Dalton Transactions

PAPER

Cite this: *Dalton Trans.*, 2014, **43**, 8230

Photophysical properties of a new water soluble tetra thiamine substituted zinc phthalocyanine conjugated to gold nanorods of different aspect ratios

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A water soluble zinc phthalocyanine substituted with thiamine is reported in this work. The aggregation of this compound in aqueous solutions causes quenching of the fluorescence quantum yields. Gold nanospheres and nanorods were linked to the phthalocyanine. X-ray photoelectron spectroscopy showed that both the amine and the sulphur groups on the thiamine substituent of the zinc phthalocyanine were involved in the linking to gold nanoparticles. The Pc showed an increase in the fluorescence quantum yields in the presence of the nanoparticles. The singlet oxygen quantum yield increased when the Pc was conjugated to the nanoparticles and even higher for larger aspect ratio gold nanorods.

Received 20th January 2014, Accepted 28th February 2014 DOI: 10.1039/c4dt00197d

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Introduction

Metallophthalocyanines (MPcs) have found diverse applications such as in non-linear optics,1 electrocatalysis2 and photodynamic therapy (PDT).³ MPcs absorb strongly in the red region of the UV-visible spectrum and produce high singlet oxygen quantum yields which make them very interesting for photodynamic therapy. Solubility of phthalocyanines is a major limitation. Substituents can be introduced at the peripheral and non-peripheral positions of the phthalocyanine ring to improve solubility.⁴ Water solubility is important for applications of Pcs in PDT. There have been a number of studies on functionalization of phthalocyanines with biomolecules including polylysine,5 carbohydrates6 and folic acid.7 These molecules provide biocompatibility and water solubility for phthalocyanines. In this work thiamine (vitamin B1) is employed as a ring substituent in order to form a water soluble zinc phthalocyanine. Thiamine is employed as a substituent for phthalocyanines for the first time.

Thiamine is a water soluble vitamin⁸ which is deficient in patients with advanced stage cancer. It has been reported that if thiamine is administered in high doses, it can reduce tumor proliferation.⁹ Thus the conjugation of thiamine to phthalocyanine is expected to enhance water solubility and improve cancer treatment through the synergistic effect.

Gold nanoparticles have been vastly studied for biological applications because they are non-toxic in the biological systems. Gold nanoparticles (AuNPs) are also known to be effective for photothermal therapy (PTT).¹⁰ Conjugation of AuNPs to Pcs should result in the combined PDT and PTT. In addition, AuNPs can facilitate photodynamic therapy by increasing singlet oxygen quantum yields through increased intersystem crossing.¹¹ Our group has reported on the conjugation of AuNPs to Pcs with improved photophysical and photochemical properties.^{12–15} The improvement in singlet oxygen quantum yield¹² in particular is of significance for PDT applications. Conjugates of AuNPs with Pcs may also be used for drug delivery.^{16,17}

Of the AuNPs, gold nanorods (AuNRs) have become more interesting because their absorption wavelength can be tuned to near infrared (NIR) regions where tissue penetration is more efficient.¹⁸ The combined PDT and PTT is expected to be more prominent for AuNRs which absorb in the NIR regions where phthalocyanines absorb, allowing for the use of one wavelength for excitation of both. In this study, we report on the conjugation of a water soluble zinc phthalocyanine containing thiamine to AuNRs for potential coupled PDT and PTT. This work reports on the effects of the aspect ratio of gold nanorods on the photophysical and photochemical properties of the thiamine substituted phthalocyanine. It has been reported that AuNRs possess superior long blood circulation time due to the anisotropic geometry.¹⁹ It is with this in mind that we study the effects of different sizes (aspect ratios) of AuNRs on the photophysical behavior of zinc tetra (4-(thiamine)phthalocyanine) (ZnTTAPc) (4).



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Experimental

Materials

Zinc chloride, gold(m) chloride trihydrate, sodium borohydride, cetyltrimethylammonium bromide, (CTAB), silver nitrate and ascorbic acid, thiamine hydrochloride, potassium carbonate and zinc phthalocyanine (ZnPc) were purchased from Sigma Aldrich. Dimethyl formamide (DMF), dimethyl amino ethanol and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were obtained from Fluka. Deionized water was used for all aqueous solution preparations. AlPcS_{Mix} (containing a mixture of sulfonated derivatives and used as a standard in the determination of singlet oxygen quantum yields in water) was synthesized according to literature methods.²⁰ 4-Nitrophthalonitrile (1) was synthesized and purified according to literature methods.²¹

Instrumentation

Infrared spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer. X-ray photoelectron spectroscopy (XPS) data were collected using a Kratos Axis Ultra DLD, using an Al (monochromatic) anode, equipped with a charge neutralizer. The operating pressure was maintained below 5×10^{-9} torr. The resolution was 10 eV pass energy in the slot mode. Mass spectral data were collected with a Bruker AutoFLEX III Smartbeam TOF/TOF Mass spectrometer using dithranol as the MALDI matrix. Details have been provided before.¹³

The concentration of gold atoms (Au^0) in AuNPs was determined using a ThermoElectron ICAP 6000 inductively coupled plasma (ICP) spectrometer with an optical emission spectroscopy (OES) detector. Gold nanoparticles were digested by addition of 3 mL of aqua regia for ICP measurements. Standard calibration was achieved at concentrations ranging from 0.5 to 5 ppm. A minimum of three measurements were taken for each sample of gold nanoparticles. Gold was analysed at wavelengths of 208, 242 and 267 nm.

Ground state electronic absorption spectra were performed using a Shimadzu UV-2550 spectrophotometer. Fluorescence excitation and emission spectra were recorded on a Varian Eclipse spectrofluorimeter. Fluorescence lifetimes were measured using a time correlated single photon counting (TCSPC) setup (FluoTime 200, Picoquant GmbH). The excitation source was a diode laser (LDH-P-670 driven by PDL 800-B, 670 nm, 20 MHz repetition rate, 44 ps pulse width, Picoquant GmbH), as described before.¹³

Transmission electron microscope (TEM) images were obtained using a Zeiss Libra TEM 120 model operated at 90 kV accelerating voltage. TEM samples were prepared by placing a drop of the nanoparticle solution on the sample grid and allowing it to dry before measurements.

Laser flash photolysis experiments were performed to determine the triplet decay kinetics. The excitation pulses were produced by a tunable laser system consisting of an Nd:YAG laser (355 nm, 135 mJ/4–6 ns) pumping an optical parametric oscillator (OPO, 30 mJ/3–5 ns) with a wavelength range of 420–2300 nm (NT-342B, Ekspla) as described in detail before.^{22,23}

The time resolved phosphorescence of singlet oxygen at 1270 nm was used to determine the singlet oxygen quantum yield of the Pc and Pc–gold nanoparticle conjugates in DMF and aqueous solution, details have been described before.²³ The singlet oxygen phosphorescence signal was compared to AlPcSmix as the standard for aqueous solutions and ZnPc as a standard in DMF.

Synthesis (Scheme 1)

Synthesis of 4-(thiamine)phthalonitrile (3). Thiamine hydrochloride **2** (6.34 g, 18.8 mmol) was added to compound **1**



Scheme 1 Synthesis of thiamine substituted zinc phthalocyanine (ZnTTAPc).

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(1.5 g, 8.67 mmol) in DMF under argon. After stirring for 15 min at room temperature K_2CO_3 was added (5 g, 36 mmol) in portions. The reaction mixture was stirred at room temperature for 2 days. The mixture was then poured into ice and the precipitate was filtered and dried. Yield: 2.4 g (65%) IR ($\nu_{max}/$ cm⁻¹): 2228 (C–N), 3032–3044 (Ar–CH), 2954 (Alph-CH), 3361 (N–H), 1594 (C=C). ¹H NMR (acetone-D₆): 8.18 (s, 1H, Ar–H), 7.97 (d, 1H, Ar–H), 7.90 (d, 1H, Ar–H), 7.79 (s, 1H, Ar–H), 7.76 (s, 1H, Ar–H), 4.33 (s, 2H, NH₂), 3.11 (t, 2H, O–CH₂), 2.89 (t, 2H, –CH₂), 2.75 (s, 2H, –CH₂), 2.36 (s, 3H, CH₃), 2.23 (s, 3H, CH₃). Anal. Calc. for C₂₀H₁₉N₆OSCl: C 61.45, H 4.86, N 21.51, S 8.19; Found: C 61.26, H 4.76, N 21.35, S 7.98.

Synthesis of zinc tetra (4-(thiamine)phthalocyanine) (ZnTTAPc) (4). A mixture of 3 (0.3 g, 0.703 mmol), anhydrous zinc chloride (0.024 g, 0.18 mmol) and DBU (1 mL, 6.3 mmol) was refluxed in dimethyl amino ethanol for 8 h. The product was cooled to room temperature and precipitated with ethanol. After filtration the product was washed repeatedly with ethanol. The product was purified in a reverse phase column using a mixture of methanol and acetonitrile (1:2) as an eluent. Yield: 0.19 g (63%). IR: 3492 (N-H), 3051, 3079 (Ar-CH), 2958, 2921 (Alph-CH), 1610 (N-H bending), 1226 (C-O-C), 1576 (C=C). ¹H NMR (DMF-D₇): 9.53–9.89 (m, 12H, Ar-H), 8.41 (s, 4H, S-CH), 7.27 (s, 4H, CH=N), 5.76 (s, 8H, CH₂-N), 3.66 (s, 8H, NH₂), 3.29 (t, 8H, O-CH₂), 2.90 (t, 8H, -CH₂), 2.47 (t, 12H, CH₃), 2.21 (s, 12H, CH₃), MALDI-TOFMS Anal. Calc. m/z 1631.27, Found: $[M]^+$: 1630.51. UV-Vis (DMF) λ_{max}/nm $(\log \epsilon)$ 358 (4.6), 617 (4.2), 686 (4.97). Anal. Calc. for C₈₀H₇₆N₂₄O₄S₄Zn: C 58.89, H 4.69, N 20.61, S 7.86; Found: C 58.49, H 4.66, N 21.09, S 7.87.

Synthesis of gold nanoparticles

Seed solution (used as nanospheres, AuNS). The seed AuNS solution was prepared following literature methods,²⁴ with slight modifications: aqueous solution of 2.5×10^{-4} M (5 mL) HAuCl₄ was mixed with 10 mL of 0.1 M CTAB solution, and the mixture was stirred for 2 min. Then, 0.6 mL of an ice cold aqueous solution of 0.01 M NaBH₄ was added, and the mixture was shaken by hand at 2 min intervals. The solution was allowed to stand for 2 h and then used for the subsequent synthesis of shaped AuNPs.

Synthesis of gold nanorods (AuNRs (2.0), AuNRs (4.7) and AuNRs (7.1)). The growth solution was prepared according to the literature²⁴ by mixing 10 mL of 0.2 M CTAB, 5 mL of 1×10^{-4} M HAuCl₄ and 0.004 M AgNO₃. The aspect ratio of the nanorods was adjusted by adding different amounts of 0.004 M AgNO₃ from 0.19, 0.22 and 0.25 mL for AuNRs with aspect ratios of 7.1, 4.7 and 2.0, respectively. To this solution, 0.07 mL of 0.1 M ascorbic acid was added and the reaction turned colourless. Then the seed solution (15 µL) prepared above was added and the mixture was shaken by hand and left undisturbed between 30 and 37 °C for 24 h. Gold nanorods were purified by centrifuging for 20 min at 2000 rpm to remove the unreacted gold and spherical gold nanoparticles. The supernatant was removed and the resulting pellet of AuNRs was dissolved in 3 mL of distilled water. Before using

for coordination to ZnTTAPc, AuNR solution was centrifuged for the third time for 5 min to remove excess CTAB. The clear liquid was discarded and the resulting gold pellet was dissolved in 1 mL distilled water. The AuNRs of different aspect ratios are represented as AuNRs (2.0), AuNRs (4.7) and AuNRs (7.1).

Conjugation of ZnTTAPc to gold nanoparticles

The solution of gold nanoparticles (1 mL) from above was mixed with 1 mL aqueous solution (pH 9) of ZnTTAPc (2 mg mL⁻¹) and this mixture was stirred for about 5 min, then further diluted with 3 mL distilled water and stirred overnight. The conjugate was purified in a size exclusion column of Bio-Beads using pH 9 buffer as an eluting solvent.

Data analysis

The total number of Au atoms per AuNP, the surface area of AuNPs and the number of surface Au atoms per AuNP were calculated using established methods.^{25–27} The extinction coefficients of AuNPs were determined using Beer's law and ICP as reported before.^{28,29}

The number of Pc molecules on AuNPs was also obtained using the Beer's law and the Q band absorption maximum of the phthalocyanines, assuming the extinction coefficient does not change upon conjugation.

Results and discussion

Characterization of Zn thiamine phthalocyanine (ZnTTAPc (4))

Thiamine was employed as a substituent for complex 4 in order to provide the solubility in aqueous media. However, the complex was not soluble in water unless at high pH. Hence pH 9 buffer was employed. Furthermore the thiamine substituent will allow for the binding with gold nanoparticles. Characterization of the compound was achieved using infra-red (IR), UVvisible, proton nuclear magnetic resonance (NMR) and matrixassisted laser desorption/time of flight (MALDI-TOF) spectroscopies and elemental analysis. ¹H NMR showed all the protons in their correct positions. The mass of the molecule was confirmed by MALDI-TOF spectrometry. Elemental analysis confirmed the absence of chloride and hence the non-ionic nature of the molecule following an extensive purification procedure.

UV-Vis spectra

The UV-Vis spectrum of the ZnTTAPc shows two absorption peaks at 638 and 696 nm due to aggregation in aqueous solutions, Fig. 1 and Table 1. The blue shifted band at 638 nm is due to the aggregates and the band at 696 nm is the monomer peak. After the addition of Triton X-100 the aggregate peak decreased and the monomer peak intensity increased, but there is still some aggregation. In DMF only one peak due to the monomer is observed showing no aggregation.



Fig. 1 UV-Vis spectra of ZnTTAPc in (i) pH 9 buffer, (ii) pH 9 buffer + Triton X-100 and (iii) DMF.

 Table 1
 Spectral properties and size of AuNPs-ZnTTAPc conjugates of ZnTTAPc and conjugates^a

Sample	Solvent	$\lambda_{\rm abs}/{\rm nm}$	$\lambda_{\rm exc}/{\rm nm}$	$\lambda_{\rm em}/{\rm nm}$	
ZnTTAPc	DMF	688	688	699	
ZnTTAPc	pH 9 buffer	638,696	696	704	
ZnTTAPc	pH 9 buffer + Triton X-100	633,696	696	704	
Pc-AuNS (1.0)	pH 9 buffer	692	693	704	
Pc-AuNR (2.0)	pH 9 buffer	694	694	706	
Pc-AuNR (4.7)	pH 9 buffer	692	692	703	
Pc–AuNR (7.1)	pH 9 buffer	692	692	712	

 $^a\lambda_{\rm abs}$ = absorption wavelength, $\lambda_{\rm exc}$ = excitation wavelength, $\lambda_{\rm em}$ = emission wavelength.

Fluorescence behaviour

The emission spectrum of the ZnTTAPc in aqueous solution was very weak due to aggregation, but improved on addition of Triton X-100, Fig. 2A. The absorption spectrum is not in agreement with the excitation spectrum for ZnTTAPc in Fig. 2A due to effects of aggregation. The emission spectrum was however the mirror image of the excitation spectrum. The excitation spectrum was in agreement with the corresponding absorption spectrum for ZnTTAPc in DMF, Fig. 2B, and the emission spectrum was the mirror image of the excitation spectrum.

It was not possible to calculate the fluorescence quantum yield ($\Phi_{\rm F}$) in aqueous media without Triton X-100 due to very weak fluorescence as a result of the highly aggregated nature of this molecule. The fluorescence quantum yield in pH 9 buffer + Triton X-100 was $\Phi_{\rm F} = 0.01$, a value lower than in DMF at $\Phi_{\rm F} = 0.09$ (Table 2). The value in DMF is still lower than is typical of unaggregated ZnPc derivatives in organic solvents,¹² due to the known³⁰ quenching of excited states by amino groups which form part of the thiamine substituent on the Pc ring.

Fluorescence lifetimes of ZnTTAPc were measured using the TCSPC method and the fluorescence decay curves are shown in Fig. 3. ZnTTAPc gave one lifetime in DMF



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Fig. 2 (i) Absorption, (ii) excitation and (iii) emission spectra of ZnTTAPc in (A) pH 9 buffer + Triton X-100 and (B) in DMF.

(Table 2). Two fluorescence lifetimes were observed for ZnTTAPc in pH 9 + Triton X-100, which is attributed to the presence of aggregates which tend to quench the fluorescence and hence the longer unquenched and shorter quenched lifetimes were obtained.³¹

Triplet quantum yields and lifetimes

The triplet decay curve for ZnTTAPc is shown in Fig. 4. Triplet quantum yields and lifetimes were obtained for the Pc alone in DMF and pH buffer + Triton X-100 (Table 2). Triplet quantum yields can be used to explain the ability of the molecules to undergo intersystem crossing to the triplet state. Triplet quantum yields were calculated using comparative methods described before³² and ZnPc in DMF ($\Phi_T^{Std} = 0.58^{33}$) and AlPcSmix in aqueous media $\Phi_T^{Std} = 0.44^{34}$ as standards. The triplet quantum yields of the Pc were 0.28 and 0.21 in DMF and pH 9 buffer + Triton X-100, respectively. Triplet lifetimes of the Pc in DMF are 35 µs and 25 µs in pH 9 buffer + Triton X-100. Both the triplet quantum yield and lifetime values are lower in aqueous media due to aggregation.

 Table 2
 Spectral properties and photophysical parameters of ZnTTAPc and conjugates

Sample	$arPhi_{ m F}$ (±0.01)	$\tau_{\mathrm{F}}^{\ a}(\mathrm{ns})$	$\tau_0 (\mathrm{ns})$	$arPsi_{ m T}$	$ au_{\mathrm{T}}(\mu \mathrm{s})$	Φ_{Δ}
ZnTTAPc (DMF)	0.09	2.43	27.0	0.28	35	0.44
ZnTTAPc (pH 9 buffer + Triton X-100)	0.01	2.71(0.94) 0.69(0.56)	293.4	0.21	25	0.19
Pc–AuNS (1.0) pH 9 buffer	0.02	2.39(0.86) 0.49(0.13)	106.0	—	_	0.23
Pc–AuNR (2.0) pH 9 buffer	0.03	2.64(0.85) 0.88(0.15)	79.2	—	_	0.23
Pc–AuNR (4.7) pH 9 buffer	0.05	2.71(0.88) 0.91(0.11)	49.7	—	_	0.25
Pc–AuNR (7.1) pH 9 buffer	0.06	2.73 (0.91) 0.83 (0.09)	42.7	—	_	0.29

^{*a*} Abundances in brackets.



Fig. 3 Fluorescence decay curves of ZnTTAPc in (a) DMF and (b) pH 9 buffer plus Triton X-100.



Fig. 4 Triplet decay curves of ZnTTAPc in DMF.

Characterization of gold nanoparticles

The aspect ratio of the Au nanospheres will be taken as one.³⁵ The surface area of the gold nanoparticles increased as the aspect ratio increased, Table 3. This has been observed before.³⁵ The total number of Au atoms as well as surface Au atoms also increased as the aspect ratio increased. The extinction coefficients of the gold nanoparticles reported in Table 1 are comparable to those found in the literature.^{28,29}

UV-Vis spectra

Spherical gold nanoparticles have a characteristic surface plasmon resonance (SPR) absorption in the visible region of the electromagnetic spectrum. Gold nanorods have two characteristic SPR peaks (transverse and longitudinal).³⁶ Fig. 5 shows the UV-Vis spectra of the gold nanospheres and nanorods with varying aspect ratios. Gold nanospheres showed one absorption peak at 518 nm, Table 3. However, for nanorods there were two absorption peaks observed and the longitudinal peak was tuned to the near infrared by increasing the aspect ratio, Table 3. The aspect ratio of the gold nanorods was controlled by the amount of silver nitrate added and was found to decrease with an increase in AgNO₃.

Transmission electron microscopy (TEM)

Fig. 6 shows TEM images of the gold nanospheres and nanorods (the latter at different aspect ratios). TEM images of gold nanospheres show the distribution of small particles with an estimated particle size of 2.1 nm (average). Gold nanorods shown in this figure have the aspect ratio of 2.0, 4.7 and 7.1 nm (Tables 1–3), represented as AuNRs (2.0), AuNRs (4.7) and AuNRs (7.1), respectively.

Characterization of ZnTTAPc-AuNPs conjugates

Gold is known to form strong bonds with amines and sulphur. ZnTTAPc contains sulphur on the thiazole group and the amine from its pyrimidine group of the thiamine. The phthalocyanine is expected to form the amine–gold or sulphur–gold bonds with the nanoparticles.

As stated above, the surface area of the nanorods was found to increase as the aspect ratio increases. The larger surface area results in an increase in the number of surface atoms which are important for attachment with the phthalocyanine molecules on the surface. The ratio of the number of phthalocyanine molecules to the number of gold nanoparticles was found to range from 27 to 243, increasing with increase in the surface area of the NPs (Table 3).

TEM images

TEM images of Pc-gold conjugates are shown in Fig. 7. The morphology of the gold nanoparticles did not change upon

 Table 3
 Properties of gold nanoparticles and loading

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Sample	λ SPR band (nm)	$E (M^{-1} cm^{-1})$	Surface area (NPs) (nm ²)	Total Au atoms	Surface Au atoms	Pcs/NPs
AuNS (1.0)	518	1.3×10^{8}	14	450	235	27
AuNR (2.0)	528,643	$2.4 imes 10^8$	477	168 214	8103	49
AuNR (4.7)	518,745	5.6×10^{8}	733	354 660	124 396	111
AuNR (7.1)	518, 825	$6.9 imes 10^8$	1271	743 236	215 671	243



Fig. 5 UV-Vis spectra of gold nanoparticles (i) nanospheres, (ii) Pc–AuNRs (2.0), (iii) Pc–AuNRs (4.7) and Pc–AuNRs (7.1).

conjugation except for the slight changes in particle sizes. The average particle size of the conjugated spherical nanoparticles is 4.6 nm, an increase from 2.1 nm of the nanospheres alone, possibly due to aggregation. The aspect ratios of the conjugated nanorods were 2.9, 4.9 and 7.8 nm for AuNRs (2.0), AuNRs (4.7) and AuNRs (7.1), respectively, showing a slight increase.

XPS studies

In order to determine if the amine or sulphur groups on the thiamine substituent on the Pc ring are coordinated to AuNPs, high resolution XPS was employed.

The high resolution scans of the N (1s) and S (2p) for the ZnTTAPc alone and ZnTTAPc-gold conjugates are shown in Fig. 8. Fig. 8A(i) shows C-NH₂, C=N and metal-N bonds present in the phthalocyanine molecule. Conjugation of the Pc to the nanoparticles resulted in the disappearance of the C-NH₂ peak, Fig. 8A(ii), which proves the participation of the amine in conjugation with gold nanorods.

The sulphur group of the thiazole ring was also analyzed with XPS to confirm the interaction with gold nanoparticles. The deconvoluted spectra for S (2p) show a doublet of $(2p_{3/2})$ and $(2p_{1/2})$ (Fig. 8B(i)) which is common for S (2p).³⁷



Fig. 6 TEM images of gold (i) nanospheres, (ii) Pc-AuNRs (2.0), (iii) Pc-AuNRs (4.7) and Pc-AuNRs (7.1).



Fig. 7 TEM images of Pc-gold conjugates (i) nanospheres, (ii) Pc-AuNRs (2.0), (iii) Pc-AuNRs (4.7) and Pc-AuNRs (7.1).



Fig. 8 XPS deconvoluted spectra of (A) N 1s and (B) S 2p of (i) ZnTTAPc and (ii) ZnTTAPc-Au.



Fig. 9 UV-Vis spectra of (A) AuNSs, (B) AuNRs (2.0), (C) AuNRs (4.7) and (C) AuNRs (7.1), and their conjugates. (i) Gold nanoparticles and (ii) Pc and (iii) gold nanoparticles–Pc conjugates in pH 9 buffer (without Triton X-100).

Upon binding to gold nanoparticles the first doublet disappeared and the second doublet was observed at higher binding energies. This shows that sulphur also played a role in the formation of the phthalocyanine–gold NP conjugate.

UV-Vis absorption spectra

Fig. 9 shows the UV-Vis spectra of the conjugates in pH 9 buffer. The conjugation of gold nanoparticles to ZnTTAPc did not significantly shift the Q band (Table 1) maxima of the monomer peak of ZnTTAPc but the aggregation was reduced as judged by the decrease in the aggregate peak near 630 nm. Thus linking of AuNPs to ZnTTAPc reduces aggregation. Disaggregation of Pc molecules has been observed before for star shaped AuNPs but not for spherical ones.¹⁵ However, in this work the spherical nanoparticles also break aggregates, Fig. 9A. The spectra of the conjugates show the characteristic SPR peaks from gold nanoparticles.

Photophysical studies

No triplet decay curves were observed for the Pc in the presence of AuNPs, probably due to the short triplet lifetime induced by the heavy atom effect. This behavior has been observed before for other Pc-AuNP conjugates.³⁸

Fluorescence behaviour. The excitation spectra of the conjugates were not similar to the corresponding absorption spectra, Fig. 10 (showing AuNRs (4.7) and AuNRs (7.1) as examples), due to remaining aggregation and the presence of SPR bands of the AuNPs. The emission spectra were all mirror images of the excitation spectra though emission was weak for gold nanospheres (figure not shown).

The fluorescence behavior of the fluorophore in the presence of nanoparticles is affected by factors such as the size of the nanoparticle and the distance separating them.³⁹ Hence, the effect of the aspect ratio of the nanorods is investigated. The fluorescence quantum yields measured in the presence of gold nanoparticles are all slightly higher than the phthalocyanine alone in pH 9 buffer + Triton X-100 due to reduced aggregation. The presence of metallic particles can modify the local field around the fluorescing molecule by causing an increase of the rate of excitation at larger distances which would result in an increase in the quantum yield.⁴⁰ Fluorescence quantum yields were found to marginally increase with increasing aspect ratio. It has been reported that the Paper



Fig. 10 (i) Absorption, (ii) excitation and (iii) emission spectra of (A) ZnTTAPc-AuNRs (4.7) and (B) ZnTTAPc-AuNRs (7.1) in pH 9.

fluorescence emission is more polarized along the length of the nanorods and the degree of polarizability by nanorods increases with their aspect ratio.^{41,42} Hence, the increase in fluorescence quantum yields could also be due to the increasing excitation rate as the aspect ratio increases. The fluorescence quantum yields in the presence of Au nanoparticles will also be affected by intersystem crossing to the triplet state due to the heavy atom effect of gold. Thus decreased aggregation will improve the values, but the heavy atom effect will decrease them.

Two fluorescence lifetimes were observed for ZnTTAPc in the conjugates, Table 2, as was the case for ZnTTAPc alone in Triton X-100. Fluorescence lifetimes ($\tau_{\rm F}$) decreased (considering the long lifetime) for the nanospheres and smaller aspect ratio nanorods (AuNRs (2.0)) when compared with the phthalocyanine alone in pH 9 + Triton X-100. The $\tau_{\rm F}$ value for AuNRs (4.7) was the same as for ZnTTAPc in pH 9 + Triton X-100, with a slight increase for AuNRs (7.1).

Radiative and non-radiative processes compete in the deexcitation of the molecules. The presence of metal nanoparticles can influence decay of the fluorophore by inducing nonradiative processes. Non-radiative processes usually quench the fluorescence and decreases the decay time. Hence, the radiative lifetime (τ_0), which is the lifetime of the molecule in the absence of the non-radiative processes, can be used to explain the quenching of lifetimes. The radiative lifetime can be calculated from the measurement of the fluorescence quantum yield ($\Phi_{\rm F}$) and lifetime ($\tau_{\rm F}$) using eqn (1).⁴³

$$\tau_0 = \tau_{\rm F} / \Phi_{\rm F} \tag{1}$$

The radiative lifetimes in Table 2 were estimated for nanospheres and different sizes of the gold nanorods. It was observed that when the aspect ratio increases, τ_0 decreases. The τ_0 values can be affected by the distance and orientation between the molecule and metal particles, and can either increase or decrease depending on the molecular dipole and the distance between the fluorophore and the metal. Both the size and shape of nanoparticles can also alter the radiative decay.44,45 Higher photonic mode density increases in the presence of nanoparticles and causes a decrease in radiative decay.46 The decrease in radiative lifetime at a larger aspect ratio is caused by the increasing photonic mode density. As the aspect ratio increased, the absorption of the nanoparticles also shifts towards higher wavelengths towards the absorption of the Pc, hence increasing the interference between their excited dipoles. The absorption and scattering coefficients of nanorods are also larger than that of spherical nanoparticles,³⁹ therefore nanorods are expected to increase the effects felt by the Pc molecules.

Singlet oxygen quantum yields. Singlet oxygen causes cell death in photodynamic therapy hence monitoring it is very important. Phthalocyanines are well known to produce singlet oxygen where they undergo intersystem crossing which makes them good photosensitizers for photodynamic therapy. Singlet oxygen was measured for the Pc alone and Pc with gold nanospheres and rods (Table 2). In DMF the singlet oxygen quantum yield of the Pc was 0.44 while in pH 9 buffer (when Triton X-100 was added) it was 0.19. There was an increase in the singlet oxygen quantum yields when the Pc is conjugated to gold NPs (Table 2). This could be due to the effect of heavy atoms which promote intersystem crossing.

The moderate increase in singlet oxygen quantum yield could have been affected by the so-called screening effect, where the AuNPs prevent the oxygen molecule from interacting with the excited triplet phthalocyanine molecule⁴⁷ and/or by the fact that the AuNPs and the Pc absorb at the same wavelength hence absorption by the former could affect the excitation of the latter.

AuNS and AuNR (2.1) produced the equal amounts of singlet oxygen ($\Phi_{\Delta} = 0.23$, Table 2). The number of Pc molecules per AuNP was slightly different for the two at 27 and 49 (Table 3), respectively. The amount of singlet oxygen produced from AuNR (4.7) at $\Phi_{\Delta} = 0.25$ was slightly larger than for AuNR (2.0) corresponding to the larger Pc molecules per AuNP at 111 and 243, respectively. The largest Φ_{Δ} value at 0.29 was observed for AuNR (7.1) with the largest number of Pcs per AuNP.

Conclusions

Water soluble zinc thiamine substituted phthalocyanine was successfully synthesized. The compound shows aggregation in aqueous solution, which led to the quenching of the fluorescence quantum yields. The photophysical properties of the Pc improved by the addition of Triton X which is a surfactant. The Pc was also conjugated to gold nanospheres and nanorods of different aspect ratios. An increase in fluorescence quantum yields in the presence of the nanorods shows that the aspect ratio has an effect on the photophysical behavior of this molecule. Singlet oxygen quantum yields were larger for complex 4 linked to AuNPs of higher aspect ratios.

Acknowledgements

This work was supported by the Department of Science and Technology (DST)/Nanotechnology (NIC) and the National Research Foundation (NRF) of South Africa, through DST/NRF South African Research Chairs Initiative for Professor of Medicinal Chemistry and Nanotechnology and Rhodes University.

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