IN VITRO AND *IN VIVO* EVALUATION OF SUSTAINED RELEASE KETOPROFEN-LOADED NANOPARTICLES

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ABSTRACT: *The purpose of this study is to i) fabricate a biodegradable nanoparticle formulation of Ketoprofen, ii) evaluate its characteristics, iii) investigate its in vitro dissolution and in vivo pharmaceutical property. The nanoparticle formulation was prepared by spray drying method using Eudragit L100 as the matrix polymer. Size and morphology of drug-loaded nanoparticles were characterized with the electron microscopes (TEM, SEM). These successfully prepared nanoparticles by spray drying method are spherical in shape and quite homologous with diameter size of 100 – 200 nm. The in vitro dissolution studies were conducted at pH 1.2 and 7.4. The results indicated that there is a significant increase in Keto concentration at pH 7.4 compared to pH 1.2. For the in vivo assessment, our Keto-loaded nanoparticles and referential Profenid were administered by oral gavages to rabbits. The results implied that Keto-loadednanoparticles remarkably increased AUC compared to Profenid.*

Keywords: *Ketoprofen, Eudragit L100, Polymeric nanoparticles, Spray drying method.*

1. INTRODUCTION

Ketoprofen is analgesics drug, classified into non-steroidal anti-inflammatory group. It is commonly used to treat rheumatism and arthritis. However, the conventional capsule formulation of Ketoprofen has several disadvantages such as the short half-life, low bioavailability and the side effects [1,2].

To overcome these disadvantages, during the last few decades, several new approaches using polymer for preparing Ketoprofen nanoparticles have been studied. Besides thesustained release ability [4-7], polymeric nanoparticle formulation presents the drug in very fine nanodroplets offering very high surface area for absorption. This helps with quick absorption of the drug, thus improves oral bioavailability. Moreover, poor permeability is also one of the major factors that limit oral bioavailability of several drugs. Owing to low bioavailability, some drugs have to be administered at significantly higher doses, whereas the improvement in bioavailability can be translated into reduction in the drug dose and dose-related side effects of many hydrophobic drugs [8]. Most of the current methods described for preparation of polymeric nanoparticles of Ketoprofen used to manufacture drug nanoparticles result in an

aqueous suspension of nanoparticles [9]. Suspensions are, however, physically unstable and common problems of suspensions are drug leakage from the particles into water phase, drug degradation, microbiological problems and physical changes such as aggregate formation in the course of time [10-13]. To increase the physical and chemical stability of the nanoparticles, dry powders would be desirable [11, 14-16]. Spray drying technique to produce nanoparticle dry powders have been studied. Spray-drying involves the conversion of a solution droplet into a dry particle by evaporation of the solvent in a one-step process [17-19]. Compared with lyophilized powder obtained from an aqueous suspension, spray dried powders can be prepared without the problems of drug leakage to another phase, and thus, the recovery of drug in the particles is quantitative [17, 20].

In this work, our laboratory has used successfully spray drying method for preparation of Ketoprofen nanoparticles with Eudragit L100, and the resultant dry powder was studied some characteristics. We pointed out several important chemical and physical properties of nanoparticles with Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM). An evaluation of the ability to release the drug at the condition of pH = 1.2 and pH = 7.4 for *in vitro* studies.Finally, an*in vivo* assessment of pharmaceutical properties was also conducted.

2. MATERIALS AND METHODS

2.1. Materials

The pharmaceutical drug, Ketoprofen (3-benzoyl-α-methylbenzeneacetic acid) is a widely used nonsteroidal anti-inflammatory drug (NSAID) [1,2], purchased from Rohm (Rohm Pharma, Darmstadt, Germany) and used without further purification. It is freely soluble in many organic solvents but practically insoluble in water at 20° C.

The polymer chosen in this study is a pharmaceutically acceptable material and has been used for oral formulation - Eudragit L100 (EUD). This agent is a copolymer consisting of methyl methacrylate and methyl methacrylic acid repeating units in a ratio of 1:1, only soluble at pH more than 6 [3]. We obtained this material from Merck (Germany) and used it as received**.**

Aerosil – a pharmaceutical excipient used in this research was purchased from GmbH (Germany).

All other chemicals usedwere procured from Merck (Germany).

2.2. Preparation of Particles

Nanoparticles containing Ketoprofen and Eudragit L100 were prepared by spray drying method**.** Briefly,the drug-polymer mixture was prepared by separately dissolving the Eudragit L100 and drug into acetone, using a magnetic stirrer and then mixing the solutions. The ratio of drug and polymer in prepared drug-polymer mixture was 1:3. The continuous process to

obtain drug loaded nanoparticles was realized with spray-drying system (Yamoto ADL 31, Japan). The parameters as dried-temperature, spraying speed and peristaltic pump speed were set up respectively at 130-140^{0}C, 22000 rpm and 12 rpm. The dry powder samples obtained were added aerosil, a pharmaceutical excipient for adding stability and anti-caking and then stored at room temperature.

2.3. Characterization of Particles

The particle size and morphological examination of the nanoparticles were performed with TEM (JEM 1400, Japan) and SEM (JSM 6480LV-JEOL, Japan) measurements. The samples were placed on carbon-coated copper grids for viewing by TEM. For SEM, the samples from dry powder particles were prepared by gently dipping copper grids into the dry nanoparticles.

2.4.Quantifying Ketoprofen by High-performance liquid chromatography

Ketoprofen concentration in unlnown samples were also determined by high-performance liquid chromatography (HPLC) using a Nucleosil ODS column (250 × 4,6 mm; 5 μm particle size) at ambient temperature. The mobile phase consisted of 40% Acetonitrile and 60% water containing 1% acid acetic and 0.3% triethylamine. The system was run isocratically at a flow rate of 1.2mL/min.

2.5. In vitro dissolution studies

The ultimate aim of this work was to develop sustained release drug delivery system of Ketoprofen. To evaluate this ability, the release tests were performed at both pH conditions in stomach and small intestine. The pH of stomach is acidic, ranging from 1.3 to 5 depending on the fed-fasted conditions. The small intestine has a significantly higher pH level ranging from 6.5 to 7.5 [21].

At each stage, an amount of 100 mg dry powder was weighed and filled into a gelatin capsule. Round-bottomed cylindrical glass vessels having a total volume of 900 mL were used as released chambers. The solutions were kept in a water bath at 37ºC ± 0.5^{0}C and stirred at a speed of 75 rpm. For the acid stage, 900 mL of HCl 0.1N was used as the release medium. Aliquot (10 mL) was withdrawn at appropriate times. Immediately after each sampling, the aliquot was filtered with a membrane filter (0.45 μm in pore diameter) and the same volume fresh fluid at 37^{0}C was supplemented to the test medium. The amount of ketoprofen released was determined as section 2.4. The measurements were performed three times; the values reported are mean values. The repeatability of the method was evaluated by analyzing three parallel samples.After two hours, we continued for the base stage. In this stage, a buffer solution at pH 7.4 was used as the release medium. The test was performed similarly for further four hours.

The percentage of Ketoprofen released was determined from the following equation:

$$\text{Release}(\%) = \frac{\text{Released Keto from Nanoparticles}}{\text{Total amount of Keto in Nanoparticles}}.100\%$$

2.6. In vivo assessment of oral administration

In vivo absorption study

Keto-EUDnanoformulations were administered by oral gavages to rabbits. All rabbits were made to fast 18 hours prior to the dose administration and remained fasting until 4 hours after dose administration. The 100 mg of Keto-EUD containing 20 % of Keto was filled in gelatin capsule and then administered to rabbits by oral gavages. Blood samples of 2.5 mL were collected into vacutainer tubes containing EDTA prior and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, 12.0, 24.0 and 36.0 hours after administration. After this collection, the blood samples were centrifuged at approximately 3500 rpm at 2–8 ºC for about 15 min. Each plasma specimen was collected and stored at -20 ºC until analysis. For the comparison, Profenid containing 32.4 % of Keto was also administered to rabbits by oral gavages following the similar procedures. The amount of Keto for oral administration was 50 mg for both Keto-EUD and Profenid.

Preparation of plasma samples for determination of Keto in rabbit plasma by HPLC

Keto in the plasma samples was determined by HPLC.Piroxicam was used as internal standard.A solution of internal standard was prepared with methanol at 50 $\mu g.mL^{-1}$.

Samples were prepared as follows: 50 µL of plasma were extracted with 1 mL of internal standard solution in polypropylene tubes containing 100 µL of H_3PO_4 and 3 mL of tertbutyl methyl ether. Samples were then sonicated for 5 min, sequent vortexed for 5 min and then centrifuged at 4,500 rpm for 5 min. Supernatants were transferred into glass test tubes. A blank (50 µL of blank plasma extracted with internal standard) and double blank (50 µL of blank plasma extracted with blank methanol) were also prepared. Samples were dried under nitrogen at 40 ºC, reconstituted with 500 µL of methanol, and transferred into a glass insert in an autosampler vial for HPLC assay.

3. RESULTS AND DICUSSION

3.1. Size and morphology of particles

The Ketoprofen was successfully incorporated into Eudragit L100 NPs by spray drying method**.** The obtained products were white dry powder samples, smooth and homogenous. Their TEM images were shown in Figure 1 which presents the successfully prepared nanoparticles. These exemplary TEM images showed solid, quite homogenous particles with size about 100 – 200 nm. Grain boundaries or crystals were not detected, therefore, it was concluded that these nanoparticles had a matrix-type structure. The particles produced are amorphous due to rapid

evaporation of the solvent from the droplets [21]. The amorphous state has higher internal energy, larger free volume and greater molecular mobility in comparison to the crystalline state [23]. These properties of the amorphous state lead to greater solubility. Amorphous solid have been used to achieve faster dissolution rates of drugs and to modify drug release [23, 24]. For morphological examinations, the SEM photographs of drug loaded nanoparticles are shown in Figure 2. It can be clearly observed from these photographs that the nanoparticles made of polymer Eudragit L100 were spherical and smooth surface.

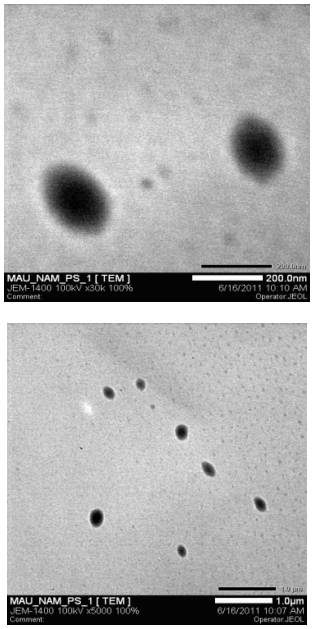

Figure 1. Exemplary TEM images of the particles fabricated.

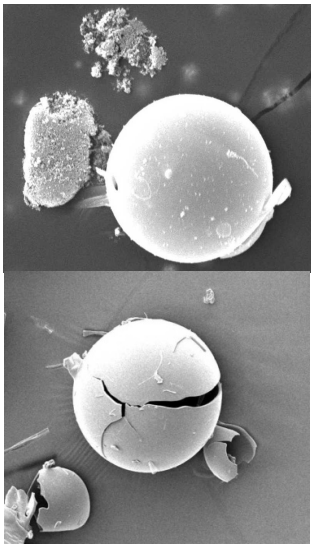

Figure 2. Exemplary SEM Images of particles fabricated

3.2. Drug release from particles

The results of *in vitro* release study in acidic and basic medium of our Keto-EUD nanoparticles (N20), referential Profenidwere shown in Table 2 and plotted in Figure 4.

The percentages of Keto released from Profenid were almost negligible (less than 5 %) after two hours. On the other hand, for the first 30 min, the percentages of Keto released from N20 were 12% and then gradually increased with the increasing time. However, the percentages of Keto released were non-linear function of time. It implies that the Keto released from Keto-EUD nanoparticles due to the diffusion of Keto from the outer shells of nanoparticles or/and due to the partial dissolution of EUD L100 in acidic medium. The diffusion of drugs from the outer shells of nanoparticles was confirmed in the previous publications [25, 26]. Keto in the outer shells

was poorly entrapped in the Eudragit L100 matrix leading to easy diffusion of Keto. However, because the Eudragit L100 is insoluble in acidic medium, the percentage of released Keto is correlative low. It can also explain why Keto released from nanoparticles but its released percentage was less than 28 % after 2 hours in acidic medium.

In basic medium, after 30 min, the N20 released 89.1% while the Profenid released 60.5% of Keto. It implies that the Keto-EUD nanoparticles released Keto faster than the Profenid when it was dissolved in basic medium. Similarly, after 1 hour, almost 100 % of Keto from both of N20 and Profenid were released**.** The percentages of Keto released from Keto-EUD nanoparticles in basic medium were significant higher than those in acidic medium with the correlative times. For example, N20 released 89.1 % of Keto at basic pH whereas only 12.09 % of Keto at acidic pH after 30 min. This can be explained based on the solubility of Eudragit L100 in basic medium. Moreover, the higher percentage of released Keto in basic medium can also be explained based on the solubility of Keto in basic environment. The carboxylic acid group in Keto is ionized in basic medium leading to the increasing solubility of Keto. As a result, Keto is released readily from nanoparticles leading to the increasing percentage of released Keto.

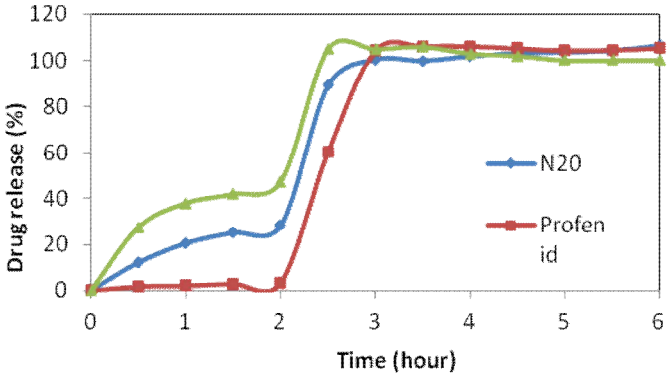

Figure 3. *In vitro* release of Keto-EUD nanoparticles, Profenid and pure Ketoin the acidic medium (in the first two hours) and in the basic medium (the continuous time)

Table 1. *In vitro* release of N20 and Profenid in the acidic medium (in the first two hours) and in the basic medium (the continuous time) [a]

Time (h)	N20 [b]	Profenid [c]	Pure Keto
0	0	0	0
0.5	12.09	1.71	27.5
1.0	20.43	2.06	38
1.5	25.17	2.86	42

2.0	28.19	3.44	47.4
2.5	89.1	60.5	105
3.0	100.43	104.26	105
3.5	99.88	106.15	106
4	101.86	106.12	103
4.5	103.13	105.27	102
5	103.57	104.27	100
5.5	104.37	104.52	100
6	106.62	105.34	100

[a]Release (%), determined by eq 1. [b]Contains 20 % of Keto. [c] Contains 32.4 % of Keto.

3.3. In vivo assessment of oral administration of Keto-EUD nanoparticles

The *in vivo* assessment of oral administration of Keto-EUD nanoparticles was tested in rabbits with the aim to investigate the absorption ability of Keto-EUD nanoparticles, the maximum Keto concentration in plasma (C_{max}), time of maximum concentration (T_{max}) and the enhancement ratio of Keto-EUD nanoparticles. Keto-EUD nanoparticles and Profenid were administered by oral gavages to rabbits with the amount of Keto of 50 mg for each rabbit. The oral administration procedures and the preparation of plasma were stated in section 2.6. The plasma concentration-time profiles of Keto after oral administration in rabbits were shown in Figure 4. A comparison of T_{max} between N20 and Profenid indicates that the concentration of Keto in plasma increased rapidly in both cases and peaks were observed after 2 hours. These results imply that Keto released in intestine from Keto-EUD nanoparticles and Profenid penetrated through intestine into blood stream of rabbits and the T_{max} of Keto-EUD nanoperticles and Profenid were almost the same.

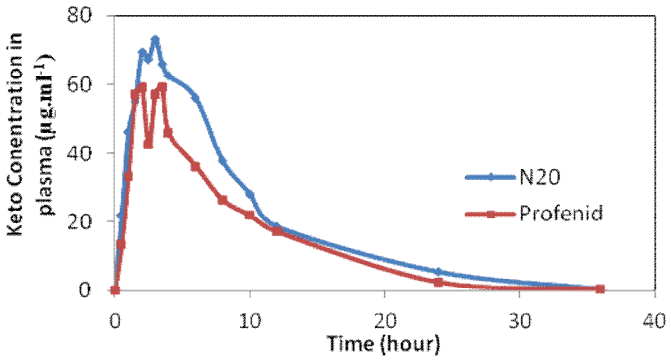

Figure 4. Plasma concentration-time profiles of Keto after oral administration in rabbits of N25 and Profenid.

Pharmacokinetic parameters following oral administration of the Keto-EUD nanoparticles and Profenid are presented in Table 2. Interestingly, the Keto-EUD nanoparticlesremarkably increased AUC and the maximum drug concentration (C_{max}) values of Keto, 1.3-fold (enhancement ratio) compared to the respective value of Profenid. The *in vivo* absorptions of Keto were significantly improved by Keto-EUD nanoparticles compared to those of Profenid.

Table 2. Pharmacokinetic parameters of Keto following oral administration of Keto-EUD nanoparticles and Profenid[a].

Drug formulation	C_{max} [b] ($\mu g \cdot mL^{-1}$)	T_{max} [c] (h)	AUC [d] ($\mu g\ h \cdot mL^{-1}$)	Enhancement ratio [e]
Profenid	59.3	2	546.89	1.0
N25	73.25	2	722.639	1.3

[a]50 mg of dose of Keto for each rabbit. [b]C_{max} denotes maximum drug concentration. [c]T_{max} denotes time of maximum concentration. [d]AUC area under the plasma concentration-time curve. [e]Determined by the equation: the ratio = the corresponding AUC/AUC of Profenid.

4. CONCLUSION

We used Eudragit L100 as a matrix polymer to prepare nanoparticle formulation of Ketoprofen. On the basis of the results of the investigations presented, it can be concluded that this formulation allows prolonged drug release. Due to its solubility, this polymer has the ability to prevent release drug from particles in the acidic medium, whereas, in pH condition of intestine, it is rapidly dissolved and shows a complete releasing the encapsulated material. Thus, this work confirms that Eudragit L100 is really a suitable matrix polymer for Ketoprofen. For the *in vivo* assessment, Keto-EUDnanoparticles and referential Profenid were administered by oral gavages to rabbits. The results implied that our Keto-EUD nanoparticles remarkably increased AUC compared to Profenid. These initial results demonstrate that nanoparticles containing Ketoprofen and Eudragit L100 can be further developed to enhance delivery.

ĐÁNH GIÁ SINH KHẢ DỤNG IN VITRO VÀ IN VIVO CỦA HẠT THUỐC NANO POLYME MANG KETOPROFEN

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TÓM TẮT: *Trong bài viết này, chúng tôi trình bày một số kết quả nghiên cứu trên hạt thuốc nano polyme mang Ketoprofen. Hạt thuốc nano Ketoprofen được chế tạo với phương pháp phun sấy từ vật liệu polyme Eudragit L100. Hạt thuốc nano thu được có hình cầu, kích thước khá đồng nhất trong khoảng 100-200 nm. Nghiên cứu độ hòa tan ở hai điều kiện pH 1.2 và 7.4 cho thấy hàm lượng Ketoprofen được phóng thích phù hợp với điều kiện viên tan trong ruột, tác dụng kéo dài. Ngoài ra các thử nghiệm sinh khả dụng trên thỏ cũng cho thấy sự phóng thích Ketoprofen vào máu có hiệu quả so với Ketoprofen nguyên liệu và thuốc Profenid.*

Từ khóa: *Ketoprofen, Eudragit, hạt thuốc nano polyme, phương pháp phun sấy.*

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