

## A colorimetry based, semi-automated portable sensor device for the detection of arsenic in drinking water

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### Abstract

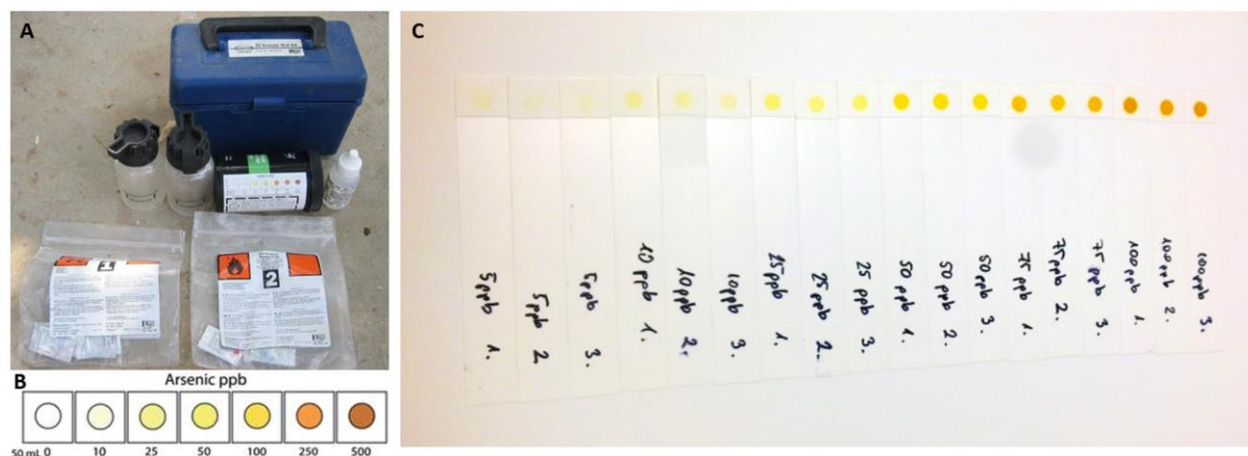
A portable colorimetric device is presented for arsenic ion detection in drinking water. The main purpose of this device – which was developed to complement a commercial water dearsenating station demanding regular (weekly to monthly) regeneration runs depending on its saturation – is to provide regular field measurements and easy, semi-automatic operation designed specifically for untrained, typically illiterate users e.g. the population of developing countries. The device exploits a commercially available, well established reagent kit, which is widely used in arsenic field-testing in many developing countries. The hereby presented custom-designed microfluidic system enhances the user-friendliness, the functionality and reliability of the test-strip based kit. The response for total inorganic arsenic ion concentrations (As(III) and As(V)) is linear in the 5-20  $\mu\text{g/l}$  (ppb) range with a sensitivity of 1  $\mu\text{g/l}$ , thus the device is capable to distinguish drinkable water based on the World Health Organization (WHO) guidelines of 10  $\mu\text{g/l}$ . The average relative standard deviation of the measurements is 8 % in the linear range and it is below 2 % for higher arsenic concentrations (above 20  $\mu\text{g/l}$ ). The hardware construction of the device and the custom microfluidic system is presented in detail. The effect of elevated water sample temperature on the kinetics and performance of the device was also investigated in order to reduce the required time for the measurements. Stable and reliable arsenic concentration values were obtained after 60 min at 22 °C (ambient temperature) and after only 25 min at 50 °C water sample temperature, but at a cost of an increased relative standard deviation to 16 %, in the linear range.

Keywords: colorimetry; electronic readout; arsenic; portable sensor; drinking water monitoring

### 1. Introduction

The arsenic contamination of drinking and irrigation water systems is one of the major environmental challenges of our century. Besides its direct toxicity, the mutagenic and carcinogenic potential of arsenic upon prolonged exposure is also reported (Hughes, 2011). Out of its four existing valence states, As(III) and As(V) are the most widespread forms in nature, with As(III) being the dominant one (Luong, 2007). The current World Health Organization limit for

maximum arsenic concentration in drinking water is 10  $\mu\text{g/l}$  for the developed countries (WHO, 2011). In some of the developing countries, where the arsenic contamination of water systems is the most severe (such as in Bangladesh or India), this limit is 50  $\mu\text{g/l}$  for drinking water (Flanagan, 2012). While there are several reliable laboratory methods for arsenic detection and quantification in environmental samples – such as high performance liquid chromatography (HPLC) (Ackerman, 2005), inductively coupled plasma mass spectrometry (ICP-MS) (Roje, 2007), atomic absorption spectroscopy (AAS) (Jakariya, 2007), and atomic fluorescence spectroscopy (AFS) (Deng, 2013) as a couple examples – these techniques require expensive and complex instrumentation with highly skilled staff (Tyson, 2013). There is clearly a need for simpler, portable (preferably handheld) devices for on field testing and monitoring. There are several candidate methods in various phases of development which aim to provide an alternative for portable arsenic sensing. In a recent review Yogarajah and Tsai (2015) collected and compared the strengths and weaknesses of several reported methods. Some of the most promising candidates for potentially portable measurements use either electrochemical methods, for example with screen printed electrodes (SPE) (Khairy, 2010), surface sensing methods, such as surface plasmon resonance (SPR) (Forzani, 2007), spectroscopic methods based on for example chemiluminescence (CL), (Hashem, 2011), or colorimetry. Colorimetric methods using various reagents (Sirawatcharin, 2014) are offering a simple and economic way to determine arsenic concentrations and some of these are already commercially available as field test kits. Yogarajah and Tsai (2015) also give a good overview regarding the available arsenic test kits, which are mostly based on the Gutzeit reaction, such as the test kit from Hach®, presented in Fig.1.



**Fig. 1.** A) The Hach® EZ arsenic field test kit with its reagents. B) The reference color series given by the manufacturer to compare the results to. C) Photograph of a series of experimental calibration results performed in triplicates by using the original reaction vessel and original reagents.

These field test kits, like the Hach EZ or the ITS Econo-Quick performed rather well and reliably in the past to distinguish drinkable water based on both the 10  $\mu\text{g/l}$  and the 50  $\mu\text{g/l}$  threshold (George et. al, 2012, Geen et. al, 2005, Geen et. al, 2014). However, there are still several disadvantages of such field test kits. They are often unreliable around or below the WHO limit of 10  $\mu\text{g/l}$  concentration, besides the human eye is not very sensitive to changes in the yellow color,

not to mention that on the field reproducible lighting conditions, the first step of any reliable optometric readings, is hardly available. This makes the quantitation of arsenic dependent on the skills of the user and thus sometimes unreliable. Also, the operation of such kits could still be complicated for ordinary untrained field users, especially for illiterate people. The determination should be made quickly since the developed yellow color can fade with time, and also the user should avoid breathing in the produced toxic gases (such as arsine).

Conclusively, the development of an easy to use device with built-in, automatic readout, data logging and evaluation could be a valuable asset, especially for developing countries. In this work we present our custom colorimetric device, developed in cooperation with S-Metalltech 98 Co. (Hungary). Our main goals were to provide a simple and reliable way for arsenic ion detection and readouts in the relevant concentration range (considering both the 10 µg/l and 50 µg/l potability thresholds) and to provide easy handling with automated measurement and evaluation. The company initiated this RTD work to ensure the most proper maintenance planning tool for their freshly commercialized well-dearsenating station demanding regular (weekly to monthly) regeneration runs depending on its saturation. Their plan was to complement their dearsenating product with the new user friendly and reliable arsenic monitoring device/method presented in this paper. By designing and developing a camera based reader electronics and a disposable microfluidic cartridge, which is prefilled with the necessary reagents, only minor actuation is required from the operator person, which makes it ideal for untrained users as well, even in the case of illiterate users.

Regarding the price comparison with existing methods and test kits it must be noted, that neither digital nor traditional electronics-free test kits are available on the market for illiterate users, thus, the hereby presented concept is definitely the most cost-effective one in its category. However, an estimation was made in this regard and the production of a series of 20 prototype devices and 100+ prefilled microfluidic cartridges indicated a 700 USD envisaged retail price for the equipment with around 1.5 USD/test cost. Compared to high-end portable devices on the market, such as the Wagtech Arsenator (Wagtech, 2016), with around 1800 USD retail price and 9 USD test cost (Safarzadeh-Amiri et. al, 2011), our concept would offer both a cheaper solution and much simpler operation. Compared to the numerous test kits, the sample cost of our concept fits well in the range of available solutions (0.4-2 USD, Yogarajah et. al, 2015), and although currently it cannot beat the low-end, we have to emphasize, that the aim if the presented device is to beat the user experience and ease of operation, not the price. More detailed comparisons of the commercially available test kits can be found in the works of Yogarajah (2015) and Kabir (2006).

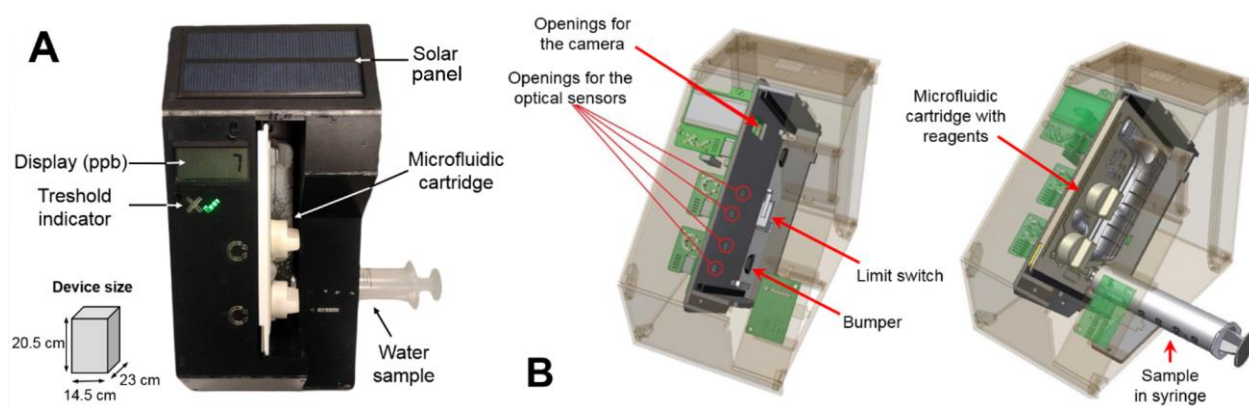
In the next sections the hardware setup and detection characteristics of the device will be presented in detail.

## **2. Hardware Construction**

### **2.1. General working principles**

Our custom device utilizes colorimetric detection and the standard reagents used by a field arsenic testing kit, namely the Hach® EZ Arsenic Test Kit (Hach, 2016; Kinniburgh, 2002; Steinmaus,

2006), shown in Fig. 1/A. The device itself is presented in Fig. 2, with the main parts and its assembly highlighted. Its small size (20.5 cm x 14.5 cm x 23 cm), portability and standalone operation makes it perfectly suitable for on-field tests. The applied reagents of the Hach® EZ Arsenic Test Kit are integrated into a small microfluidic cartridge, which is presented in Fig. 3. The method used by Hach® is based on the Gutzeit reaction, which is discussed in detail elsewhere (Brindle, 2007; Yogarajah, 2015), here only the basic steps are presented. First hydrogen sulfide is oxidized to sulfate to prevent interference, then the oxidizing environment is neutralized. Next, sulfamic acid and powdered zinc react to create strong reducing conditions in which inorganic arsenic is reduced to arsine gas ( $\text{AsH}_3$ ). The arsine gas then reacts with mercuric bromide ( $\text{HgBr}_2$ ), impregnated into a test paper to form mixed arsenic/mercury halogenides (e.g.  $\text{AsH}_2\text{HgBr}$  or  $\text{As}(\text{HgBr})_3$ ). These mixed halogenides discolor the test strip to a degree proportional to the concentration of arsenic in the sample. The color change is from white to yellow to tan to brown, as can be seen if Fig. 1 B and C, which compares photographs of experimental calibration results and the reference color series. It is important to note that the method is not selective, it measures the total inorganic arsenic ion concentration and the color change of the test strip has the same sensitivity to As(III) and As(V).



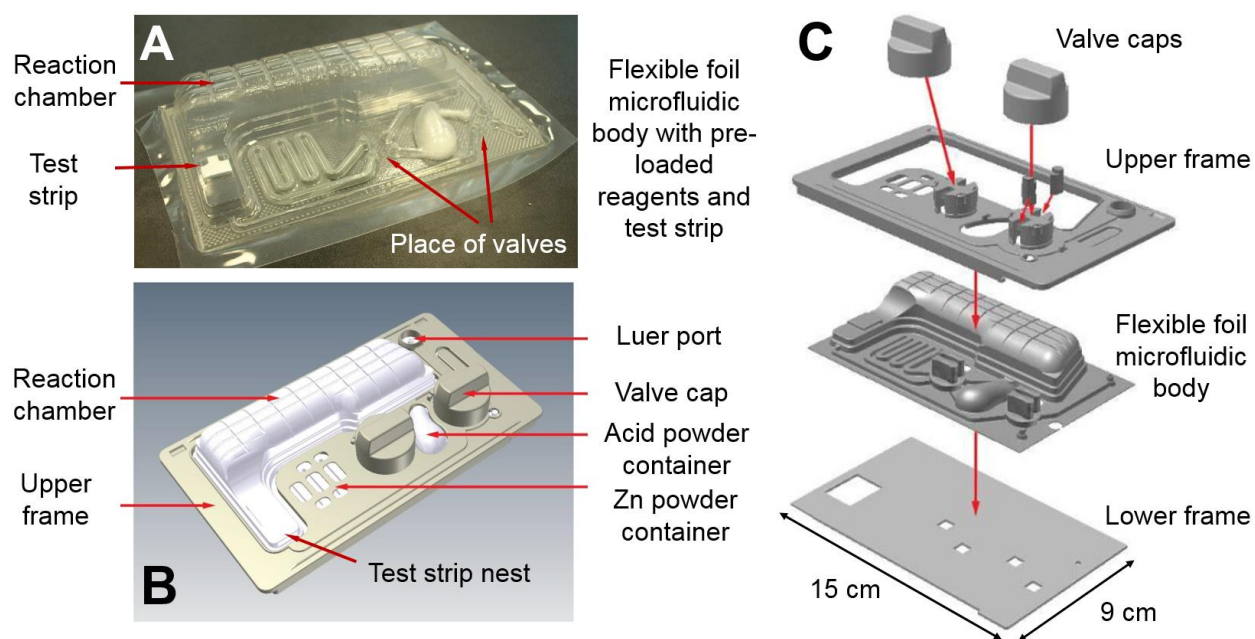
**Fig. 2.** A) Photograph of our custom-developed colorimetric device for arsenic detection. B) 3D CAD images, which present the assembly of the device with the microfluidic cartridge and the water sample.

Based on the technical datasheet provided by the manufacturer (Hach, 2016), the following substances could cause interference: acidity (below pH 5), antimony (above 250 ppb), selenium (above 1 ppm), sulfide (above 15 ppb), tellurium (possible, but not tested). Out of these, the interference of sulfide is handled, as mentioned above, besides, such high values of hydrogen sulfides would not be common for drinking waters (McNeil, 2014). Also, nitric acid interferes with the reduction step. The following conditions did not interfere with the measurement of arsenic: iron (10 mg/l), hardness and alkalinity tested with 1000 mg/l  $\text{CaCO}_3$ . More information about the detailed interference studies and real water tests (bottled, well and tap water) with the Hach method can be found in (Kroll, 2016) and in (Spear et. al, 2006).

## 2.2. Microfluidic cartridge

Since the use of the test kit can be challenging for untrained users on the field due to the difficult process which include step-by-step reagent addition, stirring and the evaluation of the color-change

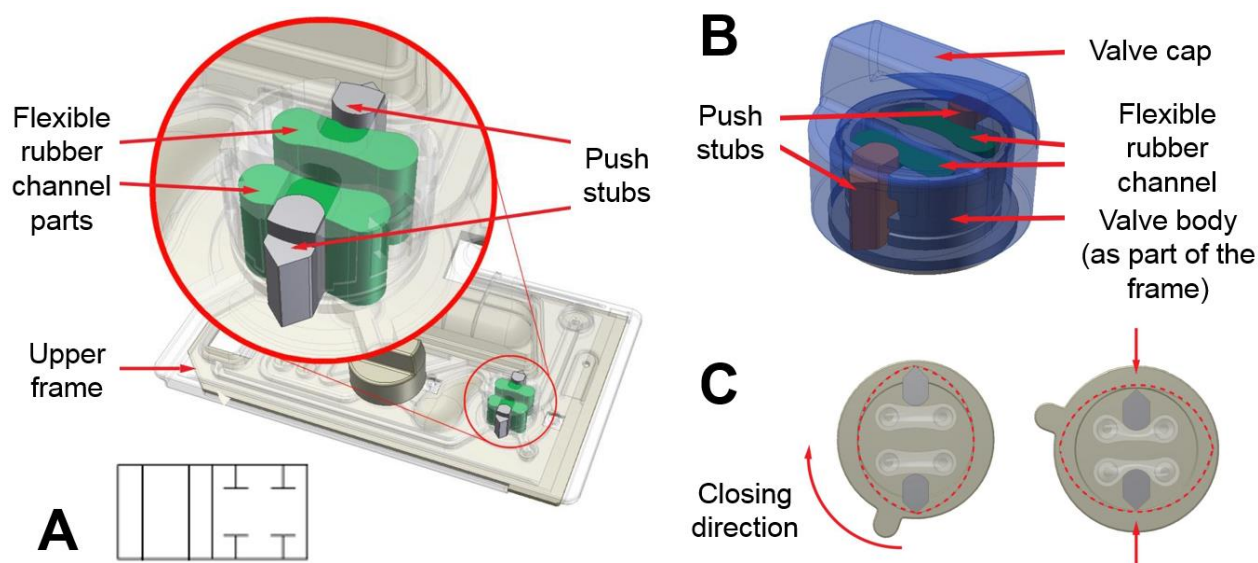
of indicator paper, our idea was to integrate the whole process into a disposable cartridge with automatic camera based color evaluation and only a minimal process actuation required from the user. As it can be seen in Fig. 3, the cartridge consists of three main parts. The upper frame is made of a two-component poly-urethane based hard plastic, fabricated by vacuum casting by using a mold made of an RTV (Room Temperature Vulcanization) two-component silicone elastomer, the lower frame is a 1.4 mm thick FR-4 (Flame Retardant) board machined with contour milling. The two frames can be assembled by sliding the lower part along a railing into the upper frame. In the middle comes the main microfluidic body, made from a flexible polymer film, which contains the pre-loaded reagents (Zn powder and acid powder from the Hach® Test Kit) and the test strip. The film is made of coextruded PET/EVOH/PE (Polyethylene Terephthalate/Ethylene-Vinyl Alcohol Copolymer/Polyester), the PE part is in contact with the sample. The polymer film microfluidic body itself (Fig. 3/A) also consists of two parts: the upper one, which defines the channels, reagent chambers and reaction chamber was fabricated by vacuum forming/thermoforming, then the structured film was bonded to a base film through hot plate welding.



**Fig. 3.** The disposable microfluidic cartridge used in the device. A) Photograph of the vacuum formed film based microfluidic body with the pre-loaded reagents and test strip piece. B) 3D CAD image of the assembled cartridge. C) 3D CAD exploded view of the main parts and assembly of the cartridge.

An important part of the microfluidic setup is the two valves, which are presented in more detail in Fig. 4. The main reason for these four-port two-position valves – and also why it is not possible to completely eliminate the need for manual actuation of the microfluidic cell – is that the two reagents must not be mixed too early, they need to react with the introduced water sample in a definite order. The early mixing and reaction of the reagents would yield false or unreliable measurement results, thus the addition of the sample and the reactions are needed to be controlled manually. Besides, the produced arsine gas is highly toxic and it should be contained in the reaction chamber. To ensure this, the channels are to be closed after the addition of the sample to avoid the possible backflow of the gas.





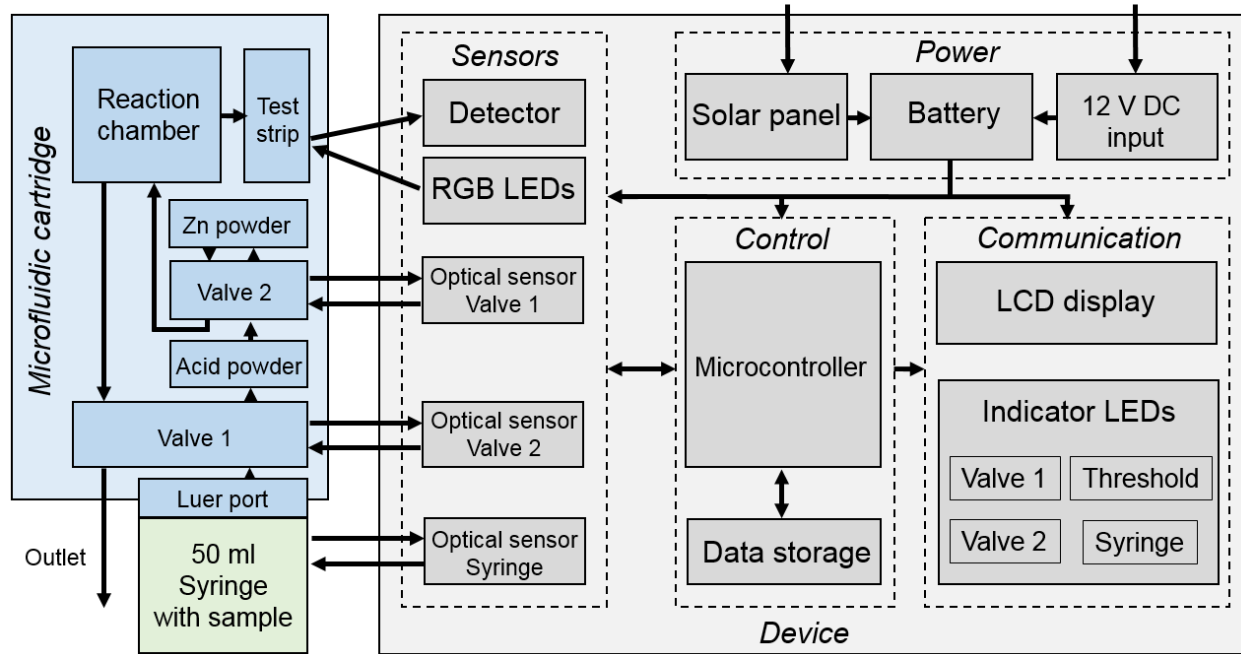
**Fig. 4.** Details of the valve system used in the microfluidic cartridge. A) The position of the valves on the cartridge with the symbol of a four-port two-position valve in the bottom left corner. B) Main parts of the assembled valve. C) Operation principle of the valve (Left: the slideable push stubs are not pressed towards the resilient channel portions, Right: the push stubs are pressed to the center by the oval inner contour of the valve cap if it is turned by 90°).

The operation of the valves is based on two patented inventions of Sántha et al. [EP2205356 (B1) and US8367019 (B2), EP2501971 (B1) and US8834815 (B2)]. The main idea is to elevate the channel above of the main plane of the fluidic system, where its diameter, thus, the flow can be controlled by mechanical pressure from the sides. This is achieved by a resilient tube portion (marked with green in Fig. 4/A) made of either in-situ casted or preformed and attached elastomer pieces and connected to the main fluidic system (i.e. the vacuum formed trenches in the film) via holes. . The tube is constricted by pressing two push stubs against them by turning the valve cap (Fig. 4 B and C). The interior design of the valve caps is oval (red dashed line in Fig 4/C), so turning the caps 90 degree clockwise closes the valves. In the open position the fluidic path is completely open along the whole cartridge, which prevents the occurrence of overpressurization during sample addition. In the closed position the valves ensure that the toxic arsine gas remains in the reaction chamber. Bumpers are placed on the valve caps and on the upper frame, so the valves can only be turned between fixed positions. There are also small holes on the bottom frame below the valves, so the device can sense the status of the valves between the two positions with optical sensors (the bottom surface of the valve caps is coated with light reflective material).

### 2.3. Operation/measurement sequence

The device can be automatically turned on simply by placing a microfluidic cartridge into its recess, thanks to the limit switch, which is positioned at the bottom of the cartridge holder (Fig. 2/B). From this point on the devices gives continuous feedback and instructions during the whole measurement process through both the LCD display and also LED illuminated instruction symbols. First, the device indicates with blinking red arrows that the valves should be turned to the open position (if they are at the correct position the arrows blink green). After setting the valves the user should

introduce the syringe (also prompted by a blinking symbol) and push 50 ml air into the system. This air blows the Zn powder into the measurement chamber. Upon completion of this step, the user should change the syringe and add the 50 ml water sample (also indicated by the blinking symbols). After adding the water, the device asks to close the valves, and the measurement begins. The remaining time from the measurement is indicated by the LCD display, which also displays the measured arsenic concentration at the end.



**Fig. 5.** The main building blocks of the device and the microfluidic cartridge.

## 2.4. Electronics

For the illumination of the measurement area the device uses four WS2812B type RGB LED-s, integrated with driving electronics, which are placed in pairs at the backside of the main PCB, against the camera. They are controlled by the microcontroller through serial, single-wire serial interface. Imaging is done with a camera module containing an Omnivision OV9655 type CMOS sensor. The microcontroller stores the obtained image, finds the outline of the test strip on the digitized picture, determines the RGB color values of the pixels in the defined measurement area and then averages them. The concentration of arsenic in the sample is determined by computing the relative proportion of the blue component, which is compared to an image made on the reference area. The original Hach® test strip was cut out in a way, that only the lower stem of a ‘T shape’ is impregnated with  $\text{HgBr}_2$ , which acts as the measurement area. The upper part of the T shape retains its white color during the whole measurement, hence it is used as the reference area for the calculations.

The interface which communicates with the user consists of the following parts. A 4-digit 7-segment LCD display, which indicates the remaining time during the measurement and also displays the results in  $\mu\text{g/l}$  (ppb). Two LED illuminated symbols called threshold indicators (a red cross and a green check mark), which are alternately blinking during the measurement, then

indicate the drinkability of the water, based on the measured arsenic concentration and the 10 µg/l WHO threshold (below – green check mark, above – red cross). Two LED illuminated arrow symbols, which instruct the user to turn one of the two valves in the indicated direction. Also, LED illuminated indicators, which are situated close to the water inlet port instruct the user to introduce the air and water samples at the appropriate time.

The LCD display only indicates the end-point value of the arsenic concentration at the end of the measurement. The detailed measurement data is stored in a 1 GB SD card, in a .txt file. The measured RGB values of both the measurement and the reference areas are saved with a 30 s sampling rate, for any future evaluation.

To monitor and control the process the microcontroller requires feedback from the status of the mechanical parts, which are actuated by the user. For this purpose both the valve caps and the side of the syringe are coated with a reflexive layer and the position of the valve and the presence of the syringe with the water sample are monitored with a CNY70 type reflective optical sensor, which consists of an LED and phototransistor pair working in the infrared range.

As power supply, the device has a built-in Li-ion battery with 750 mAh capacitance and 3.7 V nominal voltage. The battery can be charged through an external 12 V DC power adapter, or through the built-in solar panel, which is placed atop the device (see Fig. 2). The measurement LED-s require 5 V nominal voltage, which is provided by a boost (step-up) DC-DC converter during battery operation or a buck (step-down) converter during network operation. All the other hardware parts require 3 V nominal voltage, which is provided by a buck converter. To increase battery life, the separate parts of the hardware (including the illuminator LED-s) can be independently switched off by the microcontroller, while they are not used.

In order to define the relation between the relative blue component of the calculated RGB value and concentration of arsenic in the sample, an experimental calibration measurement was carried out. The calibration measurement was performed in triplicates by using the original reaction vessel and original reagents of the Hach® EZ Arsenic Test Kit. The field kit with its reagents, the reference color series and a photograph of the results is shown in Fig. 1.

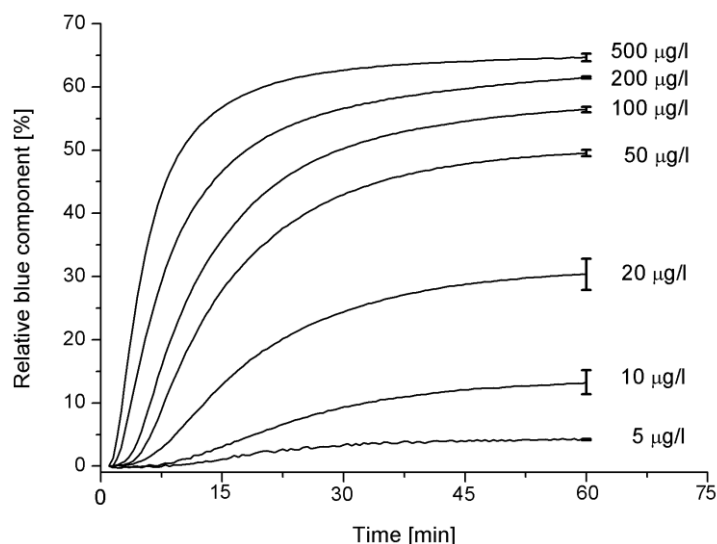
### **3. Results and Discussion**

#### **3.1. Device characteristics**

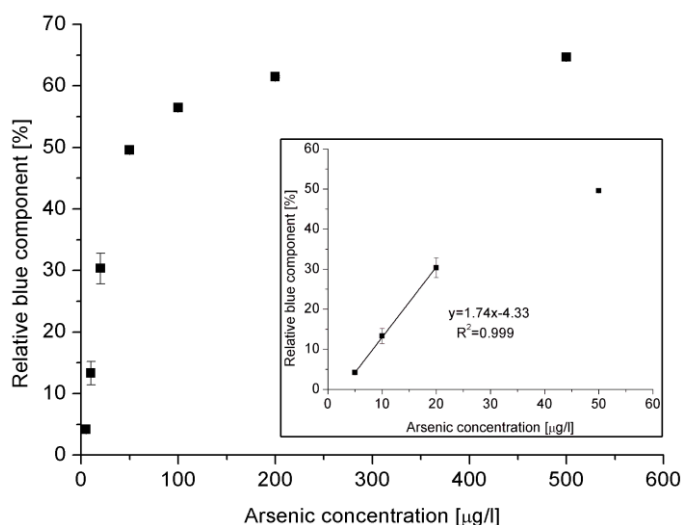
The kinetic response and the static characteristic of our integrated colorimetric device is presented in Figs. 6 and 7, respectively. All of the measurements were done on dilutions prepared by using the As(III) Arsenic Reference Standard Solution from Hach®. As can be seen, our device has a linear response to As(III) concentrations in the 5-20 µg/l (ppb) range (Fig. 7 insert), measured after 60 min, at the stable region of the curves. Considering the standard deviation of the measurements we could say, that the sensitivity of our device is around 1 µg/l with a detection limit of around 3 µg/l (based on the IUPAC definition of  $3\sigma$ ). Although this limit of detection is clearly better than the 10 µg/l, which can be achieved by using the original Hach® field kit, we have to note that maximizing either the sensitivity or the detection limit was not our primary purpose with the device. Our main goal is that it should be capable to distinguish between drinkable and not-drinkable water



based on the World Health Organization (WHO) guidelines of 10  $\mu\text{g/l}$  (ppb) for developed countries and 50  $\mu\text{g/l}$  (ppb) for some of the developing countries, which is possible with the presented characteristics with a satisfactory reliability. A slight drawback of this method is that it takes at least around 60 min to yield reliable results – based on the curves presented in Fig. 6 – in the relevant concentration range at generally occurring ambient temperatures of drinking water (e.g. between 10-25  $^{\circ}\text{C}$ ).



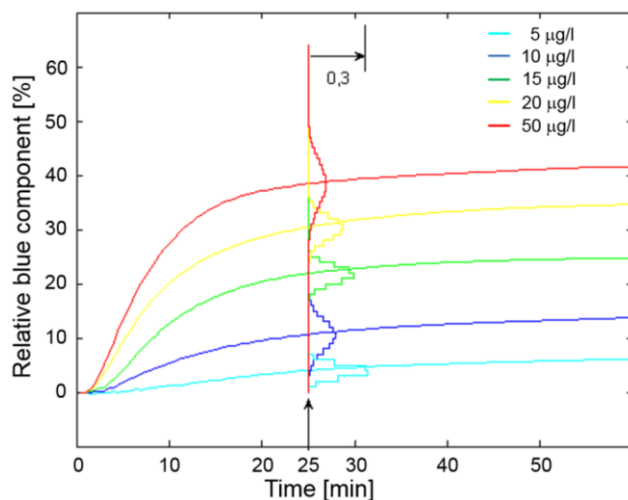
**Fig. 6.** Kinetic curves of the measurements done at laboratory ambient temperature (22  $^{\circ}\text{C}$ ) with various arsenic (As(III)) concentrations. The error bars at 60 min represent the min-max deviation of the results based on 3-5 measurements.



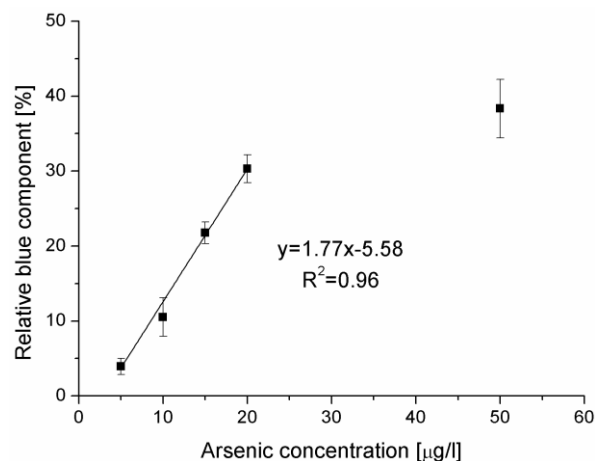
**Fig. 7.** Static characteristic of the colorimetric device. The measurements were done at laboratory ambient temperature (22  $^{\circ}\text{C}$ ) with various arsenic (As(III)) concentrations. The error bars represent the min-max deviation of the results based on 3-5 measurements. Insert: linear fit in the 5-20  $\mu\text{g/l}$  range.

### 3.2. Effect of elevated water temperatures

To reduce the response time of the device, the effect of elevated water sample temperatures was investigated for 25 °C, 40 °C, 50 °C, 60 °C and 80 °C, respectively. Temperature values refer to the temperature of the sample at the beginning of measurements, temperature control during the measurements is not integrated in the developed system. Fig. 8 presents the kinetic curves measured at 50 °C for arsenic concentrations between 5-50 µg/l. By comparing the curves with Fig. 6 we can see, that the curves stabilize significantly faster at this temperature. The plotted distributions in Fig. 8 present the spread of the obtained relative blue component values after 25 min. It can be seen, that the deviation of the obtained values increased with the elevated temperatures compared to the measurements which were performed at ambient temperature. However, based on the static characteristic plotted in Fig. 9 we can still say that the arsenic concentration values can be reliably distinguished by the associated relative blue component values after only 25 min in this elevated temperature of 50 °C. Although higher temperatures enable faster approximation of arsenic concentration, vapor formation becomes significant around and above 60 °C. Due its condensation, an overshoot appears on the kinetic curves, which renders early measurements unreliable and which increases the deviation of the obtained results above 50 °C. Sample preparation and handling also requires more care at higher temperatures. Considering these effects, we do not recommend going above 50 °C with the sample temperature.

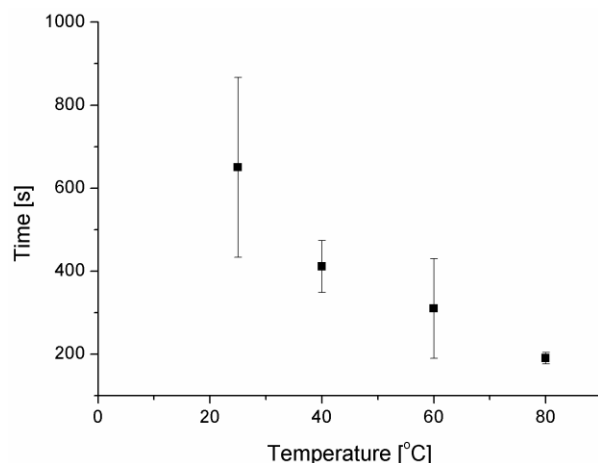


**Fig. 8.** Kinetic curves of the measurements done at 50 °C with arsenic concentrations (As(III)) between 5-50 µg/l. The superposed distributions at 25 min are calculated based on 5-13 measurements.



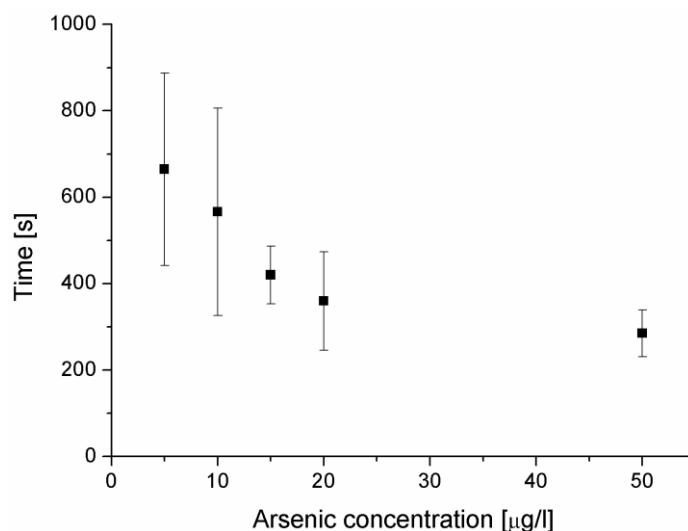
**Fig. 9.** Static characteristic of the colorimetric sensor: relative blue component in function of the arsenic concentration (As(III)) measured after 25 min at 50 °C. Average based on 5-13 measurements.

In order to reduce the measurement time even further, we investigated another concept connected to the elevated water temperatures, namely the possibility to include the determination of the inflexion point of the kinetic curves in the measurement process. In theory, the time needed for the appearance of the inflexion point of the sigmoid like kinetic curves could be reduced by increasing the temperature of the samples. For this purpose sample temperatures of 25 °C, 40 °C, 60 °C and 80 °C were used and the appearance of the inflexion point of the kinetic curves was determined at a fixed arsenic concentration of 20 μg/l. On 80 °C, only three successful measurements were performed due to the frequent leakage of the microfluidic cartridge and the vapor formation due to high temperature. The effect of the elevated water sample temperature on the appearance of the inflexion point of the kinetic curves is shown in Fig. 10. The average time until the appearance of the inflexion point at 60 °C is less than 50 % of that at 25 °C. The time until the appearance of the inflexion point of the kinetic curves at 50 °C in function of the arsenic concentration of the samples (5, 10, 15, 20 and 50 μg/l, respectively) is shown in Fig. 11.



**Fig. 10.** Appearance of the inflexion point of the kinetic curves in function of the sample temperature, measured at 20 μg/l arsenic concentration (As(III)). Average and standard deviation of 6-15 measurements, except at 80 °C with only 3 measurements.

Based on Figs. 10 and 11 the appearance of the inflexion point is a parameter which could be used to approximate the concentration of arsenic in the sample, since higher concentration implies earlier appearance. However, due to its much higher standard deviation (compared to the deviation of the measured end-point of the relative blue component), this parameter is not recommended to be used for the measurement of arsenic concentration. Thus the evaluation of the inflexion point of the curves was only used for experimental purposes and was not implemented in the final construction of the device.



**Fig. 11.** Appearance of the inflexion point of the kinetic curves in function of the arsenic concentration (As(III)) of the sample, measured at 50 °C. Average and standard deviation of 6-13 measurements.

## 4. Conclusions

The construction and the capabilities of a portable colorimetric device, designed specifically for untrained, typically illiterate users, was presented to measure the arsenic concentration of drinking water samples. To decrease the measurement time the effect of elevated water sample temperatures was investigated in the 25-80 °C range. It was found that the time until the appearance of the inflexion point in the kinetic curves correlates with the arsenic concentration, however due to its high deviation it was not included in the evaluation process. It was demonstrated that at an elevated temperature of 50 °C the arsenic concentration of the drinking water samples can be reliably obtained only after 25 min (compared to the original 60 min at ambient temperatures) and that our device has a linear response to arsenic concentrations in the 5-20  $\mu\text{g/l}$  (ppb) range. Based on this our device is capable to determine the approximate arsenic concentration of water samples with calculable probability, and reliably distinguish drinkable water based on the 10  $\mu\text{g/l}$  (and also 50  $\mu\text{g/l}$ ) WHO guideline, according to its primary purpose.

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2012-0051). Attila Bonyár is grateful for the support of the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

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