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INFLUENCE OF FORMULATION VARIABLE IN DEVELOPMENT OF FLOATING MICROSPHERES OF WATER SOLUBLE DRUG

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ABSTRACT

Floating drug delivery system for propranolol hydrochloride (PPL) was developed to prolong gastric residence time and increase drug bioavailability. The aim of present work was to prepare floating microspheres of propranolol HCl using solvent evaporation technique. Propranolol HCl is a non-selective beta adrenergic blocking agent with short elimination half life 3-5 hours. The short half life of propranolol HCl and multiple administration dose make propranolol HCl a very good candidate for formulation of floating drug delivery system. Total 27 batches were prepared by using 3^3 full factorial design, in which effect of drug polymer ratio, RPM and proportion of dispersion medium were studied. The prepared floating microspheres were evaluated for, particle size, percentage yield, in vitro buoyancy, drug content, entrapment efficiency, in vitro drug release, scanning electron microscopy and statistically analysed. Check point analysis was done to confirm the optimized batch. Stability of microspheres was studied as per ICH guidelines. It was observed that prepared microspheres were white, free flowing and spherical in shape. Formulation B25 prepared with drug:polymer ratio (1:5), RPM 1500 and proportion of dispersion medium(9:1) which exhibited higher percentage yield, in vitro buoyancy, entrapment efficiency and percentage drug release (96.09±0.17 %) after a period of 24 hrs. Results show that, an increase in drug polymer ratio, RPM and ratio of light liquid paraffin to n-Hexane affects the particle size, percentage yield, in vitro buoyancy and drug release of microspheres. It was observed that increase in drug polymer ratio increases the entrapment efficiency and mean particle size of the microspheres. Whereas, with the increase in RPM particle size of microsphere reduced and fine spherical shaped microspheres were produced. The data obtained in this study suggest that, floating microspheres of propranolol HCl with selected formulation variables are promising for sustained drug delivery which can reduce dosing frequency.

KEYWORDS: Floating microspheres, Propranolol HCl, 3^3 Factorial design, optimization.

INTRODUCTION

Microspheres are small spherical particles, with diameters in the micrometer range (typically 1 µm to 1000 µm). Microspheres can be manufactured from various natural and synthetic materials [1]. Floating systems or Hydro-dynamically controlled systems are low-density systems that remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. The drug is released slowly at the desired rate from the system. This result in an

increased Gastric residence time (GRT) and a better control of the fluctuations in plasma drug concentration (Fig 1) [2].

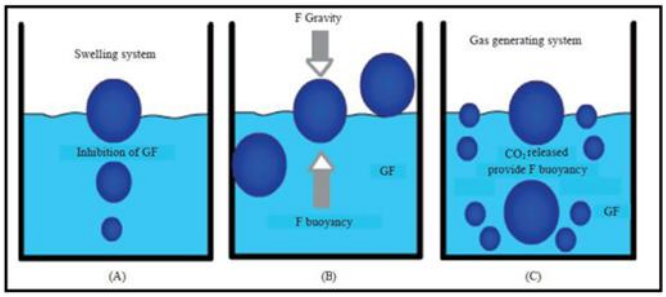

Figure 1: Mechanism of Floating System

When floating microspheres comes in contact with gastric fluid, the gel formers hydrate to form a colloidal gel barrier that controls the rate of fluid penetration into the device and consequent drug release. The air trapped by swollen polymer lowers the density and confers buoyancy to the microspheres [3].

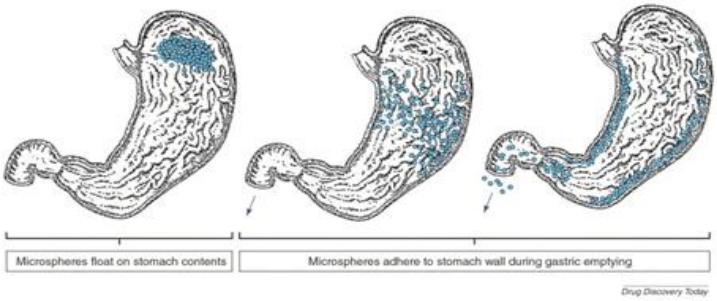

Figure 2: Floating microspheres in stomach

Formulation parameters studied under this research work are:

1. Effect of Drug: Polymer Ratio (DPR)
2. Effect of rotation speed
3. Effect of Dispersion medium

Propranolol hydrochloride is a highly hydrophilic, non-selective beta adrenergic blocking agent, has been widely used in the treatment of hypertension, angina pectoris, and many other cardiovascular disorders. Furthermore, it has a short elimination half-life of 3 h, which makes it a suitable candidate to be delivered at a controlled rate [4].

If floating microspheres are formulated without optimization of formulation parameters then it may frequently leads following drawbacks:

- Irregular shaped microspheres
- Desirable size cannot be produced
- Less stablity
- Less bio-availability

- Reduced therapeutic efficacy

The main objective of present study was to develop optimized sustained release floating microspheres by varying formulation parameters with accepted stablity. Here, in this study, influence of various formulation parameters like RPM, drug-polymer ratio and ratio of dispersion medium (proportion of light liquid paraffin to n-hexane) on properties of microspheres were studied, whereas other formulation parameters were kept constant.

MATERIALS AND METHOD:

Prpranolol HCl was obtained from Alpha Laboratories, Baroda. Ethyl-cellulose, tween 80, light liquid paraffin, n-Hexane, methanol and DCM all were obtained Sulab, Suvidhan Laboratories, Baroda.

The term preformulation is self explanatory, i.e., the study conduct at very beginning stage of the dosage form development. Before formulation of drug substances into a dosage form, it is essential that drug and excipients should be chemically and physically characterized.

Floating microspheres were prepared by emulsion solvent evaporation method in year 2014. Drug + methanol and polymer are mixed in organic solvent of dichloromethane. That mixture was added drop wise to non-aqueous solution and stirred till the organic phase completely evaporates and microspheres formed. Prepared microspheres were filtered, washed with petroleum ether and dried at room temperature [5].

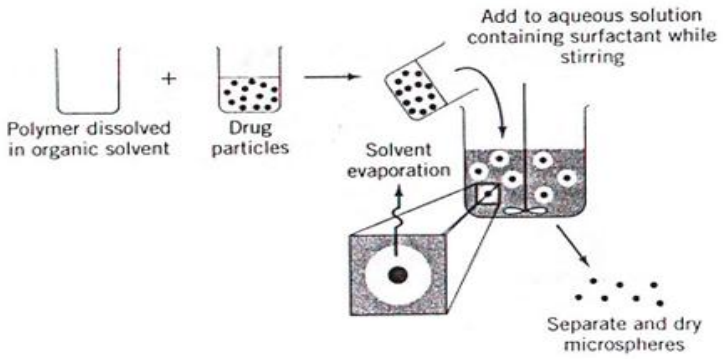

Figure 3: Solvent evaporation method for preparation of microsphere [6].

EVALUATION OF FLOATING MICROSPHERE

Production yield: Thoroughly dried microspheres were collected and weighed accurately. The percentage yield was then calculated [7].

Size distribution: Particle size of prepared microspheres was measured using an optical microscope, and the mean particle size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer [5]

Photomicroscopy: All batches of floating microspheres prepared were observed under compound microscope and photos were taken by a suitable camera.

Drug content and entrapment efficacy of microspheres: The drug content of propranolol HCl present in floating microsphere was determined by accurately weighing 50mg of Floating microspheres and extracting in 2ml of methanol to dissolve the polymer and then make up-to 50ml with water used as a solvent of drug. The solution was diluted suitably. Drug content was analyzed spectrophotometerically at 292 nm and the percentage drug entrapment was calculated [8].

$$\textbf{Drug entrapment efficiency} = \frac{\textbf{Experimental drug Content}}{\textbf{Theoretical drug Content}} \times 100$$

In vitro drug release study: The in vitro release studies of drug-loaded floating microspheres were carried out using USP dissolution apparatus in 900 ml of simulated gastric pH medium (0.1N HCl) at 37^{o}C ±2^{o}C at 100 rpm. An accurately weighed amount of microspheres (equivalent to 50 mg of drug) were added to dissolution medium and at predetermined interval 10ml of aliquots were withdrawn and replaced by an equal volume of fresh dissolution medium. Aliquots following suitable dilution were analyzed spectrophotometrically at 292nm. Dissolution studies were performed for period of 24 hours. The concentrations of propranolol in samples were calculated using regression equation of the calibration curve of propranolol HCl in 0.1 N HCl of pH 1.2 [9, 10].

In vitro buoyancy: Floating microspheres equivalent to 300 mg were dispersed in 900ml of 0.1 N hydrochloric acid solution (pH 1.2) containing 0.02% v/v Tween 80 as a dispersing agent at 37°C in an USP XXIV dissolution apparatus (type II). The medium was agitated with a paddle rotating at a speed of 100 rpm for 12 hr and after 12 hr, the layer of buoyant microspheres (Wf) was pipette out and separated by filtration simultaneously sinking microspheres (Ws) was also separated. Both microspheres type were dried at 40°C overnight and weighed. The buoyancy was determined by the weight ratio of the floating microspheres to the sum of floating and sinking microspheres [9, 11].

Data interpretation and analysis: The statistical evaluation of the results was carried out by analysis of variance using Microsoft Excel 2007. The data analysis results (p value) shows the effect of variables on various parameters such as %yield, particle size, %drug content, %drug entrapment and %CDR. Regression coefficients is statistically significant if $p < 0.05$. The

significant factors in the equations were selected for the calculation of regression analysis. The term of full model having non-significant p value have negligible contribution in obtaining dependent variables and thus neglected. The equation represents the quantitative effect of the formulation variables on responses [12].

Construction of contour graphs: Sigma plot is specifically designed to aid in documenting and publishing research, specializing in the graphical presentation of results. Plot between two variables were plotted keeping one variable constant for the effect of entrapment as well as particle size.

Evaluation of model/ check point analysis: In order to assess the reliability of the model, two experiments were conducted by varying the process variables at values other than that of the responses were estimated by using the equations and experimental procedure. The comparison between the experimental and predicted values of the responses for these addition experiments is done.

Surface morphology: The shape and surface topography of optimized batch of floating microspheres were visualized by scanning electron microscopy (SEM) [13].

Stability study: Stability studies were carried out for optimized formulation according to ICH guidelines. The samples were analyzed for physical appearance, drug content and % drug release at regular interval of 15 days by spectrophotometer at 292 nm [14].

RESULT AND DISCUSSION

In order to initiate experimental study to formulate floating microsphere of propranolol HCl first of all preformulation was done.

In this present study, 3^3 full factorial design was used to prepare floating microspheres of drug by using solvent evaporation method.

Drug- excipients compatibility studies were carried out by using FT-IR spectroscopy. The data of spectrum shows compatibility between drug and excipients because, the spectrum of drug and excipients shows no change into the peak of functional group.

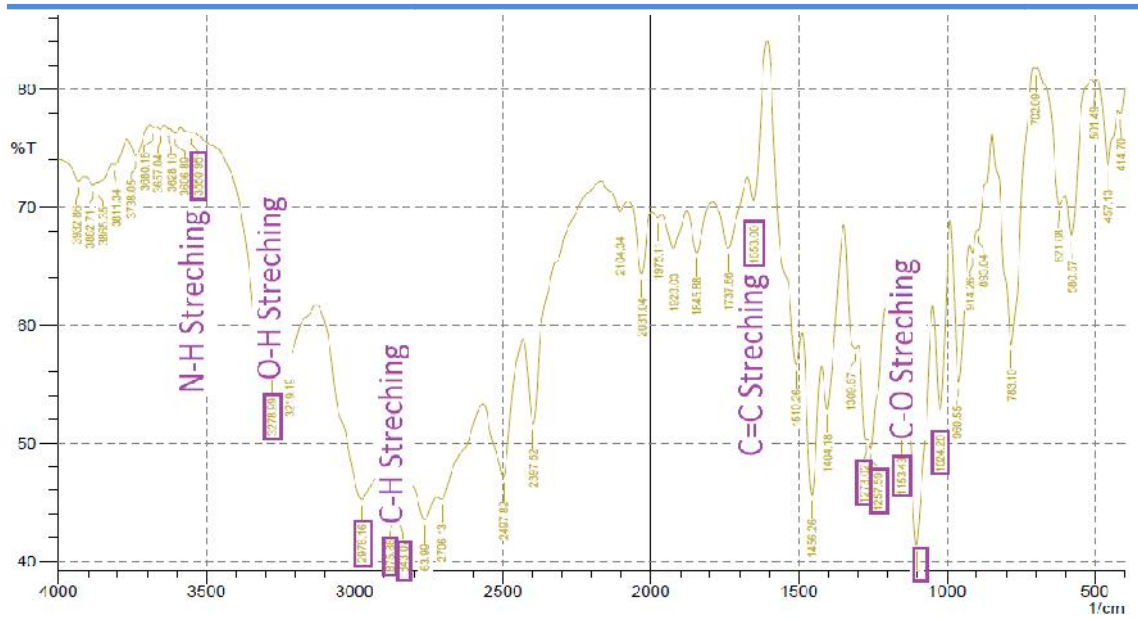

Figure 4: FT-IR Spectrum of Propranolol HCl in range of 4000 to 400 cm^{-}

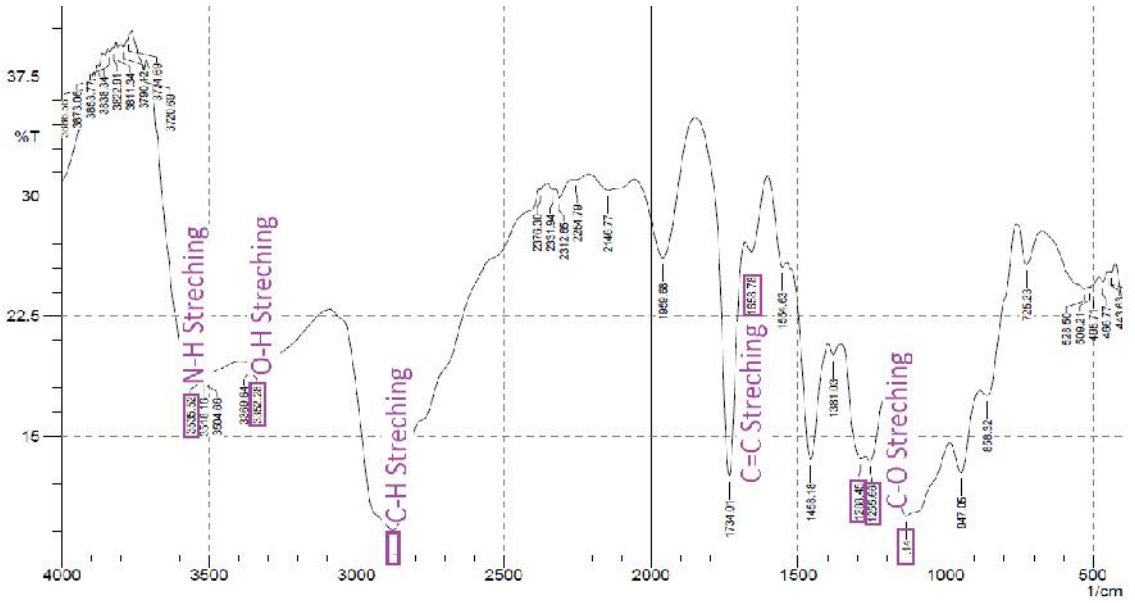

Figure 5 : FT-IR spectrum of Propranolol HCl + Ethyl cellulose + Tween 80 in range of 4000-400 cm^{-}

Peak for N-H stretching of pure was 3550.95 and for combination were 3535.52. O-H stretching peak was observed 3278.99 for pure drug and 3352.28 for combination. C-H stretching has a range of 2955-2800 and the peak observed for pure drug was 2875.07 and for combination were 2875.86. C=C stretching have a range of 1680-1620, peak for pure drug was 1653 and for combination wew 1658.78. C-O stretching peak observed for pure drug was 1103.28 and for combination was 1134.14.

In batch B1 to B9, satisfactory products were not obtained. Product was oily and lumpy in nature, possible reason may be due to lower level of Drug: Polymer ratio and less amount of polymer, proper spherical particles were not obtained. Hence, these batches were excluded from further evaluation. In batch B12, B14 and B15, again same products were obtained as in case of B1 to B9. Reason may be due to lower RPM and may be due to low drug: polymer ratio.

Production yield was observed in the range of 50.66 ± 0.985 to 96.74 ± 0.990 for the batches B10, B11, B13 and B16 to B27. From the data it can be interpreted that as the drug polymer ratio were increases from 1:1 to 1:5, satisfactory product was obtained. Batch B25 shown maximum yield.

Particle size of all the prepared batches was found to be in range of 104 ± 0.96 to 194 ± 2.1. These data indicates that as RPM increases particle size decreases. It was also found that proportion of dispersion medium also affects the particle size of product. As the proportion of n-Hexane decreases from 7:3 to 9:1, more uniform particles were obtained. On increasing RPM more than 1500 the microsphere ruptures and hence satisfactory particle size was not obtained.

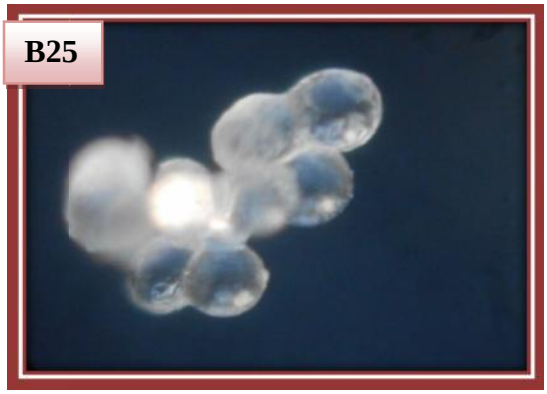

Figure 6: Optical microscopic image of floating microsphere.

Drug content indicates the amount of drug present in formulation. It was determined spectrophotometericaly by UV- Visible spectrophotometer. Entrapment efficiency (EE) is the amount of drug entrapped in formulation. % EE of Floating microsphere of propranolol HCl varies in the range of 16.6±0.2 to 82±0.54 % which indicates a fair amount of drug was entrapped by floating microspheres. It was found that EE increases as the drug- polymer ratio increased. The low value of EE was probably due to the lost of drug.

In- vitro release studies of drug-loaded floating microspheres were carried out using USP dissolution apparatus. %CDR indicates the amount of drug released by a formulation in a particular time. All batches of floating microspheres exhibited a prolonged release for about 24 hrs. Batches studied under B10 to B18 with constant drug polymer ratio (1:3) shows %CDR between 70.65±0.62 to 82.46±0.19 and %CDR of batches from B19 to B27 with constant drug

polymer ratio (1:5) was varying from 74.02±0.25 to 96.09±0.17. It was found that as the drug: polymer ratio increases, the release pattern of propranolol HCl might be decreases, which may be because of increased amount of polymer may not allow the drug release from microsphere system.

B25 formulation shows comparatively desired drug release pattern. As the ratio of polymer to drug increases, higher amount of drug was encapsulated and hence, drug release rate decreases. The retarding effect on drug release is frequently attributed to the polymer diffusional barrier between the drug and the dissolution medium.

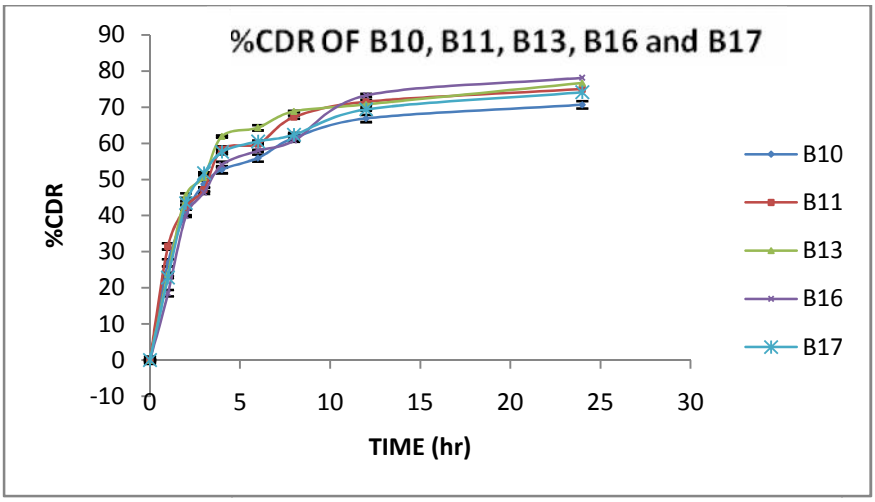

Figure 7 : In vitro dissolution profile of B10, B11, B13, B16 and B17

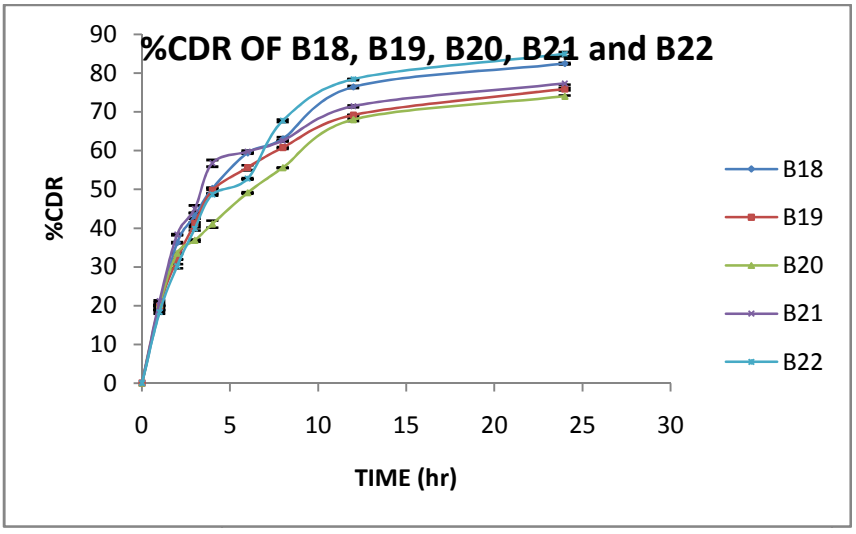

Figure 8 : In vitro dissolution profile of B18, B19, B20, B21 and B22

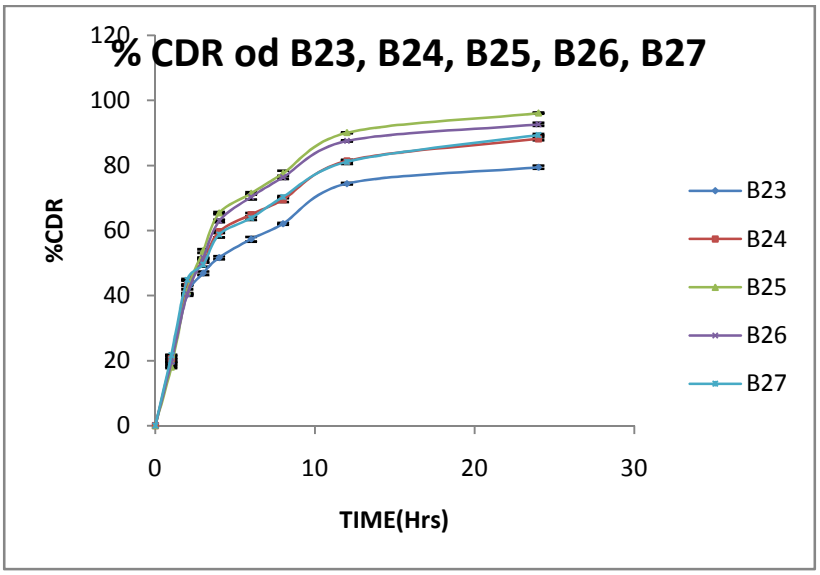

Figure 9: In vitro dissolution profile of B23, B24, B25, B26 and B27

Buoyancy percentage indicates the amount of floating microspheres remain buoyant in gastric media. It was observed that the % buoyancy of all batches was above 70%. It ranges from 70.21± 0.2 to 87.94 ±0.01. It was observed that an increase in drug polymer ratio produce increased buoyancy of floating microspheres.

In data interpretation and analysis, the factors were investigated systemically, a 3^3 factorial design was employed. As shown in equation, a statistical model incorporating interactive and polynomial terms is used to evaluate the responses.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{31}X_3X_1 + b_{123}X_1X_2X_3$$

Two dimensional contour graphs were established by sigma plot version11.0 using reduced polynomial equation, keeping one parameter stationary and varying others. It is useful to study the interaction effects of the factors on the responses. It was observed that effect of RPM and proportion of dispersion medium taking drug: polymer ratio constant was pronounced on particle size of floating microsphere. Also, it was found that Drug: polymer ratio and RPM exhibited pronounced effect on EE as compared to proportion of dispersion medium.

Check point analysis was done to confirm the optimized batch by keeping one parameter same while changing others. It was observed that batch B25 is the optimized batch. It was observed that value of a new batch was quite closer to predicted value.

SEM was performed on optimized batch B25 at 150X and 500X zooming. The particles were observed in range of 101 to 114 µm. It was found that SEM of drug loaded floating microspheres reveals uniform and spherical shape particles.

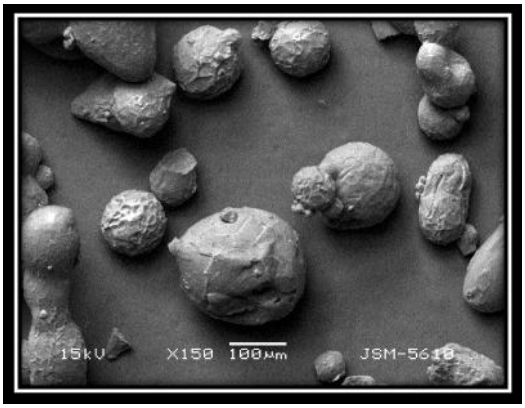

Figure 10: SEM of B25 at ×150

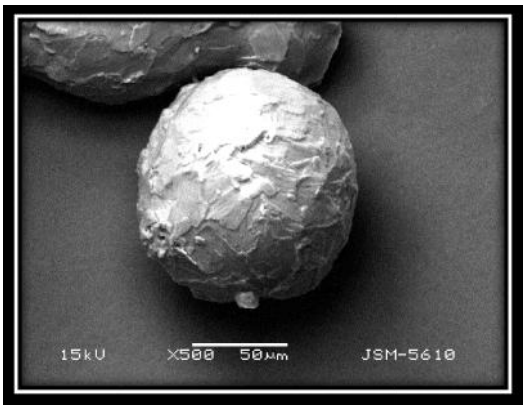

Figure 11: SEM of B25 at ×500

Stability studies were carried out for optimized formulation according to ICH guidelines. The microspheres of the optimized formulation were placed in rubber cocked; amber coloured and transparent glass vials and in open lid amber coloured and transparent glass vials. The formulations were stored in room temperature at 25 ± 2°C/ 60%RH and in stability and humidity chamber at 40 ± 2°/ 75%RH for a period of 60 days at interval of 0, 15, 30, 45 and 60 day. The samples were analyzed for physical appearance, drug content and % drug release at regular interval of 15 day by spectrophotometer at 292 nm.

It was found that no change in physical appearance observed after 60 days. Reduction in drug content observed between 2-3% i.e. under accepted criteria. No significant change was observed in In-vitro drug release. Formulation was more stable at 25^0 C ± 2^0C in amber coloured close lid vial as compared to other storage conditions i.e. after 60 days %EE was observed to be 80.39±0.13 and observed %CDR was 95.37±0.30. Hence, it is suggested to store the optimized batch at this storage condition.

CONCLUSION

The purpose of this work was to understanding effect of various formulation variables on preparation of the floating microsphere of water soluble β-blocker drug propranolol HCl. Floating microspheres were successfully prepared by emulsification solvent evaporation method by using 3^3 full factorial designs. Influence of three independent variable drug: polymer ratio, RPM and ratio of dispersion medium were investigated on dependent variable of microspheres. Results of FT-IR show that there were no drug polymer interaction appeared during the experimental conditions used in this work which allows for the formation of microspheres. The drug entrapment efficiency of prepared microspheres was directly proportional to drug: polymer ratio. Particle size decreases with increase in RPM. In-vitro drug release rate also decreases with increase in drug: polymer ratio. Optimization was done and contour plot was plotted, B25 was

found to be optimized batch. Check point analysis was carried out, which shows that there was no significant difference between predicted and experimental value. The optimized formulation was subjected for SEM and accelerated stability studies by storing at various ICH storage conditions. The sample were analysed for its physical appearance, EE and %CDR at an interval of 15 days. It shows better storage in amber coloured close lid glass vial at $25^{o}C \pm 2^{o}C$/60 %RH. It can be concluded that, B25 formulation shows better results by using drug: polymer ratio 1:5, RPM 1500 and proportion of dispersion medium 9:1 during the process of formulation. By this approach the drug release sustained and lead to avoidance of frequent drug administration.

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