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Inclusion of ketoprofen with skimmed milk by freeze-drying

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Abstract

Ketoprofen is a non-steroidal anti-inflammatory drug which is not soluble in water and creates gastrointestinal problems. In order to improve the solubility of the drug in water and enhance its dissolution rate, physical mixture (PM) and inclusion (IC) of ketoprofen with skimmed milk (SM) were prepared and investigated. Enhancement of solubility of ketoprofen was obtained by preparing its IC with SM which can be used because of its amino acid and surface active agents content and can also be used for treatment of gastric disturbance. Lyophilization technique was used to prepare the IC. Results obtained showed that the solubility of IC of ketoprofen with SM was almost four times greater than the solubility of the plain drug. Data from the dissolution rate determination have also indicated that IC of ketoprofen with SM significantly improved the dissolution rate of the drug compared with PM and the plain form. Differential scanning calorimetry (DSC), X-ray powder diffraction and scanning electron microscopic (SEM) analysis revealed the formation of IC of ketoprofen with SM.

Keywords: Ketoprofen; Skimmed milk; Physical mixture; Inclusion; Solubility; Enhancement in dissolution

1. Introduction

Ketoprofen has analgesic, anti-inflammatory and antipyretic properties and is an inhibitor of prostaglandin synthetase. It is used in rheumatic disorders and in mild to moderate pain. It is not freely soluble in water [1] and causes local or systemic disturbance in the gastrointestinal tract. Ketoprofen was found to cause gastrointestinal side-effects requiring withdrawal of treatment [2]. In order to improve the solubility of the drug in water, addition of surface active agents and formation of water soluble salts were carried out [3] and to enhance dissolution and absorption rate, reduction of the particle size and increasing the wettability have been attempted [4].

For reducing the particle size, inclusion (IC) has been proposed [5]. A freeze-drying method [6] was proposed to form IC. In order to eliminate or reduce gastro-intestinal disorders of non-steroidal drugs, amino acids have been suggested either as additives in the peroral application [7] or in the form of amino acid salts [8]. In this study, as the carrier for IC, skimmed milk (SM) has been chosen due to its surface active agent and amino acid content [9]. Additionally milk has been proposed against the gastric disturbance caused by non-steroidal drugs with anti-inflammatory effects [10]. Differential scanning calorimetry (DSC), X-ray powder diffraction and scanning electron microscopic (SEM) analysis were performed to determine the physicochemical properties of the physical mixture (PM) and the IC in comparison with the plain drug. These techniques have been known to be used for the assessment of molecular interactions occurring between solid components of pharmaceuticals [11,12].

2. Experimental

2.1. Apparatus and chemicals

Photometer: Hitachi U-1100, Tokyo UV double beam spectrophotometer flow cell; dissolution apparatus: Erweka, Germany; peristaltic pump: Desaga STA, Firma Desaga, Heidelberg, Germany; potentiometer

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recorder: Servogor, type RE 541 Firma BBC Metrawatt Nürnberg, Germany; Netz apparatus: Type Voltaraft NG 15 Firma Conrad Elektronik, Hirschau; computer: IBM-compatible AT 486/33 with VGA graphics card, Firma J. Friedrichs, Münster, Germany; Lyophilization apparatus: Lyovac GT 2 (Leybold Heraeus).

Ketoprofen (Sigma) and all other reagents and chemicals were of analytical grade; SM used had a maximum 1% fat and 4% carbohydrate content. Ketoprofen was purchased and used in micronized form.

2.2. Procedure

2.2.1. Preparation of skimmed milk powder

After freeze-drying (SM was lyophilized until the sample's humidity reduced to a maximum of 3%. Lyophilization time was chosen as 72 h to reduce humidity, according to preliminary studies), 25 ml SM yielded $\cong 2.615$ g* powder (*mean of five experiments). SM was sieved from 250 μm mesh sieves prior to the preparation of SD and PM. Powder size ≤ 10 μm.

2.2.2. Preparation of the inclusion

Ketoprofen (500 mg) was mixed in 25 ml SM in a water bath at a temperature of 50°C and stirred for 30 min using a magnetic stirrer. It was frozen by keeping it in a liquid nitrogen bath and lyophilized for 72 h. IC was sieved from 250 μm mesh sieves.

2.2.3. Preparation of the physical mixtures

The micronized drug (500 mg, particle size of 10 μm) was uniformly mixed with 2.615 g lyophilized SM using an agate mortar and pestle. The prepared mixtures were kept in a dessicator over calcium chloride (0% relative humidity) at room temperature.

2.3. Solubility studies of inclusions and physical mixtures

Ketoprofen (100 mg), its IC and PM with SM equivalent to 623 mg (including 100 mg ketoprofen) under test, was placed in a glass stoppered flask and 100 ml water were added. Then it was shaken in a water bath at 25°C for 15 h (USP XIX). The solution was filtered through blue ribbon filter paper (S&S) of pore diameter 0.45 μm. The dissolved drug was measured spectrophotometrically at 262 nm.

2.4. Dissolution rate determination

The dissolution rates of ketoprofen IC as well as the PM of the drug with SM compared with the plain drug, were determined using the USP XXII Paddle method with a dissolution apparatus. Pure ketoprofen (15 mg) or its equivalent of the IC or the PM with SM were sprinkled in 1000 ml of distilled water maintained at 37°C and stirred at 100 rpm. After certain time intervals, samples of the dissolution medium were withdrawn, filtered through Millipore membrane of 0.45 μm pore diameter and assayed for the drug content spectrophotometrically at 262 nm.

2.5. Differential scanning calorimetry studies

Samples weighing approximately 3 mg were placed in aluminum pans and analyzed using a Perkin–Elmer DSC-2°C calorimeter. The scanning speed was 10°C/min in the range 20–340°C.

2.6. X-ray powder diffraction analysis

X-ray diffraction patterns were recorded with a Philips PW 1710 diffractometer (The Netherlands). Cu Kα radiation (λ 1-5418), nickel filter, 50 kV, 40 mA.

2.7. Scanning electron microscopic analysis

Surface morphology of ketoprofen, its PM as well as its IC with SM were analyzed using a scanning electron microscope (Joel 1200 EX-11, Tokyo, Japan).

3. Results and discussion

In order to determine the interaction between ketoprofen and SM, DSC studies were performed on the PM and IC of ketoprofen with SM, as well as their individual components. As shown in Fig. 1, the DSC curve of ketoprofen showed one endothermic peak at nearly 90°C, corresponding to its melting point, whereas SM's peak has been shown to be 165°C [13]. The DSC plots of the PM and IC showed only one broad peak for each at about 70 and 180°C, respectively. Since the DSC peak value of IC is far away from the melting point of ketoprofen, it could be concluded that the formation of a bond between ketoprofen and SM occurred. Disappearance of the specific peak of the drug indicated that the drug has interacted with the carrier.

The X-ray powder diffraction pattern of the pure drug exhibits its characteristic diffraction peaks at various diffraction angles indicating the presence of crystallinity, whereas SM exhibits a diffraction spectrum, typical of mostly amorphous materials not showing any detectable diffraction peaks. In the case of IC, absence and reduction of major ketoprofen diffraction peaks indicate that mostly an amorphous form (disordered state) existed in the IC (Fig. 2). In the X-ray diffraction spectrum of the PM, it is possible to detect some crystals of ketoprofen, since the particle size is bigger than in the IC. However, the particle size of ketoprofen in PM and the plain drug are similar. Based on these

results, enhancement in solubility and dissolution rate may be attributed to the surface active agents and enzymes content of SM in the case of PM and reduction of particle size and formation of an amorphous state in IC form.

The SEM results Figs. 3–5 show that in the case of PM, the particle size of ketoprofen is approximately the same, and on some SM particles crystals of ketoprofen could be seen. In IC, ketoprofen particles are in almost amorphous form, which allows one to conclude that reduction of particle size was mostly achieved.

Solubility of ketoprofen in IC is (0.099%) nearly four times, and in PM (0.043%) 2.4 times higher than in the plain drug which has a solubility of 0.026% in water (Table 1).

The dissolution profiles of IC and PM of ketoprofen with SM are shown in Fig. 6. Ketoprofen as a plain drug was also analyzed and found to be the least soluble. Incorporation of ketoprofen with SM especially in IC form significantly improved the dissolution rate of the drug as compared with PM and the plain drug. The dissolution rate was also found to be higher and faster in IC form than in PM.

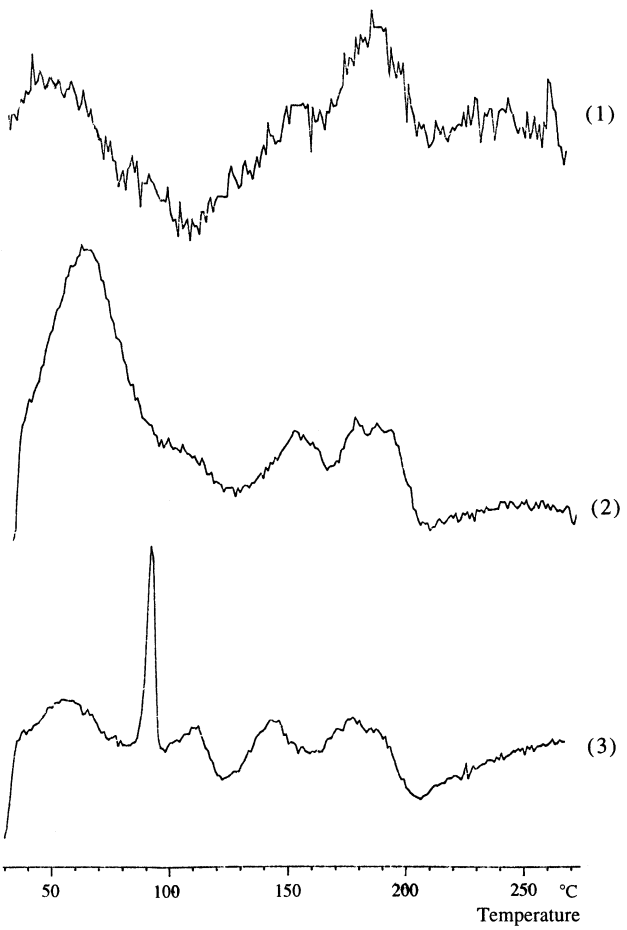

Fig. 1. DSC thermograms of IC of ketoprofen with SM (1), PM of ketoprofen with SM (2), ketoprofen (3).

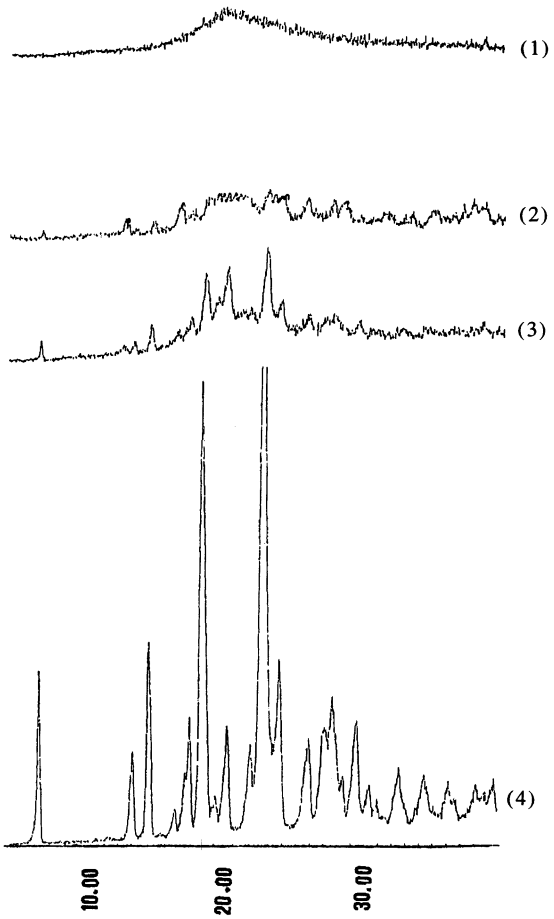

Fig. 2. Powder X-ray diffraction spectra of SM (1), IC of ketoprofen with SM (2), PM of ketoprofen with SM (3), ketoprofen (4).

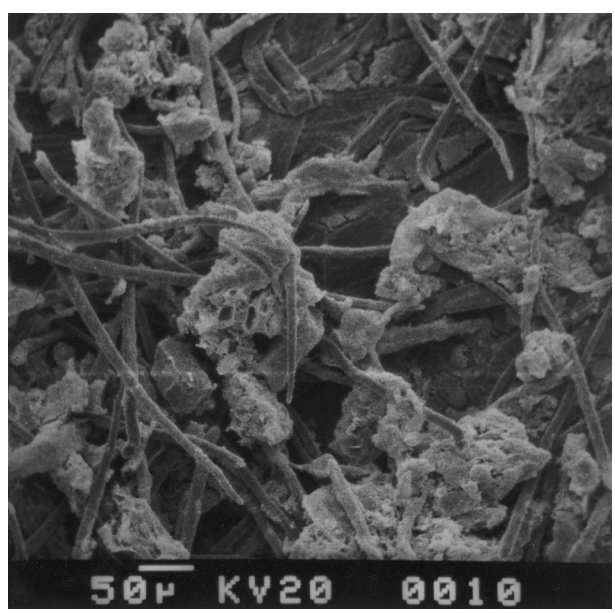

Fig. 3. SEM picture (10 000 × magnification) of ketoprofen.

Due to these results, it can be concluded that as expected IC with SM was found to be the most suitable form for ketoprofen in terms of solubility and dissolution in water, whereas the PM of ketoprofen with SM has also given better results than the plain drug. Also,

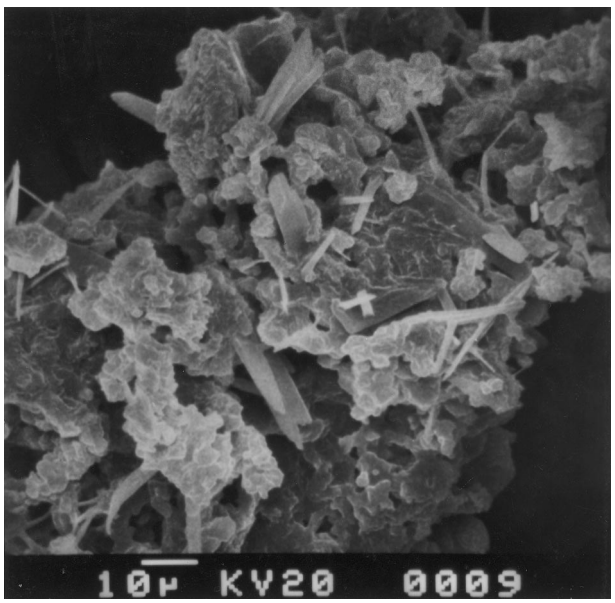

Fig. 4. SEM picture (1000 × magnification) of PM of ketoprofen with SM.

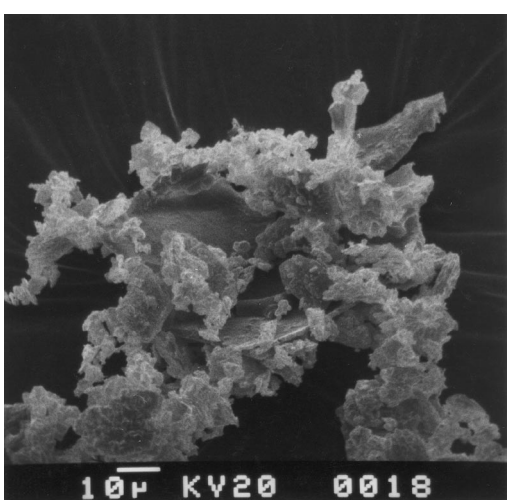

Fig. 5. SEM picture (1000 × magnification) of IC of ketoprofen with SM.

Table 1
Solubility of ketoprofen as a plain drug, in IC and PM with SM in distilled water at 25°C

Ketoprofen w/v (%)	Form of the drug	Solubility (% ± SD)
0.1	IC	0.099% ± 0.081
0.1	PM	0.043% ± 0.032
0.1	plain drug	0.026% ± 0.014

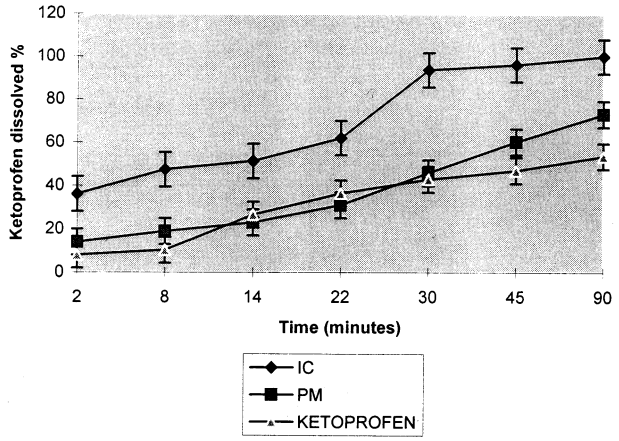

Fig. 6. Dissolution profiles of ketoprofen, its IC and PM with SM in distilled water at 37°C.

according to our investigation in view of pharmacological activity and the side-effects of gastrointestinal disturbance of other non-steroidal drugs with the same therapeutic activity, the pharmacological activity has been increased and the gastrointestinal disturbance has been decreased significantly [10,14]. These problems occur in therapy with ketoprofen, so it could be possible to adapt these results to IC of ketoprofen with SM and the same advantages could be expected for ketoprofen. Therefore, it would be possible to formulate ketoprofen with SM in IC having an eventual decreased therapeutic dose with less gastrointestinal disturbance for peroral and also parenteral applications.

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