

THE INTERACTION OF SURFACTANTS WITH ANTIMICROBIAL AGENTS

The influence of sodium dodecyl sulphate on the antibacterial and
allergenic effects of neomycin and bacitracin

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Emulsion bases containing surface active agents have recently gained ground in the topical treatment of dermatological conditions. Some surfactants may interact with certain antimicrobial agents, causing an inhibition of the antimicrobial effect and/or a change in the texture of the ointment. Published articles dealing with this problem (1, 2, 3, 4, 8, 10, 16) are somewhat controversial. The purpose of this investigation is, firstly to estimate quantitatively the possible incompatibilities between some surfactants and antimicrobial agents and secondly to determine if these incompatibilities lead to parallel changes in antimicrobial and allergenic activities.

The material for the study consisted of the anionic surface active agent sodium dodecyl sulphate (sodium lauryl sulphate) along with the antibiotics neomycin and bacitracin.

A. Chemical Aspects

Commercial neomycin is a mixture of neomycins A, B and C, neomycin B sulphate being the main component. Neomycin B ($C_{23}H_{46}N_6O_{13}$) contains six free amino groups. Sodium dodecyl sulphate (SDS), when added to neomycin solution, reacts

with the cationic amino groups and a flocculent precipitate is formed.

In this study the course of the precipitation was followed after mixing solutions of SDS¹ and commercial neomycin sulphate² in a series of molar ratios from 1 : 1 to 6 : 1. The final molar concentration of neomycin in each reaction was 0.025. According to Ford *et al.* (7) the molecular weight of neomycin B sulphate is 880. This value was used in the preparation of the solutions. The precipitate formed through mixing the SDS and neomycin solutions was filtrated, vacuum dried and weighed. The results can be found in Fig. 1, in percent of the theoretical weight. The theoretical weight was calculated with the assumption there would be complete chemical combination of SDS and neomycin base in the molar ratio of 6 : 1.

Bacitracin A³, the main component of commercial bacitracins, is a polypeptide ($C_{66}H_{103}O_{16}N_{17}S$) containing four free amino groups. These cationic groups, analogously to those found in neomycin, were expected to react with anionic surfactants, such as SDS. The addition of SDS into a bacitracin solution, however, did not produce precipitation. A simple method for demonstrating the reaction between the

¹ Sodium dodecyl sulphate, pro anal. Marchon Products Ltd., England. Mol. weight 288.33.

² Apotekernes Laboratorium, Oslo, Norway. Mol. weight ca. 880.

³ Apotekernes Laboratorium, Oslo, Norway. Mol. weight ca. 1420.

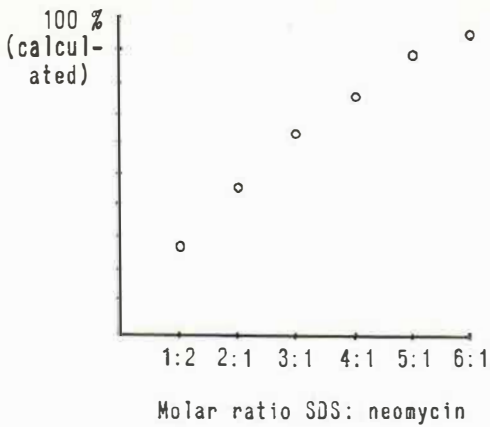


Fig. 1. The course of precipitation of neomycin when sodium dodecyl sulphate is added in increasing molar ratios.

amino groups of bacitracin and SDS employed Albustix® (Ames) slips as an indicator while titrating a given bacitracin solution with SDS. It was observed that the clearly positive colour reaction for protein found with bacitracin alone became weaker with increasing SDS concentrations. The

test became negative for protein when the molar ratio SDS : bacitracin became approximately four to one (Fig. 2). Thus it appeared that one dodecyl sulphate ion blocked one of the four amino groups of bacitracin and that in the 4 : 1 mixture all the amino groups were blocked. This mixture, however, an even a 5 : 1 mixture gave an ultraviolet spectrum characteristic for the thiazoline ring. This group has been held essential for the antibiotic activity of bacitracin (5) and according to our earlier investigations, also for its allergenic effect (15).

B. Bacteriological Aspects

Sodium dodecyl sulphate in 0.02, 0.04, 0.06, 0.08, 0.10, 0.12 and 0.14 molar solutions were mixed with equal amounts of 0.02 molar aqueous solution of neomycin. As controls both 0.02 molar neomycin and 0.14 molar SDS were diluted 1 : 2 with water. In each of the SDS neomycin mixtures a precipitate was formed. The precipitates were separated by centrifugation. A twofold

Molar ratio
SDS : bacitracin

Protein
reaction

Bacitracin alone

Positive

1 : 1

2 : 1

3 : 1

4 : 1

5 : 1

Negative

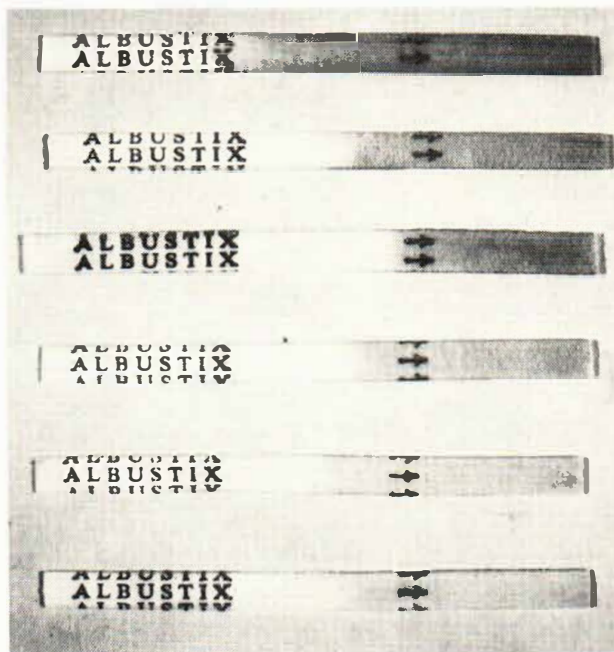


Fig. 2. The influence of sodium dodecyl sulphate on the protein reaction of bacitracin.

Table 1. The effect of sodium dodecyl sulphate on the bacteriostatic activity of neomycin

Molar ratio SDS : neomycin	Inhibition of growth of <i>Staph. aureus</i> in different dilutions of 0.01 molar neomycin solution								
	160	320	640	1280	2560	5120	10240	20480	40960
Neomycin alone	—	—	—	—	—	—	—	—	++
1 : 1	—	—	—	—	—	—	—	+	++
2 : 1	—	—	—	—	—	—	+	++	+++
3 : 1	—	—	—	—	—	+	+++	+++	+++
4 : 1	—	—	—	—	+	++	+++	+++	+++
5 : 1	—	—	—	+	++	+++	+++	+++	+++
6 : 1	—	—	+	++	+++	+++	+++	+++	+++
7 : 1	—	—	++	+++	+++	+++	+++	+++	+++
SDS alone	++	+++	+++	+++	+++	+++	+++	+++	+++

— = no growth

+-++++ = different grades of growth

Table 2. The effect of sodium dodecyl sulphate on the bacteriostatic activity of bacitracin

Molar ratio SDS : bacitracin	Inhibition of growth of <i>Staph. aureus</i> in different dilutions of 0.01 molar bacitracin solution								
	160	320	640	1280	2560	5120	10240	20480	40960
Bacitracin alone	—	—	—	—	—	++	+++	+++	+++
1 : 1	—	—	—	—	++	+++	+++	+++	+++
2 : 1	—	—	—	—	++	+++	+++	+++	+++
3 : 1	—	—	—	—	+	+++	+++	+++	+++
4 : 1	—	—	—	—	—	++	+++	+++	+++
5 : 1	—	—	—	—	+	+++	+++	+++	+++
SDS alone	++	+++	+++	+++	+++	+++	+++	+++	+++

— = no growth

+-++++ = different grades of growth

Table 3. The effect of sodium dodecyl sulphate on the bacteriostatic activity of neomycin in ointment

Ointment containing		Molar ratio SDS: neomycin	Diameters of inhibition zones in mm.	
SDS per cent	Neomycin per cent		I	II
0	0.125	—	22	23
0	0.25	—	24	25
0	0.50	—	27	27
0.50	0.50	3 : 1	23	24
1.0	0.50	6 : 1	22	21
2.0	0.50	12 : 1	19	19

dilution series was prepared from each of the supernatants in serum broth starting with a 1 : 40 dilution. Each tube of the dilution series was infected with a loop of an

18 hour broth culture of *Staphylococcus aureus* (strain 209). After 18 hours incubation at 37°C the growth was recorded.

Under the test conditions neomycin still inhibited the growth of *Staphylococcus aureus* when reduced to a molar dilution of 2.5×10^{-7} . The growth inhibiting effect of neomycin was lowered by treatment with SDS. As is seen in Table 1 the bacteriostatic effect of neomycin decreased as the molar ratio SDS neomycin increased. In concentrations used in the present experiments SDS itself did not inhibit the growth of *Staphylococcus aureus*.

Similar experiments were carried out with bacitracin. The results are presented in Table 2. SDS did not have any effect on the bacteriostatic activity of bacitracin.

Table 4. *The influence of addition of sodium dodecyl sulphate in ratio 5:1 upon skin reactions to bacitracin*

Persons tested	Testing technique	Type of reaction	Influence of SDS
Control persons	Intra-cutaneous	Wheal due to histamine liberation	No effect
Patients sensitive to bacitracin	Intra-cutaneous Epi-cutaneous (chamber)	Delayed eczematous reaction	No effect Marked depression

In order to study the interaction of SDS with neomycin in conditions corresponding more closely those on the skin, similar experiments *in vitro* were performed using SDS-neomycin mixtures in ointment. Neomycin B sulphate was added to an o/w emulsion base containing a non-ionic surfactant to prepare concentrations of 0.125, 0.25 and 0.5 per cent. SDS 0.5, 1.0 and 2.0 per cent was then added to three samples of the ointment containing 0.5 per cent neomycin. This created SDS : neomycin molar ratios 3 : 1, 6 : 1 and 12 : 1, respectively. The bacteriostatic activity of these ointments and the ointment base were studied by the cup plate method against *Staphylococcus aureus* (strain 209). Cups of four millimeter diameter in the sensitivity testing medium were filled with the ointments. After 18 hours incubation at 37°C the inhibition zones were recorded. The testing was carried out about a week after the preparation of the ointments and was made in duplicate.

The inhibition zones of the different ointments can be seen in Table 3. The zone diameter became larger with increasing neomycin concentration. The addition of increasing amounts of SDS diminished the diameter of the inhibition zones. The inhibition zone of the ointment with 0.5 per cent neomycin and 2.0 per cent SDS was smaller than the zone of the ointment containing only 0.125 per cent neomycin.

This experiment was repeated using an ointment base containing 0.5 per cent SDS

as emulgator to which neomycin B sulphate was added to make concentrations of 0.125, 0.25 and 0.5 per cent. SDS was further added to two samples of the ointment containing 0.5 per cent neomycin. The final concentrations of SDS in these samples were 1.0 and 2.0 per cent, respectively. The inhibition zones were similar to those obtained when SDS and neomycin were added in corresponding concentrations to the ointment containing non-ionic surfactant as emulgator described above. For the differences in the textures of the ointments see below (D).

C. Allergological Aspects

Four patients sensitive to neomycin were patch tested using a dilution series of solutions containing neomycin alone and SDS mixed with neomycin in molar ratios 6 : 1, 3 : 1 and 1.5 : 1. The SDS-neomycin solutions, especially that of molar ratio 6 : 1, caused reactions weaker than solution containing corresponding concentration of neomycin alone. Furthermore, the threshold concentrations that still caused positive reactions were higher.

Preliminary investigations with customary patch tests suggested that the addition of SDS into bacitracin solutions decreased the strength of the reactions in patients sensitive to bacitracin. As the supposed allergenic structure of bacitracin was preserved undamaged, as presented above, it appeared that the suppression of epicutaneous reactions was most probably due to decreased rate of penetration. To confirm this, additional skin testing using the chamber method (13) and intracutaneous tests were performed. The results of these investigations, which in more detail have been presented elsewhere (14), are seen in Table 4.

Bacitracin, when injected intracutaneously, elicits even in normal persons, an immediate type of wheal reaction. This is due to histamine liberation. The addition of SDS has no influence on this type of reaction. In addition to this reaction, delayed eczematous reactions are obtained in patients sensitive to bacitracin, regardless of whether the allergen is applied intra- or

Molar ratio
SDS : bacitracin

Bacitracin alone

2 : 1

4 : 1

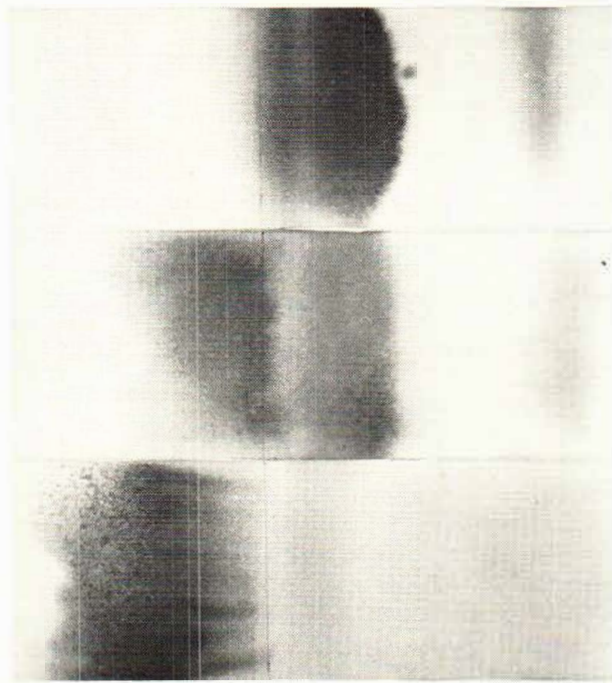


Fig. 3. The influence of sodium dodecyl sulphate on the behaviour of bacitracin in paper electrophoresis (pH 5).

epicutaneously. Addition of SDS into the bacitracin test solution has no influence upon the test results when the intracutaneous method is used, whereas in epicutaneous tests a marked depression of the reactions is observed. In further studies using paper electrophoresis it could be shown that in an acid medium, such as skin surface, SDS brings about a reversal of the electrical charge of the bacitracin molecule, from positive to negative (Fig. 3). This tends to decrease the penetration (17).

D. Pharmaceutical Aspects

The influence of the reaction between sodium dodecyl sulphate (SDS) and the antibiotics concerned on the texture of the ointments was studied preliminarily. It appeared that addition of 0.5 per cent neomycin into an o/w emulsion base containing 0.5 per cent SDS breaks the emulsion. This corresponds to the molar ratio SDS : neomycin 3 : 1.

When both SDS and neomycin were added to an o/w emulsion base containing

a non-ionic surfactant the ointment did not lose its texture. However, if the mixing was not done carefully the SDS-neomycin precipitate could be seen in clumps.

When bacitracin was added to an SDS emulsion base no changes in the texture were seen. The combining of bacitracin with SDS could be followed using Ames slips, as described above. The SDS emulsion base as such gave a negative reaction. Addition of bacitracin in molar ratio greater than 1 : 4 turned the reaction positive.

Comments

It has been reported that neomycin is chemically incompatible with some anionic substances, among others sodium dodecyl sulphate (SDS) and is precipitated by them (6, 8). The significance of the precipitation on the antibiotic activity of neomycin has, however, been doubted. In the present work it was found that the precipitation increased with increasing molar ratio SDS : neomycin. The precipitation was close to 100 per cent at the ratio 6 : 1. This is con-

sistent with the fact that neomycin B, the main component of commercial neomycin, contains six primary amino groups which can be expected to react with the DS-ion.

The antibacterial activity *in vitro* of SDS-neomycin mixtures from which the precipitate had been separated by centrifugation was found to decrease in proportion to the increasing molar ratio SDS : neomycin. This is consistent with the chemical findings. In another experiment, simulating more closely the effect of neomycin on the skin, the antibacterial activity of an ointment containing SDS and neomycin in a molar ratio 6 : 1 was shown to be less than a quarter of that of an ointment containing the same concentration of neomycin without SDS.

When SDS was added to neomycin solutions it was found that the allergenic activity of neomycin decreased with increasing concentration of SDS. It has also been reported that because of chemical incompatibility, the combination of SDS with streptomycin decreases the sensitizing effect of this antibiotic (9). These findings appear to be the reverse of some other observations to the effect that SDS, among other surface active agents, enhances the sensitization by weak sensitizers (12) and strengthens patch test reactions (10). A simple explanation is, however, that the precipitation of neomycin decreases the concentration of soluble neomycin allergen thus resulting in a decreased instead of increased penetration into the skin. The precipitation resulting from the chemical reaction of SDS and neomycin also explains the weaker antibacterial activity of the ointments containing both these substances as compared to ointments containing only neomycin.

Bacitracin contains four free amino groups which were shown to react with SDS. No precipitate, however, was formed. Furthermore, addition of SDS did not affect the antibacterial activity of bacitracin *in vitro* but decreased the allergenic effect of it in the patch test. This decrease was shown to be caused by the change in the electrical charge of the bacitracin molecule by SDS from positive to negative. The reversal of charge decreases the penetration

of bacitracin into the reacting layers of the skin.

Contrary to neomycin, bacitracin, which formed a soluble complex with SDS, did not interact with the emulsifying capacity of SDS. Therefore the SDS-bacitracin ointments remained stable.

SUMMARY

Neomycin is chemically incompatible with sodium dodecyl sulphate (SDS) and is precipitated by it. This incompatibility is reflected in the weak antibacterial and allergic activities of neomycin-SDS mixtures.

Bacitracin forms a soluble complex with SDS. This reaction has no effect on the antibacterial activity of bacitracin. The combination of SDS with bacitracin, however, changes the electrical charge of the bacitracin molecule. This change diminishes the penetration of bacitracin into the skin resulting in weaker allergic epicutaneous reactions.

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