Colloids and Surfaces B: Biointerfaces 200 (2021) 111587

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

An acetylcholinesterase-based biosensor for the detection of pesticides using liquid crystals confined in microcapillaries

Duy Khiem Nguyen, Chang-Hyun Jang *

Department of Chemistry, Gachon University, Seongnam-daero 1342, Sujeong-gu, Seongnam-si, Gyeonggi-do, 13120, Republic of Korea

ARTICLE INFO

Keywords:
Liquid crystals
Microcapillary biosensor
Acetylcholinesterase
Myristoylcholine
Pesticides
Inhibitors

ABSTRACT

Here, we demonstrate a capillary-sensing platform based on liquid crystals (LCs) confined in microcapillaries for simple and sensitive detection of acetylcholinesterase (AChE) and its inhibitors. LC droplets were formed through sequential injection of LCs and an aqueous solution into trichloro(octyl)silane (OTS)-treated microcapillaries. When the confined LC droplets make contact with a cationic surfactant solution, myristoylcholine chloride (Myr), the formation of a Myr monolayer at the aqueous/LC interface induces a horizontal orientation of the LCs at the interface along the microcapillary, producing an optical LC droplet texture of a four-petal shape. On the other hand, AChE can catalyze the hydrolysis of Myr into choline and myristic acid. The hydrolyzed Myr is unable to form a monolayer at the aqueous/LC interface, and therefore the confined LC droplets exhibit two bright-lined optical images when in contact with the pre-incubated mixture of Myr and AChE, corresponding to the homeotropic orientation of LCs at the interface. However, in the presence of AChE-inhibiting pesticides, such as fenobucarb and malathion, the activity of AChE is inhibited, and thus, the enzymatic hydrolysis of Myr cannot occur. As a result, the confined LC droplets present the four petal-shaped optical images when in contact with the pre-incubated mixture of Myr, AChE, and pesticides. Based on this principle, an LC-based microcapillary sensor was developed and utilized for the detection of pesticides. Using this sensing platform, fenobucarb and malathion were detected at limits of 5 pg/mL and 2.5 pg/mL, respectively. Moreover, the proposed biosensor was successfully applied to the determination of pesticides in real river water. Therefore, this LC-based microcapillary sensor is a promising platform for simple, rapid, and label-free detection of pesticides with very high sensitivity.

1. Introduction

Acetylcholinesterase (AChE) is an enzyme that catalyzes the hydrolysis of the neurotransmitter, acetylcholine (ACh), into choline and acetate and terminates signal transmission from the nerve, thus ensuring the normal transmission of neural signals in organisms [1,2]. Inhibition of AChE by enzyme inhibitors, such as pesticides, can lead to the accumulation of ACh in the body, causing continuous stimulation of the muscles, glands, and the central nervous system, potentially resulting in fatal convulsions in high doses [3]. Currently, pesticides are extensively used to control agricultural pests owing to their insecticidal properties [4]. However, because of the presence of toxic components, pesticides are toxic to humans, animals, and aquatic vegetation. During the application of pesticides, approximately 1% of a pesticide affects target organisms and the rest is percolated in the soil, leading to water contamination and resulting in many ecotoxicological problems [4,5]. Therefore, it is necessary to develop methods that are fast, sensitive, and precise for the detection of pesticides in real samples.

Many conventional analytical methods have been developed for the detection of pesticides, such as chromatography [6,7], electrochemistry [8,9], and colorimetry [10]. These methods, especially chromatographic techniques, are very sensitive, reliable, and capable of detecting pesticides in real samples. However, they also have several limitations: the instruments are expensive and complex, maintenance costs are high, a highly trained technician is required for operation, analysis is time-consuming, and the instruments cannot be used in the field [11,12]. Fluorescence methods have also been devised for pesticide detection [13,14]. Although these methods are considered to be simple, rapid, selective, sensitive, and capable of performing testing in the field, they are still limited because relatively few pesticides are strongly fluorescent, and labeling pesticides with a fluorogenic compound is required [11]. Therefore, the development of label-free, simple, and rapid methods for the precise detection of pesticides with high sensitivity and selectivity is greatly desirable.

* Corresponding author.
E-mail address: chjang4u@gachon.ac.kr (C.-H. Jang).

https://doi.org/10.1016/j.colsurfb.2021.111587
Received 28 September 2020; Received in revised form 28 December 2020; Accepted 19 January 2021
Available online 22 January 2021

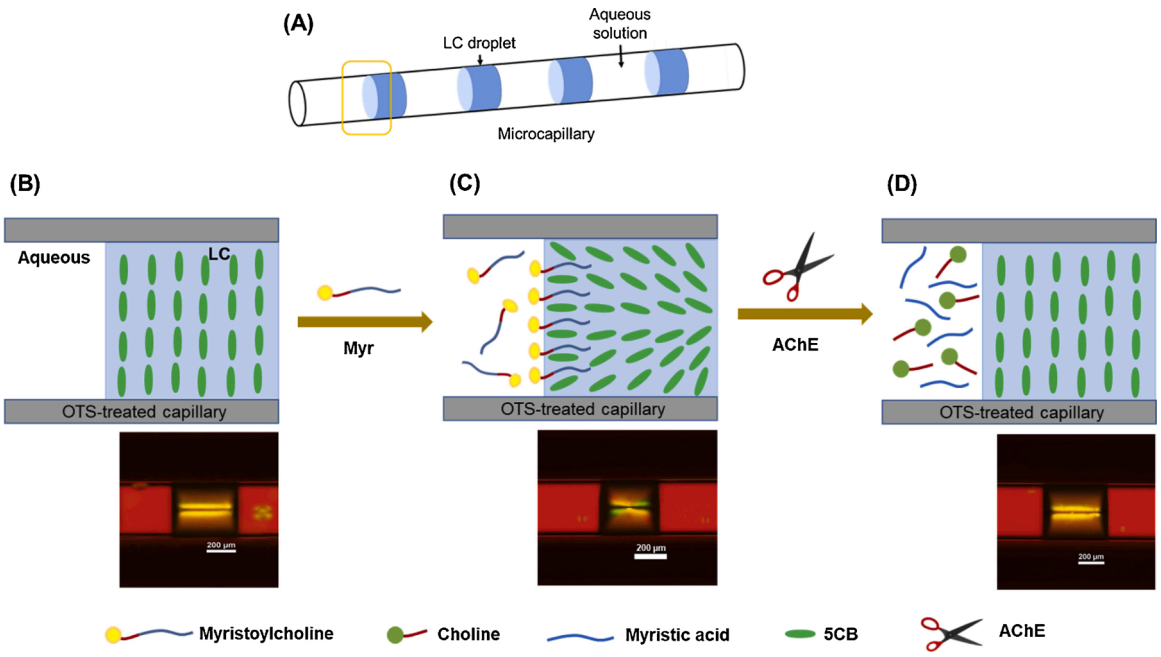

Fig. 1. Schematic illustration of the LC-based microcapillary sensor. The formation of LC droplets inside an OTS-treated microcapillary (A); the orientation transition of LC molecules generated in (B) pure PBS solution (pH = 7.4), (C) Myr aqueous solution, and (D) a mixed solution of Myr and AChE. The lower panel images represent the polarized optical microscopy images corresponding to the respective interfacial events at the aqueous/LC interface. OTS = trichloro(octyl)silane.

Over the past decade, liquid crystal (LC)-based sensors have been widely used for monitoring chemical and biological events because of their many advantages, such as easy fabrication, high sensitivity, rapid response, portability, and lack of complex instrumentation [15–18]. Moreover, these sensors can work in an ambient environment and magnify bio/chemical events at the nanoscale, such that these events can be easily visualized under a polarized light microscope (POM) without any labels on the analytes [19]. Recently, LC-based biosensors for the monitoring of AChE and its inhibitors have been developed and reported. Wang et al. constructed an LC thin film decorated with a cationic surfactant, myristoylcholine chloride (Myr), to detect AChE and its inhibitors based on the ability of AChE to hydrolyze Myr into choline and myristic acid [20]. Although this LC thin-film sensing system is simple and sensitive, some drawbacks should be addressed. Large volumes (hundreds of microliters) of solutions of interest are needed for imaging, limiting the detection of a smaller amount of substances. Further, removal of excess LCs when preparing LC thin films on copper grids wastes materials. The copper grids are easily deformed and oxidized, limiting their reusability. Moreover, because aqueous solutions are exposed to the external environment, aqueous solution/LC interfaces can be interrupted by heat, airflow, or certain vibrations. In another study, Wang et al. developed a different strategy for the sensitive detection of AChE and pesticides using an LC droplet pattern platform decorated with a cationic surfactant (Myr) [21]. This sensing system is quite simple, convenient, and highly sensitive for the detection of pesticides with a detection limit for baycarb at 1 ng/mL and dimethoate at 0.1 ng/mL. Moreover, only small amounts (~1 μL) of the solution of interest are required for imaging. However, because of the evaporation of the solutions, this system can remain stable for a few minutes at room temperature, limiting its applications.

Our group has previously developed a microcapillary sensor for imaging enzymatic activity using confined nematic LCs [19,22]. LC droplets immiscible with water were produced by sequential injection of LCs and an aqueous solution into an OTS-treated microcapillary. The orientational behavior of the confined LC droplets in the microcapillary was dependent on the properties of the aqueous solution. The LC droplet patterns showed two bright lines when in contact with non-surfactant solutions, such as pure deionized (DI) water or phosphate-buffered saline (PBS) solution (pH = 7.4), whereas four-petal shapes were obtained after introducing surfactant solutions. This new strategy has been demonstrated to be simple, rapid, and sensitive for the detection of enzymatic activity. Moreover, because it constitutes a nearly closed system, preventing evaporation of the solutions, the LC textures formed in the microcapillaries are stable for more than 120 h (5 days). Another advantage of this strategy is that OTS-treated microcapillaries can be reused at least 10 times without any significant effects [23].

Inspired by the aforementioned studies, we designed an LC-based biosensor for the simple and sensitive detection of AChE and its inhibitors using confined LC droplets in a microcapillary. The formation of LC droplets after the sequential injection of LCs and an aqueous solution into an OTS-treated capillary is depicted in Fig. 1A. As mentioned earlier, when an LC droplet made contact with a non-surfactant solution, such as PBS solution, two bright-lined textures were observed under POM, coupled to the homeotropic orientation of LC molecules at the aqueous/LC interface (Fig. 1B). Here, we used Myr (the substrate of the enzyme, AChE) as a surfactant to induce the reorientation of the LC molecules at the interface. The four-petal texture was observed after introducing the Myr solution, coupled to the horizontal orientation of the LC molecules at the interface. This suggests that the Myr molecules are anchored to the aqueous/LC interface owing to their amphiphilic properties, resulting in a change in the orientation of the LC molecules (Fig. 1C). The LC pattern turned into two bright lines upon the addition of a pre-incubated mixture of Myr and AChE, which indicates that the Myr monolayer could not form based on enzymatic hydrolysis of Myr by AChE (Fig. 1D). In addition, we also verified the detection of pesticides based on the optical response of the LC droplet patterns after introducing Myr pre-incubated with a mixture of AChE and pesticides. As typical pesticides, fenobucarb and malathion were selected to evaluate the proposed biosensor. Low limits of detection (LOD) of 5 pg/mL for fenobucarb and 2.5 pg/mL for malathion were achieved using this sensing system. These LOD values are comparable to the LOD values of other reported methods [21,24–33]. Therefore, this simple, label-free LC-based biosensor holds great promise for detecting AChE and pesticides with low cost, high sensitivity, robust stability, and reproducibility. Moreover, the practicability of the proposed sensor has also been demonstrated in the real environment of the Tancheon River.

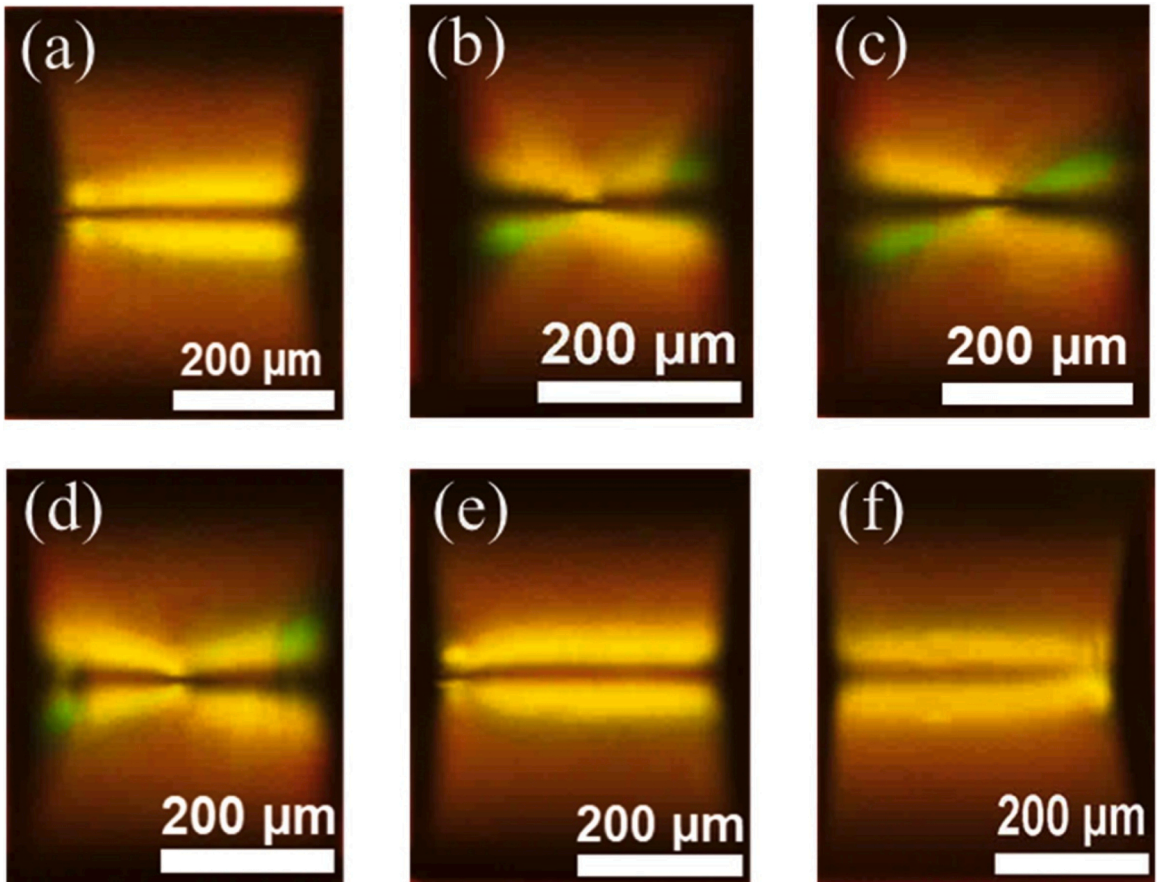

Fig. 2. Polarized optical microscopy images of LC droplets confined in a microcapillary when in contact with (a) PBS solution (pH = 7.4), and various concentrations of Myr solutions: (b) 100 μM, (c) 50 μM, (d) 30 μM, (e) 25 μM, and (f) 10 μM.

2. Materials and methods

2.1. Materials

Myristoylcholine chloride (Myr) was purchased from Toronto Research Chemicals Inc., Canada. Nematic liquid crystal 4-cyano-4′-pentylbiphenyl (5CB) was obtained from Tokyo Chemical Industrial Co., Ltd. (Tokyo, Japan). Hirschmann micropipettes (microcapillaries of 1−5 μL; inner diameter: 580 μm), acetylcholinesterase (AChE), fenobucarb, malathion, trichloro(octyl)silane (OTS), and PBS were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrogen peroxide (H_2O_2, 30% w/v), sulfuric acid (H_2SO_4, 95 %), ethanol, methanol, n-heptane (anhydrous), and methylene chloride (CH_2Cl_2) were purchased from Daejung Chemicals & Metals Co., Ltd (Daejung, South Korea). DI water (18.2 MΩ. cm) generated from a Milli-Q water purification system (Millipore, Bedford, USA) was used for all experiments in this study.

2.2. Modification of microcapillaries with OTS

OTS-modified microcapillaries were prepared following procedures reported in previous publications [19,22]. Briefly, the microcapillaries were immersed in the "piranha solution" (H_2SO_4/H_2O_2 = 7/3 (v/v)) at 80 °C for 1 h. After cooling, the microcapillaries were rinsed in turn with DI water, ethanol, and methanol, dried under a stream of nitrogen, and incubated at 120 °C overnight prior to OTS deposition. The "piranha--treated" microcapillaries were next immersed in an OTS/n-heptane (270 μL/135 mL) solution at room temperature for 1 h. After washing with methylene chloride, the microcapillaries were dried under nitrogen flow and stored in a desiccator until further use.

2.3. Preparation of the solution to be assayed

A stock solution of fenobucarb (0.1 mg/mL) was prepared in a mixture of acetonitrile-DI water (1:9 *v/v*) followed by serial dilution in PBS (pH = 7.4). Malathion was first dissolved in ethanol at a concentration of 0.1 mg/mL as a stock solution and then diluted with PBS (pH = 7.4) to obtain malathion solutions in the testing concentration range. All aqueous solutions related to enzymatic reactions were prepared in PBS (pH = 7.4). During the AChE detection assay, 30 μM Myr and AChE at various concentrations were mixed and then pre-incubated at 37 °C for 30 min. Then, the mixed solution was injected into the microcapillary using a syringe for target detection. During the pesticide detection assay, AChE with a concentration of 2.5 μg/mL and fenobucarb/malathion at different concentrations were mixed and then pre-incubated at 37 °C for 30 min. After introducing 30 μM Myr, the aqueous solutions were incubated at 37 °C for 30 min. The incubated solution was then injected into the microcapillary for target detection. To evaluate the practicability of the sensor in a real environment, the determination of pesticides in river water was performed. River water was obtained from the Tancheon River, Seongnam City, Republic of Korea. After filtering, the river water sample was spiked with different concentrations of fenobucarb followed by analysis applying the proposed assay.

2.4. Visualization of orientational behavior of LCs

A small volume of 5CB (approximately 0.5 μL) was dispensed into one end of an OTS-treated microcapillary using capillary force. Next, approximately 3 μL of aqueous solutions of interest were injected into the same end of the microcapillary using a syringe connected to a pipe, and then 5CB was automatically separated into several droplets. The

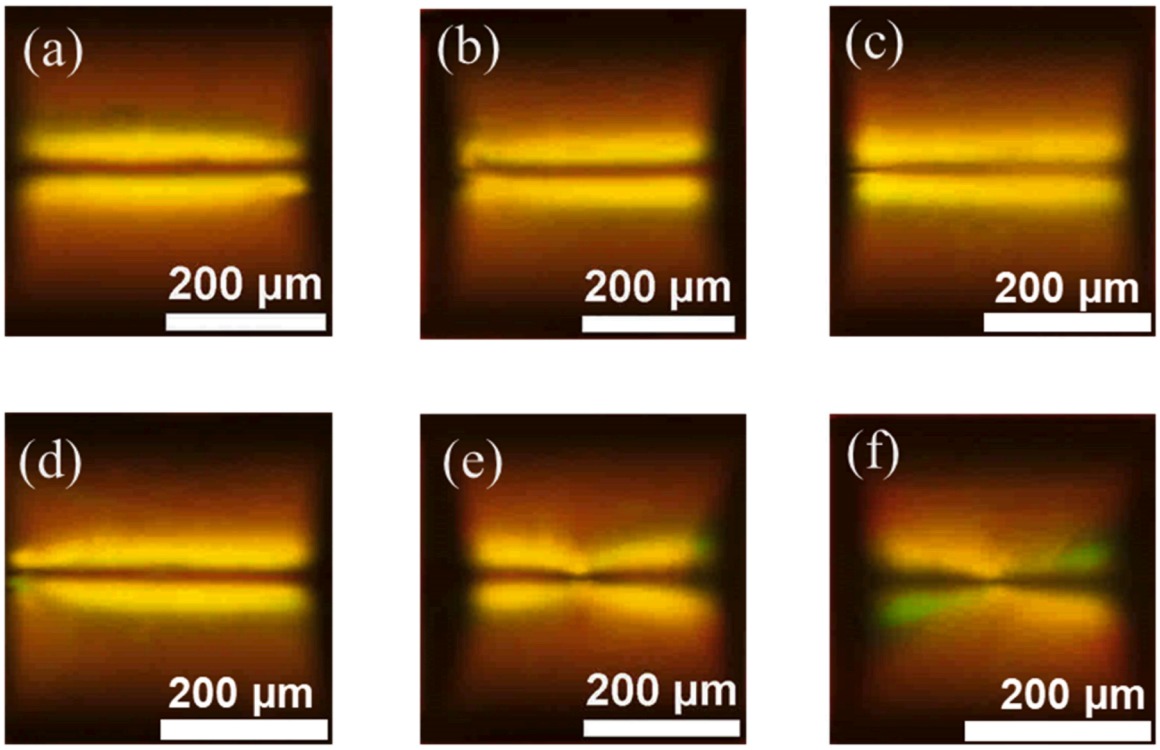

Fig. 3. Polarized optical microscopy images of LC droplets confined in a microcapillary when in contact with a pre-incubated mixture of Myr (30 μM) and various concentrations of AChE: (a) 100 μg/mL, (b) 10 μg/mL, (c) 5 μg/mL, (d) 2.5 μg/mL, (e) 2 μg/mL, and (f) 1 μg/mL.

optical appearance of nematic 5CB inside the microcapillary was visualized with a POM (Eclipse LV100 POL, Nikon, Tokyo, Japan). All optical images were taken using a digital camera (DS-2Mv, Nikon, Tokyo, Japan) attached to the microscope using a 5× objective lens, at a resolution of 2560 × 1920 pixels, a gain of 1.2× and a shutter speed of 1/25 s. Each group of experiments was repeated at least five times to avoid artifacts.

3. Results and discussion

3.1. Optical response of LC droplets confined in OTS-treated microcapillaries to Myr

The inner surfaces of the microcapillaries were coated with OTS to control the alignment of the LCs in a homeotropic orientation [22]. In addition, the hydrophobicity of the OTS-treated microcapillary interior induced the siphoning of 5CB into the microcapillaries [34]. After approximately 0.5 μL of 5CB was dispensed into one end of the OTS-treated microcapillary, the aqueous solutions of interest were injected into the same end of the microcapillary using a syringe connected to a pipe. Based on the immiscibility between 5CB and aqueous solutions, the 5CB separated into several individual droplets with a typical length of 300–500 μm. After 1 or 2 min, stable optical images were observed. The formation of LC droplets inside the OTS-treated microcapillary is illustrated in Fig. 1A. Previous studies have established that the orientational behavior of the confined LC droplets in the microcapillary was dependent on the properties of the aqueous solution [22,23]. The LC droplet patterns presented two bright-lined textures when in contact with non-surfactant solutions, such as PBS solution (pH = 7.4) (Fig. 1B), whereas four-petal shapes were observed after introducing surfactant solutions (Fig. 1C). Indeed, after injecting PBS solution (pH = 7.4) into the microcapillary containing 0.5 μL of 5CB, we observed the texture of two bright lines under POM, coupled to the homeotropic orientation of the LCs at the aqueous/LC interface, indicating that the PBS solution did not affect the alignment of the LCs at the interface (Fig. 2a). To investigate the effect of Myr on the alignment of the LCs at the aqueous/LC interface, we first studied the optical response of LC droplets confined in the microcapillary in contact with Myr solutions at various concentrations (10 μM–100 μM) (Fig. 2b–f). The four-petal texture was observed after introducing a PBS solution (pH = 7.4) containing 100 μM Myr into the microcapillary, coupled to the horizontal orientation of LC molecules at the interface (Fig. 2b). This suggested that enough Myr molecules had anchored to the aqueous/LC interface and formed a stable self-assembled Myr monolayer at the interface, causing an ordering transition of LCs at the interface from a homeotropic to a horizontal orientation. A previous study had also demonstrated that the stability of the Myr monolayer was largely affected by the pH of the aqueous solution due to the presence of the ester functional group in the structure of Myr, and the PBS solution with pH = 7.4 was the optimal pH of the enzyme and the stability of the Myr monolayer [20]. Therefore, the PBS solution with pH = 7.4 was used for subsequent experiments.

It is known that the LC-ordering transition occurs when the concentration of the surfactant anchors to the aqueous/LC interface exceeds the critical value [22,23]. Therefore, we studied the effect of decreasing the concentration of the Myr solution on the optical response of LC droplets. For concentrations of Myr from 50 to 30 μM, four-petal textures were observed, indicating that enough Myr molecules were adsorbed at the aqueous/LC interface that induced the orientational transition of the LC droplets (Fig. 2c–d). However, as the Myr concentration decreased to 25 μM or lower, two bright-lined images were observed (Fig. 2e–f), suggesting that the adsorbed Myr molecules at the aqueous/LC interface were not sufficient for inducing the orientational transition of the LCs at the interface when the Myr concentration was below 25 μM. These results indicated that the critical concentration of Myr solution to turn the LC droplets from two bright lines into a four-petal texture was roughly 30 μM. Thus, a Myr concentration of 30 μM was used for subsequent experiments regarding the detection of AChE and pesticides.

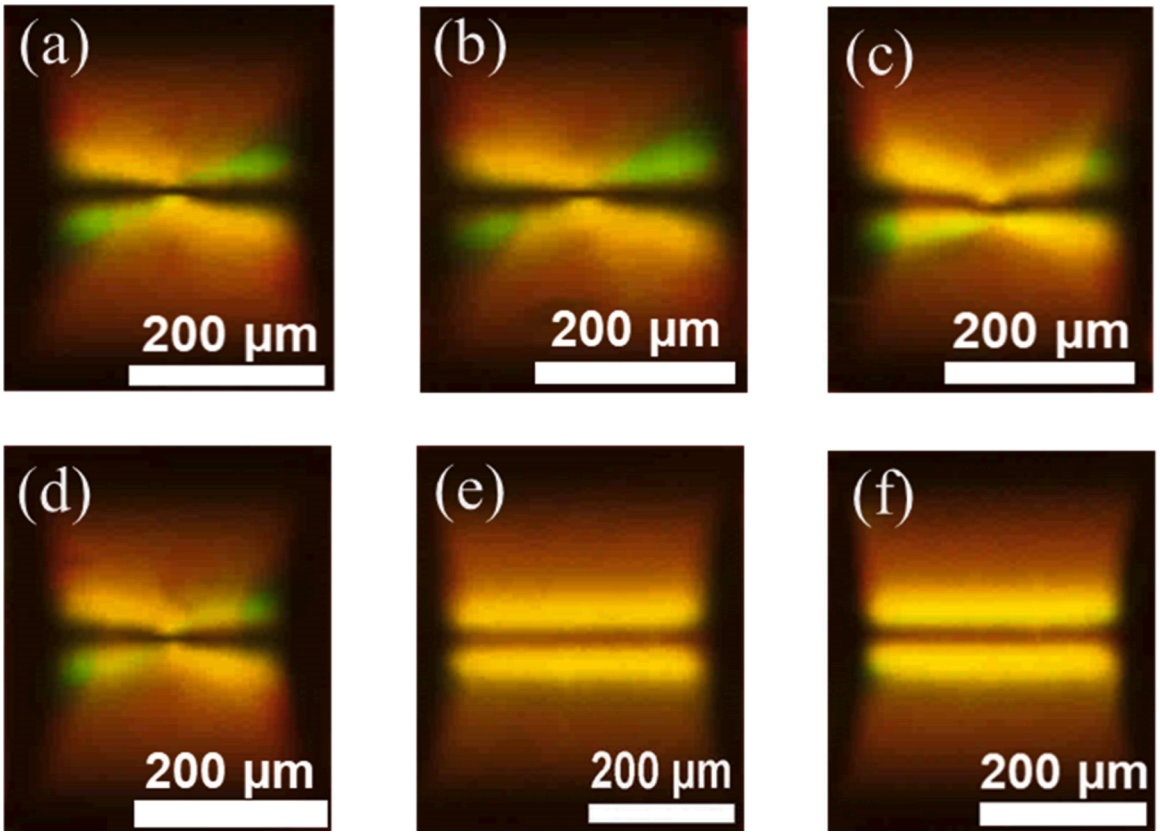

Fig. 4. Polarized optical microscopy images of LC droplets confined in a microcapillary when in contact with a pre-incubated mixture of 30 μM Myr, 2.5 μg/mL AChE and different concentrations of fenobucarb: (a) 1 ng/mL, (b) 100 pg/mL, (c) 10 pg/mL, (d) 5 pg/mL, (e) 3 pg/mL, (f) 1 pg/mL.

3.2. Detection of AChE by LC-based microcapillary sensor

Previous studies have demonstrated that AChE can efficiently catalyze the enzymatic hydrolysis of Myr into myristic acid and choline [20,35]. Therefore, we hypothesized that the optical images of LC droplets confined in a microcapillary would be changed after the introduction of AChE because the surfactant Myr will be hydrolyzed (Fig. 1C). To verify our hypothesis, we investigated the orientational behavior of LC droplets confined in a microcapillary in contact with a pre-incubated mixture of Myr and various concentrations of AChE. Here, 30 μM Myr was pre-incubated with different concentrations of AChE, ranging from 1 to 100 μg/mL, at 37 °C for 30 min before being injected into the OTS-treated microcapillary containing 5CB. The optical appearances of the LC droplets were then observed under a POM. As shown in Fig. 3a, the optical image of the LC droplets displayed two bright lines after injecting a mixture of 30 μM Myr and 100 μg/mL AChE, coupled to a homeotropic orientation of the LCs at the aqueous/LC interface. This indicated that Myr was completely hydrolyzed by AChE. After verifying the feasibility of this LC-based microcapillary sensor for the detection of AChE, the LOD for AChE was also evaluated by measuring the concentration of AChE. The optical images of LC droplets also showed two bright lines after the addition of 10 μg/mL, 5 μg/mL, or 2.5 μg/mL AChE (Fig. 3b–d). However, as the AChE concentration decreased to 2 μg/mL or lower, the orientational transition of LC droplets occurred, and four-petal textures were observed (Fig. 3e–f). This was attributed to the incomplete hydrolysis of Myr by AChE. These results indicated that AChE could be detected on the LC-based microcapillary sensor, and the LOD for AChE was 2.5 μg/mL.

3.3. Detection limit of fenobucarb and malathion pesticides in capillary sensors

Next, we explored the detection potential of the developed LC-based microcapillary sensor for the determination of pesticides in aqueous solutions. The principle for detecting pesticides using this capillary sensor is based on the inhibitory effects of pesticides on AChE. In the presence of pesticides, AChE is inhibited and therefore cannot catalyze the hydrolysis of Myr, resulting in a detectable change in the optical signal of LC droplet patterns. The inhibitory effect of pesticides on AChE is proportional to the concentration of pesticides in the samples and can be determined by the degree of enzymatic hydrolysis of Myr by AChE.

At present, carbamates and organophosphates are extensively used as pesticides because of their abundant insecticidal properties by effectively inhibiting AChE in the nerves [4,35]. In this study, fenobucarb (a carbamate pesticide) and malathion (an organophosphorus pesticide) were used as representative pesticides to evaluate the detection potential of the proposed LC-based microcapillary sensor for monitoring the presence of pesticides in aqueous solutions. First, we studied the optical response of LC droplets confined in a microcapillary after the introduction of fenobucarb. In this experiment, fenobucarb at different concentrations was mixed with 2.5 μg/mL AChE and then pre-incubated at 37 °C for 30 min. After the addition of 30 μM Myr, the mixtures were incubated at 37 °C for 30 min. The incubated solutions were then injected into a microcapillary containing 5CB, and the optical responses of LC droplets were observed. At 1 ng/mL of fenobucarb, the optical image displayed a four-petal shape, coupled to the horizontal orientation of LC molecules at the aqueous/LC interface (Fig. 4a). This result indicated that the activity of AChE was completely inhibited, and therefore the enzymatic hydrolysis of Myr by AChE could not occur, enabling Myr molecules to be anchored at the aqueous/LC interface and resulting in four-petal optical responses. Therefore, fenobucarb pesticide

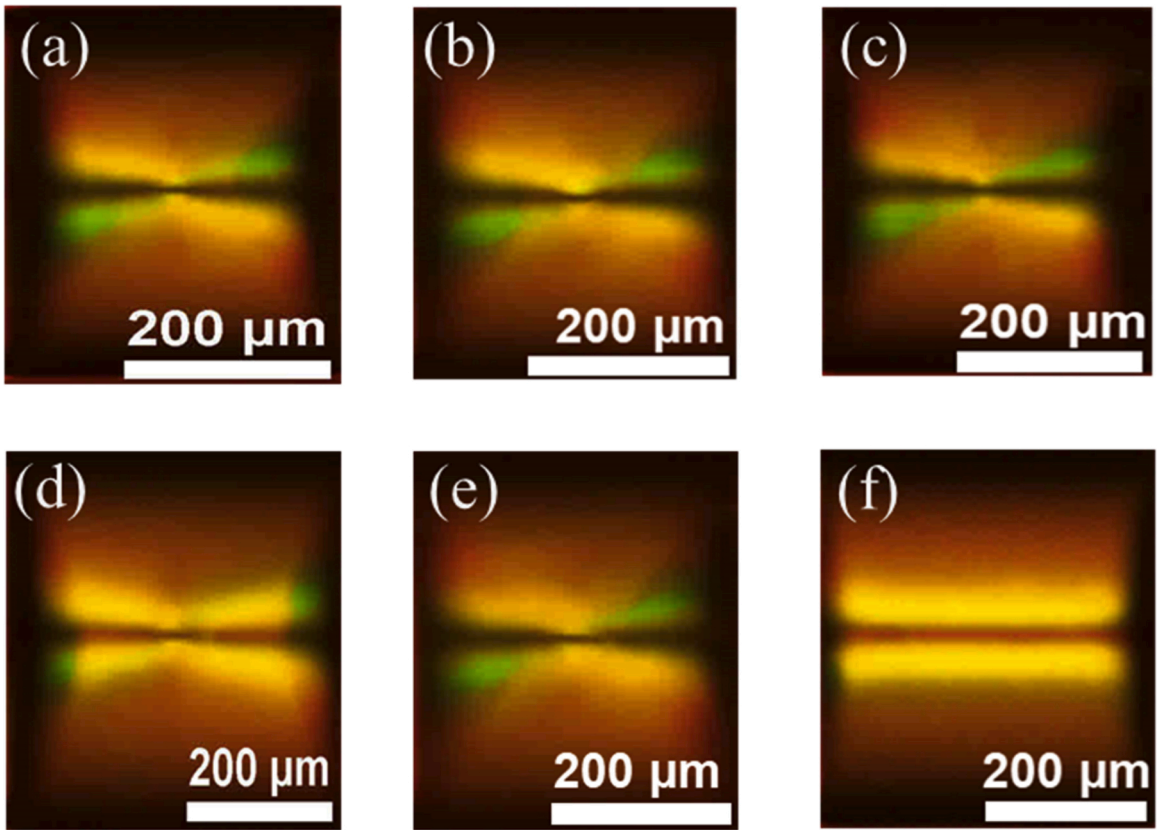

Fig. 5. Polarized optical microscopy images of LC droplets confined in a microcapillary when in contact with a pre-incubated mixture of 30 μM Myr, 2.5 μg/mL AChE and different concentrations of malathion: (a) 1 ng/mL, (b) 100 pg/mL, (c) 10 pg/mL, (d) 5 pg/mL, (e) 2.5 pg/mL, (f) 1 pg/mL.

Table 1
Comparison of various methods for the detection of fenobucarb and malathion pesticides.

Detection methods	Pesticides	Detection limit	References
LC[a]-based sensor	Fenobucarb	1 ng/mL	[21]
Colorimetry	Fenobucarb	23 pg/mL	[24]
LC[b]-ESI-MS	Fenobucarb	0.5 ng/mL	[25]
HPLC[c]-MS/MS	Fenobucarb	6.9 ng/mL	[26]
Amperometry	Fenobucarb	2.07 ng/mL	[27]
Colorimetry	Malathion	3.9 ng/mL	[28]
Electrochemistry	Malathion	17.47 ng/mL	[29]
Fluorescence	Malathion	304 ng/mL	[30]
Surface-enhanced Raman spectroscopy	Malathion	165.3 ng/mL	[31]
Electrochemistry	Malathion	224.6 pg/mL	[32]
Amperometry	Malathion	901 pg/mL	[33]
Microcapillary sensor	Fenobucarb	5 pg/mL	Present study
Microcapillary sensor	Malathion	2.5 pg/mL	Present study

[a] LC-based sensor, liquid crystal-based sensor.
[b] LC-ESI-MS, liquid chromatography-electrospray-mass spectrometry.
[c] HPLC-MS/MS, ultra-high-performance liquid chromatography-tandem mass spectrometry.

can be monitored using the developed capillary sensor. As the inhibitory effect is proportional to the concentration of pesticides, we then decreased the concentration of fenobucarb to determine the detection limit of this capillary sensor for fenobucarb. The LC droplet appearance maintained a four-petal shape when the concentration of fenobucarb varied from 100 pg/mL to 5 pg/mL (Fig. 4b–d), indicating that fenobucarb inhibited AChE and thus prevented the enzymatic hydrolysis of Myr. However, when the concentrations of fenobucarb were decreased to 3 pg/mL or lower, we observed two bright-lined textures, suggesting that fenobucarb did not completely inhibit AChE activity at these concentrations (Fig. 4e–f). These results indicated that the LOD of our developed LC-based microcapillary sensor for fenobucarb was 5 pg/mL.

The inhibitory effect of malathion on AChE was also investigated using the same experimental procedure. Fig. 5a–e shows the four-petal shapes when the LC droplets were in contact with the mixtures of 30 μM Myr, 2.5 μg/mL AChE, and various concentrations of malathion ranging from 1 ng/mL to 2.5 pg/mL. However, the LC droplets presented a two bright-lined texture when the concentration of malathion was reduced to 1 pg/mL (Fig. 5f). In this case, the amount of malathion was too low to completely inhibit AChE activity. Therefore, we concluded that the LOD for malathion is 2.5 pg/mL. The LOD values of our developed capillary sensor for pesticide detection were then compared to the LOD values of the reported sensing methods (Table 1). While other sensing methods, such as colorimetric, amperometric, chromatographic, fluorescent, and electrochemical techniques, require expensive and bulky instruments, are time-consuming, and feature complicated experimental procedures, as well as requiring professional operators, limiting their applications for on-site detection, our developed sensing strategy offers a simple, rapid, label-free, and highly sensitive method for monitoring pesticides with a lower LOD than the reported sensing methods. Therefore, this capillary biosensor is a promising platform for monitoring AChE–inhibiting pesticides in aqueous samples with high sensitivity.

3.4. Detection of pesticides in river water

The developed biosensor was employed to detect pesticides in Tancheon River water to verify the practicability of this method when analyzing real samples. After filtering, the river water sample was spiked with different concentrations of fenobucarb followed by analysis using

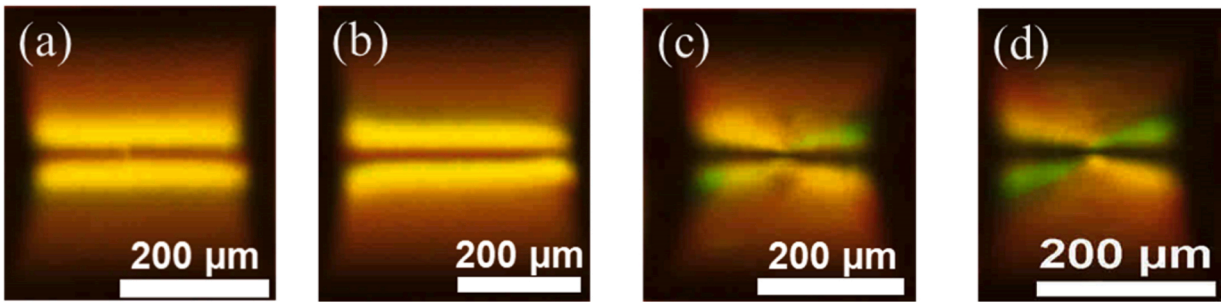

Fig. 6. Polarized optical microscopy images of LC droplets confined in a microcapillary when in contact with a pre-incubated mixture of 30 μM Myr, 2.5 μg/mL AChE and different concentrations of fenobucarb in river water: (a) 0 pg/mL (only river water), (b) 1 pg/mL, (c) 5 pg/mL, (d) 25 pg/mL.

the proposed assay. River water without the addition of fenobucarb was used as a control. Measurements were performed in triplicates. The optical images of LC droplets displayed two bright-lined textures when the concentrations of fenobucarb spiked into the river water were below 5 pg/mL (LOD for fenobucarb), confirming that there were no pesticides in the river water (Fig. 6a–b). However, when 5 pg/mL or higher concentration of fenobucarb was spiked into the river water, four-petal textures were observed (Fig. 6c–d). These results were consistent with those obtained in PBS, therefore demonstrating that the proposed LC-based capillary sensor was feasible for the practical detection of pesticides in real samples. However, if a real water sample contains other surfactants that can bind to the 5CB and induce the orientational transition of 5CB molecules at the interface, it will affect the optical textures of LC droplets, and therefore affect the LOD of pesticides. This limits the practical applications of the proposed biosensor for the detection of pesticides in real samples. Besides, the disadvantage of the proposed LC sensory platform is the difficulty of sequentially adding analytes into the platform, and thus, limit the in site detection of enzyme reactions.

4. Conclusions

In summary, we developed a microcapillary sensor for monitoring AChE and its inhibitors using LC droplets confined in an OTS-treated microcapillary. The optical appearance of the confined LC droplets presented two bright lines or a four-petal shape when in contact with non-surfactant or surfactant solutions, respectively. Based on these optical responses of LC droplets, an LC-based capillary-sensing system was designed and developed for the detection of AChE and pesticides. In particular, a four-petal texture was observed before the addition of AChE into myristoylcholine chloride surfactant solution, while a two bright-lined texture was obtained after the hydrolysis reaction of myristoylcholine chloride by AChE. However, in the presence of pesticides, such as fenobucarb and malathion, the enzymatic activity of AChE was inhibited, and thus, the LC droplets presented a four-petal appearance. A low LOD of 2.5 μg/mL for AChE was achieved using this sensing system. In addition, employing this sensing technique, the detection limits of fenobucarb and malathion reached as low as 5 pg/mL and 2.5 ng/mL, respectively. Moreover, the proposed biosensor could be successfully applied to detect pesticides in real river water. Therefore, this LC-based microcapillary sensor not only offers a simple, low-cost, rapid, and label-free method for the detection of pesticides with high sensitivity but also shows great potential for practical applications.

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 report no declarations of interest.

Acknowledgments

This work was supported by the Basic Science Research Program of the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2019R1A2C1003862).

References

[1] V.N. Talesa, Acetylcholinesterase in Alzheimer's disease, Mech. Ageing Dev. 122 (2001) 1961–1969.
[2] Y. Ohta, M. Darwish, N. Hishikawa, T. Yamashita, K. Sato, M. Takemoto, K. Abe, Therapeutic effects of drug switching between acetylcholinesterase inhibitors in patients with Alzheimer's disease, Geriatr. Gerontol. Int. 17 (2017) 1843–1848.
[3] S. Liao, Y. Qiao, W. Han, Z. Xie, Z. Wu, G. Shen, R. Yu, Acetylcholinesterase liquid crystal biosensor based on modulated growth of gold nanoparticles for amplified detection of acetylcholine and inhibitor, Anal. Chem. 84 (2012) 45–49.
[4] V. Dhull, A. Gahlaut, N. Dilbaghi, V. Hooda, Acetylcholinesterase biosensor for electrical detection of organophosphorus compounds: a review, Biochem. Res. Int. 2013 (2021), 731501, 18 pages.
[5] Z. Li, S. Qu, L. Cui, S. Zhang, Detection of carbofuran pesticide in seawater by using an enzyme biosensor, J. Coast. Res. 80 (2016) 1–5.
[6] I. Ferrer, E.M. Thurman, J.A. Zweigenbaum, Screening and confirmation of 100 pesticides in food samples by liquid chromatography/tandem mass spectrometry, Rapid Commun. Mass Spectrom. 21 (2007) 3869–3882.
[7] I. Ferrer, E.M. Thurman, Multi-residue method for the analysis of 101 pesticides and their degradates in food and water samples by liquid chromatography/time-of-flight mass spectrometry, J. Chromatogr. A 1175 (2007) 24–37.
[8] S. Viswanathan, H. Radecka, J. Radecki, Electrochemical biosensor for pesticides based on acetylcholinesterase immobilized on polyaniline deposited on vertically assembled carbon nanotubes wrapped with ssDNA, Biosens. Bioelectron. 24 (2009) 2772–2777.
[9] A.M. Al'Abria, S.N.A. Halim, N.K.A. Bakar, S.M. Saharin, B. Sherino, H.R. Nodeh, S. Mohamad, Highly sensitive and selective determination of malathion in vegetable extracts by an electrochemical sensor based on Cu-metal organic framework, J. Environ. Sci. Health B 54 (2019) 930–941.
[10] M. Pohanka, J.Z. Karasova, K. Kuca, J. Pikula, O. Holas, J. Korabecny, J. Cabal, Colorimetric dipstick for assay of organophosphate pesticides and nerve agents represented by paraoxon, sarin and VX, Talanta 81 (2010) 621–624.
[11] R. Ben-Zur, H. Hake, S. Hassoon, V. Bulatov, I. Schechter, Optical analytical methods for detection of pesticides, Rev. Anal. Chem. 30 (2011) 123–139.
[12] Z. Zheng, X. Li, Z. Dai, S. Liu, Z. Tang, Detection of mixed organophosphorus pesticides in real samples using quantum dots/bi-enzyme assembly multilayers, J. Mater. Chem. 21 (2011) 16955–16962.
[13] X. Jing, L.M. Du, H. Wu, W.Y. Wu, Y.X. Chang, Determination of pesticide residue cartap using a sensitive fluorescent probe, J. Integr. Agr. 11 (2012) 1861–1870.
[14] L. Jia, J. Pan, J. Zhu, Label-free fluorometric assay for acetylcholinesterase and inhibitor screening based on supramolecular assemblies, Anal. Methods 5 (2013) 5431–5436.
[15] Q.Z. Hu, C.H. Jang, Using liquid crystals for the label-free detection of catalase at aqueous–LC interfaces, J. Biotechnol. 157 (2012) 223–227.
[16] D.K. Nguyen, C.H. Jang, Label-free liquid crystal-based detection of As(III) ions using ssDNA as a recognition probe, Microchem. J. 156 (2020), 104834.
[17] D. Hartono, C.Y. Xue, K.L. Yang, L.Y.L. Yung, Decorating liquid crystal surfaces with proteins for real-time detection of specific protein–protein binding, Adv. Funct. Mater. 19 (2009) 3574–3579.
[18] Q.Z. Hu, C.H. Jang, Imaging trypsin activity through changes in the orientation of liquid crystals coupled to the interactions between a polyelectrolyte and a phospholipid layer, ACS Appl. Mater. Interfaces 4 (2012) 1791–1795.

[19] J.S. Rim, C.H. Jang, Detection of catalase activity with aldehyde-doped liquid crystals confined in microcapillaries, Anal. Biochem. 560 (2018) 19–23.
[20] Y. Wang, Q. Hu, Y. Guo, L. Yu, A cationic surfactant-decorated liquid crystal sensing platform for simple and sensitive detection of acetylcholinesterase and its inhibitor, Biosens. Bioelectron. 72 (2015) 25–30.
[21] Y. Wang, Q. Hu, T. Tian, L. Yu, Simple and sensitive detection of pesticides using the liquid crystal droplet patterns platform, Sens. Actuators B Chem. 238 (2017) 676–682.
[22] H.J. Kim, C.H. Jang, Micro-capillary sensor for imaging trypsin activity using confined nematic liquid crystals, J. Mol. Liq. 222 (2016) 596–600.
[23] S. Zhong, C.H. Jang, Nematic liquid crystals confined in microcapillaries for imaging phenomena at liquid–liquid interfaces, Soft Matter 11 (2015) 6999–7004.
[24] S. Qian, H. Lin, Colorimetric sensor array for detection and identification of organophosphorus and carbamate pesticides, Anal. Chem. 87 (2015) 5395–5400.
[25] L.L. El Atrache, R.B. Sghaier, B.B. Kefi, V. Haldys, M. Dachraoui, J. Tortajada, Factorial design optimization of experimental variables in preconcentration of carbamates pesticides in water samples using solid phase extraction and liquid chromatography-electrospray-mass spectrometry determination, Talanta 117 (2013) 392–398.
[26] Z. Shi, J. Hu, Q. Li, S. Zhang, Y. Liang, H. Zhang, Graphene based solid phase extraction combined with ultra high performance liquid chromatography-tandem mass spectrometry for carbamate pesticides analysis in environmental water samples, J. Chromatogr. A 1355 (2014) 219–222.
[27] M.G.F. Sales, M.C.V.F. Vaz, C. Delerue-Matos, S.A.A. Almeida, M.F. Barroso, H.A. O. Ferreira, Flow amperometric determination of carbofuran and fenobucarb, Int. J. Environ. Anal. Chem. 88 (2008) 37–49.
[28] D. Li, S. Wang, L. Wang, H. Zhang, A simple colorimetric probe based on anti aggregation of AuNPs for rapid and sensitive detection of malathion in environmental samples, Anal. Bioanal. Chem. 411 (2019) 2645–2652.
[29] L. He, B. Cui, J. Liu, Y. Song, M. Wang, D. Peng, Z. Zhang, Novel electrochemical biosensor based on core-shell nanostructured composite of hollow carbon spheres and polyaniline for sensitively detecting malathion, Sens. Actuators B Chem. 258 (2018) 813–821.
[30] H.A. Azab, A.S. Orabi, A.M. Abbas, New probe for fluorescence detection of Azinphous ethyl, Malathion and Heptachlor pesticides, J. Lumin. 160 (2015) 181–187.
[31] Y. Nie, Y. Teng, P. Li, W. Liu, Q. Shi, Y. Zhang, Label-free aptamer-based sensor for specific detection of malathion residues by surface-enhanced Raman scattering, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 191 (2018) 271–276.
[32] G. Bolat, S. Abaci, Non-enzymatic electrochemical sensing of malathion pesticide in tomato and apple samples based on gold nanoparticles-chitosan-ionic liquid hybrid nanocomposite, Sensors 18 (2018) 773, https://doi.org/10.3390/s18030773.
[33] N.F.M. Rodrigues, S.Y. Neto, R.C.S. Luz, F.S. Damos, H. Yamanaka, Ultrasensitive determination of malathion using acetylcholinesterase immobilized on chitosan-functionalized magnetic iron nanoparticles, Biosensors 8 (2018) 16, https://doi.org/10.3390/bios8010016.
[34] Z. An, C.H. Jang, Sensitive and selective method for detecting cysteine based on optical properties of liquid crystal, Sens. Actuators B Chem. 269 (2018) 135–142.
[35] R. Duan, X. Hao, Y. Li, H. Li, Detection of acetylcholinesterase and its inhibitors by liquid crystal biosensor based on whispering gallery mode, Sens. Actuators B Chem. 308 (2020), 127672.