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Design of Biologically Active Macromolecular Ligands and Their Luminescent Complexes with Terbium Ions

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Electronic Supplementary Information

Abstract Water-soluble copolymers of *p*-methacrylamidobenzoic acid (MABA) with neutral comonomers (*N*-vinylpyrrolidone (VP), *N*-methyl-*N*-vinylacetamide (MVAA), *N*-methacryloyl glucosamine (MAG)) and anionic comononer sodium styrene sulfonate (NaSS) were synthesized by radical copolymerization. The interactions between the prepared copolymers and Tb^{3+} ions in aqueous solutions were studied; the significant influence of chemical structure of a comonomer on luminescence intensity of Tb^{3+} complexes with the copolymers was revealed. The luminescence intensity of Tb^{3+} complexes with the copolymers containing *N*-vinylamide units (VP, MVAA) is three times more intense than that observed for the complexes between Tb^{3+} and MAG-containing copolymers. In the case of NaSS-containing copolymers, the luminescence intensity is controlled by the values of binding constants between Tb^{3+} and MABA and the content of MABA units in a copolymer. The studied copolymers and their complexes with Tb^{3+} have low cytotoxicity and a pronounced antiviral activity against human respiratory syncytial virus.

Keywords Luminescence; Water-soluble copolymers; Lanthanide complexes; Antiviral activity; Cytotoxicity

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INTRODUCTION

In the recent years, along with popularity of designing novel ligands capable of efficient sensibilization of lanthanide ion luminescence, there has been a growing interest in directional synthesis of macromolecular ligands with functional properties such as antiviral and antimicrobial activity.[1–3] In the current conditions, when new mutant strains of coronavirus SARS-CoV-2 cause predictable pandemic waves coinciding with seasonal waves of traditional respiratory infections, there is high demand for new antiviral agents. The search for efficient antiviral drugs that are not toxic for host cells and the studies of their interactions with viruses and cells, their pharmacokinetics, *etc*., are important tasks. In particular, of interest are macromolecular complexes of lanthanide ions (MCL), which are promising for biomedical applications.

Lanthanide ions (Ln^{3+}) are widely used as luminescent probes in biology and medicine due to some particular features of their luminescence as compared to that of organic luminophores. Among them are quasi-monochromaticity of emission (half-width of luminescence band is 5–10 nm, while for organic chromophores it exceeds 100 nm); long-term stability of luminescence; high values of the Stokes shift in complexes; long excited state lifetimes (≈1000 μs); independence of the spectrum shape on the ligand nature virtually for all lanthanides (except Eu^{3+} ions, whose spectra demonstrate the Stark splitting of *f*-levels caused by crystal field of ligands[4,5]).

Varying the structure of a macromolecular ligand and the nature of comonomer units not only allows one to change luminescence properties of MCL, but also gives a number of additional opportunities, *e.g.*, the use of copolymers with inherent physiological activity (antiviral, immunostimulating, *etc*.), prolongation of the interaction between a (co)polymer and cells or tissues, and visualization of drug action.[1,6,7]

The MCL intended for use in bioimaging should meet a number of requirements. They should possess solubility in water, bio- and cytocompatibility, bioactivity (retain their biological effect). In aqueous solutions, the first coordination sphere is completed with water molecules, whose OH groups ("OH-oscillators") are efficient luminescence quenchers.[4,6–9] Therefore, the search for ways of minimizing this effect is a topical problem.

The intense luminescence of Ln^{3+} ions in their complexes with low molecular weight derivatives of aromatic carboxylates has been observed,[10] sparking interest in synthesising

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macromolecular ligands that contain aromatic carboxylic acids as chelating units.[11–14] The effectiveness of electronic excitation energy transfer from a ligand's triplet level to the resonance level of a lanthanide ion is influenced by both the energy of the triplet level and the substituents' nature and their interaction with the environment. Additionally, the luminescence efficiency of Ln^{3+} may be significantly impacted by the surrounding environment of a chelating unit.[10]

To investigate this effect, copolymers of *p*-methacrylamidobenzoic acid (MABA) with comonomers of varying structures and natures (neutral vinylamides (*N*-vinylpyrrolidone (VP), *N*-methyl-*N*-vinylacetamide (MVAA)); *N*-vinylsaccharide *N*-methacryloyl glucosamine (MAG), and anionic monomer sodium styrene sulfonate (NaSS) were synthesized (Scheme 1). The interest in NaSS-MABA copolymers is caused by the fact that polystyrene sulfonate possesses high antiviral activity,[15,16] but does not form luminescent complexes necessary for bioimaging, while poly-MABA forms intensely luminescent complexes with Ln^{3+}. It is expected that introducing MABA chelating units into macromolecules will make it possible to create luminescent bio-probes with antiviral activity and study their interactions with cells and viruses. Radical copolymerization of NaSS with MABA was used to synthesize polyanions able to form luminescent complexes with Ln^{3+}. The interactions between the synthesized copolymers and terbium ions in aqueous solutions were studied. The influence of the comonomer nature, copolymer concentration, lanthanide ion concentration in solution, the metal/copolymer ratio on photophysical properties of complexes was revealed. The optimal conditions for preparation of stable and intensely luminescent complexes were established. Antiviral activity of the prepared copolymers and their complexes was investigated.

EXPERIMENTAL

General Considerations

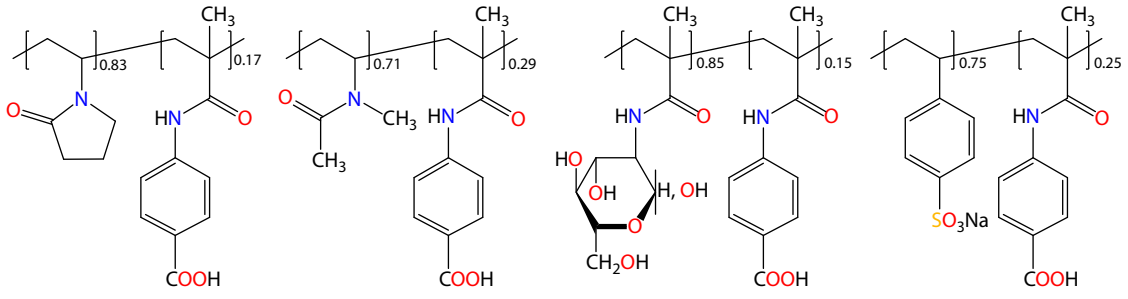
The following reagents were used in the work: VP (Aldrich, cat. No. V3409); MVAA (Aldrich, cat. No. 25,5130), NaSS (Aldrich, cat. No. 32,8596); AIBN (Porofor ChKhZ57, 99%, LDKhim Ltd.); *p*-aminobenzoic acid (99%, Aldrich, cat. No. 10,0536); methacryloyl chloride (90%, Aldrich, cat. No. 32,0374); Et_2O (analytical grade, Kuzbasorgkhim Ltd.); NaOH (reagent grade, Lenreaktiv Inc.); DMSO (reagent grade, Vekton); EtOH (GOST P 51652-2000, Vekton), DMFA (reagent grade, Vekton); Pr^{i}OH (reagent grade, Vekton), Me_2CO (reagent grade, Vekton); $TbCl_3{\cdot}6H_2O$ (reagent grade, 99.99% Aldrich cat. No. 204560); DMEM nutrient medium containing L-glutamine and glucose (Reg. Cert. FSR 2008/03103, Biolot); Tween 20 ((RFE, USPNF, BP, Ph.Eur.), for biochemistry, Biolot); phosphate-buffered saline (tablets, Biolot); tetramethylbenzidine (for biochemistry, not less than 98% (titer), «AppliChem»); penicillin-streptomycin («Gibco», USA); fetal bovine serum (FBS) («Gibco», USA), bovine serum albumin (KhIMEKS Ltd.).

Synthesis of Copolymers VP-MABA, MVAA-MABA, MAG-MABA, NaSS-MABA

MABA monomer was synthesized according to the modified technique by acylation of *p*-aminobenzoic acid with methacryloyl chloride in acetone solution[17] and purified by recrystallization from water-alcohol medium; m.p. 220 °C (literature data: average m.p. 215–219 °C). The structure of the monomer was confirmed by NMR and FTIR spectroscopy. ^{1}H-NMR spectra were registered in DMSO-d_6 solution using a Bruker AVANCE-400 NMR spectrometer. The values of chemical shifts were measured from the residual signal of DMSO-d_6 solvent (^{1}H δ=2.5 ppm) ^{1}H (DMSO-d_6, δ, ppm, *J*/Hz): 12.72 (s, 1H, COOH); 10.07 (s, 1H, CONH); 7.92 (d, 2H, H(*ortho*)); 7.83 (d, 2H, J_1=8.74, H(*meta*)); 5.85, 5.57 (both s, 1H each, $H_2C{=}C<$); 1.97 (s, 3H, Me). FTIR spectra were obtained with the aid of a Vertex70 FTIR spectrophotometer (Bruker) equipped with an ATR attachment (Pike). FTIR spectrum (KBr, ν, cm^{-1}): 3390 (—CONH); 3200–2500 (—COOH); 1650 (—CONH); 1690 (—COOH); 1620 ($H_2C{=}C<$); 1597, 1550, 1500 (Ar).

Copolymers were synthesized by radical polymerization in organic solvents (DMFA, DMSO) and water-organic mixtures (DMSO-water, DMFA-water) in argon atmosphere, in the presence of radical initiators (AIBN). The products were purified by dialysis against water followed by lyophilization. Compositions of the copolymers were determined by UV-Vis spectrophotometry and ^{1}H-NMR (Figs. S1–S5, in the electronic supplementary information, ESI).

The molecular weight of the samples and the hydrodynamic radius (R_h) of macromolecules were measured by static and dynamic light scattering in dilute solutions in 0.1 mol/L NaCl at 21.0 °C.[18] Light scattering was studied on a Photocor Complex instrument (Photocor Instruments Inc., Russia); the light source was a Photocor-DL diode laser (power, 5–30 mW; wavelength, λ=659.1 nm). The calibration of the device, that is, the determination of the instrument coefficient, was carried out using toluene (R_V=1.38×10^{-5} cm^{-1}). The measurements were performed at scattering angles θ in the range of 45°–135°. The correlation function of the scattered light inten-



Scheme 1 Structures of MABA copolymers.

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sity was recorded using a Photocor-PC_2 correlator with 288 channels and processed using DynaLS software (ver. 8.2.3, SoftScientific, Tirat Carmel, Israel). The refractive index increments d*n*/d*c* were measured using a RA620 (λ=589.3 nm) refractometer (KEM, Japan). The particle size distribution obtained by the DLS method (Table S1 and Figs. S6–S9 in ESI) was relatively narrow for all samples except sample VMAA-MABA, which suggests that the polydispersity of the samples is typical of free radical polymerization-derived polymers.

Preparation of Solutions

A weighed amount of a copolymer was dissolved in water; pH of a solution was adjusted to 7.5–8.5 by adding 0.1 mol/L NaOH. The working solution with a desired concentration of the copolymer was prepared by taking the calculated volume of the concentrated solution, adding water to bring the volume to 1 mL, and then introducing 1 mL of 2.5×10^{-4} mol/L aqueous solution of $TbCl_3$. Before measurements, the solutions were kept at room temperature for 1 h. Varying copolymer concentration at the constant $TbCl_3$ concentration allowed us to change the $[COO^-]/[Tb^{3+}]$ ratio over a wide range.

Methods

Absorption spectra of copolymer solutions were obtained with the use of an SF256 UV-Vis spectrophotometer (LOMO Fotonika Ltd., Russia). Photoluminescence excitation and emission spectra of solutions were recorded on a LS100 spectrofluorimeter (PTI, Canada). Luminescence intensity I_{544} was estimated from the maximum of the luminescence band at λ=544 nm. The I_{544} measurements were acquired under similar settings such as photomultiplier voltage and slit widths of the monochromator and were standardized to the luminescence value of a standard. The excited state lifetime of Tb^{3+} in its complexes τ_{phosph} was determined from the kinetic phosphorescence decay curve (Figs. S10–S12 in ESI). The photoluminescence spectra were registered at an excitation wavelength of 298 nm. The photoluminescence excitation spectra were registered at an emission wavelength λ_{em}=544 nm. All measurements were carried out in a quartz cuvette with an optical path length of 1 cm in a thermostatically controlled cell at a temperature of 25 °C.

Cytotoxicity and antiviral activity of the synthesized copolymers were determined using the HEp2 cell culture (human laryngeal epidermoid carcinoma) obtained from the collection of cell cultures of the Laboratory of cell cultures of Smorodintsev Research Institute of Influenza (Fig. S13 in ESI). Human respiratory syncytial virus (A2 strain) was obtained from the Laboratory of biotechnology of diagnostic preparations on April 1, 2018, then accumulated on the HEp2 cell culture and stored at −80 °C. The DMEM medium with antibiotics (penicillin-streptomycin), FBS, and L-glutamine served as the maintenance medium.

Cytotoxicity (CC_{50}) of polymers was assessed from cell viability using a microtetrazolium test.[19] Antiviral activity was determined by the enzyme immuno assay (EIA) method. A series of 3-fold dilutions of polymers was prepared, starting from ½ CC_{50}, then applied to the HEp2 cell culture in double concentration (100 μL per well). Immediately after that, the virus was added (100 μL in a series of 10-fold dilutions). Then the cells were incubated for 1 h at 37 °C in the presence of CO_2 (5%). The virus was washed off, the preparations were applied at the onefold concentration, incubation was performed for 6 days at 37 °C in the presence of 5% CO_2.

To carry out cell-enzyme-linked immunosorbent assay (cell-ELISA), the cell culture was fixed with cold 80 % acetone for 15 min, then washed with phosphate buffered saline with added Tween 20 (up to 0.05%). The solution of primary mouse antibodies to the respiratory syncytial virus F protein was applied to the culture, then the cells were incubated for 2 h at room temperature at continuous stirring.

Then the cells were washed with phosphate buffered saline, secondary mouse antibodies were applied, and the cells were incubated for 2 h at continuous stirring. The antibodies were washed off, and the substrate (chromogenic mixture with tetramethylbenzidine) was applied. The reaction was terminated in 5 min with 0.1 mol/L sulfuric acid, and optical density of the solution at λ_{max}=450 nm was measured. The wells in which the absorbance value exceeded that in the control wells by a factor of two or more were considered contaminated. The virus titer was calculated according to the Reed-Muench method.[20]

The criterion for evaluating antiviral activity is a statistically significant decrease in the virus titer in cells treated with a polymer compared to that of the control. These results were used to calculate the effective drug concentration at which the virus titer is reduced by 50%, IC_{50}.

To assess the prospects of (co)polymers as antiviral agents against respiratory syncytial virus A2, we used therapeutic index (TI), the ratio of CC_{50} to IC_{50}. The compounds with TI values of more than 8 were considered promising.

RESULTS AND DISCUSSION

Characterization of the Synthesized Copolymers

Table 1 presents the synthesis conditions and characteristics of the polymers obtained.

Photophysical Properties

Photoluminescence

Fig. 1 displays the absorption spectra of aqueous solutions of the studied copolymers. To exclude the influence of the ionization degree on absorption spectra, they were recorded at pH=9, when all COOH groups of MABA units are ionized.

The spectra exhibit one peak (λ_{max}=267–269 nm) associat-

Table 1 Conditions of copolymerization of MABA (M_2) with comonomer M_1 (*T*=65 °C, *t*=24 h) and characteristics of the obtained copolymers.

M_1	Composition of monomer mixture (mol%)		Solvent, *C* (%)	C_{AIBN} (%)	Yield (%)	Copolymer composition (mol%)		M_w (10^{-3})	R_h (nm)
	M_1	M_2				M_1	M_2		
VP	85	15	DMFA, 30	1	92	83	17	125	9.2
VMAA	83	17	DMFA, 30	1	73	71	29	70	6.1
MAG	90	10	DMFA, 20	2	84	85	15	101	7.4
NaSS	70	30	DMSO/H_2O, 16	1	83	75	25	121	10.8

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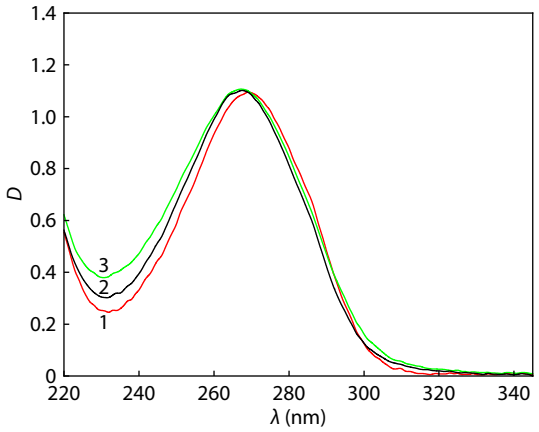

Fig. 1 The absorption spectra of solutions of MABA copolymers with VP (1), MAG (2), MVAA (3). [COO^-]=9.1×10^{-5} mol/L.

ed with π-π^* electronic transitions in the MABA benzoate ring. As can be seen in Fig. 1, the shapes and intensities of spectra are virtually independent on the nature of neutral comonomer. The addition of $TbCl_3$ (1.0×10^{-4} mol/L) does not lead to a shift in the absorption bands and a change in their intensity.

Fig. 2(a) depicts the photoluminescence excitation spectra of Tb^{3+} ions at their constant concentration (1.25×10^{-4} mol/L) in solutions of the MAG-MABA copolymer of varying concentrations. In the studied concentration range, the molar ratio [COO^-]/[Tb^{3+}] varies from 1 to 12 (spectra 1–6, respectively).

Fig. 2(b) shows the photoluminescence spectrum of the solution of the MAG-MABA copolymer with the maximum value of this ratio ([COO^-]/[Tb^{3+}]=12). The band λ_{ex}=298 nm was selected for registration of photoluminescence spectra. The positions of bands in luminescence spectra at 489, 544, 584, and 621 nm (which correspond to $^5D_4\rightarrow{}^7F_J$ (J=6, 5, 4, 3) transitions in Tb^{3+} ions) do not depend on copolymer concentration, while the luminescence intensity changes considerably. In copolymer solutions, the intensity of Tb^{3+} luminescence increases by over 30 times when compared to that of individual ion solutions. This indicates (i) the formation of the MAG-MABA/Tb^{3+} complex and (ii) efficient transfer of excitation energy from the triplet level of ligand to the resonance level of Tb^{3+} ion (antenna effect).

For all studied copolymers, the dependences of I_{544} on the concentration of MABA units (COO^- groups) are shown in Fig. 3. Firstly, the I_{544} value increases almost linearly with increasing concentration of COO^- groups, then reaches the maximum at [COO^-]=8×10^{-4} mol/L, and lastly, decreases insignificantly. This rise in the I_{544} value is caused both by an increase in the number of emitting centers (due to the increasing concentration of COO^- groups interacting with Tb^{3+} ion) and by an increase in the efficiency of intramolecular transfer of ab-

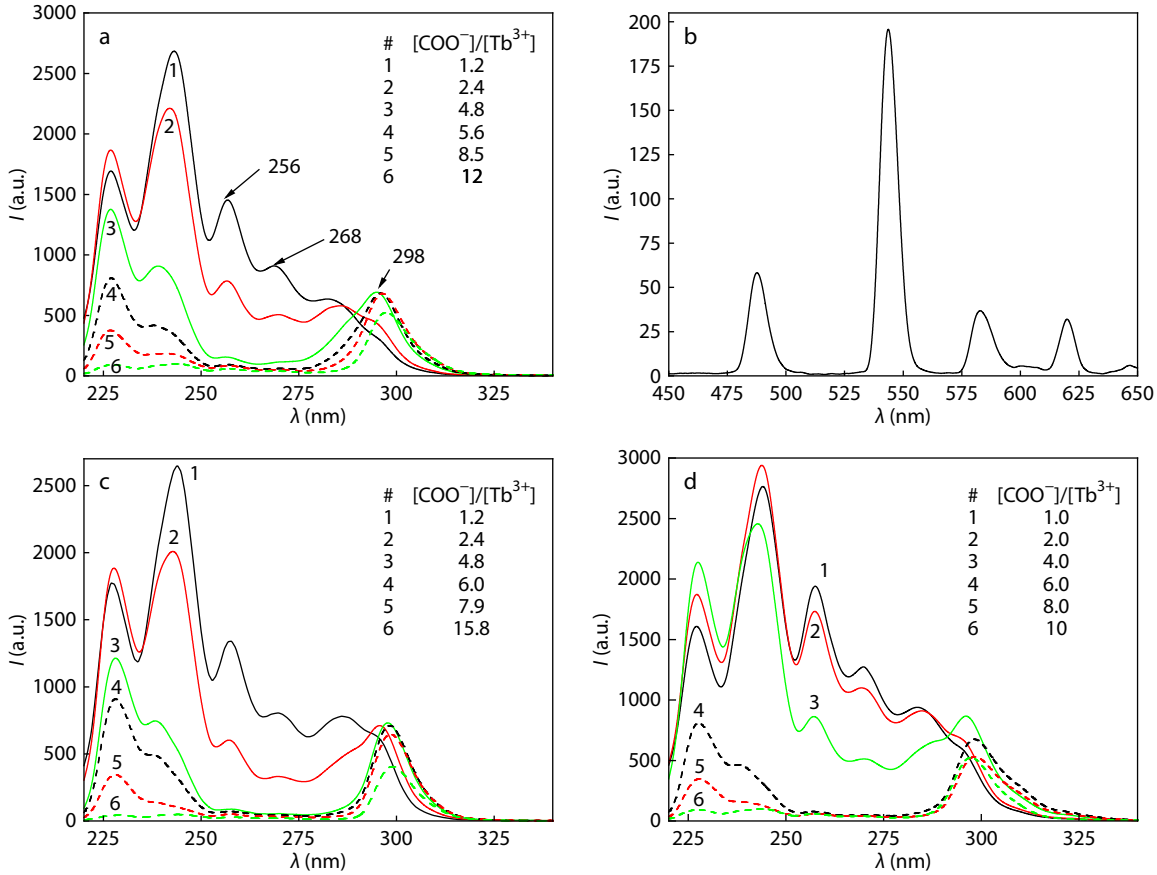

Fig. 2 (a, c, d) Photoluminescence excitation spectra (λ_{em}=544 nm) of Tb^{3+} ions at their constant concentration in solution (1.25×10^{-4} mol/L) and varying [COO^-]/[Tb^{3+}] ratio: (a) MAG-MABA, (c) VP-MABA, (d) VMAA-MABA; (b) Photoluminescence spectrum (λ_{ex}=298 nm) of Tb^{3+} ion (1.25×10^{-4} mol/L) in solution of the MAG-MABA copolymer at [COO^-]/[Tb^{3+}]=12.

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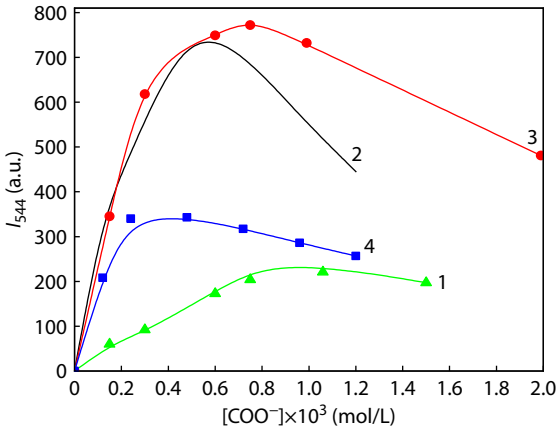

Fig. 3 Dependences of I_{544} on concentration of MABA units in solutions of copolymers: (1) MAG-MABA, (2) VMAA-MABA, (3) VP-MABA, (4) NaSS-MABA. $[Tb^{3+}]=1.25\times10^{-4}$ mol/L.

sorbed energy from the triplet level of ligand to the emitting level of Tb^{3+}. This is indicated by the absence of the bands at 256 and 268 nm related to the ligand in the photoluminescence excitation spectra (Fig. 2a, compare curves 1, 2, and 4–6).

The maximum value of I_{544} corresponds to $[COO^-]/[Tb^{3+}]\approx 7$. Since Tb^{3+} ion is able to bind only 3–4 COO^- groups, the determined ratio ($[COO^-]/[Tb^{3+}]\approx7$) indicates that a portion of COO^- groups remains free and is not included in the complex due to steric limitations (caused by polymer nature of the ligand). Since complexes of lanthanide ions usually have high coordination numbers, the first coordination sphere includes water molecules together with COO^- groups. Their hydroxyl groups are efficient luminescence quenchers (for more details, see below).

To reveal the influence of the nature of neutral comonomer on luminescence efficiency of Tb^{3+} ions, copolymers of MABA with *N*-vinylamides (VP and VMAA) were synthesized. Fig. 2 presents photoluminescence excitation spectra of Tb^{3+} ions in solutions of copolymers VP-MABA (Fig. 2c) and VMAA-MABA (Fig. 2d) of varying concentrations.

Comparison of spectra in Figs. 2(a), 2(c), and 2(d) shows that both the shapes of photoluminescence excitation spectra and positions of bands in these spectra depend on the concentration of chelating units in a similar fashion. However, the nature of comonomer units has a significant impact on I_{544}, as demonstrated in Fig. 3, where the dependence on copolymer concentration is also shown.

The results from Fig. 3 indicate that, firstly, the maximum available I_{544} depends on the comonomer nature. For the copolymers containing bulky side substituents (see Scheme 1), the maximum intensities I_{544} are observed at Tb^{3+} concentrations corresponding to the $[COO^-]/[Tb^{3+}]\approx7$ (MAG-MABA) and 5.5 (VP-MABA), while for the VMAA-MABA copolymer, $[COO^-]/[Tb^{3+}]=4.5$. The presence of large side groups in a macromolecule leads to stronger steric hindrance effects, lower mobility of polymer chain fragments; as a result, the portion of COO^- groups unavailable for interaction with Tb^{3+} increases.

Secondly, the I_{544} values for solutions of VMAA-MABA and VP-MABA are significantly higher (over three times higher) than found in MAG-MABA solutions. Possibly, the observed effect is due to the specifics of the hydration of poly-*N*-vinylamides in solutions and films. It is known that water molecules are effective luminescence quenchers in coordination complexes of lanthanides. One possible way to decrease the contribution of OH oscillatory motion into non-radiative processes is creation of rigid environment for a lanthanide ion that will prevent oscillation of the solvent.[21,22] The mechanism of hydration of poly-*N*-vinylamide molecules and the structure of solvent in the vicinity of a polymer chain are discussed.[23,Chapter 3] Through IR, NMR, spin-echo, and DSC studies, it was found that water molecules in the first and second hydration layers, close to the C=O carbonyl group of the *N*-vinylamide monomer unit, form stronger hydrogen bonds with each other compared to those farther away from the polymer chain. This leads to formation of a distorted ice-like water structure. This anomaly is not observed in the case of polysaccharides, because their hydration shell structure is little different from the structure of bulk water.[24] Therefore, it can be assumed that in the complexes of Tb^{3+} with the VP-MABA and VMAA-MABA copolymers, water molecules located in the vicinity of carbonyl groups C=O (and strongly bound by hydrogen bonds) are partially included in the coordination sphere of Tb^{3+} (Fig. 4). As a result, oscillations of OH groups are hindered, which facilitates enhancement of luminescence.

Thirdly, significant concentration quenching is observed in the solutions of copolymers VMAA-MABA and VP-MABA after reaching the maximum luminescence intensity I_{544}, whereas the I_{544} value in the solution of MAG-MABA remains almost unchanged. The absence of concentration quenching indicates that the emitting centers are located far from each other, which is apparently caused by random distribution of MABA units along the MAG-MABA copolymer chain. The distribution of MABA units in its copolymers with *N*-vinylamides

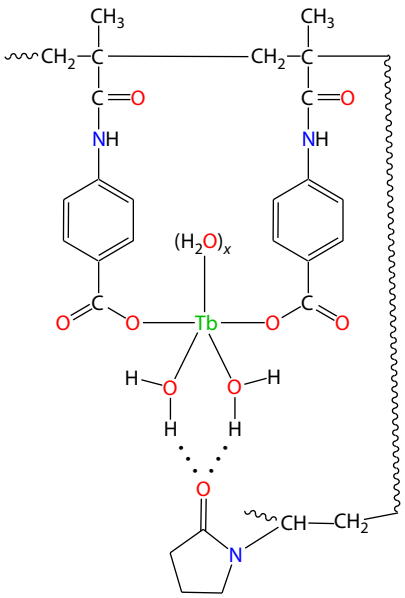

Fig. 4 Schematic representation of an intramolecular contact between the VP unit and water molecules of coordination sphere of Tb^{3+} ion coordinated to the MABA units of the VP-MABA copolymer.

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is determined by the fact that (meth)acrylates are more reactive comonomers than VP during copolymerization in aprotic solvents; the values of effective copolymerization constants differ by an order of magnitude or more.[23,25] With microblock distribution of chromophore groups, the distance between emitting centers is less than with statistical distribution, which leads to a concentration quenching. It should be noted that this microblock structure of the VMAA-MABA and VP-MABA copolymers causes a noticeable opalescence at low copolymer concentrations in solutions, which indicates the formation of intermolecular contacts. Opalescence disappears with increasing copolymer concentrations. Similar dependence of solubility of complex on the $[COO^-]/[Ln^{3+}]$ ratio was observed in the process of interaction between $CeCl_3$ and poly(acrylic acid), which contains COO^- group in each monomer unit.[26]

Copolymerization of MABA with an anionic comonomer instead of a neutral one provides an opportunity to achieve binding Tb^{3+} by both types of functional groups. Fig. 5 presents photoluminescence excitation spectra and a luminescence spectrum of Tb^{3+} ions in the solution of the NaSS-MABA copolymer. Comparison of the excitation spectra of Tb^{3+} solution and the corresponding spectra for the solutions of MABA copolymers with neutral comonomers shows that their shapes are virtually identical. An increase in copolymer concentration leads to an increase in the efficiency of energy transfer from the triplet level of ligand.

Comparison of the dependence of I_{544} on concentration of the NaSS-MABA copolymer (Fig. 3, curve 4) with the corresponding curves for MABA copolymers with neutral comonomers shows that the maximum value of I_{544} is reached at a significantly lower $[COO^-]/[Tb^{3+}]$ ratio (2.6). This suggests that Tb^{3+} ions can bind both COO^- and SO_3^- groups and form mixed complexes, where the coordination sphere includes both anions (see Scheme 2). Since styrene sulfonates are not capable of sensibilization of Tb^{3+} luminescence, luminescence intensity values of these complexes are lower than those of the complexes with coordination spheres consisting only of MABA units.

The possible structures suggested on the basis of the above results are presented in Scheme 2.

Excited state lifetime

In addition to luminescence intensity, an important characteristic of the obtained complexes is their excited state lifetime τ_{phosph}. The τ_{phosph} values were determined from the Tb^{3+} luminescence decay curve according to the equation:

$$I(\tau) = I_0 \sum_i A_i \exp(-\tau/\tau_i) \quad (1)$$

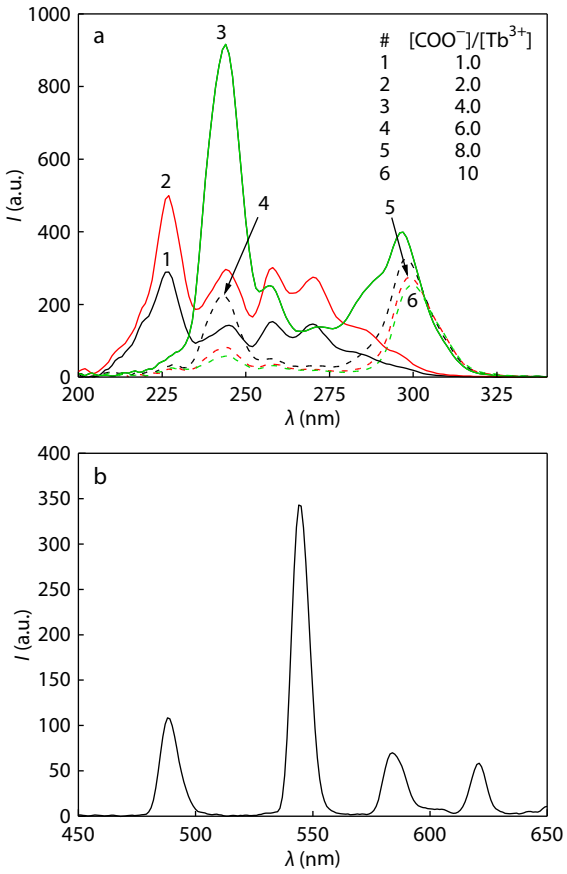
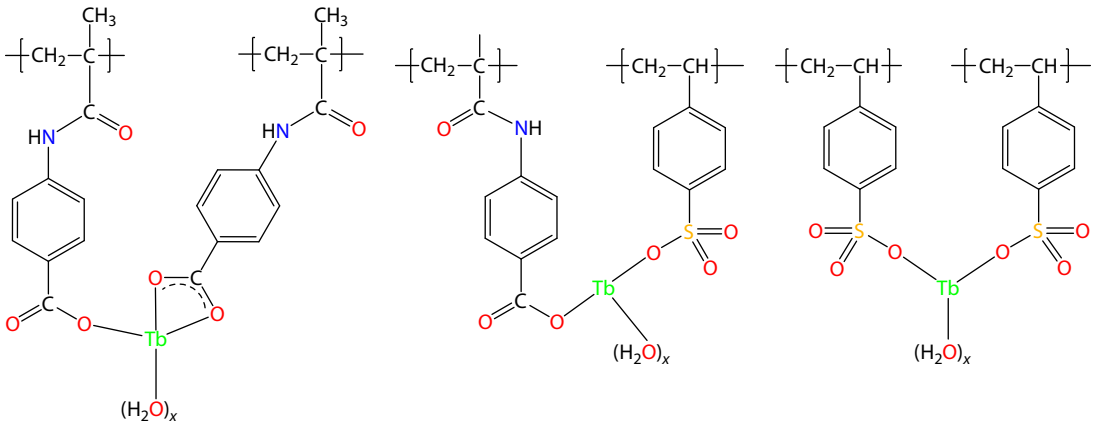


Fig. 5 (a) Photoluminescence excitation spectra (λ_{em}=544 nm) of Tb^{3+} ions at their constant concentration in solution (1.25×10^{-4} mol/L) and varying concentrations of the MABA units in the solution of the NaSS-MABA copolymer; (b) Photoluminescence spectrum (λ_{ex}=298 nm) of Tb^{3+} ions (1.25×10^{-4} mol/L) in solution of the NaSS-MABA copolymer at $[COO^-]/[Tb^{3+}]$=2.0.



Scheme 2 Fragments of the supposed structures of the NaSS-MABA/Tb^{3+} complex.

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where the constants A_i characterize the relative contribution of the components. The τ_{phosph} values were determined from the Tb^{3+} luminescence decay curve for the solutions of copolymers with the maximum I_{544} at an excitation wavelength of 298 nm and an emission wavelength of 544 nm. For all solutions, the luminescence intensity decay was approximated by a two-exponential dependence.

Table 2 lists the values of excited state lifetimes τ_1, τ_2 of Tb^{3+} ions and their contributions. The presence of two noticeably differing times τ_1, τ_2 suggests the existence of at least two types of emitting centers with different compositions of coordination sphere of the complex. These differences result from the number of bound COO^- groups and water molecules. The shortest-lived component is related to more hydrated complexes, while the longer τ_{phosph} values correspond to less hydrated structures.

Table 2 Excited state lifetimes τ_1, τ_2 of Tb^{3+} ions and their contributions in aqueous solutions of copolymers MAG-MABA, VP-MABA, VMAA-MABA, NaSS-MABA.

Copolymer	A_1	τ_1 (μs)	A_2	τ_2 (μs)
MAG-MABA	0.42	380	0.58	914
VP-MABA	0.14	341	0.86	784
VMAA-MABA	0.24	405	0.76	901
NaSS-MABA	0.12	171	0.88	531

It is seen from Table 2 that the τ_1 and τ_2 values are almost similar for all samples and do not depend on the nature of neutral comonomer; however, their contributions change significantly.

Thus, in the solution of MAG-MABA/Tb^{3+}, the contribution of short lifetimes is equal to 42%, while in the solution of VP(VMAA)-MABA/Tb^{3+} it is only 14%–24%. These changes in the contributions of τ_1 and τ_2 can be explained by the above hypothesis concerning the dependence of luminescence efficiency on the type of hydration of Tb^{3+} coordination sphere.

In aqueous solutions of the NaSS-MABA/Tb^{3+} complex, the τ_1 and τ_2 values are lower than the corresponding values calculated for the copolymers of MABA with neutral comonomers (VP and MBAA). This indicates a high probability of inclusion of sulfonate groups in the coordination sphere of the complex.

Cytotoxicity and Antiviral Activity of the (Co)Polymers and the Polymer Complex of Tb^{3+}

Table 3 presents the values of CC_{50}, the effective drug concentration at which the virus titer is reduced by 50%, IC_{50} and the therapeutic index TI for poly-NaSS and poly-MABA, copolymer NaSS-MABA, and complex NaSS-MABA/Tb^{3+}.

The data presented in Fig. 3 indicate that all homo- and copolymers as well as their complexes with Tb^{3+} have low cytotoxicity and a pronounced antiviral activity against human respiratory syncytial virus. Note that introduction of Tb^{3+} ions leads to a considerable decrease in cytotoxicity. The obtained results give grounds to expect that MABA copolymers with anionic NaSS comonomers and their complexes with Tb^{3+} will be a promising class of precursor polymers for designing new antiviral preparations.

Table 3 The values of CC_{50}, IC_{50} and TI of poly-NaSS and poly-MABA, NaSS-MABA copolymer and NaSS-MABA/Tb^{3+} complex for the cell culture of HEp2 and respiratory syncytial virus, respectively.

(Co)polymer	CC_{50} (μg/mL)	IC_{50} (μg/mL)	TI
Poly-NaSS	160	1.3	125
Poly-MABA	250	2.5	100
NaSS-MABA	260	1.7	150
NaSS-MABA/Tb^{3+}	>1000	<3.7	270

CONCLUSIONS

Copolymers of *p*-methacrylamidobenzoic acid with neutral and anionic comonomers of various natures were synthesized. Their complexation with Tb^{3+} ions in aqueous solutions was studied. The comonomers with bulky substituents demonstrate the maximum luminescence intensity at higher $[COO^-]/[Tb^{3+}]$ ratios, *i.e.*, at higher concentrations of COO^- groups that are not included in the complex. The luminescence intensity values of Tb^{3+} complexes with *N*-vinylamide containing copolymers are more than three times higher than the corresponding values for the MAG-MABA copolymers. It was assumed that in the case of VP-MABA and VMAA-MABA copolymers, water molecules located in the vicinity of amide carbonyl groups (C=O) and strongly bound by hydrogen bonds are partially included in the coordination sphere of Tb^{3+} and thus facilitate enhanced luminescence. The formation of heteroanion complexes of Tb^{3+} with NaSS-MABA copolymer leads to a decrease in the luminescence intensity of the solution. It is established that the formation of luminescent complexes occurs in dilute solutions of $c_{pol} \leq$ 0.02 wt%–0.1 wt%.

The synthesized homo- and copolymers as well as the complex of NaSS-MABA with Tb^{3+} show low (or moderate) cytotoxicity, a pronounced antiviral activity against A2 human respiratory syncytial virus, high values of therapeutic index. This promising class of polymers requires further detailed studies and can be applied in development of antiviral preparations.

Conflict of Interests

The authors declare no interest conflict.

Electronic Supplementary Information

Electronic supplementary information (ESI) is available free of charge in the online version of this article at http://doi.org/10.1007/s10118-024-3081-0.

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