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TUNING THE MECHANICAL AND ADHESIVE PROPERTIES OF TRANSDERMAL DRUG DELIVERY SYSTEMS

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Preface

During the last 20 years, the cutaneous administration of active principles has been taking a key role not only for the possibility to achieve high skin drug concentrations for the treatment of local pathologies, but also for reaching therapeutic plasmatic concentrations for systemic administration. In the field of cutaneous delivery, film-forming systems and transdermal patches are the most widely used dosage forms because they are able to firmly adhere to the skin and release the active substance(s) for the entire treatment period. Despite many products are currently available on the market, some critical aspects, such as adhesive and mechanical properties, as well as rheological behavior of these pharmaceutical dosage forms, are still scarcely studied since most of the research is overlooking their importance dealing with drug delivery, pharmacokinetic and safety evaluation. Hence, there is the need to study the most relevant issues involved in the design of both film-forming systems and transdermal patches and clarify the possible relationships between rheological pattern, adhesive properties and *in vitro* biopharmaceutical performances. This kind of relationship could provide useful information to design and optimize both film-forming systems and transdermal patches.

This doctoral thesis aimed to evaluate the effect of the formulation compositions on rheological, adhesive and mechanical properties as well as on the *in vitro* biopharmaceutical performances of different polymeric matrices, in order to design film-forming systems and transdermal patches. In particular, the experimental work was focused on:

(1) the design of film-forming systems based on an ammonium methacrylate copolymer (Eudragit® RL) solubilized in a mixture of solvents in different ratios and plasticized by different amounts of plasticizer; the effects of solvent systems as well as the addition of the plasticizer were evaluated on drying time, outward stickiness, mechanical properties and *in vitro* biopharmaceutical performances;

(2) the design of transdermal patches based on a differently plasticized ammonium methacrylate copolymer (Eudragit® RL) and the evaluation of their rheological and tack properties, primarily to better understand their debonding mechanisms and adhesive

characteristics; moreover, the main technological and *in vitro* biopharmaceutical properties were tested;

(3) the design of transdermal patches based on a styrenic copolymer (styrene-*block*-(ethylene-co-butylene)-*block*-styrene, SEBS); the effects of SEBS and tackifiers molecular weights on the rheological and adhesive properties were investigated. In particular, the debonding behavior under different operative conditions was evaluated and the technological as well as the *in vitro* biopharmaceutical performances of the formulated patches were studied.

General introduction

The relevance of transdermal dosage forms is due to the possibility to obtain a prolonged drug release over a period of time up to 24 hours. Nowadays, among the several transdermal delivery systems actually developed, film-forming systems (FFS) and transdermal patches (TP) are the most widely used because of their abilities to firmly adhere to the skin and to release the active substance(s) for the entire treatment period, thus assuring a prolonged and adequate drug permeation through the skin.

FFS are innovative drug delivery systems intended to be applied onto the skin in order to achieve a systemic effect [1]. They are composed by a vehicle (a volatile solvent or a mixture of solvents), in which the polymer and the active substance(s) can be dissolved or dispersed [2]. After the application to the skin, the volatile vehicle should evaporate resulting ultimately in an *in situ* formation of a polymeric film. Since this allows a significant quantitative change in the composition of the film, usually a plasticizer is added to overcome the brittleness of the final film [1].

TP are flexible and self-adhesive pharmaceutical preparations of varying sizes, containing one or more active substances and they are intended to be applied to the unbroken skin in order to deliver the active substance(s) to the systemic circulation after passing through the skin barrier [3]. Nowadays, the same systems are used to obtain either local or regional effects. According to their design, patches can be divided into matrix and reservoir types. Among the matrix platforms, drug-in-adhesive systems consist of an adaptable backing layer, an adhesive matrix, in which the active substance(s) is dissolved or dispersed, and a removable release liner. These systems allow to improve patient's compliance because they are thin, flexible, comfortable and conformable [4]. Furthermore, from a formulative point of view, they are easy and cheap to produce [5].

The systemic treatment by delivering a drug through the skin by using both FFS and TP is gaining an increase interest due to their several advantages. In particular, transdermal delivery provides convenient and pain-free self-administration for patients; it avoids the hepatic first-pass metabolic effects associated with the oral administration of drugs. Since it offers controlled release of the drug, it also enables a steady blood level profile, resulting in reduced systemic side effects and, sometimes, improved efficacy over other

dosage forms [6]. All these advantages lead to enhance patient compliance, especially when long-term treatment is necessary.

In order to design suitable FFS or TP some critical issues must be taken into account. In particular, drug absorption process depends on the drug partition between the dosage form and the skin and subsequently on the permeation of the active ingredient through the stratum corneum. Therefore, to assure a prolonged permeation of the active substance(s) through the skin, a whole contact between the dosage form and the intact delivery surface for the entire treatment period is critical [7]. A strongly adhesion of the dosage form to the skin determines drug delivery, therapeutic effects and patient compliance [8].

To satisfy this requirement the use of bioadhesive materials, that are defined as materials able to adhere to a biological substrate and being retained on such substrate for an extended period by interfacial forces [9], is mandatory.

In particular, focusing on FFS, the main features which should be considered during a formulative study are the time required for the *in situ* film formation (drying time), the cosmetic attributes of the formed film and, mostly, its mechanical properties.

With respect to the conventional topical dosage forms, a good and more patient-friendly formulation would be fast drying (*i.e. in vivo* a drying time lower than 5 minutes is mandatory) and the minimum film-forming temperature should be below the skin surface temperature. The formed film should be cosmetically acceptable (*i.e.* almost invisible) and non-sticky to avoid adhesion to patient's clothes. Moreover, suitable films intended for applications as transdermal drug delivery systems must be not only flexible enough to follow the body movements without breaking, but also sufficiently persistent to prevent abrasion of the film caused by cloths frictions [10].

In order to satisfy the requirements of flexibility and elongation necessary to assure a proper skin/dosage form contact, the *in situ* formed film should present a glass transition temperature below than the skin surface temperature (32 °C). Usually, a plasticizer is added to reduce the polymer glass transition temperature and the brittleness of the final film. In the formulation of FFS the selection of the proper amount of plasticizer is crucial since a certain reduction of the glass transition temperature (T_g)

is mandatory to obtain suitable mechanical properties, but, on the other hand, an excess of plasticizer could cause the formation of a sticky film on the skin.

Regarding TP, the adhesion is guaranteed by the so-called "pressure-sensitive adhesives" (PSAs), that are the main components of a drug-in-adhesive patch. They are self-adhesive polymeric materials that display an instantaneous adhesion on most surfaces, such as the skin, by applying a light pressure; moreover, they can ideally be detached without any residue [11].

Tack, shear and peel adhesion represent the main features of patch adhesion. Tack gives rise to the initial adhesion of the patch to the skin under light pressure and on brief contact. Since patients usually apply the patch slowly and with accuracy, low tack values are desired. Shear adhesion represents the resistance of the PSA to flow and provides an indication of its cohesiveness. A patch should remain attached to the skin for the entire treatment period and it should resist to the stresses due to body movements and cloths frictions; hence, high shear adhesion values (about hours) are required. Lastly, peel adhesion refers to the force required to remove the patch from the skin at the end of the treatment period. In this latter case, low values are related to the absence of both pains and adhesive residues during the peeling process [12].

Given these preliminary considerations, a patch should be sticky and exhibit an optimal balance between adhesiveness and cohesiveness.

To obtain a sticky material the knowledge of the polymer T_g is crucial since it influences the modulus of elasticity of the polymer itself. An increase in polymer T_g improves the hardness and stiffness of the material itself [13]. Polymer with high T_g value allows to produce a very strong, but brittle matrix, while low T_g value permits to obtain flexible matrixes. Furthermore, Zosel A. demonstrated that to make an uncross-linked or slightly cross-linked polymer sticky and adherent to almost any surface, its T_g should be 25-45 °C lower than the application temperature [14]. 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 when applied onto the skin and they can be used to design transdermal patches able to adhere to skin. Usually, the addition of compatible excipients, such as plasticizers, that remain homogeneously dispersed or dissolved into the final matrix

after solvent evaporation, is necessary to reduce the polymer T_g and to produce certain desirable effects on the physical properties of the final polymeric matrix: this changing transforms the polymer from a glassy and rigid material to a viscous one, making it softer and more flexible [15]. Plasticizers are low-molecular weight molecules that reduce polymer-polymer chain secondary bonding (*e.g.* hydrogen bonding), forming instead secondary bonds with the polymer chains [16]. Obviously, the plasticizer has to be compatible with the polymer and the other components of the formulation and the optimization of its concentration is a crucial factor. The plasticizer concentration influences the T_g reduction and, therefore, the characteristic of the final matrix (*e.g.* the higher the plasticizer concentration, the higher the reduction in the polymer glass transition temperature). Furthermore, according to the free volume theory [17], the higher the glass transition value, the lower the mobility of the PSA and thus the lower the drug release rate [18]. It is clear that the amount of such substance must be optimized to obtain matrices with the desired characteristics: a lower amount of plasticizer allows to produce flexible, but non-sticky films, while increasing its concentration sticky polymeric matrix can be produced. Despite that, matrices exceeding in plasticizer amount should exhibit a completely viscous character, thus resulting not suitable for the design of TP.

In addition, tackifiers, such as resins, are other excipients that can be ideally added to increase tack and stickiness of the adhesive surface [19].

To function properly, a PSA should exhibit the typical behavior of a viscoelastic material, that combines a liquid- and solid-like patter dependent of the applied frequency at a given temperature [12]. Usually, at low frequency a soft viscoelastic material behaves like a viscous liquid (the viscous modulus is higher that the elastic one), while at high frequency the elastic modulus prevails on the viscous one, denoting a solid-like behavior. The extent of viscoelasticity is crucial in order to relax stresses, easily create a molecular contact and dissipate energy upon debonding and it is strictly related to adhesive properties of a patch, as referred to bond formation as well as bond separation [14]. The interpretation of these features demonstrates that low frequencies (in the 0.005-0.05 rad/s range) can be related to tack, while high frequencies (ranged between

100 and 1000 rad/s) can be related to the peeling process [20]. As a matter of fact, during the bonding phase (tack), the adhesive matrix should behave like a viscous liquid to favor its spreading onto the skin and to form good molecular contact under a lightly applied pressure. Conversely, during the debonding process from the skin (peel), the adhesive should behave like a cohesive solid to ensure complete removal without leaving any residue [21]. Furthermore, a patch should remain attached to the skin for the entire treatment period, it should not present an overspreading of the adhesive matrix beyond the boundaries and it should not leave any residues upon removal. The shear resistance is a slightly higher rate process than tack, occurring at frequencies ranging between 0.05 and 0.5 rad/s [20].

Another common criterion should be satisfied: a suitable PSA should present an elastic modulus value at 1 Hz lower than 0.1 MPa allowing to reach a sufficient level of adhesion (Dahlquist criterion) [22]. Materials with elastic moduli exceeding this criterion have poor adhesive characteristics due to their inability to dissipate energy via viscous contributions or to deform to make good contact with the surface. They also show a high peak adhesive force, but fail quickly upon further strain (brittle failure, without fibril formation) [21].

Failure mechanisms which occurs during the detachment of the patch from the skin are strongly dependent on the rheological properties of the PSA [19]. Indeed, the viscoelastic characteristics of a PSA control its deformation and break of fibrils [23]. Regarding TP, two mains failure mechanisms can occur, namely adhesive and cohesive failures. The first one occurs at the interface between the adhesive layer and the substrate; in this case, the deformation of the layer is low with mainly lateral propagation and coalescence of the initial cavities along the interface. The latter one occurs in the bulk of the adhesive layer, allowing the debonding process to be governed by viscous flow. Since, if this latter detachment pattern occurs after the peeling process, some adhesive residues can be noticed on the skin, an adhesive failure is mandatory, so that patch will not leave any residues after the detachment.

Generally, transdermal route of administration is limited to drugs with peculiar physicochemical characteristics. Drug penetration across the skin is primarily

determined by its solubility, molecular structure and lipophilicity. As a matter of fact, smaller compounds diffuse readily across the stratum corneum than larger ones. Indeed, maximum fluxes of drugs with molecular weights greater than 500 Daltons are very low. A modest level of lipophilicity, corresponding to a log P ranging between 1-3, coupled with finite oil and water solubility are ideal characteristics for good drug skin penetration. An optimal drug diffusion through the skin is also reached with molecules that present a melting point lower than 200 °C. Finally, usually transdermal drug delivery systems are used for drugs which are extremely potent, requiring a dosage of only few mg [24].

In addition, for both the dosage forms, namely TP and FFS, the drug release rate and the subsequent extent of drug that can ideally permeate through the skin (*i.e.* bioavailability and efficacy) depend sensitively on the composition of the formulation.

The release profiles of an active substance from a polymeric matrix depend on its diffusion within the matrix itself [25]. The mobility of the polymer chains and, therefore, the grade of viscoelasticity result a key factor influencing the drug release rate [18]. In particular, a more liquid character of the formulation can weaken the interaction between the polymer chains and expand the free volume, thus resulting in a faster drug release [26].

Moreover, the possible interaction between the drug and the polymeric matrix is considered the main factor influencing the drug skin permeation [27]. In the case of FFS, both the evaporation rate of the vehicle and the thermodynamic activity of the drug influence the theoretical amount of drug that can permeate the skin. It is generally recognized that drug supersaturation in topical dosage forms can improve the permeation of the drug itself. In supersaturated formulations, the thermodynamic activity of the drug is increased, thus enhancing the skin penetration [28]. On the other hand, supersaturated formulations are thermodynamically instable, causing the drug crystallization over time, which reduces its bioavailability for the partition through the skin [29]. Since FFS become supersaturated after dose actuation, they are transiently drug supersaturated systems and the drawback of the thermodynamic instability of supersaturated systems could be overcome.

For the physical stability of the drug, the film forming polymers are chosen since they act as anti-nucleating agents and crystallization inhibitors and prevent the crystallization of the drug, even after solvent evaporation. It has been demonstrated by Cilurzo and co-authors that polymethacrylate copolymers can be potentially used as crystallization inhibitors in monolayer patches containing ibuprofen [30] and that they can be used, when opportunely plasticized, as PSA to develop suitable TP [31].

In addition to the functional features described above, there are other factor to be considered in the selection and use of a polymer for designing both TP and FFS. In particular, biocompatibility and acceptable regulatory status must be satisfied. The excipients should be compatible and mixable in the overall formulation and the entire system, namely polymer(s) and excipient(s), should be biologically inert, non-irritating and non-sensitizing to the skin, and have no toxicity. Moreover, the system should present good resistance against water and humidity and avoid an excessive occlusion of the skin.

My PhD project was focused on the study of two polymers: a poly(ethylacrylate-co-methylmethacrylate-co-trimethylammonioethylmethacrylate chloride) belonging to the class of acrylic-based adhesives and a styrene-*block*-(ethylene-co-butylene)-*block*-styrene copolymer, a styrenic-based adhesive. The former was used to design both TP and FFS, while the latter was selected to design TP.

Even if the feasibility to design topical dosage forms made of a poly(ethylacrylate-co-methylmethacrylate-co-trimethylammonioethylmethacrylate chloride), traded with the name of Eudragit® RL PO (EuRL), able to deliver several drugs has been already described, their characterization in terms of mechanical behavior and adhesive properties is not available in literature. With the aim to complete these information, several FFS and TP based on EuRL opportunely plasticized and loaded with three model drugs, namely ibuprofen, ketoprofen and flurbiprofen, were formulated. The effect of the overall formulative variables was studied, introducing also new techniques suitable to better clarify their influence of such variables on the final characteristics of the formulated dosage forms. Relationships among the mechanical and rheological behavior of the PSA and the technological properties and *in vitro* biopharmaceutical

performances of the final dosage forms were also investigated.

Aiming to confirm that these characterizations are useful in the development of a patch, a polymer with completely different physico-chemical and structural properties was selected. The feasibility to develop PSA for the design of TP based on styrene-*block*-(ethylene-co-butylene)-*block*-styrene (SEBS) copolymers was studied by compounding the polymer with an aliphatic resin as tackifier and paraffin oil as plasticizer. A systematic formulative study related to PSA mechanical and adhesive properties was performed to investigate the influence of SEBS and tackifier molecular weights on the rheological pattern and debonding behavior of such PSA. Ibuprofen and nicotine were selected as model drugs to formulate the TP and evaluate their technological characteristics and *in vitro* biopharmaceutical performances.

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1

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

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% w/w and plasticized with tributyl citrate (20 or 30% w/w 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 ibuprofen, 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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1.1 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 preformed 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 transdermal 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.

1.2 Materials and Methods

1.2.1 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.

1.2.2 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.

1.2.3 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.

1.2.4 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:

$$\sigma = F/A \qquad \textbf{Eq. 1.1}$$

where F is the force registered during the detachment and A is the probe surface area. The results are expressed as the mean ± SD of four samples for each formulation.

Tensile test - Stratum corneum strips (8 × 16 mm) or film samples (7 × 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.

1.2.5 Thermogravimetric analysis

Thermogravimetric measurements (TGA) were carried out using a TGA 2050 Thermogravimetric Analyzer (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 $\Delta m\ min^{-1}$ value obtained, the faster the solvent evaporation.

1.2.6 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.

1.2.7 *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 $K\Omega \cdot cm^2$ 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 ± 1 °C by means of a circulating water bath so that the skin surface temperature was at 32 ± 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.

1.2.8 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: HyperClone^{TM} 5μm BDS C18 130, 150×4.6 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 minutes 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).

1.2.9 Statistical analyses

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

1.3 Results and Discussion

1.3.1 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% 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 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 1.1**, the optimal concentration of both the selected plasticizers was about 20-30% w/w.

Table 1.1 - EuRL glass transition temperatures (T_g) obtained by DSC analysis dependent on the plasticizer content.

EuRL (%, w/w)	TBC (%, w/w)	TRI (%, w/w)	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

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 (**Tab. 1.2**).

Table 1.2 - Tackiness (σ) and Young's modulus (*Y*) of films obtained from FFS made of 20% EuRL in different solvent systems and plasticized by different content of tributyl citrate (TBC) or triacetin (TRI).

Form. 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.

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 (**Tab. 1.2**).

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 1.2** 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 (**Tab. 1.2**) and, therefore, an appropriate flexibility. As expected, the higher the plasticizer concentration, the lower the Young's modulus value of the formed film (**Tab. 1.2**). 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 (**Tab. 1.3**) to investigate the skin permeation process of such model drugs

Table 1.3 - 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	TBC (%)	Vol.* (µL)	Q_{24} (µg/cm^2)	J (µg/cm^2/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

* Solution loaded in the donor compartment of Franz cell.

1.3.2 *In vitro* human skin permeation

Drug permeation profiles from FFS are illustrated in **Figure 1.1a-c**.

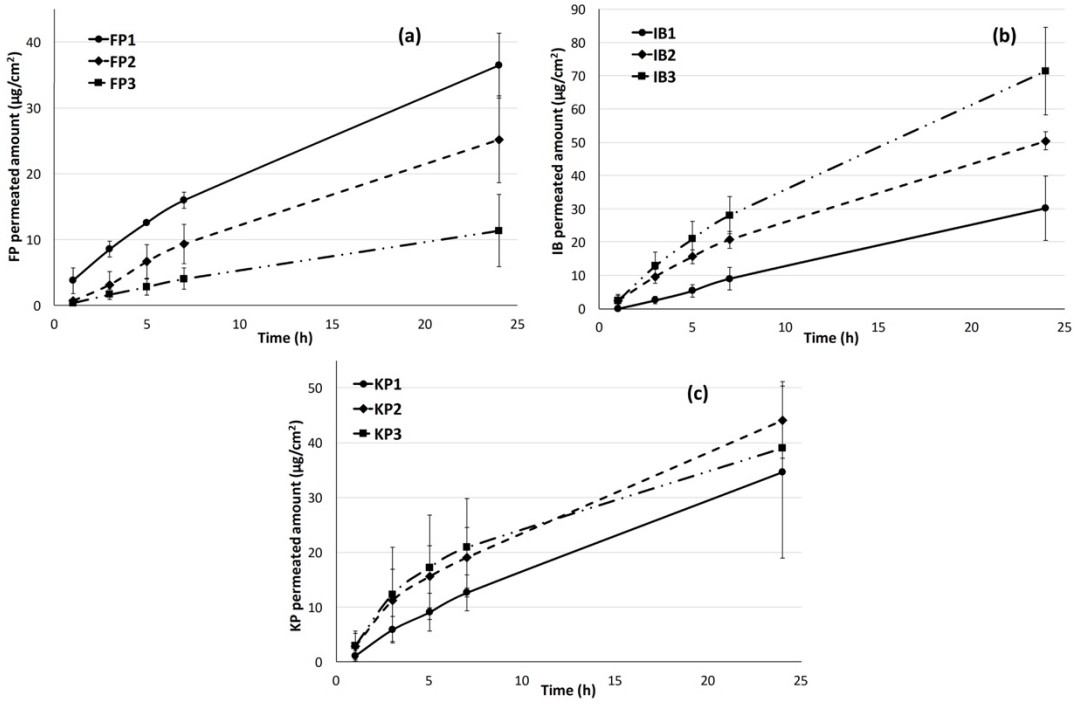

Figure 1.1 - Drug permeation profiles from FFS obtained by applying 10 µL FFS loaded by (a) FP, (b) IB and (c) KP on the Franz diffusion cells. Error bars represent standard deviation.

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, **Tab. 1.3**), 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 (**Tab. 1.3**), 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 (**Tab. 1.3**). This result cannot be related to the TGA data, since there were no significant differences between the 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 (**Tab. 1.3**). 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, **Tab. 1.3**), suggesting that only the skin barrier properties dictated the diffusion process.

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, $p < 0.05$, **Tab. 1.3**). 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 five hours following application, but it was reduced between 7 and 24 h (**Fig. 1.2**).

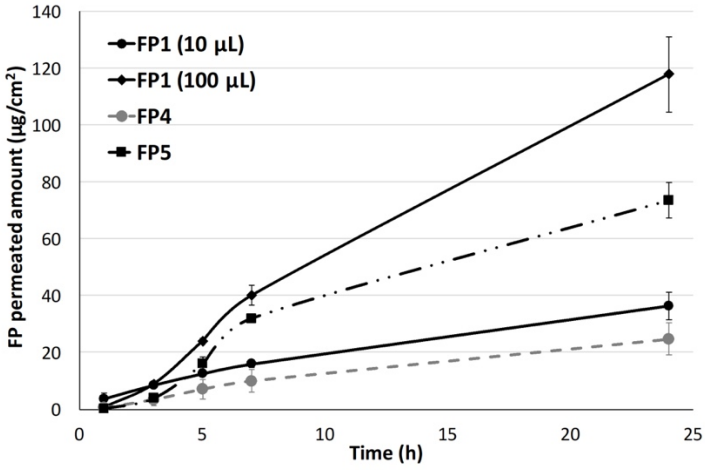

Figure 1.2 - FP permeation profiles from FFS obtained by varying the donor phase volume (10 µL or 100 µL FP1) or by varying the drug concentration in the donor phase, namely 10 µL at 2% w/w (FP4), 4% w/w (FP1) and 8% w/w (FP5). Error bars represent standard deviation.

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, **Tab. 1.3**), 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.

1.4 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.

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2

Tuning the rheological properties of an ammonium methacrylate copolymer for the design of adhesives suitable for transdermal patches

Abstract

Eudragit® RL (EuRL) matrices have been proposed to release a drug to the skin. However, no information is available on both viscoelastic and adhesive properties of such compositions. This work focuses on the evaluation of both rheological and texture properties of EuRL differently plasticized with tributyl citrate (TBC) or triacetin (TRI) in order to design a pressure sensitive adhesive suitable for transdermal patch preparation. The patch adhesive properties (*i.e.* tack, peel adhesion and shear adhesion) as well as its *in vitro* biopharmaceutical performances were determined after loading ibuprofen, ketoprofen or flurbiprofen. The addition of 40-60% w/w TBC or 40-50% w/w TRI to EuRL permitted to obtain matrices with the desired adhesive properties. Moreover, the increase of plasticizer content and loading of the drug reduced the relaxation time (τ_R). Consequently, the shear adhesion values decreased and the *in vitro* drug release constants (*k*) increased. Indeed, the *k* values from patches containing TBC were lower than the corresponding with TRI because of the lower fluidity of such matrices. In conclusion, the 60/40 EuRL/TBC binary blend is suitable for the design of transdermal patches since the *in vitro* drug permeability of the three selected drugs appeared comparable to those described in literature for marketed drug products.

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2.1 Introduction

Transdermal patches and medicated plasters are pharmaceutical preparations designed to provide a prolonged delivery of drugs to the skin to achieve a systemic or local effect, respectively. Usually, they are drug-in-adhesive systems, in which the drug is dispersed and/or dissolved in a pressure-sensitive adhesive (PSA) matrix. PSAs are defined as soft polymeric materials that display an instantaneous adhesion on almost any surface by simple contact under a light pressure and that can ideally be detached from the substrate without any residue [1].

The efficiency of the therapeutic treatment by these dosage forms is related not only to their ability to release the drug through the skin, but also to their complete skin contact over the whole delivery surface for the entire treatment period. If the patch lifts or partially detaches, the effective contact area, and thus the drug absorption, is unpredictable and therapeutic failure can occur [2].

The adhesive properties of a PSA strongly depend on their viscoelastic properties. Viscoelastic materials are needed in order to relax stresses, easily create a molecular contact and dissipate energy upon debonding. Indeed, PSAs should be soft and relax stresses to favor the contact with the substrate [3], but they should also be highly dissipative and lightly physically or chemically crosslinked to resist to the applied stress once the bond is formed. From a technological point of view, the PSA matrix is characterized by tack, peel adhesion and resistance to shear. Tack is the property that enables an adhesive to form a bond with the surface of another material upon brief contact and under light pressure; peel adhesion is the force required to peel away a patch from a surface; shear adhesion represents the resistance of the matrix to flow over long times and moderate loads [4].

The literature reports the feasibility to design PSAs able to deliver several drugs made from a poly(ethylacrylate-co-methylmethacrylate-co-trimethylammonioethylmethacrylate chloride), traded with the name of Eudragit® RL PO (EuRL) [5]. However, no information is available about either the viscoelastic behavior or the adhesive properties of such compositions.

This study focuses on the evaluation of the viscoelastic and adhesive properties of PSAs made of EuRL differently plasticized in order to identify a matrix suitable to develop a patch and clarify the possible relationships between the main rheological descriptors and the adhesive properties of such patch and the drug release pattern. This kind of relationship is rarely investigated, although it could provide useful information to design both transdermal patches and medicated plasters in the attempt to optimize their performance.

To achieve this goal, several PSA were designed by mixing EuRL in different ratios (40-60% w/w) with two plasticizers, namely triacetin (TRI) and tributyl citrate (TBC). The drug release properties of the optimal combinations were evaluated by adding three different drugs, namely ibuprofen, flurbiprofen and ketoprofen. PSAs were characterized in terms of rheological and tack properties. Probe tack tests were performed not only to evaluate the adhesive properties of the PSAs, but mainly to understand better the debonding mechanisms [6,7]. The patches were characterized by measuring shear and peel adhesion and cold flow, which refers to the dimensional change and/or deformation of the polymeric matrix of a patch beyond the boundaries [8]. The drug release pattern was determined by both *in vitro* dissolution test and *in vitro* skin permeation using human epidermis as membrane.

2.2 Materials and Methods

2.2.1 Materials

Poly(ethylacrylate-co-methylmethacrylate-co-trimethylammonioethylmethacrylate chloride), traded with the name of Eudragit® RL PO (EuRL), with molar ratio of 1:2:0.2 and molecular weight 32 kDa, was kindly donated by Rofarma Italia (Gaggiano, Italy). Tributyl citrate (Citroflex 4, TBC) was supplied by Morflex (Greensboro, USA) and triacetin (TRI), ethyl acetate (EtOAc) and isopropanol (iPrOH) were purchased from Sigma Aldrich (Milan, Italy). The release liner used for patch preparation was a siliconized polyester film from Saint Gobain kindly donated by Bouty (Cassina de Pecchi, Italy), while the backing layer was a polyester film with a thickness of 57 µm (Polifibra, Agrate Brianza, Italy). Three active ingredients were selected: S-ibuprofen (IB) was purchased from Dipharma Francis (Baranzate, Italy); ketoprofen (KP) and flurbiprofen (FP) from Farmalabor (Canosa di Puglia, Italy). All solvents were of analytical grade, unless specified.

2.2.2 Blend preparation

To obtain the EuRL organic dispersion, the powder was dispersed in the solvent, ethyl acetate or isopropanol, at the concentration of 40% w/w. The polymeric blends were prepared by adding the plasticizer (TBC or TRI) to the EuRL dispersions. The amount of TBC or TRI ranged between 40 and 60% with respect to EuRL weight. When the active ingredient (*i.e.* IB, KP or FP) was added to EuRL mixture, it was preliminarily dissolved in the plasticizer and, then, added to the polymeric dispersion. The drug content was 4% w/w calculated on EuRL weight.

The polymeric dispersion was mixed for 3 hours at 60 °C with a magnetic stirrer of 100 rpm. One night of rest was necessary in order to reduce the air bubbles formed during the stirring and to favor the full swelling of the polymeric chains.

2.2.3 Rheological properties in the linear regime

The rheological characteristics were measured on a Discovery Hybrid Instrument HR-3 (TA Instruments, New Castle, USA). The frequency-dependence of the viscoelastic moduli G' and G'' was characterized with a parallel plate geometry (diameter 20 mm), by using a crosshatched upper plate for the formulations containing the lower amount of plasticizer and a sandblasted plate for the other formulations.

Sample preparation. In order to obtain equilibrated samples of about 1 mm thickness, a special sample holder was used. The device consisted of lower plate geometry, to which a ring was fixed through a Teflon tape; this particular device allowed to put 10-15 mL (depending on the plasticizer concentration) of solution and completely dry it (slowly drying in air for 48 hours, and then 30 minutes of drying at 45 °C); the same sample holder was used to test the sample on the rheometer by simply removing the holding ring just before the analysis (after the 7 days required for matrices maturation).

Experimental setup. To evaluate the linear viscoelastic regime, a strain-sweep procedure at 1 Hz was performed; then, a frequency-sweep deformation (0.01 - 100 rad/s) was applied to the sample and the resulting response in terms of stress was measured. Each sample was analyzed first at 25 °C and then at 32 °C. The analyses were performed in triplicate to verify the reproducibility of the experimental conditions.

2.2.4 ATR-FTIR Spectroscopy

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra were recorded over the wavenumber region 4000-650 cm^{-1} with an ATR-FTIR spectrometer (Perkin Elmer, Waltham, USA), equipped with a diamond crystal. For each sample 256 scans were collected at a resolution of 2 cm^{-1}. Spectra were ATR corrected and smoothed and then analyzed by using Origin Pro (Origin Lab). The maximum absorbance of peaks in the 1650 and 1800 cm^{-1} region was assigned by second derivate.

2.2.5 Differential scanning calorimetry (DSC)

The glass transition temperature (T_g) of EuRL/plasticizer blends was evaluated by DSC (DSC1 Instrument, Mettler-Toledo, CH) according to the method previously described [9].

2.2.6 Probe tack test

Probe tack experiments were performed on a custom-designed probe apparatus adapted on a MTS 810 hydraulic testing machine, allowing the simultaneous observation of the debonding process through a transparent glass substrate [10].

Sample preparation. In order to get films of thickness 180 - 200 μm on a glass slide $2.6 \times 10 \times 0.2$ cm^3 previously cleaned and activated by a plasma technique, 2 - 3 mL (depending on their concentration) of each placebo formulation were deposited on each glass slide (using a perfectly levelled support plate). Each sample was dried slowly in air for 48 hours and then for 30 minutes in an oven at 45 °C. The PSA thickness was measured by a white light scattering technique with an optical profilometer (Microsurf 3D, Fogale nanotech, Nimes, France). The resulting PSAs were stored in a container to prevent dust for the 7 days required for the matrices maturation at room temperature.

Experimental setup. The experiments were carried out as follows: a flat-ended probe was brought into contact with the adhesive layer at a constant probe velocity of 30 μm/s until a set compression force was reached, kept at a fixed position for a given time of 10 s, and subsequently removed at a constant crosshead speed which was varied between 1 and 1000 μm/s. Experiments were conducted at room (storage) temperature (25 ± 0.5 °C) and at skin surface temperature (32 ± 0.5 °C). The probe was made of stainless steel with a diameter of 9.7 mm; this substrate was preferred because it is a common material with a simple behavior. Since surface roughness can affect probe test results, the degree of surface roughness was well controlled: the flat end of the probe was polished with several grades of abrasive paper; the same probe was used throughout a series of tests

and its flat end was cleaned with acetone. The applied contact forces were 10, 40 and 70 N for the formulations containing 60, 50 and 40% w/w of plasticizer, respectively. During each experiment, the force, the displacement of the crosshead and the time were acquired simultaneously. Since the results are influenced by the film thickness, the force-distance curve was converted into a stress-strain curve.

The tensile stress (σ) was calculated by dividing the force registered during the detachment (F) for the contact area (A), evaluated by the video streaming, as follows:

$$\sigma = F/A \qquad \textbf{Eq. 2.1}$$

Defining h as being the time-dependent thickness of the adhesive layer and h_0 as the initial adhesive layer thickness the nominal strain (ε) was calculated as follows:

$$\varepsilon = (h - h_0)/h_0 \qquad \textbf{Eq. 2.2}$$

The area under the curve recorded by the instrument software was defined as the work of separation (W).

Selected images from recorded films were digitized and analyzed. Tests were carried out on the selected placebo formulations because the low amount of drug did not affect the adhesive and debonding mechanisms of PSAs. Each formulation was analyzed in triplicate to verify the reproducibility of the experimental conditions.

2.2.7 Preparation of transdermal patches

Patches were prepared by casting, using a laboratory-coating unit Mathis LTE-S(M) (Mathis, Oberhasli, CH), equipped with a blade coater. The mixture was spread on the backing layer. The coating thickness was set at 350 µm in order to obtain a dry film of about 50 - 100 µm. The spread mixture was dried at 60 °C for 30 minutes and covered with the release liner. Finally, patches were sealed in an airtight container and stored at 25 ± 0.1 °C over a 7-day period.

2.2.8 Thickness of the matrices of the transdermal patches

A sample of 2.5 cm × 2.5 cm of the patch was placed between the jaws of the MI 1000 micrometer (ChemInstruments, Fairfield, USA). First, the whole thickness (T_W) was measured; then, the respective thicknesses of both backing layer and release liner (T_{BL+RL}) were measured.

The thickness of the matrix layer was calculated as $T_W - T_{BL+RL}$. The results are expressed as the mean of five measurements.

2.2.9 Shear adhesion

The shear adhesion was performed using an 8 Bank Oven Shear HT8 Instrument (ChemInstruments, Ichemico, Cuggiono, Italy).

Sample preparation. Each patch sample was cut in order to obtain specimens of 25 mm × 60 mm using three samples from each formulation. Each specimen was placed on the test panel center; it was applied to cover an area of 25 mm × 25 mm without added pressure. A clamp was placed on the masked free end of the specimen and was aligned in order to ensure a uniform distribution of the load.

Experimental setup. The test assembly was placed in the test stand so at an angle of 2° from vertical, minimizing the peel forces that eventually acted on the patch. A mass of 500 g was applied to the clamp [11]. The entire setup was enclosed in a controlled-temperature chamber in order to maintain the specimens at 32 ± 0.5 °C. The time required to completely detach the specimen from the test panel was recorded.

The results are expressed as the mean ± standard deviation of three specimens for each formulation.

2.2.10 Peel adhesion 180° test

The peel adhesion test at 180° measures the patch adherence when peeled at a 180° angle from a standard steel or Teflon panel [12]. The tests were performed using a tensile machine equipped with a 50N cell (Instron 5965, ITW Test and Measurement Italia S.r.l., Trezzano sul Naviglio, Italy).

Sample preparation. Each patch sample was cut to obtain strips of 12 mm $\times$ 120 mm and was folded about 5 mm from its release liner and a tape leader was placed on about 3 mm of the exposed patch with the adhesive side of the tape attached to the one of patch. The remainder of the tape leader was, then, folded on itself to form a double thickness leader. Then, the release liner was removed and the specimen was placed onto the Teflon test panel. The prepared specimen was first smoothed with a 2.04 kg roller and then stored in an airtight container at 25 $\pm$ 0.1 °C for 15 minutes.

Experimental setup. The specimen was placed into the instrument with the free end of the tape leader being placed into the instrument clamp [11]. Six specimens were pulled from the plate at a 180° angle at a peel speed of 300 mm/min.

The average force was calculated as the arithmetic mean of all values of the linear portion of the curve. The peel values are expressed in centiNewton per centimeter (cN/cm) by dividing the registered force by the width of the patch. The results are expressed as the mean $\pm$ standard deviation of six specimens for each formulation.

2.2.11 Cold flow

The cold flow was evaluated after a storage period of one month at 40 $\pm$ 1 °C. The dimensional changes in punched-out sample were measured using a graph paper.

The evaluation was performed on samples punched in order to obtain a patch of 32 mm diameter covered with a release liner of 40 mm diameter. The samples, sealed in single airtight pocket, were stored at 40 ± 1 °C for one month. The extent of cold flow was expressed as the maximum migration of the adhesive in mm on the release liner (**Fig. 2.1**).

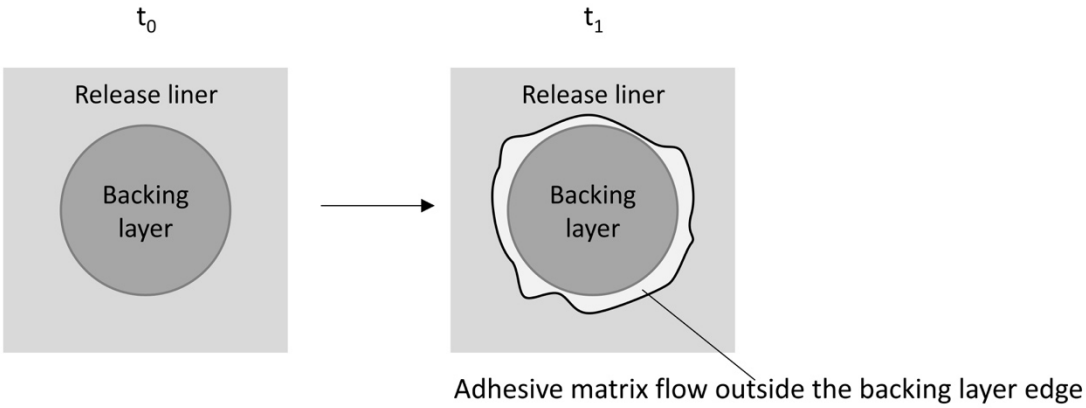

Figure 2.1 - Schematic representation of cold flow phenomenon.

The cold flow was considered negligible if the PSA was not visually detectable outside the backing layer. The analysis was performed in triplicate.

2.2.12 Drug content

An exactly weighted 2.54 cm^2 patch sample was dissolved in 20 mL methanol by sonication (UP200st, Hielscher, Teltow, Germany) and mechanically stirred. Afterwards, the samples were left at rest for three hours and diluted with 20 mL mobile phase (described below). Each value represents the average of three measurements.

2.2.13 Dissolution test

The dissolution was performed by using an apparatus SR8 PLUS dissolution test station (Hanson Research, Chatsworth, USA) according to "Dissolution test for transdermal patches" of European Pharmacopoeia 9.1 (2017) [13].
A patch sample of 8.0 cm^2 was placed flat on the disk with the release liner surface facing up. The backing layer was attached on the disk by using a cyanoacrylate adhesive. The

vessels were filled with 300 mL of pH 7.4 PBS buffer, the water bath temperature was kept at 32 ± 0.5 °C and the paddle speed was set at 25 rpm. At predetermined intervals, 5 mL samples were collected and immediately replenished with fresh medium.

The solutions were assayed by HPLC, according to the methods reported below. Each result represents the average of three determinations.

The release rate constant was calculated according to the Higuchi's equation as follows:

$$M_t/M_\infty = kt^{-0.5} \qquad \textbf{Eq. 2.3}$$

where M_t is the amount of drug released at time *t*, M_∞ is the drug loading in the matrix and *k* is the release rate constant expressed as h^{-1}.

2.2.14 *In vitro* drug permeation

Skin preparation. The permeation studies were performed using abdominal skin from female donors, who underwent cosmetic surgery and signed an informed consent for the use of biological samples for research purposes [14]. After removing the subcutaneous fatty tissue, skin was kept frozen until further use. For the sample preparation, adequate pieces of the frozen skin were immersed in water at 60 °C for 60 s, according to an internal protocol [15]. After this treatment, the epidermis was carefully removed from the underlying tissue with the help of forceps. The integrity of all tissue samples was assessed measuring their electrical resistance (voltage: 100 mV, frequency: 100 Hz; Agilent 4263B LCR Meter, Microlease, Cernusco sul Naviglio, Italy), using a modified Franz diffusion cell (PermeGear, Hellertown, USA) with an effective penetration area and a receptor volume of 0.636 cm^2 and 3 mL, respectively. Samples with an electrical impedance resistance higher than 30 kΩ·cm^2 were used for the *in vitro* permeation experiments [16].

Experimental setup. The receiver compartment of Franz diffusion cell was filled with 0.9% w/v NaCl solution. Patches of 2.5 cm^2 were used as donor phase and they were

applied with slight pressure with the adhesive layer in contact with the stratum corneum side before mounting the diffusion cell.

These tissue samples were sandwiched between the donor and receptor compartments of the diffusion cell, with the stratum corneum facing up the donor compartment. The upper and lower parts of the vertical Franz cell were sealed with Parafilm® and fastened together by means of a clamp. The system was kept at 37 ± 1 °C by means of a circulating water bath so that the skin surface temperature was at 32 ± 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 occlusive conditions. During this period, 200 µL samples were drawn at predetermined intervals and replaced by aliquots of the receptor fluid. Sink conditions were maintained throughout the experiment. Samples were analyzed by HPLC according to the methods described below.

The cumulative amount (Q) 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 steady flux (*J*) was calculated as the slope of the linear portion of the plot. Finally, the efficiency of transdermal patches (E%) in drug releasing over a 24 h period was calculated as the ratio between the Q_{24h} and drug content per cent.

2.2.15 Drug assay

The drug concentration in each patch, in the dissolution media and in the receiving media were quantified by HPLC analysis (Agilent HP 1100, Chemstation, Hewlett Packard, Santa Monica, USA). The following chromatographic conditions were used: *column*: HyperClone™ 5µm BDS C18 130, 150 mm × 4.6 mm (Phenomenex, Torrance, USA); *mobile phase*: acetonitrile/water pH 2.6 (60/40, % v/v); *flow rate*: 1.5 mL/min; *wavelengths*: 225 nm (IB), 255 nm (KP) or 246 nm (FP); *temperature*: 25 °C; *injection volume*: 20 µL. The drug concentrations were determined from standard curves in the 0.1 - 50.0 µg/mL range.

2.2.16 Statistical analyses

The performances of the matrices in terms of drug release rates were compared by analysis of the variance followed by Tukey post-analyses (Daniel's XL Tollbox 6.70). The level of significance was taken as $p < 0.05$.

2.3 Results and Discussion

2.3.1 Pressure sensitive adhesive physico-chemical characterization

The performances of a PSA are related to its ability to form a molecular contact with the adherent, to its rheological behavior and, obviously, to its adhesive properties. All of these characteristics are strongly dependent on the application temperature. It is generally recognized that when the glass transition temperature (T_g) of a lightly entangled and high molecular weight polymer is 25 - 45 °C lower than the application temperature the material becomes sticky [17]. Since the raw EuRL exhibited a T_g of about 63 °C and the skin temperature is about 32 °C, the addition of a plasticizer is necessary. Among the widely used plasticizers of poly(methyl methacrylate) derivatives, TBC and TRI were selected and in both the cases a minimum amount of 40% w/w of TBC and TRI was necessary to reach the target T_g value (*i.e.* $T_g < 0$ °C). Indeed, the T_g values of the EuRL plasticized with TBC and TRI at 40% w/w resulted -20 ± 1 °C and -7 ± 5 °C, respectively. On the basis of these data, the effect of the plasticizer on the rheological and adhesive performances of EuRL was studied in the 40-60% w/w range.

The rheological analyses revealed that, with the exception of form. no. 6, showing a distinctly liquid behavior in the considered frequency range, all the formulations were viscoelastic: at low frequency the viscous modulus (G'') was higher than the elastic modulus (G'); while at high frequency G' dominated and a clear crossover point was visible at 25 °C (the patterns are exemplifies in **Fig. 2.2**).

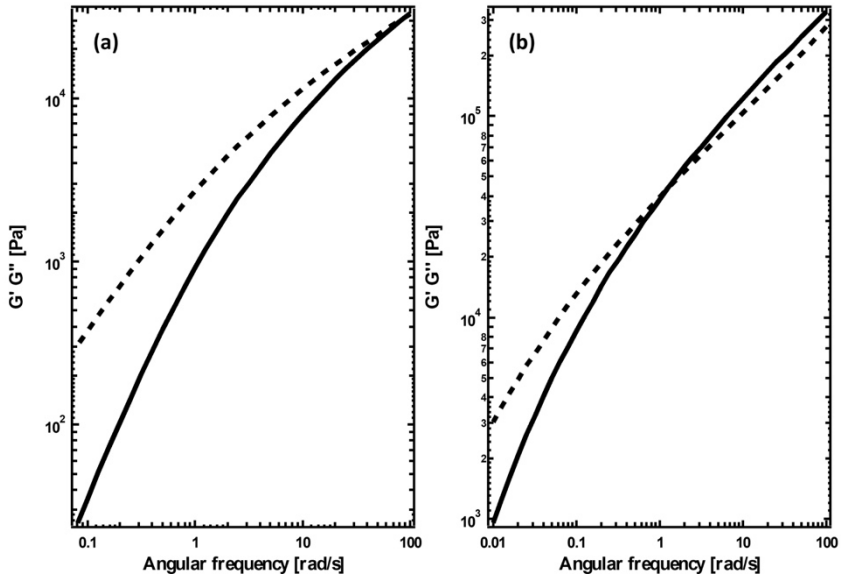

Figure 2.2 - Evolution of G' (solid line) and G'' (dashed line) as a function of frequency. (a) Form. no. 6 at 25 °C and (b) form. no. 4 at 25°C.

The influence of each variable on the rheological behavior of a PSA can be clarified by evaluating the vertical shift in elastic and viscous moduli, *i.e.* G' and G'', and the horizontal shift of the crossover point.

In order to evaluate the structure of the PSA in the in-use conditions, the experiments were also performed at 32 °C. As expected, even if each formulation maintained its rheological character, increasing the temperature from 25 °C to 32 °C the elastic and viscous moduli decreased (**Tab. 2.1**) and the relaxation time (τ_R), defined by $1/\omega$ at the crossover point between the storage (G') and viscous (G'') moduli, decreased according to an increase of the fluidity of the system.

Table 2.1 – Elastic modulus (G') as a function of frequency and temperature, relaxation time (τ_R) and G' at 1 Hz as function of temperature and adhesive properties (shear and peel adhesion) of placebo formulations.

Form. nos	Solvent	EuRL	TBC	TRI	G' (kPa)				τ_R (s)		G' (MPa)		Shear adhesion (min)	Peel adhesion (cN/cm)
					0.05 rad/s		100 rad/s				1 Hz			
					25 °C	32 °C	25 °C	32 °C	25 °C	32 °C	25 °C	32 °C	32 °C	25 °C
1	EtOAc	60	40	-	22.98	14.19	618.47	470.61	26.31	11.11	1.86×10^{-1}	1.46×10^{-1}	1004.9 ± 9.5	14.1 ± 8.0
2	EtOAc	50	50	-	4.39	2.39	177.91	140.20	3.22	1.14	5.70×10^{-2}	4.32×10^{-2}	18.2 ± 3.5	7.5 ± 2.1
3	EtOAc	40	60	-	0.59	0.27	43.00	35.56	0.45	0.16	1.25×10^{-2}	8.59×10^{-3}	2.1 ± 0.1	3.2 ± 0.5
4	EtOAc	60	-	40	4.98	1.99	330.02	239.26	0.81	0.31	9.90×10^{-2}	6.72×10^{-2}	869.6 ± 12.4	29.1 ± 5.6
5	EtOAc	50	-	50	0.44	0.12	116.09	84.09	0.13	0.05	2.92×10^{-2}	1.83×10^{-2}	12.4 ± 2.1	17.6 ± 3.7
6	EtOAc	40	-	60	-[a]	5.97×10^{-3}	33.20	26.55	0.01	-[b]	5.57×10^{-3}	3.69×10^{-3}	0.6 ± 0.4	15.0 ± 1.7
7	iPrOH	60	40	-	3.87	1.90	279.51	192.31	0.26	0.12	7.37×10^{-2}	4.93×10^{-2}	542.1 ± 13.8	8.4 ± 2.4
8	iPrOH	60	-	40	1.61	0.58	216.84	138.53	0.18	0.08	5.75×10^{-2}	3.38×10^{-2}	393.0 ± 5.1	17.8 ± 0.7

a: not determined;
b: out of range.

The increasing of the relative amount of each plasticizer (from 40 to 60% w/w) caused shifts in the elastic and viscous moduli at each frequency (**Tab. 2.1**).

Figure 2.3a shows the rheological results obtained by analyzing the formulations containing different amounts of TBC (form. nos. 1, 2 and 3) at 25 °C.

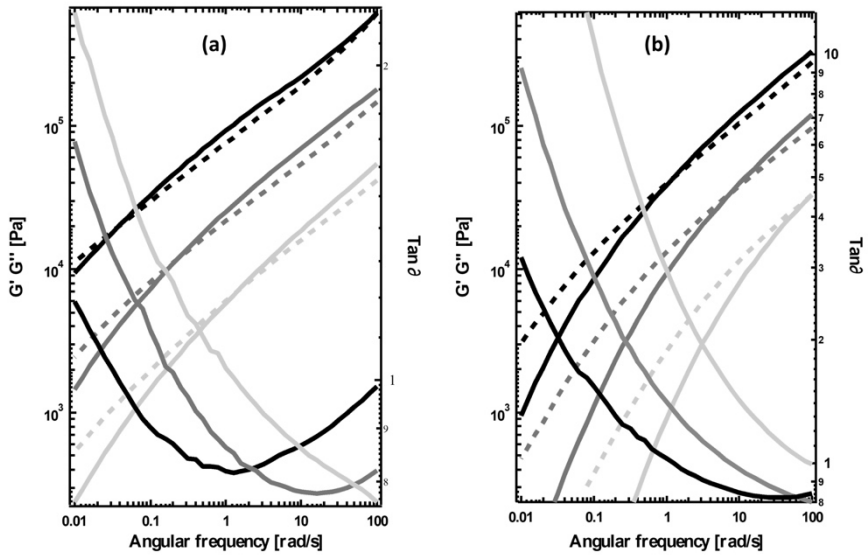

Figure 2.3 - Evolution of G' (solid line) and G'' (dashed line) as a function of frequency. (a) formulations containing TBC and (b) formulations containing TRI. (black line: formulations containing 40% w/w of plasticizer, grey line: formulations containing 50% w/w of plasticizer and light grey line: formulations containing 60% w/w of plasticizer). Tests were performed at 25 °C.

Even if all these formulations are viscoelastic fluids, the PSAs become more fluid increasing the plasticizer amount. Indeed, the observed relaxation time decreased when increasing the amount of TBC (**Tab. 2.1**).

Moreover, TRI appeared a better plasticizer of EuRL than TBC. Indeed, it was clear by frequency sweep measurements at 25 °C that formulations containing TRI are more liquid, showing higher terminal relaxation times (**Fig. 2.3b** and **Tab. 2.1**). As already stated above, at the highest concentration of plasticizer, the PSA containing TRI showed a liquid behavior, while the PSA containing TBC at the same concentration behaved as a viscoelastic material.

This effect cannot be merely ascribed to the differences in miscibility of TBC or TRI with EuRL. Indeed, the solubility parameters (∂), calculated for these plasticizers (∂_{TBC} = 18.86 $MPa^{1/2}$; ∂_{TRI} = 20.82 $MPa^{1/2}$) and for EuRL (∂_{EuRL} = 21.24 $MPa^{1/2}$) according to the Fedors method [18], resulted almost superimposable. Thus, the different effects of the selected plasticizers on the EuRL rheological behavior can only be attributed to different specific interactions. The comparison of ATR-FTIR spectra recorded on the raw materials and different PSAs showed significant modifications in the 1800-1650 cm^{-1} region attributed to the stretching of the carbonyl moieties of the copolymer and plasticizers (in **Fig. 2.4** the whole spectra are reported).

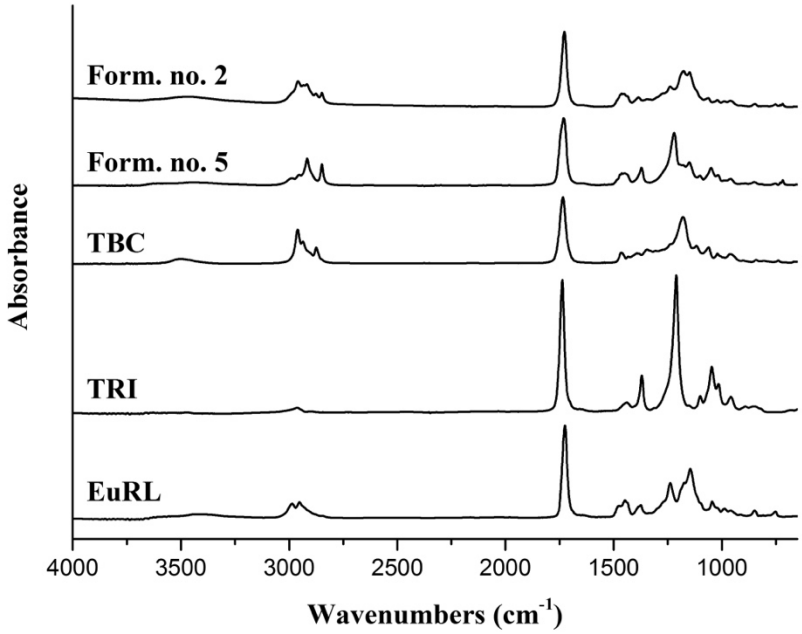

Figure 2.4 - ATR-FTIR spectra of raw materials (EuRL, TBC and TRI) and formulations containing 50% w/w of both the plasticizers.

In this region, independently of the PSA composition, the intensity of the C=O band decreased with respect to that registered on raw materials, suggesting a possible interaction between EuRL and the two plasticizers mediated by H-bonds. This reduction of the carbonyl peak intensity was accompanied by a significant shift of the band toward higher wavenumbers (**Fig. 2.5a** and **b**).

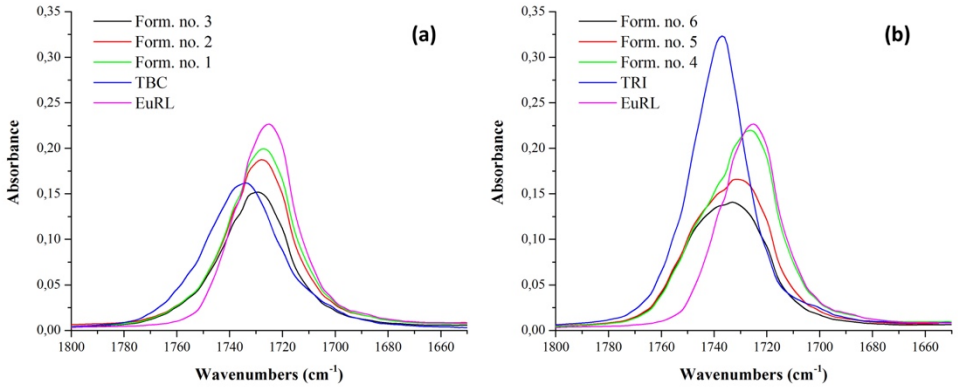

Figure 2.5 - ATR-FTIR spectra of C=O bands of the raw materials and formulations 1-6 in the 1650-1800 cm^{-1} region.

Shifts to higher wavenumbers associated with lower intensities of C=O stretching bands can be related to the formation of novel H-bonds which determines changes in electron densities on different atoms [19]. Since these events are more evident in the spectra registered on PSA made of EuRL-TRI (**Fig. 2.5b**) than on those made of EuRL-TBC (**Fig. 2.5a**), it is reasonable to hypothesize that TRI interacted with EuRL more strongly than TBC, in agreement with the higher plasticizing effect verified in the rheological studies.

Focusing now on the adhesion properties, it should be considered that the initial bonding of a PSA on an irregular surface, like the skin, is driven by the wettability and flow properties at low frequencies. Moreover, Dahlquist suggested that a sufficient level of adhesion is reached when the PSA exhibits an elastic shear modulus lower than 0.1 MPa measured at 1 Hz frequency [20]. As shown in **Table 2.1**, all the formulations fulfill the so-called Dahlquist criterion of tackiness with the only exception of form. no. 1 showing a slightly higher, but still acceptable, G' value either at room temperature and skin temperature.

The performances of the designed formulations were, then, further characterized by studying the debonding mechanism during the tack tests.

The compression force to apply during the experiment was preliminary evaluated for each sample (performing different tests at different contact forces) in order to maintain constant the indentation of the probe in the adhesive layer (maximum 15 µm); the

objective was to impose a contact force that causes an indentation that did not influence the shape of the stress-strain curve.

Generally speaking, detachment patterns in probe tests are mainly governed by the extent of plasticizer in the formulation. **Figure 2.6a** exemplifies the typical stress-strain curve observed for the formulations with the lowest plasticizer concentration.

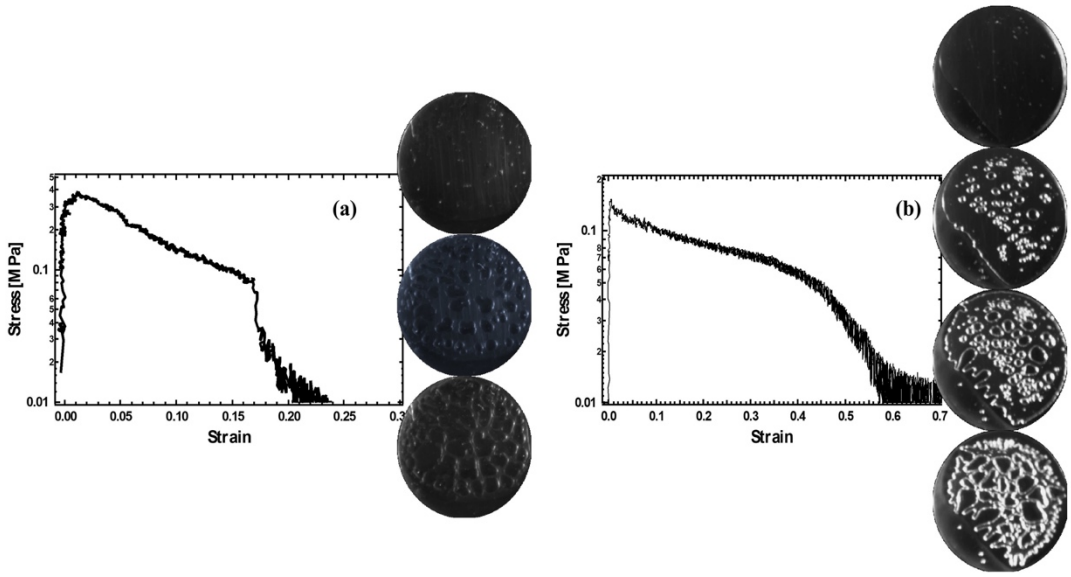

Figure 2.6 - Typical stress-strain curves, schematic and video captures of the debonding mechanism at 10 µm/s and 25 °C of (a) formulation no. 8 and (b) 6.

The detachment pattern shows an initial cavitation occurring at the interface between the probe and the PSA; the deformation of the layer during the debonding process was low with mainly lateral propagation and coalescence of the initial cavities along the interface and a decrease in nominal stress.

This mechanism of debonding and, therefore, the shape of the stress-strain curves were not influenced much by changing the type of plasticizer (form. no. 7 *vs* form. no. 8) or the solvent used for the preparation of the PSA (form. no. 7 *vs* form. no. 1 and form. no. 4 *vs* form. no. 8) as shown in **Fig. 2.7**.

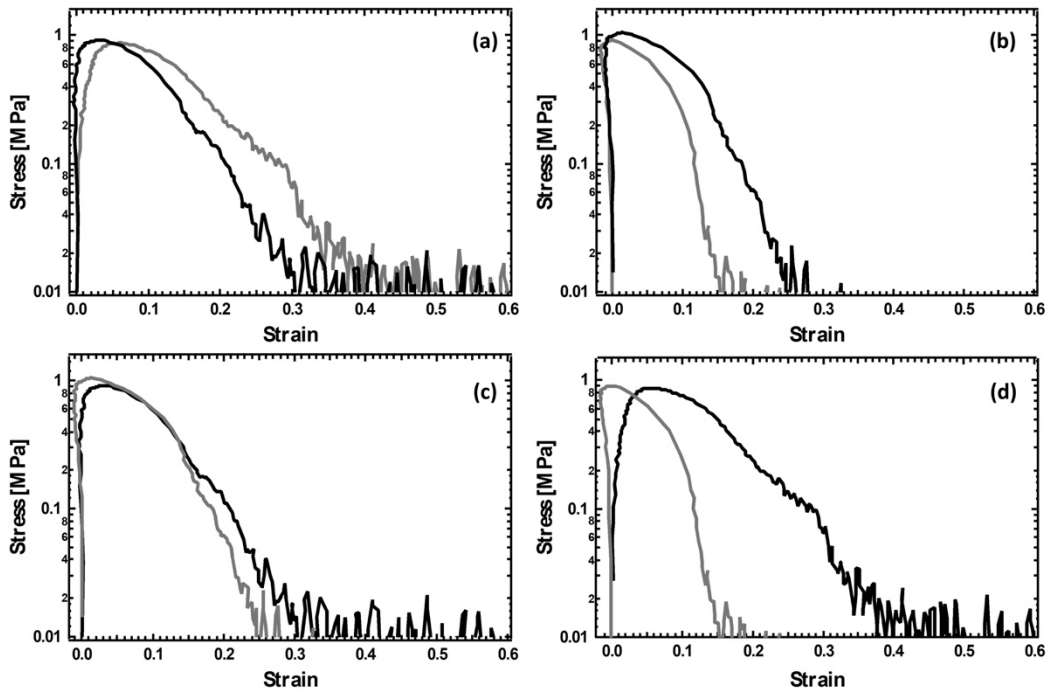

Figure 2.7 - Stress-strain curves of the formulations with (a) 40% w/w of plasticizer and ethyl acetate as solvent (black line: TBC, grey line: TRI); (b) 40% w/w of plasticizer and isopropanol as solvent (black line: TBC, grey line: TRI); (c) 40% w/w of TBC (black line: ethyl acetate, grey line: isopropanol) and (d) 40% w/w of TRI (black line: ethyl acetate, grey line: isopropanol). Tests were performed at 100 μm/s and 32 °C. Note that negative slopes in the loading part of the curve are due to an overcorrection of the compliance of the machine, but do not affect the measured adhesion energy (integral under the curve).

Increasing the debonding rate, independently of the temperature, the maximum nominal stress and the work of adhesion increased significantly (**Fig. 2.8a** and **b**), at least up to the values taken into consideration. This behavior can be attributed to the viscoelastic losses occurring in the adhesive layer, which increase with increasing deformation rate.

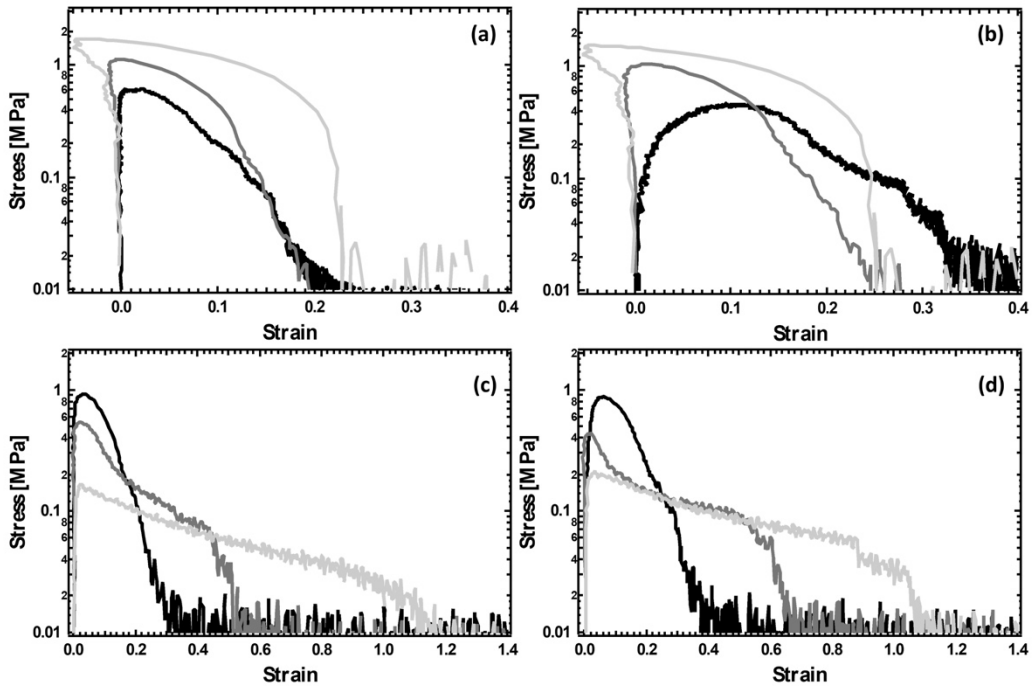

Figure 2.8 - Effect of the debonding velocity (a and b) and of the plasticizer amount (c and d) on the stress-strain curves. Formulation no. 7 tested at (a) 25 °C and (b) 32 °C. (Light grey line: 1000 μm/s, grey line: 100 μm/s, black line: 10 μm/s.). Formulations containing (c) TBC and (d) TRI. (Black line: formulations with 40% w/w of plasticizer, grey line: formulations with 50% w/w of plasticizer, light grey line: formulations with 60% w/w of plasticizer.)

In order to evaluate the behavior of each PSA in the in-use conditions, experiments were also performed at 32 °C. No significant modifications in the adhesive and detachment properties were observed.

Increasing the amount of both the plasticizers from 40% w/w to 60% w/w, the maximum stress decreased, while the maximum nominal strain increased (**Fig. 2.8c** and **d**). These trends were observed for all the tested debonding velocities, in each experimental condition.

Keeping constant the EuRL/plasticizer ratio at 40/60 w/w, the two formulations showed the same detachment pattern and very similar stress-strain curves (**Fig. 2.6b**), even if they presented somewhat different rheological properties. Indeed, the debonding process was governed by a viscous flow, independently of the debonding rate and temperature. Four different stages were identified: (i) homogeneous deformation corresponding to a rapid increase of the force and no optically visible voids; (ii)

nucleation and rapid growth of voids at the probe-film interface, close to the point where the nominal stress goes through a maximum; (iii) slow growth of these voids until they occupy most of the initial contact area; (iv) air penetration into the voids and breakage of the bonds with the formation of isolated fibrils that eventually break (cohesive failure).

It is interesting to note that both the formulations containing 50% w/w of plasticizer showed the first type of debonding mechanism at the higher debonding velocities (100 and 1000 μm/s); while at 10 μm/s they presented the second pattern, even if no residues were optically visible at the end of the experiments. This feature may be attributed to the fact that at lower debonding velocity the polymeric chains have time to re-organize and the PSA has time to relax.

2.3.2 Characterization of placebo patches

The results on the shear adhesion are shown in **Table 2.1**. They represent the resistance of the matrix to flow [21]. The shear adhesion test measures the ability of a patch to adhere to a standard steel panel under a constant shear stress. Since the detachment of the patch from the stainless steel surface occurred cohesively, the data obtained by shear adhesion test can be considered as a true measure of the cohesive strength of the PSA. The increase in plasticizer content caused a reduction in shear adhesion values, due to the dilution of the entanglements of the polymer, resulting in a flowing matrix. This effect appeared clearly with both the plasticizers, in agreement with the pattern found in the rheological measurements at 0.05 rad/s (**Tab. 2.1**). However, excluding form. no. 6, all shear adhesion values could be considered suitable for the development of a patch intended for drug administration [22].

The comparison of the shear adhesion values also revealed that the solvent used to prepare the formulation had an impact on the creep resistance of placebo formulations, since the patches prepared in isopropanol showed lower shear adhesion values (form. nos 7 and 8, **Tab. 2.1**) relative to those prepared in ethyl acetate, in agreement with the rheological studies.

Shifting from shear adhesion to peel adhesion, that represents the force required to peel away a patch from a substrate, it is noteworthy that only the patches prepared with the highest amount of plasticizer left residues on the stainless steel panel, confirming the low cohesive properties of the formulation. Consequently, in order to compare all the formulations, the experiments were repeated using a Teflon® panel which presents a lower critical surface tension [23]. In presence of TRI, the peel adhesion values appeared slightly higher than those of the corresponding patches prepared with TBC (**Tab. 2.1**). In both cases, the peel adhesion values decreased with increasing the plasticizer concentration. However, the relatively low peel force values obtained can be considered satisfactory, since higher values could cause pain or skin damage upon the patch removal.

Cold flow, a phenomenon similar to drug leakage from the edge of membrane-controlled patch, is one of the possible product quality defects [24]. It represents the migration of the PSA outside the edge of the backing layer during the storage and it occurs when the matrix flows like a very viscous liquid between the backing layer and the release liner during the storage. As an example, this negative effect may result in a patch stuck to the primary packaging container becoming unusable. Both the formulations containing 60% w/w of plasticizer presented cold flow after one month of storage at 40 °C, in agreement with the rheological data and independently of the type of plasticizer; while this phenomenon was absent when 40-50% w/w of plasticizer was used. These results on placebo patches are consistent with the data reported by Lin and co-workers [25] who reported that the minimum amount of TRI and TBC required to obtain a positive answer to the rolling ball tack test was about 35% and 40%, respectively. Similarly, 40% of di-butyl-phthalate was required. Indeed, when this plasticizer was used at lower concentrations (*i.e.* 30% and 20%) to develop transdermal patches containing risperidone [26] and verapamil [27], it would not be enough to conferee suitable adhesive properties to the patch. Indeed, since the patch was fixed to animal skin, probably, the rheological and adhesive properties were not sufficient to guarantee the residence of the patch on application site over the required time period.

2.3.3 Characterization of the drug-loaded patches

Since the drugs loaded polymeric mixtures prepared by using ethyl acetate shrunk after casting on the release liner, the drug loaded patches were prepared using only isopropanol as solvent. In all cases, the drug content complied the Ph. Eur assay for the uniformity of dosage units (**Tab. 2.3**).

The loading of the selected active ingredients, namely IB, KP and FP, to the matrices containing TRI did not significantly influenced the debonding patterns (data not shown), peel adhesion and shear adhesion values (**Tables 2.1 and 2.2**). These data were in agreement with the lack of modification on the rheological pattern suggesting that the loaded drug did not modify the interactions occurring between EuRL and TRI. Conversely, in the case of the adhesive matrices prepared using TBC, a reduction of about 20% shear adhesion values was found (**Tables 2.1 and 2.2**) probably because of a plasticizing effect exerted by the loaded drugs (**Tables 2.1 and 2.2**). It can be assumed that the drug can disrupt the interactions occurring between TBC and EuRL in favor of the formation of H-bonds between the carboxylate of the drug and the ester moieties of the copolymer [12,29].

Table 2.2 – Elastic modulus (G') as a function of frequency and temperature, relaxation time (τ_R) as a function of temperature and adhesive properties (shear and peel adhesion) of drug-loaded formulations.

Form. nos	Solvent	EuRL	TBC	TRI	Drug	G' (kPa)				τ_R (s)		Shear adhesion (min)	Peel adhesion (cN/cm)
						0.05 rad/s		100 rad/s					
						25 °C	32 °C	25 °C	32 °C	25 °C	32 °C	32 °C	25 °C
9	iPrOH	60	40	-	IB	2.85	1.37	244.96	182.75	0.25	0.10	433.0 ± 5.3	8.9 ± 0.2
10	iPrOH	60	40	-	KP	1.03	0.53	157.85	128.21	0.20	0.07	420.8 ± 1.6	5.2 ± 2.5
11	iPrOH	60	40	-	FP	1.97	0.79	215.90	134.22	0.23	0.08	430.1 ± 0.6	8.8 ± 3.1
12	iPrOH	60	-	40	IB	1.16	0.49	157.85	117.64	0.17	0.07	385.7 ± 5.4	12.2 ± 0.8
13	iPrOH	60	-	40	KP	0.90	0.33	160.91	117.86	0.13	0.05	388.2 ± 1.0	36.4 ± 2.1
14	iPrOH	60	-	40	FP	1.34	0.53	188.61	142.71	0.16	0.07	380.6 ± 2.1	9.8 ± 0.4

Table 2.3 – Drug content, drug release constants and main parameters calculated from *in vitro* skin permeation experiments (drug flux, *i.e. J*; cumulative drug amount permeated, *i.e.* Q_{24h}; efficiency of transdermal patches, E%) of drug-loaded patches.

Form. nos	Solvent	EuRL	TBC	TRI	Drug	Drug content ($\mu g/cm^2$)	k ($h^{-0.5}$)	J ($\mu g/cm^2/h$)	Q_{24h} ($\mu g/cm^2$)	E% (%)
9	iPrOH	60	40	-	IB	153.0 ± 4.2	0.58 ± 0.04	2.35 ± 0.41	51.26 ± 7.42	33.5 ± 4.8
10	iPrOH	60	40	-	KP	159.9 ± 3.8	0.57 ± 0.01	0.45 ± 0.22	24.14 ± 4.10	15.1 ± 2.6
11	iPrOH	60	40	-	FP	120.4 ± 5.1	0.38 ± 0.03	1.63 ± 0.17	29.57 ± 11.32	24.6 ± 9.4
12	iPrOH	60	-	40	IB	131.3 ± 4.3	0.67 ± 0.01	-*	-*	-*
13	iPrOH	60	-	40	KP	171.5 ± 10.9	0.78 ± 0.01	-*	-*	-*
14	iPrOH	60	-	40	FP	130.8 ± 14.7	0.51 ± 0.07	-*	-*	-*

*: not determined.

The lack of significant differences due to the loaded drug can be justified on the bases of the solubility parameters. Indeed, all the three compounds can be considered freely mixable with EuRL being their values (IB: 20.91 $MPa^{1/2}$; KP: 23.23 $MPa^{1/2}$; FP: 21.53 $MPa^{1/2}$) close to that of the placebo adhesive matrix (EuRL/TBC 60/40: 20.28 $MPa^{1/2}$). Since the therapeutic performances of a transdermal patch are affected not only by its adhesive properties, which assure the residence time of the drug on the skin surface, but also by the ability of the patch itself to release the drug, both the *in vitro* drug release and *in vitro* drug skin permeation were determined.

Table 2.3 shows the drug release constants calculated according to the Higuchi model. These data were in agreement with the rheological results on the effect of the type of plasticizer. The patches containing TRI presented significantly higher release constants ($p < 0.05$) due to the more pronounced plasticizing effect: the lower the G' values at each frequency and, more important, the shorter the relaxation time, the higher the viscous characteristic of the PSA at a given temperature and the faster the release rate (**Tables 2.2** and **2.3**). Hence, the presence of TRI increased the mobility of the polymeric chains favoring the diffusion of the drug through the matrix.

The *in vitro* drug release can be considered satisfactory for all formulations since it was almost complete within 7 hours independently of the plasticizer type (**Fig. 2.9a**). Thus, the plasticizer was selected only on the bases of the adhesive properties and, therefore, the *in vitro* skin permeation studies were carried out on formulations containing TBC (**Fig. 2.9b**).

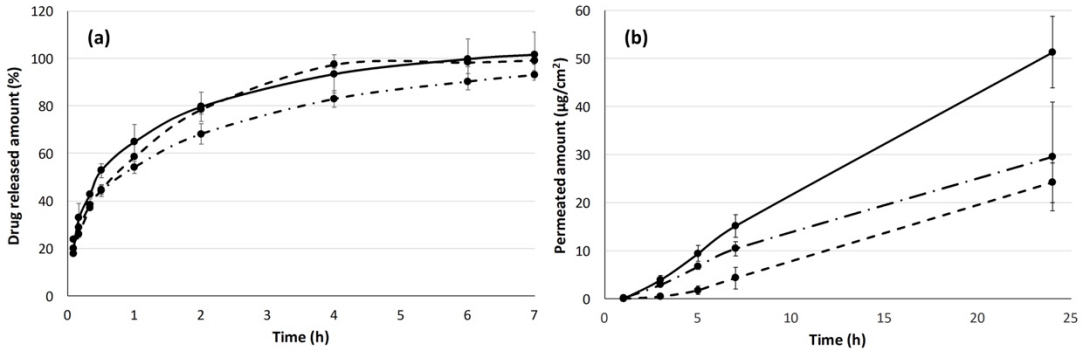

Figure 2.9 - *In vitro* release profile (a) from TBC patches and *in vitro* drug permeation profiles through human skin (b) of the tested transdermal patches. (Solid line: IB, Dashed line: KP, and Dot-dashed line: FP).

PSA made of EuRL and TBC in the 60/40 w/w ratio allowed to obtain a prolonged permeation profile of the loaded drug over least 24 h. Moreover, it should be underlined that the efficiency of transdermal patches in drug releasing over a 24 h period (E%, **Tab. 2.3**) resulted almost double with respect to commercially available plasters containing flurbiprofen (Transact®) and ibuprofen (Ibupas®) and slightly lower with respect that loaded with ketoprofen (Keplat®) [11].

The increased mobility (or lower viscosity) caused by the selected drugs did not have a significant influence on the permeation process. Indeed, the τ_R values taken as representative of the rheological pattern, did not match with the drug fluxes through the skin, suggesting that the barrier properties of the stratum corneum ruled the skin permeation process. In other words, drug permeation pattern after the application of a patch on the skin is governed by different mechanisms [30] with respect to the *in vitro* drug release. In particular, the flux determined for KP was about 5-fold lower than FP and IB, which were not statistically different ($p > 0.05$). The same trend was found by Swart and coauthors [31] using aqueous saturated solution as the reported KP flux was about 4-fold lower than those measured in the case of FP and IB, which were not significantly different. Hence, the overall data suggested that the skin permeation process was not influenced by the formulation in a PSA made of EuRL and TBC, but only by the physico-chemical characteristics of the loaded drug, which dictates the diffusion process through the stratum corneum.

2.4 Conclusions

The current work allowed defining the rheological and adhesive properties of highly plasticized EuRL. The results permitted to identify the optimal copolymer/plasticizer ratio and evidenced the relevance of the PSA rheological behavior on the adhesive properties, reflecting on debonding pattern and shear adhesion. In the specific case of EuRL, the optimal polymer/plasticizer ratio was in the 60/40-50/50% w/w range for both TBC and TRI. The selected drugs loaded at 4% w/w decreased the relaxation time, as an indication of the increase of the matrix fluidity. This behavior has a beneficial impact on the *in vitro* release, determined by dissolution test: the shorter the relaxation time, the higher the drug release rate.

In conclusion, the knowledge of viscoelastic properties of the PSA appears crucial to identify the formulation space during the development of transdermal patches and/or medicated plasters and to optimize their performances. In particular, the matrix relaxation time appears a suitable parameter to optimize the copolymer/plasticizer ratio and establish the possible influence of the active ingredient.

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3

SEBS block copolymers as novel materials to design transdermal patches

Abstract

Background: Styrene-*block*-(ethylene-co-butylene)-*block*-styrene (SEBS) copolymers are biocompatible elastomers with outstanding stability to UV radiations. This work aimed to design a pressure sensitive adhesive made of SEBS to demonstrate the potentialities of such class of materials in the design of transdermal patches.

Research design and methods: The influence of SEBS and tackifier molecular weights on rheological pattern, debonding mechanisms, technological properties (*i.e*. tack, shear and peel adhesion) as well as on the *in vitro* biopharmaceutical performances (*i.e.* drug release and skin permeability) was investigated using ibuprofen and nicotine as model drugs.

Results: Relationships among the rheological pattern and the main technological and *in vitro* biopharmaceutical properties of the prepared patches were evidenced. The higher the liquid component of the matrix, the lower its cohesiveness and the faster the drug release rate. The drug itself can affect the rheological pattern of the matrix, influencing the whole patch performances.

Conclusions: SEBS copolymers are suitable materials to design drug in-adhesive patches. SEBS-low molecular weight is the polymer worthy of consideration because of its favorable viscoelastic behavior. The *in vitro* drug permeability of ibuprofen was not limited by the polymeric matrix if compared to the commercial reference product.

3.1 Introduction

Styrene block copolymers are common thermoplastic elastomers. They consist of two polystyrene endblocks, which give characteristics of rigidity, and a linear midblock, which confers flexibility to the copolymer. Thus, they combine the mechanical properties of rubbers with the processability characteristics of thermoplastics.

Among these copolymers, styrene-isoprene-styrene (SIS) copolymers were studied for the design of pressure sensitive adhesives [1] suitable for the preparation of both transdermal patches [2] and medical devices [3]. However, because of their chemical structure, they need to be compounded with relatively large amount of antioxidants to improve their stability [4]. Conversely, styrene-*block*-(ethylene-co-butylene)-*block*-styrene (SEBS) copolymers, being hydrogenated derivatives, are elastomers with outstanding stability to UV radiations and well-known biocompatibility [5,6].

The compounding of SEBS with paraffin oil and aliphatic resins (tackifiers) allows to obtain a hot melt pressure sensitive adhesive with a very high creep resistance [7]. Tackifiers are crucial components in reducing the polymer glass transition temperature and elastic modulus, that generally is too high at room temperature to form a bond upon simple contact [8].

Taking into account all these considerations, SEBS copolymers appear potential candidates for the design of transdermal patches and/or medicated plasters. Nevertheless, the performances of the system obtained by casting technique is scantly investigated and the ability of the compounded pressure sensitive adhesive to release a loaded drug unknown.

The aim of this work was to design a pressure sensitive adhesive made SEBS in order to demonstrate the potentialities of such class of materials in the development of transdermal patches.

The adhesive properties were conferred using low molecular weight, but high glass transition temperature hydrocarbon resin as tackifier and liquid paraffin as plasticizer [9]. In particular, two resins, with similar ring and ball softening point, cloud point and glass transition temperature, but different molecular weight were selected. The

designed pressure-sensitive adhesives (PSA) were prepared by using SEBS with different average molecular weight and characterized by determining rheological pattern and adhesive properties focusing the attention on debonding behavior under different operative conditions. The *in vitro* biopharmaceutical performances of patches were assessed using ibuprofen and nicotine, as model drugs. These compounds were selected in order to explore the impact of loading cationic and anionic compounds on the designed hydrophobic matrices.

3.2 Materials and Methods

3.2.1 Materials

SEBS are available on market under the trade name of Europrene SOL TH®. Three types of SEBS were kindly gifted by Versalis SpA (San Donato Milanese, Italy): Europrene SOL TH® 2311 (three blocks, 30% bound styrene, average molecular weight 45.61 kDa, SEBS-L), Europrene SOL TH® 2312 (three blocks, 30% bound styrene, average molecular weight 64.23 kDa, SEBS-M), and Europrene SOL TH® 2315 (three blocks, 32% bound styrene, average molecular weight 176.35 kDa, SEBS-H). In order to make the system adhesive it was necessary to add a tackifier. The hydrocarbon resins Regalite™ R1100 (ring and ball softening point = 100 °C, glass transition temperature = 50 °C, cloud point = 78 °C, molecular weight = 850 g/mol) and Eastotac™ H100W (ring and ball softening point = 100 °C, glass transition temperature = 41 °C, cloud point = 81 °C, molecular weight = 1000 g/mol) were received from Eingemann&Veronelli Spa (Rho, Italy). Paraffin oil was obtained by Carlo Erba (Milan, Italy). In order to produce transdermal patches a polyester film was chosen as backing layer, while a polyester siliconized film Saint Gobain (thickness 120 µm) as release liner. S-ibuprofen (IB) and nicotine (NT) were purchased from Dipharma Francis (Baranzate, Italy) and Sigma–Aldrich (Milan, Italy), respectively. Toluene was chosen as solvent and it was purchased by Sigma Aldrich (Milan, Italy). All the solvents were of analytical grade.

3.2.2 Mixture preparation

SEBS were swelled in toluene and the mixtures were continuously maintained under stirring with a magnetic bar at room temperature for 1 hour. Then, the other components of the formulation were added and the mixture was continuously maintained under stirring with a magnetic bar at room temperature for 23 hours. When the drug-loaded formulations were prepared, IB or NT were added in the polymeric mixture, before adding tackifier and oil. To prepare PSAs and patches 24 hours were

waited in order to reduce air bubbles formed during the stirring. A pre-formulation study was performed to achieve the final compositions of the formulations, which are illustrated in **Table 3.1**.

Table 3.1 - Placebo and drug-loaded formulation compositions (%, w/w)

Form. code	SEBS-L	SEBS-M	SEBS-H	Regalite® H1100	Eastotak® H100W	Paraffin oil	IB	NT
1	10	-	-	40	-	50	-	-
2	10	-	-	-	40	50	-	-
3	-	10	-	40	-	50	-	-
4	-	10	-	-	40	50	-	-
5	-	-	10	40	-	50	-	-
6	-	-	10	-	40	50	-	-
7	9	-	-	36	-	45	10	-
8	9	-	-	-	36	45	10	-
9	-	9	-	36	-	45	10	-
10	-	9	-	-	36	45	10	-
11	-	-	9	36	-	45	10	-
12	-	-	9	-	36	45	10	-
13	9.7	-	-	-	38.8	48.5	-	3

3.2.3 Rheological properties in the linear regime

The rheological parameters were determined on a Discovery Hybrid Instrument HR-3 (TA Instruments), by using a parallel crosshatched plate geometry (diameter: 22 mm). In order to obtain equilibrated samples of about 2 mm of thickness, a special sample holder (6 $\times$ 5 cm) made of teflon was used. This device allows to put the solution and completely dry the sample. A 2-steps drying process was used: firstly, PSAs were dried under a glass cover for 24 hours at room temperature and subsequently in an oven at 45 °C under vacuum for 24 hours. After 7 days, necessary for PSA maturation, each sample was cut (diameter: 22 mm) by using a punch. Strain sweep measurements were performed at 6.28 rad/s (1 Hz) in order to define the linear viscoelastic region (LVR). Once the LVR was set, a frequency sweep deformation in the range 10^{-2} – 10^{2} rad/s was applied into the sample. The strain value was in the LVR. Each sample was analyzed first at 25 °C and then at 32 °C. Storage modulus (G'), loss modulus (G'') and phase angle (ð) were used to characterize the samples. Each experiment was performed twice to test data reproducibility.

3.2.4 Texture analysis

In order to obtain a dry PSA of 180 – 200 μm thickness, 3 mL of polymeric mixture was deposited on a standard glass slide (2.6 $\times$ 10 $\times$ 0.2 cm^3), previously cleaned and activated by a plasma technique. A 2-steps drying process was used: firstly, PSAs were dried under a glass cover for 24 hours at room temperature and subsequently in an oven at 45 °C under vacuum for 24 hours. The PSA final thickness was evaluated by a white light scattering technique with an optical profilometer (Microsurf 3D, Fogale nanotech). Probe tack experiments were performed on a custom-designed apparatus adapted on a MTS 810 hydraulic testing machine, allowing the simultaneous observation of the debonding process through a transparent glass substrate [10]. A circular flat ended probe was brought into contact with the PSA layer at a constant velocity (30 μm/s). Once the probe and the sample came into contact, a compressive force of 30 N was applied.

The probe was maintained in contact with the adhesive for 10 s. At the end of the contact time, the probe was separated from the adhesive by retracting it at a predefined speed, namely 10, 100 and 1000 µm/s. The probe was made of stainless steel with a diameter of 9.7 mm. The probe flatness was well-controlled by polishing it with several grades of abrasive paper. The entire probe tack set-up was enclosed in a controlled-temperature chamber to perform the experiments at room temperature (25 $\pm$ 0.5 °C) and at skin surface temperature (32 $\pm$ 0.5 °C). The whole force-distance curve was recorded. The detachment force and the elongation at break were measured and expressed in Newton (N) and millimetres (mm), respectively. Since the results are influenced by the PSA thickness, the force-distance curve was converted into a stress-strain curve. The tensile stress (σ) and the strain (ε) were calculated according to the following equations:

$$\sigma = F/A \qquad \textbf{Eq. 3.1}$$

$$\varepsilon = (h - h_0)/h_0 \qquad \textbf{Eq. 3.2}$$

The term F is the force registered during the detachment, A is the probe surface area, that was exactly determined by the video streaming, h(t) is the PSA elongation at time t and h_0 is the PSA initial thickness. To evaluate the fracture occurring during the debonding process all tests were filmed and recorded through a 45° mirror. For each formulation and for each experimental condition three tests were carried out.

3.2.5 Patches preparation

Patches were prepared by casting, using a laboratory-coating unit Mathis LTE-S(M) (Mathis, CH), equipped with a doctor knife. Coating thickness was set at 250 µm in order to obtain a dry matrix of about 50 µm. The mixture was spread on the backing layer, dried at 40 °C for 20 minutes and covered with the release liner. To evaluate the final

matrix thickness a MI 1000 micrometer (ChemInstrument, USA) was used. Samples were sealed in a well-closed airtight container and stored at 25 ± 0.1 °C until use.

3.2.6 Inclined ball-tack test

The inclined ball tack test allows quantifying the ability of a patch to quickly adhere to a stainless steel surface. In this test, balls characterized by different diameters are rolled on the adhesive surface of a patch placed onto an inclined rump; the largest ball that stops on the patch determines the tack value. According to the "Inclined ball tack testing" reported in the Japanese Pharmacopeia XVII (JP), a plane having an inclination angle of 30° was used. Samples were cut in order to obtain specimen larger than 10 mm in width and 70 mm in length. Each specimen was fixed in the prescribed position on the ramp with the adhesive layer side up. The upper and the lower ends of the specimen were covered with proper shits of paper, leaving 50 mm of adhesive free at the central position. The balls must be able to roll down without slipping. Several hard steel balls (from no. 2 to no. 32, JP XVII) were used. The higher the ball number, the larger the diameter and, therefore, the greater the weight. Before running, each ball was cleaned by rinsing it with acetone and wiping dry with absorbent cleaning material followed by air drying for at least 10 min. Finally, each ball was rolled over the inclined plane from the top of the ramp onto the adhesive tape. Specimens were changed for each run. The number (No.) of the largest ball that stops on the adhesive represents the value of the inclined ball tack test. The assay was performed in triplicate.

3.2.7 Shear adhesion

Shear adhesion tests were performed by using an 8 Bank Oven Shear HT8 Instrument (ChemInstruments, Ichemico, Cuggiono, Italy), in accordance with an internal method described by Cilurzo et al. [11]. The adhesive sample was pressed onto the stainless steel test panel with a bonding area of 2.5 × 2.5 cm^2 by a double pass of a 2.04 kg roller. A

mass of 500 g was hung at the end of each sample. To perform tests at 32 °C a controlled-temperature chamber was used. Each result, namely the time required to completely detach the sample from the test panel, is expressed as the mean ± standard deviation of six determinations for each formulation.

3.2.8 Peel adhesion 180° test

Peel adhesion measures the patch adherence when peeled at 180° angle to a standard steel panel. Peel strength was measured on a tensile machine equipped with a 50 N cell (Instron 5965, ITW Test and Measurement Italia S.r.l., Trezzano sul Naviglio, Italy) at room temperature. Tests were performed according to an internal method [11].
Six samples for each formulation were run by using a stainless steel panel, a peel angle of 180° and peel speed of 300 mm/min.
The average force was calculated as the arithmetical mean of the values of the linear portion of the curve. The peel values are expressed in cN per centimeter width of the adhesive patch under test. The results are expressed as the mean ± standard deviation of six determinations for each formulation.

3.2.9 Cold flow

Cold flow represents the migration of the PSA outside the edge of the backing layer during the storage and involves drug leakage from the edge of membrane-controlled patch. It occurs if the matrix flows like a very viscous liquid between the backing layer and the release liner during the storage [12]. Circular samples (PSA and backing layer) were cut by using a punch (diameter: 32 mm), maintaining the whole release liner. After one month of storage in a sealed container impervious to water and humidity at 40 ± 1 °C, samples were evaluated in terms of migration of the PSA on the release liner. The dimensional changes were measured using a graph paper and cold flow was considered negligible if the PSA was not visually detectable outside the backing layer.

The analyses were performed in triplicate.

3.2.10 Drug content

To determine IB or NT amount loaded in the patches, a specimen of 2.54 cm^2, after release liner removing, was firstly weighed and then dissolved in 50 mL of methanol. To support the complete solubilization of the adhesive matrix and the active principle, the sample was sonicated for 1 hour and then left at rest overnight. The solution was filtered through a 0.45 µm polypropylene filter (VWR International, Milan, Italy), diluted with mobile phase (1:9 v/v) and assayed by HPLC, according to the methods reported below. Each value represents the average of three determinations for each formulation.

3.2.11 Dissolution test

The IB or NT release rate was studied by using an apparatus SR8 PLUS Dissolution test station (Hanson Research, CA, USA) according to the "Dissolution test for transdermal patches" of European Pharmacopoeia 9.2 (2017) [13].
A sample of 8.04 cm^2 was cut and placed flat on a disk with the release liner surface facing up. The backing layer was attached on the disk by using a cyanoacrylate adhesive and the release liner was removed. Each vessel was filled with 300 mL of PBS buffer (pH 7.4), the water bath temperature was maintained at 32 $\pm$ 0.5 °C and the paddle speed was set at 25 rpm. Samples (5 mL) were collected at predetermined intervals and assayed by HPLC, according to the methods reported in paragraph 3.2.13. An equal volume of fresh medium was immediately added to maintain the dissolution volume.
The release rate constant was calculated according to Higuchi's equation as follows:

$$M_t/M_\infty = kt^{-0.5} \qquad \textbf{Eq. 3.3}$$

where M_t is the amount of drug released at time *t,* M_∞ is the drug loading in the matrix and *k* is the release rate constant expressed as h^{-1}. Each value represents the average of three determinations.

3.2.12 *In vitro* skin permeation

Since pig ear skin shows similar histological and physiological characteristics and close permeability properties with the respect to human skin [14,15], it was selected to study IB-permeation profile from SEBS-patches. The skin was obtained from the ears of different pigs, which were kindly provided by a local slaughterhouse (Milan, Italy).

The pig ear skin was separated from the underlying cartilage, cut into squares of about 3.5 cm^2 and sealed in evacuated plastic bags and frozen at -20 °C. Before the experiments, the samples were equilibrated at room temperature for 1 hour.

The integrity of all tissue samples was assessed measuring their electrical resistance (voltage: 100 mV, frequency: 100 Hz; Agilent 4263B LCR Meter, Microlease, Cernusco sul Naviglio, Italy) [16].

The permeation experiments were performed using modified Franz cell under occlusive conditions. They have a diffusion area of 0.636 cm^2 and a receptor compartment volume of approximately 5 mL. This compartment was filled with physiological solution. Patch sample (2.54 cm^2) was applied onto pig-ear with slight pressure. The patch-skin system was carefully mounted on the lower part of the Franz cell with the patch facing upward. The upper and lower parts of the vertical Franz cell were sealed with Parafilm® and fastened together by means of a clamp. 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. At predetermined interval times (1, 3, 5, 7 and 24 hours) 200 µL samples were withdrawn from the receptor compartment and an equivalent volume of fresh receiver medium was added. Sink conditions were maintained throughout the experiment. Samples were analyzed by HPLC according to the method described below.

The cumulative amount permeated through the pig ear skin per unit of area was calculated from the concentration of IB in the receiving medium and plotted as a function of time. The main calculated permeation parameters were the sum of the amount of drug permeated through the skin (Q_{24h}), expressed as μg/cm^2, and the steady flux (*J*), expressed as μg/cm^2/h and determined as the slope of the linear portion of the plot. The results of each permeation experiments are expressed as the mean ± standard deviation of three replicates for each formulation.

3.2.13 Drug assay

The IB and NT concentrations were quantified by HPLC analysis (Agilent HP 1100, Chemstation, Hewlett Packard, Santa Monica, USA). Regarding IB, 20 μL sample was injected at 25 °C on a HyperClone™ 5μm BDS C18 130, 150 mm x 4.6 mm (Phenomenex, Torrance, CA) column. The composition of the mobile phase was acetonitrile/water pH 2.6 (60/40, % v/v). The flow rate was 1.5 mL/min. The wavelength was set at 230 nm. IB concentration was determined from a standard calibration curves in the 0.1-50 μg/mL range.

The concentrations of NT in the medium were determined injecting 20 μL sample at 25 °C on a C18 reverse-phase column (Lichrospher, 100 RP-18E, Agilent, G). The composition of the eluent was 2.31 g/L sodium dodecyl sulphate in acetonitrile/ 13.6 g/L potassium dihydrogen phosphate solution (25/75, % v/v). The flow rate was 1.5 mL/min. The wavelength was set at 254 nm. The drug concentration was determined from NT standard calibration curves ranging between 0.1 and 50 μg/mL.

3.2.14 Statistical analyses

The performances of the patches in terms of drug release rates and adhesive properties were compared by analyses of the variance followed by Tukey post-analyses (Daniel's XL Tollbox 6.70). The level of significance was taken as $p < 0.05$.

3.3 Results and Discussion

3.3.1 Pressure sensitive adhesive characterization

Rheological oscillatory tests were performed to assess the PSA viscoelasticity.

The rheological measurements showed as the materials made of SEBS-L behaved as viscoelastic fluids over the whole range of frequencies, while the others prepared by SEBS-M and SEBS-H behaved like moderately viscoelastic solids. As shown in **Figure 3.1**, only for the form. no. 1, the elastic modulus decreased strongly at low frequency, typical trend of a material with a pronounced viscoelastic character. Therefore, only for this formulation the relaxation time (defined as the phase angle inverse at the crossover point between the storage and viscous moduli) fell within the considered range of frequencies and resulted 0.40 and 0.13 second at 25 and 32 °C, respectively. On the contrary, the storage moduli of the formulations nos. 3 and 5 were significantly higher than the loss modulus, confirming the prevalence of the elastic component over the viscous one.

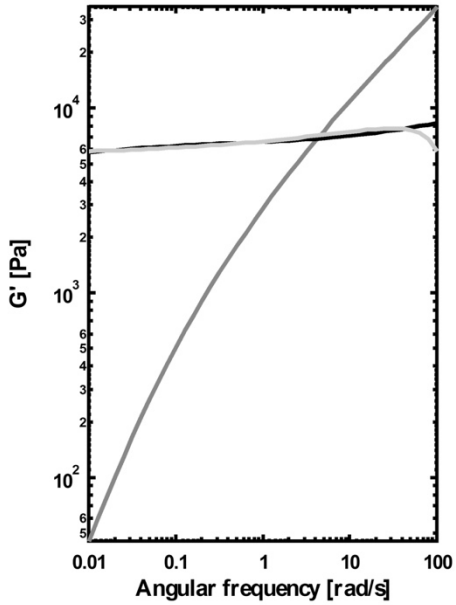

Figure 3.1 - Evolution of G' with frequency. Tests were performed at 25 °C (Grey line: form. no. 1, black line: form. no. 3, light grey line: form. no. 5).

The storage (elastic) moduli at 0.1 rad/s and 25 °C of the formulations composed by SEBS-M or SEBS-H (form. nos. 3 and 5) were closed to 6 kPa (**Tab. 3.2**). The formulation prepared by SEBS-L showed significant lower G' values at the same frequency and temperature (about 0.5 kPa). The non-significant difference among the formulations nos 3 and 5 (**Tab. 3.2**), despite the polymer molecular weight was different, is due to their elastic behavior. Indeed, the effect of the polymer molecular weight is relevant only when the material behaves like a liquid.

Table 3.2 - Elastic (storage) moduli at 0.1 rad/s and 1 Hz.

Form. code	Polymer	Tackifier	G' (kPa)	G' (MPa)
			0.1 rad/s	1 Hz
1	SEBS-L	Regalite® H1100	0.51	2.11×10^{-3}
3	SEBS-M	Regalite® H1100	5.94	6.35×10^{-3}
5	SEBS-H	Regalite® H1100	6.26	6.54×10^{-3}

In order to develop suitable transdermal patches, the matrix viscoelasticity plays an important role to assure the appropriate balance among cohesion, to avoid a too high creep compliance and obtain an adequate shear adhesion, to dissipate energy upon patch application and obtain a suitable peel adhesion during the removal of the patch from the skin [17]. In other words, pressure sensitive adhesive should behave as an elastic solid to guarantee a good resistance to shear over long times and, when subjected to the peeling process, it should exhibit a viscous pattern to dissipate energy [18]. PSA should have a low elastic modulus (G') value at low frequencies and a high G' value at high frequencies, since the bonding process usually undergoes at low shear rate and the peeling process undergoes at high shear rate [19].

However, as shown in **Table 3.2**, all the formulations satisfied Dahlquist's criterion, according to which a good PSA has to show an elastic modulus determined at the bonding frequencies (1 Hz) lower than 0.1 MPa [20].
No differences on the rheological pattern were observed by changing the tackifier. On the contrary, increasing temperature from room to application temperature of 32 °C, G' and G'' decreased as the viscoelastic PSA (form. no. 1) was considered, while taking into account the elastic PSAs (form. nos. 3 and 5) no differences were noticed.

Texture experiments were performed not only to evaluate the PSA instant adhesion properties, but also its debonding mechanism.
The compression force (30 N) applied during the experiment was preliminary evaluated and chosen, after performing different tests at different contact force, to be large enough to obtain a full contact between the PSA and the probe surface, while avoiding a probe indentation higher than 15 μm, which can influence the results.
There was no relationship between the maximum stress values measured during the texture tests at 10, 100 and 1000 μm/s debonding velocity and the elastic moduli of the formulations measured at a frequency ranging between 0.01-100 rad/s in the LVR. The most important results shown in **Figure 3.2** concern the increase in the maximum strain and adhesion energy with the SEBS molecular weight. **Figure 3.2** refers to the formulations prepared with Eastotac™ H100W for sake of clarity since the selected tackifing resins did not affect PSA characteristics neither in terms of adhesive nor debonding properties.

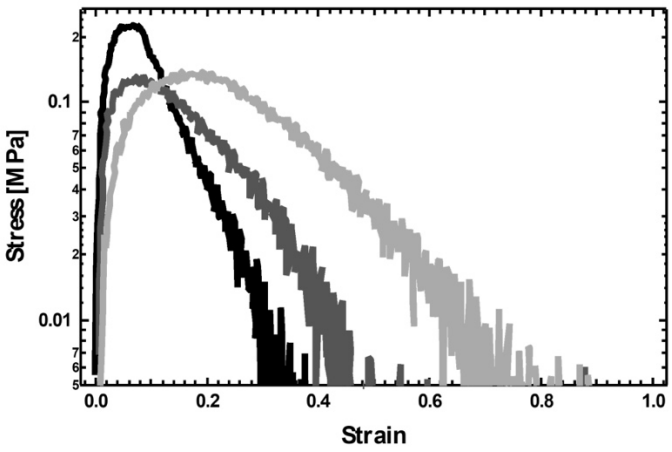

Figure 3.2 – Effect of SEBS-molecular weight on the stress-strain curves. Formulations were tested at 32 °C and 100 µm/s as debonding velocity. (Black line: form. no. 2, grey line: form. no. 4, light grey line: form. no. 6).

In order to understand SEBS-made PSAs debonding processes, their failure mechanisms were studied and by the video streaming two typical debonding mechanisms were identified.

Form. nos. 1-6 showed a liquid-like behavior at the lower debonding velocity with many digitations, because they have more time to relax and reorganize (**Fig. 3.3**, black line).

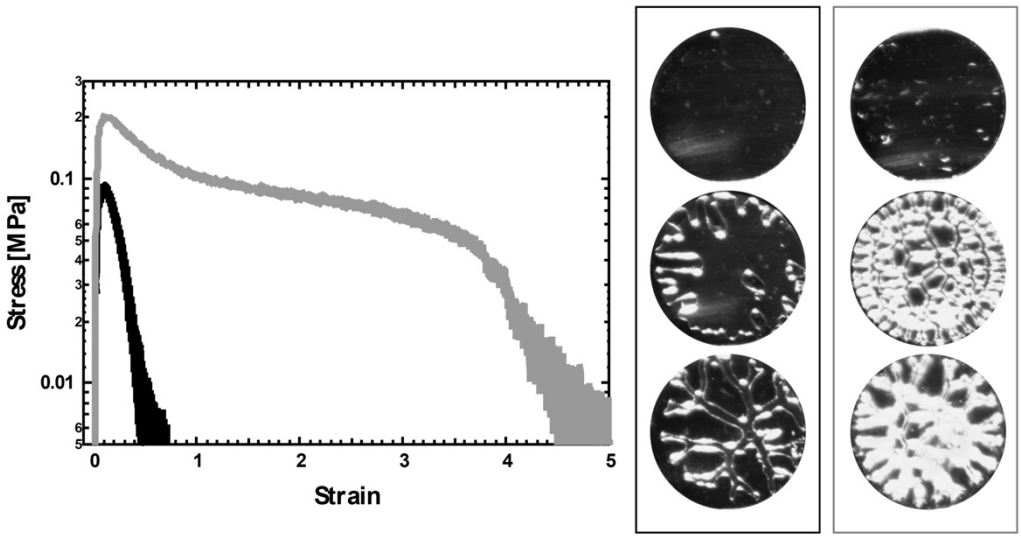

Figure 3.3 – Typical stress-strain curves, schematic and video captures of the debonding mechanisms at 10 µm/s (black line) and 1000 µm/s (grey line) and 32 °C of the formulation no. 5.

On the contrary, at the faster debonding velocity, they did not have time to relax. In these conditions, PSAs showed a more solid-like behavior and the formation of a high number of cavities was observed (**Fig. 3.3**, gray line).

Considering that in the normal usage conditions a patch must adhere to the skin for a long time, the studied PSAs will have time for relaxation, showing in the debonding phase an interfacial behavior. This latter mechanism is required in order to achieve an adhesive failure without residues of adhesive after the debonding process.

The adhesive behavior during the debonding phase as well as the shape of the stress-strain curve and the debonding mechanisms did not qualitatively change neither with polymer nor tackifier molecular weight at all the tested debonding rates.

As far as the influence of the debonding velocity on the main adhesive parameters is concerned, the maximum nominal stress and the work of adhesion increased increasing the debonding velocity, at least up to the value taken into consideration. This behavior can be attributed to the viscoelastic losses occurring in the adhesive layer which should increase with increasing deformation rate.

3.3.2 Placebo patches characterization

The thickness of both placebo and drug-loaded matrices was in the 49 - 55 μm range. Considering the accuracy of the instrument, all the matrices can be considered similar. The shear adhesion represents the ability of the matrix to flow and measures the ability of a PSA to adhere to a standard steel panel under constant stress [17]. Conversely to the results obtained in the rheological studies, the increase of the molecular weight of SEBS determined a reduction of the shear adhesion values (**Tab. 3.3**).

Table 3.3 – Adhesive properties of placebo patches: shear adhesion, peel adhesion and tack (ball no.) evaluated by inclined rolling ball test.

Form. code	Shear adhesion (min)	Peel adhesion (cN/cm)	Ball No.
1	21.2 ± 3.5	37.8 ± 9.3	5
2	23.2 ± 1.0	38.0 ± 9.0	5
3	9.4 ± 0.7	26.4 ± 2.6	8
4	10.2 ± 2.3	25.8 ± 3.3	8
5	2.4 ± 0.2	48.0 ± 9.7	12
6	3.6 ± 0.2	48.6 ± 10.3	12

This unexpected pattern can be explained considering that the patches detached adhesively (*i.e.* the adherent plate was clean after the detachment); as a consequence, the shear adhesion results can not be considered as a true measure of the PSAs internal strength [17]. The good cohesive properties of the prepared adhesive matrices are further supported by the lack of cold flow after storage at 40 °C over 1 month that was in agreement with the rheological characterization, in which the placebo PSA presented more elastic than viscous character. Indeed, it was demonstrated in a previous work that patches present cold flow when the polymeric matrix shows a more viscous character [12]. Moving the attention toward the tackifiers, an influence of their molecular weight can be noted even if this effect resulted statistically significant only in the formulations made with SEBS-H ($p < 0.05$). In particular, the higher the molecular weight of the resin, the higher the shear adhesion values.

The peel adhesion represents the force required to peel away a patch from a substrate [17]. The low peel values (**Tab. 3.3**) could be considered satisfactory, since it is well known that high values could hurt and cause skin damage upon removal. Unfortunately, there was any correlation between the formulative variables and patch peel strength.

The inclined rolling ball test provided information on the superficial properties of the adhesive. Indeed, differences in the diameter of the balls that stop on the adhesive layer

were evident changing SEBS-molecular weight (**Tab. 3.2**) and a correlation among tack and shear adhesion was highlighted: the higher the number of ball, the lower the shear adhesion.

3.3.3 Drug loaded patches performances

In order to confirm the feasibility to design transdermal patches made of SEBS, patches loaded with IB and NT were prepared. The thickness of resulted homogenous being in the 49 - 55 μm.

The trend shown by the placebo patches in terms of shear resistance performances, namely the lower the SEBS molecular weight, the higher the shear adhesion, appeared evident also in the case of the drug-loaded patches.

The IB and NT caused a general reduction in shear adhesion values suggesting that these small molecules could act as plasticizer (**Tab. 3.4**).

The significant reduction ($p < 0.05$) of the shear adhesion values correlated to the presence of both the selected drugs may be due to a reduction in the number of entanglements between the polymer chains, which determined an increase in the matrix fluidity. In the case of IB such a reduction of shear adhesion values resulted much more evident when the Eastotac™ H100W was used, suggesting that in the softer matrices also the M_w of aliphatic tackifier could play a key role in determining the shear adhesion performances of the patches. The addition of smaller amount of the cationic drug, namely the NT, determined a greater reduction of the shear adhesion values with respect to the 10% IB (**Tab. 3.4**).

Table 3.4 - Adhesive properties of drug-loaded patches: shear adhesion, peel adhesion and tack (ball no.) evaluated by inclined rolling ball test.

Form. code	Shear adhesion (min)	Peel adhesion (cN/cm)	Ball No.
7	2.6 ± 0.3	75.8 ± 9.6	12
8	5.5 ± 0.6	76.0 ± 11.7	10
9	2.9 ± 0.3	42.3 ± 2.9	12
10	5.0 ± 0.5	45.0 ± 3.8	10
11	1.3 ± 0.1	36.4 ± 10.0	15
12	1.7 ± 0.1	37.0 ± 10.1	15
13	2.2 ± 0.1	37.9 ± 1.9	12

The softening effect of the IB and NT on the SEBS based matrix also influenced the tack values (**Tab. 3.4**). Conversely, the peeling of the patches from the flat surface resulted unaffected by drug loading.

Drug content (**Tab. 3.5**) satisfied the Ph. Eur. assay for the uniformity of dosage units.

The IB or NT release was completed within 7 hours and it followed Higuchi pattern independently of the SEBS and tackifier molecular weight.

The IB release constants (**Tab. 3.5**), calculated according to the Higuchi model, highlighted as the IB constant release rate was significantly faster by using SEBS-L as polymer ($p < 0.05$), but it was not dependent on the tackifier, suggesting that the softening effect, verified in the study of the adhesive properties of the drug loaded patches, did not influence the drug diffusivity.

Table 3.5 - Drug content and main parameters calculated for *in vitro* drug release and *in vitro* skin permeation experiments: constant drug release rate (*k*), steady flux (*J)* and cumulative drug amount permeated at the end of the experiment (Q_{24h}).

Form. Code	Drug content ($\mu g/cm^2$)	*k* ($h^{-0.5}$)	*J* ($\mu g/cm^2\ h$)	Q_{24h} ($\mu g/cm^2$)
7	674.4 ± 22.8	0.44 ± 0.02	21.94 ± 0.48	465.77 ± 12.83
8	688.8 ± 11.1	0.43 ± 0.02	23.65 ± 3.78	522.80 ± 107.73
9	773.1 ± 12.4	0.36 ± 0.01	15.44 ± 2.59	307.43 ± 53.06
10	734.7 ± 30.3	0.36 ± 0.03	14.39 ± 2.04	304.32 ± 44.68
11	573.1 ± 17.5	0.33 ± 0.01	13.03 ± 3.30	243.81 ± 63.08
12	586.5 ± 5.1	0.31 ± 0.01	13.21 ± 0.86	241.84 ± 15.45
Ibupas®	-*	0.35 ± 0.01	11.79 ± 4.62	227.04 ± 7.98
13	246.8 ± 13.2	0.61 ± 0.06	-*	-*

*: not determined.

These data were in agreement with the rheological pattern: the higher the liquid character of the formulation, the faster the drug release rate. Moreover, comparing these data with the drug release results obtained by dissolution of the commercially available patch loaded with IB, namely Ibupas®, the drug release rate from form. nos. 7, 8 and 12 resulted significantly different ($p < 0.05$). In particular, form. nos. 7 and 8 exhibited faster IB release rate if compared to that of Ibupas®, while in the case of form. no. 12 it resulted slower.

The release constant obtained by patches loaded with NT confirmed its capability to increase the fluidity of the matrix that was more effective if compared to IB. By comparing *k* values of formulations nos. 8 and 13 was confirmed that the matrix fluidity plays a key role in the drug release: the lower the viscosity of the PSA at a given temperature, the faster the release rate.

Aiming to deepen the information on the biopharmaceutical performances, *in vitro* permeation studies by using Franz cell and pig ear skin as membrane were performed

on the patches loaded with IB (form. nos. 7, 8, 9, 10, 11 and 12). Moreover, IB permeability from the commercial reference product (Ibupas®) was also evaluated.

The permeated amount after 24 hours (Q_{24h}) through the pig ear skin and flux (*J*) of IB from the formulated patches are summarized in **Table 3.5**. The amount of IB permeated was influenced by polymer molecular weight, but no by the tackifier (**Fig. 3.4**): in particular, the lower the polymer molecular weight, the higher the IB permeated.

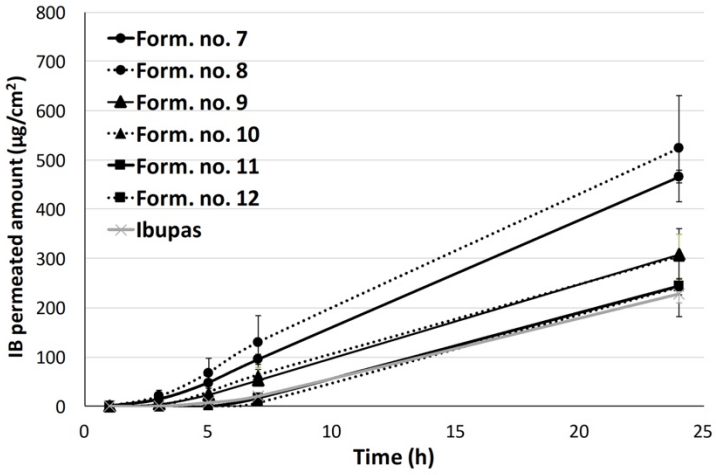

Figure 3.4 - *In vitro* IB permeation profiles through ear-pig skin from the formulated transdermal patches and from the commercial reference product (Ibupas®).

Surprisingly, the IB diffusion through the skin by the formulations nos. 7 and 8 was not limited by the polymeric matrix if compared to the commercial reference product (**Fig. 3.4**). These data correlated with obtained results regarding drug release from the matrix.

3.4 Conclusions

The overall data suggested that SEBS copolymers is a suitable material to prepare drug in-adhesive patches. The *in vitro* permeation results evidenced that SEBS based matrices are suitable to administrate drugs with different chemical-physical characteristics, such as IB and NT. However, SEBS-low molecular weight is the polymer worthy of consideration to design transdermal patches because of its favorable viscoelastic behavior and its capability to not limit *in vitro* IB permeability if compared to the commercial reference product. The resin used to make the polymer sticky did not significantly influence the rheological behavior and adhesive properties placebo SEBS-matrices. On the contrary, concerning the drug loaded patches, both the polymer and resin molecular weight and the loaded drug affected the overall technological and *in vitro* biopharmaceutical features of the final patch.

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Final remarks

This PhD thesis dealt with the investigation of the adhesive and mechanical properties of polymeric matrices in order to design transdermal dosage forms, such as film-forming systems and transdermal patches.

The overall experimental data allowed to highlight the relevance of physicochemical and technological as well as *in vitro* biopharmaceutical characterizations in defining the formulation window to design such dosage forms. In particular:

(a) by studying the mechanical properties of film-forming systems made of EuRL, the importance to evaluate the outward stickiness and elasticity of the formed film was evidenced;

(b) by *in vitro* skin permeation studies from film-forming systems made of EuRL, it was highlighted that the overall absorption process can be significantly influenced not only by the drug thermodynamic activity in the film, but also by the film forming rate, that was not previously considered in literature;

(c) by evaluating the rheological and adhesive properties of highly plasticized EuRL for the design of transdermal patches, the relevance of the viscoelasticity extent of the polymeric matrices on the adhesive properties was evidenced, reflecting on debonding pattern and shear adhesion; furthermore, the impact of the viscoelastic properties on the *in vitro* drug release was highlighted;

(d) by studying film-forming systems and transdermal patches made of EuRL, the versatility of this polymer, opportunely plasticized, in the design of both such dosage forms was confirmed;

(e) by investigating the possibility of using SEBS for the design of patches, the impact of rheological behavior on both adhesive and *in vitro* biopharmaceutical properties was confirmed also for this type of polymers.

In general, the characterization of the final dosage form in terms of both technological and *in vitro* biopharmaceutical performances and the knowledge of the main features of the polymeric matrix, namely rheological behavior and mechanical properties, are crucial to identify a formulative window for designing both film-forming systems and transdermal patches.

In the case of film-forming systems (**Chapter 1**), the evaluation of the outward stickiness resulted important to define an initial formulative space as well as the film elasticity, that plays a key role in defining a whole contact between the pharmaceutical dosage form and the skin independently of the stresses caused by body movements. The *in vitro* skin permeation studies of three model drugs, namely ibuprofen, ketoprofen and flurbiprofen, allowed to evidence that both the vehicle evaporation rate and the thermodynamic activity of the drug within the film influenced the extent of skin permeation. In particular, the evaporation rate of the vehicle, that depends not only by the volatile components, but also by the overall formulation composition (*i.e.* in the simplest formula: vehicle, polymer, plasticizer and drug), affected the initial partition of the drug through the skin. This final remark is due to the formulation metamorphosis, following the vehicle evaporation, which can lead to two different outcomes. From one side an increase of the thermodynamic activity of the drug within the final formed film can occur, promoting the permeation through the skin. On the other side, the drug can crystallize, reducing its availability for the partition from the film toward the skin and, consequently, compromising the whole permeation process.

A series of pressure-sensitive adhesives made of EuRL for the design of transdermal patches were formulated, changing the solvent used to solubilize the polymer and the type and content of plasticizer. In **Chapter 2** the effect of each formulative variable on the main rheological, adhesive and *in vitro* biopharmaceutical features was studied. Interestingly, a relationship between the extent of viscoelasticity, the adhesive properties and drug release was evidenced. In particular, the higher the liquid character of the adhesive matrix, determined in terms of relaxation time, the lower the shear resistance and the higher the *in vitro* drug release rate. Moreover, even in the case of these patches, as well as in the case of film forming systems, the effect of the loaded drugs, namely ibuprofen, ketoprofen and flurbiprofen, on the main pressure-sensitive adhesives characteristics cannot be overlooked. Indeed, a drug can behave like a plasticizer or an antiplasticizer, affecting the viscoelasticity extent of the final patch and, consequently, the other correlated features.

In the case of SEBS (**Chapter 3**), the feasibility to design patches was demonstrated. Moreover, the effects of polymer and tackifier molecular weights were studied. Even if some formulations behaved as elastic materials, the relevance of studying the rheological behavior of a pressure-sensitive adhesive used to design a patch was verified. As a matter of fact, a relationship between the main rheological parameters, the adhesive properties and the *in vitro* biopharmaceutical performances was confirmed. In this case, the higher the matrix fluidity, the faster the *in vitro* drug release and, consequently, the higher the *in vitro* drug permeated amount through the skin within 24 hours. Surprisingly, patches made of SEBS allowed to load a high extent of ibuprofen, without its crystallization, and to obtain a permeated amount of such drug after 24 hours of application higher if compared to the commercial available patch made of an acrylate copolymer.

Scientific publications and communications

Published Article	Gennari CGM, Selmin F, Franzè S, Musazzi UM, Quaroni GMG, Casiraghi A, Cilurzo F. A glimpse in critical attributes to design cutaneous film forming systems based on ammonium methacrylate. *J. Drug Deliv. Sci. Tecn.* **2017**, 41: 157-163.
Published Article	Quaroni GMG, Gennari CGM, Cilurzo F, Ducouret G, Creton C, Minghetti P. Tuning the rheological properties of an ammonium methacrylate for the design of adhesives suitable for transdermal patches. *Eur. J. Pharm. Sci.* **2018**, 111: 238-246.
Draft to be submitted	Quaroni GMG, Gennari CGM, Cilurzo F, Ducouret G, Creton C, Minghetti P. SEBS block copolymers as novel materials to design transdermal patches.
Oral communication	Quaroni GMG. On the characterization of SEBS pressure sensitive adhesives for the design of patches. XI A.It.U.N, Meeting "Clinical experience and technological innovation in pain therapy: from traditional APIs to cannabinoids", May 11-12, **2017**, Padova, Italy.
Oral communication	Quaroni GMG. Transdermal patches and film forming solutions: the case of ammonium methacrylate. Advanced School in Nanomedicine, September 25-28, **2017**, Pula, Cagliari, Italy.
Poster communication	Quaroni GMG. Studio formulativo di un cerotto costituito da Eudragit® RL. XV Scuola Nazionale Dottorale per la formazione Avanzata in Discipline Tecnologico-Farmaceutiche, September 9-11, **2015**, Fisciano, Salerno, Italy.
Poster communication	Quaroni GMG, Gennari CGM, Creton C, Ducouret G, Minghetti P, Cilurzo F. Pressure-sensitive adhesives made of Eudragit® RL: a physical and technological characterization. 4th Congress on Innovation in Drug Delivery - Site-Specific Drug Delivery, September 25-28, **2016**, Antibes Juan les Pins, France.
Poster communication	Quaroni GMG, Gennari CGM, Cilurzo F, Ducouret G, Creton C, Minghetti P. Design of SEBS-based pressure-sensitive adhesives for medicated plasters preparation. 2th European Conference on Pharmaceutics "Novel Dosage Forms & Innovative Technologies, April 3-4, **2017**, Krakow, Poland.
Poster communication	Quaroni GMG, Gennari CGM, Cilurzo F, Ducouret G, Creton C, Minghetti P. Design of SEBS-based pressure-sensitive adhesives for medicated plasters preparation. 57° Simposio AFI, June 7-9, **2017**, Rimini, Italy.

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