SOLID SELF EMULSIFYING DRUG DELIVERY SYSTEM OF QINAPRIL FORMULATION AND CHARACTERIZATION

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ABSTRACT

Objective: The objective of this work was to improve the solubility and dissolution rate of Qinapril by preparing a solid-self micro emulsifying drug delivery system (Solid-smedds). **Methods:** Liquid-self-emulsifying drug delivery system formulations were prepared by using linseed oil as oil, tween 80 as a surfactant and PEG 400 as cosurfactant. Components were selected by solubility screening studies and the self-emulsifying region was identified by the pseudo- ternary phase diagram. Thermodynamic stability study was performed for the determination of stable liquid-smedds formulation. These formulations were evaluated for self-emulsification time, drug content analysis, robustness to dilution test, particle size analysis, *in vitro* diffusion study, and Stability study. Solid self-micro emulsifying formulations were prepared by using aerosil-200 at a different ratio. Lf9S (0.65:1) was selected due to its highest drug entrapment efficiency and a decrease in particle size. It was selected for further studies into DSC, SEM, FTIR, and XRD analysis. **Results:** DSC and XRD result shows that the drug within the formulation was in the amorphous state. From the SEM study, it was observed that the drug has been uniformly distributed and having a smooth surface. From the *in vitro* dissolution study, it improved the dissolution rate of Qinapril which was 98.70% of drug release where pure drug release only 6.72%. **Conclusion:** In conclusion, a solid self-micro emulsifying drug delivery system is improved the solubility and drug release rate but also improved the stability of the formulation.

Keywords: Solid-smedds, Liquid-smedds, Adsorption technique, Aerosil 200, Solubility and dissolution, Qinapril

INTRODUCTION

Around 40% of recent drug candidates become poor water solubility and also the oral deliveries of those medicines are usually related to high intrasubject and inter-subject variability, a lack of dose proportionality, low bioavailability. To overcome these issues, several formulation ways are utilized together with specific utilization of surfactants, lipids, permeation enhancers, micronization, salt formation, cyclodextrins, solid dispersion. Newly, the lipid-based formulation has gained a lot of attention with special importance on self-emulsifying drug delivery systems (SEDDS) which is used to boost the bioavailability of orally administered lipophilic drugs. SEDDS or self-emulsifying oil formulations (Seof)

outlined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactants or instead, one or additional hydrophilic solvents and co-solvents/surfactants [1]. On delicate agitation, these systems will form fine o/w emulsions (oil-in-water) or self-micro emulsifying drug delivery system (SMEDDS) or microemulsions followed with dilution into liquid media, like epithelial duct (GI) fluids [2]. SEDDS effectively produce emulsions with a droplet size range of 100-200 nm for SMEDDS whereas SNEDDS produce less than 100 nm [3]. Qinapril is a dihydropyridine calcium channel antagonist that belongs to BCS class-II mainly applied in the treatment of hypertension and angina-pectoris administered by the oral route. It is the most common kind of calcium channel blocker (CCB) and is used for the treatment of hypertension as well as stable, unstable and prinzmetal angina.

It is known to exhibit its antihypertensive effect at a very low plasma concentration of 13.4 mg/ml [5]. Taneja *et al.* [4] had developed a pulsatile microsphere of Qinapril for the treatment of hypertension. It showed slow release of drug initially for 4 h. and at a particular lag time, it followed pulsatile release (after 6 h). In recent studies, Qinapril in combination with Valsartan and Candesartan lowers blood pressure effects in high-risk individuals. Mancia *et al.* had examined the effects of Qinapril GITS–candesartan cilexetil combination at various doses. This combination therapy has been proved that the effect of blood pressure was lowered as well as decrease the incidence of vasodilatory side effects [5]. Another achievement for the development of a microemulsion system of Qinapril and valsartan which was administered transdermally *invitro*(rat skin) to observe the permeation rate. The study was suggested that the microemulsion system improved the solubilization as well as transportation of both drugs across rate skin [6].

On another side, Qinapril is used in the treatment of angina, but at high doses, Qinapril increased the risk of out of hospital cardiac arrest due to fatal cardiac arrhythmia [7]. Another important aspect has been reported that Qinapril can promote breast cancer and should be avoided for women who suffer from breast cancer and hypertension. From the report, it was identified that the effects of Qinapril on MCF-7 cells were via the protein kinase B-endothelial constitutive nitric oxide synthase-nitric oxide axis, and on MDA-MB-231 cells via activation of the extracellular signal-regulated kinase pathway [8].

In this study, an attempt was made to improve the solubility and *in vitro* dissolution of Qinapril by formulating it as S- SMEDDS. Qinapril, Dimethyl 2, 6-dimethyl-4-(2-nitrophenyl)-1, 4 dihydropyridine-3,5-dicarboxylate, has poor aqueous solubility resulting in low and often irregular bioavailability. The present work provided in improvement in the dissolution rate for Qinapril when formulated as self-emulsifying drug delivery systems. The self-emulsifying mixture that combines good self-emulsifying properties, acceptable solubilization of Qinapril and optimum surfactant, co-surfactant/co-solvent composition was selected, evaluated for droplet size, stability, dissolution, and a Solid SMEDDS was

prepared using aerosil 200 as adsorbent.

The solid SMEDDS was further evaluated by *in vitro* dissolution studies and characterized by Scanning electron microscopy (SEM), Differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FT-IR).

Drug release is a vital and rate-limiting step, mainly for drugs with low solubility and high permeability i.e., biopharmaceutical classification system BCS class II drugs. SEDDS is the technique that can be used to enhance the solubility and dissolution rate of poorly water-soluble drugs. But Liquid SEDDS having problems like the irritating effect of a high percentage of surfactant on the gastrointestinal mucosa, lower formulation stability and plausible interaction of excipients with capsule shell. To surmount these ostensible problems associated with liquid SEDDS a new technology is investigated known as solid S-SEDDS [9, 10]. Solid SEDDS are the solidified self-emulsifying formulation which is prepared by converting liquid/semisolid SEDDS into self-emulsifying powders/nanoparticles by using various solidification techniques such as, nanoparticle technology, melt extrusion, spray drying, and adsorptions to solid carriers [11].

MATERIALS AND METHODS

Materials

Qinapril was a gift sample from G. C. Chemie Pharmie Ltd; Iraq. Almond oil, Soybean oil, castor oil, and linseed oil were supplied by Merck Pvt. Ltd. Tween 80, tween 20, tween 40, span 20 were supplied from Sisco research laboratories Pvt. Ltd. PEG 400, PEG200, propylene glycol, glycerol, PEG 600 were supplied from Sisco research laboratories Pvt. Ltd. Aerosil 200 was supplied from yarrow Chem product Pvt. Ltd.

Solubility screening study

The highest amount of drug solubility was determined by the shake flask method. 500 mg of Qinapril was added to 5 ml of each vehicle containing oil, surfactant, and cosurfactant. Then the mixture was shaken for 72 h to reach a uniform equilibrium state. It was centrifuged (Remi centrifuge equipment) for 10 min. at 5000 rpm. The supernatant was collected and diluted with phosphate buffer (pH 6.8). and quantified by spectrophotometrically at λmax of 238 nm [12, 13]

Construction of pseudoternary phase diagram

After the selection of higher drug solubility containing excipients (oil, surfactant, and cosurfactant), the pseudo ternary phase diagram was prepared at 1:1 and 1:2 ratio of the mixture of surfactants and co-surfactants. It determines the self-emulsifying region by taking the different ratios of oil and mixture of surfactant and co-surfactant [Oil: (S-COS) mix] from 9:1 to 1:9. The water was titrated into the mixture with constant stirring to observe the formation of o/w microemulsion The pseudo ternary phase diagram

was constructed using software Chemix School (ternary software) [13].

Preparation of sedds formulation of Qinapril

Different SEDDS formulations were prepared by using oil (Linseed oil), surfactant (tween 80) and co-surfactant (PEG 400). In each formulation, the amount of Qinapril (i. e-100 mg/10 ml) was constant. Each of the excipients was exactly weighed and gently mixed. Then the prepared mixtures were mixed with the help of a magnetic stirrer until a homogenous mixture was prepared. Then the prepared homogenous mixture was stored at 25^0C for further studies [14].

Thermodynamic stability studies

Thermodynamic stability study was done to observe any signs of phase separation, drug precipitation, creaming or cracking. All the smedds formulations were diluted with distilled water and centrifuged at 3500 rpm for 15 min. and check for any phase separation or clear emulsion. Then it was exposed to heating-cooling cycle (4 °C and 45 °C) and freeze-thaw stress cycle (-21 ° and+25 °C) with storage at each temperature for not less than 48 hr. All the testing was done in triplicate and observes the extent of phase separation [15].

Self-emulsification time

After thermodynamic stability testing, the stable formulations were taken for visual assessment self-emulsification efficiency. Self-emulsification efficiency study was performed in the USP XXIV type II dissolution apparatus. 1 ml of each smedds formulation was added dropwise into 500 ml of buffer pH 6.8 and 1.2. and maintained at 37 °C with a rotating speed of 50rpm. Then the time is noted for complete emulsification in two different media [16].

Drug content analysis

The liquid-smedds formulation containing 10 mg equivalent drug was taken into 100 ml volumetric flask and diluted it with phosphate buffer pH 6.8 and analyzed by U. V. Visible Spectrophotometer at the λmax of 238 nm.

Droplet size and zeta potential

The droplet size of smedds formulation was determined using a zeta sizer Nano ZS (Malvern instrument, UK) dynamic light scattering particle size analyzer at a wavelength of 635 nm and a scattering angle of 90 °C at 25 °C. The formulation (0.1 ml) was diluting with 100 times with double distilled water and sonicated for at least 30 min. for the reduction of particle size of emulsion [17, 18].

Robustness to dilution

All the formulations were taken for checking the robustness of emulsion in diluting with enzyme-free

phosphate buffer ph6.8 (simulated intestinal fluid) and 0.1N HCL (simulated gastric fluid). 1 ml of each formulation was subjected to 50, 100, 500, 1000 fold dilution and kept them for 24h. After that, all the formulations were checked for any change in physical appearance i.e. coalescence of oil droplets, drug precipitation or phase separation [19].

***In vitro* diffusion studies of smedds**

The drug release experiment was performed in USP XXIII rotating paddle method using a dialysis bag method. The dialysis membrane was shocked in dialysis media (buffer pH6.8) for 12hr. at room temperature. After that, the liquid- smedds containing 100 mg of Qinapril were filled into soaked dialysis membrane and closed both sides of the dialysis membrane by using thread. Then it was put into the vessel containing 900 ml of phosphate buffer pH6.8 carefully by which the dialysis membrane can easily rotate. The dissolution was performed at 37±0.5 °C for and rotated at 50 rpm for 120 min. At a specific time interval i.e. 15, 30, 45, 60, 75, 90, 105, 120, the aliquot of 5 ml was withdrawn and filtered through a 0.45 µm membrane filter. The same volume of the withdrawn amount should be replenished to maintain the sink condition of dissolution. The concentration of Qinapril was determined by spectrophotometrically at 238 nm. The dissolution of each formulation was performed in triplicate times. The dissolution profile of stable formulations was prepared and compared with the dissolution profile of the pure drug [20, 21].

Stability studies

The liquid-smedds sample (LF9L) was selected for stability study at 25±0.5˚C/60±5 % RH(relative humidity) and 40±0.5˚C/75±5 % RH for 3 mo. It is filled in glass vials with a rubber stopper and then placed in Stability chambers. The physical appearance, drug content analysis, and particle size were evaluated with each 1-month interval [22].

Preparation of solid-smedds

Solid self-emulsifying powder formulation was prepared by using adsorption to the solid carrier method, which is a very simple and reliable technique. The optimized formulation was taken for solid-smedds preparation. Aerosil 200 was used in a different ratio to prepare solid smedds in the ratio of 0.50:1, 0.55:1, 0.65:1(adsorbent: liquid smedds). The adsorbent and liquid smedds were mixed in a porcelain dish until a uniform homogenized free-flowing powder was obtained. Then the powder was passed through sieve no. 120 and dried at ambient temperature for further use. These formulations were evaluated for flow property. The developed optimized formulation was characterized for particle size analysis, percentage of drug content and *in vitro* dissolution study, scanning electron microscopy study(SEM), X-ray diffraction study(XRD), differential scanning calorimetry (DSC), and f. t. i. r study(Fourier transform infrared spectroscopy). The evaluation study was carried out for optimized formulation and compared with pure drug.

Flow properties of solid-smedds

Flow properties of solid smedds were determined by Carr's method. All the samples (0.50:1, 0.55:1, 0.65:1) were poured through the funnel in which the height of powder and its radius was obtained. The angle of repose was calculated using equation tan θ= H/r. The powder preparation having good flow property was selected as an optimized formulation and taken for particle size analysis and drug content analysis [23].

Drug content analysis of solid smedds

SEDDS formulation (equivalent to 10 mg of the drug) was diluted with 100 ml of pH 6.8 phosphate buffer. It was diluted suitably with Phosphate buffer pH 6.8. It was analyzed using UV/vis. Spectrophotometer at 238 nm [24].

The particle size of solid smedds

The particle size and zeta potential of the selected formulation were determined using Zetasizer Nano ZS(Malvern instrument, UV). The formulation was diluting with 100 times with distilled water then the emulsion was taken for analysis of particle size at 25^0C at 90^0C angle [24].

Solid state characterization of optimized solid self-microemulsifying formulation

The optimized solid-smedds was analyzed for ftir, DSC, XRD, and SEM analysis to investigate its solid-state properties. DSC thermogram was analyzed in Mettler Toledo DSC. F. t. i. r analyzes the compatibilities between drug and excipients present in the formulation. Each sample was scanned in a ftir spectrophotometer (Spectrum 2 FTIR spectrophotometer, Perkin Elmer) at a range of 4000-400 cm-1. The XRD analysis of the sample was analyzed in an x-ray diffractometer (Rigaku Ultima IV, Japan) and SEM of self-emulsifying powder was performed in the SEM instrument (Zeiss EVO 18 special edition) [25].

***In vitro* drug release**

The *in vitro* drug release study of solid smedds was performed in the USP XXIII dissolution apparatus at temperature 37±0.5 °C with a rotation speed of 50 rpm. The s-smedds formulations (equivalent to 100 mg) were put into the vessel containing 900 ml buffer pH 6.8(enzyme free simulated intestinal fluid). The entire dissolution was performed for 2 hr. At predetermined periods, samples were withdrawn and diluted with phosphate buffer. Then the diluted sample was filtered using a 0.45 μm membrane filter and analyzed drug concentration on UV-spectrophotometer at λmax 238 nm. The same volume of fresh media was replaced after each interval of withdrawn of the sample to maintain the dissolution media constant.

Stability studies

The stability study of optimized s-smedds was performed at 40 °C±2 °C and 75%±5% RH and

25±0.5˚C/60±5 % RH for three months. The samples were analyzed for physical appearance, particle size, and drug content analysis after each one-month interval.

Statistical data analysis

For the data analysis, a one-way analysis of variance (ANOVA) was used to compare the difference between solid- smedds, liquid smedds, and pure drugs by using mean value±standard deviation (SD).

RESULTS

Self-emulsifying preparation is a monophasic clear emulsion that contains oil, surfactant, and co-surfactant. For the analysis of solubility with Qinapril linseed oil, soybean oil, and almond oil were as oil, tween 60, tween 40, span 20 and tween 80 were as a surfactant and PEG 400, PEG 200, PPG, PEG 600 were taken as cosurfactants. The results of solubility in various vehicles were represented in fig. 1. From the analysis, it has been shown that Qinapril shows high solubility in linseed oil (61.23±0.95 mg/ml), Tween 80 (56.47±0.92 mg/ml), PEG 400 (49.98±0.15 mg/ml). Linseed oil is a natural and long-chain triglyceride oil, which have a good solvent capacity for dissolving drugs. Tween 80 is a hydrophilic nonionic surfactant that has a good solubilizing capacity and PEG 400 as co-surfactant which lowers the interfacial tension.

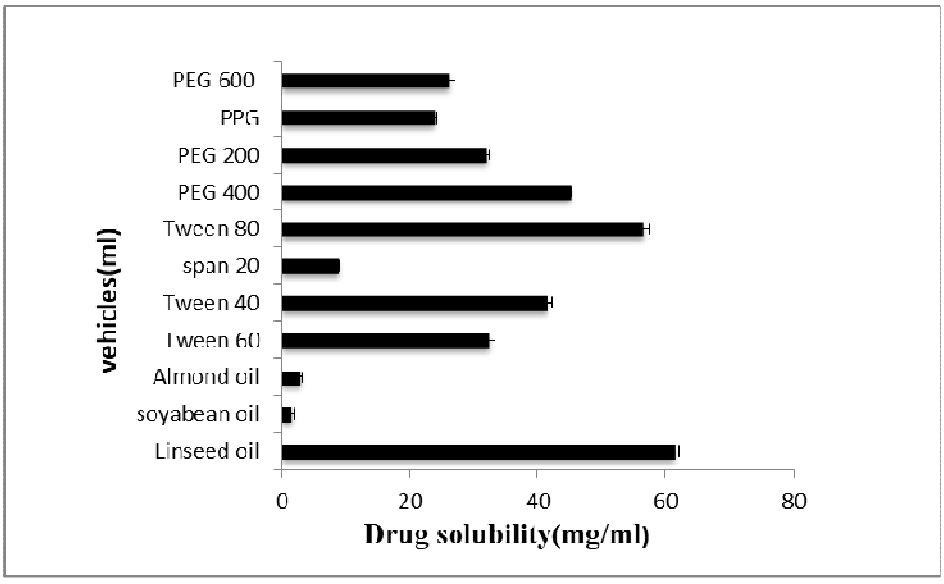

Fig. 1: Solubility studies of drug with various oils, surfactants, and cosurfactants. (mean±SD, n=3)

Pseudo-ternary phase diagram

Proper concentration of vehicles that produce stable emulsion must be essential to prepare self-emulsifying formulations. The pseudo ternary phase diagram defines the ternary phase behavior between

components and provides proper concentration to prepare a stable emulsion. After water titration, the amount of water used was noted and developed the ternary phase diagram by using ternary software which has written in the method part. Fig. 2(a) and (b) represents the ternary phase diagram of smedds between castor oil, tween 80 and PEG 400 (blue colored region indicate the region of self-emulsification produced by SCOSmix 1:1 ratio and 1:2 ratio. The ternary diagram indicates that among both the SCOSmix ratio, a 1:1 ratio provides a wide self-emulsification region. So this ratio was taken as the superlative ratio for preparation smedds.

Preparation of smedds of Qinapril

After the selection of oil, surfactant and co-surfactant and emulsification region, nine self-emulsifying formulations were prepared at 1:9 to 9:1 ratio where SCOSmix was 1:1 ratio. In all the mixture Qinapril (equivalent to 100 mg) was mixed and kept them for 24hr. at 25 °C. After 24 hr., LF1L, LF2L, LF3L, LF4L, LF5L, LF6L, and LF9L were selected as stable because they showed no sign of phase separation and unstable formulations (LF7L and LF8L) are rejected as they showed phase separation.

Thermodynamic stability study

Seven formulations showed stability i.e. there was no phase separation, the appearance of coalescence of oil droplets or any cracking appearance after keeping them for 24hr. of storage. Four formulations were remained stable after thermodynamic stability studies. But three formulations showed phase separation after freeze-thaw stress testing. So four formulations i.e. LF2L, LF3L, LF4L, and LF9L were taken for the evaluation study. Each test was done in triplicates.

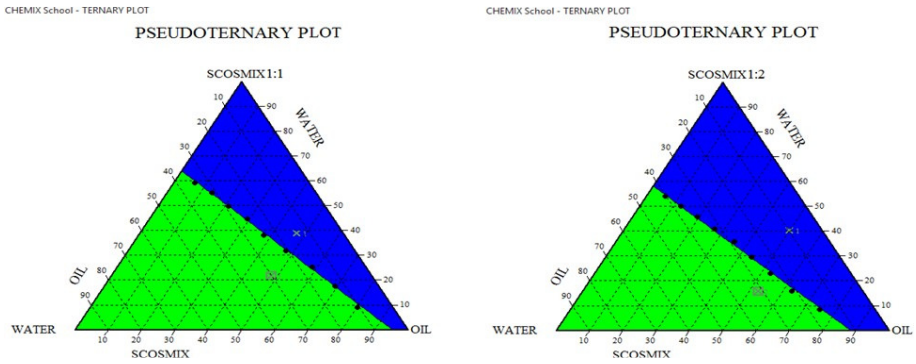

Fig. 2: Represents the ternary phase diagram between linseed oil, tween 80 and PEG 400 (green colored region indicate the self-emulsification region produced by SCOSmix 1:1 ratio (a) and 1:2 ratio (b)

Table 1: Observation of physical instabilities of sedds formulation during thermodynamic stability studies (mean±SD, n=3)

Formulations	Heating cooling cycle	Centrifugation test	Freeze-thaw stress cycle
LF1L	✓	✓	×
LF2L	✓	✓	✓
LF3L	✓	✓	✓
LF4L	✓	✓	✓
LF5L	✓	×	×
LF6L	✓	×	×
LF9L	✓	✓	✓

Droplet size and zeta potential analysis

In the self-emulsification performance, the particle size of the emulsion is a crucial factor, because it determines the rate and extent of drug release as well as drug absorption. The particle size of formulations was in the nanometer range. LF9L showed a low particle size (249.1 nm) as compared to others. An increase in the ratio of the oil phase and a decrease in the S-Cosmix ratio resulted that the increase in particle size. Smaller in particle size increases drug release and delivers larger interfacial area across which drug can diffuse into the gastrointestinal fluids and thus increases drug absorption.

Zeta potential of linseed oil formulation found to be-16.9 as a negative value which shows that due to the ionization of free fatty acids and glycols present in the oil and surfactants which improves formulation stability by preventing globule coalescence

Drug content analysis

From the drug content analysis, the result showed that three formulations, LF2L, LF3L, LF4L, and LF9L contained between 69.25% to 79.05%.

Robustness to dilution test

Robustness to dilution study is performed to observe the effect of formulation on different pH. In this study, LF2L was unstable because it showed signs of phase separation when diluted with phosphate buffer (pH 6.8) and 0.1N HCL but LF3L, LF4L, and LF9L formulations remained stable for 24hr of dilution with both pH 6.8 and 0.1N HCl. LF2L was rejected for further study due to the observation of phase separation.

***In vitro* diffusion study of optimized smedds formulation**

From the robustness to the dilution test, LF2L was not selected for the *in vitro* drug diffusion study. The diffusion study of three formulations i.e. LF3L, LF4L, LF9L was performed for 2hr. using a dialysis bag method. The diffusion of the drug from prepared smedds and the pure drug was indicated in fig. 3. The result from *in vitro* diffusion studies was indicated that LF9L formulation showed more drug release of 54% at 2hr. while pure drug release Qinapril only 6.72% at 2hr. LF3L and LF4L release 45.35% and 46.63%, which was less in comparison to LF9L formulation. Because these formulations contain more oil and less surfactant concentration which produces interruption with the release of the drug into the

dissolution media. Drug release at 120 min was compared between LF9L formulation and pure drug using one way ANOVA. From the data analysis, it was suggested that, Qinapril release from LF9L formulation much faster and higher in comparison to the pure drug ($^{*}P<0.05$). When SEDDS were exposed to aqueous media, it produces oil in water (o/w) microemulsion, having small globule size. The small globule size permitted drug release at a rapid rate from microemulsion.

Table 2: Observation of the effect of sedds formulations on dilution at phosphate buffer pH 6.8 and 1.2. (mean±SD, n=3)

Formulation code	Phosphate buffer (pH6.8)	0.1N HCl	Phosphate buffer (pH6.8)	0.1N HCl
LF2L	Phase separation (P. S.)	P. S.	No drug precipitation (D. P.)	No D. P.
LF3L	No P. S.	No P. S.	No D. P.	No D. P.
LF4L	No P. S	No P. S.	No D. P.	No D. P.
LF9L	No P. S	No P. S.	No D. P.	No D. P.

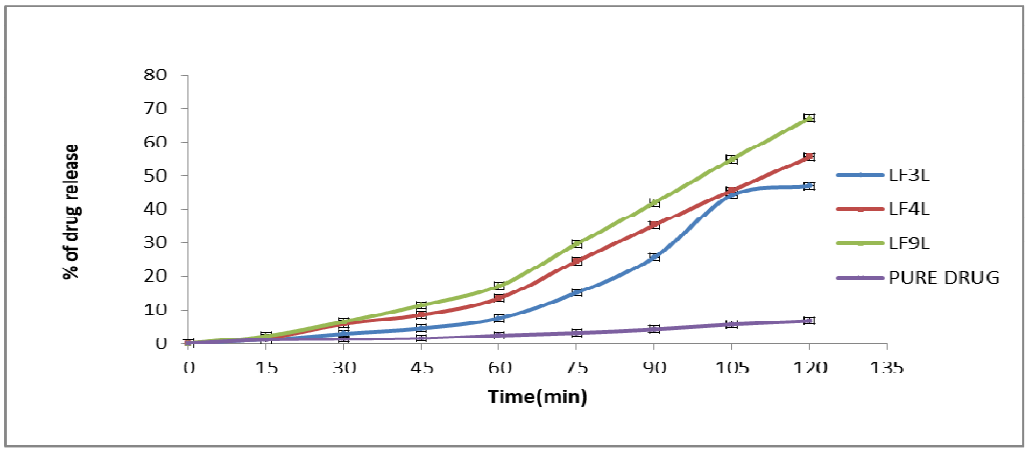

Fig. 3: *In vitro* diffusion study of LF3L, LF4L, LF9L, and Qinapril (pure drug) (mean±SD, n=3)

Stability study

Based on all evaluation LF9L was selected for stability study to observe any physical and chemical changes within the formulation. The stability study was performed according to ICH guidelines for 3 mo. Initially, the drug content (79.05%), particle size (249.1 nm) and zeta potential (-16.9mv) of LF9L were measured. After 3 mo, at accelerated condition((40±2˚C/75±5 %) the %drug content was reduced to 63% and at 25±2˚C/60±5 %, the %drug content was 69% which indicated that the instability of the formulation was observed due to decrease in assay content. After 3 mo, Particle size was 262.3 nm and zeta potential was-22.3mv at the accelerated condition. Particle size and zeta potential were measured in zeta sizer and results were represented in fig. 4(a) and (b).

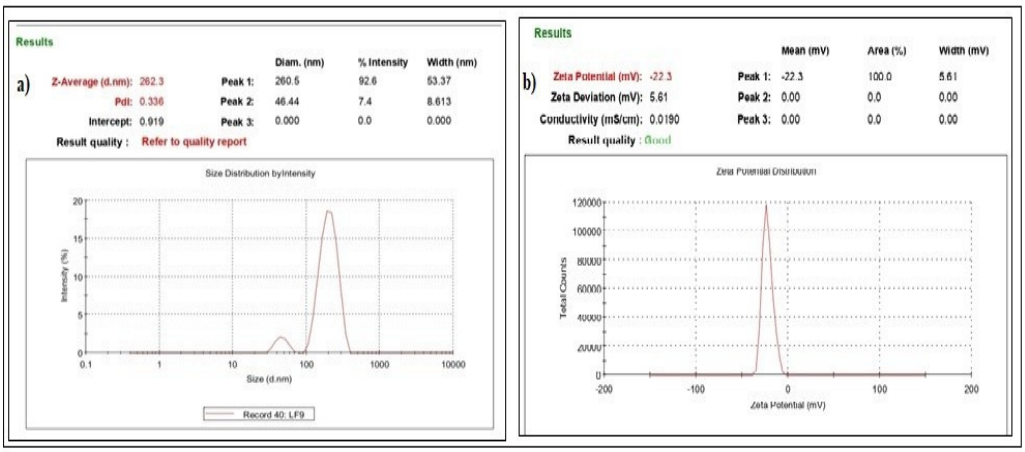

Fig. 4: Particle size (a) and zeta potential (b) of LF9L after 3 mo at accelerated stability condition

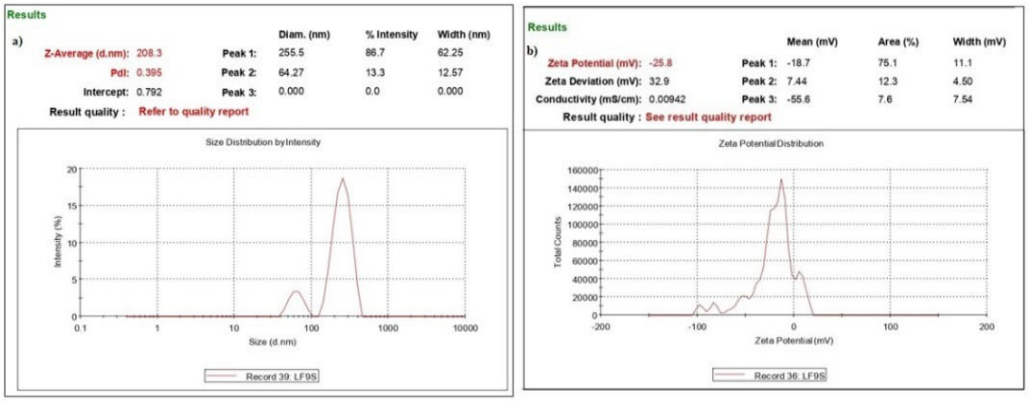

Fig. 5: Particle size (a) and zeta potential (b) of LF9S

Preparation solid smedds of Qinapril

For improving the stability, LF9L was formulated into solid smedds preparation by using adsorbent aerosil 200 at a different ratio. Three solid-smedds prepared i.e. LF9S (0.65:1), LF9S1 (0.55:1), LF9S2 (0.50:1) and taken for flow property study.

Micromeretic study

The flow property of three smedds powders was determined by calculating its angle of repose, tapped density, bulk density, Hausner's ratio, and Carr's index. Out of three formulations, LF9S has good flow property due to its value of angle of repose (22.3), Carr’s index (%) (15.6%) and Hausner's ratio (1.15).

Particle size and zeta potential of LF9S

After solidification, the particle size of LF9S was 208.3 nm and zeta potential was-25.8mv which was represented in fig. 4(a) and (b).

Drug content analysis

After solidification, the drug content analysis of LF9S was 85.26% which may be due to reduced particle size after solidification.

Solid-state characterization of LF9S

FTIR spectra of Qinapril (pure drug) and LF9S was represented in fig. 6(A). It was shown that the characteristic peaks of the pure drug were 3328.70 cm-1 (N-H stretching of amine group), 2952.90 cm-1 (C-H aliphatic stretching), 1677.73 cm-1 (C=O stretching of carboxylic group), 1622. cm^{-1} (C=C aromatic alkene stretching), 1527.07 cm-1 (NO2 stretching) which were observed in the FTIR spectra of LF9S. So it was indicated that there were no chemical incompatibilities between drug and excipients present I formulation.

The X-ray powder diffractometry (fig. 6(B)) of the pure drug shows some sharp and intense peak which indicated that the drug is present in the crystalline state. The presence of those intense small and sharp peaks was also observed in the physical mixture of drug and aerosil200 which showed that the drug present in the physical mixture is the semi- crystalline state. But in LF9S formulation, the sharp and intense peaks were disappeared which indicated that the drug present in the formulation is in an amorphous state.

DSC thermogram of Qinapril (pure drug) and LF9S was presented in fig. 6(C). DSC of Qinapril shows a sharp endothermic peak at 175 °C, corresponding to its melting points, indicating the crystalline nature of the drug. DSC of LF9S did not show any endothermic peak corresponding to its melting point. So it was concluded that the formulation was present as amorphous or solubilized form.

SEM image of pure drug and formulation LF9S represented in fig. 7. At 5,000 magnification, the SEM image of the pure drug showed that the particles are seen very large and present as an unevenly shaped form. But in the formulation, at 20,000 magnifications, the drug particles were distributed uniformly throughout the solid carrier. There was a complete absence of crystal structure which represented that the drug was present in the formulation as an amorphous state.

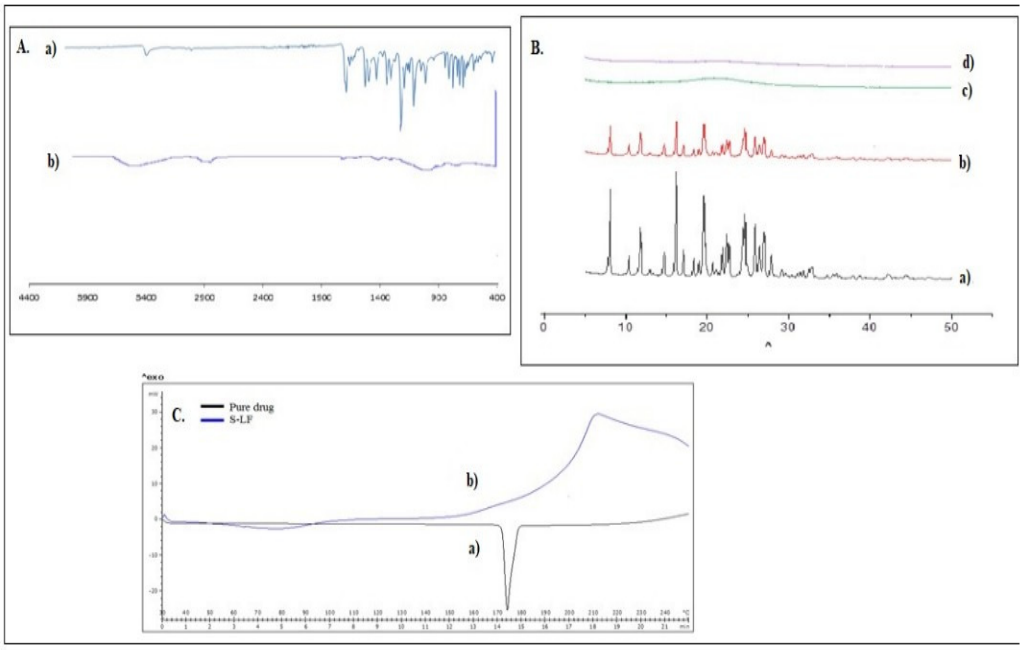

Fig. 6: A. Fourier transform infrared spectrophotometer study of Qinapril(a) and LF9S(b), B. X-ray diffractometry study of Qinapril(a), physical mixture of drug and aerosil 200(b), aerosil 200(c) and LF9S(d), C. Differential scanning calorimetry of Qinapril(a) and LF9S(b)

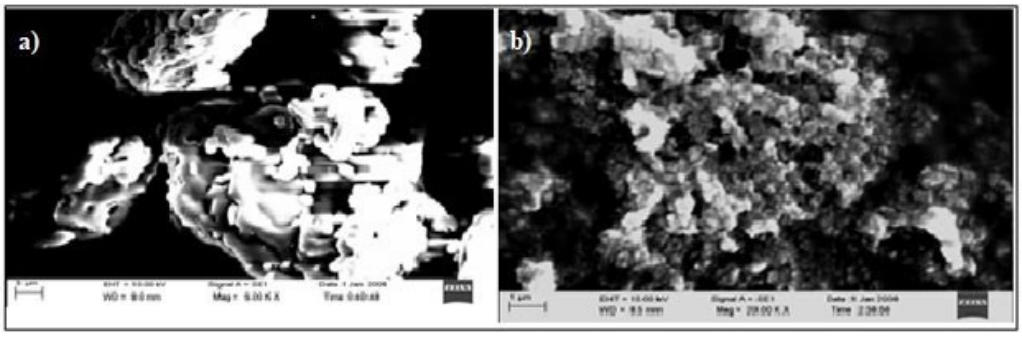

Fig. 7: Scanning electron microscope photograph of Qinapril (a) and LF9S (b)

***In vitro* drug release**

The drug release study of LF9S was performed and compared with the release pattern of pure drug and LF9L (smedds formulation). The *in vitro* dissolution profile of LF9S, pure drug and LF9 was represented in fig. 7. After 1 h of dissolution, LF9S released drugs more than 75%, in which LF9 and pure drug released only 17.22±0.26% and 2.54±0.04% respectively. At 120 min. LF9S release drug more than 97% which was significantly higher than pure drug and LF9L ($^{*}P<0.05$). Drug release from LF9S was faster due to increased surface area by use of adsorbent Aerosil 200, increasing the porosity of the formulation and may be due to transformation from crystalline to amorphous form.

Stability study

The stability of LF9S was performed according to ICH guidelines for 3 mo. Initially, the drug content (85.26%), particle size (208 nm) and zeta potential (-18.7mv) were measured. After 3 mo, at 40±2˚C/75±5 %, the % drug content was reduced to 81.39% and at 25±2˚C/60±5 %, the %drug content was to 84.67%. After 3 mo, the Particle size and zeta potential of LF9S were 249.4 nm and-32.2mv respectively (fig. 8). So there was no such significant difference observed in the assay study. After solidification, the stability is improved as a comparison to LF9 (liquid smedds).

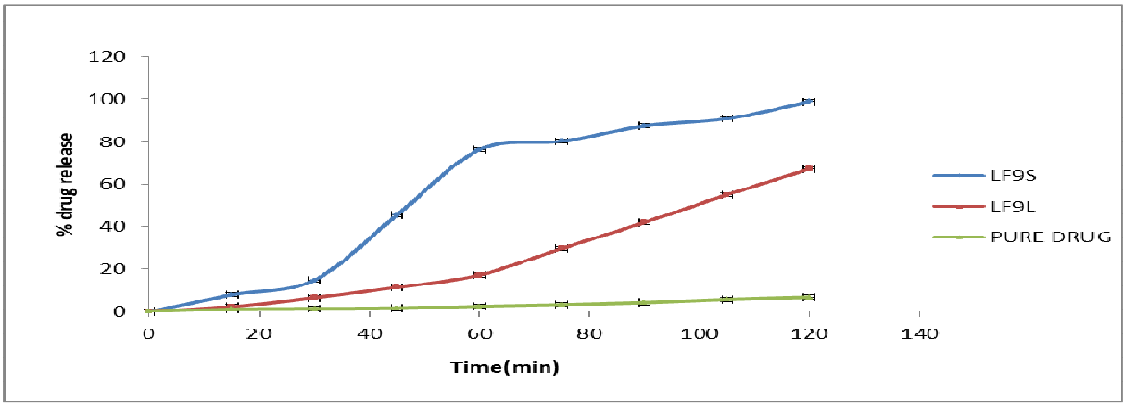

Fig. 8: *In vitro* diffusion profile of solid smedds (LF9S), smedds (LF9L) and Pure drug (Qinapril) (mean±SD, n=3)

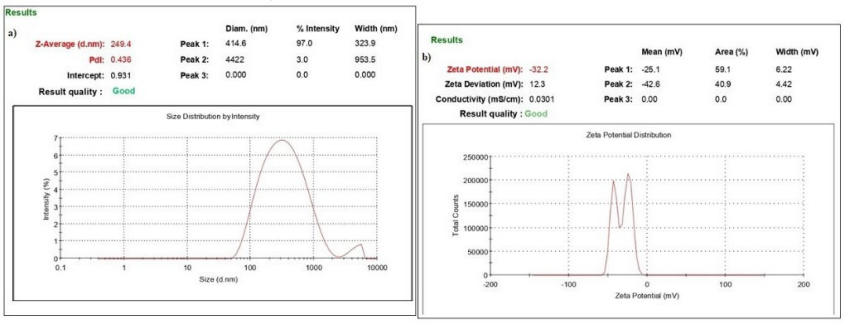

Fig. 9: Particle size (a) and zeta potential (b) of LF9S after 3 mo at accelerated stability condition

DISCUSSION

Liquid self-emulsifying drug delivery system of Qinapril was prepared by using oil, surfactant, and cosurfactant. Oil, surfactant, and co-surfactant were selected by solubility screening study. Linseed oil showed the highest solubility (61.23±0.95 mg/ml) and better than soybean oil (1.49±0.45) and almond oil (2.91±0.25). The surfactant itself an important element which stabilizes and help to solubilize drug.

Nonionic surfactants were generally used due to less toxicity and high stability. It is better than ionic and amphiphilic surfactants. [3] So among all surfactants used tween

80 exhibited highest solubility (56.47±0.92) than tween 60 (32.34±0.96), tween 40(41.34±0.88) and span 20 (8.87±0.18). The solubility of Qinapril with various cosurfactants has been investigated. PEG 400 was found (44.98±0.15) which was highest than PEG 200 (32±0.43), PPG (23.82±0.31) and PEG 600(26.10±1.01). According to solubility analytical studies, sedds formulations were developed by using linseed oil, tween 80 and PEG 400. A pseudo ternary phase diagram was developed by taking respective oil, surfactant, and cosurfactant. Surfactant and cosurfactant were mixed at 1:1 ratio and 1:2 ratios in which the mixtures of oil, surfactant, and co-surfactant were prepared at 1:9 to 9:1 ratio. From the pseudo-ternary phase diagram, SCOSmix (1:1) produced a reasonably wide self- emulsification region than a 1:2 ratio. The efficiency of emulsification was good when the composition of the surfactant/co-surfactant was taken at the same concentration. It was constructed based on the observations marked during titration after building the phase diagram of different formulations were selected at different point of the ternary phase.

Different formulations were prepared at different concentrations of oil, surfactant, and cosurfactant. Formulations containing (70-80%) oil and SCOSmix (20%-30%) were rejected due to phase separation. Phase separation was initiated because the ratio of oil and SCOSmix contained in the formulation shown incompatibilities keeping after 24h. The seven formulations were stable out of nine formulations. These formulations (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 1:9) were exposed to heating cooling process, freeze thaw stress testing, centrifugation testing (table 1).

Among those, three formulations showed phase separation after freeze-thaw stress testing because of the intolerance of excipients with a change in temperature. These three stable formulations were taken for particle size analysis, drug content analysis,and robustness to the dilution test. Robustness to dilution test was performed at different pH media pH 1.2 and 6.8 to mimic the *in vivo* conditions revealed no precipitation or phase separation indicating all the formulations were found to be robust towards different pH conditions.[11] So at both pH conditions LF3L, LF4L, LF9L shown stable i.e. no phase separation and drug precipitation after 24hr. But LF2L shown phase separation at both pH conditions. Because the robustness of formulation LF2L after dilution was decreased at both pH conditions (table 2). As the discussed previously the particle size of LF9L was 249.1 nm and zeta potential-16.9mv which shown the least particle size than other formulation.

The particle size of LF9L was decreased because the presence of the low amount of oil. As the concentration of SCOSmix is increasing the particle size is decreased. The smaller droplet size indicates more rapid absorption and improves the bioavailability of drug [26]. Due to its least particle size, the percentage of drug content is high and the percentage of drug release was more (54%) in comparison to other formulation and pure drug (6.72%). It may be due to proper composition between proportions of oil and SCOSmix which produced smaller droplet size with the highest drug content in the system. Due to its highest drug content, small droplet size and highest percentage of drug release LF9L is an optimized formulation for further study. It was exposed to different accelerated stability condition for 3 mo at 40 °C±2 °C and 75%±5% RH and 25±0.5˚C/60±5 % RH. After 3 mo the percentage of drug content, particle size, and zeta potential analysis (fig. 9) were performed. It was seen that the percentage of drug content was reduced and the particle size (262.3 nm) and zeta potential (-22.3mv) were increased due to the rise of instability in the formulation. The instability may be due to incompatibilities with the soft gelatin capsule shell and/or oxidation of lipids present in sedds [27].

So to improve stability, the solid smedds was prepared by using solid adsorbent aerosil 200 at a different ratio. Aerosill 200 is hydrophilic colloidal silicon dioxide which allows to attract and bind moisture to eliminate liquid bridges between solid particles that hinder powder flow. [28] Aerosil 200 was taken at a different ratio to determine the formulation of having good flow property. LF9S was selected due to its good flow property having angle of repose (22.3), Carr's index (%) (15.6%) and Hausner's ratio (1.15). The selected solid smedds LF9S was taken for drug content analysis, particle size, and zeta potential analysis. The percentage of drug content was more due to complete and uniform adsorption of sedds into aerosil 200. The particle size and zeta potential were 208.3 nm and-25.8mv which was less in comparison to liquid sedds (fig. 5). From the f. t. i. r study, there were no incompatibilities between drug and excipients due to presence of same characteristics peaks in formulation and pure drug (fig. 6(A)). From the x-ray diffractometric study, the drug present in the formulation is in the amorphous state. It may be due to the uniform adsorptivity of the drug throughout the adsorbent (fig. 6(B)).

The scanning electron microscopic study of formulation showed that the shape of the particle is uniform and distributed uniformly throughout the solid carrier (fig. 7). From the differential scanning calorimetry, LF9S was present in the formulation at an amorphous state because it showed a straight line within a melting point (175 °C) (fig. 6(C)). The *in vitro* dissolution study was performed for 2h. at pH 6.8 dissolution media. At 120 min. LF9S release drug more than 97% which was significantly higher than pure drug and LF9. Drug release from LF9S was faster due to increased surface area by use of adsorbent (fig. 8). Aerosil 200, increasing the porosity of the formulation and may be due to transformation from crystalline to amorphous form. After an *in vitro* dissolution study, LF9S was taken for accelerated stability study for a minimum period of 3 mo at 40 °C±2 °C and 75%±5% RH and 25±0.5˚C/60±5 % RH. The

drug content analysis, particle size, and zeta potential analysis was performed. The particle size and zeta potential were 249.4 nm and-32.2mv. Which describe that LF9S lied within micron range. There was no such significant difference observed in assay study, particle size and zeta potential analysis. After solidification, the solubility as well as stability was improved as a comparison to LF9L (liquid smedds) formulation.

CONCLUSION

Solid-self emulsifying drug delivery system of Qinapril was successfully prepared by using linseed oil as oil part, Tween 80 as a surfactant, PEG 400 as co-surfactant and Aerosil 200 as adsorbent. Nine liquid self-emulsifying formulations were prepared at a different ratio. The best self-emulsifying formulation (LF9L) which was optimized by different evaluation process and stability study. At accelerated condition after three months, the percentage of drug content was reduced which indicated that the formulation was in instability condition. So to improve stability of the liquid self- emulsifying formulation, it was converted to s-sedds by adsorption to solid carrier technique using aerosil200. After solidification, drug content was more than 85.26% and the particle size was 208 nm. From the *in vitro* dissolution study, LF9S release 98.70%, which was highest among LF9L and pure drug. From the FTIR study, it was indicated that there were no incompatibilities between drug and excipients. From the DSC and XRD study, it was shown that the drug was present in the formulation as an amorphous form. From the stability analysis, it was seen that no significant difference was observed during drug content analysis. Thus it was concluded that solid SEDDS formulation is capable to enhance solubility and dissolution of poorly water-soluble drugs like Qinapril by using aerosil 200 which improves therapeutic performance.

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