

PREPARATION AND PHYSICO-CHEMICAL CHARACTERIZATION OF POORLY SOLUBLE DRUG-LOADED LIPID NANOPARTICLES

Răzvan PRISADA¹, Cristina Dinu PÎRVU^{1,2*}, Mariana FERDEȘ², Alina ORȚAN²,
 Corina Dalia TODERESCU^{3,4}, Dumitru LUPULIASA⁴

¹Physical and Colloidal Chemistry Department, Faculty of Pharmacy,
 "Carol Davila" University of Medicine and Pharmacy, Bucharest

²University Polytechnic Bucharest

³Analytical Chemistry Department, Faculty of Medicine, Pharmacy and Dental Medicine,
 "Vasile Goldiș" Western University, Arad, Romania

⁴Pharmaceutical Technology and Biopharmacy Department, Faculty of Pharmacy,
 "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

ABSTRACT. This study presents the preparation and characterization of testosterone loaded lipid nanoparticles in order to obtain a topical system. The designed lipid nanostructures were prepared by conventional rotary evaporation method followed by mechanical dispersion in hydrating medium or by the method proposed by Touitou. The effects of formulation variables upon the liposomes' characteristics were also investigated. The experiments showed that the molar ratios of the drug, PC and Co influence the drug entrapment or, in the two case, the entrapment efficiency was affected by the percentage of ethanol, PC and TS in the formulation. The stability of the lipid vesicles was determined by the entrapment efficiency and size distribution. The studies were performed during a period of 2 months. Lipid vesicles prepared by Touitou method proved to be more stable than the first, in the same experimental conditions.

Keywords: lipid vesicles, topical application, entrapment efficiency, size distribution, stability

INTRODUCTION

Bilayer lipid vesicle is a new technology for the encapsulation and delivery of bioactive agents. The list of bioactive materials that can be incorporated into a bilayer lipid vesicle is wide and involves pharmaceuticals, cosmetics and biotechnological fields. Because lipid nano vesicles are able to enhance the performance of bioactive agents (improving their solubility and bioavailability, in vitro and in vivo stability, their biocompatibility and biodegradability, cell-specific targeting, minimizing adverse effects, being able to provide controlled release, prolonged drug therapeutic action, presenting optimum therapeutic selectivity) they have potential applications in nanomedicine, nanotherapy, cosmetics, food technology, agriculture (Barry B, 2002; Touitou E. et al, 2002)

Preformulation and optimizing of the solute pharmaceutical systems with low solubility bioactive compounds still represents a challenge in pharmaceutical research. Ideally, a "well balanced" drug molecule should be sufficiently hydrophilic to be both soluble in aqueous biological fluids, and in buffer solutions, but, at the same time, lipophilic enough to penetrate the biological membranes (Wen A. H. et al, 2006; Leucuța SE, 2002). The use of new nanotechnologies is meant to protect the active molecule in its way in between the site of administration and target, to improve the cell penetration and to increase the biological specificity for a

target. The pharmaceutical forms, correctly formulated, which containing lipids require a special attention due to their possible large scale used in pharmaceutical industry, in optimizing existing forms and in the development of new formulations (Vyas SP et al, 2002).

The low permeability of drugs through the skin barrier is one of the problems encountered in dermal/transdermal drug delivery (Cevc G., 2004). One of the possibilities for enhancing the permeation through skin is the use of special lipid vesicles as drug delivery systems (Elsayed M. et al, 2006). The aim of this study is the preparation and characterization of testosterone loaded lipid vesicles in order to obtain a therapeutic system for topical application (Honeywell-Nguyen PJ et al, 2005).

Transferosomes were prepared by conventional rotary evaporation method, followed by mechanical dispersion in hydrating medium (Cevc G., 2003). Ethosomes were prepared by Touitou method (Godin B, Touitou E, 2003). The effects of formulation variables on the liposomes characteristics were also investigated. The stability of the elastic vesicles was determined by the entrapment efficiency and size distribution.

MATERIALS AND METHODS

Materials

The phosphatidyl choline from fresh eggs (PC), sodium cholate (Co), chloroform and ethanol, all of

*Correspondence: Cristina Dinu Pîrvu, Colloidal and Physical Chemistry Department, "Carol Davila" University of Medicine and Pharmacy, Bucharest, no.6, Traian Vuia St., 020956, Tel. +40-(021)-3180751, Fax. +40-(021)-3180751, email: ecristinaparu@yahoo.com

analytical grade, all of which were purchased from Sigma. The testosterone (TS) was provided by Industrial Chemica.

Methods

Preparation of Transferosomes and Ethosomes

- Transferosomes were prepared by conventional rotary evaporation method followed by mechanical dispersion in hydrating medium (fig.1).
- Ethosomes were prepared by Touitou method (fig. 2).

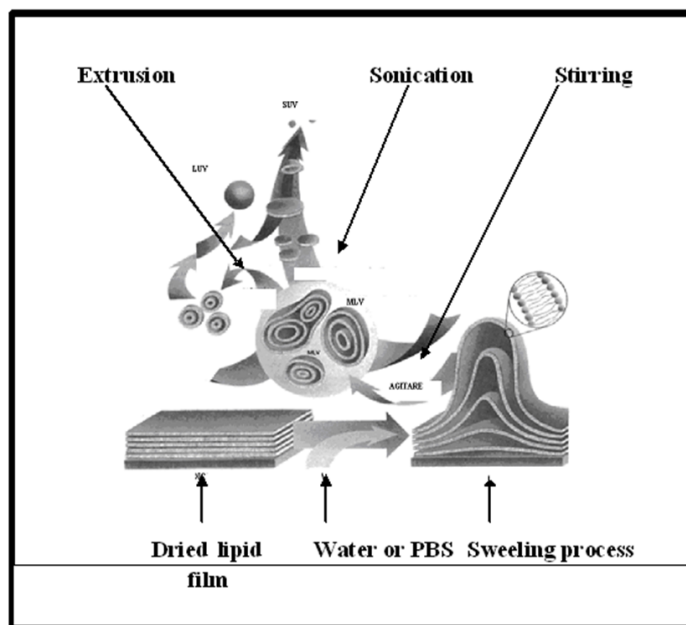


Fig. 1 The mechanism of transferosomes formulation by hydration of the lipid film method

Phospholipid and TS was dissolved in ethanol in a covered vessel at room temperature by vigorous stirring. Propylene glycol (PG) was added during stirring. This mixture was heated to 30°C in a water bath. The water

was heated to 30°C in a separate vessel and was added to the mixture drop wise in the centre of the vessel, which was stirred for 5 min at 700 rpm in a covered vessel. Ethosomes were formed spontaneously by the process.

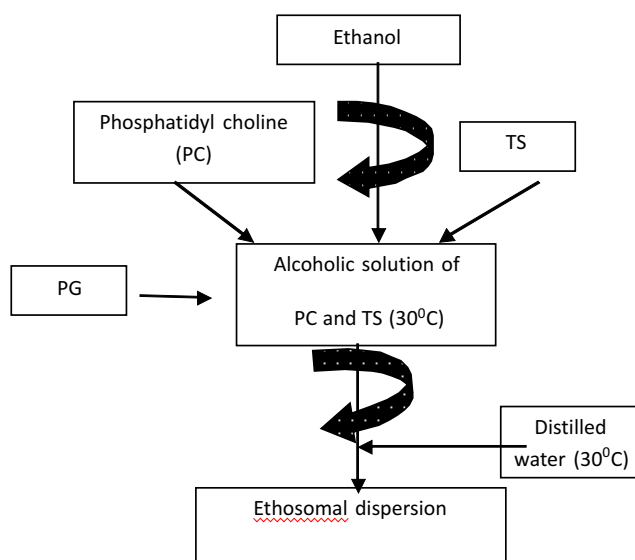


Fig. 2 The mechanism of ethosomes formulation by Touitou method

Size Distribution - Size distribution was determined with Zetasizer 2000 – Malvern instrument.

Entrapment Efficiency Determination. 0.2 ml from liposomal suspensions were separated through centrifugation at 12000 rpm at 5°C for 60 min. TS was spectrophotometrically analyzed.

Stability studies for ethosomes and transferosomes. The studies were performed during a period of 2 month;

the entrapment efficiency and size distribution were determined.

RESULTS AND DISCUSSIONS

Size distribution before sonication

Size distribution was determined with Zetasizer 2000 – Malvern instrument. The resulting histograms are represented in fig. 3:

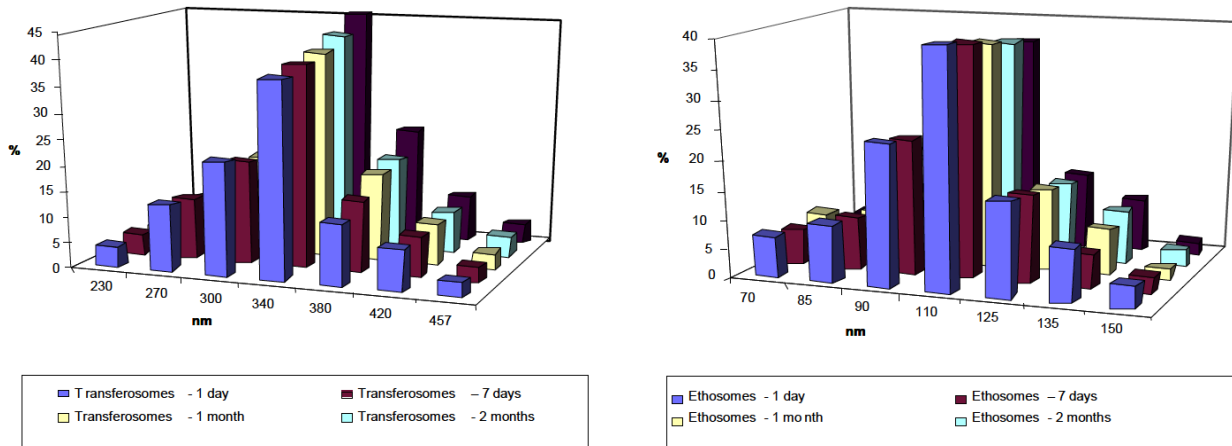


Fig. 3 Size distribution of transferosomes and ethosomes over a 2 months period

The analysis of the diagrams shows a polydispersity of vesicles, the medium dimension varying between 230 and 457 nm for the analyzed transferosomes, and 0 – 150 nm in case of ethosomes. The two histograms show a higher percentage of vesicles with dimensions between 300-380 nm, and 90-125 nm respectively.

Size Distribution after sonication

The dimensions of transferosomes population were between 50 – 90 nm, and for ethosomes between 20-70 nm. (fig. 4)

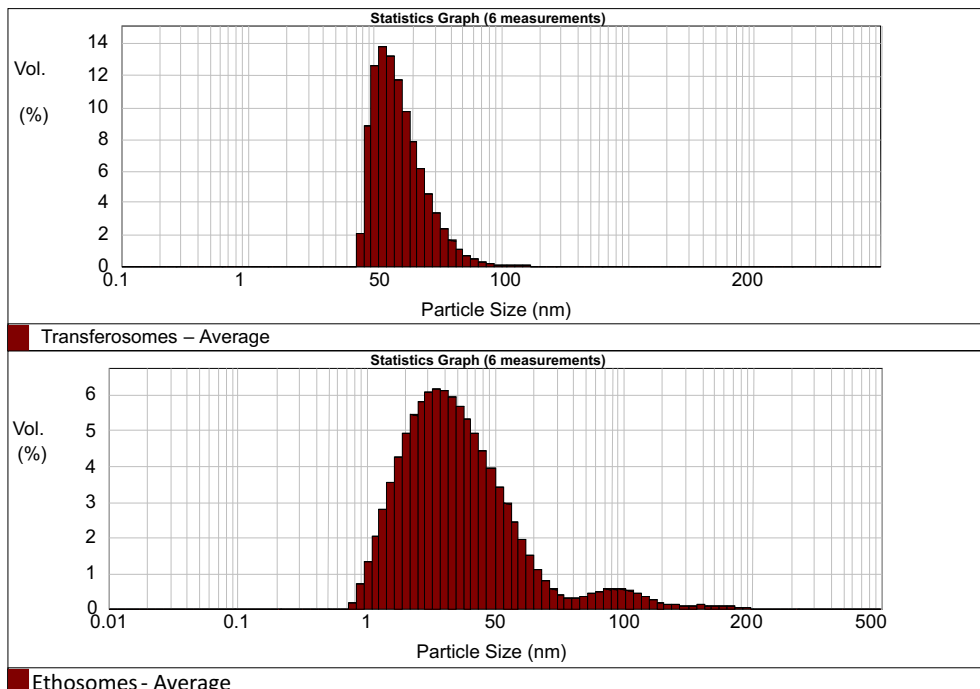


Fig. 4 Size distribution after sonication for transferosomes and ethosomes

Entrapment Efficiency Determination

The TS entrapment efficiency of nano lipid vesicles was determined by ultracentrifugation method. The entrapment efficiency was determined after separating the unincorporated drug by centrifugation at 40C at 15.000 rpm for 1h. Vesicles are destroyed using Triton-X 100 (0.1% v/v) and then analyzed spectrophotometrically (at 241 nm) to determine the drug content. Entrapment

efficiency was expressed as percentage of total drug entrapped. The entrapment capacity was calculated using the formula (Ning M. et al, 2005):

$$\%TS_{ent.} = Q_{TS} / Q_{TSexp} \times 100$$

where :

Q_{TS} = the theoretical amount of TS that was added

Q_{TSexp} = the content of drug detected only in supernatant

Table 1

The influence of transferosomes and ethosomes composition on the entrapment efficiency

Type of vesicles	Ratio m/m/m PC : Co : TS (g)	Ratio V/V ethanol : water (%)	Entrapment efficiency (%) - spectrophotometrically
TTS I	10 : 1 : 1	-	91.04
TTS II	10 : 1 : 2	-	98.57
TTS III	10 : 1 : 3	-	94.30
ETS I	-	1 : 2.5	78.13
ETS II	-	1 : 4.0	80.40

Stability studies

Lipid formulations were stored in the first case at a temperature of $4 \pm 1^\circ\text{C}$, and in a second case at $20 \pm 1^\circ\text{C}$. The stability was studied over a two months period. The storage conditions, especially temperature, were found to

have an impact on the vesicles' stability (Solanki A. et al, 2008). Table 2 presents the entrapment efficiency of the lipidic formulations stored at 40C, over a period of 2 months.

Table 2

The entrapment efficiency of transferosomes and ethosomes for 2 months at 4°C

Type of vesicles	Entrapment efficiency (%) after 1 week	Entrapment efficiency (%) after 2 weeks	Entrapment efficiency (%) after 1 months	Entrapment efficiency (%) after 2 months
TTS I	89.92	86.98	81.33	79.12
TTS II	97.68	95.00	93.89	90.92
TTS III	92.89	90.82	87.20	84.63
ETS I	77.32	76.78	75.85	74.98
ETS II	79.20	77.93	78.44	76.72

At room temperature (20°C) it was found that, after 2 weeks of storage, the loss of TS is bigger than 25%. That can be explained by a higher fusion rate of the vesicles at

this temperature. For example, table 3 presents the entrapment efficiency during storage for TTS I and ETS I formulations.

Table 3

Recovered percentage of TS in lipid formulation during storage

Period after preparation	Recovered percentage of TS	
	ETS I (4°C)	TTS I (4°C)
1 day	100 ± 0	100 ± 0
7 days	100 ± 0.25	100 ± 0.40
14 days	99 ± 0.95	98 ± 0.8
30 days	97 ± 1.20	96 ± 1.00
45 days	96 ± 2.10	95 ± 1.65
60 days	96 ± 1.35	93 ± 2.98

Modification of particle size of lipid vesicles is shown in figures 3, 4 and 5.

Results had shown that formulation were stable during that entire period of time, the loss in TS being a small one (over 95% of the initial TS was still entrapped in the

vesicles at a temperature of 4°C), while the size of the vesicles increased by 5 to 10%. We can conclude that the presence of ethanol in formulation determines an increased stability of the lipid vesicles.

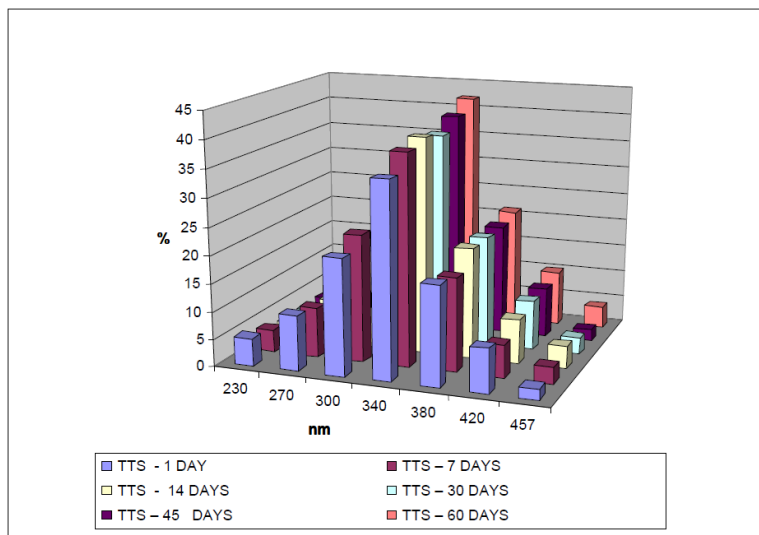


Fig. 5 Size distribution of transferosomes during storage

CONCLUSIONS

For both type of lipid vesicles we obtained a good entrapment efficiency. The size determinations showed a large polydispersity of the elastic vesicles, as the medium dimensions vary in between 70 and 150 nm in the case of the analyzed ethosomes, and between 180-500 nm in the case of transferosomes.

After sonication, the dimensions of transferosomes population were between 50 – 90 nm, and for ethosomes between 20-70 nm; also these values were approximately constant also after 2 months.

The stability of the elastic MLV vesicles is higher than the one of the SUV vesicles, which are still preferred because of the lower polydispersity of the population obtained after sonication.

The conducted experiments showed that the molar ratios of TS, PC and Co influence the drug entrapment of the transferosomes. In case of ethosomes, the entrapment efficiency was affected by the percentage of ethanol, PC and TS in the formulation.

Over the analyzed period of time, ethosomes proved to be more stable than transferosomes, in the same experimental conditions.

ACKNOWLEDGMENTS

This work has been funded by the Sectorial Operational Programme Human Resources Development 2007-2013 of the Romanian Ministry of Labour, Family and Social Protection through the Financial Agreement POSDRU/89/1.5/S/52432.

REFERENCES

- Barry B, *Transdermal Drug Delivery in Pharmaceuticals: The Science of Dosage Form Design*, ed. Aulton E. M., Churchill Livingstone, 499 – 528, 2002.
- Cevc G, Lipid vesicles and other colloids as drug carriers on the skin, in *Advanced Drug Delivery Reviews* 56, 675–711, 2004.
- Cevc G, Transferosomes: Innovative Transdermal Drug Carriers, in *Modified Release Drug Delivery Technology*, Rathbone J. M., Hadgraft J., Roberts M. S. (eds.), Marcel Dekker Inc., 533 – 547, 2003.
- Elsayed M, Abdallah O, Naggat V, Khalafalah N: Deformable liposomes and ethosomes: Mechanism of enhanced skin delivery, *International Journal of Pharmaceutics*, 322, 60-66, 2006.
- Godin B, Touitou E, Ethosomes: New prospects in transdermal delivery, *Therapeutic Drug Carriers Systems*, 20, 63-102, 2003.
- Honeywell-Nguyen PJ, Bouwstra JA, Vesicles as a Tool for Transdermal and Dermal Delivery, *Drug discovery Today: Technologies*, 2, 67-74, 2005.
- Leucuța SE, *Biofarmacie și farmacocinetică*, Ed. Dacia, Cluj Napoca, 2002.
- Ning M, Guo Y, Pan H, Chen X, Gu Z, Preparation and in vitro and in vivo evaluation of liposomal/niosomal gel delivery systems for clotrimazole, *Drug development and Industrial Pharmacy*, 31, 375-383, 2005.

- Solanki A, Parikh J, Parikh R, Preparation, Characterization, Optimization, and Stability Studies of Aceclofenac Proniosomes, Iranian Journal of Pharmaceutical Research., 7 (4), 237-246, 2008.
- Touitou E., Godin B., Weiss C., Enhanced Delivery of Drug Into and Across the Skin by Ethosomal Carriers, Drug Develop. Res., 50, 406-415, 2002
- Vyas SP, Khar RK, Targeted and controlled drug delivery: Novel carrier systems — Liposomes, CBS Publishers, 196-197, 2002.
- Wen A H, Choi MK, Kim DD, Formulation of Liposome for Topical Delivery of Arbutin, Arch. Pharm. Res., 29(12), 1187-1192, 2006.