Physico-Chemical in vitro Methods for Determination of the Skin Compatibility of Surfactants

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In order to reduce the number of animal tests there have been some efforts to establish in vitro methods for testing cosmetic products, especially for the skin compatibility of surfactants. The main principles of the published physico-chemical methods for in vitro testing of surfactants are summarized. They are mostly based on the interaction of surfactants with proteins and protein structures, e.g. isolated skin layers. Water binding capacity, adsorption, denaturation, solubilisation, penetration and other properties have been investigated.

The practical application of these methods implies thorough knowledge of their possibilities and limits. Thus the swelling behaviour of isolated pig epidermis has been studied in detail to develop an in vitro test for the skin compatibility of surfactants. The water uptake of epidermis strips in surfactant solutions was gravimetrically measured. Dependent on the surfactant structure the swelling values were different and characteristic. The effects of various types of surfactants and their mixtures are discussed and compared with skin irritation values of human and animal tests. Anionic surfactants displayed a satisfactory ranking correlation of swelling and irritation values.

As manufacturers and users of cosmetic products we, of course, do not only want to ensure that products have the desired properties, but also that they have as few side-effects as possible, i.e. that they are compatible. Besides testing for any general side-effects, their application on the skin requires the verification of local skin compatibility.

In general, cosmetic products contain several components. Almost ubiquitous ingredients are surfactants, which are used especially as cleaning and foaming agents in shampoos, soaps and bubble baths as well as solubilizers and emulsifiers in solutions and cremes.

Surfactants are interfacially active substances, which, when applied to the skin, can interact with skin structures and components. In general the use of surfactants does not cause any problems, especially "rinse off" products, which are only in contact with the skin for a short time. As, however, surfactants can remain upon the skin it is mandatory to carefully ensure the skin compatibility.

This is currently valid even for the general use of skin cosmetic products. An indication of the increasing awareness of environmental problems is the trend towards the use of cosmetics which are mild-to-the-skin.

The methods for the determination of skin compatibility presently established or under discussion are based on biologically active systems as man, animal and cultures of organs and cells on the one hand, and on passive, physico-chemical measuring methods on the other (Fig. 1). Biological methods

Fig. 1 Applied and potential methods for the determination of the skin compatibility of surfactants

- Methods on the basis of biological active reactions
  - Human (clinical tests etc.)
  - Animal (animal tests)
  - Painless living systems (cell and tissue cultures)
- Methods on physico-chemical basis
  - Physical and chemical tests
  - "Computer tests"

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are confined to toxicological laboratories, specially trained staff and a constant supply of laboratory animals and cell cultures, etc. In contrast, physico-chemical measurements can be carried out without such technical facilities and dispense with living material. But recognized methods have, up to now, been limited to animal tests, which, however, are increasingly being criticized. This discussion has lead to an intensified search for alternative testing methods. These encompass in-vitro experiments on living systems incapable of feeling pain as well as methods based on physical and chemical procedures. Using the concept of a computer experiment, the goal is a system based on mathematical techniques. Via computer programmes and based upon known toxicological data, the properties of individual structural elements and thus the toxicity of new compounds will be calculated. However, the validity of such a technique has yet to be demonstrated.

Methods that can be defined as physico-chemical methods are still under development. In publications, first and foremost interactions of surfactants with proteins and isolated skin layers are described and possible connections with the skin-toxic properties of surfactants are discussed. A short summary, which makes no claims for completeness, will be given in this report. The compilation includes methods whose results are concerned solely with skin compatibility data as well as others with a similar goal and indirectly connected with skin compatibility. Subsequently some own results will be discussed.

**Fig. -2** summarizes the most important effects resulting from the interaction of surfactants with protein substrates as a basis for method development. Research has focused especially on the permeability\(^{(1)}\), water balance\(^{(2)}\), denaturation and blocking of proteins\(^{(3)}\), adsorption\(^{(4)}\) and solubility\(^{(5)}\). In the following tables the results gained by several research groups are summarized. Thus, for example Gibson and Teall\(^{(6)}\) established that sodium laurate and lauryl sulphate exhibited a higher degree of permeation through isolated rat skin than did sodium lauroyl isethionate (Fig. -3). The following examples have to be interpreted similarly. Numerous studies based upon the water content of skin preparations are known (Fig. -4). The water content of surfactant treated preparations determined by measuring length and thickness or weight depend on the surfactant structure. The influence of surfactants on the denaturation and blocking of proteins is shown in Fig. -5. Finally, a connection between the solubilization potential of surfactants and skin compatibility can be demonstrated (Fig. -6). Especially the Zein Test\(^{(5b-d)}\), which is based on the influence of surfactants on the solubility of the maize protein Zein, has been investigated in more detail.

These publications show promising possibilities of deducing skin compatibility from the interaction

**Fig. -2** Effects of the interactions of surfactants with protein substrates on the basis of Physico-chemical methods

--- Change of permeability
--- Influence on the water content
--- Change of chemical structure
--- Adsorption
--- Change of solubility

**Fig. -3** Influence of surfactants upon the permeability of isolated skin layers

<table>
<thead>
<tr>
<th>Permeability to surfactants</th>
<th>Isol. rat skin</th>
<th>NaL &gt; NaLS &gt; NaLLs</th>
<th>Gibson, Teall (1983)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isol. epid. (human)</td>
<td>(&lt;\text{NaL})</td>
<td>(\text{C}<em>{14} &lt; \text{C}</em>{16} &lt; \text{NaLS} &lt; \text{C}_{18} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(&lt;\text{C}<em>{12}\text{NH}</em>{4}\text{Cl}^-)</td>
<td></td>
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</table>
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**Fig. -4** Influence of surfactants upon the water balance of skin and protein preparations

<table>
<thead>
<tr>
<th>Change of prep. length by water uptake or release</th>
<th>Collagenprepar. (calf)</th>
<th>C₈&lt;sub&gt;C₁₈&lt;/sub&gt;&lt;sub&gt;&lt;sup&gt;&lt;/sup&gt;&lt;/sub&gt;&lt;sub&gt;Na&lt;/sub&gt;&lt;sub&gt;LS&lt;/sub&gt;&lt;sub&gt;ABS&lt;/sub&gt;&lt;sub&gt;&lt;sup&gt;&lt;/sup&gt;&lt;/sub&gt;&lt;sub&gt;Nal₅&lt;/sub&gt;&lt;sub&gt;₅&lt;/sub&gt;&lt;sub&gt;Nio&lt;/sub&gt;. Cat.</th>
<th>Choman (1963)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isol. epid. (ox)</td>
<td>NaLS&gt;NaLS+Polyglycol</td>
<td>Götte (1950)</td>
<td></td>
</tr>
<tr>
<td>Isol. epid. (human)</td>
<td>C₈&lt;sub&gt;C₁₈&lt;/sub&gt;&lt;sub&gt;Na&lt;/sub&gt;&lt;sub&gt;LS&lt;/sub&gt;&lt;sub&gt;C₁₄&lt;/sub&gt;&lt;sub&gt;C₁₈&lt;/sub&gt;&lt;sub&gt;Na&lt;/sub&gt;&lt;sub&gt;LS&lt;/sub&gt;&lt;sub&gt;ABS&lt;/sub&gt;&lt;sub&gt;C₁₅N*(CH₂)₃Br&lt;/sub&gt;</td>
<td>Robbins. Fernee (1983)</td>
<td></td>
</tr>
<tr>
<td>Isol. s.c. (guin. pig)</td>
<td>NaLS&gt;NaLS&lt;sub&gt;ABS&lt;/sub&gt;&lt;sub&gt;&lt;sup&gt;&lt;/sup&gt;&lt;/sub&gt;&lt;sub&gt;&lt;sup&gt;&lt;/sup&gt;&lt;/sub&gt;&lt;sub&gt;&lt;sup&gt;&lt;/sup&gt;&lt;/sub&gt;&lt;sub&gt;&lt;sup&gt;&lt;/sup&gt;&lt;/sub&gt;&lt;sub&gt;&lt;sup&gt;&lt;/sup&gt;&lt;/sub&gt;&lt;sub&gt;&lt;sup&gt;&lt;/sup&gt;&lt;/sub&gt;&lt;sub&gt;Na&lt;/sub&gt;&lt;sub&gt;LES&lt;/sub&gt;&lt;sub&gt;Cationics, Nonionics&lt;/sub&gt;</td>
<td>Putterman et al. (1977)</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. -5** Change of protein structure caused by surfactants/Adsorption of surfactants

| Protein denaturation | Ovalbum. Human ser. alb. | NaLS=ABS=C₁₂N*(CH₂)₃Cl<sub>₅</sub><sub>Na</sub><sub>LES</sub>=Betaine Tauride<sub>₅</sub><sub>Amine ox</sub><sub><sup></sup></sub><sub><sup></sup></sub><sub><sup></sup></sub><sub><sup></sup></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub>=Imidazol<sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub><sub><sup></sub></sub>=Nio. | Miyazawa, Ogawa, Mitsui (1984) |
| Enzyme block. | Saccharase | NaLS>NaLES>Sulfosucc. >Protein surfactant | Wilsmann (1963) |

**Fig. -6** Solubilisation of proteins by surfactants

| Eluation of N-Cpd | Human skin | C₈<sub>C₁₈</sub><sub>ABS</sub><sub>Na</sub><sub>LS</sub><sub>C₁₄</sub><sub>C₁₈</sub><sub>Na</sub><sub>LS</sub><sub>Na</sub><sub>LES</sub> | Malaskiewicz, Gloshuber (1970) |

between the surfactant and the protein or the respective skin preparation, for rank orders were observed which were in part comparable. Some of the methods mentioned were sporadically used as in-vitro methods, e.g. the Zein Test. However, a generally acknowledged alternative method on a physico-chemical basis does not exist. One of the main reasons is certainly the restricted knowledge concerning the possibilities and limits of such methods. It is not sufficient to check a few standard surfactants. Detailed correlation studies incorporating in-vivo tests on various surfactant types, surfactant mixtures, additives and market products are lacking. Only when detailed knowledge is available will a wider, practical application as in-vitro method be possible.

in-vitro studies are rendered additionally difficult due to the uncertainties attached to the interpretation of the in-vivo data. The relation of the values and even their sequence can be altered, depending upon method chosen, type of application, surfactant concentration, contact time etc. Similarly, a general evaluation of the in-vitro data as a measure of the skin compatibility will also only be possible to a limited extent.

To collect general information about physico-chemical methods and if possible, to develop a well-tested in-vitro method, we have carried out detailed studies of the swelling of isolated epidermis in surfactant solution and its correlation to skin compatibility. For this purpose we used isolated pig epidermis from animals destined for human consumption, and determined the water uptake gravimetrically according to the following method (Fig.-7)\(^6\). Strips of epidermis are treated with surfactant solution or water under controlled conditions. After a short rinse and removal of surface water by gentle pressing between tissues, the swollen strips are weighed. They are then dried over calcium chloride and re-weighed. From the pairs of weights, dry and swollen weight, the respective quotients \(p\) and \(w\) are calculated, which express the water weight as a multiple of the weight of dry epidermis:

\[
p, w = \frac{\text{weight of swollen epid.} - \text{weight of dried epidimis}}{\text{weight of dried epid.}}
\]

\(w\) = value of water treated epidermis

\(p\) = value of surfactant treated epidermis

The percentage change of swelling caused by surfactant treatment in relation to the water treated epidermis is defined by

\[Q = \left(\frac{p}{w} - 1\right)100\%\]

\(\text{Fig. -8 Calculation and Definition}\)

A series of \(Q\)-values for some surfactants determined under standard conditions is shown in Fig. -9\(^6\). Each value is the average of eight individual values. The sequence within this series can be reproduced with correlation coefficients greater than 0.9. The absolute \(Q\)-values can differ to a greater extent, depending upon the quality of the epidermis. It is apparent that most surfactants of this series, mainly of the anionic type, result in increasing swelling, whereas amphoteric surfactants hinder swelling. Thus there is a method available which can differentiate between product properties which influence swelling of epidermis. The \(Q\)-values can be used for this purpose and can also be compared with other effects of the interaction between surfactant and skin.

Thus the \(Q\)-values of the anionic surfactants in this series were compared with the skin compatibility values which were obtained from the Duhring Chamber Test using human subjects, and which are described in the publication of Kästner and Frosch\(^5\).
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<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Change in swelling Q±s (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium lauryl sulfate</td>
<td>276±22</td>
</tr>
<tr>
<td>Monoethanolammonium lauryl sulfate</td>
<td>192±21</td>
</tr>
<tr>
<td>Sodium C_{12-14}-alkyl sulfate</td>
<td>153±12</td>
</tr>
<tr>
<td>Ammonium lauryl sulfate</td>
<td>141±13</td>
</tr>
<tr>
<td>Triethanolammonium lauryl sulfate</td>
<td>129±16</td>
</tr>
<tr>
<td>Alkyl benzene sulfonate, Na-salt</td>
<td>121±11</td>
</tr>
<tr>
<td>Sec. alkane sulfonate, Na-salt</td>
<td>103±9</td>
</tr>
<tr>
<td>Sodium lauryl ether (2 EO) sulfate</td>
<td>82±12</td>
</tr>
<tr>
<td>Triethanolammonium lauryl ether (2 EO) sulfate</td>
<td>80±10</td>
</tr>
<tr>
<td>Sodium lauryl Myristyl ether (3 EO) sulfate</td>
<td>77±23</td>
</tr>
<tr>
<td>Sodium sulfosuccinic acid ester</td>
<td>76±12</td>
</tr>
<tr>
<td>Ammonium lauryl ether (2 EO) sulfate</td>
<td>52±6</td>
</tr>
<tr>
<td>Protein-fatty acid condensate</td>
<td>17±11</td>
</tr>
<tr>
<td>Sodium/Magnesium alkyl ether (6 EO) sulfate</td>
<td>3±4</td>
</tr>
<tr>
<td>Amide ether sulfate, TEA-salt</td>
<td>2±12</td>
</tr>
<tr>
<td>Fatty Alcohol polyglycol ether</td>
<td>1±4</td>
</tr>
<tr>
<td>Cocoaamphocarboxyglycinat</td>
<td>−3±3</td>
</tr>
<tr>
<td>Cocamidopropyl betaine</td>
<td>−5±4</td>
</tr>
<tr>
<td>Coco-betaine</td>
<td>−18±2</td>
</tr>
<tr>
<td>Amidoalkyl Dimethyl amine oxide</td>
<td>−19±4</td>
</tr>
</tbody>
</table>

**Fig. -10** Comparison of rank numbers of anionic surfactants according to increasing swelling (Q) and skin irritation-values (DT)

**Fig. -9** Swelling of pig epidermis caused by aqueous solutions of various surfactants (I).

**Fig. -10** gives the rank numbers of increasing swelling and skin irritation in comparison with one another. The agreement, seen at first glance, is confirmed mathematically with a rank correlation coefficient of 0.84.

**Fig. -11** contains a second series of anionic surfactants and their Q-values. When compared to *in vivo* data, this time obtained from the intracutaneous test on hairless mice, a high degree of correlation is again found (Fig. -12).

Within the scope of the statistical accuracy found, there is hence no doubt that a clear, although not a stringent relationship exists between epidermis swelling and skin irritation in case of anionic surfactants.

Measurements using several non-ionic surfactants based upon polyglycol, sorbitol and glucose mainly showed only slight tendencies towards swelling promotion and hindrance. The relatively small differences in animals experiments only permitted limited differences in the skin compatibility to be recognized. The generally good compatibility of non-ionic surfactants, together with their low swelling values, justifies their inclusion in the scale of anionic surfactants, with, however, a degree of uncertainty.

In contrast, cationic surfactants showed primarily swelling inhibition (Fig. -13). A comparison in the sequence found, with the very major differences found in the mucous membrane compatibility on the rabbit eye permits a connection to be established

between increasing swelling and irritation, just as in the case for anionic surfactants (Fig.-14). The small differences in swelling, however, only permit a statistically certain differentiation to be made for the terminal members of the series. At a similar course of swelling, the position and extent of the Q-value scale of cationic surfactants is fundamentally different from that of anionic surfactants. Thus the Q-values of anionic and cationic surfactants have to be interpreted in their own system respectively.

Amphoteric surfactants as well inhibit swelling under standardized conditions, as has been demonstrated in the table previously shown (Fig.-9). On the whole, a connection between swelling and skin compatibility was not found after evaluation of the results of a series of seven amphoteric surfactants.

It is clear from the results obtained for the individual surfactant types that the behaviour of mixtures can only be correctly interpreted if the swelling values for the individual components have the same significance. Thus, compatibilities of mixtures of anionic surfactants, e.g. an alkyl sulphate and an alkyl ether sulphate, can be represented by means of the swelling values (Fig.-15). As in all other cases investigated, the Q-values form a nearly linear function corresponding to the mixture rule.

Good agreement in their sequence of skin swelling and compatibility was also found for anionic-/non-ionic-surfactant mixtures (Fig.-16).

Replacement of the "swelling-neutral" sodium ion by the magnesium ion, which considerably inhibits the swelling, reduced the swelling without, however, improving the compatibility to the same extent (Fig.-17). If, as in this case, a compatibility of one of the ingredients in the mixture does not correspond to the swelling effect found, then the correlation for the formulation can be disturbed.
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Additions of amphoteric or cationic surfactants to ether sulphate improved the skin compatibility synergistically in line with the declining course of swelling for anionic surfactant rich mixtures (Fig.-18). As there is no correlation for amphoteric surfactants and a different scale for cationic surfactants, deviations occurred in the case of larger additions.

It should be noted that the compatibility of am-
photeric surfactants including amine oxides is not, in general, as good as is often claimed in the literature. This class of surfactant also includes very mild and very irritant products.

This assumption is probably due to the synergistic compatibility improvement of anionic surfactants which also arises when less compatible amphoteric surfactants are added.

The swelling values which have been discussed up to now were measured under standard conditions, i.e. at 2% concentration, 40°C and a pH-value of 6.5. The necessity for fixing these conditions is shown in Fig. -19, in which the dependence upon concentration is demonstrated. The swelling rises to a maximum at approx. 0.1 mole/l (2–5%), i.e. above the critical micelle/concentration, and then decreases. A synchronous change with compatibility is only observed in the increasing Q-values. Subsequently the values move in opposite directions to each other. It is just as important to control the temperature, which strongly influences the swelling (Fig. -20). The existing trend of swelling increase or inhibition is intensified by an increase in temperature. In the presence of sodium lauryl sulphate, the epidermis is largely degraded at 60°C. In contrast, the betaine results in an epidermis with a parchment-type structure.

Finally, the pH-value also has a major influence upon the swelling (Fig. -21). Each class of surfactant exhibits a characteristic dependence. With increasing pH-value swelling decreases in the case of cationic surfactants and increases in the case of anionic surfactants. In the acid range amphoteric surfactants exhibit a characteristic dependence. With increasing pH-value swelling decreases in the case of cationic surfactants and increases in the case of anionic surfactants. In the acid range amphoteric surfactants exhibit a characteristic dependence. With increasing pH-value swelling decreases in the case of cationic surfactants and increases in the case of anionic surfactants. In the acid range amphoteric
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Surfactants behave like cationic surfactants, in the basic range like anionic surfactants. They pass through a swelling minimum which apparently relates to the isoelectric point. The marked and individual dependence upon the pH shows that the swelling phenomena arise predominantly due to ionic interactions. The epidermis itself is an amphoteric matrix, which, as a function of pH, forms salts of varying ionic type and strength, as well as complexes, thus leading to changes in its structure, hydrophilic character and swelling behaviour.

Summing up it can be said that the comparative study of epidermis swelling and skin compatibility caused by surfactants shows a partial connection which can be used for in-vitro testing of anionic surfactants and their mixtures as well as certain further combinations of surfactants. It must be taken into account that swelling-active, non-correlating components can disturb the comparability.

The correct interpretation of the swelling values as compatibility data thus requires knowledge of the surfactant types, mixtures and additives to be tested.

Of course, the individual results obtained cannot be simply transferred to the other physico-chemical methods initially discussed. Nevertheless, some of the studies possess similarities, e.g. the different behaviour of cationic and anionic surfactants. This is not surprising as these measurements are also based upon interactions with proteins and skin structures.

Considering the facts presented for in-vivo/in-vitro comparisons, a careful evaluation permits a few generalizations to be made.

Whereas experiments carried out on living systems are directly concerned with assessing the damage, and the results are therefore, almost by definition, "correct", the use of physico-chemical methods enables one to approach the problem from the direction of the product itself. One commences with the individual properties of substances, whose structure theoretically contains all the information, and attempts to understand the possible interactions occurring between the substance and organism, by means of simplified models. From these considerations one tries to draw conclusions as to the reaction of the living system. But a single method cannot completely replace human or animal experiments. The price for the renunciation of living models as complex test objects is a restricted breadth of application. This confirms the necessity of defining the possibilities and limits of such methods as carefully as possible. Bearing this precondition in mind, these methods can advantageously be used as preliminary tests for the determination of the skin compatibility of surfactants. As shown by the number of publications concerning this topic and the increasing interest for alternative methods based upon physico-chemical principles, it must be possible by combining suitable methods to increase their predictive power.

Acknowledgement

We would like to thank Mr. H. Ikeda, Henke Hakusui, for translating this lecture into Japanese.

Literature

   b. B.L. Fremaux, Rohm & Haas 1982
   b. E.K. Götte, Kolloid Z. 117, 42 (1950)
   g. B.L. Fremaux, Rohm & Haas 1982


(昭和60年8月1日受理)