Liquid Crystal Based Detection of Arsenic ions Using Aptamers as Recognition Elements

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Certificate of Examination

This is to certify that the dissertation titled "**Liquid Crystal based detection of arsenic ions using aptamers as Recognition Elements**" submitted by Ms. Vaishnavi S(Reg. No. MS13132) for the partial fulfilment of BS-MS dual degree programme of the institute, has been examined by the thesis committee duly appointed by the institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

Dr. Sugumar Venkataramani Dr. Debashis Adhikari Dr. Santanu Kumar Pal

(Supervisor)

Dated: April 20, 2018

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr. Santanu Kumar Pal at the Indian Institute of Science Education and Research Mohali. This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Vaishnavi S

Dated: April 20, 2018

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Santanu Kumar Pal

(Supervisor)

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Abbreviations

LC	Liquid Crystal
DMOAP	N,N-dimethyl-N-octadecyl-3-aminopropyltrimethoxysilyl chloride
CTAB	cetyltrimethylammonium bromide
5CB	4-cyano-4’-n-pentylbiphenyl
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
POM	Polarized Optical Microscope
CD	Circular Dichroism
DI	DeIonized

Contents

Abstract

Liquid Crystal(LC) based sensors are gaining more attention over the past few years, as they overcome few important drawbacks that are commonly found in other analytical techniques. The detection principle of LC sensors is based on the orientational changes that occur in LC molecules when there are minor disturbances in the interfacial regions. Moreover, LC sensors are capable of label free detection of analyte, do not require costly instrumentation and sample preparation.

In this project, we are using LC system to detect As(III) ions in water samples. As(III) is one of the most toxic ions present in contaminated water and causes chronic poisoning when exposed. A lot of analytical techniques have been designed to detect the presence of As(III) in contaminated water, however they require costly instrumentation, time consuming sample preparation, have portability issues, require maintenance facilities, etc. A carefully designed LC sensing system will be capable of overcoming these drawbacks and prove to be useful for on-site detection of As(III).

The first chapter gives a general introduction to Biosensing and Liquid Crystals; properties of LCs that make them good interfacial material for sensing experiments. The second chapter discusses the scheme that is used for sensing of As(III) ions and the last chapter enlists the observations made and concludes the results of the experiments.

Chapter One

Introduction

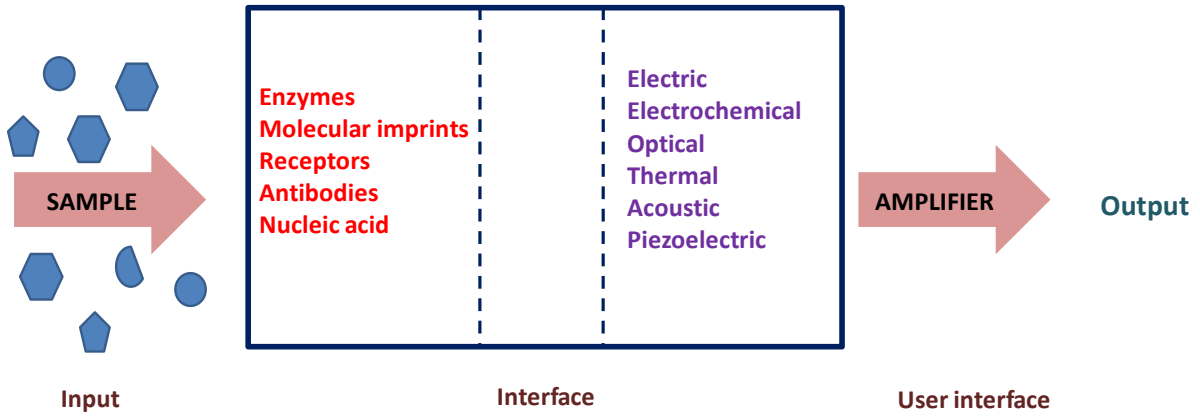
1.1 Biosensing

The core of Biosensing lies essentially with the recognition element, that is, the moiety responsible sensing the analyte. Briefly, a biosensor can be defined as an analytical device, which utilises a biological or a biologically derived recognition element to sense the analyte or target, which in turn is also associated with a physio-chemical transducer.[1] The concept of using biological or biologically derived recognition elements for analytical kits was derived from the study of natural systems and the biochemical reactions happening in them. One good example of a widely used recognition element in sensing experiments is antibodies, as their highly specific and selective to their target molecules. However, today with the development in biotechnology a lot of these moieties are designed specific to the experiments and are synthesized.

In general, a sensing system has the following components:

- Sample or input
- Recognition element
- Interfacial or Transducing element
- Transducer
- Amplifier followed by the Output or signal receiver.

Therefore, while designing a sensing system, it is very essential that all these elements cooperatively function with each other. Designing of the transducing element is very important in a sensing system, as it has the vital role of receiving and sending the message or the information that it obtains from the biological event. Hence, the sensitivity of the system directly depends, not only on the recognition element but also on the interface.



Scheme 1: General Schematic of a Biosensing system

1.2 Liquid Crystals

Liquid crystals(LCs) are states of matter that has properties intermediate between crystalline solids and isotropic liquids. They exhibit fluidity and take the shape of the vessel that contains them like liquids. However, they are not isotropic like liquids and posses some order in the arrangement of the molecules and have optical, electric and magnetic properties like crystals.[2]

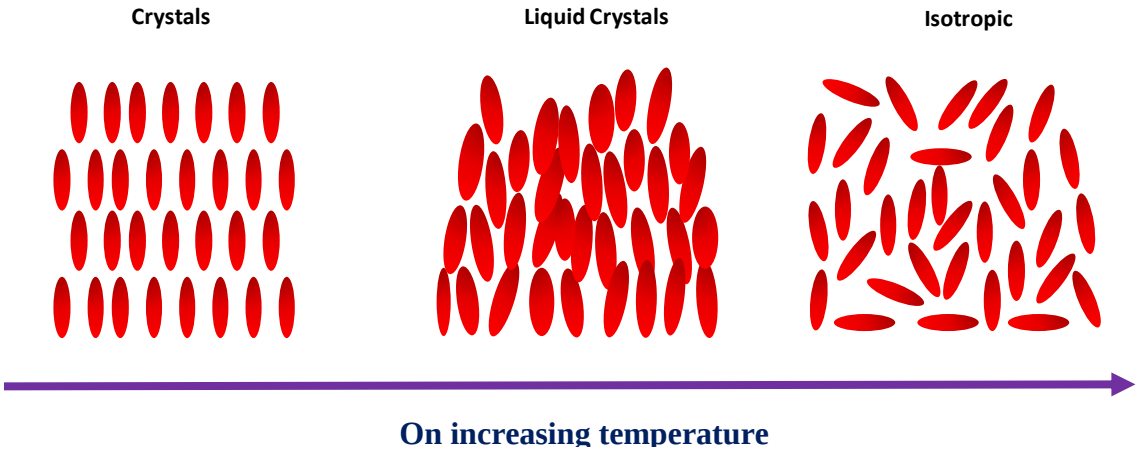


Figure 1: Existence of LC phase in-between ordered crystals and isotropic liquids

The many LC phases are categorised and characterised based on the symmetry they do or do not exhibit. The general classification of LCs is given below. Most of the LC phase has orientational order, where they are characterised by long range correlation of molecular direction. That is, on an average all the molecules in the LC phase point in a particular

direction. The most common phase of this type is the uniaxial nematic phase, which we will discuss further.

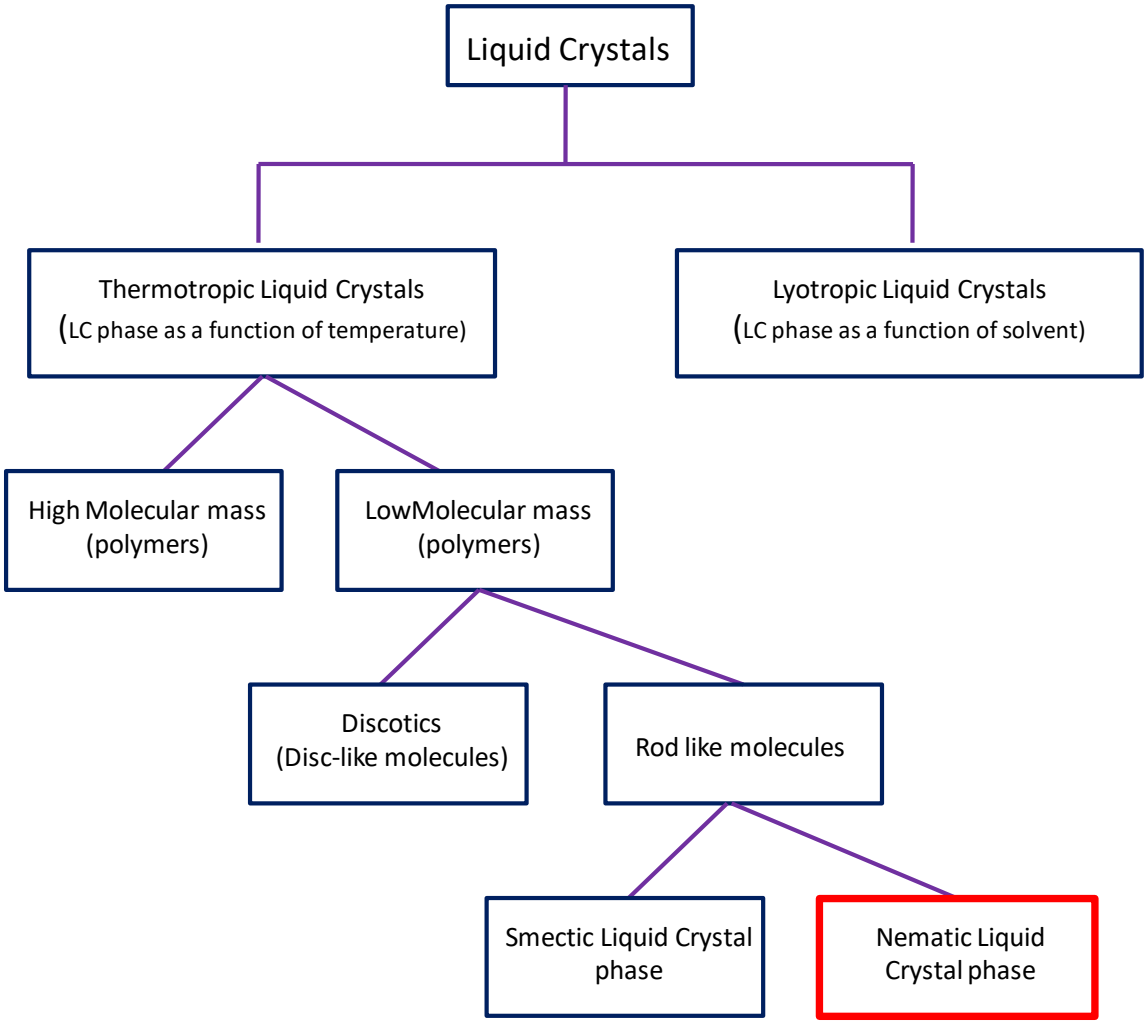


Figure 2: General classification of LCs

1.2.1 Historical perspective

The concept of using LCs as interfacial surface in sensing experiments has its origin from the biochemical reactions happening in our body. In 1888, an Australian botanist Friedrich Reinitzer isolated and studied a form of cholesterol, cholesterol benzoate. On studying this molecule, he observed the existence of two melting points at 145.5°C and 178.5°C.[3] In between these two melting points, he observed a milky liquid phase, whereas above 178.5°C, there was a clear liquid.

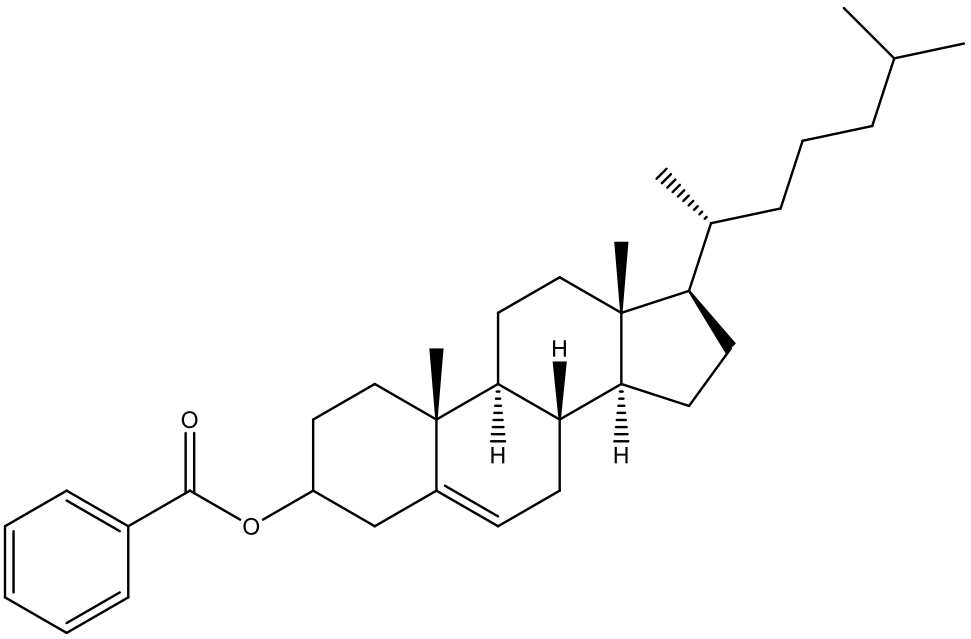


Figure 3: Structure of Cholesterol benzoate

He then contacted Otto Lehman, a physicist in Germany, who was studying crystallization of materials using Polarised Optical Microscope. On investigating the sample, he concluded that the opaque fluid observed by Reinitzer was distinct phase of matter, which had properties of both solids and liquids, and hence coined the phrase “Liquid Crystal”.

1.3 Nematic LC phase

Thermotropic LCs undergoes change in phase as a function of temperature. They are further categorised based on their shape as Calamitic (rod-like) and Discotic (disc-shaped). Nematic phase of LC is characterized by long range orientational order, where the long axis of the molecules tends to align along a particular direction.

The local orientation a molecule, denoted by a vector n(r), may vary throughout the medium and this vector is called the director. Its magnitude is taken as unity as it does not hold any significance. Nematic phase LCs do not have long range positional order, therefore the director sign also does not have any physical significance.

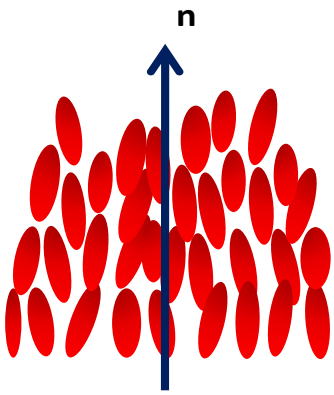


Figure 4: Nematic phase LC

The most widely used Nematic phase LCs for sensing experiments and the ones which exhibit LC phase at room are 5CB and E7. 5CB (4-cyano-4'-n-pentylbiphenyl) exhibits LC phase from 18°C upto 35°C. E7 is a mixture of four alkoxycyanobiphenyls (nCBs) with differing alkyl chain lengths: 51 wt.% 5CB, 25 wt.% 4-cyano-4'-n-heptyl-biphenyl (7CB), 16 wt.% 4-cyano-4'-n- oxyoctyl-biphenyl (8OCB), and 8 wt.% 4-cyano-4'-n-pentyl-p-terphenyl (5CT). It exhibits nematic phase between -10°C to 60°C. E7 is in general preferred for biological experiments over 5CB, as it less toxic and exhibits nematic mesophase over a wide range of temperature.

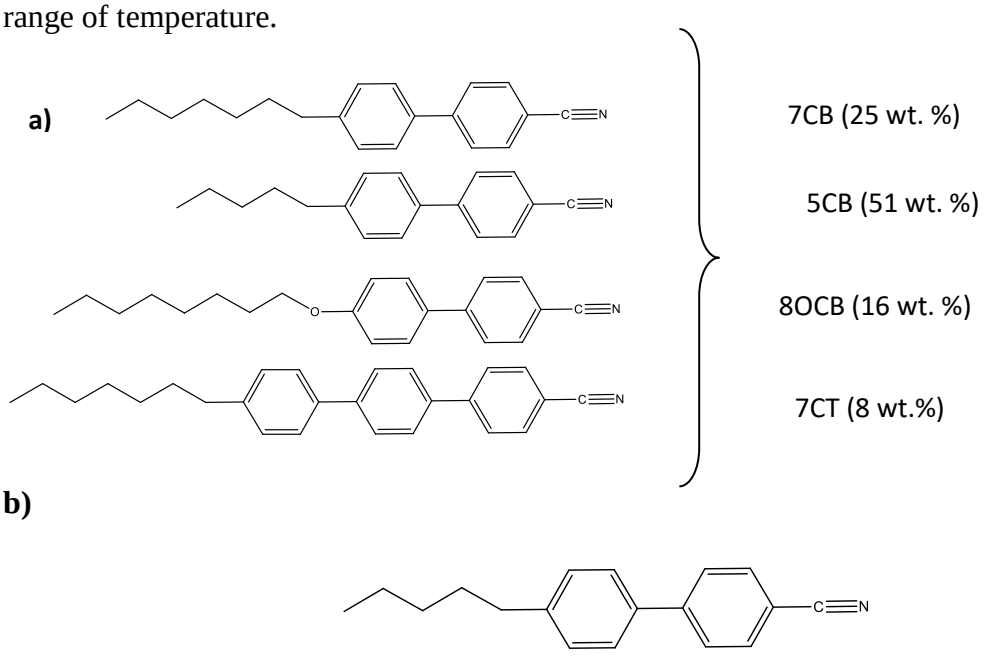


Figure 5: a) Various components of E7, b) 5CB

The orientational order parameter essentially quantifies the degree of order in Nematic phase LCs, and is denoted as S. Isotropic liquids, being completely disordered has a S value of 0, while perfectly oriented samples have a value of 1. Therefore, Liquid crystals lie in the in-between range and their typical value falls between 0.3 - 0.8.

Below is the equation to calculate the value of S, which is essentially the average of the secondary Legendre polynomial[2] and θ is the angle between the director and the local orientation of the molecules in the nematic phase.

$$\boldsymbol{S = < P_2(\cos\theta) > = < \frac{3\,cos^2\theta - 1}{2} >}$$

1.4 Behaviour of LCs at interfaces

One important advantage of using LCs in Biosensing is that, it not only acts as the interface, but it can also amplify the signal it receives from the biological event. As already mentioned, LCs have orientational order like crystals and this ordering is very sensitive to interfacial interactions. The signal so produced can be amplified to the LC bulk phase upto hundred micrometers. Not only can the signal be transmitted to the bulk phase but can also be done within tens of milliseconds owing to the elasticity and fluid like mobility of LCs.

Main properties relating to Nematic phase LCs have been discussed here, that are very relevant and important from the Biosensing point of view. These properties are:

- Birefringence
- Surface anchoring of LCs
- Anchoring angles of LCs

1.4.1 Birefringence

LCs are optically anisotropic materials. Birefringence is a property that is common to all anisotropic material whereby the refractive index of the material depends on the polarization of the light or the direction in which it propagates.[2] We will be dealing with Nematic LCs which falls in the uniaxial category. So basically when the light falls on the LCs, the speed at which it propagates in one direction is different from the other two, or in other words, its refractive index in one direction is different from that of the other two. The optical anisotropy

of material is basically the difference between the refractive indices of light polarised perpendicular and parallel to this direction (extraordinary and ordinary indices respectively)

$$\Delta n = n_{||} - n$$

Therefore, using the phenomenon that light polarized in different directions travel at different speed, the optical image of LCs are obtained using POM.

1.4.2 Surface anchoring of Liquid crystals.

The direction in which the director points depend on the environment or the boundary conditions present on the surface. Three types of alignment can be induced in liquid crystals based on the surface conditions namely, planar, homeotropic and tilted. On such a surface an easy angle is defined as the angle between the surface normal and the director in the undistorted state. The alignment of interest in our case is Homeotropic in which the nematic phase director is along the direction of the surface normal. This type of alignment is obtained by coating the substrate or the glass slide with surfactants like DMOAP or OTS and making a LC film above it. The details of the procedure are discussed in the following chapter. This will give rise to a uniformly aligned film which is very essential for LC based experiments.

Application of an external field or changing the environment of LC film will cause the director to deviate from its easy axis.[2] The amount of energy this process requires can be determined from the anchoring energy. The surface anchoring energy of liquid crystals is in the irder of 10^{-3} to 1 mJ/m^2.[4] Hence, a small change in the environment of the LC interface could disturb its ordering causing a transition and this transition can be communicated to a distance of 100μm from interface.

1.4.2 Anchoring of Liquid Crystal

The polar angle, Θ and the azimuthal angle ϕ are used to describe the anchoring of Liquid Crystals.[2] The polar angle is the angle between the director and surface normal. Polar anchoring is of three types,

i) Planar anchoring, Θ = 90°
ii) Homeotropic anchoring, Θ = 0°
iii) Tilted anchoring, 0°<Θ<90°

Azimuthal angle represents the in-plane orientation of the LC director with respect to azimuthal axis, x as shown in the figure.

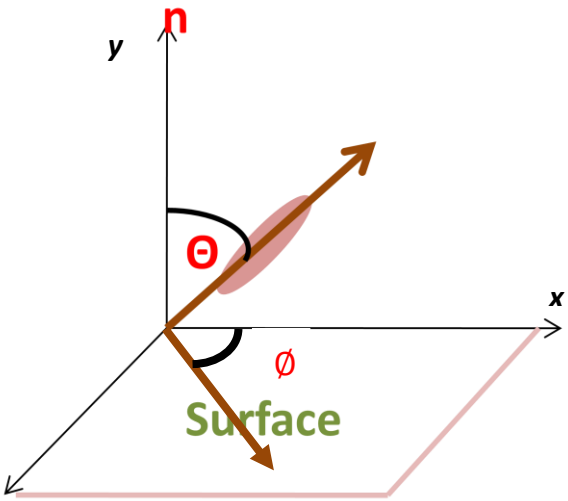


Figure 6: Schematic illustrations of a polar angle, θ and azimuthal angle, Ø

1.5 Polarized Optical Microscope(POM)

As already mentioned above LCs are birefringent material and their orientational changes can easily be analysed and observed using POM. Using POM for Biosensing, hence eliminates the requirement of complex and expensive instrumentational analysis.

Polarized Optical Microscope helps in acquiring high contrast images of optically anisotropic materials. LCs can be analysed qualitatively and quantitatively based on the orientational changes they undergo under different environmental conditions.

Its basic set up consists of an objective lens, commonly known as the polariser and an analyser which are placed before and after the sample respectively. These two lenses can be oriented at different angles with respect to each other based on the experimental requirements. In all the experiments, we have placed the lenses perpendicular to each other and this orientation is called “crossed polariser”.

The incident light is initially polarised by the objective lens in a single plane, which on falling on the birefringent sample is split into two different rays that are polarised in mutually perpendicular planes and travel at different velocities. These two components are out of phase

when they leave the sample but are recombined with constructive and destructive interference after passing through the analyser. Hence, this technique can not only provide high contrast images but can also distinguish between isotropic and anisotropic materials.

When the LCs are homeotropically oriented, the polarized light emerging from the sample completely blocked by the analyser and a dark image is observed from the POM. Whereas, when the LCs are in tilted orientation, near the surface the LCs are homeotropically aligned and in the top the molecules are parallel to the interface. When this happens, a thin film of LC exhibits birefringence and a coloured texture appears under cross polarized condition.

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Chapter Two

Experimental section

2.1 Introduction

Arsenic, the 20th most abundant element in the terrestrial crust and the 12th most abundant mineral in the human body, is extensively studies for its harmful effect on human health and its ecotoxicological consequences. Exposure to high level of arsenic causes arsenic poisoning known as arsenicosis and can primarily occur through infected water and food, which results in changes in skin pigmentation and thickening and also causes various types of cancers.[1-3]

In nature inorganic Arsenic occurs in -3, 0, +3, +5 oxidation states, though, in water it is predominantly found in the +3 and +5 oxidation states[4]. Both organic and inorganic forms of arsenic are found in water; however inorganic arsenic species poses the major toxicity and arsenite, As(III) is 60 times more toxic than arsenate,As(v).[5]

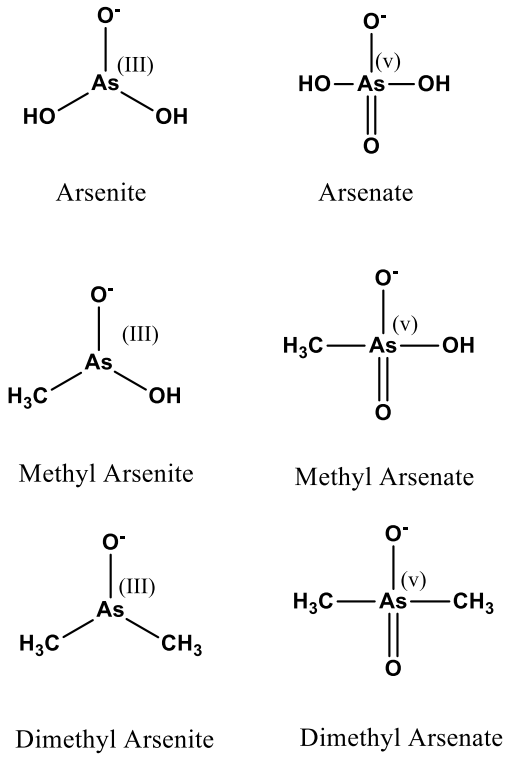


Figure 7: Inorganic and Organic arsenic species present in water

India, Bangladesh, China, Argentina, Mexico, Myanmar, Nepal, Pakistan, Vietnam and parts of the USA are few countries that are majorly affected with Arsenic contaminated water.[6,7] It has been estimated that millions of people are at the risk of drinking arsenic contaminated water.[8] The current Maximum Contamination Limit for arsenic in drinking water set by the World Health Organization is 10ppb.[9]

Various analytical techniques have since then been developed to detect the presence of Arsenic in drinking water which includes, absorption spectrophotometry (AAS), hydride generation-AAS, inductively coupled plasma, neutron activation analysis, electrochemical method, colorimetry etc.[10] However these analytical techniques have drawback of being extremely expensive, time consuming, portability issues, and the need for professional operation & maintenance.

2.2 Objective

The main objective of this project is to device a sensing system for arsenic ions in drinking water that is not so expensive and one which is capable of on-site detection, as the regions affected with arsenic contaminated water are rural. Also using LC based sensing system could overcome the portability and sample preparation issues.

Having these factors in mind we designed a LC based sensing for arsenic ions with help of biologically derived recognition element, aptamers that are highly selective and specific for As(III) ions

2.3 Detection of Arsenic and Proposed Scheme:

A lot of reports have published on Heavy metal ion detection using biologically derived recognition elements in the last few decades. The ideas behind these analytical techniques have their basis from the biological processes happening in our body. For instance, the detection of mercury has been designed based on the fact that mercury forms a coordination complex with two thymine units, Thy-Hg-Thy.[11] This kind of complex formation stabilized

two complementary strands of DNA with a Thy-Thy mismatch. Following this, was a report on the detection of lead ions which was based on DNA duplex- quadruplex conformational change. Lead ions are known for their ability to stabilize the G-quadruplex structure of DNA, causing the DNA duplex structure to unwind. This cycle was also made reversible by the addition of lead chelator to the system.[12] Therefore, on addition of fluorophore to the system, which gets activated at a particular conformation of DNA, the lead ions present in the system is quantitatively analysed.

The advantage LC based sensing system over this is that there is no requirement of a fluorescent lable, that is, it is capable of label-free detection of analyte molecules. Detection of mercury has been carried using the LC based sensing technology, again using the Thy-Hg-Thy coordination chemistry.[13] Following this a lot reports have been published on detection of analyte molecules using biologically derived recognition systems. We have tried to extend this approach towards detection of arsenic ions.

The recognition element we planned use for this project is an aptamer, Ars-3. Aptamers are *in-vitro* selected artificial oligonucleotides, like RNA or single stranded DNA or peptides that can bind to a wide range of target molecules with high affinity and specificity due to their specific three dimensional structure. They lately have been seen as replacements to antibodies owing to their several advantages, which includes thermal stability, broad target range, low cost, low immunogenicity and their ease of production and modification.

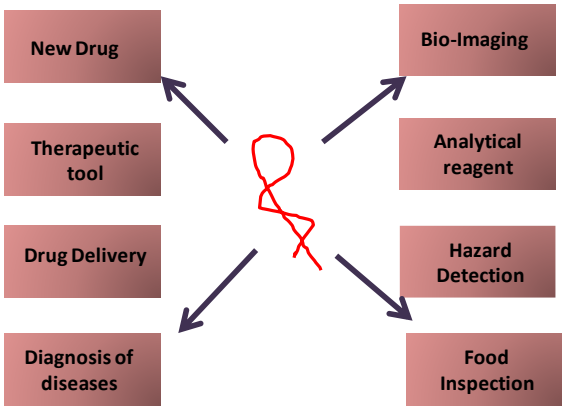


Figure 8: Picture illustrating various biological applications of aptamer

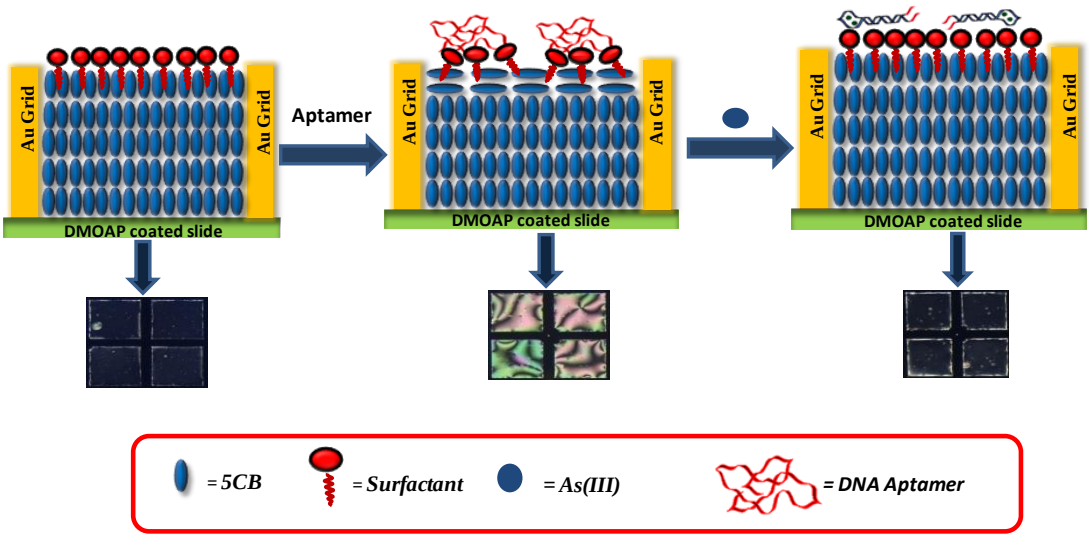
Ars-3 aptamer, highly selective and specific towards As(III) ions, was initially used to remove As(III) ions from contaminated water in Vietnam.[14] Later this aptamer has been

used in colorimetric detection of As(III) ions and has given promising results.[15,16] We have tried to use the same aptamer with carefully designed LC interface to detect As(III) ions to overcome some drawbacks existing in the previous methodologies.

Proposed Scheme:

The LC interface has been carefully designed based on the requirements of the experiment. The homeotropic alignment of LC in aqueous-LC interface is brought about by the addition of CTAB. We have chosen CTAB specifically for this experiment because CTAB is a cationic surfactant and it is known to non-specifically interact with negatively charged DNA molecule through electrostatic interactions. Therefore, this interaction causes perturbation in the interfacial area and will disturb the alignment of 5CB molecules and will orient them in planar fashion. Further on the addition of arsenic ions into the system, due to the specific interaction between arsenic ions and Ars-3 aptamer, the aptamer will no longer strongly bind to CTAB. This unbound CTAB can now align the 5CB molecules back to the homeotropic orientation.

We confirm this hypothesis by taking POM images of the 5CB molecules in the different environments. Also, to establish what kind interactions happens between aptamer, CTAB and As(III), we have performed Circular Dichroism Spectroscopy.



Scheme 2: Illustration of the propose scheme for As(III) detection using LC system

2.3.1 Circular Dichroism Spectroscopy:

Circular Dichroism Spectroscopy is a kind of absorbance spectroscopy which measures the difference in absorbance between the right circularly polarised and left circularly polarised light. This is technique is useful when a molecule contains one or more chiral chromophores.

$$\textbf{Circular Dichroism} = \Delta A(\lambda) = A(\lambda)_{LCPL} - A(\lambda)_{RCPL}$$

In this technique the CD of the molecule is measured over a range of wavelength and the CD signal is reported in units of mdeg.

The most important application of CD is in the study of large biological molecules, especially in analysing the secondary structure or conformations of macromolecules. It can be used to analyse how the structure of these molecules change when there is a change in the environment, like a change in temperature or pH. Hence, it is a very useful technique in protein interaction studies.

Measurements are taken in the visible and ultra violet range, so that electron transitions that happen can be monitored. If the molecule contains a chiral chromophores then one CPL will be absorbed to a greater extent than the other, and a corresponding peak at that particular wavelength will be observed in the CD spectrum. The value of CD can be positive or negative depending upon which CPL is absorbed to a greater or lesser extent. If L-CPL is absorbed to a greater extent, then the value is positive, otherwise it is negative. To analyse the secondary structure of peptides/oligonucleotides the spectra has to be carefully analysed within the range of 260nm to 180nm.

2.4 Materials and Methods:

The sequence of Ars-3 aptamer was synthesized by Genxbio as referenced in the previous literature. The sequence of Ars-3 aptamer is 5'-GGTAATACGACTCACTATAGGGAGATACCAGCTTATTCAATTTTACAGAACAACCAACGTCGCTCCGGGTACTTCTTCATCGAGATAGTAAGTGCAATCT-3'. The stock solution of Ars-3 aptamer was made by dissolving the aptamer in 10mM N-(2-Hydroxyethyl)piperazine-N'-2-ethanesulfonic acid (HEPES) buffer solution of pH 7.1. Hexadecyltrimethyammonium bromide (CTAB), N,N-dimethyl-N-octadecyl-3-

aminopropyltrimethoxysilyl chloride (DMOAP) and Sodium metaArsenite($NaAsO_2$) were purchased from Sigma Aldrich. Sulphuric acid and hydrogen peroxide (30% w/v) were purchased from Merck, and Ethanol from Jebsen & Jenssen GmbH and Co., Germany (Sd. Fine-chem limited). Deionization of a distilled water source was performed using a Milli-Q-system (Millipore,Bedford,MA). Fischer's Finest Premium Grade glass microscope and cover glass were obtained from Fischer Scientific (Pittsburg,PA). Gold specimen grids(20 µm thickness, 50µm wide bars, 283µm grid spacing) were obtained from Electron Microscopy Sciences (Fort Washington, PA).

2.4.1 Cleaning of glass slides:

The cleaning of glass slides were performed in accordance with published procedures using 'piranha' solution [70:30 (%v/v) H_2SO_4:H_2O_2 (30%)].[15] In short, the glass slides kept in Kaplin jar were immersed in 60 ml of Piranha solution. The setup was heated at 80°C using water bath for atleast an hour. The glass slides were then rinsed in running deionised water for 2-3 times. Finally, the slides were rinsed sequentially in ethanol and dried under nitrogen. The clean glass slides were then kept in the oven at 100°C for overnight.

2.4.2 Preparation of DMOAP coated glass slides:

The cleaned glass slides were dipped into 0.1 %(v/v) solution of DMOAP in DI water for 30 minutes at room temperature and then, were rinsed with DI water for 4-5 times to remove any unreacted DMOAP. The DMOAP coated glass slides were then purged with nitrogen gas and was kept in oven at 100°C for overnight to facilitate the crosslinking of DMOAP.

2.4.3 Procedure for As(III) detection:

50µL of 150nM Ars-3 aptamer was incubated at 25°C with 25uL of varying concentrations of As(III) ions for a 60 minutes. To this, 25uL of 7uM CTAB was added and was incubated further for a period of 30 minutes. This sample was then added on to the Liquid Crystal films to observe the time dependent orientational change of liquid crystals for varying concentrations of As(III) ions. Selectivity test for the aptamer was also performed using the same procedure, for a single concentration of 500ppb for various ions which includes, Na^+, Ca^{2+}, Mg^{2+}, Hg^{2+}, K^+, and Cd^{2+}.

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Chapter Three

Results and Discussion

The experiments were performed as discussed in the proposed scheme in Chapter two. Briefly, the main motive of this work is to device an analytical tool that is capable of on-site arsenic detection. To achieve this, we designed a LC based sensing system using aptamers as recognition element.

Initially, 5CB LCs are oriented homeotropically by introducing CTAB into the system. As already mentioned, CTAB has been reported to interact non-specifically with DNA molecules through electrostatic interactions and this causes the LC molecules to change their orientation to planar and the POM gives a bright texture. Now, on introducing As(III) ions into the system, due to the specific interaction between Ars-3 aptamer and As(III) ions, the aptamer no longer interacts strongly with CTAB as it did when there was no As(III) in the system, and hence it aligns 5CB back to the homeotropic orientation.

This was our expectation before starting the experiments. In order to test this hypothesis, we performed experiments using POM to observe the alignment of LCs in various environments. Circular Dichroism was also performed to understand what kind of interaction is there between aptamer & As(III) and aptamer & CTAB.

3.1 Initial optimization of experiments:

(i) Concentration of CTAB

All solutions were made in 10mM HEPES Buffer solution with a pH of 7.1. First, we need to determine the minimum concentration of CTAB at which 5CB molecules orient homeotropically and are stable in that orientation for the required amount of time. Below are the POM images for this experiment.

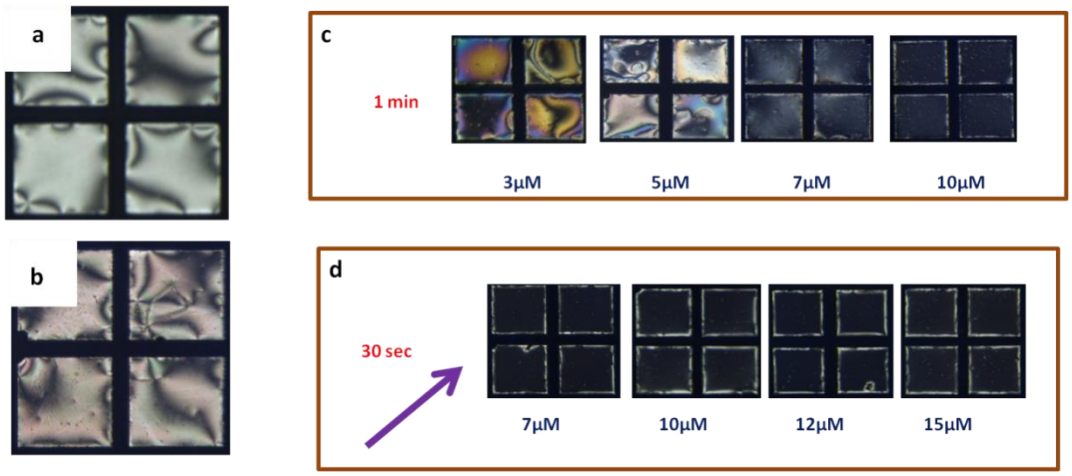

Figure 9: POM texture of 5CB a) in 10mM HEPES buffer solution, b) in 1µM CTAB after 30 minutes, c) at various concentration of CTAB after 1 minute, d) at various concentration of CTAB after 30 seconds

As all solutions are prepared in HEPES buffer, initially a control experiment was performed to check the orientation of 5CB molecules. As expected, the aligned in planar orientation as seen LC-aqueous interface. The lowest concentration at which CTAB remains in planar orientation was observed to be 1µM. Therefore, on gradually increasing the concentration, it was observed that at 7µM it changes to homeotropic orientation within 30 minutes and is stable.

Therefore, for further experiments the net concentration of **CTAB** in the sample will be **7µM.**

(ii) Concentration of Ars-3 aptamer :

To determine the concentration of Ars-3 aptamer to be used, various concentration of aptamer was added to 7µM CTAB. The ratio of CTAB to Ars-3 aptamer was taken to be 1:1. All readings were taken at room temperature.

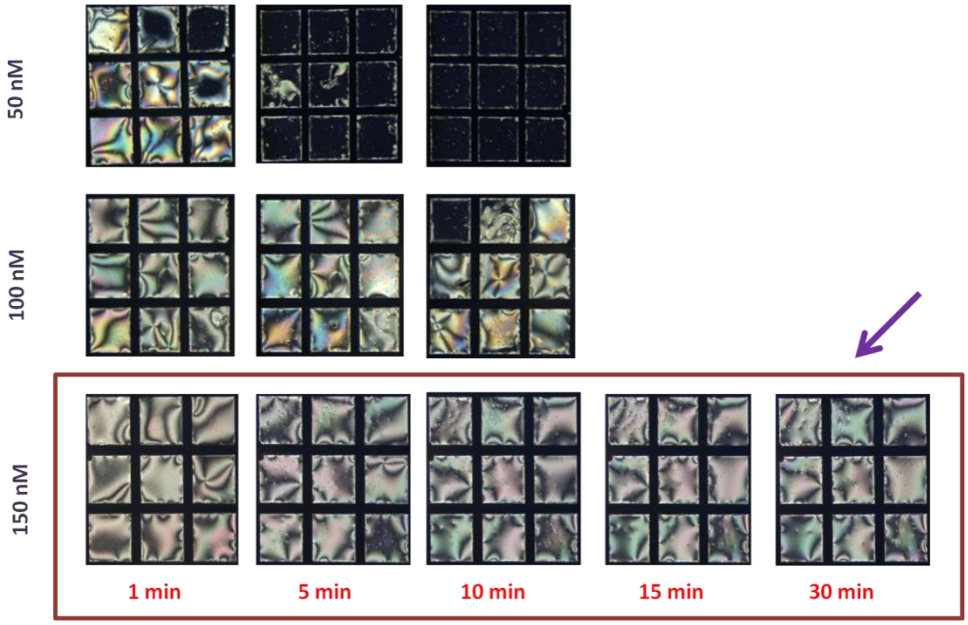

Figure 10: Image shows the behaviour of 5CB molecules over time at various concentrations of Ars-3 aptamer with a net concentration of 7µM CTAB.

As seen from the image above at 150nM of Ars-3 aptamer the 5CB molecules are oriented in planar fashion and this orientation was observed to be stable for 1 hour.

Therefore, the net concentration of **Ars-3 aptamer** to be used for further experiments is **150nM.**

3.2 **Sensitivity of the LC system to As(III) ions**

After the initial optimization experiments, now we intend to find the sensitivity of this LC sensing to detect As(III) ions. For this we observe the behaviour of 5CB at varying concentrations of As(III) over a period of 20 minutes. The sample preparation has been performed as explained in chapter 2 under materials and methods section. Following, Grey scale analysis was also performed.

Figure 11a: Behaviour of 5CB at varying concentration of As(III) ions. Experimental conditions: Varying concentration of As, 7µM CTAB and 150nM Ars-3 aptamer

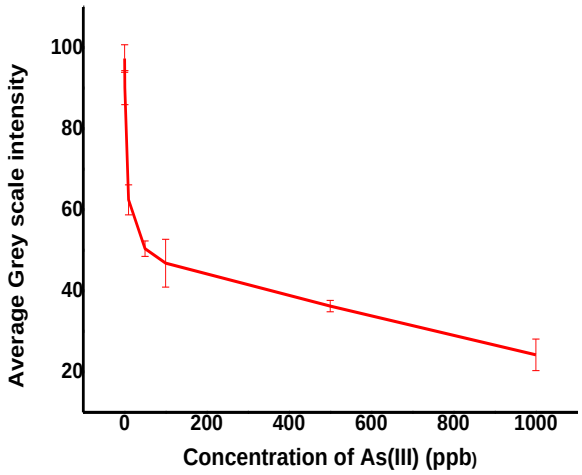

Figure 11b: The average grey scale intensity of optical images of 5CB films as a function of varying concentrations of As(III). Experimental conditions: Varying concentration of As, 7µM CTAB and 150nM Ars-3 aptamer

3.3 Selectivity of the sensing system towards As(III) ions:

Water has a lot other ions present and the ions present in the range from 0.1 to 1000 mg/L are said to be the Major Constituents of water. The major cations present in water are Sodium(Na^+), Calcium(Ca^{2+}), Magnesium(Mg^{2+}) and Potassium(K^+). The selectivity of Ars-3 aptamer for few of these ions was tested.

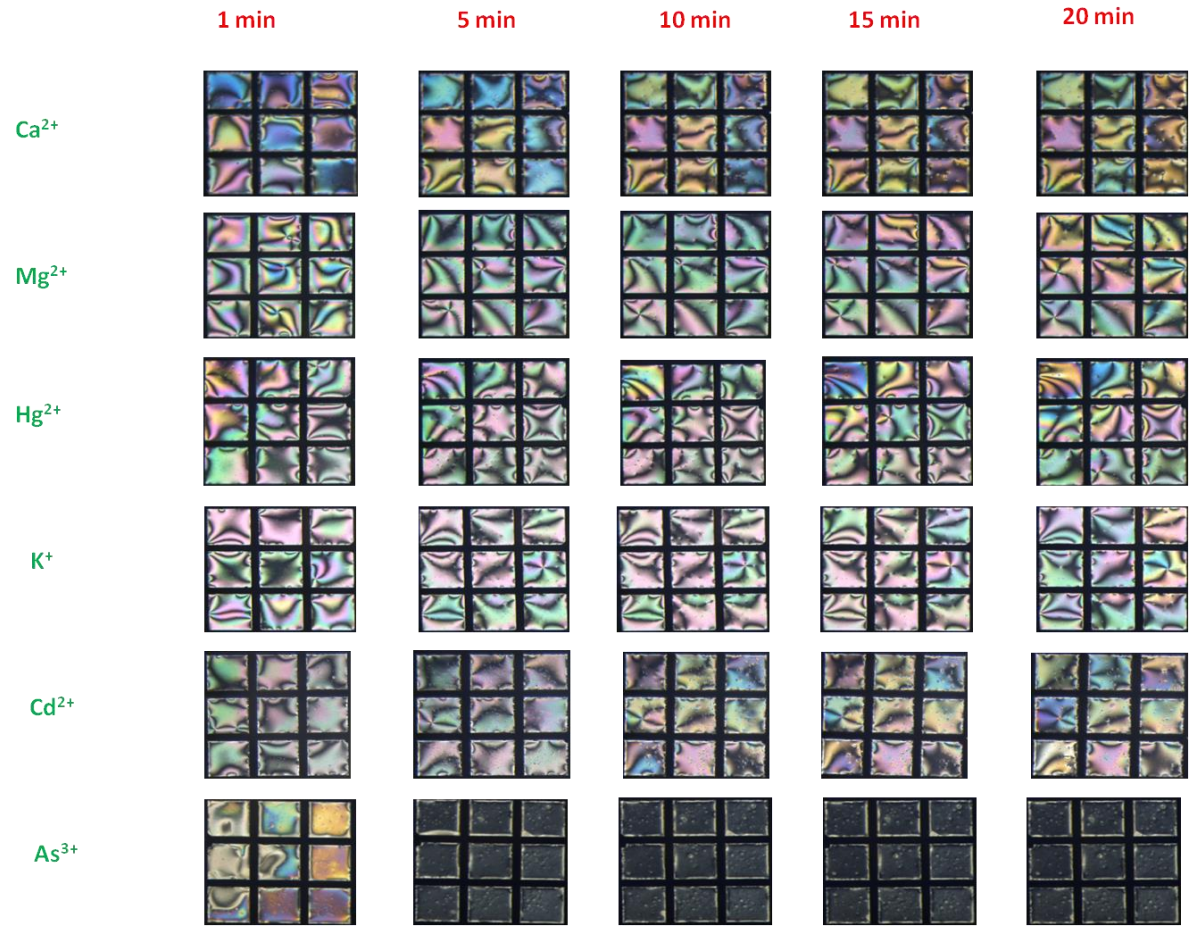

Figure 12: Selectivity of Ars-3 aptamer to other commonly found ions in water. Experimental conditions: 500ppb of various ions, 7µM CTAB and 150nM Ars-3 aptamer

It is observed that at 1ppb of As(III) the 5CB molecules align in homeotropic orientation within 20 minutes. At concentration lesser than that brighter domains are still observed. Concentrations higher than 1ppb, 5CB align in homeotropic orientation within 20 minutes. At very high concentrations of 500ppb and 1000 ppb they go to homeotropic orientation within 3 minutes. This can also be observed from the average Grey scale intensity plot.

Hence, from this data we conclude that the LOD of this sensing system is **1ppb**.

3.4 Circular Dichroism Spectra

To understand what kind of interaction happens between Ars-3 and the other components of the system, we performed CD spectroscopy. This will also give us an idea about the structure of Ars-3 aptamer and the changes it undergoes when it interacts with CTAB or As(III) ions.

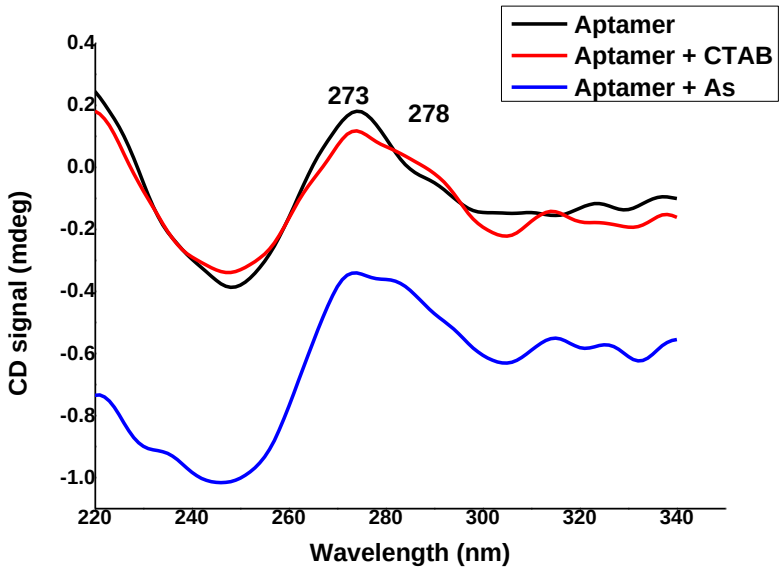

Figure 13: CD spectra of Ars-3 aptamer solutions treated with CTAB and As(III). Experimental conditions: 500 nM Ars-3 aptamer, 50 µM CTAB and 2000 ppb As(III).

The Ars-3 aptamer has a negative peak in the range of 240-260nm and a positive peak at 270-290nm which are characteristic peaks of B-DNA structures. In the presence of CTAB the CD spectra did not change much, but there is a slight shift in the peaks present in the positive region. This indicates electron transfer occurs from CTAB to Ars-3 aptamer. A decrease in CD signal has been observed in case of Ars-3 aptamer and As(III) mixture. This could be due to the interaction of As(III) ions with the some bases of the Ars-3 aptamer, leading to a change in its conformation. Therefore, the interactio of As ions happens with the hydrophobic part of the aptamer.

3.5 Conclusion

- Liquid Crystal based sensor system for detection of arsenic using aptamer has been designed and fabricated.
- This sensor is highly selective to Arsenic ion with respect to other heavy metal ions.
- The lower detection limit of the sensor is 1ppb.

3.6 Future works

To perform Real time detection of Arsenic ions

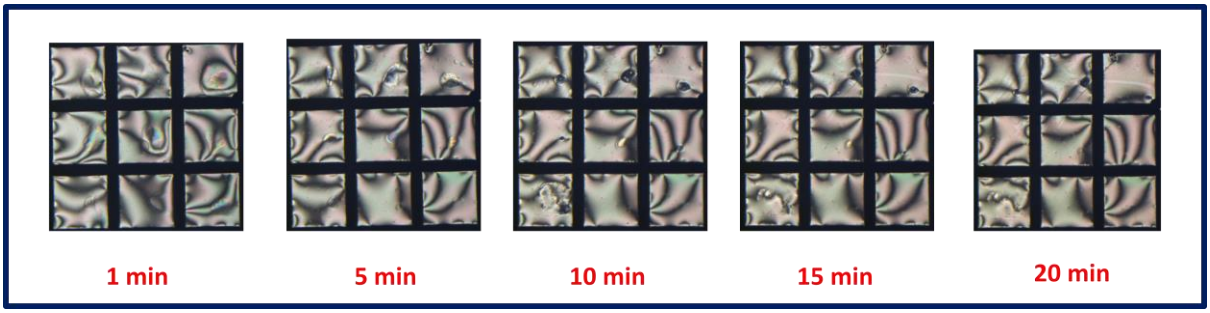

Figure 14: 5CB behaviour in the presence of tap water instead of As(III) ions in the sample.