DEVELOPMENT AND EVALUATION OF INDOMETHACIN LIPOSOMAL SYSTEMS

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(Abstract): **Aim:** Treating the inflammation with oral indomethacin might be in some situations inadequate for patients that cannot dispense the medication on their own, a fact that conduct to the necessity to develop new pharmaceutical formulations administered through different pathways, such as the transdermal application. To trespass the skin layers, this study aimed to develop and evaluate an indomethacin liposome formulation that will be included in a Carbopol gel, subsequently. **Material and methods:** To develop indomethacin liposomes egg lecithin and cholesterol were used in order to confer membrane stability and elasticity. Two technologies of liposome-sizing were used: extrusion and sonication, the obtained vesicles being incorporated in a Carbopol gel. The *in vitro* release profiles were conducted on both liposomes and gels using the Franz cell method and two types of membranes (M1 and M2). **Results:** Liposome sizing through extrusion proved to be a better method compared to the sonication one. The microscopic evaluation of the liposomal vesicles showed that through extrusion smaller liposomes are obtained, a fact that is reflected also in their capacity for API releasing. **Conclusions:** Via the *in vitro* releasing study differences of 10% were observed regarding the amounts of API liberated, through both the extruded liposomal dispersion and the gel containing this dispersion in comparison with the sonicated liposomes. The type of releasing membrane used influenced the amount of API released, observing a higher concentration of API released after 8 hours through M2 (the skin-like membrane). **Keywords:** INDOMETHACIN, LIPOSOMES, TRANSDERMAL, FRANZ CELL.

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most prescribed classes of drugs in the world for treating rheumatoid polyarthritis and osteoarthritis. Indomethacin (Ind) is a well-known NSAID used as a long-term treatment in these kinds of pathologies, thus, its main disadvantage during oral administration is represented by the gastric side effects such as ulcer, digestive hemorrhage, allergy, and respiratory and hematology disorders. Ind is an indole derived compound, with the IUPAC name 2-[1-[(4-Chlorophenyl)carbonyl]-5-methoxy-2-methyl-1H-indol-3-
acetic acid having the following physico-chemical properties: white-yellowish powder, photosensitive, with the molecular mass of 357.8 g/mol, insoluble in water, partly insoluble in alcohol. 90% of the NSAIDs users are patients over 65 years old, the fact that implies a risk regarding the side-effects increase (1-3).

To decrease the risk and to improve the bioavailability of the medicines, the pharmaceutical industry started developing new pharmaceutical formulations such as liposomes. Besides the minimization of the well-known side-effects belonging to an NSAID, the transdermal administration of formulations in which the API is processed as liposome can facilitate skin penetration, improving bioavailability. A major advantage of the liposomes is given by their capacity to encapsulate different types of API and biocompatibility (4-8). The API liposomal systems have the advantage of incorporating this API, therefore, protecting it from degradation and transformation into inactive metabolites, also, the API is vectorized to the unhealthy cells, providing a gradual release. In this manner, the toxicity caused by a conventional administration decrease, and the distribution to other organs is hindered or reduced. The liposomes do not represent a pharmaceutical formulation lacking disadvantages, the loss or fusion of the APIs entrapped, or the oxidation of the phospholipids in the composition being some of them. Liposomes, named also, microparticle lipidic vesicles, bi-layered lipidic membranes, are constituted of lipids, their lipophilic parts being oriented through the internal and external part of the membrane, forming a mixture of lipidic substances, more often being used the phospholipids (phosphatidylcholine, phosphatidylserine), octadecyl amine and cholesterol (9-14). Taking into consideration the exposed introduction, this study aimed to develop and evaluate Ind liposomes that will be furthermore processed in a Carbopol gel for transdermal administration.

**MATERIAL AND METHODS**

**Materials for liposome preparation:** indomethacin (Sigma Aldrich Milan, Italy), egg lecithin (AppliChem GmbH, Germany), cholesterol (Fagron, Greece), ethanol (Chimreactiv, Romania), phosphate saline buffer pH 7.4.

In first table is presented the composition of the Ind liposomal dispersion.

<table>
<thead>
<tr>
<th>Ingredient (g)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin</td>
<td>active pharmaceutical ingredient</td>
</tr>
<tr>
<td>Egg lecithin</td>
<td>stability and elasticity of the liposomal membrane</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>stability and elasticity of the liposomal membrane</td>
</tr>
<tr>
<td>Ethanol</td>
<td>solvent</td>
</tr>
<tr>
<td>Phosphate saline buffer</td>
<td>adjusting pH</td>
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**Liposomes preparation method:** In the first step the lecithin, cholesterol, and Ind are dissolved in ethanol under continuous stirring for 20 minutes at 300 rpm (Heidolph RYR1 stirrer, Germany, fig. 1). During the second step, the dispersion is added gradually, using an Eppendorf pipette, drop by drop, in 25 g of phosphate saline buffer pH 7.4. A liposomal dispersion of 0.4% Ind is obtained.
**Fig. 1.** Liposomes preparation method

**Decreasing the liposomes vesicle size:**

**Extrusion:** The liposomal dispersion was sized by the 8-time passage through two syringes of 250 µL belonging to the Mini Extruder (fig. 2, left), provided with a supported filter at one of the ends. The passage from one syringe to another was realized through a support membrane located between the two syringes. The extruded liposomal dispersion will be furthermore coded as Lex.

**Sonication:** The liposomal dispersion was subjected to the ultrasound action, in an iced water bath (fig. 2, right) (t=2.5-4°C) for 20 minutes, in innings of 5 minutes with pauses between them of 5 minutes. The extruded liposomal dispersion will be further coded as Lsn.

**Fig. 2.** Liposomes sizing equipment

**Liposomes aspect:** The liposome aspect was evaluated microscopically, using a digital binocular microscope with a camera (Optika Microscopes, Italy), the objective of the camera of 100 X.

**Materials for Carbopol gel preparation:** carbopol 940 (BF Goodrich), sodium hydroxide solution 10% (Merck, Germany), distilled water.

In Table II is presented the composition of the Carbopol gel.

<table>
<thead>
<tr>
<th>TABLE II. Carbopol gel composition</th>
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<tbody>
<tr>
<td>Ingredient (g)</td>
</tr>
<tr>
<td>Carbopol 940</td>
</tr>
<tr>
<td>Sol. NaOH 10%</td>
</tr>
<tr>
<td>Distilled water</td>
</tr>
</tbody>
</table>
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of the gels a rheometer Rheotest RV was used (Rheotest RV, Germany), supplied with coaxial cylinders S/S1 and 12 shear rates.

**In vitro release study:**

**Samples (1 g):** Lex; Lsn; Gex; Gsn.

**Apparatus:** series of Franz cells (PermeGear, Germany), 11.28 mm clear jacketed with flat ground joint, 8 ml receptor volume, pinch clamp, and stir bar.

**Acceptor phase:** phosphate buffer pH 7.4, maintained at 37°C±1°C.

From the acceptor compartment of the Franz cell, samples of 0.8 mL were withdrawn at different time intervals for a period of 8 hours. The acceptor phase was immediately replaced with fresh phosphate buffer maintained at 37°C±1°C.

**Tested membranes:** two types of membranes were used to evaluate the release profiles of the drug from the analyzed samples:

- M1: Teknokroma membrane filters, nylon, 0.45 µm thickness, 25 mm diameter; requires hydration before use
- M2: Strat-M Membrane, 0.45 µm thickness, 25 mm diameter; has two polysulphonated layers (corresponding to epidermis and dermis) similar to the human skin layers, and polyolefin layers that simulate the porous aspect of the skin.

The cumulative amounts from the tested formulations were quantified by a validated HPLC method at 237 nm: injected volume: 10 µL, flow rate: 1 mL/min., mobile phase: formic acid solution 0.1% (25 %) + methanol (75 %), retention time: 2.9 min, stationary phase: Nucleodur C18 Gravity 3 µm column.

**RESULTS AND DISCUSSION**

**Liposomes aspects**

The microscopic evaluation of the liposomes obtained through extrusion (fig. 3) showed that through this method smaller vesicles are obtained in comparison with the ones sized through the sonication method (fig. 4). This aspect can suggest a better dermal penetrability of the extruded liposomes.

**Rheological evaluation of Carbopol gel**

The rheological analysis of the Carbopol gel shows a plastic behavior (fig. 5) characterized by a modification of the structure only after the tangential tension reaches a certain limit (flow threshold). Over this threshold and during the shear rate is increasing the viscosity starts to decrease.
In vitro release study

The etalon scale and typical chromatograms are included in figure 6.

Through the API in vitro releasing study from the liposomal dispersions, it has been noticed after 8 hours that the extruded vesicles are easily trespassing the tested membranes in comparison with the ones obtained through sonication. Also, the type of membrane tested in the study differentiates the amount of Ind released. It can be observed that in the case of the extruded liposomes with the M2 membrane (skin-like membrane), 13% more of the API is released in comparison to the M1 membrane. Similar behavior is retrieved in the case of the sonicated liposomes, the difference in the amount of API released being 10% (fig. 7).

Ind passaging through the membrane from the carbopol gels that included liposomes maintained the same behavior manifested also at the releasing study from the liposomal dispersion, with the distinction that after 8 hours the concentration of API released was in general, lower. This behavior might be explained by the initial necessity to release API from the gels followed by the passage of the testing membrane. Also, in the case of the liposomal gels, it was observed the importance of the membrane tested during the in vitro releasing study, noticing that the gels with extruded liposomes and the ones with extruded liposomes, that via the M2 membrane a higher amount of Ind is released (10% higher) compared to M1 (fig. 8).
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These results suggest that in physiological conditions Ind might passage the skin layers to ensure a systemic anti-inflammatory effect.

Fig. 7. Release profiles of indomethacin from liposomal dispersions across M1 and M2

CONCLUSIONS

The oral anti-inflammatory treatment can be in some situations inadequate for some patients that can administer by themselves the medication. In this manner and to minimize the Ind side-effects, the formulation of transdermal gels with medicinal liposomes can be an adequate solution for the pathologies treatment that includes an inflammatory process.

The liposome-sizing through extrusion proved to be a better method compared to the sonication one. The microscopic evaluation of the liposomal vesicles showed that through extrusion smaller liposomes are obtained compared to the ones obtained through sonication, a fact that is reflected also in their capacity for API release.
Through the *in vitro* releasing the study, differences of 10% of API released were observed for both extruded liposomes and gel with extruded liposomes compared to the sonicated liposomes. The releasing membrane type also influenced the amount of API released, observing a higher concentration of API released after 8 hours through M2 (the skin-like membrane).

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest, and this work was supported by the “George Emil Palade” University of Medicine, Pharmacy, Science and Technology of Targu Mures, Research Grant No. 10127/15/17.12.2020.

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