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Colonic luminal surface retention of meloxicam microsponges delivered by erosion based colon-targeted matrix tablet

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ABSTRACT

The work was aimed at developing calcium-pectinate matrix tablet for colon-targeted delivery of meloxicam (MLX) microsponges. Modified quassi-emulsion solvent diffusion method was used to formulate microsponges (MS), based on 3^2 full factorial design. The effects of volume of dichloromethane and EudragitRS100 content (independent variables) were determined on the particle size, entrapment efficiency and %cumulative drug release of MS1–MS9. The optimized formulation, MS5 (d_{mean} = 44.47 μm, %EE = 98.73, %CDR = 97.32 and followed zero order release) was developed into colon-targeted matrix tablet using calcium pectinate as the matrix. The optimized colon-targeted tablet (MS5T2) shielded MLX loaded microsponges in gastrointestinal region and selectively delivered them to colon, as vizualized by vivo fluoroscopy in rabbits. The pharmacokinetic evaluation of MS5T2 in rabbits, revealed appearance of drug appeared in plasma after a lag time of 7 h; a t_{max} of 30 h with Fr = 61.047%, thus presenting a formulation suitable for targeted colonic delivery. CLSM studies provided an evidence for colonic luminal retentive ability of microsponges at the end of 8 h upon oral administration of MS5T2. Thus calcium pectinate matrix tablet loaded with MLX microsponges was developed as a promising system for the colon-specific delivery that has potential for use as an adjuvant therapy for colorectal cancer.

1. Introduction

Polysaccharides that are metabolized by the colonic flora, have gained popularity as colon specific drug delivery carriers due to the attractive features of being non-toxic, hydrophilic, gel-forming, biodegradable, inexpensive, abundant and are available in various grades of varying properties. Other advantages include their amenability to chemical and biochemical modifications. Pectin, a linear heterogeneous polysaccharide composed of α-(1 → 4) linked D-galacturonic acid residues, interrupted by 1,2-linked L-rhamnose residues (Ashford et al., 1993) is refractory to gastric and intestinal enzymes. However, it is completely degraded by the colonic bacterial enzymes to produce soluble oligalactorunates, and hence presents a major constraint in its application as colonic delivery carrier. This limitation may be overcome by modifying its solubility while retaining its biodegradability. Calcium pectinate, a less soluble form of pectin forms an 'egg-box' configuration in water due to restricted entry of aqueous media (Morris et al., 1982) and can be effectively used as colon specific matrix carrier.

Apart from being site specific, retention of drug or its carrier system on the colonic surface is yet another important consideration that guided the selection of microsponges® as the drug carrier system in the present project. This system that has the ability to entrap wide range of active materials due to numerous interconnected pores, and can adsorb high quantity of active pharmaceutical ingredients on its surface and/or load into the bulk of the particle (Orlu et al., 2006). Microsponges can also increase the lag time for absorption of drug as these get entrapped on the surface of the colon (Comoglu et al., 2007) and thus have the potential for being developed as colon-targeted drug delivery system.

Meloxicam, a preferential COX-2 inhibitor has been evidenced to inhibit the proliferation of colorectal cancer cell cultures. Nevertheless, the inhibitory action of meloxicam was observed specifically on the cells involved in prostaglandin synthesis in a concentration dependent manner (Goldman et al., 1998).

The authors in their research work have reported that meloxicam can reduce the expression of vascular endothelial growth factor and angiopoietin-2 at the protein and mRNA level in colon carcinoma cell line. Some workers have suggested local targeting of the meloxicam as a viable adjuvant therapeutic agent (Wolfesberger et al., 2006) for the treatment of various types of cancers.

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Based on these considerations the present project was approached (a) to optimize the modified quassi emulsion solvent diffusion method using water soluble porogen for the preparation of the meloxicam loaded microsponges, (b) to explore the utility of solubility modulated pectin as matrix forming polymer for the development of uncoated matrix tablet for colon targeting, and (c) to prove that the microsponges are resilient enough to withstand the compression forces of tablet and have colonic luminal surface retentive ability.

2. Materials and methodology

2.1. Materials

Meloxicam (MLX) was gifted by Cadila Pharma Ltd., Ahmedabad, India. Eudragit RS100 (ERS) was procured from Degussa India (P) Ltd., Mumbai, India. Pectinase was a gift sample by Shamli distillery and Chemical Works, Shamli, Uttar Pradesh, India. Pectin and sodium chloride was purchased from S.d. Fine Chem Ltd. Mumbai, India. HPMC K 30 M was procured from Sigma–Aldrich, New Delhi, India. PVPK30 and PVA were purchased from Qualikems Fine Chemicals Pvt. Ltd., New Delhi, India.

2.2. Methodologies

2.2.1. Preliminary studies

For in vitro adsorption study, MLX was dissolved in 100 ml phosphate buffer, pH 6.8 till saturation was achieved. A 10-fold weight of grounded ERS was added to form homogeneous dispersion. The dispersion was magnetically stirred (100 rpm) at $30 \pm 2\,^{\circ}C$ for 4 days. Samples were periodically drawn at 0, 1, 2, 4, 24, 48, 72 and 96 h, filtered through nylon disc filter #22 μm, diluted appropriately and assayed spectrophotometrically at 353 nm (Shimadzu, Pharmaspec 1700, Tokyo, Japan) at 361 nm. A graph of percent drug adsorbed versus time was made and paired student *t*-test was applied to select the optimum time required for stirring. Level of significance was set at ($p < 0.05$). Equilibrium solubility study was performed by the shake flask method. Based on the preliminary optimization studies (data not shown) the formulative excipients were added separately into four different flasks containing 10 ml of double distilled water followed by excess amount of drug. The flasks were allowed to shake for 72 h at 25 °C using a mechanical water bath shaker (Jindal Industries, Ambala, India). At the end of 72 h the samples were filtered using nylon disc filter (#22 μm) and analyzed spectrophotometrically after appropriate dilution.

2.2.2. Fabrication and evaluation of microsponges

2.2.2.1. Method development for the fabrication of microsponges. Previously described methods of microsponge preparation by quassi emulsion technique using porogen (Bae et al., 2009; Graves et al., 2005) were modified to employ aqueous solution of sodium chloride as porogen. 1% (w/v) aqueous solution of the porogen (NaCl) was prepared and sufficient amount of Span 80 was added to it with agitation to obtain 1% (v/v) dispersion. A solution of the ERS and MLX was prepared in DCM and the porogen solution (0.1 ml) was uniformly emulsified in it, to form a w/o emulsion. 10% (w/v) aqueous PVA solution (external phase) was prepared separately and the previously prepared w/o emulsion was emulsified in it. This w/o/w emulsion was stirred for 24 h to get microsponges that were filtered, dried at 60 °C and stored in dessicator.

2.2.2.2. Microsponge formulation. Microsponges were fabricated using 3^2 full factorial design (Table 1) and a total of nine experimental formulations (MS1–MS9) were prepared by varying both, the volume of DCM and the quantity of ERS at three different levels. The dependent variables were the particle size, entrapment efficiency and percent cumulative drug release at 8 h.

2.2.2.3. Particle size determination. The particle size of microsponge formulations was determined by Dynamic laser scattering (Beckman Coulter LS 13 320 Brea, California, US). The aqueous microsponge suspension was continuously added dropwise to the sample cell attached inside the counter till the obscuration rate of 5% was achieved. Particle size distribution and other statistical parameters of the size distribution were analyzed by the inbuilt software version 3.29.

2.2.2.4. Determination of drug content, entrapment efficacy and drug loading. Microsponges, theoretically equivalent to 10 mg of drug were crushed and extracted using methanol by vortexing. Centrifugation (R-4C, Remi centrifuge, Vasai, India) of the sample at 2000 rpm ($224 \times g$) for 10 min separated the insoluble residue and the centrifugate was analyzed spectrophotometrically at 353 nm after appropriate dilution. Percent drug content, %entrapment efficiency and %drug loading were calculated.

2.2.2.5. In vitro drug release. Microsponges equivalent to 10 mg of drug were placed in the basket (mesh #230 = 63 μm) of USP apparatus 1 and the study was performed in the 900 ml of simulated colonic fluid (SCF) without pectinase for 8 h at 50 rpm, maintained at $37 \pm 0.5\,^{\circ}C$. Aliquots were withdrawn periodically and sink conditions were maintained by adding equal amount of release medium. The samples were analyzed spectrophotometrically and %cumulative drug release (CDR) versus time plots was constructed.

2.2.2.6. Statistical analysis of data. The effect of independent variables on the responses was modeled by Design Expert software version 8.0.5.2 (Stat-Ease, Inc., Minneapolis, USA). Polynomial equations were generated for the dependent variables that were reduced by removing non-significant coefficients by applying one way ANOVA. Level of significance was set at $p < 0.05$.

2.2.2.7. Validation of experimental design. The experimental design was validated by an extra check point formulation (MS10). The predicted values for particle size, entrapment efficiency and $\%CDR_{8\,h}$ for MS10, generated by their respective polynomial equations were compared with experimental values and tested for statistical significance using pooled *t*-test at 95% confidence interval, and degree of freedom = 4 ($p < 0.05$).

2.2.2.8. Surface morphology and rheological characterization. The optimized microsponge formulation (MS5) was vizualized by scanning electron microscope (SEM, JEOL 5400, Tokyo, Japan) to assess the morphological changes in the microsponge and its surface before and after drug release. The samples were coated with gold under argon atmosphere using gold sputter module in a high vacuum evaporator (Sputter Coater unit VG Microtek, West Sussex, UK) and observed under various magnifications. Microsponges were also evaluated for angle of repose, Hausners's ratio and Carr's compressibility index (Staniforth, 2007).

2.2.3. Modification of pectin and its characterization

Pectin (PCT) was crosslinked by ionotropic gelation method. A 20% (w/v) aqueous PCT dispersion was added drop wise into gently stirred 150 ml of 20% (w/v) aqueous solution of calcium chloride pre-adjusted to pH 5.5. The gelled particles were left in the medium for 10 min, washed with deionized water and dried at 60 °C. The particles were screened through sieve no 44 and kept in dessicator to remove the traces of moisture and then evaluated

Table 1
3^2 Factorial design for preparation of meloxicam loaded microsponges.

Formulation code	Amount of drug (mg)	Volume of organic media (X_1)	Polymer content (X_2)	Dependent variables
MS1	400	−1 (5 ml)	−1 (400 mg)	Particle size (Y_1)
MS2	400	+1 (7 ml)	+1 (1200 mg)	
MS3	400	0 (6 ml)	0 (800 mg)	%Entrapment efficiency (Y_2)
MS4	400	−1 (5 ml)	+1 (1200 mg)	
MS5	400	+1 (7 ml)	−1 (400 mg)	
MS6	400	0 (6 ml)	−1 (400 mg)	
MS7	400	−1 (5 ml)	0 (800 mg)	%Cumulative drug release after 8 h (Y_3)
MS8	400	+1 (7 ml)	0 (800 mg)	
MS9	400	0 (7 ml)	+1 (1200 mg)	
MS10[a]	400	+0.5 (6.5 ml)	−0.5 (600 mg)	

[a] Extra design check point.

for micromeritic, rheological and swelling characteristics (Sharma et al., 2008) and surface morphology by SEM.

2.2.4. Formulation and evaluation of colon-targeted matrix tablet

Colon targeted matrix tablets of MLX loaded microsponges were prepared by wet granulation method (Table 2). Calcium pectinate (matrix diluent) was mixed with the optimized MS5 and aerosil (lubricant). The binder(s) was dissolved in 0.5 ml of IPA and utilized for powder blending. The wet mass was passed through sieve no.16 to obtain granules which were dried in hot air oven at 50 °C for 2 h. The dried granules were then passed through sieve no. 20 and compressed using single station electrical punching machine (Hicon Enterprises, New Delhi, India). The tablets were evaluated for various US pharmacopoeial parameters and other aspects including diameter, thickness and friability.

2.2.4.1. In vitro transit time determination. The tablets ($n = 6$) were placed in tablet disintegration test machine USP and subjected to regular up and down movement in a series of gastrointestinal fluids at 30 ± 2 cpm. The sequence followed was exposure to simulated gastric fluid (SGF, pH 1.2) for initial 2 h followed by simulated intestinal fluid (SIF, pH 7.4) for the next 6 h at 37 ± 0.5 °C. The tablets were visually observed for any damage and disintegration.

2.2.4.2. In vitro drug release. The study was performed in USP drug release apparatus 1 in various release media sequentially SGF (pH 1.2) for 2 h, followed by SIF (pH 7.4) for next 6 h and then in SCF (containing pectinase, 120 IU) for further 16 h). The test conditions were 900 ml of medium stirred at 50 rpm and maintained at 37 ± 0.5 °C. Aliquots were withdrawn at regular intervals and replaced with equal amount of the fresh medium to maintain sink condition. The samples were analyzed at 344 nm, 364 nm and 361 nm for SGF, SIF and SCF, respectively. Concomitantly the swelling characteristics and physical behavior of tablets were observed at 0, 2, 5, 8, 10, 14, 18 and 24 h and recorded visually. The systematic evaluation of MS5T1–MS5T4 tablets led to identification of optimized tablet formulation.

2.2.5. Animal studies

All the animal experiments have been conducted in full compliance with the institutional ethical and regulatory principles and as per the spirit of Association for Assessment and Accreditation of Laboratory Animal Care & International's expectations for animal care and use/ethics committees. The investigations were performed after obtaining approval by the Institutional Animal Ethical Committee of Rajiv Academy for Pharmacy, Mathura, India (IAEC no: IAEC/RAP/3248/2011).

Table 2
Formulation design of colon-targeted matrix tablets containing meloxicam loaded microsponge formulation MS5.

Code	MLX	Binder by wt		Aerosil by wt	Calcium pectinate q.s.
MS5T 1	10 mg	PVP K 30 M	10%	2%	250 mg
MS5T 2	10 mg		20%	2%	250 mg
MS5T 3	10 mg	HPMC K 30 M	10%	2%	250 mg
MS5T 4	10 mg		20%	2%	250 mg

2.2.5.1. In vivo fluoroscopy. The MLX loaded microsponges in MS5T2 tablets was partially replaced with barium sulfate microsponges and administered orally with 15 ml of water, to New Zealand white rabbits ($n = 3$; 3–3.5 kg) that were fasted overnight with free access to water. Fluoroscopic images of the abdomen of the rabbits were taken before administration (0 h) and at 1.5 h, 3.5 h, 4 h, 5 h, 7 h and 8 h to trace the in vivo movement and behavior of the tablet in the gastrointestinal tract. X-ray images of the rabbits in prone position were captured using L&T Vision 100 (C-arm) X-ray machine (Larsen & Tourbo Limited, Mumbai, India), at 64 mAs and 63 kV voltage.

2.2.5.2. Luminal surface retention of microsponges in the colon. Rhodamine B loaded microsponges were formulated, loaded in optimized tablet formulation and administered to the New-Zealand white rabbits ($n = 3$; 3–3.5 kg). After 8 h the animals were sacrificed, colon was traced, separated, opened and washed with the phosphate buffer pH 6.8. The microscopic slides were prepared for the radial and internal surface view and observed by Confocal laser scanning microscope at varying magnification (Olympus Fluoview FV 1000, Olympus, Japan).

2.2.5.3. In vivo pharmacokinetics. New Zealand white rabbits weighing 3–3.5 kg were classified in two groups namely standard and test with three animals in each group. The animals were fasted overnight with free access to the water. The standard group received the in-house developed immediate release tablet containing animal dose, while the test group received site targeted formulation (MS5T2) containing animal dose. One milliliter of the blood sample was withdrawn from the marginal ear vein at various time points of 0, 1, 2, 4, 7, 10, 15, 20, 24, 30, 36, 40 and 44 h. The blood samples were centrifuged at 4000 rpm (894 g) for 15 min to separate the plasma. The plasma was analyzed by the validated method developed by Velpandian et al. (2000). Briefly 0.5 ml plasma, 50 μl internal standard (piroxicam; 50 μg/ml in methanol) and 150 μl 1 M HCl were mixed and centrifuged at 1000 rpm for 15 min. Chloroform (5 ml) was added to the supernatant, agitated and left for equilibrium. The lower organic layer was separated and evaporated under vacuum. The obtained residue was re-suspended in 200 μl mobile phase and 50 μl was injected. The mobile phase consisted of an aqueous solution of diammonium hydrogenorthophosphate (50 mM), methanol and acetonitrile in the ratio of (5:4:1, v/v). The samples were analyzed by the HPLC (column C18 length 125 mm,

Adept Cecil CE 4201, Cambridge, England) using the UV detector at 364 nm.

3. Results and discussion

3.1. Preliminary studies

In vitro adsorption study was done to estimate the time required for maximum drug entrapment during microsponge formulation. ERS has ammonio methacrylate units that confer positive charge on the polymer surface while the drug molecule (MLX) with thiazolyl group is alkaline in character, and has minute negative charge. Therefore the drug molecule can undergo ionic adsorption on the surface of the polymer. Thus 40% adsorption was recorded in the initial 2 h thereafter the rate slowed down due to limited availability of adsorption sites and reached equilibrium within 24 h. No significant difference ($p < 0.05$) was observed in the percent drug adsorbed at 24 h and beyond 24 h (Fig. as supplementary file). Thus it can be assumed that a stirring time of 24 h during drug loading will provide the maximum drug entrapment. Equilibrium solubility study was done to resolute the levels of the excipients required for the preparation of microsponges. The study revealed that the magnitude of drug's aqueous solubility increased in the presence of all excipients except sodium chloride (Table 3).

Supplementary material related to this article found, in the online version, at doi:10.1016/j.ijpharm.2012.01.036.

3.2. Formulation and evaluation of the drug loaded microsponges

3.2.1. Method development for the preparation of microsponges

In present study, sodium chloride was used as porogen, based on the considerations that use of hydrogen peroxide (Bae et al., 2009) and sodium bicarbonate (Graves et al., 2005) as porogen(s) result in the evolution of gasses like nascent oxygen or carbon dioxide respectively, that may interact with the active material and cause its degradation. NaCl as porogen, is inert in terms of evolution of gas and its high aqueous solubility would facilitate its easy extraction in the outer aqueous phase during the microsponge formation. Therefore using sodium chloride as porogen, nine formulations (MS1–MS9) were prepared and evaluated for dependent variables and production yield.

3.2.2. Particle size

The particle size of the microsponges ranged between 44.47 μm (MS5) and 56.25 μm (MS4). On applying one-way ANOVA, it was observed that the experimental design had significant influence on the particle size. As the amount of the drug to be incorporated was kept constant, any change in particle size was influenced by the variation in the levels of ERS (polymer) and the volume of DCM. At lower level of DCM, the microsponges MS1, MS4 and MS7 of mean diameter of 53.42 μm, 56.25 μm and 55.88 μm, respectively, were obtained. However, on increasing the volume of DCM, microparticles with smaller diameter were formulated. Thus MS2, MS5 and MS8 yielded smaller microsponges of 48.73 μm, 44.47 μm and 46.19 μm respectively. It can be interpreted that the high viscosity of internal phase led to an increase in surface tension producing larger globules for solidification (Nokhodchi et al., 2005).

3.2.3. Entrapment efficiency

Analysis of the data in Table 4 revealed that level of polymer should have significant influence on the entrapment efficiency. However, statistical analysis by Design expert software 8.0.5.2 contradicted the assumption and hence factors influencing the entrapment efficiency were identified as the volume of DCM and the particle size. Apart from these two factors the porous structure of the particle can also be considered as an important factor for the entrapment of drug. The entrapment efficiency was observed to increase with an increase in the level of DCM that can be attributed to better solubilization of the drug in the organic medium. Higher volume of the organic phase resulted in the more uniform mixing of the drug and polymer resulting in a more uniform matrix with high drug entrapment efficiency.

Particle size also had an impact on the entrapment efficiency but it was not statistically significant. The observations suggested that entrapment efficiency of the microsponges increased with the decrease in the particle size. Consequently MS5 of 44.47 μm had 98.73% entrapment efficiency while the MS4 with the particle size of 56.25 μm entrapped 79% of MLX. As a well-known fact, lower the particle size more will be the availability of surface containing active sites for adsorption of drug, resulting in the better drug loading (Devrim and Canefe, 2006).

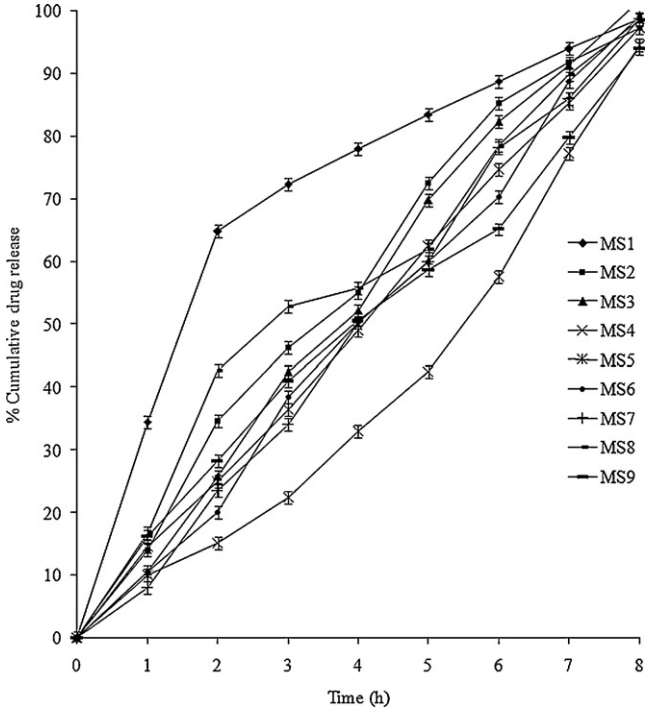

Fig. 1. In vitro drug release profile of the microsponge formulations (MS1–MS9) in 900 ml of SCF at 37 ± 0.5 °C for 8 h using USP apparatus 2 (basket type).

3.2.4. In vitro drug release

The percent cumulative drug release in the SCF was observed to range from 94% (MS9) to 101.72% (MS3) suggesting the ability of the microsponges to release the drug completely. All the formulations followed zero order release kinetics (Fig. 1) except MS1 and MS4 that followed Peppas and first order kinetics respectively (Table 4). Zero order release of drug from microsponges, has been reported by Devrim and Canefe (2006). The mechanism of drug release from microsponges as explained by various researchers is correlatable to its porous surface. The porous surface of the carrier particle enables easy penetration of the release media and its accessibility to the entrapped drug molecule. In case of MS1 and MS8, a high initial rate of the drug release in 2–3 h was observed due to the surface adsorbed drug molecule that underwent quick release to attain the equilibrium with the dissolved drug molecule.

Additionally, as suggested by various research reports, control on the drug release can also be achieved by uniform mixing of the drug with polymer to form homogenous organic phase. MS1 formulated with low levels of polymer and DCM might have resulted in the improper encapsulation of the MLX in the ERS matrix. Due to the

Table 3
Influence of various excipients used for preparation of microsponges on the solubility of meloxicam.

Drug/excipient	Concentration (% w/v)	Solubility (μg/ml)	Change in solubility	%Change in solubility	Reason	Reference
MLX	–	17.38	–	–	Poor wettability	
PVA	1	144.51	127.14	731.73	Dispersing property and hydrophilic character of PVA	Devrim and Canefe (2006)
ERS 100	2	32.68	15.31	88.06	Decrease in the drug particle size to molecular level	Demirbas et al. (2004)
Span 80	0.1	151.29	133.92	770.78	Surfactant action	
NaCl	0.1	9.85	−7.52	−43.29	Decrease in the apparent solubility product resulted in increased activity coefficient	Serajuddin et al. (1987)

abrupt encapsulation, majority of the drug molecules may reside on the particulate surface as adsorbed molecules that have the probability of undergoing quick solubilization and hence quick drug release. But after achieving equilibrium, the rate of drug release decreased. This effect was not predominant in MS8 as is constituted intermediate level of ERS and high level of DCM. While in MS4, the release fitted first order model that may be due to the fact that the drug release from the polymeric microsponge occurs on transition of the polymer, and as the amount of polymer in microsponges increases the time required for phase conversion also increases (Jain and Singh, 2010). Thus in MS4, initial drug release was due to the adsorbed molecules but with lapse of time, ERS layer underwent transition resulting in the abrupt or burst release of the MLX.

3.2.5. Statistical analysis

Statistical analysis was done by Design expert software version 8.0.5.2 (Stat-Ease, Inc., Minneapolis, USA) and the second order polynomial equations were derived. The transformed equations are,

$$\text{Particle size } (Y_1) = 50.29 + 4.89X_1 - 1.06X_1^2 + 1.52X_1X_2 - 0.81X_1^2X_2 - 1.95X_1X_2^2 + 0.87X_1^2X_2^2 \quad (7)$$

$$\%EE(Y_2) = 87.45 + 4.10X_1 - 4.19X_1^2 - 1.37X_1X_2 - 3.32X_1^2X_2 + 2.08X_1X_2^2 - 1.98X_1^2X_2^2 \quad (8)$$

$$\%CDR_{8\ h}(Y_3) = 97.83 + 0.82X_1X_2 + 0.24X_1^2X_2 - 0.54X_1X_2^2 + 1.54X_1^2X_2^2 \quad (9)$$

where X_1 and X_2 are the independent variables. The effects of various independent variables on the dependent variables are illustrated in Fig. 2 that clearly depicts the effect of varying the levels of volume of DCM and polymer content on particle size, %EE and $\%CDR_{8\,h}$.

3.2.6. Validation of the experimental design

An extra design check point formulation (MS10) was made and the predicted value and experimental values of dependent variables were compared using pooled *t*-test at 95% confidence interval, degree of freedom 4 and $p < 0.05$. No significant difference was recorded between the two values (Table 4) thereby establishing validity of the generated model.

3.2.7. Selection of optimized formulation

Eventually MS5 of smallest particle size of 44.47 ± 31.98 μm, maximum entrapment efficiency of $98.73 \pm 0.08\%$, $CDR_{8\,h}$ of $97.32 \pm 1.37\%$ and desirability factor of 0.792 was identified as the optimized formulation and was used for development of colon target tablet using calcium pectinate as the site specific matrix.

3.3. Modification of pectin to calcium pectinate

Modification of pectin was accomplished for its intended use as matrix forming agent of colon-targeted tablet. As the pectin is soluble in water, it cannot be considered suitable matrix forming agent that can shield the drug load effectively during its transit in stomach and small intestine. Thus pectin was cross-linked with Ca^{+2} so that the soluble fibrous pectin would change into the insoluble globular calcium pectinate. Calcium pectinate forms egg box configuration (Morris et al., 1982), which is metabolized by the colonic microflora (Rubinstein et al., 1993). These features guided the selection of calcium pectinate as matrix agent for colon-targeted systems and its feasibility was assessed.

PCT and calcium pectinate were compared for their swelling characteristics. Both the forms showed no signs of swelling in the SGF (pH 1.2) but PCT swelled considerably (460%) in the SIF (pH 7.4) while calcium pectinate was resistance to swelling in the SIF. The swelling of calcium pectinate was reduced by 95.65% to a value of 20%. The reduction in swelling of the calcium pectinate in SIF presents its feasibility to be used as carrier for the colon-targeted drug delivery. Earlier reports on calcium pectinate microparticles claim their potential as carrier for colonic drug delivery (Rubinstein et al., 1993; Jain et al., 2007) and floating drug carriers (Badve et al., 2007; Sriamornsak et al., 2005). Our investigation forms the first

Table 4
Compilation of the evaluation parameters of meloxicam loaded microsponges.

Formulation code	Production yield (%)	Particle size (μm)	Entrapment efficiency (%)	Drug content (%)	Drug loading (%)	% CDR after 8 h	Zero order model (r^2)
MS1	75.00	53.42 ± 36.08	96.67 ± 0.27	128.89 ± 0.36	64.45 ± 0.18	98.87 ± 0.06	0.0536
MS2	64.38	48.73 ± 34.97	71.34 ± 0.44	110.82 ± 0.68	27.71 ± 0.17	97.32 ± 0.49	0.9831
MS3	71.67	50.76 ± 38.91	86.39 ± 0.29	120.54 ± 0.39	40.18 ± 0.13	101.72 ± 1.96	0.9906
MS4	78.13	56.25 ± 39.85	79.24 ± 0.29	101.43 ± 0.10	25.36 ± 0.03	94.64 2.15	0.9631
MS5	73.75	44.47 ± 31.98	98.73 ± 0.08	133.87 ± 0.11	66.94 ± 0.05	97.32 ± 1.37	0.9992
MS6	65.00	47.14 ± 36.21	86.43 ± 0.22	132.97 ± 0.33	66.49 ± 0.17	99.19 ± 0.41	0.9962
MS7	82.50	55.88 ± 34.28	98.74 ± 0.26	119.68 ± 0.32	39.89 ± 0.11	98.74 ± 0.43	0.9963
MS8	76.50	46.19 ± 28.96	92.56 ± 0.13	120.99 ± 0.17	40.33 ± 0.06	98.60 ± 0.88	0.9603
MS9	72.75	49.81 ± 37.28	76.95 ± 0.67	105.78 ± 0.93	26.45 ± 0.23	94.05 ± 1.13	0.9899
MS10[a]	Experimental value	51.68 ± 32.75	86.53 ± 0.58	119.23 ± 0.08	47.69 ± 0.32	98.56 ± 0.41	0.9889
MS10[a]	Predicted value	51.28	86.375			98.32	

[a] Extra design check point.

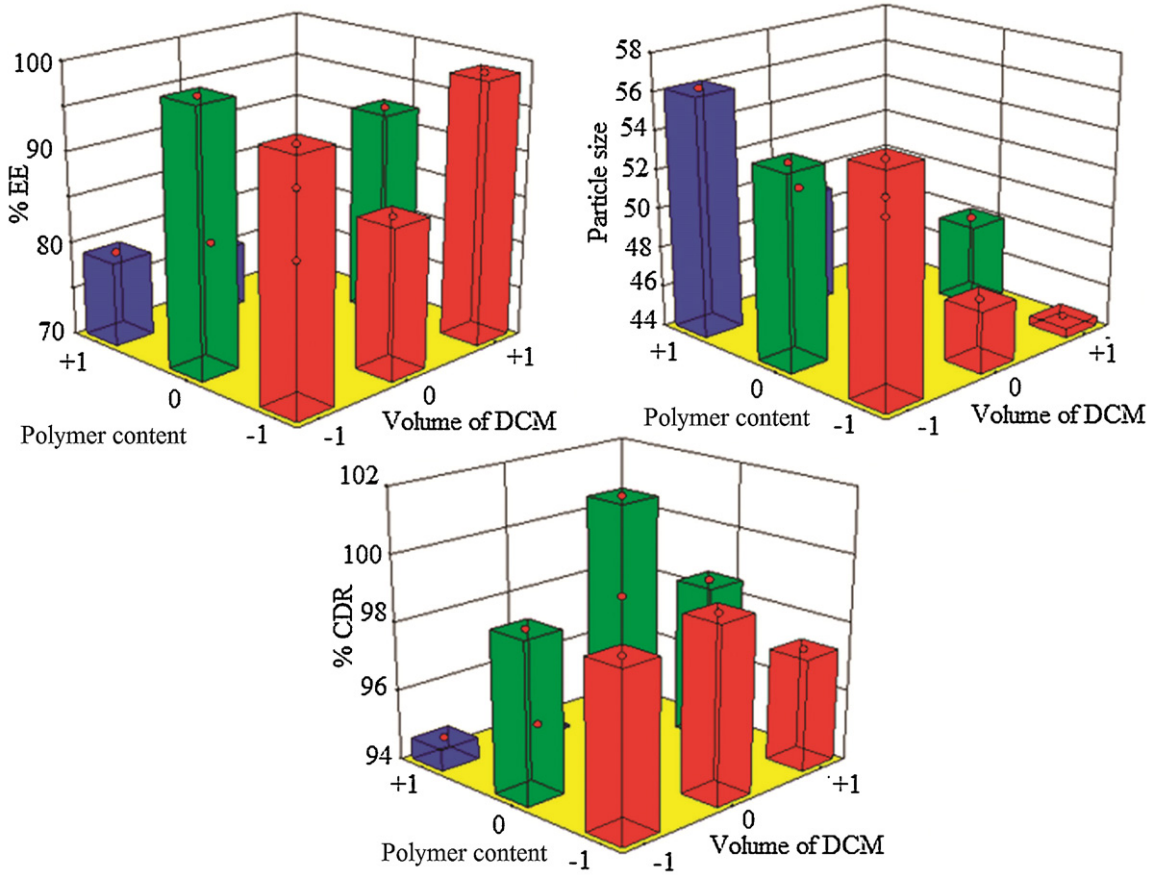

Fig. 2. 3D bar surface chart depicting the influence of independent variables over dependent variables.

report on use of calcium pectinate as tablet matrix excipient for colon-targeted system.

3.4. Characterization of MS5 and calcium pectinate

3.4.1. Micromeritic and rheological characterization

The particle size distribution curve for the MS5 and calcium pectinate showed nearly symmetrical size distribution (Fig. as supplementary file). The particle size analysis revealed lower d_{mean} of 44.47 μm for MS5 with a leptokurtic distribution (kurtosis = 2.983) than d_{mean} of 106.5 μm for calcium pectinate with platykurtic distribution (kurtosis = −0.362). Higher d_{mean} of matrix former than microsponges is assumed to be a favorable characteristic as smaller microsponges will be shielded by the carrier matrix to safely reach the colon. Analysis of the rheological characteristics of MS5 and calcium pectinate (Table 5) confirmed good rheological properties suitable for production steps.

Supplementary material related to this article found, in the online version, at doi:10.1016/j.ijpharm.2012.01.036.

3.4.2. Morphology and shape

SEM images of MS5 before in vitro release test, revealed spherical, smooth surfaced uniformly porous particles. The surface was smooth with numerous tiny pores on it (Fig. 3a and b). After the drug release for 8 h the structural integrity was not lost but the particles were no longer truly spherical (Fig. 3c). The surface view revealed numerous regular striations (Fig. 3d) due to surface erosion of microsponges that was result of phase transition of ERS caused by release of surface associated drug molecules. The SEM image of the cross-section of MS5 (Fig. 3e) revealed numerous interlinked pores throughout the particle conforming the spongy structure.

On the other hand, micrographs of PCT demonstrated smooth doughnut shaped particles more or less, of uniform size (Fig. 3f) while calcium pectinate comprised of regular, spherical particles that were smooth surfaced (Fig. 3h and j). The uniformly spherical shaped particles with smooth surfaces of both MS5 and calcium pectinate present advantageous tablet manufacturing features.

Table 5
Micromeritic and rheological characteristics of the MS5 and calcium pectinate (modified pectin).

Parameter	MS 5	Calcium pectinat
Mean diameter (μm)	44.47	106.5.5
Standard deviation (μm)	31.98	53.11
Specific surface area (cm^2/ml)	3345	1554
Skewness	1.569 (Right)	0.014 (Right)
Kurtosis	2.983 (Leptokurtic)	−0.362 (Platykurtic)
IQCS	0.2029	−0.054
Median (μm)	36.48	108.2
Mode (μm)	37.97	116.3
$d_{50\%}$ (μm)	36.48	108.2
Variance (μm^2)	1023	2821
Loose bulk density (mg/cm^3)	283.33	416.67
Tapped bulk density (mg/cm^3)	314.81	454.54
True density (mg/cm^3)	326.54	–
Hausner's ratio	1.11	1.09
Carr's compressibility index	9.99	8.33
Angle of repose	19.10 ± 3.76°	21.10 ± 3.25°

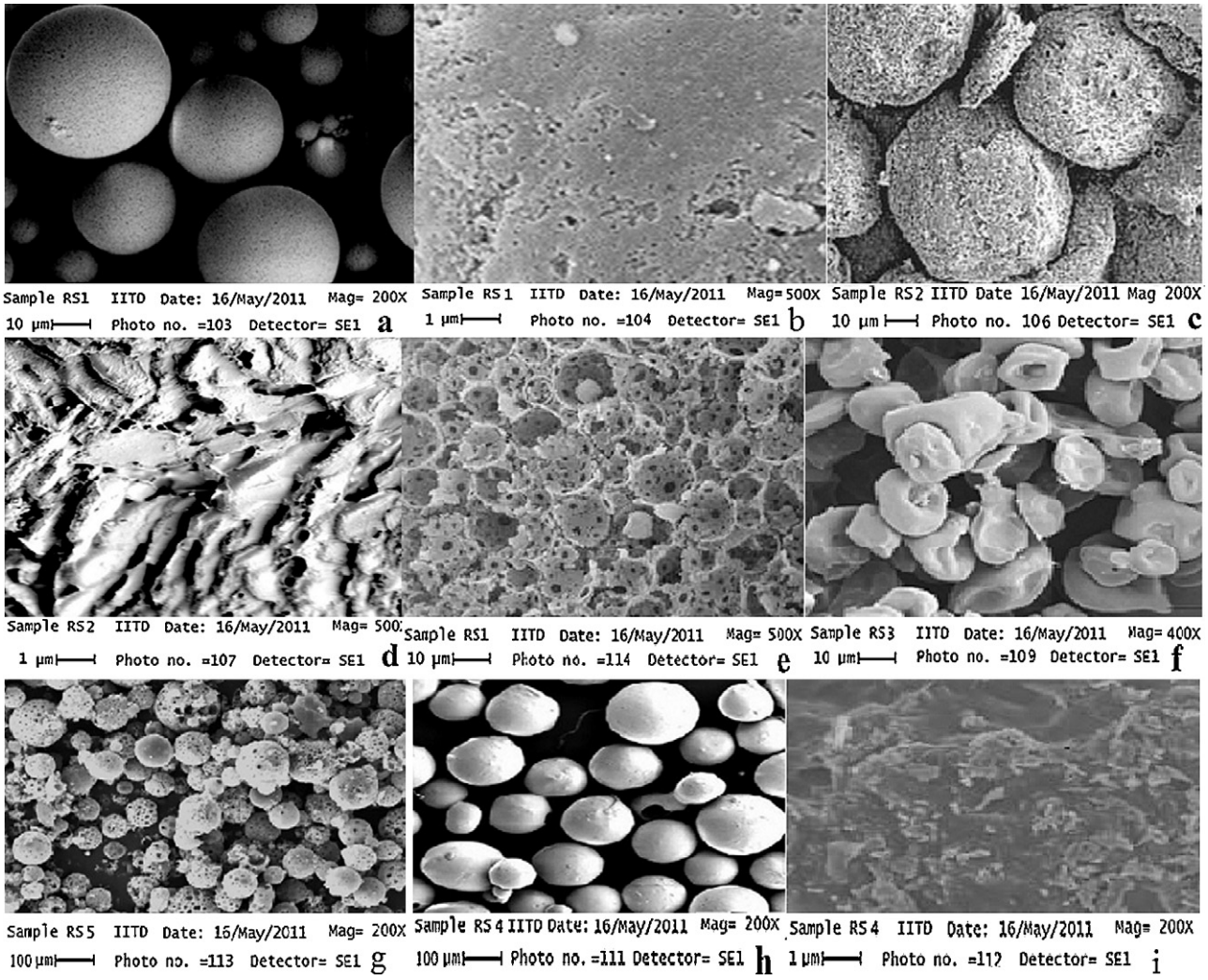

Fig. 3. SEM images of (a) MS5 before drug release, (b) surface view of MS5 before drug release, (c) MS5 after drug release, (d) surface of MS5 after drug release, (e) cross section of MS5, (f) PCT, (g) crushed tablet depicting spherical microsponges, (h) calcium pectinate and (i) surface of calcium pectinate.

3.5. Evaluation of the colon-targeted matrix tablets

3.5.1. Pharmacopoeial evaluation

Various US 2004 pharmacopoeial characteristics of the colon-targeted tablets are enlisted in Table 6 that suggests acceptable characteristics. All the formulations displayed a hardness ranging from 6.87 to 7.20 kg and friability below 0.5% that indicated sufficient mechanical strength of the tablets and also affirms the ability of calcium pectinate for use as tablet matrix. The drug content of all the formulations was more than 99%. Thus the process used for the tablet preparation can be said as precise.

3.5.2. In vitro GI transit time

The tablets were evaluated for in vitro GI transit time test to predict their fate during passage through the stomach and small intestine. For efficient site targeting for drug release to the colon the matrix must remain intact during its passage through the stomach and small intestine. Thus a matrix tablet intended for colon targeting must not disintegrate as it traverses through GIT. All the formulations, except MS5T3, were resistant to disintegration and the surface remained intact. MS5T3 that fragmented during the test was rejected and rest of the formulations were vizualized as promising colon-targeted systems.

3.5.3. In vitro drug release

The in vitro drug release profiles of MS5T1, MS5T2 and MS5T4 (Fig. 4) showed that the tablets were capable to restrict the drug release in gastric region as well as in the intestinal region as no release was documented in the initial 5 h. However, MS5T1 displayed surface fracture after 5 h causing premature drug release

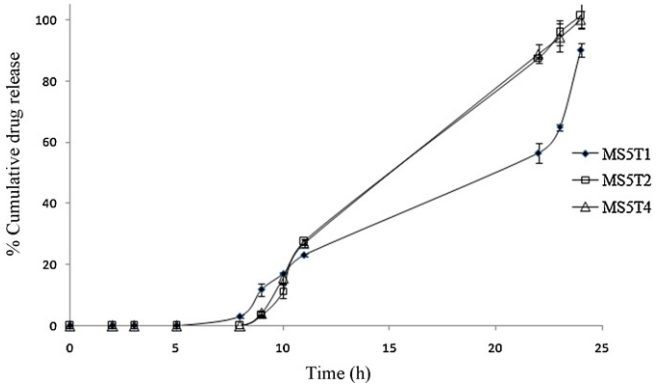

Fig. 4. In vitro drug release profile of the colon-targeted matrix tablets in various release media at 37 ± 0.5 °C, using USP apparatus 2 (basket type). Volume of media was 900 ml.

Table 6
US Pharmacopoeial characterization of colon-targeted calcium pectinate matrix tablets constituting meloxicam loaded microsponges.

Parameter	MS5T1	MS5T2	MS5T3	MS5T4
Hardness (kg)	6.87 ± 0.31	6.93 ± 0.23	6.87 ± 0.31	7.2 ± 0.4
Friability (%)	0.44	0.43	0.63	0.56
Weight (mg)	227 ± 6.57	227 ± 4.70	229.5 ± 6.05	237 ± 5.71
Tablet diameter (mm)	8 ± 0.01	8 ± 0.01	8 ± 0.01	8 ± 0.02
Tablet thickness (mm)	4.125 ± 0.03	3.8 ± 0.05	4 ± 0.01	4.4 ± 0.07
Drug content (%)	99.42 ± 0.73	99.56 ± 0.25	99.67 ± 0.31	99.47 ± 0.55

in 8 h, and hence was discarded. This observation revealed that the amount of the binder had major impact on the colon targeting ability of the dosage form. Consequently, MS5T3 and MS5T1, both with low levels of binder failed the in vitro screening tests and were abandoned. Whereas, the formulations MS5T2 and MS5T4 remained intact and showed %CDR of 100.1% and 96.78%, respectively in the SCF in 24 h. Physical observations of these formulations beyond 8 h revealed swelling of tablets till 10th hour followed by gradual erosion. The swelling of the matrix was attributed to breaking of cross-linked chains of the calcium pectinate that subsequently got eroded in the presence of pectinase. MS5T4 displayed gradual swelling and did not undergo erosion, but was fragmented as layers in 18–24 h that limited complete release of the microsponges while MS5T2 was completely eroded within 24 h with %CDR of 99.87%. The swelling and physical behavior of the tablets in various test release media are presented in Table 7 that helped in understanding the release pattern of the formulations based on their constituent binder.

HPMC in MS5T4, due to its hydrophilic characteristic can absorb the aqueous phase forming a colloid (Rowe et al., 2006) that may act as a gel layer on the matrix (Orlu et al., 2006). This gel like protective structure has the potential to limit the penetration of enzymatic media to the crosslinked pectin and avoids enzymatic metabolism. While PVP in MS5T2, due to its water soluble characteristic underwent solublization in the aqueous phase (Rowe et al., 2006) and the enzymatic media readily permeated the matrix causing its erosion. Thus MS5T2 released the microsponges more efficaciously and completely in simulated colonic fluid and was hence was selected for in vivo evaluation.

3.5.4. Microsponge resiliency after tablet compression

As the microsponges are claimed to be resilient these should not show any sign of disruption even after compression as tablet. This was verified by SEM micrographs of crushed tablet. Fig. 3g clearly shows the spherical porous particles (microsponges) without any signs of fracture or damage that were structurally similar to fresh microsponges. Thus it can be claimed that the microsponges are resilient enough to withstand the compression force applied during tablet compression, again a favorable manufacturing aspect.

3.6. Animal studies

3.6.1. In vivo fluoroscopy

The series of results of roentgenographic study are shown in Fig. 5. Fig. 5a is the X-ray image of the empty rabbit stomach, while Fig. 5b shows that the position of the tablet in the esophagus just after the administration of the tablet. Fig. 5c shows intactness of the tablet after 1.5 h in the stomach. Fig. 5d exhibits no significant difference in the integrity of tablet in comparison to Fig. 5c there by indicating intactness of the tablet on reaching the small intestine and its intactness was retained after 4th and 6th h of administration (Fig. 5e and f) i.e. throughout the small intestine. Reduction in size of tablet was seen in Fig. 5g indicating erosion of the matrix in colon and in Fig. 5h the intact tablet cannot be evidenced. These images clearly demonstrated the efficiency of the selected formulation (MS5T2) for site specific targeting of the MLX microsponges to colon.

3.6.2. Surface retention in the colonic surface

The luminal surface view and radial section of rabbit colon after 8 h showed the presence of fluorescent dye on the luminal surface and penetration of drug across colonic wall respectively. The luminal wall (Fig. 6a) revealed the presence of dye entrapped microsponges on its grooved surface and the permeation of marker across the colonic wall (Fig. 6b) evidenced the absorption of marker but the microsponges have the capacity to retain on the luminal surface of the colon. This can be considered as an advantage for a colonic drug delivery system intended for localized treatment.

3.6.3. In vivo pharmacokinetics

The in vivo pharmacokinetic study was performed to establish the ability of the microsponges to deliver and retain the active agent at the desired targeted site. For efficient local treatment in colon the drug must get retained in the colonic tissue and preferably should not enter the systemic circulation. The mean plasma drug concentration levels of MLX after per-oral administration of optimized colon-targeted matrix tablet (MS5T2) and in house produced immediate release (IR) tablets, of dose strength = 4 mg are shown in Fig. 7. The appearance of the MLX in blood plasma was documented after 4 h exhibiting the C_{max} of 3.64 μg/ml observed after 7 h (t_{max}). While in case of colon-targeted tablets, MLX appeared in plasma after 7 h (lag time) of administration with a C_{max} of 2.923 μg/ml at 30 h. A lag time of 7 h for the developed colon-targeted tablets indicated the ability of the colon-targeted formulation to prevent the release of MLX in the stomach and small intestine.

The C_{max} of colon-targeted formulation was significantly ($p < 0.001$) less than the C_{max} of IR formulation, suggested reduced systemic absorption of drug from MS5T2. This means that larger fraction of drug was available on the colonic surface for local action. $AUC_{0-\infty}$ for IR formulation and colon-targeted tablets was found

Table 7
Sequential swelling characteristics and physical behavior of selected matrix tablets in simulated gastric fluid(SGF), simulated intestinal fluid (SIF) and simulated colonic fluid (SCF).

Media	SGF		SIF		SCF			
Time (h)	0	2	5	8	10	14	18	24
MS5T1	No effect	No effect	No effect	Appearance of cracks	Swelling	Swelling and erosion	Erosion	Complete erosion
MS5T2	No effect	No effect	No effect	No effect	Swelling	Swelling and erosion	Erosion	Complete erosion
MS5T4	No effect	No effect	No effect	No effect	Swelling	Swelling	Swelling coupled with fracture initiation	Fractured as layers

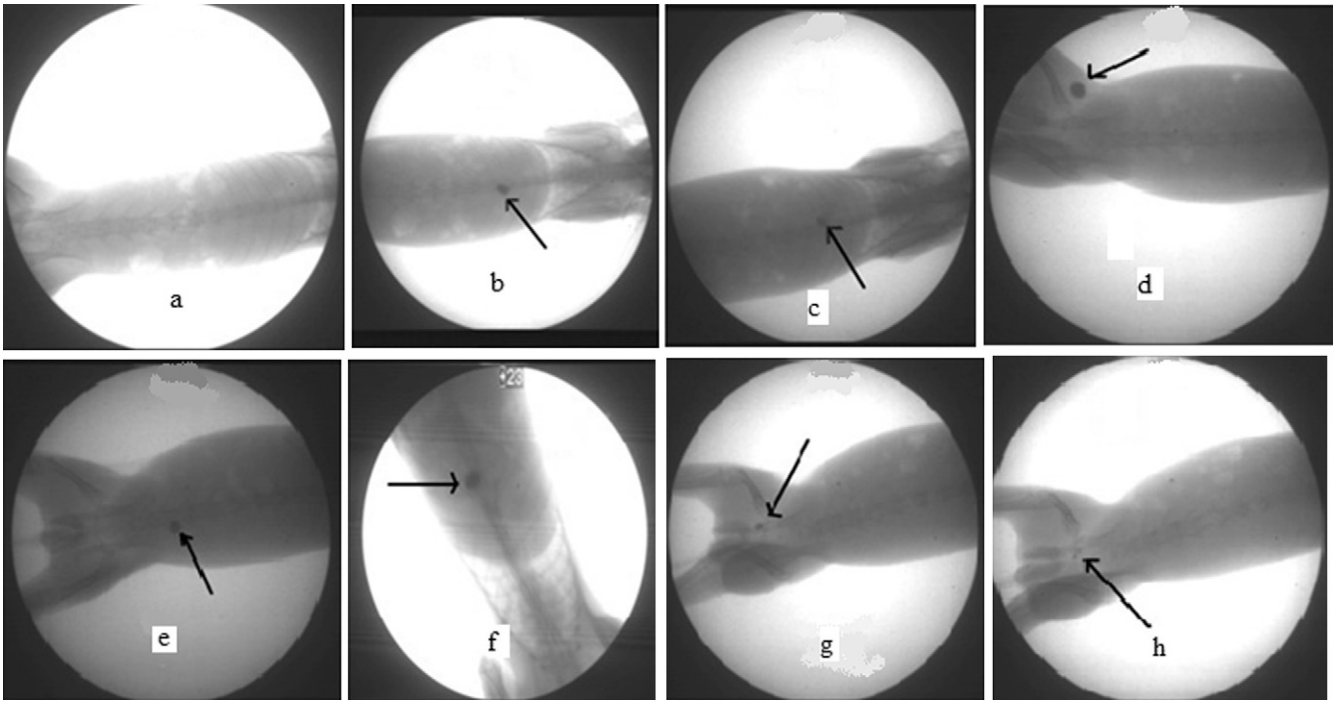

Fig. 5. In vivo fluoroscopic images (a) empty stomach, (b) just after tablet administration, (c) 1.5 h (stomach), (d) 3.5 h (pyloric junction), (e) 4 h (small intestine), (f) 5 h (intestinal–colon junction), (g) 7 h and (h) 8 h (colon).

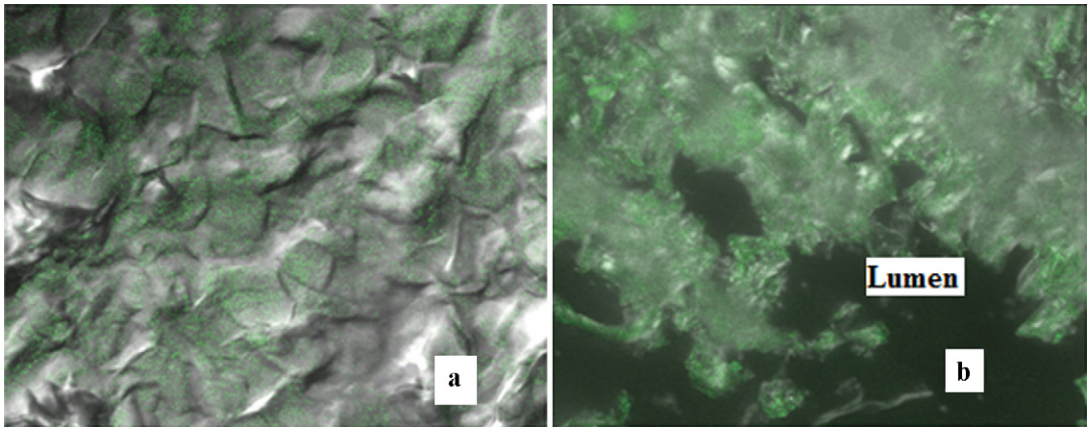

Fig. 6. CLSM images depicting the surface retentive capacity of the microsponges. (a) Surface view showing uniform distribution of the marker loaded microsponges in the groves of internal surface, (b) radial view high density of the marker on the internal surface.

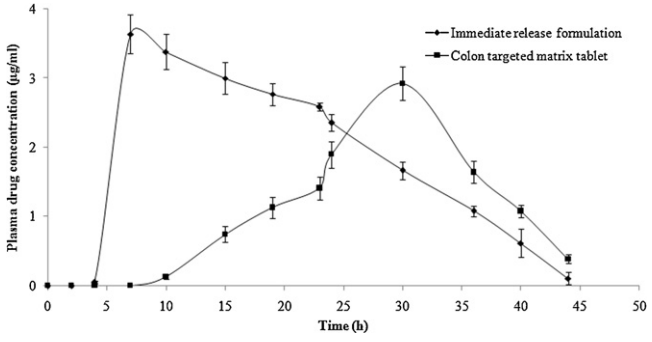

Fig. 7. In vivo pharmacokinetic profile of the MLX in New Zealand white rabbits following per-oral administration of immediate release tablets and colon-targeted matrix tablet.

to be 82.6673 μg h/ml and 50.466 μg h/ml, respectively. Consequently the Fr (relative bioavailability) of colon-targeted tablet was determined as 61.047%. These results are in confirmation with the CLSM studies that clearly evidenced retention of marker loaded microsponges on the colonic surface. Thus, in vivo pharmacokinetic studies of calcium pectinate-based colon-targeted tablets composed of the MLX loaded microsponges (MS5T2), exhibited increased lag time, delayed t_{max}, decreased C_{max} and reduced bioavailability. It can thus be concluded that the developed colon-targeted formulation has the ability to avoid the drug release in the upper GIT, but can release the active agent specifically in the colon exert local action with reduced systemic exposure.

4. Conclusion

A simple inexpensive and patient friendly dosage form of MLX was developed for colon-targeted delivery that has potential for use

as an adjuvant therapy in treatment of colorectal cancer. The colon-targeted calcium pectinate matrix comprising of microsponges demonstrated two distinct features. (1) The ability and suitability of calcium pectinate as a specific carrier for colon-targeted system and (2) the biological surface retention ability of microsponges will ensure local effect and reduced systemic exposure that can reduce peripheral side effects.

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