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Formulation, characterization, and efficacy of an adenosine-containing dissolvable film for a localized anti-wrinkle effect

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Synopsis

A water-dissolvable film was developed to topically deliver adenosine for a localized anti-wrinkle effect. The polymers used to produce the film were cellulose derivatives. An aqueous mixture of the film components was made, coated on a liner, and then dried to form a solid film. No preservatives were added and the film was shown to be stable over time. The film quickly dissolves in water to form a uniform layer at the surface of the skin, as shown by scanning electron microscopy. The film layer can still be visualized on the wrinkle six hours after being applied on the skin. A randomized, placebo-controlled, investigator-blind study was conducted in female volunteers to assess the efficacy of the 1% adenosine-containing dissolvable film. After three weeks and eight weeks, a twice daily application led to a significant decrease in the skin roughness parameters as observed using fast optical *in vivo* topometry (FOITS). These results demonstrate that water-dissolvable films may be used as novel, preservative-free, cosmetic delivery systems.

INTRODUCTION

Dissolvable, edible films have been used for years in the food industry as carriers for flavor or nutritional additives or as barriers for separation, protection, and preservation purposes (1). Usually, edible films are made of highly water-soluble polymers, such as polysaccharides or proteins (2) as well as film-forming materials such as lipids and resins or combinations of these (2,3). Recently, edible strips for breath freshening have been very successfully introduced on the market and are becoming increasingly popular. Indeed, dissolvable films can easily be carried everywhere by the consumer and represent a new type of user-friendly, single-dose formulation to administer various ingredients, ranging from food additives to over-the-counter drugs.

We investigated solid, water-soluble films to deliver cosmetic ingredients to the skin. The goal of the present study was to formulate a film that can easily be dissolved with water on the skin, dries quickly, and leaves a continuous film on the skin surface to deliver adenosine, an active anti-wrinkle cosmetic ingredient.

MATERIALS AND METHODS

FILM FORMULATION AND MANUFACTURING

Materials. The films were composed of hydroxypropylmethylcellulose (HPMC; Metolose 60 SH 50, USP, Syntapharm) and hydroxypropylcellulose (HPC, Klucel EF, pharma grade, Hercules). Glycerol (85%, pharma grade, Brenntag) was added as the plasticizer. Panthenol (D-Panthenol, USP, Roche), adenosine (Pharma Waldhof), and magnesium sulfate (Mallinckrodt Chemicals) were used as cosmetic active ingredients. The release liner consisting of polyethylene terephthalate (PET, Siphan) was obtained from Siliconature, Italy.

Preparation of the film. HPMC was dissolved in distilled water to produce a 12.5% w/w stock solution. HPC was dissolved in distilled water to produce a 25% w/w stock solution. For each batch (of 150 g), 83.6 g of the HPMC stock solution and 20.6 g of the HPC stock solution were mixed and stirred at 30 rpm using a propeller stirrer to avoid air bubble inclusion. Then, 10.9 g of glycerol, 0.3 g of adenosine, 5.8 g of panthenol, and 0.2 g of magnesium sulfate were added. Finally, 28.6 g of distilled water was poured into the mixture, which was then stirred for 30 min under the same conditions. The resulting mixture was spread on a siliconized PET release liner using a film casting knife with a gap of 250 μm, and then oven dried at 70°C for 45 min. The final film had an area weight of 50 g/m^2. Individual squares of 1.5 × 1.5 cm were punched out of the film and packed in aluminum/polyethylene pouches.

FILM CHARACTERIZATION

The dissolution of the film in water was assessed by the following method. The film (1.5 cm × 1.5 cm) was clamped with tweezers and immersed in 10 ml of water under slow stirring (20 rpm) at room temperature. The time after which no more film residue could be visually detected was recorded.

The total amount of residual water contained in the film was measured by Karl Fischer titration. The water activity, which measures the vapor pressure generated by the moisture present in the film, was determined using a Hygromer® apparatus (Rotronic, USA). The moisture sorption/desorption kinetics at 25°C was obtained using a dynamic vapor sorption apparatus comprising an ultra-sensitive microbalance (Surface Measurement Systems, UK).

The films, placed on a polyethylene terephthalate release liner and packaged in aluminum/polyethylene pouches, were also stored at 45°C for one month. The weight, water content, and adenosine concentration of the films were measured and compared to values at initial time.

VISUALIZATION OF THE DISSOLVABLE FILM ON THE SKIN

The area around the corner of the eye (crow's feet) of volunteers was moistened with water, and the film was subsequently applied on the skin wrinkles. The skin surface covered by the film (2.25 cm^2) was approximately 20 to 25 cm^2, representing about 0.5 mg of material, i.e., 5 μg of adenosine per cm^2 of skin.

The visualization of the film was performed by scanning electron microscopy (SEM) as follows:

Skin Siflo replicas were taken after one and six hours of drying time and compared with those taken before application on the same person. These negative replicas (See Figure 1, right panel) were transformed to positive ones using an epoxy resin, Araldit© 2011 (Vantico, Fr). These new imprints were then glued to a copper cylindrical sample holder using a silver-based varnish and eventually coated with a thin film of conducting platinum using an HTS 010 sputter coater (Balzers, Fr). Finally, all the observations were done in a field-emission high-resolution JEOL 6300F microscope.

EVALUATION OF ANTI-WRINKLE EFFICACY IN FEMALE VOLUNTEERS

A randomized, placebo-controlled, investigator-blind study was conducted to assess the efficacy of the dissolvable film. One hundred and twenty-six female volunteers, 45–65 years old, were enrolled with distinct wrinkles at least 2 cm long on the right and left crow's feet. The subject applied either the 1% adenosine-containing dissolvable film, a 0.1% adenosine-containing cream, or a placebo cream according to randomization, twice a day on the crow's feet areas, for eight weeks. To apply the film, the volunteer first moistened the treated area with water and then dissolved the film by gentle circular massage. Fast optical *in vivo* topometry (FOITS) measurements (4,5) were made three times for the evaluation of wrinkles: before the first application (baseline), and after three and eight weeks of the application 12 hours after the last application. FOITS classical analysis was used to record the two roughness parameters, Ra (average roughness) and Rz (average of the heights measured between the peaks and troughs of each relief feature), as previously described (4). Ra and Rz were determined on 50 parallel lines perpendicular to the axis of the main wrinkle and starting from the corner of the eye (for periorbital lines), two lines being separated by 250 μm.

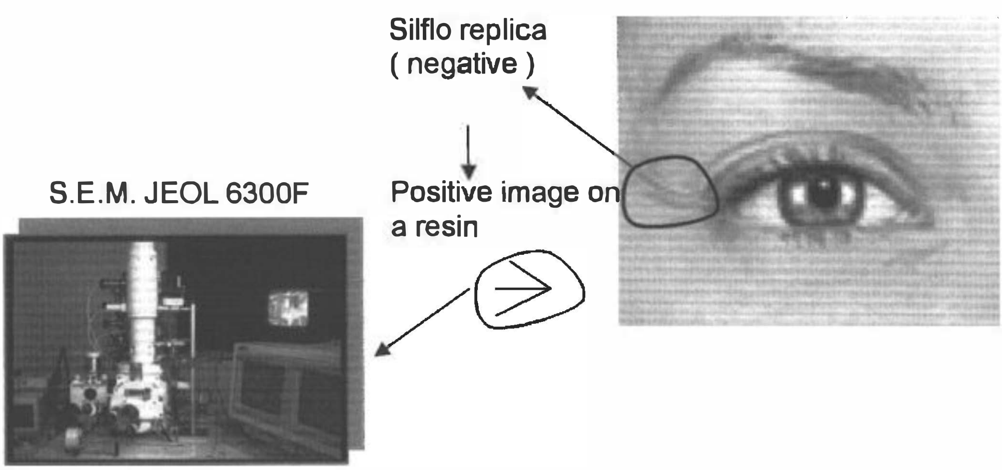

Figure 1. Methodology used for the scanning electron microscopy sample preparation.

RESULTS

FILM CHARACTERIZATION AND STABILITY

In vitro dissolution of the film in water. The time needed to fully dissolve the film ranged from 10 seconds to 15 seconds. In comparison, under the same experimental setting, the dissolution of a commercial pullulan-based breath-freshening strip (Cool Mint Listerine PocketPaks™, Warner-Lambert Consumer Healthcare) took between 35 and 40 seconds.

Water content and uptake. As the films are intended to quickly dissolve in water, their sensitivity to moisture and water uptake needs to be carefully assessed.

Figure 2 shows the moisture sorption/desorption kinetics of the films at 25°C. As relative humidity (RH) increases, the uptake of moisture increases the film weight. At 80% RH, a weight increase of approximately 50% was measured. However, no hysteresis was observed, i.e., the film weight decreased according to the same kinetics when the RH was brought down. This result indicates that moisture uptake at high RH does not induce any structural modification of the film or lead to any significant chemical degradation.

Table I shows the weight, water content, and activity variations after one month of storage at 45°C. At initial time, the water content and activity of the films were both very low, showing that the drying conditions used during the manufacturing process was efficient. After one month at 45°C, a slight, although non-significant, variation in weight was observed, while the overall water content and activity remained at initial values.

Adenosine content over time. As shown in Table I, the adenosine content per film after one month at 45°C was equal to 96% of the value at initial time.

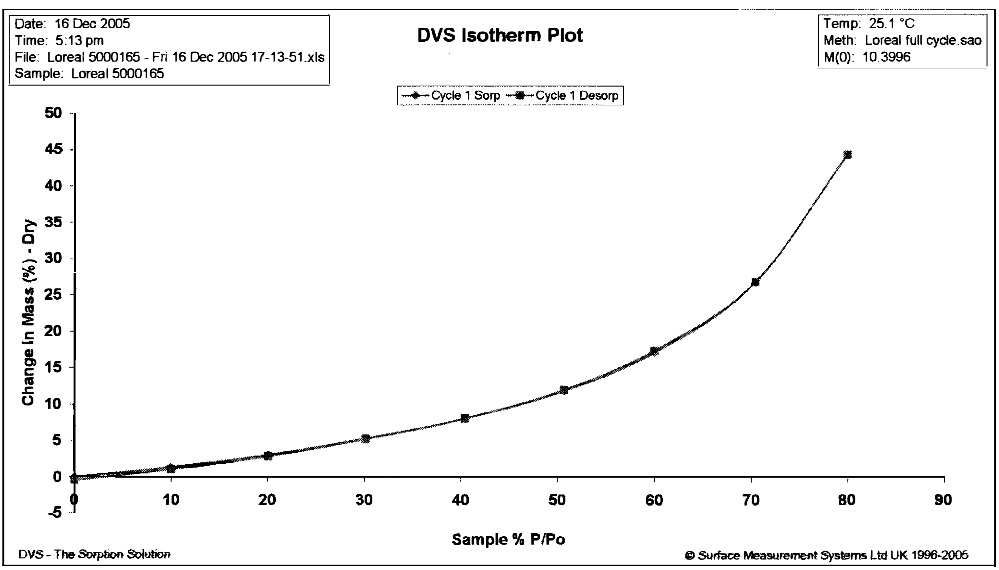

Figure 2. Moisture sorption/desorption kinetics of the dissolvable film from 0% RH to 80% RH.

Table I
Stability of the Dissolvable Adenosine-Containing Film Over Time

	Film weight (mg) (n = 20)	Water content (% w/w) (n = 15)	Water activity (n = 3)	Adenosine content (mg/film) (n = 5)
Initial time (T0)	10.6 ± 0.5	<10%	<0.35	0.104 ± 0.001
After 1 month's storage at 45°C	11.3 ± 0.8	<10%	<0.35	0.100 ± 0.001

VISUALIZATION OF FILM DEPOSITION ON THE SKIN

Scanning electron microscopy (SEM) showed that one hour after dissolution with water on the skin, the film formed a continuous and homogeneous layer fully covering the wrinkle area onto which it was applied (Figure 3B). After six hours, the film dried and the layer became partially discontinued as some solid pieces of the film detached. However, large skin surfaces were still covered with the film layer six hours after application (Figure 3C).

EVALUATION OF ANTI-WRINKLE EFFICACY IN FEMALE VOLUNTEERS

As measured by FOITS, both the Ra and Rz skin roughness parameters decreased following the application of the 1% adenosine-containing film. The reduction in roughness was evidenced as early as three weeks after starting product application and reached a level of −4.5% and −5.3% for Rz and Ra, respectively (Table II). At three weeks, the reduction of the skin roughness parameters obtained with the dissolvable film was significantly higher than that induced by the placebo cream. An improvement in the facial relief in the periorbital region was still observed at eight weeks (Table II).

DISCUSSION

The present study investigated the formulation and the efficacy of a thin, water-soluble film to deliver a cosmetic active ingredient, adenosine, onto the skin. Preformulation data showed that low-molecular-weight cellulose derivatives, e.g., hydroxypropylcellulose or hydroxypropylmethylcellulose were suitable polymers to formulate such films. Indeed, other polymers widely used to formulate oral strips, such as pullulan, are less suitable for skin application. For example, upon drying, pullulan becomes very sticky, leading to unpleasant cosmetic effects (data not shown).

The film we developed quickly dissolves in water and can be spread on the skin within seconds when applied with a drop of water. It also adsorbs moisture to a large extent when exposed to high RH. Water uptake of cellulose-based films was found to increase sharply in a high-water-activity environment (6). It is also hypothesized that the high level of glycerin present in the formulation (30%) strongly increases the water uptake of the film. It was previously reported that adding 30% glycerin in hydroxypropylcellulose films dramatically increases water permeability and film elongation (7). However, in a suitable packaging, the film appears stable over time and no degradation of the active ingredient adenosine is detected. The film is a substantially dry dosage form and hence

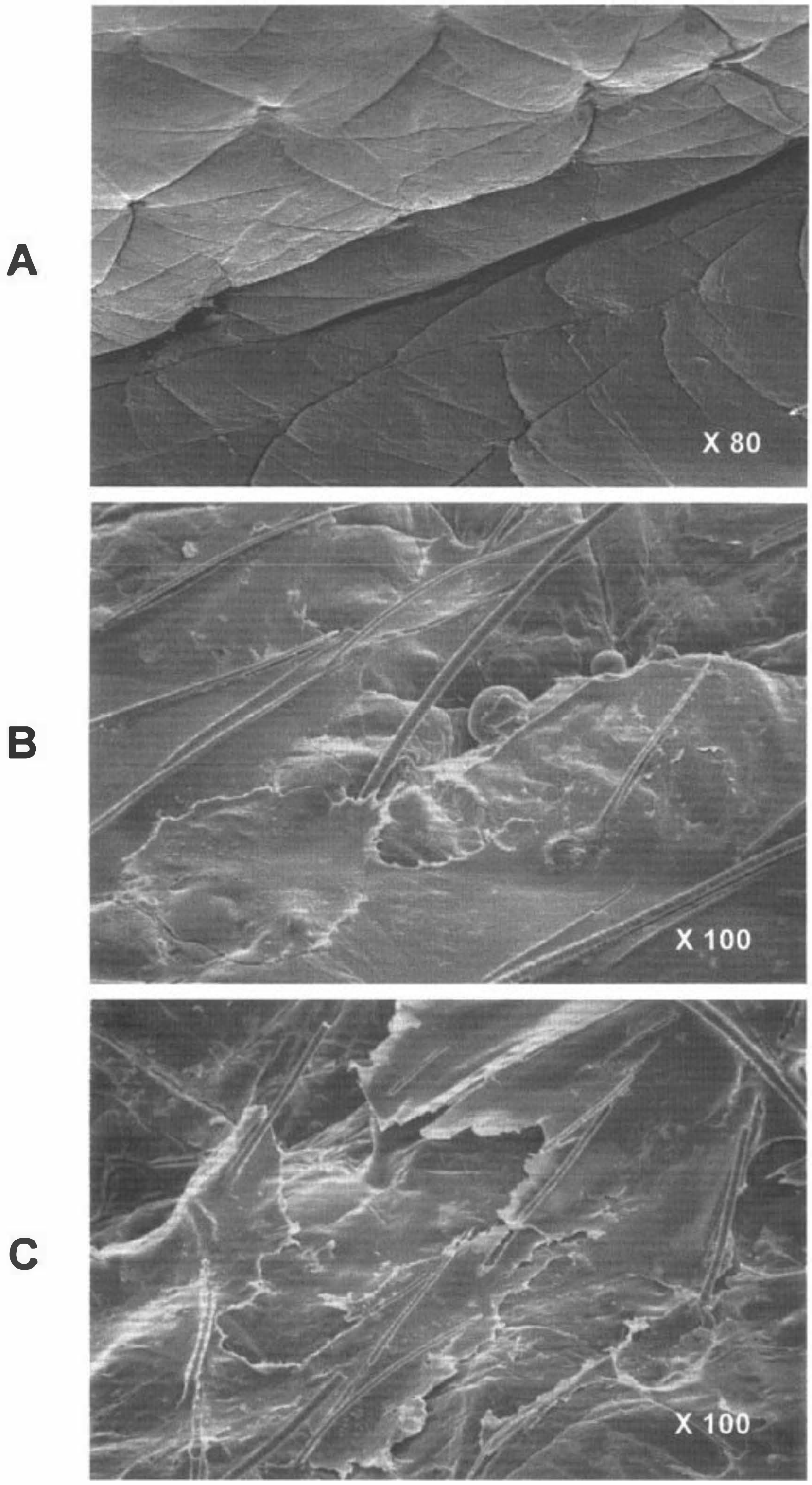

Figure 3. Scanning electron microscopy micrographs of the skin surface (A) before application, (B) one hour after application, and (C) six hours after application.

Table II
FOITS Analysis of Skin Roughness: Mean Values of the Rz and Ra Parameters After 3 and 8 Weeks of Application

Baseline (μm)	After 3 weeks (μm)	Difference (μm)	Significance vs baseline	Significance vs placebo	After 8 weeks (μm)	Difference (μm)	Significance vs baseline	Significance vs placebo
				Rz				
139.4	133.5	−5.9 (−4.5%)	S	S	137.9	−1.5 (−1.0%)	NS	S
				Ra				
24.0	22.8	−1.2 (−5.3%)	S	S	23.2	−0.8 (−3.0%)	S	S

n = 84.
S: significance, $p \leq 0.05$.
NS: no significance, $p > 0.05$.

its water activity is very low. These features allow the film to limit bacterial growth and to be preservative-free.

As shown by SEM, after dissolution on its site of action, the film forms a continuous layer on the skin for at least one hour. Even after six hours, large film pieces are still visible, indicating that the film may serve as a sustained-release dosage form of cosmetic active ingredients.

Adenosine is a rather hydrophilic molecule (clog P = −2.1) which could easily be incorporated into the film formulation at a level of 1%. Precise and localized application of the film on the wrinkle area enables a significant level of adenosine, i.e., approximately 5 $\mu g/cm^2$ to be delivered in the area to be treated.

FOITS measurements in the periorbital region of women having received both adenosine-containing formulations (cream or film) indicated that a significant anti-wrinkle effect was achieved over the placebo cream. The effect of a cream containing 0.1% of adenosine was previously reported (8). The present data demonstrate that a very simple adenosine-containing product, i.e., the dissolvable film, can also significantly reduce wrinkles. Such a result supports the hypothesis that adenosine can act as an anti-wrinkle agent, as previously suggested (9).

Some authors previously reported the use of self-adhesive films to deliver drugs topically (10). Similarly, S. Nicoli *et al.* (11) developed a bioadhesive film to enhance the transdermal penetration of caffeine. Although not designed to adhere on the skin, the film we developed is slightly tacky when exposed to low moisture, which may contribute to enhancing the delivery of adenosine onto the skin.

A direct physical effect of the film cannot also be ruled out. Indeed, SEM showed that once dissolved with water the film lies in the wrinkle depth for a period of a few hours. A slight tensile effect of the polysaccharide-type polymers that serve as the basic film composition, as well as the presence of glycerin, may also contribute to the overall anti-wrinkle efficacy of the dissolvable film.

CONCLUSION

The dissolvable films may represent a novel generation of solid, preservative-free cosmetic delivery systems that open up a new avenue for the development of active cosmetic products.

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