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IN THE FIELD OF CHEMISTRY

THERMOSENSITIVE SYSTEMS STUDIED BY ELECTRON SPIN RESONANCE SPECTROSCOPY AND CALORIMETRIC METHODS

SUMMARY OF Ph.D. THESIS

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KEY WORDS

- ESR spectroscopy;
- microcalorimetry;
- *"host-guest"* complexes;
- thermosensitive systems.

INTRODUCTION

Since the beginning of the 21st century, Electron Spin Resonance Spectroscopy (ESR spectroscopy) has undergone considerable development, both in terms of instrumentation and in terms of the valuable information that this method can provide to the field of physical-chemical research. Over the years, the pharmaceutical industry has been confronted with problems related to solubilization or controlled release of some active substances, which is why research directions have been focused on the development of methods to decrease drug lipophilicity and controlled release to optimize bioavailability. One method is the formation of *”host-guest”* systems. Some categories of chemical compounds used as *”host”* molecules are cyclic or acyclic oligosaccharides or cationic, anionic or non-ionic surfactants, while *”guest”* molecules must have the property of interacting physically, through non-covalent bonds with the *”host”* molecules forming molecular inclusion complexes that may be thermosensitive systems.

In the Institute of Physical Chemistry - Ilie Murgulescu of the Romanian Academy there are two research groups: one focused on *"Quantum Chemistry and Molecular Structure"* and the other focused on *"Chemical Thermodynamics"*. The first department focuses on molecular and structural studies of biologically relevant systems, while the second department focuses on the influence of the binding of bioactive compounds, such as polyphenols, on the chemical behavior of proteins with applications in the food and pharmaceutical fields.

Thus, in this thesis, we aimed to combine two techniques, a spectroscopic and a calorimetric one, to investigate thermosensitive systems with possible biomedical applications, since ESR spectroscopy can provide information about the dynamics of paramagnetic species in different systems, considering that spectral parameters are temperature and local interactions dependent. At the same time, calorimetric methods can provide global data on the thermal effects that accompany the processes occurring in a system as a result of temperature change. The two methods therefore complement each other in the information they provide on the molecular systems under study. Thus, the Ph.D. thesis focuses on the study of *"Thermosensitive systems studied by Electron Spin Resonance Spectroscopy and calorimetric methods"*.

PART I. THE CURRENT STATE OF KNOWLEDGE

CHAPTER 1. MOLECULAR SYSTEMS WITH THERMOSENSITIVE PROPERTIES

Thermosensitive systems can be categorized as *"smart"* materials [1] which have application in the biomedical field by their use as drug carriers and controlled release of active ingredients from a pharmaceutical formulation, ensuring bioavailability of drugs by increasing the solubility of drugs that have a marked lipophilic character [2]. Thermosensitive molecular systems could also help to decrease non-specific biodistribution, i.e. the drug to reach directly to the desired tissue where to be released to avoid other adverse effects [3].

The exposure of smart materials to physical stimuli such as temperature and chemical stimuli such as p*H*, ionic strength, involves changes in their initial properties as a response of the environment to stimuli from outside [4]. The responses consist in the possibility of changes in molecular conformation, and hence shape, water solubility and *sol-gel* transition [5], [6]. In particular, the properties of thermosensitive systems will be modified by temperature variation, but this is not the only factor.

The phase transition of the system from the stressed to the initial state involves a relaxation by removal of the stimulus that generated the change at the molecular level. The reversible effect is also known as the memory effect [7]. Thermosensitive systems may include micelles, supramolecular gels or biphasic materials that generate hydrogels. The generated systems contain amphiphilic, pluronic-type polymeric surfactants in which *sol-micelle* [8], [9] or *micelle-gel* [10], [11] transitions can be observed.

Encapsulation of molecules with the formation of *„host-guest"* systems in supramolecular chemistry is another type of thermosensitive system. It is based on the complexation of two molecules by the formation of non-covalent interactions between them, in the absence or presence of external stimuli [12]. The formation of these complexes is the result of several weak interactions, of physical nature among which can be mentioned ion-ion, ion-dipole, dipole-dipole, *van der Waals*, hydrogen bonding, etc., involving steric effects in many cases [13]. The study of these systems can be performed using spectroscopic and calorimetric methods, and the Ph.D. thesis is centered on *"Thermosensitive systems studied by electron spin*

resonance spectroscopy and calorimetric methods". References to the two techniques used to develop this Ph.D. thesis will be given below.

Spin Electron Resonance Spectroscopy (ESR) is used to obtain information on the polarity of the microenvironment surrounding the paramagnetic fragment and the dynamics of the latter (**Figure 1.1.A**) for a nitroxide radical [14].

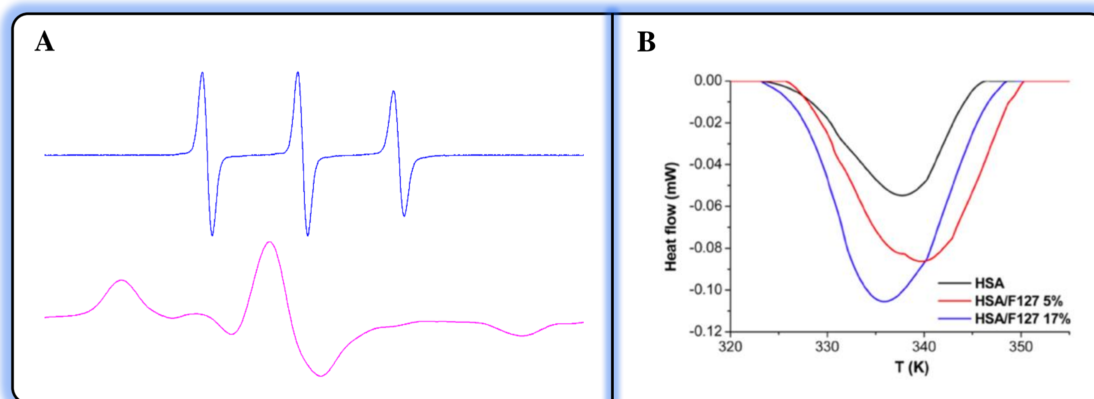

Figure 1.1.A: ESR spectra of a fast-moving (blue line) and restricted-moving (pink line) nitroxide-type free radical [14]. **B:** μDSC thermograms corresponding to protein denaturation of HSA in the absence and presence of *F127* at different concentrations [15].

Differential Scanning Microcalorimetry (μDSC) is a technique that complements the ESR method in the data it provides, as it provides both individual and interaction information on a mesoscopic scale for a thermosensitive system. One such example is the system consisting of serum protein and the block-copolymer Pluronic *F127*. The corresponding thermograms of Human Serum Albumin (HSA) in the absence and in the presence at two different concentrations of the polymer indicate different effects of Pluronic *F127* concentration on protein denaturation. Thus, at low concentration, HSA is stabilized (peak temperature has higher value) (**Figure 1.1.B**) [15]. While the μDSC method provides global information on the analyzed systems, ESR spectroscopy provides local information on the microenvironment of paramagnetic species in the analyzed systems [14].

In this thesis, a number of systems that are thermosensitive have been analyzed and studied either by ESR spectroscopy or by μDSC or using both techniques. Thus, protein complexes, *"host-guest"* complexes, self-assembly of ionic or nonionic surfactants were analyzed.

CHAPTER 2. ELECTRON SPIN RESONANCE SPECTROSCOPY

Paramagnetic species can be used in studies of phase transitions occurring in block-copolymer systems, due to the sensitivity of the hyperfine coupling constant of the nitrogen atom (a_N) as well as the correlated turnover time (τ_c) to changes in the microenvironment around the free radical induced by dehydration and self-assembly processes of polymer chains.

An example of a ESR study using the spin probe method concerns the use of two spin probes, *CAT16* and 5-*DSA*, which can bind to HSA sites, the formed system being sensitive to phase transitions of the Pluronic *F127* block-copolymer. Both free radicals have different affinities for HSA, but also for *F127* micelle, and the distortion of the spectral lines that occur indicates the types of interaction in these systems. In micellar solutions of Pluronic *F127*, the *CAT16* probe exhibits fast motion, whereas the 5-*DSA* probe exhibits slower motion [15].

The *"spin labeling method"* can be used to investigate by ESR spectroscopy the self-assembly behavior of amphiphilic copolymers in aqueous solution and the internal conformation of polymer aggregates. Mirjam *et al.* investigated the ability of encapsulation of an active substance using 4-amino-*TEMPO*-functionalized copolymers in the presence and absence of the surfactant lecithin-cholesterol. The variation of the molar ratio of lecithin and cholesterol does not influence the ESR parameters of the spin-labeled polymer [16].

ESR spectroscopy is often used in the study of the complexation of cyclodextrins (CDs) with nitroxides, as thermodynamic and structural information of the formed systems is obtained. This is possible due to the spectral changes that occur as a result of the *"host-guest"* interaction in the system. The decrease in the intensity of the high-field line and its broadening indicates the formation of the *"host-guest"* complex. Therefore, the value of τ_c is larger, indicating a slower rotation. The study of *"host-guest"* interactions at varying temperatures provides information on the dynamics and orientation of the paramagnetic group in the complex [17]. Chemical modification of CDs by their functionalization with alkyl radicals leads to the increase in the hydrophobic character of natural CDs. This aspect can be highlighted by ESR using spin probes (*TEMPO* and *DOXYL*) with variable hydrocarbon chain. The chemically modified CDs generate a more nonpolar microenvironment around the paramagnetic fragments resulting in a decrease in a_N value [18].

CHAPTER 3. THERMAL ANALYSIS. DIFFERENTIAL SCANNING MICROCALORIMETRY

The effect of the interaction between an organic ligand and a protein are examples of thermosensitive studies investigated by μDSC, as they provide global information about the systems under study. The stability of a protein can be explained by its thermal behaviour. The thermal denaturation of a protein with a native structure consists in its unfolding process.

The two essential parameters of a DSC thermogram are the denaturation temperature (T_m) and the denaturation enthalpy (ΔH) [19].

The presence of the ligands in the protein solution and their binding to BSA bring changes in the DSC thermograms by shifting T_m towards higher values. In other words, the ligand plays a protective role on the thermal denaturation of the protein by stabilizing it. The solvent may or may not contribute to the destabilization of the protein through the non-covalent interactions that can be achieved. Hydrophobic interactions or hydrogen bonding can be established to act as a co-factor on protein unfolding [19].

The thermogram corresponding to protein denaturation of BSA shows two corresponding values T_{m1} and T_{m2}. The lower value of T_{m1} can be attributed to protein unfolding due to a weak interaction with the ligand. The higher value of T_{m2} can be attributed to the domain of the protein being more stable. Quercetin is used as a ligand and can bind either to the native protein structure or to the already unfolded protein structure. By increasing the ligand:protein molar ratio an increase in the T_{m1} value and a decrease in the T_{m2} value was observed. This can be explained by the hydrophobic interaction of the quercetin molecule at the BSA binding site I. The neutral quercetin molecule transforms into an anion that can electrostatically interact with the amino acid residues in their cationic form and thus the electrostatic repulsions at site II are minimized leading to better stabilization of the protein [19].

Another μDSC study concerns the effect of gallic acid binding to BSA. Both in the presence and absence of gallic acid, the protein denaturation is irreversible. However, it is the ligand:protein ratio that is essential, because increasing gallic acid concentration causes a shift of T_{m1} and ΔH toward higher values. Even at an equimolar ratio, the protein is stabilized, in contrast, at a 25-fold higher ligand ratio, the stabilization effect is significant [20].

PART II. PERSONAL CONTRIBUTIONS PART (ORIGINAL)

CHAPTER 4. INTERACTION OF THE FLAVONOID MORIN WITH BOVINE SERUM ALBUMIN

In this study, BSA protein thermal denaturation experiments were performed using μDSC7 evo (Setaram), followed by data collection and processing using software provided by the instrument manufacturer, Calisto v.1077, using a linear baseline, with data converted to excess heat capacity using BSA concentration and working cell volume. With the PeakFit software we performed the decomposition of the excess heat capacity profiles. Thus, we determined the thermodynamic parameters of the denaturation process: T_m and ΔH. To evaluate the effect of Morin binding on the thermal stability of BSA we found that both the use of a low concentration of 1% DMSO, which plays a role in Morin solubilization, and the presence of the polyphenol lead to stabilization of the native state of the protein when thermally modulated. Thus, BSA unfolding occurs at higher temperatures in the presence of DMSO, as a stabilizing effect of the BSA structure is induced. Likewise, systems containing ligand:protein in molar ratios of 1:1 and 10:1, respectively, lead to a thermally stabilizing effect for BSA, with the best influence being in the presence of a higher concentration of Morin (**Figure 4.1.**).

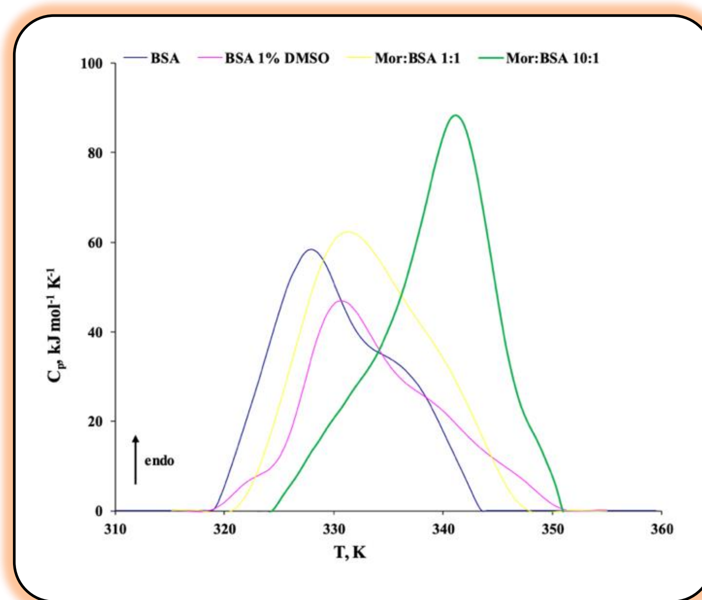

Figure 4.1. DSC thermograms for the thermal denaturation of BSA in the absence (blue) and in the presence of Morin (yellow, 1:1 ratio) and green, 10:1 ratio), 1% DMSO (pink).

CHAPTER 5. INTERACTION OF SPIN-LABELED 4-PHENOXYANILINE WITH BSA IN WATER AND IN TREHALOSE SOLUTION

In this study the molecule 4-phenoxyaniline was spin-labeled with 4-carboxy-TEMPO to obtain *4FA_4CT*, a process involving the synthesis, processing, and purification of the free radical. ESR measurements were carried out using a JEOL FA100 X-band spectrometer equipped with a TE011 cylindrical resonator and a temperature control unit. BSA samples were prepared in water or trehalose in the presence and absence of *4FA_4CT* and recorded using a μDSC7 evo (Setaram). Data processing was done using Calisto v.1077 software, the experimental DSC profiles were decomposed as a sum of two independent two-state transitions using PeakFit. In the case of the effect of trehalose on the freezing behavior of β-CD and albumin solutions, it was found that at low temperatures, in the absence of trehalose, the spin probe exhibits a spectrum in which the freezing phenomenon is associated with free radical precipitation. The presence of trehalose plays a protective role and leads, under the same conditions, to a strongly restricted motion of the *4FA_4CT* probe but is not associated with the precipitation of the radical species. Concerning the analysis of the interaction of *4FA_4CT* with β-CD the use of trehalose was done in order to highlight the effect of disaccharide on the complexation process between *4FA_4CT* with β-CD. Since there are no changes of a_N, this aspect denotes that the paramagnetic fragment is not included in the hydrophobic cavity of β-CD, instead the increase of β-CD concentration leads to the increase of τ_c values of the probe which implies the formation of the *"host-guest"* complex of the *4FA_4CT* probe with β-CD.

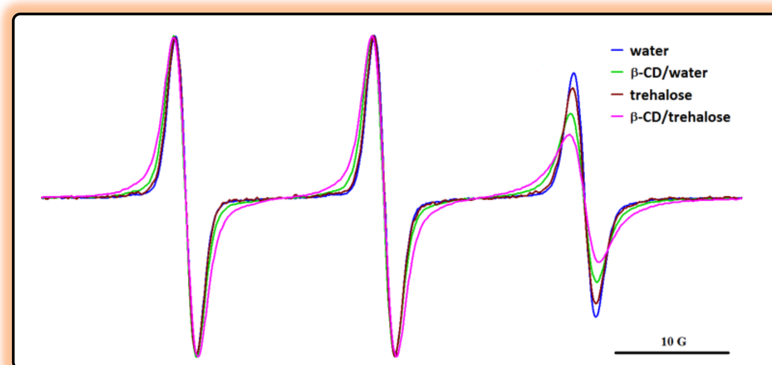

Figure 5.1. ESR spectra of the *4FA_4CT* spin probe in the absence and presence of β-CD, in water and in 20% trehalose solution; [β-CD] = 10^{-2} M.

On the interaction of spin-labeled 4-phenoxyaniline with BSA, it was observed that the spin probe motion is less restricted in BSA than in β-CD. The spectral component corresponding to a spin probe strongly immobilized in BSA disappears in the presence of β-CD, which proves the removal of the spin probe from the protein complex by formation of *"host-guest"* complex with β-CD. The effect of trehalose on the thermal stability of albumin solutions during heating consists in a protective role of the disaccharide on the protein leading to an increase in the denaturation temperature of BSA in the presence of trehalose compared to its absence. The presence of *4FA_4CT* causes a destabilizing effect on the protein due to binding to the BSA binding site. This binding causes the protein unfolding process to occur at lower temperature values. In contrast, upon addition of trehalose, it inhibits the interaction between the probe and BSA leading to higher values of the denaturation temperature.

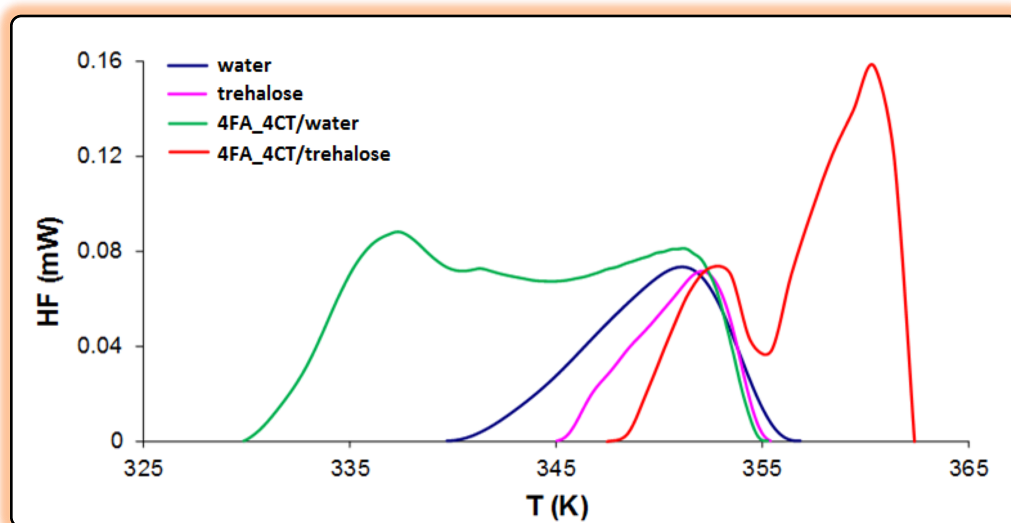

Figure 5.2. μDSC thermograms of the thermal denaturation of BSA in water and in 20% trehalose solution, in the absence and in the presence of the *4FA_4CT* probe.

Also, using circular dichroism we could show the influence of temperature on the circular dichroism spectra of BSA in the four investigated systems. The presence of trehalose does not modify the secondary structure of BSA. Upon heating, a similar behaviour of BSA/water and BSA/trehalose 20% solutions is observed, i.e., a 41- 46% loss of the helical structure. However, upon cooling the samples to 298 K, the recovery of the native secondary structure of BSA is significantly higher in trehalose (84% versus 72% in water). Binding of the *4FA_4CT* ligand to BSA results in a 3% decrease in the α-helix content of the protein, irrespective of the presence of trehalose.

CHAPTER 6. PHYSICOCHEMICAL STUDY ON THE MOLECULAR ORGANIZATION IN SYSTEMS CONTAINING DIRECT OR REVERSE PLURONICS. THE EFFECT OF HYALURONIC ACID

In this study, microcalorimetric measurements were performed obtaining thermodynamic parameters characterizing the micellization process (T_{onset}, T_{peak}, ΔH) of Pluronic in the absence and in the presence of hyaluronic acid (*HA*). It was observed that in the case of the direct Pluronic, *F127*, micelle formation is due to hydrophobic association of PPO blocks, temperature being a factor influencing the micellization of *F127*, as PPO chains exposed to water tend to associate to form micelles. Two endothermic peaks are obtained, one attributed to micellization and one to gelation. The micelle-gel transition can be explained by dehydration of the PEO chains at a temperature above that of micellization. Reverse Pluronic also show two endothermic peaks indicating the formation of molecular aggregates. In the presence of *HA*, the peak temperature of *F127* increases slightly, whereas in the situation of the reverse Pluronic, *10R5* and *17R4*, the presence of *HA* leads to a decrease in the peak temperature indicating favoring the micellization process.

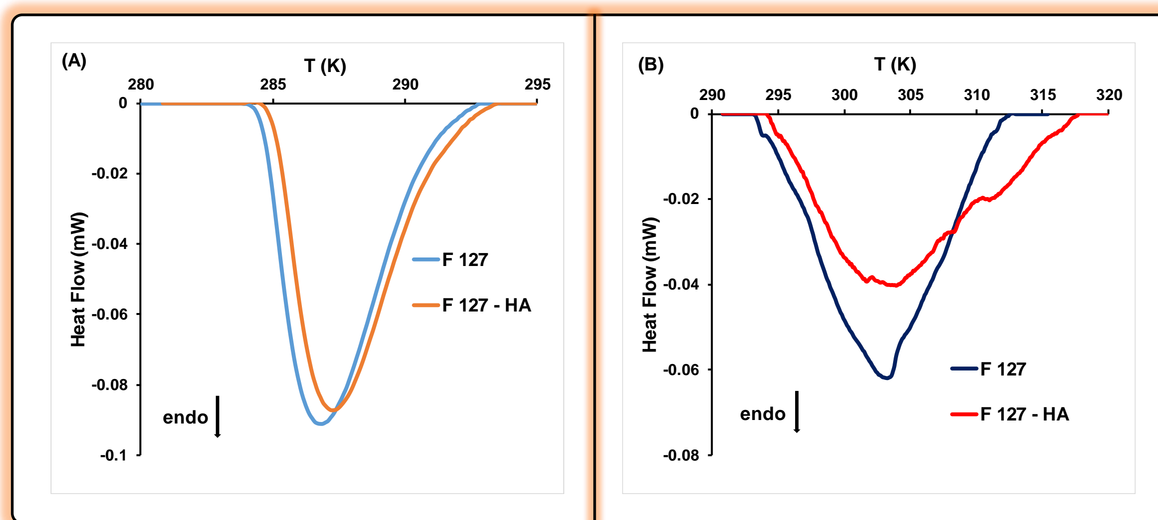

Figure 6.1. μDSC thermograms for micellization (**A**) and gelation (**B**) of Pluronic *F127* (20%) in the absence and presence of *HA*.

To investigate phase transitions in Pluronic using ESR spectroscopy, four spin probes with distinct molecular structures favouring their localization in different regions of the formed micelles/aggregates were used.

In the Pluronic *F127* and Pluronic *F127+HA* systems, the mobility of the 5-*DSA* spin probe has a strongly restricted motion indicating its localization within the micelles, both in the absence and presence of *HA*. The use of the *CAT16* probe leads to a different localization of the probe because the dynamics of the probe is much faster compared to 5-*DSA*. The use of the L62NO spin probe did not lead to record an influence of *HA* in the investigated system. Also the presence of *HA* does not lead to any visible effect in the system containing the C12NO molecular spin probe.

For the Pluronic *10R5* and Pluronic *10R5+HA* systems no probes showed significant changes in spectral parameters, as the *10R5* chain size influences the shape of the micelles as well as the interaction of the probes with the molecular aggregates. The 5-*DSA* probe is sparingly soluble in *10R5*, but the presence of *HA* slightly increases the solubility of the probe in the investigated system, not influencing the spectral parameters of the spin probe. The ESR spectra of the *CAT16*, L62NO and C12NO spin probes are similar both in the absence and presence of *HA*.

For Pluronic *17R4* and Pluronic *17R4+HA* systems containing 5-*DSA*, the presence of *HA* shows a major influence on the spin probe spectral parameters (**Figure 6.1.A.**).

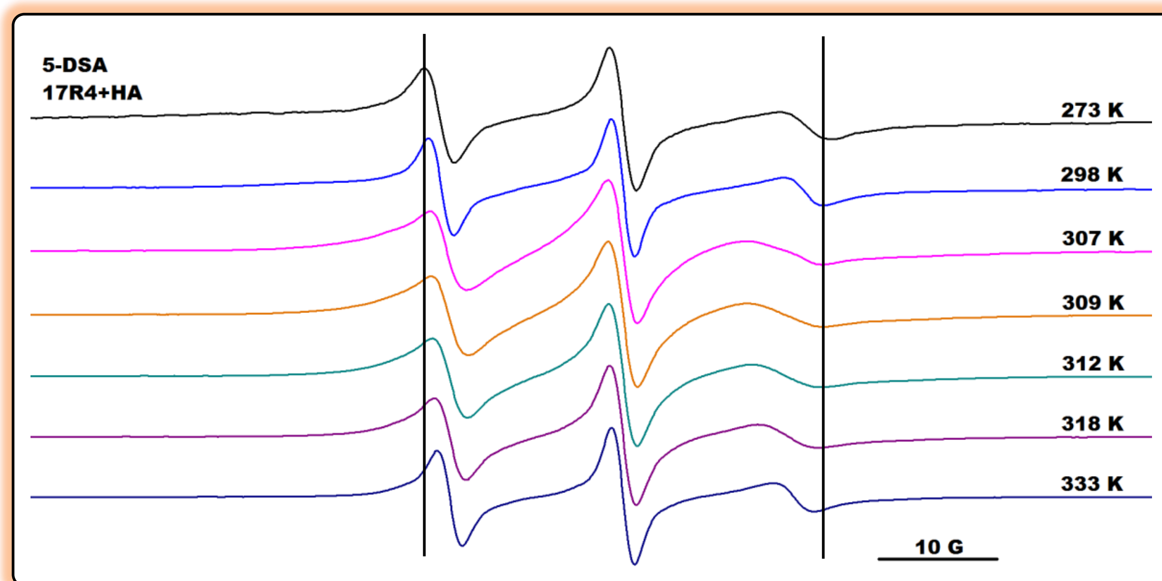

Figure 6.1.A. ESR spectra of the 5-*DSA* spin probe in Pluronic *17R4* in the presence of *HA*.

In the absence of *HA* the ESR spectrum of the 5-*DSA* probe exhibits a strongly restricted motion at low temperatures, whereas in the presence of *HA*, the motion of the probe becomes faster (**Figure 6.1.B.**).

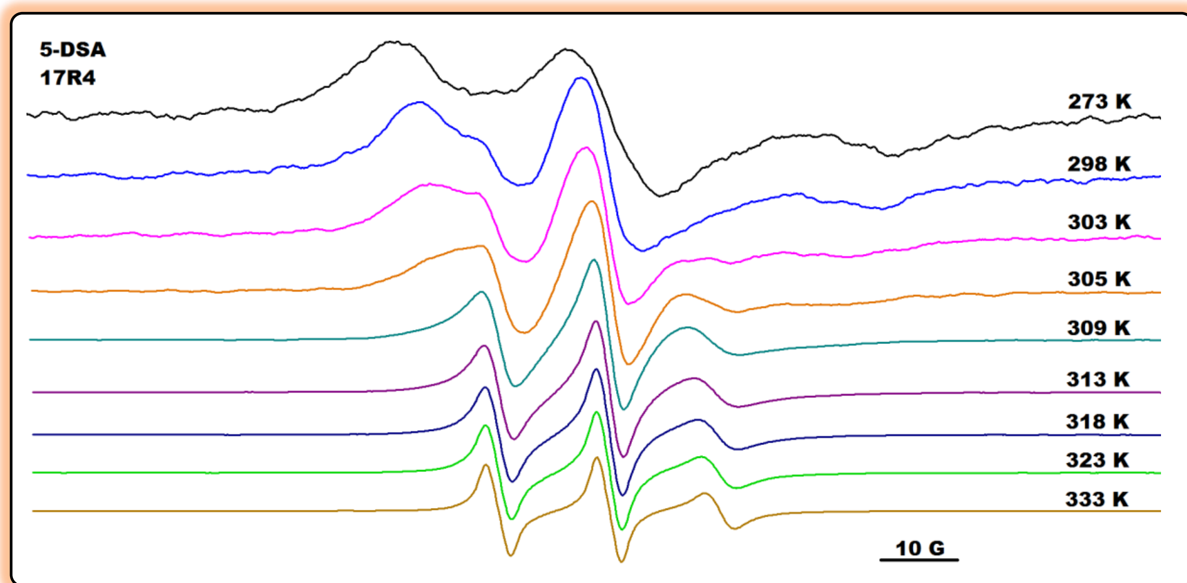

Figure 6.1.B. ESR spectra of the 5-*DSA* spin probe in Pluronic *17R4* in the absence of *HA*.

The dynamics of *CAT16*, L62NO and C12NO wells are fast and the presence of *HA* in the investigated systems does not lead to significant ESR parameter changing effects due to different well localization.

CHAPTER 7. *"HOST-GUEST"* COMPLEXES OF ALIPHATIC CHAIN FUNCTIONALIZED NITROXIDES AND CYCLODEXTRINS
β-PHOSPHORYLATED NITROXIDES

7.1. Conformational modifications of *β*-phosphorylated nitroxides - a basic tool in the study of *"host-guest"* interactions

New *β*-phosphorylated nitroxide-type molecular probes, two with cyclic (*S1*, *S2*) and two with acyclic (*S3*, *S4*) structures capable of forming molecular inclusion complexes with CDs, were used. The obtained ESR spectra were simulated with the WinSim software available from *NIEHS* using the LMB1 optimization algorithm and with the EasySpin software using the garlic function to describe a fast and isotropic motion of the paramagnetic species. The four spin probes exhibit ESR signal in water, and in the presence of CDs they change their characteristic parameters as a result of the non-covalent interactions that are established between the free radicals and the interior of the hydrophobic cavity of the *"host"* molecules.

S1 is partially included in *γ*-CD, as the ESR spectrum shows the presence of two species, a fast-moving species in solution and a slower-moving species, i.e. the CD complexed species. *S2* is sensitive in the presence of *α*-CD and *β*-CD, the change in the ESR spectrum reflecting the interaction of the paramagnetic fragment that is included in the two CDs. The flexibility of the *S3* and *S4* probes is due to the acyclic structure and the possibility of adopting a conformation that allows them to establish stronger non-covalent physical interactions with the interior of the CDs. In the case of *S4* in water, the probe presents a ESR signal consisting of six lines, while in the *S4/γ*-CD system the spectrum is significantly modified obtaining a spectrum consisting of 5 lines due to a full inclusion of the paramagnetic species in the *γ*-CD interiors (**Figure 7.1.**). The interactions that are established in the studied systems highlight the change in the relative orientation of the nitroxyl and phosphoryl groups that cause changes in the values of the coupling constants a_N and a_P. Following spectral simulation using WinSim software, a_N and a_P are not sensitive in the presence of *α*-CD, *β*-CD, and HPB, while the presence of *γ*-CD causes a decrease in the value of a_N, and the complexation phenomenon is dependent on the size of the CD cavity. The corresponding τ_c values were obtained by simulating experimental spectra using EasySpin software. In this study, competitive binding experiments were performed to reveal the affinity of *β*-phosphorylated nitroxides for the less polar cavity of CDs.

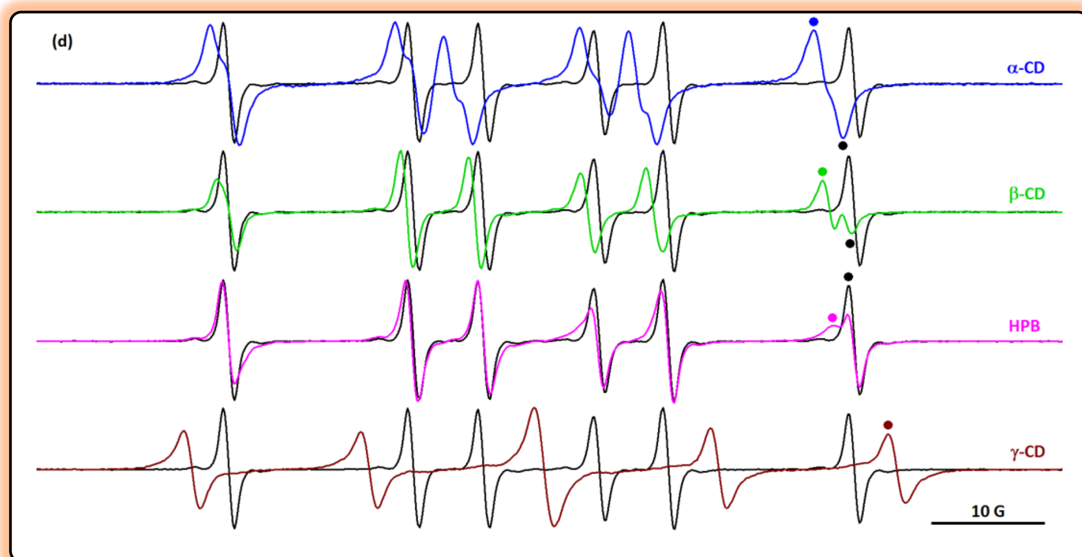

Figure 7.1. ESR spectrum of spin probe *S4* in the absence (black) and presence of cyclodextrin; spin probe concentration 2.5×10^{-4} M, cyclodextrin concentration 10^{-2} M (*β*-CD) or 10^{-1} M (*α*-CD, *γ*-CD, HPB).

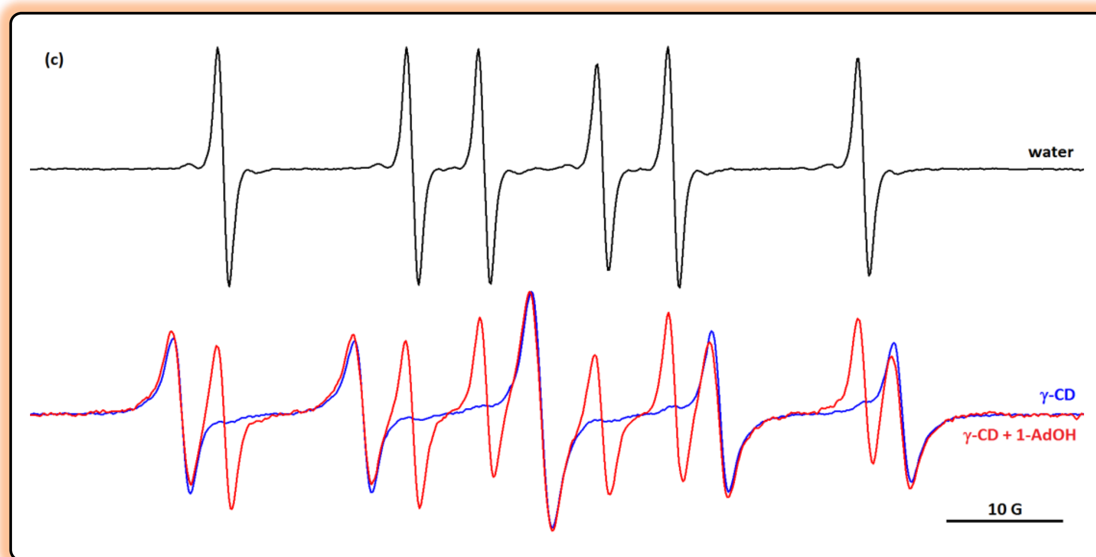

Figure 7.2. ESR spectrum of spin probe *S4* in water and in *γ*-CD solution at maximum cyclodextrin concentration (10^{-1} M), in the absence and in the presence of 1-AdOH.

Due to the higher affinity of *S4* for *α*-, *β*- and *γ*-CD cavities, upon addition of 1-adamanthanol (1-AdOH) the spin probe is expelled from inside the *α*- and *β*-CD cavities. In contrast, due to the *γ*-CD exhibiting a larger cavity size, the probe is not completely expelled by

the 1-AdOH molecules which exhibit a high affinity for CDs. Thus the ESR spectrum highlights the presence of two species, one free in solution and one complexed with γ-CD (**Figure 7.2.**).

S3 and *S4* exhibit values corresponding to the binding constants (K_a) of spin probes to CDs of order hundreds (M^{-1}). Compared to *TEMPO* derivatives, β-phosphorylated nitroxides can be used to differentiate CDs from a mixture serving as an application in the analytical field.

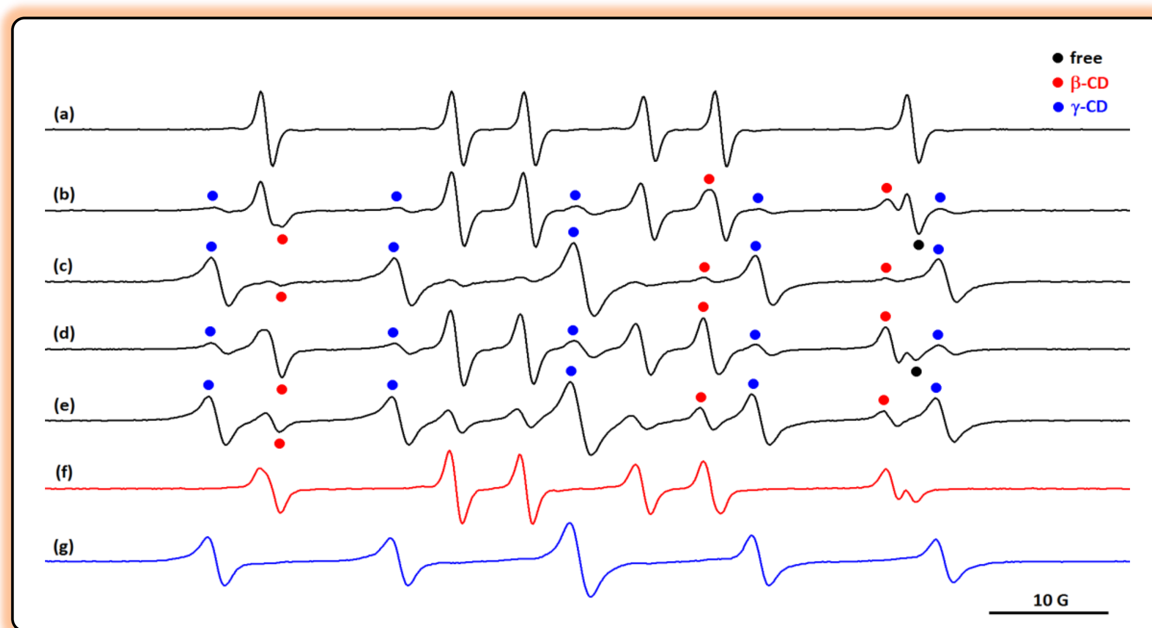

Figure 7.3. ESR spectra of the *S4* spin probe in water (**a**) and in mixtures of β-CD and γ-CD at different concentrations: (**b**) 3×10^{-3} M β-CD, 3×10^{-3} M γ-CD; (**c**) 3×10^{-3} M β-CD, 10^{-1} M γ-CD; (**d**) 10^{-2} M β-CD, 10^{-2} M γ-CD; (**e**) 10^{-2} M β-CD, 10^{-1} M γ-CD; in 10^{-2} M β-CD (**f**) and, in 10^{-1} M γ -CD (**g**). The free β-CD complexed and γ-CD complexed components in solution are marked with dots in the corresponding color. The free component is labeled only for the high-field lines where the spectral differences are most evident.

7.2. Investigation of ionic surfactant solutions in the presence of cyclodextrins using β-phosphorylated nitroxide molecular probes

Self-assembled sodium dodecyl sulfate (SDS) systems were traced in the absence and presence of CDs using three *S2-S4* spin probes. At the maximum concentration of SDS the three radicals show more pronounced spectral changes compared to their spectra at the maximum concentration of CDs. The surfactant molecules associate to form micelles whose diameter is

larger than that of the CDs. As a result, the three spin probes can be embedded inside the cavity of the SDS micelles modifying their ESR parameters due to the noncovalent interactions that are established. *S4* shows a more pronounced spectral change compared to the spectrum of the same probes in *γ*-CD, because this time the spectrum shows a signal consisting of only four lines that can also be explained by a change in geometry (**Figure 7.4.**).

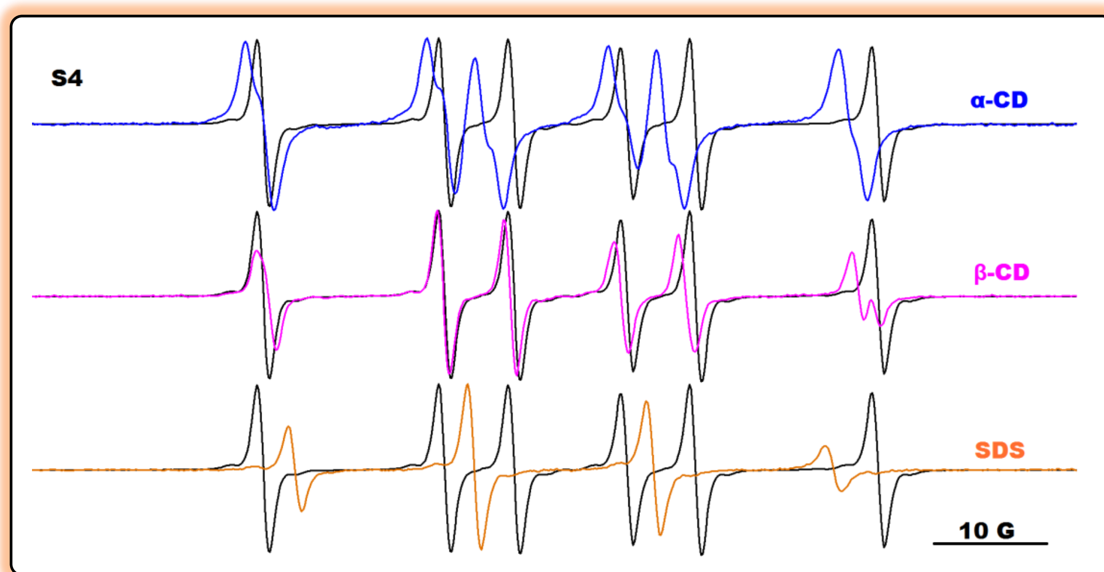

Figure 7.4. ESR spectra of *S4* spin probes in the absence (black line) and in the presence of CDs (blue line and pink line) and SDS (orange line), at 2.5×10^{-4} M molecular spin probe concentration, 10^{-2} M concentration for *β*-CD, 10^{-1} M for *α*-CD and SDS.

In the competitive experiments to study the affinity of molecular spin probes for cyclodextrins in the presence of sodium dodecyl sulfate, it was observed that at a level below the premicellar concentration, surfactant molecules show affinity for the hydrophobic interior of CDs, which is why the probes are dislocated by SDS unimers as the surfactant concentration increases. Also, the *CMC* value of the surfactant increases as some of the SDS molecules form complexes with CDs in solution. In the case of the *S2*-*α*-CD/SDS system the presence of two S2 species, one free in solution and one complexed with the SDS micelle is emphasized. Also, two species are also present in the absence of *α*-CD. In the case of the *S2*-*β*-CD/SDS system the probe is completely expelled from inside the CD, which highlights a higher affinity of the SDS unimers for *β*-CD than for *α*-CD. In the case of the *S3*-*α*-CD/SDS complex, the probe has a high

affinity for the surfactant micelle, because both in the presence and in the absence of *α*-CD the ESR signal is identical and corresponding to a single species immobilized in the micelle. Also, in the case of the *S3-β*-CD/SDS system, the probe is expelled as the surfactant concentration increases, the probe exhibits an ESR signal similar to that in water, and after micelle formation, the probe forms inclusion complexes with the micellar aggregates. The *S4-α*-CD/SDS and *S4-β*-CD/SDS systems are similar to the *S3-β*-CD/SDS complex, except that the ESR signal of the S4 probe consists of only four spectral lines leading to the idea of a strong noncovalent bond with the SDS micelle interior (**Figure 7.5.**).

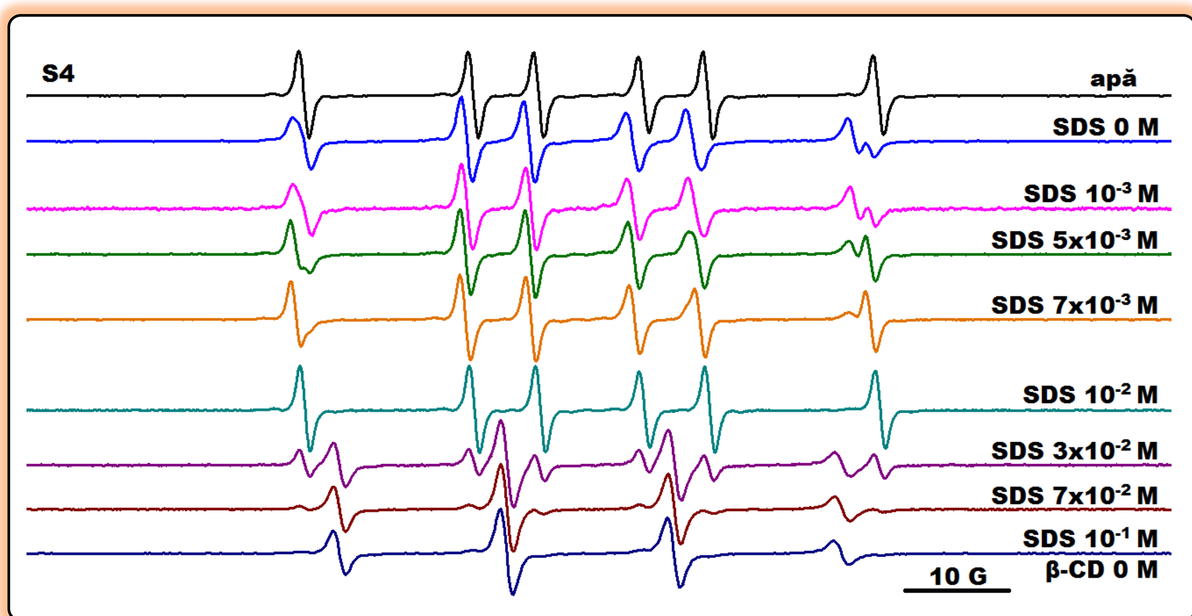

Figure 7.5. ESR spectra of the molecular spin probe *S4* in CDs (10^{-2} M for *β*-CD; 10^{-1} M for *α*-CD) in the absence and in the presence of increasing concentrations of SDS (10^{-5}-10^{-1} M).

7.3. New molecular spin probes for the study self-assembled systems

In this part, the formation of the *"host-guest"* systems of six new spin probes with CDs and SDS was pursued. The *β*-phosphorylated nitroxides *S3-1*, *S3-6* do not show ESR signal in water due to a pronounced hydrophobic character. The ESR spectra of spin probes *T-2*, *T-4*, respectively *P-3*, *P-5* highlight the formation of non-covalent bonds between them and CDs, respectively SDS by decreasing a_N values and/or line broadening at high field. Another ESR parameter indicating the formation of supramolecular inclusion systems is τ_c, as it is associated with molecular mobility. The higher its value the slower the probe motion.

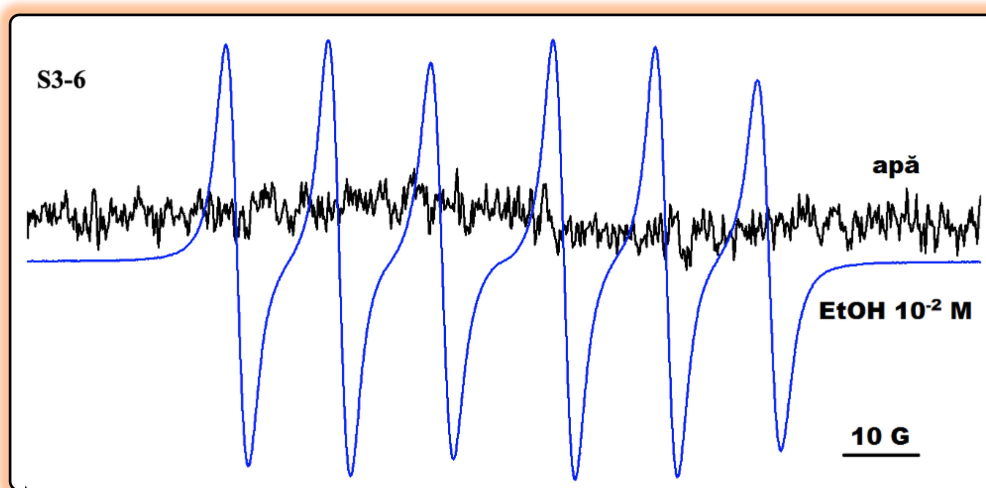

Figure 7.6. ESR spectrum of spin probe *S3-6* in water (black) and 10^{-2} M ethyl alcohol (blue).

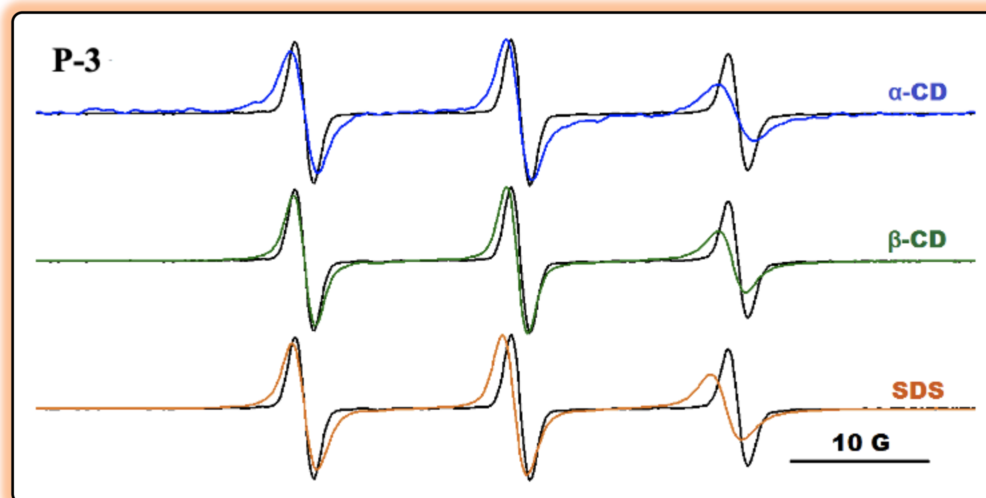

Figure 7.7. ESR spectra of the *P-3* molecular spin probe in the absence (black) and in the presence of CDs and SDS; concentration of the 2×10^{-4} M molecular spin probe, concentration of 10^{-2} M (β-CD), 10^{-1} M (α-CD) and SDS 10^{-1} M.

In competitive experiments to study the affinity of molecular spin probes for cyclodextrins in the presence of sodium dodecyl sulfate, it was observed that systems containing the two β-phosphorylated nitroxides can be used for *CMC* determination as an alternative method, because when the spin probes are ejected from the cavity of the two CDs, a line resembling their ESR spectra in water appears, and after the formation of the SDS molecules,

the probe forms a complex with the SDS molecule, and the ESR signal consisting of the six lines corresponding to the two *β*-phosphorylated nitroxides appears (**Figure 7.8.**).

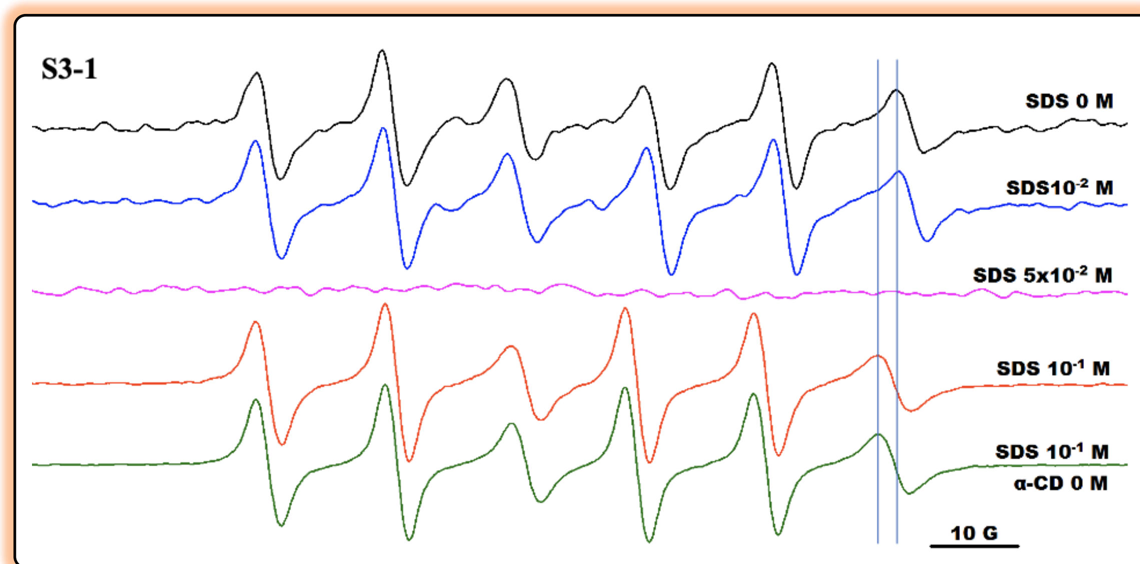

Figure 7.8. ESR spectra of the *S3-1* spin probe in CDs in CDs (10^{-2} M for *β*-CD; 10^{-1} M for *α*-CD) in the absence and in the presence of increasing concentrations of SDS (10^{-5}-10^{-1} M).

CONCLUSIONS AND PERSONAL CONTRIBUTIONS

The current experimental data obtained in the Ph.D. thesis entitled *"Thermosensitive systems studied by Electron Spin Resonance Spectroscopy and calorimetric methods"* offer the possibility to extend the studies on conformational changes of proteins in interaction with different classes of compounds. These studies can be linked to biochemical information on the formation of molecular aggregates associated with the occurrence of neurodegenerative diseases. On the other hand, different molecular vectors carrying paramagnetic groups may find applications in bioimaging, in particular if systems are found to protect the nitroxide group.

As we have shown, both micellar forms and *"host-guest"* complexes are solutions for increasing radical stability. We also propose to spin label several molecules with biomedical applications to investigate their behavior in binary and/or ternary systems of acyclic and/or cyclic oligosaccharides by ESR.

Also, in the direction of supramolecular chemistry studies, we plan to investigate self-assembled systems formed by complexation of cyclic and/or acyclic β-phosphorylated nitroxides with new *„host"* molecules (cucurbituryl, pilararene, calixarene) and new surfactants that allow the development of systems that can be thermally modulated and that can open new research directions.

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