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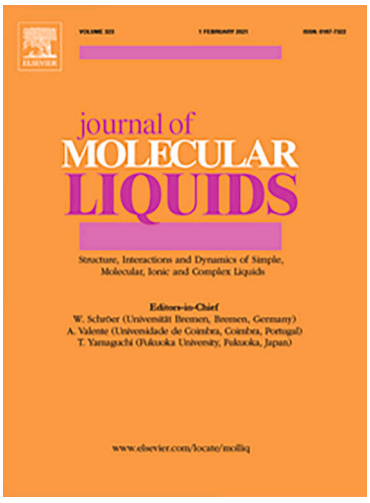

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PII: S0167-7322(22)00330-0
DOI: https://doi.org/10.1016/j.molliq.2022.118793
Reference: MOLLIQ 118793

To appear in: *Journal of Molecular Liquids*

Received Date: 22 November 2021
Revised Date: 16 February 2022
Accepted Date: 18 February 2022

Please cite this article as: Erica.M. Pachón Gómez, O. Fernando Silva, M. Der Ohannesian, M. Nú ñez Fernández, R.G. Oliveira, M.A. Fernández, Micelle-to-vesicle transition of lipoamino Gemini surfactant induced by metallic salts and its effects on antibacterial activity, *Journal of Molecular Liquids* (2022), doi: https://doi.org/10.1016/j.molliq.2022.118793

Micelle-to-vesicle transition of lipoamino Gemini surfactant induced by metallic salts and its effects on antibacterial activity

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Abstract

Gemini surfactants are amphiphilic molecules formed by two polar heads and two hydrophobic tails joined by a spacer between the polar groups. Their molecular structure gives them particular physico-chemical properties in comparison with their monomeric counterparts. In this work we synthetized a Gemini surfactant (SDDC) derived from the amino acid cystine and decanoic acid. The influence of different metallic salts on the aggregation of the surfactant was studied. SDDC forms core-shell type triaxial ellipsoidal micelles in aqueous solution, but the aggregates suffer a change in morphology, forming vesicles in the presence of copper or silver ions. This behavior was corroborated by TEM and SAXS measurements. With the last technique was possible to estimate that vesicles formed in presence of copper consisted of 7.3 stacked bilayers. It was possible to determine that Cu^{2+} was reduced to Cu^{+} in the solution containing SDDC, through EPR and UV-visible spectrophotometry measurements. The reduction of copper was accompanied with the concomitant oxidation of the cystine. The antimicrobial activity of the different aggregates was studied against *E. coli* and *S. aureus* and compared with a conventional antibiotic ampicillin. The most efficient system resulted the mixture of SDDC:$CuSO_4$/10:1 against the Gram-negative bacterium, there with the minimum concentration used (0.01 mM), was possible to inhibit 70 % of growth. The new systems characterized in this work have many possibilities for potential applications, mainly in the area of drug delivery systems, templates in the synthesis of nanomaterials or as antibacterial compounds.

Keywords: gemini surfactant; lipoamino acid surfactant; metallosurfactants; amino acid derivative surfactant; antibacterial activity; vesicles

1. Introduction

Amphiphilic compounds are able to reduce surface tension and to form different self-assembled aggregates, as micelles and vesicles, for instance. Due to their ability to control interfacial properties, surfactants are extensively used in many areas such as oil recovery, food processing and pharmaceutical industry, as well as in catalysis, biochemistry and materials science.[1,2,3] Research is currently underway to apply new kinds of surfactants in high technologic areas such as microelectronic and biotechnology (COVID vaccines for instance) between others. Therefore it is necessary to design novel surfactants with appropriate properties.

A very interesting kind of surfactants are gemini, formed by two hydrophobic tails joined by a spacer between the polar groups.[4,5,6] Their molecular structure gives them particular physico-chemical properties in comparison with their monomeric counterparts.[7,8,9,10] Normally they present low critical micellar concentration (CMC) and unusual morphologies in the aggregates that they form,[11,12] which can be utilized in potential applications.[13,14,15]

An additional challenge in the synthesis of amphiphilic compounds, is to use raw materials from renewable sources, like amino acids, sugars and natural oils, to prepare biocompatible surfactants ensuring soft reaction conditions, efficiency and multifunctional activity.[16,17] Amino acid-based surfactants are an important kind of biomolecules with different application possibilities. For instance there are examples of amphiphilic derivatives of lysine or cysteine used as antibacterial agents.[18,19]

On the other hand, metallosurfactants are amphiphilic compounds in which the polar headgroup and/or tail of the surfactant are capable to coordinate to metals. The presence of metal centers produces significant changes in the aggregation behavior of the surfactants and represents a new class of material containing a metal centre as an integral structural component. Another interesting aspect is the fact that a metal cation can bridge multiple surfactant headgroups. So, the polar groups may be closer, or more far away, depending on the chemical structure of the surfactant, and this will produce aggregates of lesser or greater curvature, respectively.[20] For instance, octadecylamine is a cationic surfactant with only one chain that forms micelles in acidic conditions. However, when it is sonicated in presence of a small amount of Ag^{+} ions, the surfactant forms vesicles.[21] Another interesting example is the work of Huang *et* al., who reported that a gemini dicarboxilic surfactant in aqueous solution formed micelles of various sizes. In the presence of a small concentration of Cu(II) (related with the surfactant concentration) the authors observed vesicles, attributed to the coordination of Cu(II) by the carboxylate groups of several molecules of surfactant.[22] These compounds make it possible to concentrate metal ions at the interfaces, where the metal gives an additional

advantage of redox properties and coordination chemistry.[23] The association of amphiphilic molecules with metals produces in some cases very attractive systems with several interesting applications and possibilities to use, for instance as supramolecular aggregates for contrast agents,[24] transport,[25] nanoscale molecular containers and sensors,[26] metal extraction strategies,[27] or catalysts that resemble hydrolytic metalloenzymes.[28,29] Many hydrolytic metalloenzymes are found in nature, and understanding their structure / function relationship motivates the design and synthesis of new metal complexes that miniaturize and mimic active sites in a hydrolytic enzyme.[30]

In previous works, we studied and characterized a gemini lipoamino acid surfactant named 3,3´-disulfanediyl bis 2-decamido propanoic acid (Figure 1, SDDC) which was obtained by an amide group formation between the amino acid cystine and a fatty acid chloride derived from decanoic acid, under aqueous alkaline conditions.[31] SDCC was used in mixtures with a non-ionic surfactant (Tween 80),[32] or as a ligand in the synthesis of silver nanoparticles.[33] The presence of several functional groups in the headgroup of SDDC makes it capable of binding to various metal cations. In this work we studied the influence of Cu^{2+} and Ag^{+} on the aggregation behaviour of SDDC also exploring the use of these new systems as potential antimicrobial agents. There is an urgent need to find new antibiotics, as well as to develop innovative antimicrobial approaches, able to kill drug-resistant bacteria, so amino acids based surfactants are currently studied as promising alternatives to conventional antimicrobial compounds.[34,35]

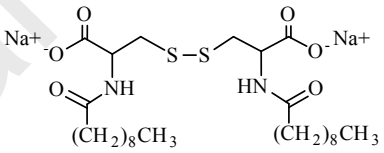

Figure 1: Structure of the gemini lipoamino acid surfactant SDDC.

2. Materials and Methods

2.1. Materials

L-cystine, decanoic chloride (Sigma Aldrich), analytical grade NaOH, $AgNO_3$ and $CuSO_4$ (Anedra), were used as received. 3,3´-disulfanediyl bis 2-decamido propanoic acid (SDDC) was synthesised by the reaction of cystine with decanoic chloride according to the literature.[31] The culture medium was brain heart infusion broth and brain hearth infusion agar (Laboratorios Britania S.A.). *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and ampicillin (Laboratorios Bagó) were used as received. Milli Q water was used in all experiments.

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2.2. Preparation of complexes SDDC:metal ions

A 3 mM solution of SDDC was used to dissolve $CuSO_4$. The dilutions were sonicated during three hours to give a colloidal suspension. In this way, solutions with 1:1, 2:1, 10:1 and 100:1 relationships of SDDC:$CuSO_4$ were prepared. These solutions presented greenish coloration.

When $AgNO_3$ was used to perform complexes with SDDC, the procedure was the same, but it wasn´t possible to obtain stoichiometric solutions, because they were not stable. Only in the cases of SDDC: $AgNO_3$, 10:1 and 100:1 stable solutions were obtained.

2.3. Vesicles morphology

Vesicles morphology was determined using transmission electron microscopy (TEM). The Jeol 1200 EX II microscope worked at an accelerating voltage of 80 kV. The solutions containing metal ions were measured without staining agent addition. A drop of vesicle suspension was placed on a carbon coated copper grid and then was left 1 min to ensure adhesion process. The excess of suspension dispersion was removed using a piece of filter paper.

2.4. SAXS experiments

SAXS measurements were performed in the SAXS-1 line at the Brazilian Synchrotron Light Laboratory, Campinas, Brazil. The incident photon wavelength was λ= 1.5 Å and the nominal sample-detector distance was 1 m. Intensity was expressed as a function of the modulus of the scattering vector $q = (4\pi \sin 2\theta/2)/\lambda$, the scattering angle being 2θ. We carried out the analysis of samples containing different concentrations of $CuSO_4$ and a constant surfactant concentration of 8 mM, to obtain different relationships $CuSO_4$: SDDC. The experiments were done at pH 7. Samples were placed in cells for liquids with mica windows (1 mm optical path) and temperature was maintained at 30 °C. The two-dimensional SAXS patterns were recorded by a Pilatus 300K X-ray detector from Dectris. The contribution of the buffer was subtracted as background from the SAXS profile and the resulting signal was radially integrated using Fit 2D.[36] The spacing of the multilamelar vesicles is equal to $2\pi n/q_{Bragg}$ where q_{Bragg} is the q value that satisfies the Bragg diffraction condition of the n^{th} order, n=1 and n=2 in our case. The correlation length was calculated from the first order diffraction peak full width at half maximum employing the Scherrer equation.

2.5. Characterization of copper in the complexes

Electron Paramagnetic Resonance (EPR) measurements were done in a spectrometer equipped with X-band microwave source (9 kHz) and a field modulation of 100 kHz. The samples were analysed at room temperature in a Brucker EMXPlus spectrometer (Department of Physics, FByCB-UNL).

2.6. In vitro antimicrobial assays

The antimicrobial activity of complexes SDDC:metallic salts was evaluated *in vitro* against *E. coli* and *S. aureus*.

A suspension of each bacterial strain was prepared in physiological solution (1.5 x 10^8 CFU/mL). 10 μL of 1/10 dilution of the initial suspension were placed in a vial containing 200 μL of the surfactant (or the surfactant/metal solution) at the corresponding concentration, and 800 μL of brain heart infusion broth. One vial was prepared for each concentration of each system with the two microorganisms. Besides, control solutions of ampicillin as antimicrobial agent were prepared for each bacteria (positive control). Control tests with each bacteria and culture medium in absence of surfactant and ampicillin were also prepared (negative control). The samples were incubated at 37°C for 24 hours. After that, the content of each vial was diluted and an inoculum was poured in a Petri dish with brain heart infusion agar, and incubated for another 24 hours, at 37°C. The bacterial growth was measured as the amount of colony forming units (CFU). The amount of CFU that grew up in the negative control was considered as the maximum growth (100% growth, 0% inhibition). All assays were carried out in triplicate.

3. Results and Discussion

3.1. Interaction of the gemini surfactant SDDC with metal ions

In this study, we examined the interaction of SDDC with $CuSO_4$ due the potential use of metallosurfactants containing Cu (II) as mimics of natural Cu enzymes, among others.[37] $CuSO_4$ is not soluble in water at pH higher than 6 (due the precipitation of hydroxides), but when the metallic salt was added to a buffer solution containing SDDC with different stoichiometric ratios (1:1, 2:1, 10:1 and 100:1, SDDC: $CuSO_4$), we obtained stables colloidal solutions at pH 5, 7 and 12. It is worth mentioning that SDDC is not soluble in water at pH below 6. These results indicate that there is an interaction between the polar head group of the surfactant and the cation that contributes to maintain both species dissolved over a wide range of pH, and the complex formed appears to be stable at least for a month after preparation without precipitation under ambient conditions.

We prepare solutions of $CuSO_4$ with L-cystine (polar moiety of the surfactant), with sodium decanoate (hydrophobic moiety of SDDC), and with the mixture of L-cystine

and sodium decanoate, in order to elucidate which are the surfactant moieties involved in complex formation with copper.

The results of these experiments are showed in Table 1 and Figure S1 (Supplementary material).

Table 1: Interaction of $CuSO_4$ with different ligands[a]

Solution	Components	Stoichiometric ratio	Results
A	SDDC:$CuSO_4$	1:1	Greenish solution
B	L-cystine: Sodium decanoate: $CuSO_4$	1:2:1[b]	Blue precipitate
C	L-cystine: $CuSO_4$	1:1	Light blue precipitate
D	$CuSO_4$	----------	White precipitate
E	Sodium decanoate: $CuSO_4$	2:1[b]	Blue soft precipitate

[a] pH 7, T=25°C.

[b] Sodium decanoate was added in double amount since SDDC contains two hydrocarbon chains

From the analysis of Table 1 and Figure S1, $CuSO_4$ precipitated in buffer pH 7 as was predicted. In other way, the simple mixture between $CuSO_4$ and molecules containing similar functional groups than those found in SDDC, also formed precipitates. These results indicate that a complex is formed between both species, so the structural features of SDDC act as the entity that controls the stability of the complex, and its formation is not due solely to the presence of the polar head or the hydrocarbon tails of surfactant.

An extra experiment using $CuCl_2$ was performed to assess the counterion effect on complex formation, but addition of chloride salt did not result in significant changes and the same greenish solution was obtained.

3.2. Characterization of the aggregates formed in mixtures SDDC: $CuSO_4$

SDDC forms core-shell type triaxial ellipsoidal micelles in aqueous solution as was determined by small-angle X-ray scattering measurements (SAXS) and previously described.[32] SDDC micelles were not observed by TEM, but SDDC in presence of the metallic salt forms vesicles that can be clearly observed by TEM, even without the addition of TEM staining agents.

Figure 2 shows TEM images of vesicles of the gemini surfactant in the presence of the metallic salt.

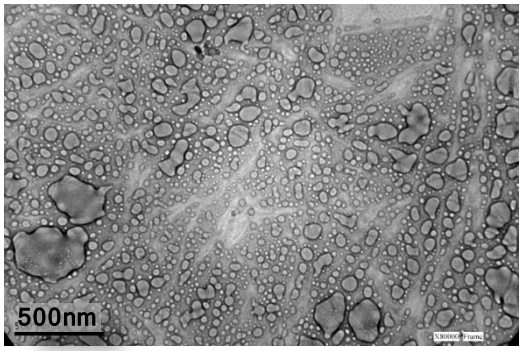

Figure 2: TEM microphotographs of SDDC:$CuSO_4$ (1:1) at pH=7, [SDDC]= 3 mM

The persistence of these aggregates at least for a month was verified taking TEM images in function of the time. (Figure S2). In addition, solutions with different stoichiometric relationships were evaluated, for instance SDDC:$CuSO_4$, at 10:1 and 100:1 ratios, and aggregates with a wide range of sizes in all solutions were observed (Figure S3). In order to confirm that the aggregates are vesicles and not micelles, dilution experiments were carried out lowering the SDDC concentration up to 0.06mM, which is far from its CMC value (0.35 mM).[32] At this concentration the aggregates are present although in small amounts (Figure S4). Considering that SDDC forms micelles in aqueous solution, it is possible to conclude that the presence of the cation induces morphological changes in aggregates, and a transition from micelles to vesicles was observed. Similar results were observed by Zhou et al. when they studied the behaviour of 3-aminopropylmethyltrisiloxane, which in water forms micelles, but in the presence of Cu^{2+} forms vesicles.[38]

The formation of complexes between SDDC and the metallic salt was also corroborated by NMR measurement (Figure S5). The spectrum of the complex shows wide distorted signals, compared with SDDC pure spectrum, which is probably due to the presence of the metal. It was previously described that the complexes formed between amino acids and transition metals having unpaired electrons produce large local magnetic fields in the immediate vicinity of nuclei, which are observed as widening of the signals.[39] The formation of vesicles could be another cause of signal broadening due to curvature of vesicles and polydispersity of the vesicular sizes.[40] The main distortion is observed in the region corresponding to the protons of the carbons directly bonded to the sulphur atoms in the amino acid, indicating that this moiety of SDDC is affected by the presence of the metal.

The suspensions formed by SDDC:$CuSO_4$ at different molar ratios were also studied by SAXS to get more information about the size and shape of the aggregates. Figure 3A shows the experimental X-ray scattering intensities I(q) of solutions prepared at a constant concentration of SDDC (8 mM) and different concentrations of $CuSO_4$, at pH 7. For SDDC alone, the scattering profile is the typical of a micellar one (horizontal at low q and with a small bump at higher q, black line). Nevertheless, in the presence of $CuSO_4$ the signal shifts to a typical one of a lamellar state with an exponential decay at low q (blue line, oblique arrow) when the relationship SDDC:$CuSO_4$ is 1:1. Besides, in the latter plot it is possible to observe two Bragg peaks at 2.02 nm^{-1} (first order, n=1) and at 4.04 nm^{-1} (second order, n=2) indicated by two blue vertical arrows in Figure 3A. The ratio between the two peaks is 2 indicating a lamellar series of bilayers. In Figure 3B is possible to observe clearly the differences in traces at low q, comparing the (null) slope for SDDC micelles (black dots) and a non zero defined slope for SDDC:$CuSO_4$/1:1 vesicles (blue dots). At low concentration ratios for SDDC:$CuSO_4$, the plots showed intermediate behaviours.

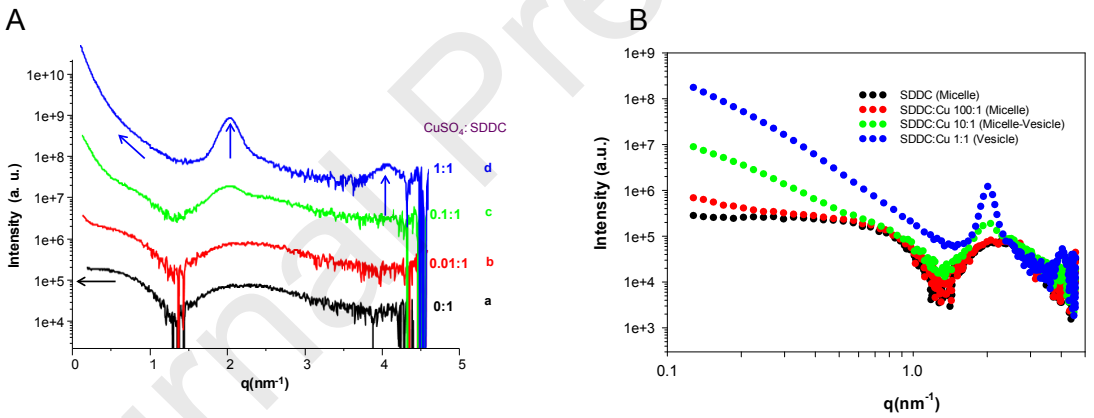

Figure 3. SAXS experimental curves of water solutions of SDDC or different mixtures SDCC: CuSO4. [SDDC]= 8mM, T =(30.0 ± 0.1) °C. (A) Semi log representation. Bragg peaks are observed only at high $CuSO_4$ concentration, with a 1:2 relation (lamellae). Black curve is characteristic of micelles. (B) Double log representation where defined slopes can be observed at low q, going from null slope (black curve: micelles) up to an slope between -2 and -3 proper of aggregated lamellae (blue curve).

The black line in Figure 3 (SDDC in absence of copper) was fitted with a model of a core-shell type triaxial ellipsoid micelle.[32] From the fit of the first order peak of SDDC:$CuSO_4$/1:1 (blue line) it was possible to estimate the number of 7.3 stacked bilayers with a periodicity of 3.11 nm from Scherrer equation.[41] These studies

corroborated the change of morphology of the aggregates of SDDC in presence of copper.

3.3. Characterization of the oxidation state of cooper in complex SDDC:$CuSO_4$

An important experimental observation of the system was that the solutions prepared with SDDC and $CuSO_4$ always showed a greenish coloration, instead the characteristic light blue color of $CuSO_4$ (Figure S1). A possible explanation could be the coordination of Cu^{2+} with functional groups in cystine moiety. It is known that when Cu^{2+} is coordinated with halogens, the complex can present greenish color.[42] An alternative explanation could be the reduction of Cu^{2+} to Cu^{+}, which leads to the aforementioned color change. To shed light on this fact we prepared a solution of SDDC with CuCl and we observed the same greenish solution. We evaluated this solution by TEM observing very similar aggregates to the previously described in solutions with $CuSO_4$ (Figure S6).

In the current study, the possibility of the reduction of Cu^{2+} to Cu^{+} was evaluated using several techniques. Among them, Electronic Paramagnetic Resonance (EPR) was used to assess the nature of the copper present. The measurements were done in solutions with different molar ratio between SDDC y $CuSO_4$ (1:1, 10:1 and 100:1). There was no EPR signal corresponding to Cu^{2+} and this result suggests that copper must be present as Cu^{+} ion. However since Cu^{+} is diamagnetic, it does not give EPR response.[43]

Therefore, some experiments were done using the molecular probe neocuproine (Neo), which is a specific Cu^{+} chelator. Neo forms a very stable complex with Cu^{+} ($[Cu(Neo)_2]^{+}$) that absorbs strongly at 450 nm, that can easily detect it at very low concentrations (0.03 µg of copper).[44] Figure 4 shows the UV-visible spectra of the complex and of individual solutions of CuCl and $CuSO_4$ at the same concentrations of copper that in the complex, in all cases in the presence of Neo.

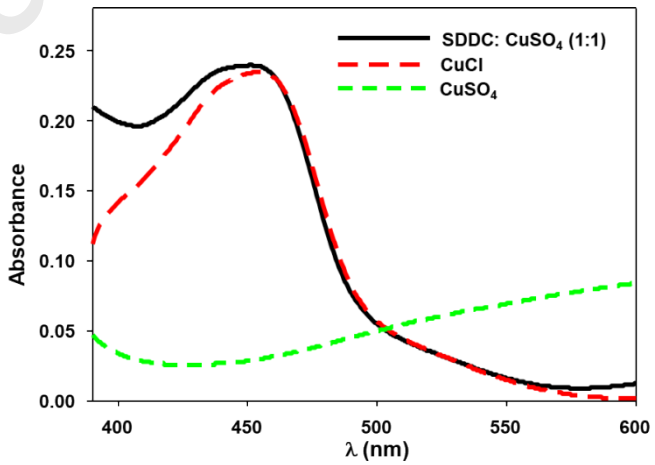

Figure 4: UV-Visible spectra of aqueous solutions of Neo in presence of SDDC:$CuSO_4$ /1:1, CuCl y $CuSO_4$. In all solutions the copper concentration was 0.7 mM. [Neo]= 3.2 mM.

The spectrum of the complex formed by Neo and CuCl (cutted red line) has a maximum at 450 nm as it was described in literature.[44] The mixture Neo-$CuSO_4$ does not present any signal at that wavelength, as was expected, whereas the mixture Neo-SDDC-$CuSO_4$ presents a band with a maximum at 450 nm, which matches with that obtained from Neo:Cu^+. These results agreed with EPR experiments and demonstrated that Cu^{2+} in the presence of SDDC is reduced to Cu^+, and it is likely to be accompanied by gemini surfactant oxidation.

3.4. Analysis of the surfactant in complex SDDC:$CuSO_4$

Considering the oxide-reduction reaction in the complex SDDC:$CuSO_4$, we analyzed possible changes in the surfactant during this reaction. It is known that disulfide bond can be broken by metal ions, but the mechanism is quite complicated; however, one possible pathway may be homolytic cleavage of the S-S bond into RSH and RSOH. Lumb et al. suggested that the thiol formed reduces Cu(II) to Cu(I), and some disulfide bond is reformed.[45] Some others authors proposed that particularly, the oxidation of the sulfur atom of cystine can occur going through different more oxidized species until reaching cysteic acid (Figure 5).[46,47] The IR spectrum of the mixture SDDC:$CuSO_4$ was compared with the spectra of SDDC and cysteic acid (Figure S7). In the region of 1250-1300 cm^{-1} there are similar signals between cysteic acid and the mixture SDDC:$CuSO_4$, that could be attributed to the stretching of R-SO_3^- and R-SO_2-OH, whereas those signals are absent in the spectrum of SDDC. In the case of the oxidation of the sulfur, the signal corresponding to S-O bond appears in the range of 600-800 cm^{-1} and were found for cysteic acid and the complex SDDC:$CuSO_4$ but it was absent in the spectrum of SDDC. In other way, in the region between 2750-3000 cm^{-1}, practically there was no differences between SDDC and SDDC: $CuSO_4$, indicating that the alkyl chains were not affected by the complex formation. The amide bonds were also unaffected, because there was no changes in the region 3000-4000 cm^{-1} and the same happened with the stretching vibration of the carbonyl group of SDDC in comparison with the complex. From the results obtained we can conclude that there are changes in the oxidation state of the species involved that can be coordinated with the copper present in the solution. It is known that copper (I) ions react with specific ligands, such as cysteine.[48] Copper binding

can occur through bridging sulfur bonds with thiols of cysteine,[49] or with (-SO_3H) group of cysteic acid, which is an important Cu^+ ligand.[50]

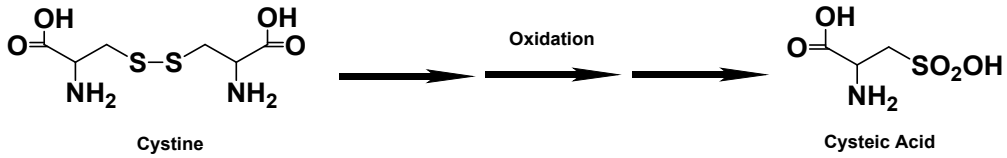

Figure 5: Proposed cystine oxidation

3.5. Interaction of SDDC with $AgNO_3$

As it is known, before the pharmaceutical antibiotic revolution, some metals such as silver and copper were employed as antimicrobial agents, hence an alternative approach was to build a complex between Ag^+ and SDDC.[51]

In mixtures of SDDC with $AgNO_3$, stable solutions were obtained at very low concentration of metallic salt (10:1 and 100:1), Figure S8. The solutions prepared were investigated by TEM, and we observed the presence of aggregates with spherical morphology and having high dispersion in size (Figure S9), in a similar way to those observed when the metallic salt was $CuSO_4$ (Figure S3). Since one of the properties of some Gemini surfactants is the formation of silver nanoparticles (AgNPs) characterized by their surface plasmon peak (SPR) around 410 nm,[52] we paid enough attention for possible appearance of AgNPs by UV–Visible spectrophotometry, looking for the presence of the SPR band, and we could not detect AgNps (Figure S10). Moreover, in order to evaluate if the absence of SPR band was due to low concentrations of AgNPs, we did some experiments of fluorescence looking for silver clusters,[53] but all studies gave negative results.

Similar SAXS results were obtained for $AgNO_3$ as for $CuSO_4$.The typical micellar shape transformed in a lamellae pattern as $AgNO_3$ concentration increased, and the periodicity of the lamellae were approximately the same as in the SDDC:copper system (Figure S11).

3.6. Study of antimicrobial activity of Gemini surfactant and its complexes with metallic salts

The antibacterial activities of the prepared complexes were evaluated using both Gram positive and Gram negative bacterial strains. A number of reports discussed the antibacterial action of copper and silver ion and, generally, they claimed that the metallic

ions generate reactive oxygen species (ROS) so cellular components undergo oxidative degradation that causes cell death.[54]

The results obtained with the use of SDDC and its complexes with metallic ions are condensed in Table 2. In addition we compared the effect of metallic salts in absence of SDDC, and ampicillin, which is a commercial antimicrobial agent. This last compound is used to prevent and treat a number of bacterial infections, such as respiratory, urinary and intestinal tract infections.[55]

Table 2: Growth inhibition percentage of Gram positive (*S. aureus*) and Gram negative (*E. coli*) bacteria in the presence of different potential antimicrobial agents.[a]

Growth inhibition percentage (%)						
[Antimicrobial agent], mM	**SDDC**	**SDDC:$CuSO_4$ (10:1)**	**SDDC:$AgNO_3$ (10:1)**	**Ampicillin**	**$CuSO_4$**	**$AgNO_3$**
	***S. aureus*[b]**					
0.01	0	0	0	41	0	0
0.06	0	0	0	60	0	0
0.12	4	46	64	71	0	0
0.18	95	95	96	83	0	0
	***E. coli* [c]**					
0.01	29	70	0	0	0	N.C.[d]
0.06	88	83	89	80	0	N.C.[d]
0.12	88	60	88	99	0	0
0.18	87	90	83	99	12	35

[a] T=36°C, incubation time: 24 h, pH=7.

[b] $[S.\ aureus]_{initial}=5x10^{-4}$ CFU/mL.

[c] $[E.\ coli]_{initial}=5x10^{-4}$ CFU/mL.

[d] N.C.= not quantified

The first observation about the results of Table 2 is the growth inhibition of Gram-positive and Gram-negative bacteria by SDDC alone and in the presence of the copper and silver salts. SDDC displayed higher antibacterial efficiency against the Gram-negative bacteria as compared with the Gram-positive one.

Particularly, in the analysis of the growth inhibition of this last microorganism, it can be seen that even at concentration 0.12 mM of SDDC, there was no inhibition, whereas at 0.18 mM, the inhibition was practically complete. With the use of the metallic salts of

SDDC there was approximately a 50% of inhibition at the first concentration. At 0.18 mM, all the systems containing the gemini surfactant were efficient in a similar way. However it is important to mention that ampicillin was more efficient at lower concentrations, although did not reach the 95 % of inhibition achieved at the maximum concentration of the amphiphilic systems. The isolated salts did not produce any effect on the growth of *S. aureus*. About this last point, it is important to take into account that $CuSO_4$ is not very soluble at the working pH.

Regarding *E. coli*, all the media containing SDDC (alone or with metallic salts) produced similar results, inhibiting 83-90 % the growth of the bacteria, even at a concentration of 0.06 mM of the surfactant. The mixture SDDC:$CuSO_4$ produces 70 % of growth inhibition at 0.01 mM while ampicillin is not effective at that concentration. The isolated salts produced a very small inhibition percentage at the maximum concentration used.

From the results in Table 2, and considering the minimum inhibitory concentration (MIC) of the antibiotics as the lowest antibiotic concentration that inhibits ≥90 % of the growth of the microorganism compared to the growth control,[56] we calculated MIC values for the different studied systems. The obtained MIC values were 106.6, 109.5, and 109.6 µg/mL for SDDC, SDDC:CuSO4 (10:1) and SDDC:$AgNO_3$ (10:1) for *S. aureus* respectively, whereas at the maximum molar concentration used for all systems (0.18 mM), the MIC of ampicillin has not yet been reached, so we only can conclude that its MIC is higher than 62.9 µg/mL. In the case of *E. coli*, on the other hand, it is only possible to calculate MIC values for SDDS and ampicillin (106.6 and 62.9 µg/mL respectively), while the gemini and metal salts systems have MICs higher than 109 µg/mL.

The difference in susceptibility of both microorganisms is presumably attributed to the differences in the cytoplasmic membrane physiology of the two bacterial types, as briefly is explained. In Gram positive bacteria *(S. aureus*), the adsorption occurs in the lipoteichonic acid layer, which is characterized by a charged nature due to asymmetric distribution of the lipids. In the Gram-negative bacteria (*E.coli*), the lipid layer is the target of the amphiphilic cationic biocides. So the mode of action of that type of compounds on different microorganisms can be attributed to the adsorption of amphiphilic molecules on the outer cellular membrane of the microorganism due to their amphipathic characteristics.[57]

In addition, the similarity between the hydrophobic chains in SDDC moieties and the lipid layers, and the building units of the cell membranes and functional groups (including a carboxylic acid and an amide group), plays a crucial role in antibacterial activity.

In literature there is evidence of the improved performance of some surfactants containing metals,[58] and the authors attributed this fact to a better interaction of the metallosurfactants with the cell membrane, producing an electrostatic unbalance,[59] with the concomitant changes in structure, protein performance and normal metabolism. However, in this system, there is no an important effect due to the complexes gemini surfactant:metallic salts. It can only be highlighted the effect of SDDC:$CuSO_4$/10:1 against *E. coli*, where with the minimum concentration used (0.01 mM), was possible to inhibit 70 % of growth, being this system more efficient even than ampicillin.

4. Conclusions

Several amphiphilic systems can coordinate metallic ions, and this coordination may produce changes in the aggregates. In the mixture SDDC:$CuSO_4$ studied in this work, two very interesting results were obtained: (i) it is possible to maintain the copper solubilized in a wide pH range in the presence of the surfactant, even at pHs where normally the cation would be precipitated. In other way, the gemini surfactant is also insoluble at pH lower than 6, whereas in presence of the metallic salt it is possible to maintain it soluble in water; (ii) the interaction of the gemini surfactant with the metallic salt produced a different morphology of the aggregates, changing from micelles to vesicles. In other way, this interaction resulted in an oxide-reduction reaction, where the reduction of copper was occurring with the concomitant oxidation of the sulfur atom of SDDC. The final vesicular system could involve the interaction of Cu^+ with the surfactant. No evidences of the same oxide-reduction reaction were found in presence of Ag^+, although the formation of vesicles was also observed.

SDDC undergoes a change in aggregates morphology upon coordination of some metal ions, which presents great potential for broad applications, for instance the use of these vesicles as carriers of active compounds, or as templates in synthesis of materials.

In other way, SDDC and their complexes with metallic salts resulted an adequate alternative as antimicrobial agents, having similar activity as a commercial antibiotic as ampicillin, both against Gram positive and Gram negative bacteria. Based on the results obtained, these systems, in particular SDDC, could be used as new antimicrobial compound derived from natural sources, forming part of disinfectants or cleaning products.

Conflict of interest

There are no conflicts to declare.

Acknowledgements

This research was supported in part by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP 112-2015-01-00242), Agencia Nacional de Ciencia y Tecnología (FONCyT, PICT 2014-2516), and Universidad Nacional de Córdoba, Argentina (SECyT, CONSOLIDAR I, 2018-2021, Res. 75/19, 99/19, 212/20, 191/21). OFS, RGO and MAF hold researchers positions at CONICET. EMPG thanks CONICET for the fellowship granted. We thank the Brazilian Synchrotron Light Laboratory (Beamline SAXS-1) for beamline under project 20150067 and also the Instituto de Física del Litoral (IFIS-CONICET-UNL) by the EPR measurements.

References

CRediT author statement

Title of the manuscript:

Micelle-to-vesicle transition of lipoamino Gemini surfactant induced by metallic salts and its effects on antibacterial activity

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Highlights

- Gemini surfactant derived from cysteine forms complexes with copper and silver salts.

- There is a change in the morphology of the aggregates induced by the metallic salts.

- The aggregates change from micelles to vesicles.

- There is a redox process involved in the association of the Gemini with Cu (II)

- The systems have antimicrobial properties against Gram positive and negative bacteria.

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