Research Article

FORMULATION AND EVALUATION OF MICROSPHERES BY CHEMICAL CROSSLINKING METHOD USING IMATINIB MESYLATE AS MODEL DRUG

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ABSTRACT

The present research deals with formulation of microspheres containing an Anti-cancer drug Imatinib mesylate reduce the frequency of dosing. Imatinib mesylate is a protein-tyrosine kinase inhibitor, useful in the treatment of various types of cancer and used in the treatment of atherosclerosis, thrombosis, restenosis, or fibrosis. Imatinib mesylate loaded microspheres were formulated by using both hydrophilic and hydrophobic polymers. Polymers are impregmented by Chemical Cross Linking method using like Sodium Alginate and HPMC K100 to develop a sustained release dosage form. The concentrations of cross-linking agent will influences loading of polymers in this formulation. The effect of concentration of cross-linking agent (Glutaraldehyde) in microspheres formulations and its properties investigated. The investigation done by following properties estimations like particle size, bulk density, angle of repose, encapsulation efficiency, percentage of drug loading, SEM ,DSC, XRD, biodegradability, and drug release kinetics. Our all formulations shows particle, bulk density, encapsulation, releasing rate in optimized way. The kinetics of Imatnib mesylate release from microspheres were analyzed using four different theoretical models, that is, Zero order, First order, Higuchi, and Peppa's models. Microspheres prepared with Glutaraldehyde showed different release kinetics depending upon polymer loading/finding capacity. Increasing the polymer concentration decreased the release rate of Imatinib Mesylate from microspheres because of formation of greater structural strength and more tightly texture with the drug. Besides, microspheres gave an adequate fit to either zero order or first order kinetic models, depending on the extent of cross linking reaction between drug and the cross linking agent.

Keywords: Imatinib Mesylate, Sodium Alginate, HPMCK100, microspheres, sustained release, Anticancer drug, Release Kinetics.

INTRODUCTION

The microparticles delivery system includes microcapsules, pellet, emulsions, microspheres, lipospheres, etc., generally given in the form of oral or topical solutions. Different types of coated particles may be obtained depending on the coating process used. The particles can be surrounded within a polymeric or proteinic matrix complex in either as solid aggregated state or a molecular dispersion, resulting in the formulation of microspheres. Alternatively, the particles can be coated by a solidified polymeric or proteinic envelope, leading to the formation of microcapsules. Imatinib Mesylate is currently registered in adults for two indications : (a) monotherapy in Chronic Myeloid Leukemia (CML) and (b) Metastatic Gastrointestinal Stromal Tumors (GISTs).

Microspheres are defined as homogenous, monolithic particles in the size range of about 1.0– 1000 µm and are widely used as drug carriers for Sustained release. Administration of the drug in the form of microspheres usually improves the action by providing the localization of the active substance at the site of action and by sustaining the release of drugs. Glutaraldehyde was used as cross-linking agent to extent the release of the drug from the formulation. It may have more advantage to deliver this drug in a sustained release dosage form. The present study was focused on development of sustained release Imatinib mesylate using Chemical Cross linking method [1-4].

MATERIALS AND METHODS

Materials

Imatinib Mesylate was procured as a gift sample from Natco pharma limited, Mumbai (India). Sodium Alginate and HPMC K100 was obtained from Safe Pharma, Narasaraopet as gift sample. All chemicals were of analytical grade and were used without further purification.

Method of preparation

A 4.0% (w/v) Polymer 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 25ml of Petroleum ether containing 8.0mlof Span 80 in a beaker at room temperature. Stirring was continued at 2000rpm using a 3- blade half moon paddle for 5 minutes (Remi Equipments, Mumbai, India). 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 2.0 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[1-4]. The microspheres were

then finally dried at room temperature and stored in vacuum desiccators.

Compatibility studies

One of the requirements for the selection of suitable carrier for pharmaceutical formulation was compatibility. Therefore in the present work a study was carried out by using FTIR spectrophotometer to find out if there are any possible chemical interactions between Imatinib mesylate, Sodium Alginate and HPMC K100[5, 6].

Angle of repose

The fractional force in the loose powder can be measured by the angle of repose. Angle of repose was calculated by static method using fixed funnel method. This was the maximum angle possible between the surfaces of the site of the powder to the horizontal plane[7].

Determination of bulk density

The bulk density was determined by 3-tap method. Bulk density is defined as, "the mass of powder divided by the bulk volume". The packing characteristics of the powder play an important role in determining physical properties of product[7].

Encapsulation efficiency

Encapsulation efficiency was calculated using the following formula[8]:

$$\text{Encapsulation Efficiency} \frac{\text{Estimated Drug Content\%}}{\text{Theoretical Drug Content\%}} \times 100$$

Determination of Drug Content

Accurately weighed microspheres equivalent to 25mg of Imatinib mesylate, crushed in glass mortar and pestle and the powdered microspheres were suspended in 100 ml of 0.1N HCl. After 24 hours, the solution was filtered and the filtrate was analyzed for the drug content[9].

***In-vitro* dissolution studies**

The drug release study was performed using USP XXIII dissolution test apparatus, the capsules filled with equivalent amount of Imatinib mesylate 100 Mg was placed in a basket. The instrument was set at 100-rpm rotation and at 32°C, 900 ml of 0.1N HCl was filled as dissolution medium for first 2 hours and phosphate buffer pH 7.4 from third hour onwards. Samples were withdrawn at predetermined intervals, from 0.5 – 24 hours filtered and analyzed spectrophotometrically at 230nm using corresponding medium as blank. After each withdrawal, the same quantity of fresh medium was replaced immediately[9].

Kinetic characteristics of the drug release

To know the mechanism of the drug release from the microspheres, the results obtained from the In-vitro dissolution process were fitted into different kinetic equations as follows[10-12]:

1. Zero - order drug release: Cumulative % drug release Vs Time.

2. First Order drug release: Log cumulative % drug retained Vs Time.

3. Higuchi's classical diffusion equation: Cumulative % drug release Vs Square root of time.

4. Peppa's Korsemeyer Exponential equation: Cumulative % drug release Vs Log time.

'n' values can be used to characterize diffusion release mechanism as :

n > 0.5	Fickian diffusion
0.5 < n < 1	Non-Fickian diffusion
n > 1	Class – II transport

Particle size analysis

The microsphere size distribution was determined by the optical microscopy method using a calibrated stage micrometer (µm)[13-15].

Stability studies

The Optimised preparation was divided into 3 sets and was stored at 4°C (refrigerator), room temperature and 40°C (thermostatic oven). After 15, 30, 60, 90 and 180 days drug content and SEM of the formulation was determined[16].

RESULTS AND DISCUSSION

Imatinib mesylate 10 formulations were formulated using 2 different polymers (refer table No:1). The characterisation of formulations are studied in particle size, angle of repose, bulk density, tapped density, percentage yield, drug content, encapsulation efficiency, cumulative percentage of drug release (refer table No:2).

Table 1: Formula for microspheres

Formulation code	Drug (mg)	Sodium Alginate	HPMC K100
CCFS1	400	200	--
CCFS2	400	400	--
CCFS3	400	600	--
CCFS4	400	800	--
CCFS5	400	1000	--
CCFS6	400	--	200
CCFS7	400	--	400
CCFS8	400	--	600
CCFS9	400	--	800
CCFS10	400	--	1000

The Sustained release microspheres of all batches were found to be spherical and moderate free flowing (refer Figure No:1,2&3). FTIR studies said that there was no incompatibility between Drug and polymers. Formulations particle size shows from 42.10±2.28 (CCFS8) – 52.82±3.07 (CCFS6) (refer Table No:2). The Angle of repose for the formulated microspheres found to be within the range (22.33+0.150 (CCFS9) - 25.43+0.147(CCFS6)) (refer Table No: 2) and showed as moderate flow characters.

Table 2: Evaluation of Formulated Microspheres

Formulation code	Particle Size (µm)	Angle of Repose (θ)	Bulk Density (gm/cm)	Tapped Density (gm/cm)	% Yield (%)	Drug Content (in25mg)	Encapsulation Efficiency (%w/w)	Cum.% Release
CCFS1	47.40±2.70	22.76+0.700	0.83+0.01	0.992+0.03	82.91+0.760	21.92+0.571	85.82+0.470	98.55±0.2914
CCFS2	49.89±2.28	24.24+1.060	0.83+0.008	0.967+0.011	85.40+0.800	23.00+0.640	84.81+0.530	98.58±0.4014
CCFS3	52.17±2.14	22.46+0.091	0.838+0.02	0.972+0.022	89.90+0.161	23.38+0.190	89.93+0.441	99.44±0.1301
CCFS4	43.41±3.76	23.62+0.812	0.826+0.01	0.969+0.009	83.44+0.161	22.53+0.794	83.91+0.723	98.57±0.5084
CCFS5	49.30±3.04	24.92+1.142	0.841+0.01	0.989+0.006	85.60+0.790	22.30+0.922	85.40+0.894	98.49±0.4989
CCFS6	52.82±3.07	25.43+0.147	0.842+0.01	0.977+0.016	85.39+1.713	21.90+1.166	84.77+0.565	96.74±0.5827
CCFS7	47.83±1.56	24.69+0.804	0.836+0.04	0.988+0.005	84.75+0.513	21.85+0.430	85.28+1.054	97.82±0.7705
CCFS8	42.10±2.28	25.04+0.594	0.824+0.009	0.963+0.025	85.03+0.839	22.16+1.33	85.22+0.895	98.67±0.3006
CCFS9	52.08±1.52	22.33+0.150	0.818+0.01	0.971+0.006	85.42+0.822	23.27+0.344	88.90+0.658	99.39±0.1474
CCFS10	47.27±3.15	24.06+0.536	0.830+0.009	0.974+0.009	83.90+0.733	22.52+0.492	85.26+0.995	98.87±0.3179

N=mean±SEM

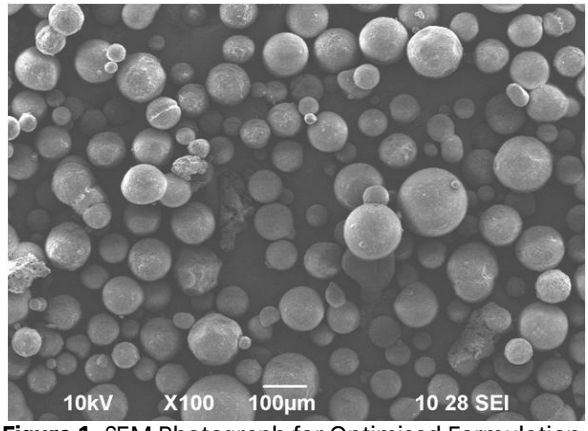

Figure 1: SEM Photograph for Optimised Formulation (CCFS3)

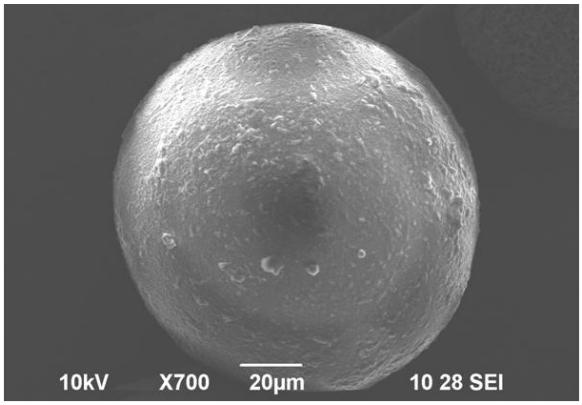

Figure 2: SEM Photograph for Optimised Formulation showing surface (CCFS3)

Figure 3: Optical Microscopic Photograph for Optimised Formulation (CCFS3)

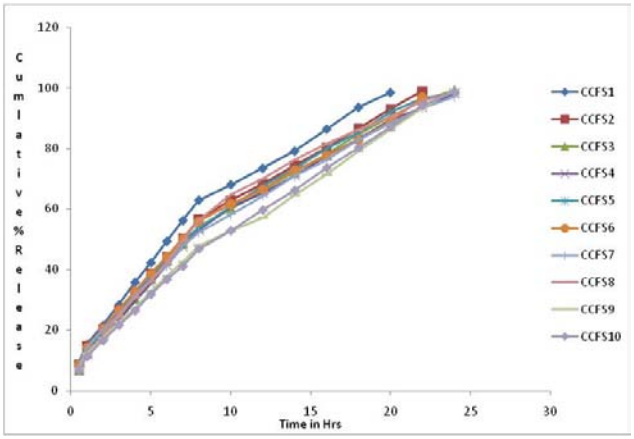

Figure 4: Cumulative % release of Formulations

The formulation shows the bulk density values less than 1.2 gm/cm^2 (0.818+0.01 (CCFS9) - 0.842+0.01(CCFS6)) & tapped density values are from 0.963+0.025 (CCFS8) – 0.992 + 0.03 (CCFS1) (refer table No:2) indicating moderate flow characteristics of microspheres. The percentage yields of formulations were 82.91 + 0.760 (CCFS1) – 89.90 + 0.161 (CCFS3) (refer table No: 2). The drug content was stared from 21.85 + 0.430 (CCFS7) – 23.38 + 0.190(CCFS3) (refer table No: 2). The Drug encapsulation efficiency of microspheres was found to be 84.81-89.93%w/w (refer table No:2). Formulations show drug content levels from 21.85 – 23.38 (refer table No:2). The percentage of yield shows from 82.91 – 89.90(refer table No:2). Stability Studies stated that there was not much different in the drug content and moderate difference in the SEM analysis (refer figure No: 1&2) and drug content. The in-vitro drug release for all formulations was found to follow Non -Fickian Diffusion release kinetics (refer table No: 2).

CONCLUSION

From our study, it was concluded that the Imatinib Mesylate (α-form) loaded microspheres prepared with cross-linking agent Glutaraldehyde, with different polymers like Sodium Alginate, HPMC K100 showed decreased in the release rate of drug from the formulation depending on the concentration of the polymers. The concentration of the polymers influenced the drug release pattern and polymer implementation depending upon cross-linking agent. The formulation with

drug and polymers in 1:3 ratio (CCFS3) with cross linking agent maximum concentration was considered best because, it showed retard drug release in pH 7.4 buffer was found to be 99.44 % almost complete and uniform after the 24hour release study. Drug encapsulation efficiency for formulation (CCFS3) was 89.93% w/w and all the remaining parameters were within the prescribed limit. Concluded that formulation CCFS3 fully satisfy all expectation.

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