

## Preparation, characterization, and in vitro release of ketoprofen loaded polymeric microspheres

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**Abstract :** The purpose of this study was to prepare and to evaluate ketoprofen microspheres fabricated from biodegradable polymers (polylactic acid and polylactic-co- glycolic acid) by oil in water solvent evaporation method in different ratios of drug to polymer. The prepared microspheres were evaluated for percentage yield, entrapment efficiency, drug loading, drug polymer compatibility and in-vitro release of the drug.

The percentage yields of different formulations were in the range of 81.61% - 96.18% while percentage of drug entrapment efficiency was in the range of 71.62%- 86.40% and found to be higher in case of PLGA based microspheres as compared with PLA based microspheres.

FT-IR spectra of the microspheres showed no interaction between the drug and the polymers.

The release profile of ketoprofen from the different formulations was pH dependent. Lower release was observed in acidic medium while in phosphate buffer, sustained drug release was observed over 24 hours. As the drug to polymer ratio decreased, the percentage of entrapment efficiency and the yield of microspheres increased while ketoprofen release from polymeric microspheres was sustained.

The study concluded that the prepared polymeric microspheres of ketoprofen may prove to be potential candidate for safe and effective sustained drug delivery.

**Keywords :** Microspheres, ketoprofen, Solvent evaporation method, Polylactic acid, Polylactic-co-glycolic acid.

### Introduction

Conventional drug delivery systems are characterized by immediate release and repeated dosing of the therapeutic agent<sup>1</sup>. However, they suffer from many limitations, including undesirable side effects due to fluctuating drug level in plasma, poor drug efficacy, and poor patient compliance<sup>2</sup> as it seems remarkable with those drugs of short biological half life.

Due to the previous drawbacks of conventional drug delivery systems, there is a need to incorporate a therapeutic agents into a new drug delivery system to enhance the therapeutic efficacy of drug, minimize its toxicity as well as improve the patient compliance<sup>3</sup>.

One of these methods is the use of microspheres as therapeutic agents carriers to manufacture an safe and effective drug delivery system which improves that patient compliance by prolonging the drug effect, reduction of dosing frequency and limiting adverse side effect by lowering drug levels fluctuation in the plasma<sup>4,5,6</sup>.

Microspheres may be defined as solid particles, almost spherical in shape varying from 1 to 1000  $\mu\text{m}$ , including dispersed drug particles in either solution or microcrystalline form that prepared to provide extended drug release to enhance bioavailability, stability, patient compliance and to target the therapeutic agent to the desired location at a predetermined rate.<sup>7,8</sup>

Microspheres loaded with biodegradable polymeric materials such as polylactic acid (PLA) and poly lactic-co-glycolic acid (PLGA) have been widely used in development of drug delivery systems due to their biodegradation properties, drug encapsulation ability and biocompatibility are well understood and a number of delivery systems based on these polymeric materials have been FDA approved<sup>9</sup>.

Ketoprofen belongs to propionic acid family of non steroidal anti-inflammatory drugs (NSAIDs) with anti-inflammatory, analgesic, antipyretic effects, and is widely described for the treatment of osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis.<sup>10</sup>

Due to its short biological half life (2-2.5 hours) and ability to cause severe gastric damages like irritation or bleeding upon exposure to stomach at high concentration, ketoprofen is regarded as good candidate for the formulation of sustained release dosage regimens.<sup>11,12</sup>

The aim of present study was to develop and evaluate ketoprofen microspheres by o/w solvent evaporation method employing PLA and PLGA 50:50 polymers as carrier for oral administration to achieve oral sustained release of the drug and to protect the gastric mucous membrane from drug irritation. The various parameters and the in vitro release rates from these microspheres were thus examined.

## Materials and Methods

### 2.1. Drugs and Chemicals :

PLA and PLGA (with ratio of lactide/glycolide 50/50) were purchased from PCAS (France). Ketoprofen was purchased from Modern Pharmaceutical Company, Sana'a (Yemen). All other chemicals used in this study were of analytical grade.

### 2.2. Preparation of ketoprofen loaded microspheres:

The polymeric microspheres of ketoprofen (Table.1 )in this study were prepared by oil/water emulsion solvent evaporation method which was modified from the method described by Tiwari & Verma (2011)<sup>13</sup>.

Polymer was dissolved in 20 milliliters of dichloromethane (DCM) as organic solvent to yield a 2 % (w/v) polymeric solution. After obtaining a clear solution, the appropriate amount of ketoprofen was added to the polymeric solution. The stirring of mixture was continued until formation of uniform drug polymer solution. The above organic phase containing ketoprofen, polymer and DCM was slowly added drop wise into 100 ml of a continuous aqueous phase containing 1% (v/v) tween 80 and was emulsified by vigorous stirring (2000 rpm) at room temperature by using a three-blade digital propeller mixer.

After the emulsification, the stirring was further continued for three hours in order to facilitate the evaporation of the volatile organic solvent (DCM). The formed microspheres were washed with distilled water for several times and collected by vacuum filtration.<sup>14</sup> The filtered microspheres were dried and stored in tightly closed containers for further investigations.

**Table.1: Formulations of ketoprofen loaded microsphere.**

Formulation Code	Polymer	Drug: Ratio	Polymer
K1	PLA	1:1	
K2	PLA	2:1	
K3	PLGA	1:1	
K4	PLGA	2:1	

## **2.3.Characterization of ketoprofen microspheres:**

### **2.3.1.Percentage yield :**

The recovered dried microspheres at the end of preparation were weighed and the microspheres yield percent was determined by using following formula<sup>15</sup>:

$$\% \text{ Yield} = \frac{\text{Actual weight of the recovered microspheres}}{\text{Theoretical weight of drug and polymer}} \times 100$$

### **2.3.2.Drug entrapment efficiency and drug loading estimation:**

The drug entrapment efficiency is defined as the ratio of the actually encapsulated amount of the drug to that theoretically used during the microsphere preparation<sup>10</sup>.

The amount of encapsulated ketoprofen in microspheres made of PLA or PLGA 50:50 polymers was assayed by high performance liquid chromatography instrument

(Waters, USA) consisted of stainless steel column 150×4.6 mm packed with end-capped octadecyl gel for chromatography (MZ-Analysentechnik, Spain), a binary pump, and a UV-Visible detector (Waters, USA). The mobile phase consisted of mixture of 55 volumes of freshly prepared phosphate buffer, 43 volumes of acetonitrile and 2 volumes of water with a flow rate of 1 ml per minute according to the method adapted from Yamada et al (2001) , Kasnia et al (2012).<sup>12,16</sup>

Suitable quantity of ketoprofen loaded microspheres was dissolved in 25 ml of mobile phase, mixed well gently and sonicated in ultrasonic bath . Twenty micro liters of filtered sample was injected into the HPLC system employing wavelength of 260nm<sup>15</sup> .

The drug entrapment efficiency (DEE) and drug loading were calculated according to the following formulas :<sup>17,18</sup>

$$\text{DEE \%} = \frac{\text{Actual drug content}}{\text{Theoretical weight of drug and polymer}} \times 100$$

$$\text{Loading \%} = \frac{\text{Actual weight of drug}}{\text{Weight of loaded microspheres (Drug + Polymer)}} \times 100$$

### **2.3.3. Drug polymer compatibility study :**

Study of drug polymer compatibility in the microspheres formulations was carried out by measuring Fourier Transform Infra Red (FTIR) spectra of pure ketoprofen, PLA, PLGA (50:50) and ketoprofen loaded microspheres. FTIR spectra were recorded by crushing and grounding of the examined particles with KBr at room temperature using FTIR model (perkin Elmer spectrum 65,USA). IR spectra were scanned between the ranges of 500 to 4000 cm<sup>-1</sup>.

### **2.3.4. In vitro release studies:**

Keoprofen loaded microspheres equivalent to 10 mg of ketoprofen were weighed and suspended in glass bottles containing 100 ml phosphate buffered saline (PBS) of pH 7.4 or simulated gastric fluid (SGF) of pH 1.2 as other release medium. Then, the bottles were kept in water bath at 37±0.5C°. Five milliliters of the drug releasing media was withdrawn at various time interval of 30 min, 1, 2, 4, 6, 8 and 24 hours, and replaced by

an equal volume of freshly prepared release medium.<sup>19</sup> The drug was estimate in each formulation by UV – Visible Spectrophotometer (Analyticjena, Germany) at 260 nm<sup>6</sup>.

## Results and Discussion

### 3.1. Characterization of ketoprofen microspheres:

#### 3.1.1. Percentage yields:

The percentage yields of different formulations were in the range of 81.61%-96.18 % as listed in table 2. The maximum yield was obtained informulation K1 (96.18 %) while the minimum value was appeared in formulation K4(81.61 %) .

**Table.2: Percentage yields of ketoprofen loaded microspheres**

Yield (%)	Drug: Polymer Ratio	Polymer	Formulation Code
96.18	1:1	PLA	K1
85.38	2:1	PLA	K2
94.27	1:1	PLGA	K3
81.61	2:1	PLGA	K4

The results showed increasing in the percentage yield of the microspheres associated with increasing of the polymer concentration which indicates a reduction in the product losing during preparation of the ketoprofen loaded microspheres. These findings correspond with results obtained by Bansode et al (2010) <sup>20</sup>.

This reduction in the percentage yield with increasing of ketoprofen: polymer ratio may be due to the loss of smallest particles during filtration and washing procedures.

The percentage yield was found to be slightly higher in case of PLA based microspheres formulations (K1 and K2 ) as compared to that of PLGA based microspheres (K3 and K4 ) with the similar ratio of drug to polymer.

#### 3.1.2. Drug entrapment efficiency and drug loading estimation :

Percentage of drug entrapment efficiency for different formulations of ketoprofen microspheres was found to vary from 71.62 % to 86.40 % as reported in table 3. The highest EE % was seen in the formulation K3 in which ketoprofen was loaded with PLGA (50:50 ) in ratio of 1:1.

**Table.3: Drug entrapment efficiency and drug loading of microspheres**

Drug loading (%)	Entrapment efficiency(%)	Drug : Polymer Ratio	Polymer	Formulation Code
39.25	78.54	1:1	PLA	K1
47.77	71.62	2:1	PLA	K2
43.20	86.40	1:1	PLGA	K3
47.60	72.42	2:1	PLGA	K4

In general, all of the prepared formulations exhibited good percentage of entrapment efficiency that may be due to poor solubility of ketoprofen in the continuous phase, high oil/water partition coefficient of ketoprofen which enhance its retention in the organic phase and festered the precipitation of solid microspheres upon evaporation of dichloromethane.<sup>21,22,23</sup>

Other factor employed significant mpact on EE % is related to the properties of the selected solvent for ketoprofen and PLA, PLGA polymers during preparation of microspheres by o/w solvent evaporation method;

DCM which is used as organic solvent during the preparation of ketoprofen loaded microspheres in this study has high water miscibility and required low heat to remove it from the preparation by evaporation at room temperature, thus improving the entrapment efficiency of the drug.<sup>13</sup>

From the results of this study, we observed that as the polymer concentration was increased, the entrapment efficiency increased as that noticed in K1 and K3 formulations with EE % of 78.54 and 86.40 respectively. These obtained results comply well with results showed in other studies.<sup>24,15</sup>

The results indicated that polymer concentration plays a major role in drug entrapment efficiency. When there was a lower ratio of polymer to drug content 400 mg :800 mg as shown in K2 and K4 formulations, the entrapment efficiency decreased as compared to the formulations contained higher ratio of polymer to drug content 400 mg: 400 mg as that seen in K1 and K3 formulations.

The higher encapsulation efficiency associated with increasing of polymer concentration may be due fast precipitation of the polymer due to its high concentration which prevents diffusion of ketoprofen across the phase boundary. Additionally, as the polymer concentration increased, viscosity of the solution increased which delayed the diffusion of within the polymer droplets.<sup>25,26,22</sup>

The results show drug entrapment efficiency was found to be higher in case of PLGA based microspheres formulations (K3 and K4) as compared with that of PLA based microspheres (K1 and K2), because the solubility of polymers in the organic solvent determines the precipitation rate during the microencapsulation process as previous described ;PLA showed a high solubility in DCM so it had affinity to remain in the organic phase. Its solidification rate was decrease, leading to low drug encapsulation values.<sup>27</sup>

Drug loading percent was varied from 39.25 to 47.75 as that seen in table .

Higher percentage of ketoprofen loading was obtained by increasing the amount of ketoprofen with respect to polymer used. This result agree well with findings of an earlier study.<sup>28</sup>

### **3.1.3. Drug polymer compatibility study:**

Fourier transform infrared spectrometry ( FTIR) was done to examine drug polymer compatibility. The individual spectra of the pure ketoprofen, PLA , PLGA 50:50 as well as the ketoprofen loaded microspheres formulations are shown in the figure.1.

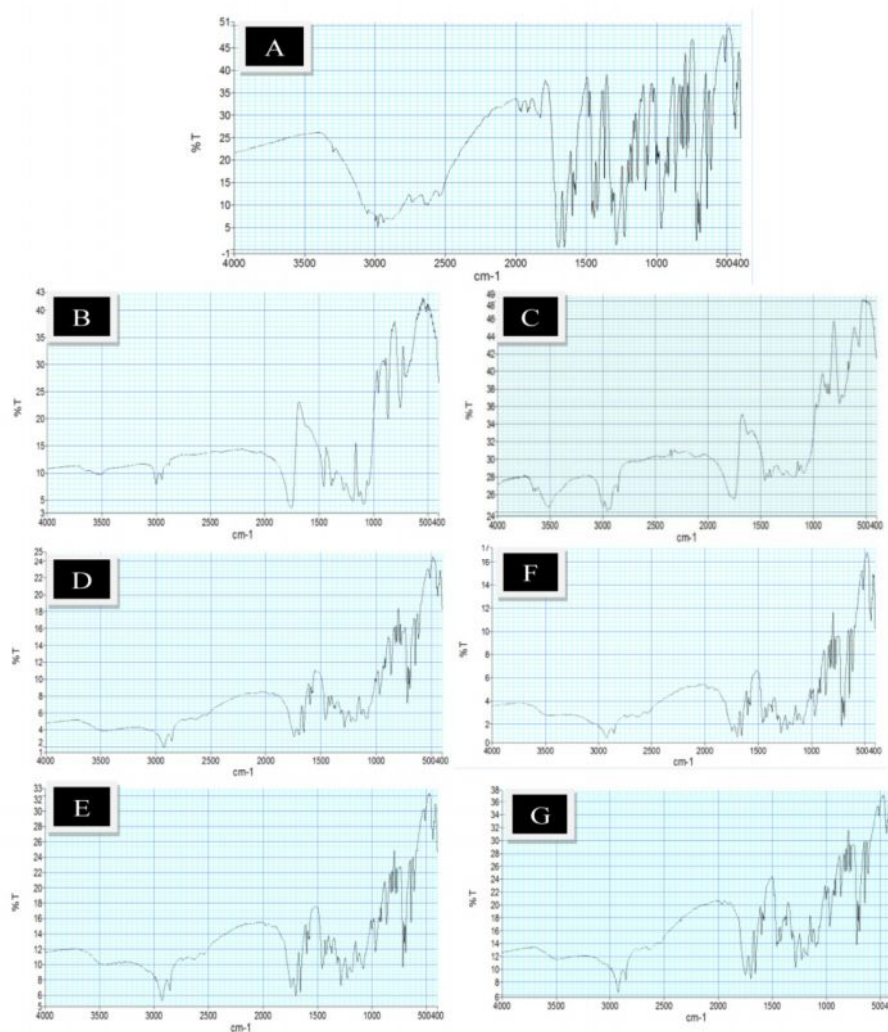
The characteristic absorption peaks of pure ketoprofen ( Figure 1.A) are observed at 3050, 2950 , 1650, 1700 and 3300  $\text{cm}^{-1}$ , indicating the presence of aromatic C-H stretch , aliphatic C-H stretch, C = O stretching of ketone , C = O stretching of acid and carboxylic acid O-H stretch, respectively.

FTIR spectrum of PLA( Figure 1.B) showed absorption bands at 2950, 1750 and 3510  $\text{cm}^{-1}$  due to stretching of aliphatic C-H , C=O vibrations of an acid group and carboxylic acid O-H stretch, respectively.

On other hand, FTIR spectrum of PLGA( Figure1.C) showed characteristic spectra in the range of 2920 - 2850  $\text{cm}^{-1}$ , indicate the presence of aliphatic C-H stretch while the absorption peaks in the range of 1800-1750  $\text{cm}^{-1}$  due to C=O vibrations of an acid group .

The absorption peaks of PLGA spectra at 3650 and 3510  $\text{cm}^{-1}$ , can be attributed to O-H alcohol and acid.

The observations indicated that there are no significant changes in the position of the principle absorption peaks of ketoprofen after incorporation into PLA (Figure1.D and E) or PLGA (Figure 1.F and G) polymers, which indicate that there is no interactions between the drug and the polymers and good compatibility of polymers with drug.



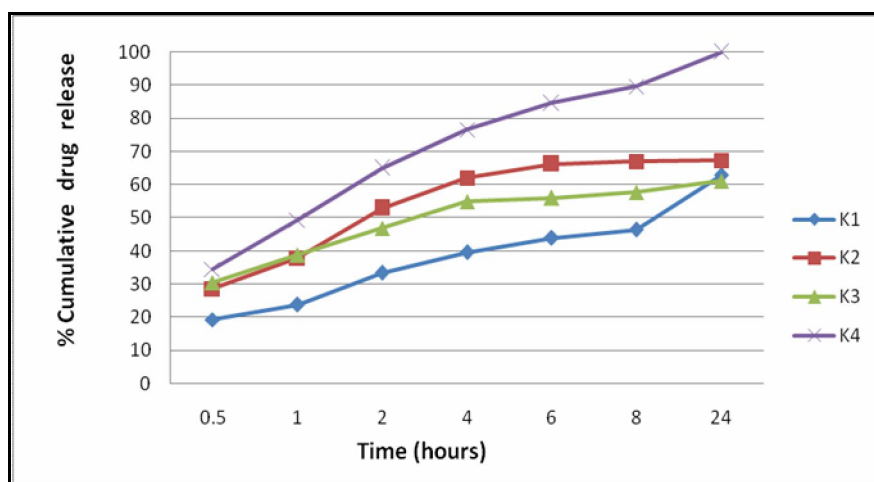
**Figure 1. FTIR Spectra of : A)Pure ketoprofen, B) PLA, C) PLGA 50:50, D)K1 , E)K2 , F)K3 and G) K4 formulations.**

### 3.1.4. In-vitro release study :

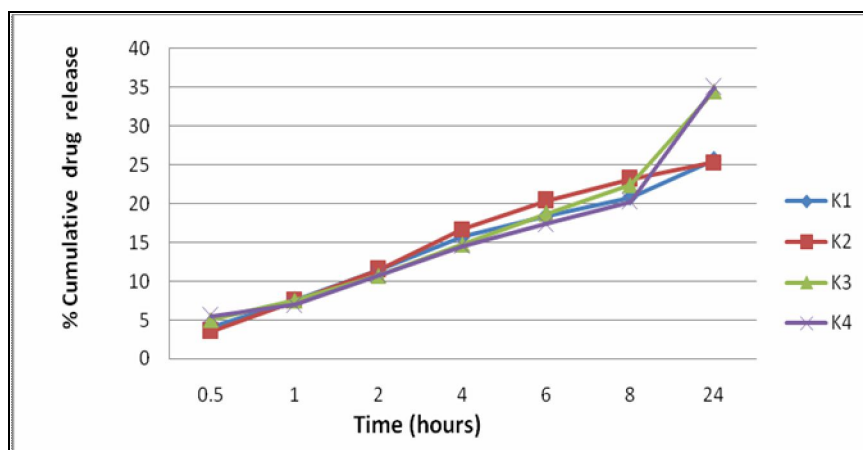
The in-vitro release of ketoprofen loaded microspheres was performed by measuring the cumulative percent release of different formulations in phosphate buffer saline (PBS) of pH=7.4 and simulated gastric fluid(SGF) of pH=1.2 for 24 hour as shown in figures 2 and 3 respectively .

Cumulative release of ketoprofen from the various formulations was found to be dependent on pH of the release medium. In SGF of pH 1.2, lower release rates were observed while the significant drug release was showed in PBS of pH 7.4.

In PBS of pH 7.4, the maximum and minimum percent of cumulative released were found to be 100 % and 61.15 %, respectively at the end of 24 hours. The release profile of the different formulations was characterized by initial burst release of ketoprofen ranged from 19.37 % to 34.48 % followed by a moderate to slow release according to the amount of drug available after the burst effect and resulted from degradation of the microspheres and diffusion of ketoprofen. Formulation K1 showed lowest burst release while formulation K4 showed highest burst release which can be reduced by increasing the polymer concentration.<sup>29,30</sup>



**Figure. 2: %Cumulative release of ketoprofen microspheres in PBS (pH =7.4 )**



**Figure. 3: %Cumulative release of ketoprofen microspheres in SGF(pH =1.2)**

The results showed a decrease in the cumulative release of ketoprofen in respect with increasing of the polymer concentration resulting in difference in the rate and extent of drug release during the first six hours in formulation K1 as compared to that in formulation K2 and in formulation of K3 as compared to K4 due to the change of drug to polymer ratio. These results indicated that ketoprofen release from polymeric microspheres was sustained as the drug : polymer ratio was decreased which may be attributed to the increase in the wall thickness of the polymeric microspheres arising, hence, leading to an elongation in the length of drug diffusion across the polymer membrane.<sup>5,31,32</sup>

Ketoprofen release was found to be slower in case of PLA based microspheres formulations (Kk1 and K2) as compared to that of PLGA based microspheres (K3 and K4) , this may attribute to the higher hydrophobicity of PLA polymer as a result of the additional methyl group on its chemical structure which reduced the penetration by the aqueous media and degradation of the PLA polymer and finally resulted in slower degradation time as compared to that of more hydrophilic PLGA polymer<sup>33</sup>.

## Conclusion

This study demonstrated that oil/water solvent evaporation method has been successfully employed to prepare ketoprofen loaded poly lactic acid and poly lactic-co-glycolic acid microspheres with desirable percentage yield, drug entrapment efficiency, drug loading, drug polymer compatibility and extended release profile. The formulation variables as drug to polymer ratio and the type of polymer used exerted a significant influence on the percentage yield, drug encapsulation as well as drug release pattern.



It can be concluded from the obtained data that the prepared polymeric microspheres of ketoprofen may present a promising approach for safe and effective sustained drug delivery which can be used to overcome the adverse effects which are caused by the localization of a high dose of the drug.

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