

Selective Ligand-Doped Liquid Crystal-Based Sensing Platform for Detection of ClO^- Ions in Aqueous Media

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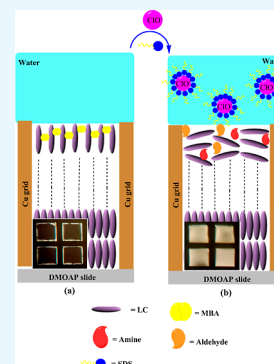


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Supporting Information

ABSTRACT: The liquid crystal (LC) detection platform has been fabricated for the detection of hypochlorite ions (ClO^-) in aquatic solutions. In this system, an imine consisting of the ligand (*E*)-2-((4-(diethylamino)-2-hydroxybenzylidene)amino)-5-methoxybenzenesulfonic acid (MBA) was doped in 4-cyano-4'-pentyl biphenyl as a selecting LC for ClO^- . When immersing the platform in a solution containing ClO^- , hypochlorite appears to react with the imine bond in the MBA, and cause it to cleave, which eventually disrupts the direction of LC and causes a dark-to-bright conversion of the LC image. The detection limit for ClO^- is $0.05 \mu\text{M}$. This sensory platform was unresponsive to NO_3^- , BrO_3^- , CH_3COO^- , CO_3^{2-} , and PO_4^{3-} ions. Our sensing platform also detected ClO^- in piped water. Since this sensory platform is colored under ambient light, it is easy for regular operators, and it can be used as a mobile tool for monitoring water quality anywhere.



INTRODUCTION

There is increasing interest in the characterization of reactive oxygen species (ROS) because of their harmful and dangerous effects on physiological and pathological processes.^{1,2} According to recent studies on ROS, cancer cells continuously produce ROS as a result of carcinogenic changes.³ During the bio-oxidative reaction pathway between hydrogen peroxide and chloride anion, myeloperoxidase produces ClO^- (hypochlorite) as one of the ROS.⁴ Different functional groups such as thiol, oxime, hydroxamic acid, imine, and acyl nitroso groups can be oxidized by the ClO^- anion as a strong oxidizing agent. The World Health Organization (WHO) has proposed a minimum residual ClO^- of $3.3 \mu\text{M}$.⁵ Numerous analytical procedures for the detection of hypochlorite such as colorimetry, electrochemistry, fluorescent chemical sensors, and chemical illumination have been reported.⁶ Although these techniques have advantages and are able to accurately detect ClO^- , as a result, their use is limited due to tedious instrumentation and lengthy sample preparation procedures. Due to these factors, it has become increasingly necessary and important to develop new sensors and probes with low cytotoxicity and high environmental compatibility.

In recent decades, liquid crystals (LCs) have had an intriguing focus on converting bio-molecular events and delicate processing into optical signs that can be comfortably distinguished by the naked eye.⁷ In contrast to common analysis methods, sensors that use the LC-based technology do not require complex instrumentation and do not require labeled molecules under ambient light.⁸ In recent years, functional molecules have been incorporated into LCs for the development of new sensors. Most LC-based sensing platforms diagnose using

chemical reactions, in which the analyte serves as the reagent. It is possible that this reaction could reorient the LC molecules, changing the color of the optical picture of the LC.⁹ According to this notion, different types of analytes, including macromolecules, small molecules, cations, and anions, have been detected using LC-based sensing platforms.^{10,11} However, there has been no study of the detection mechanism of LC-based sensing platforms to detect ClO^- . Yun et al. synthesized a chemo dosimeter ligand comprising the imine fraction, (*E*)-2-((4-(diethylamino)-2-hydroxybenzylidene)amino)-5-methoxybenzenesulfonic acid (MBA) (Scheme 1), and demonstrated that this imine bond therein selectively cleaved ClO^- ions in aquatic medium.¹²

This study tested MBA as a probe for ClO^- detection using the most frequent nematic LC, 4-cyano-4'-pentyl biphenyl (SCB)-loaded transmission electron microscopy copper grids. Doping MBA in SCB created a new LC-based sensing platform for detecting ClO^- specifically and sensitively in aquatic solutions. It is expected that the aforesaid molecule will align at the LC/water interface to provide the bulk LC layer with the homeotropic direction. Hypochlorite-ligand binding, followed by reaction and imine bond cleavage, changed the direction of the LC and the corresponding optical images. Therefore, we used a digital camera to register LC pictures using a mobile

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Scheme 1. MBA Synthesis

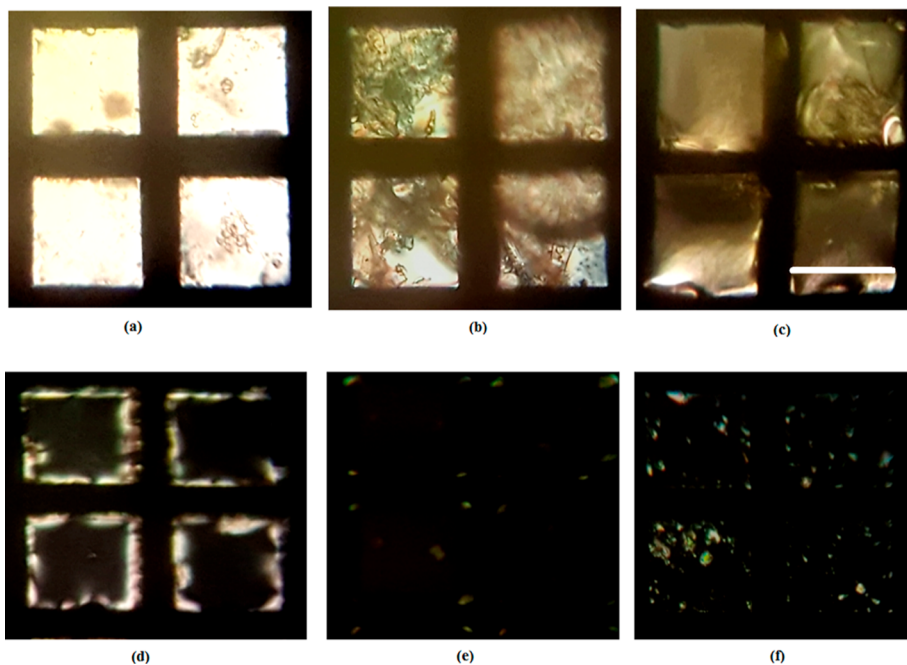
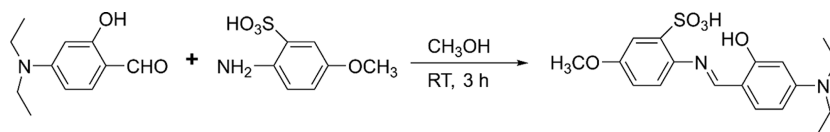


Figure 1. LC-based systems' polarized images at different weight percentages of MBA. (a) 0.2, (b) 0.4, (c) 0.5, (d) 0.6, (e) 0.8, and (f) 1. Scale bar: 150 μm .

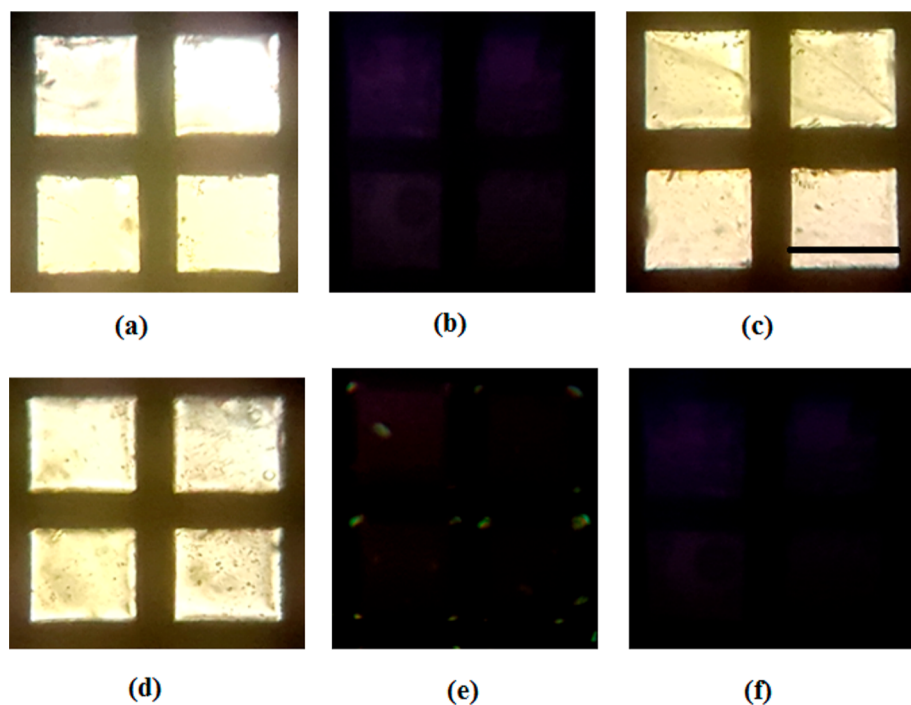


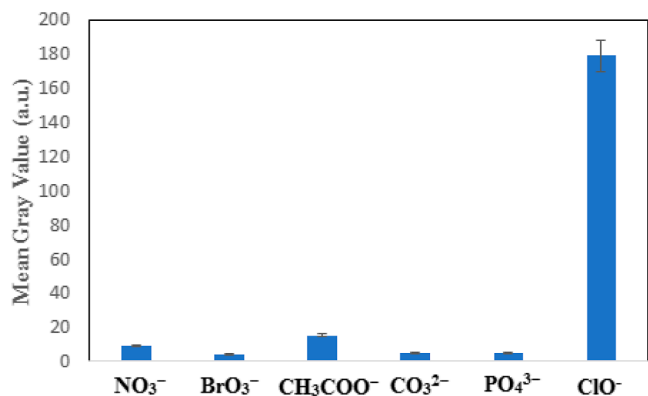
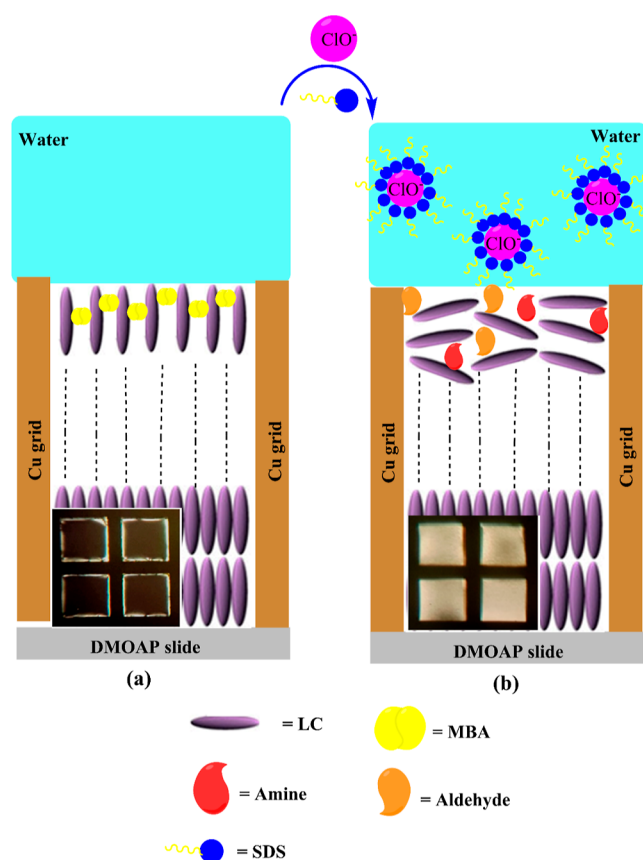
Figure 2. POPs of filled Cu grids (a) submergence of 0.6% MBA-doped 5CB in 10 μM ClO^- , (b) 0.6% MBA-doped 5CB submersed in 0 μM ClO^- solution, (c) 0.6% aldehyde-doped 5CB submersed in 0 μM ClO^- solution, (d) 0.6% amine-doped 5CB submersed in 0 μM ClO^- solution, (e) pure 5CB submersed in 10 μM ClO^- solution, and (f) pure 5CB submersed in 0 μM ClO^- solution. It indicates that bright LC picture emerged only when MBA and ClO^- are both existent in the platform. Scale bar: 150 μm .

device that loaded LC-based sensing platforms. Furthermore, LC-based sensing platforms were evaluated with regard to their abilities to detect ClO^- .

RESULTS AND DISCUSSION

MBA was synthesized by a reaction in which 4-(diethylamino)-salicylaldehyde condenses with 2-amino-5-methoxybenzenesul-

Scheme 2. ClO^- Detection by an LC-Based Sensory System; (a) At the Interface between the LC and Aqueous Phase, MBA Molecules Align LCs Homeotropically, (b) At the LC/Aqueous Junction, Oxidative Cleavage of MBA Imine Bond Disrupts LC Orientation from Homeotropic to Planar/Tilted Alignment



fonic acid (Scheme 1), as reported previously by Yun et al.¹² Yun et al. revealed that hypochlorite ion is able to react with MBA through oxidative cleavage of the imine bond.

After confirming the reaction of MBA with ClO^- , in order to detect ClO^- in aquatic medium, we constructed a LC-based sensing system using MBA. A first study was conducted to investigate the orientation response of the 5CB in the presence of varying weight percentages of prepared MBA at the water/LC junction. Cu grids containing 5CB doped-MBA were placed on ([3-(trimethoxysilyl)propyl]octadecyldimethylammonium chloride) DMOAP-coated glass slides to stabilize the connection between the LC and water phase. In air, the surface of a 5CB is observed as dark before immersion in an aqueous solution. LC is vertically anchored at the air/LC interface due to the homeotropic alignment of the LC at the interface provided by the DMOAP-coated glass slides.¹³ In de-ionized water, on a DMOAP-covered slide, we submersed a Cu grid loaded with pure 5CB. The 5CB optical image became bright after immersion in water, indicating that LC is either planar or distorted when it comes in contact with water, while its bottom surface is homeotropic. As a final step, copper grids with doped 5CB at diverse concentrations of MBA were submersed in de-ionized water to observe the structures (Figure 1). With a weight percentage of MBA less than 0.6, in 5CB, a bright image is observed, which indicates that MBA could not achieve LC homeotropic orientation at the LC/water junction up to 0.6 wt % concentration in 5CB. Bright images with dark areas are observed in high weight percent MBA (0.6 wt % doped with 5CB). The data suggest that MBA can partially orient the 5CB molecule in the LC/aqueous interface. Fully dark-stabilized optical images were obtained within 30 min after doping 5CB with 0.8 wt % MBA. In the LC/aqueous junction, the 5CB obtained a uniform dark optical image by transitioning from an orientation to a homeotropic orientation. There is a hydrophobic interaction between 5CB and MBA at the LC/water junction, which results in the homeotropic alignment of LCs. We ran all tests with a weight of 0.6 wt % MBA in 5CB. Consequently, at a concentration of MBA ≥ 0.6 wt %, one observes an absolutely dark image of the LCs.

In general, the hypochlorite solutions available commercially are prepared as aqueous sodium hydroxide solutions. First, it should be confirmed that the preparation of ClO^- solution using NaOH as a solvent does not affect the alignment of LC molecules at the 0.6% MBA-doped 5CB water/LC interface. As a matter of fact, after injecting $400 \mu\text{L}$ of NaOH solution ($10\text{--}100 \mu\text{M}$) into the 0.6% MBA-doped 5CB water/LC interface (without ClO^-) and incubating for 30 min, the LC was observed under polarized light microscopy. The dark image confirms that NaOH does not affect the alignment of LC molecules at the interface (Figure S1). In a further study, to observe whether the liquid-crystal optical image changes in the presence of ClO^- , on a DMOAP-coated slide, we placed a copper grid, then loaded it with 5CB doped with 0.6% MBA as LC selective to ClO^- . The entire system was then submerged in the 0.5% sodium dodecyl sulfate (SDS) aqueous solution with or without $10 \mu\text{M}$ ClO^- . According to Figure 2a, LC polarized optical pictures (POPs) were bright after being soaked for 10 minutes in $10 \mu\text{M}$ ClO^- solution. However, Figure 2b indicates that without ClO^- under comparable conditions, the POP of LCs was dark. After 6 h, there was no significant change in the LC images. This means that if there is ClO^- in a solution, you can tell by the way the LC pictures change from dark to bright. A schematic of the proposed LC/water detection mechanism is shown in Scheme 2. The SDS

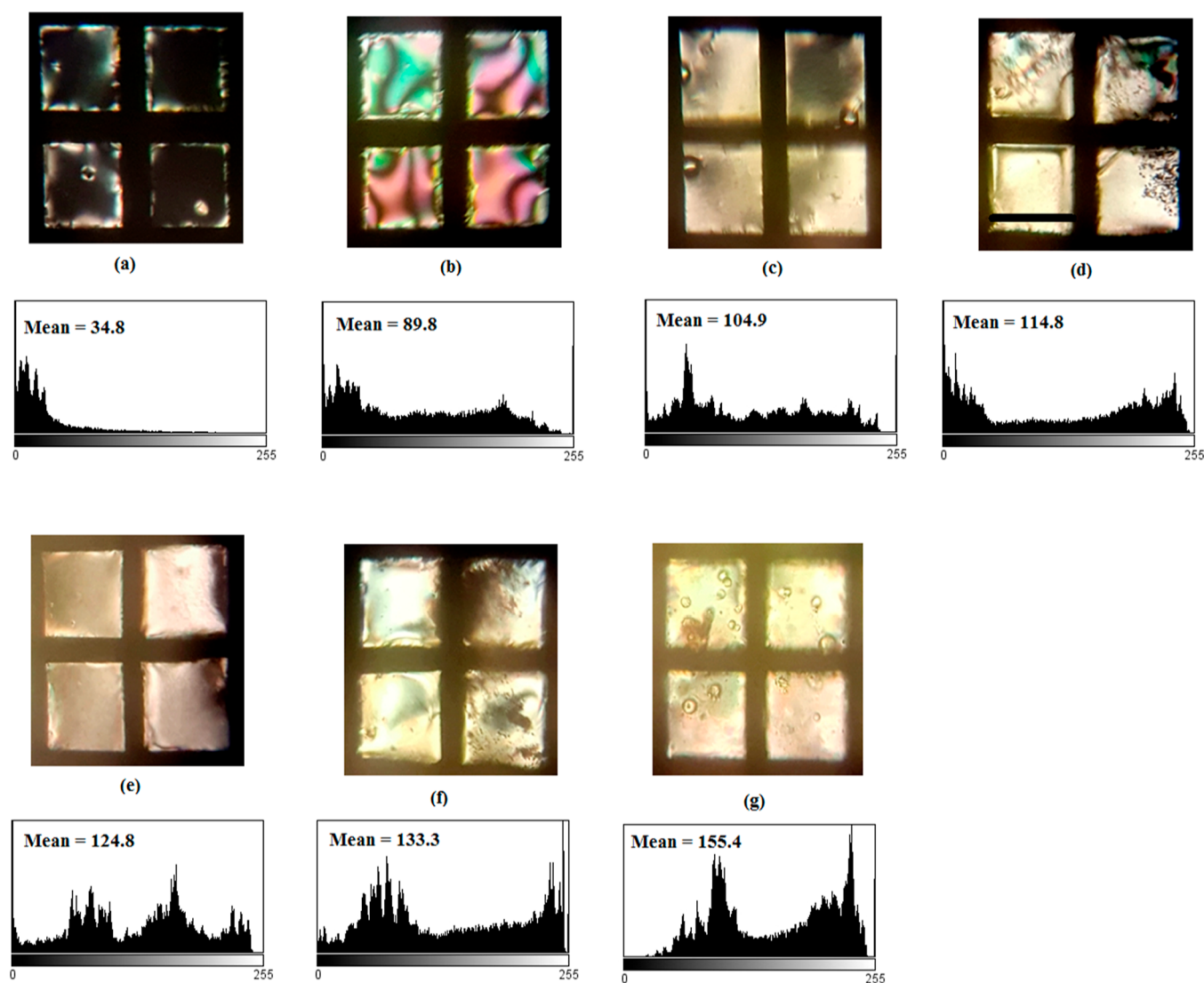


Figure 4. POPs of 0.6% MBA-doped SCB-loaded grids submerged in the aquatic solution containing (a) 0.05, (b) 0.25, (c) 0.50, (d) 5.0, (e) 10.0, (f) 100.0, and (g) 1000.0 μM of ClO^- . This platform has a LOD of 0.05 μM for ClO^- . Scale bar: 150 μm .

was added to the ClO^- solution only so that the ClO^- ions could interact with the MBA imine bond in SCB doped with MBA to initiate oxidative cleavage. A major function of it is to allow ClO^- ions to enter the organic phase from the aqueous phase (MBA–SCB) more effectively. According to common knowledge, bright textures are caused by the transition of LCs from homeotropic alignment to planar/tilted alignment at the interface between LCs and water. In the absence of ClO^- ions in solution, MBA molecules in SCB align homeotropically with LCs at the LC/aqueous interface, resulting in a dark image. In aqueous solutions containing ClO^- ions, the oxidative cleavage of the MBA imine bond occurs at the LC/water interface. As reported elsewhere, this observation was similar to that made by other LC-based sensors in aqueous media.^{11,14} The mechanism of the MBA imine bond cleavage by ClO^- ions was previously well demonstrated by Yun et al.¹² At the LC/aqueous interface, MBA imine cleavage disturbs the alignment of LCs from homeotropic to planar/tilted alignment, resulting in a bright image (Scheme 2b). To better investigate this event, we doped SCB separately with 0.6% 4-(diethylamino)salicylaldehyde and 2-amino-5-methoxybenzenesulfonic acid and considered their LC-doped POPs. It was found that none of them were able to re-orient the

LC, and their POPs were bright (Figure 2c,d). In addition, we have performed two more control studies with pure SCB in the platform. Figure 2e,f indicates that both LC pictures are dark regardless of whether ClO^- was available or not, indicating that the existence of ClO^- in the aqueous solution does not have the ability to reorient LCs. Of course, the hydrolysis mechanism of the imine bond by ClO^- cannot be considered as the complete mechanism of the sensor's action, and the following possibilities are also possible. The ClO^- ion may chlorinate or oxidize SCB at the benzylic position in the presence of a phase-transfer catalyst or hydrolyze the CN group as a blank study of SCB in the presence of ClO^- ions in the absence of MBA (Figure 2e) shows some light leakage. Also, MBA is a sulfonate that may act as a phase-transfer catalyst to accelerate oxidation or chlorination.¹⁵

Different anions including NO_3^- , BrO_3^- , CH_3COO^- , CO_3^{2-} , and PO_4^{3-} were used to evaluate the selectivity of the LC-based sensory system. Based on Figure S2, POP of LC is bright only in solutions containing ClO^- but not in solutions containing other anions. POPs' mean gray values are presented in Figure 3. Based on these results, it appears that this platform will be able to detect ClO^- over a range of anions with good selectivity.

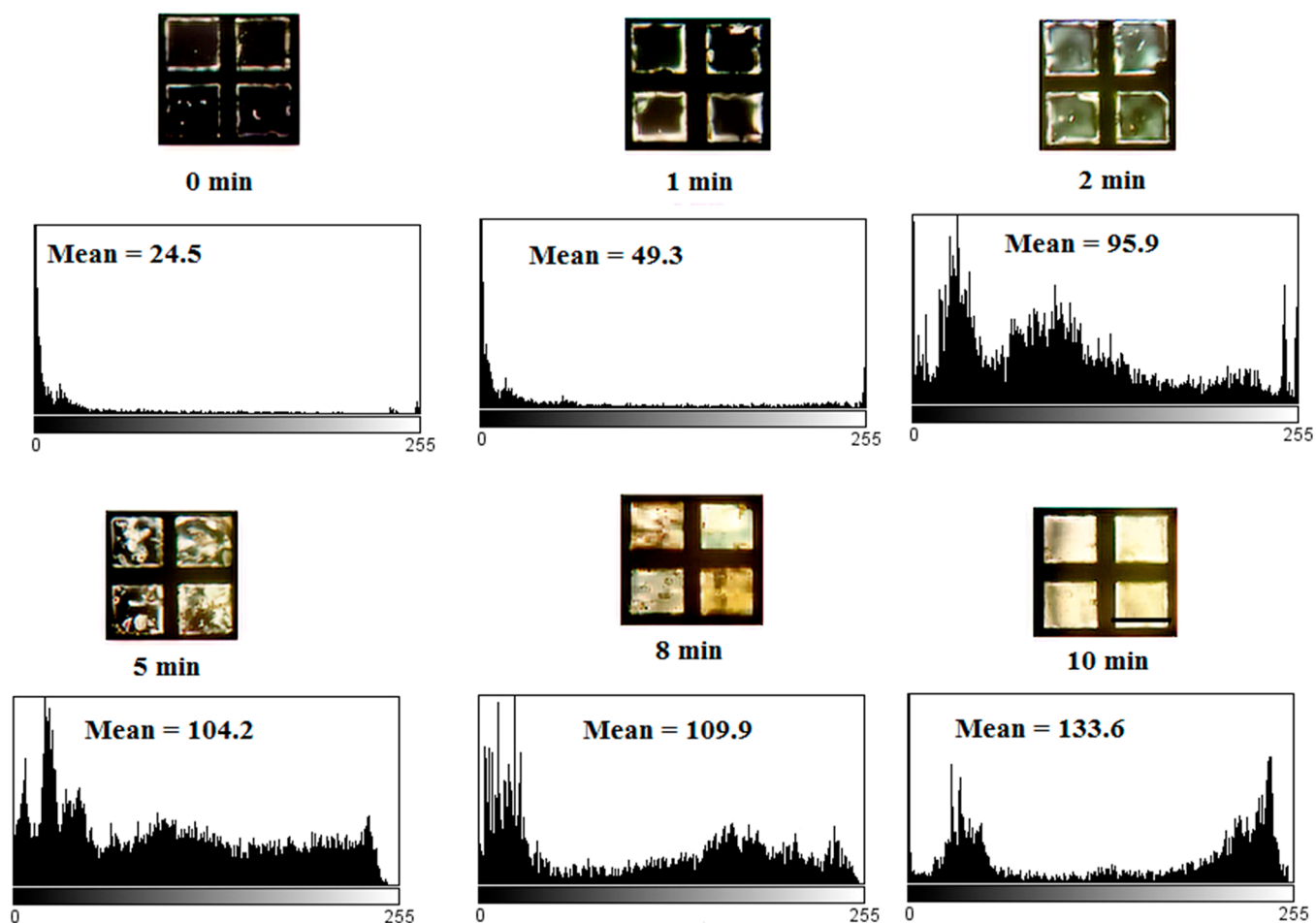


Figure 5. POPs of time profile of 0.6% MBA-doped 5CB-loaded grids submersed in the aquatic solution containing $10 \mu\text{M ClO}^-$. Scale bar: $150 \mu\text{m}$.

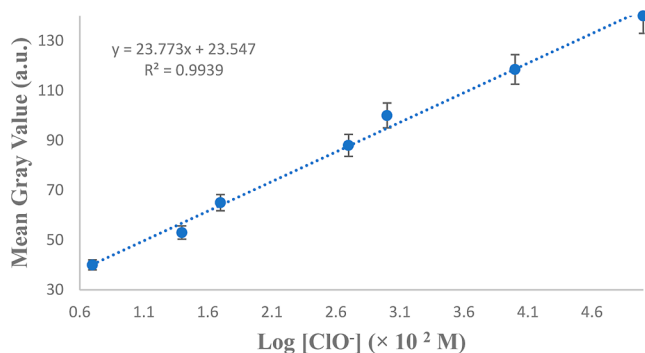


Figure 6. Graph showing mean gray value vs $\log [\text{ClO}^-]$ ($n = 3$) ($[\text{ClO}^-] \times 10^2 \text{ M}$).

Table 1. Comparison of Previously Reported ClO^- Detection Sensors with the Studied LC Sensor

sensor	method of detection	LOD (μM)	refs
p-methoxyphenol-substituted aniline compound	colorimetric	1.74	17
azobenzene acid	colorimetric	2.0	18
an imine-based chemodosimeter	colorimetric	0.95	12
a benzothiazole-based probe	fluorescence	1.74	2
a phenanthridine-based probe	fluorescence	0.008	19
a ligand-doped liquid crystal-based sensor	liquid crystal	0.05	present study

Using different concentrations of ClO^- , we then tested the limit of detection (LOD) of the system for ClO^- detection. The polarized light images and brightness quantities are illustrated in Figure 4. Figure 4 indicates that after 10 minutes when the ClO^- concentration was greater than $0.05 \mu\text{M}$, the POP of LC was bright, while it was dark when the ClO^- concentration was less than $0.05 \mu\text{M}$. Taking these findings into account, we can establish that the LOD for ClO^- using this platform is $0.05 \mu\text{M}$. As defined by the WHO, this amount is below the limit for ClO^- in drinking water ($\sim 3.3 \mu\text{M}$). Considering that the transition from dark to bright is usually not clear and may cause difficulty in detection, the time profile (0–10 min) and contrast of the transition from dark to light are quantitatively shown in Figure 5.

In order to quantify the optical signals from LCs, we utilized ImageJ software (NIH Freeware) to determine the gray scale value of the LC pictures of the area of interest. As shown in Figure 6, the mean gray value correlates with the $\log [\text{ClO}^-]$. According to the findings, ClO^- within the present project has a LOD of $0.05 \mu\text{M}$. A more accurate calculation of LOD was made using eq 1 (as per ICH Q2 guidance):¹⁶

$$\text{Limit of detection} = 3.3 \times (\text{SD of intercept/slope}) \quad (1)$$

Using this method, the detection limit was calculated at $0.08 \mu\text{M}$, which corresponds to Figure 4.

Compared to some existing sensing platforms for the ClO^- detection, our evaluation indicates high sensibility, as indicated in Table 1.

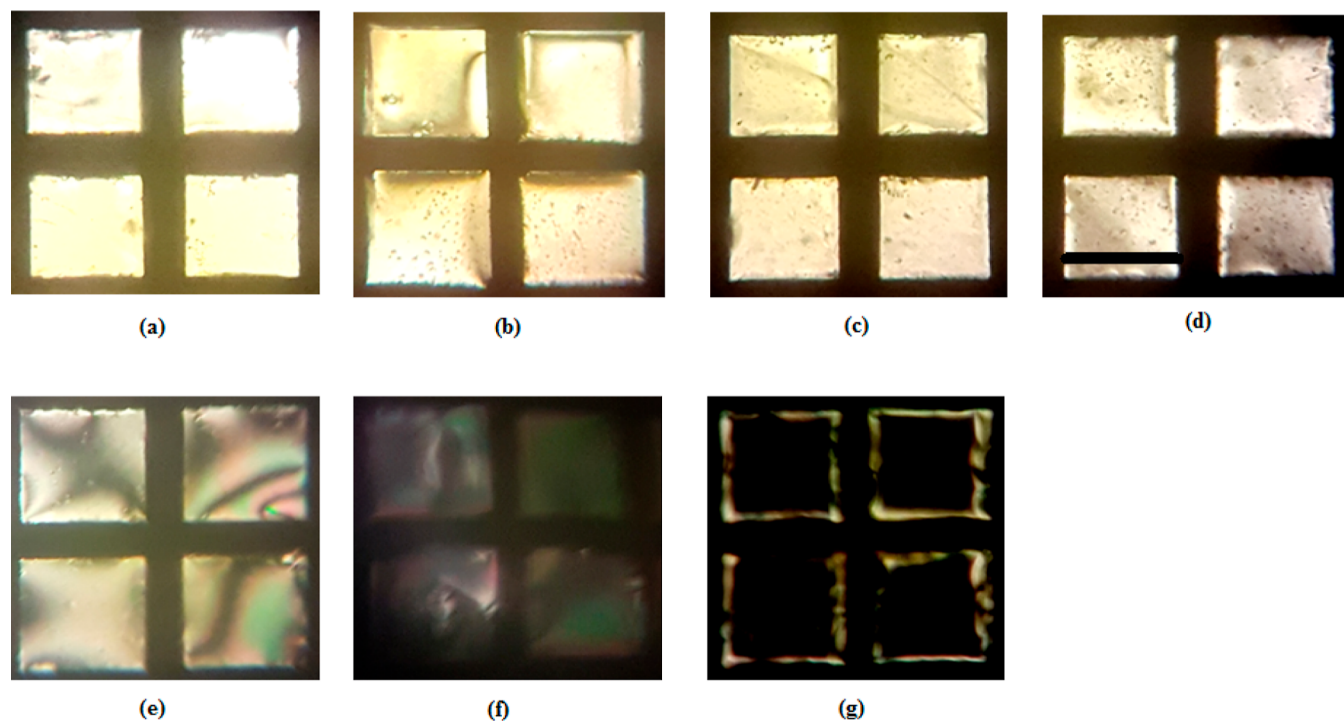


Figure 7. POPs of 0.6% MBA-doped 5CB-loaded grids submerged in tap water (a–g) comprising 1000, 100, 10, 5.0, 0.5, 0.25, and 0.05 μM of ClO^- , respectively. Scale bar: 150 μm . A test on real water samples shows that the platform can detect ClO^- at 0.05 μM .

A real water sample (piped water) was investigated as a test subject to see whether the sensor based on LCs can be used in real-life applications. This sensor can detect ClO^- in piped water up to a detection limit of 0.05 μM , as shown in Figure 7. As this amount is the same as the LOD of this system carried out in de-ionized water, possible interference from actual water samples has no effect on it. Additionally, this study showed that instantaneous detection of ClO^- in actual water can also be achieved by the LC-based sensory system.

CONCLUSIONS

We have synthesized MBA as a ligand that can react with ClO^- . With the help of this compound, a LC-based sensory system that can detect ClO^- in aquatic media rapidly and easily was fabricated. This technique employs selective oxidative cleavage of the imine bond of the MBA-doped LCs by ClO^- , which changes the reorientation of the LC and leads to a dark-to-bright conversion in optical characters that could be diagnosed by the naked eye. With this platform, ClO^- can be detected with great selectivity at 0.05 μM . In addition, we demonstrated that this sensory platform is also applicable to actual water samples like piped water.

EXPERIMENTAL SECTION

Materials. A microscopic glass slide HDA-7102 was used in this study. Sodium hypochlorite and all other salts, nematic 5CB LC (4'-pentyl-4-biphenylcarbonitrile) 98%, DMOAP, 4-(diethylamino)salicylaldehyde, 2-amino-5-methoxybenzenesulfonic acid, and Cu grids (150 mesh) were also used in this study. The Sigma Chemical Company provided sulfuric acid (99.9%) and hydrogen peroxide (30%). Commercially obtained hypochlorite solution was diluted with water. A Millipore system was used to refine the water. The campus of Islamic Azad University, Bandar Abbas Branch, provided piped water. In all aquatic solutions, de-ionized water has been used.

Synthesis of MBA. The MBA was prepared, as stated in the literature method.¹²

Method for Treating Glass Slides Coated with DMOAP.

For organic contamination removal, the slides were soaked for 1 h at 80 $^{\circ}\text{C}$ in 30% H_2O_2 and 70% H_2SO_4 (piranha solution). After washing five times with HPLC grade water and ethanol, they were dried with nitrogen and then baked at 120 $^{\circ}\text{C}$ for 3 h. In order to clean the glass slides, they were submerged in a 0.35% (v/v) DMOAP aquatic solution for 30 min, before being washed with high purity water and ethanol. The glass slides covered in DMOAP were dried with nitrogen and baked in a vacuum oven for 1 h at 100 $^{\circ}\text{C}$.

LC-Based Sensor Platform Assembly Method. The LC-based sensory system was constructed using a copper grid on a DMOAP-coated slide (6 mm \times 6 mm) filled with 2 μL of doped 5CB with MBA (isotropic state at 40 $^{\circ}\text{C}$). Following that, the excess LCs were removed using a capillary tube that had been cleansed. For the LC-based sensor cell, various concentrations of ClO^- (0.05 to 1000 μM) were prepared and then placed into the sensor for 10 min. By using a polarized optical microscope (Motic, BA 400 B-POL, Spain) in the transmission mode, the optic appearances of the samples were observed. A digital camera mounted on a microscope was used to take each photograph at 1/80 s. Three replications were performed on all experiments.

ClO^- Selectivity of the Liquid Crystal System. The specificity of the LC-based sensor platform was investigated for NO_3^- , BrO_3^- , CH_3COO^- , CO_3^{2-} , and PO_4^{3-} anions (each sample has a concentration of 10.0 μM). We replicated all the tests, and the data are represented as average \pm SD ($n \geq 3$).

Actual Sample Detection of ClO^- . To determine whether the proposed LC-based sensor works in actual water samples, piped water samples were used in this study. In order to remove coarse particles from the pre-treated piped water sample, it was filtered with a Whatman filter before analysis by the water

sample. A 5 min centrifugation at 10,000g followed, and the supernatant was collected. In order to eliminate the suspending materials, the sample was filtered five times through a 0.22 μm filter. A 100 μL volume of the real sample was incorporated into the LC-based sensing cell according to the aforementioned procedure. After 10 min of incubation, the LC-based sensing system was washed with de-ionized water, and images were taken.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.2c05250>.

POPs of filled copper grids, 0.6% MBA-doped SCB submerged in 10–100 μM sodium hydroxide solution, and testing the selectivity of the sensor in aqueous solutions containing NO_3^- , BrO_3^- , CH_3COO^- , CO_3^{2-} , PO_4^{3-} , and ClO^- (PDF)

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Notes

The authors declare no competing financial interest.

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