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Simultaneous determination of furazolidone and metronidazole in combined drug formulation by a simple electro analytical technique

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Abstract
In present study, a successful attempt has been made to develop a simple method for the simultaneous determination of Furazolidone and Metronidazole using Differential Pulse Voltammetry (DPV) technique. Quantification of Furazolidone and Metronidazole was done in Britton-Robinson Buffer of pH 4.25 using 1M KCl as a supporting electrolyte. Both Furazolidone and Metronidazole exhibit reduction cathodic peak in given pH with peak potential at -0.06 V for Furazolidone and -0.22 V for Metronidazole vs. S.C.E. 0.1N CH_3COOH was used as Solvent for the analysis. The parameters used for the method validation are linearity, accuracy, precision, robustness, ruggedness, LOD and LOQ. Proposed method was found to be simple, precise, and accurate and can be successfully applied for routine quality control analysis and simultaneous determination of Furazolidone and Metronidazole in combined drug formulations.

Keywords: Differential pulse voltammetry (DPV), furazolidone, metronidazole, britton-robinson buffer

Introduction
Determination of quantity of drugs is very important during various stages of manufacturing of drug and during its clinical trials. 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]. Little attention is given to the simultaneous determination of drugs using electroanalytical techniques. Simultaneous determination of some combinations have been reported [12].
Furazolidone, $C_8H_7N_3O_5$ that is 3-{[(5-nitro-2-furyl) methylene] amino}-1, 3-oxazolidin-2-one, (Molecular Weight: 225.16 g/mol), it has been used to in the treatment of diarrhoea caused by bacteria or protozoan infections.
Metronidazole, $C_6H_9N_3O_3$ that is 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl) ethanol, is an antibiotic, amebicide, and antiprotozoal (Molecular weight: 171.15 g/mol g/mol) It is highly effective for bacterial and protozoan infections and is available in the tablet form.
Furazolidone and Metronidazole in combined dosage form is available in the market, It is used for treating diarrhooea, bacterial and protozoal infections.

Objective
The main objective of study is to provide a simple, rapid, efficient, reliable and economic method for the simultaneous determination of Furazolidone and Metronidazole in combined pharmaceutical formulations using Differential Pulse Voltammetry technique.

Materials and Methods (Experimental)
Introduction to workstation

Electrochemical workstation- PG STAT 30 with 663 VA Electrode stand (Metrohm)
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
4) The pH measurements were made with Euiptrances model No. 610.

Reagents

Standard Furazolidone and Metronidazole was obtained from local pharmaceutical company. All the solutions were prepared in double distilled water. All the reagents use were of AR grade. Britton-Robinson buffer solutions-[100ml of 0.04M H_3BO_4 + 0.04M H_3PO_4 + 0.04M CH_3COOH]. Further the desired value of pH (4.25) was adjusted with the addition of 1M NaOH.

Analytical method development

Preparation frz standard solution

25mg of standard Furazolidone and 75mg of standard Metronidazole was accurately weighed and dissolved in 0.1N CH_3COOH and made up to a volume of 50 ml in standard flask to give stock solution (500µg/ml of Furazolidone and 1500µg/ml of Metronidazole respectively). Further all the standard solutions containing the mixture of Furazolidone and Metronidazole were prepared using this stock solution.

Preparation sample solution

Ten tablets of FRZ and MZ combination were weighed and powdered for the analysis. The amount equivalent to the half of the average weight of a tablet i. e. 0.578/2 = 0.289 g, was accurately weighed and transferred quantitatively to 1000 mL standard flask; then about 950 mL of 0.1N acetic acid was added in it and the mixture was sonicated for 10 mins with intermittent shaking. The volume was made up to the 1000 mL by adding 0.1N acetic acid. The solution was then filtered through Whatman filter paper no. 41 to remove the excipients present in the tablet.

Proposed voltammetric method

An aliquot of 20 ml made up of 18 ml Britton-Robinson Buffer adjusted to pH 4.25 by 1M NaOH + 2 ml of 1M KCl as a supporting electrolyte was placed in the dry and clean valtammetric cell. Then it was purged with highly pure nitrogen gas for 180s. A negatively directed DP scan between the potential of 0.0 V to -1.00 V vs. S.C.E was applied. The optimized operational parameters were as follows: 1] Scan rate: 15 $mVs^{-1.}$ 2] Pulse amplitude- 50mV. After recording a polarogram of blank, aliquots of (0.5ml) each of the required standard solutions was added from the standard stock solution. Resulted polarograms were recorded under the optimum experimental conditions. Peak currents were recorded. Calibration curve was prepared by plotting peak current versus concentration of Furazolidone and Metronidazole applied.

Analytical method validation

System suitability

System suitability tests are used to ensure reproducibility of the equipment. The test was carried out by recording polarogram for Furazolidone (10 µg/ml) and for Metronidazole (30µg/ml) with five replicates and the mean was used for the whole calculations. The % RSD was found to be 0.82% for Furazolidone and 1.42% for Metronidazole, which was acceptable as it is less than 2%.

Specificity

To confirm specificity of the FRZ peak, first polarogram of the sample (Combination of both MZ and FRZ) was recorded. In the same solution, 1.0 ml of standard solution of FRZ was added and again the polarogram was recorded and was overlaid on the polarogram of sample. With the addition of standard solution of FRZ there was increase in peak current of the peak at -0.06 V. This indicated that the peak at -0.06 V is specific to FRZ. Figure [1]

To confirm specificity of the MZ peak, first polarogram of the sample (Combination of both MZ and FRZ) was recorded. In the same solution, 1.0 ml of standard solution of MZ was added and again the polarogram was recorded and was overlaid on the polarogram of the sample. With the addition of standard solution of MZ there was increase in peak current of the peak at -0.22 V. This indicate that peak at -0.22 V is specific to MZ. Figure [2]

Limit frz detection and limit frz quantitation

The limit of detection (LOD) and the limit of quantification (LOQ) for FRZ and MZ were determined by signal to noise ratio of 3:1 and 10:1 respectively. The replicates for blank solution were recorded 20 times and the mean current value at the reduction potential of Furazolidone (i.e. at -0.06 V) and Metronidazole (i.e. at -0.22 V) was calculated. The concentration at which the peak current was found three times of mean blank current was taken as a limit of detection. And the concentration at which peak current was found to be ten times than the mean blank current was selected as limit of quantification.

The LOD and LOQ of Furazolidone were 0.21 µg/ml and 0.98 µg/ml Figure 3 And Metronidazole was found to be 0.73 µg/ml and 1.76 µg/ml respectively Figure 4.

Linearity and Range

The linearity for Furazolidone and Metronidazole were observed simultaneously by addition of standard solution. A good linearity was achieved in the concentration ranges 0.98 to 25.0 µg/ml for Furazolidone (Plot:1) and 1.76 to 56.25 µg/ml for Metronidazole (Plot:2). The calibration curves were constructed with peak current (ip) against concentration (c) Figure 5]. The slope, Intercept, regression equation and correlation coefficient for the Furazolidone and Metronidazole was obtained is given in Table 1.

Intraday and interday precison

The intra-day and inter-day precision was used to study the variability of the method. It was checked by recording the polarograms of standard solutions of Furazolidone and Metronidazole i.e. whole concentration range for both at intra-day (three times within 24 hour) and inter-day (three times each during 3 days intervals) to check the precision. The mean % RSD for intra-day and inter-day precision for Furazolidone found to be 0.77% and 0.23% and for Metronidazole it was 0.1.22% and 0.96%, respectively.

Assay

The developed Polarographic method was used for simultaneous determination of Furazolidone and Metronidazole from a drug formulation. The sample solutions

were analyzed by the developed method described above. Polarograms were recorded under the optimum experimental conditions. Resulting peak currents of Furazolidone and Metronidazole were measured and the amount of Furazolidone and Metronidazole calculated using already constructed calibration graph. Assay studies were carried out at three different levels lower, middle and higher levels. The percentage assay at three different levels for both Furazolidone and Metronidazole was found to be from 98.00 % to 102.00 %. The results were shown in Table 2.

Robustness

The robustness of the method was examined by the consistency of peak height and peak shape with the deliberately small changes in the experimental parameter. It is a measure of its capacity to retain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. To determine the robustness of the proposed method, the following variations were made in the analytical method-

1] Scan rate by ± 2.5 mVs^{-1}. 2] Pulse amplitude ± 10 mV

These parameters were deliberately changed one at a time and the effect of these changes on the assay studies was carried out. The proposed method was found to be robust.

Accuracy (Recovery)

The recovery was used to evaluate the accuracy of the method. Accuracy of the method was determined using the method of standard addition. A fixed volume of pre-analyzed sample of Furazolidone and Metronidazole solution was mixed with different concentrations of standard solution and mixtures were analyzed by proposed method. The percentage recovery was determined at different levels i.e. from lower, middle and higher levels. The results of percentage recovery analysis for Furazolidone and Metronidazole are shown in Table 3

Result and Discussion

In the present study simultaneous quantification of Furazolidone and Metronidazole have been done from the drug formulation using Differential Pulse Voltammetry technique. Simultaneous quantification has many advantages over individual quantification such as

1) Saving of time
2) Saving of various recourses such as chemicals, energy, analysis time etc.

The developed method was validated and the results are shown in 1 to 3

The percentage assay and percentage recovery at three different levels for both Furazolidone and Metronidazole was found to be well within the acceptable limit of 98.00 % to 102.00 %. The results were shown in Table 2 and Table 3.

Table 1: Method validation parameters for determination of FRZ and MZ

Parameters	Values	
	Furazolidone	**Metronidazole**
System suitability (n=5) %RSD	0.73 %	0.38 %
Linearity range (µg/mL)	0.98 to 25.0 µg/mL	1.76 to 56.25 µg/mL
Working Range	4.55 µg/mL to 17.74 g/mL	13.64 µg/mL to 53.23 µg/mL
Slope (m) [a)]	21.5816	22.8680
Intercept(c) [a)]	+ 54.0273	+ 57.6468
Correlation coefficient (R^2)	0.9990	0.9993
LOD (µg/mL)	0.21 µg mL^{-1}	0.73 µg mL^{-1}
LOQ (µg/mL)	0.98 µg mL^{-1}	1.76 µg mL^{-1}
Intraday precision (n=3)	0.77%	0.73%
Interday precision (n=3)	1.22%	0.96%
Assay	98% to 102%	98% to 102%
Recovery	98% to 102%	98% to 102%

a) Of the equation y = mx + c, where y is peak current (ip), m is the slope, x is the Concentration and c is the intercept.

Table 2: Results of assay studies for MZ and FRZ

Drug	Level	Labeled claim (mg/tablet)	Drug found in mg	% Assay
FRZ	Lower	100	98.52	98.52
	Middle	100	100.47	100.47
	Higher	100	101.60	101.60
	Average % Assay			100.19
	Standard Deviation (SD)			1.56
	% RSD			1.55
MZ	Lower	300	295.74	98.58
	Middle	300	297.03	99.01
	Higher	300	295.47	98.49
	Average % Assay			98.69
	Standard Deviation (SD)			0.28
	% RSD			0.28

Table 3: Results of Recovery studies for MZ and FRZ**:**

Standard	Level	Conc. Of std added [µg/mL]	Conc. of std Found [µg/mL]	Recovery (%)
FRZ	Lower	3.06	3.04	99.16
	Middle	5.77	5.85	101.46
	Higher	8.18	8.15	99.63
	Average % Recovery			100.09
	Standard Deviation (SD)			1.21
	% RSD			1.21
MZ	Lower	9.18	9.14	99.47
	Middle	17.31	17.14	99.01
	Higher	24.55	24.55	100.03
	Average % Recovery			99.51
	Standard Deviation (SD)			0.51
	% RSD		0.51	

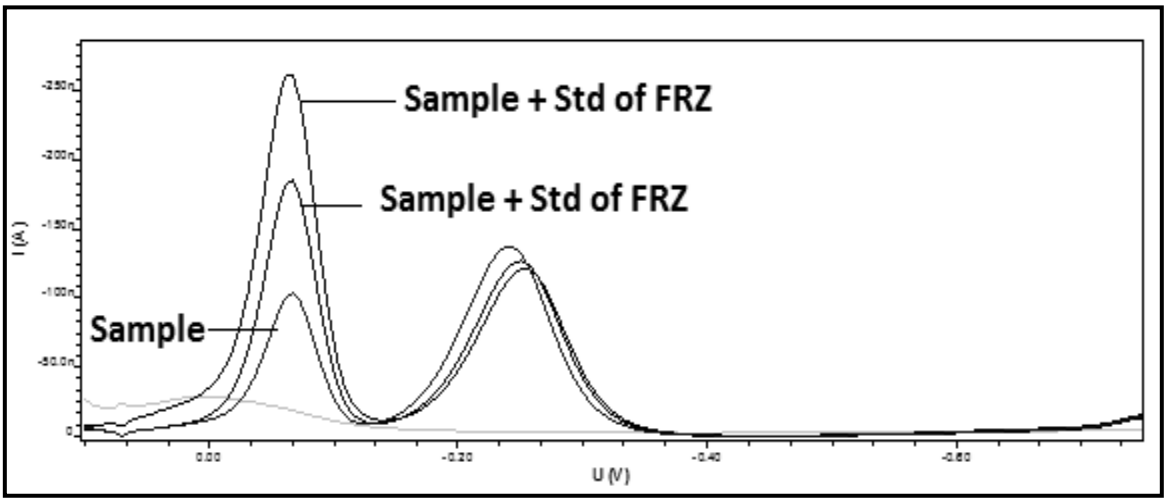


Fig 1: Shows the Overlay of the polarograms of the sample and Standard of FRZ.

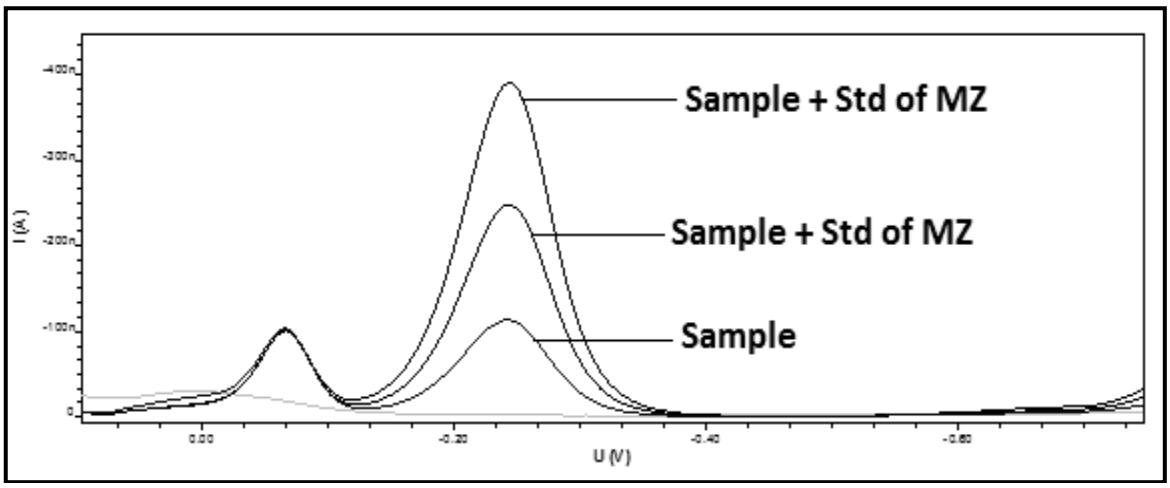


Fig 2: shows the overlay of the polarograms of the sample and Standard of MZ.

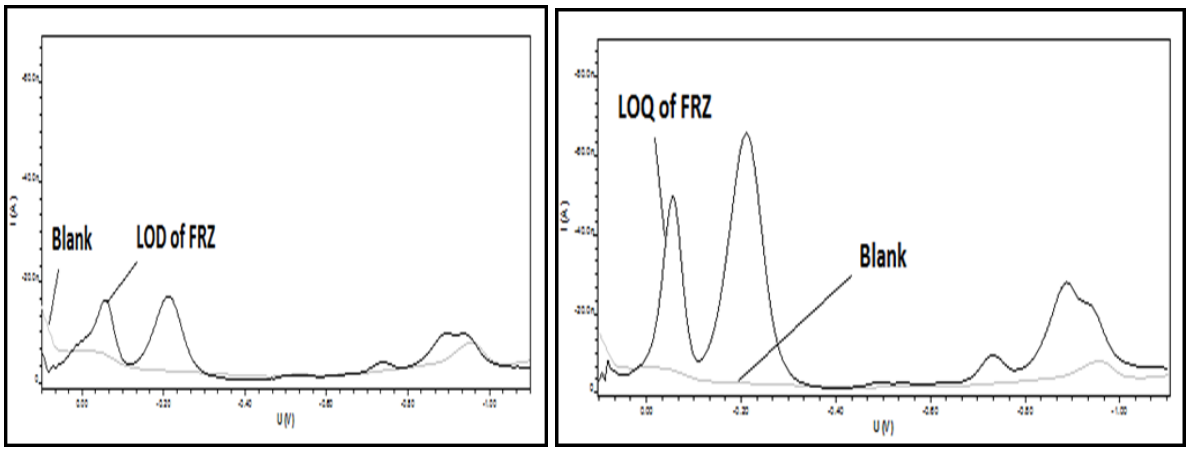


Fig 3: Polarograms showing the limit of detection and limit of quantification for FRZ

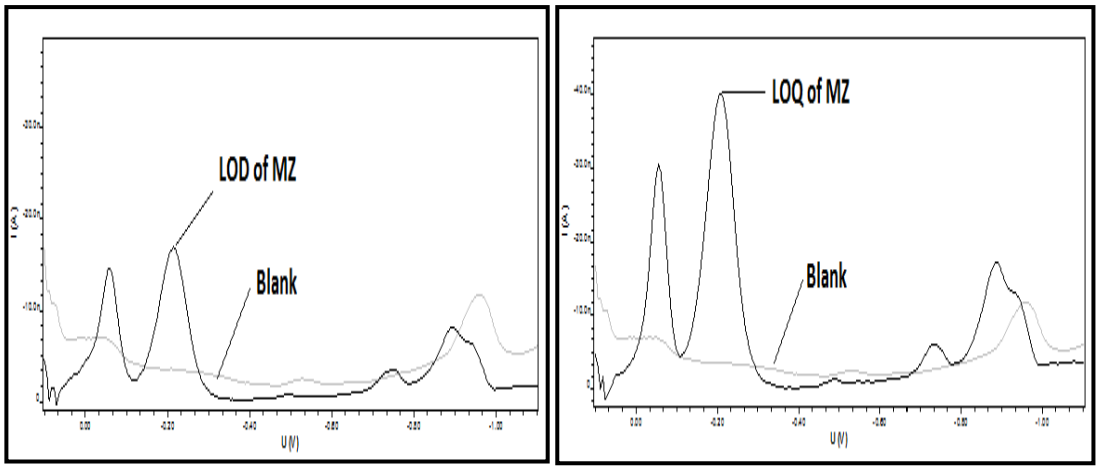


Fig 4: Polarograms showing the limit of detection and limit of Quantification for MZ

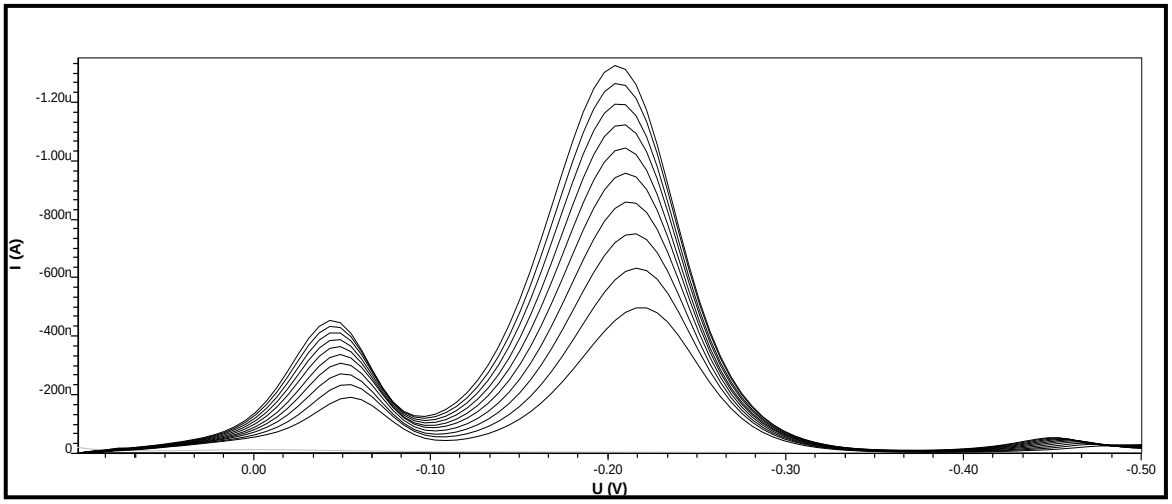
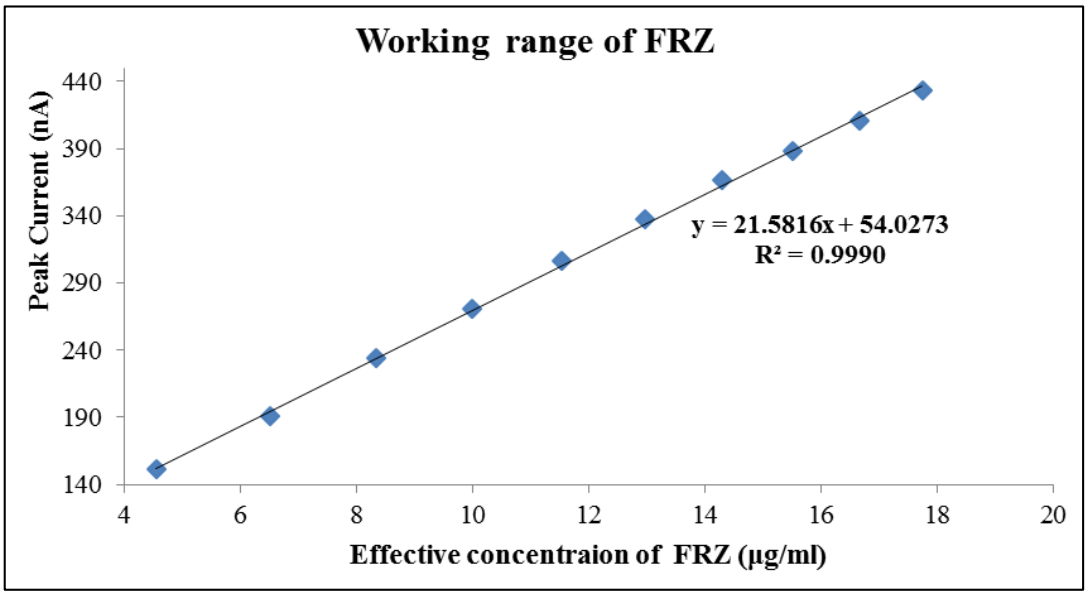
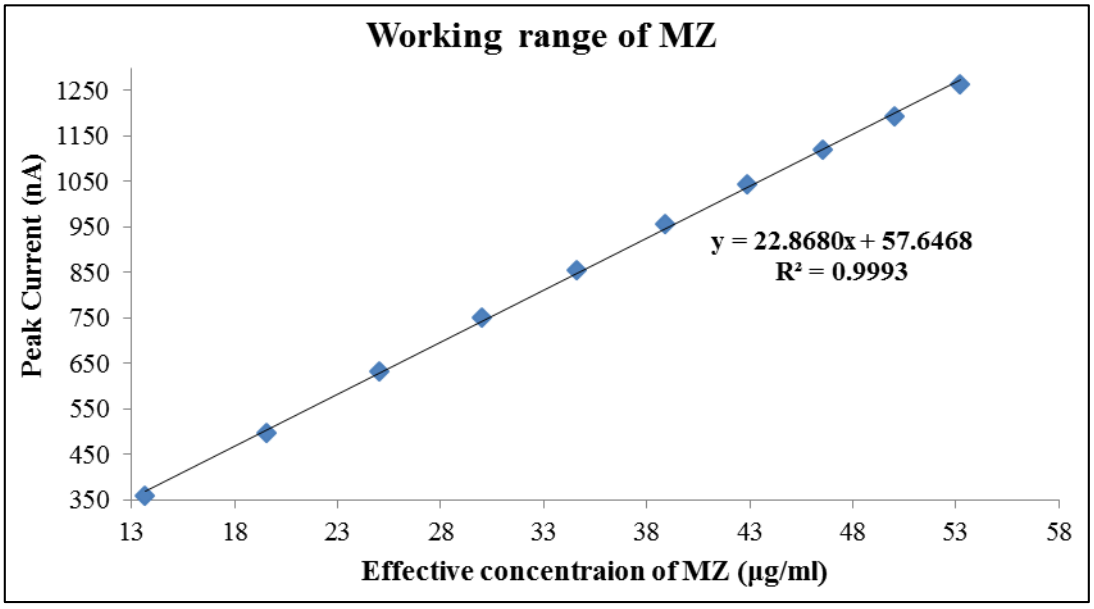


Fig 5: Polarograms of the working range for MZ and FRZ



Plot 1: The linear working range for FRZ



Plot 2: The linear working range for MZ

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