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Preparation and Characterization of Sildenafil Loaded Solid Lipid Nanoparticles: Drug Delivery System Suitable for Nebulization

Ahmed khames [1, 2]

[1]*Department of pharmaceutics and pharmacy technology, Taif University, Taif, KSA.*

[2]*Beni-suef University, Beni-suef, Egypt.*

***Corresponding author:** Ahmed khames, Department of pharmaceutics and pharmacy technology, Taif University, Taif, KSA.*

ABSTRACT

The primary indication of sildenafil is the treatment of erectile dysfunction. It is also effective in pulmonary arterial hypertension; its absolute bioavailability is 41% due to first-pass metabolism. In this work; attempt was made to prepare sildenafil solid lipid nanoparticles suitable for nebulization for direct application into the lung to increase bioavailability, and increase its local action on the pulmonary arteries. Solid lipid nanoparticles were prepared by hot homogenization- ultrasonication technique using Precirol ATO 5, Compritol 888 and Glyceryl monostearate as solid lipids. Gelucire 44/14, Pluronic f68 and Cremophor EL as surfactants. The prepared SLNs were evaluated by measuring particle size, zeta potential, and drug loading capacity. The drug release rate at simulated lung conditions were examined. The optimized SLN formula was further examined for particle shape using SEM and solid state characterization by DSC, IR and XRD. Results showed that the prepared SLN formulae had particle size in the range of 44 to 107 nm, with percentage drug of 94.39% to 89.11% and +20.3 to + 24.5 mV zeta-potential. The drug release from prepared SLN was improved with a slow prolonged rate, where percentage drug released exceeded 75 up to 95.12% from different SLN formulations within 12 hours in comparison to 26.83 % for plain drug. For the optimized formula, The drug melting peak at 189.88ºC on DSC thermogram was completely disappeared, drug main functional groups were all retained in IR spectrum while XRD pattern showed disappearance of drug characteristic peaks. SEM results showed that the prepared particles were spherical with irregular surface. Depending on these results it could be concluded that SLN formula is efficient dosage form suitable for nebulization and delivery through

the lung due to small particle size, uniform size distribution, high drug tolerating ability, compatibility and ability to improve drug release rate over extended time period.

Keywords*: Sildenafil, Solid lipid nanoparticles, zeta potential, Pluronic f68, Precirol ATO 5.*

INTRODUCTION

Sildenafil was originally developed by British scientists and then brought to market by the US-based pharmaceutical company, Pfizer (1). It acts by inhibiting cGMP-specific phosphodiesterase type 5 (PDE-5), an enzyme that delays degradation of cGMP, which regulates blood flow in the penis. Since becoming available in 1998, sildenafil has been the prime treatment for erectile dysfunction. The primary indication of sildenafil is treatment of erectile dysfunction (inability to sustain a satisfactory erection to complete intercourse). Its use is now standard treatment for erectile dysfunction in all settings, including diabetes (2). 1-((3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo (4, 3-d) pyrimidin-5-yl)-4-ethoxyphenyl) sulfonyl)-4-methylpiperazine

Sildenafil is also effective in the rare disease pulmonary arterial hypertension (PAH). It relaxes the arterial wall, leading to decreased pulmonary arterial resistance and pressure. This, in turn, reduces the workload of the right ventricle of the heart and improves symptoms of right-sided heart failure. Because PDE-5 is primarily distributed within the arterial wall smooth muscle of the lungs and penis, sildenafil acts selectively in both these areas without inducing vasodilatation in other areas of the body. Pfizer submitted an additional registration for sildenafil to the FDA, and sildenafil was approved for this indication in June 2005 (3).

Several authors have reported the use of sildenafil in the treatment of pulmonary hypertension, relaxing isolated human vessels (4) and reducing the pulmonary hypertension in animal models (5-7). Zhao and colleagues used a model of hypoxic pulmonary vasoconstriction in humans after inhalation of 11% oxygen. The control group showed a 50% increase in pulmonary artery pressures and the sildenafil group (100 mg PO) developed no pulmonary hypertension (8). Intravenous sildenafil was used in a study by Shekerdemian and colleagues in a model of meconial aspiration in the pig, which showed a 40% decrease in pulmonary vascular resistance and a 30% increase in cardiac output without change in oxygenation (9).

Perrault, Louis P et al, (10) studied the effects of inhaled and intravenous sildenafil on the pulmonary endothelium-dependent relaxations; the hemodynamic profile and oxygenation after cardio-pulmonary bypass (prior or after CPB, on pulmonary artery endothelial function). They found that; Exposure to CPB is associated with occurrence of pulmonary endothelial dysfunction, which contributes to clinically significant pulmonary hypertension and the potential for right ventricular failure with its attendant high mortality. Inhaled sildenafil in the prophylaxis of pulmonary hypertension in the postoperative setting could be an additional tool to prevent this important clinical problem. Moreover, sildenafil was able to reverse pulmonary hypertension once established. Since the effect occurs and ceases rapidly, it could be used once the pulmonary hypertension is established or in prophylaxis with continuous nebulization.

After oral single-dose administration, sildenafil was rapidly absorbed; reaching maximum plasma concentrations within approximately 1 h with elimination half-life of approximately 3 – 4 h. Food slowed the rate but minimally affected the extent of drug absorption. The absolute bioavailability was 41% due to first-pass metabolism (11–13).

Solid lipoid nanoparticles are one of the novel potential colloidal carriers (14) systems in the range of 100-150nm as alternative materials to polymers which is identical to oil in water emulsion for parenteral nutrition, but the liquid lipid of the emulsion has

been replaced by a solid lipid (15). They have many advantages such as good biocompatibility, low toxicity, high drug payload, capability of including lipophilic and hydrophilic drugs, drug targeting, controlled release (fast or sustained), and occlusive properties and lipophilic drugs are better delivered by solid lipid nanoparticles and the system is physically stable (16). Solid lipid nanoparticles may be a promising sustained – release and drug targeting system for lipophilic CNS antitumor drugs (17).

Armin Braun et al (18), studied the potential cytotoxicity of solid lipid nanoparticles (SLN) for human lung as a suitable drug delivery system (DDS). They used a human alveolar epithelial cell line (A549) and murine precision-cut lung slices (PCLS) to estimate the tolerable doses of these particles for lung cells. They concluded that; SLN20 showed lower toxic values in all test systems. Therefore, we conclude that SLN20 could be used as a suitable DDS for the lung, from a toxicological point of view.

The primary goal of this work is to incorporate sildenafil base in a safe solid lipid nanoparticle preparation suitable for delivery by nebulization to improve bioavailability through escaping liver metabolism and shorten its onset of action. Sildenafil base was selected for this work rather than the citrate salt as it is more lipophilic which is more suitable to be incorporated into SLN formulations. SLN formulations will be prepared by different lipid and surfactant combinations and the prepared formulae will be characterized and optimized for maximum drug loading ability, smaller particle size, optimum zeta potential and higher drug release rate. The optimized formula will be investigated for compatibility of components, crystalline properties and particle shape. Further subsequent in-vitro and in-vivo nebulization and bioavailability studies for the optimized sildenafil loaded SLN formula are to be completed in separate work.

MATERIALS AND METHODS

MATERIALS

Sildenafil (purchased from Sigma-Aldrich, Steinheim, Germany), Precirol ATO 5, Gelucire 44/14, Compritol 888, and Cremophor EL (gift from Gattefosse SAS, Saint-Priest Cedex, France), Pluronic f 68, Deoxycolic acid sodium, Glyceryl monostearate, Stearic acid, Palmitic acid, Lauric acid, cholesterol, bees wax, carnauba wax, Stearylamine and polysorbate 80 (Merck KGaA, Darmstadt, Germany). All other solvents are HPLC grade (Sigma-Aldrich, Steinheim, Germany) and were used as obtained from the manufacturers without further purification.

METHODOLOGY

Selection of lipid materials

Varieties of lipid materials were tested for use as lipid core in preparation of sildenafil loaded SLN, including Stearic acid, Palmitic acid, Lauric acid, Glyceryl monostearate, cholesterol, Precirol ATO 5, and Compritol 888, bees wax and carnauba wax. Lipid solubility value of sildenafil in the proposed lipid materials was employed for the selection of the suitable lipid in the preparation of sildenafil loaded SLN.

Determination of sildenafil lipid solubility

The drug lipid solubility was measured according to the method applied by Joshi M and Patravale V, where the conventional equilibrium solubility determination method was modified by measuring the drug partitioning behavior from aqueous solution to the tested lipid (19). For that, 5 ml (1% Sildenafil) aqueous dispersion was shaken with melted lipid (1g) at 80 ^{o} C for 30 minutes in a hot water bath. The mixture was cooled to room temperature, centrifuged at 15000 rpm for 30 minutes. The aqueous layer was collected by syringe and the drug content was spectrophotometrically measured at 291 nm which was previously determined as drug λ max.

Preparation of solid lipid nanoparticles

SLNs were prepared by hot emulsification—ultrasonication method (20). Sildenafil (0.5%), solid lipid [namely; Compritol 888, Precirol ATO 5 or Glyceryl monostearate (GMS)] (2%) and Deoxycolic acid Sod. (0.5%) were dissolved in 20 ml mixture of chloroform and methanol (1:1). Organic solvents were then completely removed using a rotary evaporator. The drug loaded lipid layer was melted by heating at 80^{o}C. For the preparation of aqueous phase, Gelucire 44/14, Pluronic f68, or Cremophor EL were

separately dissolved in double-distilled water to produce 3% surfactant solution sufficient to produce 20 ml of the preparation and heated to the same temperature as the molten lipid phase. Hot aqueous phase was added to molten lipid phase, and homogenization was carried out (10,000 rpm at 80ºC) for 10 minutes. Coarse hot oil in water emulsion was obtained and then was sonicated using probe sonicator for further 10 minutes. Sildenafil SLNs were obtained by allowing hot nanoemulsion to cool to room temperature. Stearylamine was used as the surface charge modifier of SLNs (positive-charge inducing agent) and was incorporated within the lipids in SLN formulations (21).

Characterization of the systems

The prepared solid lipid nanoparticles will be evaluated as follows

Particle size measurement

The average diameter and polydispersity Index (PDI) of the prepared SLN formulae were determined by Photon Correlation Spectroscopy (PCS) using a Zetasizer 4 (Malvern, UK) at a fixed angle of 90°C and at 25°C after suitable dilution with distilled water. Each value is the average of three measurements.

Z-potential measurement

The electrical properties at solid/liquid interface was measured as Zeta Potential (ZP) of the dispersed solid particles using a Zetasizer 4 at 25°C also after suitable dilution with buffered double distilled water to adequate intensity (pH = 6.8). The recorded value is average of three measurements.

Drug loading and entrapment efficiency

Free drug concentration in the dispersion medium was measured as an indicator for the entrapment efficiency of the prepared SLNs using Centrisart, where centrifugation force was applied to separate the dispersion medium through ultra-filter (poly sulfone membrane-20 kDa MWCO) mounted at the base of the inner tube (recovery chamber) placed on the top of sample. 2.5 ml undiluted sample was placed in the outer tube and centrifuged at 5000 rpm for 30 min. The drug concentration in the aqueous phase collected in recovery chamber was spectrophotometrically measured at 291 nm which was previously determined as drug λmax. Encapsulation efficiency (EE) was calculated as:

$$\mathbf{EE\ (\%) = (Fs / Ts)\ 100}$$

Where:

Fs = soluble-free drug.

Ts = initial amount of drug added during preparation of SLNs.

Determination of drug release rate from the prepared SLN formulations

In this study, the dialysis bag method (22) was applied to investigate sildenafil release rate from the prepared SLN formulae using the United State Pharmacopoeia (USP) XXIV dissolution testing apparatus II (UDT-804 paddle dissolution apparatus, Logan, USA) in 500 ml phosphate-buffered saline (PBS) containing polysorbate 80 (tPBS) as dissolution medium of simulated lung fluid properties (23) maintained at 37 ± 0.5 ºC at 50 rpm using. Formula suspension was packed into dialysis bags with a 12 kDa molecular weight cutoff (MWCO) and immersed in the dissolution medium. Five ml dissolution medium sample was withdrawn with replacement using a glass syringe at 5, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 hours, filtered (0.45-µm). The absorbance of the drug was spectrophotometrically measured at λ_{max} 291 nm (Shimadzu UV/Vis double beam spectrophotometer). The cumulative percentage of drug release was calculated using an equation obtained from previously constructed standard calibration curve. For comparison, the dissolution rate of plain drug was also determined. The mean of six determinations was considered.

Statistical analysis of dissolution data

The dissolution data was statistically analyzed using post hoc one-way ANOVA test (Tukey mode) to declare the significance of the observed difference in dissolution profile of drug from the prepared SLN formulations in comparison to plain drug at p-value > 0.05 (IBM-SPSS, Inc., Chicago, Illinois).

Optimization of the prepared solid lipid formulation

Depending on the results of the previous studies, Sildenafil solid lipid formula of the smallest particle size, maximum drug loading capacity and highest drug release rate was selected and subjected to further evaluation including the following:

Solid state characterizations and compatibility studies

The lyophilized optimized sildenafil solid lipid formula, plain drug, and formula components were subjected to the following studies.

Differential scanning calorimetric studies (DSC)

Sample (10 mg) were separately weighed into an aluminum pan of differential scanning calorimeter (Perkin-Elmer DSC4 U.S.A.), calibrated with purified indium standard (99.9%), and continuously purged together with a blank with nitrogen gas over a temperature range of (25-300ºC) at heating rate of 10°C/min. The DSC thermograms were recorded and analyzed.

Infrared Spectroscopy (IR)

Sildenafil and the selected SLN formula samples (2-4mg) were separately mixed with about 400 mg of dry potassium bromide powder and compressed into transparent disc under pressure of 10.000 to15.000 pounds/inch^2; and scanned in the range of 4000 - 500 cm-^{1} at ambient temperature using IR spectrophotometer (Shimadzu IR-435, Kyoto, Japan) their IR spectra were recorded and analyzed.

X-Ray Diffraction (XRD)

Sildenafil and the selected SLN formula samples were subject to X-ray diffraction analysis on XRD-6000 X-ray powder diffractometer (Shimadzu, Japan) coupled with a standard Cu Sealed X-ray tube with 40 kVvoltageand 40 mA current. Data collection was performed at 2- theta of 5 - 60° in steps of 0.04 and scanning speed of 0.4 degrees per step. XRD charts were recorded and investigated for any change in the drug crystalline pattern.

Scanning electron microscopy (SEM)

Following particle suspension in water, a few drops of the dispersion were placed on a slab and dried under vacuum at RT. A Sputter Coater® JFC-1100 (JEOL, Tokyo, Japan) was used to coat the dried samples with gold (~20 nm thickness), placing them onto stubs. Finally, the samples were observed under a JSM-6400® SEM (JEOL, Tokyo, Japan).

Mathematical Kinetic modeling of drug release data

To study the mechanism of sildenafil release from the prepared optimized SLN formula, KinetDS 3.0 (Aleksander Mendyk, GNU GPLv3 license, 2007) software was used to kinetically analyze the release data.

RESULTS AND DISCUSSION

In previous work sildenafil citrate was formulated as SLNs to improve its water solubility and/or bioavailability (24). In this work sildenafil base was selected to be formulated as SLNs due to higher lipophilicity to maximize the SLN drug loading ability and minimize the ionized citrate charge effect on the prepared SLNs zeta potential.

Sildenafil lipid solubility and solid lipid selection

The drug entrapment within the prepared SLN formulations is highly correlated to its lipophilicity and hence solubility in the applied solid lipid (25). For that, initial study of drug partitioning between the tested melted lipid and aqueous phase can be applied as a significant criterion for predicting the drug loading and entrapment efficacy in the prepared SLN formulation (26).

For the selection of the most appropriate lipid to be applied in the preparation of solid lipid nanoparticle formulae, the ability of different lipid varieties of different natures to tolerate sildenafil was screened by percentage of drug partitioned from aqueous solution to the fatty layer after shaking together for 30 minutes. Results presented in Table 1, indicated that Precirol ATO 5, Compritol 888 and GMS showed maximum drug tolerating ability where the percentage of partitioned drug was 89.67, 86.43 and 79.36% respectively. So, they were selected as lipid core for the preparation of solid lipid nanoparticles.

Precirol EL and Compritol 888 are mixture of mono-, di-, and triglycerides of palmitostearate and behenate respectively this resulted in loose imperfect matrix structure of high porosity allowing to accommodate more drug molecules (27), both lipids

almost have comparable lipophilicity as indicated by their similar HLB values (28). This explains the higher drug solubilizing ability which is correlated to higher drug entrapment efficacy of these two lipids from other tested lipids (29).

Table-1: Lipid solubility of sildenafil base

Tested solid lipid	Drug content (%)
Stearic acid	76.45
Palmitic acid	70.98
Lauric acid	64.54
Glyceryl monostearate	79.36
Cholesterol	75.61
Precirol ATO 5	89.67
Compritol 888	86.43
Bees wax	59.64
Carnauba wax	54.99

Characterization of the prepared SLN formulae

Table 2, shows the formula composition of the prepared sildenafil SLNs using different solid lipids and surfactants. Deoxycolic acid sodium was added to lipid mixture in all formulations to improve drug entrapment efficacy and stabilize the prepared final formulae (30). Using three solid lipids and three surfactants, nine sildenafil SLN formulations were prepared using hot homogenization- ultrasonication method which is an efficient, simple, and quick method to produce SLNs.

Table-2: Formulae composition of sildenafil SLNs

Formula Composition	Formula Code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Sildenafil	+	+	+	+	+	+	+	+	+
Precirol ATO 5	+	+	+						
Compritol 888				+	+	+			
GMS							+	+	+
Gelucire 44/14	+			+			+		
Pluronic f68		+			+			+	
Cremophor EL			+			+			+
* DOCA	+	+	+	+	+	+	+	+	+
*: Deoxycolic acid sod									

Entrapment efficacy

The characterization results for the prepared sildenafil SLNs are presented in Table 3. The entrapment efficacy of the prepared SLNs varied according to the used lipid type and to lesser extent to the applied surfactant type. Generally, it could be considered that all prepared SLN formulae have satisfied drug entrapment ability ranged from 94.39% to 89.11%.

Despite behenic acid has a longer chain length (C_{22}) than either stearic acid (C_{18}) or palmitic acid (C_{16}) of expected higher lipophilicity and hence higher drug entrapment efficacy (31), the diversity of fatty acid content of Precirol EL may explain its higher drug tolerating ability than Compritol 888. previous results including lipid solubility and entrapment efficacy reflects the positive effect of using sildenafil base in SLN formulations rather than the citrate salt.

Particle size and size distribution

Results in Table 3 showed that, all prepared SLN formula showed acceptable particle size ranged from 44 nm to 107 nm with acceptable PDI in the range of 0.327 ± 0.131 and 0.296 ± 0.112. The small recorded PDI values indicates a narrow particle size distribution, in contrast to the entrapment efficacy the applied surfactant type showed greater effect on particle size, where smaller size was recorded for formulae prepared by Pluronic f68 followed by Gelucire 44/14 where larger particles were collected when Cremophor EL was applied as surfactant.

Particle size of SLN formulations is a complicated process and affected by many factors including lipid type, concentration, emulsifying properties, surfactant type (HLB), crystallization conditions including homogenization/sonication time and speed (27). In this work lipid concentration, preparation and lipid crystallization conditions were kept constant. Higher melting point lipids have slower crystallization rates during hot homogenized with subsequent increase in the particle size (32). The proximity of melting point value of applied lipids explains the recorded results with neglecable effect of lipid type on particle size.

The surfactant effect on particle size was more significant and could be correlated to the HLB values of the applied surfactant, Figure 1, where the smaller particle size was recorded in SLN formulation prepared using Pluronic f68 of higher HLB (equals 29) followed by Gelucire 44/14 and Cremophor EL (HLB =14). These results are in accordance with that recorded by P Ekambaram and Hasan Sathali A Abdul who recorded that the particle size of Ramipril-loaded solid lipid nanoparticles increased in the order of Poloxamer 188 > Tween 80 > Span 20 (32). The higher melting point and molecular weight of Compritol 888 can describe the larger particle size of Compritol 888 based SLN formulae.

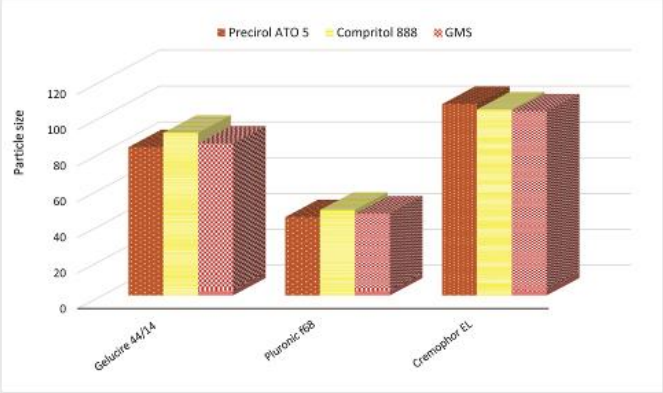

Figure-1: Histogram of the effect of surfactant type on particle size of SLNs.

Zeta potential

The high shearing conditions applied during the preparation of SLN to produce particles of smaller size generally causes undesired increase in the surface free energy at the lipid/water interface with subsequent significant decrease in physical stability as particles tend to spontaneously re-aggregate (33). For that, surfactant is added during formulation to reduce the interfacial tension and stabilize the prepared formulae. Type and concentration of applied surfactant is critical factor in SLN formulation to insure physically stable product (34).

According to results presented in Table 3, Zeta- potential was almost similar in all prepared SLN formulae in the range of +20.3 to + 24.5 with neglicable difference values and this was expected due to addition of Stearylamine as positive charge inducer and due to fixation of preparation conditions (homogenization and sonication speed and time).

SLNs with low zeta potential being unstable and being highly susceptible to aggregation on storage, it is also documented that SLNs of zeta potential values higher than – 60 mv is an indication of physically stable condition (35). For that and to have higher control on zeta potential value on the prepared SLNs, stearyl amine as charge-inducing agents was added during preparation of SLNs to be entrapped within the lipid core projecting its charged amine groups outward forming a positively charged electric repulsive layer on particle surface to prevent their aggregation and particle size change (36). Stearyl amine concentration in the prepared SLNs formulae was selected to give the optimum zeta potential value depending on results of trial experiments (not mentioned in this article).

Table-3: Characterization of the prepared sildenafil SLNs.

Code	E.E	Particle size	Zeta potential
F1	92.83	83±1.23	+ 20.3
F2	94.39	44±1.46	+ 21.4
F3	90.14	107±1.91	+20.8
F4	89.11	91±1.11	+22.7
F5	92.41	48±1.09	+23.6
F6	89.77	104±1.49	+23.2
F7	89.13	85±1.22	+24.5
F8	90.44	46±1.33	+26.4
F9	89.98	103±1.42	+ 23.9

Drug release study

Phosphate-buffered saline (PBS) containing polysorbate 80 (0.2%) [TPBS] was selected as dissolution medium to test for drug release from prepared SLNs to simulate lung fluids, the dissolution data are presented in Figure 2. The drug release profiles from the prepared SLNs generally showed high drug release rates that exceed 75% in all formulations in comparison to only 26.83% for plain drug after 12 hours, Figure 2-A & B, this could be explained partially by the presence of polysorbate 80 as a component of the dissolution medium that have a positive effect on drug release rate (37). The drug release rate was affected by formula components, for SLN formulations prepared using Precirol ATO 5 as lipid the percentage drug released was 80.92, 95.12 and 83.12% for Gelucire 44/14, Pluronic f68 and Cremophor EL as surfactants respectively after 12 hours. The drug release rate was 77.95, 92.22 and 81.79% after 12 hours from SLN formulae prepared using Compritol 888 as lipid for the different surfactants respectively. In case of SLN formulae prepared using Glyceryl monostearate as lipid, the drug release reached 75.92, 88.72 and 76.92% after 12 hours for different surfactants respectively. Previous studies concluded that use of mono and diglyceride lipids as a matrix base in SLNs could promote drug solubilization 12, this can explain the higher drug release rates from Precirol EL and Compritol 888 based SLNs than that from Glyceryl monostearate based formulae.

Results also showed that Pluronic f68 showed a higher positive effect on dug release rate where the percentage of drug release reached 95.12% in comparison to 92.22% and 88.72% after 12 hours for different lipid types (Precirol ATO 5, Compritol 888 and Glyceryl monostearate) respectively, figure 2-C. This could be explained by the high HLB value of Pluronic f68 (HLB = 29) when compared to Gelucire 44/14 and Cremophor EL (HLB =14). It is also worthy to say that the drug release profile from the prepared SLNs showed initial higher release rate followed by slower extended rate, this release profile is common in matrix based formulae where initial rapid drug release rate occurs due to solubilization of drug from outer most layer and by time the release rate declines as it is controlled by diffusion of the dissolution medium through deeper matrix layer and solubilization of the drug within, also erosion of the matrix may occur by time with subsequent increase in the viscosity of the stagnant layer that

also retard penetration of medium through the matrix and slowing the dissolution process. Similar results were recorded by many authors who studied different drug release profiles from SLN formulae (21, 38).

Statistical analysis of the dissolution data

Statistical analysis of the dissolution data of sildenafil from the prepared SLN formulae in comparison to plain drug was made according to Post hoc one-way ANOVA test. Although the results showed that the overall variance between group means was insignificant, Table 4, at the selected probability level ($p > 0.05$), further data manipulation according to post hoc (Tukey mode) analysis, Table 5 and Figure 2-D, showed that only dissolution profile of drug from SLN formula F2 was significantly different from other compared groups including plain drug.

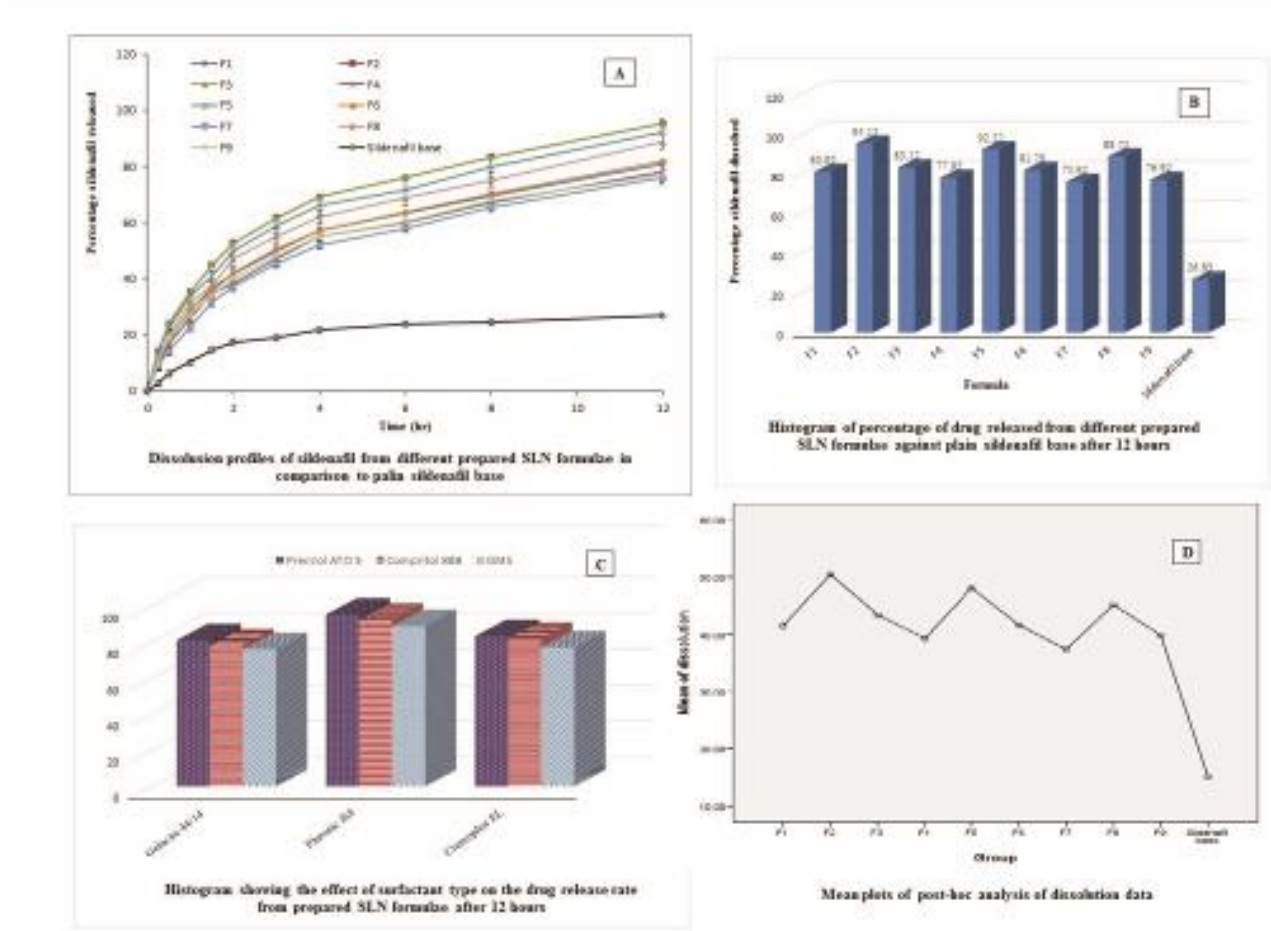

Figure-2: Dissolution data of Sildenafil from different prepared SLN formulae in comparison to plain drug

Table-4: One way ANOVA test of dissolution data

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9299.578	9	1033.286	1.613	**0.122**
Within Groups	64064.793	100	640.648		
Total	73364.371	109			

Since the overall one-way ANOVA tests the null hypothesis that all the treatments have identical mean values, it is affected by random sampling. In contrast, multiple comparison tests (post-test) tests the null hypothesis that every group pair have identical

means. For that, post-tests are considered more focused and have higher power to find differences between groups even when the overall ANOVA is not significant (39).

Table-5: Post-hoc analysis (Tukey HSD) of dissolution data (p > 0.05) with Confidence intervals.

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower	Upper
F2	F1	8.98273	10.79266	0.998	-25.9453	43.9107
	F3	7.08545	10.79266	1.000	-27.8426	42.0135
	F4	11.18636	10.79266	0.989	-23.7416	46.1144
	F5	2.34364	10.79266	1.000	-32.5844	37.2716
	F6	8.81545	10.79266	0.998	-26.1126	43.7435
	F7	13.12000	10.79266	0.968	-21.8080	48.0480
	F8	5.30909	10.79266	1.000	-29.6189	40.2371
	F9	10.62000	10.79266	0.993	-24.3080	45.5480
	Sildenafil	35.36000	10.79266	**0.045**	0.4320	70.2880

Optimization of the prepared SLN formula

Depending on the previous evaluation and characterization studies, sildenafil SLN formula No.2 (F2) prepared from Precirol ATO5 as solid lipid, Pluronic f68 as surfactant and Stearylamine as charge-inducing agent was selected as an optimum formula of higher drug entrapment efficacy, smaller particle size and higher drug release rate and subjected to further evaluation studies including solid state characterization and SEM examination.

Thermal analysis

DSC is a valuable, simple, highly trusted tool that giving an indication about drug purity, formula compatibility and also drug crystalline characteristics within the mixture which is a direct consequence of distribution within certain matrix. As presented in Figure 3-A, the DSC thermogram of plain sildenafil showed a sharp endothermic melting peak at 189.88ºC which is in consistence with the data in literature (40) indicating purity of the used rug sample. Complete disappearance of the drug characteristic sharp endothermic peak in the thermograms of the prepared SLN and decrease of melting enthalpy without appearance of any other new peaks indicates uniform drug distribution within the lipid of the formed nanoparticles in the formulation in amorphous form.

Infra- red analysis

Figure 3-B, shows the IR spectra of plain sildenafil and the selected SLN formula. The drug main functional groups of sildenafil namely; unsaturated C-H stretching (3029.68 cm^{-1}), saturated C-H stretching (2961.60), C=O stretching (1702.17 cm^{-1}), secondary N-H stretching (3299.49 cm^{-1}), and SO_2 stretching (1172.56 cm^{-1}) are all appeared in the IR spectrum of plain drug and retained in the spectrum of the selected SLN formula eliminating the possibility of any chemical interaction and insures the compatibility of the selected excipients.

XRD analysis

Figure 3-C, shows the XRD pattern of sildenafil in comparison to the selected SLN formula. The XRD pattern of plain drug exhibited sharp, intense, and less diffused peaks at 2 theta angels of 7.35, 8.09, 10.23, 13.07, 14.39, 16.23, 17.52, 19.75, 20.61, 22.70, 24.38, 22.72, and 28.46° indicating the high crystalline nature of drug sample. The XRD pattern of the optimized SLN formula showed major changes including peak disappearance and decreased intensities with attenuated diffraction at same diffraction angles ascertain the solid-state transformation of sildenafil to amorphous form within SLN formula. These results are in accordance with that recorded by DSC studies.

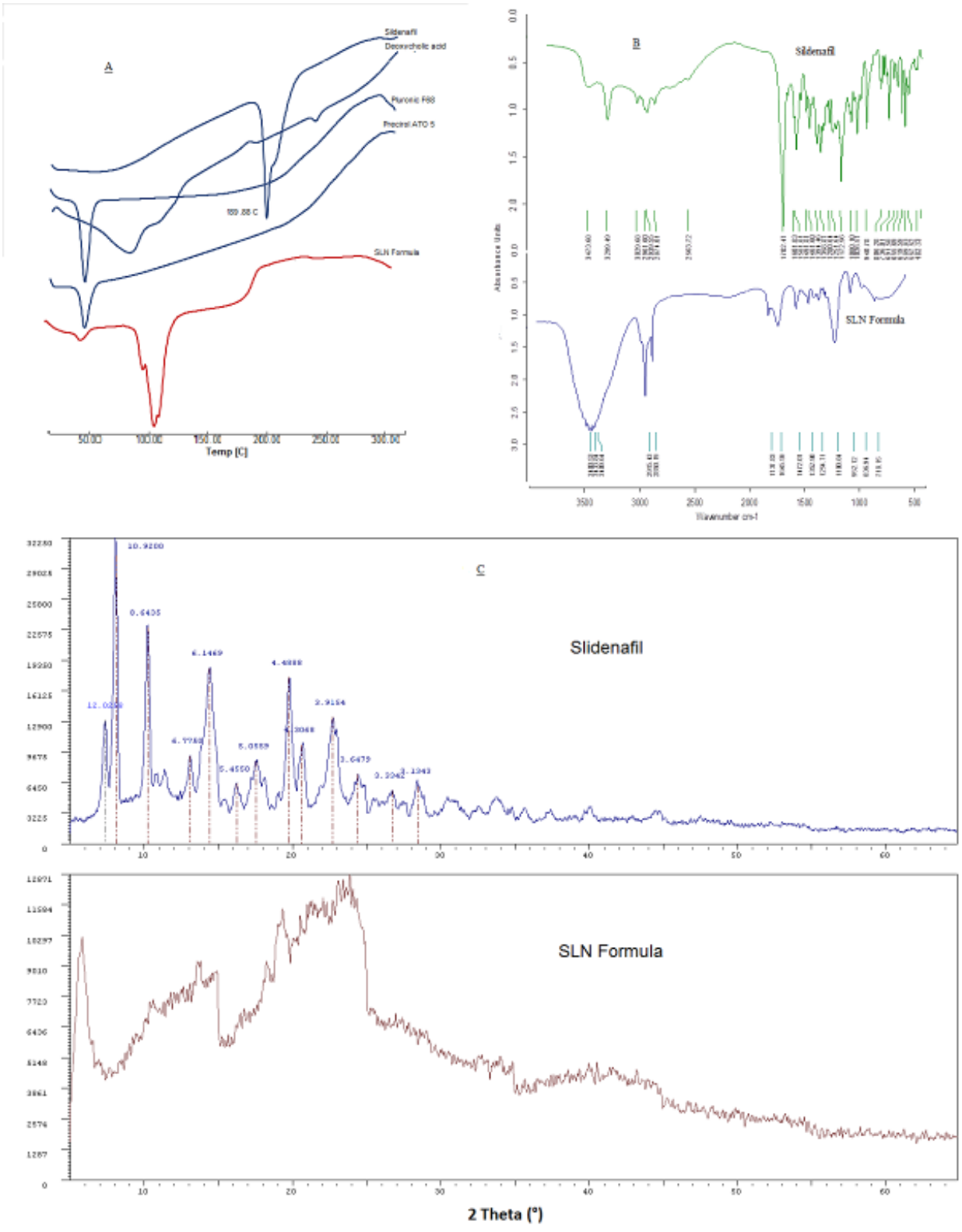

Figure-3: Solid state characterization of the optimized SLN formula using A) DSC, B) IR and C) XRD

Scanning electron microscopic examination (SEM)

SEM images of the optimized SLNs (F2) were presented in Figure 4. Sildenafil loaded SLNs were almost spherical particles with rough or irregular surfaces. Some particle appeared as aggregates in the sample that may occur due to inappropriate dilution of the sample before imaging. The sizes observed from SEM micrographs were almost the same those obtained from particle size analyzer.

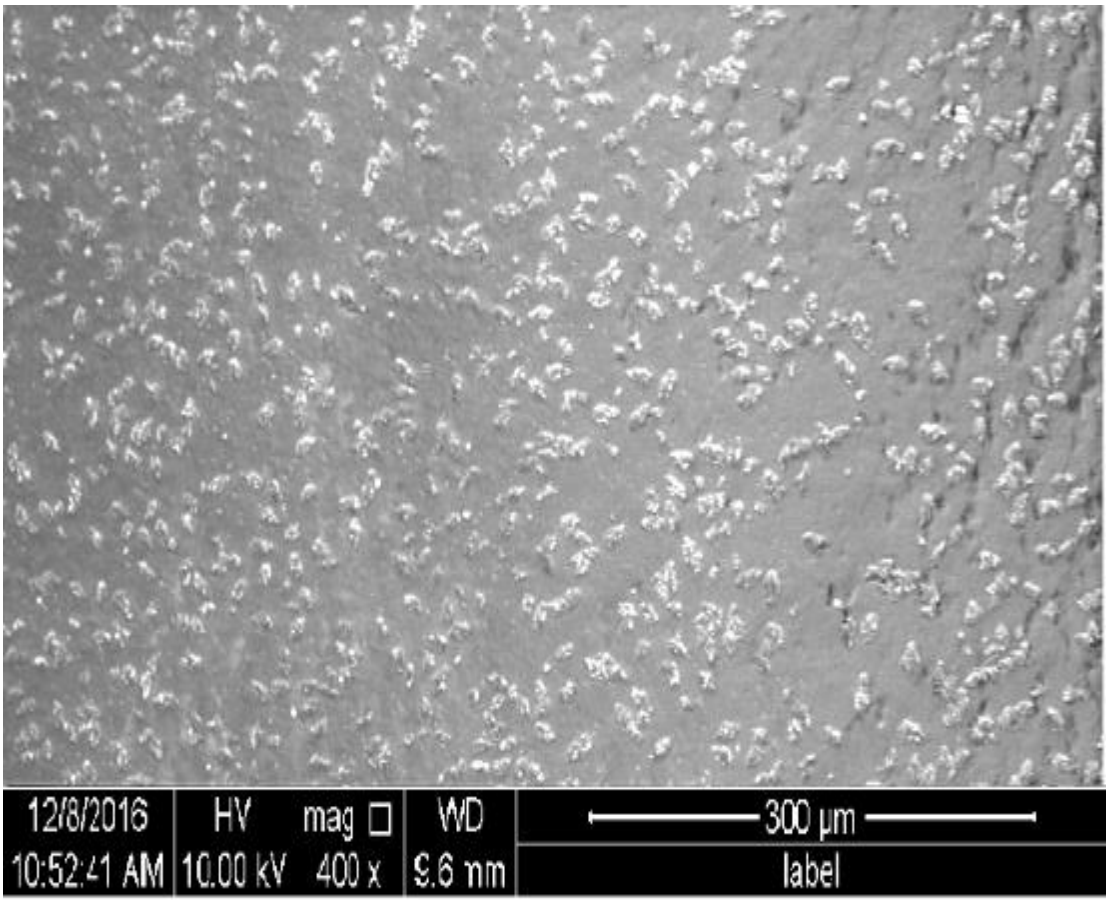

Figure 4: SEM image of the optimized SLN formula.

Kinetic analysis of the drug release data

Kinetic analysis of the drug release data from optimized SLN formula, Figure 5, showed that Sildenafil release followed Weibull diffusion model with correlation coefficient (R^2) equals 0.9936 which is usually used to describe the release profiles of matrix type drug delivery (41) and these results reflects the proposed matrix structure of the formulated SLNs with drug uniformly distributed through the matrix rather than formation of core/coat structure.

These results are also in accordance with that obtained by Maryam Hasan et al, who studied the kinetic release data from SLN formulae and concluded that Weibull model is an optimum kinetic model to be applied and generally describes the complex multi-mechanistic drug release involving dissolution, diffusion, and mixed dissolution - diffusion rate from SLN formulations (42).

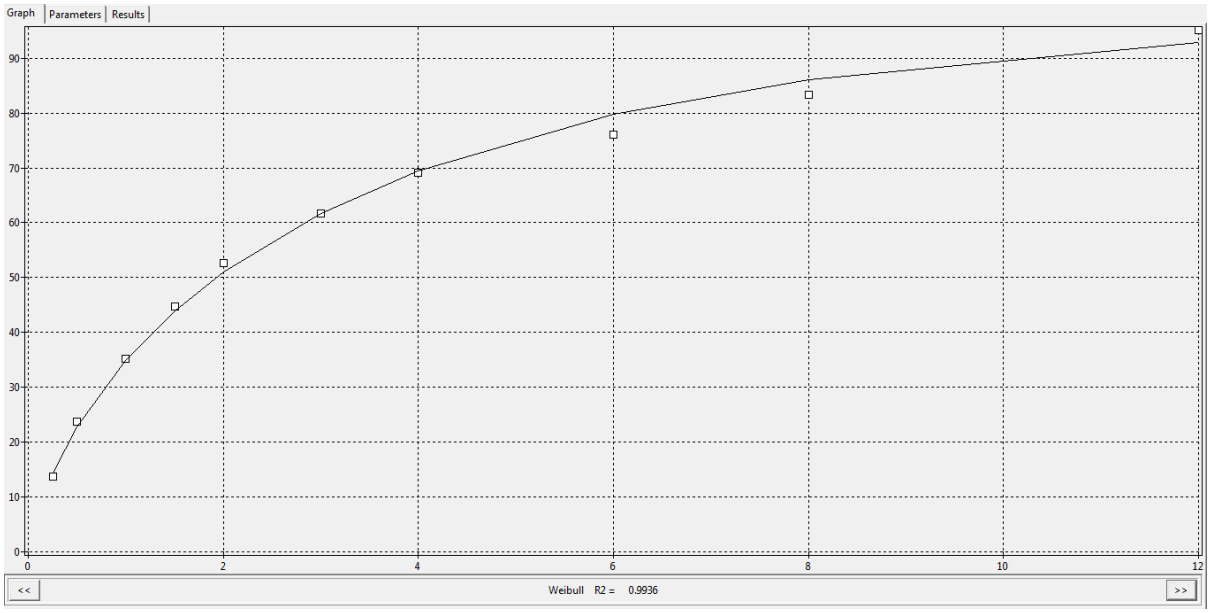

Figure-5: Kinetic analysis of sildenafil release data from optimized SLN formula

CONCLUSION

In this work sildenafil base, rather than the citrate salt was selected as the most lipophilic form of drug to be formulated into SLNs formulae. The prepared optimized formula showed small particle size, high drug entrapment capacity, optimum zeta potential extended improved drug release rate in simulated lung conditions and high compatibility. This sildenafil loaded SLN formula is suitable for nebulization and delivery through the lung due to small particle size, uniform size distribution and compatibility with the lung tissue, and needs further investigation for *in-vitro* spraying pattern and performance on cascade Impactor and *in-vivo* bioavailability studies in animals to be completed in separate work.

DECLARATION OF INTEREST

Author of the article declares no conflict of interests in relation to this study.

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