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RESEARCH ARTICLE

FORMULATION AND EVALUATION OF SUSTAINED RELEASE FLOATING MICROBALLOONS OF KETOROLAC TROMETAMOL

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The present study was aimed at the design of sustained release floating microballoons of ketorolac trometamol (ketorolac tromethamine) using two polymers ethyl cellulose and HPMC K4M with different permeability characteristics. Ketorolac microballoons were prepared by solvent diffusion method using different concentrations of both polymers and studied for *in vitro* and *in vivo* parameters. Prepared microballoons were spherical in shape, stable, float on simulated gastric fluid for more than 8 h and was significantly less ulcerogenic ($p<0.001$) than plain ketorolac trometamol.

Key words: Ketorolac trometamol, Microballoons, Sustained release, HPMC K4M, Ethyl cellolose.

INTRODUCTION

Ketorolac trometamol, is an analgesic, anti-inflammatory agent inhibit the bodily synthesis of prostaglandins by competitive blocking the enzyme cyclooxygenase (COX) non-selectively. Dose requirement of ketorolac trometamol is 60 to 120 mg/day and more frequent use can cause high incidence of GI side effects and toxicity (McDaid *et al* 2010). Therefore, continued efforts are being made to improve the formulation of ketorolac trometamol in order to achieve an optimal therapy. Various researchers have used various polymers and their combinations to formulate multiparticulate drug delivery system for sustained drug delivery (Dahiya and Gupta, 2011; Tripathi *et al* 2011; Basarkar *et al* 2013; Tyagi and Kori, 2013; Verma *et al* 2014).

Floating microballoons are spherical particles with size varying from 50 nm to 2 mm possessing a characteristic internal hollow structure and show an excellent *in vitro* floatability (Vyas and Khar, 2002). Gastric floating drug delivery system (FDDS) can overcome the problems associated with oral controlled release drug delivery systems. The FDDS is able to prolong the retention time of a dosage form in the gastro intestinal tract, thereby improving oral bioavailability of the drug, particularly useful for drugs that are primarily absorbed in the duodenum and upper jejunum segments (Shakya *et al* 2013).

MATERIALS AND METHODS

Materials

Ketorolac trometamol was obtained as a gift sample from Sun Pharma Global Inc., Vadodara, HPMC K4M was obtained as a gift sample from Colorcon Pvt. Ltd., India. Dichloromethane was purchased from Loba Chemie, Mumbai and ethyl cellulose, sodium lauryl sulphate (SLS), tween-80, ethanol, methanol were purchased from S.D. Fine Chemicals Ltd., Mumbai. All chemicals used in research work were of analytical grade.

Methods

Preparation of floating microballoons

Microballoons containing ketorolac trometamol were prepared by solvent evaporation method. Weighed quantity of both polymers was dissolved in dichloromethane at the mentioned

ratio. Magnesium stearate 5% *w/w* and ketorolac trometamol 50% *w/w* were dispersed to the above slurry and stirred in magnetic stirrer. The drug polymer dispersions were pressurized under CO_2 gas, which upon release of the pressure form cavities on the polymeric surface. The porous drug polymer dispersions were then slowly introduced into 70 ml liquid paraffin previously emulsified with 0.2% *w/v* SLS at 40°C while vortexing (1000 rpm for combination of polymers and 700 rpm for single polymer), with a three-blade propeller at room temperature (Xiao *et al* 2013). The whole system was stirred for 3 h to allow the complete evaporation of acetone.

The oil layer was decanted and microballoons were washed several times with petroleum ether (40-60°C). The washed microballoons were dried in an oven at room temperature not exceeding 25°C (El-Kamel *et al* 2001).

Compatibility studies

Infrared spectrum of the drug, drug loaded micrballoons, blank microballoons, physical mixture and empty microballoons were recorded using FTIR (Porwal *et al* 2011).

Viscosity of the polymer organic phase

In order to measure the relative viscosity of polymer solutions, different polymer concentrations were prepared in acetone and measured the viscosity using ostwald's viscometer (Obeidat and Price, 2005).

Scanning electron microscopy

The prepared microballoons were coated with gold in an argon atmosphere. The surface topography and internal textures of the microballoons were observed by scanning electron microscopy (Bodmeier and Chen, 1989).

Yield of microballoons

The prepared microballoons with a size range of 23-37 μm were collected and weighed. The measured weight was divided by the total amount of all nonvolatile components which were used for the preparation of the microballoons (Yang *et al* 2001).

% Yield = (Actual wt. of product / Total wt. of excipient and drug) × 100

Particle size analysis

Particle size of prepared microballoons was measured using an optical microscope, and the mean particle size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer (Etzler and Sanderson, 1995).

Drug entrapment efficiency

Microballoons equivalent to 100 mg of pure drug were crushed and added to 50 ml of 0.1 M HCl, pH 1.2. The resultant mixture was shaken in a mechanical shaker for 3 h to extract the drug completely (Chong-Kook *et al* 1994). The solution was filtered with a Whatmann filter paper and 1 ml of this solution was appropriately diluted to 25 ml using 0.1 M HCl, pH 1.2, and analyzed spectrophotometrically at 322 nm using UV-Visible double beam spectrophotometer (Shimadzu 1700, Japan).

% Drug entrapment = (ODC / TDC) × 100

ODC = Observed drug content

TDC = Theoretical drug content

Floating behavior (buoyancy)

Fifty mg of the microballoons were placed in 100 ml of simulated gastric fluid (pH 1.2) containing 0.02% *w/v* tween 20. The mixture was stirred at 100 rpm on a magnetic stirrer. After 4 h, the layer of buoyant microballoons was pipetted and separated by filtration; particles in the sinking particulate layer were also separated by filtration. Particles of both types were dried in a desiccator. Both the fractions of microballoons were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles (Yadav and Jain, 2012).

Buoyancy (%) $= [W_f / (W_f + W_s)] \times 100$

W_f = weights of the floating micropartcles

W_s = weights of the settled microballoons

In vitro drug release studies

The *in vitro* dissolution studies were carried out for all products in USP XXIV paddle type dissolution apparatus. Weighed amount of drug loaded floating microballoons were introduced into 900 ml simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8), used as a dissolution medium, for 11 h maintained at 37±0.5°C at a rotation speed of 100 rpm.

Two ml of aliquots were withdrawn at different time intervals and an equivalent volume of medium pre warmed at 37°C was added to maintain sink condition. Withdrawn samples

were analyzed spectrophotometrically at 322 nm using a UV-Visible double beam spectrophotometer (1700, Shimadzu, Japan). Drug release from the prepared microballoons made of ethyl cellulose, HPMC K4M and mixture of two polymers were kinetically evaluated (El-Kamel *et al* 2001, Awasthi and Kulkarni, 2014).

Stability Studies

The selected formulations were tested for a period of 10 weeks at different storage conditions of 25°C and 40°C with 60% RH and 75% RH, to evaluate for their drug content (Ma *et al* 1994).

Thermal analysis

Thermoanalytical measurements were carried out with Differential Scanning Calorimetry (DSC-60, Shimadzu, Mumbai). Selected microballoons, after 45 days at 40°C and 75% RH were placed into aluminium containers and heated at a constant rate of 10°C / min. between 40°C to 120°C under nitrogen and argon and thermogram was recorded (Mura *et al* 1995).

Zeta potential study

The surface charge of prepared microballoons was determined by the electrophoretic mobility of Microparticles in a U type tube at 25°C, using a zetasizer (Malvern, UK) (Vandervoort and Ludwig, 2002).

In vivo evaluation of microballoons

Ketorolac trometamol (KET) floating microballoons were tested for gastric irritancy (Letter no. 316/CPCSEA). Male wistar rats were taken in to group of five and they were fasted for 36 h with free access to water.

Ketorolac trometamol (100 mg/kg), plain microballoons and selected formulation were separately administered to rats orally by gastric tube in form of a suspension in 0.1% solution of tween 20.

After 10 hours of drug administration (with no access of water), the rats were sacrificed and their stomachs were excised and opened along their lesser curvature and mounted on a wax plate. The mucosal lining was examined for the appearance of any ulcer. The parameters like, ulcer index, mean ulcer index and percent protection were evaluated using formula (Akiyama *et al* 1995) given as under.

Mean ulcer index= TUI / TNR

TUI = Total ulcer index

TNR = Total no. of rats in that group

Percent protection = (UIK - UIP / UIK) × 100

UIK = Ulcer index for KET

UIP = Ulcer index for preparation

RESULTS AND DISCUSSION

Preparation of floating microbaloons

The compositions and stirring speed of the formulations to be prepared using different polymers are shown in **Table 1**.

Compatibility studies

FTIR spectra revealed that there was no interaction between the drug and the polymers used for microballoon formulation.

The principal absorption peak of ketorolac trometamol appears at 3550 cm^{-1} due to the N-H stretching of the primary amine group (-NH_2) However, a sharp peak occured at 1725 cm^{-1} [C=O stretch (acid)] 1167 cm^{-1} [C=O stretch (diaryl ketone)]. The identical peaks of N-H stretching, C-O stretching vibrations were also appeared in the spectra of KET loaded microballoons prepared with ethyl cellulose, HPMC K4M and physical mixture of both polymers with blank microballoons (**Figure 1**).

Viscosity of the polymer organic phase

HPMC K4M solution had higher relative viscosities than ethyl cellulose. Combination of HPMC K4M and ethyl cellulose imparts a synergistic increase in the relative viscosity compared to the single polymers (**Figure 2**).

Table 1. Composition of each formulation with stirring speed

Batch	Drug (mg)	Ethyl cellulose (mg)	HPMC K4M (mg)	Magnesium stearate (% *w/w*)	Stirrer speed (rpm)
A	50	100	-	5	700
B	50	-	100	5	700
C	50	50	50	5	1000
D	50	25	75	5	1000

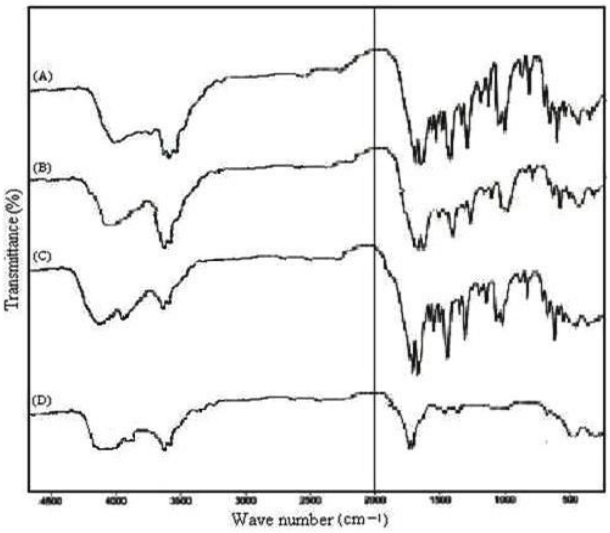


Fig. 1. Comparative study of FT-IR spectrum of ketorolac trometamol (A), ketorolac trometamol loaded microbaloons (B), physical mixture of ketorolac trometamol and blank microballoons (C), and blank microballoons (D)

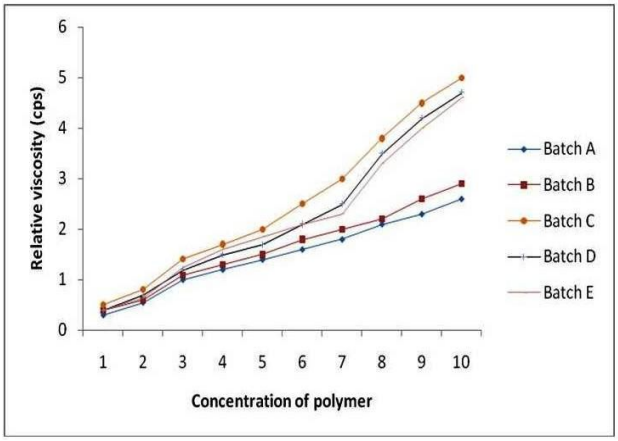


Fig. 2. Viscosity study of prepared batches

Scanning electron microscopy

SEM study showed that particles made of HPMC-K4M and ethyl cellulose were predominantly spherical in shape with smooth surface. The surface of the drug-loaded microballoons manifested the presence of drug particles, clearly visible from outside. Irregular surfaces and larger sizes were observed in the microballoons prepared from polymer to polymer ratio 1:1 at the same stirring speed. The porous nature and characteristics internal structure of the microballoons, a hollow cavity inside enclosed with the rigid shell constructed with drug and polymer was clearly evident. Presence of pores were detected on the microballoons surface which increased in size and number after dissolution indicating leaching of the drug through these channels. The porous nature and cavity formed in the microballoons would dictate the floating behavior of microballoons of ketorolac trometamol as shown in **Figure 3**.

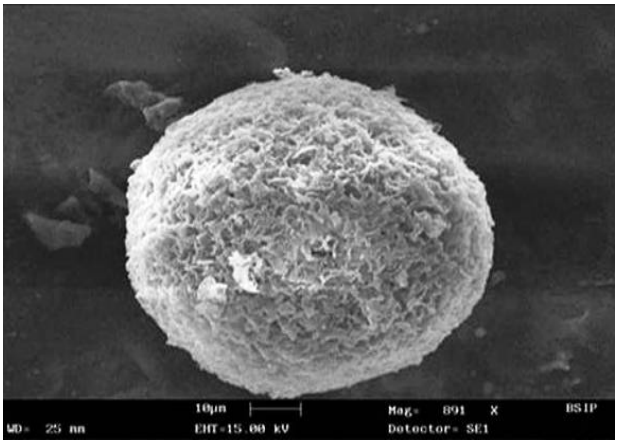


Fig. 3. SEM photograph of drug loaded microballoon made of equal concentration of ethyl cellulose, HPMC K4M and ketorolac trometamol

Yield of microballoons

All batches showed a percentage yield of greater than 60%, whereas three batches showed a yield of more than 70%.

Particle size analysis

When the polymer to polymer ratio was 1:1, there was formation of microballoons with large and irregular sizes due to increase in solution viscosity of the combined polymers. Hence, higher agitation speed is required to prepare microballoons of same sizes as compared to that of single polymer alone (**Table 2**).

Drug entrapment efficiency

The drug loading increases as the concentration of polymer is increased relative to drug concentration. The analysis of drug content showed maximum entrapment efficiency at the drug polymer ratio 1:1 (**Table 2**).

Floating behavior (buoyancy)

The formulated batches showed good buoyancy on simulated gastric fluid (pH 1.2). The buoyancy was higher with ethyl cellulose (EC) and HPMC K4M based microballoons than EC based microballoons as shown in **Table 2**.

In vitro drug release studies

In vitro dissolution studies of all batches of microballoons revealed that up to 90% drug released within 11 h in 0.1 N HCl and phosphate

Table 2. Summary of results of microballoons evaluation parameters

Formulation code	% Yield	Particle size in micron*	Drug entrapment efficiency* (%)	Buoyancy* (%)
A	75.7	37.107 ± 6.512	82.72±0.285	73±2.5
B	60.1	23.551 ± 3.059	73.35±0885	76±1.4
C	78.7	25.935 ± 5.049	92.78±0.224	76±1.7
D	68.0	31.515 ± 7.497	60.82±0.938	56±2.2
E	74.2	32.047 ± 8.725	72.95±0.851	63±1.5

* All values are expressed as mean ± SD, n=3

buffer respectively (**Figure 4, 5**). In selected batch C, addition of HPMC K4M to ethyl cellulose polymer increased the permeability of the microballoons to dissolution medium due to the swelling nature of HPMC K4M.

Moreover, the porous nature of the microballoons, water can penetrate into dosage form and leaching out the drug. The combination of polymers at polymer to polymer ratio 1:1 helped to leach out the drug from its matrices and exhibited an initial rapid drug release for the first 2 to 3 h and then slower drug release which could be best explained by Higuchi's spherical matrix release.

It can be observed from drug release kinetics that the release of ketorolac trometamol from the HPMC K4M and EC microballoons exhibited diffusional characteristics and highly correlated with Higuchi spherical matrix release, followed by first order and zero order.

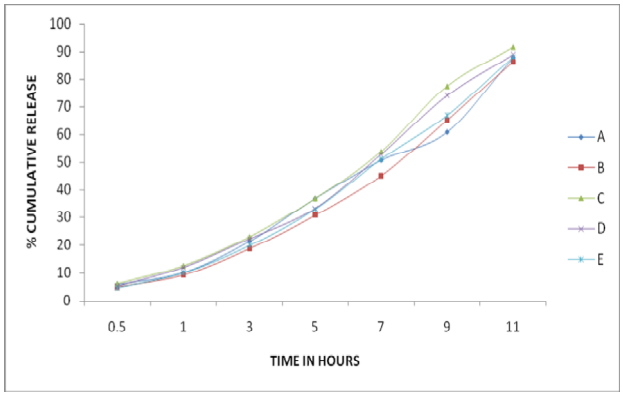


Fig. 4. Comparison between various prepared batches of drug loaded microballoons for *in vitro* drug release in 0.1 N HCl (pH 1.2)

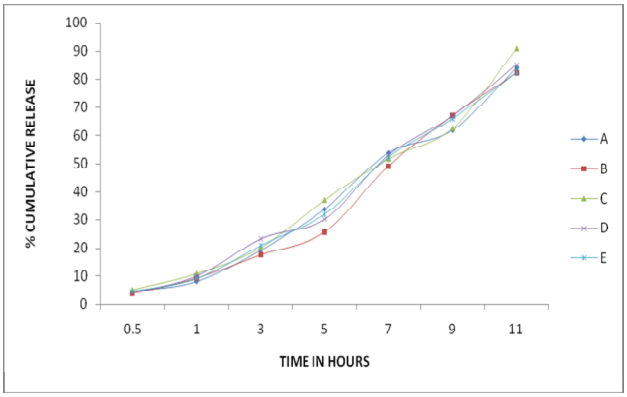


Fig. 5. Comparison between various prepared batches of drug loaded microballoons for *in vitro* drug release in phosphate buffer pH 6.8.

Table 3. Results of stability studies

Formulation	Temperature	Drug Content (%)*				
		After 2 weeks	After 4 weeks	After 6 weeks	After 8 weeks	After 10 weeks
A	At 25°C	97.85±0.16	97.15±0.05	96.20±0.09	94.40±0.24	93.30±0.09
	At 40°C	96.60±0.11	94.80±0.08	95.10±0.20	92.90±0.10	90.30±0.14
B	At 25°C	97.55±0.10	96.80±0.19	95.15±0.05	94.20±0.05	94.65±0.06
	At 40°C	96.85±0.22	94.75±0.25	93.35±0.15	92.35±0.12	91.10±0.13
C	At 25°C	97.90±0.17	96.80±0.09	95.50±0.13	94.10±0.09	93.40±0.18
	At 40°C	96.75±0.20	95.65±0.11	94.10±0.08	93.55±0.19	92.10±0.05
D	At 25°C	97.70±0.09	96.95±0.18	95.30±0.12	94.00±0.22	94.50±0.22
	At 40°C	96.55±0.07	95.45±0.13	94.20±0.17	93.45±0.18	92.60±0.19
E	At 25°C	98.10±0.15	97.60±0.06	96.40±0.24	95.50±0.13	94.90±0.15
	At 40°C	96.90±0.06	96.00±0.17	95.10±0.18	94.35±0.23	94.20±0.08

*All values are expressed as mean ± SD, n=3

Stability Studies

Prepared formulation was found to be stable, because there were no significant changes in the percentage amount of drug content after 10 weeks (**Table 3**).

Thermal analysis

The DSC analysis of microballoons showed their respective peaks at their melting point range (ketorolac trometamol, HPMC K4M, ethyl cellulose). Almost unchanged characteristic peaks of ketorolac trometamol, HPMC K4M and ethyl cellulose indicated the absence of strong interaction in examined formulation (**Figure 6**).

Zeta potential study

The presence and magnitude, or absence, of a charge on colloidal particle is an important factor in the stability of colloidal system. Zeta potential of microballoons was near to zero which suggested that particles required only a minute to charge for stabilization. Positive zeta potential value represented the force of repulsion between the particles, the system was deflocculated (**Figure 7**).

In vivo evaluation of microballoons

Scoring guideline for calculating ulcer index is mentioned in **Table 4**. Selected formulation (formulation C) and plain microballoons were significantly less ulcerogenic ($p<0.001$) than the plain ketorolac trometamol. These results indicated that gastrointestinal toxicity due to plain ketorolac trometamol was protested by formulating in form of floating microballoons (**Table 5, Figure 8**).

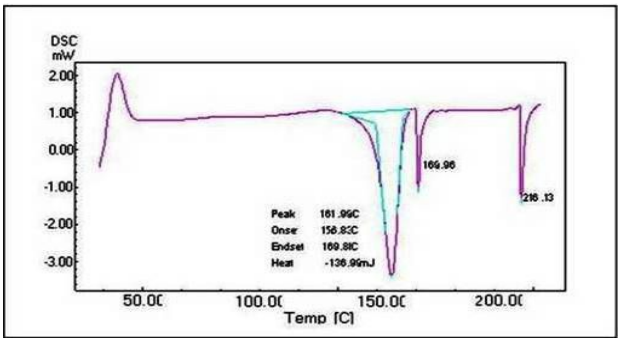


Fig. 6. DSC thermogram of physical mixture of ethyl cellulose, HPMC K4M and KET

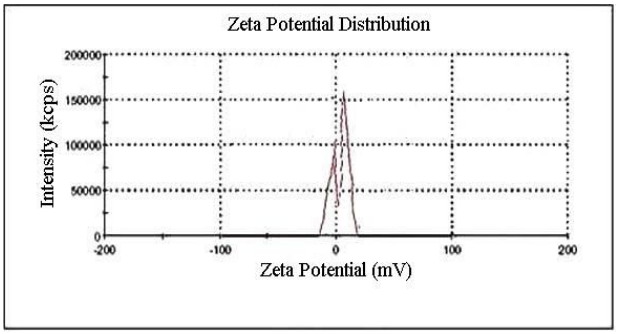


Fig. 7. Zeta potential of prepared microballoons of EC, HPMC K4M and KET (Batch C)

Table 4. Scoring guideline for calculating ulcer index

S. No.	Condition	Ulcer score
1	Normal stomach	0
2	Patchy hemorrhage	1
3	Definite hemorrhagic erosion	2
4	Very small ulcers (< 1mm diameter)	3
5	Small ulcers (1 to< 2 mm diameter)	4
6	Medium ulcers (2 to <3 mm diameter)	5

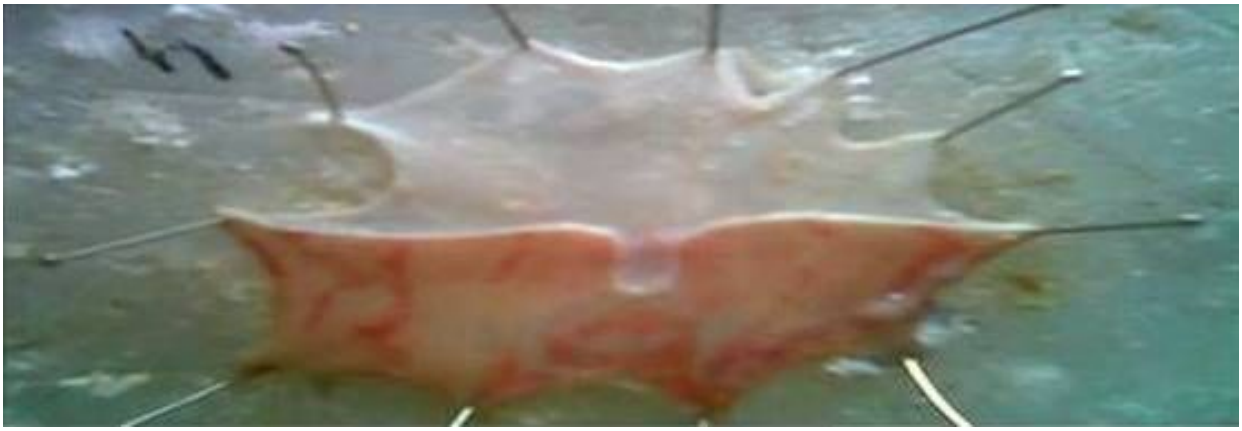
Table 5. Comparative results of ulcer protective potential of drug and microballoon formulation

Treatment	Dose (mg/kg)	Ulcer Index Mean ± SEM	Percent protection
Plain Ketorolac tromethamine	100	22.8 ± 0.86	–
Empty microballoons	–	3.6 ± 0.3*	84.2%
Formulation C	100	6.8 ± 1.4*	70.2%

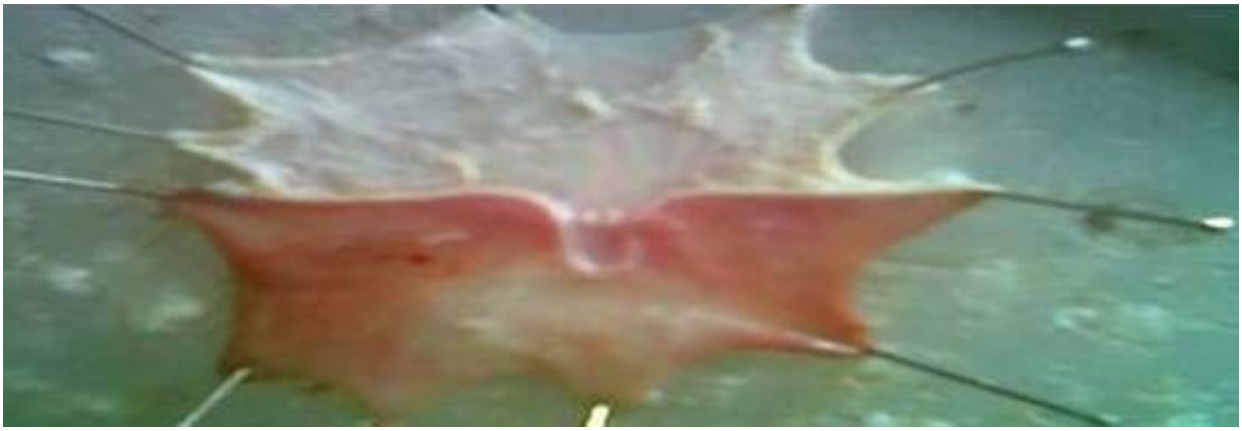
*$p<0.001$ as compared to plain Ketorolac tromethamine; $F(2, 14) = 115.85$ ($p < 0.0001$); Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's t-test as compared to control.

CONCLUSION

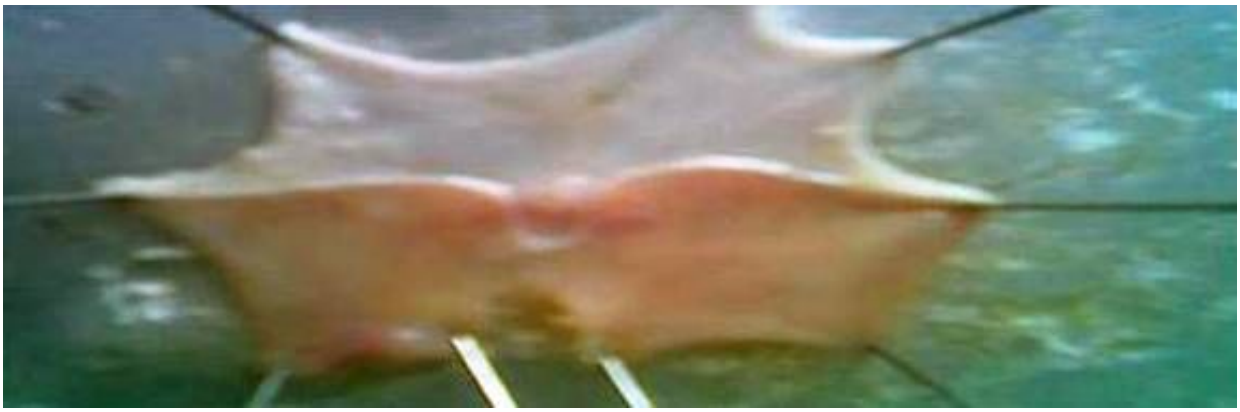
Gastric floating microcapsules of ketorolac trometamol were successfully prepared using two polymers of different permeability characteristics. HPMC K4M is insoluble at physiological pH and prepared microballoons



(A)



(B)



(C)

Fig. 8. *In-vivo* testing of (A) Plain drug ketorolac trometamol, (B) Microballoons of formulation C, (C) Empty microballoons using stomach mucosa of wistar rat

extended the drug release for longer period of time, with an initial slow release at the first one-hour and then controlled release for the rest period of time. But, microballoons made of both the polymers at 1:1 ratios (total 10% *w/w*) exhibited satisfactory drug release pattern, as it released the drug in controlled fashion for extended period of time by maintaining the buoyancy. There is no extended release microbaloon formulation in the market. Microballoon formulation offers several advantages over other sustained-release systems, especially matrix type tablets; since they can be widely distributed throughout the GI tract and produce local high concentration of drug at the absorption site.

Therefore, it may be concluded that drug loaded floating microballoons are a suitable delivery system for ketorolac with a new choice of an economical, safe and possibly more bioavailable formulation in the management of analgesia and inflammation.

REFERENCES

Akiyama Y, Nagahara N, Kashihara T, Hirai S, Toguchi H. *In vitro* and *in vivo* evaluation of mucoadhesive microspheres prepared for the gastrointestinal tract using polyglycerol esters of fatty acids and a poly(acrylic acid) derivative. *Pharm Res.* 1995;12(3):397-405. [DOI: 10.1023/A:1016208 703380]

Awasthi RR, Kulkarni GT. Development of novel gastro-retentive drug delivery system of gliclazide: Hollow beads. *Drug Dev. Ind. Pharm*. 2014;40(3):398-408. [DOI: 10.3109/03639045.2013.763817]

Basarkar GD, Shirsath GN, Patil SB. Development of microspheres containing diclofenac diethylamine as sustained release topical formulation. *Bull. Pharm. Res.* 2013;3(1):14-22.

Bodmeier R, Chen H. Preparation and characterization of microspheres containing the anti-inflammatory agents, indomethacin, ibuprofen and kitoprofen*. J. Control. Rel.* 1989;10(2):167-75. [DOI: 10.1016/0168-3659(89)90059-X]

Chong-Kook K, Mi-Jung K, Kyoung-Hee, O. Preparation and evaluation of sustained release microcapsules of terbutaline sulphate. *Int. J. Pharm.* 1994;106(3):213-9. [DOI: 10.1016/0378-5173(94)90004-3]

Dahiya S, Gupta ON. Formulation and *in vitro* evaluation of metoprolol tartrate microspheres. *Bull. Pharm. Res.* 2011;1(1):31-9.

El-Kamel AH, Sokar MS, Al Gamal SS, Naggar VF. Preparation and evaluation of ketoprofen floating oral delivery system. *Int. J. Pharm.* 2001;220(1-2):13-21. [DOI: 10.1016/S0378-5173(01)00574-9]

Etzler FM, Sanderson MS. Particle size analysis: A comparative study of various methods. *Particle & Particle Syst. Character.* 1995;12(5):217-24. [DOI: 10.1002/ppsc.19950120503]

Ma X, Santiago N, Chen Y-S, Chaudhary K, Milstein SJ, Baughman RA. Stability study of drug-loaded proteinoid microsphere formulations during freeze-drying. *J. Drug Target.* 1994;2(1):9-21. [DOI: 10.3109/10611869409015889]

McDaid C, Maund E, Rice S, Wright K, Jenkins B, Woolacott N. Paracetamol and selective and non-selective nonsteroidal anti-inflammatory drugs (NSAIDs) for the reduction of morphine-related side effects after major surgery: A systematic review. *Health Technol. Assess.* 2010;14(17):1-153. [DOI: 10.3310/hta14170]

Mura P, Manderioli A, Bramanti G, Furlanetto S, Pinzauti S. Utilization of differential scanning calorimetry as a screening technique to determine the compatibility of ketoprofen with excipients. *Int. J. Pharm.* 1995;119(1):71-9. [DOI: 10.1016/0378-5173(94)00374-E]

Obeidat WM, Price JC. Preparation and in vitro evaluation of propylthiouracil microspheres made of eudragit RL 100 and cellulose acetate butyrate polymers using emulsion-solvent evaporation method. *J. Microencapsul.* 2005; 22(3):281-9. [DOI: 10.1080/02652040500100907]

Porwal A, Swami G, Saraf S. Preparation and evaluation of sustained release microballoons of propranolol. *Daru J. Pharm. Sci.* 2011;19(3):193-201.

Shakya R, Thapa P, Saha RN. *In vitro* and *in vivo* evaluation of gastroretentive floating drug delivery system of ofloxacin. *Asian J. Pharm. Sci.* 2013;8(3):191-8. [DOI: 10.1016/j.ajps.2013.07.025]

Tripathi M, Radhika PR, Sivakumar T. Formulation and evaluation of glipizide hollow microballoons for floating drug delivery. *Bull. Pharm. Res.* 2011;1(1):67-74.

Tyagi LK, Kori ML. Formulation and *in vitro* evaluation of Eudragit® RS 100 microspheres containing lornoxicam prepared by emulsion-solvent evaporation method. *Bull. Pharm. Res.* 2013;3(3):112-20.

Vandervoort J, Ludwig A. Biocompatible stabilizers in the preparation of PLGA nanoparticles: A factorial design study. *Int. J. Pharm.* 2002;238(1-2):77-92. [DOI: 10.1016/S0378-5173(02)00058-3]

Verma S, Kumar V, Jyoti, Mishra DN. Formulation, evaluation and optimization of mucoadhesive microspheres of acyclovir. *Bull. Pharm. Res.* 2014;4(1):14-20.

Vyas SP, Khar RK. Targeted and Controlled Drug Delivery, 1st edition, CBS Publishers and Distributors, New Delhi: 2002; 417-53.

Xiao C-D, Shen X-C, Tao L. Modified emulsion solvent evaporation method for fabricating core-shell microspheres. *Int. J. Pharm.* 2013;452(1-2):227-32. [DOI: 10.1016/j.ijpharm.2013.05.020]

Yadav A, Jain DK. Formulation and characterization of sustained release floating microballoons of metformin hydrochloride. *Trop. J. Pharm. Res.* 2012;11(4):561-8 [DOI: 10.4314/tjpr.v11i4.6]

Yang Y-Y, Chung T-S, Ng NP. Morphology, drug distribution, and in vitro release profiles of biodegradable polymeric microspheres containing protein fabricated by double-emulsion solvent extraction/evaporation method. *Biomaterials* 2001;22(3):231-41. [DOI: 10.1016/S0142-9612(00)00178-2]

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