Liposomes, Formulation and Pharmacotechnical Assessment of Anti-Acne Preparations

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The present study aims at obtaining an efficient local formulation able to ensure both stability and increased penetration of the active ingredients in effective optimal concentrations without side-effects. The novelty of this study is the association in an encapsulated form (liposomes) of tretinoin to benzoyl peroxide as an innovative alternative capable of minimizing side-effects, but preserving at the same time their efficiency. The pharmaceutical forms using liposomes ensure the controlled release of medicine, as a result of the encapsulation of the active substances in the amphiphile structure-liposomes, with the possibility to diminish irritating secondary reactions in various forms of acne, and to provide efficiency, tolerability, conformity and cosmetic acceptability, to be proved in a future study.

Keywords: liposomes, tretinoin, benzoyl peroxide, acne

Acne is included in WHO’s chronic disease list and is adequately characterized by such a condition: prolonged manifestation, sequential progression with remission and recurrence periods, psychological and social impact with negative effects on the integration of the patient’s community.

Acne has a tremendous medical and social importance, as it affects over 85% of teenagers according to WHO reports, but it may also affect adults, and in rare cases newborns.

Physical changes caused by acne may have a negative effect on self-esteem, psychology and life quality.

A solution for the future could be an emulsion with encapsulated microcapsules [1-3] in view of obtaining a retard pharmacological preparation. Liposomal systems have remarkable flexibility in modifying structural and functional characteristics to adapt them to therapeutic needs compared to other colloidal transport systems and controlled release of active principles. Conventional liposomes - are the first generation of liposomes that have been used in the pharmaceutical industry. Most often they are obtained from phospholipids and / or cholesterol. Although the handling of properties such as size, number of lipid bilayers, lipid composition, the fluidity thereof is the strength in the process of obtaining these liposomes exhibit a relatively small blood circulation and is rapidly eliminated by macrophages.

Long-lasting liposomes - can remain in circulation for a longer period of time (fig. 1). It is obtained by covalently attaching to the external surface of conventional liposomes linear hydrophilic polymer chains (PEG). The liposomes thus formed are also known as sterically stabilized liposomes and exhibit excellent solubility in aqueous media.

Certain cutaneous adverse reactions require prolonged topical or systemic treatment, such as antibiotics, reparatory creams, or steroids [3-8], sometimes even anti-inflammatory drugs such as ibuprofen or other cyclo-oxygenase inhibitors, which may cause unexpected adverse reactions, like other common drugs [9-13].

Experimental part

Material and methods

Table 1 describes the materials and instruments used.

### Table 1

<table>
<thead>
<tr>
<th>Liposomes</th>
<th>Pharmaceutical forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tretinoin (Mediram T. M.)</td>
<td>Lanolin</td>
</tr>
<tr>
<td>Benzoyl Peroxide (Mediram T. M.)</td>
<td>Vaseline</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Castyl alcohol</td>
</tr>
<tr>
<td>Chiroform</td>
<td>Methylcelulose</td>
</tr>
<tr>
<td>Tween 80</td>
<td>Benzoic acid</td>
</tr>
<tr>
<td>Tampon solution (i.d. and boric acid)</td>
<td>Glycerina</td>
</tr>
<tr>
<td>Distilled water</td>
<td>Distilled water</td>
</tr>
<tr>
<td>Concentrated ethyl alcohol</td>
<td>Mcgar and pestle mill</td>
</tr>
<tr>
<td>Bandelin Sonoplus homogenizer</td>
<td>Electronic scales</td>
</tr>
<tr>
<td>Electronic scales</td>
<td>Spatula</td>
</tr>
<tr>
<td>Gas bulb</td>
<td>Watch glass</td>
</tr>
<tr>
<td>Round bottom flask</td>
<td>Berzelius and Eilenmeyer glasses</td>
</tr>
<tr>
<td>Berzelius glass</td>
<td>Plastic cases 5g</td>
</tr>
<tr>
<td>Spatulas</td>
<td>Funnel</td>
</tr>
<tr>
<td>Sterile plastic containers</td>
<td>pH-metric and filter paper și de filtru</td>
</tr>
<tr>
<td>T-tretinoin; P.B. - benzoyl peroxide</td>
<td></td>
</tr>
</tbody>
</table>

*email: buzia_olimpia@yahoo.com All authors had equal contribution to designing and writing the presented paper.

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The stages of preparing liposomes, and the corresponding pharmaceutical formulas proposed, were as follows:
- preparing liposomes by the method of lipofilm hydration;
- formulating ointments according to Roman Pharmacopoeia 10th edition;
- control of ointment quality;
- preparing tretinoin and benzoyl peroxide liposomes by the method of lipofilm hydration.

Tretinoin C20H28O2

Tretinoin is the most efficient comedolitic agent in acne. Acne pimples occur in the follicles of epithelial cells with an excess of keratine. Tretinoin helps remove the cornified cells and speed up the removal of corneocytes from the follicle, increase the myotic activity of the follicular epithelium, preventing the formation of microcomedons, the precursors of lesions in vulgar acne. Tretinoin is most commonly used in treating acne [14]. It is also used off-label to treat and reduce the aspect of stretch marks by increasing collagen production in the dermis [15].

In topical form this drug is pregnancy category C and must not be used by pregnant women [14].

Tretinoin is most commonly used to treat acne [16]. It is also used off-label to treat and reduce the appearance of stretch marks by increasing collagen production in the dermis [17].

In topical form, this drug is pregnancy category C and should not be used by pregnant women [16].

People using the local form should not use any cream or lotion that has a high drying potential, or contains alcohol, astringents, spices, chalk, resorcinol or aspirin, as these may interact with tretinoin or increase its side-effects.

Its use is limited by skin irritation, erytheme, burning sensation or increase sensitivity to sunlight in the spots where it was applied. Liposomal tretinoin is superior in point of skin irritation, but shows a considerable decrease of all associated side-effects.

Benzoyl peroxide (BPO) (C14H10O4)

Therapeutically speaking, it is a substance used in the topical treatment of acne. It may cause side-effects like dry skin, irritation, red skin.

It may be used in monotherapy, and also in association with retinoids (tretinoin). It has a powerful antimicrobial effect, does not cause resistance, and thus may be used over long periods of time, has a moderate anti-inflammatory effect and slightly regulates comedogenesis, preventing the formation of white and black spots.

Liposomal BPO leads to a decrease in the frequency of occurrence of adverse reactions, also having a significantly higher antibacterial effect.

It is considered that benzoyl peroxide is three times more active in treating acne. It is sebostatic, and inhibits P. acnes. (Propionibacterium acnes) whose pathogen form is now called Cutibacterium Acnes) [16]. In general, Acnea vulgaris is a hormone-mediated inflammation of the sebaceous glands and hair follicles. Hormonal changes trigger an increased production of keratine and sebum, which results to blocked sebaceous drainage. P. acnes has many lytic enzymes that break down the proteins and lipids in the sebum, leading to an inflammatory reaction. The free radical reaction of BPO may break down keratine, thus unblocking the sebum flow channel (comedolytic). It may trigger the nonspecific peroxidation of P. acnes, making it bactericide and supposedly decreasing sebum production; there are debates in specialized literature in this respect, including the biofilm effect [16,17]. Another recent theory approaches the alteration of microbiome diversity at cutaneous level in acne, a theory correlated to various alterations of the attached endosimbionts at skin level [18-26].

Table 2 summarizes the substances used in our study on patients affected by acne.

Preparing liposomes by the method of lipofilm hydration (fig.4)

Step 1: Preparing active substance lipophylic solutions

In order to prepare lipophylic solutions of active substance, we used 0.5 g of tretinoin and 1.5 g of benzoyl peroxide dissolved in an organic solvent (chloroform), in order to insure the homogeneity of the lipid mixture.

Step 2: Preparing the mixture of phospholipids with the active substance

Drug substances were encapsulated, when liposomes were prepared, in the lipophylic membrane.

The phospholipid mixture is made up of lecithine, tween 80 (solubility-increasing agent - it helps lipids dissolve in water), cholesterol and water, added to the active substances dissolved in chloroform.

Step 3: Transferring the mixture in a round-bottom flask and filming on water-bath.

The lipid mixture is transferred into a round bottom flask, which is kept in a water bath for 5 min until the chloroform used to dissolve the active substances completely evaporates, and the lipid film is formed.

The removal of the organic solvent by evaporation leads to the formation of a viscous gel, then the spontaneous occurrence of large vesicles, forming a multilamellar film (figs. 2 and 3).

<table>
<thead>
<tr>
<th>Class</th>
<th>Drug</th>
<th>Dose</th>
<th>Action</th>
<th>Clinical evidence</th>
<th>Adverse effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinoids</td>
<td>Tretinoin</td>
<td>0.025%</td>
<td>non-comedogenic, antinflammatory, keratolytic effect</td>
<td>Slight comedolonic acne</td>
<td>Irritative dermatitis – low degree, rash, burning, photosensitivity</td>
</tr>
<tr>
<td>Keratolitic</td>
<td>BPO</td>
<td>0.05%</td>
<td>Effect bactericidal, antinflammatory, effect slab non-comedogenic &amp; keratolytic</td>
<td>Acne inflammation, slight to moderate acne</td>
<td>Irritative dermatitis with rash, skin dryness, peeling, stinging, redness, burning, wrinking hair, clothes, beauties</td>
</tr>
</tbody>
</table>

Table 2: General presentation of drugs used in acne and associated factors.

Link: [http://www.revistadechimie.ro](http://www.revistadechimie.ro)
The lipid film being thin, the hydration and encapsulation process are more efficient.

Thus, in the case of phosphatidylcholine, in water excess (minimum 30%), mere hydration results in the fast formation of regular lipidic bistrata separated by water layers, called multimolecular vesicles (MLV). The thin film is detached from the container walls by strong stirring, yielding multilamellar vesicles of various sizes.

**Step 5: Ultrasonication of the mixture**

The mixture undergoes ultrasonication by means of the Bandelin Sonoplus sonicator – a probe-type sonicator homogenising by ultrasound irradiation, yielding small-size liposomes, used in the pharmaceutical and cosmetic industry.

Formulating ointments based on liposomes

Specialized literature proposes various concentrations of tretinoin (0.025%, 0.05% or 1%) and benzoyl peroxide (2, 5, 4, 5, 10%)

We proposed the concentrations 0.025% for tretinoin and 0.06% for benzoyl peroxide in various pharmaceutical forms: gel, ointment and cream (table 3).

<table>
<thead>
<tr>
<th>Name of substance</th>
<th>Gel – amount grams</th>
<th>Ointment – amount grams</th>
<th>Cream – amount grams</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tretinoin liposomes</td>
<td>6 ml</td>
<td>3 ml</td>
<td>3 ml</td>
<td>Anti-acne agent</td>
</tr>
<tr>
<td>Methylcellulose</td>
<td>2 g</td>
<td>-</td>
<td>-</td>
<td>Viscosity-increasing agent</td>
</tr>
<tr>
<td>Balsam Acid</td>
<td>0.5 g</td>
<td>-</td>
<td>-</td>
<td>Preservative</td>
</tr>
<tr>
<td>Glycerin</td>
<td>10 g</td>
<td>-</td>
<td>-</td>
<td>Moisturiser</td>
</tr>
<tr>
<td>Distilled water</td>
<td>q.s. ad. 100</td>
<td>-</td>
<td>q.s. ad. 100</td>
<td>Vehicle</td>
</tr>
<tr>
<td>Benzoyl peroxide liposomes</td>
<td>24 ml</td>
<td>24 ml</td>
<td>-</td>
<td>Anti-acne agent</td>
</tr>
<tr>
<td>Lanoline</td>
<td>7.3 g</td>
<td>7.3</td>
<td></td>
<td>Ointment base.</td>
</tr>
<tr>
<td>Vaseline</td>
<td>65.7 g</td>
<td>55.45</td>
<td></td>
<td>Ointment base.</td>
</tr>
<tr>
<td>Cetyl alcohol</td>
<td>-</td>
<td>2.92</td>
<td></td>
<td>A/U Emulsifier</td>
</tr>
</tbody>
</table>

Table 3 FORMULATION OF GEL, OINTMENT AND CREAM
Ointment quality control: organoleptic control, pH measurement, stability and display capacity were performed.

Results and discussions
By the lipofilm hydration method and ultrasonication, the liposomes we obtained were of the SUV-type of the smallest sizes. They are homogeneous and contain only one bistratum. Out of 5 g of tretinoin and 1.5 g of benzoyl peroxide 60 mL liposome solution were obtained. Their stability was of maximum 7 days in cold storage.

Table 4 shows the characteristics of semi-solid liposome preparations we obtained.

The organoleptic characteristics in 15, 30, and 45 days respectively, may be seen in table 5.
The pharmaceutical liposome forms conformed with the requirements of the Roman Pharmacopoeia, 10th edition, the pH ranging between 7 (liposome gel and ointment), and 8 (liposome cream).

Stability was preserved unmodified for 45 days.
The stretching capacity was determined by means of the Ojeda-Arbussa extensometer, taking into account the diameter of the circle consisting 1 g ointment after pressing with a plate weighing 440 g (G). At 1 min intervals we placed increasing mass weights on the upper plate of the extensometer (table 6 and fig. 5).

The values of the corresponding surfaces ($d/\pi$) were calculated, and the extensometric curves were drawn based on the surfaces occupied by the preparation (fig. 6), the y-coordinate representing the display values of the product, and the x-coordinate the loading values in grams.

The results obtained lead to the conclusion that in all cases the display capacity increases with the increase of the added weights, i.e., the bigger the surfaces, the better the display capacity of the products.

The analysis of the results shows that gels have superior spreading capacity as compared to creams and ointments, because of their initial consistency. It is found that the presence of cetyl alcohol in the composition of creams leads to the decrease of their display capacity.

Table 5 shows the characteristics of semi-solid liposome preparations we obtained.

Table 5 ORGANOLEPTIC CHARACTERS IN 45 DAYS

<table>
<thead>
<tr>
<th>Formulation</th>
<th>In 15 days</th>
<th>In 30 days</th>
<th>In 45 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel</td>
<td>Non-modified</td>
<td>Non-modified</td>
<td>Non-modified</td>
</tr>
<tr>
<td>Cream</td>
<td>Non-modified</td>
<td>Non-modified</td>
<td>Non-modified</td>
</tr>
<tr>
<td>Ointment</td>
<td>Non-modified</td>
<td>Non-modified</td>
<td>Non-modified</td>
</tr>
</tbody>
</table>

Table 6 THE VALUES OF DISPLAY CAPACITIES FOR LIPOSOME FORMULATIONS

<table>
<thead>
<tr>
<th>Mass (g)</th>
<th>Gel</th>
<th>Cream</th>
<th>Ointment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.5</td>
<td>6.7</td>
<td>7.1</td>
</tr>
<tr>
<td>50</td>
<td>9</td>
<td>7</td>
<td>7.3</td>
</tr>
<tr>
<td>100</td>
<td>9.3</td>
<td>7.3</td>
<td>7.5</td>
</tr>
<tr>
<td>150</td>
<td>9.7</td>
<td>7.5</td>
<td>7.7</td>
</tr>
<tr>
<td>200</td>
<td>10</td>
<td>7.7</td>
<td>7.9</td>
</tr>
<tr>
<td>300</td>
<td>10.5</td>
<td>7.8</td>
<td>8.2</td>
</tr>
<tr>
<td>500</td>
<td>11</td>
<td>8.1</td>
<td>8.5</td>
</tr>
</tbody>
</table>

Fig. 5. Display surface of liposome preparations
changed their initial smell. These characteristics persisted up to day 45, and in addition the gel’s consistency changed (it became much more viscous than on the day it was prepared ++ ). In the future topical liposome preparations may be used by including specific substances, that may sometimes be natural extracts, adapted to the clinical diagnosis and localisation in various health issues, not just acne, i.e. not only infectious, but also inflammatory conditions, or autoimmune, such as rosacea, sclero-atrophic lichen, lichen planus, alopecia areata [27-39].

Conclusions

The liposome product formulated conformed to the Pharmacopoeia, which was seen upon analysing the results obtained in the pharmaceutical technology laboratory.

According to these results, the preservation requirements for liposome preparations may be established, i.e. they should be stored in the fridge, as liposomes quickly deteriorate at room temperature.

Encapsulating anti-acne medication in liposomes is an innovative alternative in order to minimize the associated sideeffects, but preserving at the same time the efficiency of the active substances. The liposomes of tretinoin and benzoyl peroxide showed a high encapsulation efficiency, and superior physical stability. Being an innovative pharmaceutical form, we can anticipate its use by incorporating other active substances, such as antibiotics [40,41]. Today, antibiotics are used extensively in hospitals and outpatients. In this mode of liposome encapsulation, we offer the possibility of reducing the associated side effects, and especially we can obtain retarded pharmaceutical forms, providing a more convenient administration to patients.

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