Analysing the impact of the oil phase selection and curcumin presence on the nanoemulsion stabilizing layer using electron paramagnetic resonance

Jelena Đoković^{1*}, S

spectroscopy



<u>Jelena Đoković</u>^{1*}, Sotiria Demisli ², Vassiliki Papadimitriou², Aristotelis Xenakis ², Snežana D. Savić ¹

¹ University of Belgrade – Faculty of pharmacy, Department of Pharmaceutical Technology and Cosmetology,

Belgrade, Serbia



² National Hellenic Research Foundation - Institute of Chemical Biology, Athens, Greece

CONCLUSION

This study concluded that curcumin is located in the stabilizing layer of nanoemulsions, regardless of the oil phase selection, but its impact on the nanoemulsion structure depends on the type of oil used, with the stabilizing effects in fish oil formulations being particularly noticeable.

INTRODUCTION

There are many health benefits associated with the use of polyunsaturated fatty acids (PUFAs), such as a reduced risk of cardiovascular diseases, immune disorders, and poor infant development. Some effort has been made in recent years to include oils rich in eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), such as fish oil, in pharmaceutical preparations. Given their low aqueous solubility and high lipophilicity, one of the ways to best utilize their advantages was to incorporate them into colloidal carriers such as nanoemulsions (NEs). Considering one of the main factors impacting the stability and destiny of NEs upon administration is their stabilizing layer, it is crucial to determine how it is impacted by the oil phase selection.

AIM

The purpose of this study was to evaluate the effects of curcumin, an active with a wide range of potential beneficial effects as well as the use of different oils, including soybean oil, the most frequently used in commercial formulations, and fish oil, which is rich in PUFAs, on the stabilizing layer of developed NEs.

MATERIALS AND METHODS

The preparation of NEs

The NEs were prepared using the high-pressure homogenization technique. The aqueous and oil phases were prepared separately and heated at 50 °C. The premulsion was formed by mixing the phases on a rotor stator homogenizer for 1 min at 11000 rpm, and subsequently passed for 10 consecutive cycles at 800 bar at 50 °C (Figure 1.).

Electron Paramagnetic Resonance (EPR) Spectroscopy

Formulations were analysed with the EPR technique using three different spin probes with unpaired electrons: 5-, 12- and 16-doxyl stearic acid (DSA), to investigate membrane dynamics at different depths, with the 5-DSA locaized closer to the polar heads of the surfactants, and 16-DSA situated near the oil core. EPR spectra were analyzed in terms of rotational correlation time (τ R), order parameter (S), and isotropic hyperfine coupling constant (α N).

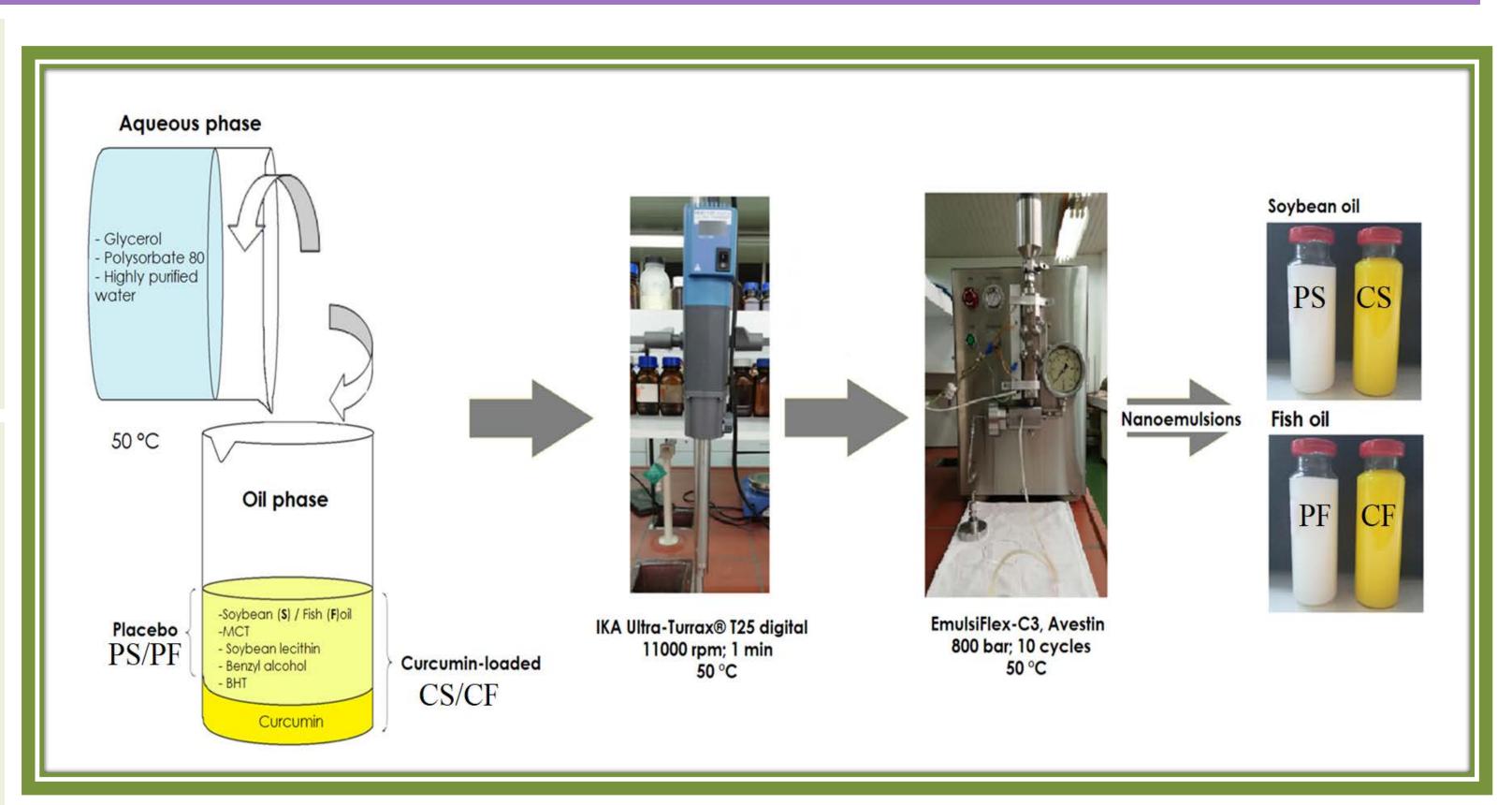


Figure 1. The preparation of NEs

RESULTS AND DISCUSSION

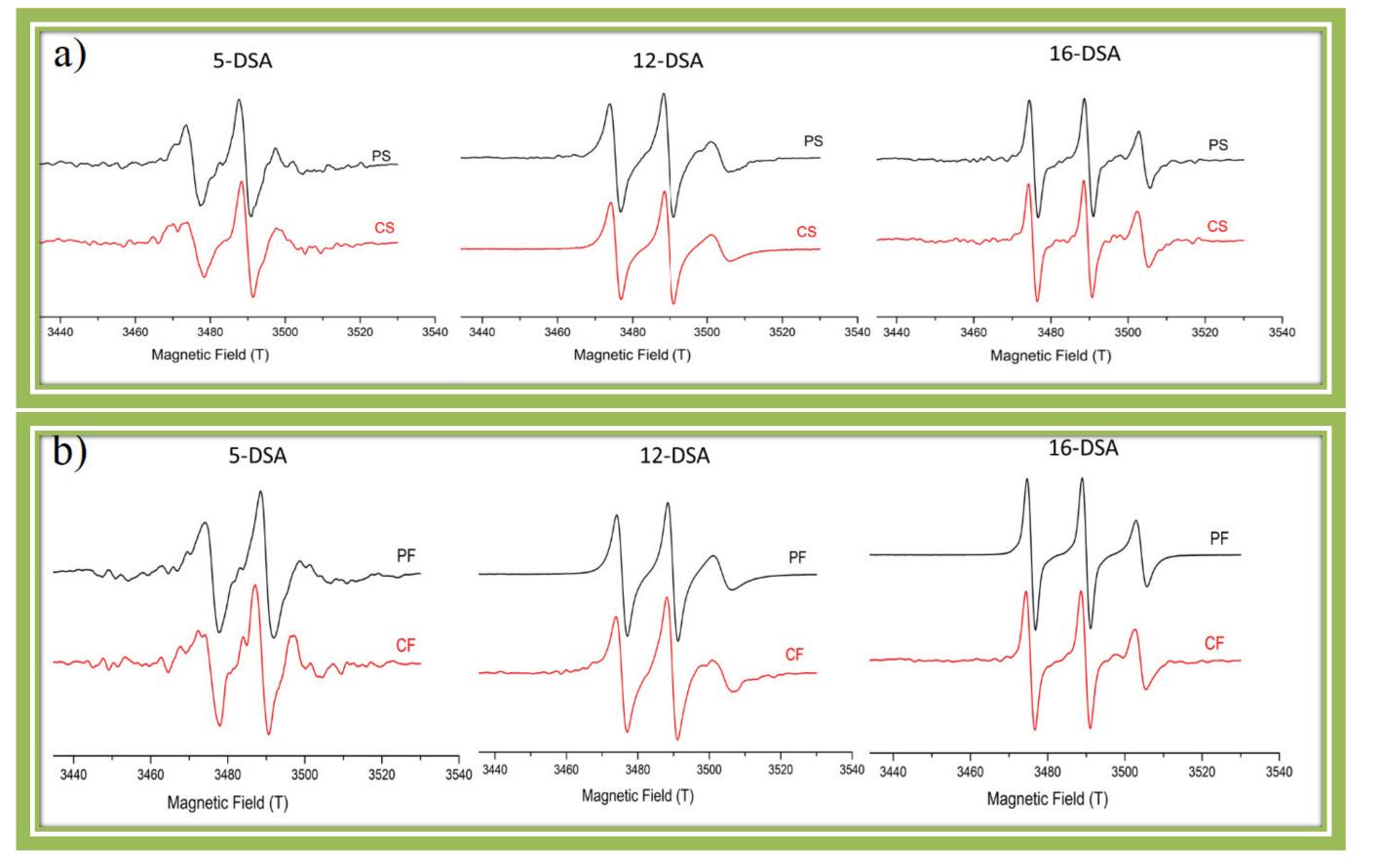


Table 1. Rotational correlation time (τR), order parameter (S), and isotropic hyperfine coupling constant (αN) of 5-DSA, 12-DSA, and 16-DSA in empty and loaded NEs with soybean (PS, CS) and fish oil (PF, CF).

Spin-probes	EPR values	Formulations			
		PS	CS	PF	CF
5-DSA	τR (ns)	2.18 ± 0.60	1.66 ± 0.61	1.19 ± 0.10	2.96 ± 0.81
	S	0.22 ± 0.04	0.29 ± 0.11	0.17 ± 0.04	0.26 ± 0.03
	αN (×10 ⁻⁴ T)	12.79 ± 0.37	13.14 ± 0.80	14.17 ± 0.38	12.79 ± 0.52
12-DSA	τR (ns)	1.95 ± 0.07	1.81 ± 0.06	1.63 ± 0.13	2.27 ± 0.19
	S	0.11 ± 0.001	0.11 ± 0.01	0.10 ± 0.01	0.13 ± 0.001
	αN (×10 ⁻⁴ T)	14.29 ± 0.55	14.08 ± 0.02	14.33 ± 0.10	14.33 ± 0.11
16-DSA	τR (ns)	0.63 ± 0.02	0.61 ± 0.03	0.55 ± 0.07	0.72 ± 0.07
	S	0.05 ± 0.001	0.05 ± 0.001	0.04 ± 0.001	0.04 ± 0.001
	αN (×10 ⁻⁴ T)	14.77 ± 0.02	14.64 ± 0.01	14.75 ± 0.05	14.67 ± 0.06

Figure 2. EPR spectra of the three spin-probes in (a) soybean and (b) fish oil NEs in the presence (red line) and absence (black line) of curcumin.

Figure 2 displays the findings of the EPR investigation, while Table 1 displays the computed values. Curcumin was localized in the stabilizing layer, as indicated by changes in τR values following the addition of the compound. The τR and the S values declined in the following order: 5-DSA, 12-DSA, and 16-DSA regardless of the oil type or the presence of curcumin, indicating a more rigid structure around the polar heads (Table 1). The stabilizing layer for both oil phases changed as a result of the addition of curcumin, as evidenced primarily by the changes in the τR values, which reduced from 2.18 \pm 0.60 to 1.66 \pm 0.61 ns and increased from 1.19 \pm 0.10 to 2.96 \pm 0.81 ns for CS and CF, respectively, for 5-DSA. Curcumin also had a more significant impact on the stability of the fish oil NEs, as evidenced by the rise in R values for all of the examined spin probes, with higher τR values indicating a more rigid structure.

REFERENCES

- 1. Nikolic, I. et al. Curcumin-loaded low-energy nanoemulsions: Linking EPR spectroscopy-analysed microstructure and antioxidant potential with in vitro evaluated biological activity. *J. Mol. Liq.* **2020**, *301*, 112479.
- 2. Griffith, O.H. and Jost, P.C. Lipid Spin Labels in Biological Membrane. In *Spin Labeling, Theory and Applications;* Berliner, L.J., Eds.; Academic Press: New York, NY, USA, 1976; pp
 454–484

ACKNOWLEDGMENT

This research was funded by the MESDT, Republic of Serbia through Grant Agreement with University of Belgrade-Faculty of Pharmacy No: 451-03-68/2022-14/200161 and supported by the Science Fund of the Republic of Serbia, GRANT No 7749108, - NanoCellEmoCog.