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# Study on spectroscopic characterization of *meso*-tetrakis (4-hydroxyphenyl) porphyrin (THPP) in β-cyclodextrin and its derivatives

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**Abstract**

*Meso*-Tetrakis (4-hydroxylphenyl) porphyrin (THPP) is a photosensitize offering an alternate approach to the treatment of cancer in the form of photodynamic therapy (PDT). The supramolecular system of THPP and β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) and carboxymethyl-β-cyclodextrin (CM-β-CD), in aqueous solution has been studied by fluorescence and UV–vis spectroscopy. The formation of inclusion complexes has been observed on the base of change of spectroscopy properties. It is noted that the addition of HP-β-CD leads to remarkably strong enhancement of fluorescence intensity (nearly 300 times) of THPP. THPP forms 1:1 inclusion complex with β-CD and 1:2 inclusion complexes with the two other derivatives. Comparative to β-CD and CM-β-CD, the strongest inclusion ability of HP-β-CD can be explained that the hydrogen bond plays significant role in the inclusion process. In addition, the UV–vis experimental showed that the cavity of HP-β-CD causes the transform of the state of THPP, which is in agreement with the effect of polarity of solvent. Comparative to the pure buffer solution, the distinct equilibrium, $H_4THPP^{2+} \rightleftharpoons H_2THPP$, has been observed in the solution of HP-β-CD. It is certain that the enhancement factor of nearly 300 of fluorescence presents a potential application in the detection of THPP in pharmacokinetics and biodistribution studies.


*Keywords:* *meso*-Tetrakis (4-hydroxylphenyl) porphyrin (THPP); Cyclodextrin; Absorption spectroscopy; Steady-state fluorescence

## 1. Introduction

The photodynamic therapy of cancer (PDT) is used a combination of photosensitize drug and light giving rise to reactive oxygen species in the tumor environment, leading to tumor death. The first-generation photosensitizes, hematoporphyrin derivative (HpD) and Photofrin-II (P-II) have been currently in clinical trials worldwide for PDT treatment of a variety of solid tumors in the last years [1,2]. However, high drug and light doses were required to get a sufficient biological effect. More recently, report has shown that by using the second-generation photosensitize such as, *meso*-tetrakis(*m*-hydroxylphenyl)porphyrin (*m*-THPP), it was possible to destroy intrahepatic tumors with better efficacy and fewer side effects [3]. It is necessary to quantity the concentration of photosensitize porphyrin in biological media in the pharmacokinetics and biodistribution studies. Whereas the reported methods, such as high-performance liquid chromatography (HPLC) [4], electrochemical analysis [5], and optical fiber fluorimetry need tissue extraction [6]. Then, the detection method is required to improve.

Cyclodextrins (CDs) are cyclic oligomers of α 1–4 linked D-glucose and are able to complex with a variety of molecules and especially water-insoluble organic ones in aqueous solution. This property leads to widespread applications involved in the field of drug delivery. And CDs has been used as carries to improve the aqueous solubility [7], stability against chemical and photochemical degradation [8] and to control drug release [9] and so on. As some of the included molecules exhibit native fluorescence due to their aromaticity, the key analytical applications were that the formation of supramolecular complexes of analytes with CDs resulted in an increase of their fluorescence quantum yield or even in the appearance of room temperature phosphorescence (RTP) [10–12]. This enhancement appears of particular interest

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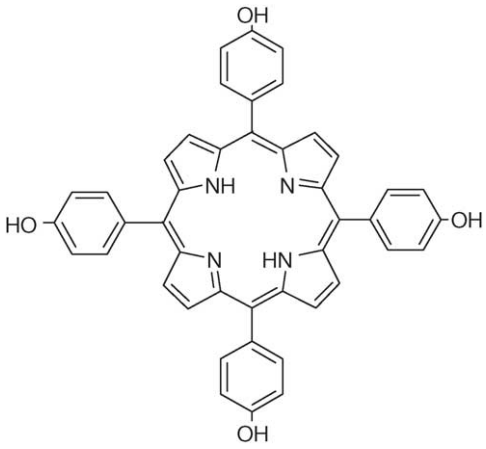


Fig. 1. The chemical structure of THPP.

from the analytical point, either for direct detection of the guest molecule or as a detection mode after separation techniques, such as chromatography and electrophoresis [13–15].

A strong fluorescence enhancement of *m*-THPP has been observed with natural and modified cyclodextrins. Especially for Me-β-CD (di-substitution DS = 1.8), an about 300 factor of fluorescence emission of *m*-THPP was obtained, which shows considerable analytical interest for direct detection of *m*-THPP [16]. In the same field of PDT, Mosinger et al. have prepared a soluble supramolecular sensitizer by the formation of inclusion complexes between *meso*-terakis(4-sulfonatophenyl)porphyrin(TPPS) and 2-hydroxypropyl-cyclodexrin (hp-CD) (mono-substitution MS = 0.6) [17].

It is well known that THPP is an amphiphilic porphyrin (Fig. 1). Among of the three isomers (*o*-, *m*-, *p*-THPP), *o*-THPP is toxic to skin. Whereas, it has been proved that *m*- and *p*-THPP, with high photosensitivity and organic selectivity [18], may be used as potential drug molecules in PDT for anticancer treatment. As mentioned above, the complexes of *m*-THPP with natural and modified cyclodextrins have been studied. The aim of this work was the synthesis of an amphiphilic porphyrin (*p*-THPP) and the characterization of the spectral properties of *p*-THPP in virtue of the formation of inclusion complex between *p*-THPP and β-CD and its derivatives. The results show that THPP forms 1:1 inclusion complex with β-CD and 1:2 inclusion complexes with HP-β-CD and CM-β-CD. A nearly 300 times of fluorescence intensity of THPP was observed in the presence of HP-β-CD. This enhancement shows potential application values in the direct detection of biological media and in the photodynamic therapy (PDT) for anticancer treatment.

## 2. Experimental

### 2.1. Reagents

β-CD (Yu-nan Gourment Factory, China) is purified by recrystallization in doubly distilled water. HP-β-CD (average MW = 1657), has a degree of substitution (DS = 9.0). CM-β-CD (DS = 4.8) are prepared based on our previous research [19]. Other reagents used are of analytical reagent grade and doubly distilled water is used throughout. THPP is synthesized according to the literature [20]. 4-Hydroxy benzaldehyde (915 mg) was dissolved in 50 ml propionic acid. The solution was heated to 140 °C. A volume of 0.52 ml newly distilled pyrrole was added slowly and the mixture was refluxed for 1 h, followed by the addition of 50 ml anhydrous alcohol. The solution was transferred into a big beaker, cooled to room temperature and placed for 1 h at −5 °C. Filtered and the filter cake was washed thoroughly with the mixture of propionic acid and alcohol (1:1), and $CHCl_3$, respectively. The obtained blue powder was dissolved with DMSO and the solution was recrystallized by heat to give violet solid of THPP. The reaction yield is 6%. $^1$HNMR $\delta_H$(DMSO-$d_6$): 9.99(s, 4H, −OH), 8.85(s, 8H, β-Pyrrole), 7.97(d, 8H, 2,6-phenyl), 7.18(d, 8H, 3,5-Phenyl), −2.92(s, 2H, NH-pyrrole). $\lambda_{max}$(DMSO): 426 nm (Soret), 520, 560, 60, 653nm. Anal. calcd. for $C_{44}H_{30}N_4O_4$: C 77.86, H 4.46, N 8.26; found: C 77.65, H 4.43, N 8.20.

### 2.2. Apparatus

All absorption and fluorescence measurements are performed with UV-265 spectrophotometer (Shimadzu) and F-4500 spectrofluorimeter (Hitachi). Excitation and emission bandwidths are set at 10 and 20 nm, respectively. The pH meter (E-201-C) is made in the factory of magnetic spectrograph in Shanghai. Elemental analysis was determined with Elementar Analysensysteme GmbH VarioEL instrument. All experiments are carried out at 20 ± 1 °C.

### 2.3. Method

A 1 ml aliquot of the stock solution ($2.5 \times 10^{-5}$ mol $L^{-1}$) of THPP in dimethyl sulfoxide (DMSO) is transferred into a 10 ml volumetric flask and an appropriate amount of 0.001 mol $L^{-1}$ β-CD (or HP-β-CD or CM-β-CD) is added. The pH is controlled by the 0.5 mol $L^{-1}$ phosphate buffer solution. The mixed solution is diluted to final volume with distilled water and shaken thoroughly, following equilibrated for 30 min at 20 ± 1 °C. The spectra are recorded or fluorescence and absorption intensities are measured.

## 3. Results and discussion

### 3.1. Formation of inclusion complexation of THPP and CDs

Fig. 2 shows the effect of HP-β-CD on the fluorescence spectra of THPP in basic media (pH = 10.00). The maximum excitation wavelengths were set at 432 nm. With the increasing concentration of HP-β-CD, the remarkable enhancement of the excitation and emission intensities were observed. The measured emission wavelength was 656 nm and kept hardly

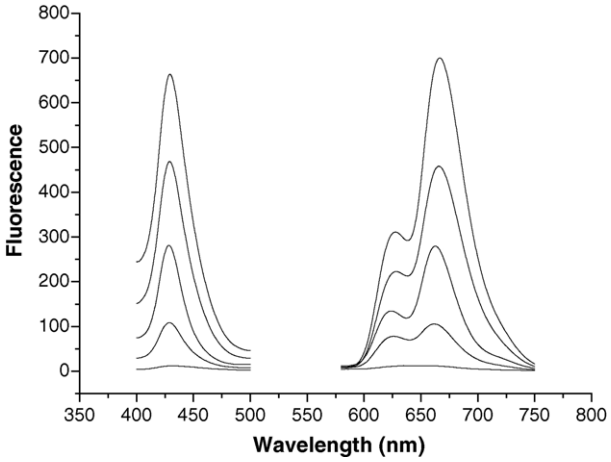


Fig. 2. Effect of HP-β-CD on excitation and emission spectrum of THPP ($2.5 \times 10^{-6}$ mol L$^{-1}$) in basic media (pH = 10.00). The concentration of HP-β-CD from bottom to top is 0, $1.0 \times 10^{-5}$, $3.0 \times 10^{-5}$, $6.0 \times 10^{-5}$, $16 \times 10^{-5}$ mol/L.

changed, which implies the formation of inclusion complexes between THPP and CDs. It should be mentioned that the fluorescence intensity of THPP in the presence of HP-β-CD was increased nearly 300 times and a increasing factor about 100 were detected forβ-CD and CM-β-CD. Fig. 3 shows the dependence of fluorescence intensity of THPP on the concentration of β-CD, CM-β-CD and HP-β-CD. Comparative to inclusion complexes, the low yield free molecular may be due to the easy formation of non-emitting oligomers of porphyrins or to quenching following charge transfer or deprotonation of phenolic groups in the excited state. However, the inclusion provides a more rigid microenvironment and restricted available space to decrease the radiationless deactivation. Furthermore, it brings some protection for molecules quenched by water or transition metal ions [21].

Fig. 4 shows the UV–vis spectra of THPP ($2.5 \times 10^{-6}$ mol L$^{-1}$) in pH3.0 buffer solution containing various

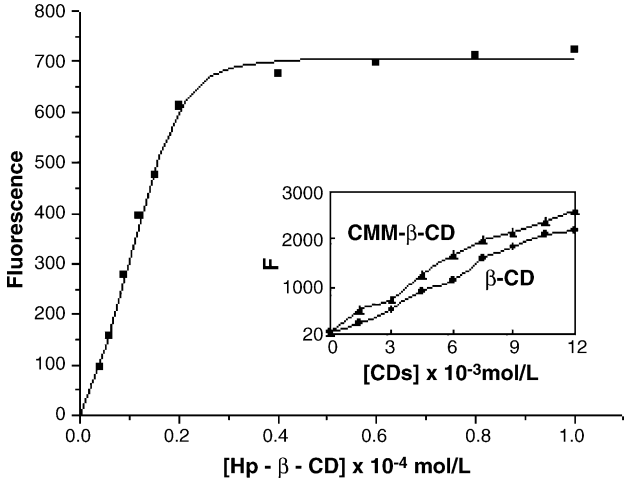


Fig. 3. Dependence of fluorescence intensity of THPP ($2.5 \times 10^{-6}$ mol L$^{-1}$) on the concentration of β-CD, CM-β-CD and HP-β-CD in pH 3.0 buffer solution.

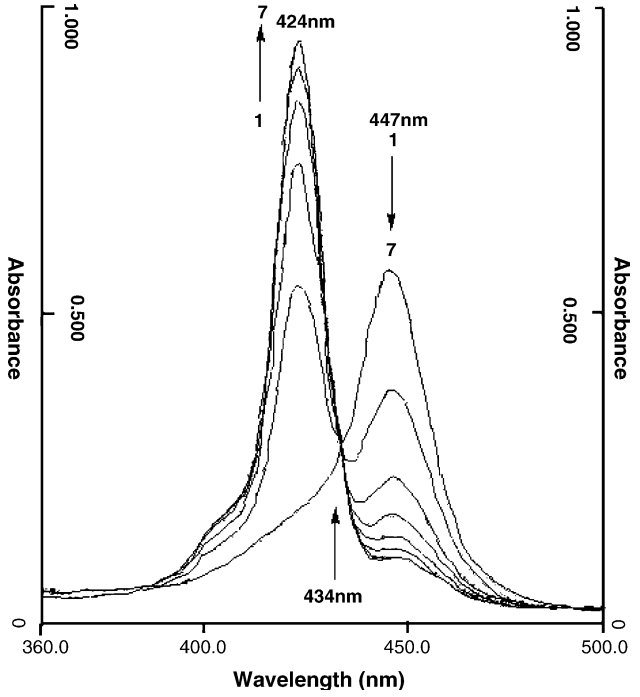


Fig. 4. UV–vis spectra of THPP ($2.5 \times 10^{-6}$ mol L$^{-1}$) containing various concentration of HP-β-CD in the acid media (pH = 3.0).

concentration of HP-β-CD. In the acid media, THPP is exists in diprotonated state ($H_4THPP^{2+}$). The Soret band is at 447 nm, which is different from with the maximum absorption wavelength of unprotonated form ($H_2THPP$) and for the latter Soret band is at 424 nm. With the addition of HP-β-CD, the maximum absorption peak is blue shifted from 447 to 424 nm, companied by decreasing absorption at 447 nm and increasing absorption at 424 nm, which demonstrates that the deprotonated form ($H_2THPP$) is more appreciate to inclusion by HP-β-CD than diprotonated form ($H_4THPP^{2+}$). It is interesting to appear the isosbestic point at 434 nm, which confirms the forms of inclusion complex and implies the dissociation equilibrium as follow: $H_4THPP^{2+} \rightleftharpoons H_2THPP$ (dissociation constant: $K_a$).

### 3.2. Effect of pH

Fig. 5 shows the effect of pH on the UV–vis spectroscopy of THPP in the presence of β-CD and HP-β-CD. It can be seen that the inclusion interaction were very depended on pH. At lower (pH < 3.0) or higher pH (pH > 10.0), there is no obvious change observed not only for absorbance but also for corresponding Soret bands. As the system was controlled by pH 3.0–10.0 buffer solution, a noticeable increase of THPP absorbance was detected with the addition of CDs, especially for HP-β-CD (Fig. 5a). At the same time, the maximum absorption peak was blue-shifted about 3–9 nm (Fig. 5b).

Table 1 provided the formation constants of THPP/2HP-β-CD in different buffer solution. As can be seen from the given data, the inclusion behavior of HP-β-CD to THPP

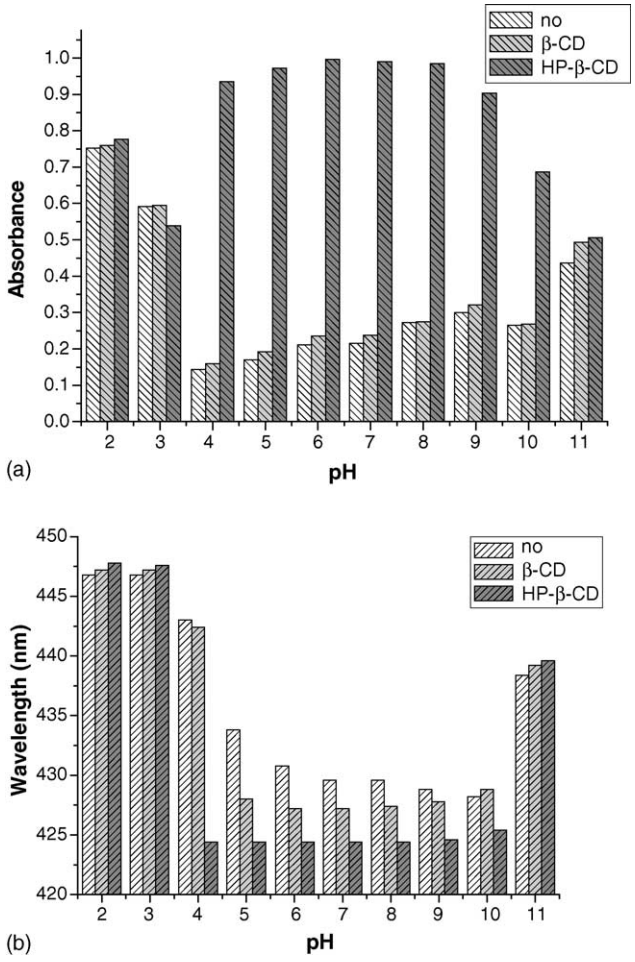


Fig. 5. The influence of pH on the absorption spectroscopy of THPP ($1.25 \times 10^{-6}$ mol L$^{-1}$) in the β-CD ($2.0 \times 10^{-4}$ mol L$^{-1}$) and HP-β-CD ($2.0 \times 10^{-4}$ mol L$^{-1}$) aqueous solution: (a) absorbance; (b) absorption wavelength corresponding to the Soret band.

is various under different buffer solution, which suggests that the pH has an influence on the inclusion interaction between THPP and HP-β-CD. The inclusion complex interaction, expressed by the formation constant, follows the order: pH = 7.0 > pH = 10.0 > pH = 3.0, which suggests that the inclusion ability of system in the neutral solution is strongest and too low or too high pH is not suitable for the interaction, which is in agreement with the result of UV–vis spectroscopy. The possible reason is that THPP is cation in acid medium and anion in basic medium, which enhance the polarity of the ground state. And it is disadvantageous compared to the hydrophobic cavity of cyclodextrins.

Table 1
Formation constants $K$ ($M^{-1}$ for β-CD and $M^{-2}$ for dCM-β-CD, HP-β-CD) of inclusion complex THPP/CDs at pH values

| Cyclodextrins | pH 3.0 | pH 7.0 | pH 10.0 |
|---|---|---|---|
| β-CD | – | 46.0 ± 1.1 | – |
| CM-β-CD × $10^4$ | – | 3.30 ± 0.82 | – |
| HP-β-CD × $10^8$ | 1.53 ± 0.70 | 4.89 ± 0.4 | 2.50 ± 0.62 |

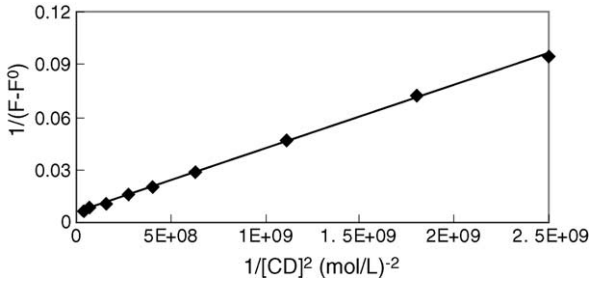


Fig. 6. Double reciprocal plots for THPP complex to HP-β-CD at pH 3.00 media.

### 3.3. Formation constants of inclusion complexes

The formation constant ($K$) is an important parameter, which represents the inclusion interaction. The formation constant can be obtained from fluorescence data by the modified Benesi–Hildebrand equation [22]:

$$\frac{1}{F - F_0} = \frac{1}{K \times k \times Q \times [P]_0} \times \frac{1}{[CD]_0{}^n} + \frac{1}{k \times Q \times [P]_0}$$

where $[P]_0$ denotes the initial concentration of THPP and $[CD]_0$ denotes that of CDs. $F$ and $F_0$ are the fluorescence intensities of THPP in the presence and absence of CDs, respectively, and $k$ is the instrument constant and $Q$ is the fluorescence quantum yield of the inclusion complex. Fig. 6 shows the double reciprocal plots of $1/(F - F_0)$ versus $1/[CD]^2$ for THPP complexed with HP-β-CD at pH 3.0. The plot exhibited good linearity (the linear correlation coefficient $R = 0.999$). This implied the formation of inclusion complexes with a stoichiometry of 1:2 between THPP and HP-β-CD. A 1:1 and 1:2 stoichiometry has been observed for THPP complex with β-CD and CM-β-CD. The association constant values were calculated assuming the existence of complexes with 1:1 or 1:2 stoichiometry. The related association constants for HP-β-CD, β-CD and CM-β-CD with THPP were $(1.53 \pm 0.70) \times 10^8$ (mol/L)$^{-2}$, $(46.0 \pm 1.1)$ (mol/L)$^{-1}$, $(3.30 \pm 0.82) \times 10^4$ (mol/L)$^{-2}$, respectively (seen in Table 1). The large difference of inclusion constants for HP-β-CD, CM-β-CD, and β-CD may be explained that comparing with native β-CD, the chemically modified β-CD are endowed with specially functional groups and the solubility and flexibility of CDs have been improved in the great degree. Moreover, the strongest inclusion capacity of HP-β-CD was caused by the strong hydrogen bond between THPP and HP-β-CD.

### 3.4. The related inclusion mechanism

Fig. 7 shows the effect of solvent polarity on the spectroscopic characterization of porphyrin in pH3.0 media. In the

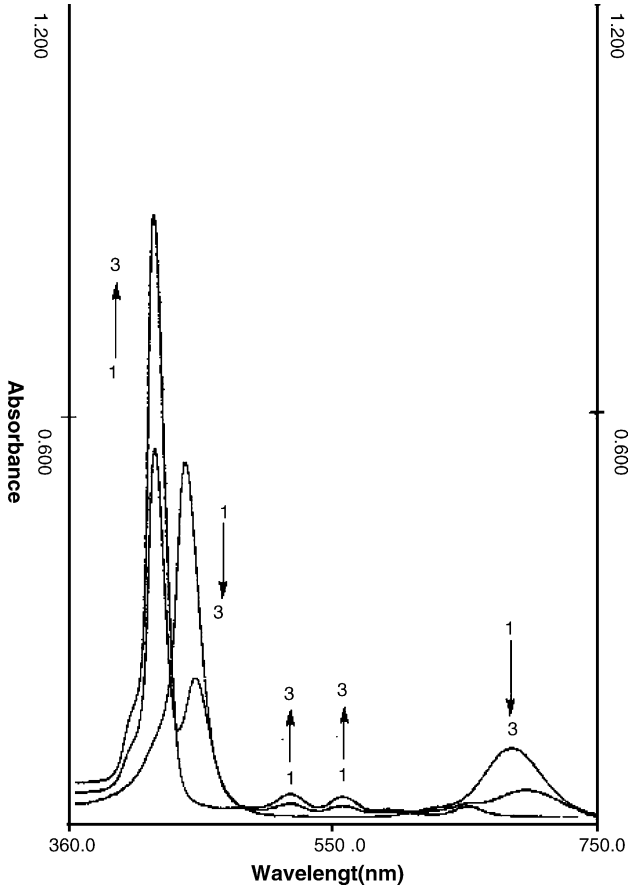


Fig. 7. Effect of solvent polarity on the spectroscopic characterization of porphyrin in pH 3.0 media: 1 → 3, $r$ is 19, 1.0, 0.67 ($r$ is the value of $V_{H_2O}$ vs. $V_{DMSO}$).

strong polarity solution $r = 9$ ($r$ is the ratio of the volume of $H_2O$ and DMSO), one Soret band and one $Q$ band, which are the characteristic peaks of diacid form of THPP, were observed at 447 and 688 nm, respectively. With the decreasing $r$, the original Soret band at 447 nm occurs split and a new peak appears at 424 nm (free base form in aqueous), followed by the increasing absorbance of later and the decreasing absorbance of former. As $r < 1.0$, the peak at 447 nm disappeared completely. At the same time, four $Q$ bands (the characteristic peaks for free base form) appeared. The results of solvent influence are very similar with the addition of HP-β-CD, which proves further fact that the cavity of HP-β-CD supplies the same hydrophobic microenvironments for the THPP molecular as DMSO. The transform of the form of porphyrin in the solvent with different polarity can be explained that, comparative to $H_2O$, DMSO belongs to typical Lewis base and has a smaller p$K_a$. Thus, the lost of proton of THPP becomes easier in DMSO. As a result, with the change of the volume of DMSO in the mixed solvent, the transform of diprotonated porphyrin to unprotonated porphyrin takes place. Comparative with the effect of HP-β-CD, it is different that no isosbestic point has been observed in the course of the change of solvent polarity. The possible reason is that HP-β-CD is able to offer better hydrophobic environments and more easily induce the equilibrium: $H_4THPP^{2+} \rightleftharpoons H_2THPP$ than DMSO.

## 4. Conclusion

The inclusion interaction between THPP and the β-CD and its derivatives has been observed by spectrometry. The fluorescence spectroscopy has shown that the HP-β-CD results in nearly 300 times enhancement of fluorescence intensity of THPP. Due to the strong hydrogen bonds, THPP is prefer to interact with HP-β-CD comparative to β-CD and CM-β-CD. A distinct equilibrium, $H_4THPP^{2+} \rightleftharpoons H_2THPP$, has been observed in the solution of HP-β-CD on the base of the UV–vis experimental. Furthermore, the results of the paper present potential application not only in analysis and in pharmacokinetics and biodistribution studies.

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