Method development and validation of a rapid silica-based smart-phone assisted device in the detection of iron in water

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Abstract— A new method of rapidly detecting iron in water using a smartphone assisted device is introduced. Currently, point-of-need devices have largely explored patterned paper as a substrate. While paper is affordable and detection is rapid, its sensitivity is compromised by its fast-wicking nature hence the need for methods of retaining and immobilizing analytes. Silica generally has a higher absorptive power than paper therefore can better immobilize analytes for better detection. The improvement of sensitivity in such devices is important in the monitoring of heavy metals such as iron that can be dangerous to plant and animal life. Testing of such metals not only requires rapid methods of detection but those with low detection limits. The pairing of these devices with smartphones contributes to their rapidity. The aim of this paper is to present findings on the method development and validation of silica-based smart-phone assisted rapid detection of iron in water. Iron standards were prepared in various amounts (0-60 ng) and converted to iron (II). A silica plate was prepared by adding 1,10-phenanthroline that would turn bright orange on reacting with iron (II). A box structure was built around the silica plate to control light and the distance of the light source (smartphone). The standards and reference material were tested by depositing on the treated silica plate before capturing with the smartphone and analyzed using a software called ImageJ. Detection limit, linear range, accuracy and precision were determined. The results showed a limit of detection of 0.2 ng, a limit of quantification of 0.6 ng, a linear range of 0.6 ng to 30.0 ng and RSD of <5%. The results also showed that for silica not only are complex immobilizing reagents not necessary but they in fact, lead to worse precision, accuracy and sensitivity. From this study we conclude that silica-based substrates make a more sensitive detection method while requiring less reagents. The settings of the software were found to be unique to the substrate.

Keywords— Silica, rapid testing, iron detection, ImageJ, heavy metals, method validation

I. INTRODUCTION

Analytical chemistry methods are an important part of everyday life including areas such as medical diagnostics, product development, quality control and environmental monitoring [1], [2]. The ability to carry out tests and analyses in and out of the laboratory is a big part of informed decision making that in many cases can be life-saving.

Currently, the detection of toxic substances like heavy metals is done through Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) and Atomic Absorption Spectroscopy (AAS) among others. These methods have low detection limits,

good sample throughput and are a reliable way of analysis in the laboratory [3]–[5]. However, the cost of using these can be very high. Cate (2016), notes that the cost of these instruments can exceed USD150 for just one sample. Additionally, transportation and sample processing drive up the costs of testing with these instruments [6]. The turnaround time for using such instruments may range from a couple of weeks to a few months [3], [7]–[10]. In the case of environmental monitoring, there is a need for high frequency testing in order to capture what is happening in real time [7], [11]. This means there is a need to increase rapidity of testing. The current methods of analysis consume significant amounts of reagents while requiring highly skilled personnel to operate. These challenges have significant implications on accessibility especially for developing countries and their communities.

In recent years, there has been a growing interest in improving environmental monitoring through lab-on-a-chip and point-of-need devices [3], [7]–[10], [12], [13]. Such devices have been around since the early 90s and researchers have been advancing on their construction and development. Point-of-need devices can use micro litre (or even less) levels of analyte and reagents. They can be rapid in detection and allow for analysis to be carried out at the outside the laboratory. Most have a designated point of introducing a solution, a pre-treatment zone and a detection zone. These zones are usually connected by small channels that have attributes of driving solutions without the need of an external driver such as gas (e.g capillary movement in paper devices). Some of the substrates commonly used in the construction of point-of-need devices include paper, carbon, alumina and silica. The method of detection may be electrochemical, chemiluminescent or colorimetric among many others.

Heavy metals make up some of the most ubiquitous and potentially toxic pollutants globally (Kaur et al. 2019)(WHO). Many of these metals can cause cancer in humans even at low concentrations (WHO). Poisoning of communities and their livestock has been reported in many instances after drinking groundwater laden with some toxic metals especially arsenic, lead, iron and chromium. Iron is one of the most significant heavy metals for its effects to human physiology and concentrations in the environment. As a micronutrient, iron is the major component in the heamoglobin molecule that helps to transport oxygen in the body [14]. Iron at higher concentrations in the environment has been reported to pollute underground

water sources [15]. This poses a threat as water reserves are said to be steadily dwindling, globally [16]. Additionally, iron plays a major role in the release and attenuation of other toxic metals such as arsenic [17]. The rapid detection of this metal in the environment is important.

The aim of this paper is therefore to present findings of method development and validation of a new rapid method of iron analysis. This paper specifically explores silica as a substrate for out-of-the-lab iron analysis in water with the assistance of a smartphone.

II. MATERIALS AND METHODS

A. Chemicals and materials

Analytical grade chemicals were used as is in this study. AAS grade Iron standard 1000ppm, hydroxylamine hydrochloride, 1,10-phenanthroline, poly (acrylic acid), TLC silica plate on aluminium base.

B. Sample Preparations

The samples were prepared in concentrations ranging 0-30 mg/L of Fe from the 1000 ppm standard solution. To each sample was then added 2mL of hydroxylamine hydroxide solutions (5g prepared in 50mL water) to convert all Fe into Fe (II).

C. Fabrication on Silica Plate

On the silica plate, circles representing detection points were drawn using an HB (hard black) lead pencil. 2H (too hard) pencils were damaging to the silica plate. 3 aliquots of 1,10-phenanthroline (0.52g in 100mL water) were deposited on these detection points. The plate was allowed to dry before each deposition. Poly (acrylic acid) was added to detection points for immobilization of the ferroin complex that would be formed from Fe reacting with 1,10-phenanthroline. The silica plate was then ready for rapid detection.

D. Fe detection

The prepared standard samples were deposited in designated detection points in triplicate. That it, one concentration had 3 detection points. The blank and a standard reference material (TraceCERT CRM Iron in water (ISO/IEC 17025)) were also included. A second silica plate that did not have poly (acylic acid) as a ferroin complex immobilizer, was done in a similar manner.

E. Image Processing

Images of the silica plate after color development, were captured using an iPhone 11 Pro® in wide camera mode, aperture f/1.8, 26 mm, 12 MP, 3024 x 4032. The images were also scanned using the smartphone's notes application scanning component for comparison. The images were then sent to a laptop through email to be processed. The image software used was ImageJ, a public domain software developed by the National Institute of Health (US). For use with this software, all images had to be converted to JPEG format. Upon being loaded on the software, images were first inverted to negatives such that the areas outside the color development are black and measure

zero in color intensity as done previously [18] [19]. After inverting, the image was set to an auto color contrast in contrary to color thresholding in previous studies [18] as this gave a clearer image. Color intensity was measured in gray intensity and integrated density for comparison.

III. RESULTS AND DISCUSSION

A. Color Development on Silica Plate

A bright orange color developed upon introducing samples to the prepared silica showing the formation of a ferroin complex. The detection points that were also treated with poly (acrylic acid) to immobilize the complex, resulted in poor color development. This is in contrast to the effect of poly (acrylic acid) in literature [18], [19]. While these studies found that treating paper substrate with poly (acrylic acid) improves the color development, in this study, it did the opposite and made the color development worse (Figure 1). Poly (acrylic acid) is said to have low mobility particularly on paper [20]. It is a weak anion therefore making is highly mobile on silica substrate [1]. This explains its failure to immobilize ferroin complex on silica.

B. Results of Image Processing

Although image processing methodology from literature was followed, results show that the type of substrate has an effect on the output of the software. Figure 2 shows the results of the image of the silica plate when color thresholding as done by [18]. It is observed that this method leads to loud background noise and is not suitable for silica substrate (Figure 2a). A better image was obtained from manually adjusting the color contrast (Figure 2b) with much of the background noise eliminated. The best image resulted from setting the color contrast to "Auto" i.e. allowing the software to correct the color contrast (Figure 2c). Through this setting, the background noise was successfully reduced to zero. This was evidenced by color intensity measurements in non-detection zones recording zero.
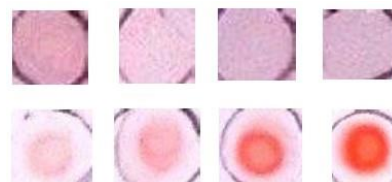

Fig. 1: Detection points of ferroin complex color development with poly (acrylic acid) (top) and without poly (acrylic acid) (bottom)
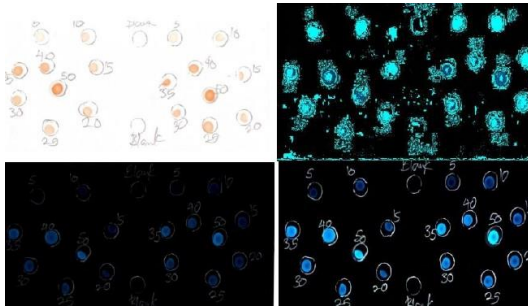
Fig. 2: Images of the silica device after color detection (a), after color thresholding (b), after manual contrast adjustment (c) and after automatic contrast adjustment (d)

C. Color Intensity Measurements

As the software had already showed specificity for type of substrate, the color intensity of the ferroin complex was measured in both mean gray and integrated density. Results show that both measurements successfully measured color intensity on silica substrate. However, there were differences noted in the two methods. Figure 3 shows calibration curves from both methods. Results show no statistical difference (p<0.05), between mean gray and integrated density. Despite this, all measurements of integrated density seemed to have a coefficient of determination (R^2) that is closer to 1 than those measure in mean gray. In other studies mean gray has been the choice of measurement in this software due to the accurate results it yielded [13], [21]–[27]. With scanty information of integrated density as a measurement for color intensity, this study found it equally accurate. A trend was also observed where images higher background noise seem best measured in mean gray while those with lower background are best measured in integrated density. According to literature [28], [29], integrated density captures bright and dim colors more accurately while mean gray "corrects" the dim colors to be brighter and the brighter colors to be dimmer.

Figure 4 presents the calibration curve from the image being scanned instead of photographed. It is clear that scanning produced better images for analysis in the improved R^2 value. This was attributed to how the scanning application automatically adjusts lighting to illuminate shadows on the silica plate. A box enclosure was therefore not as needed.

D. Method Validation

To validate the method, figures of merit were determined and are presented. Table 1 shows the relative standard deviation (RSD) was found to be under 5% in all test samples. A good analytical method is generally considered to have an RSD of less than 20% [30]–[32].
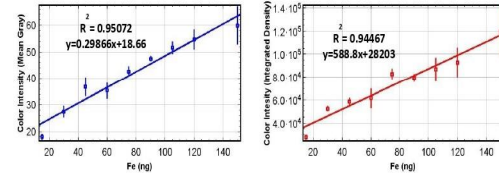

Fig. 3: Color intensity of ferroin complex measured in mean gray and measured in integrated density
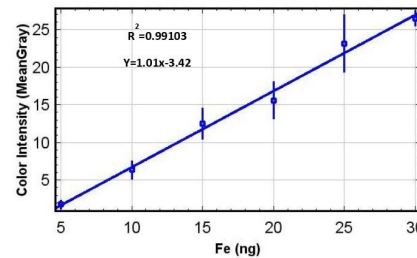

Fig. 4: Fe calibration curve measured in mean gray from scanned silica device

TABLE 1: Relative Standard Deviations of Fe standards (5 mg/L, 15 mg/L and 30 mg/L) in %

Measurement Tool	Calculated RSD in %		
	5 mg/L	*15 mg/L*	*30 mg/L*
Mean Gray	3.14	3.26	0.96
Integrated Density	3.77	2.42	2.96

N=10

TABLE 2: Certified reference material concentration determination with silica device compared to AAS analysis in mg/L

TraceCERT® CRM Iron in water (ISO/IEC 17025) (mg/L)	AAS Analysis (mg/L)	Silica Device analysis (mg/L)
20.00	20.03 ± 0.17	20.13 ± 0.29

N=10

Results show that this method was accurate as measurements of CRM was were statistically not different (p<0.05, n=10) from those of AAS (Table 2). The CRM which was Fe of concentration 20.0 mg/L was measured to be 20.09 ± 0.17 mg/L using AAS and 20.13 ± 0.29 mg/L using the silica device.

The limit of detection and limit of quantification (calculated on equation (1) and equation (2) were determined as 0.2 and 0.6 µg of Fe, respectively. This LOD is less than that previously reported for paper using the same method [33]

$$\left(\frac{3s}{m}\right) = LOD \tag{1}$$

$$\left(\frac{10s}{m}\right) = LOQ \qquad (2)$$

IV. CONCLUSION

The development and method validation of a new smartphone assisted method was successfully done. With an LOQ of 0.6 ng of Fe, good precision (RSD of <5%), accuracy against AAS using certified reference material and a linear range of 0.6 – 30.0 ng of Fe. The developed method proved more sensitive than methods in literature for paper substrate. This study also found that with silica as a substrate, ferroin complex immobilization with poly (acrylic acid) is not necessary. The software ImageJ is unique to the substrate in its settings. Going forward, the same method may be applied to other metals and environmental samples.

REFERENCES

[1] F. W. Fifield and D. Kealey, *Principles and practice of analytical chemistry*. Blackwell Science Hoboken, 2000.

[2] D. A. Skoog, D. M. West, F. J. Holler, and S. R. Crouch, *Fundamentals of analytical chemistry*. Cengage learning, 2013.

[3] N. A. Meredith, C. Quinn, D. M. Cate, T. H. Reilly, J. Volckens, and C. S. Henry, "based analytical devices for environmental analysis," *Analyst*, vol. 141, no. 6, pp. 1874–1887, 2016.

[4] G. Tyler and S. Jobin Yvon, "ICP-OES, ICP-MS and AAS Techniques Compared," *ICP Optical Emission Spectroscopy Technical Note*, vol. 5, 1995.

[5] O. Mestek, M. Loula, A. Kaňa, and M. Vosmanská, "Can ultrafast single-particle analysis using ICP-MS affect the detection limit? Case study: silver nanoparticles," *Talanta*, vol. 210, p. 120665, 2020.

[6] S. Wilbur, "A comparison of the relative cost and productivity of traditional metals analysis techniques versus ICP-MS in High Throughput commercial laboratories," *Agilent Technologies Application Note*, 2005.

[7] A. Jang, Z. Zou, K. K. Lee, C. H. Ahn, and P. L. Bishop, "State-of-the-art lab chip sensors for environmental water monitoring," *Meas Sci Technol*, vol. 22, no. 3, p. 032001, 2011.

[8] C. Dincer *et al.*, "Disposable sensors in diagnostics, food, and environmental monitoring," *Advanced Materials*, vol. 31, no. 30, p. 1806739, 2019.

[9] K. R. Rogers and E. N. Koglin, "Biosensors for environmental monitoring: an EPA perspective," in *Biosensors for Direct Monitoring of Environmental Pollutants in Field*, Springer, 1998, pp. 335–349.

[10] O. A. Sadik, A. K. Wanekaya, and S. Andreescu, "Advances in analytical technologies for environmental protection and public safety," *Journal of Environmental Monitoring*, vol. 6, no. 6, pp. 513–522, 2004.

[11] M. P. Bhat, M. Kurkuri, D. Losic, M. Kigga, and T. Altalhi, "New optofluidic based lab-on-a-chip device for the real-time fluoride analysis," *Anal Chim Acta*, vol. 1159, p. 338439, 2021.

[12] K. Pyrzynska, A. Kubiak, and I. Wysocka, "Application of solid phase extraction procedures for rare earth elements determination in environmental samples," *Talanta*, vol. 154, pp. 15–22, 2016.

[13] S. Balasubramanian *et al.*, "Digital colorimetric analysis for estimation of iron in water with smartphone-assisted microfluidic paper-based analytical devices," *Int J Environ Anal Chem*, pp. 1–18, 2021.

[14] C. Camaschella, "Iron deficiency," *Blood, The Journal of the American Society of Hematology*, vol. 133, no. 1, pp. 30–39, 2019.

[15] R. Jahanshahi and M. Zare, "Assessment of heavy metals pollution in groundwater of Golgohar iron ore mine area, Iran," *Environ Earth Sci*, vol. 74, no. 1, pp. 505–520, 2015.

[16] World Health Organization, *Guidelines for drinking-water quality*, vol. 1. world health organization, 2004.

[17] T. Pal, P. K. Mukherjee, and S. Sengupta, "Nature of arsenic pollutants in groundwater of Bengal basin-a case study from Baruipur area, West Bengal, India," *Curr Sci*, vol. 82, no. 5, pp. 554–561, 2002.

[18] M. M. Mentele, J. Cunningham, K. Koehler, J. Volckens, and C. S. Henry, "Microfluidic paper-based analytical device for particulate metals," *Anal Chem*, vol. 84, no. 10, pp. 4474–4480, 2012.

[19] D. M. Cate, P. Nanthasurasak, P. Riwkulkajorn, C. L'Orange, C. S. Henry, and J. Volckens, "Rapid detection of transition metals in welding fumes using paper-based analytical devices," *Annals of occupational hygiene*, vol. 58, no. 4, pp. 413–423, 2014.

[20] B. Giri, *Laboratory methods in microfluidics*. Elsevier, 2017.

[21] P. Rattanarat, W. Dungchai, D. Cate, J. Volckens, O. Chailapakul, and C. S. Henry, "Multilayer paper-based device for colorimetric and electrochemical quantification of metals," *Anal Chem*, vol. 86, no. 7, pp. 3555–3562, 2014.

[22] X.-L. Guo, Y. Chen, H.-L. Jiang, X.-B. Qiu, and D.-L. Yu, "Smartphone-based microfluidic colorimetric sensor for gaseous formaldehyde determination with high sensitivity and selectivity," *Sensors*, vol. 18, no. 9, p. 3141, 2018.

[23] G.-H. Chen, W.-Y. Chen, Y.-C. Yen, C.-W. Wang, H.-T. Chang, and C.-F. Chen, "Detection of mercury (II) ions using colorimetric gold nanoparticles on paper-based analytical devices," *Anal Chem*, vol. 86, no. 14, pp. 6843–6849, 2014.

[24] A. Motalebizadeh, H. Bagheri, S. Asiaei, N. Fekrat, and A. Afkhami, "New portable smartphone-based PDMS microfluidic kit for the simultaneous colorimetric detection of arsenic and mercury," *RSC Adv*, vol. 8, no. 48, pp. 27091–27100, 2018.

[25] A. K. Ellerbee *et al.*, "Quantifying colorimetric assays in paper-based microfluidic devices by measuring the transmission of light through paper," *Anal Chem*, vol. 81, no. 20, pp. 8447–8452, 2009.

[26] Y. Xia *et al.*, "Smartphone-based point-of-care microfluidic platform fabricated with a ZnO nanorod template for colorimetric virus detection," *ACS Sens*, vol. 4, no. 12, pp. 3298–3307, 2019.

[27] S. Shariati and G. Khayatian, "The colorimetric and microfluidic paper-based detection of cysteine and homocysteine using 1, 5-diphenylcarbazide-capped silver nanoparticles," *RSC Adv*, vol. 11, no. 6, pp. 3295–3303, 2021.

[28]   M. D. Abràmoff, P. J. Magalhães, and S. J. Ram, "Image processing with ImageJ," *Biophotonics international*, vol. 11, no. 7, pp. 36–42, 2004.

[29]   C. A. Schneider, W. S. Rasband, and K. W. Eliceiri, "NIH Image to ImageJ: 25 years of image analysis," *Nat Methods*, vol. 9, no. 7, pp. 671–675, 2012.

[30]   I. C. H. H. T. Guideline, "Validation of analytical procedures: text and methodology," *Q2 (R1)*, vol. 1, no. 20, p. 05, 2005.

[31]   P. Bruce, P. Minkkinen, and M.-L. Riekkola, "Practical method validation: validation sufficient for an analysis method," *Microchimica Acta*, vol. 128, no. 1, pp. 93–106, 1998.

[32]   S. Walfish, "Analytical methods: a statistical perspective on the ICH Q2A and Q2B guidelines for validation of analytical methods," *Biopharm Int*, vol. 19, no. 12, pp. 1–6, 2006.

[33]   D. M. Cate, J. A. Adkins, J. Mettakoonpitak, and C. S. Henry, "Recent developments in paper-based microfluidic devices," *Anal Chem*, vol. 87, no. 1, pp. 19–41, 2015.