Technological Study of Anticellulite Formulations

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Summary

Cellulite is an incorrect term, which is commonly used to designate a “medico-esthetical problem” that normally affects women. However, from a medical point of view, it is a real cutaneous disorder. Often, the products recommended in the treatment of the cellulite are realized based on different phytotherapeutic extracts.

With this idea in mind the main aim of this work is the development of a topical formulae containing active phytotherapeutic principles able to act effectively on all the components involved in the development of the cellulite. For this reason two phytotherapeutic assets - Hydrocotyle asia and acid salicylic - were selected. In the present work we have designed and studied two new cosmetic forms attending to the selected excipients: latex of olive oil and glucidic gel. In order to study the physical stability and characterize the viscoelastic properties of the designed formulae’s, rheological studies have been performed. Attending to the results, the latex formulae presented a plastic behaviour while pseudoplastic characteristics were obtained for the gels. Finally, for verifying the liberation profiles of the formulae’s, in vitro analysis have been performed and fitted to kinetic models.

Riassunto

La parola cellulite è un termine incorretto utilizzato per definire un “problema medico-estetico” che colpisce soprattutto le donne.

Da un punto di vista strettamente medico la cellulite è una vera disfuzione cutanea ed i relativi prodotti raccomandati per il suo trattamento sono spesso estratti fitoterapici.

Partendo da questi presupposti si è cercato di sviluppare una formulazione di uso topico contenente principi attivi fitoterapici capaci di agire su tutte le cause che intervengono nella formazione della cellulite.

A questo scopo sono stati selezionati rispettivamente Hydrocotyle asia e acido salicilico inseriti in due formulazioni cosmetiche caratterizzate dall’uso di particolari veicoli: un lattice di olio d’oliva ed
un gel glucosidico.
Sono stati condotti studi reologici per verificare la stabilità fisica delle due formulazioni e le relative proprietà viscoelastiche.
Secondo i risultati ottenuti il lattice ha mostrato caratteristiche plastiche mentre i gel hanno dimostrato caratteristiche pseudo-plastiche.
I profili di cessione dei principi attivi sono stati verificati con modelli cinetici.
INTRODUCTION

The term cellulite has been generalized to define the whole series of alterations of the conjunctive subcutaneous tissue, especially the deposit of subcutaneous fat in certain zones of the organism as the regions located below the buttocks and on the top of the thighs. In general these conjunctive disorders can be classified in two big groups: (i) those which principally concern the deposit of the adipocitary lipase, and (ii) those which affect on the micro-traffic and the perivascular space (1, 2). In a few cases it predominate over the lipase component, with an abnormal increase of fat and an increase of the number and size of the adipocytes. Since in many occasions this adipose accumulated does not accompany to a state of widespread obesity, it is talked about segmentary or located lipodystrophy. In other cases, it prevails or coexists the vascular and intermediate component, with a notable capillary fragility, edema. Attending to the kind of edema, the pharmaceutical treatment change. Specially for indicating an Anticellulite treatment, it is indispensable a good diagnosis. There is not a unique intensive Anticellulite treatment but it exists a set of effective treatments (3-5). In order to promote mutually therapeutics benefits, the synergy of diverse agents must be looked carefully. The most appropriate thing is the accomplishment of an integral treatment that includes a general action and another specific. Though the combination of both treatments improves the cellulite course. However it does not exist a treatment that treats definitively this affection and still continues being investigated to obtain new therapeutic resources that guarantee the definitive resolution of the problem (6).

The products recommended in the treatment of the cellulite are realized based on different phytocidal extracts and other drugs, among them, some vitamins. In this work, our main aim is the development of formulae of hackneyed application that contain active phytocidal principles that act in an effective form on three components involved in the development of the cellulite: the micro-traffic, the oily tissue and the connective tissue. Concretely, we appeal to the Centella asiatica (Hydrocotyle asia) as a potential of the biosynthesis of collagen and to the acid salicylic that, in an indirect way, disables the lyphogenesis inside the adiposities (7, 8). As excipients of this active principle, very used in the treatment of the cellulite, we have selected two new vehicles, and this is the innovative contribution of our work: carrier successfully the Anticellulite drugs assets up to its place of action to improve the efficiency of the preparations. With this purpose, two excipients of different nature, latex of olive oil as lipophilic excipient and a glucidic gel, have been studied (9-11).

EXPERIMENTAL

Materials

- Extract Hydrocotyle asia glycolic was supplied by Guinama (Valencia, Sapin).

Hydrocotyle asia is also known as Centella asiatica and Hygrophila spinosa.

Centella asiatica belongs to the family Umbelliferae. It is found in swampy area of India, commonly found as a weed crop fields and other waste places throughout India upto an altitude of 600 meters. The C. asiatica extract possesses antioxidant (12), antiinflammatory, immunomodulating (13), antiproliferative (14), antigenotoxic (15) properties. The extract of C. asiatica L. has certain bioactive terpene acids such as asiatic acid, madecassic acid and their respective glycoside, asiaticoside and madecassoside (16).

Asiaticoside, have wound healing activity, promotes fibroblast proliferation and increases the level of enzymatic and non-enzymatic antioxidants. There are some phenolic compounds in
the extract of C. asiatica, having the activity same as that of the alfa-tocopherol. The crude extract of C. asiatica was shown to be non-toxic in normal human lymphocytes and reduced the genotoxic effects of methyl methanesulphonate and cyclophosphamide in cultured human lymphocytes (17, 18).

- Salicylic acid
  Salicylic acid was supplied by Panreac (Barcelona, Sapin).
  Salicylic acid is the chemical compound with the formula C6H4(OH)CO2H, where the OH group is adjacent to the carboxyl group. This colorless crystalline organic acid is widely used in organic synthesis and functions latin as a plant hormone. The name derives from the Latin word for the willow tree (Salix), from whose bark it can be obtained. Also known as 2-hydroxybenzoic acid (one of several beta hydroxy acids (compare to AHA), salicylic acid is a key additive in many skin-care products for the treatment of acne, psoriasis, calluses, corns, keratosis pilaris, and warts. It treats acne by causing skin cells to slough off more readily, preventing pores from clogging up (Fig. 1).

- All chemicals were analytical quality, and manufactured by Panreac (Spain). Water used to prepare the formulations was of Milli-Q quality (Milli-Q Academic, Millipore, France).

### Formulations Preparation

- Latex of olive oil
  The formula is described in table I. The oily phase is prepared warming the olive oil up to 85.0 ± 0.1°C, and adding the emulgent, Olivem 700, until it melt. The prepared formula is leave to cool down and after n-decan is added. Finally, the watery phase is added to the oily one under constant agitation with electromagnetic agitator to 600 rpm.

- Glucidic gel
  The glucidic gel (table I) was prepared dispering the gelling agent, sodium carboxymethylcellulose, in propylene glycol and adding 86.5 ml of water under moderate mechanical stirring (1000-1200 rpm).

### METHODS

#### Viscosity

The viscosity of different formulations was determined by a Brookfield DV-II+ digital rotational viscometer immersed in a thermostatic bath at a constant temperature of 25.0 ± 0.1°C. This viscometer is a shear rate controlled system, therefore the samples are put under a sweeping of shear rate (dy/dt) at regular intervals, measuring how the viscosity and shear stress varies.

In order to obtain reproducible results, all the samples were always presheared during 60 seconds under a shear rate strong enough to break the structure of the samples, followed by a 120 seconds of waiting time.

We have made a viscosimetric study as much to the vehicles used as to the formulas with the cosmetic drugs.
**TABLE I**

**Composition of formulations.**

<table>
<thead>
<tr>
<th>OLIVE OIL LATEX</th>
<th>GLUCIDIC GEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olive oil</td>
<td>Propylene glycol</td>
</tr>
<tr>
<td>Olivem 700</td>
<td>Carboximethylcellulose sodium</td>
</tr>
<tr>
<td>n-Decane</td>
<td>Distilled water</td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
</tr>
</tbody>
</table>

**Diffusion experiments**

Most published studies involve Franz-type cells (19). In our case we performed the diffusion experiments with FDC-400 Franz cells, Vidrafoc (Barcelona, Spain). It consists of two compartments with a membrane clamped between the donor and receiver chambers. The receptor phase was phosphate-buffered saline, pH 5.6. All the experiments were performed under the same skin pH conditions. The membranes are 47 mm in diameter and 0.45 µm in pore size. In this study, two types of membranes were tested: methylcellulose (Teknocroma) and nylon (Mfd, Waters Corporation).

**Analytical methods**

The concentrations of drugs were determined by UV-spectrophotometry measurements at maximum wavelength: 231 nm for salicylic acid and 322 nm for *Hydrocotyle asiatica*, in a Perkin-Elmer UV/VIS Lambda 40 UV-spectrophotometer. The method was previously validated and verified for accuracy, precision and linearity. Standard solutions were prepared by diluting the stock solution with phosphate-buffered saline.

**RESULTS AND DISCUSSION**

**Viscoelastic properties**

The rheograms present the applied shear rate curve versus the resulting stress. Based on these data (figures 2-4), we can affirm that latex has a plastic behaviour. This happens so in concentrated suspensions that the particles tend to form three-dimensional reticule. According to, its cohesive forces give it the characteristics of solid, but when the flow limit is exceeded, the bonds break and it acts as a liquid, flowing easily (20). This shear stress knows like yield stress ($\sigma_0$). The yield stress values obtained for the latex, latex with salicylic acid and latex with *Hydrocotyle asiatica* are 2129 D·cm$^{-2}$, 1509 D·cm$^{-2}$ and 2388 D·cm$^{-2}$ respectively. Therefore, the salicylic acid decreases the $\sigma_0$ value whereas *Hydrocotyle asiatica* increases it (21, 22).

Figure 2 shows the behaviour of the glucidic gel does not change with the addition of the cosmetic drugs. In addition, we observed a pseudoplastic behaviour, characteristic in semisolids formulas as our hydrogel. These systems are characterized by an increase in shear stress led to progressively greater break down of the gel.
structure with a steady decrease in the apparent viscosity. We can determine the value of apparent viscosity like the slope of the siccative to the curve of flow in $P$ (figure 4). This is equal to the relation $\varphi/(dy/dt)_p$ (23). The values of apparent viscosity obtained are 60.42 Cp for glucidic gel, 56.32 Cp for salicylic gel and 56.32 Cp for Hydrocotyle asia gel (24, 25). However, based on these results, we can conclude that statically significant differences between the three studied samples gel do not exit ($p<0.005$).

![Fig. 2 Rheogram of the latex (△), latex with Hydrocotyle asia (○) and latex with salicylic acid (■).](image)

![Fig. 3 Rheogram of the gel (△), gel with Hydrocotyle asia (○) and latex with salicylic acid (■).](image)

![Fig. 4 Rheogram of the glucidic gel.](image)

**Membrane selection**

We selected the most suitable membrane as that which offered the least resistance to the diffusion of both drugs, in order to minimize its influence in the test (26). For this study, a 0.05 mg/ml solution of salicylic acid and 58.5 mg/ml solution of Hydrocotyle asia were used as the donor phase. Tables II and III show the amounts of drug accumulated in each type of membrane (methylcellulose and nylon). Samples were taken at predetermined time intervals from 0.03 to 1 hour.

Phosphate-buffered saline, pH 5.6 ± 0.1, was used as the solvent to prepare the drug in the donor phase. This buffer was also used as the receptor phase. We previously verified that sink conditions were maintained.

At shown in figures 5, the nylon membrane limited salicylic acid diffusion most than methylcellulose membrane. On the other hand, figure 6 shows there were no significant differences between the methylcellulose and nylon membranes for Hydrocotyle asia. Although, transfer was slightly more rapid with the methylcellulose membrane. Nevertheless, we selected nylon membrane for our diffusion studies with salicylic acid and methylcellulose membrane with Hydrocotyle asia.
### TABLE II
Accumulated amounts (mg) of salicylic acid in the receiver chamber of the Franz cell as a function of time for different type of membranes.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Nylon</th>
<th>Methylcellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.08</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>0.16</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>0.5</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>1.0</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>1.5</td>
<td>0.06</td>
<td>0.04</td>
</tr>
</tbody>
</table>

### TABLE III
Accumulated amounts (mg) of centella asiatic in the receiver chamber of the Franz cell as a function of time for different type of membranes.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Nylon</th>
<th>Methylcellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.08</td>
<td>21.37</td>
<td>6.73</td>
</tr>
<tr>
<td>0.16</td>
<td>34.78</td>
<td>18.39</td>
</tr>
<tr>
<td>0.50</td>
<td>43.25</td>
<td>24.71</td>
</tr>
<tr>
<td>1.00</td>
<td>57.12</td>
<td>40.22</td>
</tr>
<tr>
<td>1.50</td>
<td>79.11</td>
<td>68.71</td>
</tr>
</tbody>
</table>

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**Fig. 5** Accumulated amounts of drug solution (mg) released with each type of membrane for salicylic acid: nylon (●) membrane and methylcellulose (□) membrane.

**Fig. 6** Accumulated amounts of drug solution (mg) released with each type of membrane for Hydrocotyle asiatic: nylon (□) membrane and methylcellulose (●) membrane.
In vitro release studies

Once prepared, both formulations were submitted to an in vitro diffusion assay by means of Franz-type cells. This assay was performed in the same conditions as previously mentioned in the membrane selection paragraph. A pH 5.6 phosphate buffer was used, thus ensuring sink conditions (27, 28).

In figure 7 and 8, we represent the percentages of active principle yielded from both studied formulations, results that corroborate the obtained ones in the rheological study. It does not exist statistically significant differences ($p < 0.005$) in the profile of liberation observed for the Hydrocotyle asiatica. Nevertheless, the difference between the latex and the gel glucidic is notable in case of the salicylic acid since the gel yields only 30 %, whereas the latex yields 78.7 % at 4 a.m. of the test essay.

In addition, a discriminatory procedure was used to determine the model that best explained the diffusion process. One of most widely used methods is Akaike’s approach (29). It involves the calculation of the so-called AIC criterion (Akaike discriminatory criterion):

$$AIC = n \cdot \ln SSQ + 2p$$

where $n$ is the number of pairs of experimental values, SSQ is the residuals sum of squares and $p$ is the number of parameters in the fitting function.

The criterion identifies the model that best fits the data as that with the minimum value of AIC.

Table IV shows the AIC average values for different formulations. Depending on these results, we can affirm that the formulae that contains salicylic acid follow the kinetic one of square root, whereas those who contain Hydrocotyle asiatica adjust to the kinetic one of cube root. The kinetic one to which there adjust the formulae that contains salicylic acid as cosmetic drug answers to the expectations gathered in bibliography for the pharmaceutical semisolid forms.

<table>
<thead>
<tr>
<th>Model</th>
<th>Salicylic acid</th>
<th>Ceatella asiatica</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Latex</td>
<td>Gel</td>
</tr>
<tr>
<td>Order 0</td>
<td>41.80 ± 8.95</td>
<td>44.70 ± 1.35</td>
</tr>
<tr>
<td>Order 1</td>
<td>44.39 ± 4.43</td>
<td>46.39 ± 3.43</td>
</tr>
<tr>
<td>Square root</td>
<td>32.20 ± 3.32</td>
<td>39.11 ± 0.78</td>
</tr>
<tr>
<td>Cubical root</td>
<td>41.79 ± 2.95</td>
<td>41.65 ± 0.62</td>
</tr>
</tbody>
</table>

![Fig. 7 Percentage of salicylic acid released for both formulations: latex (•) and glucidic gel (△).](image)
On the other hand, the formulae with *Hydrocotyle asia* are still a model of cube root, probably because the cosmetic assets, which is in use in the shape of liquid extract, arrange in the shape of drops spherical in the bosom of the vehicles thanks to the presence of emulgents as the Olivem 700 in the latex and to the structure of double propeller (helix) of the gel glucidic.

On the basis of all the obtained results, we can affirm that both formulations present an appropriated rheological behaviour, which supposes an important advantage both in the physical stability of the systems and in a suitable extensibility of the same ones during its application. In agreement with the profiles of liberation, we can affirm that the vehicle most adapted for the salicylic acid is the latex, whereas for the *Hydrocotyle asia* both of them can be used.

![Fig. 8 Percentage of Hydrocotyle asia released for both formulations: latex (●) and glucidic gel (□).](image-url)
References


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