



Electrochemical determination of caffeine in beverage using graphene oxide modified glassy carbon electrode

E Murugan* & A Poongan

Department of Physical Chemistry, School of Chemical Science, University of Madras, Chennai 600 025, Tamil Nadu, India

E-mail: dr.e.murugan@gmail.com

Received 12 February 2021; accepted 01 September 2021

Graphene oxide (GO) has been synthesized through simple experimental method and used for surface modification of glassy carbon electrode, thus developed a new efficient GO/GCE as a sensor for identification and quantification of Caffeine in beverage samples. Common analytical techniques have been employed to establish the physiochemical properties of graphene oxide. The electro catalytic activity GO/GCE has been examined towards sensing and quantification of caffeine through Cyclic voltammetry and Differential pulse voltammetry. The results show high sensitivity with a wide linear range in concentration 3.2-183 μM and low detection limit (LOD) 0.043 μM . In addition, in order to inspect the real time application of GO/GCE, it has been also employed to determine the concentration of caffeine in cola, Red bull and coffee samples. Very low LOD has been observed for caffeine even in real sample analysis and hence this electrode can very well be expended for real time application and thus enable to maintain the quality control of the food samples.

Keywords: Beverages, Caffeine, Cyclic Voltammetry, Differential Pulse, Graphene oxide

It is well known that, the healthy growth of human cells usually depends on the proper building block of biomacro molecules including carbohydrate, protein fates, nucleic acids, vitamin and minerals. Again generation of healthy biomacro molecules/building blocks depends on occurrence of proper metabolism reactions and intake of quality food. In the current global scenario adulteration in the food are witnessed largely. Hence, analysis of foods and liquid refreshments plays a salient role in production industry. The universal consumption of beverages from past decades has increased extremely in adults and Children. So, analysis of beverages and food components is of high necessity due to food safety and security. Caffeine (CAF) (1,3,7 trimethylxanthine) was one of the common additives of various drinks and foods¹. CAF is a natural occurring alkaloids that is presenting in plants such as leaves, coffee seed, cola drinks and cocoa beans^{2,3}. It is used as a painkiller for simple headache preventing and relaxation. It is also accessible through consumption of foods. Especially CAF is mainly used as a psychoactive stimulant due to its ability to affect the central nervous system^{5,6}. Consumption of CAF 400mg/day is equal to taking up 4 to 5 cups of coffee⁷. In addition, Murugan and Kalpana reported the nanomolar detection of CAF in various beverages

samples⁸. The high dosage of CAF may lead to nausea, seizures and even cardio vascular problem^{9,10}. Hence, development of a reliable method for rapid detection of CAF is vital to avoid its excess consumption. Presently many methods are available to detect CAF which includes Mass spectrometry¹¹ and Gas chromatography¹²⁻¹⁴. In these methods there are some disadvantages like high cost and much time for detection. In contrast, the electrochemical methods are more reliable and fast method due to their rapid and accurate response.

Graphene Oxide (GO) due to its large conductivity, fast heterogeneous electron transfer and large surface area has been shown be an ideal material for electrochemical detection. GO is a more expensive electro active choice than the other carbon-based materials. It is integrated from graphite in two stages: in the primary, graphite was oxidized to graphite oxide, prompting the presentation of oxygen-containing utilitarian gatherings, like epoxy (C–O–C), hydroxyl (OH), carbonyl (C=O) and carboxyl (R–COOH) bunches into the basal plane and the edges of graphene oxide sheets. In the subsequent stage, the created graphite oxide is promptly peeled in water helped by sonication or shearing to deliver GO suspension. Different strategies were utilized to convert graphite to graphite oxide. Brodie strategy,

that was created in 1859, is the primary technique to deliver graphite oxide by adding KClO_3 to a combination of graphite and smoldering HNO_3 . Afterward, the Staudenmaier strategy was created in which a combination of H_2SO_4 and HNO_3 is utilized as the intercalant, creating graphite oxide with a higher C/O proportion. Today, the most far and wide GO amalgamation strategy is the Hummers' technique, created in 1958. In this technique, H_2SO_4 is utilized as the intercalant and $\text{NaNO}_3/\text{KMnO}_4$ as the oxidizing specialist. In any case, the Hummers' strategy has been condemned on account of the arrival of poisonous gases like NO_x and ClO_2 into the climate. All the more as of late, an improved Hummers' strategy was created by Tour by killing NaNO_3 , and utilizing a 9/1 blend of H_3PO_4 and H_2SO_4 . The Tour strategy does not kill the creation of poisonous gases, yet in addition produces a more oxidized graphite oxide with a more standard carbon structure and bigger sheet size^{15,16}. It is the hydrophilic oxidative derivative of Graphene and owing to its oxygen functionalities and direct electron transfer properties¹⁷, in the present work it was used to modify GC Electrode (GO/GCE) to detect CAF present in real samples such as Coffee, black Tea and red bull energy drink.

Experimental Section

Apparatus

All electrochemical experiments were performed using HCHI electrochemical workstation. Measurements were carried out at room temperature, using a three electrodes namely Glassy Carbon electrode as a working electrode, platinum electrode as a Counter Electrode and Ag/AgCl Electrode as reference electrode. Further the patterns of GO recorded on Bruker D8 advance. The FT-IR spectrum recorded on Bruker-Tensor-27 instruments. The Raman spectra were recorded an EZ-Raman-Enwaveoptronics and morphological studies were carried out on HR-SEM Model FEIQuanta FEG200.

Materials and Methods

Materials

Graphite microparticles (<20mm), conc. nitric acid, hydrochloric acid, sodium phosphate dibasic and monobasic sodium chloride and caffeine were purchased from SRL (Sisco Research Laboratory) Double distilled water was used throughout the experiment. Tea bags, cola and red bull were purchased from local market.

Preparation of Graphene oxide (GO)

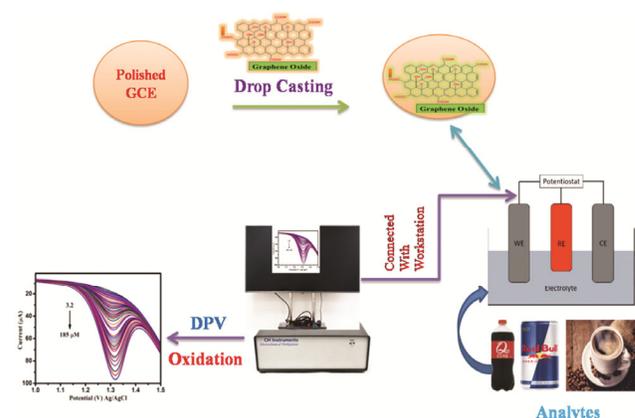
In typical procedures GO was synthesized produce using modified Hummers method¹⁸. Sulphuric Acid (25 mL) and of phosphoric acid (3 mL) were mixed and stirred for several minutes. Then, 0.225g of Graphite powder was added slowly to the solution. This mixture was stirred for 6 h until the solution became dark green. To eliminate the excess of KMnO_4 , 0.67 mL of hydrogen peroxide (H_2O_2) was added slowly and stirred for 10 min. The exo-thermal reaction occurred and allowed it to cool. HCl (10 mL) and of DD water (30 mL) were added and centrifuged for copious times. The washed GO solution was dried at 70°C for 24 h in the air oven.

Fabrication of GO/GCE

Prior to fabrication, GCE was polished to a mirror-like surface and subsequently with fine grade alumina powder having size $0.05\ \mu\text{m}$. Then the electrode was rinsed with ethanol and distilled water dried at room temperature. The 5 mg of GO was dispersed in 5 mL of DD water and its used as a stock solution. Prior GCE coating the stock solution was sonicated 10 min for uniform dispersion, and then 5mL of stock solution was drop casted on the surface of the GCE.

Real sample preparation and measurement procedure

The beverage sample was properly diluted in phosphate buffer solution (PBS) before measurement. The coffee solution was prepared by dissolving a Table spoon of coffee powder in 100 mL of boiling water. Tea solution was prepared by immersing the tea bag in 100 mL warm water. Cola beverage and energy drink were used directly without any purification.



Scheme 1

Results and Discussion

XRD analysis of GO

The synthesized GO was analyzed by X-Ray Diffraction (XRD) JCPDS-ICDD library database was used to determine the average crystal size of GO structure properties Fig. 1. Shows the spectrum of GO indicating the formation of peaks at $2\theta = 11^\circ.12$ (002). The peak $2\theta = 11^\circ.12$ indicate oxidation of Graphite to Grapheneoxide¹⁹. The average crystalline size of GO was calculated by the wellknown Debye-Scherrer equation.

$$D = K\lambda / \beta \cos\theta \quad \dots(1)$$

Where, β is the full peak width at half maximum (FWHM) expressed in radians. θ half diffraction angle of the peak corresponding to inter-layer spacing of ($2\theta = 11^\circ.12$) for GO and K a constant. The average to crystallite size of GO was calculated to 18.17nm.

FT-IR analysis of GO

The FT-IR spectra of GO is known in Fig 2. The spectrum of graphite it is also known in the same figure. The characteristic stretching vibration of C=C the C-O and C=O of GO showed peaks at 1619, 1048 and 1862 cm^{-1} respectively. This is an indication for oxidation of Graphite to GO^{20,21}. The intense and broad peak due to O-H stretching vibration of - and O-H groups occurred at 3265 cm^{-1} .

Raman analysis of GO

The Raman spectroscopy used to study the disorder and defects in the crystal structure. The disorder was determined by the intensity ratio between the D band

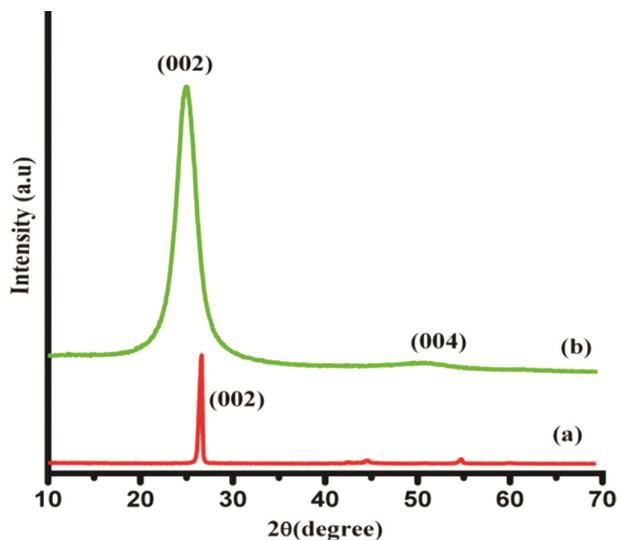


Fig. 1 — XRD pattern of (A) Graphite and (B) Graphene oxide

and the Raman allowed G band as ID/IG. Where Graphite flake as oxidized to G, the G band peak broadened and the D band intensity increased indicating the decrease in the size of the in plane sp^2 sites possibly due to oxidation process, the D band for the GO appeared at $\sim 1326\text{cm}^{-1}$ and G band at $\sim 1602\text{cm}^{-1}$ (Fig. 3).

Morphological studies of GO

The Scanning Electron Microscope (SEM) was employed to provide the structure and morphology of GO. Fig. 4.(A) shows cloth like morphology due to π - electron clouds and the oxygen containing functional groups like -O-H and -COOH and epoxides. Similar morphology for GO was also reported in the

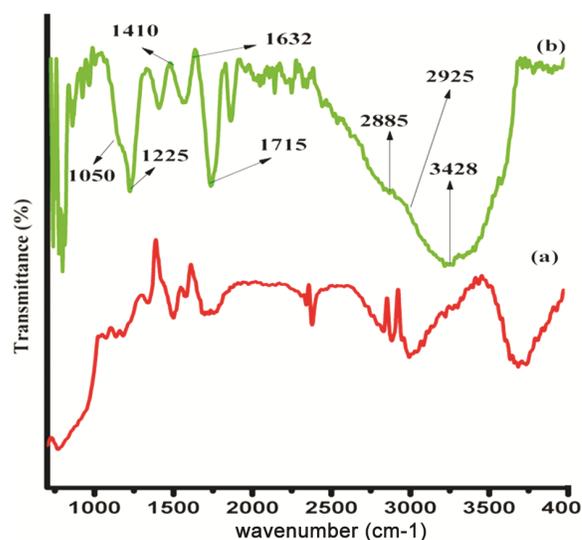


Fig. 2 — FT-IR pattern of (A) Graphite and (B) Graphene oxide

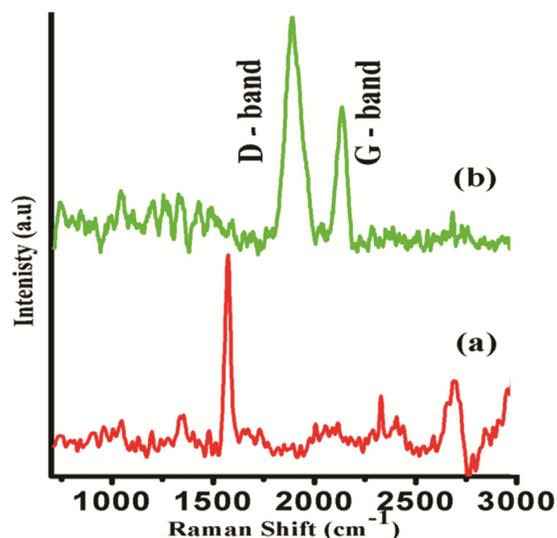


Fig. 3 — Raman spectra of (A) Graphite and (B) Graphene oxide

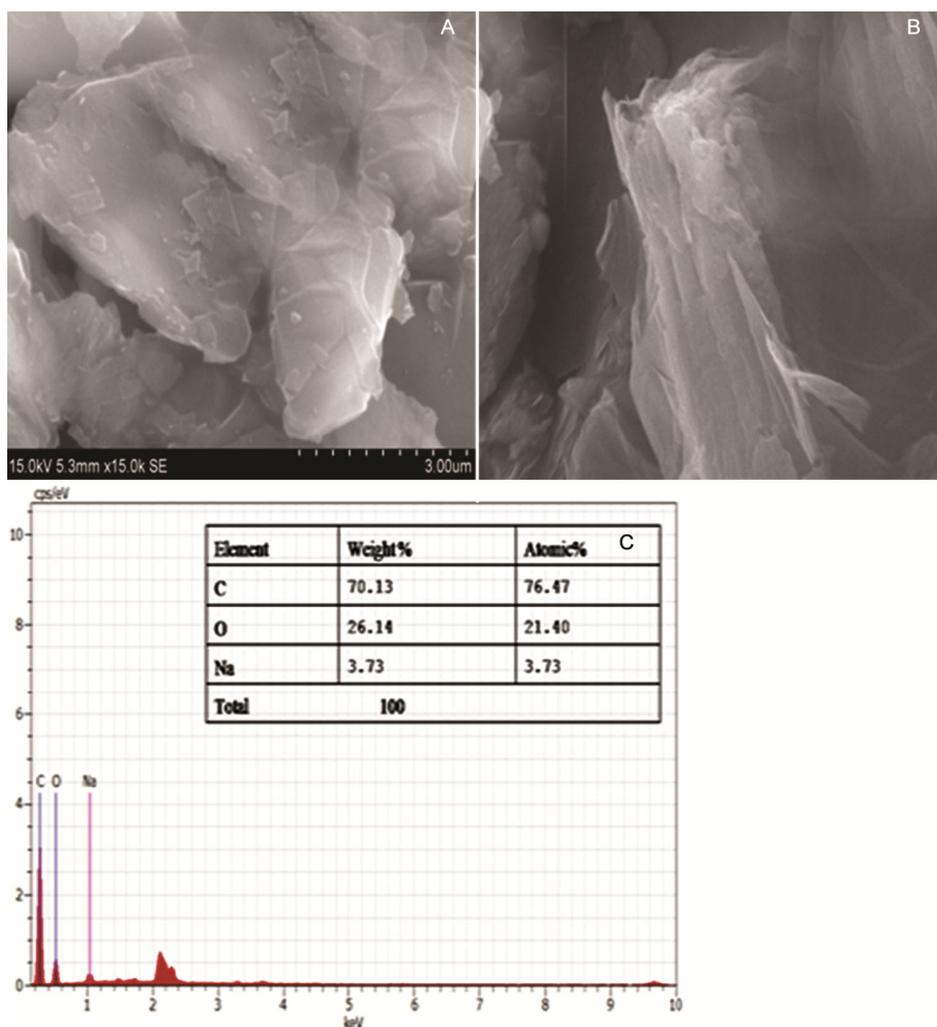


Fig. 4 — FE-SEM of (A) Graphite with EDAX and (B) GO with EDAX

literature^{22,23}. The EDAX spectrum of GO is shown in Fig.4. (B) And its results confirm the presence C, O that constitute of GO, depicted layers of Graphene, which indicate an effective exfoliation of GO.

Electrochemical characterization

The electro active surface area of working electrode was determined by $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as redox probe by Cyclic voltammetry (CV). Figure 5 (A) showed the CVs of Bare GCE and GO/GCE recorded with 1 mM of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl at 50 mV/s. A pair of redox peak was observed at the surface of bare electrode (curve a) with peak separation 225 mV/s. GO/GCE exhibited high intense redox peak with ΔE_p value 165 mV which is smaller when compared with bare GCE. The observed minimum ΔE_p and the corresponding improvement in the redox peak current at GO/GCE indicate good electron transfer kinetics and

large surface area. The active surface area of GO/GCE was calculated using Randles-Sevcik equation 2.

$$I_p = (2.69 \times 10^5) n^{3/2} D^{1/2} C A v^{1/2} \quad \dots(2)$$

Where, a represents the microscopic area of the working electrode, n the number of the electrons ($n = 1$) D , the diffusion co-efficient ($6.626 \times 10^{-6} \text{ cm}^2/\text{s}$), C the concentration of the $[\text{Fe}(\text{CN})_6]^{3-/4-}$

(1 mM) and V the scan rate (V/s). From the plot of $I_p v$ s square root of scan rate ($v^{1/2}$) (Fig.5(c)) the square root of bare GCE and GO/GCE equal to 0.03 and 0.432 cm^2 respectively was obtained.

Electrochemical behaviour of CAF

Cyclic voltammetry was used to study the electrochemical behaviour of CAF at GO/GCE. Mechanism of electrochemical oxidation of CAF is given in Scheme 2 As discussed in the experimental

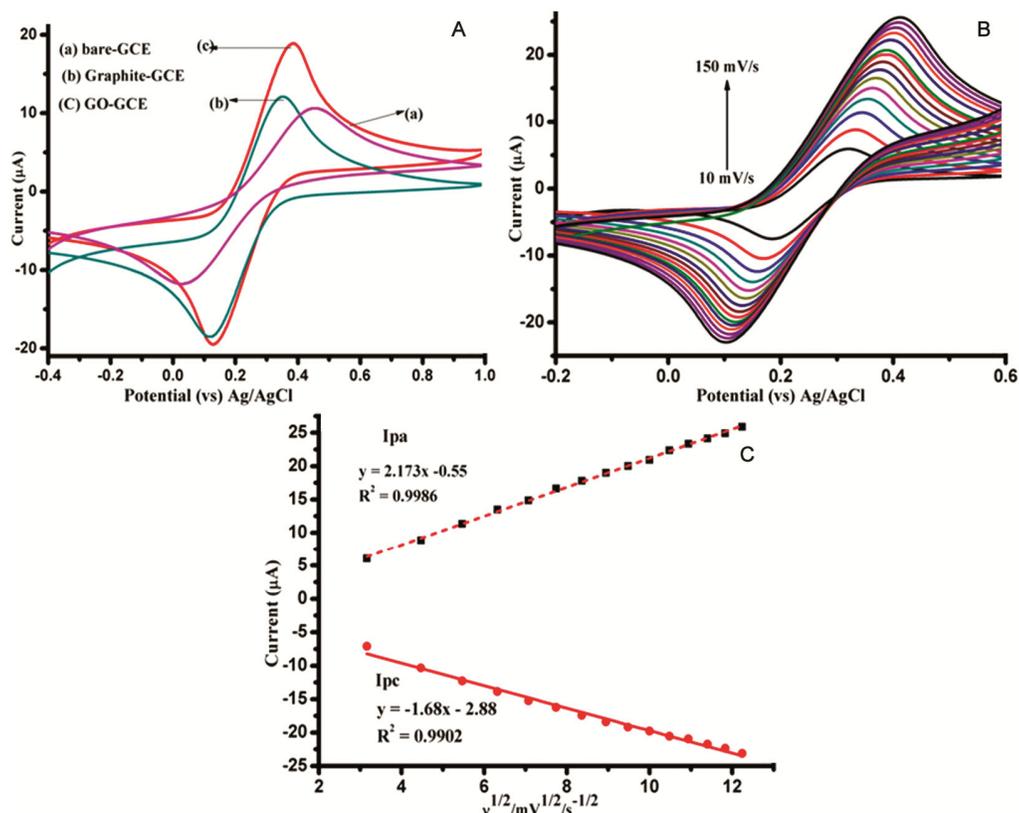
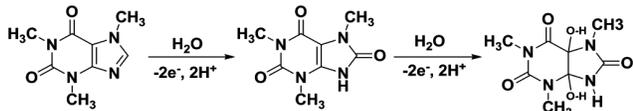


Fig. 5 — CVs of (A) different modified electrodes; (B) Effect of scan rate in 0.1 M KCl/ 0.1 M[Fe(CN)₆]^{3-/4-} at GO/GCE and (C) Plot of *i* peak vs Squire root of scan rate



Scheme 2 — Mechanism of electrochemical oxidation of CAF

Table 1 — Peak potentials and peak currents for CAF oxidation at different electrodes.

Electrodes	Epa (V)	Ipa (αA)
Bare GCE(Control)	1.45	1.04
Graphite/GCE	1.42	1.52
GO/GCE	1.32	2.04

section three types of electrode was fabricated and these are shown in Fig. 6 (A). It shows the CVs response of A) bare GCE, B) Graphite/GCE and C) Graphene Oxide/GCE. Its can seen in the absence of CAF no peak potential was observed. In contrast presence of (8.6μM) of CAF showed the oxidation peak at 1.35V. Absence of reduction peak indicated that CAF undergoes an irreversible oxidation at both bare and GO/GCE, which is agreement with previously reported works^{24,25}. The observed oxidation potentials of CAF at different electrodes are presented in Table. 1. Particularly, GO/GCE showed a higher electro-catalytic

activity toward CAF oxidation almost 1.2 times higher than bare-GCE. Also it showed higher peak current and lower potential due to easy charge transfer ability and thus improved electron transport between CAF and the working electrode.

Effect of Scan rate

To establish the transport characteristics of the GO/GCE in the influence of scan rate from 10 to 100mV/s was examined. The results are shown in Fig. 7 (a). As can be seen in the figure, the oxidative peak current shifts to higher positive value with an increase in the scan rate, thus confirming irreversibility of the oxidation yielded a CAF at the modified electrode²⁶. Plot of log *I*_p of anodic peak current vs log scan rate linear regression equation of $I_{pa}(\mu A) = 0.2578(v^{1/2}mV^{1/2}/s^{1/2} + 1.0379)$ ($R^2 = 0.9943$) as shown in Fig.7(b). The GO/GCE for oxidation of CAF followed diffusion controlled kinetics which in agreement with reported works²⁷.

Effect of pH

The effect of pH on the electrode chemical response of GO/GCE for CAF was studied in the pH range of 3 to 11 shown in Fig. 8(A). Furthermore the

pH of electrolyte can influence the peak shape and peak potential and the peak current on the GO/GCE. It is more useful for estimation of proton to electron involved in the electrode reaction. It can be seen in the Fig. the oxidation peak current increased in pH 7. Thus pH 7 was selected as the optimum pH which was used in the sub sequential experiments. The

electro chemical reaction of oxidation involves four electrons and four protons. The first step of the reaction was two electrons and two proton oxidation of the C-8 and N-9 bond giving substituted uric acid. Then eliminate two electron and two proton oxidation of the 4, 5 diol analogous of uric acid occurs, which is agreement with previously reported^{28, 29}.

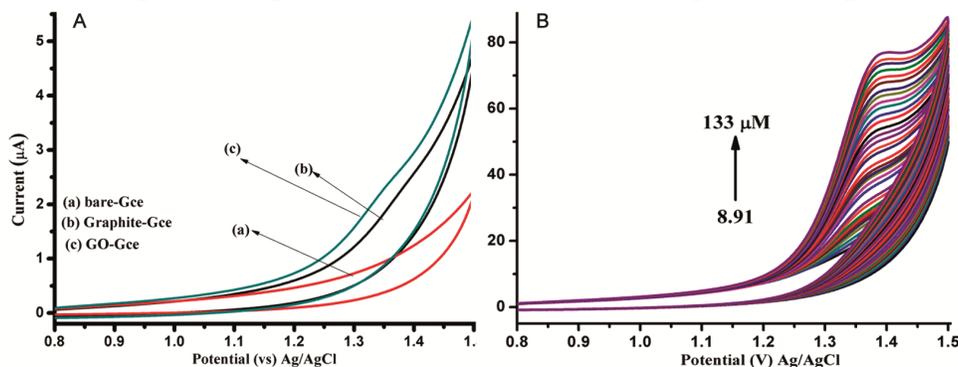


Fig. 6 — (A) CVs of (a) bare GCE, (b) Graphite/GCE; (c) GO/GCE in 0.1 M PBS containing 1mM CAF (at a scan rate of 50 mV s^{-1}) and (B) various concentration of CAF

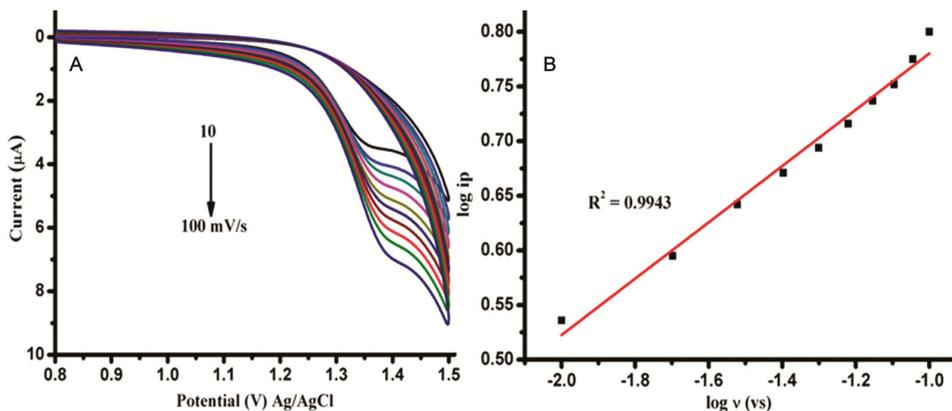


Fig. 7 — CVs of GO/GCE in 0.1 M PBS (pH 7.2) containing 1mMCAF (A) At various scan rates from 10 to 100 mV s^{-1} and (B) Calibration plot of $\log I_p$ vs \log scan rate.

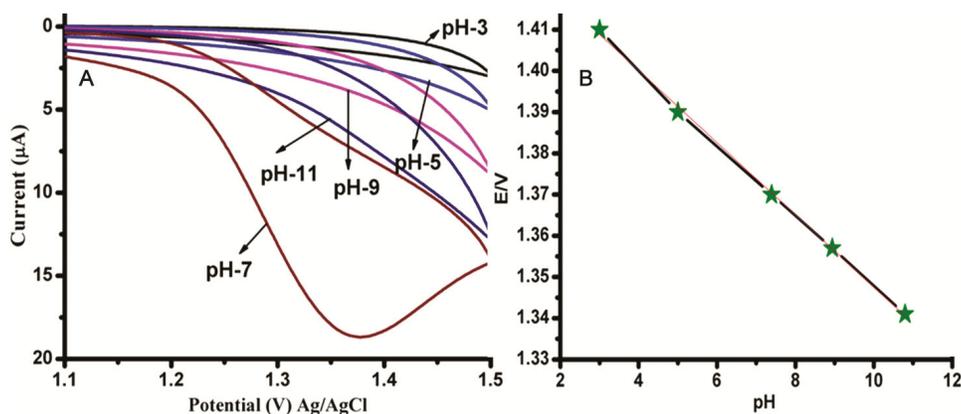


Fig. 8 — (A) Cyclic voltammograms of $8.9 \mu\text{M}$ of CAF on GO/GCE in 0.1 M PBS with different pH at a scan rate of 50 mV s^{-1} and its plot (inset) and (B) pH linear plot

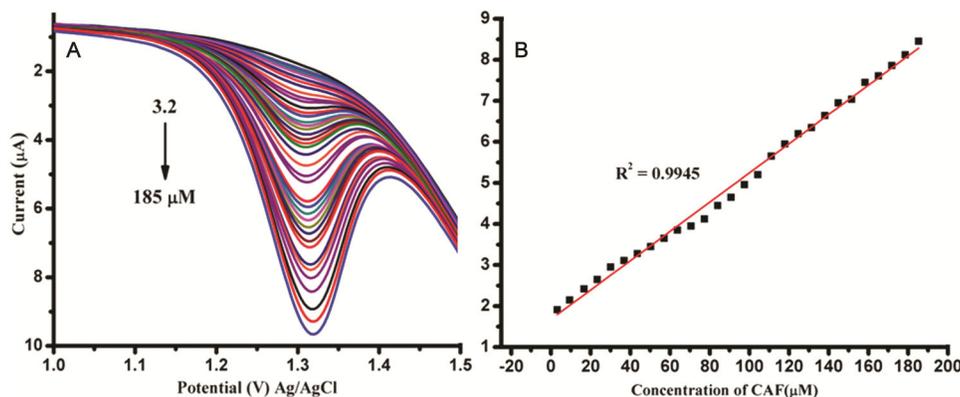


Fig. 9 — (A) Differential pulse voltammograms of GO/GCE at different concentration of CAF in 0.1 M PBS and (B) Calibration plot of peak current vs CAF concentrations.

Table 2 — Comparison of analytical parameters of electrocatalytic oxidation of CAF at different electrodes.

Various electrodes	Experimental technique	Linear range (μM)	LOD (μM)	Ref
FMWCNT/GCE	DPV	10-100	3.54	30
PST/Nafion/GCE		03-100	0.10	31
Large mesoporous/Carbon/Nafion/GCE	DPV	1.3-230	47	32
CA-ZN/GCE	DPV	39.8-458	28.5	33
Nafion/GNP/LBL/Flim	CV	50-5000	24	34
Nafion/PDDA-MWCNT/GCE	DPV	0.3-80	0.05	35
GO/GCE	DPV	0.3-182	0.043	This Work

Fig. 8 (B) showed the linear relationship between the anodic peak potential and pH with linear regression equation of $E_p (\text{V}) = -0.00876 \text{ pH} + 1.435$ ($R^2=0.9943$). According to the following Nernst equation

$$dE_p/dpH = 2.303 \text{ mRT}/nF \quad \dots(3)$$

where, F is the Faraday Constant (96485 C/mol) R the universal gas constant ($8.314 \text{ JK}^{-1}\text{mol}^{-1}$), T the temperature (298K) m number of proton and involved in the number of electron involved for CAF oxidation. It's showed that the number of e^- and proton oxidation of CAF is equal.

Voltammetric determination of CAF

The Differential pulse voltammetry (DPV) has advantages of increase in sensitivity and high resolution in quantitative analysis compared CVs. It is also used to study the linear detection range; Figure 9 (A) shows the DPV curves of the GO/GCE towards CAF 3.2 to $185\mu\text{M}$ in PBS. The anodic peak response of CAF was found to be proportional to its concentration. Figure 9(B) showed the linear regression equation of $I_{p(\mu\text{A})} = 0.03564[\text{CAF}/\mu\text{M}] + 1.68$ ($R^2= 0.9946$). The limit of detection was found to be $0.043\mu\text{M}$. The detail analytical performance was compared with previous reported sensors in Table 2.

Anti-interference study

To establish the anti-interference property of the GO/GCE, the oxidation of CAF was examined in the presence of F^- , Cl^- , Br^- , CO_3^{2-} , HCO_3^- , Mg^{2+} , Ca^{2+} , Cd^{2+} , Cu^{2+} , NH_4^+ at 10 fold concentration of CAF. No interference was observed. These results prove the suitability of the present sensor for practical applications.

Stability, reproducibility and repeatability

The GO/GCE electrode was stored for 11 days in 0.1 M PBS and $100 \mu\text{L}$ of 0.01 mM CAF at room temperature to check the long term stability. A slight change in density was observed, thus proves the electrode stability. Similarly, the reproducibility of the GO/GCE was studied by repeating the same experiments for five times (Fig. 10). The observed results showed 1.8% RSD proving the good reproducibility, thus concludes that the GO/GCE electrode can be used for practical applications.

Practical application

The prepared GO/GCE was utilized for determination of CAF in real samples such as cola and red bull energy drink. Both samples were analyzed without any pretreatment which means no pH adjustment, dilution or no filtration apart from PBS solution. The

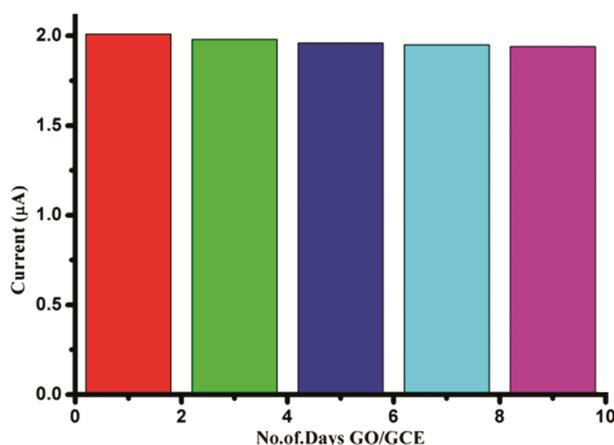


Fig. 10 — Reproducibility of GO/GC electrode

Table 3 — Analysis of Cola, Redbull and Coffee in real samples with GO/GCE

Sample s	Original (µM)	Added (µM)	Found (µM)	Recovery (%)
	CAF	CAF	CAF	CAF
S1	9.6	10	19.45	99.52
S2	9.4	10	19.41	99.47
S3	9.8	10	19.77	99.63

*Note: S1, Cola-sample; S2, Red-Bull; S3, Coffee

pH of the beverages was determined as 2.46 and 2.91 respectively, finally evaluated by coffee samples boiled and filtered for further analysis. Each sample was determined three times for relative standard deviation method (RSD) and the results are presented in Table 3 in addition the results of the CAF to stability accuracy of the method was studied the results of 101.32 confirm the efficiency of the proposed sensor towards the determination of CAF in samples with good recovery rate.

Conclusions

The GO was synthesized by modified Hummers method and characterized the same through XRD, Raman, FT-IR, SEM, with EDAX. The formation of GO was confirmed using FT-IR and XRD. Then the surface of GCE was modified with GO to obtain GO/GCE electrochemical sensor. The electrochemical efficiency of the electrode was evaluated through sensing and determination of CAF. On comparing the CV results of control electrodes such as bare GCE and Graphite/GCE, the latter showed a significant increase in oxidation current with the peak potential shifted towards positive region. Similarly, the DPV results showed a significant increase with increase in the concentration from 3.2 to 183 µM with LOD of

0.043 µM. The GO/GCE showed excellent sensitivity, and wide linear range with lower detection limit. The developed electrode was also employed for sensing and determination of CAF concentration in Cola, Red bull, and Coffee samples. The electrode showed a good recovery rate of 99.44, 99.45, and 99.77%, respectively. Therefore, the results of this study provide a way to utilize the GO/GCE for real sample analysis and quantification of CAF in various beverages.

Acknowledgement

The authors acknowledge DST-SERB-EMEQ New Delhi, Government of India, for providing financial assistance.

References

- Aklilu M, Tessema M & Redi-Abshiro M, *Talanta*, 76 (2008) 742.
- Osman H, Nasarudin R & Lee S L, *Food Chem*, 86 (2004) 41.
- Revelle W, Condon D M, Wilt J & Ramachandran V S, *Academic Press*, Please tell me volume number (2012) 423.
- Barone J J & Roberts H R, *Toxicol*, 34 (1996) 119.
- Cauli O & Morelli M, *Behav Pharm*, 16 (2005) 63.
- Nehlig A, Daval J L, Debry G & Brain, *Res Rev*, 17 (1992) 139.
- Nawrot P, Jordan S, Eastwood J, Rotstein J, Hugenholtz A & Feeley M, *Food Addit Contam*, 20 (2003) 1.
- Murugan E & Kalpana K, *Anal Chem*, 91 (2019) 5667.
- Peri-Okonny U L, Wang S X, Stubbs R J & Guzman N A, *Electrophoresis*, 26 (2005) 2652.
- Artin B, Singh M, Richeh C, Jawad E, Arora R & Khosla S, *Am J Ther*, 17 (2010) 169.
- Norton K L & Griffiths P R, *J Chromatogr A*, 703 (1995) 503.
- Tzanavaras P D & Themelis D G, *Anal Chim Acta*, 581 (2007) 89.
- Franeta J T, Agbaba D, Eric S, Pavkov S, Aleksic M & Vladimirov S, *Farmaco*, 57 (2002) 709.
- Aranda M & Morlock G, *J Chromatogr Sci*, 45 (2007) 251.
- Pumera M, *Chem Soc Rev*, 39 (2010) 4146.
- Pumera M, Ambrosi A, Bonanni A, Chng E L K & Poh H L, *Trends Anal Chem*, 29 (2010) 954.
- Santhoshkumar S & Murugan E, *Appl. Surf. Sci.* 553 (2021) 149544.
- Zaabaa N I, Foa K L, Hashima U, Tanb d, S J Wei-Wen Liua c & Voona C H, *Procedia Eng*, 184 (2017) 469.
- Hidayah N M S, *AIP Conf Proc*, 1892 (2017) 150002.
- Song J, Wang X & Chang C, *Hindawi*, 6 (2014) 276143.
- Javed S I & Hussain Z, *J Electrochem Sci*, 10 (2015) 9475.
- Murugan E, Rubavathy Jaya Priya A, Janaki Raman K, Kalpana K, Akshata C R, Santhosh Kumar S & Govindaraju S, *J. Nanosci. Nanotechnol*, 19 (2019) 7596.
- Murugan E, Dhamodharan A, Poongan A & Kalpana K, *Indian J Chem*, 59A (2020) 1313.
- Amare M & Admassie S, *Talanta*, 93 (2012) 122.
- Lange U, Roznyatovskaya N V & Mirsky V M, *Anal Chim Acta*, 614 (2008) 1.
- Meareg A & Senait A, *J Anal Methods Chem*, 3979068 (2017) 8.

- 27 Uslu B & Ozkan S A, *Anal Lett*, 40 (2007) 5817.
- 28 Svorc L u, Tom cík P, Svitková J, Rievaj M & Bustin D, *Food Chem*, 135 (2012) 1198.
- 29 Alpar N, Yardım Y & Sentürk Z, *Sens Actuators B Chem*, 257 (2018) 398.
- 30 Amiri-Aref M, Raof J B & Ojani R, *Sens Actuators B Chem*, 192 (2014) 634.
- 31 Guo S, Zhu Q, Yang B, Wang J & Ye B, *Food Chem*, 129 (2011) 1311.
- 32 Gao Y, Wang H & Guo L, *J Electroanal Chem*, 706 (2013) 7.
- 33 Arroyo Gómez J J, Villarroel-Rocha D, de Freitas-Araújo K C, Martínez-Huitile C A & Sapag K, *J Electroanal Chem*, 822 (2018) 171.
- 34 Sandra H A, Afshin T, James, McGettrick D & Paolo B, *Nanomaterials*, 9 (2019) 221.
- 35 Shu X, Bian F, Wang Q, Qin X & Wang Y, *Int J Electrochem Sci*, 12 (2017) 4251.