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Formulation and optimization of drug-loaded mesoporous silica nanoparticle-based tablets to improve the dissolution rate of the poorly water-soluble drug silymarin

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Abstract

Porous carriers have been put forward as a promising alternative for stabilizing the amorphous state of loaded drugs, and thus significantly improving the dissolution rate of poorly soluble compounds. The purpose of this study was to enhance the saturation solubility, dissolution rate and drug loading of the poorly water-soluble drug silymarin via incorporation into mesoporous silica nanospheres within a lyophilized tablet to obtain a unique formulation. A full factorial design was applied to study the effect of both independent variables, polyvinyl alcohol (PVA) as stabilizer and binder and sucrose as cryoprotectant and disintegrant; and on the dependent variables that included the mean particle size (Y1), disintegration time (Y2), tablet strength (Y3) and % of drug release after 2 minutes, R2min, Y4. The drug-loaded mesoporous silica nanospheres and the optimized formula was evaluated by different characterization methods: scanning
electron microscopy, transmission electron microscopy, differential scanning calorimetry, X-ray diffractometry and Fourier transform infrared spectroscopy; as well as drug content, saturation solubility and moisture content. The evaluation demonstrated that the loaded mesoporous silica nanospheres and the optimized formula are in amorphous state without any chemical interaction with the silica matrix or the stabilizer. Moreover, the drug was stably maintained in nanosize range with narrow particle size distribution. Furthermore, the optimized lyophilized tablets had highly porous structure, low friability (less than 1%), fast disintegration (less than 30 seconds), high tablet strength, low moisture content (less than 1%), remarkably increased dissolution rate and noticeable improvement in saturation solubility.

**Introduction**

Oral drug delivery is considered the preferred route of administration of active pharmaceutical ingredients (APIs) due to its low cost and high compliance, i.e. being the easiest and simplest way for patients to intake medicines. However, the majority of the innovative APIs display poor water solubility and low oral bioavailability, both of which are considered the most critical problems facing the formulation scientists who are working on oral drug delivery (Kesisoglou et al., 2007). Different strategies have been used to overcome these issues (Rodriguez-Aller et al., 2015)(Stegemann et al., 2007). Recently, mesoporous silica materials (MSMs) have been increasingly employed as carrier matrices for enhancing the aqueous solubility and for improving the dissolution rate of poorly water-soluble APIs and, consequently, remarkably increasing the oral bioavailability (Biswas, 2017). Several methods of preparation of MSMs with different morphologies have been reported, the most famous being the MCM and SBA families of materials (Vadia and Rajput, 2012) (Trendafilova et al., 2016)(Crean et al., 2015). MSMs are inorganic materials that are characterized by several unique features. Amorphous silica, which the materials
are made up of, is classified as a “generally regarded as safe” (GRAS) material by the FDA; since it is bioerodible, chemically inert and exhibits good biocompatibility. In addition, the silica morphology, structure and surface can be easily modified to control the pore size and volume, and functionalized chemically to optimize the drug (API) affinity during both loading and release (Maleki et al., 2017). Moreover, and perhaps most importantly, the API can be maintained in amorphous state upon loading as a result of confinement of the drug in the nanosized pores, which in general remarkably enhances the aqueous solubility (Zhang et al., 2010). Furthermore, MSMs play an intrinsic role in the stability of the drug by protection from hydrolysis, oxidation or degradation processes by restricting the impact from the surrounding environment (elevated temperature/high humidity) due to the physicochemical stability of the silica matrix. However, there are several factors that could affect the API release from MSMs; for instance particle size, pore diameter, pore length and modification of the surface, leading to drug release occurring within a few minutes or within days from the pore surface (Maleki et al., 2017). In this study, MSMs with a particle size on the nanoscale was prepared via a heterogeneous oil-water biphasic stratification approach, in which the reaction takes place in the oil-water interface for one-pot continuous interfacial growth (Li et al., 2014). In this synthesis approach, the pore diameter can be controlled according to the concentration of both hydrophobic solvents and silica source (Li et al., 2014). The model drug used in our study is silymarin (SLM); used mostly as a hepatoprotective drug albeit with other potential therapeutic activities, for instance anticancer, anti-oxidation, anti-inflammation as well as for the treatment of Alzheimer’s disease (AD) (Guo et al., 2019)(Li et al., 2019)(Kren and Walterová, 2005). However, SLM is characterized by low aqueous solubility and poor permeation across the intestinal fluid. Moreover, SLM is subject to degradation by gastric
fluids. These shortcomings have manifested themselves in slow dissolution rates as well as poor oral bioavailability (Costanzo and Angelico, 2019)(El-Samaligy et al., 2006).

Freeze drying process is considered one of the most effective drying processes for maintaining the primary physical and chemical characteristics of nanoparticles (Abdelwahed et al., 2006). Moreover, freeze drying can be exploited for formulation of lyophilized tablets. Freeze drying process includes three main steps, namely: freezing, primary drying and secondary drying steps (Fonte et al., 2016). The most intrinsic factor that affects the efficiency of the freeze drying is the type and the concentration of cryoprotectant and lyoprotectant (Fonte et al., 2016). Different cryoprotectants and lyoprotectants could be used for maintaining the particles in nanosize range, for instance different types of sugars such as mannitol, sucrose, and trehalose; as well as different types of polymers such as polyvinylpyrrolidone (PVP) and polyvinylalcohol (PVA) (Ibrahim et al., 2019)(Ibrahim et al., 2018)(Abdelwahed et al., 2006). The aim of this study was to develop a loading strategy for mesoporous silica nanospheres (MSNs) that could be further incorporated within a particular type of oral disintegrating tablet (lyophilized tablet) to obtain a unique formulation, i.e. a lyophilized, drug-loaded MSN-based tablet. These lyophilized tablets are characterized by a high saturation solubility, fast disintegration, rapid dissolution rate, sufficient tablet strength, and low friability (less than 1%). Moreover, the loaded API was maintained in amorphous state and in nanosize range, which generally would contribute to significant oral bioavailability enhancement (Riikonen et al., 2018)(Juère and Kleitz, 2018)(Bremmell and Prestidge, 2019).
Materials and methods

Materials

Silymarin (SLM), Tetraethyl orthosilicate (TEOS), hexadecyltrimethyl ammonium chloride (CTAC), tri ethanolamine (TEA), ammonium nitrate, tween 80, sodium chloride poly ethylene glycol (PEG 6000) and absolute ethanol, were purchased from Sigma-Aldrich (St. Louis, MO, USA). polyvinyl alcohol 4-88, sucrose, potassium dihydrogen phosphate and Calcium chloride were purchased from Merck (Merck KGaA, Darmstadt, Germany). polyvinylpyrrolidone (PVP K-17), hydroxypropyl methylcellulose (HPMC, Benecel™ grade E5 Pharm), hydroxyethyl cellulose (HEC, Natrosol™ grade 250M) and hydroxypropyl cellulose (HPC, Klucel™ grade EXF Pharm) were donated by Ashland (Covington, KY, USA). Sodium hydroxide was purchased from VWR chemical (VWR, Geldenaaksebaan, Leuven, Belgium).

Methods

Preparation of mesoporous silica nanospheres (MSNs)

MSNs with a 3D-dendritic pore structure were synthesized by a one-pot biphasic stratification process with slight modification (Li et al., 2014). Briefly, 24 ml CTAC, 450 mg of TEA as catalyst and 36 ml of Milli-Q water were mixed gently on magnetic stirrer at 60°C for 1 hour in a 100 ml round bottom flask resulting in pH values of 8.5. Then, 20 ml of (20 v/v %) TEOS in cyclohexane were added dropwise to the aqueous phase and maintained at 60°C for 9 hours. The products were then extracted using ammonium nitrate ethanol solution followed by washing with absolute ethanol to remove the extraction solution. The extracted 3D-dendritic MSNs products were dried (Figure 1a) and stored at room temperature for further characterization, including mean particle size, zeta potential, pore diameter and morphology.

SLM loading procedure
SLM was loaded into the MSNs using the solvent evaporation method. 200 mg of SLM was dissolved in 1 ml of absolute ethanol, then mixed with different amounts of MSNs using a spatula until seemingly dry (Figure 1b). Different ratios of drug to carrier (1:1 and 1:2) were used and dried at room temperature for 48 hours for further characterization of their mean particle size, zeta potential, pore diameter, morphology, physicochemical properties and drug loading.

**Preparation of lyophilized MSN-based tablet (LMSNT)**

Different quantities of both PVA and sucrose were dissolved in milli-Q water to prepare a polymeric solution with different concentrations of both PVA and sucrose (table 1) with addition of 0.2 w/v % of PEG as co-binder. Then, the loaded MSNs were added to the polymeric solution. The system was sonicated on bath sonicator at 25°C for 30 minutes until the product was homogeneous (Figure 1c). The product was poured into the pockets of a PVC blister pack to attain a SLM dose of 25 mg per tablet. Then the blister pack was frozen at –80°C for 6 hours followed by lyophilization process for 48 hours using Hetotrap CT 60e freeze dryer (Heto-Holten AyS, Allerd, Denmark). The obtained lyophilized MSN-based tablet, LMSNT (Figure 1d) were kept in tightly closed containers at room temperature until further use.

**Experimental design and optimization**

Screening study was applied for several types of cryoprotectants and polymers to determine the most suitable excipients to maintain the individual nanoparticles during the freeze drying processes. Subsequently, the $3^2$ full factorial design was carried out in which cryoprotectant and precipitation inhibitor is considered the most intrinsic independent variables for the lyophilization cycle to obtain a proper lyophilized tablet and to preserve the particles in nanosize range upon redispersion in water. The concentration of both sucrose and PVA were used as independent variables with 3 levels for each factor for optimization process. The dependent
variables (responses) were mean particle size ($Y_1$), Disintegration time ($Y_2$), Tablet strength ($Y_3$) and $R_{2\text{min}}$, % of drug release after 2 minutes, ($Y_4$) as elucidated in Table 2, in which the all dependent variables should be minimized except $Y_3$ and $Y_4$ should be maximized. $3^2$ full factorial design was applied using design expert 10 software that provided suitable second polynomial model equations (equation 1) for each response.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_1^2 + \beta_5 X_2^2$$ (1)

$Y$ represents the measured responses, while $\beta_0$ is the intercept, $\beta_1$ to $\beta_5$ are the regression coefficient for the second order polynomial equation and $X_1$ & $X_2$ represents the independent variables. $X_1 X_2$ represents the interaction between the main effects. $X_1^2$ & $X_2^2$ are quadratic terms of independent variables that were used to simulate the curvature of the designed sample space. Moreover, $3^2$ full factorial design showed the analysis of variance that aim for demonstration whether there was a significant effect ($P > 0.05$) of each factor individually and their interaction on the all responses. Furthermore, it displayed 3 dimensional graph of responses surface for the all dependent variables and finally exhibited the predicted value for each responses to be compared with the observed values of the optimized formula to determine the relative error as elucidated in the equation 2.

$$\text{Relative error (\%) } = \left( \frac{\text{Predicted value} - \text{Experiment value}}{\text{Predicted value}} \right) \times 100$$ (2)

**Characterization of MSNs, loaded MSNs and LMSNTs**

**Nitrogen physisorption measurements**

Nitrogen physisorption measurements of MSNs as well as SLM-loaded MSNs were carried out on an ASAP2010 instrument (Micromeritics, USA). The samples were degassed at 40 °C for at least 12 h before analysis. The surface area was calculated using the Brunauer–Emmet–Teller
(BET) method in the relative pressure range 0.05-0.20 P/Po, the pore size distribution was
analyzed using the Barett–Joyner–Halenda (BJH) model, and the total pore volume was
determined by the amount of nitrogen adsorbed at P/Po = 0.99.

**Transmission Electron Microscopy**

The surface morphology of pure MSNs, loaded MSNs and optimized LMSNT was visualized by
transmission electron microscope (TEM) (Jeol JEM-1400 plus TEM, Tokyo, Japan). Pure MSNs
and loaded MSNs were redispersed in distilled water using bath sonicator. The optimized
LMSNT was redispersed in distilled without sonication. Briefly, a drop of the resulted dispersion
was placed onto carbon-coated copper grids. Then the grid was air dried, and viewed under
different magnifications.

**Scanning Electron Microscopy**

Scanning electronic microscopy (SEM) was applied for pure MSNs and loaded MSNs to detect
the size and the surface of the particles. Moreover, SEM images were studied for cross-section
and surface view of optimized LMSNT using SEM instrument (LEO 1530 SEM Oberkochen,
Germany). The sample was mounted in resin followed by rough grind and polishing of the
opposite side to be flat that aim the sample to stick easily on the plate specimen. The sample was
coated with carbon to improve the electrical conductivity before examination. The sample was
viewed at different magnifications.

**Mean particle size, zeta-potential and poly polydispersity index (PdI)**

The particle size, PdI and zeta potential for pure MSNs, loaded MSNs before and after
lyophilization of LMSNTs were measured using dynamic light scattering and electrokinetic
measurements (Nano ZS, Malvern Instruments Limited, Worcestershire, UK). Each sample was
suitably suspended with Milli-Q water before analysis. Each sample was analyzed in triplicate and the results were recorded as the mean value of these runs ± SD.

**Differential Scanning Calorimetry (DSC)**

The DSC thermograms of pure SLM, pure MSNs, loaded MSNs (1:1 and 1:2), PVA, Sucrose, lyophilized MSNs and PVA, lyophilized MSNs and sucrose, blank LMSNTs (lyophilized tablet without drug), physical mixture (mixture of pure SLM and blank) and optimized LMSNTs were recorded using DSC apparatus (DSC Q2000, TA Instruments—Waters LLC, New Castle, Delaware, US). Approximately 1–2 mg of fresh sample was taken in an aluminium pan and crimped on lids using a press. Samples were heated at scanning rate of 10 °C/min in the range of 25 to 300 °C in presence of nitrogen at flow rate of 50 ml/min to obtain the endothermic peaks.

**Powder X-ray Diffractometry (PXRD)**

Powder x-ray diffraction measurements were applied for pure silymarin, pure MSNs, loaded MSNs 1:1 and 1:2, blank lyophilized tablet, physical mixture and optimized LMSNTs. PXRD was carried out using a Bruker D8 Discover instrument (Karlsruhe, Germany) equipped with a Cu Kα x-ray source and scintillator point detector. The samples were scanned in the 2-theta range of 10-40° using a step size of 0.04° for 4 s per step.

**Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR)**

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra were applied for all samples used for DSC was performed using a Spectrum two spectrophotometer (UTAR-FTIR spectrophotometer, PerkinElmer, Llantrisant, UK). The samples were placed on a diamond crystal then the spectra recorded in transmittance mode at 100 scans and at 4 cm⁻¹ resolution over the wavenumber region 4000–400 cm⁻¹.
**In vitro disintegration test**

According to FDA, disintegrating test could be applied using conventional disintegrating testing (McLaughlin et al., 2009). The disintegration times for the nine formulae and optimized formula were determined via Disintegration Tester (Sotax DT2, Basel, Switzerland) according to USP32-NF27 for uncoated tablets. Six tablets from each formula were selected randomly to determine the time required for complete disintegrating in the dissolution medium at 37 ± 0.5°C. All the results displayed as the mean value ± SD (n = 6).

**Friability test**

Friability test was applied for the nine batches and optimized formula using the drum of Roche friabilator USP test apparatus (Erweka type, GmbH, Germany). Ten tablets for each formula were placed in the drum and rotated for 4 minutes at 25 rpm. The tablets were weighed before and after the test to determine the percent of friability which should be lower than 1 % according to the US pharmacopeia. The friability percent were determined according to the following equation.

\[
\text{Friability} \% = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100
\]  

(3)

**Tablet crushing strength**

Tablet strength was applied to detect the breaking point and the structural integrity of the tablet and to examine its ability to withstand the abrasion during handling, packaging and shipment. The hardness of the tablet was evaluated for the nine formulae and the optimized formula. Tablet Hardness Tester (Tablet Hardness Tester TH3/500Nottingham, UK) was used to determine the tensile strength. The tablet is placed on the test platform between the test jaw and the load cell plunger. A multi-turn, low-friction hand-wheel was employed to apply load to the tablet until the
tablet is fractured. The resulting breaking force is displayed on the LCD display in grams (g). The results are presented as a mean value ± S.D. (n=5).

**Dissolution study**

The in vitro release study was performed for pure drug, nine formulae and optimized LMSNT using USP Apparatus I (SOTAX AT 7 smart, Allchwil/Basel, Switzerland). The dissolution medium was 500 ml of simulated salivary fluid at pH 7.4 containing of 0.5w/v tween 80 and keeping the temperature at 37°C ± 0.5 °C and a stirring rate at 100 rpm. The tablet equivalent to 25 mg of SLM was placed inside the basket and 1 ml of sample was withdrawn at predetermined time intervals and replenished by an equal volume of the fresh dissolution medium. All samples were analyzed spectrophotometrically (Lambda 35 UV/VIS Spectrophotometer, PerkinElmer, Singapore) at wavelength 288 nm after filtration using 0.2 µm syringe filter (VWR, Leuven), and analyzed spectrophotometrically (Lambda 35 UV/VIS Spectrophotometer, PerkinElmer, Singapore) at wavelength 288 nm. All runs were applied in triplicates to determine the mean value and standard deviation (SD).

**Drug loading and drug content studies**

Drug loading was determined for loaded MSNs while the drug content was determined for loaded MSNs and optimized LMSNTs. A quantity equivalent to 25 mg of SLM was initially weighed followed by dissolving in the dissolution medium under continuous stirring. The solutions were filtered using 0.2 µm syringe filter (VWR, Leuven) and the drug content was determined spectrophotometrically at 288 nm (Lambda 35 UV/VIS Spectrophotometer, PerkinElmer, Singapore). The measurements were repeated three times to determine the mean value and standard deviation (SD). Drug content percent could be calculated using the following equation:
Drug content % = \frac{\text{Concentration of drug measured}}{\text{Initial concentration of drug added}} \times 100 \quad (4)

While the drug loading was determined according to the following equation (Papadimitriou and Bikiaris, 2009):

\text{Drug loading} \% = \frac{\text{weight of the drug in the loaded MSNS}}{\text{weight of the loaded MSNS}} \times 100 \quad (5)

**Saturation solubility study**

The solubility study was applied by preparation of saturated solutions of both pure SLM and LMSNT in which excess amount of each is dispersed in the dissolution medium using a shaker at 37°C ± 0.5°C till reaching to the solubility equilibrium. The samples were filtered through 0.2 µm syringe filter (VWR, Leuven) for determination of SLM spectrophotometerly (Lambda 35 UV/VIS Spectrophotometer, PerkinElmer, Singapore) at 288 nm.

**Moisture Content**

The residual moisture content was determined for lyophilized tablet using moisture analyzer (moisture analyzer MAC 50/NH). The lyophilized tablets were placed in the aluminum pan. The drying mode was used to automatic finish (change of mass 1 mg/ in time range 60s) and the end point temperature of 120°C. The display set up to show values in % of mass loss. The results are presented as mean value ± SD (n = 3).

**Stability study**

The stability for the optimized LMSNTs was studied. LMSNTs were placed in well closed amber yellow container and then placed in a desiccator at 25 °C with maintaining the relative humidity at 75 % using a saturated solution of sodium chloride. Samples were taken at 0, 3 and 6...
months storage to evaluate particle size, dissolution, drug content, disintegration strength of tablet. One-way ANOVA test was used to analyze the mean ±SD of the results.

Results and Discussion

Characterization of pure SLM, pure MSNs and drug-loaded MSNs

The materials characterization performed on pure SLM, pure MSNs and drug-loaded MSNs are described and presented in the Supplementary material. Based on these characterizations, the best ratio of the drug to MSN was determined to be 1:2, which was further used for the preparation of the lyophilized tablet.

Experimental Design Optimization

The screening study (Supplementary material) revealed sucrose and PVA to be the most suitable cryoprotectant and polymer, respectively, that could reduce the aggregation of nanoparticles. Therefore, $3^2$ full factorial design was applied as elucidated in Table 1 to determine the optimum concentration of both PVA and sucrose through studying their significant effects on the dependent variables, as displayed in the 3D graph of response surface (Figure 2). Furthermore, the quadratic equations in terms of coded factors (in which the high level of factors are coded as +1 and the low level of factors are coded as -1) were used to identify the impact of the factors on the responses.

Effect of both independent variables on the mean particle size ($Y_1$)

The mean particle size was determined for nine formulae and the optimized formula, and are illustrated in Table 1. At any level of $X_1$, when the concentration of sucrose ($X_2$) increase from 5% w/v to 15%w/v, the particle size was significantly decreased ($p<0.05$) from $769.2 \pm 56.5$ nm
to 169.1 ± 1.5 nm. The reduction of the mean particle size could be observed in the 3D graphical response surface (Figure 2a) and through the following quadratic equation:

$$Y_1 (Particle\ size) = 250.29 - 21.72X_1 - 260.73X_2 + 28.68 + 25.70X_1X_2 + 199.37X_2^2 \quad (6)$$

This is due to sucrose minimizing the stress of both the freezing and drying steps, thus acting as both cryoprotectant and lyoprotectant, which consequently prevented the agglomeration during freeze drying (Fonte et al., 2016). Moreover, sucrose is characterized by its non-eutectic nature. Therefore, its optimum concentration could preserve the LMSNT by forming a matrix of amorphous nature (Wilkhu et al., 2017) (Abdelwahed et al., 2006). Furthermore, the optimum concentration of sucrose provided the lyophilized tablet with a highly porous structure that could be easily redispersed in water with preserved particle size (Fonte et al., 2016). On the other hand, PVA is a hydrophilic polymer that aimed to increase the wettability of loaded MSNs, and hence the solubility. Furthermore, PVA could act as cryoprotectant, which consequently could maintain the particles in a stable nanosize range. Moreover, it acted as precipitation inhibitor, which prevented the aggregation of loaded MSNs. PVA, being a nonionic polymer, provides steric stabilization as a result of adsorption of the polymer on the surface of the loaded MSNs through formation of hydrogen bonds (Wang et al., 2013)(Xia et al., 2010) (the interaction will be discussed in more depth in conjunction to the FTIR characterization). However, at any level of $X_2$, when the concentration of PVA was changed, there was no significant effect ($p>0.05$) on the mean particle size, as demonstrated in the 3D graph of the response surface (Figure 2a). This is due to the used PVA concentrations (4, 5 and 6 %w/v) having the same effect as a precipitation inhibitor, which consequently prevented the aggregation of loaded MSNs through their steric stabilization, simultaneously having the same effect as cryoprotectant. Although the viscosity
was elevated when the concentration of stabilizer (PVA) was increased, which may negatively
effect on the particle size before the lyophilization process, the particle size for the all batches
was not changed before the lyophilization. This may be due to the time of sonication being
sufficient to complete the redispersion of loaded MSNs for all the concentrations of PVA.

**Effect of both independent variables on the disintegration time (Y2)**

In vitro disintegration time was determined for the nine experiments and optimized LMSNTs. Disintegration time ranged from $19.5 \pm 1.9$ seconds to $171.2 \pm 8.3$ seconds. All the results were within the range of USP, in which the disintegration time for an oral disintegrating tablet should be lower than 3 minutes. However, it should be within 30 seconds according to the FDA guidelines. All the results have been summarized in Table 1. Fast disintegration of the lyophilized tablet may be due to the highly porous structure of the tablets that formed during the sublimation of the frozen water in the lyophilization step, which consequently facilitated the penetration of water through these pores and provided rapid disintegration. The porosity was also clearly observed in SEM. The effect of both independent variables on the disintegration time was demonstrated through the equation 7.

\[
Y_2(\text{Disintegration time}) = 67.71 + 50.40X_1 - 26.18X_2 + 7.73X_1^2 - 17.93X_1X_2 + 1.58X_2^2 \quad (7)
\]

Here, at any level of $X_2$, if the concentration of PVA gradually increases, the disintegration will be significantly delayed ($P < 0.05$) as exhibited in the ANOVA table and Figure 2b. This is due to that PVA acted as binding agent and exhibited adhesive properties, and upon increase of its concentration, it yielded firm tablets with slow disintegration rate (Higuchi et al., 2014). However, increasing the concentration of sucrose at any level of $X_1$, the
disintegration was extremely fast due to sucrose being a hydrophilic sugar that resulted in fast wettability of the tablet (Wilkhu et al., 2017). Moreover, sucrose was converted to amorphous form that provided rapid wettability and consequently, high solubility (will be explained in more detail later in conjunction to the DSC results).

**Effect of both independent variables on tablet strength (Y₃)**

Strength of tablet was examined for the nine formulae and the optimized LMSNT. The strength of tablets ranged from 2.1 ± 0.3 N to 17.1 ± 0.6 N. The effect of X₁ and X₂ on all dependent variables could be displayed in Table 1 and response surface 3D graph (Figure 2c). When increasing the concentration of PVA at any level of X₂, the strength of the tablet was significantly (p < 0.05) increased. Moreover, at any level of X₂, the concentration of sucrose is directly proportional to the tablet strength, which is due to both of them in high concentration being able to act as a binding agent and yielding a firm intact tablet (Figure 2c) (Higuchi et al., 2014)(Chandrasekhar et al., 2009). This may be clear also from equation 8, in which the positive sign in X₁ and X₂ indicates the highly significant effect of both PVA and sucrose for increasing the tablet strength.

\[ Y₃(\text{Tablet strength}) = 8.54 + 3.00X₁ + 4.52X₂ + 0.53X₁² + 0.25X₁X₂ + 0.28X₂² \quad (8) \]

**Effect of both independent variables on R²min (Y₄)**

Dissolution studies were applied for pure SLM, nine formulae and optimized formula (Figure 3). Pure SLM shows slow dissolution rate due to poor aqueous solubility, as expected. R²min for the nine formulae of LMSNTs ranged from 22.7 ± 2.4 % to 93.5 ± 4.2 %. For the optimized formula, R²min was 91.6 ± 5.7 as exhibited in Figure 3. The effect of both independent variables on the R²min is demonstrated in equation 9.

\[ Y₄(R²min) = 61.00 - 22.02X₁ + 17.52X₂ - 3.55X₁² - 5.6X₁X₂ - 3.75X₂² \quad (9) \]
At fixed level of X, R increases when the concentration of PVA was decreased from 6 %w/v to 4 %w/v, as elucidated in Table 1 and response surface 3D graph (Figure 2d). This improvement may be due to that PVA acted as binder and had adhesive properties. Thus, at high polymer concentration, the viscosity was increased which in turn led to increase in the thickness of the diffusion layer, which led to formation of a gel that entrapped the drug and finally could significantly (p <0.05) delay the disintegration and consequently the dissolution rate. At fixed level of X, R was significantly (P<0.05) increased when the concentration of sucrose was elevated from 5 %w/v to 15 %w/v. This is due to sucrose being a hydrophilic sugar that contribute to increasing the wettability and hence, both the disintegration and the dissolution rates were remarkably rapid. Moreover, sucrose is a very efficient cryoprotectant that maintained the particle size in nanosize range after lyophilization process, and hence the surface area was exceedingly increased, which aided in the rapid dissolution rate.

Optimized LMSNT

The optimum concentration of both independent variables could be determined by design expert software in which the optimum concentrations of both PVA and sucrose were 4.2 % w/v and 15 % w/v, respectively. The weight of the optimized LMSNT was 367.8 mg, in which the weights of drug-loaded MSNs, PVA, sucrose and PEG per tablet were 76.8 mg, 63 mg, 225 mg, and 3 mg; equivalent to 20.9 %w/w, 17.1 % w/w, 61.2 % w/w, 0.8 % w/w, respectively. The observed values of responses were 170.1 ± 0.5 nm, 22.3± 2.7 sec, 11.5 ± 1.2 N and 91.6 ± 2.8 % for the mean particle size (Y1), disintegration time (Y2), tablet strength (Y3) and of drug release after 2 minutes, R , respectively. While the predicted values were 169.7 nm, 23.1 second, 11.2 N and 93.5 % For Y1, Y2, Y3 and Y4 respectively. All results for both observed and predicted
values were in agreement with each other, and the relative error percentage was less than 5% as shown in Table 2, emphasizing the reproducibility and validity of the optimized model.

Characterization of optimized LMSNTs

Mean particle size, zeta potential, PDI, drug content, disintegration, tablet strength, friability and moisture content were determined for LMSNT as follows: 170.1 ± 0.5 nm, –20.7 ± 0.4 mV, 0.112 ± 0.007, 96.4 ± 2.1%, 22.3± 2.7 sec, 11.5 ± 1.2 N, 0.72 ± 0.09% and 1.8 ± 0.7%, respectively. The colloidal stability of the particles on the nanosize range relies mostly on the steric stabilization provided by the stabilizer (PVA). Generally, the zeta potential is in the range of -20 - -30 mV for pure silica nanoparticles (Wang et al., 2013) (Bhattacharjee, 2016) whereby a zeta potential of approximately –20 mV may be sufficient to electrostatically stabilize a colloidal dispersion (Wang et al., 2013). Moreover, the moisture content is lower than 2%, which indicated an efficient lyophilization process and also serves as an indication for long term stability (Fonte et al., 2016). Moreover, the PDI was significantly lower than 0.3, which confirmed the narrow size distribution of the sample (Wang et al., 2011). Finally, the saturation solubility results of pure SLM and optimized LMSNTs were 1324.3 ±5.74 µg/ml and 5865±18.02 µg/ml, respectively. This is due to the adsorption of drug to the available surface area of the nanoparticles, leading to exceedingly increased amount of drug which, in turn, can enhance the solubility. Moreover, PVA (hydrophilic polymer) and sucrose (water soluble sugar) provided high wettability of the loaded MSNs and hence, the saturation solubility was also remarkably increased.

Morphology of optimized LMSNTs
SEM micrographs and TEM images of pure and loaded MSNs are shown in the Supplementary material. SEM micrographs of the LMSNT could demonstrate the surface characteristics of the tablet. The cross section and the surface view (Figure 4a &b) exhibited a highly porous structure of the tablet, which formed as a result of the sublimation of the frozen water during the lyophilization step that was aimed for promoting easy penetration of water during disintegration. Upon high magnification of the SEM micrograph (Figure 4c), the spherical shape of the loaded MSNs was observed without significant change in the particle size when compared with pure MSNs. Further, TEM images (Figure 4d) of the LMSNT confirmed the SEM results and displayed the spherical shape of the loaded MSNs. Moreover, in TEM images (Figure 4d), the darkness of the spherical particles indicated that the pores of MSNs were loaded with drug and both the polymer and the cryoprotectant stabilized the particles on the nanosize range. However, the results of the particle size from DLS are higher than the results of TEM, due to that the hydrodynamic radius ($R_h$) may be increased in the presence of both hydrophilic polymer and the hydrophilic sugar.

**Physicochemical properties of optimized LMSNTs**

DSC measurements were conducted on pure SLM, pure MSNs, drug-loaded MSNs (1:1) and drug loaded MSNs (1:2) as explained in the Supplementary material. DSC was further applied to PVA, sucrose, lyophilized MSNs with PVA, lyophilized MSNs with sucrose, blank tablet (lyophilized tablet without drug), physical mixture (blank and SLM) and the optimized LMSNT, as shown in Figure 5. Sucrose showed the characteristic endothermic peaks at 189.8°C (Beckett et al., 2006)(Kawakami et al., 2006) while PVA displayed the characteristic peaks at 189.9°C (Rebia et al., 2018). However, the DSC thermogram of the lyophilized MSNs with PVA showed deviation of the characteristic peak of the PVA from 189.9 °C to 169.4 °C, which explained the
amorphous form of PVA due to formation of hydrogen bonds, as elucidated in the FTIR measurements. On the contrary, in the lyophilized MSNs with sucrose, the characteristic peak of sucrose was deviated from 189.9°C to 233.8°C which may be explained by formation of the amorphous form of sucrose upon freeze drying process (Lappalainen et al., 2006). The DSC thermogram of the blank tablet (lyophilized tablet without drug) displayed deviated endothermic peaks of PVA at 155.0°C and both endothermic peaks of sucrose at 184.4°C and 232.2°C. The thermograms of the physical mixture and optimized LMSNT are similar to that of the blank tablet, which demonstrated overlapping of the characteristic peak of the drug (148°C) with the characteristic peak of the deviated endothermic peaks of PVA (155.38°C). Therefore, DSC could not efficiently detect if the drug after loading is still in amorphous state when formulated in lyophilized tablet or if it was transformed partially or completely to crystalline state. Therefore, PXRD was also applied to pure SLM, pure MSNs, drug-loaded MSNs (1:1) and drug loaded MSNs (1:2) as shown in the Supplementary material. PXRD diffractograms were also determined for PVA, sucrose, physical mixture (loaded MSNs, PVA and sucrose), and the optimized LMSNT as shown in Figure 6. The physical mixture pattern showed reflections from crystalline SLM. However, the patterns of the blank tablet and the optimized LMSNT are both similar, i.e. they are deficient of the characteristic SLM peaks. This indicates that the drug in the optimized LMSNT is still completely in amorphous state. To support this, FTIR spectra was carried out on the same samples as DSC as interpreted in Supplementary material and on PVA, sucrose, lyophilized MSNs with PVA, lyophilized MSNs with sucrose, blank tablet (lyophilized tablet without drug), physical mixture (blank and SLM) and the optimized LMSNT as displayed in Figure 7. The PVA spectrum showed the characteristic broad stretched band of O—H at 3305.8 cm^{-1}, vibrational band of C–H at 2911.6 cm^{-1}, stretching vibration of carbonyl
(\textgreater{}C=O) at 1730.3 cm\(^{-1}\), stretching vibration of methyl (–COO–CH\(_3\)) in acetate group at 1372.7 cm\(^{-1}\) and absorption peak at frequency 1088 cm\(^{-1}\) this vibrational band is distinctive to the crystallinity of the PVA which related to the carboxyl stretching band (C–O) (Mansur et al., 2004) (Jiang et al., 2015) (Mansur et al., 2008). The spectrum of lyophilized MSNs with PVA exhibited major vibration bands of siloxane groups (Si–O–Si) at 1068 cm\(^{-1}\) and 453 cm\(^{-1}\) and silanol groups at (Si–OH) 964 cm\(^{-1}\) and a broader band at 3332 cm\(^{-1}\) (Mansur et al., 2004). Furthermore, there was a shift of the peak of PVA from 1730.3 cm\(^{-1}\) to 1713.9 cm\(^{-1}\) that may be explained as the hydrogen bond formation between the carbonyl group of PVA and the silanol groups of MSNs. The spectrum of sucrose showed two absorption peaks at 3323.3 and 3382.2 cm\(^{-1}\) and one isolated sharp peak at 3561 cm\(^{-1}\) present in the region of OH-stretching band, which was centered around 3300 cm\(^{-1}\). The fingerprint region that covers the range of approximately 1500 to 800 cm\(^{-1}\) is complex, which displayed a series of sharp overlapping absorption bands arising from CO-stretching, CC-stretching, and COH-bending vibrations. Therefore, sucrose spectra showed a high degree of homogeneity of intermolecular interactions that led to less dispersion of vibrational levels and increase in spectral resolution (Sritham and Gunasekaran, 2017). While upon lyophilization of sucrose with MSNs, the spectrum showed disappearance of the high degree of homogeneity of intermolecular interactions. In the blank tablet spectrum, there is no change in the characteristic peaks of both PVA and sucrose. On the other hand, in the spectrum of physical mixture and optimized LMSNT there was no new peaks, which indicated there is no chemical interaction and thus the pharmacophore of the drug was not affected.

**Stability Test**

Results of the stability study are presented in Table 3. Stability studies showed that there was no significant difference for the all characterizations before and after storage for 6 months (p> 0.05).
The stability of the loaded MSNs in nanosize range may be due to the effect of the precipitation inhibitor (PVA) providing steric stabilization. Moreover, the zeta potential was not affected, indicating that no (chemical) alterations of the particle surfaces had taken place. Furthermore, lyophilization process provided physical and chemical stabilization of the particles, while using of cryoprotectant avoided freezing and drying stress during the efficient freeze drying process (Abdelwahed et al., 2006). Importantly, the disintegration and dissolution rate was not affected – most likely due to the good stability of the loaded MSNs, whereby the highly porous structure of the tablet was maintained.

Conclusions
We have developed an approach for incorporating drug-loaded MSNs within lyophilized tablets. To our knowledge, this is the first time to apply this type of incorporation of MSNs within a tablet, which had the ability to enhance the solubility of the poorly water soluble drug SLM, and consequently, exceedingly improve the dissolution rate. Moreover, we studied the effect of the PVA (precipitation inhibitor and binder) and sucrose (cryoprotectant and disintegrant) on the formulation to provide an optimized LMSNT. This optimized formula showed high stability of the loaded MSNs on nanosize range, narrow particle size distribution (lower than 0.2) with sufficient zeta potential (absolute value higher than 20mV), sufficient tablet strength, low friability (lower than 1%), rapid disintegration (lower than 30 seconds) and consequently, fast dissolution rate and high saturation solubility. Therefore, the LMSNT could be taken sublingually to reach to the systemic circulation without gastric degradation and, consequently, we would anticipate the oral bioavailability to be significantly improved.

Acknowledgements
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References


Table 1. $3^2$ full factorial design with observed response values of lyophilized nanosuspension tablet.

| Batch Code | Independent variables | | | Dependent variables |
|------------|-----------------------|---|---|---------------------|---|---|---|---|
|            | PVA concentration ($X_1$) | Sucrose concentration ($X_2$) | | Particles size (After lyophilization) ($Y_1$) | Disintegration time ($Y_2$) | Tablet strength ($Y_3$) | $R_{2\text{min}}$ ($Y_4$) |
|            | %w/v | Level | %w/v | Level | Nm | Second | Newton | % |
| F1 | 4 | —1 | 5 | —1 | 704.1 ± 142.2 | 36.0 ± 2.1 | 2.1 ± 0.3 | 51.5 ± 4.8 |
| F2 | 5 | 0 | 5 | —1 | 769.2 ± 56.5 | 94.7 ± 3.4 | 4.1 ± 0.6 | 37.9 ± 3.9 |
| F3 | 6 | 1 | 5 | —1 | 600.5 ± 134.7 | 171.2 ± 8.3 | 7.8 ± 1.0 | 22.7 ± 2.4 |
| F4 | 4 | —1 | 10 | 0 | 254.7 ± 10.2 | 22.8 ± 3.3 | 6.3 ± 0.5 | 85.9 ± 4.1 |
| F5 | 5 | 0 | 10 | 0 | 210 ± 10.2 | 69.3 ± 4.4 | 8.5 ± 0.5 | 56.2 ± 5.3 |
| F6 | 6 | 1 | 10 | 0 | 228.8 ± 15.68 | 126.5 ± 6.1 | 11.9 ± 0.9 | 33.8 ± 2.6 |
| F7 | 4 | —1 | 15 | 1 | 169.9 ± 1.8 | 19.5 ± 1.9 | 10.4 ± 0.8 | 93.5 ± 4.2 |
| F8 | 5 | 0 | 15 | 1 | 170.4 ± 1.7 | 42.3 ± 2.7 | 13.6 ± 1.3 | 81.4 ± 3.9 |
| F9 | 6 | 1 | 15 | 1 | 169.1 ± 1.5 | 83.0 ± 2.4 | 17.1 ± 0.6 | 42.3 ± 2.0 |
Table 2. Illustration of predicted and observed values for all responses with their relative errors.

<table>
<thead>
<tr>
<th>Responses</th>
<th>predicted values</th>
<th>observed values</th>
<th>Relative error (%)</th>
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<tbody>
<tr>
<td>mean particle size ((Y_1)) nm</td>
<td>169.7</td>
<td>170.1± 0.5</td>
<td>0.2</td>
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<tr>
<td>Disintegration time ((Y_2)) second</td>
<td>23.1</td>
<td>22.3 ± 2.7</td>
<td>3.5</td>
</tr>
<tr>
<td>Tablet strength ((Y_3)) N</td>
<td>11.2</td>
<td>11.5 ± 1.2</td>
<td>2.7</td>
</tr>
<tr>
<td>(R_{2\text{min}}(Y_4)) %</td>
<td>93.5</td>
<td>91.6 ± 2.8</td>
<td>2.0</td>
</tr>
</tbody>
</table>
Table 3. Results of stability study

<table>
<thead>
<tr>
<th>Characterization</th>
<th>Initial</th>
<th>3 month</th>
<th>6 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean particle size (nm) ± SD</td>
<td>170.1 ± 0.5</td>
<td>173.5 ± 7.3</td>
<td>177.7 ± 12.2</td>
</tr>
<tr>
<td>Zeta potential (mV) ± SD</td>
<td>–20.7 ± 0.4</td>
<td>–21.1 ± 0.2</td>
<td>–21.8 ± 0.6</td>
</tr>
<tr>
<td>Disintegration time (sec.) ± SD</td>
<td>22.3 ± 2.7</td>
<td>22.7 ± 3.1</td>
<td>23.3 ± 3.3</td>
</tr>
<tr>
<td>Tablet strength (%)</td>
<td>11.5 ± 1.2</td>
<td>12.0 ± 1.1</td>
<td>12.3 ± 1.2</td>
</tr>
<tr>
<td>$R_{2\text{min}}$ (%)</td>
<td>91.6 ± 2.8</td>
<td>92.4 ± 3.6</td>
<td>90.9 ± 3.3</td>
</tr>
<tr>
<td>Drug content (%) ± SD</td>
<td>96.4 ± 2.1</td>
<td>95.9 ± 2.8</td>
<td>95.3 ± 3.4</td>
</tr>
</tbody>
</table>
Figure Legends

Figure 1. Images of pure MSNs (a), loaded MSNs (b), dispersion of the loaded MSNs in polymeric solution (c) and LMSNT (d)

Figure 2. 3D graphs of response surface showing the effect of both independent variables (PVA and sucrose) on (a) mean particle size, (b) In vitro disintegration time, (c) tablet strength and (d) % of drug release after 2 minutes, R_{2min}.

Figure 3. Dissolution profiles pure SLM, nine batches, and optimized LMSNTs.

Figure 4. SEM micrograph for optimized LMSNT, cross-sectional view (a) surface view (b) higher magnification of SEM micrograph (c) and TEM image for optimized LMSNT (d),

Figure 5. DSC thermograms for pure SLM, loaded MSNs, PVA, sucrose, lyophilized (MSN and sucrose), lyophilized (MSN and PVA) blank lyophilized tablet, physical mixture and optimized LMSNT.

Figure 6. PXRD patterns for pure SLM, loaded MSNs (1:2), PVA, sucrose, blank lyophilized tablet, physical mixture and optimized LMSNT.

Figure 7. FTIR spectra pure SLM, loaded MSNs (1:2), PVA, sucrose, lyophilized (MSN and sucrose), lyophilized (MSN and PVA), blank lyophilized tablet, physical mixture and optimized LMSNT.