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Study and description of hydrogels and organogels as vehicles for cosmetic active ingredients

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Synopsis

Cellulite, a clinical syndrome mainly affecting women, involves specific changes in conjunctive dermic and subcutaneous tissue, leading to vascular and hypertrophic alterations in adipose tissues and the consequent alteration of tissue structure. This paper describes the design of hydrogels and pluronic-lecithin organogels elaborated as vehicles of *Aloe vera* (*Aloe vera linné*) and *Hydrocotyle asiatica* (*Centella asiatica*) for the treatment of cellulite. The objective of this work was to carry out a complete evaluation of the proposed formulae through the study of the organoleptic and rheological properties of the formulae. Our work revealed that, in appearance, hydrogels show better organoleptic characteristics than organogels. On the other hand, from a rheological point of view, both hydrogels and organogels display a plastic behavior. However, the main difference between the two is that the more complex internal structure of the organogel bestows it with more viscosity. Finally, *in vitro* tests with Franz-type diffusion cells revealed that the release of cosmetic active principle from the tested excipients was appropriate, both in terms of magnitude and velocity.

INTRODUCTION

Cellulite, a clinical syndrome mainly affecting women, involves specific changes in conjunctive dermic and subcutaneous tissue, leading to vascular and hypertrophic alterations in adipose tissues and a consequent alteration of tissue structure (1,2). Hence, in order for a specific treatment to be effective, it is important that the formula should be capable of acting upon the three components of cellulite: microcirculation, fatty tissue, and connective tissue (3). The products used for the treatment of cellulite are based on a variety of phytotherapeutic extracts and other active substances, whose mission is to increase the excretion of urine (diuresis), to stimulate endocrinal function, to facilitate the liver metabolism of fats, and to aid circulation (4). In this study, two widely used anti-cellulite active principles, Aloe gel side 10:1 and a glycolic extract of *Hydrocotyle asiatica,* were incorporated into topical pharmaceutical formulations, with the objective of reaching the coetaneous layers where their anticellulitic action is most effective. As an anticellulitic treatment, Aloe gel aids tissue and cellular regeneration of the treated area (5) because of

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the different properties of its components. Typically, Aloe contains glycoproteins promoting re-epithelization, alantoine, and the polysaccharides aiding angiogenesis and phenolic components, which produce anti-inflammatory and antimicrobial effects (6). *Hydrocotyle asiatica,* on the other hand, is an ingredient derived from asiaticoside. It was first used to treat cellulite in the seventies, and its components produce both anti-inflammatory and venotonic effects.

However, it is very important to choose an appropriate delivery vehicle for each active principle, in accordance with how the formulation is to be applied. Absorption of the cosmetic active substance into the skin depends on its function, on the behavior of the vehicle, and on the condition of the skin. The main variables affecting the release velocity of the different active substances, or of the same substances in different vehicles, depend on the concentration of the active substance in the vehicle, the partition coefficient of the cosmetic active substance between the stratum corneum, the vehicle, and the partition coefficient (7).

For our study, a hydrogel, as a traditional vehicle, and a pluronic lecithin organogel (PLO), considered as the most advanced excipient to date, were selected as delivery vehicles. Although the organogel has been the object of many studies, due to the controversy arising from its internal structure and its jellification mechanism (8,9), it has been shown to be effective as a dermic and transdermic vehicle of medication. Consequently, we chose this substance as a new delivery vehicle, given that the active substances of the treatment must reach the deep layers of the skin (10,11). The *in vitro* release model used in this study has been shown to provide a valid assessment of the release of the cosmetic active substances from semisolid formulations, with similar parameters coming into play in the *in vivo* application of the formulae (12,13).

MATERIAL AND METHODS

MATERIALS

The products used as components of the formulations were: Aloe gel side 10:1, provided by Guinama (Valencia, Spain), made up of a dry extract (acibar) and a gel (a clear and mucilaginous liquid), consisting of water (99%) and a complex mixture of different components (1%). These components vary, depending on the plant, but typically include antranol, crisofanic acid, cianocobalamine, folic acid, monosaccarides, polysaccharides, tannins, sterols, organic acids, enzymes, vitamins, and minerals, to mention just a few. In the form of an extract (in gels and creams), it is used topically at a concentration of between 2% and 10%, as a revitalizing and regenerative agent.

Hydrocotyle asiatica glycolic extract was supplied by Guinama (Valencia, Spain). *Hydrocotyle asiatica* is also known as *Centella asiatica* and *Hygrophila spinosa. Centella asiatica*, belonging to the Umbelliferae family, is found in the low wetlands of India, as a weed on arable land and on wastelands throughout India, at altitudes of up to 600 metres. The centella asiatica extract possesses antioxidant (14), anti-inflammatory, immunomodulating (15), antiproliferative (16), and antigenotoxic (17) properties; and it contains certain bioactive terpene acids, such as asiatic acid and madecassic acid, and their respective glycosides, asiaticoside and madecassoside (18).

Asiaticoside has wound-healing activity, promotes fibroblast proliferation, and increases the level of enzymatic and non-enzymatic antioxidants. Some of the phenolic compounds in the extract of *Centella asiatica* display the same beneficial activity as α-tocopherol. The crude extract of *Centella asiatica* has been shown to be non-toxic in normal human lymphocytes and has reduced the genotoxic effects of methyl methanesulphonate and cyclophosphamide in cultured human lymphocytes (19,20). Pluronic F-127® = Lutrol F-127 = Poloxamer 407 INCI contains water <0.01%, oxypropylene-oxyethylene 71.5%–74.9%, and propylene oxide <1ppm. Poloxamers are synthetic block copolymers of hydrophilic ethylene oxide chains and hydrophobic propylene oxide chains, with the general formula of $HO–[C_2H_4O]_a–[C_3H_6O]_b–[C_2H_4O]_a–H$, where the subscripts a and b represent the number of the hydrophilic and hydrophobic chains respectively. This component was provided by Roig Farma-Fagron (Terrasa, Spain).

Isopropyl palmitate (concentration >90%) was provided by Meta (Roig Farma-Fagron). Soy lecithin in powdered form (water content 1.2%, concentration 97%, pH (sol. 1%) 7) was provided by Roig Farma-Fagron.

Lecithin is usually used as a synonym for phosphatidylcholine (PC), which is the major component of a phosphatide fraction. It is frequently isolated from soya beans and is commercially available in a highly pure state. Isolation and purification of lecithins from different sources are described in Kuksis (21) and Prosise (22). PC is a mixture of differently substituted sn-glycerol-3-phosphatidylcholine backbones. The structure of PC is variable and dependent on fatty acid substitution. In the sn-1-position, saturated acyl-groups are more common, and in the sn-2-position, unsaturated species are more common. The sn-1-chain typically shows an average of 16 C, whereas the sn-2-chain shows an average of 18 C. Water used to prepare the formulations was provided by Milli-Q Quality (Milli-Q Academic, Millipore, France).

EXPERIMENTAL METHODS

Viscosimetry. The viscosity of different formulations was determined by a Brookfield DV-II+ digital rotational viscometer immersed in a thermostatic bath maintained at a temperature of 25° ± 0.1°C. This viscometer is a shear rate controlled system; therefore the samples are put under a sweep of shear rate ($d\gamma/dt$) at regular intervals, allowing variations in viscosity and shear stress to be observed.

In order to obtain reproducible results, the samples were always pre-sheared for 60 sec, with measurements being taken after 120 sec. In addition, a viscosimetric study was carried out on both the delivery vehicles themselves and the complete formulae containing the active substances.

Diffusion experiments. Franz-type cells are commonly used in most published studies (23). The FDC-400 used in our study was supplied by Vidra-Foc (Barcelona, Spain). It consists of two compartments with a membrane clamped between the donor and receiver chambers. The receptor phase was a phosphate-buffered saline, at pH 5.6. This pH was chosen because it corresponds to the pH in the areas of the skin where the drug acts after topical administration. The membranes are 47 mm in diameter and 0.45 μm in pore size. Two types of membranes were tested: methylcellulose (Teknocroma) and nylon (Mfd, Waters Corporation).

Analytical methods. The concentrations of drugs were measured by UV-spectrophotometry at 223 nm for *Aloe vera* and at 322 for *Hydrocotyle asiatica* (λ_{max}). 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. A UV-spectrophotometer (Perkin-Elmer UV/VIS Lambda 40) was used for all measurements.

RESULTS AND DISCUSSION

PREPARATION AND ORGANOLEPTIC DESCRIPTION OF FORMULAE

In formula one, the base of the hydrogel, provided by BASF, was adapted to our cosmetic active substances (Table I). In the cold production process (4° C ± 0.1), Pluronic F-127® was completely dissolved in the formula, with water added immediately after addition of the active substances. Finally, the process of gelification was carried out at room temperature.

Aloe hydrogel at 10% is consistent, transparent, and pink in color, with a characteristic aloe scent. It is easily spread over the skin, leaving a fine, transparent film that is quickly absorbed. It does not leave behind a sticky feeling and can be washed off with water, leaving the skin soft and smooth.

The hydrogel with *Hydrocotyle asiatica* at 7% differs in color from the Aloe hydrogel. It is slightly orange in appearance and has its own distinctive aroma. Our reference for the study of the organogel was the formula published by Pince (24), which we adapted for the incorporation of the two active principles. A solution of lecithin in isopropyl palmitate and a gel of Pluronic F-127® at 30% were prepared separately and then mixed using gentle electromagnetic agitation (800 rpm). The remaining components were added during homogenization and then left to cool to obtain the final organogel. No significant organoleptic differences between the Aloe organogel and the *Hydrocotyle asiatica* organogel were observed. Both were creamy, with a viscous consistency, without bubbles, and

Table I
Composition of Hydrogels

Hydrogels	
Aloe gel side 10:1 10 g	*Hydrocotyle asiatica* 7 g
Propylene glycol 19,8 g	Propylene glycol 19,8 g
Pluronic F-127® 18 g	Pluronic F-127® 20,46 g
Distilled water 52.2 g	Distilled water 52.7 g

Table II
Compositions of Organogels

Organogel PLOs	
Aloe gel side 10:1 10 g	*Hydrocotyle asiatica* 7 g
Propylene glycol 13.5 ml	Propylene glycol 14 ml
Lecithin/IPP 19.8 ml	Lecithin/IPP 20.4 ml
Pluronic F-127® 30% gel 56.7 ml	Pluronic F-127®30% gel 58.6 ml

were easily spread and absorbed into the skin. They were ochre in color and had a penetrating soy lecithin-like smell.

The fundamental advantage of the organogel over the hydrogel system is that it facilitates the transdermic penetration of the medication to a greater degree. This is important in the case of anticellulitic treatments, given that active ingredients must be able to reach the deepest layers of the skin in order to act upon the cells where fat is accumulated at the adipose tissue level. The skin is a complex organ designed to isolate the organism from the external milieu, and thus poses a challenge to the pharmaceutical development of excipients that yield optimal permeation and absorption of the active principles.

In the early 1990s Jones and Kloesel (cited in ref. 25) developed pluronic lecithin organogel (PLO) as a transdermal drug carrier and delivery system. The most promising medical applications of PLO are for nonsteroidal anti-inflammatory drugs (26) such as ketoprofen, piroxicam, and diclofenac (27). Our findings (28) reveal that PLO has great potential in the development of transdermal drug delivery formulations, as an alternative to oral or parenteral administration. They are also easy to prepare and apply.

RHEOLOGICAL STUDY

We used the data of shear stress of each shear rate to obtain an estimated concentration for each of the three replicates. The mean shear stress and their standard deviations are included in Figures 1 and 2. The rheogram in Figure 1 shows the rheological behavior of our hydrogels. The data obtained reveals that all samples displayed a plastic behavior given that they were semisolid in consistency. However, these systems are known to become more liquid the more they are shaken. They begin to flow when shear stress reaches a limit known as yield stress (σ_0). The values of yield stress are determined as the point when the sample begins to flow after reaching maximum viscosity (29). According to this criterion, the values of yield stress obtained were 3549.67 D/cm^2 for the excipient, 2639.33 D/cm^2 for the *Hydrocotyle asiatica* hydrogel, and 1092.33 D/cm^2 for the Aloe. An explanation for these results could be the difference in pH found in the samples from

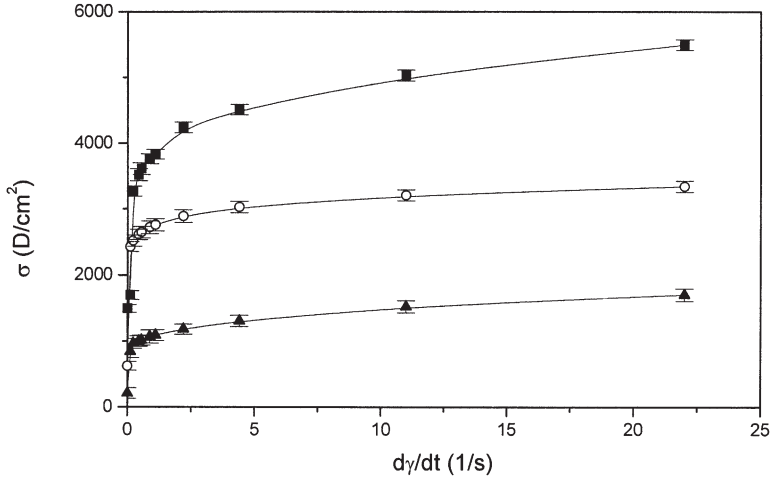

Figure 1. Rheogram of the hydrogel (■), hydrogel with Aloe (▲), and hydrogel with *Hydrocotyle asiatica* (○).

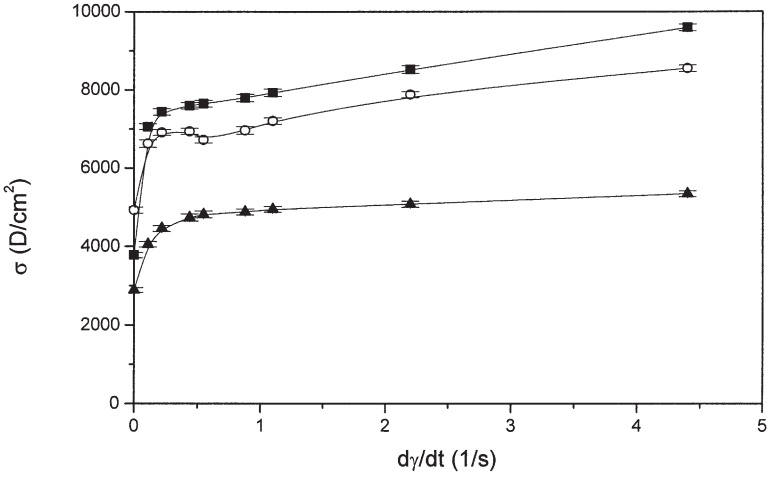

Figure 2. Rheogram of the PLO (■), PLO with Aloe (▲), and PLO with *Hydrocotyle asiatica* (○).

previous studies conducted by our research team (30). As the acidity increases, the internal structure of the system decreases, as its shear stress does accordingly. This phenomenon has been verified by several authors (31). As far as the organogels are concerned, Figure 2 shows that they display a similar plastic behavior. There are no statistically important differences ($p < 0.005$) in the values of yield stress of the excipient (7542.33 D/cm^2) and the *Hydrocotyle asiatica* organogel (6923.67 D/cm^2). However, the Aloe organogel, as with the hydrogels, is characterized by an inferior yield stress, 4805.66 D/cm^2. The values of σ_0 show that organogels present a more complex internal structure, which gives them greater viscosity. As observed in Figures 3 and 4, in these plastic systems viscosity is not constant. On the contrary, this parameter decreases as the speed of the deformation increases.

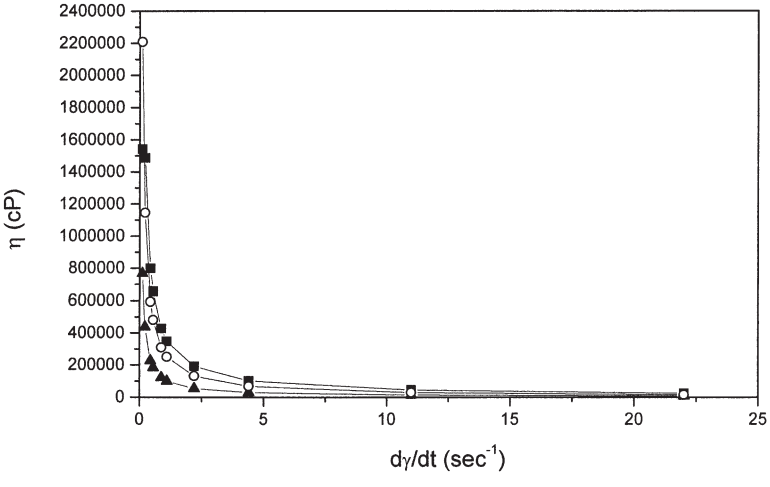

Figure 3. Viscosity as a function of shear rate for the hydrogel (■), hydrogel with Aloe (▲), and hydrogel with *Hydrocotyle asiatica* (○).

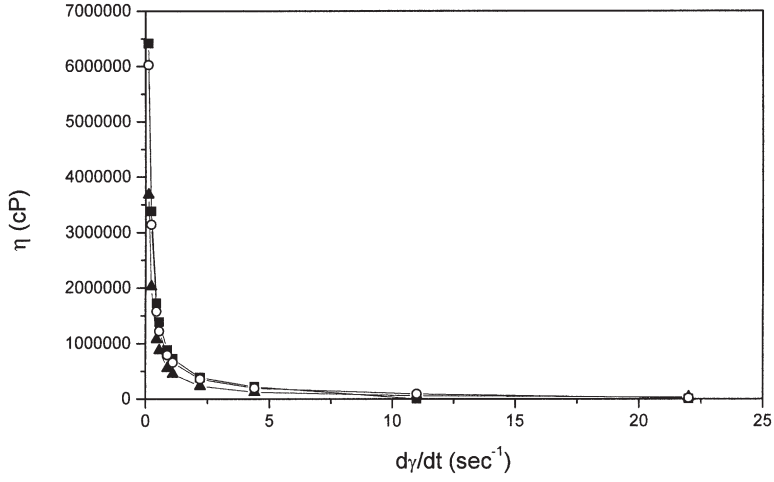

Figure 4. Viscosity as a function of shear rate for the hydrogel (■), hydrogel with Aloe (▲), and hydrogel with *Hydrocotyle asiatica* (○).

IN VITRO RELEASE STUDY

A previous step to conducting the *in vitro* release tests is the selection of the most appropriate membrane for each active principle: one offering the least resistance on application of the formula (32). This is important to ensure appropriate *sink* conditions (23) and to ensure that the *in vitro* study is subject to the same variables as the *in vivo* test. Both delivery vehicles were subjected to these tests, and as shown in Figure 5, there are no important statistical differences ($p < 0.005$) between the two membranes tested for Aloe gel variables. However, in the case of the *Hydrocotyle asiatica*, the nylon membrane offered the least resistance and was therefore chosen for use in this study (Figure 6).

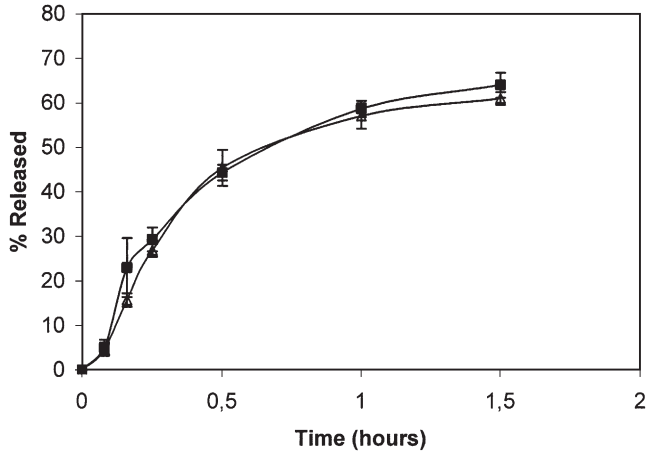

Figure 5. Percentage of drug solution released with each type of membrane for Aloe gel: nylon membrane (■) and methylcellulose membrane (△).

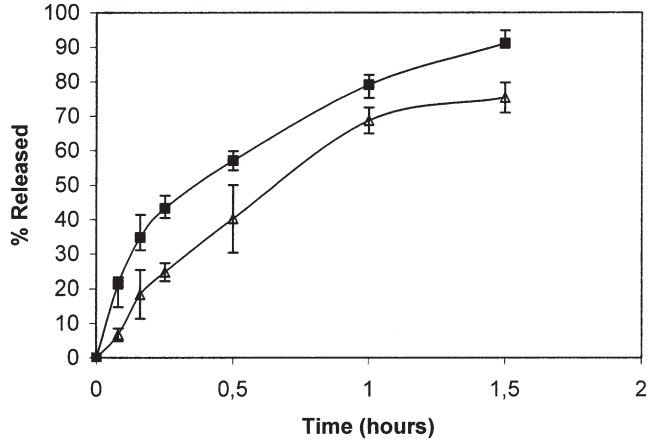

Figure 6. Percentage of drug solution released with each type of membrane for *Hydrocotyle asiatica*: nylon membrane (■) and methylcellulose membarnce (△).

Figure 7 shows the release profiles from all the samples studied, with the release percentage of active ingredient practically reaching 100% in all cases. The release of *Hydrocotyle asiatica* from both formulations was similar, both in terms of total quantity released, as well as in terms of time taken for a 100% release, taking place two hours after initiation of the test. However, differences for the Aloe samples were observed, with 100% release taking place after two hours for the organogel and after four hours for the hydrogel. However, we can confirm that the release of the active principle from the excipients used in our study was appropriate, both in terms of magnitude and rate, and that the active ingredients will be effective after application to the skin.

The results obtained from the *in vitro* release model used in this study enable us to estimate the *in vivo* release behavior of the formulae tested, subject to the same parameter variables (7,13). The differences found in the time taken for the 100% release of *Hydrocotyle asiatica* and the Aloe in the same media could be attributable to differences in

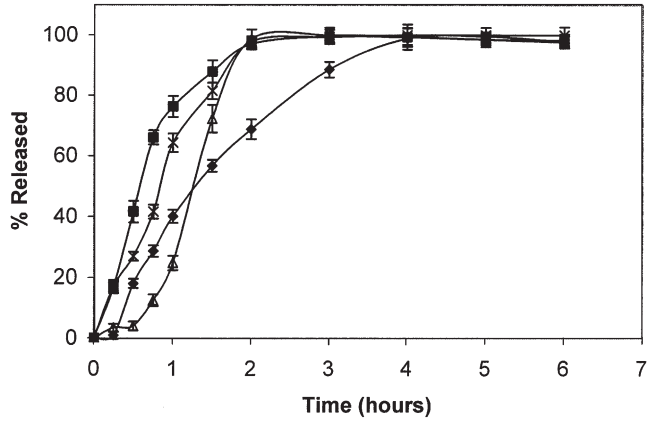

Figure 7. Percentage of drug released for different formulations: Aloe hydrogel (♦), *Hydrocotyle asiatica* hydrogel (■), Aloe PLO (△), and *Hydrocotile asiatica* PLO (×).

physical–chemical properties such as solubility and partition coefficient. In addition, pluronic gel is hydrophilic whereas the PLO base is lipophilic (28).

CONCLUSIONS

On the basis of these results, we can confirm that all of the formulations studied had appropriate organoleptic characteristics as well as a good rheological plastic behavior. Compression force values were low, despite the fact that the organogels presented higher viscosity. These properties give them a pleasant texture, allowing them to be spread easily over the skin. On the other hand, the *in vitro* release profiles reveal that the delivery of the drugs from the tested excipients is appropriate, both in magnitude and in time.

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