Printing of cutaneous patches loaded with propranolol for the treatment of infantile haemangiomas

Umberto M. Musazzi,* Chiara G.M. Gennari, Silvia Franzè, Paola Minghetti and Francesco Cilurzo

[a] Department of Pharmaceutical Sciences, Università degli Studi di Milano - via G. Colombo 71 – 20133 Milan (Italy); umberto.musazzi@unimi.it (U.M.M.), chiara.gennari@unimi.it (C.G.M.C), silvia.franze@unimi.it (S.F.),paola.minghetti@ francesco.cilurzo@unimi.it (F.C.)

* Correspondence: umberto.musazzi@unimi.it

1 Author Contributions

Conceptualization, U.M.M., F.C.; methodology, U.M.M., F.C., S.F.; formal analysis, U.M.M., C.G.M.G.; investigation, U.M.M., C.G.M.G.; data curation, U.M.M.; writing—original draft preparation, U.M.M.; writing—review and editing, U.M.M., S.F.; supervision, F.C. All authors have read and agreed to the published version of the manuscript.

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Abstract

Topical propranolol has been used in clinics for treating cutaneous infantile haemangiomas, but frequent applications of semi-solid preparations are required to maintain therapeutic drug concentrations in the skin layers over time. This work aims to study the preparation of cutaneous propranolol patches by hot-melt ram extrusion printing a novel technique suitable for the personalization of the dosage forms. The preparation steps are: i) mixing of a poly-ammonium methacrylate polymer (Eudragit RL) with a suitable amount of plasticizer (acetyl triethyl citrate (ATEC), triacetin or tributyl citrate, TBC), and the drug (propranolol base, or hydrochloride), ii) the melting in the ram extruder, and iii) the printing on the backing layer foil. All formulations released the loaded drug in a reasonable time and exhibited suitable adhesive properties. The determination of permeation profiles of the drug revealed the patch made of Eudragit RL and TBC and containing 1% propranolol hydrochloride as the most promising formulation for ensuring the drug retention on the human epidermis ($Q_{ret}/J = 1.32$) and, therefore, it can be selected when a superficial haemangioma has to be treated. Conversely, the patch made of Eudragit RL and ATEC and 1% propranolol base can be used in the case of deep haemangiomas.

Keywords

Propranolol; Infantile haemangiomas; cutaneous patch; Eudragit RL; Hot-melt extrusion; printing.

4 Introduction

Propranolol (PR) is the first-line therapy for the management of infantile haemangiomas (IH), which are the most common benign tumours of infancy and affect 3% to 10% of infants [1]. IH are characterized by rapid and intermittent growth of the tumour mass, followed by a spontaneous regression in 90% of patients by their ninth birthday. IH can be divided by their morphology into superficial, subcutaneous (deep), and mixed haemangiomas [1]. If not appropriately treated, most of them cause disfigurements or functional impairments (e.g., obstruction of airways and vision, cardiac insufficiency, and hypothyroidism) [1,2]. PR clinical efficacy was firstly documented in 2008 when Léautè-Labréze and co-workers observed a rapid regression of IH in a patient who received the β-blocker for the treatment of a pre-existent cardiovascular disease [3]. After such serendipity, oral PR has replaced existing therapies due to the higher efficacy and safety [1]. The application of topical preparations containing PR and timolol has also been proposed as an alternative treatment for superficial IH involving the skin [1]. Therefore, its topical use allowed a clinical efficacy comparable to oral PR, but with a lower risk of medium- and long-term side effects [1].

PR permeation through the skin was strongly affected by the ionic form of the drug and the design of the delivery system [4,5]. Since the topical treatment with PR administered by semi-solid preparations requires multiple daily applications [6], the design of cutaneous patches is attractive to maximize the residence of the dosage form at the absorption site, simplifying the regimen. The basic design of a cutaneous patch includes a backing layer, which protects the formulation from the outer environment, a pressure-sensitive adhesive (PSA) containing the drug, and a protective foil, which is peeled out before the patch application. A drug-in-adhesive patch can be obtained by casting technologies or by printing with the advantage to tailor the geometry of the patch according to the IH affected area without wastes, as recently demonstrated [7].

This work aimed to investigate the preparation of PR cutaneous patch by hot-melt ram extrusion printing. The preparation procedure to obtain (trans)dermal patches consists of the melting of a mixture made of all the formulation components in the ram extruder and printing the melt directly on the backing layer. Afterwards, the patches were coupled with the release liner and sealed in an airtight bag. The PSA were made of Eudragit® RL and opportunely

plasticized by triacetin (TRI), tributyl citrate (TBC), and acetyl triethyl citrate (ATEC). Such plasticizers were selected based on previous evidence [7–9] to deepen the influence of plasticizer types on patch printability and its technological properties. The influence of ionic drug species on the release and permeation performance of each PSA matrix was studied using patches loaded with PR hydrochloride (PR-Cl) or with PR base (PR-B). The drug content was set to 1% w/w based on the existing literature on topical treatments for IH and other similar cutaneous diseases [1,4,10].

5 Materials and Methods

5.1 Materials

Poly-(ethylacrylate-co-methylmethacrylate-co-trimethylammonioethylmethacrylate chloride), traded with the name Eudragit® RL PO (EuRL), with a molar ratio of 1:2:0.2, was kindly donated by Rofarma Italia (I). Acetyl triethyl citrate (ATEC) and tributyl citrate (TBC) were supplied by Morflex (US), whereas triacetin (TRI) was purchased from Sigma Aldrich (I). PR-Cl, white petrolatum, and lanolin were purchased from Farmalabor (I). The PR-B was obtained for precipitating the PR-Cl with sodium hydroxide solution. The release liner and the backing layers tested were kindly donated by IBSA (I). All solvents were of analytical grade unless specified.

5.2 Preparation of patch

The mixtures were obtained by mixing the accurately weighted amount of each component in a mortar according to the composition reported in Table 1. The final weight of each mixture was about 10 g. The mixture was immediately transferred in the hot-melt ram extrusion printer previously described [7], melted and printed at 100°C through a 0.7-mm needle. The distance from the needle tip to the surface of the backing layer was fixed at 0.3 mm to permit a suitable deposition of the melted blend and to obtain an adhesive matrix with a thickness of around 50-70 µm. The speeds of the mobile plate and the extruder ram were set at 12 and 10 mm/s, respectively. Finally, the filling angle was set at 135° to the x-axis of the baking layer. The melted materials extruded through the die was deposited on the 20 x 20 cm backing layer fixed in the mobile plate of the printer. The printing rate and the distance between the needle and backing layer were set to obtain adhesive matrices with a thickness of about 50 µm

measured by using a micrometer MI 1000 µm (ChemInstruments, US). The dimension and number of patches per each print were set up by 3D builder® (Microsoft, US) and converted in G-code. Afterwards, the patches were matched with a siliconized polyethylene film sealed in the primary packaging and stored until use without further manipulations.

5.3 Adhesive properties determination

The adhesive properties of patches were determined according to internal protocols [7,9,12], which are briefly described below.

Cold flow – The cold flow was evaluated on patch samples of 25 x 60 mm after a storage period of two weeks at room temperature (RT). The specimen complied with the test when the PSA was not visually detectable outside the backing layer. When occurring, the extent of cold flow was expressed as the maximum migration of the adhesive in millimetres on the release liner. It was measured by putting the sample, which was in any case almost transparent, on graph paper. The analysis was performed in triplicate. If the cold flow was observed, the formulation was discarded.

Probe tack test – Patch samples of 25 x 60 mm were printed from each formulation and stored at 25 ± 1°C for two weeks to assure the stabilization of the adhesive matrix [9]. The probe tack test was performed according to a standard internal procedure using a tensile testing machine equipped with a 50 N cell (Instron 5965, ITW Test and Measurement Italia, I). A strip of double-coated tape (TESA, D) having the same size as the plaster specimen was applied between the flat bottom plate of the tensile testing machine and the backing layer of the patch specimen. The patch release liner was then removed. The flat stainless-steel probe (diameter: 5 mm) was placed ~0.05 mm above the adhesive matrix. The probe was then lowered onto the adhesive 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 absence of PSA residues on the probe surface (adhesive failure) was visually determined. The whole force-distance curve (compression and traction) was recorded. The area under the curve force vs probe displacement was assumed as the work of separation (W). The tack stress (σ_{max}) values for each experiment were calculated as the maximum traction force normalized by the probe area. The results were expressed as the mean ± standard deviation of four determinations.

Shear adhesion test – Patch specimens of 25 x 60 mm were printed from each formulation (Table 1) and stored at 25 ± 1 °C for two weeks to assure the stabilization of the adhesive matrix [9]. The shear adhesion was performed using an 8 Bank Oven Shear HT8 Instrument (ChemInstruments, Ichemico, I), according to a method previously described using a 500 g mass to generate the stress [7]. The experiments were performed at room temperature (25 ± 1 °C). The results were expressed as the mean ± standard deviation of four specimens.

Peel adhesion 180° test – The tests were performed using a tensile machine equipped with a 50 N cell (Instron 5965, ITW Test and Measurement Italia, I) using an iron steel panel, accordingly to the method described by Cilurzo and co-workers [12]. Patches printed with a 12 x 120 mm size were stored in primary packaging material at 25 ± 1 °C for two weeks before use.

5.4 Drug content

An accurately weighed 2.54 cm^2 patch sample was dissolved in 50 mL of a mixture of acetonitrile and phosphate buffer solution at pH 4.5 (1:1) by mechanically shaking and sonication (UP200st, Hielscher, D). Afterwards, the samples were left to rest overnight and then diluted 1:1 with the mobile phase described below. Before the injection, samples were filtered with a 0.45 µm polypropylene filter (VWR International, I). The drug content in the patch was calculated as a function of both the matrix mass (µg/g) and area (µg/cm^2). The results were expressed as the mean ± standard deviation of three specimens for each formulation.

5.5 In vitro dissolution test

The dissolution was performed by using an apparatus SR8 PLUS dissolution test station (Hanson Research, US) according to the disk assembly method described in the "Dissolution test for transdermal patches (01/2008:20904)" of European Pharmacopoeia. A 4.91 cm^2 patch sample was placed flat on the iron disk (mesh size of the disk net: 125 µm) with the adhesive surface facing up according to the method previously described. The vessels were filled with 100 mL of dissolution medium, the bath temperature was kept at 32.0 ± 0.5 °C, and the paddle speed was set at 25 rpm. Phosphate buffer solution at pH 5.5 was used as a dissolution medium. At predetermined intervals (5, 10, 20, 30, 40, 50, 60 min), 5 mL samples were

collected and immediately replaced with fresh medium. The solutions were assayed by HPLC, according to the methods reported below. The results were expressed as the mean ± standard deviation of three specimens for each formulation. The release rate constant was calculated according to Higuchi's equation as follows:

$$\frac{M_t}{M_\infty} = K^{0.5} \qquad (1)$$

where M_t is the amount of drug released at time t, M_∞ is the drug loading in the patch matrix and K is the release rate constant expressed as h^{-1}. The K was calculated as the slope of the linear portion of the plot for M_t/M_∞ lower than 0.8.

5.6 In vitro skin permeation and retention studies

The permeation studies were performed using abdominal skin from donors, who underwent cosmetic surgery. According to an internal protocol [4], after removing the subcutaneous fatty tissue, the skin samples were immersed in water at 60 °C for 1 min, and the epidermis was carefully removed from the underlying tissue with the help of forceps. The integrity of epidermis samples was assessed by measuring their electrical resistance (voltage: 100 mV, frequency: 100 Hz; Agilent 4263B LCR Meter, Microlease, I), using a modified Franz diffusion cell (PermeGear, US). Each Franz's cell has an effective permeation area and a receptor volume of 0.636 cm^2 and 3 mL, respectively. Samples with an electrical resistance higher than 20 kΩ·cm^2 were used for the in vitro permeation experiments [13].

At the beginning of the in vitro permeation studies, a 2.5 cm^2 circular sample, obtained from a printed patch by a precision die cutter, was gently applied to the epidermis specimen. Then, the assembly was mounted on the receiver compartment of the Franz diffusion cell filled with saline solution, containing sodium azide (100 µg/mL), as a preservative, and maintained at 35 ± 1 °C, so that the skin surface temperature was 32 ± 1 °C. Special care was taken to avoid air bubbles between the epidermis and the medium in the receptor compartment. The receptor medium was continuously stirred with a small magnetic bar at 1800 rpm to assure a uniform distribution of the permeated drug. The upper and lower parts of the Franz diffusion cell were sealed with Teflon (VWR International, I) and Parafilm® (Pechiney Plastic Packaging Company, US) and fastened together using a clamp. At predetermined times (1, 3, 5, 7, 24 h), 200 µL samples were withdrawn from the receiver compartment and replaced with a fresh receiver

medium. Sink conditions were maintained throughout the experiments. Samples were analysed by HPLC according to the method 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.

At the end of the permeation experiments, the epidermis sheet was removed from the Franz diffusion cell, and each side was gently treated with 10 mL of methanol to wash out the unabsorbed drug. Subsequently, the sample was dried, thinly sliced, and placed in 5 mL of fresh methanol. The suspension was soaked in a sonicator for 30 min and then maintained for 24 h at 2–8 ° C. Finally, the supernatant was filtered at 0.45 µm and analysed by HPLC. The results were expressed as the average of parallel experiments performed in triplicate. The retained drug amount (Q_{ret}) was expressed as micrograms of PR per unit area of the epidermis.

5.7 HPLC method

The drug content and its concentration in the dissolution medium were quantified by HPLC analysis (Agilent HP 1100, Chemstation, Hewlett Packard, US), using the following chromatographic conditions: Column, InertCloneTM 5 µm ODS 100 Å, 150x4.6 mm (Phenomenex, US); mobile phase, acetonitrile/water pH 2.5 (30/70, % v/v); flow rate, 1.5 mL/min; wavelengths, 230 nm; temperature, 25 °C; injection volume, 20 µL. The LOQ of the method was equal to 0,02 µg/mL, whereas the LOD was 0,002 µg/mL. The drug concentrations were determined from standard curves in the 0.02–100 µg/mL range.

5.8 Statistical analysis

Tests for significant differences among formulations data were performed by the one-way ANOVA followed by Turkey-Kramer post-analysis (JMP® 14, SAS, US). Differences were considered significant at the $p < 0.05$ level.

6 Results and discussion

The PR-Cl and PR-B did not influence the PSA printability. The final thickness of the patches (50 ± 10 µm), and the drug contents were uniform (Table 2), exception made for the TRI-based formulations (Forms. 8 and 9). In these cases, the melt was too-fluid and impeded reproducible deposition of the adhesive matrix on the backing layer which caused the decrease of the drug content. Even if such behaviour could be partially mitigated by modulating the printing temperature, the PSA prepared with TRI and containing the drug failed the cold flow test after two weeks of storage, showing low stability of such matrix over time (Table 1). This evidence agreed with those obtained by patches prepared with other active ingredients by both printing and solvent casting techniques [7,9].

6.1 Adhesive properties

The tack parameters of placebo ATEC- and TRI-based patches resulted significantly higher than those obtained from TBC ones ($p < 0.01$; Table 1). When PR-B or PR-Cl was added to the matrix composition, the tack (σ_{max} and W-values, Table 1) could be ordered as follow: placebo < PR-B < PR-Cl. This trend was particularly evident in TBC ($p < 0.001$) and TRI series ($p = 0.046$). In particular, the σ_{max} of PR-B and PR-Cl loaded patches resulted 24- ($p < 0.002$) and 45-fold higher than placebo ($p < 0.001$), respectively. On the contrary, the addition of PR-Cl to the ATEC-PSA (Forms. 3-5) caused a slight, but non-statistically relevant, increase of the patch stickiness ($p = 0.228$). Generally speaking, all patches presented satisfactory tackiness since the values are sufficiently low to assure suitable handling by the patient at the moment of patch application onto the skin [16].

The shear adhesion of ATEC or TBC-based patches (> 1400 min) was 5-fold higher than TRI-based ones (< 300 min). The cohesivity gap increased when PR was loaded in the PSA. The TRI values dropped more than a half, whereas both TBC and ATEC showed a comparable pattern in comparison to the placebo ones. However, it is worth noting that the cohesivity of TBC-based matrices seemed more influenced by the ionic drug species than the ATEC ones (Table 1). Indeed, PR-B significantly reduced the shear adhesion of TBC-matrix in comparison to placebo ($p < 0.002$) and PR-Cl ($p < 0.03$). On the contrary, no differences were observed when ATEC was used as a plasticizer.

The results of 180° peel adhesion tests demonstrated that the forces required to peel away all the printed patches from the steel iron surface were quite low for all the formulations. In agreement with previous results obtained by using similar PSA on Teflon® surface [7] since all formulations exhibited an adhesive failure and the loaded drug did not affect the peel value. Furthermore, the absolute value of peel data indicated that the patches could overcome the frictions related to the clothes and not accidentally detached; at the same time, the patches removal can occur painlessly. These features distinguish the EuRL based PSA from other adhesives designed for hot-melt extrusion techniques which usually exhibit very high peel values [14,15].

The overall results showed that ATEC other than TBC already used in other studies can be used for the preparation of printable PSA. Indeed, all the adhesive properties values fall in the range of marketed loco-regional patches [17].

6.2 Drug release and skin permeation

The in vitro release studies demonstrated that PR was rapidly released from all formulations suggesting that the thermodynamic activity of the drug at the cutaneous patch/stratum corneum interface should be guaranteed during the application on the skin. Both the ionic drug species and the PSA composition had a slight influence on the in vitro drug release. In particular, the PR-Cl was released faster than PR-B in ATEC-based PSA (i.e., Forms. 5 and 6; Figure 1). A reduction of the drug release over time was found in the case of TBC-based PSA. It is possible to speculate that TBC creates ionic interactions with the PR, due to its basic hydroxyl group (pK_a = 11.30 ± 0.29) [18]. On the contrary, only weak interactions (e.g., van der Waals forces) can be possible between PR and ATEC due to the esterification of the hydroxyl group. Therefore, more polar species such as PR-Cl can be released faster than PR-B by the ATEC due to the lower interaction strength in comparison to TBC. Such a hypothesis agreed with the results obtained by Yang and co-workers, who demonstrated that the PR release could be controlled by modifying the number of PSA chemical groups interacting with the drug [19].

The J values, calculated from the in vitro skin permeation experiments, followed the rank order: Form. 6 < Form. 3 < Form. 2, < Form. 5 ($p < 0.001$; one-way ANOVA) evidencing that the observed differences in the release profiles were relevant only for the penetration of PR-

B. Indeed, the flux from ATEC-based PSA (Form. 5: J = 3.54 ± 0.33 µg/cm^2/h) was higher than from TBC ones (Form. 2: J = 2.42 ± 0.38 µg/cm^2/h). The faster release of the PR-B from the ATEC-based matrix permitted the drug to be promptly available at the patch/skin interface, quickly establishing the concentration gradient required to sustain the drug permeation. This hypothesis was also supported by the different time lag between the two formulations: 1.41 ± 0.26 h in the case of Form. 2, whereas it was almost equal to zero for Form. 5 (Table 2).

The permeation profiles of PR-Cl were lower than the other and almost superimposable (Figure 2). Significant differences were not observed between Forms. 3 and 6 in terms of either J ($p = 0.9842$) or lag time ($p = 0.5360$). This was expected since the ionic drug species is one of the most relevant factors in skin permeation. Indeed, the limiting step of drug permeation is the drug partition into the stratum corneum, which is a dense and lipophilic barrier that protects the lower skin layers from the environment. Lipophilic species (e.g., PR-B) can penetrate more easily than ionic ones (e.g., PR-Cl). It agrees with the trend already described for semisolid preparations [5]

As shown in Table 2, the retained amount of PR was around 0.3 µg/mg (≈ 14 µg/cm^2) for almost all tested formulations. It suggests that PR was able to saturate the epidermal layers after the partition process between patch and skin. The only exception was Form. 6 (Q_{ret} = 0.15 ± 0.02 µg/mg), which was statistically different from others ($p < 0.04$). Here again, this outliner data may be due to the prevalence of cationic PR species that limit the partition into the stratum corneum (Form. 6 vs Forms. 2 and 5; Table 2).

The comparison of the Q_{ret} of Forms. 3 and 6 seemed to suggest a different equilibrium of PR-B and its cationic species within the PSA matrix. Unlike ATEC, the hydroxyl group of TBC could shift the acid/base balance of PR towards the neutral-charged form in the adhesive matrix, with a positive impact on the drug partition.

The results showed that the permeation/retention profile of PR could be modulated by changing the composition of the PSA matrix. This aspect has significant repercussions for the extemporaneous preparation of small patch batches for the treatment of IH. In particular, the proper PSA matrix can be easily selected for treating different types of IH. In this light, the Q_{ret}/J can be a simple parameter for choosing the most appropriate formulation based on the

pathophysiology of the IH. If the patches should ensure high skin retention, the formulation with a $Q_{ret}/J > 1$ should be preferred. Otherwise, the formulation can promote drug permeation through the lower epidermal layers ($Q_{ret}/J < 1$) [19]. As shown in Table 2, Form. 3 was the most promising formulation for ensuring PR retention on the human epidermis ($Q_{ret}/J = 1.32$). Therefore, it can be selected by the compounding pharmacist when a superficial IH had to be treated. On the contrary, Form. 5 ($Q_{ret}/J = 0.09$) should be preferred every time the physicians needed to reach higher PR concentrations in the subcutis (e.g., deep IH).

Finally, the designed formulations can present some potential advantages also regarding safety and efficiency (i.e., the percentage of the loaded drug which reach the skin) which was introduced in the EMA Guideline on the quality of transdermal patches. As a matter of fact, the J-value of printed patches was at least four-time lower than PR-loaded patches designed for a systemic PR administration [5]. This evidence suggested that printed patches can be used for the loco-regional delivery of PR, with a low risk of systemic absorption and, therefore, side effects like those that are sometimes reported for oral PR [1]. Furthermore, their efficiency was higher with respect to semi-solid preparations containing a similar PR amount and designed and tested in vivo to be used for treating IH and similar cutaneous diseases [4,10]. Indeed, the PR permeated profiles of Forms. 2 or 5 were slightly better than a hydrophilic cream in terms of the technological performances: the PR permeated after 24 h from the printed patches was around 10% of the drug loading, whereas only the 2.77 ± 0.39% from the hydrophilic cream [4]. A similar trend was observed for the retained amounts. Indeed, even if Form. 6 was the worst formulation in terms of retention among printed patches, the Q_{ret} (1.16 ± 0.31 %) was comparable to that obtained by a lipophilic ointment used in clinics (1.82 ± 0.23%).

The overall results showed that printed patches permit to obtain similar *in vitro* performances of semi-solid preparations that have been already used in clinical practice. Although further studies are desirable to demonstrate the clinical efficacy and safety of PR-loaded patches with respect to semi-solid formulations, these findings suggested that the proposed approach may apply not only to the treatment of IH but also to other cutaneous diseases in which the treatment efficacy can be reduced by the low residence time of the formulation onto the absorption site or the low patient's compliance due to the frequent dose application. Indeed, both obtained results and published data on patches prepared with the same technology

demonstrated that adhesive matrices made of poly-ammonium methacrylate polymers are enough versatile to be printed at relatively low temperature and robust to obtain patches with an acceptable quality profile, independently from the drug physicochemical properties [7,20,21].

7 Conclusions

The overall results showed that PR could be effectively loaded into different low-temperature melting hot-melt PSA made of poly-ammonium methacrylate polymer. The drug did not significantly affect the adhesive properties of the patches plasticized with TBC and ATEC. Such technological platforms seem promising for the extemporaneous preparation of tailor-made (trans)dermal patches intended to treat IH and other similar cutaneous diseases (e.g., pyogenic granulomas). On the one hand, the use of patches instead of semi-solid preparations permits prolonging the resistance time of the formulation onto the damaged skin, other than to protect it from the environment. On the other hand, considering the high inter-patient variability of the IH pathophysiology, the printing technology allows compounding pharmacists to design the extemporaneous preparations based on the specific needs of patients (e.g., shape, size, strength). However, it is worth mentioning that the composition of the PSA has to be adjusted according to the possible effects of the loaded drug on the physical properties of the adhesive as well as the possible interactions occurring among the drug/s and functionality-related excipients. Indeed, the addition of a small molecule to the adhesive can affect both the printability and technological performance of the obtained patches. Furthermore, even if relatively low melting temperatures are used, the proposed method might not be feasible for thermosensitive drugs.

8 References

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Tables

Table 1. Composition (%) of placebo and drug-loaded patches used for screening the acceptable polymer/plasticizer ratio in terms of cold flow, and adhesive properties. For cold flow: N, the absence of cold flow; Y, the presence of cold flow.

Form	**Composition (%)**						**Cold flow**[1]	**Adhesive properties**			
	EuRL	*ATEC*	*TBC*	*TRI*	*PR-B*	*PR-Cl*		*Tack*		*Shear adhesion (min)*	*Peel adhesion (cN/cm)*
								σ_{max} *(kPa)*	*W (mJ)*		
1	60.0	-	40.0	-	-	-	N	5.9 ± 1.5	0.008 ± 0.001	1423 ± 157	12.8 ± 5.1
2	59.4	-	39.6	-	1.0	-	N	145.6 ± 69.3	0.049 ± 0.023	718 ± 91	15.3 ± 2.9
3	59.4	-	39.6	-	-	1.0	N	270.4 ± 68.1	0.092 ± 0.025	1100 ± 196	16.5 ± 6.8
4	60.0	40.0	-	-	-	-	N	223.5 ± 72.1	0.072 ± 0.032	> 24 h	20.8 ± 5.0
5	59.4	39.6	-	-	1.0	-	N	243.7 ± 96.2	0.093 ± 0.045	> 24 h	14.1 ± 5.7
6	59.4	39.6	-	-	-	1.0	N	313.0 ± 98.9	0.121 ± 0.032	1357 ± 370	13.2 ± 9.6
7	60.0	-	-	40.0	-	-	N	159.2 ± 81.8	0.053 ± 0.013	278 ± 667	12.5 ± 5.8
8	59.4	-	-	39.6	1.0	-	Y	241.5 ± 81.1	0.079 ± 0.034	48 ± 21	28.4 ± 14.3
9	59.4	-	-	39.6	-	1.0	Y	287.0 ± 66.4	0.098 ± 0.034	115 ± 57	23.9± 4.1

[1] RT, two weeks.

Table 2 Drug content, release rate constant (*K*), skin permeation flux (*J*), lag time, drug retained amount (Q_{ret}) of printed drug-loaded patches (Mean ± S.E.M.; n = 3; n.d.: not determined).

Form.	Drug content		*K*	*J*	Lag time	Q_{ret}	Q_{ret}/J
	(µg/mg)	*(µg/cm²)*	*($h^{-0.5}$)*	*(µg/cm²/h)*	*(h)*	*(µg/mg)*	
2	10.6 ± 0.5	68.5 ± 11.9	1.47 ± 0.22	2.42 ± 0.38	1.41 ± 0.26	0.32 ± 0.03	0.13
3	10.3 ± 0.6	74.7 ± 9.6	1.64 ± 0.13	0.28 ± 0.07	3.99 ± 0.18	0.37 ± 0.04	1.32
5	8.9 ± 0.2	83.3 ± 23.1	2.04 ± 0.57	3.54 ± 0.33	-	0.31 ± 0.02	0.09
6	9.7 ± 0.3	141.5 ± 17.0	-	0.13 ± 0.03	3.09 ± 0.91	0.15 ± 0.02	1.15
8	4.9 ± 0.2	41.1 ± 15.6	n.d.	n.d.	n.d.	n.d.	n.d.
9	9.1 ± 0.3	38.2 ± 11.5	n.d.	n.d.	n.d.	n.d.	n.d.

Figure captions

Figure 1. In vitro release profiles of PR-B (Forms. 2 and 5) and PR-Cl-loaded patches (Forms. 3 and 6) plasticized with TBC (Forms. 2 and 3) and ATEC (Forms. 5 and 6) (Mean ± S.E.M.; n =3).

Figure 2. In vitro permeation profiles of PR-B (Forms. 2 and 5) and PR-Cl-loaded patches (Forms. 3 and 6) plasticized with TBC (Forms. 2 and 3) and ATEC (Forms. 5 and 6) (Mean ± S.E.M.; n =3).