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Research paper

Optimization of skin permeation and distribution of ibuprofen by using nanostructures (coagels) based on alkyl vitamin C derivatives

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ABSTRACT

In this investigation two vitamin C-based -6-O-ascorbic acid esters (ASC_{12} and ASC_{16}), able to form liquid–crystal structures (coagels) was evaluated for their potential usefulness to promote the permeation and distribution of ibuprofen (IBU). Two coagel formulations and the same coagels added of polyethylene glycol (PEG-400) were assayed in comparison with a commercial product (Arfen®) by using hairless rat skin as model.

The ASC_{16} and ASC_{12} derivatives gave rise to stable supramolecular assemblies in water and in water/PEG mixtures (coagels), allowing the solubilization of IBU (0.85%) and producing a IBU controlled release systems, as evidenced by the dynamic dialyse test: the *n* values were near 1.0, indicative of a linear kinetic, for all coagel formulations, except for the ASC_{12}PEG/C formulation ($n = 1.51$).

Our results evidenced the enhancement activity of coagels and the synergic effect of the combination with PEG: all coagels showed a higher amount of IBU permeated through the skin compared to commercial Arfen® with an enhancement factor of 52.94 and 21.53 for ASC_{12}PEG/C and ASC_{16}/C respectively. Otherwise, coagels formulations appeared to produce a low IBU depot in the skin and in the same order of magnitude in epidermis and derma, in spite of significant increase of IBU cutaneous permeation. The positive synergic effect of the coagel–PEG mixtures was demonstrated by the high amount of IBU accumulated in the upper skin layers.

The effect of the coagels on the IBU skin permeation and distribution depending on their hydrolipophilic character could allow a rational design and an optimization of topical formulations.

1. Introduction

The amount of drug that can be transported into or through the skin (dermal or transdermal delivery) depends on the ability of drug to permeate the skin at a rate and in quantity sufficient to achieve an effective concentration in the biological tissue. Several factors are involved in this process, for instance the skin permeability, the physicochemical properties of the drug and the characteristics of the vehicle [1–4].

The penetration of a drug through the skin is hindered by the cutaneous outermost external layer, defined stratum corneum (SC), constituted of keratin-rich dead cells embedded in a complex lipid matrix base of ceramides, cholesterol, and free fatty acids.

The SC is a considerable barrier to the transport of hydrophilic substances while the viable epidermis, situated below the SC and much more aqueous in character, represents a significant barrier for very lipophilic substances [5–7].

Ibuprofen (IBU), a small molecule (MW = 206) with a octanol–water partition coefficient (log P) of around 4 [8], is a potent non-steroidal anti-inflammatory drug (NSAID) effective to control muscle and joint pain and used in rheumatoid and osteoarthritis therapy [9,10]. Because of the poor skin permeability of ibuprofen, there is a great interest for the cutaneous dosage forms that could provide appropriate drug levels at the application site avoiding the adverse effects like gastric mucosal damage and bleeding associated to oral delivery. However, high doses of drug have to be applied on the skin to obtain a therapeutic amount of drug in the target site. The total amount of delivered drug to target site might be improved by influencing the physicochemical properties of the drugs as lipophilicity, solubility, molecular weight or size, and hydrogen bonding ability, and/or with the incorporation of a penetration enhancer in the formulation. Numerous chemical compounds have been evaluated for penetration-enhancing activity. The penetration enhancers can increase drugs diffusion through the skin by modifying the intercellular lipid packing of the skin, or can increase the partition coefficient of the active agent between skin and vehicles by solubilizing into the skin [11–15].

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The 6-O-ascorbic acid alkanoates (ASC_n) behave as amphiphilic molecules in water for the presence of a hydrophobic moiety (aliphatic chain) and a polar group (ascorbic acid). Depending on the length of *n*-alkyl fatty chain, they form supramolecular aggregates at temperatures (CMT, Krafft point) in which the solubility reaches the critical micellar concentration (CMC) [16–18]. These systems (coagels), increasing the apparent solubility and the stability of several drugs and promoting the penetration of several substances, seem a promising tool for the development of pharmaceutical dosage forms [19–22]. The addition of polyethylene glycol as co-enhancer to coagel appears to increase the solubility of lipophilic compounds [23–26] and to reduce the compactness of the lamellar structures of coagels favouring a change of drug bioavailability [27,28].

In the present study, the influence of the coagels prepared with ascorbyl laurate (ASC_{12}) and ascorbyl palmitate (ASC_{16}) derivatives on cutaneous permeation and distribution of ibuprofen was investigated using rat skin as a widely accepted model for human skin [29–31]. Two coagel formulations and the same coagels added of polyethylene glycol (PEG 400) as cosolvent/enhancer were tested choosing a commercial product (Arfen®) as reference. Furthermore, a dynamic dialyse *in vitro* study was carried out to characterize the release mechanism and rate of ibuprofen from the different formulations.

2. Materials and methods

2.1. Chemicals

Palmitoyl-6-O-ascorbic acid or ascorbyl palmitate (ASC_{16}), L-ascorbic acid (AA), S-**(+)**-Ibuprofen (IBU), lauric acid (LA), polyethylene glycol 400 (PEG 400), sodium lauryl sulphate (SLS), and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Fluka (Milan, Italy). Lauroyl-6-O-ascorbic acid or ascorbyl laurate (ASC_{12}) was synthesized in our laboratories according to the procedure already reported in the literature [16].

Sulphuric acid 95%, diethyl ether, petroleum ether, and sodium sulphate (analytical grade) were purchased from Fluka (Milan, Italy) and used without purification.

Methanol (MeOH), acetonitrile (AcN), isopropyl alcohol (i-PrOH), all of HPLC-grade, were purchased from Carlo Erba (Milan, Italy). Milli-Q water was used in all experiments.

A commercial gel containing 10% w/w of ibuprofen and isopropanol according to the information provided to the consumer (Arfen®, Lisapharma, Erba, CO, Italy) was used as reference.

2.2. Synthesis of ascorbyl laurate (ASC_{12})

Ascorbyl laurate (ASC_{12}) was synthesized by condensation reaction between lauric acid (LA) and C_6OH primary group of L-ascorbic acid in sulphuric acid at 45 °C [16]. A 100 ml sample of 95% sulphuric acid was placed in an Erlenmeyer flask, and a gentle stream of nitrogen was passed through for 30 min under magnetic stirring at room temperature. Then, ascorbic acid (4.41 g, 25 mmol, AA) was completely dissolved, and after LA (5.01 g, 25 mmol) was added. The flask was then equipped with a rubber stopper and kept in a nitrogen atmosphere. A water bath at 45 °C was used to dissolve LA completely. After 16 h, the mixture was poured into a beaker containing 600 ml of ice and stirred until it reached room temperature. The solution was treated with diethyl ether several times; after, the organic phase was treated with anhydrous sodium sulphate for 30 min and then filtered. The white-yellow solid was obtained after evaporation under reduced pressure of the solvent that was washed in petroleum ether and finally re-crystallized three times from diethyl ether/petroleum ether (50:50) mixtures.

Purity was conveniently assessed through TLC and chemical–physical analysis. TLC (silica gel, diethyl ether): only one spot with $R_f = 0.41$ was observed. The melting point was 104.5–105.5 °C, and the UV–VIS spectroscopy showed an absorbance at $\lambda_{max} = 236$ nm (CH_3CN) for an ASC_{12} organic solution.

2.3. Formulations

2.3.1. Preparation of the coagels (ASC_n/C)

Coagels containing ibuprofen (IBU) were prepared by heating at 52 °C and 68 °C for ASC_{12} and ASC_{16}, respectively, both above their critical micellar temperature (CMT = ASC_{12}: 47.3 °C, ASC_{16},: 63.8 °C; [20]), a 5% w/w of aqueous suspensions of the derivatives containing IBU (0.85% w/w). The systems were kept over CMT for 2 h in order to obtain the complete solubilization of IBU, and then, they were cooled to reach room temperature obtaining the coagels ASC_{12}/C and ASC_{16}/C.

The composition of the formulations were reported in Table 1.

2.3.2. Preparation of the coagels with PEG 400

Coagels were prepared following the method previously reported and adding to aqueous suspensions of ASC_n, 30% w/w of PEG 400, to obtain the final formulations ASC_{12}PEG/C and ASC_{16}PEG/C.

The composition of the preparations were reported in Table 1.

2.3.3. Preparation of a formulation based on ASC_{16} derivative

A formulation with ASC_{16} coagel structure breakdown (ASC_{16}) was prepared by applying a shear stress of 20 Pa for 10 min to previously prepared coagel formulation (ASC_{16}/C, see Table 1) using a rotational viscosimeter (Rheostress RS 150 apparatus equipped with the cone plate combination 60/4°, Haake, Paramus NJ). The shear stress value was higher than the typical static yield for the ASC_{16} derivative as reported by Palma et al. [20].

2.3.4. Preparation of a reference suspension in PEG 400 (PEG-R)

A suspension containing 0.85% w/w IBU (PEG-R) was prepared in a PEG 400 and water mixture (70:30 w/w ratio). The suspension was maintained under magnetic stirrer for two hours at room temperature (Table 1).

2.3.5. Preparation of the reference solution (IBU-S)

A reference solution containing 0.85 w/w IBU (IBU-S) was prepared by dissolving drug in a isopropanol/pH 7.4, 66.7 mM phosphate buffer (20:80 w/w/ ratio) mixture under stirring.

2.4. Skin model

Full-thickness rat skin was obtained from 6–8 weeks old hairless male animals (HsdHan™:RNU-Foxn1 rnu, Harlan Italy srl, Correzzana, Italy). The animals were killed by cervical dislocation immediately before the experiments; the skin was carefully

Table 1
Composition of formulations containing IBU.

Formulations	Composition (% w/w)					
	IBU	ASC_{12}	ASC_{16}	i-PrOH	Water	PEG 400
ASC_{12}/C	0.85	5.00			94.15	
ASC_{16}/C	0.85		5.00		94.15	
ASC_{16}/IBU[a]	0.85		5.00		94.15	
ASC_{12} PEG/C	0.85	5.00			64.15	30.00
ASC_{16} PEG/C	0.85		5.00		64.15	30.00
PEG-R	0.85				69.15	30.00
IBU-S	0.85			19.83	79.32	

[a] ASC_{16}/IBU is the coagel with the disrupted structure.

excised, and the adhering fat and subcutaneous tissue were removed. The thickness of the prepared skin was measured with a micrometer (550–650 μm), and the skin samples were stored at −20 °C prior to use. The study performed in this section was approved by the Ethical Committee of the University of Pisa, and the protocol was compliant with the European Union Directive 86/609/EEC for the use of experimental animals.

2.5. In vitro release studies

The *in vitro* release of ibuprofen from the coagels and from the reference formulations (IBU-S, PEG-R, and commercial preparation, Arfen®) was assessed using dialysis bags (MWCO:3500; Spectra/Por 3 Membrane, Spectrum Medical Industries Inc., Houston, USA) filled with an exactly weighted amount of each formulations (10.0 g). The bags were put into 10.0 ml of 66.7 M, pH 7.4 buffer phosphate solution (PBS) maintained at 37 °C and stirred at 20 rpm. At predetermined intervals of time 1.0 ml of buffer solution were withdrawn and replaced with fresh PBS. The amount of drug in the receiving phase was determined by HPLC.

2.6. Ex vivo permeation studies

Permeation experiments through excised rat skin were carried out as previously described [32] using Gummer-type diffusion cells with an available diffusion area of 1.23 cm^2 and stratum corneum facing the donor compartment. In order to have an equal amount of drug in the donor compartment, 1.0 ml of each formulation containing ibuprofen (free acid form) or 0.14 ml of Arfen® containing the ibuprofen lysine salt were placed on the skin surface. The receptor compartment contained 5.0 ml of PBS maintained at 37 °C and stirred at 600 rpm. At 60 min intervals for the first 10 h and at 120 min intervals for the following 10 h, 5.0 ml samples were withdrawn for HPLC analysis and replaced with the same volume of fresh fluid. Each permeation test was replicated at least six times, and the solubility of IBU in the receiving fluid determined by HPLC was 1.96 mg/ml.

Linear regression analysis of pseudo steady-state diffusion data allowed calculation of the steady-state flux (J, given by $Q/\Delta t$, where Q is the amount of IBU diffusing across the area A in time t) and the apparent permeability coefficient (P_{app}) using the relationship $P_{app} = J/C_d$, where C_d is the initial drug concentration in the donor phase. The drug 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.

Enhancements factors (EF), expressing the relative activity of each formulation with respect to commercial reference Arfen®, were calculated from the ratio of P_{app} values calculated for each experimental formulation and for commercial reference (Arfen®).

2.7. Skin distribution studies

At the end of the *ex vivo* permeation experiments, the skin was removed from the cells and rinsed with deionized water. The samples were then frozen and sliced horizontally with a cryomicrotome (MEV Cryostat, Slee-Technik GMBH, Mainz, Germany). The precision of the microtome gives reproducible sections of 0–60 μm in 1-μm steps up to 10 μm, 2-μm steps up to 20 μm and 5-μm steps up to 60 μm.

Although skin was flatten with a weight of 2 kg for 1 min before sectioning, the first slices were usually incomplete because of the irregular surface of the skin samples. The mean thickness of the incomplete slices and skin residues was calculated from their weight with reference to a standard slice of known weight and thickness [32]. Ibuprofen was extracted from the exactly weighted skin slices by treatment with 1.0 ml of 2% w/v sodium lauryl sulphate aqueous solution at room temperature for 20 h. After addition of MeOH (2 ml), the mixture was maintained under magnetic stirrer for 3 h, and then it was centrifuged at 13,000 rpm for 10 min. The amount of IBU in the samples was analyzed by HPLC.

For the validation of extraction procedure, a different series of 20- or 25-μm slices of blank skin was submitted to the assay, and the retention time of endogenous compounds was compared with that of IBU to verify that there were no interferences in drug analysis. The extraction recovery was determined by computing the ratio of the amount of IBU extracted from the skin to the amount added.

2.8. Analytical methods

The quantitative analysis of IBU was carried out by HPLC (LC-10AD pump, SPD-10AV detector, 20-μl Rheodyne injector and computer integrating system, Shimadzu Corp., Kyoto, Japan). The column (150 × 4 mm) was C18 Synergi 4 μm Fusion-RP 80A (Milford, MA, USA). The mobile phase, pumped at a flow rate of 1.0 ml/min, consisted of a mixture of methanol, acetonitrile, and phosphate buffer 0.05 M adjusted to pH 2.5 with phosphoric acid (65:14:21 v/v).

The analysis was carried out at 225 nm, and the retention time was 4.2 min. The amount of IBU in the samples was determined by comparison with appropriate standard curves; in the case of biological materials, standard curves were obtained by adding increasing amounts of IBU to blank biological samples. The limit of quantitation (LOQ) was 0.025 ng/ml.

2.9. Statistical analysis

Statistical differences between the means were assessed by GraphPad Prism software (GraphPad Prism software Inc., S. Diego, CA). The evaluation included the mean of six determinations and relevant standard error. Groups comparison was performed using the Student's two-tailed unpaired t-test. Means were considered statistically different at $P < 0.05$ level.

3. Results and discussion

The *in vitro* drug release test is a standard and effective method to characterize the performance of topical dosage forms. Essential changes in the chemical–physical characteristics of the formulations or in the thermodynamic properties of a drug should be related to differences in drug release.

The mean drug release profiles were fitted according to the power law equation in order to describe the drug release mechanism from the coagel systems [33]:

$$M_t/M_\infty = Kt^n$$

where M_t and M_∞ are the amount of drug released at time t and infinite time, respectively; K is a constant reflecting the structural characteristic of the matrix-eluent system, and n is the exponent characterizing the release mechanism. When $n = 0.5$, the fraction of drug released is proportional to the square root of time (Higuchi equation) and the drug release is pure diffusion controlled; when $n = 1$, drug release is swelling controlled (zero-order release kinetics or case II transport). Values of n between 0.5 and 1 indicate anomalous transport and a superposition of both phenomena often defined non-Fickian kinetic and corresponding to coupled diffusion/polymer relaxation [34–36], while values of $n > 1$ has been regarded as Super Case II kinetics [37,38].

In vitro release parameters are reported in Table 2 for all formulations under study: data represent the mean of six

Table 2
In vitro release data of IBU from the different formulations.

Formulations	Release rate (% min^{-1} ± SE)	*n* (±SE)	*K* (min^{-n} ± SE)	$t_{30\%}$ [a] (min ± SE)	$t_{60\%}$ [a] (min ± SE)
ASC_{12}/C	0.49 ± 0.08	1.18 ± 0.01	0.22 ± 0.04	67.11 ± 10.47	120.87 ± 18.84
ASC_{16}/C	0.27 ± 0.01	1.19 ± 0.09	0.11 ± 0.04	117.19 ± 6.62	210.86 ± 21.09
ASC_{16}/IBU	0.57 ± 0.13	1.00 ± 0.01	0.56 ± 0.13	56.54 ± 15.04	113.02 ± 0.69
ASC_{12}PEG/C	0.15 ± 0.01	1.51 ± 0.07	0.02 ± 0.01	153.38 ± 12.13	243.89 ± 24.39
ASC_{16}PEG/C	0.16 ± 0.01	1.09 ± 0.05	0.10 ± 0.02	189.24 ± 13.93	359.74 ± 36.74
PEG-R	0.29 ± 0.02	1.25 ± 0.01	0.08 ± 0.02	122.86 ± 9.16	214.73 ± 21.31
IBU-S	1.12 ± 0.03	0.74 ± 0.01	3.32 ± 0.02	19.99 ± 0.43	51.36 ± 1.35
Arfen®	1.30 ± 0.05	0.70 ± 0.01	4.53 ± 0.41	14.96 ± 1.36	40.15 ± 3.08

[a] $t_{30\%}$ and $t_{60\%}$, times required to release of 30% and 60% of IBU, respectively.

replicates ± standard error (SE). The *in vitro* release profiles of IBU from the more representative formulations are illustrated in Fig. 1. Table 2 summarizes for each formulation tested, in addition to *n* and *K* values, $t_{30\%}$ and $t_{60\%}$ values (time required to release 30% and 60% of drug, respectively). Even if some calculated interpolation curves appeared to indicate a non-ideal fit, the relevant correlation coefficients (*r*) were in the range 0.967–0.998.

Coagels, except ASC_{12}PEG/C, have the *n* values near 1.0, indicative of a linear kinetic, while Arfen® and IBU-S gave 0.70 and 0.74 values, respectively, demonstrating an anomalous drug release. The capacity of the coagels to control the release rate of IBU was partly influenced by adding 30% w/w PEG as cosolvent; in fact, the *n* value was 1.09 ± 0.05 for ASC_{16}PEG/C coagel and 1.51 ± 0.07 for ASC_{12}PEG/C. Both ASC_{12}PEG/C and PEG-R showed an anomalous release with *n* values greater than 1.0 (PEG-R, $n = 1.25 \pm 0.01$). The addition of PEG seems to shift IBU release from zero-order kinetic obtained for the coagels in absence of cosolvent, probably because it can partially penetrate into the lipophilic layer, replacing part of the strictly bound water molecules and reducing the attractive interactions that keep the bilayered structure together. This behaviour resulted in a partial disruption or in a weakening of the coagel compactness [27].

The release of IBU from Arfen® and IBU-S formulations was characterized by fast diffusion: 60% of drug was released in 40.15 and 51.36 min for Arfen® and IBU-S, respectively, and with a corresponding release rate of 1.30 ± 0.05 and 1.12 ± 0.03% min^{-1}.

For the coagel formulations $t_{60\%}$ ranged between 120.87 ± 18.84 and 359.74 ± 36.74 min for ASC_{12}/C and ASC_{16}PEG/C, respectively. The release rate rose by decreasing the length of alkyl chain: 0.27 ± 0.01% min^{-1} for ASC_{16}/C with respect to 0.49 ± 0.08% min^{-1} for ASC_{12}/C, while in presence of PEG this correlation is limited (see Fig. 1).

In order to know the influence of the nanostructures (coagel systems) on the release profile of IBU, a formulation based on ASC_{16}, with its secondary breakdown structure was studied (ASC_{16}/IBU). The absence of secondary dimensional structure did not modify the release kinetic mechanism ($n \sim 1$ for both formulations). However, as highlighted in Table 2, the release rate of IBU from ASC_{16} formulation was twice higher than ASC_{16}/C (release rate: = 0.27 ± 0.01 and 0.57 ± 0.13% min^{-1}, for ASC_{16}/C and ASC_{16}/IBU, respectively).

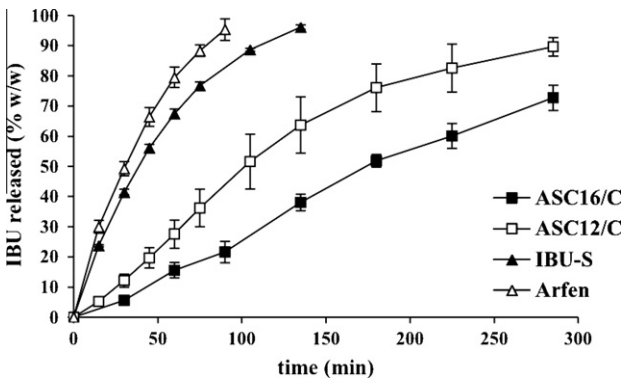

Fig. 1. *In vitro* release profile of IBU from ACS_{12}/C (□) and ACS_{16}/C (■) coagels, reference experimental solution (▲) and reference commercial formulation (△) (mean ± SE, $n = 6$).

Although human skin should be the ideal substrate for percutaneous absorption test, its limited availability forced the investigators to use alternative models. Many studies have been reported in the literature regarding the appropriate animal, reconstituted human skin models, or synthetic membranes to replace human skin in permeation and penetration studies [29–31,39]. Different results have been obtained depending on many factors, as the anatomical sites, the presence of hair follicles, and the different chemical–physical characteristics of the permeants [40]. Human skin is less permeable than rat skin and, as a consequence, human drug exposure would be probably overestimated with this model [41,42]. Hairless rat skin should be a more relevant model for prediction of percutaneous absorption in human than haired rat, even if the difference in percutaneous absorption data between rat and human skin may reach even a factor of 100 [43]. In addition, it is important to highlight that *in vitro* tests on animal models provide important tools for screening different drug formulations, skin permeation enhancing properties and for analyzing the mechanisms of action of delivery systems. Anyway, the capability of ibuprofen to permeate and penetrate the skin from different formulations has been widely studied both in past and recent years, using hairless rat skin substrate as a model for human skin [44–47] demonstrating that *in vitro* permeation studies using rat skin could provide information useful for choosing the best formulation so that the desired permeation and/or penetration of the drug across human skin would be achieved. For this reason, we decided to use rat skin in our study.

The *ex vivo* permeation parameters of IBU from formulations under study (flux, apparent permeability coefficient, lag time, IBU permeated after 20 h) are listed in Table 3. Data represent the mean ± standard error. The more representative permeation profiles of IBU using the formulations under study (see Table 1) are shown in Fig. 2.

Commercial product, Arfen®, produced the lowest values of IBU steady-state flux and P_{app} ($J = 13.97 \pm 1.47$ μg/cm^2 h and $P_{app} = 0.24 \pm 0.023$ cm $h^{-1} \times 10^3$). On the contrary, IBU-S solution containing the drug dissolved in a isopropanol:phosphate buffer mixture (20:80) appeared the most efficacious formulation on improving IBU transdermal permeation: $J = 108.93 \pm 12.89$ μg/cm^2 h; $P_{app} = 13.21 \pm 1.50$ cm $h^{-1} \times 10^3$. It is well known that isopropanol acts as the cutaneous enhancer towards lipophilic drugs, reducing the diffusional barrier by extracting stratum corneum lipids and proteins [48].

ASC_{12}/C and ASC_{16}/C coagels formulations increased IBU transdermal permeation of 21.5 and 11.9-times compared to Arfen® ($p < 0.05$), respectively, demonstrating the ability of coagels to

Table 3
Permeation parameters for IBU through hairless rat skin obtained with the different formulations.

Formulations	J (mg cm^{-2} h^{-1} ± SE)	P_{app} (cm h^{-1} × 10^3 ± SE)	Lag time (h ± SE)	Amount of IBU permeated after 20 h (mg cm^{-2} ± SE)
ASC_{12}/C	43.27 ± 11.42	5.17 ± 1.37	0.82 ± 0.20	0.83 ± 0.21
ASC_{16}/C	24.10 ± 2.33	2.86 ± 0.31	0.48 ± 0.16	0.47 ± 0.05
ASC_{16}/IBU	16.17 ± 1.56	1.90 ± 0.18	0.90 ± 0.43	0.31 ± 0.03
ASC_{12}PEG/C	103.2 ± 13.22	12.71 ± 1.73	1.49 ± 0.04	1.94 ± 0.23
ASC_{16}PEG/C	41.13 ± 3.60	4.59 ± 0.36	0.53 ± 0.26	0.80 ± 0.08
PEG-R	32.07 ± 1.56	3.66 ± 0.13	0.87 ± 0.41	0.61 ± 0.04
IBU-S	108.93 ± 12.83	13.21 ± 1.50	0.79 ± 0.07	2.10 ± 0.25
Arfen®	13.97 ± 1.47	0.24 ± 0.03	3.71 ± 0.28	0.23 ± 0.03

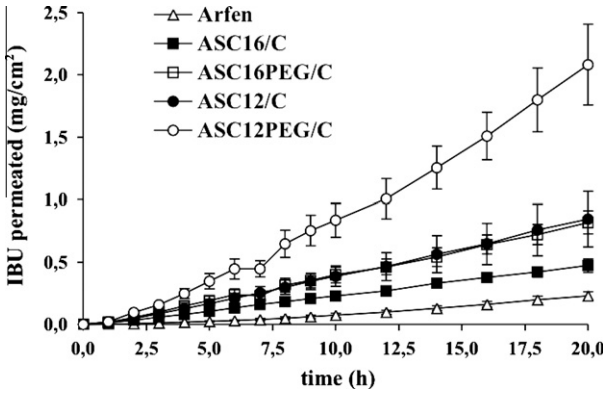

Fig. 2. Permeation profile through rat skin of IBU from ACS_{12}PEG/C (○), ACS_{12}/C (●), ACS_{16}PEG/C (□), ACS_{16}/C (■) coagels and reference commercial formulation (△) (mean SE, n = 6).

improve IBU skin permeation. The ASC_{12}/C coagel appeared the most suitable vehicle to transport IBU through the skin. Palma et al. [21] highlighted a structural similarity of ASC_{12} derivative to other chemical enhancers like Azone and oleic acid. It was suggested that Azone exists in a "spoon-shaped conformation" and may alter the properties of the SC lipids by insertion and opening-up of adjacent ceramide molecules [49]. As indicated from Palma et al. [21], ASC_{12} may adopt this "spoon-shaped" conformation and consequently be able to insert itself between ceramide molecules. Despite the bigger polar head of ASC_{12}, this surfactant might penetrate into the SC. Although the use of ASC_{16}/C did not bring any advantage compared to ASC_{12}/C ($P_{app} = 2.86 \times 10^{-3} \pm 0.31 \times 10^{-3}$ and $5.17 \times 10^{-3} \pm 1.37 \times 10^{-3}$ cm h^{-1}), the breaking down of coagel (ASC_{16}/IBU) produced a significant statistically further decrease in skin permeation ($P_{app} = 1.90 \times 10^{-3} \pm 0.18 \times 10^{-3}$ cm h^{-1}).

Adding PEG 400 to ASC_{12}/C (ASC_{12}PEG/C) allowed to increase IBU cutaneous permeability up to 12.71×10^{-3} cm h^{-1}; this data was comparable to that obtained applying the IBU aqueous/isopropanol solution without significant statistically differences (IBU-S, $P_{app} = 13.21 \times 10^{-3}$ cm h^{-1}). Permeation parameters showed a synergic enhancement effect between ASC_n and PEG 400, more evident for ASC_{12}PEG/C coagel with respect to PEG-R formulation ($p < 0.05$).

P_{app} value of PEG-R was similar to that calculated for ASC_{12}/C and ASC_{16}/C coagels: $3.66 \times 10^{-3} \pm 0.13 \times 10^{-3}$, $5.17 \times 10^{-3} \pm 1.37 \times 10^{-3}$, and $2.86 \times 10^{-3} \pm 0.31 \times 10^{-3}$ cm h^{-1}, respectively, against $12.71 \times 10^{-3} \pm 1.73 \times 10^{-3}$ cm h^{-1} of ASC_{12}PEG/C.

Lag times ranged between 3.71 ± 0.28 and 0.48 ± 0.16 h evaluated for commercial reference (Arfen®) and for ASC_{16} coagel formulation, respectively. The significant reduction in lag time could be due to the permeation enhancer effect caused either by the presence of the ASC_n derivatives or i-PrOH or PEG solvents in the formulation and by their physical structure.

As reported above, ASC_{12}PEG/C produced the same permeation parameters (flux, P_{app}, lag time) of IBU-S, but it is important to highlight that coagels have characteristics of biocompatibility more suitable to the cutaneous administration with respect to the IBU-S formulation, containing high level of isopropanol. The promotion effect of short-chain alkanols as i-PrOH on the flux of lipophilic drugs through human skin was detected both when it was used neat and in association with other permeation enhancers [48].

Table 4 summarizes and compares relative release rates (RR) calculated from the ratio between release rate of the formulation and that of Arfen® obtained from *in vitro* release studies and EF (enhancement factor) for *in vitro* permeation experiments through a natural tissue. It is interesting to highlight that RR are in almost all cases < 1, indicating that IBU release from the formulations took place more slowly than from the reference. On the contrary, EF values are remarkably over 1.0 depending on the formulation; it seems to demonstrate the existence of an interaction between the formulation and the skin that facilitates the drug permeation overcoming the IBU reduced release rate. In particular, ASC_{12}PEG/C followed from ASC_{16}/C had a high EF value (52.94 ± 7.21 and 21.53 ± 5.73, respectively) in spite of a low RR value (0.12 ± 0.01 and 0.38 ± 0.06, respectively).

Our results evidenced both the enhancement role of the ASC_n coagels and the synergic effect of the combination ASC_n/PEG. The combined use of lipophilic permeation enhancer with PEG was already investigated in *ex vivo* drug permeation studies through porcine buccal mucosa where an increase in the drug permeation adding up to 10% of PEG 200 was observed not withstand any change drug release profile [25].

The preliminary *ex vivo* penetration data are summarized in Table 5 as micrograms of IBU retained in each skin layers per milligram of skin after contact with the formulations under study for 20 h.

Except for coagels containing PEG, the IBU-S formulation produced the highest concentration of IBU retained in the rat skin, both in upper and deeper layers. IBU concentration for IBU-S at 50 μm depth (5.80 ± 1.66 μg/mg) was higher than that obtained with Arfen® (2.10 ± 0.63 μg/mg) and remarkably higher if compared to both coagels formulations (0.71 ± 0.03 and

Table 4
Comparison between permeation parameters (enhancement factor) and *in vitro* release parameters (relative rate) of IBU obtained with the different formulations.

Formulation	*EF* (±SE)	*RR* (±SE)
ASC_{12}/C	21.53 ± 5.73	0.38 ± 0.06
ASC_{16}/C	11.92 ± 1.28	0.21 ± 0.01
ASC_{16}/IBU	7.91 ± 0.77	0.44 ± 0.10
ASC_{12}PEG/C	52.94 ± 7.21	0.12 ± 0.01
ASC_{16}PEG/C	19.12 ± 1.51	0.13 ± 0.01
PEG-R	15.26 ± 0.53	0.22 ± 0.02
IBU-S	55.04 ± 6.27	0.86 ± 0.02
Arfen®	1	1

Table 5
Concentration of IBU in the skin depth following application of the formulation to hairless rat skin *in vitro* (mean μg/mg ± SE, $n = 6$).

Skin depth (μm)	ASC_{12}/C	ASC_{16}/C	ASC_{16}/IBU	ASC_{12}PEG/C	ASC_{16}PEG/C	PEG-R	IBU-S	Arfen®
50	0.71 ± 0.03	0.53 ± 0.11	2.22 ± 0.31	12.46 ± 2.90	24.23 ± 2.53	2.70 ± 0.86	5.80 ± 1.66	2.10 ± 0.63
100	0.32 ± 0.05	0.26 ± 0.04	1.57 ± 0.36	3.00 ± 0.96	8.37 ± 1.25	0.80 ± 0.25	3.11 ± 0.48	2.56 ± 0.85
150	0.23 ± 0.01	0.34 ± 0.10	0.61 ± 0.15	1.88 ± 0.28	5.36 ± 1.38	0.72 ± 0.12	2.61 ± 0.50	0.77 ± 0.20
300	0.32 ± 0.05	0.30 ± 0.02	0.48 ± 0.07	0.89 ± 0.16	3.71 ± 0.72	0.90 ± 0.17	2.35 ± 0.78	0.16 ± 0.04
600	0.30 ± 0.03	0.40 ± 0.07	0.90 ± 0.28	1.33 ± 0.32	1.88 ± 0.33	1.30 ± 0.23	1.14 ± 0.31	0.42 ± 0.21

0.53 ± 0.11 μg/mg for ASC_{12}/C and ASC_{16}/C, respectively). Coagels formulations appeared to produce a low IBU depot in the skin; IBU concentration remained in the same order of magnitude in epidermis and derma, in spite of significant increase in IBU cutaneous permeation (see Table 3). The limited amount of IBU retained in all skin layers could be related to the coagel structure; in fact, the breakdown coagel (ASC_{16}/IBU) showed a increased IBU penetration with values ranging between 2.2 and 0.9 μg/mg closed to those obtained with no structured formulations as Arfen® (see Table 5). Otherwise, IBU-S contained high percentage of organic solvent (19.83% isopropanol) that appeared to influence both drug depot and permeation.

The highest amount of IBU, between 20 and 40 times superior in the upper skin layer and with a similar value in the deeper layers of the skin was found after treatment with the coagels formulations containing PEG 400. This behaviour is a further proof of the positive synergic effect on the IBU skin permeability due to the ASC_n coagel–PEG mixture, above all for ASC_{16}PEG/C.

While the more lipophilic ASC_{16}PEG/C coagel favoured the IBU accumulation in the skin associated with lower IBU steady-state flux, an opposite behaviour was observed for the more hydrophilic ASC_{12}PEG/C.

4. Conclusion

Several factors influence the skin permeation, and consequently, the improvement in the therapeutic efficacy of topically delivered drugs among which are the release of the drug from the formulation, the drug penetration into the stratum corneum and the drug diffusion into the skin layers up to the dermis to reach the circulation.

Different strategies to increase ibuprofen penetration through the skin using both chemical and physical enhancers have been studied, although ibuprofen shows poor skin permeability. Terpenes and phospholipids [50], polyoxyethylene alkyl ether [51], supersaturated solutions, limonene [46] and menthol, propylene glycol or iontophoresis [52,53] resulted to favour the transdermal drug permeation in various extent. The enhancement activity depends on different mechanisms as induction of lipid packing disruption, formation of eutectic mixtures [54,55] or change of the ibuprofen solubility in the skin [47,56].

In this study, both the *in vitro* test to characterize the drug partition from the vehicles and the *ex vivo* drug rat skin diffusion test to analyze the complexes interactions that occur between skin and excipients proved the efficacy of a new series of derivatives of 6-O-ascorbic acid alkanoate.

The studied ASC_{16} and ASC_{12} coagels, defined as stable supramolecular assemblies in water or in water/PEG mixtures, allowed the solubilization of IBU (0.85%) and controlled the drug release rate. Our results demonstrated that ASC_{16} and ASC_{12} coagels and in particular those vehicles containing PEG, producing a higher amount of permeated drug through the skin compared to commercial Arfen®, are a promising tool as carriers for cutaneous IBU delivery. Furthermore, our results evidenced a different behaviour of the ASC_n derivatives on the IBU skin permeation and distribution depending on their hydro-lipophilic character that could allow a rational design and an optimization of a topical formulations.

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