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Study of variation in electrochemical behaviour of ofloxacin and tinidazole simultaneously by differential pulse voltammetry

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Abstract
In present study, a successful attempt has been made to study the variation in electrochemical behaviour of ofloxacin and Tinidazole simultaneously using Differential Pulse Voltammetry (DPV) technique. The effect of different pH (2-10) of Britton-Robinson Buffer on voltammogram using 1M KCl as a supporting electrolyte was studied. The optimum pH was found to be pH 4.75. Both Ofloxacin and Tinidazole exhibited reduction cathodic peak at optimum pH with peak potential at -1.21 V for Ofloxacin and -0.25 V for Tinidazole vs. S.C.E. 0.1N CH_3COOH was used as Solvent for the analysis. The variation in electrochemical behaviour of Ofloxacin and Tinidazole simultaneously at the optimized pH was studied by varying pulse amplitude and scan rate. The optimized pulse amplitude was found to be 50mV and the optimized scan rate was found to be 22.5 mV/s.

Keywords: Differential pulse voltammetry (DPP), ofloxacin, tinidazole, britton-robinson buffer, pulse amplitude and scan rate

Introduction
Individual determination of several drugs by various electroanalytical methods have been reported [1, 4]. Simultaneous determination of drugs using conventional methods such as HPLC and spectroscopy have been reported [5, 11]. Simultaneous determination of some combinations have been reported [12]. For development and validation of any method based on electroanalytical technique, the optimum parameters are very important. The optimized parameters such as pH, pulse amplitude and scan rate can be useful in the simultaneous detection and determination of pharmaceutical formulation by electroanalytical technique. It can also be used for devising electo-sensors for those pharmaceutical drugs.
Ofloxacin, $C_{18}H_{20}FN_3O_4$ that is 8-Fluoro-3-methyl-9-(4-methyl-piperazin-1-yl)-6-oxo-2,3-dihydro-6H-1-oxa-3a-aza-phenalene-5-carboxylic acid, (Molecular Weight: 361.37 g/mol), is available in single dose tablet or combined dose tablet. It is used to the treatment of various bacterial infections.
Tinidazole, $C_8H_{13}N_3O_4S$ that is 1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole, (Molecular Weight: 247.27 g/mol) is available in single dose tablet or combined dose tablet. It is used to treat or prevent a variety of bacterial infections.
Ofloxacin and Tinidazole in combined dosage form is available in the market, has gained great acceptance in diarrhea, bacterial and protozoal infections. In many cases, drugs with two active ingredients are prescribed to the patients to have an added advantage. Many of these antibacterial drugs are found in combination with antifungal and antiprotozoal drugs which are highly effective against fungal and protozoal infections.

Objective
The main objective of study is to provide optimized parameters such as pH, pulse amplitude and scan rate of volammogram for Ofloxacin and Tinidazole simultaneously which can be used in the method development and validation of Ofloxacin and Tinidazole in combined pharmaceutical formulations using Differential Pulse Voltammetry technique.
Intestines of cow and pig that could influence the performance of dosage forms.

Materials and methods

Introduction to workstation

Electrochemical workstation- PG STAT 30 with 663 VA Electrode stand (Metro hm)

It is made up of three electrode system namely-

1. Hanging Mercury Drop electrode (HMDE) as the working electrode
2. Saturated calomel electrode as the reference electrode
3. Platinum electrode as the counter electrode

The pH measurements were made with Euiptrances model No. 610.

Solution preparation

Solution A: Combined stock solution of standard TZ and standard OF (1500 µg/mL + 500 µg/mL)

150 mg of standard **TZ** and 50 mg standard **OF** was accurately weighed and transferred into 100 mL standard flask; about 80 mL of 0.1N acetic acid was added to it. The mixture was sonicated for 10 minutes to dissolve the standards with intermittent shaking. The volume was made up to the 100 mL mark by adding 0.1N acetic acid.

Preparation of Britton-Robinson buffer

0.204 g of boric acid, 2.8 mL of (85%) phosphoric acid and 2.3 mL of glacial acetic acid were transferred to three separate 100 mL of volumetric flasks and the volume of each flask was made up to the 100 mL mark with distilled water. These three solutions are then mixed in a beaker to get the solution of pH 1.8. The pH of the resulting solution was adjusted to the desired value by adding required quantity of 1M NaOH.

Preparation of the supporting electrolyte solution (1M KCl).

7.46 g of A.R. KCl were weighed and transferred into a 100 mL volumetric flask. About 80 mL of distilled water was added to dissolve the solid completely and then the volume was made up to the 100 mL mark with distilled water.

Optimization of the pH

The response of TZ and OF combination was studied over the pH range 2 to 10 in Britton – Robinson buffer. At pH = 4.5 peak shape was good but R^2 values were not satisfactory. At pH = 5.0 peak shape was not good but R^2 values were good. So pH = 4.75 was tried. At pH = 4.75 peak shape was good and R^2 values were also satisfactory, therefore pH=4.75 was selected as the optimum pH for this combination.

Effect of pH on polarogram of TZ and OF:-

Polarogram of TZ and OF combination were recorded at different pH (2-10) at fixed scan rate (22.5 mV/s) and at fixed pulse amplitude (50 mV) for same concentration of TZ and OF using 1M KCl as supporting electrolyte. It was observed that with increase in pH, peak potential shift to more negative potential i.e. right side of polarogram for both TZ and OF.

For TZ peak height increases with increase in pH till pH = 6 then peak height roughly remains constant with increase in pH. Only at pH = 7.0 small decrease in peak current was observed.

For OF no peak was obtained at pH = 2. First peak appears at pH = 3. Peak height decreases from pH = 3.0 to pH = 7. From pH = 7 to pH = 10, the response by OF was very poor and hence very low peak currents were observed. Figure [1] shows overlaid polarograms of TZ and OF combination at various pH (2-10).

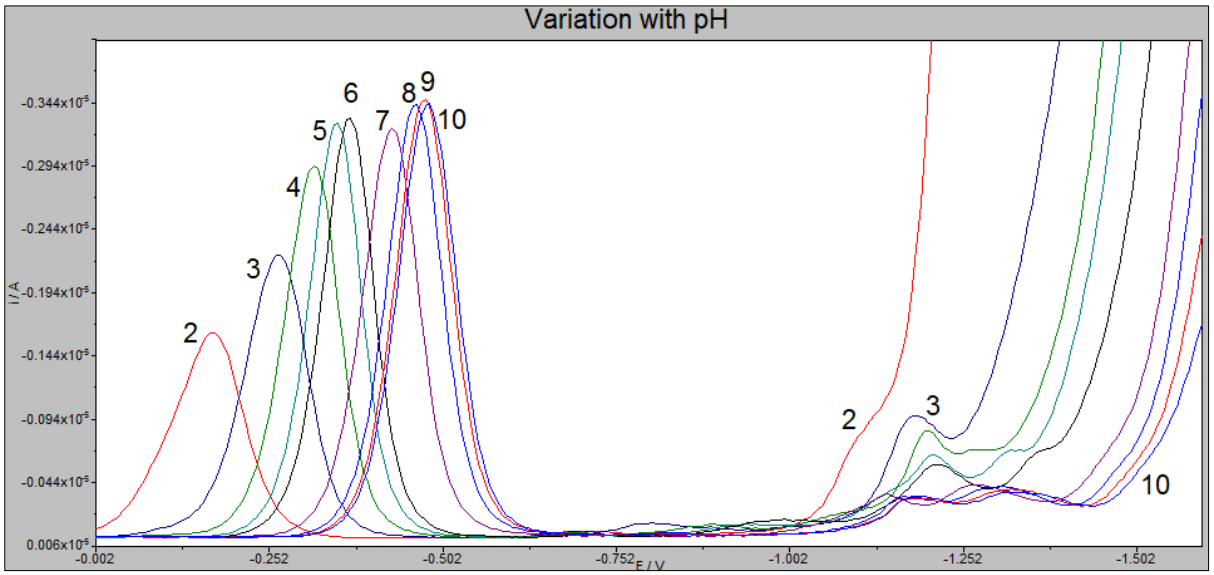

Fig 1: Polarogram of TZ and OF combination at various pH(2 to 10), with pulse amplitude of 50 mV, and the scan rate of 22.5 mV/s

Optimization of Pulse amplitude

The peak current varies linearly with the pulse amplitude in the range of 10mV to 100 mV. The pulse amplitude of 50 mV was chosen for all the analytes, because (R^2) values were not satisfactory

at higher pulse amplitudes and response was poor at lower pulse amplitudes.

Effect of pulse amplitude on polarogram of TZ and OF

Polarogram of TZ and OF combination were recorded at different pulse amplitude (10-100 mV) at fixed scan rate (22.5 mV/s) and at pH = 4.75 for same concentration of TZ and OF using 1M KCl as supporting electrolyte. It was observed that with increase in pulse amplitude, peak potential shifted slightly towards positive side i.e. towards left side of the polarogram for both TZ and OF. Shift for OF was more prominent than TZ. Peak height increases continuously with increase in pulse amplitude for both TZ and OF. Figure [2] shows overlaid polarograms of TZ and OF combination at various pulse amplitudes (10-100 mV).

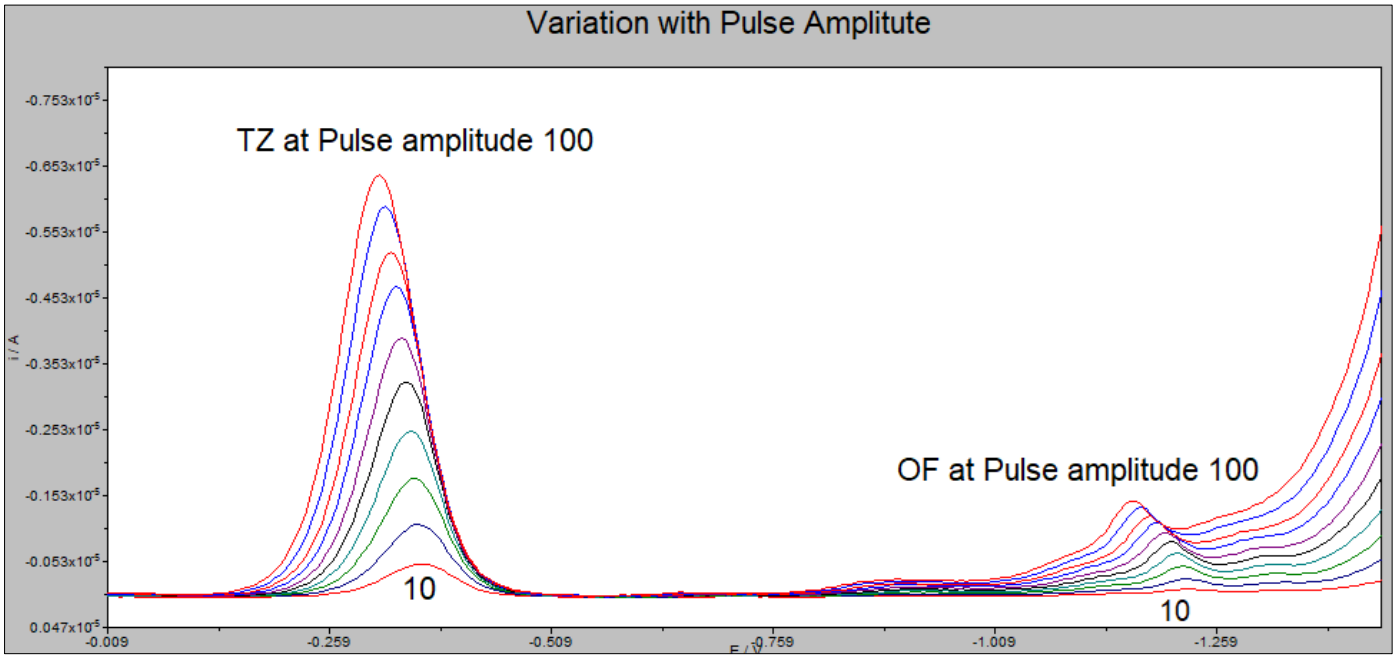

Fig 2: Polarogram of TZ and OF combination at various pulse amplitudes (10 to 100 mV), at pH = 4.75 and at the scan rate of 22.5 mV/s

Optimization of Scan rate

The polarograms for TZ and OF combination were recorded at various scan rates from 5 mV/s to 35 mV/s with the interval of 5mV/s. At scan rate of 5 mV/s and 10 mV/s the response was very low with unsatisfactory R^2 values. At scan rate of 15 mV/s peak shape was good but R^2 values were unsatisfactory. R^2 values were satisfactory from 20 mV/s to 25 mV/s. Scan rate of 22.5 mV/s was chosen as the optimum scan rate because it gave better peak shape along with good R^2 values.

Effect of scan rate on polarogram of TZ and OF

Polarogram of TZ and OF combination were recorded at different scan rate (5-35 mV/s) at fixed pulse amplitude (50 mV) and at pH = 4.75 for same concentration of TZ and OF using 1M KCl as supporting electrolyte. It was observed that with increase in scan rate, there was no shift in peak potential for both TZ and OF. Peak height increases continuously with increase in scan rate for both TZ and OF. Distorted Peak shapes were observed at scan rates greater than 25 mV/s for both TZ and OF. Figure [3] shows overlaid polarograms of TZ and OF combination at various scan rates (5-35 mV/s).

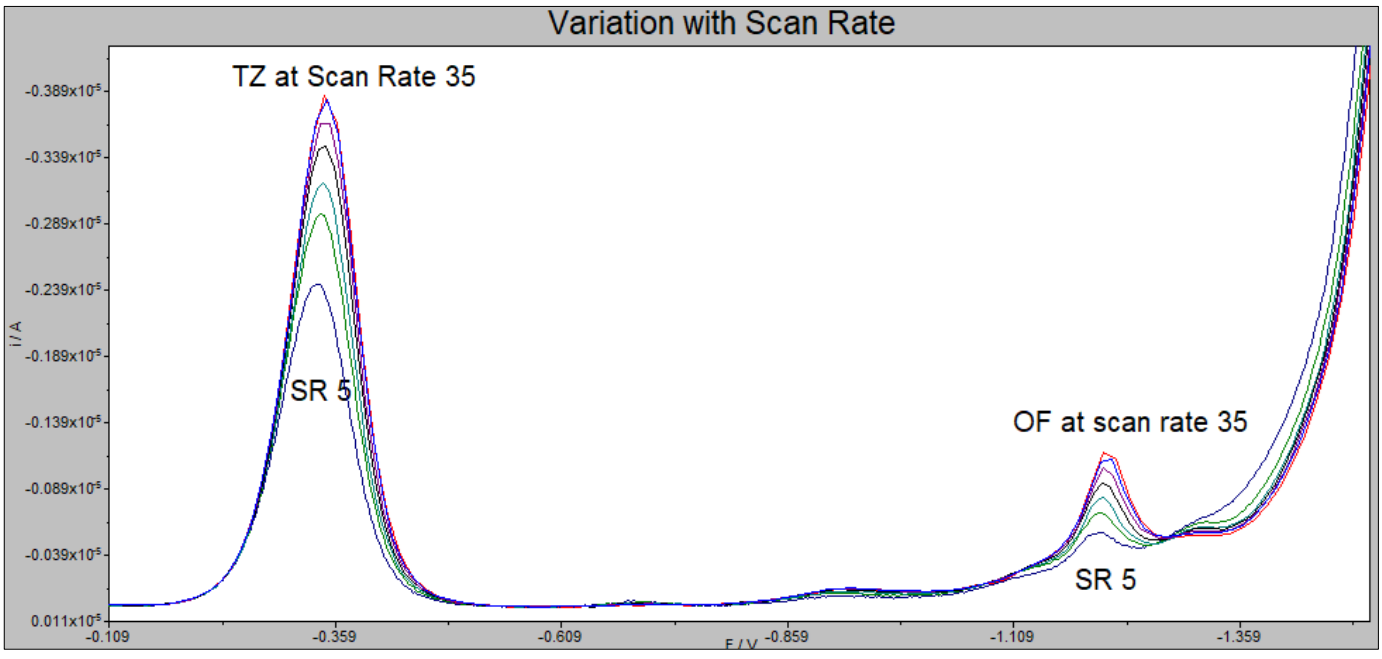

Fig 3: Polarogram of TZ and OF combination at various scan rates (5 to 35 mV/s), at pH = 4.75 and at the pulse amplitude of 50 mV.

Result and discussion

Table 1: All the optimized voltammetric parametersand instrumental parameters are as follows

Parameters	Optimum Values
Buffer	Britton – Robinson buffer
pH	4.75
Supporting Electrolyte	1 M KCl
Purge Time (Blank)	180 sec
Purge Time (Addition)	100 sec
Equilibration Time	10 sec
Start Potential	0.0 V
End Potential	-1.6 V
Pulse Amplitude	0.05 V
Pulse Time	0.04 sec
Voltage Step	0.009 V
Voltage Step Time	0.4 sec
Scan Rate	0.0225 V/sec

Conclusion

The optimized voltammetric parameters such as pH, pulse amplitude and scan rate for Ofloxacin and Tinidazole can be used for any further research involving electrochemistry of Ofloxacin and Tinidazole.

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