

Use of AF4-MALS to prove the mechanism of action of the silver-nanolipid complex



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Introduction

Silver, especially microsilver, shows anti-microbial activity due to the oligodynamic effect [1]. Upon dermal application, nanostructured lipid carriers (NLC) increase skin hydration and re-enforce the natural lipid barrier of the skin [2].

The combination of silver and NLC leads to the formation of a silver-nanolipid-complex. This complex was developed as a cosmetic product for sensitive skin. It was found that it is also active against atopic dermatitis [3].

The proposed **mechanism of action** includes the assumptions that (Figure 1):

1. Silver acts as an electrode by releasing positively charged silver ions.
2. The **silver ions adsorb to the negatively charged NLC surface**, thus forming the silver-nanolipid-complex.
3. NLCs possess an increased adhesiveness to surfaces, i.e. after dermal application they remain longer on the skin. Therefore, the adhering silver ions get closer into contact with the membranes (e.g. skin or bacteria), which promotes the increased activity [4].

However, until now the proof of this concept was not possible, since regularly applied methods e.g. zeta potential measurements and size measurements failed to prove the adsorption of silver ions onto the surface of NLC.

Aim of the study

The study was conducted to prove the adsorption of silver ions onto the surface of NLCs using asymmetric-flow field-flow fractionation coupled with multi-angle light scattering (AF4-MALS).

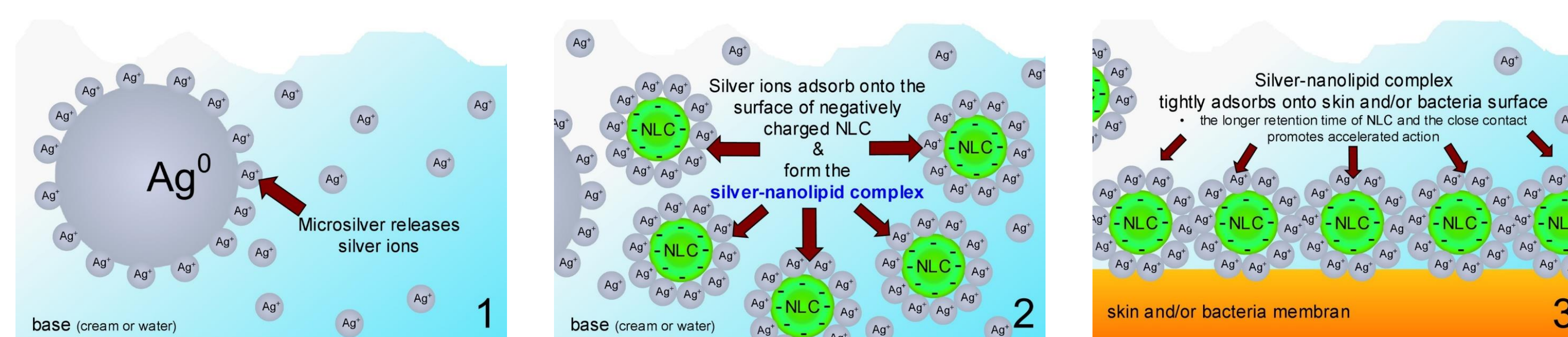


Figure 1: Silver-nanolipid complex – Theory of action

Materials

Microsilver BG was obtained from BioEpiderm GmbH (Nürnberg, Germany). The NLC consisted of cetyl palmitate (Cognis GmbH, Düsseldorf, Germany) and hemp seed oil (Gustav Hees, Stuttgart, Germany) and were stabilized with Inutec SP1 (Erbslöh Spezialchemikalien, Düsseldorf, Germany) and Tego Care 450, Franken-Kosmetik Chemiehandel, Wendelstein, Germany) and contained purified water (Milli Q, MilliQ-Plus, Schwalbach, Germany).

Methods

Production of silver-nanolipid complex: microsilver BG was dispersed in water with a magnetic stirrer (5 min, 800 rpm). NLC (composition cf. table 1) were prepared by hot high pressure homogenisation (80 °C, 2 homogenization cycles at 800 bar, [4]). Upon cooling the NLC dispersion was added to the microsilver dispersion. Table 2 shows the formulations produced.

AF4-MALS analysis: HPLC: an Agilent 1200 system was used: degasser, isocratic pump, autosampler, and a variable wavelength detector at 280 nm. For particle separation, the Eclipse® system was used (Wyatt Technology). Size and concentration were determined with a DAWN® (18 angle light scattering detector) and an Optilab® (refractive index detector), respectively (Wyatt Technology).

DLS and zeta potential do not differentiate

Sample	NLC dispersion % (w/w)	Microsilver % (w/w)
AB1	5.0	0.1
AB2	10.0	0.1
AB3	5.0	0.15
AB4	10.0	0.15

Preliminary studies

No significant differences could be obtained with regularly applied techniques (i.e. size analysis by dynamic light scattering or surface charge analysis by zeta potential measurements, data not shown). Therefore the proof of the adsorption of silver ions did not seem to be possible.

AF4-MALS shows clear differentiation

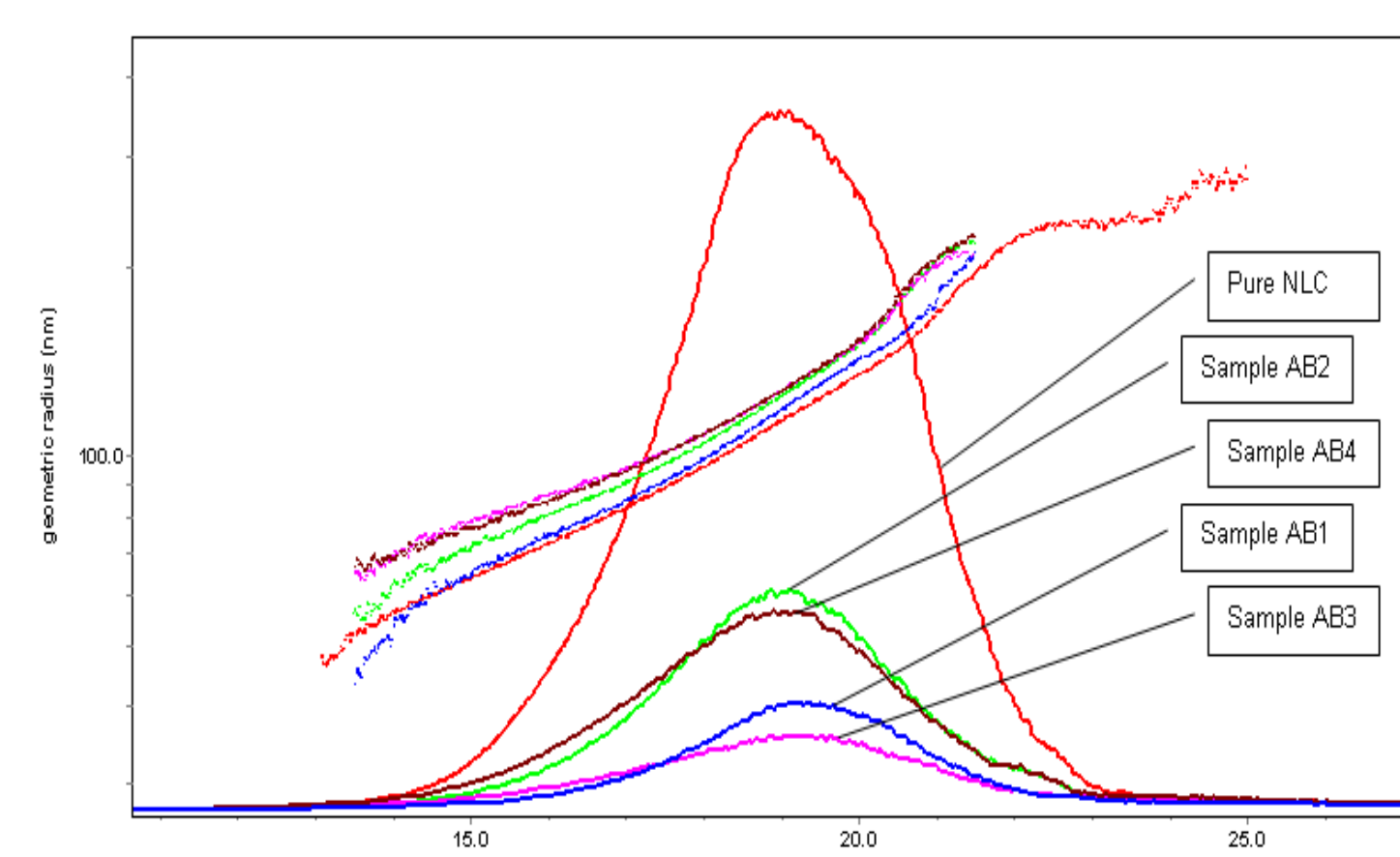


Figure 2: AF4-MALS analysis of the silver-nanolipid complex and the NLC dispersion (red curve). All formulations show a higher radius than the NLC formulation, proving the adsorption of silver ions onto the surface of the NLC (cf. Fig. 1).

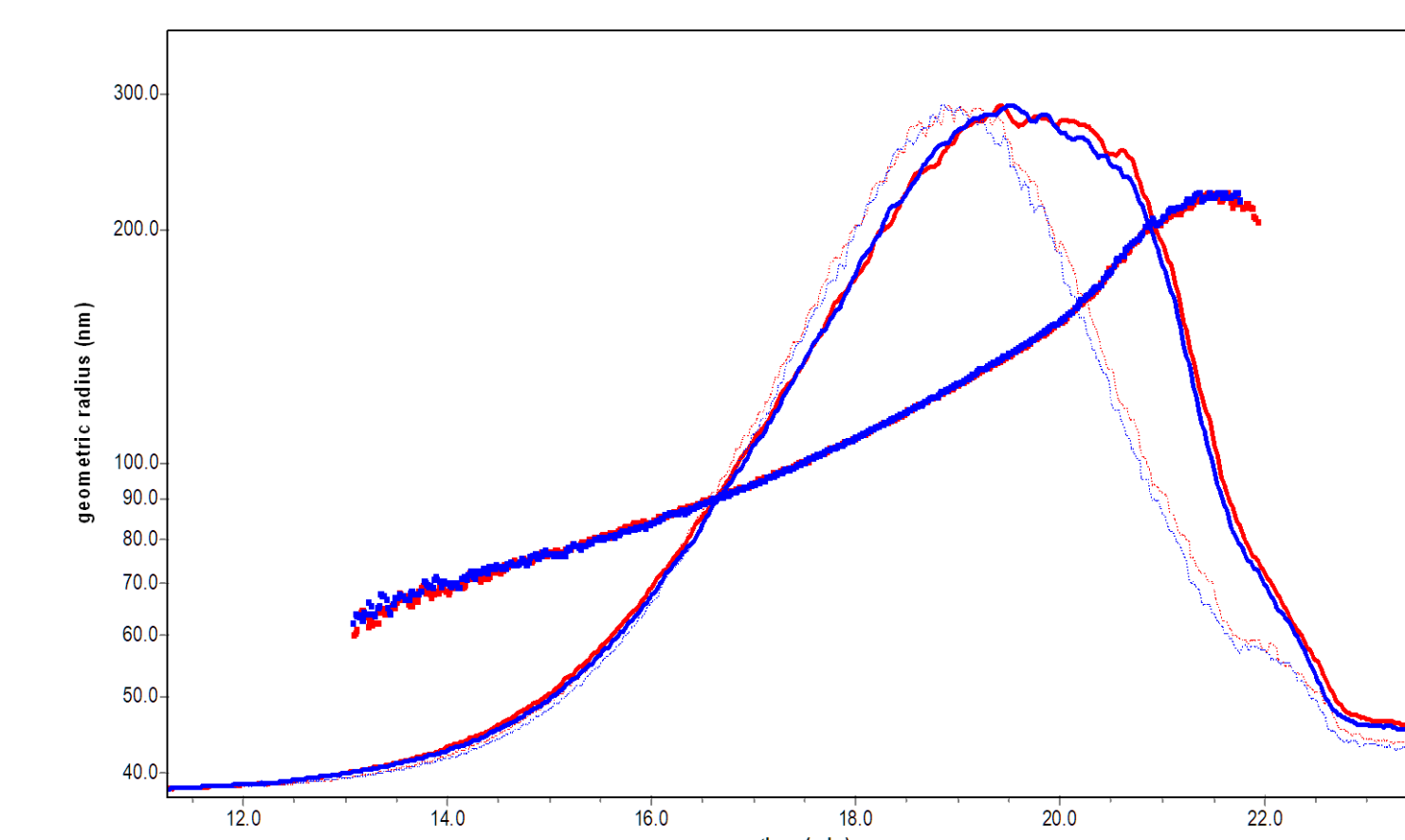


Figure 3: Overlay of two independent measurements. No differences are detected, proving good reproducibility of the method.

Results and discussion

AF4-MALS analysis

Figure 2 shows the results obtained for the different formulations (cf. Table 2) and the NLC dispersion as a reference. The light scattering signal is plotted as an overlay – no significant differences are found for the elution times. However, significant differences were found for the geometric diameters (Fig. 2 – indicated by red arrow) All silver-coated particles show a higher radius than the pure NLC dispersion (red line). These results perfectly correlate with the model of silver sticking to the outer surface of the particles, thus increasing the particle radius observed. Interestingly the diameters were different for AB1 (blue line), AB 2 (green line), but were similar for AB3 and AB4 (brown and pink lines). This indicates that both, the amount of NLC and the amount of added silver, will influence the thickness of the silver ion coating.

Reproducibility

To investigate the reproducibility and the robustness of the method, samples were subjected to several repetitions. Figure 3 shows the overlay of two independent determinations. The overlay of the two measurements shows good reproducibility of the measurements. Therefore, AF4-MALS method is highly reliable and reproducible, an important pre-requisite for reliable analysis.

Conclusion and references

Conclusion:

It was shown that the NLC particle radius increases after the formation of silver-nanolipid-complexes. Therefore the activity of the silver-nanolipid complex can be explained by the adsorption of silver ions onto the surface of the NLC.

In summary AF4-MALS provided the first evidence about the theory of action, which was not possible with standard methods.

References:

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