

Label-free liquid crystal-based detection of As(III) ions using ssDNA as a recognition probe

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ABSTRACT

We report a label-free liquid crystal (LC)-based biosensor for sensitive detection of arsenic (III) ions (As3+) in aqueous solutions, using an arsenic-binding aptamer (Ars-3 aptamer) as a molecular recognition element. In this sensing system, the cationic surfactant, cetyltrimethylammonium bromide (CTAB), was employed to induce a homeotropic orientation of the LCs at the aqueous/LC interface, a result of the self-assembly of CTAB molecules. In the absence of As3+, the addition of Ars-3 aptamers disturbed the self-assembly of CTAB at the aqueous/LC interface, due to interactions between CTAB and the Ars-3 aptamers, causing an orientational transition of LCs from homeotropic to planar. In the presence of As3+, the specific binding of the Ars-3 aptamers with As3+ led to the formation of an aptamer-As3+ complex, resulting in a conformational change of the aptamer. This change weakened the interaction between CTAB and the aptamer at the interface, causing the orientation of the LCs to remain unchanged in a homeotropic state. The changes in the orientation of the LCs caused by the interactions between the Ars-3 aptamer, As3+, and CTAB were simply converted and observed under a polarized light microscope as a shift between a bright and a dark image. A low detection limit of 50 nM (~3.7 ppb) was obtained, which is well below the maximum permissible level of As3+ in drinking water (133 nM) set by the US Environmental Protection Agency and the World Health Organization. Other chemical species (heavy metal ions) did not induce these changes, and thus, the biosensor was highly specific for As3+ ion sensing. The potential application of the developed sensor for As3+ detection in tap water was also demonstrated. Therefore, this LC-based biosensor is a promising platform for simple, rapid, label-free determination of As3+ concentration in aqueous samples with high selectivity and sensitivity.

1. Introduction

Arsenic is one of the most toxic heavy metals present in water, soil, rain, vegetables, and cereals [1]. It is a known carcinogen and is becoming a major hazard to the health of humans and ecosystems globally [2,3]. In the natural environment, arsenic exists in two forms, as trivalent arsenite (As3+) and pentavalent arsenate (As5+) [4]. Both As3+ and As5+ compounds are rapidly and extensively absorbed from the gastrointestinal tract; however, As3+ compounds are more water soluble and 60 times more toxic than As5+ compounds [3–5]. Prolonged consumption of arsenic-contaminated water or plants grown in an arsenic-contaminated area may lead to various health problems, such as skin lesions, diabetes, circulatory and cardiovascular disorders, neurological complications, many types of cancer, or even death [6–8]. Due to its high toxicity, the US Environmental Protection Agency and the World Health Organization have set the maximum permissible level for arsenic in drinking water as low as 10 ppb (10 µg L⁻¹ or 133 nmol

L⁻¹) [9].

Various conventional methods, such as high-performance liquid chromatography (HPLC) [10], atomic fluorescence spectroscopy [11], atomic absorption spectroscopy [12], inductively coupled plasma mass spectrometry [13, 14], and inductively coupled plasma atomic emission spectrometry [15], have been used for the detection of As3+ in water. Although these techniques can sensitively and accurately measure As3+ levels below 10 ppb (133 nmol L⁻¹), they have some drawbacks, such as the need for expensive and bulky equipment, chemicals, labeling of samples, and highly trained personnel [16,17]. Therefore, the development of simple and robust methods for the precise detection of As3+ levels, with high sensitivity and selectivity, is highly necessary.

Aptamers are artificial, single-stranded DNA or RNA oligonucleotides (ssDNA or ssRNA) that possess the ability to recognize a broad range of target molecules, including drugs [18], proteins [19–21], small molecules [22], and even cancer cells with high affinity and specificity

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[23]. Due to their distinctive features, such as simple synthesis and modification, stability in aqueous solutions, high selectivity, and cost-effectiveness, aptamers have been widely used as recognition elements for many novel biosensors [9]. Therefore, recently, several efficient aptamer-based sensing methods have been introduced for the determination of As^{3+} using As^{3+} -binding aptamer (known as Ars-3 aptamers) as molecular recognition elements. These include fluorescence [3–24–26], colorimetric [27–30], and electrochemical assays [1–31].

Liquid crystals (LCs) have been widely used as a promising sensing platform for monitoring chemical and biological events. The unique characteristics of LCs, such as their anisotropic properties, long-range orientational behavior, and molecular sensitivity, allow for the amplification and transduction of chemical and biological interactions into optical signals that are visible under a polarized light microscope (POM) [32–34]. The orientation of LCs is extraordinarily sensitive to small changes in the aqueous/LC interface at the molecular level. When the LC molecules are oriented perpendicular or parallel to the surface in homeotropic or planar orientations, respectively, the biochemical interactions at the aqueous/LC interface may induce a transition from a homeotropic to a planar orientation in the LC molecules [35]. This transition can be easily transduced into optical signals that can be observed under a POM [36]. Recently, the surfactant-laden aqueous/LC interface has been widely used in LC-based sensing systems for the detection of enzymatic activity [37–40], biomolecular interactions [41–43], or heavy-metal ions [44]. These sensing systems are based on the orientational transitions of LCs triggered by macromolecular binding or enzymatic hydrolysis events occurring at these interfaces, which can be easily amplified and transduced into optical signals under a POM. For example, our group recently developed a new LC-based sensing platform for the detection of heavy-metal ions using LC droplet patterns modulated by the interaction between a negatively charged surfactant and heavy-metal cations. Deprotonated stearic acid was employed as an anionic surfactant to induce homeotropic anchoring of the LCs at the aqueous/LC interface. Subsequently, the interactions of the heavy-metal ions with the deprotonated stearic acid head groups and the consequent interruption of the self-assembly of the acid molecules at the aqueous/LC interface, induce optical and orientational changes in the LC droplet patterns. However, the selectivity of this detection system is low, because the detection mechanism mainly depends on the electrostatic interaction between the positively charged heavy-metal ions and the negatively charged carboxylate groups [44].

In this study, we developed a simple LC-based biosensor for the sensitive detection of As^{3+} in aqueous solutions, using Ars-3 aptamers as molecular recognition elements. A cationic surfactant, cetyltrimethylammonium bromide (CTAB) moiety that self-assembled at the aqueous/LC interface, was employed to induce homeotropic anchoring of the LCs at the interface. In the absence of As^{3+} , the addition of Ars-3 aptamers can disturb the self-assembly of CTAB at the interface, due to interactions between positively charged CTAB and negatively charged Ars-3 aptamers, leading to a transition from a homeotropic to a planar orientation of the LCs at the interface. However, in the presence of As^{3+} , the specific binding of the Ars-3 aptamers with As^{3+} leads to the formation of an aptamer- As^{3+} complex, resulting in a conformational change of the aptamer [27,28–30]. We hypothesized that this conformational change may weaken the interaction between CTAB and the aptamer at the interface, which would cause the orientation of the LCs to remain in a homeotropic state (Fig. 1) We studied the orientational behavior of the LCs after contact with the Ars-3 aptamer at LC interfaces containing a self-assembled monolayer of the surfactant CTAB. We then examined the orientation of the LCs after transferring the Ars-3 aptamer, which had been pre-incubated with As^{3+} , onto the CTAB-coated LC interface. A low limit of detection (LOD) of 50 nM (~ 3.7 ppb) was obtained using this system. This LOD value is comparable to the LOD values of other reported methods [45–49]. In addition, this sensor showed a high selectivity for As^{3+} . Therefore, in this

label-free LC-based aptasensor is a promising platform for the real-time determination of As^{3+} in aqueous samples with high selectivity and sensitivity.

2. Materials and methods

2.1. Materials

Sodium (meta)arsenite (NaAsO_2 , $\geq 90\%$), CTAB, N-(2-hydroxyethyl) piperazine-N'-2-ethanesulfonic acid (HEPES, $\geq 99.5\%$), lead (II) chloride (98%), calcium chloride dihydrate (99%), zinc nitrate hexahydrate (98%), nickel (II) chloride hexahydrate, iron (II) chloride tetrahydrate (98%), mercury (III) nitrate monohydrate ($\geq 98.5\%$), and cobalt (II) chloride hexahydrate ($\geq 98\%$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nematic liquid crystal 4-cyano-4'-pentylbiphenyl (5CB) was obtained from Tokyo Chemical Industrial Co., Ltd. (Tokyo, Japan). A 100-mer DNA aptamer specific for arsenic (Ars-3 aptamer) was synthesized by Mbiotech (Hanam, South Korea), with the following sequence: 5'-GGT AAT ACG ACT CAC TAT AGG GAG ATA CCA GCT TAT TCA ATT TTA CAG AAC AAC CAA CGT CGC TCC GGG TAC TTC TTC ATC GAG ATA GTA AGT GCA ATC T-3'. This aptamer was chosen based on previous studies [27,28–30–50]. Before use, the aptamer was dissolved in 50 mM HEPES buffer solution, pH 7.4. n-Octyltrichlorosilane (OTS, 97%) was obtained from Alfa Aesar (Lancashire, England). Copper grids (75 mesh square, pitch = 340 μm , hole = 285 μm , bar = 55 μm) were purchased from Gilder Grids Ltd. (Lincolnshire, England). Micro glass slides were obtained from Matsunami Glass IND., Ltd (Osaka, Japan). Eight-well chamber slides were purchased from BD Biosciences (Bedford, MA, USA). Hydrogen peroxide (H_2O_2 , 30% w/v), sulfuric acid (H_2SO_4 , 95 %), ethanol, methanol, anhydrous n-heptane, and methylene chloride (CH_2Cl_2) were purchased from Daejung Chemicals & Metals Co., Ltd (Daejung, South Korea). Deionized (DI) water with a resistivity of 18.2 $\text{M}\Omega\cdot\text{cm}$ was generated using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Modification of micro glass slides with OTS

OTS-modified glass slides were prepared following procedures reported in previous publications [37–51]. Briefly, the micro glass slides were immersed in "piranha solution" ($\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2 = 7/3$ [v/v]) at 80 °C for 1 h. After cooling, the slides were washed in turn with DI water, ethanol, and methanol and were then dried under a stream of nitrogen and heated at 120 °C overnight. After this treatment, most of the organic matter was removed and hydroxyl groups (-OH) were formed on the surface of the glass slides. The "piranha-treated" glass slides were then modified with OTS. The glass slides were immersed in an OTS/n-heptane solution at room temperature for 30 min. After washing with methylene chloride, the glass slides were dried under nitrogen flow and stored in a desiccator until further use.

2.3. Preparation of optical cells

The OTS-treated glass slides were fixed to the bottom of an eight-well chamber slide using silicone glue (Shin-Etsu Silicone Korea Co., Ltd, Seoul, Korea). On each well, copper specimen grids were placed onto the surface of the OTS-treated glass slides. The grids were then filled with 1 μL of 5CB and excess 5CB was carefully removed using a 5 μL capillary tube. This procedure led to the formation of a uniform thin film of 5CB within the grid. Finally, 400 μL of aqueous solutions of interest was dispensed into the optical cell using a micropipette at room temperature (~ 25 °C). Each assay was repeated at least five times. After use, the eight-well chamber optical cells and the copper specimen grids were rinsed in turn with DI water, and ethanol and were dried in an oven at 37 °C, then were used again. The eight-well chamber optical cells and the copper specimen grids can be reused for at least 5 times

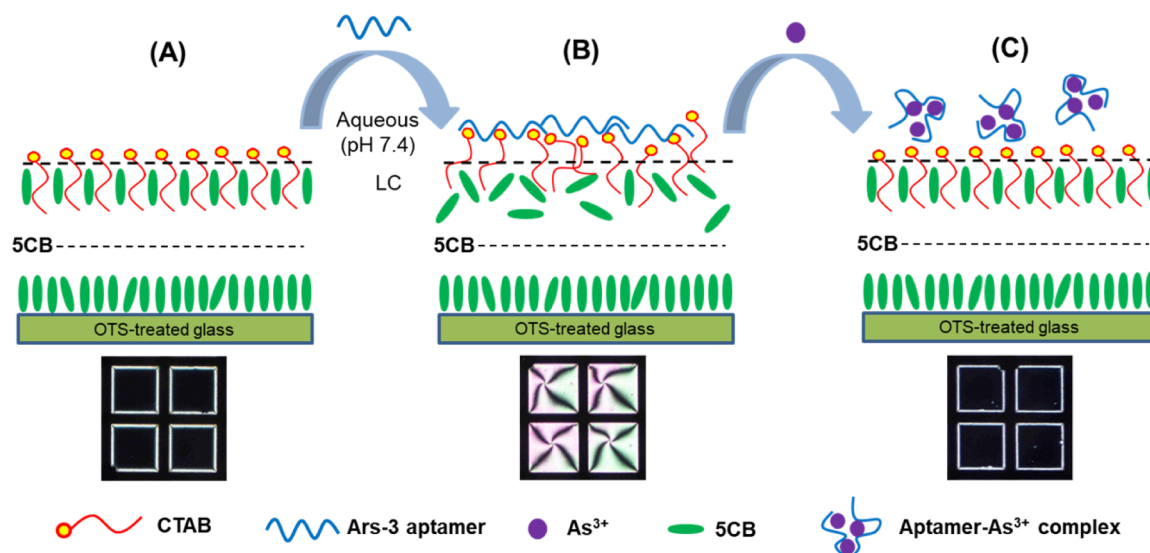


Fig. 1. Schematic illustration of the LC-based biosensor for the detection of As^{3+} ions based on the self-assembly of CTAB at the interface, influenced by a conformational change of the Ars-3 aptamer upon binding with the target. The lower images represent the polarized optical micrographs corresponding to the respective interfacial events at the aqueous/LC interface. OTS = *n*-octyltrichlorosilane.

without significant effects.

2.4. Preparation of aqueous solutions of CTAB, metal ions, and aptamers

All aqueous solutions were prepared in 1 mM HEPES buffer (pH 7.4). CTAB and metal ions solutions of different concentrations were prepared by dissolving solid powder materials in 1 mM HEPES (pH 7.4). Various concentrations of the Ars-3 aptamer solution were prepared by diluting a 100 μM Ars-3 aptamer stock solution with 1 mM HEPES (pH = 7.4). To detect As^{3+} ions, equal volumes of aqueous solutions of Ars-3 aptamer and As^{3+} were incubated at 37 $^{\circ}\text{C}$ for 2 h. After cooling to room temperature, the samples were introduced into the CTAB-laden aqueous/LC interface. To verify the selective binding of the Ars-3 aptamer to As^{3+} , other metal ions, such as Ca^{2+} , Co^{2+} , Fe^{2+} , Hg^{2+} , Ni^{2+} , Zn^{2+} , and Pb^{2+} were also tested under similar procedures. To verify the applicability of this method for the detection of As^{3+} in real samples, tap water collected from our laboratory was spiked with As^{3+} at a concentration of 1000 nM. The tap water sample was then mixed with 300 μM Ars-3 aptamer in a 1:1 volumetric ratio and incubated at 37 $^{\circ}\text{C}$ for 2 h. The final concentrations of As^{3+} and Ars-3 aptamer in the mixture were 500 nM and 150 μM , respectively.

2.5. Optical characterization of LC textures

A POM (Eclipse LV100 POL, Nikon, Tokyo, Japan), equipped with a digital camera (DS-2Mv, Nikon, Tokyo, Japan), was used to image the optical LC textures formed by polarized light transmitted through the optical cells filled with 5CB. All images were observed using 4 $\times$ objective lenses, at a resolution of 2560 $\times$ 1920 pixels, a gain of 3.4 $\times$, and a shutter speed of 1/50 s. The gray-scale intensity of all images was calculated using Adobe Photoshop CC2019 software. For each image, the gray-scale intensity of four squares of a copper grid were measured. The gray-scale intensity values were then averaged and the standard deviations were then calculated.

3. Results and discussion

3.1. LC-based sensing mechanism

In this study, we developed a simple LC-based biosensor with a cationic surfactant-laden LC thin layer on copper grids for sensitive

detection of As^{3+} in aqueous solutions, using an Ars-3 aptamer as a molecular recognition element. The self-assembled cationic surfactant layer induced homeotropic anchoring of the LCs at the aqueous/LC interface, corresponding to a dark image observed under a POM (Fig. 1A). The addition of Ars-3 aptamers disturbed the self-assembled monolayer of CTAB, due to interactions between positively charged CTAB and negatively charged aptamers, resulting in a transition from a homeotropic to a planar orientation of the LCs at the interface, which corresponded to a bright image observed under a POM (Fig. 1B). However, in the presence of As^{3+} , the specific binding of the Ars-3 aptamers with As^{3+} led to a conformational change of the aptamer, which may weaken the interaction between CTAB and the aptamer, causing the orientation of the LCs to remain in a homeotropic state, which corresponded to a dark image observed under a POM (Fig. 1C). Based on this sensing mechanism, we expected that As^{3+} in aqueous samples would be effectively detected.

3.2. Interaction between the ARS-3 aptamer and CTAB at the aqueous/LC interface

First, to monitor the optical response of 5CB to the surfactant, CTAB, 5CB was deposited into the holes of a copper grid supported on an OTS-treated glass slide to form a thin film. OTS with hydrocarbon tails can anchor the 5CB in a perpendicular (homeotropic) orientation at the glass/LC interface, due to the interactions between the hydrophobic tails of OTS and the LCs [51]. In previous studies, cationic surfactants have been shown to induce the homeotropic alignment of the LCs at the aqueous/LC interface [42,43]. Here, we used CTAB, a cationic surfactant that contains both a long hydrophobic chain and a hydrophilic head group, to regulate the orientation of 5CB at the aqueous/LC interface. The minimum concentration of CTAB required to induce the homeotropic alignment of the LCs at the aqueous/LC interface was determined. A volume of 400 μL of aqueous solutions of CTAB at different concentrations was introduced into the optical cells and incubated for 30 min. The optical images of LCs were then observed under a POM. As shown in Fig. S1, dark images of the LCs were observed as the concentration of CTAB was equal to or higher than 5 μM , corresponding to a homeotropic orientation of the LCs at the interface (Figs. S1c–1d). Thus, in subsequent experiments, a CTAB concentration of 5 μM was applied.

We next examined the change in LC optical response according to

the degree of interactions between the aptamer and the 5 μM CTAB-laden aqueous/LC interface. After incubating for 30 min, excess CTAB was removed from the bulk solution using a micropipette. Then, 400 μL of aqueous solutions of the Ars-3 aptamer at different concentrations was introduced into the CTAB-laden aqueous/LC interface and incubated for 30 min. The optical images of LCs were then observed using a POM. Fig. S2 shows the optical images of the LCs after incubating the CTAB-laden aqueous/LC interface with the Ars-3 aptamer at different concentrations for 30 min. It was first necessary to confirm that the HEPES buffer used as a solvent to dissolve the Ars-3 aptamer did not affect the alignment of the LC molecules on the CTAB-laden aqueous/LC interface. Indeed, after introducing 400 μL of 1 mM HEPES buffer solution ($\text{pH} = 7.4$) into the 5 μM CTAB-laden aqueous/LC interface and incubating for 30 min, a dark image of the LCs was observed under a POM, verifying that the HEPES buffer did not affect the alignment of the LC molecules on the interface (Fig. S2a). However, as the concentration of aptamer increased, the optical appearance of the LCs gradually changed from dark to bright, corresponding to a perpendicular-to-planar shift in the orientation of the LCs (Figs. S2b–2e). This is because the electrostatic interactions of the aptamer with CTAB increase in proportion to the aptamer concentration, leading to the disruption of the self-assembled CTAB monolayer at the interface, resulting in a transition of the LCs from a perpendicular to a planar orientation [36]. When the concentration of the Ars-3 aptamer was lower than 150 μM , the images of the LCs remained dark or not fully bright, corresponding to a perpendicular or random orientation, respectively (Figs. S2a–2c). However, as the aptamer concentration increased to 150 μM or higher, completely bright images, corresponding to a planar orientation of the LCs, were observed (Fig. S2d–2e). These LC images remained the same for at least 24 h (1 day). These results indicated that 150 μM is the minimum concentration of Ars-3 aptamer required to disturb the alignment of the CTAB molecules at the interface and induce a planar orientation of the LCs, resulting in bright images of the LCs. Thus, an Ars-3 aptamer concentration of 150 μM was used for subsequent experiments to detect As^{3+} ions.

3.3. Sensitivity of the biosensor for As^{3+} detection

Next, we determined the sensitivity and response time of the designed LC-based biosensor by investigating the orientational behavior of the LCs after transferring Ars-3 aptamer solutions that had been pre-incubated with As^{3+} , onto the CTAB-laden aqueous/LC interface. As mentioned in the Introduction section, the specific binding of the Ars-3 aptamers with As^{3+} leads to the formation of an aptamer- As^{3+} complex, resulting in a conformational change of the aptamer. This conformational change may weaken the interaction between CTAB and the aptamer at the interface, which would cause the orientation of the LCs to remain in a homeotropic state. Here, 150 μM Ars-3 aptamer was pre-incubated with different concentrations of As^{3+} , ranging from 0 to 1000 nM, at 37 $^{\circ}\text{C}$ for 2 h before being added to the optical cells containing a 5 μM CTAB-laden aqueous/LC interface. The optical appearances of LCs were observed under a POM at specific time points (Fig. 2). For As^{3+} concentrations below 25 nM, the optical appearance of the LCs began to brighten after 10 min and the bright domains of the LC images gradually expanded to the whole image area after 30 min. This result indicated that, as the concentration of As^{3+} became lower than 25 nM, the interactions between Ars-3 aptamer and CTAB were still strong enough to disrupt the alignment of the LCs at the interface, leading to a transition from a perpendicular to a planar orientation, and consequently, a transition from a dark to a bright image when observed under a POM. However, for concentrations of As^{3+} from 25 to 300 nM, the optical appearance of the LCs took at least 20 min to begin to brighten and the bright domains of the LC images did not cover the entire image area, even after 30 min. At concentrations of As^{3+} higher than 300 nM, the optical appearances of the LCs were completely dark, even after 30 min. Therefore, the time required to observe the transition

from a perpendicular to a planar orientation increased as the concentration of As^{3+} increased. This indicated that the interaction between Ars-3 aptamer and CTAB on the interface is weaker as the concentration of As^{3+} increases. Another observation was made from the data presented in Fig. 2, when comparing the optical appearances of the LCs after 30 min of incubation. As the concentration of As^{3+} increased from 0 to 1000 nM, the optical appearances of the LCs gradually changed from bright to dark after 30 min, corresponding to the transition from a planar to a perpendicular orientation of the LCs at the interface. These results can be attributed to the specific binding of the Ars-3 aptamer to As^{3+} ions, which forms an aptamer- As^{3+} complex and induces a conformational change in the Ars-3 aptamer. This is likely to weaken the interaction between CTAB and the Ars-3 aptamer at the aqueous/LC interface. This event causes the LCs at the interface to remain in a homeotropic alignment, leading to a dark optical appearance of the LCs when observed under a POM. Therefore, these findings demonstrated that As^{3+} ions in aqueous samples can be monitored using this simple and rapid LC-based sensing technique.

After we confirmed the feasibility of this LC-based sensing system for the detection of As^{3+} ions, the LOD of this biosensor was also evaluated by comparing the average gray-scale intensity (AGI) of LC images after 30 min of incubation of the 5 μM CTAB-laden aqueous/LC interface with 150 μM Ars-3 aptamer pre-incubated with different concentrations of As^{3+} . As mentioned in the Materials and Methods section, each assay was repeated at least five times and the gray-scale intensity values were then averaged and the standard deviations were then calculated. Table 1 shows that the AGI values were almost unchanged when the concentration of As^{3+} was equal to or lower than 25 nM. However, as the concentration of As^{3+} increased from 50 nM to 1000 nM, the AGI values significantly decreased. The lowest AGI values were observed when the concentration of As^{3+} was 500 nM or higher, corresponding to the completely dark LC images. Therefore, based on the AGI values, the LOD of this biosensor for As^{3+} was 50 nM, which is well below 133 nM, the maximum permissible level of As^{3+} in drinking water [9]. Table 1 also shows low standard deviations that indicate high reproducibility of the data. The LOD of the proposed LC-based sensor was then compared to that of the recently reported sensing methods for the detection of As^{3+} , as illustrated in Table 2. While other sensing methods, such as colorimetric, resonance scattering, fluorescence, and electrochemical techniques require expensive, complex instrumentation and skilled technicians to operate the bulky instruments, the proposed LC-based sensing method offers a simple, rapid, and label-free method for the detection of As^{3+} ions in aqueous solutions, with a comparable or lower LOD than current methods.

3.4. Selectivity of the LC-based biosensor

The selectivity of the LC-based biosensor was also investigated. A variety of competitive heavy metal ions, including Ca^{2+} , Co^{2+} , Ni^{2+} , Fe^{2+} , Zn^{2+} , Pb^{2+} and Hg^{2+} were tested using a similar procedure as the As^{3+} detection method. The optical images and their corresponding AGI values were collected and compared. As can be seen in Figs. 3a–h, the optical appearances of LCs changed from dark to completely bright within 30 min when 150 μM Ars-3 aptamer was incubated with 500 nM Ca^{2+} , Co^{2+} , Ni^{2+} , Fe^{2+} , Zn^{2+} , Pb^{2+} , or Hg^{2+} , while it remained dark, even after 30 min, when 150 μM Ars-3 aptamer was incubated with 500 nM As^{3+} . Moreover, after a constant 30 min of incubation of the aptamer with various metal ions, the AGI decreased to its lowest value, only when As^{3+} was present in the aqueous samples (Table 3). These findings implied that these other metal ions could not bind to the Ars-3 aptamer and thus, would not weaken the interaction between the aptamer and CTAB at the interface. These observations demonstrated that the designed LC-based biosensor was highly selective towards As^{3+} and suggested its potential application for the selective analysis of As^{3+} over other competitive metal ions.

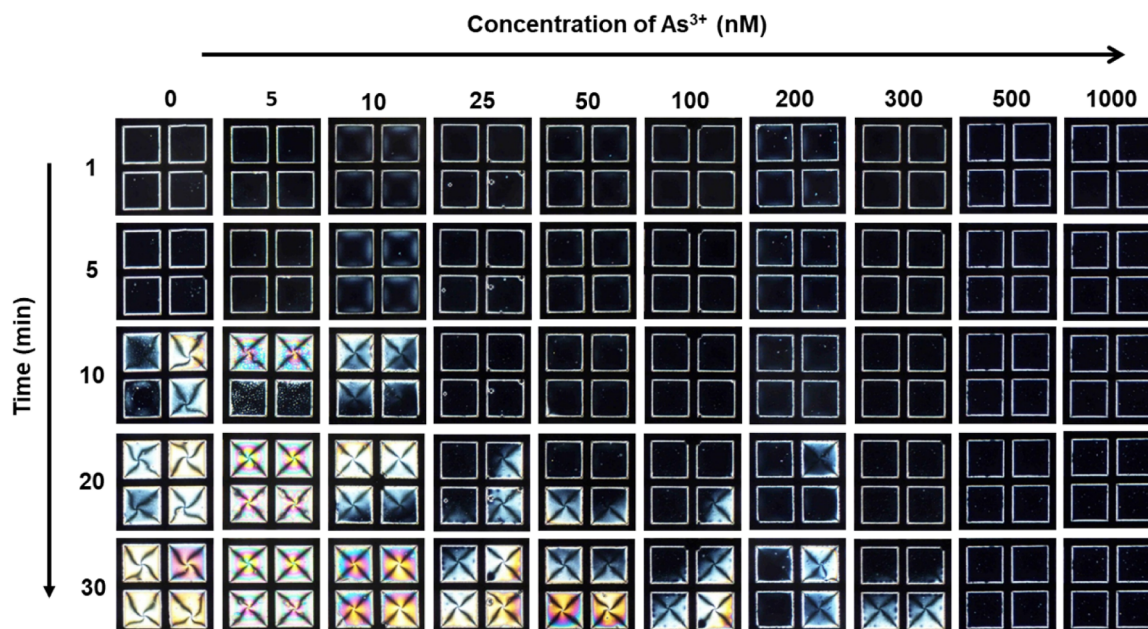


Fig. 2. Optical LC images observed under a POM, after incubating the 5 μM CTAB-laden aqueous/LC interface with 150 pM Ars-3 aptamer, pre-incubated with different concentrations of As^{3+} , for different times. The size of each hole in the copper grid was 285 μm .

Table 1

Average gray-scale intensity of LC images after 30 min of incubation of the 5 μM CTAB-laden aqueous/LC interface with 150 pM Ars-3 aptamer pre-incubated with different concentrations of As^{3+} .

Concentration of As^{3+} (nM)	Average gray-scale intensity (a.u.)	Standard deviation
0	135.18	3.5
5	129.31	6.1
10	120.07	4.8
25	119.41	7.2
50	90.1	4.7
100	87.86	2.3
200	84.1	3.9
300	66.4	5.1
500	29.91	1.9
1000	29.76	2.2

3.5. Detection of As^{3+} in tap water sample

The developed sensor was tested in spiked tap water to verify the applicability of this method for the detection of As^{3+} in a real sample. Tap water was spiked with 1000 nM As^{3+} and then mixed with 300 pM Ars-3 aptamer in a 1:1 volumetric ratio. The final concentrations of As^{3+} and the aptamer in the mixture were 500 nM and 150 pM, respectively. After pre-incubation at 37 $^{\circ}\text{C}$ for 2 h, the mixture was introduced into the CTAB-laden aqueous/LC interface and incubated for 30 min. The optical response of the LCs was observed under a POM. Tap water without the addition of As^{3+} was used as a control. Fig. 4a

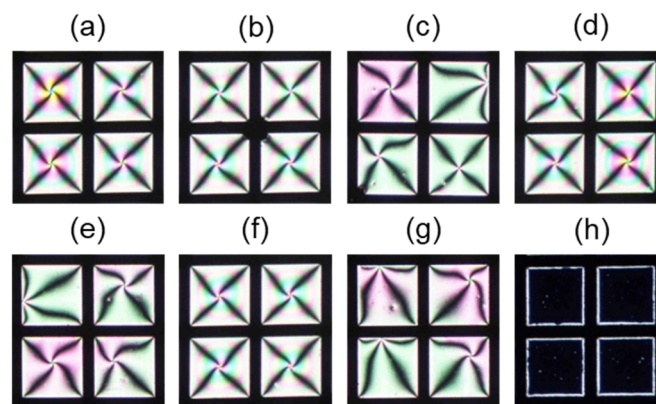


Fig. 3. Optical LC images observed under a POM after 30 min of incubation of the 5 μM CTAB-laden aqueous/LC interface with 150 pM Ars-3 aptamer pre-incubated with 500 nM different metal ions: (a) Ca^{2+} , (b) Co^{2+} , (c) Ni^{2+} , (d) Fe^{2+} , (e) Zn^{2+} , (f) Pb^{2+} , (g) Hg^{2+} , and (h) As^{3+} . The size of each hole in the copper grid was 285 μm .

shows that in the control sample, the optical appearance of LCs gradually turned bright within 30 min of incubation. However, when tap water spiked with 500 nM As^{3+} was introduced into the sensing system, the dark optical appearance of the LCs remained unchanged after 30 min of incubation (Fig. 4b). These results demonstrated the potential application of the designed LC-based biosensor for As^{3+} detection in tap water.

Table 2

Comparative analysis of arsenic sensors.

Method applied	Target	Detection range (μM)	Detection limit (nM)	Reference
Colorimetry & resonance scattering	As^{3+}	0.013–20	53	[28]
Colorimetry & fluorescence	As^{3+}	0.1–100	60	[45]
Colorimetry	As^{3+}	0.1–100	31.8	[46]
Fluorescence	As^{3+}	0.067–13.4	55	[47]
Colorimetry	As^{3+}	5–500	2000	[48]
Whole-cell	As^{3+}	66.7–186.9	100	[49]
Liquid crystal	As^{3+}	0.02–1	50	Present study

Table 3

Average gray-scale intensity of LC images after 30 min of incubation of the 5 μ M CTAB-laden aqueous/LC interface with 150 pM Ars-3 aptamer pre-incubated with 500 nM different metal ions.

Metal ion	Average gray-scale intensity (a.u.)	Standard deviation
Ca ²⁺	132.55	5.3
Co ²⁺	134.16	7.1
Ni ²⁺	133.63	4.9
Fe ²⁺	133.61	3.8
Zn ²⁺	130.41	4.7
Pb ²⁺	134.85	5.0
Hg ²⁺	128.55	2.9
As ³⁺	29.91	4.1

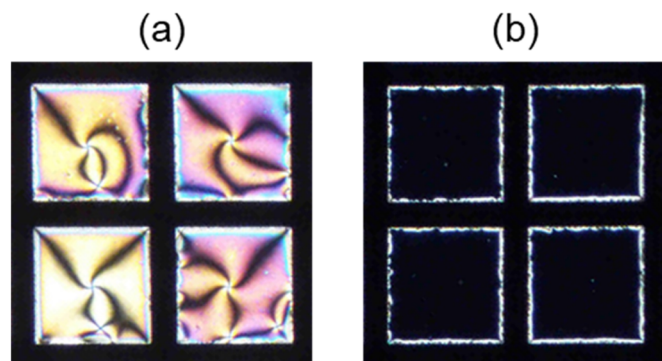


Fig. 4. Optical LC images observed under a POM after 30 min of incubation of the 5 μ M CTAB-laden aqueous/LC interface with 150 pM Ars-3 aptamer pre-incubated with (a) tap water (control), and (b) tap water spiked with 500 nM As³⁺. The size of each hole in the copper grid was 285 μ m.

4. Conclusions

In summary, we have successfully developed a novel LC-based biosensor, using an arsenic-binding aptamer as a recognition element, for the detection of As³⁺ in aqueous solutions with high sensitivity and selectivity. The principle of such a biosensor is based on the change in LC orientation, which is controlled by the unique interactions among the arsenic-binding aptamer, CTAB, and As³⁺. In the absence of As³⁺, the interactions between Ars-3 aptamers and CTAB disturb the self-assembled monolayer of CTAB at the aqueous/LC interface, resulting in a transition from a homeotropic to a planar orientation of the LCs. However, in the presence of As³⁺, the specific binding of the Ars-3 aptamers to As³⁺ leads to the formation of an aptamer-As³⁺ complex, which weakens the interaction between CTAB and the aptamer, so that orientation of the LCs remains unchanged in a homeotropic alignment. This variation in LC orientation was readily observed under a POM. The detection limit of this sensing system may be set as low as 50 nM. Moreover, the LC-based biosensor demonstrated high selectivity towards As³⁺ over other competitive metal ions. In addition, it rapidly and reliably detected As³⁺ in tap water samples. This LC-based aptamer sensing method not only offers a simple, rapid, and label-free method for the detection of As³⁺ in aqueous solutions with high selectivity and sensitivity, but may also be extended to the detection of other toxic metal ions in the natural environment.

CRediT authorship contribution statement

Duy Khiem Nguyen: Conceptualization, Methodology, Data curation, Writing - original draft, Writing - review & editing, Visualization, Investigation. **Chang-Hyun Jang:** Conceptualization, Methodology, Funding acquisition, Supervision.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.microc.2020.104834](https://doi.org/10.1016/j.microc.2020.104834).

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