

Modulation of solubility and dissolution of furosemide by preparation of phospholipid complex

Abstract

Aim: The aim of this study is to improve the solubility and dissolution of furosemide (a potent high ceiling diuretic used for the treatment of hypertension and a Class IV drug that is low solubility and low permeability drug as per the Biopharmaceutical Classification System) by preparing its phospholipid complexes or pharmacosomes. **Materials and Methods:** Furosemide was complexed with phosphatidylcholine in four different molar ratios (1:1, 1:2, 1:3 and 1:4) by conventional solvent-evaporation technique. The pharmacosomes prepared were evaluated for drug content, solubility, X-ray powder diffraction (XRPD) and *in-vitro* dissolution study. **Results:** Pharmacosomes of furosemide showed high drug content ranging from 88.30% to 100%. XRPD studies confirmed the formation of phospholipid complex and the amorphization of drug in the complex. The water solubility was found to be increased up to six-fold in the complexes. The octanol solubility also increased in the complexes indicating the probable increase in permeability. The *in-vitro* dissolution profile of the prepared complexes was found to be much better than furosemide. **Conclusion:** It was concluded that the phospholipid complexes can be effectively used for improving the solubility, dissolution, permeability and hence the bioavailability of furosemide like Class IV drugs.

Key words:


Bioavailability, furosemide, pharmacosome, phospholipid complex, solubility

Introduction

Furosemide is a potent high ceiling (loop) diuretic used for the treatment of hypertension, bronchial asthma, edema associated with congestive heart failure, cirrhosis of the liver and renal disease, including nephrotic syndrome. It works by blocking the absorption of sodium, chloride, and water from the filtered fluid in the kidney tubules, causing a profound increase in the output of urine (diuresis).^[1,2] Furosemide is 4-chloro-2-[(furan-2-ylmethyl] amino)-5-sulfamoylbenzoic acid [Figure 1a]. The drug has been classified as a Class IV drug as per the Biopharmaceutical Classification System (BCS). It is slightly soluble in water and poorly absorbed from lower intestine due to its low solubility and oral bioavailability.

Oral bioavailability of drug depends on its solubility and/or dissolution rate. The dissolution may be the rate

determining step for the onset of therapeutic activity. Poor solubility and the dissolution of drugs is the major challenge for formulation scientists and the various techniques such as solid dispersion, solvent deposition, supercritical fluid process, micronization, use of surfactants, use of salt forms, complexation etc., have been investigated for resolving solubility and dissolution issue in pharmaceutical product development. Out of these, the complexation technique has been employed more precisely to improve the solubility and the dissolution of poorly water soluble drugs.^[3-10] Among the complexation techniques the phospholipid complexation and cyclodextrin complexation are the two most widely investigated approaches for improving the solubility. In the various previous studies, it was reported that developing the drugs as lipid complexes (also called pharmacosomes) may prove to be a potential approach to improve solubility and

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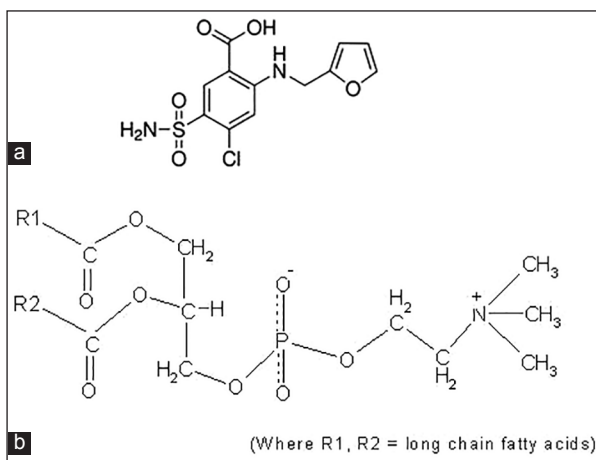


Figure 1: Chemical structure of furosemide (a) and phosphatidylcholine (b)

to minimize the gastrointestinal toxicity of drugs.^[11-15] In the phospholipid complexation the drug and a phospholipid [Figure 1b] are treated in certain molar ratio (generally 1:1 or 1:2) to yield an amphiphilic complex with improved solubility, permeability and dissolution profile.

Therefore, to improve the solubility and dissolution of furosemide its pharmacosomes or phospholipid complexes were prepared. These complexes may improve their absorption by imparting an environment of improved solubility (for dissolution) and improved lipophilicity for better permeation (across the biological membranes). Thus, furosemide-phospholipid complex were prepared and evaluated for various physical parameters (like drug content, solubility, and X-ray powder diffraction analysis [XRPD]) and the *in-vitro* dissolution study.

Materials and Methods

Furosemide was obtained from SGS Company Limited, Roorkee. Soya phosphatidylcholine (PC) (LIPOID S-80) was obtained as a gift sample from LIPOID GmbH, Germany. All other chemicals were of analytical grade.

Preparation of furosemide-phosphatidylcholine complexes

Furosemide-PC complex was prepared by refluxing furosemide with an equimolar concentration of PC (80% purity grade of soya-phospholipids). To prepare the first formulation of complex (F1), the equimolar concentration of PC and furosemide (i.e., 1:1 molar ratio) were placed in a 100 ml round bottom flask and dissolved in methanol. The solvent was evaporated off under vacuum at 40°C in a rotary vacuum evaporator (Model No. 5600, Buchi type, Perfit India Limited Mumbai,). The lipid complexes (pharmacosomes) were obtained as dried residue which were collected and placed in vacuum desiccators overnight and then subjected to characterization. Similarly, all the other formulations F2,

F3, and F4 were prepared with drug to PC ratio of 1:2, 1:3 and 1:4, respectively.

Drug content

To determine the drug content in complex, the complex equivalent to 50 mg were weighed and added in 50 ml of phosphate buffer (pH 6.8). The volumetric flask was stirred continuously for 24 h on a magnetic stirrer. At the end of 24 h, 1 ml from this solution was taken and volume was made up to 100 ml by addition of phosphate buffer. The absorbance of the solution was measured against the corresponding blank solution using an ultraviolet (UV) spectrophotometer (double beam UV-visible spectrophotometer, Lambda 25, Perkin Elmer, USA). Each sample was analyzed in triplicate.

Solubility study

To determine change in solubility due to complexation, solubility of drug and complex was determined in buffer and n-octanol by shake flask method. Fifty milligrams of furosemide drug (and 50 mg equivalent in case of complex) was taken in a 100 ml conical flask in which 50 ml of phosphate buffer (pH 6.8) was added and then stirred for 15 min. Then it was transferred to 250 ml of separating funnel with 50 ml of n-octanol and was shaken well. Separating funnel was allowed to stand for about 30 min. At the end, aqueous portion was analyzed by UV-visible spectrophotometer.

X-ray powder diffraction analysis

The crystalline state of furosemide in the different samples was evaluated with XRPD (Bruker Axs-D8 Discover Powder X-ray diffractometer, Germany). The X-ray generator was operated at 40 kV tube voltages and 40 mA of tube current, using the K-alpha lines of copper as the radiation source. The scanning angle ranged from 1° to 60° of 2θ in step scan mode (step width 0.4°/min). Furosemide, PC (80%) and furosemide-PC complex were analyzed with X-ray diffractions (XRDs).

In-vitro dissolution study

In-vitro release study was carried out using USP dissolution rate test apparatus Type II at 100 rpm and at 37 ± 0.5°C. Accurately, weighed quantities of complex equivalent to 100 mg of were taken for the dissolution study using 900 ml of phosphate buffer (pH 6.8) as media. Samples were withdrawn at different time intervals (0.5, 1-10th h) and replaced with the equal volume of fresh media for maintaining the sink condition. The withdrawn samples were filtered (through 0.45 μm Whatmann filter paper), suitably diluted with dissolution fluid and then analyzed spectrophotometrically.

Statistical analysis

Results are expressed as mean values and standard deviations (±SD) and the significance of the difference observed was analyzed by the Student's *t*-test.

Results and Discussion

In the present experiment, furosemide-phospholipid complexes were prepared by a simple and reproducible method to improve the solubility and the dissolution profile of furosemide. Furosemide and PC were taken in four different molar ratios of 1:1, 1:2, 1:3 and 1:4 for preparation of F1, F2, F3, and F4 formulations, respectively.

The drug content of furosemide-PC complex (as estimated by UV spectrophotometry at 277 nm in phosphate buffer) was found to be in the range $88.30 \pm 0.017\%$ to $100 \pm 0.066\%$. Formulation F1 and F4 showed the highest percentage of drug loading that is $100 \pm 0.066\%$ and $97.45 \pm 0.208\%$, while the F2 and F3 showed the lower drug loading efficiency of $88.30 \pm 0.017\%$ and $93.60 \pm 0.036\%$, respectively. There was a paradoxically low drug content in F2 and F3 in comparison of F1 and F4. However in general, this study support the previous studies, which have reported the maximum drug content in the complex prepared in 1:1 ratio of drug and lipid.^[15,16] The good percent loading of the drug favors the feasibility of the clinical or therapeutic delivery of drug. Furthermore, the low values of SD indicate that the drug was uniformly distributed in phospholipid complex.

Various previous studies on pharmacosomes or the lipid complexes report that in the pharmacosomes the drug is reversibly bonded chemically with the lipids and thus show not only a good percent loading, but also the better stability than liposomes.^[15]

Solubility study was performed to determine the effect of complexation on solubility for all the complex formulations in comparison with the plain drug [Table 1]. It was found that the aqueous and n-octanol solubility of the furosemide-PC complex was much higher (in water and n-octanol both) than the furosemide. The water solubility of lipid complexes F1, F2, F3 and F4 were found to be 18.33, 27.16, 35.60 and 41.33 $\mu\text{g/ml}$ than that of furosemide (7.16 $\mu\text{g/ml}$). So there was about six-fold increase in water solubility of furosemide in the complex. A significant increase in solubility was observed in the formulation with the higher percentage of lipid. This can be attributed to the surface activity of lipid.

Table 1: Solubility study of furosemide and its complex

Drug/formulation*	Solubility in aqueous layer ($\mu\text{g/ml}$)**	Solubility in n-octanol layer ($\mu\text{g/ml}$)**
Furosemide	7.16 ± 0.0003	22.0 ± 0.0003
F1	18.33 ± 0.0002	36.0 ± 0.0005
F2	27.16 ± 0.0002	41.83 ± 0.0005
F3	35.60 ± 0.0075	48.16 ± 0.0002
F4	41.33 ± 0.0002	56.33 ± 0.0004

*Furosemide-phosphatidylcholine complex with drug to phosphatidylcholine ratio 1:1 (F1); 1:2 (F2); 1:3 (F3) and 1:4 (F4); **All values represent mean \pm SD ($n = 3$); SD – Standard deviation

This reduction in surface tension increased the wetting of drug particles and as a result, increased the solubility. The water solubility of formulation F4 revealed the highest value. The complexes also increased in lipid solubility of furosemide and this may facilitate the permeation of the drug across the biomembranes and hence leading to higher bioavailability. Various previous studies of the phospholipid complexes of synthetic drugs (such as aspirin, diclofenac, and aceclofenac) and of the phytoconstituents (such as naringenin, emodin, gallic acid, baicalein, chrysophanol, rutin, quercetin etc.) support the data of the present study.^[10,11,14,16-22]

To check whether the changes in the furosemide crystal morphology correspond to a polymorphic transition and to study the solid state of furosemide-phospholipid complex, XRPD analysis was conducted. The XRD patterns of different complex formulations exhibited a series of broad and diffused peaks of low intensity similar to PC [Figure 2]. The reduction in intensity and disappearance of diffraction peaks of furosemide indicated the amorphous form of the drug in the phospholipid complex. However, there was no significant difference in the diffraction pattern of different

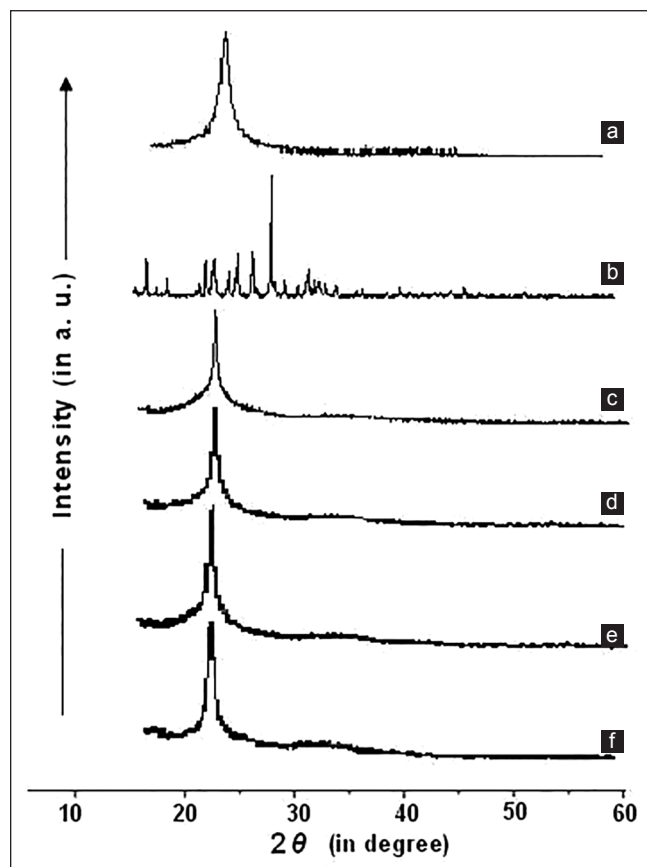


Figure 2: X-ray powder diffraction analysis spectra of phosphatidylcholine (PC) (a); furosemide (b); and furosemide:PC complex with drug to lipid ratio (c) 1:1; (d) 1:2; (e) 1:3 and (f) 1:4

formulations. This induction of amorphization in the complex might have been responsible for the increase in the aqueous solubility of the drug in the complex. These results were well-supported by the previous studies done with the phospholipid complexes of aspirin, diclofenac, aceclofenac, naringenin, emodin, gallic acid, baicalein, chrysophanol, rutin, quercetin etc.^[10-25]

The *in-vitro* drug release studies of all the formulations were performed in phosphate buffer (pH 6.8) at 277 nm. Furosemide-phospholipid complex showed better dissolution profile than the furosemide [Figure 3]. Due to poor aqueous solubility furosemide showed a total of just 69.14% cumulative percent drug released at the end of 10 h. On the other hand, the complex formulations showed drug release ranging from 85.81% to 100% at the end of 10 h in dissolution study. This may be due to the colloidal or molecular dispersion of drug and lipid. At lower concentration the amphiphiles exists in the monomer state. Further increase in concentration may lead to variety of structures that is micelles of spherical or hexagonal shape, which have the ability to solubilize drugs to a larger extent. This may be possible due to the increased wettability of drug by the carrier, particle size reduction, polymorphic transformation of drug crystals and/or chemical interactions between drug and carrier. Drug released from formulations F2, F3, and F4 was much higher than formulations F1. Unlike the other formulations, F2 achieved the highest cumulative drug release at the end of 10 h. The previous studies of phospholipid complexes of various synthetic drugs and phytoconstituents well-supported the results of the present dissolution study.^[10-25]

Conclusion

In this study, furosemide-phospholipid complexes (pharmacosomes) were prepared using the solvent- evaporation technique. The complexes with high drug

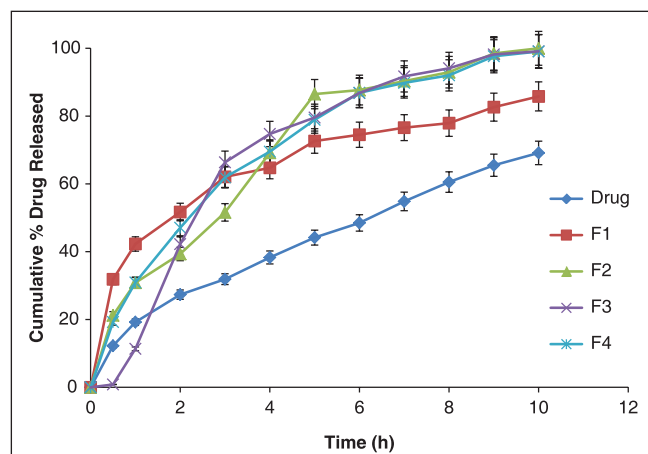


Figure 3: *In-vitro* dissolution study of furosemide and furosemide-phospholipid complexes

content were obtained. Solubility and *in-vitro* dissolution profile of furosemide were remarkably improved with the phospholipid complexes. The increase in solubility was also well supported by the XRPD study, which indicated the amorphization of drug in the complex. The increase in lipid solubility may facilitate the permeation of the drug across the biomembranes and hence leading to higher bioavailability.

It was concluded that the phospholipid-complexes of BCS Class IV drugs such as furosemide may improve the solubility, permeability and the dissolution profile of the drugs. The phospholipid complexes can be further explored for improved therapeutic applications.

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