A Screen Printed Graphene Based Electrochemical Sensor for Single Drop Analysis of Hydroquinone in Cosmetic Products

Wannida Duekhuntod[1], Chanpen Karuwan[2], Adisorn Tuantranont[2], Duangjai Nacapricha[3,4] and Saowapak Teerasong[1,4,*]

[1] Department of Chemistry and Applied Analytical Chemistry Research Unit, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand
[2] National Electronics and Computer Technology Center, National Science and Technology Development Agency (NSTDA), Pathumthani 12120, Thailand
[3] Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahidol University, Bangkok 10400, Thailand
[4] Flow Innovative Research for Science and Technology Laboratories (FIRST Labs.)
[*]E-mail: saowapak.te@kmitl.ac.th

A graphene modified carbon paste sensor (or so-called graphene based sensor) was developed for electrochemical detection of hydroquinone. The sensor was fast fabricated using screen-printing technique. By integrating three fundamental electrodes on a single device, the sensor is small and portable. A determination is based on a one drop analysis. A 60 µL-drop of sample was placed onto the sensor prior to cyclic voltammetric measurement. The method relies on green analysis since it lowers the consumption of sample and waste generation. Under the optimal conditions, a linear calibration was achieved in range of 1.0×10^{-4} to 5.0×10^{-3} M hydroquinone. The detection limit was 7×10^{-5} M, a sensitive adequate for measuring hydroquinone in cosmetic products. The sensor provided good precision (%RSD = 2.78) and accuracy (recoveries = 87-114%), with analysis times of less than a minute. The sensor was applied to determine the presence of hydroquinone in whitening creams. The results obtained from the developed sensor satisfactorily agreed with the HPLC method, indicating the reliability of the method.   Due to its advantages in terms of rapidity, low-cost and portability, the device is a viable choice for on-site screening for hydroquinone contamination in whitening products.

1. INTRODUCTION

Skin-lightening products have received a lot of attention in Asian countries including Thailand. This is due to the desire of people in some countries to have a fairer skin color. In Thailand, over 60%

of face creams sold in the market have whitening agents among their ingredients [1]. Whitening agents commonly put into lightening products include ascorbic acid and its derivatives, alpha- and beta- hydroxyl acids as well as herbal extracts. Although these compounds have regulatory and safety perspectives, efficacy on skin lightening requires long-term use. Hydroquinone is a known effective depigmenting agent, however its side effects are the subject of controversy [2]. Hydroquinone containing cosmetics are still allowed (up to 2%) in the US but have been banned in Thailand for decades [3]. Unfortunately whitening products contaminating hydroquinone are occasionally found in local Thai markets. Consequently, there is random checking by Thai Food and Drug Administration (FDA) to find products contaminated with hydroquinone.

Various techniques have been exploited to determine the presence of hydroquinone. These include high performance liquid chromatography (HPLC) [4,5], spectrophotometry [6] and electrochemistry [7-9]. Among all them, electrochemistry offers a fast response, simple operation and possible miniaturization that support the technique so that it can be suitable for field-application. In the literature, there are many attempts focused on electrode modification to improve sensitivity of hydroquinone detection. Various materials such as NiO/carbon nanotubes [7], CuO/carbon nano-fragments [8] and MoS_2/graphene oxide [9] have been proposed as electrode modifiers. These modifiers were synthesized and incorporated onto a glassy carbon electrode (GCE) using a drop casting approach. The modified GCE showed superior response and was applied for sensing of hydroquinone at low level in water samples, i.e., tap water and river water. However, a conventional GCE is relatively large in size and requires a considerable sample volume (milliliters) for analysis. The homogeneity and stability of an electrode fabricated by drop casting is also addressed [10]. The modifier that has been physically adsorbed onto the surface of an electrode may be gradually stripped off in long term operations.

Graphene is a two-dimensional carbon material, in which the carbon atoms are arranged in a hexagonal lattice resembling a honeycomb [11]. Graphene possesses a large specific surface area and has excellent electrical conductivity. This material is frequently employed as an electrode modifier to promote electron transfer between a target analyte and electrode, leading high sensitivity of detection. Karuwan et al. developed a graphene modified carbon paste electrode using screen printing approach [12]. Graphene was synthesized and mixed with carbon paste. The mixture was screen printed onto a thin substrate using a commercial inkjet printer. The screen printed graphene-based electrode exhibited good performance for analyzing various compounds. These compounds included, hydrogen peroxide, nicotinamide adenine dinucleotide and ferri/ferro cyanide. With the screen printing approach, large numbers of electrode can be obtained in one production run. The screen printed electrode has advantages over the conventional GCE in terms its cost, disposability and size. It is therefore considered a potential tool for on-site applications.

There have been reported uses of screen printed electrodes for determining levels hydroquinone in cosmetic products. Yang et al. proposed a flow injection system coupled with electrochemical detection for quantifying hydroquinone in whitening creams [13]. The detection unit was composed of an electrochemical cell with three individual electrodes. A screen printed disk carbon ring electrode was fabricated and used as a working electrode, while conventional Ag/AgCl and Pt electrodes were

employed as reference and counter electrodes, respectively. However, use of this three electrode system is sometimes inconvenient. A flow injection method typically generates considerable waste.

In this work, an electrochemical graphene based sensor was developed by combining three electrodes on the single device using a screen printing approach. The sensor was small (0.8 cm wide and 4.0 cm long). The graphene sensor enhanced sensing response compared with a carbon electrode. A determination of hydroquinone can be performed based on a one drop analysis [14,15] by directly dropping a minute volume of sample onto the device prior to cyclic voltammetric measurement. With the proposed sensor, a conventional electrochemical cell is no longer need. The method presented here is considered green and low-cost, as well as suitable for field-analyses. The performance and application of the sensor to commercially obtained cosmetic products is discussed in the following sections.

2. EXPERIMENTAL

2.1 Chemicals and instruments

Deionized water (Zeneer Up900, Korea) was used throughout the experiment. Hydroquinone was purchased from Acros Organics (Belgium). $NaH_2PO_4 \cdot H_2O$ and $Na_2HPO_4 \cdot 7H_2O$ were acquired from QRec (New Zealand) and Panreac (Spain), respectively. A 0.1 M stock solution of hydroquinone was prepared with water. A series of working standard solutions was daily prepared by appropriate dilution of the stock solution. $NaH_2PO_4 \cdot H_2O$ and $Na_2HPO_4 \cdot 7H_2O$ was used to make a 0.1 M phosphate buffer (pH 7.0) which served as a supporting electrolyte.

A potentiostat (PGSTAT 128N Metrohm, the Netherlands) was employed for cyclic voltammetric measurement.

2.2 Fabrication of screen printed graphene-based sensor

Graphene powder was synthesized by electrolytic exfoliation in a polystyrene sulfonate solution as reported elsewhere [12]. The obtained graphene powder was thoroughly mixed with the carbon paste at a weight ratio of 1 to 9.

In this work three integrated electrodes were fabricated as a single device. The electrode alignment was designed using Visio. The fabrication consisted of three steps. Firstly, the graphene/carbon paste mixture was screen printed onto a polyvinyl chloride (PVC) substrate to construct the three electrodes. The screened substrate was dried at 60°C for 5 min. Next, Ag/AgCl paste was screen printed onto one of the electrodes to form the reference electrode. Here, the two existing graphene electrodes act as working and counter electrodes. The screened substrate was again dried at 60°C for 5 min. Finally, an insulating layer of polyurethane was screened printed onto the three electrodes to define the detection area. The screened substrate was then dried at 60°C for 5 min. The fabricated sensor is shown in Fig. 1a.
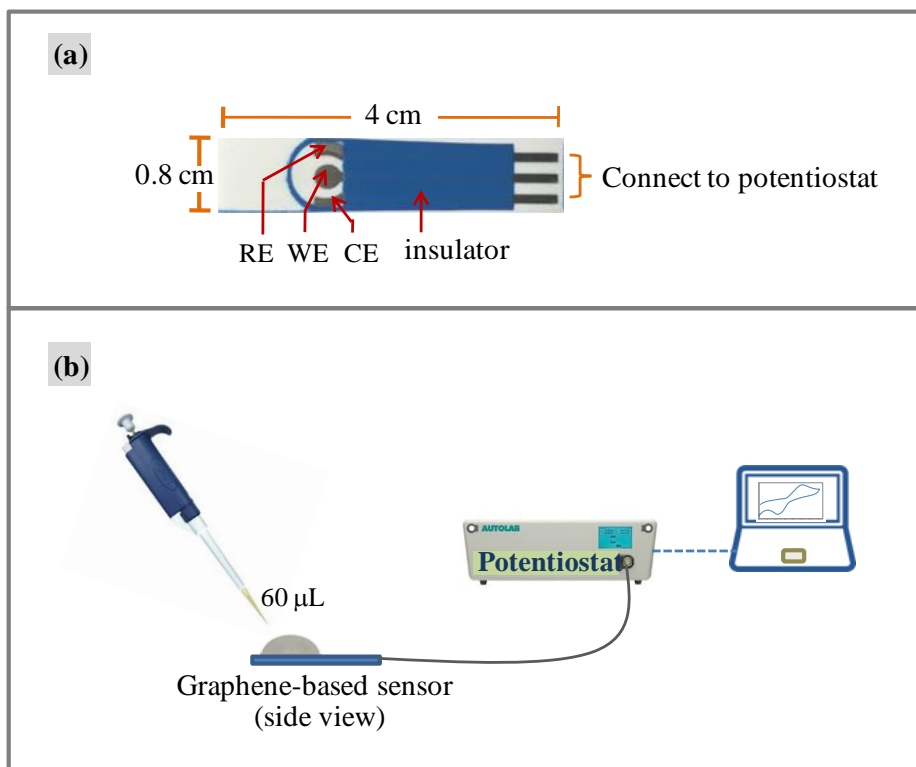
Figure 1. (a) A screen printed graphene based sensor with three integrated electrodes (top view) (WE: working electrode, RE: reference electrode and CE: counter electrode) and (b) An experimental set up for one drop analysis of hydroquinone

2.3 One drop analysis of hydroquinone

One drop analysis with cyclic voltammetric measurement was used for hydroquinone sensing. 1.00 mL of a standard or sample solution was thoroughly mixed with 1.00 mL of 0.1 M phosphate buffer in a vial. Subsequently, a 60 μL-droplet of the mixture was placed onto the screen printed graphene sensor using a micropipette. This volume of sample formed a droplet that covered all of three electrode surfaces of the sensor. The experimental set up is illustrated in Fig. 1b. Cyclic voltammetry was done by scanning three cycles over the potential range of -0.8 to +0.6 V with a scan rate of 100 mV s^{-1}.

2.4 Sample preparation

Various brands of whitening cream were randomly purchased from local night markets and from the Internet. Some of these samples did not have a FDA registration number on their packages, meaning that they were not subject to FDA approval. Three grams of cream were accurately weighed and then 25.00 mL of water was added with moderate stirring. These sample solution was incubated in a water bath at 40°C for 20 min. The extract was centrifuged at 5000 rpm for 60 min, and subsequently filtered through a 0.45 μm nylon membrane before analysis.

2.5 Comparison method

The results of hydroquinone concentration in whitening creams obtained from the developed sensor were compared to those obtained from a HPLC method [5]. Analysis was performed in triplicate using an HPLC (Waters 486 Millipore Corporation, USA) with a UV detector. Separation was carried out under the following conditions: HiQ sil C18 HS column (150 × 4.6 mm, 5 µm), isocratic elution with water:methanol (80:20 v/v) mobile phase, 1.0 mL min^{-1} flow rate, 10 µL injection volume, 289 nm absorbance measurement, and a column temperature of 25°C.

3. RESULTS AND DISCUSSION

3.1 Characterization

A graphene modified carbon paste composite was used for fabrication of the screen printed sensor. The morphology of this material was examined using scanning electron microscopy (SEM). As shown in Fig. 2, polygonal nanosheets of graphene were distributed among fine carbon nanoparticles. The graphene sheets were very thin and semi-transparent to electrons. Their dimensions (width and length) were approximately 250-400 nm. Graphene increased the active surface area of the modified electrode and electron transfer was more pronounced [12].

Figure 2. SEM image of graphene modified carbon paste composite

3.2 Electrochemical behavior of the screen printed graphene based sensor

The electrochemical performance of the screen printed graphene based sensor was investigated by comparing it with that of an unmodified carbon paste sensor. In this study, a $[Fe(CN)_6]^{3-/4-}$ solution was used as an electrochemical indicator [16]. Cyclic voltammograms of the test solution using both

sensors are shown in Fig. 3. With the unmodified sensor, lower current peaks of the redox reaction were observed, while more pronounced peaks were achieved using the modified sensor. The graphene based sensor was clearly more effective than the unmodified carbon paste sensor.
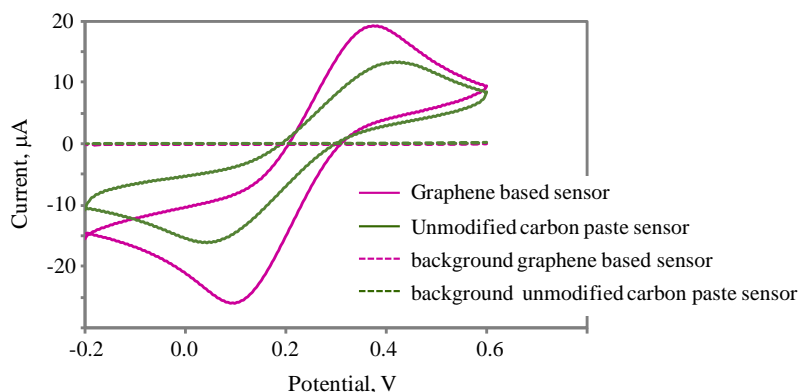

Figure 3. Cyclic voltammograms of the graphene based sensor for a 3.0×10^{-3} M of $[Fe(CN)_6]^{3-/4}$ in a 0.1 M KCl solution, compared to an unmodified carbon paste sensor
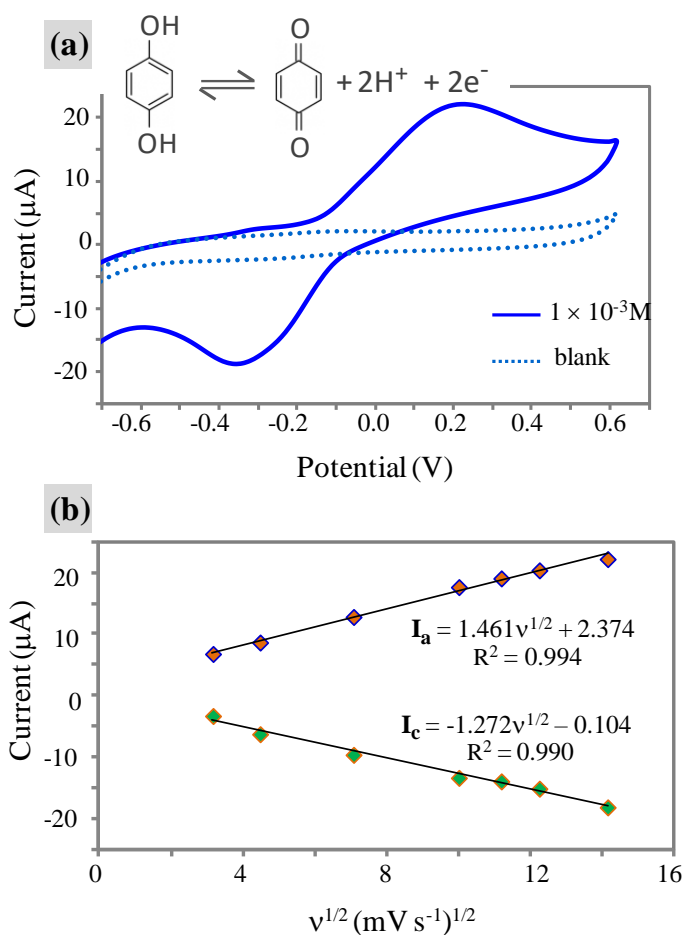

Figure 4. (a) Cyclic voltammograms of hydroquinone (0 and 1.0×10^{-3} M) in a 0.1 M phosphate buffer (pH = 7.0) on a graphene based device with scan rate of 100 mV s^{-1} and (b) A plot of anodic and cathodic peak currents *vs.* square root of scan rate ($v^{1/2}$) under the test solution, 1.0×10^{-3} M hydroquinone in 0.1 M phosphate buffer pH 7.0

The electrochemical behavior of hydroquinone in 0.1 M phosphate buffer (pH = 7.0) using graphene-based sensor was further studied. Fig. 4(a) shows well-defined anodic and cathodic current peaks of hydroquinone appearing at 0.22 and -0.36V, respectively. The reaction is reversible, which involves a two-electron transfer between hydroquinone and its oxidized form of benzoquinone [8].

The effect of the scan rate was examined in detail to elucidate the electrochemical process of hydroquinone at the graphene sensor. A solution of 1.0×10^{-3} M hydroquinone was analyzed at various scan rates ranging from 10 to 200 mV s^{-1}. It was found that both the anodic (I_a) and cathodic (I_c) peak currents increase with increasing scan rate. A linear relationship was observed between the peak currents (I_a and I_c) against square root of the scan rate ($\nu^{1/2}$) (Fig. 4(b)). The linear relationship was I_a (μA) = $1.461\nu^{1/2}$ + 2.374; R^2 = 0.994 and I_c (μA) = $-1.272\nu^{1/2}$ - 0.104 with R^2 = 0.990. This confirmed that a diffusion-controlled process dominated the redox reaction at the sensor [17].

3.3 Effect of pH and ionic strength

In previous work [7,9,18], phosphate buffer had appropriate properties for use as an electrolyte in electrochemical detection of hydroquinone. Therefore, phosphate buffer was selected as the supporting electrolyte in the current study. Since the pH and ionic strength of the buffer critically influence the sensitivity of detection, these two parameters were extensively investigated.

The effect of pH of phosphate buffer on the response of hydroquinone at the developed sensor was investigated at pH values of 5.8, 7.0 and 10.0. Fig. 5 shows that the highest sensitivity was attained at pH 7.0, which agrees with earlier reports for detection of quinones [7,9,18]. The redox reaction of hydroquinone is proton-dependent (see the chemical reaction in Fig. 4(a)). In an acidic solution (pH 5.8), excess protons may hinder the redox of hydroquinone.
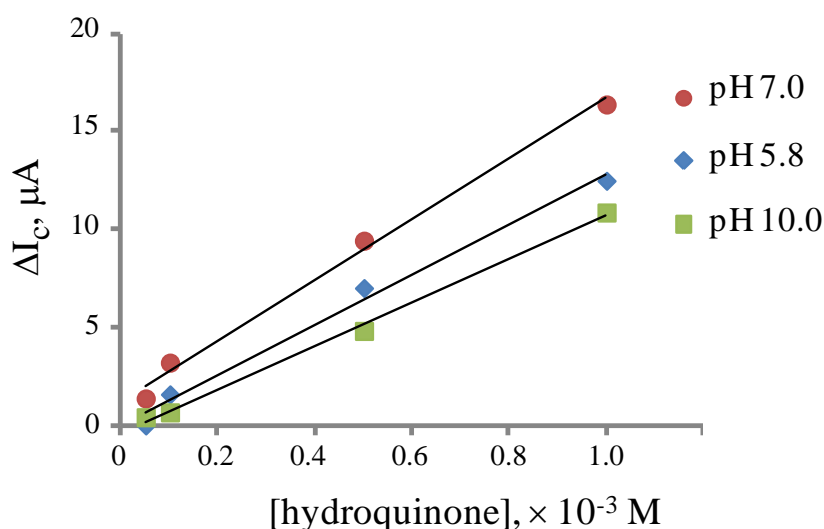

Figure 5. Effect of pH on the cathodic responses of hydroquinone, ΔI_c is a difference in cathodic signal of hydroquinone and blank solutions. The conditions were a 0.1 M phosphate buffer and a scan rate of 100 mV s^{-1}.

Alternatively, when the pH value was high (pH 10.0) hydroxyl groups on graphene structure and hydroquinone molecule may become deprotonated. Electrostatic repulsion between the negative charges of electrode and analyte caused a dramatic decrease in sensitivity. Consequently phosphate buffer at pH 7.0 was selected for subsequent experiment.

The effect of ionic strength was tested at pH 7.0. The concentration of phosphate buffer was varied at 0.01, 0.1 and 0.5 M. No significant difference in response obtained. In this work, 0.1 M phosphate buffer was chosen.

3.4 Effect of sample volume

The optimal volume of sample for one drop analysis was determined by dropping various sample volumes (i.e., 40, 60 and 80 μL) onto the sensor surface. It was visually observed that a 60 μL was the smallest volume that covered all of the electrode surface. A reproducibility of analysis was studied under this condition. A 60 μL aliquot of 1.0×10^{-3} M hydroquinone was applied to the sensor surface before voltammetric measurement. The relative standard deviation (RSD) of analysis was found to be 1.03% (three replicates). This indicated that a 60 μL-droplet was suitable for one drop analysis since it provided reproducible results. Therefore, this volume was selected for use in the next experiment.

3.5 Stability of graphene based sensor

The sensor signals attained on consecutive days were recorded to demonstrate a stability of the graphene based sensor. The same sensor was used to measure a 1.0×10^{-3} M hydroquinone solution for 10 days. When the sensor was not in use, it was stored dry at room temperature. A slight decrease in the current signal was observed after 10 days. The ten-day signal was about 95.9% that of its initial response. The stability of this sensor is comparable that of other electrodes used for detection of hydroquinone [7,9]. Here, it is noteworthy that the developed sensor offers simple fabrication and operation with good stability and reproducibility.

3.6 Performance of the graphene based sensor

After obtaining the optimal conditions, the analytical performance of the sensor was examined. A cyclic voltammogram was done, scanning from -0.8 to 0.6 V, at a scan rate of 100 mV s^{-1}. The anodic and cathodic responses were linearly proportional to the hydroquinone concentration over the range from 1.0×10^{-4} to 5.0×10^{-3} M (Fig. 6). In this work, the cathodic current was used to quantitatively analyze hydroquinone concentration, since it gave a bit higher sensitivity than that of anodic current. Linear regression was done yielding the following equation:

ΔI_c, μA = (9.98±0.35)[hydroquinone], $\times 10^{-3}$ M + (2.82±0.91)

where ΔI_c is the difference in cathodic peak current of hydroquinone and background (blank). The limit of detection (LOD) and limit of quantitation (LOQ) were estimated based on 3 times and 10 times of standard deviation of blank signal. The LOD and LOQ were found to be 7×10^{-5} M and 2×10^{-4} M, respectively. The sensitivity of the developed sensor is sufficient to quantify hydroquinone levels in cosmetics.
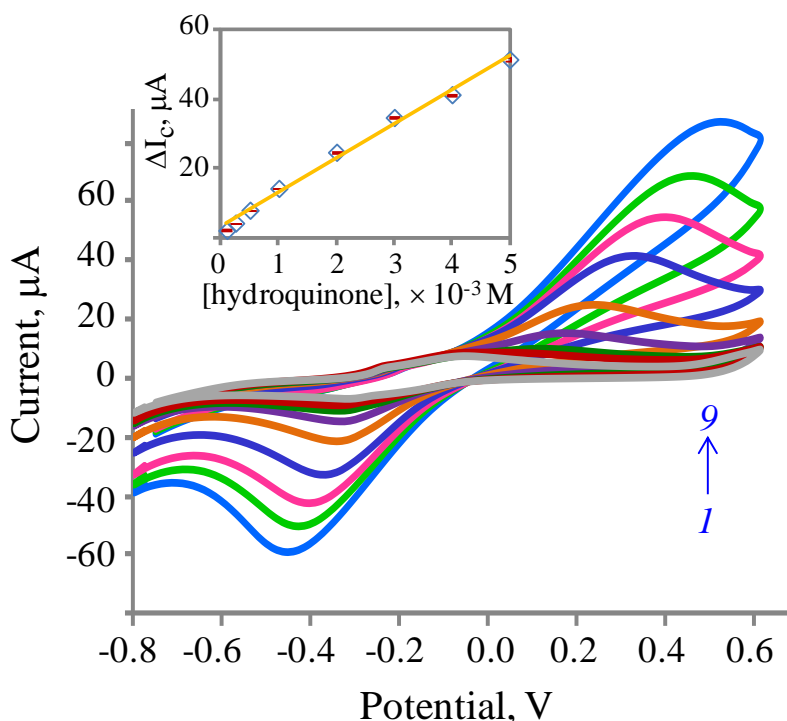

Figure 6. Cyclic voltamograms of hydroquinone with various concentrations (from *1* to *9*: 0.0, 0.1, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0×10^{-3} M). The inset shows the corresponding calibration plot. The conditions were a 0.1 M phosphate buffer pH 7 and a scan rate of 100 mV s^{-1}.

Moreover, the precision of the method was checked by measuring blank signals for five replicates. A RSD of the measurements was found to be 2.78%, indicating good precision of analysis was achieved. A voltammetric measurement can be completed within a minute, which is more rapid than other techniques such as HPLC [4,5] and spectrophotometry [6]. This sensor enables fast and inexpensive analysis with a portable device which makes it attractive for on-site applications. Table 1 lists the comparison of analytical parameters for hydroquinone using different electrodes. As the results showed, the current sensor provides a wide linear range and comparable precision of measurement to the previously reported [7-9,13,18].

Table 1. Comparison of the analytical parameters for hydroquinone detection using different electrodes

Electrode	Technique[*]	Linear range	Precision (%RSD)	Sample	[Ref.]
NiO/CNT glassy carbon electrode	DPV	0.01 – 0.5 mM	3.6	Tap water	[7]
CuO/CNF glassy carbon electrode	DPV	3 – 80 µM	-	River water	[8]
MoS2/RGO glassy carbon electrode	DPV	1 – 9 nM	3.5	Tap water	[9]
NiO/NPs/BMITFB carbon paste electrode	SWV	0.1 – 500 µM	2.2 – 3.8	Tap water Waste water	[18]
Preanodized screen-printed carbon electrode	AP	0.002 – 1.45 mM	0.8 – 2.3	Cosmetic products	[13]
Screen printed graphene based sensor	CV	0.1 – 5.0 mM	2.8	Cosmetic products	This work

[*]DPV: Differential pulse voltammetry; SWV: Square wave voltammetry; AP: Amperometry; CV: cyclic voltammetry

3.7 Application to cosmetic samples

The graphene based sensor was used to determine hydroquinone in whitening creams to verify practical application of the developed device. After sample preparation, the extracts of sample A to H were directly analyzed, whilst the extract of sample I and J needed 12-fold dilutions before analysis. The results are summarized in Table 2.

Table 2. Comparison of hydroquinone levels found in whitening creams using the screen printed graphene based sensor and HPLC method

sample	Add ($\times 10^{-3}$ M)	Found ($\times 10^{-3}$ M)[†]		Recovery (%)
		Graphene sensor	HPLC	
A	-	n.d.	n.d.	
	1.0	1.06 ± 0.05	1.06 ± 0.09	106
B	-	n.d.	n.d.	
	1.0	1.14 ± 0.04	1.17 ±0. 30	114
C	-	n.d.	n.d.	
	1.0	1.08 ± 0.02	1.37 ± 0.07	108
D	-	n.d.	n.d.	
	1.0	1.09 ± 0.01	1.35 ± 0.09	109
E	-	n.d.	n.d.	
	1.0	1.09 ± 0.01	0.97 ± 0.05	109
F	-	n.d.	n.d.	
	1.0	1.04 ± 0.01	1.00 ± 0.02	104
G	-	n.d.	n.d.	
	1.0	1.05 ± 0.04	1.14 ± 0.20	105
H	-	n.d.	n.d.	
	1.0	0.95 ± 0.02	0.68 ± 0.30	95
I	-	2.43 ± 0.01	1.33 ± 1.00	

	1.0	3.30 ± 0.02	3.46 ± 0.10	87
J	-	1.68 ± 0.05	1.57 ± 0.08	
	1.0	2.58 ± 0.01	2.21 ± 0.30	90

[†]determination was performed in triplicate

n.d. = not detect

Samples A to H were hydroquinone-free, however sample I and J were contaminated with hydroquinone. It was found that samples I and J contained 0.026 and 0.018 grams of hydroquinone per gram of cream, respectively. For comparison purposes, a HPLC method [5] was employed for determining hydroquinone in those samples. All samples were spiked with 1.0×10^{-3} M of hydroquinone and then analyzed using the graphene sensor and HPLC. Two sets of results were statistically compared using a paired *t*-test. No significant difference at 95% confidence level was found between the two methods ($t_{stat} = 0.92$, $t_{critical} = 2.20$). This revealed that the developed sensor is reliable for quantitative determination of hydroquinone. Additionally, the recovery was studied. Acceptable recoveries were obtained, in range of 87-114%, reflecting that other cosmetic ingredients did not interfere the analysis using the developed sensor.

4. CONCLUSIONS

A screen printed graphene based sensor is presented for fast and reliable detection of hydroquinone. A combination of three electrodes on a single device miniaturizes the sensor enabling easy use outside of the laboratory. Analysis of one drop of solution (60 μL) lowers the consumption of sample and waste generation, making it a green method of analysis. The sensor has enough sensitivity (LOD = 7×10^{-5} M) to detect hydroquinone in whitening products. Additionally, the developed sensor showed good stability and reproducible performance. This device was used to determine hydroquinone concentrations in commercially obtained whitening cream samples. The results correlated well with the HPLC results. Therefore it is a promising method for screening hydroquinone in adulterated cosmetics.

References

1. O. M. Secara and D. V. Sasu, *The impact of globalization in the industry of cosmetics*, Annals of Faculty of Economics, University of Oradea, (2013) Romania.
2. M. Matsumoto, H. Todo, T. Akiyama, M. Hirata-Koizumi, K. Sugibayashi, Y. Ikarashi, Y. Ono, A. Hirose and K. Yokoyama, *Regul. Toxicol. Pharm.*, 81 (2016) 128.
3. Cosmetic control group, Thai Food and Drug Administration (FDA),

http://www.fda.moph.go.th/sites/cosmetic/Pages/Main.aspx.

4. J. S. Jeon, B. H. Kim, S. H. Lee, H. J. Kwon, H. J. Bae, S. K. Kim, J. A. Park, J. H. Shim, A. M. Abd El-Aty and H. C. Shin, *Int. J. Cosmetic Sci.*, 37 (2015) 567.

5. P. López García, M. I. Rocha Miritello Santoro, E. R. M. Kedor-Hackman and A. Kumar Singh, *J. Pharm. Biomed. Anal.*, 39 (2005) 764.

6. M. Esteki, S. Nouroozi and Z. Shahsavari, *Int. J. Cosmetic Sci.*, 38 (2016), 25.

7. L. Zhao, J. Yu, S. Yue, L. Zhang, Z. Wang, P. Guo and Q. Liu, *J. Electroanal. Chem.*, 808 (2018) 245.

8. L. A. Alshahrani, L. Liu, P. Sathishkumar, J. Nan and F. L. Gu, *J. Electroanal. Chem.*, 815 (2018) 68.

9. Y. Peng, Z. Tang, Y. Dong, G. Che and Z. Xin, *J. Electroanal. Chem.*, 816 (2018) 38.

10. S. Cinti and F. Arduini, *Biosens. Bioelectron.*, 89 (2017) 107.

11. J. Xu, Y. Wang and S. Hu, *Microchim. Acta*, 184 (2017) 1.

12. C. Karuwan, A. Wisitsoraat, D. Phokharatkul, C. Sriprachuabwong, T. Lomas, D. Nacapricha and A. Tuantranont, *RSC Adv.*, 3 (2013) 25792.

13. H. -H. Yang, H. -H. Ting and Y. Shih, *Anal. Methods*, 8 (2016) 5495.

14. J. -M. Zen, T. -H. Yang, A. S. Kumar, Y. -J. Chen, J. -C. Hsu and Y. Shih, *Electroanal.*, 21 (2009) 2272.

15. Y. Zhao, L. Xu, S. Li, Q. Chen, D. Yang, L. Chen and H. Wang, *Analyst*, 140 (2015) 1832.

16. S. Reddy, B. E. K. Swamy, S. Ramakrishana, L. He and H. Jayadevappa, *Int. J. Electrochem. Sci.*, 13 (2018) 5748.

17. N. Elgrishi, K. J. Rountree, B. D. McCarthy, E. S. Rountree, T. T. Eisenhart and J. L. Dempsey, *J. Chem. Educ.*, 95 (2018) 197.

18. H. Soltani, A. Pardakhty and S. Ahmadzadeh, *J. Mol. Liq.*, 213 (2016) 63.