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Skin Irritation Potential Study of Anionic Surfactants with Electron Paramagnetic Resonance (EPR) Spectroscopy

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1. Background/ Objectives

Each day our skin is in contact with a multitude of aggressions that we need to minimize. It is very important to predict irritation potential of the chemical, which potentially contacts to our skin. For that purpose, the test should be conducted on “human”. However, it is impossible to do it from a humanitarian point of view. Accordingly, Draize test employing “animal (rabbit)”, of which procedure was developed in 1944 by Draize and his colleague, has been utilized for a long time to assess primary irritation and corrosion induced by chemicals.

The reproducibility of Draize test procedure has been questioned, and numerous modifications have been examined and proposed to improve its prediction of human experience. Criticisms of the Draize test have been embraced by the groups supporting abolition of animal testing as demonstrating that use of the method is unwarranted.

During the last few decades, in vitro alternative methodology, which is “cytotoxicity assays” employing viable cells and the other equivalent, have been extensively studied and developed for assessing toxicity of chemicals.

“Surfactant”, which has a “hydrophilic” moiety and a “lipophilic” moiety in its structure, has a tendency to reach across (bridge) the two phases: the hydrophilic phase and the lipophilic phase. Such substances have, therefore, also been called “amphiphilic”. Anionic surfactants are mainly used in cleansing products which consumers are exposed on a daily basis. Cleansing products like shampoos, bar soaps, are undoubtedly some of the irritating products, particularly because they are applied repeatedly to the body, which is sensitive. Therefore, it is not surprising that one of the most desirable claims for cleansing products is “mild” (“non-irritant” ideally).

The mechanism of surfactant inducing skin irritation has been incompletely understood. It has been discussed with respect to protein denaturation, lipid removal, inhibition of cellular proliferation and chemical mediator-release contribute to irritation. The first step of skin alteration, structural change of stratum corneum lipid, occurs when chemicals contact to the skin surface. A major barrier to chemicals diffusion through the skin is the stratum corneum, composed of corneocytes embedded in lipid domains consisting of alternately hydrophilic and lipophilic layers. Information on the molecular structure of these lipids is important in elaborating a rational design for effective penetration enhancers in transdermal drug delivery and to understand the mechanism of irritant dermatitis and other stratum corneum diseases, and various spectroscopic approaches such as thermal analysis, X-ray diffraction, and FT-IR spectroscopy have been used.

Electron paramagnetic resonance (EPR), employing nitroxide spin labels, measures the freedom of anisotropic molecular motion and the polarity of the spin labeled molecule providing structural information on biological membranes. We tried to apply this approach to characterize human stratum corneum treated with

anionic surfactants selected from the available on the market (sodium lauroyl glutamate (SLG), sodium lauryl sulfate (SLS) and some anionic surfactants and their mixtures) focusing on physico-chemical interactions between anionic surfactants and intercellular lipid layers in stratum corneum.

Patch tests on human, Draize tests and several techniques of in vitro cytotoxicity assay were also conducted to discuss the correlation between EPR spectral data (order parameter) and the clinical/ in vitro assay data.

2. Methods

2.1 Test articles

Following surfactants were used for the study.

Table 1: Test Articles (anionic surfactants)

#	Abbreviations	Chemical Name	Lipophilic moiety	Hydrophilic moiety
1	SLS	Sodium lauryl sulfate	C12	sulfate
2	SLMT	Sodium N-lauroyl-N-methyltaurate	C11	Methyltaurate (sulfate)
3	SLES	Sodium laurylethersulfate	C12	ethersulfate
4	SL	Sodium laurate	C11	carboxylate
5	SMLP	Sodium monolaurylsphosfate	C12	phosphate
6	SLEC	Sodium laurylethercarboxylate	C12	carboxylate
7	SLG	Sodium N-lauroyl-N-glutamate	C11	glutamate (carboxylate)

2.2 Safety testing methods

Human patch test [1], Draize method for skin irritation [2], MTT assay and NR assay on human keratinocyte [2], SIRC toxicity assay [2], Skintex® [2] were conducted.

2.3 Electron Paramagnetic Resonance Spectroscopic analysis with human cadaver stratum corneum [3, 4]

Human abdominal skin was obtained from fresh cadaver skin with a dermatome. The epidermis was separated from the dermis by immersing the dermatomed skin in 60^{o}C water and the stratum corneum was obtained from the epidermis by trypsin treatment. The stratum corneum was dried in a desiccator at -70^{o}C for 3~4 days.

5-DSA was used as a stearic acid spin labeling agent. Some slices of dry stratum corneum sheets (approx. 0.4 cm^2, approx.0.4 cm x approx. 1 cm) were incubated in Tris-HCl buffer solution (pH 7.4) dissolving spin labeling reagents at 10 mg/L (2.6x10^{-5} M) for 2 hour and at 37^{o}C and then dried under flow of nitrogen gas for 1 hour at approx. 25^{o}C over silica gel for laboratory convenience.

Treatment with anionic surfactants was performed as follows: spin-labeled stratum corneum (5-DSA) was immersed in the surfactant aqueous solutions prepared at designated concentration, and incubated at approx. 37^{o}C for 1 hour. After rinsing with purified water, they were dried by the same procedure as described in the above.

EPR spectrum measurements were carried out by an ER200Seris ESR spectrometer (IBM Instruments Inc., Danbury, CT, USA) with microwave power out of 25mW and spectrum data were collected by an IBM-PC system. The hyperfine splittings of labeled skin samples were determined with 100 gauss scan width, 4 x 10^2 receiver gain, 16-minutes scan time and 0.3-second time constant. Each sample was scanned several times. EPR parameter from each spectrum, which is “order parameter S”,

was calculated and averaged to give a single estimate for the example/ condition.

3. Results

Draize test, human patch test and in vitro tests showed that SLG is “mild”, SLS is “severe” and other surfactants are “moderate”.

The profiles of EPR spectra of 5-DSA labeled depend on the anionic surfactants treated. The corresponding order parameters S obtained from each EPR spectrum and *in vivo* human patch test results were summarized in Table-2, which is one of the data sets.

Table 2. Order parameters of stratum corneum treated with surfactants and clinical observations

Sample Name	Averaged Order Parameter ***S*** (mean ± SD; n=3)	Human Patch (mean ± SEM; n=15)	
		Visual Score	TEWL g/m^2/h
Control	0.86 ± 0.03	0.53 ± 0.08	13.0 ± 1.0
0.25 %wt SLS	0.70 ± 0.02	0.73 ± 0.08	22.3 ± 1.7
0.50 %wt SLS	0.66 ± 0.04	0.70 ± 0.10	22.3 ± 1.7
0.75 %wt SLS	0.64 ± 0.03	0.87 ± 0.14	22.7 ± 1.5
1.00 %wt SLS	0.56 ± 0.03	1.03 ± 0.15	25.4 ± 2.6
0.25 %wt SLS + 0.75 %wt SLG	0.81 ± 0.07	0.42 ± 0.30	20.0 ± 1.7
0.50 %wt SLS + 0.50%wt SLG	0.71 ± 0.00	0.08 ± 0.20	20.7 ± 1.9
0.75 %wt SLS + 0.25 %wt SLG	0.66 ± 0.04	0.04 ± 0.10	21.2 ± 2.6
0.25 %wt SLS + 1.00 %wt SLG	0.81 ± 0.05	**NA**	**NA**
0.50 %wt SLS + 1.00 %wt SLG	0.79 ± 0.05	**NA**	**NA**
0.75 %wt SLS + 1.00 %wt SLG	0.74 ± 0.04	**NA**	**NA**
1.00 %wt SLS + 1.00 %wt SLG	0.66 ± 0.05	**NA**	**NA**
1.00 %wt SLG	0.82 ± 0.02	0.67 ± 0.08	15.8 ± 1.1

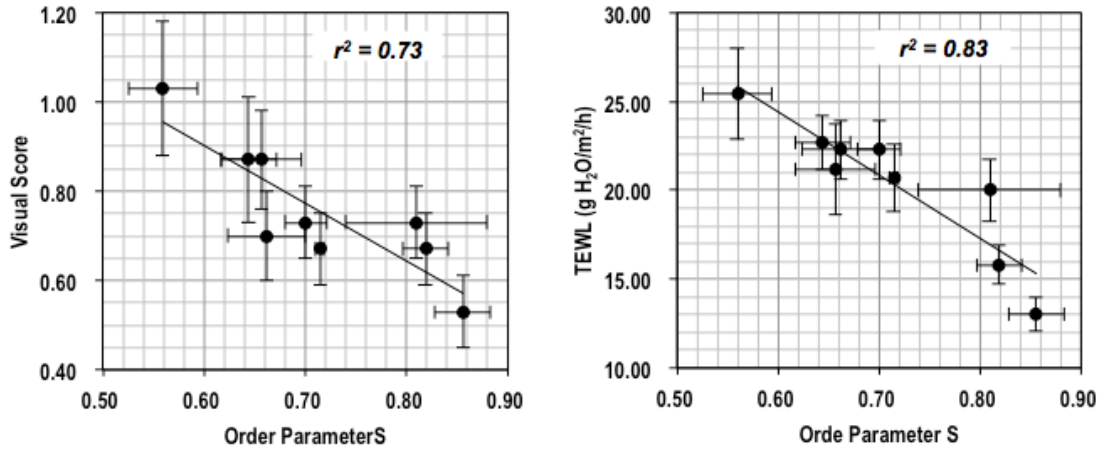
The order parameter of water treated stratum corneum (vehicle control) was 0.86 ± 0.03. Anionic surfactants as an amphiphilic molecule might be incorporated into structured lipids (lamellar structure). Order parameter (***S***) calculated from 1.0%wt SLS treated stratum corneum was 0.56 ± 0.03, indicating lipid structure disordering. On the contrary, the high ***S*** value (0.82 ± 0.02) for 1.0%wt SLG means less lipid structure disordered, which means 1.0%wt SLG almost equals to water. Treatment with 0.25%wt, 0.50%wt and 0.75%wt SLS solutions revealed intermediate levels between 1.0%wt SLG and SLS.

Each order parameters of 5-DSA labeled stratum corneum treated with SLS/SLG mixtures (total concentration is constant at 1.00 %wt) showed higher values than that of 0.25 %wt, 0.50 %wt, 0.75 %wt SLS, respectively. There were no statistically significant difference between 0.50 %wt SLS and 0.50 %wt SLS / 0.50 %wt SLG, and between 0.75 %wt SLS and 0.75 %wt SLS / 0.25 %wt SLG. ($p>0.05$)

Order parameters at each SLS concentration (0.25, 0.50, 0.75 and 1.00 %wt SLS) with 1.0%wt SLG showed higher values than those of SLS only solutions. There were statistically significant differences between with and without 1.0%wt SLG. ($P<0.05$), suggesting that the addition of 1.0%wt SLG inhibits the fluidization of intercellular lipid by SLS.

Order parameter ***S*** (EPR spectral data) correlated with the clinical readings. The correlation coefficients ($\mathbf{r^2}$) of visual score and TEWL values were 0.73 and 0.83, respectively. The order parameter ***S*** correlates to TEWL values better than to visual scores. This difference may be explainable that TEWL is a measure of water barrier function while visual scores represent total skin reactions including physical or

structural changes and physiological or biological reactions with surfactant. The order parameter measurement represents the skin water barrier function.



(a) (b)

Fig-1. Correlation between clinical data of 24 hour patch test and order parameter of 5-DSA labeled cadaver stratum corneum incubated in surfactant solution for 1 hour at 37°C: (a) correlation between order parameter and visual scores; (b) correlation between order parameter and TEWL (error bars: mean ± SD for order parameter n=3, mean ± SEM for clinical data n=15)

4. Conclusion

We demonstrated that order parameter is an easy to use and quantifiable method for predicting irritation reaction in the skin. The use of EPR spin labeling method should provide further insight into the mechanism of epidermal barrier disruption (fluidity) by surfactants. It may also aid in investigating the irritation potential of general chemicals, effects of topical penetration enhancers, drug delivery systems and skin diseases such as xerosis and atopic dermatitis. It should be also interesting to apply this technique for stripped stratum corneum instead of cadaver skin.

5. References

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