A Study on Some Factors Affecting the Preparation of Piroxicam Nanosuspension Using Hydroxypropyl Methyl Cellulose (HPMC E5)

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ABSTRACT

Background: Piroxicam is nonsteroidal anti-inflammatory drug used for the treatment of inflammatory conditions such as arthritis. Piroxicam belongs to Biopharmaceutical Classification System (BCS) class II, by its low solubility and high permeability through the biological membrane. Objectives: This study aimed to improve the water solubility of piroxicam by formulating it as nanosuspension using hydroxy propyl methyl cellulose (HPMC E5) as a main stabilizer by the mean of solvent-antisolvent precipitation method. Methods: Different factors were studied and evaluated in an attempt to obtain a monodispersed nanosuspension formula with lower particle size and optimum stability. Seventeen formulas were prepared and characterized for particle size, polydispersity index and drug entrapment efficiency percentage. Results: The results showed that the best formula (F13) has a particle size of (197) nm due to Dynamic Light Scattering Technique measurements. F13 stabilized by the synergistic effect of hydroxy propyl methyl cellulose (HPMC E5) and sodium lauryl sulphate (SLS). Conclusion: The poorly water-soluble piroxicam was successfully formulated as nanosuspension preparation by using an aqueous vehicle, that improved the drug water solubility.

Keywords: Nanosuspension, Piroxicam, Hydroxypropyl methyl cellulose.

INTRODUCTION

Nanosuspension drug delivery system (NS) represents the optimum choice for clinical treatment. Nanosuspensions are colloidal dispersions of nanosized drug particles stabilized by surfactants and polymers, in which the diameter of the suspended particles is <1 micron. Nanosuspension is the unique technique for a drug molecule with some properties like, a large molecular weight and dose, high log P and melting point and also for drugs that have low solubility in both of aqueous and lipid media.

The benefits of nanosuspension is that it is a simple technique and scale up, that can be used to formulate most drugs by handling a large dose in a small volume, can be administered by many routes as an oral, ocular, parenteral and transdermal route with low incidence of side effects and better tolerance by the patients. On the other hand a stability problems at storage due to an elevation of the settling rate of the dispersed nanoparticles. This may arise as a major problem with nanosuspension, although this problem can be avoided by the use of suitable polymers. Hydroxy propyl methyl cellulose (HPMC), is the propylene glycol ether of methylcellulose. It is a nonionic, water-soluble stabilizer, present in various grades such as E5 and E15, that vary in viscosity and degree of substitution. Various grades of HPMC are branded by an attached number.

Piroxicam (PRX) is a non-steroidal anti-inflammatory drug, used in arthritis, gout and other inflammatory joint diseases. It showed polymorphism with melting point is 198°C to 201°C.

Piroxicam belongs to class II with low solubility and high permeability based on the BCS parameters. Arthritis means inflammation of the joints, tissues surrounding it and other connective tissues.

MATERIALS AND METHODS

Piroxicam powder was purchased from Al Safa Pharmaceutical Industries (SPI), HPMC E5 (Qingdao Sinocmc Chemicals), poly vinyl alcohol (PVA), sodium lauryl sulphate (SLS), sodium lauryl ether sulphate (SLES) and tween 80 from Alpha Chemika.

Determination of piroxicam melting point

The melting point of piroxicam was measured by DSC. Pure PRX powder wrapped in a flat-bottomed aluminum pan of the differential scanning calorimeter (Shimadzu DSC-60 plus, Japan). Data collection was attained at a heating rate of 10°C/min under nitrogen gas at a flow rate of 40 ml/min.

Determination \( \lambda_{\text{max}} \) and Calibration curves of piroxicam: The stock solutions of piroxicam were prepared by dissolving 10 mg of piroxicam in 100 mL deionized water. Serial dilutions were constructed from the stock solution of concentration 100 \( \mu \)g/ml. Spectrophotometric analysis of samples was done at the wavelength of maximum absorbance of piroxicam. The measured absorbances were recorded and plotted versus the respective concentrations.

Preparation of piroxicam nanosuspension (NS): The nanosuspension formulas of piroxicam were formulated by solvent anti-solvent precipitation method. The organic phase was formed by dissolving 10 mg of piroxicam powder in (1 ml) of acetone at room temperature with sonication for 5 minutes. The stabilizers were dissolved in (10 ml) of deionized water, also at room temperature with continuous stirring using a magnetic stirrer. The organic phase dispensed into the aqueous phase in a slow rate (1 ml/min), by a plastic syringe positioned directly into the aqueous phase with continuous stirring 2 min sufficient time to evaporate the organic solvent and to precipitate the drug with stabilizers. The composition of NS formulas are shown in Table 1.
Table 1: The composition of nanosuspension formulas

<table>
<thead>
<tr>
<th>F NO.</th>
<th>Piroxicam (mg)</th>
<th>HPMC E5 (mg)</th>
<th>CMC (mg)</th>
<th>Dextrin (mg)</th>
<th>PVA (mg)</th>
<th>SLS (mg)</th>
<th>SLES (mg)</th>
<th>Poloxamer 188 (mg)</th>
<th>Poloxamer 407 (mg)</th>
<th>Tween 80 (%)</th>
<th>Stirring Rate (rpm)</th>
<th>Stirring Time (min)</th>
<th>Temperature (°C)</th>
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<td>500</td>
<td>60</td>
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<td>1</td>
<td>500</td>
<td>60</td>
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Evaluation of the prepared nanosuspension formulas

**Determination of drug entrapment efficiency (EE%)**

The measurement of the EE% was performed by centrifugation of nanosuspension formula (10 ml) 20 minutes at 3000 rpm. Then, 1ml of the supernatant solution was taken and filtered by a filter syringe (0.45 µm), diluted with deionized water up to 10 ml and analyzed by spectrophotometer to determine the amount of the free drug. EE% was calculated by the Equation 1

\[
\text{DEE} = \frac{(\text{Total drug in formula} - \text{Free drug})}{\text{Total drug in formula}} \times 100 \quad \text{...Equation 1}
\]

The procedure was repeated in triplicate and the standard deviations were recorded (15).

**Determination of particle size and polydispersity index (PDI)**

The mean particle size and PDI of nanosuspension formulas was measured by dynamic light scattering technique (DLS), which detects the light scattering difference at scattering angle 90° at room temperature (16).

**Factors affecting particle size and polydispersity index**

**Effect of drug: polymer Ratio**

Formulations were used to study the impact of the drug to stabilizer ratio at two different stirring rates (500 and 300) rpm at room temperature. F1 to F5 utilized (HPMC) in ratios (1:1, 1:2 and 1:3).

**Effect of stirring speed**

Nanosuspension formulas, from F1 to F6 were prepared at different stirring speeds (500, 300 and 700) rpm at room temperature. The impact of stirring rate was investigated using formulas (F1, F4, F6).

**Effect of Stirring time**

Nanosuspension formulas were prepared at different stirring times. (F1 to F6) were formulated for 60 minutes, F7 in 90 min and F8 formulation was performed at 30 min.

In order to determine the optimum time required to formulate a good formula, that is sufficient for the volatile solvent to evaporate, a comparison study was performed between F1, F7 and F8.
Effect of temperature
All formulas were prepared at room temperature except F9 was formulated at 70 °C in order to estimate the temperature effect on particle size and PDI.

Effect of different surfactants
Various surfactants were used in our study to evaluate their influence on the properties of the obtained nanoparticles. Surfactants used in F11 to F19 includes Dextrin, PVA, SLS, SLES, Poloxamer 188, Poloxamer 407 and Tween 80. All were used in combination with stabilizers.

Ethical Approval
The study was approved by the Ethics Board of University of Baghdad.

Statistical analysis
The recorded data were examined using statistical software for social sciences, version 20.0. (SPSS Inc., Chicago, Illinois, USA). The Student t test was used to express and compare the mean and Standard Deviation (SD) of quantitative data. To compare qualitative data that was presented as frequency and percentage, Chi2 tests were conducted. As the level of confidence was maintained at 95%, a P value of 0.05 was considered significant.

RESULTS
Differential scanning calorimetric measurement
The result of DSC analysis of melting point was 203.85 °C, as shown in Figure 1, that fitted with the references and indicated the purity of the drug (17).

![Figure 1: DSC measurement of piroxicam melting point.](https://ejhm.journals.ekb.eg/)
The $\lambda_{\text{max}}$ of piroxicam

The obtained values of $\lambda_{\text{max}}$ measurement in deionized water was 361 nm as seen in Figure 2. In calibration curves of piroxicam in, the absorbance values were plotted versus concentrations and a straight lines were obtained which suggests that the calibration curve follow Beer's Lambert law as shown in Figure 3 (18).

![Figure 2: The $\lambda_{\text{max}}$ of piroxicam in deionized water.](image1)

![Figure 3: Calibration curve of piroxicam in deionized water](image2)
Results of characterization of piroxicam nanosuspension formulas

**Table 2: The characteristics of NS formulas**

<table>
<thead>
<tr>
<th>Formula NO.</th>
<th>Particle size (nm)</th>
<th>Polydispersity index</th>
<th>Entrapment Efficiency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>244.2</td>
<td>0.1863</td>
<td>97.36 ± 4.09</td>
</tr>
<tr>
<td>F2</td>
<td>320.9</td>
<td>0.2587</td>
<td>94.34 ± 2.31</td>
</tr>
<tr>
<td>F3</td>
<td>413.4</td>
<td>0.2066</td>
<td>93.6 ± 4.43</td>
</tr>
<tr>
<td>F4</td>
<td>252.5</td>
<td>0.2617</td>
<td>93.33 ± 3.31</td>
</tr>
<tr>
<td>F5</td>
<td>318.8</td>
<td>0.3309</td>
<td>93.33 ± 1.29</td>
</tr>
<tr>
<td>F6</td>
<td>262.4</td>
<td>0.3418</td>
<td>96.12 ± 2.67</td>
</tr>
<tr>
<td>F7</td>
<td>378.9</td>
<td>0.4232</td>
<td>92.9 ± 5.21</td>
</tr>
<tr>
<td>F8</td>
<td>279.4</td>
<td>0.4548</td>
<td>93.22 ± 4.68</td>
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<td>F9</td>
<td>280.3</td>
<td>0.368</td>
<td>92.02 ± 2.57</td>
</tr>
<tr>
<td>F10</td>
<td>410.2</td>
<td>0.4247</td>
<td>91.21 ± 3.98</td>
</tr>
<tr>
<td>F11</td>
<td>3873</td>
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<td>48.05 ± 5.76</td>
</tr>
<tr>
<td>F12</td>
<td>237.7</td>
<td>0.2558</td>
<td>99.69 ± 5.21</td>
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<td>F13</td>
<td>197</td>
<td>0.2945</td>
<td>99.78 ± 1.13</td>
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<tr>
<td>F14</td>
<td>954.6</td>
<td>0.6985</td>
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</tr>
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<td>F15</td>
<td>327.9</td>
<td>0.5129</td>
<td>97.03 ± 2.56</td>
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<tr>
<td>F16</td>
<td>629.4</td>
<td>0.7705</td>
<td>96.34 ± 2.66</td>
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<tr>
<td>F17</td>
<td>343.6</td>
<td>0.5747</td>
<td>96.98 ± 2.98</td>
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</table>
Figure 4: DLS measurements of F13
DISCUSSION

The mean particle size distribution results range was between 197 nm to 3873 nm according to dynamic light scattering technique. Concurrently polydispersity index was measured by the same technique. Polydispersity index is an indicator of the particle size distribution width. The values of less than 0.05 is an indicator of a highly monodispersed system and more than 0.7 is of polydispersed system (19). The obtained results of PDI were between (0.1863 -1).

Effect of drug: polymer ratio

Different ratios were studied to evaluate the effect of this factor, (1:1, 1:2 and 1:3) in formulas (F1to F5), to F23). These different ratios were investigated at different stirring rates (300 and 500) rpm at room temperature. At the stirring rate of (700 rpm), NS formula was prepared at a drug to polymer ratio of 1:1.

As the ratio was increased, the particle size was significantly (p<0.05) increased.

The results showed that the ratio of 1:1 was optimum for obtaining a lowest mean particle size. This result was true for (F1and F4). Mean particle size in F1, F4, were 244.2 nm, 252.5nm, respectively. In this ratio the amount of stabilizers used was adequate to coat PRX particles and to maintain their stability at low size.

An increase in the amount of a stabilizer related to drug resulted in a higher viscosity of antisolvent solution that might preclude particle movement and cause more coating of drug particles (20).

Polydispersity index values in (F1 to F5) showed a homogenous dispersion that characterized as monodispersed systems.

Effect of stirring speed

The effect of stirring speed was investigated at three stirring speeds, 500 rpm in F1, 300 rpm in F4, and 700 rpm in F6, at room temperature.

At the stirring rate, 500 rpm the lowest mean particle size was obtained, 244.2 nm in F1. The particle size reduction at this rate might be as a result of high mixing efficiency improved the rate of diffusion of the organic phase into the aqueous phase that brought high homogeneous dispersion in brief time, thus fast nucleation obstructed the accumulation of particles to obtain smaller drug particles (21).

An increase in a mean particle size was observed in F4 when the stirring rate was reduced to 300 rpm. The mean particle size value was 252.5 nm.

Besides, a higher stirring rate (700 rpm) caused a larger uniform drug particles, 262.4 nm in F6.

Nanosuspension formulas were non-significantly (p>0.05) affected by the change in the stirring rate (22).

Effect of stirring time

The stirring time has an important effect in nanosuspension formulation. Formulas, F1, F7 and F8 were formulated under a stirring time of 60 minutes, 30 minutes and 90 minutes respectively.

The best particle size reduction and homogenous mixing was found in F1, 242.2 nm (at 60 min), that represents an optimum time required for PRX particles nucleation. An increase in the stirring time to a certain limit in F8 led to a significant (p<0.05) increase in the particle (23). Also, a decrease in the stirring time to 30 min in F7 caused a significant rise in particle size (p<0.05). PDI was still within a monodispersed type in all formulas.

Effect of Temperature

Temperature is the interesting factor in governing the particle size. In which F9 was prepared at 70°C compared to F1 that was formulated at room temperature. As the temperature was increased, the particle size was also increased significantly (p<0.05) from 242.2 nm in F1 to 280.3 nm in F9.

Increased particle size with temperature was due to fact that as temperature was increased the viscosity of the aqueous phase was decreased that facilitated the diffusion of the organic phase into the aqueous phase, this resulted in, the increase of the equilibrium solubility of PRX in deionized water (the aqueous phase), as mentioned solubility study, and thus the degree of supersaturation during the nanoprecipitation process decreased, as a result, the nucleation rate was decreased (24).

As temperature was increased up to 70 °C, PDI values was remained as monodispersed systems.

Effect of different surfactants

The effect of different surfactants as co-stabilizers was studied to evaluate the synergistic effect of different mechanisms. The resulted particle size measurements were between 197 nm to 3873 nm. These results were compared with F1.

Sodium lauryl sulphate (SLS) revealed a prominent significant effect (p<0.05) in particle size reduction as shown in F13, in which the mean particle size resulted values were 197 nm. SLS is an anionic surfactant which provided an electrostatic stabilization. It is desired to give retractive forces and maintenance of particle stabilization (25).

Conversely, the combination that provided a steric mechanism of tween 80 with HPMC E5 in F17 was significantly increased the particle size (p<0.05) up to 343.6 nm.

The combination of stabilizers is preferred for particle size reduction and long-term stabilization. The differences in particle size were due to different in affinity of the polymer molecules toward drug particles.

CONCLUSION

Nanosuspension is an interesting technique for the improvement of drugs solubility of poorly water-soluble drugs. Melting point is an indicator of the purity of the
drug. Piroxicam pure powder was successfully prepared as nanosuspension by solvent antisolvent precipitation method. Various factors were considered to evaluate their effect on particle size, poly dispersion index and entrapment efficiency percentage. The stirring rate, stirring time and temperature were appeared to have a non-significant effect on particle size, unlike surfactants that were affected the particle size significantly. PDI values were within a homogenous range except F11 appeared as polydispersed system. EE% was high in all formulas except F11 containing dextrin as surfactant.

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REFERENCES