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Dual-responsive and reusable optical sensors based on 2,3-diaminoquinoxalines for acidity measurements in low-pH aqueous solutions

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ABSTRACT: This work is focused on the age-old challenge of developing optical sensors for acidity measurements in low-pH aqueous solutions ($\text{pH} < 4$). We prepared halochromic (3-aminopropyl)amino-substituted quinoxalines **QC1** and **QC8** possessing different hydrophilic-lipophilic balance (HLB) and investigated them as molecular components of pH sensors. Embedding the hydrophilic quinoxaline **QC1** into the agarose matrix by sol-gel process allows for fabrication of cheap pH-responsive polymers and paper test strips. The emissive films thus obtained can be used for a semi-quantitative dual-color visualization of pH in aqueous solution. Being exposed to acidic solutions with pH in the range of 1–5, they rapidly give different color changes when the analysis is performed in day light or under irradiation at 365 nm. In compared to classical non-emissive pH indicators, these dual-responsive pH sensors allow to increase the accuracy of pH measurements in particular in complex environmental samples. pH Indicators for quantitative analysis were prepared by the immobilization of amphiphilic quinoxaline **QC8** using Langmuir-Blodgett (LB) and Langmuir-Schäfer (LS) techniques. Compound **QC8** possessing two long alkyl chains ($n\text{-C}_8\text{H}_{17}$) forms stable Langmuir monolayers at the air–water interface and these monolayers can be successfully transferred onto hydrophilic quartz and hydrophobic polyvinylchlorid (PVC) substrates using LB and LS techniques, respectively. The 30-layer films thus obtained are emissive, reveal excellent stability and can be used as dual-responsive pH indicators for quantitative measurements in real-world samples with pH 1–3. The films can be regenerated by immersing in basic aqueous solution ($\text{pH} = 11$) and reused at least 5 times.

Luminescent materials attract living interest in both fundamental and applied science holding great promise for the conversion of academic research into technological innovations.^{1–3} Molecular materials are widely studied in this field because the structure of organic emitters can be adapted to target applications, among which sensorics are of particular interest. Luminescent properties of organic compounds are known to be highly sensitive to the environment of luminophores. This limits the number of suitable solid supports and immobilization procedures appropriated for fabrication of emissive molecular materials. From a synthetic point of view, this limited choice of fabrication methods often imposes laborious structural modifications of the molecular probe in order to prepare a suitable precursor of the desired emissive sensing material. Cutting-edge developments in sensing in aqueous media are focused on the development of multi-responsive sensors in which a luminescence response on the analyte binding is accompanied by signal transduction using another channel, e.g. variations of absorbance. Dual-responsive sensors allow for solving crucial problems in sensorics such as increase of sensibility and selectivity of the analyses, for instance.

Solid-state optical sensors based on organic molecules can be prepared by immobilization of chromogenic molecules into a polymer matrix or by grafting these molecules onto inorganic supports.⁴ Another convenient synthetic approach to the sensory materials involves the prepara-

tion of Langmuir monolayers at the air–water interface followed by their assembling into Langmuir-Blodgett (LB) or Langmuir-Schäfer (LS) films.^{5–8} This method allows for a aware control on the molecular orientation both in Langmuir monolayers and on solid surfaces. A consecutive deposition of monolayers on solid supports affords structurally organized films with a tunable thickness. When efficient transfer conditions are developed, the fabrication of sensors is easy and rapid and may be performed in an aqueous medium using a minimum amount of organic solvents and an expensive organic dye. Additional benefits such as an increase in selectivity, sensitivity and shortening duration of analyses can be achieved due to an appropriate supramolecular organization of the 2D systems (monolayers) and precise control on the number of monolayers deposited onto the solid surface.⁹

Rapid and real-time monitoring of pH values is critical in a myriad of clinical, industrial and environmental situations and most of analyses should be performed in aqueous media.^{10–12} Therefore, pH indicators suitable for wide range of experimental conditions are required that probably explains constant research interest in the development of novel halochromic systems. Surprisingly, most of sensors for pH measurements in the range of 1–5 reported elsewhere in the literature are suitable only for qualitative analysis and are commonly used for gaseous samples (Table S1). As shown in Table S1, the solid optical devices for acidity measurements in low-pH acidic solutions are lim-

ited to a very few examples and most of them are irreversible devices.

Quinoxaline derivatives absorbing the light in visible region attract great attention as optical sensors. The ability of quinoxalines to change their optical properties when *N*-heterocycle is protonated was thoroughly investigated to obtain colorimetric and fluorescent pH chemosensors¹³⁻²⁷ because these compounds change the color in acidic media with $\text{pH} < 4$ in which most of pH indicators are insensitive.²⁸⁻³² Unfortunately, most of quinoxaline derivatives reported elsewhere in literature for pH monitoring can be used only in organic solvents or binary solvent mixtures and are inconvenient for real-world analyses. Water-soluble quinoxalines or solid-state devices (litmus strip, for instance) based on these compounds are required for practical usefulness. Surprisingly, halochromic materials based on quinoxalines are limited to chromogenic drop-cast films prepared by evaporation of chemosensor solutions on glass surfaces.^{18,33} These films were used only for analyses of acidic vapors probably due to their low stability in aqueous media. It is also worth noting that only one of these films reported previously was emissive and allowed for pH monitoring through PET-based sensing mechanism (ON-OFF sensor).¹⁸ Despite Langmuir monolayers formed by quinoxaline derivatives³⁴ and cavitands³⁵, dendrimers³⁶ and polymers³⁷ bearing quinoxaline moieties were investigated they never attracted much attention in sensing probably due to quenching of their luminescence in Langmuir monolayers induced by strong π - π stacking of these heteroaromatic molecules.³⁸

Recently we reported that a water-soluble push-pull 2,3-bis[4-(methoxycarbonyl)phenyl]-6,7-bis[(3-aminopropyl)amine]quinoxaline (**QC1**, Figure 1) can be used as dual-responsive optical (spectrophotometry and fluorescence) pH indicator in the pH range of 1–5.³⁹ This compound absorbs and emits visible light owing to the presence of a long and unsymmetrically substituted conjugated system in the molecule. Protonation of quinoxaline nitrogen atom is observed in pH range of 1–5 and leads to large bathochromic shifts of both absorption and emission bands.

In this work, we investigated different strategies for preparation of pH-responsive materials based on 2,3-bis[4-(alkoxycarbonyl)phenyl]-6,7-bis[(3-aminopropyl)amine]-quinoxalines to develop dual-responsive and reusable sensors for visual semi-quantitative and quantitative instrumental pH analyses.

First, cheap halochromic polymer films and paper test strips based on hydrophilic quinoxaline **QC1** were prepared by sol-gel process involving an agarose matrix. The pH indicators thus obtained were suitable for dual-color visualization of pH in the range of 1–5. Next, to increase the analysis accuracy, the amphiphilic halochromic compound **QC8** was obtained and deposited onto solid supports using LB or LS techniques. Thin film sensors thus prepared were emissive and suitable for visual dual-channel pH monitoring in the pH range of 1–3 but also allows for more accurate analyses using an appropriate instrumentation (a laboratory spectrophotometer and a fluorimeter).

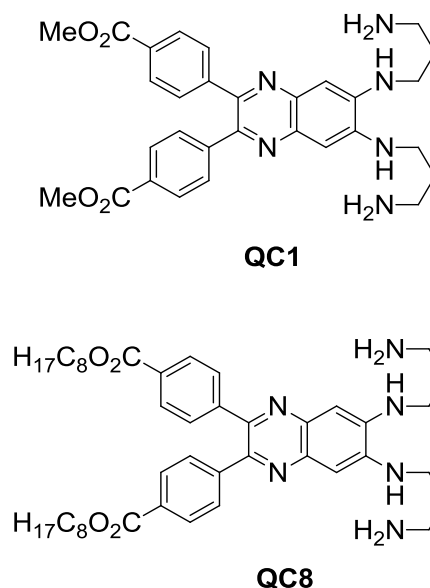


Figure 1. Structures of aminoquinoxalines **QC1** and **QC8** investigated in this work.

To our knowledge, this is the first example of preparation of *emissive* LB/LS films based on quinoxalines and their successful use as a sensor.

EXPERIMENTAL SECTION

Synthesis of chemosensors **QC1** and **QC2** can be found in the Supporting Information.

Preparation of polymer films and test strips.

A sol-gel process involving agarose was used for the preparation of polymer films and test strips from compound **QC1**. An aqueous solution ($c = 0.2$ mM) of quinoxaline **QC1** (1.1 mg), was added to a solution containing agarose, type I-B (0.15 g) and glycerol (0.5 g) in deionized water (10 mL). This solution (**Solution Q**) was heated to 90 °C and stirred at this temperature for 3 min.

Preparation of polymer films. A hot (90 °C) **Solution Q** (2 mL) was poured into a Petri dish and a flat glass disc was accurately placed on a surface of this liquid phase so that the liquid was sandwiched between the bottom of the Petri dish and the glass disc through the rubber gasket. These special conditions of cooling of the reaction mixture to room temperature allows for a control on the film thickness. The glass disk was removed after 1 h and polymer films with about 2 mm thickness were cut out and deposited in the loculi of a Teflon plate.

Preparation of test strips. Cellulose filter paper with 8–12 μm pore size ("Belaya lenta", Russia) was chosen for the preparation of pH-indicator paper test strips. Paper strips (10 × 40 mm) were immersed for 20 s into a hot **Solution Q** (90 °C, 8 mL). Then the strips were dried in air for 10 min, placed in a microwave (700 W) for 30 s and then dried in air for additional 8 h.

Preparation of monolayers and LB/LS films.

Computer controlled 1000-2 KSV Minitrough ($l \times w = 36.4 \times 7.5$ cm) model (KSV Instrument Ltd., Helsinki, Finland) with a Teflon trough and polyacetal barriers equipped with a platinum Wilhelmy plate (Sensitivity ± 0.01 mN m^{-1}) was

used for the preparation of Langmuir monolayers. All measurements were carried out at room temperature ($20 \pm 1^\circ\text{C}$). Deionized water ($18.2 \text{ M}\Omega \text{ cm}$, $\text{pH} \sim 5.5$) was used as a subphase and cleanliness of its surface was checked in blank compression experiments (surface pressure after compression process should not exceed 0.3 mN m^{-1}). Monolayers were formed by spreading a freshly prepared 0.1 mM chloroform solution of **QC1** or **QC8**. The spreading solution was deposited in $2.5 \mu\text{L}$ portions using a Gilson "Distri-man" micropipette. Delivered drops were uniformly distributed over a water surface in a checkerboard pattern. After spreading of $100 \mu\text{L}$ of this solution, chloroform was allowed to evaporate for 15 min and the monolayer was compressed at a rate of 10 mm min^{-1} .

Langmuir monolayers at the water surface with fixed pH were recorded under the same conditions using acidified or basified water as a subphase. Each compression isotherm was recorded at least three times to ensure reproducibility of results.

LB films were deposited onto quartz slides while LS technique was used to prepare films on and PVC and mica substrates. Transfers of monolayers were performed at a constant surface pressure of 20 mN m^{-1} by vertically immersing and withdrawing the solid substrate at a speed of 5 mm min^{-1} (LB films) or by horizontally deposition onto a solid substrate (LS films). The transfer ratio for the LB and LS films was no less than 0.95.

***In situ* UV-vis absorption spectroscopy of Langmuir monolayers.**

In situ UV-vis absorption spectra of Langmuir monolayers at the air-water interface were recorded in the 200–750 nm wavelength range using an AvaSpec-2048 FT-SPU (Avantes) fiber-optic spectrophotometer equipped with a 75 W DH-2000 deuterium-halogen light source and a CCD array detector. The UV-vis reflectometric probe with a fiber-optic diameter of $400 \mu\text{m}$, combined with a 6-fiber irradiating bundle, was placed perpendicular to the studied surface at a distance of 2–3 mm. The signal reflected from the deionized water surface was used as a baseline.

UV-vis absorption and fluorescence measurements of LB/LS films.

The UV-vis absorption spectra of LB/LS films deposited onto quartz and polyvinyl chloride (PVC) substrates were recorded on a Shimadzu-2450 spectrometer (Japan) in a wavelength range of 200–900 nm. The fluorescence spectra of solutions and LB/LS films deposited onto solid substrates were recorded on a Shimadzu RF-5301 spectrofluorometer (Japan) in a wavelength range of 200–900 nm. Liquid samples were analyzed at $25 \pm 1^\circ\text{C}$ in quartz cuvettes with an optical path length of 1 cm. All studied samples were irradiated at 420 nm.

Fluorescence quantum yields were measured at 25°C by a relative method using fluorescein in ethanol ($\Phi = 92\%$) as a standard.⁴⁰ The following equation was used to determine the relative fluorescence quantum yield:

$$\Phi_X = \Phi_{ST} \left(\frac{F_X \times A_{ST} \times \eta_X^2}{F_{ST} \times A_X \times \eta_{ST}^2} \right) \quad (1)$$

where A is the absorbance (in the range of 0.01–0.1), F is the area under the emission curve, η is the refractive index

of the solvents (at 25°C) used for the measurements, and the subscripts X and ST represent the studied and standard compounds, respectively. The following refractive index values are used: 1.362 for ethanol and 1.445 for chloroform.

Atomic force microscopy (AFM) investigations.

AFM measurements were performed on MultiMode Nanoscope V (Veeco, USA) operating in tapping mode. Noncontact polysilicon cantilevers with high aspect ratio were used (TipsNano, Estonia, resonant frequency – 120kHz, Q-factor – 350). Image processing was performed using Gwyddion (Chechen) software.

Visual determination of pH in aqueous solutions.

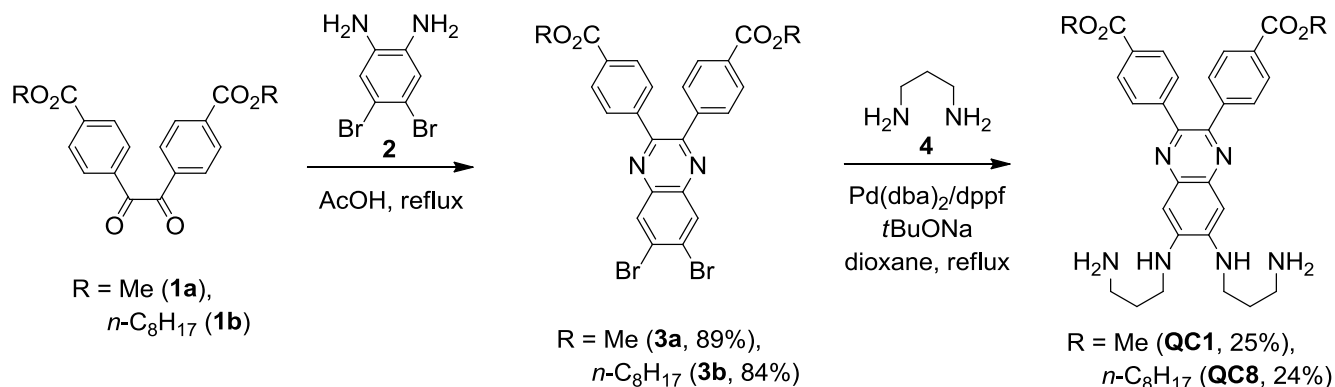
First, pH of studied solutions was measured using portable Ecotest 2000 pH-meter with combined ESK-10601/7 glass electrode. The electrode was calibrated with commercial buffers ($\text{pH} = 4.01, 6.86$ and 9.18) before to determine the pH of various samples. A series of standard solutions with $\text{pH} 1.0, 2.0, 3.0, 4.0, 5.0$ was prepared adding aqueous HCl to deionized water. Next, an aqueous solution containing 0.1 mM of $\text{K}^+, \text{Na}^+, \text{Mg}^{2+}, \text{Ba}^{2+}, \text{Ca}^{2+}, \text{Zn}^{2+}, \text{Co}^{2+}, \text{Cd}^{2+}, \text{Pb}^{2+}, \text{Ag}^+, \text{Ni}^{2+}, \text{Cu}^{2+}, \text{Hg}^{2+}$ and Al^{3+} perchlorates ($\text{pH} 4.52$) was prepared. Three others samples were a freshly prepared lemon and lime juice ($\text{pH} 2.45$ and 1.95 , respectively) and a kefir whey acidified by HCl ($\text{pH} 1.20$).

Then, the acidity of these samples was determined using dual-responsive optical sensors developed in this work. The pH-induced color changes of **QC1** in polymer films and test strips and of **QC8** in LB/LS films were evaluated visually at day light (absorption channel) or using 365 nm LED (emission channel). For polymer films deposited in the loculi of a Teflon plate, aqueous solutions with different pH (0.5 mL) were added by a Gilson "Pipetman P1000L" pipette and color of solid sensor was changed after 30 s. Test strips and LB/LS films were immersed in aqueous solutions with different pH for 30 s. The color changes of all pH indicators were observed immediately, but their exposition to analyzed solutions was continued up to 30 s to investigate material stability to decolorization under experimental conditions. An excellent agreement of all experimentally measured values was observed for each of studied samples.

RESULTS AND DISCUSSION

Synthesis of aminoquinoxalines

Aminoquinoxaline **QC8** was obtained using the reaction sequences shown in Schemes 1 and S1. First, 4,4'-bis(octyloxycarbonyl)benzil (**1b**) was prepared from methyl ester **1a** according a two-step procedure involving sequential hydrolysis and esterification reactions (Scheme S1). Then this compound was transformed in aminoquinoxaline **QC8** using synthetic strategy developed by us previously for the preparation of **QC1** (Scheme 1).³⁹ The size of the carbon chain in the alkoxy carbonyl substituent did not influence on the course of condensation and amination reactions and aminoquinoxalines **QC1** and **QC8** were obtained in similar overall yields.



Scheme 1. Synthesis of aminoquinoxalines **QC1** and **QC8**.

The newly synthesized compounds **1b**, **3b**, and **QC8** were unambiguously characterized by NMR, IR, UV-vis and HRMS (ESI) analysis (Figures S1–S10). While chromophore **QC1** is soluble in aqueous medium, **QC8** is an amphiphilic compound with structural parameters fitting well with LB and LS techniques owing to the presence of two hydrophobic alkyl chains attached to the planar heteroaromatic core and an opposite arrangement of hydrophilic and hydrophobic residues.

Electron absorption and emission spectra of **QC1** and **QC8** in chloroform were very similar as shown in Figure S11 and summarized in Table S2. Both compounds have proton sensitive heteroaromatic nitrogen atoms and are of interest for development pH materials for strongly acidic aqueous solutions. Indeed, an addition of hydrochloric acid (up to pH 1) to aqueous solutions of **QC1** and water-methanol (1:1 v/v) solutions of **QC8** induces bathochromic shifts of absorption and emission bands which are visible by the naked eye in day light or under irradiation by 365-nm LED, respectively (Figure S12).

Polymer films and test strips based on aminoquinoxaline **QC1**

One of key steps in the development of pH indicators for visual semi-quantitative pH monitoring is immobilization of halochromic molecules on surfaces to prepare solid sensors which are suitable for performing express analyses, in particular “in field” conditions. In this regard, we investigated the preparation of polymer films and paper test strips based on aminoquinoxaline **QC1** by using agarose as a gelling agent in sol-gel technique. The polymer film thus prepared was deposited on the filter paper and in the loculi of a Teflon plate. Both solid sensors thus prepared were brightly colored and changed their color immediately in the pH range of 1–5 as it was observed when a solution of **QC1** was used for pH measurements in aqueous samples. The color changes were easily observed by the naked eye in day light or under irradiation at 365 nm (Figures 2 and 3) and the immersion of sensors in an aqueous solution containing 0.1 mM of each of 14 metal cations (K⁺, Na⁺, Mg²⁺, Ba²⁺, Ca²⁺, Zn²⁺, Co²⁺, Cd²⁺, Pb²⁺, Ag⁺, Ni²⁺, Cu²⁺, and Al³⁺) to studied aqueous solution does not induce any changes in results. Moreover, solid sensors have demonstrated their effectiveness for the analysis of real-life samples such as lemon (pH 2.45) and lime juice (pH 2.45 and 1.95, respectively) as well as a kefir whey

acidified by HCl to pH 1.20. Such dual-color visualization is convenient and important because this allows for an increase of accuracy in particular for the analyses of real-world samples contaminated by many occasional organic and inorganic compounds which may disturb one of the optic responses of the pH indicator. Use of a porous paper sheet as a solid support simplifies visual analyses in the pH range of 3–5 because the color changes of paper strips covered by the polymer film are easily seen by human eye (Figures 2 and 3). Both type of sensors can be rapidly regenerated in basic solution with pH 11.0 and reused at least 5 times without loss of efficiency and any decrease in color intensity (Figure S13). However, these pH indicators are suitable only for semi-quantitative analysis since accurate spectrophotometric pH measurements cannot be performed using these flexible and ruffled polymeric films.

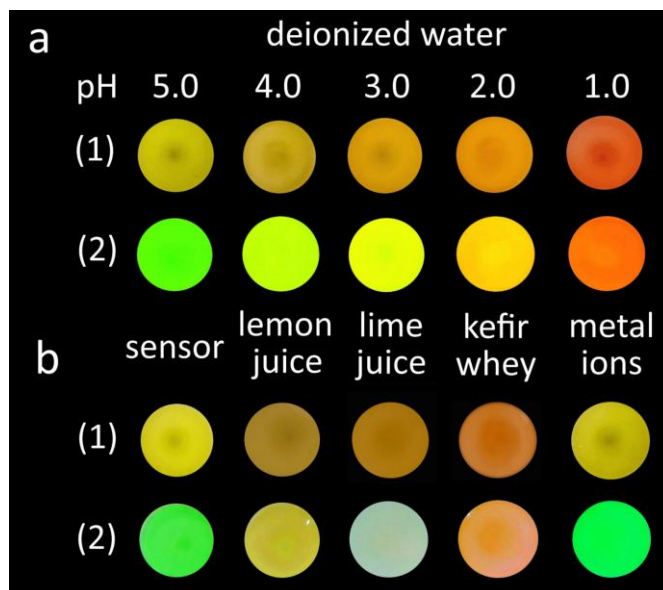


Figure 2. pH-induced color changes of polymer films based on **QC1** (1) in day light and (2) under irradiation by LED ($\lambda = 365$ nm) for (a) deionized water with different pH value and (b) for real-life samples and an aqueous solution containing metal perchlorates (0.1 mM of 14 salts).

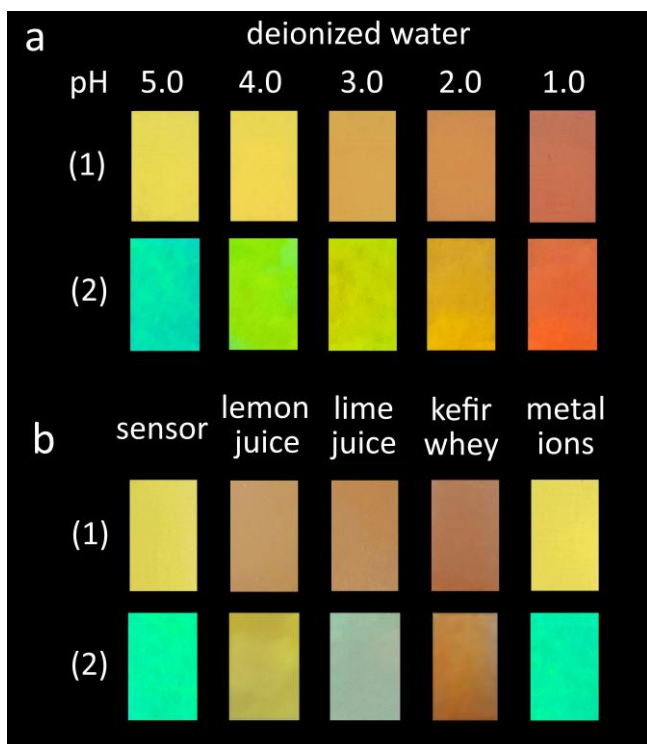


Figure 3. pH-induced color changes of paper test strips based on **QC1** (1) in day light and (2) under irradiation by LED ($\lambda = 365$ nm) for (a) deionized water with different pH value and (b) for real-life samples and an aqueous solution containing metal perchlorates (0.1 mM of 14 salts).

Monolayers of amphiphilic aminoquinoxaline **QC8** at the air-water interface and their transfer on solid supports.

To optimize the solid sensor fabrication and prepare thin films allowing for quantitative pH determination using spectrophotometric/fluorometric measurements, we investigated a deposition of halochromic aminoquinoxalines **QC1** and **QC8** onto solid surfaces through the formation of Langmuir monolayers at the air-water interface followed by their assembling into Langmuir-Blodgett (LB) or Langmuir-Schäfer (LS) films onto transparent solid supports. We have chosen this immobilization strategy because this method for film fabrication is applicable to different types of solid supports and the thickness of LB and LS film can be precisely controlled by changing the number of transferred monolayers.

Our preliminary experiments demonstrated that compound **QC1** did not form Langmuir monolayers at the air-water interface because of its high solubility in water subphase. Amphiphilic aminoquinoxaline **QC8** being deposited on water subphase with different pH (1.0–11.0) gives Langmuir monolayers which can be compressed to surface pressure of about $42 \text{ mN}\cdot\text{m}^{-1}$. Compression isotherms of **QC8** on surfaces of aqueous solutions with pH 11.0, 5.5 and 1.0 are depicted in Figure 4a. The extrapolated molecular areas corresponding to linear sections of isotherms are equalled to $140 \text{ \AA}^2/\text{molecule}$ for the monolayer formed on the surface of subphase with pH 1.0 and $130 \text{ \AA}^2/\text{molecule}$ on subphases with pH 11.0 and 5.5. This probably indicates that orientation of molecules is similar in all studied monolayers. According to computa-

tional calculations based on minimum energy equilibrium geometry estimated within Spartan package (Figure 4a, inset) a molecule of aminoquinoxaline **QC8** in the face-on orientation with respect to a surface of water should occupy ca. 225 \AA^2 and in the perpendicular arrangement should cover ca. 45 \AA^2 on the surface. Thus, we assume that **QC8** molecules adopt an inclined orientation in the monolayer or quinoxaline fragments lie parallel to the surface while two hydrophobic alkyl chains of octoxycarbonyl substituents are directed away from the water surface. The shape of compression isotherms and *in situ* UV-vis absorption spectra recorded at different compression pressures reveal the absence of structural reorganizations of studied monolayers during a compression cycle. The absorption bands are broadened as compared to those observed in chloroform solution but the absorbance maxima display about the same positions. Absorbance intensity is gradually increased when the surface pressure is risen. This spectrophotometric behaviour probably indicates that π - π stacking of heterocyclic fragments in the monolayer formed by voluminous quinoxaline molecules of **QC8** is rather weak because such intermolecular interactions generally can be observed spectrophotometrically as shifts of absorption bands. Indeed, the presence in this molecule of two aryl substituents twisted with respect the quinoxaline core³⁹ and two linear polyamine substituents is unfavourable for π - π stacking of both six-membered aromatic ring of the quinoxaline system.

Surprisingly, maxima of absorbance bands are pH independent and appear at 290 and 430 nm over the entire of pH range in contrast to that which was observed in water-methanol (1:1 v/v) solution (Figure S14). Similar results were obtained when *in situ* UV-vis absorption spectra of **QC8** monolayer were recorded at the constant surface pressure of $10 \text{ mN}\cdot\text{m}^{-1}$ with a gradual acidification of subphase from pH of 11.0 to pH of 1.0 (Figure 4b). In this experiment, two absorption bands were observed over the pH range of 11.0 to 3.0 and further acidification of the subphase led to the appearance of a shoulder at 490 nm.

This low sensitivity of **QC8** monolayers to pH changes is probably caused by a its lower basicity in the monolayer as compared to that of **QC1** and **QC8** in aqueous solutions. This is easy to understand considering a less efficient solvation of charged protonated species on the water surface.

These results revealed that fabrication of sensing films from these monolayers is challenging because transfer of the monolayers on solid supports should be performed controlling the acid-basic properties of self-assembled systems thus obtained. Therefore, we investigated the film preparation by vertical deposition (LB method) of monolayers onto hydrophilic quartz and by their horizontal deposition (LS method) onto hydrophobic polyvinylchloride (PVC) supports.

The deposition onto a quartz substrate by LB method was performed at a constant surface pressure of $20 \text{ mN}\cdot\text{m}^{-1}$ from surface of aqueous subphase with pH 11.0, 5.5 and 1.0. Light absorption properties of the films thus obtained were investigated visually (Figure S15) and spectrophotometrically (Figure S16).

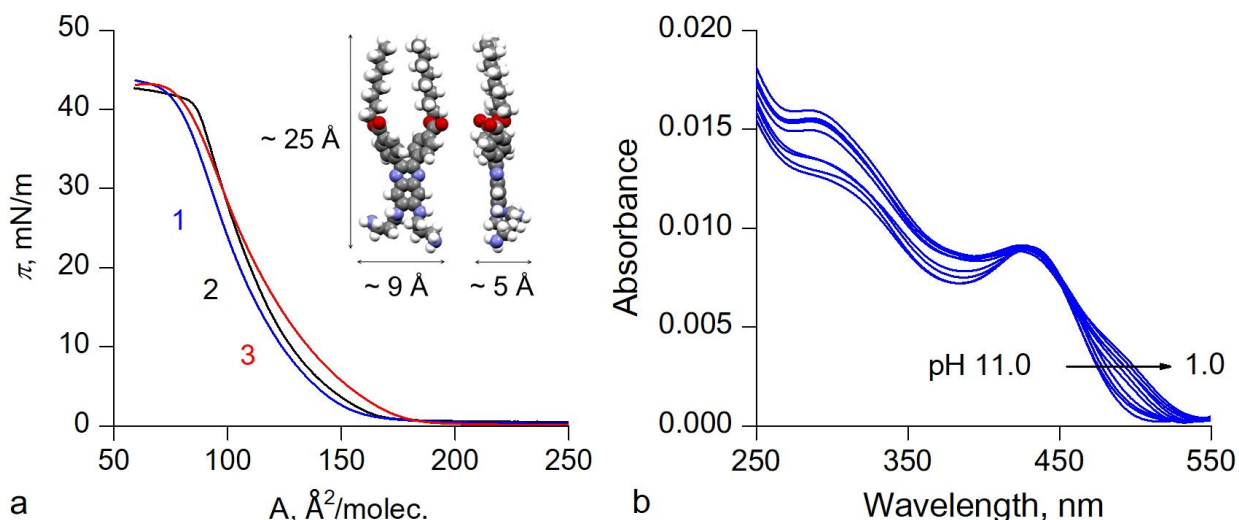


Figure 4. (a) Surface pressure vs area isotherms of **QC8** on the deionized water surface at pH 11.0 (1), 5.5 (2) and 1.0 (3). Insert shows spacefill model of **QC8** molecule optimized by the semi-empirical PM6 method.⁴¹ (b) *In situ* UV-vis absorption spectra for the monolayer of **QC8** on the pure water surface measured at 10 mN m⁻¹ upon decrease of pH value from 11.0 to 1.0.

It was observed both by naked eye and spectrophotometrically that **QC8** monolayers formed on the subphase with pH = 1.0 cannot be transferred onto a quartz surface probably due to electrostatic repulsion of positively charged species.

Naked eye inspection of the LB film transferred from the subphase with pH = 11.0 reveals that the film is inhomogeneous. The best quality of the film was observed using **QC8** monolayer formed at the surface of deionized water (pH = 5.5). This film was uniformly colored and displayed the highest absorbance intensity among three studied films. Thus, this subphase was used for further investigations and for preparation of 30-layer LB and LS films.

To get insight into the morphology of one-layer films of

QC8 by atomic force microscopy (AFM) the **QC8** monolayers were deposited by LB and LS methods at mica surface which is suitable for imaging by this technique. Good quality AFM images were obtained only for the film prepared by LS method.

The images, which are depicted in Figure 5, reveal that the films thus obtained are homogeneous at the mesoscopic scale. They are composed of micro-sized elongated species with the predominant height of 4.5 and 9 nm. This thickness is higher than that which is expected for the **QC8** monolayer film of with perpendicular orientation of quinoxaline molecules (2.5 nm, Figure 4a, insert) and corresponds to the dimension of **QC8** bilayer (4.5 nm) and aggregates formed by two such bilayers (9 nm).

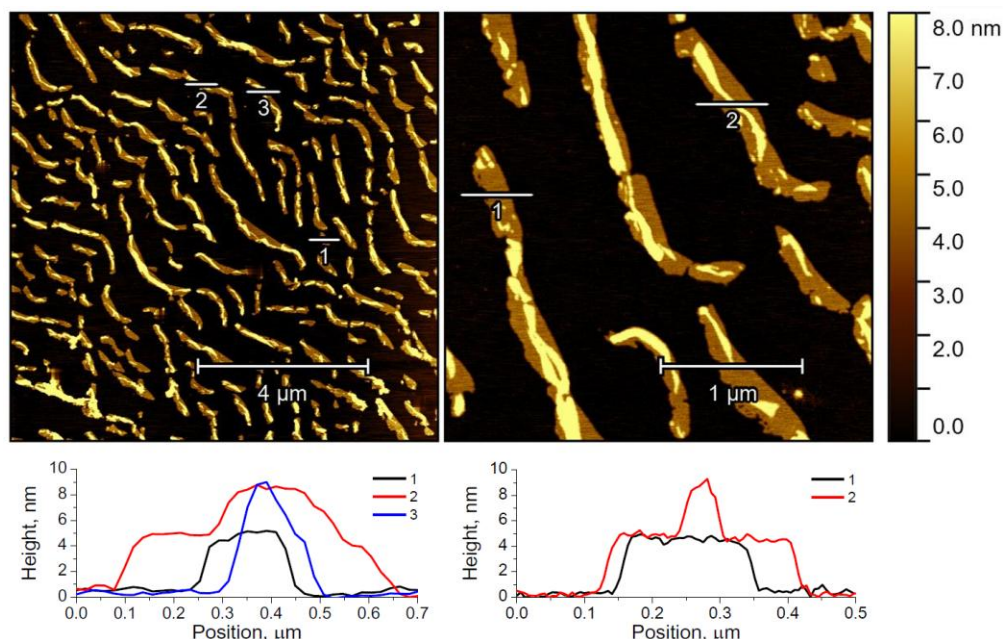


Figure 5. AFM images of one-layer LS film of **QC8** deposited onto a mica substrate at a surface pressure of 20 mN m⁻¹ from the deionized water surface and corresponding height profiles. The number 1-3 in the graph legends correspond to the regions shown in the images.

The formation of bilayer films on mica surface is currently not understood and we also cannot exclude that

the deviation of the film height from the value expected for a monolayer is caused by the presence of strongly bonded water molecules because the film formed under these conditions should be composed of doubly-protonated **QC8** molecules.³⁹ Noteworthy, amphiphilic porphyrins deposited on mica surface by LB method also formed a film with a surface roughness corresponding to a bilayer structure.⁴²

Such perforated films with a tight surface coverage by halochromic molecules held promise for fabrication of photoactive multi-layer sensing films with high surface area provided an easy accessibility of receptor units to analytes. Unfortunately, the film morphology is dependent on the nature of support and films deposited on practically important surfaces such as quartz or PVC, for instance, cannot be analyzed by AFM technique.

Next, 30-layer films were successfully prepared applying surface pressure of 20 mN m⁻¹ on both hydrophilic (quartz) and hydrophobic (PVC) substrates according to

LB and LS techniques, respectively. The films were colored and emissive and their exposure to aqueous solutions with pH lower than 3 for 30 s led to large bathochromic shifts of both absorption and emission bands in the visible region (Figure 6). Surprisingly, despite the difference in the solid supports, sensing properties of two types of films thus obtained were very similar as shown in Figure 6. Linear dependence of absorbance and emission on pH value allows for quantitative pH measurements using both pH indicators (Figures 7 and S18). Immersion of both films in aqueous solution containing 0.1 mM of various metal cations (K⁺, Na⁺, Mg²⁺, Ba²⁺, Ca²⁺, Zn²⁺, Co²⁺, Cd²⁺, Pb²⁺, Ag⁺, Ni²⁺, Hg²⁺, Cu²⁺, and Al³⁺) does not induce any changes of absorption and emission of light as compared to spectra of deionized water (Fig. S19). Important to note, that pH values obtained for all real-life samples (an acidified kefir whey, lemon and lime juices) using these calibration curves were in a good agreement with values measured by standard pH electrode (Fig. 7).

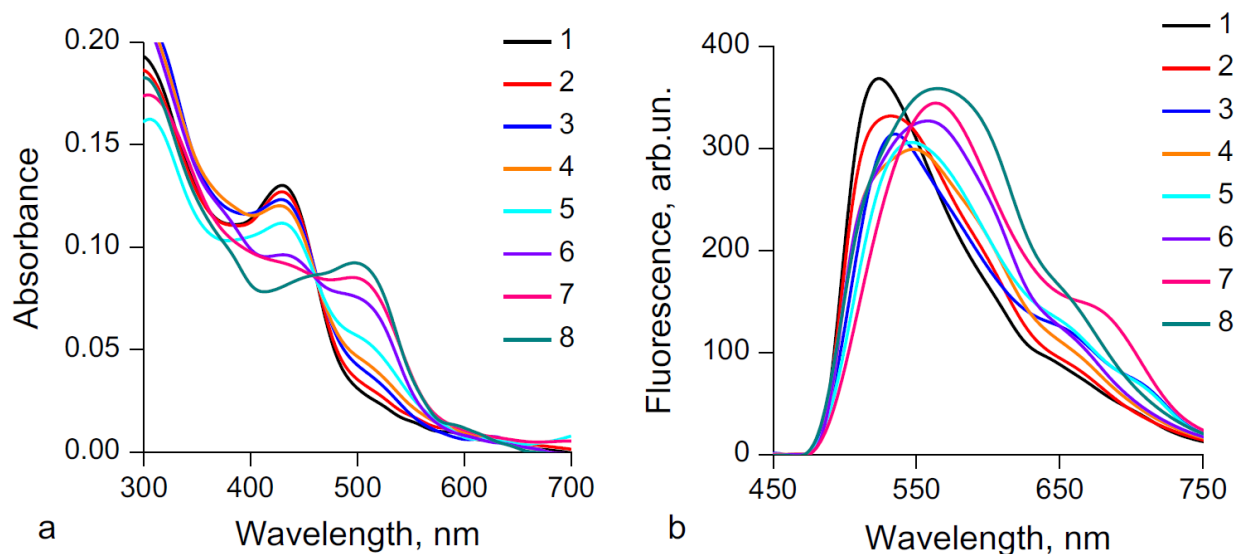


Figure 6. UV-vis absorption (a) and fluorescence (b) spectra of 30-layer LS film of **QC8** (1) transferred onto quartz (LB) and PVC (LS) substrates, at 20 mN m⁻¹ and immersed in aqueous solutions with pH 3.00 (2), lemon juice 2.45 (3), 2.00 (4), lime juice 1.95 (5), 1.5 (6), kefir whey 1.20 (7), and 1.0 (8) for 30 s, $\lambda_{ex} = 420$ nm.

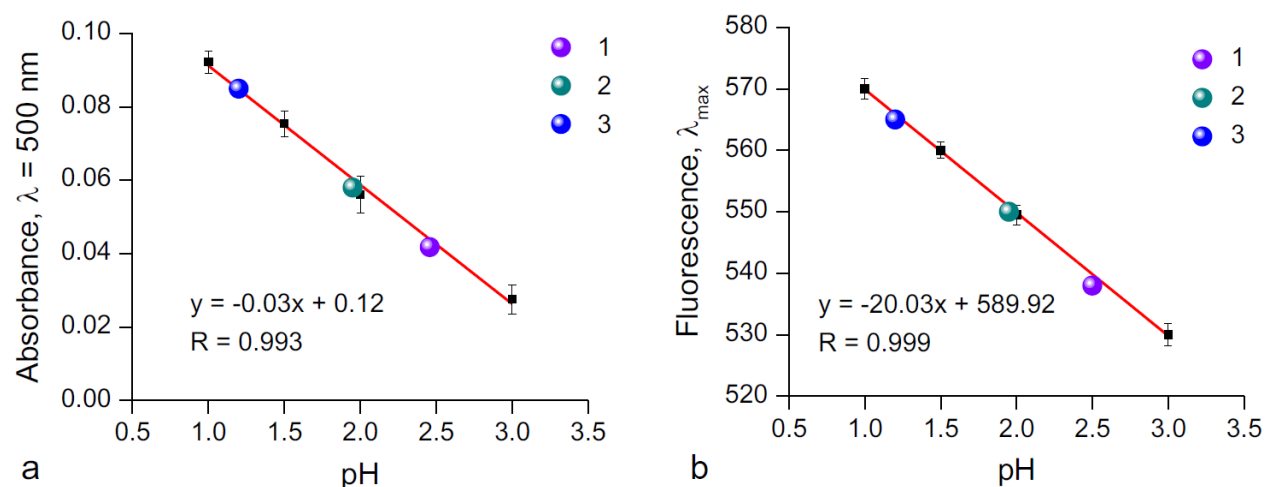


Figure 7. Dependence of the absorption intensity (a) and fluorescence λ_{max} (b) on pH value for 30-layer LS film of **QC8** transferred at 20 mN m⁻¹: lemon juice pH 2.45 (1), lime juice pH 1.95 (2), and kefir whey 1.20 (3), $\lambda_{ex} = 420$ nm.

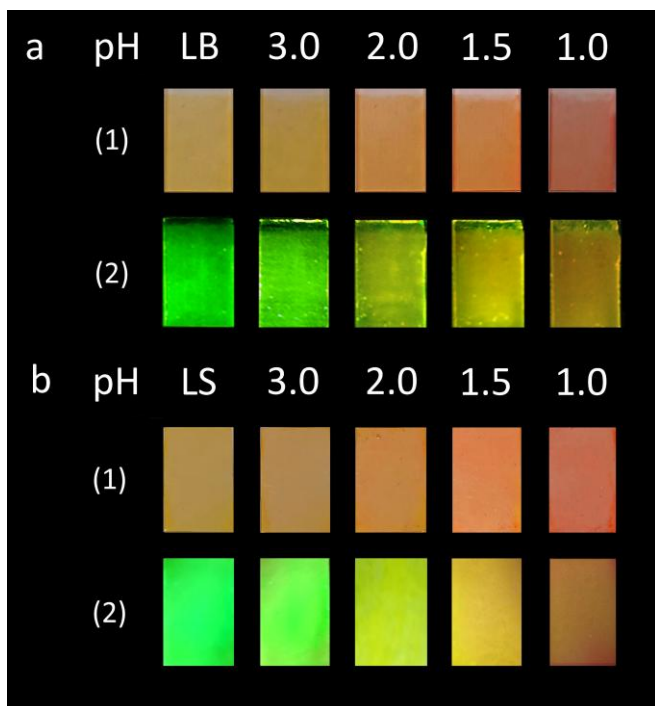


Figure 8. pH-induced color changes of 30-layer LB (a) and LS (b) films of **QC8** (1) in day light and (2) under irradiation by LED ($\lambda = 365$ nm).

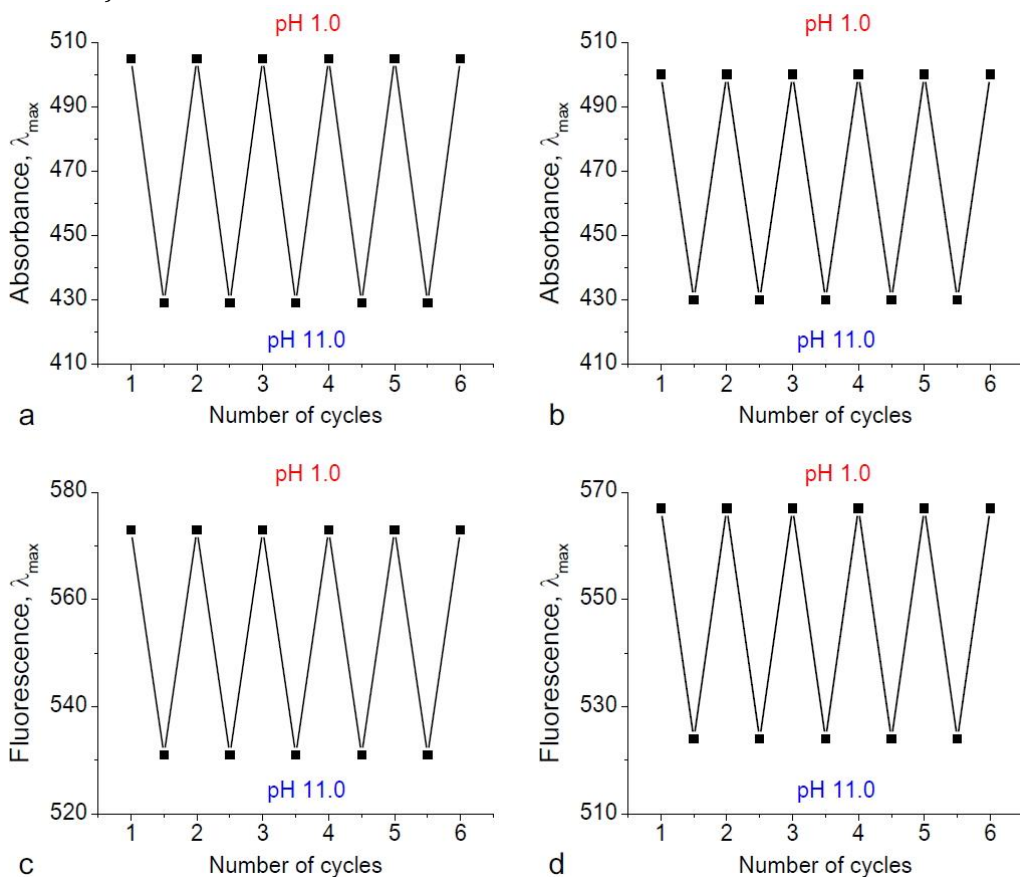


Figure 9. Changes of absorbance (a, b) and fluorescence (c, d) λ_{max} during five consecutive measurements of pH = 1.0 and recycling of the indicator at pH = 11.0 in aqueous samples by 30-layer LB (a, c) and LS (b, d) films. The spectral data were recorded after immersing of the indicators during 30 s in the acidic and basic solutions.

These spectral changes can also be observed by naked eye in day light and under UV irradiation ($\lambda = 365$ nm) (Figure 8) allowing for easy and rapid semi-quantitative pH measurements.

Both types of pH indicators exhibit excellent stability at different pH and can be easily recycled by immersing in basic aqueous solution (pH = 11.0) and reused as shown in Figures 9 and S20.

Comparing sensing properties of these pH indicators to those obtained by immobilization of quinoxaline **QC1** in agarose polymer matrix (Figures 2 and 3), one can conclude that all of them are efficient for dual-color visualization of pH and convenient for the analysis of real-life samples but they have distinct differences. Sensors obtained by immobilization of **QC8** according to LB/LS methods allow to perform quantitative pH monitoring in the pH range of 1–3 in which most known pH sensors are inapplicable. These techniques for the immobilization of quinoxalines also allow for a rational consumption of the chromogenic compound because the thin films containing only 30 layers can be used for reproducible pH measurements and can be regenerated more than 6 times.

In contrast, to perform visual semi-quantitative pH measurements, the immobilization of hydrophilic compound **QC1** in agarose matrix is the best strategy because pH indicators thus obtained rapidly give a visual response in large pH interval (1–5).

CONCLUSIONS

In this work, we report that quinoxalines are convenient molecular components for the preparation of pH-responsive materials for acidity measurements in low-pH aqueous solutions (pH < 4). Our molecular design of the dyes is based on the introduction of 1) electron donating (3-aminopropyl)amino residues at 6 and 7 positions of the quinoxaline core to obtain a long and unsymmetrical conjugated system, and 2) voluminous aryl substituents at 2 and 3 positions of the heterocycle for preparation of emissive materials. We demonstrated that HLB of these compounds can be fine tuned varying a long carbon chain in 4-(alkoxycarbonyl)phenyl substituents. According to these design principles, a water-soluble quinoxaline **QC1** and amphiphilic compound **QC8** were prepared and investigated as molecular components of solid pH sensors.

Immobilization of the hydrophilic **QC1** into the agarose matrix using sol-gel technique allows us to prepare cheap polymer films and paper test strips for semi-quantitative dual-color visualization of pH in aqueous samples with pH 1–5. Various (14 metal ions were tested) metal cations do not interfere with protons that is important for the analysis of real-world samples.

Next, focusing on quantitative pH measurements, we investigated the fabrication of nano-structured films based on quinoxalines. It was demonstrated that the introduction of long alkyl chains in the molecule is required for the fabrication of stable Langmuir monolayers at the water–air interface. The transfer of the **QC8** monolayers onto hydrophilic quartz and hydrophobic PVC was carried out using LB and LS techniques, respectively. The 30-layer films revealed excellent stability and were halochromic. Moreover, these films were emissive probably because the presence of voluminous aryl substituents and long alkyl and polyamine chains at the heterocycle periphery prevents strong π - π stacking of neighboring quinoxaline molecules. These films were successfully used as dual-responsive sensors for quantitative analysis of acidity in the pH range of 1–3 by instrumental methods (spectrophotometry and fluorescence spectroscopy). These dual-responsive sensors are of particular value for the analyses of real-world samples in which the interference of other compounds could cause erroneous responses of one of the two signaling channels. It is also worth noting that in terms of consumption of organic compounds, ease of handling and automation this immobilization technique is very seductive because the nano-structured films composed of only 30 monolayers give reproducible sensing results, can be easily stored and regenerated more than 6 times.

Thus, we developed efficient strategies for preparing cheap pH indicators for semi-quantitative and quantitative acidity measurements in low-pH aqueous solutions starting from halochromic 6,7-diaminoquinoxalines. Our ongoing studies are focused on the design and investigations of dual-responsive sensors based on sterically hindered aminoquinoxalines for various analytes including toxic metal ions and anions.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/xxx>. Experimental Procedures and characterization data provided in the Supporting Information (PDF).

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