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**Original Research Article**

# Formulation, characterization and optimization of nebivolol-loaded sustained release lipospheres

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## Abstract

***Purpose:*** *To formulate, characterize and optimize nebivolol-loaded sustained release lipospheres (LPs) using beeswax (BW) as the drug carrier.*

***Methods:*** *Nebivolol-loaded LPs were formulated using solvent evaporation technique (SET) and characterized. The impact of independent variables on responses such as percentage yield (PY), entrapment efficiency (EE) and drug release after 12 h (DR12) was assessed using central composite design (CCD). Numerical and graphical optimization techniques were also used to evaluate outcomes of the measured responses.*

***Results:*** *Twenty micron-sized (20 - 100 µm), smooth spherical LPs with good rheological properties were produced. The yield ranged from 33 (F10) to 81 % (F6), while EE ranged from 32 (F4 and F9) to 69 % (F6). The results of rheological evaluation revealed angle of repose > 24 o, Hausner's ratio > 1.5, and Carr's index ranging from 13 to 19 %. Fourier-transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC) and x-ray diffraction (XRD) revealed nebivolol and BW compatibility, and the absence of possible interactions between formulation components. Duration of nebivolol release was strongly associated with BW concentration and formulation F15 showed minimum drug release (46 %). Drug release was significantly higher in formulations with similar BW concentrations and low Tween-20 (T-20) concentrations (F1 and F11) than in formulations with high T-20 concentrations (F2, $p < 0.05$). The zeta potential of deflocculated LPs ranged from +15 to +35 mV. Nebivolol release (46 - 85 %) at pH 6.8 was significantly affected by BW concentration and it followed zero order model.*

***Conclusion:*** *The results obtained in this study have shown that BW is a suitable material for producing an effective sustained release formulation. The mechanism of drug release in nebivolol- loaded LPs is diffusion accompanied by erosion.*

***Keywords:*** *Lipospheres, Nebivolol, Beeswax, Formulation, Central composite design*



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## INTRODUCTION

Hypertension, congestive heart failure (CHF), myocardial infarction (MI), cerebral diseases and nephropathy result in the death of millions of people globally [1]. At present, the treatment strategies for cardiovascular diseases (CVDs) only serve as mere palliatives [2]. There is increasing demand for sustained delivery

systems that can promote heart function [3,4]. For this purpose, novel formulations such as LSs provide great advantages. Liposphere are sustained release formulations employed in disease conditions requiring plasma drug concentration that can be sustained for a prolonged period, and they are used for convenient delivery of semi-synthetic, synthetic and biological agents [5]. They can hold both hydrophobic and hydrophilic drugs, and they are physically stable and economically viable. In addition, LSs reduce dose frequency and side effects, and enhance patient compliance [6]. They are formulated from solid lipids characterized by low melting points (65 $^{o}$C), good biocompatibility and biodegradability, absence of toxicity and ease of production [7]. As a drug carrier, BW can sustain drug release and enhance its bioavailability [7,8].

Nebivolol, a drug used as first-line treatment for hypertension, is a selective beta-blocker which induces vasodilatation via the generation of nitric oxide (NO) [4]. Although nebivolol is highly effective and more generally accepted than other antihypertensive agents, it requires frequent dosing because of its poor solubility, bioavailability (< 40 %) and short plasma half-life (2 h) [9, 10].Therefore, BW is usually used to improve its solubility and bioavailability. The aim of this study was to formulate, characterize and optimize nebivolol-loaded sustained release LS using BW as drug carrier.

## EXPERIMENTAL

### Materials

Nebivolol was a product of Nabi-Qasim Pharmaceuticals (Pvt) Ltd. (Pakistan). Potassium dihydrogen phosphate ($KH_2PO_4$) and Tween-20 (T-20) were purchased from Merck (Germany). Infra-red grade cellulose dialysis tube and BW were obtained from Sigma Aldrich (USA), while potassium bromide (KBr) was a product of Fischer Scientific (UK). Equipment used included X-ray diffractometer (Bruker AXS, USA) and Zetasizer (Malvern Instruments Ltd., UK).

### Preparation of LS

Nebivolol-loaded LS were prepared using SET. Nebivolol and BW were dissolved in 50 ml chloroform and the resultant solution was added to T-20 preheated at 75 °C and then homogenized to obtain a pre-emulsion [12]. The pre-emulsion was mixed with cold water and stirred using a magnetic stirrer. The LS formed were recrystallized at room temperature and filtered using 0.45 µm filter paper, and dried using a desiccator. The procedure was performed in triplicates with the aqueous phase and nebivolol concentration kept constant.

### Central composite design

The CCD was performed using Design Expert (8.0.6.1) [11]. The different LS formulations were designed with concentrations of BW and T-20, and stirring speed (SS) as independent variables, while PY ($Y_1$), EE ($Y_2$) and $DR_{12}$ ($Y_3$) were the dependent variables/responses. The compatibility of nebivolol and BW was evaluated using FTIR spectroscopy, DSC and XRD. The particle sizes, rheologies, morphologies and zeta potential of the formulated LS were also determined. Release profiles of the LS were evaluated using kinetic models such as zero order, first order, Higuchi and Korsmeyer Peppas. Numerical and graphical optimization techniques were used to create conditions for producing optimum intensity of the measured responses.

### Rheological studies

Rheological evaluation was carried out on the formulated LS based on the method described by Reithmeier *et al* [13] and Carr's index, Hausner's ratio, and angle of repose were determined (Table 1).

**Table 1:** Rheological parameters of formulated LS

| Flow parameter | Equation | Range |
|---|---|---|
| Carr's index (%) | $I = [V_b - V_t/V_t] \times 10$ | 12 - 19 |
| Hausner's ratio | $HR = \rho t/\rho b$ | < 1.25 |
| Angle of repose ($^{o}$) | $Tan\theta = h/r$ | < 30 |

### Determination of PY

The final constant weight (W) of dried LS was divided by the total weight (TW) of all solid lipids used in LS formulation to obtain the yield (Y) of liposheres as in Eq 1 [14].

Y = W/TW …… (1)

### Evaluation of EE

Portions of LS were crushed and dispersed in phosphate buffer (pH 7.4) for 24 h. After dilution, absorbance was read at 282 nm using UV-visible spectrophotometer, and the EE was calculated as in Eq 2 [10,14].

EE = ADA/TDA × 100 …… (2)

where ADA is the actual concentration of nebivolol, and TDA is the theoretical concentration of nebivolol.

### Evaluation of drug release *in vitro*

This lasted 12 h and was performed using Drug Dissolution Apparatus II USP (Paddle) immersed in phosphate buffer, pH 6.8, and maintained at 37 ± 0.5 °C. The LS was put in a dialysis tube containing 5 ml of dissolution medium, and immersed in dissolution vessel containing 900 ml of dissolution medium. After a specified time interval, an aliquot (5 ml) was withdrawn and equal volume of freshly prepared medium was added to the vessel. After further dilution of the sample, absorbance was read at 282 nm [15].

### Drug release kinetics

The drug release data were analyzed using kinetic models such as zero order, first order, Korsmeyer Peppas, Hixson-Crowell and Higuchi's. The mathematical equations used are shown in Eqs 3 – 7.

$$F_t = K_0 t \quad \ldots\ldots\ldots\ldots (3)$$

$$\text{Log } F = \log F_0 - {}^{K_t}/_{2.303} \quad \ldots\ldots\ldots\ldots (4)$$

$$F = K_H t^{1/2} \quad \ldots\ldots\ldots\ldots (5)$$

$$F_0^{1/3} - F_t^{1/3} = K_{HC} \times t \quad \ldots\ldots\ldots\ldots (6)$$

$$M_t/M_\infty = K_s t^n \quad \ldots\ldots\ldots\ldots (7)$$

### Analysis of variance (ANOVA) of two-factor interaction (2FI) model

2FI model was analyzed using ANOVA at 5 % significance level. Value of $p < 0.05$ was taken as an indication that the model was significant for $Y_1$ and $Y_2$.

### FTIR spectroscopy

The FTIR spectra of nebivolol, BW and nebivolol-loaded optimized formulation (OF) were recorded and analyzed using FTIR spectrophotometer. Before analysis, the mixture of LS and KBr was pelleted. Resolution of 2 $cm^1$, hydraulic pressure of 150 $kg/cm^2$ and scanning range between 400 and 4000 $cm^{-1}$ were used [15].

### Differential scanning calorimetry

Appropriate amounts of nebivolol, BW and OF (2 mg each) were separately heated at the rate of 10 ºC/min from 0 to 220 ºC in a sealed aluminium pan under nitrogen flow rate of 20 ml/min, and analyzed using a thermal analyzer [15].

### X-ray diffraction

The samples were irradiated with monochromatized X-rays of Cu-Kα using D-8 advance X-ray diffractometer at a current of 40 mA, with scanning capacity of 2 º $min^{-1}$ (diffraction angel-2θ) from 0 - 45º [15].

### Scanning electron microscopy (SEM)

Optimized LS were positioned on a double adhesive tape on aluminum stub. Gold coating of stubs was performed under argon atmosphere, and photomicrographs of LS were obtained using scanning electron microscope (x500) at 10 kV [15].

### Zeta potential and particle size measurements

The charges on surfaces of optimized LS were measured by evaluating their electrophoretic mobilities in a U-shaped tube at 25 °C using Malvern Zetasizer [15].

### Statistical analysis

Measurement data are expressed as mean ± SD. The statistical analysis and CCD were performed using Design Expert (8.0.6.1) [16]. The optimized formulation was selected on the basis of desirability and numerical optimization functions of Design Expert. Regression analysis was also performed on the measured responses to determine the adequacy and suitability of proposed models. Where appropriate, values of $p < 0.05$ were considered statistically significant.

## RESULTS

### Formulation components and measured responses

The PY ranged from 33 (F10) to 81 % (F6). Lipospheres with high BW concentrations and low T-20 concentrations formulated at low SS (F4, F9, F10, F11, F13 and F20) had PY < 50 %. Formulations containing the same concentrations of T-20 and BW (F4 and F10) had high PY (44 %), but the PY of F4 was significantly higher than

that of F10 (33 %) ($p < 0.05$). The EE ranged from 32 (F4 and F9) to 69 % (F6).

Although F5 and F6 had the same BW concentrations and were formulated at the same SS, both formulations had different EE (39 and 69 %, respectively). Formulations such as F9, F10 and F11 which had low concentrations of BW and T-20 had minimum EE (Table 2).

**Rheological properties**

As shown in Table 3, the rheological properties of ls showed Carr's index ranging from 13 - 19 %, angle of repose > 24$^o$ and Hausner's ratio > 1.5.

**Optimization of data and model validation**

The statistical model selected 2FI for PY and EE, and the suggested relations are shown in Eqs 8 and 9 for $Y_1$ and $Y_2$, respectively.

$Y_1 = +37.23 - 22.55X_1 - 7.610X_2 + 0.014X_2 + 30.00X_1X_2 - 4.33X_1X_2 - 4.44X_2X_2$ …… (8)

**Table 2:** Formulation components and measured responses

| Formulation | Formulation component (Coded level) | Formulation component (Actual level, % w/v) | | | Response (%) | | |
|---|---|---|---|---|---|---|---|
| | | BW | T-20 | SS | $Y_1$ (PY) | $Y_2$ (EE) | $Y_3$ ($DR_{12}$) |
| F1 | Factorial (-1, -1, +1) | 0.75 | 0.50 | 3000 | 58.00 ± 2.71 | 44.00 ± 1.33 | 72.00 ± 3.13 |
| F2 | Factorial (-1, +1, +1) | 0.75 | 1.25 | 3000 | 67.00 ± 2.89 | 52.00 ± 1.76 | 56.00 ± 3.36 |
| F3 | Center (0, 0, 0) | 1.25 | 0.88 | 2250 | 51.00 ± 2.54 | 39.00 ± 1.82 | 61.00 ± 3.53 |
| F4 | Factorial (+1, -1,+1) | 1.75 | 0.50 | 3000 | 44.00 ± 2.98 | 33.00 ± 2.33 | 72.00 ± 3.19 |
| F5 | Center (0, 0, 0) | 1.25 | 0.88 | 2250 | 50.00 ± 2.74 | 39.00 ± 2.03 | 59.00 ± 3.87 |
| F6 | Axial (0, + ß, 0) | 1.25 | 1.51 | 2250 | 81.00 ± 2.88 | 69.00 ± 1.89 | 50.00 ± 3.45 |
| F7 | Center (0, 0 , 0) | 1.25 | 0.88 | 2250 | 50.00 ± 3.15 | 39.00 ± 2.24 | 60.00 ± 3.98 |
| F8 | Center (0, 0, 0) | 1.25 | 0.88 | 2250 | 50.00 ± 2.97 | 39.00 ± 2.45 | 60.00 ± 4.23 |
| F9 | Axial (0, -ß, 0) | 1.25 | 0.24 | 2250 | 40.00 ± 2.47 | 33.00 ± 2.86 | 85.00 ± 3.67 |
| F10 | Factorial (+1, -1, -1) | 1.75 | 0.50 | 1500 | 33.00 ± 2.82 | 31.00 ± 1.91 | 76.00 ± 2.73 |
| F11 | Factorial (-1, -1, -1) | 0.75 | 0.50 | 1500 | 42.00 ± 2.56 | 33.00 ± 2.34 | 80.00 ± 2.51 |
| F12 | Factorial (-1, +1, -1) | 0.75 | 1.25 | 1500 | 50.00 ± 3.11 | 42.00 ± 1.95 | 70.00 ± 3.71 |
| F13 | Axial (+ß, 0, 0) | 2.09 | 0.88 | 2250 | 44.00 ± 3.31 | 35.00 ± 2.37 | 49.00 ± 4.23 |
| F14 | Axial (-ß, 0, 0) | 0.41 | 0.88 | 2250 | 68.00 ± 2.31 | 55.00 ± 2.18 | 52.00 ± 3.22 |
| F15 | Factorial (+1, +1, +1) | 1.75 | 1.25 | 3000 | 74.00 ± 2.67 | 61.00 ± 2.07 | 46.00 ± 3.11 |
| F16 | Axial (0, 0, +ß) | 1.25 | 0.88 | 3511 | 64.00 ± 2.78 | 55.00 ± 2.24 | 55.00 ± 3.77 |
| F17 | Factorial (+1, +1, -1) | 1.75 | 1.25 | 1500 | 65.00 ± 2.42 | 54.00 ± 1.66 | 56.00 ± 3.34 |
| F18 | Center (0, 0, 0) | 1.25 | 0.88 | 2250 | 50.00 ± 3.81 | 39.00 ± 1.51 | 60.00 ± 4.03 |
| F19 | Center (0, 0, 0) | 1.25 | 0.88 | 2250 | 50.00 ± 3.63 | 39.00 ± 2.23 | 60.00 ± 3.44 |
| F20 | Axial (0, 0, -ß) | 1.25 | 0.88 | 988.6 | 45.00 ± 2.57 | 37.00 ± 2.43 | 76.00 ± 3.78 |

**Table 3:** Rheological properties of LS

| Formulation | Angle of repose ($^o$) | Hausner's ratio | Carr's index (%) |
|---|---|---|---|
| F1 | 18.00 ± 1.25 | 1.10 ± 1.88 | 16.00 ± 1.37 |
| F2 | 16.00 ± 1.66 | 1.02 ± 1.66 | 13.00 ± 1.86 |
| F3 | 20.00 ± 1.82 | 1.13 ± 0.89 | 18.00 ± 0.77 |
| F4 | 19.00 ± 0.96 | 1.11 ± 1.77 | 16.00 ± 0.97 |
| F5 | 19.00 ± 1.23 | 1.14 ± 0.98 | 19.00 ± 1.09 |
| F6 | 17.00 ± 1.89 | 1.03 ± 0.96 | 14.00 ± 1.67 |
| F7 | 18.00 ± 1.24 | 1.10 ± 1.65 | 15.00 ± 0.96 |
| F8 | 20.00 ± 2.26 | 1.12 ± 1.83 | 17.00 ± 0.94 |
| F9 | 16.00 ± 1.09 | 1.03 ± 0.89 | 13.00 ± 1.76 |
| F10 | 23.00 ± 1.04 | 1.20 ± 1.61 | 19.00 ± 1.70 |
| F11 | 20.00 ± 1.98 | 1.21 ± 1.67 | 17.00 ± 1.83 |
| F12 | 16.00 ± 0.95 | 1.01 ± 2.33 | 13.00 ± 1.86 |
| F13 | 20.00 ± 1.79 | 1.12 ± 2.66 | 18.00 ± 1.75 |
| F14 | 16.00 ± 1.56 | 1.09 ± 1.92 | 14.00 ± 0.91 |
| F15 | 17.00 ± 1.85 | 1.08 ± 1.93 | 15.00 ± 0.88 |
| F16 | 22.00 ± 1.78 | 1.11 ± 2.07 | 19.00 ± 0.93 |
| F17 | 20.00 ± 1.46 | 1.12 ± 2.03 | 18.00 ± 1.88 |
| F18 | 18.00 ± 1.95 | 1.10 ± 1.35 | 15.00 ± 0.92 |
| F19 | 16.00 ± 1.34 | 1.07 ± 1.77 | 13.00 ± 1.72 |
| F20 | 20.00 ± 1.89 | 1.12 ± 0.97 | 18.00 ± 1.37 |

$Y_2 = +9.808 + 10.75X_1 - 26.39X_2 + 1.73X_2 + 26.00X_1X_2 - 0.013X_1X_2 - 5.77X_2X_2$ ..... (9)

Drug release ($Y_3$) followed the quadratic model and suggested Eq 10 for $Y_3$.

$Y_3 = +118.08 - 24.347X_1 - 42.62X_2 + 0.02X_3 - 13.75X_1X_2 + 2.65X_1X_3 - 5.30X_2X_3 - 9.41X\frac{2}{1} + 26.07X\frac{2}{2} + 5.27X\frac{2}{3}$ ..... (10)

The maximum to minimum ratio for measured responses ($Y_1$ = 2.45, $Y_2$ = 2.07 and $Y_3$ = 1.85) was < 3.

## Regression analysis for the measured responses

Predicted $R^2$ for the measured responses were very close to their adjusted $R^2$, and signal to noise ratio measured from adequate precision was > 4 (Table 4).

**Table 4:** Outcomes of regression analysis for the measured responses

| Parameter | $Y_1$ | $Y_2$ | $Y_3$ |
|---|---|---|---|
| R-Squared | 0.8756 | 0.8488 | 0.9509 |
| Adjusted R-Squared | 0.8182 | 0.7790 | 0.9067 |
| Predicted R-Squared | 0.6285 | 0.8715 | 0.7899 |
| Adequate precision | 13.637 | 11.534 | 17.803 |
| Mean | 53.85±5.26 | 57.25±5.74 | 62.75±3.37 |
| CV (%) | 9.77 | 10.04 | 5.37 |
| PRESS | 1073.81 | 1721.35 | 847.04 |

## Three-dimensional (3D) surface graphs

The 3D graphs showing interaction effect of two factors, while keeping the third factor constant are shown in Figure 1.

## *In vitro* drug release profiles of the formulations

Nebivolol release time was strongly associated with BW concentration and F15 showed minimum drug release (46 %). Drug release was significantly higher in formulations with similar BW concentration and low T-20 concentration (F1 and F11) than in formulations with high T-20 concentration (F2) ($p < 0.05$; Figure 2).

## Outcomes of ANOVA of the measured responses

The results of ANOVA showed that the applied models were significant ($p < 0.0001$), and values of $f$ for $Y_1$, $Y_2$ and $Y_3$ were 15.25, 12.16 and 21.25, respectively. The results also showed $prob > f$ (Table 5).

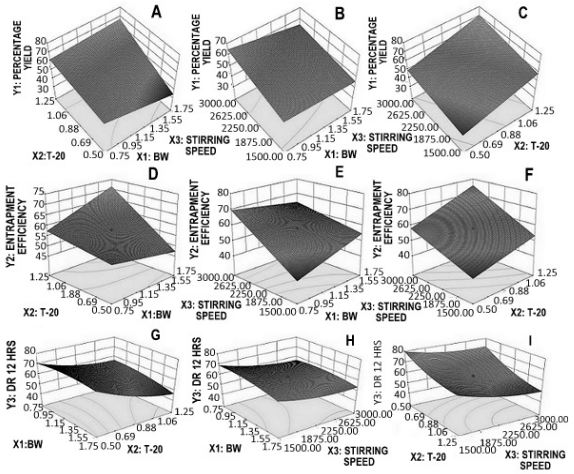


**Figure 1:** Three-dimensional (3D) response surface plots. **A:** effect of $X_1$ and $X_2$ on $Y_1$**; B:** effect of $X_1$ and $X_3$ on $Y_1$; **C:** effect of $X_2$ and $X_3$ on $Y_1$**; D:** effect of $X_1$ and $X_2$ on $Y_2$**; E:** effect of $X_1$ and $X_3$ on $Y_2$**; F:** effect of $X_2$ and $X_3$ on $Y_2$**; G:** effect of $X_1$ and $X_2$ on $Y_3$; **H:** effect of $X_1$ and $X_3$ on $Y_3$; **I:** effect of $X_2$ and $X_3$ on $Y_3$

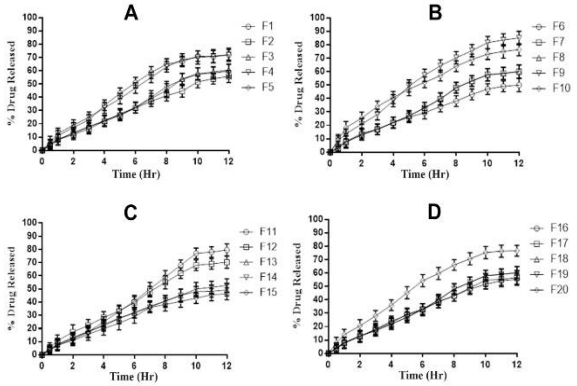


**Figure 2:** *In vitro* drug release profiles of A (F1 - F5), B (F6 - F10), C (F11 - F15) and D (F16 - F20)

## Optimized formulations

The OF suggested by optimization techniques was prepared and characterized. The desirability of measured responses was close to 1, and prediction error was at lower level (Table 6). The drug release profiles dominantly followed zero order model since obtained $R^2$ for zero order was greater when compared with $R^2$ for the other models (Table 7).

## FTIR spectra and thermal characteristics

The spectra of nebivolol and BW were compared with FTIR-spectrum of OF. Characteristic aliphatic N - H, alkanes C = C and C - H stretches were observed at 3185 $cm^{-1}$, 2319 $cm^{-}$

[1] and 1490 $cm^{-1}$, respectively in nebivolol spectrum and nebivolol-loaded OF. Carbonyl and sulphur-oxy groups were also visible at 1536 and 1074 $cm^{-1}$, respectively in the FTIR-spectrum of OF (Figure 3). The specific endothermic peaks relevant to melting point of BW and nebivolol were quite visible at 65 °C (Figure 3 B) and at 221 °C (Figure 3 C), respectively. The peak associated with melting of nebivolol in OF at 221 °C was also revealed (Figure 3 A).

## X-ray diffraction properties

The x-ray diffractograms revealed the presence of characteristic peaks of nebivolol at 2θ of 25º, 30º and 40º without any impact on diffraction positions (Figure 4B). The LS of optimized formulations were spherical in shape (Figure 5).

**Table 5:** Analysis of variance showing the effect of factors on responses

| Source | PY ($Y_1$) | | EE ($Y_2$) | | $DR_{12}$ ($Y_3$) | |
|---|---|---|---|---|---|---|
| | *f* | *p prob > f* | *f* | *p prob > f* | *f* | *p prob > f* |
| Model | 15.25 | < 0.0001 | 12.16 | < 0.0001 | 21.25 | < 0.0001 |
| BW | 4.53 | 0.0013 | 1.87 | 0.01946 | 7.06 | 0.0021 |
| T-20 | 57.95 | < 0.0001 | 21.25 | 0.0005 | 110.38 | < 0.0001 |
| SS | 19.11 | 0.0008 | 37.70 | < 0.0001 | 32.78 | 0.0002 |
| $X_1X_2$ | 9.15 | 0.0098 | 5.76 | 0.0021 | 4.40 | 0.0032 |
| $X_1X_3$ | 0.76 | 0.3980 | 5.76 | 0.0321 | 0.70 | 0.0042 |
| $X_2X_3$ | 4.519 | 0.9474 | 0.64 | 0.0438 | 1.58 | 0.0066 |
| $X_1^2$ | | | | | 7.03 | 0.0243 |
| $X_2^2$ | | | | | 16.96 | 0.0021 |
| $X_3^2$ | | | | | 11.04 | 0.0077 |
| LOF | 167.92 | 0.0006 | 79.89 | 0.0008 | 55.80 | 0.0002 |

**Table 6:** Composition of OF

| Independent variable | Optimum level | Dependent variable/response | Predicted value | Observed value | Prediction error | Desirability level |
|---|---|---|---|---|---|---|
| $X_1$ | 1.75 | Y1 | 71.75 | 75.8 | 1.54 | |
| $X_2$ | 1.25 | Y2 | 77.71 | 78.8 | 1.8 | 0.891 |
| $X_3$ | 3000 | Y3 | 43.83 | 46.7 | 1.5 | |

**Table 7:** Release kinetics of LS formulations

| Formulation | Zero order | | First order | | Higuchi | | Hixson-Crowell | | Korsmeyer-Peppas | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $R^2$ | $K_0$ | $R^2$ | $K_1$ | $R^2$ | $K_H$ | $R^2$ | $K_{HC}$ | $R^2$ | $N$ |
| F1 | 0.9949 | 7.265 | 0.8566 | 0.345 | 0.8731 | 12.716 | 0.8798 | 0.031 | 0.9617 | 0.989 |
| F2 | 0.9897 | 6.567 | 0.8966 | 0.189 | 0.9176 | 11.188 | 0.8583 | 0.022 | 0.9578 | 1.021 |
| F3 | 0.9877 | 7.355 | 0.8898 | 0.278 | 0.9027 | 17.341 | 0.8865 | 0.043 | 0.9876 | 1.022 |
| F4 | 0.9897 | 8.345 | 0.9045 | 0.176 | 0.8635 | 15.156 | 0.8758 | 0.021 | 0.9856 | 0.981 |
| F5 | 0.9912 | 9.243 | 0.9256 | 0.177 | 0.8324 | 14.765 | 0.7898 | 0.027 | 0.9798 | 0.890 |
| F6 | 0.9933 | 8.765 | 0.8763 | 0.156 | 0.8817 | 16.254 | 0.8495 | 0.031 | 0.9848 | 0.904 |
| F7 | 0.9907 | 8.135 | 0.9374 | 0.099 | 0.8557 | 14.546 | 0.8145 | 0.023 | 0.9598 | 0.887 |
| F8 | 0.9934 | 7.354 | 0.9532 | 0.343 | 0.8917 | 18.423 | 0.8732 | 0.021 | 0.9298 | 0.917 |
| F9 | 0.9812 | 7.134 | 0.8684 | 0.423 | 0.9198 | 16.675 | 0.8912 | 0.032 | 0.9842 | 0.899 |
| F10 | 0.9943 | 7.287 | 0.9067 | 0.257 | 0.9234 | 18.345 | 0.8548 | 0.024 | 0.9897 | 1.012 |
| F11 | 0.9941 | 6.287 | 0.8994 | 0.232 | 0.9334 | 17.214 | 0.8267 | 0.018 | 0.9768 | 0.932 |
| F12 | 0.9777 | 7.798 | 0.8190 | 0.231 | 0.8767 | 12.657 | 0.8187 | 0.029 | 0.9165 | 0.904 |
| F13 | 0.9944 | 7.564 | 0.8588 | 0.199 | 0.8987 | 11.089 | 0.8756 | 0.024 | 0.9698 | 1.065 |
| F14 | 0.9767 | 8.213 | 0.8744 | 0.324 | 0.8766 | 13.467 | 0.8498 | 0.034 | 0.9786 | 0.896 |
| F15 | 0.9987 | 8.378 | 0.9143 | 0.213 | 0.9033 | 17.367 | 0.8576 | 0.025 | 0.9897 | 0.916 |
| F16 | 0.9879 | 7.678 | 0.8878 | 0.254 | 0.8788 | 15.247 | 0.8687 | 0.032 | 0.9896 | 0.919 |
| F17 | 0.9989 | 8.187 | 0.8033 | 0.222 | 0.8876 | 14.876 | 0.8997 | 0.037 | 0.9765 | 0.893 |
| F18 | 0.9898 | 7.665 | 0.9189 | 0.167 | 0.8345 | 13.258 | 0.8898 | 0.019 | 0.9856 | 0.993 |
| F19 | 0.9859 | 8.987 | 0.8978 | 0.213 | 0.8934 | 20.456 | 0.8667 | 0.034 | 0.9156 | 0.985 |
| F20 | 0.9976 | 7.567 | 0.8788 | 0.213 | 0.8853 | 13.348 | 0.8745 | 0.028 | 0.9387 | 0.988 |

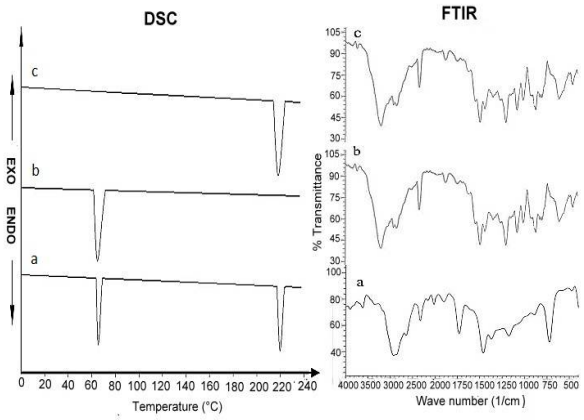


**Figure 3:** Thermograms and FTIR spectra. **A:** thermogram and FTIR spectrum of BW; B: thermogram and FTIR spectrum of nebivolol; C: thermogram and FTIR spectrum of nebivolol-loaded optimized LS

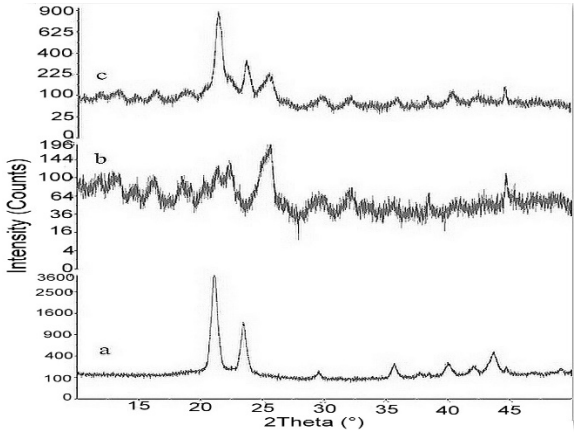


**Figure 4:** X-ray diffraction patterns of (a) BW, (b) nebivolol, and (c) nebivolol-loaded optimized LS

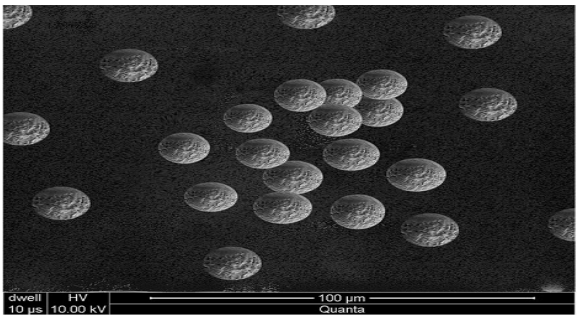


**Figure 5:** Scanning electron micrograph of OF

### *Particle size and zeta potential*

The size distribution of the LS ranged from 20 to 100 µm (Figures 6A), while the major fraction (55 %) of LS had a mean size of 50 µm. The zeta potential of OF was in the range of +15 to +35 mV (Figure 6B).

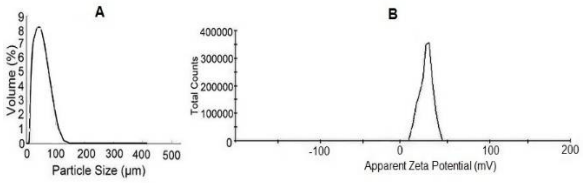


**Figure 6:** Particle size distribution and zeta potential curves. A**:** particle size distribution of OF; B**:** zeta potential curve of OF

## DISCUSSION

Hydrophilic and lipophilic drugs can be successfully delivered into deep and peripheral tissues by encapsulating them with crystalline lipids as LS. Lipospheres offer more advantages than the single-unit systems with respect to their uniform distribution in the gastrointestinal tract resulting in uniform absorption of the encapsulated drug [5]. The present study examined the formulation and characterization of nebivolol-loaded LS using BW as drug carrier. In this study, twenty micron-sized LS were produced. The results of DSC analysis and FTIR spectroscopy did not reveal absence or shift of any principal peaks of nebivolol and BW either in the spectrum of OF or individual spectra, and thermograms of nebivolol with BW. These results suggest compatibility of nebivolol and BW, and are in agreement with those previously reported [17].

The pattern of X-ray diffraction of OF revealed sharp and scattered peaks of nebivolol, an indication that nebivolol may have remained in crystalline form and that the process of formulation did not produce any negative effects on it [18]. The ratio of BW to surfactant strongly influenced rheology, morphology, PY, EE and size distribution of formulated LS. High concentrations of T-20 and SS contributed significantly to the production of free-flowing, smooth, spherical and micron-sized LSs [19].

These results are in agreement with those previously reported for lipid-based microparticles of somatostatin and oxybenzone [8,13]. The zeta potential of OF appears to suggest good stability since positive charge would naturally generate electrostatic repulsion between LSs, thereby preventing their aggregation [19]. It appears that increased concentrations of BW and T-20 are favorable conditions for producing high PY and EE, and that attainment of both requires concomitant increase in SS [17]. Low PY and EE may be associated with increased aggregation of lipids at low concentration of T-20, while high concentration of T-20 may prevent drug loss in

external phase and stabilization of lipid microparticles [17].

In this study, increased concentrations of BW, T-20, and SS resulted in increases in PY and EE (for $Y_1$ and $Y_2$). This suggests that BW, T-20 and SS may have positive impacts on PY and EE. For $Y_1$, the terms $X_1$, $X_2$, $X_3$, and $X_1X_2$ were significant, an indication that BW, T-20, and SS may significantly affect PY [11]. The interaction of BW with T-20 ($X_1X_2$,) and T-20 with SS ($X_2X_3$) were synergistic with respect to $Y_1$ and $Y_2$ [16]. High $X_1$ negatively affected $Y_3$, an indication that an increase in BW concentration may retard drug release [18]. In addition, BW interaction with T-20 ($X_1X_2$) produced sustained release nebivolol-loaded LS. However, the role of SS was critical. These results are in agreement with those previously reported [19].The drug release followed a zero order model. It is possible that the underlying mechanism of drug release involves diffusion accompanied by erosion [3,19]. The selection of OF was made on attainment of maximum PY, maximum EE and minimum $DR_{12}$ [20].

## CONCLUSION

The results obtained in this study have shown that BW is a suitable material for producing a good sustained release formulation of nebivolol. The mechanism of drug release in nebivolol-loaded LS involves diffusion and erosion.

## DECLARATIONS

### *Acknowledgement*

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### *Conflict of Interest*

No conflict of interest associated with this work.

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