

# Enhancement in Dissolution Rate of Piroxicam by Two Micronization Techniques

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## ABSTRACT

Piroxicam is a nonsteroidal anti-inflammatory drug that is practically insoluble in water. The oral absorption rate of piroxicam is dependent on its dissolution rate in the GI tract. The aim of this study was to enhance the dissolution of piroxicam by a microcrystallization technique. The preparation of microcrystals of piroxicam was done by two methods, solvent change and pH shift. In the solvent-change method, the drug was dissolved in acetone, and the stabilizer was dissolved in water. The aqueous phase was added to acetone under homogenization in an ice bath for 1 min. In the pH-shift method, the drug and stabilizer were both dissolved in 0.1 N NaOH (pH 12) using homogenization. The pH was adjusted to 3 using 0.1 N hydrochloric acid. Dissolution testing was carried out in a hydrochloric acid medium using the rotating basket method. Particle size and morphology, FTIR, DSC, XRD, and surface area of the microcrystals were studied. The effects of drug and stabilizer concentration and homogenization rate on particle size and dissolution efficiency were studied statistically using a D-optimal design. The dissolution efficiency in both methods was increased about 3- to 4-fold. The particle size in both methods was decreased in comparison with untreated drug. Maximum dissolution and minimum particle size were obtained by the solvent-change method. According to the results, both microcrystallization methods are effective in the modification of the crystalline habit of piroxicam.

## INTRODUCTION

Poor solubility is one of the major challenges in drug development today (1). An estimated 40% of drugs fall under BCS Class 2 (low solubility and high permeability) or Class 4 (low solubility and low permeability) (2, 3). These drugs show limited bioavailability because of their low solubility (4). Piroxicam, one of the most potent nonsteroidal anti-inflammatory drugs, is a BCS Class 2 drug with high membrane permeability but low water solubility (5). Different techniques have been studied to enhance the dissolution rate of piroxicam. Cavallaria et al. (6) used beta-cyclodextrin to improve the dissolution of piroxicam powder by wet granulation. Results showed advantages such as better powder flow and compressibility and improved bioavailability. Other methods such as liquid-solid compacts (5, 7), wet granulation using beta-lactose and PVP (8), and solid dispersions (9, 10) have been proposed to enhance the dissolution rate of piroxicam.

Micronization is an effective way to enhance the dissolution rate of poorly water-soluble drugs (11, 12). However, mechanical methods such as milling or grinding have inevitable drawbacks, including the tendency of the produced particles to agglomerate, reducing the available surface area (13). In addition, the potential for physical instability or chemical degradation from disruptions in the crystal lattice (14, 15) and the high energy input makes this micronization method extremely inefficient (16). Several other methods have been introduced to produce

micron-sized drug particles such as high-pressure homogenization (17), supercritical fluid micronization (18, 19), liquid-solid techniques (20), and hydrosol formation (11).

In situ micronization has been proposed recently to overcome the above-mentioned problems of mechanical techniques. Since particles are prepared directly in the micronized state (21) and the newly formed microcrystal surface is simultaneously stabilized by a stabilizer (usually a polymer), particles have a much lower tendency for crystal growth and agglomeration (11).

Two methods of microcrystallization are pH shift and solvent change. In the solvent-change method, an organic solvent and an aqueous solvent with a stabilizing agent are used. The drug microcrystals are formed by mixing the aqueous solution of the stabilizer with the organic drug solution; this leads to a reduction of interfacial tension, since the stabilizing agent would be adsorbed on the precipitated drug particles. A drug powder with a high drug load is obtained after drying this dispersion (22). Disodium cromoglycate microcrystals have been prepared for pulmonary delivery using this method (22). Zimmermann et al. (23) reported that the adsorption of pharmaceutical excipients onto microcrystals of siramesine hydrochloride changes the physicochemical properties of the drug such as particle size, morphology, and dissolution rate. Aspirin, mebutamate, and quinine sulfate microcrystals with a particle size of less than 10  $\mu\text{m}$  were prepared using the solvent-change method (24).

In the pH-shift method, an aqueous solution is used instead of organic solvent. A supersaturated solution is

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**Table 1. Different Process Variables and Respective Levels in Micronization Technique (pH-Shift and Solvent-Change Methods)**

Micronization Technique	Variables	Levels	
		Lower Limit	Upper Limit
pH shift	A: Drug concentration (%)	0.3	0.6
	B: Stabilizer concentration (%)	0.03	0.06
	C: Homogenization speed (rpm)	17500	21500
Solvent change	A: Drug concentration (%)	0.3	0.6
	B: Stabilizer concentration (%)	0.03	0.06
	C: Homogenization speed (rpm)	17500	21500
	D: Stabilizer type	Brij 35	Poloxamer 188

prepared to precipitate the drug microcrystals using pH-dependent solubility. Kim et al. (16) reported an increased dissolution rate and uniform size distribution for indomethacin, which has a pH-dependent solubility.

In the present study, two techniques of solvent change and pH shift are compared for size reduction, and hence the enhancement of dissolution and bioavailability of piroxicam.

## MATERIALS AND METHODS

### Materials

Piroxicam was supplied from Darupakhsh Company (Iran). Brij35 (Fluka, USA), Poloxamer 188 or Pluronic F68 (Sigma, USA), acetone, ethanol, HCl, and NaOH were all from Merck Chemical Company (Germany). All chemicals were of analytical grade.

### In Situ Micronization Technique

After different process variables including the concentration of the drug and stabilizer, homogenization speed, and stabilizer type were determined, two levels were established for each variable. By changing three process variables for the pH-change method and four variables for the solvent-change method (Table 1), each at two levels, eighteen and twenty-four different formulations were designed by a D-optimal design, respectively, using Design Expert software (Version 7.2, US).

#### *Preparation of Piroxicam Microcrystals Using pH-Shift Method*

Piroxicam microcrystals were prepared by reducing the pH level of the piroxicam solution to form a micron-size dispersion of the drug due to low solubility of piroxicam in lower pH values.

The drug was dissolved in 100 mL of 0.1 N NaOH (pH 12) containing Brij35 as the stabilizer. After the drug was completely dissolved using an ultra-homogenizer (Ultra Turrax-T25 Basic IKA, Germany), the pH of the solution was reduced from 12 to 3 within one minute by the addition of 0.1 N HCl in an ice bath. The lower pH (pH 3) was selected based on preliminary studies in which reducing the pH of the dispersion to lower values had not changed the

amount of precipitate. After the spontaneous formation of the micron-size dispersion of the drug, the mixture was transferred to a rotary evaporator (Buchi, Switzerland) and stirred for one hour at 40 °C. After that, the aqueous suspension was frozen at -70 °C for 24 h and freeze-dried (Christ Alpha 4.2 LD, Germany) at 0.001 bar for 24 h. Eighteen different formulations described in Table 2 were prepared by this procedure.

#### *Preparation of Piroxicam Microcrystals Using Solvent-Change Method*

The process was carried out by instantaneously mixing two liquids in the presence of Brij35 or poloxamer188 as stabilizing agents. The process was performed in an ice bath. In the first step, the drug was dissolved in 100 mL of acetone (as the solvent) and the stabilizing agent in 100 mL of water (as nonsolvent). The nonsolvent was poured rapidly into the drug solution under stirring at different homogenization speeds ranging from 17500 to 21500 rpm using an ultra-homogenizer. A micron size, fine dispersion formed spontaneously. The dispersion was dried using the previous method. Twenty-four different formulations were prepared using this procedure (Table 3).

### Scanning Electron Microscopy (SEM)

Electron micrographs of crystals were obtained using a scanning electron microscope (PHILIPS X L30, Netherlands). The specimens were mounted on a metal stub with double-sided adhesive tape and coated under vacuum with gold in an argon atmosphere prior to observation.

### Particle Size Analysis

A laser diffraction particle size analyzer (Malvern, long bed ver. 2.15, UK) was used to measure the size distribution and the mean particle size diameter of the prepared microcrystals and the pure drug powder by dispersing the particles in double-distilled water.

### X-ray Powder Diffraction

The sample holder of the X-ray diffractometer (Bruker, D8 ADVANCE, Germany) was filled with the ground

**Table 2. Particle Size and DE<sub>150</sub> of Piroxicam Microcrystals Prepared by Solvent-Change Method with a D-Optimal Design**

Formulation Code	Particle size ± SD (µm)	DE <sub>150</sub> ± SD (%)
Untreated piroxicam	15.6 ± 37.4	24.0 ± 4.4
P <sub>0.52</sub> B <sub>0.05</sub> S <sub>19500</sub>	3.5 ± 0.1	52.1 ± 6.5
P <sub>0.6</sub> P <sub>0.06</sub> S <sub>19500</sub>	1.1 ± 0.1	65.3 ± 5.5
P <sub>0.3</sub> B <sub>0.06</sub> S <sub>17500</sub>	0.9 ± 0.2	65.0 ± 4.0
P <sub>0.3</sub> B <sub>0.06</sub> S <sub>19500</sub>	3.9 ± 0.3	70.2 ± 4.2
P <sub>0.45</sub> B <sub>0.03</sub> S <sub>21500</sub>	0.4 ± 0.0	69.3 ± 5.9
P <sub>0.6</sub> P <sub>0.03</sub> S <sub>21500</sub>	14.5 ± 0.4	64.0 ± 1.8
P <sub>0.6</sub> P <sub>0.03</sub> S <sub>17500</sub>	3.9 ± 1.6	51.4 ± 3.1
P <sub>0.3</sub> P <sub>0.06</sub> S <sub>17500</sub>	3.3 ± 0.0	56.1 ± 3.3
P <sub>0.6</sub> B <sub>0.06</sub> S <sub>21500</sub>	0.7 ± 0.1	82.5 ± 2.3
P <sub>0.45</sub> B <sub>0.03</sub> S <sub>21500</sub>	1.4 ± 0.1	70.2 ± 1.4
P <sub>0.6</sub> B <sub>0.06</sub> S <sub>17500</sub>	14.2 ± 0.1	46.0 ± 3.0
P <sub>0.6</sub> B <sub>0.03</sub> S <sub>17500</sub>	2.3 ± 0.4	55.0 ± 2.2
P <sub>0.3</sub> B <sub>0.03</sub> S <sub>17500</sub>	1.8 ± 0.4	67.0 ± 1.5
P <sub>0.45</sub> B <sub>0.04</sub> S <sub>18500</sub>	12.3 ± 0.3	66.3 ± 1.2
P <sub>0.6</sub> B <sub>0.03</sub> S <sub>19500</sub>	9.3 ± 0.1	44.0 ± 0.9
P <sub>0.3</sub> P <sub>0.03</sub> S <sub>17500</sub>	1.3 ± 0.0	71.6 ± 2.3
P <sub>0.3</sub> P <sub>0.06</sub> S <sub>21500</sub>	3.3 ± 0.3	87.2 ± 6.5
P <sub>0.6</sub> B <sub>0.06</sub> S <sub>21500</sub>	0.3 ± 0.0	85.3 ± 2.4
P <sub>0.3</sub> B <sub>0.04</sub> S <sub>21500</sub>	1.4 ± 0.4	86.2 ± 0.7
P <sub>0.6</sub> P <sub>0.03</sub> S <sub>21500</sub>	9.3 ± 0.0	78.0 ± 1.6
P <sub>0.3</sub> P <sub>0.03</sub> S <sub>19500</sub>	14.1 ± 0.2	71.5 ± 5.1
P <sub>0.45</sub> P <sub>0.04</sub> S <sub>19500</sub>	10.2 ± 0.0	69.4 ± 1.3
P <sub>0.3</sub> B <sub>0.04</sub> S <sub>21500</sub>	1.5 ± 0.1	84.1 ± 0.7
P <sub>0.3</sub> P <sub>0.06</sub> S <sub>21500</sub>	1.1 ± 0.2	73.4 ± 4.0

sample powder and then smoothed with a spatula. X-ray diffraction patterns of untreated drug, microcrystals of piroxicam, and physical mixtures of the drug and excipients were obtained over a range of 5–60 2θ by exposing the samples to CuKα radiation.

#### Fourier Transform Infrared (FTIR) Spectroscopy

Fourier transform infrared spectra were obtained by FTIR spectrophotometry (Rayleigh, WQF-S10, China) over a scanning range of 450–4000 cm<sup>-1</sup>. Samples were prepared by mixing with KBr powder and compressing into a disk by hydraulic pressure.

#### Thermal Analysis

Differential scanning calorimetric tests were performed to investigate potential incompatibilities between the drug and the stabilizer and to monitor the purity of piroxicam microcrystals. The DSC thermograms of the samples (3–6 mg) were recorded using a thermal

**Table 3. Particle Size and DE<sub>150</sub> of Piroxicam Microcrystals Prepared by pH-Shift Method with a D-Optimal Design**

Formulation Code	Particle size ± SD (µm)	DE <sub>150</sub> ± SD (%)
P <sub>0.6</sub> B <sub>0.06</sub> S <sub>21500</sub>	3.2 ± 0.1	63.4 ± 3.3
P <sub>0.6</sub> B <sub>0.03</sub> S <sub>21500</sub>	3.7 ± 0.3	48.5 ± 0.9
P <sub>0.3</sub> B <sub>0.06</sub> S <sub>19500</sub>	3.1 ± 0.2	52.0 ± 1.2
P <sub>0.6</sub> B <sub>0.03</sub> S <sub>21500</sub>	6.1 ± 0.2	57.2 ± 1.5
P <sub>0.3</sub> B <sub>0.03</sub> S <sub>17500</sub>	2.7 ± 0.2	74.1 ± 0.9
P <sub>0.6</sub> B <sub>0.04</sub> S <sub>19500</sub>	3.5 ± 0.0	73.3 ± 2.3
P <sub>0.6</sub> B <sub>0.03</sub> S <sub>17500</sub>	4.7 ± 0.6	71.4 ± 1.5
P <sub>0.6</sub> B <sub>0.06</sub> S <sub>17500</sub>	2.9 ± 0.6	68.1 ± 3.6
P <sub>0.3</sub> B <sub>0.04</sub> S <sub>21500</sub>	3.6 ± 0.4	68.2 ± 4.1
P <sub>0.45</sub> B <sub>0.04</sub> S <sub>17500</sub>	3.8 ± 0.1	59.5 ± 2.0
P <sub>0.3</sub> B <sub>0.06</sub> S <sub>21500</sub>	2.4 ± 0.1	73.3 ± 2.1
P <sub>0.3</sub> B <sub>0.03</sub> S <sub>21500</sub>	6.3 ± 0.3	63.2 ± 2.1
P <sub>0.45</sub> B <sub>0.03</sub> S <sub>19500</sub>	2.5 ± 0.3	74.0 ± 5.7
P <sub>0.3</sub> B <sub>0.06</sub> S <sub>17500</sub>	5.4 ± 0.2	78.3 ± 2.2
P <sub>0.6</sub> B <sub>0.03</sub> S <sub>17500</sub>	5.3 ± 0.3	70.0 ± 2.1
P <sub>0.45</sub> B <sub>0.05</sub> S <sub>20500</sub>	2.6 ± 0.0	74.4 ± 1.6
P <sub>0.3</sub> B <sub>0.03</sub> S <sub>17500</sub>	2.3 ± 0.2	73.2 ± 1.2
P <sub>0.6</sub> B <sub>0.06</sub> S <sub>17500</sub>	3.2 ± 0.5	68.2 ± 3.3

analysis system (Mettler, TA400, Germany) calibrated with indium standard. The samples were heated at a scanning rate of 10 °C/min in an aluminum pan under a nitrogen atmosphere. A similar empty pan was used as the reference.

#### Specific Surface Area

The specific surface areas of the untreated drug and microcrystals were determined using the gas adsorption technique. Calculations were carried out according to the Brunauer-Emmett-Teller (BET) equation (25).

#### Dissolution Studies

The dissolution rate of 10 mg of piroxicam crystals was determined using dissolution Apparatus 1 (basket) method (Erweka, Germany) at 37 ± 0.5 °C and stirring at 50 rpm. The dissolution medium was 900 mL of 0.1 N HCl. Samples were withdrawn from the dissolution vessels at selected time intervals (5, 10, 15, 20, 30, 45, 60, 90, 120, and 150 min), and piroxicam was quantified at 328.8 nm with a UV spectrophotometer (Shimadzu, Japan). Each sample was replaced with fresh dissolution medium. Dissolution efficiency up to 150 min (DE<sub>150</sub>) was calculated according to (26):

$$DE\% = \frac{\int_0^t y dt}{y_{100}t} \times 100$$

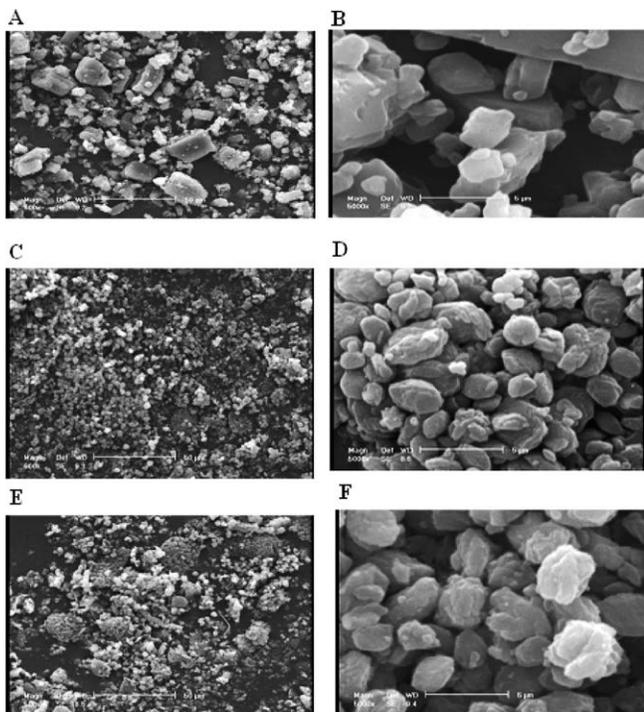


Figure 1. SEM micrographs of untreated piroxicam powder (A)  $\times 500$  and (B)  $\times 5000$ ; optimized microcrystals of piroxicam prepared by solvent-change method (C)  $\times 500$  and (D)  $\times 5000$ ; and optimized microcrystals of piroxicam prepared by pH-shift method (E)  $\times 500$  and (F)  $\times 5000$ .

## RESULTS AND DISCUSSION

### Microcrystallization of Piroxicam

Three water-miscible solvents (i.e., acetone, methanol, and ethanol) were evaluated as potential solvents of piroxicam for the solvent-change method. Acetone was selected for the microcrystallization process because it can solubilize larger quantities of the drug.

Microcrystallization produces new surfaces and an increased energy of the system. A protective stabilizer prevents aggregation by steric hindrance between the particles. Different stabilizers such as HPMC, Brij35, Tween 80, gelatin, chitosan, hydroxypropyl cellulose, poloxamer 188, PEG 6000, and polyvinyl pyrrolidone have been studied as the protective polymers (27). Among these, HPMC and Brij35 at 0.05% and 0.1% concentrations yielded smaller particles with less aggregation (27). If the precipitation process is carried out without a stabilizer, the crystal growth would be greatly accelerated. This could be prevented by the adsorption of a stabilizer to the newly formed hydrophobic surface of the microcrystals. Thus polymers with more hydrophobic groups such as HPMC and methyl hydroxyethyl cellulose have a greater ability to stabilize the smaller particles than polymers with more hydrophilic properties such as HPC (17). This is due to better interaction and more similarity between the hydrophobic surface of the stabilizer and the particles. Poloxamer has the hydrophobic group of polyoxypropylene, and

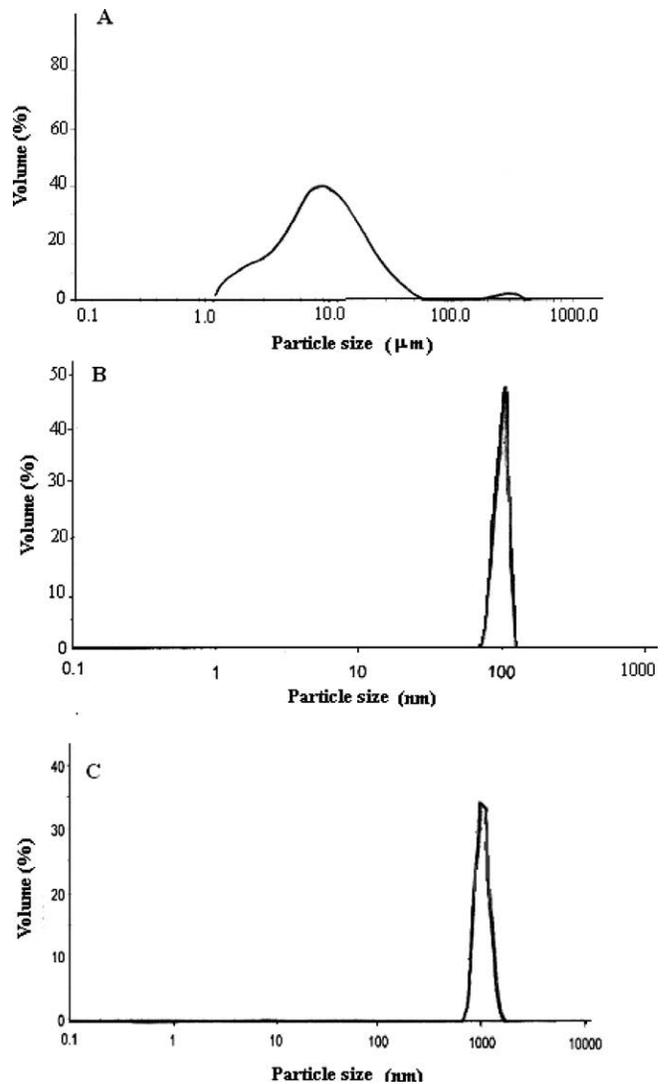


Figure 2. Particle size distribution of (A) untreated piroxicam powder, (B) optimized microcrystals of piroxicam prepared by solvent-change method, and (C) optimized microcrystals of piroxicam prepared by pH-shift method.

Brij35 is a nonionic surfactant with a hydrophile–lipophile balance (HLB) of 16.9 (28), a suitable combination for stabilization of the microcrystals. In the solvent-change method, Brij35 produced smaller particles than poloxamer, and accordingly, it was the only studied stabilizer in the pH-shift method (Tables 2 and 3).

### SEM Morphology

Micrographs of the untreated piroxicam powder and microcrystals of the optimum formulations of the two methods are shown in Figure 1. The untreated piroxicam particles are angular and cubic or rod-shaped, whereas the microcrystal particles have nearly spherical shapes and are obviously smaller. The nearly spherical microcrystals of piroxicam can result in better powder flow and fewer problems in producing solid dosage forms (27). In contrast, the pure piroxicam powder particles (Figure 1)

are angular, rod-shaped cubic, and polymorphic, causing difficulties in powder flow.

### Particle Size Distribution of Microcrystals

The size distribution of standard piroxicam powder and microcrystals of the optimum formulations of the two methods are shown in Figure 2. Tables 2 and 3 show the mean particle size of all formulations prepared by the two methods.

Untreated piroxicam powder had the largest particle size with a mean diameter of  $15.66 \pm 37.36$  nm. As shown in Table 2, the smallest mean particle size in the solvent-change method was obtained from the formulation  $P_{0.6}B_{0.06}S_{21500}$ . However, the smallest mean particle size in the pH-shift method was obtained from the formulation  $P_{0.3}B_{0.03}S_{17500}$ .

Based on a quadratic model fitted by Design Expert software to particle size data, the following equations were obtained to predict the average particle sizes in terms of different process variables for solvent change ( $Y_1$ ) and pH-shift ( $Y_2$ ) methods:

$$Y_1 = 10.21 + 0.2A - 2.59B + 0.055C + 1.66D + 8.50A^2 + 9.58B^2 - 2.40C^2 + 2.64AB - 1.50AC - 0.71AD - 3.54BC - 0.59BD + 0.81CD$$

$$Y_2 = 3.68 + 0.053A - 0.54B + 9.175E - 0.03C - 0.45AB - 0.19AC - 0.69BC$$

Positive values in these equations reflect an enhancement of the response by the experimental variable, while a negative value means the experimental variable decreased the response.

As shown in Figure 2, the size distribution of the pure standard piroxicam powder was broad, while the size distributions of the microcrystals of the optimized formulations were narrow and uniform. The same results were obtained in other studies for gliclazide (27), ibuprofen (29), itraconazole and ketoconazole (11), and indomethacin (16). The narrow and uniform size distribution of the particles is one of the important advantages of the microcrystallization process, in contrast with other particle size reduction methods such as milling (16). Statistical analysis of the particle size of microcrystals produced by the solvent-change method by a two-way ANOVA test shows that the drug and stabilizing agent concentrations and the type of stabilizer had a significant effect ( $p < 0.05$ ) on particle size, while the homogenization speed did not have a significant effect on particle size ( $p > 0.05$ ).

The statistical analysis by a two-way ANOVA shows that increasing the drug concentration from 0.03% to 0.45% or the stabilizer concentration from 0.04% to 0.06% had a lowering effect on particle size, while changing the Brij35 to poloxamer, increasing the concentration of the drug from 0.45% to 0.6%, or increasing the stabilizer concentration from 0.03% to 0.04% had an additive effect on the

particle size of the microcrystals prepared by the solvent-change method.

The two-way statistical analysis of particle size for microcrystals produced by the pH-shift method shows that drug concentration and homogenization speed had insignificant effects on particle size ( $p > 0.05$ ), while the stabilizing agent concentration had a significant effect on this variable ( $p < 0.05$ ). The analysis of variance shows that increasing the stabilizing agent concentration had a lowering effect on the particle size.

### Dissolution Studies

Dissolution profiles of untreated piroxicam and crystals obtained from the two microcrystallization methods were obtained in pH 1.2 medium (Figure 3). The  $DE_{150}$  was calculated from the dissolution data as an independent release model parameter (Tables 2 and 3). All treated samples had remarkably faster dissolution rates than the untreated drug. The highest  $DE_{150}$  value in the solvent-change method was from the formulation  $P_{0.3}P_{0.06}S_{21500}$ , while formulation  $P_{0.3}B_{0.06}S_{17500}$  had the highest value in the pH-shift method (Tables 2 and 3).

A linear model to predict the  $DE_{150}$  value ( $Y_3$  and  $Y_4$ ) of the microcrystals was suggested by the Design Expert software. According to this model, the final equations are as follows.

For the solvent-change method:

$$Y_3 = 66.89 - 5.55A + 1.10B + 9.80C + 0.41D$$

For the pH-shift method:

$$Y_4 = 67.17 - 2.32A + 0.52B - 3.74C$$

Dissolution rates of all formulations prepared by the solvent-change and pH-shift methods were faster than those of the untreated powder (Tables 2 and 3). Particle size, crystalline form, and the pH of the dissolution medium are considered the main determinant factors for dissolution rate (16). In all microcrystals of piroxicam, higher

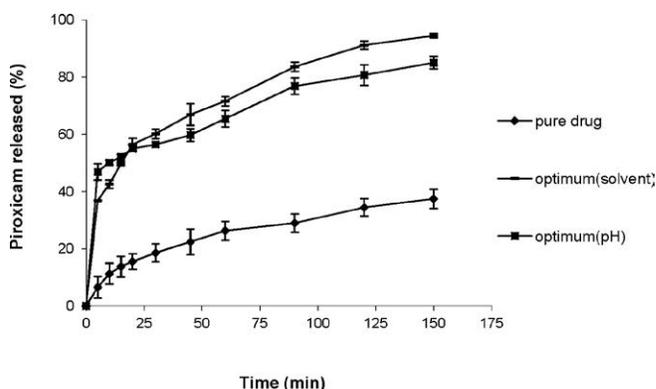


Figure 3. Drug dissolution profiles of untreated piroxicam powder compared with its optimized microcrystals prepared by solvent-change and pH-shift methods in pH 1.2 ( $n = 3$ ).

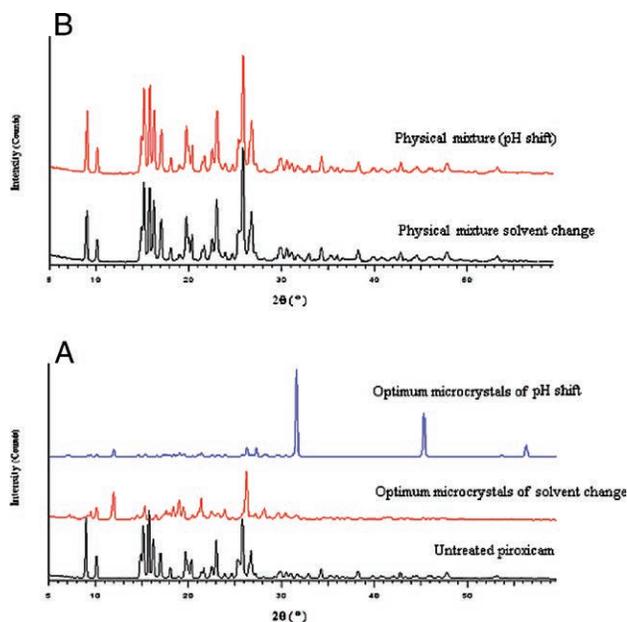


Figure 4. X-ray diffraction patterns of (A) untreated piroxicam powder and optimized microcrystals of piroxicam prepared by solvent-change and pH-shift methods and (B) the physical mixture of piroxicam with additives of each method.

dissolution rates appeared to result from reduced particle size (Tables 2 and 3) and a subsequently increased surface area, as shown in Table 4 (24).

In the solvent-change method,  $DE_{150}$  decreased with an increase in drug concentration ( $p < 0.05$ ) and increased with an increase in homogenization speed ( $p < 0.05$ ). However, the effects of the stabilizing agent concentration and the type of polymer were not statistically significant ( $p > 0.05$ ), as shown in Table 2.

The drug and stabilizing agent concentration, homogenization speed, and stabilizer type had a significant effect ( $p < 0.05$ ) on  $DE_{150}$  of gliclazide microcrystals prepared by the solvent-change method (30). A linear model was fitted to the effect of drug concentration and homogenization speed on  $DE_{150}$ . Accordingly,  $DE_{150}$  decreased linearly with increasing drug concentration at all homogenization speeds. Mixing the drug powder with polymers resulted in increased wettability and dissolution surface area and reduced interfacial tension between the dissolution medium and the hydrophobic drug surface (30).

## Optimized Formulations

Computer optimization of the results from response surface methodology by D-optimal design (Tables 2 and 3) will allow the estimation of a specific combination of variables ( $X_i$ ) that will optimize the individual responses ( $Y_i$ ) and will yield a product with desirable qualities. The variables involved in the optimization of all studied factors were particle size and  $DE_{150}$  of the microcrystals. The optimal values (i.e., 100% desirability) were predicted by Design Expert software for particle size ( $Y_1$ ,  $Y_2$ ) and  $DE_{150}$  ( $Y_3$ ,  $Y_4$ ) for the solvent-change and pH-shift methods, respectively. To achieve these levels, the predicted formulations of microcrystals were  $P_{0.6}P_{0.06}S_{21500}$  (for solvent change) and  $P_{0.3}B_{0.03}S_{17500}$  (for pH-shift method). The particle size and  $DE_{150}$  of the optimized microcrystals are shown in Table 4. Statistical analysis of the results by independent Student's *t*-test shows that the particle size obtained by the solvent-change method is significantly smaller than that obtained by the pH-shift method ( $p < 0.05$ ).  $DE_{150}$  is also significantly greater for the solvent-change method than for the pH-shift method ( $p < 0.05$ ). Consequently, the solvent-change method is preferred for the production of piroxicam microcrystals.

## X-ray Diffraction Studies

Diffraction spectra of untreated piroxicam powder, optimized microcrystal formulations from each preparation method, and their physical mixtures are shown in Figure 4. The XRD patterns of the physical mixtures are very similar to that of the standard crystalline powder. The major peaks are present in the diffractograms of the optimized formulations but have less intensity than those for the untreated crystalline drug. Figure 3 shows that the XRD patterns of the physical mixtures of the optimized formulations and the untreated drug powder are completely similar. Because peak height is influenced by crystal size and crystallinity, the reduction of the height of the peaks indicates reduction of the particle size and formation of the microcrystalline form of the drug (31). Gliclazide microcrystals also showed such a phenomenon in their XRD patterns (27).

NaCl crystals could be responsible for sharp peaks in  $2\theta = 30$  (Figure 4) from microcrystals produced by the pH-shift method, as HCl and NaOH were used to adjust the pH. Treated phenytoin powder showed more peaks in the XRD pattern than the untreated drug powder (32). This could be due to an alteration of the powder to a completely

Table 4. Results of Thermal Analysis and Surface Area of Optimized Piroxicam Microcrystals Prepared by Solvent-Change and pH-Shift Methods

Formulation Code	Particle size (nm)	$DE_{150}$ (%)	$T_m$ (°C)	$\Delta H$ (j/g)	Surface area (m <sup>2</sup> /g)	$V_m$ (mL/g)
Untreated piroxicam	15.6 ± 37.4	24.0 ± 4.4	199.4	685.5	1.98	0.0005
Optimized microcrystals of solvent-change method ( $P_{0.6}P_{0.06}S_{21500}$ )	2.17 ± 0.3	81.0 ± 5.6	197.4	630.89	2.10	0.0006
Optimized microcrystals of pH-shift method ( $P_{0.3}B_{0.03}S_{17500}$ )	3.75 ± 0.46	69.0 ± 2.9	190.5	902.58	4.39	0.001

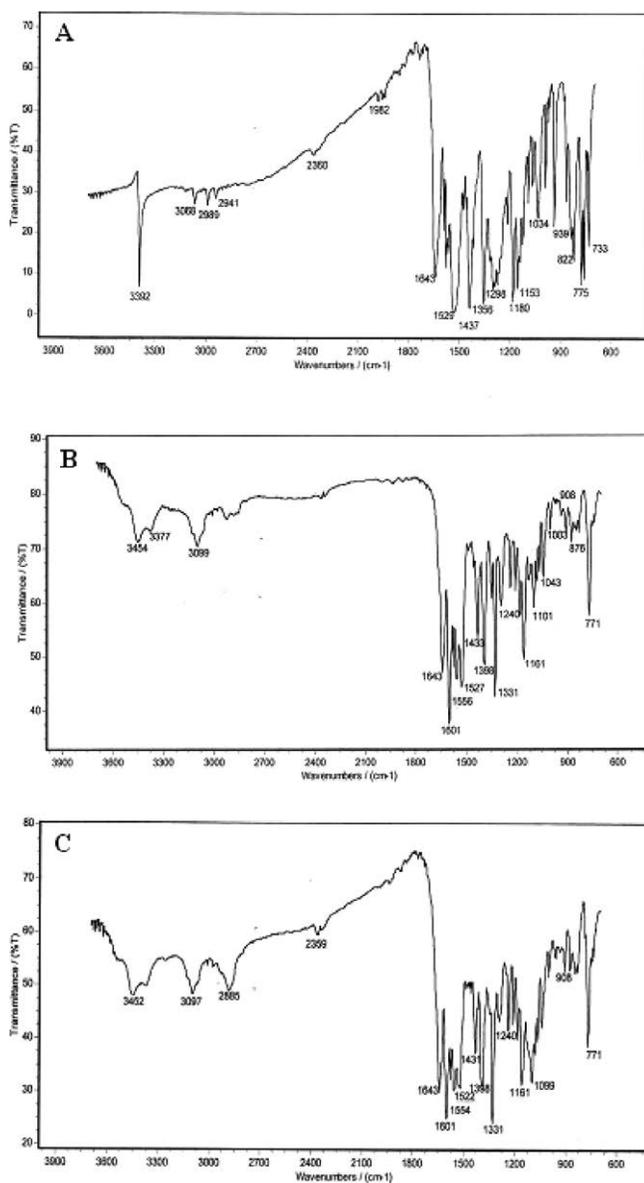


Figure 5. FTIR spectra of (A) untreated piroxicam powder, (B) optimized microcrystals of piroxicam prepared by solvent-change method, and (C) optimized microcrystals of piroxicam prepared by pH-shift method.

crystalline form or a difference in crystal orientation due to a change in crystalline habit. However, a lack of difference between the XRD patterns might suggest that there is no change in the drug crystallinity and no interaction between the drug and excipients during the procedure. This could also be understood by studying the DSC thermograms.

### FTIR Spectroscopy

FTIR spectroscopy was used to characterize possible changes in the chemical structure and drug-additive interactions in the untreated crystalline powder and the microcrystals. The spectra of all samples are similar (Figure 5). The principle absorption bands of piroxicam appear in

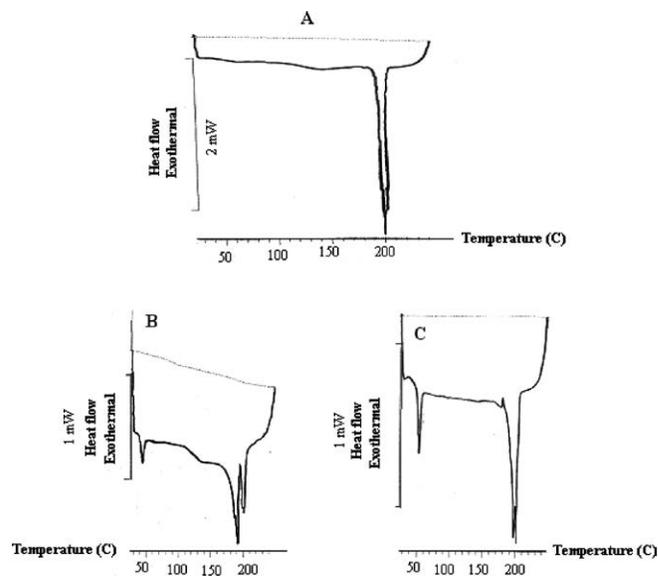


Figure 6. DSC thermograms of (A) untreated piroxicam powder, (B) optimized microcrystals of piroxicam prepared by solvent-change method, and (C) optimized microcrystals of piroxicam prepared by pH-shift method.

the regions of 3392, 1643, and 1356  $\text{cm}^{-1}$ , and these bands are related to the functional groups of OH, C=O, and S=O, respectively (Figure 5A).

These bands were slightly shifted to the regions of 3354, 1643, and 1331  $\text{cm}^{-1}$  in the FTIR spectra of microcrystals prepared by the solvent-change method (Figure 5B) and to the regions of 3452, 1643, and 1331  $\text{cm}^{-1}$  for the pH-shift method (Figure 5C). As there is no significant difference in the location of the bands, the internal structures were not different (27), and there was no incompatibility between the drug and stabilizers (33).

### DSC Studies

Figure 6 shows the DSC thermograms of untreated piroxicam and its optimum microcrystals. The final results for melting point and enthalpy are also listed in Table 4. The  $T_m$  values for the drug are similar in all experiments and consistent with the literature (34). The presence of similar endothermic peaks, which are associated with the melting points, suggests no interaction between the drug and excipients and no change in drug crystallinity (20). The endothermic peak at 200 °C relates to the melting point of the drug (Figure 6A). The  $T_m$  peak shows only a slight but insignificant change. If the main peaks were omitted, this could mean that the entire product was changed to the amorphous form (35). A slight reduction in  $T_m$  indicates that microcrystals are not completely crystalline (16). In addition, a change in the melting point may suggest the presence of polymers (17, 36, 37). Short peaks at the beginning of the heating procedure (Figure 6B,C) could be related to poloxamer and Brij melting points (28, 38, 39).

The third endothermic peak in Figure 6A might suggest the presence of small amounts of drug polymorphic form. This was also observed for the solid dispersion of carbamazepine (35).

Table 4 shows a reduction in enthalpy changes of optimized microcrystals from the solvent-change method relative to the untreated drug. The reduction in microcrystal particle size and the small amount of stabilizer might affect a reduction in enthalpy (27). Perhaps the precipitated drug is sterically stabilized against crystal growth by adsorbed polymers (Brij or Poloxamer), and the surface energy and consequently the enthalpy of the system is lowered. Accordingly, the molecularly dispersed drug is associated with particles in the required size range and simultaneously stabilized in the formed dispersion. Chow et al. (40) also have reported that the modification of the physical properties of phenytoin by recrystallization from methanol exhibited an increase in the specific surface area of the phenytoin crystals, a drastic reduction in crystallization yield, and a progressive change of the crystal habit from needles to elongated plates. Powder X-ray diffraction studies on the samples indicated essentially the same diffraction patterns and lattice spacing for both the untreated phenytoin powder and its recrystallized form, suggesting that the doped crystals did not undergo gross structural modification. However, the enthalpy of fusion,  $H_f$ , as determined by DSC, was reduced by as much as 17%, indicating a significant change in both the enthalpy and entropy of the phenytoin crystals.

Enthalpy changes of the optimized formulation of microcrystals from the pH-shift method were higher than those of the untreated drug (Table 4). The presence of dissolved impurities may affect the rate of crystallization and even change the crystal habit, provided that these impurities are surface active and become adsorbed onto the nuclei or onto growing crystals (41). The microcrystals from the pH-shift method had a higher enthalpy than the untreated drug, which might result from the presence of impurities of the NaCl produced.

### Specific Surface Area

According to the BET equation (25), the specific surface area and the monolayer adsorbed gas volume were calculated for the untreated piroxicam powder and the two optimized formulations of each preparation method (Table 4). The results show an increase in the surface area of the microcrystals in comparison with the untreated drug powder that is consistent with the increase in the specific surface area and the monolayer adsorbed gas volume (Table 4).

### CONCLUSION

The results of the present study show that microcrystallization has an effect on piroxicam crystal habit modification. Both the pH-shift and the solvent-change methods using an ultra-homogenizer and stabilizers produced

microcrystals with a higher dissolution rate than those prepared with pure piroxicam powder. The highest dissolution rate and the lowest particle size were observed using the solvent-change method.

### ACKNOWLEDGMENTS

The authors wish to thank the Vice Chancellor of Isfahan University of Medical Sciences that supported this work by project No. 386253.

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