European Journal of Pharmaceutics and Biopharmaceutics 79 (2011) 258–267

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Ex vivo decrease in uranium diffusion through intact and excoriated pig ear skin by a calixarene nanoemulsion

Aurélie Spagnul [a,b], Céline Bouvier-Capely [b], Guillaume Phan [b,*], Géraldine Landon [b], Christine Tessier [b], David Suhard [b], François Rebière [b], Michelle Agarande [b], Elias Fattal [a]

[a] Faculté de Pharmacie, Châtenay-Malabry, France
[b] Institut de Radioprotection et de Sûreté Nucléaire (IRSN), Fontenay-aux-Roses, France

ARTICLE INFO

Article history:
Received 12 January 2011
Accepted in revised form 12 May 2011
Available online 19 May 2011

Keywords:
Calixarene
Nanoemulsion
Uranium
Cutaneous contamination
Decontamination
Pig ear skin
Franz cells

ABSTRACT

Cutaneous contamination by radionuclides is a major concern in the nuclear industry. In case of skin exposure to uranium, no efficient emergency treatment is available to remove the actinide from the skin. For this purpose, we developed a nanoemulsion containing calixarene molecules displaying good chelating properties towards uranium. In this paper, we describe the ability of this formulation to trap uranium and limit its transfer from the cutaneous contaminated site into the blood. Uranium percutaneous diffusion kinetics was assessed with Franz cells over 24 h through intact and excoriated pig ear skin biopsies, after or without application of the nanoemulsion. Uranium distribution in the skin layers was analysed by SIMS microscopy. The results showed that prompt application of the calixarene nanoemulsion allows a 94% and 98% reduction of the amount of uranium diffused respectively through intact and excoriated skin. The formulation is still efficient in case of delayed application up to 30 minutes since the 24 h-uranium transfer through excoriated skin is reduced by 71%. Besides, no accumulation of uranium or uranium-calixarene chelate was observed in the different skin layers. In conclusion, this study demonstrated the efficiency of the calixarene nanoemulsion, which can be regarded as a promising treatment for uranium cutaneous contamination.

1. Introduction

Accidental cutaneous contamination by radionuclides represents, after inhalation, the second cause of internal contamination of nuclear workers and may occur because of accidental confinement disruption, mechanical damage or gloves defect, when manipulating those radionuclides through gloves-boxes [1]. According to a study concerning the following up of workers from a French nuclear facility, between 1995 and 2003, the number of contaminations after an injury was in average of 20 cases per year (11% of the total number of contaminations), whereas the number of contaminations by inhalation was around 170 cases per year (88%) [1]. In France, workers involved in the production, fabrication or reprocessing of the nuclear fuel, which is mainly composed of uranium oxide and 3–7% plutonium oxide, may be exposed to these actinides present as either solid state (oxide particles) or solubilised in solutions (mainly nitric acid) [1]. In particular, cutaneous contamination by uranium may induce a high internal exposure after translocation of the radioelement through intact or excoriated skin [2–4]. Indeed, in case of contamination of superficial injuries by soluble uranium compounds, the radionuclide can be transferred from the wound site to the bloodstream in less than 30 min [5,6]. Subsequently, uranium is partly excreted in urines and distributed in its main retention organs, *i.e.*, kidneys and bones, where respectively chemical and radiological toxicities may occur [7–10]. To our knowledge, no effective and specific dosage form is available to treat in emergency skin uranium contamination [11,12]. Indeed, the common medical care of victims only consists in a local rinsing of the contaminated wound with soaped water or 25% calcium salt of diethylene triamine pentaacetic acid (Ca-DTPA), afterwards Ca-DTPA decorporation treatments are intravenously administered at the medical unit of the nuclear site in order to enhance urinary excretion of radionuclides. Unfortunately, Ca-DTPA exhibits a poor efficiency towards uranium which means that these procedures are ineffective [13–16]. To improve the treatment of skin contamination by radionuclides, some research groups have developed topical formulations containing biphosphonates as uranium chelating agents in order to limit the

* Corresponding author. Institut de Radioprotection et de Sûreté Nucléaire (IRSN), DRPH, SDI, LRC, Postal Box 17, 92262, Fontenay-aux-Roses Cedex, France. Tel.: +33 158357028; fax: +33 158359365.

E-mail addresses: aurelie.spagnul@irsn.fr (A. Spagnul), celine.bouvier@irsn.fr (C. Bouvier-Capely), guillaume.phan@irsn.fr (G. Phan), geraldine.landon@irsn.fr (G. Landon), christine.tessier@irsn.fr (C. Tessier), david.suhard@irsn.fr (D. Suhard), francois.rebiere@irsn.fr (F. Rebière), michele.agarande@irsn.fr (M. Agarande), elias.fattal@u-psud.fr (E. Fattal).

0939-6411/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2011.05.004

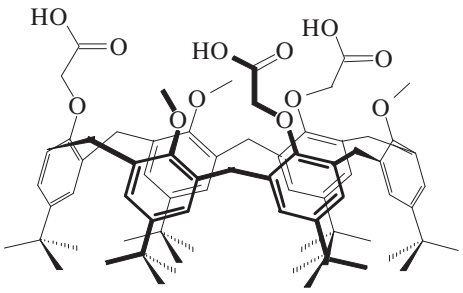

Fig. 1. Structure of 1,3,5-OCH_3-2,4,6-OCH_2COOH-*p-tert*butylcalix[6]arene.

translocation of uranium from the wound site [17–19]. However, no biphosphonate chelating effect was actually demonstrated in these studies. In this context, we have developed a new dosage form [20] consisting of an oil in water nanoemulsion containing a calixarene molecule (Fig. 1) developed in our laboratories primarily for radiotoxicological analyses purpose and known for its particular uranium chelation properties [21,22]. Previous *in vitro* experiments demonstrated that this calixarene nanoemulsion was able to extract more than 80% of uranyl ions from an aqueous contaminated solution [23].

The aim of the present work is to estimate the ability of this formulation to limit the diffusion of uranium through intact and wounded skin after contamination by a solution containing uranyl ions, which is a highly transferable uranium form. The contamination compounds used in this study, a uranyl nitrate solution (molecular weight of the salt $UO_2(NO_3)_2$: 394 g mol^{-1},very soluble in aqueous solutions), are commonly encountered in nuclear fuel cycle facilities and often manipulated as nitric solutions. Franz diffusion cells, an *ex vivo* technique commonly used in skin pharmacology and validated for radiotoxicological studies [24], were employed to study the percutaneous uranium diffusion after or without application of the calixarene nanoemulsion. In addition, ionic microscopy technique (SIMS) was used in order to localise uranium in the skin layers for *ex vivo* experiments.

2. Materials and methods

2.1. Materials

Calixarene molecule (Fig. 1) was synthesised as described in the patent by Duval and coworkers [25]. Other components used for formulation were paraffin oil (d = 0.86), (VWR, Fontenay sous Bois, France), non-ionic surfactants sorbitan monooleate (Span® 80) and polyoxyethylene glycol sorbitan monooleate (Tween® 80), purchased from Sigma–Aldrich (Saint-Quentin-Fallavier, France). Water used in all experiments was obtained from a Milli-Q® Synergy 185 water purification system (Millipore, Saint-Quentin-en-Yvelines, France). Uranium-contaminated solutions were prepared by diluting a depleted uranium standard solution (1000 mg l^{-1} in 2% HNO_3, SPEX Certiprep, Horiba Jobin Yvon, Longjumeaux, France).

2.2. Preparation of nanoemulsion

Nanoemulsions were prepared by the emulsion inversion point method as described previously [20]. Briefly, water was slowly added to a mixture of paraffin oil, non ionic surfactants and calixarene under slight stirring using a magnetic stirrer. The emulsification temperature was kept at 50 °C. Samples were then cooled at room temperature under slight stirring. The amounts of surfactants, oil and water were 5%, 20% and 75% (w/w), respectively. Thereafter, the nanoemulsions without or with calixarene (at 4 mg g^{-1}) will be referred in the text respectively as "unloaded" or "loaded" nanoemulsion. The previous physicochemical characterisation studies have shown that the oil droplet diameter is around 150 nm and that this nanoemulsion is quite homogeneous since the polydispersity index is low (PDI < 0.250), and the zeta potential is around −50 mV [20].

2.3. Skin sampling and measurements

2.3.1. Pig ear skin samples preparation

Pig ears were purchased from slaughterhouse with the veterinary department authorisation (Direction Départementale des Services Vétérinaires de Hauts de Seine, France). Female pigs were Landrace and male were Piétrain species. Pig ears were stored at −20 °C until use. Before each experiment, they were warmed to room temperature. Full thickness skin pieces were then removed from the external face of the ears [26] and prepared to fit to Franz diffusion cells for diffusion assays.

2.3.2. Wound generation by excoriation

The *stratum corneum* of pig ear skin pieces was removed by tape stripping using standard D-Squame® adhesives (Ø 28 mm, Monaderm®, Monaco) pressed onto the skin using a D-Squame® applicator providing a constant 150 g cm^{-2} pressure. Tapes were removed with one quick movement. To determine the number of adhesive application required to remove the *stratum corneum*, 0, 20, 40 and 60 tapes were applied. Skin samples were embedded in OCT compound (Optimal Cutting Temperature compound, Tissue-Tek®, Sakura Finetek, Villeneuve d'Ascq, France) and frozen. Ten micrometers cryostat skin sections were fixed and stained with Mayer's hemalun. Stained skin sections were then observed with an optical Leitz Diaplan microscope (Leica Microsystèmes, Rueil-Malmaison, France) equipped with a Coolsnap ES camera (Roper scientific, Evry, France). A minimum of 12 cryosections coming from different pig ear skin biopsies were analysed for each test (2 pig ears and 3 skin pieces per pig ear for each tape number tested).

2.3.3. Thickness measurement of skin pieces

The full-thickness pig ear skin samples were measured before diffusion assay using a thickness gage (Mitutoyo Corporation, Roissy, France). The mean thickness of intact and excoriated skin samples was respectively 1.07 ± 0.10 mm and 0.92 ± 0.05 mm (mean of 12 assays ± standard deviation).

2.3.4. Transepidermal Water Loss measurements

The Transepidermal Water Loss (TEWL) can be dramatically altered if the *stratum corneum* barrier function is perturbed by physical, chemical, therapeutic and pathological factors and can thus be a sensitive indicator of skin water barrier alterations [27–29]. The TEWL of intact and excoriated skin samples was determined before each diffusion assay by using a Tewameter TM 300® (Monaderm®, Monaco) equipped with an adapted probe. Room temperature and relative humidity were checked to meet the optimal use conditions of the apparatus. According to the apparatus specifications, TEWL values below 15–25 g m^{-2} h^{-1} can reflect the integrity of the skin barrier function, whereas TEWL values exceeding 30 g m^{-2} h^{-1} can indicate a skin water barrier disruption.

2.4. Ex vivo procedures

2.4.1. Franz diffusion cells

The Franz diffusion cells system used was a MicroettePlus™ apparatus (Hanson Research Corp., Chatsworth, California, USA). Transcutaneous diffusion was assessed according to OECD guidelines [30]. Franz cell receptor compartment was filled with 7 ml

of 0.25 M carbonate-bicarbonate pH 9.8 buffer (Sigma–Aldrich, Saint-Quentin-Fallavier, France) homogenised by magnetic stirring (400 rpm) and maintained at 33.5 °C to ensure a surface skin temperature of 32 °C [30]. Skin biopsies were placed between the donor and the receptor compartment ensuring that the dermal face was in contact with the receptor medium, whereas the epidermal face was turned to the donor compartment. The whole device was then fixed with a clamp. The contaminated solution was deposited on 1.76 cm^2 of the skin epidermal face in the donor compartment. Skin surface temperature was measured with a thermometer (Testo 925, Testo, France) equipped with an adapted probe (Testo, France). All Franz diffusion cell experiments were conducted under occlusive condition. The uranium diffusion kinetics was evaluated by automatically sampling 1 ml aliquots of receptor fluid at the following predetermined times: 0, 0.5, 2, 4, 6, 12, 18 and 24 h. Franz cell blank experiments were conducted with skin biopsies that were not contaminated by uranium in order to evaluate the release of uranium that is naturally contained into the skin samples as a function of time. The diffusion kinetics of uranium from the contaminated solution deposited onto the skin was then determined by subtracting for each time the cumulative quantity of natural uranium recovered in the receptor fluid for blank experiments to the cumulative quantity of uranium obtained after skin contamination. Uranium diffusion kinetics was represented by plotting the percentage of cumulative quantity of uranium that diffused through skin as compared with the initial quantity of deposited uranium as a function of time. The uranium diffusion steady-state flux (ng cm^{-2} h^{-1}) was determined from the plot of the cumulative diffused uranium mass per unit skin area versus time. It was estimated from the slope of this plot when the rate of diffusion was constant over time.

2.4.2. Radiocontamination of skin

The contamination was made by depositing 600 μl of 10 mg l^{-1} uranyl nitrate solution buffered at pH = 5 with a 0.01 M acetate solution on the skin biopsies placed in the Franz cells (6000 ng of uranium per skin biopsy). This contaminated solution volume corresponds to the minimal one required to cover uniformly the skin surface, which ensures optimal transcutaneous diffusion conditions by avoiding lateral diffusion phenomenon. Uranium-contaminated solution was prepared by diluting a standard depleted uranium solution (1000 mg l^{-1} in 2% HNO_3, SPEX Certiprep, Horiba Jobin Yvon, Longjumeaux, France). The contaminated solution pH was adjusted to 5 with 0.01 M acetate buffer (Normapur, VWR, Fontenay sous Bois, France) [23]. The contaminated solution was kept in contact with the skin samples during the 24 h of diffusion kinetics study.

2.4.3. Skin decontamination by calixarene nanoemulsion

To assess the calixarene nanoemulsion decontamination efficiency, a 600 μl volume of 4 mg g^{-1} calixarene loaded nanoemulsion was deposited on intact and excoriated contaminated skin biopsies in the donor compartment of the Franz cells by the mean of a syringe. The dosage form was first applied immediately after the contamination step. It was also applied 5, 15 and 30 min after the skin contamination step on excoriated contaminated skin samples in order to simulate the time which may elapse between a contamination accident and the administration of treatment (e.g. time usually needed to transfer a patient from a work place to the infirmary, where decontamination protocols are performed) [17].

2.4.4. Sample analysis

The ^{238}U content of receptor fluid samples was measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, X Series II, Thermo Electron, Courtaboeuf, France) using optimised protocols originally designed for human urine samples [31–33]. A multielemental standard stock solution containing depleted uranium at 1 μg l^{-1} (from a 10 mg l^{-1} tuning solution SPEX Certiprep, Horiba Jobin Yvon, Longjumeaux, France) prepared in 2% HNO_3 solutions (from a 67% HNO_3 solution, Normatom, VWR, Fontenay sous Bois, France) was used before each ICP-MS measurement series to optimise the ^{238}U signal and get the best instrumental conditions.

Aliquots were properly diluted in 2% HNO_3 (67% HNO_3 solution, Normatom, VWR, Fontenay sous Bois, France) and ^{209}Bi (10 mg l^{-1} single element internal standard, SPEX Certiprep, Horiba Jobin Yvon, Longjumeaux, France) was added as internal standard at 1 μg l^{-1}. As the diluted solutions were prepared by weighing, the related uncertainties can be neglected compared with the statistical ones. Five ICP-MS measurements were performed for each aliquots. The combination of the statistical errors was made using the conventional law of error propagation. In all experiments, standard deviations of ICP-MS measurements (<2%) were negligible as compared to the standard deviations of the uranium percutaneous diffusion through the skin biopsies (10 assays for each experimental condition). Considering the very low detection limit of ICP-MS for uranium (0.5 ng l^{-1} in 2% HNO_3) and the amount of deposited uranium (600 μl of U at 10 mg l^{-1}, *i.e.*, 6000 ng of U), percentages of uranium diffused through skin as low as 0.005% can be detected. The cumulative amounts of uranium permeating through the skin were expressed as a percentage of the amount of uranium (6000 ng) deposited on each skin biopsy. This percentage is corrected to take account of the previous removal of receiver samples and plotted against time. Permeability coefficient (K_p) and lag time values were calculated using the pseudo steady-state from plots of cumulative penetration versus time. The extrapolation of the linear part of the curves to the time axis gave the lag time. And the slope of the linear part of the curves yielded the pseudo steady-state flux J_{ss} (ng cm^{-2} h^{-1}). The permeability coefficient K_p (cm h^{-1}) was calculated as $K_p = J_{ss}/C$ (C is the concentration in the donor compartment).

Comparison between means of cumulative quantities of diffused ^{238}U obtained during the kinetics studies were done by using t-test nonparametric statistical test with $\alpha = 0.05$.

The calixarene content in the receptor medium aliquots was determined by High Performance Liquid Chromatography (HPLC). The HPLC system used was an Agilent 1200 Series apparatus (Agilent Technologies, Massy, France) equipped with an autosampler (1200 Series Standard Autosampler), a mobile phase delivery pump (1200 Series Quaternary Pump) associated with a degasser (1200 Series Vacuum Degasser), a UV–Visible detector (Multi-Wavelength Detector) and a data module (HPLC 2D ChemStation Software). The stationary phase was a Zorbax Eclipse XDB-C8 column (150 × 4.6 mm., 5 μm), and a mixture of acetonitrile and 0.05% trifluoroacetic acid (HPLC grade, Sigma–Aldrich Saint-Quentin-Fallavier, France) was employed as mobile phase. Samples were diluted in tetrahydrofuran (HPLC grade, Sigma–Aldrich, Saint-Quentin-Fallavier, France) in ratio 1:1 (v/v) and were analysed in duplicate. The injection volume was set at 20 μl and the absorbance measurement was performed at 210 nm, a wavelength that is suitable to detect the calixarene. Calibration curves were performed with 0.05 μg ml^{-1}–0.5 μg ml^{-1} solutions by diluting appropriate amount of calixarene powder in a 1:1 (v/v) mixture of carbonate-bicarbonate buffer and tetrahydrofuran. The detection limit of the analytical method was 0.02 μg ml^{-1} and the quantification limit was 0.05 μg ml^{-1}.

2.4.5. Localisation of uranium in skin biopsies by SIMS microscopy

At the end of the 24 h Franz cell diffusion experiments, skin biopsies were thoroughly rinsed with water and fixed in a solution

containing 6% glutaraldehyde in a sodium cacodylate buffer at 4 °C during 1 day. Skin samples were then dehydrated in various ethanol and propylene oxide baths, permeated with a propylene oxide/Epon mixture and then embedded in pure EPON-type resin [34]. Skin sections (0.9 μm) of embedded samples were transversally cut (from the *stratum corneum* to the hypodermis) and laid on silicium or gold holders for Secondary Ion Mass Spectrometry (SIMS) analysis. The SIMS analyses were performed with a CAMECA IMS 4F-E7 instrument (Gennevilliers, France).

The SIMS microscopy, which allows the elemental and isotopic analysis of a solid surface, is based on the sputtering of a few atomic layers from the surface of a sample by O^{2+} primary ions beam bombardment. The ejected secondary ions are accelerated and analysed by mass spectrometry. Then, the collected secondary ions can be measured with an electron multiplier and also sequentially converted into an image. A more detailed description of the physical phenomenon is provided in the literature [35,36]. For each skin area analysed, $^{40}Ca^+$ or $^{23}Na^+$ images gave the histological structure of the skin and $^{238}U^+$ images showed uranium localisation within the cutaneous structures.

3. Results

3.1. Wound generation by tape stripping (excoriation)

The number of tapes required to remove the *stratum corneum* was determined by a histological study. After successive application of 0, 20, 40 or 60 tapes, pig ear skin sections (10 μm-thick) were observed with an optical microscope. The results showed that the successive application of 60 tapes per skin biopsy was necessary to remove the whole *stratum corneum* from the skin explants (Fig. 2).

3.2. Comparison of the uranium percutaneous diffusion kinetics through intact and excoriated skin

The integrity of intact pig ear skin explants in Franz diffusion cells was preliminary checked by TEWL measurement on 6 intact skin explants during 24 h. The TEWL values were stable during the 24 h experiment (mean TEWL value: 5.2 ± 1.1 g m^{-2} h^{-1}), which indicated that the skin biopsies integrity was maintained under our experimental conditions. For excoriated skins, TEWL value measured before each experiment was around 40 ± 1.1 g m^{-2} h^{-1}. The uranyl diffusion kinetics through intact and excoriated skin is represented in Fig. 4. After 24 h of exposure, 0.07 ± 0.03% and 39.5 ± 13.4% of the initially deposited uranium has diffused respectively through intact (Fig. 3A) and excoriated (Fig. 3B) skin samples. Uranium was significantly ($p < 0.05$) detected in the Franz diffusion cells receptor medium from 30 min after the contamination of intact and excoriated skin. The steady-state flux of uranium was 55 times higher across excoriated skin (60.9 ng cm^{-2} h^{-1}) than across intact skin (1.10 ng cm^{-2} h^{-1}). Thus, the elimination of the *stratum corneum* greatly enhanced the uranyl diffusion and the quantity of uranium that diffused across the pig ear skin samples.

Visualisation of uranium distribution in the skin structures after 24 h of exposure by SIMS microscopy: comparison between intact and excoriated skin.

To verify the absence of interference in SIMS analysis, the mass spectra of intact skin biopsies that were not exposed to uranium were recorded around the mass of the ^{238}U isotope (Fig. 4). No significant peak at the 238 mass was observed. Similar mass spectra have been obtained for excoriated skin biopsies. These results showed that natural uranium contained in skin biopsies was not detected by SIMS and that there was no superposition of polyatomic ions at the mass of interest.

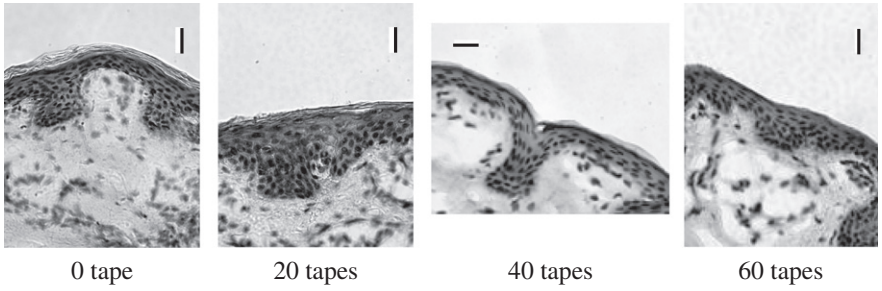

Fig. 2. Microscopic observations of 10 μm-thick stained pig ear skin cryosections after the application of 0 to 60 tapes (scale: 20 μm).

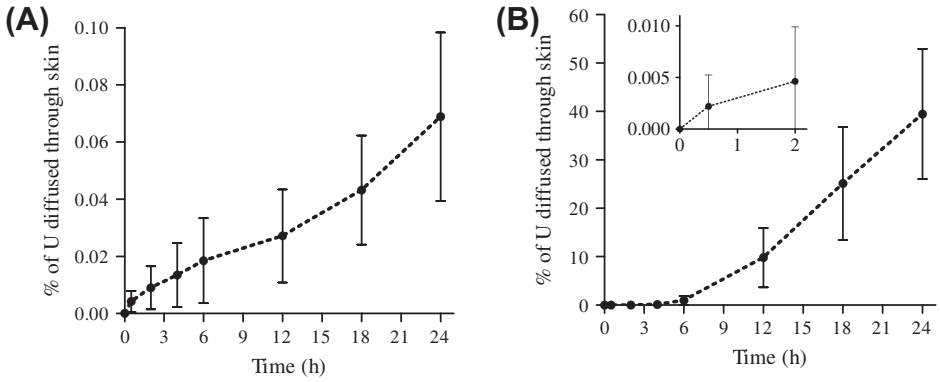

Fig. 3. Uranium percutaneous diffusion kinetics in Franz diffusion cells. Percentage of initially deposited uranium that diffused through intact (A) or excoriated (B) skin biopsies during the 24 h of exposure. Each point corresponds to the mean of 10 assays ± standard deviation.

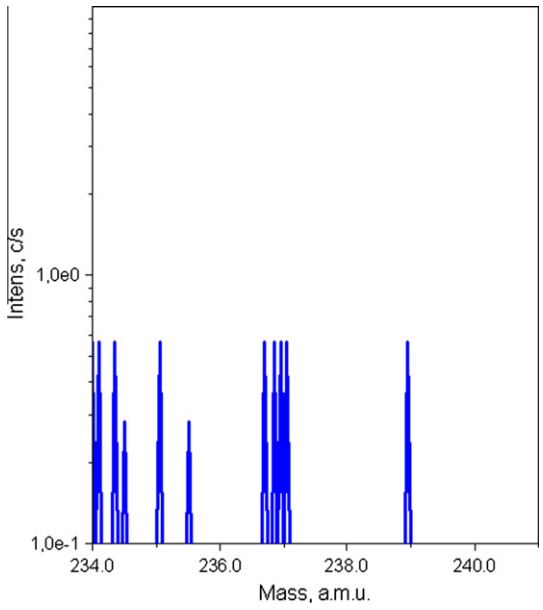

Fig. 4. Mass spectrum (recorded at mass around 238) of intact skin biopsy that was not exposed to uranium. *X* axis represents mass in atomic mass unit (a.m.u.), *Y* axis represents the signal intensity in counts per second (c/s).

After 24 h of exposure to uranium, intact and excoriated skin samples were analysed by SIMS microscopy (Fig. 5). SIMS images showed an uranium accumulation in the most external layer of the *stratum corneum* of intact skin explants (Fig. 5A), whereas no significant uranium accumulation was detected in the excoriated skin samples structures, in SIMS experimental conditions (Fig. 5B).

3.3. Calixarene percutaneous diffusion from calixarene nanoemulsion

The calixarene percutaneous diffusion was determined in Franz diffusion cell experiments prior to assessing its decontamination efficiency. A 1 ml calixarene nanoemulsion volume was deposited on intact and excoriated skin biopsies in the Franz cell donor compartment, and the diffusion of the calixarene molecule was assessed during 24 h under occlusive condition. No calixarene was detected by HPLC in the Franz diffusion cell receptor fluid during the 24 h experiments through both intact and excoriated skins.

3.4. Calixarene nanoemulsion efficiency on contaminated intact skin

The uranium diffusion kinetics was studied after the application of unloaded or loaded nanoemulsion on intact skin biopsies immediately after the contamination step. The application of the unloaded nanoemulsion did not significantly change the uranium diffusion kinetics (Fig. 6). By contrast, the application of the calixarene loaded nanoemulsion allowed reducing very significantly ($p < 0.01$) the diffused uranium quantity starting from 30 minutes following contamination. Moreover, the application of the 4 mg g^{-1} calixarene loaded nanoemulsion allowed firstly, a flux decrease in uranium from 1.10 ng cm^{-2} h^{-1} in absence of treatment to 0.02 ng cm^{-2} h^{-1} and secondly, an extremely significant decrease ($p < 0.001$) in the diffused uranium percentage after 24 h from 0.07 ± 0.03% in absence of treatment to 0.004 ± 0.003%.

At the end of the 24 h Franz cell diffusion experiments, intact skin biopsies were analysed by SIMS microscopy (Fig. 7). The images and mass spectra showed that after the application of the unloaded nanoemulsion (Fig. 7A), the uranium localisation was the same as that observed in absence of treatment (Fig. 5A), *i.e.*, uranium is accumulated in the most external layers of the *stratum corneum*. SIMS analysis revealed that, after the application of the loaded nanoemulsion on uranium-contaminated skin explants, the radionuclide was no more significantly detected in skin structures (Fig. 7B). Hence, there was a significant difference in uranium repartition in the cutaneous structures between the non-treated and the calixarene nanoemulsion-treated contaminated intact skin.

3.5. Calixarene nanoemulsion efficiency on contaminated excoriated skin

Unloaded or loaded nanoemulsion was deposited on excoriated skin biopsies immediately after the contamination step and the uranium diffusion kinetics was followed during 24 h (Fig. 8). The application of unloaded and loaded nanoemulsion allowed decreasing significantly ($p < 0.05$) the quantity of diffused uranium starting 4 h after contamination. The application of the unloaded nanoemulsion and of the loaded nanoemulsion respectively led to a 4-fold (14.1 ng cm^{-2} h^{-1}) and to a 36-fold (1.7 ng cm^{-2} h^{-1}) uranium steady-state flux reduction. The percentage of diffused uranium after 24 h was decreased from 39.5 ± 13.4% in absence of treatment to 7.5 ± 2.7% and to 1.0 ± 0.6% by unloaded and loaded nanoemulsion, respectively. The dilution effect on uranium diffusion kinetics was studied by depositing 600 μl of water in the Franz cell donor compartment immediately after the contamination step. The application of water on contaminated excoriated skins led to a 1.8-fold diminution of the uranium steady-state diffusion flux (34.0 ng cm^{-2} h^{-1}) and to an approximate 2-fold reduction of the diffused uranium percentage after 24 h (16.2 ± 8.0%). The values of pseudo steady-state flux of uranium, lag time and permeability coefficient for diffusion experiments on intact and excoriated skins are compiled in Table 1.

After 24 h of Franz cell diffusion experiments, SIMS images and mass spectra showed that no significant uranium accumulation was detected in the contaminated excoriated skin structures after the application of unloaded or loaded nanoemulsion. However, uranium traces were found to be retained in the areas, where a few residual *stratum corneum* layers remained after the tape stripping step (Fig. 9). There was thus no significant difference between these observations and those made when studying the uranium diffusion through excoriated skin in the absence of treatment (Fig. 5B).

3.6. Calixarene nanoemulsion efficiency on contaminated excoriated skin in case of delayed application

The effect of time elapsed between the skin contamination by uranium and the application of the treatment on the uranium diffusion kinetics was studied by delaying the application of the calixarene nanoemulsion by 5, 15 and 30 minutes. As illustrated in Fig. 10, uranium diffusion kinetics was roughly the same when the calixarene nanoemulsion was applied 5, 15 or 30 minutes after the contamination step. In these three cases, the calixarene nanoemulsion allowed a 2-fold uranium steady-state flux reduction (average steady-state flux of 32.4 ± 9.2 ng cm^{-2} h^{-1}) and a 3.5-fold uranium diffusion percentage decrease after 24 h (11.6 ± 2.8%) as compared to the absence of treatment.

4. Discussion

The goal of the present work was to evaluate the ability of the calixarene nanoemulsion to trap uranium on intact and excoriated skin. For this purpose, pig ear skin was chosen as model in Franz diffusion cell experiments because it is known for being

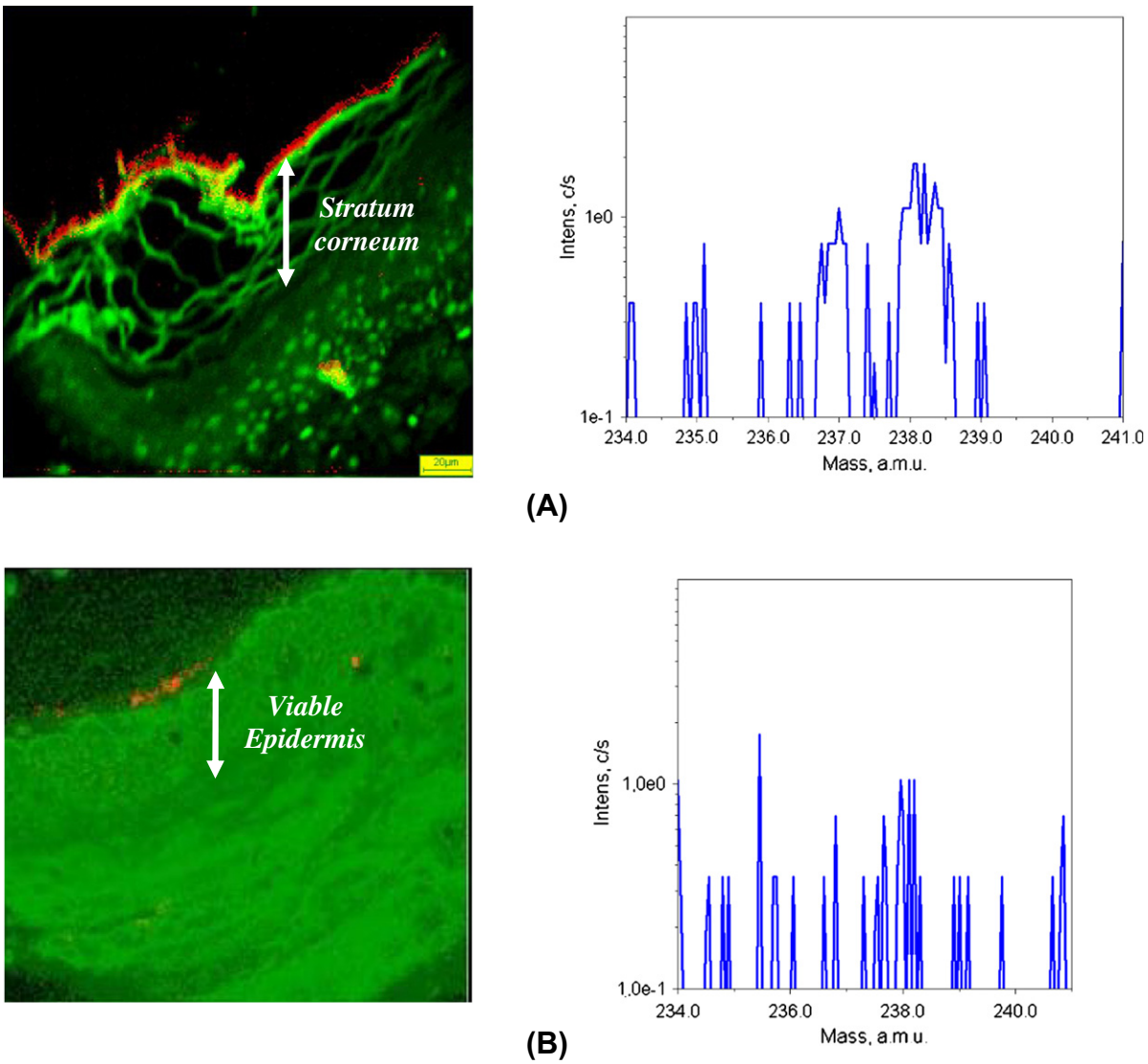

Fig. 5. Ionic images and corresponding mass spectra of intact (A) and excoriated (B) skin biopsies after 24 h of exposure to 10 mg l^{-1} uranyl solution: superposition of $^{238}U^{+}$ (red) and $^{40}Ca^{+}$ (green) images (200 µm × 200 µm image field).

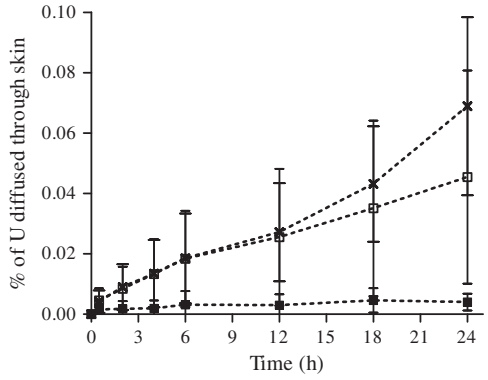

Fig. 6. Uranium percutaneous diffusion kinetics through intact skin in absence of treatment (x) or after application of unloaded nanoemulsion (□) or loaded nanoemulsion (4 mg g^{-1} calixarene) (■). The treatment was applied immediately after the contamination step and the percentage of uranium that diffused through intact skin biopsies was recorded during 24 h. Each point corresponds to the mean of 10 assays ± standard deviation.

the most representative model of human percutaneous absorption [26,37,38]. The external face of pig ear skin explants was put in contact with carbonate–bicarbonate buffer as receptor fluid. This solution ensures sink conditions and high uranium solubility, which was not the case of physiological phosphate buffer in which uranium precipitation was observed during our preliminary uranium solubility studies. Moreover, the use of 0.025 M carbonate-bicarbonate buffer allowed us reproducing the blood medium in which uranium is strongly bound to carbonate ions once it passed through the skin and penetrates into the bloodstream [39,40]. The integrity of intact skin explants in contact with this receptor medium was checked during 24 h by TEWL measurement before performing diffusion assays. The obtained TEWL values (5.2 ± 1.1 g m^{-2} h^{-1}) were in good agreement with the values recommended in the specifications of the used apparatus (Tewameter TM 300®, Monaderm®, Monaco) and in the literature [41]. All Franz diffusion cell experiments were conducted under occlusive condition to prevent evaporation and concentration phenomena of solutions deposited in the Franz cell donor compartment during the 24 h percutaneous diffusion studies.

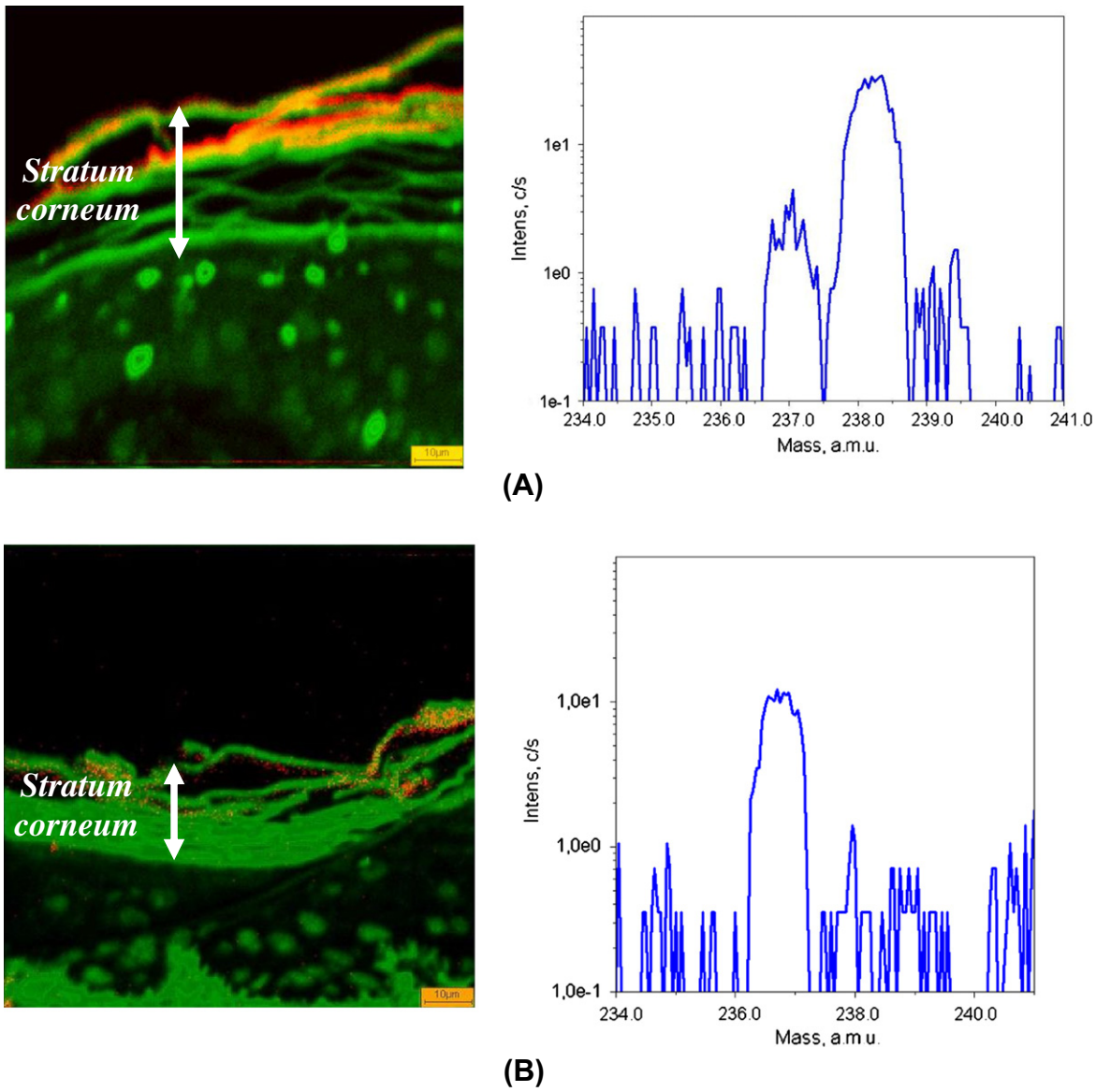

Fig. 7. Ionic images and corresponding mass spectra of intact skin biopsies treated immediately after the contamination step (10 mg l^{-1} uranyl solution) by unloaded nanoemulsion (A) or loaded nanoemulsion (4 mg g^{-1} calixarene) (B). Biopsies were analysed by SIMS microscopy 24 h after the beginning of Franz cell experiments. In these ionic images, $^{238}U^+$ image (red) was superposed to $^{23}Na^+$ (A; green) or $^{40}Ca^+$ (B; green) images (100 μm × 100 μm image field).

The low amount of uranium diffusing across intact pig ear skin without any treatment (0.07 ± 0.03% after 24 h) is close to the quantity passing through intact human skin in Franz diffusion cell experiments [24], but lower than the value determined on back rat skin model [4,24]. This confirms that pig ear skin represents a better animal skin model for uranium percutaneous diffusion study than rat skin. SIMS images of intact skin explants and corresponding mass spectra determined 24 h after contamination showed that uranium is mostly retained at the surface of the skin in the first most external *stratum corneum* layers. Uranium that is present in these layers can then be eliminated by natural skin desquamation [42]. Intact skin thus appears to be an efficient barrier against the entry of uranium into the body.

Concerning the uranium diffusion through excoriated pig ear skin, uranyl diffusion is 55-fold faster through excoriated skin than through intact skin, and the quantity of diffused uranium 24 h after the contamination step is 530-fold higher in case of excoriation, which corresponds to a percutaneous passage of around 40% of initially deposited uranium. Furthermore, contrary to the studies on intact skin, where SIMS technique allowed to observe a significant accumulation of uranium in the *stratum corneum*, no uranium accumulation was found in SIMS ionic images in the cutaneous structures of excoriated skin biopsies after 24 h of uranium contamination. Besides, percutaneous diffusion studies demonstrate that excoriation, which represents a common and superficial wound, leads to a dramatic increase in uranium diffusion flux and in the diffused uranium quantity as a function of time. This wound model, which is easy to produce by tape stripping and reproducible, thus, represents a suitable wound model for assessing the ability of local treatments for the decontamination of uranium-contaminated injured skin. Moreover, the fact that uranium can pass through both intact and superficial wounded skin in less than 30 minutes emphasises the need to treat very rapidly the cutaneous contamination.

Taking into account this fast diffusion of uranium through both intact and excoriated skin and the lack of specific and efficient

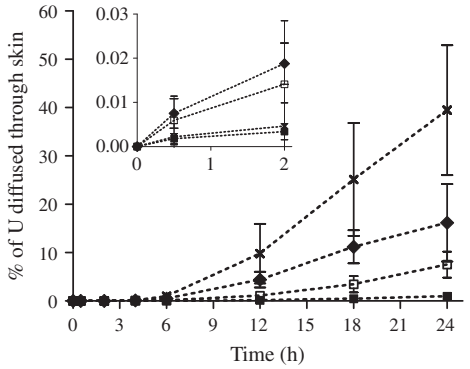

Fig. 8. Uranium percutaneous diffusion kinetics through excoriated skin in absence of treatment (x) or after application of water (♦) or unloaded nanoemulsion (□) or loaded nanoemulsion (4 mg g^{-1} calixarene) (■). The treatment was applied immediately after the contamination step and the percentage of uranium that diffused through excoriated skin biopsies was recorded during 24 h. Each point corresponds to the mean of 10 assays ± standard deviation.

topical or decorporation treatments in case of cutaneous contamination by uranium, we have designed a specific emergency treatment for contaminated intact and wounded skin and tested its decontamination ability using again pig ear skin explants.

Before the determination of the calixarene nanoemulsion potential for cutaneous uranium decontamination, the percutaneous diffusion of the calixarene molecule through intact and excoriated skin explants was preliminary investigated. Calixarene nanoemulsion was then deposited on the biopsies and calixarene content in the fluid receptor was measured during 24 h. It appeared that calixarene was not detected in the Franz diffusion cell receptor medium. Therefore, based on the detection limit of the HPLC method, less than 0.01% of the deposited calixarene quantity could have passed through both intact and excoriated skins without being detected, which represents an almost negligible amount.

The skin decontamination potential of the calixarene nanoemulsion was first evaluated on contaminated intact skin and the diffusion of uranium was compared with the one obtained in absence of treatment. From the diffusion kinetics data and SIMS images, it appears that the unloaded nanoemulsion has no significant decontamination effect on intact skin. The application of the calixarene loaded nanoemulsion on intact skin immediately after the uranium contamination step allowed a 94.2 ± 4.7% reduction of the quantity of uranium that diffused through the skin at the end of the 24 h Franz diffusion cell experiments and a 55-fold uranium steady-state flux decrease. SIMS images and corresponding mass spectra revealed that there was no uranium retention in intact skin structures. By comparing these SIMS images (Fig. 7B) to those obtained in absence of treatment (Fig. 5A) or after the application of the unloaded nanoemulsion (Fig. 7A), we can see that the treatment prevents the accumulation of uranyl ions in the *stratum corneum* and thus, its penetration through the other skin layers. According to these results obtained on intact skin and from our previous work that demonstrated *in vitro* the ability of the calixarene nanoemulsion to extract uranyl ions from a contaminated solution [23], we can conclude that the immediate application of the calixarene nanoemulsion after the cutaneous contamination step allows trapping and retaining the uranyl ions from an aqueous contaminated solution, which interferes with the uranium percutaneous transfer. Moreover, as SIMS images did not show any uranium accumulation after the contamination treatment by the calixarene nanoemulsion, it seems that the calixarene-uranium chelate does not significantly penetrate into intact skin structures. Thus, calixarene nanoemulsion constitutes an efficient treatment of skin contamination that acts at the skin surface. However, as intact skin can be considered as a sufficient barrier against the entry of uranium in the body, it is important to assess the efficiency of the calixarene nanoemulsion on a contaminated wound model.

Table 1
Pseudo steady-state flux of uranium through intact or excoriated pig skin after prompt or delayed application of treatment (mean ± SD of $n = 10$ determinations).

	Lag time (h)	Flux, J_{ss} (ng cm^{-2} h^{-1})	Permeability coefficient, K_p (cm h^{-1})
Prompt treatment			
Intact skin			
No treatment	9.7 ± 2.1	1.10 ± 1.40	$1.1 \times 10^{-4} \pm 1.4 \times 10^{-4}$
Unloaded nanoemulsion	7.1 ± 1.9	0.04 ± 0.04	$4.0 \times 10^{-6} \pm 4.5 \times 10^{-6}$
Loaded nanoemulsion (4 mg g^{-1} calixarene)	4.9 ± 3.2	0.02 ± 0.02	$2.0 \times 10^{-6} \pm 1.6 \times 10^{-6}$
Excoriated skin			
No treatment	8.4 ± 2.3	60.9 ± 40.3	$6.1 \times 10^{-3} \pm 4.0 \times 10^{-3}$
Water (1:1 v/v)	8.9 ± 1.6	34.0 ± 13.8	$3.4 \times 10^{-3} \pm 1.4 \times 10^{-3}$
Unloaded nanoemulsion	10.4 ± 1.2	14.1 ± 8.1	$1.4 \times 10^{-3} \pm 8.1 \times 10^{-4}$
Loaded nanoemulsion (4 mg g^{-1} calixarene)	10.5 ± 2.2	1.7 ± 1.4	$1.7 \times 10^{-4} \pm 1.4 \times 10^{-4}$
Delayed treatment			
Excoriated skin			
Loaded nanoemulsion (4 mg g^{-1} calixarene) 5 min after the contamination	11.0 ± 1.7	24.3 ± 12.7	$2.4 \times 10^{-3} \pm 1.3 \times 10^{-3}$
Loaded nanoemulsion (4 mg g^{-1} calixarene) 15 min after the contamination	9.5 ± 1.2	42.4 ± 20.4	$4.2 \times 10^{-3} \pm 2.0 \times 10^{-3}$
Loaded nanoemulsion (4 mg g^{-1} calixarene) 30 min after the contamination	8.9 ± 2.2	30.4 ± 22.2	$3.0 \times 10^{-3} \pm 2.2 \times 10^{-3}$

The skin decontamination potential of the calixarene nanoemulsion was then evaluated on a superficial wound model consisting in excoriated skin. It was observed in a preliminary study that the unloaded nanoemulsion allowed a 4.3-fold uranium diffusion flux diminution and a reduction of 83.0 ± 6.5% of the quantity of uranium that diffused through excoriated skin after 24 h of Franz diffusion cell experiments. This action of the unloaded nanoemulsion can be partly explained by the dilution effect of the contaminated solution due to the nanoemulsion aqueous phase. Indeed, the uranium diffusion flux obeys to the first Fick's law which stipulates that the diffusion kinetics at the steady-state is proportional to the difference in uranium concentration between the epidermal and the dermal faces of the skin [43]. So, by diluting the uranium concentration deposited on the epidermal face of skin biopsies, the percutaneous diffusion flux of the radionuclide should be decreased. This dilution effect was demonstrated by depositing pure water instead of unloaded nanoemulsion on the contaminated skin. The water application allowed a 1.8-fold diminution of the uranium steady-state diffusion flux and an approximated 2-fold reduction of the 24 h diffused uranium percentage. Moreover, it was demonstrated in a previous paper [23] that the unloaded nanoemulsion was able to extract around 20 % of uranium from a contaminated solution under the same conditions

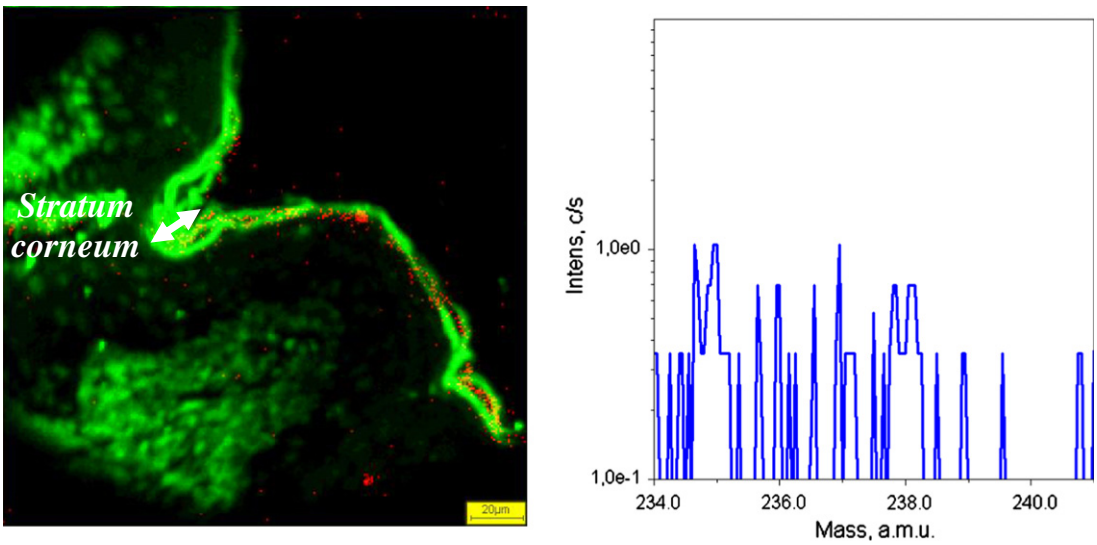

Fig. 9. ionic image and mass spectrum of excoriated contaminated skin biopsy treated immediately after the contamination step (10 mg l^{-1} uranyl solution) by loaded nanoemulsion (4 mg g^{-1} calixarene). Biopsy was analysed by SIMS 24 h after the beginning of Franz cell experiments. In the SIMS image, $^{238}U^{+}$ image (red) was superposed to $^{23}Na^{+}$ (green) image (200 µm × 200 µm image field).

contributing to the reduction of the uranium diffusion flux. The fact that the unloaded nanoemulsion effect was not significant on uranium diffusion through intact skin biopsies may certainly be explained by the low uranium diffusion and the high standard deviation observed in this case. SIMS images and corresponding mass spectra of excoriated skin biopsies 24 h after the contamination step and the application of the unloaded nanoemulsion were not significantly different from the ones obtained in absence of contamination treatment. Uranium is thus not retained in excoriated skin structures after application of the unloaded nanoemulsion on contaminated skin. The application of the calixarene nanoemulsion immediately after the contamination step induced a decrease of 97.5 ± 1.7% of the quantity of uranium that diffused through excoriated skin after 24 h of Franz diffusion cell experiments and allowed a 36-fold uranium diffusion flux diminution, which represents a 8-fold greater flux reduction than the one obtained by application of the unloaded nanoemulsion. SIMS analyses of excoriated skin biopsies at the end of the Franz diffusion cell experiment showed no uranium accumulation in the cutaneous tissues, as it was previously observed in absence of treatment of the skin contamination and after application of the unloaded nanoemulsion. From the results obtained in uranium diffusion assays and from SIMS images, we can conclude that the calixarene nanoemulsion quantitatively extracts and retains uranyl ions of a uranium-contaminated solution deposited on wounded skin. Furthermore, the calixarene nanoemulsion seems to act at the surface of the skin as no significant penetration of the calixarene-uranyl chelate was observed into the skin. In addition, it was observed that the calixarene nanoemulsion is less efficient in case of delayed application but still allows a very significant uranium diffusion decrease as compared to the absence of treatment application. This reduction of efficiency being approximately the same whatever the delay of application (5–30 min), we assume that a part of the deposited uranium fastly penetrates in the upper excoriated skin structures, saturates them and then diffused passively through the skin to later reach the receptor medium. Experiments are in progress to improve our understanding of this phenomenon. From these results, it appears that it is better to treat the cutaneous contamination as soon as possible to ensure an optimal decontamination efficiency of the calixarene nanoemulsion.

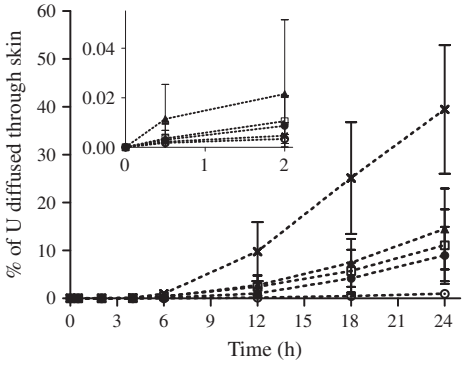

Fig. 10. Uranium percutaneous diffusion kinetics through excoriated skin in absence of treatment (x) and after either immediate application (○) or delayed application of loaded nanoemulsion (5 minutes (●), 15 minutes (▲), 30 minutes (γ)). The percentage of uranium that diffused through excoriated skin biopsies was recorded during 24 h. Each point corresponds to the mean of 10 assays ± standard deviation.

5. Conclusion

To sum it up, our study demonstrates that the calixarene nanoemulsion allows to decrease almost quantitatively the uranium percutaneous diffusion by trapping and retaining uranyl ions at the surface of the skin. This patented formulation [44] thus constitutes a promising therapeutic approach for the local treatment of intact and wounded skin contaminated by transferable uranium compounds. For optimal efficiency, the treatment should be applied as fast as possible after the contamination.

Further, *ex vivo* studies in Franz diffusion cell are in progress in order to compare calixarene nanoemulsion with other commonly used chelating molecules, such as DTPA or biphosphonates, in terms of U skin decontamination efficiency. Finally, since the calixarene molecules used in this study also efficiently chelate other actinides, we planned to evaluate the calixarene nanoemulsion efficiency on skin contaminated by other actinides such as plutonium and americium and to perform *in vivo* studies.

Acknowledgements

Research funds were granted by the Délégation Générale pour l'Armement (DGA) and the Institute for Radiological Protection and Nuclear Safety (IRSN).

References

[1] N. Blanchin, S. Desloires, L. Grappin, A.M. Guillermin, P. Lafon, A. Miele, Protocols in an occupational medical facility for the management of internal plutonium exposure incidents in a nuclear plant: development–application–analysis–validation from 1996 to 2002, Radioprotection 39 (2004) 59–75.
[2] B.M. De Rey, H.E. Lanfranchi, R.L. Cabrini, Percutaneous absorption of uranium compounds, Environ. Res. 30 (1983) 480–491.
[3] R. Lopez, P.L. Diaz Sylvester, A.M. Ubios, R.L. Cabrini, Percutaneous toxicity of uranyl nitrate: its effect in terms of exposure area and time, Health Phys. 78 (2000) 434–437.
[4] F. Petitot, A.M. Moreels, F. Paquet, In vitro evaluation of percutaneous diffusion of uranyl nitrate through intact or excoriated skin of rat and pig, Can. J. Physiol. Pharmacol. 82 (2004) 133–139.
[5] F. Petitot, S. Frelon, A.M. Moreels, M. Claraz, O. Delissen, E. Tourlonias, B. Dhieux, C. Maubert, F. Paquet, Incorporation and distribution of uranium in rats after a contamination on intact or wounded skin, Health Phys. 92 (2007) 464–474.
[6] F. Petitot, C. Gautier, A.M. Moreels, S. Frelon, F. Paquet, Percutaneous penetration of uranium in rats after a contamination on intact or wounded skin, Radiat. Prot. Dosim. 127 (2007) 125–130.
[7] R.L. Kathren, R.K. Burklin, Proposed standards for acute exposure to low enrichment uranium for compliance with 10 CFR 70.61, Radiat. Safety J 95 (2008) 122–127.
[8] R.L. Kathren, R.K. Burklin, Acute chemical toxicity of uranium, Health Phys. 94 (2008) 170–179.
[9] A. Bleise, P.R. Danesi, W. Burkart, Properties, use and health effects of depleted uranium (DU): a general overview, J. Environ. Radioact. 64 (2003) 93–112.
[10] A.C. Miller, D. Beltran, R. Rivas, M. Stewart, R.J. Merlot, P.B. Lison, Radiation and Depleted Uranium-Induced Carcinogenesis Studies: Characterisation of the Carcinogenic Process and Development of Medical Countermesures, NATO Human Factor Medicine Panel Research Task Group 099 "Radiation Bioeffetcs and Countermeasures" Meeting, 2005.
[11] ASN, The French Nuclear Safety Authority, National Guide: Medical Intervention in Case of Nuclear or Radiological Event, V3.6, 2008. <http://www.asn.fr/sites/default/files/files/Interventions_medicales_evenement_nucleaire_ou_radiologique.pdf>.
[12] C. Rojas-Palma, A. Liland, A. Naess Jerstad, G. Etherington, M. Del Rosario Pérez, T. Rahola, K. Smith, TMT Handbook – Triage, Monitoring and Treatment of People Exposed to Ionising Radiation Following a Malevolent Act, 2009.
[13] P.W. Durbin, S. Lauriston, Taylor lecture: the quest for therapeutic actinide chelators, Health Phys. 95 (2008) 465–492.
[14] P.W. Durbin, B. Kullgren, J. Xu, K.N. Raymond, Development of decorporation agents for the actinides, Radiat. Prot. Dosim. 79 (1998) 433–443.
[15] D. Muller, P. Houpert, M.H. Hengé-Napoli, H. Métivier, F. Paquet, Potential synergy between two renal toxicants: DTPA and uranium, Radioprotection 41 (2006) 413–420.
[16] N. Pavlakis, C.A. Pollock, G. McLean, R. Bartrop, Deliberate overdose of uranium: toxicity and treatment, Nephron 72 (1996) 313–317.
[17] P. Houpert, V. Chazel, F. Paquet, A local approach to reduce industrial uranium wound contamination in rats, Can. J. Physiol. Pharmacol. 82 (2004) 73–78.
[18] F. Yang, H. Guo, X. Cai, X. Chen, Syntheses and adsorption properties of novel calixarene polymers: Calix[6]-1,4-crown-4-based netty polymers, React. Funct Polym. 64 (2005) 163–168.
[19] K. Xu, W. Ge, G. Liang, L. Wang, Z. Yang, Q. Wang, I.M. Hsing, B. Xu, Bisphosphonate-containing supramolecular hydrogels for topical decorporation of uranium-contaminated wounds in mice, Int. J. Radiat. Biol. 84 (2008) 353–362.
[20] A. Spagnul, C. Bouvier-Capely, G. Phan, F. Rebière, E. Fattal, Calixarene-entrapped nanoemulsion for uranium extraction from contaminated solutions, J. Pharm. Sci. 99 (2010) 1375–1383.
[21] B. Boulet, C. Bouvier-Capely, C. Cossonnet, G. Cote, Solvent extraction of U(VI) by calix[6]arenes, Solvent Extr. Ion Exc. 24 (2006) 319–330.
[22] C. Bouvier-Capely, A. Manoury, A. Legrand, J.P. Bonthonneau, F. Cuenot, F. Rebière, The use of calix[6]arene molecules for actinides analysis in urine and drinking water: An alternative to current procedures, J. Radioanal. Nucl. Chem. 282 (2009) 611–615.
[23] A. Spagnul, C. Bouvier-Capely, M. Adam, G. Phan, F. Rebière, E. Fattal, Quick and efficient extraction of uranium from a contaminated solution by a calixarene nanoemulsion, Int. J. Pharm. 398 (2010) 179–184.
[24] H. Tymen, P. Gerasimo, D. Hoffschir, Contamination and decontamination of rat and human skin with plutonium and uranium, studied with a Franz's chamber, Int. J. Radiat. Biol. 76 (2000) 1417–1424.
[25] R. Duval, C. Cossonnet, C. Bouvier-Capely, C. Le Strat, B. Boulet, Para-Tertio-butylcalix[6]arenes Portant des Fonctions Triacides en Positions 2, 4 et 6, Membranes Liquides Supportées et Matériaux Supports les Comportant et Leurs Utilisations, Institut de Radioprotection et de Sûreté Nucléaire, French Patent, FR-2006/001105, 2006.
[26] W. Meyer, J. Kacza, N.H. Zschemisch, S. Godynicki, J. Seeger, Observations on the actual structural conditions in the stratum superficiale dermidis of porcine ear skin, with special reference to its use as model for human skin, Ann. Anat. 189 (2007) 143–156.
[27] S.J. Bashir, A.L. Chew, A. Anigbogu, F. Dreher, H.I. Maibach, Physical and physiological effects of stratum corneum tape stripping, Skin Res. Technol. 7 (2001) 40–48.
[28] H. Löffler, F. Dreher, H.I. Maibach, Stratum corneum adhesive tape stripping: influence of anatomical site, application pressure, duration and removal, Brit. J. Dermatol. 151 (2004) 746–752.
[29] H. Zhai, E. Dika, M. Goldovsky, H.I. Maibach, Tape-stripping method in man: comparison of evaporimetric methods, Skin Res. Technol. 13 (2007) 207–210.
[30] OECD, Guideline for the Testing of Chemicals. Draft Guideline 428: Skin Absorption: *in vitro* Method, 2004.
[31] N. Baglan, C. Cossonnet, F. Trompier, J. Ritt, P. Bérard, Implementation of ICP-MS protocols for uranium urinary measurements in worker monitoring, Health Phys. 77 (1999) 455–461.
[32] C. Bouvier-Capely, N. Baglan, A. Montegue, J. Ritt, C. Cossonnet, Validation of uranium determination in urine by ICP-MS, Health Phys. 85 (2003) 216–219.
[33] C. Bouvier-Capely, J. Ritt, N. Baglan, C. Cossonnet, Potentialities of mass spectrometry (ICP-MS) for actinides determination in urine, Appl. Radiat. Isotopes 60 (2004) 629–633.
[34] S. Halpern, Méthodes chimiques de préparation des échantillons, in: C. Quintana, S. Halpern (Eds.), Microanalyse en Biologie, SFME, Paris, 1983, pp. 93–104.
[35] R. Castaing, G. Slodzian, Microanalyse par émission ionique secondaire, J. Microsc. 1 (1962) 395–410.
[36] C. Tessier, D. Suhard, O. Simon, M. Floriani, F. Rebière, J.R. Jourdain, Detection and analysis of the microdistribution of uranium in the gills of freshwater Corbicula fluminea by SIMS technique, Nucl. Instrum. Methods Phys. Res. Sec. B: Beam Interact. Mater. Atoms 267 (2009) 1931–1935.
[37] SCCNFP, The Scientific Committee on Cosmetic Products and Non-Food Products Inteded for Consumers. Basic Criteria for the *in vitro* Assessment of Dermal Absorption of Cosmetic Ingredients, 2003.
[38] U. Jacobi, M. Kaiser, R. Toll, S. Mangelsdorf, H. Audring, N. Otberg, W. Sterry, J. Lademann, Porcine ear skin: an in vitro model for human skin, Skin Res. Technol. 13 (2007) 19–24.
[39] S. Chevari, D. Likhner, Complex formation of natural uranium in blood, Kompleksoobrazovanie Estestvennogo Urana v Krovi, Meditsinskaia Radiologiia 13 (1968) 53–57.
[40] P.W. Durbin, Metabolic Model for Uranium, in: Biokinetics and Analysis of Uranium in Man, Washington, 1984.
[41] J. Nikolovski, G.N. Stamatas, N. Kollias, B.C. Wiegand, Barrier function and water-holding and transport properties of infant stratum corneum are different from adult and continue to develop through the first year of life, J. Invest. Dermatol. 128 (2008) 1728–1736.
[42] M.B. Reddy, R.H. Guy, A.L. Bunge, Does epidermal turnover reduce percutaneous penetration?, Pharm Res. 17 (2000) 1414–1419.
[43] J. Zatz, Skin Permeation – Fundamentals and Application, Allured Publishing Corporation, 1993.
[44] C. Bouvier-Capely, E. Fattal, G. Phan, F. Rebière, A. Spagnul, Formulations Pharmaceutiques de Molécules de Calix[6]arènes pour le Traitement de Contaminations Cutanées par des Actinides, Institut de Radioprotection et de Sûreté Nucléaire, French Patent, FR-08/58703, 2008.