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RADIOTRACER TECHNIQUE TO PREDICT IRRITATION POTENTIAL OF SOAP

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ABSTRACT

The application of a radiotracer technique using tritiated water to predict the irritation potentials of some soap products is demonstrated. Collagen films are treated with 0.5% and 1.0% soap solutions and tritiated water then incubated at 50 degrees centigrade for 24 hours. After incubation, the uptake of tritiated water by the collagen films was measured by liquid scintillation counting.

INTRODUCTION

Skin irritation characterized by redness, swelling, and flaking, has often resulted from exposure to some detergents, soap, and other cleaning products. Surfactants or surface active agents are the main ingredients in these cleaning products which give the necessary properties of detergency, emollience, and emulsification (1).

Studies have shown (2-4) that anionic surfactants or surfactant based products induced skin irritation, the extent of which is determined from the swelling of isolated skin and epidermal membranes. Choman (5) reported that the exposure of dermal collagen and skin to various anionic surfactants resulted in expansion and contraction of these substances.

Blake-Haskins et al. (6), described the process of swelling as one that begins with the adsorption of surfactants at the solution-substrate interface. The surfactants are thought to adsorb through a combination of hydrophobic and ionic interactions with the protein. Hydrophobic interactions between surfactant chains and the protein would leave pendant ionic head groups, and swelling would occur because of the electrostatic repulsion between them. Ionic interactions between the head groups and oppositely charged sites on the substrate would also

occur. These conclusions were drawn from their study on the irritancy of different types of surfactants, wherein a radiotracer technique was used to quantitatively determine swelling. The irritancy of surfactants was predicted from the amount of uptake by a collagen film of tritiated water from a treatment solution.

This method was adopted by our laboratory to evaluate the irritation potential of some local soap products. The study was undertaken from July-August, 1987 as a service contract between the Analytical Measurements Research (AMR) of the PNRI and the Philippine Institute of Pure and Applied Chemistry (PIPAC).

MATERIALS

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The ³H standard solution supplied by the International Reference Center - World Health Organization, was obtained from the Health Physics Research (HPR). Radioactivity was measured using a Packard Model 3255 Liquid Scintillation Spectrometer with Instagel, from Packard, as the liquid scintillation cocktail. The soap solutions and powders, collagen films, 0.1 mM phosphate buffer, pH 7.0, concentrated perchloric acid and 2N sodium hydroxide were provided by PIPAC.

METHOD

Samples of pre-weighed and pre-cut collagen films (ca. 1x1

2
cm) were each placed in a 20 ml screw cap vial. Each piece of film was treated with 10 ml soap solution in 0.1 mM phosphate buffer, pH 7.0, and enough tritiated water to give approximately 1×10^5 dpm/ml. This was later incubated at 50 C for 24 hrs in a water bath. After incubation, the collagen film was removed from the solution then rinsed with deionized water from a rinsing bottle to remove any adhering tritiated water. The rinsed film was placed in a liquid scintillation vial then digested with 1 ml 2N NaOH. It was later dissolved in 10 ml of scintillation cocktail then acidified with 0.25 ml concentrated perchloric acid.

Solutions for counting background and efficiency determination were prepared by digesting in the same manner untreated collagen films. For the efficiency determination, the collagen film digest was spiked with tritiated water before addition of scintillant.

Each sample, background, and standard was counted for 1 min in 3 cycles. The swelling was measured as the milliliters tritiated water uptake per gram of dry collagen. Three replicates were done on each sample.

RESULTS AND DISCUSSION

In the work done by Blake - Haskins et.al. (6), the effects of various surfactant treatments on the swelling of a

collagen film was studied extensively. Surfactants which were widely used and had well known relative irritation potentials, varying from mild to severe, were used. A summary of their work is presented in Table I.

TABLE I
Comparison of Surfactant-Induced Skin Irritation
and Swelling of Collagen Film

Test Solution (+)	In vivo (++) Skin Irritation	In vitro (+++) Collagen Swelling (ml/gm)
LAS	severe within 1 day	10.8 ± 0.1
AEOS. 3EO	mild to moderate by 5th day	4.4 ± 0.1
AEOS. 6EO	mild to moderate by 5th day	3.5 ± 0.1
AEOS. 9EO	none after 5 days	2.7 ± 0.1
Tween 20	none after 5 days	2.6 ± 0.1
Water	-----	1.6 ± 0.1

(+), Test Solutions:

LAS = Linear dodecylbenzenesulfonate
 AEOS. 3EO = Ammonium laureth-3 sulfate
 AEOS. 6EO = Ammonium laureth-6 sulfate
 AEOS. 9EO = Ammonium laureth-9 sulfate
 Tween 20 = Polysorbate 20, a mixture of laurate esters of sorbitol and sorbitol anhydrides

(++), Irritation in vivo: Human subjects, 10% concentration, pH 7, 2 to 3 subjects, Duhring chambers, 5 days.

(+++), Collagen swelling test conditions: 2 replicates, concentration 10 mM, time 24 hours, pH 7.0.

Two sets of uptake experiments were conducted by the AMR : one using 1.0% and another using 0.5% soap solutions. Collagen

swelling test conditions were similar to the above. Results of these experiments are shown in Table II.

TABLE II
Results of Collagen Swelling Experiments on Soap Solutions

Sample Code	Collagen Swelling* (ml/gm)
I. 1.0% soap solution	
3	3.3 ± 0.6 a
4	3.1 ± 0.1 a
5	2.4 ± 0.5 b
II. 0.5 % soap solution	
B	3.6 ± 0.7 a
E	3.5 ± 0.3 a
C	3.3 ± 0.5 a
F	3.0 ± 0.4 a
A	2.8 ± 0.3 b
G	2.8 ± 0.3 b
D	2.0 ± 0.4 c
Water	1.3 ± 0.2 d

* Collagen swelling test conditions: 3 replicates, time 24 hours, pH 7.0. Values followed by different letters are significantly different according to Duncan's Multiple Range Test.

Based on the classifications derived by Blake - Haskins et al. (see Table I) from the comparison of surfactant-induced skin irritation and the swelling of collagen film, we could classify the soap samples studied into two categories: mild to moderate irritants, (a), with collagen swelling ranging from 3.0 to 3.6 ml/gm, and non-irritants, (b,c), with collagen swelling ranging from 2.0 to 2.8 ml/gm.

Results further indicated that there was no significant change in swelling with concentration. This was also observed by Blake - Haskins et. al. (6) in the case of AEOS.3EO and Tween 20 , classified as mild irritant and non - irritant respectively. This was attributed to an apparent substrate saturation at the given test conditions. Furthermore, the value obtained for water in this study is comparable to the results of Blake - Haskins.

CONCLUSION

In this study, the applicability of a radiotracer technique to investigate surfactant interactions and to predict their irritation potentials was demonstrated. This method is easy to perform which makes it very convenient to use for routine screening of surface active ingredients and products.

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