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DEVELOPMENT AND EVALUATION OF IMATINIB MESYLATE MICROSPHERES BY CHEMICAL CROSS LINKING METHOD USING CHITOSAN

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ABSTRACT

Chitosan (CS) microspheres containing Imatinib mesylate were prepared by chemical cross linking method. The incorporation efficiency of the prepared microspheres ranged between 76 % and 88%. The effect of Chitosan concentration, cross-linking ions and drying conditions was evaluated with respect to entrapment efficiency, particle size, surface characteristics and In vitro release behaviour. Infrared spectroscopic study confirmed the drug-polymer compatibility. Differential scanning calorimetric analysis revealed that the drug was molecularly dispersed in the CS microsphere matrices. Scanning electron microscopic study of microspheres showed the smooth surface due to higher concentration of drug molecules dispersed at molecular level in the chitosan matrices. The mean particle size and entrapment efficiency were found to be varied by changing various formulation parameters. The in vitro release profile could be altered significantly by changing various concentration parameters to give a sustained release of drug from the microspheres. The kinetic modeling of the release data indicate that imatinib release from the chitosan microspheres follow anomalous transport mechanism after an initial lag period when the drug release mechanism was found to be Non-Fickian diffusion controlled.

KEYWORDS : *Chitosan, microspheres, Imatinib mesylate, Chemical cross linking.*

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INTRODUCTION

New drug delivery technologies revolutionizing the drug discovery, development and creating Research focused pharmaceutical industries to increase the momentum of global advancements. Microspheres (MS) can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 µm. They are made from polymeric, waxy, or other protective materials such as starches, gums, proteins, fats and waxes and used as drug carrier matrices for drug delivery. Preparation of uniformly sized Microspheres was first reported in the late 60's and early 70's [1, 2].

Microspheres are one of the multiparticulate delivery systems and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability or stability and to target drug to specific sites. Microspheres can also offer advantages like limiting oscillation within therapeutic range, reducing side effects, decreasing dosing frequency and improving patient compliance. Imatinib mesylate [3] (IM) is used to treat cancers and act by specifically inhibiting a certain enzymes of a receptor tyrosine kinase and its characteristics of a particular cancer cell, rather than non specifically inhibiting and killing all rapidly dividing cells.

Chitosan or β-(1→4)-2-amino-2-deoxy-D-glucose is obtained by hydrolysis of the amino acetyl groups of chitin, a polysaccharide. Chitosan with excellent biodegradable and biocompatible characteristics is a hydrophilic biopolymer. Due to its unique polymeric cationic character and its gel and film forming properties, chitosan has been examined extensively used in the pharmaceutical industry for its potential in the development of drug delivery system [4-6].

MATERIALS AND METHODS

Imatinib mesylate is a gift from Natco Pharma Limited, India. Chitosan was obtained from Ajantha Pharma Mumbai, India, as a gift sample, Light Liquid Paraffin, Acetone and other chemical reagents are the analytical range from SD fine Chemicals Mumbai.

Method of preparation [6, 7]

A 4.0 %(w/v) Polymer and Drug solution in aqueous acetic acid (5.0%) was prepared. This dispersed phase was added to continuous phase (100 mL) consisting of light liquid paraffin and 25 mL of Petroleum ether containing 2.0 mL of Span 80 in a beaker at room temperature. Stirring was continued at 2000 rpm using a 3- blade half moon paddle for 5 minutes. A drop-by-drop solution of a measured quantity (2.5 mL each) of aqueous glutaraldehyde (25% v/v) saturated with toluene was added at 15, 30, 45, and 60 minutes. Stirring was continued for 3.0 hours and separated by centrifugation and washed, first with petroleum ether (60°C - 80°C) four times, once with acetone and then thrice with distilled water to remove the adhered liquid paraffin and glutaraldehyde, respectively. The microspheres were then finally dried at room temperature and stored in vacuum desiccators. The microspheres were filtered by using Whattman filter paper. The collected microspheres were dried for 1 hour at room temperature and subsequently stored in desiccators over fused Calcium chloride.

Evaluation of microspheres

Particle size determination [8-10]

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 and tabulated in Table 1.

Particle Shape and Surface morphology

The scanning electron microscopy (SEM) has revealed the structural features of microcapsules as to be varying and complex. The shape and surface morphology of the IM loaded microspheres were studied using (Jeol, JSM-6400 scanning electron microscope, Japan). The gold coated microspheres were subjected to secondary imaging technique at 15^0 tilt,15mm working distance and 25 Kv accelerating voltage. Optical microscopic photograph was also taken for optimised formulation and shown in Figure 1, 2.

Angle of repose

The angle of repose (θ) i.e., flow property of the microspheres which measures the resistance to particle flow was calculated as

$$\tan(\theta) = 2H/D$$

where,

2H/D is the surface area of the free standing height of the microspheres heap that is formed after making the microspheres flow from the glass funnel.

Drug loading Percentage

25 mg of microspheres were treated with 50 mL of phosphate buffer (pH 7.4), in 100 ml.

amber colored vial with stirring at 250 rpm. The temperature was maintained at 37 + 0.2º C. At the end of two hours it was filtered, and the filtrate was analyzed photometrically at 230 nm using UV. Visible spectrophotometer (Shimadzu, Japan). Drug loading efficiency was calculated as:

Drug loading (%) = (Weight of drug / Weight of microspheres**)** × 100

Drug encapsulation (%) = (Actual drug concentration/Theoretical drug concentration) × 100

Percentage yield

The prepared microspheres with a size range of were collected and weighed from different formulations. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres.

% Yield = (Actual weight of product / Total Weight of excipients and drug) × 100

In vitro dissolution studies [11]

Drug release studies were carried out using USP XXIII dissolution rate test apparatus (100 rpm, 37±1°C) for 2hours in0.1N HCl and Upto24hours in 7.4 pH phosphate buffer(simulated intestinal fluid). At different time intervals, 5ml of the sample was withdrawn and replaced with same amount of fresh medium. The sample was analyzed for IM directly or after appropriate dilution (5–50 ml) with the pH 7.4 phosphate buffer spectrophotometrically at 230 nm using a UV/ VIS spectrometer against a reagent blank. If the absorbance (concentration) of the released drug is beyond the calibrated range of absorbance then to make it within the calibrated range the dilution of the collected dissolution sample was done by diluting with the addition of appropriate volume (5–50 ml) of dissolution medium. That dilution factor was included in the calculation of Cumulative % drug release.

Kinetics of drug release

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing zero-order (Q v/s t), first-order (log (Q0−Q) v/s t), Higuchi's square root of time (Q v/s t1/2) and Korsemeyer peppas double log plot (log Q v/s log t) respectively, where Q is the cumulative percentage of drug released at time t and (Q0−Q) is the cumulative percentage of drug remaining after time t.

Differential scanning colorimetry (DSC)

Thermograms were obtained by using a TGA Q200 V24.4,Japan thermal analyzer at a heating rate 50ºC /min over a temperature range of 0 to 300°C. The sample was hermetically sealed in an aluminium crucible. Nitrogen gas was purged at the rate of 100 ml/min for maintaining inert atmospheres.

Stability Studies [12, 13]

Stability study was carried out for the optimised (F5) formulation by exposing it to different temperature as 4°C, Ambient temperature and 40°C for 3 months. The sample was analyzed for drug content at the regular intervals and end of 90days. It was found that no notable change in the drug content of F5formulation. This indicates that F5was stable for following temperature. SEM Analysis was also done for the optimised formulation.

RESULT AND DISCUSSION

In the present study Chitosan microspheres encapsulated with Imatinib Mesylate were prepared by chemical cross linking technique. The swelling ratio of microspheres increased dramatically when a smaller amount of cross - linking agent was used [14].Three hundred microspheres of each batch were sized by a light microscope equipped with an optical micrometer and the average percentage as plotted against size ranges (Table. 1). The mean size range of the five batches of microspheres was estimated between 105.88+0.399 µm with nearly 70% lying between 106-114 µm. As the drug to polymer ratio was increased, the mean particle size of IM

microspheres was also increased (Table 1). The significant increase may be because of the increase in the viscosity of the droplets (due to the increase in concentration of polymer solution).

The microspheres were also photographed by an optical camera and SEM (The photomicrographs are depicted in Figure no.1, 2) as it shows microspheres are spherical with quite smooth surfaces. They found that drug loading could be increased by increasing drug to polymer ratio, [Surface smoothness of MS was increased by increasing the polymer concentration, which was confirmed by SEM]. Angle of repose was found to be range from 22.44(F1) to 24.44(F2) that shows good flow nature for the prepared microspheres. Encapsulation efficiency were 76.09 ± 0.784 (F2) - 87.28 ± 1.021 (F5) showed that polymer concentration increase the encapsulation efficiency.

Drug content comes also the same way to increase the concentration of polymer really improve content level [15]. From the optimized F5 batch is considered to be the most promising formulation batch because among all the batches it shows better extent of drug release 91.28% after 24 hours period. This also found that drug release from microspheres was remarkably slower than that of the plain drug. This makes the microsphere system of Imatinib Mesylate as a sustained release (SR) delivery system which has advantages of reducing the number of dosing, providing better patient compliance, reducing drug loss due to the first pass metabolism, and finally increasing drug bioavailability due to longer contact time of the delivery system with the absorption site.

CONCLUSION

Sustained drug release in the current study indicates that the hydrophilic matrix microspheres of Imatinib Mesylate, prepared using CS, can successfully be prepared by Chemical Cross Linking technique. The results revealed that the drug-to-polymer ratio and stirring speed are imperative to acquire sustained release and entrapment efficiency. The microspheres of best batch exhibited mean particle size of 120 µm and entrapment efficiency of 87.28%. The 91% of drug were released upto 24 hours indicates that the microspheres of Imatinib Mesylate could sustain the release of the drug for more than 24 hours.

Tables and figures:

Table.1. Evaluation of microspheres

Formulation Code	Particle size	Angle of Repose	Bulk Density	% yield	Encapsulation Efficiency	Drug Content	Cumulative Release
F1	105.88±0.399	22.44±1.073	0.845±0.010	81.35±0.579	76.38±0.631	19.61±0.374	99.10±0.288
F2	107.37±1.290	24.44±0.786	0.838±0.045	85.75±0.429	76.09±0.784	19.98±0.634	98.87±0.215
F3	109.88±0.804	23.08±1.210	0.860±0.035	89.75±0.580	76.65±0.693	19.87±0.450	99.23±0.500
F4	116.76±0.562	23.24±0.935	0.872±0.019	90.87±0.343	84.28±0.845	21.81±0.382	96.61±0.192
F5	119.19±1.015	22.85±0.720	0.874±0.009	92.01±0.680	87.28±1.021	22.21±0.350	91.28±0.406

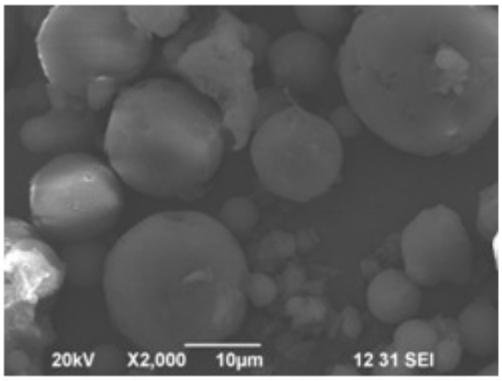

Figure. 1: SEM photograph of optimised formulation

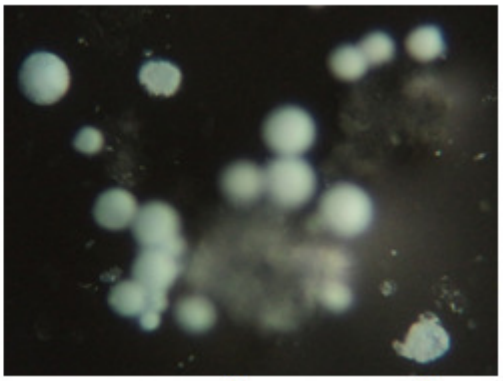

Figure.2: Optical microscopic photo

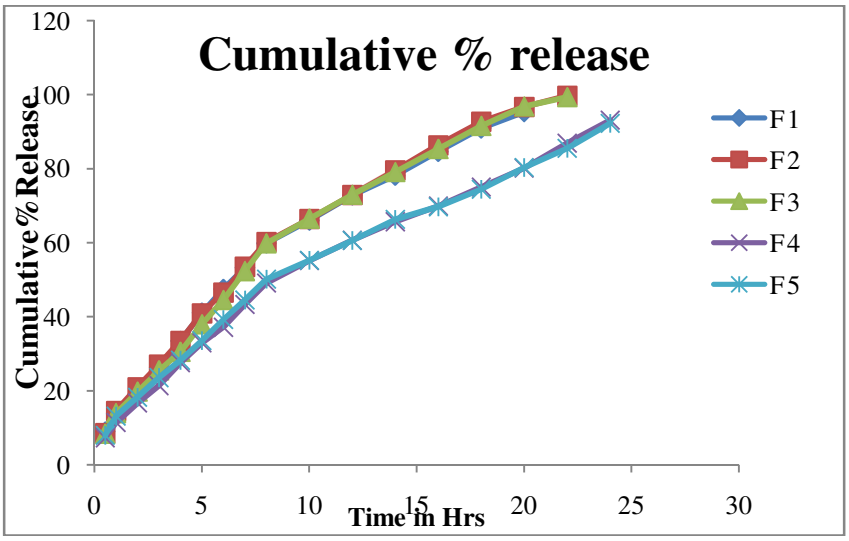

Figure. 3: Cumulative %release of microspheres

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