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Drug Excipient Interaction Studies in Dopamine Loaded Lipid-Surfactant Based Drug Carrier

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In present study, chemical and physical interaction studies were carried out between the drug dopamine HCl with lipids (cholesterol, lecithin) and surfactants (Span 20, 40, 80) and Tween 80 excipents. These excipients were an important constituent in designing liposomal drug delivery. Fourier transform infrared spectroscopical studies (FTIR) of drug showed no deviation in characteristic drug absorption peaks in presence of formulating excipients. O-H (Hydroxyl) and N-H (amine group) stretching vibrations and C-H bending vibrations (methyl group) of drug was prominent in both drug and with excipients. This explicitly showed absence of interference in eliciting drug's pharmacodynamic properties. Differential scanning calorimetric study were also performed to determine the physical interaction among the drug, lipid and surfactant excipients. The liposomal formulations with and without surfactants showed complete absence of those pure lecithin endothermic peaks particularly its melting point peak. Other characteristic peaks of lecithin appears shifted to about 5-8 ºC which indicates fluidization during the formation of lipid bilayers. The lecithin and cholesterol peak of surfactant liposomes showed very less or broad endothermic peak which is an indicative that surfactant have interacted well with the lipids during the formation of vesicles. Conversion of crystalline dopamine drug to amorphous was also proved in the differential scanning calorimetric studies.

Key Words: Dopamine hydrochloride, FTIR, Differential scanning calorimetric, Liposomes, Surfactants.

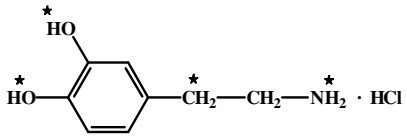
INTRODUCTION

The conventionally used lipid excipents to form liposomes were lecithin and cholesterol and these have greater potential in encapsulating both lipophilic and hydrophilic drug in their respective mileu. The main objective of this research work is to perform Fourier transform infrared spectroscopical studies (FTIR) and differential scanning calorimetric study (DSC) inorder to perdetermine any chemical and physical interactions between drug-dopamine with lipid carrier-lecithin, cholesterol and with surfactants. These liposomal carrier with modified surface activity using various non ionic surfactant has a greater potential for penetrating Blood brain barrier efficiently[1]. Inclusion of surfactants in to liposomes produces highly deformable vesicles[1,2] with transport process reported in few cases depending on the formulation. Surfactants produces flexibility favouring penetration and further increases membrane hydrophilicity and diffusability[3,4]. Cholesterol in liposomes enhances the rigidity of bilayer membrane above the phase transition temperature of the constituent phospholipids, resulting in increased elastic modulus, which inhibited curving of the bilayer[5,6]. Cholesterol is amphipathic and could be inserted in to the liposomal with its hydroxyl group oriented towards the aqueous surface and the aliphatic chain aligned parallel to the acyl chains in the center of the bilayer. When the bilayers are in fluid crystal state, above the phase transition temperature of phospholipids, the rigid steroid nucleus of cholesterol along the carbon side of phospholipids has the effect of reducing freedom of motion of these carbons[7]. This limited freedom of acyl chains causes the membranes to condense with a reduction in area in to a closer packing arrangement with decreased fluidity[8,9]. On inclusion of surfactant, this rigidity was decreased proving the increment of fluidity of liposomes. Rigidness of phospholipid carbon could be overcome by this inclusion of surfactants.

As theses surfactants can enhance the potential of a liposomal carrier for passing blood brain barrier due to high fluidity and flexibility, they along with lipid excipients should not chemically interact with the drug towards drug degradation. Thus a Fourier transform infrared spectra (FT-IR) and differential scanning calorimetric studies study (DSC) were performed and the results were reported and discussed favouring the intactness of the drug inside the carrier.

EXPERIMENTAL

Formulation of lipid carrier: Series of small unilamellar vesicles (SUV) liposomal formulation composed of lecithin (Lec) and cholesterol (Ch) were prepared for DSC studies by reverse phase evaporation technique using different non ionic surfactants such as span 20, span 40, span 80 and Tween 80 entrapping the drug dopamine hydrochloride (Dopamine HCl) were prepared with modification of Jain *et al.*[10] and Szoka *et al.*[11]. In brief, phosphotidyl choline, cholesterol at a molar ratio of 9:1were dissolved in diethyl ether. To this 5 mL of dopamine HCl drug (2 mg/mL) in phosphate buffer saline (PBS) 273 solution of pH 7.4 were added along with surfactant span 20, span 40, span 80 separately at molar concentration (Table-1) of 9:1:1 (Lec:Ch:surfactant). As Tween 80 does not favour vesicle formation, combination of span 80 and Tween 80 were tried at various molar concentration and finally the molar concentration of 9:1:2:1 (Lec:Ch:Span 80: Tween 80) were selected (as Tween 80 liposome was stable at this concentration with span 80). The optimized molar ratios of lipid and surfactant were selected, based on their stability and vesicle formation in PBS pH 7.4 followed by addition of vitamin E at 0.6 mol % (to prevent oxidative degradation of liposome). Then they were emulsified by high shear mixer using a homogenizer (Tenbroeck tissue grinder, Kontes Glass Co., Vineland, NJ) for 20 min at 5000 rpm at 50 ºC. The dispersion was then cooled to room temperature and the weight of the dispersion was adjusted before swelling. This led to the formation of a reverse (w/o type) emulsion which later forms a semi solid gel like consistency. The residual diethyl ether solvent is evaporated further by using vacuum evaporator (BUCHI EL 131 Rotavapor, Germany) under reduced pressure (260-400 mm Hg) at 60 ºC. The lipid gel so formed was collapsed and transformed in to a fluid by continuous and vigorous mechanical agitation using a vortex mixer (REAX top, Heidolph Instruments GmbH & Co., Schwabach, Germany). To this 5 mL of warm phosphate buffer saline (PBS) (pH 7.4) was added to hydrate the vesicles thus producing suspension of small unilamellar vesicle liposomes (SUV). The resulting liposomes were then sonicated using a microtip probe sonicator (Vibracell, Sonics and Materials, Inc, Danbury, CT) for 0.5 H at 40 % frequency to produce a complete homogeneous dispersion. The preparation was then flushed with nitrogen gas for 1 min to remove dissolved oxygen and to ensure complete removal of all traces of organic solvent. The weight of the liposomes was adjusted to the initial weight prior to sonication. The liposome dispersion samples were kept at 4 ºC and protected from light. Prior to use, they were filtered through Whatman filter paper [pore size: 0.2 µm (200 nm)] for sterilization and size reduction of liposomes. The formulation prepared using span 20, span 40, span 80 and combination of span 80-Tween 80 was coded as S20 and as S40, S80 and ST80, respectively for identification. The conventional drug containing liposomes (lecithin soya phosphotidyl choline:



Structure of dopamine HCl, *Functional group which shows characterisitc IR absorption peaks in FTIR spectrum

cholesterol) that do not have surfactants were prepared by the same method as described in above procedure entrapping the drug and was coded as NS.

Differential scanning calorimetry (DSC): Physical interaction studies between drug-dopamine HCl and excipients were studied by DSC. About 2 mg of each sample (cholesterol, lecithin, S20, S40, S80, ST 80 and NS) were heated separately in a sealed aluminum pan (capacity-4 µL) under nitrogen flow (30 mL/min) at a scanning rate of 5 ºC /min from 40 to 600 ºC using differential scanning calorimeter (Spectrum one, Perkin Elmer, Model Sd 10, Norwalk, CT). The empty aluminium pan was used as a reference. The heat flow as function of temperature was measured for each samples. Thermogram of the formulation was then analyzed in comparison with the spectrum of drug and the excipients to assess the interaction of drug with the excipients.

Fourier transform infrared spectroscopy (FTIR): In order to get evidence on the possible interaction of drug with excipients at molecular level, FTIR study by Disc method was carried[12]. Drug with excipients (lecithin, cholesterol, surfactant (span 20, 40, 80 and Tween 80)) in 1:1 molar ration were prepared in KBr disks and subjected to FTIR studies using Perkin-Elmer spectrum one, FTIR spectrophotometer (1600 series, Perkin-Elmer Inc, Norwalk, CT). The scanning range was from 4000-450 cm^{-1}. The FTIR spectrum of the formulation was then analyzed in comparison with the spectrum of standard dopamine HCl for evidence of any drug degradation[13,14].

TABLE-1
MOLAR CONCENTRATION OF LIPIDS AND SURFACTANT FOR DOPAMINE HYDROCHLORIDE FORMULATION

Formulation	Formulation code	Lipid composition (molar ratio)
Le:Ch:Span 20	S20	9:1:1
Le:Ch:Span 40	S40	9:1:1
Le:Ch:Span 80	S80	9:1:1
Le:Ch: Span 80: Tween 80	ST 80	9:1:2:1
Le:Ch	NS	9:1

RESULTS AND DISCUSSION

Differential scanning calorimetry: Differential scanning calorimetry (DSC) is a thermal analytical techniques which measures heat flow associated with transitions in materials as a function of temperature. DSC results provide useful information about the physical and chemical changes that involve endothermic or exothermic process or changes in heat capacity. If drug is dissolved in the aqueous layer, it would probably be in amorphous state. This was so and indicated by the shift from its pure crystalline drug (dry powder) shown by the endothermic and sharpness in peak. The melting endotherm for the drug-dopamine hydrochloride was found to be at 147.3 ºC which disappeared in the DSC of the formulation. Complete suppression of the drug peak in formulation suggested a homogeneous dissolution of the drug in the aqueous mileu. Moreover the crystallinity of the drug is lost once dissolved in the

aqueous mileu which exist in amorphous nature. Interaction of this amorphous drug with lipid and aqueous bilayer may be responsible for the loss of drugs melting point endotherm[15]. The thermal energy supplied during the scan was responsible for loosening of the crystal forces of drug and this results in amorphous finely dispersed drug in the aqueous core of the lipid formulation.

Melting point of the pure drug reduces or disappears due to small particle size (nm range), the high specific surface area and the presence of surfactant. Kelvin realized that small isolated particles would melt at a temperature lower than the melting temperature of bulk materials[16]. The depression of melting point is in proportion to the curvature 1/r (where r is the radius of the spherical particle) of a spherical nanoparticle according to Gibbs-Thomson equation[17].

Large endotherm over the temperature range of 50-100 ºC is due to water content of the formulation which gets loss during the heating.

Melting point of the lipid and the enthalpy was observed when the lipid formulated with drug. The lipid bilayer which has drug results in an increase in the number of defects in the lipid crystal lattice and hence causes a decrease in the melting point of the lipid in the final formulation[18].

Cholesterol endothermic peak was found at 147 ºC but absence or low broad peak in the pure liposomal and surfactant modified formulation signifies complete interaction of cholesterol with lecithin while forming lipid bilayers. Differential scanning calorimetry of lecithin showed a small broad endothermic peak at 40.3 ºC which indicates its change from crystalline form to Amorphous form. Endothermic peak at 92.6 ºC indicates its change from its wax like phase to viscous isotropic phase and a sharp peak at 233.2 ºC indicates its melting point[18]. In the liposomal formulation with and without surfactant showed a complete absence of those pure lecithin endothermic peaks particularly its melting point peak. Other characteristic peaks of lecithin appears shifted to *ca.* 5-8 ºC which indicates fluidization during the formation of lipid bilayers. Presence of surfactants and drug in the vesicle further broadened/shifted the peak of the lipids. Ability for the phospholipid-based surfactants to stabilize the particles depends upon the temperature of the medium within the receiving vessel. Most phospholipid vesicles exhibit a transition temperature from a rigid gel-like state to a fluid liquid-crystalline state. As temperature increases, the phospholipid chains within the vesicles go from a very rigid, ordered state, to one that is more flexible and can allow diffusion across the bilayer. Rigidity of the phospholipid tails and hence the vesicles, could affect surfactant's ability to rearrange in order to stabilize the carrier. The lecithin and cholesterol peak of surfactant liposomes showed very less or broad endothermic peak which is an indicative that surfactant have interacted well with the lipids during the formation of vesicles.

Fourier transform infrared spectroscopy (FTIR): The spectrum showed FTIR spectra of drug-dopamine HCl and the FTIR spectra of the drug with lipid and surfactant excipents. Pure drug displays an absorption peak (Table-2) characteristic for the O-H stretching vibration at the range of 3436 cm^{-1} which appeared around 3439 cm^{-1} in drug with excipient. A band with main strong peak at the range of 2925 cm^{-1} is an indicative for the N=H group stretching of amine in the drug which was also present in drug with excipient at 2926 cm^{-1}. The presence of amine (NH_3) group in the drug was evidently proved from the drug with excipient absorption peaks at 1645 cm^{-1}. C-H bending of methyl (CH_3) group of the drug showed an absorption peak in 843-840 cm^{-1} range both in pure drug and drug with excipients FTIR spectra. These results suggested that absence of drug degradation or drug- excipient molecular interaction and this favours the long stability of drug in the lipid formulation.

TABLE-2
FUNCTIONAL GROUP WITH THEIR RESPECTIVE ABSORPTION PEAKS FOR DRUG-DOPAMINE HCl

Functional group	Absorption peak	Status*
3410-3440	O–H stretching	Present
3015-3440	N=H stretching	Present
1640-1645	NH_3 group	Present
840-850	C-H bending	Present

*Presence of drug's respective absorption peak in FTIR spectrum of drug-excipient mixture.

Conclusion

This study reveals that the drug-dopamine hydrochloride showed compatibility with the conventionally used lipid excipients and surfactants for liposome carrier formulation. The DSC study evidently proved that the drug had lodged in to the aqueous mileu and intercalated well with the lipid excipients. Therefore this lipid based drug delivery if formulated with surfactants would be an ideal, safe and efficient carrier for the drug dopamine and for further research in neuronal brain targeting therapy.

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