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ORIGINAL PAPER

Low-cost, portable, on-site fluorescent detection of As(III) by a paper-based microfluidic device based on aptamer and smartphone imaging

Min Yuan[1] · Chen Li[1] · Mengxue Wang[1] · Hui Cao[1] · Tai Ye[1] · Liling Hao[1] · Xiuxiu Wu[1] · Fengqin Yin[1] · Jinsong Yu[1] · Fei Xu[1]

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Abstract

A turn-on fluorescent aptasensor based on a paper-based microfluidic chip was developed to detect arsenite via aptamer competition strategy and smartphone imaging. The chip was prepared by wax-printing hydrophilic channels on filter paper. It is portable, low-cost, and environmentally friendly. Double-stranded DNA consisting of aptamer and fluorescence-labeled complementary strands was immobilized on the reaction zone of the paper chip. Due to the specific strong binding between aptamer and arsenite, the fluorescent complementary strand was squeezed out and driven by capillary force to the detection area of the paper chip, so that the fluorescent signal arose in the detection area under the excitation wavelength of 488 nm. Arsenite can be quantified by using smartphone imaging and RGB image analysis. Under the optimal conditions, the paper-based microfluidic aptasensor exhibited excellent linear response over a wide range of 1 to 1000 nM, with a detection limit as low as 0.96 nM (3σ).

Keywords Arsenite detection · Aptamer · Fluorescence · Paper-based microfluidic chip · Smartphone imaging · RGB evaluation

Introduction

Arsenic is a well-known toxic, carcinogenic, and teratogenic substance. In the environment, arsenite is more toxic than other arsenic species (e.g., arsenate (As(V)) and organic As compounds) [1]. According to the regulations of the World Health Organization (WHO) and the U.S. Environmental Protection Agency (EPA), the maximum acceptable level for the total As in drinking water is 10 μg/L (133 nM) [2–4]. Some technologies including inductively coupled plasma-mass spectrometry (ICP-MS) [5], atomic absorption spectroscopy (AAS) [6], and atomic fluorescence spectrometry (AFS) [7] are usually used for arsenic determination. Although these methods are widely used, but they require specific large laboratory instrumentation and cannot determine arsenite (As(III)) on-site. Therefore, it has a great demand to develop the detection method and device for the portable on-site arsenite detection.

In this context, microfluidic paper-based analytical device (μPAD) [8–12] and aptamer [13–15] are both efficient and interesting approach to explore. Aptamer, as a functional single-stranded DNA or RNA sequence, demonstrates high specificity in binding to the target [16, 17]. Matsunaga K et al. [18] developed a DNA aptamer-based colorimetric method by gold nanoparticle for arsenite determination, and the detection limit was 2.1 μM. Although the method was simple and fast, but the sensitivity was low. Pan J et al. [19] used an exonuclease III-assisted fluorescent signal amplification strategy to sensitively detect As(III) in water.

Min Yuan, Chen Li, and Mengxue Wang contributed equally.

Highlights
1. A fluorescent paper-based microfluidic device based on smartphone imaging was developed for the on-site detection of arsenite.
2. The device was portable, low-cost, and environmentally friendly.
3. The device displayed a low detection limit of 0.96 nM in arsenite detection.

✉ Fei Xu
xufei8135@126.com

[1] Shanghai Engineering Research Center of Food Rapid Detection, University of Shanghai for Science and Technology, Shanghai, China

However, the exonuclease assistant aptamer biosensors need strict temperature control and a long reaction time, which is not suitable for on-site detection. The μPAD shows the advantages of low-cost, portable, and strong ability to filter impurities [20–22]. Aptamer sensors combined with paper-based microfluidic devices have become a new trend, which can achieve the purpose of on-site detection. He M et al. [23] proposed the first portable upconversion nanoparticles (UCNPs)-based paper device for road-side field testing of cocaine. Monisha et al. [24] measured Hg^{2+} by a colorimetric method using a paper-based sensor with silver nanoparticles (AgNPs) printed by smartphone inkjet.

Here, we present a paper-based microfluidic device to detect As(III) on-site based on an aptamer competition strategy and smartphone imaging. The chip was prepared with hydrophilic and hydrophobic regions through wax spray printing. The double-stranded DNA with quenched fluorescence was fixed on the reaction area of the paper chip, in which one strand was a quencher-modified aptamer and the other strand was a fluorophore-modified complementary sequence. After adding the arsenite sample, aptamer was preferentially combined with arsenite to release the complementary sequence [18, 25, 26]. Due to the capillary force-driven liquid action in the hydrophilic channel of the filter paper, the fluorescein-modified complementary strand flowed into the detection area, where the fluorescence signal was enhanced. The changes of the fluorescence signal were captured by a smartphone camera. Therefore, the proposed device indicated the advantages of portable, low cost, and less consumption. It has great potential in the field detection of pollutants.

Materials and methods

Materials

4-hydroxyethyl piperazine ethanesulfonic acid (HEPES, 99%) was purchased from Sigma-Aldrich (Shanghai) Trading Co., Ltd. (www.sigmaaldrich.com). Sodium arsenite ($NaAsO_2$), sodium phosphite (Na_2HPO_3), sodium hypophosphite (NaH_2PO_2), sodium arsenate (Na_3AsO_4), lead acetate ($Pb(CHCOO)_2$), cadmium chloride ($CdCl_2$), sodium chloride (NaCl), and potassium dichromate ($K_2Cr_2O_7$) were purchased from Shanghai Sinopharm Reagent Co., Ltd. (www.reagent.com.cn). Whatman No. 1 filter paper was purchased from Shanghai Gaoxin ChemicalGlass Co., Ltd. (www.shgxshop.com). All other reagents were of analytical grade. Ultrapure water was used throughout.

DNA aptamers designed in our work were synthesized by Shanghai Sangon Biotech Co., Ltd. (www.sangon.com), and their sequences were listed as follows (design details are in Table S1 and Fig. S1 of the Supplementary Information):

Q-Apt-34: 5′-BHQ1-ACAGAACAACCAACGTCGCTCCGGGTACTTCTTC-3′

C-FAM-11: 5′-GAGCGACGTTG-FAM-3′

Apparatus

A paper-based chip was printed by a wax jet printer (Suzhou Royston Microfluidics). A clamp-type hot press machine (MNP-001) was bought from ASONE (Japan). A smartphone (Huawei p20 pro) was bought to take the photos and then analyzed by the software ImageJ. A fluorescence spectrophotometer (RF-6000) was bought from Shimadzu (Japan). The 488 nm laser and 520 nm high transmission filter were bought from Guangzhou Fuzhe Laser Technology.

Fabrication of the paper-based chip

The paper-based chip size was 52 × 20 × 0.5 mm (Fig. 1a). It was composed of a wax jet printing layer at the top and a black hydrophobic plate at the bottom. The top layer of the paper-based chip was composed of an injection area, an aptamer fixation area, and a detection area. After pressing the wax jet printing layer with a hot-pressing plate, a black hydrophobic plate was attached to the back of the paper to make a paper-based chip (Fig. 1b). (Details are described in Supplementary Information).

Manufacture of the detection device

The self-made portable detection device was shown in Fig. 2. It was composed of a metal camera obscura, a 488 nm laser, and a 520 nm filter. The fluorescent signal photographs were taken using a smartphone through a filter window. The dimension of the metal box was 27 × 24 × 22 cm, and it was all black inside, except for a window with a filter and a door for taking the chip was left. As a fluorescent exciter, the 488 nm laser was fixed inside the camera obscura at an angle of 45° to the bottom.

Procedure for detection of As(III) using paper-based microfluidic device with smartphone

The 1.5 μM aptamer (Q-Apt-34) and complementary strand (C-FAM-11) were reacted in 25 mM HEPES buffer (containing 0.1 M NaCl, pH 7.6) at 4 °C for 10 min to form double-stranded DNA. We took 10 μL of the mixture to the fixation area of the paper chip and let it sit for 20 min in darkness, so that the DNA was completely adsorbed on the chip. Then, we added 20 μL As(III) solution in the injection area, and the liquid flowed into the fixation area and reacted with aptamer for 30 min. Finally, 40 μL of 25 mM HEPES buffer was added to the injection area,

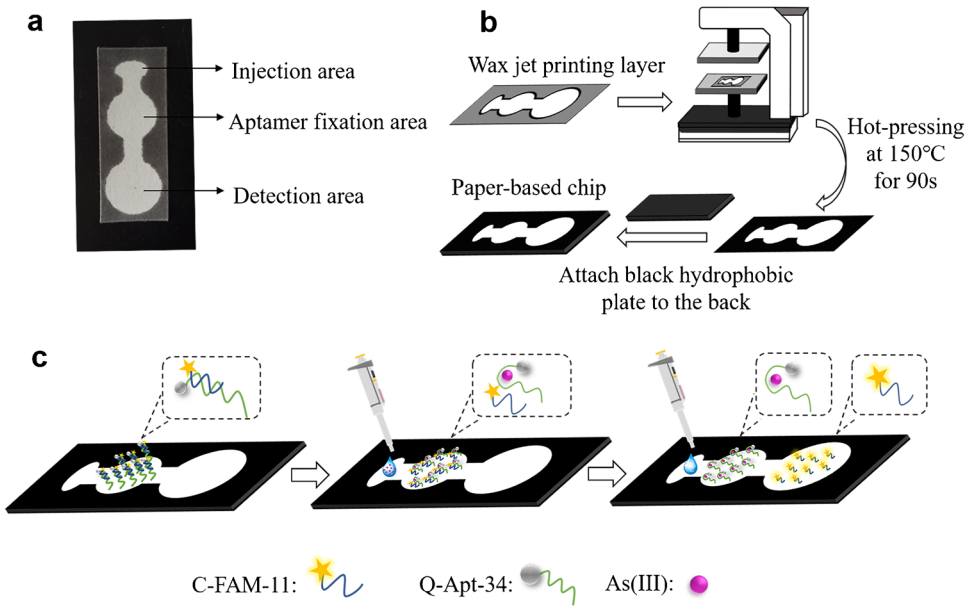

Fig. 1 The schematic representation of the paper-based chip. **a** Real photo of the chip, the size of 52 × 20 × 0.5 mm. **b** Chip fabrication process. **c** Schematically illustration of the experimental principle. The aptamer was fixed; the sample was added to the injection area, and after full reaction, the buffer was added dropwise and detected in the detection area

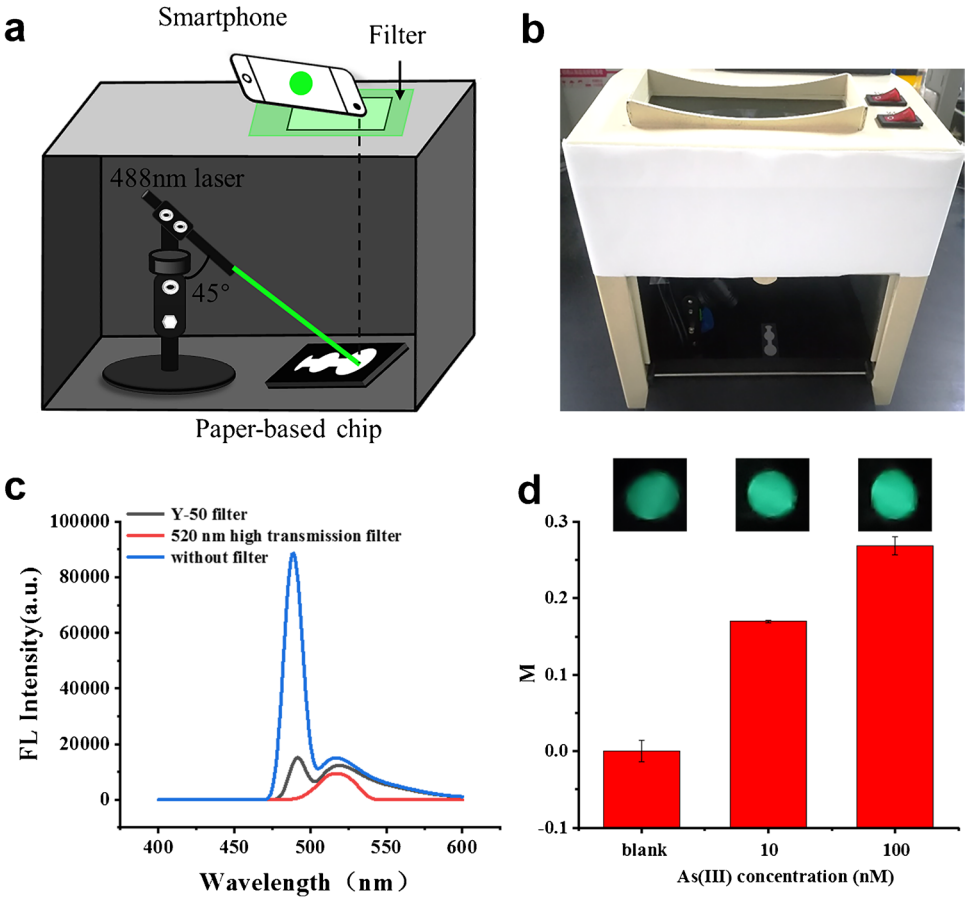

Fig. 2 **a** Schematic of the layout structure of the detection device. **b** Real photo of the detection device. **c** Selection of the suitable filter with the fluorescence spectrophotometer. The excitation wavelength was 488 nm, and the emission wavelength was 520 nm (curve blue: without filter; curve red: used the 520 nm high transmission filter; curve black: used Y-50 filter). **d** Fluorescence signals obtained by smartphone photography and the M values in the absence and presence of As(III), the fluorescence images above correspond to 0, 10, and 100 nM As(III)

which flowed into the detection area after passing through the fixation area along the channel. When the liquid filled the detection area, the chip was immediately put into the camera obscura for photo recording under the excitation wavelength of 488 nm. The photo of the detection area was analyzed for RGB values using ImageJ software.

Real samples detection

Tap water and Huangpu River water samples were tested to investigate the application of the paper-based microfluidic device. The samples were filtered by a 0.22 μm membrane and then adjusted pH to 7.6 by adding an appropriate amount of NaOH or HCl. The samples with spiked concentration of 100 nM and 1000 nM were prepared by adding 0.05 mL (10 μM/100 μM) arsenite solution into 4.95 mL tap water or river water. The prepared samples were assayed according to Method 2.5.

Results and discussions

Detection principles of paper-based chips

The paper-based chips were used to detect As(III) based on the strong binding ability of aptamer to heavy metal ions. The binding mechanism, as suggested by Kaur et al. [27], is that adenine and guanine in the aptamer promote specific binding of the aptamer to As(III) through directed unsaturated hydrogen bonds with nucleotide bases and their self-assembly-induced recognition behavior. Figure 1c is a schematic illustration of the experimental principle. The double-stranded DNA formed by quencher-labeled aptamer (Q-Apt-34) and fluorophore-labeled complementary strand (C-FAM-11) were added to the aptamer fixation area in the paper-based chip. And then, double-stranded DNA was adsorbed on filter paper with liquid deposition [28, 29]. After adding As(III) in the injection area, the solution was propelled to the aptamer fixation area through the capillary force-driven liquid action of the filter paper, and the sample was simply filtered. The specific binding of the aptamer to As(III) destroyed the double-stranded structure, and the C-FAM-11 was competed down. Since C-FAM-11 has fewer bases and a smaller molecular mass than Q-Apt-34, when sufficient buffer was added, free C-FAM-11 flowed with the buffer to the detection area, and the larger detection area can hold the free C-FAM-11.

To prevent the liquid from leaking out, a black hydrophobic plate was attached to the back of the paper. Since the fluorescent background of the paper-based chip itself was difficult to remove, it was important to choose a good hydrophobic plate. The white background will make the background fluorescence stronger. Custom-made quartz chips can effectively reduce fluorescent background, but with high cost and troublesome to use. Thus, a low-cost black PVC plate can avoid fluorescence noise background and is easy to fit with the paper chip.

Design of the portable detection device

In order to overcome the inconvenience of using conventional equipment, a portable detection device was designed (Fig. 2a, b). The detection device was composed of a camera obscura, a 488 nm laser, and the 520 nm high transmission filter. Because different filters have different properties, it is important to choose the right filter. Y-50 filter and 520 nm high transmission filter were selected for testing, and the results showed that the 520 nm high transmission filter had the best performance (Fig. 2c). (Details are shown in Supplementary Information).

Fluorescent images were collected using a smartphone through a filter window and then converting the fluorescent signal into color (RGB) data using the software ImageJ. The final fluorescence signal (M) was obtained by analyzing the data of red channel (R) and green channel (G). G_1 and R_1 represent the green and red values of fluorescence photography results in the presence of As(III). G_0 and R_0 represent the green and red values of fluorescence photography results without As(III). The calculation formula was based on the previous literature [30] and modified as follows:

Eq. (1):

$$M = \frac{G_1/R_1 - G_0/R_0}{G_0/R_0} \tag{1}$$

Feasibility of the As(III) detection based on the paper-based microfluidic device

In order to verify the feasibility of the established detection strategy, fluorescent images of the As(III) samples with concentrations of 0, 10 nM, and 100 nM were taken under the excitation wavelength of 488 nm and analyzed, respectively. As shown in Fig. 2d, the fluorescence signals of As(III) samples in the detection area of the paper-based microfluidic chip were much stronger than that of the blank control. And the M value increased with increasing As(III) concentration. It indicated that the paper-based microfluidic chip and device designed above successfully detect As(III).

Sensitivity and selectivity of the paper-based microfluidic device

In order to improve sensitivity, experimental conditions for the reaction, including the concentration of aptamer, amount of the washing fluid on the paper-based chip, and time of As(III) reacted with aptamer, were performed. The 1.5 μM of aptamer, 40 μL of HEPES buffer was added as the flushing liquid and 30 min reaction time were found to be optimum (Fig. S2 in Supplementary Information).

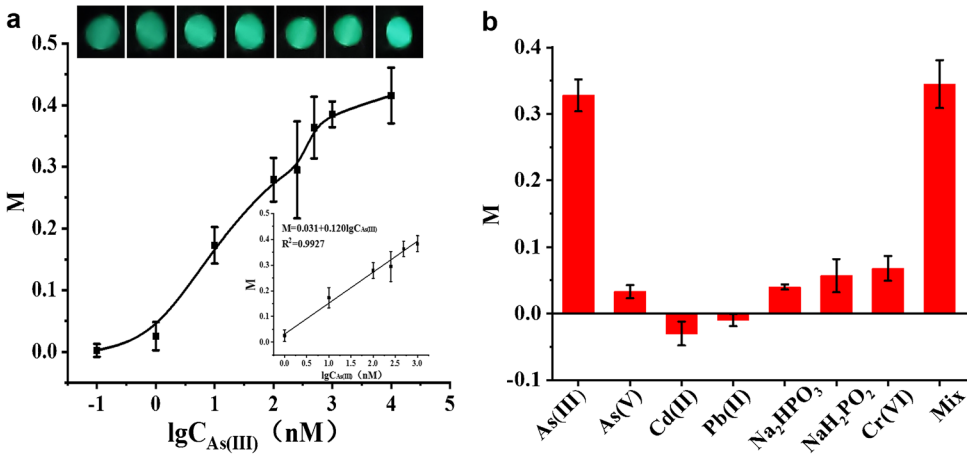

Fig. 3 Sensitivity and selectivity of the paper-based microfluidic device. **a** The signal responses of the microfluidic paper-based chip to different concentrations of As(III) under the excitation wavelength of 488 nm, inset shows the linear relationship, the fluorescence images above correspond to 0, 1, 10, 100, 200, 500, and 1000 nM As(III). **b** Specificity of the paper-based chip. The concentration of each ion was 100 nM. For the mixed sample, the final concentration of each metal was also 100 nM

Under optimal conditions, various concentrations of As(III) were examined at the excitation wavelength of 488 nm. As shown in Fig. 3a, the M value was boosted with increasing As(III) concentrations, and it was linearly correlated to the logarithm of As(III) concentration from 1 to 1000 nM. The linear regression equation was $M = 0.031 + 0.120\ \lg C_{As(III)}$ ($R^2 = 0.9927$). The limit of detection (LOD) of As(III) was calculated to be 0.96 nM (3σ).

The responses of the microfluidic paper-based chip to other metal ions (As(V), Cd(II), Pb(II), Na_2HPO_3, NaH_2PO_2, Cr(VI)), and mixed metal ions were investigated in Fig. 3b. As shown in the figure, NaH_2PO_2 and Cr(VI) had a certain response to the sensor, but compared with As(III) with the same concentration, As(III) had a more significant response. When all ions were presented simultaneously, the response signal produced was almost the same as that produced when only As(III) was presented, so that the sensor had excellent selectivity to As(III) when there were interfering ions in the environment. Such high selectivity was mainly attributed to the high specificity of the Q-Apt-34 for As(III).

The storage stability of paper-based microfluidic chips

To ensure the stability, we stored the fabricated paper-based chips at room temperature for a long time and tested the fluorescence signal (M) responded to 100 nM As(III) at 1–5 days, 15 days, and 30 days. As shown in Fig. S3, no significant decrease in fluorescence signal was observed within 30 days, indicating that the paper-based chips had good storage stability.

Real samples detection

To further verify the viability and practicability of the proposed method, recovery experiments in tap water and Huangpu River water were carried out. All the samples were filtered by 0.22 μm microporous membrane before testing. As shown in Table 1, when the samples were not spiked, As(III) was not found in tap water, and 13.84 nM of As(III) was detected in the Huangpu River water. For the tape water samples with spiked concentration of 100 nM and 1000 nM, the recoveries were 95.28% and 98.02%, respectively. And the recoveries of the Huangpu River water samples with spiked concentration of 100 nM and 1000 nM were 103.24% and 108.29%, respectively. It should be noted that the recovery values of the river water were calculated by deducting the background values. All the recoveries of the real samples were within the acceptable range with RSD < 15%. It means that the proposed method for the determination of As(III) has good suitability and reliability in real samples.

Table 1 Recovery in the water samples

Sample	Added (nM)	Total found (nM)	RSD($n=3$, %)	Recovery(%)
Tap water	0	Not found		
	100	98.03 ± 9.47	9.67	98.02
	1000	952.80 ± 124.43	13.06	95.28
River	0	13.58 ± 1.71	12.59	
	100	116.82 ± 15.59	13.35	103.24
	1000	1096.48 ± 38.27	3.49	108.29

Table 2 An overview on recently reported optical methods for the determination of As(III)

Methods	Materials	Linear range	LOD	Ref
Fluorescence	Paper-chip and Cu nanoclusters	0–1.2 μM	93 nM	[31]
	carbonaceous fluorescent nanomaterials	3.3–828.5 μg/L	0.48 μg/L	[32]
	persistent luminescent nanoparticles and AuNPs	0.067–13.4 μM	55 nM	[33]
Liquid crystal	aptamer and cetyltrimethylammonium bromide	20–1000 nM	50 nM	[34]
Fluorescence imaging	aptamer and paper-based microfluidic device	1–1000 nM	0.96 nM	This work

Performance comparison with the other optical methods

The optical method, especially the fluorescence spectrophotometer, is a popular detection method of arsenic. Herein shows an overview of the recently reported optical methods for the detection of As(III) in Table 2. Compared with the other reported methods, the fluorescence imaging method proposed in this paper exhibited a wide detection range and higher sensitivity. Moreover, the detection using smartphone imaging improves the detection portability, which is conductive to the on-site detection.

Conclusions

In this study, a portable paper-based microfluidic device was developed for the sensitive detection of As(III), in which the cost of a single detection was less than $0.3. The usage of aptamer enables the detection to maintain good selectivity in the complex environment and avoids the possible interference from other ions. And the combination of paper-based chips and smartphone imaging improves the detection portability and enables the on-site detection of samples. However, the paper-based chips used in the proposed strategy have a background fluorescent signal. Although its influence is not obvious on a turn-on fluorescent biosensor, it does limit the application in developing turn-off fluorescent biosensors. Thus, reducing the background noise and improving the detection sensitivity is one of our next research focuses. We believe that the proposed paper-based microfluidic chip and portable detection device will have broad application on the rapid on-site detection.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00604-023-05693-3.

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Data Availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

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