Designing of a phytosome dosage form with *Tecomella undulata* as a novel drug delivery for better utilization

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Abstract: In recent past scientists are moving for the novel herbal medicines for treatment of almost all diseases as they have no or lesser adverse effects as compared with modern allopathic medicines. The potency of any dosage form is depending on effective drug delivery level of any therapeutically active drug molecule. Phytosome is a novel approach to drug delivery system that produce more absorption and utilization than conventional herbal extracts and shows enhanced bioavailability. The present investigation is to prepare and evaluate phytosomes of *Tecomella undulata* using aqueous extract of its stem bark and lecithin. Solvent evaporation method was used for preparation of phytosomes. Phytosomes were studied for their evaluation parameters such as morphology, release character, drug entrapment efficiency, size of particles and charge on surface. Phytosomes were successfully developed and having unilemellar vesicles, good entrapment efficiency and drug release in nano sizes (up to 90%) and average particle size 153.2 nm with -23.7 mv charge on their surface. The results showed that the phytosomes can improve the bioavailability without resorting any pharmacological adjuvant or structural modification of the ingredients.

Keywords: Phytosomes, *Tecomella undulata*, phospholipids

INTRODUCTION

Novel plant formulations like phytosomes, liposomes, nanoparticles, polymeric nanoparticles, nanoemulsions, transferosomes, ethosomes and microspherehas prepared and reported bioactive by different scientists (Ajazuddin and Saraf, 2010). Biologically active constituents of plants are mostly water soluble or polar in nature (i.e. Flavonoids, terpenoids, glycosides, tannins etc). Water soluble constituents are having their size greater than lipid soluble constituents. Absorption of water soluble constituents is poor because of their large size. Passive diffusion is not possible due to their size limitation and they don't cross the lipid rich cell membrane, resulting low bioavailability (Manach *et al*., 2004). Isolation and purification of chemical constituents of any plant extract can cause partial or complete reduction of bioactivity and it can cause loss of synergy of natural constituents (Bala *et al*., 2011). It has generally been observed that chemical complexity is necessary for bioavailability of active constituents of crude or purified extract. Gastric environment of the body may be reduce or loss of activity of some active constituents after taken orally. Due to this reason the extract shows low bioavailability and their clinical utility also is doubtful (Pandey, 2010).

Phytosomes is novel technique emerged in 1989 to merger of aqueous soluble active constituents of plants with phospholipids for preparation of a molecular complex of lipid and phyto constituents (Bhattacharya and Kidd, 2009). Phytosomes host their polyphenolic guest, generally little soluble both in water and in lipids, at their surface, where lipophilic guest (due to their polar functionality) interact with phosphate head of phospholipids via hydrogen bonds and polar interactions. They form a little micro sphere or cell with unique arrangement and better lipid solubility (fig. 1). Structure of phospholipid molecule contains water soluble head and two oil soluble tails. Because of their solubility both in fat and water, the phospholipid perform their action as a surface active agent/emulsifier. Emulsifying action of phospholipids combine with plant extract enhanced the bioavailability for fat soluble drugs, proved by their improved and faster absorption in GIT (Parris and Kathleen, 2005). This results phytosome better absorbed on cell membrane (fig. 2). Gastro-protective property of phosphatidylcholine also protects the active chemical constituents of plants from gastric enzymes and intestinal bacterias (Cryer 2011; Mcneil, 1989). Pharmacokinetic studies also proved the higher relative bioavailability of phytosomes than plant extracts and their individual constituents at the equal dose (Bombardelli, 1989; Jain, 2010).

The *Tecomella undulata* (family-bignoniaceae) commonly *named* 'Roheda' is deciduous plant of dry and semi-dry areas (Nagpal, 2010). Distribution of the tree is circumscribed to the arid parts of the Saudi Arabia, south-east parts of Pakistan and north-west regions of India (Khare, 2004). The bark of the tree is highly astringent

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specifically used for treat internal tumors and various diseases associated with liver, spleen and abdomen. Usefulness of tecomella undulate stem bark extract is also mentioned in Charakasamhita and prescribed in treatment of hepatomegaly, splenomegaly, urinal disorders, anemia, jaundice and internal worms (Dash, 1991).

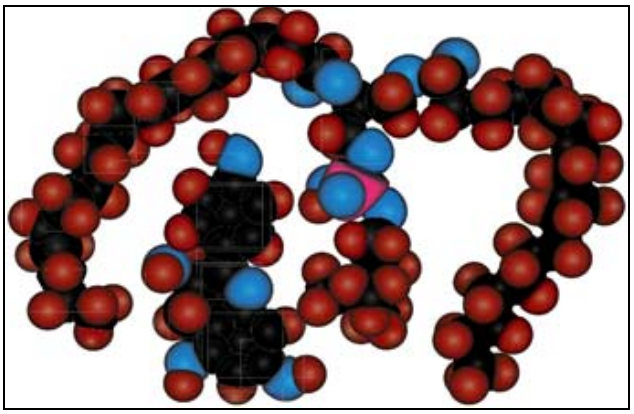

Fig. 1: Schematic of the phytosome molecular complex

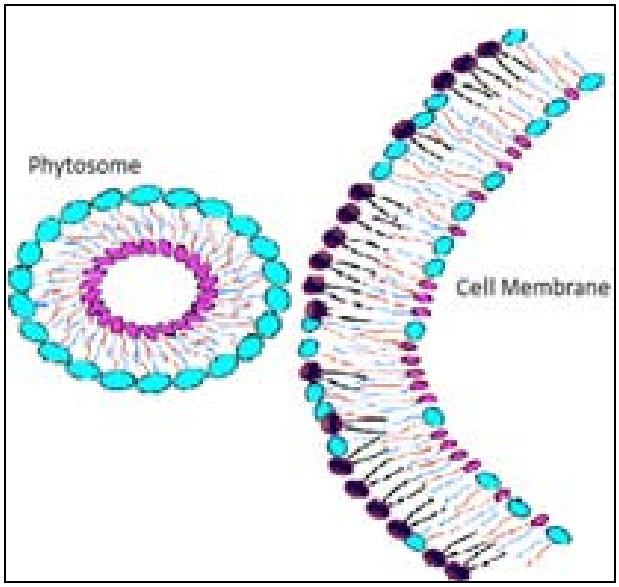

Fig. 2: Representation of a phytosome approaching a cell membrane

MATERIAL AND METHODS

Collection of plant material

The bark was stripped carefully from a cultivated tree of *Tecomella undulata* present in the field of Nohar tehsil (Rajasthan), in the month of November 2009 at morning time. The bark was identified by NISCAIR (National Institute of Science Communication and Information Resources), New Delhi (Ref. No.NISCAIR/RHMD/ Consult/2009-10/1326/128).

Phospholipids material

Lecithin soya 30% was purchased from Vinayak Ingredients (India) Private Limited, Mumbai. Cholesterol and all other analytical grade chemicals were purchased from S.D. Fine Chem., Mumbai.

Preparation of aqueous extract

The bark of *Tecomella undulata* was dried and coarsely powdered in home grinder. Decoction of the powdered bark (500g) was done with distilled water for 1 hour. Extract was filtered and concentrated by evaporate the menstruum in water bath (Ouedraogo *et al.*, 2011).

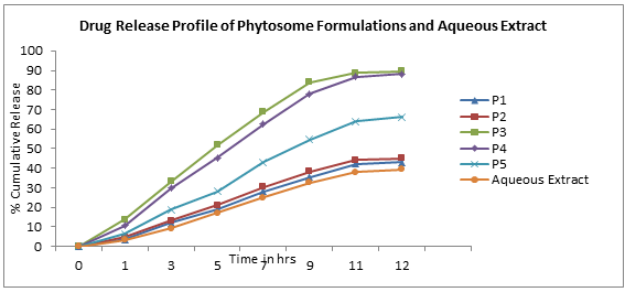

Fig. 3: *In vitro* drug release of phytosome formulations and aqueous extract

Preparation of phytosomes

a) Preparation of thin layer of phospholipids mixture

Phytosomes of *Tecomella undulata* were prepared by solvent evaporation method. Accurately weighed quantity of lecithin and cholesterol were dissolved in chloroform in round bottom flask to assure homogenous mixture of lipids. Prepared clear lipid solution was sonicated and organic solvent was evaporated in rotary evaporator for about 10 minutes with a bath temperature not exceeding 50°C. After complete removal of solvent, thin layer of phospholipids mixture was formed. The flask of rotary evaporated was attached with vacuum pump overnight. Organic solvent was evaporated and a dried phospholipid layer found. The phospholipid was added to a swirling container of dry ice-acetone bath carefully. Frozen lipid was lyophilized in high vacuum for 24 hours. The dried lipid film was stored in tightly closed container until ready to hydrate (Szoka *et al*., 1980).

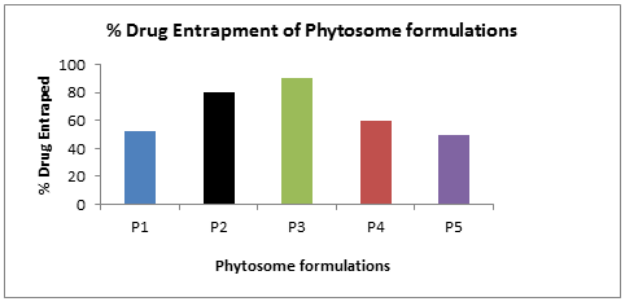

Fig. 4: Entrapment efficiency of prepared formulations

b) Hydration of lipid film

Aqueous extract of tecomella undulate heated on 37-40°C and 10ml of this hot extract was mixed to hydrate the thin film of phospholipids. Phospholipids suspension was

agitated on 37-40°C for 1 hour with vigorous shaking. After complete hydration, lipid suspension was added in a test tube and sonicated for 20 minutes by using ultrasonic bath sonicator on 37-40°C (Hui *et al*., 2011). The lipid suspension changed to slightly hazy solution. Composition of different batches is given in table 1.

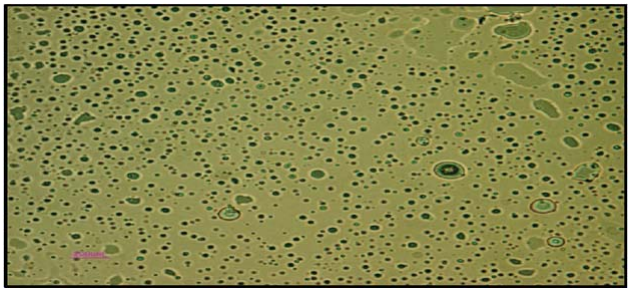

Fig. 5: Photomicrograph of phytosome formulation P3

In vitro release study

The in vitro release of prepared formulation was studied by using simple diffusion cell apparatus. The apparatus consist of glass tube with an inner diameter of 2.5cm open at both ends; one end of the tube is tied with sigma dialysis membrane (250-9U, molecular weight cut off: 12400 Dalton; Sigma, Banglore, India), 200µm in thickness, pH 5.8 to 8 and porosity 0.45µm, which serves as a donor compartment. Phytosomes equivalent to 100 mg of drug extract was taken in test tube and placed vertically in 100 ml of phosphate buffer. The temperature of medium was set on 37°C and stirring of medium (50 rpm) was done by using magnetic stirrer. 1 ml of sample was withdrawn periodically and maintained the sink conditions. The samples were assayed using UV spectrophotometer at 251nm. All diffusion studies and sample analysis were carried out three times and mean values along with standard error of mean were recorded using one way ANOVA. All analyses were performed using the Sigma Stat statistical program (Systat Software, Inc.) and differences were considered to be significant at a level of $P<0.05$. The releases of all prepared formulations were compared with pure bark extract of *Tecomella undulata* (Nagpal, 2012; Kumar, 2010).

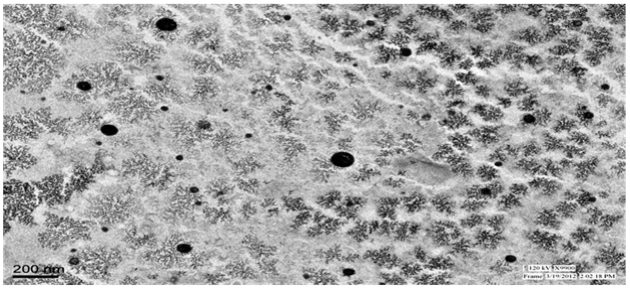

Fig. 6: TEM of phyto some formulation P3

Entrapment efficiency (Percent drug remaining entrapped)

10mg/ml sample of phytosome formulation was withdrawn and subjected to centrifugation on ultracentrifuge at 40,000 rpm for 50 min. The supernatant of the centrifuge was separated and the precipitate (pellets of Phytosomes) was redispersed in 10ml distilled water to remove remaining drug (phytoconstituents) adsorbed onto phytosomes. The process was repeated for 3 times and each collected supernatant was mixed with previously collected supernatant fraction represent the unentrapped fraction of the drug.

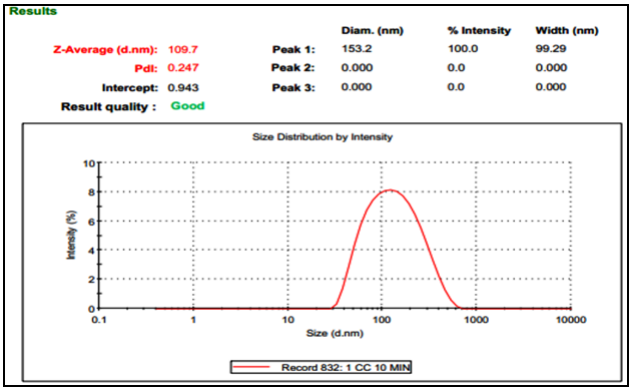

Fig. 7: Report of particle size of formulation P3

The pellets of Phytosomes were solubilized using Triton-X 100 (1% w/v) and the released phytoconstituents was the phytosome-entrapped fraction. The entrapment efficiency (EE) of phyto constituents was calculated using HPTLC. EE (%) = (amount of *T. undulata* entrapped in phytosomes/overall amount of the *T. undulata* in formulation) × 100 % (Ramana *et al*., 2010).

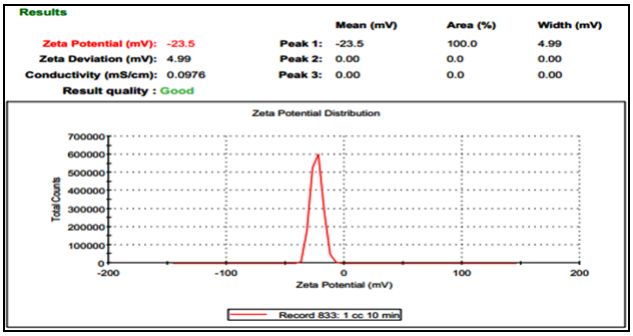

Fig. 8: Zeta potential study of formulation P3

Morphology of formulation

The morphology of prepared formulation was evaluated by the inverted microscope and Transmission Electron Microscopy (TEM). In TEM, a drop of Phytosomes was applied to a grid covered with a thick film. After leaving for five minutes to allow adsorption of Phytosomes to the grid, the excess was removed by a filter. 1% phosphotugstic acid was dropped onto the grid. Then the grid was air-dried for approximately 10 minutes and examined under a transmission electron microscope. The sample shape of formulation was photographed (Elhissi *et al*., 2007).

Table 1: Optimization of phytosome formulations

S. N	Formulation	Cholesterol (mg)	Lecithin (mg)	Chloroform (ml)	Aq. extract (*T. undulata*) (ml)	Cholesterol: lipid ratio
1	P1	15	30	5	10	1:2
2	P2	15	40	5	10	1:2.66
3	P3	15	45	5	10	1:3
4	P4	15	50	5	10	1:3.33
5	P5	15	60	5	10	1:4

Size analysis and zeta potential study

A drop of phytosomes was diluted in 10 mM of sodium chloride solution and size of Particles was measured by the photon correlation spectroscopy (Mastersizer, Malvern England) (Elhissi *et al*., 2007).

The charge present on surface of colloid particles were measured with a zetasizer (Malvern England). The formulation was diluted with distilled water, loaded into capillary cell mounted on the apparatus. (Ducat *et al*., 2011)

RESULTS

In vitro release studies

Drug release profile of prepared formulations was showed in fig. 3. *In vitro* dissolution revealed the % cumulative drug release were between 43-89.7% for phytosome formulations and was 39.4% for aqueous extract of *Tecomella undulata* bark was within 12h in phosphate buffer medium. (fig. 3).

Entrapment efficiency

Maximum entrapment efficiency (90%) was resulted with cholesterol and lecithin ratio 1:3 (batch P3). The entrapment efficiency for each batch showed in fig. 4.

Morphology of formulation

Photomicrography and TEM provided the evidence of vesicle formation and their morphology evaluation showed small, spherical and unilamellar vesicles with selected combination. (fig. 5, 6).

Size analysis and zeta potential study

The average particle size of phytosome was less than 200 nm. and the zeta potential was negative for every formulation batch. Formulation batch P3 had average particle size 153.2nm (fig. 7) and the zeta potential of batch P3 was -23.5mv (fig. 8).

DISCUSSION

Two major factors that affect the efficacy to cross the cell membrane are lipid solubility and molecular size of the dosage form. (Bhattacharya 2009) Method of preparation of lipid vesicles is not depending on the composition of lipid mixture (anionic, cationic or neutral) (Souto 2010). Quantity of lipid (10-20mg), which dissolves in organic solvent (1ml) depends on lipid solubility and acceptability of mixing. Rotary evaporation technique was used due to higher volume of organic solvent (>2ml) present in mixture.

The solvent evaporation method produced small particles with a mean diameter in the range of 109.7nm (batch P3). Pharmacokinetics and pharmacodynamics properties of any drug is depend on the size of dosage form so size and shape of phytosomes perform crucial role for an effective dosage form. Prepared phytosomesmostly had unilamillar vesicle with size less than 200 nm.

Phytosomes are specific type of drug delivery system in which drug itself in conjugation with lipids was forming vesicles so drug entrapment is higher. Transmission Electron Microscopy introduces the structural property of phytosomes and it is a useful technique for characterizing the thermodynamic, electric and mechanical properties of phytosomes. As observed, prepared pytosomes were well-identified perfect spheres and seen in disperse and aggregate collection. Presence or absence of charge on colloidal particles is an important stability parameter of colloidal system. Particle-to-particle interaction is a crucial element in determining the characteristics of colloids. One of the most important forces is electrokinetic repulsion. Charge present on particles affects the attachment of drug delivery to cell membrane. Difference in magnitude of surface charge determines the tissue binding and direct phytosomes to cellular compartment. Results showed that surface charge on phytosomes was negative and relatively high, so adjacent colloids repelled each other and tend to maintain their individuality.

As per DVLO theory, the higher negative magnitude of zeta potential shown that system stability will be good. The stability depends upon the ratio of the repulsive forces and attractive forces between the particles and at the value (-23.5mV) of zeta potential the repulsive forces is higher as compare to attractive forces between the particles and they never adhere strongly.

Results of *in-vitro* parameters are concluded that bioavailability of prepared formulation was drastically enhanced with their prolonged release.

Formulation batch P3 having cholesterol and lecithin ration 1:3, had unilamillar vesicle with average size 153.2 nm, surface charge -23.5mv, drug entrapment efficiency 90% and cumulative drug release 89.7% seems to be the best batch. Results of in vitro drug release lead us to the conclusion that phytosome drug delivery has a greater potential for sustained release of drug.

The newly developed *T. undulate* phytosomes provides a promising future treatment of various diseases of spleen, liver, abdomen, internal tumors, urinary system and others by their enhanced bioavailability.

REFERENCES

Ajazuddin and Saraf S (2010). Applications of novel drug delivery system for herbal formulations. *Fitoterapia.*, **81**: 680-689.

Bala A, Haldar PK, Kar B, Naskar S, Saha P and Kundusen S (2011). Antioxidant activity of the fractions of cleome gynandra promotes antitumor activity in ehrlich ascites carcinoma. *Asian Journal of Chemistry*, **23**(11): 5055-5060.

Bhattacharya S (2009). Phytosomes: The new technology for enhancement of bioavailability of botanicals and nutraceuticals. *Int. J. Health Res.*, **2**(3): 225-232.

Bombardelli E, Curri SB, Della RL, Del NP, Tubaro A and Gariboldi P (1989). Complexes between phospholipids and vegetal derivatives of biological interest. *Fitoterapia.*, **60**: 1-9.

Cryer B, Bhatt DL, Lanza FL, Dong JF and Lichtenberger LM and Marathi UK (2011). Low-dose aspirin-induced ulceration is attenuated by aspirin phosphatidylcholine: A randomized clinical trial. *Am. J. Gastroenterol. advance*, **106**: 272-277.

Dash B (1991). Metriamedica of ayurveda based on: Madanapala's Nighantu. New Delhi (India): B. Jain Publishers (P) Ltd. p.108.

Ducat E, Deprez J, Gillet A and Noel A (2011). Nucler delivery of a therapeutic peptide by long circulating pH-sensitive liposomes: Benefits over classical vesicles. *Int. J Pharm.*, **420**(2): 319-332.

Elhissi AMA, Faizi M and Naji WF (2007). Physical stability and aerosol properties of liposomes delivered using an air-jet nebulizer and a novel micro pump device with large mesh apertures. *Int. J Pharm.*, **334**: 62-70.

Hui Z, Zhi YW and Wei G (2011). Development and characteristics of temperature sensitive liposomes for vinorelbinebitartrate. *Int. J Pharm.*, **414**: 56-62.

Jain N, Gupta BP, Thakur N, Jain R, Banweer J, Jain D and Jain S (2010). Phytosome: A novel drug delivery system for herbal medicine. *International Journal of Pharmaceutical Sciences and Drug Research*, **2**(4): 224-228.

Khare CP (2004). Indian Herbal Remedies: Rational western therapy, Ayurvedic and other traditional usage, Botany. New Delhi (India): Society of new age herbals. p.67.

Kidd PM (2009). Bioavailability and activity of phytosome complexes from botanical polyphenols: The silymarin, curcumin, green tea and grape seed extracts. *Alternative Medicine Review*, **14**(3): 226-246.

Kumar B, Kavimani S and Jaykar B (2010). Development of formulation and *in vitro* evaluation of sterically stabilized (stealth) liposomes containing cytrabine. *Journal of Innovative Trends in Pharmaceutical Sciences*, **1**(7): 283-293.

Manach C, Scalbert A and Morand C (2004). Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.*, **79**: 727-747.

Mcneil PL and Ito S (1989). Gastrointestinal cell plasma membrane wounding and sealing *in vivo*. *Gastroenterology*, **96**: 1238-1248.

Nagpal N, Arora M, Chawla A, Rahar A and Reni K (2012). Examine the presence of chemical constituents in *Tecomellaundulata* and their interaction with lipids. *International Journal of Research in Pharmacy and Science*, **2**(4): 60-67.

Nagpal N, Arora M, Rahar S, Swami G and Kapoor R (2010). Pharmacological and Phytochemical review on T*ecomellaundulata*. *Research Journal of Pharmacy and Technology*, **2**(4): 103-108.

Ouedraogo N, Tibiri A, Sawadogo RW, Lompo M, Hay AE, Koudou J, Dijoux MG and Guissou IP (2011). Antioxidant anti-inflammatory and analgesic activities of aqueous extract from stem bark of Pterocarpuserinaceus Poir. (Fabaceae). *J. Med. Plants Res.*, **5**(10): 2047-2053.

Pandey S and Patel K (2010). Phytosomes: Technical Revolution in Phytomedicine. *International Journal of Pharm Tech Research*, 2(1): 627-631.

Parris K and Kathleen H (2005). A review of the bioavailability and clinical efficacy of milk thistle phytosome: A silybin-phosphatidylcholine complex. *Altern. Med Rev.*, **10**(3): 193-203.

Ramana LN, Sethuraman S, Ranga U and Krishnan UM (2010). Development of a liposomal nanodelivery system for nevirapine. *J. Biomed. Sci.*, **17**(57): 1-9.

Souto EB (2010). Patenting Nanomedicines: Legal Aspects, Intellectual Property and Grant Opportunities. Heidelrberg (Germany): Springer Science. p.311.

Suresh RN and Vandana SP (2008). Hepatoprotective effect of Ginkgo select Phytosome in rifampicin induced liver injury in rats: Evidence of antioxidant activity. *Fitoterapia.*, **79**: 439-445.

Szoka FJ and Papahadjopoulos D (1980). Comparative properties and methods of preparation of lipid vesicles (Liposomes). *Ann. Rev. Biophys. Bioeng.*, **9**: 467-508.

Taylor KMG and Morris RM (1995). Thermal analysis of phase transition behavior in liposomes. *Thermochimica. Acta.*, **248**(C): 289-301.