Preparation and Evaluation of Ketoprofen Nanosuspension Using Solvent Evaporation Technique

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Abstract

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. Ketoprofen is class II type drug according to (Biopharmaceutics Classification System BCS) with low solubility and high permeability. The aim of this investigation was to increase the solubility and hence the dissolution rate by the preparation of ketoprofen nanosuspension using solvent evaporation method. Materials like PVP K30, poloxamer 188, HPMC E5, HPMC E15, HPMC E50, Tween 80 were used as stabilizers in perpetration of different formulas of Ketoprofen nanosuspensions. These formulas were evaluated for particle size, entrapment efficiency of drug (EE), effect of stabilizer type, effect of stabilizer concentration and in-vitro dissolution studies. 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 9.4 nm to 997 nm. Entrapment efficiency was ranged from 79.23% to 95.41 %. The in vitro dissolution studies showed a significant ($p<0.01$) enhancement in dissolution rate of nanosuspension formulas compared to pure drug (drug alone) and physical mixture (drug and stabilizer). 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, Dissolution rate.

تحضير وتقييم معلق نانوي للكيتوبروفين باستخدام تقنية تبخير المذيب

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الخلاصة

المساحة السطحية لجُسَيْم الدواء تزداد بواسطة تقليل حجم الجُسَيْم وذلك لان الذوبان يحدث على سطح المذاب. المساحة السطحية الأكبر يرافقها المزيد والسريع في معدل الذوبان. الكيتوبروفين هو ضمن الصنف الثاني (نظام تصنيف الصيدلانيات البيولوجية) والذي يملك ذوبانية قليلة مع نفاذية عالية. الغرض من البحث هو زيادة الاذابة ومعدل الذوبان للكيتوبرفين من خلال تحضير معلق النانو بطريقة تبخير المذيب. مواد مثل بولي فنيل بيروليدون والبلوكسامير ١٨٨ و الهايدروكسي بروبل مثيل سليلوز والتوين تم استخدامها كمثبتات في تحضير صيغ مختلفة من معلق النانو للكيتوبروفين. هذه الصيغ تم تقييمها من خلال حجم الجُسَيْم وتأثير نوع المثبت وتركيز المثبت ودراسات الذوبانية بالمختبر. كل الصيغ المحضرة للجُسَيْمات النانوية للكيتوبروفين أظهرت حجم الجُسَيْم ضمن مدى النانو و ان معدل حجم الجُسَيْم لصيغ الجُسَيْمات النانوية للكيتوبرفين لوحظت بين ٩,٤نانومتر الى ٩٩٧ نانومتر . كفاءة التحميل كانت بين ٧٩,٢٣% الى ٩٥,٤١% . ان دراسات الذوبان بالمختبر أظهرت تحسن مهم ($p<0.01$) في معدل الذوبان لصيغ معلق النانو بالمقارنة للدواء وحده او الخليط الفيزيائي من الدواء مع المثبت. النتائج تبين ملائمة طريقة تبخير المذيب للكيتوبرفين مع تحسين معدل الذوبان بالمختبر وهذا ربما يعزز الفعل الأول والسريع للدواء.

الكلمات المفتاحية : كيتوبرفين، معلق نانوي ، حجم الجسيم ، معدل الذوبان .

Introduction

Bioavailability of drug depends on its dissolution and availability as a solution form at the site of absorption. Therefore, the main problems associated with poor soluble drug its low solubility in biological fluids, which results into low bioavailability after administ ration. Poor soluble drug will typically show dissolutionrate limited absorption [(1)]. Drug dissolution in biologic fluids is an important step for systemic absorption.

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The rate at which drugs with poor aqueous solubility dissolve and release from dosage form in the biologic system frequently controls the rate of systemic absorption of the drug. About 40% of different chemical entities discovered now are poorly water soluble [(2)]. Therefore, the pharmaceutical researches focus on enhancing the solubility and rate of dissolution of poorly water-soluble drugs.

According to US food and drug administration (FDA), the drug are classified into four classes based on aqueous solubility and permeation through biological membrane. These classes are follows[(3)] : class I (highly soluble, highly permeable), class II (poorly soluble , highly permeable), class III (highly soluble, poorly permeable),and class IV (poorly soluble, poorly permeable) Poor water soluble drug has many problems such as low or variable bioavailability, large dose and delayed onset of action. 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 [(4)].
The Ostwald–Freundlich equation describes the relationship between the saturation solubility of drug and its particle size:

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

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) [(5)].

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[(6)]. 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 [(7)].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.

Methods

Determination of Ketoprofen saturation solubility

Saturated solubility measurements of Ketoprofen in various solutions were determined by shake flask method. An excess amount of Ketopofen were separately introduced into stoppered conical flask containing 10 ml of

0.1 N HCl (pH 1.2), DW and buffers of phosphate (pH 6.8 and 7.4). 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 0.45µm 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 revolution 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).

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)	Tween 80 (ml)
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	50						0.1
17	50						0.2
18	50						0.3

Continued table (1)

Formula NO.	Drug (mg)	PVP k30 (mg)	Poloxamer 188 (mg)	HPMC E5 (mg)	HPMC E15 (mg)	HPMC E50 (mg)	Tween 80 (ml)
19	50	50	50				
20	50	50		50			
21	50	50			50		
22	50	50				50	
23	50	50					0.1
24	50		50	50			
25	50		50		50		
26	50		50			50	
27	50		50				0.1
28	50			50			0.1
29	50				50		0.1
30	50					50	0.1
31	100	100					
32	100	200					
33	100	300					
34	100		100				
35	100		200				
36	100		300				
37	100			100			
38	100			200			
39	100			300			
40	100				100		
41	100				200		
42	100				300		
43	100					100	
44	100					200	
45	100					300	

Effect of type and concentration of stabilizer on the size of Ketoprofen nanosuspensioins

To reach the best formula different types of stabilizers at various concentrations were used in preparation of Ketoprofen nanosuspensions. The formulas (F1-F15) prepared by using these different stabilizers and were subjected to particle size analysis. The combinations of two stabilizers (F19-F30) were also studied to determine their effect on particle size.

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**.**

Determination of entrapment efficiency of drug (EE) in Nanosuspension

The freshly prepared nanosuspensions of different drug: stabilizer ratios were centrifuged at about 20,000 rpm for 20 minutes at 4°C using cooling ultracentrifuge. The concentration of free drug was detected by measuring the absorbance an appropriately diluted sample of supernatant at 260 nm using UV- spectrophotometer [11-13]. EE was determined by subtracting the weight of free drug in the supernatant layer of solution from the initial weight of drug used. For each formula, the experiment was repeated in triplicate and the average was calculated. Percentage of entrapment efficiency (EE) could be calculated by the following equation:

$$\boldsymbol{Entrapment\ efficiency}\% = \frac{\boldsymbol{weight_{initial\ drug} - weight_{free\ drug}}}{\boldsymbol{weight_{initial\ drug}}} \times \mathbf{100} \qquad (\mathbf{2})$$

In vitro dissolution of Ketoprofen nanosuspensions

In vitro dissolution study was performed by USP dissolution apparatus-type II using 900 ml of 0.1N HCl (pH 1.2) as a dissolution medium maintained at 37 ± 0.5°C and stirring speed (50 rpm). The freshly prepared nanosuspensions (equivalent to 50 mg of Ketoprofen) of drug: stabilizer ratios were added to the dissolution medium, five-milliliter samples were withdrawn at specific intervals of time (5,10,15,20 ,30,40.50,60,70,80 and 90 minutes), then filtered through a 0.45 µm filter paper and analyzed for their drug concentrations by measuring at 260 nm wavelength. The same test was done for pure drug (free drug in dissolution media) and physical mixture of drug powder and stabilizer material (PM) [14].

***In vitro* dissolution / model independent approach**

The percent of dissolution efficiency (%DE) was detected for comprising the relative performance of Ketoprofen nanosuspension at drug: stabilizer ratios, pure Ketoprofen powder and physical mixture of drug and polymer. The %DE at 60 minutes (%DE60 min) for each formulation was computed (using Microsoft Excel program) as the percent ratio of area under the curve of dissolution up to the time *t* to that of the rectangle area described by complete dissolution (100%) at the same time [15, 16].

$$\mathbf{DE} = \{\int_{t1}^{t2} \boldsymbol{y.dt}\ \mathbf{x}\ /y_{100}\ \mathbf{x}\ (t_2\text{-}t_1)\}\ \mathbf{x}\ 100 \qquad (\mathbf{3})$$

The difference factor (f_1) evaluating the percent error between two curves over all time points is given by:

$$f_1 = \{ [\sum_{t=1}^{n} | R_t - T_t |] / [\sum_{t=1}^{n} R_t] \}\ \mathbf{x}\ 100 \qquad (\mathbf{4})$$

Where *t* is the number of dissolution sample, *n* is the dissolution times number, and R_t is the amount of the reference drug and T_t is amount of test drug dissolved at each time point *t*. The percent error is zero when the test drug and reference outlines are matching and increase correspondingly with the dissimilarity between the two dissolution profiles.

The similarity factor (f_2) is a logarithmic transformation of the sum-squared error of differences between the test *Tt* and reference *Rt* over all time points, and is given by:

$$f_2 = \mathbf{50x\ log}\ \{ [\ 1 + \frac{1}{n}\sum_{t=1}^{n} |Rt - Tt|^2\ ]^{-0.5}\ \mathbf{x}\ 100 \qquad (\mathbf{5})$$

The standards for similarity factor are 50- 100, while for dissimilarity factor are 0-15. The dissolution parameters were detected by fitting the data of dissolution using a software

program, called DDSolver, which is a menu-driven add-in program for Microsoft Excel written in visual basic for applications.

Results and Discussion

Determination of Ketoprofen saturation solubility

The saturation solubility values of Ketoprofen were found to be about 0.11mg/ml, 0.205mg/ml, 9.82mg/ml and 10.75mg/ml, in D.W, pH 1.2, pH 6.8 and pH 7.4, respectively. The results of saturation solubility of Ketoprofen were illustrated in table (2). Determination of saturated solubility of pure Ketoprofen powder in phosphate buffers (pH 6.8 and 7.4) and 0.1N HCl (pH 1.2) was necessary to maintain the sink condition that is the volume of medium at least three times greater than the necessary volume to form a solution saturated with drug substance [17].

According to biopharmaceutical classification system, Ketoprofen is an example of Class II drugs having low aqueous solubility and well absorption through gastrointestinal tract [18] 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 [7].Therefore, its solubility increase with pH increase towards alkaline medium [19] as shown in figure (1).

Table (2): Saturation solubility values of Ketoprofen in different media

Media	Solubility (mg/ml)
Distilled water D.W	0.11
0.1N HCl (pH1.2)	0.205
Phosphate buffer (pH 6.8)	9.82
Phosphate buffer (pH 7.4)	10.75

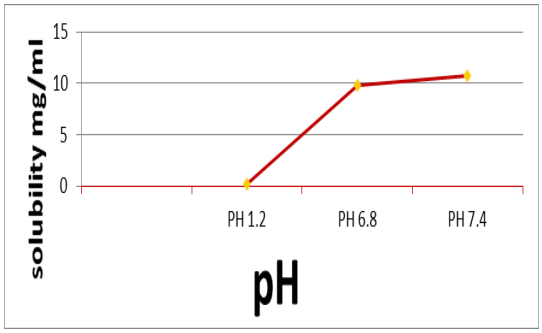

Figure (1): Solubility-pH profile of Ketoprofen powder

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 9.4 nm to 997 nm, as shown in tables (3) and (4).The smallest size 9.4 nm for F18 and the largest size 997nm for F26 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[20]. The SSA values for the prepared formulas were at range (2.07-247.47) m^2/g, the largest surface area is recorded in F18 formula and smallest surface area $2.07 m^2/g$ in F26 formula as appeared in table (3 and 4).

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 0.00466 to 0.019 depending on formulation variables. The formula F28 showed lowest PDI (0.00466), as seen in table (3), 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) [21].

Effect of stabilizer type (optimization of polymer used)

The effect of using one stabilizer

The average particle size for formulas (F1-F18) at drug: stabilizer was ranged from 9.4 nm-676.5 nm as seen in table (3). PVP K-30, poloxamer188, HPMC (E5, E15, and E50) and Tween 80 were used as stabilizers in these formulas (F1-F18). PVP K-30 and poloxamer188 are polymeric nonionic stabilizers for nanosuspensions, they form physical barrier on the surface and interrupt the contact of the close particles [22]. The non-ionic stabilizers with amphiphilic moieties are usually employed to give steric stabilization, which is dominated by wetting effect.

When non-ionic stabilizers are incorporated into nanosuspensions, they are adsorbed onto the surface of the drug particlesth rough an anchor part that is strongly interacted with the suspended particles [23], while the other part that is well-solvated tail will extend into the dispersion medium. HPMC was frequently used as a stabilizer due to its alkyl substituent, which has higher affinity towards the hydrophobic surface of drug particle [24].Figure (2), shows the effect of polymer type on particle size.

Generally HPMC at 1:1 of drug: stabilizer ratio shows smaller submicron size (9.4nm) when compared with PVP K30 and poloxamer188 because the hydrophobic moiety of HPMC has a good affinity toward the drug particles and thereby it is able to provide active steric barrier against particles growth. On the other hand, the large size of particle (426.5nm) was obtained in formula F4 that contains poloxamer 188 as a stabilizer at drug: stabilizer 1:1. These results are in agreement with that obtained by El-Badry M *et.al*. in preparation of Albendazol nanosuspension[25].

The size of other formulas lies between the average sizes ranges, since the differences in particle size are due to different in affinity of the polymer molecules toward drug particles**.**

PDI for formulas (50mg of drug and 50mg of stabilizer) F1, F4, F7, F10, F13 and F16 (50mg of drug and 0.1 ml of Tween 80) ranged from 0.008 to 0.016, therefore all these formulas have monodisperse standard.

SSA of the formulas F1, F4, F7, F10, F13 and F16 was ranged from 21.02m^2/g to 5.34 m^2/g. larger surface area was found to be 21.02m^2/g in formula F16 which contain Tween 80 as a primary stabilizer, because it had smaller particle size. In contrast to this result, a smaller surface area 5.34 m^2/g was obtained in formula F4 that contain poloxamer188 as a stabilizer, since it had lager particle size.

Table (3) :Particle size, polydispersity index and specific surface area of Ketoprofen formulas using different types and amounts of stabilizers

Formula No.	Average Particle size (nm)	PD	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
F16	95.1	0.016	21.02
F17	88	0.012	24.97
F18	9.4	0.017	247.47

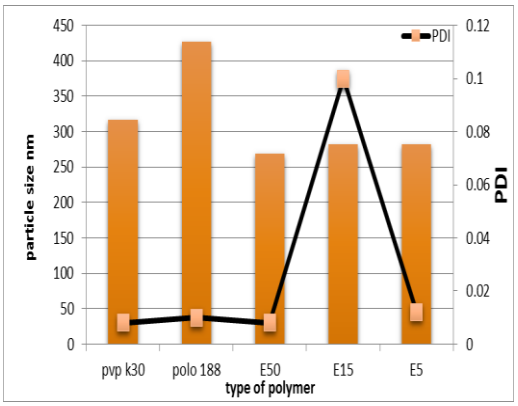

Figure (2): Effect of using different types of polymers on particle size at ratio 1:1 (F1, F4, F7, F10 and F13).

The effect of Co- stabilizers

Combination of stabilizers was preferred for long-term stabilize. Based on the Stokes'principle, drug nanocrystals tend to precipitate in the production media like water or other vehicles [23] .The appearance of agglomeration was largely due to the small particle size, which leads to increment surface-volume ratio that can produce a large amount of Gibbs free energy. Slowly, the particle will collective automatically to decrease the extra surface energy. When it comes to crystal growth, the theoretical background is Ostwald ripening. For that reason, a stabilizing agent is used to solve this problem. Stabilizers can efficiently decrease the surface activity energy to inhibit aggregation that decrease dissolution rate [23]. The critical parameter of stabilizer is the stabilization ability by enhancement of the physical stability of nanoparticulate system and maintaining the smallest size of particles. [26]. The average particle size of F19-F30 was ranged from 49 nm to 997 nm as shown in table (4).

Table (4): Particle size, polydispersity index and specific surface area for ketoprofen formulas using combined stabilizer system

Formula	Type of mix polymers	Average Particle size (nm)	PDI	SSA (m^2/g)
F19	PVP+polo188	49	0.008	45.40
F20	PVP+ E5	399	0.012	6.13
F21	PVP +E15	503	0.071	4.39
F22	PVP+E50	564	0.018	3.91
F23	PVP+tween80	99.9	0.019	24.73
F24	Polo188+E5	399	0.007	5.75
F25	polo188+E15	797	0.008	2.73
F26	Polo188+E50	997	0.005	2.07
F27	Polo188+tween80	84.85	0.011	31.45
F28	E5+tween 80	111	0.00466	21.62
F29	E15+tween80	119	0.01	17.87
F30	E50+tween80	141	0.01	15.09

According to the above results, a smaller particle size (49 nm) was achieved in a combination between PVP K30 and poloxamer188. Since this combination show significant ($p<0.05$) reduction in particle size as when compared with other combinations, and it may be because of the highest affinity to the drug molecules, the same data was reported by XueMing Li *et.al*. in Fenofibrate nanosuspension[27]. A combination of PVP_K30 and HPMC (E5, E15, and E50) gave a large particle size as when compared with each polymer separately, this result was in agreement with Shahzeb Khan *et.al* in preparation of Ibuprofen nanocrystal[28].

Furthermore combination of poloxamer188 and HPMC (E5, E15, E50) in formulations F24, F25 and F26 shows larger average particle size with significant differences($p<0.05$), these results due to that the combination lead to increase viscosity of the disperse media, so it is ineffective combination and cannot stabilize the nanoparticulate system.

On the other hand, a mixture of Tween 80 with each polymer separately gave good reduction in particle size as seen in figure (3).From above results formula F19 was select as a best formula for further evaluation tests.

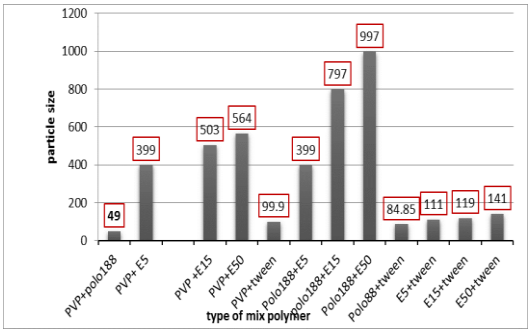

Figure (3): Effect of use of mixed polymers on particle size of Ketoprofen

A combination of Tween 80 and PVP K30 or poloxamer188 yields nanoparticles with average size not different significantly ($p>0.05$) from Tween 80 as single stabilizer. This is due to PVP K30 and poloxamer188 are polymeric molecules, have ability to adsorb on the particle surface and act as a steric barrier, preventing close contact of the particles. Furthermore, it may be because both of them are non-ionic stabilizers so no ionic repulsion occurs. Similar results were gained by P. Kocbek *et.al* in preparation of Ibuprofen nanosuspension [29]. The size of particles in other combination of Tween 80 with HPMC polymer types was larger than that obtained from combination of Tween with poloxamer or PVP K 30. PDI of formulas (F19-F30) was varied from 0.00466 to 0.019, therefore, all these formulas have monodisperse standard. Depending on formulation variables, the formulation F28 (HPMC E5 +Tween 80) showed lowest PDI (0.00466) that indicates good uniformity in average particle size distribution. A narrow size distribution is essential to prevent particle growth, due to Ostwald ripening phenomenon[30]. Formula F21 (PVP K30+HPMC E15) exhibited a highest PDI (0.071) so that is in the range of nearly monodisperse.

SSA of formulas (F19-F30) was ranged from 2.07m^2/g to 45.40 m^2/g. F19 formula (PVP K30+poloxamer188) showed large surface area about 24.73 m^2/g, since it had showed small particle size. F26 formula (poloxamer188+ HPMC E50) exhibited small surface area, due to large particle size of this formula, as particle size decrease, the surface area per unite mass increase and vice- versa.

Effect of stabilizer concentration

The concentration of stabilizer may give negative effect (decrease particle size) or positive effect of on particle size (increase particle size). It can also influence on the adsorption affinity of non-ionic stabilizers to particle surface. In general, as the concentration of stabilizer increases the particle size decreases at fixed drug concentration, which indicated that the drug particle surface was sufficiently enveloped by the stabilizer molecules [31].

Concentration of stabilizer was played a role in maintaining stability of nanosuspension, if used too low concentration lead to aggregation of particle, and if used high concentration lead to enhance Ostwald ripening [25].

As shown in table (3) the size range of particles is decrease in the sequence of F1 (317nm) > F2 (313 nm) > F3 (282.5nm) that correspond to 1:1, 1:2 and 1:3 of drug: stabilizer (PVP K30) ratio, respectively, these results indicated that mean size of particles showed a regular decrease with increasing the concentration of pvpk30. The same result was observed with poloxamer188. The size range of particles is also decreased in the sequence F4 (426.5nm)>F5 (317nm)>F6 (282.5nm) that correspond to 1:1, 1:2 and 1:3 of drug: stabilizer (poloxamer188) ratio, respectively. Figures 4 and 5 explain the effect of PVP K30 and

poloxamer188 concentration on average particle size correspondingly. These effects may be due to a process of a primary covering of the newer surfaces competing with the aggregation of the uncovered surfaces. Hence, an elevation in ratio of surfactant in the primary dispersion results in rapid enclosing of the newly formed particle surfaces. There was an optimum concentration of surfactant, above which the increase in concentration did not result in a decrease in particle size due to saturation point; these results are in agreement with that obtained by Chander Parkash Dora *et.al* in preparation of Glibenclimid nanoparticle when poloxamer188 was used as stabilizer at different ratios [32]. Poloxamer188 (pluronic F68)® is a block co-polymer, can act as a surfactant, responsible for the hydrophobic association with the molecules of drug. The inhibition of the crystal growth is mainly related to the hydrophobic part (polypropylene oxide group PPO) in the pluronic polymer, while the second chain which is (the hydrophilic polyethylene oxide) (PEO) can provide steric hindrance against particles aggregation[26].

On the other hand, as the concentration of HPMC increases the particles size increases in different ratios of grades. For grade E15 the size range is increase in sequence of F10 (282.5nm) <F11 (426.5nm) < F12 (479nm), that correspond to 1:1, 1:2 and 1:3 of drug: stabilizer (HPMC E15) ratio, respectively. The same results was observed when compared the size of particles for other grades (E5 and E50), as illustrated in figures 6, 7 and 8. These findings may be due to the addition of polymer produce a coat around drug particles until a certain concentration where all drug particles are coated with polymer, then an increasing of polymer concentration would lead to increment the thickness of the polymer coat around each particle or may be lead to aggregation of many particles. Yuancai Dong, *et.al*, obtained the opposite result when HPMC was used as a stabilizer in preparation of nanoparticles of spironolactone using antisolvent precipitation technique[33].

Furthermore, Tween 80 was also used as stabilizer in preparation of nanoparticles, as the amount of Tween 80 increased the average particle size decreased. It act as a wetting agent at low concentration or below its CMC (critical micelle concentration) and as solubilizing agent at concentration above CMC [34], since surfactant adsorption at solid-liquid interface can lead to decrease surface tension and increment in nucleation rate and lead to decrease particle size, as shown in figure (9). Sameer V. Dalvi and Rajesh N. Dave, et.al, observed similar results[35] .

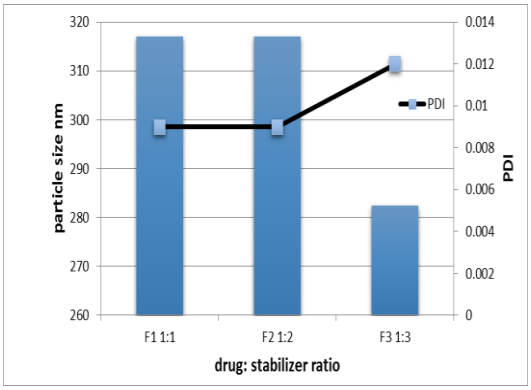

Figure (4): Effect of PVPk30 concentration on particle size

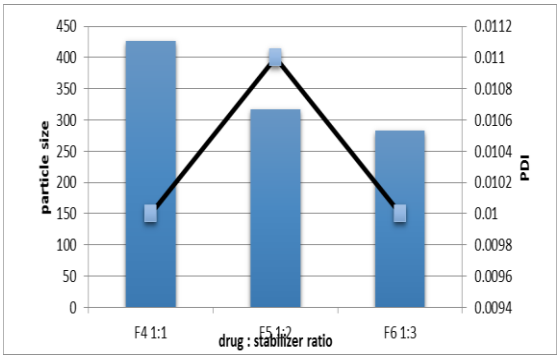

Figure (5): Effect of poloxamer188 concentration on particle size

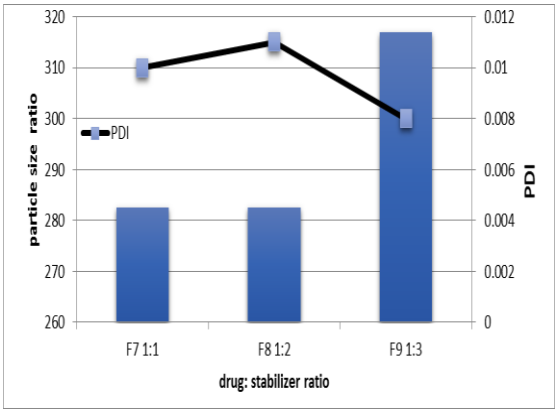

Figure (6): Effect of HPMC E5 concentration on particle size

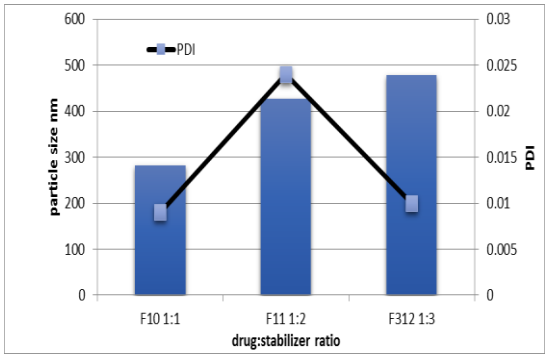

Figure (7): Effect of HPMC E15 concentration on particle size

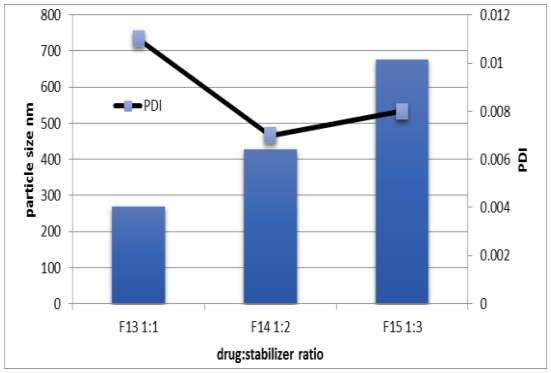

Figure (8): Effect of HPMC E50 concentration on particle size

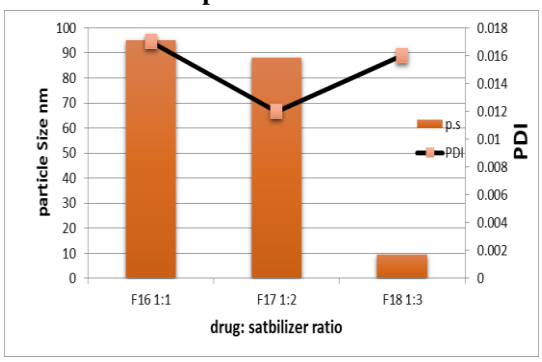

Figure (9): Effect of Tween 80 concentrations on particle size

The effect of entrapment efficiency

The encapsulation efficiency of different formulas of nanoparticles prepared from different stabilizer concentrations were evaluated, the data are illustrated in table (5), which shows that entrapping efficiency ranged from 79.23% (F7formula) to 95.41 % (F15formula) It is clear that the increase in stabilizer concentration increased the drug entrapment efficiency[36].

The higher encapsulation efficiency of F15 formula (HPMC E50 at 1:3 drug: stabilizer ratio) may be because of optimum type and concentration of polymer used. The encapsulation efficiency may be dependent on hydrophobic part of HPMC E50, which has a high affinity to hydrophobic Ketoprofen .

In-vitro dissolution study and model independent analysis of nanosuspension

The dissolution profile of prepared nanoparticles formulations was carried out for formulas F1 to F15 and for F19, F31 and F45 formulas at 1:1, 1:2 and 1:3 of drug: stabilizer ratio, because they provide the optimum entrapment efficiency. The dissolution of the prepared formulas was done in 0.1N HCl (pH 1.2) at 37°C ±0.5, the release profile was also carried out for physical mixture (drug and polymer) and for drug alone (pure drug).

Table (5) summarize the dissolution parameters for all formulas such as DP10 min (percent drug dissolved within 10 minutes), similarity and dissimilarity. These dissolution parameters were done for all formulas by fitting the dissolution data using a software program, called DDSolver[37]. A comparative study was done, since the dissolution profiles of Ketoprofen from different samples was made using *f*1 and *f*2. According to the food and drug administration's guidelines, *f*1 values lower than 15 (0–15) and *f*2 values greater than 50 (50–100) mean similarity of the dissolution profiles [38]. Figures (10, 11, 12, 13, 14 and 15) illustrated the release of drug from formulas at ratio 1:1 ,1:2 and 1:3 for single stabilizer [F1-F3(PVP K30), F4-F6(poloxamer188) , F7-F9(HPMC E5) , F10-F12 (HPMC E15) and F13-F15 (HPMC E50)] , Co-stabilizer F19 (PVP K30 and poloxamer188) , respectively.

The results shown in table (5) indicate that there is significant ($p<0.01$) enhancement in dissolution rate of all nanosuspension formulas compared to pure drug (drug alone) and physical mixture (drug and polymer). This confirms the superiority of the prepared of nanoparticles formulas compared to that of physical mixture and drug alone, since these nanoparticles provide a large surface area than others.

On comparison with free drug (pure drug), the cumulative percentage of drug release for F19, F31 and F45 formulas were estimated in 0.1N HCl (pH 1.2). The cumulative % of drug

release of these formulas was 100 % within 10 minutes.

On the other hand, a low percentage of release was obtained from pure drug (52.7%) in the same time (10 minutes) and maximum cumulative % of drug release was reached (100 %) at 80 minutes. So there is a significant differences ($p<0.01$) in release of drug between pure drug and other formulas, as shown in figure (16). These results are consistent with study, which reported by Monzurul Amin Roni *et.al* [39] .The reasons for such results that some of drug particle may be convert from crystalline to amorphous state. In addition, it is mainly attributed to the rule, which states that the reduction of drug particle size can result in larger surface area and consequently enhanced the contact of nanoparticles with the dissolution medium. The gained results are coordinated with Noyes–Whitney equation, in which the increment in saturation solubility and the decrement in particle size can lead to an enhanced dissolution rate [40].

Table (5) :Dissolution Parameters for F1 to F15, F19, F31 and F45 Formulas

Formula	DP at 10 min	DE 60 min	Dissimilarity factor *f1*	Similarity factor *f2*	Entrapment efficiency ±SD
F1	100	94.8	24.36	29.43	91.4 ± 0.152
F2	72	90.3	17.75	38.64	92.92 ± 0.014
F3	73.5	90.9	18.25	37.82	91.83 ± 0.007
F4	82.4	89.5	18.8	35.15	94.99 ± 0.045
F5	84.3	92.77	21.49	36.33	91.13 ± 0.015
F6	75	94.7	23.97	33.71	88.13 ± 0.040
F7	83.4	91.2	19.44	36.14	79.23 ± 0.040
F8	83	90.9	19.01	36.7	83.56 ± 0.080
F9	87.34	92.9	21.73	32.84	86.26 ± 0.136
F10	97.7	90.9	19.46	35.36	84.03 ± 0.020
F11	84.6	90.9	18.88	36.76	82.84 ± 0.102
F12	86.6	91.1	19.20	36.45	82.4 ± 0.050
F13	86	94.3	28.52	34.05	83.91 ± 0.055
F14	91	96	25.82	32.75	85.25 ± 0.045
F15	100	96.9	27.17	30.75	95.41 ± 0.015
F19	100	93.4	22.48	31.96	91.6 ± 0.076
F31	100	95.1	24.78	28.84	90.43 ± 0.025
F45	100	97.7	28.31	29.66	93.01 ± 0.037

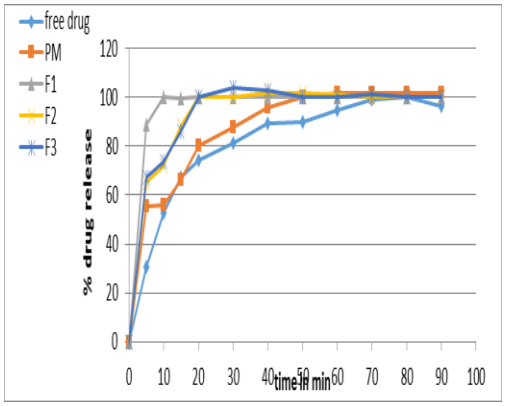

Figure (10): Dissolution profile of the prepared Ketoprofen nanoparticles (F1, F2 and F3) compared to physical mixture and free drug in 0.1N HCl (pH 1.2) at 37 C^0± 0.5.

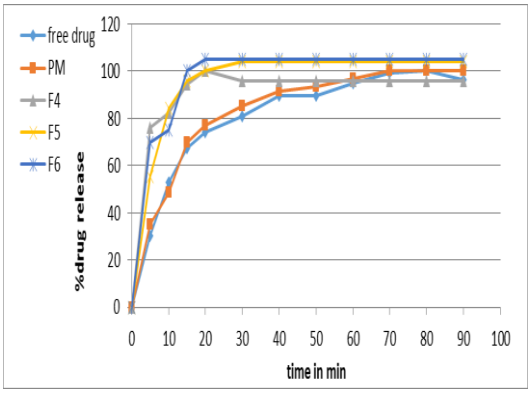

Figure (11): Dissolution profile of the prepared Ketoprofen nanoparticles (F4, F5 and F6) compared to physical mixture and free drug in 0.1N HCl (pH 1.2) at 37 C^0± 0.5.

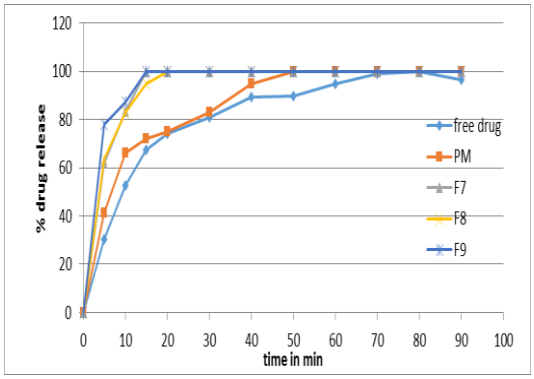

Figure (12): Dissolution profile of the prepared Ketoprofen nanoparticles (F7, F8 and F9) compared to physical mixture and free drug in 0.1N HCl (pH 1.2) at 37 C^0± 0.5.

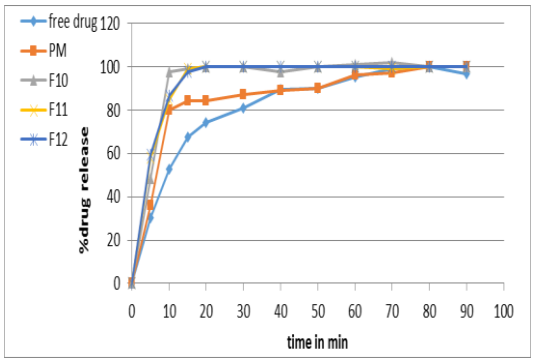

Figure (13): Dissolution profile of the prepared Ketoprofen nanoparticles (F10, F11 and F12) compared to physical mixture and free drug in 0.1N HCl (pH 1.2) at 37 C^0± 0.5.

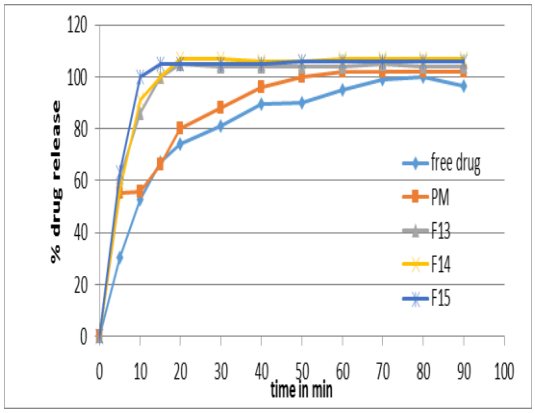

Figure (14): Dissolution profile of the prepared Ketoprofen nanoparticles (F13, F14 and F15) compared to physical mixture and free drug in 0.1N HCl (pH 1.2) at 37 C^0± 0.5.

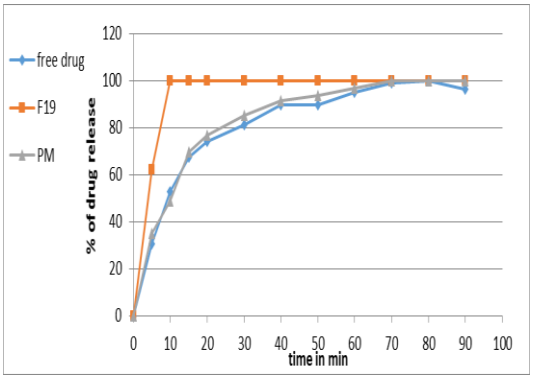

Figure (15): Dissolution profile of the prepared Ketoprofen nanoparticles F19 compared to physical mixture and free drug in 0.1N HCl (pH 1.2) at 37 C^0± 0.5.

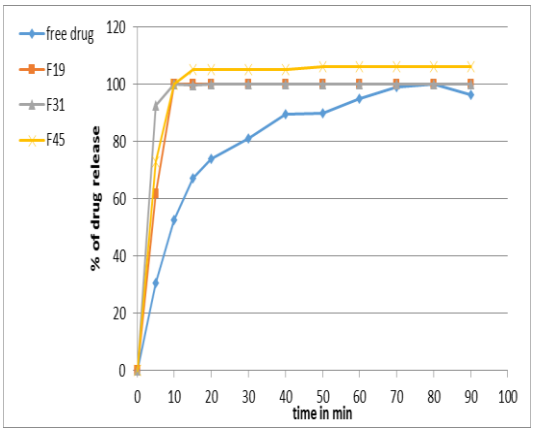

Figure (16): Dissolution profile of the prepared Ketoprofen nanoparticles (F19, F31 and F45) compared to free drug in 0.1N HCl (pH 1.2) at 37 C^0± 0.5.

Conclusion

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. Ketoprofen nanosuspensions were successfully prepared using different types of stabilizers at drug: stabilizer ratios 1:1, 1:2 and 1:3. All the prepared formulas have small particle size within Nano size range. The formed nanoparticles can significantly potentiate the dissolution rate of drug.

References

1. Ishwar D.,. Enhancement of dissolution of poorly water-soluble drug by solid dispersion technique. International Journal of Pharmaceutical Sciences Review and Research, 2017;43 (1):155-160.

2. Heo MY, Piao ZP, Kim TW, Cao QR, Kim A, Lee BJ.,. Effect of solubilizing and microemulsifying excipient in polyethyleneglycol 6000 solid dispersion on enhanced dissolution and bioavailability of ketoconazole. Archives of pharmacal research, 2005;28:. 604-611.

3. Ku, M.S. and Dulin, W., A biopharmaceutical classification-based Right-First-Time formulation approach to reduce human pharmacokinetic variability and project cycle time from First-In-Human to clinical Proof-Of-Concept. Pharmaceutical development and technology, 2012.17(3): 285-302

4. **4**-Leon Shargel, Susanna Wu-Pong, Andrew B.C. Yu. Applied Biopharmaceutics and Pharmacokinetics, 5th Edition. 2004; chapter 14.

5. Shaal, L.A., Müller, R.H. and Keck, C.M.,. Preserving hesperetin nanosuspensions for dermal application. Die Pharmazie-An International Journal of Pharmaceutical Sciences,2010, 65(2): 86-92

6. **6**-Kumar, A., Chen, F., Mozhi, A., Zhang, X., Zhao, Y., Xue, X., Hao, Y., Zhang, X., Wang, P.C. and Liang, X.J. . Innovative pharmaceutical development based on unique properties of nanoscale delivery formulation. Nanoscale, 2013,5(18):8307-8325.

7. **7**- Pharmacopoeia, B. British Pharmacopoeia Commission London; the Department of Health. Social Services and Public Safety, 2013; 1: 719-720.

8. Mudit D, Keshvaraon K P. A novel technique to enhance the solubility and dissolution of ketoprofen using freeze dryer. International research Journal of Pharmacy, 2011; 2(12):249-252

9. Moorthi, C. and Kathiresan, K. Fabrication of highly stable sonication assisted curcumin nanocrystals by nanoprecipitation method. Drug Invention Today, 2013; 5(1):66-69.

10. Vpass A Sh. Formulation development and optimization of nitrendipine nano suspension with improved pharmacokinetic characteristics. International Journal of Pharmaceutical Sciences and Nanotechnology , 2013;6(2) :2053- .

11. Yadav, P.S., Kumar, V., Singh, U.P., Bhat, H.R. and Mazumder, B. Physicochemical characterization and in vitro dissolution studies of solid dispersions of ketoprofen with PVP K30 and d-mannitol. Saudi Pharmaceutical Journal, 2013; 21(1): 77-84.

12. Sahu, B.P. and Das, M.K. Nanosuspension for enhancement of oral bioavailability of felodipine. Applied Nanoscience, 2014;4(2): 189-197.

13. Banchero, M., Ronchetti, S. and Manna, L.,. Characterization of ketoprofen /methyl-β-cyclodextrin complexes prepared using supercritical carbon dioxide. Journal of Chemistry, 2013.

14. Abd-Elrahman, A.A., El Nabarawi, M.A., Hassan, D.H. and Taha, A.A. Ketoprofen mesoporous silica nanoparticles SBA-15 hard gelatin capsules: preparation and in vitro/in vivo characterization. Drug delivery, 2016 ;23(9):3387-3398.

15. **15**- Sahoo, N.G., Kakran, M., Shaal, L.A., Li, L., Müller, R.H., Pal, M. and Tan, L.P. Preparation and characterization of quercetin nanocrystals. Journal of pharmaceutical sciences, ., 2011;100(6):2379-2390.

16. Medina, J.R., Salazar, D.K., Hurtado, M., Cortes, A.R. and Domínguez-Ramírez, A.M.Comparative in vitro dissolution study of carbamazepine immediate-release products using the USP paddles method and the flow-through cell system. Saudi Pharmaceutical Journal, 2014 ;22(2):141-147.

17. Gupta, A., Gaud, R.S. and Ganga, S. Development of discriminating dissolution method for an insoluble drug: nisoldipine. International Journal of Pharm Tech Research, 2010;2:931-9.

18. Goenawan, J., Trisanti, P.N. and Sumarno, , December. The influence of dissolved H2O content in supercritical carbon dioxide to the inclusion complexes formation of ketoprofen /β-cyclodextrin. In AIP Conference Proceedings ,2015 ;1699(1); p. 040012. AIP Publishing.

19. Donthi, M.R., Dudhipala, N.R., Komalla, D.R., Suram, D. and Banala, N. Preparation and Evaluation of Fixed Combination of Ketoprofen Enteric Coated and Famotidine Floating Mini Tablets by Single Unit Encapsulation System. Journal of Bioequivalence and Bioavailability, 2015; 7(6), p.279.

20. Shinde, V., Amsa, P., Tamizharasi, S.Karthikeyan, D., Sivakumar, T. and Kosalge, A. Nanosuspensions: a promising drug delivery strategy. Research Journal of Pharmacy and Technology, 2010 ; 3(1):39-44.

21. Gadad, A., Chandra, P.S., Dandagi, P. and Mastiholimath, V. Moxifloxacin loaded

polymeric nanoparticles for sustained ocular drug delivery. International Journal of Pharmaceutical Sciences and Nanotechnology , 2012; 5:1727-1734.

22. Sun, W., Tian, W., Zhang, Y., He, J., Mao, S. and Fang, L. Effect of novel stabilizers—cationic polymers on the particle size and physical stability of poorly soluble drug nanocrystals. Nanomedicine: Nanotechnology, Biology and Medicine, 2012; 8(4):460-467.

23. Wu, L., Zhang, J. and Watanabe, W. Physical and chemical stability of drug nanoparticles. Advanced drug delivery reviews, 2011; 63(6) :456 - 469.

24. Rasenack, N. and Müller, B.W. Dissolution Rate Enhancement by in Situ. Pharmaceutical research, 2002; 19(12) : 1894 -1900.

25. El-Badry12, M., Fetih, G., Salem-Bekhit, M.M. and Shakeel, F. Formulation and evaluation of nanosuspension of albendazole for dissolution enhancement. Nanoscience and Nanotechnology letters, 2013; 5(9): 1024-1029.

26. Cerdeira, A.M., Mazzotti, M. and Gander, B. Formulation and drying of miconazole and itraconazole nanosuspensions. International journal of pharmaceutics, 2013;443(1):209-220

27. Li, X., Gu, L., Xu, Y. and Wang, Y. Preparation of fenofibrate nanosuspension and study of its pharmacokinetic behavior in rats. Drug development and industrial pharmacy, 2009;35(7):827-833.

28. Khan, S., Matas, M.D., Zhang, J. and Anwar, J. Nanocrystal preparation: low-energy precipitation method revisited. Crystal Growth and Design, 2013; 13(7:2766-2777.

29. Kocbek, P., Baumgartner, S. and Kristl, J. Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs. International journal of pharmaceutics, 2006;312(1):179-186

30. Lindfors, L., Skantze, P., Skantze, U., Rasmusson, M., Zackrisson, A. and Olsson, U. Amorphous drug nanosuspensions. 1. Inhibition of Ostwald ripening. Langmuir, 2006; 22(3):906-910.

31. Liu, Y., Sun, C., Hao, Y., Jiang, T., Zheng, L. and Wang, S. Mechanism of dissolution enhancement and bioavailability of poorly water soluble celecoxib by preparing stable amorphous nanoparticles. Journal of Pharmacy and Pharmaceutical Sciences, 2010;13(4):589-606

32. Dora, C.P., Singh, S.K., Kumar, S., Datusalia, A.K. and Deep, A. Development and characterization of nanoparticles of glibenclamide by solvent displacement method. Acta Polonia Pharmceutica , 2010; 67(3):283-90.

33. Dong, Y., Ng, W.K., Shen, S., Kim, S. and Tan, R.B. Preparation and characterization of spironolactone nanoparticles by antisolvent precipitation. International journal of pharmaceutics, 2009;375(1):84-88.

34. Brown, C.K., Chokshi, H.P., Nickerson, B., Reed, R.A., Rohrs, B.R. and Shah, P.A. Dissolution testing of poorly soluble compounds. Pharm. Tech., 2004; 28:56-43.

35. Dalvi, S.V. and Dave, R.N. Controlling particle size of a poorly water-soluble drug using ultrasound and stabilizers in antisolvent precipitation. Industrial and Engineering Chemistry Research, 2009 ; 48(16):7581-7593.

36. Rajalakshmi, R., Venkataramudu, T., Kumar, R.A., Sree, K.D. and Kiranmayi, M.D. Design and characterization of valsartan nanosuspension. International Journal of Pharmacotherapy, 2012;2(2):70-81.

37. Zhang, Y., Huo, M., Zhou, J., Zou, A., Li, W., Yao, C. and Xie, S. DDSolver: an add-in program for modeling and comparison of drug dissolution profiles. The AAPS journal, 2010;12(3):263-271.

38. FDA Guidance for Industry, 1997. Dissolution testing of immediate release solid oral dosage forms.

39. Roni, M.A., Islam, M.S., Kibria, G., Sadat, S.M.A., Rony, R., Rahman, H. and Jalil, R.U. Effects of poloxamer and HPMC on the dissolution of clonazepam-polyethylene glycol solid dispersions and tablets. Indian journal of pharmaceutical education and research, 2011; 45(2):139.

40. Li, W., Yang, Y., Tian, Y., Xu, X., Chen, Y., Mu, L., Zhang, Y. and Fang, L. Preparation and in vitro/in vivo evaluation of revaprazan hydrochloride nanosuspension. International journal of pharmaceutics, 2011; 408(1), :157-162.