DESIGN, DEVELOPMENT, PHYSICOCHEMICAL CHARACTERISATIONS AND *IN VITRO* EVALUATION OF KETOPROFEN MICROSPHERES

Varshney H.M.*1, Tanwar Y.S.*2, Chauhan B.S.[1], Sharma P.K. [1]

*[1] **Jaipur College of Pharmacy, Jaipur (Raj.),**

*[2] **B.N. (PG) College of pharmacy, Udaipur (Raj.),**

ABSTRACT

Background and the purpose of the study: Generally, drug release from biodegradable polymer depends upon the drug solubility in the polymer, the chemical composition of the polymer and drug particle size. Ketoprofen exhibits short half-life, poor compressibility, caking tendency, gastrointestinal irritation & ulcerogenic effect. Hence, it is desirable to formulate into sustained release dosage form. Microspheres of ketoprofen were developed as oral sustained release dosage form using ethyl cellulose as the coating/ matrix forming material or release retarding material, influence of non-solvent used for the polymer solution i.e. acetone and dichloromethane, employed in the formulation was studied on the drug release from ethyl cellulose microspheres.

Methods: Ketoprofen microspheres were formed using solvent evaporation technique in different ratios and by using different stirrer speed. The microspheres were evaluated for size distribution, surface characteristics, drug content, drug entrapment efficiency, drug release characteristics.

Results and major conclusion: The use of different ratio of ethyl cellulose polymer, different mixing amount of solvent ratio and different stirring speed clearly indicates that the formulations can delay the drug release prior to microspheres. The maximum release of the ketoprofen microsphere was found with FM-3, they can be arranged as increasing order- FM-3 > FM-4 >FM-5 > FM-2 > FM-1. Poorly water soluble ketoprofen loaded microspheres safely improve the solubility and/or absorbability of drug.

Keywords**:** Ketoprofen, Ethyl cellulose, Acetone, Dichloromethane

INTRODUCTION

Generally, drug release from biodegradable polymer depends upon the drug solubility in the polymer, the chemical composition of the polymer and drug particle size. The drug release from the polymer is influenced by the presence of other additives in the formulation and may result in an increase or decrease in the rate of release depending on the nature of the polymer and that of the additives and its concentration (1). Many sustained release dosage forms are designed to release the drug at slow rates maintaining uniform selective therapeutic drug levels for an extended period of time. There are several techniques used to produce polymeric microspheres drug delivery systems, which include physicochemical processes (such as solvent evaporation method or phase separation method), mechanical processes (such as spray drying), and a non-solvent addition process (2).

Ketoprofen is an NSAID having prominent anti-inflammatory, analgesic and antipyretic properties. Ketoprofen is an arylpropionic acid derivative. Ketoprofen is one of the most powerful inhibitors of cyclooxygenase at concentrations well within the range of therapeutic plasma concentrations (EC50 2µg/l). This inhibits ion results in a reduction in the tissue production of prostaglandins such as PGE2 and PGF2a. In addition to its effect on cyclooxygenase, Ketoprofen inhibits the lipoxygenase pathway of the arachidonic acid cascade. Ketoprofen is also a powerful inhibitor of bradykinin, an important chemical mediator of pain and inflammation. It also stabilizes lysosomal membranes against osmotic change and prevents the release of lysosomal enzymes that mediate tissue destruction in inflammatory react ions (3, 4).

Corresponding author:
HIMANSHU MOHAN VARSHNEY
Ph.D. Scholar, M.Pharm, (Pharmaceutics)
Sr. Lecturer
***242, Shiv Vihar, Opp. Road No. 5, ***
Vishvakama Thana, Chaumu Road, Jaipur (Raj.)-302013, India
Ph. No. 01416459818 (R), 09887587443 (M)
Email: hmvmpharm2005@yahoo.com

Ketoprofen is a NSAID readily absorbed from the gastrointestinal tract and peak plasma concentrations occurs in about 1–2 h after dosing, but it causes certain level of irritation in the gastrointestinal mucous membrane. The half-life of Ketoprofen in plasma is about 2–2.5 hours. The short half-life and the low single dose administration make Ketoprofen a very good candidate for the formulation of sustained release dosage forms (5). It is used in musculoskeletal and joint disorders such as ankylosing spondylitis, osteoarthritis, and rheumatoid arthritis, in soft-tissue disorders such as sprains and strains, for postoperative pain, in mild to moderate pain including dysmenorrhoea and migraine (1,6,7).

The objective of the study is to develop microsphere of Ketoprofen by using non-biodegradable polymer and study the effect of drug-polymer concentration on drug release with a view to avoid loss of drug due to first pass effect and to uncover toxic effects and produce safe and effective dosage form and safely improve the solubility and/or absorbability of poorly soluble drug.

Table. 1.: Preparation of different ketoprofen microspheres

Code	Core Material : Coating Material Ratio	Solvent Ratio	Stirring Speed (rpm)
FM-1	1:1	5:5	**450**
FM-2	1:1.5	4:6	**550**
FM-3	1:2	3:7	**650**
FM-4	1:2.5	2:8	**750**
FM-5	1:3	1:9	**850**

MATERIALS AND METHODS:

Ketoprofen was purchased from Yarrow Chem Products, Wadala (E), Mumbai, Ethyl cellulose was purchased from Ases Chemical Works, Jodhpur, Acetone and Dichloromethane (Merck, India), polyvinyl alcohol (Merck, India). All other chemicals and reagents were used of analytical grade.

Preparation of Ketoprofen Microspheres:

The details of the formulations are given in Table 1. Ketoprofen microspheres were prepared by using Emulsion Solvent Evaporation method. Ketoprofen, as core material and ethyl cellulose, as coating/ matrix forming material in different ratios were dissolved in different ratio of acetone and dichloromethane and prepared homogenous polymer solution was kept in an ultrasonifier for 10 minute. Prepared 1% poly vinyl alcohol (PVA) solution. Then drug-polymer solution poured slowly in 1% PVA solution at different rpm using a propeller stirrer with constant stirring for 2 hour at ambient conditions until all acetone-dichloromethane solvent completely evaporated from the aqueous solution. Then microsphere filtered and washed with water 3-4 times and dried in oven at 40^0C for 1-2 hour. Dried microspheres were stored at room temperature (8, 9).

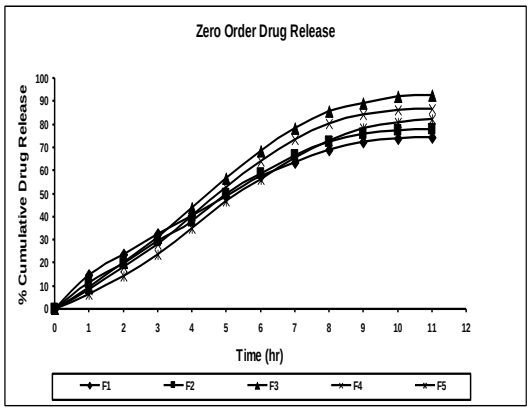


Fig. 2. - Zero order drug release profile of different microsphere formulations

Characterization of Microspheres:

Micromeritics of microspheres (Particle size, shape and surface morphology analysis):

All the microspheres were evaluated with respect to their size and shape using optical photomicroscope fitted with an ocular micrometer, a stage micrometer and camera. An average of 100 microspheres were used for the study and the mean particle size (arithmetic mean diameter) was consider to be the deciding factor in selecting optimum formulation conditions for each variable parameter studied (10, 11). The angle of repose, bulk density and tapped density were measured and shown in table 2.

Drug content determination in microspheres:

Drug loaded microspheres (100 mg) from each batch were crushed and make finely powdered in a glass mortar, then dissolved in 100 ml of Phosphate buffer solution pH 7.4 and the resultant dispersions were exposed to ultrasonic treatment for 20 min. The ultrasonic treatment was repeated 3 times with a resting period of 30 min. between

the treatments. Filtered this solution through 0.45 µ filter and prepared solution with suitably dilution and the absorbance was determined spectrophotometrically at 258 nm (Shimadzu 1700 UV Visible Spectrophotometer). The Ketoprofen content of the microspheres was calculated using a standard calibration curve (12). The drug content estimated from different batches is shown in table 2.

Drug Entrapment Efficiency:

Accurately weighed quantity of drug loaded microspheres were suspended in methanol to extract the drug from microspheres and then shaken in a mechanical shaker. After 24 hrs., the filtrate was analysed spectrophotometrically at 258 nm for drug content against methanol as blank (13, 14, 15). Corresponding drug concentration in the sample was calculated from the calibration plot generated by regression of the data. Preliminary UV scanning showed that the presence of the polymers did not interference with the absorbance of Ketoprofen at 258 nm and drug entrapment efficiency estimated from different batches is shown in table 2. Drug entrapment efficiency was calculated by using the formula:

Drug entrapment efficiency =

$$\frac{\text{Estimated drug content (\%)}}{\text{Theoretical drug content (\%)}} \times 100$$

Swelling Ability:

Swelling ability of microspheres in physiological media was determined by swelling them to their equilibrium. Accurately weight amounts of microspheres were dissolved in little excess of phosphate buffer and kept for 24 hrs. the excess surface-adhered liquid drops were removed by blotting and the swollen microspheres were weighed. The microspheres were then dried in an oven at 60^0C for 5 hrs. until there was no change in the dried mass of the sample (16, 17). Swelling index estimated from different batches is shown in table 2. Swelling index of the microspheres was calculated by using the formula:

Swelling Index =

$$\frac{\text{Mass of swollen microspheres – mass of dried microspheres}}{\text{Mass of dried microspheres}} \times 100$$

Table.2.: Physico-chemical characterization of ketoprofen microspheres

Code	Shape	Bulk Density (g/cm^3)	Tapped Density (g/cm^3)	Angle of Repose (°)	Average Particle Size (µm)*	% Drug Content*	Drug Entrapment Efficiency (%)	Average Swelling Index#	Percen-tage yield (%)
FM-1	Spherical	0.3292	0.3821	31.34	169.52 ± 0.58	50.28±0.12	49.09	1.12±0.34	**79.54**
FM-2	Spherical	0.3454	0.3974	27.76	178.78± 0.62	72.12±0.58	70.87	1.19±0.39	**82.69**
FM-3	Spherical	0.3280	0.3741	28.96	199.54± 0.74	79.51±0.21	78.71	1.25±0.67	**87.36**
FM-4	Spherical	0.3373	0.3923	29.88	217.76±0.85	74.27±0.43	71.42	1.33±0.72	**91.41**
FM-5	Spherical	0.3496	0.4169	32.82	261.98±0.72	73.29±0.18	69.88	1.38±0.56	**93.49**

* values expressed as Mean ± SD, n=100.

values expressed as Mean ± SD, n=3.

In vitro release studies:

In vitro drug release studies were carried out using USP dissolution apparatus I (Electrolab, Mumbai, India) at 100 rpm. In flask, 900 ml of Phosphate buffer solution pH 7.4 was used as a dissolution medium. The temperature was maintained at 37±0.5 ^{0}C. An accurately weighed amount of the prepared microspheres equivalent to 100 mg of the drug were filled in hard gelatine capsules and putted into the basket. The samples (10 ml) were withdrawn at predetermined interval of 1 hour for 11 hour and filtered through 0.45 µ membrane filter. The same volume (10 ml) of the

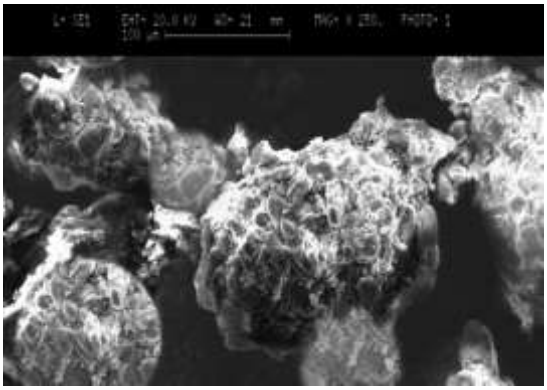
dissolution medium was replenished immediately. Then absorbance was determined spectrophotometrically (18).

Kinetics of drug release

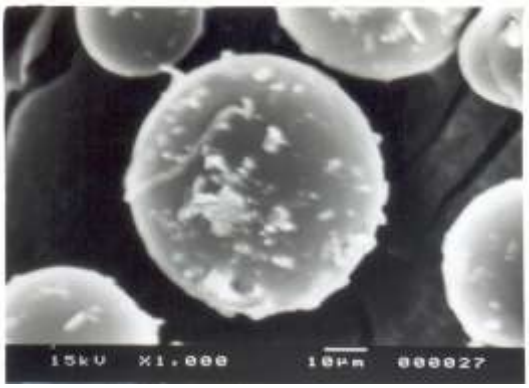
To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models represent in zero order, first order. In short, the results obtained from *in vitro* release studies were plotted in two kinetic models of data treatment as follows:

Cumulative percentage drug release v/s Time (zero order rate kinetics) shown in Fig. 2.

Log cumulative percentage drug retained v/s Time (first order rate kinetics) shown in Fig. 3(19).



(a)



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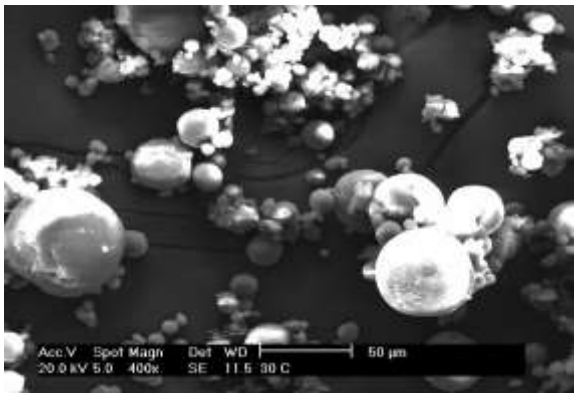

(c)

Fig.1. SEM of Ketoprofen microspheres:
(a) Microspheres prepared below at 650 stirrer speed (b) Microspheres prepared at 650 stirrer speed
(c) Microspheres prepared above at 650 stirrer speeds.

RESULTS AND DISCUSSION:

Ketoprofen must be administered at least three times in a day. In the usual oral administration of NSAIDs, the tablets and capsules have let to peptic ulceration and anorexia. Its physicochemical characteristics (weak acid) are responsible for the adverse effect on the gastro intestinal tract resulting in an increased incidence of gastric irritation.

Ethylcellulose has been reported as one of the important matrix forming polymeric materials used in microspheres. This is due to its high safety, good stability, easy fabrication and cheapness. In present work sustained release ethyl cellulose microspheres containing ketoprofen were prepared by a emulsion solvent evaporation method employing two solvents for the polymer solubilisation namely acetone and dichloromethane. All the microspheres were found to be discrete, spherical with smooth surface, glossy in nature and free flowing. The effect of various process variables like stirring speed, amount of cross-linking agent and core: coat ratio on particle size of microspheres is prepared and tabulated in Table-1 and evaluated for physical properties like particle size, bulk and tapped density, angle of repose, drug entrapment efficiency, swelling index and *in vitro* drug release study are tabulated in Table-2.

The experiments were carried out and the results of % yield of ketoprofen microspheres were 79.54% to maximum of 93.49%. The maximum yield was obtained with formulation FM-5.The

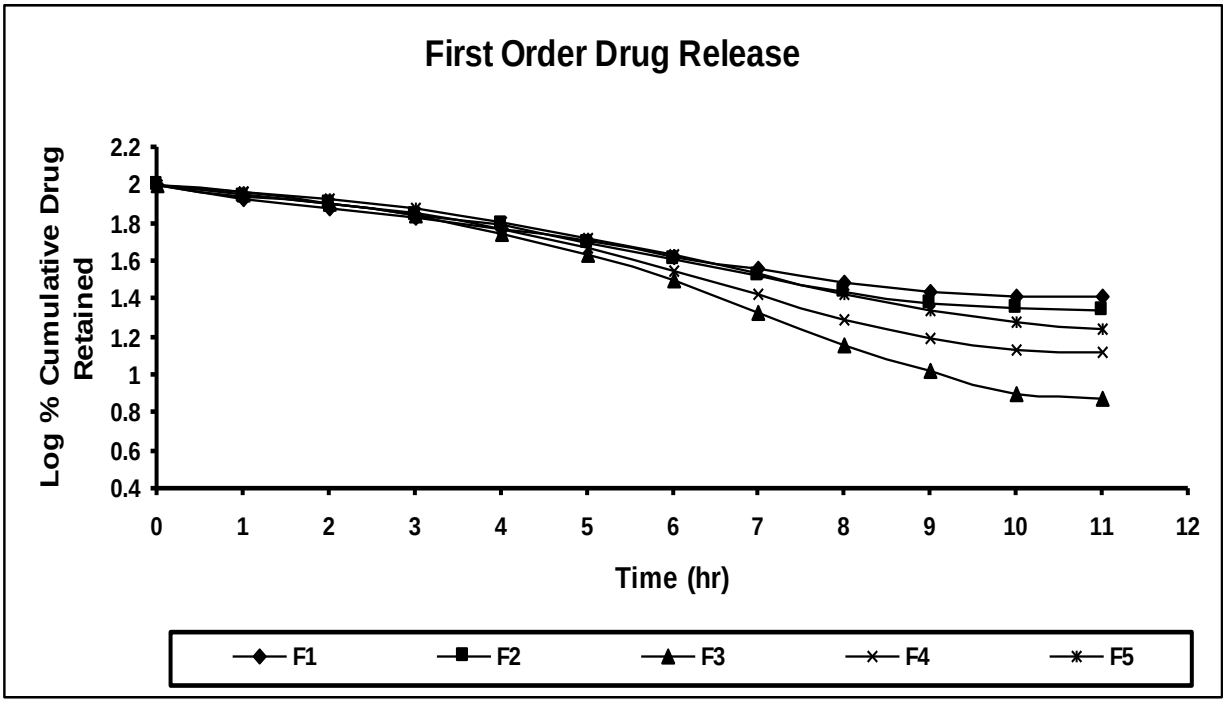

Fig. 3. - First order drug release profile of different microsphere formulations

microspheres vary in size range from 169.52 – 261.98 µm. The particle size distribution was uniform and narrow. The angle of repose of microspheres was found to be less than 40° i.e. 27.76 – 32.82° (very good flow) which indicate free flowing nature. The bulk density and tapped density were found to be less than 1.25 g/cm^3 i.e. 0.3280 – 0.3496 and 0.3741 – 0.4169 g/cm^3 respectively which indicate good packing and flow characteristics.

It was found that percent drug content of Ketoprofen was between 50.28-79.51% depending on core: coat ratio. The effect of core: coat ratio on percent drug entrapment efficiency was also studied. The total drug encapsulated was found to be less than theoretical yield i.e. 49.09 – 78.71. This may be due to uneven or non-uniform distribution of drug in microspheres. The microspheres prepared in 1:2 ratio of drug to EC were found to contain highest drug encapsulated as compared to other batches.

It was found that swelling index was between 1.12 – 1.38% due to core: coat ratio and the amount of polymer plays an important role in solvent transfer. It can be concluded from data shown in table 2 that with an increase in polymer concentration, the time for maximum swelling index increases. Thus, we can concluded that amount of polymer directly affects the swelling index. During the swelling process, two phenomenon were involved, i.e. swelling and thus increase in particle size; and dissolution and then decrease in particle size of microspheres. Competitions in the two processes determine the final behaviour of microspheres. EC microspheres swell quickly within 40 minutes, but after this time, size decreases owing to the prevalence of dissolution. Maximum swelling was observed with the microspheres with less cross-linking agent and swellability decreases with increase in cross-linking density and amount of polymer. This could be due to increasing cross-linking of hydroxyl group of the polymer with the cross-linking agent.

In vitro dissolution studies were performed and plot of different order of drug release were obtained in Fig. 2, 3. Drug release followed a biphasic pattern i.e. initial fast release called as burst effect and later on a sustained release which may be due to swelling of coating polymer. The initial fast release of drug may be due to the porous surface. The present study reveals that 1:2 (drug: polymer) ratio was suitable for producing the ideal spherical microspheres and gives better sustained release for 11 hrs. & about 92% of drug was released at the end of 11 hrs. Resultant microspheres did not have any surface irregularities and are non-aggregated. As the drug:

polymer ratio was decreased, the yield was reduced and the resultant microspheres were irregularly shaped and were highly aggregated in nature and highly impossible to distinguish as individual microspheres. In order to avoid the formation of irregularly shaped larger particles, in the present method, (1:2) drug: polymer ratio was best used. In an attempt to investigate the effect of stirring speed on the particle size distribution and drug release profile from the microspheres.It was observed that with the increase in the stirring speed from 650 rpm there was a decrease in the formation of the spheres and recovery yield of the microspheres. It is due to small sized microspheres, which were lost during successive washings. When the stirring speed was lower than 650 rpm, gel like slime was formed (shown in Fig.). When the stirring time was lower than 30 minute, little amount of melted material adhered to the sides of the beaker during the cooling process, results in reduction of yield. About 92% of the microspheres of different batches were found to be in the size range of 160-280µm. The drug was released at a uniform rate from the microspheres but the highest drug release was obtained at 650 rpm stirring speed in all batches. It was found that as the polymer concentration increases at maximum level drug release was found to be retarded. With respect to release rate different formulation can be arranged at - FM-3 > FM-4 >FM-5 > FM-2 > FM-1.

To investigate the influence of solvent ratio on the drug release, various batches were prepared using different ratio of solvent blend of acetone and dichloromethane. The polymer to drug dispersion was found to be more translucent in nature with this solvent blend. The drug release in the first hour from the microspheres of batch FM-5 reduced to 6% as compared to 14% from batch FM-1. the results reveal that the change in solvent blend did have significance influence on the burst effect. The probable reason is the difference in solubility of the drug in the two solvent systems. In order to investigate the effect of polymer to drug ratio, the performance of different batches were compared. The drug was released at a faster rate from the microspheres of batch FM-3 and at a relatively slower rate from the microspheres of batch FM-1. The probably reason for slower drug release is the change in the tourtuosity of the matrix. It may be concluded that by choosing an appropriate polymer to drug ratio, one can engineer the release of drug.

Microscopic evaluation:

SEM photographs showed that the microspheres were spherical in nature, had a smooth surface with inward dents and shrinkage, which is due to the collapse of the wall of the microspheres. Photographs reveal that as the concentration of the polymer was increased the surface of the microspheres was found to be smooth and the surface shrinkage reduced, indicating uniform distribution of the drug within the microspheres. The rate of solvent removal from the microspheres exerts an influence on the morphology of the final product. (20) Photomicrographs of Ketoprofen microspheres were presented in Fig.1. All microspheres appeared porous in nature.

CONCLUSIONS:

Ethyl cellulose microspheres loaded with Ketoprofen (FM-3) can be prepared by the use of the solvent evaporation method. The procedure gave satisfactory results in terms of the size, shape, and size distribution of the microspheres. The dissolution experiment showed that the release of drug is in Phosphate buffer solution pH 7.4 media and shows good release profile.

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