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Alteration of room temperature phosphorescence lifetimes of quinine and quinidine by chiral additives

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Received (in Cambridge, UK) 23rd September 2003, Accepted 28th November 2003
First published as an Advance Article on the web 18th December 2003

Room temperature phosphorescence (RTP) lifetime differences between quinine and quinidine were observed in micellar solutions and the ability to alter these differences based on the use of chiral additives was demonstrated.

The ability to recognize/quantify enantiomeric forms of chiral molecules is of great importance in chemical, biological and pharmaceutical sciences. In the pharmaceutical industry, the characterization and monitoring of the enantiomeric purity of drugs have attracted much attention in recent years, as enantiomeric forms of drugs can show different pharmacological/toxicological activity and the demand for single-enantiomer drugs has increased substantially worldwide.[1] Fluorescence spectroscopy has been demonstrated to be a potentially powerful technique for the rapid and reliable analysis of chiral molecules.[2] Based on the use of phase-modulation resolved fluorescence,[3] it was recently demonstrated that fluorescence lifetime differences can be used to resolve and quantify a pair of pseudo-enantiomers with significant pharmaceutical interest, *i.e.*, quinine (QN) and quinidine (QD), without the need for physically separating QN and QD.

The recent development of room temperature phosphorescence (RTP) has opened up new opportunities for the application of phosphorescence-based techniques in routine chemical analysis.[4] Since the first observation of RTP in sodium dodecyl sulfate (SDS) micellar solutions by Kalyanasundaram *et al.* in 1977,[5] RTP has been developed into a relatively simple and effective analytical technique for the determination of trace amounts of many organic and inorganic compounds with high signal to noise ratio, good selectivity and a wide linear dynamic range.[6] To the best of our knowledge, however, the feasibility of employing RTP-based methodologies for chiral analysis has never been reported. In the present work, the observation of RTP lifetime differences between QN and QD in deoxygenated SDS micellar solutions using thallium as an external atom was demonstrated and the ability to alter these lifetime differences was made possible by the addition of chiral "modifiers", such as chiral counter-ions and surfactants, to the SDS micellar solutions.

Fig. 1 shows the fluorescence and long-lived emission/RTP spectra of QN and QD. Relatively intense fluorescence signals were obtained for QN and QD in aqueous solution with the emission maximum centered at *ca.* 379 nm. With an addition of thallium nitrate (heavy atom perturber), sodium sulfite (chemical deoxygenator), and SDS micelles (organized/stabilizing medium) to the aqueous sample solution containing QN and QD, the fluorescence intensity of this pair of pseudo-enantiomers was found to decrease with a concomitant increase of the RTP signal. Note that long-lived emissions observed for QN and QD in Fig. 1 with wavelength maximum centered at *ca.* 379 nm were likely due to delayed fluorescence.[7] Optimal experimental conditions employed for the generation of RTP intensity and lifetime for QN and QD, with wavelength maximum centered at *ca.* 543 nm as shown in Fig. 1, were as follows: thallium nitrate (12.5 mM), sodium sulfite (3 mM) and SDS micelles (50 mM) at pH = 8.0. Note that different forms of QN and QD (*i.e.*, as dication, monocation and/or neutral molecule) are known to exist as a function of pH.[8] The distribution of QN and QD species under the present experimental conditions requires detailed investigations in future studies. In the present work, it was found that at pH greater or less than 8.0, the RTP intensity and lifetime decreased significantly for both QN and QD (*e.g.*, decreased by more than 30% at *ca.* pH = 7 or 10).

A critical parameter that governs the suitability of employing luminescence lifetime spectroscopy for the resolution and quantitation of chiral molecules is the magnitude of lifetime differences. Using water as the solvent, the fluorescence lifetime for QN and QD was found to be 4.429 ± 0.268 ns and 4.464 ± 0.272 ns, respectively and the lifetime difference between QN and QD was *ca.* 0.8%.[9] On the other hand, using optimal RTP conditions in terms of the concentrations of thallium nitrate, sodium sulfite, SDS micelles, as well as pH, the RTP lifetime for QN and QD was determined to be 106.02 ± 3.41 ms and 107.35 ± 3.58 ms, respectively; however, although the lifetime for QN and QD was markedly prolonged in RTP measurements, the lifetime difference was similar to that obtained in fluorescence measurements, *i.e.*, *ca.* 1%.

Although QN and QD alone showed very small fluorescence lifetime differences, Navas Díaz *et al.*[3] found that the formation of a diastereomeric ion pair between QN and QD with a chiral counter-ion, (+)-10-camphorsulfonic acid (CSA), yielded two diastereomeric complexes with different stabilities and relatively large fluorescence lifetime differences (*i.e.*, up to *ca.* 5%). As shown in Table 1, the addition of (+)-10-CSA to the sample solution for the measurement of the RTP lifetimes of QN and QD provided comparatively large increases in lifetime differences (*i.e.*, up to *ca.* 15%). Note that the addition of (−)-10-CSA or another type of chiral counter-ion, (+)-tartaric acid, also increased the lifetime differences between QN and QD, but the magnitude (% difference) of these increases was significantly lower, *i.e.*, almost halved, compared to (+)-10-CSA under optimized conditions. Besides the addition of chiral counter-ions, the addition of chiral surfactants as a novel approach for the alteration of lifetime

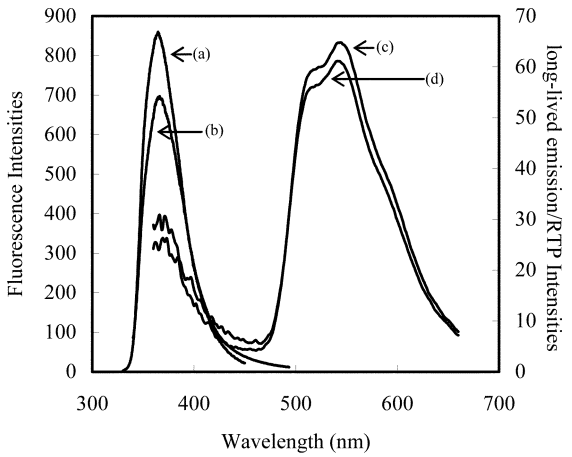

Fig. 1 Fluorescence spectra of (a) QD and (b) QN; long-lived emission/RTP spectra of (c) QD and (d) QN. The concentration of QD and QN was 5×10^{-5} M and 8×10^{-5} M, respectively. In long-lived emission/RTP measurements, λ_{exc} = 331 nm, delay time = 0.04 ms, and gate time = 0.26 ms.

† An Area of Excellence of the University Grants Committee (Hong Kong).

DOI: 10.1039/b311667k

Table 1 RTP lifetime difference between QN and QD in the presence of different chiral modifiers

Chiral modifiers	Concentration[a]/ mol L^{-1}	RTP lifetime/ms[b] QD	RTP lifetime/ms[b] QN	Lifetime difference/ms	Lifetime difference (%)
(+)-10-CSA	1×10^{-3}	42.30 ± 2.25	36.52 ± 1.98	5.78 ± 3.0	14.67
(−)-10-CSA	7×10^{-4}	33.99 ± 2.18	31.68 ± 2.06	2.31 ± 3.0	7.04
(+)-Tartaric acid	7×10^{-4}	39.22 ± 2.13	36.81 ± 2.02	2.41 ± 2.9	6.34
Digitonin	1×10^{-3}	80.86 ± 3.01	72.52 ± 2.56	8.34 ± 3.9	10.87
GCA	1×10^{-4}	52.36 ± 2.35	45.57 ± 2.01	6.79 ± 3.1	13.87

[a] Concentration of chiral modifiers at which maximum lifetime differences were observed. [b] An average of five determinations. [QD] = 5×10^{-5} M and [QN] = 8×10^{-5} M. λ_{exc} = 331 nm and λ_{em} = 543 nm.

Table 2 Stern–Volmer quenching constants for QN and QD in the presence of different chiral modifiers

Chiral modifiers	K_{SV} (QN)/ L mol^{-1}	K_{SV} (QD)/ L mol^{-1}	K_{SV} (QD)/ K_{SV} (QN)	Linear range/mol L^{-1}	Coefficient r^2
(+)-10-CSA	1599	1812	1.13	$5 \times 10^{-5} - 1.0 \times 10^{-3}$	0.996
(−)-10-CSA	5239	5057	0.96	$8 \times 10^{-5} - 1.0 \times 10^{-3}$	0.995
(+)-Tartaric acid	2676	2714	1.01	$5 \times 10^{-5} - 9 \times 10^{-4}$	0.999
Digitonin	251.3	283.4	1.13	$5 \times 10^{-5} - 2.5 \times 10^{-3}$	0.997
GCA	11288	13059	1.16	$5 \times 10^{-6} - 1.75 \times 10^{-4}$	0.996

differences between chiral molecules was examined. The data in Table 1 indicate that the addition of chiral surfactants, *i.e.*, digitonin and glycyrrhizic acid (GCA), was effective in extending the RTP lifetime differences between QN and QD, with lifetime difference (%) similar to that obtained using (+)-10-CSA as the chiral additive.

Unlike fluorescence measurements as reported by Navas Díaz *et al.*[3] in which lifetime differences between QN and QD were increased based on intrinsic differences in the fluorescence lifetimes of the corresponding ion-pair diastereomers, the appearance of lifetime differences in RTP measurements is likely based on different mechanisms. As shown in Table 1, all of the chiral modifiers added into the sample solution produced a decrease in the RTP lifetimes for QN and QD, and the extent of lifetime differences between QN and QD was dependent on the concentration and chemical nature of the chiral additives. For (+)-10-CSA, (−)-10-CSA and (+)-tartaric acid, which are capable of forming ion-pair diastereomers with QN and QD, the decrease in RTP lifetimes could be related to the ability of these counter-ions in quenching the triplet formation of individual QN and QD molecules. On the other hand, chiral surfactants such as digitonin and GCA, which are capable of forming mixed micelles with SDS,[10] could alter the RTP lifetime by changing the micellar microenvironment of QN and QD, *i.e.*, altering the restriction placed upon the internal motions of QN and QD.

The decrease in RTP lifetimes for QN and QD due to the addition of the various chiral organic modifiers shown in Table 1 was examined by fitting the data to the Stern–Volmer equation: $\tau_0/\tau = 1 + K_{SV}[Q]$, where τ_0 and τ are the RTP lifetimes of QD or QN in the absence and presence of a quencher (Q), respectively. A plot of τ_0/τ *versus* [Q] yielded the corresponding Stern–Volmer quenching constants (K_{SV}) as shown in Table 2 for QN and QD in the presence of different quenchers (chiral modifiers). As indicated by the correlation coefficients, the quenching data (decrease in RTP lifetimes) fitted well with the Stern–Volmer treatment within the concentration ranges studied for both groups of quenchers (chiral counter-ions and surfactants). Note that the ratio of the K_{SV} values between QD and QN (Table 2) appeared to show good correlation with RTP lifetime differences (Table 1), *i.e.*, the three organic modifiers, (+)-10-CSA, digitonin and GCA, with K_{SV} ratios which deviated furthest from unity (1.13 and 1.16), also have the largest lifetime differences (%), whereas (−)-10-CSA and (+)-tartaric acid, with K_{SV} ratios of 0.96 and 1.01, respectively, gave somewhat lower lifetime differences (%).

In conclusion, the observation of RTP lifetime differences between QN and QD and the ability to alter these differences based on the addition of chiral counter-ions or surfactants are reported for the first time. The feasibility of exploiting RTP lifetime differences for the rapid chiral analysis of a variety of diastereomers/ enantiomers should be explored in future studies. Also, the possibility of increasing RTP lifetime differences between chiral molecules *via* the use of different types of chiral additives should be pursued based on more comprehensive and in-depth investigation of the basic mechanisms responsible for the alteration of the RTP intensities and lifetimes of various chiral molecules.

Financial support from the Areas of Excellence Scheme established under the University Grants Committee (UGC) of the Hong Kong Special Administrative Region, China (Project No. AoE/P-10/01) and the Research Grant Council of the UGC (HKBU 2018/99P) is gratefully acknowledged.

Notes and references

1 S. C. Stinson, *Chem. Eng. News*, 2001, **79**, 79.
2 M. A. Fox and N. J. Singletary, *Tetrahedron Lett.*, 1979, **20**, 2189; T. Yorozu, K. Hayashi and M. Irie, *J. Am. Chem. Soc.*, 1981, **103**, 5480; T. D. James, S. K. R. A. Sandanayake and S. Shinkai, *Nature*, 1995, **374**, 345; T. Grady, S. J. Harris, M. R. Smyth, D. Diamond and P. Hailey, *Anal. Chem.*, 1996, **68**, 3775; G. Beer, J. Daub and K. Rurack, *Chem. Commun.*, 2001, **12**, 1138; M.-H. Xu, J. Lin, Q.-S. Hu and L. Pun, *J. Am. Chem. Soc.*, 2002, **124**, 14239.
3 A. Navas Díaz, F. García Sánchez and M. C. Torijas, *Anal. Chim. Acta*, 1999, **381**, 11.
4 R. J. Hurtubise, *Anal. Chem.*, 1993, **55**, 669A; H. A. Ackerman and R. J. Hurtubise, *Anal. Chim. Acta*, 2002, **474**, 77.
5 K. Kalyanasundaram, F. Grieser and J. K. Thomas, *Chem. Phys.*, 1977, **51**, 501.
6 A. S. Carretero, C. Cruces-Blanco, M. Sanchez-Polo, J. C. Avila-Roson and A. F. Gutierrez, *Anal. Chim. Acta*, 2002, **474**, 91; R. Pereiro Garzía, Y. M. Liu, M. E. Díaz Garzía and A. Sanz-Medel, *Anal. Chem.*, 1991, **63**, 1759.
7 C. A. Parker and C. G. Hatchard, *Trans. Faraday Soc.*, 1961, **57**, 1894; D. Levy and D. Avnir, *J. Photochem. Photobiol., A: Chem.*, 1991, **57**, 41.
8 S. G. Schulman, R. M. Threatte, A. C. Capomacchia and W. L. Paul, *J. Pharm. Sci.*, 1974, **63**, 876; D. Pant, H. B. Tripathi and D. D. Pant, *J. Lumin.*, 1992, **51**, 223.
9 Fluorescence and RTP lifetimes were measured in the time domain using a TimeMaster lifetime spectrometer (Photon Technology International), with a time resolution of less than 200 ps. Note that two different detection systems housed within the TimeMaster instrument were employed for fluorescence and RTP lifetime measurements.
10 K. Otsuka and S. Terabe, *J. Chromatogr., A*, 2000, **875**, 163.