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Preparation, Characterisation and In-Vitro Study of Microspheres Containing Imatinib Mesylate by Solvent Evaporation Technique Using Ethyl Cellulose.

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Research Article

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ABSTRACT

The purpose of this research was to prepare and evaluate microparticulate drug delivery system of Imatinib Mesylate (IM). The microspheres were prepared by emulsification solvent evaporation technique using ethyl cellulose (EC) as a release rate controlling polymer in the ratios 1:0.5, 1:1, 1:1.5, 1:2, and 1:2.5. The prepared microspheres were evaluated for drug-polymer compatibility, micromeritic properties, drug entrapment efficiency, in-vitro drug release studies. The mean particle size increased with increase in the polymer concentration, when compared to pure drug and it was lying between 113.26 ± 0.870 – 132.15 ± 0.474µm. The micromeritic properties were found to be improved when compared to pure drug .Scanning electron microscopy confirmed the hollow structure with smooth external surface. The drug and polymer were found to be compatible as seen in IR studies. The entrapment efficiency considerably decreased with increase in the polymer concentration ranging from 66.29%. The microspheres up to 24 hrs over the gastric buffer medium and the prepared microspheres exhibited prolonged drug release for more than 24 hrs. The mechanism of drug release was found to be a combination of both peppa's and zero order release kinetics. The developed microspheres of Imatinib may be used for prolonged drug release for at least 24 hrs for maximizing the therapeutic efficacy along with patient compliance.

INTRODUCTION

Conventional dosage (oral) form [1] does not usually provide any controlled release or target specificity because of its immediate release. Many shortcomings of the conventional dosage form may be overcome by microspheres technology There are a number carriers – Microspheres, nanoparticles, liposomes and others for which optimized technologies are under development to enhance the performance of products that have already been delivered with some success via that route and modulates the release and absorption characteristics of the drugs. Dosage forms that can precisely control the release rates and target drugs to a specific body site have created enormous impact on the formulation and development of novel drug delivery systems [2, 3]. Controlled drug delivery occurs when a polymer, whether natural or synthetic, is with care combined with a drug or other active agent in such a way that the active agent is released from the material in a predesigned manner. Among these systems, a microparticulate system has been proven to be better than single-dosage forms as it has more expected and reproducible gastrointestinal transit time and less local irritation/side effects[4] Various biocompatible polymers are currently in use, such as cellulose derivatives [5, 6, 7].

MATERIALS AND METHODS

Materials

Imatinib Mesylate [8] was obtained as a gift sample from Natco Pharma Limited, Mumbai. Ethyl cellulose from S.D fine chemicals was purchased. All the reagents were of analytical grade.

Method

Microspheres were prepared by Solvent Evaporation Method. 10mL of acetone used as solvent of Imatinib Mesylate and polymer. Imatinib Mesylate and Ethyl Cellulose were dissolved completely in common solvent consisting of acetone by stirring at 500

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rpm for 10 minutes with magnetic stirrer to the resulting mixture and sonicated for complete dispersion. The resulting mixture was poured into the 100 mL of liquid paraffin agitation at 1500 rpm by the resulting emulsion was heated to 35ºC gradually (1ºC/min) and stirred at this temperature for 3h. During this period, acetone was completely removed by evaporation. The solidified microspheres were filtered using Whattman filter paper, washed four times with 25 ml of *n*-hexane and two times with distilled water and dried at a room temperature for 24 h and stored in desiccators containing $CaCl_2$.

Micromeritic properties [9, 10]

The microspheres were characterized for true density, tapped density, Carr's index; Hausner's ratio (HR) The tapping method was used to determine the tapped density and Carr's index as follows.

$$D_O = \text{True density} = W/V_O$$

$$Dt = \text{tapped density} = W/V_t$$

$$\text{Hausner's ratio (HR)} = \text{Tapped density / true density}$$

$$\text{Carris index} = (\text{Tapped density} - \text{True density} / (\text{Tapped density}) \times 100$$

V_O and V_t are the true volume and tapped volume respectively.

Angle of repose

Fixed funnel method was employed for determining angle of repose. The angle of repose (θ) for samples was calculated using the formula,

$$\text{Angle of repose } (\theta) = \tan^{-1} (h/r)$$

Percentage yield for microspheres

The yield of microspheres was calculated from the amount of microspheres obtained divided by the total amount of all non-volatile components

$$\% \text{ Yield} = \frac{\text{Actual weight of the microspheres}}{\text{Total weight of all non-volatile components}} \times 100$$

Surface Morphology [11]

The surface morphology of F5 was examined using Scanning electron microscopy (SEM JEOL JSM6400, Tokyo, Japan). Prior to examination, the samples were fixed on a brass stub and coated with a gold-palladium layer under argon atmosphere using a gold sputter module in a high vacuum evaporator. The pictures were then taken in the instrument set at an excitation voltage of 20 kV. The surface morphology of microspheres was investigated before and after the in vitro drug release study.

Differential Scanning Calorimeter (DSC) analysis [12]

Differential scanning calorimetry (DSC) analyses were performed on various samples (Drug, polymers, physical mixture and microspheres) with a Pyris diamond TGA Q200 V24.4 analyzer, instruments with a thermal analyzer. Under nitrogen flow of 25 ml/min, approximately 5-10 mg of the sample was placed in a sealed aluminum pan and heated at a scanning rate of 10°C/min over the temperature range of 30-300°C.

Fourier-Transform Infrared spectroscopy (FTIR) [13]

The Infrared spectrum of the drug, microspheres containing the drug were obtained in potassium bromide discs (0.5% w/w) using a FTIR spectrophotometer.

Particle size analysis and morphological studies

Particle size was determined by using an optical microscope under regular polarized light, and the mean particle size was calculated by measuring 300 particles with a help of a calibrated ocular microscope.

Determination of Drug loading and Encapsulation efficiency [14, 15]

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Drug Encapsulation Efficiency [DEE] is defined as the percentage of the actual mass of drug encapsulated in the polymeric carrier relative to the initial amount of drug loaded. In the determination of the EE, accurately weighed quantity (50 mg) of microspheres was dissolved in 1 ml of DMSO by vortexing until the microspheres dissolved completely.

10 ml of 0.1 N HCl was then added to precipitate the polymer and centrifuged at 2000 rpm. The supernatant was collected and filtered through 0.2µ (Millipore) filter and then analyzed using UV spectrophotometer (Syntronics) at λ_{max} 230 nm. These results were further used to determine the percentage of drug loading. Each sample was analysed in triplicates.

The encapsulation efficiency (EE) was calculated from the following equation

$$DEE = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100$$

***In-vitro* study** [16,17]

In vitro drug release study of all the batches were carried out by paddle method using USP type 2 apparatus using 900 ml of 0.1 N HCl as dissolution medium at 100 rpm and 37±0.5°C for the first 2 hrs, afterwards using pH7.4 Phosphate Buffer. A quantity of microspheres containing 100mg equivalent of Imatinib Mesylate was placed in the dissolution medium. The samples were withdrawn at a predetermined time interval, diluted approximately and were analyzed spectrophotomatrically at 230nm against reagent blank. As shown in Fig. 1

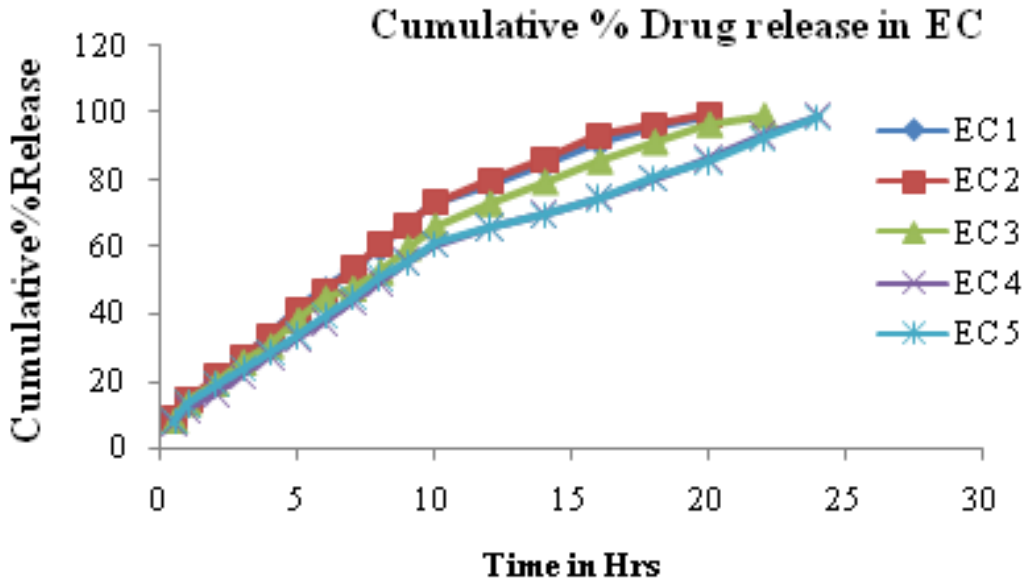

Figure 1: In-vitro drug release of Imatinib Mesylate (F1-F5)

Release Kinetics [18]

To determine the order of drug release from microspheres, the dissolution data was fitted into zero-order, first order, and Higuchi equation. The dissolution data was also fitted in an exponential equation (korsmeyer-Peppas) often used to describe drug release behaviour from polymeric systems when the mechanism is not well known or when more than one type of release phenomenon is involved.

Stability Studies [19]

Stability studies were performed under various temperatures in a period of 90days. At regular intervals, the microspheres were subjected to drug content assay. At higher temperature (40 °C), the percentage of drug content was found to be 92.08%, whereas 97.49% was obtained at 5 °C at the end of 90 days. This result suggests that the microspheres were more stable at 5 °C. The in vitro release profiles of optimized formulation (F5) before and after stability studies are illustrated in Figure 2.

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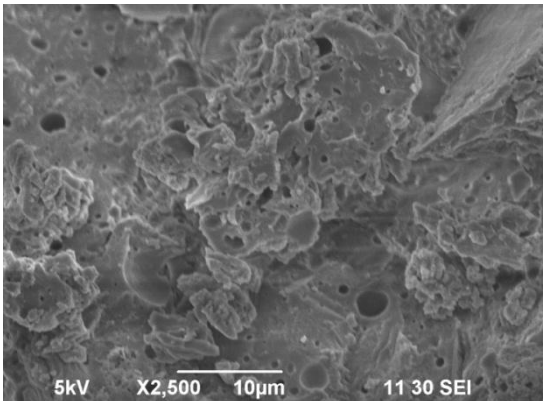

Figure 2: SEM after 40°C

Result and Discussion

Emulsion solvent evaporation method was used to prepare Imatinib Mesylate microspheres. When Drug: Polymer ratio was too low (1:1, w/w) the release rate was near equal to conventional dosage form, Drug: Polymer ratio was 1:2.5% showed better release even after 24 hrs. These results show that the amount of solid, thus the viscosity of the inner phase is an important factor for the preparation of microspheres. Keeping the drug amount and the solvent amount volume constant, spherical particles were obtained as the amount of polymer increased to give a polymer drug ratio (1:2.5). Two examples of the scanning electron micrographs of the microspheres prepared are shown in Figure 2 and 3.

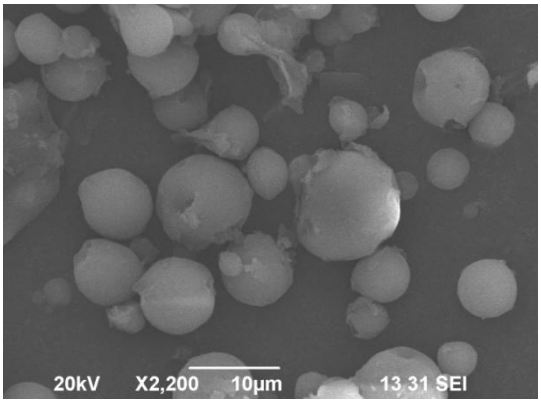

Figure 3: SEM of optimised formulation

On the other hand, Drug Entrapment Efficiency was found to increase with increase in polymer concentration was found to be maximum (74.57 ± 2.96). Most of the microspheres obtained were collected in the size range of 100-200 µm by all formulation (Table 1).

Table1: Micromeritic Evaluation of microspheres

Formulation Code	Particle size	Angle of Repose	Bulk Density	Tapped Density	Carr's index	Hausner's ratio
EC1	113.26±0.870	23.02±0.702	0.810 ±0.231	0.982 ±0.014	17.42 ±3.186	1.21 ±0.049
EC2	117.97±0.768	24.38 ±0.246	0.837 ±0.019	0.986 ±0.009	15.10 ±2.608	1.18 ±0.035
EC3	124.45±0.881	23.75 ±0.484	0.844 ±0.006	0.975 ±0.018	13.37 ±2.145	1.16 ±0.032
EC4	127.99±0.680	23.69 ±0.161	0.863 ±0.007	0.967 ±0.019	10.72 ±2.330	1.12 ±0.029
EC5	132.15±0.474	22.35 ±0.276	0.813 ±0.090	0.972 ±0.009	16.43 ±1.230	1.20 ±0.021

Increasing the Drug: Polymer ratio caused the mean particle size to shift towards a higher particle size. Higher concentration of polymer produced a more viscous dispersion which formed larger droplet and consequently larger microspheres. Increasing the stirring speed decreased the particle size of microspheres. The yield of preparation and Imatinib Mesylate Entrapment Efficiencies were higher for all formulations and maximum for optimized formulation (F5). The drug release rates from microspheres were studied using the USP type 2 paddle method. The in vitro drug release profile was biphasic with an initial burst release (19.22 %) in 1 hr attributed to surface associated drug, followed by a slower release phase as the entrapped drug slowly diffuse out into the release medium. 85.23 % drug release after 24 hrs there was a sustain release of drug at a constant rate and shown in Table 2.

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Table 2: Evaluation of microspheres

Formulation Code	Percentage yield	Encapsulation Efficiency	Drug Content	Cumulative Release
EC1	78.20±1.370	66.29±0.513	17.01±0.297	96.77±0.570
EC2	82.58±1.135	73.87±0.578	19.13±0.245	95.35±0.580
EC3	85.53±1.508	78.95±0.513	20.81±0.253	93.44±0.721
EC4	87.30±1.891	82.57±0.900	21.07±0.672	88.87±1.243
EC5	93.13±3.284	89.38±1.810	22.76±0.380	85.23±0.451

The absorbed molecules on surface of particle are rapidly desorbed when in contact with the dissolution medium. The diffusion of drug, the erosion and degradation of polymer are the main mechanism for the drug release. Kinetics model further support the above statement. Zero order, First order, Korsmeyer Peppas, Higuchi plot were applied on optimized formulation. The studied showed that drug release from all formulations was not found to be statistically significant. But on the basis of required size, shape, drug entrapment efficiency of floating microspheres EC 5, Drug: Polymer ratio (1:2.5) was found to be optimum batch.

CONCLUSION

Microsphere of Imatinib Mesylate were prepared by novel o/w emulsion solvent evaporation technique, using Ethyl Cellulose in order retain drug in body for longer period of time to increased bioavailability. Ethyl Cellulose based Microspheres show there buoyancy for more than 24 hours, required for sustained therapeutic activity due to their more smooth structural nature. EC5 formulation showed good result among all other formulations. It could be concluded that the variation observed in entrapment efficiency, production yield, mean particle size and the drug release behaviour among the formulations are the result of the drug polymer ratio employed. Their release profile of drug molecule. From the present work, it was concluded that Imatinib Mesylate microspheres based on Ethyl Cellulose offer a most suitable dosage form to improve bioavailability of Imatinib mesylate.

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