Development of a liposomal suspension to increase the skin penetration of Diclofenac

Gherrak Fouzia[1], Djilali Khadija[1], Hadj Sadok Abdelkader[1]
1: Laboratory of Functional Analysis of chemical processes, University of Blida1, gfoguerra@hotmail.fr

Abstract

The objective of this work was to develop a liposomal suspension encapsulating a non-steroidal anti-inflammatory which will constitute the dispersed phase of a gel for dermic use. Diclofenac preparations for dermal application exist only under the gel and emulgel forms. This liposomal form has been proposed in order to increase the skin penetration or even the availability of Diclofenac due to the phospholipid bilayer of the liposome having the same structure as the skin for cutting the barrier (horny layer).

Liposome was formulated from soy lecithin, cholesterol, by the inverse phase evaporation method then optimized and characterized. After then a study of the influence of the excipients used in the basic formulation on certain physicochemical properties (particle size distribution and zeta potential) of the system, was carried out. An experimental design and a response surface methodology were carried out using MODDE 6.0 software. The determined optimum was characterized by a maximum amount of ethanol in relation to water combined with maximum levels of cholesterol and lecithin, promoting the production of small-sized liposomes containing a relatively high surface charge (zeta potential) and having only a narrow polydispersity, thus meeting the stability criteria favoring transposition on an industrial scale.

Keywords: *Formulation, liposomes, encapsulation, bioavailability, stability.*

I. Introduction

To be efficient, an active substance must be delivered at the correct site of action at an effective concentration and for a sufficient period of time. These objectives constitute the real challenges of an aimed formulation mode to modify the skin permeability. Several formulation strategies for modifying the skin permeability and for improving the activity of an active compound can be envisaged.

Diclofenac diethylamine, a non steroidal anti-inflammatory drug is widely prescribed to treat mild and moderate pains, inflammation and osteoarthritis by inhibiting the enzyme cyclooxygenase. It is already marketed in various forms (gel, emulgel, ophthalmic solution, immediate and prolonged release tablet, suppositories and intramuscular injection). Furthermore 40% of Diclofenac administered orally undergoes first pass metabolism and does not reach the systemic circulation [1]. Because of its low solubility in water and in the acidic environment of the stomach, Diclofenac has a low availability and a short half-life of about two hours. [1] A topical application for the treatment of rheumatic diseases is therefore suggested to increase bioavailability and decrease toxicity [2]

Diclofenac diethylamine is not readily absorbed during trans dermal application; the use of liposomes, spherical particles composed of lipid bilayers closed on themselves, as encapsulation system, is proposed.

Liposomes are vesicles of tens to a thousand nanometers (nm) in diameter. These vesicles are composed of one or more lipid bilayers, most often of a phospholipid nature, making it possible to isolate one or more aqueous internal compartments from the external aqueous medium. Liposomes have long been considered synthetic analogs of living cells [3]. Their interest in the pharmaceutical field lies mainly in the possibility of vectorizing substances either by inclusion in the lipid membrane or by encapsulation in the internal space. It is therefore possible to encapsulate active ingredients of very different solubility (hydrophilic, amphilic, lipophilic). Knowledge of their behavior in vivo then allowed their use in the treatment of certain pathologies [4].

On the other hand, several advantages are linked to the encapsulation of an active ingredient in liposomes such as: time-controlled release, maintenance of active ingredient integrity, decrease in the toxicity of the active ingredient, and solubilization of the active ingredient poorly soluble in water.

In the 1970s, several studies established the concept that liposomes can encapsulate substances and be used as transport systems [5- 7]. Other studies have shown that liposomes can change the in vivo distribution of encapsulated drugs [8, 9]. In parallel, methods have been developed to allow the preparation of large unilamellar liposomes (LUV) with improvements in encapsulation efficiency and homogeneity [10]. The production of LUVs by extrusion of multilamellar vesicles (MLVs) through polycarbonate membranes with pores of dimensions of 100 nm or less has been a very important advance. [11]

In the 1980s and 1990s, advances in the formulation of liposomes were obtained in order to prolong their lifetime in the bloodstream. Stealth liposomes having a steric barrier created around the lipid bilayer were most often distinguished by hydrophilic polymers such as polyethylene glycol (PEG) coupled by covalent bonds to membrane phospholipids [12, 13]. Immuno liposomes that carry antibodies specific to antigenic targets on their surface and can also be furtive [14, 15], specifically bind to the surface of target cells (eg tumor cells) [14].

At the same time, LUVs have been used as a membrane model to study the effect of certain molecules of biological, pharmacological or therapeutic interest [17,18,19] or certain factors such as the size and composition of liposomes [20,21] on the permeability of the biological membrane. They are also used to study the effect of certain bioactive molecules on the morphology and surface properties of lipid membranes [22].

A robotic approach for the construction of an artificial cellular model at the molecular level has recently been proposed [23]. This model is based on the giant liposomes whose membrane is known as the simplest model of living cell membrane [24]. The so-called "molecular robotics" system consists of a giant liposome (GUV) encapsulating a gene to allow the synthesis of membrane proteins, an artificial DNA-based molecular formula used to attach the liposomes to the membrane of Living cells and membrane and artificial peptide molecules that enter the surface recognition and cell signaling processes [23].

II. Material and method

All the raw materials used in the formulation were as follows: Soy Lecithin, Cholesterol, Ethanol 96%, Diclofenac Diethylamine, Purified Water and Propylene Glycol.

An experimental protocol was established following the inverse phase evaporation method where the organic phase containing the soya lecithin and the cholesterol dissolved in ethanol was prepared at the same time as the aqueous phase containing the Diclofenac dissolved in the purified water and the propylene glycol with stirring.

The organic phase was then incorporated into the aqueous phase dropwise by means of a syringe while maintaining the stirring until a milky solution was obtained. This was then sonicated for 5 minutes before evaporating the ethanol in a rotary evaporator at 55°C. The liposomal suspension obtained at the end was stored at 4°C to undergo the characterization test

The experimental design method with objective surface modeling was used. The factors being independent, it was opted for a fractional factorial box Bhenken type for which the criteria were statistically met. This plan proposed 15 tests, three of which were repeatable and which were located at the center of gravity. The corresponding polynomial model is of quadratic type with interaction of order 1 and is expressed as follows:

$$\boldsymbol{Yi = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2} \quad (1)$$

where: $\mathbf{Y}_{i:}$ represents the response, $\mathbf{b}_{i:}$ represents the model coefficients, X_1 X_2 X_3 are the reduced centered variable factors respectively associated with reporting: ethanol / water, lecithin / ethanol, cholesterol / lecithin, respectively.

The area of interest of the controlling factors is: cholesterol [10% - 50%], soy lecithin [1% - 5%], ethanol [5% - 100%].

In the present case the study of the influence of three parameters was considered. Others factors such as manufacturing process, amount of the aqueous phase and amount of Diclofenac diethylamine, were frozen.

Using the procedure described above, we performed the tests of this matrix.

II.1 Scanning Electron Microscopy (SEM) observations

To confirm the formation of the liposomes, it was necessary to use an adequate technique to be able to observe morphologically the vesicles.

II.2 Size, polydispersity, and zeta potential

The 10-fold dilutions in distilled water were made on samples taken from the obtained liposomal suspensions. Measurements of the average diameter (nm) of these samples were determined at 25°C and at a diffraction angle of 90 degrees. The polydispersity index (PI) was also determined. The results were expressed for each sample by the mean or the Z-average.

II.3 Encapsulating rate of diethylamine Diclofenac

The incorporation rate was determined after separation of vesicles in an ultracentrifuge type SIGMA 3-30 K at high speed 15500 rpm for 6 hours at a temperature maintained at 4°C.

Standard solutions of Diclofenac diethylamine were prepared with a concentration of 30 mg in 100 ml of methanol. The amount of Diclofenac in the sediments was determined after solubilization of the vesicles in 10 ml methanol which were then filtered and injected. The supernatant was filtered and then injected directly.
In a first stage, the mobile phase was allowed to circulate in the column of the apparatus for at least 30 minutes. Following this, a volume of 20 µl was injected separately as a standard solution, along with 5 µl of solution containing the sample, starting with the pellet and then the supernatant, using a suitable micro-syringe. The same procedure was applied for the other samples.

II.4 Calculation procedure

The peak areas of the supernatants of the samples and of the standard solution were measured. The amount of Diclofenac not trapped in the supernatants is calculated by the following formula:

$$[\mathbf{Diclofenac}]_{\mathbf{sample}} = \frac{\mathbf{4} \times \mathbf{S_{sp}} \times [\mathbf{C}]_{\mathbf{std}}}{\mathbf{S_{std}}} \times \mathbf{50} \quad (2)$$

With S_{std} the surface of the standard peak, S_{sp} the sample peak area, $[C]_{std}$ the concentration of standard in mg /mL and $[Diclofenac]_{sample}$ the concentration of the sampled in mg / 50 mL, the factors 4 and 50 are the volume multiplication coefficient and the total volume of the liposomal suspension, respectively.

The trapping efficiency was determined by the following equation:

$$\%\ encapsulation = \left(\frac{totale\ Qt - non\ encapsulated\ Qt}{totale\ Qt}\right) \times 100 \quad (3)$$

With Total Qt and Non encapsulated Qt the theoretical amount of drug used to prepare the formulation (2g) and the amount of drug in the supernatant, respectively.

III. Results and discussions

The SEM image shows a deformable spherical form and of nanometric sizes. The deformation is due to the collapse of the liposomes, deposited on the plate, subjected to the solid (glass) - liquid (liposome) interfacial tension force and to the action of gravitation, like a drop of d Water deposited on a clean support of relatively low interfacial tension.

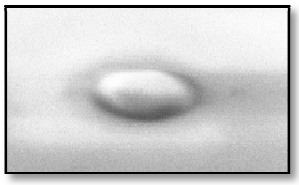

Figure 1. SEM image of a liposomal vesicle

II.5 Study of the effects and interactions of factors on responses

At the end of the responses surface modeling it was proposed to determine the effect of the interaction of the factors (cholesterol, soy lecithin and ethanol) on each characteristic retained in the liposomal suspensions formulated.

• Ethanol / cholesterol interaction

Figure (2.a) shows that at low levels of cholesterol, the ethanol ratio decreased the size and its effect was positive. When the cholesterol level reached its maximum value, the same effect was found, but with a less pronounced slope, the cholesterol-ethanol interaction was negative.

• Ethanol / lecithin interaction

Figure (2.b) shows that at low level of lecithin, the ethanol/water ratio had no effect on the size because this size remained constant. When the level of lecithin rose to its maximum value, there was a

very remarkable decrease in the size of the liposomes and therefore the interaction between ethanol and lecithin was beneficial.

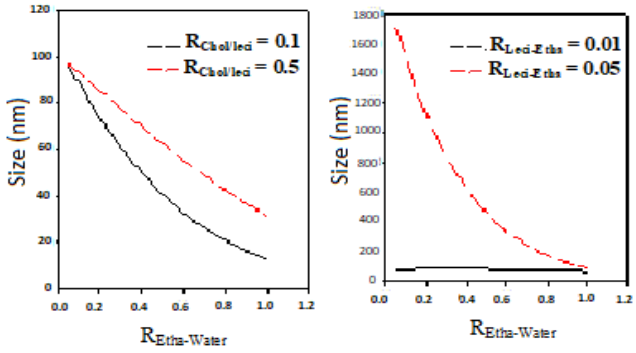

Figure 2. Effect of ethanol with lecithin and cholesterol on the size.

Similarly, the effects of the interactions of the three factors on the other responses (zeta potential, polydispersity index) were determined and the three other responses (size, surface charge and polydispersity) are illustrated by the iso-response contours associated to determine the optimal area:

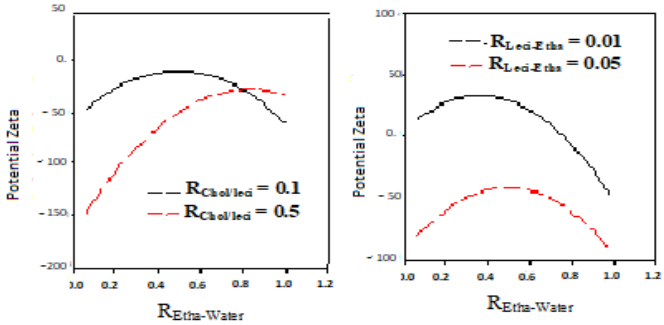

Figure 3. Effect of ethanol with lecithin and cholesterol on zeta potential.

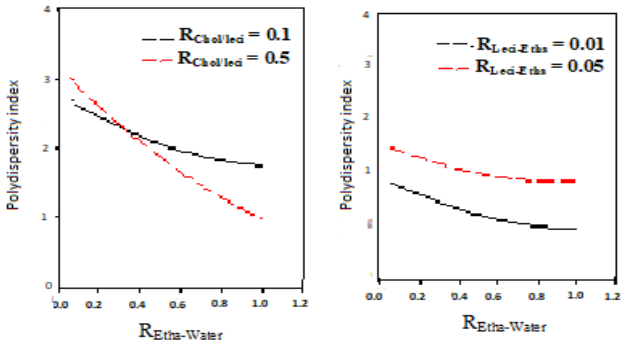

Figure 4. Effect of ethanol with lecithin and cholesterol on polydispersity.

II.5.1 Optimum at low Ethanol/water ratio

Following the phase of surface modeling, a scan of the three control factors was carried out, in order to identify the optimal areas that correspond to:

- Minimum sizes to allow penetration through the skin;
- Zeta potential values greater than 30 mV in absolute value, to confirm a satisfactory stability for liposomes, electrostatic repulsive interactions dominate van der Waals attractive ones;
- Areas with satisfactory polydispersity indices with the lowest possible values.

The optimal zones were identified by the software MODDE6.0 and are presented in the form of maps on which iso-response curves (contours) are plotted. It is then proposed to represent these contours at the three ethanol levels *i.e.* low, medium and high, which gives in numerical values:

Low ethanol/water = 0.05

Ethanol/water medium level = 0.525

Ethanol/water high level = 1

The region, in which the size must have relatively small values, in order to facilitate the penetration of the liposomes through the skin, is very narrow. It corresponds to the domains of the two factors: cholesterol/lecithin ratio of 0.40 and lecithin/ethanol ratio <0.022

For the zeta potential, and in order to avoid the field in which this magnitude was less than 30mV, in absolute value, it was noted that the only possibility available, in view of the mapping of the zeta potential, was delimited in the area of cholesterol / lecithin ratio <0.40 and lecithin/ethanol ratio of 0.020

Finally, on the basis of the polydispersity iso-index mapping, no solution was available since all values of IP were greater than 2.

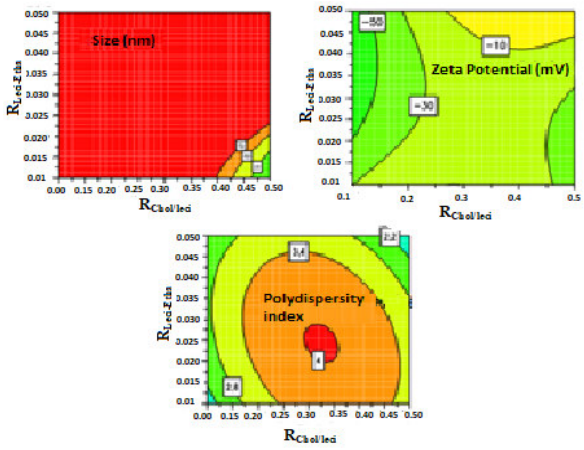

Figure 5. Iso-response contours at low Ethanol level

Consequently the results showed that when using a low ethanol-water ratio, there was no optimum.

II.5.2 Optimum for medium Ethanol/Water ratio

The region, in which the size must have relatively small values, less than 50nm, corresponds to two domains characterized by a cholesterol to lecithin ratio($R_{Chol/Etha)}$ greater than 0.42 or less than 0.15 and a lecithin to ethanol ratio ($R_{leci/Etha}$) greater than 0.015 or less than 0.040

For the zeta potential, the domains are characterized by $R_{Chol/leci>}$ 0.40 or <0.25 and $R_{leci/ Etha}$ <0.0125.

Finally, in terms of iso-polydispersity index, a very narrow area matching IP 1, the domain is characterized by $R_{Chol/leci>}$ 0.45 and $R_{leci / Etha>}$ 0.045.

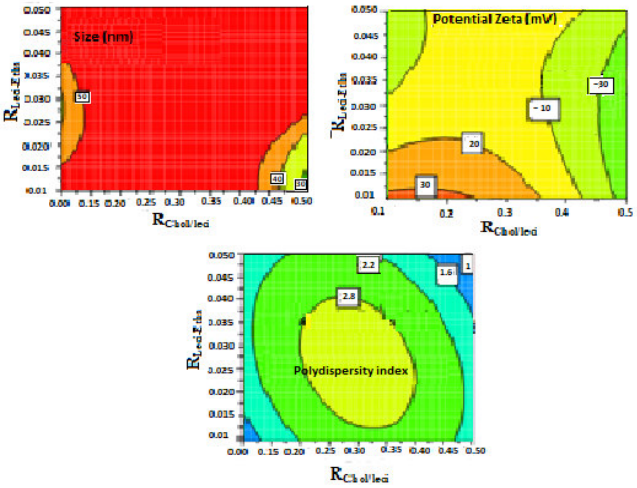

Figure 6. Iso-response contours at medium Ethanol level

In order to satisfy the three optimal domains simultaneously, at a medium Ethanol level their intersection had to be determined, but no solution was obtained.

II.5.3 Optimum for high Ethanol/Water ratio

The region in which the size must have relatively small values <50 nm corresponds to several domains made of all the iso-regions except those shown with a red color.

With respect to the zeta potential the optimum is in the region characterized by $R_{Chol/leci}$ <0.30 and $R_{leci/Etha}$ <0.024.

With respect to the PI the optimum region was characterized by $R_{Chol/Leci>}$ 0.40 and $R_{leci/ Etha>}$ 0.035.

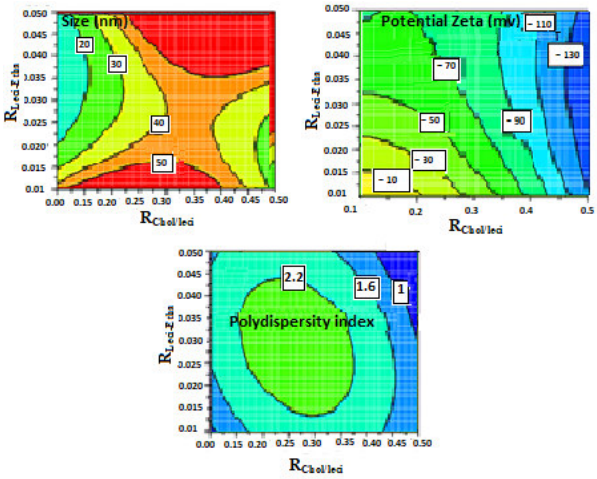

Figure 7. Iso-response contours at high Ethanol level

To satisfy the three optimal domains their intersection had to be considered and it is characterized by $R_{Cho/Leci>}$ 0.40 and $R_{Leci/ Etha>}$ 0.035.

III. Conclusion

Basing on the surface modeling responses, using the PLS (Partial Least Square) method, predictive surface response models were obtained, for the hydrodynamic size of the liposomes, the zeta potential and the polydispersity index. These three responses are all important and strongly condition the bioavailability, stability and homogeneity of the dispersed system, respectively. It should be noted that in this work, the response rate of encapsulation was not retained, given the obtained results which were not in adequation with the ratio: liposome sediment and supernatant, separated by ultracentrifugation. This technique was inspired from the monograph of a commercially available EMULGEL® emulsion but was not successful.

The use of the three RSM models, by numerical simulation, made it possible to highlight the effects of each factor and its interactions on the three responses and the cartographies in terms of iso-response contours. It would seem that a maximum amount of ethanol with respect to water combined with maximum levels of cholesterol and lecithin favored the production of small liposomes containing a relatively high surface charge (zeta potential) and having only a narrow polydispersity.

This study was far from completed it would be interesting and necessary to improve the method of analysis in order to optimize its operating conditions for the liposomal form and to propose a galenic form for dermal use of semi-solid type to

the optimum formulation, referring to the physicochemical and rheological characteristics.

Acknowledgments

The authors would like to thank Professors Khelifa Professor and Ait Mesbah of the University of Blida 1 as well as Mr Rekab of the quality control laboratory of PRODIPHAL.

Abbreviations

HPLC: High Performance Liquid chromatography.
IR: Infrared
PI: Polydispersity index
LUV: Large Unilamellar Vesicles.
MLV: multilamellar Vesicles
MVV: Multivesicular Vesicles.
nm: nano-meter.
OLV: Oligolamellar Vesicles
Qt*:* quantity
REV: Reverse Phase Evaporation Vesicles.
SUV: Small Unilamellar Vesicles.

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