Effect of micellar and sol–gel media on the spectral and kinetic properties of tetracycline and its complexes with Mg(2+)

ARTICLE *in* PHOTOCHEMICAL AND PHOTOBIOLOGICAL SCIENCES · DECEMBER 2013

Impact Factor: 2.27 · DOI: 10.1039/c3pp50314c · Source: PubMed

5 AUTHORS, INCLUDING:

Benedetta Carlotti
Università degli Studi di Perugia
40 PUBLICATIONS **296** CITATIONS
SEE PROFILE

Catia Clementi
Università degli Studi di Perugia
35 PUBLICATIONS **598** CITATIONS
SEE PROFILE

Raimondo Germani
Università degli Studi di Perugia
136 PUBLICATIONS **1,718** CITATIONS
SEE PROFILE

Fausto Elisei
Università degli Studi di Perugia
193 PUBLICATIONS **3,318** CITATIONS
SEE PROFILE

Photochemical & Photobiological Sciences

PAPER

Effect of micellar and sol–gel media on the spectral and kinetic properties of tetracycline and its complexes with Mg^{2+}†

Cite this: *Photochem. Photobiol. Sci.*, 2014, **13**, 509

Alessio Cesaretti, Benedetta Carlotti, Catia Clementi, Raimondo Germani and Fausto Elisei*

The spectroscopic and photophysical properties of the broad-spectrum antibiotic tetracycline (TC) and its Mg^{2+} complexes were studied in organized media attained by means of three iso-structural quaternary ammonium surfactants able to self-assemble in water at low c.m.c. values, thus giving spherical micelles and sol–gel media upon increasing the concentration. Specific protonated forms of TC and its complexes were introduced in these micro-heterogeneous environments and then investigated through steady-state (both in absorption and emission) and pulsed (up to femtosecond resolution) spectroscopic techniques. Free TC showed minor spectral and kinetic variations while complexes remained unchanged in the presence of spherical micelles, meaning that TC is likely to be placed at the interface between the micelle and the bulk aqueous solution, without altering its bioactivity. Ultrafast transient absorption spectroscopy proved to be a powerful tool to gain deep insight into the distribution of the investigated species between the heterogeneous structure of sol–gel media. In fact, according to the polarity and net charge of free TC and its complexes, these species can be mostly found in the hydrophobic (intertwined worm-like micelles) or in the hydrophilic domains (basically aqueous pools) that the sol–gel is made up of. In the first case, the properties are dramatically altered (highly enhanced fluorescence and lengthened lifetime of the first singlet excited state up to the nanosecond time scale), leading to the improved traceability of the drug.

Received 9th September 2013,
Accepted 1st November 2013

DOI: 10.1039/c3pp50314c

www.rsc.org/pps

Introduction

Confined environments, such as micellar and sol–gel media, have gained increasing interest in the last few years thanks to their ability to interact with a variety of molecules, thus providing them with higher viscosity and lower polarity than the bulk aqueous solution.[1] This kind of organized media results in an environment capable of altering the photophysical behaviour of entrapped molecules, affecting both their spectral properties and their deactivation dynamics.[2–6] These features earned micelles and sol–gels the title of drug delivery systems: they can be loaded with various compounds, poorly water-soluble drugs as well as inorganic ions, thus carrying them through the body and up to the desired sites.[7–9]

Both micelles and sol–gels develop starting from surfactant solutions as precursors. It is well known that when the surfactant concentration rises above a critical value, namely the critical micellar concentration (c.m.c.), aggregates are formed.[10] At first, micelles are like spheres, but as the concentration of surfactants continues to rise, their shape can turn from spherical to worm-like. It has been found that micellar sphere-to-rod growth can occur when the Coulombic repulsion between the charged hydrophilic heads is reduced by the presence of a sufficient amount of an additional salt providing definite counterions.[11–15]

The worm-like aggregates can then interact through intermolecular forces to give an integrated network (sol–gel) whose rheology resembles that of solids rather than that of liquids.[16–20] Such media are able to immobilize drugs and hence can act as carriers for pharmacological purposes. Moreover, these viscoelastic systems can be employed not only as drug delivery vehicles, but also as a wide range of agents: detergents, cosmetics and household products, just to mention a few.[21]

Therefore, many organic chemistry groups have focused their attention on the synthesis of specific surfactants capable

Chemistry Department, and Centro di Eccellenza sui Materiali Innovativi Nanostrutturati (CEMIN), University of Perugia, via Elce di Sotto 8, 06123 Perugia, Italy. E-mail: fausto.elisei@unipg.it; Fax: +39-075-5855598; Tel: +39-075-5855588

†Electronic supplementary information (ESI) available: Additional graphs, which are numbered consecutively starting with Fig. S1 as referred to in the text, and additional tables (Tables S1 and S2). See DOI: 10.1039/c3pp50314c

of giving gelation at lower concentration: this is the case of cationic surfactants and their derivative zwitterionic amine-oxide surfactants.[22,23] The latter have small highly polar head groups whose micelles have zero net-charge, thus being able to grow with no addition of salts. Amine-oxide surfactants have proven to be extremely interesting for their biodegradability and biocompatibility, as well as for their not needing any solvent other than water in order to form the gel.[22–27]

For all these reasons, the work herein described deals with the interactions of tetracycline, an important antibiotic, with a class of ammonium and amine-oxide surfactants having the same hydrophobic tail. Tetracyclines have been widely studied since their discovery in the 1940s because of their ability to bind more specifically the bacterial ribosome than the eukaryotic one.[28,29] Their complexes with divalent metal cations are able to inhibit the protein synthesis within the bacterial cell thus leading to cell death, making them suitable for application as broad antimicrobial-spectrum antibiotics.[29–31] The photophysical behaviour of tetracyclines in water, alone[32,33] and in the presence of biologically important ions,[34–36] has been the subject of several studies in the last few decades. It has been proven that the presence and position of additional hydroxyl groups in the upper peripheral region of the compound are responsible for a peculiar affinity towards magnesium(II) cations. The effect of the divalent metal cations on the complexation process has been elucidated by probing the interaction of tetracycline with calcium(II) and copper(II), as well as magnesium(II). The association mechanism was found to be strictly dependent on the protonated form of tetracycline prevailing at each pH value explored.

Tetracyclines have already been investigated in micellar and sol–gel media in a few scattered reports. In particular, micelles have been found to promisingly enhance the fluorescence of complexes between tetracyclines and europium(III),[5,37] thus fostering their traceability. Also, new very interesting frontiers have been opened up by the findings of the first recent investigations on the inclusion and controlled release of tetracyclines in sol–gel matrixes[38] as well as in bioactive glasses.[39] Moreover, to the best of our knowledge, no systematic studies have been carried out on the pH-dependent behaviour of complexes of tetracycline with divalent metal cations fundamental for their bioactivity, in micelles and sol–gels. For all these reasons, the present work aims at the spectroscopic and photophysical characterization of tetracycline and its complexes with the biologically relevant Mg^{2+} cations in micellar and sol–gel environments, established by means of ammonium and amine-oxide surfactants in water buffered at different pH values.

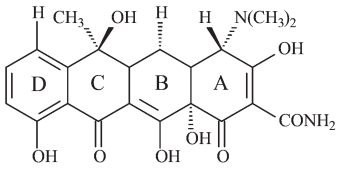
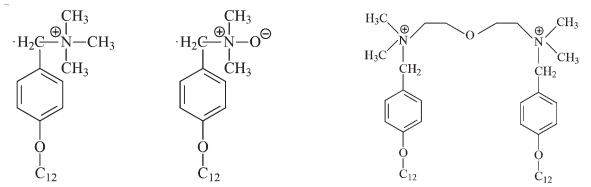


Chart 1 Tetracycline (TC) molecular structure.



A *B* *C*

Chart 2 Molecular structures of the surfactants used in the various experiments. (A) *p*-Dodecyloxybenzyltrimethylammonium bromide (*p*DoTABr), (B) *p*-dodecyloxybenzyldimethylamine oxide (*p*DoAO) and (C) bis-[2-(*N*,*N*-dimethyl-*N*-*p*-dodecyloxybenzylammonium bromide)-ethyl]-ether (Gemini).

Experimental section

Chemicals and samples

The investigated compound, tetracycline (TC, Chart 1) from Fluka BioChemika, was used without any further purification. Solutions of the metal cations Mg^{2+} at specific concentrations were prepared by dissolution in water of $MgCl_2$ anhydrous salt furnished by Fluka. The pH of aqueous solutions was obtained by Britton buffers in the pH range 2–11 for the various experiments. Britton buffers were prepared by mixing an acid solution (H_3BO_3 0.04 M, H_3PO_4 0.04 M and CH_3COOH 0.04 M) with a 0.2 M solution of NaOH, adjusting the ionic strength of the final solution to 5 mM.

Cationic and zwitterionic surfactants (Chart 2)[23–25] were added in aqueous solutions in order to establish micellar and sol–gel environments. The cationic surfactant *p*DoTABr (c.m.c. = 5.99×10^{-4} M) was used at 0.005 M or higher to form micelles and enclose TC, whose interactions with the micelles and with both types of metallic cations in micellar medium were studied. The isostructural zwitterionic *p*DoAO (c.m.c. = 1.61×10^{-5} M) and the more complex Gemini (c.m.c. = 2.09×10^{-5} M) surfactants were used at 0.067 M to create sol–gel and provide a more rigid environment for the substrates and their complexes. Depending on the pH, different concentrations of Mg^{2+} were added to a TC solution (5×10^{-6}–5×10^{-5} M) in order to promote as much as possible the association process and, thus, study the properties of the completely complexed drug.

When dealing with sol–gel systems, initial solutions of surfactants 0.1 M were prepared; the solutions were then stirred and warmed to dissolve the surfactants, forming a viscous gel. These sol–gels were heated again in order to be fluidified and to be put in suitable cuvettes for spectroscopic analysis with a 1 cm optical path. Appropriate ratios of TC and cation solutions (for a total amount of 1 mL) were then added to 2 mL of gels straight into the cuvette, where the solutions were homogenized by means of further heating. Sol–gels were left to cool down to room temperature for at least 4 hours and then used for spectroscopic studies.

Photophysical measurements

Absorption spectra were recorded with a Perkin Elmer Lambda 800 spectrophotometer. In order to establish the equilibrium constants of the complexation process between TC in micellar medium and Mg^{2+}, as well as the concentration profiles and spectra of the intermediate species, global fitting of multivariate spectrophotometric data was carried out by the ReactLab Equilibria software (Jplus Consulting).[40] The parameters sum-of-squares (ssq) and standard deviation for the residuals (σ_r) were used to evaluate the accuracy of the fits.

Fluorescence emission spectra were recorded with a Fluorolog-2 (Spex, F112AI) spectrophotofluorometer that provides corrected spectra for the monochromator response and detector sensitivity. Fluorescence quantum yields (experimental error of *ca.* 7%) were determined from the emission spectra of samples recorded at an absorbance lower than 0.1 at the excitation wavelength, to avoid self-absorption effects. TC in aqueous solution at various pHs was used as a reference[34] to calculate fluorescence quantum yields of the same substrate at the same pH in the presence of micelles formed by the cationic surfactant. In sol–gel medium fluorescein was used as a standard: as its fluorescence yield in sol–gel at pH 9 was found to be higher than in aqueous solution, where it is 0.97 at the same pH,[41] $\Phi_F = 1$ was estimated in this medium, avoiding the drawback of not knowing the refractive index of the gel. The error relative to the determination of fluorescence quantum yields (Φ_F) is about 10% when $\Phi_F \geq 10^{-3}$ and 20% when $\Phi_F < 10^{-3}$. The fluorescence lifetimes (τ_F) were measured by a spectrofluorometer based on the time correlated single photon counting technique (TC-SPC), equipped for the systems under investigation with a LED source centred at 370 nm using an interference filter centred at 366 nm in the excitation line and a long-pass filter in emission at 488 nm. The resolution time of the experimental set-up is 0.5 ns when LED is used.[42] All the data acquired were analyzed using the IBH Data Analysis software; the program allows us to deconvolve the decay profile of the source from the sample decay and provides the fitting of the data whose goodness is given by means of residuals distribution and χ^2 value.

The experimental set-up for ultrafast spectroscopic and kinetic measurements has been widely described elsewhere.[43,44] The 400 nm excitation pulses of *ca.* 40 fs were generated by an amplified Ti:sapphire laser system (Spectra Physics, Mountain View, CA). The transient absorption set-up (Helios, Ultrafast Systems, Sarasota, FL) is characterized by a temporal resolution of *ca.* 150 fs and a spectral resolution of 1.5 nm. Probe pulses for optical measurements were produced in the 470–750 nm range by passing a small portion of 800 nm light through an optical delay line (with a time window of 3200 ps) and focusing it into a 2 mm thick Ti:sapphire window to generate a white-light continuum. The chirp inside the sample cell was determined by measuring the laser-induced Kerr signal of the solvent. All the measurements were carried out under magic angle in a 2 mm cell and at an absorbance of 0.3–1.0 at 400 nm. To avoid any possible photoproduct interference, solutions were stirred during the experiments, while cuvettes holding samples in sol–gel were kept in movement at a constant speed of 0.5 mm s^{-1} by a translational sample holder (Ulltrafast Systems) controlled by two NSC200 controllers (Newport, Irvine, CA), for horizontal and vertical displacement respectively, to prevent local photodegradation of the non-diffusing medium. Transient absorption data were analyzed using the Surface Xplorer PRO (Ultrafast Systems) software where it was possible to perform singular value deconvolution of the 3D surface into principal components (spectra and kinetics) followed by global analysis.

Results and discussion

Absorption and fluorescence properties

Micelles (*p*DoTABr). TC in water can undergo several deprotonation equilibria, involving the functional groups bound to its linearly fused four-ring skeleton, whose dissociation constants have been broadly characterized ($pKa_1 = 3.5$; $pKa_2 = 7.3$; $pKa_3 = 9.5$).[32,45,46] As a result, TC can exhibit in aqueous solution either a positive or a net negative charge according to the pH, the prevailing form being cationic, zwitterionic, monoanionic and di-anionic at pH 2, 5, 9, 11, respectively.

Nonetheless, as has already been described before for cationic surfactants,[47,48] the inclusion of molecules within a micellar medium can alter the acid–base behaviour of the drug by increasing its acid dissociation constant values as a consequence of a local pH increase, and the same was found for TC. In fact, the spectral properties of the latter are affected by changes in the protonation state (Fig. S1†). These pH-dependent variations, acquired in the pH range 2–12 in the presence of *p*DoTABr micelles, are well fitted by considering three dissociation points at 3.1, 6.5, 9.7, respectively (Fig. S2†): the first two are slightly lower than those detected in pure water and the third is nearly unaffected by the new environment. Moreover, the acidity constants in the excited state, retrieved from pH-dependent changes in the fluorescence spectra, have been found to be very close to the corresponding values in the ground electronic state, as previously revealed in pure water solution,[32] meaning that no proton transfer equilibrium occurs upon excitation. These findings allowed the photobehaviour of TC to be studied at pH values of about 2, 5, 9 and 11, as they are still distant enough from the newly-calculated equilibrium constants in micellar medium, being the same differently protonated form the one prevailing at each pH both in absorption and in emission.

The interaction between TC and the cationic surfactant (*p*DoTABr) was then studied by means of spectrophotometric and fluorimetric titrations. Starting from buffered aqueous solutions of TC, increasing concentrations of the surfactant were added, up to two orders of magnitude above its c.m.c. As for the spectrophotometric titration, the spectral changes observed at pH 5 upon addition of *p*DoTABr are shown in Fig. 1A (the results obtained at pH 9 and 11 are shown in Fig. S3–S4†). Zwitterionic TC is characterized by two

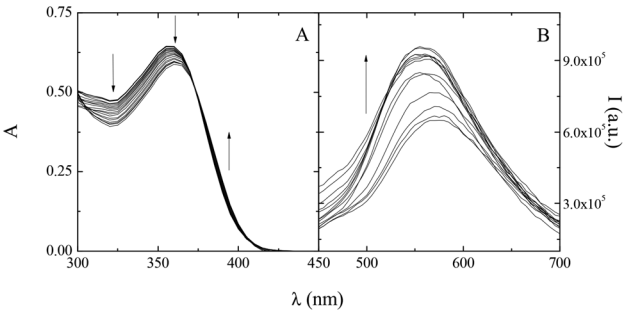


Fig. 1 (A) Absorption spectra of TC (5.2×10^{-5} M) in aqueous solution at pH 5 alone and in the presence of increasing amounts of *p*DoTABr up to 0.0375 M. (B) Fluorescence spectra of TC (9.0×10^{-6} M) in aqueous solution at pH 5 alone and in the presence of increasing amounts of *p*DoTABr up to 0.0110 M.

absorption bands centred at 355 nm and 275 nm, the latter being covered by the absorption of the ammonium surfactant, which is not negligible at 300 nm. As the concentration of the surfactant begins to rise, some variations are detected: the long wavelength band is red-shifted to a new maximum at about 361 nm, while its absorbance is reduced to a small degree and a deeper minimum is formed around 320 nm. Fig. 1B reports fluorescence spectra of TC at the same pH: zwitterionic TC shows a broad emission band and the interaction between the drug and the surfactant is proven by a slight enhancement of the emission intensity together with a shift towards a shorter wavelength that results in a reduced Stokes shift.

The mechanism of complexation between TC and Mg^{2+} cations in the presence of *p*DoTABr micelles was then investigated at pH 5 (Fig. S5–S6†) and pH 11 (Fig. S7–S8†) through spectrophotometric and fluorimetric titrations, as previously done in water.[34] Increasing amounts of Mg^{2+} were added to the micelle containing solution and this provided changes in both absorption and emission spectra (red shifted absorption, blue shifted emission, reduced Stokes shift and enhanced fluorescence intensity). As observed for other molecules (flavonols and anthraquinoids)[49–51] where the complexation site is a hydroxyl oxygen adjacent to a carbonyl oxygen (β-ketol position), forming a six-membered chelate ring structure with the metal ions, the appearance of emission and the reduction of the Stokes shift can be attributed to the lack of excited state vibrational relaxation through hydrogen bonding with the solvent, similarly to what happens for the deprotonated forms. The analysis of the data revealed the presence of two subsequent complexation equilibria, with the formation of two complexes in stoichiometries 1 : 1 and 1 : 2 (TC : metal ions), whose interaction sites can be attributed according to previous literature reports.[34–36]

Association constants between TC and Mg^{2+} cations obtained at pH 11 from spectrophotometric titrations in micelles and in aqueous solution are listed in Table 1 (results obtained at pH 5 are shown in Table S1†). Even though in the organized medium the association constant values are lower than those retrieved in pure water solutions, no interference in the complexation between TC and magnesium cations seems to have been brought about by the micelles. The data obtained at pH 5 cannot be considered completely reliable due to the very low values of the association constants.

Table 1 Association constants (K_{ass}) obtained by absorption and fluorescence titrations and interaction sites of the complexes between TC and Mg^{2+} cations in aqueous solutions at pH 11 and in *p*DoTABr micelles ([*p*DoTABr] = 5×10^{-3} M)[a]

			Log K_{ass}	
pH	System	Complex	Solution	Micelle
11	TC–Mg^{2+}	1 : 1	4.581 ± 0.006	2.48 ± 0.03
		1 : 2	1.74 ± 0.02	1.23 ± 0.09

[a] Data in solution retrieved from ref. 34. Values in micellar medium refer to this work.

Absorption and emission properties of TC and its complexes with Mg^{2+} cations in the presence of *p*DoTABr micelles at different pHs are shown in Table 2. The same properties revealed in aqueous solutions are given therein for comparison. Irrespective of the pH, what can be readily seen is a reduction of the Stokes shift for unassociated TC in micellar with respect to aqueous medium. Complexes with Mg^{2+}, on the other hand, experience less pronounced shifts both in absorption and emission, resulting in a smaller reduction of the Stokes shift. Moreover, by increasing the pH, free TC exhibits bathochromic shifts in absorption and hypsochromic shifts in emission, as a consequence of the prevailing acid–base form present in solution. Though less important, the same trend is followed by the complexes with magnesium. According to fluorescence quantum yields (Table 2), emission is still a minor pathway to the excited state deactivation of the drug, but it becomes greater by 30–40% for unassociated TC in micellar medium compared to the same data calculated in water. Furthermore, fluorescence yield does not seem to vary on going from the solution to the micellar medium for the complexes with magnesium.

These results suggest that the presence of Mg^{2+} ions affects more deeply the spectral and photophysical properties of TC with respect to the effect of the *p*DoTABr surfactant.

Sol–gel (*p*DoAO and Gemini). Sol–gel media have been attained by means of an amphoteric zwitterionic surfactant (*p*DoAO, Chart 2B), isostructural to the cationic one used to establish the micellar environment, but able to give a firm gel (Gel(+/−)) when its concentration rises above 0.05 M. In order to investigate the effect of the surfactant structure, a second more rigid sol–gel, named Gel(gemini), was employed, using a cationic dicephalic surfactant (Gemini, Chart 2C) made up of two subunits resembling *p*DoTABr and linked together by an ether bridge.

Sol–gels, in which TC and its complexes were dissolved, were first prepared with deionized water and then with aqueous solutions buffered at the typical pH values of about 2, 5, 9 and 11. Samples in buffered Gel(+/−) and Gel(gemini)

Table 2 Absorption and fluorescence properties of TC and its complexes with divalent metal cations in aqueous solutions and in *p*DoTABr micellar medium ([*p*DoTABr] = 5×10^{-3} M) at different pHs[a]

		λ_{abs} (nm)		λ_{em} (nm)		Stokes shift (cm^{-1})		Φ_F	
pH	System	Solution	Micelle	Solution	Micelle	Solution	Micelle	Solution	Micelle
2	TC	355	357	605	595	11 700	11 200	0.00060	0.00070
	TC–Mg^{2+}	350	356	595	580	11 800	10 800	0.00070	0.001
5	TC	355	361	600	560	11 500	9800	0.00085	0.0012
	TC–Mg^{2+}	375	372	530	535	7800	8100	0.013	0.014
9	TC	370	374	530	530	8200	7900	0.0018	0.0024
	TC–Mg^{2+}	375	375	530	530	7800	7800	0.018	0.0093
11	TC	380	381	530	520	7500	7000	0.0030	0.0042
	TC–Mg^{2+}	375	377	520	510	7500	6900	0.020	0.019

[a] Data in solution retrieved from ref. 34. Values in micellar medium refer to this work.

Table 3 Absorption and fluorescence properties of TC and its complexes with Mg^{2+} at different pHs in *p*DoAO sol–gel ([*p*DoAO] = 0.067 M) and in Gemini sol–gel ([Gemini] = 0.067 M)[a]

		λ_{abs} (nm)		λ_{em} (nm)		Stokes shift (cm^{-1})		Φ_F	
pH	System	Gel(+/−)	Gel(gemini)	Gel(+/−)	Gel(gemini)	Gel(+/−)	Gel(gemini)	Gel(+/−)	Gel(gemini)
2	TC	362	358	555	Broad	9600	n/a	0.0072	$\sim 10^{-3}$
	TC–Mg^{2+}	—	—	—	—	—	—	—	—
5	TC	365	360	545	555	9000	9800	0.0074	$\sim 10^{-3}$
	TC–Mg^{2+}	374	—	525	—	7700	—	0.045	—
Deionized H_2O	TC	370	377	535	520	8300	7300	0.0098	$\sim 10^{-3}$
	TC–Mg^{2+}	375	—	515	—	7200	—	0.10	—
9	TC	375	376	525	510	7600	7000	0.033	0.015
	TC–Mg^{2+}	375	375	515	525	7200	7600	0.11	0.012
11	TC	380	380	520	505	7100	6500	0.058	0.014
	TC–Mg^{2+}	376	376	510	510	7000	7000	0.10	0.022

[a] No data could be acquired in the case of the complexes at acid pHs and in deionized water because of the precipitation of the Gemini surfactant as a consequence of the salting out phenomenon.

were characterized in terms of absorption and emission spectra (Table 3).

As can be easily seen from the comparison with the same properties detected in water[34] (*cf.* Table 2), Gel(+/−) is able to confine the drug and its complexes in a constrained environment, whose effect is to reduce the Stokes shift, by moving the absorption band towards longer wavelength (Fig. S9†) and causing the blue shift of the emission spectrum (Fig. 2).[4] These features reveal that TC experiences a more viscous environment, in analogy to previous studies where the same spectral modifications were observed in solvents of increasing viscosity.[32] This effect is quite clear for free TC, whereas the Stokes shifts of complexes with magnesium are less affected. Moreover and as well as in aqueous solution, a monotone trend is revealed by increasing pH: absorption moves to the red (or stays unchanged in the case of complexes) while fluorescence moves to the blue.

Dealing with Gel(gemini), absorption wavelengths are very close to those detected in Gel(+/−), both for free TC and for complexes with magnesium. But when it comes to fluorescence, different behaviour emerges whether there is Mg^{2+}

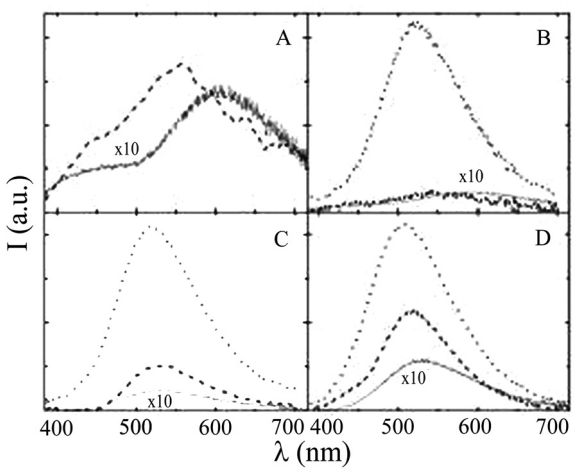


Fig. 2 Emission spectrum of TC ($\sim 5 \times 10^{-6}$ M) in aqueous solution (full line) and in Gel(+/−) ([*p*DoAO] = 0.067 M) alone (dashed line) at pH 2 (A). Emission spectra of TC ($\sim 5 \times 10^{-6}$ M) in aqueous solution (full line) and in Gel(+/−) ([*p*DoAO] = 0.067 M) alone (dashed line) and in the presence of Mg^{2+} (dotted line) in concentration 0.17 M at pH 5 (B), and in concentration 0.0067 M at pH 9 (C) and 11 (D).

or not: in the case of unassociated TC, a greater effect on the emission maximum, which moves hypsochromically (up to 505 nm at pH 11), is revealed, while the fluorescence band position of the complexes is almost unaltered by this medium with respect to aqueous solutions.

As to Gel(+/−), a major effect is detected on fluorescence quantum yields (Φ_F). Compared to their behaviour in water, Φ_F of free TC is subjected to an increase[4] by one order of magnitude or more at basic pHs, while it is enhanced by a factor of five for the complexes. Fluorescence yields of unassociated drug increase with pH on going from cationic to anionic forms. In the case of complexes with magnesium, as long as almost complete complexation of the fluorophore is reached (namely at pH 9 and pH 11), the same value of about 10% for the emission efficiency is observed, whereas at pH 5, where a different mechanism of complexation is known to occur,[34] Φ_F is not so high. On the other hand, fluorescence quantum yields in Gel(gemini) are again enhanced for free TC, but there is a slight increase over Φ_F in pure water, in comparison with the other sol–gel. Furthermore, complexes with magnesium are not affected by this viscous medium and their fluorescence efficiencies approach the values found in aqueous solutions.

To try and explain these peculiarities, the nature of the medium has to be invoked: sol–gels are surfactant solutions organized in worm-like micelles able to establish a heterogeneous environment, made up of aqueous domains and hydrophobic ones.[3] Given that the substrate can spread out into these different areas, it displays a behaviour more or less resembling that in water according to its allocation ratios. The polarity of the drug and its electrostatic interactions with micelle head groups play a key role in its distribution between the two domains: free TC shows either a positive or a negative charge, depending on pH, while complexes, because of the chelation of divalent metal cations, are always positively charged. Therefore, unassociated TC properties are greatly modified by the sol–gel, as the free drug is always able to make its way through the hydrophobic domains to a large extent, as opposed to complexes with magnesium, which are more likely to be still in water pools. This could be the reason why complexes in both sol–gels are partially located in a basically aqueous environment, especially in the case of Gel(gemini) where micelles themselves are positively charged and the complex has to get over the electrostatic repulsion to be placed in between the worm-like micelles.

Femtosecond transient absorption

Micelles (*p*DoTABr). Since TC and its pH-dependent complexes with divalent metal cations show a fast decay in pure water, the excited state dynamics of the free molecule and its Mg^{2+} complexes in micellar solutions buffered at various pH values (2, 5, 9 and 11) were investigated by means of ultrafast pump–probe absorption spectroscopy.

Concerning free TC, data are primarily characterized by negative signals due to stimulated emission, whose position shifts towards the red part of the spectrum with pH on going from the cationic to the di-anionic form of the drug, as the stationary fluorescence does (Fig. 3 and Table 4). Similarly to what has already been observed in pure water,[32,34] TC dynamics in *p*DoTABr micelles is well described by bi- or tri-exponential functions according to which protonated form prevails at the investigated pH value (Table 4). Apart from a short component, ascribed to a fast solvent equilibration (SE)[52,53] or a vibration cooling phenomenon (VC),[54] a medium lived component (M), whose time constant ranges from 19 ps to 25 ps, is the most intense at all pHs and it can be assigned to the first excited singlet state of the drug, S_1(TC). As previously observed in aqueous solutions between pH 6.7 and 10.2,[32] again in basic buffers (pH 9 and pH 11), a third longer-lived component (L), being a differently protonated or tautomeric form of the lowest excited singlet state S_1, is observed (τ_L = 140–280 ps). The evidence of a keto-enolic tautomerism has been foreseen in TC by semiempirical and quantum mechanical calculations[55,56] and experimentally observed in the analogous doxycycline in aqueous solutions at different pHs.[57] A more reliable assignment of the L transient would require a more detailed study of the possibility of tautomerism also in the excited state, which is beyond the aim of the present work.

The behaviour of magnesium complexes changes with pH as the mechanism of complexation changes. Concentration

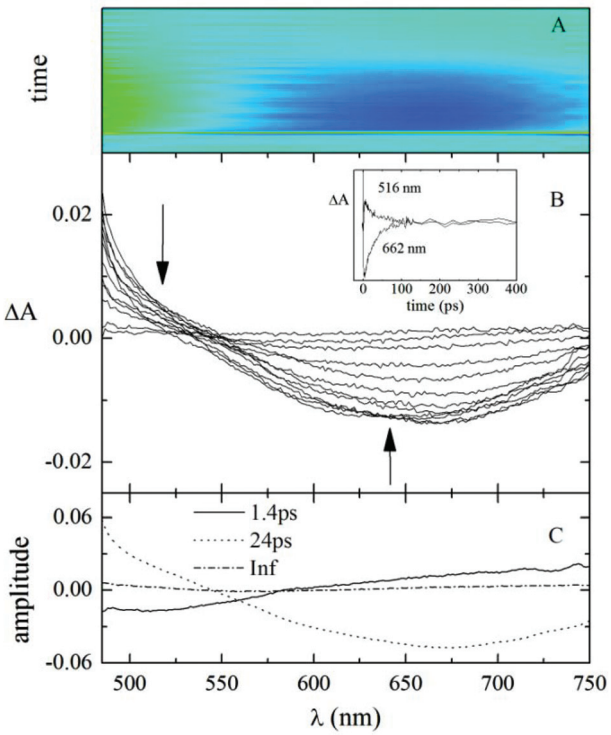


Fig. 3 Pump–probe absorption spectroscopy of TC in a micellar environment at pH 5 (λ_{exc} = 400 nm; [*p*DoTABr] = 5 × 10^{-3} M): (A) contour plot of the experimental data; (B) time resolved absorption spectra recorded 0.7 (a), 1.1 (b), 1.9 (c), 2.8 (d), 6.0 (e), 8.8 (f), 12 (g), 20 (h), 30 (i), 47 (j), 81 (k) and 410 (l) ps after the laser pulse. Insets: decay kinetics recorded at meaningful wavelengths and (C) amplitudes of the decay components obtained by SVD and global analysis.

Table 4 Spectral and kinetic properties of the excited states of TC and its complexes (1 : 1 and 1 : 2) with divalent metal ions in water and in *p*DoTABr micellar medium at different pHs (λ_{exc} = 400 nm; [*p*DoTABr] = 5 × 10^{-3} M)[a]

		Solution		Micelle		
pH	System	λ (nm)	τ (ps)	λ (nm)	τ (ps)	Assignment
2	TC	550(−), 700(+)	1.8	<580(−), 700(+)	0.98	SE
		525(+), 660(−)	20	<575(+), 675(−)	19	S_1(TC)
		Broad	Inf	—	—	T_1
	TC–Mg^{2+}	680(+)	1.0	640(+)	0.29	SE
		500(+), 660(−)	20	<565(+), 670(−)	26	S_1(TC)/1 : 1 (A)
5	TC	530(−), 680(+)	2.0	510(−), 720(+)	1.4	SE
		525(+), 680(−)	22	<545(+), 670(−)	24	S_1(TC)
		Broad	Inf	Broad	Inf	T_1
	TC–Mg^{2+}	500(−), 610(+)	2.5	<500(−), 695 (+)	1.1	SE
		510(+), 640(−)	20	555(−)	27	1 : 1 (A)
		565(−), >630(+)	200	565(−)	290	1 : 2 (A,BCD)
		—	—	670(+)	Inf	T_1
9	TC	510(−), 670(+)	3.5	500(−), 730(+)	4.2	VC
		<500(+), 570(−)	20	530(−), 720(+)	25	S_1(TC1)
		510(+), 620(−)	200	575(−), 740(+)	280	S_1(TC2)
		650(+)	Inf	530(+)	Inf	T_1
	TC–Mg^{2+}	470(−), 610(+)	1.6	<500(−), 580(+)	1.4	SE
		—	—	<500(+), 540(−), >660(+)	20	S_1(TC)
		440(+), 570(−)	250	<500(+), 555(−), >660(+)	280	1 : 1/1 : 2 (BCD,A)
11	TC	580(+), 700(−)	6.0	500(−), 725(+)	5.0	VC
		<480(+), 570(−)	19	535(−), 725(+)	25	S_1(TC1)
		510(+), 620(−)	150	550(−), >630(+)	140	S_1(TC2)
		660(+)	Inf	Broad	Inf	T_1
	TC–Mg^{2+}	520(−), 650(+)	4.0	<590(−), 715(+)	1.0	VC/SE
		—	—	540(−), 715(+)	14	S_1(TC)
		<470(+), 570(−)	280	555(−), 715(+)	300	1 : 1/1 : 2 (BCD,A)

[a] Data in solution retrieved from ref. 32 and 34. Values in micellar medium refer to this work.

ratios ([Mg^{2+}]/[TC]) were chosen in order to have a significant formation of the complexes, pushing to its maximum the coordination of magnesium ions on TC sites. Nonetheless, it has to be highlighted that magnesium salt concentrations were kept slightly lower than that achieved in pure water to prevent the precipitation of the surfactant as a result of salting out. Hence, together with solvent equilibration ($\tau_S \sim 1$ ps at all pHs), what can be detected is some unassociated TC and its complexes TC–Mg^{2+} in stoichiometries 1 : 1 and 1 : 2 (Table 4 and Fig. 4).

To summarize, the effect of *p*DoTABr micelles on the deactivation dynamics of TC and TC–metal ion complexes is negligible: time constants of excited states are usually slightly lengthened but not markedly altered by the presence of the surfactant molecules. As stationary measurements pointed out, even transient absorption spectroscopy revealed that the introduction of micelles in solution makes the emission shift towards shorter wavelengths, as a result of the confinement of TC and its complexes at the water–micelle interface.[58] The effect is very clear at basic pHs, where both unassociated TC and its complexes with Mg^{2+} experience a blue shifted stimulated emission. At pH 9, for instance, stimulated emission for free TC is centred at about 540 nm (Fig. S10†) while in pure water it was detected at 570 nm, whereas TC–Mg^{2+} complexes emit at 555 nm (Fig. S11†) in the presence of micelles as opposed to 570 nm in their absence (Table 4).

Sol–gel (*p*DoAO and Gemini). In the case of *p*DoAO sol–gel medium, since Φ_F values are greater than those detected in water and the emitting excited state deactivation is expected to be slowed down by the restrained environment,[59] the fluorescence decay kinetics of free and complexed TC were successfully measured by TC-SPC. Although stationary data suggest that the Gemini sol–gel is not able to confine TC–Mg^{2+} complexes efficiently, longer fluorescence lifetimes (τ_F) were determined also in this medium, probably due to some molecules still placed in the hydrophobic domains.

Irrespective of the complexation, all the fluorescence decays are well fitted by a bi-exponential function, made up of a shorter component with a time constant approaching the instrument temporal resolution and a longer one, described by a lifetime ranging from 1.3 to 2.8 ns (Table 5). In the case of Gel(+/−) the relative amplitudes of the two components vary markedly in step with pH, both for the free drug and for its complexes with magnesium. In deionized water as well as in basic buffer solutions, 60% of the emission is associated with the longer-lived species, while the relative amplitudes at acid pH are such that the shorter component prevails over the other. On the other hand, the emitting species with a lifetime on the order of nanoseconds is always the least important in Gel(gemini), in agreement with the stationary data, which revealed the difficulty of TC to be placed in the hydrophobic domains, especially for its complexes with magnesium: longer

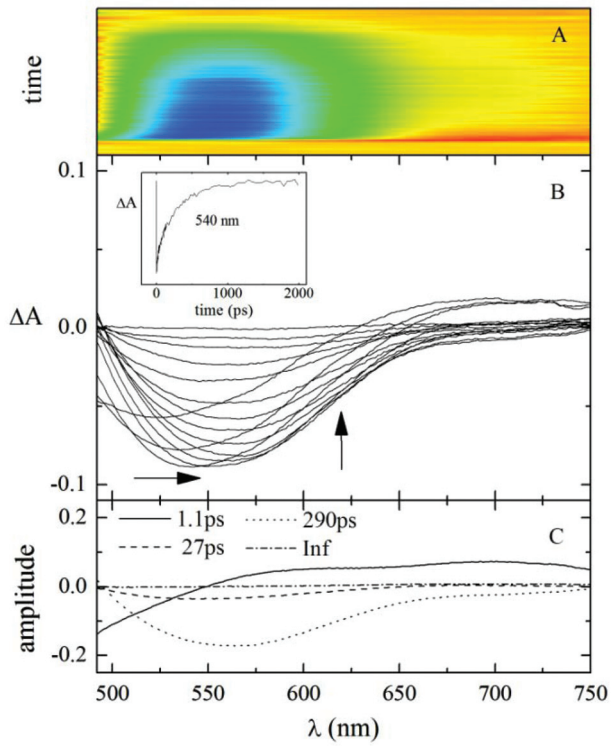


Fig. 4 Pump–probe absorption spectroscopy of TC in a micellar environment at pH 5 (λ_{exc} = 400 nm; [*p*DoTABr] = 5 × 10^{-3} M) in the presence of Mg^{2+}: (A) contour plot of the experimental data; (B) time resolved absorption spectra recorded 0.1 (a), 0.2 (b), 0.4 (c), 1.5 (d), 3.3 (e), 9.9 (f), 22 (g), 50 (h), 80 (i), 110 (j), 230 (k), 350 (l), 470 (m), 730 (n) and 1100 (o) ps after the laser pulse. Insets: decay kinetics recorded at meaningful wavelengths and (C) amplitudes of the decay components obtained by SVD and global analysis.

lifetimes are indeed due to the confinement of the molecule in between the worm-like micelles, and the amplitude of this component could prove how much of the drug is attached to the micelle walls.

Nonetheless, it was through femtosecond transient absorption spectroscopy that a clear assignment of these components could be achieved, based on the transient spectral shapes determined together with lifetimes. Time resolved spectra were acquired exclusively in deionized water, pH 5 and pH 9 to test the changes in the deactivation dynamics inferred by the different mechanisms of complexation known to take place at these two pHs.

The pump–probe absorption spectroscopy set-up is able to acquire spectra on a 3200 ps time scale, making it difficult to estimate accurately the lifetime of the long-lived transient, especially when it was found to be longer than 2 ns. Therefore, during the fitting procedure, the time constant of the long-lived component was fixed at the value retrieved from TC-SPC measurements to improve the goodness of the fit itself.

As long as Gel(+/−) is concerned (Table 6), free TC data are again characterized by mainly negative signals and they are well fitted by considering together with a rest absorption three components whose lifetimes and positions change significantly with pH, thus leading to a slightly different interpretation of the transients probed. At pH 5 (Fig. S12†), a short (4.0 ps) transient with a negative band at 505 nm and a positive maximum centred at 640 nm is detected; its time constant and spectral shape indicate that it could be a vibrational cooling. Yet, the other two transients are revealed and they are very different from the transient absorption signal of the free drug in water: both transients are described by stimulated emission negative spectra, with hypsochromically shifted minima compared to TC in water, and longer time constants of 72 and 2400 ps, respectively. Such changes in lifetimes and spectral properties are typical of molecules confined in the hydrophobic cores of the micro-heterogeneous sol–gel medium, and the two species can be considered as two differently protonated or tautomeric forms of the lowest excited singlet state.

In deionized water and basic buffered sol–gels (measurement at pH 9 shown in Fig. S13†), no transient ascribable to a relaxation phenomenon is revealed: the shortest component shows lifetimes of tens of picoseconds (τ = 15 ps in deionized water, τ = 17 ps at pH 9) and stimulated emission bands centred at about 530 nm; these features are very close to those of free TC in micelles, so that it could be some TC placed in the water pools,[60] which can be found in the sol–gel, but able

Table 5 Fluorescence lifetimes (obtained using the TC-SPC technique) and relative amplitudes of TC and its complexes with Mg^{2+} in *p*DoAO sol–gel ([*p*DoAO] = 0.067 M) and in Gemini sol–gel ([Gemini] = 0.067 M) at different pHs

		pH									
		2		5		Deionized H_2O		9		11	
System		τ (ns)	Relative amplitude	τ (ns)	Relative amplitude	τ (ns)	Relative amplitude	τ (ns)	Relative amplitude	τ (ns)	Relative amplitude
Gel(+/−)	TC	<0.5	61.9%	<0.5	72.1%	0.57	39.1%	<0.5	38.8%	<0.5	37.4%
		2.3	38.1%	2.5	27.9%	2.4	60.9%	1.7	61.2%	1.6	62.6%
	TC–Mg^{2+}	—	—	<0.5	86.7%	<0.5	34.7%	<0.5	45.8%	<0.5	41.3%
		—	—	1.3	13.3%	2.0	65.3%	1.9	54.2%	1.9	58.7%
Gel(gemini)	TC	—	—	<0.5	58.1%	<0.5	62.5%	<0.5	69.5%	<0.5	67.0%
		—	—	2.4	41.9%	2.5	37.5%	2.8	30.5%	2.6	33.0%
	TC–Mg^{2+}	—	—	—	—	—	—	<0.5	82.2%	<0.5	64.8%
		—	—	—	—	—	—	1.6	17.8%	1.6	35.2%

Table 6 Spectral and kinetic properties of the excited states of TC and its complexes (1 : 1 and 1 : 2) with divalent metal ions Mg^{2+} ([TC]/[Mg^{2+}] are given in brackets) in *p*DoAO sol–gel at different pHs (λ_{exc} = 400 nm)

pH	System	τ (ps)	λ (nm)	Assignment
5	TC	4.0	505(−), 640(+)	VC
		72	585(−)	S_1(TC) gel
		2400	560(−)	S_1(TC tautomer) gel
		Inf	540(+)	T_1
	TC–Mg^{2+} (1 : 200)	1.5	500(−), 640(+)	VC
		60	540(−)	S_1(1 : 1 A) gel
		290	575(−)	S_1(1 : 2 A,BCD) H_2O
		1300	560(−)	S_1(1 : 2 A,BCD) gel
		Inf	535(+)	T_1
H_2O	TC	15	530(−), 675(+)	S_1(TC) H_2O
		140	605(−)	S_1(TC) gel
		2400	550(−)	S_1(TC tautomer) gel
		Inf	broad	T_1
	TC–Mg^{2+}	18	520(−), 680(+)	S_1(TC) H_2O
		250	555(−)	S_1(1 : 1 BCD/1 : 2 A,BCD) H_2O
		1900	560(−)	S_1(1 : 1 BCD/1 : 2 A,BCD) gel
		Inf	550(+)	T_1
9	TC	17	525(−), 675(+)	S_1(TC) H_2O
		140	<520(+), 605(−)	S_1(TC) gel
		1700	545(−), 700(+)	S_1(TC tautomer) gel
		Inf	515(+), 640(+)^{sh}	T_1
	TC–Mg^{2+} (1 : 350)	34	520(−), 670(+)	S_1(TC) H_2O
		360	555(−)	S_1(1 : 1 BCD/1 : 2 A,BCD) H_2O
		1900	550(−), 690(+)	S_1(1 : 1 BCD/1 : 2 A,BCD) gel
		Inf	510(+)	T_1

to interact with the nearby surfactant molecules. Longer-lived transients display time constants of 140 ps and 2–3 ns, respectively, and they could be assigned to different forms of the molecule in the hydrophobic domains, as previously discussed for pH 5. The main difference occurring on going from the zwitterionic to the mono-anionic form of TC (namely from pH 5 to pH 9) is that the zero net charged species is essentially allocated in between the worm-like micelles, whereas negative TC, being more polar, can still be found even in basically aqueous domains. Moreover, the different contributions of the two longer transients revealed by TC-SPC and supported by ESA (excited state absorption) amplitudes, as they are both assigned to molecules occupying the same environment (hydrophobic domains), can be explained by the peculiar relative stabilization given to these differently protonated or tautomeric forms[55] by the Gel(+/−).

In the case of free TC in Gel(gemini) (Table 7), the behaviour is very similar. However, the distribution of the drug between the hydrophobic and the aqueous domains has to be taken into account: this time already at pH 5 some molecules spread preferentially in the water pools. This is not surprising considering the di-cationic nature of the Gemini surfactant: despite hydrophobic interactions favoured by the poor polarity of its zwitterionic form, TC needs to overcome a higher electrostatic repulsion with the surfactant to lie between the worm-like micelles in this kind of sol–gel.

On introducing magnesium ions, the picture becomes more complex. At pH 5 (Fig. S14†), Mg^{2+} is likely to form a first complex in stoichiometry 1 : 1 involving the chromophore localized on the A ring and not affecting the first excited singlet state time constant, and some 1 : 2 (TC–Mg^{2+}) complex, where TC coordinates a second ion on the fluorophore BCD, leading to an excited state lifetime lengthening.[34] These two complexes differ in the total net positive charge given by the cations, which happens to be higher after the second complexation. Therefore, the 1 : 1 (A) complex would be preferentially allocated in the hydrophobic domains (τ = 60 ps), while the 1 : 2 (A,BCD) complex inside the water pools (τ = 290 ps). Nevertheless, a further transient, whose lifetime is lengthened up to 1300 ps, is detected and can thus be ascribed to some of these 1 : 2 (A,BCD) chelates confined even in between the micelles. At pH 9 (Fig. 5), Mg^{2+} is known for being able to interact with the BCD fluorophore at first and only afterwards with the A ring. The prompt complexation on the BCD ring system is

Table 7 Spectral and kinetic properties of the excited states of TC and its complexes (1 : 1 and 1 : 2) with divalent metal ions Mg^{2+} in Gemini sol–gel at different pHs (λ_{exc} = 400 nm)

pH	System	τ (ps)	λ (nm)	Assignment
5	TC	34	670(−)	S_1(TC) H_2O
		140	600(−)	S_1(TC) gel
		2500	Broad	S_1(TC tautomer) gel
9	TC	42	520(−), 675(+)	S_1(TC) H_2O
		380	<500(+), 610(−)	S_1(TC) gel
		2500	<600(−), 670(+)	S_1(TC tautomer) gel
		Inf	510(+)	T_1
	TC–Mg^{2+}	2.9	515(−), 680(+)	SE + VC
		220	<490(+), 560(−)	S_1(1 : 1 BCD/1 : 2 A,BCD) H_2O
		1600	570(−)	S_1(1 : 1 BCD/1 : 2 A,BCD) gel
		Inf	580(+)	T_1

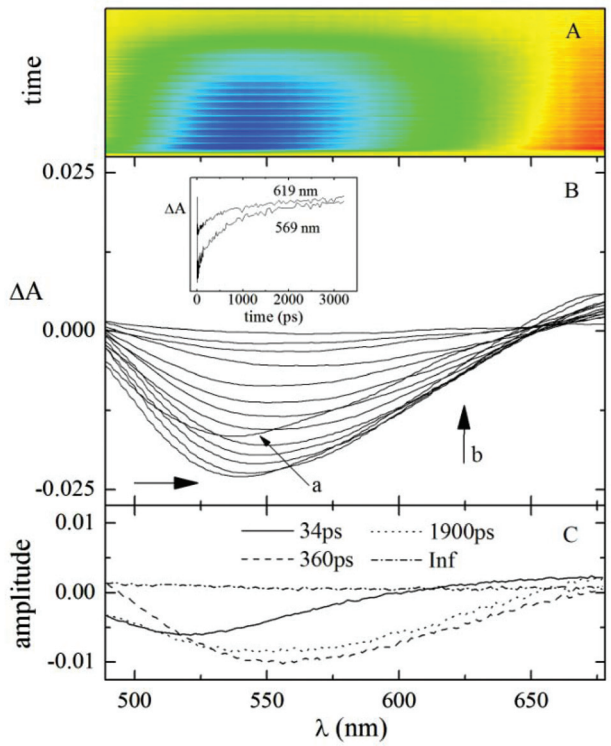


Fig. 5 Pump–probe absorption spectroscopy of TC in Gel(+/–) at pH 9 (λ_{exc} = 400 nm; [*p*DoAO] = 0.067 M) in the presence of Mg^{2+}: (A) contour plot of the experimental data; (B) time resolved absorption spectra recorded 0.3 (a), 1.2 (b), 3.6 (c), 8.1 (d), 18 (e), 41 (f), 95 (g), 160 (h), 320 (i), 520 (j), 990 (k), 1600 (l), 2300 (m) and 3200 (n) ps after the laser pulse. Insets: decay kinetics recorded at meaningful wavelengths and (C) amplitudes of the decay components obtained by SVD and global analysis.

responsible for the typical lengthening of the time constant, both for TC in gel (τ = 1900 ps) and for the drug in solution (τ = 360 ps), but no evidence of a second complexation on the A chromophore can be deduced from the ESA measurements. The fitting of the data gives back three transients: the longer-lived ones are complexes involving the chelation on the BCD fluorophore, 1 : 1 (BCD) or 1 : 2 (A,BCD), in the two different domains, whereas the shortest transient (τ = 34 ps and λ_{min} = 520 nm) could be some unassociated TC in the water pools. The reported assignments were strengthened by the investigation of the effect of magnesium concentration on the ultrafast excited state dynamics in Gel(+/–) (results reported in ESI, Table S2†). In the case of Gel(gemini), just like in Gel(+/–), long lifetimes assigned to complexes concerning the BCD chromophore in the two different environments are found in the presence of magnesium at basic pH.

In light of femtosecond spectroscopy findings, the relative amplitudes of the emitting components revealed by the TC-SPC technique (Table 5) for complexes with magnesium in sol–gel media can be now fully understood: the longer one is always assigned to a BCD-chelating complex in hydrophobic domains, while the other is due to the same complex in water. This type of complex is the first to be formed at basic pHs, but it is attained only after the second complexation under acid conditions; as a result, its distribution in between the hydrophobic worm-like micelles is preferential at basic pHs; that is when a less charged 1 : 1 (BCD) complex can be found. Nevertheless, the amplitude of the component assigned to a complex placed in the hydrophobic domains is always the least important in Gel(gemini), as a consequence of the electrostatic repulsion of the positively charged complexes with the Gemini cationic surfactant.

Conclusions

The work herein described was aimed at the investigation of the spectroscopic and photophysical properties of the widely known antibiotic TC and its pH-dependent Mg^{2+} complexes in organized environments ranging from micellar to sol–gel systems. Three surfactants belonging to the same family but characterized by different charges and structures were employed in order to establish utterly different media. To get a deeper insight into the drug features, the analysis was carried out at four pH values (*i.e.* 2, 5, 9 and 11), chosen in keeping with TC dissociation constants, so that only a specific protonated form (*i.e.* cationic, zwitterionic, monoanionic and dianionic) prevails over the others. The investigation was performed by means of stationary and pulsed techniques, pushing the envelope up to femtosecond resolution, which, to the best of our knowledge, has been rarely used to broadly study the photophysics of a drug in these confined and complex media.

The spectral changes due to micellar medium are consistent with the idea of a molecule which is not entrapped within the micelle core, but which is able to interact with the surfactant head groups, being placed at the interface between the micelles and the bulk aqueous solution. The complexation mechanism with Mg^{2+} and the properties of its chelates are therefore barely affected by the presence of *pDoTABr* micelles: they are able to interact with TC without affecting its mode of action, which requires its efficient complexation with divalent metal ions.

Sol–gel media were established by means of the zwitterionic amine-oxide surfactant *p*DoAO and the dicephalic cationic surfactant Gemini. As for Gel(+/–) the spectral properties of TC were found to be clearly different from those in water. Femtosecond transient absorption measurements proved to be revealing, allowing the whole experimental data to be understood in terms of drug distribution between the hydrophobic and hydrophilic domains, depending on the variable polarity of the drug, the total net charge of its complexes, and that of the surfactant. TC and its complexes in the hydrophobic portions are described by higher fluorescence efficiency and a longer-lived excited state. The latter two are suitable features of the ammonium surfactant gels to be used as carriers, as they make the drug easier to trace.

This work revealed the noteworthy changing properties of TC in organized media. It would be therefore particularly

appealing to probe the behaviour exhibited in this kind of media by other molecules belonging to the family of TCs whose mechanism of complexation with metal cations differs as a consequence of the presence and position of additional polar hydroxyl groups in the upper peripheral region. Hydroxyl groups could be indeed crucial as their presence could change the dipole moment of the drug, thus affecting its spatial distribution within micelles and sol–gels. In fact, further studies in this direction have already been in progress. Moreover, in order to evaluate the effect of the mere confinement in the different environments and to compare the behaviour in micellar medium with those in sol–gel, it could be also remarkable to carry out some experiments in the presence of a neutral surfactant, so that no electrostatic interactions are expected to contribute to the spectral and kinetic variations detected.

Notes and references

1 J. K. Thomas, *Chem. Rev.*, 1980, **80**, 283–299.
2 C. Ghatak, V. G. Rao, S. Mandal, S. Ghosh and N. Sarkar, *J. Phys. Chem. B*, 2012, **116**, 3369–3379.
3 S. K. Pal, D. Sukul, D. Mandal, S. Sen and K. Bhattacharyya, *J. Phys. Chem. B*, 2000, **104**, 2613–2616.
4 D. Sahoo, P. Bhattacharya and S. Chakravorti, *J. Phys. Chem. B*, 2009, **113**, 13560–13565.
5 A. Sanz-Medel, R. F. de la Campa and J. I. G. Alonso, *Analyst*, 1987, **112**, 493–497.
6 A. F. Silva, H. D. Fiedler and F. Nome, *J. Phys. Chem. A*, 2011, **115**, 2509–2514.
7 J. Rios-Doria, A. Carie, T. Costich, B. Burke, H. Skaff, R. Panicucci and K. Sill, *J. Drug Del.*, 2012, **2012**, 1–8.
8 W. Wang, D. Cheng, F. Gong, X. Miao and X. Shuai, *Adv. Mater.*, 2012, **24**, 115–120.
9 N. Nasongkla, X. Shuai, H. Ai, B. D. Weinberg, J. Pink, D. A. Boothman and J. Gao, *Angew. Chem.*, 2004, **116**, 6323–6327.
10 B. Lindman and H. Wennerström, *Top. Curr. Chem.*, 1980, **87**, 1–83.
11 F. Kern, P. Lemarechal, S. J. Candau and M. E. Cates, *Langmuir*, 1992, **8**, 437–440.
12 H. Rehage and H. Hoffmann, *J. Phys. Chem.*, 1988, **92**, 4712–4719.
13 P. A. Hassan, S. J. Candau, F. Kern and C. Manohar, *Langmuir*, 1998, **14**, 6025–6029.
14 T. Shikata, M. Shiokawa and S.-I. Imai, *J. Colloid Interface Sci.*, 2003, **259**, 367–373.
15 S. R. Raghavan, G. Fritz and E. W. Kaler, *Langmuir*, 2002, **18**, 3797–3803.
16 W. Cai, G. T. Wang, Y. X. Xu, X. K. Jiang and Z. T. Li, *J. Am. Chem. Soc.*, 2008, **130**, 6936–6937.
17 A. Ajayaghosh, P. Chithra, R. Varghese and K. P. Divya, *Chem. Commun.*, 2008, 969–971.
18 X. Zhang, Z. Chen and F. Würthner, *J. Am. Chem. Soc.*, 2007, **129**, 4886–4887.
19 M. J. Boerakker, N. E. Botterhuis, P. H. Bomans, P. M. Frederik, E. M. Meijer, R. J. Nolte and N. A. Sommerdijk, *Chem.–Eur. J.*, 2006, **12**, 6071–6080.
20 S. J. George and A. Ajayaghosh, *Chem.–Eur. J.*, 2005, **11**, 3217–3227.
21 J. Yang, *Curr. Opin. Colloid Interface Sci.*, 2002, **7**, 276–281.
22 S. K. Singh, M. Bajpai and V. K. Tyagi, *J. Oleo Sci.*, 2006, **55**, 99–119.
23 L. Brinchi, R. Germani, P. Di Profio, L. Marte, G. Savelli, R. Oda and D. Berti, *J. Colloid Interface Sci.*, 2010, **346**, 100–106.
24 L. Goracci, R. Germani, J. F. Rathman and G. Savelli, *Langmuir*, 2007, **23**, 10525–10532.
25 A. Di Crescenzo, R. Germani, E. Del Canto, S. Giordani, G. Savelli and A. Fontana, *Eur. J. Org. Chem.*, 2011, 5641–5648.
26 C. A. Finch, *Industrial Applications of Surfactants*, Royal Society of Chemistry, Cambridge, 1991, pp. 1–440.
27 M. T. Garcìa, E. Campos and I. Ribosa, *Chemosphere*, 2007, **69**, 1574–1578.
28 M. L. Nelson and M. Y. Ismail, *The Antibiotic and Nonantibiotic Tetracyclines in Comprehensive Medicinal Chemistry II*, Elsevier, 2007, pp. 598–617.
29 I. Chopra and M. Roberts, *Microbiol. Mol. Biol. Rev.*, 2001, **65**, 232–260.
30 I. Chopra, P. M. Hawkey and M. Hinton, *J. Antimicrob. Chemother.*, 1992, **29**, 245–277.
31 D. Schnappinger and W. Hillen, *Arch. Microbiol.*, 1996, **165**, 359–369.
32 B. Carlotti, D. Fuoco and F. Elisei, *Phys. Chem. Chem. Phys.*, 2010, **12**, 15580–15591.
33 H. Morrison, G. Olack and C. Xiao, *J. Am. Chem. Soc.*, 1991, **113**, 8110–8118.
34 B. Carlotti, A. Cesaretti and F. Elisei, *Phys. Chem. Chem. Phys.*, 2012, **14**, 823–834.
35 M. O. Schmitt and S. Scnheider, *Phys. Chem. Commun.*, 2000, **9**, 42–55.
36 S. Schneider, M. O. Schmitt, G. Brehm, M. Reiher, P. Matousek and M. Towrie, *Photochem. Photobiol. Sci.*, 2003, **2**, 1107–1117.
37 R. D. Jee, *Analyst*, 1995, **120**, 2867–2872.
38 L. Chen, J. Wu, L. Yuwen, T. Shu, M. Xu, M. Zhang and T. Yi, *Langmuir*, 2009, **25**, 8434–8438.
39 A. L. Andrade, D. M. Souza, W. A. Vasconcellos, R. V. Ferreira and R. Z. Domingues, *J. Non-Cryst. Solids*, 2009, **355**, 811–816.
40 G. Pizzoli, M. G. Lobello, B. Carlotti, F. Elisei, M. K. Nazeeruddin, G. Vitillaro and F. De Angelis, *Dalton Trans.*, 2012, **41**, 11841–11848.
41 G. R. Fleming, A. W. E. Knight, J. M. Morris, R. J. S. Morrison and G. W. Robinson, *J. Am. Chem. Soc.*, 1977, **99**, 4306–4311.
42 A. Romani, C. Clementi, C. Miliani, B. G. Brunetti, A. Sgamellotti and G. Favaro, *Appl. Spectrosc.*, 2008, **62**, 1395–1399.

43 B. Carlotti, A. Spalletti, M. Šindler–Kulyk and F. Elisei, *Phys. Chem. Chem. Phys.*, 2011, **13**, 4519–4528.

44 T. Del Giacco, B. Carlotti, A. Barbafina, S. De Solis and F. Elisei, *Phys. Chem. Chem. Phys.*, 2011, **13**, 2188–2195.

45 A. Amat, S. Fantacci, F. De Angelis, B. Carlotti and F. Elisei, *Theor. Chem. Acc.*, 2012, **131**(5), 1218–1231.

46 Z. Qiang and C. Adams, *Water Res.*, 2004, **38**, 2874–2890.

47 E. Sapelli, T. A. S. Brandão, H. D. Fiedler and F. Nome, *J. Colloid Interface Sci.*, 2007, **314**, 214–222.

48 C. A. Bunton, F. Nome, F. H. Quina and L. S. Romsted, *Acc. Chem. Res.*, 1991, **24**, 357–364.

49 A. Amat, C. Clementi, C. Miliani, A. Romani, A. Sgamellotti and S. Fantacci, *Phys. Chem. Chem. Phys.*, 2010, **12**, 6672–6684.

50 A. Romani, C. Clementi, C. Miliani and G. Favaro, *Acc. Chem. Res.*, 2010, **43**(6), 837–846.

51 C. Grazia, C. Clementi, C. Miliani and A. Romani, *Photochem. Photobiol. Sci.*, 2011, **10**, 1249–1254.

52 R. Jimenez, G. R. Fleming, P. V. Kuman and M. Maroncelli, *Nature*, 1994, **370**, 263–269.

53 S. Vajda, R. Jimenez, S. J. Rosenthal, V. Fidler, G. R. Fleming and E. W. Castner Jr., *J. Chem. Soc., Faraday Trans.*, 1995, **91**, 867–873.

54 S. A. Kovalenko, R. Schanz, V. M. Farztdinov, H. Hennig and N. P. Ernsting, *Chem. Phys. Lett.*, 2000, **323**, 312–322.

55 H. A. Duarte, S. Carvalho, E. B. Paniago and A. M. Simas, *J. Pharm. Sci.*, 1999, **88**, 111–120.

56 O. G. Othersen, F. Beierlein, H. Lanig and T. Clark, *J. Phys. Chem. B*, 2003, **107**, 13743–13749.

57 W. Naidong, S. Hua, E. Roets, R. Busson and J. Hoogmatens, *Int. J. Pharm.*, 1993, **96**, 13–21.

58 S. K. Pal, D. Sukul, D. Mandal, S. Sen and K. Bhattacheryya, *Chem. Phys. Lett.*, 2000, **327**, 91–96.

59 A. Rei, G. Hugerford, M. Belsey, M. I. Ferreira and P. Schellenberg, *Int. J. Spectrosc.*, 2012, **2012**, 271435.

60 R. Baumann, C. Ferrante, E. Kneuper, F. W. Deeg and C. Brauchle, *J. Phys. Chem. A*, 2003, **107**, 2422–2430.