A glimpse in critical attributes to design cutaneous film forming systems based on ammonium methacrylate

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ABSTRACT

A film forming system based on Eudragit® RL (EuRL) was designed aiming to evidence the relevance of formulative variables on the following critical attributes: film forming rate, outward stickiness, Young modulus (Y) and *in vitro* drug skin permeation. Different solvent mixtures (acetone and isopropanol in the range from 10:90 to 40:60 v/v), polymer concentrations (10—30% w/w), and plasticizer types and concentrations (triacetin or tributyl citrate, up to 50% of EuRL) were evaluated. EuRL dissolved in 80/20 or 70/30 v/v isopropanol/acetone mixtures at the concentration of 20% and plasticized with tributyl citrate (20 or 30% with respect to polymer) gave films with negligible stickiness and Y lower than 3 MPa. This value should assure an intimate and prolonged contact with the skin since it was significantly lower than Y of human stratum corneum (55 MPa). The optimized formulations were able to sustain the skin permeation of ibubrofen, ketoprofen and flurbiprofen and evidenced the importance of each formulative variable. In particular, relatively slow solvent evaporation rate can determine an initial "burst" effect and can influence the drug permeation in the initial hours. Conversely, when the solvent evaporation rate is not discriminant, the thermodynamic activity remains the main parameter driving the skin permeation.

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1. Introduction

The passive transport rate of a molecule through the skin is proportionally related to its degree of saturation in the applied vehicle [1]. Therefore, drug supersaturation in topical formulations can be induced to improve the penetration into stratum corneum. Systems that are transiently drug supersaturated, namely those systems which become supersaturated only after dose actuation, seem to be more promising as dosage forms compared to pre-formed drug supersaturated patches, since the latter need to maintain the supersaturated state during their entire shelf-life. Transient supersaturation entails the reduction of drug solubility in the vehicle that is applied on the skin surface and this is most commonly achieved through solvent evaporation [1]. The simplest approach to achieve this goal consists in the design of polymeric film forming systems (FFS) which comprises a film-forming polymer dissolved in a volatile and skin tolerated solvent. When they are applied and/or sprayed on the surface of the skin, the rapid solvent evaporation leads to the formation of a polymeric film *in situ* [2]. The potential advantages of these dosage forms reside not only in the possibility to overcome the issue related to the physical instability of a supersaturated system, but also in a possible enhancement effect related to the solvent skin penetration during the metamorphosis of the formulation [3,4]. The last claimed advantage of FFS is related to the cosmetic attributes of the film. Indeed, many patients complain about the high visibility of trans-dermal patches, which are considered cosmetically unattractive, while the formed film is supposed to be almost invisible.

Moving to the formulative requirements, a film-forming solution should exhibit some peculiar features related to both the applied dosage form (i.e. the polymeric solution itself) and the final film. Firstly, the novel dosage form should quickly dry on the skin and the minimum film forming temperature should be below the skin surface temperature (about 32 °C). Secondly, the mechanical properties of the formed film should overcome the tangential stress due to the body movements. Finally, the formed film is required to be non-sticky to avoid adhesion to the patient's clothes.

To satisfy these requirements, a broad range of polymers (e.g. acrylates, polyurethane-acrylates, cellulose derivatives, poly(vinyl pyrrolidones) and silicones) were tested [5,6]. Among them, the use

of methyl methacrylate copolymers appears of particular interest [5,7–10], even if the literature reports contrasting results on Eudragit® RL (EuRL) when it was compared to another widely used film forming material, namely hydroxyethyl cellulose. As an example, the skin permeability of estradiol from EuRL based films resulted significantly lower than that obtained with the cellulose ether [11]. Nevertheless, the use of EuRL allowed to overcome the mechanical issues associated to films made of hydroxyethyl cellulose. Indeed, it was demonstrated that both tensile strength and percent elongation at break of the films were improved by mixing in appropriate ratio cellulose and EuRL [12]. However, a systematic study of the formulation variables, namely solvent composition, polymer concentration, nature and amount of plasticizers, on the FFS properties is still lacking.

The current work aimed to study the effect of formulation compositions on technological and biopharmaceutical properties of FFS based on EuRL solubilized in a mixture of acetone and isopropyl alcohol in different ratios. This volatile vehicle was selected since both solvents have a regulatory approval for topical use.

The effects of solvent systems as well as the addition of the plasticizer, namely triacetin or tributyl citrate, were preliminary evaluated on drying time, outward stickiness and mechanical properties. In particular, since a reference for the tensile properties of the formed film is not established, the elasticity of human stratum corneum was preliminary determined and used as reference.

The performances of the optimal formulations were further investigated studying the skin permeation of three different drugs, namely flurbiprofen, ibuprofen and ketoprofen.

2. Materials and methods

2.1. Materials

Eudragit® RL PO (poly(ethyl acrylate-co-methyl methacrylate-co-trimethylammonioethyl methacrylate chloride); molar proportions of the monomer units 1:2:0.2; weight average molar mass 32 kDa, EuRL) was kindly supplied by Rofarma Italia (Italy). Tributyl citrate (TBC) and triacetin (TRI) were provided by Morflex (USA) and Sigma Aldrich (Italy), respectively. Isopropanol and acetone were purchased by VWR International (Italy). Flurbiprofen (FP) and ketoprofen (KP) were purchased from Farmalabor (Italy) and S-ibuprofen (IB) from Francis (Italy).

All solvents were of analytical grade, unless specified.

2.2. Preparation of polymeric FFS

Film-forming systems (FFS) were prepared by adding 10, or 20, or 30 (%, w/w) EuRL to different mixtures of isopropanol and acetone (ratios: 90:10, 80:20, 70:30, 60:40%, v/v) with or without the selected plasticizer. Each solution was stirred overnight to ensure the complete swelling of the polymer in the solvent blend.

FP, or IB, or KP were dissolved in the FFS at a concentration of 4% w/w.

2.3. Characterization of the polymeric FFS

The preliminary screening of placebo compositions was carried out keeping in consideration the FFS drying time, the stickiness and cosmetic attributes of the formed film. Briefly, a small volume of the formulations was applied with a micropipette onto a plastic liner and the solvent was allowed to evaporate to form the film. The applied volume was fixed at 35 μL/2.5 cm^2 as this amount is small enough to be applied without flowing away from the application site. No-sticking films formed within 10 min and showing good cosmetic attributes were considered adequate for the aim of this

work. The drying time was visually checked by evaluating the formation of a fingerprint on the film surface. This approach has been selected since the other method reported in literature, namely the use of a glass slide [5,6], did not permit to discriminate the formation of a dried, but sticky film.

The adhesive properties were preliminary evaluated by a thumb tack test [13] on the dry films according to the following score system: no adhesion, poor adhesion and good adhesion.

Afterwards, TBC or TRI were added to the most promising FFS in order to evaluate the effect of the plasticizers on the flexibility of films. To select the plasticizer concentration, the glass transition temperature (T_g) of films made by casting a polymeric mixture in isopropyl alcohol, containing the selected plasticizer in different ratios, was evaluated by differential scanning calorimetry (DSC) analysis (DSC1 Instrument, Mettler-Toledo, CH). Briefly, 20 mg (±0.01 mg) exactly weighted samples were sealed in aluminum pans and heated in inert atmosphere (70 mL min^{-1} of N$_2$). The reference was a pan containing aluminum oxide [10]. The equipment was calibrated with an indium sample. Films were scanned at 20 K/min from 20 to 80 °C in order to erase polymer thermal history, then cooled down to −50 °C at 20 K/min and re-heated up to 80 °C at 20 K/min. T_g was calculated as the inflection point in the second heating ramp.

2.4. Mechanical testing

Human stratum corneum isolation - The permeation studies were performed using the abdominal skin from female donors, who underwent cosmetic surgery and signed an informed consent for the use of the biological sample for research purposes [14]. After removing the subcutaneous fatty tissue, the skin was kept frozen until further use. For the stratum corneum isolation, skin sections were cut into squares of about 2.5 cm^2 and were immersed in water of 60 °C for 60 s according to an internal protocol [15]. Afterwards, the epidermis was carefully removed from the underlying tissue with the help of forceps and visually inspected for defects. Then, the epidermis samples were incubated for 24 h at 37 °C in a 0.1% w/v trypsin solution in pH 7.4 phosphate buffer [15]. After digestion, the underlying tissue of epidermis was scraped away and the remaining stratum corneum was washed in cold MilliQ® water. The stratum corneum samples were cut in 8 × 16 mm specimen, transferred into Petri dishes and left to equilibrate in a humidifier at 25 °C and 75% relative humidity using a saturated solution of sodium chloride, over a 12 h period.

Film preparation - Placebo films were prepared by a solvent casting technique by using a laboratory-coating unit Mathis LTE-S(M) (Mathis, CH), equipped with a blade coater. The coating thickness was set in order to obtain a dried film of about 50 μm. The FFS was spread on the release liner and dried at 32 ± 1 °C for 20 min. Film samples were cut in 7 × 20 mm specimen and stored at 25 °C until use.

Probe tack test - Probe tack test measures the force required to separate the test probe tip from the film sample by using a tensile testing machine equipped with a 50 N cell load transducer (Instron 5965, ITW Test and Measurement Italia S.r.l., Italy). The experiments were set according to an internal standard procedure [16]. Briefly, a flat stainless steel probe (diameter: 6 mm) was placed about 0.05 mm above the sample. Then, the probe was lowered onto the film surface and a constant force of 0.05 N was applied onto the sample for 5 s and, finally, the probe was removed at the debonding rate of 0.1 mm/s. The stress (σ) values for each experiment were calculated according to the following equation:

$$\sigma = F/A$$

where F is the force registered during the detachment and A is the probe surface area.

The results are expressed as the mean $\pm$ SD of four samples for each formulation.

Tensile test - Stratum corneum strips (8 $\times$ 16 mm) or film samples (7 $\times$ 20 mm) were positioned between two pneumatic jaws of the tensile testing machine, separated at a distance of 8 mm. The lower jaw remains fixed, whilst the upper jaw connected to the load cell mounted on top of the crosshead rises at a speed of 2 mm min^{-1}. Young modulus (Y) was calculated as the slope of the linear portion of the stress-strain curve. The results were expressed as force per unit area (MPa). Individual experiments were performed on four samples of stratum corneum or film.

2.5. Thermogravimetric analysis

Thermogravimetric measurements (TGA) were carried out using a TGA 2050 Thermogravimetric Analyser (TA Instruments, USA). Samples of 80 μL FFS were held at 32 °C under nitrogen atmosphere and mass losses versus time were measured over 1 h. The higher the Δm min^{-1} value obtained, the faster the solvent evaporation.

2.6. Polarized optical microscopy

Crystallization of drugs from the polymeric FFS was evaluated by a polarized optical microscopy (Axiolab E re, CarlZeiss, Germany) equipped with 10x objective. A suitable volume of each solution was spread on a microscope glass slide and allowed to dry in a water vapor saturated chamber at 32 °C, to mimic the conditions of the skin penetration experiments. The presence or absence of drug crystals was noted.

2.7. In vitro drug permeation

The human epidermis samples were prepared as described above. Prior to experimental use, the integrity was assessed measuring their electrical resistance (voltage: 100 mV, frequency: 100 Hz; Agilent 4263B LCR Meter, Microlease, Italy). Samples with an electrical impedance resistance higher than 30 kΩ cm^2 were used for the in vitro permeation experiments [15].

The epidermis sample was mounted on the Franz diffusion (PermeGear, USA) cell with an effective penetration area of 0.636 cm^2. The receptor compartment (volume: ~3.0 mL) was filled with degassed 0.9% w/v NaCl solution. Special care was given to avoid air bubbles between the buffer and the epidermis in the receptor compartment. The upper and lower parts of the vertical Franz cell were sealed with Parafilm® and fastened together by means of a clamp. Volumes of 10 μL FFS were applied uniformly on the epidermis sample as donor phase. The system was kept at 37 $\pm$ 1 °C by means of a circulating water bath so that the skin surface temperature was at 32 $\pm$ 1 °C and the receiver medium was continuously maintained under stirring with a magnetic bar.

The experiments (three replicates per formulation) were performed over a 24 h period under non-occlusive conditions. During this period, 200 μL samples were drawn at predetermined intervals and replaced by aliquots of fresh receptor fluid. Sink conditions were maintained throughout the experiment. Samples were analysed by HPLC according to the methods described below.

The cumulative amount permeated through the skin per unit of area was calculated from the concentration of each substance in the receiving medium and plotted as a function of time.

The average flux (J) was calculated over the 1—5 h period of time.

2.8. Drug assay

The drug concentrations in the receiving media were determined by HPLC assay (HP 1100, Chemstation, Hewlett Packard, USA). The following chromatographic conditions were used: Column: Hyper-Clone™ 5 μm BDS C18 130, 150 $\times$ 4.6 mm (Phenomenex, USA); mobile phase: acetonitrile/pH 2.6 water (60/40, v/v); flow rate: 1.5 mL/min; wavelengths: 246 nm (FP), 225 nm (IB) or 255 nm (KP); temperature: 25 °C; injection volume: 20 μL. The drug concentrations were determined from standard curves in the 0.1—50.0 μg/mL range. The retention time was approximately 2.5 min for FP, 3.0 min for IB and 1.8 min for KP. The method provided good precision and linearity in the required concentration range (R^2 = 1.00000 for FP, R^2 = 0.99995 for IB, R^2 = 0.99999 for KP).

2.9. Statistical analyses

Tests for significant differences between means were performed by ANOVA followed by Tukey post hoc analyses (OriginPro 2015, OriginLab, USA).

3. Results and discussion

3.1. Formulation study

The preliminary screening of placebo compositions was carried out *in vitro* keeping in consideration the FFS drying time, the stickiness, measured qualitatively by thumb tack test, and the cosmetic attributes of the formed film. No-sticking films formed within 10 min and showing good cosmetic attributes were considered adequate for the aim of this work.

The formulations prepared with the highest EuRL concentration or an acetone content higher than 30% w/w required more than 10 min to completely dry. On the other hand, the lowest polymer concentration did not allow the formation of a uniform film. Thus, 20% w/w EuRL solutions in isopropanol/acetone at the ratios in the 90:10—70:30 v/v range were considered worthy to design a FFS formulation.

Since EuRL presents a T_g at about 63 °C, the addition of a plasticizer is mandatory to decrease T_g below the skin surface temperature (~32 °C), in order to satisfy the requirements of flexibility and elongation necessary to assure a proper skin/dosage form contact. On the other hand, an excess of plasticizer concentration in the formed film can affect its properties since it is generally recognized that when the T_g is about 25—45 °C lower than the application temperature the material can become sticky [17]. Since the skin surface temperature is about 32 °C, it is reasonable to suppose that materials with a T_g lower than -10 °C are sticky [8]. On the bases of these considerations we established that the T_g of the formed film should be in the $+30$ °C to -5 °C range.

In order to avoid the influence of the solvent and the drying process, T_g was calculated on the second heating ramp. As summarized in Table 1, the optimal concentration of both the selected plasticizers was about 20—30% w/w. However, the outward stickiness can be influenced not only by the extent of plasticizer, but also by the solvent composition, which can lead to a different three dimensional organization of the polymeric chains during the *in situ* formation of the film. Hence, the stickiness of the preformed films was also determined by probe tack test (Table 2). Formulations at the highest isopropanol content caused the formation of films adhesive on the outer surface, independently of the plasticizer content. A similar behavior was evident for films plasticized by 30% w/w TBC or TRI obtained from a 70:30 v/v isopropanol/acetone solution and, therefore, the formulations nos 1—4, 7, 8, 10—12 were discarded (Table 2).

Table 1
EuRL glass transition temperatures (T$_g$) obtained by DSC analysis dependent on the plasticizer content.

EuRL (%)	TBC (%)	TRI (%)	T$_g$ (°C)
100	–	–	63 ± 1
90	10	–	43 ± 4
80	20	–	27 ± 1
70	30	–	19 ± 1
60	40	–	−20 ± 1
50	50	–	−26 ± 2
90	–	10	40 ± 2
80	–	20	18 ± 1
70	–	30	13 ± 2
60	–	40	−7±5
50	–	50	−16 ± 1

The tensile properties of the selected formulations were determined and compared to those of the human stratum corneum. In this case the acceptance criterion was defined so that the elastic modulus of the formulation should not exceed that of the stratum corneum to assure an intimate and prolonged contact after topical application. Indeed, the film should possess tensile properties that allow to accommodate normal skin mechanical responses due to tangential stresses related to body motions [9,18].

When comparing data reported in literature about mechanical properties of skin, there is a quite evident variation, because of the specimen location, the variability of biological tissues and the experimental set ups (in vitro or in vivo). Overall, the results enlisted in Table 2 are included within the ranges found in literature for the in vitro tensile tests. Indeed, depending on the anatomic origin of samples and operative conditions, the in vitro values of elastic modulus ranged from 3–150 MPa [19].

Therefore, a maximum value of 3 MPa was established for the selection of the suitable formed film in agreement with the threshold selected for other applications of films on the skin and, therefore, assumed as ideal to cover the range of elastic skin response [18].

The addition of 20% w/w of TBC or TRI allowed obtaining films with the Young's moduli lower than that of the skin (Table 2) and, therefore, an appropriate flexibility. As expected, the higher the plasticizer concentration, the lower the Young's modulus value of the formed film (Table 2). The data also underlined that the Young's modulus was not influenced by the organic solvent composition, but only by the type and content of plasticizer; in particular, TBC resulted more effective in reducing the Young's modulus values. In general, the current data were in agreement also with the results obtained by using nanoindentation to determine the elastic moduli of various films prepared with Eudragit® RS dissolved in ethanol, with or without a plasticizer and/or betamethasone 17-valerate [9].

Moreover, the authors provided that the presence of the drug in these formulations had no significant effect on the mechanical properties of the films [9]. Despite the elastic moduli determined by AFM (Y = 0.3 GPa) were different an order of magnitude to that determined by texture analysis in the current set of experiments (Y = 55 MPa), the minimum plasticizer concentration needed to obtain a flexible formulation was comparable, independently of the technique. However, in the cited work the adhesive properties were not taken in consideration. Our results suggest that in the formulation of a FFS three different techniques, namely DSC, probe tack test and tensile test, should be combined to identify the optimal range of each main formulative variables (i.e. the polymer concentration, the type and extent of plasticizer and volatile solvent composition).

The optimal FFSs resulted the formulations nos 5, 6 and 9. They were loaded with 4% w/w FP, IB or KP (Table 3) to investigate the skin permeation process of such model drugs.

3.2. In vitro human skin permeation

Drug permeation profiles from FFS are illustrated in Fig. 1a–c. In case of FFS loaded with FP and IB, both the solvent mixture and the plasticizer influenced the drug permeation profiles, but in different ways. The highest amount of FP permeated was obtained using the isopropanol/acetone mixture at the volume ratio of 80:20 v/v (Formulation FP1, Table 3), which showed also an initial "burst" effect. This result could be explained on the basis of the mechanism of drug deposition from a volatile solvent system which carries the drug into the upper layer of the stratum corneum prior to evaporation. The longer the contact time, the deeper the penetration of the solvent and drug into the epidermis. A too fast evaporation rate leads consequently to a short residence time that can limit the drug penetration [20,21]. In other words, the drug absorption rate can be expected to be inversely proportional to the evaporation rate of the volatile solvent. Comparing the permeability results of FP loaded formulations to the volatile solvent evaporation data obtained by TGA analysis (Table 3), FP1 showed a lower evaporation rate than FP2 and FP3, representing the main driving force influencing drug permeability. The above-suggested mechanism of drug delivery from a FFS vehicle can also help to explain the permeation profile from FP1: the initial burst effect can be ascribed to the slow evaporation rate of the solvent system that subsequently enhances the drug flux and the total drug permeated amount. Moreover, the FP permeation appears negatively influenced by the plasticizer content: the higher the plasticizer content (FP3), the lower the permeated amount (Table 3). This result cannot be related to the TGA data, since there were no significant differences between the

Table 2
Tackiness (σ) and Young's modulus (Y) of films obtained from FFS made of 20% w/w EuRL in different solvent systems and plasticized by different content of tributil citrate (TBC) or triacetin (TRI).

Formulation code	Isopropanol/acetone	TBC (%, w/w)	TRI (%, w/w)	Drying time (min)	σ (kPa)	Y§ (MPa)
1	90/10	20	–	<5	3.61 ± 0.52	n.d.
2	90/10	30	–	<10	4.96 ± 1.63	n.d.
3	90/10	–	20	<5	5.50 ± 0.18	n.d.
4	90/10	–	30	<10	10.37 ± 2.88	n.d.
5	80/20	20	–	<5	0.62 ± 0.16	3.0 ± 0.3
6	80/20	30	–	<10	0.96 ± 0.33	0.5 ± 0.0
7	80/20	–	20	<5	0.66 ± 0.16	31.9 ± 10.5
8	80/20	–	30	<10	0.69 ± 0.23	<0.1
9	70/30	20	–	<5	0.31 ± 0.00	3.7 ± 0.5
10	70/30	30	–	<10	12.63 ± 3.24	n.d.
11	70/30	–	20	<5	0.28 ± 0.01	40.5 ± 13.1
12	70/30	–	30	<10	6.05 ± 2.53	n.d.

n.d.: not determined.
§: Young's modulus of the human epidermis = 55.4 ± 13.0 MPa.

Table 3
Permeation data obtained by applying as donor phase FFS loaded with flurbiprofen (FP), ketoprofen (KP) or S-ibuprofen (IB) and drying rate expressed as mass variation (Δm) determined by TGA over time.

Form. code	Drug conc. (%, w/w)	Isopropanol/acetone (v/v)	TBC (%, w/w)	Vol.[a] (μL)	Q_{24} (μg/cm²)	J (μg/cm²/h)	Δm/t (% min⁻¹)
FP1	4	80/20	20	10	36.43 ± 4.89	2.19 ± 0.51	0.991 ± 0.031
				100	117.83 ± 13.34	5.78 ± 0.14	
FP2	4	70/30	20	10	25.23 ± 6.57	1.49 ± 0.40	1.366 ± 0.008
FP3	4	80/20	30	10	11.39 ± 5.50	0.62 ± 0.27	1.383 ± 0.015
FP4	2	80/20	20	20	24.80 ± 5.69	1.64 ± 0.86	1.139 ± 0.040
FP5	8	80/20	20	10	73.50 ± 6.14	3.90 ± 0.60	1.167 ± 0.079
KP1	4	80/20	20	10	34.62 ± 15.71	1.98 ± 0.78	1.208 ± 0.042
KP2	4	70/30	20	10	40.49 ± 4.13	3.19 ± 0.97	1.327 ± 0.008
KP3	4	80/20	30	10	39.06 ± 0.52	3.58 ± 1.68	1.071 ± 0.093
IB1	4	80/20	20	10	30.21 ± 9.63	1.33 ± 0.46	1.242 ± 0.016
IB2	4	70/30	20	10	50.35 ± 2.74	3.31 ± 0.29	1.172 ± 0.004
IB3	4	80/20	30	10	71.33 ± 13.14	4.62 ± 0.87	1.063 ± 0.024

[a] Solution loaded in the donor compartment of Franz cell.
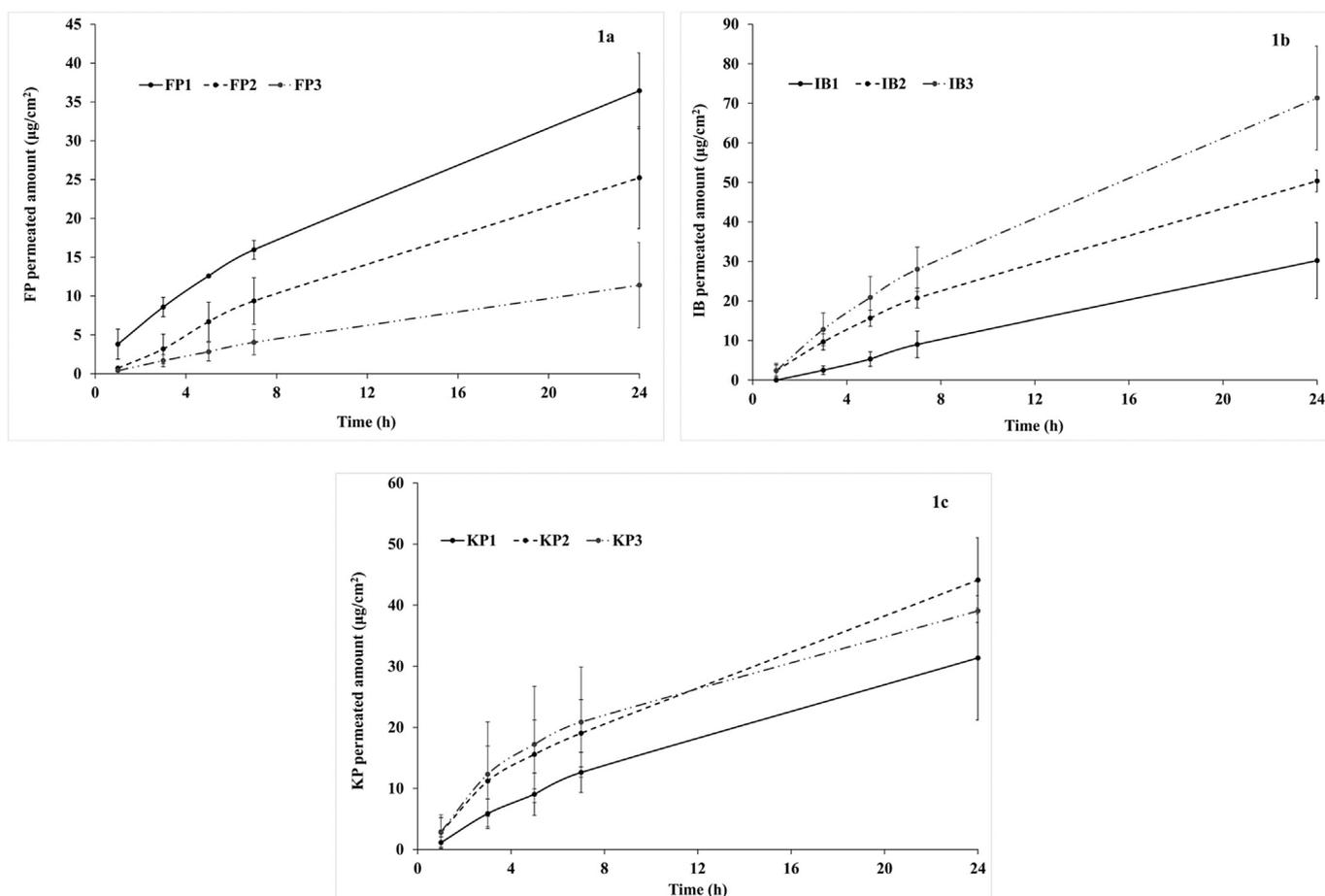
Fig. 1. Drug permeation profiles from FFS obtained by applying 10 μL FFS loaded by FP (a), IB (b) and KP (c) on the Franz diffusion cells. Error bars represent standard deviation.

solvent evaporation rate of FP3, namely the formulation with the highest plasticizer content, and FP2, namely the formulation prepared with the highest acetone content. Therefore, this feature might be explained taking into account the solubilizing effect of the plasticizer, which reduced the drug thermodynamic activity in the film and, consequently, decreased its flux through the skin (Tukey test, p = 0.048).

In the case of IB loaded FFS, the drug permeation was positively influenced by the plasticizer concentration (IB3) and the drug permeation profiles followed the rank order: IB3 > IB2 > IB1 (Tukey test, p < 0.01). Since IB is freely mixable with EuRL [22], the key factor governing the extent of skin permeation in the first hours appears the solvent evaporation rate. Indeed, the faster the evaporation rate, the lower the permeated amount (Table 3). It can be also supposed that the addition of TBC could affect the solubility of the drug in the matrix, causing an improvement of the partition of IB from the film toward the skin.

Finally, the three formulations loaded with KP, which presented the same evaporation rate, showed no significant differences in their drug permeation profiles (One way ANOVA p = 0.29, Table 3), suggesting that only the skin barrier properties dictated the diffusion process.
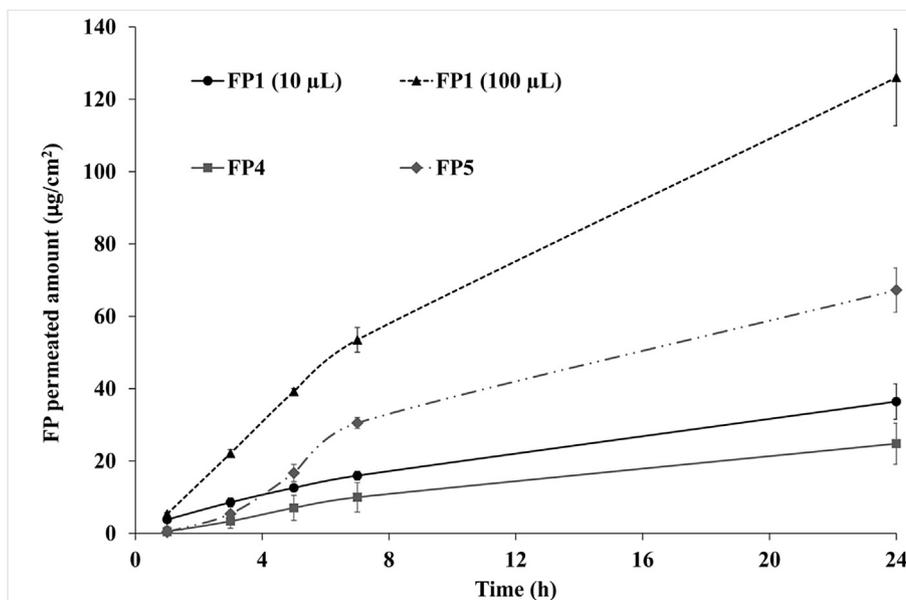
Fig. 2. FP permeation profiles from FFS obtained by varying the donor phase volume (10 μL or 100 μL FP1) or by varying both the applied volume and the drug concentration in the donor phase, namely 20 μL at 2% w/w (FP4), 10 μL at 4% w/w (FP1) and 10 μL at 8% w/w (FP5). Error bars represent standard deviation.

Aiming to investigate the effect of drug concentration on the performances of drug loaded FFS, the formulation FP1, which presented a "burst" effect, was selected to carry out further *in vitro* experiments, varying or the donor phase volume or the drug concentration in the FFS. In particular, the following modifications of the experimental protocol were considered: (a) increasing the applied volume to 100 μL, while maintaining constant the drug concentration at 4% w/w; or (b) increasing the volume of the donor phase (20 μL instead of 10 μL) and decreasing the drug concentration (2% w/w) in the FFS; or (c) increasing the drug concentration (8% w/w) and applying 10 μL of FFS as donor phase. As expected, both the increase of drug concentration and the applied volume led to an improvement of the FP amounts permeated through the human epidermis (Tuckey test p < 0.05, Table 3). It is noteworthy that the flux of FP by the film containing highest drug concentration changed over time. Indeed, the FP flux was high over the first 5 h following application, but it was reduced between 7 and 24 h (Fig. 2). A similar pattern was also reported for methylphenidate films having similar composition [23] and it was attributed to a drug crystallization, which reduced the drug availability to the partition from the donor compartment toward the stratum corneum. Indeed, the film, after dismounting from the Franz diffusion cell, showed some signs of cloudiness and small crystals were observed by light microscopy on dried film 8 h after having deposited on glass slide a FFS loaded by 8% w/w (data not shown).

Conversely, the concomitant increase of the applied volume and the decrease of the drug concentration, to apply the same dose (see formulations FP1 (10 μL) and FP4 Tukey test p = 0.025, Table 3), significantly affected both the FP flux and the amount permeated after 24 h. This might be attributed to a lower thermodynamic activity of the drug in the film, confirming that it is the more relevant feature influencing skin permeation, when the evaporation rate can be assumed comparable.

4. Conclusions

This work allowed to individuate the formulative space to design a film forming solution based on EuRL and evidenced that not only the drug thermodynamic activity, but also the solvent evaporation rate significantly influenced the skin permeation from FFS. The relevance of these two formulation parameters is strictly related to the loaded drug, even within the same class of compounds (e.g., in our case the aryl-propionic acids). In particular, the vehicle composition, apart from its function to solubilize the other excipients and the drug, can influence the initial delivery of drug into the skin, according to the solvent evaporation rate: the lower the evaporation rate, the higher the "burst" effect and the flux in the initial hours following drug application.

The second investigated aspect deals with the influence on skin permeation of the drug concentration and the applied amount of dosage form. In this case the main criticism is related to the drug crystallization that can occur also in relatively short period of time affecting the whole permeation process through the skin, as evidenced in the case of the FSS loaded with 8% w/w FP.

Finally the obtained data evidenced that the critical attributes that should be considered in the design of film forming formulations, are the outward stickiness, which was scantly investigated till now, and the elasticity of the formed film, other than the film forming rate and the biopharmaceutical performances.

Conflict of interest statement

The authors declare no conflicts of interest in this work.

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