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# Preparation and Evaluation of Inhalable Sustained Release Sildenafil Citrate Solid Lipid Microparticles Dispersions

By AA Yas
*Tikrit University*

*Abstract-* This is a preliminary study utilizing drug targeting approach for developing sildenafil citrate (SFC) pulmonary delivery system, a first – line for pulmonary arterial hypertension (PAH) treatment and hence reducing the dose and the side effects. The closed melt method was employed for preparing SFC – solid lipid microparticles dispersions (SFC – SLMDs), a non – solvent technique aid in the production of drug – matrix dispersions with sustained release properties. Glyceryl behenate (GB) (Compritol® 888 ATO) was used as the retarding matrix and the results shown that as its ratio increase there was a decrease in the fine particle fraction, an increase in the drug content and a prolong drug release pattern. The best model fit the release data was Higuchi – Matrix model which indicates drug diffusion – controlled releasing mechanism. Thus, inhaled SFC – SLMDs dry powder will improve PAH treatment via drug localization at low doses and reducing the administration frequency.

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## I. Introduction

Pulmonary arterial hypertension (PAH) is a chronic disease characterized by increased pulmonary vascular resistance and pulmonary arterial pressure resulting from blood flow restriction in the pulmonary arterial circulation, and hence shortens lifespan by leading to right – sided heart failure. The most common form is idiopathic with unknown risk factor. Although, PAH pathobiology is not well understood, the pathologic abnormalities of vascular endothelial and smooth muscle cells result from excess cellular proliferation and apoptosis resistance together with inflammation, vasoconstriction and in situ thrombosis contribute to the distal pulmonary arterioles narrowing [1]. Sildenafil citrate (SFC) has been approved by the Food and Drug Administration (FDA) and the European Medicine Agency (EMA) as first – line for PAH treatment. It acts on the NO pathway by inhibition of phosphodiesterase type 5 (PDE – 5) responsible for cyclic guanosine monophosphate (cGMP) degradation which play a role in vasodilatation. NO acts intracellularly within the smooth muscle cells by allosteric binding to the soluble guanylate cyclase (sGC) prosthetic heme group. The subsequent sGC activation catalyzes the conversion of guanosine triphosphate (GTP) to cGMP leading to vasodilatation. SFC is administered for PAH treatment as 20 mg oral tablet, 10 mg / ml oral suspension and 0.8 mg / ml intravenous (IV) formulation. They will produce the same SFC plasma concentration at their usual doses [2].

Pulmonary targeting of SFC will be promising for local treatment of PAH due to skipping liver first – pass effect, reduction in the dose and side effects [3], and improving pediatric patients' compliance [4]. Pulmonary route offers many advantages over other routes, such as high surface area and vascularization. Solid lipid nanoparticles (SLNs) consist of phospholipid: triglyceride 30: 70 ratio aqueous nanoscale suspensions is one of the colloidal drug delivery systems that is ideal platform for hydrophobic drugs, physiologically compatible and with typical pulmonary applications [5]. In vitro and ex vivo toxicological testing of SF – loaded SLNs support system suitability for the PAH treatment via pulmonary delivery [6]. Nanosuspensions formulation prepared by SFC monohydrate being complexed with cyclodextrins (α – CD, HP – β – CD and γ – CD respectively), where SF piperazine moiety formed an inclusion in the cavity of the CDs, enhancing its water solubility by a bottom – up process using dried ethanol as a solvent and HFA – 134a as an antisolvent and propellant in order to form pressurized metered – dose inhaler (pMDI) [7].

Pulmonary localizing drug release by preparing dry powder inhaler (DPI) formulation containing particles that microscaled enough to be inhalable, in the same time a release – modifying matrix should exist in order to control drug release after delivery. The difficulty inherited in the micro – sized particles production, where a size reduction always accompanied by an increment in surface areato – mass ratio; subsequently the difficulty will be escalated in the production of a controlled release profile and efficient release agent to be incorporated [8]. The present study aim is to improve SFC delivery characteristics using solid lipid microparticles dispersions (SLMDs) that utilize glyceryl behenate (Compritol ® 888 ATO) as the lipid matrix and closed melt method as a technique for dispersing / incorporating SFC, i.e.; SFC – loaded SLMDs

*Author: Department of Pharmaceutics, College of Pharmacy, Tikrit University, Tikrit – P. O. Box (42), Saladin – Iraq.*
*e-mails: dryasbiopharm@gmail.com , dr_yas@tu.edu.iq*

formulation in vitro aerodynamic and release profile evaluation.

## II. Materials and Methods

*a) Materials*

Sildenafil citrate (SFC) was obtained from the State Company for Drug Industries and Medical Appliances (SDI) (Samarra / Iraq). Gattefosse´ (Lyon / France) kindly donated glyceryl behenate (GB) (Compritol® 888 ATO). All other chemicals / solvents used were of analytical grade.

*b) High – Performance Liquid Chromatography (HPLC)*

The HPLC instrument (Shimadzu LC 20A / Japan) was equipped with a reversed – phase $C_{18}$ column (25 cm X 4.6 mm; particle size = 5 μm). The isocratic mobile phase, acetonitrile: 0.2 M phosphate buffer (70: 30, v / v, pH 7.4) was run at a flow rate of 1 ml / min at 25 °C and the column effluent was monitored by UV detector at 293 nm. A 20 μl of each sample was injected manually into the analytical column. The calibration curve of peak area versus SFC concentration was (Y = 1351313.56 X – 31213.43) under SFC concentration of 2 – 10 mg %. The retention time was 4.077 ± 0.32 min ($R^2$ = 0.999; limit of quantification = 2 – 10 μg / ml; accuracy = 99.85 %) [9].

*c) Preparation of Physical Mixtures*

Physical mixtures (PMs) of SFC and GB in powder form were mixed in mortar and passed through 60 – mesh screen (Retsch / Germany). The PMs were prepared in the following ratios; SFC: GB of 0.1: 1, 0.1: 2, 0.1: 3, 0.1: 4, and 0.1: 5.

*d) Sildenafil Citrate Solid Lipid Microparticles Dispersions (SFC – SLMDs) Prepared by Closed Melt Method*

The closed melting technique was employed in the preparation of solid dispersions (SDs). Weight of 2 gm from each PM was placed into an ampoule, sealed, heated at 80 °C for 10 minutes and then opened and dried for another 10 minutes at the heating temperature to remove the moisture. The collected sample from each ampoule kept overnight, triturated and passed through 625 – mesh screen (Retsch / Germany). The SDs were then stored in well closed containers until further use [10].

*e) In – Vitro Microparticles Aerodynamic*

Andersen cascade impactor (ACI) (Graseby – Andersen / USA) is employed in the fine particle fraction (FPF) determination in order to evaluate the in vitro deposition profiles of SFC. Samples of 30 mg were manually loaded into Rotahaler® and the ACI was operated at flow of 28.3 l / min for 10 seconds. The ACI stages effective cutoff aerodynamic diameter are as follows; stage 0, 9 μm; stage 1, 5.8 μm; stage 2, 4.7 μm; stage 3, 3.3 μm; stage 4, 2.1 μm; stage 5, 1.1 μm; stage 6, 0.65 μm; and stage 7, 0.43 μm. The definition of FPF is the amount of powder with an aerodynamic size ≤ 5 μm divided by the nominal dose [11].

*f) Drug Content and Percent Yield*

Accurately weighed SLMDs equivalent to 10 mg of SFC were added to 1000 ml of distilled water, heated up to 10 °C above excipients melting point on hotplate magnetic stirrer and then stir at 1500 rpm for 5 min to extract SFC. After being cooled to room temperature, the extract is filtered through 0.2 μm millipore filter, the drug content was determined using the previously detailed HPLC method and the percentage yield of SDs was also determined [12].

*g) In Vitro Release Study*

The conventional dissolution procedures utilizing large dissolution medium volumes will results in uncorrelated data in case of inhaled drugs, because the volume of surface liquid in the respiratory tract is relatively low. Therefore; in order to study SFC release from the SLMDs a dispersion method is being employed. Test tubes each contain 10 mg of each formulation suspended in 10 ml phosphate buffer pH 7.4 and incubated in a shaker at 37 °C on 50 rpm. Samples were withdrawn at time intervals of 0.25, 0.5, 1, 2, 4, 8 and 12 hours and SFC concentration was determined according to the HPLC method above [13].

## III. Results and Discussions

*a) In – Vitro Microparticles Aerodynamic*

There is a decline in the fine particle fraction as the GB ratio increases, as shown in table 1. The reason for the initial increment is due to the SFC amount add to the zeta potential, but as the GB amount further increased, the zeta potential is reduced [14]. In addition, because GB microparticles undergo phase transformations at low temperatures and their irregular morphologies, results in an instability state and higher interparticulate adhesion [15].

*Table 1 :* Effect of SFC: GB – PMs Ratios on the SFC – SLMDs Fine Particle Fraction

| SFC: GB – PMs | SFC – SLMDs | % FPF |
|---|---|---|
| PM 1 = 0.1: 1 | SLMD 1 | 23.32 ± 5.46 |
| PM 2 = 0.1: 2 | SLMD 2 | 19.33 ± 3.58 |
| PM 3 = 0.1: 3 | SLMD 3 | 14.60 ± 1.15 |
| PM 4 = 0.1: 4 | SLMD 4 | 11.20 ± 1.19 |
| PM 5 = 0.1: 5 | SLMD 5 | 8..27 ± 0.34 |

*b) Drug Content and Percent Yield*

Although SFC is an amphoteric drug and has pH – dependent characteristics, i.e. different level of ionization will affect its partition coefficient in both aqueous and oil phases, the entrapped amount of SFC was high and increased as the GB ratio increased, as shown in table 2. This is due to the method of preparation employed, where SFC solubility further increased in the melted GB resembling its solubility in oils which is higher than in solid lipids [9]. Also, the complexity feature of the GB which consists of varying 12 – 18 % mono -, 52 – 54 % di – and 28 – 32 % tri – esters of glycerol and behenic acid provides less ordered lipid crystals and hence high SFC quantity loaded [16].

*Table 2 :* Effect of SFC: GB – PMs Ratios on the SFC – SLMDs Drug Content and Percent Yield

| SFC: GB – PMs | SFC – SLMDs | % Drug Content | % Yield |
|---|---|---|---|
| PM 1 = 0.1: 1 | SLMD 1 | 95.24 ± 2.45 | 96.67 ± 1.37 |
| PM 2 = 0.1: 2 | SLMD 2 | 96.45 ± 1.46 | 97.30 ± 2.65 |
| PM 3 = 0.1: 3 | SLMD 3 | 97.51 ± 1.57 | 98.34 ± 1.89 |
| PM 4 = 0.1: 4 | SLMD 4 | 98.24 ± 0.53 | 99.32 ± 1.87 |
| PM 5 = 0.1: 5 | SLMD 5 | 99.41 ± 0.74 | 99.24 ± 1.08 |

*c) In Vitro Release Study*

The release profiles of SFC from SLMDs are sustained as shown in figure 1. They all have an initial slight burst followed by a sustained release over the 12 hours period. The reason for the former burst release is due to the free non – incorporated SFC amount accumulates on the surface of the SLMDs particles, whereas the reason for the latter sustained release is due to the closed melt technique employed in the SLMDs preparation which results in a drug solid solution incorporation model in a low crystallization degree GB matrix [17]. The cumulative percentage SFC released decrease as the GB ratio increased and hence more prolonged sustained release due to the steps govern the drug release from the SLMDs; entrance of the dissolution medium into the SLMDs matrices, dissolution of the dispersed SFC and diffusion of the dissolved SFC through the inert SLMDs matrices [18]. The release data were fitted to the zero – order, first – order, Higuchi – Matrix, Hixson – Crowell and Korsmeyer – Peppas release kinetic models to find the best fitting equation using DDSolver program [19]. The best fit was Higuchi – Matrix model with the highest correlation coefficient which predicts the drug diffusion – controlled releasing mechanism [20].

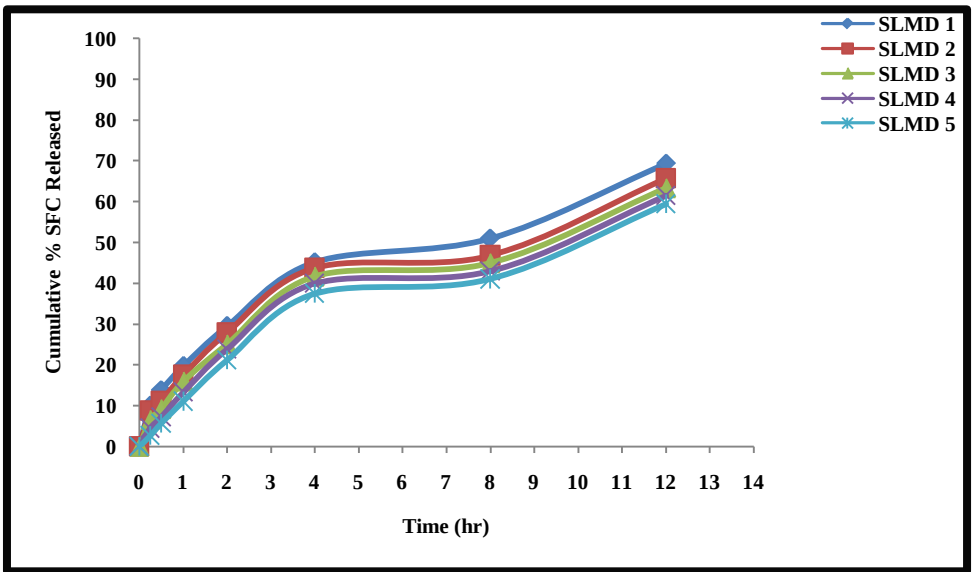


*Figure 1 :* In Vitro Cumulative Release Profiles of SFC from SFC – SLMDs in Phosphate Buffer pH 7.4/37 ˚C

## IV. Conclusions

The solid solution incorporation model of SFC within the GB matrix improves the prolonged release phenomenon which aid in the reduction of the dose used and the side effects. The varying percentages of mono -, di – and – tri – glycerides in GB produce less ordered lipid crystals which aid in SFC loading capacity and release retardation. Also, this GB complexity affects fine particle fraction and drug content and release. The sustained release of SFC was further improved by the closed melt technique that employed in the preparation of SLMDs which create a dry powder inhaler that best suited for PAH treatment.

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