Available online at: www. ljcoe.org

## Determination of ammonium in river water using the combination of paper-based analytical device and smartphone app

#### Ahmad Manbohi

Department of Marine Science, Iranian National Institute for Oceanography and Atmospheric Science, Tehran, Iran, Manbohi@inio.ac.ir

#### **ARTICLE INFO**

Article History: Received: 22 Jun. 2022 Accepted: 14 Feb. 2023

#### Keywords:

Paper-based analytical device Smartphone app Ammonium detection On-site analysis River water

#### ABSTRACT

The concentration of ammonium has been greatly increased in freshwaters flowing into the coastal zone. In this paper, the combination of a paper-based analytical device (PAD) and a smartphone application (app) for on-site quantification and mapping of ammonium (NH<sub>4</sub>-N) in river water samples have been described. The developed app can capture, analyze and map the PAD colorimetric outputs. The developed app used an image processing algorithm for analyzing color intensity and relating the RGB elements to the ammonium concentration. The GPS-tagged data can be sent via social networks. Under optimized conditions, our method provided linear range between 0.2 and 10 mg L<sup>-1</sup> with R<sup>2</sup>=0.9998. The limit of detection (LOD) of the method for quantification of ammonium was 0.10 mg L<sup>-1</sup>. The developed technique was effectively applied to determine and map the ammonium concentration in Chalus river samples (Iran). The obtained results were approved by the labbased conventional methods using spectrophotometer.

#### **1. Introduction**

Nutrient contamination is a key driver of harmful algal blooms and eutrophication in coastal waters and estuaries [1]. Although, this contamination is not a new problem, but it is among the most persistent. The main nutrients causing eutrophication are ammonium, orthophosphate and nitrate. Overloading with these nutrients can result in a series of undesirable effects such as hypoxia or anoxia, increasing the amount of organic matter, death of aquatic animals, and reducing the diversity and abundance of benthic invertebrates [2].

The concentration of nutrients such as ammonium has been usually determined using **UV-Vis** spectrophotometry [3]. However. this routine laboratory-based analysis needs large volume of reagents, requires highly trained personnel, and is time consuming and expensive. Furthermore, determination of the nutrients using the laboratory-based analysis is based on several steps including sample collection, transport, storage and analysis of the sample in a laboratory [4, 5].

Paper-based device combined with colorimetric detection using a smartphone is a powerful method capable of on-site quantification of analytes (Dungchai et al. 2009). Paper-based devices have attractive features including disposability, simplicity, rapid analysis, and portability [6-8]. The employment of PAD and smartphone-based app for detection of

ammonium in river waters have not been reported, until now. Herein, a combination of PAD and smartphonebased app have been used as a powerful tool that allowed for the quantification of NH<sub>4</sub>-N in river water samples.

#### 2. Materials and methods 2.1. Chemicals and apparatus

Ammonium chloride was purchased from Merck (Germany). Suitable amount of the ammonium chloride was dissolved in water to obtain 10 mM ammonia stock solution. Working standard solutions of the ammonia were freshly prepared by diluting the stock solution with deionized water. The reagents including tri-sodium citrate dihydrate, disodium EDTA, NaOH, phenol, sodium nitroprusside dehydrate and dichloroisocyanuric acid were purchased from Merck (Germany).

2.2 Design and fabrication of the PADs

Adobe illustrator CS4 was used to design the stamp pattern. A stainless steel stamp comprise 16 circular zones with 0.5-cm diameter was machined using CO<sub>2</sub> laser cutter (Figure 1(a)). Therefore, for each patterning, 16 PADs were constructed. The stamp pattern was used to fabricate PADs for the detection of ammonium in river water samples (Figure 1(b)). The PADs which containing hydrophobic and hydrophilic units were fabricated using wax printing method according to the previously reported method ([7, 9]. Briefly, white wax (Parsin Shimi, Iran) was heated using a hotplate until the wax melted and, then, the temperature being kept at 100 °C. Afterward, Whatman #1 paper was immersed into the wax for 30 s. Next, this paraffined paper was removed from the wax, allowed to solidify at room temperature, and then placed on the nonparaffined (original) paper. To have a repeatable pattern and by using a tape, the paraffined and nonparaffined papers were attached to the laboratory desk. Finally, the preheated stamp was brought in contact with the paraffined-nonparaffined papers. The wax was thermally transferred from the paraffined paper to nonparaffined one.



Figure 1. The stainless steel stamp (a), the produced PADs (b), and the PADs after adding different concentrations of the ammonium (c)

#### 2.3. Development of the new mobile application

A user-friendly customized Android app, termed On-Site Analyzer, was developed to allow efficient on-site ammonium measurement of river water samples, and sharing the results via social networks. To obtain RGB values for colorimetric detection of the analyte in water sample, a photo was taken using a smartphone camera (16 megapixels, Samsung Galaxy A8+) with both autoexposure and auto-focus modes. The distance between the paper-based device and the camera was eight cm. In order to run the ammonium measurements using our platform, the smartphone app was calibrated for ammonium ranging from 0.2 to 10 mg L<sup>-1</sup>. The photo can be taken either via a third-party image capture app on the device or within our app and loaded into our app ('Load Image' option). After selecting and confirming the photo, the analysis process run by pressing the "Measure" button in which each photo was split into RGB values (Figure 2 (a)). In the 8-bit color scale which values ranging from 0 to 255, white corresponds to a color intensity of 255 and black corresponds to zero. The blue channel of the RGB values was the most sensitive channel for NH<sub>4</sub>-N, which presented the highest sensitivity and best linearity.

Although we did not use gray value in this research, because our App is a generic colorimetric detector, the grayscale intensity calculation was added using the conversion from the RGB color space to grayscale intensity:

$$Gray = 0.3R + 0.59G + 0.11B$$
 (1)  
Using the predefined calibration coefficients (a=-  
13.795 and b=174.34), signal value was then  
automatically calculated and converted into the

ammonium concentration (in mg/L) displayed on the screen. After that, the RGB, the calibration coefficients (a and b values of the calibration equation), and image (that was analyzed), name and concentration of the analyte appeared in the next page of the app (Figure 2 (b)). By pressing 'Save' button, final results together with a stamp of time and geographical coordinates will save in the app database (Archive) for further use. By selecting 'Share' button, these data can be sent via social networks. The total time taken for calculating the ammonium concentration using the proposed app is 7 s. To remove or minimize the stray light during image acquisition, a closed chamber with a fixed focal distance and homogeneous LED lighting inside was constructed.



Figure 2. Screen shots of the detection app running on an Android smartphone as an example of using the proposed app: (a) split the captured image into the RGB values and calculating the concentration using the calibration equation; (b) display and share the results

## 2.4. Colorimetric detection of ammonium using PADs-app device

То determine the ammonium concentration, colorimetric method was used according to the MOOPAM standard procedure (with some modifications) [10]. The buffer solution was prepared by mixing 240 g tri-sodium citrate dihydrate, 20 g of disodium EDTA and 0.4 g NaOH in 600 ml distilled water. The phenol solution was prepared by mixing 80 g phenol, 300 ml ethanol, 600 ml distilled water and 600 mg sodium nitroprusside dehydrate. This solution was dilute to 1000 ml. The hypochlorite reagent was prepared by mixing 1 g dichloroisocyanuric acid and 8 g NaOH in distilled water. This solution was dilute to 500 ml.

During ammonium detections, 30 µL of ammonium solution was added to the detection zone. Then, 1.2 uL phenol reagent, 0.6 µL buffer solution and 1.2 µL hypochlorite reagent were add to the detection zone. The volumes of the reagents were optimized (data not shown here). Finally, the reagents on the PADs allowed to dry in a dark container. The method is specific for ammonium and is based on the formation of the blue colored indophenol complex, by phenol and hypochlorite in the presence of the ammonium [10]. In moderately alkaline solution ammonia reacts with hypochlorite to form monochloramine which, in the presence of phenol, catalytic amount of nitroprusside ions and excess hypochlorite, gives indophenol blue (Figure 1 (c)). After incubation for a defined time, the PAD was scanned by our proposed app. The blue channel of RGB was used for ammonium detection. 2.5 Spectrophotometry analytical method

For the reference measurements of ammonium, the Grasshoff method was used for water sampling, stabilization, transportation of the samples to the central laboratory, and storage [11]. To assess ammonium concentration in the river waters, the samplings from the surface waters were performed in triplicate. Colorimetric method was used to analyze the analytes according to the MOOPAM standard methods [10]. An Analytik-jena spectrophotometer was used for reference measurements of ammonium (SPECORD-210). BrandTech cuvettes (Sigma Aldrich, Ireland) were used with optical path lengths of 10 millimeters.

#### 3. Results and discussion

#### **3.1 Fabrication of paper-based device**

Various approaches exist to print a pattern on a paper [12, 13]. Herein, three methods including stampingbased wax printing, wax dipping and patterning by permanent ink pen were tested. To test the efficacy of the permanent pen ink, we drew a circle and let the ink to dry for 5 min. After that, 50 µL of distilled water was spotted inside the pattern. Permanent ink can produce hydrophobic barriers on paper. However, the pattern was not reproducible. The wax dipping approach, as described elsewhere [14], utilizes melted paraffin to cover a hydrophobic part on a paper, while the hydrophilic part is protected by a metal mold. A chromatography paper was introduced between a stainless-steel piece and the complement of pattern. Also, a magnet was put behind the piece. A tinny iron plate was attached to the front side of the pattern complement. This complex was inserted into the melted paraffin for 1 s. However, the producing a pattern was critical and eliminating the complement of pattern from the paper was not reproducible. Also, the approach needs excess of wax.

In the stamping-based process, as described elsewhere [7, 15], a stamp was machined using CO<sub>2</sub> laser in less than 10 min at a local shop. The stainless steel stamp is lightweight, resistant to the air oxidation, durable, and

cheap. Hence, the PADs can be produced using limited resources. To produce PADs, we used chromatography paper and paraffin. Chromatography paper was chosen as the substrate for spotting the reagents due to extensive availability and flexibility. Furthermore, as paraffin (wax) is low cost, resistant to most chemical compounds and white, it was chosen to generate hydrophobic barrier [8, 9]. The produced stamping-based PADs were used as substrate in colorimetric determination of the ammonium in the river water samples (Fig 1).

#### **3.2** Ammonium detection using the developed app

On-site monitoring of water quality has been always an significant challenge for public health departments and water laboratories [<u>16</u>]. For colorimetric determination of an analyte, a photo can be captured and processed by smartphones which show the most potential for on-site analysis. Smartphone-based detection devices are inexpensive, small in size, and portable.

The RGB is the main color space that has been used in the field of on-site detection [<u>17</u>]. It should be noted that due to focal length and pixel differences, the R, G and B values are different in various smartphone models. Therefore, calibration (the concentration relationships of ammonium with respect to the related signals) should be performed for each specific smartphone.

#### **3.3 Figure of merits**

The colorimetry was used for the detection of ammonium. To build a working range for the detection of ammonium, the concentration relationship of ammonium with respect to the related signal was investigated in a wide range (Figure 3). The B values were linear to the ammonium concentrations from 0.2to 10 mg/L with  $R^2$  equal to 0.9998. The regression equation was y = -13.795x + 174.34, where x was concentration of ammonium (mg/L). The LOD was calculated according to the formula  $3s_b/m$ , where  $s_b$  is standard deviation of the blank signal and m is the slope of the calibration curve. The LOD for ammonium was 0.1 mg L<sup>-1</sup>. The repeatability values of the colorimetricbased PADs (three detection zones RSD) for ammonium was found to be 4.5%. Also, to study the reproducibility of the PADs (PAD-to-PAD RSD), three PADs were prepared under the same conditions and used to detect ammonium. The RSD value of 6.2% was obtained. In the experiments, the concentration of ammonium was 2 mg/L. The low RSD values, obtained from repeatability and reproducibility tests, indicate that the developed PADs-app array was a reliable and reproducible system to achieve quantitative determination of ammonium. The linear dynamic range of the proposed PADs-app array cover the possible levels of the ammonium in the river water samples. The figures of merit of the developed PADs-app array were compared with some published methods for the determination of ammonium (Table 1). In one reported method, scanner was used for image capturing which,

then, the images were processed using a computerbased software such as ImageJ or Adobe Photoshop [<u>18</u>]. Conversely, in our developed PADs-app array, a user-friendly app was developed to allow on-site and GPS-tagged ammonium determinations of river waters, and share the results via social networks. As it can be seen, our developed PADs-app array can be used in wide linear ranges. Furthermore, the LOD of our method is comparable with the reported ones.



Figure 3. Calibration curve of ammonium obtained by using PAD-app array

#### 3.4 Selectivity of the PADs-app array

Although one of the worthy features of the ammonium colorimetric reaction is the high selectivity [10, 11, 19], the selectivity of our developed PADs-app array for the colorimetric determination of ammonium was further investigated (Figure 4). To test the ammonium PADsapp array selectivity over interferences, potential interfering species including phosphate, sulfide, silicate, and chloride were tested as negative controls. As illustrated in Figure 4, the ammonium response is showing obvious difference from other species and the developed PADs-app array response for ammonium is meaningfully higher. Furthermore, by the addition of a complexing reagent (citrate), the precipitation of calcium and magnesium hydroxides in the solution is avoided. This complexing reagent keeps the calcium and magnesium ions in solution. Also, for any given concentration of ammonium, the blue color produced in seawater is less intensive than in distilled water [10]. Thus, for each sample a correction has to be made with respect to its salinity and the resulting pH. In many situations, a simple correction may be used where the correction is given by [10]:

$$NH_{3(corr)} = [1 + 0.0073S_s]NH_{3(uncorr)}$$
(2)

where  $S_s$  is the salinity of the sample. However, as the salinity of our river water samples was low, the difference between uncorrected and corrected concentrations was negligible.



Figure 4. Selectivity of PAD-app array toward ammonium detection. The concentration of ammonium, phosphate, silicate, chloride, and sulfide were 1.5 mg/l, 59  $\mu$ g/l, 0.283 mg/l, 10 mg/l, and 1 mg/l, respectively

### **3.5** The cost and time consuming in the PADs-app array analysis

Small volume size, low fabricating cost and low power consumption are three essential features for the PAD devices [20]. Our developed on-site PADs-app array is auicker and consumes fewer reagents than conventional method using spectrophotometer. Also, as small amounts of reagents consumed in this approach, the final price of a PADs-app array will be significantly cut down. The estimated price for each PAD (price of the reagents, fabrication of PAD, and a closed box with two LEDs) is about 1 US dollar, which is lower than standard method using spectrophotometer.

Table 1.	Comparison of	f our proposed	PAD-app arra	y with other assays	s for the dete	ection of ammonium
	1	1 1	11			

Method	Matrix	Linearity (mg/L)	LOD (mg/L)	Main features	Ref.
Compact hydrodynamic sequential injection system	River water	0.30–4	0.27	Determination of	[25]
				ammonium in laboratory	
Capillary zone electrophoresis with direct UV	River water	0-5	0.24	Determination of	[26]
detection				ammonium in laboratory	
Miniaturized flow-injection-analysis (µFIA) system	River water	0-0.50	0.10	Determination of	[27]
with on-line chemiluminescence detection				ammonium in laboratory	
PAD-camera phone- office scanner-ImageJ software	industrial	0-0.50	3.34	Determination of	[18]
	wastewater			ammonium in laboratory	
PAD-Smartphone based app	River water	0.20-10	0.10	On-site determination of	Proposed
				ammonium	method

# **3.6 Case study: Determination of ammonium in Chalus river water (Iran) using the PADs-app array** The Caspian Sea is the largest lake in the world [21, 22]. Chalus river is the major river of central–northern

Iran. It originates in the Kandovan and Taleghan mountains and passes through the city of Chalus in Mazandaran Province. The river flows northward through the central Alborz mountain range, to its river mouth on the southern Caspian Sea [23].

High level of ammonium in coastal waters can be a sign of eutrophication [24]. Since our developed PADs-app array had good selectivity and sensitivity for the determination of ammonium, it was used to detect the ammonium in river water samples. The surface water samples were collected from Chalus river waters at 4 different locations. After that, the samples were detected on-site by the proposed PADs-app array (for more information, see section "Colorimetric detection of ammonium using PADs-app device") (Figure 5). The ammonium concentrations were in the ranges of 1.21-3.95 mg L<sup>-1</sup>, which were in good agreement with the obtained ammonium levels using conventional spectrophotometric method (Table 2). The recovery percent for ammonium were 95-106, which shows that the determination of ammonium can be accomplished without any significant effect from the matrixes. Remarkably, by using the proposed PADs-app array, the results can also be shared via social networks.



Figure 5. On-site determination of ammonium using the PAD-App. The device comprises a closed chamber which contain the PAD and reagents; electronic section of the device; and the smartphone. Table 2. Performance of the proposed method for the detection of ammonium from the Chalus river water samples

in arter stamping				
Station Latitude		Reference Method	PAD-app	
	Longitude	(Spectrophotometry)	Method	

А	36.65937,	1.18±0.41	1.21±0.54
	51.43655		
В	36.65230,	1.90±0.18	1.85±0.32
	51.42221		
С	36.64763,	4.04±0.22	3.95±0.64
	51.41227		
D	36.63672,	1.71±0.42	1.62±0.17
	51.40958		

#### 4. Conclusion

In this work, ammonium was detected using a selective colorimetric PADs-app array. An Android-based app was developed to on-site capture, process, and measure the PAD colorimetric outputs. The linear dynamic ranges observed for our proposed method covered the possible relevant levels of ammonium in river waters. The developed method was successfully applied for ammonium detection in river waters samples. The upper limit of the manufacturing cost was approximately 1 \$ per a PAD. Overall, compared with other method, our findings demonstrated that the designed PADs-app array is low cost and has several advantages including simplicity, wide dynamic linear range, high selectivity and short detection time. Furthermore, compared with the lab-based conventional colorimetric method, which used several milliliters of reagents, the proposed PADs-app array used only a few microliters of reagents. Also, there is no need for transport, storage and subsequent analysis of the sample in laboratory.

#### Acknowledgement

I gratefully acknowledge the Iranian National Institute for Oceanography and Atmospheric Science (INIOAS) for supporting this project.

#### **5. References**

- K. Davidson, R.J. Gowen, P.J. Harrison, L.E. Fleming, P. Hoagland, G. Moschonas, (2014) Anthropogenic nutrients and harmful algae in coastal waters, Journal of Environmental Management, Vol. 146, p. 206-216.
- [2] P. Zhang, C. Peng, J. Zhang, J. Zhang, J. Chen, H. Zhao, (2022) Long-Term Harmful Algal Blooms and Nutrients Patterns Affected by Climate Change and Anthropogenic Pressures in the Zhanjiang Bay, China, Vol. 9, p.
- [3] A. Manbohi, S. Gholamipour, (2020) Utilizing chemometrics and geographical information systems to evaluate spatial and temporal variations

of coastal water quality, Regional Studies in Marine Science, Vol. 34, p. 101077.

- [4] G. Duffy, (2017) Development and optimisation of colourimetric microfluidic sensors for water quality monitoring, School of Chemical Sciences, Dublin City University, Dublin.
- [5] D.W. Schindler, (2012) The dilemma of controlling cultural eutrophication of lakes, Proceedings of the Royal Society B: Biological Sciences, Vol. 279, p. 4322-4333.
- [6] Y. Jiang, Z. Hao, Q. He, H. Chen, (2016) A simple method for fabrication of microfluidic paper-based analytical devices and on-device fluid control with a portable corona generator, RSC Advances, Vol. 6, p. 2888-2894.
- [7] A. Manbohi, S.H. Ahmadi, (2019) Sensitive and selective detection of dopamine using electrochemical microfluidic paper-based analytical nanosensor, Sensing and Bio-Sensing Research, Vol. 23, p. 100270.
- [8] A. Manbohi, S.H. Ahmadi, (2020) Chitosan–Fe3O4 nanoparticle enzymatic electrodes on paper as an efficient assay for glucose and uric acid detection in biological fluids, Chemical Papers, Vol. 74, p. 2675-2687.
- [9] P. de Tarso Garcia, T.M. Garcia Cardoso, C.D. Garcia, E. Carrilho, W.K. Tomazelli Coltro, (2014) A handheld stamping process to fabricate microfluidic paper-based analytical devices with chemically modified surface for clinical assays, RSC Advances, Vol. 4, p. 37637-37644.
- [10]ROPME, (2010) MOOPAM, Fourth Edition, Kuwait: Regional Organization for the Protection of the Marine Environment, Kuwait.
- [11]K.K. Klaus Grasshoff, Manfred Ehrhardt (1999) Methods of seawater analysis., 3rd ed,
- [12]D.M. Cate, J.A. Adkins, J. Mettakoonpitak, C.S. Henry, (2015) Recent Developments in Paper-Based Microfluidic Devices, Analytical Chemistry, Vol. 87, p. 19-41.
- [13]N.A. Meredith, C. Quinn, D.M. Cate, T.H. Reilly, J. Volckens, C.S. Henry, (2016) Paper-based analytical devices for environmental analysis, Analyst, Vol. 141, p. 1874-1887.
- [14]T. Songjaroen, W. Dungchai, O. Chailapakul, W. Laiwattanapaisal, (2011) Novel, simple and lowcost alternative method for fabrication of paperbased microfluidics by wax dipping, Talanta, Vol. 85, p. 2587-2593.
- [15]T. Akyazi, L. Basabe-Desmonts, F. Benito-Lopez, (2018) Review on microfluidic paper-based analytical devices towards commercialisation, Analytica Chimica Acta, Vol. 1001, p. 1-17.
- [16]S. Srivastava, S. Vaddadi, S. Sadistap, (2018) Smartphone-based System for water quality analysis, Applied Water Science, Vol. 8, p. 130.
- [17]S. Ozeh, A.G.A. Nnanna, J.C. Ndukaife, (November 9–15, 2018) Smartphone-Based Device

for Monitoring Chemical Pollutants in Water, Proceedings of the ASME 2018 International Mechanical Engineering Congress and Exposition, ASME, Pittsburgh, Pennsylvania, USA, p. V003T004A021.

- [18]N.L. Nxumalo, L.M. Madikizela, H.G. Kruger, S.C. Onwubu, Mdluli, P. Selby, (2020) Development of a paper-based microfluidic device for the quantification of ammonia in industrial wastewater %J Water SA, Vol. 46, p. 506-513.
- [19]K. Grasshoff, K. Kremling, M. Ehrhardt, (1999) Determination of nutrients, Methods of Seawater Analysis, p. 159-228.
- [20]K. Xu, Q. Chen, Y. Zhao, C. Ge, S. Lin, J. Liao, (2020) Cost-effective, wireless, and portable smartphone-based electrochemical system for onsite monitoring and spatial mapping of the nitrite contamination in water, Sensors and Actuators B: Chemical, Vol. 319, p. 128221.
- [21]S. Ataei, A, Jabari, A. M. Khakpour, M. Adjami, S. A. Neshaei, (2018) Investigation of Caspian Sea Level Fluctuations Based on ECMWF Satellite Imaging Models and Rivers Discharge, International Journal of Coastal & Offshore Engineering, Vol. 3, p. 21-30.
- [22]S. Eskandari, D. Mansouryei, (2022) Spatial and temporal variations of the electrical conductivity and magnetic field of the Caspian Sea using Princeton Ocean Model, International Journal of Coastal & Offshore Engineering, Vol. 7, p. 31-42.
- [23]<u>https://en.wikipedia.org/wiki/Chalus\_River</u>, (2022) Chalus River, Wikipedia.
- [24]Gunni Ærtebjerg, (2001) Eutrophication in Europe's coastal waters, European Environment Agency, p. 86.
- [25]W. Khongpet, S. Pencharee, C. Puangpila, S.K. Hartwell, S. Lapanantnoppakhun, J. Jakmunee, (2019) A compact hydrodynamic sequential injection system for consecutive on-line determination of phosphate and ammonium, Microchemical Journal, Vol. 147, p. 403-410.
- [26]K. Fukushi, H. Ito, K. Kimura, K. Yokota, K. Saito, K. Chayama, S. Takeda, S.-i. Wakida, (2006) Determination of ammonium in river water and sewage samples by capillary zone electrophoresis with direct UV detection, Journal of Chromatography A, Vol. 1106, p. 61-66.
- [27]B.R.M. Al-Gailani, G.M. Greenway, T. McCreedy, (2007) Miniaturized flow-injection-analysis (μFIA) system with on-line chemiluminescence detection based on the luminol-hypochlorite reaction for the determination of ammonium in river water, International Journal of Environmental Analytical Chemistry, Vol. 87, p. 425-436.

#### INTERNATIONAL JOURNAL OF COASTAL, OFFSHORE & ENVIRONMENTAL ENGINEERING