Formulation Development and Optimization of Sustained Release Microspheres of Acebrophylline

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Authors' contributions

This work was carried out in collaboration among all authors. Author NS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AS and HJ managed the analyses of the study. Author HJ managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Objectives: Aim of present work is to prepare and evaluate Sustained release microspheres of Acebrophylline for treatment of Asthma.

Experimental work: In present investigation, attempt was made to prepare sustained release microspheres of Acebrophylline with different polymer ratio using Ionic gelation method. Drug-excipient compatibility studies were performed by FTIR. The best suited Microspheres formulation was found on the basis production yield, entrapment efficiency and in vitro release study. Optimized batch of microspheres (B2) was characterized for FTIR, DSC, and SEM analysis. The drug release data of optimized batch was fitted into different release kinetic models. The optimized batch of microspheres (B2) was subjected for the short term stability study at 40 ± 2°C with RH of 75% for a period of 1 month.

Results and discussion: There was no interaction found between drug and excipients. Sodium alginate (2%) concentration, Eudragit RS-100 (1:2) ratio gave highest sustainable property and CaCl₂ (2.5%) concentration had a good cross linking property. This observation done on the basis of production yield, entrapment efficiency and In vitro release study. The Microspheres prepared from Ionic gelation method had Drug: Eudragit RS100 (1:2), 2 % Sodium alginate and 2.5 % CaCl₂.
(B2) give 99.2% drug release over the periods of 12 hr. The drug release from optimized microspheres formulation (B2) follows first order release kinetic. DSC study showed the melting behavior of drug present into microspheres. SEM studies showed that optimized microspheres were spherical and rough surface. Stability study proved that optimized formulation (B2) was stable.

Conclusion: Drug: Polymer ratio and Volume of CaCl₂ had significant effect on % Entrapment efficiency and Drug release. From the Scanning Electron Microscopy (SEM) study observed that microspheres was spherical and rough surface. Non Fickian diffusion was the mode of drug release from Acebrophylline- loaded microspheres. After stability study no physical changes & almost same drug release was observed in microspheres. Hence, the formulation B2 was stable.

Keywords: Acebrophylline; sustained release; sodium alginate; eudragit RS100; ionic- gelation method.

1. INTRODUCTION

The goal in designing sustained delivery systems is to reduce the frequency of the dosing or to increase effectiveness of the drug by localization at the site of action, reducing the dose required or providing uniform drug delivery. So, sustained release dosage form is a dosage form that release one or more drugs continuously in a predetermined pattern for a fixed period of time, either systemically or to a specified target organ.[1] Sustained release dosage forms provide a better control of plasma drug levels, less dosage frequency, less side effect, increased efficacy and constant delivery.[2] These systems also provide a slow release of drug over an extended period of time and also can provide some control, whether this be of a temporal or spatial nature, or both, of drug release in the body, or in other words, the system is successful at maintaining constant drug levels in the target tissue or cells. Sustained release indicates an initial release soon after administration, and then a gradual release over an extended period.[3]

1.1 Micro Particles

It is defined as a microscopic or tiny particles ranging in size from 1 μm to 1000 μm. Micro particulate delivery system are intended for oral and topical use.

There are two types of micro particles F1) Microcapsules: Micrometric Reservoir systems 2) Microspheres: Micrometric Matrix systems.

Microspheres are small spherical particles, with diameters 1 μm to 1000 μm. They are spherical free flowing particles consisting of proteins or synthetic polymers which are biodegradable in nature.[4]

Acebrophylline, chemically designed as ambroxol theophylline-7-acetate, is a compound with potent bronchodilator, mucosecretolytic, and anti-inflammatory activity. It is used to treat bronchial asthma and chronic obstructive pulmonary disease.

2. MATERIALS AND METHODS

2.1 Materials

Acebrophylline was obtained as a gift sample from Kores India, Ltd., Sodium alginate was a gift sample from Signet Chemical Co, India. All other ingredients used were of Analytical grade.

2.2 Method of Preparation

2.2.1 Preparation of microspheres by Ionic gelation method

Specified quantity of sodium alginate was dissolved in sufficient quantity of distilled water to form a homogeneous polymer solution, and then specified quantity of polymer was added to it and uniformly mixed with the help of magnetic stirrer. Lastly the drug Acebrophylline was added to polymer solution and mixed to form a smooth viscous dispersion, the resulted dispersion was added drop wise into 5% CaCl₂ solution by using 20G needle. The Droplets retained in CaCl₂ solution to complete curing the reaction & to produce spherical rigid particle. Then it was filtered and dried [5,6,7].

2.2.2 Composition table for optimization of CaCl₂ Concentration

Below is the table showing the quantity of ingredients to be taken for Ionic gelation method.
Table 1. Composition table for optimization of CaCl$_2$ Concentration

| Ingredients                      | Ionic-Gelation |
|----------------------------------|----------------|
| ACB : Eudragit RS 100            | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 |
| Sodium Alginate (%)              | 2  | 2  | 2  | 2  | 2  | 2  | 2  | 2  | 2  |
| Calcium chloride (%)             | 2.5| 2.5| 2.5| 5  | 5  | 5  | 7.5| 7.5| 7.5|

(ACB - Acebrophylline)

3. EVALUATION STUDY

3.1 Preformulation Parameters

3.1.1 Organoleptic characteristics

These are preliminary characteristics of any substance which is useful in identification of specific material. Following physical properties of Acebrophylline were studied: i) Color ii) Odor.

3.1.2 Identification of drug by FTIR

The FTIR studies were carried out by the pressed pellet technique using a KBr press in which the KBr was taken and kept in a hot air oven for two hours for the removal of any moisture. The above dried KBr was taken in the preparation of pellets of drug, and optimized batch of microspheres. The prepared pellet was placed in the sample holder and kept in the instrument and scanning the sample in the wave number range 400 - 4000 cm$^{-1}$ and obtained fingerprint was compared with the reference standard.

3.1.3 Differential scanning calorimetry of drug

Assessment of possible incompatibilities between active drug substance and different excipients forms an important part of the Preformulation stage. DSC allows the fast evaluation of possible incompatibilities, because it shows changes in the appearance, shift of melting endotherms and isotherms, and/or variations in the corresponding enthalpies of reaction. The DSC thermo gram of pure drug and optimized batch of microspheres was recorded. Accurately weighed samples (5mg) were loaded into aluminum pans and sealed. All samples were run at a heating rate of 10$^\circ$C/min. over a temperature range 0-350$^\circ$C in the atmosphere of nitrogen.

3.1.4 Solubility study of drug

The drug solubility study was carried in water and different buffer solutions with pH 1.2, 6.8. The excess amount of drug was added in the buffer solution to make saturated solution. Then saturated drug solutions were sonicated thrice, each time for 10 min. The solutions of Acebrophylline were kept overnight for attainment of equilibrium with solvent. Prepared solutions were filtered using what man filter paper no 42. The filtrate was analyzed spectrophotometrically at 271 nm using the respective medium as the blank. The amount of drug dissolved was quantified from the calibration curve.

3.1.5 Flow properties

The powder sample of Acebrophylline/Microspheres was evaluated for their flow properties like Bulk density, Tapped density, Carr’s index, Hausner’s ratio, and Angle of repose [8,9,10,11]

4. FORMULATION PARAMETERS

4.1 Flow Properties

All prepared microspheres were evaluated for their flow properties.

4.1.1 Particle size analysis

Particle size distributions of the microspheres are determined by optical microscopy using calibrated ocular eyepiece. Product dispersed in light liquid paraffin and a smear of the dispersion is observed under compound microscope. The size of 100 microspheres is measured in each case against a calibrated eyepiece in micrometer.

4.1.2 % Practical yield

The yield of microspheres was determined by comparing the whole weight of microspheres formed against the combined weight of the copolymer and drug.

% Practical yield = (Mass of microspheres obtained / Total weight of drug and polymer used) X 100
4.1.3 Drug entrapment efficiency

Microspheres equivalent to 20 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 6.8 Phosphate buffer repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using 6.8 PB. The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically at 271 nm against 6.8 Phosphate buffer as a blank. The amount of drug entrapped in the microspheres was calculated by the following formula,

\[
\% \text{ Drug Entrapment} = \left( \frac{\text{Calculated drug content}}{\text{Theoretical drug content}} \right) \times 100
\]

Drug loading was calculated by the following formula,

\[
\% \text{ Drug Loading} = \left( \frac{\text{Weight of drug in microspheres}}{\text{Weight of microspheres}} \right) \times 100
\]

4.1.4 In vitro drug release studies

In Vitro drug release studies was performed using USP dissolution test apparatus (Type 1). The dissolution studies was performed in 900ml of dissolution medium which is stirred at 50 rpm at 37±0.5°C following a pH progression method. i.e. pH 1.2 for first 2h, pH 6.8 for rest of studies. Aliquots were withdrawn periodically and replaced with fresh medium.

4.2 Release Kinetics

In order to investigate the mode of drug release from microspheres the release data are analyzed with the following mathematical models: zero-order, first-order, and Higuchi and Korsmeyer poppas’model.

4.2.1 Optimization of microspheres by using 3² factorial design method

For the purpose of optimization,

\[X_1= \text{Concentration of Eudragit RS 100(\%)}\]
\[X_2= \text{Concentration of CaCl}_2(\%)}\]
\[Y_1= \text{Entrapment Efficiency (\%)}\]
\[Y_2= \text{Drug release (\%)}\]

4.2.2 Characterization of optimized batch of microspheres by using different techniques

FTIR & DSC: Prepared Optimized batch of microspheres were characterized by FTIR & DSC as described

4.2.3 Scanning electron microscopy

The surface structure of microspheres was examined using scanning electron microscopy (SEM- JSM 5000) technique. The prepared microspheres were coated with gold palladium under an argon atmosphere at room temperature, and then SEM images were recorded at the required magnification.

4.3 Stability Study

Stability study was conducted for prepared microspheres formulation batch B2 as per ICH guidelines, kept at 40 ± 2°C with RH of 75% for a period of 30 days in stability chamber. The optimized batch of microspheres were placed in USP type-1 flint vials and hermetically closed with bromo butyl rubber plugs and sealed with aluminum caps. Formulation was evaluated after one month period for entrapment efficiency and drug release.[12,13,14,15,16].

| Variables                      | -1 | 0  | +1 | Responses |
|--------------------------------|----|----|----|-----------|
| \(X_1= \text{Concentration of Eudragit Rs100(\%)}\) | | | | \(Y_1= \text{Entrapment Efficiency (\%)}\) |
| \(X_2= \text{Concentration of CaCl}_2(\%)}\) | | | | \(Y_2= \text{Drug release (\%)}\) |

5. RESULTS AND DISCUSSION

5.1 Preformulation Studies

5.1.1 Organoleptic properties

| Properties       | Results           |
|------------------|-------------------|
| Description      | White Crystalline Powder |
| Odor             | Odorless          |
| Color            | White             |
| State            | Solid             |
5.1.2 Identification of drug by FTIR

Fig. 1. FTIR of Acebrophylline

Fig. 2. FTIR spectra of physical mixture of Drug + Sodium alginate

Fig. 3. FTIR spectra of physical mixture of Drug + Eudragit RS 100

Fig. 4. FTIR spectra of physical mixture of Drug + Sodium alginate+ Eudragit RS
5.2 Interpretation

Table 4. Interpretation of FTIR graphs

| Functional groups | Drug          | Drug + Sodium alginate | Drug + Eudragit RS 100 | Drug + Sodium alginate + Eudragit RS 100 |
|-------------------|---------------|------------------------|------------------------|------------------------------------------|
| -N-H group        | 1229.42       | 1229.22                | 1229.54                |                                          |
| -N-H2 group       | 3449.46       | 3448.53                | 3449.32                |                                          |
| -OH group         | 3288.17       | 3286.85                | 3288.36                |                                          |
| -C=O Stretch      | 1703.27       | 1703.05                | 1703.34                |                                          |
| -COOH group       | 2948.45       | 2947.69                | 2948.28                |                                          |

5.2.1 Differential scanning calorimetry study of drug

The thermo gram of API exhibited sharp endothermic peak at 217.39°C indicated melting point which is reported in literature.

![Fig. 5. DSC Thermo gram of Acebrophylline](image)

5.2.2 Physical properties of drug

| Property                        | Observations |
|---------------------------------|--------------|
| **Physico-mechanical properties** |              |
| Bulk density                    | 0.27±0.0057 g/cm³ |
| Tapped density                  | 0.483±0.028 g/cm³ |
| Hausner’s ratio                 | 1.36±0.577   |
| Angle of repose                 | 38.8±0.507   |
| Compressibility index           | 29.6±0.57 %  |
| Melting Point                   | 217.39 ºC    |
| **Solubility (mg/ml)**          |              |
| Water                           | 14.7         |
| 0.1 N HCl                       | 18.432       |
| Phosphate buffer 6.8 pH         | 32.128       |
|                                 |              |

5.3 Formulation Parameters

5.3.1 Flow properties of batches B1 to B9

Table 5. Flow properties of batches B1 to B9

| Batch no. | Bulk density (g/cm³) | Tapped density (g/cm³) | Hausner’s ratio | Carr’s Index (%) | Angle of repose (θ) |
|-----------|----------------------|------------------------|-----------------|------------------|---------------------|
| B1        | 0.512±0.006          | 0.589±0.02             | 1.15±0.01       | 13.07±0.02       | 27.7±0.34           |
| B2        | 0.398±0.03           | 0.435±0.06             | 1.09±0.03       | 8.50±0.006       | 24.8±0.34           |
| B3        | 0.486±0.01           | 0.538±0.04             | 1.10±0.06       | 9.66±0.05        | 25.01±0.61          |
| B4        | 0.459±0.05           | 0.519±0.06             | 1.13±0.006      | 11.5±0.07        | 29.3±0.25           |
| B5        | 0.473±0.002          | 0.541±0.06             | 1.08±0.007      | 12.5±0.006       | 22.09±0.42          |
| B6        | 0.450±0.03           | 0.500±0.03             | 1.08±0.05       | 10.0±0.06        | 31.03±0.14          |
| B7        | 0.350±0.02           | 0.417±0.01             | 1.19±0.03       | 16.06±0.03       | 33.4±0.51           |
| B8        | 0.508±0.08           | 0.570±0.02             | 1.12±0.04       | 12.4±0.04        | 34.09±0.59          |
| B9        | 0.467±0.006          | 0.538±0.02             | 1.15±0.05       | 13.9±0.02        | 28.9±0.71           |

*Each observation is the mean ±S.D. of three determinations
5.3.2 Characterization of microspheres

Table 6. Characterization of microspheres batches B1 to B9

| Batch no | Mean Size* (μm± S.D.) | Production Yield (%) | Entrapment Efficiency * (% ± S.D.) | Drug Loading* (% ± S.D.) |
|----------|------------------------|-----------------------|------------------------------------|-------------------------|
| B1       | 74.6±0.15              | 88.2                  | 81.3±1.18                          | 28.3±0.2                |
| B2       | 84.3±0.20              | 95.0                  | 87.6±1.21                          | 24.7±0.03               |
| B3       | 98.5±0.20              | 89.6                  | 86.4±2.39                          | 17.5±0.04               |
| B4       | 73.4±0.05              | 81.2                  | 79.8±1.12                          | 38.4±0.05               |
| B5       | 80.3±0.26              | 88.1                  | 81.2±2.28                          | 28.8±0.04               |
| B6       | 88.8±0.36              | 93.1                  | 85.7±2.70                          | 22.2±0.01               |
| B7       | 73.5±0.30              | 85.0                  | 77.2±2.76                          | 29.4±0.02               |
| B8       | 79.6±0.32              | 91.8                  | 80.6±2.12                          | 21.7±0.07               |
| B9       | 87.8±0.65              | 94.6                  | 83.2±1.40                          | 17.6±0.1                |

*Each observation is the mean ±S.D. of three determinations

From the above evaluation study it was concluded that the increase in Eudragit RS100 concentration, Particle size and Drug entrapment efficiency was increased. As concentration of CaCl$_2$ was increased, % entrapment efficiency of decrease. It indicated that as the concentration of CaCl$_2$ it formed rigid structure and cracks created on the surface of microspheres because of this entrapment efficiency decrease. The production yield and Drug entrapment efficiency of the ionic gelation method was found to be 81.2-95.0, 81.2-87.6% respectively. Amongst all batches batch B2 having 2.5 % CaCl$_2$, having drug polymer ratio (1:2) shows good Drug entrapment efficiency.

5.3.3 *In-vitro* drug release study (Batch B1-B9)

From the above evaluation study, it was concluded that with increase in Eudragit RS100, concentrations, sustained release pattern was obtained. As the concentration of CaCl$_2$ increase drug release decrease. So, CaCl$_2$ (2.5%) concentration was optimized based on entrapment efficiency and % CDR.

5.3.4 Release kinetic profile of optimized batch

\[ y = 7.8344x + 15.936 \]
\[ R^2 = 0.9057 \]

Fig. 6. First order model
Fig. 7. Zero order model

\[
y = -0.165x + 2.2952 \\
R^2 = 0.9237
\]

Fig. 8. Peppas model

\[
y = 37.508x - 23.173 \\
R^2 = 0.9601
\]

Fig. 9. Higuchi model

\[
y = 0.8066x + 1.1902 \\
R^2 = 0.9619
\]
Drug release mechanisms were determined by fitting drug release data of B2 to various kinetic models. By comparing the correlation coefficient values as shown in Table 7 from the applied models, the First order was shown the most appropriate to describe the kinetics of B2 formulation. So, the release study (B2) followed Peppas model and non-Fickian diffusion mechanism.

5.3.5 Optimization of microspheres by using 3² factorial design method

Table 8. Layout of optimization (A)

| Variables                                      | -1 | 0  | +1 | Responses               |
|------------------------------------------------|----|----|----|-------------------------|
| X1= Concentration of Eudragit Rs100 (%)        | Numeric | 1 | 2 | 3                       |
| X2= Concentration of CaCl₂ (%)                 | Numeric | 2.5 | 5 | 7.5                     |

Table 9. Layout of optimization (B)

| Batch Code | Coded Value | Actual Value |
|------------|-------------|--------------|
| Run        | X1 | X2 | X1 | X2 |
| B1         | -1 | -1 | 1  | 2.5 |
| B2         | 0  | -1 | 2  | 2.5 |
| B3         | +1 | -1 | 3  | 2.5 |
| B4         | -1 | 0  | 1  | 5  |
| B5         | 0  | 0  | 2  | 5  |
| B6         | +1 | 0  | 3  | 5  |
| B7         | -1 | +1 | 1  | 7.5 |
| B8         | 0  | +1 | 2  | 7.5 |
| B9         | +1 | +1 | 3  | 7.5 |
5.3.6 ANOVA for % entrapment efficiency (response Y1)

Table 10. ANOVA table for response Y1: entrapment efficiency

| Source         | Sum of square | df | Mean square | F-value | P-value | P-value |
|----------------|---------------|----|-------------|---------|---------|---------|
| Model          | 82.25         | 2  | 41.12       | 20.92   | 0.0020  | Significant |
| A-Eudragit Rs100 | 48.17       | 1  | 48.17       | 24.50   | 0.0026  |          |
| B-CaCl₂        | 34.08         | 1  | 34.08       | 17.34   | 0.0059  |          |
| Residual       | 11.79         | 6  | 1.97        |         |         |          |
| Cor Total      | 94.04         | 8  |             |         |         |          |

5.3.7 Contour plot of % entrapment efficiency (response Y1)

Fig. 11. Contour plot showing the effect of Eudragit RS100 (X1) and CaCl₂ (X2) on response Y1 (% Entrapment Efficiency)

5.3.8 Response surface plot of % entrapment efficiency (response Y1)

Fig. 12. Response surface plot of % entrapment efficiency (Response Y1)

5.3.9 Polynomial equation of % entrapment efficiency (response Y1)

Coded Equation: % EE= +79.72+1.42*A-2.38*B

Actual Equation: % EE= +81.65556+2.83333*Eudragit RS100-0.95333* CaCl₂
5.3.10 ANOVA for % CDR (response Y2)

Table 11. ANOVA table for % CDR (response Y2)

| Source     | Sum of square | Df | Mean square | F-value | P-value | Significant |
|------------|---------------|----|-------------|---------|---------|-------------|
| Model      | 256.70        | 2  | 128.35      | 23.46   | 0.0015  | Significant |
| A-Eudragit Rs100 | 178.21    | 1  | 178.21      | 32.57   | 0.0013  |             |
| B-CaCl₂   | 78.48         | 1  | 78.48       | 14.35   | 0.0091  |             |
| Residual   | 32.83         | 6  | 5.47        |         |         |             |
| Cor Total  | 289.52        | 8  |             |         |         |             |

5.3.11 Counter plot of % drug release (response Y2)

![Contour plot showing the effect of Eudragit RS100 (X1) and CaCl₂ (X2) on response Y2 (% Drug release)](image)

Fig. 13. Contour plot showing the effect of Eudragit RS100 (X1) and CaCl₂ (X2) on response Y2 (% Drug release)

5.3.12 Response surface plot of % Drug release (Response Y2)

![Response surface plot of % Drug release (Response Y2)](image)

Fig. 14. Response surface plot of % drug release (Response Y2)

5.3.13 Polynomial equation of % Drug release (Response Y2)

Coded Equation: Drug release = +96.39-2.72*A-3.62*B

Actual Equation: Drug release = + 109.07778-5.45000*Eudragit RS100-1.44667* CaCl₂
5.3.14 Overlay plot

![Overlay plot](image)

Fig. 15. Overlay plot of Eudragit RS100 & CaCl$_2$

5.3.15 Suggested solution formula given by the design expert

| Formulation | Parameters         | Predicted value | Observed value | % Error |
|-------------|--------------------|-----------------|----------------|---------|
| CP1 (B2)    | Entrapment Efficiency (%) | 82.19           | 85.3           | 3.7     |
|             | % CDR              | 98.19           | 99.2           | 1.02    |

From the results of checkpoint batch B2 (optimized batch), the predicted values of responses were found to be near to the observed value. By this, the validity of the optimization procedure was proven.

5.4 Characterization of Optimized Batch of Microspheres by using Different Techniques

5.4.1 FTIR of optimized batch of microspheres B2

![FTIR spectra](image)

Fig. 16. FTIR spectra of optimized batch B2

5.5 Interpretation

| Functional group | -N-H group | -N-H2 group | -OH group | -C=O Stretch | -COOH group |
|------------------|------------|-------------|-----------|--------------|-------------|
| Drug             | 1229.42    | 3449.46     | 3288.17   | 1703.27      | 2948.45     |
| B2               | 1243.48    | 3350.84     | 3282.60   | 1672.63      | 2932.75     |

Table 13. Interpretation of FTIR graph of B2
5.5.1 Differential scanning calorimetry

The DSC thermogram of prepared formulation B2 shows sharp endothermic peak at 197.55°C.

![DSC thermogram of B2](image)

Fig. 17. DSC thermo gram of B2

5.5.2 Scanning electron microscopy of optimized batch

From the above figures it was observed that microspheres were spherical and rough surface, the rough surface was due to the higher concentration of drug uniformly dispersed at the molecular level in alginate matrices.

5.5.3 Comparison of marketed formulation with optimized formulation

**Brand name:** ABphylline SR  
**Strength:** 200 mg  
**Batch no:** BSR1054  
**MFG. Date:** 04/2016  
**EXP. Date:** 03/2018  
**Manufacturer name:** Sun Pharma laboratories ltd

![SEM of optimized batch B2](image)

Fig. 18. SEM of optimized batch B2 (At 35 X)

**Table 14. Comparison of in-vitro dissolution of the optimized batch and marketed tablet**

| Time (hr) | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   |
|-----------|------|------|------|------|------|------|------|------|------|------|------|------|
| **B2**    | 15.8 | 22.2 | 35.4 | 49.7 | 69.5 | 78.2 | 82.1 | 85.5 | 89.8 | 94.4 | 97.2 | 99.2 |
| **Marketed Tablet** | 10.3 | 15.4 | 27.2 | 36.7 | 43.3 | 52.9 | 67.1 | 73.6 | 80.1 | 89.9 | 96.2 | 100.1 |
Fig. 19. Comparison of *in-vitro* dissolution of the optimized batch and marketed tablet

5.6 Stability Study

Table 15. Characterization of B2 after stability

| Parameters                  | Initial | After 1 month |
|-----------------------------|---------|---------------|
| Particle size               | 84.3    | 83.9          |
| % Entrapment Efficiency     | 87.6    | 86.3          |
| % CDR                       | 99.2    | 98.1          |

Fig. 20. *In vitro* drug release of B2 after stability study

From results, it was observed that the formulation showed no significant change in % Entrapment Efficiency and % drug release. So, the prepared formulation was found to stable at accelerated.

6. CONCLUSION

In the present study, an attempt was made to design and characterize sustained release microspheres formulation which intentionally delayed the drug absorption from therapeutic point of view in the treatment of asthma.

Microspheres were prepared by different techniques like Ionic gelation and Emulsification crosslinking method. The prepared microspheres were evaluated for various properties like entrapment efficiency, production yield, and drug release.

Prior to formulation, pre-formulation studies were carried out in order to establish compatibility between drug and polymers by FTIR spectroscopy. The results revealed that the drug and polymers were satisfactorily compatible, without any significant changes in the chemical nature of the drug.

Among all the formulation F5 was found best in polymer and its concentration. From the result it was observed that entrapment efficiency and production yield of Ionic gelation method was higher than the Emulsification crosslinking method. So ionic gelation method was selected for further study.

Further the microspheres were optimized using $3^2$ factorial design. All the prepared batches were evaluated for entrapment efficiency, production yield, and drug release. Batch B2 was selected
as a optimized batch on the basis of entrapment efficiency and drug release and the optimized batch was further characterized by FTIR, DSC and SEM studies.

FTIR spectra show that there was no interaction between drug and polymer in microspheres formulation.

SEM study observed that microspheres were spherical and rough surface.

Stability studies was carried out at 40 ± 2°C with RH of 75% for a period of 1 month in order to known the influence of temperature and relative humidity on % Entrapment efficiency and drug release. From the stability studies, it was observed that there were no significant changes in the entrapment efficiency and In vitro release study of optimized B2 formulation, and therefore the formulation is stable.

CONSENT
Not applicable.

ETHICAL APPROVAL
Not applicable.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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