Determination of Iron Ion in the Water of a Natural Hot Spring Using Microfluidic Paper-based Analytical Devices

Kazuma OGAWA and Takashi KANETA[†]

Department of Chemistry, Graduate School of Natural Science and Technology, Okayama University, Okayama 700-8530, Japan

Microfluidic paper-based analytical devices (μ PADs) were used to detect the iron ion content in the water of a natural hot spring in order to assess the applicability of this process to the environmental analysis of natural water. The μ PADs were fabricated using a wax printer after the addition of hydroxylamine into the detection reservoirs to reduce Fe^{3+} to Fe^{2+} , 1,10-phenanthroline for the forming of a complex, and poly(acrylic acid) for ion-pair formation with an acetate buffer (pH 4.7). The calibration curve of Fe^{3+} showed a linearity that ranged from 100 to 1000 ppm in the semi-log plot whereas the color intensity was proportional to the concentration of Fe^{3+} and ranged from 40 to 350 ppm. The calibration curve represented the daily fluctuation in successive experiments during four days, which indicated that a calibration curve must be constructed for each day. When freshly prepared μ PADs were compared with stored ones, no significant difference was found. The μ PADs were applied to the determination of Fe^{3+} in a sample of water from a natural hot spring. Both the accuracy and the precision of the μ PAD method were evaluated by comparisons with the results obtained *via* conventional spectrophotometry. The results of the μ PADs were in good agreement with, but less precise than, those obtained *via* conventional spectrophotometry. Consequently, the μ PADs offer advantages that include rapid and miniaturized operation, although the precision was poorer than that of conventional spectrophotometry.

Keywords Microfluidic paper-based analytical device, environmental analysis, iron ion, hot spring

(Received July 6, 2015; Accepted September 1, 2015; Published January 10, 2016)

Introduction

Metal ions are a major pollutant of the global environment since they frequently accumulate in plants¹ and fish,² which results in overconsumption by the animals that feed on them. Metal ions also exist in particulate matter in the air,³ and are produced by industrial and life activity. Therefore, the monitoring of metal ions is an important task for protecting and improving the global environment.

In the analysis of metal ions, atomic absorption spectrometry, inductively coupled plasma optical emission spectrometry, and inductively coupled plasma mass-spectrometry are employed conventionally for routine analysis since they are highly selective and sensitive.³ However, operations that use these instruments are, in general, limited to the laboratory so that samples must be carefully taken and stored in the field with appropriate pretreatment, such as the addition of acid, the use of clean bottles, and cooling. Thus, proper on-site measurement simplifies the sampling process and prevents contamination of the samples.

Recently, microfluidic paper-based analytical devices (μ PADs) have attracted a great deal of interest in several fields including bioanalysis and environmental analysis due to their simple structure, easy fabrication, weight reduction, inexpensive materials, and rapid analysis.^{4,5} μ PADs have shown promise for

their use in point-of-care testing during bioanalysis, as described in many publications.^{6–16} When used in environmental analysis, μ PADs have been successfully applied to the measurement of metal ions in particulate matters (PMs),^{17–19} as well as being useful for measuring the oxidative activity of PMs.²⁰ We also reported the rapid use of μ PADs in acid-base titrations during the on-site analysis of acidic water in a natural hot spring.²¹

During the environmental analysis of PMs, Henry's group used the high sensitivity of μ PADs to determine that metal ions are major components.^{17–19} The degree of precision in that research was relatively poor, however, with relative standard deviations in the determination of iron ion (Fe^{3+}) that were 6 to 12% for standard solutions and 26% for a practical sample.¹⁷

In the present study, we evaluated the precision and accuracy of μ PADs for the determination of Fe^{3+} in order to apply them to the analysis of the water in a natural hot spring. We determined the concentration of Fe^{3+} in the hot spring water using the μ PADs, and compared the results with those obtained by conventional spectrophotometry.

Experimental

Materials

All reagents were of analytical grade and were used without further purification. All solutions were prepared with deionized water purified by means of an Elix water purification system (Millipore Co., Ltd., Molsheim, France). Sodium acetate, 1,10-phenanthroline (phen) monohydrate, hydroxylamine, and

[†] To whom correspondence should be addressed.
E-mail: kaneta@okayama-u.ac.jp

an iron standard solution (1000 ppm) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Acetic acid was obtained from Kanto Chemical (Tokyo, Japan). Nitric acid was purchased from Mitsubishi Chemical (Tokyo, Japan). A poly(acrylic acid) solution (average $M_w \sim 250000$, 35 wt% in water) was obtained from Sigma-Aldrich (St. Louis, MO). A stock solution of 6.3 M acetate buffer (pH 4.7) was prepared by dissolving appropriate amounts of acetic acid and sodium acetate in water. Solutions of hydroxylamine and phen were prepared by dissolving appropriate amounts in the acetate buffer to concentrations of 0.1 g mL^{-1} and 16 mg mL^{-1} , respectively. A 5.6 mg mL^{-1} poly(acrylic acid) solution was prepared by diluting the 35% solution with water. For conventional spectrophotometry, 5% hydroxylamine, 5.4 mM phen, and 1 M acetate buffer were prepared with deionized water.

μ PAD fabrication

The μ PADs used in the present study were fabricated according to a procedure reported by Henry's group.¹⁷ The pattern of the μ PAD was drawn using Microsoft Office Power Point 2013 so as to locate a sample reservoir at the center with four detection reservoirs arranged radially in a $30 \times 30 \text{ mm}$ square. The μ PADs were printed on a sheet of filter paper ($200 \times 200 \text{ mm}$, Chromatography Paper 1CHR, Whatman™, GE Healthcare Lifesciences, UK) using a wax printer²² (ColorQube 8570DN, Xerox, Norwalk, CT), followed by heating at 150°C for 2 min in a drying machine (ONW-300S, AS ONE Corp.). The backside of the printing surface was covered with clear packing tape to prevent the solution from leaking out underneath the μ PAD. The μ PADs were prepared by applying appropriate volumes of reagent solutions to the detection reservoirs *via* a microsyringe or a micropipette; $1 \mu\text{L}$ of 0.1 g mL^{-1} hydroxylamine and $0.3 \mu\text{L}$ of 5.6 mg mL^{-1} poly(acrylic acid) were added to the four detection reservoirs, followed by two $0.2\text{-}\mu\text{L}$ aliquots of 16 mg mL^{-1} phen in 6.3 M acetate buffer (pH 4.7). The μ PAD was dried completely between each addition of reagent solution.

Analytical procedure

In the analysis, $25 \mu\text{L}$ of a sample solution was introduced into the center of the μ PAD using a micropipette. After all the channels and reservoirs were filled with the sample solution, the μ PAD was dried, and then scanned (CanoScan LiDE 500F, Canon, Tokyo, Japan) to obtain electronic images. The color intensity of each detection reservoir in the scanned images was measured using the public domain software ImageJ (National Institutes of Health).¹⁷ In general, each sample was introduced into 5 μ PADs printed on a piece of paper so that the color intensities of the 20 reservoirs could be averaged for the sample. The average intensity was plotted against the concentrations of Fe^{3+} and their logarithms in order to construct the calibration curve. A schematic illustration of the experimental procedure including fabrication of the μ PADs and operations is shown in Fig. 1(A).

Analysis of the water from a natural hot spring

An acidic water sample from a natural hot spring was collected at the source of the Tsukahara Onsen hot spring, Yufu, Oita, Japan. Water was taken from a pool near a spa (40°C) and was mixed with 2 M HNO_3 at a volume ratio of 1:1 to avoid the precipitation of Fe^{3+} .

In the spectrophotometric measurements, the hot spring water sample was diluted 10-fold with water. The diluted sample and the standard solutions containing 1, 3, 5, 7, and 10 ppm of Fe^{3+} were reacted with phen as follows: 10-mL aliquots of the sample

Fig. 1 Schematic illustration of the experiments. (A) Fabrication and operation of the μ PADs. (B) Measurements of a standard solution by the μ PADs. Sample concentrations: 100 ppm Fe^{3+} , sample volume; $25 \mu\text{L}$ for each device. The experimental conditions are given in the text.

solution and the standard solutions were taken into 25 mL volumetric flasks, and then 5 mL of 5% hydroxylamine and 5 mL of 5.4 mM phen were added to each volumetric flask. Finally, the volume was adjusted to 25 mL with 1 M of acetate buffer (pH 4.7). Absorbance at 510 nm was measured by the spectrophotometer using a 1-cm cuvette.

Results and Discussion

Calibration curve

Initially, we constructed calibration curves of Fe^{3+} to assess the sensitivity and precision in the determination of Fe^{3+} using the μ PADs. The results for a standard solution of 100 ppm Fe^{3+} are shown in Fig. 1(B). The average color intensity for the 20 detection reservoirs in Fig. 1(B) was measured at $(16.17 \pm 0.73) \times 10^4$, which would be acceptable for practical analysis since the relative standard deviation was less than 5%.

The relationship between the concentration of Fe^{3+} and the color intensities ranging from 20 to 1000 ppm is also shown in Figs. 2(A) and 2(B). The relationship between the logarithm of the concentration and the color intensity was linear and ranged from 100 to 1000 ppm (Fig. 2(A)). The dependence of the color intensity on the concentration also was linear and ranged from 40 to 350 ppm with a correlation coefficient (R^2) of 0.9945, while the saturation of the color intensity was above 350 ppm (Fig. 2(B)). The limit of quantification (LOQ) was estimated at 40 ppm, which was the minimum concentration that could be distinguished by the image processing software when the threshold of the color intensity was adjusted to the minimum

Fig. 2 Calibration curves of Fe^{3+} . (A) Semi-log plot, (B) linear plot. The experimental conditions are given in the text. Error bars show the standard deviations.

value of zero, which was the intensity of the blank. The LOQ was comparable to that reported by Mentele *et al.*¹⁶ ($1.5 \mu\text{g}$ in $30 \mu\text{L}$, 50 mg L^{-1}) while Asano *et al.* found a lower LOQ ($13.8 \mu\text{M}$, 0.771 mg L^{-1}) using a paper device with test spots where no diffusion happened since a single spot was used as a reservoir.²³ Thus, the spot-type paper device was preferable for enhancing the sensitivity, although the throughput was lowered since a sample had to be added to several spots for multiple measurements, which meant that Asano's device took four-times longer than either Mentele's or ours during the respective sample introductions. In terms of the sample volume, Asano *et al.* needed $20 \mu\text{L}$ for a single measurement, whereas we used $25 \mu\text{L}$ of a sample to acquire four types of data ($6.25 \mu\text{L}$ for a single measurement), which was similar to Mentele's device that required $30 \mu\text{L}$ for a device with four detection reservoirs. Therefore, the sample consumption of our device was comparable to Mentele's device and was much lower than Asano's device.

To evaluate the stability of the μPADs , intraday and day-by-day precisions were assessed by successive measurements during four days. In the measurements, we employed two types of μPADs each day; one type was freshly prepared and the other was prepared on the first day then stored in the dark. The calibration curves obtained by the freshly prepared μPADs and the stored μPADs are shown in Table 1 (the standard solutions contained 40, 100, 200, and 350 ppm Fe^{3+}). The calibration curves varied considerably from day to day whereas the slopes between the freshly prepared μPADs and the stored μPADs showed fewer significant differences during the day. For example, the relative difference in the slopes was -0.4% for the third day and 0.8% for the fourth day, although it was slightly

Table 1 Reproducibility of calibration curves for Fe^{3+}

	Freshly-prepared	Stored
1st day	$y = 0.142x - 6.238$ ($R^2 = 0.987$)	—
2nd day	$y = 0.194x - 1.587$ ($R^2 = 0.999$)	$y = 0.175x - 6.96$ ($R^2 = 0.997$)
3rd day	$y = 0.269x + 1.474$ ($R^2 = 0.997$)	$y = 0.270x - 11.891$ ($R^2 = 0.994$)
4th day	$y = 0.247x - 1.6245$ ($R^2 = 0.974$)	$y = 0.245x - 10.710$ ($R^2 = 0.980$)

R is the correlation coefficient.

Table 2 The concentration of Fe^{3+} in the hot spring water sample

	Spectrophotometry ($n = 3$), ppm	μPAD ($n = 4$) ^a , ppm
Mean	107.8	112.1
Standard deviation	2.3	11.3
Confidence interval (95%)	± 2.6	± 11.1

a. The sample was determined in four successive days in which the calibration curve was constructed each day. Five μPADs consisting of four detection reservoirs were employed for the measurement of the sample and the mean value was obtained using the results of 20 detection reservoirs. Thus, the number of measurements represents the number of days to measure the sample.

larger for the second day (9.8%). Conversely, the slopes for different days varied from 0.142 to 0.269 (89%) in the freshly prepared μPADs and from 0.175 to 0.270 (54%) in the stored μPADs . It should be noted that the color intensities of the freshly prepared μPADs were always greater than those of the stored μPADs , which may imply that the μPADs were degraded slightly, even when stored in the dark. These results suggest that the calibration curve must have been constructed on the day the samples were determined.

To optimize the experimental conditions, we measured 60, 200, and 500 ppm Fe^{3+} solutions at temperatures of 30, 40, 50, 60, 70, and 80°C . We also measured the absorbance of a $\text{Fe}(\text{phen})_3^{2+}$ complex at 0, 20, and 80°C using absorption spectrometry. In both methods, the differences in the absorbance, or the color intensity, of the $\text{Fe}(\text{phen})_3^{2+}$ complex was almost independent of temperature change. These results are quite reasonable in terms of the thermodynamic parameters of the complex formation between Fe^{2+} and phen. Calorimetry²⁴ showed the enthalpy and entropy of the complex formation to be $-28.5 \text{ kcal mol}^{-1}$ and $-15.4 \text{ cal mol}^{-1} \text{ K}^{-1}$, and the stability constants of the complex ($\log \beta_3$) were 22.96 at 1°C and 17.07 at 80°C , respectively. These constants were so large that the complex formation took place quantitatively even at a high temperature. Therefore, there was no need to control the temperature in the measurement of Fe^{3+} , and the measurements could be carried out at room temperature.

Analysis of the water from a natural hot spring

To demonstrate the applicability of μPADs to the analysis of natural water, the Fe^{3+} in a sample of water from a natural hot spring was determined at room temperature and the results were compared with those from conventional spectrophotometry, as shown in Table 2. Assuming a 95% confidence interval, the concentration was determined to be $107.8 \pm 2.6 \text{ ppm}$ by

conventional spectrophotometry and 112.1 ± 11.1 ppm by the μ PADs. Therefore, the results of the μ PADs were comparable to those from conventional spectrophotometry, although the precision of the μ PADs was slightly poorer. These results indicate that the μ PADs would be advantageous based on facile and rapid operation, which makes them suitable for rough estimations of concentration.

Conclusions

We assessed the precision and accuracy of μ PADs when used in the determination of Fe^{3+} . It was clear that calibration curves must be constructed for each measurement since the slope showed day-by-day fluctuations. We also demonstrated that the μ PADs were applicable to the determination of Fe^{3+} in the water of a natural hot spring. The μ PADs were useful for a simple assay of the Fe^{3+} in natural water samples because of easy operation, lightness and affordability, although conventional spectrophotometry provides a more precise and sensitive determination.

Acknowledgements

This research was supported by The Yakumo Foundation for Environmental Science and Grants-in-Aid for Scientific Research, Scientific Research (B) (No. 26288067) from the Japan Society for the Promotion of Science.

References

1. P. C. Nagajyoti, K. D. Lee, and T. V. M. Sreekanth, *Environ. Chem. Lett.*, **2010**, 8, 199.
2. M. I. Castro-Gonzalez and M. Mendez-Armenta, *Environ. Toxicol. Pharmacol.*, **2008**, 26, 263.
3. G.-C. Fang, Y.-S. Wu, S.-H. Huang, and J.-Y. Rau, *Atmos. Environ.*, **2005**, 39, 3003.
4. A. W. Martinez, S. T. Phillips, M. J. Butte, and G. M. Whitesides, *Angew. Chem., Int. Ed.*, **2007**, 46, 1318.
5. D. D. Liana, B. Raguse, J. J. Gooding, and E. Chow, *Sensors*, **2012**, 12, 11505.
6. A. K. Yetisen, M. S. Akram, and C. R. Lowe, *Lab Chip*, **2013**, 13, 2210.
7. D. M. Cate, J. A. Adkins, J. Mettakoonpitak, and C. S. Henry, *Anal. Chem.*, **2015**, 87, 19.
8. A. W. Martinez, S. T. Phillips, G. M. Whitesides, and E. Carrilho, *Anal. Chem.*, **2010**, 82, 3.
9. H. Noh and S. T. Phillips, *Anal. Chem.*, **2010**, 82, 8071.
10. S. Li, J. A. Hagen, and I. Papautsky, *Lab Chip*, **2012**, 12, 4240.
11. K. M. Schilling, D. Jauregui, and A. W. Martinez, *Lab Chip*, **2013**, 13, 628.
12. G. G. Lewis, J. S. Robbins, and S. T. Phillips, *Anal. Chem.*, **2013**, 85, 10432.
13. J. I. Hong and B.-Y. Chang, *Lab Chip*, **2014**, 14, 1725.
14. R. Gerbers, W. Foellscher, H. Chen, C. Anagnostopoulos, and M. Faghri, *Lab Chip*, **2014**, 14, 4042.
15. K. Yamada, S. Takaki, N. Komuro, K. Suzuki, and D. Citterio, *Analyst*, **2014**, 139, 1637.
16. M. M. Mentele, J. Cunningham, K. Koehler, J. Volckens, and C. S. Henry, *Anal. Chem.*, **2012**, 84, 4474.
17. P. Rattanasarat, W. Dungchai, D. M. Cate, W. Siangproh, J. Volckense, O. Chailapakul, and C. S. Henry, *Anal. Chim. Acta*, **2013**, 800, 50.
18. J.-Y. Sun, C.-M. Cheng, and Y.-C. Liao, *Anal. Sci.*, **2015**, 31, 145.
19. D. M. Cate, P. Nanthasurasak, P. Riwkulkajorn, C. L'Orange, C. S. Henry, and J. Volckens, *Ann. Occup. Hyg.*, **2014**, 58, 413.
20. Y. Sameenoi, P. Panymeesamer, N. Supalakorn, K. Koehler, O. Chailapakul, C. S. Henry, and J. Volckens, *Environ. Sci. Technol.*, **2013**, 47, 932.
21. S. Karita and T. Kaneta, *Anal. Chem.*, **2014**, 86, 12108.
22. E. Carrilho, A. W. Martinez, and G. M. Whitesides, *Anal. Chem.*, **2009**, 81, 7091.
23. H. Asano and Y. Shiraishi, *Anal. Chim. Acta*, **2015**, 883, 55.
24. G. Andregg, *Helv. Chim. Acta*, **1963**, 46, 2813.