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Anti-oxygen-quenching room temperature phosphorescence stabilized by deoxycholate aggregate

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Abstract

Oxygen is the most effective dynamic quencher in liquid room temperature phosphorimetry (LRTP). Using oxygen scavenger to achieve significant or more sensitive RTP signals may bring about kinds of trouble in many cases and is apparently not very convenient in procedures. To date a few examples on LRTP without deoxygenation have been reported. Herein, we present a new named anti-oxygen-quenching RTP system using deoxycholate as a rigid medium. It can induce both lipophilic α-bromonaphthalene or 1-bromo-2-methylnaphthalene (BrMeN) and strongly water-soluble meso-tetrakis-(4-trimethylaminophenyl)porphyrin palladium (Pd-TAPP) to produce strong RTP emission under sodium deoxycholate of 4 mmol l^{-1} and incubation time of half an hour. The maximum excitation and emission wavelengths respectively lie at 408 and 680 nm, with phosphorescence lifetime of 0.39 ms for Pd-TAPP, and at 285 and 515 nm, with lifetime of 4.9 ms for BrMeN. It is supposed that the hydrophobic and oxyphobic sandwich structure formed by two deoxycholate molecules with a phosphor in the case of deoxycholate or the interdiction fully of oxygen molecule diffusion into the microenvironment where the phosphor exists in the case of cyclodextrin is a key factor in inducing anti-oxygen-quenching RTP.

Keywords: Room temperature phosphorescence; Anti-oxygen quenching; Deoxycholate; Aggregate

1. Introduction

Room temperature phosphorescence (RTP) has much more advantages over other luminescence methods, e.g., large stokes shift, high signal-to-noise ratio, good selectivity and easily measurable luminescence lifetimes, etc. [1,2], so it has been widely used in many fields such as pharmaceutical analysis, environmental monitoring of pesticide residue and polyaromatic hydrocarbons [3–6]. Recently, RTP also found use in studying interaction mechanism of small-molecule drugs with biological target molecules such as nucleic acid or protein as well as protein structure and conformation dynamics, etc. [7]. However, the most tiring thing is that RTP can be quenched by molecular oxygen during measurement. There-

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fore, a wide variety of deoxygenation means have been carried out, removing oxygen by purging N_2 [8], by CO_2/H_2 [9] produced in situ from the reaction of Na_2CO_3/active Zn and HCl, and by the chemical oxygen scavenger sodium sulfite [10]. Especially, the latter made it greatly convenient to detect RTP. But there are still kinds of trouble to treat such as the changeable acidity against the addition of the chemical oxygen scavenger and deposits caused by the reaction of sodium sulfite and some metal ions. Therefore, it is a significant project to attempt to establish a non-deoxygenated RTP measurement system.

To date a few examples on liquid room temperature phosphorimetry (LRTP) without deoxygenation have been reported. Wei and Liu [11] noticed that 7-methylquinoline, in the presence of β-cyclodextrin (β-CD) and dissolved oxygen, could produce significant phosphorescence after the addition of 1-bromocyclohexane (BCH). Jin et al. [12] observed that the strong RTP emission of some polyaromatic hydrocarbons and nitrogen heterocycles could be conveniently detected in β-CD/cyclohexane system without removing oxygen and with no heavy atom perturber, and proposed the "inclusion complexation-suspension microcrystals" twice rigidization mechanism. Recently, Sanz-Medel's group found that 6-bromo-2-naphthyl sulfate or α-bromonaphthalene (BNS or BrN) encapsulated in a rigid sol–gel matrix could display significant RTP emission in aqueous media without deoxygenation, and developed the first phosphorescent pH sensor based on energy transfer from immobilized pH-insensitive phosphor (BNS or BrN) molecules to pH-sensitive mixed dyes [13]. Trace mercury in water was also determined in β-CD/BCH dithizone system without removing oxygen [14]. The results of our recent experiments have shown that α-BrN in deoxycholate medium could produce strong RTP intensity (I_{RTP}) [15]. Herein, the results further indicated that lipophilic 1-bromo-2-methylnaphthalene (BrMeN) and strongly water-soluble meso-tetrakis-(4-trimethylaminophenyl)porphyrin palladium (Pd-TAPP) could also produce analyzable phosphorescence which was named as anti-oxygen-quenching or non-deoxygenated RTP.

2. Experimental

2.1. Apparatus and reagents

Fluorescence spectra were recorded on F-4500 spectrophotometer (Hitachi) at room temperature and all corrected RTP spectra were performed on LS-50B spectrophotometer (Perkin–Elmer Co.) with a xenon-pulsed lamp. Obey-Decay application program was used for phosphorescence lifetime measurements. The excitation and emission slits were typically set at 15 and 20 nm, respectively. The delay time and the gate time were set at 0.02 and 2 ms, respectively. The accurate acidity was measured on pHS-2 acidometer (Shanghai).

The stock solution of 1×10^{-4} mol l^{-1} Pd-TAPP was prepared in situ [6,16] and diluted to a certain concentration when used. Sodium deoxycholate (NaDC, $>98\%$), sodium taurodeoxycholate (NaTDC, $>98\%$) and BrMeN were from ACROS ORGANICS Co. and BrMeN was purified further by distillation. Water used here was doubly distilled. All other reagents were of analytical grade.

2.2. Procedures

Typically, 0.2 ml of Pd-TAPP aqueous solution (1×10^{-4} mol l^{-1}) or 0.5 ml of BrMeN methanol solution (1×10^{-4} mol l^{-1}) was transferred into a comparison tube of 5 ml and deoxycholate of appropriate volume was added. The working solution was stood over half an hour and then transferred into a 10-mm quartz cell with a cover. It should be noticed that Pd-TAPP–NaDC system was lactescent. Despite this, the depositing speed was so slow that it would cause little effect on the whole process of RTP measurement and the whole experiment had an excellent precision. As a matter of fact, similar study was once carried out in the lactescent CD system [12,17]. To avoid much difference, adequate shaking was done for all the working solution before measurements. While in the transparent BrMeN–NaDC system, it was not necessary to do the similar treatments. The acidity and ionic strength of all the samples were not tuned by any buffer solution except for investiga-

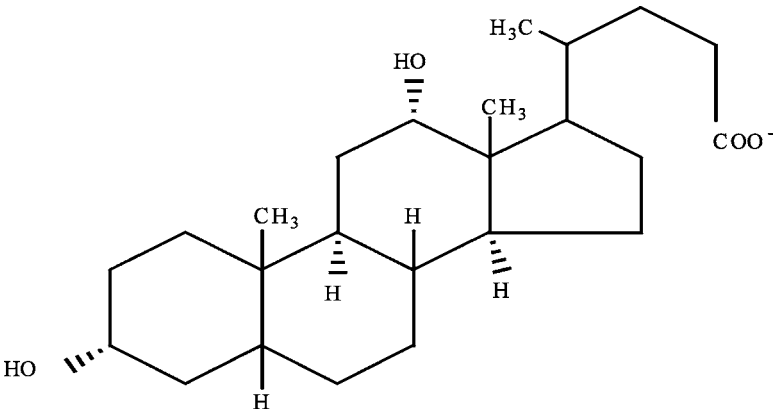

Fig. 1. The structure of NaDC.

tion on purpose. pH value was ca. 6.0 without special statement in the whole paper.

3. Results and discussion

3.1. Phosphorescence characteristics of Pd-TAPP in NaDC

Even if molecular oxygen is absent, Pd-TAPP could not produce phosphorescence in fully aqueous solution. After the removal of dissolved oxygen by Na_2SO_3, the water-soluble Pd-TAPP could be used as sensitive phosphorescent probe when it interacted with anionic surfactant SDS or calf thymus DNA (ctDNA) [4,7]. Here, without deoxygenation, Pd-TAPP also displayed easily detectable phosphorescence in NaDC medium, which provided a rigid enough environment for the phosphor. And the maximum excitation and emission peaks lied at 408 and 680 nm, respectively. However, no RTP emission was observed in NaTDC because of its much looser structure [18] in comparison with that of NaDC.

When [Pd-TAPP] was fixed at 4 μmol l^{-1}, I_{RTP} of the probe increased gradually with the titration of NaDC and reached its maximum when [NaDC] was 4 mmol l^{-1} (shown in Fig. 2a). Then, phosphorescence would decay till it was almost completely disappeared when [NaDC] reached over 8 mmol l^{-1} (shown in Fig. 2b).

The plot of phosphorescence lifetimes (shown in Fig. 3) indicated that the lifetime (τ) of the probe increased at first and then decreased with the increasing [NaDC]. The maximum lifetime was 0.39 ms (when [NaDC] was 4 mmol l^{-1}), which was far less than that of the probe in SDS and ctDNA ($\tau_{SDS} = 0.56$ ms, $\tau_{DNA} = 0.81$ ms) [19],

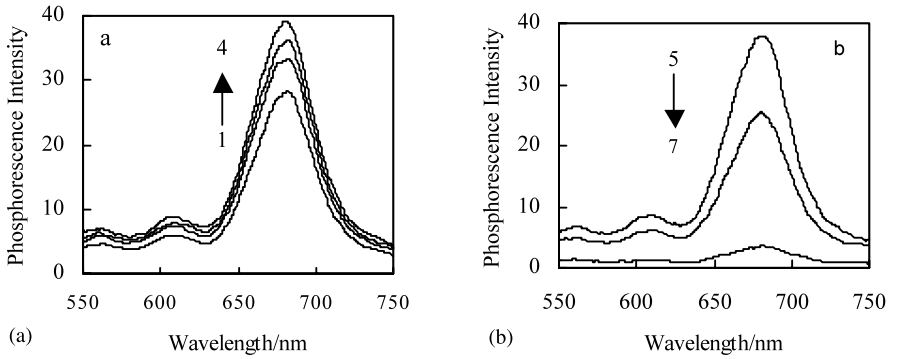

Fig. 2. RTP emissions spectra of Pd-TAPP in the presence of NaDC ([NaDC] from 1 to 7: 1, 2, 3, 4, 5, 6, 7 mmol l^{-1}).

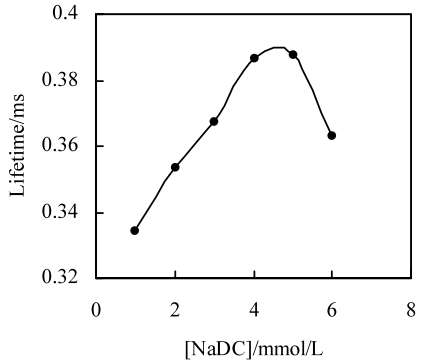

Fig. 3. Phosphorescence lifetime curve in the presence of NaDC.

while the quantum yield was only 1/5 of that in SDS or 3/4 of that in ctDNA. It was indicated that probe molecule experienced the whole process from thoroughly oxygen-exposed without NaDC to non-oxygen and further to oxygen-exposed environment in higher concentration of NaDC.

3.2. The effect of ionic strength on RTP of Pd-TAPP–NaDC

Fig. 4 showed that I_{RTP} of Pd-TAPP–NaDC system obviously decreased with the increasing [NaCl], and the optimal [NaDC] moved to lower values those were correspondent with different maximum intensities. For example, when [NaCl] was 30 mmol l^{-1}, I_{RTP} reached its maximum with [NaDC] of 3 mmol l^{-1} instead of 4 mmol l^{-1}. When [NaCl] was 70 mmol l^{-1}, phosphorescence

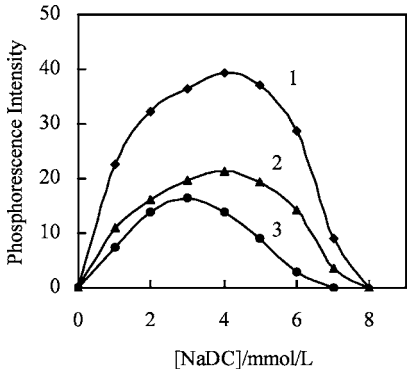

Fig. 4. Effect of I_{RTP} of Pd-TAPP–NaDC by NaCl.

Table 1
Effect of salt concentration on RTP maximum intensity

[NaCl] (mM)	Optimal [NaDC] (mM)	Maximum I_{RTP}[a]
0	4	1.0
10	4	0.52
30	3	0.38
70	–	–

[a] The first I_{RTP} was selected as the base value of 1.0 and the others were the comparison values.

signals were almost quenched thoroughly. The corresponding results were listed in Table 1.

3.3. The RTP of BrMeN in NaDC

Compared with water-soluble Pd-TAPP, the smaller and lipophilic probe BrMeN was selected to further elucidate the non-deoxygenation phosphorescence behavior in deoxycholate medium. BrMeN gave extremely strong RTP without the removal of oxygen (shown in Fig. 5), and the maximum excitation and emission wavelengths lied at 285 and 515 nm, respectively. The varying trend of its phosphorescence intensities (a) and lifetimes (b) with NaDC concentration was almost similar to that of Pd-TAPP, as shown in Fig. 6. Surprisingly, the maximum lifetime of BrMeN reached 4.9 ms, which was very close to 5.0 ms of BrN in sol–gel vitreous body [13] and was over 10 times than hydrophilic Pd-TAPP in the same medium, while the quantum yield of BrMeN was over 30 times than that of Pd-TAPP. The results showed that BrMeN was probably surrounded in a very rigid microenvironment.

3.4. Fluorescence comparison of Pd-TAPP and BrMeN probe in NaDC

The fluorescence characteristics of the two obviously different probe molecules in structure and polarity or solubility in NaDC were investigated and the results showed that the fluorescence intensity reached its maximum for each probe when [NaDC] was 4 mmol l^{-1}. Then, with the titration of NaDC, fluorescence intensity decreased gradually till it disappeared thoroughly (shown in Fig. 7).

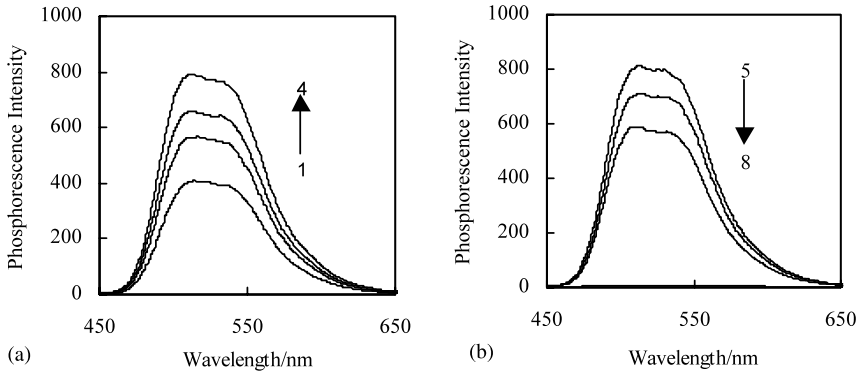

Fig. 5. RTP emission spectra of BrMeN in the presence of NaDC ([BrMeN] = 10 μmol l^{-1}; [NaDC] from 1 to 8: 1, 2, 3, 4, 5, 6, 7, 9 mmol l^{-1}).

3.5. The effect of standing time

In order to investigate the dynamic interaction between NaDC and phosphor, I_{RTP} of Pd-TAPP and BrMeN was detected every certain time. The results shown in Fig. 8 indicated that the intensity increased dramatically at the very beginning and the increasing scale was more evident in the case of BrMeN. When standing time was over half an hour, the interaction between Pd-TAPP and NaDC reached saturation, while I_{RTP} of smaller molecule probe BrMeN still increased slowly. A platform in the curve (I/I_0 vs standing time) of BrMeN appeared when standing time was over 8 h. The results meant that a very slow dynamic process appeared after a quite fast interaction between the probe and NaDC aggregate; however, eventually the probe would dwell in the rigid environment provided by NaDC.

3.6. The effect of pH

The effect of pH from 1.28 to 12.48 on RTP of Pd-TAPP–NaDC was studied in Britton-Robison buffer solution. The status of Pd-TAPP–NaDC system under different acidity was listed in Table 2.

When pH value was not quite low, the solution of Pd-TAPP–NaDC was cloudy and could emit fairly strong RTP. In contrast to that, almost no RTP could be detected when pH was over 7. As shown in Fig. 9, Pd-TAPP produced faint RTP when pH was very low, but the I_{RTP} increased rapidly with the acidity decreased until I_{RTP} reached the maximal value (pH 5.14). The optimal pH range was 4–6. But here, the acidity of the whole experiment was not tuned by any buffer solution because NaDC was easily deposited against higher ionic strength.

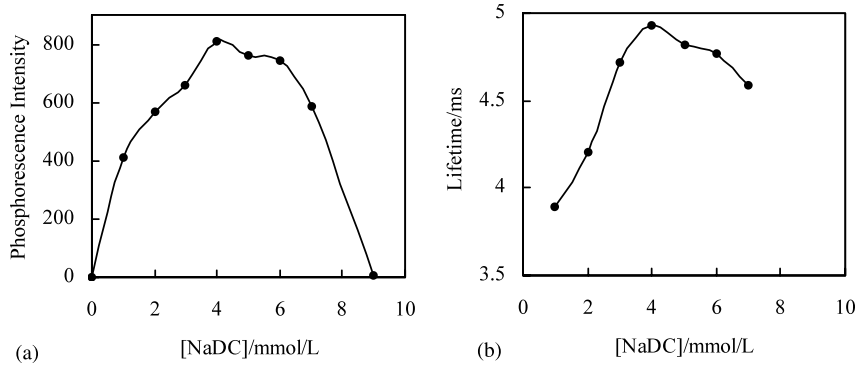

Fig. 6. Phosphorescence intensities and lifetimes of BrMeN–NaDC.

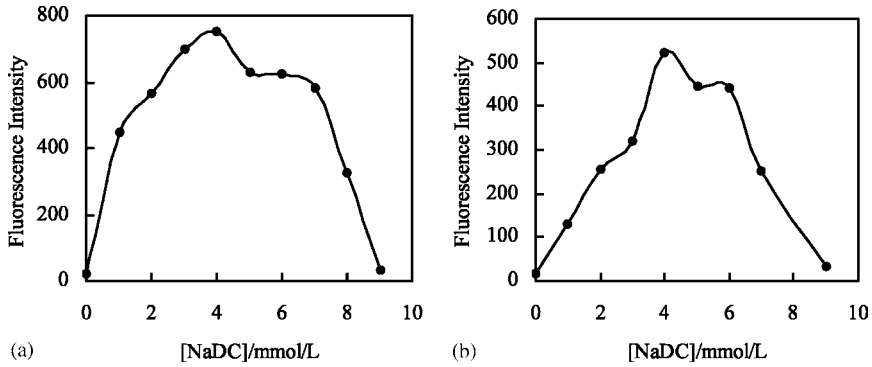

Fig. 7. Fluorescence characteristics of Pd-TAPP (a) and BrMeN (b) in the presence of NaDC ([Pd-TAPP]: 4 μmol l^{-1}; [BrMeN]: 10 μmol l^{-1}).

3.7. Effect of dilution

The effect of diluting the two phosphorescent systems on I_{RTP} was studied to further understand the behaviors of NaDC clustering. As mentioned above, in NaDC of 8 mmol l^{-1} no phosphorescence of Pd-TAPP was observed at all. However, after dilution, phosphor fast regained its RTP emission as shown in Table 3. The data meant that the structural change of NaDC aggregates from dimer to helical aggregate was rapid and convertible.

3.8. Discussion

Two possible mechanisms were proposed in inducing anti-oxygen-quenching RTP. One was the interdiction fully of oxygen diffusion into the microenvironment that protected the phosphor from being quenched and the other the formation of oxyphobic domain where the probe dwelt in. Non-deoxygenated phosphorescence in CD system might belong to the former [11,12,20] and herein NaDC system might belong to the latter.

Deoxycholates, a kind of biological surfactant, consist of steroid skeleton substituted by hydroxyl group with a negatively charged tail chain [21] (shown in Fig. 1). The molecules have special 3D structure: steroid skeleton with methyl groups position on the convex side and hydroxyl groups lie in the concave side. The resulting molecular conformation provides a hydrophilic *surface* on one side and a hydrophobic *surface* on the other. The conventional surfactants generally have hydrophilic *head groups* and long hydrophobic *tail*

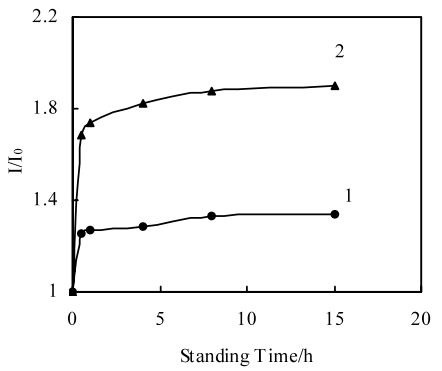

Fig. 8. Effect of standing time on RTP of Pd-TAPP (1) and BrMeN (2) ([Pd-TAPP]: 4 μmol l^{-1}; [BrMeN]: 10 μmol l^{-1}; [NaDC]: 4 mmol l^{-1}). I_0 and I: the intensities of samples without and with standing time, respectively.

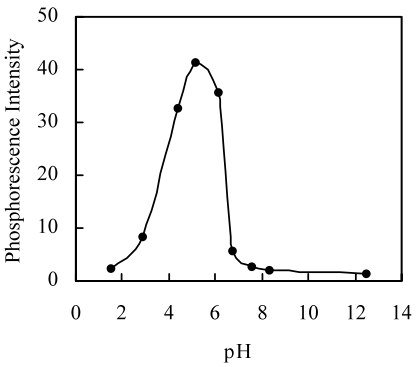

Fig. 9. Effect of pH on I_{RTP} of Pd-TAPP–NaDC ([Pd-TAPP]: 4 μmol l^{-1}; [NaDC]: 4 mmol l^{-1}).

Table 2
Status of Pd-TAPP–NaDC system under different acidity

pH	Status of Pd-TAPP–NaDC system[a]
> 6.70	Transparent solution
~ 6.70	Transparent vitreous gel
5.50–6.70	Lactescence
< 5.50	Delaminated system (top: cloudy; bottom: transparent)

[a] [Pd-TAPP]: 4 μmol l^{-1}; [NaDC]: 4 mmol l^{-1}.

chains, so they usually form globular micelles with the hydrophilic heads extended into the bulky water phase and the tail groups aggregated together to form the hydrophobic core. The micelle has an obvious critical micelle concentration (CMC), in which solubilization sites only include the surface of micelle, palisade layer and hydrophobic core [21]. But deoxycholate one has a very broad CMC range. The clustering process is complicated and generally in step-wise manner (from the initial dimer or trimer to larger micellar aggregation units), and the hydroxyl and tail groups play a vital role in forming the aggregates [22].

The above-mentioned results indicated that the phosphorescence intensities, lifetimes and the fluorescence intensities of Pd-TAPP and BrMeN probes increased firstly and then decreased till thoroughly disappeared at last with the titration of NaDC. NaDC gradually formed dimers, which might have the characteristics of both hydrophobicity and oxyphobicity (the latter requires further proving), with the increasing [NaDC] (lower than CMC). The dimers and the probes sandwichedly combined by hydrophobic and/or electrostatic interactions, which protected probe molecules from being quenched. NaDC and the hydrophobic moieties of BrMeN or Pd-TAPP formed a sandwich structure, which provided BrMeN a more rigid environment because of its smaller size than Pd-TAPP for producing RTP [18]. So BrMeN possessed quite long phosphorescence lifetime under the same experimental conditions, which was over 10 times than that of Pd-TAPP. When [NaDC] was over 4 mmol l^{-1}, dimer might begin to transit towards new aggregate and this inference is supported by NMR data which will be reported in another paper written by our research group. We supposed that the new aggregate should be helical micelle [23]. The narrow platform domains existed in the curves of the two probe molecules (intensity vs [NaDC]) whatever they were for phosphorescence or fluorescence measurement, which indicated that there might be a transition state namely the conversion between dimer and helical micelle was not obviously separated. The helical NaDC aggregate resulted that the probe molecules slowly broke away from protection and was exposed to outside environment as well as getting adequate touch with dissolved oxygen because of its loose structure and too large cavity of new clusters with poor hydrophobicity and oxyphobicity [15,24], so that its fluorescence or phosphorescence decayed fast. When [NaDC] was over CMC (6.4 mmol l^{-1}) [25], NaDC formed quite stable helices and both bigger and smaller probe molecules thoroughly broke away from the protection of the deoxycholate. Perhaps when [NaDC] was over 9 mmol l^{-1}, the aggregation of helical micelle was completely carried out and all molecules were exposed to more outside environment, so phosphorescence or fluorescence disappeared, as shown in Fig. 6.

Table 3
Effect of dilution on I_{RTP}

	[NaDC] (mmol l^{-1})	[Pd-TAPP] (μmol l^{-1})	After dilution, I_{RTP} for different standing time		
			0 min	10 min	30 min
Original sample	8	4	0	0	0
After dilution	4	2	49.8	52.1	54.0
Comparison sample	4	2	40.9	48.7	47.9

Lots of experiments have proved that inorganic salts can affect the clustering process of deoxycholates [26]. According to the above experimental results, NaCl contributed a lot to facilitate clustering or aggregating of NaDC, as phosphorescence intensities obviously decreased with the increasing concentration of inorganic salts, which further indicated that the probes gradually got touch with dissolved oxygen and finally experienced more outside environment because of large cavity of the helical aggregate. And NaCl played a significant role in forming loose "helical" structure of NaDC.

In summary, we obtained an anti-oxygen-quenching RTP system quite different from CD media or sol–gel matrix and expected that it could be applied to RTP measurements for pharmaceutical analysis in formulation or clinic or pesticide residue in environment samples.

Acknowledgements

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