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# Preparation and characterization of ketoprofen loaded eudragit RS polymeric nanoparticles for controlled release

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**Abstract**
Nanospheres containing ketoprofen (Keto) and polymer eudragit RS were prepared using an emulsion solvent evaporation method. The ultrasonic probe (VCX500, vibracell) was used as a tool to disperse oil phase into aqueous phase leading to water/oil emulsion. Nanoparticles were successfully prepared and their morphologies and diameters were confirmed by transmission electron microscope (TEM) and dynamic light scattering (DLS), respectively. The result showed that particles were spherical with submicron size. The particle size was dependent on the RS concentration, emulsification tools and the types of organic solvents. For the encapsulation ability, Keto-loaded RS nanoparticle showed 9.8% of Keto in nanoparticle, which was evaluated by high-performance liquid chromatography (HPLC). Moreover, the drug release behavior of Keto-loaded eudragit RS nanoparticle was also investigated *in vitro* at pH 7.4 and compared to referential profenid.

**Keywords:** nanoparticles, ketoprofen, eudragit RS, emulsion solvent evaporation

**Classification numbers:** 2.05, 4.02

## 1. Introduction

Today, arthritis affects millions of people of all age groups. Approximately 355 million people worldwide have arthritis; most of them are in the developed countries. For example, nearly 40 million people in the United States are affected by arthritis, causing disadvantages for jobs, labor productivity and life. In the US, the cost of arthritis is estimated to be about $ 65 billion per year [1, 2].

The most common forms of arthritis are osteoarthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, gout and lyme. The conditions for the development of arthritis come from many reasons, but fortunately it is not a contagious disease [1]. Publications on arthritis and the investigations on the solutions for arthritis treatment have attracted researchers, governments and especially medical companies. Hence, the first 10 years of the 21st century have been selected by the World Health Organization (WHO) as osteoarthritis decade (Bone & Joint Decade) [3].

Among drugs used in the treatment of arthritis, ketoprofen (Keto) (phenyl propionic acid, see scheme 1) which belongs to a group of drugs called nonsteroidal anti-inflammatory drugs has been widely used. It acts by inhibiting the production of prostaglandin with analgesic and antipyretic effects. However, the disadvantages of Keto are short half-life time, about 1.5–2 h after using oral dosage form, and irritation caused in the gastrointestinal mucous membrane [4].

In order to avoid the side effects and enhance the usage of drugs, drug nanoparticles have been developed and investigated since the 1970s. Nanoparticles with submicron size (10–1000 nm) have more advantages than conventional

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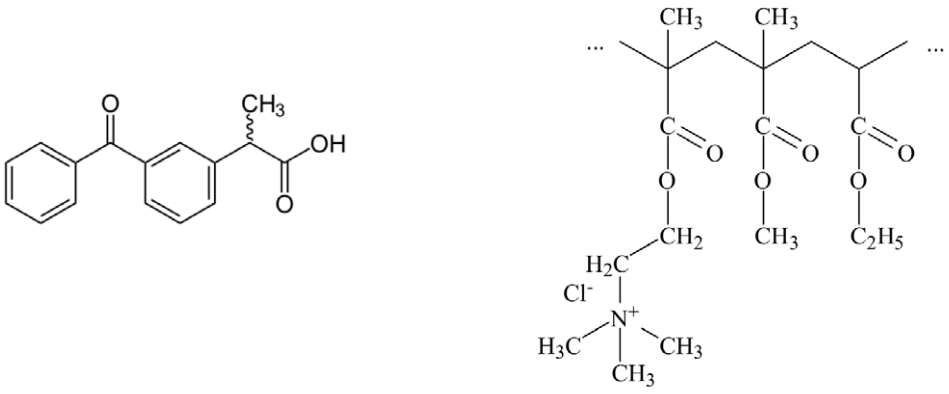

**Scheme 1.** The chemical structure of (left) Keto and (right) eudragit RS.

dosage formulations. They include improved efficacy, reduced toxicity, increased ability and controlled drug delivery [5,6].

In this paper, nanoparticles containing Keto and polymer eudragit RS were prepared using an emulsion solvent evaporation method. Their structures, morphologies and sizes were investigated using transmission electron microscope (TEM) and dynamic light scattering (DLS). The particle sizes of nanoparticles were controlled by changing types of solvents and polymer concentrations. The percentages of Keto in polymeric nanoparticles were evaluated using UV-Vis and high-performance liquid chromatography (HPLC). Moreover, the *in vitro* drug release assessment of RS–Keto was also conducted at pH 7.4.

## 2. Experimental

### *2.1. Materials*

Ketoprofen (Keto), (2-(3-Benzoylphenyl) propionic acid), was purchased from Rohm Pharma Darmstadt (Germany). Polymer eudragit RS was obtained from EVONIK Rohm GmbH (Germany). Organic solvents as acetone, ethanol and tetrahydrofuran (THF) were used for synthesis grade (Merck, Germany). Deionized (DI) water was used as aqueous medium.

### *2.2. Preparation of the Keto-loaded RS nanoparticles*

A typical procedure for the preparation of RS–Keto nanoparticles is described as follows (run 1, table 2): the oil phase containing RS and Keto is prepared by dissolving Keto (1 g) and RS (5 g) in 60 ml of acetone at room temperature. The solution is then dripped drop by drop into 300 ml DI water. The Keto/RS (1/5) and oil/water (1/5) are maintained at standard conditions. The emulsion is formed using ultrasonic probe and magnetic stirrer. Organic solvents are removed by the rotary evaporator at 40 °C for 4 h. The suspension is then centrifuged at 9500 rpm for 30 min and sequentially filtrated, washed with DI water and dried overnight under low vacuum.

### *2.3. Characterization of nanoparticles*

*2.3.1. Nanoparticles morphology.* The morphology of nanoparticles was analyzed by TEM (JEM 1400, Japan). The dry particles were gently spread into the carbon-coated copper grid many times.

*2.3.2. Particle size and size distribution.* Nanoparticles were dissolved into DI water, and would be performed by the particle size (LB550—HORIBA, Japan). Using DLS method, the particle distribution analysis was calculated with polymer particle refractive index as 1.380.

*2.3.3. Infrared spectroscopy.* Using a Fourier transform infrared (FTIR) spectrometer (TENSOR$^{TM}$37—BRUNER), the absorption spectral of raw materials and nanoparticles were recorded from 2200 to 400 $cm^{-1}$. Resolution used in the scans was 1 $cm^{-1}$, and the spectra were averaged over three scans.

*2.3.4. UV-Vis spectra.* With a UV-Vis spectrometer (CARY100, Germany), the absorption speak of drug could be investigated in the wavelength region 200–400 nm.

*2.3.5. Amount of Keto in nanoparticles determination by high performance liquid chromatography (HPLC).* 50 mg pure ketoprofen and RS–Keto nanoparticles were dissolved in methanol 75% to make standard and test sample. The samples were analyzed by HPLC (Hitachi D7000, Japan). The amount of drug in nanoparticles was calculated as follows:

$$W = \frac{K}{M} \times 100\%, \tag{1}$$

where $W$, $K$ and $M$ are the percentage of Keto concentration in nanoparticles, the amount of Keto in nanoparticles, and the total weight of nanoparticle powder sample, respectively.

### *2.4.* In vitro *dissolution studies*

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 1000 ml were used as release chambers. The solutions were kept in a water bath at 37 ± 0.5 °C and stirred at a speed of 75 rpm. For the test in base medium, 700 ml of phosphate buffered saline pH 7.4 was used as release medium. Aliquot (10 ml) was withdrawn

**Table 1.** Particle sizes depend on emulsification probes and polymer concentrations.[a]

| Tools | Polymer concentration[d] (g ml$^{-1}$) | | | |
|---|---|---|---|---|
| | 5 | 10 | 15 | 20 |
| Ultrasonic probe[b] | 110 | 266 | 350 | 518 |
| Magnetic stirrer[c] | 103 | 276 | 577 | 962 |

[a] Measured by DLS, unit: nm, Keto/RS = 1/5.
[b] Intensity of 40%.
[c] Speed of 400 rpm.
[d] In acetone.

at appropriate times and immediately replaced with fresh medium equilibrated at 37 °C. The amount of released Keto was determined by measuring UV absorption at wavelength of 258 nm. The percentage of released Keto was determined from the following equation:

$$\text{Release}(\%) = \frac{\text{Release Keto from NPs}}{\text{Total amount of Keto in NPs}} \times 100\%. \quad (2)$$

The measurements were performed three times and the values reported are mean values. The repeatability of the method was evaluated by analyzing three parallel samples. The RS–Keto nanoparticle containing 9.8% of Keto (N9.8) was a sample in this study. The release behavior of N9.8 was also compared to that of referential profenid.

## 3. Results and discussion

### *3.1. Preparation of Keto-loaded RS nanoparticles*

The Keto-loaded RS nanoparticles were prepared using emulsion solvent evaporation method. In this paper two types of emulsification tools were utilized, namely ultrasonic probe and magnetic stirrer. The particle sizes were different with two types of tools. In addition, the tendencies to change in the particle sizes by changing polymer concentrations were also different with two tools. Moreover, the particle sizes were dependent on different types of solvents. The effect of these processing parameters will be discussed thoroughly in the following sections.

*3.1.1. Effect of polymer concentrations and emulsion tools.* Two types of tools were utilized to disperse oil phase into water phase. The volume ratio of oil and water phases was maintained at 1 : 5 (v/v) for all experiments. The polymer concentrations were in the range of 5–20% with a step of 5%. The results are shown in table 1.

The results indicated that the increase in RS concentration leads to increasing particle size in both cases using ultrasonic probe and magnetic stirrer. This phenomenon can be explained based on the viscosity of dispersed phase. The increasing polymer concentration is usually accompanied by increasing viscosity of dispersed phase. As a result, the droplet is formed bigger leading to the bigger nanoparticle diameters. The control of nanoparticle sizes by changing polymer concentrations has previously been reported [7, 8]. It was explained that the increase in polymer concentration leads to an increase in the viscous forces resisting droplet breakdown by sonication. The viscous forces oppose the shear stresses in the organic phase and the final size and size distribution of particles depends on the net shear stress available for droplet breakdown [9]. Figure 1 illustrates this effect.

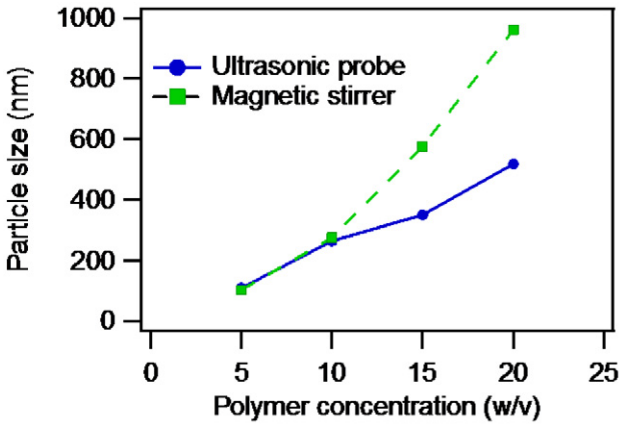


**Figure 1.** Effect of emulsification tools and polymer concentrations on particle size of RS–Keto.

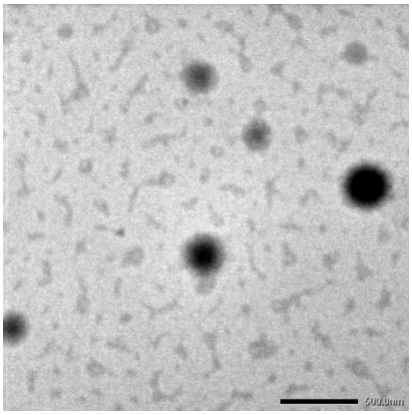

**Figure 2.** TEM image of RS–Keto nanoparticles prepared by ultrasonic probe. Conditions: Keto/RS = 1/5; oil/water = 1/5; polymer concentration 10 g ml$^{-1}$.

Although particle sizes increased with increasing RS concentration when using both ultrasonic probe and magnetic stirrer for emulsification, the tendencies to change in the particle sizes were different when comparing between ultrasonic probe and magnetic stirrer. It is not hard to see in figure 1 that the change in particle size by changing RS concentration in the case of magnetic stirrer is larger than that in the case of ultrasonic probe. Figure 1 also implies that the magnetic stirrer gives bigger particle sizes compared to ultrasonic probe. Since the control of particle size when using ultrasonic probe was easier than when using magnetic stirrer, ultrasonic probe will be used in the following sections.

For the morphology, the obtained RS–Keto was confirmed to be spherical, smooth and nano-size using TEM. Figure 2 presents a TEM image of Keto-loaded RS prepared by ultrasonic probe (conditions: Keto/RS = 1/5; oil/water = 1/5; polymer concentration 10 g ml$^{-1}$). The result indicates that RS–Keto nanoparticles have a mean size of 253 nm. The mean hydrodynamic diameter of RS–Keto nanoparticles determined by DLS was 276 nm (see table 1) which is slightly bigger than that determined by TEM (253 nm, see figure 2).

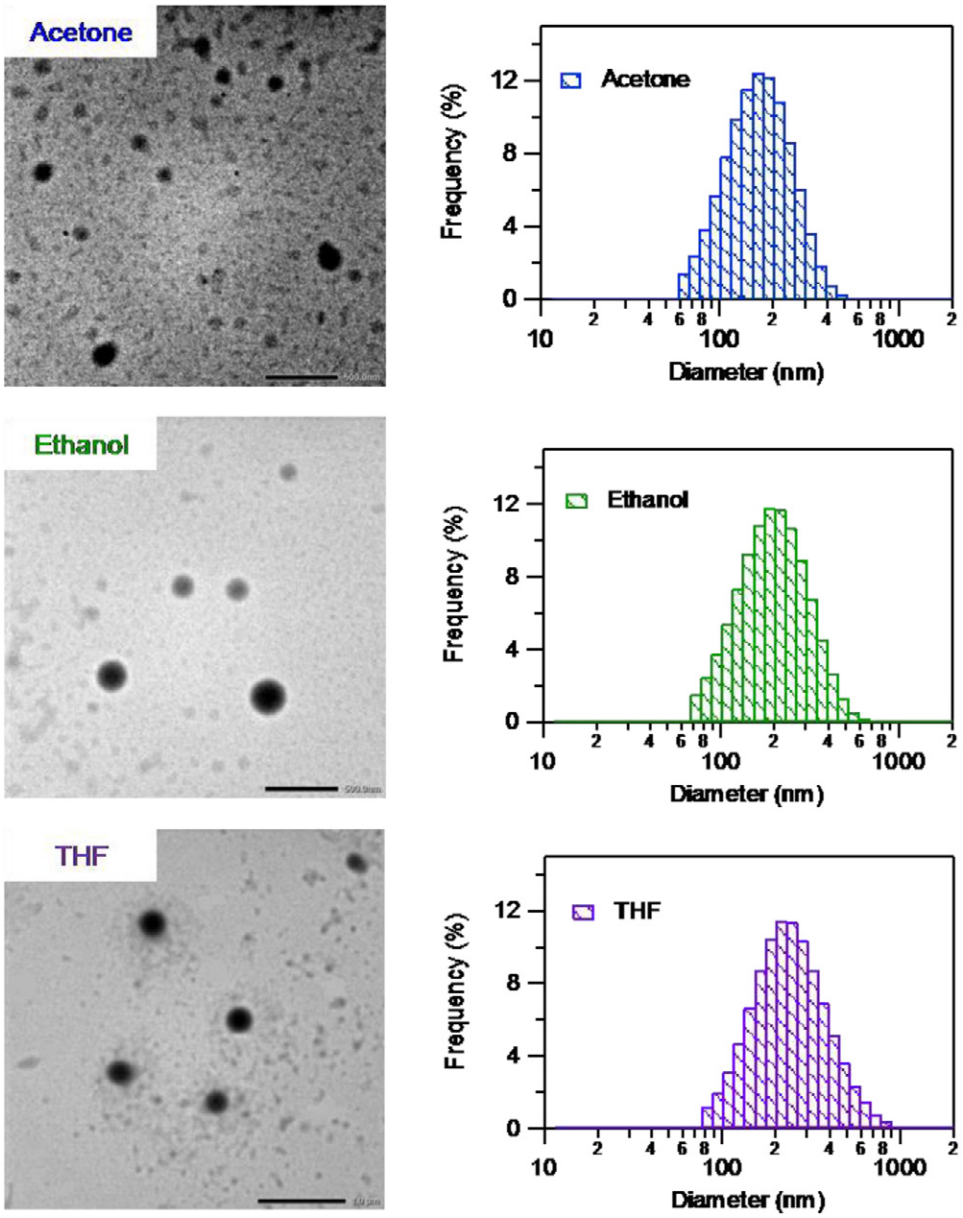


**Figure 3.** TEM image (left) and particle distribution by DLS (right) of RS–Keto nanoparticles prepared by ultrasonic probe.

This result is quite reasonable since particle size determined by DLS represents its hydrodynamic diameter, whereas that obtained by TEM is related to the collapsed nanoparticles after water evaporation.

*3.1.2. Effect of solvents.* In emulsion solvent evaporation method, there is a thermodynamic equilibrium between organic solvent phase and water phase. The particles are formed during the transportation of organic solvent that diffuses to the external phase and their size may be dependent upon the types of organic solvents. Here in this section, the effect of the types of organic solvents on the mean particle size was investigated. To evaluate the effect of organic solvents, various organic solvents were used for the preparation of RS–Keto nanoparticles, namely acetone, ethanol and THF. Table 2 presents the effect of organic solvents on particle size measured by DLS and TEM. The results showed that the mean particle size of RS–Keto was smallest when using acetone as organic solvent. The biggest particle size was observed when using THF as organic solvent. Figure 3 presents size distribution by DLS (right) and TEM images of RS–Keto nanoparticles prepared by different organic solvents. The particle sizes measured by DLS were slightly bigger than those measured by TEM which was already explained in the previous section.

**Table 2.** Effect of solvent on particle size[a]

| Run | Solvent | Particle size (nm) | |
|---|---|---|---|
| | | DLS | TEM |
| 1 | Acetone | 163 | 132 |
| 2 | Ethanol | 192 | 171 |
| 3 | THF | 295 | 268 |

[a] Conditions: Keto/RS = 1/5, oil/water = 1/5, RS/solvent = 1/12 g ml$^{-1}$.

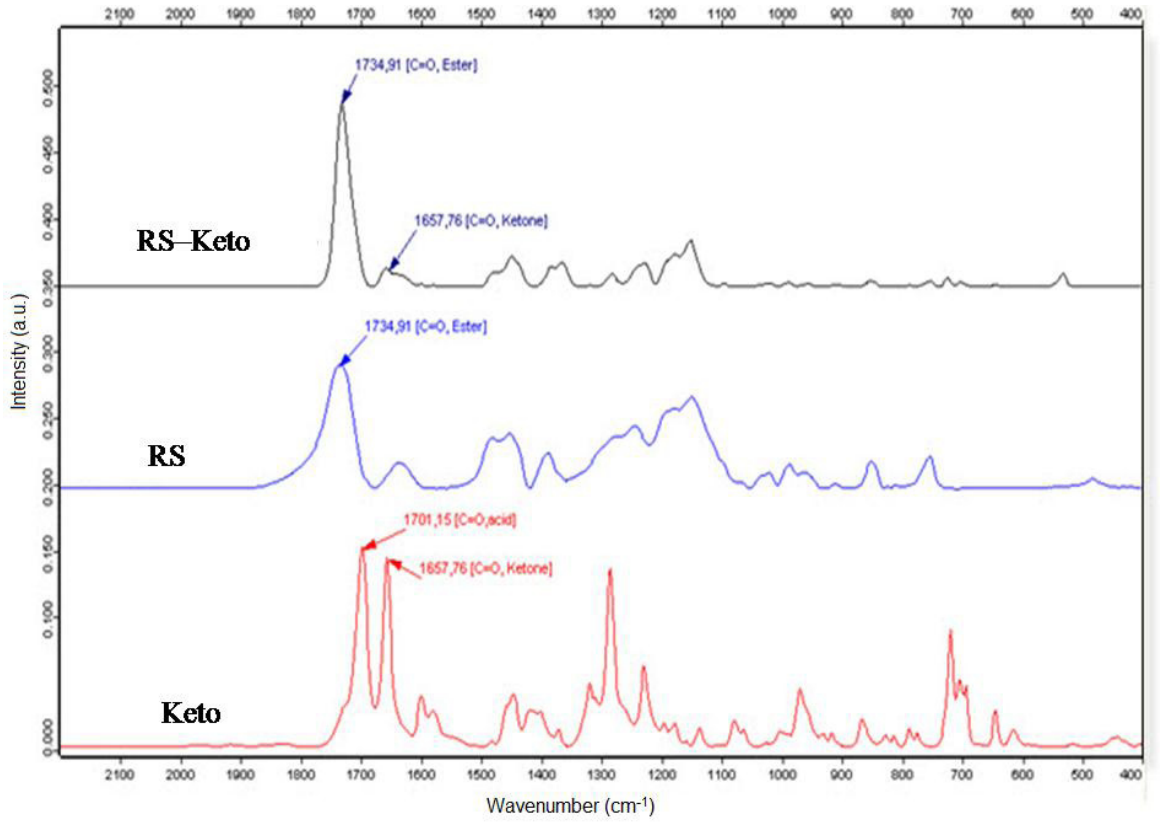


**Figure 4.** FTIR spectra of Keto, RS and RS–Keto nanoparticles. Conditions: Keto/RS = 1/5, oil/water = 1/5, RS/acetone = 1/20 mg ml$^{-1}$.

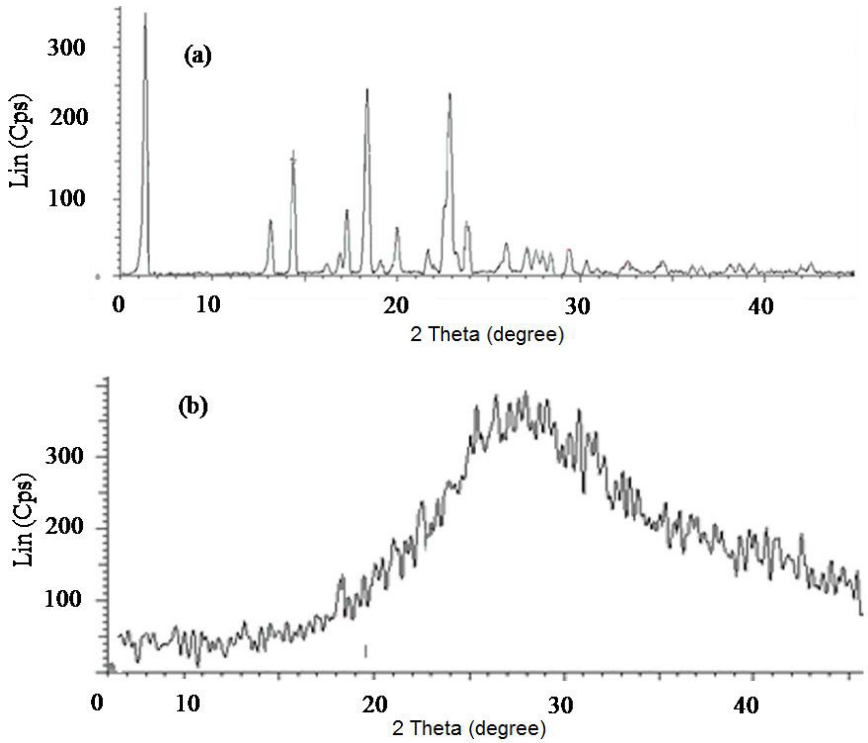


**Figure 5.** XRD patterns of (a) Keto and (b) RS–Keto nanoparticles. Conditions: Keto/RS = 1/5, oil/water = 1/5, RS/acetone = 1/20 mg ml$^{-1}$.

### *3.2. Characterization of RS–Keto nanoparticles*

*3.2.1. FTIR spectroscopy.* FTIR spectroscopy was used to study the interactions between the Keto and the RS and the result is presented in figure 4.

Keto has a carboxylic acid group with two peaks recorded at 1701 and 1657 cm$^{-1}$ corresponding to acid carbonyl group and ketonic carbonyl group, respectively. For the spectrum of the RS, the strong vibration of the carbonyl group could be identified at 1734 cm$^{-1}$. However, for polymer nanoparticles

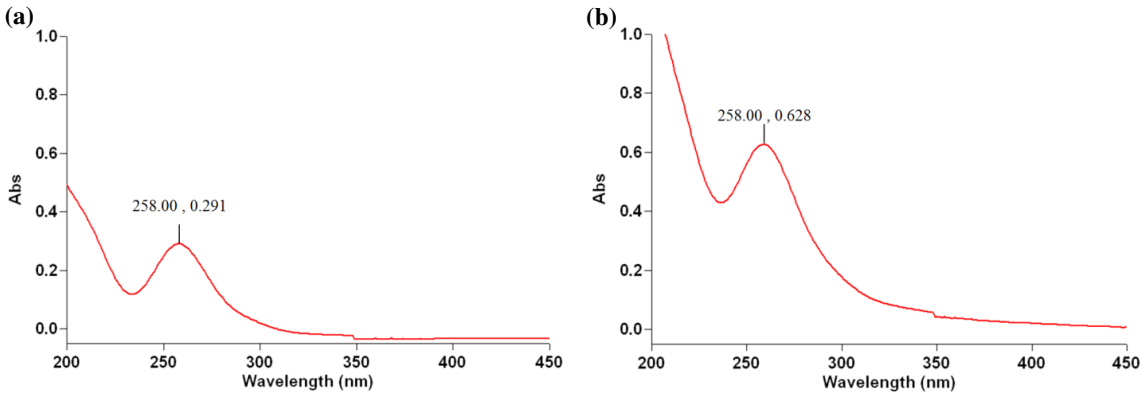


**Figure 6.** UV-Vis spectra of (a) pure Keto and (b) RS–Keto. Conditions: Keto/RS = 1/5, oil/water = 1/5, RS/acetone = 1/20 mg ml$^{-1}$.

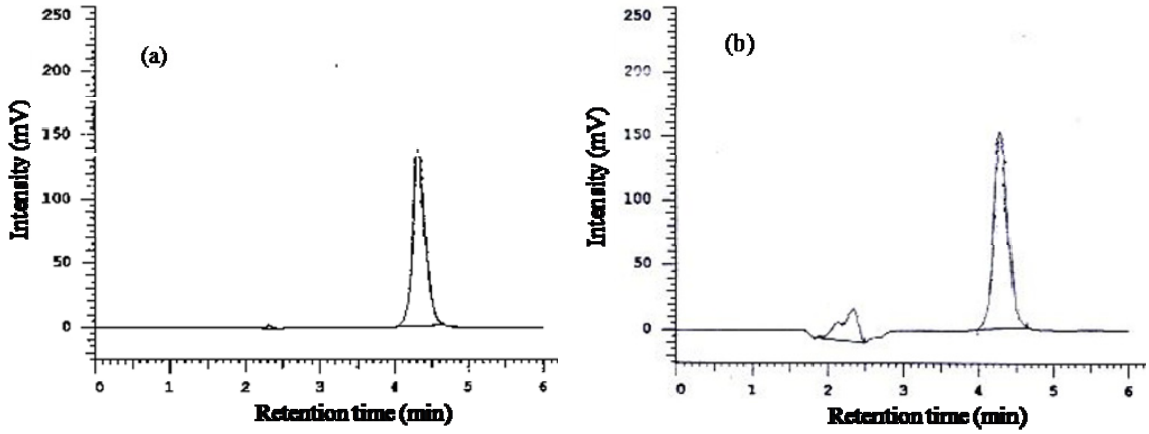


**Figure 7.** The HPLC curve of (a) pure Keto and (b) RS–Keto. Conditions: Keto/RS = 1/5, oil/water = 1/5, RS/acetone = 1/20 mg ml$^{-1}$.

containing Keto (RS–Keto), the FTIR spectra showed a very strong peak at 1734 cm$^{-1}$ and ketonic carbonyl group of Keto. The peak corresponding to the acid carbonyl group of Keto at 1701 cm$^{-1}$ was not seen. It could be understood that the carboxylic acid group of Keto interacted with the RS, leading to the overlap of acid carbonyl group of Keto and the ester group of the polymer.

*3.2.2. X-ray diffraction (XRD).* Figure 5 shows XRD patterns of Keto and RS–Keto nanoparticles. On the left, XRD shows diffraction with many peaks for crystal structure of raw Keto (at 6.5°, 18.3°, 22.9° etc). However, in the RS–Keto nanoparticle, no peak was seen. We could conclude that the RS–Keto nanoparticles were amorphous, so the peaks corresponding to diffraction from crystal lattice drug were not detected.

*3.2.3. UV-Vis spectra.* The pure Keto and the polymer nanoparticles were used as the standard and test samples, respectively. The UV-Vis spectral was studied in the region 200–450 nm and the result is shown in figure 6.

*3.2.4. The determination of amount of Keto in nanoparticles by HPLC.* The percentage of Keto in nanoparticles was investigated using HPLC. According to equation (1), the percentage of Keto in the nanoparticles was calculated to be 9.8% using HPLC (figure 7). This result is similar to that measured by UV-Vis.

**Table 3.** *In vitro* release of CB [6]—Keto nanoparticles and profenid in the basic medium (pH 7.4).[a]

| Time (h) | N9.8[b] | Profenid[c] |
|---|---|---|
| 0 | 0 | 0 |
| 0.5 | 35.8 | 60.5 |
| 1.0 | 39.8 | 104.3 |
| 1.5 | 45.6 | 106.2 |
| 2.0 | 48.4 | 106.1 |
| 2.5 | 52.7 | 105.3 |
| 3.0 | 55.4 | 104.3 |
| 3.5 | 59.5 | 104.5 |
| 4.0 | 62.5 | 105.3 |

[a] Release (%), determined by equation (2).
[b] Content 9.8% of Keto.
[c] Content 32.4% of Keto.

*3.3.* In vitro *drug release at pH 7.4*

The results of *in vitro* release study in basic medium of RS–Keto nanoparticles and referential profenid are shown in table 3 and plotted in figure 8. The profenid released almost 100% of Keto after 1 hour while RS–Keto N9.8 released 39.8% of Keto. This implies that the profenid released Keto

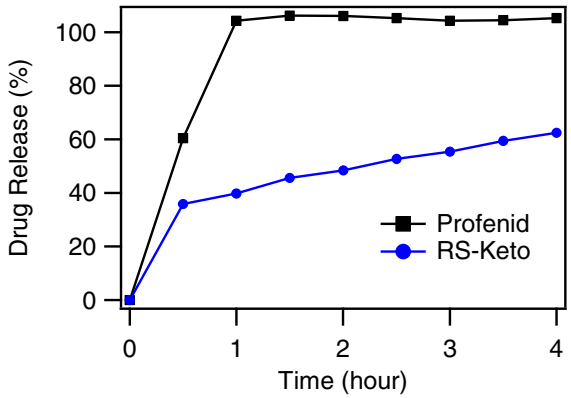


**Figure 8.** *In vitro* release of RS–Keto nanoparticles and profenid in the basic medium.

faster than the RS–Keto nanoparticles when it was dissolved in basic medium. The released percentage of Keto was then gradually increased with the increasing time. However, the percentages of Keto released were a nonlinear function of time. This implies that the Keto released from RS–Keto nanoparticles was due to the diffusion of Keto from the outer shells of nanoparticles. The diffusion of drugs from the outer shells of nanoparticles has been confirmed in previous publications [10, 11]. Keto in the outer shells was poorly entrapped in the RS matrix leading to easy diffusion of Keto. Because the solubility of RS is irrespective of pH, the release of Keto is just probably dependent on the diffusion of Keto from RS matrix. Therefore, the released percentage of Keto was 62.5% after 4 h.

## 4. Conclusion

Keto-loaded RS nanoparticles were successfully prepared by emulsion solvent evaporation method. The obtained RS–Keto particles were confirmed to be spherical, smooth and submicron size using TEM and DLS. The particle sizes of RS–Keto were controlled by changing the RS concentrations and the types of solvents. The results showed that the particle sizes increased with the increasing RS concentration and acetone is a good solvent to obtain smallest particle size. The *in vitro* dissolution studies of RS–Keto nanoparticles were conducted at pH 7.4. The results indicated that the profenid released Keto faster than the RS–Keto nanoparticles when it was dissolved in basic medium. Because the solubility of RS is irrespective of pH, the release of Keto is just probably dependent on the diffusion of Keto from RS matrix.

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