

Research article

Low Cost Paper-based Electrochemical Sensing Platform for the Determination of Hydrogen Peroxide

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Abstract

Keywords

low-cost device;
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paper-based device;
electrochemical device;
modified graphite-based electrode

A rapid, precise, low cost, selective and sensitive paper-based electrochemical device for the determination of H₂O₂ in milk is described here. Commercially available varnish and a simple hand drawing method were used to develop the hydrophobic pattern to generate a hydrophilic detection zone on the filter paper. The electrode system was fabricated on the detection zone in order to detect H₂O₂ electrochemically. A commercially available graphite pencil and conductive silver ink were used to fabricate the counter electrode and pseudo-reference electrode, respectively. A paste of Prussian blue (PB) modified graphite, unmodified graphite and phenol-formaldehyde polymer were used to fabricate a PB modified graphite working electrode on paper. This modified electrode showed electrocatalytic activity towards the reduction of H₂O₂ and it was successfully used for the chronoamperometric detection of H₂O₂ at 0 V vs Ag reference electrode in 0.1 mol l⁻¹ phosphate buffer, buffered at pH 6.0 in 0.1 mol l⁻¹ KCl. Under optimum conditions, the calibration curve for the H₂O₂ determination was linear from 5 to 50 mmol l⁻¹ with a detection limit (LoD = $\bar{x} + 3SD$) of 4.0 mmol l⁻¹. In addition, the PB modified graphite electrode showed selectivity for H₂O₂ detection in the presence of ascorbic acid, sucrose and citric acid.

1. Introduction

Paper-based devices have evolved as good alternatives to traditional analytical instruments for point-of-care testing because they are easy to use, portable, require small volumes of reagents and samples, inexpensive, provide rapid analysis and disposable [1, 2]. The fabrication of paper-based devices involves creating channels within the paper substrate in order to constrain fluid flow. Such microchannels on the paper can be fabricated by patterning hydrophilic channels on the paper that are separated by water repellent barriers. Physical and chemical methods have been reported in the

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literature for fabricating patterns on paper. Photolithography, plasma treatment, and inkjet printing are some of the chemical methods used in creating hydrophobic barriers in the paper [1, 3]. All these methods require chemicals such as photoresists, which are expensive. The first two methods mentioned above have the disadvantage of needing to use a mask for the patterning. The design of the mask takes a long time, and this makes the method expensive. Inkjet printing does not require a mask, however, the designing of suitable ink requires more research. The most common physical patterning methods are wax patterning, plotting, paper cutting and shaping, and screen printing [4]. Although these methods do not require expensive chemicals, some of them, such as screen printing, require masks. Plotting, paper cutting and shaping require precision printers, making the methods expensive. Wax patterning, consisting of wax printing, wax dipping and wax screen printing, is the most economical of the other methods; however, all these methods require an oven or a hot plate to melt the wax. Moreover, when melted, the spreading wax is hard to control [3, 5, 6].

In this work, a drawing method with commercially available varnish is utilized for the fabrication of hydrophobic barriers on paper-based devices. Varnish is a combination of a drying oil, resin, and a thinner. It leaves a transparent, hydrophobic film that can be spread in a controlled way on the paper. Unlike other patterning methods, it does not require any expensive instruments, as it is an air-drying liquid. Therefore, varnish becomes an attractive option due to its low cost and ease of handling.

Amperometric detection was used in the development of paper-based devices. A Prussian blue modified hand drawn electrode is used as the working electrode in order to achieve selectivity. Here, graphite powder is modified with PB by *in situ* chemical synthesis. This PB modified graphite is carefully hand mixed with unmodified graphite powder and phenol formaldehyde polymer in order to prepare a PB modified graphite paste that is employed to fabricate a working electrode on paper [7-9]. In the literature, modified carbon paste electrodes were constructed using mineral oil and solid paraffin [10, 11]. These electrodes experienced modifier leakage due to their poor mechanical strength. In the present work, phenyl formaldehyde replaces the mineral oil and solid paraffin, improving the mechanical strength. Further, a commercially available Ag pen drawn electrode is used successfully as a pseudo reference electrode and a graphite pencil is used to draw the counter electrode.

The objective of this work is to present the new concepts of using varnish in fabrication of channels in paper-based devices, and then introducing a phenyl formaldehyde polymer based carbon paste electrode, a Ag pen drawn pseudo reference electrode and a pencil drawn counter electrode system for amperometric detection. The use of H_2O_2 as a preservative is prohibited in many countries, however, some countries allow to include H_2O_2 as a preservative additive in liquid milk, at up to 0.05% (w/w) [12]. As a consequence, a paper-based device was developed to determine H_2O_2 . To evaluate the efficiency of the developed novel paper-based device, H_2O_2 was electrochemically detected in milk using the new device, and the results were compared to those done by the traditional redox titration method of H_2O_2 detection.

2. Materials and Methods

2.1 Reagents

Hydrogen peroxide (H_2O_2 , 35%, w/w) was purchased from Sigma Aldrich. Varnish was purchased from Lankem Ceylon PLC, Sri Lanka. All other chemicals were analytical grade and used as received. For pH effect studies, acetate buffer ($0.1 \text{ mol l}^{-1} \text{ CH}_3\text{COOH}/\text{CH}_3\text{COONa} + 0.1 \text{ mol l}^{-1} \text{ KCl}$, pH 3.0-5.0) and PBS ($0.1 \text{ mol l}^{-1} \text{ Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4 + 0.1 \text{ mol l}^{-1} \text{ KCl}$, pH 6.0-9.0) were used.

Standard working solutions of H_2O_2 were freshly prepared. Double distilled water was used to prepare all solutions.

2.2 Synthesis of Prussian blue modified graphite

Graphite (2.0 g) was suspended in an equimolar mixture (40 ml) of iron(III) chloride and potassium ferricyanide (0.1 mol l^{-1}) containing 10 mmol l^{-1} HCl. The resultant mixture was stirred for 10 min. The particles with adsorbed PB were then collected by filtration and then washed with 10 mmol l^{-1} HCl until the washing solution became colorless. The washed particles were then dried in oven at 100°C for 1.5 h. The PB modified graphite was stored in dark in a desiccator at room temperature.

2.3 Preparation of phenol-formaldehyde polymer

Phenol (6.4 g) and formaldehyde (16 ml) were mixed with oxalic acid (0.6 g dissolved in 5 ml of distilled water) until the solution became clear. The clear mixture was heated for a period of 1 h at 90°C . The resulting milky solution was allowed to stand for 30 min in order to allow it to separate into two layers. The lower layer, which contained phenol-formaldehyde was collected, and the upper layer was discarded.

2.4 Construction of paper-based electrochemical device

Whatman No.1 filter paper was used to construct the paper-based electrochemical device. A hydrophilic circular region was defined on the paper using a hydrophobic circular barrier patterned with varnish. Electrodes were drawn on the detection zone of the hydrophilic circular region. The reference electrode and its conductive pad was drawn using conductive silver ink (conductive silver ink pen CW2200MTP (Georgia, USA)). For the counter electrode and its conductive pad, commercially available graphite pencil (6B) was employed. PB modified graphite, unmodified graphite (in different ratios) and phenol formaldehyde polymer were mixed with graphite to polymer ratio of 2:1 in a mortar and then used to fabricate the working electrode and its conductive pad. A cardboard stencil was used to maintain uniform working electrode features. Three electrodes were drawn at 2 mm from one another. The layout of this electrode system of the paper-based electrochemical device is shown in Figure 1. A $20 \mu\text{l}$ volume was used as the sample volume. In all cases, phosphate buffer system (PBS, 0.1 mol l^{-1} $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$, $\text{pH} = 6.0$) containing 0.1 mol l^{-1} KCl was used as the supporting electrolyte.

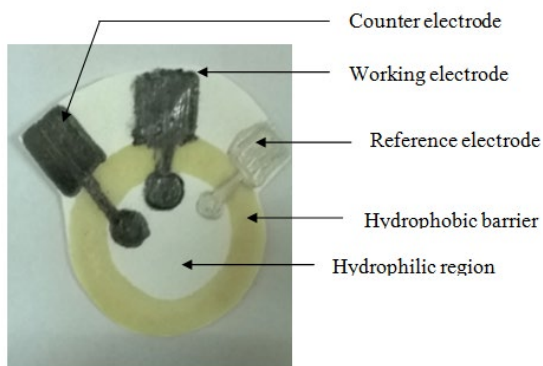


Figure 1. Three electrode paper based electrochemical device

2.5 Real sample preparation and analysis

Volumes of 3.0 ml milk, H₂O₂, 5.0 ml of 0.1 M PBS buffer in 0.1 M KCl were mixed and diluted up to 10.0 ml to develop the H₂O₂ spiked milk samples with 5, 10, 20, 30, 40 and 50 mM of H₂O₂ for the development of calibration plot. A milk sample with 25 mM H₂O₂ was prepared using the same method for the validation process. Fourteen and ten replicates were performed using a single paper-based device and the standard potassium permanganate titration methods, respectively, for the 25 mM H₂O₂ spiked milk sample.

2.6 Apparatus

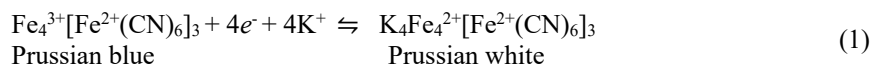
Cyclic voltammetry (CV) and chronoamperometry experiments were performed using WaveDriver 20 Bipotentiostat/Galvanostat (Pine Research Instrumentation, Durham, USA) equipped with AfterMath scientific data analysis software (Pine Research Instrumentation, Durham, USA). All measurements were carried out under controlled room temperature.

3. Results and Discussion

3.1 Optimizing the composition of the working electrode

The optimum composition of the working electrode was investigated by mixing percentages of 10, 20 and 30% of PB modified graphite (Figure 2) and unmodified graphite with phenol-formaldehyde polymer (with graphite to polymer ratio at 2:1). Composites containing higher amounts of PB modified graphite were not tested due to the Ohmic drop effect, as PB modified graphite has lower conductivity.

Reversible electrochemical behavior was observed for the supporting electrolyte at pH 6.0 on the PB modified graphite working electrode [13]. The redox reaction of insoluble PB particles can be expressed as in equation (1) given below.



The corresponding electrochemical peaks were observed around -0.1 V (vs. Ag) and around 0.3 V (vs. Ag). Both cathodic (I_{pc}) and anodic peak current (I_{pa}) increases with the increase in the percentage of the PB modified graphite and I_{pa}/I_{pc} ratio remained close to 1.0 for all three percentages of PB modified graphite electrodes. However, when the percentage of PB modified graphite was increased, the difference between the anodic and cathodic peak potentials (ΔE_p) increases indicating slower electrode kinetics at the working electrode. Accordingly, 10% of PB modified graphite was selected to fabricate the working electrode.

3.2 Optimization of variables at PB modified graphite working electrode

In order to verify the electrocatalytic activity of the PB modified graphite working electrode for the reduction of H₂O₂, cyclic voltammetry was conducted in the presence and absence of H₂O₂. As observed in Figure 3, well-defined cathodic and anodic peaks were obtained. Increments in cathodic peak current was observed with the addition of 1, 10 and 30 mmol l⁻¹ of H₂O₂ relative to the background current. This increase confirmed that the PB modified graphite working electrode had the catalytic capability to reduce H₂O₂.

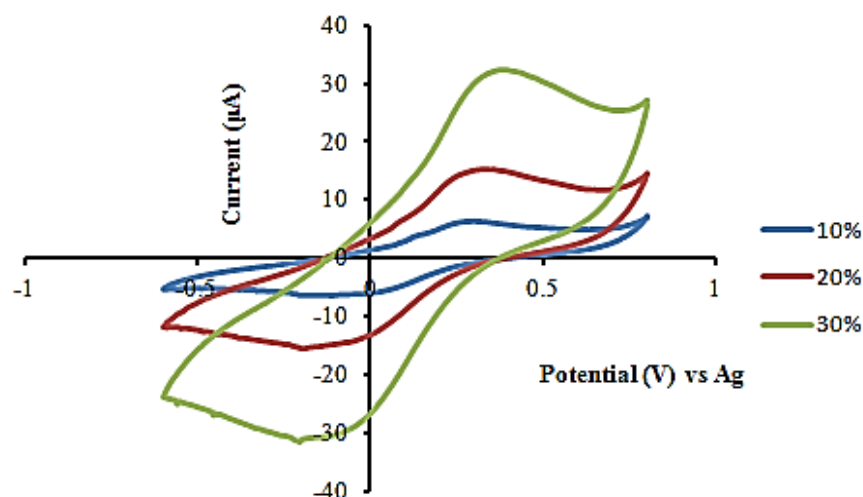


Figure 2. Cyclic voltammograms of PB modified graphite electrode at different percentages of PB modified graphite in 0.1 mol l⁻¹ PBS + 0.1 mol l⁻¹ KCl, pH 6.0 at the scan rate of 50 mV s⁻¹

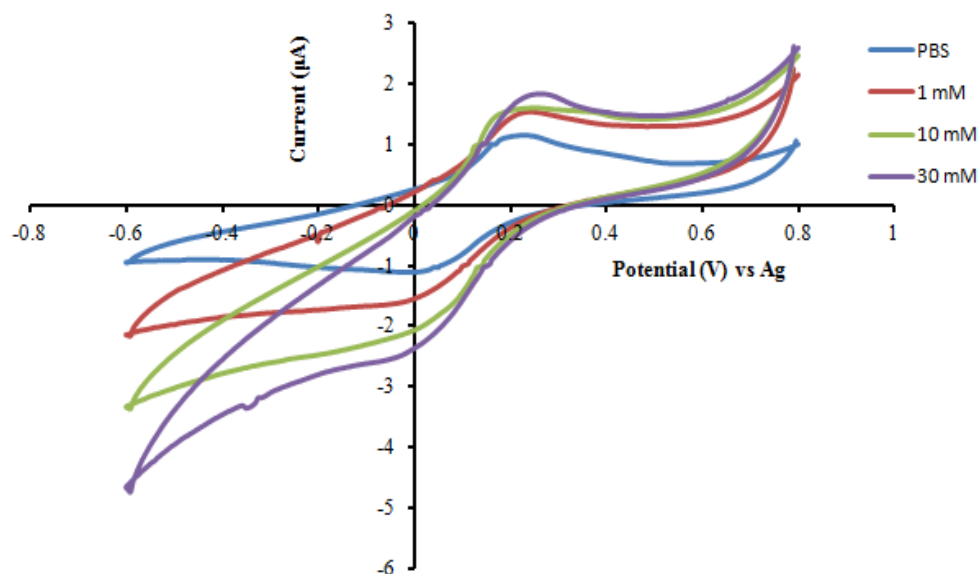


Figure 3. Cyclic voltammograms of PB modified graphite working electrode in the absence (PBS) and presence of 1, 10 and 30 mmol l⁻¹ H₂O₂ between 0.8 V to -0.6 V at the scan rate of 50 mV/s

Potential optimization was carried out in the region from -0.2 to 0.1 V (vs. Ag) where the catalytic reaction of H₂O₂ occurs. As is apparent from Figure 4(A), both cathodic current (signal) and background current increased when the potential decreased from 0.1 to -0.2 V. Therefore, signal/blank ratio was studied (Figure 4(B)). The signal/blank ratio increased only up to -0.1 V and then it started to decrease. Since the signal/blank ratio was highest at -0.1 V (vs. Ag), this potential was selected for further studies.

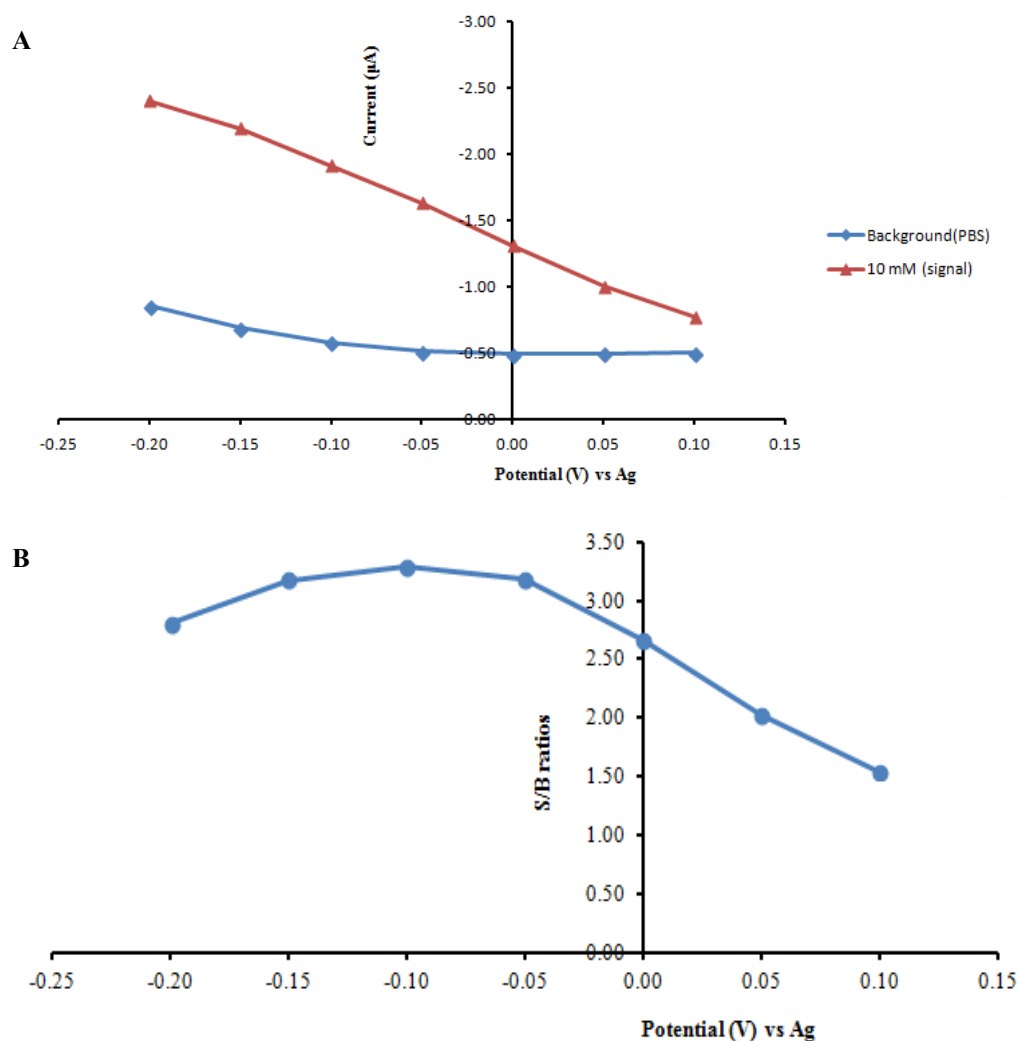


Figure 4. (A) The graph of current vs potential (vs Ag) for background (PBS) and 10 mmol l^{-1} H_2O_2 (B) The graph of signal-to-background ratios at different potentials in the potential range of 0.1 V to -0.2 V

The pH stability of the electrode was also tested in solutions containing 10 mmol l^{-1} H_2O_2 at different pH values, which varied from pH = 3.0 to 9.0. The modified electrode showed an electrocatalytic activity towards H_2O_2 in all tested pH solutions. Peak current reached a maximum value at pH 6.0 and started to decrease at higher pH values. At high pH values, high concentrations of hydroxyl ions break the Fe–CN–Fe bond, solubilizing the PB. This is attributed to the hydrolysis of Fe^{3+} to $\text{Fe}(\text{OH})^{2+}$ in alkaline media. On the other hand, at low pH, protons can block the electrochemical reactivity of PB, which results in a decrease in the sensitivity [11]. Therefore, the optimal pH value of the electrolyte solution was pH 6.0 as it produced the best response current for H_2O_2 reduction.

3.3. Response of the PB sensor for H₂O₂ calibration

A plot of current versus H₂O₂ concentration is given in Figure 5, and it shows linear response in the range 10-60 mmol l⁻¹ with a linear regression coefficient (R^2) of 0.9989. The detection limit of the sensor is 4.0 mmol l⁻¹ based on a signal to noise ratio of 3. The device to device reproducibility of the signal was tested by measuring the reduction current of 20 mmol l⁻¹ H₂O₂ in PBS on 10 different paper-based devices. The relative standard deviation of the measurements was found to be 12%. The possible interferences for the determination of H₂O₂ with the paper-based device with PB modified graphite working electrode were investigated and the resultant current ratios of interferences to H₂O₂ (30 mmol l⁻¹) are summarized in Table 1. According to Table 1, sucrose and ascorbic acid in their tested concentrations do not significantly interfere with the H₂O₂ signal response. However, citric acid interferes as there is a considerable increase in the H₂O₂ signal.

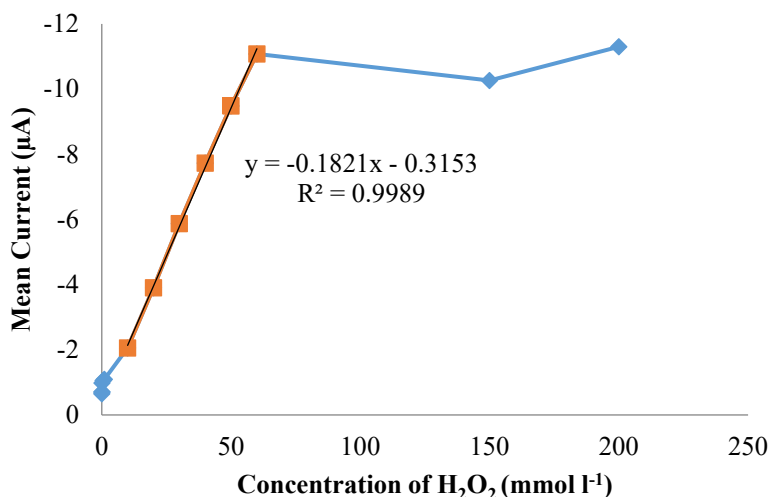


Figure 5. The plot of mean current vs concentration of H₂O₂ in the range between 0.01 to 200 mmol l⁻¹ in 0.1 mol l⁻¹ PBS + 0.1 mol l⁻¹ KCl, pH 6.0

Table 1. Effect of interfering species on H₂O₂ determination using paper-based device*

Interfering species	The concentration ratio of H ₂ O ₂ : interferences	Mean current (µA)	Current ratio
Sucrose	1:1	-11.72	1.123
	1:2	-9.71	0.931
	2:1	-10.58	1.014
Citric acid	1:1	-15.89	1.523
	1:2	-16.35	1.567
	2:1	-15.83	1.517
Ascorbic	1:1	-9.64	0.924
	1:2	-8.73	0.837
	2:1	-11.62	1.114

*The current response observed for 30 mmol l⁻¹ H₂O₂ was -10.43 µA

3.4. Validation and analytical application

In order to validate the paper-based electrochemical device, a concentration of 20 mmol l⁻¹ H₂O₂ standard solution was determined using the device under optimum conditions. The concentration of the standard solution was also determined using the classical potassium permanganate titration method. The results are shown in Table 2.

Table 2. Determination of the concentration of 20 mmol l⁻¹ H₂O₂ standard solution*

	Concentration (mmol l ⁻¹ ± SD)	Replicates
paper based electrochemical device	18.38 ± 2.38	14*
KMnO ₄ Titration	18.68 ± 0.16	10

*This data was collected using 14 different devices

The two-sample t-test assuming unequal variance was used to validate the paper-based electrochemical device against the potassium permanganate titration. No significant difference was found at 95% confidence level. Therefore, the concentration determined using the developed paper-based electrochemical device can be accepted.

The concentration of the H₂O₂ in ultra-high-temperature (UHT) processed milk was determined using the device to verify the practical application. Analysis of H₂O₂ in milk sample (spiked) was carried out by the standard addition method under the optimum conditions. The resultant chronoamperograms for the milk samples spiked with H₂O₂ concentrations of 5-50 mM are shown in Figure 6, and the resultant calibration plot is given in Figure 7.

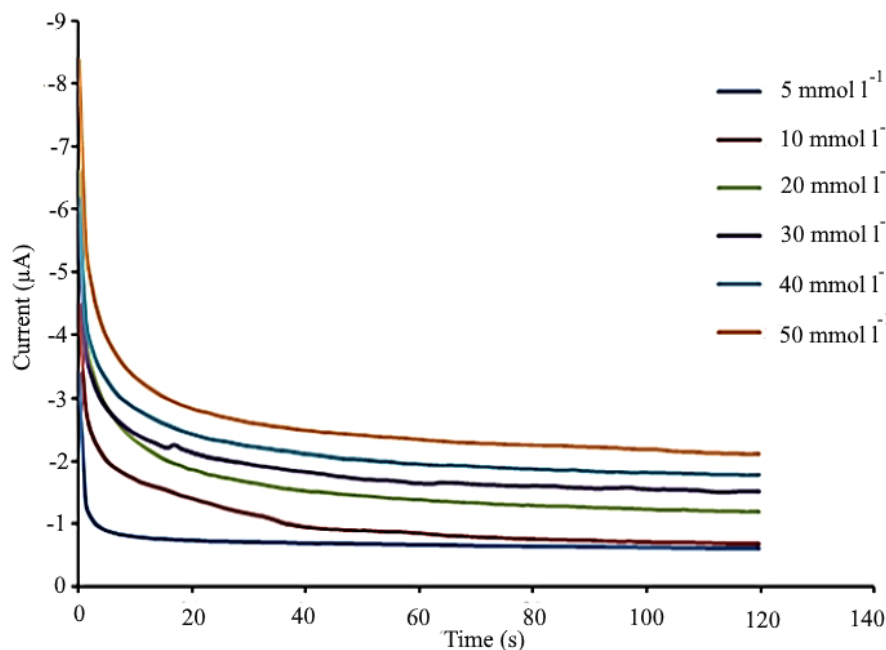


Figure 6: Chronoamperograms collected at -0.1 V for the buffered (pH = 5) milk samples spiked with H₂O₂ concentrations of 5-50 mM

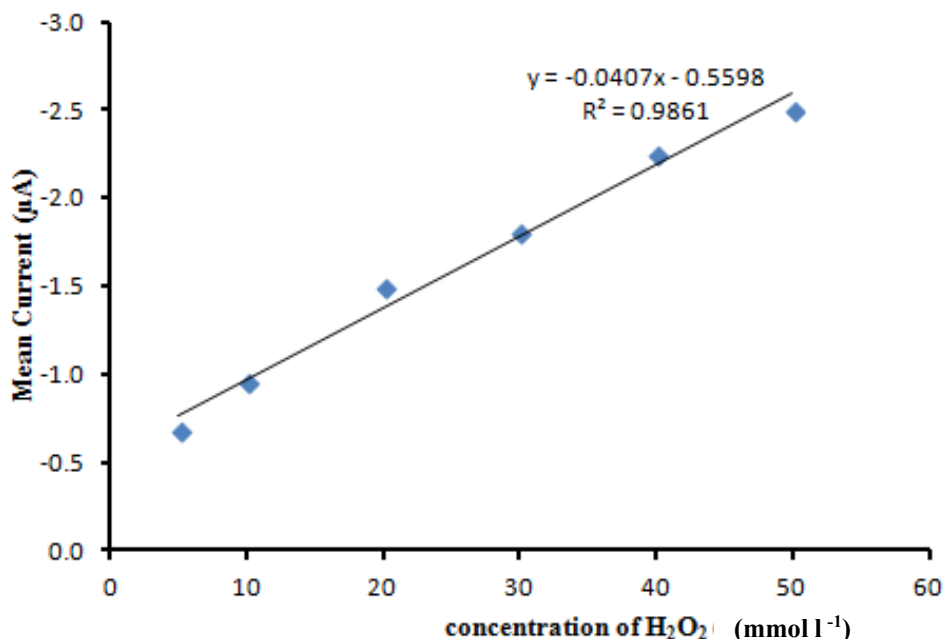


Figure 7. The plot of current vs concentration of H_2O_2 for UHT milk sample

As shown in Figure 7, the current increased linearly in the range of 5 to 50 mmol l⁻¹ concentrations with a regression equation; $y = -0.0407x - 0.5598$, where y represents the current in μA , x represents the H_2O_2 concentration in mmol l⁻¹, and R^2 was 0.9861. The limit of detection (LoD) was determined using average response, \bar{x} and the standard deviation, the SD of 10 blank samples. The LoD, 4.0 mmol l⁻¹ was calculated using $LoD = \bar{x} + 3SD$. LoD values of similar electrochemical methods reported recently are given in Table 3.

Table 3. LoD values of similar electrochemical methods reported recently for the detection of H_2O_2 in milk

Electrochemical Method	Detection limit	Reference
Electrochemiluminescence	1.2×10^{-9} mM	[13]
Amperometric (nonenzymatic)	0.47 μM	[14]
Conductivity	0.16 μM	[15]

The determination of H_2O_2 in milk was also validated against the potassium permanganate titration method using UHT milk spiked with H_2O_2 (25 mmol l⁻¹). The mean concentrations determined using the paper-based device and the potassium permanganate titration were 24.75 ± 1.68 mM and 24.49 ± 0.53 mM, respectively for 10 replicate measurements. There was no significant difference between the concentrations determined using the two methods at 95% confidence level of two sample t-Test assuming unequal variance. Thus, the concentration of H_2O_2 in milk analyzed using this proposed paper-based method can be accepted.

4. Conclusions

This work demonstrates the development of an amperometric paper-based device for the determination of H_2O_2 . A paste consisting of PB modified graphite, unmodified graphite and phenol-formaldehyde polymer was used in fabrication of the working electrode along with a conductive silver ink reference electrode and a graphite pencil drawn counter electrode on paper. The PB modified graphite electrode possesses excellent electrocatalytic activity for the reduction of H_2O_2 and exhibits excellent sensitivity and stability for H_2O_2 determination at low potentials. The linear range for the determination of H_2O_2 was from 5 to 50 mmol l^{-1} and the detection limit was 4.0 mmol l^{-1} . The developed paper-based electrochemical device was also successfully applied to the determination of H_2O_2 in aseptically package UHT milk spiked with H_2O_2 . By providing a useful avenue for the determination of H_2O_2 in milk, this work expanded the scope of the analytical methods for H_2O_2 determination, and it can be further developed as a platform for routine analysis.

References

- [1] Nurak, T., Praphairaksit, N. and Chailapakul, O., 2013. Fabrication of paper-based devices by lacquer spraying method for the determination of nickel (II) ion in waste water. *Talanta*, 114, 291-296.
- [2] Puiu, M., Mirceski, V. and Bala, C., 2021. Paper-based diagnostic platforms and devices. *Current Opinion in Electrochemistry*, 27, 100726, <https://doi.org/10.1016/j.coelec.2021.100726>
- [3] Liana, D.D., Raguse, B., Gooding, J.J. and Chow, E., 2012. Recent advances in paper-based sensors. *Sensors*, 12(9), 11505-11526.
- [4] Cate, D.M., Adkins, J.A., Mettakoonpitak, J. and Henry, C.S., 2015. Recent developments in paper-based microfluidic devices. *Analytical Chemistry*, 87(1), 19-41.
- [5] Nery, E.W. and Kubota, L.T., 2013. Sensing approaches on paper-based devices: A review. *Analytical and Bioanalytical Chemistry*, 405(24), 7573-7595.
- [6] Mahadeva, S.K., Walus, K. and Stoeber, B., 2015. Paper as a platform for sensing applications and other devices: A review. *ACS Applied Materials & Interfaces*, 7(16), 8345-8362.
- [7] Silva, R.A.B., Montes, R.H.O., Richter, E.M. and Munoz, R.A.A., 2012. Rapid and selective determination of hydrogen peroxide residues in milk by batch injection analysis with amperometric detection. *Food Chemistry*, 133(1), 200-204.
- [8] Ping, J., Mao, X., Fan, K., Li, D., Ru, S., Wu, J. and Ying, Y., 2010. A Prussian blue-based amperometric sensor for the determination of hydrogen peroxide residues in milk. *Ionics*, 16(6), 523-527.
- [9] Ping, J., Wu, J., Fan, K. and Ying, Y., 2011. An amperometric sensor based on Prussian blue and poly(o-phenylenediamine) modified glassy carbon electrode for the determination of hydrogen peroxide in beverages. *Food Chemistry*, 126(4), 2005-2009.
- [10] Siimenson, C., Kruusma, J., Anderson, E., Merisalu, M., Sammelselg, V., Lust, E. and Banks, C.E., 2012. Prussian blue modified solid carbon nanorod whisker paste composite electrodes: Evaluation towards the electroanalytical sensing of H_2O_2 . *International Journal of Electrochemistry*, 2012(1), <https://doi.org/10.1155/2012/238419>.
- [11] Moscone, D. and D'ottavi, D., 2001. Construction and analytical characterization of Prussian blue-based carbon paste electrodes and their assembly as oxidase enzyme sensors. *Analytical Chemistry*, 73(11), 2529-2535.
- [12] Lima, L.S., Rossini, E.L., Pezza, L. and Pezza, H.R., 2020. Bioactive paper platform for detection of hydrogen peroxide in milk. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 227, 117774, <https://doi.org/10.1016/j.saa.2019.117774>.

- [12] Karyakin, A., 2001. Prussian blue and its analogues: Electrochemistry and analytical applications. *Electroanalysis*, 13(10), 813-819.
- [13] Karimi, A., Husain, S.W., Hosseini, M., Azar, P.A. and Ganjali, M.R., 2018. Rapid and sensitive detection of hydrogen peroxide in milk by enzyme-free electrochemiluminescence sensor based on a polypyrrole-cerium oxide nanocomposite. *Sensors and Actuators B: Chemical*, 271, 90-96.
- [14] Gulera, M., Turkoglu, V., Kivrak, A. and Karahan, F. 2018. A novel nonenzymatic hydrogen peroxide amperometric sensor based on Pd@CeO₂-NH₂ nanocomposites modified glassy carbon electrode. *Materials Science and Engineering*, 90, 454-460.
- [15] Borràs-Brull, M., Blondeau, P. and Riu, J., 2021. The use of conducting polymers for enhanced electrochemical determination of hydrogen peroxide. *Critical Reviews in Analytical Chemistry*, 51(3), 204-217.