Functional Materials 20, No.1 (2013)

Luminescent temperature sensor based on $[Ru(bpy)_3]^{2+}$ incorporated into chitosan

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Received November 12, 2012

A novel luminescent temperature sensor using ruthenium(II)-tris(2,2′-bipyridil) ($[Ru(bpy)_3]^{2+}$) incorporated into chitosan polymer matrix was developed. The sensor exhibits completely reversible and stable luminescent response (λ_{exc} = 455 nm, λ_{em} = 605 nm), strong temperature sensitivity (−2.0 %/°C), significant luminescence intensity decrease (~10×) in the range of 25–75°C, and stable construction. Ruthenium(II) complex oxygen sensitivity was avoided by use of matrix (chitosan) with low oxygen permeability coefficient. In the absence of oxygen the sensor shows a little higher accuracy and stability of luminescence response than under oxygen conditions. Moreover, this sensor, due to biocompatibility of the used ingredients and the emission in the red VIS-region, which is transparent for tissues, is promising for use in biological applications.

Разработан новый люминесцентный сенсор температуры, использующий рутений (II)-трис(2,2′-бипиридил) ($[Ru(bpy)_3]^{2+}$), введенный в полимерную матрицу хитозана. Сенсор проявляет полностью обратимый и стабильный люминесцентный отклик (λ_{exc} = 455 нм, λ_{em} = 605 нм), сильную температурную чувствительность (−2,0 %/°C), значительный спад интенсивности люминесценции (~10×) в диапазоне 25–75°C, а также стабильное строение. Вклада от кислородной чувствительности комплекса рутения (II) удалось избежать путем использования матрицы (хитозана), имеющей низкую проницаемость для кислорода. В отсутствии кислорода, сенсор показывает несколько большую точность и стабильность люминесцентного отклика по сравнению с условиями в присутствии кислорода. Полученный сенсор, благодаря биосовместимости использованных ингредиентов и испусканию в красной части видимой области, прозрачной для тканей, является перспективным для биологических применений.

1. Introduction

Luminescent sensors use (i) luminophores that change their optical properties due to influence of certain parameter (e.g. oxygen concentration, pressure, pH, temperature) and (ii) matrix (polymer matrix in the most cases) dissolving luminophores and being a solid support of the luminescent sensor; such mixture forms sensor film used in painting [1–4].

Observing current trends in luminescent sensors technology, a creation of luminescent sensor, consisting of luminophores (absorbing and emitting in the VIS-region) incorporated into a biocompatible matrix turns out to be an attractive aim. Hence, three novel luminescent temperature sensors based on biocompatible polymer matrices arise: (i) europium(III)- tris(thenoyltrifluoroacetonato)-(diantipyrylpropylmethane) ($[Eu(tta)_3(dapm)]^{3+}$) incorporated into chitosan [5], (ii) $[Eu(tta)_3(dapm)]^{3+}$ incorporated into cellulose triacetate, [6] and (iii) ruthenium(II)-tris(2,2′-bipyridil) ($[Ru(bpy)_3]^{2+}$) incorporated into chitosan (presented here).

Ruthenium(II) polypyridyl complexes are temperature sensitive luminophores (TSL) used in luminescent sensors technology [7–11]. Such Ru(II) complexes play role as TSL due to their (i) high stability, (ii) high luminescence quantum yield, (iii) high molar absorption coefficient, and (iv) high temperature sensitivity of their luminescence [12]. The used $[Ru(bpy)_3]^{2+}$ complex is classified to mentioned group of polypyridyl complexes.

Ruthenium(II) complexes absorb and emit visible light, [13] facilitating excitation and registration methods of Ru(II) complexes luminescent response, which is oxygen and temperature dependent simultaneously (cross-sensitivity) [14]. Cross-sensitivity signifies that luminescence intensity and lifetime of $[Ru(bpy)_3]^{2+}$ decrease in function of temperature and oxygen concentration increase. In the presented case, incorporating $[Ru(bpy)_3]^{2+}$ into chitosan matrix allowed to avoid oxygen sensitivity of the luminophore. Molecular structures of the used luminophore and matrix are shown in Fig. 1.

Chitosan is non-toxic, biodegradable and biocompatible polysaccharide and chitin derivative from chitin deacylation [5]. Due to its properties, numerous sources in nature, and low cost, chitosan is a commonly used material in many fields, e.g. industry, agriculture, cosmetics, medicine and pharmacy [5, 15].

This polymer is soluble in water at pH < 6.3. In such conditions, chitosan amine groups are protonated and positively charged [16, 17]. Moreover, this type of polycationic structure may be used as ion conductor [18] and can interact with various negatively charged important biological molecules, e.g. antibodies, nucleic acids and enzymes [19–21].

Chitosan exhibits electric conductivity and very good properties for thin films formation, which was used in creation of electrochemiluminescent sensors [22]. Additionally, chitosan shows low oxygen ($7.2{\cdot}10^{-5}$ $cm^3/m{\cdot}day{\cdot}atm$ [23]) and carbon dioxide ($2.5{\cdot}10^{-8}$ $cm{\cdot}cm^3/cm^2{\cdot}s{\cdot}cmHg$ [24]) permeability coefficients. Therefore, the mentioned biocompatible polymer makes a barrier for these gases [25]. Hence, chitosan may be included to polymer matrices suitable for cross-sensitive TSLs.

2. Experimental

Ruthenium(II)-tris(2,2′-bipyridil) complex ($[Ru(bpy)_3]^{2+}$), acetic acid (CH_3COOH) and methanol (CH_3OH) were obtained from

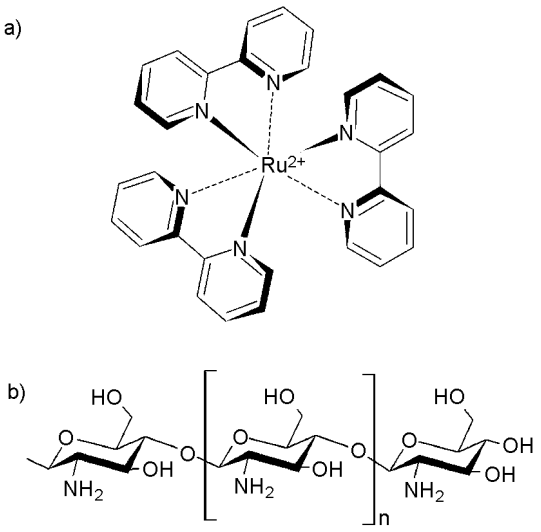

Fig. 1. (a) Ruthenium(II)-tris(2,2′-bipyridil) ($[Ru(bpy)_3]^{2+}$) (various counterions) (luminophore) and (b) chitosan (matrix) molecular structures.

Aldrich (www.sigmaaldrich.com), and chitosan was from Sea Institute of Fishing in Gdynia (Poland) (Morski Instytut Rybacki w Gdyni) with constant deacylation of 73 % and viscosity of 256 mPa·s.

Amount of 0.75 g of chitosan was dissolved in 1 % solution of acetic acid in distilled water. Then the [Ru(bpy)3]2+ solution in methanol was prepared. The weight ratio of 5 % of Ru(II) complex to chitosan mass solution was obtained by mixing appropriate amounts of Ru(II) complex/CH_3OH (luminophore) and chitosan/$CH_3COOH_{(aq)}$ (matrix) solutions.

Prepared luminophore/matrix solution was cast in appropriated amount on smooth glassy or poly(methyl methacrylate) base and was slowly dried at 40°C for 24 h. The obtained films were homogenous and transparent. Film thickness was dependent on amount of casted luminophore/matrix solution. The films of 5–100 μm thickness were obtained by this method.

Absorption measurements were carried out in the spectrophotometer Shimadzu UV-2401PC (Shimadzu, www.shimadzu.com) with spectral slit set of 1 nm.

Luminescent measurements (excitation, emission and kinetics) were carried out after Xe 75 W lamp excitation in the spectrofluorimeter Fluorat-02-Panorama (Lumex, www.lumex.ru) with spectral slit of 15 nm. The settings of the device were as follows: λ_{exc} = 455 nm, λ_{em} = 605 nm, strobe delay = 0.05 μs, strobe duration = 20.00 μs, av-

eraging = 25 of flashes. During luminescence kinetics measurements: strobe duration = 0.20 μs.

Six temperature measurements by the luminescence of the sensor film were carried out: three under oxygen conditions (*a*,*b*,*c*) and three in oxygen absence (*d*,*e*,*f*). Every temperature measurement includes one measurement cycle, consisting "before" phase (heating from 25°C to 75°C) and "after" phase (cooling down from 75°C to 25°C). Luminescence intensity is read for intervals of 10°C simultaneously during these phases. All three measurements under oxygen conditions were carried out on a one sensor sample. Next three measurements (in oxygen absence) were carried out on another sample of the same sensor film. Oxygen absence conditions were obtained by inserting a sensor sample between two quartz glasses, creating triplex.

3. Results and discussion

Excitation and luminescence spectra are presented in Fig. 2. Absorption and excitation spectra are highly similar. Therefore, the luminescence spectrum is independent of used wavelength, and absorption band at 455 nm (the VIS-region) allows us to avoid expensive excitation sources.

Absorption, excitation and luminescence maxima are relatively broad. The excitation spectrum was obtained by the device with comparatively large spectral slit equal to 15 nm. However, the excitation and luminescence spectra studies on this device are validated, because absorption spectrum shape obtained by the device with smaller spectral slit equal to 1 nm is highly similar to the excitation spectrum shape (15 nm).

The luminescence spectrum of $[Ru(bpy)_3]^{2+}$ incorporated into chitosan is similar to luminescence spectrum of $[Ru(bpy)_3]^{2+}$ in water [26]. Presence of chitosan hydroxyl groups in Ru(II) complex coordination outer sphere is probably a reason

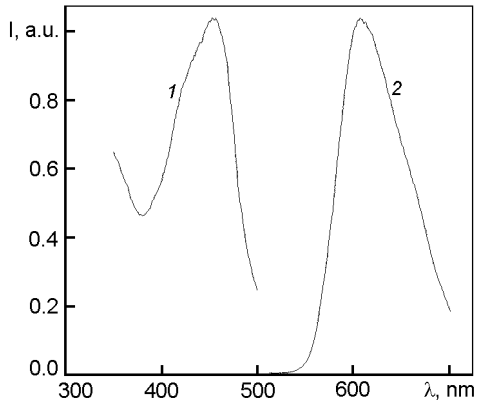

Fig. 2. Excitation and emission spectra of $[Ru(bpy)_3]^{2+}$/chitosan at 25°C. Indications: λ_{exc} — used excitation wavelength (*1*), λ_{em} — used emission wavelength (*2*).

of this similarity; anion counterions influence on $[Ru(bpy)_3]^{2+}$ was not observed. A divergence is present in reported maximum emission wavelengths of Ru(II) complexes solutions of the same kind, e.g. maximum λ_{em} for $[Ru(bpy)_3]^{2+}$ in water varies from 608 nm [27] to 628 nm [28]. Such hesitations arise probably due to use of photomultiplier tubes with different sensitivities or inappropriate correction coefficients for these spectra.

Comparison between obtained luminescence lifetime of $[Ru(bpy)_3]^{2+}$ incorporated into chitosan, and luminescence lifetimes of $[Ru(bpy)_3]^{2+}$ in other mediums is presented in Table. The obtained value of luminescence lifetime of $[Ru(bpy)_3]^{2+}$ incorporated into chitosan is relatively high and arises due to low oxygen permeability coefficient of chitosan. A weak oxygen quenching of luminescence is in chitosan matrix. Hence, a high luminescence quantum yield of $[Ru(bpy)_3]^{2+}$ is expected in chitosan.

The luminescence intensity and lifetime of $[Ru(bpy)_3]^{2+}$ incorporated into chitosan decrease in function of temperature increase. As mentioned above, Ru(II) complex

Table. Luminescence lifetime comparison of $[Ru(bpy)_3]^{2+}$ in different media

Luminophore	Medium	τ [μs]	Ref.
$[Ru(bpy)_3]^{2+}$	water	0.12	[29]
	organic solvents	< 2.2	[29, 30]
	PVPy	1.4	[31]
	PAA	1.6	[31]
	chitosan	2.2	–
	cellulose triacetate	0.9	[30]

oxygen sensitivity was avoided due to use the matrix with low oxygen permeability coefficient. The temperature in range of 25–75°C was measured by luminescence intensity and lifetime of presented sensor. However, the time resolution (1 μs) of used device was too low to treat luminescence lifetime temperature sensing as validated.

In the temperature range of 25–75°C arises an intense $[Ru(bpy)_3]^{2+}$ luminescence decrease (~10×) (Fig. 3). This temperature dependent decrease is more intense than in the case of $[Ru(bpy)_3]^{2+}$ in acetonitryl (~4×) or deaerated water (~ 2×) [32]. Hence, the luminescence of $[Ru(bpy)_3]^{2+}$ incorporated into chitosan exhibits strong temperature sensitivity equal to −2.0 %/°C. Temperature sensitivity of presented sensor is comparable to temperature sensitivities of certain Eu(III) complexes (e.g. −2.2 %/°C [33], −4.4 %/°C [34]), being the most commonly used TSLs. Moreover, the temperature sensitivity of luminescence of $[Ru(bpy)_3]^{2+}$ incorporated into chitosan is stronger than temperature sensitivities of luminescence of other Ru(II) complexes, e.g. temperature sensitivity of ruthenium(II)-tr is (4,7-diphenyl-1,10-phenanthroline) ($[Ru(dpp)_3]^{2+}$) incorporated into silicone layer is equal approximately −0.33 %/°C, but the main aim of this sensor was the oxygen sensing [35].

A small shift to longer wavelengths of luminescence spectrum of Ru(II) complex arises during temperature increase in the range of 25–75°C. The maximum emission wavelength of $[Ru(bpy)_3]^{2+}$ incorporated into chitosan is in the region of 595–610 nm at 25°C, but at 75°C the maximum emission wavelength is in the region of 600–620 nm. The luminescence spectrum is broad and its shift occurs in the range of 15 nm, which overlaps the spectral slit of the used device (15 nm). Hence, temperature measurements were carried out at constant wavelength (605 nm), being the maximum emission wavelength at 25°C.

The aim of carrying out measurements in different conditions (oxygen presence and absence) is observation of oxygen influence on temperature sensing by luminescence of $[Ru(bpy)_3]^{2+}$ incorporated into chitosan. Luminescent properties mentioned above occur in both conditions. Differences in luminescent properties are shown in comparison of temperature measurements under oxygen conditions (*a*,*b*,*c*) and in oxygen absence (*d*,*e*,*f*) presented in Fig. 4.

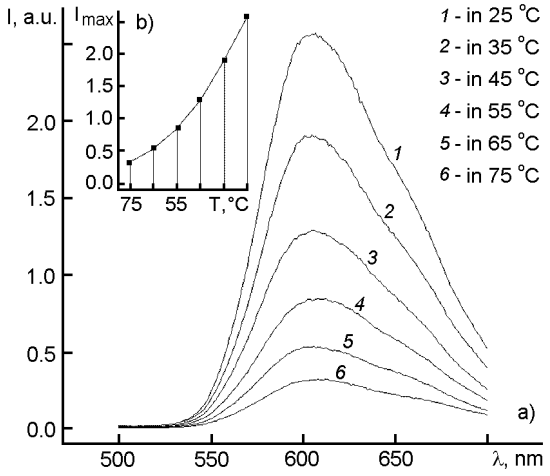

Fig. 3. Temperature measurements by luminescence of the $[Ru(bpy)_3]^{2+}$/chitosan at λ_{exc} = 455 nm (a) .Comparison of maxima of emission intensity of the luminescence spectra at λ_{em} = 605 nm (Imax) at measured temperatures (b).

At the first temperature measurement under oxygen conditions (*a*), the luminescence intensity increases in the "after" phase of the measurement, and enlarges the intensity from the "before" phase. As it is known, water is responsible for luminescence quenching. Hence, the mentioned increase arises due to escape of water traces from the sensor sample during heating ("before" phase). Anyway, the sensor luminescent response for temperature changes in the range of 25–75°C is reversible and stable, which is seen at the next measurements (*b*,*c*). To get the most stable sensor luminescent responses under oxygen conditions, one measurement cycle is required to be carried out.

The temperature measurements by luminescence of $[Ru(bpy)_3]^{2+}$ incorporated into chitosan in oxygen absence (*d*,*e*,*f*) exhibit lower values of luminescence intensity than values obtained under oxygen conditions (*a*,*b*,*c*) (Fig. 4). Anyway, the temperature sensitivity is constant (−2.0 %/°C). The sensor sample is tightly closed in triplex, consisting two quartz glasses (oxygen absence conditions). Therefore, escape of water traces is highly difficult. Hence, sensor luminescence intensity is lower in oxygen absence than under oxygen conditions. This effect is referred to humidity sensitivity of $[Ru(bpy)_3]^{2+}$ incorporated into chitosan. This phenomenon depends on luminescence intensity decrease in function of humidity increase [36]. Anyway, in the tem-

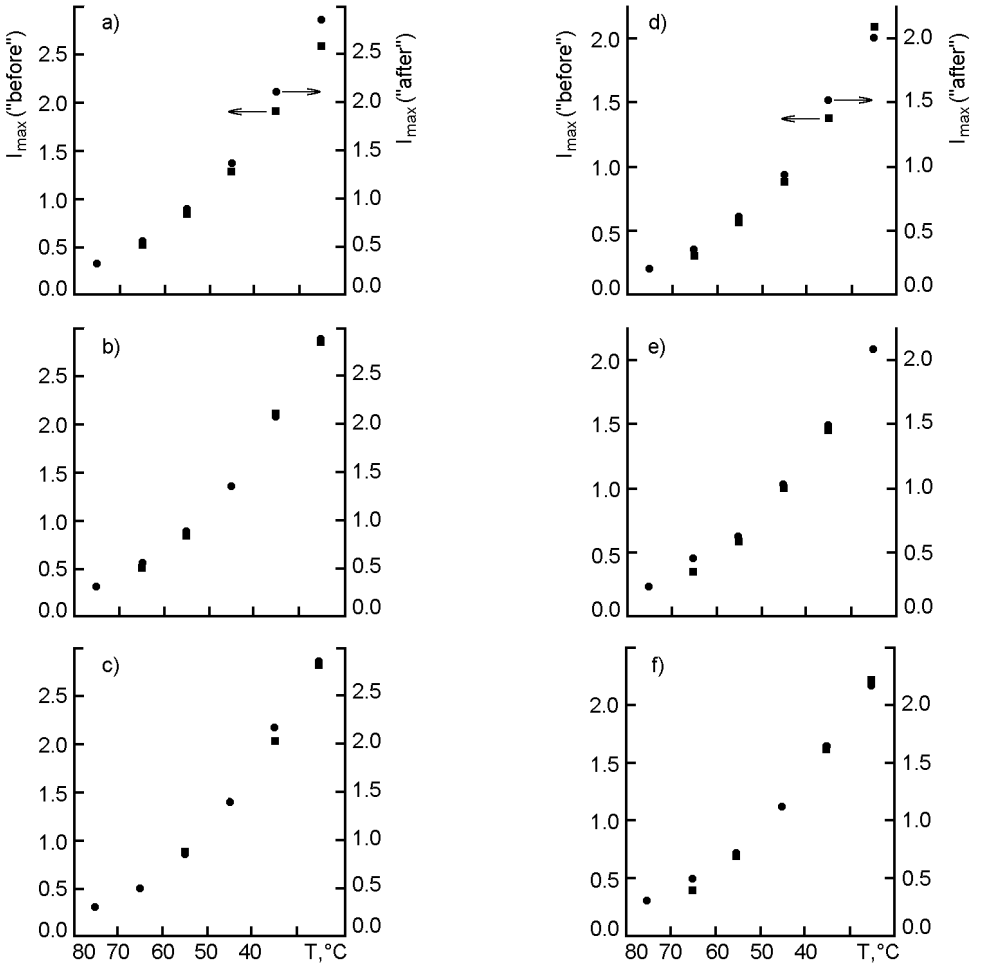

Fig. 4. Temperature measurements by luminescence of $[Ru(bpy)_3]^{2+}$/chitosan at λ_{exc} = 455 nm. Temperature measurements are presented as maxima of emission intensity (I_{max}) for every measured temperature under oxygen conditions (*a*,*b*,*c*) and in oxygen absence (*d*,*e*,*f*). Measurements in oxygen absence were carried out on a new sensor sample.

perature measurements in oxygen absence, the sensor luminescent response is reversible. Additionally, these measurements show a little higher accuracy and stability than measurements under oxygen conditions. It is seen at the first temperature measurement in oxygen absence (*d*), where results between "before" and "after" phases are a little more overlapped than in the case of results obtained under oxygen presence (*a*).

4. Conclusions

The temperature luminescent sensor based on $[Ru(bpy)_3]^{2+}$ incorporated into chitosan exhibits complete reversibility of the stable luminescent response (λ_{exc} = 455 nm, λ_{em} = 605 nm), relatively strong temperature sensitivity (−2.0%/°C), significant luminescence intensity decrease (~10×) in the range of 25–75°C and stable construction easy to reproduce. Oxygen sensitivity of Ru(II) complex was avoided by use the matrix (chitosan) with low oxygen permeability coefficient. In oxygen absence the sensor shows a little higher accuracy and stability of its luminescent response than under oxygen conditions. Moreover, since biocompatibility of used components and sensor red visible emission (transparent for tissues), the sensor is promising for use in biological applications.

Acknowledgements. This work was partly supported by the research project from the Polish Ministry of Science and Higher Education (Grant No.NN313).

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Люмінесцентний температурний сенсор на основі $[Ru(bpy)_3]^{2+}$, інкорпорованого в хітозан

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Розроблено новий люмінесцентний температурний сенсор, що використовує рутеній(II)-трис(2,2′-біпіридил) ($[Ru(bpy)_3]^{2+}$), інкорпорований у хітозанову полімерну матрицю. Сенсор показує повністю оборотній та стабільний температурний відгук (λ_{exc} — 455 нм, λ_{em} — 605 нм), високу температурну чутливість (–2,0 %/°C), значне зниження інтенсивності люмінесценції (~10×) у діапазоні 25–75°C, а також стабільну будову. Чутливість до кисню комплексу рутенію(II) оминули застосуванням матриці (хітозану) з низьким коефіцієнтом проникності кисню. За відсутності кисню, сенсор виявляє дещо вищу точність та стабільність люмінесцентного відгуку у порівнянні з умовами наявності кисню. Більше того, цей сенсор, завдяки біосумісності використаних інгредієнтів та випромінюванню у червоній частині видимої області (прозорої для тканин), є перспективним для біологічних застосувань.