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Design and evaluation of colon specific drug delivery system containing flurbiprofen microsponges

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Abstract

The purpose of this study was to design novel colon specific drug delivery system containing flurbiprofen (FLB) microsponges. Microsponges containing FLB and Eudragit RS 100 were prepared by quasi-emulsion solvent diffusion method. Additionally, FLB was entrapped into a commercial Microsponge® 5640 system using entrapment method. Afterwards, the effects of drug:polymer ratio, inner phase solvent amount, stirring time and speed and stirrer type on the physical characteristics of microsponges were investigated. The thermal behaviour, surface morphology, particle size and pore structure of microsponges were examined. The colon specific formulations were prepared by compression coating and also pore plugging of microsponges with pectin:hydroxypropylmethyl cellulose (HPMC) mixture followed by tabletting. In vitro dissolution studies were done on all formulations and the results were kinetically and statistically evaluated. The microsponges were spherical in shape, between 30.7 and 94.5 μm in diameter and showed high porosity values (61–72%). The pore shapes of microsponges prepared by quasi-emulsion solvent diffusion method and entrapment method were found as spherical and cylindrical holes, respectively. Mechanically strong tablets prepared for colon specific drug delivery were obtained owing to the plastic deformation of sponge-like structure of microsponges. In vitro studies exhibited that compression coated colon specific tablet formulations started to release the drug at the 8th hour corresponding to the proximal colon arrival time due to the addition of enzyme, following a modified release pattern while the drug release from the colon specific formulations prepared by pore plugging the microsponges showed an increase at the 8th hour which was the time point that the enzyme addition made. This study presents a new approach based on microsponges for colon specific drug delivery.

Keywords: Microsponge; Flurbiprofen; Pore structure; Quasi-emulsion solvent diffusion method; Colon specific drug delivery

1. Introduction

Colon, as a site, offers distinct advantages on account of a near neutral pH, a much longer transit time, reduced digestive enzymatic activity and a much greater responsiveness to absorption enhancers (Edwards, 1993; Sinha and Kumria, 2003). Colon specific drug delivery systems have been the focus of increasing interest due to the importance of this region of the gastrointestinal tract, not only for local but also for systemic therapy. Additionally, colonic delivery of drugs may be extremely useful when a delay in drug absorption is required from a therapeutic point of view, e.g. in case of diurnal asthma, angina pectoris and arthritis (Rubinstein, 1995; Kinget et al., 1998). Conventional oral dosage forms are ineffective in delivering drugs to the colon due to absorption and/or degradation of the active ingredient in the upper gastrointestinal tract (Mrsny, 1992). Several triggering mechanisms utilizing the gastrointestinal transit time of various formulations and the change in pH, bacterial concentration and pressure in the gastrointestinal tract have been reported to achieve colon specific drug delivery (Chavan-Patil and Mishra, 1999; Leopold, 2001). In general, four primary approaches have been proposed for colon specific drug delivery, namely prodrugs (McLeod et al., 1993; Pellicciari et al., 1993; Uekama et al., 1997; Minami et al., 1998; Davaran et al., 1999; Jung et al., 2000; Wiwattanapatapee et al., 2003), pH-dependent systems (Rao and Ritschel, 1992; Ashford et al., 1993; Ishibashi et al., 1998; Rodriguez et al., 1998; Khan et al., 1999; Lamprecht et al., 2004), time-dependent systems (Gazzaniga et al., 1994; Pozzi et al., 1994; Steed et al., 1997; Murata et al., 1998; Fukui et al., 2000; Sangalli et al., 2001; Yang et al., 2003a) and microflora-

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activated systems (Ashford et al., 1994; Lorenzo-Lamosa et al., 1998; Krishnaiah et al., 2002; Siew et al., 2004; Sinha et al., 2004). Every system has advantage as well as shortcoming. Prodrugs, as being considered as a new chemical entity from regulatory perspective, the similarity in pH between the small intestine and the colon and also the highly variable retention times make the mentioned strategies less reliable (Yang et al., 2002). However, microflora-activated systems formulated making use of non-starch polysaccharides are highly promising because the polysaccharides remain undigested in the stomach and the small intestine and can only be degraded by the vast anaerobic microflora of the colon (Rubinstein, 2000). Furthermore, this strategy exploiting the abrupt increase of the bacteria population (400 distinct species of bacteria) and corresponding enzyme activities will also accomplish greater site-specificity of initial drug release (Sinha and Kumria, 2003). The polysaccharides are also inexpensive, naturally occurring and abundantly available for colonic drug delivery (Vandamme et al., 2002).

Advances in preparing microparticles have caused a renewal of interest in delivering of drugs to the colon. While the monolithic forms such as tablets provide uniform transit time through gastrointestinal tract, the particulate pharmaceutical forms such as microsponges show several advantages such as uniform distribution at the target region and smaller risk of dose dumping (Lorenzo-Lamosa et al., 1998; Villar-López et al., 1999; Amorim and Ferreira, 2001).

Microsponges are porous, polymeric microspheres that are used mostly for topical (Nacht and Katz, 1990; Embil and Nacht, 1996) and recently for oral administration (Çomoğlu et al., 2002). Microsponges are designed to deliver a pharmaceutical active ingredient efficiently at the minimum dose and also to enhance stability, reduce side effects and modify drug release (Embil and Nacht, 1996).

FLB, a non-steroidal anti-inflammatory drug, effectively used for treatment of inflammation, pain or rheumatoid arthritis, was selected as a model drug. FLB has a plasma half-life of 3–6 h. Its administration rate is frequent due to its short half-life (Kean et al., 1992; Davies, 1995).

The aim of this study was to design novel colon specific drug delivery system containing FLB microsponges. This investigation consisted of preparation and evaluation of FLB microsponges and colon specific tablet formulations containing microsponges.

The microsponge formulations containing FLB were prepared by quasi-emulsion solvent diffusion method with Eudragit RS 100 (Kawashima et al., 1989, 1992; Ré and Biscans, 1999; Perumal, 2001; Çomoğlu et al., 2003). As a different approach, flurbiprofen was entrapped into the commercially available microsponge system, namely Microsponge® 5640.

The effects of drug:polymer ratio, inner phase solvent amount, stirring time and speed and stirrer type on the formation of microsponges were investigated. The thermal behaviour, surface morphology, particle size and pore structure of microsponges were examined.

Two different approaches were investigated for providing colon specific drug delivery. In the first one, the core tablets of microsponges were prepared by direct compression method. The prepared core tablets were coated with pectin:HPMC mixture which was found to be resistant to gastric and intestinal fluids but could be degraded by colonic microbial flora. As a second method, the pore openings of the prepared microsponges were plugged with the pectin:HPMC mixture and lyophilized in the freeze-dryer. Then, the lyophilized mixture was compressed in order to obtain tablets delivering the drug specifically to the colon.

The compression coated and pore-plugged tablets were tested in vitro for their suitability as colon specific drug delivery system. Kinetic and statistical evaluations were held for comparison between the applied approaches.

2. Materials and methods

2.1. Materials

FLB was donated by Adilna & Sanovel Pharmaceuticals Ind. Inc. (Turkey). Microsponge® 5640 (methyl methacrylate/glycol dimethacrylate crosspolymer) was kindly gifted by Cardinal Health (USA). Pectinex Ultra SP-L (26,000 FDU/mL) was purchased from Novozymes AG (Switzerland). Methocel® K100MCR Premium EP (hydroxypropylmethyl cellulose, HPMC) was supplied by Colorcon (UK). Eudragit RS 100 was from Degussa-Röhm GmbH & Co. (Germany). Polyvinylalcohol 30,000–70,000 (PVA), pectin (from citrus fruits, methoxy content 9.4%) and sodium carboxymethyl cellulose (Na-CMC) were from Sigma (USA). All other chemicals used for analysis were analytical grade.

2.2. Preparation of FLB microsponges by quasi-emulsion solvent diffusion method

The microsponges containing FLB were prepared by quasi-emulsion solvent diffusion method using the different polymer amounts. The processing flow chart is presented in Fig. 1a.

To prepare the inner phase, Eudragit RS 100 was dissolved in ethyl alcohol. Then, FLB was added to solution and dissolved under ultrasonication at 35 °C. The inner phase was poured into the PVA solution in water (outer phase). Following 60 min of stirring, the mixture was filtered to separate the microsponges. The microsponges were dried in an air-heated oven at 40 °C for 12 h and weighed to determine production yield (PY).

$$\text{PY}\ (\%) = \frac{\text{Practical mass (microspheres)}}{\text{Theoretical mass (polymer + drug)}} \times 100 \qquad (1)$$

Each formulation was carried out in triplicate.

2.2.1. Optimisation of formulation parameters and process factors

2.2.1.1. Effect of drug:polymer ratio. Different drug:polymer (FLB:Eudragit RS 100) ratios (11:1, 10:1, 5:1, 4:1 and 3:1) were investigated to prepare the microsponge formulations. In each formulation, the amounts of drug (2.5 g), ethyl alcohol (3 mL), PVA (50 mg), distilled water (200 mL) and inner phase temperature (35 °C) were kept constant. The microsponge formulations were prepared using centrifugal stirrer at a stirring rate

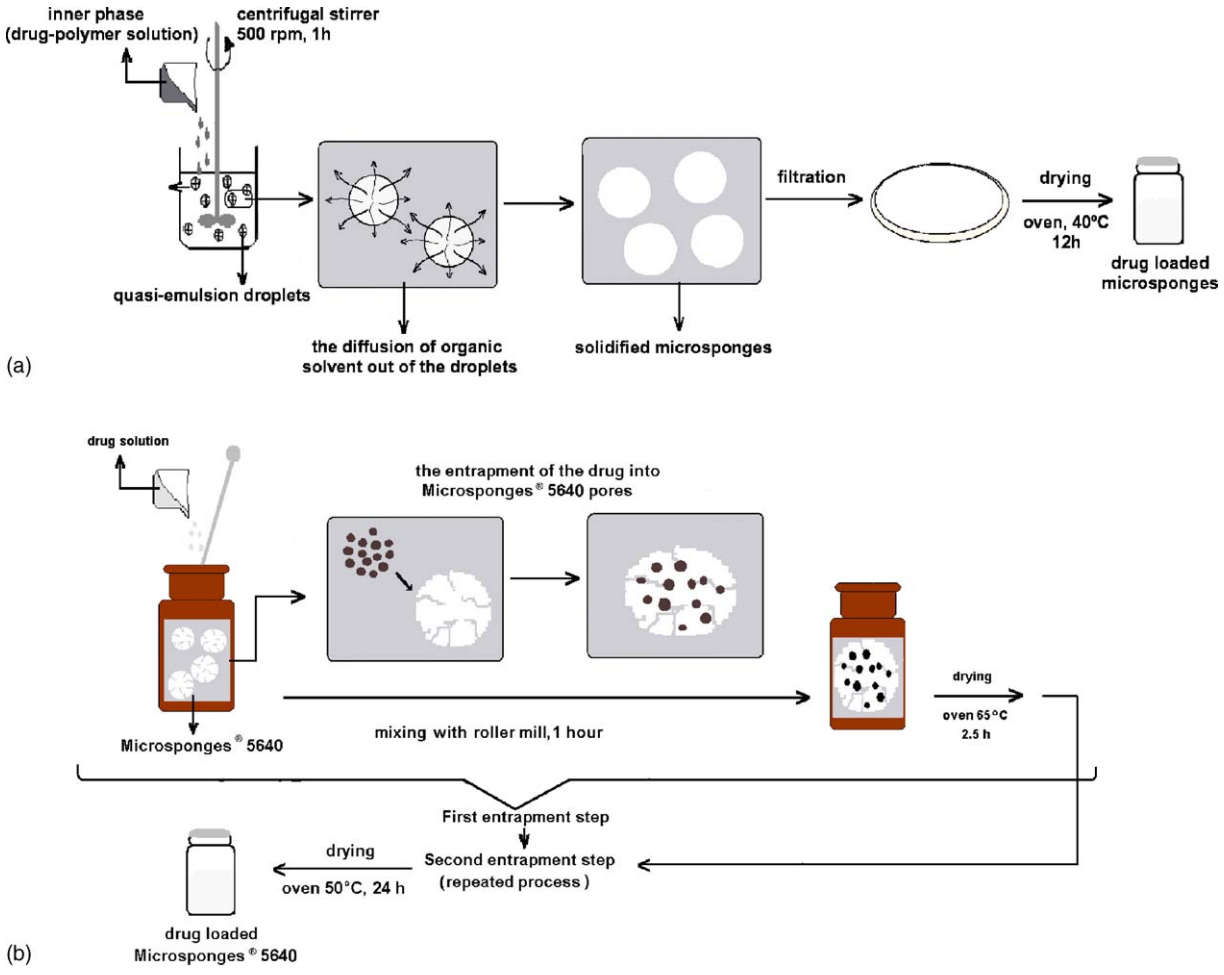

Fig. 1. Preparation of FLB microsponges by: (a) quasi-emulsion solvent diffusion method and (b) entrapment process.

of 500 rpm for 60 min. The microsponge formulations possessing drug:polymer ratios 3:1, 4:1 and 5:1 that could be obtained (FM1, FM2 and FM3) are given in Table 1.

2.2.1.2. Effect of inner phase solvent amount. The effect of inner phase solvent amount was investigated by using the formulation parameters defined for FM1 except the amount of ethyl alcohol. Three different solvent amounts were chosen as 3, 5 and 10 mL.

Table 1
Microsponge formulations prepared by quasi-emulsion solvent diffusion method

Constituents	Microsponge formulations		
	FM1	FM2	FM3
Inner phase			
FLB (g)	2.5	2.5	2.5
Eudragit RS 100 (g)	0.833	0.625	0.500
Ethyl alcohol (mL)	3	3	3
Outer phase			
Distilled water (mL)	200	200	200
PVA 30,000–70,000 (mg)	50	50	50

2.2.1.3. Effect of stirring time and speed. Different stirring times (30, 60 and 120 min) and speeds (350, 400, 450 and 500 rpm) were employed for FM1 formulation.

2.2.1.4. Effect of stirrer type. In order to determine the appropriate stirrer type, FM1 formulation was prepared with different stirrers (centrifugal stirrer, three and four blade propeller stirrers) at 500 rpm for 60 min.

2.3. Preparation of FLB microsponges by entrapment process

FLB microsponge formulation (FM4) was obtained by entrapping the drug into Microsponge® 5640 as represented in Fig. 1b.

1.5 g of FLB was dissolved in 3 g ethyl alcohol. The first half of the drug solution was added to the 1.5 g blank Microsponge® 5640 in an amber bottle. Bottle was arranged on a roller mill and mixed for 1 h. The mixture was dried in an oven at 65 °C for 2.5 h. This process was repeated for a second entrapment step for the remaining drug solution and the drying process was held at 50 °C for 24 h.

Table 2
Core tablet formulations containing FLB microsponges

Core tablet formulations	Microsponge formulations				Na-CMC (mg)	Magnesium stearate (mg)
	FM1 (mg)	FM2 (mg)	FM3 (mg)	FM4 (mg)		
CT1	139.0	–	–	–	41.0	9.0
CT2	–	132.0	–	–	48.0	9.0
CT3	–	–	125.0	–	55.0	9.0
CT4	–	–	–	134.0	46.0	9.0

2.4. Characterization and evaluation of microsponge formulations

2.4.1. Fourier transform infrared (FTIR) analysis

FTIR spectra of the FLB, Eudragit RS 100, empty Microsponge® 5640 and FM1–FM4 were measured in potassium bromide disks using a Perkin-Elmer Model 1600 FTIR spectrometer (USA).

2.4.2. Differential scanning calorimetric (DSC) analysis

Thermal analysis using a DSC method were carried out on FLB, physical mixture of FLB and Eudragit RS 100, empty Microsponge® 5640 and FM1–FM4 employing differential scanning calorimeter (Mettler Toledo DSC 822^e, USA). Samples (approximately 5 mg) were accurately weighed into aluminium pans and sealed. All samples were run at a heating rate of 15 °C/min over a temperature range 25–430 °C in atmosphere of nitrogen.

2.4.3. Morphology and particle size studies

Particle size analyses were performed on microsponge formulations by Malvern Mastersizer (Malvern Instruments, Mastersizer 2000, UK). The results are the average of three analyses. The values (d_{50}) were expressed for all formulations as mean size range.

For morphology and surface characteristics, prepared microsponges were coated with gold–palladium under an argon atmosphere at room temperature and then the surface morphology of the microsponges was studied by scanning electron microscopy (SEM) using a JEOL JXA 840A (USA).

2.4.4. Characterization of pore structure

Porosity parameters of microsponges such as intrusion–extrusion isotherms, pore size distribution, total pore surface area, average pore diameters, shape and morphology of the pores, bulk and apparent density were determined by using mercury intrusion porosimetry (AutoPore IV 9500, Micromeritics, USA). Incremental intrusion volumes were plotted against pore diameters that represented pore size distributions. The pore diameter of microsponges was calculated by using Washburn equation (Washburn, 1921).

$$D = \frac{-4\gamma \cos\theta}{P} \tag{2}$$

where D is the pore diameter (μm); γ the surface tension of mercury (485 dyn cm^{-1}); θ the contact angle (130°); and P is the pressure (psia).

Total pore area (A_{tot}) was calculated by using Eq. (3).

$$A_{tot} = \frac{1}{\gamma \cos\theta} \int_0^{V_{tot}} P \cdot dV \tag{3}$$

where P is the pressure (psia); V the intrusion volume (mL g^{-1}); V_{tot} is the total specific intrusion volume (mL g^{-1}).

The average pore diameter (D_m) was calculated by using Eq. (4):

$$D_m = \frac{4V_{tot}}{A_{tot}} \tag{4}$$

Envelope (bulk) density (ρ_{se}) of the microsponges was calculated by using Eq. (5):

$$\rho_{se} = \frac{W_s}{V_p - V_{Hg}} \tag{5}$$

where W_s is the weight of the microsponge sample (g); V_p the empty penetrometer (mL); V_{Hg} is the volume of mercury (mL).

Absolute (skeletal) density (ρ_{sa}) of microsponges was calculated by using equation:

$$\rho_{sa} = \frac{W_s}{V_{se} - V_{tot}} \tag{6}$$

where V_{se} is the volume of the penetrometer minus the volume of the mercury (mL).

Finally, the percent porosity of the sample was found from Eq. (7):

$$\text{Porosity (\%)} = \left(1 - \frac{\rho_{se}}{\rho_{sa}}\right) \times 100 \tag{7}$$

Pore morphology was characterized from the intrusion–extrusion profiles of mercury in the microsponges as described by Orr (1969).

2.4.5. Actual drug content and encapsulation efficiency

The weighed samples of drug loaded microsponges (30 mg) were dissolved in 100 mL, pH 6.4, phosphate buffer under ultrasonication for 4 h at 30 °C. The samples were filtered using 0.2 μm membrane filter and absorbances of samples were read at 248 nm against blank using spectrophotometer. The actual drug content and encapsulation efficiency were calculated as given below. All analyses were carried out in triplicate.

$$\text{Actual drug content (\%)} = \frac{M_{act}}{M_{ms}} \times 100 \tag{8}$$

$$\text{Encapsulation efficiency (\%)} = \frac{M_{act}}{M_{the}} \times 100 \tag{9}$$

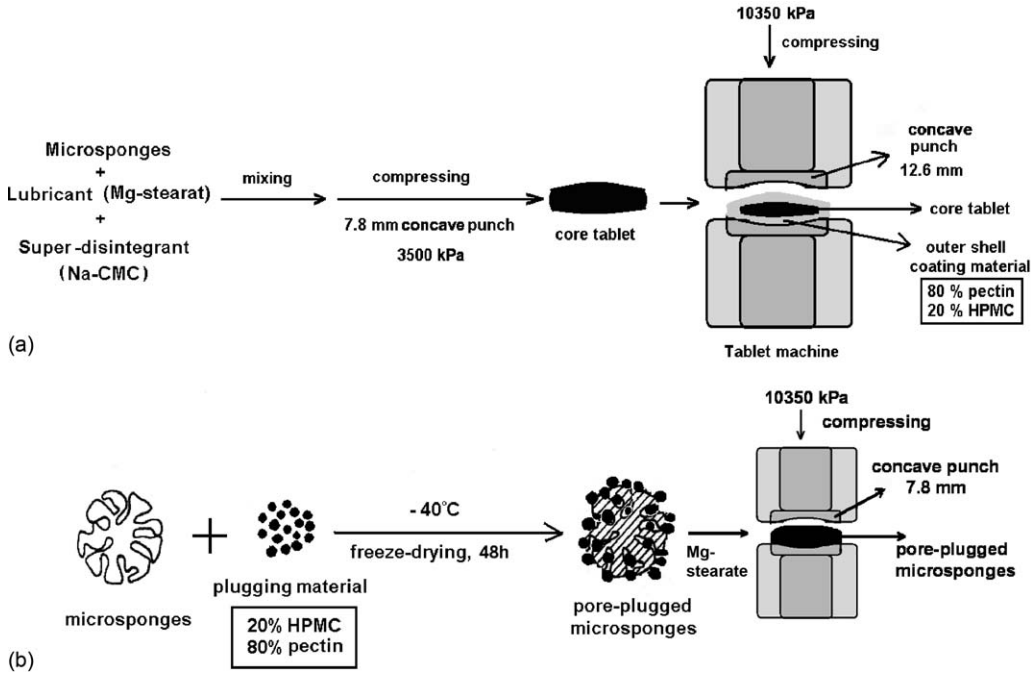

Fig. 2. Preparation of: (a) core and compression coated tablets and (b) tablets based on pore-plugged microsponges.

where M_{act} is the actual FLB content in weighed quantity of microsponges, M_{ms} is the weighed quantity of powder of microsponges and M_{the} is the theoretical amount of FLB in microsponges calculated from the quantity added in the process.

2.5. Preparation of colon specific tablet formulations

2.5.1. Preparation of compression coated tablets

The core tablets consisting of microsponges containing 100 mg FLB, a super disintegrant Na-CMC and magnesium stearate as a lubricant were prepared by direct compression method. All tablet constituents were weighed and mixed in a cubic mixer for 15 min. Final powder mixture was compressed using 7.8 mm concave punches on a laboratory scale single punch tabletting machine using 3500 kPa compression pressure. Core tablet formulations are given in Table 2.

Pectin:HPMC (80:20) mixture was used as outer shell for compression coating. The coat weight was optimised as 400 mg. Fifty percent of coat weight was placed in the die cavity followed by carefully centering the core tablet and the addition of the remainder of the coat weight. The coating material was compressed around the core tablet at an applied pressure of 10,350 kPa using round concave punches (12.6 mm) on the same tabletting machine.

The concept of this system is shown schematically in Fig. 2a.

2.5.2. Preparation of tablets containing pore-plugged microsponge formulations

To prepare the tablets (PPT1, PPT2, PPT3 and PPT4) containing pore-plugged microsponge formulations, FM1, FM2, FM3 and FM4 formulations were used, respectively.

800 mg pectin and 200 mg HPMC were swollen in 10 g distilled water overnight and added to prepared microsponges in the ratio of 1:1 (w/w) (polymer:microsponge) and mixed continuously for about 15 min. Polymer mixture was incorporated to the pores of the microsponges. The obtained bulk was frozen at −40 °C and freeze-dried over 48 h at 0.1 mPa. The solid mixture was passed through a 250 μm sieve. Magnesium stearate as a lubricant (0.5%) was added and mixed in a cubic mixer for 15 min and compressed at an applied pressure of 10,350 kPa using 7.8 mm concave punches on a single punch tabletting machine.

The concept of this system is shown schematically in Fig. 2b.

2.6. Drug release studies

2.6.1. Microsponges and core tablet formulations

In drug release studies of microsponges and core tablet formulations, the changing pH media, Method 1, USP 24, for delayed release tablets was used. Dissolution test was conducted in USP rotating paddle apparatus with a stirring rate of 50 rpm at 37 ± 0.5 °C. Initial drug release studies were done in 750 mL of 0.1N HCl for 2 h. Then, 250 mL of 0.2 M trisodium phosphate solution was added to the dissolution media and the pH was adjusted to 6.8 with 2N HCl for 8 h. Samples were withdrawn after regular intervals of time to evaluate drug release and analyzed spectrophotometrically at a wavelength of 248 nm. Dissolution tests were performed at least three times for each sample.

2.6.2. Colon specific formulations

The drug release studies were done with the same method used for microsponges and core tablets. Additionally, Pectinex Ultra SP-L was added to the dissolution medium at 8th hour in order to simulate the enzymatic action of the colonic bacteria.

2.7. Statistical analysis

The data obtained from each experiment were subjected to statistical analysis using one-way analysis of variance (ANOVA) followed by Newman–Keuls multiple comparisons test. $p < 0.05$ was considered to be indicative of significance.

3. Results and discussion

3.1. Optimisation of quasi-emulsion solvent diffusion method

The preparation methods of microsponges are limited in the means of complexity and cost. The suspension polymerization is the known process to prepare the commercially available microsponges. Quasi-emulsion solvent diffusion method serves an alternative way for preparing microsponges (Kawashima et al., 1989, 1992; Perumal et al., 1999; Ré and Biscans, 1999; Perumal, 2001; Çomoğlu et al., 2003; Yang et al., 2003b). This method seems to be promising for the preparation of flurbiprofen microsponges with being easy, reproducible, rapid method and has an advantage of avoiding solvent toxicity (Ré and Biscans, 1999).

In this study, two different methods were used for the preparation of microsponges. For the optimisation of the formulations and process factors of microsponges prepared by quasi-emulsion solvent diffusion method, the effects of drug:polymer ratio (11:1, 10:1, 5:1, 4:1 and 3:1), inner phase solvent amount (3, 5 and 10 mL), stirrer type (centrifugal stirrer, three and four blade propeller stirrers), stirring time (30, 60 and 120 min) and stirring speed (350, 400, 450 and 500 rpm) on the formation of microsponges were investigated.

Microsponges could not be obtained with the drug:polymer ratios 11:1 and 10:1 and free FLB crystals were seen in the investigation done by optical microscope while the drug:polymer ratios 3:1, 4:1 and 5:1 gave spherical microsponges. It was previously reported that ibuprofen microsponges in the drug:polymer ratio between 5:1 and 2:1 (Kawashima et al., 1989) and ketoprofen microsponges in the drug:polymer ratio between 11:1 and 3:1 (Çomoğlu et al., 2003) could be prepared with Eudragit RS 100.

In quasi-emulsion solvent diffusion method, the formation of the microsponges could be described in the following processes: the formation of quasi-emulsion droplets, the diffusion of the ethyl alcohol and the solidification of the droplets. The rapid diffusion of ethanol (good solvent for the polymer and drug) into the aqueous medium might reduce the solubility of the polymer in the droplets, since the polymer was insoluble in water. The instant mixing of the ethanol and water at the interface of the droplets induced precipitation of the polymer, thus forming a shell enclosing the ethanol and the dissolved drug. Counter diffusions of ethyl alcohol and water through the shell promoted further crystallization of the drug in the droplets from the surface inwards. The finely dispersed droplets of the polymer solution of the drug were solidified in the aqueous phase via diffusion of the solvent (Ré and Biscans, 1999). In this respect, the effect of the ethyl alcohol amount on the formation of microsponges was investigated. Milky phase was formed with high amount of inner phase solvent (5 and 10 mL). The microsponges could be prepared by decreasing the amount of ethyl alcohol to 3 mL. Kawashima et al. (1992) described that the recovery of microsponges decreased sharply with increase in ethyl alcohol amount. This result is related to the decrease in the concentration of drug in the inner phase solvent.

Table 3
Optimum microsponge production parameters with quasi-emulsion solvent diffusion method

Specification	Optimum values
FLB:Eudragit RS 100 ratio	3:1–4:1–5:1
Amount of drug (g)	2.5
Emulsifying agent	PVA 30,000–70,000
Amount of emulsifying agent (mg)	50
Inner phase solvent	Ethyl alcohol
Amount of inner phase solvent (mL)	3
Amount of water in the outer phase (mL)	200
Temperature of inner phase (°C)	35
Stirrer type	Centrifugal stirrer
Stirring rate (rpm)	500
Stirring time (min)	60

The effects of stirrer type, stirring time and speed on the formation of the microsponges were also determined. Aggregates were formed when three and four blade propeller stirrers were used for the preparation of microsponges. The aggregate amount was very low with two blade centrifugal stirrer and microsponges showed homogenous particle size distribution. Yang et al. (2003b) found that the increased mechanical shear force, produced by increasing the stirring speed, divided the suspension of drug and polymer into small droplets rapidly. In our study, the stirring speed applied between 350 and 450 rpm caused fibrous aggregates. However, the microsponges prepared with 500 rpm had smaller particle size and homogenous size distribution. In this respect, 500 rpm was selected as the optimum stirring speed. The stirring time had significant effect on the formation of microsponges. It was found that the solidification of microsponges was not enough in 30 min of stirring. One hour stirring was appropriate for the preparation and additional stirring time did not affect the formation of microsponges. In this respect, the optimum stirring time was selected as 1 h.

According to the results of preformulation studies, optimum microsponge production parameters are given in Table 3.

3.2. Characterization of microsponges

Fig. 3a gives the FTIR spectra of FLB, Eudragit RS 100 and FM1–FM3. In FTIR spectra of FLB powder, a carbonyl stretching band at 1700 cm^{-1}, a hydroxyl stretching peak related with carbonyl group at 3450 cm^{-1} and C–F stretching peak at 1216 cm^{-1} were seen as observed in previous literature (Lacoulonche et al., 1997). Eudragit RS 100 also showed an ester C=O stretching peak around 1734 cm^{-1} as in accordance with the given data (Pignatello et al., 2002). All characteristic peaks of FLB were observed in the IR spectra of FM1–FM3 formulations. Fig. 3b gives the FTIR spectra of FLB, empty Microsponge®

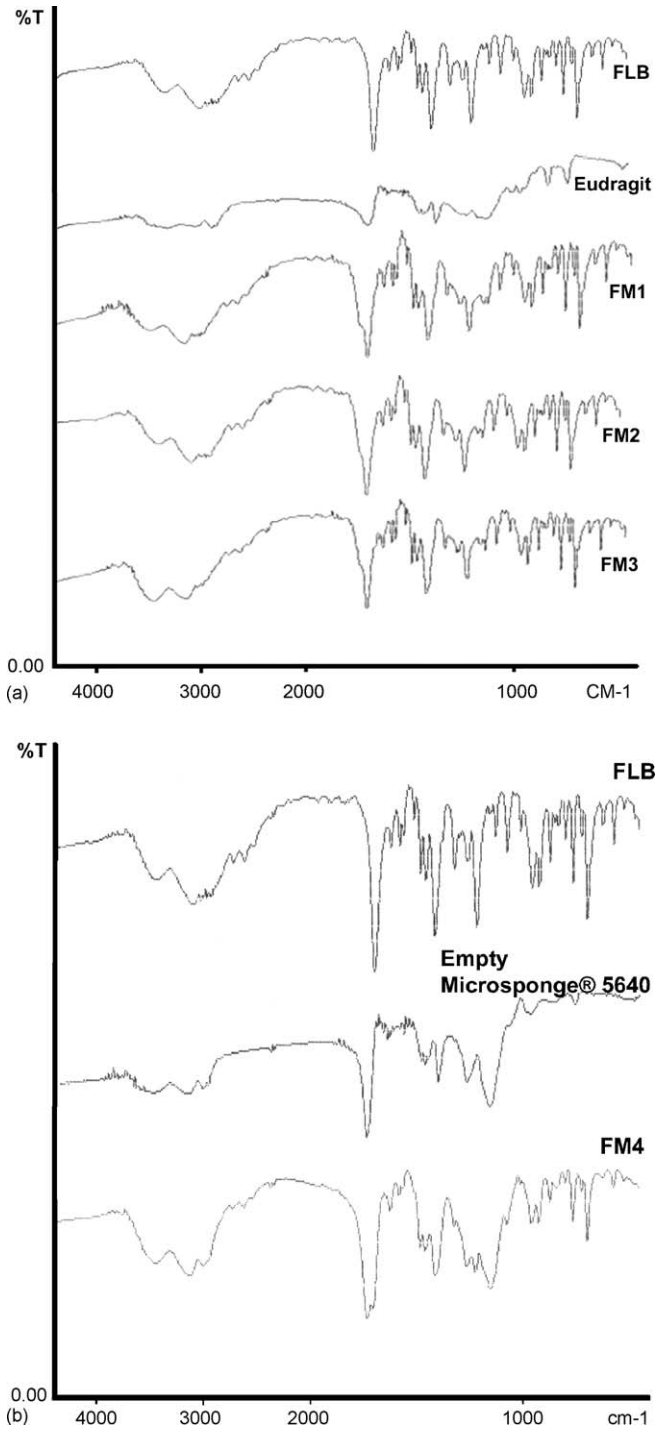

Fig. 3. FTIR spectra of: (a) FLB, Eudragit RS 100 and FM1–FM3 microsponge formulations and (b) FLB, empty Microsponge® 5640, FM4 microsponge formulation.

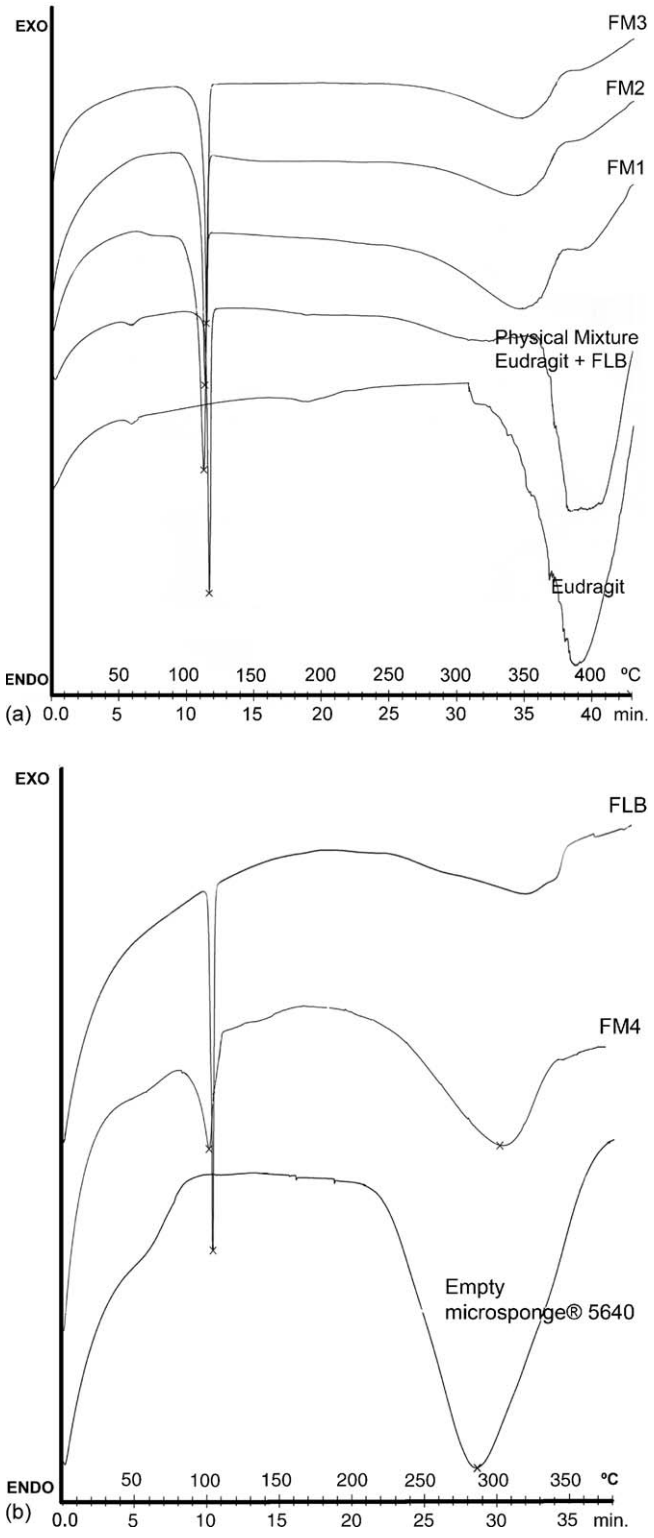

Fig. 4. DSC thermograms of: (a) Eudragit RS 100, physical mixture of FLB and Eudragit RS 100 and FM1–FM3 microsponge formulations and (b) FLB, empty Microsponge® 5640 and FM4 microsponge formulation.

5640 and FM4. Characteristic peaks of FLB were observed in the spectra of FM4 formulation. These results showed that there was no chemical interaction or changes during microsponge preparation and FLB was stable in all microsponge formulations.

DSC provides information about the physical properties of the sample as crystalline or amorphous nature and demonstrates a possible interaction between drug and other compounds in microsponges (Ford and Timmins, 1989). The thermal behaviour of FLB, Eudragit RS 100, empty Microsponge® 5640 and prepared microsponge formulations are shown in graphs presented in Fig. 4a and b. According to the thermograms, FLB presented a sharp endothermic peak at 117 °C corresponding to the melt-

Table 4
Production yield, actual drug content, encapsulation efficiency and mean particle size of FLB microsponges ($n = 3$)

Formulation	Polymer type	Drug:polymer ratio	Production yield* (% ± S.D.)	Theoretical drug content (%)	Actual drug content* (% ± S.D.)	Encapsulation efficiency** (% ± S.D.)	Mean particle size† (μm ± S.D.)
FM1	Eudragit RS 100	3:1	75.29 ± 2.14	73.90	71.85 ± 0.01	97.23 ± 0.02	46.47 ± 4.86
FM2	Eudragit RS 100	4:1	74.17 ± 3.13	78.74	75.83 ± 0.02	96.30 ± 0.02	68.81 ± 6.97
FM3	Eudragit RS 100	5:1	78.10 ± 2.59	81.97	80.08 ± 0.02	97.69 ± 0.03	94.49 ± 7.23
FM4	Microsponge® 5640	1:1	91.67 ± 2.19	50.00	48.06 ± 0.02	96.12 ± 0.02	30.72 ± 3.42

* No significant differences between FM1, FM2 and FM3 ($p > 0.05$) and significant differences between FM4 and other formulations (FM1–FM3) ($p < 0.05$).
** No significant differences between all formulations ($p > 0.05$).
† Significant differences between all formulations ($p < 0.05$).

ing point of drug in the crystalline form. Eudragit RS 100 in the amorphous form showed a broad endothermic peak at 385 °C. The obtained results were in accordance with literature (Henck and Kuhnert-Brandstatter, 1999; Eerikäinen et al., 2004). In the DSC curves of physical mixture (FLB:Eudragit RS 100; 1:1) and FM1–FM3 formulations, characteristic peaks of FLB and Eudragit RS 100 were seen. The thermograms of physical mixture and FM1–FM3 formulations showed that drug was in its crystalline form and also there was no interaction between FLB and Eudragit RS 100. Microsponge production process used for FM1–FM3 formulations did not change the nature of the drug in microsponges. The similar result was also observed for the microsponge formulation prepared by the entrapment process. Empty Microsponge® 5640 presented an endothermic degradation peak at 286 °C. In the DSC curve of FM4 formulation, the endothermic melting peak concerning FLB and the degradation peak concerning Microsponge® 5640 were observed. According to these data, there was no interaction between FLB and empty Microsponge® 5640.

The morphology of the microsponges prepared by quasi-emulsion solvent diffusion method and entrapment method were investigated by SEM. The representative SEM photographs of the microsponges are shown in Fig. 5. It was observed by SEM analysis that the microsponges were finely spherical and uniform and no entire flurbiprofen crystals were observed visually. A cross-sectional view of a microsponge (FM1) (Fig. 5i) revealed that the characteristic internal structure was a spherical cavity enclosed by a rigid shell constructed from drug and polymer. The inner structure consists of void spaces.

The production yield, actual drug content, encapsulation efficiency and mean particle size of FLB microsponge formulations are given in Table 4. The production yield was between 74.17–78.10% for FM1–FM3 and 91.67% for FM4. The actual drug content of microsponges, expressed as a percentage of the amount of FLB entrapped in the microsponges, varied between 71.85–80.08% for FM1–FM3 and 48.06% for FM4 (Table 4). On increasing the amount of polymer, the actual drug content of microsponges decreased. While there was not a significant difference between formulations FM1–FM3 ($p > 0.05$), the difference between FM4 and the other formulations was significant ($p < 0.05$). The encapsulation efficiency was found to be high in all formulations since it exceeded 96%. The relatively high drug content and encapsulation efficiency of microsponges also indicated that both methods were suitable for preparing the microsponge formulations. These results were in accordance with the previous literatures (Nacht and Katz, 1990; Kawashima et al., 1992).

The mean particle size (d_{50}) of FM1–FM3 ranged from 46.47 to 94.49 μm. It was found that the mean particle size increased with the decrease in the polymer amount. FM4 possessed the lowest particle size corresponding to 30.72 μm.

Quasi-emulsion solvent diffusion method could fuel interest in the preparation of microsponges, thus providing a high percent of porosity (Kawashima et al., 1992). Intrusion volume of mercury is a function of total porosity (Webb and Orr, 1997; Mehta et al., 2000; Mattsson and Nyström, 2001; Rahman and Sablani, 2003). In Fig. 6, the cumulative intrusion volume is plotted against pore diameters showing the intrusion–extrusion profiles of FM1–FM4. According to intrusion and extrusion curves, the majority of the pores presented in FM1–FM3 were spherical type pores, whereas mainly cylindrical hole type pores were seen in FM4. Table 5 shows the total intrusion volume, aver-

Table 5
Total intrusion volume, average pore diameter, envelope and apparent densities, total pore area and porosity of microsponge formulations ($n = 3$)

	Pore morphology	Total intrusion volume* (mL g^{-1})	Average pore diameter** (μm)	Envelope (bulk) density* (g/mL)	Apparent (skeletal) density* (g/mL)	Total pore area** (m^2/g)	Porosity** (%)
FM1	Spheres	1.27 ± 0.14	0.21 ± 0.02	0.48 ± 0.08	1.23 ± 0.14	24.01 ± 0.43	60.91 ± 2.11
FM2	Spheres	1.42 ± 0.16	0.24 ± 0.03	0.44 ± 0.06	1.17 ± 0.11	25.27 ± 0.39	62.56 ± 1.94
FM3	Spheres	1.60 ± 0.10	0.26 ± 0.05	0.41 ± 0.09	1.17 ± 0.10	26.94 ± 0.42	65.24 ± 2.01
FM4	Cylindrical holes	2.10 ± 0.13	0.07 ± 0.01	0.34 ± 0.04	1.21 ± 0.13	125.33 ± 0.51	71.83 ± 2.05

* No significant differences between all formulations ($p > 0.05$).
** No significant differences between FM1, FM2 and FM3 ($p > 0.05$) and significant differences between FM4 and other formulations (FM1–FM3) ($p < 0.05$).

age pore diameter, envelope and apparent densities, total pore area and porosity of all formulations. According to the comparison of FM1–FM3 formulations, total intrusion volume, average pore diameter, total pore area and porosity values showed small increase due to the increase in drug:polymer ratio but no significant difference was seen between these results ($p > 0.05$). On the other hand, FM4 exhibited a five-fold increase in total pore area and four-fold decrease in average pore diameter when

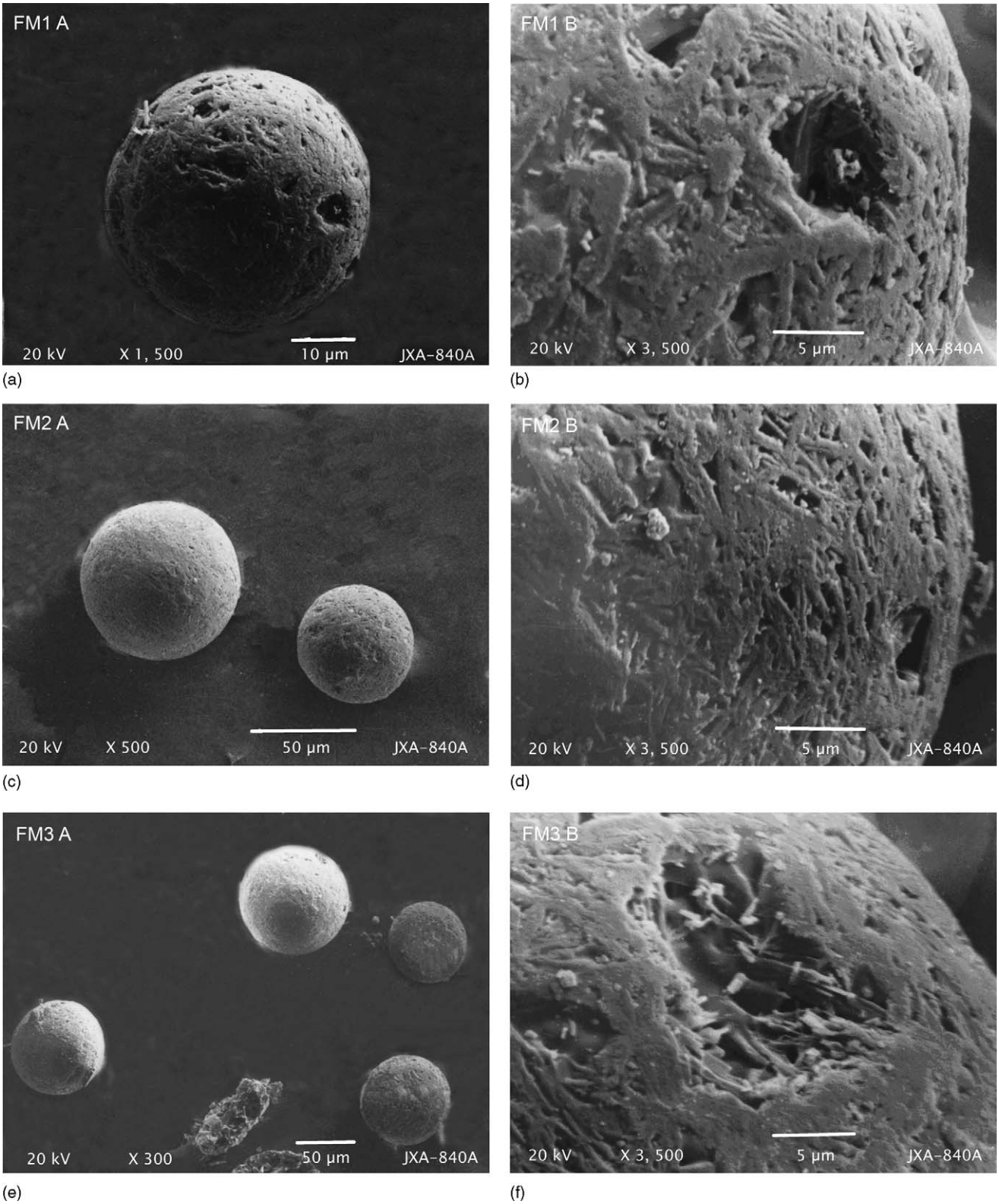

Fig. 5. (a–i) SEM photographs of microsponge formulations. The photograph coded 'A' represents whole image; 'B' represents surface photographs; the photograph coded as *"pore"* shows the cross-section of FM1 formulation.

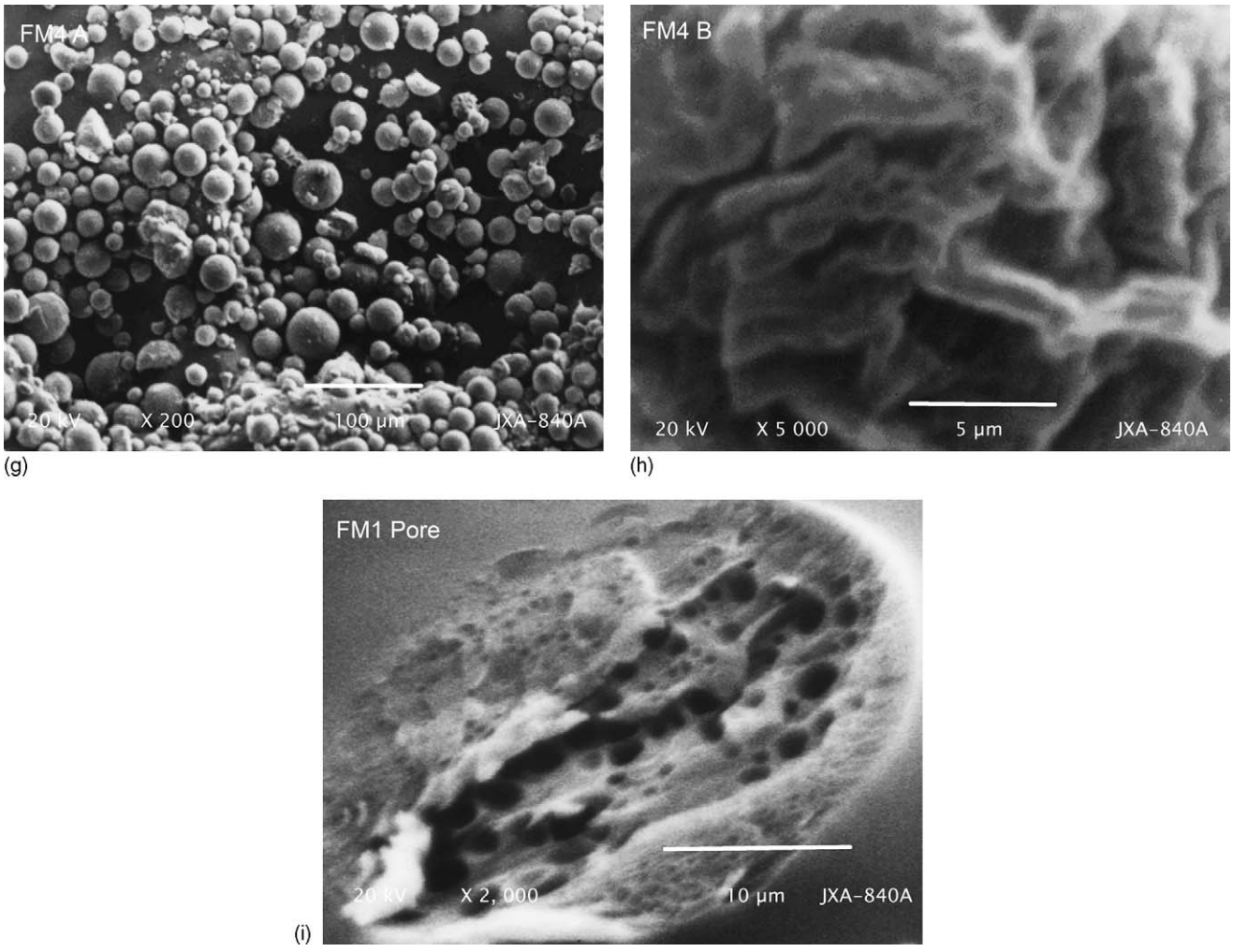

Fig. 5. (*Continued*).

compared with FM1–FM3 ($p < 0.05$). The percent of porosity of FM1–FM3 ranged between 60.9 and 65.2% ($p > 0.05$), of FM4 was 71.8% and the difference between FM4 and the other formulations was statistically significant ($p < 0.05$). Fig. 7 shows the incremental intrusion volume as a function of the pore diameter of the microsponge formulations with different drug:polymer ratios. Using Figs. 6 and 7, average pore diameters of microsponges can be characterized. The data indicated

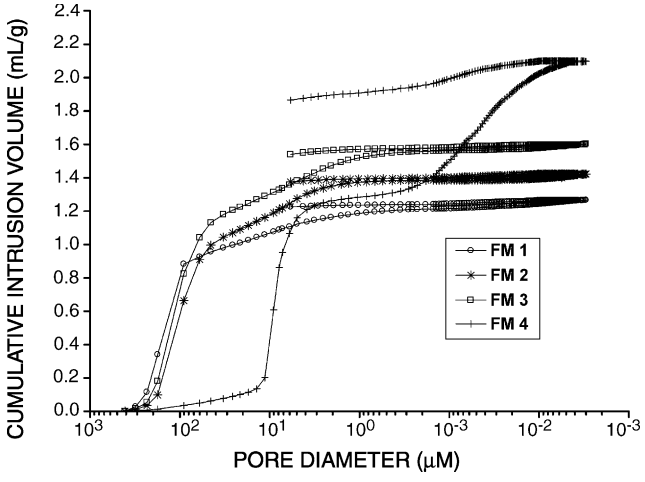

Fig. 6. Cumulative intrusion volume vs. pore diameter curve of microsponge formulations.

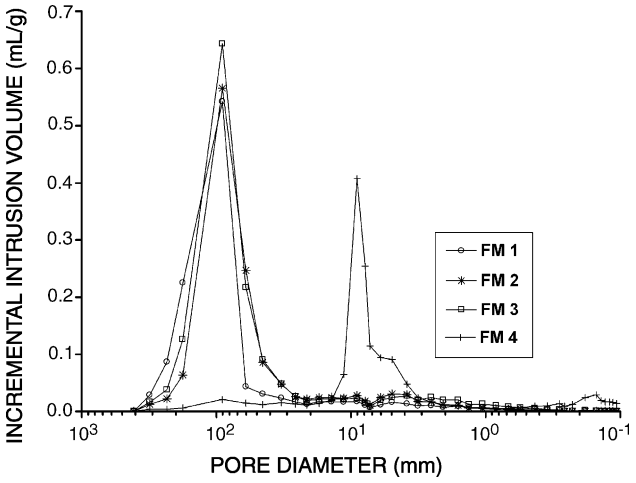

Fig. 7. Pore size distribution of microsponges.

that as the drug:polymer ratio increased from 3:1 to 5:1, the average pore diameter increased.

3.3. Optimisation of tablet formulations containing microsponges

In order to prepare the compression coated tablet formulations, core tablets were prepared as the first step. Microsponges show good flowability and direct compressibility properties (Anderson et al., 1994). The homogenous granular characteristic of microsponges is due to their highly porous structure and in these means, microsponges have the compressibility to produce strong tablets (Çomoğlu et al., 2002; Kawashima et al., 2002).

The disintegration time of core tablets was retarded because of the insoluble polymers used in the preparation of microsponges. When the core tablets did not include disintegrating agent, the disintegration time was found to be about 2 h at pH 6.8. Therefore, Na-CMC was added to the formulation in order to make the core rapidly disintegrating. The disintegration time of tablets containing Na-CMC decreased to 2–3 min. This would allow the core tablets to disintegrate rapidly once the coat material is digested by the resident microflora of the colon. The obtained all core tablet formulations showed high strength (110–134N) and the friability results (<0.5%) were in accordance with *The European Pharmacopeia* standards (2005).

Among the different approaches to achieve colon specific drug delivery, the use of polymers, specifically biodegraded by colonic bacteria, holds great promise. Working on this rationale, a drug release-retarding ingredient falling under the category of polysaccharides, pectin, which is known to retard drug release considerably (Ahrabi et al., 2000; Ashford et al., 1994; Liu et al., 2003) was selected for our study. Pectin alone has been earlier found to be insufficient to protect the drug until it reaches the proximal colon and in addition it has poor compactibility properties (Kim et al., 1998). So, pectin was used in combination with HPMC (Turkoglu et al., 1999; Turkoglu and Ugurlu, 2002), chitosan (Fernández-Hervás and Fell, 1998), HPMC–chitosan mixture (Macleod et al., 1999a,b) and ethyl cellulose (Wakerly et al., 1996, 1997) for colon specific drug delivery. In this study, the pectin–HPMC envelope was selected for colon specific drug delivery and it was found to be promising drug delivery system for those drugs to be delivered to the colon, as already mentioned by Turkoglu and Ugurlu (2002).

In order to prepare compression coated colon specific formulations, the different ratios of pectin:HPMC (50:50, 70:30, 80:20 and 90:10) were investigated as coating material. The tablet formulations prepared using compression coating in the ratio of 90:10 exhibited high friability (1.75%) and drug release started at the 6th hour. The formulations compressed using the ratios of 50:50 and 70:30 showed low friability (<0.5%) and started to release the drug at 15th and 12th hours, respectively. On the other hand, the drug release from the colon specific tablet formulations, compression coated in the ratio of 80:20, started at the beginning of the 8th hour corresponding to the colon arrival time. Therefore, the pectin:HPMC combination in the ratio of 80:20 was selected for the preparation of colon specific tablet formulations.

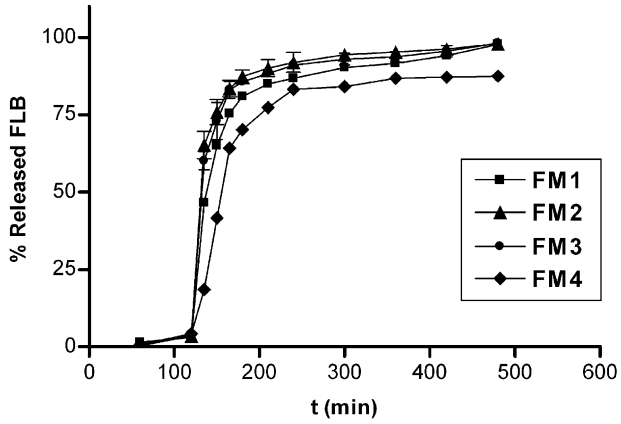

Fig. 8. In vitro drug release profiles of FLB from microsponge formulations.

3.4. In vitro drug release studies from microsponge and colon specific tablet formulations

Drug delivery to the large intestine has become attractive to researchers whose main interest is the treatment of colonic disorders and systemic drug administration for diurnal diseases (Leopold, 2001). There are currently four approaches that are pursued to achieve colon specificity, namely pH-controlled drug release, enzyme-controlled drug release, time-controlled drug release and prodrugs. In the recent years, microflora-activated systems formulated making use of non-starch polysaccharides such as pectin (Ashford et al., 1994; Ahrabi et al., 2000; Liu et al., 2003), chitosan (Tozaki et al., 1997; Lorenzo-Lamosa et al., 1998; Shimono et al., 2002; Zhang et al., 2002; Hejazi and Amiji, 2003) and guar gum (Krishnaiah et al., 2002; Sinha et al., 2004) are highly promising because the polysaccharides remain undigested in the stomach and the small intestine and can only be degraded by the vast anaerobic microflora of the colon.

Advances in preparing microparticles have caused a renewal of interest in delivering of drugs to the colon. However, there was no published literature concerning delivery of microsponges for colon specific drug delivery.

The drug release studies of microsponges and core tablet formulations were held using the same procedure done for the colon specific tablets. The drug release profiles of the microsponge formulations are illustrated in Fig. 8. While the in vitro drug release amount was maximum 4% in the first 2 h for all formulations (pH: 1.2), burst effect was observed (46.5–60.1% for FM1–FM3 and 18.4% for FM4) following 15 min after changing pH from 1.2 to 6.8. High initial release was observed in all formulations. The burst effect could be due to two reasons: the first was the drug near or on the surface of the microsponges and the second was the well known porous nature of microsponges, the pores provided a channel for release of the drug (Mandal et al., 2001). The drug release from FM1–FM3 formulations finished at the 8th hour while the 87.5% of drug released from FM4 at the same time. According to the obtained data, the microsponge

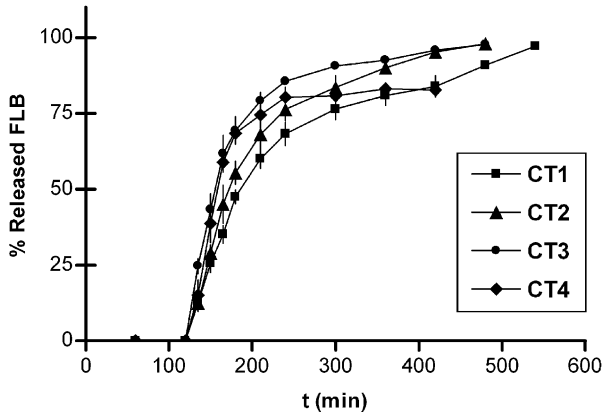

Fig. 9. In vitro drug release profiles of FLB from core tablet formulations.

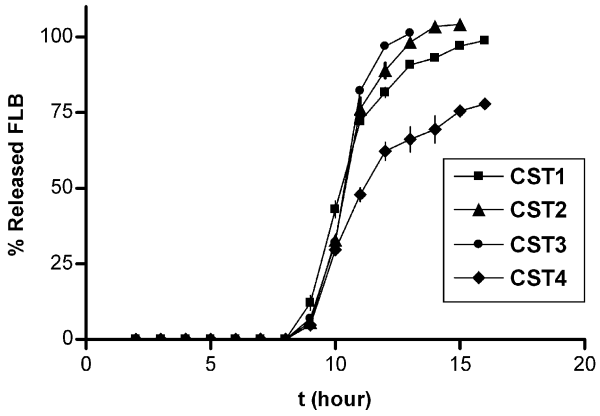

Fig. 10. In vitro drug release profiles of FLB from compression coated tablet formulations.

formulations which have similar pore diameter prepared by quasi-emulsion solvent diffusion method (FM1–FM3) did not differ in their drug release rate ($p > 0.05$). Due to smaller pore diameter of the microsponge formulation prepared by entrapment method, in vitro drug release from FM4 showed slower release compared with the other microsponge formulations. However, these differences between FM4 and other formulations were not statistically significant ($p > 0.05$). The microsponges differ from regular microspheres with their highly porous surface. This characteristic gives property to release the drug at a faster rate through the pores. Kawashima et al. (1992) reported that microsponges having a more porous internal structure exhibited a faster drug release rate than that of rigid microspheres.

In vitro release profiles of FLB from core tablet (CT1–CT4) are displayed in Fig. 9. Kawashima et al. (1992) reported that plastic properties of microsponges allowed them to be more easily compressed by direct compression to produce a mechanically strong tablet than the physical mixture of drug and polymer.

Çomoğlu et al. (2002) described that 1000–2000 kgf/cm^2 pressure did not cause the structure deformation of microsponges. It was observed that the in vitro drug release results of microsponge and core tablet formulations were similar and did not differ significantly ($p > 0.05$). This finding was attributed to the unique compression property of microsponges and it was assumed that structure deformation has not been seen due to applied compression pressure.

Drug release was not seen from CST1 to CST4 in the first 8 h (Fig. 10). After this lag time, due to the addition of the Pectinex Ultra SP-L to the dissolution medium, the drug release started at 9th hour and continued up to 16th hour for CST1 (98.9%), 14th hour for CST2 (103.5%), 13th hour for CST3 (101.3%) and 16th hour for CST4 (77.8%), following almost a Rosin–Rimmler–Sperling–Bennet–Weillbull (RRSBW) kinetic model. No significant differences were detected among CST1–CST4 in the values obtained for the in vitro release rate ($p > 0.05$). It was assumed that the optimum ratio of pectin:HPMC (80:20) would protect the cores up to 8th hour corresponding to the time to reach the colon (the cores remained constant until the addition of the enzyme) and after that under the influence of the enzyme, the system would degrade faster and deliver the drug to the proximal colon that forms the main site of bacterial carbohydrate metabolism. So, the results were in accordance with the triggering mechanism due to the very active metabolism in the proximal part compared with the distal part of colon and pectin could find the appropriate environment to be degraded (Edwards, 1993).

The same triggering mechanism worked for the pore-plugged formulations (PPT1–PPT4). The pectin:HPMC complex plugged the pore openings and sealed the drug inside the pores. The complex was degradable only by the action of bacteria that were specific to the colon. The degradation of the complex by these bacteria resulted in the removal of the complex from the pore openings and consequently the release of the drug. As a different approach, incorporation of polymers into the microsponge was compared to the compression coating of microsponges. The pore-plugged tablets (PPT1–PPT4) did not show drug release in 2 h. FLB release took place at a highly retarded rate till the enzymatic breakdown occurred at the 8th hour (approximately 25%). The amount of FLB released was 92.6–100% from PPT1 to PPT4 at 14th hour (Fig. 11). The drug release results of the first 2 h were not taken into consideration in the kinetic evaluation due to the fact that no release was seen in pH 1.2. A good correlation was observed indicating that the drug release

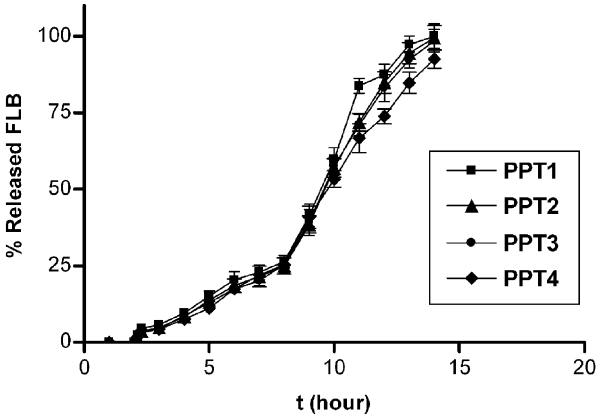

Fig. 11. In vitro drug release profiles of FLB from pore-plugged tablet formulations.

from all pore-plugged tablets (PPT1–PPT4) followed zero order kinetic model. No significant differences were detected among PPT1–PPT4 in the values obtained for the in vitro drug release rate ($p > 0.05$).

4. Conclusion

This study presents new approaches for the modification of microsponges as well as a new system with a great potential for colonic drug delivery. The unique compressibility of microsponges offers a new alternative way for producing mechanically strong tablets owing to the plastic deformation of sponge-like structure.

The colon specific tablet formulations, including microsponges, were prepared based on two approaches. Both two approaches, using the triggering mechanism of microflora-activation, represented interesting forms for delivering the drug to the proximal part of the colon, avoiding release in the small intestine. The particulate form (microsponges) has been used to provide more uniform distribution of the drug in the colon and help the drug to spread on the colon surface in an appropriate way.

The obtained microsponges exhibited spherical shape, high porosity, good flowability and compressibility properties. The rapid drug release from microsponge and core tablet formulations can be attributed to the highly porous structure of microsponges. The compression coated formulations prepared using pectin and HPMC in the ratio of 80:20 protected the drug from being released in the stomach and small intestine under in vitro conditions mimicking mouth-to-colon transit.

The colon specific formulations prepared by pore plugging the microsponges with the same polymer mixture released approximately 25% of the drug in the dissolution medium before the addition of the enzyme and carried the remained drug specifically to the colon. These formulations released the drug for 14 h in the colon following zero order kinetic model.

This study presented a novel colon specific drug delivery system containing flurbiprofen (FLB) microsponges. It is concluded that both the microsponges prepared by quasi-emulsion solvent diffusion method and Microsponge® 5640 can be used successfully in the systems designed for colon specific drug delivery. In vivo studies are planned to assess the relative usefulness of the microsponge formulations to carry drug specifically to the colon.

Acknowledgments

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