at the end of all the oligosaccharide side chain of the mucin network, the terminal moieties are either sialic acid or L-fucose (Carsledt & Sheehan, 1984). Since the pKa of sialic acid is 2.6, it is completely ionized at a physiological pH of 7.4. Together with the presence of sulfate residues, the mucin network is known to carry a substantial negative charge at physiological pH. It is interesting that because PCL/RS nanoparticles have a positive potential, they are likely to interact with mucin glycoprotein and facilitate the intestinal absorption of insulin or other peptides/proteins that could also be encapsulated.

Quantity of Entrapped Insulin

The data describing the relationship between the amount of entrapped insulin and the three factors are shown in Table 3d.

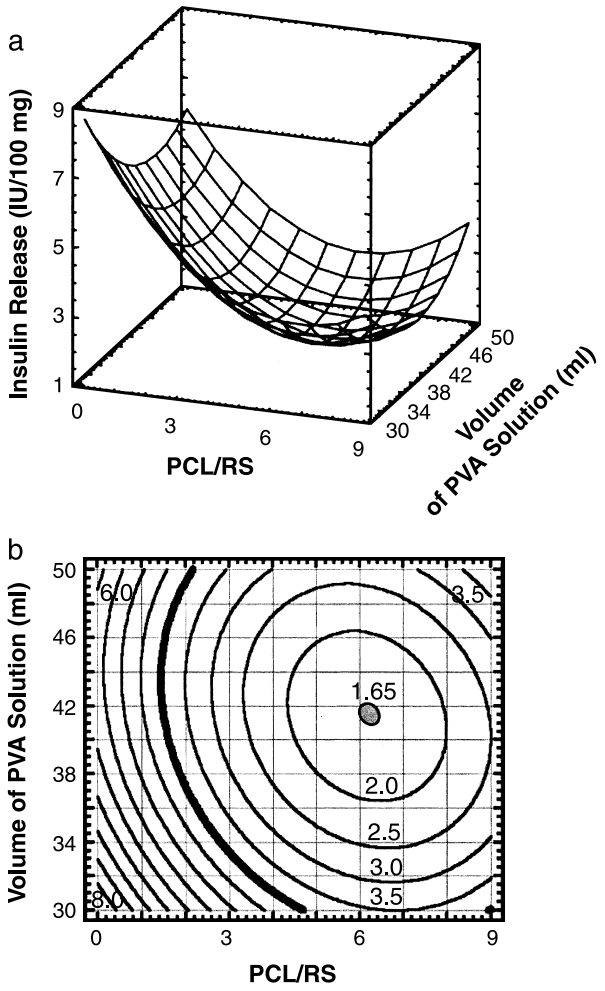

FIGURE 4 **Three (4a) and Two-dimensional (4b) Plots for the Quantity of Insulin Released after 7 hours as a Function of the Formulation Variables: PCL/RS Ratio and Volume of PVA Solution (pH=5.2).**

The volume of PVA plays a highly significant role, and the model is characterized by a strong linear effect and smooth quadratic influence. The pH is only influent through the quadratic term. The PCL/RS ratio reveals no significant effect on the quantity of entrapped insulin. As shown in Fig. 3a and b, a saddle point (minimax) is observed for a volume of 45 mL and pH 5.2 leading to 26.5 IU of entrapped insulin per 100 mg of polymer. In the overall experimental field, the highest entrapment (31 IU/100m g) is obtained at pH 5.2 with 30 mL of PVA solution.

For optimal entrapments observed at pH 5.2, an increase of the PVA volume from 30 to 50 mL decreases slightly the quantity of entrapped insulin approximately from 31 to 27 IU per 100 mg of polymer.

Several possible explanations are possible for the loss of insulin from nanoparticles. The difference in osmotic pressure between the internal and the external aqueous phases could be responsible for the slight decrease in entrapment efficiency, but could also lead to rupture of the nanoparticles and favors an exchange between the internal and the external aqueous phases, with a consequent loss of insulin. Due to the very hydrophilic nature of insulin, there is probably a faster and more important release towards larger volumes (50 mL) of PVA aqueous solutions during the solvent evaporation process. It is also possible that the loss in entrapment efficiency at larger volumes is due to the dilution of the entrapping polymers, reducing their ability to prevent loss. Although the polymers are not water soluble, they have some water solubility, and this change in volume may decrease the organic phase concentration of the polymers to a slight degree.

Insulin loss is observed on both sides, increasing or decreasing the pH from this level of 5.2. When the pH of PVA solution is fixed at 5.2, the volume of PVA solution set at 40 mL, and a low PCL/RS ratio is chosen, it is possible to encapsulate 27 to 31 IU of insulin per 100 mg of polymer. This is equivalent to 75% of entrapment efficiency. For further investigations, pH beyond 5.2 are excluded because there are not favorable to optimal particle size and polydispersity index.

Quantity of Released Insulin

The dissolution profiles of the 23 formulations (not shown) corresponding to the experimental design runs are characterized by an initial burst release in the first

TABLE 4 **Predicted vs. Observed Values for PCL/RS 50/50 Formulation (Volume of PVA Solution: 40 mL, pH=5.2)**

PCL/RS 50/50	Predicted response	Observed response	Relative error (%)
Size (nm)	322	350	8.7%
Polydispersity index	0.22	0.21	4.6%
Entrapped insulin (IU/100 mg)	25.7	25.0	2.7%
Quantity released after 7 hours (IU/100 mg)	4.7	4.8	2.1%
Zeta potential (mV)	+43	+44	0.2%

15 minutes (between 5% and 15% depending on the formulation) followed by a plateau during 24 hours. The statistical results are presented in Table 3e. This initial burst effect leads to rapid insulin release and is followed by a steady state reached after 7 hours of release for almost all of the produced batches. Significant differences are observed for the quantity of released insulin. This response variable has been successfully described by a mathematical model. The model was found to generate a correlation coefficient R^2 of 0.912. Except for pH, all the other controlled variables (PCL/RS ratio and volume of PVA solution) display highly significant linear and quadratic effects.

At pH 5.2, a minimum of release (1.65 IU/100 mg) is obtained for PCL/RS=6.3 and 42 mL of the PVA solution (Fig. 4a and b). This could be due to the pH value below the isoelectric point.

With low amounts of PCL, the highest levels of released insulin ranged from 6 (PVA volume: 50 mL) to 8 IU of insulin per 100 mg of polymer (PVA volume: 30 mL). On the contrary, with high amounts of PCL, a lower release of insulin is observed owing to the high hydrophobicity of PCL that reduced nanoparticle wettability and consequently decreased the quantity of insulin released.

The interesting operating conditions leading to a release of at least 4IU/100 mg is represented below the thick isoresponse contourline in Fig. 4b.

CONCLUSION

The formulation of insulin-loaded nanoparticles has been studied and optimized using the response surface methodology method. Based on the use of experimental designs, this approach allows efficient quantification, with the minimum number of experiments, of the effects of three controlled variables on the main characteristics of the particles. It was found that a PCL/RS ratio below 4.6, a volume of PVA solution between 30 to 40 mL, and a pH of the PVA solution between 3.5 and 5.2 were necessary to obtain "small" nanoparticles with a narrow particle size distribution, containing at least 25 IU of insulin per 100 mg of polymer and delivering at least 4 IU after 7 hours of release. The results estimated by the models and those observed after performing the experiments are summarized in Table 4. They show a good fit for all the response variables, highlighting the good predictability of the models. Under these optimized conditions, the zeta potential still remains highly positive. This result would favor intimate contact with the gastrointestinal tract and therefore the absorption of insulin (Gershanik et al., 1998). Based on results already obtained with similar nanoparticles incorporating heparin (Jiao et al., 2002), insulin-loaded nanoparticles prepared by the emulsification and evaporation process with blends of biodegradable and positively charged, nonbiodegradable polymers show promise as new oral insulin delivery systems. An interesting optimized formulation, nanoparticles manufactured with the blend PCL/RS 50/50 is now under in vivo investigation.

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