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Quantitative Structure-activity Relationship of Surfactants on Eye Irritation Predicted by Hemoglobin Denaturation

HAYASHI, T.[1], ITAGAKI, H.[2], FUKUDA, T.[1], TAMURA, U.[1] and KATO, S.[2]

[1]Shiseido Product Research Center, [2]Shiseido Safety & Analytical Research Center, 1050 Nippa-cho, Kohoku-ku, Yokohama 223, Japan

Summary

Hemoglobin denaturation property of 21 surfactants was measured spectrophotometrically and hemoglobin denaturation ratio (HDR) were calculated. HDR of each surfactant was processed by an equation obtained by the multi-regression analysis to predict eye irritation (HDR method; corneal and total Draize score were estimated with the accuracy of r=0.878 and r=0.861 respectively by the equation composed by HDR at two or three concentration level of tested surfactant).

On the other hand, 21 surfactants were classified on the basis of structural feature (hydrophilic group; six categories, counter ion; two categories, and additional hydrophilic group; nine categories). Predicted eye irritation score (equivalent to Draize total score) was subjected to quantification theory category one (enable us to predict quantitatively described criterion variable, in this case eye irritancy predicted by HDR basis, by using categorically described explanatory variables, in this case structural classification) to identify the quantitative structure-activity relationship (QSAR) of surfactants on eye irritation.

Results of the analysis indicated that very high correlation was observed between the structural features and the estimated eye irritation scores (r=0.92) although mechanism is still obscure. This method provides us the useful quantitative evaluation of the irritancy of surfactants without any experimental efforts.

Introduction

The Draize eye irritation test[1)] has been criticized from the viewpoint of animal welfare in recent years. Thus a number of materials, such as EYTEX[2)], red blood cell[3)], albumin[4)], SIRC and HeLa cells[5)], chorioallantoic membrane of fertile egg[6)] and liposomes[7)] have been used in *in vitro* test systems for predicting eye irritancy.

In the previous report, we have built the hypothesis that eye irritation is primarily determined by protein denaturation and destruction of the cellular plasma membrane system, and presented a regressional quantitative evaluation method (HDR method) using hemoglobin denaturation[8)]. In this work, we tried to utilize the method and tried to determine QSAR of surfactants.

QSAR approach to primary eye irritation of chemicals has been reported by Sugai *et al*[9)10)]. However the report emphasized a substructural features and few examination were done on a series of chemical groups, such as hydrophilic group (terminal hydrophilic group in the molecule, for example, amido bond, ether bond, ester bond, etc.), counter ion, and additional hydrophilic group (hydrophilic group other than terminal hydrophilic group in the molecule). Further, the report did not mention much about surfactants. The

*Correspondence to:Mr. Toshikatsu Hayashi,Shiseido Research Center,Research planning and coordination division, 1050 Nippa-cho, Kohoku-ku, Yokohama 223, Japan.
Fax: (Japan) 045-543-3436

QSAR methodology used in that report is adaptive least-squares (ALS) method[11)12)]. In this study, quantification theory category one[13)14)] which is basically the same method as aLS method was applied to analyze the relationships. The quantification theory category one does not assume any particular distribution for the data of structural features of chemicals and employs quantitative data for biological activity. However, the theory is quite understandable and useful for various purpose as far as the criterion variable is quantitative and explanatory variables are categorically described.

Materials and Methods

1) Chemicals

Twenty-one kinds of anionic surfactants (Table 1) are concerned for the hemoglobin denaturation measurement to investigate the structure-activity relationship between surfactant and eye irritation on the bases of hemoglobin denaturation.

All surfactants were obtained commercially and used without purification.

Table 1. List of tested surfactants (hemoglobin denaturation measurement)

Name of surfactant	Abbreviation
Sodium laurate	S1
TEA laurate	S2
Sodium lauryl ether carboxyrate	S3
Sodium hydroxy ethr carboxylate	S4
Sodium acyl sarcosinate	S5
TEA acyl sarcosinate	S6
Sodium acyl alaninate	S7
Sodium hydroxy ether sulfonate	S8
Sodium acyl methyl taurate	S9
TEA acyl methyl taurate	S10
Sodium alkyl sulfo carboxylate	S11
Sodium dodecyl sulfate	S12
Sodium POE dodecyl ether sulfate	S13
TEA POE dodecyl ether sulfate	S14
sodium amide ether sulfate	S15
Sodium mono glyceryl sulfate	S16
Sodium acyl glutamate	S17
TEA acyl glutamate	S18
Sodium alkyl sulfo succsinate	S19
Sodium alkyl ether sulfo succsinate	S20
Sodium mono alkyl phosphate	S21

2) Determination of eye irritation score using hemoglobin denaturation test

Hemoglobin was dissolved in the standard phosphate buffer (pH 6.86) at 0.05% (w/w) concentration. Surfactants were diluted with ionexchange water to make 2% (w/w) solution. 100 μl of surfactant solution at 12 concentrations (from 0% to 2%, w/w) according to the serial twofold dilution method were placed in 8 replicates on the 96 well microplate (Sumitomo Bakelite co., Ltd., Tokyo). An equal amount of hemoglobin/buffer solution was added to each well of four lines immediately followed by buffer solution to the remaining four lines. Organized micro plate was incubated five minutes at 25°C and optical absorbance at 418 nm was measured by an microplate reader (BIO-RAD Model 3550). The absorbance data (n=4) were processed in accordance with the following equation (Formula 1) and hemoglobin denaturation ratio (HDR) at each concentration was calculated.

$$HDR=100-\{Abs(SHB)-Abs(SB)\}/\{Abs(WHB)-Abs(WB)\}\times 100(\%)$$

(Formula 1)

where Abs(SHB); Absorbance of surfactants mixed with hemoglobin/buffer solution,

Abs(SB); Absorbance of surfactants mixed with buffer solution,

Abs(WHB); Absorbance of ionexchange water mixed with hemoglobin/buffer solution,

Abs(WB); Absorbance of ionexchange water mixed with buffer solution.

Dt (estimated eye irritation score equivalent to draize total score on the basis of HdR) was calculated according to the equation below (Formula 2). The equation was obtained by the multi-regression analysis and

Corneal draize score (Dc) and total Draize score (Dt) were estimated with the accuracy of r=0.878 and r=0.861 respectively by the equation composed by HDRs at two or three concentration level of tested surfactant.

Dt=14.922+0.686×HDR% at 2%+0.496×HDR% at 0.063%

(Formula 2)

14.922 is a constant and 0.686, 0.496 are coefficients. For the prediction of total draize score, two HDRs (at 2% concentration of surfactant and 0.063%) were used.

3) Classification of surfactants

Twenty-one surfactants were classified on the basis of structural feature. They are classified by their terminal hydrophilic group (six categories), counter ion (two categories), and additional hydrophilic group other than terminal hydrophilic group (nine categories) as are shown on Table 2. Classification of each surfactant together with the Draize equivalent total eye irritation score (Dt) of the surfactant is shown on Table 3.

4) Statistical calculation (QSAR analysis by using quantification theory category one)

In order to quantify the effect to the irritancy of the structure, we postulate that eye irritancy (Ie) is explained by the linear addition model composed by a category (k; detail classification of the structure of each item) of an items (j; terminal hydrophilic group, counter ion, and additional hydrophilic group). For describing each surfactant's belongings to the items and the categories, we define a dummy function; $f_i(jk)$ as $f_i(jk)=1$ when surfactant i is classified k category of j item and $f_i(jk)=0$ when surfactant i is classified as the other. Then the eye irritancy of each surfactant (Ie_i) can be described as following equation (Formula 3; ejk is a category score explaining the irritancy effect of the structural category of the item).

$$Ie_i=\sum\sum ejk f_i(jk)$$

(Formula 3)

For the sake of predicting Iei with the highest accuracy, least squares method is applied to minimize the gap between the predicted eye irritation (Pe_i) and Ie_i as follows (Formula 4).

$$\sum(Pe_i-Ie_i)^2 \text{---}>\text{minimize}$$

(Formula 4)

This can be achieved by solving partial differential equation with the assist of a computer. The calculations wer done by the Lotus 1-2-3 program with an add-in package program for statistical analysis (Lotus 1-2-3 Multi-variate analysis v 1.0) provided by Audemain, Tokyo.

Results

Classified structural features and estimated Draize equivalent total eye irritation score (Dt) were subjected to the quantification

Table 2. Structure classification of surfactants

Number of categories	Hydrophilic group	Counter ion	Additional hydrophilic group
1	-COO	Na	none
2	-COO, -COO	TEA	-CONH-
3	$-SO_3$		$-CON(CH_3)-$
4	$-OSO_3$		-COO-
5	$-PO_4$		-EO-
6	-COO, $-SO_3$		-O-, -OH
7			-CONH-, -cO-
8			-COO-, -OH
9			-COO-, -EO-

Table 3. Structure classification of surfactants and their estimated eye irritation scores (Dt)

Surfactant	Hydrophilic group	Counter ion	Additional hydrophilic group	Dt (HDR% oriented estimated eye irritation score)
S1	-COO	Na	none	27.7
S2	-COO	TEA	none	28.8
S3	-COO	Na	-EO-	29.3
S4	-COO, -COO	Na	-O-, -OH	27.2
S5	-COO	Na	$-CON(CH_3)-$	27.7
S6	-COO	TEA	$-CON(CH_3)-$	21.8
S7	-COO	Na	$-CON(CH_3)-$	25.3
S8	$-SO_3$	Na	-O-, -OH	26.5
S9	$-SO_3$	Na	$-CON(CH_3)-$	20.6
S10	$-SO_3$	TEA	$-CON(CH_3)-$	22.0
S11	$-SO_3$	Na	-COO-	37.2
S12	$-OSO_3$	Na	none	29.7
S13	$-OSO_3$	Na	-EO-	23.8
S14	$-OSO_3$	TEA	-EO-	27.0
S15	$-OSO_3$	Na	-CONH-, -EO-	16.4
S16	$-OSO_3$	Na	-COO-, -OH	29.7
s17	-COO, -COO	Na	-CONH-	40.6
s18	-COO, -COO	TEA	-CONH-	27.1
S19	-COO, $-SO_3$	Na	-COO-	28.9
S20	-COO, $-SO_3$	Na	-COO-, -EO-	32.5
S21	$-PO_4$	Na	none	35

theory category one to identify the QSAR of surfactants. Table 4 shows the coefficients (category scores) assigned to the structural features in the equation for the estimation of eye irritancy potential.

Contribution of each categories to the estimated eye irritation score could be discussed by coefficients. The bigger score, the more irritant. Range of a category also indicates the contribution of an item to decide irritancy of a surfactant.

According to the results, additional hydrophilic group showed the biggest contribution. On the other hand, contribution of counter ion had very small influence toward Dt. Hydrophilic group had a medium effect.

Coefficients of a category of each item (structural group) can be compared to discuss the effect of each category to eye irritation. But comparison must be done among the item. In order to compare the strength of contribution among each item, coefficients of each category should be standardized. Standardized effects towered the eye irritation

Table 4. Structure-activity relationship obtained by the quantification theory category one

Item	Category	Coefficient	Range
Hydrophilic group	-COO	11.444	16.322
	-COO,-COO	9.000	
	$-SO_3$	8.300	
	$-OSO_3$	10.219	
	$-PO_4$	16.322	
	-COO, $-SO_3$	(0)	
Counter ion	Na	2.939	2.939
	TEA	(0)	
Additional hydrophilic group	none	-13.822	26.319
	-CONH-	-6.181	
	$-CON(CH_3)-$	-18.031	
	-COO-	-3.600	
	-EO-	-15.447	
	-O-, -OH	-14.300	
	-CONH-, -EO-	-26.319	
	-COO-, -OH	-13.019	
	-COO-, -EO-	(0)	

Constant: 29.561
Correlation coefficient: 0.916

Table 5. Standardized structure-activity relationship obtained by the quantification theory category one

Item	Category	Standardized Coefficient	Range
Hydrophilic group	$-PO_4$	7.108	16.322
	-COO	2.229	
	$-OSO_3$	1.004	
	-COO, -COO	-0.214	
	$-SO_3$	-0.914	
	-COO, $-SO_3$	-9.214	
Counter ion	Na	1.469	2.939
	TEA	−1.469	
Additional hydrophilic group	-COO-, -EO-	12.302	26.319
	-COO-	8.702	
	-CONH-	6.121	
	-COO-, -OH	-0.717	
	none	-1.520	
	-O-, -OH	-1.998	
	-EO-	-3.145	
	$-CON(CH_3)-$	-5.729	
	-CONH-, -EO-	-14.017	

Constant: 27.943(Average of 21 surfactants)
For the standardization, results of the analysis were processed as follows;
1) calculate a gap between average coefficient of a category and a coefficient to obtain standardized coefficient.
2) average coefficient of each category were totaled and added to constant to obtain standardized constant.

activity were summarized on Table 5 and visualized in Fig. 1.

For the standardization, results of the analysis were processed as follows;

1) calculate a gap between average coefficient of a category and a coefficient to obtain standardized coefficient.
2) average coefficient of each category were totaled and added to constant to obtain standardized constant.

According to the result shown in Table 5 and Fig. 1, it became clear that the surfactant which has certain heterogeneous hydrophilic group (-COO group and $-SO_3$ group) would show the biggest effect to decrease the irritancy potential (Dt) among the hydrophilic group. The magnitude of the contributions of other hydrophilic group to Dt was low.

Difference of the counter ion did not show the noticeable effect but TEA was slightly better than Na. As for additonal hydrophilic group, -CONH- group together with -EO- group would show the best performance for decreasing Dt and $-CON(CH_3)-$ was the second. On the contrary, existence of both -COO- and -EO-, -COO-, and -CONH- bond worsened the score relatively.

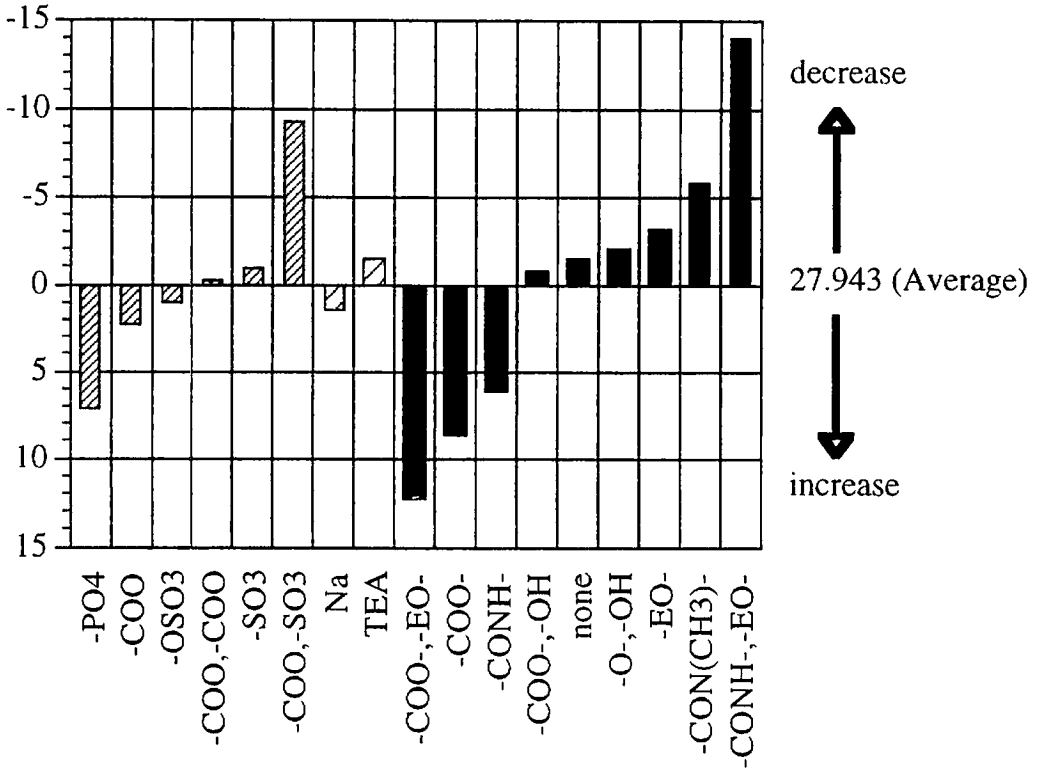

Fig. 1. Effect of each structure toward the eye irritation activity (Dt)

Discussions

Quantification theory category one could provide the accurate structure-activity relationship for the estimation of eye irritancy for 21 surfactants. Results of the analysis (Table 4) indicated that very high correlation (r=0.92) was observed between the structural feature of surfactant and Dt. This suggests the possibility that there is a certain relationship between the structure of surfactant and its hemoglobin denaturation property which is related to the *in vivo* eye irritation property measured by Draize eye irritation test.

From the analysis, coefficients of eye irritancy score could be obtained for each structure group. Therefore any chemical's eye irritation score (Dt) can be calculated as long as the chemical contains classified structure group by using the coefficients assigned.

And as far as the analysis of 21 anionic surfactants was concerned, desirable structure of the chemical could be settled according to the coefficients obtained. For example, though it may be little audacious, imaginary chemical which is expected to show the lowest irritation score would contain heterogeneous terminal hydrophilic group (-COO, $-SO_3$), TEA as the counter ion an additional -CONH- bond and -EO- chain in a molecule. On the contrary, the chemical which has -COO group as the terminal hydrophilic group, Na counter ion and ester bond (-COO-) together with POE chain (-EO-) is supposed to show high irritation score.

For decreasing Dt, both -COO group and $-SO_3$ group would effect good among the hydrophilic group and another hydrophilic group did not show the remarkable effect. On the other hand, $-PO_4$ showed the increasing effect. As for additional hydrophilic group, -CONH- group togethr with -EO- group showed the best performance and $-CON(CH_3)-$ was the second. On the contrary,

existence of -COO- and -EO-, -COO-, and -CONH- bond worsened the score relatively.

There may be three possible mechanisms explaining these result; hydrophobicity, conformation of a molecule, and its electronic effect. Those factors effects the affinity of chemicals to hemoglobin and that causes the deference of irritation features. Further study is necessary to make these mechanisms clear although rational indexes of hydrohpobicity, conformation of molecule, and its electronic effect are very difficult to chose and measure. Possible index may be hydrophile lipophile balance (HLB) for hydrophobicity, molecule weight and electronegativity or electrochemical affinity for conformation of molecule, and $_pK_a$ for electronic effect.

Apart from the mechanism, hemoglobin denaturation test and QSAR clarified by quantification theory category one provide us the useful quantitative evaluation of the irritancy of surfactants. Although hemoglobin denaturation test (HDR method) is easy to perform and sterilized condition is not required, structure-activity relationship offers easier and quicker irritancy estimation of chemicals, for it requires no experimental efforts. Although structure-activity relationship analyzed in this report is not matured yet and further research is necessary, we believe this information about structure-activity relationship helps to know the irritancy of anionic surfactant.

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