https://doi.org/10.17952/37EPS.2024.P1262

# Low-Molecular α-Amino Acid-Based Derivatives as Nanocarriers of Plant Growth Regulators

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## Purpose:

The development of nanocarriers, suitable to deliver biologically active compounds, is a modern interdisciplinary field. These substances can be designed as carriers of active compounds for delivery in plants, for example plant growth regulators. Our research is related to design and synthesis of α-amino acid-based amphiphiles and study of their ability to form supramolecular complexes in aqueous solution under specific conditions. Here, we present newly synthesized derivatives of L-Asp and the decanoic acid, which have the structure of either a monomeric or a gemini surfactant [1, 2]. The supramolecular complexes of the sodium salt of the gemini derivative were loaded with thidiazuron (TDZ) and tested *in vitro* on *Stevia rebaudiana* Bertoni to evaluate the effectiveness of the release of the regulator and to monitor the result growth changes. The same surfactants have been used as carriers of silver cations and have shown beneficial effect on the plant development [3].

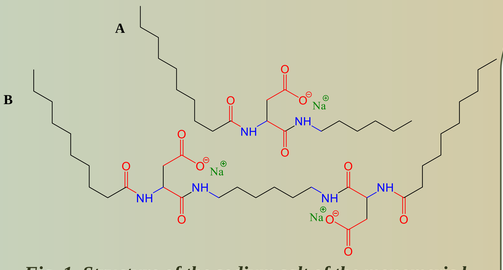


*Fig. 1. Structure of the sodium salt of the monomeric l-Asp surfactant MA6N1CNa (A) and the double sodium salt of the gemini L-Asp surfactant A6N10CNa (B).*

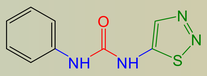


*Fig. 2. Structure of thidiazuron (TDZ).*

## Materials and methods:

The compounds used for this study have been synthesized applying TBTU condensing approach in solution [1, 2]. As sodium salts, the L-Asp derivatives (Fig. 1) behave as anionic surfactants in aqueous media. The gemini surfactant representative was loaded with the plant growth regulator thidiazuron (TDZ, Fig. 2) through a dispersion technique. Supramolecular complexes formed by surfactant solely and the surfactant with TDZ were investigated and confirmed by DLS analysis (Fig. 3). Our experiments showed that the best results for nanoaggregates are obtained at concentrations of the surfactant three times higher than its critical aggregation concentration [1] and for complexes with TDZ in molar ratio: 3:1. The work solutions were analyzed with UV-Vis spectroscopy (Fig. 4). The solid obtained from the solution with the supramolecular complexes of TDZ after solvent removal was applied as an additive to the culture medium Murashige and Skoog for *in vitro* experiments on plant species in several concentrations.

*Tab. 1. The average size and z-potential values of the complexes, according the data obtained from the DLS analysis.*

| Compound | Average size [nm] | | Average z-potential [mV] | |
|---|---|---|---|---|
| | 3xCAC | 6xCAC | 3xCAC | 6xCAC |
| MA6N10CNa | 361.3 | 128.5 | -67.47 | -59.23 |
| A6N10CNa | 134.5 | 2.6 | -52.55 | -61.26 |

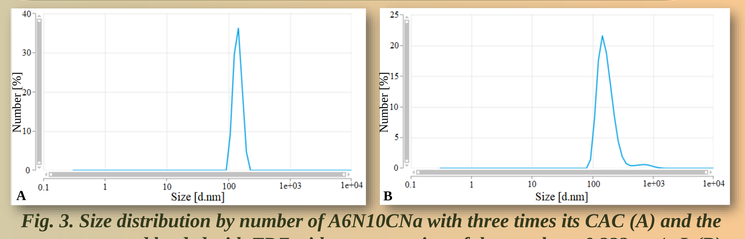


*Fig. 3. Size distribution by number of A6N10CNa with three times its CAC (A) and the same compound loaded with TDZ with concentration of the regulator 0.333 mg/mL (B).*

## Results and discussion:

Low-molecular weight organic compounds derived from L-Asp that belong to the anionic monomeric (Fig. 1A) and gemini surfactants (Fig. 1B) were synthesized. The study of the supramolecular structures by DLS is summarized in Tab. 1, which presents the average size of the complexes distributed by number along with their average z-potential values. It can be well noticed that for both of the compounds, the higher the concentration, the lower the sizes of the aggregates, which may be due to the number of sodium cations in the solution and the concentration of the compound. More experiments should be carried out to clarify the reason for these results.

The gemini surfactant A6N10CNa was chosen for further experiments. The size distribution by number of the aggregates of either A6N10CNa alone and the complex A6N10CNa-TDZ, are shown on the graphics in Fig. 3 A and B. In both cases the majority of the aggregates are of around 130 nm in diameter.

The UV-Vis spectra of the compounds TDZ and A6N10CNa, as well as of the complex A6N10CNa-TDZ are presented in Fig. 4. The UV-Vis analysis of the loaded molecules showed that there is a significant change in the absorption signals of TDZ after the addition of the nanocarriers, such as a bathochromic effect along with a hyperchromic effect of the TDZ peak in 290 nm. This confirms the effectiveness of the loading technique.

The results of the *in vitro* testing of the loaded nanocarriers show insignificant change in the growth of *Stevia rebaudiana* Bertoni at the lowest concentration (1 mg/L), while at higher concentrations there is a negative effect on the plants growth compared to the control plant (Fig. 5). Also, some non-typical morphological changes appear as a result of the treatment, such as thick and curved leaves.

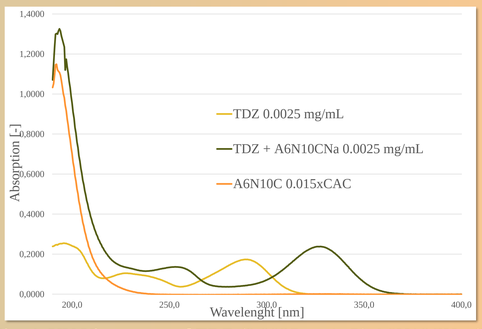


*Fig. 4. UV-Vis spectra of TDZ (0.0025 mg/mL) A6N10CNa (0.015xCAC) and A6N10CNa loaded with TDZ (0.0025 mg/mL).*

## Conclusions:

The sodium salt of the newly synthesized A6N10C self-aggregates into supramolecular complexes in the nanoscale. The changes of the spectra of the probes loaded with TDZ suggest that the molecules of the compound act as potential nanocarriers of TDZ, although the *in vitro* analysis shows that the complex A6N10C-TDZ has a negative effect on the plants growth. Our previous studies with the same organic compound used as a carrier of Ag+ show that the effect of the carrier is not limited only to transferring an active compound, but the process is more complicated and depends also on the complexes formed. More experiments are planned to be carried out in order to explain these results.


*Fig. 5. Photographs of in vitro plants of Stevia rebaudiana Bertoni after three weeks treatment with TDZ (A) and A6N10CNa loaded with TDZ – 1 mg/L (B), 10 mg/L (C), 50 mg/L (D).*

## References:

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[3] Sichanova, Mariana, et al. "Influence of the Abiotic Elicitors Ag Salts of Aspartic Acid Derivatives, Self-Organized in Nanofibers with Monomeric and Dimeric Molecular Structures, on the Antioxidant Activity and Stevioside Content in Micropropagated Stevia rebaudiana Bert." *Plants* 12.20 (2023): 3574.

## Acknowledgments:

This research was funded by the National Science Fund at the Bulgarian Ministry of Education and Science, Project КП-06-Н56/8 12.11.21. The authors also acknowledge prof. G. Staneva's group at the IBPhBME-BAS for the opportunity to study solution nanoformations by applying DLS analysis.