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Research Article

# FABRICATION AND EVALUATION OF SUSTAINED RELEASE MICROSPHERES OF KETOROLAC TROMETHAMINE

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**ABSTRACT**

The aim of the present paper was to study preparation of methacrylate microparticles for delivery of ketorolac tromethamine via the oral route. Drug was encapsulated within polymethacrylate copolymer (Eudragit RS100 and Eudragit RL100), by solvent evaporation method. Magnesium stearate was used as droplet stabilizer in concentration of 0.3% (v/v). Selected formulations were characterized for their entrapment efficiency, particle size, surface morphology and release behavior. *In vitro* dissolution tests were performed by using dissolution media with two different pH. All the selected formulations exhibited a prolonged release for almost 24 hr. The mean particle size of microspheres ranged from 75 to 225 µm and encapsulation efficiency ranged from 72.72 to 95.88% (w/w). Scanning electron microscopy of microspheres revealed a spherical and uniform appearance with rough surface. Mechanism of release was found to be Higuchi type. *In vivo* study of microspheres in albino wistar rats demonstrated significant analgesic and anti-inflammatory activities of microspheres for longer period of time compared to the parent drug. This study indicated that eudragit microspheres containing ketorolac tromethamine could be prepared successfully by using an emulsion solvent evaporation technique, which would not only sustain the release of drug but also minimize the side effects of this drug.

**Keywords:** Ketorolac tromethamine; Microspheres; Eudragit; NSAID(S).

## INTRODUCTION

Ketorolac tromethamine is non-steroidal anti-inflammatory drug (NSAID), which has potent analgesic and anti-inflammatory activity due to prostaglandin related inhibitory effect of drug[1]. This drug, like other NSAID(S), may produce gastrointestinal side effects. After oral administration it is rapidly eliminated from blood exhibiting a short biological half life of 4-6 hr.

Microspheres are one of the microparticulate delivery systems which are widely accepted to achieve oral controlled drug delivery. Microspheres can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects due to decrease in dosing frequency and improving patient compliance[2]. Among the various methods developed for the formulation of microspheres, oil in oil solvent evaporation gained much more attention due to its ease of fabrication without compromising the activity of drug.[3-5]

Eudragit polymers are series of acrylate and methacrylate polymers available in different ionic forms. Eudragit RS100 and Eudragit RL100 are referred to as aminomethacrylate copolymers with the former having 5% functional quarternary ammonium groups and later having 10% functional quarternary ammonium groups which are responsible for permeability of water in polymer matrix.[6-8]

The aim of this study was to prepare eudragit microspheres containing ketorolac tromethamine by solvent evaporation method to achieve a controlled drug release profile. Investigation of the effect of various processing and formulation factors such as drug to polymer ratio, stirring speed, phase ratio and surfactant concentration on the shape, mean particle size, yield of production, particle size distribution, encapsulation efficiency, surface properties and release rate of drug from the microspheres were performed. After examination of all the formulation variables on the microspheres properties, the optimized batch was selected and *in-vivo* study performed. By monitoring its analgesic and anti-inflammatory effects sustained release of drug from microsphere formulation was confirmed.

## MATERIALS AND METHODS

### Materials

Ketorolac tromethamine was obtained as a gift sample from Dr. Reddys Laboratories, India. Eudragit RS100 and RL100 were obtained from Rohm Pharma, GmbH, Darmstadt, Germany. All other reagents and solvent used were of pharmaceutical or analytical grade.

### Methods

#### Preparation of microspheres

Ketorolac tromethamine microspheres were prepared by solvent evaporation method. In this method a combination of Eudragit RS100 and Eudragit RL100 (in different ratios) was dissolved in a mixture of solvents containing acetone (5.0ml) and methanol (3.0ml) in a 100 ml of beaker with the help of magnetic stirrer. After complete dissolution, this solution was added with drug (100 mg). Magnesium stearate, as dispersing agent, was dispersed in drug and polymer solution with the help of sonicator. Resulting dispersion was poured in another 250ml beaker, containing mixture of paraffin oil light (60ml) and n-hexane (6.8ml), with continued stirring at 500-1500 rpm. Stirring was continued for 2 h until acetone evaporated completely. After evaporation of acetone, formed microspheres were filtered and residue was washed 4-5 times in 50 ml petroleum ether, each. Microspheres were dried at room temperature for 24 h. All the microsphere formulations were prepared in triplicate.

#### Scanning electron microscopic analysis

The shape and surface characteristics of microspheres were analyzed by scanning electron microscopy (SEM). Sample was dusted on a double-sided adhesive tape applied previously to an aluminium stub. Excess sample was removed and stub coated (Polaron Sputter 7040) with 30 nm layer of gold-palladium observed with a scanning electron microscope (Leo 0430, Leica Cambridge Ltd., Cambridge, UK).

#### Particle size analysis

Prepared microspheres (10 mg) were dispersed in water and particle size and particle size distribution of microspheres were determined by laser light scattering method (Malvern Mastersizer, Malvern Instruments, UK).

#### Percentage yield

The percentage yield value of microspheres was determined from the ratio of amount of solidified total microspheres to total solid material used in the inner phase.

**Determination of encapsulation efficiency**

Microspheres were crushed and powdered by using a mortar. Accurately weighed 100 mg of this powder was extracted in 100 ml of water. After 24 hr solution was filtered and a sample of 2 ml was withdrawn from this solution, diluted to 50 ml with water and assayed spectrophotometrically at 322 nm to determine Ketorolac tromethamine content of microspheres.

**In-vitro release study**

Drug dissolution test of microspheres was performed by USP II paddle type apparatus. Microspheres equivalent to 20 mg of drug were added to 400ml dissolution media. The content was rotated at 100 rpm at 37°C ± 0.5°C. The pH of dissolution media were kept 1.2 for 2 hr using 0.1N HCl, then 480ml of phosphate buffer and 20ml of 2MNaOH added to adjust the pH to 7.2 and maintained up to 24 hr. 5ml of each samples were withdrawn from the dissolution medium at various time intervals and replaced by an equal volume of dissolution medium. After filtration and suitable dilution, the samples were analyzed spectrophotometrically at 322 nm. The concentration of Ketorolac tromethamine in sample was calculated based on calibration curves of Ketorolac tromethamine taken in both the acidic 0.1NHCl media (n=3, $R^2$ = 0.999), and basic media (n=3, $R^2$ = 0.999).

**Biological evaluation**

**Procurement, identification and housing of animal**

Albino rats of wistar strain (200-250 g) of either sex were procured from the central animal house of the institute. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2°C; relative humidity 60-70%) in a 12 hr light-dark cycle. The rats were given a standard laboratory diet and water ad libitum. Food was withdrawn 12 hr before and during the experiment. Protocols were approved by the Institutional Animal Ethics Committee, registered under CPCSEA.

**Anti-inflammatory activity**

The animals were divided into three groups. Acute inflammation was produced by subplantar injection of 0.1 ml of 1% suspension of carrageenan in normal saline, in the right hind paw of the rats. After induction of inflammation, drug was administered after one hour of oral administration of the drug, the paw volume was measured plethismographically at a time interval of 0, 0.5, 1, 2, 4, 6, 8, 10 hr. The difference between the two readings was taken after carrageenan injection as the volume of oedema and percentage anti-inflammatory activity was calculated. Ketorolac tromethamine (5 mg/kg) solution in water was used as standard drug.

**Analgesic activity**

**Tail flicking method**

The prescreened animals were divided in three groups. Ketorolac tromethamine (5 mg/kg) solution in water was used as standard drug. The drugs were given orally and after one hr of administration of drug, tail flicking latency period was measured by analgesiometer (Almicro, India). The strength of current passing through the wire was kept constant at 6 amps. The distance between the heat source and the tail skin was 1.5 cm, measured from the route of the tail. The cut-off time was fixed at 20 sec to avoid tissue damage.

$$\% \text{ Inhibition of pain} = \frac{V_{control} - V_{treated}}{V_{control}} \times 100 \qquad \text{(Eq. 1)}$$

Where:

$V_{controlled}$ = mean time of tail flick in controlled group.

$V_{treated}$ = mean time of tail flick in test and standard group.

**Statistical analysis**

The data obtained from the particle size, encapsulation efficiency, and release rate determination studies of ketorolac tromethamine were analyzed statistically with one-way ANOVA and Newman-Keuls test.

**RESULTS**

The resulting microspheres formulated by solvent evaporation method were found to be spherical and free flowing in nature. The mean particle size of microspheres ranged from 75.11 µm to 212.92 µm. It was noticed that mean particle size increased with increase in polymer concentration and decrease in magnesium stearate concentration. The encapsulation efficiency was recorded 77.28 to 95.88 % (w/w). The encapsulation efficiency was also found to be dependent on nature of polymer used in the formulation. From the *in vitro* drug dissolution studies it was found that the sustaining effect of microspheres depends on the polymer concentration and type of polymer used.

**DISCUSSION**

For the preparation of microspheres various methods i.e. melt dispersion, double emulsion and solvent evaporation were utilized. Melt dispersion method was not the method of choice with Eudragit because of visible changes in physical properties on increasing the temperature. In double emulsion method, after analysis of globule size and shape, a large variation in size with lesser yield was evident. This may be attributed to instability of primary emulsion since droplets agglomerate on addition of the second phase.

Comparatively smoother surface and uniform size of microspheres were obtained for microspheres using solvent evaporation process. In this method, first trial was made to prepare microspheres by using a solvent evaporation technique in water phase like acetone/PVA solution or methanol/PVA solution but low yield of microspheres was obtained. Then (acetone: methanol)/liquid paraffin system was used and various formulation prepared. To select the optimum method, the effect of various experimental parameters such as surfactant concentration, stirring speed, phase ratio, drug/polymer ratio on the morphology and the size of microspheres were investigated.

Combinations of Eudragit RS100 and Eudragit RL100 displayed better effect on release rate of the drug. Upon increasing the proportion of Eudragit RL100, there was increase in release rate of drug which was significant ($p < 0.001$) as shown in study up to 10 hr. After 12 hr and 24 hr the difference was insignificant in case of formulation 17 and 18 ($p > 0.05$). This is due to the fact, that the amount of quarternary ammonium compound in eudragit RL100 is higher than in Eudragit RS 100, which makes it more permeable to water, so that release is less retarded and maximum amount of drug releases before 12 hr of dissolution of drug[8]. Similar results were reported by Dortunc et al.[9]

At low concentration of polymer (1%, w/w) no microsphere product was obtained. Keeping the drug amount and the solvent volume constant, spherical but larger particles were obtained as the amount of polymer was increased to give a polymer: drug ratio of 2:1, 3:1, 4:1, 5:1 ($p < 0.001$). An increase in concentration of the polymer in a fixed volume of organic solvent resulted in an increase in encapsulation efficiency with increasing the polymer:drug ratio ($p < 0.001$), as reported in research article[10].

The influence of stirring rate on the average size of microspheres is evident from data given in the Table No.1. An increase in stirring rate causes a decrease in average particle size; this finding is in accordance with reports of other researcher group[11]. At a higher stirring rate a finer emulsion is obtained since shear force is greater. Significance of influence was confirmed by one way ANOVA ($p < 0.001$).

**Table 1: Effect of fabrication variables on the particle size and encapsulation efficiency**

| Parameters | Fabrication variables | Batch code | Mean diameter (µm), n=3 | Encapsulation efficiency (%w/w), n=3 |
|---|---|---|---|---|
| Eudragit | 2.8:0.2 | F1* | 101.38± 0.35 | 84.90±0.548 |
| RS100:Eudragit | 2.5:0.5 | F2 | 115.67± 0.26 | 81.86±0.789 |
| RL100** | 2.3:0.7 | F3 | 106.43± 0.56 | 78.52±0.549 |
| Drug/Polymer | 1:2 | F4 | 125.37± 2.22 | 81.43± 1.7 |
| Ratio(%) | 1:3 | F5* | 153.22± 2.6 | 86.52± 1.25 |
| | 1:4 | F6 | 139.29± 2.8 | 90.77± 1.55 |
| | 1:5 | F7 | 212.92± 3.3 | 92.21± 1.6 |
| Stirring speed | 500 | F8 | 150.51± 1.8 | 83.63± 0.47 |
| (rpm) | 1000 | F9 | 112.46± 0.5 | 82.36± 0.34 |
| | 1500 | F10* | 100.62± 1.4 | 85.01± 0.38 |
| Surfactsnt | 0.2 | F11 | 148.95± 3.3 | 77.28± 3.5 |
| concentration | 0.3 | F12* | 97.48± 2.7 | 95.88± 1.52 |
| (%w/w) | 0.4 | F13 | 155.68± 1.17 | 85.90± 1.8 |
| | 0.5 | F14 | 201.63 ±1.15 | 80.89± 2.0 |
| Phase ratio | 1.5 | F15 | 112.35± 1.6 | 93.46± 1.0 |
| (%v/v) | 1.10 | F16* | 95.67± 3.6 | 89.42± 3.1 |
| | 1.15 | F17 | 84.98± 1.4 | 85.31± 0.89 |
| | 1.20 | F18 | 75.11± 2.3 | 81.42± 0.9 |

*Optimized formulation **For 1:2 ratio of drug /polymer

Magnesium stearate was added to the formulation as a droplet stabilizer to overcome the problem of droplet coalescence during solvent evaporation. It was noticed that on lowering the quantity of magnesium stearate an increase in globule size and subsequently decreased surface area resulted in lesser entrapment of drug, present in the external phase ($p > 0.05$).

The effect of Continuous phase/Dispersed phase (CP/DP) ratio on properties of microspheres was investigated and no significant difference in particle size was observed ($p > 0.05$). However the encapsulation efficiency increased remarkably with increasing CP/DP ratio ($p < 0.05$) Similar phenomenon has been reported for Progesterone microspheres (Yang et al, 2000). Larger CP/DP ratio displays a higher incidence of burst release required to produce minimum effective concentration as reported by researchers[3]. Properties surfaces of microspheres were investigated on decreasing quantity of continuous phase.

Scanning electron micrographs are shown in Fig. 1a and Fig. 1b. The surface of the loaded microspheres appeared spherical but rough. After dissolution, there were increased number of pores on the surface Fig. 1d in comparison to SEM image of microspheres before dissolution Fig. 1c.

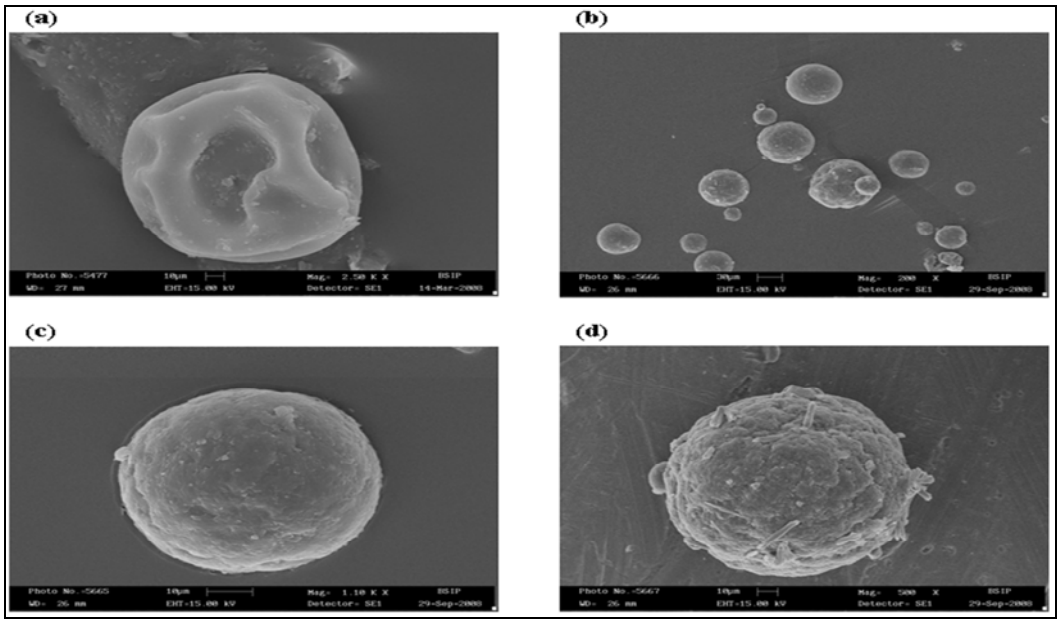


**Fig. 1: Scanning electrone micrographs of optimized formulation a. Placebo batch. b. Loaded with drug. c. Before dissolution. d. After dissolution.**

The dissolution rate of drug from the microspheres was studied at pH 1.2 and pH 7.2 using USP II paddle type apparatus. Ketorolac tromethamine is a weakly acidic drug and its solubility is higher at high pH, as expected. The release profile of ketorolac tromethamine from eudragit microspheres is as shown in Figure 2. All the microspheres showed biphasic release,initially a fast release followed by a slower release. An initial burst observed, was due to the release of the drug present on the surface and solubility of

Ketorolac tromethamine in dissolution medium[12]. Release profile followed Higuchi model ($R^2$ = 0.924). It appears that mechanism of drug release from microspheres was diffusion controlled. The influence of stirring rate on the release kinetics can also be explained by difference in porosity. Microspheres produced at higher stirring rate are more porous, exhibit fast release of drug[13]. It is also evident that the rate of drug release from the microspheres depends on the polymer concentration and ratio of polymer used. The decrease in release rate with increasing concentration of polymer can be explained by a decreased amount of drug present close to the surface ($p < 0.001$).

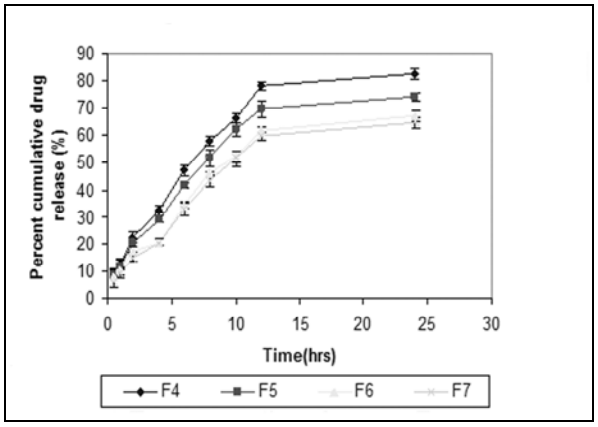


**Fig. 2: In-vitro dissolution profile of formulations F4 to F7.**

**Tail flick method**

The pharmacodynamic studies were conducted using tail flick method. The tail-flick is considered to be a spinally mediated reflex[14]. After administration of pure drug a rapid increase in tail flicking latency period was observed within 0.5 hr of oral administration (Fig. 3). Maximum inhibition of 90% ($p < 0.001$), was observed after which that tail flicking recovered within 6 hr. In case of Ketorolac tromethamine microspheres reduction in tail flick was slow and it started to decrease significantly 196% ($p < 0.05$) within 1 hr, and reached to maximum reduction of 239% ($p < 0.001$) within 6 hr, after oral administration. Significant % inhibition after oral administration of Ketorolac tromethamine, was maintained only from 0.5 - 6 hr where as in case of microspheres, it was maintained for a period of 2 - 10 hr ($p< 0.001$).

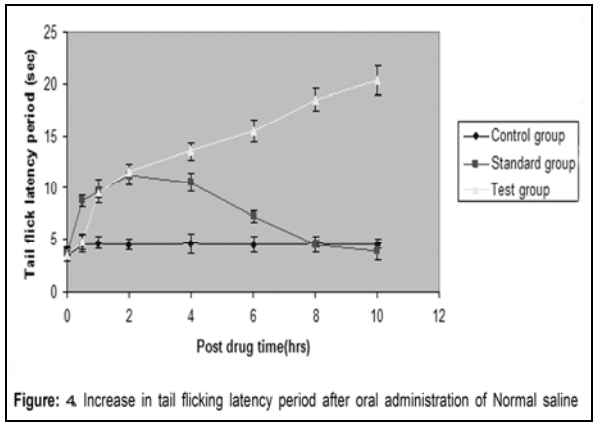


**Fig. 3: Plot of tail flicking latency period against post drug time of optimized formulation F16.**

**Carrageenan-induced paw oedema method**

Carrageenan-induced hind paw oedema is the standard experimental model of acute inflammation[15].The present study demonstrated that, the Ketorolac tromethamine microspheres induced time dependent reduction of paw edema in rat, and produced significant inhibition of paw oedema for a longer period, as compared to standard drug, in dose of 5 mg/kg by oral route.

Treated animals showed significant increase in percent inhibition of paw volume (Fig. 4), compared to the control group ($p < 0.05$). Lesser effect was observed after one hour (29%) when the formulation F16 was administered in comparison to the pure drug (38%). After 2 hr the anti-inflammatory activity (43%) was observed in case of microspheres which was equal to the time taken for pure drug. Overall, the anti-inflammatory activity of pure drug was maintained only for 0.5 hr, which was fond to be subsided by 6 hr. But in case of our mcrospheres the analgesic activity was 56% at the end of 10 hr, which was maintained almost up to 12 hr and thus was significant.

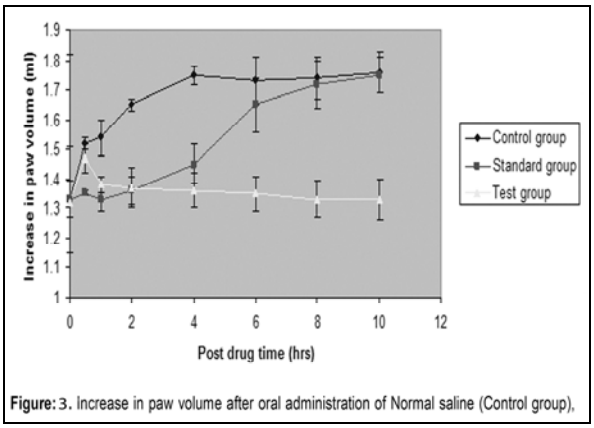


**Fig. 4: Plot of increase in paw volume against post drug time of optimized formulation F16.**

This data clearly shows sustained and uniform release of Ketoprofen tromethamine over a longer period, which proves that Ketoprofen tromethamine sustained release microspheres were significantly more effective than standard drug. In conclusion, formulation F16 (containing polymer: polymer ratio of 2.7:0.3, drug polymer ratio 1:3 and phase ratio 1: 8) achieved the aim of the present study.

**CONCLUSION**

Microspheres were prepared successfully using solvent evaporation method. The surface structure of the microspheres was spherical and rough. Optimization of all the parameters was required to obtain a better formulation with good encapsulation efficiency and reduced size. Release rate of Ketorolac tromethamine from the microspheres were dependent on combination of polymer and the amount of polymer used. The drug release profile, aimed for peroral administration could be obtained by optimizing ratio of Eudragit RS100 and Eudragit RL 100. Release pattern was found to be of Higuchi type. The in vivo study demonstrated significant analgesic and anti inflammatory activity of microspheres. Sustained release without initial peak level achieved with these microsphere formulations can reduce the dosing frequency, decreased side effects and improve patient compliance.

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**REFERENCES**

1. Muriel P, Gallo MO, Hernandez GC, Urizer JP, Agurre AP. Ketorolac pharmacokinetics in experimental cirrhosis by bile duct ligation in the rat. Annal Hepatol. 2003; 2: 175-181.
2. Vyas, SP, Khar RK. Targeted and controlled drug delivery novel carrier system. I$^{st}$ed. CBS Publication and distributors, Delhi; 2007.
3. Kissel T, Schaper A, Li L, Xu TJ, Shi Y, Mao S. Effect of process and formulation parameters on characteristics and internal

morphology of poly (D,L-lactide-co-glycolide) microspheres formed by the solvent evaporation method. Int. J. Pharm. 2008; 68: 214-223.

4. Giandalia G, Caro Viviana DC, Cordone L, Giannola LI. Trehalose-hydroxyethylcellulose microspheres containing vancomycin for topical drug delivery. Eur. J. Pharm. Biopharm., 2001, 52 (1), 83-89.
5. Gopferich A, Herrmann J, Reithmeier H . Development and characterization of lipid microparticles as a drug carrier for somatostatin. Int. J. Pharm. 2001; 218: 133-143.
6. Price JC, Obeidat WM. Preparation and in vitro evaluation of propylthiouracil microspheres made of eudragit RL100 and cellulose acetate butyrate polymers using the solvent evaporation method. J. Microencapsul. 2005; 22: 281-289.
7. Baykara T, Yuksel N, Kilicarslan M, Horoz BB. Influence of aluminium tristearate and sucrose stearate as the dispersing agents on the physical properties and release characteristics of eudragit RS microspheres. AAPS Pharm.Sci.Tech. 2006; 7: 16.
8. Baykara T, Kilicarslan M. The effect of the drug/polymer ratio on the properties of the varapamil HCL loaded microspheres. Int .J. Pharm. 2003; 252: 99-109.
9. Haznedar S, Dortunc B. Preparation and in-vitro evaluation of eudragit microspheres containing Acetazolamide. Int. J. Pharm. 2004; 269: 131-140.
10. Bahera BC, Sahoo SK, Dhal S, Barik BB, Gupta BK. Characterization of Glipizide-loaded polymethacrylate microspheres prepared by an emulsion solvent evaporation method. Trop. J. Pharm. Res. 2004; 7: 879-885.
11. Sahoo SK , Mallick AA, Barik BB, Senapati P. Formulation and in-vitro evaluation of Eudragit microspheres of satavudine. Trop. J. Pharm. Res. 2005; 4: 369-375.
12. Bozdag S, Calis S, Kas HS, Peksoy MT, Hincal A. In-vitro evaluation and intra-articular administration of the biodegradable microsphere containing Naproxen Sodium. J. Microencapsul. 2001; 18: 443-456.
13. Mateeovic T, Kriznar B, Bogataj M, Mrhar A. The influence of stirring rate on biopharmaceutical properties of Eudragit RS microspheres. J Microencapsul. 2005; 19: 29-36.
14. Jurgensen S, Dalbo S, Angers P, Roberto A, Soares S and Ribeiro-do-valle RM. Involvement of 5- HT2 receptors in the antinociceptive effect of uncaria tomentosa. Pharmacol Biochem behave. 2005; 81: 466-477.
15. Chakrabort A, Devi RKB, Rita S. Preliminary studies on anti-inflammatory and analgesic activities of Spilanthes acmella in experimental animal models. Ind. J. Pharmacol. 2004; 36: 148-150.
16. Sudjarwo. The potency of piperine as anti-inflammatory and analgesic in rats and mice. Folia Medica Indo. 2005, 41: 190-194.