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Glibenclamide Loaded PLA Nanoparticles using Single Emulsion O/W Solvent Evaporation Method: A Factorial Design Approach

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Abstract—Glibenclamide loaded poly-lactic acid nanoparticles were prepared using 3-level factorial design. The variables such as PLA (X_1), PVA (X_2) were optimized for maximum encapsulation efficiency (Y) using response surface methodology (RSM). RSM analysis predicted that both PVA and PLA were significant for drug encapsulation efficiency ($p < 0.05$). The nanoparticles were characterized by FESEM, FTIR, and particles size. Encapsulation efficiency of optimized nanoparticles was obtained about 79.21%. The nanoparticles consists two phase drug release, fast release phase within the first 15 min, followed by a slow release phase in the next hours. Size of nanoparticles was found in the range of 400–600 nm.

Keywords: Factorial design; glibenclamide; nanoparticles; PLA; solvent evaporation method.

1. Introduction

Hyperglycemia is happen due to a relative or absolute lack of insulin hormone. Diabetes mellitus occurs in two main forms classified as type 1 and type 2 [1]. As a result, the non-insulin dependent diabetes mellitus may be controlled by diet and oral hypoglycemic agents such as glibenclamide. Despite of the limitations of very short half life and poor solubility in water, glibenclamide is very popular oral hypoglycemic drug and widely use as mainstays in the management of type-2 diabetes [2, 3]. Due to its short half life, it needs many dosages in a day and its poor water solubility leads less oral bioavailability. Control release tablet and solid dispersion (fast release) formulation separately can solve both the problems of high dosing frequency and poor oral bioavailability [4] while, controlled/sustained release nanoparticles can solve both the problems.

Factorial analysis (FA) has been widely adopted for optimization of various parameters in drug formulation and development [5]. This is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes in which a response of interest is influenced by multiple variables and the objective is optimize this response [6, 7]. Apart from that, it also reduces the number of experimental runs required to generate statistically-validated results [8, 9].

In this work, glibenclamide loaded PLA nanoparticles were developed by single emulsion solvent evaporation method. And the encapsulation efficiency influenced by PLA and polyvinyl alcohol was evaluated by using response surface methodology (RSM). The present study also deals with the investigation of morphologies and release behaviors of the nanoparticles.

2. Materials and Methods

2.1 Materials

Glibenclamide was received as a gift sample from Wockhard Research Centre, Aurangabad, Maharashtra India. Dichloromethane (DCM) and methanol was commercially obtained from Merck specialties Ltd and RFCL Ltd, India respectively. Poly vinyl alcohol (PVA) was purchased from S.D fine-Chem. Ltd, India.

2.2 Methods

2.2.1 *Preparation of Glibenclamide Loaded Nanoparticles*

Nanoparticles containing glibenclamide as a core material was prepared by single emulsion (o/w) solvent evaporation method [10, 11]. Specific quantity of glibenclamide (100 mg) and PLA was dissolved in equal volume of DCM and methanol (5ml each) to obtain organic phase. O/W emulsion was prepared by drop wise addition of organic phase into the aqueous phase containing PVA as a stabilizing agent, under the high speed Homogenizer (OMNI International *TH*) at 20,000 rpm for 10 min subsequently 35,000 rpm for 3 min

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at 4° C. The emulsion was subjected under lab stirrer (Remi, Mumbai) at 1,000 rpm for 3 h for evaporation of the organic solvents. Nano particles were collected by centrifugation at 15,000 rpm (cooling centrifuge, Remi Mumbai, India) followed by lyophilization (Labogen, scanvac).

2.2.2 *Encapsulation Efficiency*

10 mg of freeze dried nanoparticles was dissolved in mixture of 10 ml of organic solvents (DCM and methanol, 5ml each). After suitable dilution, it was analyzed using UV spectrophotometer (Hitachi, U 2900). Glibenclamide entrapment efficiency (EE) in the nanoparticles was calculated from the following equation.

$$EE\ (\%) = (\text{Mass of drug in nanoparticles} / \text{Mass of drug added}) \times 100 \quad (1)$$

2.2.3 *Factorial Design*

In order to limit the number of experiments, a 3^2 full factorial design was employed using the Design - Expert® Software (Version-8.0.7.1, Stat-Ease Inc., Minneapolis, MN). Amount of PLA (X_1, mg) and PVA (X_2, % w/v) was selected as independent variables. The levels of independent variables and experimental condition are shown in the Table 1. RSM was used to analyze the results and the model graphs were generated.

Table 1. Experimental condition

Factor	Factor Level used (coded)	
	Low level (–1)	High level (+1)
X_1	300	600
X_2	0.1	0.5

Experimental run	X_1	X_2	Y
1	600	0.3	73.23
2	300	0.1	70.23
3	300	0.5	92.99
4	450	0.3	78.21
5	600	0.1	60.22
6	600	0.5	80.03
7	300	0.3	82.32
8	450	0.1	65.25
9	450	0.5	88.43

X_1= PLA (mg) and X_2 = PVA (%), Y= Encapsulation Efficiency(%)

2.2.4 *Characterization of Nanoparticles*

Characterization of PLA nanoparticles was performed for Particle Size analysis (Malvern zetaseizer, Nano ZS), FT-IR studies, conducted on a FT-IR spectrometer (FT-IR-8400; Shimadzu, Asia Pacific Pvt. Ltd. Singapore). The spectra of samples were measured by a KBr pellet method. Spectra were recorded in the scan range of 4000–400 cm^{-1} in transmission mode and the surface morphology of nanoparticles was observed by Field emission scanning electron microscope (Hitachi, Model-S4800 type II). The nanoparticles were mounted on metal stub with the help of double sided adhesive tape and coated with gold for 80 second under vacuum.

2.2.5 *Dissolution Study*

The drug release behavior of the nanoparticles was investigated by using fully calibrated six station dissolution test apparatus (USP type II) (Eletrolab Mumbai, India) at a stirring rate of 100 rpm and 37 ± 0.5 °C. Dissolution study was performed initially 2 h in acidic media (pH 1.2) subsequently in phosphate buffer (pH 7.4) for the next 10 h. The nanoparticles were dispersed in phosphate buffer (10ml) and filled with pre-activated dialysis membrane (HIGHMEDIA, India). Dialysis membrane containing nanoparticles was placed into dissolution media. At specific time intervals, dissolution samples (10 ml) were collected and a replacement was made with same fresh dissolution media. Collected samples were assayed spectrophotometrically at 300 nm.

3. Result & Discussion

3.1 Surface Morphology and Particle Size Distribution (PSD)

FE-SEM and particle size analyzer are powerful tools to characterize the surface morphology and size of polymeric nanoparticles. Fig. 1(i) showed a FE-SEM image of glibenclamide loaded nanoparticles. All the nanoparticles were spherical in shape with smooth surfaces. Currently, one of the essential features of the nanoparticles for clinical use is size. The average size of nanoparticles was found in the range of 400-600 nm as measured by PALS technique, Fig. 1 (ii).

3.2 Infrared Spectrophotometric (FT-IR) Analysis

FT-IR spectral data of pure Glibenclamide, PLA and glibenclamid Loaded PLA nanoparticles were performed (Fig 2, (i)). The principal peak of glibenclamide was obtained at wave number 3457, 3368 cm^{-1} attributed to –NH stretching. 542, 609 cm^{-1} attributed to –Cl bending deformation, 1715 cm^{-1} is due to –C=O bending, 2849, 2918, 2956 cm^{-1} attributed to –CH (fundamental) stretching (Fig. 2 (i) A) It was found that there was no disappearance and shifting of FT-IR peak in the spectra of the nanoparticles (Fig. 2 (i) C). Thus the study confirmed that there is no interaction between drug and polymer.

3.3 Factorial Design & Statistical Analysis of Data

A total of 9 runs were performed for the formulation of nanoparticles and investigated for the effects of independent variables (X_1 & X_2) on the dependent variable (Y) using factorial design (Table 1). Linear, cross-product contribution (2FI), quadratic and cubic models were generated in the response by the software. Table 2 showed the model summary statistics of the response (Std. Deviation, R^2 and Adjusted R^2). Linear model showed a best fit for the response. The results are expressed linear order polynomial equation that fitted to the data is as follows:

$$Y = 76.360 - 0.03563\ X_1 + 54.7916\ X_2 \quad (2)$$

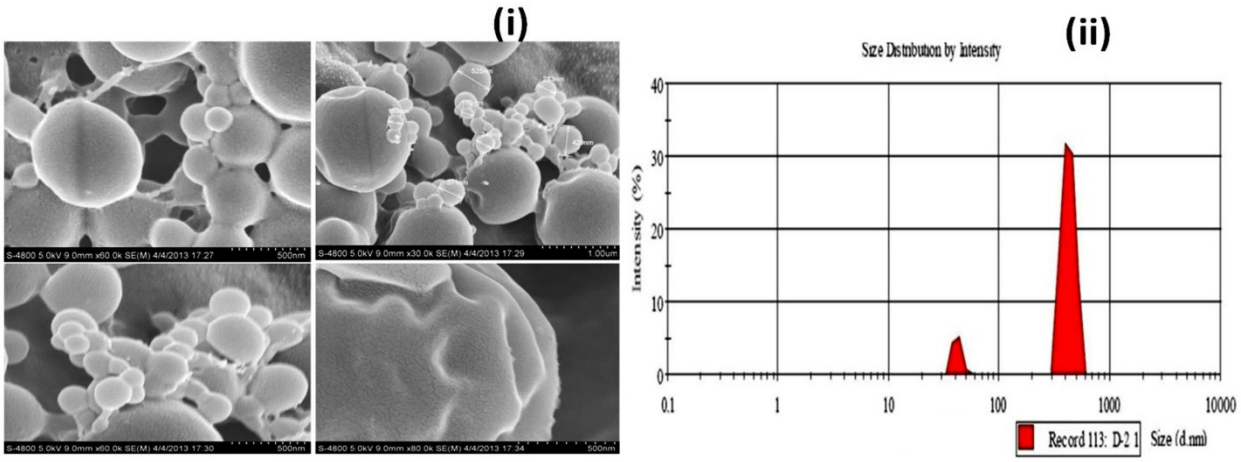

Figure 1. (i) FESEM Image and (ii) Particles size distribution of PLA Nanoparticles.

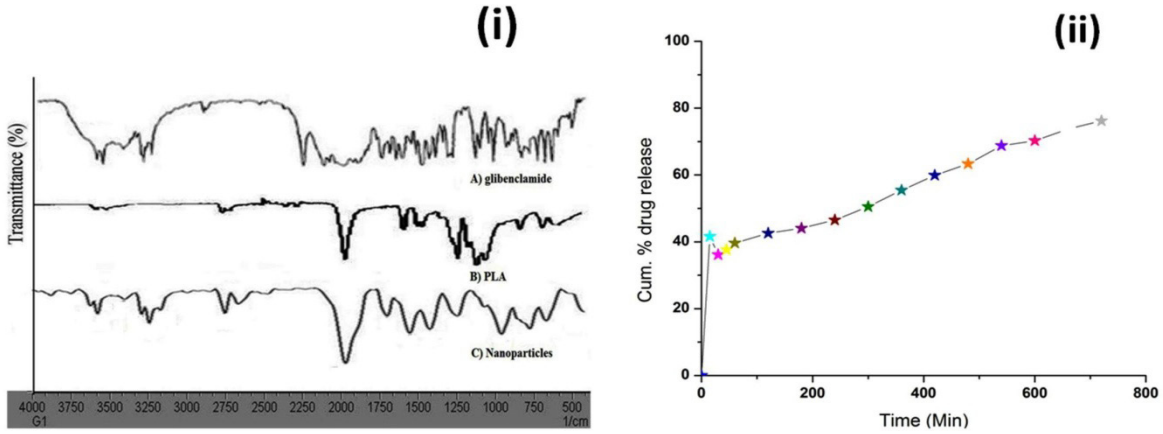

Figure 2. (i) FT-IR spectra of A] pure Glibenclamide, B] pure PLA polymer and C] nanoparticles (ii) Cumulative % drug release from Glibenclamide-Loaded PLA Nanoparticles.

The equation represents the quantitative effects of factor (X_1 & X_2) upon the response (Y). By the Eq. 2, it can be concluded that encapsulation efficiency was maximum influenced by PVA concentration. A significant factors which affecting the response (Y) were X_1 (PLA concentration, p-value-0.0001) and X_2 (PVA concentration p- value < 0.0001).

3.4 Effect of PVA Concentration and Polymer on Encapsulation Efficiency

The role of PVA as a surfactant/stabilizer in development of polymeric nanoparticles has been widely studied. Since, PVA concentration is a key factor in controlling particle size and release kinetics [12], variation of PVA concentration provides an opportunity to study the drug encapsulation efficiency. A 3-D response surface and 2-D contour plots were generated. The 3-D response surface plot (Fig. 3 A) showed about the main effects of PVA and PLA concentration on the drug encapsulation efficiency of nanoparticles. Whereas, 2-D contour plot provided a visual representation of values of the response (Fig. 3 B). It was observed that the increase in PVA concentration increases the drug encapsulation efficiency. But, the increase in polymer concentration decreased

Table 2. Model summary statistics of response and Coefficient estimate and p-values of each factor for the measured response.

Response			Model	Std. Dev.	R^2	Adjusted R^2	Significance
Y			Linear	1.4517	0.98601	0.98135	Suggested
Factor	**Coefficient estimate**	**p-value**	2FI	1.4470	0.98842	0.98147	---
X_1	-5.3433	0.0001	Quadratic	1.0388	0.99642	0.99045	---
X_2	10.9583	<0.0001	Cubic	0.3583	0.99985	0.99886	---

Y = encapsulation efficiency

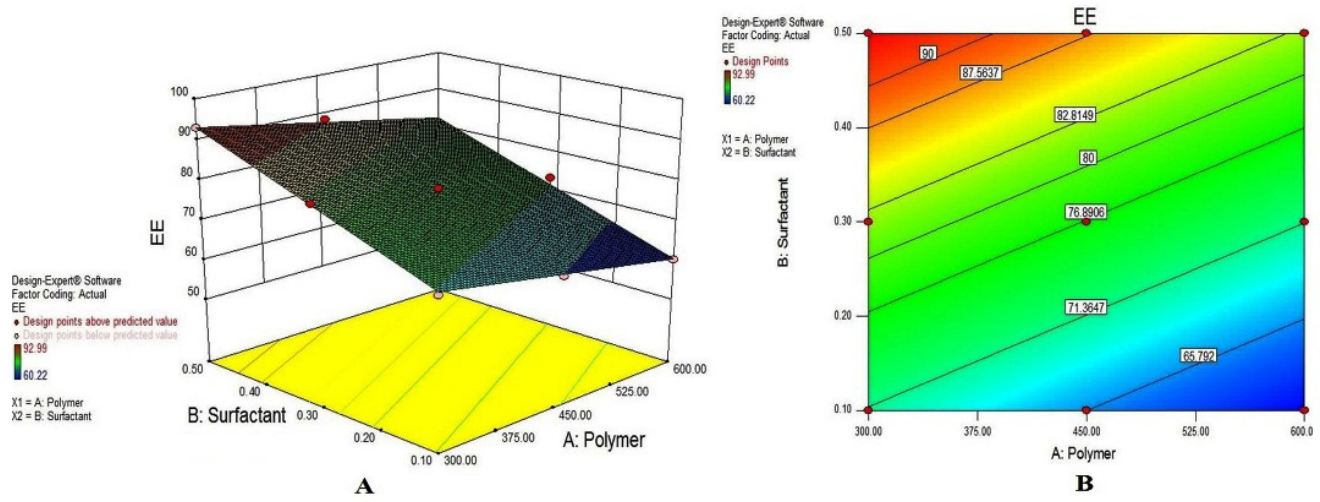

Figure 3. (A) 3-D model and (B) 2- D contour graph of Effect of surfactant and polymer concentration on entrapment efficiency of Glibenclamide-loaded PLA nanoparticles

the drug encapsulation efficiency of the nanoparticles. With increasing PVA concentration, it is known to reduce the interfacial tension and may be based on increasing surface area of PLA nanoparticles which lead more diffusion of the drug. Ultimately, it may decrease the encapsulation efficiency [13-15]. Conversely, in our finding, it increased. Increase in encapsulation efficiency might be due to free drug available on the surface in place of entrapment in nanoparticles. This can be confirmed by higher burst release (Fig. 2). While percent encapsulation efficiency decreases with an increase in the amount of polymer. This may be due to the more time taken for the precipitation of polymer which was in higher amount [23].

3.5 Optimization of Variables

In order to generate optimal conditions for encapsulation efficiency. A feature of the Design-Expert® Version 8.0.7.1 software was applied. The independent parameters used in numerical optimization include polymer (450 mg) and PVA (0.3 %) whose concentration were set within the range. The obtained encapsulation efficiency (79.21 %) is well in agreement with the predicted value (76.77 %), as the experimental error is less than ±5%, it can be concluded that the proposed statistical model was adequate for predicting the encapsulation efficiency.

3.6 In-Vitro Release Profile of Nanoparticles

In- vitro drug release study of the optimized formulation is shown in Fig. 2 (ii) Glibenclamide release of nanoparticles appeared in two phases of drug release. An initial exponential phase in which about 40% (Fig. 2 ii) of drug release was observed within 15 min followed by a slow phase drug release in the next 10 h. The initial release phase of glibenclamide was probably due to glibenclamide which was adsorbed or associated with the surface of the nanoparticles. Finally, the drug release was slow and continued. The total drug release was observed nearly 70% in 12 h. An increase in the polymer concentration decreases the drug release as well as bust release of the nanoparticles.

Conclusion

In this study, glibenclamide loaded PLA nanoparticles were developed with high encapsulation efficiency and a specific glibenclamide release pattern was investigated. It has been observed that an increase in concentration of PVA increases the encapsulation efficiency. While, an increase in the polymer concentration decreases the drug release. The FE-SEM analyses confirmed that formulated nanoparticles were of spherical in shape with smooth surface. The concentration of polymer and PVA, both have significant effect on encapsulation efficiency.

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References

[1] Michael U. Adikwu, Yukako Yoshikawa, Kanji Takada (2004). Biomaterials; 25, 3041–3048.
[2] Sunny R. Shah, Rajesh H. Parikh , Jayant R. Chavda , Navin R. Sheth (2013). Powder Technology; 235, 405–411.
[3] Sarath Chandiran Irisappan, Balagani Pavan Kumar, Korlakanti Narasimha Jayaveera (2013). Journal of Pharmacy Research; 7, 766–773.
[4] Jaime Salazar, Rainer H. Muller, Jan. P (2013). Eur. J. Pharm. Sci.; 49, 565–577.

[5] R. M. Ribeiro-Costa et al (2009). Powder Technol; 190, 107–111.
[6] Vinod J mokale et al (2013). International Journal of drug delivery. 5, 300–308.
[7] Sunil k Yadava et al (2014). Journal of Sol-Gel Science and Technology. 71, 60–68.
[8] Gokul Khairnar, Vinod Mokale, Jitendra Naik (2014). Journal of Pharmaceutical Investigation, DOI 10.1007/s40005-014-0118-3.
[9] Vinod mokale et. al., (2014). DIO 10.1007/s40005-014-0122-7
[10] J. B Naik et al (2012). International journal of Pharma & biosciences; 3, 573–590.
[11] Moritz Beck-Broichsitter et al (2012). J. Control. Release; 157, 469–477.
[12] Yang Y, Gao Y, Mei X (2013). Pharm. Dev. Technol; 18, 1169–74.
[13] Yeonsoon Choi et al (2011). B. Kor. Chem. Soc.; 32, 867.
[14] Yoon Yeo and Kinam Park (2004). Arch. Pharm. Res.; 27, 1–12.
[15] Jayesh Shivaji Patil et al (2013). International Journal of Pharmacy; 3, 843–852.