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# Potential Use of Ascorbic Acid–Based Surfactants as Skin Penetration Enhancers

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**ABSTRACT** 6-O-Ascorbic acid alkanoates (ASCn) are amphiphilic molecules having physical-chemical properties that depend on the alkyl chain length. The derivatives of low molecular weight ($n < 11$) have enough aqueous solubility to produce self-assemblies at room temperature (≈25°C), while those with longer alkyl chains possess a critical micellar temperature (CMT) higher than 30°C. At higher temperatures ($T° > CMT$), ASCn aqueous suspensions turn into either micellar solutions or gel phases, depending on the length of the hydrophobic chain. On cooling, coagels are produced, which possess a lamellar structure that exhibit sharp X-ray diffraction patterns and optical birefringence. The semisolid consistency of such coagels is an interesting property to formulate dermatological pharmaceutical dosage forms able to solubilize and stabilize different drugs. The objective of the present study was the evaluation of the enhancing permeation effect of ASCn with different chain lengths and to correlate permeability changes with histological effects. With this purpose, ASCn coagels containing anthralin (antipsoriasic drug) or fluorescein isothiocyanate (FITC, hydrophobic fluorescent marker) were assayed on rat skin (ex vivo) and mice skin (in vivo), respectively. Also, histological studies were performed aimed at detecting some possible side effects of ASCn. No inflammatory cellular response was observed in the skin when ASCn coagels were applied, suggesting non-irritating properties. Light microscopy indicated slight disruption and fragmentation of stratum corneum. The penetration of ASCn through rat skin epidermis was very fast and quantitatively significant. The permeation of anthralin was significantly increased when the drug was vehiculized in ASCn coagels, compared to other pharmaceutical systems. The results indicated that ASC12 seems to have the highest enhancing effect on FITC permeation. ASC12 appears to be the compound that possesses the highest capacity to enhance the penetration of the drugs. Furthermore, it has the highest permeation of the serie.

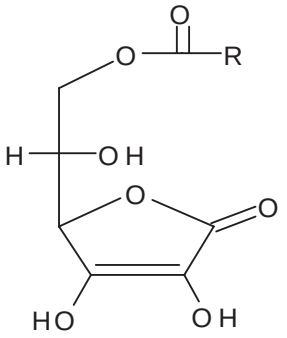
**KEYWORDS** Ascorbic acid derivatives, Anthralin, Enhancer, Coagel, Dermatological formulations

## INTRODUCTION

The stratum corneum (SC) is a highly organized structure and constitutes a formidable barrier both to water transportation out of the body and to inward chemical permeation. SC also limits the transdermal delivery of drugs. This route of administration presents many advantages such as better control of blood levels, reduced incidence of systemic toxicity, absence of hepatic first-pass metabolism, etc. Many strategies have been suggested in order to overcome the low permeability of drugs through the skin. A useful alternative is the use of penetration enhancers (or accelerants) which reduce temporarily the permeability barrier of the SC (Williams, 2004). The practical use of enhancers requires a careful balance between the toxicity on the skin and permeation enhancement benefits. Among the substances that have been shown to increase drug permeation are water (in general, increased tissue hydration appears to increase transdermal delivery of both hydrophilic and lipophilic permeant), sulphoxides and similar chemicals, azone, pyrrolidones, fatty acids, alcohols, fatty alcohols and glycols, surfactants, urea, essential oils, terpenes and terpenoids, phospholipids, solvents at high concentrations, and others (Williams, 2004).

6-O-Ascorbic acid alkanoates (ASCn, Fig. 1) possess both a hydrophobic moiety (aliphatic chain) and a polar group (ascorbic acid) (Lo Nostro, 1997; Palma, 2002a) and behave as amphiphilic molecules in water, and consequently it is to be expected that they would show enhancing properties. Some of these derivatives were synthesized in our laboratories (Palma, 2002b) with the main aim of obtaining amphiphiles that could combine the powerful antioxidant properties of ascorbic acid with the capacity to produce supramolecular aggregates.

These compounds show physicochemical properties that depend on the alkyl chain length. The solubility of




$R = (CH_2)_x\text{-}CH_3$

| x | ASC *n* |
|---|---|
| 6 | (ASC $_8$) |
| 8 | (ASC $_{10}$) |
| 9 | (ASC $_{11}$) |
| 10 | (ASC $_{12}$) |
| 12 | (ASC $_{14}$) |
| 14 | (ASC $_{16}$) |

**FIGURE 1** **Chemical Structure of ASCn.**

ASCn decreases with alkyl chain length and increases with temperature. In practice, this means that the longer the alkyl chain, the higher the critical micellar temperature (CMT; the temperature where solubility reaches the critical micellar concentration [CMC]). At higher temperatures (>CMT), ASCn aqueous suspensions turn into either micellar solutions or gel phases, depending on the length of the hydrophobic chain (Palma, 2003a). Then, on cooling, coagels are produced. These hydrated semicrystalline phases, also defined as "opaque suspensions of crystals," "poorly hydrated multilamellar polycrystalline suspensions," "hydrated solids," or "biphasic mixtures containing crystals," possess a lamellar structure that exhibits sharp X-ray diffraction patterns and optical birefringence. Thus, ASCn of higher molecular weight ($n > 11$) have CMTs above 30°C, while those of shorter chain length have enough aqueous solubility to produce self assemblies at room temperature (Palma, 2003b; 2003c). In earlier works we evaluated the potencial utilization of ASCn coagels as drug delivery systems (Palma, 2002b; 2003d). The semisolid consistency of such coagels is adequate to prepare dermatological pharmaceutical dosage forms able to solubilize and stabilize different drugs (Palma, 2003c; 2003d).

Following this up, the objective of the present study was the evaluation of the enhancing permeation effects of ASCn for different chain lengths, and to correlate permeability changes with histological effects. Coagels from ASC12, ASC14, and ASC16 were selected owing to the fact that these systems show adequate rheological properties for use in the design of semisolid pharmaceutical dosage forms made to be applied on the skin. To this purpose, ASCn coagels containing anthralin (antipsoriasic drug) or fluorescein isothiocyanate (FITC, hydrophobic fluorescent marker) were assayed on rat skin (ex vivo) and mice skin (in vivo), respectively. Histological studies were also performed, aiming to detect any possible side effects of ASCn.

## MATERIALS AND METHODS

### Materials

Alkanoyl-6-O-ascorbic acid esters were synthesized in our laboratories according to a previously described procedure (Capuzzi, 1996) (ASCn, with n = 12, 14 and 16). Purity was assessed through TLC and elemental analysis. All reactants (analytical grade) were purchased from Fluka (Milan, Italy) and used without

further purification. Bidistilled water was purified with a MilliQ apparatus.

Reagents: FITC was purchased from Sigma-Aldrich (St Louis, MO, USA) and anthralin from Parafarm (Buenos Aires, Argentina).

## Animals

The experiments were performed using female 3-month-old BALB/c mice or Wistar rats. The animals were originally obtained from the Comisión Nacional de Energía Atómica (CNEA), Argentina. The Institutional Care and Use of Animals Committee (exp N° 15-05-56272) approved animal handling and experimental procedures.

## Sample Preparation

Drug-free coagels were prepared by heating ASCn (ASC12, ASC14 and ASC16) aqueous suspension to above the phase transition temperature and allowing the temperature to fall to room temperature, thus yielding the respective coagels (Coa12, Coa14, and Coa16). To incorporate anthralin or FITC in the coagels, ASCn aqueous suspensions were heated to above their CMT (Palma, 2002b) until the gel phase was formed, and then precisely weighed amounts of anthralin (0.15% W/V) or FITC (0.2% W/V) were incorporated as finely divided solids to obtain ASCn-A or ASCn-F series, respectively for n = 12, 14, and 16. The systems were kept under these conditions for 2 h in order to obtain the complete solubilization of the drugs. After this period, the colloidal dispersions were filtered (0.45 μ) and the filtrate was allowed to reach room temperature, so as to yield the coagels. In all cases the concentration of ASCn was 5% (W/V).

## *In vitro* Diffusion Studies

The abdominal hair of Wistar rats was shaved using an electric razor. Twenty-four hours later, the rats were sacrificed, and abdominal skin was surgically removed and collected in pH:7.4-saline solution. In vitro skin permeation experiments were carried out using a modified Franz diffusion cell (6.72 cm2 surface area). The shaved abdominal skin of Wistar rats was mounted on the receptor compartment with the stratum corneum side facing upwards toward the donor compartment. The donor mediums were Coa12, Coa14, Coa16, Coa12-A, Coa14-A, and Coa16-A, respectively. The receptor medium was 10 mL of distilled water, which was removed at 10-min intervals and replaced with a fresh one. All samples were evaluated through UV-VIS measurements (λ = 365 nm) using a Shimadzu UV-160 spectrophotometer. All experiments were made in triplicate (Palma, 2003e).

## *In vivo* Topical Application

Appropriate amounts of the sample (ASCn-F series) were applied uniformly on the dorsal sides of mice ears. The animals (two for each sample) were sacrificed 6 h after application and the ears were excised. The ventral sides of the ears were used as controls.

## Histological Examination by Light Microscopy

The ears were removed after samples (Coa12, Coa14, and Coa16) were applied. The tissue samples were processed for microscopic evaluation by fixing in 10% pH 7.4 buffered formalin solution, cleaned with xylol, embedded in paraffin, sectioned (5 μm sections) and stained with hematoxylin and eosin, and examined under a light microscopy. All histological observations were performed in a blind way.

## Histological Examination by Fluorescent Microscopy

This assay was performed using Coa12-F, Coa14-F, and Coa16-F. Tissue samples of ears were frozen in OCT (Miles, Elkart, IN) and 8-μm cryostat sections were prepared. Sections were fixed at room temperature for 1 min and dried in air. Next, the samples were mounted in a medium of 90% glycerol with 10% PBS and evaluated by UV microscopy. We used a microscope equipped with filters for FITC fluorescence. All observations of tissue sections were performed immediately after coverslips were mounted.

# RESULTS

## Qualitative Evaluation of ASCn Enhancer Properties: Permeation of FITC

ASCn coagels containing FITC (Coa12-F, Coa14-F, or Coa16-F) were assayed on mice skin as previously

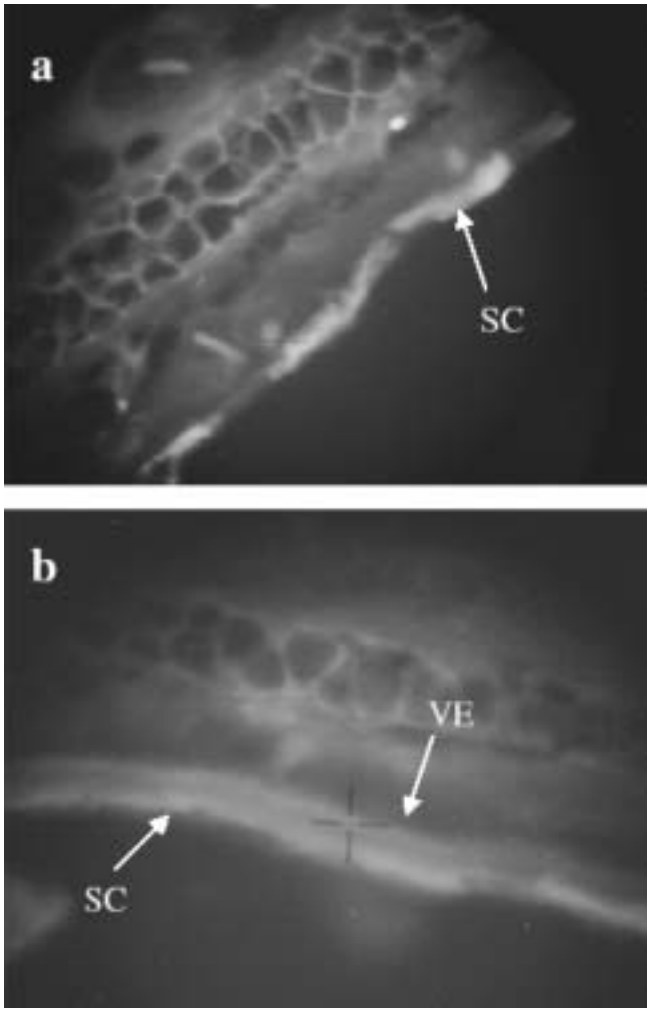



**FIGURE 2** **Fluorescent Microscopy of ASCn Coagels Containing FITC. a) ASC14, b) ASC12 (SC = stratum corneum, VE = viable epidermis).**

described. Fluorescent microscopy showed that Coa16-F exhibited a thin external fluorescent line. With Coa14-F the marker was only observed in the SC, while for Coa12-F the fluorescence was detected in the whole epidermis (Fig. 2a and 2b, respectively). In all cases the fluorescent marker was not visualized in the dermis. These results indicate that Coa12 seems to have the highest enhancing effect on FITC permeation. This result is consistent with that observed for the permeation of anthralin (see next section).

## Permeation Studies

The penetration of anthralin through rat skin was measured using ASCn coagels as vehicle. Also, the permeation of ASCn under the same conditions was evaluated through cumulative amount-time profiles.

**TABLE 1** **Permeation of Anthralin and ASCn Through Rat Skin**

| | Flux ($\mu g/cm^2/h$) | |
|---|---|---|
| Coagel | Anthralin | ASCn |
| ASC12 | 67.2 ± 4.2 | 331.8 ± 16.2 |
| ASC14 | 25.9 ± 1.5 | 146.4 ± 7.5 |
| ASC16 | 34.3 ± 1.3 | 49.5 ± 3.9 |

Fluxs ($\mu g/cm^2/h$) reported in Table 1 were calculated from the slopes of the resulting linear segment of plots. The results are also depicted in Figs. 3 and 4, respectively.

Penetration of ASCn through rat skin epidermis was very fast and quantitatively significant. ASC12 appears to be the compound that possesses the highest capacity to enhance the penetration of the drug as well as for self-penetration through the epidermis. The ability of these compounds to permeate the rat skin is related to their chemical composition, since the flux of ASCn decreases as alkyl chain length increases. Furthermore, a burst effect was observed with ASC12, which could be important for the enhancing effect on anthralin.

## Histological Evaluation of ASCn Side Effects

Histological examination by light microscopy was used to assess the effects of ASCn on skin and to explore the mechanism of biological responses. For this reason, it is very instructive to make a comparison of histological changes versus the penetration enhancement visualized after coagel application. No inflammatory cellular response was observed in the skin when Coa12, Coa14, or Coa16 were applied, suggesting non-irritating properties for these coagels. Light microscopy indicated a slight disruption and fragmentation of SC in ASC12, ASC14, and ASC16 treated skin, which may have contributed to the capacity of coagels in enhancing the permeation of anthralin. A typical example of a light micrograph taken after 6 h is shown in Fig. 5.

## DISCUSSION

In the permeation of drugs through the skin, two pathways may be identified: the transcellular route

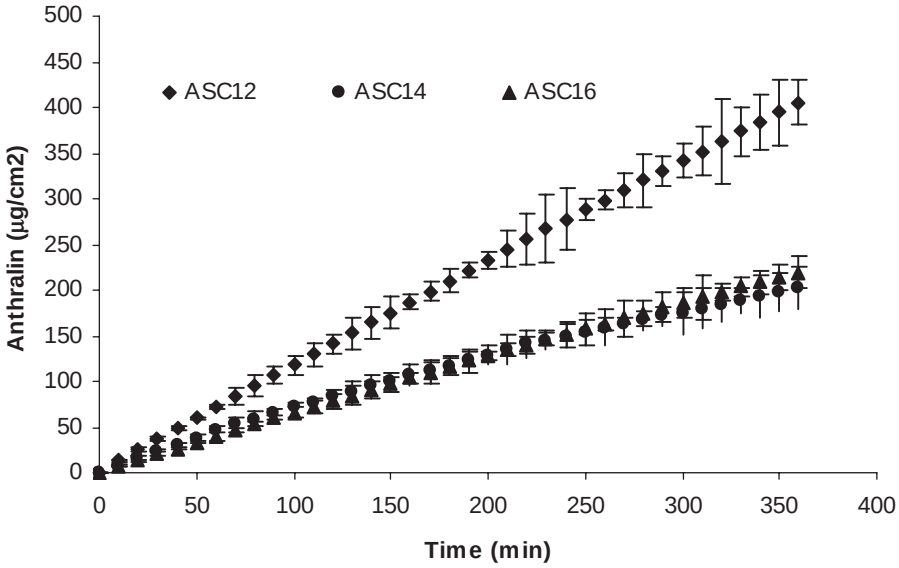



**FIGURE 3** **Permeation of Anthralin Through Rat Skin Using ASCn Coagels. (✦ ASC12, • ASC14 and ■ ASC16).**

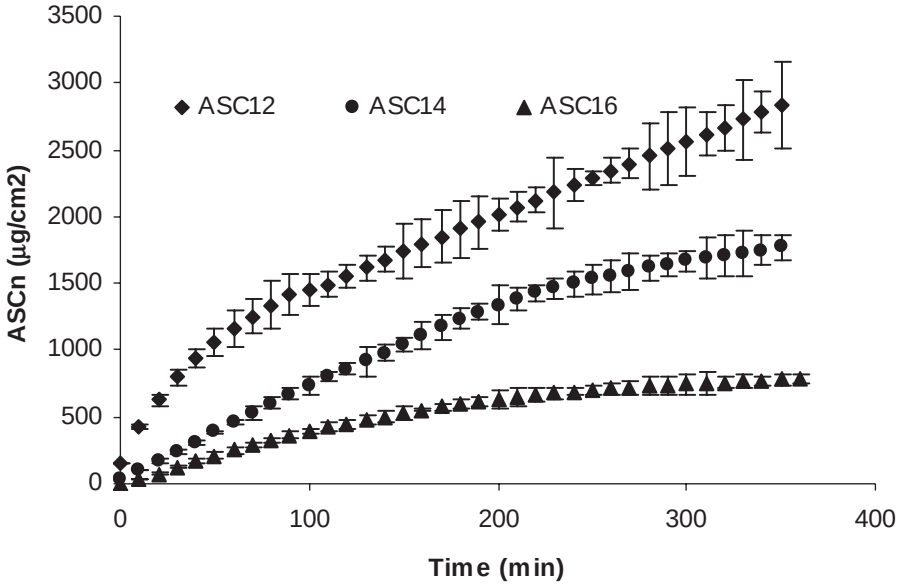



**FIGURE 4** **Permeation of ASCn Through Rat Skin. (✦ ASC12, • ASC14 and ■ ASC16).**

crossing through the corneocytes, and the paracellular route between corneocytes. In both cases, the permeant must diffuse through the intercellular lipid matrix. In this way, any substance (for example, a surfactant) that alters the configuration of this domain could influence the penetration of drugs. As previously mentioned, the use of chemical enhancers is a current technical resource for increasing permeation. Based on Fick's first law, an increase in penetration would be mainly produced by raising the diffusion coefficient of the drug and/or by increasing the drug solubility in the membrane (Moser, 2001). ASCn are relatively lipophylic compounds (ASC16: log $P_{o/w}$ = 1.9) having decreasing aqueous solubility for increasing molecular weight. The permeation of anthralin was significantly increased when the drug was vehiculized in ASCn coagels, compared to other pharmaceutical systems (Agarwal, 2001). According to Fig. 4, ASCn can penetrate the epidermis of rat skin quickly, especially in the case of ASC12.

ASC12 has a structural similarity to other chemical enhancers like Azone® (Fig. 6b) and oleic acid, where

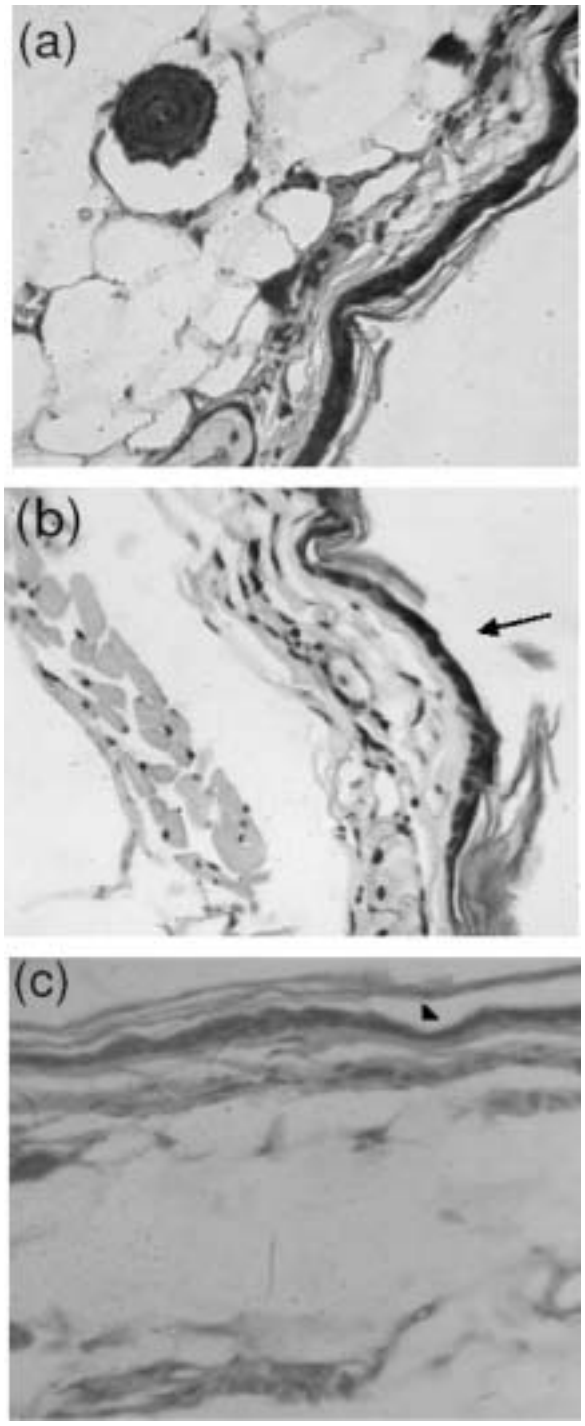



**FIGURE 5** **Light Micrograph of Mice Skin (400x). a) Untreated Skin, b) and c) Skin After 6 h Treatment with Coa12. Note the Absence of Inflammatory Response and Disruption (Head Arrow) and Fragmentation (Arrow) of SC.**

the difference lies in the polar head. It was suggested that Azone® exists in a "spoon-shaped conformation" and may alter the barrier properties of the SC lipids by insertion and opening-up of adjacent ceramide molecules (Williams, 2004; Hoogstraate, 1991). However, unlike ASC12, Azone® is unable to penetrate further into the SC principally due to its high lypophilicity (log $P_{o/w} = 6.2$) (Williams, 2004). As can be realized from Fig. 6a, ASC12 may adopt this spoon-shaped-like conformation and consequently be able to insert itself between ceramide molecules. Nevertheless, the bigger polar head of ASC12 and its lower lipophilicity might allow this surfactant to penetrate deeply into the SC.

ASCn provokes reversible changes in the SC structure that make possible itself penetration and the permeation of other molecules. This is clearly observed when hydrophobic molecules, such as FITC and anthralin, are vehiculized in ASCn coagels. Among the derivatives studied, ASC12 showed the highest enhancer effect. This behavior was also observed with other structurally related surfactants, such as Azone® and fatty acids (Williams, 2004), where the chemical structure containing an alkyl chain of 12 atoms of carbon was the most effective. Apparently, ASCn permeate very quickly the SC by intercalating in the lipid domain and thus producing a reversible and transient rearrangement (for example lipid fluidizing). In agreement with this, when anthralin is administrated in an ASCn coagel, the penetration of the surfactant produces changes that allow the permeation of the drug. This fact could explain the lack of lag time (Fig. 3), which is usually observed for the penetration of drugs co-administrated with surfactant enhancers (Shokri, 2001; Nokhodehi, 2003).

It would be desirable to evaluate the enhancing effect of ASCn coagels compared to other commercial formulations. Unfortunately, these types of medicine (Antraderm®) were removed from the list of available medicines in Argentina some years ago.

However, some results published obtained under similar conditions (Agarwal, 2001) were used for comparison. In this work it was reported that the topical administration of vesicular systems (liposomes and niosones) containing anthralin (dithranol) exhibited enhanced permeation through the mice skin in comparison with the conventionally prepared cream base formulation of anthralin. The authors reported flux values of 23.13 $\mu g/cm^2/h$ for the liposomal system and 7.78 $\mu g/cm^2/h$ for the niosomal system, compared to 4.10 $\mu g/cm^2/h$ obtained for the conventional cream. If such results are compared to those obtained in our work (see Table 1), it can concluded that ASCn exhibit a higher efficiency as permeation promoters of anthralin.

Other important features of ASC12 performance (Fig. 4) is the observed burst effect. Although the

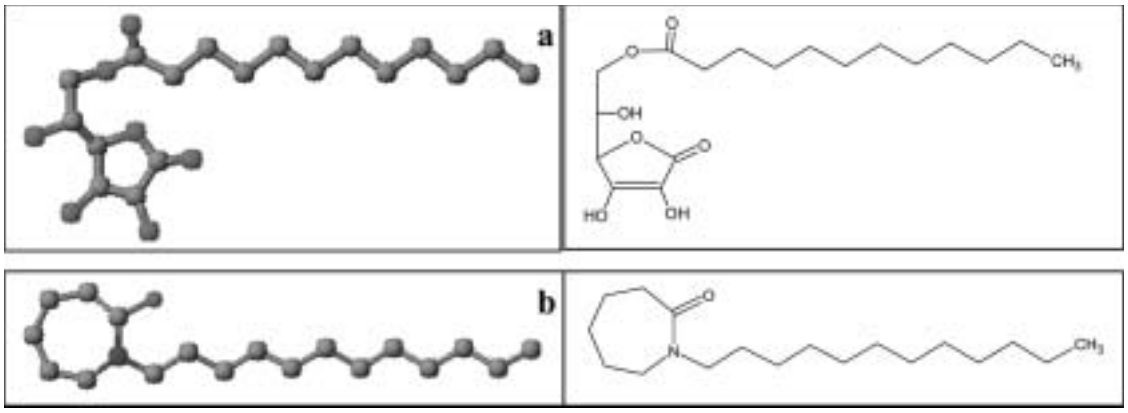



**FIGURE 6** **Molecular Structures of a) ASC12 and b) Azone®.**

results of this work are not enough to propose any possible explanation, it could be hypothesized that the initial rapid hydration that the coagels produce on the SC promotes the fast permeation of ASC12. On the basis of these observations; some assays should be performed to obtain a better understanding of the absorption promoter mechanism of ASC12. Some questions could be answered, for example: is the monomer or the aggregate (lamella) responsible for enhancing effect? Do ASCn and anthralin diffuse together across the skin? Finally, it is important to emphasize the absence of side effects observed in the epidermis after the application of ASCn coagels.

## CONCLUSIONS

ASCn conform an interesting group of compounds with promising pharmaceutical applications. The coagels are pharmaceutical carrier systems for unstable and low solubility drugs. According to the results of this paper, it could be possible to increase the permeation of lipophilic drugs. In this way, these surfactants would be very useful by improving the biopharmaceutical properties of a great variety of therapeutically relevant drugs to be administrated by different routes, where drug absorption is required.

## ACKNOWLEDGEMENTS

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