DEVELOPMENT OF A FLUIDIC PLATFORM FOR AUTOMATED ANALYSIS OF HEAVY METALS

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ABSTRACT

DEVELOPMENT OF A FLUIDIC PLATFORM FOR AUTOMATED ANALYSIS OF HEAVY METALS

Heavy metals are part of Earth's crust and the significant problem is accumulation of them in the ground waters. They have harmful results to body even they are at low concentration. Arsenic is one of the heavy metals which cause serious health problems such as; cancer, diabetes etc. Most of the developing countries are lack of detecting arsenic amount into drinking water. Therefore, especially in Bangladesh 1 out of 100 people die due to arsenic related cancer. According to World Health Organization, the maximum arsenic concentration in the drinking water must be 10 μg/L but the concentration amount reaches 50 μg/L or more at the developing countries. However, existing methods cannot detect arsenic at this range or the ones that can detect arsenic at the standard have so high prices that developing countries cannot afford. Therefore, the aim of this project is to develop a device for arsenic detection. In this thesis, a microfluidic chip was developed, and gold nanoparticles was used to detect arsenic in samples using absorbance spectroscopy. The detection principle was designed based absorption of arsenic and then gold on thiol-modified surfaces. The different concentration of arsenic samples was injected into microfluidic chip and 1.3 mg/L arsenic concentration could be detected. Then, syringe pump was added to the system and flow applied. As a result, the developed microfluidic chip is able to detect arsenic at 2.2 μg/L.

ÖZET

AĞIR METALLERİN ANALİZİ İÇİN OTOMATİKLEŞTİRİLMİŞ BİR AKIŞKAN PLATFORMUN GELİŞTİRİLMESİ

Ağır metaller, dünya kabuğunun bir parçasıdır ve yeraltı sularında birikmeleri önemli bir problem haline gelmiştir. Düşük konsantrasyonlarında bile vücutta ciddi zararlı sonuçları vardır. Arsenik de ciddi sağlık sorunlarına neden olan ağır metallerden biridir örneğin; kanser, diyabet vb. Gelişmekte olan ülkelerin çoğunda arsenik miktarının içme suyundaki tespiti yetersizdir. Bu nedenle, özellikle Bangladeş'te 100 kişiden 1'i arsenik kaynaklı kanser nedeniyle ölmektedir. Dünya Sağlık Örgütü'ne göre, içme suyunda maksimum arsenik konsantrasyonu 10 µg / L olmalıdır ancak gelişmekte olan ülkelerde bu miktar 50 µg/L ve üzerine çıkmaktadır. Bununla birlikte, mevcut yöntemler bu aralıkta arsenik tespit edememektedir veya standartta arsenik tespit edebilenler, gelişmekte olan ülkelerin karşılayamayacağı kadar yüksek fiyatlara sahiptir. Bu nedenle, bu projenin amacı arsenik tespiti için bir cihaz geliştirmektir. Bu projede, bir mikroakışkan çip geliştirildi ve metalik özelliklerinden dolayı sonuç elde etmek için altın nanoparçacıklar kullanıldı. İlke, As-S bağı ve Au-S bağı temel alınarak tasarlanmıştır. Farklı arsenik örneklerinin konsantrasyonu mikroakışkan çipin içine enjekte edildi ve 1.3 mg/L arsenik konsantrasyonu tespit edilebildi. Daha sonra sisteme şırınga pompası eklendi ve akış uygulandı. Sonuç olarak, geliştirilen mikroakışkan çip, 2.2 µg / L'de arsenik tespit edebiliyor.

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CHAPTER 1

INTRODUCTION

One of the most important life sources is “water” for plants and animals and is generally get from either lakes or ground water. Water can absorb, adsorb, dissolve and suspend lots of distinct compounds due to its unique property such as hydrogen bonds and its polarity [(Momodu & Anyakora, 2010)]. Therefore, water cannot keep its pure clean form and contamination increases because of humans and animals. The most striking environmental problem is the pollution of ground water. Heavy metal contamination leads the maximum concern because of its toxicity even if it is at low concentration. Heavy metals are part of Earth’s crust and mostly placed in formation of rocks [(Sharma & Agrawal, 2005)]. The extreme usage of heavy metals in industry increase to spread out the toxicity to environment. For heavy metals, being water soluble make them more toxic [(M. Arora et al., 2008)]. Even when they are at low concentration, they have devastation results in human and animal body, because they cannot be eliminated from the body. Additionally, they are not biodegradable in environment and they have ability to accumulate in the body [(Suruchi & Khanna, 2011)]. Eight common heavy metals are considered; arsenic, barium, cadmium, chromium, lead, mercury, selenium and silver [(M. Arora et al., 2008)].

Arsenic (As), which name is coming from the Greek word “arsenikon” means potent, is one of the heavy metals [(S.Y. Choong, Luqman Chuah, Yunus, Koay, & Idris, 2007), (Martin & Griswold, 2009)]. Arsenic is odorless and tasteless therefore it is difficult to detect arsenic in water. Arsenite (As (III)) and arsenate (As(V)) are inorganic arsenic forms and they most found in water and they are highly toxic for human and plants therefore they have priority for detection [(Yogarajah & Tsai, 2015)]. Arsenic occurs naturally in the environment however it can largely be discharged through volcanic movements, erosion of rocks, forest fires and human action. Arsenic is likewise found in paints, colors, metals, medications, cleansers and semi-channels.

Additionally, some fertilizers, pesticides and provender can release high amount of arsenic, also copper industry, mining and coal burning industries also release arsenic to the environment [(Martin & Griswold, 2009)].

Arsenic toxicology can be divided into two groups as acute and sub-acute. The acute poisoning is a result of ingestion of contaminated food or drink and needed immediate medical consideration. The early symptoms can be listed as; occurring mouth and throat burning and dryness, vomiting, diarrhea, muscular cramps, facial edema and because of promptly loss of water shock can be occurred [(S.Y. Choong et al., 2007)].

In general, four stages of arsenic poisoning;

i. Preclinical: Arsenic can be noticed in urine samples even there will not be any symptoms.
ii. Clinical: According to World Health Organization (WHO) to reach this stage, human need to exposure arsenic for 5-10 years. The symptoms can be observed on the skin for instance; darkens of skin and dark spots on chest and back. Edema is observed in hands and feet. The most severe manifestation is hardening of skin into nodules.
iii. Complications: At this stage, arsenic starts to influence internal organs. It is recorded that growth of liver, kidneys and spleen. Bronchitis, diabetes and conjunctivitis can also be observed according to some researches.
iv. Malignancy: Last stage of arsenic poisoning. At this stage, organs are affected by tumors or carcinoma. [(S.Y. Choong et al., 2007)]

Arsenic cannot be devastated, and it can just be converted into other forms of itself. Arsenic contamination is a problem that affects all corners around the world. According to WHO the maximum arsenic concentration in water must be 10 µg/L. However, in the most of developing countries, concentration of arsenic is so much higher than the standards [(WHO, 2001)]. Argentina, Bangladesh, Nepal, Pakistan, China, India, Taiwan, Mexico, Chile, part of USA and Vietnam are the arsenic infected areas shown in Figure 1(Yogarajah & Tsai, 2015)].

Smith et.al. indicated that from 125 million citizen of Bangladesh, 35 million to 77 million are under at danger because of exposure of contaminated water [(Smith, 2000)]. Also, a study shows that in Bangladesh 20% of deaths are arsenic related, between the 2000-2009 years and the arsenic concentration in drinking water is 50 µg/L [(Yogarajah & Tsai, 2015)]].

Figure 1. Map indicated the global arsenic circumstance; red highlighted part are arsenic affected aquifiers, red dots show the arsenic related to mining operations and green dots indicate the geothermal waters .(Source: Yogarajah & Tsai, 2015)

In Chile, 12% of citizens have skin symptoms related with arsenic consumption in water and in Taiwan, exposure of arsenic causes “blackfoot disease” which means gangrene [(Lu, 1990),(Borgono & Greiber, 2019)].WHO indicated that in Europe, the arsenic concentration is reported as below 10 µg/L [(WHO)].

1.1 Arsenic Detection Methods

Nowadays, expensive laboratory techniques, arsenic kits, colorimetric methods and microfluidic devices are used for arsenic detection. The laboratory techniques that are used for arsenic analysis are; atomic absorbance spectroscopy (AAS), atomic fluorescence spectroscopy (AFS), atomic emission spectroscopy (AES), mass spectroscopy and chromatography.

1.1.1 Standard Laboratory Tests for Arsenic Detection

The most common techniques are compared in Table 1. The limit of detection (LOD) of these techniques are 1 µg/L [(Yogarajah & Tsai, 2015),(U. Bose, 2011)]. The performance of the techniques is high however the costs of these techniques are above $ 30,000; without considering well-educated technicians and upkeep costs. [(Yogarajah & Tsai, 2015)]

Table 1. Comparison of the most common laboratory methods for arsenic detection [(Yogarajah & Tsai, 2015),(Raj Behari & Prakash, 2006)]

	LABORATORY DETECTION METHODS		
	AAS	**ICP-MS**	**AFS**
LIMIT OF DETECTION RANGES (µg/L)	0.0009-1	0.0003-1	0.0003-10
REPRODUCIBILITY(%RSD)	<10	<10	<10
SAMPLE SIZE (µL)	>1000	2-200	20-200
TIME REQUIRED	~30 minutes	~30 seconds	~10 minutes
COST OF DEVICE (USD)	~$60,000	~$200,000	~$60,000
SKILL REQUIREMENT	Simple to handle for educated technician	Difficult first method improvement even for technician	Simple to handle for educated technician

AAS was first used in 1980s to detect arsenic [(U. Bose, 2011)]. While detecting the trace amount of arsenic, assembly of AAS with vapor generation is a common technique. The principle starts with reducing As (V) to As (III) for true analysis. AAS can only detect and make absorption signal if there is free As atoms. Therefore As (III) is reduced to arsine gas (AsH_3) and free As atoms at last.

Behari Raj J. et al., shows that, to be successful, the vapor generation system was linked to AAS, which has two channels, one is acid channel with HCl and other is reduction channel with sodium borohydride ($NaBH_4$). Sample is reduced by two ways: (i) sample can be reduced previously by using potassium iodide (KI) and (ii) sample is let in the instrument and then KI is added to reduction channel through sodium borohydride [(U. Bose, 2011),(Raj Behari & Prakash, 2006),(Aggett & C Aspell, 1976)].

AAS is also combined with hydride generation (HG) again to increase the selectivity, sensitivity and to decrease the limit of detection [(U. Bose, 2011)]. This technique also includes the reduction of arsenic into the AsH_3 with acid and sodium borohydride [(Sigrist, Albertengo, Beldomenico, & Tudino, 2011),(Wahed et al., 2006)]. The arsine gas fed into the separator where liquid phase and gas phase are separated, and gas phase is transmitted to absorption cell. Then, by using software, peak heights show the quantitation [(Wahed et al., 2006)]. On the other hand, Niedzielski P. et al., indicated that by using AAS-HG system the arsenic species go through the graphite tubes, which are covered with modifier, and caught by the walls of tubes. Total arsenic species can be detected with AAS-HG with the help of *in situ* trapping graphite tube walls, and this technique provides to decrease the limit of detection approximately 10 times compared to other AAS methods. [(Niedzielski & Siepak, 2005)].

AFS is one of the fluorescence methods and therefore it has constitutional high sensitivity, by this way the detection limits are improved [(U. Bose, 2011). While real samples are considered with AFS, interferences occur. The major challenges are light scattering and background since sample matrix (M. Arora, Megharaj, M., & Naidu, R., 2008; Pacquette, Elwood, Ezer, & Simeonsson, 2001). By using HG system which was firstly started to use in 1990s, arsine gas is isolated from the sample matrix, and just arsine gas reached to AFS detector. So, interference effect is importantly defeated, and the limit of detection is improved adequately.[(M. Featherstone, C. V. Butler, V. O'Grady, & Michel, 1998)]

Inductively coupled plasma (ICP) method was first founded in the beginning of 1960s [(U. Bose, 2011)]. The principle of this technique starts with using plasma to ionize ingredients, the acidified sample is sprinkled into the plasma. The plasma ionises and atomises the all types of arsenic with high temperature plasma. Generally, ICP is connected to other techniques as mass spectrometry (MS). By this connection, it is not needed any time to for sample preparation [(Q Hung, Nekrassova, & G Compton, 2004)]. ICP-MS is one of the most common and crucial technology for detecting arsenic.

For the low concentration of real arsenic samples, HG can be connected to the system to increase sensitivity, selectivity and it decreases the interference effect [(U. Bose, 2011),(A Francesconi & Kuehnelt, 2004)]

AES is the other analytical techniques to detect arsenic and combining it with HG provides to lower detection limit below the 0.1µg/L [(U. Bose, 2011),(Jain & Ali, 2000)]. ICP-AES is also one of the combination techniques, however interference effect is higher for real samples this method. Therefore, AES can be combined with HG to decrease interference effect. Terlecka E. indicated that, corporation with ICP and HG increase the sensitivity and reduce the interference effect [(Terlecka, 2005)].

In chromatography techniques, liquid chromatography (LC) and ion chromatography are the most common techniques. Thompson et al., indicated that LC are used with the combination of ICP-MS for arsenic detection in order to lower the detection limit with the range of 0.1-0.2 ng concentration [(U. Bose, 2011), (Beauchemin, W.M. Siu, W. McLaren, & S. Berman, 1989)]. High-performance liquid chromatography (HPLC) is an effective separation technique [(Thomas & Sniatecki, 1995)]. Ion chromatography (IC) is one of the effective techniques while detecting arsenic because it can isolate either inorganic, organic or free atoms. Interacting the charged analytes with functional groups on the stationary phase gives the working principle of IC. The most important parameters while detecting arsenic are pH and ion strength. If pH has higher value than the sample pKa, analytes become anions. Liu Ming Y. et al., indicated that pKa values of arsenic species are lower than pH, therefore an anion exchange column is used for arsenic separation [(M. Arora, Megharaj, M., & Naidu, R., 2008; Sheppard, 1992)),(Ming Liu & Sanz-Medel, 1993)]. In order to obtain high capacity IC is also connected with ICP and MS [(M. Arora, Megharaj, M., & Naidu, R., 2008),(Xie, Johnson, Spayd, S Hall, & Buckley, 2006)].

1.1.2 Available Kits for Arsenic Detection

The contamination of drinking water affects millions of people in developing countries. Organizations such as UNICEF, WHO and the WorldBank decided to find out a solution for arsenic detection.

For instance, arsenic field kits which are portable and economic, and millions of US dollars have been spent for these test kits.Some of these arsenic test kits are; NIPSOM, Merck, AAN, Hach EZ, Arsenator. The arsenic field kits detect arsenic based on the most common Gutzeit method [(Yogarajah & Tsai, 2015),(Rahman et al., 2002),(M. Arora, Megharaj, M., & Naidu, R., 2008)]. The Gutzeit method principle is, reducing arsenic to more toxic arsine gas by the addition of zinc granules which then get into reaction with mercuric bromide filter paper. Finally, embedded paper changes its color, according to intensity of color, arsenic amount is determined [(Brindle, 2007; Effluents, 1956)].

Rahmann et al., showed that these test kits have less reliability. False negative results were higher than 68% and false positive results reached 35%. More than two thousand samples were analyzed, and it was found that 44.9% of samples show arsenic concentration lower than 50 µg/L while the concentration was higher than this value [(M. Arora, Megharaj, M., & Naidu, R., 2008),(Rahman et al., 2002)]. Even the maximum arsenic concentration in drinking water should be 10 µg/L according to WHO, generally these field kits are unreliable below 70 µg/L concentration as can be seen in Table 2.

Pande et al., studied about the arsenic field kits and showed that, Merck can only detect the arsenic concentration above 50 µg/L as AAN and NIPSOM can detect concentration above the 20 µg/L, both concentrations are higher than the WHO standard [(M. Arora, Megharaj, M., & Naidu, R., 2008),(P. Pande, Deshpande, & N Kaul, 2001)]. Van Geen et al., analyzed the Hach EZ test kit and found out that, kit gives result between the concentrations of 50-100 µg/L. Many of the results are underrated as including the lower concentration of 50 µg/L. Additionally, it was indicated that, for Hach EZ kit, 40 minutes waiting for arsenic detection gives better results than 20 minutes waiting [(M. Arora, Megharaj, M., & Naidu, R., 2008),(van Geen et al., 2005),(M Steinmaus, M George, A Kalman, & H Smith, 2006)].

Moreover, Steinmaus et al., demonstrated that for Hach EZ kit, when the arsenic concentration of sample is higher, hydrogen sulphide interference is a possibility [(M Steinmaus et al., 2006)]. On the other hand, Jakariya et al., illustrated that, some wrong allocations occur in detecting arsenic because of analytical and human errors, especially, in the range of 10-24 µg/L and 50 -99.9 µg/L arsenic concentrations, which is crucial for health [(M. Arora, Megharaj, M., & Naidu, R., 2008),(Jakariya et al., 2007)]. Moreover, the electronic kit Wagtech Arsenator improved the correctness of the sample detection.

Table 2. General comparison of portable test kits; the theoretical, practical LOD and their reliability [7,(M. Arora, Megharaj, M., & Naidu, R., 2008),(P. Pande et al., 2001),(van Geen et al., 2005),(M Steinmaus et al., 2006)].

	PORTABLE ARSENIC TEST KITS				
	NIPSOM	**Merck**	**AAN**	**Hach EZ**	**Arsenator**
THEORETICAL LOD (µG/L)	10	10	10	10	0.5-2
PRACTICAL LOD (µG/L)	>20 µg/L	>50 µg/L	>20 µg/L	>50 µg/L	<20 µg/L
RELIABILITY (µG/L)	Unreliable <70 µg/L	Unreliable <70 µg/L	Unreliable <70 µg/L	Only identify samples 50 µg/L	More reliable for lower concentrations

Sankararamakrishnan et al., claimed that the correctness percentage of arsenator is 85% and it is available for determining the concentration range between 5-100 µg/L, and has low precision in the range of 20-100 µg/L. However, for some researchers the calibration and algorithm of arsenator cause challenges [(Sankararamakrishnan, Chauhan, T Nickson, M Tripathi, & Iyengar, 2008),(Safarzadeh-Amiri, 2011)].

The other problems of field kits can be listed as below;

i. Determination of the field kits is colorimetric, the intensity of occurred yellow color is variable for person to person. Thus, misclassification is inevitable. In addition to that, this color dims with time. The analysis must be done fast.
ii. Generated arsine gas is more toxic than arsenic.
iii. Most of the test kits are sensitive to hydrogen sulphide (H_2S) interference.
iv. Zinc cause some difficulties; zinc is used as reducing agent in the system, but zinc has arsenic inherently, so it may cause some errors during detection. Kinniburgh et al., claimed that, zinc make arsenite form of arsenic to arsine gas immediately, but arsenate reduction needs time. Thus, converting arsenate to arsenite requires pre-reduction step.
v. Finally, most of the kits can only detect the arsenic concentration above 50 µg/L, and few above 20 µg/L [(Yogarajah & Tsai, 2015),(M. Arora, Megharaj, M., & Naidu, R., 2008),(G Kinniburgh & Kosmus, 2002)].

1.1.3. Biological-Based Arsenic Detection

Most bacteria have protection system against to arsenic species therefore they can be used for arsenic detection. Diesel et al., reported that when the protein called ArsR in the bacterial cell meets the arsenite, it changes its conformation and starts the defense system. In the absence of arsenic, the specific DNA sequence and its overlapping RNA polymerase sequence (the promoter) bind and so this protein enforce the response. However, if arsenic is present in the cell, ArsR loses its affinity and RNA polymerase start to transcribe arsDCAB genes as defense system (Figure 2). In order to make this a bioassay, another copy of operator-promoter DNA and reporter protein are equipped to the cell. When arsenic species are felt by the cell, the reporter gene will also be released, and reporter protein will be synthesized. The response of reporter protein is directly proportional with arsenic concentration in the cell [(Yogarajah & Tsai, 2015),(Diesel, Schreiber, & Roelof van der Meer, 2009)].

In developing countries, it is needed to develop a simple colorimetric test for arsenic detection. Stocker et al., improved a strip-based colorimetric test for field applications. In general principle, arsenic is detected with X-gal and β-galactosidase. Cell cultures are prepared as arsenic biosensors by cutting out the arsenic specific fragment from one vector and ligated with lacZ including vector, then vector is transferred to bacteria.

Cell cultures are placed on the specific marked area on fabricated paper-strips. The analysis is achieved firstly, strips are incubated with arsenic solution for 30 minutes and then X-gal solution was dropped on the specific area including bacteria lacZ gene. Color change is observed, and the change of the blue color is proportional with arsenic amount in the solution. By this method, arsenic concentration level can be detected between 5-100 μg/L [(Stocker et al., 2003)].

Theytaz et al. designed a closed and non-returnable microfluidic device with bacteria. The principle of this device is with a filter in the channel, so that bacteria traps and accumulate while flow continues.

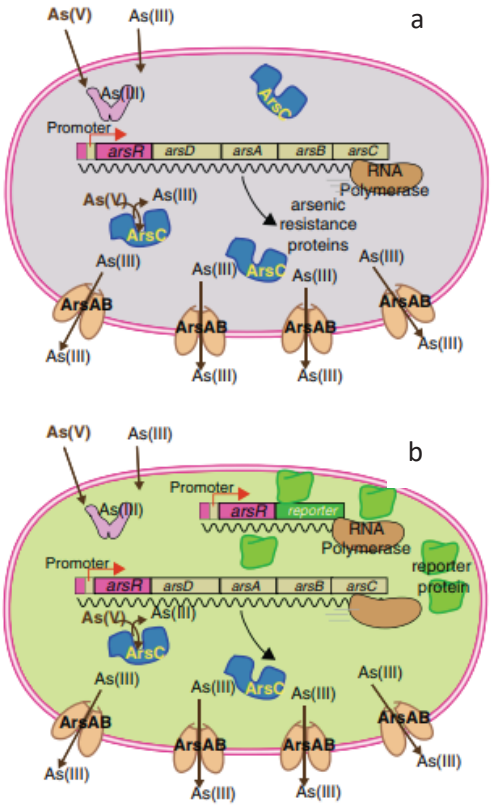

Figure 2. Principle of arsenic detection method with bacteria sensor; a) The sensing procedure of arsenic molecules b) Measuring arsenic samples with reporter protein. (Source:Diesel et al., 2009)

The bacteria and arsenic solution are mixed and fed into the device, bacteria start to immobilize while arsenic flow continuous, and then bacteria, which express GFP when expose to arsenic, accumulate in the filter. So, the response can be obtained with bright field and fluorescence as shown in Figure 3 [(Theytaz et al., 2009)].

1.1.3 Chemical Reaction Based Arsenic Detection

Colorimetric detection method is an effective option for arsenic detection since it is portable, simple, fast, inexpensive and most practical method for field applications.

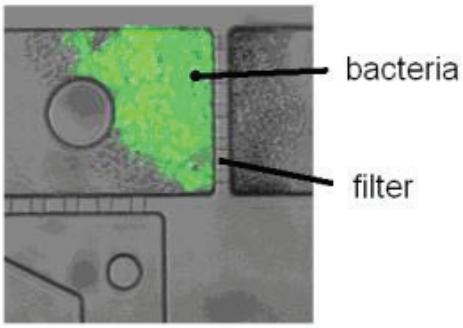

Figure 3. Fluorescence image of microfluidic device (Soırce:Theytaz et al., 2009)

Colorimetric method is efficient even not using sophisticated instruments therefore it has been introduced for arsenic detection lately [(Yogarajah & Tsai, 2015), (L. Johnson & Pilson, 1972), (Tsang, Phu, Baum, & Poskrebyshev, 2007)]. [(Sirawatcharin et al., 2014)].

Johnson and Pilson improved a method for detection of arsenite, arsenate and phosphate with molybdenum blue complexes with absorbance values. The procedure starts with reducing agent to lower the arsenate (As(V)) to arsenite (As (III)) since arsenite cannot make molybdenum complex. This approach provides observing only concentration of phosphate (H_3PO_4) molybdenum complex without affecting arsenate. Then, arsenite is converted to arsenate by using oxidizing agent. The total concentration (phosphate and inorganic As) is determined according to change of absorbance values and inorganic arsenic was calculated by the difference between two values [(Yogarajah & Tsai, 2015), (L. Johnson & Pilson, 1972),(Tsang et al., 2007)].

Arsenate and phosphate have similar chemical properties and they are both consist in water together, and also phosphate concentration is generally higher than the As concentration. Thus, the important disadvantage of this method is interference of arsenate and phosphate in drinking water [(Tsang et al., 2007)].

Rupasinghe et al., developed a system to get rid of the interference by combining molybdenum blue method with pervaporation flow injection (PFI). By PFI, arsenic can be detected by the form of arsine gas when phosphates in the sample did not volatilized. The detection limit of this system is 1-15 µg/L, however, not available for *in situ* detection. [(Yogarajah & Tsai, 2015), (Rupasinghe, J Cardwell, W Cattrall, Potter, & D Kolev, 2004)].

Table 3. General comparison of chemical reaction based arsenic detection

	Sensitive and selective	Detection time	Portable	Health and environmental
MOLYBDENUM BLUE (PFI)	LOD; 1-15 µg/L, Not interference	30 min	Not portable	Toxic arsine gas produced.
MOLYBDENUM BLUE (PKA EFFECT)	LOD; 4-8 µg/L Not interference	7-10 min.	Potentially portable	Yes, arsenic is always in the solution.
MOLYBDENUM BLUE (ETHYL VIOLET)	LOD; 10-25 µg/L Interference; P	30 min	Potentially portable	Yes, arsenic is always in the solution
METHYLENE DYE	LOD; 10-100 µg/L Not interference	6 min	Potentially portable	Arsine gas is generated buy always stays in the solution
SULFANILIC ACID-NEDA	18 µg/L	30 min	Portable	Yes, arsenic is always in the solution
PAPER-BASED SENSOR	1 µg/L		Portable	Yes

Dasgupta et al., reported a different strategy to eliminate the phosphate interferences. Difference in acidities between As(III), As(V) and P is considered and can be separated and evaluated independently. The sample can be evaluated as two experiment; first whole pentavalent form and then trivalent form of arsenic are examined, and finally total arsenic concentration is detected.The detection limit of this system is nearly 4-8 µg/L [(Yogarajah & Tsai, 2015), (Dasgupta, Huang, Zhang, & Cobb, 2002)].

Morita et. al,developed more sensitive method for lower concentration of arsenic in water.

Ethyl violet dye and molybdenum blue get into reaction and forms colored nanoparticles and the particles turn into different intensity of purple color according to arsenic concentration. The limit of detection of this method is approximately 25µg/L [(K. Morita & Kaneko, 2006),(Yogarajah & Tsai, 2015),(K. Morita, & Kaneko, E. (2006), 2006)].

Another developed chemical reaction method to detect arsenic in ppm values is methylene blue (MB) which is based on the reaction of arsenic and cationic color bleaching methylene blue the organic dye. The method is simple, fast and environmentally healthy, and the most important benefit of this method is not interference with phosphate [(Yogarajah & Tsai, 2015)]. Additionally, during the process the generated toxic arsine gas stays in the solution therefore decrease the exposure of the user. The protocol of method is, arsenite or arsenate are reduced with sodium borohydride ($NaBH_4$) and arsine gas is generated, and methylene blue become colorless in micellar media. Pal et al., reported that reduction cannot occur only with arsine gas or sodium borohydride, it is also be needed to region them in selected micelle [(Pal et al., 1998)]. Kundu et al., tested different surfactants such as CTAB, TX-100 and SDS, it was found that SDS give the best result because methylene blue and SDS have contrary charges and electrostatic attraction occurs between them which gives better results. The color reducing intensity is directly proportional to the arsenic concentration. The detection occurs rapid and 10 samples can be analyzed in 2 hours ,(Ghosh, Kundu, Mandal, & Pal, 2002)]

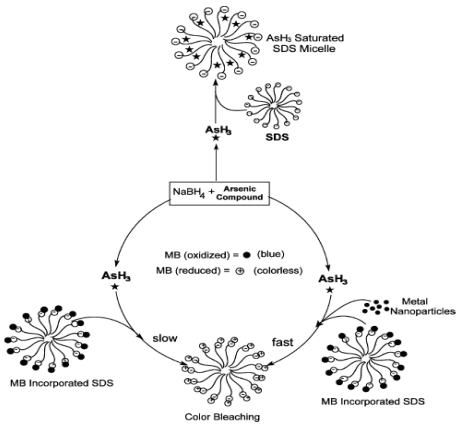

Figure 4. The schematic representation of reduction of arsine gas in a micellar medium (Source:Kundu, Ghosh, Mandal, Pal, & Pal, 2002)

Metal nanoparticles can behave as an efficient catalyst in redox reactions (Figure 4) [(Pradhan, Pal, & Pal, 2001), (Pal et al., 1998)]. Jana et al. claim that metal nanoparticles can be used as catalyst in order to increase the reaction rate. The reason of getting higher reaction rate is, nanoparticles deliver electrons between arsine gas and MB with their large surface area. In the beginning, whole reactants bind to surface of the metal nanoparticles, later, reductant lose an electron on the metal surface and MB gets the electron and reduced. Therefore, metal nanoparticles behave as a catalyst [(Jana, Sau, & Pal, 1999),]. Moreover, Henglein et al., showed that silver nanoparticles have better catalyst properties than gold nanoparticle since it is observed that gold nanoparticles make bound with MB better and as a result the surface of gold nanoparticles are covered with dye, that blocks the electron relay between dye and arsine gas. Thus, silver nanoparticles are more efficient as a catalyst [(Kundu et al., 2002),(Ghosh et al., 2002), , (Henglein, 1993)].

One of the chemical reactions based arsenic detection method is reported by Sharma et al., by using sulfanilic acid and *N*- (1-naphtyl) ethylene diamine hydrochloride (NEDA). This technique purposes to detect arsenic inexpensive, fast, sensitive and simple. The initial step of protocol is sulfanilic acid reduction by arsenic and then the generated product make reaction with NEDA to form magenta-color. The whole process was carried out by restraining the reactants on thin layer chromatography (TLC). When arsenic, sulfanilic acid and NEDA react to each other purple to magenta stains formed which then analyzed by MATLAB software. The benefits of this sensor are not interfering with phosphate because only selective for As(III) species and portable since paptode strips. It is reported that, 30 minutes is needed for analysis and the LOD of the system is 18 μg/L [(Yogarajah & Tsai, 2015),(Dubey Sharma, Joshi, & Amlathe, 2011; Ruchi Dubey Sharma, 2012)].

1.1.5. Gold Nanoparticle Based Arsenic Detection

In many researches, nanoparticles especially gold nanoparticles (GNPs) obtain high attention for various applications because of their size, shape and optical properties.

For instance; developed absorption area, scattering, biocompatibility and improved bioconjugation protocols, which makes them different from other bulk materials [(Weinrib, Meiri, Duadi, & Fixler, 2012),(Marinakos, Chen, & Chilkoti, 2007)].In order to reduce the toxic arsine gas exposure, gold nanoparticles are started to use as a sensor in colorimetric methods since the size, shape and optical properties of gold nanoparticles [(P. Nath, Arun, R. K., & Chanda, N., 2014),(Domínguez, González Varela, & Bermejo-Barrera, 2014)].

Gold nanoparticles have so much benefits as biosensors, initially, they have high stability and they can be produced many different chemical or biological ways; while in the Turkevich method gold nanoparticles are synthesized with a surface of citrate coated, in Brust Schiffirin method, they have produced with using biological agents (algae, fungus, bacteria). Second advantage of GNPs is their high surface to volume ratio which improves the sensitivity and selectivity. Thirdly, they exclusive optical and electrochemical properties that are depend on the size and shape of GNPs [(Priyadarshini & Pradhan, 2016)]. GNPs have been used for colorimetric assays for a large range of area from catalysis to biomedicine for example DNA detection, antibody/antigen protocols and even heavy metal analysis towards environmental pollution [(Marinakos et al., 2007),(Priyadarshini & Pradhan, 2016),(Gianini Morbioli, Mazzu, M. Stockton, & Carrilho, 2017)].

For biomolecular reactions, it is always needed to find out an easy, reproducible and sensitive methods and devices. In biosensors world, localized surface plasmon resonance is one of the impressive techniques [(Huang, 2009)]. LSPR is a phenomenon that collective oscillation of surface electrons is excited by the lights in gold nanoparticles and the cause peaks in the extinction spectrum of the nanoparticles [(N. Nath & Chilkoti, 2004),(Peng, Chen, Wang, Shen, & Guo, 2016)]. The characteristic of the LSPR peak is dependent on the size, shape and natural composition of the material [(N. Nath & Chilkoti, 2004),(Peng et al., 2016),(N. Nath & Chilkoti, 2004)]. When surface plasmon resonance (SPR) based biosensors are compared with traditional sensor, they have plenty of important benefits; ultra-high refractive index sensitivity, rapid response, real-time analysis and label-free approach [(Cao, Sun, & Grattan, 2014)].

If a thin metallic film, such as glass, is deposited on a dielectric medium, SPR technology is used. Resonance occurs and then the electrons on the film begin to oscillate. On the other hand, for metal nanoparticles, because of their small size, oscillation of the electrons is restricted (Figure 5).

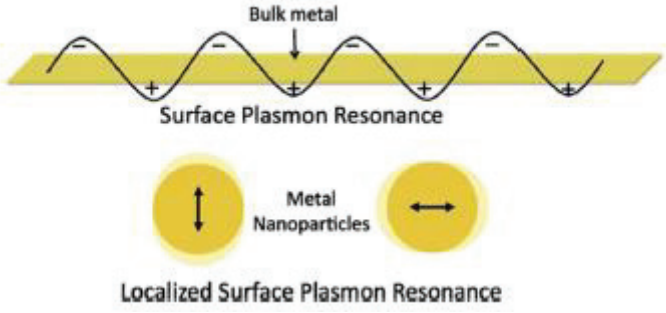

Figure 5. Schematic representation of SPR and LSPR phenomenon (Source:B.D. Borah et al., 2015).

Therefore, by using LSPR, when resonance happens, the metallic nanoparticles indicate improvement at the local electromagnetic area and give intense absorption band [(B.D. Borah, Bora, Baruah, & Dutta, 2015)].

On the other hand, there are plenty of developed methods for arsenic detection. Boruah et al. reported polyethylene glycol (PEG) functionalized gold nanoparticles work for arsenic detection. PEG is nontoxic and biocompatible biopolymer; therefore, it is a good choice for arsenic detection in aqueous medium. The functionalized AuNPs solution was added to arsenic solution and the red color is changed to blue due to aggregation of nanoparticles.

The principle of this method is based on the electrostatic interaction of arsenic and AuNPs. When PEG molecules bind to nanoparticles and nanoparticles become negative charged. Functionalized AuNPs and positive arsenic come in to contact and so electrostatic interaction begins. PEG binds nanoparticles with hydrogen bond and arsenic senses the -OH group on PEG and binds to AuNPs. Finally, gold nanoparticles start to aggregate, and color change is observed. With this method, minimum 5 µg/L arsenic concentration can be detected [(Boruah, 2018)].

Ge et al., improved colorimetric probe with dithiothreitol (DTT) modified gold nanorods to detect arsenic. DTT molecules have two thiol ends, one binds to gold nanorod and the other one binds to As ions. The principle of this colorimetric probe is; one end of DTT make covalent Au-S bond with gold nanorods and the other end makes As-S bond and trigger the aggregation of gold nanorods as shown in Figure 6 [(Ge et al., 2018)]. Ge and co-workers also indicated another thing.

When arsenic binds to DTT- the absorption peak will be lower than DTT-AuNRs. According to decrement into the absorption peaks, arsenic concentration is determined [(Ge et al., 2018)].

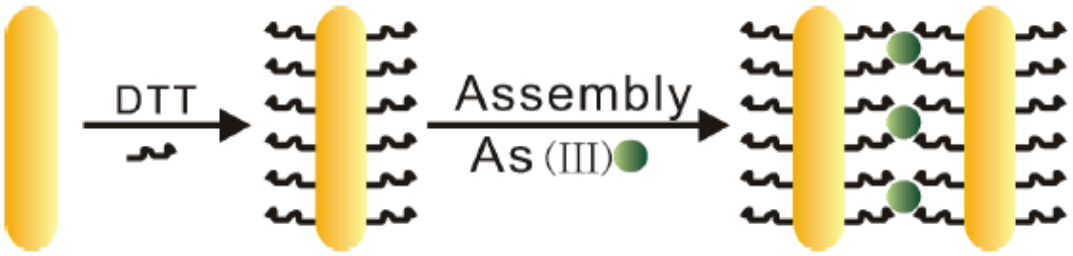

Figure 6. The representation of DTT-AuNRs aggregation (Source:Ge et al., 2018).

1.1.6 Microfluidic Based Arsenic Detection

Microfluidic has significant advantages to manipulate less amount of volumes and high surface to volume ratio. Also microfluidics increase the mass transfer and so provides the rapid chemical processes which is important for sensing mechanisms[(Stroock, 2008),(Halldorsson, Lucumi, Gómez-Sjöberg, & Fleming, 2014)].

Alternative method for arsenic detection is paper based sensors. This technology is inexpensive, easy to use, fast and portable. Nath et al. reported Y-shaped design by using Whatman filter paper, for detecting As (III) with LOD of 1 µg/L which is lower than the arsenic standard limit of WHO, with modified gold nanoparticles (AuNPs). This protocol based on the modification of gold nanoparticles with thioctic acid (TA) and thioguanine (TG) and produces Au-TA-TG gold nanosensor. When arsenic and modified gold nanoparticles meet with each other, gold nanoparticles aggregate, and visible bluish-black color observed on a paper substrate as shown in Figure 7 [(Yogarajah & Tsai, 2015),(P. Nath, Arun, R. K., & Chanda, N., 2014)].

The procedure starts with binding gold nanoparticles with TA and the with TG when EDC and NHS are in the environment. It is known that arsenic make bond with -SH group (As-S bond), the -SH group is kept free.

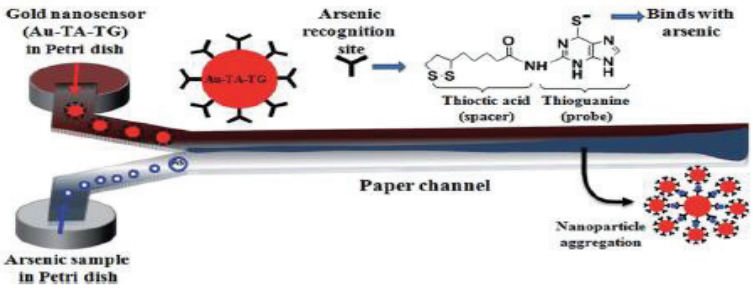

Figure 7. The schematic representation of paper-based sensor by using modified gold nanoparticles (Source: P. Nath, Arun, R. K., & Chanda, N., 2014)

In the system thioctic acid is used as spacer in order to reduce the steric hindrance and thioguanine is used as a probe to bind arsenic and cause aggregation of AuNPs and later then bluish-black color is observed [(P. Nath, Arun, R. K., & Chanda, N., 2014)].

Paper-based sensor methods are considered for microfluidic researches due to their self-driven flows and capillary action in the channels. Arun et al. reported that the flow rate is 2-3 µL/min, that low flow rate provides to detect arsenic in low concentrations and arsenic has enough time in the channels to react with the nanosensors and signal can be observed [(P. Nath, Arun, R. K., & Chanda, N., 2014),(Jakariya et al., 2007; Li, Ballerini, & Shen, 2012),(Arun, Halder, Chanda, & Chakraborty, 2014)]. Nath et al. indicated that, at low concentration of arsenic, the visual color difference cannot be observed because arsenic amount would not be enough to form AuNPs aggregation. However, in order to decrease the detection limit, microfluidic channel can be used by increasing the interaction of AuNPs and arsenic. Last but not least property of paper-based sensor is, interfere is not occurred between other metal ions, thus this nanosensor has high selectivity for arsenic [(P. Nath, Arun, R. K., & Chanda, N., 2014)].

Chowdury et al. developed a paper-based method for detection of arsenic in groundwater. It is understood that, the method, which Nath et al. developed, has Fe interferences in the groundwater. Chowdury and coworkers handled this problem by changing pH level in the microfluidic paper based analytical device. The device is based on gold nanoparticle functionalization with thioctic acid and thioguanine (Au-TA-TG). T-shaped paper was used for this method in order to supply steady and specific detection area during sample flows and contact with AuNPs [(Chowdury, Walji, Mahmud, & Macdonald, 2017)].

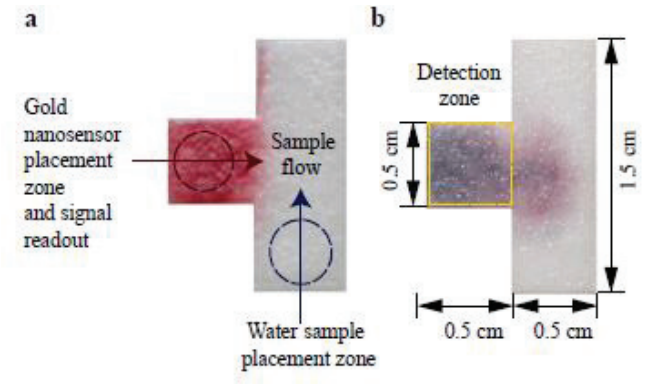

Figure 8. The T-shaped microfluidic device. (a). 0 µg/L concentration of arsenic. (b).50 µg/L concentration of arsenic (Source: Chowdury et al., 2017)

The arsenic sample and functionalized gold nanoparticles were placed on the areas that labelled in Figure 8a. Chowdury et al. indicated that when arsenic sample and gold nanosensors meet, the red color turns into blackish color because of aggregation as can be seen in the Figure 8b [(Chowdury et al., 2017)].

In researches, miniaturized devices are integrated in a microfluidics system to design arsenic bioassays to achieve high performance, portable and no need to pre-preparation for samples. Microfluidics system has other benefits such as; decreasing sample size, faster sample analysis and less power needed. Rothert et al. created a microfluidic biosensor with PMMA compact disk with a special design that done with CNC machines as can be seen in Figure 9. This device has two parts, first one is adjusting microfluidics with motors, power supply and controllers. And the second part is fluorescence observing setup with spectrophotometer [(Rothert et al., 2005)].

The device has two inlet reservoirs, and one mixing channel that ends in the detection reservoir. By spinning the disk shape device centrifugal force is generated in order to make reagents flow. Regulatory protein of arsenic was used to produce green fluorescent protein GFP which is reporter protein for this system. The cells and the activator solution are put into the device and mixed. Then the bacterial GFP response is observed with fluorescence microscopy which are directly proportional with arsenic amount [(Rothert et al., 2005)].

While many researches have been done for arsenic detection, according to Yogarajah and co-workers the ideal arsenic sensor has five crucial criteria [(Yogarajah & Tsai, 2015)].

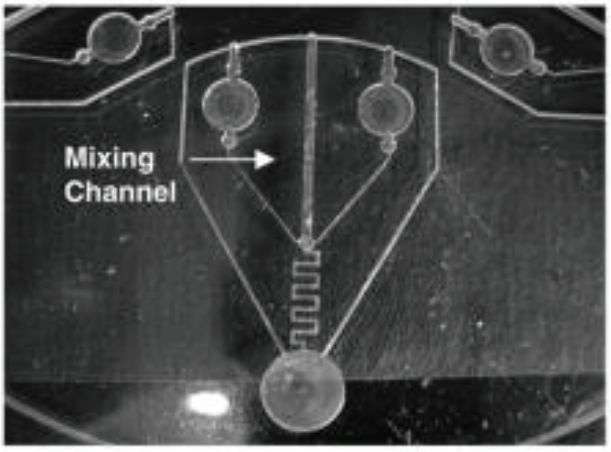

Figure 9. The compact disk with reservoirs and mixing chamber (Source: Rothert et al., 2005)

i. Arsenic detection sensor must be sensitive and selective; It is needed to have a device to measure arsenic concentration in the range of WHO standards. Additionally, arsenic is found in drinking water with other interfering species, it is also desirable a sensor with arsenic selectivity.

ii. Arsenic detection should be done rapid and reproducible; So many samples are tested especially in the developing countries thus the assay should have high yield.

iii. The sensor must be tough and portable; The test kit should be tough enough to cope with all challenges at the detection area and the detection should be done in situ thus while it reduces the processing, consciousness will increase in the local area.

iv. The procedure should be eco-friendly and healthy; Chemical process should decrease the arsenic exposure for users and eliminate the poisoning environment.

The system should be inexpensive and easy to use; In order to make local people use these kits, they should be simple enough.

Considering the properties of ideal arsenic biosensor, it was aimed to develop a sensor which is cheap, portable, non-toxic to user and environment, and most importantly can detect arsenic concentration at the range of WHO standards.

CHAPTER 2

MATERIALS AND METHOD

2.1 Materials

The 39436 coded arsenic standard solution for AAS, 741981 coded gold nanoparticles and 175617 coded (3-Mercaptopropyl) trimethoxysilane (3-MPS) were obtained from Sigma-Aldrich. Acetone with 20066 code was obtained from VWR, I.075.00.006 coded, 24×50 mm cover glasses were taken from INTERLAB. SYLGARD 184 Silicone Elastomer Kit was taken from Dow Corning. The Clear V2 FLGPCL04 photoreactive resin that was used for 3D Printer was get from Formlabs. The SolidWorks drawing program was used for designing microfluidic chip molds. Observing absorbance peaks of result is done with bright field microscopy that combined with Ocean Optics Spectrometer (HR4000CG UV-NIR) as used for absorbance spectroscopy. For activating surface of cover glasses oxygen plasma was used (Diener Plasma Cleaner). Syringe pump (New Era Pump Systems, NE 1600) was used for injecting samples at a specific flow rate.

2.2 Method

We designed a microfluidic chip for arsenic detection by using gold nanoparticles. The detection principle of our method is based on the As-S and Au-S bond. Arsenic binds to -SH groups on the cover glass surface, the -SH group amounts will decrease, also gold nanoparticles bind to -SH groups, therefore if there is arsenic in the environment, there would be less amount of -SH groups and gold would be able to bind less.

2.2.1 Silanization of Cover Glasses

Cover glasses are cleaned in sonicator with 70% ethanol solution for 10 minutes at room temperature. After sonication, they dried with nitrogen gas and surface is activated with oxygen plasma operated at 0.5 mbar pressure for 2 min at 100 W.

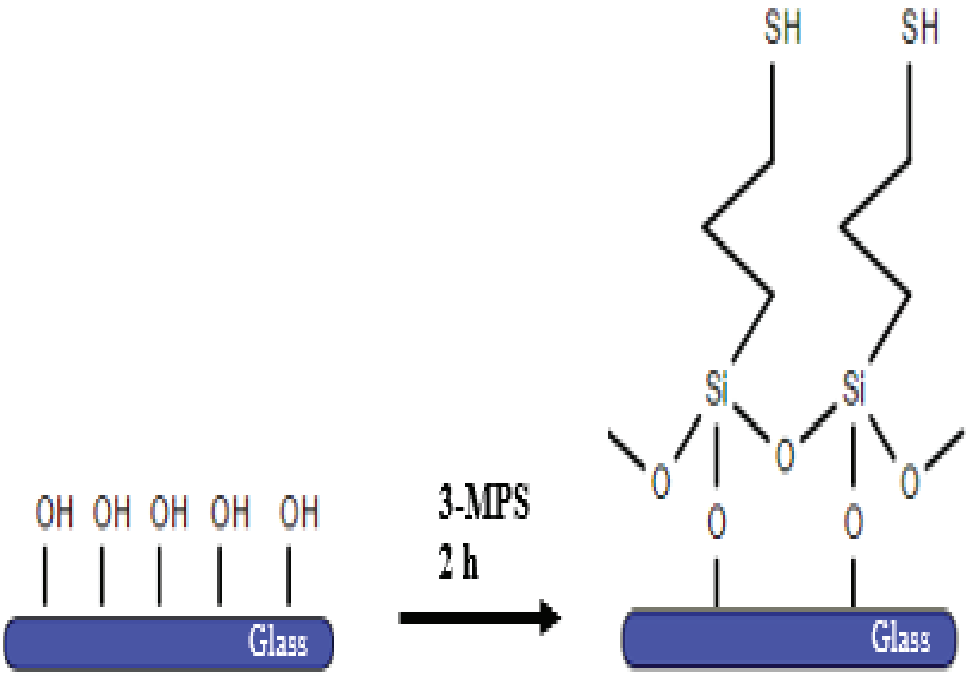

Figure 10. Schematic illustration of silanization procedure on glass.

Then, cover glasses are silanized with 3-MPS in acetone with 2.5% (w/v) ratio for 2 hours in the dark to cover the surface with -SH groups as in Figure 10. Again, nitrogen drying is done after silanization process.

After silanization process is done microfluidic chip production was carried out by using silanized glasses.

2.2.2 Production of Microfluidic Chip

The microfluidic chip for arsenic detection was produced by soft lithography technique with synthetic polymer poly(dimethylsiloxane) (PDMS).

PDMS is a good option for our technique due to its nontoxicity, biocompatibility, elasticity, transparency and durability.

PDMS provides gas permeability so diffusion of oxygen and carbon dioxide is allowed. However, PDMS has hydrophobic surface, therefore before using it for our experiment, PDMS should be treated with oxygen plasma to make the surface hydrophilic [(Subramaniam & Sethuraman, 2014)].

During experiment, three different PDMS molds were used; with three channels mold, with two channels mold and with two channels which were designed as thinner at the beginning and end of the channels. The reason of three different mold is; firstly, at the three channeled microfluidic chip, one channel is used as a control channel and one of the other channels is reference solution channel and sample channel. So that, at the first channel 100 μL of arsenic in distilled water, at second channel just distilled water were injected. Samples were waited for 10 min at channels and then gold nanoparticle was introduced at the both channels. At the third channel, just gold nanoparticle was injected. Then, the control channel was removed by considering the results and channel number was decreased to two and same procedure was applied. At the last and best channel design, the beginning and the end of the channels are made thinner in order to increase the sample amount at the area where measuring is taken. The microfluidic chips were designed and drawn by the SolidWorks 2015 drawing program as shown in Figure 11 and were printed out with 3D printer with 0.1 mm resolution. The dimensions of the chip were designed according to dimensions of cover glass. Channels for the 3rd design are length 35 mm, width of the thinner parts is 1 mm, and the larger parts are designed as 5 mm. The angle between he thinner and larger part of channel is designed as 135 degree. The 3D printer operates FORMLABS FORM2 software program and uses Clear V2 FLGPCL04 photoreactive resin. PDMS is prepared with elastomer and agent with 1:10 ratio. Then mixed for 10 minutes and occurred bubbles are eliminated with desiccator under vacuum. The PDMS is then poured into the microfluidic molds and in order to cure the PDMS.

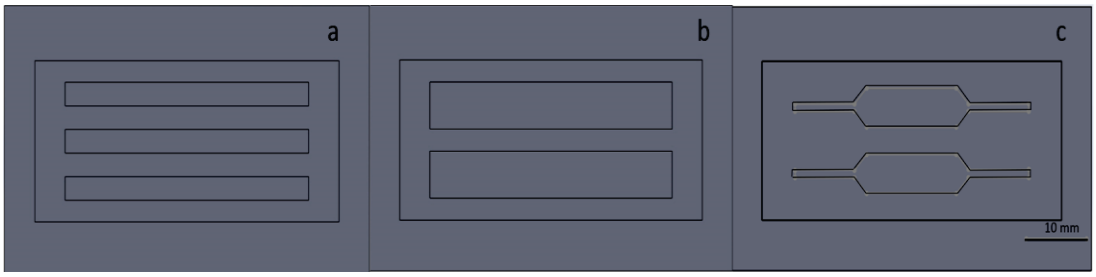

Figure 11. The SolidWorks image of three different microfluidic molds. (a) mold with three channels. (b). mold with two channels and (c) is the mold with thinner inlet and outlets.

They are waited in the oven at 60 degree Celsius, overnight. Then, inlets and outlets are formed with 1.4 mm diameter of needles. The prepared microfluidic PDMS chip is then treated with oxygen plasma to activate the surface and attached on to silanized cover glasses and they clamped with screw from top and bottom platforms to prevent the leakage As can be seen in Figure 12 the platforms were drawn with the dimensions PDMS and designed as blank in the middle of both bottom and top (light comes from top and objective is in the bottom) in order to be able to measure the absorbance values into the channels with microscopy. And there are blanks at the inlets and outlets point to be able to inject solutions into the channels.

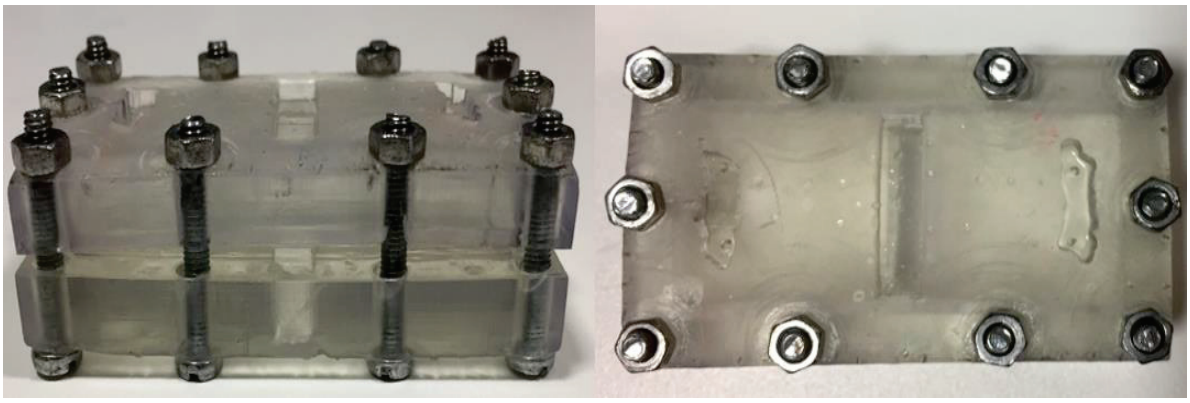

Figure 12. The image of microfluidic chip platform.

2.2.3 Arsenic Detection Procedure Without Flow

Meanwhile, gold nanoparticles are centrifuged at 12000 rpm for 15 minutes and the excess solution is removed and without dilution distilled water (DI) is added.

When the microfluidic chip is ready, 100 µL of distilled water is injected into the both channels for 2 times to eliminate any impurities. At the next step, 100 µL Tris(2-carboxyethyl) phosphine hydrochloride (TCEP) solution is introduced into the channels and waited for 10 minutes. TCEP is a water-soluble disulfide reducing agent [(Burns et al., 1991)]. The concentration of TCEP solution is calculated according to -SH bonds on the surface as taking reference of Schimid et al. work. Schmid and co-workers calculated the thiol amount of cover glass, when ratio of silanization is 2.5% (w/v), as 0.27 molecules/nm [(L. Schmid, A. Keller, Dienes, & Vogel, 1997)]Therefore, as the dimensions of the cover glass that uses in this project is 24*50 and as a result 3 mg TCEP was calculated and mixed with 1 mL of distilled water. In our experiment, TCEP is used in order to eliminate the disulfide bonds on the silanized surface of cover glasses. After that, channels are washed with 100 µL of distilled water for 2 times again.

For detection process, different concentration of arsenic solution is injected into the one channel and just distilled water is injected into the other channel and waited. Finally, gold nanoparticles are introduced to both channels and waited for different time periods. Lastly, the channels are washed with 100 µL of DI.

The principle is; at the first channel that contains arsenic, since arsenic binds to -SH groups on the cover glass surface, the -SH group amounts will decrease, while at the other channel that does not contain arsenic sample, the -SH group amount will stay constant. Therefore, at the first channel the gold nanoparticles would bind to the surface less than the second channel as the illustration indicated in Figure 13.

The results are measured using absorbance spectroscopy. The absorbance difference at the 530 nm, which is the specific peak for gold nanoparticle as seen in Figure 14, between the channels is directly proportional with the concentration of arsenic sample. The channel which does not contain arsenic, is substracting from the channel that contains arsenic. So that, with this method different concentration of arsenic molecules can be calculated.

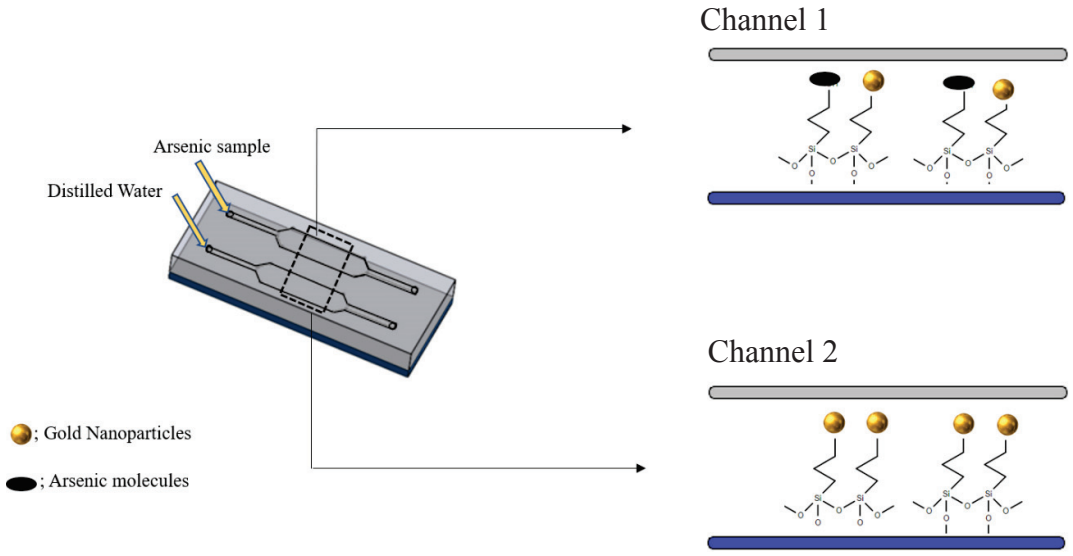

Figure 13. The schematic illustration of the procedure. At channel 1, arsenic sample is injected to the channels and arsenic makes As-S bond, therefore number of -SH bonds decrease. At channel 2, number of -SH bonds stay constant, so more gold particles will be binded in this channel and the absorbance value of channel two will be measured higher than the absorbance value of the channel one.

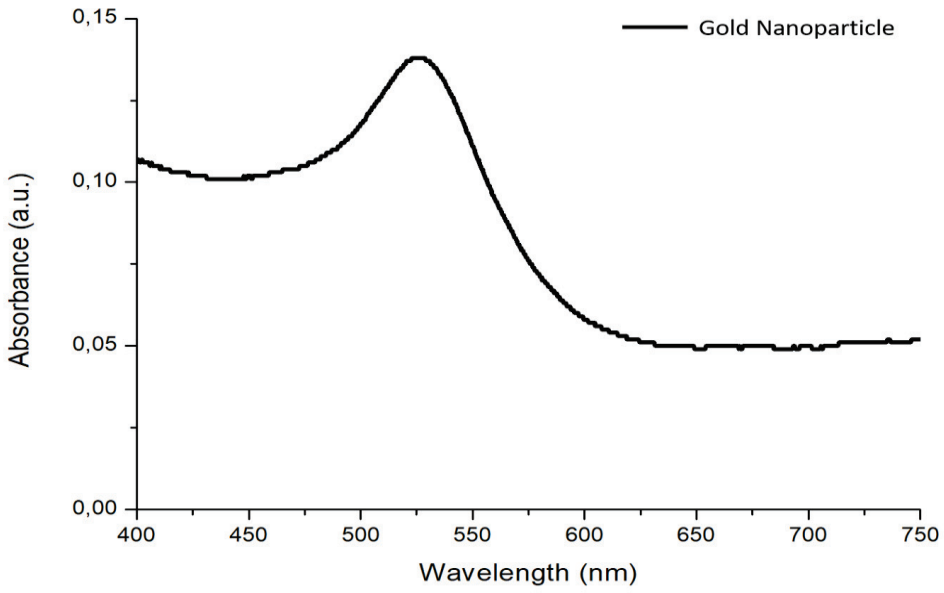

Figure 14. The absorbance of gold nanoparticle

2.2.4 Arsenic Detection with Flow

The experiments were carried out with drinking water in order to determine the selectivity of our method.

For these experiments, syringe pump was used in order to drive samples in microfluidic channels for increasing sample volume and the bonding of arsenic molecules onto the silanized glass surfaces. Silanized glasses and PDMS microfluidic chip was assembled and again washed with 100 µL of distilled water for 2 times. Then, same concentration of 100 µL TCEP solution was injected into the both channels and after waited for 10 min, channels were washed with 100 µL of distilled water for 2 times. For this process, different concentration of arsenic solutions was diluted with drinking water with ÖZSU brand and includes; Aluminum; <5 µg / L, Ammonium; <0.05 mg / L, Chloride: 13.8 mg / L, Iron; <5µg / L, Manganese; 15µg / L, Sulfate; 3.1 mg / L and Sodium; 17.3 mg / L, Apart from the above procedure, the solutions were introduced to the channels with syringe pump. At channel 1, 100 mL of arsenic solution was introduced and in the other channel 100 mL of just drinking water was injected.

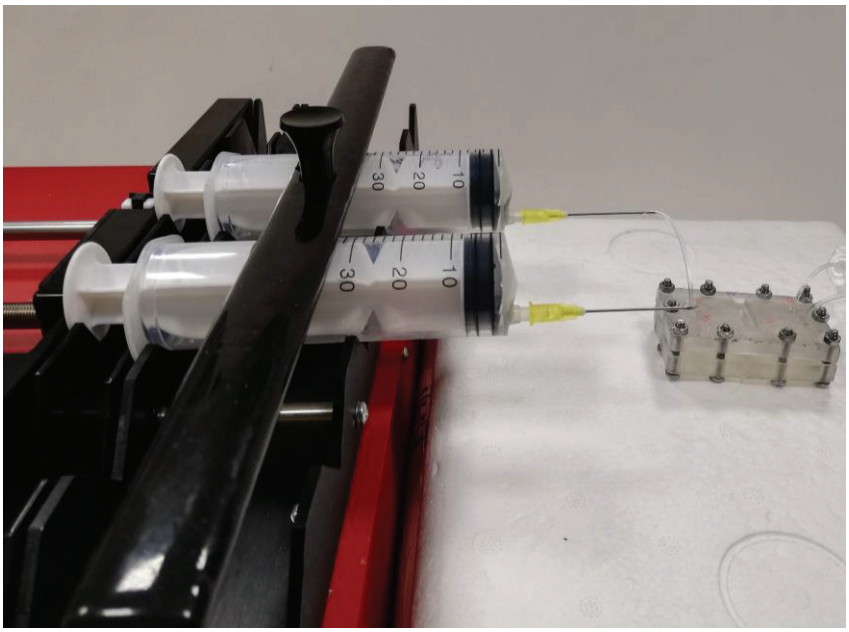

Figure 15. The image of syringe pump process.

This process was done with the help of diameter of 28.57 mm syringes and tubings as can be seen in Figure 15. The samples that obtained from outlets are gathered in beakers. The flow rate was adjusted to 10 mL/min with the help of syringe pump. After that, microchip was separated from syringe pump and by pipetting ,channels were washed with 100 µL of distilled water for 3 times in order to eliminate the pH effect of drinking water. Finally, gold nanoparticles were injected to the channels and waited for 2 hours and the results were measured with absorbance spectroscopy.

CHAPTER 3

RESULTS AND DISCUSSION

Firstly, optimizations of the process were completed and then experiments continue based on optimized conditions. Firstly, silanization of the cover glass results were controlled in order to be sure about the silanization Then TCEP material was investigated.After that, waiting times of arsenic sample and gold nanoparticles war optimized. Then different concentration of arsenic samples was examined to detect the lowest arsenic concentration. Moreover, syringe pump was added into the process to aim the decrease noticeable arsenic concentration limits.

3.1 Surface Control of Cover Glasses

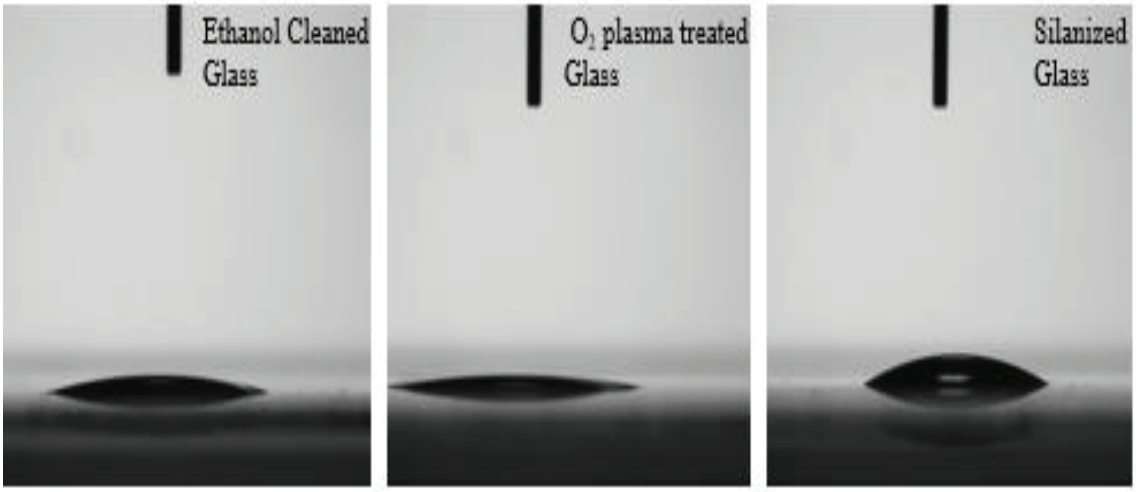

Figure 16. The contact angle values of ethanol cleaned glass, oxygen plasma treated glass and silanized glass.

The surface of cover glasses was analyzed with contact angle measurement device. As shown in Figure 16, the contact angle values of clean glass (using sonicator with ethanol), silanized glass and O_2 plasma treated glass and they are different. The contact angle values of glasses are 10, 5 and 30 degree, respectively. As expected, the oxygen plasma treated glass has more hydrophilic surface than just ethanol cleaned glass. Additionally, silanized glass has the highest contact angle value due to -SH bonds on the its surface.

3.2 Detection Protocol Optimization

While optimization procedure, several parameters were considered and optimized. For instance; waiting gold nanoparticles at different time periods, waiting arsenic samples at different time periods and pH adjustment of gold nanoparticle solution. Firstly, TCEP optimization was carried out due to the first step of the experiment. Then, since detection time is important, arsenic molecules waiting and gold nanoparticles waiting optimization experiments were carried out.

3.2.1 TCEP Optimization

Firstly, after silanization procedure, injecting TCEP solution into the channels was controlled. If TCEP solution would not be used, -SH bond on the surface will bind to each other and form disulfide bonds so that binding of arsenic molecules and gold nanoparticles will decrease. In the process, after silanization of cover glasses, again PDMS is assembled with glass and then washed with 100 µL of DI water 2 times. Then TCEP solution was injected into the one channel and just DI water was injected into the other channel and waited for 10 minutes. Without injecting arsenic sample, gold nanoparticles were injected and waited for 2 hours As shown Figure 17, using TCEP solution has higher absorbance value due to its feature of reducing disulfide bonds.

Therefore, for following experiments, it is decided to use TCEP for this system.

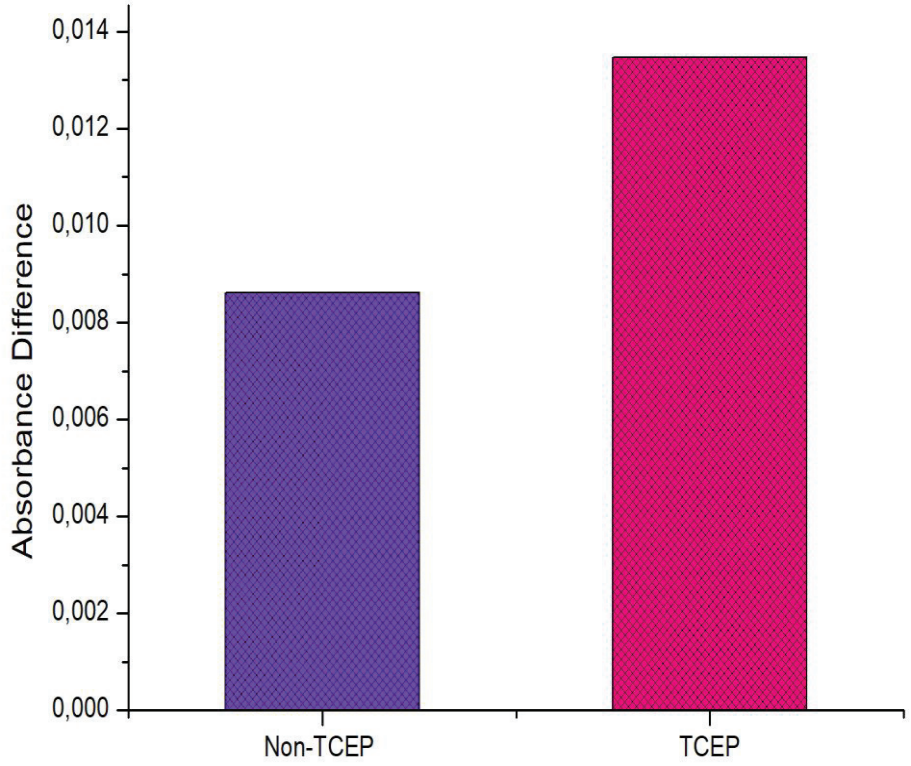

Figure 17. The effect of using TCEP solution.

3.2.2 Waiting of Gold Nanoparticles at Different Time Periods

To optimize the required time for gold nanoparticles to attach the -SH bonds on the glass surface, gold nanoparticles were waited for different time periods in the channels.

Firstly, background signal was measured by injecting distilled water into the both channels and subtracting the absorbance values between two channels. After that, in one channel 100 µL of 100 mg/L arsenic was injected and in the other channel just DI water was injected. Then, 100 µL of gold was waited for 10 min, 30 min, 60 min and 120 min. And channels were washed with 100 µL of DI water for 2 times. As can be seen from Figure 18, the difference between the two channels (channel with arsenic sample and without arsenic sample) is highest when gold nanoparticles are waited for 120 mins.

3.2.3 Waiting of Arsenic Molecules at Different Time Periods

In order to understand the needed time for arsenic sample to bind the silanized surface, 100 µL of 100 mg/L arsenic samples were waited for different time periods in the channels as 10 second, 1 min, 10 min and 100 min.

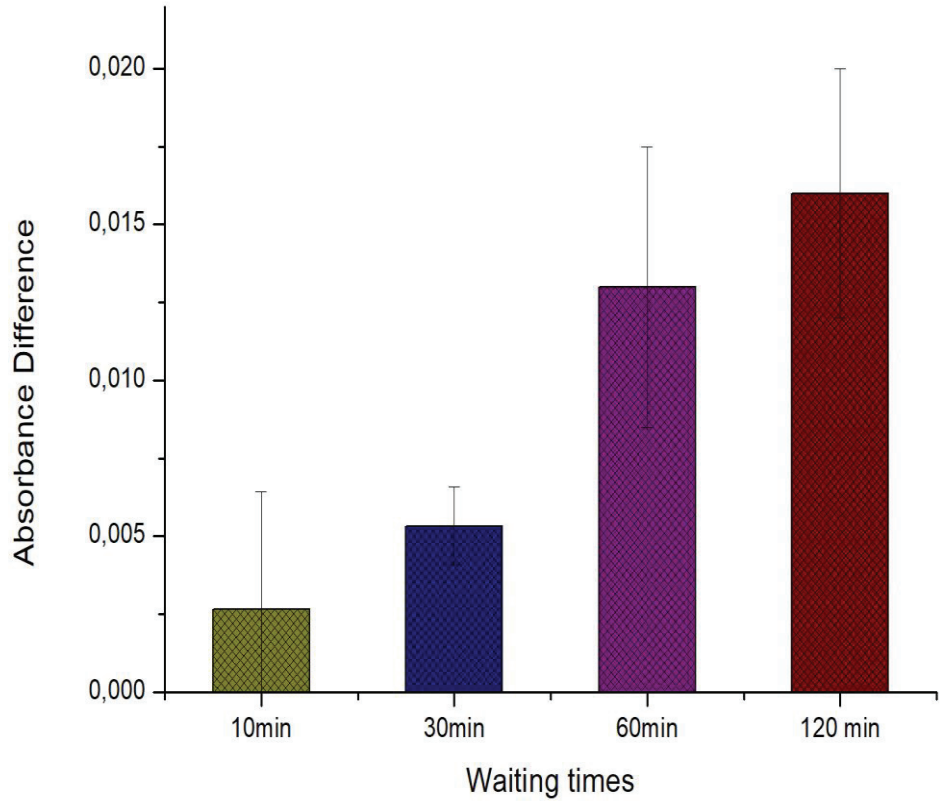

Figure 18. The comparison of waiting of gold nanoparticles at different time periods.

The same procedure was applied again, which is, subtracting the absorbance values between two channels. As can be seen from Figure 19, the optimum required time for arsenic samples to bind the surface is 10 minutes that gave the highest absorbance difference.

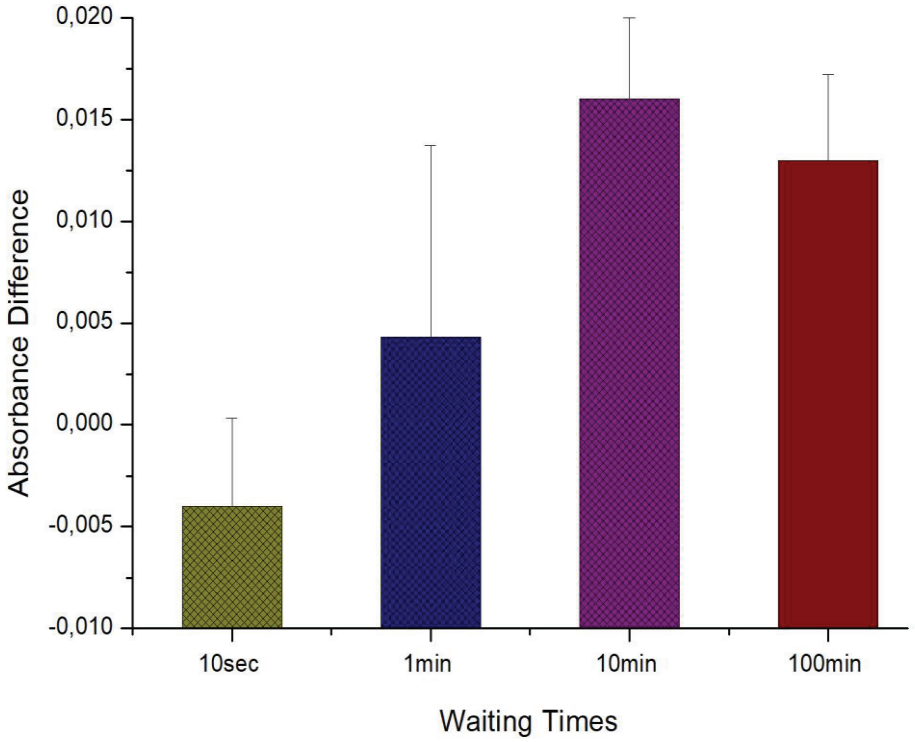

Figure 19. The comparison of waiting arsenic samples at different time periods.

3.2.4 The pH Effect on the Method

One of another important parameters for our method is the pH of the gold particle. Because pH changes the binding property of gold nanoparticles. The pH of gold nanoparticles was adjusted with HCl and NaOH as acidic and basic respectively. Firstly, pH was adjusted below 4, but then it is observed that, gold nanoparticles start too aggregate at that value. Therefore, pH values are adjusted to 4 ,5, 8 and 9. Also the experiment was carried out when pH is equal the DI water which is approximately 6.5. As mentioned in method section, gold nanoparticles are centrifuged, excess solution is removed, and DI water is added. In process, TCEP solution is added and washed,then 100µL of 1000 mg/L arsenic solution was injected into the one channel and DI was injected into the other. As shown in Figure 20, the highest absorbance value is when pH equals to DI water. It is understood from the results that, acidic and basic pH values affect the system adversely.

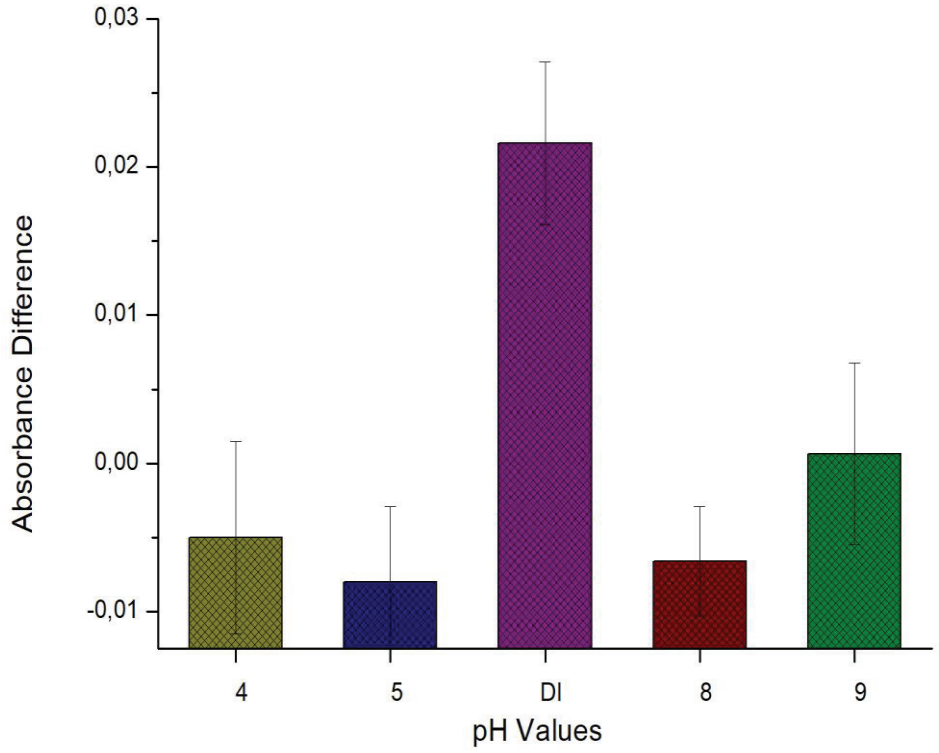

Figure 20. Comparison of absorbance signal using different pH values for gold nanoparticle solution.

Because the absorbance values were calculated negative except pH=9, but again the absorbance difference between channels is below the background signal. The reason of negative results may be the acidity and basicity cause the disappearance of -SH bonds on the surface. Or else, distinguish pH values affect the structure of gold nanoparticles and inhibit to bind the surface. As a result, the optimum pH value is found out as 6.5 and the experiments were carried out on this way.

3.3 Arsenic Detection Without Pumping

Different concentration of arsenic samples was measured in the microfluidic chip without using syringe pump. Experiments were carried out with different concentrations. These are;1000 mg/L, 100 mg/L, 10 mg/L, 5 mg/L, 1 mg/L and finally 100 µg/L. And 0 µg/L concentration of arsenic was injected into the one channel as a background signal.

The absorbance difference between two channels is summarized in Figure 22. The absorbance difference between two channels starts from 0.021 and decreases till 0.0033, for 1000 mg/L and 100 µg/L respectively. The measured absorbance differences were resulted as expected. Because, when the arsenic concentration is higher, the amount of arsenic sample that binds onto the silanized surface, which is composed of -SH bonds, increases. Therefore, the available -SH bonds for gold nanoparticles to bind decrease, and so gold nanoparticles bind less onto the surface. As a result, the absorbance value of the channels that contains arsenic sample has lower absorbance value which means inversely proportional with arsenic concentration.

The limit of detection (LOD) of the arsenic concentration experiment was calculated according to background signal and was found as 1.3 mg/L as used below formula, shown Figure 21 and Figure 22.

$$LOD = (3 * STD\ of\ Background\ Signal) + Mean\ of\ Background\ Signal \quad (3.1)$$

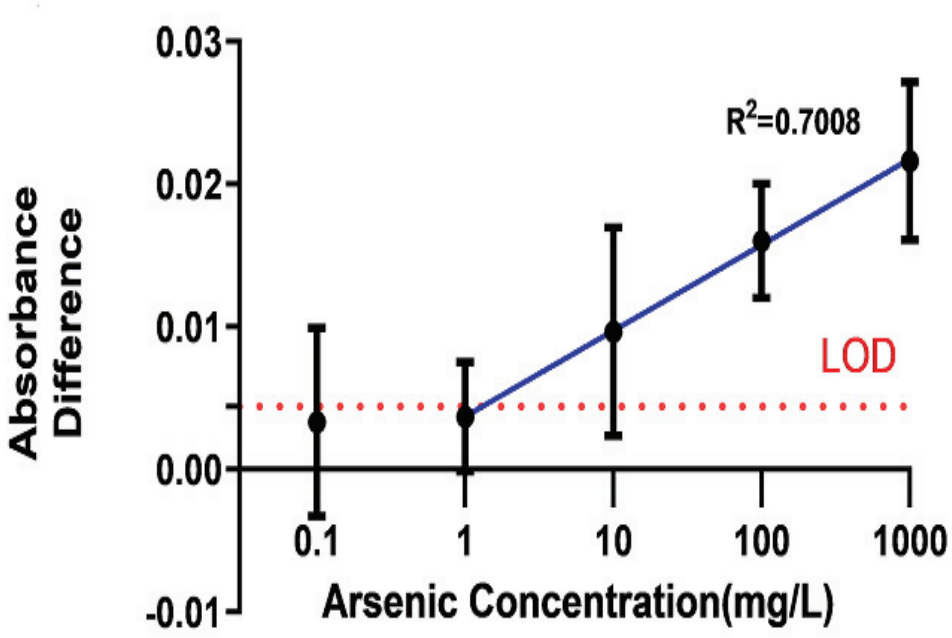

Figure 21. The representation of different concentration of arsenic samples without flow and their standard deviation with the comparison of LOD.

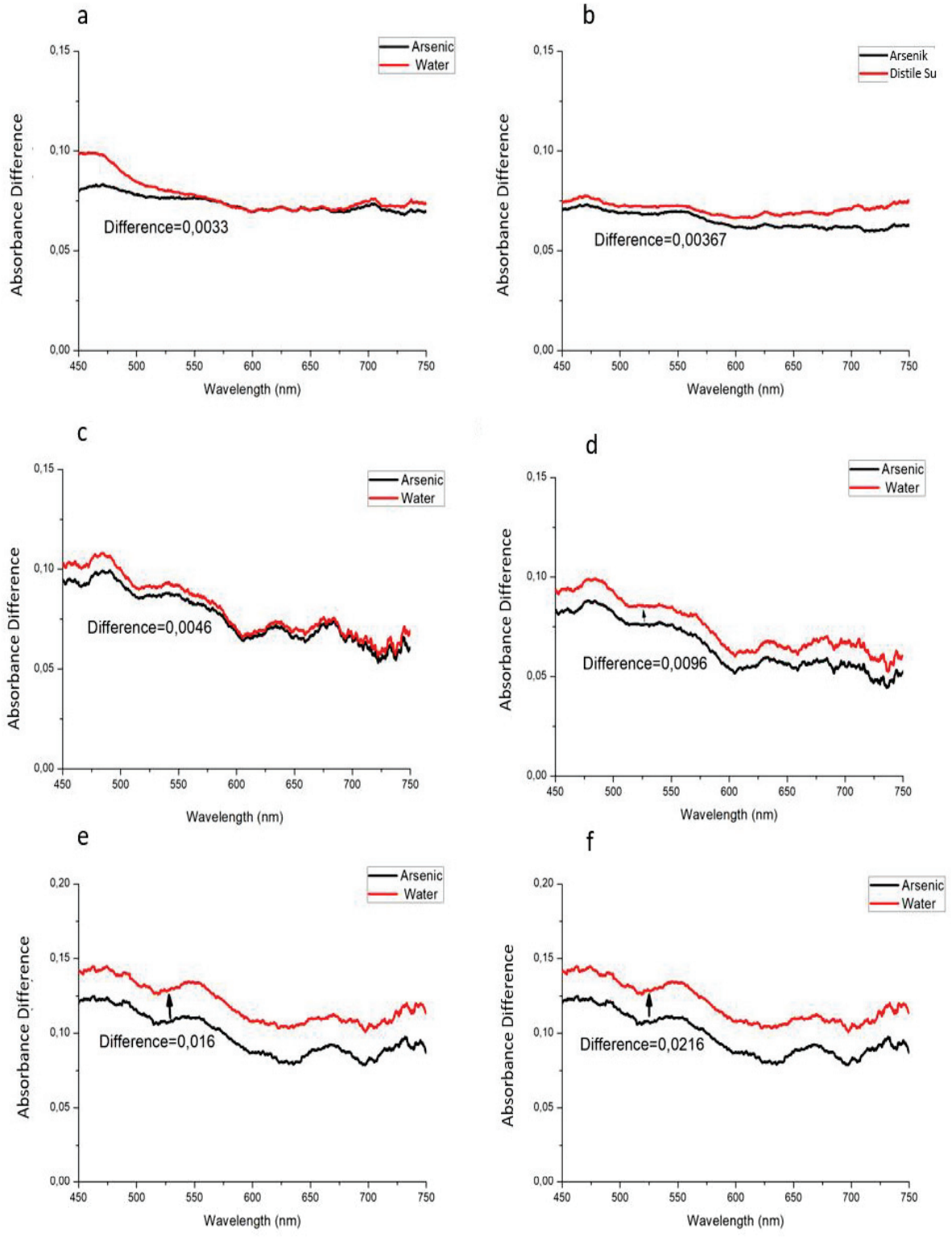

Figure 22. The absorbance difference between two channels results for different concentration of arsenic, a) 100 µg/L, b) 1 mg/L, c) 5 mg/L, d) 10 mg/L, e) 100 mg/L, f) 1000 mg/L.

3.4 Arsenic Detection using Syringe Pump

According to WHO, the maximum arsenic concentration in drinking water must be maximum 10 µg/L. At the above procedure, 100 µg/L sample was injected into the channels and waited for 10 minutes. In order to increase the binding of arsenic samples onto the surface, sample volume was increased. Therefore, it was decided to add flow into the system in order to be able to detect the arsenic concentration at standards. In 10 minutes, 100 mL of arsenic sample was injected into the one channel and 100 mL of drinking water was injected into the other channel. The flow was applied with syringe pump, with 10mL/min flowrate.

The concentration of arsenic samples is adjusted as, 1 µg/L, 10 µg/L, 100 µg/L, 1 mg/L and 10mg/L. And 0 µg/L concentration of arsenic was injected into the one channel as a background signal. The absorbance differences between channels are illustrated at Figure 24, as can be seen from the graphs, the 10 mg/L concentration of arsenic sample has 0.017 absorbance value whereas 1 µg/L concentration of arsenic sample has 0.004. Which is expected due to the relationship between arsenic concentration and absorbance difference.

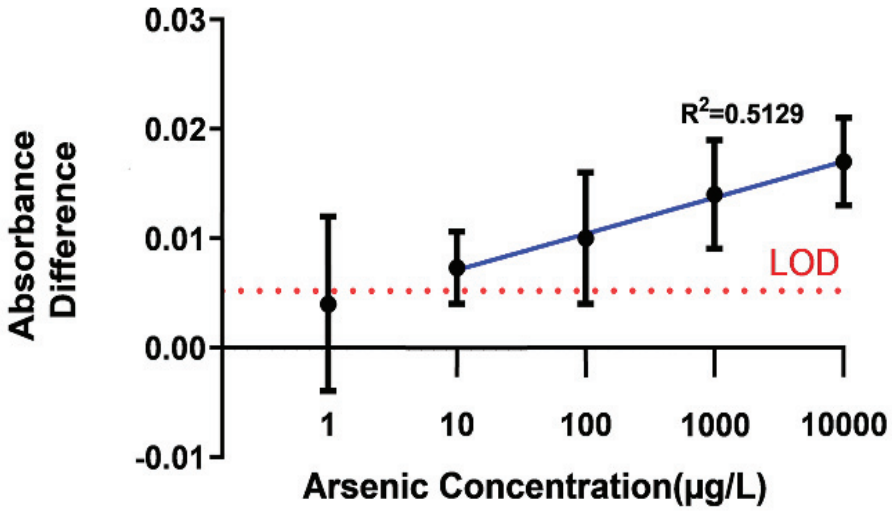

Figure 23. The representation of different concentration of arsenic samples with flow and their standard deviation with the comparison of LOD

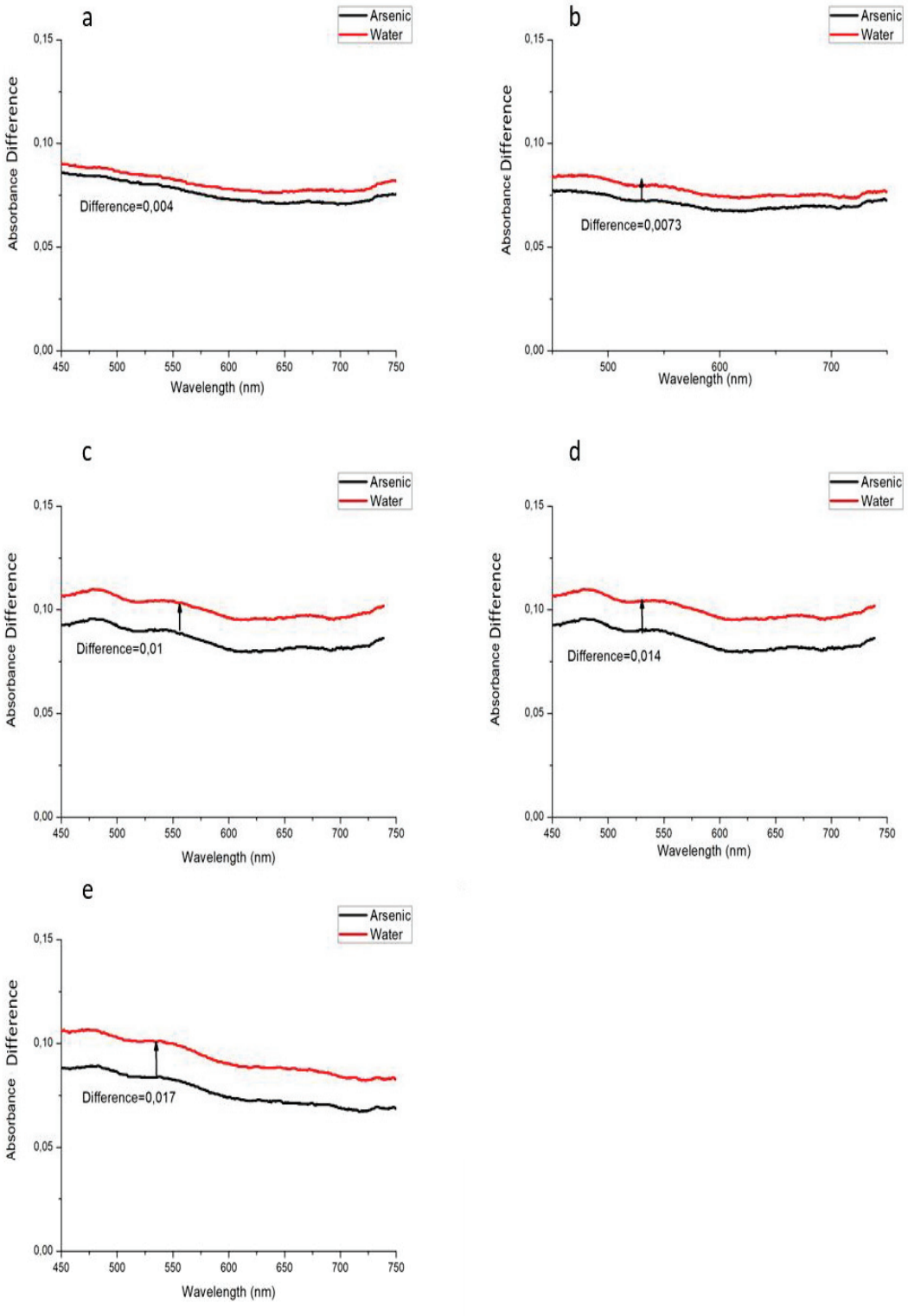

Figure 24. The absorbance difference between two channels results for different concentration of arsenic, a) 1 µg/L, b) 10 µg/L, c) 100 µg/L, d) 1 mg/L, e) 10 mg/L.

When LOD was calculated from the syringe pump background signal it was found 2.22 µg/L, as shown in Figure 23. However, more important, as shown in Figure, with syringe pump experiments, the arsenic concentration can be detected at the range of WHO standards.

If the results were compared with previous arsenic detection method, the developed system with syringe pump can detect arsenic at 2.2 µg/L which means flow increased the binding onto the silanized surfaces.

3.5 Withdrawing & Pumping with Syringe Pump

Solutions were pumped and then withdrawed with syringe pump by this way same solution passed through channel for 100 times and so it is aimed to increase the binding of arsenic molecules and gold nanoparticles onto the surface. This procedure has two sections.

Firstly, arsenic samples were withdrawed and pump into the system. Which means, after TCEP solution was injected into the both channels, 1 mL of arsenic solution and 1mL of DI water was pumped with 10mL/min flowrate into the system and gathered into separate eppendorfs. Then same solutions were withdrawed into the system. This procedure continues for 100 times (50 times pumping, 50 times withdrawing) and totally 100 mL of samples would be injected into the system. Therefore, it is able to compare with pumping 100 mL of sample for one time and 1 mL of sample for hundred times.This experiment was done with 10 µg/L arsenic concentration which is the maximum concentration according to WHO. The absorbance value of withdrawing-pumping experiment is 0.0106 whereas just pumping experiment result is 0.0072 for 10 µg/L. It is understood that, flowing same solution into the channels increase the binding of samples onto the system. Also, as shown in Figure 25, pumping and withdrawing of same solution gives higher absorbance value and higher than background signal of the system. Additionally, with this experiment, sample volume was decreased 100 times and makes this detection microfluidic chip as more convenient for point of care testing. On the other hand, as mentioned above the absorbance value of without flow experiments for even 100 µg/L is not higher than background signal.

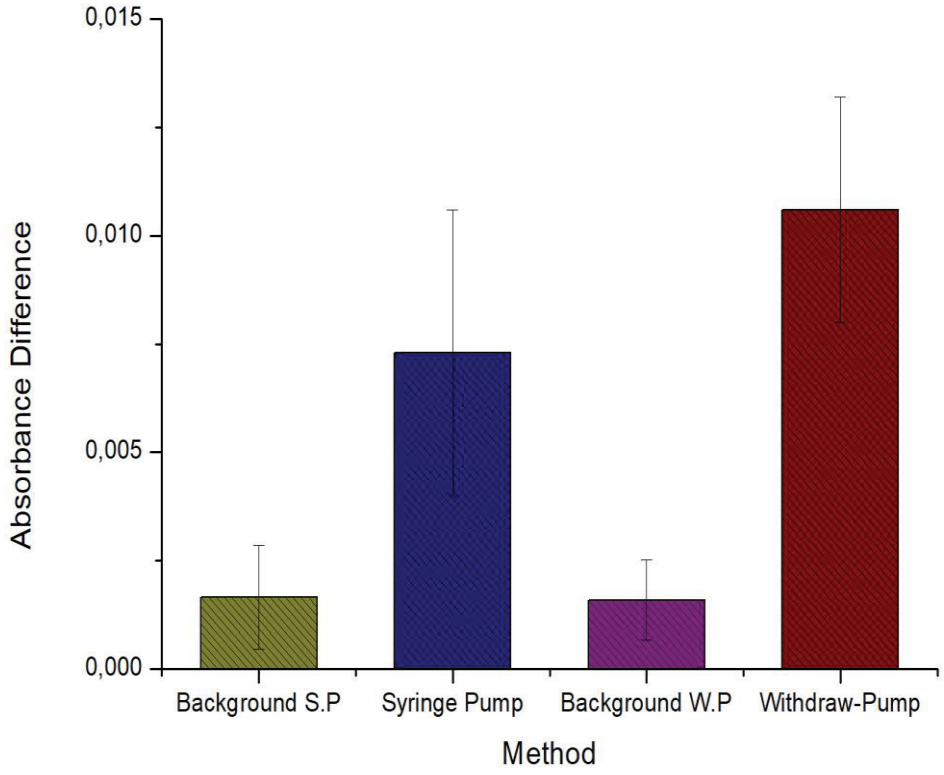

Figure 25. The comparison of two methods; syringe pump and withdraw-pump based on background signal.

Therefore, it was not included into comparison.

Secondly, withdrawing and pumping (WP) of gold nanoparticles experiment was carried out with syringe pump. The procedure again starts with TCEP injecting and waiting for 10 minutes for arsenic sample in one channel and DI water sample in the other channel. Then, different from the previous method, instead of waiting 2 hours, for gold nanoparticles to bind the surface, 100 µL of gold nanoparticle solution was injected into the system for 100 times (50 times pumping, 50 times withdrawing) again with 10 mL/min flowrate. This experiment was carried out for 100 mg/L arsenic concentration in order to compare with waiting 2 hours to bind. As can be seen from Figure 26, while waiting gold nanoparticles for 2 hours has an absorbance value at 0.016, withdrawing-pumping system has 0.007 absorbance value. They are both higher than the background signal and higher than the LOD as calculated however, it is understood that waiting of gold nanoparticles for 2 hours increase binding onto the surface. Additionally, for this experiment one-way- ANOVA analysis was done. The results are as shown in Figure 26.

There is significant difference between gold waiting for 2 hours experiment and the background of that experiment. On the other hand, withdraw-pump results have no significant difference between them.

3.6 Fourier Transform Infrared Spectroscopy (FTIR) Results

Also, the FTIR characterization (Figure 27) of the cover glasses were done for whole process; silanization, arsenic binding and gold nanoparticle binding.

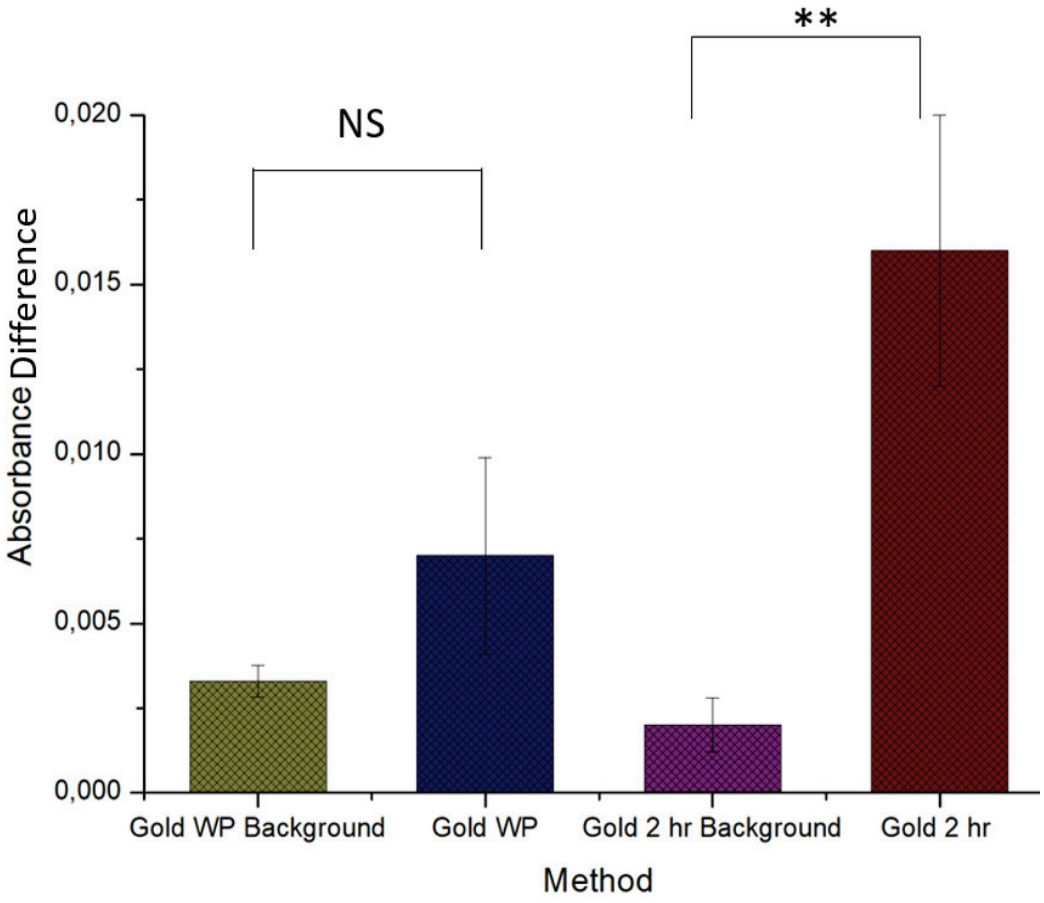

Figure 26. The comparison of different binding methods of gold nanoparticles as waiting 2 hours and withdrawing-pumping with syringe pump and their ANOVA results.

For cover glasses the peaks are observed at the wavelength of 1000-2000, firstly, the O2 plasma treated cover glasses were compared with silanized cover glasses and the peak differences between two samples are at the wavelengths of 1451, 1643,2645 and 3419 (Figure 28) [(Hunt) accessed on 20.05.2019]. It is understood from the literature that, 1451,3419 and 1643 peaks are coming from acetone, since silanization was done with acetone. Additionally, it is found that the peak that was occurred at the wavelength of 2645 is due to the –SH bonds on the surface [[(LibreTexts, 2014) accessed on 20.05.2019;("ATR-FT-IR spectrum of Acetone (4000 – 225 cm–1)," 2015) accessesd on 20.05.2019,(Biorad, 2019 accessed on 07.08.2019]

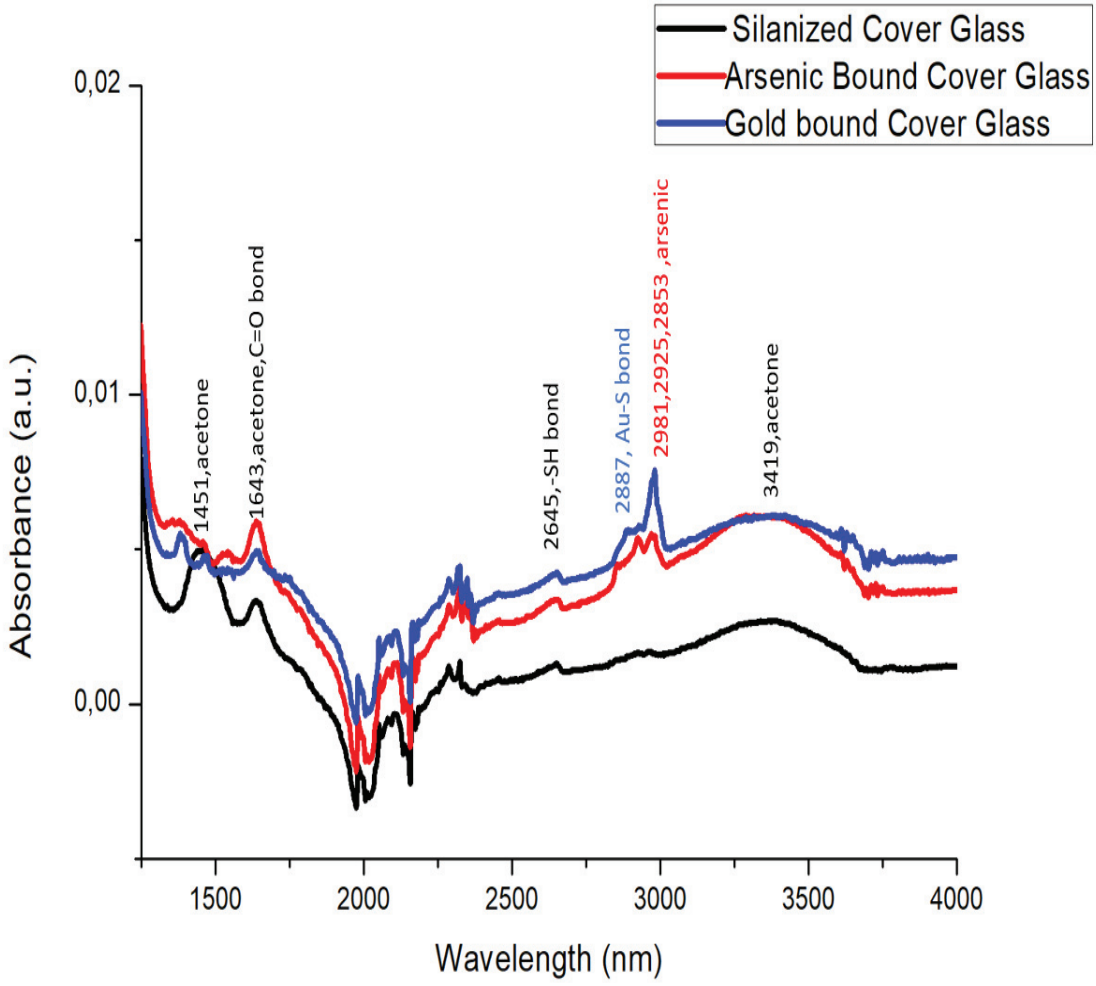

Figure 27. The FTIR Results.

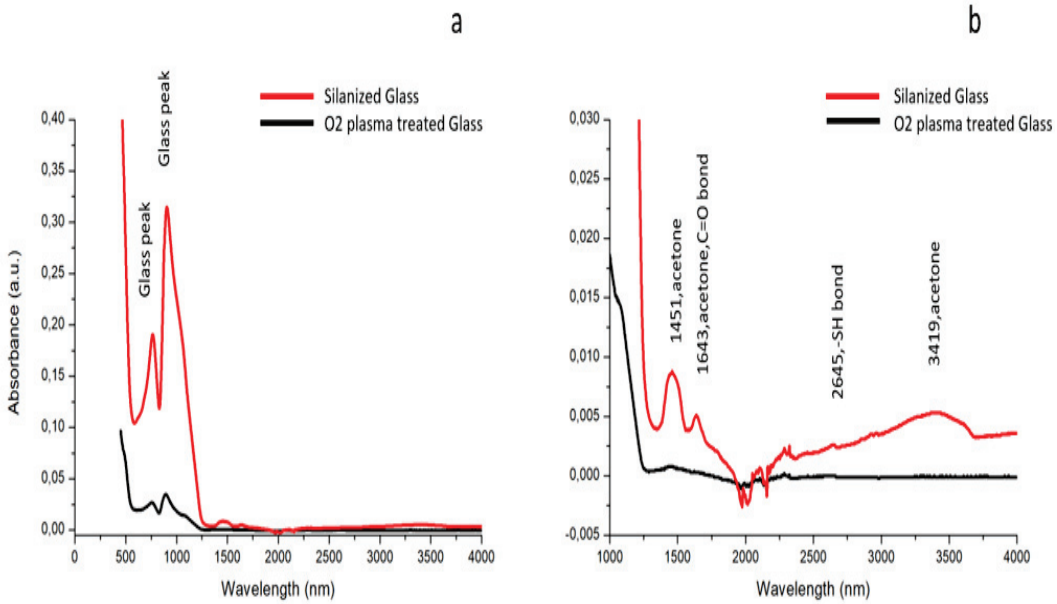

Figure 28. The comparison of silanized cover glass and O_2 plasma treated glass a.) Common glass peaks b) Zoomed in to observe silanization peaks.

On the other hand, the arsenic (As2O3) bound cover glass have peaks at the wavelengths of 2981, 2968 and 2853. These peaks are coming from arsenic peaks; therefore, it is understood that arsenic binds onto the surface successfully [(Commerce, 2018) accessed on 20.05.2019].

At the next step, gold nanoparticles are bound onto the surface. As can be seen from the graph, similar peaks with arsenic was observed. This is because, during the process the gold nanoparticle binding step is after the arsenic binding. As shown, at the wavelengths of 2887, 2980 and 2926 peaks are observed for gold nanoparticle bound surface. However, as can be seen from Figure 24 gold nanoparticle has extra peak at 2887 wavelength. It is figured out that this peak is coming from the Au-S bond due to the alkanethiols [(Shengfu & Du, 2002),(Moon, Kusunose, & Sekino, 2009)].

Finally, whole results are compared to each other and previous works. Firstly, it is known that the cost of arsenic detection methods is crucial especially for the developing countries. Laboratory methods have high sensitivity to detect arsenic however it is needed to well-educated persons to use the devices and devices have incredibly high prices.

So, it is proved that developed arsenic detection method in the thesis is so much cheaper than the laboratory methods.

For one microfluidic chip, the cost was calculated by considering cover glasses, gold nanoparticles, PDMS, acetone and 3-MPS and found as 10,7 Turkish Liras. The cost of available arsenic kits is reasonable, but they are not able to detect arsenic below the 50 μg/L concentration which is higher than the WHO standards. As expressed, the LOD of the arsenic detection microfluidic chip is 2.2 μg/L when flow was added to the system. Furthermore, the chemical reactions methods have also reasonable price however they are toxic to both environment and the user which is again different from the developed system.

Additionally, the enzymatic detection system is more complex compared to developed chip. Moreover, whole previous works except available kits are not portable which is an important parameter for ideal arsenic sensor different from the developed microfluidic chip. Speed is also the other parameter for arsenic detection, arsenic available kits and detection with chemical reactions are rapid procedures, but they have selectivity and non-healthy challenges and compared to developed microfluidic chip, it is portable, rapid and non-toxic.

Secondly, after optimization experiments are done, the experiment continues with the most optimized way such as; using TCEP, waiting 10 minutes for arsenic binding onto the silanzied cover glass surface and waiting 2 hours for gold nanoparticle binding and using DI water due to its pH. After compared the As concentrations without flow, minimum 1.3 mg/L concentration can be detected which is not enough for the standards therefore syringe pump was added to the system. And then, it is understood from the results, using syringe pump advances the arsenic detection principle and decrease the detectable concentration till 2.2 μg/L. Then system also developed one more and using syringe pump more effective by withdrawing and pumping arsenic sample. Finally, the best result for the developed microfluidic chip is using syringe pump with withdrawing and pumping system.

In general, the assay time of this project is nearly 150 minutes, and arsenic can be detected at 2.2 μg/L by using 100 mL sample. The general comparison of the developed microfluidic chip with methods in the literature as seen in Table 4. In Table 4, the advantages and disadvantages of whole methods can be cleary seen.

Table 4. The general comparison of the developed microfluidic chip with methods in the literature.

	Developed microfluidic chip	**Arsenator arsenic kit**	**Molybdenum blue method**	**AAS**	**Paper based microfluidic sensor**
ASSAY TIME	150 minutes	20 minutes	30 minutes	30 minutes	5 minutes
SAMPLE AMOUNT	100 mL	50mL	30mL	>1mL	3mL
LOD	2.2 µg/L	50 µg/L	15 µg/L	1 µg/L	1 µg/L

CHAPTER 4

CONCLUSION

Arsenic is a major concern worldwide due to its properties that are harmful for humans. Detection of arsenic can be done with many choices but none of them can lead the ideal arsenic sensor features which are, cheap, rapid, selective and sensitive, non-toxic to environment and the user, easy to handle and portable. Therefore, with this thesis, it was aimed to produce a microfluidic chip for arsenic detection. For the first time, arsenic was detected with 2 channels of microfluidic chip without mixing arsenic sample and recognition sample (for this thesis gold nanoparticles), arsenic was detected by the difference of absorbance values between channels. Microfluidics is used because of advantages such as; low amount of sample, high surface to volume ratio and small size to be a portable device for arsenic determination and gold nanoparticles helped with their metallic surface properties for analysing arsenic. To have the best result, plenty of experiments were carried out. By without flow experiments, arsenic can be detected with 100 μL solution but detectable concentration was 1.3 mg/L. Then, flow was added and with 100 mL of arsenic sample, 2.2 μg/L concentration of arsenic could be detected. Both processes can be completed in 150 minutes.

In the future, same method can be applied to a fully automated platform for arsenic detection. Colorimetric agents can be added to the system and colorimetric detection can be done. With automated platform and the colorimetric detection system, the arsenic detection procedure will be so much easier. Last but not least, arsenic contamination is a global problem and unawareness cause many deaths, therefore in my opinion arsenic analysing devices should be developed in reasonable prices and higher detection limits, and precautions must be done.

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