Differential Pulse Anodic Voltammetric Determination of *Dapsone* in Pharmaceutical Preparation Using Carbon Paste and Glassy Carbon Electrodes: Application to Quality Control

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Abstract: The voltammetric behavior of *Dapsone* has been carried out at carbon paste electrode (CPE) and glassy carbon electrode (GCE) using cyclic voltammetry and differential pulse voltammetry. *Dapsone* undergoes a well-defined irreversible oxidation peak at both electrodes. The proposed methods were successfully utilized for the determination of *Dapsone* in various dosage forms without interference from matrix with acceptable precision (RSD < 1.46 %) and accuracy (RE < 1.2 %). Forced stability studies of drug in acid, basic and oxidative media were monitored. Moreover, the CPE was used to study the *in vitro* release of *Dapsone* tablet, and testing tablet uniformity. The kinetic studies reveals first order with diffusion controlled release. The results compare favorably with data obtained by the official volumetric method.

Keywords: *Dapsone*; Cyclic voltammetry; Differential pulse voltammetry; Stability studies; *In-vitro* release.

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Introduction

Dapsone is 4,4'-diaminodiphenyl sulfone (DDS) (see Scheme 1) [1,2]. It is antileprotic drug used in the treatment of paubacillary leprosy [3,4]. This drug is an official medicament in the BP and USP lists [5,6].
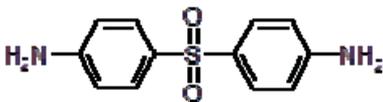
Scheme 1:
The chemical structure of Dapsone.
$M(C_{12}H_{12}N_2O_2S) = 248.302$ g mol^{-1}

The methods available for analysis of the drug in pharmaceutical dosage forms and biological fluids include volumetry (classical titrations [7]), spectrophotometry [8-12], electrochemical [13,14] and HPLC [15,16].

Carbon and its derivatives, as the high performance material, occupy a special place in electrochemistry due to its extreme properties [17]. Carbon paste electrodes (CPEs) had found their place in modern electrochemistry, due to its versatility, ease of preparation and composition modification, low cost of ingredients and instrumentation, and high sensitivity. Glassy carbon electrode (GCE) is a class of non-graphitizing carbon. It is used very commonly because of its excellent mechanical and electrical properties, impermeability to gases, and extremely low porosity. In general, in pharmaceutical analysis, these electrodes have many applications [18-20].

A literature survey revealed that no attempt has been made to study the differential pulse voltammetric behavior of DDS for stability study and in vitro release of *Dapsone* tablet. Therefore, the aim of this study was to establish and optimize the experimental conditions for the determination of DDS in drug substance and product at carbon paste electrode, and glassy carbon electrode using cyclic voltammetry (CV), and differential pulse voltammetry (DPV). Moreover, the stability of drug was monitored under different stress conditions, acid, basic, and oxidative. The *in vitro* release of drug from its tablet dosage form was investigated which revealed first order kinetics with diffusion controlled release. To the author's knowledge, these studies were not investigated before.

Experimental

Chemicals, Reagents, Stock and Standard Solutions

Dapsone was kindly supplied from El-Nile Co., Egypt. Its purity was found to be 99.80 % according to the official volumetric method [5]. *Dapsone*® tablet, labeled to contain 50 mg DDS per tablet, El-Nile Co., Egypt (Batch No. 16226), was purchased from the market. Standard stock solution (2.48×10³ µg mL^{-1}) was prepared by dissolving appropriate weight of the drug standard powder

(62 mg) in 25.0 mL methanol under continuous stirring until complete dissolution of the drug. The standard solution was then kept in a refrigerator.

Working standards were freshly prepared just before assay by dilution of the standard stock solution using an appropriate amount of Britton-Robinson (B-R) buffers in the range from pH 2.26 to 10.50, which served as supporting electrolyte. Unless otherwise stated, all solutions were prepared using doubly distilled water and analytical grade reagents. Also, all potentials were measured against and referred to Ag/AgCl reference electrode.

Electrochemical Apparatus and Other Instrumentation

Cyclic voltammetry and differential pulse voltammetry were performed using an electrochemical workstation. A three compartment electrochemical cell, incorporating the working electrode (CPE or GCE) was used. The carbon paste of uniform graphite particles was mixed with a paraffin binder (for use in aqueous media). The reference electrode was the Ag/AgCl (3 M KCl) and a Pt wire was used as an auxillary electrode. The operating conditions for the DPV were 50 mV pulse amplitude, 30 ms pulse width and a scan rate of 10 mV s^{-1}. The working procedures and preparation conditions of both the CPE and GCE were always the same.

Preparation of the Working Electrodes

A paste was prepared by mixing graphite powder (0.5 g) with Nujol® (0.3 mL) in a mortar using a pestle. The carbon paste was packed into the hole of the electrode body and smoothed on a filter paper, until it has a shiny appearance. The glassy carbon electrode was polished with 0.5 mm diameter alumina powder on a smooth polishing cloth. The electrode was rinsed with ethanol, then with water, dried and fitted to electrochemical cell.

Electroanalytical Measurements and Related Procedures

The potential scan was carried out between -0.4 and +1.4 V at a scan rate of 10 mVs^{-1} for a large number of scans. Generally, 10 min of potential cycling were enough to produce a reproducibly clean surface. The cell was then filled with 5 mL B-R buffer as the blank solution and the potential scan started from -0.4 to +1.4 V. After recording the voltammetric data of the blank solution, an appropriate amount of DDS was added and the voltammetric response at the working electrode was recorded. The measurements were carried out at constant room temperature of 25±2 ^{o}C and the peak heights were evaluated by the tangent procedure [21]. The pH of the solution was found to have a significant effect on the voltammetric drug response.

Calibration Graph

A 5 mL of the electrolyte solution was transferred into the voltammetric cell. After measurement of the blank solution, aliquots equivalent to 2.48 – 124 µg mL^{-1}, and 12.4 – 74.4 µg mL^{-1} of DDS were added for CPE, and GCE respectively and the potential cycling was carried out under constant operational parameters (pH and scan rate). The peak heights were evaluated as the difference between each voltammogram and the background electrolyte voltammogram. All measurements were carried out at room temperature (25±2 ^{o}C).

Application of the Proposed Differential Pulse Voltammetry

Application to Drug Product. Six tablets of DDS were finely powdered and weighed, and the average mass per tablet was determined. A portion of finely powder needed to obtain 2.48×10^3 µg mL^{-1} DDS was accurately weighed, transferred to a 100 mL volumetric flask, and 80 mL methanol was added. The flask was sonicated for 30 min and made up to the volume with the same solvent. The solution was filtered to separate out the insoluble excipients. The first portion of the filtrate was rejected and the general procedure was followed.

Stress Stability Studies. The drug was dissolved in aqueous 2 M HCl, 1 M NaOH and exposed to 30% H_2O_2 in concentration 1 mg mL^{-1} at 90 ^{o}C for 2 hrs in thermostatic water bath. Every 15 min 1 mL of each solution was neutralized and evaporated on water bath at 90 ^{o}C. Then the electrochemical behavior was followed by using CPE.

In Vitro Release Study of Dapsone Tablet. Effectiveness of DPV method in estimating DDS was confirmed by comparing with UV spectrophotometric method. Dissolution studies of the released of *Dapsone* from the tablet matrix were conducted and the samples obtained were analyzed by both methods. In vitro release of *Dapsone* from its tablet dosage form (50 mg/tablet) was followed according to the official method [5] using apparatus 1 (rotating basket). The bag was rotated at 100 rpm in 900 mL of the freshly prepared 0.1 M HCl solution dissolution medium and thermostatically adjusted at a temperature of 37±5 ^{o}C. At predetermined time intervals (every 15 min for 2 hours), aliquot of 20 mL of the release medium was withdrawn from each vessel and equal volume was replaced with fresh 0.1 M HCl aqueous solution. The samples were filtered and analyzed for DDS content by UV spectrophotometric method and DPV method (n = 3). The absorbance was measured at 289 nm using aqueous 0.1 M HCl as a blank for UV spectrophotometric method. While for DPV method the recommended procedure was followed using CPE. The percentage release of *Dapsone* from tablet was calculated from the previously constructed calibration curve.

The data obtained from the in vitro release studies were kinetically analyzed by plotting the relation between the remaining percentage of DDS against concentration to determine the mechanism and the order of drug release.

Results and Discussion

Preliminary investigation of the oxidation of a 12.4 µg mL $^{-1}$ solution of DDS in acid medium at CPE (pH 5.1) and GCE (pH 2.26) shows one well irreversible peak at about 0.966 and 1.01 V for CPE and GCE respectively. From the structure of DDS it can be concluded that the oxidation peak obtained is due to NH_2 group.

The prepared samples were subjected to a series of investigations to optimize the determination conditions of the pharmaceutical preparation. Different supporting electrolytes, namely HCl, acetic acid/sodium acetate, phosphate buffer and Britton-Robinson (B-R) buffer were investigated. From all those supporting electrolytes the B-R buffer solution was found to give the best and most reproducible results and was used in all investigations.

The voltammetric behavior of DDS was found to be affected by the solution pH and the type of supporting electrolyte. The effect of the above mentioned supporting electrolytes and the pH of the solution on the peak current of the drug were recorded. The different investigations were then carried out in the best supporting electrolyte, i.e. the B-R buffer over the pH range 2.26 – 10.50 with different scan rates. Typical cyclic voltammograms of 2.48×10^2 µg mL^{-1} drug in B-R buffer of pH 5.1 recorded with 100 mV s^{-1} scan rate at CPE are presented in Fig 1. Similar results were obtained using 0.744×10^2 µg mL^{-1} DDS at the GCE with pH 2.26.

Dapsone was found to give a well-defined anodic peak at pH 5.1 for CPE and 2.26 for GCE. In both cases no cathodic peak in the reverse scan was recorded, which means that the oxidation of the tested drug is irreversible.

The optimum concentration of the B-R buffer at the respective pH was found to be 0.04 mol L^{-1}. It was observed that the peak potential shifts to the positive direction when the solution pH decreased. On both the CPE and GCE electrodes, the peak potential shows a semi-linear relation between pH 5.1 and 2.26. As found out, when the pH has increased, the peak current oppositely decreased.

The decrease of the peak current with the increase of the solution pH is attributed to the fact that the electroactive species of the drug occur in the basic form and the drug molecules are exchanging protons during the redox process.

The rate of formation of the basic form of the drug conjugate acid it reaches its diffusion controlled limiting value at higher pH, where the rate of transformation of the conjugate acid to the basic form reaches its maximum.
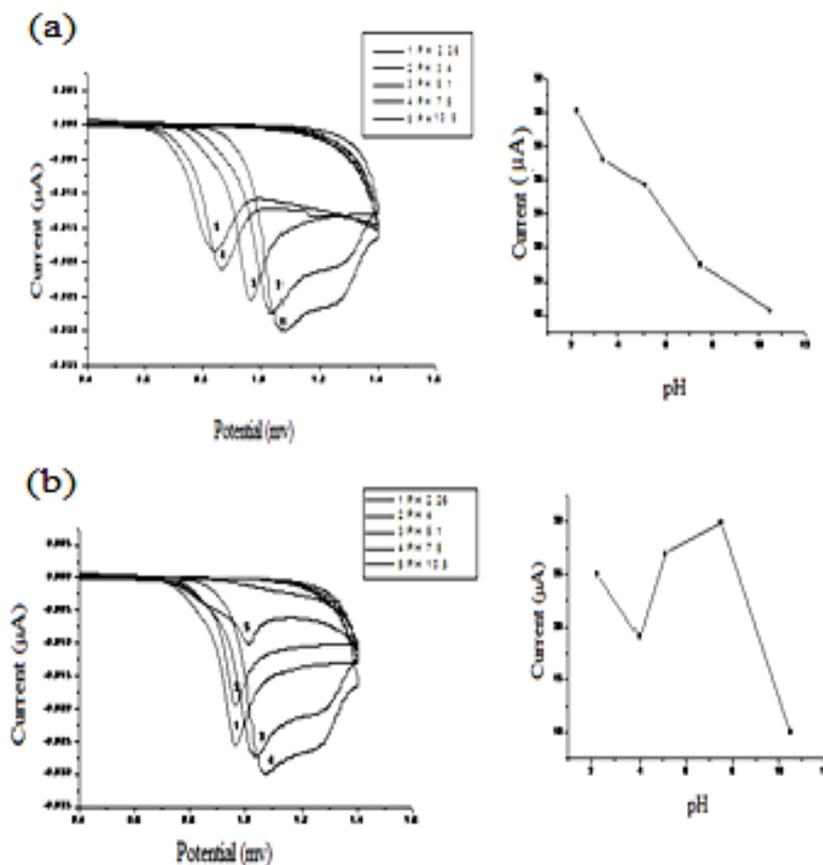
Fig. 1: *Cyclic voltammograms of Dapsone in B-R buffer of different pH 2.26, 3.4, 5.1, 7.5, 10.5 at scan rate 100 mV s⁻¹*: **a**) CPE, 2.48×10^2 µg mL⁻¹, **b**) GCE, 0.74×10^2 µg mL⁻¹.

The scan rate was found to affect both peak potential and peak current. By increasing the scan rate, the peak potential shifts in the anodic direction. The positive shift is accompanied by an increase in the peak current. The peak current (Ip) increases with the scan rate (v) as logarithmic function in the range between 10 and 250 mV s⁻¹.

When considering a reduction process at the potentials positioned positively from the redox potential, there is no faradaic reaction in response to the pulse, so the difference current reaches a maximum, and decrease to zero as the current becomes diffusion controlled [22]. A typical example of the variation of the peak current of DDS with scan rate is then presented by the corresponding sets of voltammograms in Fig. 2.
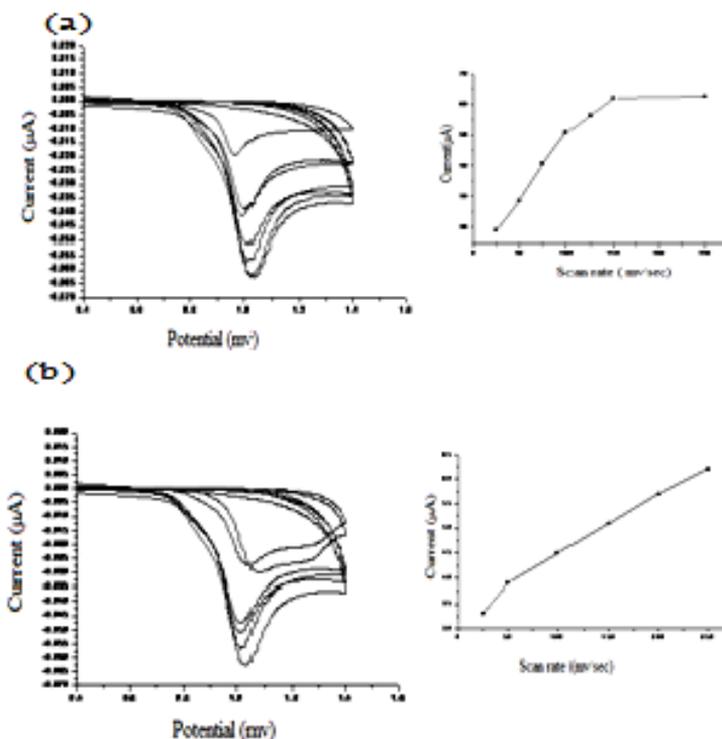
Fig. 2: *Cyclic voltammograms of Dapsone in B-R buffer*. **a**) pH 5.1 for CPE 2.48×10^2 μg mL^{-1}, and **b**) pH 2.26 for GCE 0.74×10^2 μg mL^{-1} at different scan rates (25 – 250 mV s^{-1}).

The relation between "Ip" and "v" can then be formulated as

$$\text{Log Ip} = A + B \log v \tag{1a}$$

$$\log \text{Ip} = -0.061 + 0.37 \log v, \text{ for CPE and} \tag{1b}$$

$$\log \text{Ip} = -0.50 + 0.44 \log v, \text{ for GCE}; \tag{1c}$$

where "A" is the intercept and "B" the slope of the linear relation. An increase in the scan rate is accompanied by a positive shift in the peak potential and an increase in the peak current. The increase of Ip with v is obeying the above log – log relation. The value of the slope of the obtained linear relations is around 0.5, which implies that the participating species are transported by a diffusion process [22]. This means that the electrode surface was immediately covered completely with the electroactive species. From different investigated scan rates, the 100 mV s^{-1} gave the best voltammograms and higher selectivity.

Analytical Aspects

Calibration Graph. A scan rate of 10 mV s^{-1} gave well defined DPV peaks and reproducible results. The peak current increases with successive additions of the drug. The DPV voltammograms for determination of different concentrations of the drug at CPE and GCE surfaces were recorded. Standard measurements were carried out and calibration curve was constructed for DDS. Typical examples of the differential pulse voltammograms of DDS recorded at the CPE and GCE are presented in Fig. 3a and b, respectively.
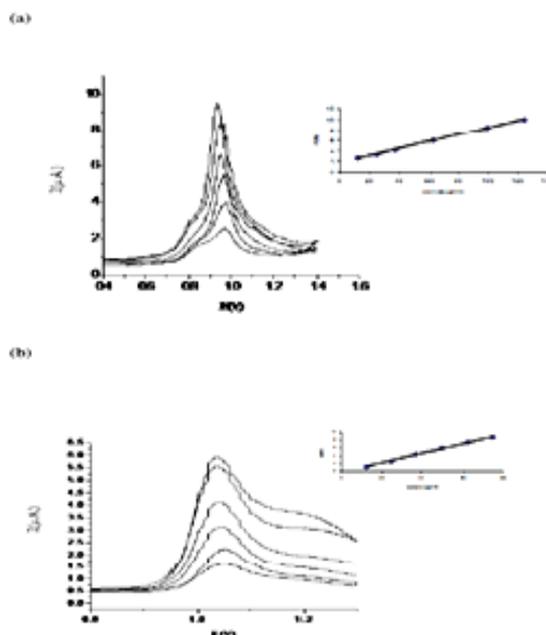

Fig. 3: *Calibration curves of anodic peak current **a)** for CPE in range 2.48 - 124 µg mL^{-1}, and **b)** for GCE in range 12.4 - 74.4 µg mL^{-1}, pulse amplitude = 40 mV, scan rate = 10 mV s^{-1}.*

The corresponding calibration curve is presented as inserts in the respective voltammograms. The calibration plots were described by the following equations:

$$Y = 0.0669\,c + 1.7454; \quad r = 0.9993; \quad \text{for CPE} \qquad (2a)$$
$$Y = 0.1252\,c - 0.3579; \quad r = 0.9986; \quad \text{for GCE} \qquad (2b)$$

Where Y – current (Ip) in µA, c = concentration (µg mL^{-1}), and r = correlation coefficient.

Method Validation. The limits of detection (LOD) and quantification (LOQ) were calculated according to the following equations [23]:

$$\text{LOD} = 3 \times \text{SD} / \text{slope}, \quad \text{LOQ} = 10 \times \text{SD} / \text{slope} \tag{3a,b}$$

Where SD is the standard deviation of intercept. The calculated values for the drug at both the CPE and GCE are presented in Table I.

Table I: *Validation report for differential pulse voltammetric method for determination of Dapsone using CPE and GCE.*

Parameters	CPE	GCE
Linearity & range ($\mu g \ mL^{-1}$)	2.24 – 124	12.4 – 74.4
LOD ($\mu g \ mL^{-1}$)	0.96	1.82
LOQ ($\mu g \ mL^{-1}$)	3.21	6.06
Regression		
Slope±SE	0.067±0.0012	0.125±0.0032
Intercept±SE	1.750±0.084	0.357±0.158
Correlation coefficient	0.9993	0.9986
SE of estimation	0.114	0.170

The precision and repeatability were carried out three times. The relative standard deviation was calculated to be less than 1.6 indicating the high precision of the method and the confidence in its repeatability (Table II).

Table II: *Intra and inter-day precision and accuracy of the proposed differential pulse voltammetric assay for Dapsone in drug substance.*

Concentration ($\mu g \ mL^{-1}$)		Precision[a] RSD [%]				Accuracy[a] RE [%]			
		Intra		Inter		Intra		Inter	
CPE	GCE	CPE	GCE	CPE	GCE	CPE	GCE	CPE	GCE
24.8	12.4	1.230	1.364	0.408	1.178	0.67	1.20	-0.33	-0.43
37.2	37.2	0.428	0.246	0.543	0.398	-1.80	-2.00	-1.47	-1.36
62.0	62.0	1.090	0.354	0.649	0.935	-0.93	1.16	1.10	0.23

Legend: a) n = 3.

The robustness of the proposed method is evaluated by the constancy of the peak area values with the deliberated small changes in the experimental parameters, which was realized by the method. The time between preparation of the solutions and the measurement gives an indication about this factor.

The results obtained by applying electroanalytical method for the analysis of DDS in drug substance and product based on CPE and GCE were statistically compared with the official volumetric method. The values of the calculated F and t test are less than tabulated ones, which reveals that no significant difference with respect to precision and accuracy [24] as stated in Table III.

Table III: *Statistical comparison between the proposed differential pulse voltammetry and Pharmacopoeia methods.*

Values	Proposed DPV method		Official Method[a]
	CPE	GCE	
Mean	99.80	100.02	100.29
SD	0.6	1.45	1.166
SE	0.24	0.59	0.47
Variance	0.36	2.1	1.36
n	6	6	6
F (5.05)[b]	1.540	3.780	-
t (1.8125)[b]	0.355	0.916	-

Legend: a) Official volumetric method [5]; b) values in parentheses represent the declared
t and F values at P = 95%.

The percent recovery was obtained by the standard addition technique, where different levels of standards were added to previously analyzed sample. Then the percent recovery was calculated and the mean recovery was obtained. The results are presented in Table IV.

The specificity of the method was confirmed by investigation of the voltammograms of both the standards and the drug test solution. Identical voltammograms were obtained. The addition of the standard drug solution to the test solution did not change the characteristics of the differential pulse voltammogram.

Application to Dapsone Tablet. The proposed DPV method was successfully applied to determine DDS in its tablet dosage form. The results were compared with those obtained with the official method.

Table IV: *Results of application of standard addition technique to Dapsone® tablet (50 mg/tablet) by the proposed differential pulse voltammetric method using CPE and GCE.*

Amount taken (μg)		Pure added (μg)		Found of claimed amount[a] (recovery±RSD [%])	
CPE	GCE	CPE	GCE	CPE	GCE
24.8	12.4	12.4	12.4	99.70±1.17	99.80±1.36
24.8	12.4	24.8	24.8	100.60±1.22	100.58±0.79
24.8	12.4	37.2	37.2	99.50±1.28	102.00±0.71
24.8	12.4	49.6	49.6	99.90±0.84	100.90±1.17
24.8	12.4	74.4	62.0	98.50±0.74	98.82±1.14

Legend: a) n = 3.

The obtained mean percentage recoveries and the relative standard deviations (RSD) based on the average of five replicate measurements were found to be between 98.66 and 101.66% and between 0.61 and 1.46, respectively. The small value of relative standard deviation indicates that the proposed method is highly accurate, precise and reproducible.

Stability Studies. *Dapsone* was subjected to acid, base and 30% (w/v) H_2O_2 at 90 ^{o}C for 2 hrs. The significant change occur upon exposure of *Dapsone* to 30% H_2O_2, where significant change of the peak current occur with appearance of two signals at different current and potential (Fig. 4). This is due to oxidation of the two amino group (NH_2) into hydroxyl amine (NHOH), which is the metabolite of drug [13]. This means that DPV method can discriminate DDS in presence of its oxidative degradants, which affects safety and efficacy of the drug.

In Vitro Release Study of Dapsone Tablet. The proposed voltammetric method was applied for quantitative estimation of DDS release from its commercial dosage form (*Dapsone* tablet). The content uniformity test indicates 99.0 % of the tablet amount and a standard deviation less than 2 % (n = 6). Dissolution tests at 100 rpm in 900 ml of 0.1 M HCl (stimulated gastric fluid) were also made using both the voltammetric and spectrophotometric measurements at 289 nm. With the voltammetric method, the current values were constantly recorded at 15 min time intervals and compared with calibration graph. Calibration curve of drug in dissolution medium was constructed and the regression equation was computed and found to be
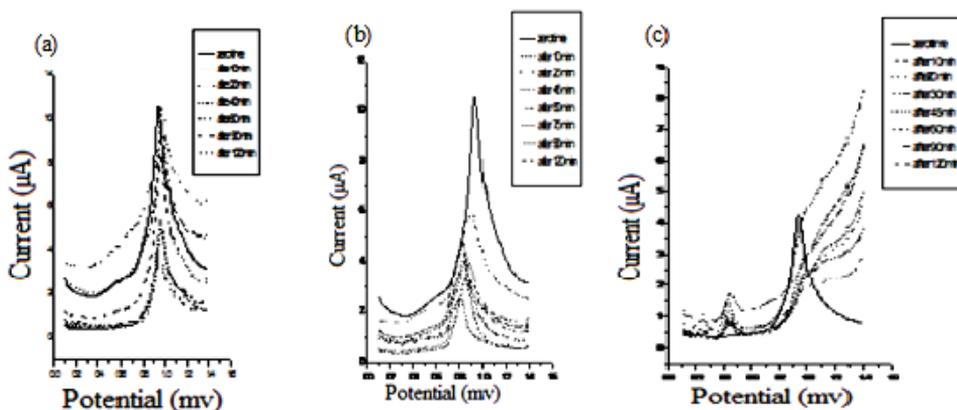
$$Y = 0.073\ X + 0.082; \quad r = 0.9950 \tag{4}$$

Fig. 4: *Differential pulse voltammetry as a tool for monitoring of the effect of stress stability studies*: **a**) acid 1 M HCl, **b**) base 2 M NaOH, **c**) 30% H_2O_2 at 90 ^{o}C for 2 hr. at CPE, pulse amplitude = 40 mV, scan rate = 10 mV s^{-1}.

For the UV spectrophotometric assay fixed volumes of the dissolution medium were withdrawn, diluted with 0.1 M HCl, measured at 289 nm and compared with a calibration graph. The drug release profiles obtained by both methods, indicating that the profiles generated by both methods are similar. In conclusion, the proposed DPV method is almost identical (see Fig 5).
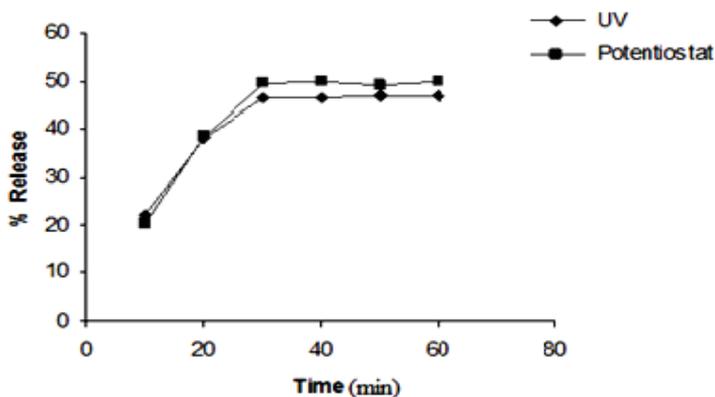

Fig. 5: *In-vitro release of Dapsone using UV spectrophotometry and differential pulse voltammetric methods.*

The use of voltammetric method, however has the advantage of *in situ* monitoring. The data obtained from the release studies were investigated and revealed first order kinetics with diffusion controlled release.

Conclusions

Experimental comparison of carbon paste and glassy carbon electrodes for determination of *Dapsone* reveals that CPE displays best performance characteristics and high sensitivity. In general, the voltammetric method described in this work using CPE and GCE are sufficiently simple and specific for the quantitative determination of *Dapsone* at the concentration level as low as 2.48 μg mL^{-1}.

The method, beside its low cost, can be applied for the drug analysis in any dosage form without special separation or sample preparations. It is very selective without any interference from solution constituents. The recommended method is concerned with the analysis of DDS in presence of its oxidative degradants. Moreover, the DPV method suggested in the article was applied for determination of in vitro release of *Dapsone* tablet. The dissolution profile reveals first order kinetics with diffusion controlled release.

Last but not least, regarding the use of carbon paste-based electrode, also our study has confirmed its suitablity for pharmaceutical analysis, offering quick and effective surface renewal, sufficient sensitivity and selectivity, a hig affinity to lipophilic molecules of numwerous pharamaceuticals (due to the hydrophobic nature of the binder that coforms the carbon paste mixture), some specific surface treatments, or highly effective surface and bulk modifications. And all these aspects contributes to the fact that both unmodified and chemically / biologically modified CPEs still belong among the most popular voltammetric working electrodes, potentiometric indicators, or even various detection systems for analysis in flowing streams (see *e.g.* [17,25-30).

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