

FORMULATION AND EVALUATION OF FLOATING-MUCOADHESIVE MICROSPHERES OF NOVEL NATURAL POLYSACCHARIDE FOR SITE SPECIFIC DELIVERY OF RANITIDINE HYDROCHLORIDE

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ABSTRACT

Objective: Localization of ranitidine hydrochloride (RH) into the upper part of the intestinal tract is beneficial for better drug bioavailability. Present work described the method of preparation of novel plant polysaccharide based floating microspheres for delivery of the drug into the stomach.

Methods: Polysaccharide was extracted from the seeds of plant *Tamarindus indica* (TI). Extracted polysaccharide was evaluated for some physicochemical parameters. Floating-mucoadhesive microspheres were prepared by using extracted polysaccharide as mucoadhesive excipients while eudragit as a release controlling polymers by using emulsion crosslinking method. Chemical crosslinking was done by using epichlorohydrin. Prepared microspheres were evaluated for their drug-polymer compatibility study by using fourier transform infrared spectroscopy (FT-IR). Further characterization such as size, surface properties, swelling index, percentage encapsulation, *in vitro* buoyancy and drug release was performed.

Results: FT-IR study confirms the chemical crosslinking of extracted polysaccharide and also drug stability during processing of microspheres. The size of microspheres was in the range of 5.38 to 7.84 μm . SEM images revealed that all batches were of spherical in size and smooth surface. The swelling index showed better swelling in the range of 158-257 percentages. Encapsulation efficiency was found to be decreased by decreasing the concentration of polysaccharide. *In vitro* buoyancy study possesses that formulation F1 showed better floating ability as compared to the others. Finally, *in vitro* drug release study revealed that prepared microspheres were able to release the 100% drug within 8-12 h, indicating sustain release behavior.

Conclusion: Present study concludes that polysaccharide of TI may be used as excipients for the preparation of floating-mucoadhesive microspheres.

Keywords: *Tamarindus indica*, Ranitidine hydrochloride, Floating microspheres, Natural polymer

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INTRODUCTION

RH is a commonly used antacid as H₂ receptor antagonist which shows maximum absorption at the initial part of small intestine. Moreover, colonic metabolism is also responsible for poor oral drug bioavailability [1]. Effective treatment can be achieved by administration of 200 mg of RH, three times a day, which leads to a patient in compliance. In order to improve therapeutic action and patient compliance, it is required to design a formulation which can increase the retention of the drug into the upper part of along with sustain release action up to 12 h [2].

The floating system is used for localized drug release into the specific region of the gastrointestinal tract (GIT). This system can be effervescent or non-effervescent depends on ingredients used in the delivery system. The most commonly used excipients in non-effervescent floating drug delivery system are either swellable or gel forming hydrocolloids and polysaccharide. These systems are also known as plug type system due to the tendency to remain lodged near the pyloric sphincter [3]. Nowadays, a large number of plant polysaccharide has been investigated for their potential role in the floating drug delivery system. These polymers have a tendency to swell on gastric media and float, therefore, increasing the residential time of formulation into the upper part of GIT. Additionally, this plant polysaccharide has some advantages such as biodegradable, biocompatible, low cost and relatively free availability [4].

With the same context, in the present work drug loaded floating mucoadhesive microspheres were developed to improve the residential time of RH into upper GIT. A novel plant polysaccharide

was investigated for their application in the mucoadhesive delivery system. The polysaccharide was isolated from the seeds of TI. The polysaccharide of TI is nonionic, neutral, branched structure consist of cellulose like backbone which carries xylose and galactoxylose subunits. Polysaccharide has been investigated for many applications such as gelling agent, tablet binder, stabilizer and thickening agent [5, 6]. So far, the polysaccharide is not evaluated in the floating drug delivery system. Aim of present work was to investigate the role of novel TI polysaccharide in development of floating mucoadhesive microspheres for localized drug delivery of RH specifically into upper parts of GIT.

MATERIALS AND METHODS

RH was obtained as generous gift sample from Hetero labs, Hyderabad, India. Seeds of TI were purchased from local market of Kanpur (UP). All other materials such as Eudragit, castor oil, epichlorohydrin, and sodium bicarbonate were of analytical grade and were procured from commercial sources.

Polysaccharide extraction

TI polysaccharide was extracted as per reported methods in various publications [7]. The seed were process by separating the brown peels from the kernel seed with the blender and plastic sieve were used to separate the seed. The seed were powdered and boiled with distilled water. Mucilaginous juice was separated by filtration through muslin cloth under vacuum and precipitated by ethanol. Precipitate was dried at 50 °C for 2 d. Further, the extracted dry polysaccharide was characterised in terms of purity and viscosity.

The purity of the polysaccharide was evaluated by testing the presence of carbohydrate, by performing the molisch's, ruthenium, and iodine test. The viscosity of 2% w/v aqueous solution of polysaccharide was determined by using rheometer (Brookfield R/S plus, Massachusetts, USA) of cone and plate geometry equipped with C-25 din measuring spindle at a shear rate 10 (s⁻¹) for 100 sec at 34±0.5 °C temperature.

Preparation of microspheres

Microspheres were prepared by using cross-linking emulsification method described by Nitin *et al.* [8]. The accurately weighed amount of RH was dissolved into distilled water to make 20 mg/ml drug solution.

Varied amount of extracted polysaccharide and eudragit were dispersed into drug solution to make 2% w/v dispersion of both excipients (TI polysaccharide and eudragit) (table 1). Sodium bicarbonate (0.1:1with TI polysaccharide) was dissolved into aqueous phase.

Aqueous phase was allowed for complete hydration till 4 h under stirring using a magnetic stirrer. Hydrated aqueous phase was emulsified in castor oil (1:10 aqueous to oil phase ratio) using span 80 (1.0% v/v) at high-speed homogenizer (2000 rpm, Yamato, LT400, Tokyo, Japan). Sulphuric acid (0.2 ml) and epichlorohydrin (as chemical crosslinker, 4.0% v/v) were added. And stirring was continued till 18 h at 45 °C. Finally, microspheres were collected by centrifugation followed by its washing using isopropyl alcohol and dried at 60 °C for 5 h.

Table 1: Batch detail and characterization of *Tamarindus indica* polysaccharide based floating-microspheres

Formulation code	TI Polysaccharide (%w/w)	Eudragit (%w/w)	Particle size (µm)	Swelling ratio (%)	In vitro buoyancy (%w/w)	Encapsulation efficiency (%)
F1	100	-	7.84±0.34	257±26	87.35±12.43	88.47±17.44
F2	75	25	3.51±0.11	212±31	81.72±18.29	86.57±11.29
F3	50	50	6.48±0.36	187±13	78.05±9.55	85.49±9.34
F4	25	75	5.38±0.52	158±39	76.40±5.49	82.57±10.29

*Percentage of TI polysaccharide and eudragit was for total content of both excipients to make 2% w/v dispersion into aqueous phase

Characterization of microspheres

FT-IR study

The FT-IR spectra of RH, extracted polysaccharide and prepared microspheres were recorded to determine any possible chemical changes of drug during microspheres preparation. Dried KBr was used as diluent and samples were scanned over the range of 4000 to 400 cm⁻¹ by using FT-IR spectrophotometer (Shimadzu 84005, Japan).

Morphology

Surface morphology of prepared microspheres was studied using scanning electron microscopy (SEM). The samples were sprinkled on an aluminium stub by using double sided adhesive tape and then gold sputter coated at 50mA under an argon atmosphere (POLARON model SC-76430). The thickness of the coating was 200 Å. Images were captured in the magnification range of 3000 X by using electron microscope (LEO-430, UK).

Particle size analysis

The particle size determination was done by using phase contrast microscope (Radical Instruments, Ambala Cant, India). Prepared microspheres were suspended into light paraffin oil. Few drops (2-3) of this suspension were shifted over the glass slide and covered with a coverslip. The diameter of approximate 300 microspheres was measured and the average diameter was calculated.

Swelling ratio

Swelling property of prepared floating microspheres was studied as per the method described by Rana *et al.* [9]. Briefly, known weight (25 mg) of microspheres were soaked at 37±0.5 °C in phosphate buffer (pH 1.2) solution. After a certain time, period microspheres were and excess media was removed by blotting with suitable media. Swollen microspheres were weighed by using electronic balance (Shimadzu AUX220, Japan). The degree of swelling (α) was calculated by using equation 1.

$$\alpha = \frac{W_g - W_o}{W_o} \times 100 \quad (1)$$

Where W_o is the initial weight of microspheres and W_g is the weight after swelling.

In vitro buoyancy study

In order to explore the floating ability of prepared microspheres *in vitro* buoyancy study was performed according to the method describe by Dey *et al.* [10]. The study was carried out in 0.1 N HCl containing 0.02% v/v tween 80. Prepared microspheres were dispersed into the 900 ml of media at 37±0.5 °C. Paddle was rotated

at 100 rotations per minute. Each fraction of microspheres floated on the surface and those settled down were recorded at a predetermined time interval. Settled microspheres were collected, dried and weighed.

Encapsulation efficiency

These parameters were estimated by dispersing accurately weighed quantity of crushed powder of prepared microspheres (25 mg) into 25 ml distilled water. The dispersion was kept overnight with under continuous shaking for complete release of RH from microspheres. Debris of microspheres was removed by filtration and centrifugation. The absorbance of the filtrate was measured after suitable dilution using UV-visible spectrophotometer (Shimadzu 1700, Japan) at 313 nm. Experiments were carried out in triplicate and the encapsulation efficiency and drug content were calculated by using equations 2.

$$\text{Encapsulation efficiency (\%)} = \frac{\text{actual amount of drug}}{\text{theoretical amount of drug}} \times 100 \quad (2)$$

In vitro dissolution studies

For determination of in vitro dissolution study, accurately weighed the quantity of prepared microspheres (equivalent to 300 mg of RH) was transferred in the beaker having 900 ml phosphate buffer (pH 1.2), temperature 37±1 °C; stirring rate 100 rpm. At predetermined time intervals, aliquots of 1 ml were withdrawn, replaced by equal volume of fresh media and centrifuged at 4000 g for 15 min. Finally, the supernatant was estimated for drug content by using UV-visible spectrophotometer at 313 nm. Further, the exact mechanism of drug release from the microspheres was calculated by fitting release data different release kinetic models such as zero-order, first-order, Higuchi's and Korsmeyer-Peppas equations. Data of release kinetics such as of kinetic rate constant (k), correlation coefficient (r²) and release exponent (n) were calculated for the determination of best fit model [11].

RESULTS AND DISCUSSION

The polysaccharide of TI was extracted by using precipitation method with percentage yield of 13.59 %w/w. The brownish red dry polysaccharide was characterized for their purity and viscosity. As a result, it was found that extracted polysaccharide was of carbohydrate in nature. Presence of mucilage and absence of any other ingredients indicates the high level of purity of extracted polysaccharide. Similarly, 2.0% w/v aqueous solution showed 304±21 mPas viscosity at shear rate 10 (s⁻¹) and 37±0.5 °C temperature. Junginger *et al.*, (2007) suggested a relation between viscosity and mucoadhesion of polymers [12]. According to this relation a mucoadhesion property of polymer is only triggered by its swelling behavior. For effective mucoadhesion, polymer need to swell first, followed by interpenetration into biological tissue. Therefore, high value of viscosity indicates that the

extracted polysaccharides may be considered as excipients for mucoadhesive drug delivery. Emulsification cross-linking method was found to be a suitable method for the preparation of RH loaded TI based microspheres. The percentage yield of prepared microspheres was in the range of 78-81% w/w. Epichlorohydrin was used as a chemical cross linking agent, which has been used for cross-linking of various carbohydrates. Chemically, it is an organochlorine compound (an organic compound containing at least one covalent bonded atom of chlorine) and an epoxide (cyclic ether with three ring atom).

FT-IR study

The FT-IR spectra of RH, extracted polysaccharide and prepared microspheres are shown in fig. 1. The characteristic peaks of RB and

extracted polysaccharide were studied by using the previously reported literature [13]. Obtained FT-IR spectra of RH were compared with spectra of microspheres. The study indicates that all characteristic peaks of RH were present in the spectra of microspheres, which revealed that the drug was unchanged during the preparation of microspheres.

However, some new peaks were observed in the spectra of microspheres at frequencies 1164 and 867 cm^{-1} , which may be due to the formation of mono and diether linkage as the result of chemical cross-linking between extracted polysaccharide and epichlorohydrin. The absence of any new peak in the FT-IR spectra of microspheres also confirmed the absence of any by-product through epichlorohydrin mediated polysaccharide cross linking.

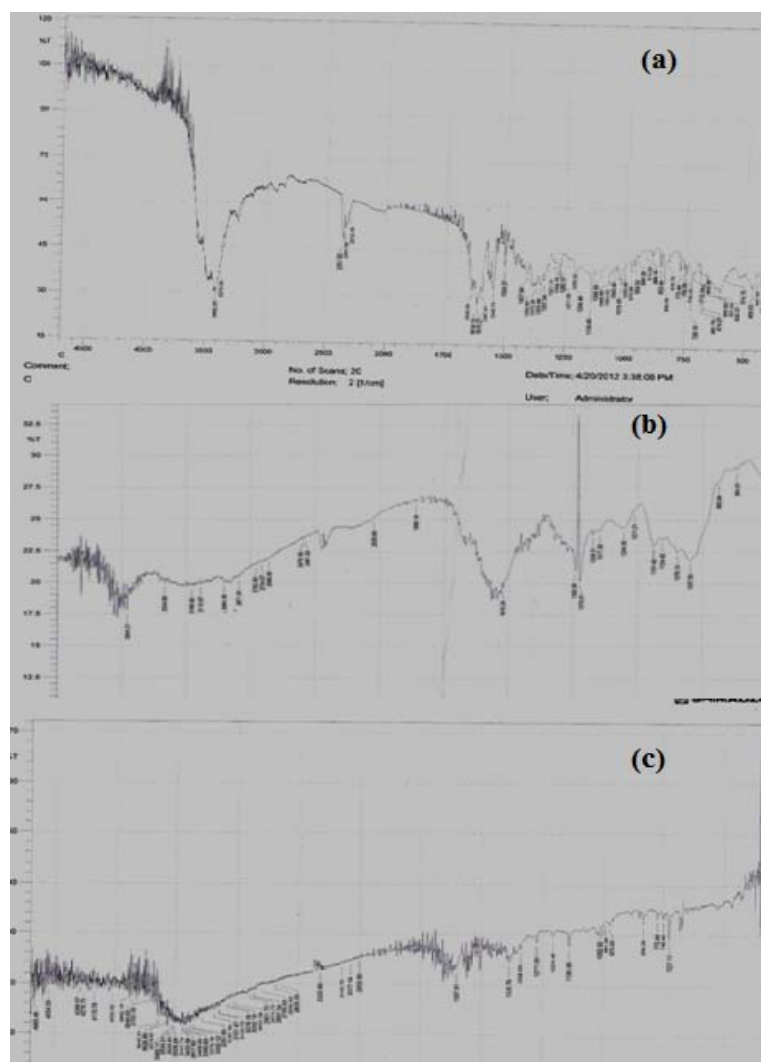


Fig. 1: FTIR spectra of drug RH (a), extracted plant polysaccharide (b) and prepared floating-mucoadhesive microspheres

Surface morphology

Surface characteristics of different batches of prepared TI polysaccharide based microspheres were examined by using scanning electron microscopy (SEM). Fig. 2 indicates the SEM images of prepared microspheres of all batches. Visual examination of the SEM pictures indicated that all batches of microspheres were of spherical shape with varied surface roughness. Present investigation indicates that there were no any significant effects of variables on surface morphology of microspheres.

Particle size

Particle sizes of all batches of drug loaded TI polysaccharide based microspheres were evaluated by using simple microscopic methods.

Particle size of all batches of microspheres was in the range of 5.38-7.84 μm (table 1). The present study indicated that there was not any remarkable effect of concentration of excipients on the particle size of prepared microspheres.

Swelling ratio

Swelling behavior of microspheres defines the extent of mucoadhesion and drug release pattern through it. Therefore, evaluation of this study becomes essential while preparing any mucoadhesive formulations. The effect of varied concentration of TI polysaccharide and eudragit on the swelling index of the microspheres was evaluated (fig. 3). Fig. indicates that swelling index was gradually decreased by decreasing the concentration of TI

polysaccharide. Batch F1 showed highest swelling, while batch F4 showed minimum swelling. This might be due to insufficient polysaccharide content to swell. Highest swelling index F1 may be due to highest polysaccharide concentration.

Since, polysaccharides are consist of large numbers of polar compound which leads to water absorption and holding capacity and further more swelling property of prepared microspheres [8].

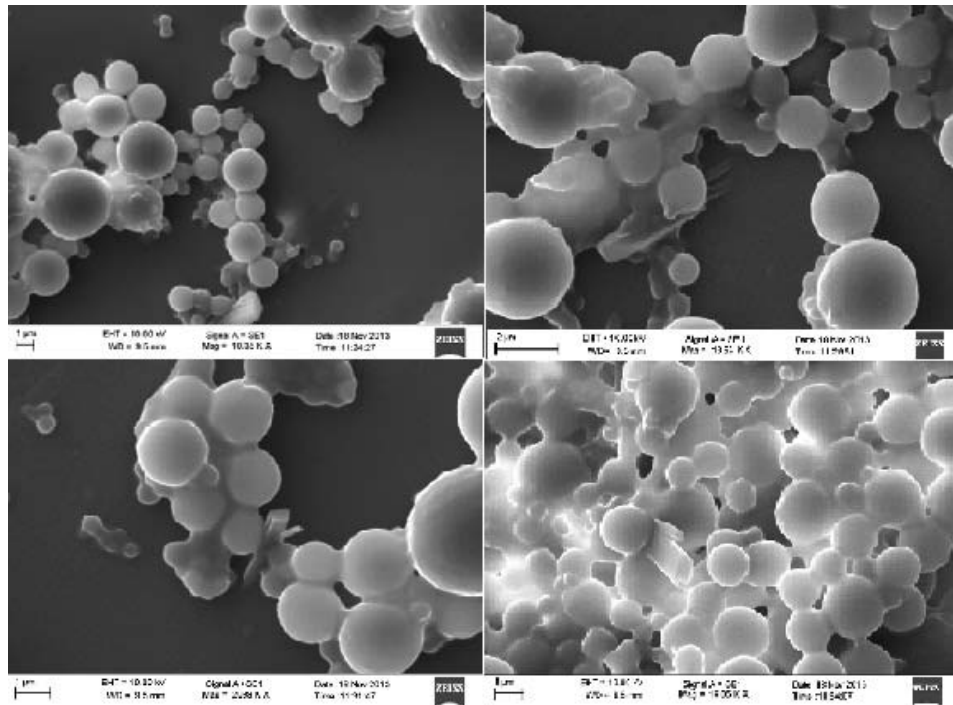


Fig. 2: SEM images of all batches of microspheres prepared by using extracted TI polysaccharide

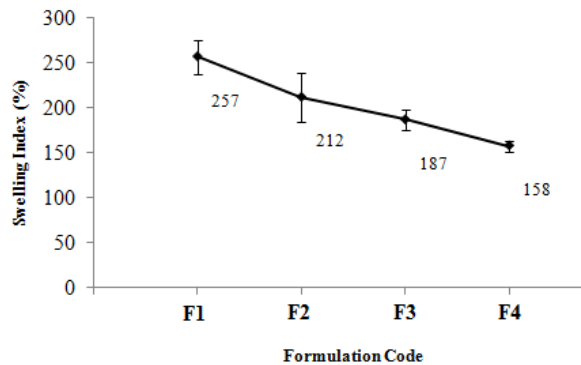


Fig. 3: Percentage swelling index of TI polysaccharide based floating-mucoadhesive microspheres measurement was done in triplicates (n=3, mean±SD)

In vitro buoyancy study

The purpose of preparing floating microspheres was to extend the gastric residence time of the drug. Floating ability of prepared microspheres was determined by using *in vitro* buoyancy study in 0.1 N HCl containing 0.02% v/v tween 80. Tween 80 was added to counteract the downward pulling at the liquid surface by lowering surface tension. The prepared microspheres floated immediately on the medium, and the floating lag time (min.) was zero for all the formulations. The absence of floating lag time is ideal to prevent dosage form from the transition to the intestine which could be due to low density of prepared microspheres [14]. Obtained data of *in vitro* buoyancy study are given in table 1.

Formulation F1 possesses best floating ability (87.35% w/w) while it gradually decreased by replacing the concentration of TI

polysaccharide by eudragit. Floating ability of prepared microspheres may be co-related as per the swelling characteristic of microspheres. Due to the highest concentration of TI polysaccharide in formulation F1, these particles swell to its highest extent and become floatable. Since eudragit are not swellable therefore by increasing the concentration of eudragit, microspheres started to sediments.

Encapsulation efficiency

Data of encapsulation efficiency of prepared microspheres are shown in table 1. Values of percentage encapsulation efficiency were increased by increasing the TI polysaccharide concentration in respect to the concentration of eudragit. The reason may be, increase in polysaccharide concentration leads to increase in the viscosity of aqueous phase and highly viscous phase shows high drug retention capacity by preventing the migration of drug from droplets to surrounding media which result to more encapsulation efficiency [12].

In vitro dissolution studies

The *in vitro* releases of RH from all batches prepared by using a mucoadhesive TI polysaccharide and a rate controlling polymer (eudragit) are illustrated in fig. 4. All the formulations showed drug released pattern till 12 h course of study. Initially, at 30 min 30.73%w/w, 24.16%w/w, 21.90%w/w and 17.75%w/w drug was released from the formulations F1, F2, F3 and F4 respectively. The rate of drug release was found gradually decreased by increasing the concentration of eudragit concentration into the prepared microspheres. Increased concentration of eudragit into microspheres make an impermeable three-dimensional network through which drug migration become difficult and as a result suppress the drug release. Since eudragit are insoluble at acidic pH, therefore at highest concentration (F4) drug release was found to be decreased. Moreover, all prepared TI polysaccharide based formulations were found to be release 100%w/w of RH from 8 to 12

h into acidic media. Rationally, the prepared microspheres can be delivered the drug into the upper part of GIT up to 12 h.

The exact mechanism of drug release from prepared microspheres was calculated by fitting the release data into several models. The criterion of selection of best model was based on the goodness of fit. It was observed that drug release from microspheres followed Higuchi square root kinetics for the formulations F2, F3 and F4. While the formulation F1 (solely containing TI and not containing Eudragit) followed korsmeyer peppas kinetics having the value of n was 0.352. This shows that the release drug release kinetics of the TI microspheres (F1) was a fickian. However, the formulations F2, F3 and F4 followed diffusional kinetics according to Higuchi's equation, all the plots were linear with correlation coefficient values (r^2) in the range of 0.8914–0.9452 (table 2), indicating diffusion controlled drug release. Finally, in order to know the exact mechanism of drug diffusion through prepared microspheres, the release data was fit into Korsmeyer–Peppas equation. All the plots were found to be linear with r^2 values in the range of 0.9764–0.9936 (table 2). The release exponent (n) values for all batches were found to be more than 0.45, indicating Fickian release.

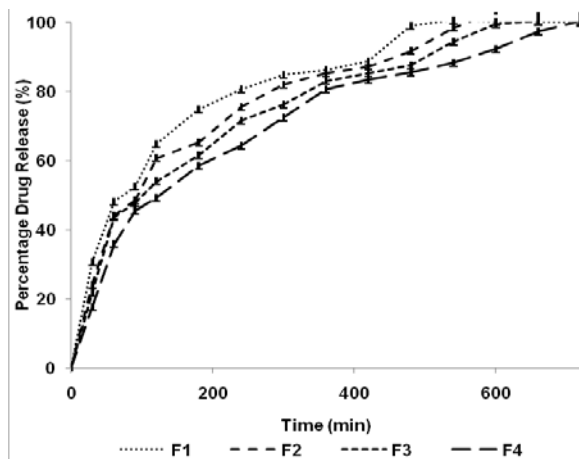


Fig. 4: Percentage release of RH from TI polysaccharide based floating-mucoadhesive microspheres ($n=3$, mean \pm SD)

Table 2: Values of kinetic rate constant (k), correlation coefficient (R^2) and release exponent (n) from different batches of prepared microspheres

Formulations	Zero-order		First-order		Higuchi		Korsmeyer-Peppas		
	K	R^2	k	R^2	k	R^2	K	R^2	n
F1	0.107	0.751	-0.107	0.751	3.565	0.928	10.881	0.955	0.352
F2	0.116	0.819	-0.116	0.819	3.762	0.963	2.832	0.961	0.643
F3	0.115	0.843	-0.115	0.843	3.699	0.973	2.6143	0.923	0.648
F4	0.115	0.864	-0.115	0.864	3.687	0.981	2.1149	0.940	0.659

CONCLUSION

The present study concludes that the extracted plant polysaccharide of TI can be considered as excipients for the development of floating-mucoadhesive drug delivery. Extracted polysaccharide possesses an optimized value of viscosity and extraction procedure was found suitable with optimum yield. Prepared microspheres found suitable in buoyancy study with high encapsulation efficiency.

Finally, *in vitro* release study indicates that microspheres were able to release the RH up to 12 h into acidic media. Further investigations such as stability studies, scale-up studies and *in vivo* pharmacokinetic studies are needed for the establishment of *Tamarindus indica* polysaccharide as pharmaceutical excipients for microspheres drug delivery systems.

CONFLICTS OF INTERESTS

Declare none

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