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Membrane-lytic actions of sulphonated methyl ester surfactants and implications to bactericidal effect and cytotoxicity

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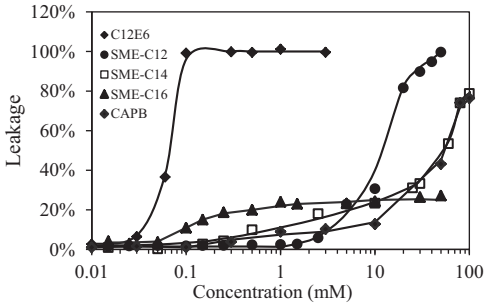
GRAPHICAL ABSTRACT



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ABSTRACT

Surfactants are multifunctional molecules widely used in personal care and healthcare formulations to cleanse, help disperse active ingredients (e.g., forming emulsions) and stabilise products. With increasing demands on improving biosafety, there is now mounting pressure to understand how different surfactants elicit toxicities at molecular and cellular levels. This work reports the membrane-lytic behaviour of a group of sulphonated methyl ester (SME) surfactants together with representative conventional surfactants. All surfactants displayed the clear rise of lysis of the model lipid bilayer membranes around their CMCs, but the two ionic surfactants SDS and C_{12}TAB even caused measurable lysis below their CMCs, with membrane-lytic actions increasing with monomer concentration. Furthermore, whilst ionic and nonionic surfactants could achieve full membrane lysis once above their CMCs, this ability was weak from the SME surfactants and decreased with increasing the acyl chain length. In contrast to the conventional anionic surfactants such as SDS and SLES, the protein solubilizing capability of the SME surfactants was also low. On the other hand, MTT assays against 3T3 fibroblast cells and human chondrocyte cells revealed high toxicity from SDS and C_{12}TAB against the other surfactants studied, but the difference between SME and the rest of conventional surfactants was small. Similar behaviour was also observed in their bactericidal effect against *E. coli* and *S. aureus*. The trend is broadly consistent with their membrane-lytic behaviour, indicating little selectivity in their cytotoxicity and bactericidal action. These results thus reveal different toxicities implicated from different surfactant head groups. Increase in acyl chain length as observed from SME surfactants could help improve surfactant biocompatibility.

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1. Introduction

Surfactants are widely used in personal care, healthcare and hygiene products [1]. They are also widely used in skin based drug delivery systems and formulation [2]. Their main physical role is to help disperse components that may have poor water solubility and improve product stability, irrespective of their appearances, e.g., dispersions, foams, emulsions or gels. In addition to product stabilization, surfactants can also work as emollients and moisturisers to soften skin by reducing moisture evaporation. For the products that are dedicated to cleansing, their contacts with skin are short. In many personal care and healthcare applications, however, where the products are left on skin (so-called leave-ons), their mildness or biocompatibility must be more carefully assessed as prolonged skin contact might cause skin irritancy or toxicity.

A typical formulated personal care or infection control cream often contains more than a dozen of ingredients that have different levels of toxicity, but surfactants are usually the most abundant. Surfactants and other ingredients can be synthetic or naturally occurring. Because of increasing demands on biosafety and environmental concern, there is now growing requirement for understanding which type of surfactants is better suited for a particular use. The European Union (EU) has the most restrictive regulations to control chemicals used in personal care, healthcare and hygiene; products sold in the EU must comply with these regulations [3]. Despite these restrictions, new surfactant based products may still be developed by using existing and newly developed chemicals with biosafety information available, demonstrating that benefits outweigh hazards [4].

Human skin acts as a barrier to resist the penetration of many molecules, particularly those with molecular weights (MWs) below 500 Dalton [5,6]. Because most surfactants currently used in personal care and healthcare have MWs below 500 Da they have been examined by various test models investigating their effects in mediating permeation across the skin barrier. Extensive research has provided evidence to support the view that most known contact allergens are under 500 Da and that larger ones usually can't act as contact sensitizers. In addition, common pharmacological agents for topical skin treatment are usually under 500 Da [5]. In contrast, immune suppressants aimed at topical applications such as cyclosporine, tacrolimus and ascomycins have MWs above 500 Da, thus augmenting this point from the opposite side [6]. However, it should be noted that marking the MW of 500 Da as the limit is largely empirical as there are some known allergens that have MWs above 500 Da. On the other hand, the exact chemical allergens can be person specific. Various dermatological tests have been developed including the routine patch test series advised by the International Contact Dermatitis Research Group (ICDRG) to diagnose contact allergy from potential sensitizing agents [6].

Extensive research over the past 2 decades using cell models and clinical studies have revealed controversial results suggesting that certain cosmetic ingredients such as parabens, aluminium salts, phthalates, or bisphenol A could be carcinogenic and mutagenic to humans [7]. They could act as xenoestrogens to disrupt the normal metabolism of the natural estrogen and result in DNA damage in animal and human mammary epithelial cells. In contrast, surfactants such as nonyl phenol ethoxylates (the Triton series) have also been reported to be toxic to mammalian cells and aquatic species by lysing cell membranes [8]. In vitro and in vivo tests on different skin models have suggested that cationic surfactants are more toxic than anionic ones whilst nonionic surfactants were not-toxic for the skin [9–12].

In addition to membrane disruption, the irritancy of ionic surfactants could be enhanced by their ability to bind to keratin and lead to membrane swelling because ionic surfactant molecules can initiate their binding to proteins through electrostatic attraction and the process is then promoted by hydrophobic interaction [13]. The nature of the polar head group appears as a significant factor governing the irritancy. Whilst both anionic and cationic surfactants can bind to protein molecules due to the presence of cationic and anionic amino acids in their structure the exact strength of binding and structural disruption is also dependent on the proteins concerned and their physical properties such as the isoelectric points, the net numbers of positive and negative amino acids and their structural stability (tertiary structure). On the other hand, ionic surfactants with different sizes and CMCs may impose different extent of interaction, resulting in different skin irritancy and cytotoxicity [4].

In spite of extensive studies of biosafety of surfactants used in personal care and healthcare, there is still a lack of understanding of how surfactant structures affect their cytotoxicities. Furthermore, as surfactants can attack bacterial membranes and kill them as well, it would be highly desirable to understand how to optimize their actions against bacteria whilst minimizing their side effects on host cells [14,15]. The sulfonated methyl esters (SME) have recently been reported to show attractive surface adsorption behavior [16–18]. Methyl esters are shown to be easier to degrade than other conventional surfactants [19]. They could thus be considered as alternatives to replace some conventional surfactants but a key criterion that must also be considered is their cytotoxicity. In this study we investigated the mildness or biocompatibility of SME-C_n (where n stands for the number of carbon atoms in the fatty acid chain, n = 12, 14, 16) against other conventional surfactants. Through measurements of their lysis of model vesicles, capacity in solubilizing zein and their ability to kill representative bacteria and mammalian cell models, the working mechanism underlying membrane-lytic actions and potential benefits from these surfactants are discussed.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals and surfactants

The acyl sulfonated methyl esters (SME) were prepared by sulphonation of methyl esters with different fatty acid chains (dodecanoic, myristic and palmitic acids) and denoted as SME-C12, SME-C14 and SME-C16, respectively. They were provided by KLK Oleo, with their molecular structures shown in Scheme 1. At ambient conditions, SME-C12 appeared in the form of thick pastes, SME-C14 in the form of dry powders and SME-C16 in the form of flakes. They were used as received without any further purification. These SME samples were of the same batches as used by Danov et al. [16,17] who showed the purity above 98% and 96.0% for SME-C14 and SME-C16, respectively by liquid chromatography–mass spectrometry (LC/MS) analysis. They suggested that the samples might contain a small amount of unsulfonated methyl esters and other compounds as impurities. However, the LC-MS characterisations revealed a small amount of homologues with neighbouring chain lengths in each sample but with only traces of unsulfonated methyl esters present. These observations were further confirmed by their combined measurements of surface tension and electric conductivity, as will be explained later.

Other surfactants including sodium dodecylsulphate (SDS), dodecyltrimethylammonium bromide (C_{12}TAB), hexaethyleneglycol monododecyl ether ($C_{12}E_6$) and Triton X-100 (octylphenol ethoxylates, used as reference in membrane lysis) were all analytical reagents from Sigma-Aldrich. Sodium lauryl ethoxylate sulphate (SLES), linear benzyl-alkyl sulphonate (LAS) and