

Fluorescent Nano pH Indicators Based on Supramolecular Interactions

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Z. Naturforsch. 2010, 65b, 291–294; received October 21, 2009

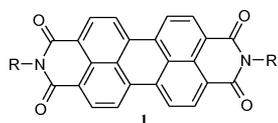
Dedicated to Professor Rolf W. Saalfrank on the occasion of his 70th birthday

Lipophilic pH-sensitive perylene derivatives were combined with detergents in a supramolecular arrangement to obtain nanomicelles that can be applied as fluorescent pH indicators for the aqueous phase.

Key words: pH Indicators, Nano Particles, Fluorescence, Supramolecular Chemistry

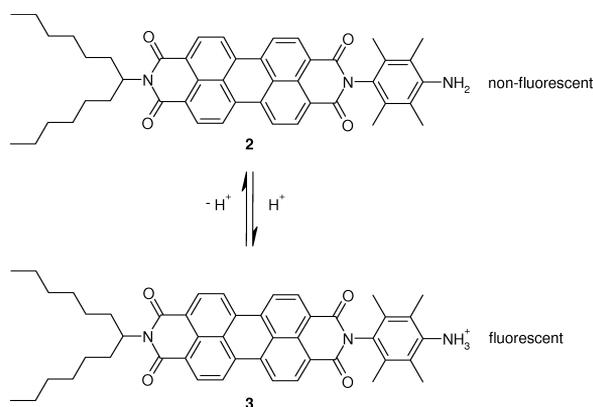
Introduction

Highly hydrophilic switchable dyes are widely applied [1] as optical pH indicators. On the other hand, the basic chromophoric structure of such indicators commonly consists of extended olefinic or aromatic structures, where lipophilic properties are dominating [2], and special measures are necessary for solubilisation in water, such as the introduction of a charge and the attachment of hydrophilic substituents to the periphery. However, the lipophilicity of the core still remains and may cause problems such as aggregation in solutions with high ionic strengths. Further problems arise for permanently coloured solutions where the change in the colour of the indicator is difficult to see. It would be of interest to apply lipophilic indicators directly to the aqueous phase.



Results

We generated local lipophilic compartments in the aqueous phase by means of nanomicelles to incorporate non-hydrophilic chromophores [3] as the indicators. Changes in their fluorescence properties were targeted to generate an indicator system applicable even to coloured solutions. The strongly fluorescent and lipophilic perylene dyes **1** [4] were used as the basic chromophore not only because of their strong fluorescence and chemical stability, but also because



there are orbital nodes [5] at the nitrogen atoms in the HOMO and the LUMO which cause an electronic decoupling of attached substituents from the colour-generating structure. Thus, we attached the long-chain *sec*-alkyl group 1-hexylheptyl (“swallow-tail substituent”) to one nitrogen atom for solubilisation in lipophilic media and attached the 4-amino-2,3,4,5-tetramethylphenyl substituent for pH monitoring to the other N-atom to obtain derivative **2**. The latter substituent, which is relatively electron-rich owing to the electron-donating amino group, is turned orthogonal to the plane of the chromophore because of steric interactions. This geometry causes a further electronic decoupling.

Its electronically high-lying HOMO causes fluorescence quenching of the chromophoric perylene structure in **2** by means of an electron transfer into the hole of the HOMO of the electronically excited chromophore according to Fig. 1 (compare ref. [6]).

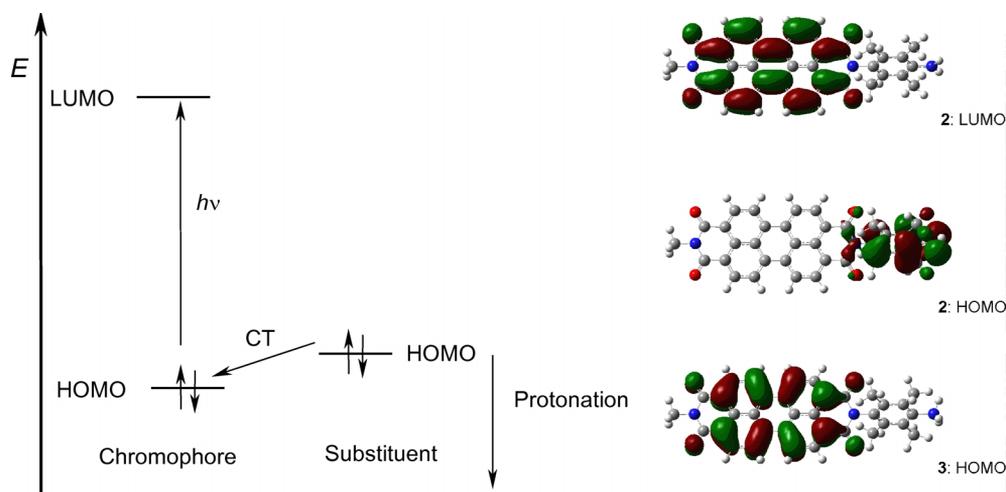


Fig. 1. Left: Terms of HOMO and LUMO of the perylene chromophore and HOMO of the attached aminotetramethylphenyl substituent (CT: charge transfer). Protonation of the substituent lowers its HOMO. Right: Calculated orbitals of **2** and **3** (DFT B3-LYP).

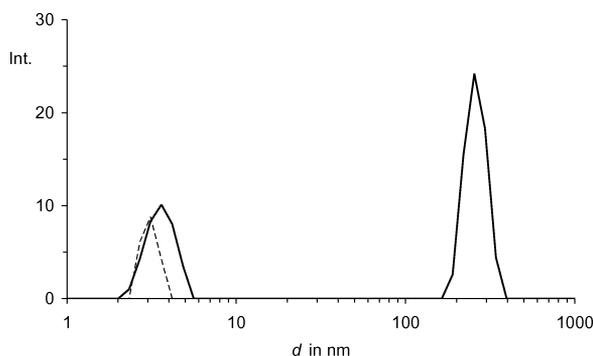


Fig. 2. Size distribution (d in nm) of micelles from sodium 1-dodecyl sulphate as the detergent in water by dynamic light scattering (DLS). ---: Pure detergent; —: supramolecular material composed of **2** and the detergent, buffered at $\text{pH} = 1.73$. Intensity is in arbitrary units.

A protonation of the amino group in **2** to form **3** lowers the energy of the HOMO of the substituent by electron depletion and inhibits the electron transfer for quenching (see Fig. 1). As a consequence, fluorescence is switched on to high quantum yields. The $\text{p}K_a$ of **3** is in a region relevant for the aqueous phase.

Sodium 1-dodecyl sulphate (SDS) [7] was applied for the generation of micelles in the aqueous phase. Thus, a gel of this material was prepared and spread in water. Micelles with an average size of some 3 nm were obtained as is shown in Fig. 2 (compare ref. [7e, f]). The indicator **2** [8] was incorporated into the gel, and micelles were prepared in the same manner as was described before. The chromophore **2** changes the ar-

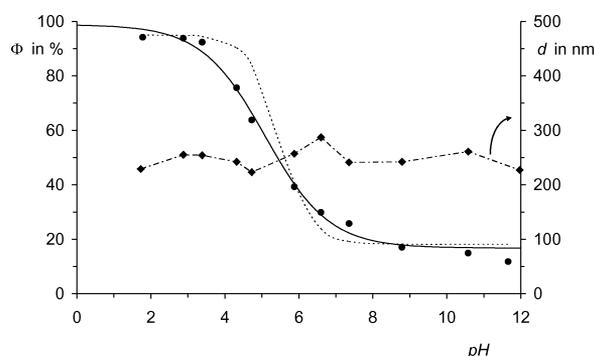


Fig. 3. Micelles of **2** and sodium 1-dodecyl sulphate at various pH values. Circles: Fluorescence quantum yields with compensating curve (—). ···: Optimal approach according to the law of mass action; --- and diamonds: size of the nanoparticles by DLS.

range of the micelles by supramolecular interactions to increase the size to some 250 nm (compare ref. [9]); the formation of small micelles was still observed (Fig. 2). The size of the indicator-doped micelles is essentially independent of the pH value of the aqueous phase (see diamonds in Fig. 3).

The UV/Vis-absorption spectra of the micellar solution are identical with the spectra in non-polar media such as chloroform indicating isolated chromophores being situated in the lipophilic region of the micelles; no exciton interactions of chromophores were observed in the spectra. The high fluorescence quantum yield of the protonated amine **3** in the micelles is a further indicator for isolated chro-

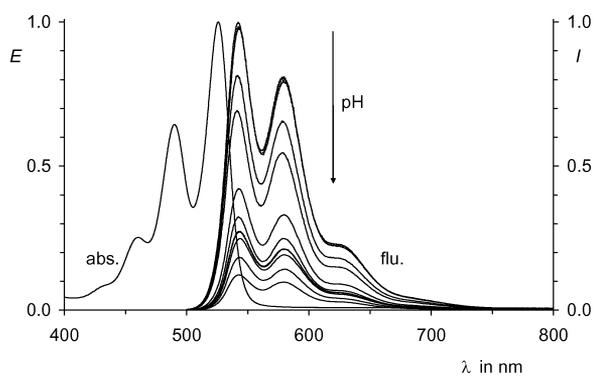


Fig. 4. Absorption (E) and fluorescence (I) spectra of **2** in micelles. The absorption spectrum is invariant to the increase of the pH value, whereas the fluorescence intensity decreases (from bottom to top: pH = 11.97, 10.58, 9.05, 8.79, 7.36, 6.60, 5.88, 4.73, 4.32, 3.39, 2.88 and 1.73).

mophores, as are the non-perturbed fluorescence spectra.

The fluorescence intensity of **2** in micelles strongly decreases with increasing pH (Fig. 4). Only the intensity is changed, but not the line shape. This is interpreted in terms of an equilibrium between **2** and **3**. However, the Henderson-Hasselbalch equation, would require a steeper curve (---) than the experimental results in Fig. 3 (circles). This is interpreted by the existence of more than a single arrangement of **2** and **3**, respectively, in the micelles. Dynamic processes in these complex structures [10] may be a further cause for the diminishing the steepness of the curve in Fig. 3.

The titration curve in Fig. 3 remains steep enough to apply the micellar system of **2** as an indicator; no changes of the micellar solution was observed upon storage. The switching from the non-fluorescent **2** to the fluorescent **3** can be easily recognised visually. The response of the nanomicelles to a change of pH is immediate so that applications for titration are possible; a pK_a value of about 5.5 was found as the point of inflection in the curves in Fig. 3.

Conclusion

Micellar arrangements of lipophilic pH-sensitive dyes can be applied as indicators for the aqueous phase. A switch in fluorescence allows the titration even of coloured solutions.

Experimental Section

General

UV/Vis spectra: Varian Cary 5000; fluorescence spectra: Varian Eclipse. The dye **2** was prepared and purified according to the literature [8].

Preparation of nanoparticles in the aqueous phase

Sodium 1-dodecyl sulphate (460 mg) and distilled water (1.7 g) were heated to 50 °C to form a colourless gel. The perylene derivative (1 mg) and chloroform (60 mg, *ca.* 10 drops) were added at 40 °C with subsequent ultrasonification for 10 min, treatment with distilled water (30 mL) and filtration (D5 glass filter). The nanoparticles in water remain unaltered for many months. Neither flocculation nor degradation of the strong fluorescence could be observed.

Buffer solutions

The pH values reported in Fig. 3 were stabilised with buffer solutions: Citrate buffers were applied for 1.73, 2.88 and 3.38, acetate buffers for 4.32 and 4.73, phosphate buffers for 5.88, 6.60 and 7.36, tris buffer for 8.79, tampon for 9.05, phosphate buffer for 10.58 and tampon for 11.97. Standard buffers from Honeywell Specialty Chemicals Seelze GmbH (Riedel-de-Haën) were applied.

Acknowledgements

We are grateful to the Fonds der Chemischen Industrie and CeNS, Center for NanoScience, for financial support and Prof. Dr. Thomas Bein for help with DSL measurements. T. P. thanks Degussa Evonik for a scholarship.

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