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, tollerability, 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 wiew 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-inflammatories such as ibuprofen or other cyclo-oxygenase inhibitors, which may cause unexpected adverse reactions, like other common drugs [9-13].

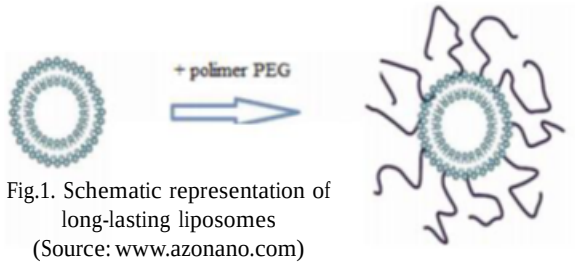

Fig.1. Schematic representation of long-lasting liposomes (Source: www.azonano.com)

Experimental part

Material and methods

Table 1 describes the materials and instruments used.

Table1
MATERIALS AND EQUIPMENT USED IN THE STUDY

Liposomes	Pharmaceutical forms
Tretinoin (Medchim T.M.)	Lanolin
benzoyl Peroxide (Medchim T.M.)	Vaselin
Cholesterol	Cetyl alcohol
Chloroform	Methylcelulose
Tween 80	Benzoic acid
Tampon solution (a.d. and boric acid)	Glycerin
Distilled water	Distilled water
Concentrated ethil alcohol	Mortar and pestle mill
Bandelin Sonoplus homogenizer	Electronic scales
Electronic scales	Spatula
Gas bulb	Watch glass
Round bottom flask	Berzelius and Erlenmeyer glasses
Berzelius glass	Plastic cases 5g
Spatulas	Funnel
Sterile plastic containers	pH-metric and filter paper și de filtru

T-tretinoin; P.B.- benzoyl peroxide

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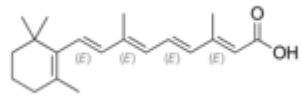
All authors had equal contribution to designing and writing the presented paper.

The stages of preparing liposomes, and the corresponding pharmaceutical formulas proposed, were as follows:

- preparing liposomes by the method of lypofilm hydration;
-formulating ointments according to Roman Pharmacopoeia 10th edition;
- control of ointment quality;
- preparing tretinoin and benzoyl peroxide liposomes by the method of lypofilm hydration.



Tretinoin C20H28O2

Tretinoin C20H28O2

It 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 mytotic 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 the collagen production in the dermis[15]. In topical form this drug is pregnancy category C and must not be used by pregnant women [14].

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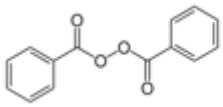
In topical form, this drug is pregnancy category C and should not be used by pregnant women [16].

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, eritema, 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.



($C_{14}H_{10}O_4$)

Benzoyl peroxide (BPO) ($C_{14}H_{10}O_4$)

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 lythic 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).

Class	Drug	Dose	Action	Clinical evidence	Adverse effects
Retinoids	Tretinoin	0.025 % 0.05%	non -comedogenic, antiinflammatory, keratolytic effect	Slight comedonian acne	Irritative dermatitis – low degree, rash, burning, photosensitivity
Keratolitic	BPO	0.6%	Efect antibacterian, antiinflamator, efect slab non - comedogenic și keratolitic	Acne inflammation, slight to moderate acne	Irritative dermatitis with rash, skin dryness, peeling, stinging, redness, burning, whitening hair, clothes, bedsheets

Table 2
GENERAL PRESENTATION OF DRUGS USED IN ACNE AND ASSOCIATED FACTORS

The lipid film being thin, the hydration and encapsulation process are more efficient.

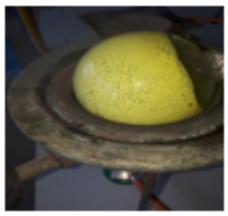


Fig. 2. Formation of lipofilm with tretinoin

Fig. 3. Formation of lipofilm with BPO

Step 4: Lipofilm rehydration

An aqueous solution tamponated with boric acid (*p*H = 7) is added in the round-bottom flask to hydrate the lipids, stirring gently, and some of the lipids make up unique complexes in point of structure. The temperature of the hydrating environment should be higher than the gel-liquid transition temperature in order to obtain liposomes that are as stable as possible.

Thus, in the case of phosphatidilcoline, in water excess (minimum 30%), mere hydration results in the fast formation of regular lipidic bistrata separated by water layers, called multimollecular 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).

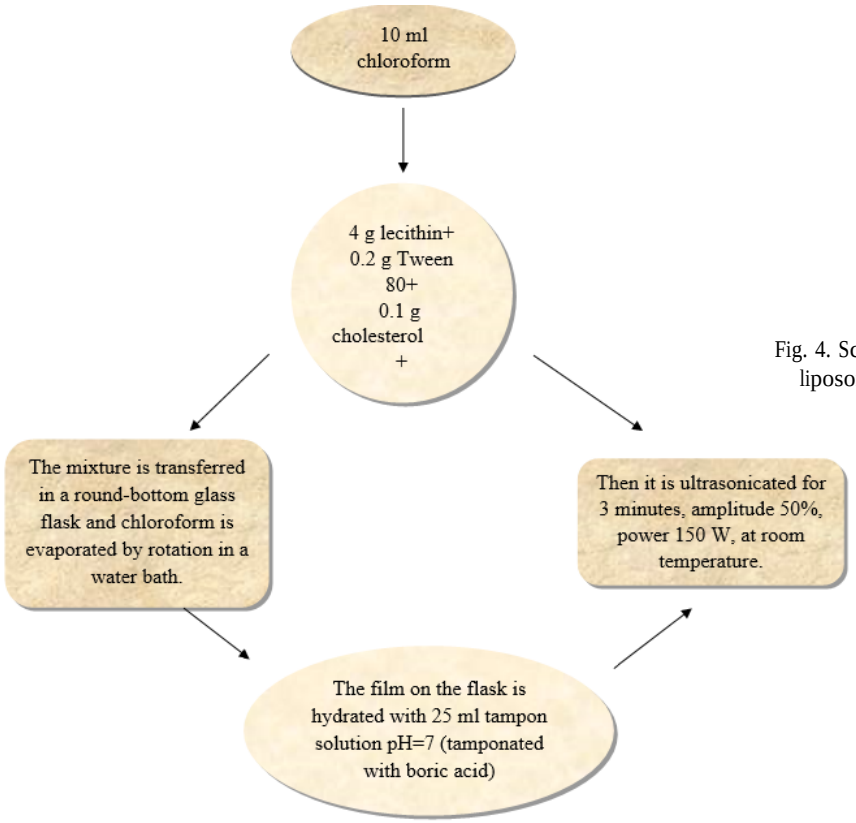


Fig. 4. Scheme used in preparing liposomes in lab conditions

Name of substance	Gel –amount grams	Ointment- amount grams	Cream – amount grams	Rol e
Tretinoin liposomes	6 ml	3 ml	3 ml	Anti-acne agent
Methylcellulose	2 g	-	-	Viscosity-increasing agent
benzoic Acid	0.5 g	-	-	Preservative
Glycerin	10 g	-	-	Moisturiser
Distilled water	q.s.ad.100	-	q.s ad 100	Vehicle
Benzoyl peroxide liposomes		24 ml	24 ml	Anti-acne agent
Lanoline		7.3 g	7.3	Ointment base.
Vaseline		65.7 g	55.48	Ointment base
Cetyl alcohol		-	2.92	A/U Emulsifier

Table 3
FORMULATION OF GEL, OINTMENT AND CREAM

Ointment quality control: organoleptic control, pH measurement, stability and display capacity were perrformed.

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 homogenous 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 *p*H 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 1min intervals we placed increasing mass weights on the upper plate of the extensometer (table 6 and fig. 5)

The values of the corresponding surfaces ($\eth d^2/4$) 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 ,in other words the bigger the surfaces, the better rge display capacity of the products.

The analysis of the results shows that gels have superior spreasing 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.

Stability of preparations over time, at different temperatures (table 7)

As seen in table 7, the preparations kept refrigerated did not undergo organoleptic changes over the periods under study (-). On the other hand, when stored at room temperature, in a month's time they showed the first colour alterations (+) in ointments and creams, while gels

Formulation	*Aspect*	*Consistency*	*Colour*	*Smell*	*pH*
Gel	Translucent	Fluid	Yellow	Specific	7
Cream	Translucent	Viscous	White-yellowish	Specific	8
Ointment	Translucent	Viscous	Light yellow	Specific	7

Table 4
CHARACTERISTICS OF SEMI-SOLID LIPOSOME PREPARATIONS

Formulation	*In 15 days*	*In 30 days*	*In 45 days*
Gel	Non-modified	Non-modified	Non-modified
Cream	Non-modified	Non-modified	Non-modified
Ointment	Non-modified	Non-modified	Non-modified

Table 5
ORGANOLEPTIC CHARACTERS IN 45 DAYS

Mass (g)	Gel		Cream		Ointment	
	d (cm)	S (cm²)	d (cm)	S (cm²)	d (cm)	S (cm²)
0	8.5	56.74	6.7	35.25	7.1	39.59
50	9	63.61	7	38.48	7.3	41.85
100	9.3	67.92	7.3	41.85	7.5	44.17
150	9.7	73.89	7.5	44.17	7.7	46.56
200	10	78.53	7.7	46.56	7.9	49.01
300	10.5	86.59	7.8	47.78	8.2	52.81
500	11	95.03	8.1	51.52	8.5	56.74

Table 6
THE VALUES OF DISPLAY CAPACITIES FOR LIPOSOME FORMULATIONS

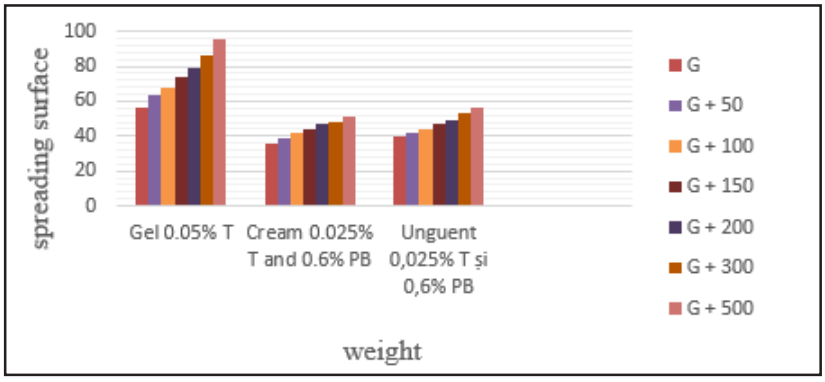
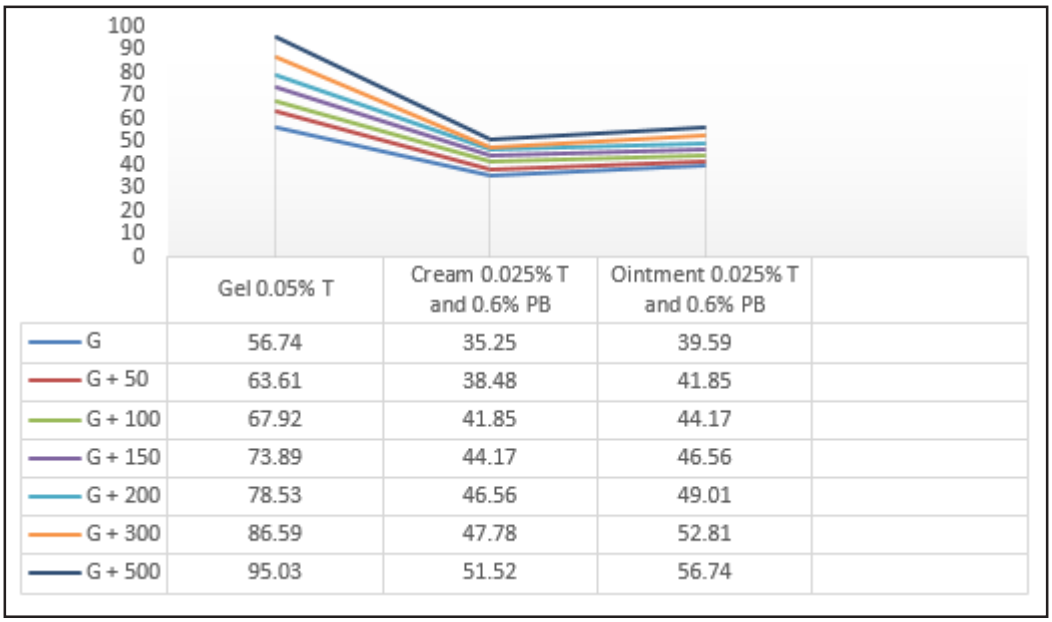


Fig. 5. Display surface of liposome preparations



	Gel 0.05% T	Cream 0.025% T and 0.6% PB	Ointment 0.025% T and 0.6% PB
G	56.74	35.25	39.59
G + 50	63.61	38.48	41.85
G + 100	67.92	41.85	44.17
G + 150	73.89	44.17	46.56
G + 200	78.53	46.56	49.01
G + 300	86.59	47.78	52.81
G + 500	95.03	51.52	56.74

Fig. 6. Extensometric curve for liposome preparations

	REFRIGERATED (4° C)			ROOM TEMPERATURE (25±2°C)		
	Gel	cremă	unguent	gel	Cremă	Unguent
15 DAYS	-	-	-	-	-	-
30 DAYS	-	-	-	+	+	+
45 DAYS	-	-	-	++	+	+

Table 7
STABILITY OF PREPARATIONS OVER TIME AT VARIOUS TEMPERATURES

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 topcal 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 side-effects, 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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