Determinaition of Curcumin in Turmeric Sample Using Edge Plane Pyrolytic Graphite Electrode

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Abstract
Phenolic compounds are an important class of the antioxidants and found in several natural products. Research on the phenolic compounds having antioxidant properties present in natural products like fruits, spices and herbs are increased in recent years. Electroanalytical methods have low detection limits very short analysis time and require less budge as well. These advantages make the electroanalytical methods favorable and voltammetric analysis methods are one of the preferred methods in determination of the compounds having antioxidant properties present in different matrices. In this study, curcumin in turmeric samples were quantitatively determined by using edge plane pyrolytic graphite electrode (EPPG) and differential pulse voltammetry. Electrooxidation behaviour of curcumin was also examined by using cyclic voltammetry method. A three electrode electrochemical cell was used for voltammetric analysis. Edge plane pyrolytic graphite (EPPG), was used as working electrode, saturated calomel electrode (SCE) was used as reference electrode and Pt wire electrode was used as counter electrode in the cyclic voltamety and differential pulse voltametry studies. A linear relationship between anodic peak current and curcumin concentration was observed between 0.325 μM to 1.95μM at EPPG electrode with differential pulse voltammetry. Detection limit was calculated as 0.296 μM and the method successfully applied for detection of curcumin amount in a turmeric sample.

Keywords — Curcumin, Cyclic Voltammetry, Differential Pulse Voltammetry, Edge Plane Pyrolytic Graphite Electrode, Extraction, Turmeric

1. Introduction
Curcumin is a natural phenolic compound which has antioxidant [1,2] antibacterial [3] and anti-inflammatory [4] properties. Turmeric is a curcumin-rich spice which is commonly used in Indian food and medicine [1]. In literature, there are numerous studies on separation and analysis of curcumin in spices, performed by high performance liquid chromatography (HPLC) [6-10], microemulsion electrokinetic chromatography [11,12], capillary electrophoresis[13,14], liquid chromatography coupled to mass spectroscopy (LC/MS) [15,16], nuclear magnetic reonance (NMR) [16,17], infrared (IR) [17,18], raman [18], ultraviolet-visible (UV-VIS) spectroscopy [17,19], fluorescence spectroscopy [20].

Electrochemical methods are known to have low detection limits up to 10^{-12} M and they are usually faster and less expensive than the spectroscopic methods [21]. These advantages make the electroanalytical methods very important in analytical chemistry. Escarpa [22] indicated that electrochemical approaches can be used for food analysis effectively. Voltammetric techniques have also been used to determine curcumin in various spices. [23-36]. Carbon electrodes are relatively low cost, chemically inert and can be used in various types of stable surface modifications [37]. Edge plane pyrolytic graphite electrode (EPPGE) is one of the carbon electrodes which has favourable background current [38], fast response due to high electron transfer rate, low passivation on repeated voltammograms and low fouling effect [39], similar electrocatalytic properties to carbon nanotubes [40] and low detection limits [41,42]. Banks and Compton indicated that EPPGE could be optimal electrode [43] that can be replaced for carbon nanotube modified electrodes for routine analysis without modifications [44,45]. Scientists still have interest in
EPPGE for electroanalysis. Ostatná et al. [46] compared carbon, carbon paste, screen-printed, edge and basal plane pyrolytic graphite for analysis of human serum albumin and reported that EPPGE has better electroanalytical response than the other electrodes. Recently, unmodified edge plane pyrolytic electrode was used as a voltammetric pH sensor [47]. To the best of our knowledge, edge plane pyrolytic graphite has never been used for the electroanalytical determination of curcumin in turmeric. Various conventional methods have been used for curcumin extraction including hydro distillation and soxhlet extraction [49], pressurized hot water extraction at high temperature up to 197°C [50] or overnight soaking process [51]. Recent methods such as supercritical carbondioxide or microwave extraction were also used for extraction of curcumin from natural products [48]. On the other hand, ultrasonic extraction allows for a decrease in extraction time and temperature [52-55]. We used the EPPGE as working electrode in the cyclic voltammetry (CV) and differential pulse voltammetry (DPV) analysis of curcumin and extracted the curcumin in the turmeric samples by ultrasonic extraction method.

2. Materials and Methods
Curcumin (65%), curcumin analytical standard (98%), ethanol (99.8%), potassium chloride (99.5%), and hydrochloric acid (37%) were obtained from Sigma Aldrich, and sodium bicarbonate (99.9%) was obtained from VWR. CH Instruments electrode polishing kit was used for cleaning operations of electrodes. Chemicals were used without any further purifications. Curcuma longa rhizome powder (turmeric) was obtained from local store. A conventional three electrode electrochemical cell and CH Instruments 842B electrochemical analyser were used for voltammetric analysis. Edge plane pyrolytic graphite (d: 3 mm) was used as working electrode. Saturated calomel electrode (SCE) was used as a reference electrode and a platinum wire was used as counter electrode. All solutions were degassed for 5 minutes with argon before the measurements. EPPGE was electrochemically activated with 0.1M bicarbonate solution until stable voltammogram was obtained after mechanical cleaning procedure. Cyclic voltammograms were recorded at a scan rate of 100 mVs⁻¹. In differential pulse voltammetry, a pulse height of 50 mV and pulse width of 0.05 s were selected. All measurements were carried out at room temperature.

2.1 Preparation of stock and supporting electrolyte solutions
Curcumin is soluble in organic solvents like acetone, ethanol, dichloromethane. Wakte et al. reported that extraction with acetone is more efficient than ethanol [48]. On the other hand, GlaxoSmithKline’s solvent selection guide indicates that ethanol is a more greener solvent than acetone [56]. A stock solution of 6.5x10⁻⁴ M curcumin was prepared in ethanol and diluted to the required concentrations to be used as calibration standards.

It was shown that at pH > 7, curcumin alkaline degradation occurs and ferulic acid is formed together with condensation products [57]. Therefore, quantitative analysis of curcumin by DPV was performed at acidic and neutral pH values. 0.1M KCl solutions were used for pH 2. Hydrochloric acid was used to adjust desired pH values. 0.1M phosphate buffer solution was used at pH 7 for CV.

2.2. Extraction of curcumin in turmeric samples
0.5 gram turmeric powder was dissolved in 20 mL ethanol and placed at 55 °C in ultrasonic bath for 30 minutes. The mixture was then centrifuged at 5500 rpm for 5 minutes and the supernatant was collected. The remaining solid was extracted twice more by following the same procedure. The supernatant solutions were gathered and diluted with buffer solution to reduce the matrix effect prior to electrochemical analysis. We assumed that all the curcumin in turmeric sample were extracted after these three extraction.

3. Results and Discussion
3.1 Cyclic Voltammetry of curcumin
Electrooxidation of curcumin at edge plane pyrolytic graphite electrode is an irreversible and pH dependent process and peak current of curcumin oxidation is strongly affected by pH values. Highest oxidation peak 2a shown on CV of 1.3x10⁻⁴ M curcumin solution in Figure 1 was chosen to use in quantitative analysis of curcumin by DPV. Quite high peak current values were obtained by EPPG electrode at pH 2. Electrochemical response is directly related to the electrode material, edge sites on the surface of EPPG electrode also provide an increased peak current as response for curcumin concentration.

![Figure 1. CV voltammogram of 1.3x10⁻⁴ M curcumin with EPPG electrode in 0.1M pH 2 KCl buffer.](Image)
3.2. Extraction and determination of curcumin in turmeric sample

Standard solutions of curcumin were prepared and the anodic current values were measured by DPV. (Fig. 2a) Higher current values were obtained at pH 2 KCl solution, so it was selected for quantitative determination of curcumin. A quasi-linear relationship between anodic peak current and curcumin concentration was observed. A calibration curve was plotted between 0.325 μM to 1.95μM at EPPG electrode with differential pulse voltammetry. Detection limit was calculated as 0.296 μM. The regression equation was ip (μA) = 3,90C - 3,1 x10⁻⁷ (R²=0.9915). Detection limit was calculated according to equation of C = 3Sy/x /b, (where Sy/x is y- residuals and b is the slope of the calibration curve) [58,59].

Conventional soxhlet extraction, maceration and stirring are time consuming and extraction procedure of curcumin takes hours [60].

Supercritical carbon dioxide extraction of curcumin [48] needs high pressure which has flow systems. Pressured hot water extraction need high temperature and pressure [50]. Ultrasonic extraction has provided advantages in extraction time, temperature and solvent volume. Ultrasonic extraction process applied for turmeric reduced the extraction time to 90 minutes without any complex extraction system.

Tayyem et al [5] have analyzed different turmeric samples and explained that average curcumin content was 1.51 % and reported that their turmeric products contain 0.58% at lowest and 3.14% at highest. In this study, extraction of curcumin in the turmeric samples was repeated 3 times. It was assumed that the total amount extracted by these repeated extractions were 100 percent, hence in first, second and third extractions extraction yields were calculated as 85.8%, 11.5% and 2.7 % respectively. Percentage amount of curcumin in the turmeric sample were found 1.03% (first extraction), 0.138 % (second extraction), 0.03 % (third extraction) and 1.198% as total. (Fig. 2c)

At table 1, working electrodes, electrochemical methods, working ranges and detection limits for the analysis of curcumin in the literature were given.

In literature, researchers generally selected carbon based and modified electrodes for determination of curcumin.
Since nanoparticles or conductive polymer modified electrodes provides increase in current response while adsorptive stripping voltammetry methods, differential pulse voltammetry and square wave voltammetry provides low detection limits, modified electrode studies found in literature generally had better quantitative results than unmodified ones as expected. When we look at unmodified working electrode studies, although Gholivand et al [33] have found lower detection limits than this study, they used stripping voltammetry. In addition, HDME is not used commonly in routine analysis because of its toxicity and handling problems. This method and EPPGE electrode was successfully applied to the detection of curcumin in turmeric.

| Working electrode | Electrochemical Method | Working range(mol/L-1) | Detection Limit(M) | Reference |
|-------------------|------------------------|------------------------|--------------------|-----------|
| MWCNT/GCE         | FLE-FFTSWV a           | 1.0x10^-8 - 1.0x10^-6  | 5.0x10^-9          | 44        |
| DyNW/CPE b        | FLE-FFTSWV             | 2.0x10^-9 - 1.0x10^-6  | 5.0x10^-10         | 44        |
| PdNps/Poly(Pr)/GE c | SWV                   | 5.0x10^-9 - 1.0x10^-7  | 1.2x10^-9          | 45        |
| Poly-ACBK/GCE d   | DPV                    | 1.0x10^-7 - 7.0x10^-5  | 4.1x10^-8          | 35        |
| GR/GCE e          | LSV                    | 5.0x10^-8 - 3.0x10^-6  | 3x10^-8            | 46        |
| Modified Electrodes |                        |                        |                    |           |
| HDME              | DPAdSV f               | 5.76x10^-3 - 4.83x10^-6 | 9.6x10^-7 - 1.08x10^-5 (for anodic peak) | 444 |
| HDME              | DPAdSV f               | 5.76x10^-4 - 2.74x10^-6 | 9.6x10^-7 - 4.84x10^-5 (for cathodic peak) | 444 |
| HDME              | AdCSV g                | 5x10^-9 - 2.8x10^-7    | 1.5x10^-9          | 39        |
| GCE               | CV                     | 9.9x10^-6 - 1.07x10^-4 | 4.1x10^-6          | 38        |
| Carbon-SPE        | AdSV                   | 2.2 x10^-6 - 2.8 x10^-4 | 4.9x10^-6          | 37        |
| EPPGE             | DPV                    | 3.25x10^-7 - 1.95x10^-6 | 2.96x10^-7         | This work |

4. Conclusions
Electrochemical oxidation of curcumin was investigated on edge plane pyrolytic graphite by CV and EPPGE and shown that detailed information similar in the literature could be obtained by this working electrode which had good sensitivity for determination of low amounts of curcumin. Ultrasonic bath extraction is readily applicable to the extraction of phenolic compounds in the natural products and provides required concentration of curcumin for voltammetric analysis, Differential pulse voltammetry is a powerful method for determination of micromolar concentrations and does not require preconcentration step as in the stripping methods. This method which provided high current response and low detection limit for curcumin analysis without stripping procedure or any modification of EPPG electrode, is suitable for routine analysis.

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