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Preparation and Evaluation of Ketoprofen Nanoparticles

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ABSTRACT

Ketoprofen is class II type drug according to (Biopharmaceutics Classification System BCS) with low solubility and high permeability. The effective surface area of drug particle is increased by a reduction in the particle size. Since dissolution takes place at the surface of the solute, the larger the surface area, the further rapid is the rate of drug dissolution. The aim of this investigation was to increase the solubility and hence the dissolution rate by the preparation of ketoprofen nanoparticles using solvent evaporation method. Materials like PVP K30, poloxamer 188, HPMC E5, HPMC E15 and HPMC E50 were used as stabilizers in perpetration of different formulas of Ketoprofen nanoparticless. These formulas were evaluated for particle size, effect concentration of drug and effect of injection volume ratio. All of the prepared Ketoprofen nanosuspensions formulas showed a particle size result within Nano range. The average particle size of Ketoprofen nanosuspensions formulas was observed from 31.2 nm to 676.5 nm. SEM images for Ketoprofen nanoparticle showed a reduction in an average particle size compared to pure powder and DSC DSC illustrated that the crystallinity of Ketoprofen was partially lost in lyophilized powder and converted to an amorphous form. The results indicate the suitability of solvent evaporation method for Ketoprofen with improved in vitro dissolution rate and thus perhaps enhance fast onset of action for drug.

Keywords: Ketoprofen; Nanosuspension; Particle size; Solubility

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INTRODUCTION

For a drug molecule the solubility may be an important issue determining the dissolve and so the amount obtainable for absorption. Compound that has little water solubility may be exposed to slow dissolution rate and inadequate absorption in the gastrointestinal residence time. In biopharmaceutical terms, the importance of the solubility has been decorated by Biopharmaceutical Classification System (BCS) styled by Amidon in 1995, in which drugs have been classified into four groups [1]. Poor water soluble drug has many problems such as low or variable bioavailability, large dose and delayed onset of action [2]. There are various approaches available for overcoming the solubility of poorly soluble drugs for examples, modification of the crystal habit, self-emulsification, solid dispersion, solubilization by surfactant, salt formation, pH modification, co-crystallization, use of co-solvent, micronization and nanosization. In general, the rate of the drug solubility is related to particle size, as a particle gets smaller one, as (the surface area: volume) ratio increases. The larger surface area allows a better interaction with the solvent, which cause increase in dissolution rate. Since dissolution takes place at the surface of drug particle, the high dissolution rate of drug is associated with large surface area of drug particles. Many drugs are active intravenously but are not effective when taken orally, because of low oral absorption. Reduction of the particle size for drugs with low aqueous solubility to a micronized form has improved the oral absorption of Griseofulvin, nitrofurantoin, and many steroids. In addition smaller particle size enhances water penetration into the particles [3].
The Ostwald–Freundlich equation describes the relationship between the saturation solubility of drug and its particle size:

$$log \frac{Cs}{C\infty} = \frac{2\,\sigma\,V}{2.303\,RT\,pr} \text{ ----------1}$$

Where C_S is the saturation solubility, C∞ is the solubility of the solid particles with large sizes, σ is the interfacial tension, V is the molar volume, R is the gas constant, T is the absolute temperature, ρ is the solid density, and r is the radius. It is clear that the saturation solubility (C_S) of certain drugs will increase by decreasing the particle size (r) [4].

Nanosization is a method, where a drug particle is converted into nanoparticles having size less than 1μm. Nanotechnology allowed drug delivery system with improved physical, chemical and biological properties. The main purposes in designing nanoparticles as delivery systems are to control the particle size, surface properties and dissolution of drug, then to reach the action site at a perfect rate and dose level [5]. The selection of suitable method for the formulation of nanoparticles depends on the physicochemical characteristics of used polymer and the drug to be loaded. However, the nanosuspension form has an advantage in their ability to increase dissolution rate and to enhance the bioavailability of poor soluble drug. Ketoprofen is an example of class II drug. It is a white crystalline powder and practically insoluble in water [6]. It is a non-steroidal anti-inflammatory drug with analgesic and antipyretic properties. The objective of study was to increase its solubility and then the dissolution rate by preparation of nanosuspension using antisolvent precipitation method.

MATERIALS AND METHODS

Materials

Ketoprofen was purchased from Lishui Nanming Chemical CO.,Ltd (China). Polyvinylpyrrolidone (PVP K30), Poloxamer 188, and Hydroxypropyl methylcellulose (HPMC) E5, E15 and E50 were purchased from Shanghai Send Pharmaceutical Technology Co.Ltd (China). Methanol was obtained from Gailand Chemical Company (UK). Disodium hydrogen phosphate and Potassium Dihydrogen Phosphate were supplied by BDH Laboratory Supplies (England) and SPINE- CHEM. Limited, respectively.

Calibration Curves of Ketoprofen

Calibration curves for Ketoprofen in 0.1 N HCl (pH 1.2) and DW were constructed by preparing serial dilutions of the drug from a stock solution (1 mg/ml) for each one, and the prepared samples were analyzed spectrophotometrically at its detected λmax. The determined absorbances were recorded and plotted versus their concentrations [7].

Determination of Ketoprofen Saturation Solubility

Saturated solubility measurements of Ketoprofen in various solutions were determined by shake flask method. An excess amount of Ketopofen was separately introduced into stoppered conical flask containing 10 ml of 0.1 N HCl (pH 1.2) and DW. The sealed flasks were shaken for 24 hours at 37°C**.** Visual inspection was made to check the precipitation of drug particles in the sample. An aliquot of solution was passed through 0.45 μm filter paper and the filtrate was suitably diluted and analyzed on a UV/visible spectrophotometer at 260 nm wavelength [8].Three determinations were carried out to calculate the solubility of Ketoprofen.

Preparation of Ketoprofen Nanosuspensions

Nanosuspensions of Ketoprofen were prepared by the solvent evaporation technique, which is also termed as antisolvent precipitation method. Ketoprofen powder was dissolved in methanol (2.5 ml) at room temperature preparing drug concentrations (20 and 40 mg/ml). The resultant organic solution of drug (organic phase) was added drop by drop by means of a plastic syringe positioned with the needle directly into aqueous solution of stabilizer [9,10]. The mixture of drug solution and stabilizer was kept at 50°C and subsequently agitated at stirring speed of 500 revolutions per minute (rpm) on a magnetic stirrer for about one hour to permit methanol to evaporate [11]. Ketoprofen being insoluble in water, therefore, it will precipitate with stabilizer. The ratios (weight: weight) of drug to stabilizer used to prepare the nanosuspension were 1:1, 1:2 and 1:3. Tween 80 was also used at different volumes as explained in Table 1.

Effect of Drug Concentration on the Size of Nanosuspensions

The objective of this study was to prepare different formulas of Ketoprofen nanoparticles (F16-F30) to show the effect of drug concentration on particle size and aqueous solubility. Different concentrations of drug were used in preparing various formulas of nanosuspension containing different types of polymers, polyvinyl Pyrrolidone PVP k30 (F16-F18), poloxamer188(F19-F21),hydroxyl propyl methyl cellulose HPMC grade E5(F22-F24), hydroxyl

propyl methyl cellulose HPMC grade E15(F25-F27) , and hydroxyl propyl methyl cellulose HPMC grade E50(F28-F30).

Effect of Injected Volume Ratio

The effect of ratio of the injected drug solution (organic phase) to aqueous phase containing stabilizer on the size of the nanoparticles formed were also studied to optimize the volume of drug solution which can be used in preparation of nanoparticles. Several formulas (F31-F45) were utilized to study this effect, as shown in Table 2.

Table 1: Compositions of ketoprofen nanosuspensions using different stabilizers at different drug: stabilizer ratios with constant volume of injection of organic solution (2.5 ml)

Formula No.	Drug (mg)	PVP k30 (mg)	Poloxamer 188 (mg)	HPMC E5 (mg)	HPMC E15 (mg)	HPMC E50 (mg)
1	50	50				
2	50	100				
3	50	150				
4	50		50			
5	50		100			
6	50		150			
7	50			50		
8	50			100		
9	50			150		
10	50				50	
11	50				100	
12	50				150	
13	50					50
14	50					100
15	50					150
16	100	100				
17	100	200				
18	100	300				
19	100		100			
20	100		200			
21	100		300			
22	100			100		
23	100			200		
24	100			300		
25	100				100	
26	100				200	
27	100				300	
28	100					100
29	100					200
30	100					300

Table 2: Composition of ketoprofen nanosuspensions using 5 ml of methanol as organic solvent with different types of stabilizers at 1:1, 1:2 and1:3 drug: stabilizer ratio

Formula No.	Ketoprofen (mg)	PVPK30 (mg)	Polo188 (mg)	HPMC E5(mg)	HPMC E15(mg)	HPMC E50(mg)
31	50	50				
32	50	100				
33	50	150				
34	50		50			
35	50		100			
36	50		150			
37	50			50		
38	50			100		
39	50			150		
40	50				50	
41	50				100	
42	50				150	
43	50					50
44	50					100
45	50					150

Characterization of the Prepared Nanosuspension

Particle size and surface area:

Determination of particle size was done using ABT-9000 nano laser particle size analyzer (Angstrom Advance Inc. USA), which is apparatus of a dynamic light scattering, acts by measuring the light intensity that is scattered by the molecules sample as a time function, at scattering angle (90°) and constant temperature (25°C) without dilution of samples. The particle size can be determined by placing samples of formulas in the analyzer. The average diameters and polydispersity index of samples were measured for each formula**.**

Freeze drying of nanosuspension:

To obtain dried nanoparticle from nanosuspension, freeze drying method has been most commonly used. The basic principle of this technique is to remove water from sample by sublimation and desorption under vacuum [12]. The selected formulas have been transferred to a cell (tube) of lyophlizer and stored in a deep freeze (-40^{o}C) for duration of 24 hr and then the frozen samples have been put in lyophilizer (LABCONCO) for 72 hr at a condenser temperature (-40^{o}C) and pressure (0.9 mbar), the obtained powders have been put in cup tightly closed and covered with a parafilm at room temperature for further tests.

Determination of saturation solubility of the lyophilized powder:

Saturated solubility of the lyophilize powder was determined separately in DW and 0.1 N HCl (pH1.2) using a volumetric flask. Flasks were stoppered to avoid evaporation of media and shaken for 24 hr in thermostatically controlled shaker water bath maintained at 37^{o}C. The samples were withdrawn, filtered and diluted and then absorbance of each was measured [13]**.** The solubility was measured in triplicate.

Visualization by scanning electron microscopy (SEM):

Scanning electron microscope (INSPECT S50) has been used for detect the morphological nature and surface topography of particles for pure drug and lyophilize powder of selected formulas. The procedure has been confirmed by direct statement of powder on carbon tape (double-sided) and coated with gold [13].

Differential scanning calorimetry (DSC):

DSC can be used to detect the physical compatibility among the drug and polymer, by determining the thermal behavior of pure ketoprofen, PVPk30, poloxamer188, physical mixture of Ketoprofen+PVPk30, physical mixture of Ketoprofen + PVPk30+poloxamer 188 and selected formulas.

Differential scanning calorimetry has been accomplished in a METTLER DSC30 device on 5 mg samples located in aluminum pans by increment the temperature from 30 to 120^{o}C at a rate of heating 10^{o}C/min beneath a nitrogen gas stream [14].

RESULTS AND DISCUSSION

Determination of Calibration Curves

Figures 1 and 2 show the calibration curves of Ketoprofen in 0.1 N HCl (pH 1.2 and DW, respectively. Straight lines were obtained as a result of plotting the absorbance versus concentrations with high regression coefficient which indicates that these curves obey Beer-Lamberts law within the concentrations used.

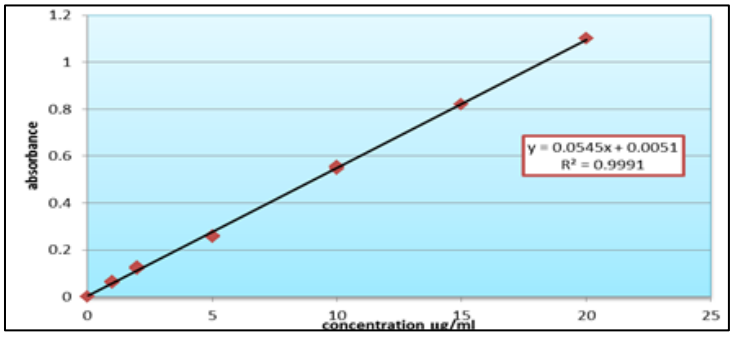

Figure 1: Calibration curve of ketoprofen in 0.1 N HCl (pH 1.2)

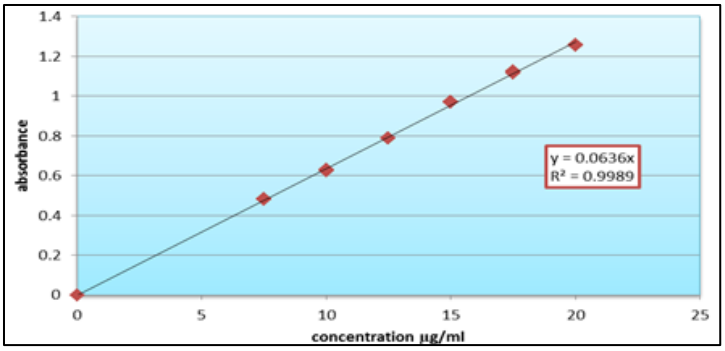

Figure 2: Calibration curve of ketoprofen in DW

Determination of Ketoprofen Saturation Solubility

The saturation solubility values of Ketoprofen were found to be about 0.11 mg/ml, 0.205 mg/ml in D.W and pH 1.2, respectively. The results of saturation solubility of Ketoprofen were illustrated in Table 3. According to biopharmaceutical classification system, Ketoprofen is an example of Class II drugs having low aqueous solubility and well absorption through gastrointestinal tract [15] due to high permeability and lipophilicity. Ketoprofen is a weak acid drug and will be ionized at higher pH; it is practically in soluble in water [6].Therefore, its solubility increase with pH increase towards alkaline medium [16].

Table 3: Saturation solubility values of ketoprofen in different media

Media	Solubility (mg/ml)
Distilled water DW	0.11
0.1N HCl (pH1.2)	0.205

Particle Size and Surface Area

The average particle size of all the prepared formulas using ABT-9000 Nano laser particle size analyzer. All of the prepared Ketoprofen nanoparticles formulas showed a particle size result within Nano range. The average particle size of Ketoprofen nanoparticles formulas was observed from 31.2 nm to 676.5 nm, as shown in Tables 4 and 5. The smallest size 31.2 nm for F16 and the largest size 676.5 nm for F15 formula. The specific surface area (SSA) of the particles is the summation of the areas of the exposed surfaces of the particles per unit mass. Where the particle size is inversely related with the surface area [17]. The SSA values for the prepared formulas were at range (4.02-71.39) m^2/g, the largest surface area is recorded in F16 formula and smallest surface area $4.02m^2/g$ in F27 formula as appeared in Table 5.

Polydispersity Index Analysis

Polydispersity index is a parameter used to define the particle size distribution obtained from the particle size analyzer. Polydispersity index gives degree of particle size distribution at range from 0.007 to 0.241 depending on formulation variables. The formula F14 showed lowest PDI (0.007), as seen in Table 4, that indicate good uniformity of nanoparticle size. Uniformity of particle size is determined by polydispersity index values in which the low value means the best uniformity. The range of PDI values (0-0.05) means (monodisperse system), 0.05-0.08 (nearly monodisperse), 0.08-0.7 (mid-range polydispersity), and >0.7 (very polydisperse) [18].

Effect of drug concentration

Formulas (F16-F30) were utilized to study the effect of drug concentration on average particle size at different types of polymers and various ratios of drug to polymer (1:1, 1:2 and 1:3) as shown in Table 5.

In general, a sufficient supersaturating is required to get precipitate of drug particles in nanoparticulate range, the higher concentration (higher supersaturation) leads to a faster nucleation rate with small particles. Moreover, at very high concentration of drug (very high supersaturation) the particle size increases (particles growth by promoting condensation and/or coagulation) [19].

The nanoparticles were prepared using different diffusing drug concentration (50 -100 mg\50 ml). When we compare between (Tables 4 and 5), we see that the average particle size varies with the change in drug concentration as shown in formula F16 at 1:1 of drug to stabilizer ratio (drug: PVP k30), a decrease in average particle size from 317 nm to 31.2 nm, was seen with increasing drug concentration. These results are in agreement with previously published study by Dong et al., where 33.3% in particles size reduction from (300 nm to 200 nm) was observed, when drug concentration increased from 10 to 100 mg/ml in preparation of Spironolactone nanoparticles [20].

Table 4: Particle Size, polydispersity index and specific surface area of ketoprofen formulas using different types and amounts of stabilizers

Formula No.	Average Particle size (nm)	PDI	SSA (m^2/g)
F1	317	0.012	6.75
F2	313	0.009	7.1
F3	282.5	0.009	7.65
F4	426.5	0.01	5.34
F5	317	0.011	6.88
F6	282.5	0.01	7.88
F7	270	0.008	8.23
F8	282.5	0.011	7.65
F9	317	0.01	6.84
F10	282.5	0.01	7.91
F11	426.5	0.024	5.58
F12	479	0.009	4.63
F13	269	0.008	8.23
F14	426.5	0.007	5.24
F15	676.5	0.011	4.17

The average particle size exhibited a slight upward trend (from 317 nm to 338 nm) with increase concentration of drug in F17 formula at 1:2 of drug: stabilizer ratio. Additionally, at 1:3 ratios the average particle size has no remarkable change. On the other hand, the average particle size of formulas F19, F20 and F21 using poloxamer188 were (426.5 nm – 527 nm), (317 nm – 349 nm) and (282.5 nm – 332 nm), respectively. This increase in average particle size may be attributed to the increased nucleation rate, the higher supersaturating and then increase of particle growth that is associated with larger particles size [21].

Formulas (F22 to F30) were utilized to study the effect of different drug concentrations on different grades of HPMC at various drug to stabilizer ratios. All formulas showed that an increase in diffusing drug concentration gave an increase in average particle size except for E50 at ratio 1:3, which exhibited a reduction in average particle size from 676.5 nm to 99.5 nm, this may due to slow mobility of the nuclei/particle because of high viscosity in presence of high concentration of polymer, that firstly reduce the average particle size for a short time (less than 1 hour). The resultant system was instable and larger particles are formed after a few minutes of preparation resulting in the formation of particles aggregates. This may be occurred because of amount of HPMC E50 used is enough for arresting particle size, but not effective to inhibit the aggregation of particles [20].

Additionally, HPMC E5 at 1:2 and 1:3 ratios for formulas F23 and F24, respectively, there is a slightly increase in average particle size was observed from (282.5 nm – 294 nm) and from (317 nm – 354 nm), respectively. These results indicate that there is an optimum drug and polymer concentrations at these ratios. Furthermore at 1:1 ratio no change in particle size was observed.

Table 5: Particle size, polydispersity index and specific surface area of ketoprofen formulas at drug: stabilizers ratio 1:1, 1:2, 1:3

Formula No.	Average Particle size (nm)	PDI	SSA (m2/g)
F16	31.2	0.009	71.39
F17	338	0.009	6.59
F18	282	0.01	7.88
F19	527	0.007	4.22
F20	349	0.009	6.37
F21	332	0.011	6.71
F22	274	0.01	8.11
F23	294	0.011	7.58
F24	354	0.01	6.28
F25	324	0.01	6.86
F26	468	0.019	4.77
F27	553	0.008	4.02
F28	324	0.012	6.87
F29	627	0.016	4.56
F30	99.5	0.241	21.5

Effect of Injection Volume

Formulas (F31 – F45) were prepared to study the effect of aqueous/organic phase ratio on the mean size of the nanoparticles as explained in Figures 3- 7. The average particle size was increased in all formulas for all stabilizers,

except for HPMCE50 at 1:2 and 1:3 (drug to stabilizer ratio). When the volume of antisolvent increases from 2.5 ml to 5 ml as shown in Table 6.

To explain that, the change in aqueous/organic phase ratio may causes change in the viscosity of the dispersion system and the particle size gradually decreases as the solvent content is decreased gradually by increasing the antisolvent to solvent ratio [22]. The increment ratio between solvent and antisolvent may lead to increase in saturation solubility and nucleation rate of small particles. An increase in nanoparticle size with increase of antisolvent to solvent ratio has also been reported by Dalvi SV and Dave RN [22].

Table 6: Particle size, polydispersity index and specific surface area for formulas (F31-F45) at drug: stabilizers ratio 1:1, 1:2 and 1:3

Formula No.	Average Particle size(nm)	PDI	SSA (m^2/g)
F31	432	0.028	18
F32	381	0.01	5.85
F33	482	0.01	4.61
F34	496	0.009	4.49
F35	253	0.009	8.8
F36	560	0.015	3.98
F37	278	0.009	7.99
F38	300	0.009	7.42
F39	436	0.042	5.16
F40	352	0.018	6.34
F41	392	0.019	5.7
F42	499	0.019	4.48
F43	300	0.009	7.4
F44	327	0.035	6.92
F45	544	0.006	4.09

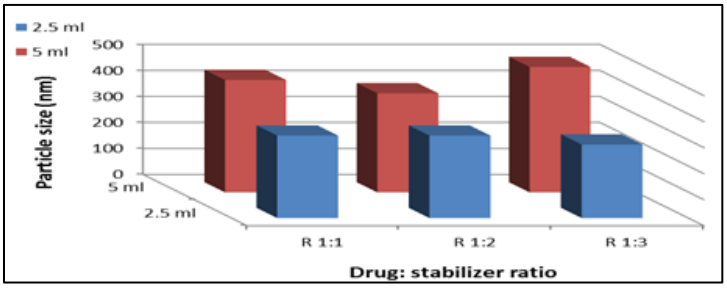

Figure 3: Effect of aqueous/organic phase ratio on the particles size of ketoprofen formulas using PVP k30 at 1:1, 1:2 and 1:3 ratios

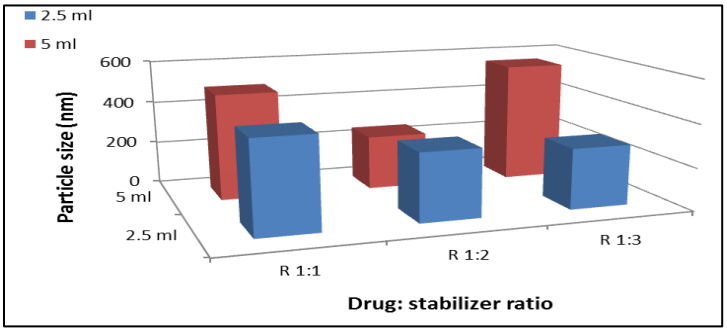

Figure 4: Effect of aqueous/organic phase ratio on the particles size of ketoprofen formulas using poloxamer188 at 1:1, 1:2 and 1:3 ratios

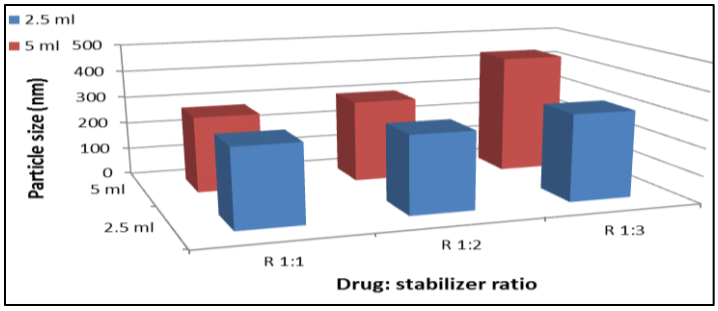

Figure 5: Effect of aqueous/organic phase ratio on the particles size of ketoprofen formulas using HPMCE5 at 1:1, 1:2 and 1:3 ratios

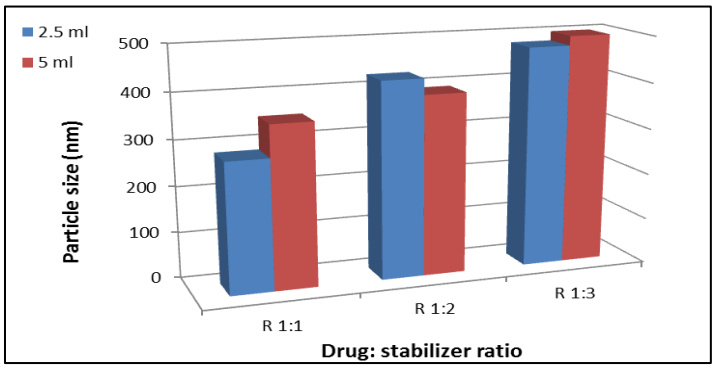

Figure 6: Effect of aqueous/organic phase ratio on the particles size of ketoprofen formulas using HPMCE15 at 1:1, 1:2 and 1:3 ratios

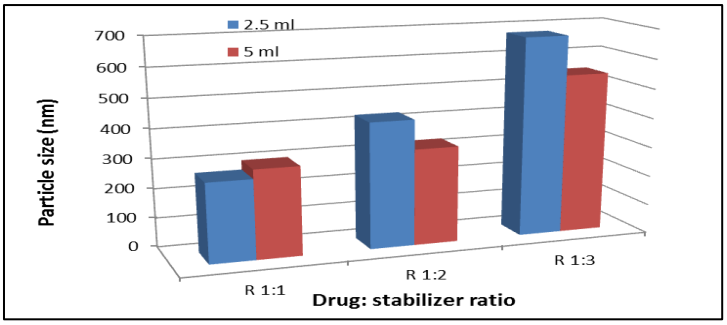

Figure 7: Effect of aqueous/organic phase ratio on the particles size of ketoprofen formulas using HPMCE50 at 1:1, 1:2 and 1:3 ratios

Saturation Solubility of Lyophilized Powder

Saturation solubility was detected for the selected formulas F16 (smallest particle size) as shown in Figure 8. Saturation solubility was tested in two media 0.1 N HCl (pH 1.2) and DW at 37^{o}C ± 0.5 for 24 hours. Saturation solubility of Ketoprofen for F16 in distilled water was 0.696 mg/ml. Therefore, saturation solubility of drug in these formulations was increased about 6.5 folds than the solubility of pure drug in distilled water (0.11 mg/ml). These results were in agreement with Dixitn Mudit [22] .

Furthermore, the solubility of drug was also increased in 0.1N HCl (pH1.2). The saturation solubilities of drug nanoparticle in formulation F31 was 0.478 mg/ml. So solubility of drug in that formula was also increased and higher than the solubility of pure drug in 0.1N HCl (0.205 mg/ml).

The saturation solubility studies showed increase in drug solubility in the prepared nanoparticles as compared to pure drug. This is mainly due to the reduction in average particle size. Such results were also reported by Hecq *etal*, when they prepared nifedipine nanocrystales and found that nanonization lead to increase saturation solubility in water from 19.5 ± 0.1µg/ml to 25.9 ± 1.4 µg/ml [23].

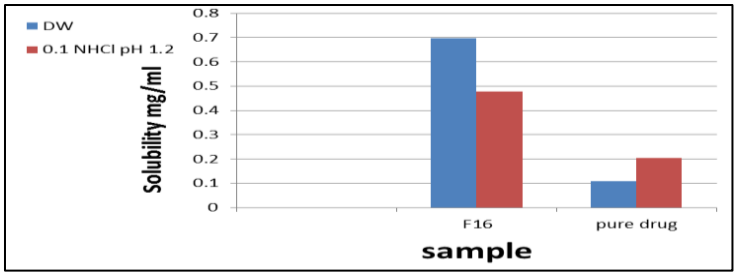

Figure 8: Saturation solubilities of ketoprofen powder, F19 and F31 formulas in DW and 0.1N HCl (pH 1.2) at 37°C ± 0.5

Scanning Electron Microscope (SEM)

Particles morphology was investigated by scanning electron microscope. Figure 9 shows SEM images of pure Ketoprofen particles (3.42 kx, and 17.11 kx magnification). They revealed that particles of drug were appeared as large particles with crystal shapes.

Morphology of precipitated drug nanoparticles in lyophilized form for F16 formulas was illustrated in Figure 10. Particles in this formula are discrete with uniform distribution of particles and there is no sign of agglomerations.

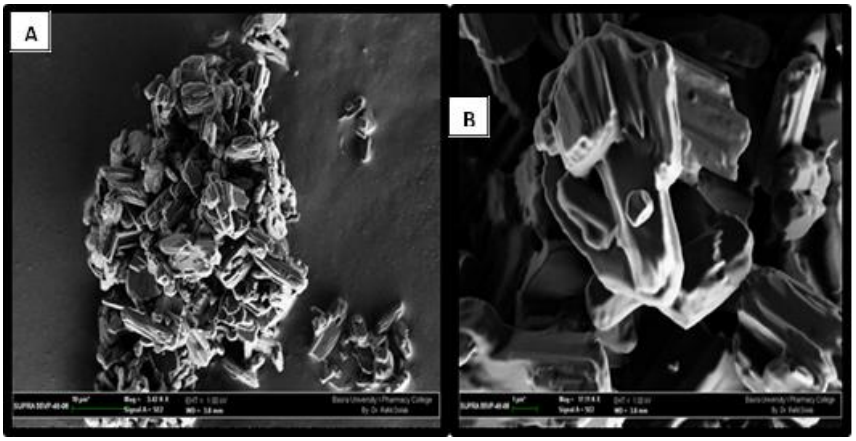

Figure 9: SEM micrographs for pure ketoprofen powder: A) at 3.42 KX magnification, B) at 17.11 KX magnification

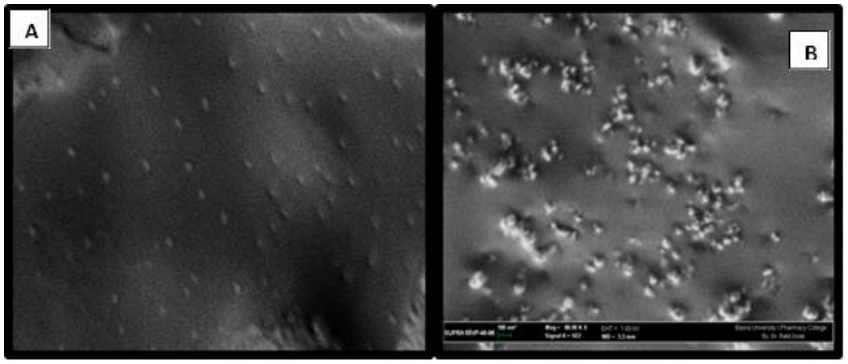

Figure 10: SEM micrographs for lyophilized ketoprofen formulas: A) F16 at 14.33 KX magnifications; B) F16 at 88.90 KX magnifications

Differential Scanning Calorimetry (DSC)

In order to confirm the physical state of materials in formulation, DSC was performed for Ketoprofen, lyophilized powder of best formula F19. DSC scan of Ketoprofen showed a single sharp endothermic peak at 97.28°C corresponding to the melting point of drug, which indicated the crystalline state of drug [24], as shown in Figure 11. Figure 12 showed DSC for PVP k30.

In case of lyophilized powder of selected formula, the crystalline structure had been decreased in nanoparticles formulation as revealed the melting endothermic peak was shifted from 97.28°C to 90.16°C in formula F16 as illustrated in Figure 13. DSC study proved that crystallinity of Ketoprofen was reduced in nanoparticles formulation. Moreover, it can be concluded that Ketoprofen and polymers do not interact with each other.

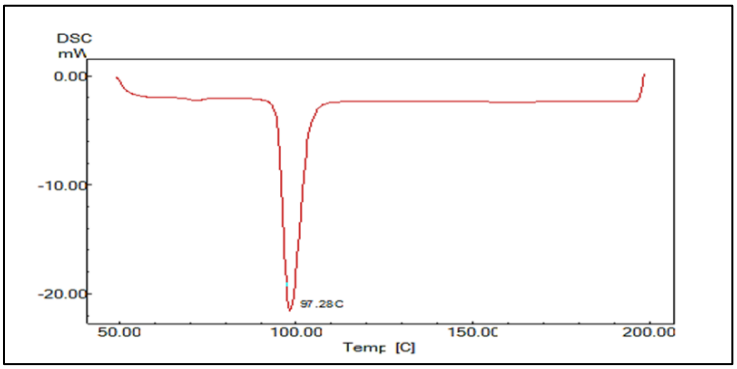

Figure 11: DSC thermogram of pure ketoprofen powder

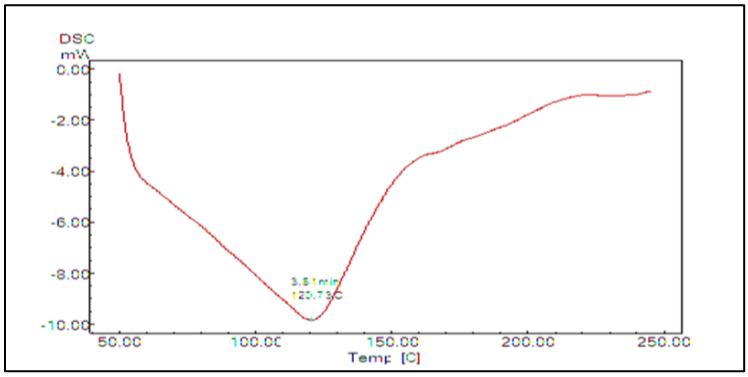

Figure 12: DSC thermogram of PVP K30 powder

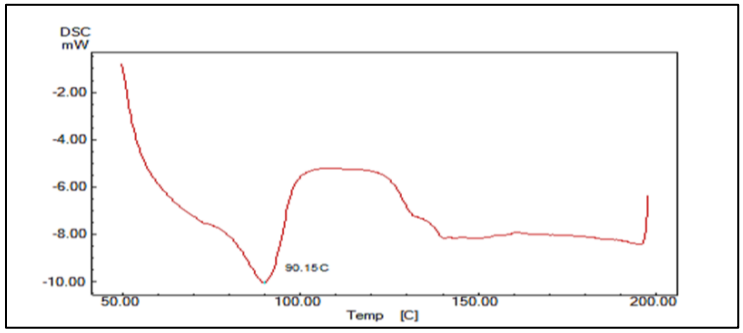

Figure 13: DSC thermogram of lyophilized ketoprofen nanoparticles formula (F16)

CONCLUSION

Ketoprofen nanoparticles were successfully prepared using different types of stabilizers at different drug: stabilizer ratios 1:1, 1:2 and 1:3. The data confirm that Antisolvent precipitation method is an effective method to prepare drug nanoparticles, and it is cost effective, easy to operate and can be easily scaled up for industrial production of drug nanoparticles. Solvent:Antisolvent ratio of 1:20 gives the best results regarding average particle size and dissolution rate. DSC confirms that the crystallinity of Ketoprofen and it was partially lost when the drug was converted into nanoparticles. SEM images showed uniform average particle size distribution which no particles agglomeration.

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