Drug Development and Industrial Pharmacy, 34:1269–1276, 2008

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040802026095

informa
healthcare

Vesicular Lipidic Systems, Liposomes, PLO, and Liposomes–PLO: Characterization by Electronic Transmission Microscopy

M. Adolfina Ruíz, Beatriz Clares, M. Encarnación Morales, and Visitación Gallardo

Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Granada, Granada, Spain

Situations exist in which rapid administration of treatment, as well as maintenance of efficient concentrations for the longest possible time, turns out to be essential. In view of the previous treatment, the elaboration of liposomes, PLO (pluronic lecithin organogel), and the mixture of both is described, as well as their characterizations by electronic transmission microscopy, with the aim of finding out precisely the type of structure for both controlled release systems, its composition, size, homogeneity, and integrity. The period of study has been 90 days. Multilaminar and unilaminar vesicles smaller than 1 μm in diameter were seen in the liposomes, PLO, and liposomes–PLO formulations on transmission electron microscopic (TEM) observation. The technique of characterization reveals the progressive aggregation of the liposomas along the period of study. However, all the vesicles of PLO maintain a defined structure and only a light aggregation 60 days after the elaboration. Changes of morphology and aggregation of liposomas decreased after the incorporation of cholesterol (CH) to the liposomal matrix. The best results were obtained with the formulas liposomes–PLO, which maintain their individuality and integrity during the whole period of study. The combined formulation of liposomas and PLO showed an increase of stability of both lipid systems.

Keywords liposomes; PLO; transdermal; reservoir effect; controlled release

INTRODUCTION

In the treatment of physiological processes, the administration of active principles should be achieved in such a way so as to ensure that the drug reaches the area of activity (biophase) at concentrations and within effective therapeutic dosage ranges. Such dosage concentrations should remain constant throughout the time necessary to bring about changes in the physiopathological process. However, this administration seems limited by resistance to the drug, uptake of the drug by other cells, the inability of the drug to penetrate cells or tissues under treatment, or a lack of specific tropism, resulting in a distribution that is not only limited to the biophase but also to other cells and tissues. Consequently, to obtain sufficient concentration levels at the target cells, it is necessary to administer relatively high doses, which may lead to undesirable toxic and immunological effects (Puisieux & Delattre, 1985).

In recent years, a great importance has been attached to using lipids as vehicular systems and permeants of active principles through the skin.

From a galenic point of view, the solution to these problems could lie in the chemical association of the active substance with an appropriate delivery preparation capable of specificity of action. In this way, vectoring is defined as the attainment of maximum efficacy of a drug, by increasing its release in the area where its pharmaceutical receptors are found, thereby minimizing its concentration in other areas of the organism, reducing the adverse effects (Torrado & Cadorniga, 1989).

Among the prolonged release systems for topical application, we can include the liposomes (Puisieux 1985). Liposomes have the ability to bind to determined types of tissues and cells and can therefore be used to both increase the therapeutic effectiveness of the drug and minimize its undesirable effects (Banciu, Schiffelers, Fens, Metselaar, & Storm, 2006). Similarly, they can be programmed to achieve controlled delivery, over a longer period of time (Jain et al., 2005). Because of a chemical similarity to other cells that circulate in the blood stream, they are biologically compatible and are also easily metabolized be the organism and constitute a useful medium for the protection of biologically active labile substances. Effective doses of drug can by dramatically reduced when administered within a liposomal preparation (Maierhofer, 1988). Also the encapsulated active substances show prolonged release (Jain et al., 2005).

Address correspondence to M. Adolfina Ruíz, Pharmacy and Pharmaceutical Technology Department, School of Pharmacy, University of Granada, 18071 Granada, Spain. E-mail: adolfina@ugr.es

However, its therapeutic efficacy is fundamentally limited because of the difficulty for the active substance in accessing the deep tissues and blood, giving rise to inefficient drug levels. In this way, the transdermal administration of drugs has a series of advantages of great therapeutic potential, leading to the medical development of a new formula known as PLO (pluronic lecithin organogel). The innovation and versatility of this vesicular lipidic system lies in the attainment of a direct deposit of the active substance in the area of activity, at systemic or neuromuscular level, to stimulate the effect rapidly and efficiently (Willian, Walde, Luisi, Gazzaniga, & Stroppolo, 1992).

Scientific evidence is still limited today and little is known about the physicochemical properties of these vehicles. For this reason, this work has dealt with different aspects of these excipients, their structure, composition, size, homogeneity, and integrity with respect to time and the efficiency of their method of attainment.

The joining of both systems would allow the initialization and constant maintenance of drug levels. For this reason, we will describe and analyze the effect of PLO on structure and stability of liposomes. Therefore, the technique for production of these vectors is fine tuned. Likewise, different formulations of liposome are produced to determine the most ideal components for inclusion in PLO. Also evaluated is the role played by the cholesterol (CH) in the fluidity of the lipidic bilayers.

MATERIAL AND METHODS

The different formulations trialled for the preparation of controlled release methods, as well as for concentrations of components are shown in Tables 1 and 2. Number 1 corresponds to liposome formulations, number 2 to the base PLO.

Material

Components of the formulations have been selected which will form the base of the lipidic matrix:

1. L-α-phosphatydylcholine (PC) from fresh eggs, type IX-E, batch number 128H8003, supplied by Sigma Chem. Co.
2. CH, batch number 0401203, supplied by Roig Farma-Fagron (Terrassa, Spain)
3. Chloroform, batch 0311184, supplied by Roig Farma-Fagron
4. Distilled de-ionized water, Interapothek (Murcia, Spain).

TABLE 1
Composition of Liposomes

No.	Formula	Molar Proportion	mmoles/100 mL
1	PC	1	0.230
2	PC : CH	1:0.5	0.230/0.115

PC, phosphatidylcoline; CH, cholesterol.

TABLE 2
Composition of PLO (pluronic lecitin organogel)

Formula	Components	%
PLO		
Oil phase	Soy lecithin	10
	Isopropyl palmitate	10
Aqueous phase	Gel Pluronic F-127 c.s.p 100 mL (Pluronic F127® 20% distilled water c.s.p 100 mL)	

The products used as components of PLO were

1. Distilled de-ionized water, Interapothek
2. Pluronic® F-127: Batch number 0507548, water content <0.01%, Ratio oxypropylene–oxyethylene 71.5–74.9%, propylene oxide content <1 ppm. Supplied by Roig Farma-Fagron.
3. Isopropyl Palmitate: Batch number 0406166, Concentration > 90%, supplied by Meta (Roig Farma-Fagron).
4. Granulated Soya Lecithin: Batch number 0501092, water content 1.2%, concentration 97%, pH (sol. 1%) 7. Supplied by Roig Farma-Fagron.

Methods

Two liposome formulations have been designed whose constitution, molar proportions, and quantities of each component, expressed in mmoles/100 mL and mg/100 mL; are shown in Table 1. These formulae are chosen based on the optimum results previously obtained (Clares & Medina, 2007). With the aim of obtaining maximum stability, liposome formulations of PC of eggs were prepared which contained CH in their lipidic matrix, at a molar concentration of 50% with respect to the proportion of phospholipid.

PLO is formed of Pluronic F-127 gel to 20% distilled water and lipids coming from soya lecithin. In PLO, there are two phases: one aqueous phase (20%) composed of the previous gel and a lipidic phase composed of granulated soya lecithin and isopropyl palmitate (80%) (Table 2).

Samples Preparation

Liposomes. The multilaminar liposomes have been obtained by the Bangham method (Puisieux, 1978), attempting to normalize conditions, under which, finally, came the preparation. This method consists of different phases. The first phase consists fundamentally of solubilizing all the components of the lipid wall. The solvent used should be volatile, to allow its elimination during the second phase of the method. Chloroform was used as a volatile organic solvent in a sufficient quantity for completely dissolving the lipids. A chloroform solution of the phospholipid is obtained as a

result (phosphatidylcholine of egg) and of the rest of the components of the lipidic bilayer (CH). Evaporation of the solvent is carried out in a Büchi rotavapor (model R-110), operating under vacuum at maximum speed of turn of the apparatus, for the time necessary for completely drying the lipids, and forming a lipid film clinging to the walls of the matrix. The working conditions remain constant in all cases, the temperature of the thermostatic bath at 37°C. Finally, the aqueous phase of the system was added (water). Hydration of the lipid system was brought about by continuous stirring at 300 rpm for 30 min, at a temperature greater than that of the transition phase of the gel to phospholipid liquid crystal (37°C for the phosphatidylcholine of egg), producing hydration of the lipid film that fell from the walls of the receptacle, with the formation of a liposome suspension in the excess aqueous solution, which was left to stand for 24 h at 4°C to facilitate vesicle formation and growth.

PLO. The preparation of the gel is finally carried out, adding the Pluronic® F-127 to the distilled water, leaving it to stand in a fridge at 4°C, stirring periodically for 24 h to ensure it dissolves. After 24 h, the volume of the solution is corrected with distilled water. As far as the preparation of the PLO is concerned, to obtain the oily phase, the granulated soya lecithin is mixed with the isopropyl palmitate at room temperature and left to settle until it completely dissolves. The aqueous phase is incorporated into the oily phase by rapid stirring. It is important that the aqueous phase is cold when mixing it with the oily phase.

Liposomes–PLO. Two formulations have been designed with respect to the presence or absence of CH in the liposomal suspension. The liposomes and PLO are added in equal parts. On suspension of the liposomes already prepared, Pluronic F-127 is added and left to stand in a fridge at 4°C, stirring slowly for 24 h to ensure it dissolves. After 24 h, the volume of the solution is corrected with distilled water. The aqueous phase is incorporated into the mixture of granulated soya lecithin and the isopropyl palmitate at room temperature.

Samples Characterization

The formulations have been characterized by negative staining and later inspection by electronic transmission microspcopy. The structure has been observed and the measurements assessed for the forms of controlled release, object of the trial.

For characterization, prepared samples are taken 24 h, 7, 30, 60, and 90 days after their preparation, with the aim of observing the effect of time on the structure, dimensions, and level of aggregation of the liposomes, vesicles of PLO, and the mixture of both. The samples have been kept at 4°C until the moment of characterization.

For transmission electron microscopic (TEM) observation, samples were prepared with negative staining with uranyl acetate according to a modification of a previously described method (Hayat, 1989). Copper grids coated with formvar (Ernest F. Fullam, Latham, NY, USA) were incubated with liposomes or PLO for 4 min, then washed in two 1-min changes of ultrapure water. The grids were then stained with a 2% aqueous solution of uranyl acetate for 1 min and dried at 37 C. All samples were observed in a Zeiss TEM 902 transmission electron microscope at 7,000–14,000×.

RESULTS AND DISCUSSION

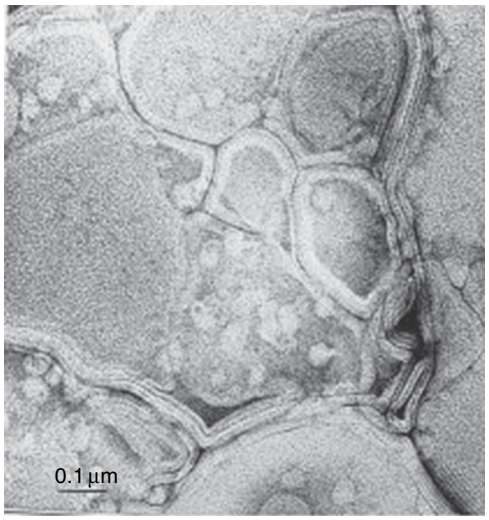
Characterization using negative staining and TEM has brought the following results. In Table 3, the average values of size of particle (μm) evaluated for the trial formulations, accompanied by their respective standard deviations, are shown. The diameters have been determined, at the time of preparation and 30, 60, and 90 days after the same, with the aim of checking the effect of passing time on the dimensions of these vectors. The number of measurements taken has been 100 per formula, series, and period of time. The samples have been kept at refrigeration temperature (4–6°C) and protected from light until the moment of their characterization.

Figure 1 corresponds to a microphotograph of a suspension of multilaminar liposomes made up of PC 24 h after preparation. These vectors have an average diameter of 0.25 ± 0.13 μm.

TABLE 3
Changes in Vesicle Size in Liposomes and in Vesicle Size in Pluronic Organogel Formulations
(μm ± *SD*/*n* = 100)

Liposomes (PC)	0.25 ± 0.13	0.3 ± 0.14	0.34 ± 0.27	0.16 ± 0.06
Liposomes (PC:CH)	0.40 ± 0.10	0.42 ± 0.18	0.45 ± 0.17	0.31 ± 0.14
PLO	0.043 ± 0.0046	0.16 ± 0.0056	0.61 ± 0.083	0.88 ± 0.13
PLO–liposomes				
Liposomes 1	0.18 ± 0.12	0.2 ± 0.10	0.3 ± 0.14	0.15 ± 0.09
PLO	0.3 ± 0.16	0.53 ± 0.14	0.5 ± 0.13	0.64 ± 0.10
Liposomes 2	0.33 ± 0.12	0.3 ± 0.11	0.40 ± 0.14	0.2 ± 0.08
PLO	0.35 ± 0.15	0.50 ± 0.06	0.6 ± 0.10	0.7 ± 0.17
Time	24 h	30 days	60 days	90 days

CH, cholesterol; PC, phosphatidylcoline; PLO, pluronic lecitin organogel.

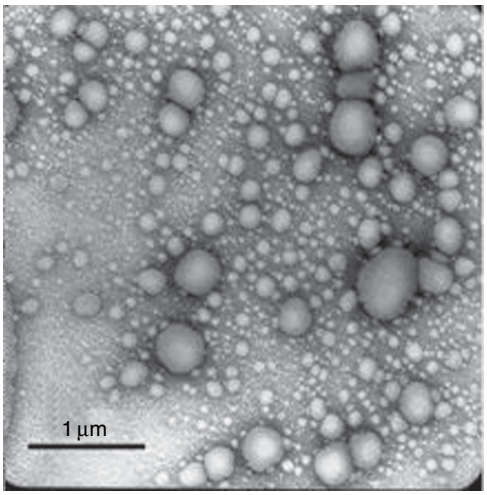


(1)

FIGURE 1. Transmission electronic microscopy photomicrograph of liposomes 24 h after preparation (140,000×).

In the figure, the phospholipid bilayers can be seen alternating with aqueous compartments, as well as a central polar area. The diffusion of the coloring used (uranyl acetate at 2%) reveals the existence of transparent areas on electron bombardment (areas in which the coloring has not been able to reach) and electrodense areas that denote the presence of heavy metal.

For this reason, the hydrophobic layers of the vesicles behave as though transparent, whereas the polar parts, concentric to the previous ones, correspond to the electrodense areas, which appear dark to us. On the contrary, Figure 2 corresponds to a microphotograph of a PLO sample 7 days after preparation. These vesicles have an average diameter of 0.10 µm. A hydrophilic layer is observed, which is composed of molecules of Pluronic F-127 around a central lipid compartment made up of soya lecithin chains. The diffusion of the coloring used (uranyl acetate at 2%) reveals the existence of transparent areas on electron bombardment (areas in which the coloring has not been able to reach) and electrodense areas that show the presence of heavy metal. For this reason, the central compartment behaves as though transparent, whereas the polar part corresponds to the electrodense areas, which appear dark to us.



(2)

FIGURE 2. Transmission electronic microscopy photomicrograph of pluronic lecithin organogel 7 days after preparation (20,000×).

The design of an efficient formulation requires a deep knowledge of the structure of liposomes and PLO; therefore, after the study of characterization, we will know where the active principles will be located depending on their solubility.

In liposomes, the hydrophilic substances occupy the aqueous compartment, whereas the lipophils are situated between the phospholipids of the wall, and the anphiphilics are joined by the rest of the lipophils at the bilayers (Puisieux, 1978).

Contrarily in PLO, on incorporating the soya lecithin into a solution of Pluronic F-127, the surfactant micelles group up around the hydrophobic tails of the phospholipids, forming a vesicle with a hydrophilic surface, where the hydrosoluble active principles are situated, around a hydrophobic compartment that would be capable of carrying liposoluble active principles (Kim, Jeong, Bae, & Lee, 1997).

After clarifying the structure of both formulations, we can get a better idea on the interactive mechanism with the skin. In the intercellular spaces of the stratum corneum, a lipidic bilayer is found that is mainly in gel phase and makes up a barrier, limiting passage of molecules to the skin (Elias, 1983). The intercellular spaces are very narrow, which is not sufficient for letting particles pass through with an important diameter like those of the liposomes. However, owing to their composition, the liposomes can modify the permeability of the lipid bilayer and as a consequence the cutaneous penetration of the active molecules.

It is worth mentioning that in this way, the structure of these transporter systems modifies the subcutaneous lipid fluidity, facilitating absorption of drugs into the skin. The unsaturated fatty acids present in phospholipids (such as phosphatidylcholine surfactants) present in the soya and egg lecithin are those responsible for a greater absorption than the active principle (Valenta, Wanka, & Heidlas, 2000; Valljakka-Koskela, Kirjavainen, Mönkkönen, Urtti, & Kiesvaara, 1998).

Generally, it is important to consider that the pluronic F-127 molecules, as well as the phospholipids, which are found on the vesicle walls and which form PLO, alter the binding structure of the stratum corneum, thus facilitating the passage of the active substance. To facilitate penetration of the active substance, the lecithin, the barrier function of the skin is modified so as to (Hoffman, Yoder, & Trepanier, 2002)

- eliminate the hydrolipidic layer: the soya lecithin can exfoliate the stratum corneum over time;

- facilitates absorption: the soya lecithin has considerable surface activity given its surfactant properties, for which reason the lipid barrier of the stratum corneum can become distorted, thereby increasing its permeability.

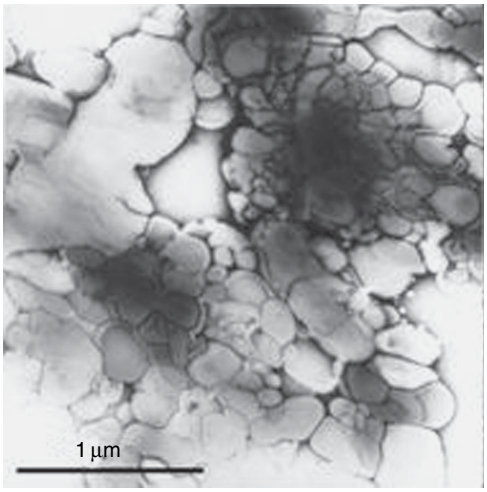
In principle, the liposomes have a stable structure given that they have phospholipids present in water to the most stable state thermodynamically. However, their conservation is a complex phenomenon. Physically, the main problem consists of the aggregation of liposomes, which modifies the permeable properties of the lipid wall. We observe, as after 30 days, that the liposomes aggregate progressively, losing their individuality (Figure 3). The same does not occur with PLO. All the PLO vesicles are characterized by a smooth outline. The grade of vesicular fusion is negligible throughout the period of study; however, 60 days after preparation, a delicate aggregation occurs and, in spite of everything, destruction of the vesicles is not seen and the vesicles keep their well-defined structure (Figure 4).

On the contrary, when CH is included in the lipid matrix of the liposomes of the previous formula the aggregation is slightly less 30 days after preparation (Figure 5). From the above it may be deduced that the changes in the structure and morphology of the liposomes are reduced in part because of the incorporation of this sterol.

The formulae with CH are characterized as having a lower breaking index than the vesicles and a smoother outline; the preparations without CH show a bumpy, porous outline on their lipid bilayers (Figure 6).

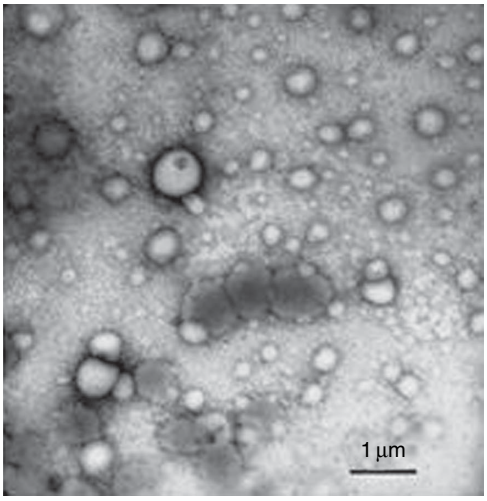
With the aim of improving stability of the system, CH is usually incorporated into the lipid bilayer, detecting a decrease in permeability of the liposome membrane (Kunikazu, Kazuo, & Motoharu, 2000; Virtanen, Ruonala, Vauhkonen, & Somerharju, 1995). Therefore, the liposomes tend to fuse together, even with this sterol, 90 days after their preparation (Figure 7).

The topical administration of active principles on the skin with the aim of achieving a systemic and reservoir effect has led to pharmaceutical development of a new form of dosage made from liposomes and PLO. The method employed demonstrates the existence of the two forms, that are the object of this trial, in one same formula (Figure 8). These multilaminar and unilaminar structures, characteristic of the liposomes and PLO,



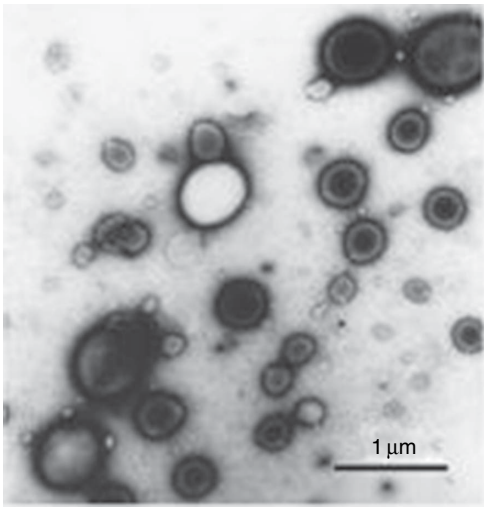
(3)

FIGURE 3. Transmission electronic microscopy photomicrograph of liposomes (PC) 30 days after preparation (30,000×).



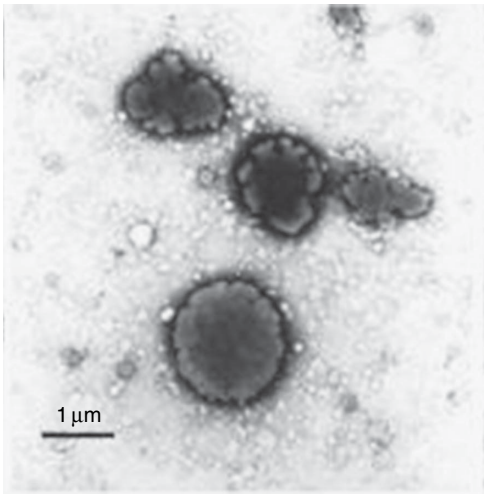
(4)

FIGURE 4. Transmission electronic microscopy photomicrograph of liposomes–PLO (pluronic lecithin organogel) 90 days after preparation (12,000×).



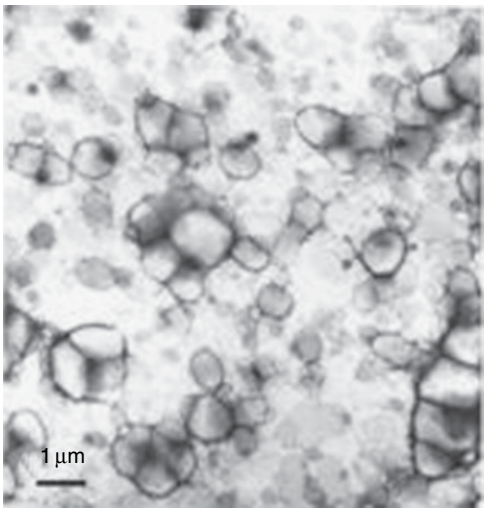
(5)

FIGURE 5. Transmission electronic microscopy photomicrograph of liposomes (PC:CH) 30 days after preparation (20,000×).



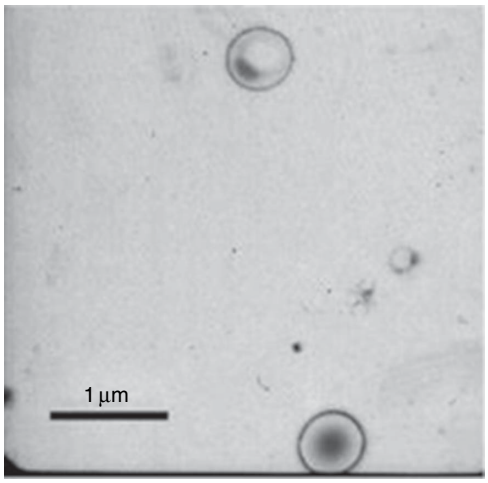
(6)

FIGURE 6. Transmission electronic microscopy photomicrograph of liposomes (PC) 90 days after preparation (12,000×).



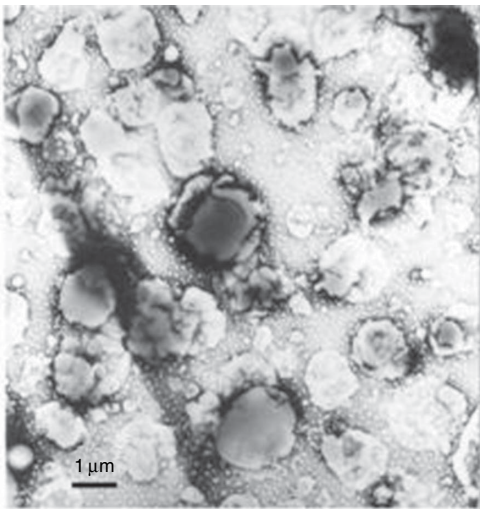
(7)

FIGURE 7. Transmission electronic microscopy photomicrograph of liposomes (PC:CH) 90 days after preparation (7,000×).



(8)

FIGURE 8. Transmission electronic microscopy photomicrograph of liposomes–PLO (pluronic lecithin organogel) 24 h after preparation (12,000×).



(9)

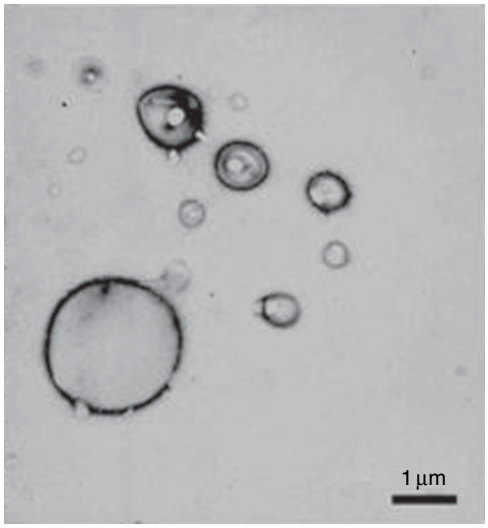
FIGURE 9. Transmission electronic microscopy photomicrograph of liposomes 1 year after preparation (12,000×).

continue with the passing of time. The results of characterization do not show any structural liposomes–PLO interaction.

The final microphotographs selected are a representative example of the effects that time has had on the structure and stability of the transport system. With the naked eye, we can see the change produced on the liposome membrane: extremely rough 12 months after preparation (Figure 9). On the contrary, the vesicles of PLO maintained a more defined structure than the liposome formulae (Figure 10) although their size is bigger at 1 μm.

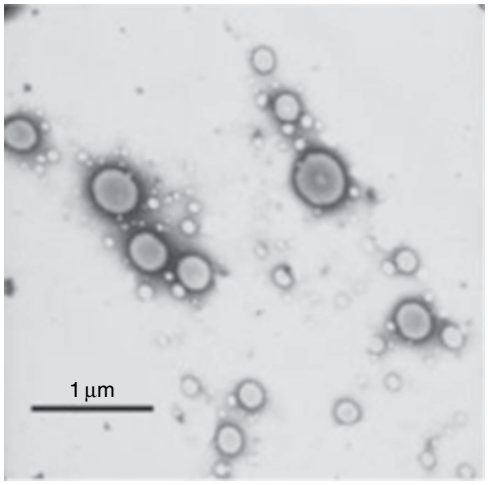
The liposome formulae, along with the PLO, keep their individuality and integrity during the whole trial period (Figure 11). That is, in the presence of PLO, the liposomes, as much those with CH as without sterol, keep their structure defined and their homogeneity. Therefore, the stability of the liposomas increases when they are formulated next to PLO, and PLO presents a more reduced size when it is formulated next to liposomas.

The study of characterization of liposomes and PLO as controlled release systems has included, as well as their microscopic inspection, determination of size of particle. The aim of



(10)

FIGURE 10. Transmission electronic microscopy photomicrograph of PLO (pluronic lecithin organogel) 1 year after preparation (3,000×).



(11)

FIGURE 11. Transmission electronic microscopy photomicrograph of liposomes–PLO (pluronic lecithin organogel) 90 days after preparation (4,400×).

this analysis is to evaluate the possible incidence of components in the formulation or interaction between both systems on the size and state of aggregation of different vesicles.

It has been shown how the average diameter of individualized liposomes, formulae with or without CH, and their stability properties vary from one sample to the next. Generally, the liposome formulae increase in diameter with time; however, 90 days later, the diameters decrease owing to rupture. Therefore, in formulae with CH, the bilayer is more fluid and so the formation of small pores and ruptures is reduced.

In both formulae, significant differences can be seen in average diameter with passing of time, reaching values from 0.043 to 0.88 μm and 0.25 ± 0.13 to 0.16 ± 0.06/0.40 ± 0.10 to 0.31 ± 0.14 for PLO and the liposomes with or without CH, respectively.

On analyzing the influence of PLO over the size of the liposomes, this is slightly less than when they are formulated separately.

CONCLUSIONS

Characterization by electronic transmission microscopy has allowed verification of the formation of the hydrolipids, unilaminar vesicles for PLO and plurilaminar vesicles in the case of liposomes, denoting the efficacy of the release method employed. Likewise, information has been gleaned on the structure, dimensions, and stability of these drug transporters. Given their structure, both systems can transport as many liposoluble active principles as hydrosoluble ones.

In accordance with the methods carried out, they experience an increase in size with the passing of time, not exceeding 1 μm in any case (90 days). Furthermore, the liposomes prepared with CH keep a higher level of individuality and a more spherical shape during the trial period. Equally, the formulation of PLO and Liposomes turns out to be stable, with the observation of defined, homogeneous, and nonfusioned structures. The structural integrity of the formula liposomes–PLO stays during long periods of time (18 months).

Of the abovementioned, it is deduced that the changes in the structure and morphology of the liposomas decrease partly thanks, to the incorporation of PLO.

ACKNOWLEDGMENTS

Part of this work was supported by MEC Spain and Feder founds, under Project MAT 2005-07746C02-02 02 and project of Excellence FQM 410.

REFERENCES

Banciu, M., Schiffelers, R. M., Fens, M. H., Metselaar, J. M., & Storm, G. (2006). Anti-angiogenic effects of liposomal prednisolone phosphate on B16 melanoma in mice. *J. Control. Release, 113*(1), 1–8.

Clares, B., & Medina, M. M. (2007). Liposomal triamcinolona acetónido: Influence of composition in performance and stability. *Ars Pharmaceutica., 48*(1), 19–29.

Elias, P. M. (1983). Epidermal lipids, barrier function and desquamation. *J. Invest. Dermatol., 80*, 445–495.

Hayat, M. A. (1989). *Principles and techniques of electron microscopy: Biological applications* (3rd ed.). London: Macmillan press published.

Hoffman, S. B., Yoder, A. R., & Trepanier, L. A. (2002). Bioavailability of transdermal methimazole in a pluronic lecithin organogel (PLO) in healthy cats. *J. Vet. Pharmacol. Ther., 25*(3), 189–193.

Jain, S. K., Jain, R. K., Chourasia, M. K., Jain, A. K., Chalasani, K. B., Soni, V., & Jain, A. (2005). Design and development of multivesicular liposomal depot delivery system for controlled systemic delivery of acyclovir sodium. *AAPS Pharm. Sci. Tech., 6*(1), 35–41.

Kim, S. W., Jeong, B., Bae, Y. H., & Lee, D. S. (1997). Biodegradable block copolymers as injectable drug-delivery system. *Nature, 388*, 860–862.

Kunikazu, M., Kazuo, M., & Motoharu, I. (2000). Spectroscopic investigation of the molecular state of nystatin encapsulated in liposomes. *Int. J. Pharm., 15, 201*(1), 37–49.

Maierhofer, G. (1988). Introducción a una nueva tecnología y descripción de técnicas preparativas. *Farm. Clin., 5*, 658–680.

Puisieux, F. (1985). Microparticulate and nanoparticulate vectors. In P. Buri, F. Puisieux, E. Doelker, & J. P. Benoit (Eds), *Formes pharmaceutiques nouvelles*. Paris: Technique & Documentation (Lavoisier).

Puisieux, F. (1978). Les liposomes: Classification et obtention. *Lab. Pharma Probl. Tech., 281*, 899–904.

Puisieux, F., & Delattre, J. (1985). Technological problems posed by the use of liposomes as vectors of medicinal substances, encapsulation, sterilisation, conservation. In *Les liposomes, applications thérapeutiques*. Paris: Technique & Documentation (Lavoisier) pp. 73–113.

Torrado, J. J., & Cadorniga, R. (1989). Nuevos sistemas de administración de medicamentos. *Vectorizacion. CIF, 8*, 242–248.

Valenta, C., Wanka, M., & Heidlas, J. (2000). Evaluation of novel soya-lecithin formulations for dermal use containing ketoprofen as a model drug. *J. Control. Release, 63*, 165–173.

Valljakka-Koskela, R., Kirjavainen, M., Mönkkönen, J., Urtti, A., & Kiesvaara, J. (1998). Enhancement of percutaneous absorption of naproxen by phospholipids. *Int. J. Pharm., 175*, 225–230.

Virtanen, J. A., Ruonala, M., Vauhkonen, M., & Somerharju, P. (1995). Lateral organization of liquid-crystalline colesterol-dimyristoyl-phosphatidylcholine bilayers. Evidence for domains with hexagonal and centered rectangular cholesterol superlattices. *Biochemistry, 34*, 11568–11581.

Willian, H., Walde, P., Luisi, P. L., Gazzaniga, A., & Stroppolo, F. (1992). Lecitin Organogel as matrix for transdermal transport of drugs. *J. Pharm. Sc., 81*, 871–874.