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FABRICATION OF TWEEN 80 LIGAND COATED CHOLESTEROL-SOYA LECITHIN BILAYERED NANOLIPOSOMES FOR IMPROVED RELEASE OF MEROPENEM AGAINST ARTIFICIAL BLOOD BRAIN BARRIER

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Keywords:

Soya lecithin, Cholesterol, Meropenem, Blood-brain barrier, FT-IR

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ABSTRACT: The blood-brain barrier is the most challenging physiological obstacle for drug moieties for reaching the central nervous system. Meropenem is a drug used against meningitis, however crossing BBB is also a challenging realm of problem for the same. Therefore attempt has been undertaken to strategically design Meropenem loaded cholesterol-soya lecithin nanoliposomes (MCSLN). MCSLN was prepared by nano-emulsification technique with tween 80 as its surface ligand. Molecular docking studies revealed that tween 80 interacts with apolipoprotein E through π-π interactions and hydrogen bonding, which might be its mechanism for transmembrane permeation across BBB. After characterization, *in-vitro* release study was carried out to evaluate cross-walking efficiency of liposomal drug delivery system through the simulated blood-brain barrier. Entrapment efficiency estimation showed that about 80% of the drug has been successfully entrapped within the liposome. Dynamic light scattering study exhibited bimodal distribution of particle with size ranges 70-75 nm and 300-350 nm. Zeta potential reveals good stability of the formulation. FTIR studies reveal that Meropenem has been successfully entrapped within the formulation. On a normalized scale, *in-vitro* release displays about five times improvement of the drug from liposome compared to free drug. These results suggested that ligand coated MCSLN may permeate BBB successfully and deliver the drug within the intrathecal fluid. The study, if successful, could potentiate a device through which molecules could reach brain cerebrospinal fluid to exert its action irrespective of molecular chemistry, size, shape and diameter.

INTRODUCTION: The penetration of suitable bacteria across the "blood-brain barrier" develops several cerebrospinal fluids (CSF) accorded infections, one of them being meningitis.

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It results in inflammation of the leptomeninges and subarachnoid space. Acute purulent (pyogenic) meningitis is caused by the bacterial origin and some of the common pathogens causing meningitis are *Escherichia coli*, Group-B *Streptococcus, Listeria monocytogenes, Streptococcus pneumoniae, Haemophilus influenza, Neisseria meningitides* [1].

Meningitis due to *Pseudomonas aeruginosa* is uncommon and it generally occurs in hospital-onset cases. It is also related to neurosurgery, with or

without an extraventricular drain (EVD) or shunt insertion. However, *P. aeruginosa* shows resistance, which limits the treatment options [2]. Out of over 7000 drugs in the comprehensive medicinal chemistry (CMC) database, only 5% of all drugs treat the CNS, and these CNS active drugs only treat depression, schizophrenia, and insomnia [3]. Thus drug delivery to brain is always very challenging. These challenges are classified into recently; many ligands are efficiently being used to deliver drug into the brain like glutathione [4], scopine [5], the OX26 [6] or anti-transferrin [6, 7] and monoclonal antibody 2C5 [8]. The use of these enhancers has been found to enhance the drug uptake by 14.25 fold. Bio-active agents can be encapsulated and delivered by a new technology known as nanoliposomes (submicron bilayer lipid vesicle). Because of their biocompatibility and biodegradability, these nanoliposomes have gained potential applications in a vast range of fields for delivery of substances and mostly used to achieve desired delivery of drug (bioactive agents) to overcome the limitation in drug delivery in certain cases [9].

Thus liposomes could be extensively used as drug carriers [10, 11]. Because of their closed vesicles, which are composed of an internal aqueous core and an external lipophilic bilayer of phospholipids molecules form an excellent carrier. Amongst an array of ligands mentioned above, ligands are still in search of efficient transportation of drugs across the blood-brain barrier. For an easy commercialization view, easily accessible ligands within economic purview are always under scientific interest. In pursuit, tween 80 has already been reported to augment transmembrane transport of biological molecules [12, 13, 14]. As a probabilistic mechanism of action, tween 80 has been acknowledged to bind with apolipoproteins E and B (apoE and apoB), which are one of the carrier proteins present in blood-brain barrier (BBB). In this study, we have undertaken tween 80 to coat the polymeric vesicle in its outer layer for facilitating the transport of Meropenem across the BBB.

In this study, we have undertaken the fabrication of solid-lipid nanoliposomes with some modifiers such as tween 80 to develop value-added formulation that can efficiently cross the blood-brain barrier. We have chosen Meropenem as the drug of choice against meningitis. Our study deals with the work of enhanced improvement of penetration of drug across the artificial cerebrospinal fluid to give valuable information on drugs meant to treat infections residing in CSF.

MATERIALS AND METHODS:

Reagents and Chemicals: Food grade Soya lecithin **Fig. 1A** was procured from the local food industry. Cholesterol **Fig. 1B** was purchased from HiMedia Labs, Mumbai, India. Meropenem **Fig. 1C** was generously gifted by Mission Hospital, Durgapur. Evion 400 and tween 80 have been procured from Merck Limited, Goa, India; Evion 400 being the source of vitamin E **Fig. 1D**.

All other chemicals and reagents were procured and used from the analytical laboratory of Dr. B.C. Roy College of Allied Health Health Sciences, Durgapur, WB, India. Centrifuge and thermostatic magnetic stirrer (Remi, Maharastra, India) have been used for this study. For liposome preparation, standard hypodermal syringe (Dispovan, Hindustan Syringes & Medical Devices Ltd.) with 22 gauge needle has been procured. All spectroscopic observations have been recorded in Shimadzu UV-1700 double beam spectrophotometer (Shimadzu, Japan).

Experimental Methods:

Density Measurement: The density of the soya lecithin under investigation was measured by aliquoting a known volume of soya lecithin within a specific gravity bottle and weighing it onto analytical balance (Metler Toledo, Columbus, Ohio, USA). The determinations were made in triplicate and the resultant output was accepted as average ± standard deviation.

Nanoliposome Preparation: Self-assembled nanoliposomes of lecithin and cholesterol were prepared *via* nano - emulsification technique. Soya phosphatidylcholine (Soya Lecithin): cholesterol: vitamin E (100: 25: 4) was used as the fabricating polymers and was dissolved in chloroform: methanol (9:1). The solvents were then evaporated by gentle heating under reduced pressure. The polymer mixture was then re-dispersed with minimum solvent volume followed by dropwise addition of Meropenem solution (aqueous) with controlled stirring at 50~60 °C. The primary

emulsion was concentrated in a rotary evaporator, and tween 80 was introduced into this intermittently *via* aqueous solution under controlled stirring. The resulting emulsion was constantly agitated for about 20 min at 50 ºC until it formed a milky white liquid. Sucrose has been added as cryoprotectant with cryoprotectant: phospholipid mass ratio as 2:1. Finally the resulting formulation was magnetically stirred for 3-4 h followed by sonication for 15 min. This resulted in the formation of opaque liposomal formulation, which was finally lyophilized to obtain dried complex bi-layered nanoliposomes.

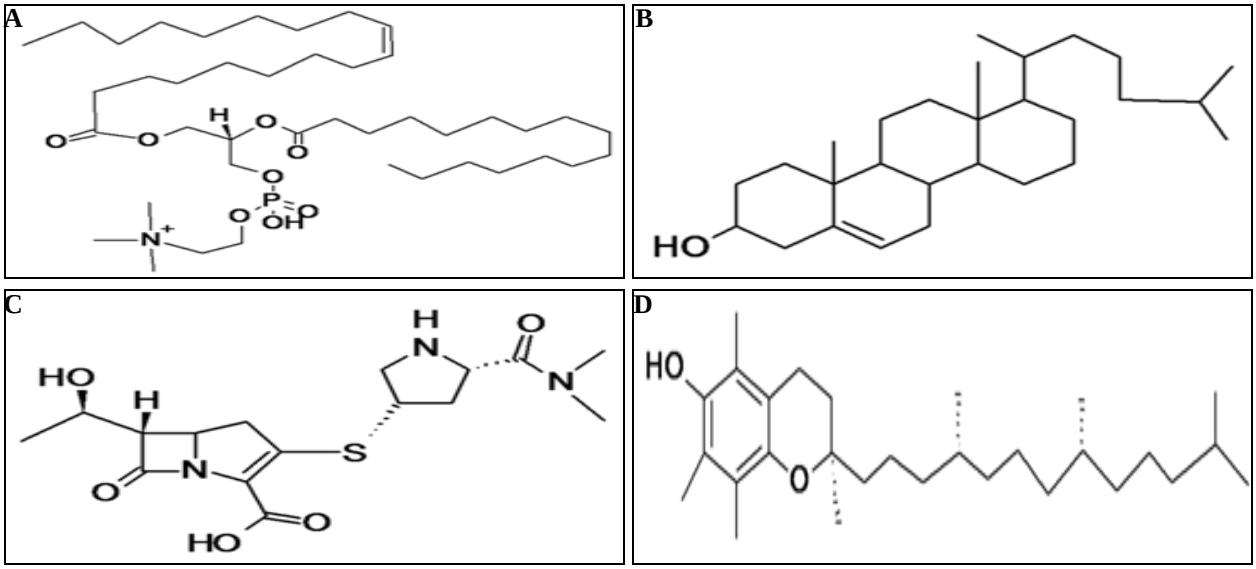

FIG. 1: CHEMICAL STRUCTURES OF COMPOUNDS. A) Soya phosphatidyl lecithin B) Cholesterol C) Meropenem D) vitamin E (Tocopherol)

Evaluation of Entrapment Efficiency: The entrapment efficiency or the encapsulating efficiency of Meropenem in lecithin cholesterol nanoliposomes was determined by removing the loosely bound/free drug outside the lecithin cholesterol nanoliposome by taking a known quantity of the prepared formulation and washing it with double distilled water.

In the process, the loosely bound or free drug present on the extraneous surface of the nanoliposomes dissolved in water which was further collected as a supernatant layer after centrifugation at 10000 rpm, 4 ºC for 10 min. The unbound drug in the supernatant was quantified by measuring the absorbance at 297 nm, the extinction co-efficient being 10940 M^{-1} cm^{-1} for Meropenem. The entrapment efficiency of the drug inside liposomes was calculated by the following formula:

Entrapment efficiency = Total amount of drug is taken – Free drug outside liposome / Total amount of drug taken × 100

Characterization: The Meropenem - liposome bound complex was characterized by Dynamic Light Scattering (DLS) and Field Emission Scanning Electron Microscopy (FESEM) for its size, shape, and morphology. The complex was further evaluated for any real interaction between the drug and the bio-polymers by FT-IR spectroscopy. Zeta potential measurement was taken to assess the stability of the liposomes. Compound microscopic observations were performed in order to assess the multi-layered anatomy of the fabricated liposome.

Microscopic Image of Prepared Nanoliposomes: A small amount of the prepared nanoliposomes was completely redispersed in water. Using a glass slide and a drop of the diluted nanoliposome solution was put on the clean glass slide and covered it carefully with a coverslip. The liposomes were further observed under the microscope.

Fourier Transform Infrared Spectroscopy (FT-IR) Study: FT-IR spectra of individual components of the formulation, as well as the whole liposome, were acquired by Shimadzu-Prestige-21 (Shimadzu, Japan) FT-IR spectrophotometer using potassium bromide (KBr) pellets. KBr pellets were set up by tenderly

blending the components (component: KBr, 1:100). The obtained spectrum was scanned from 4000 to 400 cm^{-1}.

Dynamic Light Scattering (DLS): The hydro-dynamic diametric estimate (z-normal) and the polydispersity index of the Meropenem-solid lipid liposomes were estimated by Photon Correlation Spectroscopy (PCS) utilizing a Dynamic Light Scattering System (Zetasizer Nano ZS, Malvern Instrument, Malvern, UK)

Zeta Potential: New suspensions of solid-lipid liposomes were set up by suspending it in water with tween 80 as a surfactant. The suspensions were additionally re-diluted with water as required and examined at 25 ºC against a 4mw He-Ne laser bar, 633 nm, and a back scrambling edge of 173°. Zeta estimation depended on the molecular electro-kinetic performance in fluid medium. The estimations of molecule size and zeta potential were recorded in triplicate.

Differential Scanning Calorimetry (DSC): The thermal stability and interaction analysis were carried out by Differential Scanning Calorimetry (DSC). The pure cholesterol, soya lecithin, Meropenem and finally the formulation were subjected to heat treatment under inert gas atmosphere within a temperature bandwidth of 40 ºC to 400 ºC. The heating ramp was set as 10 ºC/min and the samples were heated on platinum crucibles inside Pyris Diamond TG/DTA using alpha-alumina powder as reference standard.

Scanning Electron Microscopy (SEM): The morphology of fabricated nanoliposomes with dried particle size was observed under JEOL-6360 LV SEM assembly after coating onto a glass slide, dehydration and subsequent gold sputtering before putting into cryogenic liquid nitrogenous atmosphere inside the vacuum. The electron bombardment was carried out under 17 KV potential difference and the backscattered electrons were trapped onto the detector for signal generation.

***In-vitro* Release Study:** The *in-vitro* release study of Meropenem from the formulation was performed using simulated (artificial) blood-brain barrier prepared by treated cellophane membrane and artificial CSF as reported earlier [12].

Preparation of Artificial CSF: The artificial CSF was prepared following the protocol as described earlier [15]. The sterile water autoclaved at 121 ºC and 15 psi pressure were used as vehicle to abort any potential biogenic contamination in the study. The artificial CSF mixture contained NaCl 8.66 g/L, 0.224 KCl g/L, $MgCl_2$ 0.7637 g/L, $CaCl_2$ 0.1556 g/L, Na_2HPO_4 0.1134 g/L and NaH_2PO_4 0.0235 g/L. First, NaCl, KCl, $MgCl_2$, and $CaCl_2$ were mixed in 500 ml of sterile water (solution A); while $Na_2HPO_4$and NaH_2PO_4 were taken in another conical flask in the same volume of water (solution B). Finally, both the solutions were mixed homogeneously using a magnetic stirrer at 100 rpm to make a final volume up to 1000 ml.

Cellophane Membrane Treatment-Simulation of Blood-Brain Barrier: The artificial blood-brain barrier was prepared as described by [16]. Briefly, the cellophane membrane no. 300 was immersed into distilled water and kept overnight for hydration. After that the hydrated membrane was treated with increasing concentration of zinc sulfate (9, 10 and 11 % w/v), each gradient was treated for at least 1 h. Afterward, the preliminarily treated cellophane membrane was re-treated with 10% and 11% (w/v) zinc sulfate respectively for 1 h under room temperature and thence washed thoroughly with distilled water to remove excess zinc sulfate. This treated membrane artificially simulates our biological semi-permeable barrier.

***In-vitro* Release Study:** *In-vitro* release study was performed simulating the passive diffusion through the artificially modified cellophane membrane simulating the blood-brain barrier. Dialysis bags were fabricated with the treated cellophane membrane and a known weight of Meropenem loaded liposomal formulation (reconstituted in water with stirring and sonication) was taken inside the dialysis bag. The resulting closed system was immersed in a beaker with 200 ml of artificial CSF and stirred with magnetic bead at 100 rpm and 37 ºC temperatures. A parallel control system was run with similar model, the difference being that equal weight of raw Meropenem was taken inside dialysis bag instead of the formulation. Sink condition was maintained throughout the study and aliquots were withdrawn from both the systems while fresh CSF was used to replenish the withdrawn fluid in the system. Finally, the release

profile of drug was represented as cumulative % release *vs.* time (min) where the concentration of Meropenem was analyzed at 297 nm.

Release Kinetics of Drug and Model Fitting: The release profile of both the systems was modeled with conventional kinetics patterns of molecular release from stable polymeric matrix [17]. To note a few, The models such as Korsmeyer-Peppas [18] model (up to 60% of cumulative drug release), Weibull model, Hixson-Crowell, Higuchi, Baker-Lonsdale, Michaelis-Menten and Hill equation have been used for the study of drug release profile. Order of release kinetics have also been determined. The kinetics and modeling study has been performed by KinetDS [19]. The dissolution efficiency (DE) a calculated by the following formula [20].

$$\%DE = \left(\frac{\int_0^t y.dt}{y_{100}.t} \right) 100$$

Molecular Docking: To study molecular interactions between apolipoprotein and tween 80, molecular docking studies were performed. 2D structure of tween 80 was downloaded from the PubChem database and stabilized in its minimum energy conformation (MEM) by Chem3D (ChembridgeSoft Corporation, USA).

Apolipoprotein E (apoE) was procured from protein data bank (PDB, PDB id: 1NFN) and tailored in Discovery Studio Visualizer (ver 3.5) by removing its endogenous ligand and water molecules. Allosteric docking was performed in Autodcok Vina allowing whole apoE as the receptor with the following specifications center_x = 25.423, center_y = 38.073 and center_z = 56.117. Nine conformers of the ligand were generated by docking, out of which the best one having maximum binding energy was chosen for further studies. The polar, apolar, hydrogen bond and van der Waals interactions were mapped in Discovery Studio Visualizer (ver 3.5) while the distance of hydrogen bonding between donor (apoE residue) and acceptor (Tween 80 heteroatom) was mapped in Chimera. A length of 5 Å was set as the cutoff between the donor and acceptor hydrogen bond distance.

RESULTS:

Density Measurement: The density of soya lecithin was found to be 1.2 ± 0.4 gm/ml at room temperature (29 ± 0.01 ºC).

Evaluation of Entrapment Efficiency: Since total amount of initial drug taken was 2600 mg and total unbound drug was estimated as 506 mg hence entrapment efficiency as per the above formula was found as 80.538%

Characterization: The formulation was characterized by various microscopic, spectroscopic or thermal methods as mentioned in materials and methods section.

Anatomy by Compound Microscopy: Compound microscopic introspection of isolated droplets of the emulsion revealed that bilayered liposomes have been formed with the lipid layer as the outer core. Two distinct lipid layers are visible both in 10x and 40x magnifications (**Fig. 2A**, **2B,** respectively) where outer layer is thicker than the inner layer. The liposomes were revealed as spherical globules.

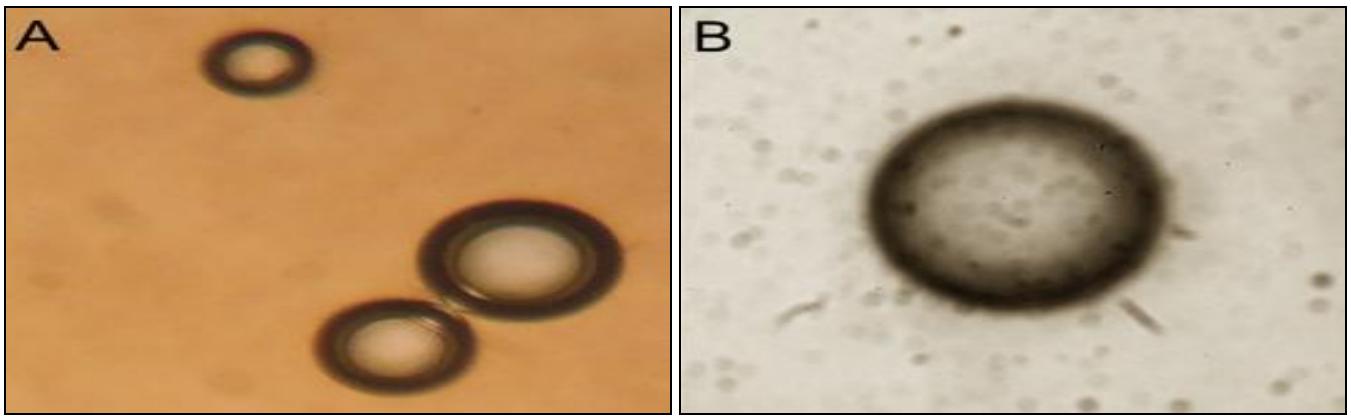

FIG. 2: MICROSCOPIC IMAGES OF LIPOSOME. A) 10x magnification, B) 40x magnification

FT-IR Study: The FT-IR study revealed lot of selected peaks appeared on the formulation spectrogram while the others from the ingredients had been suppressed. For example, sharp –OH peak is visible at 3422 $cm^{-1,}$ which is similar to the characteristic –OH peak of cholesterol. The shape, morphology, and size of the peak indicate that the –OH of cholesterol has remained non-bonded and prominent in the formulation which also suggests that cholesterol has remained on the outermost core of the formulation. The peak at 2925 cm^{-1} provides the suggestion of hydrocarbon tail which is present in all components of the formulation.

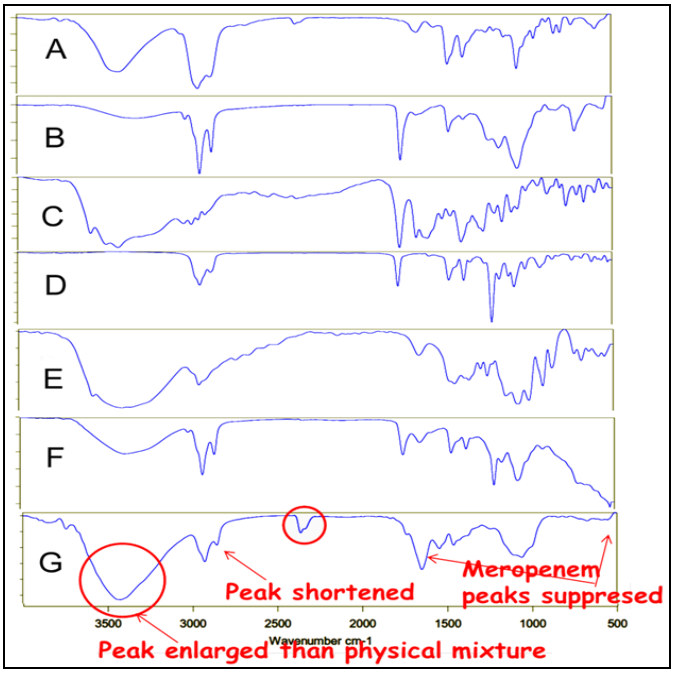

FIG. 3: FT-IR IMAGES OF INGREDIENTS AND FORMULATION. A) Cholesterol; B) Soya Lecithin; C) Meropenem; D) Vitamin E; E) Sucrose; F) Physical mixture; G) Formulation

However, this tail is smaller in height or width than that of soya lecithin which suggests that soya lecithin is in the inner layer of the bilayered liposome; thus the signal is trapped in the FT-IR spectrum. Again, most of the peaks of Meropenem have been suppressed in formulation indicating that Meropenem has been encapsulated inside the formulation core, which is the hydrophilic cavity of the liposome. Thus the FT-IR studies suggest that the anatomy of the liposome is as cholesterol-soya lecithin-meropenem (CSM) from outside to inside respectively. The FT-IR spectrum of all the parent components, as well as the formulation, have been summarized in **Fig. 3A-3G**.

DLS Study and Zeta Potential: The DLS study of the formulation revealed that CSM liposomes have bimodal distribution; one group having hydrodynamic particle size 70-75 nm while the other group having particle diameter 300-350 nm **Fig. 4A**. The polydispersity index (PDI) of the formulation was found to be 0.524 suggesting a sub-heterogeneous population of CSM nanoliposomes. Zeta potential of CSM liposomes in water revealed a strong peak at -44.4 mV **Fig. 4B** which indicates sufficient inter-particular repulsive force in between the electric double layers of colloidal liposomal interfaces present inside solution. Thus we propose that the formulation has considerable stability when suspended or emulsified in aqueous media.

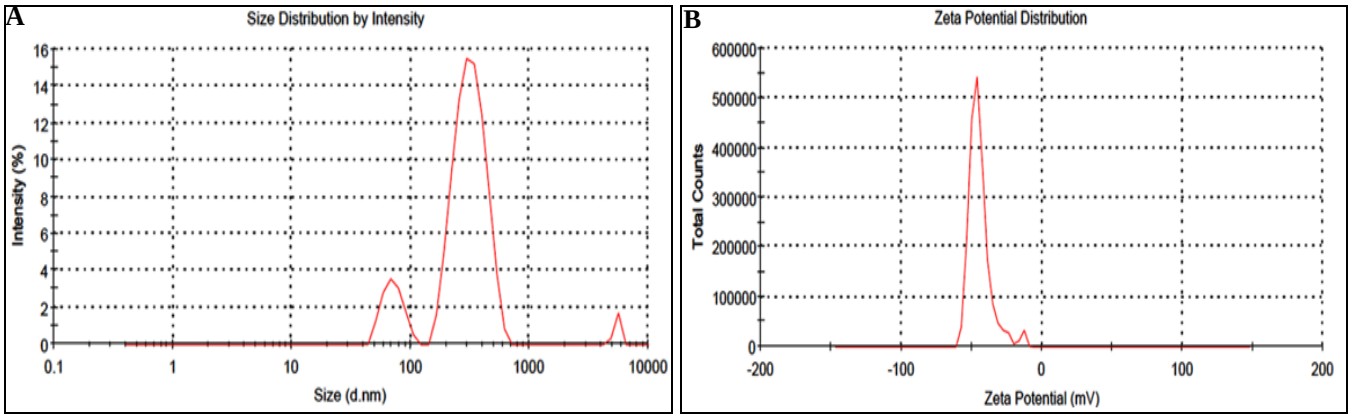

FIG. 4: DLS AND ZETA POTENTIAL OF CHOLESTEROL-SOYA LECITHIN-MEROPENEM LIPOSOMES. A) DLS B) Zeta potential

DSC Studies: DSC studies revealed that thermograms of the formulation **Fig. 5D** has been changed considerably especially the phase transition temperature (in this study the melting endotherm). First of all, the melting endotherm of the formulation has been found at 82.80ºC **Fig. 5D**

while the same for cholesterol, soya lecithin, and Meropenem has been revealed at 148.97 ºC **Fig. 5A**, 189.43 ºC **Fig. 5B** and 109.10 ºC **Fig. 5C** respectively. Thus, it can be proposed that the core-chemical bonding of the formulation have been changed from the parent components resulting in change of heat of fusion of the former. Thus, at a lower enthalpy of exogenous heat, the formulation melts while the other components remain at the same phase as previous.

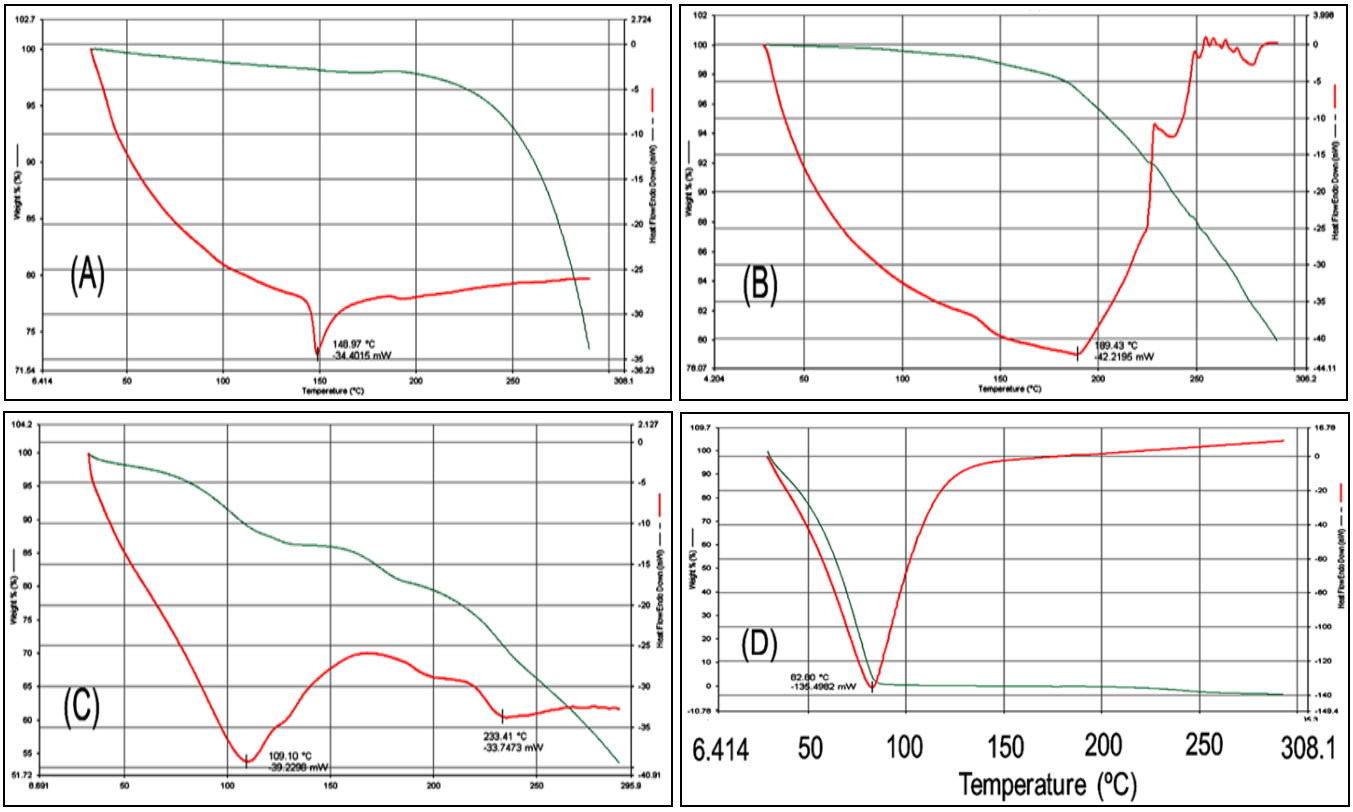

FIG. 5: DSC THEROGRAMS OF INGREDIENTS AND CHOLESTEROL-SOYA LECITHIN-MEROPENEM LIPOSOMES. A) Cholesterol; B) Soya Lecithin; C) Meropenem D) Formulation

Second, the peak morphology of the formulation reveals sharp and precise peak which clearly indicates crystalline bonding interactions have occurred between the drug of choice as well the encapsulating lipid layers. Except for Meropenem, while all other mother ingredients exhibited broad and obtuse peaks, the quick, sharp endotherm of the formulation suggested stable, however strong dipolar bonding inside the liposomal core where Meropenem has been entrapped ensheathed by lipid bilayers.

SEM Study: The scanning electron microscopy of the liposomes revealed a spherical morphology of the same with a smooth surface, which is a characteristic of liposomes **Fig. 6**.

***In-vitro* Dissolution:** The *in-vitro* dissolution of liposomal formulation with the control solitary meropenem revealed that the release of the liposomal drug in the sink compartment has been five times higher than the control one **Fig. 7**. The non-kinetic model fitting of the drug's release displayed that the drug's release could be best explained by Weibull model with a lag time (T) where the model equation has been $M = M_0 (1 - e^{(t-T/a)b})$ [16]. M is the amount of drug released at time t, M_0 is the amount of drug present at initial time (t_0), constant a depends on time of release (t) while constant b depends on the shape factor of the dissolution curve. The model fitting of the drug revealed regression coefficient (r^2) as 0.999 and hence considered as best model fitting for this equation. The *in-vitro* dissolution efficiency (DE) is a parameter that proposes the amount of a substance dissolved at a particular time (t) concerning the maximum dissolution of the same drug within a particular period. Hence increase in dissolution efficiency suggests improved release of drug from the reservoir within the same period. Herein, DE of Meropenem in the sink compartment

from the nanoliposome has been 0.3845 whereas the same from the raw Meropenem has been 0.0731. Thus the DE from the nanoliposome has been found five times higher compared to the solitary drug in the sink condition.

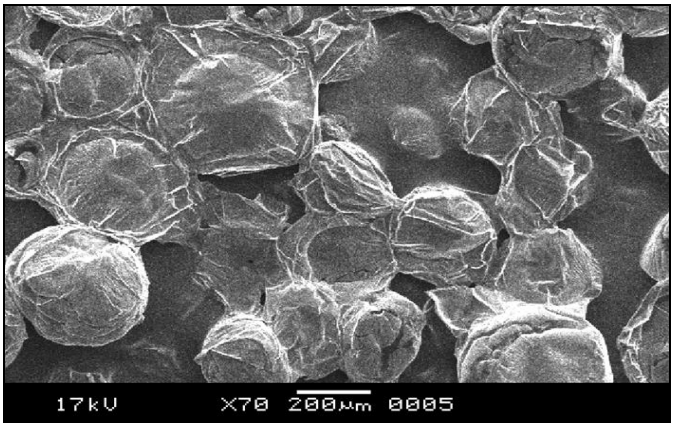

FIG. 6: SEM IMAGE OF FORMULATION

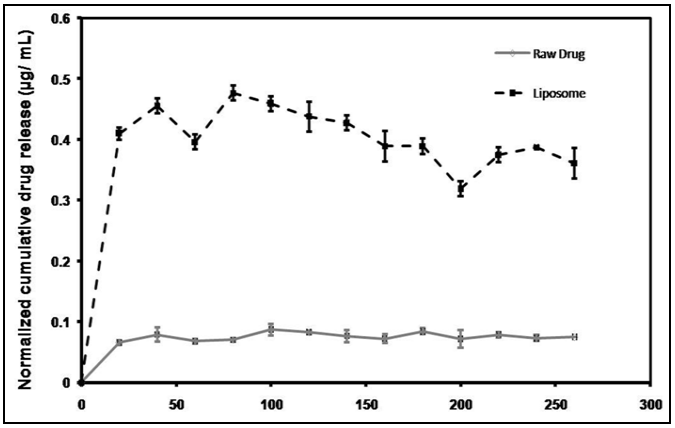

FIG. 7: NORMALIZED *IN-VITRO* RELEASE OF DRUG FROM THE FORMULATION. Yellow line-from liposome; White line- from the raw drug, each data has been represented as a mean ± standard deviation of three technical replicates (n=3).

***In-silico* Docking:** The docking studies revealed the binding site of tween 80 engulfed by the amino acids Arg32, Asp35, Trp39, Gln46, Glu59, Leu63, Thr42 and Ser44 in Apo E **Fig. 8**. The binding interactions revealed all kinds of interactions such as polar, apolar, hydrogen bonding and weak π-π or sigma bond interactions **Fig. 8**.

Ser44, Trp39, Asp35, and Thr42 revealed polar contacts while other amino acids mentioned above revealed apolar interactions with tween 80. The hydrogen bond interactions lie between OH of the ligand and Trp39 whereas the π-π interactions yield in between the aromatic center of Trp39 and tween 80. Furthermore, we assumed a hydrogen bond distance lesser than 5 Å means closer vicinity with a plausible approximation of finding stable H-bonds between protein and ligand. Following this cutoff, we found three H-bonds between tween 80 and apoE as 1.06, 3.48 and 4.06 angstroms **Fig. 9**. However, all the H-bonds were generated in between Trp39 and tween 80 heteroatoms (mainly oxygen), suggesting Trp39 plays the most crucial roles in binding the tween 80 with apoE.

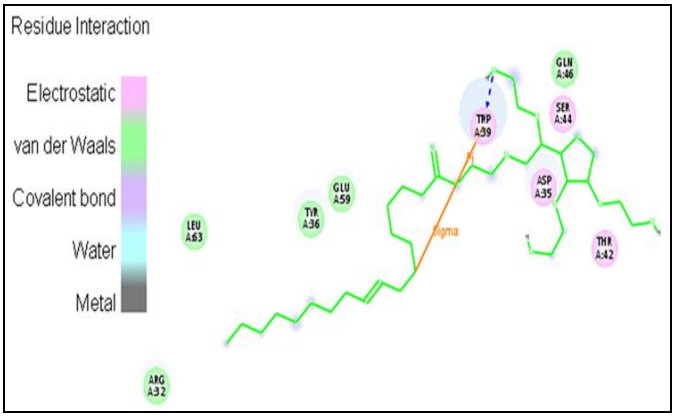

FIG. 8: DOCKED IMAGE, POLAR AND APOLAR CONTACTS OF TWEEN 80 WITH APOLIPOPROTEIN E

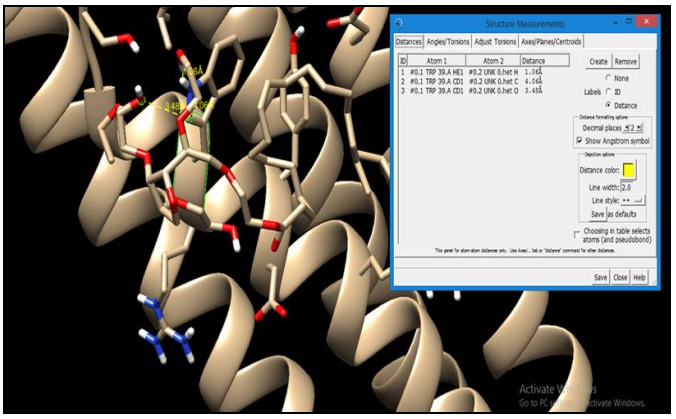

FIG. 9: HYDROGEN BONDING INTERACTIONS IN BETWEEN TWEEN 80 AND APOE. The hydrogen bonds having distances less than 5 angstroms have been taken as principle contacts.

DISCUSSION: Zahra *et al.*, 2016 reported [21] that solid lipid nanoliposomes loaded with meropenem lowers its minimum inhibitory concentration (MIC) more than four times while targeted against clinical isolates of *Pseudomas aeruginosa*. Thus, it has been promising approach in the context of meningitis treatment since *Pseudomas aeruginosa* has been one pathogen responsible for meningitis. However, one critical bottleneck is still under question if these Meropenem loaded nanoparticles could cross the blood-brain barrier to reach CSF and release the drug over there. In this study we have undertaken this problem to elucidate the

answer and thus add one more step towards better treatment of meningitis. Thus, we have fabricated soya lecithin-cholesterol nanoliposomes and tested its competence in crossing artificial biological membrane and thus release the drug in CSF.

We have undertaken soya lecithin and cholesterol because the lipophilic outer core of liposome will facilitate the diffusion of liposome through the lipophilic blood-brain barrier. Sucrose is added as cryoprotectant [22] since, liposomes needed to be lyophilized to make it stable and prevent it from drug leach. Vitamin E has been added as antioxidant which is another strategy for liposome stabilization because atmospheric oxygen might rancidify the unsaturated phospholipid present in the outer core of liposome. Tween 80 addition has been performed because according to earlier reports [23], for larger size of liposomes, tween 80 facilitates the permeation of the formulation by disrupting the pores of the blood-brain barrier.

Not only this, but tween 80 also facilitates the molecular cross walking across the BBB by binding with carrier apolipoprotein E [apoE, 12-14]. Thus, in our study, we tried to dissect the binding pattern of tween 80-apoE by molecular docking studies. Herein, we found that various kinds of bondings involving polar, apolar and hydrogen bondings are involved in tween 80-apoE binding where Tryptophan 39 plays the most crucial role for conjugating tween 80 with apoE. With all these modifications, when we charged our liposomes through artificial biological membrane and artificial CSF, the release of the drug shoot considerably higher than that of solitary drugs. Hence, we claim that our solid-lipid Meropenem nanoliposomes could be a potential approach to cross the blood-brain barrier.

CONCLUSION: In this study, solid-lipid bilayered nanoliposomes have been strategically designed to improve drug permeation through blood-brain barrier. FT-IR, DSC studies have revealed that Meropenem has been successfully loaded inside the liposomes while DLS studies revealed that a cluster of liposomal particles has been in nm range (70-75 nm). Tween 80 guided *in-vitro* release through the artificially simulated blood-brain barrier and simulated CSF, showed that the drug penetrated to CSF more than five times higher than the solitary crude Meropenem. Hence, it can be proposed that this drug delivery model could be successfully modified further to achieve a clinically valid brain targeting device that can not only deliver Meropenem but other drugs also in CSF in the required condition.

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CONFLICTS OF INTEREST: There is no conflicts of interest for this study.

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