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Der Pharmacia Lettre, 2016, 8 (20):197-208
(http://scholarsresearchlibrary.com/archive.html)

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ISSN 0975-5071
USA CODEN: DPLEB4

Development of *Trigonella foenum-graecum* polysaccharide based floating microspheres for localized delivery of ranitidine hydrochloride

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ABSTRACT

This paper describes the preparation and evaluation of floating-mucoadhesive microspheres, using Trigonella foenum-graecum polysaccharide as a novel carrier for safe, localized and effective delivery of ranitidine hydrochloride into upper GIT. The polysaccharide was extracted from the dried seeds of Trigonella foenum-graecum and floating-mucoadhesive microspheres were prepared by emulsification, followed by crosslinking method using epichlorohydrin. Prepared

microspheres were characterized for drug compatibility by using FT-IR and PXRD method which indicated the stability of drug during processing. Prepared microspheres were evaluated for particle size, surface morphology, swelling properties, mucoadhesive strength, encapsulation efficiency and in-vitro drug release. Result indicates that microspheres were of spherical in shape with the particle size range from 4.56±1.4 to 1.23±0.71 µ m. In-vitro buoyancy study confirm floating ability in range from 78.12±1.86 to 86.59±1.23 percentage. In-vitro drug release of ranitidine hydrochloride indicates that prepared microspheres can be prolong the release of drug upto 12 h time period.

KEYWORDS: Trigonella foenum-graecum, ranitidine hydrochloride, floating microspheres, natural polymer

INTRODUCTION

A large numbers of novel drug delivery systems are investigating day by day for drug administration through various routes. After that, oral route of drug administration constitutes the most convenient and preferred means of drug delivery to systemic circulation. However, oral administration of most of the drugs in conventional dosage forms has short-term limitations due to their inability to restrain and localize the system at gastrointestinal tract (1). In order to minimize these limitations, a large numbers of innovative smart formulation are coming into accounts with improved drug features such as efficacy, bioavailability and safety into the systemic circulation.

A large number of synthetic and natural polymers have been used to achieve the objectives of such formulations. The utilization of synthetic polymers is associated with the high risk of local and even systemic toxicity (2). Some synthetic polymers such as methyl methacrylates and poly-(methyl methacrylate) are well known for their carcinogenic property (3). On the other hand naturally obtained polymers are considered to be safe and equally effective due to their biocompatible or at least biodegradable nature. Researchers are also moving towards the

utilization of natural plant polymers. A wide range of natural polymers such as collagen, alginate and chitosan have been utilized as pharmaceutical excipients in different types of formulations (4).

Ranitidine hydrochloride (RHC), an H2 receptor antagonist competitively inhibits the interaction of histamine with its receptors. It shows maximum absorption at upper part of GIT. Moreover, colonic metabolism is also responsible for poor oral drug bioavailability (5). Effective treatment can be achieved by administration of 150 mg of RHC, two time a day, which leads to patient incompliance. In order to improve therapeutic action and patient compliance it is required to design a formulation which can increased the retention of drug into upper part of GIT along with sustain release action upto 12 h (6). The present work was designed to enhance the therapeutic effect of ranitidine hydrochloride by increasing the residential time into upper part of GIT. Additionally, patient compliance was also targeted through making a sustained delivery of drug which allow a prolong release upto 12 h. This objective was achieved through floating-mucoadhesive drug delivery system which comprised of a novel natural polysaccharide. Polysaccharide was obtained from the seeds of Trigonella foenum-graecum (TFG, Family: Fabaceae). Chemically this polysaccharide consist of five components such as kaempferol 3-O-β-d-glucopyranoside, kaempferol 7-O-glucoside, kaempferol 3-O-α-l-rhamnosyl (1→2) β-d-xyloside, kaempferol 7-O-β-d-glucopyranosyl (1–4) β-d-glucopyranoside and kaempferol 3-O-β-glucosyl (1→2) (6′-O-acetyl)-β-d-galactoside (7). To the best of our knowledge the application of polysaccharide in floating-mucoadhesive drug delivery is investigated first time in the present work.

MATERIAL AND METHODS

Ranitidine hydrochloride (RHC) was obtained as generous gift sample from Hetro labs, Hyderabad, India. Seeds of Trigonella foenum-graecum were purchased from local market of Kanpur (UP). All other materials such as Eudragit RS100, castor oil, epicholorhydrin, and sodium bicarbonate were of analytical grade and were procured from commercial sources.

Polysaccharide extraction

The seeds of TFG were purchased from local market of kanpur. Polysaccharide was extracted as per reported methods by Sharma et al (2). Briefly, 200 g of seeds of TFG were washed with double distilled water to remove any adherent material. About three volumes of water was added into slightly crushed seeds and heated at 60°C on a water bath for about 4 h until the slurry was prepared. Mucilaginous solution was then filtered and filtrate was diluted with three volumes of water and kept undisturbed overnight in a refrigerator, so that most of the undissolved portion settled down. The clear supernatant portion was decanted and concentrated at 60±1°C. The concentrate was cooled to room temperature and precipitated in about three volumes of acetone. The precipitate was washed repeatedly with acetone and dried at 50±1°C for about 2 days. The dried material was ground by a mechanical grinder and passed through#80 mesh sieve and kept in a desiccator till further use.

Characterization of TFG polysaccharide

Extracted polysaccharide was evaluated for their organoleptic properties such as color, odor and taste. In order to confirm chemical nature and purity, extracted polysaccharide was undergone through several chemical test. Finally, polysaccharide was evaluated for their pH, viscosity and mucoadhesion properties. Viscosity of 1% (w/v) aqueous solution of extracted polysaccharide was measured by Brookfield viscometer at 37 ±1°C at five different speeds of 10, 20, 30, 60 and 100 rpm. The mucoadhesive strength of extracted polysaccharide was determined by measuring the force required to detach the aqueous solution of extracted polysaccharide from epithelial membrane of goat GIT. At room temperature, fixed amount of 1%w/v solution of TFG was placed on the lower probe. The probes were equilibrated and maintained at 37°C. A probe with the epithelial tissue was lowered until the tissue contacted the surface of the sample. Immediately, a force of 0.1 N was applied for 2 min to ensure intimate contact between the epithelial membrane and the samples. The probe was then moved upwards at a constant speed of 0.15 mm/s. The mucoadhesive force, expressed as the detachment stress in dyne/cm2, was determined from the

minimal weights that detached the tissues from the surface of each formulation using the following equation.

Detachment stress = m.g/A

Where, m = weight added to the balance in gram; g = acceleration due to gravity taken as 980 cm/s^2; and A = surface area of nasal mucosal tissue in cm^2.

Microspheres preparation

Microspheres were prepared by using crosslinking emulsification method described by Hamdi et al., 2001 (8). Different batches of microspheres were prepared by changing the concentration of extracted polysaccharide and eudragit RS100 (Table 1). Aqueous phase was prepared by dissolving RHC into required amount of distilled water to get 20mg/ml drug solution. Varied amount of extracted polysaccharide (as mucoadhesive polymer) and eudragit RS100 (as rate controlling polymer) were dispersed into drug solution to make 2%w/v dispersion. Sodium bicarbonate (0.1:1with TFG polysaccharide) was dissolve into aqueous phase. Mixture was kept for 4 h under magnetic stirring at 500 rpm for complete hydration of polysaccharide. Aqueous phase was dispersed into castor oil using span 80 (1.0% v/v) as an emulsifying agent. The aqueous and oil phase ratio was remained 1:10 throughout the experiment. The emulsion was homogenized with an addition of 0.2 mL H2SO4 using high speed mechanical stirrer (Yamato, LT400, Tokyo, Japan) at 2000 rpm. Epichlorohydrin (4% v/v) was added as a crosslinking agent. Stirring was continued for 18 h at 40oC. Prepared microsphere was separated by sedimentation. Microspheres were washed five times using isopropyl alcohol and dried at 50^{o}C.

Table 1: Batch detail and characterization of Trigonella foenum-graecum polysaccharide based floating-microspheres.

Formulation Code	TFG Polysaccharide (%w/w)	Eudragit RS100 (%w/w)	Particle size (µm)	*In-vitro* mucoadhesion (min)	*In-vitro* Buoyancy (%w/w)	Encapsulation efficiency (%)
F1	100	-	4.56 ±1.44	473±43	86.59±1.23	91.38±8.59
F2	75	25	3.98±2.04	375±12	81.48±2.74	89.48±4.58
F3	50	50	2.76±0.84	302±32	79.75±2.18	86.52±11.46
F4	25	75	1.23± 0.71	278±24	78.12±1.86	83.71±10.23

Characterization of microspheres

FT-IR study

The FTIR spectra of drug (RHC), extracted polysaccharide (TFG) and drug loaded microspheres was recorded using a Fourier transform infrared (FTIR) spectrophotometer (Shimadzu 84005, Japan), between ranges 400 to 4500 cm-1. The samples were gently triturated with small amount of potassium bromide (KBr) powder (300 mg) and compressed into pellets/discs by applying 6000 kg/cm2 pressure, using a manual hydraulic presser.

Powder X-ray diffraction (PXRD) study

To determine the changes in the crystalline nature of the drug, PXRD patterns of ranitidine hydrochloride, extracted TFG polysaccharide and prepared microspheres were recorded by using

X-ray diffractometer (Bruker AXS D8 advance, Germany) equipped with Cu radiation. Each sample was scanned between 5-120o 2θ.

Surface morphology

Surface characteristic of prepared microspheres was evaluated by scanning electron microscopy (SEM, LEO-430, UK). The samples were prepared by lightly dusting the samples of microspheres on a double sided tape on an aluminium stub. The stubs were then coated with gold to a thickness of ~ 300 Aº using a sputtering coater (POLARON model SC-76430), and examined in microscope fitted with a scanning electron detector and the photograph of samples were taken.

Particle size

The particle size of microspheres of each batch was analyzed by using optical microscopy (Radical Instruments, RXL.5T Ambala Cant, India) fitted with an ocular and stage micrometer. A suspension of microspheres was prepared in light paraffin oil. Few drops of suspension was transferred onto glass slide and covered with a cover slip. At least 100 particles were examined in all measurements. The geometric diameter of microspheres of each batch was measured and the average diameter was calculated.

Swelling property

Swelling behaviour of prepared microspheres was evaluated in phosphate buffer (pH 6.8) by using a reported method (9). Microspheres were spread over the series of 10 glass slide and few drops of phosphate buffer (pH 6.8) were added over the microspheres for swelling of microspheres. Diameter of microspheres was periodically measured at the interval of 2 min by using a digital phase contrast microscope (Radical instruments, RXL.5T Ambala Cant, India) with attached software for measurement of size. The particles size of microspheres was determined until erosion of the particle surface. The percentage of swelling was determined at different time intervals by

the difference between diameter of microspheres at time t (Dt) and initial time (t = 0 [D0]) as calculated from the following equation 2 and averaged from three determinations was calculated

Percentage of Swelling = $(Dt-Do)\ Do \times 100$ (2)

Where Dt is the mean diameter of microspheres at time t (t= 2, 4, 6, 8…. min). D0 is the diameter of microspheres at t = 0. A percentage swelling Vs time curve was prepared to study the swelling behavior of each batch.

Mucoadhesion study

In-vitro mucoadhesion ability of prepared drug loaded microspheres was evaluated by using rat stomach mucosa as per the method described by Rajinikanth et. al (10) with slight modification. Briefly, rat stomach mucosa (1x1 cm2) was collected immediately after the scarification of animal. Microspheres from each batch were spread onto the rinsed stomach mucosa and kept inside a stability chamber under the specific conditions, relative humidity 80%; temperature 25oC; time 15 min. After that the microspheres were washed with phosphate buffer (pH 1.2, temperature 37o C) with flow speed of 2ml/min using simple pneumatic syringe. Finally, Time required for the complete washing of microspheres from mucosal surface was recorded and result is noted as an average of triplicates.

In-vitro Buoyancy study

In order to determine the floating properties of prepared microspheres in-vitro buoyancy study was performed according to the method describe by Dey et al (11). Microspheres were dispersed in to the 900 ml of media (0.1 N HCl containing 0.02% v/v tween 80) at 37±0.5oC. Paddle was rotated at 50 rotations per minute. Each fraction of microspheres floated on the surface and those settled down were recorded. Buoyancy (%) was calculated as the ratio of the mass of the microspheres that remained floating to the total mass of the microspheres, expressed as a percentage.

Encapsulation efficiency

Drug Encapsulation efficiency is defined as the amount of added drug (in percent) encapsulated in the formulation of microspheres. Encapsulation efficiency was calculated in terms of the ratio of drug in the final formulation to the amount of added drug. An accurately weighed amount (10 mg) of the formulation of microspheres was dispersed in 10 ml of phosphate buffer (pH 1.2) and ultrasonicated for 3 consecutive periods of 5 minutes each, with 5 minutes of resting period. The sample was then left to equilibrate for 24 hours at room temperature. 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. The drug encapsulation efficiency of each sample was determined in triplicate. Drug encapsulation efficiency was determined using the formula given in Equation (3).

Encapsulation efficiency (%) = [actual amount of drug/ theoretical amount of drug]×100 (3)

In- vitro drug release studies

In-vitro release study of drug from microspheres is conducted by using modified dissolution method. Accurately weighed quantity of prepared microspheres equivalent to 300 mg of RHC was suspended in 900 ml phosphate buffer of pH 1.2. Temperature of the buffer was maintained at 37±0.5°C on magnetic stirring (50 rpm). At various time intervals, l ml adequate were withdrawn, diluted and centrifuged for 15 min at 2000 rpm. The supernatant was removed and analyzed for drug content by using UV spectrophotometer at 313 nm.

RESULT AND DISCUSSION

Polysaccharide extraction and characterization

Percentage yield of extracted polysaccharide was found 17.58%w/w. Further characterization of mucilage was carried out for various parameters. Extracted polysaccharide was of yellowish brown in color without order and characteristic taste. As a result of chemical test, 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. The pH values of 1%w/w solution of TFG polysaccharide were found to be slightly acidic (6.4). The Viscosity of 1%w/w aqueous solutions of extracted polysaccharide was found to be 375±12, 472±32, 497±26, 786±53 and 1284±15 mPas at shear rate 10, 20, 30, 60 and 100 rpm. Similarly, detachment stress of TFG polysaccharide was found to be 1599.033±327 dyne/cm2. Junginger et al., (2007) suggested a relation between

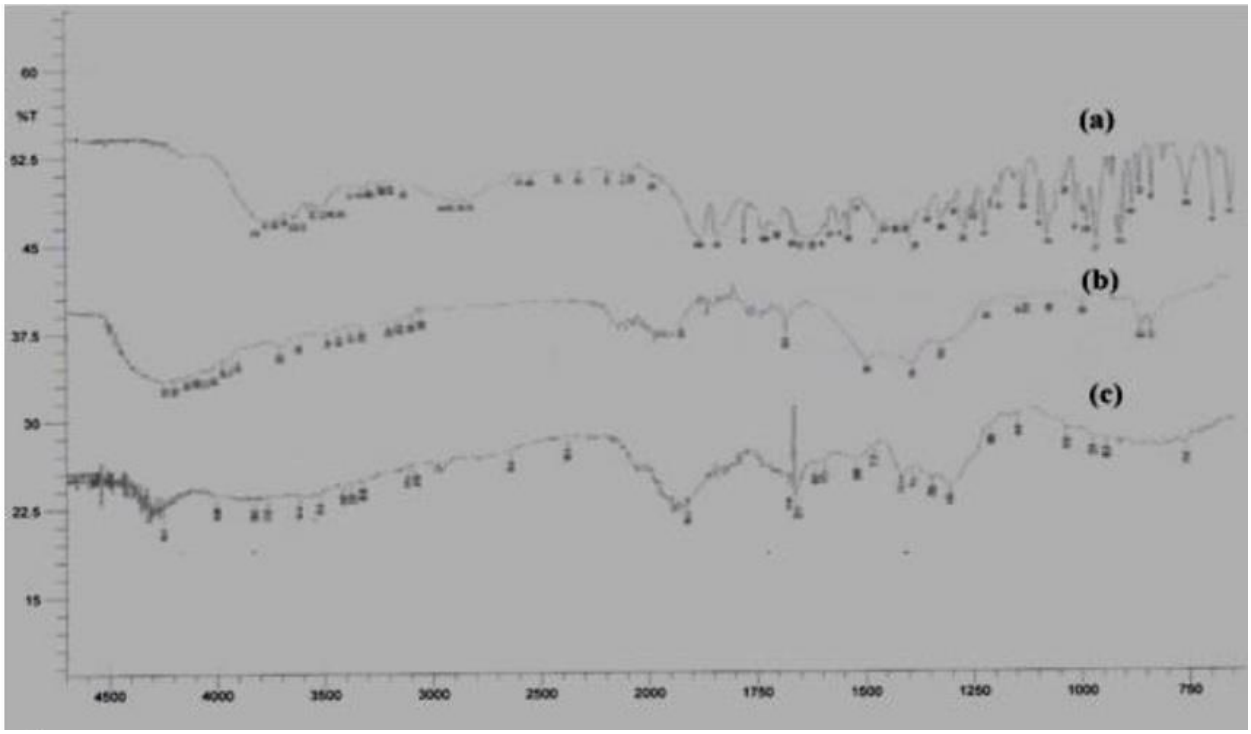
viscosity and mucoadhesion of polymers (12). According to this relation a mucoadhesion property of polymer is only triggered by its swelling behavior. For effective mucoadhesion, polymer need to swell first, followed by interpenetration into biological tissue. Therefore, high value of viscosity indicates that the extracted polysaccharides may be considered as excipients for mucoadhesive drug delivery.

FT-IR study

FT-IR spectra of drug, polysaccharide and prepared microspheres are illustrated in figure 1. Figure confirmed characteristic absorption peaks of RHC, indicating that no chemical interaction occurred between the drug and the polysaccharide. Similarly, the study indicates that all characteristic peaks of RHC were present in

the spectra of microspheres, which indicates that the drug remain stable during the preparation of microspheres (13). However, some new peaks at frequencies 1196 and 834 cm-1, which may be due to the formation of mono and diether linkage due to chemical crosslinking between, TFG polysaccharide and epichlorohydrin. Moreover, absence of new peak in the spectra of microspheres also confirmed the absence of any biproduct of crosslinking process.

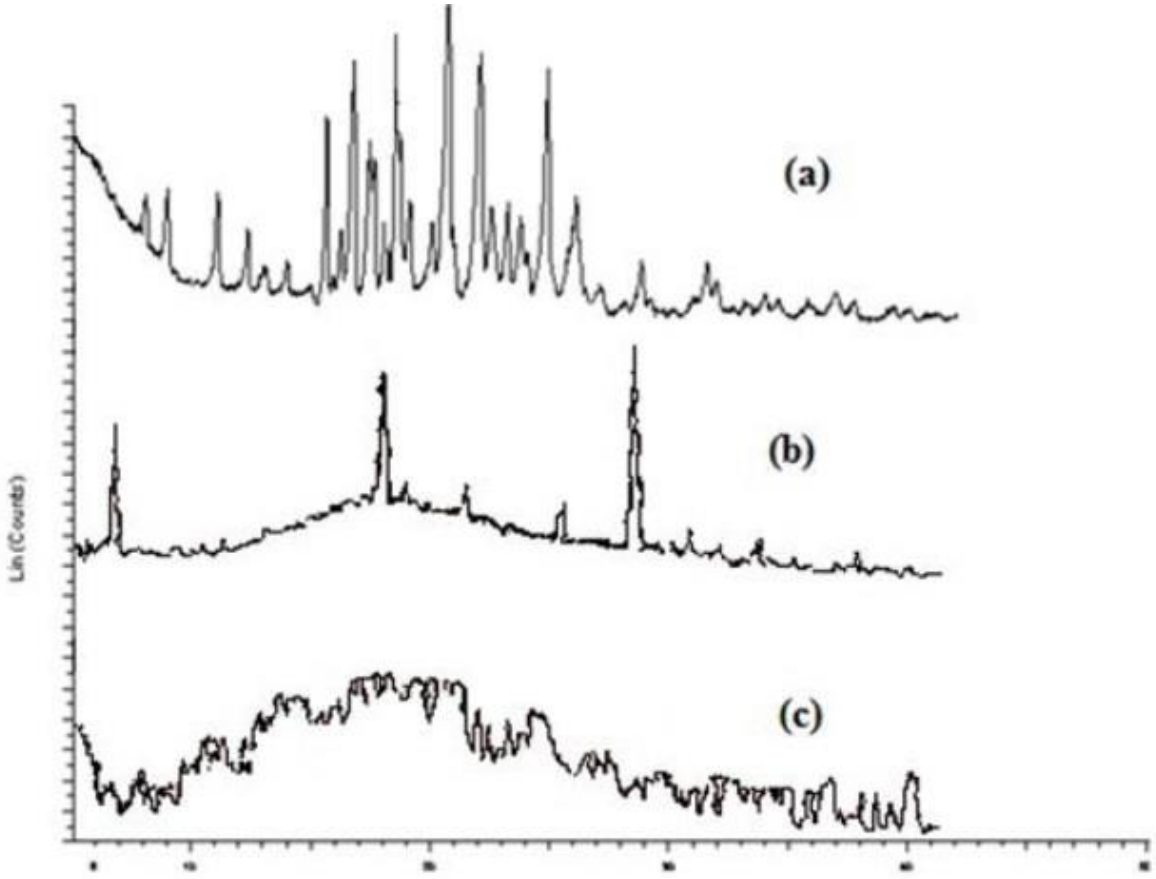
Figure 1: FTIR spectra of drug Ranitidine hydrochloride (a), extracted TFG polysaccharide (b) and prepared microspheres (c).



PXRD study

Diffractrogram of pure drug, RHC (a), drug loaded microspheres (b) and extracted TFG polysaccharide (c) are shown in figure 2. Pure drug exhibit highly intense and sharp peak at around 2θ of 24.9, 26.12, 27.98, 32.41 and 40.93. This shows that drug was in crystalline nature, but prepared microspheres shows diffused hallow pattern with peak of low intensity, which revealed drug was masked in microspheres formulation, due to amorphous dispersion of drug in to polysaccharide matrix.

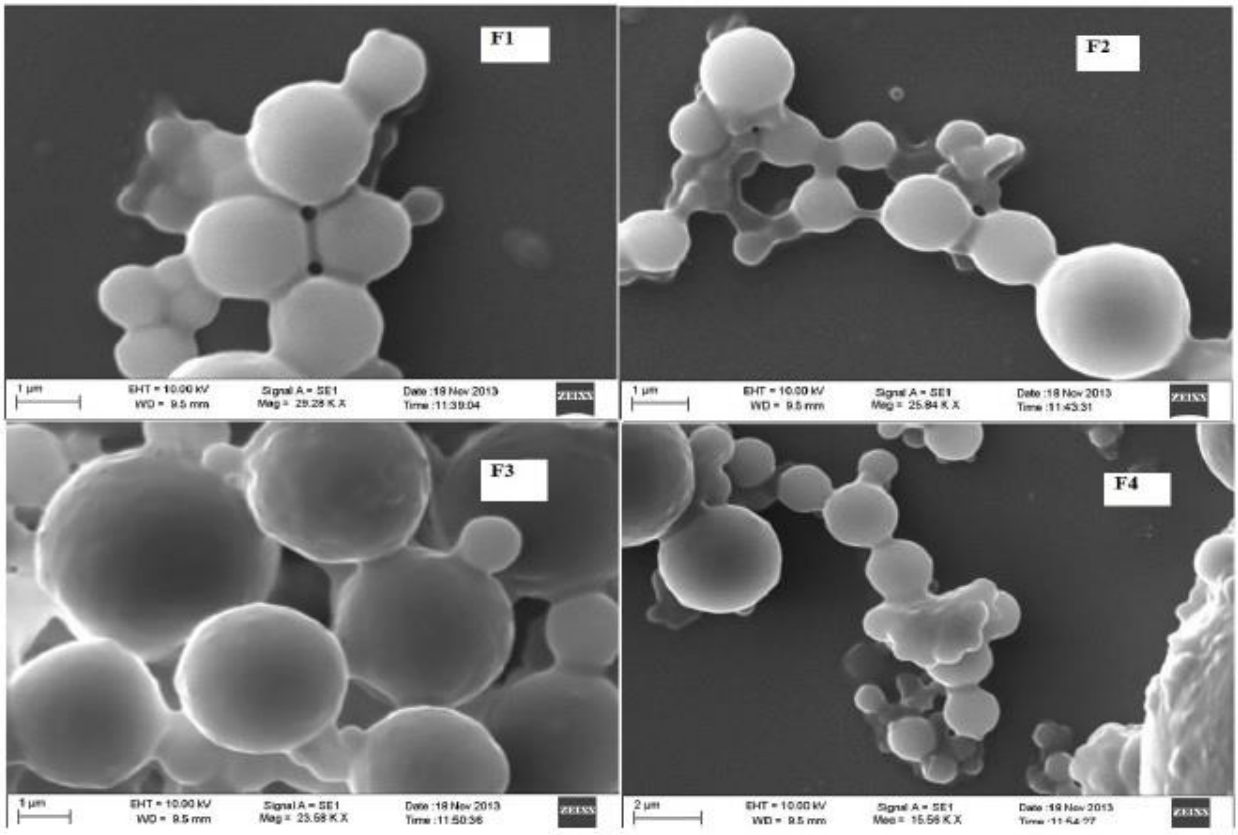
Figure-2: PXRD of drug RHC(a), prepared microspheres formulation(b)and extracted polysaccharide of Trigonella foenum-graecum (c).



Surface morphology

Shape and surface morphology of the prepared microspheres using different concentration of extracted polysaccharide and eudragit RS100 are shown in figure 3. Visual examination of the SEM pictures indicated that all microspheres formulations were found to be discrete, uniform and spherical with a smooth surface. Present investigation indicated that variables have no effect on surface morphology of microspheres.

Figure-3: SEM images of all batches of microspheres prepared by using extracted TFG polysaccharide.



Particle size

The geometric particle size of the microspheres prepared by changing the concentration of extracted polysaccharide was determined by using an optical microscopy and obtained data are depicted in Table 1. Particle size of prepared microspheres was found to be decreased from 4.56 ±1.4 to 1.23± 0.71 µ m by increasing the extracted polysaccharide concentration. This might be due to the formation of larger droplets during emulsification, because of higher viscosity of polysaccharide solution at higher concentration (14).

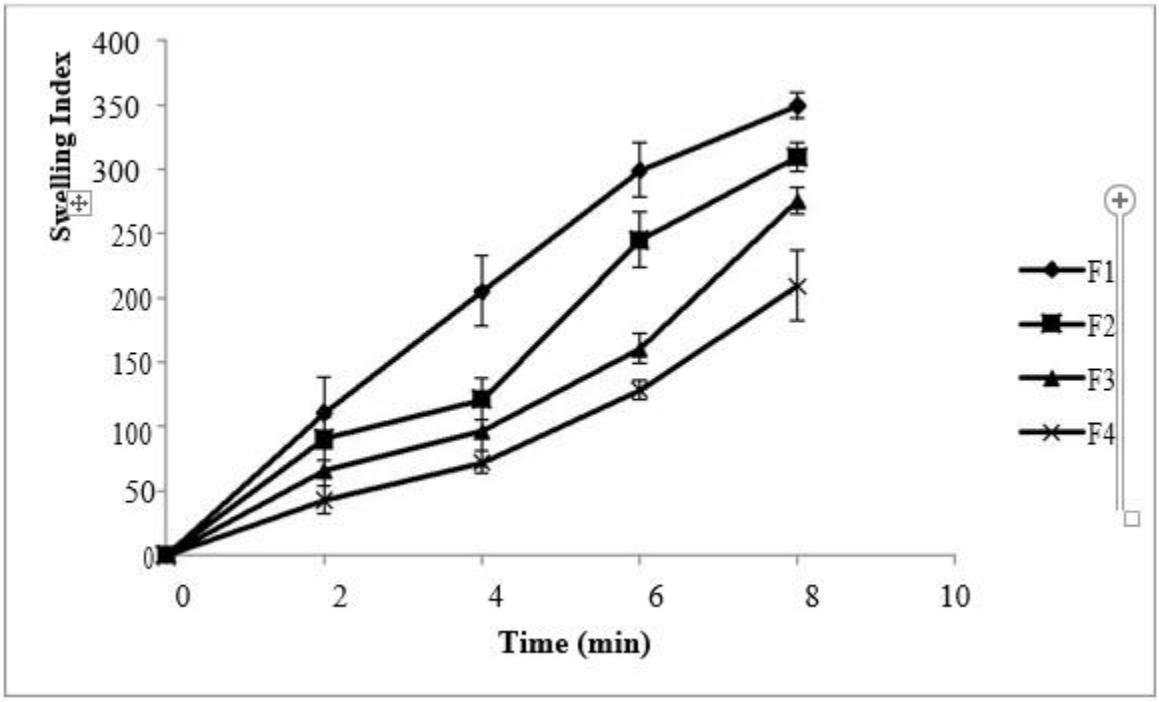
Swelling behaviour

From the data of swelling study, it may be concluded that the prepared microspheres exhibited high percentage swelling upto 10-12 min and then started to show erosion. The degree of swelling increased marginally as the concentration of TFG polysaccharide was increased in the microspheres (Figure 4). The swelling index

was found significantly high ($p<0.01$) in formulation F1 as compared to formulation F4. It may be due to the increased concentration of TFG polysaccharide in the prepared microspheres. Higher concentration of TFG

polysaccharide exhibited large number of polar group which increased water uptake and hence, increased swelling index (14).

Figure-4: Swelling behaviour of different batches of Trigonella foenum-graecum polysaccharide based RHC loaded microsphere.



Mucoadhesion study

Mucoadhesion study was carried out to ensure the adhesion ability of prepared microspheres to the mucosa at the site of absorption (upper part of GIT). Result of mucoadhesion will reflect the behavior of microspheres in terms of in-vivo performance. Mucoadhesion data of all prepared formulation are given in table

It was observed that mucoadhesion time decreased significantly ($p<0.01$) from 473±43 to 278±24 by decreasing the concentration of extracted polysaccharide from 100 to 25%w/w. It may attributed by the fact that the polysaccharide being hydrophilic absorbs higher amounts of water at higher concentrations; due to this, the microspheres get converted into gel and adhere strongly with the mucosal epithelium.

In-vitro Buoyancy study

Buoyancy Studies were performed by using 0.1N HCl at 37±0.5oC under stirring condition. Buoyancy for all the formulations was found more than 78.12%w/w after 12 h of time course study. It was also observed that percentage buoyancy was decreased by decreasing the concentration of extracted polysaccharide from

formulation F1 to F4 (Table 1). Microspheres of batch F1 containing highest concentration of polysaccharide possess highest value of percentage buoyancy (86.59) while formulation F4 with least concentration of polysaccharide possess lowest value of buoyancy (78.12). Result of buoyancy of microspheres may be accordance the swelling characteristic of microspheres. Due to the highest concentration of TFG polysaccharide in formulation F1, these particles swell to its highest extent and become floatable. On the other hand eudragit RS100 are not swellable at acidic pH therefore by increasing the concentration of eudragit RS100, microspheres started to sediments.

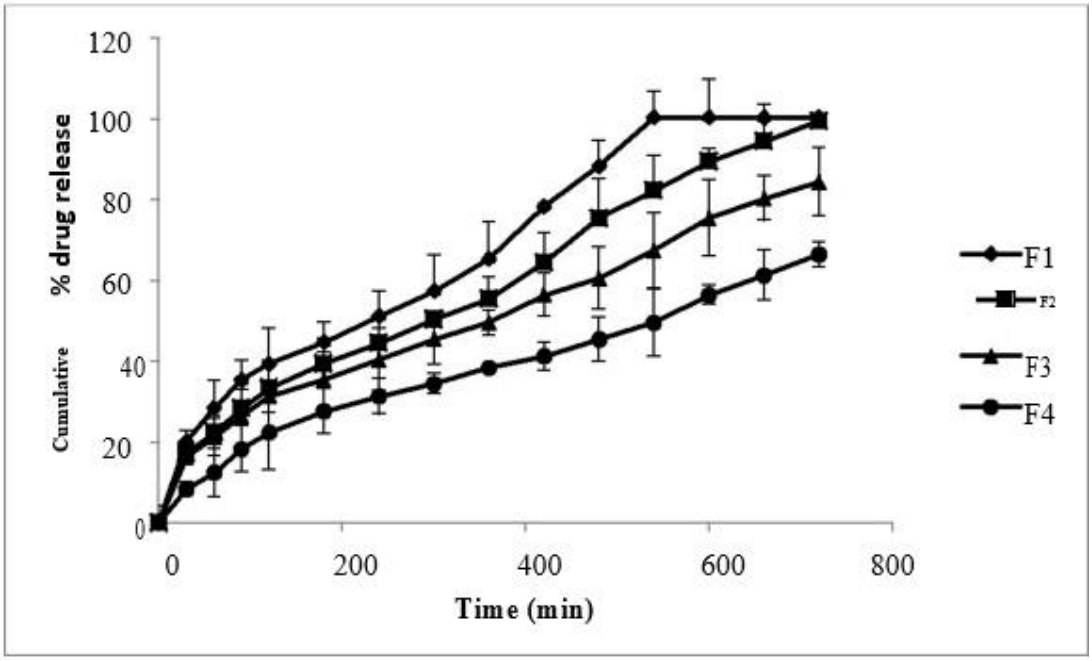
Encapsulation efficiency

The values of entrapment efficiency of all batches prepared by using TFG polysaccharide are shown in Table 1. Entrapment efficiency of formulation F1 was highest followed by the formulation F2, F3 and F4. The obtained data shows that the entrapment efficiency was increased as the concentration of polysaccharide increased in the formulation. The reason of increase in entrapment efficiency may be due to the increased in viscosity of emulsion droplets caused by increased concentration of extracted polysaccharide, due to the high viscosity of droplets the migration of drug from droplets to surrounding media was suppressed, which cause the increased entrapment efficiency.

In-vitro drug release

Release profiles of different batches of Trigonella foenum-graecum based microspheres were shown in table figure 5. All batches of microspheres were found to release 50% drug within time range of 240 to 540 min. It was observed, that content ratio of TFG polysaccharide and eudragit RS100 in prepared microspheres modulates the release of drug. At a specific time interval (540 min) it was observed that formulation F1 (without eudragit) possess a complete release of RHC with in 8 h, whereas formulation F4 (with highest proportion of eudragit) release only 66.495%w/w of drug upto 12 h course study. It was observed increased concentration of eudragit RS100 retarded the diffusion of drug through a complex structure of polymeric network. It may be due to the insolubility of eudragit RS100 at acidic environment. Moreover, the formulation F2 possess a optimized release profile of RHC upto 12 h time course.

Figure 5: Cumulative percentage release of RHC from TFG polysaccharide based floating- microspheres



CONCLUSION

On the basis of the present work, Trigonella foenum-graecum polysaccharide can be considered as a promising alternative for synthetic carriers in the preparation of floating mucoadhesive microspheres for localized drug delivery. Polysaccharide can be potentially used in the preparation of floating microspheres preparation with similar profile of synthetic polymers for the same purpose. The cross linking method was found suitable with high yield of microspheres. Characterization of prepared microspheres confirmed that extracted polysaccharide can be utilized as carrier for the development of floating- mucoadhesive microspheres. Further investigations such as stability studies, scale-up studies and in vivo pharmacokinetic studies are needed for establishment of polysaccharide as pharmaceutical excipients of microspheres preparation.

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