# Nitroxide Radicals Interacting with Polyamine-Phenolic Ligands and Their Metal Complexes

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We have analyzed the interactions of a series of nitroxide radicals with ligands containing a phenol moiety coupled with different polyamine fragments, and their Cu$^{II}$ and Zn$^{II}$ complexes, by EPR experiments. Nitroxide radicals are good mimics of the properties of pollutant molecules, e.g., organic compounds having different polarities and charges, surfactants, and polyaromatics. Charged, polar, and low-polarity sites in the ligands and complexes are all available for interactions; in particular, the ability to entrap the radicals in a vice-like structure, by the formation of phenyl sandwich complexes, determines the type of selective interaction that occurs when a chain or planar aromatic structure is present. For closely packed complexes, Zn$^{II}$ has a cooperative effect in the interaction with the radical; it allows both the entrance of low-polarity radicals at the low-polarity sites of the complex and the interactions of polar or charged radicals at the polar sites of the complexes. Conversely, Cu$^{II}$ coordinates to the amino groups of the ligands and blocks the entry of radicals, which, thus, impedes their insertion into the structure of the complexes. The opposite behavior, with respect to that of Cu$^{II}$ and Zn$^{II}$, was found for the complexes that have more open structures. The complexes that have cyclic ligands exhibit a poor ability to interact with radicals, but, for the ligands in the absence of the coordinating metals, the interactions are significantly enhanced as a result of the entrance of the radicals into the cyclic structures. In all cases, stronger interactions occur when both hydrophilic and hydrophobic sites are involved. The neutral-surfactant probe forms aggregates mainly with the cooperative effect of the copper complexes. UV/Vis measurements confirmed the results obtained by EPR and indicate that weak interactions occur between the radicals and the complexes or ligands; they also confirmed the formation of 1:1 adducts.

## Introduction

Transition metal complexes can host and bind various organic and inorganic species, which, thus, allows their recognition, transport, and activation.[1−5] In this respect, the most important property of the metal complex is its readiness to interact with the host species through an unsaturated coordination site, which, in turn, is affected by the nature of the metal and the degree of preorganization of the complex that allows it to adapt to the presence of the new species. Because many natural biological sites, such as the active centers of various metalloenzymes, are produced by two transition metal ions, many dinuclear metal complexes have been synthesized and used successfully as devices for the recognition and assembly of external species that have different properties, e.g., inorganic and organic species; therefore, synthetic receptors for metal ions can be used to mimic enzymes' active centers.[6−10] Control over the distance between the two metal ions is crucial to allow the cooperation of both metal ions and, thus, the ability to mimic the reactions of active centers such as those present, for example, in oxygen receptors, activators, and carriers.[11,12] Moreover, the cooperation of the two metal ions permits a stronger complexation of the guest, which is usually coordinated by both metal centers.

Recently, we reported the synthesis and properties of several macrocyclic and noncyclic ligands containing a phenol moiety inserted into a polyamine skeleton.[13−17] Solid-state and solution studies highlighted the ability of these ligands to form complexes with M$^{II}$ cations and, hence, to match the properties above; therefore, these systems may be suitable for interacting with secondary species that are extracted from the environment and then linked at the metal−ligand active sites. In particular, the dinuclear metal complexes we synthesized present the two transition metal ions in close proximity, which allows them to bind cooperatively to at least one substrate that is linked in a bridge disposition between them. In this paper, we report a series of ligands containing phenol moieties coupled with different polyamine fragments, which we term MODEN, DIDEN, ANIDEN, ANITR, and ANIPT (Scheme 1). With the exception of ANITR, which contains a tetraamine chain, these ligands are obtained from a combination of

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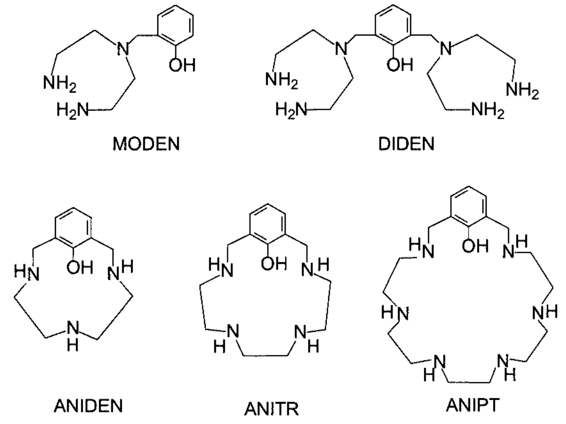
one or two diethylentriamine (dien) chains linked in different positions to the 2,6-dimethylphenol moiety. For example, ANIDEN can be viewed as the macrocyclic counterpart of MODEN, while ANIPT is the macrocyclic counterpart of DIDEN. ANITR forms only mononuclear species with transition metal ions such as Cu$^{II}$ or Zn$^{II}$ in aqueous or alcoholic solution, whereas the other ligands mainly form dinuclear species that are the only species existing in alcoholic solution. The dinuclear species of ANIPT and DIDEN possess ligand/metal molar ratios of 1:2, while those for ANIDEN and MODEN are 2:2.



Scheme 1

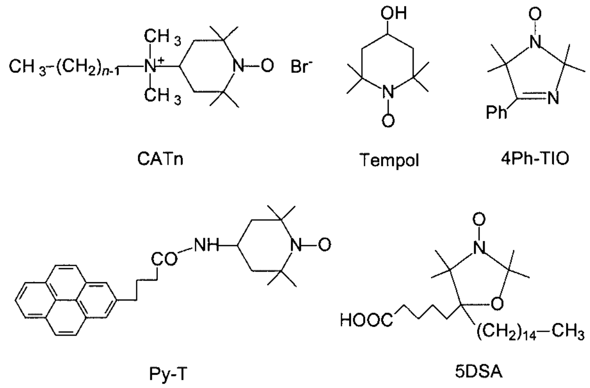
Electron paramagnetic resonance (EPR) spectroscopy is a powerful tool for the analysis of the binding of nitroxide radicals to coordination sites and ligand groups.[18,19] Several studies have dealt with the magnetic and structural properties of complexes formed from metal complexes and nitroxide radicals.[20−25] To the best of our knowledge, however, no efforts have as yet been devoted to studying the interactions of heterocyclic ligands, and their dinuclear metal complexes, with differently structured nitroxide radicals.

Therefore, in an effort to analyze the ability of these ligands — and, more specifically, their complexes with Cu$^{II}$ or Zn$^{II}$ — to interact with pollutant molecules extracted selectively from the environment, in this study we applied the EPR technique while using selected nitroxide radicals that mimic well the properties of pollutant molecules, e.g., organic species that have different polarities and charges, surfactants, and polyaromatics (their formulas and acronyms are presented in Scheme 2).

## Results and Discussion

### EPR Spectroscopy

Figure 1 shows examples of the EPR spectra obtained for the complexes in the presence of the CATn radicals (radical concentration, 1 mM; complex concentration, 8 mM) at 263 K: (a) CAT1 with DIDEN-Cu, DIDEN-Zn, MODEN-Cu, and MODEN-Zn and (b) CAT12 with DIDEN-Zn and MODEN-Zn. The full lines in the Figure are the experimental spectra and the dashed lines represent the computed ones. The computation of the spectral line shape was performed by means of a well-established procedure that was reported by Budil et al.[26] The **g** tensor components (for the coupling between the electron spin and the magnetic field) were assumed to be constants ($g_{ii}$ = 2.009, 2.006, 2.003). The **A** tensor components (for the coupling between the electron spin and the nuclear spin of the nitrogen atom) mainly reflect the variations in polarity of the nitroxide environment: an increase in $A_{ii}$ values (accuracy: 3%) corresponds to an increase in the environmental polarity. For instance, the $A_{ii}$ values found for CAT1 are greater than those evaluated for CAT12; the presence of the hydrophobic chain attached to the positively charged CAT group favors the localization of the nitroxide group in a less polar environment. An increase in line width arises from spin−spin interactions; the presence of paramagnetic copper ions in the vicinity of the nitroxide moiety may be responsible for this effect. A partial self-aggregation of surfactant radicals is also responsible for spin-exchange interactions. The variation in line width among the samples is very small, between 1 and 2 G, which gives, on average, a value of 1.5 G (reported in Figure 1). This value indicates that the spin−spin effects are quite negligible in the CAT*n* samples that we analyzed in this study. The NO group, therefore, does not approach the paramagnetic Cu$^{II}$ center.



Scheme 2

A Brownian, or Jump, model for the diffusional rotation of the nitroxide unit was considered and provided the correlation-time components, perpendicular and parallel, of $\tau_i = 1/(6D_i)$ and $\tau_i = 1/D_i$, respectively. These values (accuracy of 3% on the basis of the computation) are the most important parameters for analyses of complex−radical interactions; indeed, an increase in the correlation time for motion reflects a decrease in the radical mobility that is due to the interaction between the radical and the complex, or the ligand. This interaction is mainly physical, that is, dipole−dipole and van der Waals interactions prevail, but ion−dipole interactions are certainly the main driving forces that move the radicals towards the ligand sites.

The spectra recorded at complex/radical molar ratios < 8:1 (not shown) present higher mobilities (lower correlation time for motion) than those recorded at an 8:1 ratio (Figure 1); molar ratios > 8:1 result in almost equivalent spectra (also not shown) as that at an 8:1 molar ratio. We found, however, that already at a 1:1 molar ratio between the com-

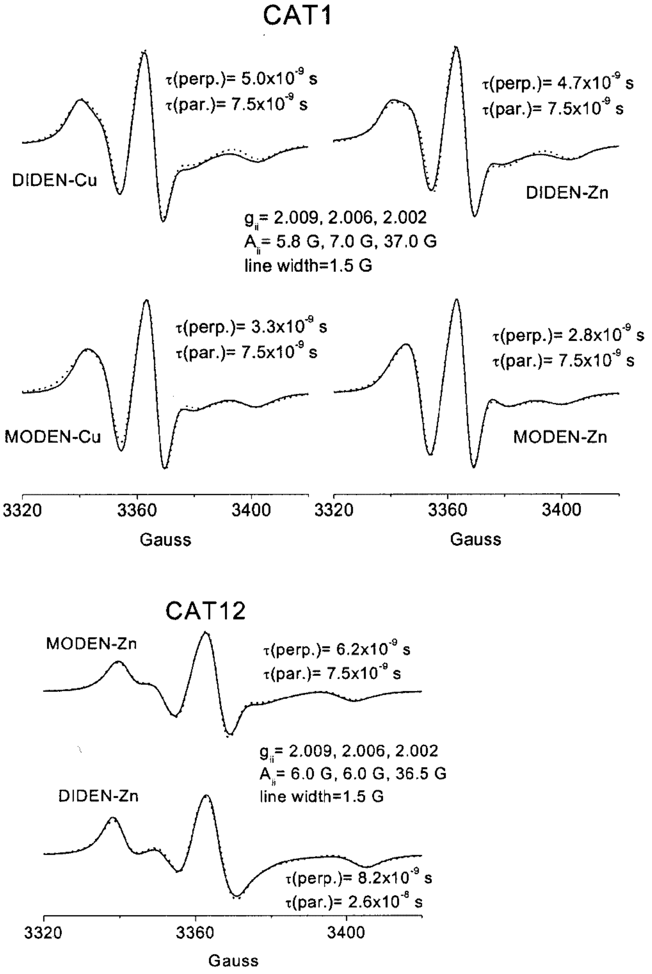


Figure 1. Examples of the EPR spectra obtained for the complexes in the presence of the CAT*n* radicals (radical concentration 1 mM; complex concentration 8 mM) at 263 K; (a) CAT1 with DIDEN-Cu, DIDEN-Zn, MODEN-Cu, and MODEN-Zn; (b) CAT12 with DIDEN-Zn and MODEN-Zn; the full lines in the figure are the experimental spectra, the dashed lines are the computed ones

plex and the radical, a small, but perceptible, quenching of the mobility occurs with respect to that of the radical in the absence of the complexes. On the other hand, we were mainly interested in the differences among the various systems (radicals + complexes or ligands), which showed equivalent trends at the different complex/radical molar ratios. For this reason, we simply compared the spectra obtained for the various radical/complex (or radical/ligand) systems at molar ratios of 1:8.

The first observation we made based on the parameters listed in Figure 1 was a decrease in the mobility on proceeding from CAT1 to CAT12, when comparing the same complex. It must be remembered that a similar difference in rotational mobility is found between the two probes in solution in the absence of the complexes, which occurs as a result of the different structures (size and shape) of the probes. In any case, this difference leads to a lower sensitivity of CAT12 with respect to its ability to differentiate among the various systems; when the interactions are strong, the mobility is very low and all the spectra appear to be similar. Computation of the line shape, however, easily reveals the differences. Problems may arise because the spectral computation of the different samples often requires a different anisotropy of motion, in which case a direct comparison of the perpendicular or parallel correlation times for motion does not work. Similarly, the average correlation time is not a good parameter for comparing the samples because different diffusional models yield different weights to the perpendicular and parallel components. Therefore, for an easier comparison, we simply used $2A_{zz}'$ splitting, i.e., the distance between the low- and high-field absorptions (in several cases, the second derivatives of the signals allowed more-accurate evaluations of this parameter: accuracy within 2−3%), which is related directly to the increased resolution of the anisotropic components of the hyperfine tensor; in turn this increased resolution is due to a progressive quenching of radical mobility: an increase in $2A_{zz}'$ reflects a decrease in mobility of the radical because of its interactions with the complexes or the ligands.

Figure 2 displays the variation of $2A_{zz}'$ for the various CAT*n* complex (or ligand) systems as histograms. Analyses of these histograms allowed us to define some general trends:

1. The DIDEN-Zn complex interacts the most with CAT*n*.
2. MODEN-Zn interacts less than does MODEN-Cu.
3. Cu complexes also interact more than do Zn complexes for the ANITR-M and ANIPT-M systems.
4. Conversely, DIDEN-Zn interacts more than does DIDEN-Cu.
5. ANITR-M and ANIPT-M interact less strongly than do the other complexes.
6. ANIDEN-Cu also interacts quite strongly.

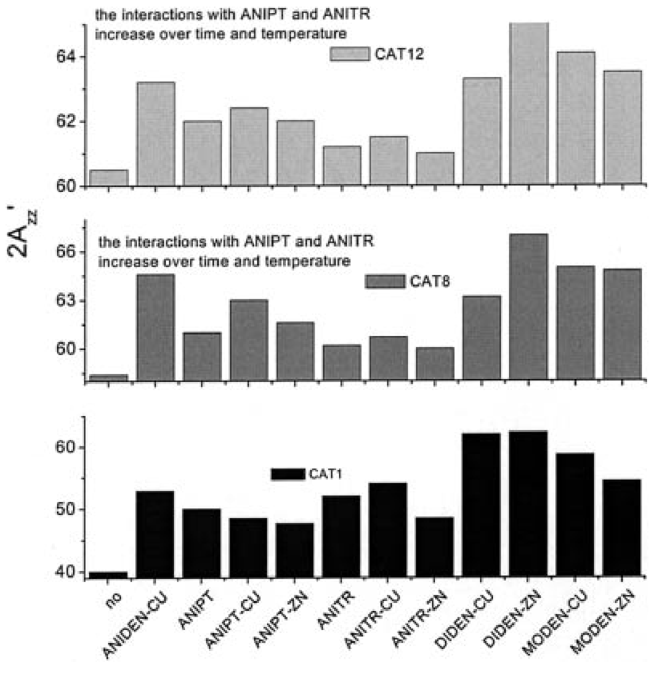


Figure 2. Histograms of the values of $2A_{zz}'$ for the various CAT*n* complex (or ligand) systems

Before discussing these results, we also stress that different behaviors were found for ANITR and ANIPT (the ligands lacking coordinated metal ions), which interact quite strongly with CAT1, but, initially, the interaction is relatively weak with CAT8 and CAT12. However, increasing the temperature or leaving the samples to equilibrate for longer times leads to a progressive increase in the strength of binding because of the slow kinetics of the interaction.

Since CAT1 behaved similarly to CAT8 and CAT12, we hypothesized that the interaction takes place mainly by means of the charged CAT group; the driving force for the interactions certainly can be ascribed to this moiety. Therefore, we supposed that the hydrophobic chains of CAT8 and CAT12 do not play a substantial role in the binding between the radical and the complexes, but rather they affect only the entrance of ANITR and ANIPT into the macrocycles, as indicated by the slow kinetics of the interactions. Consequently, it is surprising to note that the similar trends observed for CAT*n* also hold for the hydrophobic radicals Py-T and 4Ph-TIO, as is shown in the histograms in Figure 3. Nevertheless, the lower values of $2A_{zz}'$ arose from the much faster mobility of these radicals with respect to the CAT*n* radicals, because 4Ph-TIO is more mobile than Py-T. This faster rotational mobility, in turn, allows a better differentiation of one sample from another. Some further discrepancies were found, however, between the CAT*n* samples and the 4Ph-TIO and Py-T samples:

1. The difference in probe mobility between MODEN-Cu and MODEN-Zn is relatively high.
2. MODEN-Cu exhibits the maximum interaction with 4Ph-TIO.
3. ANITR-Zn interacts more strongly than does ANITR-Cu.
4. The interaction with the ligands ANITR and ANIPT is relatively strong and is constant over time and at different temperatures.

Before discussing the rationale implicit in these results, and the connections with the structures of the complexes, the results from the last two radicals shown in the histograms in Figure 4, 5DSA and Tempol, deserve separate considerations. First of all, the parameter used for describing the interaction with Tempol is not the $2A_{zz}'$ splitting, but rather the intensity ratio between the two components superimposing to form the overall signal (results not shown). These two components, which arise because the radicals exist in two motion regimes, simply termed fast and slow, originate from the two different radical environments, i.e., in water (bulk, not interacting radicals) and in the complexes (interacting radicals). As has already found in previous studies,[27] the increase in the intensity ratio between the slow and fast components (Is/If) can be interpreted as being due to the an increase in the interactions of the radicals with the complexes or the ligands. These intensities were calculated by a subtraction/addition procedure of the components themselves to separate them and obtain, by double integration, the values of their intensities. The histogram in Figure 4 for Tempol shows the values of Is/If for the different ligands and complexes. It is interesting that, on the basis of the higher Is/If ratio, the interaction of Tempol is stronger for DIDEN-Cu and ANITR-Zn, with respect to the other complexes; this finding is the opposite of the preferential interactions occurring for the other radicals and indicates that the mechanism of interaction is also different.

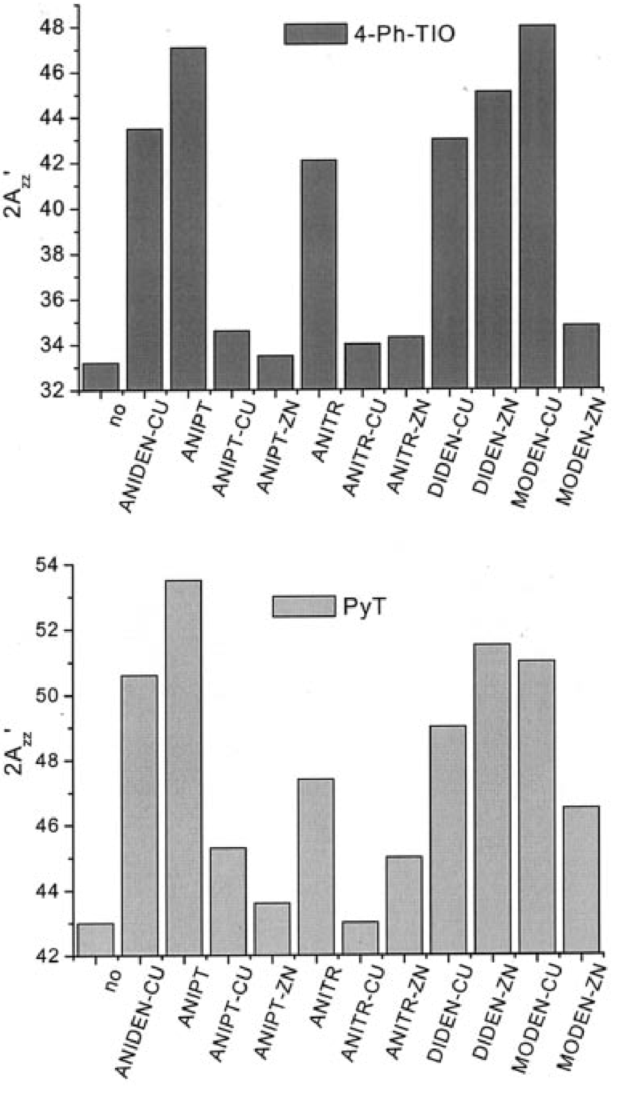


Figure 3. Histograms of the values of $2A_{zz}'$ for the Py-T and 4Ph-TIO complex (or ligand) systems

An introductory description is also needed for the results obtained from 5DSA. In this case, the self-aggregation ability of this radical provides a new contribution appearing as a single line (not shown for brevity). This component, termed the agg. component, arises from spin−spin exchange interactions due to the high local concentration of self-aggregating radicals; since this component is absent in the absence of the complex or the ligand, this observation implies that the complex or the ligand offers an aggregation site for these radicals. This behavior can be described in terms of a cooperative interaction in which the ligand or the complex, by interacting with the radicals, cooperates with them in the formation of the aggregates. This aggre-

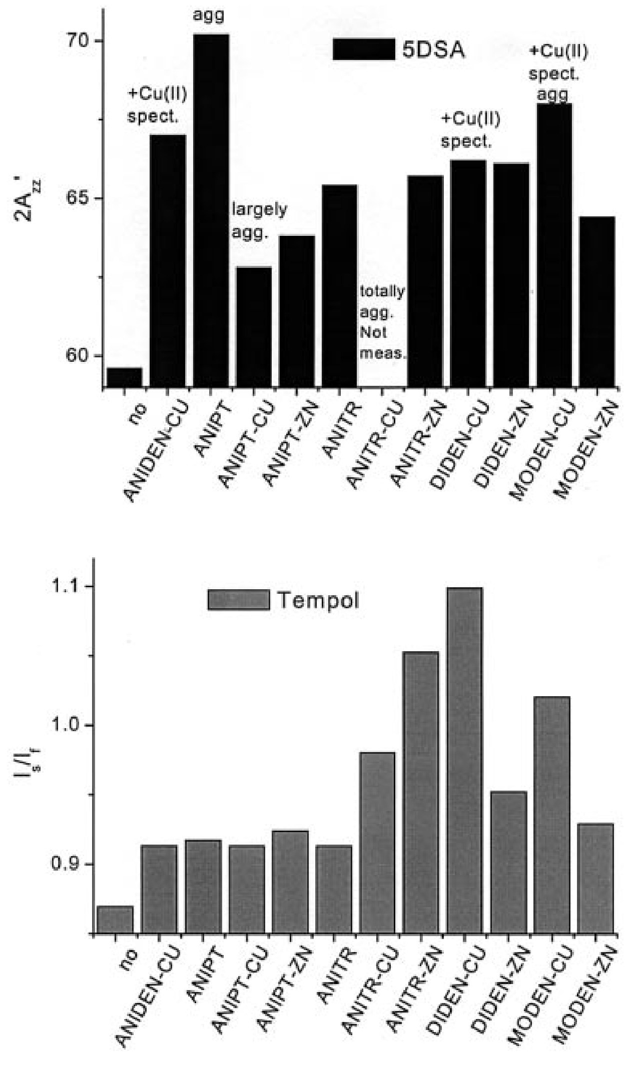


Figure 4. Histograms of the values of $2A_{zz}'$ for the 5DSA and Tempol complex (or ligand) systems

gation, however, is also favored by the repulsive effect of the charged metal sites: the hydrophobic chains escape from contact with the ions by forming aggregates. In cases in which the agg. component contributes to the EPR spectrum, we indicate the presence of aggregated surfactants by the "agg" label at the top of the pertinent bar in the histogram showing the $2A_{zz}'$ values for the different ligands or complexes (Figure 4). Although the strongest interaction for 5DSA is exhibited by ANIPT, which also shows the formation of 5DSA aggregates, the preferential formation of aggregates is presented by ANIPT-Cu and ANITR-Cu, which, conversely, show a weak interaction, as evidenced by the $2A_{zz}'$ values. This finding demonstrates that the mechanism of interaction of 5DSA with these complexes is different from that of the other complexes. Also, it is interesting that the interaction of the copper complexes with 5DSA leads to the appearance of a signal characteristic of monomeric copper complexes. This observation implies that, when interacting with the complex, this surfactant radical functions as a separator of the two $Cu^{II}$ ions. This phenomenon may be related to both a physical separation and an electron transfer between the radical and $Cu^{II}$. This result holds true also for DIDEN-Cu and ANIDEN-Cu, which show the strongest interactions with the radical. For DIDEN-Cu, the high $2A_{zz}'$ value, the appearance of a signal from monomeric $Cu^{II}$ complexes, and the presence of aggregates characterize the spectrum and are indicative of a very strong interaction.

This interaction can be understood quite easily by considering that it arises from an acid−base interaction between the COOH group of the probe and the amino groups of the ligand. Such an interaction may also involve the $Cu^{II}$ ions, which coordinate well to deprotonated carboxylic groups, and, hence, may be responsible for the appearance of the monomeric copper complexes.

All of the results described above for the different radicals can be analyzed, for each complex or ligand, by proposing a suitable mechanism of interaction.

The structure of DIDEN-M characterizes a quite strong interaction with almost all the nitroxide radicals: charged, polar, and low-polarity interacting sites are all available for the interaction, but, mainly, the ability to entrap the radicals in a vice- or pliers-like structure determines the preferential interactions of the radicals with this complex. MODEN-M and ANIDEN-M may also create a similar interacting effect, but, in these cases, the possibility of forming phenyl sandwiches with the radicals also enhances the interactions.

Perhaps surprisingly, DIDEN-Zn generally interacts more readily than does DIDEN-Cu, whereas the opposite holds true for MODEN-M (i.e., the Cu complex interacts more readily than does the Zn complex). For DIDEN-Zn, the high ability to interact arises from two synergic effects: (1) $Zn^{II}$ has a cooperative effect in the interaction with the radical and allows both the entrance of the low-polarity radicals at the low-polarity binding sites of the complex and (2) the interactions of the polar or charged radicals at the polar sites of the complex; conversely, $Cu^{II}$ readily coordinates to the amino groups of the ligands and blocks the entrance of the radicals, which, thus, impedes their insertion in the complex structure. The Cu and Zn complexes of MODEN behave in an opposite way because the more open MODEN structure allows the entrance of the radicals into the structure of the Cu complex. Essentially, the coordinative requirement of $Cu^{II}$ is not saturated by the donor atoms of the ligands and the radical behaves as an extra ligand. On the basis, however, of the strength of the interaction, as measured by EPR spectroscopy, the binding site of the radicals is not located directly at the NO moiety.

In the cases of ANITR and ANITP, their monomeric nature does not allow either a sandwich- or vice-like interaction. Therefore, the presence of the chain for the CAT or 5DSA does not favor the interaction and the ANITR-M and ANTP-M complexes show a weak interaction with the probes. Nevertheless, it is interesting that ANITP-M, which has the larger cycle, increases its interacting ability with respect to ANITR-M, by increasing the chain length of the

CAT*n* probes, because both hydrophilic and hydrophobic interactions become active.

Two other interesting features in the interactions of the radicals with the ANITR and ANITP ligands and complexes are noteworthy.

(a) Cu complexes are more interacting than Zn complexes for CAT*n*, but this trend is reversed for ANITR-M with respect to the hydrophobic probes (ANITR-Zn is more interacting than ANITR-Cu for 5DSA, Py-T, and 4Ph-TIO); this observation means that the mechanism of interaction is different because ANITR-Cu is more selective for the interaction with hydrophilic substances and ANITR-Zn is more selective in respect to the hydrophobic molecules. In relation to the different mechanisms of interaction of the copper and zinc complexes, the behavior of 5DSA is very informative: the copper complexes promote the self-aggregation of the probes, i.e., it is favored by repulsion of the charged copper site.

(b) In the absence of the metal ion, the ligands ANITR and ANIPT show a significant interaction with the hydrophobic probes; in these cases, the macrocycles offer a comfortable binding site for the probes that have a prevalently hydrophobic nature. The CAT*n* probes are less interactive, but it is interesting to note that, in the presence of the chain, a slow kinetic process (which takes hours at room temperature) allows the progressive entrance of these probes so that they can be hosted by the macrocycles. As expected, an increase in temperature accelerates this process.

With regard to the mechanism of the interaction process, further information was provided by Tempol, which, in several cases, behaved in a manner opposite to that of the CAT*n* probes. We propose that, for Tempol, the interactions occur through the hydroxyl moiety, whereas the CAT*n* probes promote the interactions via their positively charged group.

### UV/Vis Spectroscopy

The interaction of the radicals with the metal complexes was also supported by UV/Vis spectroscopy experiments, in which we observed the d−d electronic transitions of the coordinated $Cu^{II}$ chromophore in the complexes in the absence and presence of the radicals. Table 1 shows $\lambda_{max}$ (nm) and $\varepsilon_{\lambda max}$ ($mol^{-1}dm^{3}cm^{-1}$) values of DIDEN-Cu in methanol solution in the absence and in the presence of Tempol, CAT1, and Py-T. This complex in methanol shows a band at $\lambda_{max}$ = 623 nm for the d−d transitions ($\varepsilon$ = 150 $mol^{-1}dm^{3}$ $cm^{-1}$), while a shift towards lower energy was observed in the presence of Tempol and, moreover, for CAT1, but the effect was lower for Py-T, which is in agreement with the EPR spectroscopic results. Figure 5 shows the UV/Vis electronic spectra of the system Tempol-DIDEN-Cu upon addition of different quantities of Tempol to a methanol solution of the DIDEN-Cu complex. A constant profile was obtained at high Tempol/DIDEN-Cu molar ratio, which indicates the weak complexation of Tempol by DIDEN-Cu, as we had found also by EPR spectroscopy. The increase in absorbance at 669 nm, as a function of the number of equivalents of Tempol added, provided evidence for the formation of a Tempol/DIDEN-Cu adduct having 1:1 stoichiometry. CAT1 and Py-T provided similar results to those obtained with Tempol, as a function of the relative molar ratio with respect to the complex.

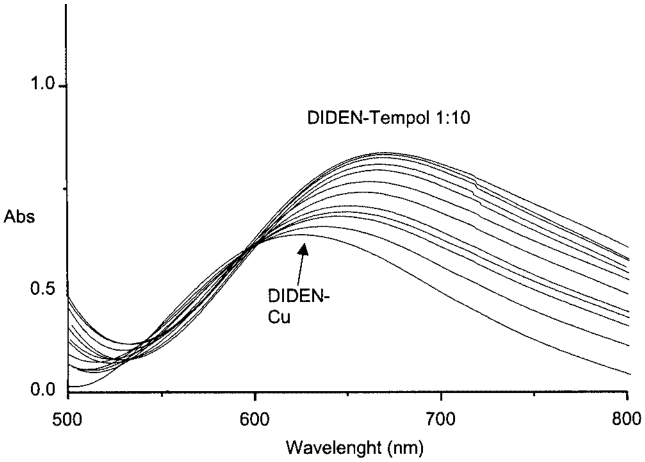


Figure 5. UV/Vis electronic spectra obtained upon addition of different quantities of Tempol to a methanol solution of the DIDEN-Cu complex; [DIDEN-Cu] = 5 × $10^{-3}$ M

The shift in the d−d band was slightly smaller for ANIPT-Cu and ANITR-Cu, with respect to those of DIDEN-Cu, ANIDEN-Cu and MODEN-Cu, but this variation was not as significant as expected on the basis of the EPR spectroscopy results, which indicates that the "copper side" (tested by the UV/Vis spectroscopic analysis) was less sensitive to the complexation behavior of the radicals with respect to the "radical side" (tested by the EPR analysis).

## Conclusions

We have demonstrated that ligands containing a phenol moiety coupled to different polyamine fragments, namely DIDEN, MODEN, ANIDEN, ANIPT, and ANITR

Table 1. Values of $\lambda_{max}$ [nm] and $\varepsilon_{\lambda max}$ [$mol^{-1}$ $dm^{3}$ $cm^{-1}$] obtained from the UV/Vis spectra of DIDEN-Cu in methanol solution in the absence and presence of Tempol, CAT1, and Py-T

| DIDEN-Cu[a] | | DIDEN-Cu/Tempol | | DIDEN-Cu/CAT1 | | DIDEN-Cu/Py-T | |
|---|---|---|---|---|---|---|---|
| $\lambda_{max.}$ | $\varepsilon$ | $\lambda_{max.}$ | $\varepsilon$ | $\lambda_{max.}$ | $\varepsilon$ | $\lambda_{max.}$ | $\varepsilon$ |
| 623 | 150 | 669 | 350 | 683 | 360 | 663 | 200 |

[a] Charges have been omitted for clarity.

(Scheme 1), and their $Cu^{II}$ and $Zn^{II}$ dinuclear complexes, bind differently to nitroxide radicals, namely Tempol, 4Ph-TIO, CAT1, CAT8, CAT12, 5DSA, and Py-T (Scheme 2), that mimic well the properties of pollutant molecules, e.g., organic species having different polarities and charges, surfactants, and polyaromatics.

Computer-aided analysis of the EPR spectra provided indications of the different strengths of interactions as a function of the complex or ligand structure, and of the structure, polarity, and charge of the radical. The interactions between the radicals and the complexes or the ligands were dynamic in nature.

The structure of DIDEN-M allows a quite strong interaction with almost all the nitroxide radicals: charged, polar, and low-polarity interacting sites are all available for the interaction, but, mainly, the ability to entrap the radicals in a vice- or pliers-like structure determines the preferential interaction of the radicals with this complex. Also, MODEN-M and ANIDEN-M may also create a similar interaction effect, but, in these cases, the possibility of phenyl sandwiches existing also enhances the interactions.

In DIDEN-Zn, the metal ion has a cooperative effect in the interaction with the radical and allows both the entrance of the low-polarity radicals at the low-polarity interacting sites of the complex and the interactions of polar or charged radicals at the polar sites of the complexes; conversely, in DIDEN-Cu, the metal readily coordinates the amino groups of the ligands and, because of the packing of the resulting structure, it blocks the entry of the radicals, which, thus, impedes their insertion into the structure of the complexes. The more open MODEN structure allows the entrance of the radicals in the structure of the Cu complex; MODEN-Cu is able to interact more strongly than is MODEN-Zn. Essentially, $Cu^{II}$ presents a coordinative requirement that is not saturated by the donor atoms of the ligand and the radical functions as an extra ligand. On the basis of the strength of interaction as measured by EPR spectroscopy, however, the binding site of the radicals is not on the NO side.

ANITR-M and ANIPT-M complexes undergo weak interactions with the nitroxide probes. ANIPT-M, however, which has the larger cycle, has increased interacting ability with respect to ANITR-M when the chain length of the CAT*n* probes is increased, because, in these cases, both the hydrophilic and hydrophobic interactions become active. ANITR-Cu was revealed to be more selective for interactions with hydrophilic substances, whereas ANITR-Zn was more selective in interactions with hydrophobic molecules. For the ANITR and ANIPT ligands, in the absence of the coordinating metal ions, the interactions are significantly enhanced because of the entrance of the radicals in the cyclic structure. The kinetics of the entrance processes are slow (it takes hours at room temperature) for CAT8 and CAT12 radicals.

With the copper complexes of MODEN, DIDEN, and ANIDEN, the interaction of 5DSA induces the appearance of the EPR spectroscopic signal of a monomeric Cu complex, which is due to magnetic separation and/or electron transfer occurring at the two $Cu^{II}$ ions. For the copper complexes of ANITR and ANIPT, the main effect of the ion is to promote the self-aggregation of the 5DSA radicals.

The interactions between Tempol and the complexes or the ligands are weak and occur through the hydroxyl moiety, which has a negative potential for interaction, whereas CAT*n* promotes interactions via the positively charged group.

The positively charged moiety of the radicals undoubtedly promotes the interactions with the complexes and the ligands, but stronger interactions occurred when both hydrophilic and hydrophobic sites were involved.

UV/Vis spectroscopic measurements confirmed the results obtained by EPR spectroscopy, i.e., they indicate that weak interactions occur between the radicals and the complexes or the ligands, and they also confirm the formation of 1:1 adducts.

## Experimental Section

**General:** The ligands MODEN,[13] DIDEN,[14] ANIDEN,[15] ANITR,[16] and ANIPT[17] (Scheme 1) and their metal complexes used in the present study were synthesized as previously reported. 5-Doxylstearic acid (5DSA) and 4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl (Tempol) were purchased from Sigma and used without any further purification. 4-Trimethylammonio-Tempo bromide (CAT1), its derivatives bearing $C_8$ and $C_{12}$ chains (CAT8 and CAT12, respectively), and pyrene-Tempo (Py-T) were kindly provided by Dr. X. Lei, Columbia University, NY. 4-Phenyl-2,2,5,5-tetramethyl-3-imidazolin-1-yloxy (4Ph-TIO) was kindly provided by Prof. L. Greci, University of Ancona, Italy. Methanol solutions of the complexes or the ligands (initial concentration: 0.05 M) and of the radicals (initial concentration: 5 mM; deoxygenated and stored in a refrigerator under nitrogen in the dark) were added together to obtain molar ratios, between the complex or ligand and the radical, from 1:1 to 10:1 at a constant radical concentration of 1 mM. The complex solutions in the absence of the radicals were also analyzed by EPR spectroscopy for the sake of comparison. The solutions were processed by EPR spectroscopy (glass tube: 2 mm diameter; sealed and inserted in the EPR spectrometer's cavity) immediately after preparation and 1 and 2 d after preparation. The EPR spectra were recorded with an EMX-Bruker spectrometer operating at the X band (9.5 GHz) and interfaced to a PC (Bruker software) for data acquisition and handling. The temperature was controlled by means of a Bruker ST3000 variable-temperature assembly. Experiments were carried out as a function of temperature (between 293 and 163 K) and a progressive quenching of the mobility was observed as the temperature decreased. The room-temperature spectra, however, did not indicate a significant variation in mobility among the various samples (results not shown), which suggests that the interactions between the complexes (or ligands) and the radicals were dynamic in nature, i.e., the radical undergoes fast exchange between the ligand site and the bulk solution, and/or the formed complex structure allowed fast rotation of the radical at the ligand site. The spectra recorded at 263 K, however, still do not indicate a frozen environment, but rather slow-exchange conditions that suggest a wide range of mobilities exist between samples; these spectra permitted an analysis of the behavior of the different interactions of the different samples. Both progressive cooling and direct cooling at 263 K in the EPR cavity were performed. The latter procedure ensured a reproducible response and, therefore, a better comparison could be made among the samples.

**Electronic Spectroscopy:** UV absorption spectra were recorded at 298 K with a Varian Cary-100 spectrophotometer equipped with a temperature control unit. The samples tested by UV/Vis spectroscopy were obtained using a constant concentration of the complex in methanol (between 1 and 5 mM) and variable concentrations of the radicals, which allowed us to obtain radical/complex molar ratios from 10:1 to 1:1.

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