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

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Microfluidic paper-based analytical devices (µPADs) 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 µPADs were fabricated using a wax printer after the addition of hydroxylamine into the detection reservoirs to reduce Fe3+ to Fe2+, 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 Fe3+ 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 Fe3+ 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 µPADs were compared with stored ones, no significant difference was found. The µPADs were applied to the determination of Fe3+ in a sample of water from a natural hot spring. Both the accuracy and the precision of the µPAD method were evaluated by comparisons with the results obtained via conventional spectrophotometry. The results of the µPADs were in good agreement with, but less precise than, those obtained via conventional spectrophotometry. Consequently, the µPADs 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

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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 (µPADs) 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 µPADs 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, µPADs 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 µPADs 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 µPADs 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 (Fe3+) 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 µPADs for the determination of Fe3+ in order to apply them to the analysis of the water in a natural hot spring. We determined the concentration of Fe3+ in the hot spring water using the µPADs, 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...
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<sub>n</sub> ~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<sup>–1</sup> and 16 mg mL<sup>–1</sup>, respectively. A 5.6 mg mL<sup>–1</sup> poly(acrylic acid) solution was prepared by diluting the 35% solution with water. Acetic acid stock solution of 6.3 M acetate buffer (pH 4.7) was prepared by dissolving appropriate amounts in the acetate buffer (pH 4.7). Five percent hydroxylamine, 5.4 mM phen, and 1 M acetate buffer were prepared with deionized water.

**μPAD fabrication**

The μPADs used in the present study were fabricated according to a procedure reported by Henry’s group. The pattern of the μPAD 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 × 30 mm square. The μPADs were printed on a sheet of filter paper (200 × 200 mm, Chromatography Paper 1CHR, Whamton<sup>18</sup>, GE Healthcare Lifesciences, UK) using a wax printer<sup>22</sup> (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 μPAD. The μPADs were prepared by applying appropriate volumes of reagent solutions to the detection reservoirs via a microsyringe or a micropipette; 1 µL of 0.1 g mL<sup>–1</sup> hydroxylamine and 0.3 µL of 5.6 mg mL<sup>–1</sup> poly(acrylic acid) were added to the four detection reservoirs, followed by two 0.2-µL aliquots of 16 mg mL<sup>–1</sup> phen in 6.3 M acetate buffer (pH 4.7). The μPAD was dried completely between each addition of reagent solution.

**Analytical procedure**

In the analysis, 25 µL of a sample solution was introduced into the center of the μPAD using a micropipette. After all the channels and reservoirs were filled with the sample solution, the μPAD 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). In general, each sample was introduced into 5 μPADs 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<sup>3+</sup> and their logarithms in order to construct the calibration curve. A schematic illustration of the experimental procedure including fabrication of the μPADs and operations is shown in Fig. 1(A).

**Results and Discussion**

**Calibration curve**

Initially, we constructed calibration curves of Fe<sup>3+</sup> to assess the sensitivity and precision in the determination of Fe<sup>3+</sup> using the μPADs. The results for a standard solution of 100 ppm Fe<sup>3+</sup> are shown in Fig. 1(B). The average color intensity for the 20 detection reservoirs in Fig. 1(B) was measured at 16.17 ± 0.73×10<sup>4</sup>, which would be acceptable for practical analysis since the relative standard deviation was less than 5%.

The relationship between the concentration of Fe<sup>3+</sup> 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<sup>2</sup>) 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
value of zero, which was the intensity of the blank. The LOQ was comparable to that reported by Mentele et al., 16 (1.5 μg in 30 μL, 50 mg L⁻¹) while Asano et al. found a lower LOQ (13.8 μM, 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 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 μL for a single measurement, whereas we used 25 μL of a sample to acquire four types of data (6.25 μL for a single measurement), which was similar to Mentele’s device that required 30 μL 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 precision 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 Fe3⁺). 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 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 Fe3⁺ solutions at temperatures of 30, 40, 50, 60, 70, and 80°C. We also measured the absorbance of a Fe(phen)₃²⁺ complex at 0, 20, and 80°C using absorption spectrometry. In both methods, the differences in the absorbance, or the color intensity, of the Fe(phen)₃²⁺ complex was almost independent of temperature change. These results are quite reasonable in terms of the thermodynamic parameters of the complex formation between Fe³⁺ and phen. Calorimetry showed the enthalpy and entropy of the complex formation to be –28.5 kcal mol⁻¹ and –15.4 cal mol⁻¹ K⁻¹, and the stability constants of the complex (log β₁) 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 Fe³⁺, 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 Fe³⁺ 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 ± 2.6 ppm by
conventional spectrophotometry and 112.1 ± 11.1 ppm by the μPADs. Therefore, the results of the μPADs were comparable to those from conventional spectrophotometry, although the precision of the μPADs was slightly poorer. These results indicate that the μPADs 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 μPADs when used in the determination of Fe³⁺. 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 μPADs were applicable to the determination of Fe³⁺ in the water of a natural hot spring. The μPADs were useful for a simple assay of the Fe³⁺ in natural water samples because of easy operation, lightness and affordability, although conventional spectrophotometry provides a more precise and sensitive determination.

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