

Does sodium lauryl sulfate concentration vary with time?

M. SUGÁR, E. SCHNETZ AND M. FARTASCH

Department of Dermatology, University of Erlangen, Germany

When performing skin irritation tests with sodium lauryl sulfate (SLS), the quality of the test solution is of major importance for the reproducibility and comparability of the results. The influence of 4 different storage parameters (concentration, duration, temperature, material of the storage vials) on the stability of aqueous SLS solutions was investigated under non-sterile conditions. SLS solutions at 5 different concentrations (0.001%, 0.01%, 0.1%, 0.5%, 1%) were analysed by mobile phase ion chromatography. Analyses were performed after a storage time of 3 h, 1 week, and 4 weeks. Storage was carried out at different temperatures: -18°C , 6°C , and 23°C . The storage containers were of 2 different materials (glass and polypropylene). After a storage of 1 week, no decrease in SLS concentration was observed, regardless of the chosen conditions. After 4 weeks at 6°C and 23°C , the SLS concentration was found to be significantly decreased for the 2 lowest concentrations (0.001% and 0.01%). At higher concentrations and lower temperatures, no decrease in SLS concentration had occurred. In parallel to the loss of SLS, contamination with bacteria was found in the solutions, especially at the 2 lowest concentrations. Bacterial growth was not observed at higher concentrations. The possibility of biodegradation of SLS has to be taken into account, especially when dealing with low concentrations of SLS.

Key words: SLS; stability; storage parameters; skin irritation testing. © Munksgaard, 1999.

Accepted for publication 29 October 1998

Results of skin irritation tests with sodium lauryl sulfate (SLS) are not only influenced by intra- or interindividual variability, but also by parameters like the size and the type of the test chambers or the SLS concentration (1–5). In recent years, excellent guidelines on SLS exposure tests have been published, with the ultimate goal of a uniform and standardised approach to SLS testing in man (6). These guidelines mention the danger of different results at various laboratories due to inter-instrumental variability (6). Regarding the quality of the SLS, the chemistry and purity of SLS have been a subject of interest in the past (7). These observations raise the question as to whether there are other parameters, besides the above-mentioned, that could influence the results of SLS irritation tests. There are no studies, however, which analyze the impact of storage conditions on the quality of SLS solutions. The SLS concentration may be influenced by time, temperature and the material of the vial. These parameters were investigated and addressed in the present study.

Materials and Methods

LAB Grade SLS (Merck, purity $>99\%$) was used for the studies.

The parameters SLS concentration, duration of storage, storage temperature, and material of the storage container were varied. The solutions were prepared with deionized water under non-sterile conditions and at 5 concentrations: 0.001%, 0.01%, 0.1%, 0.5% and 1%. On the day of analysis, the solutions had been stored for 3 h, 1 week, and 4 weeks. The storage temperatures were -18°C , 6°C , and room temperature (23°C). Storage containers of 2 different materials were used: glass autosampler vials (Macherey & Nagel) with a volume of 1.5 ml and polypropylene (PP) reaction vessels (Eppendorf Safe-Lock), also with a volume of 1.5 ml. Both types of container were supplied with leakproof lids.

The analysis method was mobile phase ion chromatography (MPIC). This method combines the advantages of reversed-phase ion-pair chrom-

atography and conductivity detection. The method is well suited for quantitative analysis of many kinds of ionic surfactants like SLS. Quantification of the surfactant was carried out by integration of the SLS peak areas. A Dionex DX500 chromatography apparatus with a Dionex GP40 gradient pump and a Dionex CD20 conductivity detector with on-line conductivity suppression was used for this purpose. The chromatography column was a Dionex NS1, the guard column was a Dionex NG1, and the suppressor was a Dionex ASRS-II. The eluent mixture consisted of a 0.01 M potassium hydroxide solution and acetonitrile in a ratio of about 1:1. The flow rate was 1 ml/min and the injection volume was 50 μ l. Analysis of all samples was performed on the same day. Samples that were stored in PP vials had to be transferred into glass autosampler vials immediately before the analysis of the sample. The freshly-prepared samples had an average storage time of 3 h when analysis was performed. They served as control standards and were analysed after every 6th sample in order to control the reproducibility of the results. In total, each of the fresh samples was analysed 6 $\times$. A total of 225 samples were analysed. For the experiments, 1 stock solution with a volume of 100 ml had been prepared for each of the concentrations and at 3 different points of time. From these stock solutions, 1.5 ml were transferred into the storage containers. Each sample was generated in triplicate. For analysis, the mean values of each of the 3 samples were generated. The size of the storage containers was not varied. In a previous pilot study, we had found that this parameter did not influence SLS stability. During the storage, the test solutions had been light protected. For analysis, solutions with concentrations $>0.1\%$ were diluted in a 1:10 ratio for reasons of analytical exactness. The water used for the preparations was fully deionized.

In addition, randomly chosen samples of each

Table 1. Reduction in concentration of a 0.001% SLS solution after storage in glass vials

Temp.	3 h	1 week	4 weeks
-18°C	0.001%	0.000998%	0.000962%
6°C	0.001%	0.000996%	0.000199%
23°C	0.001%	0.000996%	0

Table 2. Reduction in concentration of a 0.01% SLS solution after storage in glass vials

Temp.	3 h	1 week	4 weeks
-18°C	0.01%	0.01006%	0.00999%
6°C	0.01%	0.01004%	0.01004%
23°C	0.01%	0.00996%	0.00713%

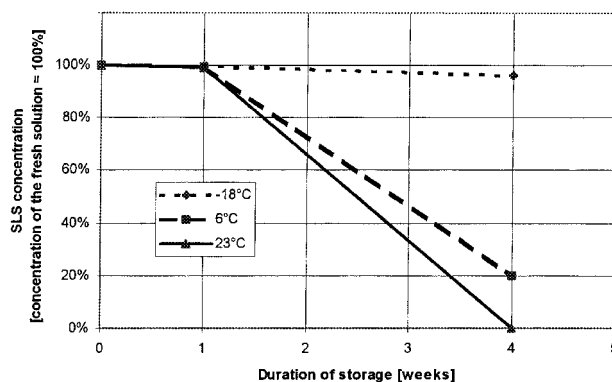


Fig. 1. Reduction in concentration of a 0.001% SLS solution after storage in glass vials.

concentration and at each temperature were analyzed regarding their contamination with bacteria after storage.

Results

SLS concentrations

The concentrations of the solutions with a storage time of 3 h (fresh samples) were set at 100%.

The standard deviations of the analysis results of the 6 control standards varied between 1.79% for the solution with an SLS concentration of 1%, and 0.27% for the solution with an SLS concentration of 0.1%. Due to these results, changes of SLS concentrations after storage of more than 2% were regarded as significant.

The solutions with SLS concentrations of 0.1%, 0.5%, and 1% did not show any changes in their SLS content.

Changes of the SLS concentration were seen with 0.001% and 0.01% under certain conditions and duration of storage. After 1 week, no significant decrease in SLS concentration had occurred regardless of the storage conditions. After 4 weeks of storage, the lowest concentration (0.001%) showed a significant decrease in SLS concentration. When stored at -18°C , the reduction of the concentration after 4 weeks was 3.8%. At 6°C the reduction of the initial SLS concentration was 80% after 4 weeks. When stored at room temperature the SLS was no longer detectable after 4 weeks. The results are shown in detail in Table 1 and Fig. 1. The SLS concentration was not influenced by the material of the vials. Due to this fact, only the results of storage in the glass vials are shown in detail. For the SLS solutions with concentrations of 0.01%, significant changes were found after a 4-week storage at room temperature. The relative decrease in SLS concentration was

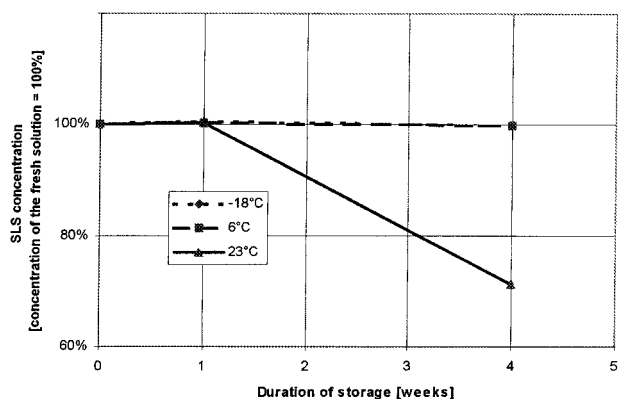


Fig. 2. Reduction in concentration of a 0.01% SLS solution after storage in glass vials.

28.7%. The results are shown in detail in Table 2 and Fig. 2.

Bacterial culture

During the 1st week, bacterial growth was found to be significantly suppressed in the SLS solutions with concentrations above 0.01%. In solutions with SLS concentrations below 0.1%, various kinds of bacteria were found even after only 1 day. These were *Pseudomonas pickettii*, gram-negative rods, and aerobic sporiparous microbes. After 4 weeks, contamination with bacteria could also be found in the 0.1% SLS solution.

Discussion

Due to its high irritant potential, SLS is often employed as a model substance for skin irritation tests. In recent years, it has been shown that, besides individual differences, many experimental parameters like amount of test solution per mm² of skin (4), temperature of SLS solution (8), purity of SLS (7), and water hardness of the solution (9) have to be considered, when performing skin irritation tests with SLS. Larger amounts of test solution per mm² of skin (4) and treatment at higher concentrations (2) caused stronger reactions, and the skin response to SLS of high purity was much stronger than the response to SLS of lower purity (7). Additionally, the influence of water hardness on the mildness of a synthetic detergent was demonstrated (9), showing that the detergent was milder when diluted with deionized water.

In this study, we have examined the influence of a variety of storage conditions on the quality of SLS solutions. We found that the concentration of SLS solution, the duration of storage and the temperature of storage all had an impact on the sta-

bility of SLS. In contrast, the material of the storage container did not seem to have any influence.

The results have shown that there were time-dependent changes in SLS concentration under certain conditions. With increasing storage time and temperature an increasing reduction of SLS concentration was seen. This reduction was especially observed at very low SLS concentrations (0.001% and 0.01%) and not at concentrations above 0.01%. To our knowledge, 0.01% is the lowest concentration used in SLS irritation tests (4).

At very low SLS concentrations (0.001% and 0.01%), growth of bacterial populations was observed in the solutions. SLS is known to possess high biodegradability (10), hence, biodegradation of SLS could be the reason for the decrease in SLS concentration with time. In a primary degradation step, it is converted to sulfate and lauryl alcohol, which means a loss of surface-active properties and, thereby, a loss of detectability by means of MPIC. In the ultimate degradation step, biodegradation, carbon dioxide, and water are produced (10). The hypothesis that biodegradation is of major importance for the decrease in SLS concentration is underlined by the fact that decrease is slowed down at 6°C and does not take place at -18°C. Decrease in SLS concentration and the observed microbiological growth seem only to occur at low concentrations, suggesting an antimicrobial effect of SLS at higher concentrations, probably due to its known high protein binding and denaturing abilities (11). Adsorption to the container walls as a cause for the disappearance of the SLS is not reasonable. In this case, a state of equilibrium would be expected to be reached much more quickly than in one week.

Besides the above-mentioned storage parameters, concentration, duration, temperature, and material of the storage vials, there could also be other influences on SLS stability during storage. One might be the effect of light. In this study, the samples were light-protected and the influence of light was not examined. Therefore exposure to light could still be an additional factor leading to SLS decomposition.

Our results show that the storage of SLS solutions, especially at very low concentrations, should be at low temperature and, preferably, in sterile vials. If very high preciseness of concentration is required, solutions should always be prepared on the day of the experiment.

Acknowledgment

The authors would like to thank Beiersdorf AG for the opportunity to perform the analysis of SLS in its R&D facilities in Hamburg, Germany.

References

1. Frosch P J, Kligman A M. The Duhring chamber. *Contact Dermatitis* 1979; 5: 73–81.
2. Agner T, Serup J. Sodium lauryl sulphate for irritant patch testing – a dose-response study using bioengineering methods for determination of skin irritation. *J Invest Dermatol* 1990; 95: 543–547.
3. Novak E, Francom S F. Inflammatory response to sodium lauryl sulfate in aqueous solutions applied to the skin of normal human volunteers. *Contact Dermatitis* 1984; 10: 101–104.
4. Tupker R A, Willis C, Berardesca E, Lee C H, Fratasch M, Agner T, Serup J. Guidelines on sodium lauryl sulphate (SLS) exposure tests. A report from the standardization group of european society of contact dermatitis. *Contact Dermatitis* 1997; 37: 53–69.
5. Fartasch M, Schnetz E, Diepgen T L. Characterization of detergent-induced barrier alterations – effects of barrier cream on irritation. *J Invest Dermatol Symposium Proceedings* 1998; 3: 121–127.
6. Pinnagoda J, Tupker R A, Agner T, Serup J. Guidelines for transepidermal water loss (TEWL) measurement. *Contact Dermatitis* 1990; 22: 164–178.
7. Agner T, Serup J, Handlos V, Batsberg W. Different skin irritation abilities of different qualities of sodium lauryl sulphate. *Contact Dermatitis* 1989; 21: 184–188.
8. Berardesca E, Vignoli G P, Distanti F, Brizzi P, Rabbiosi G. Effects of water temperature on surfactant-induced skin irritation. *Contact Dermatitis* 1995; 32: 83–87.
9. Warren R, Ertel K D. Hard Water. *Cosmetics & Toiletries* 1997; 112: 67–74.
10. Guhl W. Systematik der ökologischen Produktsicherung am Beispiel von Wasch- und Reinigungsmitteln. *SÖFW-Journal* 1997; 123: 210–221.
11. Schwuger W J, Bartnik F G. Interactions of anionic surfactants with proteins, enzymes, and membranes. In: *Anionic surfactants – biochemistry, toxicology, dermatology* 1990: 1–49.

Address:

Manigé Fartasch
Department of Dermatology
University of Erlangen
Hartmannstr. 14
D-91052 Erlangen
Germany