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Dyes and Pigments 74 (2007) 357–362
DYES and PIGMENTS
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Absorption and EPR spectra of some porphyrins and metalloporphyrins

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Received 28 January 2006; accepted 13 February 2006
Available online 2 May 2006

Abstract

Novel smart materials can be synthesized and designed at the molecular level by attaching well characterized and tailored functionalities to be used in in vitro and in vivo oximetry. A series of porphyrins and metalloporphyrins were synthesized and their EPR and UV–vis spectra studied. The steric hindrance and electronic effects of the substituted groups and the characteristics of different metal ions were the major contributors in influencing the properties of the compounds. As a result, their EPR and UV–vis spectra changed. Through studying the EPR spectroscopic properties of these compounds, we hope that novel EPR oximetry materials can be found and this will help to extend the applications of EPR oximetry in vivo.

Keywords: EPR and UV–vis spectra; Porphyrins; Metalloporphyrins

1. Introduction

The application and importance of porphyrins and metalloporphyrins in the field of biomedicine have increased significantly. They can be used as photosensitizing drugs in photodynamic therapy [1–3] when coupled to the unprotected peptide [4], DNA [5], etc. Some metalloporphyrins are used as therapeutic drugs to correct the disorder of heme metabolism and suppress tumors [6,7]. Magnetic resonance imaging of porphyrins and metalloporphyrins is the important characteristic often used in biomedical field, especially in the treatment of cancer [8]. Porphyrins and their derivatives are basic moieties that upon photoexcitation produce paramagnetic transients which are important to many biological processes as well the light-energy conversion in material science. Porphyrins and the related macrocycles display highly conjugated arrays and have rich electron atmosphere. The extended π-conjugated structures that lead to the electronically excited singlet and triplet states can be studied by EPR spectroscopy when the following are involved: (i) intramolecular electron transfer in photoexcited donor–acceptor systems and (ii) intermolecular magnetic interactions between the photoexcited porphyrin triplets and the free radicals [9]. In addition, molecular oxygen can react with porphyrin [10,11]. Therefore, it can be suggested that porphyrins and their derivatives can be used as potential EPR oximetry materials in vitro and in vivo.

Within the past few years there has been a significant amount of progress using low frequency EPR based on the measurement of pO_2, which has resulted in the availability of the instrumentation to make EPR measurements directly in vivo on animals [12–14]. Although the technology was useful and has led to the sensitive measurement of pO_2, up to now, EPR oximetry materials are not available on a large scale [15].

In our lab, we have done a large amount of careful research about porphyrin compounds. Compared to many other literature describing the specificity of the porphyrins, our research was carried out in a more systematic and detailed way including the porphyrins with different substituted groups and also the metalloporphyrins complexed with different transition metal ions. Their UV-vis spectrum and EPR spectrum were

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doi:10.1016/j.dyepig.2006.02.018

studied. Through our molecular design, different substituted groups were attached onto the porphyrin ring, which led to the change of electron atmosphere of the conjugated system. When different transition metal ions were selected and complexed, the electromagnetic performance of the compounds was also changed. Examination of the UV-vis and EPR spectra showed that substituted groups and transition metal ions in the porphyrin structures influenced the characteristics of these compounds. According to the EPR spectroscopic findings of these measured compounds, we hope to find one or several types of novel EPR oximetry materials to extend the applications of in vivo EPR oximetry.

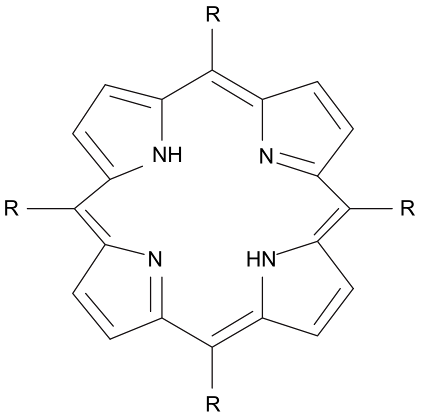
2. Experimental

2.1. Materials

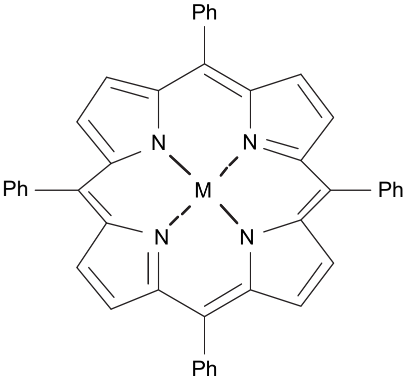
Porphyrins and metalloporphyrins detailed in this paper were synthesized in our lab, according to the methods of Alder [16,17] and others [18–23]. The syntheses of porphyrins and metalloporphyrins were mainly according to the Adler Method, while some of the procedures were modified to give higher yield under gentler reaction conditions and easier purification. All the samples were characterized by ^{1}H NMR, elemental analysis, mass and FT-IR, etc. The molecular structures of these compounds are presented in Schemes 1 and 2. Furthermore, all the molecule modeling and computation processes were recorded on the platform of SCI O_2 graphic workstation through Cerius^2 software. The stereostructures of four compounds were computed, and their optimized molecular structures were obtained (Fig. 1). The software was not applicable to give the stereostructures of the metalloporphyrins because of the existence of the metal ions.

2.2. Adsorption spectra and EPR spectroscopy

The UV—vis spectra of porphyrin solutions were recorded on a UNICO UV-2102PC spectrophotometer. The UV-vis absorption spectra of these compounds were studied in DMF solution.



Scheme 1. Porphyrin compounds. 1. *meso*-Tetraphenylporphine: R_{1-4}-Ph; 2. *meso*-Tetra(*p*-hydroxylphenyl) porphine: R_{1-4}-PhOH; 3. *meso*-Tetra(*p*-carboxylphenyl) porphine: R_{1-4}-PhCOOH; 4. *meso*-Tetra(*p*-nitrophenyl) porphine: R_{1-4}-$PhNO_2$; 5. *meso*-Tetra(*p*-aminophenyl) porphine: R_{1-4}-$PhNH_2$.



Scheme 2. Metalloporphyrin compounds. 6. Cu^{2+}-*meso*-tetraphenylporphine (Cu—TPP); 7. Li+-*meso*-tetraphenylporphine (Li—TPP).

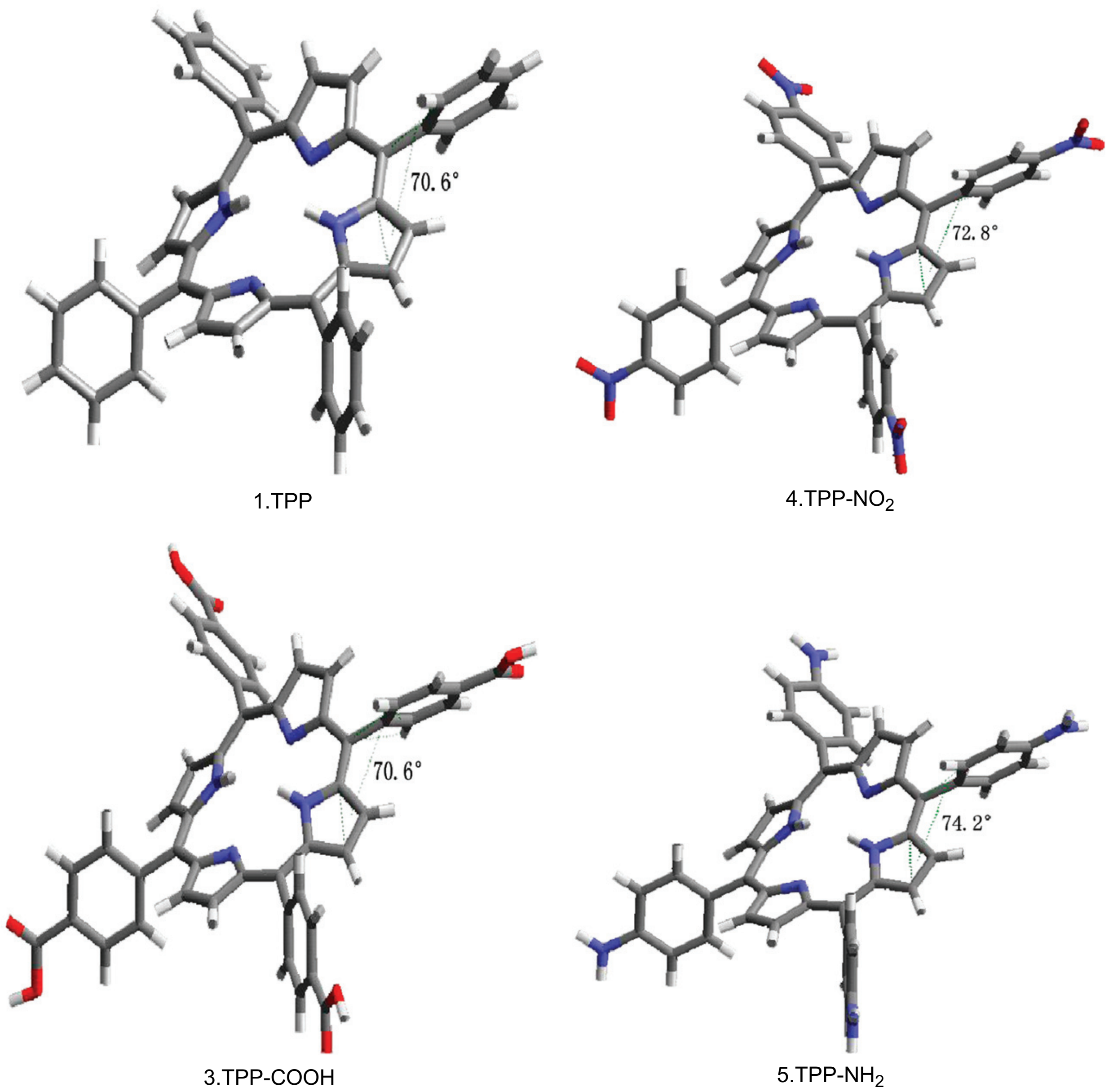
EPR spectra were measured at Bruker X-Band EPR spectrometer. All experiments were conducted at room temperature and atmospheric pressure. The solid samples were put into a quartzose tube in air. Frequency: 100 kHz, microwave power: 9.79 GHz, and time constant: 500 ms.

3. Results and discussion

3.1. UV-vis absorption spectrum

Generally speaking of porphyrins, the most significant characteristics of electron are one Soret and several Q UV-vis absorption bands [24]. In Figs. 2 and 3, we can see the intense Soret band and also one to four characteristic visible absorption bands of studied TPP and its derivatives. The former is between ultraviolet and visible spectrum (410–440 nm), called S bands; while the latter named Q band generally consists of four sub-straps (450–700 nm). Their intensity was changed with different compounds, so each different porphyrin (Tables 1 and 2) gave its own characteristics. For Q bands, the four peaks, named as I, II, III and IV straps from the longest wavelength, respectively, are generated from $\pi-\pi^*$ electrons' transition of porphyrin macrocycleπ-bands system. In Q bands, the λ values of different peaks were determined by the structures of the porphyrin macrocycles.

According to the experimental results, both steric hindrance and electron effects of the functional groups could influence the UV-vis absorption of studied compounds [25,26]. Figs. 2 and 3 indicate that the TPP of Soret bands of the studied *para*-substituted *meso*-tetraphenylporphine derivatives moved slightly toward short wavelength. When the substituted groups were added, the steric hindrance became stronger. This can be found from the structures shown in Fig. 1. As the substituted phenyl and the porphyrin rings were not in the same plane, the conjugated effect between the phenyl substitutes and porphyrin ring was relatively weak, so the influence of the substitutes to the Soret bands was weak. When the *para*-substitutes were pull-electron groups, such as nitro and carboxyl, they could decrease the density of conjugated electron



1.TPP

4.TPP-NO_2

3.TPP-COOH

5.TPP-NH_2

Fig. 1. Stereostructures of some compounds.

clouds, but comparing to the steric hindrance effect, the influence of electron effects is weaker. Therefore, the Soret bands red shift 3–5 nm. In contrast, when *para*-substituted groups are push-electronic, as hydroxyl and amino, the Soret bands further red shift about 4–18 nm, caused by the steric hindrance and electron effects.

Comparing the TPP in the case of metalloporphyrin complexes, their ultraviolet and visible absorption spectra changed significantly. The most remarkable difference was the absence of some Q bands (see Fig. 3). D-orbit electrons of the metal ions and the porphyrin macrocycleπ-band system react with each other, resulting in an increase in the energy for π–π^* electrons' transition. And when the protons on the N atoms in pyrrole rings were substituted by metal ions, the symmetry of the molecule was changed, therefore their absorption spectra were changed to a different extent.

3.2. Electron paramagnetic resonance (EPR) spectrum

All electron paramagnetic resonance (EPR) experiments were conducted under normal conditions. The solid samples were measured directly. We have not added any spin-trapping materials into the porphyrins because the free radicals' signal of the porphyrins oneself could be measured. As a result, strong simple signals of compounds were obtained (Figs. 4–6), which suggested that the free radicals produced by the porphyrins could be found under normal conditions by EPR spectrum. It can be the important factor to be used in the biosensors, and even online measurement. Unpaired electrons were in the π-orbit of porphyrin macrocycle, and there was one macro-conjugate system in the porphyrin molecule. In addition, the paramagnetic center was protected by both resonant effect and the functional groups. Therefore, the free

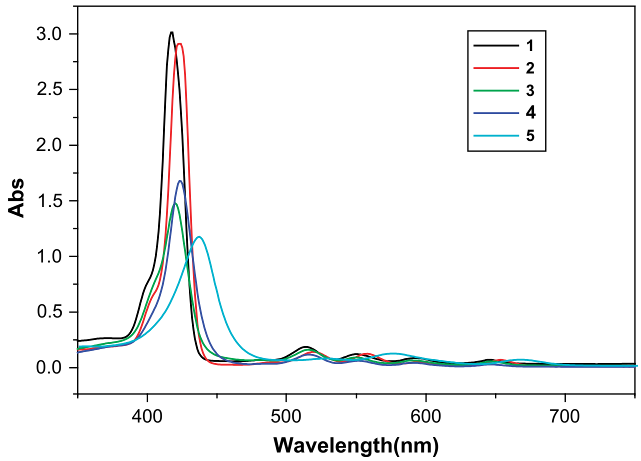


Fig. 2. UV–vis spectra of porphyrins with different substituted groups.

radicals produced by porphyrins were stable, which could even be measured by EPR spectrum directly at room temperature. There was no need to add the spin-trapping materials into the analyzed system. The porphyrin compounds were among the best choices due to the property that enables them to act as sensors without additional testing materials or using specific testing conditions.

It was observed that the EPR signals of porphyrins originated from the two paramagnetic centers: the single delocalized electron of porphyrin ring and the oxygen dependent radical [27] as shown in Figs. 4–6, especially in the EPR spectrum of the TPP. The former created the hyperfine structure of EPR spectrum. The hyperfine structure was due to the interaction of the unpaired electron with the magnetic cores (nitrogen and hydrogen atoms), mainly nitrogen atoms of the porphyrin macrocycle [28]. Because there were four nitrogen atoms in the porphyrin ring and all of them could interact with the unpaired electron, one could potentially observe nine resonances [29]. The room temperature EPR spectrum of TPP exhibited nine distinct lines due to the coupling between the porphyrin's nitrogen atoms (see Fig. 4).

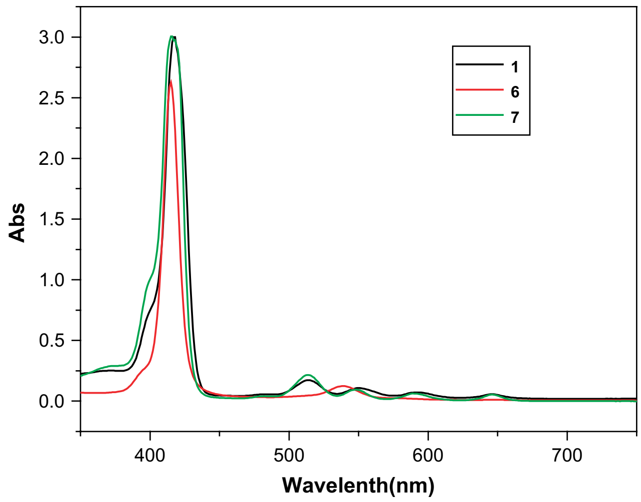


Fig. 3. UV–vis spectra of metalloporphyrins complexed with different metal ions.

Table 1
Data of UV–vis spectra of porphyrins with different substituted groups

Compounds	λ (nm)					
	S	*ε*	Q1	Q2	Q3	Q4
1	417.0	2.30×10^5	514.0	550.0	593.0	646.0
2	424.0	4.23×10^5	519.0	558.0	596.0	653.0
3	420.0	1.66×10^5	516.0	550.0	593.0	647.0
4	423.0	2.47×10^5	516.0	551.0	591.0	646.0
5	436.0	3.48×10^5	–	575.0	–	666.0

For the oxygen dependent radicals, their EPR line intensity depended on the saturation effect of the microwave power related to the isoelectric point (pH value). The photochemical reaction was of zero order with respect to the oxygen dependent radical, and was controlled by the diffusion of the triplet oxygen to the surrounding site of the porphyrin in the excited state [28]. In Figs. 4–6, there is one strong and sharp EPR signal in porphyrin EPR spectra, which was created by the reaction between the porphyrins and the oxygen molecule. As we already knew, the porphyrins were able to generate $^1O_2$, and their efficiency was strongly dependent on their chemical structures [30].

The measurement of the EPR data showed the main factors that influenced the signals of porphyrins, and these are listed below:

(i) The properties of functional groups linked to the porphyrin macrocycle;
(ii) The conditions of electron array of metal ions in the center of porphyrin macrocycle; and
(iii) The electron effects, steric hindrance and crystal structures of the compounds.

The hyperfine structures of TPP were obviously shown in its EPR spectrum (Fig. 4), while for other compounds with different *meso*-substituted groups, the hyperfine structures were very weak, or even untraceable in the EPR spectra (Fig. 4). The hyperfine structures were generated by the reaction of the atom electron spinning and the paramagnetic cores. According to Qingsong Lin et al. [28], the hyperfine structure of the porphyrins was due to the interaction of the unpaired electron with the magnetic cores (nitrogen and hydrogen atoms). However, the basic shapes of the EPR spectrum of all compounds were the same, i.e., all were the strong simple

Table 2
Data of UV–vis spectra of metalloporphyrins complexed with different metal ions

Compounds	λ (nm)					
	S	*ε*	Q1	Q2	Q3	Q4
1	417.0	2.30×10^5	514.0	550.	593.0	646.0
6	415.0	4.43×10^5	542.0	–	–	–
7	416.0	2.76×10^5	515.0	549.0	591.0	649.0

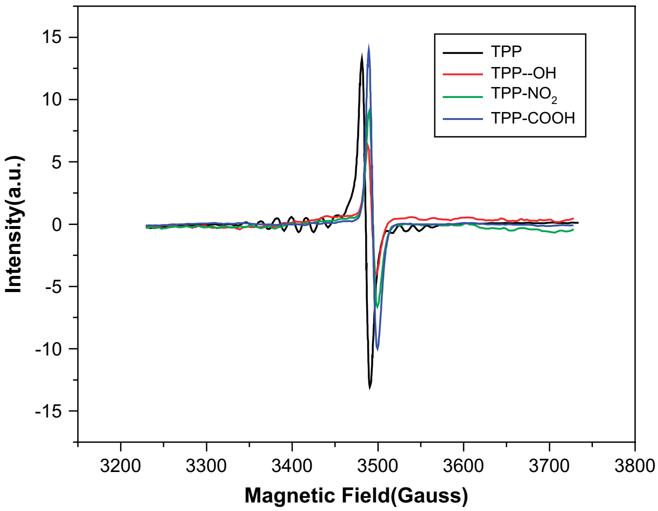


Fig. 4. EPR spectra of porphyrins with different substituted groups.

signals generated by the oxygen dependent radical. When the *meso*-substituted groups are sterically similar, the amount of O_2 absorption could be considered approximately. The bigger groups were shown to protect the paramagnetic center better, so more stable and stronger EPR signals were observed. In Fig. 1, both TPP and TPP−COOH have same bond angles (70.6°), but the *meso*-substituted carboxyl groups have larger volume; the measurement result showed that the TPP−COOH had higher EPR sensitivity (see Fig. 4 and Table 3). In addition, the carboxyl group, compared to the other *meso*-substitutes, has larger volume (Fig. 4). The EPR signal of the *meso*-tetra(*p*-carboxylphenyl) porphine was more sensitive than that of the others. When stereo effects were less, electron effects would exhibit obvious influence on the EPR signals. In similar absorbed O_2, the stronger signal intensity of EPR would be created by those compounds capable of producing superoxide anion $O_2\bullet^-$ more easily.

Fig. 5 and Table 3 show that EPR signals generated by Li−TPP and TPP are similar. The EPR signals could only be produced by the free radicals due to the unpaired electrons of Li^+ itself, and had higher EPR sensitivity. However, the TPP complexed by Cu^{2+} exhibited strong metal properties shown in Fig. 6 and Table 3. There was a significant characteristic about the EPR signals generated by Cu^{2+}, and the response was very sensitive to the complex condition. Cu was able to exhibit strong paramagnetic property because its D electron orbit is unpaired. The intensity of the EPR signals generated by Cu^{2+} was enough to cover up the free radicals' signals. Therefore, only the EPR signals of the metal ion could be measured in the EPR spectrum of Cu−TPP.

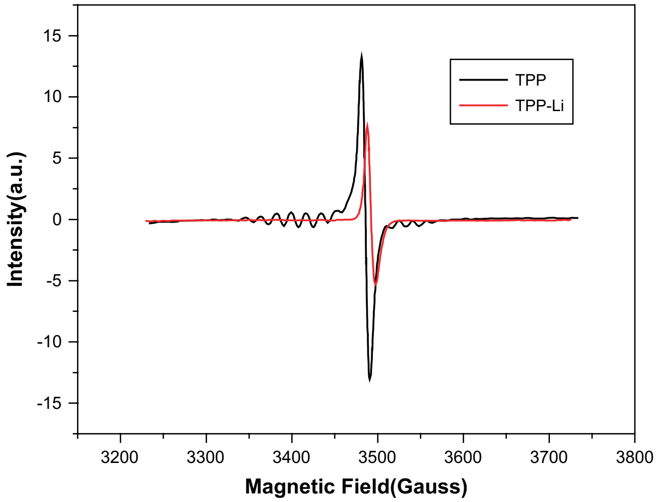


Fig. 5. EPR spectra of metalloporphyrins complexed with different metal ions.

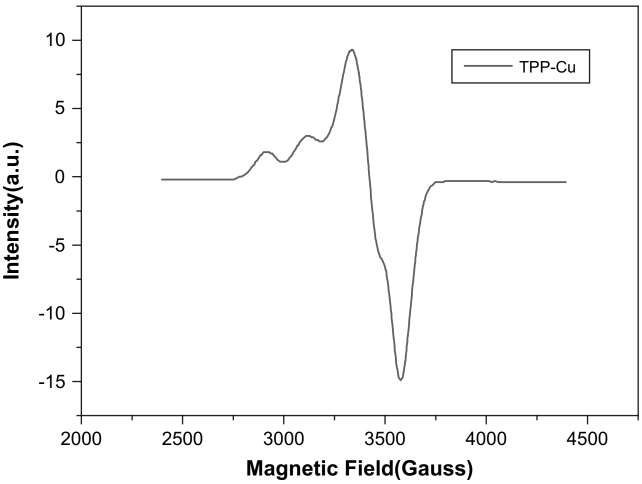


Fig. 6. EPR spectra of metalloporphyrins complexed with Cu^{2+}.

4. Conclusion

In this work, a series of porphyrins and metalloporphyrins were synthesized and their EPR and UV−vis spectra were studied. Functional groups and transition metal ions could influence the UV-vis absorption and the EPR signals of the porphyrins. The porphyrins and the related compounds prepared in our lab have optimum EPR spectroscopic properties, i.e., strong simple signals. Some conclusions have been obtained: The nature of the functional groups and their steric hindrance can change the electron atmosphere of the whole conjugated system, and affect the interactions between the photoexcited porphyrin triplets and the oxygen as well as their EPR signals. Furthermore, in fact, the steric hindrance and electron effects of the substituted groups and the characteristics of different metal ions are functional parameters influencing EPR and UV-vis spectra. At present, EPR oxygen

Table 3
The parameters of EPR spectra of the compounds

Compounds	Gain	Mid-range (G)	Scan range (G)
1	1.25×10^5	3480	500
2	1.25×10^5	3480	500
3	4×10^4	3480	500
4	1.25×10^5	3480	500
6	2×10^3	3400	2000
7	1.25×10^5	3480	500

sensitivity of the porphyrins and the related compounds is under further study. The investigation in biological oximetry showed that the size of the crystal plays a critical role in the oximetry property.

Acknowledgments

This project is supported by National Natural Science Foundation of China (contract No. 20446001), Shanghai Nano-Technology Promotion Center (SNPC), the Science and Technology of Shanghai Municipality (STCSM), Trans-Century Training Programme Foundation for the Talents by the State Education Commission, and the EU Framework Programme 6 Integrated Project Novel and Improved Nanomaterials, Chemistries and Apparatus for Nano-biotechnology (NACBO) (contract No. 500804).

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