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ABSTRACT

Accidents with toxic industrial chemicals and threats posed by chemical warfare agents, as organophosphorus nerve agents and blistering agents, has become an increasing concern over the last decades. Intoxication is not only due to inhalation but dermal route can also be important and can quickly lead to death. It is therefore crucial to have suitable means of personal protection and decontamination. Some are already commercially available but often present drawbacks. Efforts have been made to develop new systems and several studies have shown the interest of metallic nanoparticles. The challenge remains to entrap the particles in an adapted matrix to limit their diffusion in the immediate environment, while preserving the bare particles decontamination efficacy. In this study, an easy to handle peelable gel, incorporating CeO_2 nanoparticles has been developed for efficient skin decontamination against toxic chemicals. The main objective has been to render it suitable in any context of application (battlefield or civil exposure). Nano-octahedra (NO) CeO_2 particles synthetized in this study have been able to increase the degradation of paraoxon, a simulant warfare agent in liquid media. NO have then been incorporated in a peelable gel, the formulation has been optimized through design of experiments and its physico-chemical properties characterized. Even after incorporation to the gel, NO retained their neutralization properties. The formulation efficiency towards neutralization and skin decontamination have been evaluated *in vitro* on Franz diffusion cells. The interest of the peelable gel have been clearly demonstrated with a decrease by about 5-fold of the quantity of POX absorbed into the skin and a decrease of 3 to 4-fold in the viable skin, which was much higher than the use of cotton or nanoparticles as powder.

KEY WORDS

Cerium dioxide nanoparticle - Peelable gel – Skin decontamination – Paraoxon

1 INTRODUCTION

In an international context strongly marked since 2013 by the use of chemical weapons, in war or terrorist attacks [1], the development of effective countermeasures against organophosphorus nerve agents - OPs (VX, sarin, soman, tabun) and blistering agents (organochlorines - OCs) such as sulphur mustar appears essential both for battlefield or civil situation [2]. Intoxication is not only due to inhalation but dermal route can also be important and can quickly lead to death [2]. VX is the best known and most widely used of OPs because of its very low volatility and high toxicity which is explained by the presence of a P=O bond. This bond inhibits the enzyme acetylcholinesterase (AChE), an essential enzyme in the transmission of nerve signals, [1], [2], [3]. The oily appearance and lipophilic nature of VX allows it to penetrate the skin and, a single drop can cause death very quickly. It is therefore crucial to have the appropriate personal protection and decontamination equipment [4]. The objective of decontamination is to eliminate and ideally to degrade the chemical substances present on the victim's skin surface, clothing or surrounding surfaces in a very short time in order to limit intoxication and contamination transfer. The ideal decontamination systems should be effective on a wide spectrum of chemicals, easy to handle and prevent subsequent recontamination.

Different decontamination systems have been proposed and vary according to the nature of the materials used, their form (solid, liquid,...), their mechanism of action (displacement/adsorption of the toxicant or degradation into safer by-products), etc. [5]. Washing the skin with water or soap and water is probably the simplest and least expensive decontamination strategy. It neutralizes the contaminant by dilution and sometimes by partial hydrolysis. However, recent studies have shown that these methods often result in incomplete decontamination [6] and, may also elicit contaminant absorption due to the “wash-in” effect [7], [8]. Indeed, water associated or not to soapy solutions or chemicals is known to be a conventional enhancer that may increase skin permeability of molecules probably due to the temporary skin hydration leading to a reduction in skin barrier properties [9]. Zhu et al. [10] have recently shown that the penetration of benzoic acid and paraoxon into the stratum corneum

increased with the stratum corneum hydration levels. Other drawbacks of such strategy are the need of large volumes of water and the fact that they do not fully degrade the toxicant. Therefore, it generates large quantities of toxic waste and increases secondary contamination risks [6], [7]. Fuller's earth has been frequently used as decontaminant. This safe powder is an effective adsorbent for various chemicals as VX and mustard gas and has been shown to reduces their penetration into the skin [11], [12], [13], [14]. Fuller earth leads to a rapid decontamination, is inexpensive and stable over time. However, the powder is relatively volatile and inhalation of contaminated powder remains a risk. To overcome this drawback, a glove retaining the powder can be used. Nevertheless, Fuller's earth does not degrade toxics once absorbed at its surface, which increases the risk of cross contamination [11], [12], [13], [14]. Decontamination systems able to degrade the toxics into safer by products exist as the Reactive Skin Decontamination Lotion (RSDL), which is the most widely used liquid decontaminant for skin. It is currently considered as a standard decontamination lotion as such or soaked in a sponge for easier application [4], [11], [15], [16], [17], [18]. However, the product cannot be applied on wounds and eyes, is relatively expensive and not very stable over time.

In the context of the development of new effective strategies for skin decontamination, research has focused on systems inexpensive and easy to handle, able to displace and degrade toxic compounds, to prevent any spread and to facilitate waste treatment. Several studies have shown the interest of metal oxides (TiO_2, MgO, ZnO, Al_2O_3...) [19], [20], [21], [22], [23], [24] for adsorption and neutralization of chemicals into safer by-products. For example, we have previously synthesized ceria (CeO_2) nanoparticles and demonstrated *in vitro* their interests for the degradation of paraoxon (POX), a pesticide and model for OPs, in aqueous solution [25], [26], [27], [28]. *In vitro* experiment performed with Franz cells have also showed that CeO_2 suspensions and RSDL were the most efficient to remove POX from the skin surface and decrease skin absorption compared to the control (not decontaminated skin) [27]. Other studies have focused on the use of ceria for the degradation of various chemicals as VX, Soman or sulfur mustard [27], [29], [30], [31] demonstrating its interest in this context. CeO_2 nanoparticles have found application in the field of biomedicine owing to their low toxicity. They have

demonstrated good tolerance in both *in vitro* and *in vivo* biological models, as indicated by Das et al. [32]. This favorable profile supports their potential use in skin decontamination systems. However, it is crucial to thoroughly investigate the safety of ceria particles upon skin application, as it can significantly impact their practical application. Forest et al. conducted an in vitro study on macrophages from the RAW264.7 cell line to explore the impact of ceria nanoparticles [33]. The results revealed no reactive oxygen species (ROS) production, but there was a dose-dependent increase in LDH release and TNF-α production with rod-like nanoparticles, while no variation was observed with cubic/octahedral nanoparticles. Hence, the nanoparticle shape plays a pivotal role and must be carefully considered in the development of CeO_2-based skin decontaminant systems. In our study, we specifically chose nano-octahedra particles (NO). Additionally, the penetration of nanoparticles into the skin is a critical parameter. Generally, nanoparticle penetration is strongly influenced by their size. Nanoparticles with a size below 4 nm can penetrate and permeate intact skin, those between 4 and 20 nm can potentially permeate intact and damaged skin, and those between 21 and 45 nm can penetrate and permeate only damaged skin. Nanoparticles larger than 45 nm cannot penetrate or permeate the skin [34]. To prevent the penetration of CeO_2 nanoparticles into the skin, larger sizes may be employed, or nanoparticles can be entrapped in a matrix to hinder their penetration. This strategic approach is the focus of our current study.

Most of the time, metal oxides are used as powder but extremely fine powders should be avoided to limit dispersal and possible inhalation and pulmonary effects. The technological issue is therefore to entrap metal oxide particles in a system able to limit their diffusion in the immediate environment, while preserving the bare particles decontamination efficacy. Only few solutions have been proposed so far. Systems with metal oxide particles adsorbed onto tissues have been designed. For example, $ZnTiO_3$ and $AgNO_3$ have been incorporated into polyester wipes and their efficiency evaluated on diethylchlorophosphate and sulfur mustard simulants [35]. Metal oxides have also been embedded in gloves as Fast-act, a glove combining titanium dioxide (TiO_2) and magnesium oxide (MgO), and efficient against various chemicals [12], [13]. Only few other studies have focused on other type of formulations

such as metal oxide incorporated into a cream [36], [37], [38] or suspension of Fe_3O_4 grafted onto polymers [39]. However these formulations were either used as skin protectant and not as decontaminant ([36], [37], [38]) or their skin efficiency has not been studied [39]. In this context, the objective of this study was to develop an innovative CeO_2-based formulation for efficient skin decontamination against toxic chemicals. Our scientific investigation presents several distinctive features that differentiate it from prior research. The formulation designed here: 1) allows absorption and degradation of the toxic agent, and limits waste (on the contrary to the use of water or Fuller's Earth) ; 2) enables easy application and removal; it is adapted to the context of application, battlefield or civil exposure (unlike volatile powders or solutions requiring a sponge) ; 3) limits the amount of water to avoid a wash-in effect; 4) allows its use on damaged skin (unlike RSDL). A peel-off gel incorporating CeO_2 nanoparticles in its network has been developed. It has continuous thick consistency and can be applied on the skin. It is allowed to dry in few minutes and then it is peeled off. The framework of our study includes nanoparticles synthesis and characterization, formulation and a thorough understanding of interactions and optimization through design of experiment. Finally, the formulation has been optimized and its properties characterized. Its efficiency towards neutralization and skin decontamination of a simulant warfare agent (paraoxon, POX) has been evaluated *in vitro* on Franz diffusion cells.

2 MATERIAL & METHODS

2.1 MATERIALS

Cerium nitrate hexahydrate $Ce(NO_3)_3{\cdot}6H_2O$ (99.5%) was purchased from Alfa Aesar and ammonium hydroxide NH_4OH (ACS grade) from Carlo Erba. POX (O,O-diethyl p-nitrophenyl phosphate, purity 90%) was purchased from Sigma. Ethanol (96.3%) and P-nitrophenol (PNP, 99% purity) from Acros Organics Company, methanol and glacial acetic acid (HPLC grade) were purchased from Fisher Scientific. Kollidon® SR (physical mixture of 80% polyvinyl acetate + 19% povidone, Kollidon® 30) and Lutrol F127

(polyoxypropylene-polyoxyethylene block copolymer or poloxamer 407) were a gifted by BASF. Klucel HF Pharm (hydroxypropyl cellulose, HPC, MW= 1 150 kDa), Klucel EF Pharm (HPC, MW = 80 kDa) were a kind gift from Ashland. Cekol 30 000 (carboxymethyl cellulose, CMC, MW = 750 kDa) were gifted from Cp Celco. Carboxymethyl cellulose (CMC, MW = 90 kDa) were purchased from Acros Organics. Ultrapure water (resistivity > 18 MΩ.cm) was used for formulation and analysis. All chemicals were obtained and used without further purification.

2.2 METHODS

2.2.1 Nano-octahedra (NO) CeO_2 particles synthesis and characterization

The nanoparticles synthesis was performed by hydrothermal process as described in the literature [26]. Briefly, 0.0255 mol of $Ce(NO_3)_3 \cdot 6H_2O$ was dissolved in 20 mL of distilled water. This solution was added to a 130 mL solution containing 0.102 mol of ammonium hydroxide NH_4OH at room temperature under strong stirring. After stirring for 20 min, the solution was transferred into a Teflon-lined stainless-steel autoclave and heated at 180 °C (10.5 bars) for 24 hours. After hydrothermal treatment and autoclave cooling, the precipitate present in the reaction medium was then recovered by centrifugation, washed four times with distilled water and dried in an oven at 60°C for 10 hours in ambient air. 4.4 g of nano-octahedra powder were obtained for 11.1 g of cerium nitrate hexahydrate used (one batch, about 100% yield). A heat treatment was then carried out at 500°C for 2 hours. The NO obtained were characterized by transmission electron microscopy (TEM) to evaluate the size and shape of the nanoparticles, by the gas adsorption technique (BET) for the determination of the specific surface area and by X-ray diffraction to lattice parameters and crystallite size determination. The particles were observed by transmission electron microscopy (TEM) using a JEOL 2100 HT operating at 200 kV. High-resolution transmission electron microscopy (HRTEM) images were also performed. The TEM samples were prepared by drop-casting the suspension onto TEM grids. The size of the nanoparticles was determined from TEM images by counting 100 particles according to the procedure described by Trenque et al. [25]. The specific surface area of the as-prepared samples was calculated

from the adsorption/desorption isotherms of nitrogen at 77 K using a BELSORP-max instrument (Passy, France). The Brunauer–Emmett-Teller (BET) method was used for surface area calculation. X-ray diffraction (XRD) patterns were recorded on a Bruker D8 Advance diffractometer equipped with a sealed Cu X-ray tube (40 kV, 40 mA) and a linear Lynxeye XE detector. Data were collected over the range 5–90° with 0.0205° step size. The lattice parameters and the crystallite sizes were extracted from XRD patterns by profile matching (LeBail fit) using the elementary pseudo-Voigt function with the FullProf program packages.

2.2.2 POX degradation in solution

The *in vitro* degradation of POX and study of the apparition of PNP was performed (n=3-5). Suspension of NO in water (pH=7) or EtOH were prepared and temperature adjusted at 32°C. At t=0, a solution of POX was added to NO suspension to reach a POX concentration of 0.055 $g.L^{-1}$ (200 mg NO, 2 mL). Suspension were stirred at 37°C for 5 h. At different time point of contact between POX and NO, supernatant was collected, diluted by 10 in water, filtrated on 0.2 µm to remove NO and analyzed by HPLC. A one-order kinetic model has been used to fit the data [28]. A POX solution without NO was taken as control.

2.2.3 Quantification of POX and PNP by HPLC

POX and PNP were analyzed from samples using high-pressure liquid chromatography equipped with a XTerra® MS-C18 column (5 µm, 4.6 mm×250 mm) as already described [25], [27]. An HPLC Waters Alliance 2695 coupled with a 2998 photodiode array detector (PDA), working at 269 and 314 nm wavelength, was used. The column oven temperature was maintained at 40°C. The calibration curve was prepared between 0 and 100 µg/mL. The elution of 10 µL of the samples with methanol/water (acidified with 0.5% (v/v) acetic acid) at a flow rate of 0.7 $mL.min^{-1}$ gave a retention time of 7.5 min for POX and 5.5 min for PNP. For all experiments, samples were always filtrated on 0.45 µm membrane disc filter before injection.

2.2.4 Peelable gel formulation

2.2.4.1 Choice of raw materials

A peel-off gel is a topical product that dries to form an occlusive film layer that can be peeled off after application. Peel-off gel contains film-forming and gelling agents. The gel must have good skin adhesive properties, a shear thinning behavior and, a short drying time. It must stay flexible even after the drying process. In this part, different tests were carried out to select raw materials giving a final product with appropriate properties for use as a skin decontamination product. Various cellulose derivatives were selected and tested at different molecular weights as gelling agents for their mucoadhesive properties and because they provide high viscosities (carboxymethyl cellulose CMC, hydroxypropyl cellulose HPC). Different film-forming agents were also tested: Kollidon® SR (mixture of 80% of polyvinyl acetate and 19% povidone), pure PVP, PVP-hexadecene copolymer and PVP-eicosene copolymer. Finally, different amount of NO particles synthetized as reported in 3.2.1 were added.

A typical formulation procedure was as following: the solvents were mixed with a deflocculating stirrer at 300 rpm (Rayneri VMI) (10 mL ethanol and 2 mL water). Kollidon® SR (3 g), poloxamer 407 (1 g), carboxymethyl cellulose (0.3 g) were added progressively parts by parts under stirring. The mixture was then covered with an aluminum foil to avoid solvent evaporation. Then, the NO particles (1 g) were gradually dispersed in the gel and the stirring process was continued for 4 h to ensure a homogenous dispersion. Gels were stored in an airtight bottle.

All formulations were characterized before drying in order to determine their properties in terms of texture, stability, and drying time as described below in part 2.2.5. The texture analyzer measurements and rheology studies were performed to link sensory parameters and physicochemical properties.

2.2.4.2 Design of experiment

The formulation was optimized by using a 2^{k-1} fractional factorial design where k represents the number of experimental factors to study (k=5), each at two levels [40]. The chosen experimental domain or compounds used in the design of experiments (DOE) were selected according to the

preliminary tests. The variable experimental factors with their two levels and the fixed parameters are reported in Table 1.

Table 1: Fixed and variable factors used for the experimental design.

Variable parameters		
Coded level	(-1)	(+1)
Kollidon® SR quantity (X_1)	2 g	3 g
Poloxamer 407 quantity (X_2)	0.5 g	1.5 g
Type of thickening agent (X_3)	HPC MW = 1 150 kDa (Klucel HF)	HPC MW = 80 kDa (Klucel EF)
Quantity of thickening agent (X_4)	0.3 g	0.8 g
Ratio EtOH/Water (X_5)	80/20	99/1
Fixed parameters		
Nanoparticles quantity	1 g	
Total solvent	9.89 g	

The 16 runs defined by the experimental design are described in the Table 2. The DOE objective was to improve the formulation of the gel regarding its use on skin. Results of the different characterizations are presented in the Supplementary Information (Table S1).

Table 2: Formulations prepared according to a 2^{5-1} fractional factorial design.

N°Exp	Kollidon® SR (g)	Poloxamer 407 (g)	Type of thickening agent	Quantity of thickening agent (g)	Ratio EtOH/Water
F1	2	0.5	HPC 1 150 kDa	0.3	99/1
F2	3	0.5	HPC 1 150 kDa	0.3	80/20
F3	2	1.5	HPC 1 150 kDa	0.3	80/20
F4	3	1.5	HPC 1 150 kDa	0.3	99/1
F5	2	0.5	HPC 80 kDa	0.3	80/20
F6	3	0.5	HPC 80 kDa	0.3	99/1
F7	2	1.5	HPC 80 kDa	0.3	99/1
F8	3	1.5	HPC 80 kDa	0.3	80/20
F9	2	0.5	HPC 1 150 kDa	0.8	80/20
F10	3	0.5	HPC 1 150 kDa	0.8	99/1
F11	2	1.5	HPC 1 150 kDa	0.8	99/1
F12	3	1.5	HPC 1 150 kDa	0.8	80/20
F13	2	0.5	HPC 80 kDa	0.8	99/1
F14	3	0.5	HPC 80 kDa	0.8	80/20
F15	2	1.5	HPC 80 kDa	0.8	80/20

F16	3	1.5	HPC 80 kDa	0.8	99/1

The use of such DOE consists in approximating the experimental gel characteristics to be improved (Y_i) by a two-factor factorial model written as:

$$\hat{Y}_i = b_0 + \sum_{i=1}^{5} b_i X_i + \sum_{i,j=1}^{5} b_{ij} X_i X_j \quad (1)$$

where $\hat{Y}_i$ is the predicted response (with gel hardness (Y_1), relaxation (Y_2), stickiness (Y_3), stability score (Y_4)), b_0 is the intercept, the coefficient b_i is the main effect of the coded factor X_i and the coefficients b_{ij} are the two-factors interaction effects. The coefficients of Eq. (1) were determined for each response by ordinary least square regression. Multiple linear regression analyses were performed using NemrodW® software (LPRAI, Marseille, France).

2.2.5 Peelable gel characterizations

2.2.5.1 Characterization of the gel, before drying

Stability of the formulations was evaluated by keeping the gel in an air-tight vial at room temperature or 45°C for 6 months and checking for any visual change in the formulation (sedimentation, gel syneresis). A score from 0 to 10 was then given to rank the formulations according to their stability, 10 corresponding to the most stable formulations (no change detected) and 0 to the less stable formulations (sedimentation of gel syneresis detected after preparation and no return to an homogeneous suspension after manual mixing for 30 s). The texture analyzer was used to determine the hardness, elasticity and stickiness of the gels and to link sensory parameters to physicochemical properties in order to optimize the formulation. Different parameters (choice of probe, probe approach and return speed, probe insertion distance and relaxation time) were optimized in order to obtain repeatable results. Characterizations of the gels were performed with the TX-700 texture analyzer (LAMY Rheology) using a flat probe (d = 4 cm) and an extrusion cell. Parameters for the

compression and relaxation study were: speed: 1.5 mm/s, depth: 5 mm and relaxation time: 30 s. For the traction study, parameters were: manual approach at the gel surface, 60 s relaxation before traction, traction speed: 0.5 mm/s. According to preliminary tests, a satisfactory texture should have hardness values around 5 N, stickiness close to 0 N and a percentage of relaxation (which is inversely proportional to the elasticity of the product) above 90%. The film drying process was evaluated by spreading the gels on glass slides and on membranes (Strat-M® membranes, Milipore) as interactions were different for glass or membrane and placing them at 32°C to mimic the skin surface temperature. The film drying was observed until detachment of the glass side or membrane was possible.

2.2.5.2 Characterization of the film obtained after drying

The flexibility of the films obtained after drying of the peelable gel was characterized with the TX-700 texture analyzer (LAMY Rheology) using a film compression bench and a compression method with a probe insertion of 20 cm in the film. Images of the films as well as a mapping of the cerium atoms were obtained by using a Scanning Electron Microscope (FEI Quanta 250 FEG) with copper or carbon metallization (deposition = 10 nm) and an acceleration voltage of 10 kV coupled with energy dispersive X-ray spectroscopy (EDX).

2.2.6 Degradation of POX by peelable gel

POX (25.4 µL) was gently mixed in the formulation F2 (prepared with and without NO particles, 0.5 g). After 30 min at room temperature, 40 mL of EtOH was added, and the solution was vortexed for 1 min to extract POX from the formulation. Solution was then diluted in EtOH, filtrated on 0.45 µm nylon membrane disc filter and analyzed with HPLC. Experiment was performed in triplicate. Results are reported as mean ± SD.

2.2.7 Evaluation of skin decontamination efficiency

Pig-ears were collected immediately after animals were killed (Ecole de Chirurgie, University Lyon 1, France). They were stored flat at -20°C for a maximum period of 6 months. The skin was prepared following the procedure described in litterature [41]. 5 replicates for each decontamination systems

were performed. On the day of the experiment, the skin samples (1.2 ± 0.1 mm thick) were thawed and mounted on Franz-type static glass diffusion cells with a surface area of 2.54 cm^2. Ultrapure water was used as acceptor medium. The cells were maintained at 37°C in a water bath in order to reach the *in vivo* skin surface temperature of 32 ± 1°C. Skin integrity was assessed by measuring the transepidermal water loss (Tewameter TM300, Courage & Khazaka). Only skin explants with transepidermal water loss values ranging between 3 and 10.$g.h^{-1}.m^{-1}$ were used in the penetration studies.

For decontamination study, 25.4 µL of pure POX divided into microdroplets were applied on the pig-ear skin surface in the donor compartment of the cell. This resulted in an applied dose of Q_0 = 13 mg/cm^2 (or 10 µL/cm^2 or 46 µmol/cm^2) and corresponds to a full coverage of the skin. The solubility of POX in the acceptor medium (2.4 mg/mL at 25°C) ensured that sink conditions were applied since the highest POX concentration in the acceptor medium after the series of experiment was 5 µg/mL. Consequently, it was not a rate-limiting step in skin absorption. After 30 minutes of exposure to the toxic, the skin was decontaminated by applying the different decontamination systems: peelable gel containing NO particles, peelable gel without NO particles, NO particles as powder and cotton only. Some skin samples were not decontaminated and are referred as control in the following. The course of experiment was 24h.

For gel (containing NO or not), 0.5 g of gel was deposited which corresponds to 34 mg of NO. This quantity of gel leads to a total coverage of the skin surface, while maintaining a correct thickness. This corresponds to the use of the system in real-life conditions. Gel was massaged on the surface in a circular motion on each skin disc for 20 seconds with a cotton swab covered with part of a glove to mimic a manual application. The cotton swab and glove were then added to 20 mL EtOH for POX extraction and HPLC analysis. 60 min after the contamination (ie 30 min after application of the decontamination systems), gels were removed and added to 20 mL EtOH for POX extraction and HPLC analysis. The skin surface was then gently wiped with 3 cotton balls (2 slightly damp and one dry) to

297 remove the last residues of gels. Cottons were then added to 20 mL EtOH for POX extraction and HPLC
298 analysis. Simulants permeation assay was continued for 24 hours to ascertain the impact of the skin
299 decontamination process on dermal penetration and percutaneous absorption. For decontamination
300 with the NO particles powder, the same protocol was followed but 34 mg of NO were directly
301 deposited by sprinkling the entire surface with the powder. Tests were also performed following the
302 same protocol but without addition of gel or powder (samples are called cotton in the following).
303 Finally, some skin samples were not decontaminated and are referred as control in the following
304 (n = 5).

305 24 h after POX exposure, skin surface was washed 3 times with 3 mL of distilled water and dried with
306 absorbing paper. The three washings and absorbing papers were each added to 17 mL EtOH, filtered
307 and analyzed by HPLC analysis. Then the acceptor medium was collected. Skin samples were removed
308 from the diffusion cells. The first strip S1 was quickly removed using D-Squame tape (Monaderm) and
309 placed in a vial with 3 mL of EtOH. S1, the washing water and absorbing papers at 24 h represent the
310 POX remaining on the surface of the skin after decontamination and is called surface (S) in the rest of
311 the article. The stratum corneum was separated from the viable epidermis and dermis using the
312 stripping method and, stripes were pooled three by three and placed in different vials each containing
313 3 mL of ethanol (12 strips were used during this step). Then, the viable epidermis (E) was separated
314 from the dermis by heat treatment (45 s in water at 60 °C), cut into small pieces and deposited in a vial
315 containing 2 mL of ethanol. The vials were placed for 2h under magnetic stirring at room temperature
316 to extract POX. The dermis (D) was cut into small pieces and placed in a vial filled with ethanol. It was
317 extracted 4 times under magnetic stirring at room temperature, 1 time with 4 mL EtOH and 3 times
318 with 2 mL EtOH. After each extraction, aliquots were diluted if needed and filtered on a nylon
319 membrane disc filter (0.45 µm pore-size, 25 mm diameter) before HPLC quantification. It was verified
320 that this protocol led to a 100 % recovery of POX. The 100% recovery was verified on samples not
321 decontaminated at all, samples decontaminated with cotton only and samples decontaminated with
322 gel not containing NO particles (ie: all the decontamination systems which don't involve a chemical

degradation of the POX). In all cases, the total POX recovered from all the fractions was above 85% of the initial dose deposited.

The mean and standard error (SD) of n = 5 determinations were calculated. Statistical comparisons were made using nonparametric test of Mann-Whitney (with the two-sided level of significance α = 0.05).

Three ratios were calculated to compare the different decontaminant systems with the control (no decontamination):

$$Ratio = \frac{\%\ of\ POX\ for\ the\ control}{\%\ of\ POX\ for\ the\ decontaminant\ system} \quad (2)$$

Ratio 1 corresponds to total amount of POX recovered at the surface (S) and into the skin (stratum corneum (SC)+ epidermis (E) + dermis (D)+ AM (acceptor medium)) for the control comparatively to the decontaminant system. Ratio 2 corresponds to the same ratio for the percentage of POX absorbed into the skin (SC+E+D+AM). Ratio 3 corresponds to the ratio for the percentage of POX accumulated in the viable skin (E+D+AM).

3 RESULTS

3.1 NO CeO_2 PARTICLES SYNTHESIS AND CHARACTERIZATION

Synthesis of CeO_2 nano-octahedra (NO) was performed as described in part 3.2.1 and in previous publication via a solvothermal pathway [26]. NO characteristics are shown in Figure 1 and in Table 3 and 4. Small nanoparticles (6-37 nm) with {111} facets were obtained. Their potential for the degradation of organophosphorus (OPs) was evaluated in this study using paraoxon (POX). This compound has physicochemical properties and chemical functions similar to VX but is less toxic and has been shown to be an interesting simulant [41], [42], [43]. Degradation of POX with or without NO was studied in water and ethanol, the solvents used in the formulation of the peel-off gel. Fit of the

data by a pseudo-first order decay model for POX without nanoparticles was not possible as almost no degradation was detected in 5 h. The degradation of POX increased in the presence of NO particles. As expected, an increase in para-nitrophenol (PNP), one of the POX degradation products, was detected. These results demonstrate the interest of using such nanoparticles for decontamination purposes.

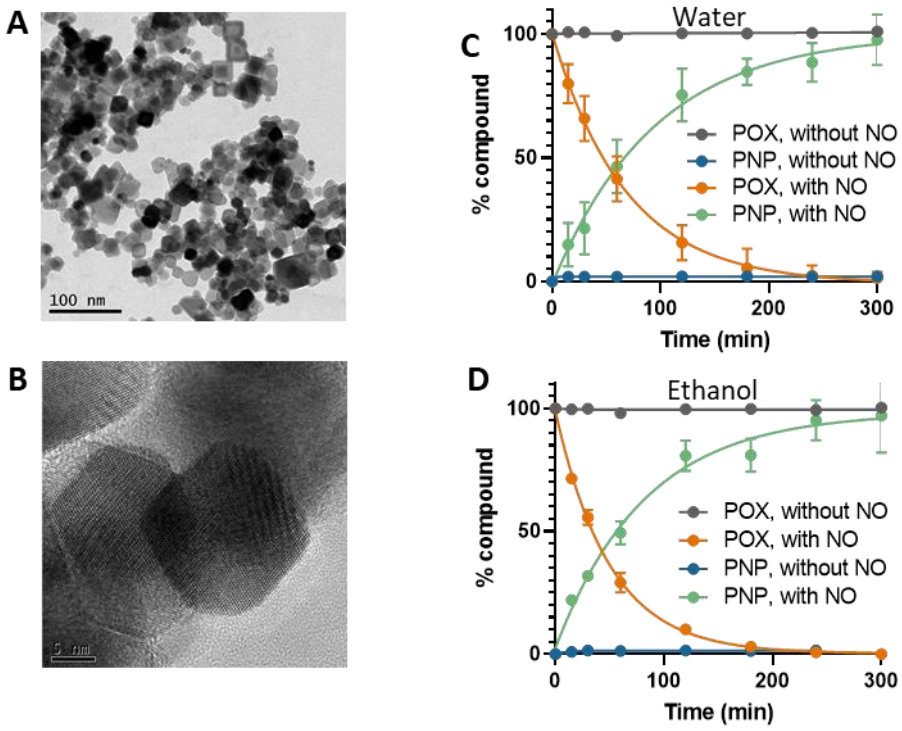

Figure 1: NO CeO_2 particles. A) *TEM image.* ***B)*** *HRTEM image.* ***C)*** *Degradation of POX in aqueous solution without or with NO.* ***D)*** *Degradation of POX in ethanol solution without or with NO. Results are expressed as the quantity of POX or PNP recovered expressed as % of the initial number of mol of POX added (n=3-5, representation of mean ± SD).*

Table 3 : NO CeO_2 particles characterizations.

Crystal faces	d_{MET} (nm)	S_{BET} ($m^2.g^{-1}$)	Vpores ($cm^3.g^{-1}$)	Mean pore diameter (nm)
{111}	6-37	60.3	0,1704	11,306

***Table 4 : Degradation of POX with NO**. Parameters extracted from the kinetic models of degradation of paraoxon with NO CeO_2 particles. k is the rate constant $t_{t1/2}$ the half-life of POX in solution and R^2 is the reliability factor of the pseudo-first order decay function used for the modeling of the degradation.*

pH	Water	EtOH
k (min^{-1})	0.015	0.020
$t_{1/2}$ (min)	48	34
R^2	0.973	0.997

3.2 PEELABLE GEL FORMULATION AND CHARACTERIZATION

Formulation was firstly based on a peelable gel called DDgel, primarily developed by Cao *et al.* and [15], [44], [45] and then optimized. DDgel is a soft jelly-like material, which can dry on the skin in few minutes after application and, can then be peeled off. It has shown great skin decontamination efficiency in removing and back-extracting topically applied chemicals such as chemical warfare simulants and other model chemicals. DDgel contains bentonite (0.5g) and Fuller's earth (2.5 g) which have high adsorption abilities and can therefore adsorb and remove toxic chemicals from skin surface. However, they do not present any degradation properties towards toxics. In the present study, powders were replaced by NO particles to provide degradation properties towards toxics to the formulation. The other components of DDgel are Lutrol F127 (1 g), carboxymethylcellulose (MW = 90 kDa, 0.3 g), water (2 mL) and ethanol (10 mL). The formulation also contains Kollidon® SR (3 g), a physical mixture of lipophilic polyvinyl acetate (PVAc) and more hydrophilic povidone (PVP).

3.2.1 Preliminary results

Peelable-gels are generally prepared by adding film forming agents (as Kollidon® SR and cellulose in DDgel), thickeners (as cellulose and poloxamer 407 in DDgel) to volatile solvent (as ethanol in DDgel). In a first step of the study, different tests were carried out to optimize the choice of excipients (thickeners, film forming agents, NO particles,…) in the formulation giving a final product with appropriate properties for use as a skin decontamination product: presence of NO particles, stability at room temperature (no visible sedimentation of NO), easy spreading of the formulation on the skin, drying of the gel in few minutes leading to a film easy to peel-off. All formulations were characterized

before drying in order to determine their properties in terms of texture, stability, and drying time. The texture analyzer measurements and rheology studies were performed to link sensory parameters and physicochemical properties.

When possible, the film obtained after drying of the formulation was characterized by texture analysis and SEM. SEM images were recorded to determine if the repartition of the NO particles was homogenous or not. EDX analysis was also performed to confirm the presence of Ce atoms. As can be seen in Figure 2, the particles are distributed on the whole gel even if aggregates of about 50 nm in size are visible.

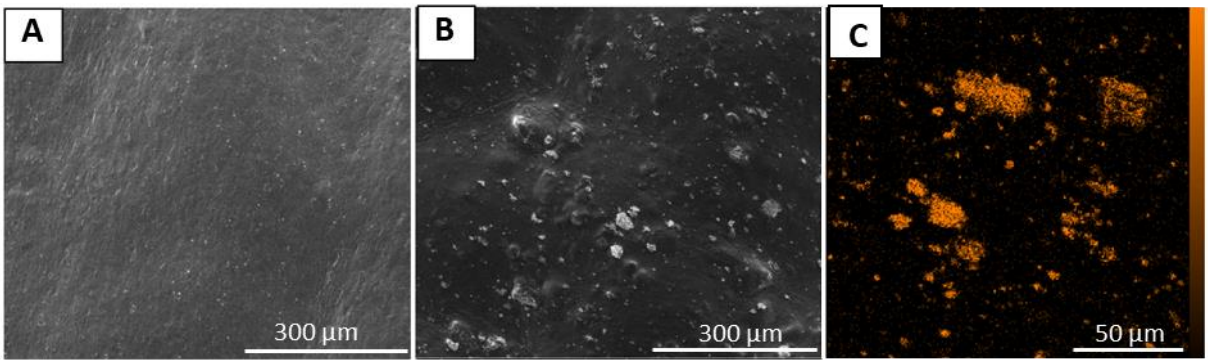

***Figure 2: Microscopy analysis of the film obtained after the gel has dried**. **A)** SEM images of formulation without particles. **B)** SEM images of formulation with particles. **C)** EDX mapping of a film containing ceria nanoparticles (orange color = cerium atoms).*

Addition of NO particles to the formulation (instead of Fuller's earth and bentonite) tends to make the mixture sticky and elastic and therefore difficult to apply. Several NO quantities were tested (from 0.1 g to 3 g) but, a sedimentation was noticeable after few minutes for the highest amount of NO. Thus, an intermediate quantity (1 g) was chosen to maintain the stability of the mixture, while ensuring the degradation properties of the gel provided by the presence of NO particles.

Various cellulose derivatives were selected and tested at different molecular weights (carboxymethyl cellulose, CMC and hydroxypropyl cellulose, HPC). It appeared that the most stable gel was obtained with hydroxypropyl cellulose (HPC, MW = 1150 kDa). The high HPC molecular weight combined to its

role as a thickener and film-former allowed for better stabilization of the nanoparticles in the mixture. Actually, among cellulose derivatives, HPC appears as the most suitable because it has a good solubility in water and many organic solvents such as ethanol. The highest molecular weight (1 150 kDa) gives higher viscosity for lower polymer concentration while HPC with the lower molecular weight (80 kDa) is more suitable in polar solvent. Therefore, two derivatives (HPC 1 150 or 80 kDa) were selected for the optimization of the formulation of the peel-off gel in the following.

When Poloxamer 407 (Lutrol F127) was added as a co-thickener, gels with better consistency were obtained (easier spreading of the gel) and it was maintained in the formulation. Poloxamers are usually used for their gelling properties which depends on their molecular weight but also to modulate the viscosity of liquid formulation at lower concentration. Poloxamer 407 can also be used as solubilizer for certain substances or oils.

Then, different film-forming agents were tested: pure PVP, PVP-hexadecene copolymer and PVP-eicosene copolymer and Kollidon® SR. The texture obtained were generally unsatisfactory (polymer poorly dispersed in the mixture for example) and the gel did not form a film after drying. Kollidon® SR proved to be the most interesting film-forming agent. This compound is particularly interesting because lipophilic polyvinyl acetate (PVAc) and more hydrophilic povidone (PVP) give to Kollidon® SR binding ability with chemicals either lipophilic or hydrophilic. Besides, water-soluble PVP creates micropores in the framework of the PVA through which water can penetrate the matrix. Finally, it offers flexible and wash resistant benefits. It can also be noted that hydrogel containing PVP and cellulose can be also used as a wound dressing which indicates that the formulation may be appropriate for use on damaged skin [46].

To conclude, the compounds chosen in the formulation were: NO (1g), hydroxypropyl cellulose (HPC, 1 150 or 80 kDa), poloxamer 407, Kollidon® SR, water and EtOH.

3.2.2 Design of experiments

The results of the design of experiments were very useful to reach the better formulation related to its handling and final properties. Detailed results are presented in the Supplementary Information (Table S1). The gel must be easy to apply but sticky enough to avoid any breaking at skin removal without slowing down the drying process. The results obtained for the gel hardness are presented in Figure 3. The coefficients of Equation 1 are represented as bars with their 95% confidence interval (determined from repeated runs): the bars in orange correspond to statistically significant effects on hardness for 5% risk (Figure 3A). The factors that had the most influence were the amount of poloxamer 407, the type of thickener (HPC of MW = 1 150 kDa or HPW of MW = 80 kDa) and its amount. When the amount of HPC increases from 0.3 g to 0.8 g, the hardness increases by an average of 3.84 N (b_4=1.92) which is consistent as HPC acts as a thickening agent. When the lower molecular weight HPC is used (HPC of 80 kDa) instead of the higher one (HPC of 1 150 kDa), the hardness decreases in average of 6.3 N (b_3=-3.15) that may be explained by the decrease in the molecular weight. However, when the quantity of poloxamer 407 increases from 0.5 to 1.5 g, the hardness decreases by an average of 5.42 N. Fakhari *et al.* showed that an high addition of ethanol (>25% v/v) to an aqueous solution of poloxamer 407 partially avoided gel formation [47]. Thus, it is likely here that in the near absence of water, the poloxamer 407 did not solvate well. Concerning the two-factor interactions, the most important are X_2X_3 and X_3X_4. From X_2X_3 representation in Figure 3C, we observed that the effect of the amount of thickening agent on gel hardness depends on the quantity of poloxamer 407 indicating a synergistic effect between both factors. Indeed, if HPC of MW = 1 150 kDa is used, the effect of the quantity of poloxamer 407 on the hardness is very important: a decrease of about 10.68 N is observed when the quantity of poloxamer 407 increases from 0.5 to 1.5g. If HPC of MW = 80 kDa is used, there is no significant effect of the quantity of poloxamer 407 on the hardness (1.4 to 1.24 N). This could be due to poor hydration of the high molecular weight HPC to the benefit of poloxamer 407 under the conditions studied here. Indeed, the presence of water is very low (20 or 1%) in order to allow a quick drying of the formulations. This effect would be less visible for the HPC with

a lower molecular weight (80 kDa). Finally, if HPC of MW = 1 150 kDa is used, the effect of the quantity of HPC on the hardness is very important (Figure 3B): an increase from 3.53 to 11.7 N is observed when its amount rises from 0.3 to 0.8 g. On the contrary, if HPC with MW = 80 kDa is used, there is no significant effect of the quantity of HPC on the hardness (1.58 to 1.07 N). The effect of HPC amount depends on the HPC molecular weight indicating a synergistic effect of both factors.

The other DOE responses (Y_2, Y_3 and Y_4) were exploited as previously described for the gel hardness (results not shown).

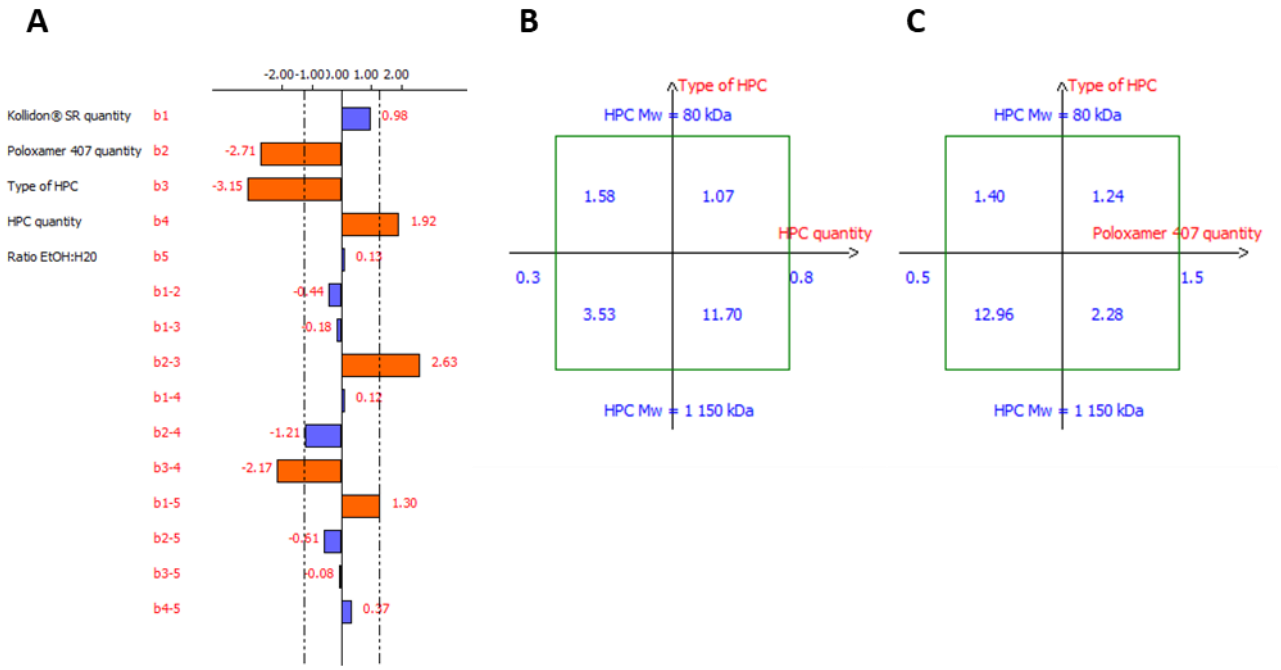

Figure 3: Results of the DOE obtained for the gel hardness. A) *Main effects and two-factor interactions coefficients calculated for the gel hardness(Y1). The dotted lines correspond to the 95% confidence interval.* ***B)*** *Representation of X3X4.* ***C)*** *Representation of X_2X_3 interactions.*

The percentage of relaxation (Y_2), which is inversely proportional to the elasticity of the product, was determined. However, among the 16 developed gels, a lot of similar relaxation values were obtained (13 values above 80%) making the statistical exploitation of the DOE difficult. Nevertheless, several interesting information can be obtained. Only two formulations (F5 and F7) exhibited very different elastic behavior with relaxation values below 55% (55 and 43% respectively) when all the other

formulations had relaxation values between 70 and 96%. In both cases, a combination of low amounts of Kollidon® SR (2 g) and HPC of MW = 80 kDa (0.3 g) was used indicating that a low molecular weight and low amount of polymer led to a less dense and more elastic network. More generally, the higher the amount of Kollidon® SR, the more the relaxation increases and thus the elasticity decreases. Results also indicate that HPC with high molecular weight (1 150 kDa) promotes an increase of relaxation thus providing less elasticity to the gel compared to HPC with low molecular weight (80 kDa). Moreover, the increase of Kollidon® SR quantity in the formulation enhances the relaxation when HPC with low molecular weight is used, while it has no influence with high molecular weight HPC. Finally a synergistic effect also appears between the ratio EtOH:H_2O and the quantity of poloxamer 407. Gel elasticity increases with the amount of poloxamer 407 with a 99:1 EtOH/H_2O ratio but remained constant for the ratio 80:20. The poor hydration of compounds in the mixture 99:1 may be an explanation of such a phenomenon.

Stickiness (Y_3) is correlated to the hardness and the same conclusions were obtained: stickiness is higher if 0.5 g of poloxamer 407 and 0.8 g of high molecular weight HPC are used. For the stability score (Y_4) (at room temperature or 45°C), the main factor influencing the stability is the type of thickening agent (high or low molecular weight HPC), the higher molecular weight giving the better results. Besides a value of 0.5 g of poloxamer 407 improved the stability, probably linked to a decrease of hardness as mentioned previously. Finally, out of all the formulations only 7 of them led to film formation after drying (F1 to F4, F6, F9 and F12) and film flexibility obtained with the texture analyzer were almost all similar (data not shown).

Based on these results, the use of high molecular weight HPC appears to be interesting as it brings less elasticity but also a good stability, certainly due to its high molecular weight allowing suspending correctly the particles in the mixture thereby avoiding their sedimentation. Nevertheless, this thickener makes the formulation compact, so a low quantity (0.3 g) should be used to counterbalance this effect. Besides, a small amount of poloxamer 407 (0.5 g), a large amount of Kollidon® SR (3 g) and

494 a EtOH/water 80:20 ratio seem more suitable. Considering all the gel characteristics obtained and
495 regardless of the POX absorption and degradation aspects, F2 formulation (3 g Kollidon® SR, 0.5 g
496 poloxamer 407, 0.3 g of HPC of MW = 1 150 kDa and 80/20 EtOH/water, 1 g of NO) seems to fulfill all
497 the desired criteria for a skin decontamination peelable gel and has been further investigated.

498 3.3 POX DEGRADATION BY THE PEELABLE GEL.

499 In a second step, F2 peelable gels containing NO or not were put into contact with POX. We observed
500 that POX had a good affinity with the gel and penetrated fairly quickly into the formulation. This agrees
501 with observations made by the team of Cao *et al.* who demonstrated extraction of the toxicant from
502 the skin by DDGel [15], [44], [45]. Besides, when POX is mixed into the formulation, a yellow color
503 quickly appears, characteristic of the formation of para-nitrophenol, one POX degradation product as
504 shown Figure 4A. This indicates that NO particles maintain their degradation activities towards POX.
505 This was confirmed by extraction of POX from the gel after 30 min of exposure and quantification by
506 HPLC. 103 $\pm$ 7% of POX was quantified when the gel didn't contain NO CeO_2 particles whereas only 65
507 $\pm$ 4 % was found when the gel did contain NO. In parallel, an increase of PNP is detected with the use
508 of the gel containing NO (24.3 ± 14.5 %) in comparison to the use of the gel without NO (2.2 ± 1.3 %).
509 (Figure S1). These results demonstrate the neutralization ability of the NO-loaded gel.

3.4 SKIN DECONTAMINATION EFFICIENCY OF THE PEELABLE GEL INCORPORATING NO

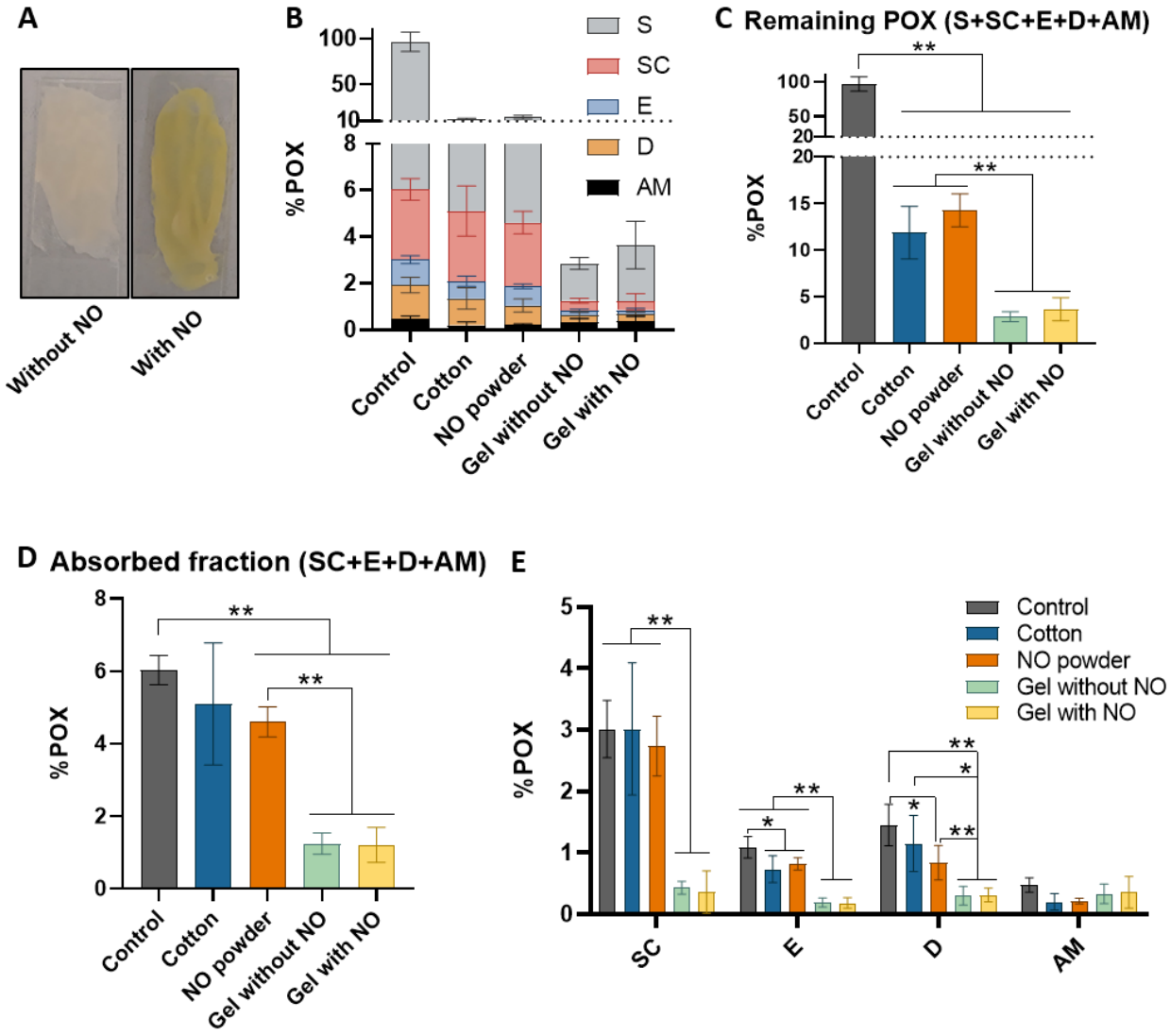

Figure 4: Degradation of POX by the peel-off gel and in vitro decontamination efficiency. A) *Picture of the peelable gels containing NO or not, 30 min after exposure to POX, the yellow color is characteristic of the POX degradation product (PNP).* ***B) and C)*** *Franz cell studies evaluation. Percentage of POX recovered at the surface (S), in the stratum corneum (SC), in the viable epidermis (E), in the dermis (D) and in the acceptor medium (AM).* ***D)*** *Percentage of POX absorbed into the skin at 24h (quantity recovered in the SC, E, D and AM).* ***E)*** *Percentage of POX recovered in the different compartments of the skin at 24h. Representation of the mean ± SD, n = 5. Significant differences are indicated, Mann-Whitney test (*: p< 0.05 ; **: p<0.01).*

Skin decontamination efficiency was evaluated by the Franz cell experiments using pig-ear skin as it was shown to be relevant model to predict the human skin permeability [48]. The POX applied dose was 13 mg/cm² (or 10 µL/cm²) and allowed a full skin coverage. This dose corresponds to severe skin contamination and also to the one recommended in the AFNOR NF X 52 122 standard. 30 min after contamination of the skin with POX, decontamination systems were applied. This time point was

chosen as it corresponds to a late decontamination procedure, realistic in real use condition. The total quantity of POX recovered during the different step of the experiment, POX remaining at the surface (S), in the stratum corneum (SC), in the viable epidermis (E), in the dermis (D) and in the acceptor medium (AM) was quantified. The non-absorbed fraction (total quantity of chemical present on the skin surface, S) is the fraction considered as the removable amount from the skin surface. On the other hand, the absorbed fraction (i.e., sum of the quantities from the SC to R) is the quantity of chemical that can potentially move into the blood. The skin decontamination results are reported in Figure 4B-E, Table 5 and Figure S2.

Table 5: In vitro distribution of POX through pig-ear full thickness unclipped skin 24 h after an exposure to POX with a decontamination at 30 min *using cotton, NO as powder, gel without NO and gel with NO. Results are expressed as % of the POX applied dose (means ± SD, n = 5). Ratio 1 represents the reduction of the fraction of POX in (S+SC+E+D+AM) in comparison to control, Ratio 2 represents the reduction of the fraction of POX absorbed into the skin (SC+E+D+AM) in comparison to control, Ratio 3 represents the reduction of the fraction of POX in the viable skin (E+D+AM) in comparison to control. R = %control / %sample.*

	Control	Cotton	NO powder	Gel without NO	Gel with NO
Total quantity of POX recovered	96.64 ± 10.28	92.00 ± 5.84	67.16 ± 9.54	92.69 ± 7.92	61.17 ± 12.84
Skin surface (S)	90.60 ± 10.42	6.77 ± 1.23	9.67 ± 2.04	1.60 ± 0.26	2.44 ± 1.02
Stratum Corneum (SC)	3.02 ± 0.47	3.02 ± 1.08	2.74 ± 0.49	0.43 ± 0.10	0.36 ± 0.34
Viable epidermis (E)	1.09 ± 0.17	0.73 ± 0.22	0.82 ± 0.10	0.19 ± 0.07	0.18 ± 0.09
Dermis (D)	1.45 ± 0.34	1.15 ± 0.46	0.84 ± 0.28	0.30 ± 0.15	0.31 ± 0.11
Acceptor medium (AM)	0.48 ± 0.12	0.20 ± 0.13	0.21 ± 0.05	0.33 ± 0.16	0.36 ± 0.26
Absorbed fraction	6.04 ± 0.40	5.10 ± 1.68	4.61 ± 0.42	1.25 ± 0.29	1.21 ± 0.48
Ratio 1	-	8.14	6.77	26.46	33.90
Ratio 2	-	1.18	1.31	4.98	4.82
Ratio 3	-	1.45	1.61	3.55	3.68

The total amount of POX recovered (Figure S2A and Table 5) was over 85% for systems without NO (control, cotton, gel without NO). It was 67.16% and 61.17% for NO powder and gel with NO respectively, which clearly indicates the capacity for POX degradation by the particles and is in line

543 with what was observed in the paragraph 3.3. As can be seen in the Figure 4B, the amount of POX in
544 the different skin compartments was different depending on the decontamination method used. The
545 total POX amount recovered after 24 h on the surface and in the skin (S + SC+ E + D + AM) was in all
546 cases, lower for decontaminant systems compared to the control (Figure 4C). For cotton and NO
547 powder about 7-8 times less POX was recovered after 24 hours compared to the control ($p<0.01$) in
548 and onto the skin and 26-34 times less for gels containing or not the NO particles ($p<0.01$) (ratio R1).
549 Gels were statistically always more efficient than other decontamination systems ($p<0.01$). These
550 results demonstrate their higher efficiency for adsorbing and eliminating the toxic from the skin. No
551 significant difference was detected between NO powder and cotton but it should be noted that the
552 quantities of powder used in this study are relatively small and did not allow a total coverage of the
553 skin surface, which probably limited their effectiveness. We can assume that an increase in the amount
554 of NO powder would reduce the amount of POX remaining related to the adsorption and degradation
555 capacities of NO towards the toxicant.
556 Regarding the amount of toxicant absorbed into the skin (SC+E+D+AM, Figure 4D), a significant
557 difference ($p<0.01$) was observed for the treatment with NO powder compared to control, which was
558 not the case for cotton decontamination alone, demonstrating the interest of CeO_2 nanoparticles.
559 Moreover, the efficiency and the interest of the gels was clearly demonstrated with a decrease by
560 about 5 of the quantity of POX absorbed into the skin ($p<0.01$). The same observation was noticed for
561 the viable skin (E+D+AM) with a 3 to 4-fold decrease in the quantity of POX when gels were used in
562 comparison to the control ($p<0.01$).

563 Looking more deeply at the distribution of POX in the different layers of the skin (Figure 4E), it can be
564 noted that POX was mainly distributed in the stratum corneum and the gels greatly reduced the
565 amount of POX found in this compartment ($p<0.01$). Similarly, they allowed decreasing the quantity of
566 POX in the viable epidermis and dermis ($p<0.01$).

4 DISCUSSION

Over the last decade, concern about the chemical warfare agents threat has increased due to several reported attacks as an assassination through the use of VX in Kuala Lumpur in 2017, a murder attempt with nerve agent ("Novichok") in the United-Kingdom in 2018 and the use of toxics reported several times during the Syrian conflict [1]. Therefore, the development of efficient decontamination systems, especially for skin, and their evaluation has become crucial. To limit the use of war gases in the laboratory many chemicals have been used as simulants [27], [42], [43], [49], [50], [51], [52]. In the case of VX, paraoxon has been identified in the literature [43] and used in this study. It presents physicochemical properties and chemical functions similar to VX and is easily detected thanks to the benzene ring. It is a lipophilic substance which therefore tends to be stored in the upper layers of the skin. It has been used as a simulant because of its molecular weight and logP close to those of VX (275.2 $g.mol^{-1}$ against 267.4 $g.mol^{-1}$ and logP = 1.8 against 2.09 respectively for POX an VX). However, it should be noted that VX penetrates faster and earlier (lower lag time) than POX into the skin. The skin penetration rate on pig skin is 15 times faster for VX than POX [43].

In the context of toxic chemicals degradation, several studies have shown the interest of metallic nanoparticles and especially cerium oxide nanoparticles [27], [29], [30], [31]. CeO_2 is from a group of lanthanoids with fluorite type structure and present various application in catalysis, fuels cells, solar cells, pharmacy and medicine. Its catalytic activity towards the hydrolysis of various types of organophosphates (pesticides plasticizers and biologically active molecules) has been studied [53]. Different methods have been described in the literature to prepare CeO_2 nanoparticles [25], [26], [27], [30]. In this study, the hydrothermal method has been chosen as it enables large quantities of particles to be obtained quickly and efficiently. In addition, synthesis parameters can be optimized to obtain particles with controlled properties (size, morphology, etc.) [25], [26]. The degradation properties of CeO_2 nanoparticles depends on various factors. Recently, we have demonstrated the influence of CeO_2 NPs size, morphology and crystallographic planes on the degradation of paraoxon, with a higher

activity of the {111} facets as compared to the {100} facets, or ill-defined surfaces [25]. When an annealing step is performed after synthesis, the degradation activity is mainly driven by the specific surface area and the shape of particles appears as a second order effect [26]. In these two studies, nano-octaedra ceria nanoparticles (NO), which are those used in the present study, have appeared really interesting for the degradation of POX in aqueous solution. Other factors have been shown to have an effect on the ceria properties as the ratio of Ce^{3+} and Ce^{4+}, number of hydroxyl groups at the surface, surface defects abundance [25], [26], [31], [53]. A mechanism has been proposed to explain the hydrolysis properties of CeO_2 towards OPs and is represented in the Figure 5 [31]. First, a coordination takes place between a phosphate group and a Ce^{4+} cation followed by a nucleophilic attack by the Ce^{3+} activated hydroxyl group on the phosphorus atom. The unique redox switching ability of cerium cations, combined with the high mobility of oxygen in the cerium oxide lattice would facilitates the dynamic creation and regeneration of this particular spatial arrangement on the cerium oxide surface (Ce^{3+}, Ce^{4+}, OH group).

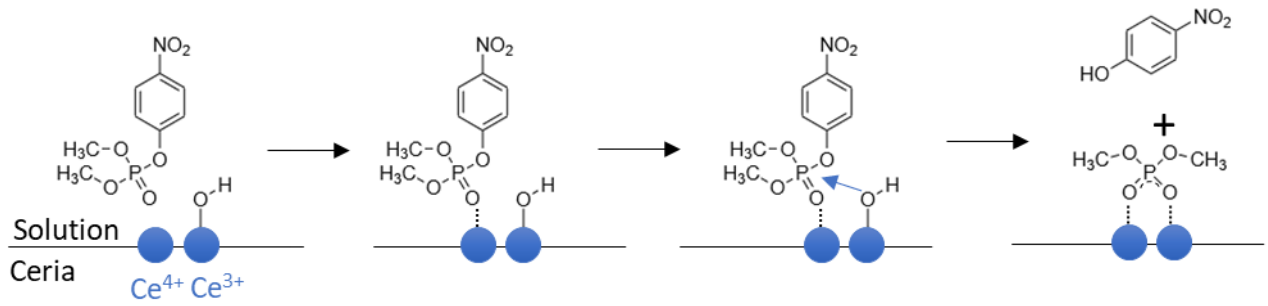

Figure 5: Degradation of POX to PNP by the CeO_2 surface.

As any powder, cerium oxide particles must be formulated to allow an easy decontamination of the skin and to avoid the spreading or inhalation of the toxicant-loaded particles after decontamination. In addition, nanoparticles in powder form are very difficult to remove from the skin after application, and thorough washing of the skin is required to remove them, resulting in high waste production. Few systems based on cerium oxide nanoparticles for skin decontamination purposes have been

613 formulated. We have previously evaluated the skin decontamination efficiency of CeO_2 nanoparticles
614 as powder or suspension containing 10 wt% CeO_2 and 1 wt% of Guar gum to prevent nanoparticles
615 sedimentation. Suspension have been almost 10 times more efficient than powder regarding the
616 decrease of POX absorption into the skin [27]. Zenerino *et al.* have also developed new topical skin
617 protectants [37] containing ceria nanoparticles and compared an oil in water emulsion and a
618 suspension of a fluorinated thickening polymer (HASE-F) grafted with these NPs. Both skin protectants
619 have decreased the penetration of POX by 3 to 4-fold factors when they have been applied onto the
620 skin before contamination. Bignon *et al.* have also worked on a barrier cream based on CeO_2
621 nanoparticles [38]. They have demonstrated, that the efficiency of the topical skin protectants is due
622 to the presence of ceria particles and depends on their quantity in the formulation as well as their
623 grafting onto a perfluorocarbon thickening polymer and, finally the formulation of the new grafted
624 polymer. These last two formulation are topical skin protectants and therefore destined to be applied
625 on the skin before the contamination on the contrary to the formulation developed in this paper
626 designed to remove the toxic from the skin after decontamination.

627 The choice of the CeO_2 formulation type for skin decontamination purpose is not straightforward as
628 they are often unstable (sedimentation for suspension, possible coalescence for emulsion, syneresis
629 for gels). The goal of this study has been to develop a new system containing CeO_2 nanoparticles but a
630 limited amount of water to avoid a “wash-in” effect, easy to apply and to remove from the skin, able
631 to absorb the toxic agent and to degrade it. Our work has been inspired by the work of Cao et al, and
632 their work on an efficient peelable gel, the DDgel [15], [44], [45]. Cao *et al.* have demonstrated through
633 *in vitro* skin decontamination experiment that DDGel can simultaneously clean chemical contaminant
634 from the skin surface, back-extract toxicant from SC, decrease the quantity of contaminant available
635 for penetrating into the skin and reduce chemical penetration and subsequent systemic absorption. It
636 is interesting to note that DDGel did not peel off SC layers but back- extracted chemicals from SC as
637 demonstrated on different chemical warfare agent simulants for OPs and blistering agents [15], [45].
638 In their studies, Cao *et al.* have found that the percentage of chemicals absorbed into the skin

639 (SC+E+D+AM) and viable skin (E+D+AM) ranged between 2.7 and 6 % and between 2 and 5.5%
640 respectively. This corresponds to a decrease of 4.5 to 12-fold in comparison to non-decontaminated
641 skins [15], [45]. In order to improve the formulation and provides degradation properties, CeO_2
642 nanoparticles have been incorporated and the formulation has then been optimized in this study. The
643 challenge has been to maintain POX access to the CeO_2 nanoparticles entrapped in the gel matrix for
644 efficient degradation of POX. The *in vitro* results have demonstrated that the gel itself was indeed no
645 obstacle to the access of POX to the surface of CeO_2 nanoparticles and maintained their ability to
646 degrade it into safer by-products. In our study, the efficiency of the optimized peel-off gel has been
647 clearly demonstrated with a percentage of POX absorbed into the skin (SC+E+D+AM) and viable skin
648 (E+D+AM) decreasing from 6% of the applied dose without decontamination to 1.21% and 0.85%
649 respectively with the peel-off gel. This corresponds to a decrease by about 5-fold of the quantity of
650 POX absorbed into the skin (SC+E+D+AM) and a decrease of 3.5-fold in the viable skin (E+D+AM) in
651 comparison to non-decontaminated skins, which is much more than the use of cotton or nanoparticles
652 as powder. It can also be noted that the gel without NO particles has been effective in removing the
653 toxicant but has not degraded it. These results are consistent with the results of Cao *et al* described
654 above.

655 In the case of the CeO_2-based peel-off gels developed in this study, the decontamination occurs due
656 to physical interactions or chemical neutralization. Concerning physical interactions, bringing the toxic
657 compound into contact with the gel on the skin enables the toxic compound to be absorbed into the
658 formulation. Depending on the compound, hydrophilic or hydrophobic interactions can take place into
659 the gel, related to the presence of Kollidon SR (a physical mixture of lipophilic polyvinyl acetate (PVAc)
660 and more hydrophilic povidone (PVP) as presented in section 3.2). The gel can then be dried on the
661 skin by evaporation of ethanol, leading to a toxicant-retaining film. This film can then be peeled off,
662 thus retaining the toxicant and facilitating waste removal. It should also be noted that Cao and his
663 team have shown that DDgel, with a formulation very similar to the one developed here, can not only
664 remove the toxicant from the skin surface, but can also back-extract the toxicant from the SC. This is

665 due to the similar or higher partition coefficient of each simulant for the gel than for SC [45]. As their
666 formulation is close to the one developed in this paper, the same mechanism is likely to occur. The
667 efficacy of the gel, thanks to the physical interactions between the gel and the toxicant, is
668 demonstrated in our study. Actually, a very good skin decontamination efficacy for the gel without
669 nanoparticles is observed. Moreover, in the case of the CeO_2-based formulation developed in this
670 study, chemical neutralization can take place. Once the POX has been absorbed into the formulation,
671 it comes into contact with the NO particles, which degrade it into the less toxic by-product PNP
672 (illustrated on Figure S1). The effectiveness of our formula stems from the physical and chemical
673 interactions between the gel and the toxic substance.

674 To finish, CeO_2 on one hand and DDgel on the other have already been shown to be effective for skin
675 decontamination of several chemicals. Therefore, it can be expected that the CeO_2-based formulation
676 prepared in this research will be efficient against various compounds as OPs and blistering agents
677 which should be validated in future studies.

678 5 CONCLUSION

679 Skin contamination with toxic agents remains a great concern and new decontamination systems able
680 to eliminate the toxic from the skin and to degrade it need to be developed and evaluated. Cerium
681 oxide nanoparticles appear promising in this context because of their catalysis properties resulting
682 from their abundant surface defects, large number of surface hydroxyl groups and low redox potential
683 between Ce^{3+} and Ce^{4+} cations. To take full advantage of the degradation potential of CeO_2 toward
684 toxic agents, these nanoparticles must be formulated in an appropriate system. In this study, they have
685 been incorporated into a gel that can easily be applied on the skin. This formulation is easier to handle
686 than powder or liquid formulation and avoid dispersion or cross contamination. After application, the
687 gel can quickly dry to form a strippable film. *In vitro* studies have demonstrated the efficiency of such
688 formulation for skin decontamination and its potential to reduce local and systemic absorption, and

689 decrease chemical toxicity. As CeO_2 and the gel are known to be efficient against various toxic, the
690 potential of the formulation developed in this study should be evaluated on other compounds.

691 CRediT AUTHORSHIP CONTRIBUTION STATEMENT

692 Eloise Thomas: Funding acquisition, Resources, Conceptualization, Methodology, Investigation,
693 Validation, Formal analysis, Supervision, Writing - original draft, Writing – review & editing,
694 Visualization, Project administration.

695 Claire Bordes: Formal analysis, Methodology, Writing - original draft, Writing – review & editing

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700

701 DECLARATION OF COMPETING INTEREST

702 The authors declare that they have no known competing financial interests or personal relationships
703 that could have appeared to influence the work reported in this paper.

704

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FIGURE CAPTIONS

Figure 1: NO CeO_2 particles. **A)** TEM image. **B)** HRTEM image. **C)** Degradation of POX in aqueous solution without or with NO. **D)** Degradation of POX in ethanol solution without or with NO. Results are expressed as the quantity of POX or PNP recovered expressed as % of the initial number of mol of POX added (n=3-5, representation of mean ± SD).

Figure 2: Microscopy analysis of the film obtained after the gel has dried. **A)** SEM images of formulation without particles. **B)** SEM images of formulation with particles**. C)** EDX mapping of a film containing ceria nanoparticles (orange color = cerium atoms)

Figure 3: Results of the DOE obtained for the gel hardness. **A)** Main effects and two-factor interactions coefficients calculated for the gel hardness(Y_1)**.** The dotted lines correspond to the 95% confidence interval. **B)** Representation of X_3X_4. **C)** Representation of X_2X_3 interactions.

Figure 4: Degradation of POX by the peel-off gel and in vitro decontamination efficiency. A) Picture of the peelable gels containing NO or not, 30 min after exposure to POX, the yellow color is characteristic of the POX degradation product (PNP). **B) and C)** Franz cell studies evaluation. Percentage of POX recovered at the surface (S), in the stratum corneum (SC), in the viable epidermis (E), in the dermis (D) and in the acceptor medium (AM). **D)** Percentage of POX absorbed into the skin at 24h (quantity recovered in the SC, E, D and AM). **E)** Percentage of POX recovered in the different compartments of the skin at 24h. Representation of the mean ± SD, n = 5. Significant difference are indicated, Mann-Whitney test (*: $p< 0.05$; **: $p<0.01$).

Figure 5: Degradation of POX to PNP by at the CeO_2 surface.

TABLE CAPTIONS

Table 1: Fixed and variable factors used for the experimental design.

Table 2: Formulations prepared according to a 2^{5-1} fractional factorial design.

Table 3: NO CeO_2 particles characterizations.

Table 6 : Degradation of POX with NO. Parameters extracted from the kinetic models of degradation of paraoxon with NO CeO_2 particles. k is the rate constant $t_{t1/2}$ the half-life of POX in solution and R^2 is the reliability factor of the pseudo-first order decay function used for the modeling of the degradation.

Table 7: *In vitro* distribution of POX through pig-ear full thickness unclipped skin 24 h after an exposure to POX with a decontamination at 30 min using cotton, NO as powder, gel without NO and gel with NO. Results are expressed as % of the POX applied dose (means ± SD, n = 5). Ratio 1 represents the reduction of the fraction of POX in (S+SC+E+D+AM) in comparison to control, Ratio 2 represents the reduction of the fraction of POX absorbed into the skin (SC+E+D+AM) in comparison to control, Ratio 3 represents the reduction of the fraction of POX in the viable skin (E+D+AM) in comparison to control. R= %control / %sample.