PLGA Nanoparticles Loaded with the Non-Steroid Anti-Inflammatory: Factor Influence Study and Optimization Using Factorial Design

Lynda Lamoudi, Jean Claude Chaumeil, and Kamel Daoud

***Abstract*—The aim of this study is to develop a polymeric drug delivery system for a non-steroid anti-inflammatory. To achieve this objective, Piroxicam loaded poly (DL-lactide-co-glycolide) (PLGA) nanoparticles were prepared by nanoprecipitation method and characterized. Formulations were prepared by using experimental design to study the effects of process and formulation variables on response of nanoparticle drug loading (TE) and yield of nanoparticles (TP); for all formulations the volume diameter is less than 1 μm. The physical characteristics of PLGA nanoparticles were evaluated using particle size analyzer and a UV–visible spectrophotometer. The results of optimized formulations showed a large yield of nanoparticles about 72%, and a drug loading more than 67%.**

***Index Terms*—PLGA, nanoparticles, nanoprecipitation technique, piroxicam, experimental design.**

I. Introduction

Micro- and nanoparticles are frequently made of poly(lactic acid) (PLA) or its copolymer with glycolic acid (PLGA), due to the biocompatibility and biodegradability of these materials [1]. Other frequently applied synthetic polymers are poly (ε- caprolactone) (PECL), poly(alkylcyanoacrylates) (PACA) and Eudragit® [2], [3].

Also natural polymers like gelatine, albumin or chitosan can be employed [4], [5]. Polymeric nanospheres composed of PLGA as a biocompatible and biodegradable compound have lower toxicity compared to other polymers and have more stability than liposome and other drug delivery systems in biological environment. PLGA is composed of lactic and glycolic acids linked together by ester bonds. The polymer degradation proceeds with formation of free carboxylic end groups. Several current reports have indicated the presence of low micro environmental pH in large specimens of PLGA [6]. The reported methods of preparing nanoparticles from biodegradable polymers include solvent evaporation, monomer polymerization, nanoprecipitation and salting out procedure [7]. The nanoprecipitation method developed by Fessi *et al.* [8] represents an easy and reproducible technique and very often used to prepare colloidal carriers both matricidal (nanosphere) and vesicular type (nanocapsules). This method was based on the interfacial deposition of a polymer followed by diffusion of a semi-polar and miscible solvent in the aqueous medium with the presence of a surfactant [9]. Several important factors contribute to the effectiveness of this method in preparing particles with acceptable size range, shape and the percentage of the drug load, namely the amount of polymer, percentage of surfactant and volume of organic and aqueous phases. It is difficult to study the effect of the variables individually or in combination, therefore we used a mathematical model in achieve to study a quantitative relationship between the formulation variables [10]-[13].

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II. Materials and Methods

A. Materials

Poly(DL-lactide-co-glycolid) (PLGA, 50:50 MW 12,000, inherent viscosity of 0.16–0.24 dl/g) was obtained from Boehringer Ingelheim Co. (Ingelheim, Germany) in the form of Resomer(R) 502H. Pluronic F68 was purchased from BASF Company (Ludwigshafen, Germany). Piroxicamis a gift from Saidal company. All chemicals and reagents used were of analytical grade.

B. Preparation of the Nanoparticles

Nanoparticles were prepared by nanoprecipitation method as described by Fessi et al. [13]. The preparation procedure was as follows: exact quantity of PLGA polymer and piroxicam (10 mg), were accurately weighted and dissolved in acetone and dichloromethane, respectively. The organic phase was added to an aqueous solution of pluronic with moderate stirring.

The desolvation of polymeric material occurred instantaneously in form of colloidal particles.

Organic solvent was then evaporated from the colloidal suspension by an adapted system.

Nanoparticles suspension was filtered through a 0.8 μm nitrocellulose membrane (Millipore) and concentrated to a final volume of 10 ml by removal of water under the same conditions. The nanoparticles suspension was lyophilized.

C. Experimental Design

An experimental design is frequently employed for the planning of a research because it provides the maximum of informations, requiring the least experiments [14]-[17].

In this study, an orthogonal experimental design was introduced to optimize the formulation of nanoparticles. In

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order to optimize the preparation of formulations, the amount of PLGA (X_1), the amount of Pluronic (X_2), and the speed agitation (X_3), were chosen as independent factors. These three factors have certainly an effect on the nanoparticles formulation and three levels of each factors were selected (Table I). Possible interactions between X_1–X_2 and X_2–X_3 were also studied. The drug loading (TE) and the yield of nanoparticles (TN) used as dependent variables (responses).

MODDE version 6 software (SIGMA PLUS, France) was used for generation and evaluation of the statistical experimental design.

TABLE I: INDEPENDENT VARIABLES AND THEIR CORRESPONDENCE BETWEEN REAL AND ORTHOGONAL VALUES DESIGN FOR NANOPARTICLE FORMULATION

Independent variable	−1	0	+1
(X1) PLGA amount (mg)	100	125	150
(X2) Pluronic amount (mg)	200	250	300
(X3) speed agitation (rpm)	100	300	500

D. Statistical Analysis

To compare each of the nanoparticles formulations quantitatively, the method defined by Wehrle *et al.* [13] was used.

Multiple regressions are based on the general theory of least squares that allows the calculation of a very simple mathematical model. According to this mathematical model, the polynomial equation includes the first order terms and the second order term of interaction variables. Thus, the equation can be described by a more complex model taking into account the quadratic effects of each of the controlled variables Eq. (1).

$$y = k + aX_1 + bX_2 + cX_3 + dX_1X_2 + eX_2X_3 + fX_1X_3 + gX_1^2 + hX_2^2 + tX_3^2 \quad (1)$$

A multiple regression procedure was introduced to find the relationships between the three studied variables described above.

The software MODDE 6 was selected to evaluate the model coefficients. Variables were introduced into the model gradually, while insignificant variables which were tested by t-test (P< 0.05) were eliminated out of the model.

E. Particle Size Analysis

Measurements of particle size were determined by laser diffraction using a Mastersizer® 2000 (Malvern Instruments, UK), which is also known as low angle laser light scattering. Nanoparticles were analyzed in duplicate with five readings for each nanoparticle sample, suspended in bi-distilled water.

F. Drug Loading of the Nanoparticles

Ten milliliters of the hydrochloric acid in methanol (1 in 1200) was added to 10 mg of lyophilized Piroxicam nanoparticles and analysed by spectrophotometer at 233 nm (UV-1202-Shimadzu).

III. RESULTS AND DISCUSSION

A. Statistical Analysis

The controlled variables studied were the amount of acetone, molecular weight of the polymer and the type of surfactant. Computed results to describe the relationships among the factors on dependent variables are expressed by Eqs. (2) and (3).

$$TN\,(\%) = 73.8 + 10.13X_1 - 2.46X_2 + 3.59X_3 - 3.22X_1^2 - 6.08X_2^2 - 6.37X_3^2 - 2.67X_1X_2 - 12.9X_1X_3 - 0.75X_2X_3 \quad (R^2 = 0.746;\ F\text{-}value = 8724.56;\ p < 0.05). \quad (2)$$

$$TE\,(\%) = 59.5 + 1.39X_1 + 13.89X_2 - 1.19X_3 - 2.37X_1^2 - 0.02X_2^2 + 4.7X_3^2 + 5.54X_1X_2 + 3.19X_1X_3 + 2.6X_2X_3 \quad (R^2 = 0.854;\ F\text{-}value = 3485.63;\ p < 0.05). \quad (3)$$

The results of ANOVA indicated that all models were significant (p < 0.05) for all response parameters investigated. Model simplifications were carried out by eliminating non-significant terms (p > 0.05) in polynomial equations, and we obtain:

$$TN\,(\%) = 73.8 - 12.91X_1X_3 \quad (4)$$

$$TE\,(\%) = 59.5 + 13.89X_2 - 0.02X_2^2 \quad (5)$$

B. Factors Effect Study on Responses

The effect of a factor is derived from models in response surface. Thus, two factors are set to their low and high levels, and then the third factor is varied in the study.

1) Copolymer PLGA effect on TP and TE

The Fig. 1 showed that, copolymer PLGA has a discontinuous effect on TP. This effect is more pronounced when the mixing speed is less than middle value.

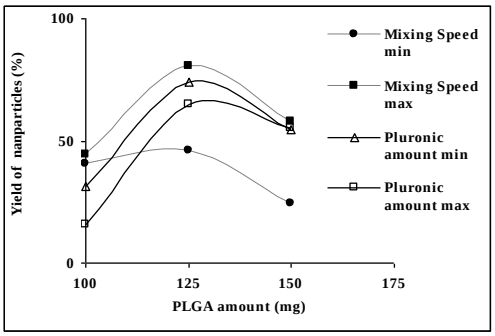

Fig. 1. Effect of PLGA amount on TP

In more, the change of Pluronic amount from the minimum to maximum level does not appear to disturb this effect.

This non-linear influence of copolymer PLGA may be explained by the increasing of TP in the first step and in the second step, the viscosity generated by this copolymer produces an opposite effect on the process of nanoparticles formation.

In addition, the increasing in speed gives a more important TP due to the increasing in repulsion shear forces.

In Fig. 2, we notice that when the speed increase, the effect of PLGA copolymer reduce, because the active ingredient is not encapsulated by copolymer PLGA due to the high shear effect.

In more, the increasing of pluronic amount does not affect the influence of copolymer PLGA.

Finally, the copolymer PLGA appears to have a major effect on obtaining nanoparticles and encapsulation.

Also these two effects are dependent on the mixing speed; simultaneously, the increasing of speed involves the growing of nanoparticles and decrease TE.

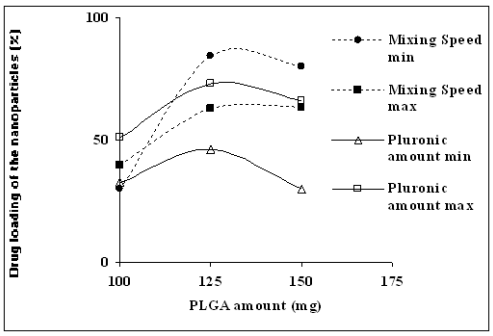

Fig. 2. Effect of PLGA amount on TE

2) Effect of pluronic on the TP and TE

In Fig. 3, pluronic amount improve the TP but the process is depending on the mixing speed; in this case, the amount of copolymer PLGA (average value) is enough and does not generate a significant increasing of the viscosity.

For a minimum level of copolymer PLGA, pluronic seems to have a positive effect on the TP.

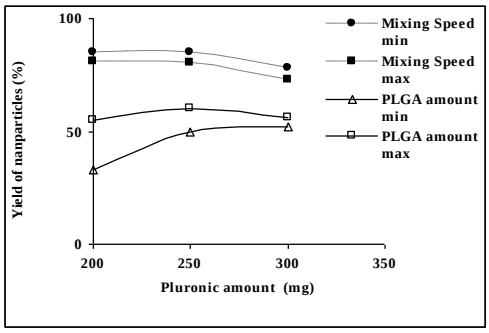

Fig. 3. Effect of Pluronic amount on TN

In Fig. 4, the effect of Pluronic on TE is positive and does not depend on the mixing speed. This means that this effect is independent of forces ratio between viscous friction of copolymer PLGA and shear forces.

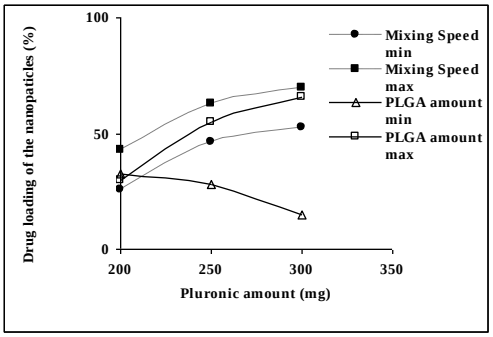

Fig. 4. Effect of Pluronic amount on TE

On the other hand, the effect of Pluronic is strongly related to the quantity of PLGA used. When the copolymer PLGA is on minimum level, the surfactant is developing a negative influence on the TE. This is due probably to the competition with the active ingredient to occupy the very low number of nanoparticles.

Also, it appears that pluronic doesn't affect the quality of TP and can therefore not be considered in relation to this response; however, for TE, the effect is important and therefore must be taken into account.

C. Optimization

To obtain the composition of the optimal formula, we represent the two iso-reponses, TP and TE on the same graph based on three factors, Fig. 5 and Fig. 6.

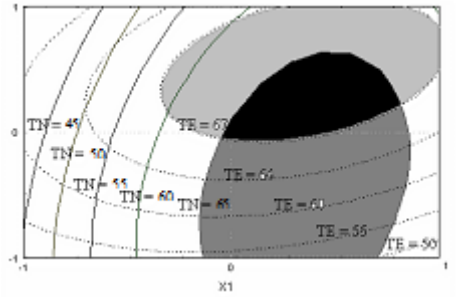

Fig. 5. Contours iso-TP and iso-TE in response surface

In Fig. 5, the optimal field is given by the intersection of iso-responses for which TE is higher than 67% and TP is greater than 65%. Thus, the interval factors x_1 and x_2 involved in this optimal field are:

$$x_1 = [0, 0.75] ;$$

$$x_2 = [0, 0.75]$$

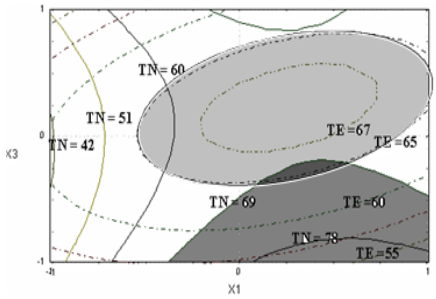

Fig. 6. Surrounders iso-TP and iso-TE in response surface

In more, from the Fig. 6, the optimal field corresponding to TE more than 65% and TP is between 69% and 78%.

Areas of factors x_1 and x_3 corresponding are:

$$x_1 = [0.1 , 0.6]$$

$$x_3 = [- 0.4 , - 0.2]$$

Therefore the optimal area for which the properties of granulation and encapsulation interesting are:

PLGA amount: $x_1 = [0.1, 0.6]$

Mixing Speed: $x_3 = [- 0.4, - 0.2]$

In order to validate the optimal field obtained by MODDE 6, we propose to do an analysis for the sample obtained in these conditions. The analytical characterization of this test is reported in the Table II.

If we make a comparison with different formulas made, we find that this test appears to be the better formulation because it presents the most important properties compared to other

tests. We noticed that the error between the experimental data and values obtained by the simplified polynomial model is less than 10%. Finally, we consider the model is validated.

TABLE II: CHARACTERIZATION OF OPTIMIZED FORMULATION

PLGA amount (mg)	Pluronic amount (mg)	Mixing Speed (rpm)
125	230	300
TP experimental (%)	TP predicted (%)	Error (%)
72,17	67,37	6,6
TE Experimental (%)	TE predicted (%)	Error (%)
67,58	70,20	3,9

IV. CONCLUSION

The nanoprecipitation method was used to prepare biodegradable nanoparticles of reproducible sizes in the range (less than 1 µm) by addressing the effects of processing parameters.

The use of a experiments design allow us to study the influence of different factors of formulation and manufacturing process on the formulation process, and thus reduce the number of experiments.

The copolymer PLGA appears to have a major effect on obtaining nanoparticles and encapsulation. The effect of Pluronic on TE is positive and does not depend on the mixing speed. This means that this effect is independent of forces ratio between viscous friction of copolymer PLGA and shear forces.

This work allows us to optimize the formulation of PLGA nanoparticles loaded with the non-steroid anti-inflammatory Piroxicam by using the experimental design.

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