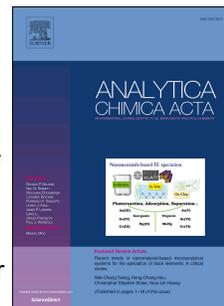


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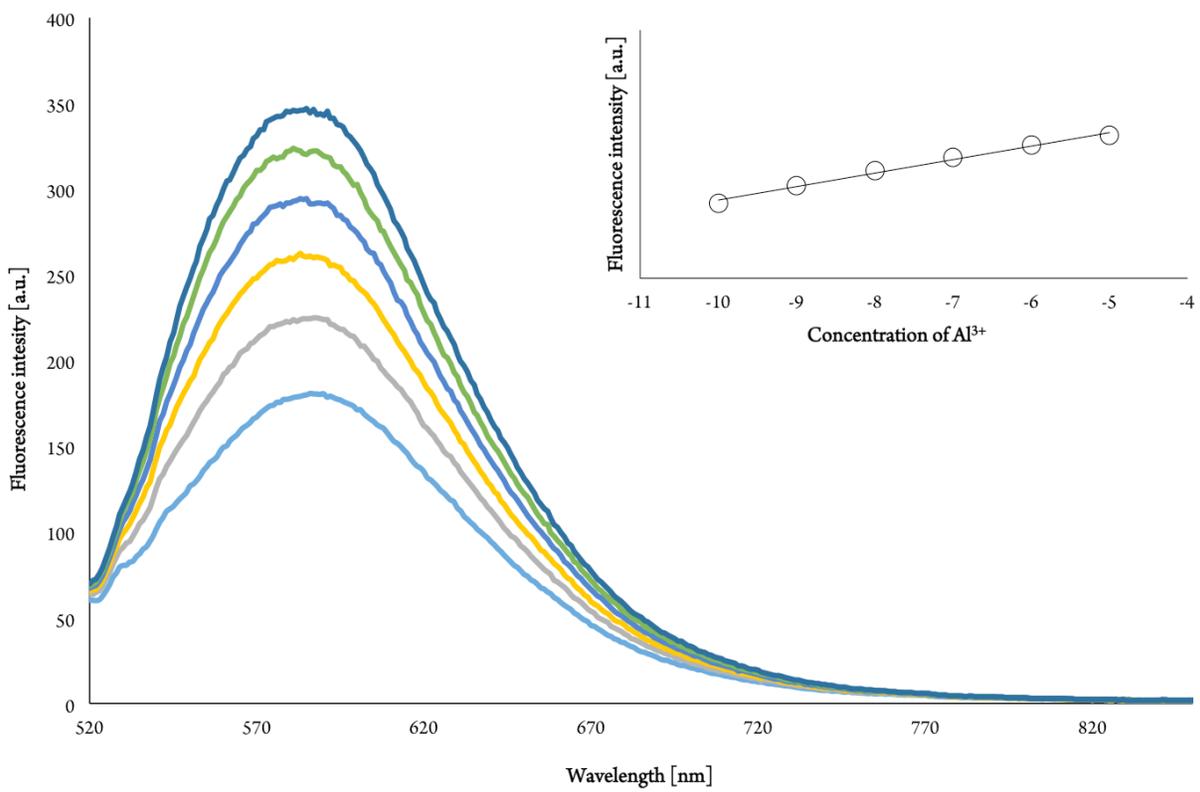
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Self-plasticized, lumogallion-based fluorescent optical sensor for the determination of aluminium (III) with ultra-low detection limits

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ABSTRACT: Aluminium-selective ion optical sensor based on covalently attached lumogallion methacrylate was synthesized and investigated in this study. Lumogallion based derivatives were copolymerized with various methacrylate monomers via a simple one step free radical polymerization to produce a "self-plasticized" copolymer. The mechanical properties of the copolymer were suitable for the fabrication of plasticizer-free optical membranes. We demonstrate that covalently attached lumogallion moieties provide adequate functionality to the optical film thus achieving a very simple, one component sensing membrane. Fluorescence experiments demonstrated excellent sensitivity towards aluminium (III) ions with the detection limits found at 4.8×10^{-12} M. Furthermore, proposed sensor displays high selectivity towards aluminium over a number of biologically relevant cations. Moreover, the synthesized copolymer was used for the fabrication of nanoparticles that exhibit strong fluorescence upon contact with aluminium (III) ions. It is anticipated that lumogallion-based copolymers may form the basis for the development of highly sensitive and robust aluminium selective sensors capable of *in situ* measurements.

In recent years, several staining methodologies have been suggested for the detection of metal ions in environmental and clinical samples.¹⁻⁵ However, the vast majority of these techniques are not suitable for real-time or *in situ* measurements.^{6,7}

Today, aluminium is used extensively in almost countless applications. For instance, in chemical analysis its presence can successfully point to whether or not the corrosion processes of aluminium-based alloys are taking place.⁸ However, under acidic conditions, Al^{3+} can be released to the environment and thus having adverse effects on agriculture and marine ecosystems⁹⁻¹²

Over the past decade, there has been growing number of studies demonstrating the neurotoxic effect of aluminium on living organisms. Aluminium toxicity has already been identified as potential cause of anemia, myopathy and bone and joints disorders.¹³⁻¹⁵ More recently, high concentration of aluminium found in the brain of individuals with early onset of Alzheimer disease was recognized as possible contributor to disease aetiology.¹⁶⁻¹⁸ This concept was even further reinforced by demonstrating that aluminium is directly involved in the formation of amyloid fibrils in the brain tissue therefore increasing the risk of developing Alzheimer disease.^{7,19-21} This creates a demand for cheap and practical analytical methods capa-

ble of measuring free aluminium in environmental and clinical samples.

Fluorescent techniques, which rely on the complex formation between targeted ion and the fluorophore, have been receiving considerable current attention as they often exhibit superb sensitivity, selectivity, rapidity and portability.²² Morin is a well-studied type of a fluorophore that is predominantly used for the detection of aluminium in solutions with recent attempts focused on chemical modifications for sensing purposes.²³⁻²⁶ However, resulting optodes often did not yield required sensitivity and selectivity towards Al^{3+} as such dye readily binds other metal ions that are commonly encountered in physiological samples such as magnesium or calcium.^{10,27} Furthermore, small binding affinity for Al^{3+} at circumneutral pH makes it unsuitable for real-time clinical measurements.²⁸ Other fluorescent probes based on the presence of coumarin,²⁹ 5-[(2-Hydroxynaphthalen-1-yl)methylene]amino]pyrimidine-2,4(1H,3H)-dione,³⁰ thiophene-2-carboxylic acid hydrazide,³¹ piperidine carboxylic acid dithiocarbamate³² amongst many others have been reported. However, new analytical methodology is required if we are to develop very sensitive and robust sensors for aluminium determination.

Fluorometric detection based on the presence of lumogallion has gained a widespread acceptance due to

its low detection limits and good selectivity to Al^{3+} .³³ It has been routinely employed in separation science as a pre-column derivatization dye³⁴ or in plant chemistry³⁵ as aluminium tracing agent. Moreover, recent study carried out by Mold et al report the first use of lumogallion for the unequivocal determination of aluminium adjuvants in monocytic T helper 1 (THP-1) cell line.³⁶ Lumogallion is an azo dye known to form 1:1 stoichiometric complexes with the soluble Al^{3+} fraction and if irradiated with the wavelength of 500 nm it produces fluorescence with the emission signal found at 590 nm (orange fluorescence).^{37,38} Despite its excellent properties as a staining agent in solutions, there has been only limited number of studies involving the use of lumogallion for the development of optical probes.³⁹ This could be attributed to the poor solubility of the dye in various organic solvents required for the preparation of optodes as well as to its predominantly hydrophilic character that would cause the diffusion of lumogallion from the more hydrophobic polymeric membrane into the aqueous solution. These significantly shorten the lifetime of resulting probes and limit their practical application to the destructive analysis in which sample contamination is not an issue.⁴⁰

Over several years, large advancements in analytical and organic chemistry offered a vast number of approaches to minimize the extent to which the active components diffuse out of the sensing layer and consequently to improve the overall performance of the polymer based chemical sensors.⁴¹ The most logical and intuitive approach involved chemical modifications of sensing components such as addition of long alkyl chains in order to improve the overall hydrophobicity of studied molecules.⁴² However, changes in the solubility of the functionalized species may result in their macroscopic phase separation from the polymeric matrix. One, very promising approach to enhance the overall stability of homogenous sensing layer is to attach all sensing components to the polymer backbone.⁴³ This may significantly reduce or even fully eliminate leaching of sensing components out of the polymeric optical film. Permanent immobilization of fluorescent moieties also prevents aggregates formation by reducing the intermolecular interactions and can improve the intensity of emission signal by eliminating collisions of a fluorophore with the solution molecules.⁴⁴

The range of monomers carrying fluorescent properties that have already been reported in the literature is very extensive and includes all commonly employed classes of dyes.⁴⁵ This includes polycyclic aromatic hydrocarbons, azo dyes, coumarins, fluorescein, cyanine dyes and rhodamines where all of the derivatized dyes are fully compatible with commonly applied polymerization techniques such free radical polymerization, atom transfer radical polymerization,⁴⁶ reversible addition-fragmentation chain transfer (RAFT) polymerization,⁴⁷ living anionic and cationic polymerization and ring opening metathesis polymerization.⁴⁸ In recent years, several optical sensors based on plasticizer-free membrane have been investigated and the response behavior of such films was evaluated in terms of their selectivity, sensitivity and

robustness towards various physiologically relevant cations and anions. Methacrylic-acrylic random copolymers synthesized via free radical polymerization are particularly attractive candidates as their physico-chemical properties can be easily modified by choosing either different combinations of monomers or polymerization routes.⁴⁹ It has been previously reported that ion selective electrodes based on the methacrylic-acrylic polymer backbone are suitable for trace analysis of ionic species.^{50,51}

This paper describes a new polymerizable lumogallion based derivative that has been covalently attached to a hydrophobic self-plasticized polymer backbone for practical applications in ion optical sensors. In this study, we also report on the first single element copolymer for the detection of aluminium (III) ions with ultra-low sensitivity (pM concentrations) and superior selectivity over various biologically relevant ions demonstrating its great potential as a sensor for environmental or *in vivo* and *in vitro* measurements.

EXPERIMENTAL

Materials

Methacryloyl chloride, 2,2'-azobis(2-methylpropionitrile) (AIBN), sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), poly(ethylene glycol) methacrylate (PEGMA), lauryl methacrylate (LMA), polyethylene glycol methyl ether methacrylate (PEGMEM), methyl methacrylate (MMA), Brij L23 and tetrahydrofuran (THF) were purchased from Sigma-Aldrich. 2,2',4'-Trihydroxy-5-chloroazobenzene-3-sulfonic acid (lumogallion) was obtained from TCI Chemicals. DropSens Dual Carbon Screen-printed Electrodes (C110) were purchased from Metrohm USA. AIBN was recrystallised from cold methanol prior to use and each monomer was passed through neutral alumina column to remove the inhibitor and then was stored in the freezer under N_2 atmosphere until further use. All other reagents were of the highest commercially available purity and were used as received. Solutions of metal ions were prepared in ultra-pure water obtained from a Pico Pure 3 water system. Working solutions of different activities were prepared by serial dilutions of a 1 M stock solution.

Synthesis of lumogallion methacrylate (LuMA)

In a flamed-dried, argon-purged, round-bottomed flask, lumogallion (200 mg, 0.58 mmol) was mixed with anhydrous dimethylformamide (5 mL DMF), cesium carbonate (284 mg, 0.87 mmol) and triethylamine (58.7 mg, 0.58 mmol). The following reaction mixture was stirred for 2h and then cooled in an ice bath. Methacryloyl chloride (60.6 mg, 0.58 mmol) was added dropwise while the mixture was constantly stirred. After addition, the mixture was warmed to room temperature and stirred for an additional 16 h. The volatiles were then removed under reduced pressure and the obtained reaction mixture was dried under the high vacuum. The resulting precipitate was added to the heterogeneous mixture of ethyl acetate (5 mL) and dilute hydrochloric acid (5%, 5 mL). The organic phase was then washed with water (3 x 10 mL) and

the organic solvent was removed via rotatory evaporation to yield the product (34%). ¹H NMR (200 MHz, CDCl₃, δ): δ 4.14 (d, *J* = 2.42 Hz, 2H, CH₂C≡C), 3.68 (b, 2H, CH₂OH), 3.57 (t, *J* = 4.69 Hz, 2H, CH₂OC), 2.84 (s, 1H, OH), 2.43 (t, *J* = 2.39 Hz, 1H, C≡CH). IR spectrum (neat liquid, NaCl plates, ν, cm⁻¹): 3391 (br, O-H stretching), 3289 (m, ≡C-H stretching), 2116 cm⁻¹ (w, C≡C stretching).

General copolymer synthesis procedure

All copolymers were synthesized via thermally initiated free radical solution polymerization. Calculated amounts of LuMA, MMA and either PEGMEM or PEGMA were added to the anhydrous mixture of THF (3 mL) and DMF (2 ml) before addition of AIBN (1 wt%). The homogenous solution was purged with a stream of nitrogen for 25 min. Polymerization was then carried out at 75 °C under vigorous stirring for 24h. After the reaction was completed, the excess of solvent was removed using rotatory evaporation, dried under the high vacuum and the dark colored precipitate was collected. The residue obtained was dissolved in dichloromethane (DCM) and re-precipitated by excess cold methanol. The same procedure was repeated three times and the resultant copolymer was dried under ambient laboratory conditions. The peak intensities for MMA, PEGMA and LuMA obtained from ¹H NMR spectroscopy were used to calculate final concentrations of each monomer in a copolymer. ¹H NMR (200 MHz, CDCl₃, δ): δ 7.76 (br, 1H, NCH=C), 4.67 (br, 2H, OCH₂C=C), 4.39 (br, 2H, COOCH₂), 4.14 (br, CH₂N), 3.93 (br, 2H, COOCH₂), 3.76 (br, 2H, CH₂CH₂O), 1.92 – 0.96 (m, COOCH₂(CH₂)₁₀CH₃; CH₂CH₂N; NCH₂CH₂CH₂CH₃; NCH₂CH₂CH₂CH₃).

UV/Vis and fluorescence measurements

Ultraviolet-visible (UV/Vis) spectra were recorded using a Unicam UV500 double beam spectrophotometer (Unicam Instruments Ltd, UK) and fluorescent measurements were collected using a Cary Eclipse fluorescent spectrophotometer (Agilent Technologies, USA) with 1000 W xenon lamp and a photomultiplier tube (PMT) set at the voltage of 600 V. Quartz 1 cm x 1 cm x 4.5 cm cuvettes were used for all cuvettes measurements. The excitation wavelength was determined from UV/Vis measurements (the absorption maxima after the complex formation between aluminium and lumogallion based copolymer) and set at 485 nm while the emission wavelengths were monitored from 510 nm to 900 nm. All measurements were carried out at ambient temperature.

Membranes preparation

Each glass slides were prepared by cutting standard microscope slides into dimensions of 1.5 cm x 1.5 cm using a diamond cutter. The resulting slides were then rinsed with ultrapure water and acetone and then dried under nitrogen gas. Optode membranes were prepared by dissolving 100 mg of lumogallion-based copolymer in 0.5 mL of THF. After the complete dissolution of the copolymer the aliquot was spread out onto each glass slide by using a spin coater at 2000 rpm for 30 sec to yield membranes with the approximate thickness of 50 μm and then each membrane was left at room temperature to dry overnight. Subsequently, prepared optode membranes were im-

mersed in the acidified solutions of Al³⁺ ions (pH 3.5, to ensure that only free aluminium (III) ions are present) for 30 min, mounted into the quartz cuvette and then their fluorescence characteristics were determined.

Selectivity measurements

Optical films used for selectivity determination were prepared according to the protocol described in Membranes preparation. Each membrane was then left at room temperature to dry overnight. The following day, the lumogallion based optical films were placed for 24h in the 0.1 M solution of interfering ions (Mg²⁺, Ca²⁺, Zn²⁺, Na⁺, K⁺, Fe³⁺), and their optical responses toward all ions were recorded.

Fabrication of lumogallion based nanoparticles

Fabrication of polymeric particles via a solvent displacement method was adapted and modified from Bychkona and Shvarev (2009).³² 100 mg of PEGMA-MMA-Lumogallion based copolymer was dissolved in 1:1 heterogeneous mixture of ethylene glycol and THF (total volume – 1 ml). A small glass container (10 mL) was filled with a 0.02% solution of the surfactant (Brij L23) in ultrapure water. The resulting solution was stirred for 10 min at approximately 150 rpm. A small aliquot (500 μL) of the copolymer solution was drawn into a disposable syringe with an attached needle. The syringe was held approximately 5 mm above the surface of the liquid and the copolymer solution was rapidly injected into the vial. Precipitation of the polymer was observed within seconds after the injection of the mixture into the surfactant solution. The resulting nanoparticles were directly transferred onto new glass slide and were instantly subjected to microscopic examination.

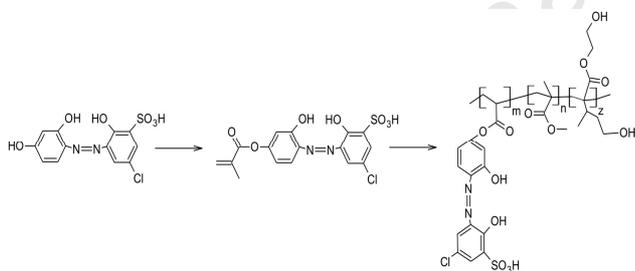
Fluorescence microscopy

Lumogallion based nano-particles were analyzed using an Olympus BX50 fluorescence microscope with a BX-FLA reflected light fluorescence attachment, equipped with a mercury burner and a vertical illuminator. For lumogallion imaging a U-MNIB3 fluorescence filter cube was used (bandpass excitation filter: 470 –495 nm, dichromatic mirror: 505 nm, longpass emission filter: 510 nm) (both from Olympus, UK). All images of copolymer based micro-particles were recorded at × 40 magnification using a ×100 Plan-Fluorite oil immersion objective (Olympus, UK) in combination with low auto-fluorescence immersion oil (Olympus immersion oil type-F). The exposure settings for the lumogallion analyses were fixed at 1 s with fixed light transmission values. Digital images were obtained using the Cell^D software (Olympus, Soft Imaging Solutions, GmbH) package and fluorescent and light channels were overlaid by use of Photoshop (Adobe systems Inc. USA).

RESULTS AND DISCUSSION

Prior to the synthesis of lumogallion-based derivatives, it was observed that a small fraction of this fluorescence dye (1% wt, as recommended for sensing membranes) can be successfully dissolved in the mixture of PVC and DOS

(33% wt, and 66% wt respectively) in THF carrying a promise for its application as optical sensor. However, upon the contact of the sensing film with the aqueous phase, almost instantaneous diffusion of lumogallion from the sensing membrane in the direction of sample took place rendering this sensor unsuitable for further testing. This was achieved by monitoring the increasing absorbance of the dye in the solution at 500 nm using UV/Vis spectroscopy (data not shown). Similarly lumogallion has been introduced into the polymeric matrix composed of the copolymerized LMA-MMA as such membranes display much lower diffusion coefficient when compared to conventional plasticized PVC membranes. However, on both occasions severe leaching of the fluorescence dye has occurred. Therefore, the most intuitive approach was to chemically modify lumogallion either by adding e.g. long alkyl chains or modifying already existing functional groups to reduce the overall polarity of this molecule. In this study, one component optical sensor based on the presence of lumogallion, which can form fluorescent complexes with aluminium (III) ions, has been proposed. This derivative contains a methacrylate moiety instead of the hydroxyl group at the para position relative to the azo group to allow simple attachment to the polymer backbone. Methacrylate based monomers were selected to yield plasticiser free copolymers with varying degree of hydrophilicity to find the best candidate for the Al^{3+} determination. This would ensure high robustness of a sensor during spectroscopic experiments and such factor has been a driving force in the development of lumogallion-based sensors.



Reagents and conditions: (i) Lumogallion, $\text{C}_4\text{H}_9\text{ClO}$, CsCO_3 , DMF, 85°C , 24h (ii) LuMA, PEGMA, MMA, AIBN, DMF, 75°C , 24h

Scheme 1. Experimental pathway for the synthesis of the copolymer consisting of MMA, PEGMA and LuMA moieties.

Sensitivity

The excitation and emission wavelengths of the resulting copolymer were found at 485 nm and 580 nm respectively. The small hypsochromic shift from the values reported in the literature (excitation at 500 nm for dissolved lumogallion) could be attributed to structural modifications introduced during the synthetic process. The synthetic pathway for the formation of lumogallion-based copolymers is illustrated in scheme 1. More detailed investigation of spectral properties show that the lumogallion based copolymer emits fluorescence that increases linearly with increasing Al^{3+} concentration (between 1.0×10^{-10} M

and 1.0×10^{-5} M) making it suitable for a sensing application. In this study, the minimum concentration of aluminium (III) ions that could be successfully measured was 4.8×10^{-12} M ($S/N = 3$). The low detection limit obtained in this study when compared to the other reported optodes based either on the use of lumogallion or morin, demonstrates that the presence of copolymerized lumogallion significantly enhances the sensitivity of the developed optical sensor. In this case, permanent immobilization of lumogallion protects the fluorophores from quenching by non-radiative processes for example via collisions with solution molecules. This results in the improvement of the emission intensity and increased sensitivity. Also, it can be hypothesized that covalent attachment of lumogallion suppresses intermolecular interactions between adjacent dye molecules and therefore prevents further lumogallion stack formation. Relatively polar character of the unattached fluorescent dye may result in the formation of reversed micelles when introduced in more hydrophobic polymeric matrix. This would reduce the overall number of available binding sites on the fluorophore and therefore limit its sensitivity towards the analyte of interest.

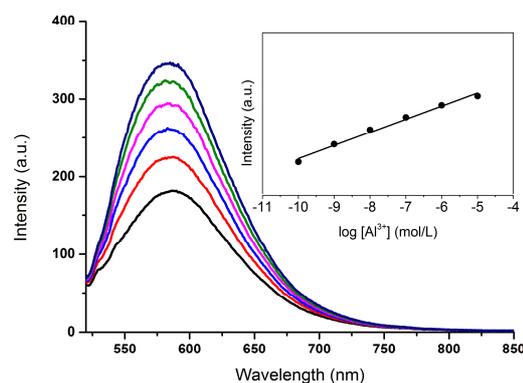


Figure 1. The response of the optical sensor to aluminium (III) ions. The data are represent as the fluorescence intensity with the excitation wavelength of 485 nm and emission equal to 580 nm. The inset demonstrates the linear response range with R^2 equal to 0.99 for Al^{3+} concentrations ranging from 1.0×10^{-10} M to 1.0×10^{-5} M. Note that the relative errors are $<5\%$ for each data point, but the error bars are too small to be visible.

Reversibility

The reversibility of the lumogallion-based copolymer was examined by repeating three cycles of the sensor with 1.0×10^{-2} M aluminium (III) ions and regenerating the optode with firstly 0.1 M HNO_3 in order to protonate hydroxyl groups (involved in binding) and consequently to release Al^{3+} back into the aqueous solution. However, when the same optical film was placed into the aqueous solution containing analyte of interest, no ion binding was observed. This could be due to the limited affinity of hydroxyl groups towards Al^{3+} in the presence of high concentration of hydrogen ions. This could be overcome by

washing the polymeric membrane with slightly more alkaline solution such sodium hydroxide and then rinsing the same sensor with ultrapure water to remove any excess of the base and therefore to prevent precipitation of aluminium upon contact with very alkaline medium. The findings from this study are demonstrated in Figure 2. Even though, the complex formation between lumogallion and aluminium is fully reversible, further attempts should be undertaken to shorten the number of steps required to regenerate the optical sensors and therefore to make it fully operational for practical applications. On some occasions, this relatively disadvantageous property of the optode can be further exploited and such polymeric film can be used for other purposes than its intended primary application as a sensing device. It was observed that the same optical film when exposed to 0.1 M HNO_3 solution used to regenerate the sensor causes a release of Al^{3+} into the aqueous solution. This is interesting as such device could be potentially applied for the selective ion extraction and controlled Al^{3+} release with the possibility to monitor in real time the efficiency of such process by looking at changes in the fluorescent signal of bound and unbound forms of the dye. However, this is not the primary scope of this paper and any further examination regarding aluminium (III) ion extraction has not been carried out.

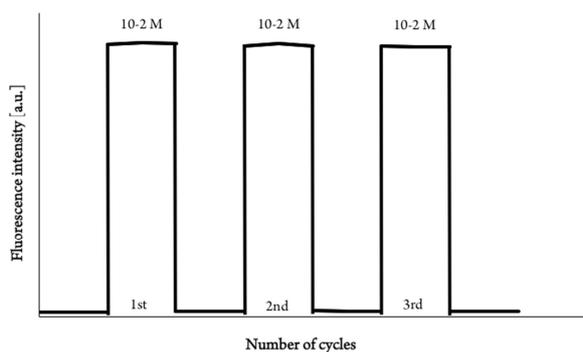


Figure 2. Reversible optical response of the proposed sensor after the regeneration with 0.1 M HNO_3 and 0.1 M NaOH .

Selectivity

In order to demonstrate usability of herein synthesized lumogallion-based copolymer for *in vivo* and *in vitro* Al^{3+} determination, the selectivity of resulting copolymer was measured against several biologically relevant ions such as Na^+ , K^+ , Fe^{3+} , Mg^{2+} , Ca^{2+} , and Zn^{2+} .

In each case, prepared lumogallion based membranes were exposed to 0.1 M of interfering ions for 24h and their fluorometric responses were measured. Only membranes preconditioned in 1.0×10^{-4} M Al^{3+} solution produced fluorescence response while no emission was recorded for other interfering ions. These findings demonstrate that covalently attached lumogallion retains its excellent selectivity towards Al^{3+} and it could be potentially used for its determination in very complex matrixes such as cellular environment. All selectivity experiments are summarized in Figure 3.

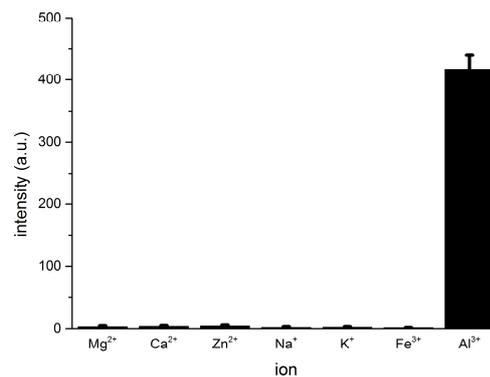


Figure 3. Fluorometric response of proposed lumogallion-based sensor to primary and interfering ions.

Response time

Another important characteristic that defines the functionality of optical sensors is their response time towards the analytes of interest. Since the response of traditional optical films result from a complete ion exchange in the bulk of the polymeric membrane, mass diffusion through such medium limits the response time and makes it much slower when compared for example ISEs. In this research several lumogallion-based copolymers with different monomer feeds were prepared and analyzed to find the most optimal Al^{3+} optical sensor. In order to ensure that any observed differences arise specifically from the presence of various monomers and their feeds, optical films were spin coated onto a glass substrate to produce polymeric membranes with reproducible thicknesses ($50 \mu\text{m} \pm 3 \mu\text{m}$). The reported response time was defined as 95% of the time required to reach steady state. Independent of the type of monomer used for membrane preparation, each optical film displayed similar LDLs when exposed to aluminium (III) ions – approximately 100 pM. However, large differences in the response time between each prepared membranes were observed. Optical films composed of LuMA (1%) copolymerized with LMA-MMA (40% wt and 59% wt respectively) exhibited the longest response times when exposed to Al^{3+} ions (up to 36 h). As the MMA content of the copolymer decreased, softer self-plasticized membranes were obtained (70% wt of LMA, 29% wt of MMA and 1% LuMA) and the response time of a resulting optode was shortened by 12 h. However, analyzed copolymers with high LMA content had poor adhesive properties and were mechanically unstable. Moreover, very long response times of the copolymers rendered these sensors unsuitable for practical applications and further experiments involving the use of such membranes were not carried out in our laboratory. These poor response characteristics were attributed to the overall hardness of the membrane as self-plasticized copolymers often exhibit reduced diffusion coefficients if compared to traditionally used PVC and DOS membranes. Additionally, a predominantly hydrophobic (apolar) character of LMA-MMA-LuMA copolymer could further limit the diffusion of relatively hydrophilic Al^{3+} ions from the aqueous solution into

the bulk of the membrane slowing sensor's response time. In order to even further shorten the response time while retaining suitable mechanical properties for sensor manufacture, only LMA moieties were replaced with monomers of varying polarity. Initially, LMA was replaced with PEGMEM moiety to produce more hydrophilic self-plasticized copolymers (MMA-PEGMEM-LuMA). Such modifications instantly resulted in shortening the response time of the prepared optical films down to 50 min. When the PEGMEM building block was replaced with even more polar PEGMA monomer, the response time was even further reduced (composition of resulting copolymer - MMA-PEGMA-LuMA). The findings of this study are summarized in Table 1. Improvement in a response time of synthesized copolymer could be due to a combination of factors such as: a) increase in overall polarity of the membrane and b) water uptake by the membrane. As the hydrophilicity of the membrane increased, a diffusion of Al^{3+} ions from the aqueous solution into the membrane became more energetically favorable improving significantly the response characteristics of such sensors. Moreover, it can be hypothesized that the increase in polarity of the membrane also facilitated a water uptake by the membrane resulting in an enhanced extraction of relatively hydrophilic aluminium (III) ions and shortened response time. Such phenomenon may also explain very poor/slow behavior of lumogallion based membranes composed of LMA and MMA moieties as their more hydrophobic character repels water from the membrane bulk more readily and therefore produces sensors with longer response time. On many occasions, water uptake can be considered as an issue in the case of traditionally prepared optical sensors or ISEs. As the water content increases in such membranes, exudation of the plasticizer from its bulk into the aqueous solution is facilitated. This not only carries the risk of deteriorating mechanical stability of ISEs/optodes but it may also promote the diffusion of sensing components such as ionophore or ionic sites in the direction of the sample. If sufficiently exposed, these membranes may be rendered inactive for practical applications. However, one component membranes that were used in this study were expected to exhibit much improved robustness as covalent linkage of the fluorescent dye to the polymer backbone would prevent its leaching upon contact with the aqueous solution. This hypothesis was further tested and the findings of this study are described in more detail in Supplementary Information - Robustness.

Table 1. Response time of synthesized lumogallion based copolymers. LuMA contributed 1% towards the overall polymer yield.

Copolymer composition	LMA + MMA (40% and 59%)	LMA + MMA (70% and 29%)	PEGMEM + MMA (40% and 59%)	PEGMA + MMA (40% and 59%)

Response time	36 h	24 h	50 min	15 min

Lumogallion-copolymer based nano-particles

PEGMA-MMA-lumogallion copolymer was selected for the fabrication of fluorescent nano-particles as we demonstrated that this copolymer exhibited the shortest response time, ultra-low detection limits and superior selectivity to Al^{3+} . Since nano-particles fabricated from the copolymer solution in 1:1 mixture of THF and ethylene glycol exhibited on average smaller diameter (see Supplementary Information and accompanied discussion), they were subjected for further examination using fluorescence microscopy. This is demonstrated in Figure 4. It is apparent that generated lumogallion based nano-spheres in the absence of aluminium (III) ions exhibit none/very faint background fluorescence that can be only recorded if the exposure time exceeds 10000 ms. This is important if precise fluorescence intensity measurements are required for instance either by using microspectrophotometer or via the extraction of intensities of red, green and blue (RGB) channels from the image.⁵³ The latter has been recently utilized in manufacturing of portable sensing devices for point of care diagnostics.⁵⁴⁻⁵⁷



Figure 4. Bright field (a) and fluorescence images (b) and (c) of nano-particles fabricated from the copolymer dissolved in THF and diethyl glycol. Fluorescence photographs were obtained using 510 nm emission and 470 - 495 nm excitation filters with 1000 ms (b) and 15000 ms (c) exposure time. All images were captured under 40 x magnification.

To further assess the functionality of synthesized nano-particles for aluminium detection, the same nano-beads were exposed to 1.0×10^{-6} M solution of aluminium nitrate and analyzed in both bright field and fluorescence modes. There were no observed changes in the appearance of nano-spheres exposed to aluminium ions as to particles that were stored in ultra-pure water and visualized in bright field mode (Figure 4a, and Figure 5a). However, as soon as the sample was analyzed in the fluorescence mode, characteristic orange emission originating from the nano-particles was observed (Figure 5b). This confirms that the proposed lumogallion-based copolymer can be used for the preparation of aluminium selective fluorescence based nano-particles.

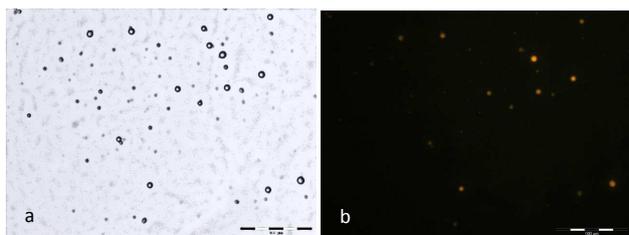


Figure 5. Bright field photograph (a) and fluorescence image (b) of the polymeric particles incubated for 15 min in 10^{-6} M solution of aluminium nitrate (exposure time 1000 ms; 40 x magnification).

CONCLUSIONS

Quantitative analysis of the free ion concentration within a biological sample is highly critical to the advancement of accurate disease screening and personalized medicine. Moreover, there is an on-going demand for the development of precise analytical tools for environmental monitoring of metal ions such as Al^{3+} . Therefore, a single piece, color changeable, fluorescent polymer membrane based sensor has been proposed for the highly selective and sensitive determination of aluminium (III) ions in aqueous environments. The sensing layer of the probe consists of a lumogallion derivative that is covalently attached to the methacrylate based polymer backbone. This fully eliminates the diffusion of the fluorophore from the sensing membrane into the aqueous solution that previously limited the development of optodes based on this relatively hydrophilic molecule. Such proposed optical sensor also exhibited excellent robustness over the period of two weeks as no leaching of the fluorophore was observed. The same optical film upon the contact with Al^{3+} ions produces a specific emission peak at 585 nm even for aluminium concentrations as low as 100 pm. Moreover, the performance of the resulting polymer was not inhibited by the presence of other interfering metal cations showing excellent selectivity towards Al^{3+} . Finally, these findings demonstrate an excellent potential of self-plasticized copolymers as sensing materials for the development of optical sensors that can be directly used for *in situ* detection of environmentally or biologically relevant ions.

ASSOCIATED CONTENT

Supporting Information

Further experimental details. This material is available free of charge via the Internet at <http://ees.elsevier.com>

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Self-plasticized, lumogallion-based fluorescent optical sensor for the determination of aluminium (III) with ultra-low detection limits

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Highlights

- Lumogallion has been modified and co-polymerized
- Lumogallion-based polymeric film exhibits linearity between 1.0×10^{-10} M and 1.0×10^{-5} M of Al^{3+}
- covalently attached lumogallion retains its excellent selectivity towards Al^{3+}
- co-polymerization with PEGMA monomer, response time has been reduced to 15 min
- Lumogallion-PEGMA – based nano-beads were produced and functionality demonstrated

Author statement

This work was possible due to the work of all co-authors. More specifically:

Lukasz Mendecki: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing – Original Draft, Writing – Review and Editing, Visualization

Sergio Granados-Fosil: Conceptualization, Methodology, Formal Analysis, Resources, Writing – Original Draft, Supervision,

Martin Jendrlin: Validation, Formal Analysis, Investigation, Data Curation

Matthew Mold: Validation, Formal Analysis, Investigation, Data Curation

Aleksandar Radu: Conceptualization, Methodology, Formal Analysis, Resources, Writing – Original Draft, Writing – Review and Editing, Supervision, Project Administration, Funding Acquisition

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: