Colloids and Surfaces A: Physicochem. Eng. Aspects 469 (2015) 166–179

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

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

Skin toxicity of surfactants: Structure/toxicity relationships

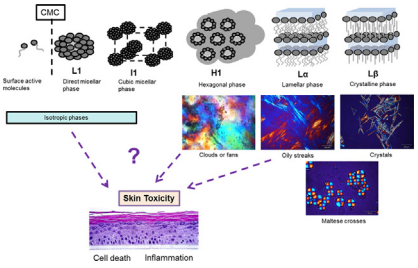
Emmanuelle Lémery[a,b,c,d], Stéphanie Briançon[a,b,c], Yves Chevalier[a,b,*], Claire Bordes[a,e], Thierry Oddos[d], Annie Gohier[d], Marie-Alexandrine Bolzinger[a,b,c]

[a] Université de Lyon, F-69008 Lyon, France
[b] Université Lyon 1, Laboratoire d'Automatique et de Génie des Procédés, UMR CNRS 5007, F-69622 Villeurbanne, France
[c] Université Lyon 1, Institut des Sciences Pharmaceutiques et Biologiques, Laboratoire de Dermopharmacie et Cosmétologie, F-69008 Lyon, France
[d] Johnson & Johnson Santé Beauté France, Département des Nouvelles Technologies, Campus de Maigremont, F-27100 Val de Reuil, France
[e] Université Lyon 1, Institut des Sciences Analytiques, UMR CNRS 5280, F-69622 Villeurbanne, France

HIGHLIGHTS

- Skin irritancy of surfactants is related to their physico-chemical properties.
- Surfactants can be split into two well-separated classes: toxic and mild.
- Ionic surfactants can be mild; non-ionic surfactants can be toxic.
- The order parameter is a universal molecular descriptor of surfactants.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 24 October 2014
Received in revised form 9 January 2015
Accepted 12 January 2015
Available online 20 January 2015

Keywords:
Surfactants
Skin toxicity
CMC
Order parameter
Principal component analysis

ABSTRACT

The skin toxicity of four ionic surfactants and fourteen non-ionic surfactants was investigated so as to disclose structure/toxicity relationships. The skin toxicity was assessed by means of four *in vitro* assays, MTT and LDH test of cell viability, and detection of the inflammation markers IL-1α and IL-8. Several descriptors of the physicochemical properties of the surfactants were measured in order to find out those molecular descriptors that correlate with the toxicity measured on skin. Principal component analysis and analysis of the matrix of Pearson's correlation coefficients were used for the search of the molecular descriptors having the highest relevance. There was a definite difference between ionic and non-ionic surfactants. Ionic surfactants are the most toxic if they are soluble in water. Crystalline ionic surfactants of low solubility show low toxicity. The sign of the charge, anionic or cationic, does not matter. The value of the CMC that has been put forward as a highly relevant parameter does not account for the full skin toxicities observed; the CMC of non-ionic surfactants is not a parameter of relevance. For non-ionic surfactants, the nature of the chemical bond linking the polar head group and the alkyl chain has a significant impact on skin toxicity; PEG ethers appear more toxic than PEG esters. The results revealed the mildness of polyoxyethylene sorbitan esters whatever be their alkyl chain length. On the other hand, for sucrose ester surfactants, C12 alkyl length resulted in the greatest skin toxicity. Since the molecular parameters of ionic, non-ionic, water-soluble and crystalline surfactants are different, a universal parameter was

Abbreviations: CMC, critical micellar concentration; CTAC, cetyl trimethyl ammonium chloride; DC, distearyldimonium chloride; EO, ethylene oxide; HLB, hydrophile lipophile balance; IL, interleukin; LDH, lactate dehydrogenase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide tetrazolium dye; O/W, oil in water; PCA, principal component analysis; PEG, poly(ethylene glycol); PEO, poly(ethylene oxide); RHE, reconstructed human epidermis; SC, stratum corneum; SLS, sodium lauryl sulfate; SSL, sodium stearoyl lactylate; TEWL, transepidermal water loss.

* Corresponding author at: Laboratoire d'Automatique et de Génie des Procédés (LAGEP), Université Lyon 1, 43 bd 11 Novembre, F-69622 Villeurbanne, France. Tel.: +33 472 43 18 77; fax: +33 472 43 16 82.
E-mail address: chevalier@lagep.univ-lyon1.fr (Y. Chevalier).

http://dx.doi.org/10.1016/j.colsurfa.2015.01.019

introduced, the order parameter describing the orientation ordering of surfactant molecules at interfaces. The highly ordered organization of crystalline surfactants associated with their low solubility in water makes them very low-irritant surfactants.

1. Introduction

Topical formulations of either pharmaceutical or cosmetic products often contain surfactants; they are mainly used as emulsifiers or detergents, and many of them may elicit skin reactions such as irritant contact dermatitis or may cause inflammation. Though emulsions are often used to treat inflammatory skin disorders such as eczema or atopic dermatitis, emulsions may also cause skin disorders because of the presence of surfactants added as stabilizers [1]. The toxicity/irritancy properties of surfactants toward skin show a wide variability; some surfactants are recognized as having strong irritant potency whereas skin can much better withstand other surfactant molecules termed as "mild" [2]. Some surfactants show moisturizing properties because they are able to supplement a lack of endogenous lipids in the stratum corneum (SC) [3]. The mildness is one property that is considered when a surfactant is selected in the design of a topical formulation. Although experimental data do exist and rough rules have been established, there is no clear rationale pertaining to the irritant properties of surfactants so that empirical knowledge prevails. It is claimed that cationic surfactants are much more irritant than anionic and non-ionic; but there are many exceptions to this crude rule. In order to go beyond empirical knowledge, structure–activity relationships are needed, which is the purpose of the research reported in the present paper. Surfactants have been extensively studied under *in vitro* and *in vivo* conditions to determine such structure–activity relationships [4]. These studies pointed out the role of the nature of the polar head group of the surfactant and especially the presence of a charge which favors interaction with SC proteins, leading to a swelling of the SC [5,6]. There is a consensus that non-ionic surfactants have the least potential for irritancy [7]. In her safety assessment of PEGs and their derivatives, Fruijtier-Pölloth [8] drew attention to the fact that there were only few cases of a sensitization reaction for preparations containing PEG and PEG derivatives such as PEG ethers and PEG esters. These surfactants did not cause ocular or dermal irritation and had extremely low acute and chronic toxicities.

Since it is believed that the bioavailable part of the full surfactant content is the free molecules (monomers) and that the surfactants involved in micelles do not contribute to the irritancy, skin toxicity is also linked to the ability of a surfactant to self-assemble as micelles. The interaction of single molecules with SC proteins is stronger than that of micelles with SC proteins. On this basis, irritancy is related to the critical micellar concentration (CMC); surfactants with high CMC being more toxic than those with low CMC [6,9]. This is a general trend only however; no definite conclusions of a general bearing can be drawn because there are so many exceptions to this trend. Moreover, once dispersed in an emulsion, the situation is much more complicated because the surfactant exists in three states, free molecules, micelles and adsorbed on oil droplets; the contribution of the oil droplets to irritancy or inflammation has never been addressed. The emulsifier adsorbed at the oil–water interface is available, though sparsely, for interaction with the SC components. As summary, both the surfactant molecules and the other excipients of the formulation contribute to the skin irritancy.

The topic is made even more complex because there are several mechanisms by which surfactant can cause irritancy. Surfactants can have a detergent activity that causes removal (washing off) of the SC lipids. They can penetrate the skin and associate with the SC lipids, causing a fluidization of the SC barrier materials. They can penetrate the skin deeper into the viable layers and cause immune reactions. They can associate and denaturate biological materials such as proteins. Since there are so many mechanisms of action, several complementary testing methods should be associated for a significant overview of the surfactant activity can be reached. Indeed, several authors who tried to sort detergents according to their skin irritancy concluded that the irritancy ranking was dependent on the choice of the type of exposure method and the type of disturbance. As an example Tupker et al. [10] evaluated the skin irritancy ranking of an anionic detergent by several methods and pointed out the influence of the evaluation method when compared with the outcome of the irritancy method. Moreover depending on the method used and the kind of disorder (lipid removal or interaction with proteins as example) no definite link was found between two definite methods such as blood flow and TEWL [1]. *In vitro* tests evaluate both irritancy and cytotoxicity. These tests have become more widely used following the 6th amendment of the European Union Cosmetics Directive [11] which drew attention to alternative methods developed to replace animal testing for irritancy assessment. These are more complex methods using biochemical markers for studying the irritancy and cytotoxicity of skin. Development of *in vitro* skin models has grown exponentially in recent decades, starting from keratinocyte monolayer and extending to human reconstructed epidermis or living skin equivalent models. Ponec et al. [12] reported that these models are equivalent to native skin tissues, based on architecture, lipid composition and homeostasis measurements. Furthermore, these models are recommended by the European authorities for skin irritation assessment [13]. It is recognized though that reconstructed skin models have inferior skin barrier function than real skin [14,15].

Skin toxicity studies are based on several available tests, both *in vitro* and *in vivo*. All such tests provide indirect information on skin toxicity; they all give complementary pieces of information. *In vivo* tests more generally aim at measuring irritation than assessing cytotoxicity. After application of patch tests or soap chamber tests over various exposure times, skin is observed by macroscopic methods, such as visual and histological examination [16], measurements of skin redness with a chromameter [17], evaluation of skin blood flow and erythema [18]. Other physical measurements can provide information on surfactant action on the SC barrier such as transepidermal water loss (TEWL) and corneometry, which reflect alteration of the skin barrier against water diffusion and disturbance of the lipid matrix of SC [19–21].

Cell viability is often evaluated by means of the MTT assay that makes use of the absorbance of the MTT tetrazolium dye (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) [22]. The classical MTT assay reflects the mitochondrial enzyme activity [23–26]. It is generally coupled with an LDH assay [27], giving complementary data on membrane alteration [28–30]. As acknowledged in a workshop of the European Center for the Validation of Alternative Methods, there is indeed a correlation between irritant potential and reduced cell viability, but other important factors, such as cytokine release, must also be considered [31]. Cell viability measurements can be combined with interleukin assay as inflammation markers, IL-1α and IL-8, are released in the case of inflammation [30,32]. The inflammatory mediator IL-1α initiates the inflammation process [33]. IL-1α is expressed