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RESEARCH ARTICLE

Preparation & Characterization of Olmesartan Medoxomil Nanosuspensions Prepared By Emulsion Diffusion Technique

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ABSTRACT

The aim of present study was to prepare nanosuspensions of Olmesartan medoxomil (OM), an antihypertensive drug with low water solubility, by the emulsion-diffusion technique using three different stabilizers like Pluronic F-68, Polyvinylpyrolidone K-30 (PVP K-30) and Sodium lauryl sulphate (SLS) with the help of ultrasound. Formulations were characterized by particle size analysis, zeta potential determination (ZP), Diffuse reflectance infrared fourier transform spectroscopy (DRIFT), powder X-ray diffraction (PXRD), scanning electron microscopy imaging (SEM), saturation solubility, dissolution testing and stability studies. Optimization of the technological parameters (organic solvents, stabilizers, sonication procedure and recovery of particles) allowed the formation of nanosuspensions with a particle size of 50-500 nm. SEM imaging confirmed the nanosized drug particles and particle morphology was influenced by choice of stabilizers. DRIFT studies indicate absence of hydrogen bonding between drug, stabilizer and lyoprotectant. PXRD patterns of the formulation revealed crystalline nature of drug particles was reduced as a result of nanoprecipitation, ultrasonication and lyophilization. Both nanosuspensions and freeze dried nano-sized powders of olmesartan medoxomil showed a dramatic increase of dissolution rate and extent compared to pure drug. Prepared nanosuspensions and freeze dried powders were found to be stable for the period of 3 months at room temperature, accelerated stability conditions and refrigeration conditions.

KEYWORDS

Olmesartan Medoxomil, Nanosuspensions, Dissolution, Freeze Drying

INTRODUCTION

Olmesartan medoxomil (OM) is a novel selective AT1 subtype angiotensin-II receptor antagonist that is approved for the treatment of hypertension.[1] OM, which is administered as a prodrug, is rapidly and completely de-esterified to the active metabolite olmesartan (RNH-6270) during absorption from the gastrointestinal tract.

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Following the conversion of OM to olmesartan, virtually no further metabolism occurs[2]. The bioavailability of OM is approximately 26% due to low solubility in water and unfavorable breakage of ester drug to a poorly permeable parent molecule in the gastrointestinal fluids[3]. Efflux pumps in gastrointestinal tract also interfere with drug absorption[4]. OM dose-dependently reduces blood pressure through arterial vasodilation and reduced sodium retention. The volume of distribution of OM is approximately 17 liters. Thus, improving solubility of OM can increase clinical efficacy,

reduce the oral dose required to achieve the same effect and hence reduce the side effects.

Oral bioavailability of poorly water-soluble drugs depends on their dissolution rate at the site of absorption. In recent years it has been estimated that up to 40% of the new drugs discovered by the pharmaceutical industry are poorly soluble or lipophilic compounds[5]. This study examines the critical issues regarding engineering of a nanosuspensions tailored to increase drug dissolution rate and its transformation into dry powder. Nanosuspensions are sub-micron colloidal dispersions of solid drug particles in a liquid phase which are stabilized by surfactants[6]. Nanosuspensions in aqueous or non-aqueous vehicles can be prepared by four important methods like "bottom up" methods where nanosuspensions are built up from dissolved drug particles, "top down" methods where the raw material is broken down by using milling or high pressure homogenization method, "emulsion diffusion method" where emulsion of drug is prepared by using volatile organic or partially water miscible solvents.

Dilution of emulsion with water causes complete diffusion of the internal phase into the external phase, leading to instantaneous formation of the nanosuspension. Last method is preparation of microemulsion as templates; these are thermodynamically stable and isotropically clear dispersions of two immiscible liquids, stabilized by an interfacial film of surfactant and co-surfactant[7]. The reduced particle size within the nanometer range leads to an enhanced dissolution rate not only because of increased surface area but also because of increased saturation solubility as described by Feundlich–Ostwald equation[8].

Nanosuspensions of OM by media milling technique were reported by Thakkar et al.[9]. But media milling suffers from many disadvantages like method is time consuming, difficult to scale up batch, fractions of particle are in micrometer range, at high shear rate degradation of some of the ingredients may occur. In this experiment Nanosuspensions of OM were produced by the emulsion-diffusion method using three different stabilizers (Pluronic F68, PVP K-30 and SLS) using the partially water-miscible organic solvents like ethyl acetate, triacetin and benzyl alcohol by applying ultrasound. Nanosuspensions were freeze died by using mannitol and characterized by particle size analysis, ZP determination, DRIFTS, PXRD, SEM, saturation solubility, dissolution testing and stability studies.

MATERIALS AND METHOD

Materials

OM was received as gift sample from Glenmark Pharmaceuticals Ltd, Mumbai, India; Pluronic F68 and Polyvinylpyrolidone K-30 (PVK K30) obtained from BASF, Mumbai, India; Sodium lauryl sulphate (SLS) obtained from Loba chemicals, Mumbai, India; Mannitol obtained from Molychem, Mumbai, India; Ethyl acetate and triacetin obtained from Merk, Darmstadt, Germany; Benzyl alcohol obtained from Research lab, Mumbai, India. All other chemicals used were of analytical grade & used as received.

Methods

Determination of OM Solubility

The solubility of raw OM in water was determined by adding about 500 mg of drug in 10 ml of water on magnetic stirrer for 24 hrs at room temperature, then filtered (0.45 µm, Pall Corporation, Mumbai, India), and the content of dissolved drug was analysed spectrophotometrically at 252 nm by using UV-visible spectrophotometer (Jasco V-530, Japan). Experiment was performed in triplicate.

To determine apparent solubility of OM in different partially water soluble organic solvents like ethyl acetate, benzyl alcohol and triacetin, 1 mg of drug was accurately weighed and 0.5 ml aliquots of partially water soluble organic solvents were gradually added until the OM was judged by visual inspection to have completely dissolved. Benzyl alcohol proved to be the most promising organic solvent for production of OM nanosuspensions.

Preparation of OM Nanosuspensions

OM nanosuspensions were obtained by emulsion diffusion technology[10], using partially water miscible organic solvent benzyl alcohol with high intensity probe sonicator. OM dissolved in benzyl alcohol was added to aqueous solutions of different stabilizers under ultrasound. Water was added under same sonication condition to dilute the emulsion, resulting in the precipitation of drug particles.

Stabilization with PLURONIC F68(NP) Accurately weighed 100 mg of OM was dissolved in 4 ml benzyl alcohol and rapidly injected into 32 ml aqueous solution of 0.5% Pluronic F68 with probe sonicator (Sonics and materials Inc., Vibra Cell, Model VCX 750, Connecticut, USA) for 3 min & 50% amplitude to form coarse emulsion. This emulsion was diluted with 150 ml water and further sonicated for 3 min. Temperature of processing solution was maintained at 15^0C using ice bath.

Stabilization with SLS and PVP K30(NPS)

100 mg of OM dissolved in 4 ml benzyl alcohol was injected into 0.1 % aqueous solution of SLS and PVPK30 (1:1) and sonicated for 3 min & 50% amplitude. The emulsion was diluted with 150 ml water and sonicate for 3 min at 15^0C

Conversion of Nanosuspension into Solid Matrix

Spray drying and freeze drying are the two ways to convert nanosuspension into solid matrix. Boiling point of benzyl alcohol is 205^0C. So it was impossible to spray dry nanosuspension, and these samples were therefore lyophilized immediately after dilution by adding 1 gm of mannitol at pressure 180 millitorr for 24 hours at room temperature by using freeze dryer (Virtis, Benchtop K). Lyophilized Nanosuspension containing Pluronic F68 is referred as Lyo NP & with SLS & PVP as Lyo NSP in following text.

Characterization of Nanoparticles

Particle Size Analysis

Nanosuspensions were characterized for average particle size (measurements were performed in triplicates) using laser diffraction technique (Malvern Mastersizer 2000 SM, Malvern Instruments Corp., U.K). Laser obscuration was kept 1%. Particle size distribution is given by d (0.9), d (0.5) and d (0.1) which is the particle size diameters determined at 90th, 50th and 10th percentile of particle undersized, respectively.

Percentage Drug Content

Samples of optimized batches of nanosuspensions and lyophilized product were assayed by dissolving 10 mg of powder in 10 ml of methanol. The solutions were filtered, diluted with distilled water and their drug content was determined (in triplicates) spectrophotometrically at 252 nm, using UV spectrophotometer (Jasco V-530, Japan).

Zeta Potential Determination (ZP)

The surface charge of the particles was assessed in triplicates by zeta potential measurements using the Malvern zetasizer (Malvern Instruments, UK). The zetasizer measures the electrophoretic mobility of the particles, which was converted into the zeta potential using the Helmholtz –Smoluchowski equation built into the Malvern zetasizer software. The samples were analyzed as such without any dilution.

Redispersibility of Lyophilized Nano-suspensions

Lyophilized powders were redispersed in distilled water and analyzed for average particle size using laser diffraction technique (Malvern Mastersizer 2000 SM, Malvern Instruments Corp., U.K). (All measurements were performed in triplicates and their average was considered.)

Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS)

The DRIFTS spectra of pure drug and lyophilized nanosuspensions were obtained, after appropriate background subtraction; using FTIR spectrometer (FTIR-4100, Jasco, Japan). The sample was mixed with dry potassium bromide and was scanned from 4000cm^{-1} to 400cm^{-1}, Jasco spectra manager ver. 2 was used for data acquisition and analysis.

Scanning Electron Microscopic Analysis (SEM)

For surface characterization, few drops of nanosuspensions were dried on a glass slide and subjected to SEM analysis. JEOL JSM-6360A analytical scanning electron microscope (Japan) was used for SEM imaging. Samples were placed on a carbon specimen holder, and then coated with platinum in an auto fine coater (JEOL JFC 1600).

Powder X-ray Diffraction Patterns (PXRD)

PXRD patterns of pure drug and lyophilized nanosuspensions were recorded using an X-ray Brucker AXS diffractometer (Model: D8 Advance, USA) with Cu line as the source of radiation. The samples were analyzed in the 2θ angle range of 5 to 50^0C. The range and the chart speed were 1 x 10^3CPS and 10 mm/^{o} 2θ, respectively.

Saturation Solubility Study

Saturation solubilities of pure drug, nanosuspension and lyophilized nanosuspensions were determined by equilibrating excess liquid/powder in phosphate buffer (pH 6.8) for 24 hours on a mechanical stirrer at room temperature. The samples were filtered through 0.45 µm membrane filter. The resulting filtrate was collected and assayed for drug content (in triplicates) spectrophotometrically at 252 nm.

In Vitro Release Studies

The dissolution studies of pure drug and lyophilized nanosuspensions were performed using USP type II dissolution test apparatus (Electrolab TDT-08L, Mumbai, India). The samples equivalent to 40 mg of OM were placed in the dissolution vessel containing 900 ml phosphate buffer (pH 6.8) maintained at 37±0.5^{0}C and stirred at 50 rpm.

Samples were collected periodically and replaced with a fresh dissolution medium to maintain sink conditions. After filtration through 45 µm filter, concentration of OM was determined spectrophotometrically at 252 nm. Analysis of data was done using PCP-Disso software (V3, Poona College of Pharmacy, Pune, India.).

Stability Studies

Stability studies were carried out for liquid nanosuspensions at accelerated stability conditions (40^{o}C / 75 %RH), room temperature (30^{o}C / 60 %RH) and refrigeration temperature (2 to 8^{0}C) for 3 months as specified in the ICH guidelines by using stability chamber (Thermolab, Mumbai, India). Each withdrawal of nanosuspension sample was characterized for particle size and drug content at regular intervals. (All measurements were performed in triplicates and their average was considered).

RESULTS AND DISCUSSION

Selection of the Solvent for Nanosuspension Preparation

The solubility of OM in water, partially water miscible solvents and the solubility of selected organic solvents in water are presented in Table 1. A drug which is suitable candidate for nanosuspension preparation by emulsion diffusion method should be very poorly soluble in water (<10-3 to 10-4 mol/l) and well soluble in organic solvent[10].

OM is very poorly soluble in water (0.10 ± 0.03 mg/ml) and better soluble in organic solvent. So Emulsion diffusion which is one of the techniques to prepare stable nanosuspension was chosen for the study. In this method surface energy of the system increases during the process of crystallization.

Small particles, which spontaneously aggregate to decrease surface energy, were stabilized by a layer of surfactant or / and protective polymer[11]. OM has highest solubility in benzyl alcohol and was therefore selected for the preparation of nanosuspensions. Ultrasonication is used to prepare emulsion and suspension by diluting emulsion.

Influence of Stabilizers on Nanosuspension Formation

The choice of stabilizer is specific to each drug candidate and each formulation procedure. The stabilizer or mixture of stabilizers should exhibit

sufficient affinity for the droplet surface to enable preparation of emulsion and for the particle surface in order to stabilize the nanosuspension. In preliminary experiments, different stabilizers were screened in different concentrations for nanosuspension preparation by solvent diffusion method.

Table 1: Solubility Testing of OM

Sr. No.	Solvent	Approximate OM solubility (mg/ml)*	Reported Solubility of solvent in water (%w/w)
1.	Water	0.10 ± 0.03	--
2.	Benzyl alcohol	30 ± 2	3.5[20]
3.	Ethyl acetate	2 ± 0.5	7.7[20]
4.	Triacetin	1± 0.3	7.1[21]

*Mean ± S.D., n=3 S.D. Standard deviation

When the influence of different stabilizers was investigated, the emulsions were prepared with a fixed concentration of the drug. Three different stabilizers like Pluronic F68, SLS and PVPK30 were tested. Suspension stabilized with 0.5% Pluronic F68 resulted into minimum particle size i.e. 192 nm (Table 2). So this batch was selected for further study. SLS produced nanosuspension with particle size range of 200 to 300 nm. But this suspension was unstable after 20 days due to increase in particle size. As reports suggest that combination of stabilizers was preferred over one single stabilizer for long term stabilization of nanosuspension[12,13], combinations of these stabilizers were tried. The particle size of nanosuspension stabilized with 0.1% SLS : PVPK30 (1:1) was significantly less than suspension stabilized with SLS & PVPK30 alone. Rationale for using combination of SLS and PVPK30 based on combined electrostatic and stearic stabilization. As reported by Rabinow[13], both steric and electrostatic mechanisms are enabled by combining non-ionic and ionic surface modifiers, which therefore complement each other.

Important function of stabilizer is that they can form a substantial mechanical & thermodynamic barrier at the interface that reduces cohesive forces between individual emulsion droplets[14]. Nonionic surfactants like Pluronic F68 offer an advantage over other polymers in that they have a higher adsorption potential on the droplet surface and act as a steric barrier, preventing close contact of droplets and later particles than an equal-chain-length polymer[15]. Ionic surface modifiers like SLS formed electrostatic repulsive layer to prevent aggregation of particles in the process of size reduction. Both ionic as well as non-ionic stabilizers would prevent Ostwald's ripening in nanosuspensions. But ionic stabilizers inhibited both nucleation and crystal growth of nanoparticles while non-ionic stabilizers arrested crystal growth only[13].

Effect of Ultrasound on Nanosuspension Formation

Ultrasound intensifies mass transfer and initiates important phenomenon called cavitation. When cavitation bubble implodes, a localized hot spot is formed releasing powerful shock wave. This cavitation phenomenon can induce primary nucleation at lower supersaturation levels by reducing the induction period and metastable zone width[16,17]. Ultrasound uniformly mixes organic and aqueous phase resulting in formation of stable emulsion. After dilution of emulsion drug particles were precipitated and suspension is formed. Due to application of ultrasound to suspension, solubility of drug particles in organic and aqueous phase was reduced sharply and immediately reached its maximum supersaturation so that primary nucleation and crystal growth were implemented rapidly. These effects bring considerable benefits to crystallization process, such as induction of primary nucleation, reduction of crystal size, inhibition of agglomeration and manipulation of crystal size distribution[18]. Ultrasound is known to suppress agglomeration of product. Process variables of high intensity probe sonicator (amplitude, duration, pulse) were least significant as they

had no effect on size reduction and stability of nanosuspension.

Particle Size Analysis

Particle size and particle size distribution analysis was done by laser diffraction (LD) technique is mentioned in Table 2. As per laser diffraction (LD) technique, liquid nanosuspensions showed nanosized particles with 90% particles having size less than 1 µm. As uniformity was less than 1, uniform nano colloidal dispersion was obtained**.** Nanosuspensions NP and NSP exhibited relatively smaller nano sized particles (192±10nm and, 348± 15nm respectively) and were stable. So, these batches were selected for further studies.

Table 2: Particle Size Analysis of Nanosuspensions by Laser Diffraction (Ld) Technique

Batches	d (0.9) µm ± SD*	Uniformity ± SD*
Pure drug	23.773±7.351	5.27±0.470
0.25% Pluronic F68	0.512±0.030	0.963±0.092
0.50% Pluronic F68 (NP)	**0.192±0.010**	**0.323±0.073**
0.75% Pluronic F68	0.307±0.015	0.715±0.049
0.25% PVPK30	0.680±0.092	1.084±0.912
0.50% PVPK30	0.545±0.010	0.863±0.212
0.75% PVPK30	0.513±0.032	0.957±0.079
0.20% Pl F68+PVPK30 (1:1)	0.639±0.034	0.814±0.056
0.50% Pl F68+PVPK30 (1:1)	0.597±0.076	0.642±0.087
0.75% Pl F68+PVPK30 (1:1)	0.580±0.085	0.850±0.096
0.25% SLS	0.246±0.052	0.605±0.045
0.5% SLS	0.247±0.055	0.694±0.052
0.75% SLS	0.342±0.062	0.924±0.023
0.02%SLS+PVPK30 (1:1)	0.684±0.095	0.768±0.099
0.1 % SLS+PVPK30 (NSP) (1:1)	**0.348±0.015**	**0.711±0.045**
0.2 % SLS+PVPK30 (1:1)	0.716±0.082	0.984±0.056

* Mean ± S.D., n=3 S.D. Standard deviation

Percentage Drug Content

Percentage Drug content of all the optimized batches is shown in Table 3. In case of NP and NSP, some amount of drug must have remained in soluble form in the stabilizer solution, and some was loosed in processing. Therefore all the batches were found to be about 90 to 95%.

Table 3: Percentage Drug Content of Optimized Batches by UV Spectroscopy

Sr. No.	Batch	Drug content (% ± SD)*
1	NP	93.27±2.13
2	NSP	95.10±3.81
3	LyoNP	92.50±4.39

* Mean ± S.D., n=3 S.D. Standard deviation

Zeta Potential Determination (ZP):

Table 4 shows Zeta potential of pure drug suspension and optimized batches of nanosuspensions. The determination of the zeta potential (property related to the double electric layer on the surface of colloidal particles) of a nanosuspension is essential as it provides an indication about the physical stability of nanosuspension as reported by Patrawale et al[19]. The magnitude of the zeta potential gives an indication of the potential stability of the

colloidal system. If all the particles have a large negative or positive zeta potential they will repel each other and there is dispersion stability. If the particles have low zeta potential values then there is no force to prevent the particles coming together and there is dispersion instability. A dividing line between stable and unstable aqueous dispersions is generally taken at either +30 or -30 mV. Particles with zeta potentials more positive than +30 mV are normally considered stable. Particles with zeta potentials more negative than -30mV are normally considered stable. Pluronic F68, a non-ionic surfactant is used as a stabilizer, which provides steric stabilization. So, negative zeta potential is attributed to drug nanocrystals. From zeta potential values it was confirmed that batch NSP was stabilized by combined electrostatic and steric effect and batch NP was stabilized by steric effect.

Table 4: ZP of Pure Drug Suspension and Optimized Batches of Nanosuspension

Sr. No.	Batch	Zeta potential determination (mV ± SD)*
1	Pure drug suspension	+15.8 ± 0.8
2	NP	-23.5 ± 0.6
3	NSP	-27.0 ± 0.5

* Mean ± S.D., n=3 S.D. Standard deviation

Redispersibility of Lyophilized Nano-suspensions

Lyophilization techniques were used to convert liquid nanosuspensions into dry powders. But these dry powders must be redispersible to original liquid nanosuspensions on dilution with a suitable media. After redispersing dry powders in distilled water, particle size distributions of lyoNP & lyoNSP are shown in Table 5.

On redispersion, dry powders showed submicron particle size and uniformity similar to that of liquid nanosuspensions (NP and NSP) when analyzed with laser diffraction (LD) technique. There was no significant change in the nanoparticle size after redispersion of lyophilized nanosuspensions. Thus, freeze drying technique could successfully incorporate liquid OM nanosuspension into solid matrix.

Table 5: Particle Size Analysis of Lyophilized Nanosuspensions on Redispersion

Sr. No.	Batches	d (0.9) µm ± SD*	Uniformity ± SD*
1	LyoNP	0.502±0.040	0.708±0.032
2	LyoNSP	0.658±0.023	0.790±0.070

* Mean ± S.D., n=3 S.D. Standard deviation

Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS)

IR spectra of pure OM, lyoNP & lyoNSP are shown in Figure 1. DRIFT analysis was performed to study any possible interactions between drug, carrier and lyoprotectant.

The spectrum of OM was characterized by peaks at 3650 cm–1 (OH stretching), 3290 cm–1 (NH stretching), 2927 cm–1 (aliphatic CH stretching), 1830 cm–1 (ester stretching), 1703 cm–1 (acetate stretching) and 1527-1446 cm–1(C=C stretching). IR spectra of lyoNP and lyoNSP also showed similar peaks indicating absence of any interaction of OM with exicipients after lyophilization of nanosuspensions IR spectra exhibited few peaks other than those observed in group frequency and finger print region of pure drug owing to presence of mannitol.

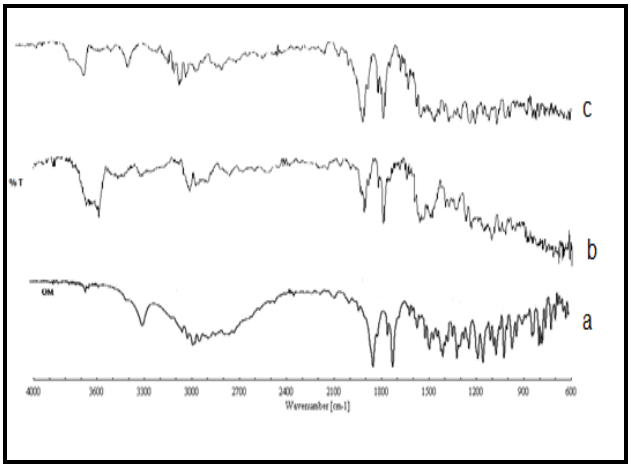


Figure 1: DRIFT spectra of a) pure drug OM b) lyoNP c) lyoNSP

Scanning Electron Microscopic Analysis (SEM)

SEM images of pure drug, air dried NP and air dried NSP are shown in Figure 2. The SEM image revealed nanosized particles of OM in contrast to micronized particles of raw OM. Nanosuspension shown in Fig. 2 did not contain mannitol, since OM nanoparticles were not visible in the SEM images of OM dried in the presence of mannitol. SEM imaging confirmed the nanosized drug particles and particle morphology was influenced by choice of stabilizers.

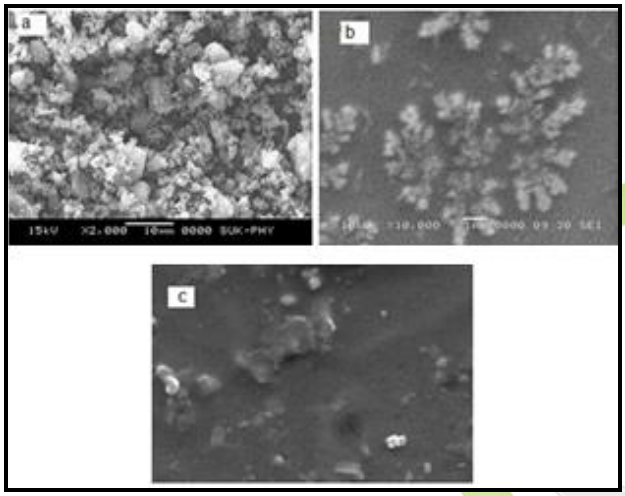


Figure 2: SEM images of a) pure drug OM b) Air dried NP nanosuspension c) Air dried NSP nanosuspension

Powder X-ray diffraction analysis (PXRD)

Figure 3 shows PXRD patterns of pure drug, lyoNP and lyoNSP. XRPD of pure OM showed numerous distinctive peaks in the region of 5 to 40^0 (2θ) at 7.10^0, 15.13^0, 18.95^0, 21.82^0, 22.10^0, 24.95^0, 29.60^0 indicating that pure OM is highly crystalline in nature**.** Intensity of these peaks was reduced in the PXRD spectrum of the Lyophilized nanosuspensions (lyoNP and lyoNSP)**.** The crystalline nature of drug particles was reduced as a result of nanoprecipitation, ultrasonication and lyophilization. Reduction in crystalline status of drug would improve its solubility. Other peaks observed in the PXRD spectra of lyophilized nanosuspensions could be ascribed to the presence of crystalline drug and excess amount of mannitol.

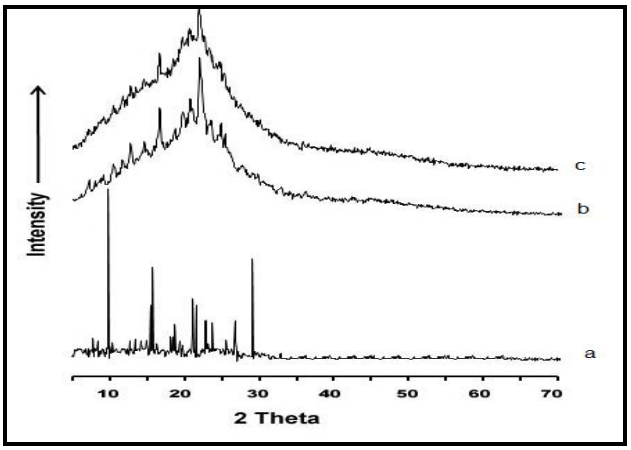


Figure 3: PXRD patterns of a) pure drug OM b) lyoNP c) lyoNSP

Saturation Solubility Study

Saturation solubility of optimized batches of nanosuspensions and lyophilized powders in water & in phosphate buffer pH 6.8 is shown in Table 6.

Reduction in particle size and crystallinity increases saturation solubility of OM. Saturation solubility of drug was improved around 20 fold when formulated as nanosuspension. Lyophilized nanosuspensions showed lower solubility improvement compared to liquid nanosuspensions due to particle size enlargement during lyophilization.

Table 6: Saturation solubility of optimized batches of nanosuspensions and lyophilized powders in water & in phosphate buffer pH 6.8

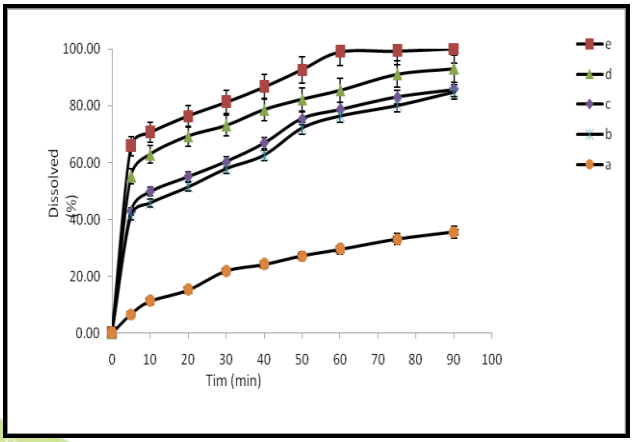
Sr. No.	Batch	Saturation Solubility in distilled water (mg/ml ± SD)*	Saturation Solubility in phosphate buffer pH 6.8 (mg/ml ± SD)*
1	Pure Drug	0.10±0.03	0.18±0.04
2	NP	2.01±0.12	2.34±0.15
3	NSP	1.88±0.16	2.04±0.18
4	LyoNP	1.84±0.14	1.97±0.22
5	LyoNSP	1.65±0.13	1.71±0.16

* Mean ± S.D., n=3 S.D. Standard deviation

In Vitro Release Studies

Figure 4 shows in vitro release profiles of pure drug OM, nanosuspension, and lyophilized products. Pure OM was characterized by only 29.48% drug release within 60 min. When formulated as a liquid nanosuspensions (NP and NSP), its dissolution rate was significantly ($p<0.05$) enhanced. This must be attributed to the increased surface area of the drug and possible better contact between nanosuspensions and dissolution medium. According to Noyes – Whitney equation, an increase in saturation solubility and decrease in particle size lead to an increased dissolution rate[19]. Nanosized particles can increase solution velocity and saturation solubility because of vapour pressure effect. In addition, the diffusional distance on the surface of drug nanoparticles is decreased, thus leading to an increased concentration gradient. The increase in surface area and concentration gradient lead to more pronounced increase in dissolution velocity as compared to micronized formulation. So, formulation of poorly water-soluble drugs as nano-sized drug particles had a dramatic effect on dissolution rate and drug solubility.

Lyophilized nanosuspensions showed lower drug release compared to liquid nanosuspensions. This must be attributed to increase in particle size during lyophilization. In first 10 min, nanosuspensions presented about 70% in solution and lyophilized products presented about 50%. In vitro release studies are in accordance with saturation solubility study findings.



* Mean ± S.D., n=3 S.D. Standard deviation

Figure 4: *In vitro* release profiles of a) pure drug OM b) lyoNSP c) lyoNP d) NSP e) NP

Stability Studies

Particle size distribution in stability studies of nanosuspensions is given in Table 7. No significant increase in particle size was observed, indicating stable nanosuspension formulations.

Table 7: Particle Size Distribution in Stability Studies of Nanosuspensions

Conditions for stability study	Particle size d (0.9) µm ±SD*				
	Batches	0 day	1 month	2 month	3 month
Accelerated stability condition (40^0C/ 75%RH)	NP	0.192±0.010	0.205±0.035	0.218±0.056	0.228±0.082
	NSP	0.348±0.015	0.362±0.040	0.372±0.038	0.388±0.022
Room temperature (25^0C)	NP	0.192±0.010	0.195±0.021	0.202±0.042	0.216±0.032
	NSP	0.348±0.015	0.351±0.020	0.362±0.035	0.388±0.040
Refrigerator temperature ($2 – 8^0$C)	NP	0.192±0.010	0.216±0.012	0.245±0.010	0.258±0.025
	NSP	0.348±0.015	0.367±0.046	0.375±0.050	0.492±0.045

* Mean ± S.D., n=3 S.D. Standard deviation

CONCLUSION

OM nanoparticles can be prepared using an emulsion-diffusion method with Pluronic F68, PVPK30 and SLS stabilizer followed by lyophilization Ultrasound uniformly mixes organic and aqueous phase resulting in formation of stable emulsion which on dilution produces stable suspension. Ultrasound intensifies mass transfer and initiates important phenomenon cavitation. This effect brings considerable benefits to crystallization process, such as reduction of crystal size, inhibition of agglomeration which can help in manipulation of crystal size distribution. Nanosuspension prepared under high intensity ultrasonication successfully resulted into nanosized, uniformly dispersed stable nanosuspension. Nano-sized OM dissolved much more rapidly than micronized. SEM images reveal distinct differences in the morphological structure of nanoparticles influenced by the stabilizers. PXRD results showed decrease in crystalline nature of drug particles as a result of nanoprecipitation, ultrasonication and lyophilization. Clearly, these findings indicated the suitability of emulsion diffusion techniques for preparation of nano-sized poorly water soluble drug with significant improvement of the in vitro dissolution rate, and thus possibly improve their oral bioavailability.

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