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Iron(III) - selective materials based on catechol-bearing amide for optical sensing

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Abstract: The synthesis and ion-binding properties of new amide **L** derived from 3,4-dihydroxybenzoic acid were described. Due to the presence of catechol unit, the compound interacts selectively with iron(III) in organic solvent (DMSO) what is followed by a color change from pale yellow to green. Incorporation of ligand **L** into polymeric matrices or its encapsulation into surfactant-based spheres enables also the analyte detection in aqueous solutions. The influence of the ligand environment, i.e. organic solvent, polymeric membrane or micelle, on the sensing materials properties was analyzed and compared.

1. Introduction

One of the most currently studied area of supramolecular chemistry is the development of sensitive, selective, and fast-responsive chemical sensors for detection and determination of diverse ions in biological, environmental, and industrial samples.[1] Among chemical sensors vast popularity have attracted optical sensors for which a signal (change of absorbance and/or fluorescence intensity) is generated as a consequence of molecular recognition between a chromogenic (and/or fluorogenic) compound and complementary to it ion.[2] Color change occurring upon host-guest interactions allows for quantitative analysis and even for non-instrumental, "naked-eye" analyte detection. Most of the chromogenic ligands available today, efficiently binding ions in organic solvents, are poorly soluble in aqueous solutions, what may limit their applications in biological and environmental probes. Introduction of hydrophilic groups into the structure of the ligands may increase its solubility in water, but this costs additional work and time. Another, simpler way is incorporation of a ligand into a polymeric sensing phase known as "optode".[3] These sensing materials found applications in metal cations[4] as well as anions[5] detection. The sensing layer usually consists of: an ionophore responsible for the analyte recognition, a lipophilic pH indicator playing the role of an optical reporter, a lipophilic salt that maintains membrane electroneutrality, a polymer and its plasticizer.[6]

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Systems where receptor and reporter units are parts of one compound are also known.[7] Each component of the sensing phase has a certain influence on the resulting sensor properties, this is why many efforts are made to determine optimal composition of the material. An important issue is also a way of ligand incorporation into the matrix. Materials with covalently attached ligands[8] may have a longer lifetime than membranes with physically entrapped receptors[9], however when moieties responsible for molecular recognition are involved in formation of covalent bonds with a matrix, a change and decrease of selectivity can be observed. Miniaturization of optodes to the micro- and nanoscale may result in a decrease of response time due to increased surface to volume ratio and in consequence faster mass transport in comparison to bulk optode. It may also open door for the sensing of small volumes as in intracellular ion imaging applications. Bakker *et al.*[10] described nanospheres containing valinomycin and solvatochromic dyes for *in vitro* analysis of potassium in human plasma. Nanoemulsions with 15-, 16-, 18-, and 20-membered pyrene-based macrocyclic polyethers were used as fluorescent sensors selective for potassium cations over other metal cations up to the milimolar concentration range. Linear response of the nanosensors towards the analyte was found in the range from 10^{-7} to 10^{-5} M in the broad pH range (from 4 to 10).[7a] Huang *et al.*[11] described DSPE-PEG (1,2-disearoyl-sn-glycero-3-phosphoethanolamine-*N*-[metoxy-(polyethylene glycol)]) based nanomicelles for Al^{3+} detection in THF:H_2O (2:3, v/v) solution with a detection limit of 2.3×10^{-8} M. The sensing material was also tested for Al^{3+} imaging in living cells. Xie *et al.*[12] compared a series of nanoscale ion-selective optodes based on Si-containing particles including PEGylated organosilica nanoparticles, PDMS (polydimethylsiloxane) nanospheres, and SiO_2 microspheres with diameters around 50 and 100 nm, and 5 μm respectively. It was shown that PEGylated and SiO_2 spheres bearing the same potassium- or lithium-selective ionophore have better selectivity than PDMS nanospheres.

Having an ionophore of known complexing properties, several sensing materials can be obtained by changing a sensor size or particular components. This prompted us to design and synthesize a receptor for biologically and environmentally important ions and to study the influence of the ligand environment (i.e. solvent, polymeric matrix) on its sensing properties. As a target iron(III) cations were chosen due to their important role in biological systems. Among other, iron provides oxygen-carrying capacity of heme and acts as cofactor in many enzymatic reactions.[13] Its deficiency leads to anemia and liver damage, whereas an increased level of this cation is associated with hemochromatosis and Parkinson's disease.[14] This is why, the obtainment of receptors capable to effective iron recognition can be significant for the control of iron concentration within a human body. Moreover, the receptors may also find

application in removal of a toxic plutonium from an organism, as this radioactive element has affinity to binding sites similar that iron(III).[15] One of the most effective iron cations chelators are naturally occurring siderophores. Many of them possess catechol units that perform this metal-binding function. Enterobactin, which is produced by enteric bacteria, has three catechol moieties that are involved in iron(III) coordination *via* phenolic oxygen atoms with high value of stability constant (K 10^{49}).[16,17] Taking inspiration from nature we obtained a new amide **L** bearing catechol unit that may potentially enable iron(III) recognition (Scheme 1). Combining of chromogenic and binding units within one compound resulted in formation of a chromoionophore and elimination from the sensing phase of an additional compound – pH indicator. The sensing properties of the ligand towards the analyte were studied in DMSO as well as in aqueous solution after amide incorporation into polymeric matrices or encapsulation into micelles. The influence of the environment on the properties of sensing materials was investigated and compared.

2. Experimental

2.1 General

All chemicals of the highest available purity were purchased from commercial sources and used without further purification. The reaction progress was monitored by TLC using aluminum sheets covered with silica gel $60F_{254}$ (Merck). ^{1}H NMR spectra were recorded on Varian apparatus at 200 or 500 MHz. Chemical shifts are reported as δ [ppm] values in relation to TMS. FTIR spectra were registered on a Nicolet iS10 apparatus. UV-Vis titrations in DMSO (POCH) were carried out using an UNICAM UV 300 spectrophotometer. For spectrophotometric measurements 1 cm quartz cuvettes were used. In experiments carried out in aqueous solution, deionized water (conductivity < 1 $\mu S \cdot cm^{-1}$, Hydrolab, POLAND) was used. The pH of aqueous solutions containing nitric acid or sodium hydroxide was determined with the use of CPC-511 pH-meter combined with glass electrode (Elmetron, Poland). The size of the prepared nanospheres was measured by dynamic light scattering method (DLS) with Zetasizer Nano apparatus (Malvern Instruments Ltd.). Molar conductivity of ligand and its complex with iron(III) were measured in DMSO (c ~ 10^{-3} M) at room temperature using a CPC-511 conductivity meter combined with an ECF-1 conductivity sensor (Elmetron, Poland).

2.2 Synthesis

Details about synthesis and spectral characterization (Fig. ESI1-3) of amide **L** are included in Electronic Supplementary Information (ESI).

2.3 Preparation of sensing materials

Optode 1: the membrane components: amide **L** (9.6 mg, 1.7% w/w), cellulose triacetate (42.4% w/w), and triethylene glycol (55.9% w/w) were dissolved in chloroform (6 mL). The mixture was magnetically stirred for several hours at room temperature till a homogenous solution was obtained and poured onto a clean Petri dish for the solvent evaporation. A "blank" optode (without the ligand) was prepared similarly.

Optode 2: the membrane cocktail was prepared by weighing: potassium tetrakis(4-chlorophenyl)borate (0.7% w/w), amide **L** (1.7% w/w), cellulose triacetate (43.4% w/w), and 2-nitrophenyl octyl ether (NPOE, 54.2% w/w), and then dissolving all components in chloroform (6 mL). The procedure of the optode preparation was similar as described in the case of optode 1.

Optode 3: the membrane cocktail was prepared by weighing: potassium tetrakis(4-chlorophenyl)borate (0.4% w/w), amide **L** (1.5 mg, 0.8% w/w), polyvinyl chloride (PVC 32.1% w/w) and NPOE (66.7% w/w). The components were dissolved in 1 mL of THF and deposited (90 μL) onto glass plates (9×49 mm). After solvent evaporation (24 h) the resulting sensing membrane were used in spectrophotometric studies. "Blank" optode was also prepared.

Nanospheres 4: potassium tetrakis(4-chlorophenyl)borate (4.1% w/w), amide **L** (1.65 mg, 11.3% w/w), Pluronic F-127 (29.8% w/w), and NPOE (54.8% w/w) were dissolved in acetone (1 mL). Then 0.1 mL of the organic mixture was transferred into deionized water (4 mL) and placed in an ultrasound bath for 10 minutes. Subsequently, the mixture was injected on a vortex with vortexing speed of 1300 rpm. To remove solvent argon was blown through the surface of the resulting emulsion.

Nanospheres 5: the material was prepared as described above for nanosphere **4**, however here bis(2-ethylhexyl) sebacate (DOS) instead of NPOE was used.

Test strips: amide solution (3×10^{-3} M) in acetone was poured into a chromatographic chamber, into which a strip of glass filter was placed, similarly to the procedure with the TLC plates. After adsorption of amide solution on the strip, it was taken out and left for the solvent evaporation.

2.4 Ligand-ion complexation studies

In UV-Vis experiments nitrate salts were used. Iron(II) was used as $FeSO_4\times H_2O$. The stoichiometry of **L**-iron(III) complex was determined by Job method. Readout of absorbance was taken as a signal after reaching an equilibrium state (constant absorbance), which was about 1 hour for single measurement. Experiments for polymeric optodes and for nanospheres were carried out in aqueous solutions at pH 2.9. In the competition studies the absorbance of the

optode in the presence of iron(III) nitrate (c = 5.47×10^{-4} M) at pH 2.9 was measured before (A_0) and after (A) the addition of interfering metal cations of 10-fold higher concentration than the concentration of the analyte. The influence of metal cations on membrane response towards iron(III) is expressed as a relative response according to the equation: $\%RR = [(A-A_0)/A_0]\times100\%$. The detection limit of iron(III) by proposed sensors was estimated following the equation: $DL = 3\sigma/k$, where σ is standard deviation of blank membrane and k is a slope of linear function $A = f(c_{Fe})$.

2.4.1 Complex preparation for spectroscopic and spectrometric analysis

Ligand **L** (2.7 mg, 0.01 mmol) and iron(III) nitrate (4.0 mg, 0.01 mmol) were dissolved in acetone : methanol mixture (1 : 1, v/v). The solution was magnetically stirred at 50°C for 3 hours. After solvents evaporation FTIR spectra were recorded with transmission technique (KBr pellet). For comparison, spectra of "free" ligand and salt were also registered.

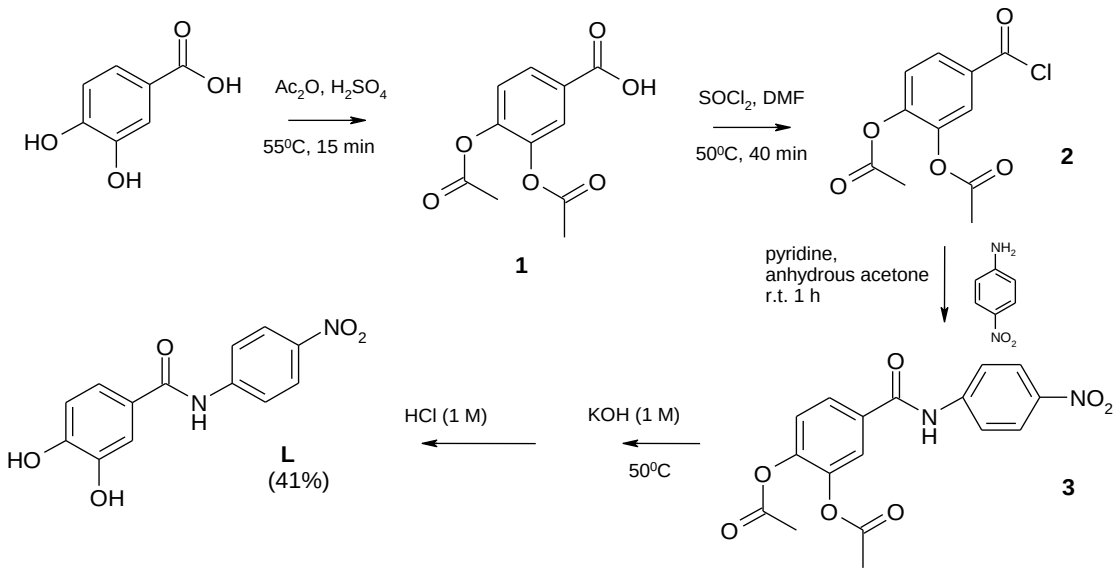
2.4.2 Detection of iron(III) in tap water and synthetic mixtures

To demonstrate the nanosensors ability to detect iron(III) in samples with high ionic background, synthetic solutions containing interfering salt at high concentration (~ 10^{-2} M) were prepared and the spectrophotometric response of the sensor towards the analyte in the presence of these solutions was measured. Iron(III) was also detected in the tap water samples at pH 2.9 (pH fixed by addition of nitric acid and controlled by pH-meter). First, two calibration curves were obtained for iron(III) in a concentration range of $5.56\times10^{-6} – 1.37\times10^{-4}$ M. A known amount of iron(III) nitrate was added to 3 samples of tap water and the final concentration was determined spectrophotometrically with the use of nanospheres with NPOE and, for comparison, ammonium thiocyanate solution. Two calibration curves for the thiocyanate-based method were also obtained. The concentration of iron(III) in each sample was determined using calibration curves based on the average of two calibration curves.

3. Results and discussion

3.1 Synthesis

Amide **L** was obtained in multistep procedure as shown in Scheme 1. Acetylated acid chloride was transformed into amide in reaction with *p*-nitroaniline according to procedure adapted from literature.[18] After deprotection pure amide was obtained with 41% of yield. To the best of the authors knowledge the compound has not been described in the literature so far (according to Chemical Abstracts).



Scheme 1. Synthetic route for amide **L** preparation.

3.2 Ligand-ion complexation studies

The presence of catechol moiety in the amide **L** structure may potentially enable recognition of iron(III) ions. To prove this statement spectrophotometric experiments were carried out. In UV-Vis spectrum of the ligand solution in highly polar DMSO in the presence of iron(III) ions a new band around 700 nm was observed, what was accompanied by a change of color from pale yellow to green (Figure 1). The appearance of the new band might be a consequence of ligand to metal charge transfer, characteristic for catechol – iron(III) interactions.[16, 19] It may suggest that ligand **L** coordinates iron(III) *via* oxygen atoms of hydroxyl groups.

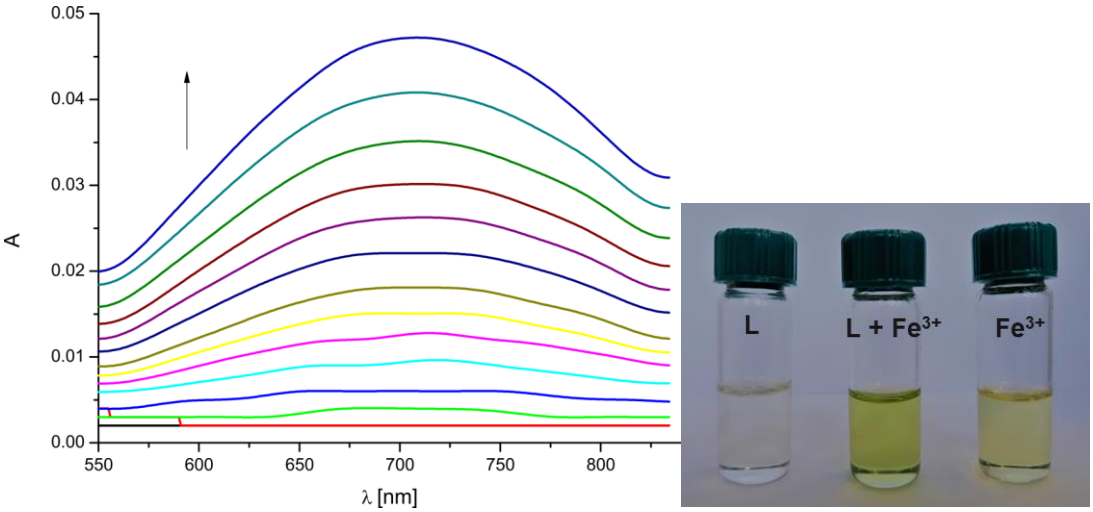


Fig. 1 Changes in UV-Vis spectrum of ligand **L** solution ($c = 5.08\times10^{-5}$ M) in DMSO in the presence of iron(III) nitrate ($c = 0\text{-}3.77\times10^{-5}$ M); photo: from left: "free" ligand **L** ($c = 5.08\times10^{-5}$ M), ligand **L** in the presence of iron(III) nitrate ($c = 3.77\times10^{-5}$ M), iron(III) nitrate ($c = 3.77\times10^{-5}$ M) in DMSO.

Determination of reliable strength of interactions between amide **L** and iron(III) in DMSO was not possible under measurement conditions due to kinetic reasons. Even after one hour after addition of iron(III) nitrate (equimolar amount to the ligand), the equilibrium was not reached (Fig. ESI4). Under these conditions, amide **L** seems not to be an interesting candidate for the colorimetric detection of iron(III) cations. Despite this, the Job method was used to estimate probable binding mode. Due to obtained results (Fig. ESI5) under measurement conditions complexes of 1:1 stoichiometry are formed with binding constant logK~5, however taking into account equilibrium process, this should be treated as an estimated value. A similar pattern of spectral changes is observed in the presence of iron(II) (Fig. ESI6). In this case also, upon addition of the salt, absorption spectra were changing within the timescale of the experiment.

The mechanism of **L**-iron(III) interactions in a solid state were studied with the use of infrared spectroscopy. In the spectrum of "free" ligand **L** (Fig. 2, ESI7) two, well separated bands at 3527 and 3386 cm^{-1} are observed which can be ascribed to ν(O-H) vibrations of two catechol hydroxyl groups: "free" and engaged in formation of intramolecular hydrogen bonds.[20] In the spectrum of **L**-Fe^{3+} complex these bands are shifted to higher wavenumbers ($\Delta\nu$: 13 and 9 cm^{-1} respectively) and the band of the "free" hydroxyl group decreased its intensity in comparison to the spectrum of "free" amide. Spectral changes are also observed in the region of bending vibration of O-H groups in the plane (~1400-1330 cm^{-1}) what may suggest (similarly as in UV-Vis spectrum) that iron(III) cation is coordinated *via* hydroxyl groups of catechol moiety. Cohen *el al.*[21] described X-Ray confirmed coordination of iron(III) by phenolic oxygen atom and amide oxygen atom (salicylate binding mode) of hexadentate ligands serving as enterobactin models. In FTIR spectra of the studied there compounds, the band of carbonyl stretching vibrations shifts to lower frequencies upon iron(III) complexation, what is in opposition to observed by us results. The I amide band of ligand **L** is shifted to higher frequencies in the presence of iron(III) ($\Delta\nu$ = 10 cm^{-1}), what rather excludes participation of carbonyl oxygen atom in complexation process. Due to the position of carbonyl and hydroxyl groups in aromatic ring of the compound **L**, the salicylate binding mode in this case is impossible. Moreover, the band of N-H stretching of amide group seems to be unaffected by the presence of iron(III). A support for above hypothesis brings also analysis of ^{1}H NMR spectra of the ligand and its complex with iron(III) (Fig. ESI8). Although ferromagnetic properties of iron, ^{1}H NMR spectroscopy to some extend can be helpful in studies of the mechanism of iron-catechol derivatives interactions.[22] A broad signal seen in the free ligand spectrum at c.a. 9.5 ppm, that can be assigned to protons of OH groups, in the complex spectrum separates into two signals, what may suggest the involvement of oxygen atoms in iron(III) complexation, however this participation of the both

atoms is not equal. The positions of the rest protons (i.e. aromatic and of the amide group) are not changed in the presence of iron(III), (but they are all widen). Similarly, as observed in infrared spectra, there is no evidence of amide group involvement in the guest recognition.

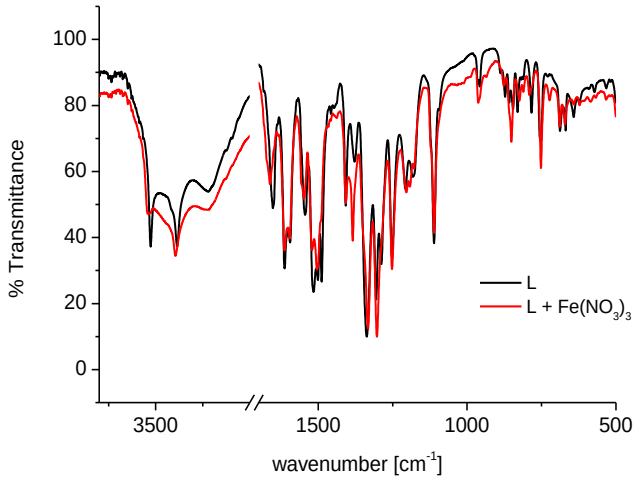


Fig. 2 Comparison of FTIR (KBr pellet) spectra of free amide **L** and **L**-Fe^{3+}complex (ligand to salt ratio 1:1).

As determination of iron in the organic solvent was not possible due to kinetic reasons, in next step it was tested if the change of ligand environment by its incorporation into a polymeric matrix would influence on complexation equilibrium. Two polymers were chosen: cellulose triacetate (optode **1** and **2**) and lipophilic PVC (optode **3**). The optode **2** containing the lipophilic salt and NPOE clouded up when it came into the contact with aqueous solution, this is why it could not be tested by UV-Vis spectrophotometry. Subsequent experiments were carried out only for optodes **1** and **3**. In a pH range of 3-10 the absorbance of both optodes is quite stable, however lesser influence of pH is seen in the case of the cellulose triacetate-based membrane **1** ($\Delta A_1 = \pm 0.003$, Fig. ESI9). Because of a tendency for iron(III) ions to hydrolyze,[23] all experiments were carried out at a fixed pH of around 3. Both optodes immersed into a solution of nitric acid ($c = 10^{-3}$ M) for 1 hour showed stability of the signal (standard deviation of absorbance ~ 8×10^{-4}). One of the important feature of the sensor is its response time. The time-dependent characteristics of membranes **1** and **3** immersed into 1 mM iron(III) nitrate at pH 3 is shown in Fig. 3a. For optode **3** based on PVC the time after which the sensor achieved 95% of stable response t_{95} is much shorter (around 30 seconds) than for the cellulose triacetate-based membrane **1** (5 minutes). Regardless the type of applied polymer, the kinetics of **L**-Fe^{3+} interactions in the case of both optodes is more favorable than for the ligand dissolved in DMSO. Similarly like in the organic solvent, the molecular recognition in both optodes is

accompanied by the change of color from yellow to green. In UV-Vis spectra a new band at around 700 nm is seen (as an example in Fig. 3b the spectrum of optode **1** is shown).

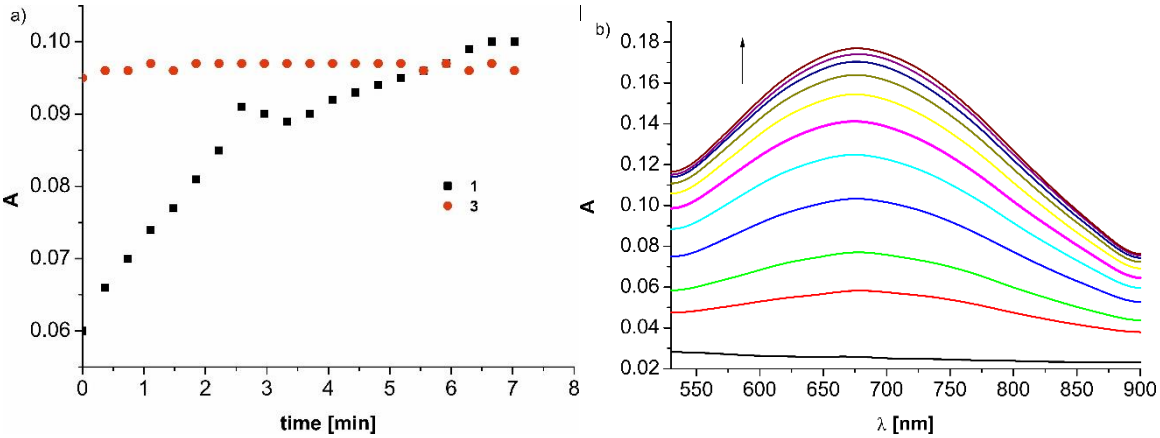


Fig. 3 a) Changes in absorbance of optode **1** (cellulose triacetate) or **3** (PVC) with amide **L** in the presence of iron(III) nitrate (c = 1 mM) *vs.* time at pH 2.9 (λ = 700 nm); b) Changes in absorption spectrum of membrane **1** with amide **L** in the presence of iron(III) nitrate (c = 0-1.35×10^{-3} M) in nitric acid solution (pH 2.9).

The linear response range for the optode **1** (0-8.16×10^{-4} M, R^2 = 0.986) is wider than for the PVC-based membrane **3** (0-8.95×10^{-5} M, R^2 = 0.979), what can be a consequence of higher concentration of amide **L** in sensor **1** (Fig. ESI10). The detection limit, determined as a concentration of the analyte generating a signal equal to three times of standard deviation of blank membrane[24] is 1.58×10^{-5} for optode **1** and 1.01×10^{-5} M for optode **3** respectively. In order to determine ligand selectivity in the membrane, the influence of several metal cations on the sensors response was tested (Fig. 4).

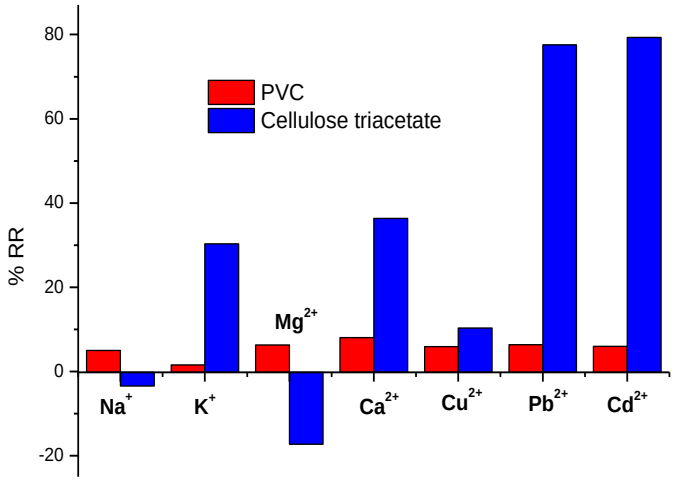


Fig. 4 The influence of metal cations (10-fold molar excess in relation to the analyte) on the spectrophotometric response of optode **1** (based on cellulose triacetate) or **3** (based on PVC) with ligand **L** towards iron(III) nitrate ($c = 5.47\times10^{-4}$ M) in nitric acid solution (pH 2.9).

The selectivity of the optode based on cellulose triacetate is quite low. Heavy metal cations: lead(II) and cadmium (used in 10-fold molar excess in relation to the concentration of the analyte) have a significant influence on the membrane **1** response towards iron(III) cations. Among tested metal cations only sodium cations have a negligible impact on iron(III) recognition (%RR within the measurement error ± 5%[25]). The change of the ligand environment, i.e. change of polymeric matrix and plasticizer has a significant influence on the optode selectivity – for the PVC-based membrane determined values of %RR are smaller than for cellulose triacetate optode. In this case the most significant influence on membrane **3** response towards iron(III) cations have calcium ions (%RR = 8%). Binding of iron(III) by ligand **L** in membranes is reversible. Regeneration of the optodes can be achieved by their immersion into 0.1 M EDTA solution.

As polyvinyl chloride is not an ideal candidate for potential biological application, a polymer-free approach was also proposed, where ligand was encapsulated into micelles formed by non-ionic surfactant Pluronic F-127. For sensing material preparation two kinds of plasticizers were used: NPOE and DOS. According to DSL experiments nanospheres containing NPOE **4** have size of 308.02±0.67 nm, whereas micelles loaded with aliphatic plasticizer **5** were much smaller: 160.55±0.07 nm. As for the polymeric membranes, all measurements were carried out at pH 2.9. Time after which the sensing material bearing NPOE shows stable response to the analyte presence (t_{95}) is short – 24 seconds. In the case of the second applied plasticizer longer time required for signal stabilization is needed – 132 s. Similarly, as for tested bulk optodes and experiments in DMSO, a color change of sensing material in the presence of the analyte was seen (Fig.5), however spectral changes observed in the UV-Vis spectrum were less significant (Fig. ESI10a). The response of NPOE-containing spheres is proportional to iron(III) nitrate concentration in the range 0-1.17×10^{-4} M ($R^2 = 0.997$) (Fig. ESI11b). Similar results were obtained for nanospheres **5** having DOS as a plasticizer (linear response range: 0-1.81×10^{-4} M, $R^2 = 0.996$). However, in this case a little bit higher detection limit of iron(III) was determined in comparison with sensor **4** (4.72×10^{-6} M and 3.25×10^{-6} M respectively). It is worth mentioning that both these values are lower than the one recommended by World Health Organization concerning iron(III) detection in drinking water (i.e. 5.36×10^{-6} M).[26] Encapsulation of amide **L** into micelles containing NPOE improved sensor selectivity in comparison with the presented

above bulk optodes (Fig. 5). None of tested metal cations (used in 10-fold molar excess) has significant influence on iron(III) complexation (% RR < 5%). Changing the plasticizer to aliphatic DOS decreases the selectivity of nanospheres towards the analyte. The most interfering ions are divalent metal cations: copper and lead. The values of %RR for most of tested metal cations are higher than for NPOE-PVC system **3**. In the case of ion-selective electrodes better selectivity towards multivalent metal cations is often observed for an NPOE-PVC membrane than for a DOS-PVC, which in turn has sometimes better selectivity towards monovalent cations.[27] The reason of this can be the difference in the plasticizers' lipophilicity (NPOE: logP 5.9, DOS: logP 10.1[28]). Perhaps in the case of the colorimetric sensors analyzed here, differences in selectivity of the tested materials are connected with system lipophilicity and the mobility of the ionophore in the proposed materials. According to the obtained results the best system for iron(III) recognition in applied conditions seems to be nanospheres **4** with NPOE. For "blank" nanospheres (without ligand) no significant changes were observed in UV-Vis spectrum in the presence of tested metal cations (Fig. ESI12), what proves that the presence of amide **L** is crucial for iron(III) sensing.

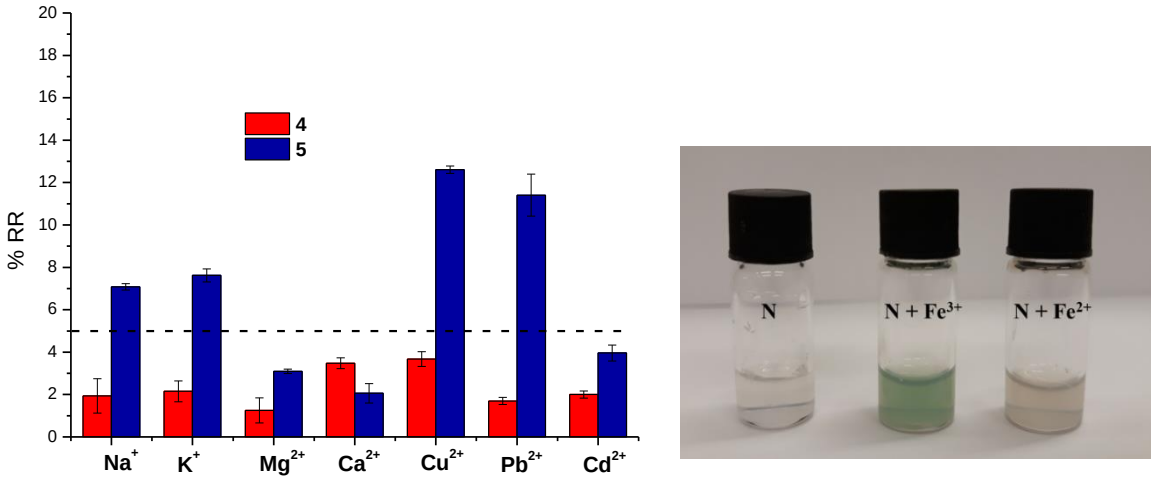


Fig. 5 Left: The influence of metal cations (10-fold molar excess in relation to the analyte) on spectrophotometric response of nanospheres **4** (containing NPOE) or **5** (containing DOS) with ligand **L** towards iron(III) nitrate ($c = 5.44\times10^{-5}$ M) in nitric acid solution (pH 2.9); right: nanospheres **4** (N) in the presence of iron(III) nitrate ($c = 5.98\times10^{-5}$ M) and iron(II) sulfate(VI) ($c = 5.53\times10^{-5}$ M) at pH 2.9.

Contrary to the changes observed in DMSO, in the spectrum registered for nanospheres **4** no changes were observed in the presence of iron(II) sulfate(VI). Addition of iron(II) salt to nanoemulsion did not cause significant change of the sensor color (addition of iron(III) salt in the concentration similar to concentration of iron(II) salt caused naked-eye noticeable change

of color) as it can be seen in Fig. 5. It means that under the measurement condition, the proposed sensing material discriminates between iron(III) and iron(II).

Sensitivity of NPOE-bearing nanospheres, defined as concentration of the analyte causing 1% of signal intensity (absorbance) changes,[29] was determined as $7.33\pm0.56\times10^{-7}$ M. In the samples with high ionic background concentration (in the presence of high molar excess of one of the selected interfering cations $c\sim10^{-2}$ M) sensitivity towards iron(III) did not change significantly, however in a more complex mixture, containing all tested, biologically important metal cations: sodium, potassium, magnesium, and calcium, it decreases twofold (Fig. 6).

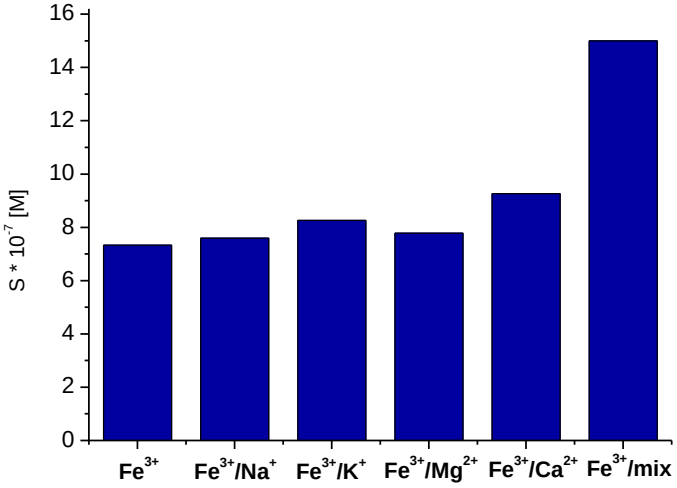


Fig. 6 Changes of sensitivity (S) towards iron(III) nitrate in the presence of metal perchlorate ($c\sim10^{-2}$ M) and mixture of all salts at pH 2.9.

In order to check possibility for the potential application of the proposed sensing material in real sample analysis, subsequent experiments were carried out in tap water. The results are summarized in Table 1. Iron(III) concentration in tap water determined by nanospheres **4** at pH 2.9 was 13.51±0.93 μM. For the sake of comparison, similar experiments were carried out using ammonium thiocyanate as a colorimetric reagent. Comparable results were obtained i.e. 14.23±0.93 μM, showing that nanospheres **4** can be considered for practical applications.

Tab. 1 Iron(III) concentration determined by nanospheres **4** and NH_4SCN solution in tap water samples at pH 3.

	Fe^{3+} added [μM]	found	Recovery [%]	RSD (n=3) [%]
nanospheres **4**	0	0	-	-
	13.77	13.51±0.93	98.1	6.8

SCN^-	0	0	-	-
	13.77	14.23±0.93	103.3	6.5

For fast, qualitative analysis, test strips with adsorbed amide **L** were prepared. As described above, one of the most interfering ions for iron(III) recognition are lead(II) cations (Fig. 4 and 5). This is why, response of the test strips towards the analyte was tested in the presence of lead(II) in concentration 100-fold higher than iron(III) salt. In the presence of lead(II) nitrate (0.1 M) at pH 2.9 the test strip with **L** changed its color from slightly yellowish to more intense yellow (Fig. 7). In the presence of iron(III) nitrate green color was observed in the concentration range 10^{-2} – 10^{-4} M. The "naked-eye" detection limit of the analyte is 10^{-4} M. After contact of the test strip with a solution containing iron(III) and lead(II) nitrate (c: 10^{-3} and 10^{-1} M respectively) a color characteristic for the analyte recognition appeared. This means that iron(III) can be detected by this simple analytical tool in the presence of interfering lead(II) cations.

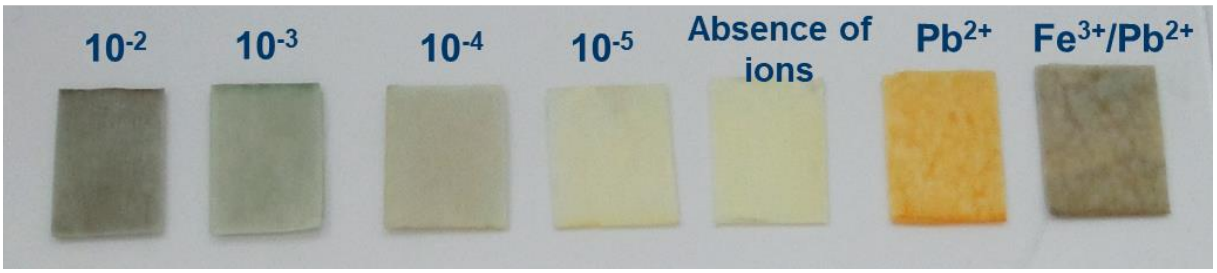


Fig. 7 Changes of color of amide **L** (c = 3×10^{-3} M) adsorbed on the test strip in the presence of different molar concentrations of iron(III) nitrate (from left) and in the mixture of lead(II) and iron(III) nitrate (c: 10^{-1} and 10^{-3} M respectively) at pH 2.9 (in nitric acid solution).

4. Conclusions

Described here, for the first time, amide **L** being derivative of 3,4-dihydoxybenzoic acid reveals affinity to iron(III) in organic solvent (DMSO) and in aqueous solutions. On the basis of spectroscopic experiments it was concluded, that in the complex formation hydroxyl oxygen atoms of **L** are involved. As ligand-ion interactions are followed by "naked-eye" seen color change (from yellow to green), several sensing materials with amide **L** were prepared. Determination of the iron(III) in aqueous solution at pH 2.9 was possible after ligand incorporation into polymeric matrices (PVC and cellulose triacetate) or after encapsulation into Pluronic F-127 based micelles. In DMSO the complexation process was too slow for sensing applications. Sensors **3** and **4** plasticized with NPOE show shorter response time (c.a. 30 s) than

cellulose triacetate bearing material **1** and nanoemulsion **5**. According to the obtained results the most promising sensing material seems to be NPOE-bearing nanospheres **4** that enable iron(III) determination with detection limit lower that the one recommended by WHO in drinking water (3.25×10^{-6} M *vs.* 5.36×10^{-6} M). Sensor **4** shows also the best selectivity towards iron(III) among presented sensing materials. None of tested metal cations has a significant influence on the sensor response towards iron(III) (% RR < 5%).

Conflicts of interest

There are no conflicts to declare.

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