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# DEVELOPMENT AND CHARACTERIZATION OF CANAGLIFLOZIN LOADED POLYMERIC NANOPARTICLES USING BOX-BEHNKENDESIGN

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**Abstract:**

*The purpose of this study was to development and characterization of Canagliflozin loaded polymeric nanoparticles using box-behnken design. Canagliflozin loaded polymeric nanoparticles, was prepared by solvent diffusion (Nano precipitation) method. Box-behnken design was introduced to optimize the formulation of polymeric nanoparticles Results: Fourier Transform Infra-Red (FTIR) studies indicate that the excipients added were compatible with the drug. The value of zeta potential Zeta potential value -28.99 is essential for effective stability and to inhibit aggregation of particles. The optimum formulation OPT-CN-PNP with entrapment efficiency 92.16±2.17% and particle size 132.23±7.26nm. Scanning electron microscopy and transmission electron microscopy images of the optimal OPT-CN-PNPs depicted that the particles appeared to be of spherical uniform shape. The size of most of the particles was found to be less than 200 nm.*

*Key words: polymeric nanoparticles, solvent diffusion, Box-behnken design, Scanning electron microscopy & Transmission electron microscopy.*

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## INTRODUCTION:

Nanotechnology is a leading scientific technique that offers sensing technologies and miniature devices to diagnose disease accurately and within time. There is wide range of applications of nanotechnology in the field of drug delivery and furthermore, to simplify the oral absorption of proteins and peptides Nano carriers are modified with specific ligands [1]. Nanoparticles are used to deliver RNA and proteins to diagnose the disease as well as to monitor the progression of disease. Pulmonary means of drug delivery is also an efficient route other than Nano carriers[1-3]. Diabetes mellitus is characterized by increased thirst, excessive weight loss, excessive desire to eat and increased urge for urination thus resulting in abnormal increase in blood glucose level. Depending on the reason for high blood sugar, it can be classified as Type 1, Type 2 or gestational diabetes mellitus. In type 1 diabetes, body fails to produce insulin due to loss of β-cells which is caused by T-cell mediated autoimmune attack. In type 2 diabetes, body becomes insulin-resistant combined with insulin deficiency. Insulin replacement therapy is prescribed for type 1 diabetes patients which includes injections of long-acting insulin at mealtimes. Regulation of meals and exercise is preferred for the initial treatment of type 2 diabetes. Insulin injections and glucose test can be painful and time consuming for diabetic patients. To overcome the drawbacks of injection therapy, several technologies have been developed like continuous glucose monitors and insulin pumps to improve patient compliance [4-6].

## MATERIALS AND METHODS:

### Materials

Canagliflozin was obtained from Sisco RL Pvt Ltd. Mumbai. All other reagents are of either laboratory/analytical grade as per the requirement.

### Methods

### Formulation of Canagliflozin Loaded Nanoparticles

Canagliflozin loaded PLGA nanoparticles (NPs) were prepared using solvent diffusion (Nano precipitation) method (Fessi et al; 1989). The optimized formulation was prepared by dissolving PLGA (25 mg) and drug (10 mg) in 2.5 ml of acetone. The organic phase was added at the rate of 0.5ml/min into 5 ml of aqueous phase containing 0.25%w/v Pluronic F68 with continuous stirring on magnetic stirrer at room temperature. Stirring was continued until the complete evaporation of organic solvent. The NPs suspension was ultrasonicated (Sonics vibra) at different interval (3-7 min at 60-80 kHz) for one cycle and allowed to cool; nanoparticles were further processed for characterization study collected[7-9].

### Box-Behnken Experimental Design:

The statistical experimental design (Box–Behnken Design Expert version 12.0.1; Stat-Ease, Inc., Minneapolis, MN) was employed to optimize the formulations. The study used three factors; three levels design to investigate the effect of independent variables on the dependent variables [28]. The different levels of independent variables A: Polymer concentration (mg), B: surfactant concentration, C: sonication time (minute) was taken for formulation development. The coded levels of independent variables used were +1 (high), 0 (medium) and -1 (low). The effect of these factors was observed on dependent variables Y1=Particle size (nm), Y2=Entrapment Efficiency[10-14].

### Characterization of Optimized Canagliflozin Loaded Nanoparticles

### Particle Size

The size analysis and polydispersity index of the NPs were determined using a Malvern Zetasizer Nano ZS (Malvern Instrument, Worcestershire, UK). Each sample was diluted ten times with filtered distilled water to avoid multi-scattering phenomena and placed in disposable sizing cuvette. Polydispersity index was noted to determine the narrowness of the particle size distribution. The size analysis was performed in triplicate and the results were expressed as mean size ± SD[15].

### Entrapment Efficiency and Drug Loading

The drug content in the NPs was determined by dissolving 10 mg of lyophilized NPs in 10 ml of acetonitrile and analyzed by HPLC after filtration through 0.22 µ and appropriate dilution with mobile phase. Drug loading was calculated as follows,

P

ercentage drug loading=A/B×100

Where A is the drug content in the NPs and B is the weight of NPs.

EE was estimated by calculating amount of drug entrapped in NPs with respect to total drug added during preparation of formulation.

The PDE was calculated according to following formula:

EE (%) = (ED/TD) × 100 where, TD is total amount of drug added and ED is entrapped drug (drug content in NPs).

### Zeta Potential

Zeta potential distribution was also measured using a Zetasizer (NanoZS, Malvern instrument, Worcestershire, UK). Each sample was suitably diluted 10 times with filtered distilled water and placed in a disposable zeta cell. Zeta limits ranged

from -200to +200 mV. The electrophoretic mobility (µm/sec) was converted to zeta potential by in-built software using Helmholtz-Smoluchowski equation. Average of 3 measurements of each sample was used to derive average zeta potential[16-19].

**Transmission Electron Microscope Studies**

A sample of NPs (0.5mg/ml) was suspended in water and bath sonicated for 30 s. 2 µl of this suspension was placed over a formvar coated copper TEM grid (150 mesh) and negatively stained with 2 µl uranyl acetate (1%) for 10 min, allowed to dry and the images were visualized at 80 kV under TEM (Philip Tecnai 20, USA) and captured using Gatan Digital Micrograph software[20].

**Stability Studies**

The stability Canagliflozin loaded NPs were studied at 2-8°C and room temperature for 3months. Periodically, samples were withdrawn and the particle size as well as drug content was determined.

## RESULTS & DISCUSSION:

**Table 1: Independent variable and Responses**

| Formulation | X1 | X2 | X3 | Particle size(nm) | EE% |
|---|---|---|---|---|---|
| **CN-PNP1** | **+1** | 4.5 | 3 | 36.56±2.21 | 126.62±4.16 |
| **CN-PNP2** | **+1** | 4.5 | 5 | 29.43±1.05 | 131.33±6.13 |
| **CN-PNP3** | **0** | 2 | 5 | 21.03±2.28 | 140.83±1.41 |
| **CN-PNP4** | **0** | 2 | 3 | 50.92±1.86 | 127.33±2.68 |
| **CN-PNP5** | **+1** | 2 | 4 | 48.71±2.34 | 136.36±4.53 |
| **CN-PNP6** | **0** | 7 | 3 | 38.23±2.43 | 139.73±2.68 |
| **CN-PNP7** | **0** | 4.5 | 4 | 51.13±1.49 | 159.73±3.72 |
| **CN-PNP8** | **-1** | 4.5 | 5 | 57.33±1.19 | 163.56±3.91 |
| **CN-PNP9** | **0** | 4.5 | 4 | 40.32±0.71 | 176.91±1.69 |
| **CN-PNP10** | **-1** | 4.5 | 3 | 61.66±2.56 | 144.13±2.13 |
| **CN-PNP11** | **-1** | 2 | 4 | 55.47±1.34 | 148.03±1.98 |
| **CN-PNP12** | **-1** | 7 | 4 | 47.47±1.04 | 152.63±6.23 |
| **CN-PNP13** | **0** | 4.5 | 4 | 83.37±1.42 | 155.63±4.61 |
| **CN-PNP14** | **0** | 7 | 5 | 73.26±1.35 | 159.63±3.73 |
| **CN-PNP15** | **+1** | 7 | 4 | 92.16±2.17 | 132.23±7.26 |

**Characterization:**

**Particle Size & Entrapment Efficiency**

The values of both particle size and drug entrapment were found to be acceptable around the intermediate levels of lipid and surfactant. The lower particle size and higher drug entrapment of CN-PNP-15 can be attributed to the high levels of lipid and low levels of surfactant which were unable to emulsify the drug completely. Similar results have been obtained in previously reported studies where low amounts of surfactant and high levels of lipid led to the formation of SLNs with high particle size and low drug entrapment.

**Zeta potential**

The values of zeta potential of all formulations prepared as per the experimental design. The formulations CN-PNP-15 and CN-PNP-4 exhibited high values of zeta potentials ostensibly ascribed to the presence of high levels of lipid and intermediate to high levels of surfactant.

**In-vitro Drug Release**

In vitro drug release studies from pure drug solution and Canagliflozin loaded PNPs is shown in Figure 5.52 and 5.53. The pure drug solution released more than 40 % drug in 1h and within 4h nearly 100% of drug was released. Whereas, from drug loaded PNPs 25.96±3.254 % of drug was released in 4h, followed by 56.89±4.259% in 24h and 98.2± 2.371% of drug released at the end of 24h.

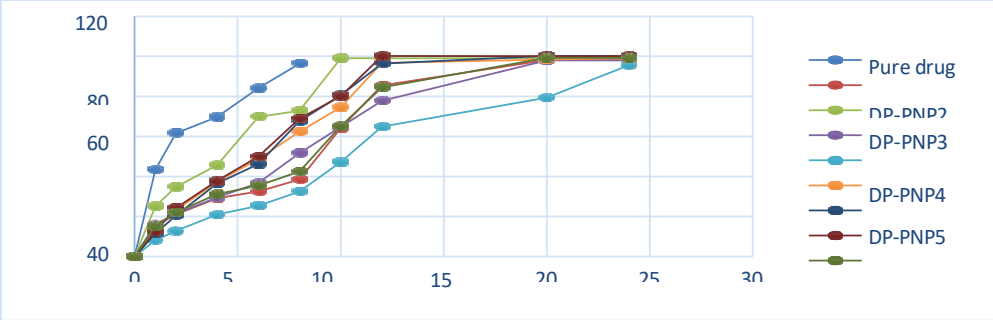


**Fig. 1: In-vitro drug Release for (CN-PNP-1 to CN-PNP8)**

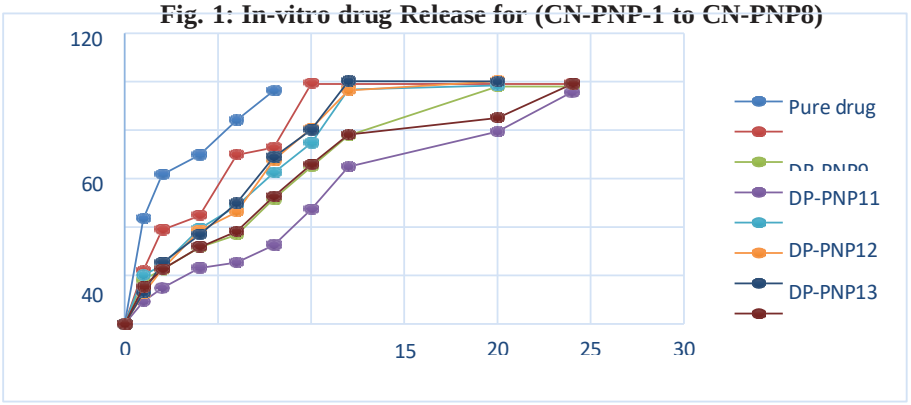


**Fig. 2: In-vitro drug Release for (CN-PNP-9 to CN-PNP15)**

**Optimized formulation**

From the results, the optimum levels of independent variables were screened by multiple regression analysis. Our desirability criteria were maximum entrapment with minimum particle size (less than 200 nm). Since PS and EE were taken into consideration simultaneously, the batch with smallest particle size of 126.6±4.16 nm exhibited EE near to 22 % (at X1 = -1, X2 = -1.0, X3= -1.0) while that with highest EE of 95.7±2.43 % produced particle size greater than 200 nm (at X1= 1, X2= 0.0 X3= -1). Hence, the optimum formulation OPT-DPPNP with EE 92.16±2.17% and particle size 132.23±7.26nm found at 1.0, -1, and -1 levels of X1, X2 and X3 respectively was selected. The above formulation was selected based on our desirability criteria and also, we wanted a formulation with good entrapment at lowest concentration of polymer.

**Scanning Electron Microscopy and TEM**

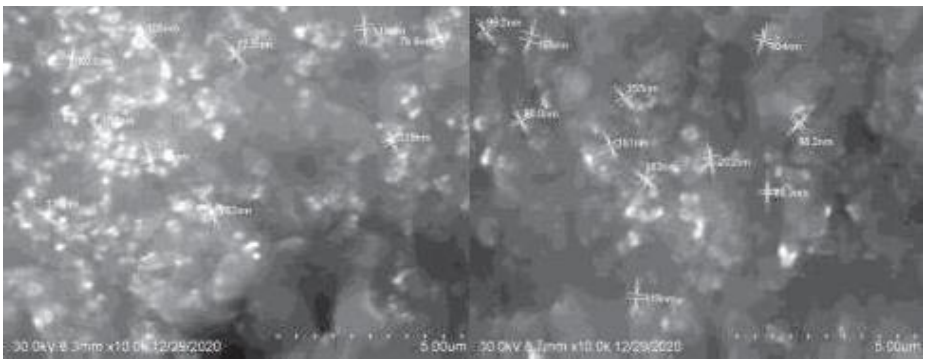

**Fig.3: SEM Images of Canagliflozin loaded PNP (CN-PNP)**

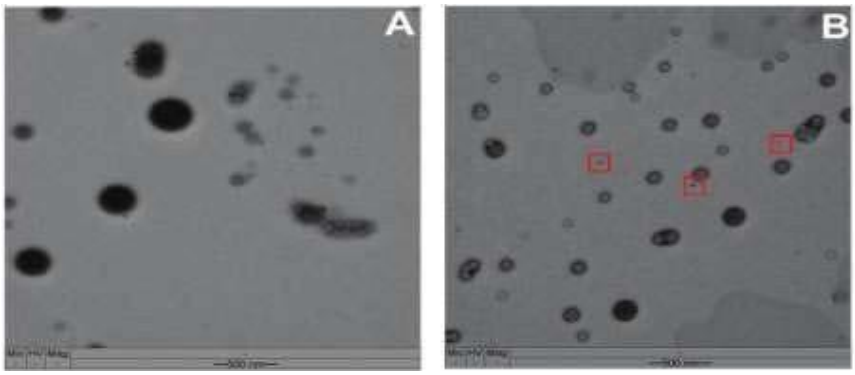


**Fig. 4: TEM Images of Canagliflozin loaded PNP (CN-PNP)**

The SEM images of the optimal OPT-CN-PNPs are shown as Figure 5.60. As depicted in the images, the particles appeared to be of uniform shape. The size of most of the particles was found to be less than 200 nm.
TEM images showed (fig 5.61) that the Canagliflozin loaded PNP, in despite of the different lipid matrices, had spherical morphology with a well-delimited surface (Fig. 2). In addition, encapsulation of drug loading did not affect the integrity of the nanoparticles.

**Stability Study of Optimized Formula OPT-CN-PNP**

Stability of optimized OPT-CN-PNP performed at both Refrigeration temperature (**5 ± 2°C**) and 25JH/60± 5%RH for six months) and result was depicted in Table 5.50. The formulation did not show any caking and phase separation during whole six-month stability study. No significant ($p<0.05$) variation was found in PS, EE% and zeta potential.

The formulation stored at long term stability, i.e., 25 ± 2°C/60 ± 5% RH, however, exhibited increased particle size from along with a significant decrease in drug entrapment efficiency. There was no remarkable ($p< 0.05$) variation was found in responses Particle size distribution, indicated that uniform distribution particle. The finding concluded that OPT-CN-PNP formulation was agreeable stable over an entire period of stability.

## CONCLUSION:

➢ Canagliflozin loaded PLGA nanoparticles (NPs) were prepared using solvent diffusion (Nano precipitation) method (Fessi et al; 1989). The optimized formulation was prepared by dissolving PLGA (25 mg) and drug (10 mg) in 2.5 ml of acetone. The organic phase was added at the rate of 0.5ml/min into 5 ml of aqueous phase containing 0.25%w/v Pluronic F68 with continuous stirring on magnetic stirrer at room temperature.

➢For PS, at fix level (-1) of X1, at all levels of X2 and X3, particle size of less than 160 nm could be achieved. At fix level of X2, plot between X1and X3explains that X1has major influence on particle size, but particle size of 140 nm could be achieved at less than 0.5 level of X1, at all levels of X3. At -1 level of X3, lower particle size could be achieved atlower levels of both the factors, as both factors causes major influence on increase in particle size.

 The optimum formulation OPT-CN-PNP with EE 92.16±2.17% and particle size 132.23±7.26nm found at 1.0, -1, and -1 levels of X1, X2 and X3 respectively was selected. The above formulation was selected based on our desirability criteria and also we wanted a formulation with good entrapment at lowest concentration of polymer. The formulation stored at long term stability, i.e., 25 ± 2°C/60 ± 5% RH, however, exhibited increased particle size from along with a significant decrease in drug entrapment efficiency. There was no remarkable ($p<0.05$) variation was found in responses Particle size distribution, indicated that uniform distribution particle. The finding concluded that **OPT-CN-PNP** formulation was agreeable stable over an entire period of stability.

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**Conflict of Interest**

The authors declare no conflict of interest, financial or otherwise.

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