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Research Article

ELECTROCHEMICAL ANALYSIS OF ZINECARD IN PHARMACEUTICAL AND BIOLOGICAL SAMPLES

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ABSTRACT

Zinecard is used to prevent a toxic effect to heart caused by certain medicines that are used to treat cancer. Zinecard is also used to treat tissue damage caused by the leakage of certain medicines that are used to treat cancer. Differential pulse polarographic method developed for the quantitative determination of zinecard gives a peak at -0.32 V at DME. From the structural point of view zinecard contains a >C=O- moiety which can be electrochemically reduced at universal buffer (pH 4.0). Millicoulometric experiment is performed successfully in estimating the number of electrons and proton to understand reduction mechanism. The differential pulse polarographic peak was adequately well-resolved, reproducible and linear dependent with the zinecard concentration. For quantification the calibration plot for zinecard concentrations ranging between 1.0×10^{-5} mol dm^{-3} to 1.0×10^{-8} mol dm^{-3} at pH 4.0 was selected. The proposed differential pulse polarographic method was successfully applied to the determination of zinecard in pharmaceutical formulations and urine samples.

Key words: Zinecard, DPP, pharmaceutical formulations and urine samples.

INTRODUCTION

Zinecard (dexrazoxane for injection) is a sterile, pyrogen-free lyophilizate intended for intravenous administration. It is a cardioprotective agent for use in conjunction with doxorubicin. Chemically, dexrazoxane is (s)-4-4'-(1-methyl-1,2-ethanedilyl)bis-2,6 piperazinedione. The structural formula is $C_{11}H_{16}N_4O_4$ and Molecular weight 268.28. It is dexrazoxane a potent intracellular chelating agent is a derivative of EDTA. Dexrazoxane is a whitish crystalline powder which melts at 191°C to 197°C. It is sparingly soluble in water and 0.1N HCl, slightly soluble in ethanol and methanol and practically insoluble in nonpolar organic solvents. Zinecard is available 250 mg and 500 mg single use only vials. Each 250 mg vial contains dexrazoxane hydrochloride equivalent to 250 mg dexrazoxane (McEvoy, 2005). Dexrazoxane is a cyclic derivative of EDTA that readily penetrates cell membranes. Results of laboratory studies suggest that dexrazoxane is converted intracellularly to a ring-opened chelating agent that interferes with iron mediated radical generation thought to be responsible, in part, for anthracycline induced cardiomyopathy.

Dexrazoxane is clinically used to reduce doxorubicin-induced cardiotoxicity (Hasinoff *et al.,* 1994b). Dexrazoxane is the (+)-(S)-enantiomer of racemic razoxane (ICRF-159), which was originally developed as an antitumor agent (cvetkovic *et al.,* 2005). Spectrophotometric and high-pressure liquid chromatography (HPLC) studies (Hasinoff *et al.,* 1994a and 1994b) showed that under physiological conditions dexrazoxane hydrolyzed to B and C with a $t_{1/2}$ of 9.3 hours at 37°C and pH 7.4, whereas the final hydrolysis product ADR-925 was produced with a $t_{1/2}$ of 23 hours according to the kinetic scheme.

The pharmacokinetics of dexrazoxane has been studied in advanced cancer patients with normal renal and hepatic function. Generally, the pharmacokinetics of dexrazoxane can be adequately described by a two-compartment open model with first-order elimination. Dexrazoxane has been adminis-

tered as a 15 minute infusion over a dose-range of 60 to 900 mg/m^2 with 60 mg/m^2 of doxorubicin, and at a fixed dose of 500 mg/m^2 with 50 mg/m^2 doxorubicin.

Following a rapid distributive phase (~0.2 to 0.3 hours), dexrazoxane reaches post-distributive equilibrium within two to four hours. The estimated steady-state volume of distribution of dexrazoxane suggests its distribution primarily in the total body water (25 L/m^2). The mean systemic clearance and steady-state volume of distribution of dexrazoxane in two Asian female patients at 500 mg/m^2. Dexrazoxane along with 50 mg/m^2 doxorubicin were 15.15 L/hours/m^2 and 36.27 L/m^2, respectively, but their elimination half-life and renal clearance of dexrazoxane were similar to those of the ten Caucasian patients from the same study. Qualitative metabolism studies with dexrazoxane have confirmed the presence of unchanged drug, a diacid-diamide cleavage product, and two monoacid-monoamide ring products in the urine of animals and man. The metabolite levels were not measured in the pharmacokinetic studies. Urinary excretion plays an important role in the elimination of dexrazoxane. Forty-two percent of the 500 mg/m^2 dose of Dexrazoxane was excreted in the urine. It is used to protect the heart against the cardiotoxic side effects of anthracyclines (Lipshultz *et al.,* 2004) such as doxorubicin (Bjelogrlic *et al.,* 2007). FDA has also approved a dexrazoxane hydrochloride drug, brand name Totect, for use as a treatment of extravasation resulting from IV anthracycline chemotherapy (Kane Robert *et al.,* 2008). Extravasation is an adverse event in which chemotherapies containing anthracylines leak out of the blood vessel and necrotize the surrounding tissue.

Dexrazoxane is a bisdioxopiperazine that readily enters cells and is subsequently hydrolysed to form a chelating agent analogous to EDTA, the two-ring opened metabolite, ICRF-198 / ADR-925. This chelating property is proposed to be its mechanism of action in the prevention of anthracycline induced iron-dependent free radical oxidative stress on the cardiac muscle. Dexrazoxane is also a catalytic inhibitor of DNA topoisomerase II. Dexrazoxane has been in clinical use since 1981. Initially, it was considered as an antineoplastic agent but the antineoplastic potential was insufficient for further development. It was then found to protect against the cardiotoxicity of anthracyclines. In Europe, since 1992, there is a marketed formulations of dexrazoxane licensed for the prevention of cardiomyopathy (Wiseman *et al.,* 1998) associated with doxorubicin administration.

Researches conducted since 1991 indicate that dexrazoxane is also a specific catalytic inhibitor of DNA topoisomerase II in that it is able to lock the enzyme's N-terminal clamp while simultaneously inhibiting its ATPase activity. The new results indicate that dexrazoxane blocks the induction of DNA strand-breaks by anthracyclines and that may be involved in the amelioration of anthracycline toxicity (Cvetkovic *et al.,* 2005).

In the present study, a simple and rapid method was developed for the determination of zinecard in pharmaceutical formulations and urine samples by DPP. Therefore, electrochemical behavior of zinecard in aqueous buffered media to arrive at the information on the mechanistic aspects and the electrode kinetics concerned and an attempt has also been made to determine the title compound in pharmaceutical formulations and urine samples by using differential pulse polarography without any prior separations.

METHODOLOGY

Materials

All chemicals were reagent grade chemicals (Merk, India), doubly distilled water was used in preparation of all solutions. Stock solution (1.0×10^{-3} mol dm^{-3}) was prepared by dissolving zinecard in dimethylformamide. All dilute solutions were freshly prepared daily from the stock solution. Universal buffer solution ranging from pH 2.0 to 12.0 were prepared using 0.2 mol dm^{-3} boric acid, 0.01 mol dm^{-3} citric acid and 0.1 mol dm^{-3} trisodium orthophosphate.

Instrumentation

Analysis were carried out with an Elico Model CL-362, three electrode system consisting of a dropping mercury electrode (DME) as the working electrode, an Ag/AgCl reference electrode and platinum counter electrode. It was outfitted with a Model LX-

Table I. Typical D.C.polarographic data of zinecard Concentration: 1.0×10^{-5} mol dm^{-3} Drop time: 3 sec

pH of the supporting electrolyte	$-E_{1/2}$ V	i_d µA	α_{na}	$D\times10^6$ V	$k^o_{f,h}$ Cm s^{-1}
2.0	0.12	6.20	0.92	3.32	6.02×10^{-11}
4.0	0.24	6.30	0.98	3.20	4.42×10^{-12}
6.0	0.46	6.15	0.94	2.08	4.82×10^{-12}
8.0	0.58	5.90	0.84	2.02	4.98×10^{-12}
10.0	0.62	4.85	0.86	1.86	6.16×10^{-11}
12.0	0.76	4.45	0.82	1.78	6.29×10^{-11}

Table II. Typical cyclic voltammetric data of zinecard Concentration: 1.0×10^{-5} mol dm-3 Scan rate: 40 mVs^{-1}

pH of the supporting electrolyte	$-E_p$ V	i_p µA	α_{na}	$D\times10^6$ Cm^2 s^{-1}	$k^o_{f,h}$ Cm s^{-1}
2.0	0.14	6.22	0.94	3.42	5.34×10^{-9}
4.0	0.22	6.25	0.96	3.56	6.24×10^{-9}
6.0	0.38	5.46	0.90	2.80	6.44×10^{-9}
8.0	0.48	5.86	0.88	2.12	6.46×10^{-9}
10.0	0.56	4.82	0.82	1.86	5.76×10^{-9}
12.0	0.66	4.73	0.78	1.84	6.64×10^{-9}

300^+ X–Y recorder. All the solutions were degassed prior to analysis by bubbling purified nitrogen gas through the cell for 10 min. All the experiments were performed at 25 ± 1°C, pH measurements were carried out with a Hanna instruments (Italy) pH meter.

Recommended procedure

A recommended standard stock solution ($1.0x10^{-5}$ mol dm^{-3}) was prepared by dissolving the required amount of the zinecard in dimethylformamide. Standard solutions are prepared by dilution of the stock solution with suitable amount of dimethyl formamide. In voltammetric cell 1 mL of standard solution is transferred and made up to 10 mL with 9 mL of the universal buffer of pH 4.0 and deoxygenated with pure nitrogen gas for 15 min. After obtaining the voltammogram small additions of (0.2 mL) of standard solutions are added and voltammogram recorded after each addition under similar experimental parameters. The optimum conditions for the determination of zinecard in pH 4.0 is found to be a drop time of 2 sec, a pulse amplitude of 25 mV and applied potential of -0.32 V.

RESULT AND DISCUSSIONS

Single well defined waves have been observed for the reduction of zinecard in universal buffer of pH 4.0. A typical D. C polarogram is presented in Figure 3. From the Tomes criterion, log-Plot analysis and dependence of half-wave potentials on drop time, the reduction process is identified to be irreversible. The obtained linear plots of i_d vs. $h^{1/2}$ passing through origin (as shown in Figure 4) in all the supporting electrolytes except pH 5.0 indicate that the reduction process is adsorption free in all the supporting electrolytes except in pH 2.0, where weak adsorption of the reactant is observed. The results observed have been used for evaluation of kinetic parameters such as diffusion coefficient, transfer coefficient and heterogeneous forward rate constants for the reduction process of carbonyl group which are reported in table I.

The peak current (I_p) is dependent on scan rate (v) and pH of the supporting electrolyte. The linear plot of I_p vs. $v^{1/2}$ (Figure 5) passing through origin indicates the electrode process to be mainly diffusion controlled adsorption on the electrode surface

Table III. Typical differential pulse polarographic data of zinecard Concentration: 1.0×10^{-5} mol $dm^{-3;}$ Drop time: 3 sec; Pulse amplitude:50mV

pH of the supporting electrolyte	$-E_m$ V	i_m µA	α_{na}	$D\times10^6$ $Cm^2\ s^{-1}$	$k^o_{f,h}$ $Cm\ s^{-1}$
2.0	0.12	5.54	0.89	3.04	6.14×10^{-9}
4.0	0.25	5.39	0.86	2.96	5.24×10^{-9}
6.0	0.44	5.22	0.84	2.82	5.64×10^{-10}
8.0	0.58	5.02	0.78	2.64	6.45×10^{-10}
10.0	0.64	4.88	0.74	2.32	6.02×10^{-9}
12.0	0.76	4.64	0.72	2.12	6.78×10^{-9}

Table IV. Determination of zinecard in pharmaceutical formulations by DPP Concentration: 1.0×10^{-5} mol dm^{-3}; Drop time: 3 sec; Pulse amplitude:50 mV

Name of the formulation	Labeled amount (mg)	Amount found (mg)	Recovery (%)	Standard Deviation	% RSD
	5	4.99	99.80	0.02	0.40
	10	9.88	98.80	0.05	0.50
Dexrazoxane	15	14.75	98.33	0.08	0.54

Table V. Determination of zinecard in urine samples by DPP Concentration: 1.0×10^{-5} mol dm^{-3} ; Drop time: 3 sec; Pulse amplitude:50 mV

Sample	Labeled amount (mg)	Amount found (mg)	Recovery (%)	Standard Deviation	% RSD
1	2.0	1.99	99.50	0.02	1.00
2	4.0	3.96	99.00	0.04	1.01
3	6.0	6.00	100.0	0.01	0.16

in all the pH ranges studied. The reduction process is found to be reversible and presence of anodic peak on the reverse scans in cyclic voltammetry as well as obedience of Tomes' criterion confirmed the electrode surface process to be reversible. Two protons have participated in the rate determining step for the reduction of keto group. Typical cyclic voltammograms is shown in figure 2.

In the present investigation, the differential pulse polarographic measurements are carried out in the supporting electrolyte at pH 4.0. Only one well defined peak is observed in all the supporting electrolytes employed. The variation of peak potential with concentration indicates the irreversible nature of the electrode process. The plots of i_m vs. $t^{2/3}$ (Figure 6) being linear and not passing through origin in all the buffers and in both solvent-water mixtures, show that adsorption complication is involved. Kinetic parameters are evaluated and reported in table III. Typical differential pulse polarogram is presented in figure 1.

The linear plot of i_p vs $v^{1/2}$ passing through the origin, indicate the electrode process to be mainly diffusion controlled and adsorption controlled. The irreversibility of the electrode process is verified by logarithmic analysis of the peak. Absence of anodic peak in the reverse scan, indicates the over all electrode processes to be irreversible. The shift in E_p values towards more negative potentials with an increase in concentration of electroactive species also suggests the irreversible nature of the peaks. The peak potentials of zinecard are found to be pH dependent and shift towards more negative values with increase in pH

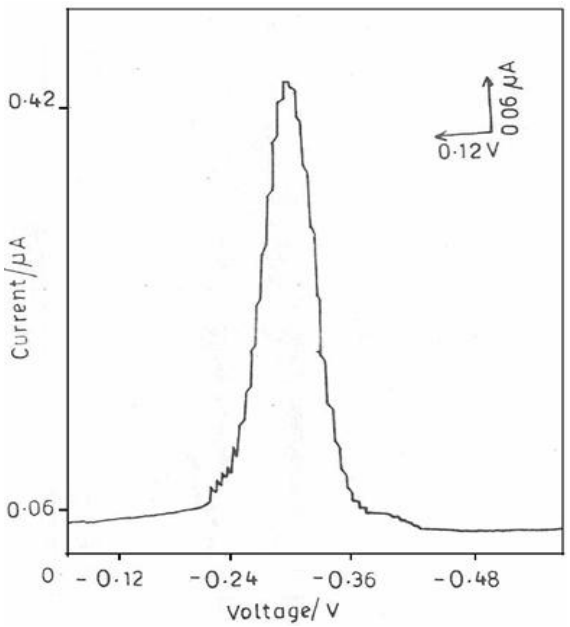

Figure 1 Typical Differential pulse polarogram of zinecard at pH 4.0, Concentration: 1.0×10^{-5} mol dm^{-3}, Pulse amplitude: 60 mV. Drop Time: 2sec.

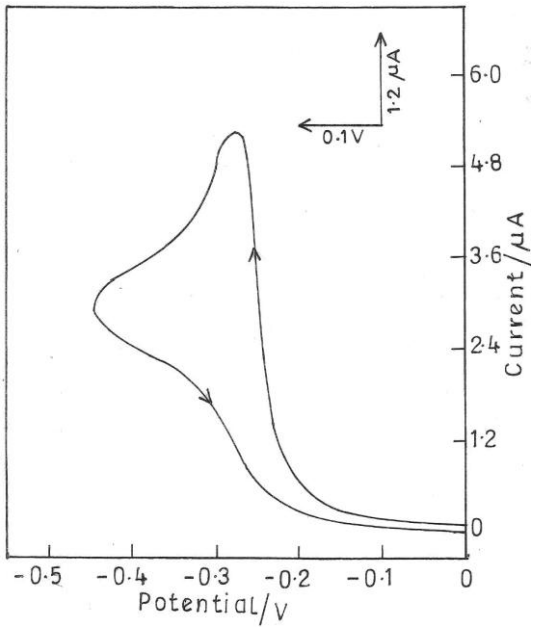

Figure 2 Typical cyclic voltammogram at zinecard at HMDE accumulation time: 60sec; pH: 4.0; accumulation potential: -0.34V; rest time: 10sec; stirring rate: 400rpm; pulse amplitude: 25 mV, concentration: 1.0×10^{-4} mol dm^{-3}; scan rate: $40mVs^{-1}$.

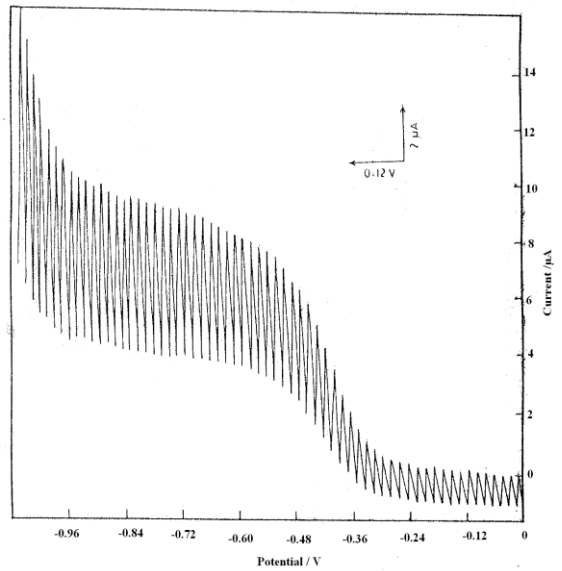

Figure 3 Typical D.C. polarogram of zinecard at pH 4.0 Concentration: 1.0×10^{-5} mol dm^{-3}; Drop time: 3 sec.

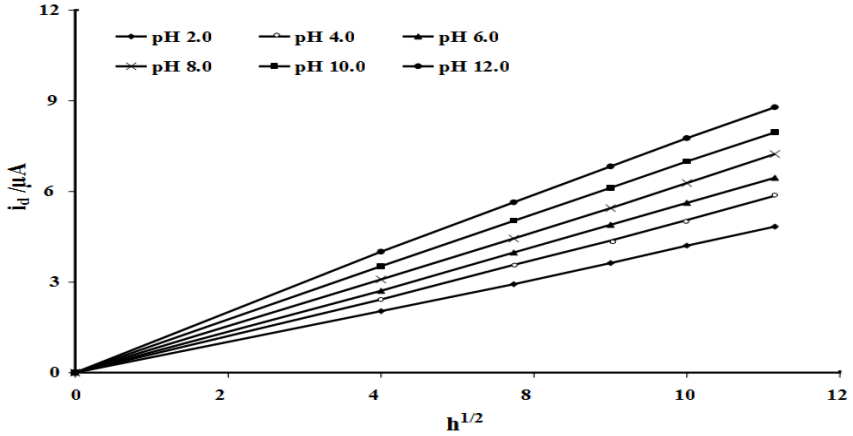

Figure 4 i_d vs $h^{1/2}$ plots of zinecard, Concentration; 1.0×10^{-5} mol dm^{-3}

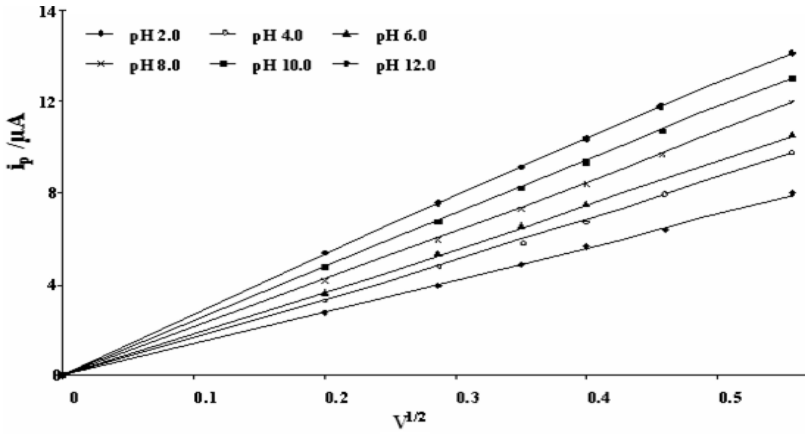

Figure 5 i_p vs $V^{1/2}$ plots of zinecard, concentration; 1.0×10^{-5} mol dm^{-3}

indicating the involvement of protons in the reduction process. The α n_a values for the reduction of carbonyl group in zinecard is found to be in the order of 0.96. The number of protons participated in the rate determining step is found to be four (Figure 9).

The total number of electrons involved in the electrode process has been established experimentally by millicoulometry. From the comparison of the peak heights observed, the number of electron involved in the reduction process of the zinecard is eight. Controlled potential electrolysis experiments are carried out at -0.32 V vs SCE and pH 4.0 also agree with the above results. The results of our investigations as well as on data from the literature, the electrode mechanism is proposed for electrochemical reduction of zinecard.

Kinetic data

Typical kinetic parameters evaluated from cyclic voltammetric technique are reported in the table II. The diffusion and adsorption controlled nature of the electrode process is clearly evidenced from the nearly equal diffusion efficient values obtained from the cyclic voltammetric technique. The 'D' values are seen to gradually decrease which account for decrease in diffusion current with increase in pH due to less availability of protons. The forward rate constant values are observed to be low in basic media (pH 8.0) when compared to that obtained in acidic

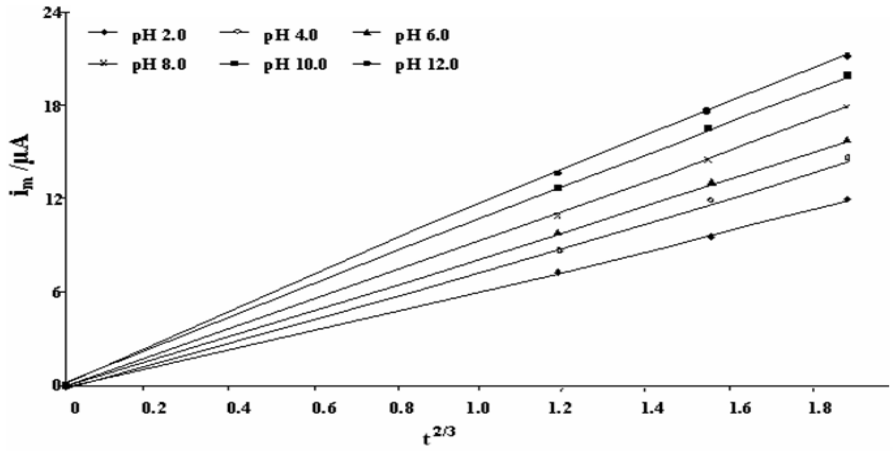

Figure 6 i_m vs $t^{2/3}$ plots of zinecard, Concentration; 1.0×10^{-5} mol dm^{-3}.

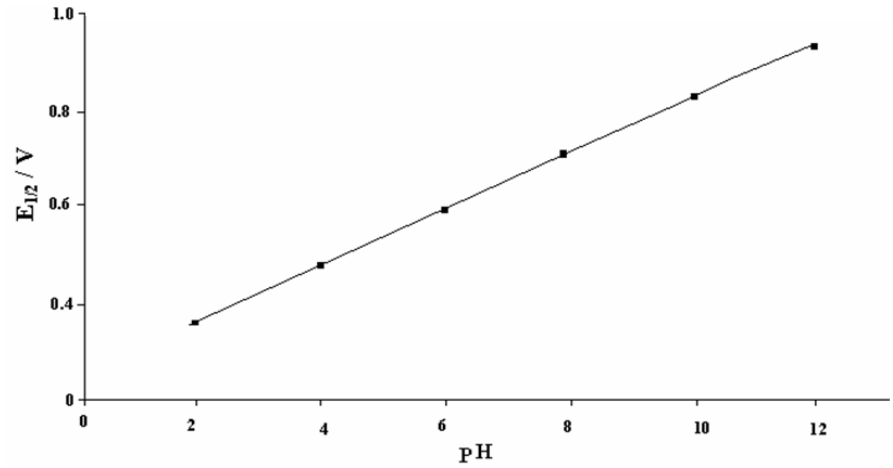

Figure 7 $E_{1/2}$ vs pH plots of zinecard Concentration; 1.0×10^{-5} mol dm^{-3}; Drop time: 3 sec.

media, since the unprotonated azomethine being the electroactive species in the acidic region. The $k^0_{f.h}$ values are found to decrease with increase in pH indicating that the electrode reaction becomes more and more irreversible with increase in pH of the solution.

The pH was investigated in the concentration range of 2.0-12.0 and the maximum peak is obtained at pH 4.0 (Figure 4), the peak potentials have been shifted towards more negative values, indicating proton participation in the reduction process.

Recommended analytical procedure

The stock solution ($1.0\text{x}10^{-5}$ mol dm^{-3}) of zinecard is prepared by dissolving the required quantity of the electroactive species in dimethyl formamide. Standard solutions are prepared by dilution of stock solution with a suitable amount of dimethylformamide. 1.0 mL of the standard solution is transferred into the voltammetric cell and made up 9 mL with the supporting electrolyte for achieving the required concentration and then deoxygenated by bubbling oxygen free nitrogen gas for 15 min.

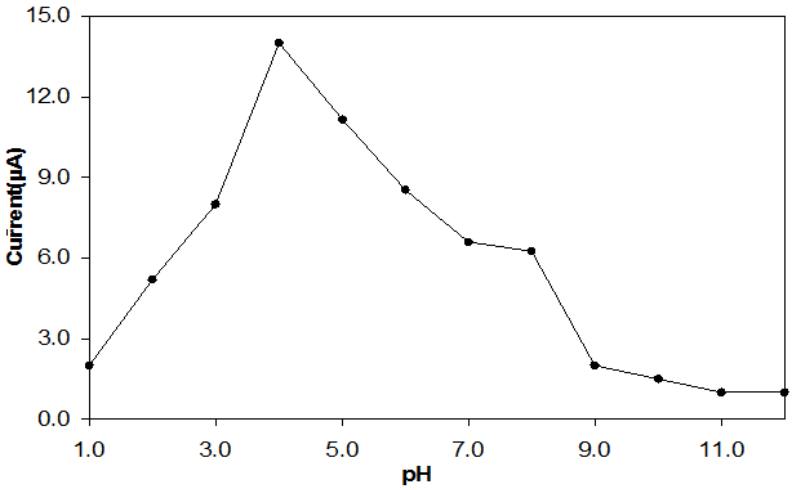

Figure 8. Effect of pH on the DPP of zinecard

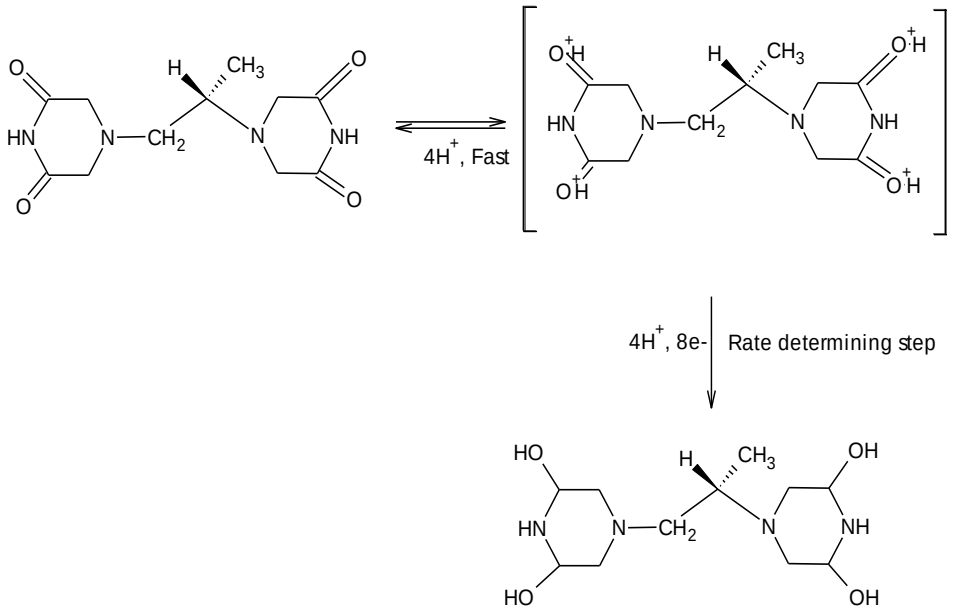

Figure 9. Reduction mechanism of zinecard

After recording the voltammogram, small increments of the standard solutions are added and then voltammograms are recorded for each addition under similar conditions. The optimum conditions for the analytical determination of zinecard at pH 4.0 are found to be a drop time of 2 sec, pulse amplitude 25 mV and applied potential of –0.32V vs saturated calomel electrode.

Zinecard in pharmaceutical formulation namely dexrazoxane containing a 250 mg of compound mass of approximately 500 mg has been analysed in order to examine the applicability of the method. A portion equivalent to 10 mg of the compound was weighed accurately and dissolved in dimethlformamide and transferred into 10 mL calibrated flasks. 1.0 mL of the solution is

diluted with 9 mL of supporting electrolyte (pH 4.0) and resulted solution was subjected to voltammetry. With the help of calibrated graph, the amount of zinecard in portion of the sample was calculated. From the recovery studies on formulations containing zinecard, the assay results for zinecard in pharmaceutical formulation at pH 4.0 are given in table 4. The recoveries are in the range 98.33% to 99.80% for dexrazoxane for samples respectively.

The reliability of the method for that determination of zinecard in urine is checked by using different urine samples. Direct determinations in undigested urine may yield for certain probands unreliable data due to trace metal trapping by natural chelating substances. An aliquot of 1.5 mL of urine without treatment were diluted with water to 25 mL in a volumetric flask; a 0.1 mL aliquot of this solution was transferred into a 25 mL calibrated flask; 5 mL of universal buffer of pH 4.0 were added and diluted to the mark with water. The samples were measured according to the above described procedure. The voltammograms of samples without zinecard do not show any signal that can interfere with direct determination.

The intention of our experiments with urine samples is to study various possibilities to overcome a biometrics effect and to find the best solution but to explore the way for simple, reliable, fast determination of zinecard in urine by its dilution on employing differential pulse polarography. The potential interference of some urine ingredients both organic and inorganic salts is checked without adding depolarizer. None of these ingredients did affect the determination zinecard. The recoveries are in the range of 99.00% to 100.00% (Table V).

CONCLUSION

In conclusion a simple low cost and low sample consumption adsorptive stripping voltammetric method can be used satisfactorily for determination of urine and pharmaceutical formulations. The result obtained revealed that the proposed procedure as good accuracy. Theproposed method using DPP not only is one of the most sensitive methods for the determination of zinecard but also excellent interms of selectivity and simplicity.

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