Preparation and Evaluation of Dapsone Nanoparticles

Yasamin Abdulhadi Sallal * , Ahmed Najim Abood**

*Department of Pharmaceutics, College of Pharmacy, Kufa University, Iraq.
**Department of Pharmaceutics, College of Pharmacy, Basra University, Iraq.

Keywords: Dapsone, Nanoparticles, Anti-solvent precipitation method.

Abstract:

Objective: Preparation of Dapsone nanoparticles to get optimized solubility, rate of dissolution and then more bioavailability.

Methods: Dapsone nanoparticles were prepared using solvent-antisolvent precipitation method. A Certain amount of drug was dissolved in water-miscible solvent (ethanol or methanol), then this solution was injected at certain speed (ml/min.) into water containing stabilizer. Upon injection, precipitation of Dapsone nanoparticles will occur immediately accompanied with stirring for 30 min. at 50˚C, and then the selective formula is lyophilized. Different formulas of Dapsone nanoparticles were prepared at different polymer types, drug: polymer ratios and different organic solvent types. Characterization of these formulas involve measurement of particle size, particle morphology, saturation solubility, release profile, crytallinity, physical and chemical compatibilities between the drug and involved stabilizers.

Results: Results show that the best formula of nanoparticle (F3) prepared by dissolving 16.6 mg/ml of Dapsone in ethanol, then 3ml was injected into to a 25 ml of water with PVP (polyvinyl pyrrolidone) as a stabilizer at ratio 1:4 (drug: stabilizer) then lyophilized to obtain nanoparticles. The best formula characterized by particle size around 28.5 nm, poly-dispersity (= 0.009), specific surface area (= 78.18), without physical or chemical incompatibility, and the dissolution rate was significantly higher than that of the raw Dapsone powder.

تحضير وتقييم الجسيمات النانوية للدابسون

مفتاح الكلمات: دابسون, الجسيمات النانوية,طريقة الترسيب للمذيب المضاد.

الخلاصة:

الهدف من هذه الدراسة: تحضير الجسيمات النانوية لدواء الدابسون من اجل زيادة معدل ذوبانيتة وسرعة تحرره مما يزيد التوافر الحيوي.

طريقة العمل: تم تحضير الجسيمات النانوية للدابسون باستخدام طريقة الترسيب بواسطة المذيب / المذيب المضاد .حيث تم اذابة كمية معينة من الدواء في محلول قابل للامتزاج مع الماء (بأستخدام الايثانول او الميثانول)، ثم تم حقن هذا المحلول في الماء التي يحتوي على المثبت. عند الحقن ، يحدث ترسيب للجسيمات

النانوية للدابسون على الفور باستخدام درجة حرارة 50 مئوية وتحريك لمدة 30 دقيقة. ثم يجفف بالتجميد، نماذج مختلفة تم تحضيرها بأستخدام مثبتات مختلفة, نسب عقار: مثبت مختلفة , ومذيبات عضوية مختلفة . بعدها

تم تقييمها من خلال تحديد حجم الجسيمات , الشكل العام , مقدار الذوبانية , نمط التحرر , الطبيعة البلورية مع تشخيص مقدار التداخل الفيزيائي والكيميائي بين الدواء والمثبت المستخدم.

النتائج: تظهر النتائج ان افضل صيغة للجسيمات النانوية للدابسون نتجت عن طريق اذابة (16.6) ملغ/مل من الدابسون في الايثانول ، ثم يحقن 3مل من هذا المحلول في 25 مل من الماء الذي يحتوي بولي فينيل يايروليدون كمثبت بنسبة 4:1 (الدواء:المثبت) ، ثم يجفف بالتجميد للحصول على مسحوق الجزيئات النانوية للدابسون.

الصيغة الافضل تتميز بحجم الجزيئات يتراوح (28.5) نانوميتر , متعدد التوزيع (0.009) , والمساحة السطحية المحددة (78.18) مع غياب التداخلات الفيزيائية والكيميائية بين العقار والمثبت المستخدم ومعدل الانحلال اعلى بكثير من ذلك الخاص بمسحوق الدابسون الخام.

1.INTRODUCTION

Dapsone (4,4'-diamino-diphenylsulfone, DAP) is a synthetic sulfone drug with bacteriostatic activity, mainly active against Mycobacterium leprae. The bacteriostatic activity acts by competitive inhibition of the enzyme dihydrofolate synthetase and dihydrofolate reductase, key enzymes in the folate biosynthesis pathway in Mycobacterium (1–3). DAP APis DAPDAP a

The present study aimed to prepare DAP amorphous nanoparticles involving freeze-drying after solvent evaporation method. The NPs were subsequently characterized regarding their crystallinity, morphology, molecular interactions and In Vitro drug dissolution study.

2. MATERIAL AND METHODS

2.1 Materials and Instruments

Dapsone (DAP), PVP k30, poloxamer, and HPMC E50 were purchased from Shanghai send pharmaceutical technology co. Ltd. China. Methanol was purchased from GCC analytical reagent UK, Ethanol was obtained from Hayman.Ltd.UK, HCl got from BDH Laboratory supplies England, Potassium dihydrogen orthophosphate was purchased from SD fine-Chem Limited.MUMBAI.and Disodium hydrogen phosphate obtained from BDH Laboratory Supplies. England.

2.2 Methods

2.2.1 Spectrophotometric scanning of Dapsone

Several stock solutions of 10 mg of drug per 100 ml of 0.1 N HCl (pH 1.2) and UV .DAP

2.2.2 Preparation of calibration curves

The calibration curves for DAP in 0.1 N HCl (pH 1.2) and 0.2 M phosphate buffer solution (pH 6.8) were measured separately, the absorbances of different concentrations of DAP solution were measured and plotted against their respective concentrations(13).

2.2.3 Preparation of Dapsone nanoparticles

Dapsone nanoparticles were prepared by the precipitation technique which is also called solvent/ antisolvent precipitation method(14). DAP, was dissolved in 3 ml of organic solvent (ethanol or methanol) at room temperature, then the resultant organic solution of drug was added to 25 ml of water containing certain type of stabilizer with subsequent agitation at an stirring speed (500 round per minute) (rpm) by a magnetic stirrer for about 30 minutes for evaporating the organic solvent. Addition of organic solvents at a certain rate(ml/min.) by means of a syringe positioned with the needle directly into aqueous stabilizer solution. Variable drug to stabilizer ratios were used (1:1,1:2 and 1:4), to prepare eighteenth formulas (F1-F18) which are demonstrated in table (1) with their compositions.

Table (1) Compositions of dapsone formulas that prepared from different types of stabilizers (PVP, Poloxamer or HPMC E50) with Different organic solvents (Ethanol or Methanol).

Formula no.	Dapsone wt. (mg)	Pvp wt. (mg)	Poloxamer wt. (mg)	HPMC E50 wt. (mg)	Ethanol volume (ml)	Methanol volume (ml)	Water volume (ml)
1	50	50			3		25
2	50	100			3		25
3	50	200			3		25
4	50		50		3		25
5	50		100		3		25
6	50		200		3		25
7	50			50	3		25
8	50			100	3		25
9	50			200	3		25
10	50	50				3	25
11	50	100				3	25
12	50	200				3	25
13	50		50			3	25
14	50		100			3	25
15	50		200			3	25
16	50			50		3	25
17	50			100		3	25
18	50			200		3	25

2.2.4 Evaluation of Dapsone nanoparticles

- Analysis of particle size

Analysis of Particle size for the prepared DAP formulas (F1-F18) was done using (Analyzer of nano laser particle size ABT- 9000) at 25ºC without dilution of the samples at an angle of scattering about 90º. The average particle size (D) (3, 4) was measured for all the prepared formulas. In addition, for each formula the poly-dispersity index (PDI) was determined as a measurement for the size distribution width. PDI is an index of variation within the size distribution of particles. The analyzer can also detect the specific surface area for each formula.

- Freeze drying of the prepared Dapsone nanoparticles

The selected formula with the best average particle size and PDI was lyophilized using a freeze dryer system at a controlled temperature of (- 40 ˚C) with pump operating at a pressure of 0.5 mbar over a period of (48–72) hours. The lyophilized powder was further investigations (15).

-Determination of Dapsone solubility

Solubilities of pure DAP, physical mixture (DAP+PVP) and the best formula from study were determined using shake-flask method for different media (water, 0.1N HCl pH 1.2 and phosphate buffer solution pH 6.8) in which an excess amount of drug was placed in contact with 10 ml of medium with stirring 99 rpm at 37°C. The mixture was shaken for 48hr. and the sample was withdrawn, filtered then diluted with solubility medium to measure the absorbance by spectrophotometer at a specific wavelength for each media to get the amount of drug that dissolved(15).

equivalent to 50 mg of DAPDAP. All these samples were grounded and combined with potassium bromide. The run was between the wave number of 4000 - 400 cm-1,**-In Vitro dissolution study**

In Vitro dissolution study was performed using USP dissolution apparatus-II (Paddle assembly) in 0.1N HCl (pH 1.2) and phosphate buffer solution (pH 6.8) as dissolution media at 37 ± 0.5°C and 50 rpm. It was performed for powder of the pure drug, physical mixture (drug and stabilizer) and the selected formula (F3) in a flask (900 ml in volume). Samples (5ml) were withdrawn at (2, 4, 6, 8, 10, 15, 20, 30, 60, 90, 120 and 150) minutes and replaced with fresh dissolution medium. Samples were filtered through syringe filter 0.22 μm and assayed spectrophotometrically at the determined wave length (15,16) .

-Fourier Transform Infrared Spectroscopy (FTIR)

Spectra were obtained using FTIR spectroscopy for pure Dapsone powder, PVP k30 and lyophilized powder of selected formula (F3) (17).

- Differential scanning calorimetry (DSC)

DSC can be utilized to detect the compatibility between DAP and polymer. It can also give idea about the crystallinity of DAP especially if prepared as nanoparticles. Thermal properties of the same samples that are previously evaluated by FTIR, were determined using an automatic thermal analyzer system (Shimadzu. DSC–60.Japan). Precisely weighed samples (5mg) were put in non- closed

hermetically aluminum pans and exposed for heating rate (20 ˚C/minute) against an empty aluminum pan as a reference one covering a range of temperature range about (50 - 300 ˚C) (18,19).

It is6 to the lyophilized selected formula **-Powder X-ray diffraction (XRD)**

It is used to detect the atomic and molecular structure of crystalline compounds such as drugs and additives . The crystalline nature of tested samples can

be confirmed by patterns of X-rays diffraction (diffractograms). Therefore, these information can be used to verify whether the tested samples are crystalline or amorphous. The analysis was achieved by using powder X-ray diffraction (XRD-6000, Shimadzu, Japan 220V/50Hz) at continuous scan range of $2\theta = 5 - 50^{o}$, the operating voltage and current were 40 (kV) and 30 (mA) respectively (20,21).

-Surface morphology study:

Scanning electron microscopy (SEM)

Scanning electron microscope of pure DAP and lyophilized formula selected were confirmed by direct deposition of powder on double-sided carbon tape and coated with gold, The samples were visualized using a scanning electron microscope operated with a secondary detector at different acceleration voltage and at different magnification(22,23).

Atomic force microscopy (AFM)

The surfaces of substances can be scanned using AFM in controlled environmental conditions which is complementary to SEM imaging. It can also measure the size and surface morphology of the nanoparticles accurately. Wetted sample (droplets) from the selected formula was deposited on freshly cleaved mica and dried 15 minutes in the oven by the technique of droplet evaporation. Particle size, 3D-dimension graph, and a histogram of particle size distribution were obtained by this test (24,25).

3. Results and discussion

3.1 Determination of λMax.

Scanning of solutions that contains 10 μg /1ml of DAP in 0.1N HCl and phosphate buffer solution using UV spectrophotometer at 200-400 nm gave the spectrum shown in figures (1) with a peak for λ max. at 288.4 and 291nm respectively. The results were in agreement with the reported ones (11,12).

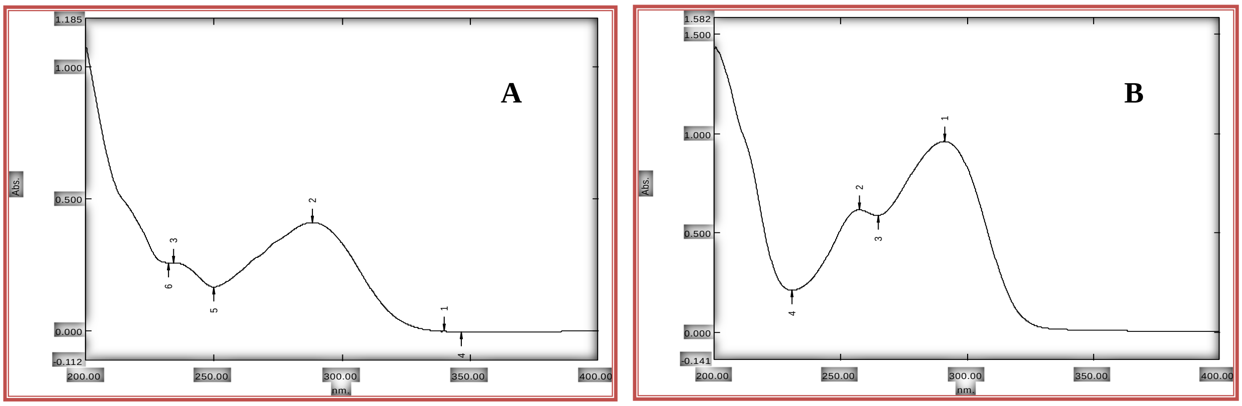

Figure (1): Dapsone spectrum in HCl 0.1N(pH 1.2) (A) and phosphate buffer solution (pH 6.8) (B).

3.2 Calibration Curve of Dapsone

Figure (2) A and B shows the calibration curves of DAP in 0.1N HCl and phosphate buffer solution respectively. A straight line was obtained by plotting the absorbance versus concentration. This indicates that calibration curve obeys Beer's-Lambert law within the range of used concentrations (26,27).

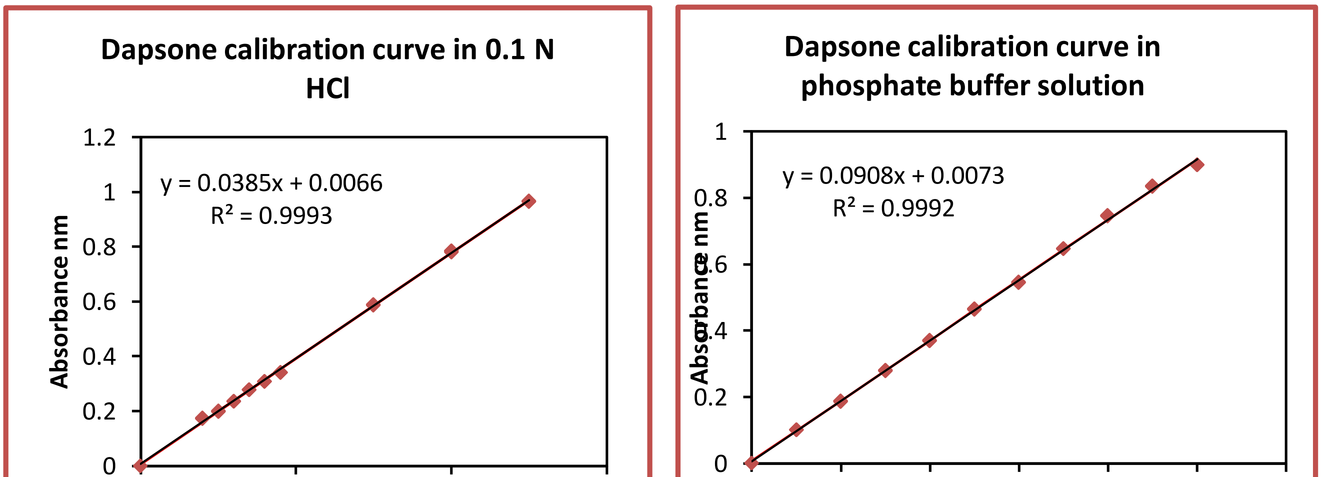

Figure (2): Calibration curves of Dapsone in 0.1 N HCl (A) and in phosphate buffer solution (B) .

3.3 Measurement of particle size of the prepared formulas

-Effect of change of organic solvent type

In this study two types of organic solvent were used, ethanol (F1-F9) and methanol (F10-F18) containing three different types of stabilizer in three different drug:stabilizer ratio (1:1,1:2 and 1:4).The results showed that the particle size was larger when methanol used as organic solvent in compare with ethanol. This may be because of variation in evaporation rate (methanol faster than ethanol) and higher solubility of DAP in methanol rather than than ethanol, so higher drug concentration generates higher super saturation which results in more aggregation possibility and thereby larger particles. Also, on an increase of drug concentration, the viscosity is also increased, which will hinder the diffusion within solvent and anti-solvent, leading to non-uniform super saturation, slower nucleation rates, and increased particle agglomeration, and hence, larger particle (28).

-Effect of using different ratios of different types of stabilizer

Some of the prepared DAP nanoparticles formulas showed a particle size result within nano range as shown in table (2). It has been shown that formula (F3) that used ethanol as organic solvent has the lowest particle size, this may be because of the high PVP concentration (at 1:4 drug: stabilizer ratio) which can cause decrease aggregation of drug particles and more finely dispersed within the solvent used, the PVP has high affinity for both surfaces (hydrophilic and hydrophobic ones) (20). And this means that PVP has a higher affinity to adsorb DAP rather than other stabilizers (Poloxamer 188 and HPMC) .The particle size is inversely proportion to

the surface area so the smallest particle size result in high specific surface area that can calculated by equation (1) :

$$S.S.A = \frac{6}{Diameter} \quad \text{----------------------(1)}$$

When the surface area increased, the dissolution rate also increased according to (2), so this formula was subjected for further evaluation (31,32)

$$\frac{dC}{dt} = \frac{DS}{Vh}(C_s - C) \quad \text{----------------(2)}$$

Table (2): Average particle size, poly-dispersity index and specific surface area of Dapsone nanoparticles formulas using constant volume of organic solvent.

Formula no.	Particle size D[4.3]	PDI	S.S.A
1	516	0.007	4.31

2	737	0.022	3.03
3	28.5	0.009	78.18
4	648	0.454	4.64
5	505	0.186	5.05
6	1883	0.009	1.18
7	229	0.069	10.26
8	4720	0	0.47
9	1605	0.028	1.39
10	764	0.006	2.91
11	613	0.007	3.63
12	12.3	0.023	182.51
13	1507	0.002	1.47
14	1185	0	1.87
15	940	0.003	2.36
16	312	0.36	9.07
17	4256	0.003	0.52
18	4720	0	0.47

-Saturation solubility of Dapsone

Different saturation solubility values of DAP powder were obtained in 0.1 N HCl pH (1.2), phosphate buffer pH(6.8) and water at 37±0.5°C.As seen in figure (3), the resulted solubilities were 2.326 ± 0.004 and 0.326 ± 0.002 and 0.141±0.001 mg / ml in pH 1.2, pH6.8 and water respectively. These solubility values can be interpreted on the basis that DAP is a basic drug and it has pKa value of 1.3, 2.5. So it's more soluble in acidic media than basic media because at lower pH, the amino group will be protonated so the solubility increased while in pH 6.8 and water the solubility is low because of the maintenance of the molecular structure and these result agrees with that obtained by previous studies (29,30). The result of saturation solubility of the selected formula (F3) was increased 7.26 times in water (pH about 5.8) and 3.8 times in phosphate buffer (pH 6.8) while in 0.1 N HCl (pH1.2) was 10 times as shown in figure (3)

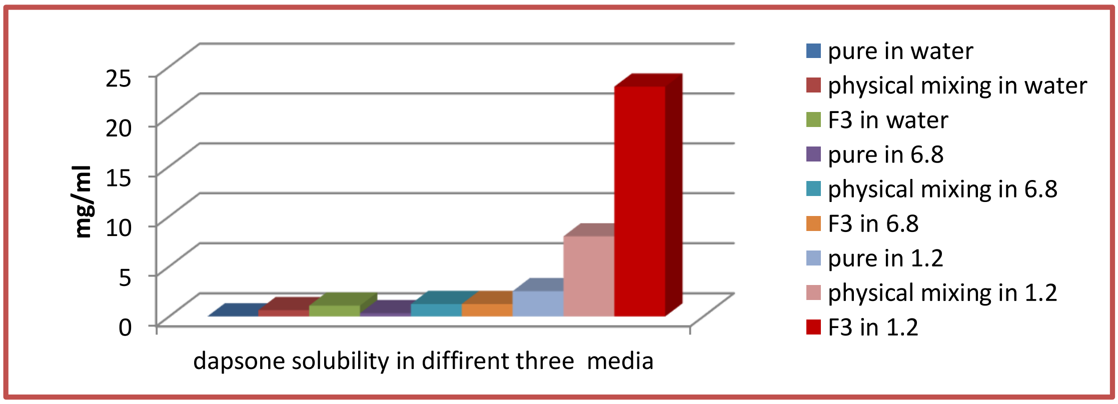

Figure (3): Solubility values of pure Dapsone powder, physical mixture and F3 in three different media.

-In Vitro dissolution study

From the release study and its parameters,F3 is the best formulas according to DP10,%DE, MDT, *f1* and *f2* factors presented in table (3).The release of DAP in F3 was compared with the pure drug powder in both media (0.1N HCl and phosphate buffer) as in figure (4).The maximum cumulative percentages of drug release from formula F3 were 99.24 % and 85.3 % within 10 minutes in 0.1N HCl and phosphate buffer solution respectively, wherease the pure drug has % release of 40% and 11 % within 10 minutes in 0.1N HCl and phosphate buffer solution respectively. This may be because of reduction of in particle size can result in larger surface area and consequently enhancing the contact of nanosized particles with the dissolution medium. The obtained results were in good accordance with Noyes–Whitney equation which states that when saturation solubility increases and particle size decreases, this can lead to an increased dissolution rate (31).

Table (3) The dissolution data for pure Dapsone powder, physical mixture and F3 in the dissolution media

Sample type	Dissolution media	DP10	MDT	%DE	F1	F2
Pure drug	Phosphate buffer	11.05	28.228	17.783		
F3	=	85.3	5.271	89.05	80.845	9.123
physical mixture	=	11.7	24.684	16.46	11.985	79.269
Pure drug	0.1N HCl	40.216	23.065	60.9		
F3	=	99.24	0.77	97.208	44.719	16.343
physical mixture	=	35.865	27.011	53.54	11.168	61.219

the DAP(-

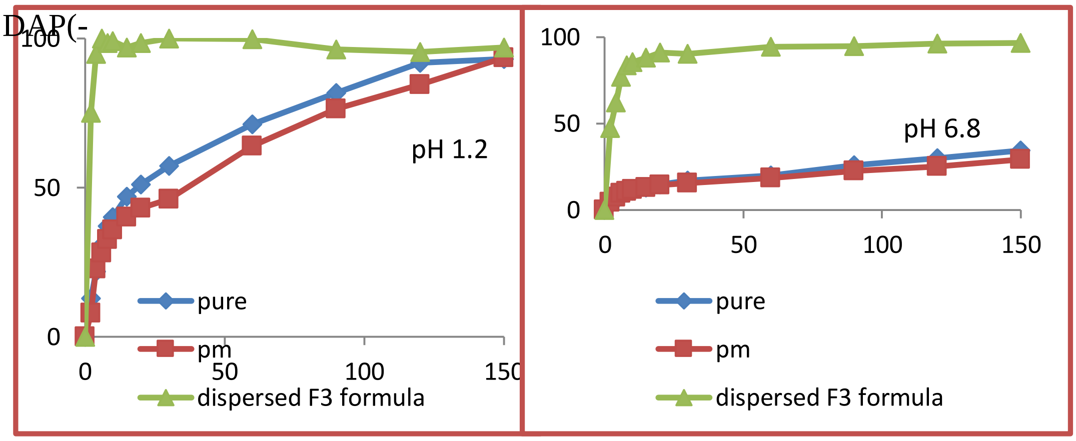

Figure (4): In Vitro dissolution of best formula in compare with the dissolution of pure and physical mixture in 0.1 N HCl and phosphate buffer solution.

-Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is one of the most widely reported spectroscopic techniques for solid-state characterization. The characteristic absorption band of Dapsone can be observed at a band of 3300–3400 cm−1 corresponding to the stretch of the amine group (N H), and peaks corresponding to bending vibration of NH2 groups between 1590 and 1550 cm−1. The bands at 1143 and 1180 cm−1 are ascribed to the symmetric and asymmetric vibrations of the sulfone group (SO2) as in figure (5)(15,17).

For the stabilizer, PVP K-30 showed a strong absorbance band at 1660 cm–1 due to the carbonyl group of tertiary amide, and at 2956.97 cm-1 due to C-H stretching. A very broad band was also seen at 3296.46-3483.56 cm−1 due to O-H stretching vibrations of absorbed water as in figure(6) confirming the broad endothermic peak detected in the DSC experiments(32,33).

The spectrum of F3 powder as showed in figures (7) was equivalent to the spectrum of the drug and stabilizer indicating no chemical interaction or complexation occur in F3 formula between DAP and stabilizer.

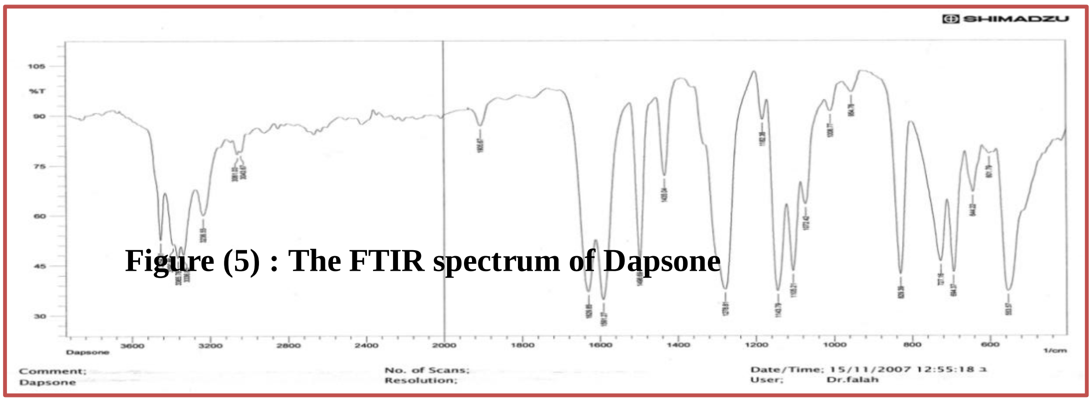

Figure (5) : The FTIR spectrum of Dapsone

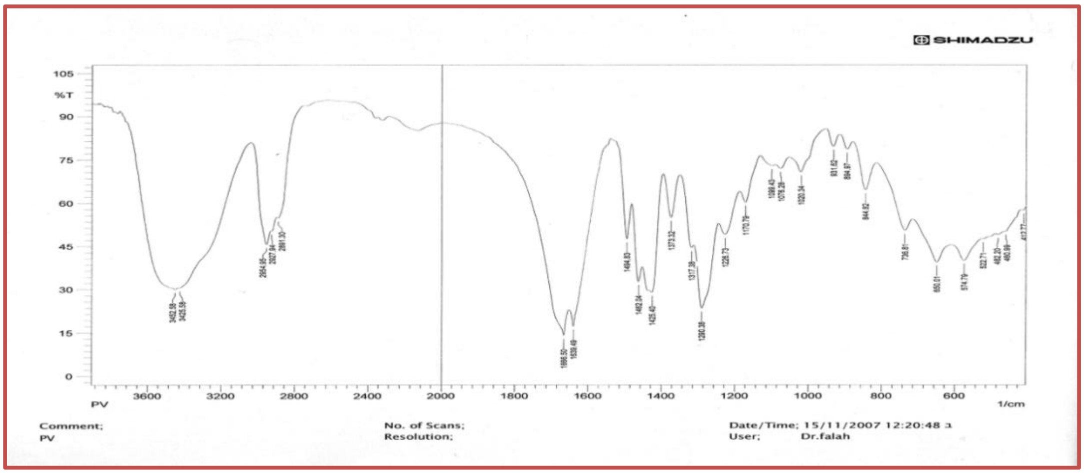

Figure (6): The FTIR spectrum of Pvp k30

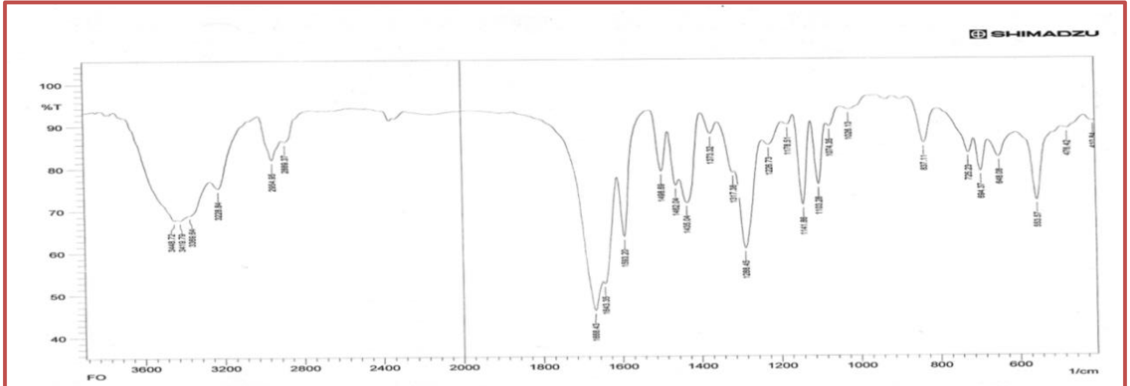

Figure (7): The FTIR spectrum of F3

-Differential scanning calorimetry (DSC)

Figure (8A) demonstrates DSC thermogram of DAP. The drug showed a sharp characteristic endothermic peak at 179 °C and this agrees with published results(12). This gives an indication that the drug has crystalline nature with high purity. PVP K-30 did not show a sharp melting peak, however, it showed a broad endotherm at 120 °C as in figure (8B) , because it is an amorphous polymer has an broad endotherm ranging from 60 °C to 120 °C (34). Physical mixture of DAP and PVP K-30 showed melting point of each one as in figure (8C) with little change in the position except for PVP K-30 which shifted to 114 °C because it is an amorphous polymer, indicating that stabilizers do not change the physical state of DAP in the physical mixture. For lyophilized powder (F3), the melting point of DAP disappeared as in figure (8D) giving a strong indication that the drug lost the crystallinity state and converted to an amorphous form(39).

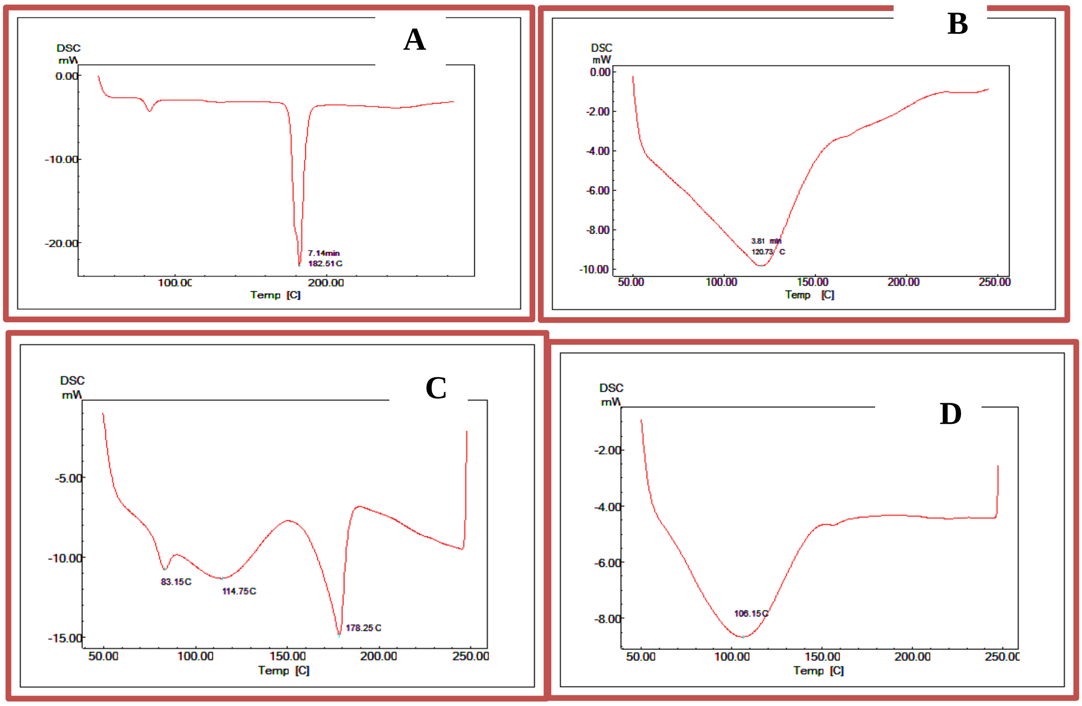

Figure (8): DSC thermograms of pure Dapsone (A), PVP k30 (B), the physical mixture (C) and F3 (D).

-Powder x-ray diffraction (XRD):

The X-ray diffractogram of the pure DAP powder, figure(9A) appeared characteristic diffraction with a numerous (narrow and symmetrical) peaks with high intensity. This indicates the crystalline structure of the drug , while PVP K-30 is dominantly amorphous in nature as indicated in its diffractogram and characterized by low intense peak as show in figure (9B). While the physical mixture, figure (9C) displayed the presence of numerous narrow and symmetric characteristic diffraction peaks with lower intensity that indicates mixing of crystalline with amorphous but the XRD of F3, figures (9D) showed no sharp peak and diffraction peak with less intensity when compared to that of raw drug, indicating that the crystalline structure of DAP was lost and converted into amorphous form. These results are coordinated with other studies in which Junghanns *et al* stated that processing of drug microcrystals into nanoparticles can lead to either crystalline or an amorphous product, especially if precipitation is applied (39).

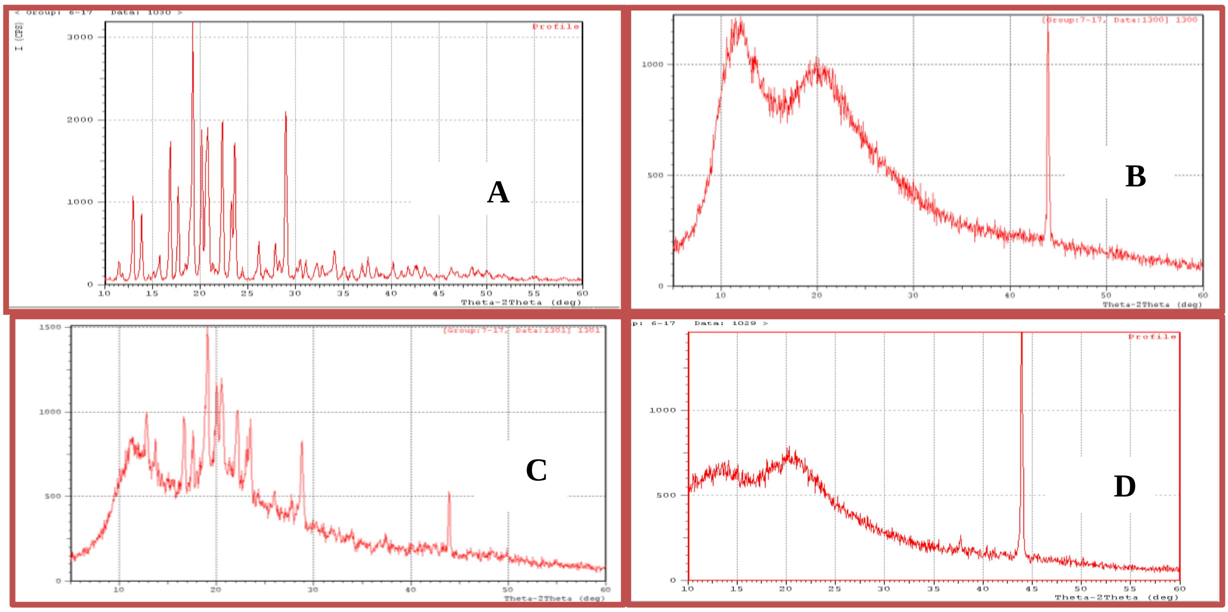

figure (9): X-ray diffractograms of pure Dapsone (A), PVPk30 (B), the physical mixture (C) and F3 (D).

-Scanning electron microscopy (SEM)

The shapes of pure DAP particles and nanoparticles obtained from F3 were visualized by scanning electron microscope as in the figure (10).

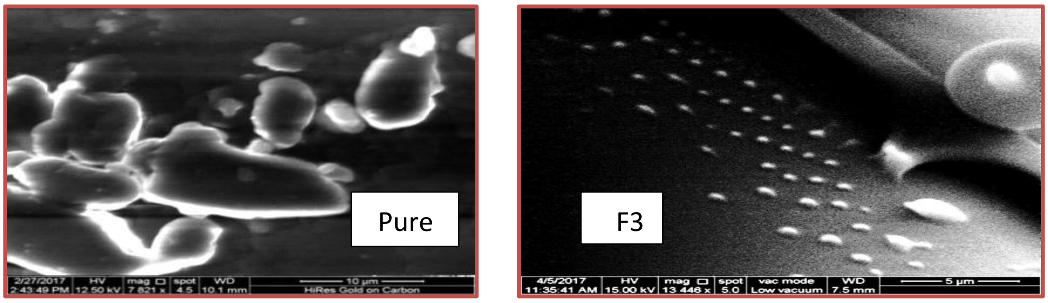

Figure (10): SEM photographs of pure Dapsone and F3

-Atomic force microscopy (AFM)

The morphological analysis and particle size of (F3) performed by AFM showed regular spherical shaped nanoparticles with a size of 56 nm as seen in figure (11) and approved by the histogram of particle size distribution in figure (12A) .While the particle size that measured by nano laser particle size analyzer was about (28.5 nm) as in figure (12B) . The particle size value of F3 obtained using AFM was larger because the nano laser particle size analyzer is not capable of giving information based on the real size distribution but based on the volumetric mean diameter of a great number of particles. For accurate determinations of single particle dimensions, size and distribution , the other microscopic methods are used (40)**.**

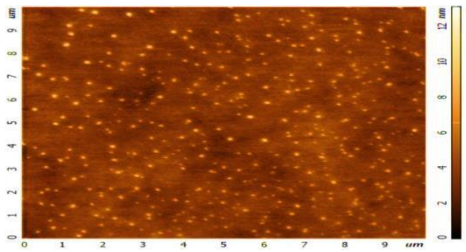

Figure (11): AFM of F3

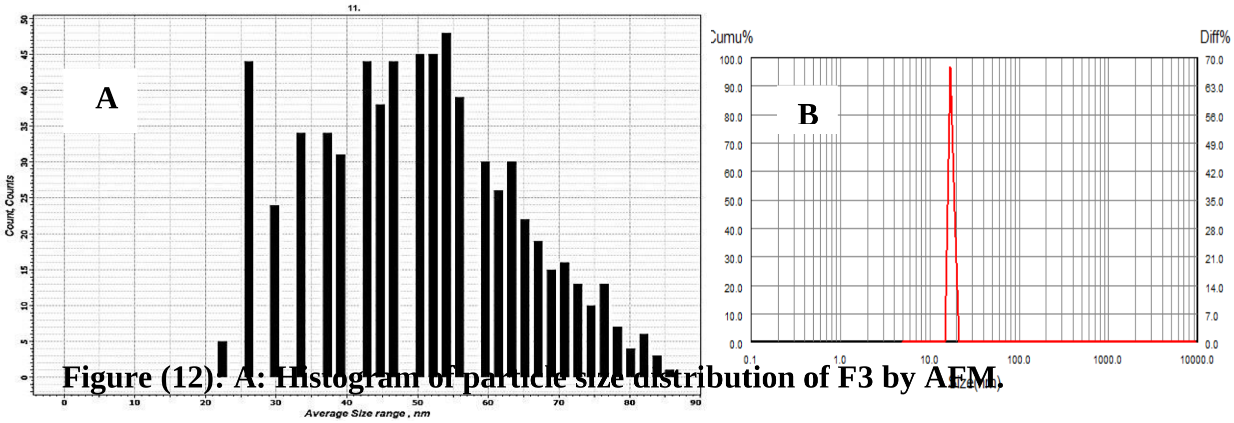

Figure (12): A: Histogram of particle size distribution of F3 by AFM.

B: Particle size distribution of F3 by particle size analyzer ABT-9000

4. Conclusions

Based on the obtained results from this study, we found that solvent-antisolvent precipitation method is an efficient method and it is cost effective, easy to operate and can be easily scaled up for industrial production of drug particles.

High amount of PVP k30 can give the best formula of DAP nanoparticles regarding particle size and dissolution rate to be formulated into different dosage forms without physical or chemical incompatibility and good homogeneity and morphological properties**.**

5. Recommendations for future

Incorporation of the best formula of nanoparticle within a topical hydrogel formulation and making clinical study to evaluate in vivo anti-leprotic effect to get *in vitro-in vivo* correlation and confirm the effectiveness of gel.

References:

1. Kar HK, Gupta R. Treatment of leprosy. Clin Dermatol. 2015;33(1):55–65.

2. Anusuya S, Natarajan J. The eradication of leprosy: molecular modeling techniques for novel drug discovery. Expert Opin Drug Discov. 2013;8:1239–51.

3. Yamasaki PR, do Nascimento DC, Chelucci RC, de Faria Fernandes Belone A, Rosa PS, Diório SM, et al. Synthesis and evaluation of novel dapsone–thalidomide hybrids for the treatment of type 2 leprosy reactions. Bioorg Med Chem Lett. 2014 Jul [cited 2017 Apr 1];24(14):3084–7.

4. Coleman MD. Dapsone: Modes of action, toxicity and possible strategies for increasing patient tolerance. Br J Dermatol. 1993;129(5):507–13.

5. Widdop B.,Moffat A.C&Osselton M.D. Clarke’s Analysis of Drugs and Poisons. fourth edi. Vol. 27, The Serials Librarian. USA: Pharmaceutical Press; 2011. 87-102 p.

6. Bergström CAS, Andersson SBE, Fagerberg JH, Ragnarsson G, Lindahl A. Is the full potential of the biopharmaceutics classification system reached? Eur J Pharm Sci. 2014;57(1):224–31.

7. Jiang L, Huang Y, Zhang Q, He H, Xu Y, Mei X. Preparation and solid-state characterization of dapsone drug-drug co-crystals. Cryst Growth Des. 2014;14(9):4562–73.

8. Guilherme Soares dos Santos ; Gabriela Garrastazu Pereira ; Eduardo André Bender ; Letícia Marques Colomé ; Sílvia Stanisçuaski Guterres ; Daniel Canema Manhães de Carvalho ; Gilberto Weissmüller. Development And Characterization Of Lipid Nanoparticles For Dapsone Topical Application. Chem New. 2012;35(3):627–33.

9. Grebogi IH, Tibola APO V, Barison A, Grandizoli CWPS, Ferraz HG, Rodrigues LNC. Binary and ternary inclusion complexes of dapsone in cyclodextrins and polymers: Preparation, characterization and evaluation. J Incl Phenom Macrocycl Chem. 2012;73(1–4):467–74.

10. Monteiro LM, Lione VF, do Carmo FA, do Amaral LH, da Silva JH, Nasciutti LE, et al. Development and characterization of a new oral dapsone nanoemulsion system: Permeability and in silico bioavailability studies. Int J Nanomedicine. 2012;7:5175–82.

11. Borges VR de A, Simon A, Sena ARC, Cabral LM, de Sousa VP. Nanoemulsion containing dapsone for topical administration: A study of in vitro release and epidermal permeation. Int J Nanomedicine. 2013;8:535–44.

12. Khadka P, Ro J, Kim H, Kim I, Kim JT, Kim H, et al. Pharmaceutical particle

technologies: An approach to improve drug solubility, dissolution and bioavailability. Asian J Pharm Sci. 2014;9(6):304–16.

13. Junyaprasert VB, Morakul B. Nanocrystals for enhancement of oral bioavailability of poorly water-soluble drugs. Asian J Pharm Sci. 2015;10(1):13–23.

14. Convention Inc. The United State Pharmacopoeia USP36-NF31.Rockville,MD20852.

15. Othman NS, Khaleel N a. Spectrophotometric and High Performance Liquid Chromatographic Methods for Determination of Metoclopramide in Pharmaceutical Preparations. Raf J Sci. 2011;22(3):39–56.

16. Mansouri M, Pouretedal HR, Vosoughi V. Preparation and Characterization of Ibuprofen Nanoparticles by using Solvent / Antisolvent Precipitation. Open Conf Proc J. 2011;2:88–94.

17. Chaves LL, Vieira ACC, Ferreira D, Sarmento B, Reis S. Rational and precise development of amorphous polymeric systems with dapsone by response surface methodology. Int J Biol Macromol. 2015 Nov;81(October):662–71.

18. Sadeghi F, Ashofteh M, Homayouni A, Abbaspour M, Nokhodchi A, Garekani HA. Antisolvent precipitation technique: A very promising approach to crystallize curcumin in presence of polyvinyl pyrrolidon for solubility and dissolution enhancement. Colloids Surfaces B Biointerfaces. 2016;147:258–64.

19. Vuković GD, Tomić SZ, Marinković AD, Radmilović V, Uskoković PS, Čolić M. The response of peritoneal macrophages to dapsone covalently attached on the surface of carbon nanotubes. Carbon N Y . 2010 Sep;48(11):3066–78.

20. Govindasamy G, Krishnamoorthy K, Rajappan M. Selection Of Excipients For Nanoparticles Formulations Of Nateglinide Through Drug-Excipients Compatibility Study. 2013;5(2):371–7.

21. Dhana UM, Poovi G, Kishore N, Reddy PN. In vitro characterization and in vivo toxicity study of repaglinide loaded poly (methyl methacrylate) nanoparticles. Int J Pharm. 2010;396(1–2):194–203.

22. Ige PP, Baria RK, Gattani SG. Fabrication of fenofibrate nanocrystals by probe sonication method for enhancement of dissolution rate and oral bioavailability. Colloids Surfaces B Biointerfaces. 2013;108:366–73.

23. Venkateswarlu V, Manjunath K. Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. J Control Release. 2004;95(3):627–38.

24. Tran TH, Poudel BK, Marasini N, Chi SC, Choi HG, Yong CS, et al. Preparation and evaluation of raloxifene-loaded solid dispersion nanoparticle by spray-drying technique without an organic solvent. Int J Pharm. 2013;443(1–2):50–7.

25. Gonugunta P, Vivekanandhan S, Mohanty AK, Misra M. A Study on Synthesis

and Characterization of Biobased Carbon Nanoparticles from Lignin. World J Nano Sci Eng. 2012;2(September):148–53.

26. Dolenc A, Govedarica B, Dreu R, Kocbek P, Srčič S, Kristl J. Nanosized particles of orlistat with enhanced in vitro dissolution rate and lipase inhibition. Int J Pharm. 2010;396(1–2):149–55.

27. Abbas SI, John HT, Fraih AJ. Preparation of Nano Crystalline Zinc – Ferrite as Material for Micro Waves Absorption by Sol-Gel Methods. Indian J Sci Technol. 2017;10(21):1–6.

28. Wang HY, Xu LX, Xiao Y, Han J. Spectrophotometric determination of dapsone in pharmaceutical products using sodium 1,2-naphthoquinone-4-sulfonic as the chromogenic reagent. Spectrochim Acta Part A Mol Biomol Spectrosc. 2004 Oct [cited 2017 Mar 13];60(12):2933–9.

29. Anjan De, Suddhasattya Dey, Prasanna Kumar Pradhan, Falguni Chaudhari MP. Estimation Of Dapsone In Bulk & Dosage Form By Uv Spectroscopic. Indo Am J Pharm Res. 2014;4(1):312–9.

30. Krishna K, Hiral K, Navin S, Mahesh D. The impact of critical variables on properties of nanosuspension: A review. Int J Drug Dev Res. 2015;7(1):150–61.

31. Kinnarinen T, Tuunila R, Häkkinen A. Reduction of the width of particle size distribution to improve pressure filtration properties of slurries. Miner Eng. 2017 Mar;102:68–74.

32. Sinko. P. J. Martin’s physical pharmacy and pharmaceutical sciences. sixth edition. LippincottWilliams &Wilkins, aWolters Kluwer business; 2011. 1-1230 p.

33. Zecevic DE, Meier R, Daniels R, Wagner KG. Site specific solubility improvement using solid dispersions of HPMC-AS/HPC SSL - Mixtures. Eur J Pharm Biopharm. 2014;87(2):264–70.

34. Aulton MEKMGT. Aulton’s Pharma c e ut ic s The Des ign and Manufacture of Medicines. fourth edi. USA: churchill livingstone elsevier; 2013.

35. Kaialy W, Shafiee M Al. Recent advances in the engineering of nanosized active pharmaceutical ingredients : Promises and challenges. Adv Colloid Interface Sci. 2015;288(December):71–91.

36. Robert M. Silverstein, Francis X. Webster DJK. Spectrometric identification of organic compounds. seventh ed. john wiley & sons,INC. 2005. 512 p.

37. Wegiel LA, Mauer LJ, Edgar KJ, Taylor LS. Mid-infrared spectroscopy as a polymer selection tool for formulating amorphous solid dispersions. J Pharm Pharmacol. 2014;66(2):244–55.

38. Patel G V, Patel VB, Pathak A, Rajput SJ. Nanosuspension of efavirenz for improved oral bioavailability: formulation optimization, in vitro, in situ and in vivo evaluation. Drug Dev Ind Pharm. 2014;40(1):80–91.

39. Junghanns JUAH, Müller RH. Nanocrystal technology, drug delivery and clinical applications. Int J Nanomedicine. 2008;3(3):295–309.

40. Sitterberg J, Özcetin A, Ehrhardt C, Bakowsky U. Utilising atomic force microscopy for the characterisation of nanoscale drug delivery systems. Eur J Pharm Biopharm. 2010;74(1):2–13.