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Design and Evaluation of Imatinib Mesylate Loaded Microspheres for Stomach Specific Drug Delivery

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RMV designed the study, wrote the protocol, and supervised the entire work. Author BLNR performed the study and carried out analysis of the study. Author VKR managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The aim of the present work was to design and evaluate the Imatinib mesylate microspheres using natural and semi synthetic polymers for stomach specific drug delivery.
Study Design: Design and Evaluation of Imatinib Mesylate loaded microspheres
Place and Duration of the Study: The study was carried out in Department of Pharmaceutics, JKKMMRF's Annai JKK Sampoorani Ammal College of Pharmacy, between November 2012 and July 2013.
Methodology: The microspheres were prepared using Sodium alginate as a polymer by Emulsification Ionic Gelation Technique. Copolymers of natural origin namely Guar gum, Karaya gum, Chitosan and semi synthetic origin namely Hydroxy propoylmethyl cellulose K4M, K15M, K100M are used as mucoadhesive polymers. The prepared microspheres were evaluated for their percentage yield, entrapment efficiency, degree of swelling, particle size, surface morphology and *in-vitro* mucoadhesion, drug release studies. Drug release kinetics was determined from drug release data. Selected formulations are subjected to stability studies under varying conditions of temperature and humidity.

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Results: The production yields of microspheres were found to be between 76.74 to 88.18%. Percentage drug entrapment efficiency of Imatinib mesylate microspheres was ranged from 65.51 to 88.78%. Particle size of the prepared microspheres was in the size range of 440-810µm. SEM analysis revealed that all the prepared microspheres were discrete, spherical in shape. The *in-vitro* mucoadhesive study demonstrated that Hydroxy propoylmethyl cellulose adhered to the mucus to a greater extent than other polymers. The *in-vitro* release study shows that, retarded release with increase in percentage of polymers. The release of drug from the microspheres followed Krosmeyer Peppas kinetics. After the stability studies, the formulations remained stable at the end of storage period.

Conclusion: Based on the results, it was concluded that, the formulations with natural polymers were found to be best than semi synthetic polymers for the oral delivery of Imatinib mesylate.

Keywords: Imatinib mesylate, mucoadhesive microspheres, Emulsification Ionic Gelation, stomach specific delivery, natural polymers, semi synthetic polymers.

1. INTRODUCTION

Gastro retentive drug delivery systems are primarily controlled release drug delivery systems, which gets retained in the stomach for longer period of time, thus helping in the absorption of drug for the intended duration of time. These dosage forms are known to extend the absorption phase of the drug in the proximal part of the small intestine where narrow absorption window drugs are preferentially absorbed due to the large surface area, in comparison to the colon; or because of the enhanced solubility of the drug in the stomach as opposed to more distal parts of the gastrointestinal tract. Several strategies have been proposed to modify the GI transit of oral pharmaceutical formulations. One such approach is to design a formulation, which can adhere to the lining of the stomach, thus retaining the drug at the target absorption site for a prolonged period of time [1]. Mucoadhesive microspheres exhibit a prolonged residence time at the site of application or absorption, facilitate an intimate contact with the underlying absorption surface, and thus contribute to improved and / or better performance of drug [2]. Some of the disadvantages were found to be as follows:

- The release from the formulations may get modified.
- The release rate may vary from a variety of factors like food and the rate of transit though gut, mucin turnover rate etc.
- Differences in the release rate can be found from one dose to another.
- Any loss of integrity in release pattern of the dosage form may lead to potential toxicity.
- These kinds of dosage forms cannot be crushed or chewed.
- Some drugs may damage the mucus when it is adhered to the mucus membrane [3].

The investigation was concerned with design and characterization of Imatinib Mesylate mucoadhesive microspheres for controlled release in order to improve efficacy. Imatinib Mesylate is an anti-cancer agent which is used to treat chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs) and a number of other malignancies. It is the first member of a new class of agents that act by inhibiting particular tyrosine kinase

enzymes, instead of non-specifically inhibiting rapidly dividing the cells. For the present study Imatinib Mesylate is selected as drug candidate, it fulfills the following characteristics which indicate its suitability for fabrication into the mucoadhesive drug delivery system.

- Formulation of mucoadhesive microspheres containing Imatinib Mesylate as a drug candidate which would remain in stomach for prolonged period of time, therefore the maximum drug release is maintained at desired site because Imatinib Mesylate has good absorption in GIT.
- Imatinib Mesylate having low pKa which remain unionized in stomach for better absorption.
- Imatinib Mesylate is a site specific drug.
- To reduce the dose related side effects which are caused by Imatinib Mesylate long term therapy, and as well as to reduce total dose amount of the drug in the formulation [4,5].

By considering the above facts, Imatinib Mesylate microspheres were designed and characterized in order to improve the bioavailability. Hence, the Imatinib Mesylate mucoadhesive microspheres were prepared by emulsification ionic gelation method using the different concentration of polymers.

2. MATERIALS AND METHODS

2.1 Materials

Imatinib Mesylate is obtained as gift sample from Hetero Drugs Ltd, Hyd. Sodium alginate was purchased from Thomas Baker chemicals. Guar gum, Karaya gum, HPMC K4M, HPMC K15M and HPMC K100M were purchased from Yarrow Chem Products. Chitosan was purchased from Cochin Fisheries Department. All chemicals were of analytical grade and were used without further purification.

2.2 Compatibility Study

Compatibility between the drug and the polymers was determined by Fourier transform infrared spectroscopy and High performance liquid chromatography [6,7].

2.3 Preliminary Studies

The preliminary studies were carried out by preparing various batches of microspheres with different process parameters in an effort to optimize the formulations for obtaining microspheres with proper physical characteristics and particle size which are ideal for oral cavity.

2.3.1 Effect of stirring speed

The speed of the propeller was varied to get the particle size suitable for oral delivery. Four batches of microspheres were prepared with a stirring speed of 500, 1000, 1500 and 2000 rpm respectively. The other process variables were kept constant.

2.3.2 Effect of different cross linking agents

Three batches of microspheres were prepared with three different cross linking agent Calcium chloride, Barium chloride and Aluminium sulphate respectively. The other process variables like concentration of cross linking agent (5.0%w/v) and rpm (2000) was kept constant. The prepared microspheres were evaluated for percentage entrapment efficiency and particle size.

2.3.3 Effect of concentration of cross linking agent

Four different formulations prepared by varying the cross linking agent (Barium chloride) concentration from 2.5%, 5.0%, 7.5% and 10% w/v respectively, while keeping all other process variables constant. The prepared microspheres were evaluated for entrapment efficiency and particle size.

2.3.4 Effect of amount of cross-linking agent

Four different formulations were prepared by varying the amount of cross linking agent (Barium chloride) was varied as 25, 50, 75 and 100ml, while keeping all other process variable like stirring speed and concentration of cross linking agent constant. The prepared microspheres were evaluated for entrapment efficiency and physical appearance.

2.4 Preparation of Mucoadhesive Microspheres

Based on the results of preliminary investigation, the different process parameters like stirring speed, different cross linking agents, concentration of cross linking agent and amount of cross linking agents were optimized and final formulations were designed by varying polymer to drug ratio as mentioned in Table 1.

2.4.1 Emulsification ionic gelation method

Mucoadhesive alginate microspheres containing *Imatinib mesylate* were prepared by emulsification ionic gelation technique. Sodium alginate and copolymers were dispersed in deionised water (30 ml) separately with continuous stirring to form homogenous polymer dispersion and both the dispersions were added in different ratio as mentioned in formulation chart. *Imatinib mesylate* was added to polymer dispersion and mixed thoroughly to form a viscous suspension. The dispersions were sonicated for 30 mins to remove any air bubbles that may have been formed during stirring. The stream of smooth viscous suspension was added to light liquid paraffin in the form of a thin stream. Stirring of the above mixture was done in a beaker placed on mechanical stirrer. Then 100 ml of Barium Chloride solution (5% w/v) was added slowly while stirring for ionic gelation reaction. The stirring was continued for 15 minutes. The mixture was allowed to settle and product was separated. Obtained microspheres were washed several times with Petroleum ether to remove the adhering paraffin and dried in room temperature [8].

Table 1. Composition of Imatinib mesylate mucoadhesive microspheres

Ingredients		Formulation code	Drug: Sodium Alginate: Polymer ratio
Drug	**Polymers**		
Imatinib mesylate	Sodium alginate	IMS1	1:1:0
		IMS2	1:2:0
		IMS3	1:3:0
	Guar gum	IMG1	1:1:1
		IMG2	1:1:2
		IMG3	1:1:3
	Karaya gum	IMK1	1:1:1
		IMK2	1:1:2
		IMK3	1:1:3
	Chitosan	IMC1	1:1:1
		IMC2	1:1:2
		IMC3	1:1:3
	HPMC K4M	IMH1	1:1:1
		IMH2	1:1:2
		IMH3	1:1:3
	HPMC K15M	IMH4	1:1:1
		IMH5	1:1:2
		IMH6	1:1:3
	HPMC K100M	IMH7	1:1:1
		IMH8	1:1:2
		IMH9	1:1:3

2.5 Evaluation of Mucoadhesive Microspheres

2.5.1 Production yield (%)

The production yield of microspheres of various bathes were calculated using the weight of the final product after drying with respect to the initial weight of the drug and polymer used for the preparation of microspheres and percentage production yield was calculated as per the following formula [9]:

$$\textbf{Percentage yield}\ (\%) = \frac{\textbf{Practical mass (Microspheres)}}{\textbf{Theoritical mass (Drug+Polymer)}} \times \mathbf{100}$$

2.5.2 Drug entrapment efficiency

To determine the amount of drug encapsulated in microspheres, a weighed (25mg) of microspheres were crushed in a glass mortar and pestle and the powdered microspheres were suspended in 100 ml of 0.1 N HCl. After 24 hours the solution was filtered and 1 ml of filtrate was pipetted out and diluted to 25 ml and analyzed for the drug content using UV-Specrophotometer at 255 nm.

The drug entrapment efficiency was calculated using the following formula:

$$\textbf{Drug entrapment efficiency}\ (\%) = \frac{\textbf{Estimated drug content}}{\textbf{Theoritical drug content}} \times \mathbf{100}$$

Theoretical drug content was determined by calculation assuming that the entire *Imatinib* present in the polymer solution used gets entrapped in *Imatinib mesylae* microspheres, and no loss occurs at any stage of preparation of *Imatinib mesylate* microspheres [10].

2.5.3 Particle size analysis

Many methods are available for determining the particle size, such as optical microscopy, sieving, sedimentation and particle volume measurement. Optical microscopy is most commonly used for particle size determination. The optical microscope is fitted with an ocular micrometer and stage micrometer. The eyepiece micrometer was calibrated. The particle diameters of more than 200 microspheres were measured randomly by optical microscope.

The average particle size is determined by using Edmondson's equation:

$$D_{mean} = \frac{\sum nd}{\sum n}$$

Where,

n - Number of microspheres observed.
d - Mean size range.

2.5.4 Shape and surface morphology

The shape and surface characteristics of the prepared microspheres were evaluated by means of scanning electron microscopy.

2.5.5 Degree of swelling

Accurately weighed 100 mg of microspheres were immersed in slight excess of 0.1N Hydrochloric acid for 24 hours and washed. The degree of swelling was calculated using the following formula:

$$\alpha = \frac{Ws - Wo}{Wo}$$

Where,

α is the degree of swelling
W_O is the weight of the microspheres before swelling
W_S is the weight of the microspheres after swelling.

2.5.6 In-vitro mucoadhesion studies

The *in-vitro* mucoadhesion study of microspheres was assessed using falling liquid film technique. A strip of sheep intestinal mucosa was mounted on a glass slide and about 50 microspheres was placed on the mucosa. Few drops of 0.1N Hydrochloric acid used to hydrate the microspheres. After 5 min, support with intestine inclined to 50° using a stand. The intestinal mucosa at room temperature was washed at a rate of 23 ± 2 ml/min using flow controlled tubes (I.V infusion set). The tip of tube carrying buffer solution was placed over the tissue with the help of rubber bands so that liquid flows evenly over the mucosa. The washings are collected into a beaker. After 45 minutes, particles that reached measurement point (2cm from original applied place) and detached collected in receiver and particles remained in the applied area used to quantify the bioadhesion [11].

Percentage mucoadhesion was calculated using the formula:

$$\%\ \boldsymbol{Mucoadhesion} = \frac{\boldsymbol{Initial\ no.of\ \ microspheres - No.of\ microspheres\ detached}}{\boldsymbol{Initial\ no.of\ \ microspheres}} \times \mathbf{100}$$

2.5.7 In-vitro drug release studies

Dissolution studies were carried out by using USP type - I dissolution assembly in stimulated gastric fluid pH 1.2. A weighed amount of microspheres equivalent to 400 mg drug were dispersed in 900 ml of 0.1 N HCl (pH 1.2) maintained at 37 ± 0.5°C and stirred at 100 rpm. Five ml of aliquots were withdrawn at 60 minutes intervals and filtered. The required dilutions were made with 0.1 N HCl and the solutions were analyzed for the drug content by UV spectrophotometer against suitable blank at 255nm. From this the percentage of drug released was calculated and plotted against function of time [8].

2.5.8 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 [12,13,14,15,16]

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.

2.5.9 Stability studies

Stability studies were carried out at 5°C/Ambient, 25°C / 60% RH and 40°C / 75% RH for three months using programmable environmental test chamber. The selected formulations were packed in amber colour glass containers and are tightly closed with the cap. They were stored at the stated conditions for three months. Samples were analyzed after 0, 30, 60 and 90 days and they were evaluated for % drug entrapment efficiency, % mucoadhesion and *in-vitro* drug release studies [17].

3. RESULTS AND DISCUSSION

3.1 Compatibility Study

The IR spectra of pure drug alone and along with the polymers indicate no interaction between the drug and the polymers when compared with the infrared spectrum of pure drug as all functional group frequencies are present.

The retention time of *Imatinib mesylate* obtained by HPLC shows that there was no potential of interaction between the drug and excipients because there was no significant difference between the retention time of pure drug alone and in combination with the polymers (Table 2).

Table 2. Retention time of Imatinib mesylate along with polymers

Sl. No	Sample	Ratio	Rt (Mins)
1	Imatinib mesylate	-	1.51
2	Imatinib mesylate + Sodium alginate	1:01	1.53
3	Imatinib mesylate + Guar gum	1:01	1.48
4	Imatinib mesylate + Karaya gum	1:01	1.54
5	Imatinib mesylate + Chitosan	1:01	1.53
6	Imatinib mesylate + HPMC K4M	1:01	1.52
7	Imatinib mesylate + HPMC K15M	1:01	1.52
8	Imatinib mesylate + HPMC K100M	1:01	1.53

3.2 Preliminary Studies

3.2.1 Effect of stirring speed

The microspheres obtained at 2000 rpm were uniform, free flowing and were in the size range of 350-450µm as shown in Table 3, which are suitable for oral delivery. Hence, in our study, the stirring speed was optimized for 2000rpm.

Table 3. Effect of stirring speed on particle size

Formulation code	Stirring speed (rpm)	Concentration of cross linking agent %w/v	Particle size in µm
PSF1	500	5	640.21µm
PSF2	1000	5	521.47µm
PSF3	1500	5	485.17µm
PSF4	2000	5	424.26µm

3.2.2 Effect of different cross linking agent

The effect of different cross linking agent was studied using Calcium chloride ($CaCl_2$), Barium chloride ($BaCl_2$) and Aluminium sulphate $Al_2(SO_4)_3$ as cross linking agents and concentration of cross linking agent was kept constant. Results were shown in Table 4. Based on the results Barium chloride was selected as cross-linking agent for the study.

Table 4. Effect of different cross linking agents on % drug entrapment efficiency and particle size

Formulation code	Different cross linking agent	Concentration of cross linking agent %w/v	% Drug entrapment efficiency	Particle size in µm
PSF5	$CaCl_2$	5%	68.7	638.4µm
PSF6	$BaCl_2$	5%	78.4	570.7µm
PSF7	$Al_2(SO_4)_3$	5%	58.2	680.2µm

3.2.3 Effect of concentration of cross linking agent

The concentration of cross-linking agent was varied from 2.5, 5, 7.5 and 10%w/v. Barium chloride was used as cross linking agent, increase in concentration from 2.5% to 10% w/v exhibited increase in particle size.

The physical characteristics of the microspheres were studied using an optical microscope and the results were shown in table 5. Based on the observations in the present work, the concentration of cross linking agent was optimized to 5% w/v.

Table 5. Effect of concentration of cross linking agent on % drug entrapment efficiency and particle size

Formulation code	Concentration of cross linking agent (ml)	Stirring speed (rpm)	% Drug entrapment efficiency	Particle size in µm
PSF8	2.5	2000	47.4	521.15µm
PSF9	5	2000	77.8	631.25µm
PSF10	7.5	2000	67.8	701.38µm
PSF11	10	2000	57.7	784.65µm

3.2.4 Effect of amount of cross-linking agent

Table 6. Effect of amount of cross-linking agent

Formulation code	Amount of cross linking agent (ml)	% Drug entrapment efficiency	Physical Characteristics
PSF12	25	47.12	Irregular
PSF13	50	56.47	Slightly irregular
PSF14	75	74.23	Slightly irregular
PSF15	100	81.57	Spherical, Free flowing

The amount of cross-linking agent was varied as 25, 50, 75 and 100ml. The higher amount of cross-linking agent appears to favour the cross-linking reaction and hence free flowing microspheres. The amount of cross-linking agent also showed significant effect on % drug entrapment efficiency. Batches PSF12, PSF13 and PSF14 showed less % drug entrapment efficiency was below 75%. Batch PSF15 showed more than 80% drug entrapment efficiency. The observed results were tabulated in Table 6.

The microspheres formed at lower amount of Barium chloride were irregular in shape and mass obtained were sticky, whereas microspheres obtained with 100ml of Barium chloride were quiet spherical in shape, uniform in size and free flowing. Hence based on these observations in the present work, the amount of cross linking agent was optimized to 100ml.

3.3 Evaluation Tests

3.3.1 Production yield

The production yields of microspheres prepared by emulsification ionic gelation method were found to be between 76.74 to 88.18%. It was observed that as the polymer ratio in the formulation increases, the product yield slightly decreases. The probable reason behind this may be the high viscosity of the solution which decreased its syringeability resulting in blocking of needle and wastage of the drug- polymer solution which ultimately decreased the production yields of microspheres (Table 7).

Table 7. Evaluation results of Imatinib mesylate microspheres.

Formulation code	Percentage yield	Entrapment efficiency	Degree of swelling	Average particle size	Percentage mucoadhesion
IMS1	88.18	65.51	0.81	633.75	67.33
IMS2	86.67	69.79	0.87	651.25	73.33
IMS3	82.25	73.67	0.92	675.25	78.00
IMG1	85.49	71.43	0.89	730.75	71.33
IMG2	83.71	73.88	0.96	764.75	78.00
IMG3	80.17	77.69	1.16	810.75	83.33
IMK1	86.24	76.12	0.84	538.25	70.00
IMK2	84.16	85.71	1.02	570.5	78.66
IMK3	81.49	89.93	1.3	592.5	82.00
IMC1	87.78	66.67	0.99	441.25	68.66
IMC2	84.37	72.31	1.22	458.25	72.00
IMC3	82.19	75.58	1.43	510.00	80.66
IMH1	85.76	74.22	0.88	670.25	71.33
IMH2	82.24	79.8	1.13	690.75	75.33
IMH3	80.31	85.99	1.33	723.25	86.67
IMH4	83.46	75.92	0.98	712.25	74.00
IMH5	81.17	83.67	1.24	735.75	78.66
IMH6	78.46	87.96	1.56	776.5	88.66
IMH7	82.18	76.53	1.09	754.25	77.33
IMH8	79.46	84.76	1.37	779.75	82.66
IMH9	76.74	88.78	1.67	787.25	91.33

3.3.2 Drug entrapment efficiency

Percentage drug entrapment efficiency of *Imatinib mesylate* microspheres prepared with different polymers was ranged from 65.51 to 88.78%. The drug entrapment efficiency of the prepared microspheres increased progressively with an increase in proportion of the respective polymers (Table 7).

3.3.3 Particle size analysis

Mean particle size of the prepared microspheres was in the size range of 440-810µm. The mean size increased with increasing polymer concentration which is due to a significant increase in the viscosity, thus leading to an increased droplet size and finally a higher microspheres size (Table 7).

3.3.4 Degree of swelling

With an increase in polymer concentration, the degree of swelling also increases (Table 7).

3.3.5 In-vitro mucoadhesion test

The *in-vitro* mucoadhesive study demonstrated that microspheres using HPMC adhered to the mucus to a greater extent than the microspheres using sodium alginate alone and also microspheres using other copolymers (Table 7).

3.3.6 Scanning electron microscopic analysis

SEM analysis of the microspheres revealed that all the prepared microspheres were discrete, spherical in shape and had satisfactory surface morphology (Fig. 1)

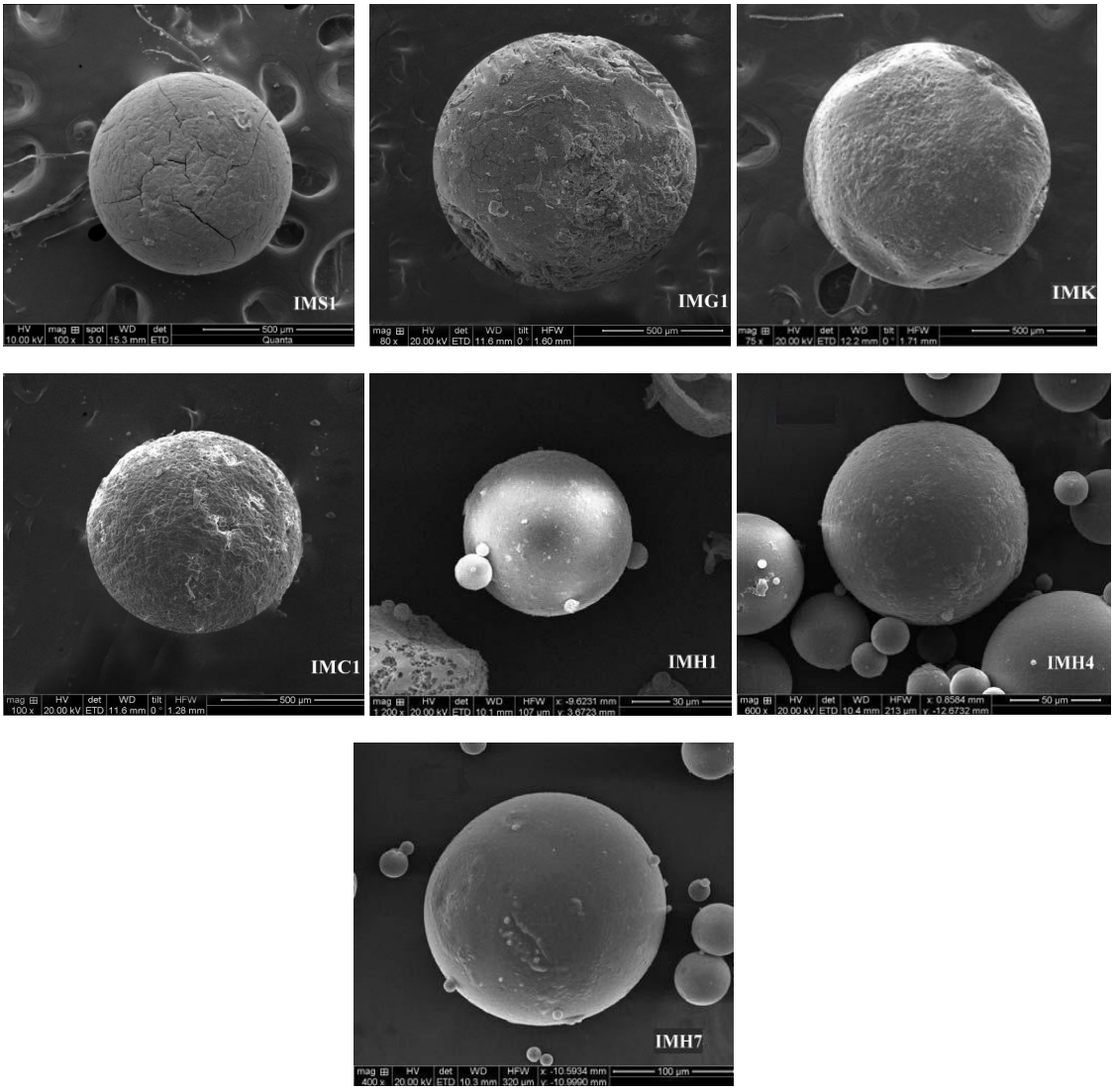

Fig. 1. Scanning electron microscopy pictures of Imatinib mesylate microspheres.

3.3.7 In-vitro drug release studies

Results of drug release study of all the formulations were tabulated in Tables 8 and 9 and shown in figures 2 and 3. As the polymer to drug ratio was increased the extent of drug release decreased. A significant decrease in the rate and extent of drug release is attributed to the increase in density of polymer matrix that results in increased diffusion path length

which the drug molecules have to traverse. Additionally, the larger particle size at higher polymer concentration also restricted the total surface area resulting in slower release.

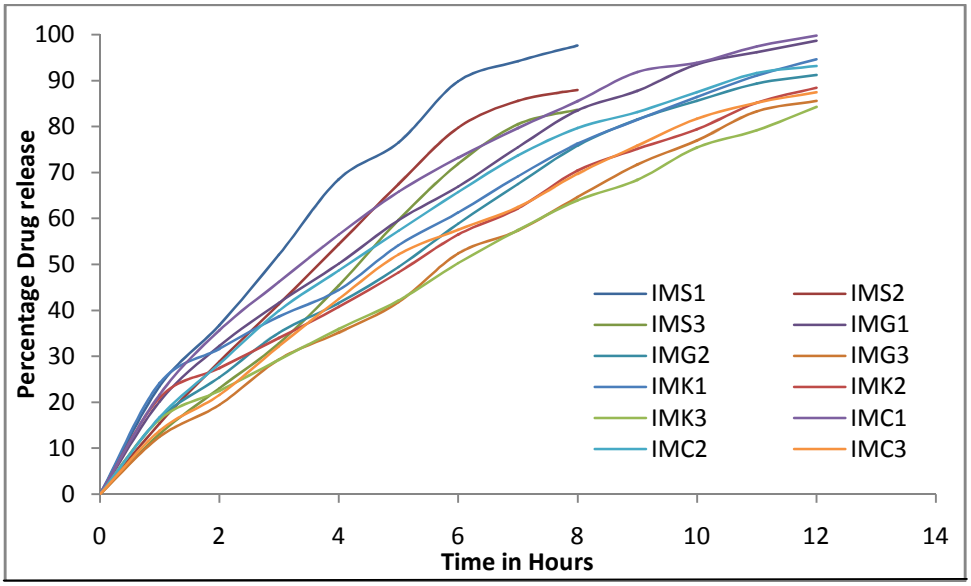

Fig. 2. *In-vitro* drug release date of Imatinib mesylate microspheres using natural polymers

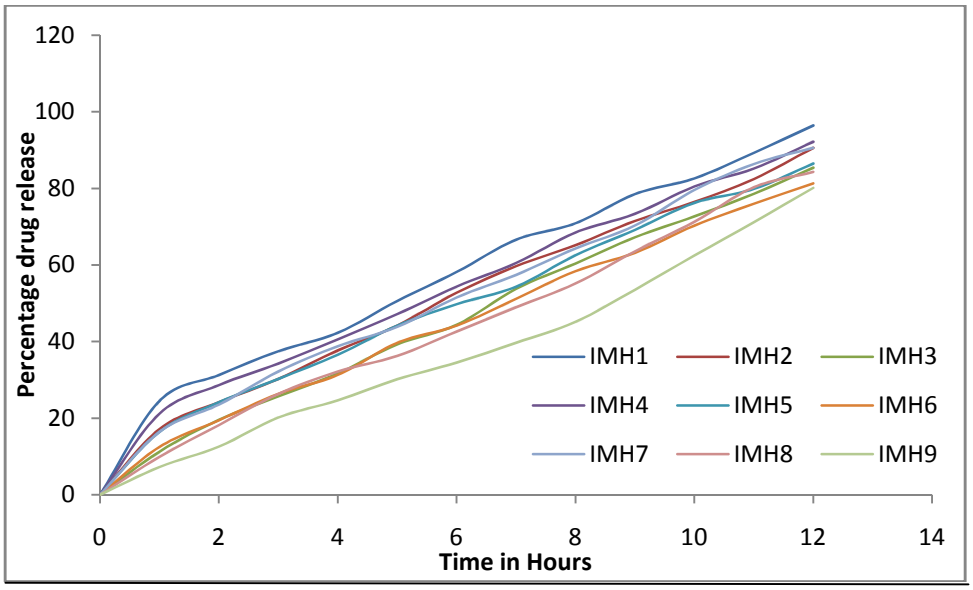

Fig. 3. *In-vitro* drug release date of Imatinib microspheres using semi synthetic polymers

3.4 Kinetics Study

From the dissolution profile of formulations, the R values of Korsmeyer peppas model were close to 1. The diffusion coefficients (n) values ranged from 0.5793 to 0.9499. The observed

diffusion coefficient values were indicative of the fact that the drug release from the formulation follows non-Fickian transport mechanism.

Table 8. Data for analysis of drug release mechanism from Imatinib mesylate microspheres

Formulation Code	Zero order	First order	Matrix	Peppas		Best fit model
	R	R	R	R	n	
IMS1	0.9575	0.9423	0.9744	0.9910	0.7212	Peppas
IMS2	0.9777	0.9749	0.9503	0.9939	0.8716	Peppas
IMS3	0.9901	0.9640	0.9231	0.9938	0.9399	Peppas
IMG1	0.9566	0.9109	0.9867	0.9972	0.6572	Peppas
IMG2	0.9729	0.9754	0.9721	0.9957	0.7259	Peppas
IMG3	0.9896	0.9712	0.9582	0.9967	0.8072	Peppas
IMK1	0.9660	0.9491	0.9849	0.9856	0.5877	Peppas
IMK2	0.9742	0.9739	0.9798	0.9858	0.6198	Peppas
IMK3	0.9867	0.9755	0.9673	0.9900	0.6981	Peppas
IMC1	0.9292	0.8323	0.9914	0.9916	0.6200	Peppas
IMC2	0.9491	0.9853	0.9839	0.9919	0.7030	Peppas
IMC3	0.9706	0.9861	0.9742	0.9929	0.7722	Peppas
IMH1	0.9748	0.8486	0.9780	0.9808	0.5793	Peppas
IMH2	0.9882	0.9321	0.9659	0.9898	0.6907	Peppas
IMH3	0.9965	0.9520	0.9454	0.9967	0.8215	Peppas
IMH4	0.9807	0.9270	0.9737	0.9829	0.6112	Peppas
IMH5	0.9878	0.9557	0.9661	0.9920	0.6825	Peppas
IMH6	0.9946	0.9664	0.9535	0.9953	0.7697	Peppas
IMH7	0.9902	0.9255	0.9606	0.9923	0.7042	Peppas
IMH8	0.9952	0.9290	0.9323	0.9955	0.8485	Peppas
IMH9	0.9879	0.8979	0.8889	0.9917	0.9499	Peppas

3.5 Stability Studies

After the stability studies percentage drug entrapment efficiency, *in-vitro* drug release profile and *in-vitro* mucoadhesion tests shows that the formulations remained stable at the end of storage period. Results were tabulated in Tables 9, 10 and 11.

3.5.1 Percentage drug entrapment efficiency of the formulations

Table 9. Percentage drug entrapment efficiency of the selected formulations

Stability condition	Sampling (days)	% Drug entrapment efficiency						
		IMS3	IMG3	IMK3	IMC3	IMH3	IMH6	IMH9
5^0 C/Ambient	30	73.67	77.57	89.48	75.84	85.47	87.47	88.48
	60	73.61	77.34	89.21	75.54	85.34	87.24	88.37
	90	73.58	77.21	89.08	75.32	85.31	87.19	88.21
25^0C / 60 % RH	30	73.28	77.48	89.67	75.48	85.74	87.48	88.56
	60	73.25	77.35	89.41	75.21	85.47	87.24	88.41
	90	73.19	77.40	89.21	75.07	85.21	87.21	88.37
40^0C / 75 % RH	30	73.38	77.65	89.36	75.79	85.54	87.37	88.74
	60	73.30	77.48	89.19	75.67	58.36	87.25	87.57
	90	73.05	76.80	88.67	75.04	57.50	86.12	87.17

3.5.2 In-vitro drug release studies

Table 10. *In-vitro* drug release profile of the selected formulations at the end of 90 days

Stability condition	Sampling (days)	% Drug release						
		IMS3	IMG3	IMK3	IMC3	IMH3	IMH6	IMH9
5^0 C/Ambient	90	83.57	85.58	82.54	87.41	85.34	78.41	74.18
25^0C / 60 % RH	90	83.09	84.87	82.09	86.57	84.29	78.09	72.25
40^0C / 75 % RH	90	84.14	85.09	83.54	86.17	86.79	77.14	72.49

3.5.3 Percentage mucoadhesion

Table 11. Percentage mucoadhesion of the selected formulations

Stability condition	Sampling (days)	Percentage Mucoadhesion						
		IMS3	IMG3	IMK3	IMC3	IMH3	IMH6	IMH9
5^0 C/Ambient	30	80	84	80	84	88	92	90
	60	78	82	78	84	86	92	88
	90	80	82	76	80	86	90	88
25^0C / 60 % RH	30	80	84	80	82	86	92	92
	60	80	80	80	82	86	90	92
	90	76	80	78	78	84	90	88
40^0C / 75 % RH	30	78	84	82	84	88	92	88
	60	76	80	80	84	88	92	88
	90	78	80	80	82	84	90	86

4. CONCLUSION

The results of this investigation indicate that ionic cross linking technique ionotropic gelation method can be successfully employed to fabricate *imatinib mesylate* loaded alginate microspheres. The technique provides characteristic advantage over conventional microsphere method, which involves an “all-aqueous” system, avoids residual solvents in microspheres. Other methods utilize larger volume of organic solvents, which are costly and hazardous because of the possible explosion, air pollution, and toxicity and difficult to remove traces of organic solvents completely.

Based on the results of evaluation tests and stability tests, formulations with natural polymers were found to be best formulations than semi synthetic polymers for the oral delivery of *imatinib mesylate* that complied with all the parameters. From the study it was concluded that natural polymers were found to be best carriers than semi synthetic polymers for oral drug delivery of microspheres.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

The authors state no conflicts of interest and have received no funding for the research or in the preparation of this manuscript.

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