Release and Permeation Kinetics of Caffeine From Bioadhesive Transdermal Films

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ABSTRACT

The aim of this work was to investigate, in vitro, the kinetics of release and permeation of caffeine, chosen as model drug, from bioadhesive transdermal films. These films are not self-adhesive but become adhesive when applied to wet skin. Permeation experiments were performed from films with different drug loadings using rabbit ear skin as barrier. In order to characterize the release kinetics of caffeine from the film, a polyethylene membrane, impregnated with isopropyl myristate was employed. The data obtained in the present work suggest that caffeine release from transdermal bioadhesive films was controlled either by the permeability characteristics of the skin or by the film itself, depending on drug loading. When drug loading is low (ie, caffeine is dissolved in the polymers constituting the film), the control resides in the skin. When caffeine loading exceeds its solubility in the film, the permeation profile is not linear, but shows a sort of burst effect in the early times of permeation, probably owing to the presence of solid drug and/or to a certain degree of ''conserved supersaturation'' in the solid phase.

KEYWORDS: transdermal, caffeine, permeation kinetics, controlled release, skin

INTRODUCTION

The skin as a route for systemic drug administration has become very attractive since the introduction of transdermal therapeutic systems (TTS) in the form of patches. The significant implementation introduced by TTS, compared with traditional topical formulations, was the control of the area of application, of the amount applied, and of the release kinetics as well as the prolongation of the application time. Moreover, the administration of systemic drugs using a transdermal patch represents a noninvasive route, with improved patient compliance; additionally, this route of administration prevents passage through the gastrointestinal tract and maintains constant plasma levels for prolonged periods of time.

Among the different types of dermal and transdermal therapeutic systems, the drug-in-adhesive products (in which the drug is included in the adhesive in contact with the skin) are very commonly used, being thin, conformable, and comfortable. Transdermal patches are generally occlusive (ie, they do not allow water to be released from the skin surface), and this is often the reason for skin irritation.[1]

We have recently described a water-based and vapor-permeable bioadhesive film for transdermal delivery.[2-4] The film is not adhesive when dry and, therefore, the skin has to be wetted before film application. The film has been shown to be able to release high percentages of the active included.

The aim of this study was to investigate in vitro the kinetics of release and the permeation of caffeine from bioadhesive films. Caffeine was chosen as the model drug for its therapeutic[5-6] and cosmetic applications.[7] Caffeine is commonly used topically in cosmetics for its stimulating activity on fat metabolism and, in therapy, for the treatment of headache and neonatal apnea. The half-life of caffeine has been reported to be 4 to 5 hours in humans, but it increases significantly in early infancy.[8] In premature neonates, caffeine is usually administered by intravenous infusion, since the plasma concentration should be fairly constant to avoid toxic manifestations such as hypertonia, tachycardia, cardiac failure, and so on.[9] Thus, transdermal administration could be considered as an interesting alternative, since it allows constant plasma levels to be maintained for prolonged periods of time.

Permeation experiments were performed from 2 films with different drug loading, using rabbit ear skin as barrier. In order to characterize the release kinetics of caffeine from the film, a polyethylene membrane impregnated with isopropyl myristate was employed.

MATERIALS AND METHODS

Materials

Caffeine (MW = 194.19) was a gift from Merck (Darmstadt, Germany). Eudragit E100 used for the preparation of the adhesive Plastoid E35H was purchased from Rofarma (Gaggiano, Milan, Italy) and polyvinyl alcohol 49 000 (PVA) (degree of polymerization, 1000; degree of hy-

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drolysis, 86-89 mol%) was purchased from Fluka (Fluka Chemical, Buches, Switzerland). Isopropyl myristate (IPM) was obtained from ACEF (Fiorenzuola d'Arda, Italy). All chemicals used were of analytical grade.

For high-performance liquid chromatography (HPLC) analysis, acetonitrile (HPLC grade) and distilled water were used.

The synthetic membrane used for the permeation experiments was a microporous polyethylene film (n° 9711 CoTran Membrane; caliper, 51 μm; void volume, 78%; bubble point, 0.18 μm) and was a gift of 3M (St Paul, MN).

Plastoid E35H was prepared according to the protocol of Rofarma: Eudragit E100 (15.9% wt/wt), lauric acid (9.2% wt/wt), and adipic acid (1.8% wt/wt) were added to hot water (72.1% wt/wt), (temperature, ∽80°C). The mixture was stirred, maintaining the temperature at ∽80°C, until a clear solution was formed. The solution was cooled to 60°C and glycerol (1.0% wt/wt) was added. The mixture was then gradually cooled to room temperature while stirring.

Methods

Film Preparation

The transdermal films were prepared as described in Nicoli et al.[4] Briefly, caffeine (740 mg or 145 mg) and PVA (9.3 g) were dissolved in hot water (21.7 mL). This solution was mixed with 13.5 g of Plastoid E35H and 2 g of sorbitol (70% solution). The resulting mixture was spread on siliconized paper (Rexam Release, Apeldoom, The Netherlands), using a film-casting knife (BYK Gardner, Silver Spring, MD) with a gap of 300 μm, and then oven dried at 80°C for 30 minutes.

Film Characterization

The film was characterized for weight, thickness, caffeine content, and water content according to Nicoli et al.[4]

To assess the presence of crystals of caffeine, the film was observed with a polarized light microscope (Labophot II Nikon, Tokyo, Japan) and a color video camera (XC-003P Sony, Tokyo, Japan).

The permeability to water vapor was determined in vivo, using the transepidermal water loss (TEWL) measurement. The film was applied on the skin, previously wetted with ∽15 μL/cm^2 of water, of 5 volunteers, and TEWL was measured using a Tewameter 210 (Courage, Koln, Germany) over 24 hours.

Caffeine Analysis

Caffeine analysis was performed using a validated HPLC method,[4] with a Perkin Elmer instrument (Norwalk, CT) and a Novapak C18 column (Waters, Milford, MA). The mobile phase was a mixture of water and acetonitrile (90:10 vol:vol) at 0.9 mL/min. The UV detector was set at 276 nm.

Permeation Experiments

Permeation experiments were conducted in Franz-type diffusion cells (Disa, Milan, Italy), with an exposed surface area of 3.9 cm^2 (when patches were used as donor) or 0.6 cm^2 (when solution was used as donor). Preliminary experiments have shown that the geometry of the cell does not modify the results obtained. Rabbit ear skin or a microporous polyethylene film impregnated in IPM were used as barrier. Rabbit skin was excised postmortem from the inner part of rabbit (6 months old) ears obtained from a local slaughterhouse. If not used immediately, the skin was kept refrigerated (2°C-5°C) and used within 3 days.

The microporous synthetic membrane was soaked overnight in IPM at 37°C and stored in aluminum foil at room temperature until use.

The donor compartment was filled with 1 mL of a saturated solution of caffeine in water at room temperature (18 mg/mL) or with 1 film containing caffeine (area 3.9 cm^2). Since the film is adhesive only in the presence of water, the exposed skin or IMP-impregnated membrane area was wetted with a measured volume of water (15 μL/cm^2) before application. During the course of the experiment, the donor compartment was left open, to simulate real use conditions. Ambient relative humidity was 46% to 50%.

The receptor phase was 0.9% NaCl solution thermostated at 37°C and magnetically stirred in order to prevent any boundary layer effects. At predetermined time intervals the receptor solution was sampled and analyzed by HPLC for the determination of caffeine permeation.

Each permeation experiment was replicated at least 4 times.

Determination of IPM/Water Partition Coefficient

IPM and water were preventively mutually saturated overnight. Two milliliters of an IPM-saturated water solution of caffeine (20 μg/mL) and 8 mL of water-saturated IPM were equilibrated in sealed centrifuge tubes. The tube content was shaken magnetically at room temperature (21°C-22°C) for 1 hour (this time interval was enough to guarantee the equilibrium, as demonstrated by preliminary experiments). After separation of the 2 phases, drug concentration in the aqueous phase was determined by HPLC. Drug concentration in the organic phase was calculated as the difference between initial and final concentration of caffeine in the aqueous phase. The partition coefficient

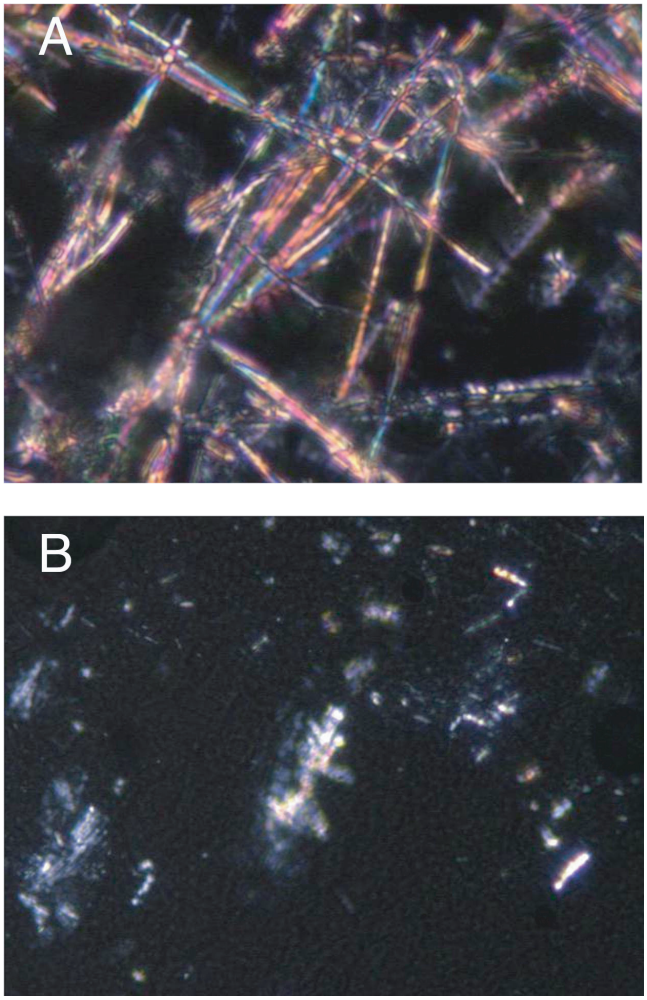

Figure 1. (A) Image of 5.5% caffeine film under polarized light microscope; (B) the same film observed after contact with water.

was calculated as the ratio of IPM and water concentration of caffeine at equilibrium.

Determination of Caffeine Solubility in IPM

An excess amount of caffeine was added to 2 mL of IPM and incubated over 24 hours at both room temperature and 37°C. After centrifugation (10 minutes, 1500 rpm) and dilution (1:100) with ethanol, the concentration of the drug in the supernatant was determined by UV analysis at 273 nm.

RESULTS

Film Characterization

Two films, containing different amounts of caffeine, were prepared. Both weighed ∽6 mg/cm^2 and had an average thickness of ∽65 μm. The water content, measured with the Karl Fisher technique, resulted ∽3.5% (wt/wt) for both films.

The 2 films differentiated for caffeine content, which was 0.89% ± 0.04% and 5.54% ± 0.24% (wt/wt) corresponding to 54.8 ± 1.2 μg and 334.4 ± 2.6 μg of caffeine per cm^2, respectively. The 2 films will be coded as 0.9% and 5.5% film throughout the article, referring to the percentage (wt/wt) of caffeine contained in the formulations.

The film containing the 5.5% of caffeine was white in color; the observation of the film under a polarized light microscope showed long caffeine crystals (Figure 1A). No crystals were present in the 0.9% film.

Figure 2 shows 1 representative profile of the time course of TEWL over 24 hours of film wearing, compared with the control site. It can be observed that after the application of the film, TEWL increased suddenly and after approximately 30 minutes, the value decreased to reach the control value and remained similar to the control up to 24 hours.

Permeation Across IPM-impregnated Polyethylene Membrane

In order to characterize the system, IPM/water partition coefficient as well as caffeine solubility in the organic solvent were measured. The equilibrium solubility of caffeine in IPM at 37°C result was 0.99 ± 0.07 mg/mL. The IPM/water partition coefficient of caffeine at room temperature result was 0.076, lower than the value (0.95) reported for octanol.[10]

Initially, the permeation of caffeine across the polyethylene membrane impregnated with IPM was studied from a saturated solution of caffeine in water (concentration 18 mg/mL). The permeation curve is shown in Figure 3. From the permeation profiles, caffeine flux was calculated as the slope of the linear regression line in the time interval

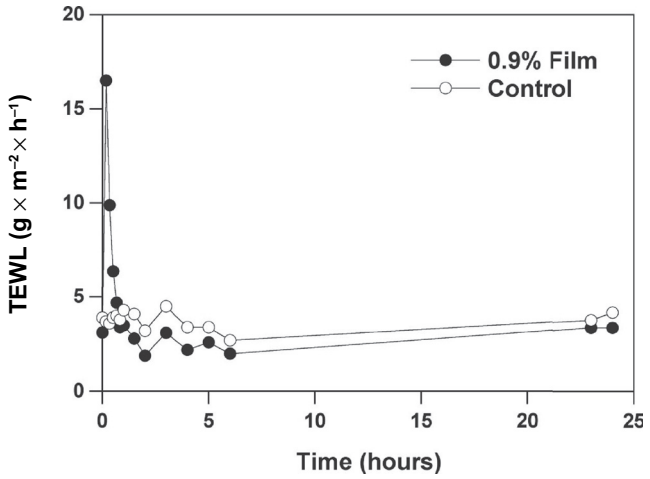

Figure 2. Transepidermal water loss measured, on 1 volunteer, across the bioadhesive film applied on the skin surface compared with the control site.

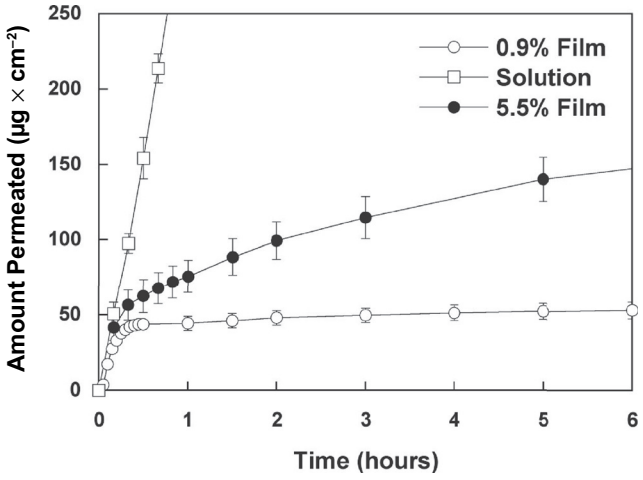

Figure 3. Permeation profiles of caffeine through an artificial membrane impregnated in IPM starting from a saturated water solution, the 0.9% film, and the 5.5% film. Mean values ± SEM.

1 to 8 hours. The permeability coefficient (P) was calculated using the following relationship:

$$J = P\ C_d, \qquad (1)$$

where J is caffeine steady-state flux across the IPM-impregnated membrane, and C_d is drug donor concentration. The permeation parameters of caffeine across the IPM-impregnated membrane are reported in Table 1.

Figure 3 also illustrates the comparison between the profiles obtained across the artificial membrane from the 0.9% and 5.5% films. When the 0.9% film was applied to the IPM-impregnated membrane, the permeation of the drug was very fast, and, after 1 hour, more than 80% of the applied dose (54.8 μg/cm^2) had permeated the membrane. When the 5.5% film was applied to the impregnated membrane, the profile showed again a burst delivery, but only 20% of loaded caffeine (334.4 μg/cm^2) crossed the skin after the first hour.

Permeation Across Rabbit Ear Skin

The transdermal permeation of caffeine through rabbit ear skin was measured starting from 0.9% and 5.5% caffeine films. The profiles are shown in Figure 4.

The profile obtained using the 0.9% film was almost linear with time, with no detectable time lag. The percentage permeated after 7 hours was 15%.

When the 5.5% film was applied on wetted skin, the caffeine profile did not show the typical time lag of transdermal patches but, on the contrary, revealed the presence of a burst effect in the first 3 hours. Then, the permeation rate of caffeine tended to decrease over time. Moreover, the percentage of caffeine permeated reached 50% of the applied dose after 24 hours, a value unusually high for transdermal patches.

DISCUSSION

It has been previously shown[3] that the bioadhesive film reported in this paper is permeable to water vapor, as assessed by in vitro measurements. The in vivo permeability to water was assessed by measuring the TEWL of the skin upon application of the 0.9% film on the skin surface. After application of the film on the skin surface, TEWL increased suddenly due to the evaporation of water used for film application (Figure 2). After approximately 30 minutes, the value decreased to reach the control value and remained similar to the control up to 24 hours.

The film described in this article is quickly dissolved by water and, for this reason, it is impossible to study the release of caffeine using the classical release experiments such as the US Pharmacopoeia tests. The only possibility is to interpose a membrane between the film and the receptor solution. Porous membranes, such as dialysis membranes, proved to be not useful since water could move backward from the receptor solution toward the donor compartment. A porous polyethylene membrane was then impregnated

Table 1. Permeation Parameters of Caffeine From Aqueous Solution Across Different Types of Membranes*

	Donor Solution	J (μg × cm^{-2} × h^{-1})	Time Lag (hours)	P (cm × h^{-1})
Rabbit skin‡	Saturated	3.53 ± 0.32	1.36	1.96 10^{-4}
IPM-impregnated polyethylene membrane§	Saturated	226.40 ± 4.02	0.02	1.87 10^{-2}
IPM-soaked silicone membrane‖	Saturated	57.60 ± 1.10†	1.63	2.58 10^{-4}
Human epidermis‖	Saturated	6.54 ± 0.16†	1.87	2.23 10^{-4}
Hairless mouse skin¶	10 mg/mL	2.60 ± 0.40	0.56	2.60 10^{-4}

*All values are mean ± SEM.
†Standard deviation.
‡Data from Nicoli et al.[4]
§Data from this study.
‖Data from Dias et al.[11]
¶Data from Monti et al.[10]

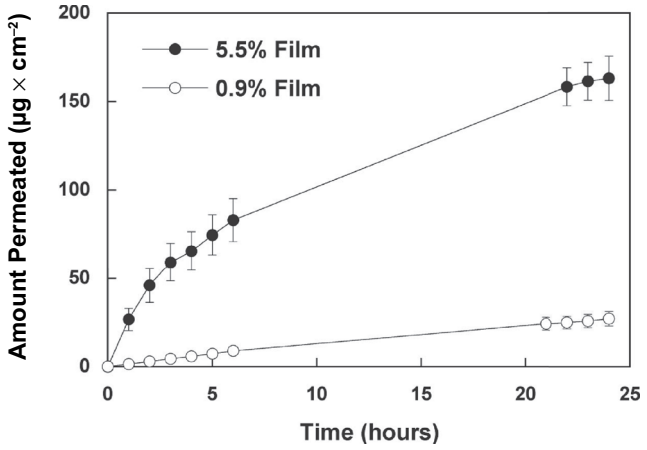

Figure 4. Permeation profiles of caffeine through rabbit ear skin starting from the 0.9% film and from 5.5% film. Mean values ± SEM.

with IPM, reported[11] to be more similar to skin lipids than octanol, and used as barrier for release experiments.

Preliminarily, caffeine permeation parameters across IPM-impregnated membrane were calculated from diffusion experiments starting from a saturated solution of the molecule in water (Figure 3). In agreement with the data obtained across cellulose acetate membrane impregnated with IPM,[11] the permeation of caffeine through IPM-impregnated polyethylene membrane was very rapid, and no time lag was detectable. The flux and time lag obtained (see Table 1) reflect the rapid permeation, when compared with the values achieved using rabbit ear skin.[4] In fact, the permeability coefficient across rabbit ear skin was 2 orders of magnitude lower than through the IPM-impregnated artificial membrane. On the contrary, Dias et al[11] reported that the permeability coefficient of caffeine across silicone membranes presoaked in IPM was of the same order of magnitude as across rabbit, human,[11] and mouse skin[10] (Table 1). Therefore, the IPM-impregnated polyethylene membrane does not represent a barrier for caffeine permeation and can be used to characterize the release of caffeine from the films. Two films containing 0.9% and 5.5% wt/wt of caffeine, respectively, were studied. When the 0.9% film was used (Figure 3), caffeine permeated the membrane very quickly, and 80% of the drug present in the film was released within 1 hour. The use of the 5.5% film led to a rapid permeation in the early times followed by a sustained nonlinear release; the percentage permeated reached 40% of caffeine loaded after 6 hours. If the permeation profiles from the 2 films are compared with that obtained from the solution, it appears that the amount of caffeine permeated after 10 minutes is comparable for the 3 formulations. Thereafter, the profile from solution continues growing in a linear way owing to the infinite-dose conditions of the donor (1 mL of a saturated solution 18 mg/mL), while the profiles from the films tend to level off. The permeation profile of the low-loading film (0.9%) can be easily explained considering drug depletion from the film since, as already mentioned, 80% of the drug permeates the membrane in 1 hour. However, in the case of 5.5% film, depletion alone cannot explain the profile. The presence of crystals of caffeine in the highly loaded film can play a role. However, since the film is applied in the presence of water, that can dissolve the solid drug, it is difficult to estimate the real importance of the presence of solid caffeine. Microscopic observation of the film placed on a glass slide wetted with water demonstrated that caffeine crystals present on the surface of the film rapidly dissolved and disappeared almost completely (Figure 1). Another possibility is that the dry film contains a supersaturated solution of caffeine in the constitutive polymers as the result of the manufacturing procedure (ie, heating of the mixture to 80°C during the drying step). This process could lead to a degree of ''conserved supersaturation'' in the solid phase, which explains the rapid permeation of caffeine in the early times.

The comparison of the permeation profiles across the 2 kinds of membranes allows for further considerations on the kinetics. The low-loading film shows, across the skin, an almost linear profile, which is comparable to the profile obtained by Nicoli et al[4] from a saturated solution of caffeine in water, at least in the first 7 hours of permeation. On the contrary, when the artificial membrane was used, all caffeine present permeated very quickly, since the membrane itself is highly permeable to caffeine.

The 5.5% film shows the same permeation profile across the skin and the IPM-impregnated artificial membrane, although the permeability of the 2 barriers toward caffeine is significantly different. In fact, the permeability coeffi-

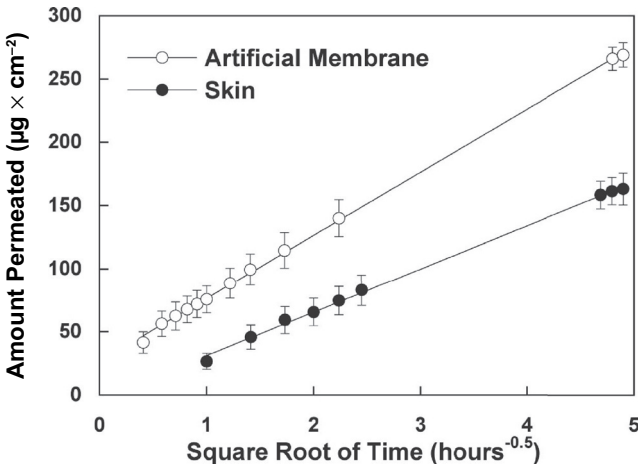

Figure 5. Amount of caffeine permeated from the 5.5% film through rabbit ear skin and through an IPM-impregnated membrane as a function of the square root of time.

cient of caffeine from saturated solution was 1.96×10^{-4} and 1.87×10^{-2} cm $\times$ h^{-1} across rabbit skin[4] and IPM-impregnated membrane, respectively. Despite the 2 orders of magnitude difference in permeability, the amount permeated across the artificial membrane was only 40% higher than through the skin.

When the amount of caffeine permeated from the 5.5% film was plotted as a function of the square root of time, the profiles, illustrated in Figure 5, were linear for both rabbit skin and for the artificial membrane. This finding suggests a matrix-type control of caffeine permeation. Additionally, the slopes of the regression lines (which give an indication of the permeation rate) were not statistically different ($P > .05$), being 34.28 ± 0.67 $\mu g \times cm^{-2} \times h^{-0.5}$ for rabbit skin and 48.72 ± 4.81 $\mu g \times cm^{-2} \times h^{-0.5}$ for the IPM-impregnated membrane, despite the 2 orders of magnitude difference in barrier permeability (Table 1). This result suggests that the film plays a role in controlling the permeation of caffeine, and in particular that drug release from the film was slower than permeation through both types of membranes. However, a diffusion control by the film is not likely to be the reason, since the 0.9% showed different kinetics across the 2 different barriers, with different permeability. Three hypotheses can be formulated: (1) the diffusion coefficient of caffeine within the film is concentration dependent, being lower for the more concentrated film; (2) the 5.5% film contains a supersaturated solution of caffeine in the solid state; or (3) the control mechanism is not diffusion but dissolution of solid caffeine crystals present in the 5.5% film (Figure 1). The last 2 hypotheses seem both to be acceptable, since they could also justify the burst effect shown by the 5.5% film across both types of membranes. In fact, the film, which may contain a supersaturated solution of caffeine, is applied on the skin or IPM-impregnated membrane surface in the presence of water, which can partly dissolve caffeine crystals present in the film, leading to the formation/maintenance of a supersaturated solution of caffeine, which sustains the permeation of the drug in the early times. The subsequent decrease in the permeation rate observed can be attributed to the decreased thermodynamic activity of the drug.

CONCLUSION

The data presented here support the potential application of the bioadhesive film described as a new drug delivery system for transdermal application. The film is nonocclusive on the skin and shows unusual permeation kinetics. In particular, caffeine release can be controlled either by the permeability characteristics of the skin or by the film itself, depending on drug loading. When drug loading is low (ie, caffeine is dissolved in the polymers constituting the film), the control resides in the skin. When caffeine loading exceeds its solubility in the film, the permeation profile is not linear but shows a sort of burst effect in the early times of permeation owing to the presence of solid drug and/or to a certain degree of ''conserved supersaturation'' in the solid phase. This hypothesis is supported by the similarity of the delivery rate obtained across IPM-impregnated polyethylene film and rabbit skin.

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REFERENCES

1. Zhai H, Maibach HI. Occlusion vs skin barrier function. *Skin Res Technol.* 2002;8:1-6.

2. Colombo P, Catellani PL, Padula C, Santi P, Colombo G. Film for Dermal and Transdermal Administration of Drugs.WO 02/30402. April 18, 2002.

3. Padula C, Colombo G, Nicoli S, Catellani PL, Massimo G, Santi P. Bioadhesive film for the transdermal delivery of lidocaine: in vitro and in vivo behavior. *J Control Release.* 2003;88:277-285.

4. Nicoli S, Amoretti V, Colombo P, Santi P. Bioadhesive transdermal film containing caffeine. *Skin Pharmacol Appl Skin Physiol.* 2004;17:119-123.

5. Amato M, Isenschmid M, Huppi P. Percutaneous caffeine application in the treatment of neonatal apnea. *Eur J Pediatr.* 1991;150:592-594.

6. Conney AH, Lu YP, Lou YR, Huang MT. Inhibitory effects of tea and caffeine on UV-induced carcinogenesis: relationship to enhanced apoptosis and decreased tissue fat. *Eur J Cancer Prev.* 2002;11:S28-S36.

7. Bertin C, Zunino H, Pittet JC, et al. A double-blind evaluation of the activity of an anti-cellulite product containing retinol, caffeine, and ruscogenine by a combination of several non-invasive methods. *J Cosmet Sci.* 2001;52:199-210.

8. Mandel HG. Update on caffeine consumption, disposition and action. *Food Chem Toxicol.* 2002;40:1231-1234.

9. Anderson BJ, Gunn TR, Holford NH, Johnson R. Caffeine overdose in a premature infant: clinical course and pharmacokinetics. *Anaesth Intensive Care.* 1999;27:307-311.

10. Monti D, Giannelli R, Chetoni P, Burgalassi S. Comparison of the effect of ultrasound and of chemical enhancers on transdermal permeation of caffeine and morphine through hairless mouse skin in vitro. *Int J Pharm.* 2001;229:131-137.

11. Dias M, Farinha A, Faustino E, Hadgraft J, Pais J, Toscano C. Topical delivery of caffeine from some commercial formulations. *Int J Pharm.* 1999;182:41-47.