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Pickering emulsions for skin decontamination

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ABSTRACT

This study aimed at developing innovative systems for skin decontamination. Pickering emulsions, *i.e.* solid-stabilized emulsions, containing silica (S-PE) or Fuller's earth (FE-PE) were formulated. Their efficiency for skin decontamination was evaluated, *in vitro*, 45 min after an exposure to VX, one of the most highly toxic chemical warfare agents. Pickering emulsions were compared to FE (FE-W) and silica (S-W) aqueous suspensions. PE containing an oil with a similar hydrophobicity to VX should promote its extraction. All the formulations reduced significantly the amount of VX quantified on and into the skin compared to the control. Wiping the skin surface with a pad already allowed removing more than half of VX. FE-W was the less efficient (85% of VX removed). The other formulations (FE-PE, S-PE and S-W) resulted in more than 90% of the quantity of VX removed. The charge of particles was the most influential factor. The low pH of formulations containing silica favored electrostatic interactions of VX with particles explaining the better elimination from the skin surface. Formulations containing FE had basic pH, and weak interactions with VX did not improve the skin decontamination. However, these low interactions between VX and FE promote the transfer of VX into the oil droplets in the FE-PE.

1. Introduction

Chemical warfare agents (CWA) such as the nerve agent sarin and the vesicant sulfur mustard, have been used not only in conflicts, but also in terrorist acts targeting civilian populations (Okumura et al., 2009; Yanagisawa et al., 2006). Liquids or droplet aerosol forms of CWA can be absorbed through the skin, the eyes, or the respiratory tract (Wartell et al., 1999) if there is no sufficient protection of the body surface. The nerve agent VX is one of the most highly toxic compounds following skin exposure (estimated $LD_{50(\text{human})} \sim 0.04$–$0.14\ \text{mg}\cdot\text{kg}^{-1}$ (Munro, 1994)). VX inhibits the acetylcholinesterase, an enzyme involved in the transmission of nerve impulses. The consequence is a continuous stimulation of the nervous system. The first signs of percutaneous intoxication appear quickly (2–3 h after exposure) in the form of severe functional disturbances at respiratory, cardiovascular, muscular, pupilar, digestive levels (Voicu et al., 2010; Van Heel and Hachimi-Idrissi, 2011) which can lead to death. A rapid body surface decontamination is therefore crucial to prevent victims poisoning. It reduces the amount of contaminant on the skin surface and thus, decreases the penetration rate and the extent of intoxication.

Shower decontamination with or without surfactants is a standard decontamination procedure but it may be limited by the 'wash-in effect' which could increase the systemic exposure (Klinger, 2007; Moody and Maibach, 2006). Moreover, a serious drawback of this method is that, although most contaminants are removed and diluted, not all of them are neutralized or destroyed.

Different decontaminant systems are currently available for skin decontamination. Some systems act by adsorption and displacement of the toxic agent such as Fuller's Earth (FE), zeolites (Vucemilović et al., 2008, 2009), even household products such as flour and talc have been tested (Van Hooidonk et al., 1983). Other systems act by neutralization (chemical degradation) such as the Canadian Reactive Skin Decontaminant Lotion (RSDL), the American kit M291, the kit IPB-80 'desprach' and hypochlorite 0.5% (Bannard et al., 1991; Chilcott et al., 2001; Gold et al., 1994; Matousek, 1999; Sawyer et al., 1991). New systems have been developed with metal oxide nanoparticles: $Zr(OH)_4$ (Bandosz et al., 2012), Ge^{4+} doped TiO_2 (Štengl et al., 2012), SiO_2 (Saxena et al., 2012; Davis et al., 2014), CuO and ZnO (Praveen Kumar et al., 2013).

These systems demonstrated efficiency on *in vivo* models when they were applied in the first minutes (Braue et al., 2011; Hamilton et al., 2004; Taysse et al., 2007) and up to 45 min after contamination (Bjarnason et al., 2008).

In agreement with previous studies, FE and RSDL are the most efficient systems for skin decontamination following a chemical warfare

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agent exposure (Bjarnason et al., 2008; Braue et al., 2011; Chilcott et al., 2001; Taysse et al., 2010). Using RSDL *in vitro* as a skin decontaminant, on a pig-ear skin and 45 min after an exposure to VX, has removed more than 96% of VX on and into the skin (Rolland et al., 2013). The efficacy of decontamination was compared to aqueous dispersion of raw particles classical systems (FE and silica raw particles) in a skin delayed decontamination protocol, *i.e.* 45 min following an exposure to VX. However, they have some drawbacks. RSDL has a potential systemic toxicity of an active ingredient (2,3-butanedione monoxime) (Jager and Stagg, 1958) and its efficacy against unthickened sulfur mustard in the *in vivo* experiments was relatively low (Van Hooidonk and Langenberg, 1995). FE is a dusty agent that effectively adsorbs CWA. However, if the contaminated particles are dispersed in air; this can lead to contamination spreading. Thus, the aim of the present study was to design, formulate and characterize a new liquid decontaminant, which could be effective and easy to handle in case of mass contamination, the liquid form avoiding the resuspension of contaminated particles in the air.

Pickering emulsions (PE), *i.e.* solid-stabilized emulsions, may represent an alternative to FE and RSDL. In this kind of emulsion, the classical emulsifier has been replaced by solid particles which adsorb onto the surface of oil droplets and stabilize the oil/water interface. Such emulsion labeled "surfactant-free" (Chevalier and Bolzinger, 2013; Aveyard et al., 2003; Pickering, 1907; Ramsden, 1903) limits the adverse effects of the presence of surfactants. In particular the skin penetration enhancer effect of surfactants would be prevented. This emulsion would combine the amphiphile behavior of emulsions as decontaminant systems where the toxic agents can solubilize in both the aqueous and the oil phases, and the adsorption potency of the stabilizing particles. Moreover the liquid emulsion droplets should ensure a better spreading on the skin and continuous surface covering (Alvarez-Román et al., 2001, 2004). The use of particles with high specific surface area like fumed silica and FE for the stabilization of the emulsion could be better suited for absorption of the toxic agent and thus, for skin decontamination (Štengl et al., 2012, Verma et al., 2015). Solid particles such as silica or clay have been successfully used to stabilize oil-in-water (o/w) emulsions (Eskandar et al., 2007; Frelichowska et al., 2009a, 2010; Guillot et al., 2009; Lagaly et al., 1999; Prestidge and Simovic, 2006). The major component of FE is montmorillonite, a T-O-T (tetrahedral-octahedral- tetrahedral) clay where each platelets consists in a central sheet of octahedral alumina or magnesia sandwiched between two sheets of tetrahedral silica (Swartzen-Allen and Matijevic, 1974; Saunders et al., 1999). Montmorillonite swells when it is immersed in water; the primary platelets shift apart (Saunders et al., 1999), thereby increasing the surface area. Thus, the dispersion of FE in water could be advantageous for increasing the absorption of the contaminant. Adding an oil phase and an active ingredient (an oxime for example) in the formulation may be interesting for decontamination purpose. Due to their hydrophilic character, FE and fumed silica promote the formation of o/w Pickering emulsions. Spagnul et al. (2010) showed the interest of an oily phase to extract uranium from skin under certain conditions and thereby the interest of emulsions as skin decontamination systems that combine an oily phase and surfactants. Indeed the nerve agent VX is a molecule of medium polarity as expressed by its midrange partition coefficient ($\log P$ or $\log K_{\text{o-w}} = 0.7$ (Czerwinski et al., 2006)), so that it can partition between aqueous and oil phases. The fatty ester diethyl adipate having a polarity close to VX ($\log P = 0.7$) was selected as an oil for the present application to skin decontamination. Furthermore, this oil has already been used in the formulation of stable Pickering emulsions, combined with hydrophilic fumed silica (Frelichowska et al., 2009a). Thus, Pickering emulsions containing water, diethyl adipate and either hydrophilic fumed silica or FE have been developed and evaluated with regards to skin decontamination in the present study. The aim of the experiments was to determine the efficacy of such Pickering emulsions for skin decontamination, using *in vitro* experiments on excised pig-ear skin model mounted in Franz diffusion as already described in a previous work (Rolland et al., 2011). The efficacy of decontamination was compared to aqueous dispersion of raw particles (FE and silica raw particles) in a skin delayed decontamination protocol, *i.e.* 45 min following an exposure to VX.

Table 1
Physicochemical characteristics of powders.

Particles	Silica	FE
Specific surface area ($m^2 \cdot g^{-1}$)	194.5	110.2
Mean pore size (nm)	12.3	13.3
Combined volume of pores between 1.7 and 300 nm ($cm^3 \cdot g^{-1}$)	0.46	0.32
Particle shape from TEM observations	Spherical	Sheets
Elementary particles size from TEM observations	20 nm	10–15 µm

2. Materials and methods

2.1. Materials

Hydrophilic fumed silica powder Aerosil® 200 was purchased from Evonik (France). According to information from the suppliers, specific area of silica was 200 $m^2 \cdot g^{-1}$ for the Sigma fumed silica grade Aerosil® 200. Fuller's earth (FE) was supplied by Sté Paul Boyé (France). RSDL package containing a sponge soaked with the lotion was purchased from E-Z-EM, Inc. (Lake Success, NY, USA). For each powder, physicochemical properties were evaluated (Table 1). The fatty ester diethyl adipate was a gift from Stéarinerie Dubois (France). *O*-Ethyl-s-[2(diisopropylamino)ethyl]methyl thiophosphonate (VX, 97.7% pure, CAS Registry Number 50782–69-9) was synthesized by the Centre d'Études du Bouchet (CEB, Vert-le-petit, France). The receptor fluid was composed of Hanks's Balanced Salt Solution (HBSS, pH 7.4) containing 1% of penicillin–streptomycin. Horse butyrylcholinesterase and butyrylthiocholine iodide were provided by Sigma (Saint Quentin Fallavier, France). (See Table 2.)

2.2. Formulation

Concentrated emulsions were prepared at 40 wt.% oil fraction and 10% of FE or silica to ensure a high viscosity and thereby an easier skin application (Frelichowska et al., 2009b). Water wet particles tend to stabilize o/w emulsions. Therefore, the first step was the dispersion of 10% silica or FE in water with an ultrasound disperser Sonics VibraCell of 500 W power (BioBlock Scientific, France) for 2 min at 40% amplitude. These intermediate forms (FE/water (FE-W) or silica/water (S-W) suspensions) were also tested in the decontamination experiments. The oil and aqueous phases were then mixed with an UltraTurrax® T25 rotor-stator device equipped with S25N18G shaft (IKA, Germany) rotating at 2400 rpm during 10 min, yielding a Pickering emulsion.

2.3. Physicochemical characterization of FE and silica particles

Transmission Electron Microscopy (TEM) was performed with JEOL 2100 microscope operating at 200 kV acceleration at the 'Centre Technologique des Microstructures' (CTµ) at University of Lyon (Villeurbanne, France). A drop of liquid sample was deposited on the TEM carbon grids, the excess liquid was then removed with an absorbent paper, and the sample was dried in open air before observation by TEM.

Table 2
Physicochemical characteristics of suspensions and emulsions.

Particles	Silica	FE
Median diameter $D(0.5)$ (µm) of agglomerates in aqueous suspension (10 wt.%)	0.21	12.09
pH of the aqueous suspensions	4.36	8.28
pH of Pickering emulsions	4.70	8.20