Combination of argan oil and phospholipids for the development of an effective liposome-like formulation able to improve skin hydration and allantoin dermal delivery

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ABSTRACT.

Allanoin is traditionally employed in the treatment of skin ulcers and hypertrophic scars. In the present work, to improve its local deposition in the skin and deeper tissues, allantoin was incorporated in conventional liposomes and in new argan oil enriched liposomes. In both cases, obtained vesicles were unilamellar, as confirmed by cryo-TEM observation, but the addition of argan oil allowed a slight increase of the mean diameter (~130 nm versus ~85 nm). The formulations, especially those containing argan oil, favoured the allantoin accumulation in the skin, in particular in the dermis (~8.7 μg/cm^2), and its permeation through the skin (~33 μg/cm^2). The performances of vesicles as skin delivery systems were compared with those obtained by water dispersion of allantoin and the commercial gel, Sameplast®. Moreover, in this work, for the first time, the elastic and viscous moduli of the skin were measured, underlining the different hydrating/moisturizing effects of the formulations. The application of ARGliposomes seems to provide a softening and relaxing effect on the skin, thus facilitating the drug accumulation and passage into and trough it.

KEYWORDS: liposomes; argan oil; phospholipids; pig skin; Turbiscan Lab; skin rheology; skin hydration.

1. Introduction

Allantoin is the final product obtained from the oxidation/metabolism of uric acid metabolism of some vertebrates; it is also presents in different plants and currently can be easily chemically synthesized. In pharmaceutical and cosmetic fields, it is traditionally employed in the treatment of skin ulcers (Fu et al., 2006; Henderson, 1946) thanks to its capability to remove necrotic tissue promoting cell proliferation and skin epithelization (A.S. Durmus, M. Yaman, 2012; Braga et al.,

2012). It is also used as keratolitic agent in the treatment of the hypertrophic scar. Despite it being used in a wide range of pharmaceutical and cosmetic products for topical application, its skin penetration ability is very low due to its low logP ~3.14 and consequently, it beneficial effect is limited (Oliveira et al., 2014). A strategy to ameliorate its topical performances, prolonging the residence time at the action site and improving the local accumulation, may be the incorporation in liposome-like systems. In order to achieve this purpose, in the present work allantoin has been incorporate in liposomes and alternatively in liposomes enriched with argan oil (ARGliposomes). The addition of argan oil to liposomes was never previously tested and it is expected to improve the vesicle ability to modify stratum corneum lamellar assembly and its hydration favouring the allantoin skin delivery. Argan oil has been traditionally used in Morocco for centuries as a beauty oil or cosmetic ingredient, mainly for its ability to eliminate skin pimples as well as juvenile acne and to reduce dry skin matters and wrinkles (Guillaume and Charrouf, 2011a). It is mainly composed of acylglycerides (~99%), carotens, tocopherols, triterpene alcohols and xantophylls (1%). Oleic and linoleic acids are the most abundant unsaturated fatty acids that significantly contribute to its favourable properties (Charrouf and Guillaume, 2008; Guillaume and Charrouf, 2011b).

The skin is the main barrier which avoid the passage of drugs topically applied and is formed by different layers: the stratum corneum, consisting of several layers of corneocytes (dead and flattened) embedded in a lipid-water lamellar matrix; the viable epidermis, consisting on living keratinocytes strongly joined with desmosomes junctions; the dermis, which is the support of the skin and it is formed by collagen and elastin fibres containing few fibroblasts; finally the hypodermis composed of fibroblasts, adipose cells, and macrophages. Due to its structure, the skin, in particular the stratum corneum, acts as main barrier, which controls the passage of

66 foreign and endogenous molecules. The predominant pathway for drug passage through the skin
67 is the paracellular way across the lipid domain between the epidermal cells, where the
68 assembling of stratum corneum matrix and the hydration status of the skin, are key parameters
69 which depend on human race, age, sex, skin type, anatomical location and humidity of the
70 environment (Darlenski and Fluhr, 2012). Also cosmetic ointments and pharmaceutical
71 preparations can affect the above mentioned properties as a function of formulation composition
72 (Edwards and Marks, 1995; Esposito et al., 2007). In particular, nanocarriers, such as
73 phospholipid vesicles and liposome-like systems due to their ability to deeply penetrate into the
74 skin, are supposed to strongly modify such parameters (Castangia et al., 2015; Manca et al.,
75 2016, 2015, 2014c, 2013a, 2013b; Zaru et al., 2012).
76 Rheological study represents an innovative tool to evaluate the skin status, particularly its
77 hydration and elasticity. Moreover, an adequate modelling to evaluate the viscoelastic properties
78 of excised skin is of paramount interest in medical and cosmetic applications because it can aid
79 to predict the modifications caused by topical preparations and their effect on its barrier function,
80 providing an important support to select the most suitable formulations. Nevertheless its
81 significance, actually few literatures reported detailed information regarding the rheological
82 properties of excised skin and the effects of topical formulations. In this work, for the first time,
83 we studied the rheological properties of excised skin and the influence of formulations in its
84 behaviour. Due to the strong junctions and complementary structure of the main strata, the skin
85 has been considered as a full and continuum layer thus microscopic properties of its components
86 and modifications of the ordered structure may be reflected in its macroscopic viscoelastic
87 behaviour.

In this work, allantoin liposomes were prepared and, as an alternative, ARGliposomes were formulated and characterized. Moreover, for the first time to our knowledge, the skin hydration effect of water, liposomal nanoformulations and Sameplast® gel was evaluated by rheological analyses and results were compared with those obtained performing *in vitro* allantoin permeation and penetration study.

2. Material and methods.

2.1. Materials

Soy lecithin (SL) was purchased from Galeno (Prato, Italy). Allantoin (AL), argan oil (ARG) and all the other products were purchased from Sigma-Aldrich (Milan, Italy). Sameplast® gel (Savoma Medicinali s.p.a.) was purchased in a drugstore.

2.2. Vesicle preparation

Empty or drug-loaded vesicles were prepared by weighing soy lecithin (60 mg/ml) allantoin (10 mg/ml) and argan oil (5 mg/ml), when appropriate, in a glass test tube, adding water and leaving the samples one night at room temperature to facilitate the swelling of the phospholipids. The dispersions were then sonicated, 20 cycles (2 sec ON and 2 sec OFF) repeated 4 times, with a Soniprep 150 ultrasonic disintegrator (MSE Crowley, London, United Kingdom) at an amplitude of 15 microns. Dispersions were purified from the non-incorporated drug by dialyzing (Spectra/Por®membranes, 3 nm pore size; Spectrum Laboratories Inc., Rancho Dominguez, United States) them against water at 25°C for 4 hours (replacing the water once). Entrapment efficiency (EE) was expressed as the percentage of the drug amount found after dialysis versus that initially used. Allantoin content was determined by high performance liquid chromatography (HPLC) after disruption of the vesicles by dilution in methanol (1/100) and analysis of limpid solutions, was performed at 220 nm using a Thermo Scientific (Madrid,

111 Spain) chromatograph. The column was a Waters C18, and the mobile phase was a mixture of
112 methanol and water (5:95 v/v). The injection volume was 20 µl and the flow rate was 1 ml/min.
113 A standard calibration curve (peak area of allantoin versus drug concentration) was built up by
114 using standard solutions (range 1.0 - 0.01 mg/ml). Calibration graphs, plotted according to the
115 linear regression analysis, gave a correlation coefficient value (R^2) of 0.999. The allantoin
116 retention time was 2.7 minutes. The limit of detection was 2 ng/ml while the limit of
117 quantification was 5 ng/ml.

118 **2.3. Vesicle characterization**

119 Vesicle formation and morphology were evaluated by cryo-TEM analysis. A thin film of each
120 sample was formed on a holey carbon grid and vitrified by plunging (kept at 100% humidity and
121 room temperature) into ethane, maintained at its melting point, using a Vitrobot (FEI Company,
122 Eindhoven, The Netherlands). The vitreous films were transferred to a Tecnai F20 TEM (FEI
123 Company), and the samples were observed in a low dose mode. Images were acquired at 200 kV
124 at a temperature ~ -173 °C, using low-dose imaging conditions with a CCD Eagle camera (FEI
125 Company).
126 The average diameter and polydispersity index (P.I.), of each sample, were determined by
127 Photon Correlation Spectroscopy using a Zetasizer nano (Malvern Instrument, Worcestershire,
128 United Kingdom). Zeta potential was estimated using the Zetasizer nano by means of the M3-
129 PALS (Phase Analysis Light Scattering) technique. Before the analysis both liposomes and ARG
130 liposomes (100 µl) were diluted with water (10 ml).
131 The stability of the vesicles was evaluated by using the optical analyser Turbiscan Ageing
132 Station (Formulaction, L'Union, France) equipped with an ageing station with three thermo-
133 regulated blocks for the storage of 54 samples. Turbiscan technology is based on Static Multiple

Light Scattering for the analysis of concentrated dispersions (without mechanical stress or dilution). In our experiments, 10 ml of each sample were placed in a cylindrical glass cell and stored in the Turbiscan for 7 days at 25, 40 or 60°C. The detection head was composed of a pulsed near-infrared light source (λ = 880 nm), two synchronous transmission (T) and back scattering (BS) detectors. The T detector receives the light, which crosses the sample (at 180° from the incident beam), while the BS detector receives the light scattered backwards by the sample (at 45° from the incident beam). The detection head scanned the entire height of the sample cell (65 mm longitude), acquiring T and BS each 40 µm. The measuring principle is based on the variation of the particle volume fraction (migration) or diameter (coalescence), resulting in a variation of BS and T signals. The stability of each sample was evaluated on the basis of the variation of back scattering (ΔBS). For a comparative evaluation between the different samples we exploited the Turbiscan Stability Index (TSI) computation, that provides a key number related to the general behaviour of the formulation. Samples were significantly different for ΔTSI values greater than 0.4

2.4. *In vitro* skin delivery studies

Studies were carried out using newborn skin of Goland-Pietrain hybrid pigs (1.2 kg), provided by a local slaughterhouse. The stored skin was pre-equilibrated in saline solution at 25°C, 12 hours before the experiments. Experiments were performed non-occlusively in Franz diffusion cells (diffusion area of 0.785 cm^2) sandwiching the full thickness skin specimens (n=6) between the donor and receptor compartments. The receptor compartment was filled with 5.5 ml of saline solution, which was continuously stirred and thermostatted at 37±1°C to achieve the physiological skin temperature (i.e. 32±1°C). Each sample (100 µl) was placed onto the skin surface and, at regular intervals (1, 2, 4, 6 and 8 hours), the receiving solution was withdrawn

157 and drug content was analysed by HPLC. After 8 of experiment, the skin surface was washed
158 and the epidermis, dermis and subcutaneous tissue were separated with a sterile surgical scalpel;
159 the method was previously validated by histological examination. Each skin specimen was
160 placed in methanol (2 ml), sonicated for 2 minutes in order to extract the drug, and then assayed
161 for drug content by HPLC.

162 **2.5. Rheological study of skin**

163 Rheological measurements were carried out at 25±1°C, using a Haake RheoStress 300 Rotational
164 Rheometer, equipped with a Haake DC10 thermostat and data acquisition and elaboration
165 software RheoWin; a cross-hatch plate device (Haake PP35 TI: diameter = 35 mm) was used.
166 Measurements were carried out on newborn pig skin samples, treated alternatively with water,
167 liposomes, ARGliposomes and Samplast®, the commercial preparation containing allantoin (1%)
168 in a gel of hydroxyethylcellulose and xanthan gum. Each sample was deposited on the top of the
169 skin and the specimens were stored for 12 h at 25°C. After that, the formulation was removed
170 from the top of the treated skin by gently wiping using absorbent paper. The skin was then
171 deposited on the lower plate of the geometry. The upper plate was then lowered and put in
172 contact with the skin until slippage phenomena were avoided, according to a previous procedure
173 developed for hydrogels (Palumbo et al., 2012; Pescosolido et al., 2010). To allow the sample
174 relaxing the stresses undergone during the loading procedure, all the samples were leaved to rest
175 for 5 minutes at 25°C. Frequency sweep tests were performed in the range of 0.01-10.0 Hz and a
176 shear strain of 0.0003 was used. Mechanical spectra (storage (G') and loss (G") moduli were
177 registered in the above mentioned frequency range and G' at 1 Hz was used to characterize the
178 viscoelastic properties of the skin treated with the different formulations. Before frequency
179 sweep experiments, amplitude sweep tests were carried out for each sample to assess the linear

viscoelastic region where the values of the moduli are independent from the applied deformation. All measurements were carried out at 25°C, using, at least, three skin specimens for each formulation.

2.6. Statistical analysis of data

Results are expressed as the means±standard deviation. Multiple comparisons of means (ANOVA) were used to substantiate statistical differences between groups, while Student's t-test was used to compare two samples. Significance was tested at the 0.05 level of probability (p). Data analysis was carried out with the software package XLStatistic for Excel.

3. Results and Discussion

3.1. Vesicle characterization and stability

Just few studies were performed to evaluate the deposition or permeation of allantoin in the skin after topical application, and only a small number of them reported the suitability of allantoin incorporation in liposomes or other lamellar vesicles intended for skin delivery (Arno et al., 2014; Chan et al., 2014). Due to the lack of literature, in this work, aimed at improving its accumulation and passage in and through the skin, allantoin was entrapped in liposomes and liposome-like systems and their carrier ability was evaluated. Referring to the allantoin concentration in the Sameplast® gel (1%), which is the commercial formulation used as reference, firstly, the minimum amount of soy lecithin (60 mg/ml) able to load 10 mg/ml of allantoin, forming stable, small in size (≤100 nm), homogeneously dispersed (≤0.25) vesicles and able to retain such amount of drug, avoiding its precipitation, was determined. Secondly, increasing amounts of argan oil were added to liposome formulation and the maximum oil concentration (5 mg/ml) able to avoid vesicle aggregation and simultaneously to ensure the most suitable properties for skin delivery such as small and homogenous sized vesicles, was assessed

(Table 1). Argan oil is a natural oil extracted from the kernels trapped in the stones of the fruit of the argan tree (Argan spinosa L. Skeels) and, to the best of our knowledge, it has never been added to liposomes. This oil is traditionally used in Africa for its restoring properties mainly related to its composition. Indeed, it contains high amount of oleic and linoleic acid, responsible for the emolliating and moisturizing properties, and a small amount of unsaponifiable matter, which can neutralize free-radicals and improve skin elasticity (Charrouf and Guillaume, 2008; Guillaume and Charrouf, 2011a, 2011b). Taking into account its favourable properties, the addition of argan oil to allantoin loaded liposomes, intended for topical application, appeared a proper combination and it was expected to ameliorate the vesicle performances. Empty formulations were also prepared and characterized to elucidate the effect of allantoin on bilayer-assembling features. Vesicle assembling and morphology were evaluated by direct observation using a cryo-TEM. Pictures showed that liposomes and ARGliposomes were very similar, spherical and unilamellar vesicles (Figure 1) with size ranging from ~50 to ~140 nm.

The size of samples was measured immediately after their preparation, using the dynamic laser light scattering that provided the value of the mean size and size distribution as a function of particles ability to scatter the light (Table 2). Empty vesicles were always similar to the corresponding allantoin loaded vesicles ($p>0.05$), indicating a complete and effective intercalation of allantoin molecules avoiding the modification of the bilayer assembly. Liposomes were sized ~85 nm whereas ARGliposomes ~ 132 nm; all the samples were homogeneously dispersed with an adequate size distribution ($PI\leq0.26$) and a zeta potential strongly negative (~ -60 mV) (Haidar et al., 2008). Sample analyses were always repeatable as confirmed by the low standard deviation values obtained from at least six repetitions. Argan oil induced an increase of both vesicle size and zeta potential, the last toward more negative values,

denoting an important effect on bilayer assembling and structure, which favoured the formation of vesicles with a higher curvature radius.

Allantoin was entrapped in high amount in both liposomes and ARGliposomes (EE% 66±8 and 84±6 respectively, Table 2). In this case, the combination of argan oil and phospholipids may facilitate the distribution of allantoin into the vesicles in higher amount, thus avoiding the drug leakage.

Size, P.I. and surface charge were monitored for 90 days keeping the samples at 25°C (Figure 2); the values were constant for all the time span studied, showing a good stability of the systems. Results were confirmed by Turbiscan measurements, previously used to evaluate the stability of different nanoparticle dispersions (Carbone et al., 2014; Manca et al., 2014a, 2014b). As reported in Figure 3, a little transmission variation in the middle of the cell was observed for both allantoin loaded samples stored at 25°C related to a certain particle size change. The intensity of the aggregation phenomenon was higher when samples were stored at 40 and 60°C (ΔBS % >2). On the basis of the TSI global results, the stability of allantoin loaded vesicles was not affected by the addition of the oily components, since the difference between TSI of both liposomes and ARGliposomes at 1, 3 or 6 days was not significant (ΔTSI ≤0.4) (Table 3).

3.2. *In vitro* skin delivery studies

In vitro penetration/permeation studies of allantoin were performed using Franz diffusion cells and newborn pig skin (Gillet et al., 2011; Manca et al., 2014b, 2013b; Manosroi et al., 2004; Verma, 2003). For each formulation the drug accumulation in the different skin layers and its permeation was quantified (Figure 4). Allantoin in water dispersion and in gel formulation (Sameplast® gel), were used as comparison. The last one is a commercial product containing allantoin 1%, dispersed in a gel of hydroxyethylcellulose and xanthan gum prepared in water and

glycerol. Using the water dispersion, the amount of allantoin accumulated in the different skin strata was always lower: ~1.6 μg/cm^2 in the epidermis and ~0.7 μg/cm^2 in the dermis and subcutaneous tissue. This low drug deposition is probably related to the poor ability of the water to offset transepidermal water loss and to significantly alter the skin hydration status, thus the barrier effect of the stratum corneum, which hampered the penetration of allantoin. Using the commercial formulation Sameplast® gel the drug accumulation in the different strata, including the dermis, was comparable with that obtained treating the skin with the water dispersion (~2.6 μg/cm^2 $p>0.05$ versus that provided by dispersion). Probably, the application of the commercial gel for 8 hours was able to avoid the water loss of the skin, as reported for gel, but was unable to effectively alter the assembling of lipid matrix and promote the accumulation/passage of allantoin into and trough the skin. Moreover, in this case, the accumulation of the drug in the skin layers is limited by both, the barrier effect of the stratum corneum and its partitioning between the stratum corneum and the gelled vehicle. Differently, when allantoin was loaded in conventional liposomes its accumulation in the dermis was double (~3.4 μg/cm^2, $p<0.05$) with respect to that provided by Sameplast® gel. When the drug was entrapped in ARGliposomes, an important drug accumulation improvement was observed in the epidermis (~2.2 μg/cm^2 $p<0.05$ versus all) and mostly in the dermis (~8.7 μg/cm^2 $p<0.01$ versus all). Additionally, liposomes and ARGliposomes, provided a significant increase of the amount of drug permeated through the skin and recovered in the receptor fluid which was ~33 μg/cm^2 ($p<0.05$ versus dispersion and Sameplast® values) while it was significantly lower (~17 μg/cm^2) when the water dispersion or Sameplast® gel were used. We can hypothesize that liposomes, thanks to the well-known phospholipid affinity to the interlamellar matrix of stratum corneum, fused with it, decreasing the skin barrier function. The high deposition provided by ARGliposomes, especially in the dermis,

suggested their direct passage in the stratum corneum matrix modifying its lamellar assembling and perturbing its ordered structure due to the additional action of argan oil probably able to further modify the intercellular pathway (Kirjavainen et al., 1999).

3.3. Effect of allantoin formulations on skin rheological behaviour

In order to elucidate the effect of the different formulations (water dispersion, Sameplast® gel, liposomes and ARGliposomes) on skin elasticity and hydration, the excised newborn pig skin was treated for 8 hours with the various formulations and a detailed rheological study was carried out. Mechanical properties of a material are strictly related to its morphology and structure. In the case of skin, many layers are overlapped but, from a mechanical point of view, at first, it can be treated as a whole material, whose properties are due to the combinations of the properties of each layer and interlayer junctions. Probably, the dermis, due to its higher thickness and fibrotic structure, can mainly affect the whole skin behaviour. Rheological experiments, i.e. mechanical properties in shear conditions, are chosen, assuming that in the skin the mechanical effects can be amplified by transversal solicitations, as the various formulations can act in a different extent on the various skin layers.

At first, stress sweep experiments were carried out at 1 Hz. In order to ascertaining to be in the linear viscoelastic regime, the G' evolution (at 1 Hz) of each sample, the waveforms of the stress applied and the deformations observed were registered. Indeed, sinusoidal waveforms are obtained from samples in the linear viscoelastic regime whereas out of this range more complex waveforms (data not reported) are obtained (Lapasin, 1995). For each skin treatment (formulation), measurements were repeated using, at least, three independent skin specimens and the collected curves laid in a range of ±10%. A representative curve for each skin treatment (water dispersion, Sameplast® gel, liposomes and ARGliposomes) is reported in Figure 5. The

295 data evidenced that the response of all skin specimens, independently of the treatment, showed a
296 deviation from the linearity starting at deformation in the range 0.003-0.006. The slope of the
297 various curves, after this point, clearly indicated that different effects are produced in the skin as
298 a function of the treatments (formulations). The curves can be grouped in two classes: specimens
299 treated with water and liposomes (first class) and those treated with Sameplast® gel and
300 ARGliposomes (second class). The behaviour of the first class was characterized by a sharp
301 decrease of G' after the linear regime which, from the rheological point of view, can be ascribed
302 to a disruption of the skin structure as a consequence of its deformation. Such lack of structure
303 (decrease) was stronger in the first class with respect to that observed in the second class using
304 the same stress. In fact, the second class was able to preserve part of the elastic behaviour of the
305 skin, and the curves showed a softer decrease respect to that of the first class. Mechanical spectra
306 of the skin, after each treatment, were recorded in the linear viscoelastic regime in the range
307 0.01-10 Hz, i.e. the frequency range normally experienced by humans in skin care applications.
308 G' (elastic component) and G'' (viscous component) were collected and their profiles as a
309 function of frequency were analysed (Figure 6). The profile of the mechanical spectrum of skin
310 wetted with water evidenced a G' higher than G'' with a positive slope, different for the two
311 moduli, indicating a dependence that, at high frequency could lead to a crossover. The G'
312 remained roughly constant for the skin treated with gel and liposomes, but an evident cross-over
313 point appeared, at 0.1 Hz for Sameplast® and at 0.01 Hz for liposomes. In the case of
314 ARGLiposomes, the G' modulus is lowered of about half-order of magnitude, and an evident
315 cross-over point was observed at 0.1 Hz. The rheological results showed that the skin behaviour
316 depended on the used formulation: the Sameplast® gel was able to relax the tissues more than
317 water and liposomes, whereas ARGliposomes were the most effective in softening the tissues,

318 reducing, at the same time, the rigidity of the skin. Taking into account the allantoin deposition
319 provided by the formulations, we can hypothesize that the better softening and relaxing effect of
320 ARGliposomes correspond to a higher drug deposition in epidermis and mainly in the dermis,
321 probably because the less rigidity of the skin favoured the passage of allantoin up to the dermis
322 thanks to the supplemental action of argan oil in addition to that of phospholipids which exerted
323 a synergic skin fluidification. On the opposite, conventional liposomes did not allow the
324 softening and relaxing effect and only provided a slight increase of allantoin deposition in the
325 dermis (with respect to the water) due to the phospholipid effectiveness as penetration enhancers.
326 Sameplast® gel was able to partial relax the skin structure thanks to its capacity to avoid water
327 loss promoting lipid swelling.

328 **4. Conclusions**

329 Liposomes offer distinct advantages as dermal drug delivery systems, but recently it has been
330 found that some modifications in their composition can greatly improve their therapeutic
331 potential. In this work, combining (trans)dermal results and rheological data, we underline that
332 the association of argan oil to phospholipid vesicles was able to ameliorate the dermal delivery
333 of allantoin. Moreover, evaluating the skin rheological behaviour, we can argue that such
334 improvement was depending to a relaxing and softening effect provided by these vesicles on the
335 skin. The argan oil behaves as a key component of ARGliposomes and thanks to its
336 dermophilicity and moisturizing power confers to vesicles optimal skin-favourable properties
337 facilitating the drug accumulation in the dermis.

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435

Figure Captions

Figure 1. Cryo-TEM micrographs of allantoin loaded liposomes (A,) and ARGliposomes (B).

Figure 2. Variation of mean diameter ~~(A)~~, polidispersity index ~~(B)~~ and zeta potential ~~(C)~~ of allantoin loaded liposomes and ARGliposomes during 90 days of storage at room temperature (25°C). Mean values±standard deviation (SD) are reported (n=6).

Figure 3. Turbiscan backscattering profiles of allantoin loaded liposomes and ARGliposomes at 25, 40 and 60°C.

Figure 4. On the left: cumulative amount of drug accumulated in stratum corneum (SCT), epidermis (EP), and dermis (D) after 8 h application of allantoin water dispersion, allantoin in Sameplast gel, allantoin loaded liposomes and ARGliposomes. Data represent the means±standard deviation (SD) of at least six experimental determinations.
On the right: Allantoin deposition in the skin strata and permeation in the receptor fluid during 8 hours of application. Mean values (n=6) ± standard deviation (error bars) are reported.

Figure 5. G' vs deformation in shear stress sweep experiments carried out at 25°C on new born pig skin treated with water and with the various formulations prepared: ARGLiposomes, Liposomes and Sameplast® gel.

Figure 6. Mechanical spectra registered in shear experiments at 25°C on new born pig skin treated with water and Liposomes (left) and with ARGLiposomes and Sameplast® gel (right).