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Dedicated to Professor Victor-Emanuel Sahini on the occasion of his 85th anniversary

EPR STUDY ON ORGANOGELS BASED ON 12-HYDROXYSTEARIC ACID

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A series of nitroxide type spin probes (amino-TEMPO, carboxy-TEMPO, a biradical bearing two TEMPO units linked by a triethylene glycol chain, and spin labeled 12-hydroxyl stearic acid) have been used as reporters for the formation of organogels based on 12-hydroxystearic acid in benzyl benzoate or toluene. The diffusion of spin probes into gels was followed by EPR spectroscopy at room temperature. The results have revealed that changes in EPR parameters attributed to the interaction between the gel network and spin probes depend upon the structural particularities of the spin probes. The most sensitive spin probe to the gel formation was 4-amino-TEMPO, while in the case of the biradical a slow hydrolysis process in the gel phase was noticed.

INTRODUCTION

Gels represent a class of soft materials which have attracted broad interests in the research and application fields.[1,2] Depending upon the bonds nature employed in the gelation process, the gels are divided into chemical and physical gels. While chemical gels are thermally irreversible because the network is formed through covalent bonds, the physical gels are thermally reversible due to the weakness of physical interaction between the gelator molecules.[3] Independently of their nature, gels are able to confine large volumes of solvent in the solid-like fibre network.

The formation and properties of gels are usually studied by microscopic observation, rheological measurements, and electronic microscopy, providing information on the macroscopic expression of the gelation. Information on changes at the molecular level which take place during gelation can be obtained using various spectroscopic methods.

The spin probe method of EPR spectroscopy has been recognized as a powerful technique to study the changes which occur in various systems at the molecular level, due to its sensitivity to changes in polarity and microviscosity around the paramagnetic group. Thus literature data provides a considerable amount of work regarding investigation by EPR spectroscopy on micellar systems,[4,5] liquid crystals,[6,8] and host-guest supramolecular systems,[9-11] all involving a molecular organization based on non-covalent interactions. EPR spectroscopy has been only rarely used to investigate gel systems. X-band EPR spectroscopy was previously introduced to study gels based on physical interaction like gelatin gels[12,13] or organogels based on low molecular weight gelators.[14-16] The synthesis of a gel based on a covalent network resulting from the reaction of isocyanate end – capped PEG derivatives and β-cyclodextrin was studied by EPR spectroscopy.[17]

In this study, using EPR spectroscopy, we aimed to obtain information regarding the

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formation of an organogel resulting from the self-assembly of 12-hydroxystearic acid (12-HSA) in benzyl benzoate and toluene, and to demonstrate the diffusion of spin probes into the gel systems. Gelation of 12-HSA in various solvents (benzyl benzoate,[18] toluene,[19] hexafluorobenzen,[20] cyclohexane,[21] and vegetable oil[22]), has already been reported and gels characterized. The nitroxide radicals presented in Fig 1, differing in molecular size, hydrogen bonding affinity and hydrophilic/hydrophobic balance, were used as spin probes to gain information on the gelation and the diffusion process into the gel system.

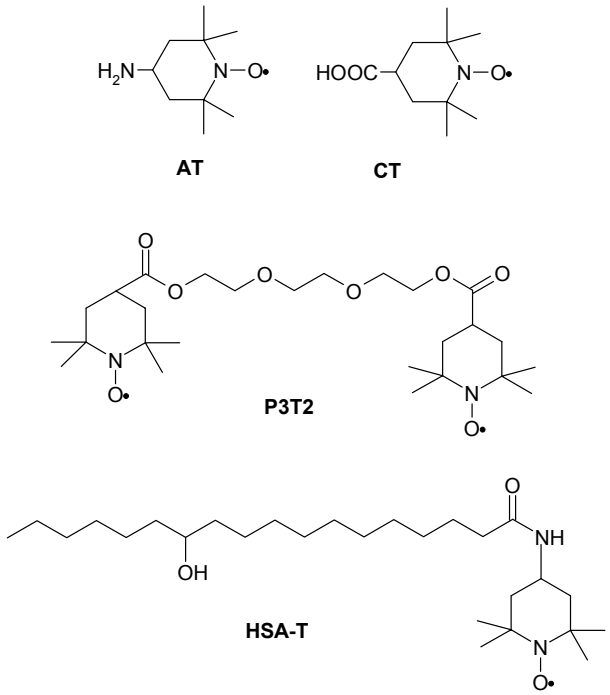

Fig. 1 – Spin probes used in the experiments.

EXPERIMENTAL

12-hydroxy-stearic acid and benzyl beanzoate were purchased from Alfa Aesar. The spin probes amino-TEMPO (**AT**) and carboxy-TEMPO (**CT**) were obtained from Aldrich. Synthesis of **P3T2** was previously described.[23] The spin-labelled 12-HSA (**HSA-T**) was obtained following the next procedure: 4-amino-TEMPO (188 mg, 1.1 mmol) was added to a solution of 12-hydroxy stearic acid (300 mg, 1 mmol) in dichloromethane, followed by the addition of dicyclohexylcarbodiimide (297 mg, 1.2 mmol). The reaction mixture was stirred for 24 h and then was washed successively with 0.1 M aq. HCl and 0.1 M aq. $NaHCO_3$. The organic layer was filtered off and the solvent was removed on a rotary evaporator. The pure **HSA-T** was obtained by thin layer chromatography on silica gel, using as eluent dichloromethane/ methanol (10:1 v/v). $R_f = 0.53$. ESI-HR-MS data for HSA-T: calc. for [C27H53N2O3 + H^+] = 454.4126 found 454.4129.

The EPR spectra of spin probes were recorded at room temperature on a Jeol Jes FA 100 spectrometer with the general settings as follows: center field 3356 G, sweep field 80 G, frequency 100 kHz, gain in the range 100- 200 sweep time 480 s, time constant 0.3 s, modulation width 1 G, microwave power 1 mW.

For the EPR measurements, in each case 5 μL of a spin probe solution in ethanol 10^{-2} M were put in a sample tube and the solvent was evaporated. Then 500 μL of solvent or mixture of 12-HSA /organic solvent was added. After sonication at room temperature, the gelator/solvent mixture samples were put in a water bath and the temperature was increased to 70 ^{0}C in order to solubilize12-HSA in the solvent (benzyl benzoate or toluene). The solution was taken in a capillary tube, and then samples were allowed to cool down at room temperature. Two series of gels corresponding to the mentioned solvents with following 12-HSA concentration were prepared: 0.5, 1.0, 1.5, 2.0, 2.5 mol %.[18]

For diffusion experiments a 0.5 mL solution of spin probe (10^{-4}M) in the solvent used to prepare gel was left in contact over night with the gel (aproximately 200 mg).

The EPR spectra of spin probes used in the study revealed a dynamic situated in the fast regime motion and the rotational correlation times (τ) were calculated based on Kivelson theory[24] and using the relation:

$$\tau = 6.5\times10^{-10}\Delta H_0\left[\sqrt{\frac{h_0}{h_{-1}}}+\sqrt{\frac{h_0}{h_{+1}}}\right]-2 \qquad (1)$$

in which ΔH_0 is the peak-to-peak width (in Gauss) of the central line, h_{-1}, h_0 and h_1 are the heights of the low field, central and the high field lines, respectively.

RESULTS AND DISCUSSION

It is assumed that the fibre network of a small molecular weight gelator, like in this case, 12-HSA, is the result of molecular assembly controlled by noncovalent interaction. In the molecule of 12-HSA there are two functional groups which can be involved in hydrogen bonding in organic solvent – the hydroxyl and the carboxyl group. Within the gel there is a balance between the dynamics of gelator-gelator and gelator-solvent interaction[25] and we can assume that when another molecule (a guest) is entrapped within the gel its properties can be affected if interactions gel fibre/guest, solvent/guest or gelator/guest are possible. For our experiments, the guests are spin labels which can be present from the beginning of gel formation or can diffuse into the gel after formation and thus EPR parameters can be influenced by possible interaction with the gelator or the solvent entrapped.

We used a variety of spin probes, but only **AT** (Fig. 1) was sensitive to the gel formation and the diffusion process. In Table 1 are summarized the rotational correlation times of **AT** in the gel systems analised and using as solvent benzyl beanzoate, as this EPR parameter reports on the dynamic of a spin probe.

In the case of **AT** a specific interaction with carboxyl group of 12-HSA is possible. In order to see if this spin probe can be incorporated in the gel fibres during gelation, five samples containing 0.5, 1, 1.5, 2 and respectively 2.5 % 12-HSA in benzyl benzoate with **AT** (10^{-4} M) were prepared and heated up to 70 ^{0}C. Then samples were left to cool down at room temperature to afford gelation. The EPR spectra indicate a small change in τ value (from 0.64×10^{-10} s corresponding to **AT** in benzyl benzoate to 1.5×10^{-10} s for **AT** in gel) which suggests a weak interaction with gel fibres and also prove that an insertion of spin probe into the gel network does not occur. The influence of gelator concentration on EPR spectra of **AT** was not noticed.

The diffusion experiments of **AT** from benzyl benzoate into the gel containing 2.5 % mol 12-HSA revealed a stronger interaction between gel fibres already formed and the spin probe. The spin probe was not completely uptake by the gel. In Fig. 2 are shown the EPR spectra of **AT** in benzyl benzoate, in supernatant after equilibration, and adsorbed into the gel.

Table 1

The τ values of AT observed in systems in which the solvent is benzyl benzoate

System	benzyl benzoate	in HSA gel (AT introduced in the systems previously to gel formation)	in HSA gel, after diffusion	in supernatant, after difussion in gel
$\tau\times10^{10}$ (s)	0.64	1.50	1.08	2.03

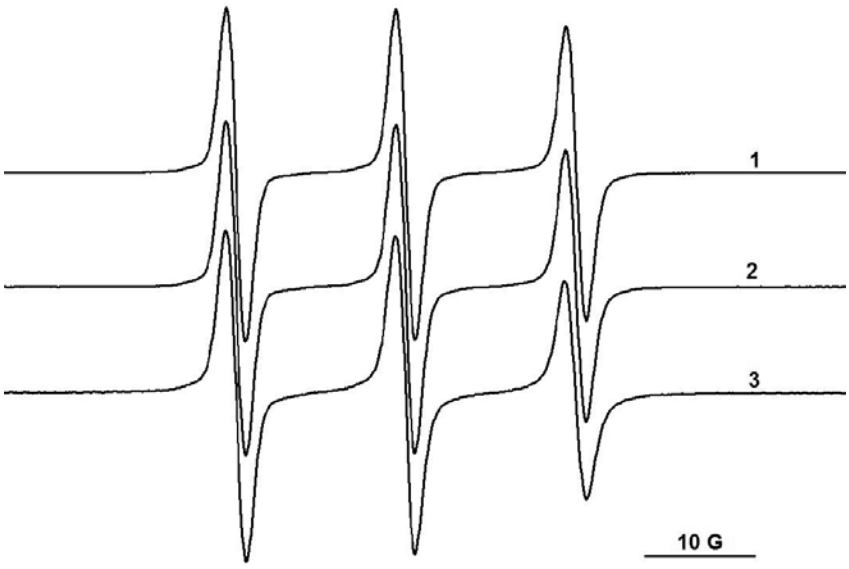

Fig. 2 – EPR spectra of AT in benzyl benzoate (1), in supernatant after diffusion in the gel, solvent benzyl benzoate (2) and adsorbed into the 12-HSA gel 2.5% mol, solvent benzyl benzoate) (3).

The τ value of **AT** increases from 0.64×10^{-10} s (fig 2-1) to 1.08×10^{-10} s (fig. 2-2) in supernatant and to 2.03×10^{-10} s in gel (Figs. 2-3). A slight increase was noticed in $2a_N$ value when **AT** is adsorbed into the gel (from 31.18 G to 31.27 G). The change is minimal, but suggests that the spin probe is placed in a more polar environment when it is taken up by the gel. The increase of τ value corresponding to the spectra of **AT** can be explained by an acid-base interaction between carboxylic moiety from 12-HSA molecules which build the gel network and the amino group from the spin probe. The fact that the motion of **AT** is still in the fast regime after diffusion into the gel can be explained if we assume that the interaction takes place at the edges of fibres, where the network might be more mobile. The changes in τ value can be due to the interaction between free 12-HSA in solvent entrapped in the gel network and the spin probe. This hypothesis is sustained by the fact that **AT** in benzyl benzoate which is in equilibrium with the gel has a more restricted motion comparatively with pure **AT** in this solvent. This slower motion can be explained by the existence of an association between **AT** and free 12-HSA from the system. In order to check if the acido-base interaction between an acid and a base can be described by changes in EPR spectra, we chose **CT** as acid. The EPR spectra of pure **CT** (10^{-4} M), **AT** (10^{-4} M) and equimolar mixture of **CT** and **AT** in benzyl benzoate were recorded. The molecular tumbling of **CT** (τ value is 1.59×10^{-10} s) in benzyl benzoate is slow compared with **AT**. For the solution containing **CT** and **AT** the calculated τ is 0.83×10^{-10} s. This proved that in the presence of **AT**, the interaction **CT** with the solvent became weaker. In the same time, **AT** and **CT** are not close enough in benzyl benzoate to evidence at room temperature the broadness of EPR lines due to spin-spin interactions. This result prompted us to see what the effect is when an amine is added to the initial mixture of solvent/gelator. Thus we prepared some samples containing 1 % HSA and 1 % dodecyl amine in benzyl benzoate or in toluene and those were heated up to 70 ^{0}C. After the samples were cooled it was noticed that in benzyl benzoate a weaker gel can be formed, while in toluene the gelation did not occur.

The diffusion of **AT** from toluene into 12-HSA gel (corresponding to 2.5 % molar concentration of HSA in toluene) leads to a slower motion of the spin probe (Fig. 3).

The EPR experiments on formation of gel and diffusion into the gel using **CT** and **AT** did not show an important immobilization within the gel network. For this reason, we decided to prepare a spin probe with a similar structure to the gelator, **HSA-T** (Fig. 1). The EPR spectra of **HSA-T** in the pure solvent and in the gel systems shown that this spin probe is not trapped in the network during the gel formation process and when diffusion take place into a gel already formed, as the parameters are not changed. These results also suggest that the formation of gel fibres is a recognition process and impurities are not held in the gel network.

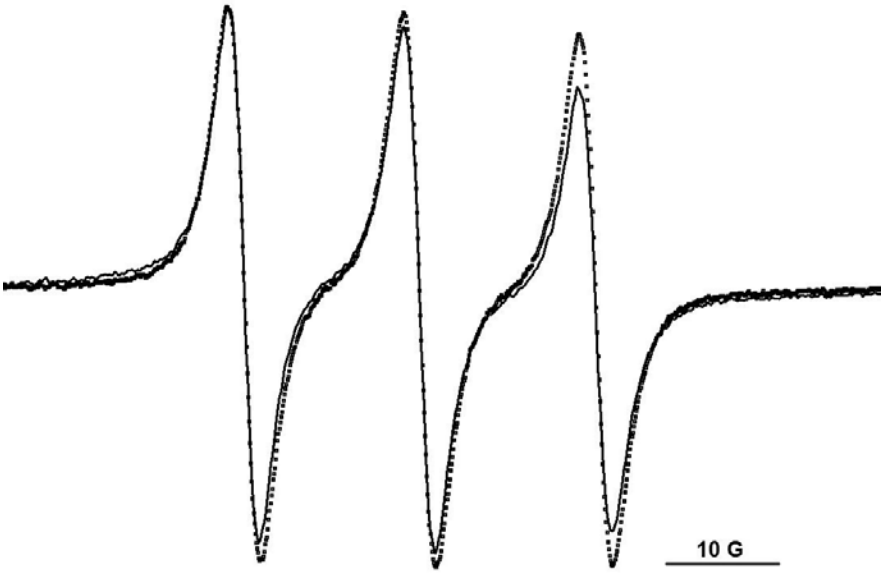

Fig. 3 – EPR spectra of **AT** in toluene (scattered line)
and in the gel 12-HSA 2.5% (molar) in toluene (full line).

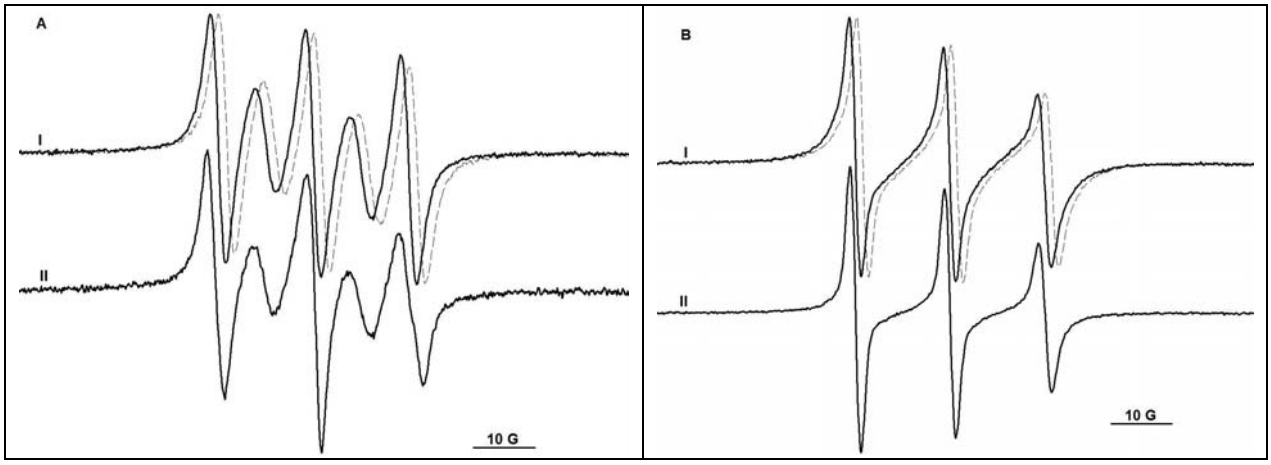

Fig. 4 – A) I - EPR spectra of P3T2 in toluene (full line) and in gel formed in toluene (dashed line); II – EPR spectra of the radical in gel formed in toluene, after 5 days; B) I- EPR spectra of **P3T2** in benzyl benzoate (full line) and in gel formed in benzyl benzoate (dashed line); II – EPR spectra of the radical in gel formed in benzyl benzoate, after 5 days.

In general, the EPR spectrum of a biradical spin probe is dependent on the solvent nature. For example, in the case of biradical **P3T2**, consisting of two paramagnetic moieties linked to trietlylene glycol through esther groups, this shows an EPR spectrum with three broad lines in benzyl benzoate, while in toluene it shows five lines revealing the spin-spin interactions (Fig 4). Independently of the solvent nature, the EPR parameters of **P3T2** (hyperfine splitting constant a_N, rotational correlation time or the ratio of the central line height and that due to the exchange interaction) are not sensitive to the gel formation or to the formation of gel fibres (Fig. 4 A-I and B-I) in both solvents.

The esther groups from molecule **P3T2** are sensitive to the presence of compounds with acid or base moieties. We observed that in time the EPR spectra of **P3T2** in gels with benzyl benzoate changed and the lines became sharper. This may be due to the hydrolysis of the biradical and the releasing into the gel of **CT**. In fact the EPR spectra shown in Fig. 4B-II correspond to the **CT** spectrum in benzyl benzoate. In the case of the system containing toluene as solvent, a decrease of the ratio of the lines due to hyperfine splitting and the line due to exchange interaction was noticed (Fig. 4A-II). This proves that hydrolysis of **P3T2** is not complete in toluene. However, this result can be further exploited to monitor by EPR measurements chemical processes which take place in gels.

CONCLUSION

In summary, the results of this EPR study on gels formed using as gelator 12-HSA show that the interaction between spin probes and gelator or gelator fibres is not accompanied by changes in dynamic type of the spin probe. The structural particularities of the spin probes play a role in the interaction with the gel system. The spin probe **AT** was the most sensitive to the changes occurring in these gel systems. Even the motions of **AT** remained in the fast regime after were entrapped in the gels a fast exchange between the molecules residing near the gel fibres and those moving freely in the solvent entrapped cannot be excluded. Our EPR experiments at room temperature show that the EPR spectra of the spin probe are independent of the 12-HSA concentration and in consequence are not sensitive to the gel fibre density of this gel. The gelation process is affected by the presence in the system of other molecules in concentration similar to that of the gelator.

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REFERENCES

1. S. Banerjee, R. K. Das and U. Maitra, *J. Mater. Chem*., **2009**, *19*, 6649-6687.
2. N. M. Sangeetha and U. Maitra, *Chem. Soc. Rev*., **2005**, *34*, 821-836.
3. P. Terech and R. G. Weiss, *Chem. Rev*., **1997**, *97*, 3133-3159.
4. N.V. Lebedeva, A. Shahine and B. L. Bales, *J. Phys. Chem. B*., **2005**, *109*, 19806-19816.
5. A. Caragheorgheopol and H. Caldararu, "EPR Spin Labelling and Spin Probe Studies of Self-assembled Systems" in Electron Paramagnetic Resonance, vol. 17, 2000, p. 205-245.

6. J. M.; Rawson, C. S. Clarke and D. W. Bruce, *Magn. Reson. Chem.,* **2009**, *47*, 3-8.
7. Y. Noda, S. Shimono, M. Baba, J. Yamauchi, Y. Uchida, N. Ikuma and R. Tamura, *Applied Magn. Res.*, **2008**, *33*, 251-267.
8. J. S. Hwang and G. A. Oweimreen, *Applied Magn. Res*., **2004**, *26*, 387-393.
9. I. Kirilyuk, D. Polovyanenko, S. I. Semenov, I. Grigor'ev, O. Gerasko, V. Fedin and E. Bagryanskaya, J. *Phys. Chem. B*, **2010**, *114*, 1719-1728.
10. P. Franchi, M. Lucarini and G. F. Pedulli, *Curr. Org. Chem*., **2004**, *8*, 1831-1849.
11. G. Ionita, A. Caragheorgheopol, H. Caldararu, L. Jones and V. Chechik, *Org. Biomolec. Chem*., **2009**, *7*, 598-602.
12. D. G. Gillies, L. H. Sitcliffe, X. Wu and P. S. Belton, *Food Chem*. **1996**, *55*, 349-355.
13. H. Caldararu, G. S. Timmins and B. C. Gilbert, *Phys. Chem. Chem. Phys*., **1999**, *1*, 5689-5695.
14. P. Terech, R. Ramasseul and F. Volino, *J. Colloid Interface Science* **1983**, *91*, 280-282.
15. P. Terech, *J. Colloid Interface Science* **1985**, *107,* 244-255.
16. M. Kveder, M. Andreis, J. Makarevic´, M. Jokic´ and B. Rakvin, *Chem. Phys. Lett*., **2006**, *420*, 443-447.
17. G. Ionita and V. Chechik, *Chem. Commun*. **2010**, *46(43)*, 8255-8257.
18. J-l. Li, R-Y. Wang, X-Y. Liu and H-H. Pan, *J. Phys. Chem. B*, **2009**, *113*, 5011-5015.
19. P. Terech. D. Pasquier, V. Bordas and C. Rossat, *Langmuir,* **2000**, *16*, 4485-4494.
20. T. Tamura and M. Ichikawa, *J. Am.Oil Chem. Soc.,* **1997**, *74*, 491-495.
21. P. Terech, V. Rodriguez, J. D. Barnes and G. B. McKenna, *Langmuir*, **1994**, *10*, 3406-3418.
22. M. A. Rogers, A. J. Wright and A. G. Marangoni, *Soft Matter*, **2008**, *4*, 1483-1490.
23. G. Ionita, V. Meltzer, E. Pincu and V. Chechik, *Org. Biomol. Chem*. **2007**, *5*, 1910-1914.
24. B. L. Bales "Spin Labeling: Theory and Application" in Biological Magnetic Resonance, Plenum Publishing Corp., New York, vol. 8, 1989, p. 77-130.
25. L. A. Estroff and A. D. Hamilton, *Chem. Rev*. **2004**, *104*, 1201-1218.