Research Article CODEN: IJPRNK ISSN: 2277-8713
Sara S. Barakat, IJPRBS, 2016; Volume 5(1): 111-122 IJPRBS

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

NANOLIPOSOMES CONTAINING PENETRATION ENHANCERS FOR THE INTRANASAL DELIVERY OF THE ANTIEMETIC DIMENHYDRINATE

SARA S. BARAKAT[1], MAHA NASR[2], SABRY S. BADAWY[1], SAMAR MANSOUR[2,3]

1. Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Misr International University, Egypt
2. Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, Egypt.
3. Pharmaceutical Technology Department- German University in Cairo. Egypt.

Accepted Date: 06/02/2016; Published Date: 27/02/2016

Abstract: This study involves the preparation of nanoliposomes containing penetration enhancers (PEVs) for the intranasal delivery of dimenhydrinate (DMH) for rapid treatment of nausea and vomiting. The nanovesicles were prepared using different amounts of phosphatidylcholine and a 1:1 ratio of the penetration enhancers (labrasol and transcutol) using the reverse phase evaporation technique (REV). The PEVs prepared were then characterized for their entrapment efficiency, particle size, zeta potential and polydispersity index (PDI). Their stability following refrigeration storage was also investigated after 3 months. The morphology of the selected formula was examined using transmission electron microscopy (TEM). Results showed that the reverse phase evaporation technique was able to produce vesicles in the nanometer range (59.46 ± 1.6 nm to 266.5 ± 20.5 nm), which were also able to incorporate dimenhydrinate at high entrapment levels (ranging from 82.95-95.35%). The formula prepared using large amount of phosphatidylcholine showed maximum stability after storage, manifested by insignificant changes in particle size, zeta potential and polydispersity index ($P<0.05$). TEM confirmed the nanosize and spherical morphology of the selected vesicular formula. These results show that penetration enhancer containing vesicles (PEVs) can be used as a successful carrier for the possible intranasal delivery of dimenhydrinate.

Keywords: dimenhydrinate, intranasal delivery, penetration enhancer containing vesicles, motion sickness.

PAPER-QR CODE

Corresponding Author: DR. SARA S. BARAKAT

Access Online On:

www.ijprbs.com

How to Cite This Article:

Sara S. Barakat, IJPRBS, 2016; Volume 5(1): 111-122

INTRODUCTION

Motion sickness is a syndrome occurring upon exposure to certain types of motion, and resolving after its cessation **[1]**. The major signs of motion sickness include nausea, salivation and vomiting. However, nausea is the hallmark symptom for motion sickness. **[2]**

It was postulated that motion sickness was mainly due to cholinergic stimulation, while the adrenergic activation suppresses it. That's why the central anticholinergics are the most effective anti-motion sickness drugs in addition to antihistamines with antimuscarinic activity. Dimenhydrinate, cyclizine, meclozine, and promethazine are the antihistamine agents most widely used for prophylaxis and active treatment of motion sickness **[3]**.

Dimenhydrinate (DMH) is an OTC drug used for the prevention and treatment of nausea, vomiting, dizziness, and vertigo associated with motion sickness. It is used for the prevention of postoperative vomiting and drug induced vomiting **[4,5]**. DMH has an antihistaminic H1 and antimuscarinic action acting mainly in the central vestibular nuclei and vomiting center. Despite its usefulness when administered via the oral route, rapid action requires the use of an alternative route that allows the transmission of the drug directly to the brain; a virtue which can be achieved using the intranasal route, owing to the direct nose to brain shunt provided by the olfactory region **[6]**.

Vesicular delivery systems are one of the most promising systems designed for targeting and controlling the delivery of drugs. Vesicles are spheres consisting of one or more concentric lipid bilayers that are formed by placing certain amphiphilic molecules such as phospholipids or surfactants in water. Liposomes are type of vesicles consisting of one or more concentric lipid bilayers separated by water or aqueous buffer compartments, with size ranging from 10 nanometers to 20 micrometers. Recently, Penetration enhancer containing vesicles (PEVs) have been developed as a new elastic vesicular system prepared using penetration enhancers such as oleic acid, Transcutol® and Labrasol® **[7,8]**. Therefore, the aim of the current work was to test the feasibility of incorporating the antiemetic drug DMH within PEVs for possible intranasal delivery for rapid antiemetic effects.

MATERIALS AND METHODS

Materials

Dimenhydrinate was kindly supplied by Alkahira Pharmaceuticals & Chemical Industries Company, Cairo, Egypt. Sodium chloride (NaCl), Sodium dihydrogen orthophosphate 1 – hydrate, Polyethylene glycol 400 (PEG), Disodium hydrogen orthophosphate anhydrous,

methanol, chloroform and Diethyl ether were purchased from El-Nasr Chemical Company, Cairo, Egypt. Dialysis membrane (Spectra / Por) 12.000 – 14.000 molecular weight Cut off was purchased from Spectrum Laboratories Inc, Rancho Dominguez, Canada. Capryl-caproyl macrogol 8-glyceride (Labrasol®) and 2-(2-ethoxyethoxy) ethanol (Transcutol®) were kindly gifted by Gattefosse' Company, France. Phosphatidylcholine (Epikuron 200) was kindly provided by Cargill Texturizing solutions, Deutschland GmbH & Co., Hamburg, Germany. Acetyl Uranil (Uranyl acetate -2- hydrate) was purchased from Allied signal, Riedel- dehaen, Germany).

Methods

Preparation of dimenhydrinate loaded PEVs

The dimenhydrinate-loaded PEVs were prepared using the reversed phase evaporation technique (REV) using different amounts of phospholipid in the presence of the penetration enhancers labrasol and transcutol at a ratio of 1:1, as shown in table (1) **[9]**. The phospholipids were dissolved in chloroform: methanol mixture (2:1, v/v) **[10]**; in a round bottomed flask, followed by evaporation of the organic solvent mixture using a rotary evaporator (Rotavapor R-210/215, Buchi, Switzerland) at 40°C resulting in the formation of lipid thin film on the inner wall of the flask. The film was re-dissolved in 12 ml diethyl ether, followed by the addition of 6 ml phosphate buffer saline pH 7.4 **[9,11]** containing different amounts of DMH solubilized in 0.5 ml PEG 400, with the penetration enhancers labrasol and transcutol included in the buffer at a ratio of 1:1 v/v. The system was swirled by hand, and the formed emulsion was then placed in the rotary evaporator where the organic solvent was removed under reduced pressure to fully remove the organic solvent followed by the addition of 4 ml phosphate buffered saline. The resultant vesicular dispersion was then sonicated at 40°C for one hour using sonicator (Transsonic model: TI-H 5, Germany) to reduce their particle size **[12]**. The composition of the prepared liposomal formulations is demonstrated in table (1).

Separation of unentrapped dimenhydrinate from the prepared PEVs

Purification of PEVs from the non-encapsulated drug was done by exhaustive dialysis, in which PEVs formulae were placed in a dialysis tubing (Dialysis membrane Spectra / Por 12.000 – 14.000 molecular weight). The dialysis was carried out against 1000 ml distilled water for 2 hours **[13]**, which was found appropriate for the removal of the non-entrapped dimenhydrinate in the medium, as shown by preliminary experiments (data not shown).

Characterization of the prepared PEVs

Determination of dimenhydrinate entrapment efficiency in the PEVs

To determine the amount of dimenhydrinate entrapped in the dialyzed vesicles, the vesicles were disrupted using methanol. Five hundred microliters of PEVs were mixed with 4.5 ml of methanol to obtain a clear solution, which was covered well with a parafilm to prevent methanol evaporation. The concentration of dimenhydrinate in methanol was determined spectrophotometrically at the predetermined λmax after appropriate dilution using ultraviolet spectrophotometer (Evisa-Shimadzu model: UV-1650PCUV-1650PC, Europe). No interference was found from PEVs at this wavelength.

The entrapment efficiency values were further confirmed by measuring the amount of free dimenhydrinate obtained in the distilled water, which was then subtracted from the total amount initially added in the formula to determine the amount of dimenhydrinate entrapped in the vesicles. The entrapment efficiency was calculated through the following relationship **[14, 15]**

$$\text{Entrapment Efficiency Percentage} = \frac{\text{Entrapped drug} \times 100}{\text{Total drug}} \quad \textbf{(Equation 1)}$$

Determination of the particle size and zeta potential of PEVs

The size, polydispersity index (PDI) and charge of the prepared dimenhydrinate PEVs were determined by photon correlation spectroscopy (PCS) using Zetasizer nano-ZS (Nano ZS 3600, Malvern Instruments Ltd., WorcesterShire, UK), after appropriate dilution **[13,16,17]**.

Stability study for the prepared vesicles

The prepared dimenhydrinate loaded PEVs were stored for three months at refrigeration temperature 2-8ºC. After the three months storage period, the samples were inspected visually for their homogeneity and consistency. The particle size, zeta potential and polydispersity index (PDI) of the PEVs were also re-measured.

Determination of the morphology of PEVs using transmission electron microscope (TEM)

The characterization of the vesicles shape was done for the selected PEVs formula by transmission electron microscopic (TEM) analysis (JEM-100 S, Joel, Tokyo, Japan) **[18]**. The analysis was done by depositing one drop of the diluted sample on a film coated 200-mesh copper grid, followed by uranyl acetate staining (1%) and drying **[8]**. Before examination any excess fluid was removed with filter paper.

Statistical analysis

The obtained data was statistically analyzed using Graph pad Instat program. Data were expressed as the mean ± standard deviation (S.D.). Comparison of the mean values was done using one way analysis of variance (ANOVA), followed by Tukey – Kramer Multiple Comparisons Test. Statistical significance was set at p-value ≤ 0.05. All measurements were conducted in triplicate.

RESULTS AND DISCUSSION

Determination of dimenhydrinate entrapment efficiency in the PEVs

PEVs were prepared using the reversed phase evaporation technique (REV) using soybean phosphatidylcholine as bilayer forming lipid. The choice of the method was based on the fact that REV was more suited for encapsulation of hydrophilic drugs, such as DMH with a log P -0.39 **[19, 20]**. Although the effect of penetration enhancers in promoting the drug absorption is well established; the precise mechanism of their action is not known, but it is suggested that they promote drug absorption by increasing the membrane fluidity, expanding the dimensions of the paracellular pathway to solute transport and also they form reverse micelles in the cell membrane thus creating transient pores in the membrane **[21]**. The selected penetration enhancers to be incorporated in the vesicles were labrasol and transcutol.

Labrasol (capryl-caproyl macrogol 8-glyceride) is a well-defined mixture of mono-, di- and triglycerides and mono- and di-fatty acid esters of polyethyleneglycol, with caprylic and capric acids being the predominant fatty acids. It was suggested that labrasol has a tight junction opening action leading to increased membrane permeability for water-soluble drugs **[22]**. On the other hand, transcutol (diethylene glycol monoethylether) is an ethylene oxide derivative with a long history of safe use as a solvent in many products including pharmaceuticals, cosmetics, and food applications, thus, presenting itself as strong solubilizer with low toxicity **[23]**. Additionally, the presence of Transcutol HP in intranasal formulations was found advantageous in enhancing the bioavailability of drugs **[24]**. PEG 400; which is also a penetration enhancer on its own, was incorporated in the hydrating buffer for the solubilization of DMH. It was reported to enhance the penetration of drugs intranasally by improving the vesicular bilayer fluidity thus facilitating the penetration of the highly fluidized vesicles **[25]**.

The EE% results of the prepared PEVs are shown in table (2). The EE% of dimenhydrinate in PEVs ranged from 78.915 ± 3.46 % to 95.35± 0.15%. These acceptable values of the entrapment efficiency (EE%) of the prepared formulae could be attributed to the polar nature of dimenhydrinate (log p = - 0.39) which allows it to be entrapped in the large hydrophilic core of

the PEVs which are prepared by the reverse phase evaporation technique. The aforementioned technique is known to result in a high aqueous space-to-lipid ratio and increase the capability of the vesicles to entrap a large percentage of hydrophilic drugs **[19, 20, 26]**. The high entrapment efficiencies can also be ascribed to the presence of the penetration enhancers labrasol and transcutol which possess hydrophilic natures **[7]**.

By further inspection of EE% values in table (2) and figure (1), it was clear that in formulae (1-3) containing the same amount of phospholipid (300 mg), the increase in the amount of drug from 50 mg in formula (1), to 100 and 150 mg in formulae (2) and (3) respectively resulted in a significant decrease in EE% of DMH ($P<0.05$). This could be attributed to the higher concentration gradient of DMH created by its increase in concentration, resulting in more diffusion of the drug upon conduction of dialysis. The preserving of this EE% upon increasing the amount of DMH to 200 mg with an accompanying increase in phospholipid amounts to 900 mg in formula (4), is probably attributed to the increased particle size of the vesicles with increased phospholipid amounts (to be displayed in the particle size section), creating more space for accommodation of the drug, leading to significant increase in the loaded DMH amounts.

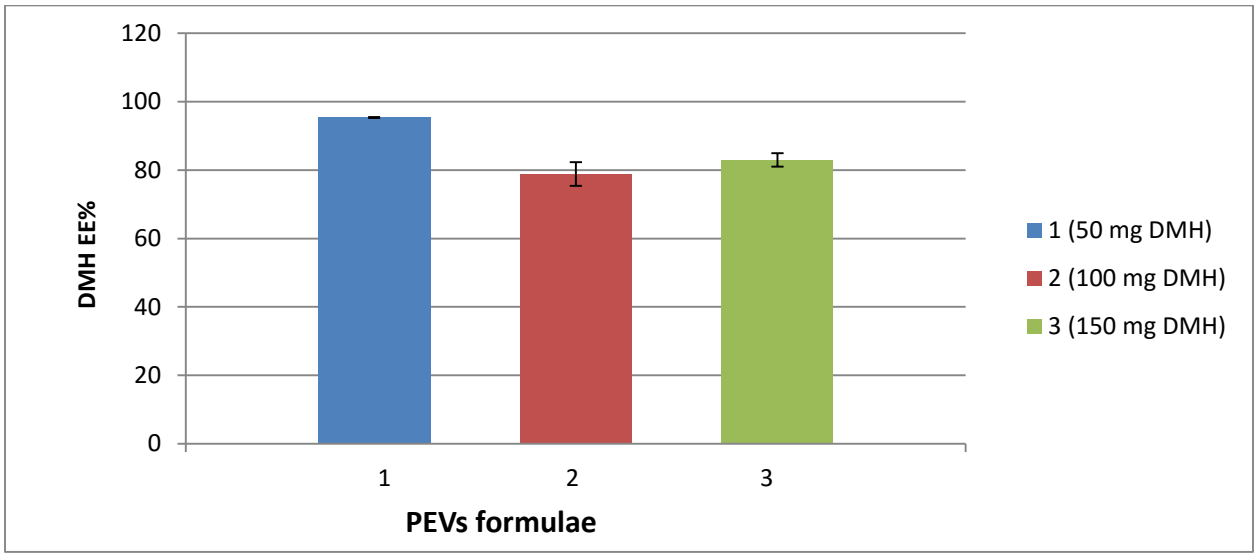

Figure (1): Effect of the amount of DMH drug on the entrapment efficiency (EE%) of the prepared PEVs.

Determination of the particle size and zeta potential of PEVs

The results of the particle size of the vesicles are shown in table (3). The particle size of the prepared PEVs ranged from 59.46 ± 1.6 nm to 266.5 ± 20.5 nm. The small particle size range of the vesicles was also reported by other authors who prepared PEVs using labrasol and transcutol **[17, 27, 28]**. Formulae 1-3, prepared using different amounts of DMH, displayed non-significant changes in particle size ($P<0.05$) (Figure 2). This suggests the solubilized nature of DMH in the aqueous core of the vesicles rather than the phospholipid bilayer coat. It was also clear that by increasing the amount of phospholipids the particle size significantly increased ($p<0.05$), in which formula (4) prepared using 900 mg phospholipids displayed a particle size of 266.5. This came in accordance with Jacquot et al., 2014 **[29]**, and could be ascribed to the increase in the phospholipid bilayer thickness upon increasing its amount in the preparation.

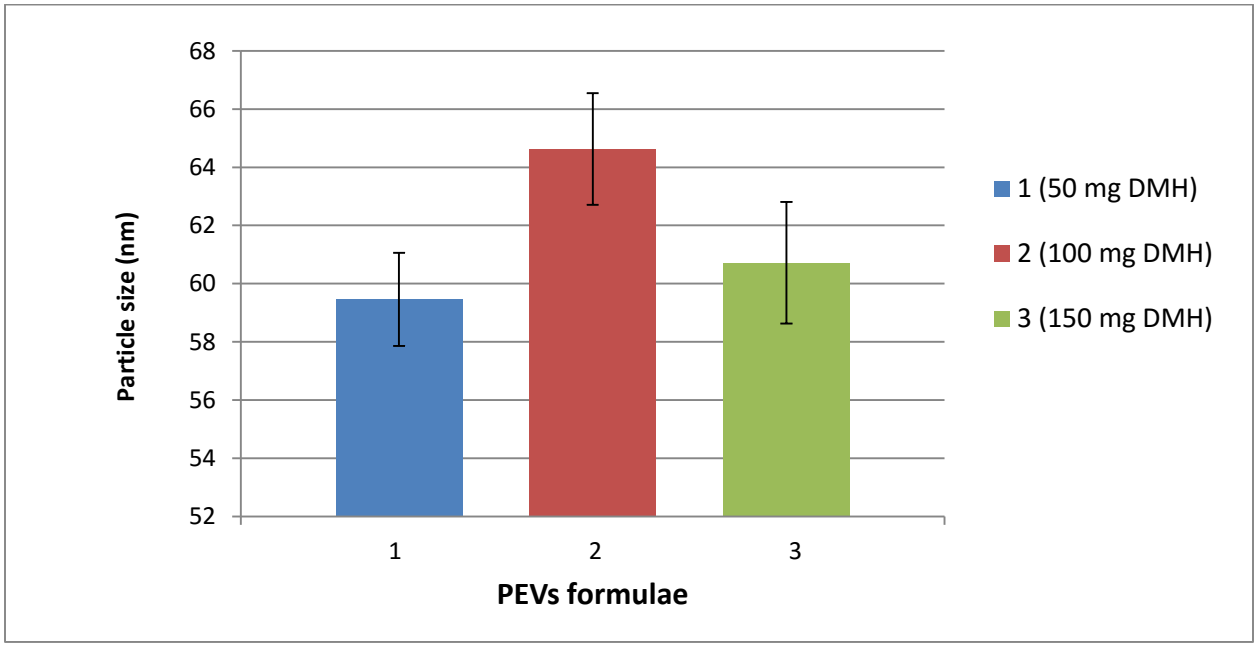

Figure (2): Effect of the amount of DMH on the particle size of the prepared PEVs.

Regarding polydispersity index, all formulae had a generally low PDI values (0.3-0.49), with a near neutral surface charge, owing to the almost neutral nature of the phospholipids, and the non-charged nature of the utilized penetration enhancers **[31]**.

Stability study of the prepared DMH PEVs

Table (3) shows the effect of refrigeration storage for 3 months on the particle size, zeta potential and PDI of the PEVs respectively.

Interestingly, the formulae prepared using 300 mg phospholipids (1-3) displayed a significant increase in their particle size and PDI upon storage ($P<0.05$) indicative of vesicular swelling, while that prepared using 900 mgs phospholipids (4) displayed an insignificant increase in particle size ($p>0.05$). This may be explained by the fact that labrasol was reported to cause destabilization of the phospholipid bilayers, and transcutol was reported to interact with the polar heads of the phospholipids **[8]**; with this interaction being accentuated at the low phospholipid:PE ratios achieved with (1-3). No significant changes in zeta potential was observed for all vesicular formulations upon storage ($P<0.05$).

Since formula 4 displayed the best storage properties, it was chosen for morphological examination using TEM.

Determination of the morphology of PEVs using transmission electron microscope (TEM)

As evident in figure 3, the PEVs displayed spherical morphology, with particle size in the nanometer range.

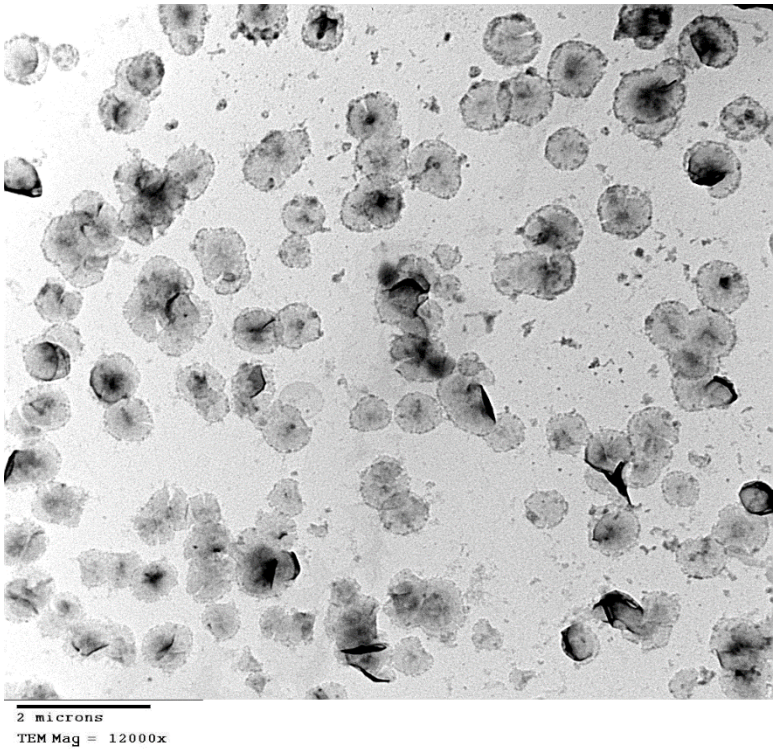

Figure (3): TEM micrograph of formula 4 at a magnification of 120000X.

CONCLUSION

Penetration enhancer containing vesicles (PEVs) can be considered as a promising system for encapsulating the antiemetic dimenhydrinate at high loading values. Their content of labrasol and transcutol in combination with the reverse phase evaporation technique contributed to their nanometer size, suggesting their potential for intranasal delivery of dimenhydrinate.

ACKNOWLEDGEMENTS

The authors are grateful to Alkahira pharmaceutical company, Egypt; Gattefosse' Company, France; Cargill Texturizing solutions, Deutschland GmbH & Co., Hamburg, Germany for their kind supply of dimenhydrinate, (Labrasol and transcutol) and phosphatidylcholine (Epikuron 200) respectively.

DECLARATION OF INTEREST

The authors report no conflicts of interest

Table (1): The composition of dimenhydrinate loaded PEVs.

Formulation code *	Amount of Drug (mg)	Total volume of PBS pH7.4 (ml)	Amount of Penetration Enhancer (ml)		Amount of phospholipid (mg)
			Labrasol	Transcutol	
1	50	7.5	1	1	300
2	100	7.5	1	1	300
3	150	7.5	1	1	300
4	200	7.5	1	1	900

Table (2): Entrapment efficiency and drug loading for different DMH loaded PEVs

Formulation code	Entrapment efficiency % (EE%) Mean ± S.D	Loaded amount of Dimenhydrinate (mg)
1	95.35 ± 0.15	47.68
2	78.915 ± 3.46	78.91
3	82.96 ± 1.95	124.44

4	83.37 ± 2.20	166.74

Table (3): Effect of storage on the stability of the PEVs.

Formula Code	P.S. of the freshly prepared vesicles (nm)	P.S. of the vesicles after 3 months storage (nm)	Zeta potential of the freshly prepared vesicles (mV)	Zeta potential stability of the vesicles after 3 months storage (mV)	PDI of the freshly prepared vesicles	PDI stability of the freshly prepared vesicles
1	59.46 ± 1.6	136 ± 8.3	0.94 ± 0.64	0.976 ± 0.08	0.36 ± 0.01	0.98 ± 0.02
2	64.63 ± 1.92	129 ± 9.42	0.629 ± 0.08	0.414 ± 0.05	0.3 ± 0.006	1
3	60.72 ± 2.09	118 ± 3.5	0.373 ± 0.11	0.593 ± 0.09	0.48 ± 0.02	0.66 ± 0.05
4	266.5 ± 20.5	284.5 ± 16	0.934 ± 0.56	0.61 ± 0.59	0.49 ± 0.12	0.46 ± 0.14

REFERENCES

1. Brainard A and Gresham C. Prevention and Treatment of Motion Sickness. American Family Physician. 2014; 90, 1: 41 – 46.
2. Sherman CR. Motion Sickness: Review of Causes and Preventive Strategies. Journal of Travel Medicine. 2002; 9, 5: 251–256.
3. Shupak A and Gordon CR. Motion Sickness: Advances in Pathogenesis, Prediction, Prevention, and Treatment. Aviation, Space, and Environmental Medicine. 2006; 77, 12: 1213 – 1223.
4. Martindale, 1977. The Extra Pharmacoepia, 27th ed. The Pharmaceutical Press, London, pp. 1299.
5. Desai J, Alexander K, Riga A. Characterization of polymeric dispersions of dimenhydrinate in ethyl cellulose for controlled release. International Journal of Pharmaceutics. 2006; 308: 115–123.
6. Nasr M. Development of an optimized hyaluronic acid-based lipidic nanoemulsion co-encapsulating two polyphenols for nose to brain delivery. Drug Delivery. 2015; Epub ahead of print.

7. Bseiso EA, Nasr M, Sammour O, Abd El Gawad NA. Recent advances in topical formulation carriers of antifungal agents. Indian Journal of Dermatology, Venereology, and Leprology. 2015; 81, 5: 457-463.
8. Bsieso EA, Nasr M, Sammour O, Abd El Gawad NA. Could nanovesicles containing a penetration enhancer clinically improve the therapeutic outcome in skin fungal diseases?. Nanomedicine (Lond.) 2015; 10, 13: 2017-2031.
9. Nasr M, Taha I, Hathout RM. Suitability of liposomal carriers for systemic delivery of risedronate using the pulmonary route. Drug Delivery. 2013; 20, 8: 311–318.
10. El Maghraby GMM., Campbell M, Finnin BC. Mechanisms of action of novel skin penetration enhancers: Phospholipid versus skin lipid liposomes. International Journal of Pharmaceutics. 2005; 305: 90-104.
11. Gariepy ER , Leclair G, Hildgen P, Gupta A, Leroux JC. Thermosensitive chitosan-based hydrogel containing liposomes for the delivery of hydrophilic molecules. Journal of Controlled Release. 2002; 82: 373–383.
12. Srisuka P, Thongnopnuab P, Raktanonchaic U, Kanokpanontd S. Physico-chemical characteristics of methotrexate-entrapped oleic acid-containing deformable liposomes for in vitro transepidermal delivery targeting psoriasis treatment. International Journal of Pharmaceutics. 2012; 427: 426– 434.
13. Manconi M, Caddeo C, Sinico C, Valenti D, Mostallino MC, Lampis S, Monduzzi M, Fadda AM. Penetration enhancer-containing vesicles: Composition dependence of structural features and skin penetration ability. European Journal of Pharmaceutics and Biopharmaceutics. 2012; 82: 352–359.
14. Gulati M, Grover M, Singh M, Singh S. Study of azathioprine encapsulation into liposomes. Journal of Microencapsulation. 1998; 15: 485-494.
15. Aggarwal D, Kaur IP. Improved pharmacodynamics of timolol maleate from a mucoadhesive niosomal ophthalmic drug delivery system. International Journal of Pharmaceutics. 2005; 290: 155-159.
16. Mura S, Manconi M, Sinico C, Valentia D, Fadda AM. Penetration enhancer-containing vesicles (PEVs) as carriers for cutaneous delivery of minoxidil. International Journal of Pharmaceutics. 2009; 380: 72–79.
17. Manconi M, Sinico C, Caddeo C, Vila AO, Valenti D, Fadda AM. Penetration enhancer containing vesicles as carriers for dermal delivery of tretinoin. International Journal of Pharmaceutics. 2011; 412: 37–46.
18. Maestrelli F, Gonz´alez-Rodr´ıguez ML, Rabasco AM, Mura P. Preparation and characterisation of liposomes encapsulating ketoprofen–cyclodextrin complexes for transdermal drug delivery. International Journal of Pharmaceutics. 2005; 298: 55–67.

19. Szoka F and Papahadjopoulos D. Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. Proceedings of the National Academy of Sciences, USA. 1978; 75, 9: 4194-4198.
20. Chen G, Li D, Jin Y, Zhang W, Teng L, Bunt C, Wen J. Deformable liposomes by reverse-phase evaporation method for an enhanced skin delivery of (þ)-catechin. Drug Development and Industrial Pharmacy. 2014; 40, 2: 260–265.
21. Patel R.B. Patel M.R., Bhatt K.K., Patel B.G. Paliperidone-Loaded Mucoadhesive Microemulsion in Treatment of Schizophrenia: Formulation Consideration. Journal of Pharmaceutical Innovation. 2013: 1-10.
22. Koga K, Kusawake Y, Ito Y, Sugioka N, Shibata N, Takada K. Enhancing mechanism of Labrasol on intestinal membrane permeability of the hydrophilic drug gentamicin sulfate. European Journal of Pharmaceutics and Biopharmaceutics. 2006; 64, 82–91.
23. Sullivan Jr DW, Gad SC, Julien M. A review of the nonclinical safety of Transcutol, a highly purified form of diethylene glycol monoethyl ether (DEGEE) used as a pharmaceutical excipient. Food and Chemical Toxicology. 2014; 72, 40–50.
24. Hosny KM., Hassan AH. Intranasal in situ gel loaded with saquinavir mesylate nanosized 2 microemulsion: Preparation, characterization, and in vivo evaluation. International Journal of Pharmaceutics. 2014; 1-7.
25. Caddeo C, Díez-Sales O, Pons R, Fernàndez-Busquets X, Fadda AM, Manconi M. Topical Anti-Inflammatory Potential of Quercetin in Lipid-Based Nanosystems: In Vivo and In Vitro Evaluation.Pharmaceutical Research. 2013; 31, 4: 959-968.
26. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, Samiei M, Kouhi M, Nejati-Koshki K. Liposome: classification, preparation, and applications. Nanoscale Research Letters. 2013; 8:102.
27. Manconi M, Mura S, Sinico C, Fadda AM, Vila AO, Molina F. Development and characterization of liposomes containing glycols as carriers for diclofenac. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2009; 342: 53–58.
28. Manconi M, Caddeo C, Sinico C, Valenti D, Mostallino MC, Biggio G, Fadda AM. Ex vivo skin delivery of diclofenac by transcutol containing liposomes and suggested mechanism of vesicle–skin interaction. European Journal of Pharmaceutics and Biopharmaceutics. 2011; 78: 27–35.
29. Jacquot A, Francius G, Razafitianamaharavo A, Dehghani F, Tamayol A, Linder M, Arab-Tehrany E. Morphological and physical analysis of natural phospholipids-based biomembranes. A Peer-Reviewed Open Access Journal. 2014; 9, 9: e107435.
30. Manca ML, Castangia I, Matricardi P, Lampis S, Fernàndez-Busquets X, Fadda AM, Manconi M. Molecular arrangements and interconnected bilayer formationinduced by alcohol or polyalcohol in phospholipid vesicles. Colloids and Surfaces B: Biointerfaces. 2014; 117: 360–367.

31. Gala RP, Khan I, Elhissi AM, Alhnan MA. A comprehensive production method of self-cryoprotected nanoliposome powders. International Journal of Pharmaceutics. 2015; 486: 153-158.
32. Song J, Shi F, Zhang Z, Zhu F, Xue J, Tan X, Zhang L, Jia X. Formulation and Evaluation of Celastrol-Loaded Liposomes. Molecules. 2011; 16: 7880-7892.