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Research Article

Development and *In vitro* Evaluation of Azithromycin Microspheres by Solvent Evaporation Technique

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Abstract

The present investigation was concerned with formulation and evaluation of microspheres for Azithromycin using Ethyl cellulose as a release retarded material by solvent evaporation technique. Nine different batches of Microspheres were prepared using three different solvents at three different Drug: polymer ratios (1:1, 1:1.5, 1:2) The microspheres were characterized for drug content, percentage yield, particle size analysis and surface morphology. The results of all the physicochemical tests of all formulations were found to be favorable. The *in vitro* % drug release was found to be in range of 59.13 to 80.13 %. At the end of 12 hrs. Optimized formulation C3 was evaluated for FTIR, XRD and SEM. XRD and FTIR studies showed that the nature of pure drug Azithromycin remains unaffected till the completion of process of microspheres formation. SEM photographs showed that the Floating microspheres were spherical in nature with smooth surface and uniform distribution of the drug within the microsphere. The *in vitro* data were fitted to different Kinetic order. The formulated tablets exhibited Fickian to anomalous transport drug release kinetics approaching Zero-order as the value of release rate exponent (n) varied between 0.524 to 0.784.

1. INTRODUCTION

Microencapsulation is a process whereby relatively thin coating of polymers are applied to small particles of solid or droplets of liquid and dispersions. The microencapsulation processes produce small particles ranging in size from 1 to 1000 μm . microcapsules are made of one or multiple core substances (solid or liquid) that are surrounded by a distinct capsule wall, whereas micromatrices are polymeric matrices in which the encapsulated substances are homogeneously dispersed^{1,2}. Azithromycin is a macrolide antibiotic that has been used for more than a decade to treat urinary tract, bronchial tract, lungs and sinus infections. The unique pharmacokinetics of Azithromycin-rapid absorption and extensive distribution in tissue allows for short 3-day (500 mg/day for 3 days) or 5-day (500 mg on day 1 followed by 250 mg on day 2-5) course of therapy. Preclinical studies have shown that Azithromycin efficacy related to AUC/MIC ratio that improved efficacy could result if the therapeutic courses were given all at once as single dose.

Azithromycin when incorporated into sustained release microspheres, release the drug slowly. And the drug is released into the lower gastrointestinal tract, reducing gastrointestinal side-effects, and allowing for a higher dose to be administered. The unique pharmacological properties and extremely long half-life of Azithromycin make this drug well suited to single-dose administration. Azithromycin dihydrate is insoluble in water and also bitter taste by making in microsphere formulation taste masking can be done, so fast onset of action achieved and potential for avoidance of hepatic first pass metabolism of susceptible drug and causes increase in the bioavailability.

Azithromycin has low bioavailability also having large dose which may cause GI side effect hence, by making in sustained release form it will released in lower GI tract and minimizes side effect. Aim of this study was an attempt taken for preparation of sustained released microspheres using ethyl cellulose, which will reduce the dosing frequency³.

2. MATERIALS AND METHODS

Azithromycin and Ethyl cellulose (50 cps) was obtained as gift sample by Zim Laboratories Ltd, Nagpur (Maharashtra) India. All other materials and solvents used were of analytical grade.

2.1 Formulation of Microspheres

The formulations were prepared by using different solvents (ethyl acetate dichloromethane, chloroform) and different drug: polymer ratio (1:1, 1:1.5, 1:2) in each solvent. In the O/W emulsion solvent evaporation method, the polymer (ethyl cellulose) was dissolved in internal organic phase or solvent. Accurately weighed quantity 1g of Azithromycin was dispersed or dissolved in the polymer solution. This resulting mixture was poured slowly with stirring into 100 ml of a 0.015% w/v aqueous solution of polyvinyl alcohol. The emulsion was then stirred continuously at 700 rpm for 1 hr to evaporate the solvent. The microspheres were recovered by vacuum filtration, washed with 200 ml of deionized water and dried at room temperature^{4,5}.

| S. No. | Formulation code | Drug : Polymer Ratio | Internal Organic Phase |
|--------|------------------|----------------------|------------------------|
| 1 | A1 | 1:1 | Chloroform |
| 2 | A2 | 1:1.5 | |
| 3 | A3 | 1:2 | |
| 4 | B1 | 1:1 | Dichloromethane |
| 5 | B2 | 1:1.5 | |
| 6 | B3 | 1:2 | |
| 7 | C1 | 1:1 | Ethyl Acetate |
| 8 | C2 | 1:1.5 | |
| 9 | C3 | 1:2 | |

2.2 Evaluation of Microspheres

2.2.1 Angle of Repose

Angle of repose is defined as the maximum angle possible between the surface of pile of powder and horizontal plane. The angle of repose for the microspheres of each formulation was determined by the funnel method⁶. The microspheres were allowed to flow out of

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the funnel orifice on a plane paper kept on the horizontal surface. It forms a pile of microspheres on the paper. The angle of repose was calculated by substituting the values of the base radius 'R' and pile height 'H' in the following equation

$$\tan \theta = H / R$$

Where, H = Pile Height.
R = Radius of Pile.

2.2.2 Bulk Density

Bulk density of all batches of microspheres was determined by pouring gently 2 g of sample through a glass funnel into a 10 ml graduated cylinder. The volume occupied by the sample was recorded. Bulk density was calculated as per given formula:

$$\text{Bulk density} = \frac{\text{Weight of sample}}{\text{Volume occupied by the sample}}$$

2.2.3 Tapped Density

The tapped density was determined by pouring 2 g of microspheres through a glass funnel into a 10 ml graduated cylinder. The cylinder was tapped from height of 2 inches until a constant volume was obtained. Volume occupied by the sample after tapping was recorded. The values for tapped density was calculated as per given formula:

$$\text{Tapped density (g/ml)} = \frac{\text{Weight of sample}}{\text{Volume occupied by the sample}}$$

2.2.4 Compressibility Index

The compressibility indices of the formulation blends were determined using Carr's compressibility index formula.

$$\text{Carr's Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

2.2.5 Hausners Ratio

It provides an indication of the degree of densification which could result from vibration of feed hopper. Lower the Hausner ratio better is the flowability. It was calculated as per given formula.

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

2.2.6 Determination of Particle Size

The particle size was determined using stage micrometer. The diameters of about 300 microspheres were measured and the average particle size was determined⁷.

2.2.7 Estimation of Drug Loading

For determination of drug content, microspheres equivalent to 100 mg were weighed and dissolved in 100ml of acetone. After suitable dilutions were with phosphate buffer (pH 6.0), the resulting solution was analysed spectrophotometrically at 244 nm^{8,9}.

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

$$\text{Drug loading (\%)} = \frac{\text{Weight of drug}}{\text{Weight of microspheres}}$$

$$\% \text{ Yield} = \frac{\text{Weight of microspheres}}{\text{Total expected weight of drug and polymer}} \times 100$$

2.2.8 In-vitro Dissolution Study

Accurately weighed microspheres equivalent to 200 mg of Azithromycin were taken in muslin cloth and it was kept in baskets. Dissolution study was carried out in phosphate buffer pH 6.0 at 50 rpm at temp 37 ± 0.5 °C. During dissolution study 10 ml of aliquot was withdrawn at a time intervals of 1 to 12 hr and same was replaced with equal volume of fresh medium. The withdrawn samples were filtered through Whatmann filter paper and

absorbances were measured at 244 nm. Drug concentration in the samples was determined from the calibration curve¹⁰.

2.3 Characterization of Microspheres

2.3.1 Differential Scanning Calorimetry

The DSC measurements were performed on a differential scanning calorimeter with thermal analyzer. All accurately weighed samples (about 10 mg of Azithromycin, ethyl cellulose, physical mixtures, and formulation) were placed in sealed aluminium pans, before heating under nitrogen flow (10 ml/min) at a scanning rate of 20 °C per min from 100 to 300 °C an empty aluminum pan was used as reference^{12,13}.

2.3.2 X-Ray Diffractometry

The powder X-ray diffraction pattern of Azithromycin and polymer were obtained using Phillips X-ray diffractometer with a Ni-filtered CuKα-radiation at a scanning speed of 10 °/min at 2θ. The graph was plotted in 2 theta angle versus intensity count¹⁴.

2.3.3 Surface Morphology

The microspheres were coated with Platinum by ion sputtering using Autofine coater. The microspheres were kept on the sample holder and the scanning electron micrographs were taken¹⁵.

2.3.4 Stability studies

The optimized formulation was subjected to study the effect of temperature. The study was carried out by storing the microspheres in glass bottle 40 °C ± 2 °C and 75% ± 5% RH for 30 days. These samples were collected on 7th, 14th, 21st, 28th day and analyzed for changes drug content and *in-vitro* dissolution studies¹⁸.

2.3.5 Kinetic Treatment to Dissolution Data

The dissolution data for all formulations was fitted to various drug release kinetic models like Zero order, First order, Higuchi Matrix and Korsmeyer Peppas, Hixon-Crowel model. Rate constants (K), correlation coefficients (R) obtained for various models. And Release exponent (n) values obtained in Korsmeyer Peppas model was studied^{16, 17, 19}.

3. RESULTS AND DISCUSSION

IR spectra for Azithromycin, ethyl cellulose and physical mixture of Azithromycin and ethyl cellulose are given in fig.1-3. Major functional groups of Azithromycin (CH₃-N Stretching) at 1377, (CH₂ Scissoring) at 1454, (C=O stretching) at 1720, (COOH) at 2891, (C-H Stretching of methyl and methylene) 2912, (O-H Stretching Vibration) at 3560 can be seen in spectra of individual drugs as well as in spectra of physical mixture. So there is no interaction between Azithromycin and ethyl cellulose.

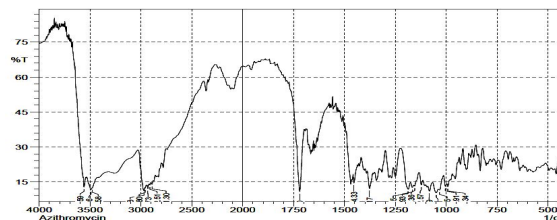


Figure-1: IR spectrum of Azithromycin

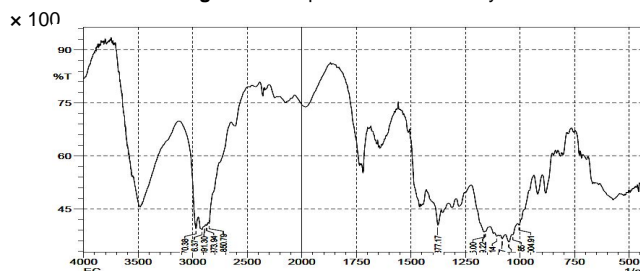


Figure-2: IR spectrum of Ethyl cellulose

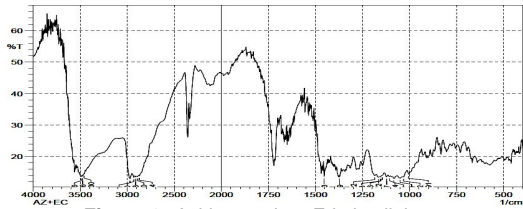


Figure-3: Azithromycin + Ethyl cellulose

All the formulations show angle of repose value in the range of 15.22 - 20.42. The values for bulk density were found in the range of 0.333 to 0.455. Tapped density was found to range from 0.380 to 0.536. Compressibility index were found in the range of 11.76 to 15.11 respectively. Hausner's ratio was ranging from 1.13 to 1.17, i.e., all the preparation showed that they had good flow properties.

Table 2: Physical parameters of Azithromycin Microsphere

| Formulation code | Angle of Repose (θ) | Bulk Density (g/ml) | Tapped Density (g/ml) | Carr's Compressibility Index (%) | Hausner's Ratio |
|------------------|---------------------|---------------------|-----------------------|----------------------------------|-----------------|
| A1 | 16.19±0.754 | 0.429±0.039 | 0.504±0.064 | 14.88±0.024 | 1.17±0.0675 |
| A2 | 15.22±0.534 | 0.405±0.024 | 0.462±0.036 | 12.33±0.032 | 1.14±0.064 |
| A3 | 15.51±0.633 | 0.391±0.024 | 0.445±0.014 | 12.13±0.075 | 1.13±0.023 |
| B1 | 17.19±0.644 | 0.423±0.035 | 0.490±0.064 | 13.67±0.075 | 1.15±0.046 |
| B2 | 18.36±0.352 | 0.333±0.063 | 0.380±0.047 | 12.36±0.035 | 1.14±0.067 |
| B3 | 19.41±0.656 | 0.455±0.062 | 0.536±0.024 | 15.11±0.013 | 1.17±0.045 |
| C1 | 19.72±0.353 | 0.352±0.044 | 0.414±0.042 | 14.97±0.064 | 1.17±0.025 |
| C2 | 20.38±0.755 | 0.431±0.023 | 0.507±0.045 | 14.99±0.034 | 1.17±0.052 |
| C3 | 20.42±0.353 | 0.450±0.035 | 0.510±0.074 | 11.76±0.042 | 1.13±0.025 |

As the polymer concentration increases, viscosity also increases which influences the interaction between disperse phase and dispersion medium that affects the size distribution of particle. Result indicated that increase in the amount of polymer concentration increases relative viscosity which results into increased mean particle size. The average particle size of microcapsules is found to be within 112.546 to 171.342 μm. Results were given in figure 4.

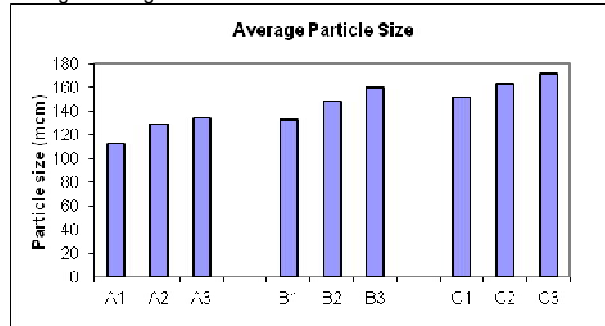


Figure-4: Graphical presentation of average particle size

The percent encapsulation efficiency of ethyl cellulose microsphere shown in table 3. The drug: polymer ratio showed significant effect on the encapsulation efficiency of microsphere. The increase in concentration of polymer showed the increase in drug encapsulation efficiency. The microsphere formulated using ethyl acetate as internal organic phase or solvent showed better encapsulation efficiency than other formulations. The % encapsulation efficiency is found to be in the range of 63.61 ± 0.7 to 75.60 ± 1.32 %.

From the *in vitro* drug release study it can be seen that the C1 batch showed sustained cumulative % drug release 80.13% in 12 hrs. Although formulation A3 showed cumulative % drug release of 59.13 % in 12 hrs and formulation B3 showed cumulative % drug release of in 67.18 % in 12 hrs, their release was insufficient. As the polymer concentration increased the drug release was decreased. The batches of microsphere formulated using ethyl acetate as solvent showed higher release rate than other solvents. The batches of microsphere formulated with drug: polymer ratio 1:1 showed higher release rate than other ratios. Results were Given in figure 5.

Table 3: Data for Percentage yield, percentage loading and encapsulation efficiency of microspheres

| Formulation Code | Drug : Polymer | Theoretical loading (%) | Actual Drug Loading (%) | Encapsulation Efficiency (%) | Yield (%) |
|------------------|----------------|-------------------------|-------------------------|------------------------------|-----------|
| A1 | 1:1 | 50 | 52.16 ± 0.905 | 64.48 ± 1.81 | 95.85 |
| A2 | 1:1.5 | 40 | 45.02 ± 0.455 | 65.76 ± 1.13 | 87.68 |
| A3 | 1:2 | 33.33 | 34.42 ± 0.355 | 66.25 ± 1.06 | 96.83 |
| B1 | 1:1 | 50 | 51.89 ± 0.35 | 63.61 ± 0.7 | 96.35 |
| B2 | 1:1.5 | 40 | 45.02 ± 0.45 | 64.76 ± 1.13 | 88.84 |
| B3 | 1:2 | 33.33 | 34.34 ± 0.44 | 64.82 ± 1.33 | 97.23 |
| C1 | 1:1 | 50 | 52.08 ± 0.202 | 74.40 ± 0.405 | 96.0 |
| C2 | 1:1.5 | 40 | 44.44 ± 0.53 | 75.60 ± 1.32 | 90.0 |
| C3 | 1:2 | 33.33 | 34.47 ± 0.38 | 73.50 ± 1.14 | 96.34 |

n = 3

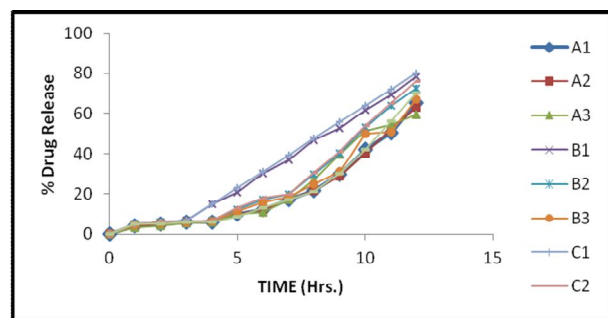


Figure-5: % Cumulative drug release of microspheres

The dissolution data for formulations A1, A2, A3, B1, B2, B3, C1, C2, and C3 was fitted to various drug release kinetic models like Zero order, First order, Higuchi Matrix and Korsmeyer Peppas, Hixon-Crowel model. Rate constants (K), correlation coefficients (R) obtained for various models are listed in table 4. with Release exponent (n) values obtained in Korsmeyer Peppas model. The model that gives high 'R' value is considered as the best fit model for the release data. It was found that as Korsmeyer Peppas best fit model for all the formulations tested. All the formulations showed

diffusion exponent (n) varying from 0.524 to 0.784. From the n values of all formulations it can be concluded that as the concentration and viscosity of polymer was increased the value of diffusion exponent also increases. This was because as the concentration of polymer increases, the rate of dissolution of disentangled chains decreases and diffusion path length of

aqueous channel increases. This leads to increase in diffusion exponent value and results in shifting of the mechanism of drug release from fickian diffusion to anomalous transport thus overlapping of different types of phenomena, potentially including drug diffusion and polymer swelling.

Table 4: Drug release kinetic parameters of microspheres.

| Batch Code | Zero Order | | First Order | | Matrix | | Korsemeyer Peppas | | | Hixon-Crowel | |
|------------|------------|-------------------|-------------|-------------------|--------|-------------------|-------------------|-------------------|-------|--------------|-------------------|
| | (K) | (R ²) | (K) | (R ²) | (K) | (R ²) | (K) | (R ²) | (n) | (K) | (R ²) |
| A1 | 8.447 | 0.914 | -0.166 | 0.994 | 24.71 | 0.994 | 22.68 | 0.995 | 0.539 | -0.042 | 0.990 |
| A2 | 7.257 | 0.950 | -0.120 | 0.993 | 21.09 | 0.988 | 17.06 | 0.996 | 0.600 | -0.033 | 0.992 |
| A3 | 6.432 | 0.966 | -0.975 | 0.992 | 18.62 | 0.980 | 14.14 | 0.996 | 0.629 | -0.028 | 0.994 |
| B1 | 7.708 | 0.981 | -0.139 | 0.977 | 22.19 | 0.968 | 14.77 | 0.994 | 0.692 | -0.037 | 0.992 |
| B2 | 6.526 | 0.984 | -0.109 | 0.993 | 18.76 | 0.965 | 11.48 | 0.997 | 0.734 | -0.028 | 0.996 |
| B3 | 5.725 | 0.984 | -0.081 | 0.989 | 16.43 | 0.959 | 9.042 | 0.996 | 0.785 | -0.024 | 0.996 |
| C1 | 9.244 | 0.891 | -0.206 | 0.985 | 27.14 | 0.996 | 25.75 | 0.998 | 0.524 | -0.050 | 0.993 |
| C2 | 8.511 | 0.931 | -0.166 | 0.991 | 24.84 | 0.993 | 19.69 | 0.998 | 0.615 | -0.043 | 0.995 |
| C3 | 7.633 | 0.968 | -0.133 | 0.994 | 22.08 | 0.978 | 15.51 | 0.997 | 0.668 | -0.036 | 0.994 |

For the structural, crystal and physical state characterization, DSC studies of Azithromycin, Ethyl cellulose, their physical mixture and microspheres were carried out. The DSC thermograms are given Figure 6, 7, 8. And 9 show the DSC curves of Azithromycin, ethyl cellulose, physical mixture and microspheres (C1) respectively. DSC curve of Azithromycin shows a single endothermic peak at 121 °c, due to melting of the drug. Optimized formulation C1 which contains Azithromycin and ethyl cellulose, the thermogram indicates characteristic peaks for melting of Azithromycin at 121 °c. This indicates absence of drug - polymer interactions. This observation further supports the IR spectroscopy results.

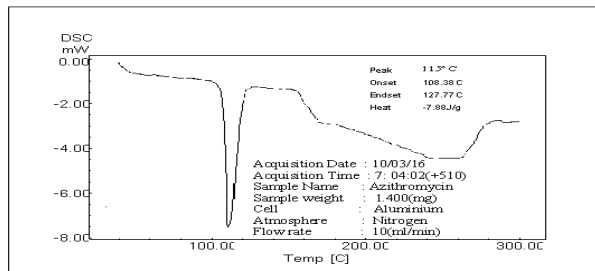


Figure-6: DSC Thermogram of Azithromycin

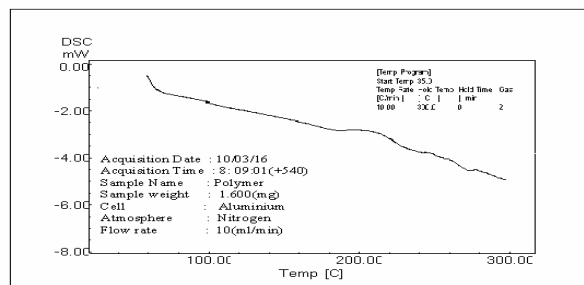


Figure-7: DSC Thermogram of Ethyl Cellulose

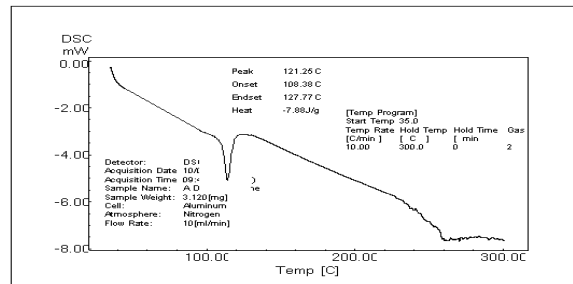


Figure-8: DSC Thermogram of Physical mixture of Azithromycin+Ethyl Cellulose

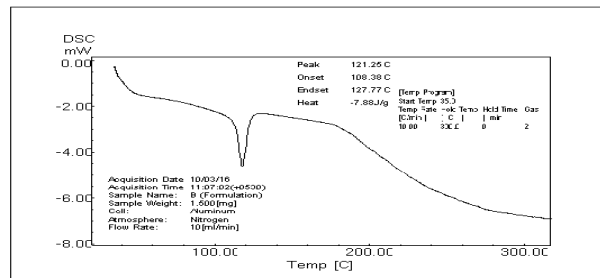


Figure-9: DSC Thermogram of Microspheres (C1)

Figure 10, 11, 12, 13 represents the XRD diffraction pattern of Azithromycin, Ethyl cellulose, their physical mixture and microspheres (C1). The XRD scan of Azithromycin showed intense peaks of crystallinity. Diffractogram of azithromycin showed high intensity peaks between 2θ of 10-20° values demonstrating the crystalline nature of drug. No intense peaks were observed in diffractogram of ethyl cellulose which indicates amorphous nature. The XRD pattern of formulation exhibited halo pattern with less intense and denser peaks compared to plain Azithromycin. This indicated that Azithromycin is dispersed at the molecular level in the blend of polymeric matrix.

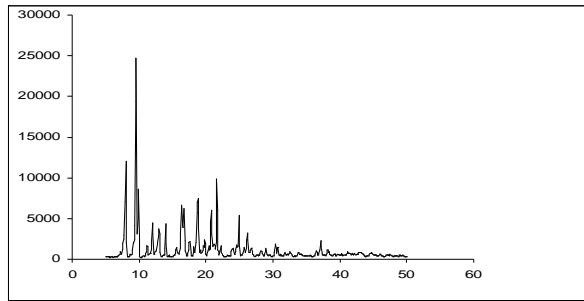


Figure-10: XRD pattern of Azithromycin

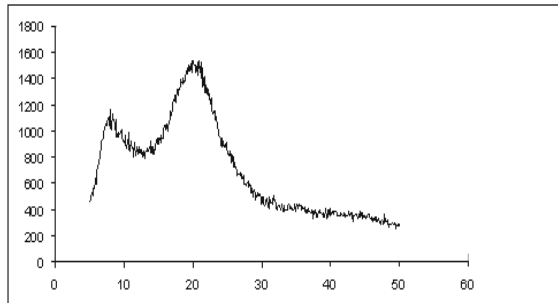


Figure-11: XRD pattern of Ethyl cellulose

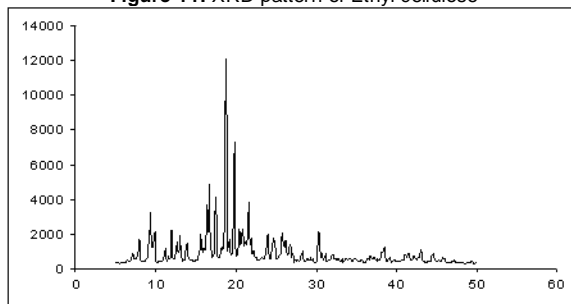


Figure-12: XRD pattern of Physical mixture

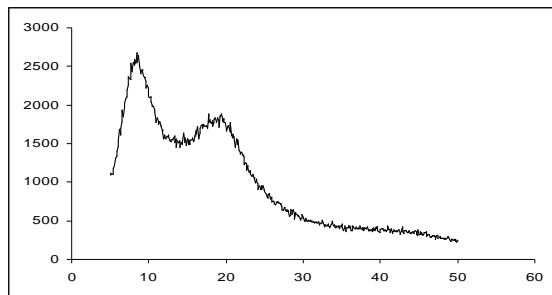


Figure-13: XRD pattern of microspheres (C1)

Figure No: 14, 15 shows the SEM photographs of various images of prepared formulation. SEM photographs showed that the microspheres were spherical in nature and had a smooth surface. SEM photographs revealed the absence of crystals of drug on the surface of microspheres and uniform distribution of the drug within the microspheres.

Accelerated stability studies (AST) was carried for optimized batch (C1) by exposing it to $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$, $75\% \pm 5\%$ RH for one month and analyzed the samples at the interval of 7,14,21,30 days and the samples was analyzed for drug content and *in-vitro* dissolution study. The stability studies show that there were no significant changes in observed drug content and percent cumulative drug release¹⁸.

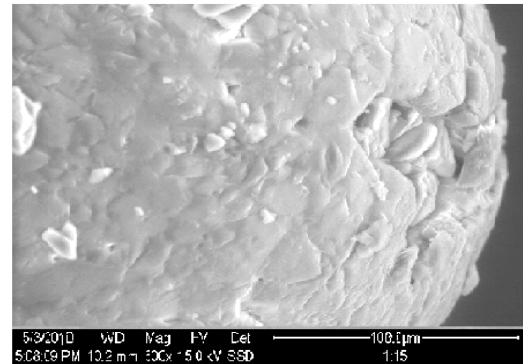


Figure-14: External surface of microsphere

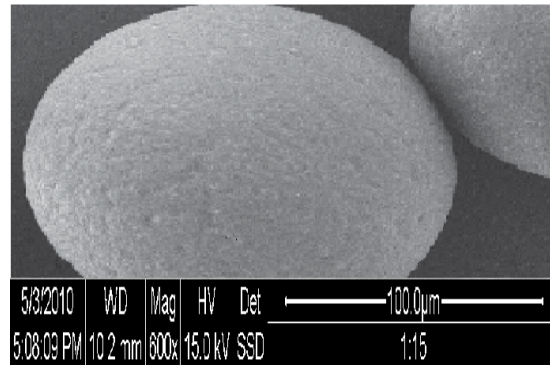


Figure-15: Whole image of microsphere

Table 5: Stability studies parameters

| Parameters | Days | | | |
|-----------------------------------|--------------|--------------|--------------|--------------|
| | 7 | 14 | 21 | 30 |
| Drug content (%) | 74.41 ± 1.32 | 73.95 ± 1.30 | 74.52 ± 1.31 | 74.14 ± 1.34 |
| <i>In-vitro</i> dissolution study | 80.11 ± 0.24 | 79.34 ± 0.23 | 79.55 ± 0.21 | 80.55 ± 0.22 |

5. CONCLUSION

From the investigation carried out and results obtained, it was observed that the % of drug released from microspheres was sustained. As the GI side effects of Azithromycin are related to its high dose which can be minimized by formulating as microspheres. It would also helps to improve bioavailability because of absorption window and microencapsulation helps to mask the bitter taste of drug. Hence from this investigation, we can propose that the objective of the study was achieved.

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