INTRODUCTION

In the body, many physiological functions are regulated by transient release of bioactive principles at a specific time. Hence, to mimic the functions of living organisms, advances in research are aiming toward synchronizing the drug delivery in a manner consistent with the body's circadian rhythms to bring both commercial and therapeutic value to health care. The last decade has witnessed the emergence of chronotherapeutic drug delivery system for several diseases. Large data-based analysis and epidemiological studies have demonstrated that many cardiovascular events such as myocardial infarction, stroke, and sudden cardiac death cluster during early morning at around 6 am and then at 12 noon. It has been attributed to the fact that there are marked circadian rhythms in blood pressure (BP) that accounts for a sharp rise in BP during early morning and then in the afternoon; after that, BP declines during night time falling 15–20 mm Hg between 8:00 pm and 2:00 am. This rise in BP is mainly ascribed to enhanced plasma-renin activity [1-3]. Thus, one of the newest advances in antihypertensive therapy is the design of drugs according to the chronotherapeutic system that would deliver the drug in highest concentrations at the time of maximum need (in early morning hours) after an initial lag phase characterized by a period of no drug release.

Thus, the aim of the present investigation is to design a delayed release multiparticulate chronotherapeutic capsule drug delivery system containing losartan potassium microspheres. Losartan potassium is an angiotensin receptor blocker that works by antagonizing the rennin-angiotensin-aldosterone system; later being one of the contributing factors for the marked rise in early morning BP. Hence, an attempt has been made to develop a newer dosage form of losartan potassium to be dosed at bedtime and release the drug after a lag time of 2 h. Moreover, losartan potassium tends to remain lesser solubilized in simulated gastric fluid (SGF) pH 1.2 that accounts for its low absorption while its solubility increases in SIF pH 6.8. The drug would be released in SIF pH 6.8 in a controlled manner; thereby, plasma drug levels would rise during the early morning when BP is at its highest (as administration of dosage form is not feasible so early); thus, preventing the major cardiovascular events [4-7]. Hence, the system could benefit from the known circadian rhythms of the disease to control the BP for once a day therapy.

The microspheres of losartan potassium were prepared using polymethacrylate polymer, Eudragit RS100, by emulsion solvent evaporation method through the application of statistical design approach. Eudragit RS100 is being increasingly employed for the development of oral sustained release microspheres [8,9]. It is a low permeability neutral polymer that is insoluble in water and digestive juices but swells due to the presence of quaternary ammonium groups; thus, releases the drug by diffusion [10]. The optimized microsphere formulation was filled into hard gelatin capsule shells. To synchronize the drug release with the circadian rhythms for hypertension, the shell was coated with Eudragit L100 [11] to obtain the desired lag time based on preliminary trials conducted to optimize the ratio.

MATERIALS AND METHODS

Materials

Losartan potassium was obtained as a gift sample from Theon Pharmaceuticals Pvt., Ltd., Baddi. Eudragit RS 100, L100 was procured from Theon Pharmaceuticals Pvt., Ltd., Baddi. Isopropyl alcohol, Light liquid paraffin, n-hexane, methanol, and dichloromethane were obtained.
from Loba Chemicals Pvt. Ltd. PEG 6000, Talc, Span 80, potassium dihydrogen orthophosphate, sodium hydroxide, and concentrated hydrochloric acid were purchased from S. D. Fine Chemicals.

Methods

Preparation of microspheres: Statistical design approach

Statistical experimental designs are powerful and systematic tools in multiple factor optimizations in fewer experimental trials. Hence, for preparation of microspheres, response surface methodology employing central composite design (CCD) was applied. The microspheres of Losartan potassium were prepared by varying the drug: polymer ratio and codissolving them at room temperature into a mixture methanol-acetone (1:1 v/v) with continuous stirring using Remi's mechanical stirrer (model: RQ - 121/D). The drug: polymer ratio and stirring speed were varied as per the CCD experimental design as shown in Table 1. The resulting dispersion was slowly introduced into liquid paraffin containing span 80 (1% w/w) as an emulsifying agent. Light liquid paraffin was then decanted, and the microspheres were separated by filtration followed by washings with n-hexane to remove paraffin. The resulting microspheres were dried at room temperature for 24 h and evaluated for various in vitro parameters [12-14].

Optimization and validation model

To optimize the formulation variables, factors selected for study were drug: polymer ratio (A) and stirring speed (B) taken at five different levels, namely – alpha, low, center point, high level, and +alpha level. Diverse batches of losartan potassium loaded Eudragit microparticles were prepared as per the CCD, and a total of 13 runs were presented by the Design Expert® software [15-18]. The response or dependent variables studied were mean particle size (Y1), drug entrapment efficiency (Y2), percent yield (Y3), and drug release at 8th h (Y4). The design matrix along with the investigated response variables is shown in Table 1. Experimental findings were analyzed using analysis of variance (ANOVA) by fitting the response figures in the run design.

The response (Yi) in each testing run was calculated by carrying out a regression analysis to develop equations for dependent variables (Y1-Y4).

\[ Y_i = \beta_0 + \beta_A A + \beta_B B + \beta_{AB} AB + \beta_A^2 A^2 + \beta_B^2 B^2 \]  

Eq. (A.1)

Where, Yi is estimated response of dependent variables, \( \beta_0 \) is the intercept of the polynomial equation, \( \beta_A \) and \( \beta_B \) represent the estimated regression coefficient for factor A and B, and \( \beta_{AB} \) are the coefficients corresponding interaction and \( \beta_A^2 \) and \( \beta_B^2 \) represent the quadratic effects.

ANOVA was also used to obtain the F-values, p-values, and multiple correlation coefficients (R²); adjusted and predicted R²; lack of fit and PRESS value for authenticating the suitability of models. Exhaustive matrix seeks over the experimental domain was performed to find out the solutions and then checkpoint formulations were selected to assess the optimization capability of the model generated through CCD [19,20]. The experimentally obtained results were then compared with the predicted responses obtained from the equations of the model and residuals were calculated.

Evaluation of microspheres

Microparticles were evaluated for micromeritic properties by measuring the angle of repose, bulk density, tapped density, Carr’s index, and Hausner ratio using standard reported procedures [21]. The average particle size of microspheres was analyzed by simple optical microscopy method using the ocular lens and stage micrometer. Approximately 300 microspheres were counted and the average equivalent spherical diameter was measured [22-25]. The drug entrapment efficiency was determined by taking accurately weighed microspheres equivalent to 25 mg of losartan potassium and crushed to obtain a fine powder. The powder was dissolved in 100 ml of phosphate buffer pH 6.8 in a conical flask. The solution obtained was filtered, suitably diluted and analyzed spectrophotometrically using ultraviolet-visible (UV-VIS) double beam spectrophotometer at 235 nm. All the readings were taken in triplicate.

Drug content was determined by the following formula:

\[ \text{Yield} = \frac{\text{Actual weight of the microspheres obtained}}{\text{Total weight of the excipients and drug}} \times 100 \]

Eq. (A.3)

Table 1: Central composite design matrix, the observed responses, and actual or coded values

| Runs | Independent variables | Dependent variables |
|------|-----------------------|---------------------|
|      | Drug: polymer Ratio (A), w/w | Mean particle size (Y1), μm | Entrapment efficiency (Y2), % | Percent yield (Y3), % | Drug release at 8th h (Y4), % |
| 1.   | 1                     | 169.2±1.23           | 71.3±1.23                     | 65.49±0.74                  | 76.97±2.62                       |
| 2.   | 0                     | 121.2±2.38           | 79.7±1.09                     | 76.49±1.73                  | 76.55±1.29                       |
| 3.   | −1                    | 102.2±2.03           | 70.3±1.54                     | 75.15±2.47                  | 75.94±0.47                       |
| 4.   | 1                     | 140.6±0.86           | 74.1±1.25                     | 74.59±1.74                  | 74.61±1.20                       |
| 5.   | 0                     | 120.4±1.01           | 78.4±0.89                     | 76.68±1.26                  | 75.35±1.40                       |
| 6.   | −1                    | 119.6±1.03           | 69.3±2.62                     | 77.98±1.74                  | 76.68±0.82                       |
| 7.   | 0                     | 145.6±0.36           | 73.0±0.63                     | 70.26±1.23                  | 64.19±2.95                       |
| 8.   | −1.41421              | 100.96±1.47          | 67.9±1.78                     | 76.85±2.05                  | 77.19±2.40                       |
| 9.   | 1.41421               | 163.7±1.59           | 73.6±1.78                     | 67.95±0.86                  | 73.89±1.23                       |
| 10.  | 0                     | 120.0±1.15           | 79.1±0.83                     | 67.45±0.55                  | 80.64±2.29                       |
| 11.  | 0                     | 119.4±1.33           | 80.7±1.02                     | 69.64±1.32                  | 66.55±3.54                       |
| 12.  | 0                     | 120.5±0.38           | 78.8±1.90                     | 65.29±1.96                  | 80.95±2.78                       |
| 13.  | 0                     | 113.6±0.59           | 75.9±2.44                     | 62.56±1.08                  | 79.25±1.40                       |

| S. No. | Independent variables | Coded and actual levels |
|--------|-----------------------|------------------------|
| 1.     | Drug: polymer ratio (w/w), A | −1 (low) 0 (middle) 1 (high) (+1.41421) alpha level |
| 2.     | Stirring speed (rpm), B   | 1.58 717 1.2 800 1.3 1000 1.4 1200 1.4 1282 |

SD: Standard deviation
On the basis of the optimization and validation model, the optimized formulation was selected for further analysis. Fourier-transform infrared (FTIR) spectra of pure drug and optimized formulation were recorded using attenuated total reflection-FTIR spectrophotometer to investigate any possible interaction between drug-loaded microspheres. The morphology of optimized formulation of microspheres was examined by scanning electron microscope (SEM) analysis using SEM; Jeol JSM-6400, Japan. To analyze the existence condition of LP in the microspheres, digital signature certificate (DSC) of the LP. LP microspheres were carried out.

In vitro drug release study was carried out in simulated physiological pH 6.8 using USP Type I dissolution apparatus at 37±0.5°C to calculate the amount of drug released from microspheres. The assembly was operated at 100 rpm under sink conditions thereby maintaining the constant dissolution volume of 900 ml. The sample was withdrawn at suitable time intervals and replaced with fresh equal volume of dissolution medium. The samples withdrawn were analyzed using double beam UV-VIS spectrophotometer at 235 nm to determine the percent drug released. The dissolution data so obtained were fitted to various drug-release kinetic models such as zero-order, first-order, Higuchi, and Korsmeyer-Peppas model. The release kinetics was assessed by comparing the values of the regression coefficient (r²) [28,29].

Filling of capsule shell with optimized microparticle formulation

After optimization of coating, the uncoated capsule bodies were taken and filled with optimized microsphere formulation equivalent to 25 mg of losartan potassium. The capsule was sealed by fixing the cap of shell over capsule body.

Coating of filled capsule shell

The whole capsule system containing optimized microparticle formulation was optimally coated with plasticized 10% w/v Eudragit RS100 in isopropyl alcohol: water plasticized with 10% PEG 6000 containing talc as a glidant. The coating was done by dip coating method, and the number of coatings was varied from 1 to 4 to attain 8–10% weight gain [11,30-33]. The design scheme for the development of capsule-based chronotherapeutic system has been shown in Fig. 1.

Evaluation of coated capsule shells containing optimized formulation

Ten capsules were selected randomly; thickness of uncoated and coated capsules was measured using digital screw gauge. Coating thickness was determined by measuring the difference between thicknesses before and after coating. The time taken by the coating to dissolve as indicated by the bursting of capsule shell was reported as lag time and evaluated using dissolution test apparatus for first 2 h in SGF pH 1.2 and SIF pH 6.8 for remaining time. A dissolution study was carried out using USP type I apparatus. Coated capsules were immersed in 900 ml of SGF pH 1.2 maintained at 37±0.5°C. Dissolution was carried out for 2 h at 100 rpm and then shifted to pH 6.8 medium by adding concentrated high pH media in the same vessel. This pH shift simulated the transition from stomach to intestines for remaining dissolution testing until 10 h [34]. Aliquots of 5 ml were withdrawn at suitable time intervals from the dissolution assembly and replaced with an equal amount of fresh buffer. The samples taken were analyzed at 235 nm spectrophotometrically and percent drug released was calculated.

RESULTS

Formulation optimization of microparticles of losartan potassium

For the preparation of microcapsules, emulsion solvent evaporation was employed using a mixed solvent system (methanol-acetone) as the dispersed phase and light liquid paraffin as an immiscible continuous phase stabilized with span 80 as an emulsifier. Judicial selection of solvent system was done based on their dielectric constants. The solvents having dielectric constant above 10 and below 40 reflect poor miscibility in light liquid paraffin; therefore, panfifin was preferred as a continuous phase in which both losartan potassium and Eudragit RS100 are scantily soluble [35]. 13 formulations were designed by applying CCD to optimize the composition and process variables.

Statistical data analysis and model validation

Models for different responses were generated by means of Design Expert® software. The calculated values of the response variables are shown in Table 1. Linear, quadratic, cubic, and cross-product (2FI) models were generated by the software for the responses, and the fit summary has been represented in Table 2.

The equations generated (Table 3) were used to quantify the response data and carried factors along with the coefficients. Positive sign of coefficient indicated synergistic effect (increase in response) while the negative sign indicated antagonistic effect (decrease in response) as the factor was changed from low to high level [36]. The values of coefficients of linear equations indicated that the formulation variables had a controlling effect on the microsphere properties.

The ANOVA for the regression model demonstrated that the quadratic model was greatly significant as indicated by p<0.0001 and R² value as shown in Table 4.

Mean particle size

The values for mean particle size showed a broad variation ranging from 100.96±1.47 to 169.20±1.23 μm. The data, as well as model, signified that the independent variables drug: polymer ratio (A) and stirring speed (B), their interaction term (AB) and the quadratic terms (A² and B²) showed a significant effect on particle size (Y1). p<0.0001. Expanding the drug: polymer proportion brought about the increase in stirring speed as indicated by the negative coefficients of A. On the other hand, mean particle size decreased with increase in stirring speed as indicated by the negative coefficients of B, p<0.0001. It was observed that size decreased with increase in stirring speed and time up to a certain mid-level and then onward it remained constant or higher.

Entrapment efficiency

The values of drug entrapment efficiency (Y2) for the various experimental runs ranged between 67.95±1.78 and 80.74±1.02%. The
model showed that drug: polymer ratio (A) and stirring speed (B) had a positive effect on entrapment efficiency, (p<0.0001). With the increase in independent variables, the entrapment efficiency increased to the middle level than it started decreasing on further increase in A and B.

Percent yield

The yield values for all the runs demonstrated wide difference going from a lowest of 62.56±1.08 to highest of 77.98±1.74%. The data indicated that percent yield was strongly dependent on the independent variable.

In vitro drug release

In vitro drug release data for all the experimental runs ranged between 64.19±2.62 and 80.95±2.78%.

Validation by checkpoint formulation (CPF) and optimization

To approve the model equations resulting from regression analysis, four CPFs were selected randomly as recommended by the listing of solutions and CPFs were formulated. Table 5 shows the predicted and experimental values of both the responses and the value of residuals obtained.

Numerical optimization method was applied to arrive at the optimized formulation through the desirability function approach. With the view of keeping in mind to use the smallest size of dosage form, so as to incorporate in the capsule shell, the desirable levels of responses were constrained to mean particle size “in range,” “maximum” percent yield and entrapment efficiency, and drug release at 8th h up to 80%. On analyzing the various dependent variables and far-reaching assessment of practicality of exhaustive matrix seek, the accompanying blend of independent variables was recommended by the software with desirability function of 0.840. The desirability plot for the optimized losartan potassium microparticles (OLPM) formulation has been shown in Fig. 2.

Batches of losartan potassium microspheres (OLPM) were developed using these optimum process variable settings and evaluated for responses. The results obtained for response variables were mean particle size of 112.41±1.27, entrapment efficiency of 78.67±1.62%, percent yield of 76.27%, and drug rel at 8th h around 77.61±1.05% with an error value <1. To graphically envision the effect of formulation/operation variables on the output variables, contour and response surface plot were constructed using the software as shown in Fig. 3.

Formulations of microspheres indicated that good flow properties as were observed from the various characteristics evaluation parameters. The values for angle of repose lied between 26.78°±0.64 and 34.57±0.36, Carr’s compressibility index for various formulations was in range 12.03–23.08% while the data obtained for Hausner ratio ranged between 12.03–23.08% while the data obtained for Hausner ratio ranged between 1.14 and 1.30. The optimized formulation was in range 12.03–23.08% while the data obtained for Hausner ratio ranged between 1.14 and 1.30. The optimized formulation OLPM was selected for further experimental evaluation. The Characteristic FTIR peaks of the drug were obtained at wave numbers 3197.48 cm⁻¹ (O-H stretching), 763.61 cm⁻¹ (C-Cl stretching), 1459.60 cm⁻¹ (C-H stretching). FTIR studies of optimized formulation showed that the drug was encapsulated in the microparticles. The spectrum of drug sample and microspheres has been shown in Fig. 4. SEM study revealed that the discrete, uniformly shaped spherical microspheres were obtained (Fig. 5). The DSC trace of LP showed a sharp thermographic peak at 279.8 and a broad endothermic peak at the same temperature as shown in Fig. 6.

In vitro drug release study for optimized formulation was carried out in phosphate buffer pH 6.8 so as to mimic the physiological pH. The
### Table 4: ANOVA responses for surface quadratic model

| Source                                      | Sum of squares | Df  | Mean square | F value | p-value | Prob>F | Significance |
|---------------------------------------------|----------------|-----|-------------|---------|---------|--------|-------------|
| **Response for (Y1) mean particle size**   |                |     |             |         |         |        |             |
| Model                                       | 5399.47        | 5   | 1079.89     | 810.11  | <0.0001 | Significant     |
| A-Drug: polymer ratio                       | 3904.85        | 1   | 3904.85     | 2929.31 | <0.0001 | Significant     |
| B-Stirring speed                            | 1040.11        | 1   | 1040.11     | 780.26  | <0.0001 | Significant     |
| AB                                          | 30.97          | 1   | 30.97       | 23.23   | 0.0019  |        |             |
| A2                                          | 292.86         | 1   | 292.86      | 219.70  | <0.0001 | Significant     |
| B2                                          | 184.03         | 1   | 184.03      | 138.05  | <0.0001 | Significant     |
| Residual                                    | 9.33           | 7   | 1.33        |         |        |        |             |
| Lack of fit                                 | 7.60           | 3   | 2.53        | 5.87    | 0.0601  | Not significant |
| Pure error                                  | 1.73           | 4   | 0.43        |         |        |        |             |
| Cor total                                   | 5408.80        | 12  |             |         |        |        |             |
| **Response for (Y2) entrapment efficiency**|                |     |             |         |         |        |             |
| Model                                       | 214.98         | 5   | 43.00       | 40.44   | <0.0001 | Significant   |
| A-Drug: polymer ratio                       | 24.32          | 1   | 24.32       | 22.89   | 0.0020  |        |             |
| B-Stirring speed                            | 8.08           | 1   | 8.08        | 7.55    | 0.0286  |        |             |
| AB                                          | 0.74           | 1   | 0.74        | 0.70    | 0.4318  |        |             |
| A2                                          | 148.54         | 1   | 148.54      | 139.71  | <0.0001 | Significant   |
| B2                                          | 53.51          | 1   | 53.51       | 50.32   | 0.0002  |        |             |
| Residual                                    | 7.44           | 7   | 1.06        |         |        |        |             |
| Lack of fit                                 | 4.26           | 3   | 1.42        | 1.78    | 0.2894  | Not significant |
| Pure error                                  | 3.18           | 4   | 0.80        |         |        |        |             |
| Cor total                                   | 222.42         | 12  |             |         |        |        |             |
| **Response for (Y3) percent yield**         |                |     |             |         |         |        |             |
| Model                                       | 337.33         | 5   | 67.47       | 46.97   | <0.0001 | Significant   |
| A-Drug: polymer ratio                       | 10.21          | 1   | 10.21       | 7.11    | 0.0322  |        |             |
| B-Stirring speed                            | 30.81          | 1   | 30.81       | 21.45   | 0.0024  |        |             |
| AB                                          | 0.99           | 1   | 0.99        | 0.69    | 0.4338  |        |             |
| A2                                          | 262.31         | 1   | 262.31      | 182.62  | <0.0001 | Significant   |
| B2                                          | 60.98          | 1   | 60.98       | 42.45   | 0.0003  |        |             |
| Residual                                    | 10.05          | 7   | 1.44        |         |        |        |             |
| Lack of fit                                 | 5.97           | 3   | 1.99        | 1.95    | 0.2635  | Not significant |
| Pure error                                  | 4.08           | 4   | 1.02        |         |        |        |             |
| Cor total                                   | 237.38         | 12  |             |         |        |        |             |
| **Response for (Y4) drug release at 8^th^ h**|                |     |             |         |         |        |             |
| Model                                       | 324.10         | 5   | 64.82       | 48.80   | <0.0001 | Significant   |
| A-Drug: polymer ratio                       | 292.55         | 1   | 292.55      | 220.24  | <0.0001 | Significant   |
| B-Stirring speed                            | 0.043          | 1   | 0.043       | 0.033   | 0.8618  |        |             |
| AB                                          | 0.30           | 1   | 0.30        | 0.23    | 0.6478  |        |             |
| A2                                          | 29.41          | 1   | 29.41       | 22.14   | 0.0022  |        |             |
| B2                                          | 4.14           | 1   | 4.14        | 3.11    | 0.1210  |        |             |
| Residual                                    | 9.30           | 7   | 1.33        |         |        |        |             |
| Lack of fit                                 | 7.28           | 3   | 2.43        | 4.80    | 0.0820  | Not significant |
| Pure error                                  | 2.02           | 4   | 0.51        |         |        |        |             |
| Cor total                                   | 333.39         | 12  |             |         |        |        |             |

ANOVA: Analysis of variance

### Table 5: Validation of model with checkpoint formulations

| S. No. | Composition | Response variables | Experimental values | Predicted values | Residuals |
|--------|-------------|--------------------|---------------------|------------------|-----------|
| CPF 1  | 2.88 1068   | Mean particle Size (Y1), µm | 113.57±1.43 | 114.68 | -1.11 |
|        |             | Entrapment efficiency (Y2), %  | 77.49±1.02 | 79.11 | -1.38 |
|        |             | Percent Yield (Y3), %          | 75.03±0.93 | 76.72 | -1.69 |
|        |             | Drug Rel at 8^th^ h (Y4), %    | 77.58±1.57 | 76.91 | 0.67 |
| CPF 2  | 3.64 1023   | Mean particle Size (Y1), µm | 137.46±0.38 | 135.77 | 1.69 |
|        |             | Entrapment efficiency (Y2), %  | 76.98±1.24 | 78.70 | 1.72 |
|        |             | Percent Yield (Y3), %          | 74.13±0.26 | 75.04 | -0.991 |
|        |             | Drug Rel at 8^th^ h (Y4), %    | 70.27±1.36 | 71.56 | -1.29 |
| CPF 3  | 3.09 1034   | Mean particle Size (Y1), µm | 119.50±1.37 | 120.77 | -1.47 |
|        |             | Entrapment efficiency (Y2), %  | 78.35±1.86 | 79.60 | -1.25 |
|        |             | Percent Yield (Y3), %          | 77.30±0.68 | 76.94 | 0.36 |
|        |             | Drug Rel at 8^th^ h (Y4), %    | 74.65±2.72 | 75.68 | -1.03 |
| CPF 4  | 2.74 1093   | Mean particle Size (Y1), µm | 113.02±0.86 | 111.24 | 1.78 |
|        |             | Entrapment efficiency (Y2), %  | 77.86±1.65 | 78.44 | -0.58 |
|        |             | Percent Yield (Y3), %          | 75.20±1.46 | 76.14 | -0.99 |
|        |             | Drug Rel at 8^th^ h (Y4), %    | 76.97±2.01 | 77.59 | -0.59 |

±SD. (n=6). SD: Standard deviation
optimized formulation showed maximum drug release of 78.36±1.05% in 8 h. It was observed that as the drug: polymer ratio was increased that the drug release was sustained from the formulation. The dissolution data so obtained were then fitted to various drug-release kinetic models. The release mechanism was assessed by comparing the values of the regression coefficient ($r^2$). The value of the regression coefficient was found to be higher for Korsmeyer–Peppas and zero-order model, i.e. $r^2=0.976$ and 0.980, respectively.

Evaluation of filled coated capsule shells
The coated capsules were found to be of uniform weight and thickness. The coating thickness for these ranged between 0.053 and 0.069 ± 0.001 mm. The in vitro drug release study was carried out in physiological buffers to analyze both lag time in drug release and in vitro dissolution data. A period of no drug release was observed for 2 h in SGF pH 1.2 followed by the release of microspheres in the dissolution medium phosphate buffer pH 6.8. The dissolution study was carried out for a period of 12 h, and prolonged release was observed from microspheres after a lag period of 2 h. Comparative drug release profile from coated
capsule shell containing OLPM aimed at stimulating chronotherapeutic release of the drug; matching the circadian rhythms and marketed formulation (Czartan - 25 mg capsule, Macleods Pharmaceuticals Ltd.) is shown in Fig. 7.

**DISCUSSION**

In the present study, the formulation design protocol was executed in three steps; first being the formulation optimization of microparticles of losartan potassium followed by its filling into the hard gelatin capsule shells. The last step consisted of applying a polymeric coating over the filled capsule shell to achieve the necessary lag time of around 2 h.

Emulsion solvent evaporation was found reliable for preparation of microparticles of losartan potassium. Span 80 was supplemented as an emulsifier to prevent coalescence of droplets during solvent evaporation. The data generated by statistical experimental design clearly signified that the independent variables (A) and (B), their interaction term (AB), and the quadratic terms (A² and B²) showed a significant effect on response variables (Y1-Y4); (p<0.0001). Higher polymer concentration produced more viscous dispersion, and therefore, shearing effect was less at impeded circulation that formed larger droplets [23,24]. Mean particle size decreased with increase in stirring speed producing uniform spherical microparticles while in case of entrapment efficiency (Y2), it was experiential that an increase in polymer concentration in a fixed volume of organic solvent resulted in augmenting the entrapment efficiency. In the case of percent yield (Y3), it was observed that finer particles formed during the preparation of microparticles tend to coalesce to give bigger particles with better percent yield. The model applied for drug release at 8th h (Y4) showed that the drug release retarded from the formulations as diffusional path length for drug release increased while with an increase in stirring speed the interfacial area per unit volume increased, so, the drug release also increased [19,37-40].

The reliability of the optimization model was further assessed by CPFs. The reasonably lower values of residual between predicted and observed experimental values for all the four response variables indicated a pragmatic agreement between the predicted and trial values. Hence, the mathematical model was well fitted. Numerical optimization technique using the desirability function approaching...
1 for obtaining optimized formulation indicated desirable range for independent variables A and B to get an optimum response. From the contour and response surface plots, it was observed that increasing the drug: polymer ratio had a positive effect on all the response variables while with increase in stirring speed (B), (Y2) and (Y3) increased initially followed by decline or constancy state on further rise in both the independent parameters while drug release decreased.

FTIR studies of optimized formulation indicated the absence of undesirable chemical interaction between the drug and polymer. SEM study revealed that the discrete, uniformly shaped spherical microspheres were obtained. DSC results indicated that there is no change of LP in a pure state, formulation of microspheres. LP existed in an amorphous as a molecular dispersion in the polymeric matrix. In vitro drug liberation study for optimized formulation proposed a sustained release of drug from microparticles indicating the efficient applicability of Eudragit RS100 polymer in controlling the drug release of hydrophilic drugs.

Eudragit L100 was found to be optimum for delaying drug release. The inclusion of PEG 600 and talc further improvised the coating capability by enhancing coating adherence, integrity thus, providing flexible coating film with uniform thickness and smoothness after drying. Dissolution studies suggested that optimized Eudragit coating provided protection against emptying of capsule shell contents for a period of 2 h after which the coating dissolved exposing the capsule shell to a simulated physiological buffer that resulted in burst of capsule shell thus releasing the formulation components followed by a sustained release of losartan potassium from Eudragit RS100 coated microspheres.

CONCLUSION
The capsule-based system designed for chronotherapeutic management of hypertension dealing with early morning pathophysiology influenced by circadian rhythm was successfully prepared and optimized through a statistical experimental design approach.

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AUTHORS’ CONTRIBUTIONS
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CONFLICTS OF INTEREST
No conflicts of interest associated with this work.

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