Original Article

NOVEL GASTRO-RETENTIVE POLYMERIC MICROSPHERES: AN APPROACH FOR INCREASED BIOAVAILABILITY AND AN ONCE DAILY DOSING OF TERBUTALINE SULPHATE

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ABSTRACT

Objective: The purpose of this study was to develop multi-unit alginate-copolymer adhesive microspheres to achieve a sustained release of terbutaline sulphate (TBS) and overcome the hepatic first pass effect so as to enhance its bioavailability.

Methods: The microspheres were prepared using inotropic gelation method and different concentration of sodium alginate alone or in combination with other polymers as well as using chitosan as a coating polymer in some formulations. All of the prepared microspheres were evaluated for yield, size, encapsulation efficiency, *in vitro* release and mucoadhesivity. The selected formulations (F11 and F19) were further subjected to differential scanning calorimetry, Fourier transform infrared spectroscopy, stability and *in vivo* bioavailability studies.

Results: The prepared microspheres exhibited quite widely varying encapsulation efficiencies from 20 to 74. 8 % and its mean diameter was in range of 963. 3-1. 635 µm. The *in vitro* release study showed a sustained release profile. The selected formulations were further subjected to differential scanning calorimetry and FTIR which confirm the absence of any incompatibility. X-ray diffraction suggests the amorphous nature of the drug after encapsulation. The selected formulation F11 and F19 showing encapsulation efficiency higher than 55 %, an amount of drug released within 50-60 % after 8 h and a relative bioavailability of 283. 84 % and 202. 04 % respectively compared with the marketed oral Aironyl® tablets.

Conclusion: The prepared microspheres were significantly efficient to achieve a sustained release of terbutaline sulphate with a higher relative bioavailability in comparison with the oral marketed tablet.

Keywords: Composite microspheres, Ionotropic gelation, CO-Polymer, Chitosan coating, Sustained release, Relative bioavailability

INTRODUCTION

The formulation of sustained drug delivery systems is important to achieve better clinical efficacy and patient compliance [1]. Such systems are highly desirable for drugs that have a short half-life to avoid unnecessary side effects, burst effect or overdose [2, 3]. In addition, sustained release dosage forms ensure optimum and uniform supply of drug, reduce the frequency of intakes [4, 5], enhance stability [6], modify solubility, and increase absorption of some drugs [7].

Microencapsulation is promising in the control of the release of many drugs, and one of the suggested options to achieve encapsulation is polymeric matrix microspheres [8]. An ionotropic gelation method using alginate natural polymer is proposed for producing small diameter microcapsules in large quantities [9]. Ionotropic gelation depends on the ability of polyelectrolytes to cross link in the presence of counter ions to form hydrogel beads known as microspheres [10]. Recently, the use of natural polymers, such as sodium alginate (NaAlg), in the design of drug delivery formulation has received much attention due to their excellent biocompatibility and biodegradability [11]. It has the ability to move from sol to gel state under mild conditions through ionic interactions of the carboxylate anions rich chain region, the 'egg box junctions', and the bivalent or trivalent cations [12, 13]. Moreover, the mucoadhesive properties of alginate may increase the residence time of microspheres in stomach and reduces the drug metabolism [14, 15]. These properties are conducive to the widespread use of alginate beads in gastro-retentive sustained dosage forms [16].

Terbutaline sulphate (TBS) is a selective β2 adrenoceptor agonist and act as short-acting bronchodilator which can be given orally, parenterally or by inhalation. It is widely used in the acute and long-term treatment of bronchial asthma, chronic bronchitis, emphysema, and indicated for the prevention of the preterm labor in pregnancy [17]. Orally administered TBS is incompletely absorbed [18] due to first pass metabolism in the gut wall and liver, so its bioavailability is only 15 % [19] and its elimination half-life is 3 to 4 h [20]. And as many scientists argued about the harmful effects of aerosol bronchodilator therapy [21, 22], many attempts to develop controlled drug delivery systems of TBS have been suggested. TBS is a freely water soluble drug and there were many challenges face its encapsulation as alginate microspheres such as low encapsulation efficiency (EE) due to the leakage of drug particles from the wet beads during cross-linking, fast disintegration of the beads in intestinal fluid and their high porosity, resulting in a rapid drug release [23]. However, the enteric coating with chitosan (CS) was achieved to improve the encapsulation efficiency (EE) of the microspheres [24, 25]. CS is a polycationic and non-toxic mucoadhesive polymer, which is safe and aids the prolonged interaction between the drug and the natural membrane epithelia [26]. An alternative approach to improve the EE and to modulate the drug release characteristics involves the using of hydrophilic co-polymers such as sodium carboxymethyl cellulose (NaCMC), carbopol and hydroxylpropylmethyl cellulose (HPMC).

Emulsion-solvent evaporation method was used to prepare oral TBS microspheres using different polymers namely; Eudragit RS [27] and ethyl cellulose [28, 29]. Another several attempts were made to encapsulate TBS using bovine serum albumin and the emulsion polymerization method was used for passive lung targeting of the prepared microspheres [30]. In addition, ethyl cellulose and HPMC were used to coat TBS loaded pellets which were prepared by extrusion/spheronization method [31]. Ionotropic gelation was used to prepare alginate hydrogel beads loaded with TBS and coated with chitosan and Eudragit [32].

The major objective of this study was to develop a novel TBS loaded polymeric microspheres coated with chitosan, using ionotropic gelation method, in order to be gatro-retentive and to achieve sustained release drug delivery effect. The prepared formulations have been well-characterized by a variety of techniques and

investigated for their *in vitro* release. An *in vivo* study was conducted using the best selected formulations.

MATERIALS AND METHODS

Materials

Terbutaline sulphate (TBS) (Kindly supplied by SEDICO and SED company,Cairo, Egypt), Alginic acid sodium salt from brown algae (NaAlg) (Sigma-Aldrich, U. S. A), Sodium carboxymethyl cellulose (NaCMC)(Loba Chemi), Carbopol 934P (carbomer) (Sigma-aldrich, U. S. A), hydroxylpropylmethyl cellulose (HPMC) 2910(viscosity of 2 % aqueous solution is 15 cps) (sigma-aldrich, U. S. A), Calcium chloride dehydrate ($CaCl_2$. $2H2O$) (LOBA CHEMI), Chitosan (Sigma-Aldrich, U. S. A), Glacial acetic acid (analytical grade, EL-Nasar Pharmaceutical chemical company) and commercially available immediate release tablet, Aironyl®. Water was used for high purity deionized and double distilled.

Methods

Preparation of TBS microspheres

Twenty different formulations were prepared as shown in (table 1). The calculated amount of NaAlg and the used co-polymers (NaCMC, carbopol and HPMC) in different concentrations were dissolved in distilled water under magnetic stirring for 15 min. TBS was added to the polymeric solution with continuous stirring until a homogenous solution was obtained. The solution was sonicated for 30 min using ultrasonicator (Sonix TV ss-series ultrasonicator, USA) to remove any air bubble. The drug–polymer solution was extruded through 21 G syringe needle into the gelation medium consisted of 5 % $CaCl_2$ with or without chitosan. The content was stirred slowly for 10 min using magnetic stirrer to cure the prepared alginate microspheres. The formed beads were then filtered using stainless steel grid, washed three times with distilled water and oven-dried at 40 °C for 4 h.

Table 1: Composition of different TBS microspheres

Formulation code	Drug: polymer ratio	Polymer concentration (%w/w)	NaAlg (%w/w)	CMC (%w/w)	Carbopol (%w/w)	HPMC (%w/w)	Chitosan (%w/w)
F1	1:2	6	100	–	–	–	–
F2	1:1	6	100	–	–	–	–
F3	1:2	10	100	–	–	–	–
F4	1:2	10	100	–	–	–	0. 5
F5	1:1	10	100	–	–	–	–
F6	1:1	10	100	–	–	–	0. 5
F7	1:2	6	75	25	–	–	–
F8	1:2	6	75	25	–	–	0. 5
F9	1:1	6	75	25	–	–	–
F10	1:1	6	75	25	–	–	0. 5
F11	1:2	10	90	10	–	–	0. 5
F12	1:1	10	90	10	–	–	0. 5
F13	1:2	10	90	–	10	–	–
F14	1:1	10	90	–	10	–	–
F15	1:2	10	70	–	30	–	–
F16	1:1	10	70	–	30	–	–
F17	1:2	10	90	–	–	10	–
F18	1:1	10	90	–	–	10	–
F19	1:2	10	70	–	–	30	–
F20	1:1	10	70	–	–	30	–

***NaAlg, sodium alginate; CMC, carboxy methyl cellulose, HPMC, hydroxylpropyl methyl cellulose. , *$CaCl_2$ (5 %) was used in all formulations as a cross-linking agent.

Characterization of the prepared TBS microspheres

Percentage yield value

The percentage yield value was defined as the quantity of beads produced as a function of loaded drug and polymer and calculated as mentioned by [33]:

$$\% \text{ Yield} = \left(\frac{\text{wieght ofprepared microspheres}}{\text{initial wieght of polymers+initial wieght of drug}} \right) \times 100 \ldots\ldots\ldots 1)$$

Drug content and encapsulation efficiency

The determination of drug content in TBS microspheres was done using the method described by [23]. An accurately weighed amount of the prepared microspheres (50 mg) were crushed and 50 ml distilled water were then added, the mixture was kept under magnetic stirring for 48 h and sonicated up to 60 min (Sonix TV ss-series ultrasonicator, USA). The solution was centrifuged (SIGMA 3-30K, Steinheim, Germany) and filtered through a 0. 45 µm membrane filter. The clear solution was analyzed spectrophotometrically at λmax 275 nm using UV-VIS Ultraviolet spectrophotometer (Jasko V-530, Japan) to determine the drug concentration. The % drug loading DL and EE were calculated as reported by Angadi *et al.* , 2012 [23]:

$$\% \text{ Drug loading} = \frac{\text{weight of drug in microspheres}}{\text{wieght of microspheres}} \times 100 \ldots\ldots\ldots 2)$$

$$\% \text{ Encapsulation efficiency} = \frac{\text{actual drug loading}}{\text{Theoretical drug loading}} \times 100 \ldots\ldots\ldots 3)$$

Morphology and particle size of TBS microspheres

Scanning Electron Microscopy (SEM) was used to visualize the shape, porosity and surface of the microspheres. Beads were sputtered with gold and placed on a copper stub. The mean size as mean diameter of microspheres was determined. Ten microspheres were selected randomly to be measured and the average value was taken [23, 34].

Swelling test

Swelling studies were performed gravimetrically in double distilled water [23]. Accurately weighed (10 mg) of prepared beads were immersed in distilled water and allowed to swell for 24 h. Beads were separated from the medium, wiped gently with soft tissue paper and weighed using electrical balance (Shimadzu, Japan). The swollen beads were put in oven at 60 °C until complete drying. The % swelling of the beads was calculated as follows:

$$\% \text{ Swelling} = \left[\frac{Ws-Wd}{Wd} \right] \times 100 \ldots\ldots\ldots 4)$$

Where Ws is weight of beads in the swollen state and Wd is weight of the dried beads. All experiments were done in triplicate and the average value was calculated.

Wash-off test

For evaluation of the mucoadhesion of microspheres, freshly slaughtered goat stomach was freshly prepared and washed with normal saline, cut into squares (1. 5 cm × 1. 5 cm) and attached to a microscopic slide using adhesive glue keeping the mucosal surface upward. Twenty microspheres were brought into direct contact with the mucus layer using a pressure of 5 g on the glass slide for 15 min to ensure complete adhesion of the microspheres. After that, the slide was connected to the arm of the disintegration apparatus (Hanson research, Chatsworth, USA) and the wash-off of the microspheres was induced by the reciprocating motion of the disintegration apparatus in a way that ensured up and down movement of tissue specimen in 8000 ml of 0. 1N HCl (pH 1. 2) and then to PBS (pH 6. 8) at 37±0. 5 °C. The time required for the microspheres to detach from the goat stomach tissue was recorded as the mucoadhesive performance [31, 35].

In vitro release study

Drug release from TBS microspheres was investigated at 37 °C and 50 rpm. The rotating basket dissolution test apparatus 2 (Hanson Research, SR 8 Plus model, Chatsworth, USA) was used under sink condition [36]. The pre-weighed amount of each sample was placed in dissolution medium (0. 1N HCl, pH 1. 2) for 2 h then in (phosphate buffer, pH 6. 8) for 6 h [37]. At periodic time intervals, 5 ml of the dissolution medium was withdrawn and measured spectrophotometrically at 275 nm. The sample volume was replaced by fresh dissolution media to maintain the sink conditions. All studies were performed in triplicate and the average value was calculated.

Kinetic studies of the release data

The data obtained from the release studies were kinetically analyzed and the order of drug release was determined. Zero-order and first-order kinetics as well as the Higuchi diffusion model were employed and the correlation coefficient values (R^2) were determined.

Selection and characterization of the best formulations

Microsphere formulations achieved high EE and a sustained release of TBS after 8 h were chosen and subjected for further evaluation.

Differential scanning calorimetry (DSC)

DSC analysis of the pure TBS, polymers and the TBS-loaded composite beads (F11 and F19) were carried out using DSC (TA-60WSI, Shimadzu, Japan) to detect any possible physical incompatibility. The instrument was calibrated using purified Indium (99. 99%). Samples (5 mg) were sealed in a flat bottomed aluminum pan (Shimadzu DSC-60, Japan). The pan was placed in the DSC instrument and scanned from 0 °C to 250 °C at a rate of 10 °C/min. Dry nitrogen was used as a carrier gas to eliminate the oxidative and pyrolytic effects with a flow rate of 10 ml/min. The melting and transition point measurements were performed using the software provided with the device.

Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra were obtained to investigate any possible chemical interactions of TBS with the polymers. Sample of 5 mg of each of the two selected formulations were mixed with 100 mg potassium bromide and compressed into discs under pressure of 10 000 to 15 000 pounds per square inch. The IR spectra were recorded using Infrared Spectrophotometer (IR435-U-04, Shimadzu Koyoto, Japan).

X-ray diffraction (XRD)

For detection of the drug polymorphism after encapsulation, the X-ray powder diffraction patterns of the drug, plain and loaded microspheres (F11) were plotted using X-ray diffractometer (XRD-610, Shimadzu, Japan). Samples were exposed to Cu Kα radiation at a scan rate of 5 °C/min over the 2Jb range of 4 °C to 70 °C. The operating voltage and current were 40 kV and 55 mA, respectively. The receiving beam slit was 0. 2 mm. The peak height (intensity) versus 2 Jb was then obtained.

Stability study

Short-term stability study of the best selected formulations F11 and F19 were carried by storing the microspheres in PVC blisters covered with aluminum foil at 40 and 60 °C in ovens for a period of 12 w. Samples were withdrawn periodically at 1, 2, 4, 6, 8 and 12 w and examined for any physical changes, drug release as well as for their drug content using HPLC stability indicating method.

In vivo testing

Animals

This study was approved by the local animal ethical committee of Beni Suef University. Six healthy male albino rabbits weighing between 2-2. 5 kg were fasted overnight, dosed and held in restrainers during blood sampling.

Study design

The study was assigned with single-dose; in a randomized crossover fashion, based on a 3X3 Latin square sequence with 1-week wash out period. Each rabbit received an oral dose from each of the three formulations (F11, F19, and the reference standard tablets, Aironyl®) with a sufficient amount of water [38]. At pre-determined time intervals (1, 2, 4, 8 and 12 h) post dosing, one ml blood samples were withdrawn from the marginal ear vein. The samples were collected in heparinized tubes and centrifuged at 3000 rpm for 15 min and stored at-20 °C. The plasma samples were analyzed by high performance liquid chromatography HPLC (Shimadzu, Koyoto, Japan) and the drug concentration was computed using Shimadzu Controller Version Analyst 1. 6.

Chromatographic conditions

A modified HPLC method was used for determination of the amount of drug in plasma [39]. The mobile phase was a mixture of acetonitrile and 0. 1 % formic acid (80:20 %v/v). It was delivered at a flow rate of 1 ml/min into the Sun Fire Column (waters) 50*4. 6 mm 5Um and the injection volume was 20 μl.

Preparation of samples for analysis

Drug was extracted by adding 4 ml tertiary butyl methyl ether to 0. 5 ml of plasma and vortex mixed for 5 min. The resultant mixture was centrifuged under vacuum at 4000 rpm for 15 min to evaporate the organic layer. Finally, 0. 25 ml mobile phase was added and injected to the HPLC column. The unknown concentration of TBS in each sample was calculated as follow: Q= R/A±B, where Q is the TBS concentration, R is the peak area ratio (Drug/Internal Standard), A is the slope of calibration curve and B is the Y-intercept.

Pharmacokinetic study

The various pharmacokinetic parameters of TBS were calculated using, WinNonlin® (version 1. 5, Scientific Consulting, Inc. , Rockville, MD).

Statistical analysis

All data were expressed as mean±standard deviation. The mean difference between groups was analyzed statistically by one-way ANOVA followed by Tukey post hoc analysis or two way ANOVA followed by Tukey post hoc analysis. Significance level was set at *$p < 0. 05$ or **$p < 0. 001$. All calculations were made using the computer program SPSS 16. 0 (SPSS, Chicago, IL).

RESULTS AND DISCUSSION

Percentage yield value

The percentage yield of the prepared coated microspheres ranged from 60 % to 93. 7 % (table 2). It was observed that the production yield of the microspheres is decreased by increasing the co-polymer concentration as the viscosity would be increased. And with respect to copolymer type; NaAlg/NaCMC composite beads showed the highest yield (93. 7 %), while those composed of NaAlg/carbopol and NaAlg/HPMC showed the lowest one (70. 2 % and 70. 1 % respectively) due to a concomitant increase in viscosity [40].

Drug content and encapsulation efficiency

The various polymeric concentrations and the drug to polymer ratio were all based on preliminary experiments and were modified according to the results of the characterization parameters obtained.

The (% EE) of TBS in the composite beads vary widely from 20 to 74. 8 % (table 2) which may be attributed to the nature of the polymer matrix and co-polymers used. Drug loading and encapsulation efficiency of microspheres containing only NaAlg, as a polymer, were found to be very low (F1, F2). This phenomenon could be a result of insufficient cross-linking between $CaCl_2$ and NaAlg, which led to high porosity microspheres with an increased chance of outside drug leakage during and after gelation [3, 41].

In a trial to improve the % EE, it was observed that there was a direct relationship between polymer concentration and % EE. The amount of the drug in microspheres was increased with raising of the NaAlg concentration from 6 to 10 % (w/w) in formulations coded (F3, F5). Indeed, that might be due to the increasing in viscosity of drug-polymer solution [36]. Statistical analysis of data showed that this increase was statistically significant (**p<0. 001) when F3 and F5 were compared with F2 and insignificant when compared with F1 (p>0. 001).

Moreover, the addition of chitosan (CS) into crosslinking solution could significantly (*p<0. 05) improve drug loading and % EE (F4, F6) due to the formation of a strong polyelectrolyte barrier membrane on the surface of microspheres which block up the large pores on the surface of the Ca-Alg microspheres. This barrier which prevent diffusion of TBS outside the microspheres was formed from the electrostatic binding between negatively charged carboxyl groups (-COO^-) of NaAlg and the positively charged amine groups (-$NH3^+$) of chitosan CS [3, 23].

Another way to get a better % EE was the use of co-polymers. The rigid network interaction between two different polymers, offered a protective layer which could increase the ability of microspheres to keep the drug inside and consequently increased the %EE [42]. Furthermore, the results reported that the type and concentration of co-polymers could affect the % DL and % EE [43].

In this research, NaCMC (F7, F8, F9, F10, F11 and F12), carbopol (F13, F14, F15 and F16) and HPMC (F17, F18, F19 and F20) were used with NaAlg. Actually, there was a variation co-polymers, on its ability to entrap the drug with a combination of NaAlg. The % EE was ranged between (23. 5 %-59 %) for NaCMC containing microspheres, (34. 5 %-50 %) for carbopol microspheres and (31. 5 %-74. 8 %) for HPMC microspheres. These variations were sometimes significant (*p<0. 05) depending on the viscosity of the polymer used, as the viscosity was increased the entrapment efficiency was also increased [41].

On the other hand, the increasing of drug to polymer ratio from 1:1 into 1:2, could lead to fast solidification rate of the prepared microspheres [28, 44] and hence an increase in thickness of coating layer [45], which in turn resulted in a decrease in the leaching of drug out of the microspheres.

It was noticed that there was a reverse relationship between the curing time and the % EE. The increase in curing time led to an increase in the loss of hydrophilic drug to the external medium [40, 46].

Morphological properties and particle size of microspheres

SEM images of the microspheres and their surfaces, taken at 200×, 1000× and 6000× magnifications were studied for surface morphology (fig. 1). Uncoated loaded NaAlg microspheres nearly had spherical shapes with rough surfaces as seen in (fig. 1, A). The surface of these microspheres exhibited an "orange peel" appearance with corrugations and wrinkles which may be attributed due to partial collapsing of the polymeric gel network during rapid drying [36].

The chitosan-coated calcium–alginate beads (fig. 1, B) were characterized by a heterogeneous structure, a dense surface, and a loose core because of the heterogeneous gelation mechanism that resulted in bead collapsing during the drying process [23]. Compared to NaAlg microspheres, blend microspheres containing NaCMC (fig. 4, C), carbopol (fig. 4, D) or HPMC (fig. 4, E) showed almost spherical, oval or disk shape with a smooth surface and uniform texture [41]. The surface topography of the blend microspheres exhibited comparatively less roughness than the

uncoated ones. The collapsed shape was attributed to their heterogeneous structures.

The mean diameter of the prepared microspheres was determined using scanning electron microscopy. The mean particle size of the beads was ranged from 963. 3 to 1653 μm (table 2). There was a direct relationship between the used polymer ratio and the particle size of microspheres. This relation could be explained by the increase in the viscosity, cross-linking and hence the matrix density of the prepared microspheres with the raise in the amount of polymer used producing larger microspheres [47, 48].

Moreover, the particle size was dependent on % (w/w) of the enteric coating chitosan polymer. Coated particles (F4 and F8) exhibited smaller sizes when compared to uncoated microspheres (F3 and F7), probably due to stronger ionic interaction between the cationic CS and the anionic NaAlg or NaCMC components of the composite beads; this might have led to the shrinkage of the beads [23].

The ratio and type of the co-polymer also affected the particle size of TBS microspheres [41]. As the co-polymer concentration increased, there was a marked increase in the average particle size of the microspheres. The effect of the polymer type may be related to differences in molecular weights and structures which in turn resulted in polymeric solutions of different viscosities [49].
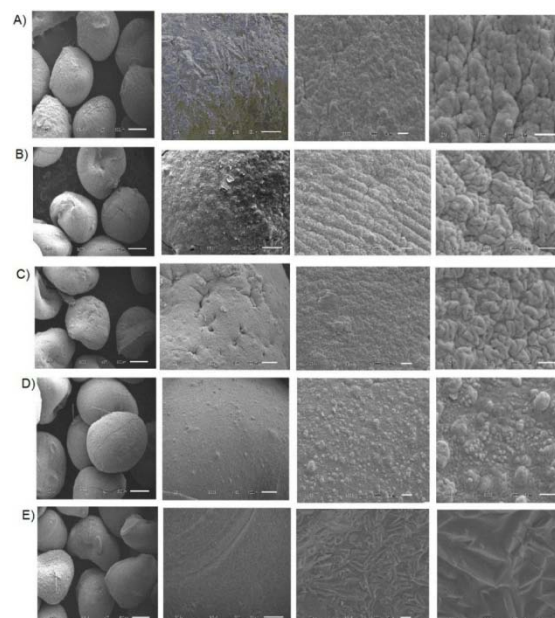

Fig. 1: Scanning electron micrographs of different formulations of TBS microspheres and its surface at 200X, 1000X and 6000X (A) F3, (B) F7, (C F11, (D) F14 and (E) F19

Swelling study

The swelling behavior of drug-loaded microspheres after rehydration (table 2) was well studied as it has a great impact on drug release [23].

The hydration of hydrophilic groups of NaAlg, CS and co-polymers was the main cause of the swelling of the dry microspheres [48]. From the swelling behavior of drug-loaded microspheres, it can be observed that the ability of dry calcium–alginate beads to swell in water was decreased after coating with CS (F4, F6, F8 and F10) which might be attributed to a couple of reasons. Firstly, the formation of an entangled system by blending of NaAlg and CS [50] and hence the water permeability of the microspheres has been reduced. In addition, a fraction of hydrophilic groups at the surface of dry calcium alginate microspheres form a polyelectrolyte complex with the amino groups of CS and hence it does not contribute to the entrapment of water molecules within the microspheres [51].

Also, It was evident that the swelling ratios was increased by increasing polymer or co-polymer concentration [47], thus suggesting that a composite matrix had absorbed a higher amount of water from the aqueous media but that increase was not statistically significant (p>0. 05) for all co-polymers used [41]. The beads containing NaCMC had the greatest swelling percentage.

In vitro release study

The release of TBS from the prepared microspheres (fig. 2) was showing biphasic release pattern. The first stage was a burst release depicted in acidic medium pH 1. 2 from 0 to 2 h, characterized by fast release due to rapid swelling of beads and diffusion of drug out of them. The second stage was found in alkaline medium pH 6. 8, where swelling of the beads was constant and the release rate was slow providing a sustained release rate of the drug.

The *in vitro* release data are discussed under the effect of many factors including polymer concentration, coating with chitosan, polymer blend composition and drug to polymer ratio.

As displayed from (table 2), the amount of TBS released from formulations containing low concentration of NaAlg 6 % (F1and F2) was fast (82 % and 85 % respectively). It referred to the high solubility of TBS and insufficient cross-linking between NaAlg and CaCl2. So, the resulting beads have a highly porous surface allowing fast release of drug out of the beads [3]. Furthermore, the formation of smaller particles, that have a larger surface area exposed to the dissolution medium, could be another reason for the higher dissolution rate of the drug [47]. However, a slight improvement in retarding the drug release was observed in the microspheres prepared from a higher polymer level 10 % (F3andF5) but this retardation in the drug release was not significant (p>0. 05) when compared with F1 and F2. This release delay might be due to the higher crosslinking, greater binding of the drug with the polymer and the larger amounts of drug adhered tightly to the polymer matrix [45]. Also, the increase in the density of the matrix at higher polymer concentrations resulted in an increase in the diffusional path length which may decrease the overall drug release from the polymer matrix [28, 52].

For a more sustained release from the microspheres, two different approaches were adopted. The first one was the addition of chitosan CS, a coating agent, to the cross-linking solution before the extrusion of microspheres into it (F4, F6, F8 and F10). This addition aimed to form a stronger membrane barrier on the surface of microspheres by electrostatic interaction between alginate and chitosan [3, 23]. That NaAlg-CS complex has blocked the large pores of Ca-Alg gel matrix, consequently, increased the encapsulation efficiency and retarded the drug release from the microspheres. By comparing the CS-coated microsphere with uncoated one, no significance difference on retardation of the drug release was appeared (p>0. 05).

The other approach was the use of co-polymers in a combination with alginate in the preparation of the microspheres to modify the mode of release of TBS from the beads and to increase the encapsulation efficiency [41]. (table 1) show that formulations containing NaAlg/NaCMC in a polymer concentration of 6 % at ratio 75:25 % w/w (F7, F8, F9 and F10) and formulations containing NaAlg/NaCMC in a polymer concentration of 10 % at ratio 90:10 % w/w (F11 and F12) have a more retardation effect on drug release than formulations containing NaAlg only (F1 and F2). The percentage of drug release was ranged from 53 % to 64 % after 8 h. There was no significance difference (p>0. 05) between F7, F8, F11 and F12 when compared with F1and F2, while there was a significance difference between F9 and F10 when compared with F1and F2.

In the case of microspheres (F13, F14, F15 and F16), the replacement of part of NaAlg with carbopol showed a significant decrease (*p<0. 05) in drug release rate when compared to NaAlg microspheres as was discussed by [34] and about 43–52 % of a drug released within 8 h. The influence of the percentage of Carbopol on the drug release profile was attributed to the effect of this polymer on the structure of the microspheres. Carbopol might increase the density of the beads and hence the prepared microspheres with higher Carbopol concentration were more compact, less porous and containing a denser network structure than those prepared with low polymer concentration [53, 54]. Moreover, carbopol could form a gel layer which could be considered as a barrier hindering the penetration of the dissolution medium inside the swelled beads, thereby retarding the diffusion of TBS outside them [55, 56].

The use of a combination between NaAlg and HPMC (F17, F18, F19 and F20) also showed a significant retardation in drug release rate when compared with (F1 and F2) (*p<0. 05). The values of percentage of TBS released after 8 h were between 35 and 44 %. That retardation could be attributed due to a couple of factors; the ultimate low solubility of HPMC in water and the production of a thick gel layer around the beads that could substantially reduce the penetration of dissolution medium into the beads [41]. The increase of HPMC concentration to 30 % (F19 and F20) in compared to 10 % in (F17 and F18) had led to an insignificant decrease in the rate of drug release (p>0. 05). Finally, it could be assumed that alginate microspheres were able to sustain the drug release for 8 h; whereas, co-polymer microspheres were able to sustain the drug release for up to 24 h.

In addition, the drug release was affected by the change in drug to polymer ratio. The alteration of the porosity of microspheres and in turn the rapid liquid penetration resulted in a fast initial drug release from microspheres prepared at higher drug to polymer ratio (1:1) [45, 57].

Kinetic study of drug release data

In an attempt to describe the drug release pattern appropriately; linear regression analysis of the *in vitro* release data were fitted to different kinetic models. Data revealed that TBS followed Higuchi diffusion mechanism for polymeric systems except formulae (F4, F5, F6, F8, F13 and F17) which followed zero-order kinetics, as well as formulae (F1, F2, F15 and F19) followed first-order kinetics.
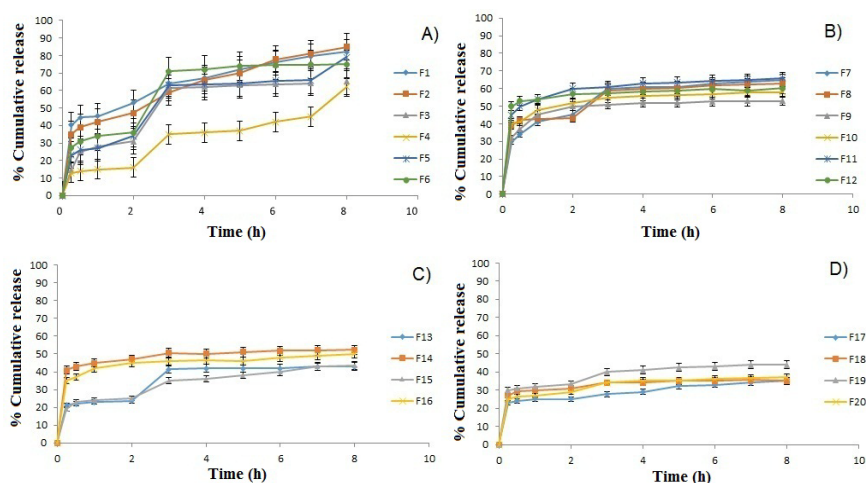

Fig. 2: Release profile of TBS from different composite microspheres: A) F1-F6, B) F7-F12, C) F13-F16 and D) F17-F20
*Each sample was analyzed in triplicate (n = 3), mean±SD

Table 2: Evaluation tests of TBS composite microspheres

F	Yield (%)	Drug loading (%)	Entrapment Efficiency (%)	Mean Size (µm)	Swelling (%)	% of release after 8hr
F1	92. 0±9. 63	13. 0±0. 26	39. 0±0. 78	963. 3±7. 89	102. 0±2. 04	82. 0±1. 47
F2	93. 5±9. 78	10. 0±0. 20	20. 0±0. 40	978. 0±8. 23	107. 0±2. 14	85. 0±1. 53
F3	75. 6±15. 7	13. 4±0. 26	40. 0±0. 80	1574±5. 56	117. 0±2. 34	65. 0±1. 17
F4	78±14. 85	16. 4±0. 32	49. 3±0. 98	1485±1. 04	109. 0±2. 18	62. 0±1. 11
F5	73. 6±16. 0	21. 4±0. 42	43. 0±0. 86	1606±2. 76	120. 0±2. 42	79. 0±1. 42
F6	77. 0±15. 5	25. 0±0. 50	50. 2±1. 00	1550±9. 44	111. 0±2. 22	75. 0±1. 35
F7	90. 6±9. 88	19. 0±0. 38	57. 0±1. 14	988. 2±6. 72	558. 8±11. 1	64. 0±1. 15
F8	93. 7±9. 72	17. 6±0. 35	53. 0±1. 06	972. 0±4. 83	476. 0±9. 52	63. 0±1. 13
F9	89. 0±9. 89	11. 7±0. 23	23. 5±0. 47	989. 5±9. 12	280. 0±5. 63	53. 0±0. 95
F10	85. 0±9. 42	12. 8±0. 25	25. 7±0. 51	942. 1±5. 23	265. 0±5. 30	58. 0±1. 04
F11	83. 0±12. 2	19. 7±0. 39	59. 0±1. 18	1227±6. 48	152. 0±3. 04	66. 0±1. 18
F12	80. 0±13. 0	20. 0±0. 40	40. 0±0. 81	1305±10. 4	158. 8±3. 17	60. 0±1. 08
F13	70. 1±10. 4	11. 5±0. 23	34. 5±0. 69	1043±7. 33	153. 0±3. 06	43. 0±0. 77
F14	79. 0±12. 4	24. 2±0. 48	49. 2±0. 98	1240±8. 34	150. 0±3. 00	52. 0±0. 93
F15	71. 0±12. 1	11. 3±0. 22	35. 0±0. 70	1216±9. 74	143. 0±2. 86	43. 7±0. 78
F16	70. 8±13. 5	25. 0±0. 50	50. 0±1. 00	1352±5. 48	132. 0±2. 64	50. 0±0. 90
F17	75. 0±14. 6	24. 9±0. 49	74. 8±1. 49	1466±11. 2	125. 0±2. 50	35. 0±0. 63
F18	75. 4±15. 9	15. 7±0. 31	31. 5±0. 63	1590±8. 63	134. 0±2. 68	36. 0±0. 64
F19	70. 2±16. 0	22. 6±0. 45	67. 8±1. 35	1600±11. 1	129. 0±2. 58	44. 0±0. 79
F20	72. 5±16. 3	20. 1±0. 40	40. 3±0. 81	1635±10. 5	112. 0±2. 24	37. 0±0. 66

Data are mean values (n=3)±SD.

Mucoadhesion time

The adhesion of the microspheres to the stomach mucosa and intestinal gut at pH 1. 2 and 6. 8 were shown in (table 3). The microspheres showed a stronger mucoadhesiveness to intestinal mucosa than stomach mucosa.

Mucoadhesion is a property of the polymeric microspheres, which allows it to adhere onto the mucus membrane [58]. Special consideration needed to be taken while designing an oral mucoadhesive drug delivery system. As the mucoadhesivity improves the retention time of the formulation at the site of absorption in GI tract, so it helps a sustained release effect for a longer period [47, 59, 60].

Table 3: Differential mucoadhesion time of TBS composite microspheres

Formula No.	Mucoadhesion time (min)	
	PH 1. 2	PH 6. 8
F1	75. 00±1. 35	245. 0±5. 39
F2	66. 00±1. 18	230. 0±5. 06
F3	80. 00±1. 44	256. 0±5. 63
F4	116. 0±2. 08	350. 0±7. 70
F5	65. 00±1. 17	224. 0±4. 92
F6	79. 00±1. 42	316. 0±6. 95
F7	116. 0±2. 08	295. 0±6. 49
F8	119. 0±2. 14	305. 0±6. 71
F9	109. 0±1. 96	273. 0±6. 01
F10	112. 0±2. 01	316. 0±6. 95
F11	330. 0±5. 94	360. 0±7. 92
F12	290. 0±5. 22	344. 0±7. 56
F13	160. 0±2. 88	329. 0±7. 23
F14	120. 0±2. 16	203. 0±4. 46
F15	133. 0±2. 39	275. 0±6. 05
F16	146. 0±2. 62	261. 0±5. 74
F17	120. 0±2. 16	265. 0±5. 83
F18	125. 0±2. 25	240. 0±5. 28
F19	175. 0±3. 15	300. 0±6. 60
F20	158. 0±2. 84	288. 0±6. 33

Data are mean values (n=3)±SD. , *Mucoadhesivity is expressed in terms of retention time of microspheres on mucosal surface. , Based on the results of the different characterization procedures, two formulations were selected (F11 and F19) for further studies due to the following reasons

It was found that the microspheres at acidic medium have a weak mucoadhesion and had washed off from the mucosal surface within 1-2. 9 h due to the shielding off of the intrinsic negative charges of the microspheres, which might play a critical role in the adhesion of the microspheres with the mucus membrane. However, the mucoadhesion time of the microspheres was increased at basic medium pH 6. 8 to be ranged from 3-6 h. At neutral pH, the charges of the microspheres tend to be more exposed, allowing stronger electrostatic interactions and thus showing a greater mucoadhesivity [35].

Using a co-polymer had to increase the mucoadhsivity of the microspheres. Also, it was observed that the higher level of the polymer, the longer the mucodhesion time. This increase in the mucoadhesion time may be attributed to the higher viscous gel produced and also to the increased number of amino groups available for binding with the sialic acid residues in mucus membrane [47]. In addition, it was found that the microspheres coated with chitosan exhibited slower wash-off and remained attached to mucosa than uncoated ones [60].

DSC studies

The DSC thermogram of pure TBS, polymers, and the DSC of the selected formulations (F11 and F19) were illustrated in (fig. 3). It was observed that the thermogram of the pure drug showed a sharp endothermic peak in the range of 244-248 °C. The endothermic peak confirmed the crystalline nature of the drug. DSC studies of the above mentioned formulations realized that there was no incompatibility with the excipients used.
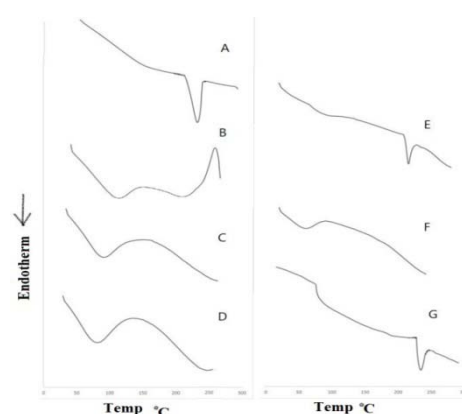

Fig. 3: DSC thermograms of (A) pure terbutalinesulphate, (B) NaAlg, (C) NaCMC, (D) Chitosan, (E) F11, (F) HPMC and (G) F19

FTIR study

Drug/polymer chemical interaction was studied by FTIR spectroscopy (fig. 4). The FTIR spectrum of pure TBS showed characteristic peaks at 3338 cm^{-1} (OH stretch), 3058 cm^{-1} (aromatic CH stretch), 2974 cm^{-1} (methyl asymmetric stretch), 1612and1487 cm^{-1} (aromatic ring stretch), 1388 cm^{-1} (t-butyl symmetric bend), 1061 cm^{-1} (secondary alcohol stretch) [61]. In the FTIR spectrum of sodium alginate, a wide band at 3430. 74 cm^{-1} was appeared due to the −OH stretching vibrations. A characteristic principal peaks were also seen at 1616. 06 and 1418. 39 cm^{-1} for asymmetric and symmetric–C=O stretching vibrations of–COO^{-}anions, respectively [23, 36]. FTIR spectrum of NaCMC showed a strong peak at 3435. 56 cm^{-1} due to O–H stretching vibration, while the band around 1057–1110 cm^{-1} was assigned to ether bonds. The absorption peak at 1620 cm^{-1} is related to carboxylate, while the peak at 2910 cm^{-1} was for the methylene groups [23, 62].

In the FTIR spectra of chitosan, characteristic principal peaks were found at 3436. 53 cm^{-1} for O–H stretching. The peak near 1156. 12 cm^{-1} was due to the asymmetric vibrations of-CO. The peaks near

1078 cm^{-1} were assigned to the CO of the ring-COH,-COC and-CH$_2$OH. The peak near 896. 737 cm^{-1} matched to the wagging of the saccharide structure of chitosan [47].

For Carbopol, the FTIR spectra showed a peak in the 3000–2950 cm^{-1} range, representing OH stretching vibration and intramolecular hydrogen bonding. The prominent peak between 1750 and 1700 cm^{-1} was assigned to carbonyl C=O stretching band while the peak at 1450 to 1400 cm^{-1} was assigned to–C-O/-O-H. The band at 1250 to 1200 cm^{-1} was assigned to C-O-C of acrylates. The ethereal crosslinking, is indicated by the prominent peak at 1160 cm^{-1}, represented a stretching vibration of C-O-C group. The band between 850 and 800 cm^{-1} indicated out of plane bending of C=CH [63]. The FTIR spectrum of the HPMC indicated the characteristics peak of 3474. 13 cm^{-1}OH stretching, 2934. 16 cm^{-1} C-H stretching alkanes and 1121. 4 cm^{-1} aliphatic C-O stretching [64].

The FTIR spectra of the two selected formulations illustrated the characteristic peaks of TBS with some broadening and reduction in intensity, indicating the absence of any chemical interactions between drug, polymers or counter ions after the formation:
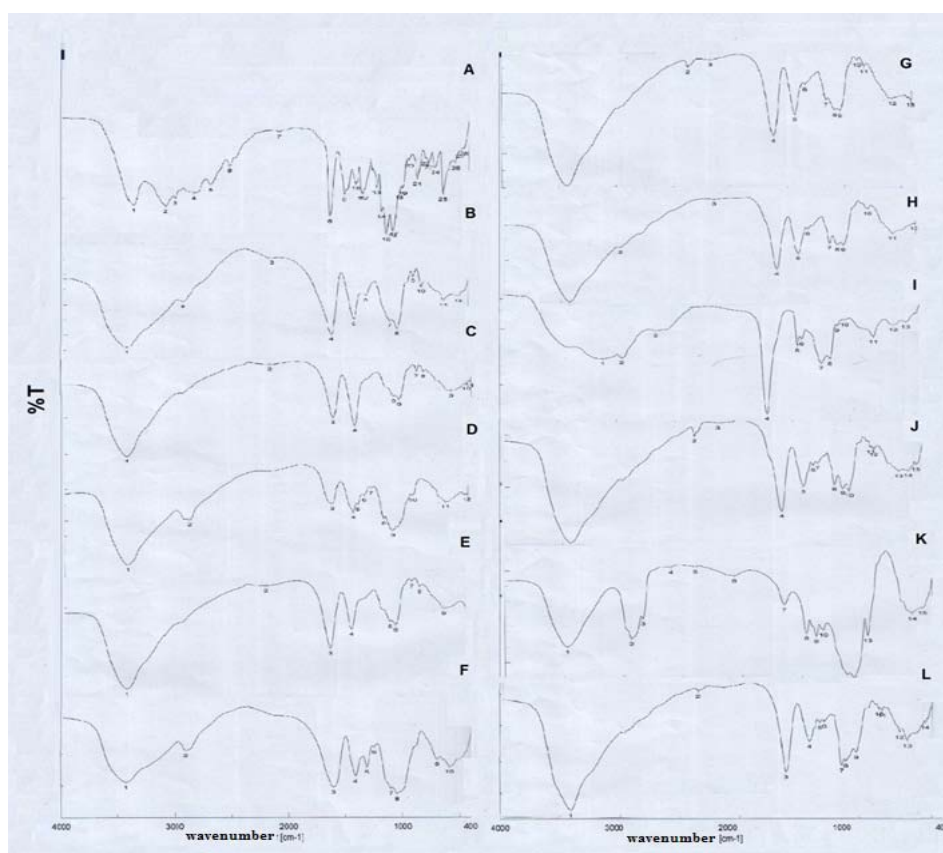

Fig. 4: FTIR spectra of (A) pure terbutalinesulphate, (B) NaAlg, (C) NaAlg microspheres F1, (D) Chitosan CS, (E) NaAlg/CS microspheres F4, (F) NaCMC, (G) NaAlg/NaCMC microspheres F7, (H) NaAlg/NaCMC/CS microspheres F11, (I) Carbopol, (J) NaAlg/Carbopol microspheres F17, (K) HPMC and (L) NaAlg/HPMC microspheres F19

X-ray diffraction (XRD)

X-ray diffractogram was used to study the polymorphism of drug after encapsulation [23]. (fig. 5) illustrated the X-ray diffraction of TBS, placebo, and drug loaded composite beads. XRD of pure terbutaline sulphate showed that the drug was crystalline in nature as demonstrated by characteristic peaks observed at about 8. 4 °, 11 °, 18. 3 °, 20 °, 23. 56 °, 24. 6 °, 25. 7 °and 27. 5 ° (2θ). Peaks at 18. 3^0 and 23. 56^0 were used to compare the XRD pattern of drug with beads. Upon analysis of the TBS-loaded beads (F11), the prominent peaks of the drug retained their positions in the loaded formulations

but with decreased intensities indicating polymorphism. The relative reduction in diffraction intensity at these angles suggested the amorphous nature of the formulation [35, 65].

Stability study

The selected microspheres of formulae F11 and F19 stored at 40 and 60±0. 5 °C for a period of 12 w exhibited no change in color or physical appearance throughout the storage period. However, the percent remaining of TBS was slightly decreased, as shown in (fig. 6), but still within the permitted limits by the USP (90–110 %) up to the end of the storage period. Also, no alteration in the drug release was observed.
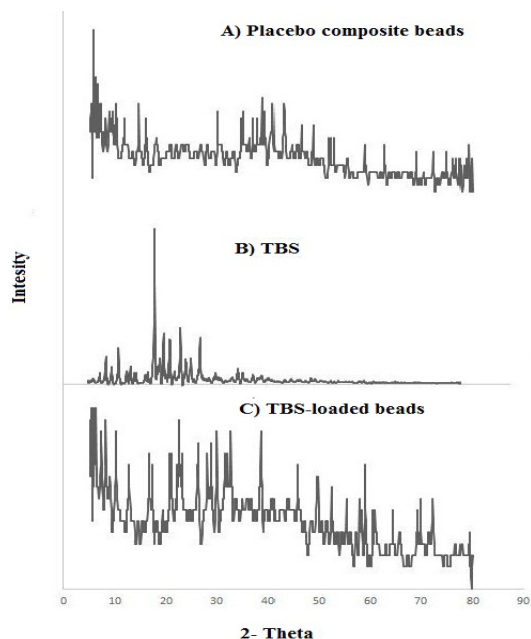
Fig. 5: XRD spectra of (A) placebo composite beads, (B) TBS and (C) TBS-loaded composite beads (F11)
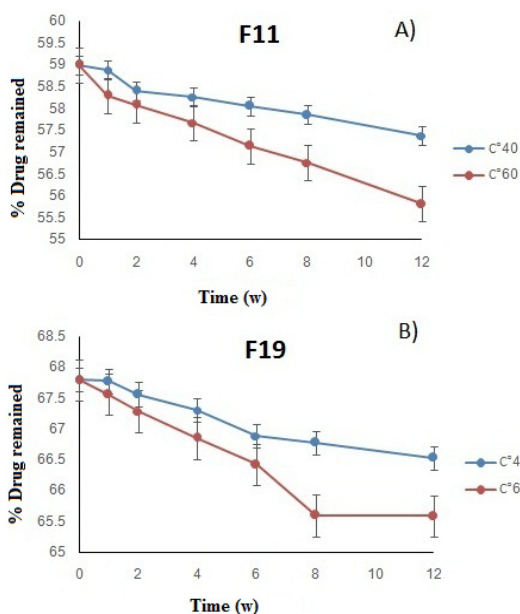

Fig. 6: Effect of storage at 40 °C and 60 °C on drug remaining at: A) F11 and B) F19. *Each sample was analyzed in triplicate (n = 3), mean±SD

In vivo bioavailability study

The pharmacokinetic parameters of TBS in rabbit plasma were used to understand the *in vivo* behavior of the selected microspheres F11 and F19 compared with oral immediate release Aironyl® (2. 5 mg) tablets. A good linearity from 0. 001 to 10 ng/ml was obtained with acceptable intra-day and inter-day reproducibility. The mean plasma drug concentration–time profiles after administration of the F11 and F19 versus Aironyl® were illustrated in (fig. 7) and the corresponding pharmacokinetic parameters were shown in (table 4).

The pharmacokinetic study revealed that the administration of TBS as controlled release microspheres had modified the pharmacokinetic profile and improved the bioavailability relative to the marketed oral tablet. The tmax of TBS microspheres was significantly higher (**p<0. 001) than the oral marketed tablet, which was 12±0. 24 h and 8±0. 16 h for F11 and F19 respectively while it was only1. 33±0. 02 h for Aironyl® tablets. This could be attributed to the preparation of TBS, which is freely water soluble drug, as microspheres retarded its absorption through the release of the encapsulated TBS in a controlled manner resulting in a delayed tmax. It can be observed that the release pattern of the drug was mostly affected by the properties of the microsphere rather than the physicochemical properties of the drug molecules. The mean AUC_{0-24} (ng h/ml) was found to be 0. 020±0. 005 ng h/ml and 0. 025±0. 005 ng h/ml for F11 and F19 respectively compared with 0. 028±0. 007 ng h/ml for marketed oral tablets. Differences between group means were considered significant at **p<0. 001. From these results, it could be concluded that, the relative bioavailability of TBS from the selected formulae F11 was 283. 84 % and 202. 04 % for F19 compared with marketed oral tablets. This higher relative bioavailability of TBS can be attributed to reduced first pass metabolism when administered as oral microspheres.
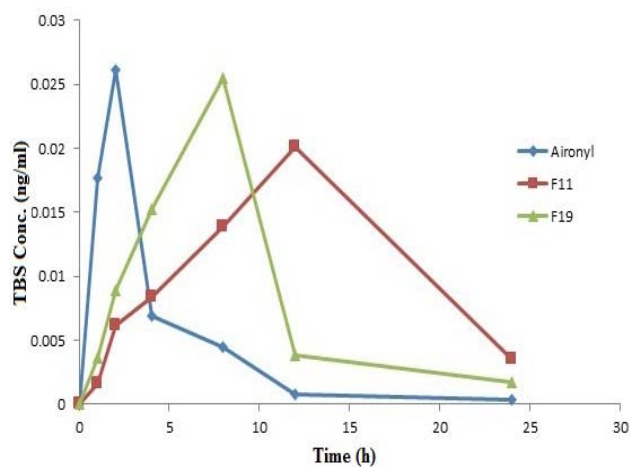

Fig. 7: Mean TBS concentrations in plasma of rabbits after administration of F11, F19 and oral Aironyl® tablets

Table 4: Pharmacokinetic parameters for the selected TBS microspheres and oral Aironyl®tablets in rabbit's plasma

Pharmacokinetic parameter	Formulation		
	F11	F19	Marketed oral tablets
t_{max} (hr)	12. 000±0. 000	8. 000±0. 000	1. 333±0. 516
C_{max} (ng/ml)	0. 020±0. 005	0. 025±0. 005	0. 028±0. 007
Ke (hr-1)	0. 100±0. 036	0. 137±0. 026	0. 175±0. 028
$t_{1/2}$ (hr)	7. 993±3. 125	5. 189±0. 913	4. 047±0. 698
$AUC_{(0-24)}$ (ng. hr/ml)	0. 277±0. 072	0. 204±0. 049	0. 104±0. 023
$AUC_{(0-\infty)}$ (ng. hr/ml)	0. 333±0. 099	0. 218±0. 057	0. 108±0. 022
% Relative bioavailability	283. 84	202. 04	

Each value represents the mean of 6 rabbit±SE for six determinations. Statistical analysis was determined using one-way ANOVA followed by Tukey Post Hoc Tests.

CONCLUSION

In this study, the increase in the polymer ratio in relation to the drug and the total amount of polymer used in microsphere formulations had effectively affected the amount of the drug encapsulated. In addition, coating with chitosan (F11) and the using of HPMC co-polymer in combination with NaAlg (F19) was an effective way to encapsulate TBS within the composite beads, using an inotropic gelation technique. All the previous four factors had successfully controlled the release of TBS and hence elongated the plasma half-life from 4 h to 24 h, providing a once daily dosing of the bronchodilator. The *in vivo* study on rabbits showed that the optimized formulae had an enhanced relative bioavailability by 283. 84 % and 202. 04 % in comparing with the marketed products.

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CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Abdul M, Rao AS, Martha S, Sirisha Y, Chandrika PU. Development of a floating multiple unit controlled-release beads of zidovudine for the treatment of AIDS. JOPR J Pharm Res 2012;6:78–83.
2. Biswal I, Dinda A, Das D, Si S, Chowdary KA. Encapsulation protocol for highly hydrophilic drug using non-biodegradable polymer. Int J Pharm Pharm Sci 2011;3:256–9.
3. Ma N, Xu L, Wang Q, Zhang X, Zhang W, Li Y, *et al.* Development and evaluation of new sustained-release floating microspheres. Int J Pharm 2008;358:82–90.
4. Basak SC, Kumar KS, Ramalingam M. Design and release characteristics of sustained release tablet containing metformin HCl. Br J Pharm Sci 2008;44 Suppl 3:477–83.
5. Patel PN, Patel MM, Rathod DM, Patel JN, Modasiya MMK. Sustain release drug delivery: a theoretical prospective. J Pharm Res 2012;5:4165–8.
6. Raffin RP, Colomé LM, Schapoval EES, Pohlmann AR, Guterres SS. Increasing sodium pantoprazole photostability by microencapsulation: effect of the polymer and the preparation technique. Eur J Pharm Biopharm 2008;69 Suppl 3:1014–8.
7. Öˮ zyazici M, Sevgi F, Ertan G. Micromeritic studies on nicardipine hydrochloride microcapsules. Int J Pharm 1996;138:25–35.
8. Chambin O, Dupuis G, Champion D, Voilley A, Pourcelot Y. Colon-specific drug delivery: influence of solution reticulation properties upon pectin beads performance. Int J Pharm 2006;321:86–93.
9. Ahmed MM, El-Rasoul SA, Auda SH, Ibrahim MA. Emulsification/internal gelation as a method for preparation of diclofenac sodium-sodium alginate microparticles. Saudi Pharm J Publ Saudi Pharm Soc 2013;21:61–9.
10. Patil P, Chavanke D, Wagh M. A review on ionotropic gelation method: Novel approach for controlled gastroretentive gelispheres. Int J Pharm Pharm Sci 2012;4 Suppl 4:27–32.
11. Shu XZ, Zhu KJ. The release behavior of brilliant blue from calcium-alginate gel beads coated by chitosan: the preparation method effect. Eur J Pharm Biopharm 2002;53:193–201.
12. Josef E, Zilberman M, Bianco-Peled H. Composite alginate hydrogels: an innovative approach for the controlled release of hydrophobic drugs. Acta Biomater 2010;6:4642–9.
13. SmidsrØd O, Skja G. Alginate as immobilization matrix for cells Trends. Biotechnol 1990;8:71–8.
14. Elzatahry AA, Eldin MS, Soliman EA, Hassan EA. Evaluation of alginate-chitosan mucoadhesive beads as a drug delivery system for the controlled release of theophylline. J Appl Polym Sci: Appl Polym Symp 2009;111:2452–9.
15. Patil SB, Sawant KK. Development, optimization and *in vitro* evaluation of alginate mucoadhesive microspheres of carvedilol for nasal delivery. J Microencapsul 2009;26:432–43.
16. Gadad AP, Patil MB, Naduvinamani SN, Mastiholimath VS, Dandagi PM, Kulkarni AR, *et al.* Sodium alginate polymeric floating beads for the delivery of cefpodoxime proxetil. J Appl Polym Sci 2009;114:1921–6.
17. Sweetman SC. The complete drug references. In: Martindale: Extra-pharmacopoeia. 34th ed; 2005.
18. Tripathi KD. Essentials of medical pharmacology. 5th ed. New Delhi: Jaypee Brothers Medical Publishers; 2004.
19. Borgstrom L, Nyberg L, Jonsson S, Lindberg C, Paulson J. Pharmacokinetic evaluation in man of terbutaline given as separate enantiomers and as the racemate. Br J Clin Pharmacol 1989;27:49–56.
20. Rao NR, Panchal H, Hadi MA. Formulation and evaluation of biphasic drug delivery system of Montelukast sodium for chronotherapy. World J Pharm Res 2012;1:757–75.
21. Multu GM, Moonjelly E, Chan L, Olopade CO. Laryngospasm and paradoxical bronchoconstriction after repeated doses of beta 2-agonists containing edetate disodium. Mayo Clin Proc 2000;75:285–7.
22. Nagabhushana S. Harmful effects of aerosolized bron-chodilator therapy in broncholitis. Indian Pediatr 2000;37:684–7.
23. Angadi SC, Manjeshwar LS, Aminabhavi TM. Novel composite blend microbeads of sodium alginate coated with chitosan for controlled release of amoxicillin. Int J Biol Macromol 2012;51:45–55.
24. Polk A, Amsden B, Yao KD, Peng T, Goosen MFA. Controlled release of albumin from chitosan–alginate microcapsules. J Pharm Sci 1994;83:178–85.
25. Sezer AD, Akubaga J. Release characteristics of chitosan treated alginate beads: I. Sustained release of a macromolecular drug from chitosan treated alginate beads. J Microencapsul 1999;16:195-203.
26. Torrado S, Prada P, Paloma M, Torrado S. Chitosan-poly(acrylic) acid polyionic complex: *in vivo* study to demonstrate prolonged gastric retention. Biomaterials 2004;25:917–23.
27. Kim CK, Kim MJ, Oh KH. Preparation and evaluation of sustained-release microspheres of terbutaline sulfate. Int J Pharm 1994;106:213–9.
28. Kotadiya R, Patel V, Patel H, Salaniya B. Evaluation of terbutaline sulphate encapsulated ethylcellulose microspheres : a factorial approach. Int J PharmTech Res 2009;1:1271–8.
29. Rajgor NB, Patel M, Shah VM, Bhaskar VH, Rajput GC. Preparation and characterization of terbutalin sulphate microsphere. Der Pharm Lett 2010;2:450–9.
30. Sahin S, Selek H, Ponchel G, Ercan MT, Sargon M, Hincal AA, *et al.* Preparation, characterization and *in vivo* distribution of terbutaline sulfate loaded albumin microspheres. J Controlled Release 2002;82:345–58.
31. Hadi MA, Srinivasa Rao A, Abhinetri V, Rao AHP. Design and *in vitro* evaluation of multiparticulate drug delivery system of terbutaline sulphate for the treatment of nocturnal asthma. Der Pharm Lett 2012;4:1777–85.
32. Kota RB, Srikrishna S, Kumar MS, Subrahmanyam KV. Design and characterization of hydrogyl beads of terbutaline sulphate. Int J Innovations Pharm Sci Res 2013;1:383–97.
33. Prasad S, Mukesh T, Ashok S, Monit S, Ashish S. Preparation and optimization of oral floating alginate gel beads famotidine. J Pharmakin 2011;3:1–15.
34. López-Cacho JM, González-R PL, Talero B, Rabasco AM, González-Rodríguez ML. Robust optimization of alginate-carbopol 940 bead formulations. Sci World J 2012:1-15. Doi:10. 1100/2012/605610. [Article in Press]
35. Agarwal T, Narayana SNGH, Pal K, Pramanik K, Giri S, Banerjee I. Calcium alginate-carboxymethyl cellulose beads for colon-targeted drug delivery. Int J Biol Macromol 2015;75:409–17.
36. Jana S, Samanta A, Nayak AK, Sen KK, Jana S. Novel alginate hydrogel core-shell systems for combination delivery of ranitidine HCl and aceclofenac. Int J Biol Macromol 2015;74:85–92.
37. Jelvehgari M, Mobaraki V, Montazam SH. Preparation and evaluation of mucoadhesive beads/discs of alginate and algino-

pectinate of piroxicam for colon-specific drug delivery via oral route. Jundishapur J Nat Pharm Prod 2014;9:e16576.

38. Chanda R, Roy A, Bahadur S, Saha S, Das S, Choudhury A. Formulation of terbutaline sulphate mucoadhesive sustained release oral tablets from natural materials and *in vitro-in vivo* evaluation. Asian J Pharm Sci 2010;5:168–74.

39. Kumar A, Nanda S. A validated high performance liquid chromatographic method for estimation of bromhexine and terbutaline in bulk and tablet dosage forms. Pharam Methods 2011;2:218–22.

40. Abd-Ellatif A. A pharmaceutical study on extended release solid dosage forms of certain antidiabetic drugs, "MSc. Thesis", Faculty of pharmacy, Beniseuf University; 2009.

41. Abou El Ela AESF, Hassan MA, El-Maraghy DA. Ketorolac tromethamine floating beads for oral application: characterization and *in vitro/in vivo* evaluation. Saudi Pharm J Publ Saudi Pharm Soc 2014;22:349–59.

42. Rokhade AP, Agnihotri SA, Patil SA, Mallikarjuna NN, Kulkarni PV, Aminabhavi TM. Semiinterpenetrating and, polymer network microspheres of gelatin Ketorolac, sodium carboxymethyl cellulose for controlled release of tromethamine. J Carbohydr Polym 2006;65 Suppl 3:243–52.

43. Sriamornsak P, Thirawong N, Puttipipatkhachorn S. Emulsion gel beads of calcium pectinate capable of floating on the gastric fluid: effect of some additives, hardening agent or coating on release behavior of metronidazole. Eur J Pharm Sci 2005;24:363–73.

44. Pandit SS, Hase DP, Bankar MM, Patil AT, Gaikwad NJ. Ketoprofen-loaded Eudragit RSPO microspheres: an influence of sodium carbonate 195on *in vitro* drug release and surface topology. J Microencapsul 2009;26:195–201.

45. El-Say KM, El-Helw ARM, Ahmed OA, Hosny KM, Ahmed TA, Kharshoum RM, *et al.* Statistical optimization of controlled release microspheres containing cetirizine hydrochloride as a model for water soluble drugs. Pharm Dev Technol 2014;20:738–46.

46. Patel YL, Sher P, Pawar AP. The effect of drug concentration and curing time on processing and properties of calcium alginate beads containing metronidazole by response surface methodology. AAPS PharmSciTech 2006;7 Suppl 4:E24-E30.

47. Dhankar V, Garima Garg KD, Awasthi R. Preparation, characterization and evaluation of ranitidine hydrochloride-loaded mucoadhesive microspheres. Polim Med 2014;44:75–81.

48. Hoffman AS. Hydrogels for biomedical applications. Adv Drug Delivery Rev 2012;64:18–23.

49. Gattani Y, Kawtikwar P, Sakarkar D. Formulation and evaluation of gastro retentive multi particulate drug delivery system of aceclofenac. Int J ChemTech Res 2009;1:1–10.

50. Lin YH, Liang HF, Chung CK, Chen MC, Sung HW. Physically crosslinked alginate/N,O-carboxymethyl chitosan hydrogels with calcium for oral delivery of protein drugs. Biomaterials 2005;26:2105–13.

51. Pasparakis G, Bouropoulos N. Swelling studies and *in vitro* release of verpamil from calcium alginate and calcium alginate–chitosan beads. Int J Pharm 2006;323:34–42.

52. Kamel S, Ali N, Jahangir K, Shah S, El-Gendy A. Pharmaceutical significance of cellulose: a review. J Express Polym Lett 2008;2 Suppl 11:758–78.

53. Chun MK, Cho CS, Choi HK. Mucoadhesive microspheres prepared by interpolymer complexation and solvent diffusion method. Int J Pharm 2005;288:295–303.

54. Silva CM, Ribeiro AJ, Ferreira D, Veiga F. Insulin encapsulation in reinforced alginate microspheres prepared by internal gelation. Eur J Pharm Sci 2006;29:148–59.

55. Al-Kassas RS, Al-Gohary OMN, Al-Faadhel MM. Controlling of systemic absorption of gliclazide through incorporation into alginate beads. Int J Pharm 2007;341:230–7.

56. Hua S, Ma H, Li X, Yang H, Wang A. pH-sensitive sodium alginate/poly(vinyl alcohol) hydrogel beads prepared by combined Ca^{2+}crosslinking and freeze-thawing cycles for controlled release of diclofenac sodium. Int J Biol Macromol 2010;46:517–23.

57. Velasco MV, Ford JL, Rowe P, Rajabi-Siahboomi AR. Influence of drug: hydroxypropylmethylcellulose ratio, drug and polymer particle size and compression force on the release of diclofenac sodium from HPMC tablets. J Controlled Release 1999;57:75–85.

58. Shaikh R, Singh TRR, Garland MJ, Woolfson AD, Donnelly RF. Mucoadhesive drug delivery systems. J Pharm Bioallied Sci 2011;3:89.

59. Ahuja A, Khar RK, Ali J. Mucoadhesive drug delivery systems. Drug Dev Ind Pharm 2008;23:489–515.

60. Suknuntha K, Tantishaiyakul V, Worakul N, Taweepreda W. Characterization of muco-and bioadhesive properties of chitosan, PVP, and chitosan/PVP blends and release of amoxicillin from alginate beads coated with chitosan/PVP. Drug Dev Ind Pharm 2011;37:408–18.

61. Mahajan AN, Pancholi SS. Formulation and evaluation of timed delayed capsule device for chronotherapeutic delivery of terbutaline sulphate. Ars Pharm 2010;50:215–23.

62. Bao Y, Ma J, Li N. Synthesis and swelling behaviors of sodium carboxymethyl cellulose-g-poly (AA-co-AM-co-AMPS)/MMT superabsorbent hydrogel. Carbohydr Polym 2011;84:76–82.

63. Sahoo S, Chakraborti CK, Naik S, Mishra SC, Nanda UN. Structural analysis of ciprofloxacin-carbopol polymeric composites by X-ray diffraction and fourier transform infra-red spectroscopy. Trop J Pharm Res 2011;10:273–80.

64. Sahoo S, Chakraborti CK, Behera PK. Spectroscopic investigations of a ciprofloxacin/HPMC mucroadhesive suspension. Int J Appl Pharm 2012;4:1–8.

65. Narkar M, Sher P, Pawar A. Stomach-specific controlled release gellan beads of acid-soluble drug prepared by ionotropic gelation method. AAPS PharmSciTech 2010;11:267–77.

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