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Effect of Different Coagels of 6-O-Ascorbic Acid Alkanoates on Permeation of Ibuprofen Through Hairless Mouse Skin

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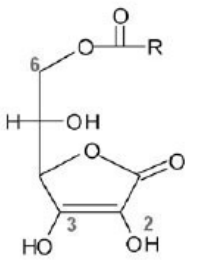
SUMMARY. The purpose of the present investigation was to evaluate 6-O-palmitoyl ascorbic acid (ASC16) and 6-O-lauril ascorbic acid (ASC12) for their capacity to promote permeation of Ibuprofen (IBU) through hairless mouse skin *in vitro*. The permeation of Arfen® (IBU 10% P/P, commercial product), IBU solution (0.85% P/P, pH 7.4, 66.7 mM phosphate buffer: Isopropyl alcohol; 80:20) and IBU (0.85% P/P) vehiculized in ASCn coagels (5% w/v) were measured and comparatively analyzed. Although IBU release from the formulation was faster in the case of Arfen® in comparison to ASCn coagel, the permeation of IBU was significantly increased when the drug was vehiculized in these systems, being ASC_{12} the most effective as enhancer. This result demonstrated the potential usefulness of the lamellar liquid crystalline system obtained from ASCn auto-aggregation for topical administration of drugs.

INTRODUCTION

Non-steroidal antiinflamatory drugs (NSAIDs) are one of the most interesting group of compounds to be formulated in topical dosage forms [1]. The choice of the most appropriate active depends on a number of factors such as potency, lack of local skin toxicity and ability to permeate the stratum corneum [2,3]. The greatest obstacle for transdermal drug delivery is the stratum corneum that forms a primary rate limiting barrier to the permeation of drugs across the skin. Ibuprofen (IBU) is a well studied NSAIDs and the improvement of permeation through skin has been intensely investigated. Different approaches such as eutectic mixtures [4], supersaturation [5,6] and the use of permeation promoters [7] have been attempted. More recently, novel formulations of IBU, such as microemulsions [8] and microgels [9,10] have been developed.

Since few years ago, we have been studying a series of surfactant compounds which have shown very interesting properties [11]. 6-O-Ascorbic acid alkanoates (ASCn) are amphiphilic molecules having physical-chemical and self-aggregation properties that depend on the alkyl chain length. ASCn are obtained through the esterification of hydroxyl group in position 6 of vitamin C (VC) with fatty acids of variable length chain (Fig 1). The acid strength of the derivatives remains similar to that of VC, with pKa = 4.2 for –OH group in position 3 and pKa = 11.6 for –OH group in position 2. Also the redox properties of VC are maintained in its amphiphilic 6-O-alkanoates.



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

x	n
6	(ASC 8)
8	(ASC 10)
10	(ASC 12)
12	(ASC 14)
14	(ASC 16)

Figure 1. 6-O-Ascorbic acid alkanoates (ASCn) chemical structures.

KEY WORDS: Ibuprofen, Liquid crystals, Skin permeation.

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The derivatives with six or eight atoms of carbon have enough solubility at room temperature to form stable supramolecular aggregates in water while for n > 10 it is necessary to raise the temperature until the solubility reaches the critical micellar concentration (CMC). This temperature dependence of surfactants' solubility indicates the existence of the so-called Krafft temperature or Krafft point (critical micellar temperature, CMT) [12,13]. The CMT of ASCn derivatives increases as the length of hydrocarbon chain becomes longer and, as can be expected, the kind of aggregates that can be formed above CMT is also dependent of the structure of derivative [14].

The derivatives with hydrophobic alkyl chains longer than 12 atoms of carbon possess a critical micellar temperature (CMT), which is in all cases higher than 30 °C. At temperatures higher than CMT, ASCn aqueous suspensions turn into either micellar solutions or gel phases, depending on the length of the hydrophobic chain. On cooling, coagels are yielded, which possess a lamellar structure that exhibit sharp X-ray diffraction patterns and optical birefringence [15] (Fig. 2).

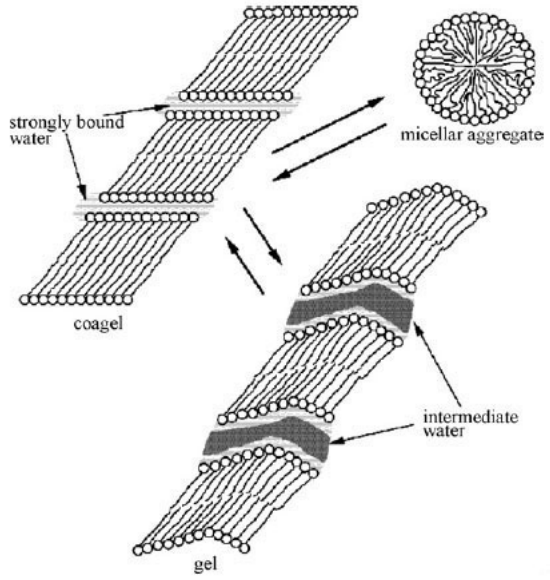

Figure 2. Self assembly properties of ASCn.

Besides, low solubility and unstable drugs were successfully formulated in ASCn coagels, whereas the permeation of some drugs through rat skin was increased [16]. All this properties, as well as the semisolid consistency of the systems, make ASCn coagels very attractive systems for formulating dermatological pharmaceutical dosage forms containing IBU. In this article we presented the results concerning the formulation of IBU in 6-O-palmitoyl ascorbic acid (ASC16) and Ascorbyl laurate (ASC_{12}) coagels. We evaluated the release of IBU through semi-permeable membrane and hairless mouse skin. A marketed drug product (Arfen®) and IBU suspended in isopropanol (IBU*i*-p) were assayed also for comparison.

MATERIALS AND METHODS

Materials

Ibuprofen (IBU), ascorbic acid (AA), 6-O-palmitoyl ascorbic acid (ASC16) and lauric acid (LA). All reactants (analytical grade) were purchased from Fluka (Milan, Italy), and used without purification. Sulfuric acid 95%, diethyl ether, petroleum ether, isopropanol and sodium sulphate were analytical grade and used as received.

Synthesis of Ascorbyl laurate (ASC12)

Ascorbyl laurate (ASC_{12}) was synthesized according to the procedure already reported in the literature, which involves the reaction in concentrated sulfuric acid between LA and the primary –OH group in position 6 of ascorbic acid [17]. The reaction product was conveniently characterized by TLC, ^{H}RMN, DSC and UV-Vis spectroscopy.

Preparation of coagels

Drug free coagels were prepared by heating ASCn (ASC_{12} and ASC_{16}) aqueous suspension to above the phase transition temperature (CMT) and allowing the temperature to fall to room temperature thus yielding the respective coagels (Coa12 and Coa16). To incorporate IBU (acidic form) in the coagels, ASCn aqueous suspensions were heated to above their CMT (CMT_{Coa12}: 47.3 °C; CMT_{Coa16}: 63.8 °C)[18] until the gel phase was formed, and exactly weighed amounts of IBU (0.85 % W/V) were incorporated as finely divided solids to obtain ASCn series. The systems were kept under these conditions for 2 h in order to obtain the complete solubilization of IBU. After this period, the colloidal dispersions were allowed to reach room temperature. In both cases the concentration of ASC_n was 5% (W/V).

In vitro release of Ibuprofen

In vitro drug release was assessed by means of Franz-type cells of the "GH" design specified by Gummer [20]. The donor and the receptor chambers were separated by dialysis membrane

(Spectra/Por® Membrane MWCO: 3,500) The donor phase consisted of the preparations under study, while the receiving phase (5 ml) was isotonic, 66.7 mM, pH 7.4 phosphate buffer containing 0.003% w/v sodium azide to prevent bacterial growth. In all experiments, 5 ml of the receptor solution were withdrawn for analysis at suitable time intervals, and were replaced with equal volume of fresh solution; the amount of permeated IBU was quantified by HPLC using a liquid chromatograph with LC 6A Pump and 20 µl Rheodyne injector, SPD-10A array detector and computer integrating system, Shimadzu Corp., Kyoto, Japan. The column (150 x 4 mm) was C18 Synergi 4u Fusion–RP 80A (size 5 µm, Milford, MA, USA). We used two different mobile phases (flow rate 1.00 ml/min). For ASCn: MeOH:CH_3CN:PBS pH 2.5 0.05M (65:14:21) and for IBU: CH_3CN:H_2O pH 2.6 with phosphoric acid (60:40). The retention time and the detection wavelength for IBU were 6.5 min and 225 nm, respectively; and 13.5 min for ASC12 and 22.5 min for ASC16. In both case the wavelength was 265 nm.

The concentration of permeate in each sample was determined from standard curves, obtained by plotting the concentration of solutions of known concentration versus the corresponding peak areas of HPLC chromatograms. pH 7.4 phosphate buffer saline isotonic solution was used as the receptor phase. Appropriated calculations demonstrated that satisfactory sink conditions were maintained throughout all experiments. Each release test was replicated at least three times.

Hairless mouse skin permeation experiments

Permeation tests of IBU through excised skin of 4-7-week-old hairless mice (Strain MF1-hr/hr/Ola, Nossan Srl, Correzzana, Milano) were carried out as described in a paper published elsewhere [19] using Franz-type cells of the "GH" design specified by Gummer [20], with an effective diffusion area of 1.23 cm^2. The donor phase consisted of the preparations under study. The receiving phase (5 ml) was isotonic, 66.7 mM, pH 7.4 phosphate buffer containing 0.003% w/v sodium azide to prevent bacterial growth. In all experiments, 5 ml of the receptor solution were withdrawn for analysis at suitable time intervals, and were replaced with equal volumes of fresh solution. The amount of permeated IBU was measured by HPLC as previously described. To ensure sink conditions, the concentration of IBU in the receiving solution was always kept approximately equal to 1/10 of the IBU solubility. The permeation experiments were continued for 20 h, and each test was replicated at least four times.

Linear regression analysis of pseudo steady-state diffusion data allowed calculation of *J,* the steady-state flux (given by *Q/At,* where *Q* is the amount of the permeate diffusing across the area *A* in time *t*). The apparent skin permeability coefficients (P_{app}) for IBU were obtained using the relation (Eq. **1**):

$$Papp = J/C_d \qquad [1]$$

derived from Fick's first law, where C_d is the initial drug concentration in the donor phase. The relative apparent skin permeability coefficients (P_{app} relative) reflect the relative permeability of the formulations comparative to Arfen®. The permeation lag times (indicating the time taken by the drug to saturate the skin and to reach the receiving compartment) were calculated from the x-axis intercept values of the regression lines.

RESULTS AND DISCUSSION

Ibuprofen release in vitro

The *in vitro* release profiles of ibuprofen from different formulations through semi permeable membrane are illustrated in Figure 3. We included in the study a commercial formulation of IBU, Arfen®, and IBU*i*-p for comparison.

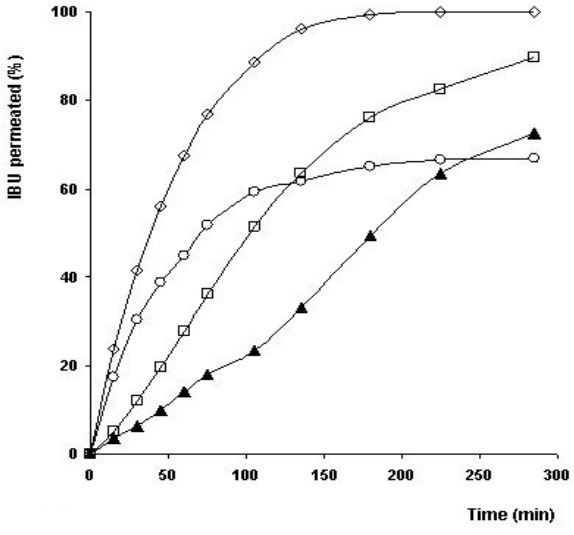

Figure 3. Permeation of IBU through semi-permeable membrane formulated in: IBU*i*-p (◇),Coa12 (❑), Coa16 (▲), Arfen® (○).

Formulation	J (mg cm^{-2} h^{-1} 103 ± S.E.)	P_{app} (cm h^{-1}.103 ± S.E.)	Lag Time (h ± S.E.)	Amount of Ibu permeated after 20 h (mg cm^{-2} ± S.E.)
ASC12/CIbu	43.27 ± 11.42	5.17 ± 1.37	0.82 ± 0.20	0.83 ± 0.21
ASC16/CIbu	24.10 ± 2.33	2.86 ± 0,31	0.48 ± 0.16	0.47 ± 0.05
Arfen	13.97 ± 1.47	0.24 ± 0.03	3.71 ± 0.28	0.23 ± 0.03
IBU*i*-p	108.93 ± 12.83	13.21 ± 1.50	0.79 ± 0.07	2.10 ± 0.25

Table 1. Indicative parameters of IBU permeation through hairless mouse skin.

As expected, IBU*i*-p showed the fastest release, whereas IBU release from Arfen® was slower. Both of them presented a behaviour that it is characteristic for a diffusion based release kinetic, which was also corroborated by calculating the diffusion exponent "n", according to the following semiempyrical relationship (Eq. **2**):

$$M_t/M_{inf} = kt^n \qquad [2]$$

where M_t/M_{inf} represents the fraction of drug released at time *t, k* is a constant, characteristic of the system, and the exponent *n* is indicative of the release kinetics [21,22]. A value of n = 0.5 indicates the occurrence of Fickian diffusion, while n = 1 corresponds to zero-order kinetics. Values of n between 0.5 and 1 indicate anomalous (non-Fickian) transport.

In the case of ASC_{12} (Coa12) and ASC_{16} (Coa16) coagels, IBU release was sustained as indicated by the diffusion coefficient, 1.0 and 1.1, respectively, IBU release from Coa16 was slightly lower than that of Coa12. This difference could be attribute to the higher viscosity of Coa16 compared to Coa12 [23].

When we evaluated the IBU permeation through hairless mouse skin, we could observe a noticeable increment in permeation in the case of ASCn coagels, especially for Coa12 (Fig. 4). The penetration of IBU vehiculized in isopropanol was very high owed mainly to skin disruption provoked by the solvent which produces an alteration of the arrangement of stratum corneum lipids. It has been reported that isopropanol/water mixtures may increase the fluidity of lipophilic alkyl chains along with decreased melting points of crystallized lipid fractions [24].

The permeation of IBU from Arfen® was lower than Coa12 and Coa16. About lag time, Arfen® needed 4.5 h to saturate the skin, while Coa16 and Coa12 could cross the skin in 45 min and 60 min, respectively. The relative P_{app}

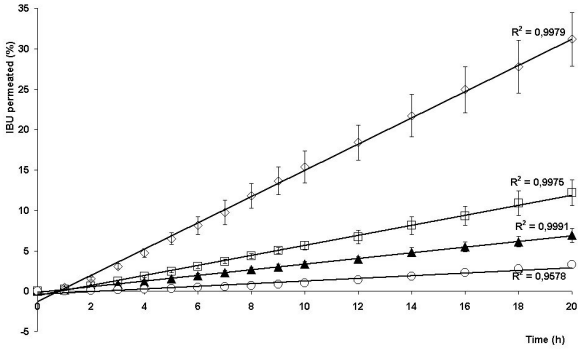

Figure 4. Permeation of IBU through rat skin formulated in: IBU*i*-p (◊), Coa12 (❑), Coa16 (▲), Arfen® (❍).

showed in Table 1 quantitatively indicate the magnitude of the increment in permeation. We have previously corroborated the enhancing activity of ASC_{12} without noticeable injurious effects on the skin [16].

CONCLUSIONS

Lamellar liquid crystals obtained from autoaggregation of ASCn have shown to possess desirable properties as vehicle for transdermal administration of drugs. In this case, Coa16 and particularly Coa12 increased the permeation of IBU comparative to the commercial formulation Arfen®.

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