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# PREPARATION AND CHARACTERIZATION OF KETOPROFEN NANOSUSPENSION FOR SOLUBILITY AND DISSOLUTION VELOCITY ENHANCEMENT

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## ABSTRACT

Nanosuspension technology has been developed as a promising candidate for efficient delivery of hydrophobic drugs. It could maintain the required crystalline state of the drug with reduced particle size, leading to an increased reporting on dissolution rate and therefore improved bioavailability. In this paper, we report on the preparation of ketoprofen (KTF) nanosuspension by high-pressure homogenization (HPH). The aim is to obtain a stable nanosuspension with an increased drug saturation solubility and dissolution velocity. The morphology and particle size distribution of the modified nanosuspensions were characterized by the means of several analyses that included: photon correlation spectroscopy (PCS), polarized light microscopy (PLM), scanning electron microscopy, differential scanning calorimetry (DSC), and powder X-ray diffractometry (XRD). The obtained results revealed that HPH can be employed to produce aqueous drug nanosuspensions with fine solubility and dissolution properties, which render the produced particles stable up to one month. In addition, the prepared nanosuspensions possessed a high drug-loading efficiency (10%). The recoded zeta potential values (≈ - 27 mV) indicated that the prepared nanosuspensions possess a higher degree of long-term stability. PCS data showed narrow size distribution with average size 322.7 nm. Morphologically, as indicated from results, the produced nanosuspensions have a homogenous distribution even after redispersion, indicating the stability of the product.

**KEY WORDS: Nanosuspension- high-pressure homogenization – Photon correlation spectroscopy – ketoprofen**

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# INTRODUCTION

Poorly water-soluble drugs are especially challenging, as they cannot achieve dissolution and , therefore, they have a very difficult pass through the dissolving fluid to contact the absorbing mucosa and to be absorbed. If the dissolution rate of the drug molecule is slow, due to the physicochemical properties of the drug molecules or formulation factors, the dissolution process will be the rate-limiting step in drug absorption and consequent bioavailability[1,2]. Ketoprofen, as a nonsteroidal antiinflammatory drug, has a very poor water solubility characteristic, which is the reason for its poor bioavailability. For such kind of drugs, micronization[1-4], nanonization[5-7], inclusion complexation (e.g. cyclodextrins)[8-13], preparation of liposomes[14-16] and amorphous solid dispersions[17-21] have been proposed to increase the rate of dissolution; especially the drug stability and bioavailability after oral administration for systemic drug absorption. An alternative and promising approach is the production of drug nanosuspensions, which has been utilized to improve the solubility and bioavailability. The major advantages of this technology are: its simplicity and general applicability to the majority of the insoluble drugs. Nanosuspensions are defined as colloidal dispersions of nano-sized drug particles that are produced by a suitable method and stabilized by a suitable stabilizer. It consists of the poorly water-soluble drug, without any additional materials, suspended in dispersion and stabilized by surfactants[22, 23]. There is a number of different dispersion media that can be used such as pure distilled water, aqueous solutions and nonaqueous media. Via nanosuspension formulation, the problems associated with the delivery of either poorly water-soluble or lipid-soluble drugs can be solved. Nanosuspensions differ from nanoparticles[24], which are polymeric colloidal drug carriers (Nanospheres and nanocapsules), as well as it differs from solid-lipid nanoparticles[25] (SLN), which are lipidic drug carriers. Preparing nanosuspensions are preferred for the compounds which are non-water insoluble but are oil-soluble with high log P value. The term drugs nanocrystals implies crystalline state of the discrete particles. In this study, a production of nanosuspensions on a laboratory scale is presented. Special features such as increased saturation solubility and dissolution velocity are discussed. In addition, physicochemical feature of drug nanocrystals was characterized by different analysis including polarized light scattering spectroscopy (PLS), photon correlation spectroscopy (PCS), differential scanning calorimetry (DSC) and X-ray diffractometer

# EXPERIMENTAL

### *Materials*

All chemicals were used as received from the suppliers, Ketoprofen was brought from Drifen Pharm. Co.(Turkey), Purified soy phosphatidylcholine (Phospholipon® 80) was obtained from Phospholipon GmbH (Cologne, Germany), Poloxamer 188(Lutrol® F68), Sodium dodecyl sulfate (SDS) and PVP K-30 were purchased from BASF AG (Ludwigshafen, Germany), Mannitol, sodium bicarbonate, citric acid, tartaric acid, fumaric acid and other excipients were of analytical grade and were purchased from Merck KGaA (Darmstadt, Germany), and Purified water was obtained using Milli-Q water purification system from Millipore (schalbach, Germany).

### *Equipment*

A Micron LAB 40 homogenizer (APV Deutschland GmbH, Unna, Germany), Ultra-Turrax T25 (Janke & Kunkel GmbH) (Staufen, Germany), Zetasizer 3000 HAS (Malvern, Instruments GmbH, Germany). Orthophlan microscope (Leitz, Wetzlar, Germany), Shaking water bath (InnovaTM 4230, New Brunswick Scientific Edision, NJ-USA), Mettler DSC 822e1200 (Mettler Toledo, Germany), X-ray diffractometer (STOE Stadi P, STOE & Cie GmbH, D-64213 Darmstadt, Germany), USP

XXIV rotating paddle apparatus with a Pharma test PTW SIII (Pharma Test, Hamburg, Germany), Filters (Sartorius AG, Goettingen Germany), Glass syringe (Poulten & Graf GmbH, Germany) and Thermostated sonicator (Bandelin RK 514, Berlin, Germany).

### *Metodology*

### *(i) Preparation of nanosuspensions*

There are different ketoprofen nanosuspension formulations that have been prepared using high pressure homoginization technique (HPH)[26]. A screening formulation was carried out to identify the most suitable formulation for further processing. As a typical procedure, the drug (ketoprofen) was dispersed in distilled water containing stabilizers (illustrated in table 1). The homogenizer was operated at pressure varying from 100 – 2000 bars with volume capacity of 40 ml. To avoid the blocking of homogenization gap during the preparation, we started with micronized drug particle size less than 250 µm. Hence, it is essential to prepare a presuspension of the micronized drug in a surfactant solution by stirring using high speed stirrer, an Ultra-Turrax T25 which operated at 8000 rpm for 10s. The suspension was processed using 2 homogenization cycles of 150 bars and 2 homogenization cycles of 500 bars as pre-milling. The final nanosuspensions were produced by applying 20 homogenization cycles at 1500 bar. Dispersed drugs in the form of microparticles passed through a continuous Micron Lab 40 at room temperature applying the variable pressure profiles, as described above. Every fifth homogenization cycle at 1500 bars, samples were withdrawn for the sake of particle size analysis.

**Table 1**
***Composition of ketoprofen Nanosuspensions***

| Formulat-ion | Ketoprofen (% w/w) | Phosphol-ipon 80 (% w/w) | Poloxamer 188 (% w/w) | SDS (% w/w) |
|---|---|---|---|---|
| A | 10% | 0.2% | ---- | ---- |
| B | 10% | 0.1% | 0.1% | ---- |
| C | 10% | ---- | ---- | 0.2% |
| D | 10% | 0.1% | 0.1% | ---- |
| E | 10% | 0.1% | ---- | 0.1% |

### *(ii) Water Removal*

The freeze-dryer could produce high percentages of the final dried ketoprofen. In brief, nanosuspension formula which consists of 10% (w/v) ketoprofen of and 1% (w/v) Mannitol as cryoprotectant was frozen at - 80 $^{o}$C. Afterwards, the frozen nanosuspension was directly placed in the freeze dryer chamber and allowed to be lyophilized over 48 hours at *-20 $^{o}$C* and 0.03 mbar pressure.

### *Characterization of drug nanosuspensions*

### *(iii) Size distributions and the zeta potentials*

The size distribution and the zeta potentials of nanoparticles were analyzed by photon correlation spectroscopy (PCS) using a Zetasizer 3000 HAS . The samples were placed into disposable plastic cuvette, and all measurements were carried out at 25 ºC.

### *(iv) Polarized light scattering microscopy (PLSM)*

The degree of crystallinity of the prepared nanocrystals was investigated by light microscopy which performed using an Orthophlan microscope. The employed magnification was 1000 fold (oil immersion) and each sample was investigated three times, since the degree of crystallinity was examined using polarized light.

### *(v) Solubility measurement*

Solubility studies were performed using a shaking water bath. Typically, in 40 ml capped

dark vials, an excess drug was dispersed in 20 ml distilled water and sonicated using a thermostated sonicator for 2 min. Vials were sealed to avoid changes in temperatures which may be caused due to water evaporation. The mixtures were shaken for 1 week at 25 ± 0.01$^{o}$C. After reaching the equilibrium, suspensions were filtered through Sartorius® 0.2 µm filters . An aliquot from each vial was withdrawn and the drug concentration was analyzed using UV-spectroscopy at a maximum wavelength of 260 nm. The taken sample volume was not refreshed by new solvent. Dilution was intentionally avoided to prevent any possible interference with the chemical equilibrium, particularly considering the presence of colloidal particles. All values were calculated from at least three independent experimental runs and the results are given as mean ± standard deviation.

***(vi) Differential Scanning Calorimetry (DSC)***

DSC analysis was performed using a Mettler DSC 822e1200. The instrument was calibrated with indium as calibration standard for melting point and heat of fusion. Analysis was performed under a nitrogen purge (20 ml/min) and standard aluminum sample pans of 40 µl were used. In such pans, 2 mg samples were placed for analysis and a heating rate of 5$^{o}$C/min was employed. An empty pan was used as a reference.

***(vii) Powder X-ray diffractometry (PXRD)***

Powder X-ray diffraction patterns of the dried suspension, physical mixture and the individual components were measured by a powder X-ray diffractometer under the following conditions: nickel-filtered Cu-Kα radiation (λ = 0.7107 Å); Voltage, 40KV; current, 40mA; scanning speed, 0.6 per minute and scan range of $2\theta$ = 2 - 40$^{o}$.

***(viii) In vitro dissolution behavior***

The dissolution test was performed using a USP XXIV rotating paddle apparatus with a Pharma test PTW SIII at 37 °C and a rotating speed of 100 rpm in 900 ml of water. Certain amounts of drugs were dispersed in the dissolution medium. At interval times, two ml samples were withdrawn from the dissolution chamber. The samples were filtered through Sartorius® 0.2 µm filters. An aliquot from each vial was withdrawn with a 1ml glass syringe and assayed by UV spectroscopy at λmax=260 nm to evaluate the drug concentration.

***(ix) Physical stability study***

Physical stability of the obtained ketoprofen nanosuspension was investigated at 25 ºC. The changes in appearance, particle size and polydispersity index were recorded over the period of 30 days. Moreover, settling behavior of samples was monitored by light microscope.

# RESULTS AND DISCUSSION

***1. Screening of different modified Formulations***

Five different formulations (A-E) of ketoprofen nanosuspensions have been prepared using three different surfactants (phospholipon 80, poloxamer 188 and sodium dodecyl sulfate). The composition of each medicated nanosuspension formula was listed in table 1. The prepared nanosuspension was analyzed by PCS. The results indicated that all formulations have different volume size distribution. A narrow size distribution is essential to prevent particle growth, due to Ostwald ripening phenomenon that is caused by different saturation solubility in the vicinity of differently sized particles. Amongst all the prepared formulae, only formula (C) revealed a nanosuspension with a mean particle size of 322.7 nm. While, formulae (A) and (B) could not generate a nanosuspension. Phospholipon 80, used as stabilizer in formula A, could not properly disperse KTF particles in the aqueous solution. Therefore, it could not be used as single stabilizer for the nanosuspension. The use of phospholipon combined with other stabilizers (for example, poloxamer 188 or SDS) may disperse drug particle into aqueous solution. Combining poloxamer 188 with SDS as stabilizer was not appropriate for ketoprofen nanosuspensions, due to the combination resulted in particles aggregation (as clearly observed by light

microscopy) during production. Therefore, both of single phospholipon 80 (formula A) and the combination SDS and poloxamer 188 (formula B) are not suitable to function as stabilizers for the ketoprofen nanosuspensions. Based on the previous observations, 10% of ketoprofen nanosuspension stabilized by 0.2% SDS could be chosen as the best formula for producing a highly concentrated ibuprofen nanosuspension.

### *2. Process Parameters—Cycle Numbers and Pressure*

The suspension passes the very small homogenization gap with a very high velocity. Due to the narrowness of the gap, the streaming velocity of the suspension increases tremendously, which makes the dynamic fluid pressure increase. Simultaneously, the static pressure on the fluid decreases below the boiling point of water at room temperature. Consequently, water starts boiling at room temperature due to the high pressure, gas bubbles are formed which implodes when the fluid leaves the homogenization gap. These cavitation forces are strong enough to break the drug microparticles to drug nanoparticles [28]. The power density ($w/m^3$) is the factor which determined achievable dispersions, i.e., the fineness of the drug nanocrystals. The power density Pv is defined as the energy W dissipated in the homogenization volume V related to the time t: $Pv = W/tV$ [29]. Based on this equation, the factors determining Pv are: the homogenization pressure and the width of the homogenization gap. Thus, larger pressure and longer time (more cycles) can lead to smaller size. However, the applied pressure should be increased step by step from lower pressure to the maximal one to avoid block of the gap, i.e., 2 cycles were performed at100 bar, 5 cycles at 500 bar, and finally 15 cycles at 1500 bar. In order to obtain a narrow size distribution, it is necessary to run several processes through the homogenizer (homogenization cycles). A typical number of homogenization cycles reported for nanosuspension are between 10 and 20, depending on the hardness of the drug to be processed. Nanosuspensions are characterized in similar ways as those used for conventional suspensions such as appearance, color, odor, assay, related impurities, etc. Apart from the aforementioned parameters, the nanosuspensions should be evaluated for their particle size, zeta potential, crystalline status, dissolution studies and *in-vitro* studies.

### *3. Zeta Potential*

Measurement of the zeta potential allows predictions about the storage stability of colloidal dispersion[30]. In general, particle aggregation is less likely to occur if particles possess enough zeta potential to provide sufficient electric repulsion or enough steric barriers to provide sufficient steric repulsion between each other. According to the literature, a zeta potential of at least –30 mV for electrostatically and –20 mV for sterically stabilized systems is desired to obtain a physically stable nanosuspension[31]. The zeta potential of formulation C was - 26.74 ±2.68 mV (n = 9). Thus, formula C expected to be a stable preparation.

### *4. Morphology of the Particles and Particle Distribution*

Nanosuspensions can undergo a change in the crystalline structure, which may be to an amorphous form or to other polymorphic forms because the impact of high-pressure homogenization. The morphological characterization of nanosuspension particles was determined by using techniques like polarized light microscopy, scanning electron microscopy (SEM), and X-ray diffraction analysis in combination with differential scanning calorimetry (DSC). Figure 1 shows the light microscopic images of the modified KTF nanosuspension (image B) compared to unmodified drug (image A). The results indicated that the very hard crystals of ketoprofen raw material (figure, A) were successfully homogenized using the HPH technique.

A B

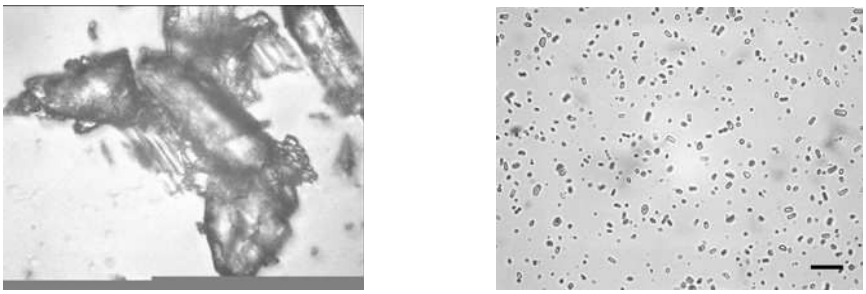

**Figure 1**
***Light microscopic pictures of ketoprofen: (a) raw materials (b) ketoprofen nanosuspension (formula C) which prepared by HPH using 20 homogenization cycles.***

The fine crystalline KTF nanosuspensions were homogenously distributed as single particles (figure, B). Furthermore, no agglomerated/aggregated particles were found. Consequently, the resulting nanosuspensions tended to be stable. Particle size distribution determines the physiochemical behavior of the formulation, such as saturation solubility, dissolution velocity, physical stability, etc. The particle size distribution was determined by photon correlation spectroscopy (PCS). Figure 2 shows the particle size distribution of the modified KTF-nanosuspensions in water.

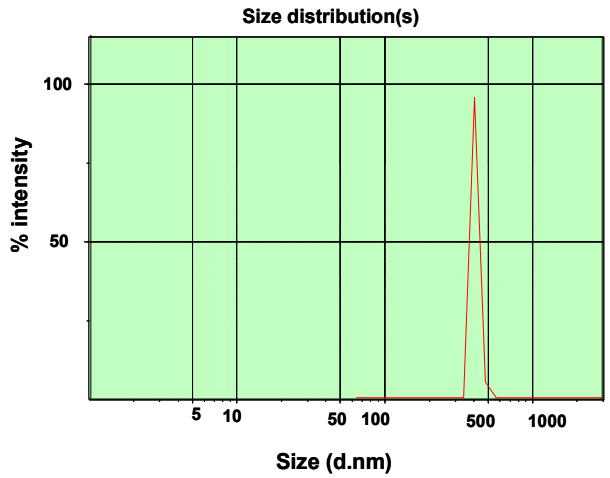


**Figure 2**
***Particle size distribution of ketoprofen nansospension***

The obtained results indicated a narrow size distribution of the KTF nanocrystals in the suspension with Z-average of 322.7 nm. Noteworthy, a narrow size distribution is essential to prevent particle growth caused by different saturation solubility in the vicinity of differently sized particles, which was explained in Ostwald ripening[32]. Put differently, the uniform size or narrow size distribution was very important for the long-term stability of the nanosuspensions.

***5. Differential Scanning Calorimetry (DSC)***

When nanosuspensions produced, the material phases of the drug and the excipients might change. The drug might present as a solid solution or dispersed among the adjuvant as a molecular or a crystalline[32]. The studies on the thermal behavior and crystallinity could help to ascertain the physicochemical status of the entrapped drug inside the excipient and to assess the interaction amongst different components during the fabrication process. The results of DSC, as illustrated in figure 3, demonstrate that the nanosuspension was quite

different from the simple mixture of its ingredients (KTF and SDS). For instance, an endothermic peak could be observed at temperature degree ranged from 91.5 to 98.5°C (figure 3, D) which is characteristic of KTF, and an endothermic peak at 75.0 ~ 87.5°C (figure 3, C), which is corresponding to SDS, could also be, noticed. Whereas, in the DSC results of nanosuspensions, the peaks mentioned above had disappeared or changed greatly, and presented some new characteristic peaks, such as the two new endothermic peaks at 54.7 ~ 63.3°C and 241.4 ~ 270.8°C. According to the DSC analysis, it could be concluded that the material phases of the nanosuspensions were quite different from the simple mixture of KTF and SDS. The formation of the new material phases testified the formation of nanosuspensions. This result suggested that KTF was not in a crystalline state but in an amorphous state, in which the drug was likely to have higher energy and therefore showed increased solubility, dissolution rates and, hence, higher bioavailability [34,35].

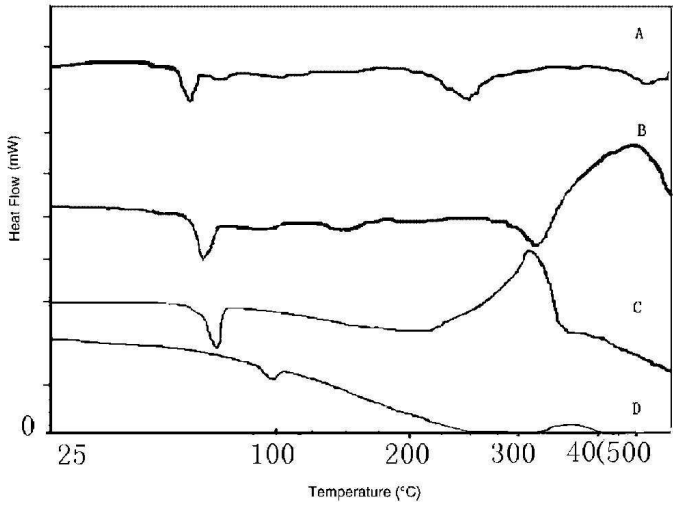


**Figure 3**
***DSC thermograms of (A)-Lyophilized Powder; (B) Physical Mixture of ketoprofn and SDS; (C) SDS, and (D)-Ketoprofen***

### *6. X-Ray Diffractometry*

The results of the X-Ray diffractometry (figure 4) demonstrate that the KTF-nanosuspensions were quite different from the simple mixture of its ingredients (i.e., SDS and ketoprofen). Based on the analysis of the simple mixture by X-Ray scattering, the characteristic peaks of SDS, Ketoprofen could be observed obviously. For example, we could find two diffraction peaks at 18.980° (*d* = 4.6720 Å) and 23.200° (*d* = 3.8380 Å), as the characteristic peak of SDS, and five diffraction peaks at 7.800° (*d* = 11.3253Å), 9.780° (9.0365 Å), 11.140° (7.9361 Å), 12.400° (7.1324 Å) and 15.280° (5.7939 Å), as the characteristic peaks of ketoprofen.

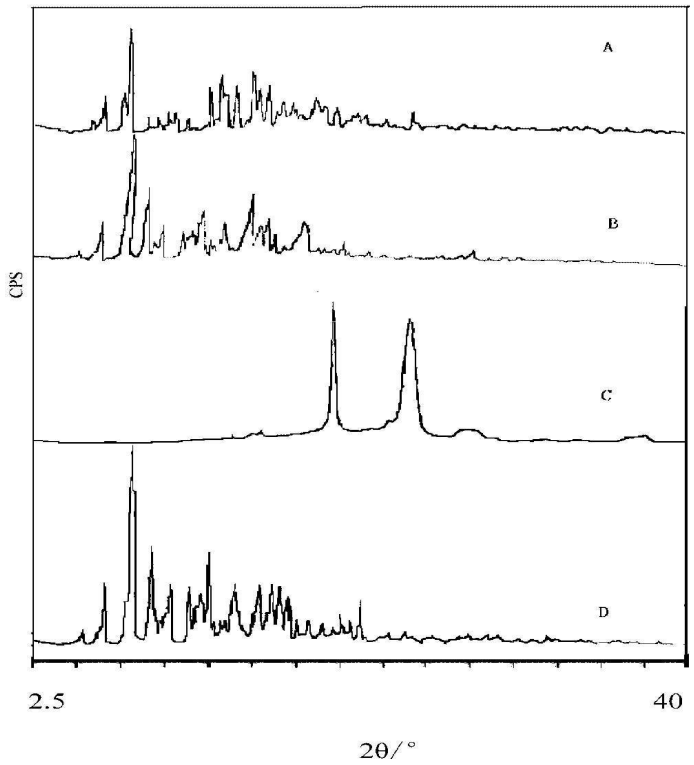


**Figure 4**
***X-ray Diffraction Patterns of A -Lyophilized Powder; B Physical Mixture of ketoprofen and SDS; C-SDS ; D-Ketoprofen***

In the case of ketoprofen nanosuspensions, some characteristic peaks of ketoprofen and SDS disappeared. The parameters of other characteristic peaks, such as the peak intensity, peak position and inter planar spacing were changed. Examples of these changes are: such as the diffraction peaks at 11.920° (7.4185 Å), 16.300° (*d* = 5.4336 Å), 19.700° (*d* = 4.5028 Å), 30.300° (*d* = 2.9474 Å). Other new peaks appeared and could be easily observed. It could be said that the X-ray diffraction confirmed the formation of the new material phases which is in agreement with that of DSC results.

### *7. Solubility study*

Chemically, KTF is one of the propionic acid derivatives and considered as a weak acidic drug. Therefore, in a basic solution it will form salts and the solubility will be increased. Solubility of the KTF nanocrystals was compared with KTF microcrystals in buffer solution having a pH of 6.8. In this solution, KTF will form the salt and solubility will be increased. The particle size reduction influenced the saturation solubility of KTF in buffer at pH 6.8. Fig. (5) shows the enhanced solubility of KTF nanocrystals in comparison to that microcrystals. In this medium, the saturation solubility of drug nanocrystals was 1724±8.4 µg/ml, only slightly higher than the solubility of ketoprofen microcrystals, which showed a solubility of only 1451±6 µg/ml. The difference in saturation solubility between ketoprofen nanocrystals and microcrystals was assumed by the difference in particle size of both ketoprofen.

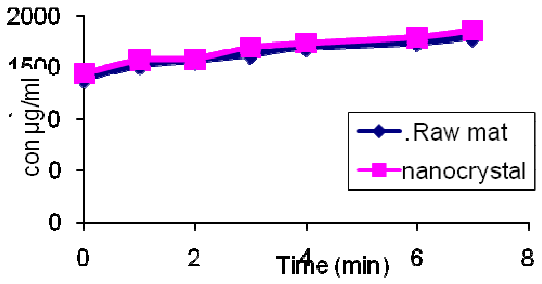


**Figure 5**
***Saturation solubility of ketoprofen nanocrystal and raw material in PH 6.8 at 25C***

***8. In Vitro dissolution study***

Figure 6 shows the dissolution profiles of KTF nanosuspensions (formula C) in comparison to the unmodified ketoprofen; both were studied in phosphate buffer pH 6.8. The dissolution rate was remarkably enhanced by the reduction of particle size since the complete dissolution of drug was obtained after about 20 minutes in the case of KTF-nanocrystals. Only 62% was dissolved in the case of unmodified drug. This may be attributed to the fact that the reduction of drug particle size caused the surface area to increase and consequently to enhance the contact between nanosuspensions and dissolution medium. The obtained results are in good accordance with Noyes–Whitney equation which states that; the increase in saturation solubility and the decrease in particle size lead to an increased dissolution rate[36]. Since the bioavailability of KTF is dissolution rate limited, particle size reduction can significantly improve the performance or the bioavailability of the drug.

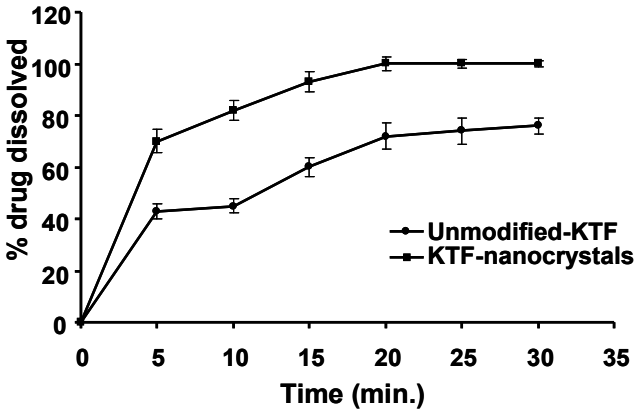


**Figure 6**
***The dissolution profile of unmodified ketoprofen (a) and KTF-nanocrystals (b) in phosphate buffer pH 6.8 at 25 ºC***

***9. Stability studies***

Physical stability of the medicated nanosuspensions was intensively evaluated over 30 days period. Figures 7 and 8 show the particle size distribution of the ketoprofen nanosuspensions over 30 days storage.

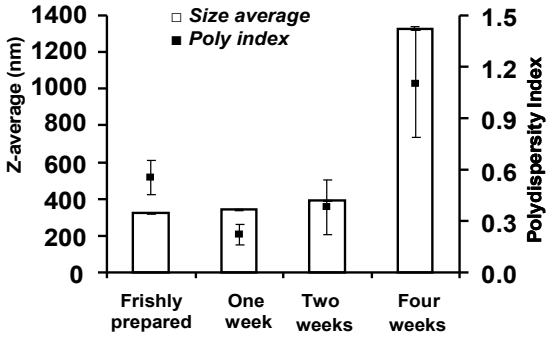


**Figure 7**
**Effect of storage time on the particle size and polydispersity index of formula C. Values are given as mean ± standard deviation**

According to the obtained results, the ketoprofen nanosuspension could retain the particle size distribution in the nanometer range within 14 days. Afterwards, the particle size began to increase and within 30 days the particle size of the ketoprofen nanosuspensions had grown rapidly to be very big particles. The particle growth was due to the existence of aggregated particles which follows Ostwald ripening phenomenon.

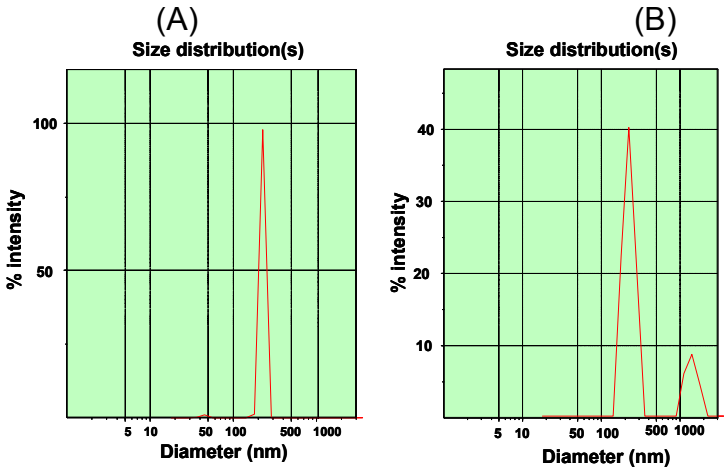


**Figure 8.**
***(A) Particle size distribution of freshly prepared ketoprofen nanosuspensions .and (B) Appearance of ketoprofen nanosuspensions, 4 weaks after preparation***

Moreover, the prepared nanocrystals were investigated by light microscopy. Figure 9 (a) shows the pictures of modified nanocrystals formula C as a function of storage time. The morphology of freshly prepared nanocrystals (Figure 9, a) showed that the particles are uniformly and separately distributed. After 14 days, these fine particles of ketoprofen grew into larger particles with very minute extent of aggregation (figure 9, b). However, within 30 days the drug particles showed the existence of large single crystals together with smaller ones, indicating the possibility of aggregation or agglomeration after one month (figure 9, c). Accordingly, the obtained results indicated the instability of the ketoprofen nanosuspensions within 30 days.

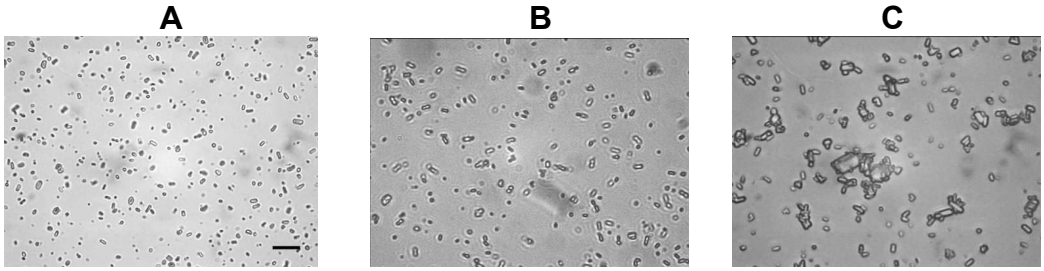


**Figure 9**
***Light microscopic pictures of formula C as a function of storage time, (a) freshly prepared (b)14 days storage (c) 30 day storage at room temperature (Magnification power 1000 times)***

## CONCLUSION

It could be concluded that it was possible to obtain ketoprofen nanosuspensions with fine solubility and dissolution properties, and the nanosuspensions possessed a high drug-loading (10%), which could reduce the administration dosage and gastrointestinal side effects. High pressure homogenization can be employed to produce aqueous drug nanosuspensions that are stable up to one month. The zeta potential values are about -30 mv or higher, i.e. in the range for a long-term stable suspension. Aqueous nanosuspension can be converted to dry nanocrystals by lyophilization. Dried KTF nanocrystals offer superior physicochemical properties. The very fine particles of the dried nanocrystals re-disperse completely in water. This characteristic is critical in improving the kinetic solubility and the dissolution behavior of drugs.

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