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Process, optimization and characterization of mebeverine hydrochloride loaded guar gum microspheres for irritable bowel syndrome

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ABSTRACT

In the present investigation, guar gum microspheres containing mebeverine hydrochloride were prepared and characterized for local drug release in colon. Formulation variables studied include guar gum concentration, drug to polymer ratio, concentration of emulsifier, amount of cross linking agent, rotational speed and cross linking time. *In vitro* studies of microspheres depict a premature release of drug in the stomach and small intestine. As enteric coating of microspheres is difficult to achieve, they were compressed to tablets. Drug release studies and surface morphological characteristics of tableted microspheres revealed that the microspheres remained intact during the compression process. *In vivo* roentgenographic evaluation in rabbits revealed that the system remained intact until it reached to the lower part of the gastrointestinal tract, where microspheres were dispersed after the dissolution of the enteric coat, thus, revealing the potential of developed system for colonic targeting.

1. Introduction

Colon targeted drug delivery systems (CoDDS) have attracted a considerable interest in recent years for the treatment of systemic (angina pectoris, nocturnal asthma, rheumatoid arthritis, etc.) as well as local disease (inflammatory bowel disease, colorectal cancer, irritable bowel syndrome (IBS), infectious disease, etc.). Over the last few years, various different approaches have been reported to achieve CoDDS viz., pH dependent, time dependent, microflora or enzyme activated system and pressure controlled based systems (Patel, Shah, & Amin, 2007). It has been reported that the system coated with pH dependent polymer lacks site specificity for drug release in the colon, and may either lead to a premature release of drug in the small intestine or no drug release in the colon (Ashford, Fell, Attwood, Sharma, & Woodhead, 1993; Ashford, Fell, Attwood, & Woodhead, 1993; McConnell, Short, & Basit, 2008). For time dependent systems, the location of initial drug release predominantly depends on the transit times of the gastrointestinal tract (GIT). Though the transit time of small intestine is relatively constant (3–4 h) (Davis, Hardy, & Fara, 1986), a large variation in the gastric emptying time was observed which may lead to either a premature release of drug in small intestine or a delayed release far down in the colon (Lee, Wilson, & Mukherji, 2003; Matsuda et al., 1996).

A more precise and accurate strategy for targeting drugs to the colon uses the ecosystem of the specific microflora present in the large intestine i.e. microbially triggered CoDDS. Natural polysaccharides are the most promising and commonly explored carriers for CoDDS which are specifically hydrolyzed by the colonic microflora. Unfortunately, as most of the natural polysaccharides are highly hydrophilic, there is a difficulty in controlling release of drug from these materials (Ji, Xu, & Wu, 2007). Polysaccharide based matrix tablets were prepared by many researchers (Krishnaiah, Veer Raju, Dinesh, Bhaskar, & Satyanarayana, 2001; Tugcu-Demiroz, Acarturk, Takka, & Konus-Boyunaga, 2004). The main drawback of this system is the undesired premature release of the drug in the gastric and small intestinal regions, which is unfavorable when a strict rate controlling for colonic delivery is required. Compression coated tablet systems, using polysaccharide were also proposed (Krishnaiah, Styanarayana, DineshKumar, & Karthikeyan, 2002; Krishnaiah, Styanarayana, & Rama Prasad, 1998; Turkoglu & Ugurlu, 2002; Ugurlu, Turkoglu, Gurer, & Akarsu, 2007). These systems were able to protect the drug release until they reach the colon, but the major drawback associated with it was the special tableting equipment, centering of the core tablet and high amount of compression material required. Also microbial degradation of these system takes place more slowly due to the compactness of the outer coating (Krishnaiah, Styanarayana et al., 2002; Krishnaiah et al., 1998), which is disadvantageous for diseases affecting the proximal colon. Hence in order to target the drug specifically to the colon and to overcome the drawback of pH dependent and microbially triggered system, a novel approach of using combination of both pH dependent (prevent the premature

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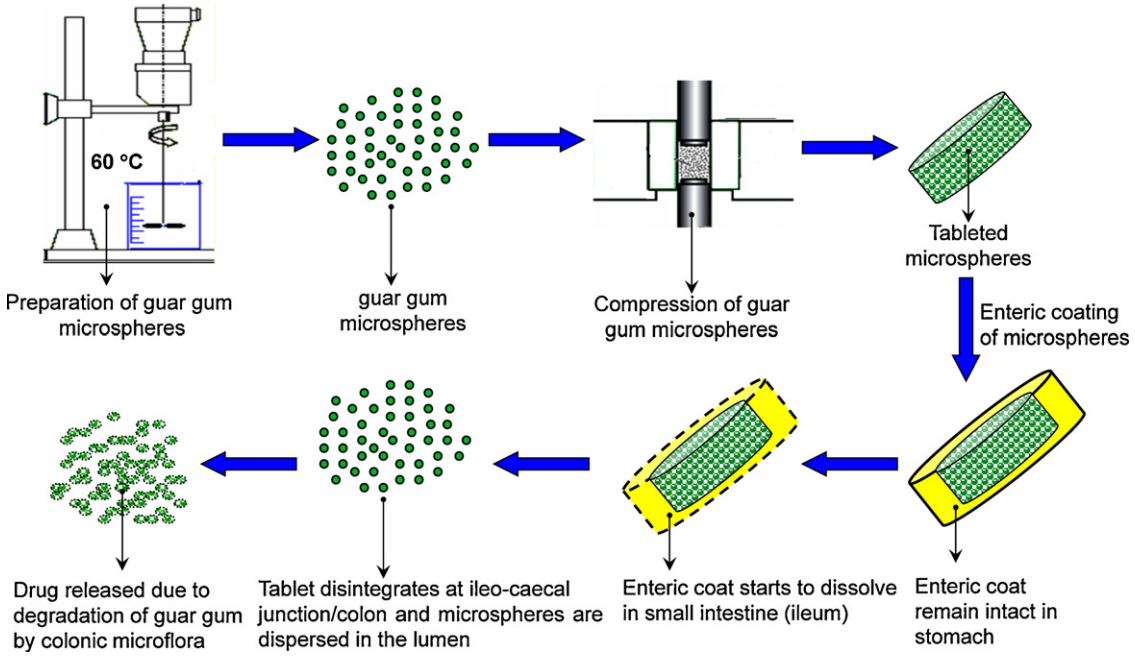

Fig. 1. Schematic diagram representing formation and performance of MBH CoDDS.

release of drug in the upper part of GIT) and microbially triggered system (ensures site specific release of drug in the colon) was employed.

IBS is one of the most commonly encountered gastrointestinal disorders, consisting of abdominal pain and an erratic bowel habit (Drossman et al., 1990). Mebeverine hydrochloride (MBH) is a smooth muscle relaxant which has long been used to treat patients with IBS (Washington et al., 1998). The aim of the present investigation was to develop a multiparticulate system for localized delivery of MBH for the treatment of IBS. Multiparticulate system was chosen since it offers slower rate of passage through the colon and its higher surface area is advantageous for microbially triggered drug release and for local treatment in colon. MBH is readily absorbed from the GIT, and its release should be protected till it reaches to the lower part of GIT. It is also reported that when MBH is delivered locally to the lumen of the lower GIT, it provides greater antispasmodic activity compared to that achieved with normal per-oral doses of MBH, which gets absorbed into the systemic circulation from the upper GIT, leading to undesirable side effects (Gary & James, 1990). Thus the objective of the present invention is to delay the release of MBH from the dosage form in the upper GIT and to achieve drug release in the colonic region.

Guar gum, a polysaccharide was used for the preparation of microspheres. Coating of multiparticulate systems i.e. microspheres with enteric polymers has been reported by many researchers at a core to coat ratio of 1:5 or 1:10 (Chourasia & Jain, 2004a; Lorenzo-Lamosa, Remunan-Lopez, Vila-Jato, & Alonso, 1998; Paharia et al., 2007). Though enteric coating can be achieved, this approach seems to be difficult to apply in scale-up, when a larger batch size is to be coated. Also during the enteric coating process of microspheres, there are chances of leaching of drug dispersed in the microspheres into the organic solvent system in which the enteric polymer is dissolved, which ultimately leads to reduction in the drug content of the microspheres (Krishnamachari, Madan, & Lin, 2007). It is also difficult to achieve reproducible results, wherever coating of microspheres is involved. Considering the advantages provided by the tablet type of dosage form (high reproducibility and industrial applicability), an attempt was made to prepare tablets from the guar gum microspheres. The enteric coat on the tablet will protect the microspheres in the upper part of GIT and thus prevent the premature release of the drug. As soon as the enteric coat dissolves, the tablet will get disintegrated and the microspheres will get dispersed into the lower part of the GIT (where the drug is released due to degradation of guar gum by colonic microflora) (Fig. 1).

2. Experimental

2.1. Materials

Mebeverine hydrochloride was obtained as a gift sample from Zydus Cadila, Ahmedabad, India. Eudragit® S100 was received as a gift sample from Evonik Röhm GmbH, Darmstadt, Germany. Guar gum, Span® 80, Tween® 80 and glutaraldehyde were purchased from Central Drug House Pvt. Ltd., New Delhi, India. Other excipients used were of standard pharmaceutical grade and all chemicals and reagents used were of analytical reagent grade and were used without any further purification.

2.2. Preparation of guar gum microspheres

The microspheres of the polysaccharide, guar gum were prepared by the emulsification method (Chourasia & Jain, 2004b). Guar gum dispersion (4%, w/v) was prepared by mixing guar gum with Tween® 80 (0.2%, w/w) using distilled water followed by the addition of drug solution (therapeutic dose of MBH is 135 mg, and 20 such doses were used during preparation). Concentrated hydrochloride acid (0.2 ml) was added and the aqueous dispersion was poured into castor oil (200 ml) containing Span® 80 (5%, w/w), kept at 60 °C. The system was kept under agitation using three blade propeller stirrer at 4000 rpm for 10 min. Glutaraldehyde (2 ml) was then added and stirring was continued for 1 h. The microspheres were collected by vacuum filtration and then washed with several fractions of petroleum ether to remove traces of oil. The final formulation was a free-flowing powder consisting of spherical micronized particles.

Various formulation and process variables that affect the preparation and properties of the microspheres were identified and optimized to get discrete, uniform and spherical microspheres.

2.3. In vitro characterization of microspheres

2.3.1. Determination of particle size, shape and surface morphology

The particle size of the cross linked guar gum microspheres was determined by optical microscopy using calibrated ocular eye-piece. The shape and surface morphology of the guar gum microspheres and tableted microspheres was characterized using scanning electron microscope (Philips, XL30 ESEM with EDAX model, Netherlands). Small amounts of microspheres were spread on double adhesive tape which was kept on a stub. The stub containing sample was then placed in the microscope chamber. The scanning electron photomicrographs were taken at the acceleration voltage of 30 kV at chamber pressure of 0.7 torr.

2.3.2. Determination of drug loading and percentage entrapment efficiency

The amount of drug loaded in the microspheres was determined as follows: The guar gum microspheres were first crushed using a glass mortar pestle. An accurately weighed amount (100 mg) of powdered microspheres was dispersed in 100 ml of pH 6.4 phosphate buffer. The resultant dispersion was exposed to ultrasonic treatment for 30 min. The ultrasonic treatment was repeated three times with a resting period of 15 min between the treatments. The sample was then shaken for 48 h at room temperature using a shaker bath. After centrifugation (2000 rpm for 15 min), the supernatant was diluted appropriately with pH 6.4 phosphate buffer and analyzed for concentration of MBH using UV absorption spectroscopy at 263 nm. The drug loading was determined for each batch in triplicate.

The drug loading and percentage entrapment efficiency (PEE) were calculated using following equation:

$$\text{drug loading} \quad (\%) = \left(\frac{\text{wt. of drug in microspheres}}{\text{wt. of microspheres}}\right) \times 100 \qquad (1)$$

$$\text{PEE} = \left(\frac{\text{drug present in the final formulation}}{\text{amount of drug added}}\right) \times 100 \qquad (2)$$

2.3.3. Differential scanning calorimetry

Differential scanning calorimetry (DSC) curves were recorded using a scanning calorimeter equipped with a thermal analysis data system (DSC 60 Schimadzu, Asia Pacific, Japan). Samples were heated in a sealed aluminium pan from 50 to 300 °C at a scanning rate of 20 °C/min, with an empty aluminium pan as reference.

2.4. Tableting of the guar gum microspheres

The optimized guar gum microspheres were compressed into tablets using microcrystalline cellulose (Avicel® PH 301) as a diluent. Polyvinyl pyrollidone (PVP) and cross linked PVP was used as binder and disintegrating agent respectively. Magnesium stearate was used as a lubricant and aerosil as flow promoter. Composition of the tableted microspheres (Batches TM1–TM3) is shown in Table 1. All the ingredients were mixed in the required amount and the tablets were compressed as per direct compression technique using Rotary tablet machine (Rimek, Ahmedabad, India), equipped with 13 mm concave punch. Average weight of tablets was 800 mg. Tablets were evaluated for thickness, weight variation, hardness, disintegration, friability and drug content.

Table 1
Composition of tableted microspheres.

	Batch TM1	Batch TM2	Batch TM3
Ingredients[a] *(%, w/w)*			
MBH guar gum Microspheres	44.37	44.37	44.37
Avicel® PH 301	51.12	48.12	46.12
Polyvinyl pyrollidone (K30)	–	3	5
Tablet evaluation parameters			
Average thickness (mm) ± SD	6.5 ± 0.03	6.6 ± 0.02	6.6 ± 0.03
Average weight (mg) ± SD	797 ± 2	803 ± 2	801 ± 4
Friability (%)	0.83	0.53	0.31
Average hardness (kg/cm^2) ± SD	3.6 ± 0.4	4.3 ± 0.4	5.8 ± 0.6
Drug content (%) ± SD	102.71 ± 0.12	98.53 ± 0.53	101.45 ± 0.42
Average disintegration	58 ± 3 s	1 min, 33 ± 5 s	2 min, 11 ± 3 s

[a] Amount of cross polyvinyl pyrollidone, magnesium stearate and aerosil in all batches were 3, 1 and 0.5% (w/w) respectively.

2.5. Enteric coating of tableted microspheres

Tablets containing guar gum microspheres (cores) were then coated with Eudragit® S100 (ES), using a pan coating equipment. Briefly, 5% (w/v) solution of ES was prepared in an organic solvent (acetone:isopropyl alcohol – 80:20). The plasticizer, triethyl citrate (20%, w/w, based on dry polymer weight) was added to the polymeric solution. An opacifier, titanium dioxide (0.5%, w/v) and an antiadherent, talc (50%, w/w, based on dry polymer weight) were also added to the coating solution to prevent adhering of tablets during the coating process.

The pan coating procedure was carried out by spraying the coating solution on a pre-warmed tablet bed (30 °C). The tablets were coated and dried using inlet air (temperature 35–40 °C) at 30 rpm. The process of coating was performed till the desired coating level was achieved. At the end of each stage of coating, the tablets were cured in the coating pan for 15 min and then in a tray drier at 40 °C for 24 h. The percentage coating level of the tablets after coating was assumed to be indicative of the coat thickness.

2.6. In vitro dissolution studies

2.6.1. In vitro drug release studies of guar gum microspheres (mimicking upper part of GIT)

The guar gum microspheres were evaluated for their integrity in the physiological environment of stomach and small intestine under conditions mimicking mouth to small intestine transit. These studies were carried out using a USP dissolution apparatus I (basket method, Electrolab, TDT–06 T, Mumbai, India) at 100 rpm, 37 °C ± 0.5 °C using 900 ml dissolution medium. Guar gum microspheres equivalent to 135 mg drug were weighed and placed in basket of dissolution apparatus. The basket was further covered with muslin cloth and immersed into the dissolution medium. The microspheres were tested for drug release at pH 1.2 for 2 h, as the average gastric emptying time is about 2 h which was then replaced with pH 6.0 dissolution medium (duodenum pH) and kept for 1 h. This medium was again replaced by pH 7.2 dissolution medium (ileum pH), and dissolution was carried out for 2 h (Ashford, Fell, Attwood, & Woodhead, 1993; Patel, Patel, Bhadani, Shah, & Amin, 2009; Patel, Shah, Amin, & Shah, 2009). Samples were withdrawn at regular time intervals, filtered using Whatman filter paper (45 μ) and were estimated using UV/VIS spectrophotometer (Shimadzu UV 2450, double beam UV/VIS Spectrophotometer, Shimadzu Corporation, Japan) at 263 nm for MBH estimation in pH 1.2 and other phosphate buffer media. The cumulative percentage release for MBH was calculated (mean ± SD, $n = 3$) over the sampling times using Beer Lambert's curve generated in the respective dissolution medium.

2.6.2. In vitro drug release of guar gum microspheres in presence of rat caecal contents (ex-vivo dissolution of guar gum microspheres using rat caecal contents)

The susceptibility of guar gum to the enzymatic action of colonic bacteria was assessed by continuing the drug release studies in 100 ml of pH 6.4 phosphate buffer containing 4% (w/v) of rat caecal contents (according to protocol no. IPS/PCEU/PhD09/003 approved by the Institutional Animal Ethics Committee at Nirma University, Wistar rats were used in this study, provided by Zydus Cadila, Ahmedabad, Gujarat, India). The caecal contents were obtained from Wistar rats (male/female) (weighing 150–200 g) after pre-treatment for 7 days with guar gum dispersion. It has been reported that the presence of 4% (w/v) rat caecal contents in pH 6.4 phosphate buffer obtained after 7 days of pre-treatment of rats with 1 ml of 2% (w/v) aqueous dispersion of guar gum provide the best conditions for *in vitro* evaluation of guar gum (Rama Prasad, Krishnaiah, & Satyanarayana, 1998). Thirty minutes before the commencement of drug release studies, rats were killed by spinal traction. The abdomen was opened, the caecai were isolated, ligated at both ends, dissected and immediately transferred into pH 6.4 phosphate buffer, previously bubbled with CO_2. The caecal bags were opened; their contents were individually weighed, pooled and then suspended in pH 6.4 phosphate buffer to give a final caecal dilution of 4% (w/v). As the caecum is naturally anaerobic, all these operations were carried out under CO_2 (Rama Prasad et al., 1998).

The drug release studies were carried out in USP dissolution rate test apparatus (apparatus I, 100 rpm, 37 °C ± 0.5 °C) with slight modification. A beaker (capacity 250 ml) containing 100 ml of dissolution medium (pH 6.4 phosphate buffer) was immersed in the water contained in the 1000 ml vessel, which was, in turn kept in the water bath of the apparatus. The microspheres were placed in the baskets of the apparatus and immersed in the dissolution medium containing rat caecal contents. The experiment was carried out with continuous CO_2 supply into the beakers to simulate anaerobic environment of the caecum. The drug release studies were carried out for 12 h and 1 ml samples were taken at different time intervals without a pre-filter and replaced with 1 ml of fresh pH 6.4 phosphate buffer bubbled with CO_2. The volume was made up to 10 ml with pH 6.4 phosphate buffer, centrifuged and the supernatant was filtered through a bacteria-proof (0.22 μm) filter and the filtrate was analyzed for MBH content at 263 nm as described above. The above study was also carried out without caecal matter in pH 6.4 phosphate buffer (control) (Rama Prasad et al., 1998).

2.6.3. In vitro dissolution of uncoated and coated tableted microspheres

In vitro dissolution of uncoated and coated tableted microspheres was carried out by same procedure as that of guar gum microspheres.

2.7. In vivo roentgenographic study

An *in vivo* roentgenography study was performed in order to provide a *proof of concept* for the developed CoDDS. According to protocol no. IPS/PCEU/PhD09/004 approved by the Institutional Animal Ethics Committee at Nirma University, three New Zealand white rabbits, weighing 3–3.5 kg, were used in this study. For roentgenographic evaluation, the guar gum microspheres were prepared by partly replacing the drug MBH with radio-opaque compound barium sulphate. These microspheres were used for the preparation of tablets. The coating of tablets was carried out similarly to that of the optimized batch. Prior to administration of tablets to the rabbits for *in vivo* roentgenography studies the tablets were subjected to *in vitro* dissolution studies, to determine the intactness of the coat. Tablets were examined visually for the intactness of the coat at regular time intervals. It was observed that the coat remained intact for nearly 5 h. All rabbits were placed on overnight fasting with free access to water under 12 h light/dark cycles. After an over night fasting, tablets were administered to rabbits with 10 ml of water. X-ray images (of abdomen) were periodically taken to trace the movement and behavior of the tableted microspheres in the GIT. In order to know the actual position of the tablet in the GIT of the rabbits, a barium meal study was conducted by administering 10 ml of standard barium meal to the rabbit. *In vivo* roentgenography study was performed at Mangalam Digital X-ray, Colour Doppler and Sonography Clinic, Ahmedabad, India. X-ray images were captured using Siemens X-ray machine, with 64 MAS and 63 KV techniques. The X-ray imaging was carried out by placing the rabbits in prone position.

2.8. Stability studies

Stability studies for optimized batch was carried out by storing at 40 °C ± 2 °C/75% ± 5% RH for 6 months at a testing frequency of 0, 3 and 6 months (Mathews, 1999; Krishnaiah, Reddy et al., 2002; The international conference on harmonisation of technical requirements for registration of pharmaceuticals for human use.(2011). Available from http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html. Accessed 15.08.10). At the end of the study period, the tablets were observed for change in physical appearance, color and drug content. The tablets were also subjected to *in vitro* dissolution studies. The dissolution profiles of the optimized batch, subjected to the stability testing were compared by calculating the similarity factor (f_2).

3. Results and discussion

Guar gum microspheres were prepared by the emulsification method using castor oil as an external phase. Hardening of microspheres was performed by chemical cross linking using glutaraldehyde as well as by using temperature induced cross linking. The process was carried at 60 °C temperature as reported by Chourasia and Jain (2004b). The acidic medium required for the process of cross liking was imparted by using concentrated hydrochloric acid. Tween® 80 was used for the purpose of wetting and uniform distribution of guar gum.

3.1. Optimization of guar gum concentration

A series of guar gum concentrations (0.5, 1, 2, 3, 4, 5%, w/v, Batches GG1–GG6 respectively) was employed for preparation of blank microspheres. The results revealed that at lower concentrations of guar gum, microspheres obtained possessed poor sphericity and aggregation. This might be due to the presence of higher amount of water in less concentrated solution, which evaporates slowly, causing the particles to come in contact with each other (Chourasia & Jain, 2004b). It was observed that a 4% (w/v) solution was necessary to obtain spherical microspheres. Solution with concentration higher than 4% (w/v) was too viscous to handle and hence could not be used in the preparation of microspheres.

3.2. Effect of various formulation parameters on particle size, percentage entrapment efficiency and in vitro drug release

3.2.1. Effect of drug to polymer ratio

Different drug to polymer ratios (1:1, 1:2 and 1:3, Batches DP1, DP2 and DP3 respectively) were tried for the preparation of guar gum microspheres. The PEE of the microspheres increases from 75.50 ± 2.1% to 87.40 ± 1.1% with increase in the drug to polymer ratio from 1:1 to 1:3 respectively (Table 2). This increase in the

Table 2
Effect of formulation variables on microspheres formation.

Formulation variables	Value	Yield (%)	Drug loading (%)	PEE	Particle size (μm)	Microspheres formation[a]
Drug to polymer ratio	1:1	77.03 ± 1.7	49.03 ± 1.9	75.50 ± 2.1	82.53 ± 1.6	Yes (+++)
	1:2	75.06 ± 1.1	37.50 ± 1.6	84.40 ± 1.7	84.64 ± 2.1	Yes (+++)
	1:3	77.77 ± 1.4	32.09 ± 2.3	87.40 ± 1.1	86.75 ± 2.5	Yes (+++)
Concentration of Span® 80 (% w/w)	1	–	–	–	–	No
	2	–	–	–	–	No
	3	73.21 ± 1.3	31.74 ± 2.1	69.7 ± 1.1	112.5 ± 1.4	Yes (+)
	4	75.03 ± 1.2	32.96 ± 1.5	74.2 ± 1.3	97.4 ± 1.3	Yes (++)
	5	76.06 ± 1.8	36.64 ± 1.8	83.6 ± 1.5	84.84 ± 1.1	Yes (+++)
	6	78.77 ± 1.3	36.82 ± 1.9	87.0 ± 2.5	71.70 ± 1.7	Yes (+++)
Amount of glutaraldehyde (ml)	0.5	77.32 ± 2.5	33.29 ± 1.1	77.21 ± 1.4	83.31 ± 2.6	Yes (+++)
	1	74.12 ± 1.7	36.21 ± 1.5	80.51 ± 2.1	83.63 ± 1.4	Yes (+++)
	2	76.01 ± 1.5	37.54 ± 1.7	85.61 ± 1.5	81.23 ± 2.1	Yes (+++)
	3	78.01 ± 1.6	37.22 ± 2.1	87.11 ± 1.2	84.51 ± 1.3	Yes (+++)
Rotational speed (per minute)	2000	–	–	–	–	No
	3000	76.12 ± 1.4	35.03 ± 1.6	80.00 ± 1.5	106.8 ± 1.3	Yes (++)
	4000	78.41 ± 1.1	36.13 ± 1.4	85.00 ± 1.3	81.70 ± 2.1	Yes (+++)
	5000	75.32 ± 1.5	34.52 ± 1.8	78.00 ± 1.1	56.57 ± 1.5	Yes (broken/fractured) (++)
Cross linking time (h)	1	75.53 ± 1.3	37.16 ± 2.1	84.20 ± 1.5	84.33 ± 1.4	Yes (+++)
	2	74.06 ± 1.7	37.18 ± 1.7	82.60 ± 1.9	83.61 ± 2.2	Yes (+++)
	3	76.77 ± 2.1	37.12 ± 1.9	85.50 ± 2.1	83.55 ± 2.4	Yes (+++)

[a] (+) slightly spherical, (++) spherical, (+++) completely spherical, values shown are mean ± SD ($n = 3$). Underlined figures indicate optimized values.

PEE may be attributed to the fact that with increase in the drug to polymer ratio more amount of polymer is available to encapsulate the same amount of drug present in the system, which means more denser network is formed which further increases the diffusion path length for the drug to escape in the external phase. With increase in drug to polymer ratio from 1:2 to 1:3, no significant increment ($p > 0.05$) in PEE was observed (The PEE was statistically analyzed using their mean ± SD and performing unpaired t-test (SPSS version 16), a p-value < 0.05 was considered as significant).

Fig. 2(a) depicts that the drug to polymer ratio had a significant effect on the *in vitro* drug release characteristics of the microspheres. Batches DP1, DP2 and DP3 showed 37.87 ± 1.85, 31.40 ± 1.32 and 26.37 ± 2.13% of drug release respectively at the end of 5th hour (which is the expected time for the arrival of dosage form in the colon). The decrease in the drug release with increase in amount of polymer was attributed to the swelling properties of guar gum. As the amount of the swellable polymer increases in the formulation, the thickness of gel layer formed surrounding the

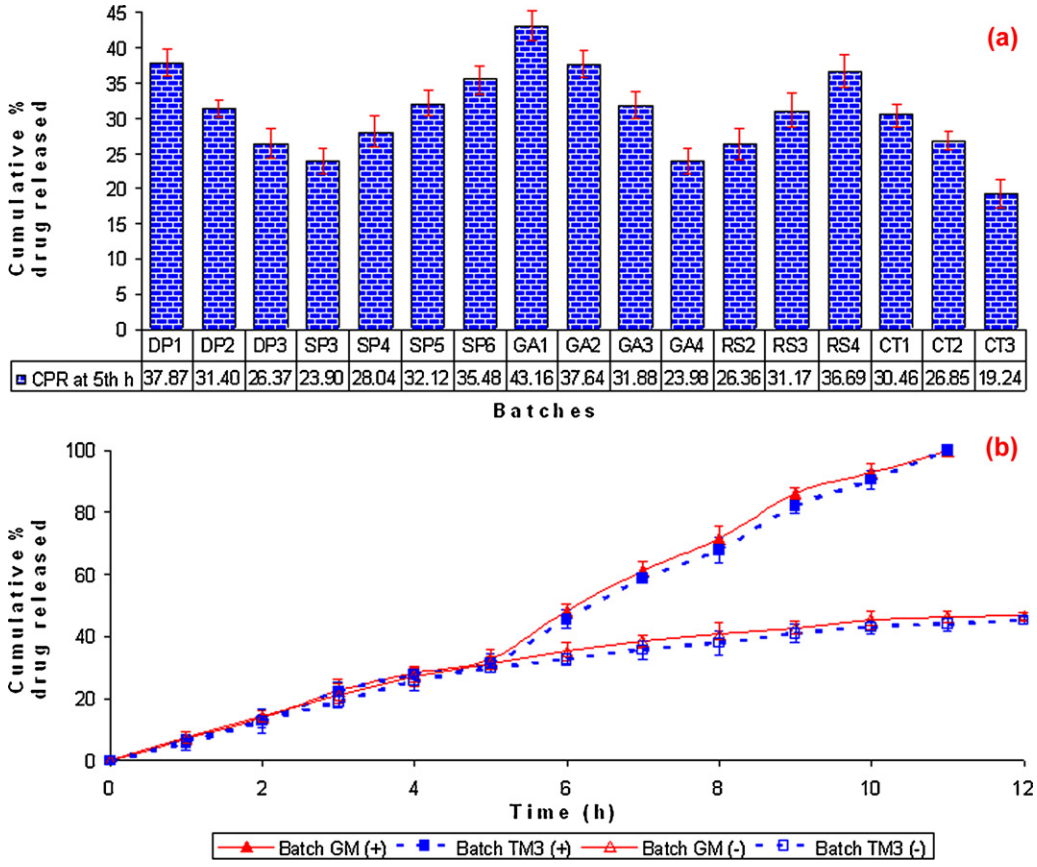

Fig. 2. (a) Cumulative percentage drug released from guar gum microspheres at the end of 5th hour (in absence of rat caecal content). (b) Dissolution profile of Batch GM and Batch TM3 in absence (–) and presence (+) of rat caecal contents.

microspheres increases. This increases the diffusion path length for penetration of intestinal fluid and hence decreases the drug release from the microspheres.

3.2.2. Effect of emulsifier (Span® 80) concentration

Span® 80 was used as an emulsifier for the preparation of W/O emulsion of guar gum solution and castor oil. Increasing the emulsifier concentration from 1 to 6% (w/w) exhibited a reversal in trend between particle size and PEE of MBH loaded guar gum microspheres (Table 2). Batches SP1 and SP2 prepared with 1 and 2% Span® 80 could not produce any microspheres, while those prepared with 3–6% of Span® 80 (Batches SP3–SP6) produced microspheres with increasing sphericity. Microspheres prepared with 3% Span® 80 had the largest particle size and the lowest PEE while those prepared with 6% Span® 80 showed the lowest particle size and the highest PEE. The decrease in the particle size and increase in the PEE at increasing emulsifier concentrations could be attributed to the decreased emulsion droplet size during the formation of microspheres (Castellanos, Carrasquillo, de Jesus Lopez, Alvarez, & Griebenow, 2001; Krishnamachari et al., 2007). When added in small concentrations, the emulsifier may not have been able to cover the entire aqueous droplet surface. Thus, some of the droplets would tend to fuse and produce larger globules because of insufficient lowering in interfacial tension. This would also explain the formation of smaller droplet size at the higher emulsifier concentrations resulting in a greater surface area for rapid solvent evaporation and rapid hardening of the microspheres, and further decreased drug diffusion in the external phase. As a result, the higher PEE was obtained at higher emulsifier concentrations (Krishnamachari et al., 2007). Also due to small particle size, the surface area available for cross linking with glutaraldehyde increases, as a result of which rapid cross linking of the particles takes place which further leads to decrease in the diffusion of the drug into the external phase and thus increases the PEE.

In vitro evaluation also revealed that with increase in concentration of Span® 80, the drug release increases. Batch SP3 prepared at 3% concentration showed 23.90 ± 1.78% drug release, while Batch SP6 prepared at 6% concentration showed 35.48 ± 2.01% of drug release at the end of 5th hour (Fig. 2(a)). This increase in drug release can be attributed to the decrease in the particle size at higher emulsifier concentrations, as a result of which the surface area of the guar gum microspheres exposed to the dissolution medium increases, which ultimately leads to increase in drug release.

3.2.3. Effect of amount of cross linking agent

The amount of cross linking agent glutaraldehyde was varied from 0.5 to 3 ml in Batches GA1 to GA4 respectively (Table 2). It was observed that the PEE increases with increase in the amount of cross linking agent. Batch GA1 prepared with 0.5 ml of glutaraldehyde had PEE of 77.21 ± 1.4%, while Batch GA4 prepared with 3 ml of glutaraldehyde showed 87.11 ± 1.2% PEE. With increasing the amount of glutaraldehyde from 2 to 3 ml, no significant improvement in the PEE observed ($p > 0.05$). Amount of glutaraldehyde had a significant effect on the dissolution characteristics of the microspheres. It was observed that with increase in the amount of the glutaraldehyde, the drug release from the microspheres was prolonged. Batch GA1 prepared with 0.5 ml of glutaraldehyde showed 43.16 ± 2.11%, while Batch GA4 prepared with 3 ml of glutaraldehyde showed 23.98 ± 1.79% of drug release at the end of 5th hour (Fig. 2(a)).

The increase in PEE and the decrease in drug release could be attributed to the fact that with increase in the amount of glutaraldehyde the cross linking density increases. As a result of which drug leaching into the external phase during microspheres formation decreases and hence the PEE increases. Glutaraldehyde causes cross linking by reacting with the hydroxyl group of galactose and the mannose unit in guar gum, thus interfering with the free access of water to the hydroxyl group of guar gum (Chaurasia et al., 2006; Soppirnath & Aminabhavi, 2002). This significantly reduces the hydration rate of the microspheres and consequently the penetration of the solvent into the microspheres. As a result of which the drug release was reduced with increasing the amount of glutaraldehyde.

3.2.4. Effect of rotational speed

The mean particle size of the microspheres decreased from 106.8 ± 1.3 to 56.57 ± 1.5 μm with increase in the rotational speed from 3000 rpm to 5000 rpm (Table 2). This may be attributed to the fact that with increase in the rotational speed at a fixed value of other influencing factors, the droplet size of the emulsion decreases due to high shear force, hence the size of the microspheres decreases. It was also observed that at the rotational speed ≤ 2000 rpm, no microspheres formation occur, this may be attributed to the poor dispersion of the guar gum solution in the external phase due to low shear force. The PEE also significantly varies by changing the rotational speed. Batch RS2 prepared at 3000 rpm had PEE of 80.00 ± 1.5% while Batch RS3 prepared at 4000 rpm showed significant increment ($p < 0.05$) in PEE (85.00 ± 1.3%). This increase in PEE might be due to decrease in the size of the droplets, which leads to increase in the surface area of the droplets as a result of which rapid evaporation of the solvent and cross linking of the microspheres takes place which further decreases the diffusion of drug into the external phase (Krishnamachari et al., 2007; Paharia et al., 2007). But this was not the case with the Batch RS4 prepared at 5000 rpm at which the PEE was found to decrease significantly ($p < 0.05$) to 78.00 ± 1.1% compared to Batch RS3. The decrease in PEE may be attributed to the influence of very high shear force of the blade which results in either breakdown of microspheres or formation of microspheres with fractured surface, these further results in diffusion of the drug in the external phase and thus decrease in the PEE (Chourasia & Jain, 2004b).

The effect of rotational speed on *in vitro* drug release is shown in Fig. 2(a). It was observed that with increase in rotational speed the drug release increases. Batch RS2 prepared at 3000 rpm showed 26.36 ± 2.16% while Batch RS4 prepared at 5000 rpm showed 36.69 ± 2.24% of drug release at the end of 5th hour. This increase in drug release can be attributed to the decrease in the particle size and formation of fractured microspheres at 5000 rpm, as a result of which the surface area of the guar gum microspheres exposed to the dissolution medium increases, which ultimately leads to increase in drug release.

3.2.5. Effect of cross linking time

It was observed that the cross linking time do not have any significant ($p > 0.05$) effect on the PEE (Table 2). The *in vitro* drug release studies revealed that with increase in the cross linking time from 1 h to 3 h (Batches CT1–CT3), the amount of drug released at the end of 5th hour decreases from 30.46 ± 1.64% to 19.24 ± 1.93% (Fig. 2(a)) respectively. This might be because increasing the cross linking time will lead to increase in the rigidity of the cross linked microspheres, which further decreases the penetration of the fluid into the microspheres, ultimately leading to decrease in the drug release.

Ex-vivo dissolution study of optimized batch of guar gum microspheres (Batch GM) revealed that the cumulative drug release was significantly higher in presence of caecal content than in the control system (Fig. 2(b)). The amount of drug released at the end of 12th hour from the guar gum microspheres without rat caecal content was 46.64 ± 2.53% while a complete drug release was observed in presence of rat caecal content. This could have been due to the enzymes present in the caecum (secreted by various anaerobic bac-

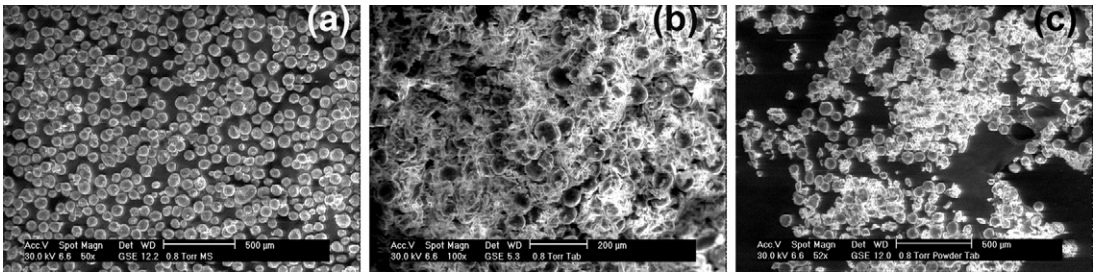

Fig. 3. Scanning electron photomicrographs of (a) cross linked guar gum microspheres, (b) tableted microspheres, and (c) dispersed tableted microspheres.

teria), which are responsible for the digestion/degradation of guar gum and which lead to release of drug from the microspheres. The similarity factor (f_2) was found to be 26.514, revealing that the *in vitro* dissolution profiles in absence and presence of rat caecal contents was not similar ($f_2 < 50$).

Shape and surface morphology were investigated using scanning electron microscopy. Fig. 3(a) indicates that the cross-linked guar gum microspheres possessed a nearly smooth surface and spherical shape.

DSC analysis depicts the change in thermal behaviors as a result of interactions during microspheres preparation (Jose, Prema, Chacko, Thomas, & Souto, 2011; Xu, Bovet, & Zhao, 2008). Hence, in order to determine the stability of a drug molecule, and possible interaction between the polymer and the cross linking agent, DCS analysis was performed (Fig. 4). Using DSC, transition temperature of guar gum (a) and MBH (b) were observed at 111.52 °C and 137.25 °C respectively. Both the peaks were also distinctly visible in their physical mixture (c). The peak of the blank guar gum microspheres (d) was changed compared to the pure guar gum (a), indicating cross linking of the guar gum microspheres by the glutaraldehyde. Also, the peak for pure drug was not found in the drug loaded microspheres (e), it could be attributed to the fact that the incorporated drug was embedded in a molecular dispersed form inside the cross-linked particle matrix (Lamprecht, Yamamoto, Takeuchi, & Kawashima, 2004).

3.3. Preparation of tableted microspheres

The *in vitro* evaluation of the guar gum microspheres revealed that, guar gum when used alone was not found suitable for achieving colonic targeting; these findings are in good agreement with the literature which revealed that an enteric coat would be required to prevent the premature release of drug in the upper part of the GIT (Ji et al., 2007; Ji, Xu, & Wu, 2009). Now, as discussed earlier enteric coating of microspheres is difficult if large amount of microspheres are to be coated. Also, it was always difficult to achieve reproducible results, wherever coating of microspheres is involved. Taking this into consideration, an attempt was made to prepare tablets from the guar gum microspheres, since it could be easily manufactured with high reproducibility and could be easily coated with enteric polymers compared to the microspheres. The enteric coat will protect the microspheres in upper part of GIT and thus prevent the premature release of the drug. As soon as the enteric coat will dissolve, the tablet containing microspheres will get disintegrated, thus dispersing the microspheres into the lower part of the GIT, where the drug will get released due to digestion/degradation of guar gum by colonic microflora.

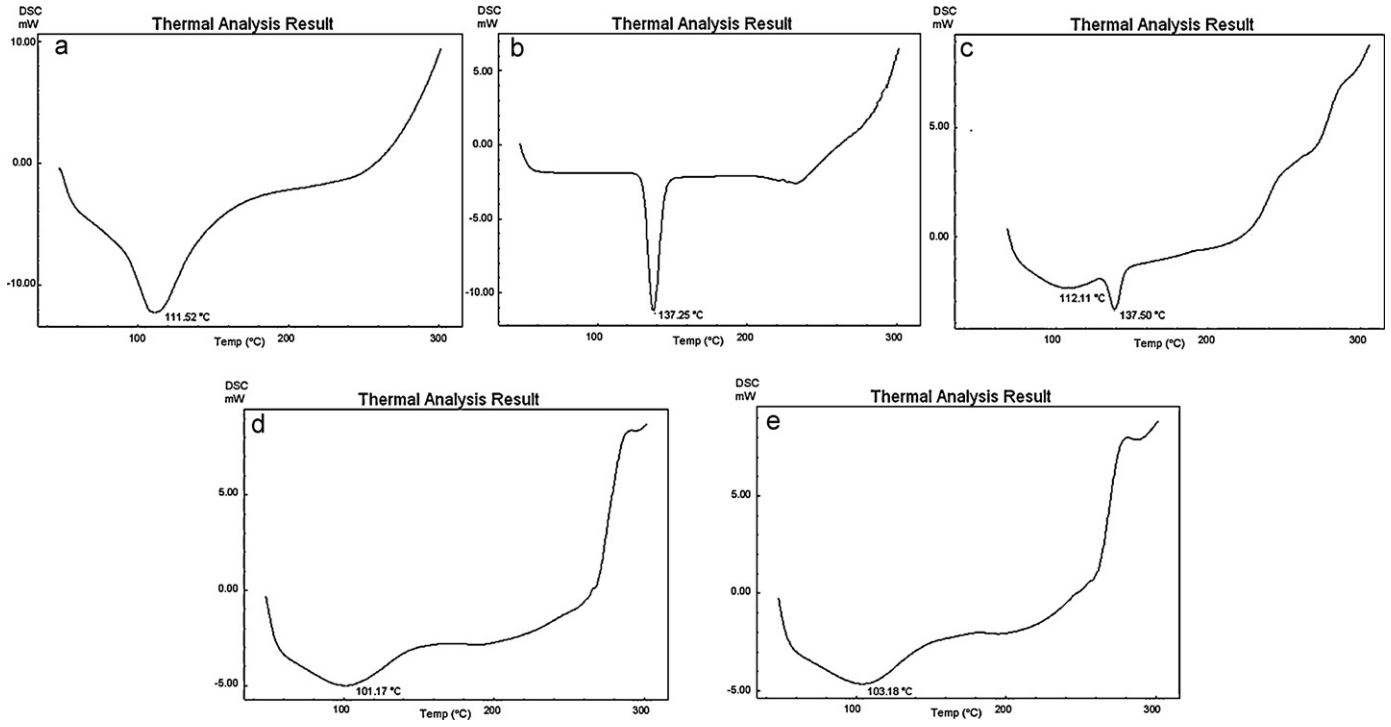

Fig. 4. DSC of (a) pure guar gum powder, (b) pure drug, (c) physical mixture of guar gum and drug, (d) cross linked blank guar gum microspheres, and (e) cross linked drug loaded guar gum microspheres.

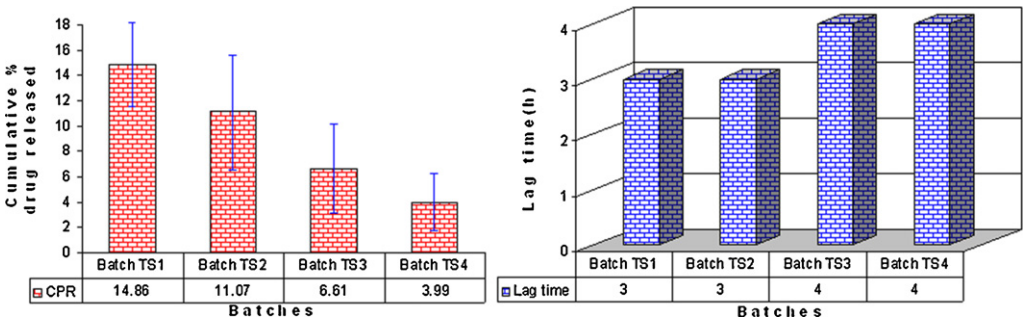

Fig. 5. Dissolution profile and lag time profile of Batches TS1–TS4.

Guar gum microspheres were tableted using Avicel® PH301 as a directly compressible diluent. The tablets were subjected to various evaluation parameters, such as thickness, weight variation, hardness, disintegration, friability and drug content. The results of these tests are given in Table 1. Batch TM1 prepared without PVP showed lower average hardness ($3.6 \pm 0.4\,kg/cm^2$) and disintegration (58 ± 3 s) values with higher % friability value (0.83%). Hence in order to impart sufficient hardness required for the preparation of coated tablets, Batches TM2 and TM3 were prepared containing 3 and 5% (w/w) PVP (by keeping the position of the hardness adjusting knob fix at a particular point, in order to maintain constant force of compression for all the batches). The hardness of Batches TM2 and TM3 was found to be 4.3 ± 0.4 and $5.8 \pm 0.6\,kg/cm^2$ respectively. With the increase in hardness, the disintegration time increases and the % friability decreases. As Batch TM3 showed hardness sufficient for maintaining tablet integrity during the coating process, this batch was used for further optimization of CoDDS.

Avicel® was used as a diluent since it has been reported to have a superior protective effect against the damage caused by the compression force and this is believed due to its excellent plastic deformability (Hasegawa, Nakagawa, & Sugimoto, 1984). Avicel® PH301 grade was selected, taking into consideration the density and particle size of the guar gum microspheres in order to avoid segregation. The bulk density of the Avicel® and guar gum microspheres was found to be 0.43 and 0.52 g/ml respectively. The particle size of the Avicel® PH301 (50 μm) was less than that of the guar gum microspheres (84.84 ± 2.1 μm). It has been reported that the small size particles would provide more protective effect than that of the larger ones (Yoa, Yamada, Yamahara, & Yoshida, 1998). The additives with smaller particle size would deform plastically without fragmentation. So, (i) when the microspheres are blended with the micronized additive (Avicel®), each microsphere would be covered by its small sized to form a sort of ordered mixture, and thus, a direct contact between the microspheres would be effectively prevented during the compression process, (ii) by mixing the microspheres with small additive particles, the mixture would tend to deform plastically, which might result in reducing the damage to the microspheres during compression, (iii) additive could also act as a cushion (hence microspheres and Avicel® are used at a ratio of 50:50) and the cushioning effect might increase with decrease in additive particle size (hence Avicel® with 50 μm particle size was used) (Torrado & Augsburger, 2008; Vladyka, Erkoboni, Sweriduk, & Christopher, 2005; Wallace, 1989; Yoa et al., 1998; Yuasa, Kanaya, & Omata, 1990). In order to re-disperse the microspheres from the tablets as soon as the enteric coat is dissolved, cross PVP was used to facilitate quick disintegration.

3.3.1. *In vitro evaluation of tableted microspheres*

The dissolution profile of optimized guar gum microspheres (Batch GM) and tableted microspheres (Batch TM3) was found to be very much similar (Fig. 2(b)), with an f_2 value of 80.82 (in absence of rat caecal content) and 79.84 (in presence of rat caecal content). The fact of similarity between the dissolution profiles of guar gum microspheres and tableted microspheres demonstrated that the tableted microspheres remained intact after the compression of the guar gum microspheres, because if the microspheres would have been broken, the dissolution values would have increases with a decrease in f_2 value (Xu et al., 2008). Fig. 3(b) and (c) shows scanning electron photomicrographs of the tableted microspheres and the dispersed tableted microspheres respectively, which reveal that the guar gum microspheres remained intact even after the compression. The amount of drug released at the end of 5th hour from tableted microspheres was found to be 31.32 ± 2.39%, depicting a strict control in drug release for colonic targeting. Hence enteric coating of tableted microspheres was performed.

3.3.2. *Enteric coating of tableted microspheres*

Tableted microspheres were enteric coated with ES using a pan coating technique. Approximately 5, 10, 15 and 20% (w/w) (Batches TS1–TS4) of coating was applied to study the effect of concentration of ES on drug release in the gastric fluid. Drug content of enteric coated tableted microspheres (99.01 ± 0.52%) was found to be similar to that of uncoated tableted microspheres, which revealed that there was no loss of drug during the coating process of tableted microspheres, unlike observed in case of enteric coating of microspheres by Krishnamachari et al. (2007).

In vitro dissolution of Batches TS1–TS4 was carried as discussed earlier which reveals that a 5% (w/w) of coating level was required to impart an enteric effect. At 5% (w/w) coating level the percentage of drug released at the end of 5th hour was found to be 14.86 ± 3.36% (Fig. 5). Increasing the coating thickness to 10, 15 and 20% (w/w) (Batches TS2, T3 and TS4 respectively) reduced the drug release to 11.07 ± 4.56, 6.61 ± 3.51 and 3.99 ± 2.32% respectively after a period of 5 h. Further, it was observed that the dissolution rate was inversely proportional to the thickness of the coat applied. These results are in good agreement with the results of the authors reported in previous studies that an increase in the coat thickness of enteric polymer, exhibited a decrease in the dissolution rate of mesalamine. This can be explained by the fact that increasing the coat concentration makes the coat more impermeable and drug release is retarded. Slowly as the coating solubilizes, drug dissolution is facilitated (Patel, Patel et al., 2009; Patel, Shah et al., 2009).

The lag time profile of Batches TS1–TS4 revealed that increasing the coating level of ES increases the lag time for drug release (Fig. 5). It was observed that the lag time for drug release at 5 and 10% (w/w) coating level was found to be 3 h, which increases to 4 h at 15 and 20% (w/w) coating level. It was observed that the lag time was same at 5 and 10% (w/w) coating level, and at 15 and 20% (w/w) coating level, the only difference was the % drug released from the respective batches. As Batch TS4 showed a maximum lag time (4 h)

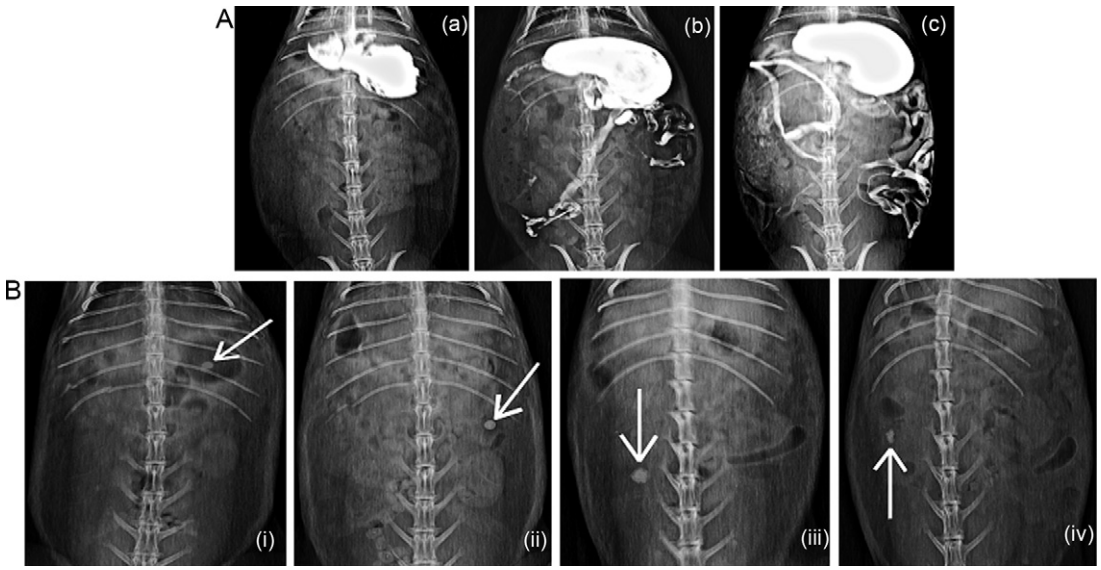

Fig. 6. Roentgenography study of CoDDS in rabbits. (A) Barium meal study of rabbit GIT depicting, (a) stomach (b) small intestine and (c) entire GIT along with colon. (B) Gastrointestinal transit of the developed tableted microspheres coated with ES in rabbits, (i) 1st hour, intact tablet in stomach, (ii) 4th hour, tablet is intact in the small intestine, (iii) 6th hour, microspheres were dispersed in the colon due to tablet disintegration, and (iv) 8th hour, small amount of microspheres were detected in the colon.

with a minimum drug release at the end of 5th hour ($3.99 \pm 2.32\%$), this batch was considered as optimum for targeting a drug molecule to the colon.

3.4. In vivo evaluation

The performance of the CoDDS to deliver MBH was validated by conducting *in vivo* studies. Rabbits were selected as an animal model, as the change in GIT pH of rabbits is similar to that of humans (pH of stomach, small intestine and colon is reported to be 1.5–2.0, 7.2 and 6.5 respectively) (Gidenne & Lebs, 2006). Also the mean colonic arrival time in rabbits (total time: 4–6 h, stomach transit time: 2–4 h, small intestine transit time: about 2 h) is almost similar to that of humans which facilitates the *in vivo* evaluation of CoDDS (Gidenne & Lebs, 2006). As it is difficult to administer the similar size tablet for human use to the rabbits for colon targeting, a smaller size tablet (6 mm) with a corresponding dosage reduction was administered to rabbits (Ghosh, 2008, chap. 24; Patel & Amin, 2011a, 2011b).

For roentgenographic evaluation, the guar gum microspheres were prepared by partly replacing the drug MBH with radio-opaque compound barium sulphate. These microspheres were used for the preparation of tablets. The coating of tablets was carried out similarly to that of the optimized batch. The formulation was first subjected to *in vitro* evaluation. The results of *in vitro* dissolution were in accordance to that of optimized coated tableted microspheres. The result of roentgenographic study is shown in Fig. 6, and the actual positions of the tablet in the GIT of the rabbit could be ascertained by comparing it with the barium meal data (Fig. 6A(a)–(c)). Fig. 6B(i)–(iv) revealed that the tableted microspheres coated with ES remain intact in stomach and small intestine. The tablet gets disintegrated and the microspheres get dispersed from the tablet in the colon, revealing that the formulated system could target the drug specifically to the colon. The time required to reach colon was about 5 h.

The *in vivo* test demonstrated that the CoDDS could deliver the drug to the colon successfully and highlighted the potential of this system for colonic drug delivery. Thus, we can conclude that the coated tablet remained intact in the upper part of the GIT, that is, stomach and small intestine, and reaches the colon after a lag time of about 5 h.

3.5. Stability studies

In view of the potential utility of optimized formulations for targeting MBH to colon, stability studies were carried out at $40\,^{\circ}C \pm 2\,^{\circ}C/75\% \pm 5\%$ RH for 6 months (accelerated testing) to assess their long term stability (Krishnaiah, Reddy et al., 2002; Mathews, 1999; The international conference on harmonisation of technical requirements for registration of pharmaceuticals for human use.(2011). Available from http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html. Accessed 15.08.10). When the optimized formulations were stored at $40\,^{\circ}C \pm 2\,^{\circ}C/75\% \pm 5\%$ RH for 6 months there appeared no change either in physical appearance, color or in drug content ($n = 6$, mean $\pm$ SD) (3 months: $99.75 \pm 0.62\%$ and 6 months: $100.73 \pm 0.56\%$). The dissolution study ($n = 12$) conducted in the simulated physiological environment of stomach, small intestine and colon demonstrated no significant difference (similarity factor f_2 was found to be 83.64 and 82.70 after storing for 3 months and 6 months respectively which is >50) in the cumulative percent of MBH released from stored tablets, when compared to the tablets of same batch before storage. The insignificant change either in the physical appearance, color, drug content or in dissolution profile of optimized formulations after storage indicated that developed formulation is stable and could provide a good shelf life.

4. Conclusions

The results of this investigation indicated that emulsification method can be successfully employed to fabricate MBH loaded guar gum microspheres. The entrapment efficiency and the *in vitro* drug release were found to be greatly influenced by the formulation variables such as drug to polymer ratio, concentration of emulsifier, amount of glutaraldehyde, rotational speed and cross linking time. Furthermore, it was observed that polysaccharide alone cannot be used for targeted delivery to lower part of GIT. This can be achieved by enteric coating of microspheres with ES. Since enteric coating was found to be difficult with microspheres, tablets were prepared successfully from them without affecting the integrity of the microspheres. The optimized enteric coated tablet formulation was found to prevent the drug release from the core tablets in acidic as well as

at the intestinal pH conditions. The *in vivo* results revealed that the optimized batch released its content in the colonic region, revealing the potential of developed system for colonic targeting.

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