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 cellulose.

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) 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-Kamal et al 2001).

**Compatibility studies**

Infrared spectrum of the drug, drug loaded microballoons, 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$_s$)] × 100

W$_f$ = weights of the floating microparticles
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 microballoons**
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 (-NH₂). However, a sharp peak occurred 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>
<thead>
<tr>
<th>Batch</th>
<th>Drug (mg)</th>
<th>Ethyl cellulose (mg)</th>
<th>HPMC K4M (mg)</th>
<th>Magnesium stearate (% w/w)</th>
<th>Stirrer speed (rpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>50</td>
<td>100</td>
<td>-</td>
<td>5</td>
<td>700</td>
</tr>
<tr>
<td>B</td>
<td>50</td>
<td>-</td>
<td>100</td>
<td>5</td>
<td>700</td>
</tr>
<tr>
<td>C</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>5</td>
<td>1000</td>
</tr>
<tr>
<td>D</td>
<td>50</td>
<td>25</td>
<td>75</td>
<td>5</td>
<td>1000</td>
</tr>
</tbody>
</table>
**Fig. 1.** Comparative study of FT-IR spectrum of ketorolac trometamol (A), ketorolac trometamol loaded microballoons (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.
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.

**Table 2. Summary of results of microballoons evaluation parameters**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>% Yield</th>
<th>Particle size in micron*</th>
<th>Drug entrapment efficiency* (%)</th>
<th>Buoyancy* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>75.7</td>
<td>37.107 ± 6.512</td>
<td>82.72±0.285</td>
<td>73±2.5</td>
</tr>
<tr>
<td>B</td>
<td>60.1</td>
<td>23.551 ± 3.059</td>
<td>73.35±0085</td>
<td>76±1.4</td>
</tr>
<tr>
<td>C</td>
<td>78.7</td>
<td>25.935 ± 5.049</td>
<td>92.78±0.224</td>
<td>76±1.7</td>
</tr>
<tr>
<td>D</td>
<td>68.0</td>
<td>31.515 ± 7.497</td>
<td>60.82±0.938</td>
<td>56±2.2</td>
</tr>
<tr>
<td>E</td>
<td>74.2</td>
<td>32.047 ± 8.725</td>
<td>72.95±0.851</td>
<td>63±1.5</td>
</tr>
</tbody>
</table>

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

**Table 3. Results of stability studies**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Temperature</th>
<th>Drug Content (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After 2 weeks</td>
<td>After 4 weeks</td>
</tr>
<tr>
<td>A</td>
<td>At 25°C</td>
<td>97.85±0.16</td>
</tr>
<tr>
<td></td>
<td>At 40°C</td>
<td>96.60±0.11</td>
</tr>
<tr>
<td>B</td>
<td>At 25°C</td>
<td>97.55±0.10</td>
</tr>
<tr>
<td></td>
<td>At 40°C</td>
<td>96.85±0.22</td>
</tr>
<tr>
<td>C</td>
<td>At 25°C</td>
<td>97.90±0.17</td>
</tr>
<tr>
<td></td>
<td>At 40°C</td>
<td>96.75±0.20</td>
</tr>
<tr>
<td>D</td>
<td>At 25°C</td>
<td>97.70±0.09</td>
</tr>
<tr>
<td></td>
<td>At 40°C</td>
<td>96.55±0.07</td>
</tr>
<tr>
<td>E</td>
<td>At 25°C</td>
<td>98.10±0.15</td>
</tr>
<tr>
<td></td>
<td>At 40°C</td>
<td>96.90±0.06</td>
</tr>
</tbody>
</table>

*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).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Condition</th>
<th>Ulcer score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal stomach</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Patchy hemorrhage</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Definite hemorrhagic erosion</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Very small ulcers (&lt; 1mm diameter)</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>Small ulcers (1 to &lt; 2 mm diameter)</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Medium ulcers (2 to &lt;3 mm diameter)</td>
<td>5</td>
</tr>
</tbody>
</table>

![Fig. 6. DSC thermogram of physical mixture of ethyl cellulose, HPMC K4M and KET](image)

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

Table 5. Comparative results of ulcer protective potential of drug andmicroballoon formulation

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

*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...
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 microballoon 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.

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
REFERENCES


