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Supramolecular assemblies of triblock copolymers with hexanuclear molybdenum clusters for sensing antibiotics in aqueous solutions via energy transfer

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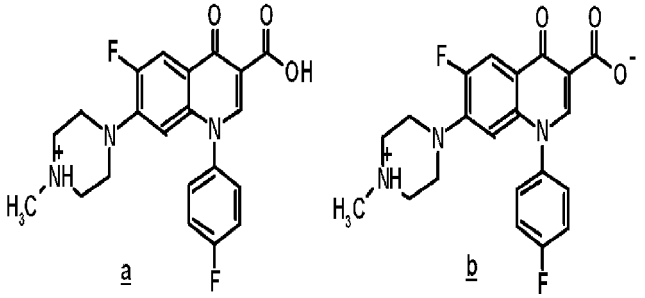
The work introduces the supramolecular assembly of triblock copolymers, namely $(PEO)_{13}(PPO)_{30}(PEO)_{13}$ (L64), $(PPO)_{14}(PEO)_{24}(PPO)_{14}$ (17R4), $(PPO)_8(PEO)_{22}(PPO)_8$ (10R5) and $(PEO)_{21}(PPO)_{67}(PEO)_{21}$ (P123) with novel cluster complexes $[K(diglyme)(CH_3CN)]_2[Mo_6I_{14}]$ (**1**) and $[K_2(diglyme)(CH_3CN)_5][Mo_6I_{14}]$ (**2**) as a route to increase their water solubility. Dynamic light scattering and photophysical measurements reveal the decisive influence of the arrangement of PEO and PPO blocks and of their length on both colloidal and photophysical properties of these solutions. ES-MS data reveal $[Mo_6I_{14}]^{2-}$ clusters as the predominant form in aqueous solutions of L64 and P123. The steady state and time resolved luminescence data indicate concentration dependent sensitizing of the Mo-centered luminescence through the energy transfer from difloxacin to $[Mo_6I_{14}]^{2-}$ mediated by the ion-pairing. The impact of both arrangement and length of PEO and PPO blocks in the luminescent response of $[Mo_6I_{14}]^{2-}$ to difloxacin is discussed. The aqueous solutions of L64 at pH 4 provide the optimal conditions for the sensing of difloxacin through the cluster luminescence.

Introduction

The triblock copolymers containing polyethyleneoxide (PEO) and polypropyleneoxide (PPO) moieties in various arrangements are efficient building blocks for supramolecular aggregates, which find wide application in modern bioanalysis and medicine.[1-3] Moreover their amphiphilic and colloid properties can be easily tuned by changing both the length and mutual arrangement of the hydrophilic PEO and hydrophobic PPO fragments of the triblock copolymers.[4] In particular, the different aggregation capacity of PEO–PPO–PEO (Pluronics or P-type triblock copolymers) and PPO–PEO–PPO (commonly known as Reverse Pluronics or R-type triblock copolymers) is well documented.[5-9] The middle-length triblock copolymers with diverse arrangements of PEO and PPO blocks, namely, $(PEO)_{13}(PPO)_{30}(PEO)_{13}$ (L64), $(PPO)_{14}(PEO)_{24}(PPO)_{14}$ (17R4), and $(PPO)_8(PEO)_{22}(PPO)_8$ (10R5), are able to aggregate only when their aqueous solutions are heated up to certain temperatures,[5-9] while triblock copolymers with longer PEO and PPO fragments, such as $(PEO)_{21}(PPO)_{67}(PEO)_{21}$ (P123), are able to form micellar aggregates in dilute aqueous solutions already at room temperature.[10-12] The present work introduces the supramolecular assembly of triblock copolymers with potassium salts of fluorescent hexanuclear molybdenum halide clusters (in this case, $[Mo_6I_{14}]^{2-}$) as a route to gain in their water solubility for sensing applications. Thus the conditions necessary to achieve the substrate induced fluorescent response are also presented in this work.

The luminescence of hexanuclear molybdenum halide clusters ($[Mo_6X_{14}]^{2-}$) has gained much attention since the first reports in early eighties.[13,14] The origin of their luminescence is well understood.[13-18] Spectroscopic and theoretical studies of various $[Mo_6(\mu_3\text{-}X)_8L_6]^{2-}$ complexes have established the HOMO and LUMO orbitals to be primarily metal-centered,[13,19,20] the nature of both inner halide (μ_3-X) and six terminal (apical) ligands, nevertheless, affects the emission wavelength.[18-22] Thus the contributions from ligand-based orbitals to the cluster HOMO should be also taken into account.[23] The factors guiding the luminescence of a $[Mo_6(\mu_3\text{-}X)_8X_6]^{2-}$ species in solutions include, in particular, energy transfer from the lowest triplet state of the cluster ion to the underlying acceptor levels.[15,16] This mechanism is operative in the collision-induced quenching by dioxygen.[17,18] The available data highlight the shift of the emission band and the quenching[18,19] or sensitizing[21,22] of the molybdenum cluster-centered luminescence, when halide apical ligands are substituted by thiolates, pyridine derivatives or carboxylates. The outer-sphere environment of the cluster core is yet another factor affecting luminescent properties. In particular, the work by Tanaka et al.[16] makes evident both the possibility of photochemical dissociation of the Mo-apical halide bonds and the effect of solvent on this process. Moreover, the identity of the counter-ions also has a significant effect on the photophysical characteristics of $[Mo_6X_{14}]^{2-}$.[24,25] The work by Grasset et al.[26] is worth noting as a fine example of the improved photo-stability of the halide clusters by their encapsulation into a silica matrix, although energy transfer to silica environment is also responsible for non-radiative decay. Thus both apical ligands substitution and outer sphere interactions with counter ions constitute a promising basis of substrate-induced luminescence response. To be of practical use, the response to a substrate should be rapid, which raises the question about the viability of the ligand exchange as the main sensing mechanism of substrate induced fluorescent response. The essential inertness of the apical ligands and the ways to increase it have been discussed in literature,[27-34] but applications of the hexanuclear molybdenum halide clusters in sensing organic substrates are scarce if any. Moreover, the majority of biorelevant analytes being water soluble, the problem of water solubility of $[Mo_6I_{14}]^{2-}$ and its hydrolytic and photo-stability should be emphasized as the key points in the development of substrate responsive luminescence.

The present work introduces aqueous solutions of L64, 10R5, 17R4 and P123 as a convenient media for dissolution of new, easily available salts $[K(diglyme)(CH_3CN)]_2[Mo_6I_{14}]$ (**1**) and $[K_2(diglyme)(CH_3CN)_5][Mo_6I_{14}]$ (**2**). Their synthesis and structure are presented. The effect of the nature of triblock copolymers on the kinetic stability, colloid formation and photophysical properties of $[Mo_6I_{14}]^{2-}$ cluster ions in aqueous solutions reveals diverse modes of their interaction with the triblock copolymers. The work also presents substrate induced sensitizing of the $[Mo_6I_{14}]^{2-}$ luminescence by a fluoroquinolone antibiotic (FQ) difloxacin (Scheme 1).



The FQs are of great interest from the viewpoint of their widespread application,[35] which in turn makes their analytical determination a rather challenging task.[36-43] The impact of the triblock copolymers nature in the substrate-induced luminescence is also discussed. The effect of zwitter-ionic and protonated forms of difloxacin on the steady state and time resolved luminescence of $[Mo_6I_{14}]^{2-}$ is compared with the related effect of acetate anions to reveal the main driving force and mechanism of the substrate induced sensitizing of $[Mo_6I_{14}]^{2-}$ luminescence in aqueous solutions of L64.

Experimental section

Materials

Triblock copolymers $(PEO)_{13}(PPO)_{30}(PEO)_{13}$ (L64), $(PPO)_{14}(PEO)_{24}(PPO)_{14}$ (17R4), $(PPO)_8(PEO)_{22}(PPO)_8$ (10R5), $(PEO)_{21}(PPO)_{67}(PEO)_{21}$ (P123), diglyme, difloxacin hydrochloride and potassium acetate were used as commercially available (Sigma-Aldrich) without further purification. Mo powder was pre-treated with H_2 (700˚C) to remove oxides, KI was dried in vacuum at 100˚ C for several hours prior to use, I_2 was freshly sublimed.

Synthesis

$[K(diglyme)(CH_3CN)]_2[Mo_6I_{14}]$ (**1**). Mo powder (0.70 g, 7.30 mmol), I_2 (1.85 g, 7.30 mmol), KI (0.40 g, 2.40 mmol) were placed together into a quartz ampoule, which was evacuated and sealed. The ampoule was heated in an oven for two days at 650°C. After cooling the ampoule was opened and the melt was extracted into 50 ml of diglyme at 160˚C for two days under argon. Hot solution was filtered and reduced to 3 ml. Another 3 ml of CH_3CN were then added followed by the addition of 10 ml of diethyl ether, producing a crop of dark-red crystals. Yield 1.16 g (34 %). Analysis. Found. C:H:N (%) 6.91:1.23:1.0, Calc. 7.40:1.41:0.92. IR (4000-100 cm^{-1}, KBr): 2989 w, 2882 s, 2820 m, 1725 w,1450 s, 1376 w, 1351 s, 1284 m, 1245 s, 1200 s, 1112 vs, 1081 vs, 1016 s, 938 m, 863 s, 836 m, 540 w, 196 m, 155 m, 141 m, 105 s.

$[K_2(diglyme)(CH_3CN)_5][Mo_6I_{14}]$ (**2**). Complex **1** (50 mg, 0.02 mmol) was heated with 3 ml of CH_3CN at 100˚ C in a high-pressure vessel for 10 h. The solution was filtered and the filtrate was allowed to be saturated with diethyl ether vapors. A crop of single crystals of

2 was obtained. Yield 80 %. The IR spectrum of **2** virtually coincides with that of **1**.

Methods

The dynamic light scattering (DLS) measurements in aqueous solutions containing **1** and triblock copolymers were carried out with a Malvern Mastersize 2000 particle analyzer. The measured autocorrelation functions were analyzed with the Malvern DTS software and the second-order cumulant expansion methods. All the samples were dissolved in twice-distilled water and purified by filtration through a PVDF membrane using Syringe Filter (0.45 μm). The diffusion coefficient values were measured at least three times for each sample. The average error was ca. 4%.

The steady-state emission spectra were recorded on a FL3-221-NIR spectrofluorometer (Jobin Yvon) under 430, 380 and 350 nm excitation wavelengths.

Luminescence decay measurements were performed on a Horiba Jobin Yvon Fluorolog -3-221 spectrofluorometer with SPEX FL-1042 phosphorimeter accessory using a xenon flash lamp as the photon source with following parameters: time per flash 50.00 ms, flash count 200 ms, initial delay 0.05 ms, and sample window 2 ms. Excitation of samples was performed at 430 and 380 nm, and emission was detected at 725 nm with 5 nm slit width for both excitation and emission. The steady state and time resolved luminescence measurements were performed under oxygenated conditions. The τ-values of **1** and **2** in deoxygenated acetonitrile were close to the value reported by Kirakci et al[21] for $[Mo_6I_{14}]^{2-}$ (about 50 μs). All the measurements were performed within one hour after the sample preparation. The samples for steady state and time resolved measurements of **1** at various concentrations of difloxacin have been prepared without buffer system in order to avoid counter-ion effect of latter. The pH values in the aqueous solutions of L64 (0.35 mM) with various amounts of difloxacin hydrochloride added varied within 5.0-5.5. The pH 4.0-4.1 in the same solutions was adjusted by the addition of required amounts of HCl.

MALDI measurements were performed on a Ultraflex III TOF/TOF (Bruker Daltonics, Germany) mass spectrometer equipped with a Nd:YAG laser and a collision cell. The mass spectra of negative ions were measured in the reflector mode. Trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) was used as matrix. The dried-droplet spotting technique (matrix, analyte) was applied. The data were processed using the FlexAnalysis 3.0 software from Bruker Daltonics.

ESI measurements were performed on an AmazonX (Bruker Daltonics, Germany) mass spectrometer in the negative mode. Nitrogen (15 psi) was used for desolvation and nebulization. The sample introduction rate was 0.2 ml/min. The ESI source conditions were as follows: capillary voltage 4500 V, capillary exit voltage -140 V, the dry gas temperature 300°C. Mass spectra were acquired at m/z range from 1000 to 3000. The concentration of **1** in acetonitrile and aqueous solutions of triblock copolymers was 0.01 mM.

Crystallographic data and refinement details are given in Table 1S in Supporting Information. The diffraction data were collected on a Bruker X8Apex CCD diffractometer with MoKα radiation (λ = 0.71073 Å) by doing φ and ω scans of narrow (0.5°) frames at 150 K. Structures of all complexes were solved by direct method and refined by full-matrix least-squares treatment against |F|2 in anisotropic approximation with SHELXTL programs set.[44] Absorption corrections were applied empirically with SADABS program.[45] The hydrogen atoms were refined in their geometrically calculated positions; a riding model was used for this purpose. Main distances and confirmation with the literature data are summarized in Table 2S. Further details may be obtained from the Cambridge Crystallographic Data Center on quoting the depository numbers CCDC 957964, 957965. This information may be obtained free of charge from http://www.ccdc.cam.ac.uk.

Results and Discussion

Synthesis and characterization of new clusters

In this work we have prepared $[Mo_6I_{14}]^{2-}$ by direct reaction from Mo, I_2 and KI at 700°C. The reaction melt was extracted into hot diglyme, from which two new salts, $[K(diglyme)(CH_3CN)]_2[Mo_6I_{14}]$ (**1**) and $[K_2(diglyme)(CH_3CN)_5][Mo_6I_{14}]$ (**2**) were isolated after addition of CH_3CN (**1**) and repeated recrystallization of **1** from neat CH_3CN (**2**). The bond lengths of the $[Mo_6I_{14}]^{2-}$ in **1** and **2** are nearly identical. In **1** they are: Mo-Mo 2.675(1) Å, Mo-I^i 2.834(2) Å, Mo-I^a 2.770(2) Å, in **2** - Mo-Mo 2.679(2) Å, Mo-I^i 2.852(2) Å, Mo-I^a 2.774(2) Å.

In the cationic part of **1** K^+ is coordinated with diglyme, acting in the tridentate mode, one CH_3CN molecule, and with terminal iodines from $[Mo_6I_{14}]^{2-}$ (Fig.1).

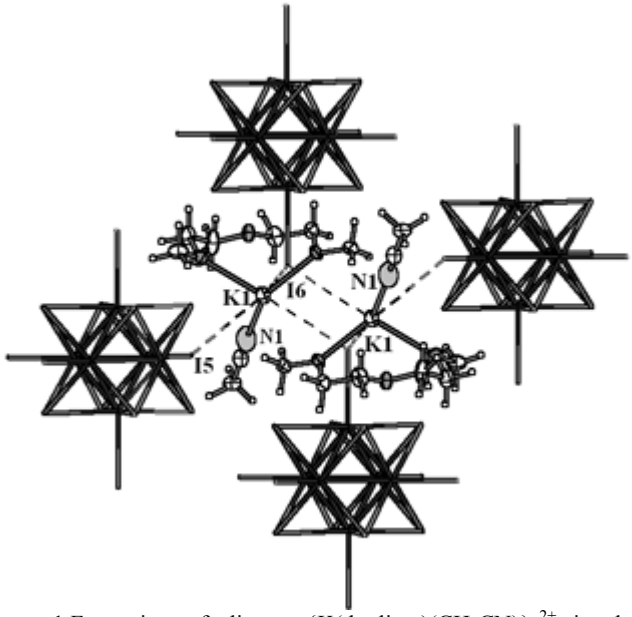


Figure 1.Formation of dimers $\{K(dyglime)(CH_3CN)\}_2^{2+}$ in the crystal structure of **1**.

Corresponding distances are: K-N 2.878(1) Å, K-O 2.708(2), 2.724(2), and 2.960(2) Å, K-I 3.717(1), 3.729(2), and 3.828(2) Å (the sum of the ionic radii $K^+ + I^-$ is 3.53 Å). An interesting structural feature of **2** is the presence of two types of potassium cations which differ in their respective coordination environments (Fig.2).

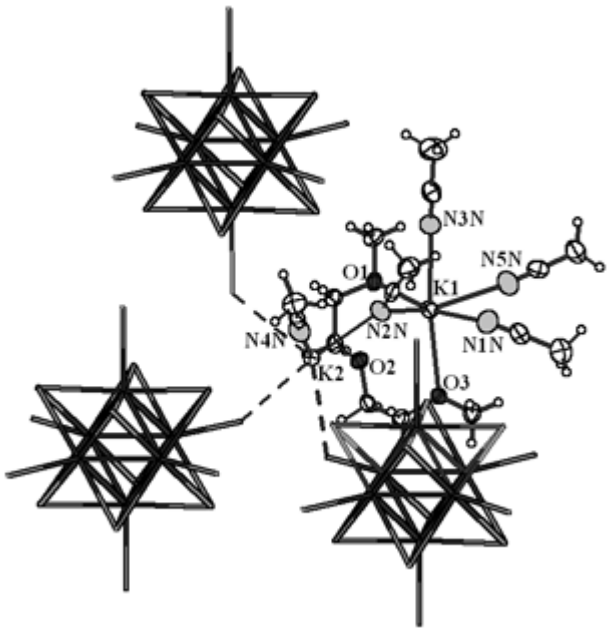


Figure 2. Formation of a dimers $\{(CH_3CN)_3K(dyglime)(\mu\text{-}CH_3CN)K(CH_3CN)\}^{2+}$ in the crystal structure of **2**.

One of them is uniquely coordinated only by the organic solvent molecules (one tridentate diglyme, three terminal CH_3CN and one bridging CH_3CN). Another K^+ coordinates one terminal CH_3CN, one bridging CH_3CN, and the central oxygen atom from diglyme, which thus behaves as bridging ligand. The coordination sphere is completed by three terminal iodides from $[Mo_6I_{14}]^{2-}$ (Fig.2). The distances are: K(1) – O 2.858(1), 2.799(1), K(1) – O(br) 2.930(1), K(1) – N(br) 2.799(1), K(1) – N 2.902(2), 2.985(2), 2.784(1); K(2) – I 3.453(1), 3.495(1), K(2) – N 2.774(2), K(2) – N(br) 2.797(2), K(2) – O(br.) 2.933(1) Å. In the crystal structure of **1** the $\{K(diglyme)(CH_3CN)\}_2^{2+}$ dimers and cluster anions form layers running along crystallographic direction [110] (Fig.S1 in SI). In the crystal structure of **2** $\{(CH_3CN)_3K(diglyme)(\mu\text{-}CH_3CN)K(CH_3CN)\}^{2+}$ and cluster anions combine into infinite columns running along crystallographic axis *b* (Fig.S2 in SI). The interactions in the crystal structures of **1** and **2** were described based on the literature data[46].

The solvated potassium salts **1** and **2** are soluble practically in all polar organic solvents (diglyme, acetone, CH_3CN, ethanol, DMF) to at least 10^{-2} M level. They are also soluble in water, but initial clear red-orange solutions rapidly become cloudy, and an amorphous precipitate separates. Nevertheless the water solubility of **1** and **2** is enhanced in the comparison with other well known salts of hexamolybdenum iodine clusters, such as $Cs_2[Mo_6I_{14}]$ and $(Bu_4N)_2[Mo_6I_{14}]$,[21,25] which indicates the effect of aquation of K^+, and of the coordination with diglyme revealed from the X-ray data (Figs.1, 2) for **1** and **2** on their solubility in water.

Dissolution of clusters in aqueous solutions of triblock copolymers

The analysis of the solubility of **1** and **2** in solutions of triblock copolymers and kinetic stabilities of the obtained solutions reveals aqueous solutions of P123 as the best media for both clusters. Dissolution of **1** and **2** (at 0.035 mM level) is already achieved in rather diluted solutions of P123 (0.35 mM). Moreover, the kinetic stability of the solutions was the highest among the studied triblock copolymers and independent on the concentration of P123. The dissolution behavior in the solutions of P123 reveals no appreciable difference between **1** and **2**, but this difference does become significant for other triblock copolymers studied in this work: both the solubility of **1** and kinetic stability of these solutions were found to be significantly higher than those of **2**. For example, complete dissolution of **2** (at 0.035 mM level) requires 10 mM concentration of L64, while **1** dissolves already at 0.35 mM concentration level of L64. The solutions of **2** were generally less stable (depositing a precipitate within one hour) than those of **1**. These differences explain our choice of **1** for further photophysical measurements.

The diverse aggregation behavior of the triblock copolymers is worth noting as the explanation of the observed difference between them. The triblock copolymers L64, 10R5 and 17R4 exist in the form of unimers in a wide concentration range at room temperature,[5-9] while P123 tends to aggregate even under ambient conditions in diluted solutions.[10-12] Thus aqueous solutions of P123 within the studied concentration range (0.35-1.00 mM) develop 14-16 nm sized aggregates as can be deduced from DLS data. The presence of **1** and **2** (0.035 mM) shows no effect on the size of the aggregates. These observations strongly suggest that inclusion of the clusters ions into micelles of P123 or so-called solubilization is the reason of the enhanced solubility of **1** and **2** in the solutions of P123.

The triblock copolymers L64, 10R5 and 17R4 exemplify another type of interactions with **1** and **2** as driving force for enhanced solubility. The DLS measurements in solutions of L64, 10R5 and 17R4 revealed the presence of unimers in all cases, while after the dissolution of **1** the DLS patterns are different for R- and P-type triblock copolymers. The L64 molecules appear as 3-4 nm sized unimers both in the absence and in the presence of **1** (Fig.S3 in SI), which can be taken as an indication that the dissolution of **1** in solutions of L64 involves interactions on the molecular level. The comparison of the above mentioned sizes with the 2.5 nm size reported for hexamolybdenum clusters in acidic ethanol solutions[26] indicates wrapping of the cluster ions by L64 molecules. The binding of L64 with **1** may proceed in the outer or the inner sphere modes. The latter could result from the substitution of apical iodines by oxygen donor atoms of the polyethyleneoxide chains. In order to check the latter possibility electrospray mass spectrometry (ESI-MS) was performed for solutions of **1** in neat acetonitrile and in aqueous solutions of L64 and P123 (0.35 and 2.00 mM, respectively). The peaks corresponding to $[Mo_6I_{14}]^{2-}$ at *m/z* 1176 dominate the spectra obtained from both solutions (Fig.3 and Fig.S4 in SI).

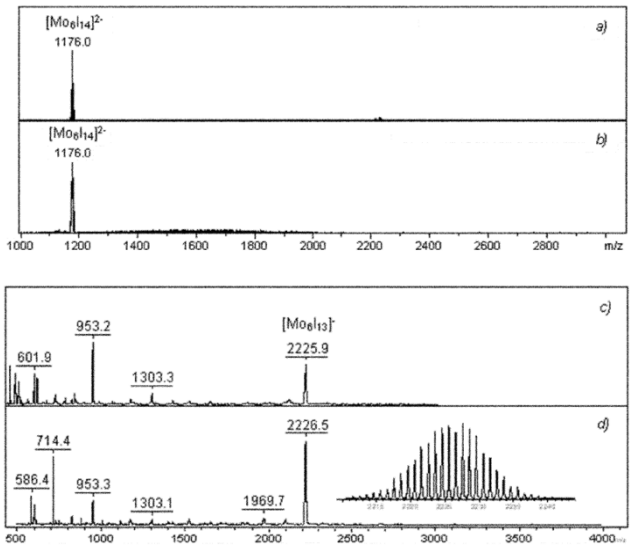


Figure 3. ESI (a,b) and MALDI (c,d) mass spectra of **1** (0.01 mM) in acetonitrile (a, c) and aqueous solutions of L64 (b, d), pH 6, concentration of L64 is 0.35 mM.

The matrix-assisted laser desorption/ionization (MALDI) mass spectra reveal the peak corresponding to $[Mo_6I_{13}]^-$ at *m/z* 2226 under all studied conditions, which is in a good agreement with the different modes of the ionization applied in both methods (Fig.3). Thus both the inclusion of **1** into micelles of P123 and its interaction with the molecules of L64 occur without detectable substitution of apical iodines by oxygen atoms of polyethyleneoxide chains and must involve cationic solvation instead.

The kinetic stabilities of the solutions of **1** in the presence of L64 tend to decrease with the growth of L64 concentration. For example, at 10 mM concentration level of L64 a precipitate is deposited within a few hours, while in more diluted solutions (0.35 mM by L64) no precipitate appears for one day at least. Time resolved DLS reveals aggregation of these complexes prior to precipitation: the increased averaged size (D_{av}) of the aggregates of about 300-700 nm preceded the precipitation from the kinetically unstable solutions of **1** at 10 mM of L64 **(**Fig.S5 in SI**).**

The dissolution of **1** in the presence of 17R4 and 10R5 requires higher concentrations of the latter (2.00 mM and more). Kinetic stabilities of these solutions tend to increase on both going from 10R5 to 17R4 and with the increase in their concentrations. For example, precipitation is observed within only a few minutes after dissolving **1** (0.035 mM) in 2.00 mM solutions of 10R5, while for 17R4 similar solution is stable within several hours. The concentration level of 17R4 should be 10 mM at least to prolong the kinetic stability of the solutions to one day. DLS measurements indicate increased averaged size (D_{av} 100-300 nm) and high polydispersity indices (PDI within 0.2-0.3) after dissolving **1** (0.035 mM) in 10 mM solutions of 10R5 **(**Fig.S6 in SI**)**. The aggregates in the solutions of **1** in the presence of 17R4 (10 mM) were more uniform in size (about 90 nm) with PDI ≤ 0.1 (Fig.4).

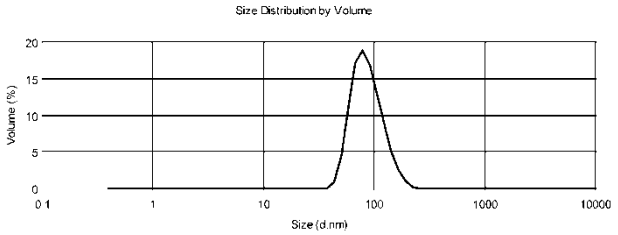


Figure 4. The size distribution by volume in aqueous solutions of **1** (0.035 mM) at 10 mM level of 17R4 from the DLS data (D_{ev}=90 nm, PDI=0.1).

These results point out to three types of the intermolecular interactions as the reason of the enhanced water solubility of **1**. The first type is the solubilization of the cluster ion into micellar aggregates of P123, which accounts for the significant kinetic stability of these solutions. The second type is the interaction at the molecular level, which is exemplified by L64, when the amphiphilic molecules serve as outer sphere ligands. These complexes also exhibit high kinetic stability within a definite concentration range of L64. Finally, the R-type triblock copolymers exemplify formation of colloids instead of true solutions, where nanosized particles of **1** are stabilized by the triblock copolymers 17R4 and 10R5. High kinetic instability of these solutions confirms this assumption. The diversity in the structures of the triblock copolymers may constitute the main reason for this difference.

Luminescence of $[Mo_6I_{14}]^{2-}$ in aqueous solutions of triblock copolymers

The luminescence data of **1** and **2** are identical in neat acetonitrile, as well as in aqueous solutions of P123 (0.35-1.00 mM) and L64 (10 mM). Nevertheless, the measurements of steady state and time resolved luminescence were always performed in aqueous solutions of **1** (0.035 mM) due to their higher kinetic stabilities and better solubility of **1**. The emission spectra (Fig.5a) were recorded upon excitation at 430 nm in accordance with the excitation spectrum (Fig.5b).

The results (Fig.5a and Table) indicate that both the intensities of the emission band and τ values of **1** in solutions of P123, L64 and 17R4 are higher than those in CH_3CN. The emission intensities and τ values of **1** are the highest in solutions of L64 within the concentration range 0.35-1.00 mM for L64, but tending to decrease when the concentration of L64 increases to 10 mM (Table).

Table. The lifetimes of the excited state (τ) of **1** (0.035 mM) in aqueous solutions of the triblock copolymers and acetonitrile (AN), as well as the brief description of colloid properties and kinetic stability of the solutions.

Medium	C, M	τ[a], μs	Colloidal properties	Kinetic stability
P123	0.35 mM	8.10±0.15	micellar solution	high stability
17R4	10 mM	7.88±0.26	colloids	low stability
10R5	10 mM	7.5±0.2	colloids	low stability
L64	10 mM	7.7±0.05	[b]	low stability
L64	0.35 mM	11.8±0.3	true solution	high stability
AN		5.0±0.12	true solution	high stability

[a] - τ-values were measured in the oxygenated conditions.

[b] – true solution immediately after dissolution, which turns to turbid with time.

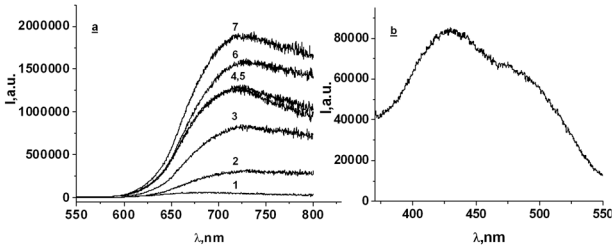
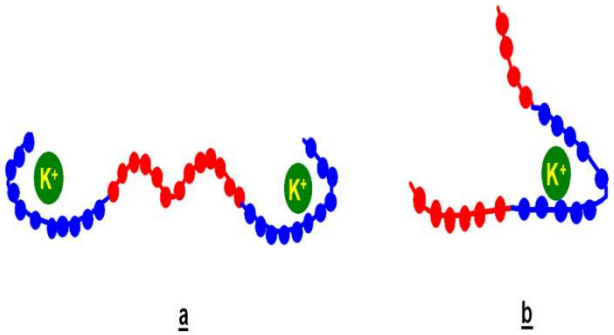


Figure 5. (a) The emission spectra of **1** (1-7) and **2** (2-4) in various solutions**:** 1- 10 mM of 10R5, 2- CH_3CN, 3- 0.35-1 mM of P123, 4- 10mM of L64, 5- 10 mM of 17R4, 6- 5 mM of L64, 7- 0. 35 mM of L64. The concentrations of **1** and **2** are 0.035 mM, pH is within 5.5-6.0, λ_{ex}=430 nm. (b) The corresponding excitation spectrum of **1** in the aqueous solutions

The brief description of colloid properties and kinetic stabilities of the measured solutions presented in Table indicates that a similar trend is observed in the kinetic stabilities of these solutions. P123 is the peculiar case, since both luminescence and kinetic stability of the

solutions stay unchanged over a wide concentration range of P123. The lack of any detectable shift of the emission wavelength on going from CH_3CN to aqueous solutions of the triblock copolymers confirms the outer sphere mode of their interaction with $[Mo_6I_{14}]^{2-}$ as revealed from the ESI-MS data (Fig.3, Fig.S4 in SI). The steady state and time resolved luminescence of **1** in solutions of 17R4 and 10R5 have been measured at 10 mM concentration level of the copolymers due to kinetic instability of the solutions under more diluted conditions. The lack of the correlation between steady state luminescence intensities and τ values for **1** in solutions of 10R5 and 17R4 at 10 mM concentration level (Fig.5a and Table) indicates enhanced contribution of some static quenching mechanism in the solutions of 10R5, in contrast to 17R4.[47] The light scattering effect from the larger-sized colloidal particles in the 10R5-based solutions as compared with 17R4 **(**Fig.S6, SI**)** can be assumed as the reason of the decreased emission intensity in the former solutions.

The effect of the structure and concentration of the triblock copolymers on the emission intensities and τ values (Fig.5a and Table) confirms the impact of the outer sphere environment of $[Mo_6I_{14}]^{2-}$ in the radiationless decay of the cluster luminescence. For example, 0.35 mM aqueous solutions of L64 provide the best water solubility of **1** combined with the lowest contribution of the radiationless decay and convenient kinetic stability of the solutions. Both emission intensities and τ values tend to decrease in solutions of the R-type triblock copolymers and P123. This tendency can be explained by the concentration induced self-quenching effect, when **1** is concentrated in colloid nanoparticles in the solutions of 17R4 and 10R5 or within the micelles of P123. Thus the observed regularities reveal the impact of both the arrangement of PEO and PPO blocks and of their length in solubility, photophysical properties of **1** and kinetic stability of the solutions**.** In particular the P-type arrangement of PEO and PPO blocks favors the above mentioned properties in the comparison with the reverse triblock copolymers. The binding of potassium ions with PEO chains can be assumed as one of the driving forces of the supramolecular assembly of **1** with the triblock copolymers. The above mentioned impact of diglyme coordination with potassium ions on the water solubility of **1** confirms this assumption. The diverse conformation freedom of PEO blocks which are exterior in the P-type and interior in the R-type triblock copolymers is a well-known factor affecting their different aggregation capacity in aqueous solutions.[5-9] In particular the aggregation of reverse triblock copolymers requires the looping geometry of the interior PEO block, which is the reason of the restricted aggregation behavior of these copolymers.[5-9] Schematic representation of the possible binding modes of potassium ions with R- and P-type triblock copolymers illustrates the looping geometry of PEO block as the prerequisite for the binding of potassium ions (Scheme 2).



Scheme 2. Schematic representation of the probable binding modes of potassium ions with P-type (a) and R-type (b) triblock copolymers, red and blue circles represent PPO and PEO blocks correspondingly.

The looping geometry in turn should be easier for the exterior PEO blocks of L64 than for the interior one of 17R4 and 10R5. Thus more efficient binding of potassium ions by PEO blocks of L64 should result in their less tight ion-pairing with the cluster anions, which can be assumed as the reason of the enhanced water solubility and kinetic stability of **1** in solutions of L64. The lengthening of PEO and PPO moieties on going from L64 to P123 improves the solubility of **1**, but adversely affects its photophysical properties due to the micellization of P123.

The fluorescent response of $[Mo_6I_{14}]^{2-}$ on the additives in aqueous solutions

The presence of both carboxylic and nitrogen-bearing heterocyclic groups in the structure of FQs (Scheme 1) is the reason of the zwitter-ionic structure due to the intramolecular proton transfer from acidic (carboxylic) to basic (nitrogen-containing) groups.[48-51] Both anionic carboxylate and heterocyclic moieties of difloxacin may interact with $[Mo_6I_{14}]^{2-}$. Keeping this possibility in mind the effect of acetate anions on the luminescence of $[Mo_6I_{14}]^{2-}$ in aqueous solutions of triblock copolymers was also addressed and compared with the related effects of zwitter-ionic difloxacin. The effects of difloxacin and potassium acetate on the fluorescence of **1** in triblock copolymer based aqueous solutions are illustrated with Figs. 6a, 7.

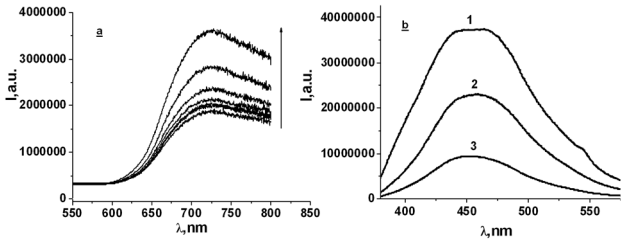


Figure 6. (a) Steady state luminescence spectra of **1** (0.035 mM) in aqueous solutions of L64 (0.35 mM) upon increase in the concentration of difloxacin from 0.005 to 0.12 mM, λ_{ex}=430 nm. (b) The luminescence spectra of difloxacin (0.035 mM) itself (1) and in the presence of **1**: 0.0175 mM (2) and 0.035 mM (3) in the aqueous solutions of L64 (0.35 mM), λ_{ex}=350 nm.

Fig.6a shows the steady state luminescence spectra of **1** at various concentrations of difloxacin in solutions of L64 (0.35 mM), while Fig.7 presents the steady state and time resolved luminescence data under all studied conditions. These data (Fig.7) are represented in the form of I/I_0 and τ/τ_0 versus concentration of the additive, while varying the nature and concentration of the triblock copolymers. The values of I and τ were measured at various concentrations of additives to aqueous solutions of **1** (0.035 mM) and triblock copolymers, I_0 and τ_0 refer to initial solutions. The results indicate that the fluorescent response of $[Mo_6I_{14}]^{2-}$ depends on the structure and concentration of both the additive and triblock copolymer. First of all, no effect is observed when any amounts of difloxacin or potassium acetate are added to **1** in acetonitrile and solutions of P123 (0.35-1.00 mM). On the contrary, the enhancement of the emission intensities and τ values of $[Mo_6I_{14}]^{2-}$ is observed upon the increase in difloxacin concentration, when concentration of L64 is 0.35 mM (Figs.6a, 7). The effect of difloxacin becomes less pronounced at 10 mM level of 17R4, and is insignificant at 10 mM level of L64 (Fig.7).

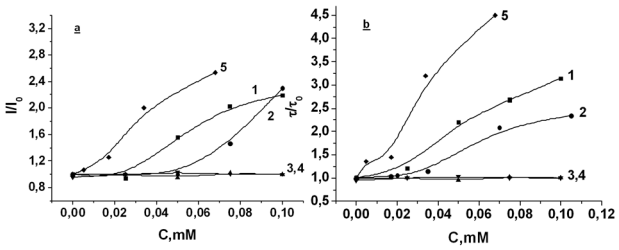


Figure 7. The I/I_0 (a) and τ/τ_0 (b) values in aqueous solutions of **1** (0.035 mM) at 0.35 mM of L64 (1, 5), 10 mM of 17R4 (2), 0.36 mM of P123 (3) at various concentrations of difloxacine and potassium acetate (4) at 0.35 mM of L64, λ_{ex}=430 nm and 380 nm, pH=5.0-5.5 (1-4), pH=4 (5).

No shift of the emission wavelength is observed at the addition of difloxacin (Fig.6a), which indicates that the enhancement of the cluster luminescence does not result from the substitution of apical iodines by the carboxylate moiety of difloxacin.[21,22] The absence of the luminescence response to added acetate confirms that carboxylate coordination to apical positions is not the reason of the luminescence response to difloxacin. The ion-pairing of the ammonium moiety of difloxacin with $[Mo_6I_{14}]^{2-}$ is the most probable channel of their interaction at 0.35 mM concentration level of L64 at pH 5.5. In accordance with the acid/base behavior of fluoroquinolones[48-51] difloxacin exists as the equilibrium mixture of the zwitter-ionic and protonated cationic forms under these conditions. It is worth noting that the measurements were performed within one hour after the sample preparation. Thus the proposed mode of the interaction between difloxacin and $[Mo_6I_{14}]^{2-}$ is restricted by the kinetic conditions, which were applied in this work. The luminescence response becomes insignificant when the cluster is trapped within the micelles of P123, as well as at increased concentrations of L64, while at 10 mM level of 17R4 the luminescence enhancement was similar, but somewhat less in the comparison with the 0.35 mM level of L64. These results indicate the impact of the triblock copolymers structure on the availability of $[Mo_6I_{14}]^{2-}$ to the ion-pairing with the protonated part of difloxacin. It is worth noting that the ion-pairing becomes less efficient when the cluster anions are trapped inside the colloid particles in the solutions of 17R4, coming to nothing in the micelles of P123. This tendency highlights the supramolecular assembly with L64 molecules as the best water soluble form of the cluster for sensing purposes.

The same increase of the I/I_0 and τ/τ_0 values at 0.35 mM level of L64 with the growth of the difloxacin concentration indicates that dynamic mechanism is responsible for the sensitization. The reasons for this mechanism are worth discussing. First of all, the energy transfer from difloxacin to $[Mo_6I_{14}]^{2-}$ is possible, taking into account the upper-laying triplet level of fluorquinolones (450-500 nm).[36,39,43] This tendency is exemplified by the results of the work[25] highlighting the decreased emission of imidazolium cation due to the energy transfer to $[Mo_6Cl_{14}]^{2-}$ cluster anion. The emission of difloxacin at various amounts of **1** was analyzed to reveal the contribution of the energy transfer mechanism. The emission of fluoroquinolones occurs at about 450 nm at the excitation by 300-350 nm light.[36,39,43] The emission spectra of difloxacin tend to decrease in intensity with the increase of the concentration of **1** upon the excitation by 350 nm (Fig.6b). The luminescence of the hexamolybdenum halide clusters can be excited by 350 nm, although the Mo-centered emission is in this case less efficient than the luminescence excited by a longer wavelength light (430 nm), which finds good confirmation in the excitation spectra (Fig.5b). The excitation by 380 nm is optimal to sensitize the luminescence of both $[Mo_6I_{14}]^{2-}$ and difloxacin, thus the luminescence spectra recorded at various cluster : difloxacin concentration ratio (Fig. S7 in SI) brings the additional confirmation of the difloxacin-to-cluster energy transfer as the major mechanism of the fluoroquinolone induced sensitization of the Mo-centered luminescence.

It is worth noting that the difference in pH values, which change from 5.5 to 5.0 when the amount of the added difloxacin hydrochloride increases, explains the deviation from the linearity of the I/I_0 versus concentration of difloxacin (Fig.7, curve 4). The acidification of the solutions to pH 4 results in more enhanced and linear increase of both I/I_0 and τ/τ_0 values within the same difloxacin concentration range (Fig.7, curve 5). These data highlight the ion-pairing of the protonated difloxacin with $[Mo_6I_{14}]^{2-}$ as the driving force of the energy transfer from difloxacin to the cluster ion.

Conclusion

To summarize, interactions with triblock copolymers offer an easy and convenient route to increase water solubility of the cluster $[K(diglyme)(CH_3CN)]_2[Mo_6I_{14}]$ **(1)** without substitution of apical iodines, efficiently preventing hydrolysis of the cluster. The solubility of **1** in aqueous solutions of triblock copolymers, kinetic stabilities and photophysical properties of these solutions are highly dependent on both the structure and concentration of the triblock copolymers. Moreover, the lifetimes of the excited state and steady state intensities can be run up to the two and seven fold increase respectively on going from acetonitrile to aqueous solutions of L64 at 0.35 mM concentration level of the latter. Comparison of the data obtained for the triblock copolymers with diverse structure identifies the P-type arrangement of PEO and PPO blocks and their length as the key factors affecting kinetic stability and photophysical properties of the aqueous solutions of $[Mo_6I_{14}]^{2-}$**.** The ion pairing of $[Mo_6I_{14}]^{2-}$ with the protonated difloxacin sensitizes Mo-centered luminescence due to the energy transfer from the triplet level of the outer sphere ligand to the excited levels of $[Mo_6I_{14}]^{2-}$. It is worth noting the impact of the supramolecular assembly with triblock copolymers in both the water solubility and the luminescence response of $[Mo_6I_{14}]^{2-}$ on difloxacin. These results open new routes of the applicability of $[Mo_6I_{14}]^{2-}$ and its analogues as sensors in aqueous solutions.

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Notes and references

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Water soluble hexanuclear molybdenum cluster assembled with triblock copolymer gives luminescent response on ion-pairing with difloxacin through energy transfer.