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Research Article

Furosemide Loaded Silica-Lipid Hybrid Microparticles: Formulation Development, *in vitro* and *ex vivo* Evaluation

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Abstract

Purpose: The main objective of the current research work was to formulate and evaluate furosemide loaded silica lipid hybrid microparticles for improved oral delivery. A novel silica-lipid hybrid microparticulate system is used for enhancing the oral absorption of low solubility and low permeability of (BCS Class IV) drugs. Silica-lipid hybrid microparticles include the drug solubilising effect of dispersed lipids and stabilizing effect of hydrophilic silica particles to increase drug solubilisation, which leads to enhanced oral bioavailability.

Methods: The slica lipid hybrid (SLH) microparticles were composed of poorly soluble drug (furosemide), dispersion of oil phase (Soya bean oil and miglyol) in lecithin (Phospholipoid 90H), non-ionic surfactant (Polysorbate 80) and adsorbent (Aerosol 380). Saturation solubility studies were performed in different oils and surfactants with increased concentration of drug revealed increased solubility of furosemide.

Results: *In vitro* dissolution studies conducted under simulated gastric medium revealed 2-4 fold increase in dissolution efficiencies for SLH microparticles compared to that of pure drug (furosemide) and marketed formulation Lasix®. *Ex vivo* studies showed enhanced lipid digestibility, which improved drug permeability. Solid-state characterization of SLH microparticles by X-ray powder diffraction and Fourier transform infrared spectroscopic analysis confirmed non-crystalline nature and more compatibility of furosemide in silica-lipid hybrid microparticles.

Conclusion: It can be concluded that the role of lipids and hydrophilic silica based carrier highlighted in enhancing solubility and permeability, and hence the oral bioavailability of poorly soluble drugs.

Introduction

Oral route of drug delivery is the most convenient and noninvasive method of drug administration which is having the highest degree of patient compliance. For well oral absorption, a drug should be sufficiently soluble in the gastrointestinal fluids and it should be easily permeate across the GI membrane without presystemic metabolism.[1,2] According to recent approximation, nearly 30% of the oral immediate-release drug products and 40-70% of the new chemical entities are poorly soluble in water.[3] Drugs with poor aqueous solubility, poor dissolution rate and poor permeability are not suitable for oral delivery as it produces low and variable bioavailability, which leads to erratic biological effects.[4] In order to improve the solubility and permeability limited bioavailability, lipid-based formulations have emerged as an effective and versatile technology.[5-7]

Some potential mechanisms by which silica-based materials enhance the oral absorption of drugs are mainly via the preservation of the drug amorphous or molecularly dispersed form, as well as increased wettability in the aqueous medium, which lead to enhanced dissolution or release kinetics.[1,8]

Hence the current research was aimed to develop a novel formulation (i.e. silica-lipid hybrid microparticles) for improving the absorption of poorly water-soluble and poorly permeable drug furosemide (BCS Class IV drug) based on appropriately selected lipid excipients and the utilization of nanoparticle technology.[9]

Materials and Methods

Materials

Furosemide is a sulfonamide, potent loop diuretic obtained as gift sample from Aventis Pharmaceuticals Ltd, Ankaleshwar, Phospolipoid 90H and Soyabean oil were obtained from Lipoid, Germany, Miglyol 812 was obtained from Hamilton laboratories, Australia, Aerosil® 380 was obtained from Evonik degussa, Germany and the remaining excipients used were of analytical grade.

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Methodology

Saturation Solubility Studies

Saturation solubility studies of pure drug (furosemide) was done to select suitable solvent for the formulation in various oils and surfactants such as Transcutol P, Cremophor EL, Captex 355, Captex 200, Capmul MCM, soya bean oil, Miglyol 812 and Tween 80. To each of these oils and surfactants, excess amount of drug was added and filled in vials. The solutions were equilibrated under continuous shaking on rotary shaker for 48 h. And after 48 h, samples were centrifuged, the supernatant was suitably diluted and filtered through whatman filter paper to obtain a clear solution and estimated for furosemide concentration using UV spectrophotometry at 276 nm after appropriate dilution. Three determinations were carried out for each sample to calculate the solubility of furosemide.[10]

Formulation of Silica Lipid Hybrid (SLH) Microparticles of Furosemide

SLH microparticles were prepared with two different oils such as soya bean oil and miglyol 812.

SLH microparticle formulations of F1-F5 and F11-F15 were prepared by taking known amount of drug (20 mg), lipid (Phospolipoid 90H) (5 mg), different oil:surfactant (soya bean oil/ miglyol 812:Tween 80) ratio of 2:1,4:1,6:1,8:1 and 10:1, respectively. Formulations F6-F10 and F16-F20 were prepared by keeping the oil:surfactant ratio constant at 10:1, and by increasing the lipid concentration from 5 to 10 mg.[11-13] Compositions of various formulations are given in the (Table 1 and 2).

Table 1. Formulation of SLH microparticles containing soya bean as oil

FORMULA	Drug (mg)	Phospholipid 90 H (Lipid)	Soya bean oil	Oil:surfactant ratio	Surfactant Tween 80	Carrier Aerosil 380
F1	20	5	20	2:1	10	40
F2	20	5	40	4:1	10	50
F3	20	5	60	6:1	10	75
F4	20	5	80	8:1	10	95
F5	20	5	100	10:1	10	125
F6	20	6	100	10:1	10	125
F7	20	7	100	10:1	10	125
F8	20	8	100	10:1	10	125
F9	20	9	100	10:1	10	125
F10	20	10	100	10:1	10	125

Table 2. Formulation of SLH microparticles containing Miglyol 812 as oil

FORMULA	Drug(mg)	Phospholipid 90 H (Lipid)	Miglyol 812	Oil:surfactant ratio	Surfactant Tween 80	Carrier Aerosil 380
F11	20	5	20	2:1	10	50
F12	20	5	40	4:1	10	75
F13	20	5	60	6:1	10	100
F14	20	5	80	8:1	10	150
F15	20	5	100	10:1	10	150
F16	20	6	100	10:1	10	150
F17	20	7	100	10:1	10	150
F18	20	8	100	10:1	10	150
F19	20	9	100	10:1	10	150
F20	20	10	100	10:1	10	150

Evaluation of SLH Microparticles

Micromeritic properties:

Angle of repose: Angle of repose of powder blend was determined by using funnel method, the diameter of the powder cone was measured and angle of repose was calculated using the following equation.

$$\tan\theta = h/r$$

Where h and r are the height and radius of the powder cone, θ is the angle of repose.

Determination of bulk density and tapped density: The bulk density and the tapped density were calculated using the following formulae.

$$\text{Bulk density} = W/V_0$$

$$\text{Tapped density} = W/V_f$$

Where, W = Weight of the powder; V_0 = Initial volume; V_f = final volume

Carr's index/ Carr's consolidation index/ Compressibility index (CI): It is an important measure that can be obtained from the bulk and tapped densities.

In theory, the less compressible a material the more flowable.

$$CI = (TD-BD) \times 100/TD$$

Where, TD is the tapped density and BD is the bulk density.

Hausner's ratio: Hausner's ratio is the ratio of tapped density to bulk density. Hausner found that this ratio was related to interparticle friction and, as such, could be used to predict powder flow properties. Generally a value less than 1.25 indicates good flow properties, which is equivalent to 20% of Carr's index.

Assay of SLH micropartcles: SLH microcapsules of soya bean oil and miglyol 812 were determined for assay. An accurately weighed portion of the powder equivalent to about 100 mg was transferred to a 100 ml volumetric flask containing SGF phosphate buffer. It was kept on rotary shaker for 1 h. After this time period, the solutions were centrifuged, filtered through membrane filter 0.45 µm and 1 ml of filtrate was diluted to 10 ml with buffer and absorbance was measured against blank at 276 nm by UV Spectrophotometry. Each content determination was performed in triplicate and the average and standard deviations were calculated.

In vitro dissolution study: Dissolution studies of pure furosemide, SLH microparticles and marketed formulation were performed by using the US Pharmacopeia (USP) XXIV type II apparatus (Electrolab, Mumbai, India) at the paddle rotation speed of 50 rpm in 900 mL of SGF as dissolution media at 37±0.5 °C. A sample equivalent to 20 mg furosemide of the prepared systems was placed in dissolution medium. During the release studies, samples of 5 ml were collected after 5, 10, 20, 25, 30, 45, 60, 90 and 120 min using a syringe and were replaced with the same volume of phosphate buffer. The samples were subsequently filtered using 0.45 µm pore size membrane filters. Then Filtrate (1 ml) was diluted to 10ml with phosphate buffer and absorbance was measured against blank at 276 nm using a UV Spectrophotometry. Each content determination was performed in triplicate and the average and standard deviations were calculated.

In vitro diffusion study: Comparative diffusion study for pure drug and selected formulation of SLH microparticles was conducted using gelatin membrane by Franz diffusion. SLH microparticles with equivalent to 20 mg of drug dissolved in 2 ml of buffer and it was then introduced into the donar compartment. The receptor compartment consists of 18 ml of 7.4pH phosphate buffer. Samples were collected at predetermined time intervals and replenished with equal volume of medium and the samples were filtered through membrane filter 0.45 µm and quantified by using UV spectrophotometry at a wavelength of 276 nm.

Ex vivo permeation studies: Goat intestinal membrane was collected from slaughter house. It was flushed with Krebs-Ringer solution to remove the mucus and adhered intestinal contents. One end of the intestine segment was tied and the formulation equivalent to 20 mg of drug was dispersed in 2 ml of buffer and it was then introduced into the intestinal lumen and was tightly closed. The tissue was placed in an organ bath with continuous aeration and maintained at a temperature of 37 °C. The receptor compartment consists of 200 ml of 7.4pH phosphate buffer. Comparative studies of pure drug, selected batch of SLH microparticles, marketed formulation were done. Samples were collected at predetermined time intervals and replenished with equal volume of medium and the samples were filtered through membrane filter 0.45 µm and quantified by using UV spectrophotometry at a wavelength of 276 nm.

Solid State Characterization

Solid state study was performed for drug (furosemide), carrier (Aerosil 380) and for selected batch of SLH microparicles.

Fourier Transform Infrared Spectroscopy: Fourier transform infrared (FT-IR) spectra were obtained using a IR prestige 21 Shimadzu model FTIR spectrometer which was employed to characterize the possible interactions between the drug and the carrier in the solid state. The samples are prepared by the KBr pellet method and the spectrum was recorded in the range of 4000–400 cm^{-1}.

X- Ray Diffraction: SLH microparticles were analyzed using an X'Pert PRO MPD diffractometer (PANalytical, Almelo, the Netherlands) with a copper anode (Cu Kα radiation, λ = 0.15406 nm, 45 kV, 40 mA). The diffraction pattern was measured with a step size of 0.017° and a dwell time of 45 s at each step between 3 and 50 2θ at ambient temperature.

Results and Discussion

Saturation Solubility Studies

Saturation solubility studies of furosemide pure drug was done in various oils and surfactants such as Transcutol P, Cremophore, Captex 355, Captex 200, Capmul MCM, soya bean oil, Miglyol 812, Tween 80. The results obtained are shown in bar graph, which is shown in Figure 1. It was found that furosemide is having high solubility in Miglyol 812, Tween 80 and soya bean oil. Hence these solvents were selected for the formulation. Miglyol 812 and soya bean oil were selected as solvents and Tween 80 was selected as surfactant.

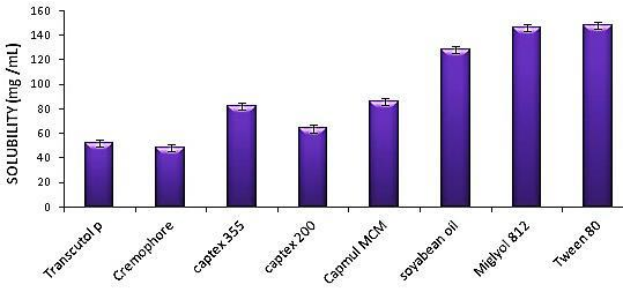

Figure 1: Saturation solubility studies of furosemide in various oils and surfactants

Micromeritic Evaluation

SLH microparticles were characterized with respect to angle of repose, bulk density, tapped density, Carr's index, Hausner's ratio & drug content. The results obtained are given in Table 3.

Table 3. Micromeritic properties of SLH microparticles containing soya bean oil

Formulation	Bulk density* (g/ml)	Tapped density* (g/ml)	Carr's index* (%)	Hausner's ratio*	Angle of repose* (θ)	Drug content* (%)
F1	0.46±0.07	0.36±0.04	13.02±0.06	0.91 ± 0.01	25 ± 0.12	84.3±0.47
F2	0.39±0.06	0.30±0.17	9.08 ± 0.12	0.95 ± 0.08	27 ± 0.06	89.6±1.36
F3	0.47±0.13	0.29±0.18	14.50 ±0.04	0.92 ± 0.02	28 ± 0.04	96.3±0.75
F4	0.45±0.17	0.41±0.07	16.45 ±0.09	0.92 ± 0.06	27 ± 0.12	94.8±1.34
F5	0.34±0.08	0.30±0.14	9.21 ± 0.07	0.91 ± 0.06	24 ± 0.02	99.5±1.03
F6	0.46±0.09	0.37±0.08	15.33 ±0.18	0.93 ± 0.07	28 ± 0.17	97.7±1.49
F7	0.42±0.12	0.32±0.07	9.04 ± 0.14	0.96 ± 0.02	26 ± 0.13	96.6±0.29
F8	0.34±0.18	0.36±0.04	16.40 ±0.08	0.91 ± 0.06	25 ± 0.09	97.4±0.43
F9	0.32±0.04	0.39±0.06	12.39 ±0.06	1.12 ± 0.04	26 ± 0.14	89.7±1.33
F10	0.36±0.08	0.38±0.03	11.51 ±0.08	0.93 ± 0.02	27 ± 0.09	94.2±0.48
F11	0.34±0.04	0.32±0.07	10.02±0.05	0.95 ± 0.02	26 ± 0.20	86.3±1.55
F12	0.46±0.03	0.41±0.04	13.08±0.06	0.91 ± 0.07	25 ± 0.07	91.6±1.43
F13	0.45±0.07	0.46±0.03	16.50±0.01	0.93 ± 0.03	28 ± 0.05	93.3±0.65
F14	0.47±0.03	0.42±0.03	14.45±0.02	0.92 ± 0.05	27 ± 0.08	94.8±1.86
F15	0.33±0.02	0.30±0.04	9.21 ± 0.04	0.91 ± 0.06	24 ± 0.03	99.7±1.07
F16	0.42±0.06	0.37±0.03	15.33±0.08	0.94 ± 0.08	28 ± 0.07	95.7±1.34
F17	0.46±0.03	0.41±0.04	10.18±0.02	0.93 ± 0.02	26 ± 0.16	87.6±0.39
F18	0.34±0.04	0.29±0.03	12.40±0.03	0.91 ± 0.08	29 ± 0.02	96.4±0.75
F19	0.36±0.07	0.30±0.03	16.39±0.02	0.95 ± 0.01	27 ± 0.07	89.7±1.36
F20	0.39±0.05	0.34±0.04	9.51 ± 0.06	0.99 ± 0.01	25 ± 0.12	95.2±0.47

*****Each value represents Mean±standard deviation (SD) of 3 observations

In vitro drug release studies

Comparitive *in vitro* drug release studies of SLH microparticles of soya bean oil and Miglyol 812 were done.

In vitro drug release of SLH microparticles containing soya bean oil

A graph was plotted by taking % drug release on Y-axis and time on X-axis, which is shown in Figure 2.

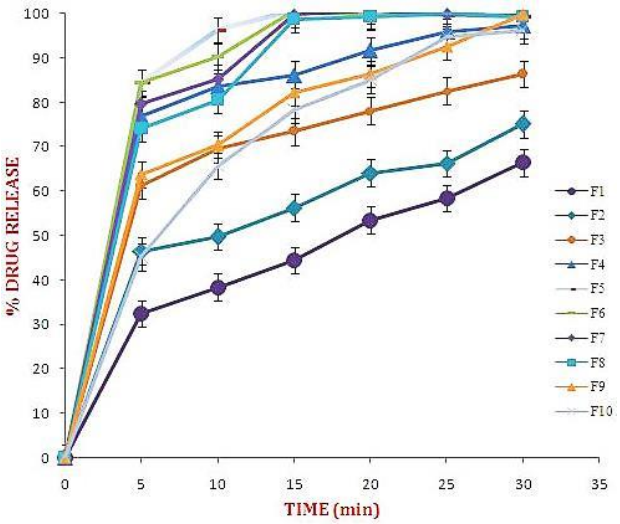

Figure 2: *In vitro* drug release profile of SLH microparticles containing soya bean oil

It was found that F5 formulation has shown faster drug release than the remaining formulations. It was observed that 84.5±0.7% of drug was released within 5 minutes and 99.3±1.8% of drug was released within 30 minutes. Hence, it was chosen as the best ratio of oil to surfactant, and the remaining formulations F6 to F10 were prepared by keeping this ratio at constant level and increasing the lipid concentration up to 10 mg. It was found that the formulations F6 to F8 have shown good drug release characteristics similar to F5 irrespective of lipid concentration. However, the drug release was found to be gradually reduced in F9 and F10 formulations. This might be due to the higher concentration of lipid. Hence F5 and F8 formulations were finally selected for the permeability studies.

In vitro drug release of SLH microparticles containing miglyol 812

A graph was plotted by taking % drug release on Y-axis and time on X-axis, as shown in Figure 3. It was found that F15 formulation has shown faster drug release than the remaining formulations. It was observed that 85.4±1.6% of drug was released within 5 minutes and 99.2±1.2% of drug was released within 30 minutes. Hence, it was chosen as the best ratio of oil to surfactant, and the remaining formulations F16 to F20 were prepared by keeping this ratio at constant level and increasing the lipid concentration up to 10 mg. It was found that the formulations F16 to F17 have shown good

drug release characteristics similar to F15 irrespective of lipid concentration. However, the drug release was found to be gradually reduced in F18 to F20 formulations. This might be due to the higher concentration of lipid. Hence F15 and F17 formulations were finally selected for the permeability studies.

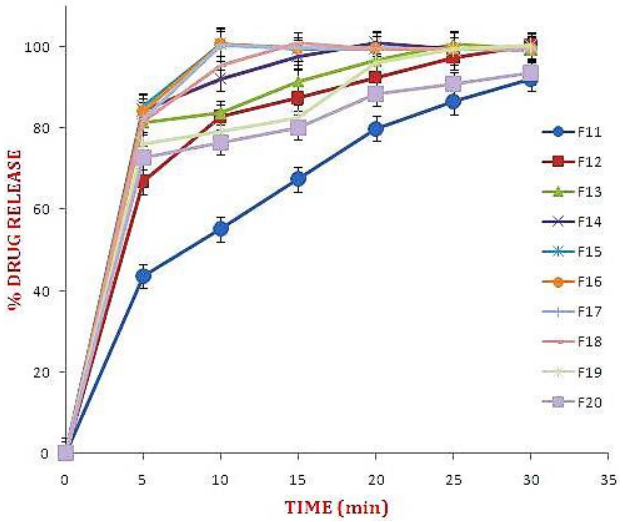

Figure 3: *In vitro* drug release profile of SLH microparticles containing Miglyol 812

However, among the SLH micropaticles of soya bean oil and Miglyol 812, SLH microparticles containing Miglyol 812 (F15) was found be the best formulation for the *in vitro* drug release studies, as 100% of drug was released within 10 minutes.

Comparative in vitro drug release profile

SLH microparticle formulations were subjected to diffusion studies at 7.4 pH phosphate buffer to determine the release profile of SLH microparticles. A graph was plotted by taking % drug release on Y-axis and time on X-axis as shown in Figure 4. Comparative diffusion studies were carried out for pure drug and best-selected formulations. It was found that F17 formulation has shown faster diffusion than pure drug and other remaining formulations. For F17 formulation, as 26.4±1.2% of drug was diffused within 15 minutes and 83.7±2.1% of drug was diffused within 120 minutes.

Comparative ex vivo permeation studies

SLH microparticle formulations were subjected to *ex vivo* permeation studies at 7.4 pH phosphate buffer to determine the release profile of SLH microparticles. A graph was plotted by taking % drug release on Y-axis and time on X-axis as shown in the Figure 5. Comparative *ex vivo* permeation studies were carried out for pure drug, marketed formulation and best-selected formulation. It was found that F17 formulation has shown faster diffusion than pure drug and marketed formulation. For F17 formulation, as 27.2±1.6 % of drug was diffused within 10 minutes and 86.3±0.9% of drug was diffused within 90 minutes. Whereas for pure drug, 7.4±0.9% of drug was diffused within 10 minutes and 49.8±2.0% of drug was diffused within 90 minutes. Whereas, for marketed formulation 15.8±2.0% of drug was diffused within 10 minutes and 67.2±0.9% of drug was diffused within 90 minutes. Hence F17 formulation was finally selected as best formulation when compared with pure drug and marketed formulation.

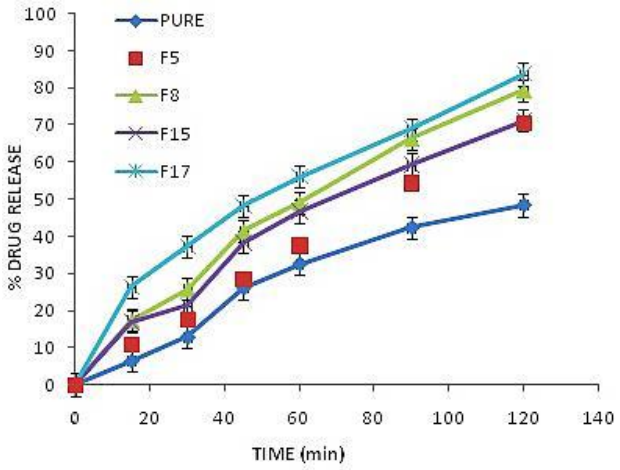

Figure 4: Comparative diffusion studies of pure drug and selected SLH microparticles

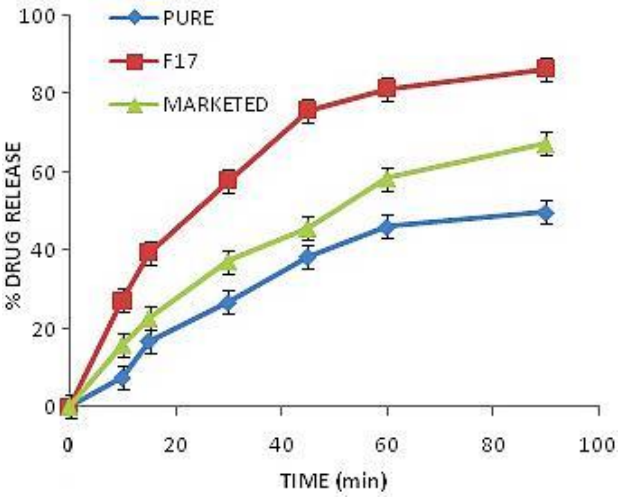

Figure 5: Comparative *ex vivo* permeation studies of pure drug, selected SLH microparticles and marketed formulation

Solid State Characterization

X-RAY Diffraction

Powder X-Ray Difraction scans of pure drug and optimized formulation were conducted to understand the crystallnity of pure drug and any loss or modification of pure drug crystalinity after its formulation into SLH microparticles. The X-ray diffractograms of optimized SLH microparticles formulation (Figure 6) showed only broad peak at 2-Theta of 20° due to its amorphous nature and no peak was seen at 2-Theta of 22°. Whereas presence of certain pure drug peak in the formulation is due to the fact that after saturation of absorption process adsorption occurs

on the surface of carrier. It can be concluded that no significant interaction between drug and excipients.

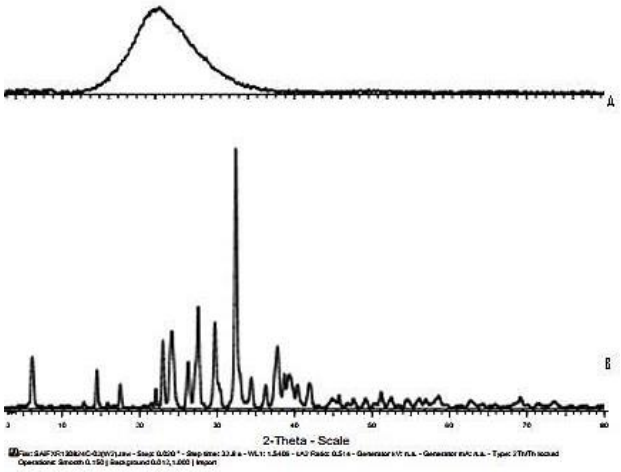

Figure 6: X-ray diffractograms A) Optimized SLH microparticles formulation B) Pure Drug

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR studies were done for pure drug and optimized formulation to determine compatibility between pure drug and excipients.

Thus results of FTIR studies suggest that a(Figure 7) reduction in intensity of the characteristic absorption bands of furosemide were observed in SLH microparticles formulations, which might be attributed to the hydrogen bonding interaction between the furosemide and of the carrier. It can be concluded from the results that there was no interaction between drug and excipients.

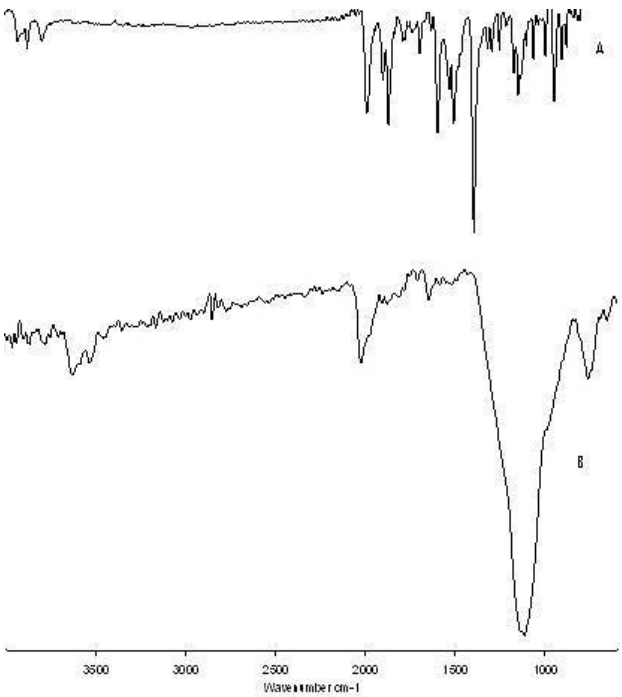

Figure 7: FTIR spectra A) Pure Drug B) Optimized SLH microparticles formulation

Conclusion

Furosemide SLH microparticles were prepared by selecting Phospolipoid 90 H as lipid, Miglyol 812 & soya bean oil as oil phase, Tween 80 as surfactant and Aerosil 380 (Hydrophilic fumed silica) as inert carrier. All characterizations were performed and finally optimized formulation was obtained. SLH Micropaticles of Furosemide containing Miglyol 812 (oil), (F15) showed enhanced solubility, dissolution rate and (F17) showed enhanced permeability when compared to other formulations and marketed drug. The results indicated that it is possible to conclude that SLH microparticles are better than pure drug and marketed drug formulation. It can also be concluded from the drug-excipient compatibility studies (FTIR, DSC) that there was no interaction between the drug and the excipients.

Acknowledgments

The satisfaction that accomplished the successful completion of any task would be incomplete without the mention of the people who made it possible and whose constant guidance and encouragement crown all the effects with success. We would like to acknowledge Aventis Pharmaceuticals Ltd, Ankaleshwar for providing furosemide as gift sample. We are grateful to Lipoid, Germany for providing the gift sample of Phospolipoid 90H and Soyabean oil. We are thankful to Evonik Degussa, Germany for providing Aerosil® 380. We are also thankful to Hamilton laboratories, Australia for providing Miglyol 812 used in the study.

Ethical Issues

Not applicable.

Conflict of Interest

It is here by declared that the authors do not have any declaration of interest.

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