IJPSR (2017), Volume 8, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 March, 2017; received in revised form, 22 May, 2017; accepted, 27 May, 2017; published 01 October, 2017

POLYSACCHARIDE-MICROSPONGE BASED MATRIX TABLET FOR COLON TARGETING OF KETOPROFEN: *IN VITRO* AND *IN VIVO* EVIDENCE

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Keywords:

Ketoprofen, Controlled release, Chitosan, Ulcerative colitis, Colon targeting, Matrix tablet

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ABSTRACT: The present research was aimed to formulate erosion based matrix tablets of ketoprofen micro-sponges for spatial-colon targeting. For the optimization, the effects of independent variables like solvent volume and concentration of chitosan were studied on the mean particle size, percent entrapment efficiency (% EE) and *in vitro* drug release. Scanning Electron Microscopy (SEM) image of the micro-sponge formulation revealed that the surface of the micro-sponge was nearly spherical and porous. Optimized micro-sponge formulation was further formulated into erosion based matrix tablet and evaluated for quality control parameters. *In vitro* release studies revealed that both the micro-sponge formulation and micro-sponge matrix tablet (MST) had restricted the drug release of ketoprofen in gastric pH followed by gradual release up to 12 hr. The release kinetics data, after fitting into various models revealed that both formulations were best fitted to zero order, indicating controlled release property. The pharmacokinetic evaluation of colon targeted MST in rats revealed the presence of drug in plasma after lag time of 4 hr, t_{max} of 4 ± 0.23, AUC (0-12hr) of 33.10 ± 3.68 with $t_{1/2}$ 3.85 ± 0.31. The developed MST of ketoprofen has the ability for spatial and temporal delivery, thus could be employed to treat various colonic diseases.

INTRODUCTION: Ulcerative colitis and Crohn's disease are the two major chronic inflammatory bowel diseases (IBDs) involving the large intestine or colon.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(10).4250-60

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (10).4250-60

Conventional therapy, for the treatment of IBDs employing anti-inflammatory drugs, is associated with problems *viz.* absorption and degradation in the upper gastro-intestinal tract (GIT), and also some serious side-effects [1-3]. Colon-specific drug delivery systems has been widely used owing to their special advantages *viz.* drug targeting, dose reduction, less systemic side effects and enhanced drug efficacy [4-5]. Colon-targeted drug delivery is necessary for the local treatment of different bowel diseases *viz.* ulcerative colitis, cirrhosis, amebiasis,

colonic cancer and other colonic pathologies [6]. In the past, several non-steroidal anti-inflammatory drugs (NSAIDs) have been used for the treatment of IBD. Ketoprofen is one of them and is often prescribed in the treatment of IBDs [7]. Oral route is the most preferred route for the administration of active drug. Owing to its low cost of rehabilitation and ease of administration, this may lead to higher level of patient acceptance [8-9].

However, oral administration of ketoprofen poses several problem *viz.* high first-pass metabolism and short half-life, and hence, demands increase in dosing frequency to maintain the optimum therapeutic concentration [10]. Moreover, ketoprofen also causes other gastrointestinal (GI) problems like stomach ache and indigestion, which can lead to decrease in patient-compliance [11]. Thus, there is a need to develop an efficient delivery system of ketoprofen which would deliver the drug only at the specific site. Further, it would prevent the drug from early degradation, as the drug is enclosed inside the micro-sponge and also the frequency of dosing could also be decreased *via* controlled delivery of drug over longer period of time and hence the patient compliance.

Earlier, various novel drug delivery systems, *viz*. nanoparticles, micro-particles, microspheres, nano-spheres and micro-sponges have been exploited for site-specific drug delivery [12-13]. These systems when made of pH sensitive polymers *viz.* Eudragit, chitosan could prevent the drug release at certain pH and release of drug only at desired site. Micro-sponge is highly porous, polymer based, cross linked, spongy spheres, having particle size in the range of 5 to 150µm and offers targeted and controlled release of drug by the way of polymers [14, 13]. Compared to other delivery systems, micro-sponge offers high drug payload and cost effectiveness [15]. Further, micro-sponge also has the ability to retain on the surface of colon for longer period [14] and can increase the drug absorption. Currently, the micro-sponge has widely been exploited in the topical delivery systems mainly for cosmetics [16].

Controlled release dosage forms are the systems which delivers the drug in a predetermined manner. The purpose of controlled release dosage forms is to deliver the drug at the constant rate to maintain the drug concentration in the blood or at target site [17]. Polymeric matrix systems are widely used in oral sustained or controlled release drug delivery systems due to its ability to obtain a desirable drug release profile, cost effectiveness, and wide range of acceptance [6, 18].

Hence, considering the advantages of micro-sponge systems for colon-targeted delivery and polymeric matrix system for modified release, a dual approach of ketoprofen MST has been conceived in the present work. Hitherto, only one research paper on micro-sponge formulation of ketoprofen employing Eudragit as a polymer has been reported; however, no reports are traceable on ketoprofen-loaded MST. Herein, different micro-sponge formulations were optimized on the basis of concentration of polymer, and solvent, *i.e.* dichloromethane (DCM), and the best composition was formulated into the MST employing pectin as a matrix forming material.

Finally, the optimized formulation was evaluated employing acetic acid induced ulcerative colitis model in rats. The pharmacokinetic studies was also employed and various parameters were determined *viz.*, V_{max}, $t_{1/2}$, AUC (0-12h) and C_{max} respectively.

MATERIALS AND METHOD:

Materials: Ketoprofen was procured *ex gratis* from M/s Odian Pvt. Ltd, Solan (H.P). Acetone, sodium chloride (NaCl), polyvinyl alcohol (PVA), sodium dihydrogen phosphate, potassium dihydrogen phosphate, sodium hydroxide (NaOH) and pectin were procured from M/s Nice Chemical Pvt. Ltd., Cochin, while ethanol, DCM and hydrochloric acid (HCl) were purchased from M/s) Merck Specialities Pvt. Ltd., Mumbai. Span 80 was purchased from M/s Qualikems laboratory reagents, New Delhi; and chitosan from M/s Hi Media Laboratory Pvt. Ltd., Mumbai. PVP K30 and microcrystalline cellulose (MCC) were obtained from M/s Loba chemical Pvt. Ltd., Mumbai.

Fabrication of Microsponges: Microsponges were prepared based on 3^2 full factorial design and total nine formulations were prepared, *i.e.* [F1–F9]. Formulations were varied for volume of DCM (solvent) and chitosan amount at three different levels as showed in **Table 1**.

Micro-sponges were prepared by quasi emulsion technique, herein; aqueous solution of NaCl was used as a porogen. Firstly, w/o emulsion was formed by mixing aqueous phase and organic phase on magnetic stirrer. For aqueous phase, 1% (w/v) of NaCl solution containing sufficient quantity of Span 80 was taken. For organic phase, chitosan was first dissolved in glacial acetic acid solution and was added into the DCM containing dissolved ketoprofen. Primary w/o emulsion so formed was then added into 5% w/v solution of PVA on continuous stirring leading to the formation of w/o/w emulsion. This emulsion was continuously stirred for 2 hr on mechanical stirrer to result into the formation of micro-sponges. Lastly, micro-sponges were dried at 60°C and stored in desiccator until further use [19].

TABLE 1: 3^2 FACTORIAL DESIGN OF KETOPROFEN- LOADED MICROSPONGE FORMULATION

Sr. no.	Formulations	PVA (g)	Chitosan (mg)	Solvents (mL)
1	F1	5	400	5
2	F2	5	600	6
3	F3	5	800	5
4	F4	5	400	7
5	F5	5	600	5
6	F6	5	800	7
7	F7	5	400	6
8	F8	5	600	7
9	F9	5	800	6

Drug = 400mg was taken in all the formulations

Characterization of Micro-sponges:

Fourier Transform Infrared Spectroscopy (FTIR) Analysis: FTIR spectra were observed (FTIR Instrument of Agilent Technologies 630 Cary) in order to examine the possible drug-excipient chemical interactions or compatibility. I.R. spectra of ketoprofen, PVA, chitosan alone and physical mixture containing all the excipients were taken in 1:1:1 ratio. Spectra were obtained by running Micro Lab software and interpretation was made.

Micromeretics: Mean particle size of drug-loaded micro-sponges was determined employing Zeta-Seizer (M/s Malvern Instruments, Mastersizer 2000, UK) at University Institute of Pharmaceutical Sciences (UIPS), Chandigarh, India. All the samples were diluted 50 times before analysis. The samples were placed into cuvettes and intensity of fluctuation of laser beam was recorded and correlated with the particle size of the dispersed phase [20].

Morphology: To observe the surface of micro-sponge, dried samples were mounted on a metal stub using double-sided adhesive tape and sputter coated with gold for 1 minute under vacuum and then observed under (SEM) at 10 kV (M/s QUANTA 250, FEI Makers, Singapore) [21].

Determination of Percent Entrapment Efficiency (%EE) and Percentage Yield (% Y): Ketoprofen-loaded micro-sponges (100mg) were crushed and extracted by 10mL of ethanol. The solution was then sonicated on ultra-sonicator (M/s Citizone-ultrasonic-Model-YJ5120) for 30 minutes followed by filtration. Filtrate containing entrapped drug was analyzed by UV spectrophotometer (M/s Systronics-Model-2202) at 258 nm. The amount of % EE and % Y was calculated by using following equations (1) and (2) respectively [22].

$$\% EE = \frac{\text{Mass of drug in micro-sponge}}{\text{Initial mass of drug}} \times 100 \text{-----(1)}$$

$$\%Y = \frac{\text{Mass of obtained micro-sponges}}{\text{Initial mass of drug + Initial mass of polymer}} \times 100 \text{----(2)}$$

***In vitro* Drug Release:** Dissolution study of all the prepared micro-sponge formulation was carried out employing USP–Type-II dissolution apparatus for a period of 12 hr. Temperature was maintained at 37 ± 5 °C, while stirring speed was kept at 100 rpm. The study was performed at different pH (pH 1.2, for 2 hr, and pH 6.8 and 7.4 for subsequent hours) to simulate the same conditions of GIT. After 12 h, samples were analyzed in UV spectrophotometer (M/s Systronics-Model-2202) at 258nm against 0.1 N HCl (pH 1.2), phosphate buffer (pH 6.8 and pH 7.4) respective controls [23-24].

Formulation and Characterization of Erosion Based MST of Ketoprofen-Loaded Micro-sponges: The best optimized micro-sponge formulation was formulated into erosion based matrix tablets prepared by direct compression method. All the powdered excipients Pectin, PVPK30 and MCC were weighed accurately and mixed together. Micro-sponges were crushed separately into a fine powder. Then, micro-sponges containing 75 mg of drug were added and mixed together. The mixture was passed through sieve no 22. This powder was directly compressed into tablets employing Rota-tory Tablet Punching machine (M/s Cadmech. Pvt. Ltd.) [25].

Characterization of MST: Different Pharma-copoeial tests were carried out for evaluation of MST like weight variation, hardness, friability according to IP [26].

***In vitro* Drug Release of MST:** *In vitro* dissolution study of ketoprofen from MST was evaluated employing same procedure as described in above section 2.3.4.

Kinetics of Release: In general the release of the drug from matrix system depends upon several factors *viz.* drug solubility, porosity, polymer system and matrix shape and size. Release data with the best optimized micro-sponge formulation and its MST were fitted to various mathematical models to study the drug release mechanisms. Zero order (% cumulative drug release vs. time) (equation 3), first order (log % drug release vs. time) (equation 4), Higuchi model (% cumulative drug release vs. square root of time) (equation 5), and Peppas model (log % drug release vs. log time) (equation 6). The kinetic model was selected based on best fit with highest value of regression coefficient (r^2) [25].

$$Q_t = k0t \text{---------- (3)}$$

$$\ln Q_t = \ln Q_{\alpha+} k_1 t \text{---- (4)}$$

$$Q_t = k\sqrt{t} \text{ ---------- (5)}$$

$$Q_t = k_k t \text{ ---------- (6)}$$

Here Q_t is the released amount of drug at time t, Q_α is initial amount of drug, whereas, k_0, k_1, k and k_k are the corresponding release rate constants for zero-order, first-order Higuchian and Korsmeyer-

In vivo pharmacodynamic study a separate section *in vivo* pharmaco-dynamic study.

Acetic Acid Induced Experimental Ulcerative Colitis in Colon: Wistar Albino rats of either sex (160–200 g), were selected and caged individually with food and water *ad libitum*. All the studies were conducted with prior approval of Institutional Animal Ethical Committee of Panjab University (PU/IAEC/2014/95). The rats were distributed randomly into four groups with each group comprised of 5 animals. Group-I received normal saline without drug, group II received ketoprofen alone, group III and IV received ketoprofen loaded micro-sponge, and MST of ketoprofen, respectively. Colitis was induced in rats by intra-rectal administration of 1mL (4%) (v/v) of acetic acid which resembled with the inflammatory bowel disease in all groups [27-28]. Rats were housed for three days without any treatment to maintain the development of full IBD model. Each group received the treatment orally in 1% carboxymethyl cellulose (w/v) solution. Group 1 received vehicle only, group 2 received pure ketoprofen suspension (6.25mg/kg), group 3 received ketoprofen loaded micro-sponges (20mg/kg) and group 4 received MST of ketoprofen containing animal dose.

Pharmacological Assessments: Animals were sacrificed after 24 hr of last dose, colon part was removed, and ulcer projections were visualized and evaluated on the basis of the inflammatory scales; *i.e.*, 0 = normal colored colon, 0.5 = red coloration, 1 = spot ulcer, 1.5 = hemorrhagic streaks, and 2 = hemorrhagic ulcer

***In vivo* Pharmacokinetic Studies:** The *in vivo* pharmacokinetic study was performed to establish the ability of the micro-sponges 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. In order to investigate the plasma drug level of ketoprofen, pure ketoprofen suspension (6.25mg/kg) in normal saline, optimized micro-sponge formulation (MS) (20mg/kg) and (MST) containing animal dose were administered orally. Approximate 500µl of blood was collected after regular interval 0, 1, 2, 4, 6, 8, 10 and 12 hr from the retro orbital plexus using a heparinized glass capillary.

Each blood samples were centrifuged for 2 min at 14000 g at 4 °C. Supernatant was collected and filtered through a 0.22µ membrane filter. Then 50µL plasma was separated and transferred to centrifuge tubes containing 10µL potassium dihydrogen phosphate (1mol/L) [29]. The filtrate was quantified for the drug concentration in plasma by HPLC at λ_{max} 258nm [30].

Stability Study: The stability of optimized ketoprofen micro-sponge formulation and MST were carried out according to International Conference on Harmonization (ICH) guidelines at accelerated conditions. The formulations were kept at 40 °C ± 2 °C and 75% ± 5% RH for three months. After 3 months micro-sponges were analyzed for physical appearance and drug content

Statistical Analysis: The data obtained are reported as mean ± SD. The data obtained from different formulations were compared for statistical significance by the one-way analysis of variance (ANOVA) followed by Student's t-test using Microsoft Excel. A difference was considered statistically significant at the level of $p < 0.05$.

RESULTS AND DISCUSSION:

FT-IR Spectra: Compatibility study was done by IR spectroscopy. In overlay spectra, it was found that there was no change in major peak of drug after adding excipients. FTIR spectroscopy showed the structural stability of drug and compatibility with other excipients. FTIR of ketoprofen **Fig. 2**. showed band stretching of C=O at 1695 cm-1, aromatic C=O stretching at ~1655 cm-1 and 1595 cm-1, -CH-deformation of aromatic ring ~ 860 cm-1 and 690 cm-[1]. Observed overlay IR spectra is shown in **Fig. 1**.

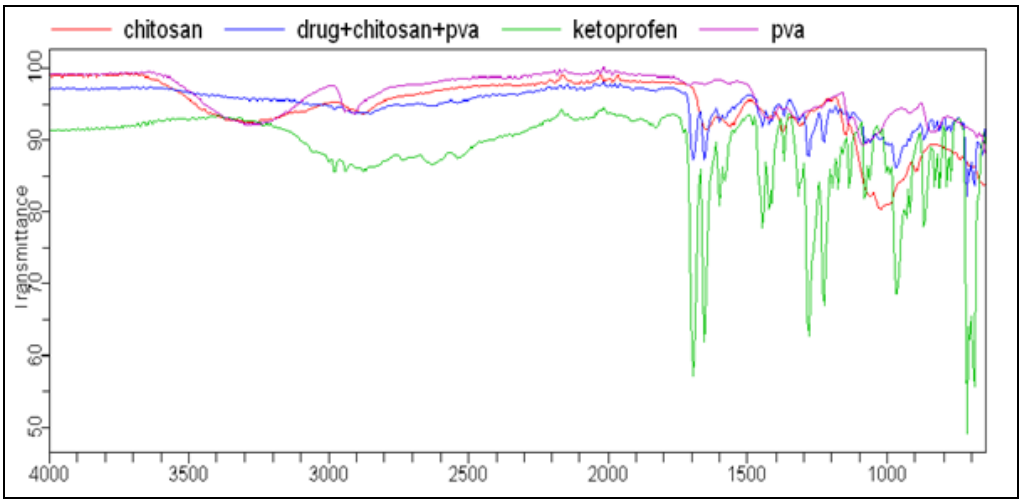

FIG. 1: IR SPECTRA OF KETOPROFEN WITH OTHER EXCIPIENT

Formulation of ketoprofen-loaded Micro-sponges: 3^2 factorial design was used to formulate the various batches of ketoprofen-loaded chitosan micro-sponge by quassi emulsion technique. In the present study chitosan was used as a polymer for the fabrication of micro-sponge due to its ability to release the drug specifically at colon pH. The formation of micro-sponge was facilitated by the prompt mixing of the DCM and water at the interface which had resulted in precipitation of the chitosan. The latter being insoluble in water had led to the formation of shell like structures enclosing DCM and drug [11]. Further, NaCl was used as porogen. The higher aqueous solubility of NaCl could facilitate its easy extraction in the outer aqueous phase during the micro-sponge formation [31, 19]. Different formulations were prepared by varying the solvent (DCM) content and polymer level to get the best optimized formulations. All the formulations (F1–F9) were then evaluated for % EE, %Y and average particle size.

Particle Size and Surface Morphology: Particle size was influenced by the variation in the levels of chitosan (polymer) and the volume of DCM (solvent). The average particle size of all the formulations were found to be in the range of 9.27 to 52.42µm as showed in **Table 2**. The DCM had more pronounced effect on the particle size of micro-sponge formulation in comparison to

chitosan. It was observed that on increasing the level of DCM in microsponge formulations, particle size decreased appreciably and the formulation F4 revealed the smallest particle size among all the formulations.

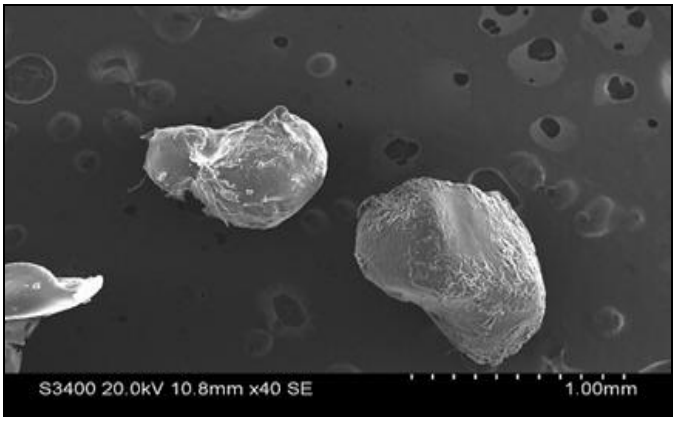

FIG. 2: PHOTOMICROGRAPH OF KETOPROFEN MICRO-SPONGES

This could be attributed to the fact that higher level of solvent leads to decrease in the viscosity and hence, results in the formation of smaller particle size [8]. For the surface morphology, micro-sponge formulation F4 was visualized by SEM. The image revealed nearly spherical and porous surface as depicted in **Fig. 2.**

Determination of (% EE) and (% Y): % EE and % Y values of all the prepared micro-sponge formulation have been shown in **Table 2**. All the formulations revealed high % EE, which could be attributed to the porous structure of micro-sponge formulations. Contrary to the particle size, on increasing the DCM solvent content, % EE was found to be increased. This is related to the fact that the ketoprofen is highly soluble in DCM, therefore, increase in the DCM content leads to better solubilization of the drug in the solvent. Moreover, appropriate mixing of the drug and polymer results in more uniform matrix and thus, high % EE. The particle size also had an impact on entrapment efficiency.

The formulation having smaller particle size revealed better % EE. This could be attributed due to the fact that smaller is the particle size; more is the surface area and hence, more is the entrapment of the drug [19]. Varying chitosan concentration has not revealed any significant impact on % EE.

TABLE 2: COMPILATION OF VARIOUS EVALUATION PARAMETERS OF KETOPROFEN-LOADED MICRO-SPONGES

Sr. no.	Formulation code	Entrapment Efficiency (%EE)	Particle size (µm)	Percentage Yield (%Y)	% CDR after 12 hr
1	F1	45 ± 0.74	50.14	35.57 ± 0.6	52.89 ± 0.11
2	F2	60.71 ± 0.56	47.33	61.89 ± 0.4	54.05 ± 0.62
3	F3	59.88 ± 0.64	49.43	40.43 ± 0.2	53.89 ± 0.06
4	F4	79.52 ± 0.24	9.27	70.05 ± 0.5	71.11 ± 0.20
5	F5	62.28 ± 0.53	52.42	66.94 ± 0.11	51.07 ± 0.04
6	F6	70.96 ± 0.19	38.74	51. 89 ± 0.04	61.56 ± 0.33
7	F7	57.5 ± 0.32	43.57	54.13 ± 0.13	53.37 ± 0.69
8	F8	62 ± 0.53	41.47	60.18 ± 0.2	57.87 ± 0.25
9	F9	61.09 ± 0.21	45.33	53.48 ± 0.51	56.78 ± 0.41

(n=3), ± S.D.

***In vitro* Release Study of Ketoprofen Micro-sponges:** *In vitro* release study of ketoprofen microsponge was conducted in different dissolution media, *i.e.* pH 1.2, pH 6.8 and pH 7.4 to simulate the *in vivo* conditions. The *in vitro* release of drug from micro-sponges is shown in **Fig. 3**. Drug release mechanisms from micro-sponge are correlated to its porous surface. The latter permits easy penetration of the release media and its approach ability to the entrapped drug [19]. It was observed that the micro-sponge formulations were able to restrict the drug release in gastric pH during initial 2hr. This could be related to the pH-sensitive property of chitosan, which might had prevented the drug release in gastric pH followed by significant release at higher pH, *i.e.* pH 6.8 and pH 7.4. Moreover, the maximum drug release was obtained from formulation F4 and could be attributed to its smaller particle size and high % EE. Smaller particle size offers more surface area, and hence, improves the contact between particles and dissolution medium. Also, according to Noyes–Whitney equation smaller particle size offers high surface area and leads to an increased dissolution

rate [32]. Further, due to high entrapment efficiency, majority of the drug molecules could have been adsorbed on the particulate surface, thus, facilitating the quick solubilization and release of the drug. The chitosan concentration also influenced the drug release as the formulation having high chitosan concentration revealed the slower release compared to the formulation having low chitosan concentration. This can be correlated to the fact that release of drug from the polymer matrix takes place after whole swelling of the polymer, and as the amount of polymer in the formulation increases, so the time required to swell also increases, and hence, slower drug release [33].

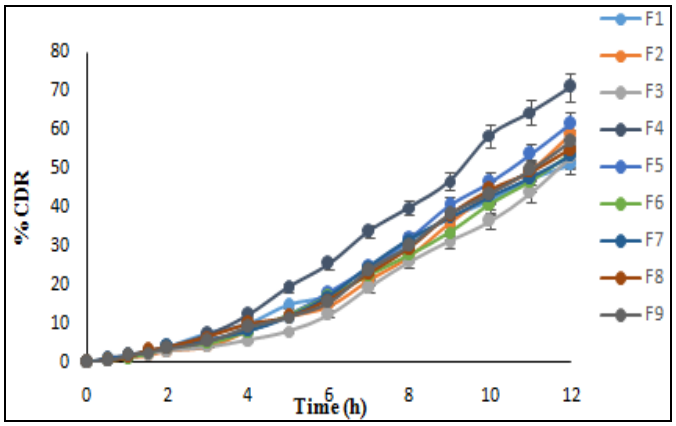

FIG. 3: DISSOLUTION PROFILE OF KETOPROFEN MICROSPONGES

Selection of Optimized Formulation: Finally F4 was selected as the optimized formulation having minimum particle size, *i.e.* 9.98µm, maximum % EE, *i.e.* 79.52 ± 0.24, and % CDR_{12h} of 71.11 ± 0.20. The optimized formulation was employed for development of matrix tablet using pectin as the site specific matrix. Pectin has the ability to act specifically in the colon. As the dosage form passes into colon, pectin get eroded in the presence of enzymes, and thus releases the drug into the vicinity of bio environment of colon [34].

Evaluation of MST:

Pharmacopoeial Evaluation: Pharmacopoeial characteristics of the matrix tablets are shown in **Table 3** and suggest its satisfactory characteristics. All the formulations exhibited a hardness of 5.16 ± 0.13kg/cm^2 and friability below 0.69 ± 0.33 % which indicated enough mechanical strength of the tablets. Average weight of the twenty tablets were found to be 499 ± 1.35 which is well within the IP limit of weight variation *i.e.* (± 5%).

TABLE 3: EVALUATION PARAMETERS OF MST

Parameters	Values
Average weight (n=20) mg	499 ±1.35
Friability test (%) (n=10)	0.69±0.33
Hardness (n=5) (Kg/cm^2)	(5.16±0.13)

***In vitro* Release of the MST of Ketoprofen:** % CDR of optimized microsponge formulation F4 was compared with the % CDR of MST.

However, MST showed more controlled and sustained release of the drug through the matrix as compare to drug loaded microsponges. *In vitro* release study of matrix tablets and optimized microsponge F4 formulation are shown in **Fig. 4**. The tablets were also capable to restrict the drug release in gastric region in the initial 2 hr. This is because of slow swelling property of the pectin followed by gradual release of the drug from the matrix tablets.

The swelling of the matrix results from the breaking of cross-linked chains of the pectin. PVP due to its water soluble property could help in the solubilization in the aqueous phase [35] and helps in the permeation of dissolution media through the matrix causing its erosion.

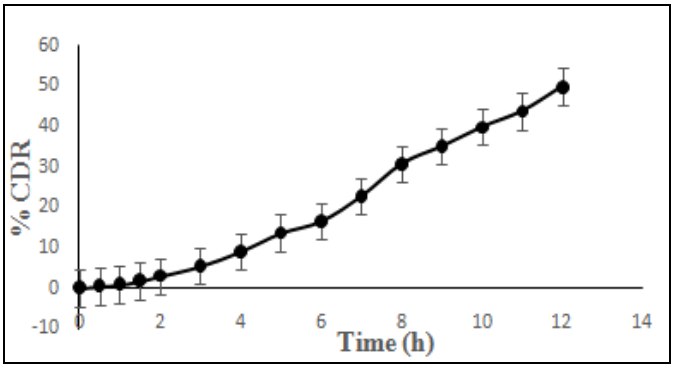

FIG. 4: % CDR (CUMULATIVE DRUG RELEASE) OF MATRIX TABLET

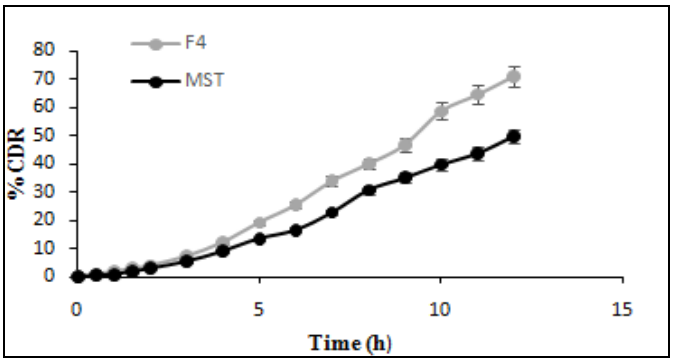

FIG. 5: COMPARISION OF % CDR BETWEEN F4 FORMULATION AND MST TABLET

Kinetic Release Analysis of Microsponge Formulations:

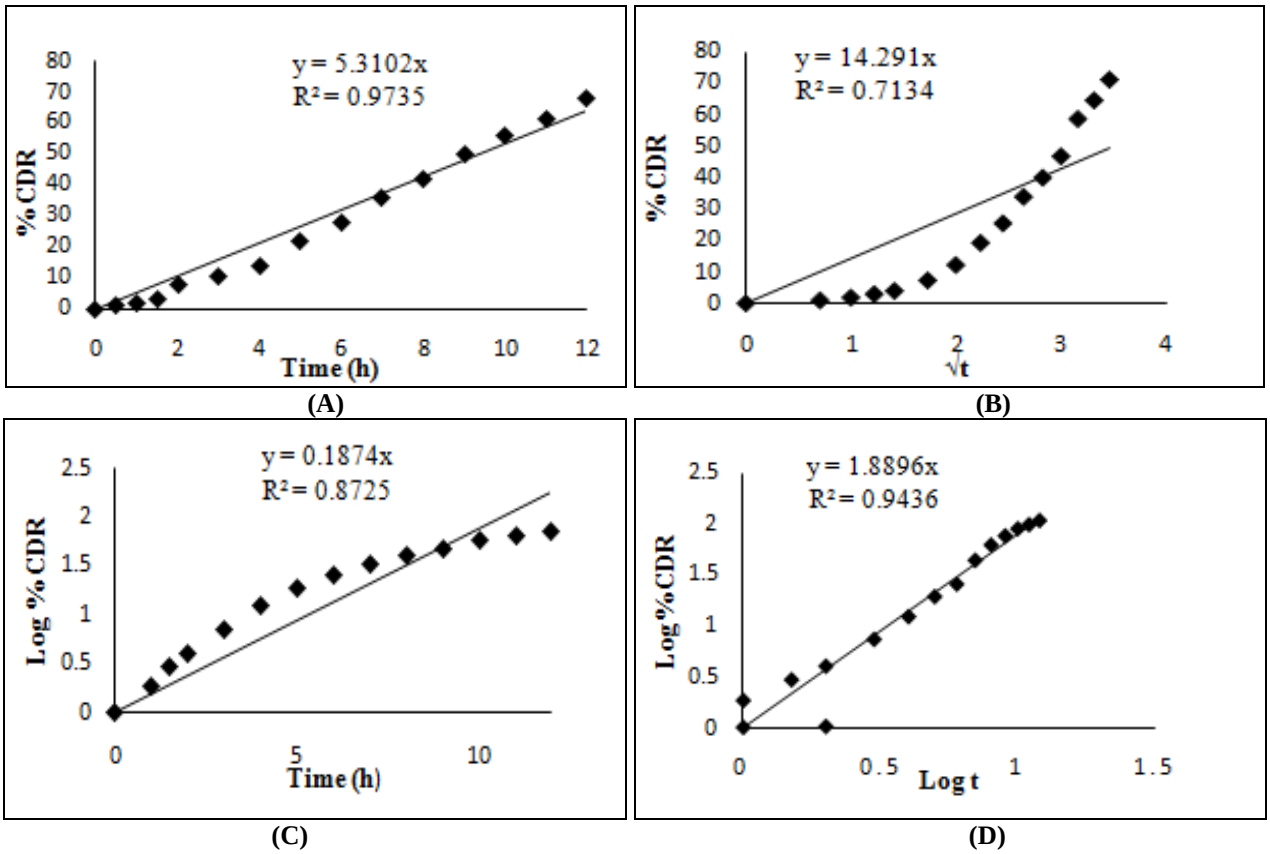

FIG. 5: RELEASE KINETIC OF OPTIMIZED MICRO-SPONGE FORMULATION, WHERE (A) ZERO ORDER RELEASE (B) FIRST ORDER RELEASE (C) HIGUCHI MODEL (D) PEPPAS MODEL

TABLE 4: RELEASE KINETIC OF OPTIMIZED FORMULATION

Kinetic models	R^2 value	
	Regression cofficient (r^2) of microsponge formulation	Regressiojn cofficient (r^2) of microsponge loaded matrix formulation
Zero order	0.973	0.975
First order	0.872	0.928
Higuchi	0.713	0.847
Pappas	0.943, n= 1.886	0.6148, n= 1.18

Different dissolution models were applied to drug release data in order to evaluate release mechanisms and kinetics. R^2 were determined by linear regression analysis [36]. For both the formulations, (r^2) values were best fit within zero order as shown in **Table 4** and **Fig. 5A, B, C** and **D** in comparison to first, Higuchi and Peppas model. The zero order rate defines the systems in which the drug release rate is independent of its concentration. R^2 of micro-sponge formulation and matrix tablet formulation was found to be 0.9735 and 0.975.

***In vivo* Pharmacodynamic Study:** *In vivo* study using acetic acid induced colitis model in rat revealed that ulcers in ketoprofen loaded MST treated group were recovered up to an appreciable extent as compared to control group. While in pure ketoprofen and ketoprofen loaded micro-sponges treated groups the healing was mild as shown in **Table 5.**

TABLE 5: MACROSCOPIC EVALUATION OF COLONIC LESION OF RATS

Groups	Colonic erosion score				
	0	0.5	1	1.5	2
Saline Control	-	-	1	1	2
Pure drug	-	1	3	1	-
Ketoprofen micro-sponges	-	2	2	1	-
Matrix tablet	-	3	2	-	-

*n = 5 in each group; 0 = normal colored colon, 0.5 = red coloration, 1 = spot ulcer, 1.5 = hemorrhagic streaks, and 2 = hemorrhagic ulcer.

***In vivo* Pharmacokinetic Studies:** The peak plasma level (C_{max}) and the time to the peak plasma level (T_{max}) were determined from the individual curves. The areas under the plasma level versus time curve from 0 to 12 hr (AUC0-12h) were calculated by the trapezoidal rule. Mean plasma concentration time profile of ketoprofen loaded micro-sponges and MST is illustrated in **Fig. 6**. The pharmacokinetic parameters **(Table 6)** for both the formulations were compared with ANOVA and student t-test respectively.

TABLE 6: PHARMACOKINETIC PARAMETERS OF KETOPROFEN AFTER PER ORAL ADMINISTRATION IN RATS

Formulation	C_{max} (µg/mL)	T_{max} (h)	AUC (µg/h/mL)	$T_{1/2}$ (h)
Ketoprofen suspension	6.68 ± 0.81	2 ± 0.36	50.75 ± 2.55	1.65 ± 0.13
MS	4.56 ± 0.72	6 ± 0.65	41.54 ± 5.33	2.85 ± 0.29
MST	2.13 ± 0.34	4 ± 0.23	33.10 ± 3.68	3.85 ± 0.31

From the **Fig. 6**, it can be seen as the plasma concentration of pure ketoprofen reached maximum at 2 hr, *i.e.* 6.68 ± 0.81 with t_{max} of 2 ± 0.36, post administration and then decreased rapidly after 12 hr, of drug administration. In case of micro-sponge formulation (MS), ketoprofen was obtained in the blood plasma only after 2 hr and C_{max} was found to be 4.56 ± 0.72 µg/mL with T_{max} of 6 ± 0.65 hr, as compared to pure drug. Whereas, in case of drug loaded MST the lag time of approximately 4 hr was achieved with C_{max} of only 2.13 ± 0.34 µg/mL. The lag time of 4hr for the MST tablets suggested the ability for the spatial delivery of the drug to the colon, and thus the former resisted the release of ketoprofen in the stomach. The C_{max} of colon-targeted MST was significantly ($p < 0.005$) less than the C_{max} of MS.

This indorsed that reduced systemic absorption of drug from matrix tablet results in availability of larger fraction of drug on the colonic surface for local action. Similarly, AUC (0-12) was found to be 50.75 ± 2.55, 41.54 ± 5.33 and 33.10 ± 3.68 for pure drug, ketoprofen loaded MS and MST with $t_{1/2}$ of 1.65 ± 0.13, 2.85 ± 0.29 (h) and 3.85 ± 0.31 (h), respectively. Both the formulations, *i.e.* (MS and MST) yields sustained release action for the period of 12 hr as expected in accordance with the *in vitro* release studies. Thus, *in vivo* pharmacokinetic studies exhibited that pectin-based colon-targeted matrix tablets composed of ketoprofen loaded micro-sponges increased lag time, delayed T_{max}, decreased C_{max} and reduced bioavailability of the drug, as compared to the micro-sponge formulation and pure drug. Thus, it can further be concluded that the developed colon-targeted matrix tablet formulation has the ability to resist the drug release in the upper GIT, but can release the active agent specifically in the colon to exert the local action with reduced systemic exposure.

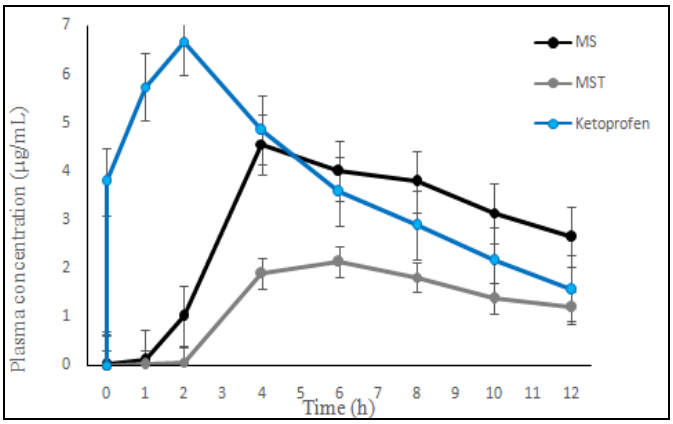

FIG. 6: *IN VIVO* PHARMACOKINETIC PROFILE OF THE KETOPROFEN IN RATS FOLLOWING PER-ORAL ADMINISTRATION OF MS AND MST CONTROL RELEASE TABLET. EACH CROSS BAR INDICATES AVERAGE VALUE ± SD (n = 3)

Stability Studies: The optimized micro-sponge formulation F4 and MST was subjected to 3-month accelerated stability study and was analyzed for physical appearance, and drug content. After 3 months the formulations were found to show no significant change in physical appearance and drug content thus, indicating the stable nature of micro-sponge and MST tablet formulation indicating good shelf life.

CONCLUSION: The aim behind formulating an oral polysaccharide based micro-sponge matrix system was to deliver ketoprofen in a controlled manner for an extended period of time so as to decline rate of administration and to improve its local bioavailability in the colon. *In vitro* drug release kinetic of micro-sponge and MST showed highest (r^2) value for the zero order, *i.e.* 0.9735 and 0.975, and therefore, indicated its controlled release

property. The *in vivo* studies also revealed better therapeutic outcomes as compared to pure ketoprofen. The combined approach of drug loaded micro-sponge and matrix tablets could be successfully used in the spatial and temporal colon targeting of ketoprofen for the treatment of various colonic pathologies.

ACKNOWLEDGEMENT: Nil.

CONFLICTS OF INTEREST: Nil.

REFERENCES:

1. Talaei F, Atyabi F, Azjdarzadeh M, Dinarvand R and Saadatzadeh V: Overcoming therapeutic obstacles in inflammatory bowel diseases: A comprehensive review on novel drug delivery strategies. European Journal of Pharmaceutical Sciences 2013; 49(4): 712–722.
2. Sareen R, Nath K, Jain N and Dhar KL: Curcumin Loaded Microsponges for Colon Targeting in Inflammatory Bowel Disease: Fabrication, Optimization, and *In vitro* and Pharmacodynamic Evaluation. Bio Med Research International 2014.
3. Sareen R, Nath K, Jain N and Dhar KL: An insight to colon targeted drug delivery system. Drug Delivery Letters 2013; 3(2): 127–135.
4. Challa T, Vynala V and Allam KV: Colon specific drug delivery system: A review on primary and novel approaches. International Journal of Pharm. Sciences Review and Research 2011; 7: 171-81.
5. Sreelatha D and Chandan KB: Colon targeted drug delivery. Journal of Global Trends in Pharmaceutical Sciences 2013; 4: 1174-83.
6. Maestrelli F, Zerrouk N, Cirri M and Mura P: Comparative evaluation of polymeric and waxy microspheres for combined colon delivery of ascorbic acid and ketoprofen. International Journal of Pharmaceutics 2015; 485: 365–373.
7. Kulkarni SK: Editor and book of experimental pharmacology, Vallabh Prakashan, New Delhi 1999; 148-50.
8. Pawar AP, Gholap AP, Kuchekar AB, Bothiraja C and Mali AJ: Formulation and Evaluation of Optimized Oxybenzone Microsponge Gel for Topical Delivery. Journal of Drug Delivery 2015.
9. Orlu M, Ceveher E and Arman A: Design and evaluation of colon specific drug delivery system containing Flurbiprofen microsponges. International Journal of Pharmaceutics 2006; 6(2): 103-117.
10. Kantor TG: Ketoprofen: A review of its pharmacologic and clinical properties. Pharmacotherapy Journal of Human Pharmacology and Drug Therapy 1986; 6: 93-102.
11. Nief RA and Hussein AA: Preparation and Evaluation of Meloxicam Microsponges as Transdermal Delivery System. Iraqi Journal of Pharmaceutical Sciences 2014; 23(2).
12. Ghosh A and Kumar PM: Development and evaluation of metronidazole loaded microsponge based gel for superficial surgical wound infections. Journal of Drug Delivery Science and Technology 2015; 30: 15-29.
13. Osmani RAM, Aloorkar NH, Thaware BU, Kulkarni PK and Moin A: Microsponge based drug delivery system for augmented gastroparesis therapy: Formulation development and evaluation. Asian Journal Pharmaceutical Sciences 2015; 10: 442-451.
14. Srivastava R and Pathak K: Microsponges: a futuristic approach for oral drug delivery. Expert Opinion on Drug Delivery 2012; 9: 863–878.
15. Aloorkar N, Kulkarni A, Ingale D and Patil R: Microsponges as Innovative drug delivery systems. International Journal of Pharmaceutical Sciences and Nanotechnology 2012; 5: 1597-1606.
16. Maiti S, Kaity S, Ray S and Biswantha SA: Development and evaluation of xanthan gum-facilitated ethyl cellulose microsponges for controlled percutaneous delivery of diclofenac sodium. Acta Pharmaceutica 2011; 61: 257–270.
17. Qiu Y: Understanding design and development of modified release solid oral dosage forms. Journal validation Technology 2011.
18. Kumar V, Soni GC and Prajapati SK: Sustained Release Hydrophilic Matrix Tablet of Ibuprofen: Influence of Polymers on *In-Vitro* Release and Bioavailability. International Journal of Pharmaceutical Research and Sciences 2012; 1: 4.
19. Srivastava R, Kumar D and Pathak K: Colonic Luminal surface retention of meloxicam microsponges delivered by erosion based colon-targeted matrix tablet. International Journal of Pharmaceutics 2012; 427: 153-62.
20. Perge L, Robitzer M, Guillemot C, Devoisselle J, Quignard V and Legrand P: New solid lipid microparticles for controlled ibuprofen release: formulation and characterization study. International Journal of Pharmaceutics 2012; 422: 59–67.
21. Comoglu T, Gonul N and Baykara T: Preparation and *in vitro* evaluation of modified release ketoprofen microsponges. Il Farmaco2003; 58: 101-106.
22. Shinde AJ, Paithane MB and Sawant SS: Development and Evaluation of Fenoprofen Microsponges and its Colonic Delivery using Natural Polysaccharides. Asian Journal of Pharmaceutical Sciences and Nanotechnology 2014; 1: 27-42.
23. Shah V, Jain H, Krishna J and Patel G: Microsponge drug delivery: A Review. International Journal of Pharmaceutical Research and Sciences 2013; 1(2): 212-18.
24. Nath B, Nath LK: Design, development and optimization of oral colon targeted drug delivery system of azathioprine using biodegradable polymer. Pharmaceutical Development and Technology 2013; 18: 1131-1139.
25. Harsh S, Patel K and Upadhyay UM: Formulation and evaluation of controlled release colon targeted micro sponge of Aceclofenac. Pharma Innovation 2014; 3(10): 81-87.
26. Indian Pharmacopoeia. Indian Pharmacopoeial Commission, Ghaziabad 2014; 1(7).
27. Tahan G, Aytac E and Aytekin H: Vitamin E has a dual effect of anti-inflammatory and antioxidant activities in acetic acid induced ulcerative colitis in rats. Canadian Journal of Surgery 2011; 54(5): 333–338.
28. Kandhare AD, Raygude KS and Ghosh P: Effect of hydroalcoholic extract of *Hibiscus rosasinensis* Linn. leaves in experimental colitis in rats. Asian Pacific Journal of Tropical Biomedicine 2012; 2(5): 337–344.
29. Zhao Y, Zhai D, Chen X, Yang J, Song X, He H, Yu Q and Xing Y: Ketoprofen glucuronidation and bile excretion in carbon tetrachloride and alpha-naphthyl-isothiocyanate induced hepatic injury rats. Toxicology 2007; 230: 145–150.
30. Yamada T, Onishi H and Machida Y: Sustained release ketoprofen microparticles with ethyl cellulose and carboxy

methylethyl cellulose. Journal of Controlled Release 2001; 75: 271–282.
31. Bae SE, Son JS, Park K and Han DK: Fabrication of covered porous PLGA microspheres using hydrogen peroxide for controlled drug delivery and regenerative medicine. Journal of Control Release 2009; 133: 37–43.
32. Chiao CSL and Price JC: Formulation, preparation and dissolution characteristics of propranolol hydrochloride microspheres. Journal of Micro-encapsulation 1994; 11(2): 153–9.
33. Jain V and Singh R: Development and characterization of Eudragit RS100 loaded microsponges and its colonic delivery using natural polysaccharides. Acta Poloniae Pharmaceutica Drug Research 2010; 67: 407–415.
34. Sande SA: Pectin based oral drug delivery to colon. Expert Opinion in. Drug Delivery 2005; 2(3): 441-450.
35. Rowe RC, Sheskey PJ and Owen SC: Pharmaceutical application of polymers for drug delivery. Pharmaceutical Press 2005; 347(5): 61.
36. Shoaib MH, Tazeen J, Merchant HA and Yousuf RI: Evaluation of drug release kinetics from ibuprofen matrix tablets using HPMC. Pakistani Journal of Pharmaceutical Sciences 2006; 19(2): 119-124.

How to cite this article:
Rathore C, Jain N, Garg N, Mahindroo N, Sharma G and Negi P: Polysaccharide-microsponge based matrix tablet for colon targeting of ketoprofen: *in vitro* and *in vivo* evidence. Int J Pharm Sci Res 2017; 8(10): 4250-60.doi: 10.13040/IJPSR.0975-8232.8(10).4250-60.