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SHORT COMMUNICATION

Electrochemical Analysis of Natural Chemopreventive Agent *(Curcumin)* in Extracted Sample and Pharmaceutical Formulation

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ABSTRACT

Curcumin has anti-oxidative and anticarcinogenic activities. This study shows the electrochemical behaviour of curcumin using polarography, i.e., DC polarography and differential pulse polarography (DPP) methods. In ammonium tartrate as supporting electrolyte, the differential pulse polarogram of curcumin shows two conjugated peaks with peak potential (E_p) –1125 mV and –1275 mV *vs* SCE. However, the direct current polarogram shows only one polarographic wave with $E_{1/2}$ which was –1275 mV. The developed electrochemical methods have been standardised for the determination of curcumin in extracted sample of natural origin and its pharmaceutical formulation. The electrochemical analysis has been supplemented by ultraviolet and infrared spectral analyses of the samples.

Keywords: Curcumin, DPP, anticarcinogenic, pharmaceutical formulation, differential pulse polarography

1. INTRODUCTION

Experiments, *in vitro* and epidemiological studies have shown that some compounds present in the diet have antimutagenic and anticarcinogenic properties[1]. Curcumin [1, 7-bis (4, hydroxy–3–methoxy phenyl)–1, 6-heptadiene–3, 5–dione] is the major yellow pigment extracted from turmeric[2] a commonly used spice derived from *Rhizome* of herb *Curcuma longa* (*Linn.*). Curcumin has shown cancer chemopreventive, antineoplastic, anti-inflammatory properties[3-7]. Curcumin acts as scavenger of oxygen species, such as hydroxyl radical, superoxide anion and singlet oxygen[8] and it interferes with lipid peroxidation[9-11]. Through metal binding curcumin protects against lead and cadmium induces lipid per oxidation in rat brain homogenates.

Curcumin has proved not to have toxic genotoxic or teratogenic properties[12-14]. Biological activity of curcumin has been attributed to the hydroxyl group substituted to the benzene rings and also to the diketonic structure. The â-diketo moiety of curcumin undergoes a keto-enol tautomerism. Crystal study has shown that symmetric structure of curcumin leads to statistically even distribution of the enol proton between the two oxygen atoms.

The HPLC, GLC, potentiometric, and spectro-photometric methods were used for the studies of curcumin analysis[15, 16]. However, the literature is silent on the use of polarographic methods for the analysis of curcumin in, different samples.

The present study was undertaken to authenticate DCP and differential pulse polarography (DPP) methods for an accurate analysis of curcumin extracted from natural origin extended to pharmaceutical formulation. The UV and IR spectroscopic methods have been used to supplement the developed polarographic method.

2. MATERIALS AND METHODS

The chemicals used were of analytical reagent grade. Curcumin was procured from Sigma Chemical Company (St. Louis, Mo.). Turmeric powder was purchased from local market; Double distilled water and ethanol were used for the preparation of experimental solutions. Turmeric powder, soxhlet assembly, distillation unit, alcohol (95 %), benzene, $Na(OH)_2$, dilute *HCl*, and concentrated H_2SO_4 were used for extraction of curcumin from turmeric powder.

2.1 Extraction of Curcumin from Turmeric Powder

Curcumin from turmeric was extracted as described by Kokate[17]. Turmeric powder of 50 g was taken with 95 per cent alcohol in a soxhlet assembly until all the colouring matter was extracted and alcoholic extract was distilled off to a semisolid brown mass. The crude extract was dissolved in 50 ml benzene and extracted twice with equal volume of 0.1 per cent *HCl*, a yellow coloured precipitate was formed. It was allowed to settle for about 15 min, and after setting the precipitate, the extract was concentrated by boiling on water bath at the same time dissolving precipitate in boiling water during this process of boiling. The resinous material would agglomerate, then filter the solution in hot condition and concentrate the filtrate to very small volume and finally cool to get curcumin. After extraction, the sample was analysed by DCP, DPP, UV, and IR.

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2.2 Instrumentation and Conditions

2.2.1 Polarography

The DCP and DPP studies, a µp-polarographic analyser [(Elico, India) model CL–362] was used. An Elico digital p*H* meter (model 335) was used for p*H* measurement. The polarographic cell consisted of three electrodes–SCE as reference electrode, platinum electrode as auxiliary electrode, and dropping mercury electrode as the working electrode.

2.2.2 Spectroscopy

Perkin Elemer Spectrum One FTIR spectrometer was used for IR spectroscopic measurement equipped with a universed ATR accessory. Perkin Elmer Lambda 3 B UV/VIS spectrophotometer equipped with Perkin Elmer R-100 A recorder was used for ultraviolet spectroscopic measurements.

2.3 Sample Preparation for Polarography

2.3.1 Authentic and Extracted Samples

Authentic curcumin (0.368 g) was dissolved in 100 ml ethanol. Sets of solutions containing varying concentration of curcumin were prepared in 1M overall concentration of ammonium tartrate at p*H* 8.1±0.1 in 25 ml analyte. Similar procedure was followed for the preparation of the analyte for extracted sample from natural origin.

2.3.2 Pharmaceutical Formulation

Turmeric Force™ (from Jeevan Annad Pharma Company, Indore) is a pharmaceutical formulation containing curcumin. Turmeric Force (3 ml) along with 5 ml of ethanol was transferred to a polarographic cell containing 12 ml of ammonium tartrate of p*H* 8.1 ± 0.1 for qualitative and quantitative analyses.

3. RESULTS AND DISCUSSIONS

The authentic curcumin sample, in 1M ammonium tartrate at p*H* 8.1±0.1, produced a well-defined DCP curve. Figure 2 with half wave potential ($E_{1/2}$) = –1275 mV *vs* SCE. Whereas the DPP response of the solution resulted in two well-defined peaks at Fig. 3 with peak potential (E_p) = –1125 mV and –1275 mV *vs* SCE. The DPP shows two peaks because its sensitivity is more than that of DCP (10^{-8} µM for DPP, 10^{-5} µM for DCP).

3.1 Effect of p*H* and Supporting Electrolyte

The p*H* of test solution is a key factor to get better polarographic waves. In the present experiment, p*H* 8.1± 0.1 of the test solution has been used for the determination of curcumin in authentic natural origin samples and pharmaceutical formulation.

At this p*H* of test solution, observed DCP/DPP peaks, were well-defined and diffusion current/peak current of the polarogram were found to be proportional to the curcumin concentration. However, in the analysis of pharmaceutical formulation and extracted sample, the minimum matrix effect was found to be at p*H* 8.1 in comparison to at other p*H*, in ammonium tartrate as supporting electrolyte. In other supporting electrolytes, the matrix effect was found to be predominant. As such, the determination of curcumin in the extracted and pharmaceutical samples was done at p*H* 8.1± 0.1.

3.2 Interpretation

Curcumin involves two electron reduction processes at p*H* 8.1±0.1 shown in Fig. 1. The two double bonds which are by the side of the *OH* and *C=O* groups are reduced to give a doublet using DPP.

The peak height of both the peaks in the case of DPP of the polarogram was found to be proportional to the curcumin concentration. It was also noted that there was no change in half wave potential $E_{1/2}$ (DCP) and peak potential E_p (DPP) values of the resulting polarograms with increasing curcumin concentration, thus, enabling the qualitative as well as quantitative use of the developed electrochemical procedure for the analysis of curcumin in different samples.

The developed procedure was, therefore, applied for the analysis of curcumin in the extracted sample which showed two well-defined peaks at –1125 mV and –1275 mV *vs* SCE, respectively, indicating the presence of curcumin in turmeric extract. Method of standard addition was used

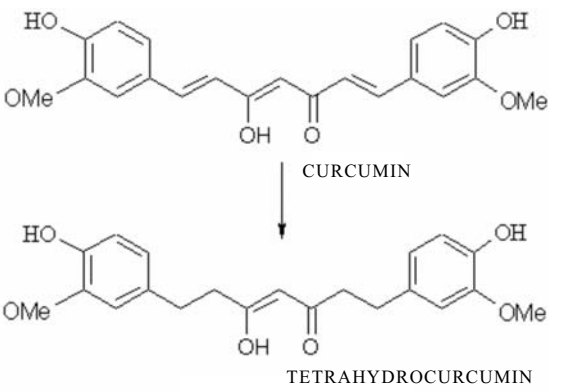

Figure 1. Schematic diagram of reduction of curcumin.

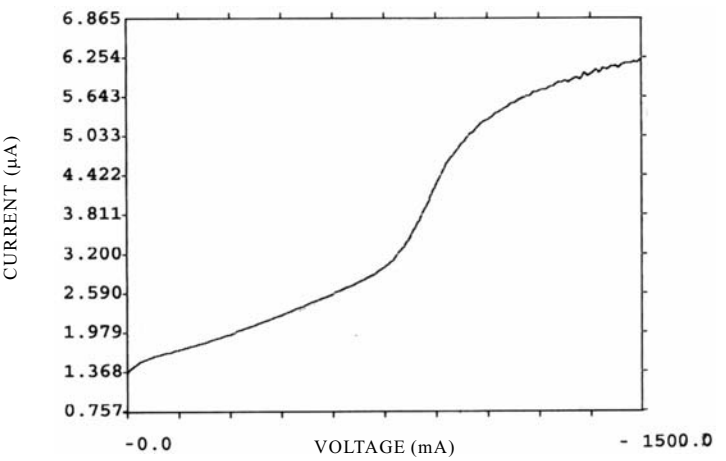

Figure 2. DCP of 0.0012 M curcumin in 1 M ammonium tartrate at p*H* 8.1 ± 0.1.

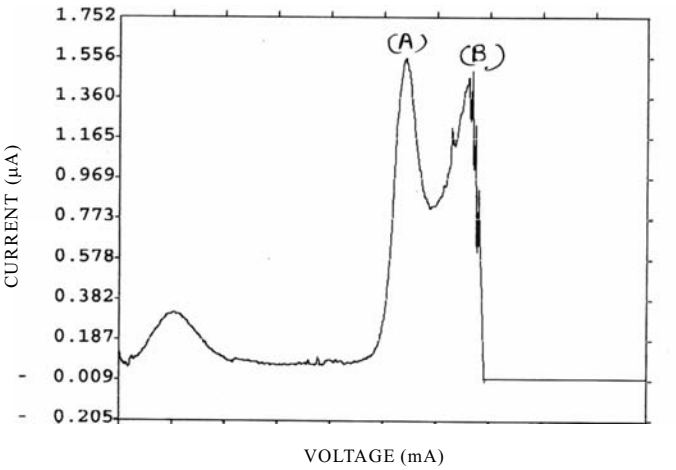

Figure 3. DPP of 0.0012 M curcumin in 1 M ammonium tartrate at p*H* 8.1 ± 0.1.

for the quantitative analysis of curcumin used in the extracted mass. The resulting DPP curves of spiked analyte showed two peaks (A and B) with no change in peak potential *Ep* values but the peak height was increased, thus, confirming the presence of curcumin in the extracted sample and also enabling the possible use of the developed procedure for an accurate qualitative analysis of curcumin in natural origin extracts. The results of analyses have been tabulated in Table 1. Method of standard addition was used for quantitative analysis of curcumin in the extract from natural origin. The per cent recovery was found to be more than 99 per cent in each case

This procedure was applied for the pharmaceutical formulation of curcumin, Turmeric Force™ at p*H* 8.1±0.1.

Method of external spiking was used to validate the presence of curcumin in the formulation, Turmeric Force™ at p*H* 8.1±0.1.The results of the analysis are tabulated in Table 1. For curcumin in pharmaceutical formulation under studies the curcumin concentration was 0.35 mg/ml which is in excellent agreement with that reported by the manufacturer.

The UV spectra of authentic curcumin (ethanolic solution) and extracted curcumin are similar in both the spectrum at λ_{max} 419.5nm and IR spectroscopy has been used for the characterisation of the sample of curcumin. The FTIR spectra of authentic curcumin using ATR accessory clearly

Table 1. Polarographic analysis (DPP) of curcumin in extracted sample

Extracted sample (ml)	Amount of curcumin (mg/ml)		Per cent recovery
	Added	Found	
5	-	0.415	98.40
	0.184	0.590	
15	-	0.871	100.0
	0.184	1.060	
20	-	2.020	100.0
	0.184	2.196	
In pharmaceutical formulation (Turmeric Force™)			
3	-	0.213	99.50
	0.184	0.395	

shows following characteristic signals at: (a)1627 cm^{-1} which is a characteristic peak for *C=O* (enolic), (b) 1520 cm^{-1} shows the presence of *C–C* group, (c) 1250 cm^{-1} shows the *C–O* stretching, and (d) 3547 cm^{-1} shows the presence of OH group present in the molecule, respectively, corresponding to the presence of curcumin extracted from turmeric powder also showed signal at the same wave numbers. Thus confirming the presence of [1, 7-bis (4, hydroxy–3–methoxy phenyl)–1, 6-heptadiene–3, 5–dione] in the extracted sample. The standard deviation and coefficient of variance never exceeded, respectively. Thus, confirming the reliability of the observed data.

4. CONCLUSIONS

The established method in this study has successfully determine curcumin in turmeric by optimising the parameters such as p*H*, viscosity of the solution, supporting electrolyte from the result of determination, the amount of curcumin in extracted sample and pharmaceutical formulation, their determine quantity. Polarographic methods are to be used in analysis of curcumin in extracted and pharmaceutical formulation.

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