Improved sensing of capsaicin with TiO_2 nanoparticles modified epoxy graphite electrode

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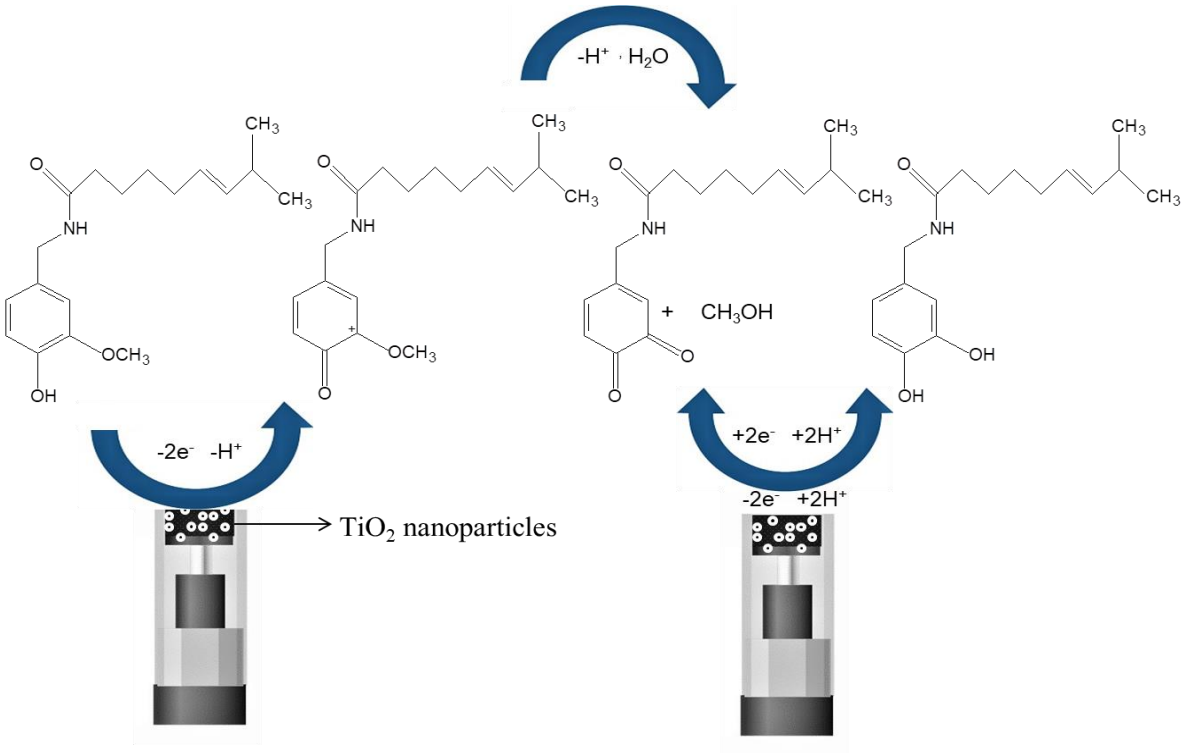
Abstract. The present research focuses on the electrochemical determination of capsaicin, a lipophilic alkaloid which is responsible for the main reason of hotness in chili peppers. An electrochemical sensor based on epoxy-graphite composite with the modification of titanium dioxide (TiO_2) nanoparticles is developed for the determination of capsaicin. The measurements were carried out in glycine buffer at pH 2.5 using cyclic voltammetry. Two linear concentration ranges were obtained from 6 to 75 μM (R = 0.99) and 12 to 138 μM with a detection limit of 5.34 μM and 11.3 μM capsaicin, for 1st and 2nd oxidation peak, respectively. The main advantage of developed sensor is its repeatability with a relative standard deviation (RSD) value of 2.5% (n=10). This voltammetry platform has successfully been applied to quantify capsaicin in various real samples such as hot chili sauce and pharmaceutical preparations.

Keywords: Capsaicin, TiO_2 nanoparticles, hot pepper sauce, analgesic, cyclic voltammetry.

1. Introduction

Chili peppers are highly valued horticultural commodity, used in culinary purposes for adding pungency or hot, spicy flavor to the dishes. The pungency of chili peppers is imparted by the naturally occurring lipophilic alkaloids present, broadly known as capsaicinoids [1-2]. The two major capsaicinoids present in most varieties of hot peppers are capsaicin and dihydrocapsaicin, constituting 90% or more of the total capsaicinoids [3]. The remaining 10% are nordihydrocapsaicin, norcapsaicin, homocapsaicin, homodihydrocapsaicin, nornorcapsaicin, nornornorcapsaicin, and nonivamide [4]. Capsaicin is present in large quantities in the placental tissues of chilli peppers that hold the seeds in fruits and may acts as a repellent against herbivores [5]. Chemically, capsaicin is trans-8-methyl-N-vanillyl- 6-nonenamide and is a fat soluble, odourless, pungent tasting, off-white solid with a melting point of 62–65°C and a molecular weight of 305.4 $g \cdot mol^{-1}$



Scheme 1: Electrochemical oxidation/reduction reaction of capsaicin.

Besides its usage in food preparations, capsaicin has widespread application in pharmaceutical industry due to its several pharmacological properties [5-7]. It is well-known to have anti-bacterial [8], anti-carcinogenic [9], anti-tumoral [10], anti-mutagenic [11] properties and it is a good anti-oxidant [12]. Capsaicin is also used as an ingredient in drugs to control obesity [13], cholesterol [14] and in topical formulations used for pain management [15-16]. In addition, capsaicin based pepper sprays are used as tools for skin conditioning self-defense and also to control mob violence by security authorities [17].

Since its first isolation and determination of its chemical structure in 1919, several advancements have been made in extraction and quantitative estimation of this alkaloid [18]. Its widespread usage calls for capsaicin estimation methods which are simple, fast and accurate. Initial pungency of hot sauces and peppers were estimated by a classical method known as Scoville's organoleptic test [19]; this uses a technique where a solution of pepper extract is diluted in a solution of sugar water until the heat or pungency is no longer sensible by taste. In addition to this traditional semi-quantitave method, a lot of other analytical techniques have been established for estimation of capsaicin. Among these are high performance liquid chromatography (HPLC) [20-22], immunoaffinity chromatography combined with liquid chromatography-tandem mass spectrometry [23-24], enzyme immunoassay [25-28], capillary electrophoresis [30], micellar electrokinetic capillary chromatography [3], HPLC coupled with electrochemical detection [31], photo diode array and mass spectrometry [32], colorimetric method [29], spectrophotometry [34-35], fluorescence spectroscopy [33], etc. Although some of these variants e.g. HPLC coupled with enhanced detection like mass spectrometry give satisfactory results with respect to sensitivity and resolution, these techniques need to face complex procedures which includes requirement of expensive instrumentation, long response time, difficulty in sample preparation etc. Therefore, to overcome these difficulties, new techniques need to be established with advantages like less expensive instrumentation, ease of use, portable, favorable sensitivity towards capsaicin, etc. Electrochemical sensors provide an excellent tool for performing on-site analysis at a reasonably cheap price with fast

and robust results. Electrochemical analysis seems to be a promising technique for the proper quantitative estimation of capsaicin since Compton et al. first demonstrated a electrochemical technique which involved its voltammetric determination using a carbon nanotube based electrochemical sensor [36]. Later on, extensive research on electrochemical analysis for capsaicin have been improving performance on each attempt. All these research use some modifiers in the electrode to improve the sensing of capsaicin. Sensors used include boron-doped diamond electrode [37]; carbon paste electrodes modified with amino-functionalized mesoporous silica [38], mesoporous cellular foams [39], β-cyclodextrin [40]; glassy carbon electrode [41]; glassy carbon electrode modified with graphene-titania-nafion composite film [42], ruthenium nanoparticles decorated carbon nanotubes [43], platform polypyrrole/Bi_2O_3/graphene oxide [44]; graphite pencil electrode [45-46]; single-walled and multi walled carbon nanotubes based screen printed electrode [47-48]; screen printed electrode modified with Ag/Ag_2O nanoparticles/reduced graphene oxide [49], poly(sodium 4 styrenesulfonate) functionalized graphite [50]; paraffin-impregnated graphite electrode [51]; polyaniline electrode [52]; biosensor based on horseradish peroxidase enzyme-capsaicin reaction mediated by ferrocene [53]; enzyme biosensor using ammonia-lyase enzyme [54]. Mechanistic steps involved in the electrochemical reactions of capsaicin are shown in scheme 1 [36].

It is observed that carbon based electrodes lead to fouling effect in the cyclic voltammetric measurements of capsaicin. This fouling results in unstable baselines with decreasing oxidation peak. In order to overcome this problem, extensive search of electrode modifiers, mainly of nano technological origin, has been made. Metal oxide nanoparticles have been used in the modification of electrochemical sensors from a long time due their interesting sensory properties like functional biocompatibility, chemical stability, controllable size, bio-safety and catalytic effects. Along with enhanced electron transfer kinetics, they may also offer adsorptive possibilities for stripping voltammetric variants. Specially, if incorporated to electrodes in nanopowder form, these materials present interesting advantages over other conventional materials on account of electron transfer and immobilization of biomolecules for enhanced chemical and biological sensor operation [55]. Here, in this research, we have developed a sensitive, rapid and efficient electrochemical sensor based on epoxy-graphite composite with the modification of Titanium dioxide (TiO_2) nanoparticles for the determination of capsaicin. TiO_2 nanoparticles were incorporated into the sensor by adding the nanoparticles into the mixture of epoxy graphite composite during the fabrication of the sensor. This developed sensor is then farther used for successful determination of capsaicin content in various hot pepper sauce and pharmaceutical products with enhanced reproducibility features and minimal fouling effect.

2. Experimental

2.1. Reagents

All reagents were of analytical reagent grade and were used as received without any further purification. Capsaicin as it is commercially available was purchased from Sigma-Aldrich (St. Louis, MO, USA). All the chemicals for the preparation of the buffers were purchased from Merck (Darmstadt, Germany). Titanium (IV) oxide (< 25 nm) nanoparticles, which were used for the modification of electrodes, were purchased from Sigma-Aldrich. Capsaicin mother solutions were prepared in 48% ethanol as it is not completely soluble in water. All the buffer solutions were prepared in ultrapure water purified by a MilliQ System (Millipore, Billerica, MA, USA). Graphite powder (particle size <50μm) used for the construction of the electrodes were purchased from BDH (BDH Laboratory Supplies, Poole, UK) and the corresponding resin and the hardener H77 is obtained from Epoxy Technologies (Billerica, MA, USA). For the real sample analysis two commercially available sauce samples and a pharmaceutical cream were purchased. These are Tabasco Habanero hot pepper

sauce from McIlhenny company (Avery Island, LA, USA), Delhuerto original hot pepper sauce (Peru) and one analgesic cream named "Alacapsin" from Alfasigma company (Spain).

2.2. Apparatus

All the cyclic voltammetric measurements were carried out in a PGSTAT 30 Autolab potentiostat (EcoChemie, The Netherlands) with GPES 4.7 version software (EcoChemie). For the pH adjustments during pH study and preparation of the electrolyte solutions, a Crison micro pH 2002 pH-meter (Crison instruments, Barcelona, Spain) was used. Electrochemical measurements were carried out in a conventional 3 electrode cell arrangement where a combined electrode (Crison 5261, Barcelona, Spain) was used, made up of metallic platinum wire and Ag/AgCl electrode, as an auxiliary and reference electrode, respectively.

2.3. Construction of working electrode

Graphite epoxy composite electrode (GEC) modified with TiO2 nanoparticles was used as a working electrode. Working electrodes were constructed with the help of a 6 mm i.d. PVC tube [56]. First of all a shaped copper disc is soldered to an electrical connector. This couple is then put into the PVC tube as shown in the Figure 1.A, resulting in a cylinder cavity. A paste is then made by mixing 54% graphite powder, 4% titanium oxide nanopowder and the corresponding epoxy resin and the hardener; this paste is then used to fill the cavity and cured at 40 °C for 48 h. The electrode surface is then polished with emery papers of decreasing size until a flat, shiny surface appears, see Fig 1.C. This prepared electrode is then used as a working electrode for the electrochemical measurements of capsaicin. Whenever needed the repolishing of the surface regenerates the electrode recovering any loss of the response.

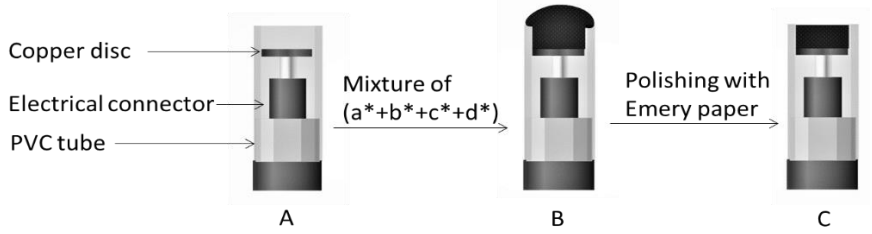


Fig 1 : Graphite-epoxy composite electrode construction.
a*: graphite powder, b*: Titanium oxide nanopowder, c*: Epoxy resin d*: Hardener.

2.4. Sample and electrolyte preparation

The supporting electrolyte solution for preparation of the capsaicin samples and for calibration used in this study was 0.2 $mol \cdot l^{-1}$ glycine buffers with 0.1 $mol \cdot l^{-1}$ KCl as saline background. Glycine buffer at pH 2.5 with KCl as saline bachground is selected in this study as a result of optimization of pH. Britton Robinson buffer with 0.1 $mol \cdot l^{-1}$ KCl was prepared for optimization of pH measuring conditions.

2.5. Extraction of capsaicin from the real samples.

For the extraction of capsaicin from the sauce samples, specific procedure was used as capsaicin is not totally soluble in water. An aliquot of 2.5 g of sauce is first weighed in a beaker containing 35 mL of ethanol, and then the beaker is sonicated for 15 min and then magnetically stirred for 2 h. This

capsaicin extracted solution is then filtered using glass fiber filter paper (GMFA SCHARLAU Ø 90 mm) into a 50 mL volumetric flask; after filtration, the funnel containing the solid part of the sauce was rinsed with some aliquots of ethanol until filling up of the volumetric flask up to the mark. This rinsing procedure was followed so that all amount of capsaicin had been extracted from the remaining solid parts of sauce in the filter. This extracted solution was then used for voltammetric measurements. The same extraction procedure was also followed for the extraction of capsaicin from the pharmaceutical cream sample.

2.6. Analytical procedure

Complete voltammograms were recorded for capsaicin by cycling the potential between -0.9 V and +1 V vs. Ag/AgCl with a step potential of 0.01 V and a scan rate of 100 mV•s-1. After each measurement chemical cleaning of the electrode surface was carried out; this involves rinsing the surface in specific media (48% ethanol:water, v/v) for 60 seconds with stirring. For the voltammetric characterization of the electrode, sample solutions were prepared first by adding standard solution of capsaicin into a solution containing 1 part of 96 % ethanol and 3 part glycine buffer at pH 2.5. Cyclic voltammetric measurements were carried out between -0.9 V and +1 V vs. Ag/AgCl with a step potential of 0.01 V and a scan rate of 100 $mV \cdot s^{-1}$.
For pH study, sample solutions at same concentration of capsaicin and at different pH values ranging from pH 2 to pH 7 were prepared by adding standard solution of capsaicin to a 20 mL of solution containing 24% ethanol and 76% Britton Robinson buffer. The three electrode system were transferred to a blank buffer solution at each pH value and cyclic voltammograms were recorded at scan rate value of 100 $mV \cdot s^{-1}$. Measurements were carried out like the same way with all the prepared samples at different pH values.
For the repeatability test of the electrodes, 10 measurements of capsaicin were carried out repeatedly on same concentration of capsaicin. To check the stability of the electrode 35 µM capsaicin were measured in a cyclic manner for 14 cycles. Before each sample measurement a blank buffer measurement was taken. Each cycle was followed by a chemical cleaning of the electrode surface which involves rinsing the surface in specific media (48% ethanol) for 60 s with stirring.
For the real sample analysis, the extracted sample described in the section 2.5 was diluted to 4 times with 0.266 $mol \cdot l^{-1}$ glycine buffer so that the final sample solution will have concentration of 0.2 $mol \cdot l^{-1}$ glycine buffer containing 24% ethanol i.e. the same background electrolyte as the previous measurements during this entire capsaicin study. For doing this 25 mL of the extracted sample was transferred to a 100 mL volumetric flash and filled up to the mark with glycine buffer. Cyclic voltammograms of 25 mL of this prepared sample were then recorded. Experimental conditions adopted in this measurement were same as mentioned in section 2.5.

3. Results and discussions

3.1. Voltammetric behavior of the prepared electrodes towards capsaicin

Cyclic voltammogram obtained for 60 µM capsaicin solution by following the analytical procedure described in the section 2.6 is shown in the Figure 2. It is observed that capsaicin gives rise to two well defined oxidation peaks in the potential of +0.17 V and +0.43 V, respectively. Corresponding reduction peak was observed at -0.05 V. It is observed that the oxidation as well as reduction peaks appear at lower potentials with this TiO_2 modified electrode in comparisons to the previous studies obtained in the literature. [38, 40, 70, 61]. This means then, that study of capsaicin with our developed

electrode needs lower energy then before for the electrochemical reactions of capsaicin, originated in some catalytic effect.

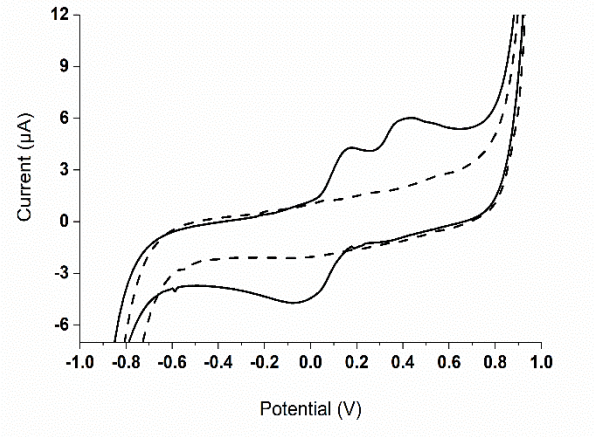


Fig.2. Cyclic voltammograms obtained from the 2nd scan of background buffer (dashed lines) and 60 µM capsaicin (solid line) prepared in glycine buffer at pH 2.5, using the TiO_2 modified graphite epoxy resin electrode.

3.2 Optimization of working pH

To obtain the optimum pH for the determination of capsaicin, effect of pH was investigated by measuring of 40µM of capsaicin. Voltammograms obtained from the 2nd scan of 40µM capsaicin solution prepared in different pH solutions starting from pH 2 to 6 are shown in the Figure 3(A). Figures 3(B) and 3(C) show plots of potential vs. pH value and peak current corresponding to 1st oxidation peak vs. pH. It is observed from 2(B) that the peak potential of oxidation peak of capsaicin varies linearly with the pH and shifts to the negative potential by 51 mV per unit increase in the pH with a regression equation (Ep= -0.051pH + 0.323, R^2=.99). This implies that protons participate in the electrochemical process. The calculated value of the slope is close the the theoretical value of 59 $mVpH^{-1}$. This suggests that equal amount of protons and electrons are exchanged during the oxidation reaction of capsaicin. From the figure 2(C) it is observed that the oxidation peak current decreases linearly with the increase of the pH value from pH 2 to pH 6. So to avoid extreme acidity on electrode surface a pH of 2.5 (accomplishable with glycine buffer) was selected for the subsequent experiments.

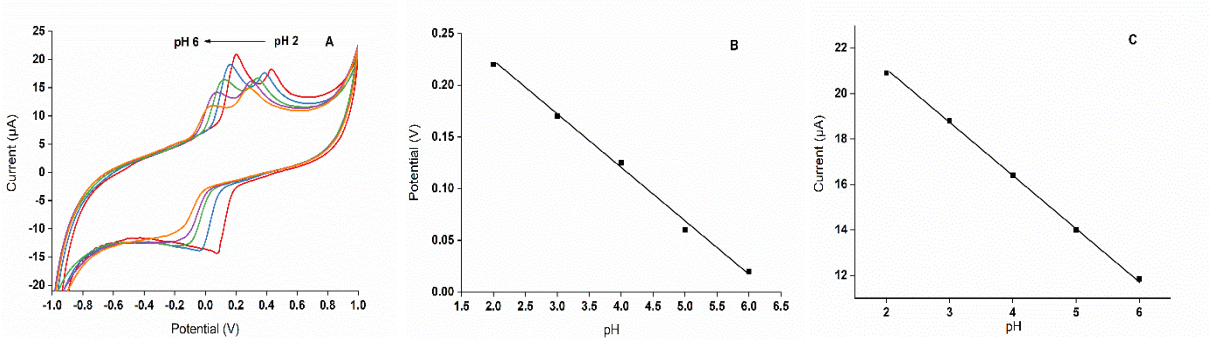


Fig.3. Current *vs*. Potential curve for 40 µM capsaicin at different pH of the Britton Robinson buffer solution (A), Potential of the 1st oxidation peak vs. pH curve (B), Maximum current for the 1st oxidation peak vs. pH curve (C)

3.3 Characterization of analytical properties

To check the analytical performance of the electrode, a calibration experiment was carried out in 0.2 $mol \cdot l^{-1}$ glycine buffer with 0.1 $mol \cdot l^{-1}$ KCl saline background. Figure 4 shows the voltammograms obtained upon additions of capsaicin. From the two oxidation peak, two calibration curves were obtained by plotting peak current vs. added concentration of capsaicin. Calibration line on Figure 4(B), obtained from the oxidation peak at +0.17 V is linear within the concentration range 6 to 75 µM with a regression equation I(µA) =0.044(±0.0021)·C(µM) + 1.63(±0.11) having correlation coefficient 0.993 (n=12). Calibration Line on Figure 4(C), obtained from the oxidation peak at +0.43 V is linear within the concentration range 12 to 138 µM with a regression equation I(µA) =0.075(±0.0037)·C(µM) + 1.64(±0.31) having correlation coefficient 0.996 (n=17). Limit of detection values were calculated as 5.34 and 11.3 µM for the oxidation peak at +0.17v and at +0.43 respectively (using the formula of $3.3S_{y/x}/m$).

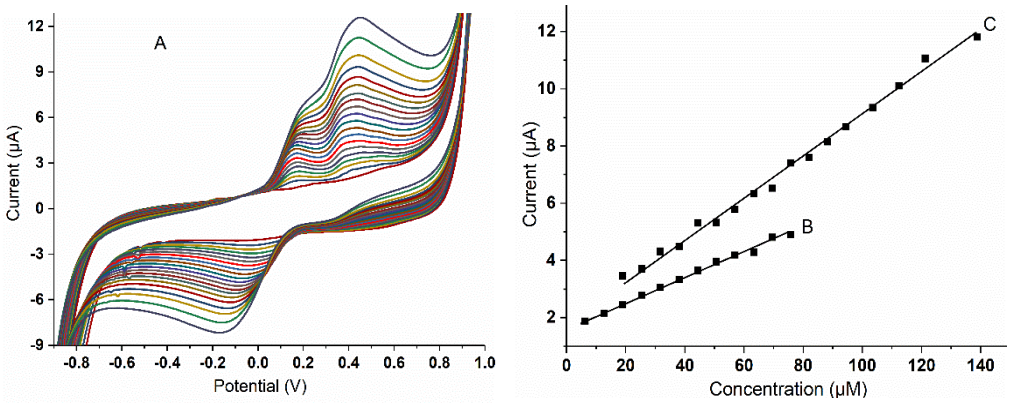


Fig.4. Cyclic voltammograms in glycine buffer, pH 2.5 at increasing concentration of capsaicin, (6-138 µM) (A); Calibration line of capsaicin for the 1st oxidation peak (+0.17V), (B); Calibration line of capsaicin for the 2nd oxidation peak (+0.43 V), (C).

To check the repeatability of our developed electrode, ten measurements of 23.33 µM capsaicin in glycine buffer were carried out in series by following the experimental conditions described in the section 2.5. The relative standard deviation of responses was obtained as 2.53% which indicates a good repeatability of the electrode responses towards capsaicin. From the stability of the capsaicin standard study as explained in the analytical procedure in section 2.6, RSD values of 3.41% and 2.31% were obtained for the capsaicin and blank buffer solutions, respectively. When this experiment was reproduced with the standard graphite epoxy resin electrode without any modification, RSD values obtained for capsaicin and blank solutions were 14.29% and 9.46%, respectively, even considering the electrode surface was rinsed with ethanol/water solution after each cycle. These values illustrate the significant fouling effect associated to the capsaicin determination, as already commented. Figure 5 illustrates the results obtained in the stability study, when the TiO_2 modified electrode was used, where a correct recovery of the response can be observed. To the best of our knowledge this attempt of checking fouling carried out by capsaicin is reported for the first time, and demonstrates how important this issue can be when not using disposable electrodes. With our TiO2 modified electrode and with a simple rinsing step, electrochemical determination of capsaicin results in a very stable series of blank measurements, indicating there is no memory effect, and with a controlled variability for the series as capsaicin standard measurements, demonstrating the minimization of the fouling effect. . Farthermore reproducibility of polishing was also carried out for both GEC and TiO_2. Modified electrode and RSD values were found to be as 3.19 % and 3.27 % respectively for capsaicin which implies that our developed sensor have similar RSD values for both

polishing and cleaning with ethanol and water mixture whereas GEC does not have it. Hence our sensor can be used for capsaicin determination by cleaning only instead of using time consuming polishing procedure. A scenario of comparison of our developed sensor with another previously developed sensors found in the literature is shown in Table 1, where it is observed that the value for repeatability of measurements (RSD) is lowest for our developed sensor. Although LOD is not among the best in the table, it is to be commented our procedure is by direct voltammetry while best sensitivities are obtained in stripping conditions.

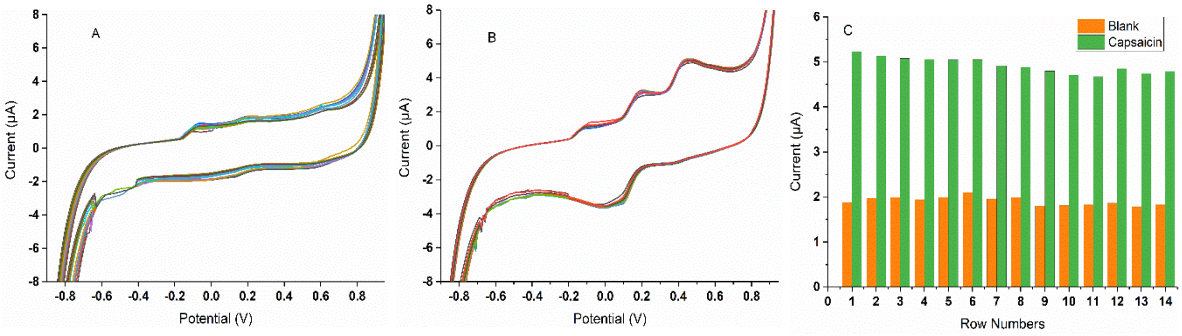


Fig 5. Current Vs. Potential curves for repetitive measurements of blank after each cycle (A), repetitive measurements of 35µM capsaicin in a buffer/capsaicin cycle manner (B), comparisons of blank and capsaicin measurements (C).

Table 1 Comparison of analytical properties of different electrodes in the literature used for the voltametric determination of capsaicin

Working electrode	Electrochemical technique [a]	pH media	Preconcentration time	LOD (µM)	Linearity range µmol L^{-1}	(RSD) %	Ref
Multi walled carbon nanotubes based screen printed electrode	CV	1.0	60 s	0.45	0.5–35	–	36
Boron doped diamond electrode	SW	1.0	90 s	0.039	0.16–20	4.44	37
Amino-functionalized mesoporosus silica	LSV	3.0	180 s	0.02	0.040–0.4, 0.4–4	–	38
Graphite pencil electrodes	ASV	9.0	120 s	0.0037	0.016–0.32	7.1	45
Mesoporous cellular foams	DPV	1.0	60 s	0.08	0.76–11.65	–	39
Ag/Ag_2O poly(sodium4-styrensulfonate) reduced graphene	DPV	1	60 s	0.4	1–60	9.70(*1)	49
Graphene-titania-nafion	LSV	1	10 min	0.0086	0.03–10	–	42
Carbon nanotubes and ruthenium nanoparticles	SW	4	–	0.0025	0.01–0.4	5(*2)	43
Graphite pencil electrodes	CV	9	–	0.1	0.1–100	–	46
Carbon paste electrodes modified by β-cyclodextrin	CV	1 mol·l^{-1} $HClO_4$	–	0.065	1.44–33	–	40
Graphite epoxy resin electrode with TiO_2 nanoparticles	CV	2.5	–	5.34	5.34-138	2.53	this work

[a] Cyclic voltammetry (CV), linear sweep voltammetry (LSV), adsorptive stripping voltammetry (ASV), differential pulse voltammetry (DPV), square wave voltammetry (SW),
(*1) peak current decreased by 9.70% after five hundred times determination.
(*2) peak current changes by less than 5% after measuring two weeks

3.4 Application to real samples

To show final application of the developed sensor towards real samples containing capsaicin, measurements were first carried out in the diluted samples of extracted solutions of capsaicin as explained in the section 2.6. After measuring the diluted samples, to obtain an accurate concentration of capsaicin in the real samples, standard additions of capsaicin were performed in each sample and voltammograms were recorded. It is observed that the voltammograms obtained from this extracted samples of capsaicin are not exactly similar with respect to shape and size to that had been obtained for the pure capsaicin samples shown in the Figure 1. This matrix effect may be because of interfering agents present in the real sample. This problem is overcome by doing baseline corrections to the voltammograms obtained from the real samples using OriginPro 2017 software after measuring. Additionally, determinations were carried out at the second oxidation potential (+0.43 V) as the obtained voltammograms are clearer to interpret. Figure 6 and their insets depicts the voltammograms obtained in the real sample Delhuerto hot pepper sauce (A) and Alacapsin (B) and the corresponding addition curve, respectively. Average of concentrations obtained from 3 replicates of each real sample is given in the Table 2

Table 2. Results of calculated concentrations of capsaicin in different real samples

Pepper samples	Amount of capsaicin determined (μM)	Confidence level (95%)	Actual amount of capsaicin
Delhuerto hot pepper sauce	15.59	±1.49	-
Tabasco habanero pepper sauce	24.68	±2.12	-
Alacapsin	29.06	±2.44	30.7

4. Conclusions

The developed capsaicin electrochemical sensor has shown interesting response features versus capsaicin and can be a good alternative to human-subjective Scoville organoleptic test; it can also be further developed as a portable electrochemical sensor for the estimation of capsaicin content in food and pharmaceutical products. In addition to this, to the best of our knowledge, our proposed sensor employing titanium nanoparticles is the first sensor to be developed which show very reduced fouling effect with capsaicin, this is accomplished through the use of chemical cleaning of the electrode surface which involves rinsing the surface in specific media (48% ethanol). The developed sensor demonstrated that can be applied in the repeated measurements of capsaicin samples without renewing the electrode surface between consecutive measurements.

Acknowledgments

Financial support for this work was provided by Spanish Ministry of Economy and Innovation, MINECO (Madrid) through projects CTQ2016-80170-P. M. S. thanks to AGAUR, Generalitat de Catalunya and to European Social Fund, European Union for FI fellowship. M. d. V. thanks the support from program ICREA Academia.

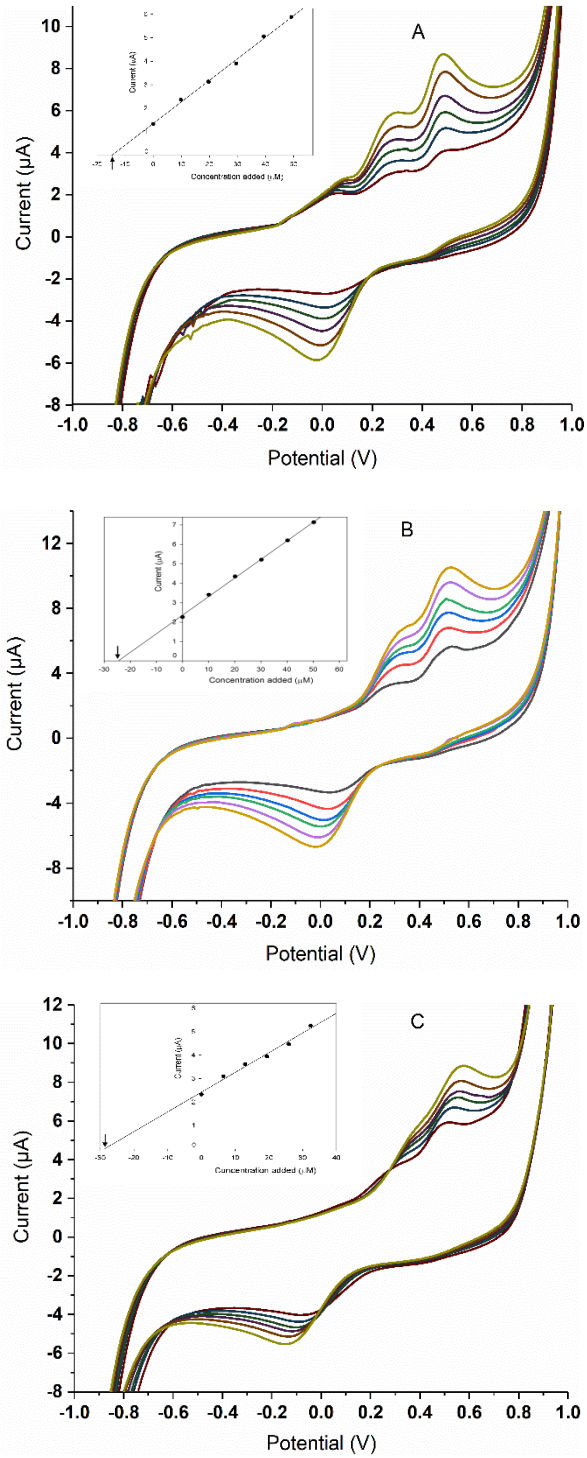


Fig 6. Cyclic voltammetric signals for additions of 10, 20, 30, 40 and 50 µM capsaicin on extracted Delhuerto hot pepper sauce (A); 10, 20, 30, 40 and 50 µM capsaicin on extracted Tabasco habanero pepper sauce (B); 6.5, 13, 19.5,26, and 32.5 µM capsaicin on the extracted Alacapsin. (C); All the measurements were carried out in glycine buffer solution (25% EtOH) pH 2.5 at open circuit condition, υ = 100 mV·s^{-1}. The first scans show the signal of diluted real sample before the additions of capsaicin.

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