



*Dedicated to Prof. Ion Grosu  
on the occasion of his 70<sup>th</sup> anniversary*

## SYNTHESIS AND SOLUBILITY PROPERTIES OF SPIN-LABELED CURCUMIN

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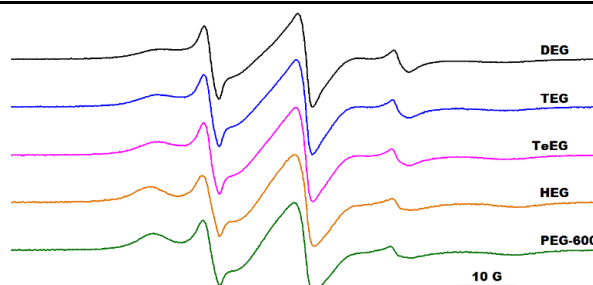
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Electron Paramagnetic Resonance (EPR) spectroscopy is a suitable method to characterize various systems using the parameters of free radicals. In this study we propose to analyze spin-labeled curcumin's behaviour in different solutions by EPR spectroscopy. In this regard, Pluronic ionic surfactants and oligoethylene glycols were used to evaluate the solubility characteristics of spin-labeled curcumin. The obtained EPR spectra have shown that spin-labeled curcumin in oligoethylene glycols leads to the formation of molecular aggregates which are more prone to exhibit a restricted internal motion. Solubilization of spin-labeled curcumin occurs by encapsulation in surfactant micelles which in EPR spectra is evidenced by the decrease of hyperfine splitting constants and the increase of rotational correlation time.



### INTRODUCTION

Curcumin is a natural polyphenol with diarylheptanoid structure (Fig. 1). There are numerous studies reported in literature referring to its pharmaceutical or therapeutic properties such as anti-inflammatory, antibacterial, anticancer, antioxidant, or wound healing.<sup>1–6</sup> The reactive methylene group can generate new derivatives of curcumin with a pronounced antitumor activity.<sup>7</sup> However, it is most likely that the high interest shown for this compound in the pharmaceutical field may stem from its wide use in food industry,

due to its properties as a coloring, spice, flavoring, or preservative agent.<sup>8</sup> Curcumin is extracted from turmeric plant, part of *Zingiberaceae* family, in mixture with other curcuminoid derivatives illustrated in (Fig. 1).<sup>9</sup>

Curcumin is a labile molecule that exhibits keto-enolic tautomerism depending on the pH value of the solution in which it is located.<sup>10</sup> The keto tautomer is mostly found in acidic or neutral conditions, while the enol tautomer is found in basic environmental conditions.<sup>6</sup> A drawback of curcumin relies in its poor solubility in water, of about 0.6  $\mu\text{g}/\text{mL}$ <sup>11</sup> which results in a reduced bioavailability.

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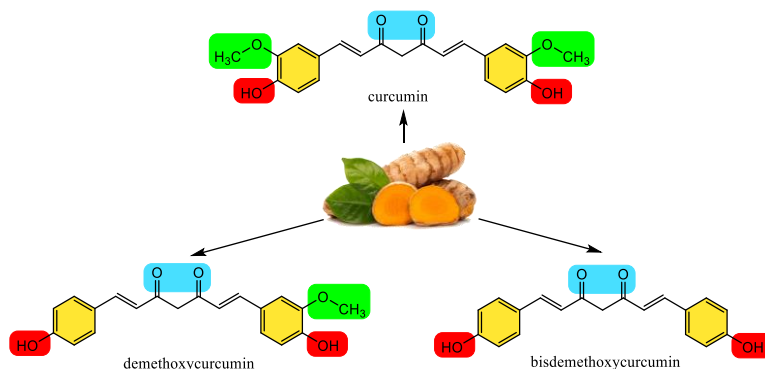


Fig. 1 – The three curcuminoids of turmeric plant.

Numerous studies<sup>12–14</sup> are devoted to improve this parameter either by derivatization of curcumin or by encapsulation in different molecular carrier systems. In line with this approach, a new spin labeled-curcumin (SLC) was synthesized and characterized and its behaviour in solutions of Pluronics (F127, 10R5 and 17R4), sodium dodecyl sulphate (SDS), diethylene glycol (DEG), triethylene glycol (TEG), tetraethylene glycol (TeEG), hexaethylene glycol (HEX) and polyethylene glycol-600 (PEG-600) has been investigated through Electron Paramagnetic Resonance (EPR) spectroscopy.

## RESULTS AND DISCUSSION

### Synthesis of (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dioxo-4-amino-2,2,6,6-tetramethylpiperidin-1-oxyl (SLC)

Spin-labeled curcumin was obtained by reaction of curcumin solubilized in glacial acetic acid with 4-amino-TEMPO under inert atmosphere according to the scheme presented in (Fig. 2). This procedure was applied by taking into account the high reactivity of the methylene group from the curcumin structure.

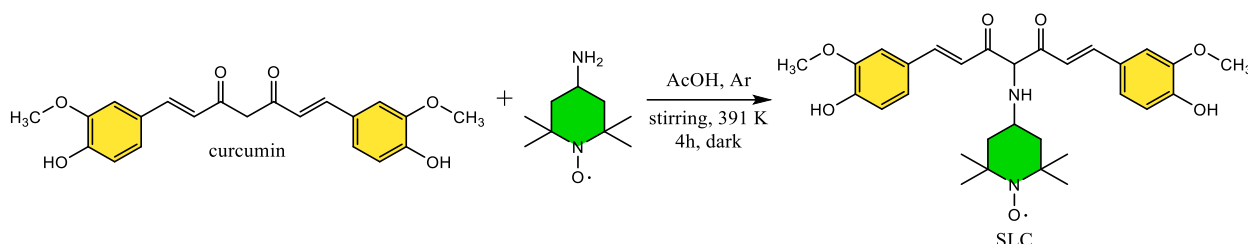


Fig. 2 – Synthesis of a newly spin labeled-curcumin.

Formation of SLC was confirmed by electrospray ionization-mass spectrometry (ESI-MS).

Although the SLC has shown a weak EPR signal

in water, a higher solubility in methanol was observed (Fig. 3). The EPR spectra of SLC in these solvents are similar, with the line at high magnetic field showing low intensity.

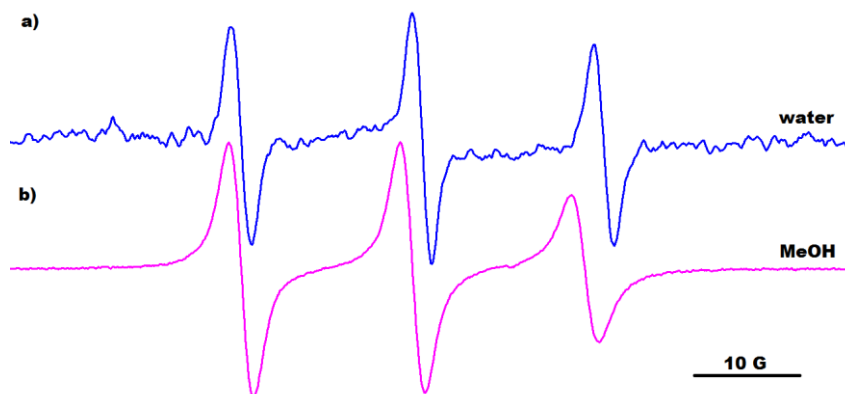


Fig. 3 – The EPR spectra of SLC in a) water and b) in methanol.

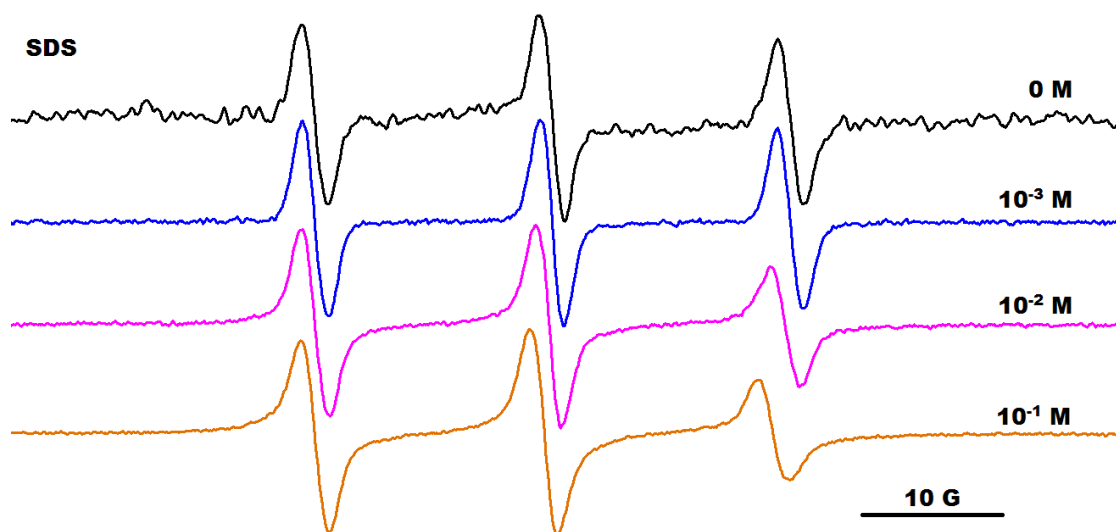
Along with the mass spectra that clearly demonstrate the obtaining of SLC, the characteristics of the EPR spectra of the new compound in water and methanol also support the SLC formation, taking into account that the lines in the high field are broader and less intense. As expected, the finding of hyperfine nitrogen splitting,  $a_N$  has higher value in water (17.1 G) compared to methanol (16.22 G) due to the higher polarity of water. The covalently attachment of TEMPO moiety to the curcumin backbone determines a slower dynamic both in water and methanol ( $\tau_{\text{water}} = 1.76 \times 10^{-10}$  s and  $\tau_{\text{methanol}} = 4.38 \times 10^{-10}$  s). The SLC has a lower solubility in water, compared with organic solvents such methanol or DCM. However, these solvents cannot be used in the formulation of curcumin for biomedical or food applications. Therefore, we have tested its behavior in ionic and nonionic surfactants and as well in oligoethylene glycols.

### The behaviour of SLC in ionic surfactants

Surfactants are often used to solubilize non-polar compounds in water at concentrations above the critical micelle concentration (CMC). Although the structure of curcumin has polar groups, its low solubility in water is due to the formation of intramolecular hydrogen bonds (*i.e.* between OH and methoxy groups attached to the phenyl rings). In this study, an anionic surfactant (sodium dodecyl sulfate – SDS) and a cationic surfactant (cetyl trimethyl ammonium bromide – CTAB) were used. The CMC of these surfactants have values of

approximately  $8 \times 10^{-3}$  M and  $10^{-3}$  M, respectively. It is important to mention that these values but these obviously also depend on the physico-chemical methods used to determine the critical micelle concentrations.<sup>15,16</sup> Moreover, the CMC values of surfactants increases in the presence of other compounds. In our experiments the SLC concentration is  $5 \times 10^{-4}$  M, while the surfactant concentrations are 10– 100 times higher. The aggregation numbers of CTAB and SDS in micelles are similar. For instance, literature survey shows values of 62 or 61 units/CTAB micelle,<sup>13</sup> and SDS are 68 units/ micelle.<sup>17</sup>

In Figure 4 are presented the EPR spectra of SLC at premicelle and above CMC of SDS (Fig. 4A) and CTAB (Fig. 4B). It can be noticed for both cases that the  $a_N$  of SLC decreases by increasing the surfactant concentration. Considering the data reported in literature at concentration of  $10^{-1}$  M ionic surfactants, the number of micelles is found above the concentration of SLC molecules. Therefore, at concentration between  $8 \times 10^{-3}$  M (CMC) and  $10^{-1}$  M in each system there is free SLC and SLC included in the micelle. As the exchange between free and included is fast, the EPR spectrum shows an average value. Another observation which can be made is that SLC experiences different environment polarities in SDS and CTAB micelles. This can be explained by different organization of SDS and CTAB micelles. While in the case of SDS a spherical shape of the micelles it is assumed, the shape of CTAB is rodlike<sup>18</sup> which can be an argument for smaller variation of  $a_N$  in CTAB micelles (16.84 G) compared with SDS (16.44 G).



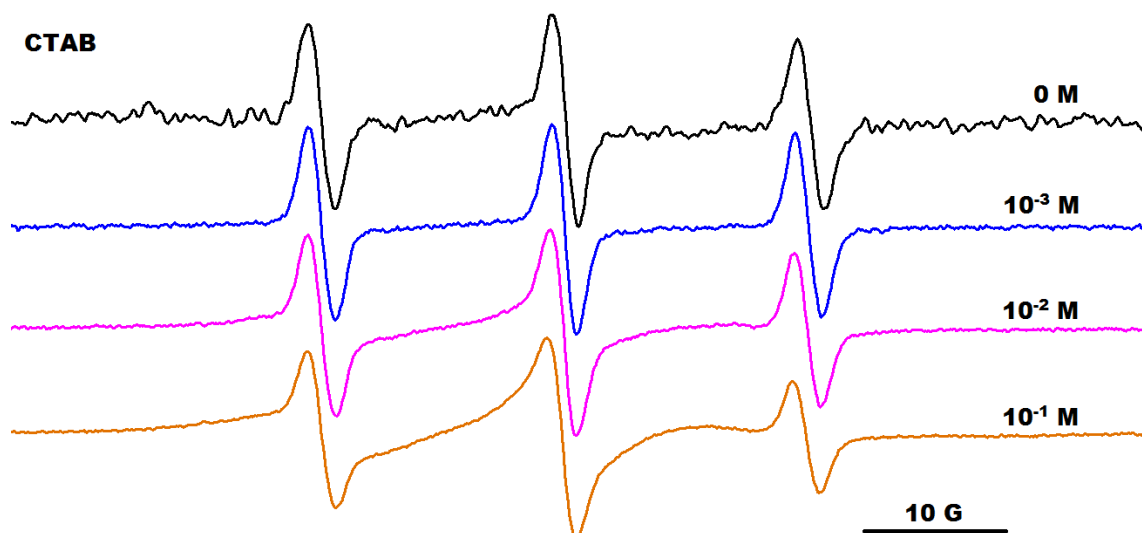


Fig. 4 – The EPR spectra of SLC in SDS and CTAB solutions.

The nonionic surfactants considered in this study are direct Pluronic F127 and two reverse Pluronics 10R5 and 17R4 in solutions of 3%, 10% and 20% concentrations. In the case of Pluronic F127 at 3% and 10% concentrations the micelles are formed due to dehydration of polypropylene glycol chains.<sup>19</sup> By increasing the concentration of F127 to 20% at room temperature the micelle to gel transformation occurs, a process that involves the dehydration of polyethylene chains which favor their entanglements.<sup>20</sup> Reverse Pluronics exhibit a  $(\text{PPO})_m\text{-(PEO)}_n\text{-(PPO)}_m$  configuration that makes the micellization process less favorable.<sup>21</sup> They can form various self-assembled aggregates, including flower-type micelles or a random network. This behaviour is due to the outer hydrophobic chain determined by PPO chains that can assembly exposing the hydrophilic chains of PEO to water environment. However, the assembly process is not likely to occur at room temperature.

F127 forms micelle in water at room temperature at 3 % and 10 % concentration,<sup>22</sup> while in the case of 20 % concentration forms a gel phase.<sup>23</sup> The EPR spectroscopy has been used to study phase transformation in Pluronic systems from sol to micelles and then to gel using various spin probes that can access different region in the micelles or gel phase. In this way it was proved that Pluronic micelles are characterized by a gradient of polarity between the more hydrophobic core delimited by PPO chains and the corona delimited by PEO chains.<sup>24-27</sup> In all Pluronic solutions the EPR parameters of SLC do not change significantly and the EPR spectra exhibit only one-component feature. In Table 1 are presented the  $a_N$  values of the spin probe SLC in Pluronic systems. The increase of the rotational correlation time ( $\tau$ ) in solutions of Pluronics are rather due to the viscosities properties.

Table 1

The EPR parameters of SLC in F127, 10R5, 17R4

Solution	water	F127			10R5			17R4		
Concentration %		3	10	20	3	10	20	3	10	20
$a_N$ (G)	17.08	16.92	16.90	16.84	16.93	16.90	16.80	17.05	16.93	16.84

The  $a_N$  values for the SLC presented in Table 1 indicate a gradual decrease with increasing concentrations of nonionic surfactant. However, this decrease is not very large (max 0.2 G), leading to the conclusion that the spin probes are located in hydrophilic regions of the micelles labeled by the PEG chains. This is explained taking into account

the SLC structure which has polar phenolic groups. In the case of nonionic reverse surfactants, the decrease in  $a_N$  is observed at 20% concentrations, which may be an indication of the dependence of self-aggregation processes on high concentrations. Correlational rotational time of SLC is another EPR parameter that may provide information on the

targeted region of the micelles Pluronics by SLC. It is observed that the spin probe motion is more

restricted in the gel phase of F127 occurring at 20% concentration.

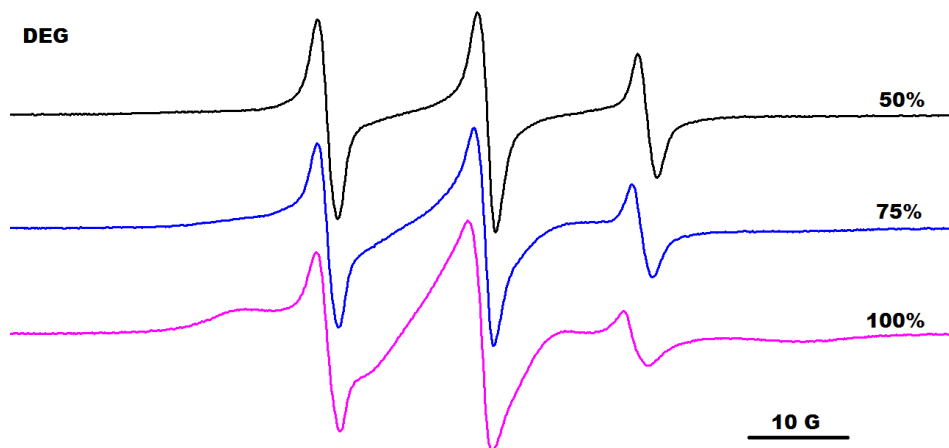


Fig. 5 – EPR spectra of the spin probe SLC in solutions with 50%, 75% and 100% concentration of diethylene glycol (DEG).

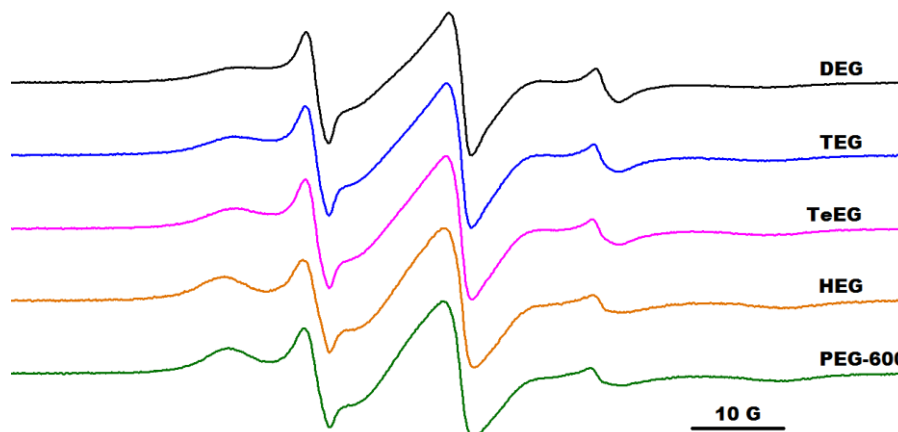


Fig. 6 – EPR spectra of the spin probe SLC recorded in diethylene glycol (DEG), triethylene glycol (TEG), tetraethylene glycol (TeEG), hexaethylene glycol (HEG) and polyethylene glycol 600 (PEG-600).

An interesting behaviour of SLC was observed in pure oligoethylene glycols and in water solutions (in 50% and 75% concentration). Oligoethylene glycols do not have self-assembly properties. Nevertheless, there are some studies reported in literature that prove the induction of oligoethylene assembly in the presence of hydrophobic molecules.<sup>28</sup> In solution of oligoethylene glycol with concentration less than 50% the EPR spectra exhibit only a one-component feature. As concentration of oligoethylene glycol increases, The EPR spectra of SLC exhibit two-component feature which proves the inhomogeneities of these systems. Ethylene glycol units are hydrated, and in diluted solution prevent self-association. In concentrated solution, SLC can induce locally the formation of aggregates that contain oligoethylene glycol molecules and SLC. This is translated by the

appearance of the second, highly immobilised component in the EPR spectra. This is exemplified in the (Fig. 5) that shows the EPR spectra of SLC in diethylene glycol (DEG) solutions and pure DEG. The degree of immobilization slightly increases with the length of oligoethylene chain. Thus the  $A_{zz}$  values are: 59 G (DEG), 58.8 G (TEG), 59.7 G (TeEG), 62 G (HEG) and 61.2 G (PEG600). The spectra showing two-component feature were simulated using EasySpin and NLSL programs. Simulation of the EPR spectra shown in (Fig. 6) provided the ratio between the component with fast motion and the component with highly restricted motion for SLC dissolved in oligoethylene glycols. In the same time, the values of the rotational correlation time of the immobilized component increases with the length of the oligoethylene chain (Table 2).

Table 2

The values of the rotational correlation time of the two components of SLC EPR spectra and the corresponding ratio of the two components

System	Fast component		Slow component	
	$\tau \times 10^{10}$ (s)	%	$\tau \times 10^9$ (s)	%
DEG	7.29	8.2	$5.95 \times 10^{-9}$	91.8
TEG	7.78	8.9	7.04	91.1
TeEG	8.31	7	7.66	93
HEG	9.44	19	9.21	81
PEG600	9.53	19	13.8	81

## MATERIALS AND METHODS / EXPERIMENTAL

Curcumin (purity  $\geq 90\%$ ) was purchased from ROTH (Germany), 4-Amino-TEMPO, Pluronic 10R5, diethylene glycol (DEG), triethylene glycol (TEG), and hexaethylene glycol (HEG) were purchased from Sigma Aldrich (USA), sodium dodecyl sulphate (SDS), tetraethylene glycol (TeEG), and polyethylene glycol-600 (PEG600) were purchased from Alfa Aesar (USA), dichloromethane, acetic acid, and methanol were purchased from Chimreactiv (Roumania), and sodium bicarbonate was purchased from SC SILAN TRADING SRL (Roumania).

### Synthesis of spin-labeled curcumin

Curcumin (0.7 mmol) was dissolved in 3.5 mL of glacial acetic acid and heated in an inert argon atmosphere until complete dissolution. Then 4-amino-TEMPO (1 mmol) was added, and the mixture was refluxed at 391 K for 4 hours in a dark place. The monitoring was carried out through a silica gel plate, using a mixture of toluene:acetone in a molar ratio of 3:7 as eluent. The  $R_f$  value for the product of interest is 0.65. The crude mixture was neutralized with a saturated sodium bicarbonate solution to neutralize the glacial acetic acid and then washed with distilled water ( $\eta=55\%$ ). The product of interest was purified by extraction with dichloromethane ( $\eta=72\%$ ) and will hereafter be referred to as SLC. MS SLC = 537.3.

### Sample preparation

Stock solution of SLC spin probe ( $10^{-2}$  M) was prepared in methanol. Appropriate volume of this solution was evaporated and the spin probe was

redissolved in water to reach a concentration of  $6 \times 10^{-4}$  M, in the absence or in the presence of Reverse Pluronic 10R5 (3–20%), SDS ( $10^{-3}$  M –  $10^{-1}$  M), DEG, TEG, TeEG, HEX, and PEG-600 (50–100%).

### Instruments

The EPR spectra were recorded on an X-band JEOL FA100 EPR spectrometer equipped with a cylindrical type of resonator TE011. The following parameters were used for measurements at room temperature: frequency modulation 100 kHz, microwave power 0.998 mW, modulation amplitude 0.5 G, sweep width 150 G, sweep time 4 min, time constant 0.1 s.

The EPR spectra were simulated with the NLSL<sup>29</sup> software to obtain the proportions and Easyspin software<sup>30</sup> using the garlic core function for isotropic and fast-motion continuous wave EPR spectra, and chili core function for tumbling spin systems in the slow-motional regime.

Mass spectra were recorded on a 310-MS Varian triple quadrupole mass spectrometer fitted with an electrospray ionization interface.

## CONCLUSION

In this study, the behaviour of spin-labeled curcumin in ionic and nonionic surfactants and as well as in oligoethylene glycols was evidenced by EPR measurements. The results shown that SLC can be uploaded in ionic micelles and in the aggregates of non-ionic surfactants at high concentration (20%). Analysis of the EPR parameters in Pluronic systems shows that SLC targets the hydrophilic region of the micelles or gels, as changes in  $a_N$  are not significant in these systems. An interesting

result is represented by the behaviour exhibited in concentrated solution or pure oligoethylene glycols as the EPR spectra of SLC have two-component feature. One component is characterized by a cvasiisotropic motion explained by the high viscosity of these solutions. The immobilized component stands for the existence of oligoethyne glycols probably induced by the SLC. These results can be a starting point in finding suitable systems carrying the curcumin derivatives.

## REFERENCES

1. J. Lal, S. K. Gupta, D. Thavaselvam, D. D. Agarwal, *Eur. J. Med. Chem.*, **2013**, *64*, 579–588.
2. S. Mishra, K. Karmodiya, N. Suroliab, A. Surolia, *Bioorg. Med. Chem.* **2008**, *16*, 2894–2902.
3. L. Ding, S. Ma, H. Lou, L. Sun and M. Ji, *Molecules*, **2015**, *20*, 21501–21514.
4. K.S. Parvathy, P.S. Negi, P. Srinivas, *Food Chem.*, **2010**, *120*, 523–530.
5. A. de Moura, C. Gaglieri, L. C. Silva-Filho, F. J. Caires, *J. Therm. Anal. Calorim.*, **2021**, *146*, 587–594.
6. A. Celebioglu, T. Uyar, *Food Chem.*, **2020**, *317*, 126397.
7. I. Ali, A. Haque, K. Saleem, M. F. Hsieh, *Bioorg. Med. Chem.*, **2013**, *21*, 3808–3820.
8. S. Winter, N. Tortik, A. Kubin, B. Krammer, K. Plaetzer, *Photochem. Photobiol. Sci.*, **2013**, *12*, 1795–1802.
9. X.-Y. Xu, X. Meng, S. Li, R.-Y. Gan, Y. Li, H.-B. Li, *Nutrients*, **2018**, *10*, 1–33.
10. B. Zheng, D. J. McClements, *Molecules*, **2020**, *25*, 1–25.
11. B. T. Kurien, A. Singh, H. Matsumoto, R. H. Scofield, *Assay Drug Dev Technol*, **2007**, *5*, 567–576.
12. T. Chandrasekar, N. Raman, *J. Mol. Struct.*, **2016**, *1116*, 146–154.
13. M. Chaudhary, N. Kumar, A. Baldi, R. M. Chandra, A. Babu, J. Madan, *J. Biomol. Struct. Dyn.*, **2020**, *38*, 200–218.
14. P. Z. Li, Z.-Q. Liu, *Eur. J. Med. Chem.*, **2011**, *46*, 1821–1826.
15. A. Cifuentes, J. L. Bernal, J. C. Diez-Masa, *Anal. Chem.*, **1997**, *69*, 4271–4274.
16. S. P. Moulik, Md. H. Haque, Emdadul, P. K. Jana and A. R. Das, **1996**, *100*, 701–708.
17. V. P. Arkhipov, R. V. Arkhipov, N. A. Kuzina, A. Filippov, *MagnReson Chem.* **2021**, *59*, 1126–1133.
18. M. A. Bahri, M. Hoebeke, A. Grammenos, L. Delanaye, N. Vandewalle, A. Seret, *Colloids Surf., A*, **2006**, *290*, 206–212.
19. P. Alexandridis, T. A. Hatton, *Colloids Surf. A: Physicochem. Eng. Asp.*, **1995**, *96*, 1–46.
20. B.K. Lau, Q. Wang, W. Sun, L. Li, *J. Polym. Sci. Part B Polym. Phys.*, **2004**, *42*, 2014–2025.
21. E. Larraneta, J. R. Isasi, *Langmuir*, **2013**, *29*, 1045–1053.
22. F. E. Antunes, L. Gentile, C. O. Rossi, L. Tavano, G. A. Ranieri, *Colloid Surf. B: Biointerfaces*, **2011**, *87*, 42–48.
23. C. Chaibundit, N. M. P. S. Ricardo, F. de M. L. L. Costa, S. G. Yeates, C. Booth, *Langmuir*, **2007**, *23*, 9229–9236.
24. A. Caragheorgheopol, H. Caldararu, I. Dragutan, H. Joela, W. Brawn, *Langmuir*, **1997**, *13*, 6912–6921.
25. H. Caldararu, A. Caragheorgheopol, M. Vasilescu, I. Dragutan, H. Lemmetyinens, *J. PhysChem*, **1994**, *98*, 5320–5331.
26. R. Shvartzman-Cohen, I. Monje, M. Florent, V. Frydman, D. Goldfarb, R. Yerushalmi-Rozen, *Macromolecules*, **2010**, *43*, 606–614.
27. M. Micutz, E. Matalon, T. Staicu, D. Angelescu, A. M. Ariciu, A. Rogozea, I. M. Turcu, G. Ionita, *New J. Chem.*, **2014**, *38*, 2801–2812.
28. A. Azri, P. Giamarchi, Y. Grohens, R. Olier, M. Privat, *J. Colloid Interface Sci.*, **2012**, *379*, 14–19.
29. D. E. Budil, S. Lee, S. Saxena, J. H. Freed, *J. Magn. Reson.*, **1996**, *120*, 155–189.
30. S. Stoll, A. Schweiger, *J. Magn. Reson.*, **2006**, *178*, 42–55.

