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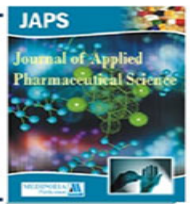


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# Development and Characterization of Controlled Release Ketoprofen Microspheres

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## ABSTRACT

Microspheres ketoprofen (KP) were prepared by o/w emulsion solvent evaporation technique using Eudragit®RS100 as a polymeric material. The effect of process variables such as drug: polymer ratio, stirring rate, emulsifier concentration, internal organic solvent and dispersing medium volumes were examined. The prepared formulations were characterized for particle size distribution, percent yield, entrapment efficiency, in vitro release and in vivo studies behavior. The study revealed that the mean particle size ranged from 293.06 to 438.63 µm, the KP loading efficiency varied from 60.19-87.63% of the theoretical amount incorporated, all microspheres patches exhibited a prolonged release for almost 24 hrs and the release pattern was diffusion model. The pharmacokinetic parameters, $C_{max}$, $T_{max,}$ $AUC_{0-24}$ and $AUC_{0-\infty}$ were calculated from the plasma drug concentration-time data. Plasma KP concentrations and pharmacokinetics parameters were statistically analyzed. The test formulation exhibited controlled and prolonged absorption ($T_{max}$) of 6.79 ± 0.0.39 vs. 2.03 ± 0.08 and 3.32 ± 0.24 hs; $C_{max}$ of 14.42 ± 0.50 vs. 18.78 ± 0.95 and 16.58 ± 1.02 µg/ml) when compared to the plain drug and marketed product, respectively. In-vivo studies revealed that the relative bioavailability of the KP increased by more than 1.3 times by formulating it into microspheres compared to plain drug.

**Keywords:** Microspheres, Ketoprofen, Solvent evaporation technique, Eudragit RS100, Pharmacokinetics.

## INTRODUCTION

Ketoprofen (KP) is a poorly water-soluble non-steroidal anti-inflammatory, antipyretic and analgesic drug, frequently used for the treatment of rheumatoid arthritis, osteoarthritis (Rother *et al.*, 2007), anklylosing spondylitis, a variety of other acute and chronic musculoskeletal disorders and mild to moderate pain (Beltran *et al.*, 1998). Ketoprofen is a potent non-steroidal anti-inflammatory drug that inhibits prostaglandin synthetase cyclooxygenase. Its oral administration is associated with a high risk of adverse effects such as irritation, ulceration of the gastrointestinal tract, oedema, dizziness, and peptic ulceration when taken orally for a prolonged period (Lanza *et al.*, 1998). These attributes make KP a good candidate for controlled release dosage forms. Microspheres are one of the multiparticulate drug delivery systems and are prepared to obtain prolonged drug delivery, improve bioavailability or stability and target drug to specific sites (Sudhamani *et al.*, 2010). They have played a vital role in the development of controlled/sustained release drug delivery systems (Deore *et al.*, 2009).

Microspheres can be defined as solid, approximately spherical particles ranging from 1 to 1000µm, containing dispersed drug in either solution (or) microcrystalline form (Vyas and Khar, 2006). These microspheres are suitable alternatives for conventional dosage forms (Malaekeh-Nikouei *et al.*, 2005).

Several methods, including Emulsion solvent evaporation technique (Karal-Yılmaz *et al*. 2011), phase-separation or coacervation method (El-Bagory *et al.*, 2007), emulsification diffusion method (Liu *et al.*, 2010), and spray drying method (Huh *et al*., 2010) are commonly used for the preparation of microspheres. The solvent evaporation method has gained much attention due to its ease of fabrication without compromising the activity of drug. This technique offers several advantages and is preferable to other preparation methods such as spray drying, sonication and homogenization because it requires only mild conditions such as ambient temperature and constant stirring. Thus, a stable emulsion can be formed (Basu and Adhiyaman, 2008) and microspheres are formed by the evaporation of an organic solvent from dispersed oil droplets containing both polymer and drug (Lakshmana *et al.*, 2009).

Several publications have described drug-containing microspheres using the Eudragit series as the encapsulating materials. The Eudragits are a family of polymers based on acrylic and methacrylic acids suitable for use in orally administered drug delivery systems. They have been used in the microencapsulation of drugs (Rassu *et al.*, 2008). Some dissolve rapidly at clearly defined pH values, whereas two grades, Eudragit ® RL and RS, are insoluble in aqueous media water and digestive juices, but swell and are permeable, which means that the drugs can be released by diffusion (Kibbe, 2000). Therefore, the permeability of drug through Eudragit RS and/or RL is independent on the pH of the digestive tract (Apu *et al*., 2009).

Eudragit®RS100 is a water-insoluble polymer that is widely used as a wall material for sustained release microspheres due to its biocompatibility, good stability, easy fabrication and low cost.

The main objective of this work was to investigate the possibility of obtaining a sustained release formulation of ketoprofen microspheres by the solvent evaporation method using Eudragit®RS100. Investigation of the effect of various processing and formulation factors such as drug to polymer ratio, stirring speed, surfactant concentration and others on the shape, mean particle size, yield of production, particle size distribution, encapsulation efficiency, and in-vitro release rate of drug from the microspheres were performed. Also, the bioavailability of ketoprofen from different formulations using rabbits as experimental animals was assessed.

## MATERIALS AND METHODS

### Materials

Ketoprofen (KP) was a gift sample kindly supplied by Amriya Pharmaceutical Industries, Alexandria, Egypt. Flurbiprofen (FP) was a gift kindly supplied by the Egyptian International Pharmaceutical Industries Co., (EPICO) El-Asher of Ramadan city, Egypt. Eudragit® RS100 (ERS100) was kindly supplied by Rohm Pharma, Darmstadt, Germany. Dichloromethane, ethanol, and sodium lauryl sulphate were purchased from El-Gomhorea Chemical Company, Cairo, Egypt. HPLC grade acetonitrile and methanol were purchased from Sigma-Aldrich Chemie, Germany. Cal-Heparine 5000 I. U. was purchased from Amoun Pharmaceutical Co., El-Obour City, Cairo, Egypt. Phosphoric acid, Potassium- dihydrogen phosphate, and Hydrochloric acid were obtained from El-Nasr Pharmaceutical Chemical Co., Cairo, Egypt. All other chemicals were obtained from El-Nasr Pharmaceutical Chemical Co., Cairo, Egypt.

### Preparation of ketoprofen-loaded microspheres

The ketoprofen loaded Eudragit microspheres were prepared by the conventional emulsion solvent evaporation method which was adapted from the process described by Mao *et al.* (2008). KP and Eudragit were used in ratios 1:1, 1:2, 1:3, 1:4 and 1:5 to obtain significant different characteristics. The required amount of the polymer was dissolved in 10ml of a mixture of dichloromethane and ethanol (1:1 v/v). The calculated amount of KP powder was dissolved in the polymeric solution. The prepared dispersion was slowly poured into 100 ml of 0.2 % w/v SLS aqueous solution and was emulsified by vigorous stirring at (800, 1200, and 1600rpm) at room temperature using a three-blade mechanical stirrer. The dispersed drug and polymer were immediately transformed into fine droplets, which were subsequently solidified into rigid microspheres due to solvent evaporation (Trivedi *et al*., 2008). Stirring was continued for 3-4 hrs until all solvent was evaporated. The formed microspheres were allowed to settle, filtered and washed several times with distilled water (Deveswaran *et al.*, 2010). The microspheres were dried and stored in air tight containers until further analysis. Formulations with different drug to polymer ratios were prepared as shown in table (1).

**Table. 1:** The composition of different microspheres formulations.

| For-mulation. No. | Drug: polymer ratio. | Stirring rate (rpm). | Surfactant. Conc (w/v). | Internal phase volume (ml). | External phase volume (ml). |
|---|---|---|---|---|---|
| 1 | 1:1 | 800 | 0.2% | 10 | 100 |
| 2 | 1:2 | 800 | 0.2% | 10 | 100 |
| 3 | 1:3 | 800 | 0.2% | 10 | 100 |
| 4 | 1:4 | 800 | 0.2% | 10 | 100 |
| 5 | 1:5 | 800 | 0.2% | 10 | 100 |
| 6 | 1:1 | 1200 | 0.2% | 10 | 100 |
| 7 | 1:2 | 1200 | 0.2% | 10 | 100 |
| 8 | 1:3 | 1200 | 0.2% | 10 | 100 |
| 9 | 1:4 | 1200 | 0.2% | 10 | 100 |
| 10 | 1:5 | 1200 | 0.2% | 10 | 100 |
| 11 | 1:1 | 1600 | 0.2% | 10 | 100 |
| 12 | 1:2 | 1600 | 0.2% | 10 | 100 |
| 13 | 1:3 | 1600 | 0.2% | 10 | 100 |
| 14 | 1:4 | 1600 | 0.2% | 10 | 100 |
| 15 | 1:5 | 1600 | 0.2% | 10 | 100 |
| 16 | 1:5 | 800 | 0.1% | 10 | 100 |
| 17 | 1:5 | 800 | 0.4% | 10 | 100 |
| 18 | 1:5 | 800 | 0.7% | 10 | 100 |
| 19 | 1:5 | 800 | 0.2% | 5 | 100 |
| 20 | 1:5 | 800 | 0.2% | 15 | 100 |
| 21 | 1:5 | 800 | 0.2% | 10 | 50 |
| 22 | 1:5 | 800 | 0.2% | 10 | 150 |

**Yield analysis of the recovered microspheres**

The relative yield was calculated based on the amount of microspheres of each formulation obtained relative to the amount of solid materials used in the dispersed phase (Trivedi *et al.*, 2008). The percentage yield was calculated according to the following equation (Najmuddin *et al.*, 2010).

$$\text{Yield (\%)} = \frac{\text{Actual weight of microspheres}}{\text{Total weight of drug and polymer}} \times 100$$

**Particle size analysis**

The mean particle sizes of the microspheres were determined by sieving method (Gupta *et al.*, 2009). ERS100 microspheres containing KP were placed on a set of standard sieves of size range 150-850 um and shaken for 10min using mechanical sieve shaker. The resulting fractions remaining on the sieves were weighed to determine the particle size distribution (El-Helw *et al.*, 2008). The mean microspheres diameter was calculated after sieving. Results were reported as means ± S.D. The mean particle size of microspheres was calculated using the following formula (Ansel *et al.*, 1995; Behera *et al.*, 2008).

$$\text{Mean Particle size} = \frac{\Sigma(\text{Mean particle size of the fraction} \times \%\text{weight fraction})}{\Sigma\ \%\text{Weight fraction}}$$

**Entrapment efficiency**

The entrapment efficiency % of the prepared microspheres was evaluated using the method of Gangadhar *et al.* (2010) with certain modification. About 25mg of the obtained microspheres were crushed into powder and were completely dissolved in 100ml of PBS (pH 7.4) and agitated in mechanical shaker for 6hrs then kept for 24hrs. Then 5ml of the solution was filtered and concentration of the drug was determined after certain dilution spectrophotometrically at 260nm (Deveswaran *et al.*, 2010). The actual drug loading and encapsulation efficiency (EE %) were calculated using the following equations (Mao *et al.*, 2008; Yerriswamy *et al.*, 2010).

$$\text{Theoretical drug loading (\%)} = \frac{\text{Drug (total)}}{\text{Drug (total)} + \text{polymer}} \times 100$$

$$\text{Actual drug loading (\%)} = \frac{\text{Drug (entrapped.)}}{\text{Drug (total)} + \text{polymer}} \times 100$$

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \times 100$$

***In-vitro* dissolution studies of KP-loaded microspheres**.

Since the permeability of a drug through Eudragit®RS100 is independent on the pH of the digestive tract (Apu *et al.*, 2009), the pH value of the tested dissolution medium was set at 7.4 (Kim *et al.*, 2002). Dissolution of KP from the prepared microspheres was carried out using microspheres equivalent to 50mg of the drug. The dissolution apparatus with 100rpm paddle rotational speed was used in the studies**.** 500 ml of PBS (pH 7.4) was used as a dissolution medium and maintained at $37^0C \pm 0.5^0C$ (El-Nahas *et al.*, 2010). At various time intervals, 4ml samples of the dissolution medium were taken and replaced with an equal volume of pre-warmed fresh medium to maintain constant volume (Raval *et al.*, 2010). The drug concentration and the percentage drug released were determined with respect to time spectrophotometrically at 260nm (Yerriswamy *et al.*, 2010). The in-vitro dissolution studies were performed in triplicate for each of sample and the results were reported as means ± S.D.

**Morphology of microspheres**

The morphology of the obtained microspheres was examined by a light microscope (Zeiss, Me 63 C, West Germany) with varied magnification powers. One drop of the freshly prepared microsphere suspension was poured onto a slide and sealed with a cover glass. Photomicrographs were captured using Samsung digital camera (Chaisri *et al.*, 2009). The morphology, size uniformity and aggregation or coalescence of the microspheres were studied.

***In-vivo* study**

***Construction of the standard calibration curve for ketoprofenin plasma***

The plasma samples were analyzed using validated HPLC method described previously (Onishi *et al.*, 2005; Heo *et al.*, 2008) with certain modification. The mobile phase consisted of a mixture of 0.02 M phosphate buffer pH 3 and acetonitrile in ratio of 55: 45 v/v, respectively. A stock solution of KP was prepared at a concentration of 10 mg/ml using the same mobile phase. Standard solutions containing 5, 10, 20, 30, 40, 50, and 60 µg/ml were prepared by diluting the stock solution with the mobile phase and were analyzed using 50 µl of flurbiprofen (20 µg/ml) as internal standard (IS) (Jin *et al.* 2008). Standard calibration curve was established in plasma by adding 100 µl aliquots of each of the above dilutions to 100 µl blank plasma in 10 ml centrifuge tube. The spiked plasma samples were processed as described below. A standard calibration curve was constructed by plotting the peak area ratios of KP to IS against KP concentrations in plasma.

100 µl of 1 M Hcl aqueous solution and 50 µl of IS were added to 100 µl of the plasma (spiked plasma and unknown plasma) in 10 ml centrifuge tube equipped with a stopper and were shaken vigorously using vortex mixer for 1 min. 2.5 ml of diethyl ether was added, and the mixture was shaken vigorously for 1 min. After centrifugation of the mixture at 2000 rpm for 5 minuts. 2 ml of the organic layer was taken and evaporated at $40\pm2^oC$ to dryness in an oven. The residue was dissolved in 200 µl of the mobile phase and the produced solution was filtered through a Millipore filter (0.22 µm). A volume of 20 µl of the reconstituted sample was injected into the column of HPLC apparatus (Temperature column oven 480, fixed with reverse phase C 18 column (Chromonith® Performance RP-18E, 100x4.6 mm. Merck, Germany).

***Bioavailability study of ketoprofen in experimental animals***

Male rabbits (weighing 1.5-2 kg) were used for the bioavailability study. Animals were housed under standard laboratory conditions. Animals were housed in the standardized conditions at the animal house of the Faculty of Pharmacy, Zagazig University, Egypt. All animals were acclimatized and kept under constant temperature ($25 \pm 2^oC$). All animal procedures were performed in accordance to the approved protocol for use of experimental animals set by the standing committee on animal care of the Faculty of Pharmacy, Zagazig University, Egypt. Animals were divided into three groups of three rabbits in each group. Twelve hours before drug administration, food was withdrawn until 24 hrs post-dosing, and the rabbits had free access to water throughout the experiment. The study was designed as a single oral dose. All groups received an equivalent of 10 mg KP/ kg body weight of rabbits (Valliappan *et al*., 2006). Group 1 received KP alone, group 2 received ketoprofen marketed product (Ketofan® capsule), and group 3 received formulation I (the best microspheres formulation that exhibited the maximum EE% and the slowest release rate, formulation no 5). One hard gelatin capsule was administered to each rabbit through a stomach tube with the aid of distilled water. Blood samples (about 1ml) were withdrawn from the sinus orbital into heparinized tubes at 0, 0.5, 1, 2, 3, 4, 6, 8, and 24 hours after each administration. The blood samples were centrifuged immediately at 3000 rpm for 10 min to obtain the plasma samples and were stored at $-20^0C$ for subsequent assay.

**HPLC analysis for ketoprofen concentration in plasma samples**

A simple and rapid HPLC assay was developed for determination of ketoprofen in rabbit plasma. Waters Instrument, Germany system consisting of HPLC pump (pump A and pump B), mixing pump, multiport computer, HPLC self injector, and oven controller. Temperature column oven 480, fixed with reverse phase C 18 column (Chromonith® Performance RP-18E, 100x4.6 mm. Merck, Germany), HPLC detector with range of 0.2, resp. time 0.05, remote, monitor and data system computer. The plasma samples were thawed at room temperature. 100 microliters of plasma were mixed with 50 µl of flurbiprofen solution in the mobile phase (20µg/ml) as an internal standard. The procedure was then completed as mentioned before. The ketoprofen concentration in the rabbit plasma samples was calculated using the calibration curve.

**Pharmacokinetics Analysis**

The pharmacokinetics parameters were calculated from the plasma level data obtained for the individual rabbit per each group and were presented as means ± S.D. the maximum plasma concentration ($C_{max}$, µg/ml) and the time required to reach maximum plasma concentration ($T_{max}$, hr) after oral administration were calculated directly from the plasma concentration-time curve using Win-nonlin Nonlinear Estimation Program. The area under the plasma concentration-time curve from time 0 to 24 hrs ($AUC_{0-24}$, µg $ml^{-1}$ h) was calculated using linear trapezoidal rule (Gibaldi, 1991; Valliappan *et al.*, 2006). The area under the plasma concentration-time curve from time 0 to ∞ hrs ($AUC_{0-\infty}$) was determined by adding the last measured plasma concentration divided by the elimination rate constant ($K_{el}$) to $AUC_{0-24}$ according to the following equation:

$$AUC_{0-\infty} = AUC_{0-24} + C_{24}/ K_{el}$$

Where, $C_{24}$ is the concentration of KP in plasma after 24 hrs. The elimination rate constant ($K_{el}$) was obtained from the slope of the terminal phase of the profiles by plotting log C against time, where the slope was equal to $-K_{el}/2.303$. The elimination half life ($t_{1/2}$) was calculated from the value of $0.693/K_{el}$

The relative bioavailability was calculated from the comparison of the $AUC_{0-24}$ of each formula with that of the control.

$F_{rel}$= ($AUC_{0-24}$ for test / $AUC_{0-24}$ for control)X100

**Statistical analysis**

All results were represented as mean ± S.D. One way analysis of variance (ANOVA) was employed to assess the significance of the difference between the tested microsphere formulations and the control at a level ($p< 0.05$) using SPSS program (Meyyanathan *et al*., 2008).

**RESULTS AND DISCUSSION**

**Particle size analysis of KP loaded microspheres**

The particle size of prepared microspheres was in the range of 293.06 ± 11.49 to 438.63 ± 4.29 as shown in table (2). The data obtained showed that, the mean diameter was increased significantly (P=0.001) as the drug: polymer ratio was varied from 1:1 to 1:5. Low concentration of ERS100 resulted in a low viscosity of the polymer solution which in turn resulted in smaller emulsion droplets in the aqueous phase (Chaisri *et al*., 2009; Parashar *et al*., 2010). The results also indicated that as the stirring rate increased from 800 rpm-1600 rpm, the mean particle size was decreased significantly ($P<0.05$) This may be attributed to the formation of an emulsion of small droplets by increasing the mechanical stress at high stirring rate (Ghazy *et al*., 2003).

**Table. 2:** Particle size distribution of ERS100 microspheres.

| Formulation No. | Mean diameter (µm)± S.D. |
|---|---|
| 1 | 329.56±6.65 |
| 2 | 356.35±8.56 |
| 3 | 376.35±9.65 |
| 4 | 399.65±10.25 |
| 5 | 416.95 ± 7.96 |
| 6 | 293.06 ± 11.49 |
| 7 | 321.24 ± 14.45 |
| 8 | 352.65 ± 13.93 |
| 9 | 383.97 ± 4.88 |
| 10 | 397.78 ± 6.47 |
| 15 | 334.43 ± 14.46 |
| 16 | 438.63 ± 4.29 |
| 17 | 370.47 ± 10.71 |
| 18 | 336.26 ± 6.94 |
| 19 | 435.02 ± 10.85 |
| 20 | 371.91± 8.57 |
| 21 | 389.38 ± 10.99 |
| 22 | 432.74 ± 8.36 |

Each result is the mean of 3 determinations ± S.D.

Increasing the concentration of surfactant from 0.1 % to 0.7 % resulted in a significant decrease (P<0.05) in the mean diameter of microspheres. This can attributed to that the lower concentration of emulsifier may not be sufficient to cover the droplets of emulsion resulting in coalescence, leading to an increase in microspheres aggregation and fusion of the formed droplets (Maia *et al.*, 2004). Increasing the internal phase volume from 5 ml to 15 ml resulted in a significant decrease in microspheres mean diameter size (P=0.001) as shown in table (2). This may also be due to the increase in the volume of organic solvent leads to decrease in viscosity of the internal phase which was an effective factor in the droplet size of the emulsion in the aqueous medium (Lakshmana *et al*., 2009). Increasing the volume of the external aqueous phase from 50 ml to 150 ml caused a significant increase (P= 0.003) in size of the microspheres. A probable explanation is that with increasing the external volume, the shear forces to break the emulsion droplets become smaller yielding larger emulsified droplets which in turn results in larger ERS microspheres (Chaisri *et al.*, 2009).

**Analysis of entrapment efficiency and percentage yield of microspheres**

The % yield of different microspheres varied from (72.93%±1.51 to 94.03±2.06) when the drug: polymer ratio was changed from 1:1 to 1:5, respectively at 1200rpm. The reduction in the percentage yield with increasing drug: polymer ratio may be due to the loss of smallest and lightest particles during filtration and washing processes**.** Trivedi *et al*. (2008) reported that, as the polymer ratio in the formulation increases, the product yield also increases**.** The results also indicated that the EE% was increased significantly (P<0.05) by varing the drug: polymer ratio from 1:1 to 1:5 (table (3)). It was noticed that drug loading was increased considerably when polymer concentration was increased relative to drug concentration in the internal phase, which can be explained by the increased viscosity of the organic phase and the dense internal structure, therefore less drug was lost during evaporation (Mao *et al*., 2008; Gupta *et al.*, 2009).

Increasing the concentration of SLS from 0.1 to 0.7 results in a significant decrease (P=0.001) in the EE% of the prepared microspheres, table (3). This could be attributed to the fact that, at low concentration of surfactant, the surface of the microspheres is smooth and intact. Increasing surfactant concentration results in microspheres with brittle surfaces, which may lead to a drug loss on washing microspheres (Lin *et al.*, 1991). The increase in the amount of surfactant resulted in a decrease in the percentage production yield as well.

A non-significant decrease in the encapsulation efficiency (P=0.64) was noticed by increasing the internal phase volume, table (3). The drug encapsulation efficiency decreased non-significantly (P=1.00) when the external aqueous phase volume increased from 50ml to 150ml as shown in table (3). This was expected since a greater volume of the aqueous external phase allows large amount of drug to be dissolved in this phase (Chaisri *et al.*, 2009).

**Table. 3:** The EE % and percentage yield of KP-loaded ERS microspheres.

| Formulation No | Yield (%) ± S.D. | Entrapment efficiency (%) ± S.D. |
|---|---|---|
| **1** | 80.67±1.51 | 69.42±1.13 |
| **2** | 85.07±1.01 | 74.95±1.48 |
| **3** | 87.03±0.93 | 80.88±1.03 |
| **4** | 91.47±1.67 | 83.19±1.43 |
| **5** | 92.22±1.39 | 87.63±1.68 |
| **6** | 76.67±1.89 | 64.21±2.06 |
| **7** | 81.87±0.71 | 69.29±2.24 |
| **8** | 83.40±1.25 | 74.00±1.65 |
| **9** | 85.33±0.46 | 78.01±2.54 |
| **10** | 86.44±1.68 | 80.06±0.78 |
| **11** | 72.93±1.51 | 60.19±1.56 |
| **12** | 76.80±2.01 | 64.08±2.86 |
| **13** | 79.53±0.50 | 66.39±2.79 |
| **14** | 81.15±1.90 | 70.87±2.76 |
| **15** | 82.67±1.76 | 71.99±1.34 |
| **16** | 93.57±2.43 | 93.47±1.32 |
| **17** | 87.63±1.25 | 84.52±1.71 |
| **18** | 82.32±1.36 | 76.83±2.44 |
| **19** | 94.03±2.06 | 90.99±1.12 |
| **20** | 81.09±1.72 | 86.52±2.44 |
| **21** | 91.87±1.99 | 91.39±1.97 |
| 22 | 89.55±2.06 | 87.88±1.41 |

Each result is the mean of 3 determinations ± S.D.

The results also showed that at high stirring rate the percentage production yield was decreased. This may be due to the adherence of the polymer to the paddle by the turbulence created within the external phase leading to a reduction in the percentage production yield (Mazumder *et al*., 2009).

Increasing the stirring rate for preparation of KP loaded ERS100 microspheres resulted in a significant decrease (P>0.05) in the EE%. A probabl explanation is that, the surface area of large particles is smaller which may lead to less transport of the drug into the external aqueous phase (Chaisri *et al.*, 2009). This was confirmed by photomicrographs, figure (1).

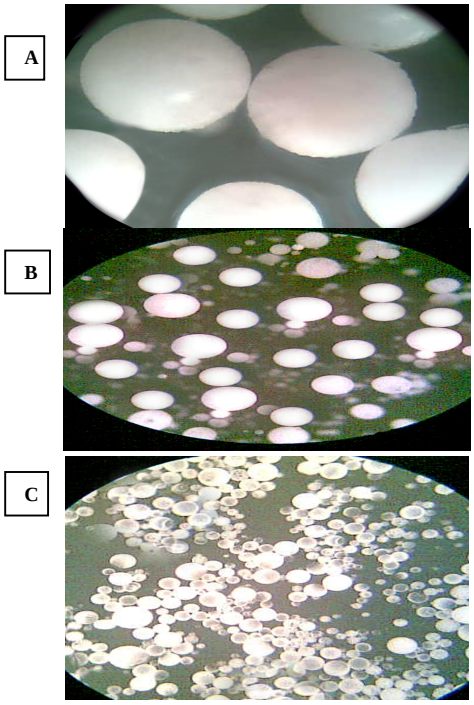


**Fig. 1:** Photomicrographs of KP loaded ERS microspheres at different stirring rates (x=40). A (800 rpm), B (1200 rpm), C (1600 rpm).

***In-vitro* release studies of KP loaded Eudragit microspheres**

The in-vitro release of KP from ERS microspheres exhibited biphasic release, initially a fast release followed by a slower release which spread over the extended period of time.

The release profiles of KP from ERS microspheres prepared from different drug: polymer ratios are shown in figure (2). The obtained data showed that the release of the pure drug was faster in comparison to that released from ERS100 microspheres. The results indicated that KP release from ERS100 microspheres was greatly extended and delayed as the drug: polymer ratio was decreased. These results might be attributed to the increase in the wall thickness of the microspheres arising due to the increase in the polymer: drug ratio leading to an increase in the the length of diffusion pathway through the polymer membrane (Prasanth *et al.*, 2011).

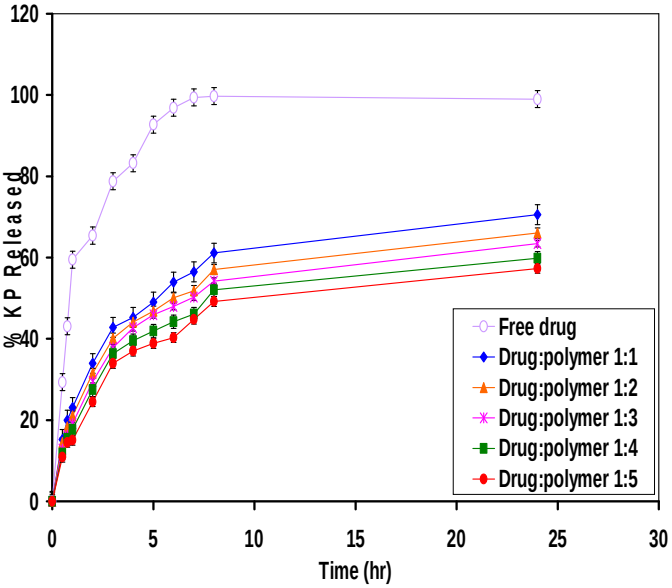


**Fig. 2:** Effect of drug: polymer ratio on the release rate of KP from ERS100 microspheres (prepared at 800 rpm).

The release profiles of KP from ERS microspheres prepared at different stirring rates into PBS pH(7.4) are shown in figure (3). The results indicate that, increasing the stirring rate resulted in a significant increase ($P<0.05$) in the rate of drug release. These results can be attributed to that smaller particle size microspheres are produced at higher stirring rates. Smaller particles possess a large surface area leading to higher release rate (Haznedar and Dortunc, 2004).

The in-vitro release studies reveal that the rate and amount of drug release is increased as the concentration of the surfactant is increased at constant drug: polymer ratio as shown in figure (4). This is due to the increase in wettability and better solvent penetration as the concentration of surfactant is increased. Increasing the surfactant concentration may also lead to an increase in amount of drugs deposited at the surface (Pachuau and Mazumder, 2009). The results obtained were fitted into various models (zero order, first order, and Higuchi diffusion model) to know which model is best fitting the release profile. All the tested formulations provided good fit to the Higuchi model. According to this model, the drug release from these formulations may be controlled by diffusion through the micropores.

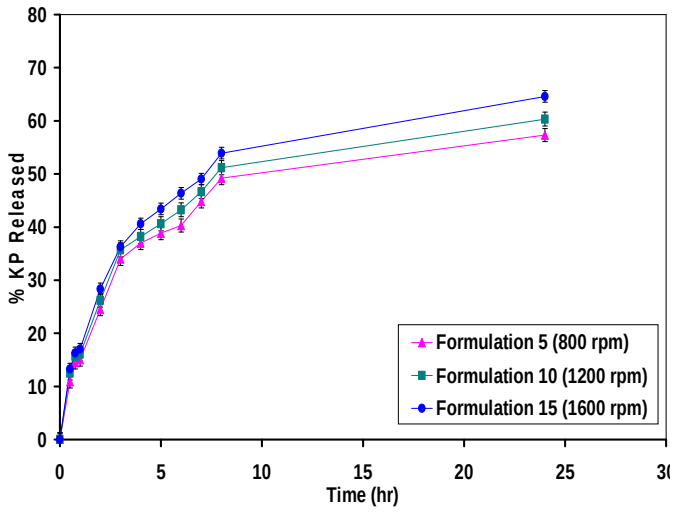


**Fig. 3:** Effect of stirring rate on the release rate of KP from ERS100 microspheres.

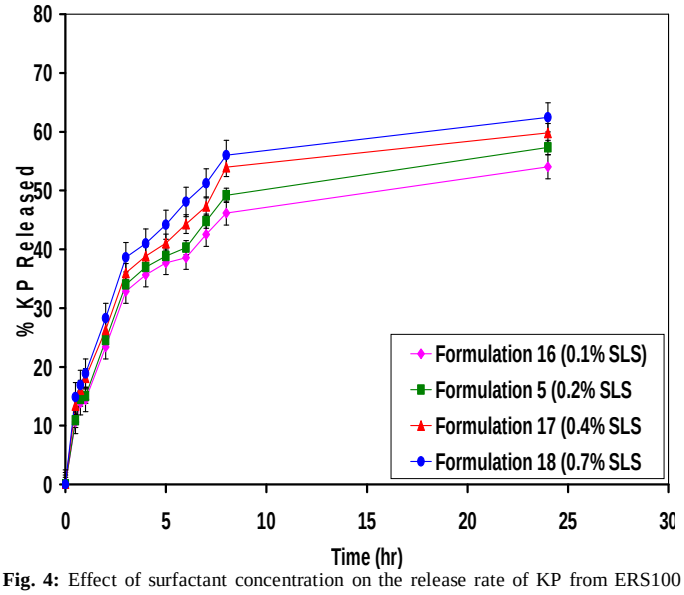


**Fig. 4:** Effect of surfactant concentration on the release rate of KP from ERS100 microspheres.

***In-vivo* study**

**Bioavailability of ketoprofen after oral administration**

The mean plasma concentrations as a function of time for ketoprofen after oral administration of plain KP and different ketoprofen formulations are illustrated in figure (5). From the obtained results, there was a noticeable difference in the $T_{max}$ between the drug and the tested formulations. The mean pharmacokinetic parameters of ketoprofen from different formulations represented by the value of $C_{max}$ (µg/ml), $T_{max}$ (h), $K_{el}$ ($hr^{-1}$), $t_{1/2}$ (hr), $AUC_{0-24}$ (µg. hr. $ml^{-1}$) and $AUC_{0-\infty}$ (µg. hr. $ml^{-1}$) are summarized in table (4). From the obtained data, it was observed that, the absorption of plain ketoprofen was rapid and reached its peak plasma concentration in (2.03±0.08 hr), whereas, the mean

$T_{max}$ for the marketed formulation and the tested formulation were 3.32±0.24 and 6.79±0.39 hr, respectively. The mean peak plasma concentrations ($C_{max}$) were 16.58±1.02 µg/ml for marketed formulation, 14.42±0.50 µg/ml for formulation I compared to 18.78±0.95 µg/ml for plain KP. The increase in the mean $T_{max}$ and the decrease in the mean $C_{max}$ of the test formulation compared to the plain drug indicated the controlled release effect of the microsphere formulations.

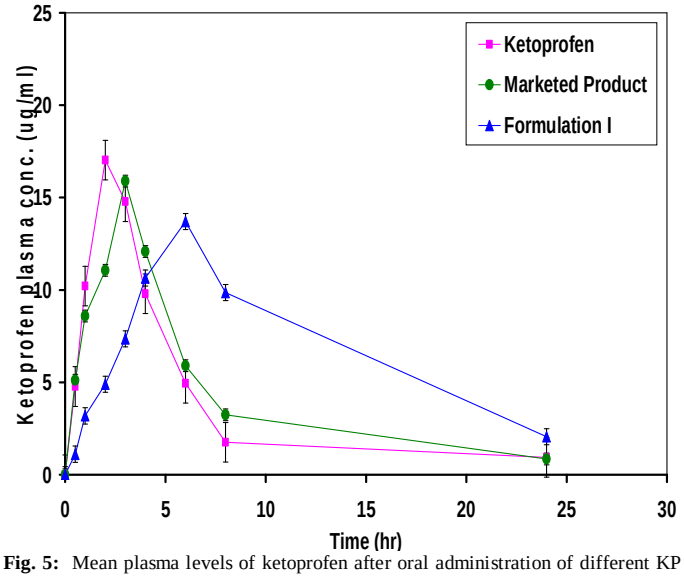


**Fig. 5:** Mean plasma levels of ketoprofen after oral administration of different KP formulations (equivalent to 10 mg/ kg) in rabbits.

**Table. 4:** Pharmacokinetics parameters after oral administration of KP in various formulations.

| Parameters | Ketoprofen | Formulation Marketed formulation | Formulation I |
|---|---|---|---|
| $C_{max}$ (µg/ml) | 18.78±0.95 | 16.58±1.02 | 14.42*±0.50 |
| $T_{max}$ (hr) | 2.03±0.08 | 3.32*±0.24 | 6.79*±0.39 |
| $K_{el}$ ($hr^{-1}$) | 0.488±0.015 | 0.406*±0.055 | 0.191*±0.032 |
| $t_{1/2}$ (hr) | 1.42±0.04 | 1.71*±0.02 | 3.69*±0.69 |
| $AUC_{0-24}$ ($\mu g.ml^{-1}.hr$) | 79.84±3.38 | 86.29*±2.83 | 105.21*±10.86 |
| $AUC_{0-\infty}$ ($\mu g.ml^{-1}.hr$) | 89.98±5.91 | 101.82*±7.37 | 164.45*±12.11 |
| Relative bioavailability | ------- | 108.07 | 131.78 |

(*) the mean difference is significant at the 0.05 level
Each result is the mean of 3 determinations ± S.D.

The mean $AUC_{0-24}$ was found to be 105.21±10.86 $\mu g.hr.ml^{-1}$ for formulation I compared to 79.84±3.38 $\mu g.hr.ml^{-1}$ for plain KP. These results confirmed the prolonged release of the two tested formulations.

From the obtained data, it was found that, the relative bioavailability of ketoprofen was increased by more than 1.3 times by formulating it into microspheres compared to control (plain drug). One of the main reasons for such a big difference between sustained-release ketoprofen microspheres and ketoprofen is that the absolute bioavailability of ketoprofen was extremely low because of the poor solubility of the drug (Cui *et al.*, 2003). Also, the increase in the relative bioavailability may be due to the slow release of the drug from the microspheres which led to a longer absorption and distribution period for the drug loaded microspheres than that of the free form (Shavi *et al.*, 2011).

## CONCLUSION

From the obtained data, it could be concluded that ketoprofen loaded microspheres exhibited prolonged and controlled release effect compared to control. This controlled effect is confirmed by low $C_{max}$ and high $T_{max}$ values. Ketoprofen loaded microspheres showed higher bioavailability in comparison to the plain drug and marketed formulation.

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