



## A LOW-COST PAPER-BASED MICROFLUIDIC IMPEDIMETRIC DEVICE FOR THE DETECTION OF WATER HARDNESS

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### ABSTRACT

A microfluidic paper-based impedimetric device was developed as a water hardness sensor. This device is capable of performing the analysis with a sample volume of a few microliters with no prior treatments. A phenol-formaldehyde graphene electrode modified with ethylenediaminetetraacetate was used as the working electrode. Ag pseudo reference and carbon electrodes were used to fabricate the device. Current simultaneous metal ion detection sensors are based on complex and expensive electrode setups. The proposed inexpensive, quick and portable device is capable of detecting  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  simultaneously. Electrode double layer-based charge transfer resistance and the maximum negative imaginary impedance produced a linear correlation with each metal ion concentration. The calculated limits of detection for  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  were 0.31 and 0.24 ppm, respectively. A set of samples containing  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  with a hardness of 2 ppm (as calcium carbonate) were used to test the device. The proposed tool is suitable as a semi-quantitative device for the determination of hardness in water.

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## 1. Introduction

Miniature microfluidic paper-based devices ( $\mu\text{Pad}$ ) can be used effectively to monitor parameters in many fields such as environmental, clinical, food and pharmaceutical applications.<sup>[1]</sup> One major advantage is that the paper can wick a hydrophilic mobile phase and this phenomenon can be used to pump the mobile phase inactively through the paper to the detector.<sup>[2]</sup> These devices offer several advantages over conventional analytical devices because they are cost-effective, easy to dispose of, ready to use, and have quick response times.<sup>[2]</sup> Colorimetric<sup>[3]</sup> and amperometric<sup>[4]</sup> methods are common detection methods used in  $\mu\text{Pad}$ . Such a paper-based device for the determination of water hardness can reduce the cost of the water hardness testing method. Also, this method can be used as an on-site sensor. Atomic absorption spectroscopy and complexometric titration methods are commonly used for the determination of water hardness. Ion-selective electrode<sup>[5]</sup> based potentiometric methods also can be used for the determination of water hardness. Compared to these methods, the proposed paper-based method is inexpensive. Also, the proposed device is completely based on solid components. Compared to most other ion-selective electrode methods that are based on liquid or gel fillings, the proposed paper-based device has a greater shelf life and this impedimetric  $\mu\text{Pad}$  can be directly used without any initial setup steps.

The use of impedimetric methods as the detection technique in paper-based devices for metal ions

has not been previously reported. However, few paper-based impedimetric devices were reported previously for the detection of biological parameters.<sup>[6],[7]</sup> Work reported in this paper describes  $\mu$ Pad that uses impedimetric based detection for the simultaneous detection of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions. Phenol-formaldehyde graphene electrode modified with ethylenediaminetetraacetate (EDTA) is used as the working electrode to fabricate the three-electrode system. This EDTA-modified phenol-formaldehyde-graphite electrode platform trap metal ions may increase the charge density of the electrode double layer when exposed to  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions. Compared to the double layer without chelated  $\text{Ca}^{2+}/\text{Mg}^{2+}$  ions, a layer chelated with metal ions has a lower double-layer capacitance. This type of capacitance change was observed with divalent copper and nickel ions for an electrode with a modified layer containing nitrilotriacetic acid groups.<sup>[8]</sup> Change in the double layer charge density due to metal ion chelation can be detected as an impedance change using electrochemical impedance spectroscopy (EIS).<sup>[9]</sup> Nyquist plots can be used to calculate this parameter.<sup>[9]</sup> The possibility of using the highest imaginary impedance (highest  $-Z''$ ) to estimate the concentration of the targeted analyte is reported in this paper. The selectivity of the detector can be improved by introducing selective chelating agents, varying pH, or adding masking agents. The ion density of the working electrode double layer may change in the presence of metal ions that can chelate with EDTA.

## 2. Materials and Methods

### 2.1. Preparation of working electrode material

Oxalic acid (0.6 g) was dissolved in a minimum amount of deionized water. The oxalic aqueous solution and a solution of phenol (phenol 6.4 g and formaldehyde 16 mL) were mixed and heated at 90 °C for an hour. The resultant mixture was cooled and the bottom phenol-formaldehyde polymer layer was collected. Phenol-formaldehyde, graphene and  $\text{Na}_2\text{EDTA}$  (4:2:1) were mixed to develop the modified working electrode material and the bare working electrode material was developed by mixing phenol-formaldehyde and graphite (2:1) only. Graphite powder (200  $\mu\text{m}$ ) was obtained from Kahatagaha Graphite Lanka, Sri Lanka.

### 2.2. Development of the paper-based device

Commercially available varnish was used to draw the hydrophobic barrier on a Whatman No. 01 filter paper to construct the paper-based device platform. Silver conductive ink (CircuitWorks® Silver Conductive Pens) and 6HB pencil were used to fabricate the reference and counter electrodes, respectively. Phenol-formaldehyde-graphite- $\text{Na}_2\text{EDTA}$  (4:2:1) mixture was used to fabricate the working electrode. The developed device is shown in Figure 1. The working electrode developed with Phenol-formaldehyde-graphite (unmodified) was used as the control device.

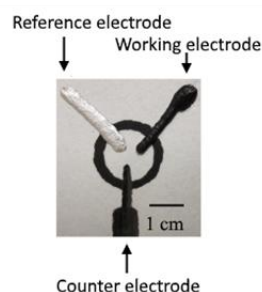


Figure 1. Top view of the proposed paper-based device. The hydrophobic barrier with a diameter of 1 cm restricts the movement of the sample.

### 2.3. Reagents

All reagents were analytical grade and all solutions were prepared using deionized water unless otherwise stated. A buffer solution (pH = 7.0) was used to prepare ion solutions of  $K^+$  from KCl (Techno PharmChem, India),  $Ca^{2+}$  from  $CaCl_2$  (Vickers, UK),  $Mg^{2+}$  from  $MgCl_2 \cdot 6H_2O$  (Merck, India) and  $Fe^{3+}$  from  $FeCl_3$  (Merck, India).

### 2.4. Electrochemical characterization of the microfluidic paper-based electrochemical device

Electrochemical characterization was performed using ZIVE SP5 workstation (WonATech, Korea). Cyclic voltammograms for the solutions (2, 4, 6, 8, 10 mM) of  $K_4Fe(CN)_6$  in 0.1 M KCl were collected using the developed  $\mu$ Pad at a scan rate of 25 mV/s. The peak current variation with the concentration of  $K_4Fe(CN)_6$  was plotted.

### 2.5. Measurement procedure of electrochemical impedances

Electrochemical impedance spectroscopy (EIS) studies were performed using ZIVE SP5 workstation (WonATech, Korea). Each run was conducted using a fresh sensor and a volume of 50  $\mu$ l was used to collect EIS response of each solution in the range of 1 Hz to 1 MHz, with the amplitude potential of 60 mV. All electrochemical impedance spectrums were collected under the 0 V biased potential against the Ag pseudo reference electrode.

### 2.6. Preparation of laboratory samples

The proposed  $\mu$ Pad was tested using the laboratory prepared solutions shown in Table 1. All solutions listed in Table 1 were prepared with a hardness of 2 ppm (as calcium carbonate) and buffered at pH = 7. A volume of 50  $\mu$ l from each solution was tested as explained above.

Table 1. Concentrations of  $Ca^{2+}$  and  $Mg^{2+}$  in laboratory prepared samples.

Sample solution number	$Ca^{2+}$ concentration /mM	$Mg^{2+}$ concentration /mM
1	0.02	-
2	0.01	0.01
3	0.005	0.015
4	0.015	0.005
5	-	0.02

## 3. Results and Discussion

The Whatman No. 01 was used as the base and commercially available hydrophobic paint was used to fabricate the paper-based device. The selection of these materials reduces the cost of a device. The electrochemically inactive phenol-formaldehyde polymer was used as the electrode matrix for both working and counter electrodes. Impedimetric paper supported immunosensors<sup>[10]</sup> and graphene paper impedimetric sensors<sup>[11]</sup> are previously reported. However, EIS detection using a modified electrode fabricated on paper-based devices is never been reported previously. An image of a developed microfluidic paper-based device ( $\mu$ Pad) is shown in Figure 1. The device only requires 50  $\mu$ L of sample for the detection of metal ion concentration. Compared to the other water hardness detection methods, the use of smaller sample volumes in these proposed methods reduces the use of chemical resources. This can be considered as a major advantage in this method. Compared to the other electrochemical<sup>[12]</sup> metal ion sensing methods, the proposed paper-based method is simple, small in size and inexpensive (cost per device = 0.1 USD).

Peak current variation with the concentration of  $K_4Fe(CN)_6$  graph is shown in Figure 2. The proposed  $\mu$ Pad is capable of producing a linear relationship between the concentration of  $K_4Fe(CN)_6$

and the peak current. The developed  $\mu$ Pad is capable of functioning as a typical electrochemical setup under standard electrochemical conditions.

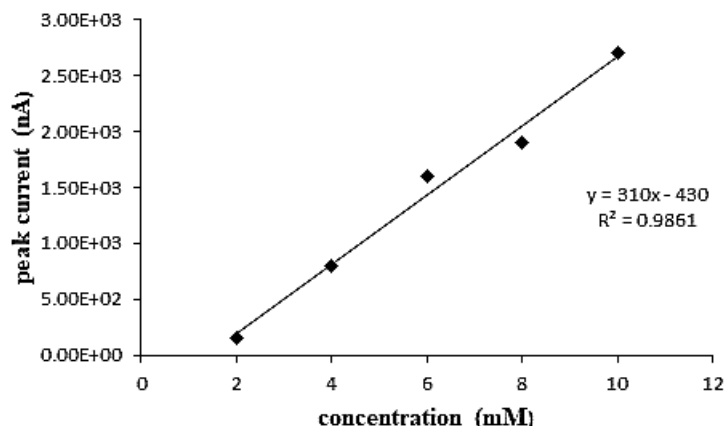


Figure 2. Peak current of the proposed  $\mu$ Pad device with EDTA modified working electrode against the  $K_4Fe(CN)_6$  concentrations of 2, 4, 6, 8, 10 mM in 0.1 M KCl at a scan rate of 25 mV/s.

All the electrochemical impedance spectrums were collected keeping the bias potential of the working electrode kept at 0 V to minimize the ion migration due to the applied electric field. The typical electrochemical impedance spectrums collected using the paper-based tool with EDTA modified electrode for  $Fe^{3+}$  ion solutions with the concentrations of 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 ppm are shown in Figure 3.

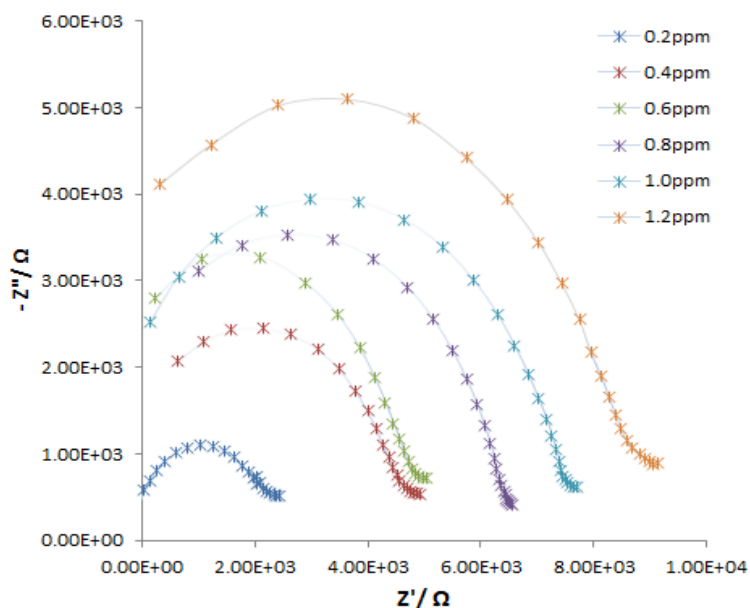


Figure 3. Impedance spectra (negative imaginary impedance,  $-Z''$  vs real impedance,  $Z'$ ) of phenol-formaldehyde graphite electrode modified with EDTA for  $Fe^{3+}$  concentrations of 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ppm buffered at pH 7.0.

The charge transfer resistance ( $R_{ct}$ ) and the highest negative imaginary impedance (highest  $-Z''$ ) of 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 ppm  $Fe^{3+}$  solutions were recorded. These values were compared with the values obtained for the same set of solutions using an unmodified electrode. Both highest  $-Z''$  and  $R_{ct}$  recorded using  $\mu$ Pad with EDTA modified electrode showed greater responses and produced a linear variation against  $Fe^{3+}$  concentration. Since the highest  $-Z''$  showed a linear relationship with  $Fe^{3+}$  concentration, the highest  $-Z''$  responses of 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ppm  $Fe^{3+}$  were used to build

the correlation between  $\text{Fe}^{3+}$  concentration and the highest  $-Z''$  of the Nyquist plot constructed for each  $\text{Fe}^{3+}$  concentration. The calibration graph for these Fe(III) concentrations is shown in Figure 4.

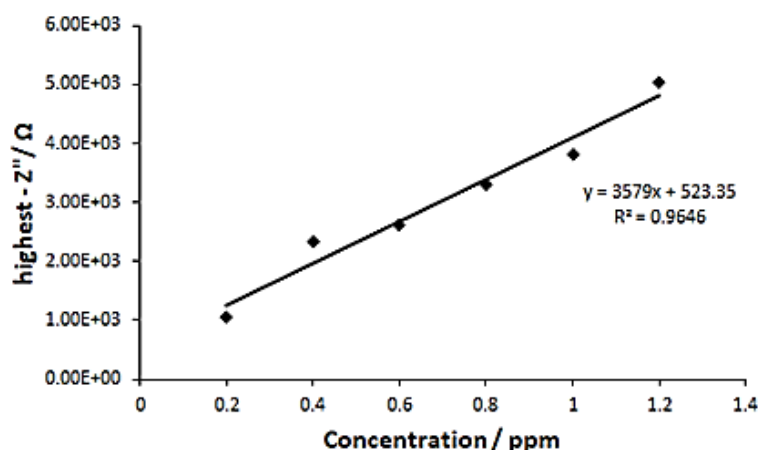


Figure 4. Variation of the highest  $-Z''$  for the impedimetric  $\mu\text{Pad}$  device with EDTA modified working electrode against the  $\text{Fe}^{3+}$  concentrations of 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ppm.

Electrodes modified with EDTA can chelate with the metal ions in the solution. Chelation of metal ions increases the charge density of the electrode double layer of the working electrode. The accumulation of  $\text{Fe}^{3+}$  ions on the modified electrode surface increases the impedance ( $-Z''$ ) and the Rct of the double layer compared to unmodified electrodes. Electrode interfacial charge-transfer rate between the solution and the electrode is controlled by the Rct. The proposed  $\mu\text{Pad}$  with the modified electrode is capable of producing a change in the impedance in the presence of metal ions that can chelate with EDTA. Both  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  chelate with EDTA and these two metal ions are responsible for water hardness. Hardness in water can be estimated by determining the total  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ion concentrations. Both these group 2 metal ions can chelate with the EDTA modified working electrode of the proposed  $\mu\text{Pad}$ . The suitability of the proposed  $\mu\text{Pad}$  to determine water hardness was evaluated. Rct and highest  $-Z''$  produced by the  $\mu\text{Pad}$  with  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  solutions of varying concentrations buffered at  $\text{pH} = 7$  are shown in Figures 5 and 6, respectively. Limit of Detection (LoD) of the device was determined based on the standard deviation of the response ( $S_y$ ) of the curve and the slope of the calibration curve ( $s$ ) of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  calibration curves using the equation  $\text{LoD} = 3.3 \left( \frac{S_y}{s} \right)$ . The calculated LoDs of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  are 0.31 and 0.24 ppm, respectively.

Both Rct and highest  $-Z''$  produced linear correlations with  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations. The increase observed in the highest  $-Z''$  and the Rct is due to the accumulation of metal ions on the working electrode surface. Rct and  $-Z''$  responses of  $\text{Mg}^{2+}$  solutions with the EDTA modified electrode were greater than those of  $\text{Ca}^{2+}$  solutions, due to the greater charge density of  $\text{Mg}^{2+}$  ions when chelated with EDTA.

The ability of the proposed device for the determination of water hardness when both  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  are available was tested using laboratory samples containing  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . These solutions were prepared with a hardness of 2 ppm (as calcium carbonate hardness). The hardness of each solution was determined using the proposed  $\mu\text{Pad}$  device and the recorded highest  $-Z''$  and Rct and values are shown in Figure 7.

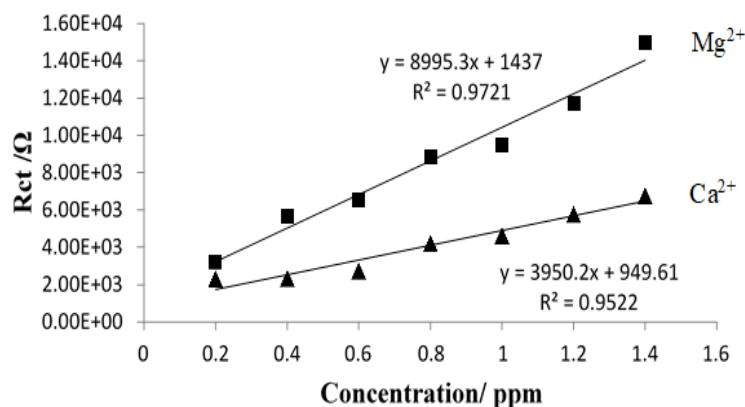


Figure 5. Variation of the  $R_{ct}$  for the impedimetric  $\mu$ Pad device with EDTA modified working electrode against the  $Mg^{2+}$  and  $Ca^{2+}$  concentrations of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 ppm.

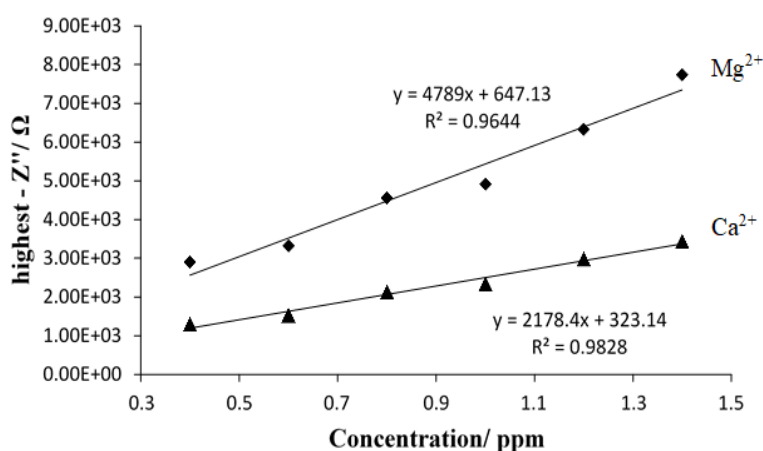


Figure 6. Variation of the highest  $-Z''$  for the impedimetric  $\mu$ Pad device with EDTA modified working electrode against the  $Mg^{2+}$  and  $Ca^{2+}$  concentrations of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 ppm.

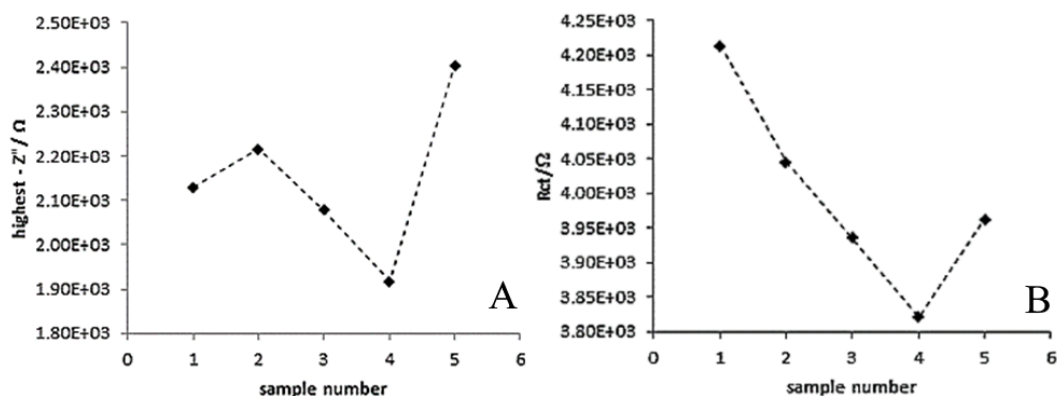


Figure 7. A: Variation of the highest  $-Z''$  for the impedimetric  $\mu$ Pad device with EDTA modified working electrode against the laboratory prepared samples with different concentrations of  $Mg^{2+}$  and  $Ca^{2+}$ , B: Variation of the  $R_{ct}$  for the impedimetric  $\mu$ Pad device with EDTA modified working electrode against the laboratory prepared samples with different concentrations of  $Mg^{2+}$  and  $Ca^{2+}$ .

Even though all five samples had a hardness of 2 ppm (as calcium hardness), the recorded  $R_{ct}$  and highest  $-Z''$  values showed variations with the amounts of Ca and Mg ions in the solution. Hence the tool is not suitable for the determination of total hardness when both Ca and Mg are present. However, when both Ca and Mg are present, the device can be used as a semi-quantitative tool to determine



whether the total hardness is larger than a pre-decided threshold value (priority previously decided  $-Z''$  value can be used as the threshold value). Advantages of such determination using the proposed device are; determination can be performed within a short period and no prior calibration is required. Further, only a minute volume (50  $\mu\text{l}$ ) is required for a single analysis. Based on these results, it can be observed that both  $R_{ct}$  and highest  $-Z''$  produced similar correlations with the tested metal ion concentrations. This justifies the use of the highest  $-Z''$  in Nyquist plots to correlate directly to the total concentration of metal ions that can chelate with the EDTA-modified electrode surface. The hardness of samples contacting either Ca or Mg, with no significant amounts of other metal ions that can chelate with EDTA, can be estimated with this proposed  $\mu\text{Pad}$ .

#### 4. Conclusion

The proposed impedimetric  $\mu\text{Pad}$  can be used as a sensor for the detection of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  with the detection limits of 0.31 and 0.24 ppm, respectively. The device can be used to detect a single metal ion, however, the proposed impedimetric  $\mu\text{Pad}$  is not ideal to detect the total  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  metal ion concentration of a solution. The device can be used successfully for the qualitative detection of hardness.

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