International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-2, 2014 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Formulation and Evaluation of Microparticles Containing Anti asthmatic Drug

Patel R*, Patel D, Patel KN, Patel PA, Nayak BS

Shree Swaminarayan Sanskar Pharmacy College, Zundal, Gandhinagar, Gujarat, India.

Manuscript No: IJPRS/V3/I2/00189, Received On: 14/04/2014, Accepted On: 21/04/2014

ABSTRACT

The present study involves development and optimization of microparticles containing Montelukast Na as anti-asthmatic drug by solvent evaporation method. Montelukast is a leukotriene receptor antagonist (LTRA), it blocks the action of leukotriene D4 (and secondary ligands LTC4 and LTE4) on the cysteinyl leukotriene receptor. This reduces the bronchoconstriction. Results of DSC and FT-IR study have shown that there was no interaction between drug and excipients. M1 to M15 batches were formulated by using different drug polymer ratio of Polycaprolactone and Ethyl cellulose having poly vinyl alcohol as an emulsifying agent supported by response surface methodology using Box-Behnken factorial design. The prepared microparticles were examined for various evaluation parameters like flow properties, % yield, % drug loading, particle size analysis, *in vitro* drug release at 12 hr. *In-vitro* release studies were performed in 0.5 % w/v SLS. There was an effect on mean particle size by altering drug polymer ratio and stirring speed. The observed responses were coincided well with the predicted values given by the optimization technique. The optimization of formulation was done by using box-behnken design. The optimized formulations were subjected to stability studies as per ICH guidelines at 40°C temperature and 75% relative humidity. The optimized batch M 19 showed the highest % yield (87.98 %), % drug loading (66.65%), % CDR at 12 hr (99.03 %). The average particle size of optimized batch M19 was 19.25 µm. The result of kinetic models of optimized batch M19 show fickian diffusion kinetics. No significant change was found in drug content by performing stability study on optimized batch M19 as per ICH guidelines.

KEYWORDS

Montelukast Na, Ethyl Cellulose, Polycaprolactone, Solvent Evaporation Method

INTRODUCTION

Now a day's conventional dosage forms of drugs are rapidly being replaced by the new and the novel drug delivery systems. Amongst, these the controlled release/sustained release dosage forms have become extremely popular in modern therapeutics. The oral route administration is mostly adopted route because of its comfortable dosage form, design and patient care. Several parameters should be kept in mind before formulating sustain release dosage form which includes various pH in GIT, the gastrointestinal motility, the enzyme system and its effect on the dosage form and the drug.

Most of sustained release dosage form follows the mechanism of diffusion, dissolution or combination of both, to produce slow release of drug at predetermined rate. Hypothetically, a sustained release dosage form should release the drug by a zero-order mechanism which maintains drug plasma level time similar to intravenous infusion. The goal in designing

***Address for Correspondence:**
Rina S. Patel
Shree Swaminarayan Sanskar Pharmacy College,
Zundal, Gandhinagar, Gujarat, India.
E-Mail Id: rinapatel9008@gmail.com

sustained release drug delivery system is to reduce the frequency of dosing

- Increase effectiveness of the drug by localization at site of action
- Reduce the dose required
- Providing the uniform drug delivery

Sustained release is dosage form thus designed to achieve prolonged therapeutic effects by continuously releasing medication over an extended period of time after administration of single dose.

Oral drug administration is by far the most preferable route for taking medications. However, their short circulating half-life and restricted absorption via a defined segment of intestine limits the therapeutic potential of many drugs. Such a pharmacokinetic limitation leads in many cases to frequent dosing of medication to achieve therapeutic effect. This results in pill burden and consequently, patient complains.

Rational approach to enhance bioavailability and improve pharmacokinetic and pharmacodynamics profile is to release the drug in a controlled manner and site specific manner.

Microparticles are the polymeric entities falling in the range of 1-1000µm. Microparticles covering two types of the forms such as microcapsules and microspheres

Microcapsules are micrometric reservoir systems. Microcapsules may be spherical or non-spherical in shape. Microcapsules are small particles, which composed of coating shell containing an active agent or core material. Microcapsule size 100 to 150µm.

Microspheres are micrometric matrix systems. Microspheres are matrix systems and essentially spherical in shape. Microsphere size 1µm to 1000µm.

Morphology of microparticles: Micro-encapsulation is a method and in that entraps solids, liquids, or gases inside a polymeric matrix. Microparticles are particulate dispersions or solid particles. Because change in size of microparticles can be differentiated microcapsules and microspheres.[1,2,3]

MATERIALS AND METHOD

Materials

Montelukast Na was gifted by Zydus Cadila Polycaprolactone was acquired from Sigma Aldrich Chemicals. Ethyl Cellulose obtained from Colorcon asia Pvt. Ltd. Dichloromethane purchased from Merck Industries Ltd. Methanol, Liquid paraffin and was purchased from RFCL Pvt. Ltd. Poly vinyl alcohol and Poly vinyl Pyrrolidone purchased from SD Fine Chem Pvt. Ltd.

Identification of Montelukast Na by FTIR-Spectroscopy

Pellets of drug and potassium bromide was prepared using hydraulic pellet pressure at a pressure of 7 to 10 tones. FT-IR was scanned from 4000 to 400 cm^{-1} by using Analytical technological Ltd. [4]

Identification of Montelukast Na by DSC

Thermal properties of the particles were studied by differential scanning calorimetry DSC (Shimadzu Corporation, model no. DSC-60).Approximately 5 mg of particles were compressed and loaded onto standard aluminium pans. The samples were purged with pure dry nitrogen at a flow rate of 5 ml/min. The analysis was carried out at a temperature heating rate of 10 ˚C/min and a temperature range of 50^0C-300^0 C.[5]

Drug- Excipient Compatibility Study by FT-IR

Fourier-transform infrared **(**FT-IR) spectra were obtained using an FT-IR spectrometer (Analytical technological Ltd). For the study Compatibility of Montelukast Na and mixture of montelukast Na and Excipient (1:1) were previously crushed and mixed thoroughly with Potassium Bromide. Make 1:5 (Sample: KBr) ratio. Samples are scan from 4000 to 400 cm^{-1}.

Drug- Excipient Compatibility Study by DSC

Thermal properties of the particles were studied by differential scanning calorimetry DSC

(Shimadzu Corporation, model no. DSC-60).Approximately 5 mg of Montelukast Na and Polycaprolactone mixture were compressed and loaded onto standard aluminium pans. The Montelukast Na and Polycaprolactone mixture were removed with dry N_2 at a flow rate of 5 ml/min. It was carried out at a temperature heating rate of 10 ˚C/min and a temperature range of 50^0C-300^0 C.

Formulation of Microparticles of Montelukast Na [6]

Preparation of Drug Polymer Solution

Depending upon the different ratio of 1:1, 1:2 and 1:3, Weighed amount of the drug and polymer were dissolved in the organic solution containing 20 ml of Dichloromethane.

Preparation Aqueous Phase Solution

For PVA solution: Based on the concentration of the PVA (0.3%, 0.4% and 0.5%), Weighed amount of PVA dissolved in the Distilled water.

For Liquid Paraffin: 100 ml of Liquid paraffin used as secondary Phase

For Poly vinyl pyrrolidone Solution: 0.5gm of Poly vinyl pyrrolidone dissolved in 100ml of Distilled water.

Method for Preparation of Microparticles

Preparation of microparticles include formulation of Drug Polymer Solution depending drug polymer ratio (1:1, 1:2, 1:3) which is added drop wise continuously with Syringe with different adding time (5min, 10min and 15 min) in aqueous solution kept for continuous stirring. The solution allowed to stir for 2 hour until the complete evaporation of organic phase. Solution is filtered and washed with water. Filtered Microparticles finally air dried over a period of 12 hour and stored in desiccator.

Box-Behnken Design[7, 8]

Optimization of Variables Using Box-Behnken Design

A 3-factor, 3-level Box-Behnken statistical design was used as standard protocol for optimization and evaluation main, quadratic, and interaction effects of various formulation ingredients of sustained release microparticles of Montelukast Na. Various dependent and independent variables along with their actual and coded levels used in this study are given in Table 1. A design matrix comprising 15 experimental runs was constructed, for which the nonlinear computer-generated quadratic model is defined as:

$$Y= b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

Where, Y is the measured response associated with each factor level combination; b_0 is constant; b_1, b_2, b_3 are linear coefficients, b_{12}, b_{13}, b_{23}, are interaction coefficients between the two factors and are computed from the observed experimental values of Y from experimental runs; and X_1, X_2, and X_3 are the coded levels of independent variables.

The terms X_1X_2 (i = 1, 2 or 3) represent the interaction effect and X_1^2, X_2^2, X_3^2 represent the curvature effects. The concentration range of independent variables under study is shown in table 5.5 along with their low and high levels, which were selected based on the results from preliminary experimentation.

The Drug and polymer ratio (X_1), Organic phase addition time (X_2) and Concentration of PVA (X_3) used to prepare the formulations.

The data transformation simplifies the calculations for model development. The data generated by the experimental design was utilized for drawing contour plot, to obtain an optimized region within the factorial space, and thereby produce an optimized formulation.

Evaluation of Microparticles[9, 10, 11, 12, 13, 14]

Percentage Yield

Microparticles after drying were weighed to calculate the percentage yield of Microparticles using the following formula:

$$Percentage\ yield = \frac{Total\ Weight\ of\ Microparticles}{Total\ Weight\ of\ drug\ and\ polymer} \times 100$$

Table 1: Box Behnken Layout and Data Transformation for Design Batches

Batches	Drug Polymer Ratio (mg)	Adding Time (min)	PVA (%w/v)
M01	1:1	5	0.4
M02	1:3	5	0.4
M03	1:1	15	0.4
M04	1:3	15	0.4
M05	1:1	10	0.3
M06	1:3	10	0.3
M07	1:1	10	0.5
M08	1:3	10	0.5
M09	1:2	5	0.3
M10	1:2	15	0.3
M11	1:2	5	0.5
M12	1:2	15	0.5
M13	1:2	10	0.4
M14	1:2	10	0.4
M15	1:2	10	0.4

Percentage Drug Loading

Microparticles equivalent to 10 mg were weighed accurately and crushed in mortar pestle. Crushed particle were soaked in 100 ml 0.5% w/v SLS. Solution than sonicated and is to be stirred for 24 hour. The solution is than to be filtered and filtrate is appropriately diluted and measure the absorbance in U.V visible spectrophotometer at λmax 345 nm.

$$\%\ Drug\ Loading = \frac{Actual Content}{Therotical\ Content} \text{ x } 100$$

Particle Size Analysis

Particle size distribution of the Microparticles was determined by optical microscopy using calibrated ocular eyepiece. Product dispersed in light liquid paraffin and a smear of the dispersion was observed under compound microscope.

In Vitro Drug Release

In vitro drug release study is to be carried out as per Indian Pharmacopoeia, Drug-loaded microparticles is to be placed in an empty cellophane bag which is consequently should be place into 900ml 0.5 percent w/v solution of SLS in water as a dissolution medium. Bath temperature is to be maintained at 37 ± 1◦C throughout the study. Speed is adjusted to 100 rpm. The dissolution is carried out for 12 hours in an interval of 2 hours with replacement of 5ml fresh medium and analyzed by UV-visible spectrophotometer at 345 nm.

Scanning Electron Microscopy

It provides vital information about the porosity and microstructure of these drug delivery systems. The most common technique used is scanning electron microscopy (SEM). The sample prepared for this method should be dehydrated as vacuum field is necessary for image generation in SEM. Primary the samples are coated with electron dense coating materials such as gold, palladium or a combination of both to take photomicrograph. The coating can be done by sputter coating or thermal vacuum evaporation.

Flow Properties

Flow properties such as tapped density, bulk density, compressibility index, angle of repose are also studied.

Statistical Analysis

Box-Behnken Design was applied with three formulation variables at 3 different levels were used to study the effects on dependent variables. All batches of microparticles were statically (confidence level 95 % or $P < 0.05$) evaluated with regard to % yield, average particles size, % CDR using design expert software. To graphically demonstrate the influence of each factor on the response, the response surface plots were generated using the design expert software.

Check Point Analysis

A checkpoint analysis was performed to confirm the role of the derived polynomial equation and

contour plots in predicting the responses. Values of independent variables were taken at 3 points, 1 from each contour plot, and the theoretical values of % yield, average particle size and % CDR were calculated by substituting the values in the polynomial equation. Microparticles were prepared experimentally at 3 checkpoints and evaluated for the responses.

Optimization of Formulation by Box Behnken Design

The optimized formulation was obtained by applying constraints (goals) on dependent (response) and independent variables (factors). The models were evaluated in terms of statistically significant coefficients and R^2 values.

Various feasibility and grid searches were conducted to find the optimum parameters. Various 3-D response surface graphs were provided by the design expert (Version 8.0.6.1, Stat-Ease Inc). The optimized checkpoint formulation factors were evaluated for various response properties. The resultant experimental values of the responses were quantitatively compared with the predicted values to calculate the prediction error.

Application of Kinetic Models

To analyse the mechanism of release and release rate kinetics of the dosage form, the data obtained were fitted into Zero order, First order, Higuchi matrix, Peppas and Hixson Crowell model. Based on the n value, the best-fit model was selected.

To analyse the mechanism of the drug release rate kinetics of the dosage form, the data obtained were graphed as:

1. Cumulative percentage drug released Vs Time *(In-Vitro* drug release plots)
2. Cumulative percentage drug released Vs Square root of time (Higuchi's plots)
3. Log cumulative percentage drug remaining Vs Time (First order plots)
4. Log percentage drug released Vs Log time (Peppas plots)

Stability Study

For all the pharmaceutical dosage forms it is important to determine the stability of the dosage form .The stability studies were carried out for the most satisfactory formulation as per the ICH guidelines to estimate the stability of the prepared drug dosage formulation.

In the present study, stability studies were carried out at 40 ± 2°C / 75 % ± 5% RH for a specific time period up to 1 months for selected formulations. For stability study, the microparticles were sealed in Aluminium packaging coated inside with polyethylene. These sample containers were placed in humidity chamber maintained at 75% RH. At the end of studies physical appearance, % yield, average particle size, % CDR, drug loading (%) parameters were evaluated to the samples.[15]

RESULTS AND DISCUSSION

Identification of Montelukast Na by FT-IR

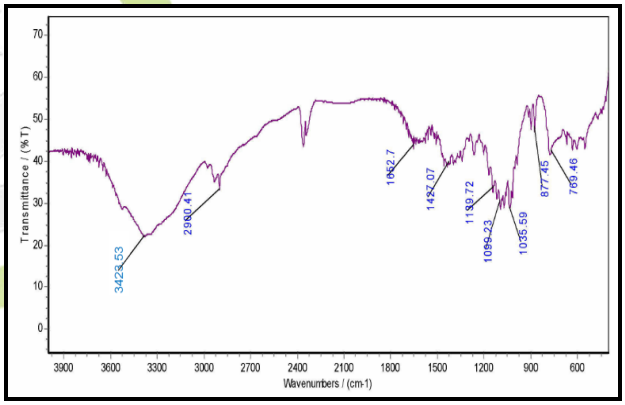


Figure 1: FT-IR of Montelukast Na

Table 2: Interpretation of FT-IR Spectra

Wave number (cm^{-1})	Interpretation
3423.53	O-H stretching
2900.41	C-H stretching
1652.70	C=O stretching
1139.72	C-N stretching
1099.23	C-O stretching

FT-IR of Montelukast Na showed peak at 3423.53cm^{-1}(OH stretching), 2900.41cm^{-1} (CH stretching), 1652.70 cm^{-1}(C=O stretching), 1139.72 cm^{-1}(C-N stretching), 1099.23 cm^{-1}(C-O stretching).

Identification of Montelukast Na by DSC

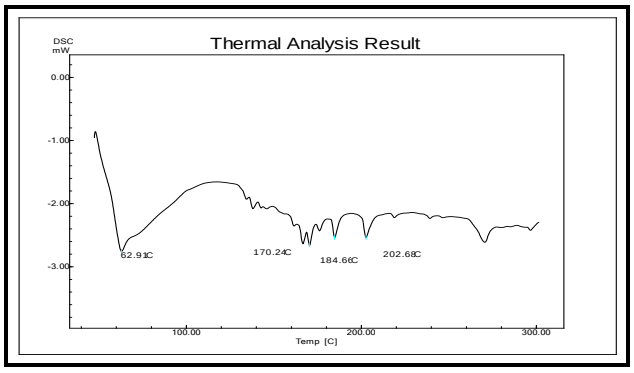


Figure 2: DSC of Montelukast Na

DSC study was performed as per figure 2 in order to investigate the nature and intermolecular interactions. The melting point of Montelukast Na was observed at 115.70 ˚C as the endothermic peak. The reported range of melting point of Montelukast Na is 112-119 ˚C, it was concluded that the test sample of Montelukast Na was pure.

Drug - Excipients Compatibility Study by FT-IR

The FT – IR studied were carried out for drug (Montelukast Na), the combination of Montelukast Na and Polycaprolactone, Montelukast Na and ethyl cellulose, Montelukast Na and Polyvinyl Alcohol to evaluate drug polymer interaction. The FT – IR graphs were as follows

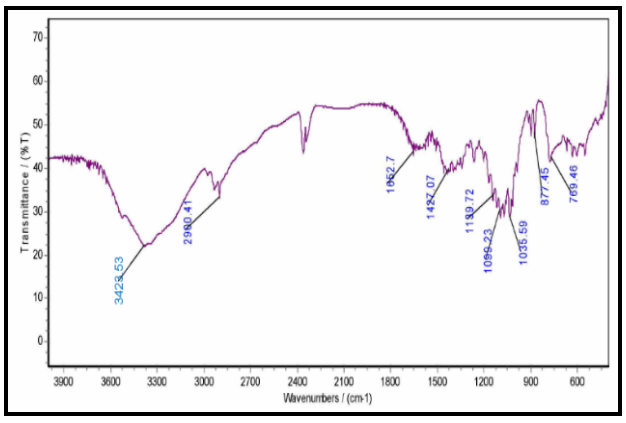


Figure 3: FT-IR of Montelukast Na

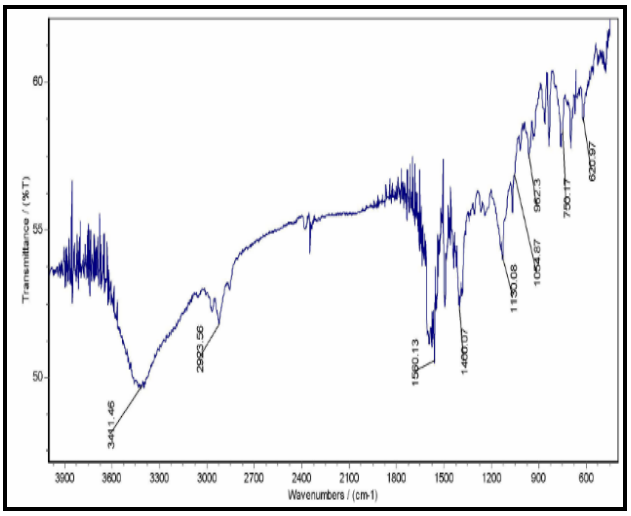


Figure 4: FTIR spectra of combination of MNKT Na and Polycaprolactone

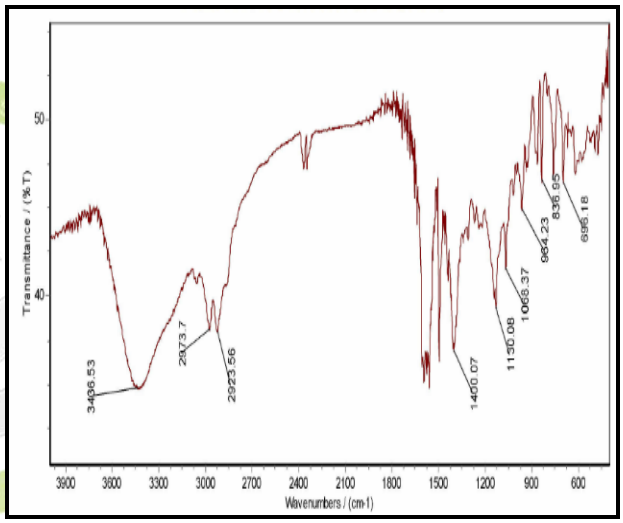


Figure 5: FTIR spectra of combination of MNKT Na and Ethyl Cellulose

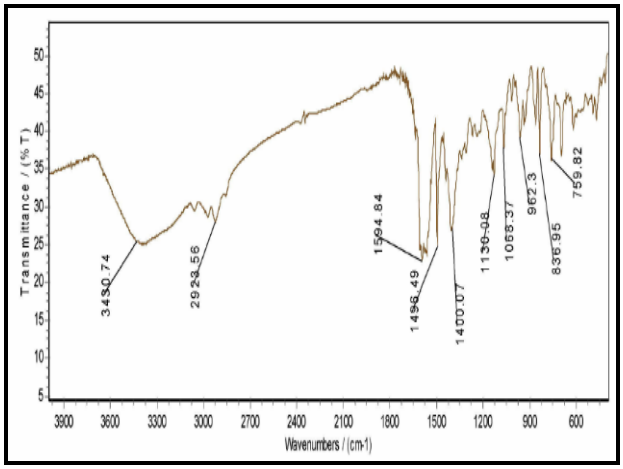


Figure 6: FTIR Spectra of combination of MNKT Na and Poly vinyl alcohol

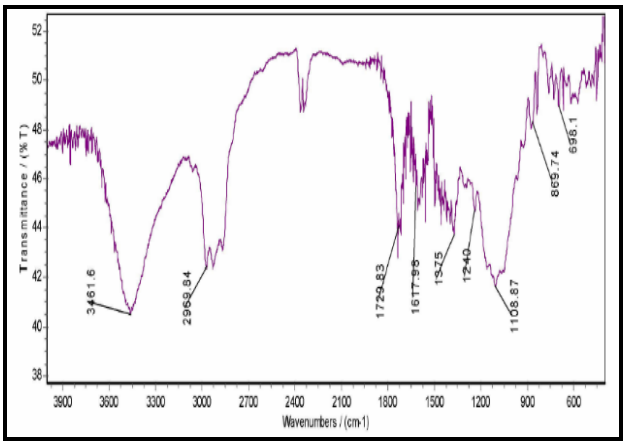


Figure 7: FTIR Spectra of MNKT Microparticles

The FTIR for drug and drug-polymer complex were shown above.

Major frequencies of functional groups of pure drug remained unchanged in presence of polymer under study; hence there is no major interaction between the drug and polymer used in the study. The interpretation was given in Table 3.

Drug – Excipients Compatibility Study by DSC

The drug exhibited a sharp melting endotherm at 115.70 °C. The DSC thermogram of Montelukast Na and Polycaprolactone is depicted in figure 6.10. No change in the melting peak of drug was observed in mixture. From this it was concluded that there was absence of interaction between drug and selected excipients.

Table 3: Interpretation of FT-IR of Drug with all Excipients

Group	MNKT Na (cm^{-1})	MNKT+PCL (cm^{-1})	MNKT+EC (cm^{-1})	MNKT+PVA (cm^{-1})	MNKT Microparticles (cm^{-1})
O-H stretching	3423.53	3411.46	3436.53	3430.73	3461.60
C-H stretching	2900.41	2923.56	2923.56	2923.56	2969.84
C=O stretching	1652.70	1623.53	1646.32	1618.80	1617.98
C-N stretching	1139.72	1130.08	1130.08	1130.08	1108.87
C-O stretching	1099.23	1054.87	1068.37	1068.37	1087.21
C-Cl stretching	877.45	750.17	836.95	836.95	869.74

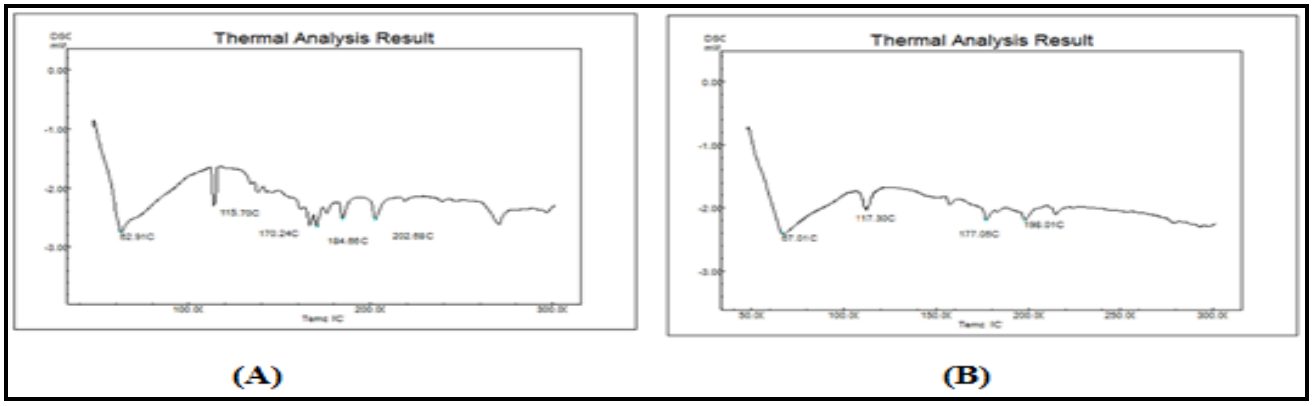


Figure 6: DSC Thermogram of (a) Standard MNKT Na (b) MNKT Na and Polycaprolactone

Evaluation of Experimental Design Batches

Percentage Yield

Microparticles prepared with PCL and Ethyl Cellulose alone was found to have much less % Yield. The % Yield was found to be increased when both PCL and Ethyl Cellulose were used together so Microparticles prepared using combination of PCL and Ethyl Cellulose show highest % yield (88.66%) in batch M13.

Table 4: Percentage Yield of Montelukast Na Microparticles

Batches	% Yield
M1	68.04 ± 1.61
M2	83.47 ± 1.42
M3	66.92 ± 1.76
M4	82.52 ± 0.96
M5	61.92 ± 2.49
M6	71.55 ± 1.03
M7	65.69 ± 0.94
M8	88.23 ± 1.04
M9	80.22 ± 4.49
M10	77.61 ± 1.16
M11	82.53 ± 1.20
M12	71.18 ± 1.69
M13	88.66 ± 1.10
M14	87.23 ± 1.12
M15	88.45 ± 1.11

*All the reading were calculated as mean value and with standard deviation where n=3

% Drug Loading

Microparticles prepared with PCL and Ethyl Cellulose alone was found to have much less % drug loading. The % drug loading was found to be increased when both PCL and Ethyl Cellulose were used together so the microparticles prepared using combination of PCL and Ethyl Cellulose show highest % Drug Loading (65.27%) in batch M13.

Table 5: Percentage Drug Loading of Montelukast Na Microparticles

Batches	% Drug Loading
M1	44.63 ± 0.81
M2	64.32 ± 1.00
M3	48.32 ± 1.56
M4	67.27 ± 2.33
M5	39.09 ± 1.44
M6	48.27 ± 1.86
M7	41.84 ± 2.61
M8	66.64 ± 2.89
M9	62.32 ± 1.76
M10	60.02 ± 2.66
M11	63.23 ± 1.55
M12	54.06 ± 3.08
M13	67.65 ± 1.20
M14	66.03 ± 1.63
M15	65.27 ± 2.97

*All the reading were calculated as mean value and with standard deviation where n=3

Flow Property

The loose bulk density and tapped bulk density used to assess the packability of the formulations. The pure drug was more bulky, which was indicated by the lowest loose bulk density value. In contrast, the microparticles exhibited higher loose bulk density. The high tapped density value of pure drug indicates a high inter-space between drug crystals. These results indicated good packability of the prepared microparticles as compared to Montelukast Na. The Hausner's ratio was used to access compressibility property of drug. Hausner's ratio of Montelukast Na has indicated poor flow property. From the table 6, it was observed that Hausner's ratio of all the batches of microparticles were in the range of 1.08± 0.06 to 1.54± 0.3 which indicates good flow property. The value of angle of repose was used to understand flow property. Montelukast Na shows good angle of repose. Also, all the prepared microparticles exhibit good flow property in the range of 19.99 ± 0.7 to 29.01 ± 0.5 which indicates free flowing nature of microparticles.

Table 6: Flow Property of Montelukast Na Microparticles

Batch no	Bulk Density* (gm/ml)	Tapped Density* (gm/ml)	Hausner's Ratio*	Carr's Index	Angle of Repose*
M1	0.54± 0.3	0.61± 0.9	1.24± 0.5	11.48 ± 0.2	22.56± 0.6
M2	0.67± 0.2	0.78± 0.5	1.45± 0.5	14.10 ± 0.1	19.99± 0.7
M3	0.51± 0.3	0.57± 0.7	1.34± 0.2	10.53 ± 0.5	26.54± 0.8
M4	0.49± 0.5	0.59± 0.9	1.04± 0.7	16.95 ± 0.2	29.01± 0.5
M5	0.50± 0.8	0.63± 0.4	1.54± 0.3	20.63 ± 0.3	25± 0.9
M6	0.51± 0.2	0.58± 0.1	1.39± 0.3	12.07 ± 0.1	22.49± 0.9
M7	0.47 ± 0.1	0.57 ± 0.5	1.21 ± 0.1	17.54 ± 0.2	25.36 ± 0.3
M8	0.37 ± 0.1	0.53 ± 0.3	1.43 ± 0.4	30.19 ± 0.3	24.76 ± 0.5
M9	0.46 ± 0.3	0.58 ± 0.3	1.08 ± 0.6	20.69 ± 0.6	26.38 ± 0.7
M10	0.44 ± 0.6	0.53 ± 0.6	1.20 ± 0.2	16.98 ± 0.2	22.87 ± 0.4
M11	0.48 ± 0.2	0.59 ± 0.4	1.22 ± 0.3	18.64 ± 0.1	25.49 ± 0.8
M12	0.39 ± 0.6	0.51 ± 0.2	1.30 ± 0.1	23.53 ± 0.4	23.83 ± 0.7
M13	0.45 ± 0.4	0.52 ± 0.1	1.15 ± 0.5	13.46 ± 0.5	24.12 ± 0.9
M14	0.41 ± 0.1	0.51 ± 0.5	1.24 ± 0.2	19.61 ± 0.5	26.59 ± 0.5
M15	0.39 ± 0.6	0.51 ± 0.2	1.29 ± 0.1	23.53 ± 0.4	21.83 ± 0.7

*All the reading were calculated as mean value and with standard deviation where n=3

Particle Size Determination

Particle size analysis was performed by optical microscopy. Microparticles of prepared batches have different mean particle size within range of 19 to 29.76 µm.

***In-vitro* Drug Release Study**

Microparticles were evaluated for *in-vitro* drug release study as per Indian Pharmacopoeia. Result of *in-vitro* drug release study listed in table 6.14 and graph of % CDR vs Time was shown in figure 10. The highest cumulative % drug release was 99.69% for M13 batch and lowest cumulative % drug release was found 92.35% in M7 batches at a time interval of 12 hours. The cumulative % drug release was found between the above ranges for all the batches. All the batches shows control release for Montelukast Na drug up to 12 hours.

Table 7: Mean Particle Size of Montelukast Na Microparticles

Batch Code	Mean Particle Size* (µm)
M1	30.47 ± 1.01
M2	27.36 ± 3.36
M3	40.57 ± 1.25
M4	34.62 ± 1.08
M5	23.45 ± 2.06
M6	24.52 ± 1.22
M7	22.93 ± 1.68
M8	25.77 ± 1.27

M9	34.06 ± 3.38
M10	39.57 ± 2.33
M11	31.76 ± 1.37
M12	33.37 ± 1.00
M13	18.23 ± 0.88
M14	19.19 ± 1.61
M15	17.58 ± 1.08

*All the reading were calculated as mean value and with standard deviation where n=3

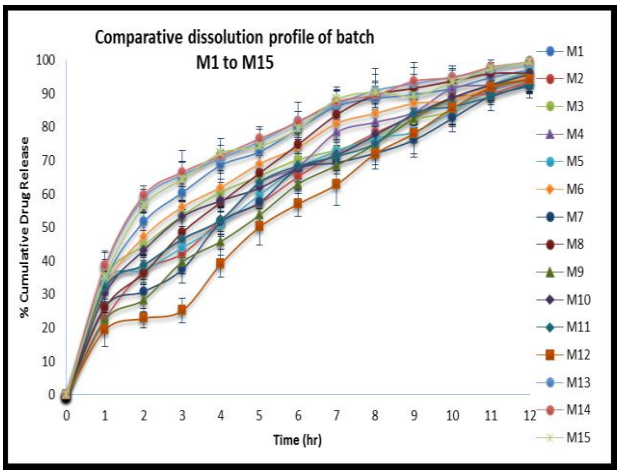


Figure 10: *In-vitro* Dissolution study of batch M1 to M15

Table 8: Results of Effect of Independent Variables on Responses

Batch Code	**X_1**	**X_2**	**X_3**	**Y_1**	**Y_2**	**Y_3**
1	1:1	5	0.4	68.04	30.47	97.15
2	1:3	5	0.4	83.47	27.36	93.68
3	1:1	15	0.4	66.92	40.57	96.24
4	1:3	15	0.4	82.52	34.62	94.63
5	1:1	10	0.3	61.92	23.45	96.89
6	1:3	10	0.3	71.55	24.52	95.07
7	1:1	10	0.5	65.69	22.93	92.35
8	1:3	10	0.5	88.23	25.77	96.03
9	1:2	5	0.3	80.22	34.06	94.03
10	1:2	15	0.3	77.61	39.57	95.91
11	1:2	5	0.5	82.53	31.76	92.98
12	1:2	15	0.5	71.18	33.37	94.15
13	1:2	10	0.4	88.66	18.23	99.69
14	1:2	10	0.4	87.23	19.19	99.36
15	1:2	10	0.4	88.45	17.58	99.46

X1 = Drug Polymerratio (mg)
X2 = Organic phase addition time (min)
X3 = Concentration of PVA(%w/v)

Y1 = % Yield
Y2 = Average particle size
Y3 = % CDR

Effect of Drug: Polymer Ratio

From the Figure 10, it was observed that batch M1 (drug: polymer ratio 1:1) % CDR was higher compared to M2 (drug: polymer ratio 1:3). So, it was concluded that change in the polymer concentration alter the release rate profile.

Effect of Organic Phase Addition Time

From the figure 10, it was observed that %CDR was higher in M13 (organic phase addition time 10 min) compared to M9 (organic phase addition time 5 min). So, it was concluded that addition of solvent at too slow speed results in slow release rate as too fast organic phase addition time solvent may diffuse into the aqueous phase before stable emulsion droplets developed and aggregation of microparticles droplets occurs which slower the release rate.

Effect of Concentration of PVA

From the figure 10, it was observed that %CDR was higher in M10 (concentration of PVA 0.3% w/v) compared to M12 (concentration of PVA 0.5% w/v). So, it was concluded that too low concentration of PVA and too high concentration of PVA results in change in the release rate profile.

Statistical Analysis

Box-behnken design was applied with three formulation variables at 3 different levels were used to study the effects on dependent variables.

Table 9: DOE Analysis and Model Selection for Response Y_1

Model Type	Sequential p-value	PRESS	Adjusted R2	Predicted R2	Remarks
Linear	0.0649	1045.1	0.323	0.1341	Suggested
2FI	0.8391	1708.64	0.1574	-0.4157	Nil
Quadratic	0.0204	1496.04	0.7807	-0.2396	Suggested
Cubic	0.0188	-	0.9931		Aliased

Table 10: ANOVA for Response Surface Quadratic Model (% Yield)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F
Model	1112.39	9	123.6	6.54	0.0262
X_1	499.44	1	499.44	26.42	0.0036
X_2	32.15	1	32.15	1.7	0.249
X_3	33.37	1	33.37	1.77	0.2414
$X_1 X_2$	6.67E-03	1	6.67E-03	3.53E-04	0.9857
$X_1 X_3$	41.69	1	41.69	2.21	0.1977
$X_2 X_3$	19.13	1	19.13	1.01	0.3607
X_1^2	330.3	1	330.3	17.47	0.0087
X_2^2	43.14	1	43.14	2.28	0.1913
X_3^2	171.1	1	171.1	9.05	0.0298
Residual	94.53	5	18.91		
Lack of Fit	93.34	3	31.11	52.28	0.0188
Pure Error	1.19	2	0.6		
Cor Total	1206.91	14			

Statistical Analysis for Response Y_1

DOE Analysis and Model Selection for Response Y_1

Various model such as linear; 2FI, quadratic and cubic models were fitted to data for the dependent response simultaneously using design expert software.

Evaluation of Response by Analysis of Variance (ANOVA)

The mathematical relationship of response Y_1, Y_2 ,Y_3 and independent variable (X_1,X_2,X_3) using quadratic model can be obtained which mentioned in equation 1.

ANOVA for drug release for % yield shown in table 10.

From the table 10, it was concluded that the model F-value of 6.54 implies the model is significant. There is only a 2.62% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob> F" less than 0.0500 indicate model terms are significant. In this case X_1, X_1^2, X_3^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

***Final Polynomial Equations in Terms of Coded Factors*:**

$Y_1 = 88.12 + 7.90 * X_1 - 2.00 * X_2 + 2.04 * X_3 + 0.041 * X_1 X_2 + 3.23 * X_1X_3 - 2.19 * X_2X_3 – 9.46 * X_1^2 + 3.42 * X_2^2 - 6.81 * X_3^2$--------- (1)

Reduced Model Equation

$Y_1 = 88.12 + 7.90 * X_1 – 9.46 * X_1^2 - 6.81 * X_3^2$

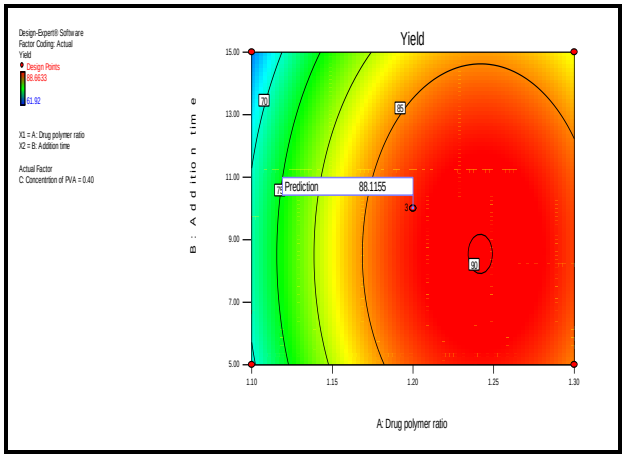
Contour Plot and Response Surface Plot for % Yield (Y_1)

Contour Plot and Response Surface Plot were drawn using design expert software version 8.0.5.

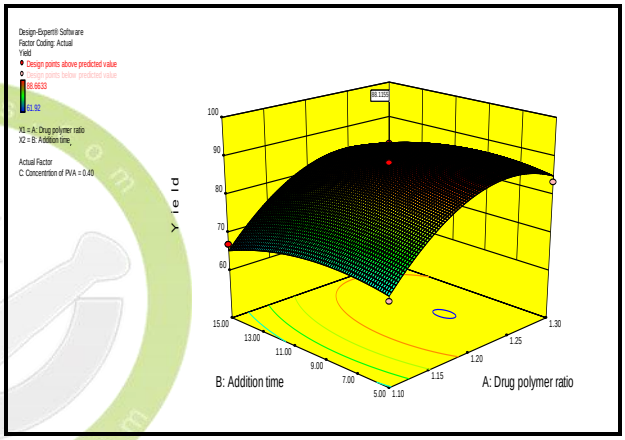
Statistical Analysis for Response Y_2

DOE Analysis and Model Selection for Response Y_2

Various model such as linear, 2FI, quadratic and cubic models were fitted to data for the dependent response simultaneously using design expert software.

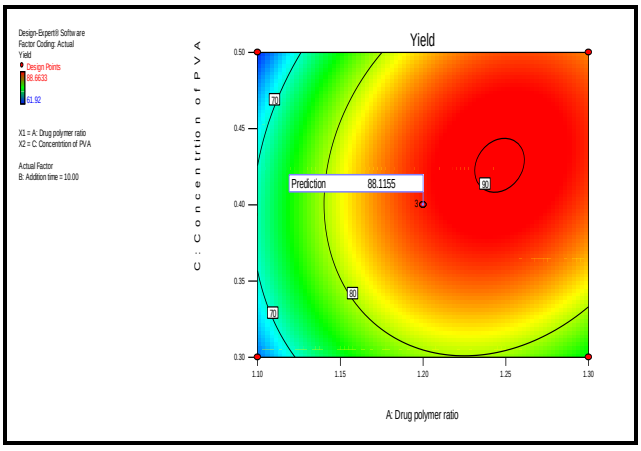


(A)

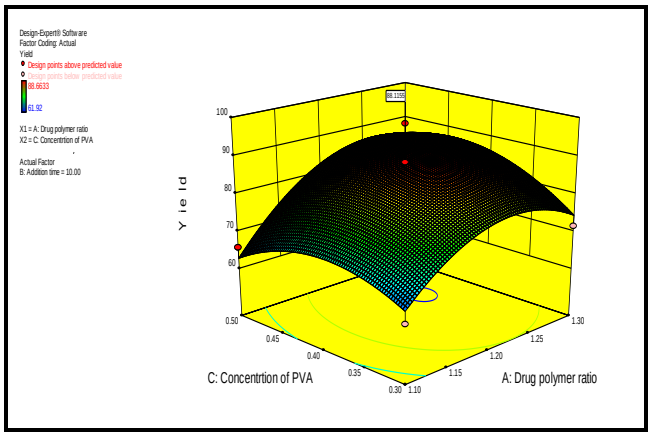


(B)

Figure 13: Contour Plot (A), Response Surface Plot (B) Showing the Effect of Drug: Polymer Ratio (X_1) and Organic Phase Addition Time (X_2) on yield (Y_1)

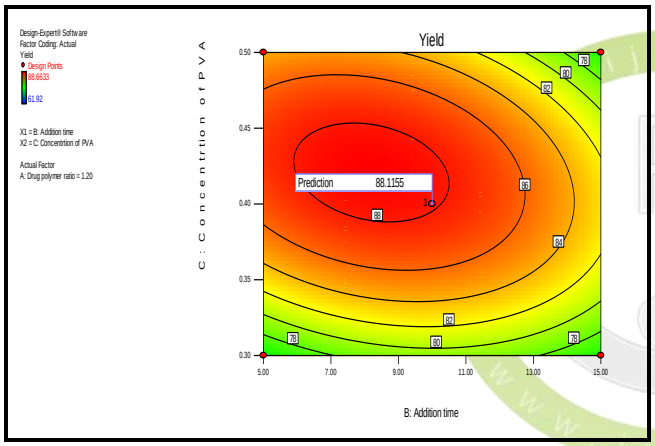


(A)

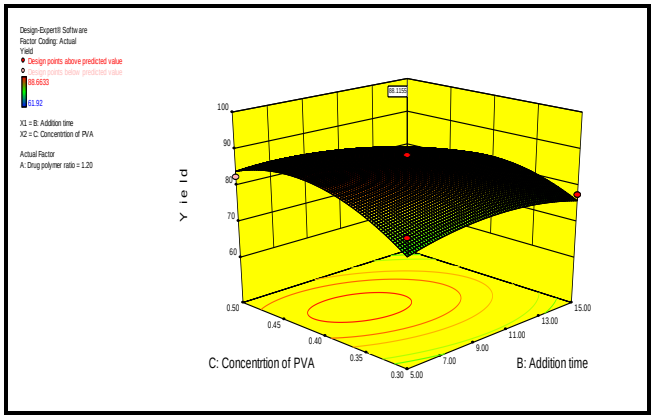


(B)

Figure 14: Contour Plot (A), Response Surface Plot (B) Showing the Effect of Drug: Polymer Ratio (X_1) and Concentration of PVA (X_3) on yield (Y_1)



(A)



(B)

Figure 15: Contour Plot (A), Response Surface Plot (B) Showing the Effect of Organic Phase Addition Time (X_2) and Concentration of PVA (X_3) on yield (Y_1)

Evaluation of Response by Analysis of Variance (ANOVA)

The mathematical relationship of response Y_1, Y_2, Y_3 and independent variable (X_1, X_2, X_3) using quadratic model can be obtained which mentioned in equation 2.

ANOVA for drug release for % yield shown in table 13. From the table 13, it was concluded that the model F-value of 8.68 implies the model is significant. There is only a 1.42% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case X_2, X_2^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

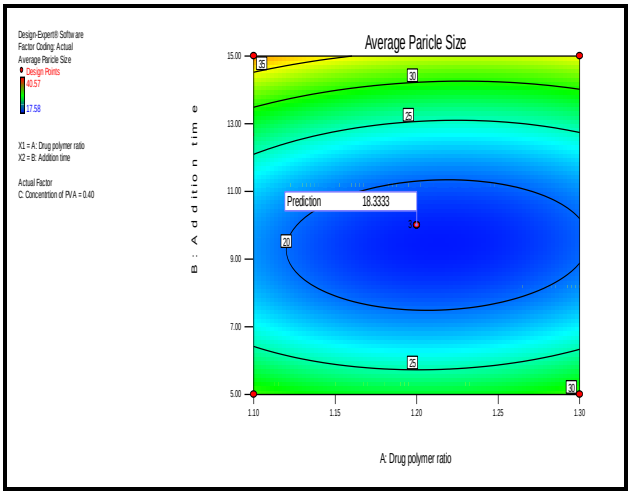
Final Polynomial Equations in Terms of Coded Factors:

$$Y_2 = 18.33 - 0.64 * X_1 + 3.06 * X_2 - 0.97 * X_3 - 0.71 * X_1X_2 + 0.44 * X_1X_3 - 0.98 * X_2X_3 + 2.20 * X_1^2 + 12.72 * X_2^2 + 3.63 * X_3^2 \text{ -------- (2)}$$

Reduced Model Equation

$$Y_2 = 18.33 + 3.06 * X_2 + 12.72 * X_2^2$$

Contour Plot and Response Surface Plot for Average Particle Size (Y_2)



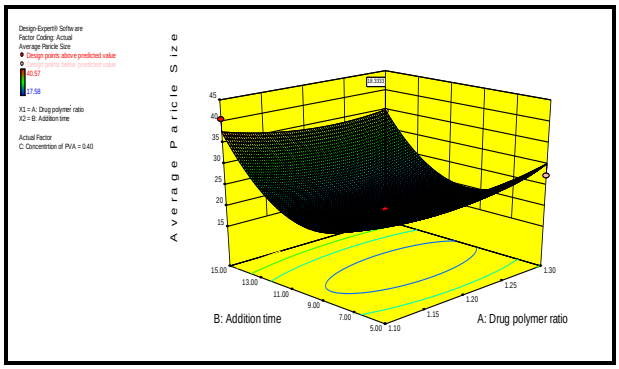
(A)

Table 12: DOE Analysis and Model Selection for Response Y_2

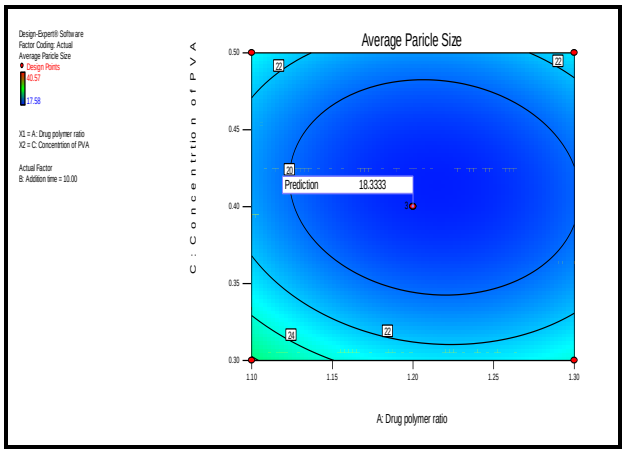
Model Type	Sequential p-value	PRESS	Adjusted R^2	Predicted R^2	Remarks
Linear	0.7144	1164.03	-0.1303	-0.5191	Nil
2FI	0.9938	2355.71	-0.539	-2.0744	Nil
Quadratic	0.0024	719.49	0.8316	0.061	Suggested
Cubic	0.0424	-	0.988		Aliased

Table 13: ANOVA for Response Surface Quadratic Model (Average particle size)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F
Model	720.15	9	80.02	8.68	0.0142
X_1	3.32	1	3.32	0.36	0.5749
X_2	74.91	1	74.91	8.13	0.0358
X_3	7.55	1	7.55	0.82	0.4071
X_1 X_2	2.02	1	2.02	0.22	0.6597
X_1 X_3	0.78	1	0.78	0.085	0.7824
X_2 X_3	3.8	1	3.8	0.41	0.549
X_1^2	17.86	1	17.86	1.94	0.2227
X_2^2	597.61	1	597.61	64.82	0.0005
X_3^2	48.78	1	48.78	5.29	0.0698
Residual	46.1	5	9.22		
Lack of Fit	44.78	3	14.93	22.75	0.0424
Pure Error	1.31	2	0.66		
Cor Total	766.25	14			

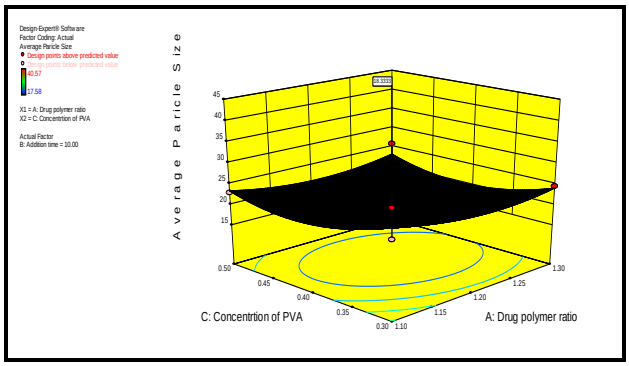


(B)



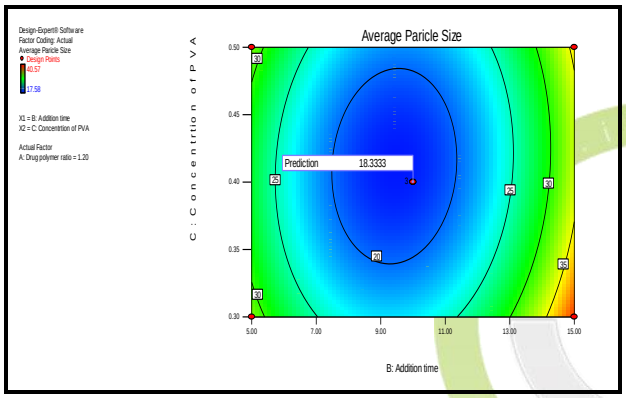
(A)

Figure 16: Contour Plot (A), Response Surface Plot (B) Showing the Effect of Drug: Polymer Ratio (X_1) and Organic Phase Addition Time (X_2) on Average Particle Size (Y_2)

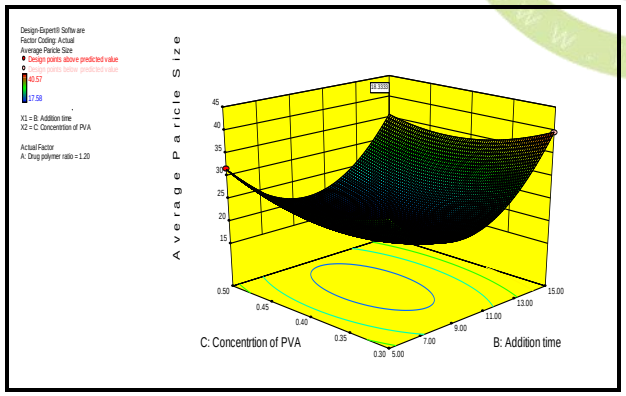


(B)

Figure 17: Contour Plot (A), Response Surface Plot (B) Showing the Effect of Drug: Polymer Ratio (X_1) and Concentration of PVA(X_3) on Average Particle Size (Y_2)



(A)



(B)

Figure 18: Contour Plot (A), Response Surface Plot (B) Showing the Effect of Organic Phase Addition Time (X_2) and Concentration of PVA (X_3) on Average Particle Size (Y_2)

Statistical Analysis for Response Y_3

DOE Analysis and Model Selection for Response Y_3

Various model such as linear, 2FI, quadratic and cubic models were fitted to data for the dependent response simultaneously using design expert software.

Evaluation of Response by Analysis of Variance (ANOVA)

The mathematical relationship of response Y_1, Y_2, Y_3 and independent variable (X_1, X_2, X_3) using quadratic model can be obtained which mentioned in equation 3.

ANOVA for drug release for % yield shown in table 16.

From the table 16, it was concluded that the model F-value of 8.68 implies the model is significant. There is only a 1.42% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case X_2, X_2^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

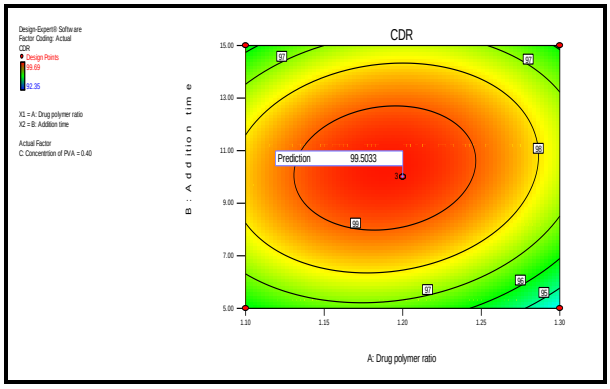
Final Polynomial Equations in Terms of Coded Factors

$$Y_3 = 99.50 - 0.40 * X_1 + 0.39 * X_2 - 0.80 * X_3 + 0.46 * X_1X_2 + 1.38 * X_1X_3 - 0.18 * X_2X_3 - 1.63 * X_1^2 - 2.45 * X_2^2 - 2.79 * X_3^2 \text{ -------- (3)}$$

Reduced Model Equation

$$Y_3 = 99.50 + 0.39 * X_2 - 2.45 * X_2^2$$

Contour Plot and Response Surface Plot for % CDR (Y_3)



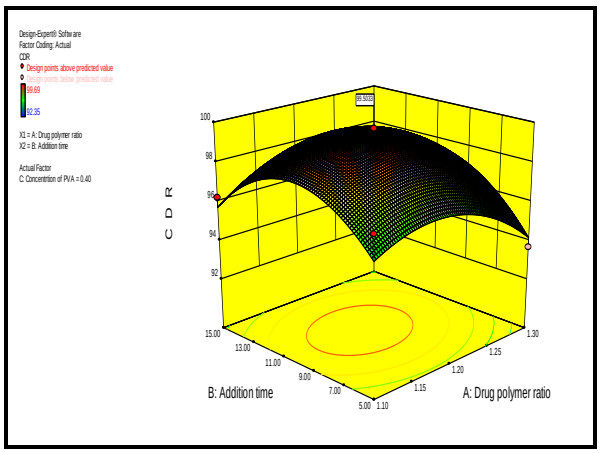
(A)

Table 15: DOE Analysis and Model Selection for Response Y_3

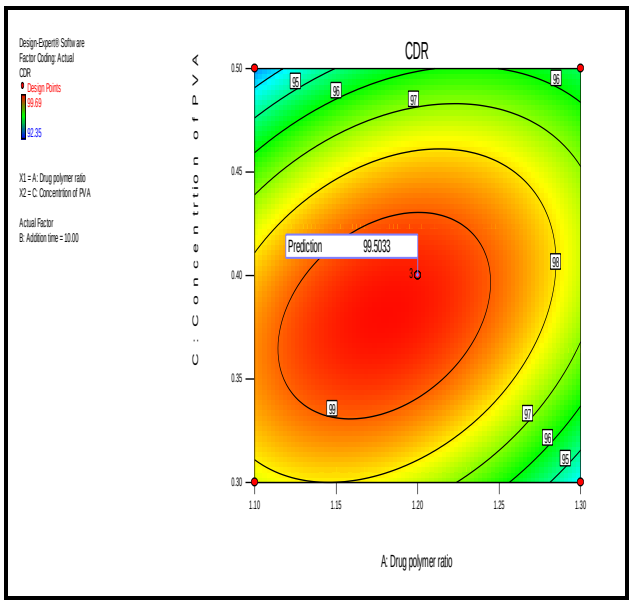
Model Type	Sequential p-value	PRESS	Adjusted R2	Predicted R2	Remarks
Linear	0.7532	107.65	-0.1465	-0.4062	Nil
2FI	0.7718	153.38	-0.3809	-1.0036	Nil
Quadratic	0.0098	115.76	0.7336	-0.5122	Suggested
Cubic	0.0118	-	0.9948		Aliased

Table 16: ANOVA for Response Surface Quadratic Model (% CDR)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F
Model	69.27	9	7.7	5.28	0.0407
X_1	1.3	1	1.3	0.89	0.3889
X_2	1.19	1	1.19	0.82	0.4069
X_3	5.1	1	5.1	3.5	0.1201
X_1 X_2	0.86	1	0.86	0.59	0.4758
X_1 X_3	7.56	1	7.56	5.19	0.0717
X_2 X_3	0.13	1	0.13	0.087	0.7805
X_1^2	9.82	1	9.82	6.74	0.0485
X_2^2	22.13	1	22.13	15.19	0.0114
X_3^2	28.7	1	28.7	19.7	0.0068
Residual	7.28	5	1.46		
Lack of Fit	7.23	3	2.41	84.13	0.0118
Pure Error	0.057	2	0.029		
Cor Total	76.55	14			

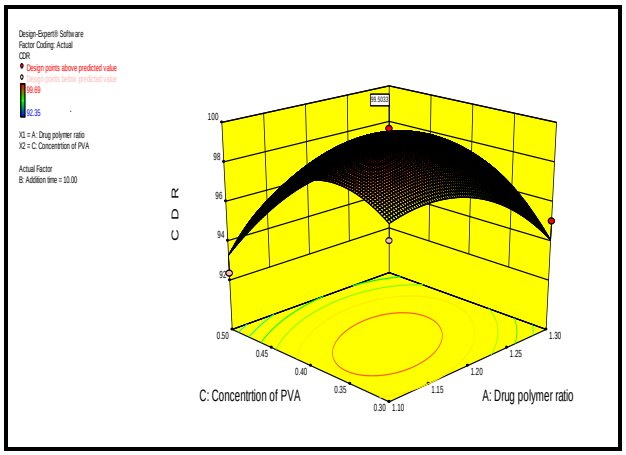


(B)



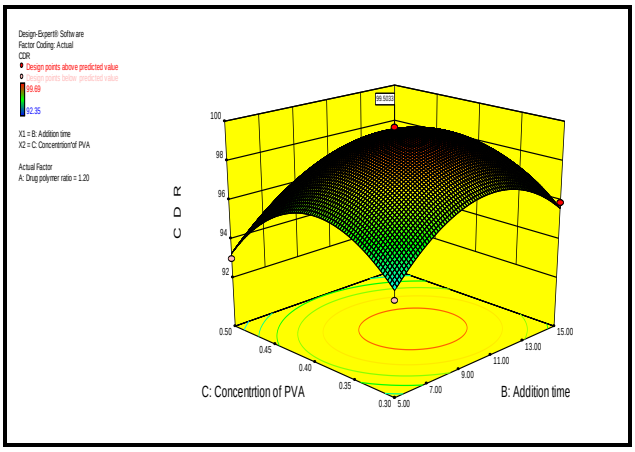
(A)

Figure 19: Contour Plot (A), Response Surface Plot (B) Showing the Effect of Drug: Polymer Ratio (X_1) and Organic Phase Addition Time (X_2) on %CDR (Y_3)



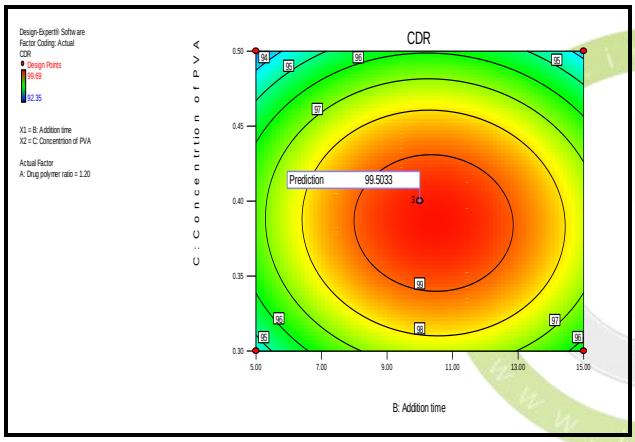
(B)

Figure 20: Contour Plot (A), Response Surface Plot (B) Showing the Effect of Drug: Polymer Ratio (X_1) and Concentration of PVA (X_3) on % CDR (Y_3)



(B)

Figure 21: Contour Plot(A), Response Surface Plot (B) Showing the Effect of Organic Phase Addition Time (X_2) and Concentration of PVA (X_3) on % CDR (Y_3)



(A)

Check Point Analysis

Besides understanding the main and interaction effects on the responses, the experimental design approach is helpful in obtaining the optimized formula in which the levels of X_1, X_2 and X_3 were decided. As a confirmation of process, a new formulation was prepared at the optimum levels of the independent variables and evaluated. The observed value of responses of Y_1, Y_2 and Y_3 gave a close agreement with the predicted values. Three checkpoint batches were prepared and evaluated for % yield, average particle size and % CDR as shown in table 6.26.

Table 18: Validation of Design by Using Check Point Analysis

Batch No	Independent variables			Observed Response			Predicted Response		
	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_1	Y_2	Y_3
M16	+0.5	-0.5	0	87.62	21.66	99.25	89.64	19.51	98.15
M17	+0.5	0	-0.5	88.03	20.38	97.58	86.19	19.77	98.25
M18	0	-0.5	+0.5	87.67	22.43	98.54	88.13	20.64	97.64

Results indicate that the measured % yield, average particle size and % CDR values were as expected. Thus we can conclude that the obtained mathematical equation was valid for predicting the responses.

Optimization of Box Behnken Design

Validation of box behnken design is necessary for confirmation of applied model. Optimized batch M19 contains drug: polymer ratio 1:2, organic phase addition time 10 min, Concentration of PVA 0.4 % was formulated and evaluated for different physico chemical parameter to calculate the design. All the parameters of optimized batch are as per requirement.

With multiple responses it is necessary to find regions where requirements simultaneously meet the critical properties. Graphical optimization displays the area of feasible response values in the factor space. The area that satisfies the constraints will be yellow, while the area that does not meet the criteria is grey.

Table 19: Composition of Optimized Batch M19

Ingredients	M19
Drug: Polymer Ratio (mg)	1:2
Organic phase addition time (min)	10 min
Concentration of PVA (% w/v)	0.4%
Solvent	Dichloromethane

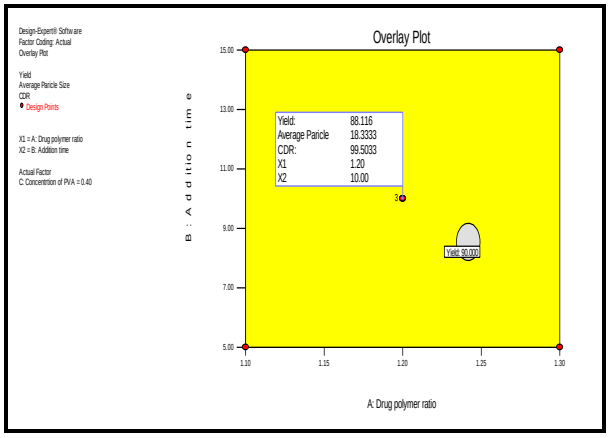


Figure 22: Overlay Plot for Optimized Batch

From the box behnken design it is expected that the % yield, average particle size and % drug release value of the check point batch can be 88.11, 18.33 and 99.50% respectively. It indicates that the result can be obtained as expected. Thus, it can conclude that the statistical model is mathematically valuable.

Surface Topography of Optimized Batch

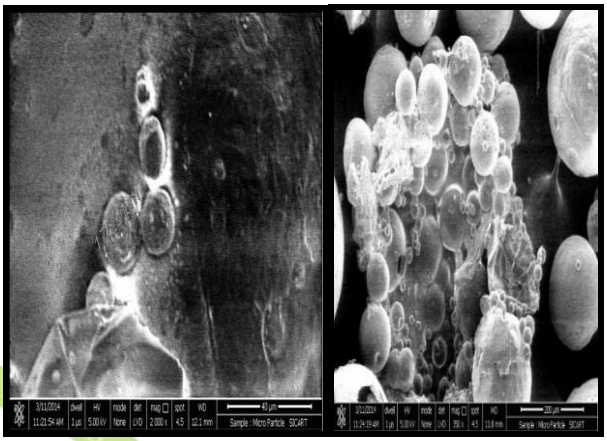


Figure 23: SEM Micrograph Showing the Morphology of Montelukast Na Microparticles

Surface morphology study was performed by Scanning Electron Microscope (SEM) for final formulation of Montelukast Na microparticles. Microparticles shows particles in good shape.

Particle Size Analysis of Optimized Batch

It shows X_{10}= 15.81µm indicate that 10% particles are of 15.81µm and 90% are of greater than 15.81µm. X_{50}= 87.08µm indicate that 50% particles are of 87.08µm and 50% are of greater than 87.08µm. X_{90}= 286.92µm indicate that 9% particles are of 286.92µm while 10% are of greater than 286.92µm.

Application of Release Kinetic Model

The zero-order rate describes the systems where the drug release rate is independent of its concentration. Figure 24 shows the cumulative amount of drug release v/s time for zero-order kinetics.

The first order which describes the release from systems where the release rate is concentration dependent is illustrated by Figure 25, which shows the log cumulative percent drug remaining v/s time.

Higuchi's model describes the release of drugs from an insoluble matrix as a square root of a time-dependent process based on Fickian diffusion. Figure 26 illustrates the Higuchi square root kinetics, showing the cumulative percent drug release v/s the square root of time. The dissolution data were also plotted in accordance with the Hixson-Crowell cube root law, the applicability of the formulation to the equation indicated a change in surface area and diameter of the tablets with the progressive dissolution of the matrix as a function of time (Figure 27).

To evaluate the mechanism of drug release from microparticles, The drug release were plotted in Korsmeyer equation as log cumulative percentage of drug released v/s log time (Figure 28), and the exponent n was calculated through the slope of the straight line. The value of n indicates the drug release mechanism related to the geometrical shape of the delivery system, if the exponent n = 0.5, then the drug release mechanism is Fickian diffusion. If n <0.5 the mechanism is Quasi-Fickian diffusion, and 0.5 < n < 1.0, then it is non-Fickian or anomalous diffusion and when n = 1.0 mechanism is non Fickian case II diffusion, n> 1.0 mechanism is non Fickian super case II. Here, the n value for M19 batch was <0.5 (Table 6.25) so it indicate that release mechanism from montelukast Na microparticles followed by Fickian transport.

Table 20: Results of Model Fitting for Optimized Batch

Model	**Parameters Used**				
	R^2	**r**	**k**	**SSR**	**AIC**
Zero-order	0.3252	0.8868	10.608	6456.4341	116.0468
First-order	0.9695	0.9892	0.340	291.4716	75.7743
Higuchi	0.9308	0.9796	31.812	661.7116	86.4328
KorsmeyerPeppas	0.9932	0.9966	44.313 n=0.33508	64.6786	58.2026
Hixson Crowell model	0.9242	0.9794	0.089	725.7258	87.6332

Table 21: Stability Study of Optimized Batch

Parameters	**At 0 month**	**At 1 month**
Physical appearance	Fine microparticles with no agglomeration	No change
% yield	87.98 ± 1.15	86.29 ± 2.35
Average particle size	19.25 ± 0.73	20.43 ± 1.47
% CDR	99.03 ± 4.47	98.87 ± 5.49
Drug Loading (%)	66.79 ± 2.59	65.39 ± 3.59

Stability Study of Optimized Batch

To assess the drug and formulation stability, stability studies were done according to ICH guidelines. The optimized formulation M19 was selected for stability study. In the present study, stability studies were carried out at 40°C/75% RH in closed high density polyethylene bottles for 1 months. The samples were withdrawn after periods of 1 month evaluated for physical appearance, % yield, average particle size, % CDR, drug loading (%) during the stability studies as shown in table 21.

CONCLUSION

Montelukast Na microparticles containing can be prepared successfully by using a solvent evaporation method. By varying the drug: polymer ratios, is found to influence the size, % Yield and release characteristics of the microparticles. The release kinetics discovered that drug release from microparticles was found to be Quasi-Fickian diffusion. The morphological analysis by scanning electron microscopy revealed that the polymers used in the formulations conferred particular surface characteristics to the polymeric microparticles. These characteristics play a critical role in assessing the drug release. Thus, it is possible to suggest that the polymeric microparticles offer a good system to control drug release, being an attractive alternative for essential anti-asthmatic treatment.

REFERENCES

1. Varshney H., & Sonagra P. (2012). Sustained Release Oral Drug Delivery System - An Overview. *International Journal of Pharmaceutical Research and Bio- science*, 1(6), 27-43.
2. Patel H.K., & Patel P.R., Brahmbhatt T.J. (2011).Sustained release microparticle: a review. *American Journal of Pharmatech Research*, 1(4), 108-126.
3. Satheesh N.V., & Kala M.S. (2011). Review on Microparticulate Drug Delivery System. *International Journal of PharmTech Research*, 3(3), 1242-1254.
4. Basu S.K., Kunchu K., & Mani R. (2011). Evaluation of Ionotropic Cross-Linked Chitosan/Gelatin B Microspheres of Tramadol Hydrochloride. *AAPS Pharm SciTech*, 12(1), 28-34.
5. Ozlem Y.C., & Emel O.C. (2012). In vitro release kinetics of polycaprolactone encapsulated plant extract fabricated by supercritical antisolvent process and solvent evaporation method. *The Journal of Supercritical Fluids*, 62, 219– 225.
6. O'Donnell P.B., & McGinity J.W., (1997). Preparation of microspheres by the solvent evaporation technique. *Advanced Drug Delivery Reviews*, 28 (1), 25- 42.
7. Kate V. K., & Payghan S.A. (2013). Development of Directly Compressible Mucoadehsive Fast Disintegrating Sublingual Tablet System of Piroxicam Using 3 factor, 3 Level Box Behnken Design.*Asian Journal of Biomedical and Pharmaceutical Sciences*, 3(27), 19-29.
8. Shah C.V., & Patel H.K. (2012).Design, development and optimization of valsartan liquisolid tablets using box-behnken design" *Indian Journal of Pharmaceutical Science and Research*, 3(8), 2741-2753.
9. Singh A., Sharma R., Jamil F. (2012). Sustained release drug delivery system: a review. *International Research Journal of Pharmacy*, 3(9), 21-24.
10. Sathali A.H. (2012). Preparation and Evaluation of Montelukast Sodium Loaded Solid Lipid Nanoparticles. *Journal of Young Pharmacists*, 4(3), 129-137.
11. Giri P.B., Das P. (2011). Formulation and Evaluation of Chewable tablets of Montelukast. *Indian Journal of Research in Pharmacy and Biotechnology***,** 1(1) , 29-34
12. O'Donnell P.B. & McGinity J.W. (1997). Preparation of microspheres by the solvent evaporation technique. *Advanced Drug Delivery Reviews*, 28(1), 25- 42.
13. Patel S. B., & Patel V.R. (2012). Formulation and Evaluation of

Microparticles for Controlled Delivery of Tramadol Hydrochloride. *International Journal for Pharmaceutical Research Scholars*, 1(2), 136-144.

14. Rajesh M., & Kumar B. K. (2013). Formulation and Evaluation of Metformin Hydrochloride Microspheres by Solvent Evaporation Method. *International Journal for Pharmaceutical Research Scholars*, 2(1), 122-127.
15. ICH GUIDELINES Q1A(R2), Guidance for industry, Stability testing of new drug substance and product, World Health Organization, WHO Technical Report Series, No 953, 2009. http:/www.ich.org.

