Silica nanoparticles with a substrate switchable luminescence

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Abstract. Silica nanoparticles with visible (Tb and Ru doped), near IR (Yb doped) and dual visible-near IR luminescence (Ru-Yb doped) were obtained by reverse w/o microemulsion procedure. Plenty of luminescent complexes (from 4900 to 10000) encapsulated into each nanoparticle ensures the intensive luminescence of nanoparticles and their applicability as biomarkers. The silica surface decoration by definite anchor groups is the required step for the gaining to these nanoparticles marking and sensing functions. Thus covalent and non-covalent surface modification of these nanoparticles was developed to provide the binding with biotargets and sensing of anions. The dicationic surfactant coating of negatively charged Tb(III)-TCAS doped silica nanoparticles was chosen as the basis for the anion responsible system. The reversible insertion of the quenching anions (namely phenol red) into the surfactant based layer at the surface of luminescent nanoparticles switches off the Tb-centered luminescence. In turn the reversible reestablishment of the luminescence results from the competitive insertion of the non-quenching anions into the surfactant layer at the silica/water interface. The hydrophobic anions exemplified by dodecylsulfates versus hydrophilic ones (hydrophosphates) are preferable in the competition with phenol red anions.

1. Introduction
Smart colloidal systems, which are temperature, pH, or substrate responsive, gain much attention due to their possible application in bioanalysis, medicine and technology [1]. The appeal of silica nanoparticles results from the well-developed procedures of their synthesis with controlled size and uniformity, as well as nontoxicity [2]. The metal complex-doped nanoparticles have an advantage over a single complex, since the silica shell protects the metal complex from photodegradation, while the surface of the nanoparticle can be functionalized by various anchoring groups. Lanthanide complexes have gained a great deal of attention as dopants [3] owing to their unique spectroscopic characteristics, including long fluorescence lifetime, large Stokes shift, and sharp line-like emission bands [4]. These properties are particularly attractive because they enable temporal and spectral discrimination against background fluorescence often associated with commonly used fluorophores in chemical biology, leading to excellent detection sensitivity. The near-infrared (NIR)-emitting lanthanide complexes are of particular interest because biological tissues are transparent in this spectral range. Combination of luminescent and binding functions within nanoparticles is required for their application as biomarkers. The use of silica matrix, doped with luminophoric molecules and decorated by various anchor groups is a well known way of getting multifunctional nanoparticles for biolabelling [5]. The silica surface decoration is aimed to increase the colloidal stability of nanoparticles, as well as to develop their
receptor properties towards biotargets. The decoration of the silica surface through the non-covalent interactions represents more convenient alternative to covalent anchoring due to the lack of multistep purification procedures [6]. For example the decoration of silica nanoparticles by adsorption of cationic surfactants enables to get non-aggregated positively charged silica nanoparticles [7] for a development of nanoparticles, exhibiting sensing function to anions. Anions play an important role in living organisms and even phospholipids membranes of cells are negatively charged.

The present report outlines the data concerning the development of highly luminescent and colloidal stable system with sensor function to anions on the basis of lanthanides complexes doped silica nanoparticles with the modified surface.

2. Materials and methods

2.1. Materials
Tetraethyl orthosilicate (TEOS) 98%, ammonium hydroxide (28-30%), n-heptanol 98%, cyclohexane 99%, tris(2,2’-bipyridyl)chlororuthenium(II) hexahydrate (99%) and ytterbium(III) nitrate hexahydrate (99.9%) from Acros; terbium(III) nitrate hexahydrate (99.9%) from Alfa Aesar, Triton X-100, cetyltrimethylammonium bromide (CTAB), Na₂HPO₄, sodium dodesylsulfate (SDS) and phenol red from Sigma-Aldrich were used as purchased without further purification. The synthesis of p-sulphonatothiacalix[4]arene tetrasodium salt (TCAS) was carried out according to the known procedure [8]. Dicationic surfactant gemini 16-6-16 (chart 1) was synthesized in analogy with the procedure [9].

![Chart 1. Gemini 16-6-16.](image)

Luminescent Tb(III) and Yb(III) complexes with p-sulphonatothiacalix[4]arene and [Ru(dipy)₃]Cl₂ were used as dopants to silica nanoparticles. Synthesis of such silica nanoparticles has been performed according to reverse microemulsion procedure presented in the work [10]. Synthesis of silica coated Yb(III) and Yb(III)-Ru(II) nanoparticles at various Yb:Ru molar ratio has been performed with the addition to the reaction mixture of 7.8 mM of Yb(III)-TCAS and concentrations of [Ru(dipy)₃]Cl₂ varying from 3.9 mM to 15.6 mM.

| Synthetic conditions of Yb, Ru and Yb-Ru doped silica nanoparticles. |
|-------------------------|-----------------|-----------------|
| SiO₂ Ru: Yb | C₉Ru complexes, mM | C₂Yb complexes, mM |
| 0:1 | 0 | 7.8 |
| 1:0 | 7.8 | 0 |
| 2:1 | 15.6 | 7.8 |
| 1:1 | 7.8 | 7.8 |
| 0.9:1 | 7.02 | 7.8 |
| 0.5:1 | 3.9 | 7.8 |

Amino- and carboxy-modified silica nanoparticles were synthesized in analogy with the procedure [11].

2.2. Methods
The transmission electron microscopy (TEM) images were obtained using JEOL JEM 100 S microscope, Japan.
The dynamic light scattering (DLS) measurements were performed by means of the Malvern Mastersize 2000 particle analyzer. A He-Ne laser operating at 633 nm wavelength and emitting vertically polarized light was used as a light source. The measured autocorrelation functions were analyzed by Malvern DTS software and the second-order cumulant expansion methods. The effective hydrodynamic radius (RH) was calculated by the Einstein-Stokes relation from the first cumulant: $D = \frac{k_B T}{6 \pi \eta R_H}$, where $D$ is the diffusion coefficient, $k_B$ is the Boltzmann constant, $T$ is the absolute temperature, and $\eta$ is the viscosity. The diffusion coefficient was measured at least three times for each sample. The average error in these experiments was approximately 4%. All samples were prepared from the bidistilled water with prior filtering through the PVDF membrane using the Syringe Filter (0.45 μm).

Zeta potential “Nano-ZS” (MALVERN) using laser Doppler velocimetry and phase analysis light scattering was used for zeta potential measurements. The average error in these experiments was approximately 10%.

The steady-state emission spectra were recorded on a spectrofluorometer FL3-221-NIR (Jobin Yvon) under 330 nm (slit 2), 320 nm (slit 14) and 450 nm (slit 2) excitation for recording Tb-centered, Yb-centered and Ru-centered luminescence correspondingly.

All samples with nanoparticles were ultrasonicated within 30 min before measurements.

All measurements have been performed at least three times. The pH = 7.0, pH = 8.2 and pH=9.2 were adjusted by Tris (2.5 mM).

3. Results and discussion

3.1. Characterization of Tb(III)-TCAS and Yb(III)-TCAS doped silica nanoparticles

The nanoparticles were prepared according to the known procedure by hydrolysis of TEOS with ammonium hydroxide in a W/O microemulsion [10], containing terbium(III) and ytterbium(III) complexes with $p$-sulphonatotiacalix[4]arene (Tb(III)-TCAS, Yb(III)-TCAS). The lanthanides complexes doped silica nanoparticles were characterized by transmission electron microscopy (TEM). As shown in figure 1 nanoparticles are spherical and uniform in size (40 ± 5 nm for Tb(III)-TCAS doped and 32±5 nm for Yb(III)-TCAS doped silica nanoparticles).

![Figure 1. TEM images of Tb(III)-TCAS doped (a) and Yb(III)-TCAS doped (b) silica nanoparticles.](image)

The aqueous dispersions of nanoparticles were analyzed by emission spectroscopy and dynamic light scattering (DLS) methods. These nanoparticles are negatively charged with $\zeta$-value being about -33±5 mV in neutral aqueous solutions. DLS measurements performed for the aqueous dispersions of nanoparticles reveal the high monodispersity (polydispersity index is within 0.1-0.3) with the particle size being 223±4 nm for Tb(III)-TCAS doped and 262±4 nm for Yb(III)-TCAS doped silica nanoparticles, which stays unchanged within the concentration range from 0.0175 to 0.67 g L⁻¹ (table 1). The gap between the sizes determined from the TEM (40±5 nm and 32±5 nm) and DLS data is...
rather high and can not be explained by the thickness of the hydration shell [7, 12]. Thus Ln(III)-TCAS doped nanoparticles are somewhat aggregated in aqueous dispersions.

| Ln(III)-TCAS complex inside nanoparticle | C (g·L\(^{-1}\)) | d (nm) | PDI  | ζ (mV) |
|----------------------------------------|-----------------|--------|------|--------|
| Tb(III)-TCAS                           | 0.0175          | 237±2  | 0.190| -33    |
|                                        | 0.028           | 223±4  | 0.243| -33    |
|                                        | 0.07            | 232±2  | 0.186| -35    |
|                                        | 0.67            | 300±5  | 0.115| -40    |
| Yb(III)-TCAS                           | 0.0175          | 234±7  | 0.265| -33    |
|                                        | 0.028           | 262±4  | 0.287| -33    |
|                                        | 0.07            | 273±9  | 0.315| -32    |
|                                        | 0.67            | 268±2  | 0.292| -32    |

The aqueous dispersions of these nanoparticles exhibit very efficient Tb- (visible) and Yb-centered (near IR) luminescence, which confirms the formation of lanthanides doped silica nanoparticles (figure 2). The comparison of emission spectra of Tb(III)-TCAS and Yb(III)-TCAS complexes in aqueous solutions and within the silica core reveals that Ln(III)-TCAS in both above-mentioned cases show the same spectrum patterns \[^{5}D_{4} \rightarrow ^{7}F_{6}(489\text{nm}), \quad ^{5}D_{4} \rightarrow ^{7}F_{5}(543\text{nm}), \quad ^{5}D_{4} \rightarrow ^{7}F_{4}(582\text{nm}), \quad ^{5}D_{4} \rightarrow ^{7}F_{3}(620\text{nm})\] for Tb(III)-TCAS and \[^{2}F_{5/2} \rightarrow ^{2}F_{7/2}(976\text{nm})\] for Yb(III)-TCAS without any emission band shift for Tb-doped and small one for Yb-doped nanoparticles (figure 2). The comparison of luminescence intensities of Ln(III)-TCAS doped silica nanoparticles with Ln(III)-TCAS complexes in aqueous solution reveals that each nanoparticle is doped with 4900 Tb(III)-TCAS complexes and 10000 Yb(III)-TCAS complexes, which ensure the intensive luminescence of nanoparticles and their applicability as biomarkers.

| Figure 2. Emission spectra of (a) aqueous solutions of Tb(III)-TCAS (1) (C = 0.5 μM) and Tb(III)-TCAS doped silica nanoparticles (2) (C = 0.028 g·L\(^{-1}\)) [10]; and (b) aqueous solution of Yb(III)-TCAS (1) (C = 0.01 mM) and Yb(III)-TCAS doped silica nanoparticles (2) (C = 0.28 g·L\(^{-1}\)) at pH = 7.0. |

It is also worth noting that 3 days of stirring of an aqueous dispersion of Tb(III)-TCAS doped nanoparticles (C = 0.028 g·L\(^{-1}\)) provides no more than 10% decrease of Tb(III)-TCAS luminescence, while 2-fold decrease results from 7 days stirring of aqueous dispersions (figure 3). This decrease indicates the self-leakage of Tb(III)-TCAS complexes, which are rather unsteady in aqueous solutions due to photobleaching processes.
3.2. Ru-Yb doped silica nanoparticles

The insertion of two luminophores into silica nanoparticles should provide more accurate recognition of the examined substrate through the ratiometric analysis. The variation of the ratio of luminescence intensities in the case of two luminophores can be measured more accurately than the change of the intensity of single luminophore system.

Silica nanoparticles exhibiting dual visible-NIR luminescence were obtained by the insertion of two type of complexes: tris(2,2'-bipyridyl)chlororuthenium(II) ([Ru(dipy)_3]Cl_2) and Yb(III)-TCAS into silica matrix using reverse W/O microemulsion procedure. The synthesized nanoparticles were doped with different ratio of Ru:Yb (2:1, 1:1, 0.9:1, 0.5:1, 0.3:1) and were analyzed by fluorimetry, electrophoresis, dynamic light scattering, microscopy and atomic emission spectroscopy methods [13].

The fluorometric measurements have been performed for single components [Ru(dipy)_3]Cl_2 and Yb(III)-TCAS, as well as their mixture at various molar ratio in aqueous solutions and in silica nanoparticles in order to compare the luminescent properties of luminophores in aqueous solutions with those within nanoparticles (figure 4). The obtained data indicate the quenching effect of [Ru(dipy)_3]^{2+} on Yb(III)-centered luminescence of [Yb(III)-TCAS]^{4+} in aqueous solutions (figure 4a) resulted from their ion pairing (chart 2).

![Figure 3. Emission spectra of Tb(III)-TCAS doped silica nanoparticles (C = 0.028 g·L^{-1}) in time: immediately after solution preparation (1), after 1 hour of stirring (2), after 3 days of stirring (3), after 7 days of stirring (4) at pH = 7.0](image)

![Chart 2. Schematic representation of the ion pair between [Ru(dipy)_3]^{2+} and [Yb(III)-TCAS]^{4+}.](image)

The analysis of Yb-centered emission intensity of nanoparticles indicates no correlation between the intensity of Yb-centered luminescence and Yb:Ru molar ratio (figure 4b). Moreover the deviation of the intensity of Yb-centered luminescence for nanoparticles with the mentioned above Yb:Ru molar ratio is not dramatic and does not exceed the experimentally observed deviation for Yb nanoparticles.
It should be concluded that Yb-centered emission in silica nanoparticles is not quenched by $[\text{Ru(dipy)}_3\text{Cl}_2]$ in these cases (figure 4b).

Keeping in mind the quenching of Yb-centered luminescence in aqueous solutions due to the ion-pairing between $[\text{Yb(III)-TCAS}]^{4-}$ and $[\text{Ru(dipy)}_3]^{2+}$, the obtained data indicate that Ru(II) and Yb(III) complexes within nanoparticles are not in close proximity to each other. It is worth noting that the formation of silica nanoparticles occurs within aqueous nanodroplets of reversed micelles. The conditions of tetraethylorthosilicate hydrolysis should be taken into account to explain the restricted ion-pairing between $[\text{Yb(III)-TCAS}]^{4-}$ and $[\text{Ru(dipy)}_3]^{2+}$. The excess amounts of $\text{NH}_4^+$ and $\text{OH}^-$ ions, which are weakening the electrostatic attraction between $[\text{Ru(dipy)}_3]^{2+}$ and $[\text{Yb(III)-TCAS}]^{4-}$ is the most probable reason of their doping as $[\text{Ru(dipy)}_3]\text{X}_2$ and $[\text{Yb(III)-TCAS}]\text{Y}_4$, where X=Cl or OH and Y=Na or $\text{NH}_4$[13].

### 3.3. Covalent surface modification of Tb(III)-TCAS doped silica nanoparticles

The surface modification of the nanoparticles by suitable functional groups is the required task for their binding with biotarget. There are two type of surface modification: covalent and non-covalent. The covalent modification of the silanol groups at the silica interface by the required anchor groups with further bioconjugation with fit biosubstrates is preferable for the application as biomarker, when the efficient binding with biotarget is required.

![Figure 5. TEM images of amino- (a) and carboxy-modified (b) silica nanoparticles.](image)
In particular Tb(III)-TCAS doped silica nanoparticles with amino surface groups were obtained by the known procedure[11]. TEM data (figure 5) indicate the enhanced aggregation of amino-modified silica nanoparticles, which restricts their size determination. The carboxy-modified nanoparticles, which can be obtained from the amino-modified ones according to scheme 1, exhibit more colloidal stability. Their TEM image indicates more uniform distribution on the copper grids with the size being about 43±5 nm.

Scheme 1. Synthesis of amino- and carboxy-modified Tb(III)-TCAS doped silica nanoparticles

DLS data confirm low colloidal stability of amino-modified nanoparticles in aqueous dispersions. Silica nanoparticles with amino surface groups are characterized by ζ = 0.82 mV and by the size of about 1300 nm in solution (pH = 8.2) by the DLS method. The surface charge neutralization due to the protonation of amino-groups at the silica surface (pKₐ = 9.0) is the main reason of the enhanced aggregation of the amino-modified nanoparticles in neutral media. Further modification of such nanoparticles by carboxyl groups results in ζ = -37 mV and in the size of obtained nanoparticles of about 220 nm (pH = 8.2). The appearance of the charged C(O)O⁻ groups due to the partial dissociation of carboxyl groups C(O)OH (pKₐ = 4.9) is the main reason of colloidal stability of carboxy-modified nanoparticles in neutral media. High negative surface charge prevents from the aggregation of carboxy-modified silica nanoparticles.

It is worth noting that the covalent surface modification of silica nanoparticles undergoes without any loss of luminescence (figure 6). The enhancement of the Tb-centered luminescence intensity is observed for the modified nanoparticles, which can be explained by the additional shielding of luminescent complexes from the quenching by the aqueous environment.
3.4. Non-covalent surface modification of Tb(III)-TCAS doped silica nanoparticles

However, a development of nanoscale sensors is of the enhanced significance for the bioanalytical application. Unlike biomarkers, biosensors should both bind substrate and report about this binding through “off-on” switching of luminescence. Anions play an important role in living organisms and even phospholipids membranes of cells are negatively charged. Our goal was to obtain highly luminescent, colloidal stable system with sensor function to anions. The main point of our approach is the following. The luminescence of silica nanoparticles should be quenched through the interaction with some quenching anions with further reestablishment of luminescence through the competitive interactions with non-quenching, but biorelevant ones.

| Table 2. The averaged hydrodynamic diameters (d), ζ-potential values and polydispersity index (PDI) of Tb(III)-TCAS doped silica nanoparticles (C = 0.028 g·L⁻¹) at various concentrations of [Co(dipy)]₃³⁺ without and in the presence of various concentrations of Na₂HPO₄ at pH = 8.2. |
|-----------------|-----------------|--------|------|------|
| [Co(dipy)]₃(ClO₄)₃ | C_{Na₂HPO₄} (mM) | d (nm) | PDI  | ζ (mV) |
| 1·10⁻³           | 216,6 ± 1,65    | 0,201  | -26,6|
| 2·10⁻³           | 275,6 ± 4,13    | 0,233  | -22,9|
| 3·10⁻³           | 724,8 ± 24,09   | 0,281  | -20,3|
| 4·10⁻³           | 1105 ± 48,36    | 0,382  | -19,3|
| 5·10⁻³           | 1900 ± 101,3    | 0,319  | -17,4|
| [Co(dipy)]₃(ClO₄)₃ + | 1·10⁻³ | 210,8 ± 4,54 | 0,230 | -27 |
| 3·10⁻³           | 223,1 ± 4,48    | 0,215  | -28,1|
| Na₂HPO₄          | 3·10⁻³ | 394,2 ± 6,67 | 0,424 | -32,5|

It is known that positively charged complexes of cobalt(III) are quenchers of terbium luminescence [14]. Indeed the terbium luminescence of Tb(III)-TCAS doped silica nanoparticles is switched off when cobalt(III) complexes are adsorbed by their surface. More detailed description of these quenching measurements is presented in our previous work [10]. Herein we represent our efforts to develop the luminescent nanoparticles with sensor function using [Co(dipy)]₃³⁺ ions as quenchers. Adsorption of [Co(dipy)]₃³⁺ (C = 3·10⁻³ mM) changes the ζ-potential of silica nanoparticles from -33 mV to -20 mV (table 2) and quenches their Tb-centered luminescence (figure 7).
It is known that phosphate anions $\text{HPO}_4^{2-}$ can form stable ion-pairs with $[\text{Co(dipy)}_3]^{3+}$ in aqueous solution. But the addition of phosphate anions $\text{HPO}_4^{2-}$ to silica nanoparticles with adsorbed $[\text{Co(dipy)}_3]^{3+}$ does not reestablish Tb-centered luminescence (figure 7). Moreover electrophoretic measurements (table 2) reveal the increased negative charge at the silica/water interface ($\zeta$ is about -30 mV) due to the deposition of phosphate anions as the second layer at the $[\text{Co(dipy)}_3]^{3+}$ ions adsorbed by the silica surface (chart 3). So, the layer by layer deposition of the opposite charged ions restricts the use of unmodified luminescent silica nanoparticles as the basis for the development of the nanoscale sensor to anions.

The decoration of silica nanoparticles with various surfactants is well developed way to modify the aggregation stability and binding ability of nanoparticles [6, 7, 15]. The adsorption of surfactants at a silica/water interface is contributed by van der Waals, electrostatic interactions and multiple hydrogen bonding [16]. Taking into account that in neutral media silica surface is negatively charged due to the dissociation of Si-OH groups ($pK_a=8.4$) [17], cationic surfactants are most convenient for adsorption at a silica/water interface. The aggregation of cationic surfactants at the silica nanoparticles is exemplified in literature in most details by cetyltrimethylammonium bromide (CTAB) [7, 15], which is monocationic surfactant. Amphiphilic cations cethyltrimethylammonium (CTA⁺) form doubly charged bilayer at the silica/water interface, resulting in the recharging of the silica surface from minus to plus [7, 18]. Taking into account that electrostatic interactions play the key role in the formation of the doubly charged bilayer, gemini surfactant with two cationic groups per molecule, namely hexalidene-bis(dimethylammonium) bromide (16-6-16, chart 1) was chosen to recharge silica surface. Though the effects of CTAB and 16-6-16 are similar, there are some differences between them. In particular the change of $\zeta$-values of silica nanoparticles from -33 mV to +22 mV occurs at $3\cdot10^{-3}$ mM of 16-6-16, while higher concentration of CTAB ($3\cdot10^{-2}$ mM) is required for the similar recharging (figure 8) [19]. This tendency is in good agreement with literature data concerning adsorption and

Figure 7. Emission of Tb(III)-TCAS doped silica nanoparticles (0.028 g·L$^{-1}$) (1) in presence of Na$_3$HPO$_4$ ($C = 1$ mM) (2) and [Co(dipy)$_3$]$^{3+}$ (C = $3\cdot10^{-3}$ mM) (3) at the addition of Na$_3$HPO$_4$ : $1\cdot10^{-3}$ mM (4), 0.01 mM (5), 0.1 mM (6) and 1 mM (7) at pH = 8.2.

Chart 3. Schematic representation of layer-by-layer deposition of the opposite charged ions on the silica surface.
aggregation of cationic and dicationic surfactants at a silica surface [16]. The modified nanoparticles possess good colloidal stability and affinity towards anions.

![Figure 8. The ζ-potential values of Tb(III)-TCAS doped silica nanoparticles (C=0.028 g·L\(^{-1}\)) versus concentration of CTAB (1) and 16-6-16 (2) in aqueous solutions at pH = 9.2.](image)

The quenching effect of dyes on lanthanide centered luminescence is also well known [20] and applied in bioanalysis [21] or pH measurements [22]. According to well-known conceptions the quenching effect results from the intermolecular energy transfer from lanthanides to chromophores according to the so-called Förster mechanism [20]. The distance between luminophoric and quenching molecules greatly affects the intermolecular energy transfer [23], thus luminophoric and quenching molecules should be drawn together by binding or collisional process. So, the detectable quenching of silica coated luminophores is possible as long as the quenching molecules locate at the silica/water interface of nanoparticles.

![Chart 4. Schematic representation of the packing mode of dicationic surfactant 16-6-16 molecules at the silica/water interface and the penetration of phenol red anions (pink ovals) (a) and dodecylsulfate anions (blue molecules) (b) into the 16-6-16 bilayer.](image)

The choice of phenol red molecules as quenching ones is determined by several reasons. The first reason is their quenching effect on the luminescence of lanthanide complexes in solutions [20]. The second reason is the efficient penetration of dye molecules into micellar aggregates [7]. The insertion of phenol red anions into surfactant bilayer at silica/water interface (chart 4) results in the quenching of Tb(III)-centered luminescence of the nanoparticles (figure 9). It is also worth noting that the quenching effect of dye anions can be decreased or come to nothing at the competitive binding of gemini modified nanoparticles with various anions.

It is anticipated that a hydrophobicity of anions plays a key role in their localization within micellar layer. Therefore both highly hydrophilic anions, such as HPO\(_4^{2-}\) and anionic surfactants, namely sodium dodecylsulfate (SDS) as a source of DS\(^{-}\) anions with high affinity towards cationic micelles were chosen. The widespread use of phosphate buffers in bioanalysis is another reason of the choice of hydrophosphate as an example of hydrophilic anions.
Figure 9. Emission spectra of Tb(III)-TCAS doped silica nanoparticles (0.028 g·L$^{-1}$) in aqueous solution of 16-6-16 (5·10$^{-2}$ mM) (1); after quenching by phenol red (5·10$^{-3}$ mM) (2) and at the addition of Na$_2$HPO$_4$: 0.1 mM (3), 1 mM (4) and 10 mM (5) at pH = 9.2.

The figures 9 and 10 illustrate the effect of various amounts of Na$_2$HPO$_4$ and SDS respectively on the emission spectra of gemini modified Tb(III)-TCAS doped nanoparticles in the presence of quenching anions (C = 5·10$^{-3}$ mM). The addition of various amounts of HPO$_4^{2-}$ does not reestablish the Tb(III) centered luminescence of the nanoparticles (figure 9), but results in some decrease of the positive surface charge of nanoparticles (table 3). Taking into account high hydrophilicity of phosphate anions they should locate in the Shtern layer of cationic micelles without significant insertion into the micellar core. So, hydrophilic HPO$_4^{2-}$ anions can not substitute hydrophobic anions of phenol red from hydrophobic interior of gemini bilayer at the silica/water interface, therefore no reestablishment of the Tb-centered luminescence of nanoparticles occurs in this case, while detectable decrease of $\xi$-value is observed.

Table 3. The averaged hydrodynamic diameters (d), $\zeta$-potential values, and polydispersity index (PDI) of gemini modified Tb(III)-TCAS doped silica nanoparticles (C = 0.028 g·L$^{-1}$) at various concentrations of Na$_2$HPO$_4$ and SDS at pH = 9.2.

| C$_{NaHPO_4}$ (mM) | C$_{SDS}$ (mM) | d (nm) | PDI | $\zeta$ (mV) | Peak Means$^a$ (nm) |
|-------------------|----------------|--------|-----|-------------|-------------------|
| 0.1               | -              | 278 ± 2| 0.311| 27          | aggregation$^b$   |
| 1                 | -              | 314 ± 4| 0.249| 16          |                   |
| 10                | -              | -      | 0.878| 19          |                   |
| -                 | 0.001          | 246±4  | 0.183| 35          |                   |
| -                 | 0.005          | 236±3  | 0.179| 42          |                   |
| -                 | 0.01           | 216±3  | 0.166| 34          |                   |
| -                 | 0.02           | 216±2  | 0.151| 43          |                   |
| -                 | 0.03           | 227±4  | 0.175| 45          |                   |
| -                 | 0.04           | 271±4  | 0.237| 46          |                   |
| -                 | 0.05           | 325±4  | 0.225| 47          |                   |
| -                 | 0.06           | 369±5  | 0.251| 47          |                   |
| -                 | 0.07           | 420±4  | 0.243| 46          |                   |
| -                 | 0.08           | 494±6  | 0.287| 43          |                   |
| -                 | 0.09           | -      | 1.000| 18          | aggregation$^b$   |
| -                 | 0.1            | -      | 1.000| 0.49        | aggregation$^b$   |
| -                 | 0.15           | -      | 0.474| -25         | 79±3 (30%), 362±24 (70%) |
| -                 | 0.2            | -      | 0.385| -27         | 271±14 (100%)     |
| -                 | 0.3            | -      | 0.411| -29         | 279±12 (100%)     |
| -                 | 0.4            | -      | 0.473| -32         | 76±37 (30%), 360±160 (70%) |
| -                 | 0.5            | -      | 0.462| -33         | 47±4 (23%), 295±30 (77%) |
| -                 | 1              | -      | 0.429| -34         | 45±5 (18%), 256±17 (82%) |

$^a$ it is more correct to use Peak Means instead of averaged hydrodynamic diameters at PDI > 0.300

$^b$ the individual peaks are indistinguishable
In case of hydrophobic DS- anions the Tb-centered luminescence is still quenched, when the concentration of the added SDS is less than $5 \cdot 10^{-2}$ mM, while at $10^{-1}$ mM of SDS the increase of Tb(III) luminescence occurs (figure 10) along with the enhanced aggregation due to the charge neutralization ($\xi$-value is close to zero) (table 3). The enhanced aggregation of nanoparticles is evident from the visible precipitation. The precipitated aqueous dispersion exhibits very poor emission, therefore the intense mixing is required to observe luminescence. The reestablishment of Tb-centered luminescence is observed at the increase of SDS concentration to $5 \cdot 10^{-1}$ mM (figure 10), followed by the recharging of nanoparticles from positive to negative (table 3). The DLS data (table 3) confirm the enhanced aggregation of nanoparticles at $10^{-1}$ mM of SDS and reveal the appearance of two sized particles (260±30 and 45±4 nm) at $5 \cdot 10^{-1}$ and 1 mM of SDS. The size being 260 nm should be obviously referred to silica nanoparticles with gemini-SDS aggregates at the surface, while the aggregates being 45 nm concern to mixed gemini-SDS aggregates. Thus, DS- anions substitute phenol red anions (chart 4) leading to reestablishment of Tb(III)-centered luminescence [19].

![Figure 10](image)

Figure 10. The $I/I_0$ of Tb(III)-TCAS doped silica nanoparticles (0.028 g·L$^{-1}$) in aqueous solution of 16-6-16 (5·10$^{-2}$ mM) in the presence of 5·10$^{-2}$ mM of phenol red (PhR$^-$) at the varied concentration of SDS at pH = 9.2. $I_0$ is the emission intensity without phenol red [18].

So, the “on-off-on” switching luminescent system was obtained. The insertion of hydrophobic dye anions (namely phenol red), which are efficient quenchers of terbium luminescence, into surfactant bilayer at silica/water interface, results in the quenching or “on-off” switching of luminescence of the nanoparticles. The displacement of dye anions by dodecylsulphate anions is manifested by the “off-on” switching of luminescence.

### 4. Summary

In summary the following points should be mentioned:

- Both Tb-TCAS and Yb-TCAS complexes are enough luminescent and thermodynamically stable to withstand synthetic conditions of water-in-oil microemulsion procedure without significant degradation. Thus the efficient insertion of Tb-TCAS and Yb-TCAS complexes into silica matrix enables to obtain uniformly sized nanoparticles with efficient visible (Tb-TCAS) and near IR (Yb-TCAS) luminescence. The synthesized nanoparticles are characterized by the slowed down leakage, and thus their luminescence is stable in time, which is of particular importance for their practical application. These nanoparticles for the first time exemplify the use of calixarene complexes as luminophoric dopants to silica nanoparticles. The covalent surface modification of these nanoparticles undergoes without any loss of luminescence, which is also of great importance for their bioanalytical application.

- The convenient and reproducible synthetic procedure has been developed to obtain dual NIR-visible luminescent nanoparticles.
• The luminescent nanoparticles, which are able to give luminescent response on the addition of definite substrates, have been developed. The Tb-TCAS doped nanoparticles modified by the dicationic surfactant coating are the basis for the substrate responsible system. The reversible insertion of the quenching anions (namely phenol red) into the surfactant based layer at the surface of luminescent nanoparticles switches off the Tb-centered luminescence. In turn the reversible reestablishment of the luminescence results from the competitive insertion of the non-quenching anions into the surfactant layer at the silica/water interface.

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