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## Nanomaterial Assisted Electrochemical Detection of Isolated Piperine: a Phytochemical From Long Pepper

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Herein, piperine, a phytochemical present in long pepper was quantitatively analyzed *via* an electrochemical technique using a chemically modified electrode. Mesoporous nanomaterial was utilized as a base matrix to carry out the experiments. The piperine was isolated from crude long pepper through a standard procedure. Glassy carbon electrode was chemically modified with mesoporous carbon matrix and isolated piperine designated as GCE/GMC@piperine. The cyclic voltammetry response gave a perfect redox response of piperine at  $E' = +0.2$  V vs. Ag/AgCl at  $50 \text{ mV s}^{-1}$  in pH 7 PBS. Effects of scan rate and solution pH were studied. Further, it was observed that a change in concentration of piperine is directly proportional to the redox peak current obtained. Therefore, this study could act as a key for quantitative analysis of piperine, a naturally occurring phytochemical in natural products such as pepper, long pepper, white pepper, etc. This is a prototype study and can be further extended to disposable screen printed electrodes for portable analysis.

**Keywords:** Piperine, Mesoporous carbon, Quantitative, Long pepper, Isolation

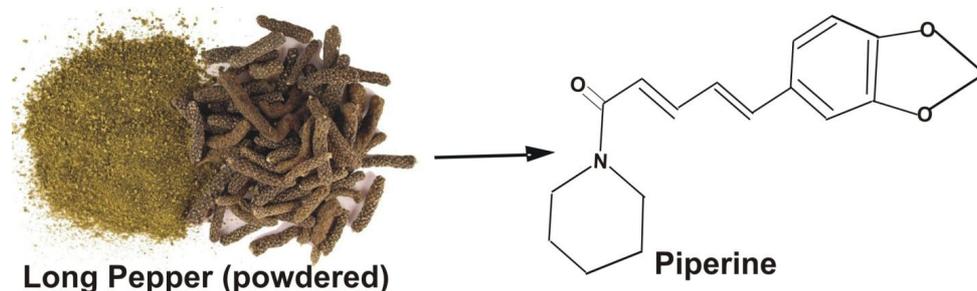
### INTRODUCTION

Nanotechnology has emerged as a cutting edge tool in various broad way applications such as medicinal, environmental, pharmaceutical, industrial, health management, information technology, electronics, engineering, etc. [1]. Employing nanotechnology, the researchers are successful to develop structured materials with target specified uses, for instance, nano material assisted drug delivery [2]. In addition, several other aspects including designing of energy storage devices [3], energy harvesting devices [4], anti-corrosive coatings [5] electrochemical/biosensors [6-9], greatly depend upon the usage of fabricated nano structures. They have also shown promising results as diagnostic tools, imaging and therapeutic substances for various diseases and their biomarkers [10]. Recent advanced application of nanomaterial is in phytochemistry. Herein, numerous well known

nanomaterials such as carbon nanotubes [11], metal-based nanoparticles [12], graphene oxide [13], mesoporous carbons [14], etc. are being utilized for detection of phytochemicals and phytotoxins [15].

Phytotoxins are the chemicals produced internally by the plants that can be hazardous to human. For example, chemicals such as cyanides, terpenes, metabolites, alkaloids, etc. which are essential for plant growth can be toxic to humans especially if consumed in high dose. Similarly, there are several bio-active phytochemicals provided by plants that are highly beneficial to human health [16]. These are generally referred as secondary metabolites produced *via* different mechanisms happening in chemical reactions of plant metabolism. The studies conducted showed that a massive group of phytochemicals can play a key role in functioning of human cells [17]. Many studies have proven that phytochemical rich foods help in improving health [18]. Furthermore, many phytochemicals are reported to be useful as the therapeutic agents [19]. Drugs developed from significant phytochemical are prevailing the pharmaceutical market in recent times. Some of the well known

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*Scheme 1.* The image of crude long pepper and the chemical structure of piperine

phytochemicals with known therapeutic value are curcumin [20] from turmeric, eugenol [21] from cloves, zingerone [22] from ginger, carotene [23] from carrot, capsaicin [24] from capsicum, and allicin [25] from garlic. One of the simple phytochemicals present in every house hold kitchen is piperine [26]. It is a significant bio-active phytochemical present in black pepper, long pepper, white pepper.

Piperine is an alkaloid in pepper. Hans Christian Orsted, in 1819, was the first person to isolate and extract piperine. It is a yellow crystal compound. The IUPAC name of piperine is 1-(5-[1,3-benzodioxol5-yl]-1-oxo-2,4-pentadienyl) piperidine [27]. Scheme I shows a typical structure of piperine. It is basically a weak base which decomposes to piperic acid upon acid/basic hydrolysis [28]. Some of the therapeutic uses of piperine include antioxidant property, anti-inflammatory, anti-bacterial, anti-fungal, anti-ulcer, anti-diabetic, anti-septic, anti-asthmatic, anti-cancer effects, cold, flu, fever, diuretic, and gastrointestinal disorders [29]. Although, piperine's therapeutic, culinary, and biological activities are well known, very few reports are available exploring the piperine electrochemical activity. The present work focuses on isolation of piperine from long pepper through traditional method and studying its electro-active nature *via* nanomaterial-based electrodes. Long pepper, also known as "Pipli" or "Pipal" is a well known therapeutic agent in Ayurveda [30]. Scheme 1 gives a typical image of long pepper. The major constituent of long pepper is alkaloids which impart pepper like pungency. The maximum amount of piperine is present in the underground part of the stem and roots of long pepper. The piperine is slightly more in amount in long pepper in comparison to black pepper [28]. A conventional Soxhlet

apparatus is employed here for a selective isolation of piperine from long pepper. The isolated piperine is further explored for electro-chemical redox behavior through graphitized mesoporous carbon (GMC) nano material matrix. GMC is a porous carbon encompassing shallow graphitic networks. The pore size is (2-50 nm) amid the range of microporous (which is < 2 nm) and macroporous (which is > 50 nm) materials [31]. The usage of electrochemical techniques [32,33] for quantification of phytochemicals is very effective in comparison to other conventional methods. It offers features like high selectivity, reduced analysis time and reproducibility. Wang *et al.* reported electrochemical quantification of piperine extracted from black pepper corns utilizing a bare GCE. However, they avoided usage of nanomaterial, because of which they got an irreversible peak of piperine [34]. Nevertheless, the incorporation of the nanomaterial in the present work, assisted the piperine to undergo stable redox reaction, resulting in a perfect redox response of piperine. To the best of our knowledge, it is the first study utilizing piperine from long pepper. This study is a prototype, wherein, various phytochemicals, having potential to undergo oxidation/reduction reaction can be explored. In future, this approach can be a potential tool for quantification of phytochemicals in natural products with respect to their electro-active redox behavior.

## EXPERIMENTAL

### Chemicals Required

Long pepper was purchased from a local super market in Hyderabad. 95% ethanol was procured from Jolly

industries, Hyderabad, India. Potassium hydroxide (KOH), graphitized mesoporous carbon (GMC), multiwalled carbon nano tube (MWCNT), single walled carbon nanotube, double walled carbon nano tube (DWCNT), carbon nano fiber (CNF), graphite nano powder (GNP), and piperine were obtained from Sigma Aldrich. Phosphate buffer solution of pH 7 was prepared by monobasic/di-basic sodium hydrogen phosphate. All the other chemicals used were of analytical grade and used as received.

### Apparatus and Glassware

The apparatus and glassware used include Soxhlet from Borosil, CHI 660 instrument from Sinsil, magnetic stirrer, Whatman filter paper, glassy carbon electrode, platinum wire, Ag/AgCl electrode, electrochemical cell, water bath, beakers, round bottom (RB) flask, and funnel.

### Procedure

**Isolation of piperine.** The isolation of piperine was done by a reported standard procedure with slight modifications [35]. 50 gram of long pepper was weighed and crushed into fine powder and packed. It was inserted into the Soxhlet apparatus which was fixed to a RB flask. The RB flask was filled with approximately 250-300 ml of 95% ethanol. The entire set-up was kept on reflux for 3 h. Then, the ethanol was distilled and 50 ml of warm ethanolic-KOH solution (2%) was added. The mixture was stirred using a magnetic stirrer, and the insoluble part was dissolved in 4-5 min. After which, the mixture was filtered using a Whatman filter paper to eliminate the undissolved particles. The filtrate was warmed on the hot water bath for 15 min and 20 ml of distilled water was added. At this point, turbidity appeared and the yellow needle like particles of piperine formed were isolated. It was left over night to evaporate and then filtered again to obtain needle like crystals of piperine [35].

**Electrochemical studies.** The electrochemical studies were carried out using cyclic voltammetry (CV) in a standard conventional three electrode system comprising of a working electrode (WE); *i.e.*, glassy carbon (GCE) modified-carbon nano material, reference electrode (RE); *i.e.*, Ag/AgCl, counter electrode (CE), and platinum wire in 10 ml working volume of pH 7 phosphate buffer solution

(PBS). The set potential window was -0.5 to +0.8 V *vs.* Ag/AgCl at a scan rate of 50 mV s<sup>-1</sup>. Scheme 2a gives a representation of the electrochemical set up.

### Preparation of GCE/carbon-nanomaterial electrode.

GCE@carbon-nanomaterial electrode was prepared by drop casting 5  $\mu$ l dispersion of 2 mg of specified carbon nano material dispersed in 500  $\mu$ l of ethanol and sonicated for 15 min in an ultrasonicator bath. The electrode was left for air drying for about 5 min at room temperature.

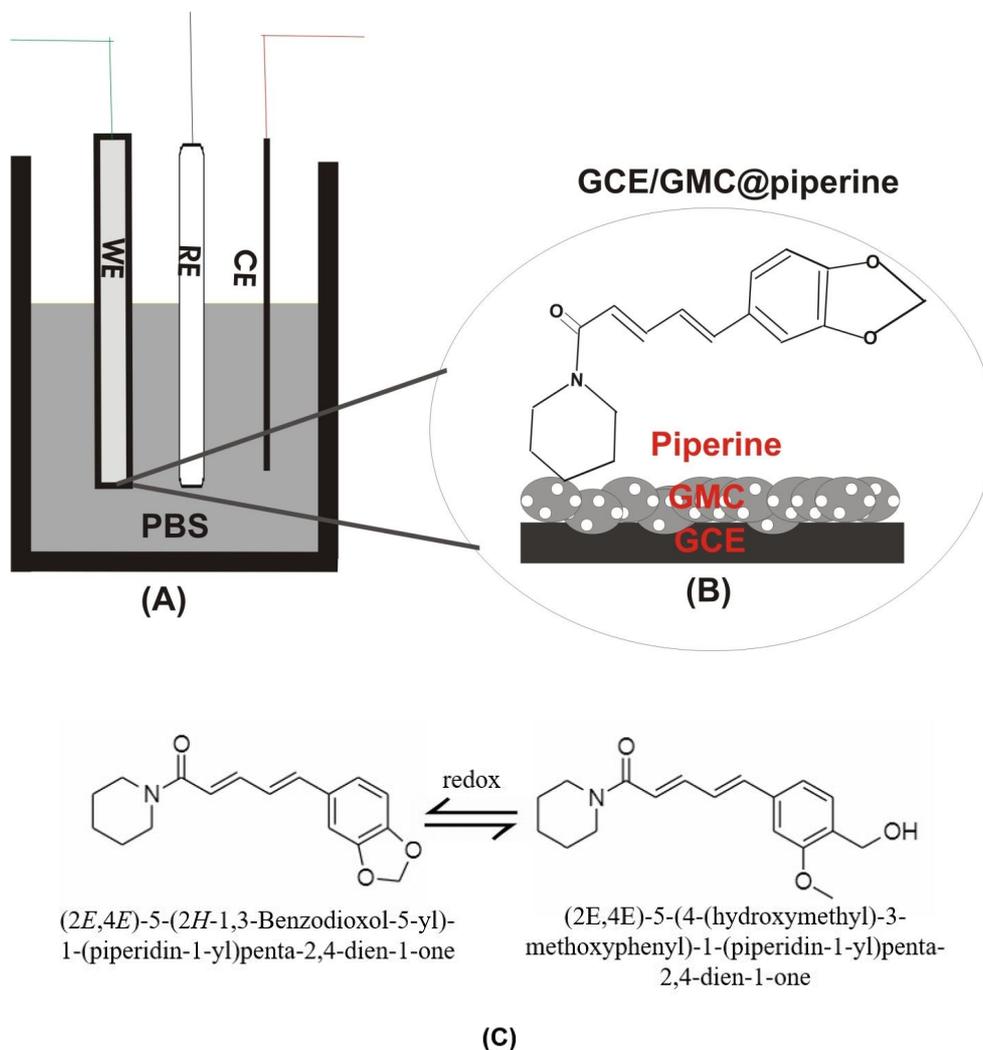
### Preparation of GCE/carbon-nanomaterial@piperine electrode.

2 mg of the obtained piperine through Soxhlet was dissolved in 1 ml of acetone. 5  $\mu$ l of this solution was top coated on the GCE/carbon-nanomaterial electrode. It was air dried for 5 min. Scheme 2b gives pictorial representation of the modified electrode.

## RESULTS AND DISCUSSION

### Screening of Various Carbon Nanomaterials

The phytochemicals are usually the concoction of polyhydrocarbons which are expected to be electroactive in nature and give electron transfer when a specified potential is applied. However, not all the phytochemicals have a feasibility to undergo electron transfer mechanism. Therefore, in such scenarios, nanomaterial matrix enhances the ability of phytochemicals to undergo oxidation and reduction. Piperine was expected to undergo electrochemical redox reaction when CV is performed. When CV was performed in the specified aforementioned parameters (50 mV s<sup>-1</sup>, for 10 cycles at -0.5 to 0.8 V *vs.* Ag/AgCl), piperine alone (GCE@piperine) failed to give any redox reaction as can be seen in Fig. 1a, wherein, glassy carbon electrode (GCE) was drop casted with 5  $\mu$ l of piperine, air dried and CV was performed. No current signal or peak is observed authenticating that, GCE alone could not support the electro activity of piperine. Nevertheless, when various carbon nanomaterial matrixes-based GCE were modified with piperine, a feeble response was observed. As observed in Figs. 1b-f, various nano materials such as DWCNT, SWCNT, MWCNT, GNP and CNF were tested. These nanomaterials assisted a little bit of electron transfer of piperine but could not give a perfect redox response.

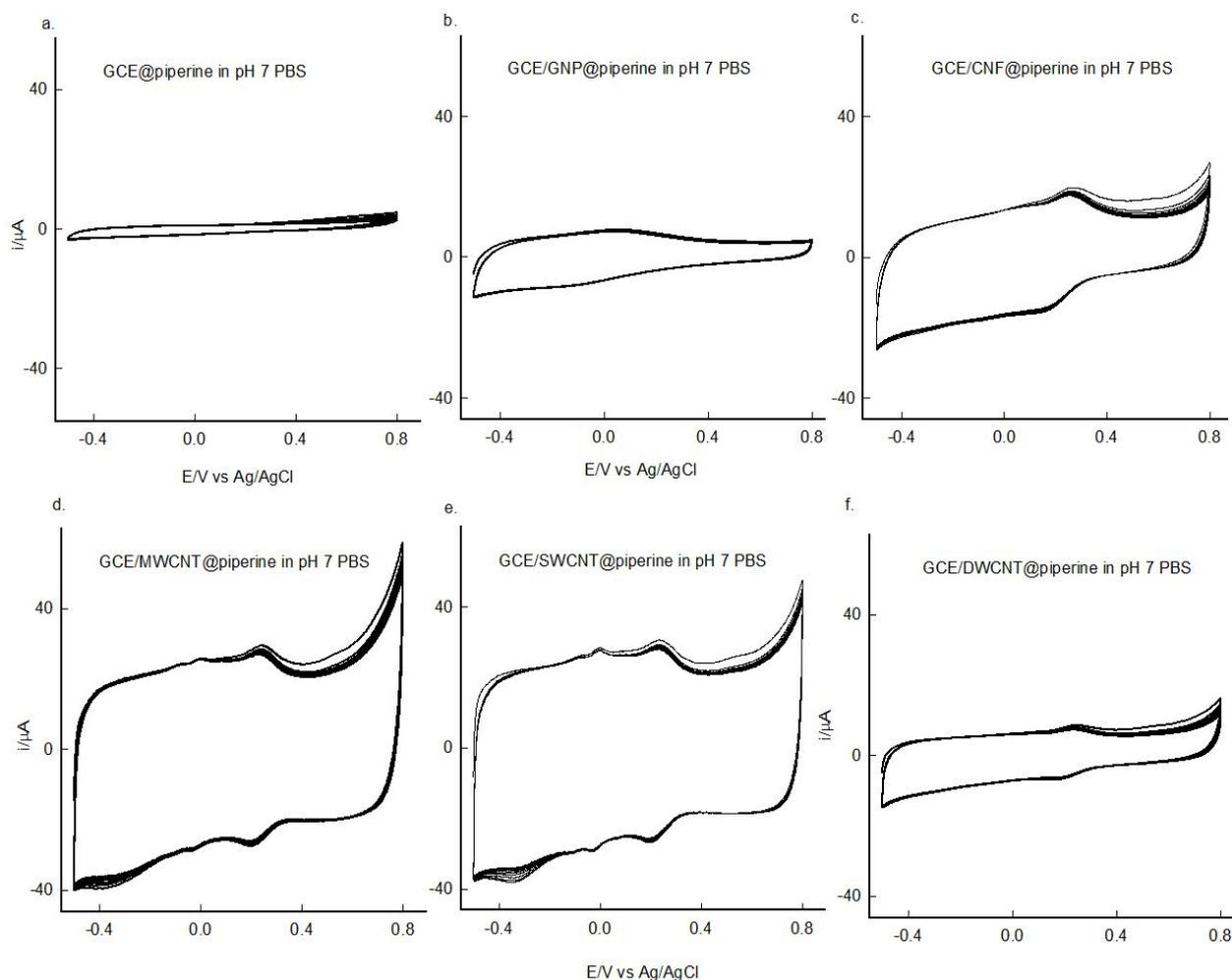


*Scheme 2.* (A) Cartoon for the experimental set up. (B) Schematic representation of GCE/GMC@piperine. (C) Plausible mechanism of the redox reaction of piperine

### Optimized Carbon Nanomaterial Matrix

Figure 2a shows the optimal carbon nano material suitable to derive electro-activity of piperine. Graphitized mesoporous carbon (GMC) was the only carbon nanomaterial amongst all the tested materials that gave a perfect redox response of piperine. Probably, owing to the mesoporous nature,  $\pi$ - $\pi$  interactions [22] between the pores of carbon nanomaterial and piperine lead to the electron transfer behavior giving a perfect redox peak. CV response (Fig. 2a) showed a well-established, stable redox peak at

$E' = +0.2$  V vs. Ag/AgCl at  $50 \text{ mV s}^{-1}$  in 10 ml of pH 7 PBS with a surface excess  $\Gamma_{\text{pip}}$  value as  $19.8 \times 10^{-9} \text{ mol}^{-1} \text{ cm}^2$ . In order to get the confirmation that the obtained response was due to isolated piperine alone and not due to any other impurity, a control experiment was performed wherein commercially available piperine was subjected to CV in the same manner as that with the isolated one. It was found that the experimentally isolated piperine from long pepper and commercially purified bought piperine both give the redox peaks at the same peak potential coinciding with each other



**Fig. 1.** A typical CV response of (a) GCE alone, (b) GNP, (c) CNF, (d) MWCNT, (e) SWCNT and (f) DWCNT@piperine in pH 7 PBS at  $50 \text{ mV s}^{-1}$ .

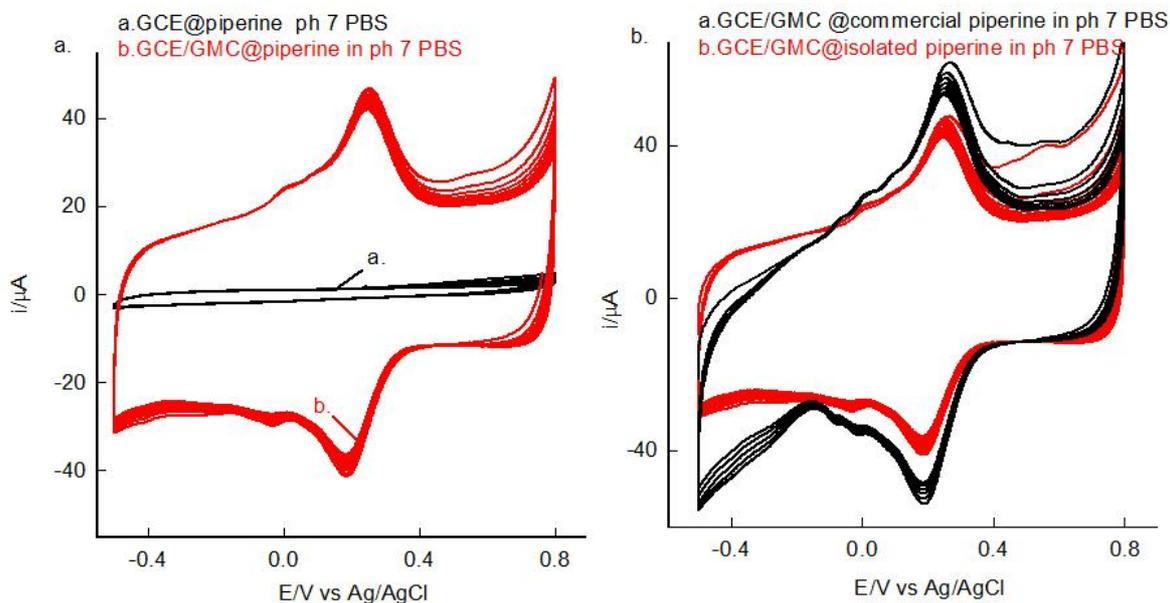
(Fig. 2b). Hence, it was confirmed that the electro active redox behavior recorded was of isolated piperine alone and not due to any other contaminant or constituent of long pepper. Scheme 2C is the plausible mechanism that piperine molecule undergoes. While applying the forward potential, the oxypiene moiety possibly undergoes oxidation giving an oxidation peak and the same undergoes reduction upon reversing the CV potential. Thus, a redox response with a stable peak is obtained. The chemical in-vitro oxidation mechanism gets mimicked over the electrode surface electrochemical redox mechanism [36].

### Microscopic Characterization

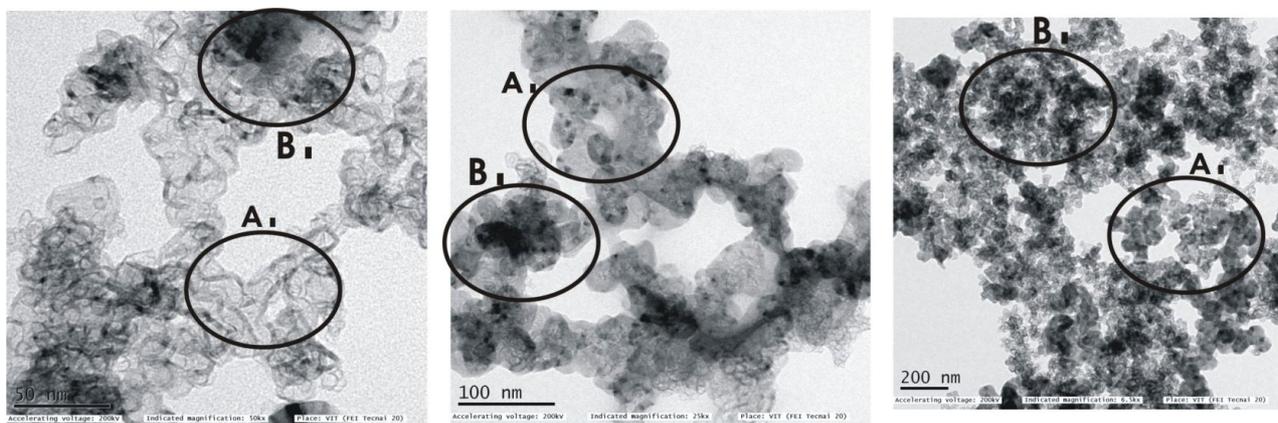
Figure 3 shows the microscopic characterization of the modified electrode with piperine. Herein, TEM images were taken at different magnifications including 50 nm, 100 nm and 200 nm. The mark A shows the areas with porous nature of GMC, B area is dark masked spots wherein piperine got adsorbed. The porous structures were masked by dark spots indicating the interaction of pores of GMC and isolated piperine.

### Effect of Potential Scan Rate

Effect of variable potential scan rate on the CV response



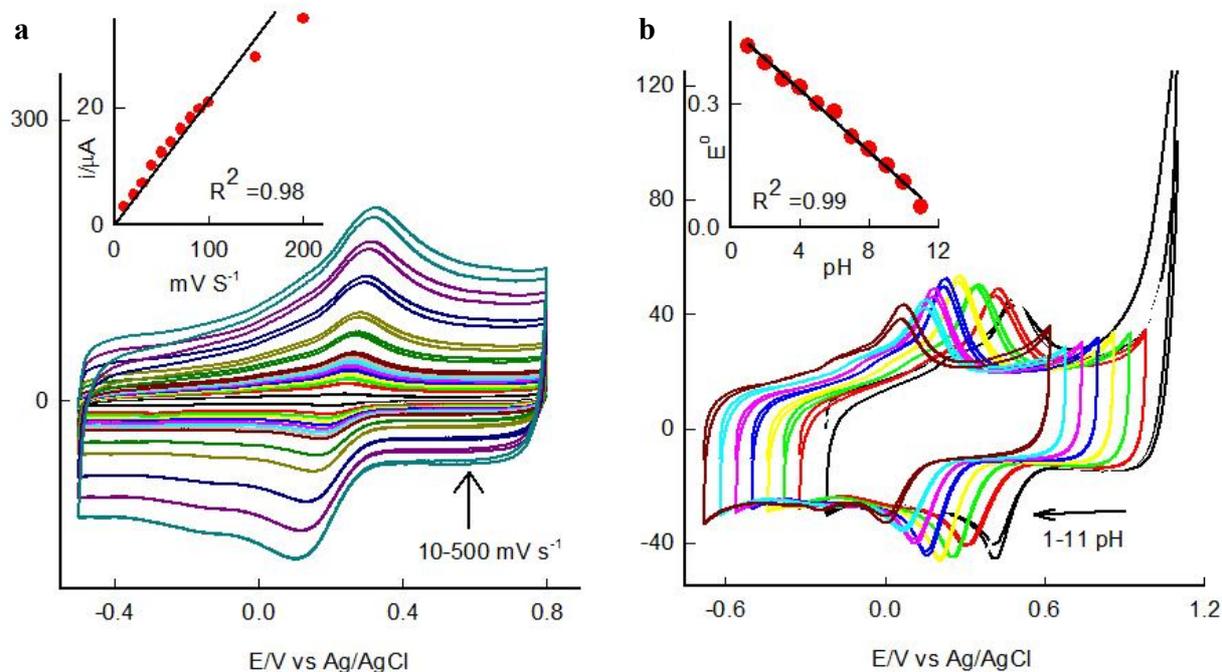
**Fig. 2.** (a) A comparative CV response of (a) GCE@piperine, and (b) GCE/GMC@piperine in pH 7 PBS at  $50 \text{ mV s}^{-1}$ ,  $n = 10$ . (b) A comparative CV response of GCE/GMC@commercial piperine and GCE/GMC@isolated piperine in pH 7 PBS at  $50 \text{ mV s}^{-1}$ ,  $n = 10$ .



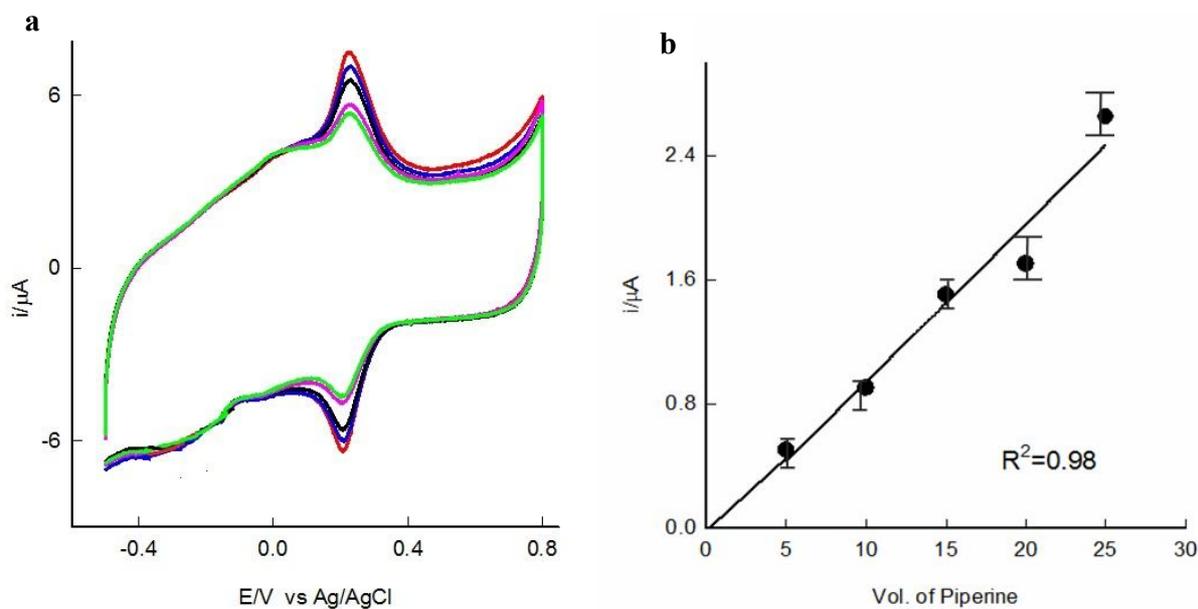
**Fig. 3.** TEM image of GCE/GMC@piperine at different magnifications. (a) The unmasked porous structure, and (b) the dark spots due to the piperine immobilization.

of GCE/GMC@piperine was also analyzed. Figure 4a demonstrates a comparative CV of various scan rates ranging from  $10\text{-}500 \text{ mV s}^{-1}$ . A well-defined, stable, ordered increment in both the anodic and cathodic peak current value was obtained (Fig. 4a). A base-line corrected

calibration plot of peak current vs. different scan rates ( $\text{mV s}^{-1}$ ) showed a linearity starting from the origin representing a surface-confined electron-transfer mechanism of the modified electrode (Inset Fig. 4a). This behavior also demonstrates an exceptional immobilization,



**Fig. 4.** (a) CV response of effect of various scan rates ( $10\text{-}500\text{ mV s}^{-1}$ ) of GCE/GMC@piperine in pH 7 PBS. Inset, base-line corrected calibration plot. (b) CV response of GCE/GMC@piperine in various pHs of PBS (1-11). Inset, base-line corrected calibration plot.



**Fig. 5.** (a) CV response of various volumes of piperine-modified GCE/GMC taken in pH PBS at  $5\text{ mV s}^{-1}$  (slow scan rate) for  $n = 4$ . (b) Corresponding base-line corrected calibration plot with triplicated experiment and error bars.

and an active electron-transfer mechanism of the isolated piperine in accordance with GMC. The results observed are accordance to the following equation which is followed by a reversible electrochemical reaction [37].

$$I_{pa} \text{ or } i_{pc} = n^2 F^2 A \Gamma_{pip} \nu / 4RT \quad (1)$$

Herein,  $n$  is the number of electrons involved,  $F$  = faraday constant (96500),  $A$  = geometrical surface area of the electrode,  $\Gamma_{pip}$  is the calculated surface excess value,  $\nu$  is the scan rate.

### Variable Solution pH Effect

The effect of varying pH of the PBS on the redox activity of GCE/GMC@Piperine was examined by taking CV in various pHs of PBS (1-11 pH). Figure 4b depicts a CV response from 1-9 pHs of PBS solutions which demonstrated a negative Nernstian shift of the redox potential peak with respect to pH [38]. A base-line corrected calibration plot of  $E^0$  vs. pH (inset Fig. 4b) shows linearity with a slope value of  $-49.75 \pm 1$  mV  $\text{pH}^{-1}$ . This clearly portrays a characteristic Nernstian behavior wherein equal number of protons and electrons are participating in the electron transfer reaction [38].

### Effect of Changing Piperine Concentration

Figure 5 shows a typically comparative CV response wherein different volumes of piperine were top coated on the GCE/GMC. CV was recorded at a slow scan rate of  $5 \text{ mV s}^{-1}$  in pH 7 of PBS. Quantitative analysis is preferred in slow scan rate. The volumes of piperine solution drop coated were  $5 \mu\text{l}$ - $25 \mu\text{l}$ . As the volume increased, the concentration of piperine was also increased. Subsequently, the peak current of the redox peak also increased linearly (Fig. 5a). However, due to slow scan rate, the peak current is low and slight disturbance is observed which is in an acceptable limit. The base-line corrected calibration plot (Fig. 5b), volume of piperine vs. redox peak current gave linearity with a regression square of 0.98. This observation, evidently authenticated that the change in concentration of piperine is directly proportional to the redox peak current obtained. Hence, this technique could be utilized for quantitative phytochemical analysis; *i.e.*, piperine in long pepper.

## CONCLUSIONS

A highly stable and reproducible electrochemical sensor for an isolated piperine phytochemical from long pepper was fabricated here. Graphitized mesoporous carbon assisted chemically modified electrode decorated with isolated piperine, designated as GCE/GMC@piperine, was used as the electrode. A conventional three-electrode-based system containing GCE/GMC@piperine as a working electrode, platinum as a counter electrode and Ag/AgCl as a reference electrode, in 10 ml working volume of pH 7 PBS was used at  $50 \text{ mV s}^{-1}$ ,  $n = 10$ . The fabricated GCE/GMC@piperine gave a highly stable, reversible redox peak at  $E' = +0.2 \text{ V vs. Ag/AgCl}$  at  $50 \text{ mV s}^{-1}$  in 10 ml of pH 7 PBS with a surface excess  $\Gamma_{pip}$  value of  $19.8 \times 10^{-9} \text{ mol}^{-1} \text{ cm}^2$ . The microscopic characterization by TEM revealed the interaction of piperine with GMC. Parameters such as the effects of potential scan rate, and solution pH were also examined. Further, quantitative analysis of various piperine concentrations was performed with respect to the redox peak current. This work is a prototype of electrochemical quantitative detection of phytochemicals in natural products.

## ACKNOWLEDGEMENTS

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### Conflict of Interest

No conflicts to declare.

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