Reducing power evaluation of antioxidant drugs by potentiometric titration

A.M. El-Kosasy, L. A. Hussien and M. H. Abdel-Rahman

Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Abbassia, 11566 Cairo, Egypt
monahamdyph@yahoo.com

Abstract: An accurate and precise potentiometric automatic titration technique is applied based on redox reaction between reducing drugs (Paracetamol and dl methionine) as antioxidants and standard potassium permanganate as an oxidizing agent, which is employed in acidic medium. The titration is monitored with a Platinum indicator electrode and carried out until the greatest jump of potential from one drop of titrant appears. %RSD values were 1.12 for Paracetamol and 1.336 for dl methionine. The proposed method was successfully applied for the determination of Paracetamol in pharmaceutical formulation with accuracy 101.50 ± 0.985%. The method was robust to deliberate changes in temperature (30^0C ± 5). Paracetamol was found to be more reducing to potassium permanganate than dl methionine.

[A.M. El-Kosasy, L. A. Hussien and M. H. Abdel-Rahman. **Reducing power evaluation of antioxidant drugs by potentiometric titration**. *J Am Sci* 2013; 9(7): 114-118]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 13

Keywords: antioxidant, titration, Paracetamol, Potentiometric, platinum.

1. Introduction:

Paracetamol and dl methionine are antioxidant drugs. Paracetamol reduces aspirin-induced gastric mucosal injury. The gastroprotective effect of Paracetamol is comparable to the protective effect of PGE_2 on gastric mucosa and is accompanied by significant decrease of the gastric lipid peroxidation (evaluated as MDA-generation)[1]. It reduces the amount of lipid peroxides generated in LDL during mononuclear cell–mediated oxidation by 69%. Marit*et al.*[2] indicated that Paracetamol protects LDL against Cu^{2+}-induced, azo compound–initiated, and mononuclear cell–mediated oxidative modification *in vitro* and that this may be due to the radical scavenger capacity of Paracetamol which was tested by using Diphenyl picryl hydrazyl radical scavenging activity, also, Paracetamol protects erythrocytes from oxidative stress[3,4]. Paracetamol significantly reduces KCN-induced superoxide anion generation as well as KCN-induced lipid peroxidation, it was found that Paracetamol has the potential to limit the undesirable effects of superoxide anion[5].It is an efficient inhibitor of potent oxidants hypochlorous acid (HOCl) and hypobromous acid (HOBr) from halide ions generated by isolated heme peroxidase enzyme myeloperoxidase which released by activated neutrophils and monocytes. These oxidants have been implicated as key mediators of tissue damage in many human inflammatory diseases including atherosclerosis, asthma, rheumatoid arthritis, cystic fibrosis and some cancers[6].Dl methionine is a precursor amino acid for important antioxidant molecules such as glutathione, cysteine and taurine which protect the cells from oxidative damage and play vital role in preventing cell detoxification[7] In addition, dl methionine has been shown to chelate lead and remove it from tissues[8].Homocysteine (Hcy), a thiol formed by demethylation of DL methionine is, at moderately high levels, a known independent risk factor for atherosclerosis and increased vascular dysfunction[9]. However, it displays an antioxidant effect on cellular systems at micromolarlevels[10].Also, L-methionine leads to a concentration-dependent induction of the antioxidant proteins heme oxygenase-1 (HO-1) and ferritin in cultured endothelial cells[11].The radical scavenging activity of methionine has been reported in literature using hydroxyl radical scavenging assay[12,13]. For d methionine it is considered as a superoxide radical suppressor[14]. In the work presented here, a novel electrochemical method for evaluation of antioxidant activity is proposed. An automatic titration technique based on redox reactionis pursued by means of potentiometry. Platinum indicator electrode is applied whose potential changes markedly at the equivalence point which is easily obtained from the titration curve by applying first and second derivative where the first derivative, $\Delta E/\Delta mL$ is maximal and its second derivative $\Delta^2E/\Delta mL^2$equals zero. The reducing power of Paracetamol and dl methionine could be determined by this method by calculating number of electrons obtained by one molecule of the two studiedantioxidant drugs. Potassium permanganate acts as an oxidizing agent in acidic medium by the following equation[15]:

$$MnO_4^- + 8H^+ + 5e^- \rightleftharpoons Mn^{2+} + 4\,H_2O \qquad E^0 = 1.51\ V.$$

2. Experimental

2.1. Instrumentation

Mettler Toledo automatic compact titrator model G20 with Labx software version 3.1 with reference electrode. Platinum wire

2.2. Samples

2.2.1. Pure samples

Pure Paracetamol and dl methionine was kindly supplied by HIKMA Pharmaceutical Company (6th October, Egypt).

2.2.2. Market samples

Abimol tablets, batch no., were manufactured by GSK Company (Cairo, Egypt).

2.2.3. Chemicals and Reagents:

Potassium permanganate (0.025 N), (Adwic) prepared according to European Pharmacopoeia[16]. The stock solution was standardized with oxalic acid and was prepared daily. Diluted sulfuric acid (10% v/v) (Adwic).

2.2.4. Standard solutions:

Stock standard solutions

Paracetamol (2.00 mg/mL)

It was prepared by accurately weighing pure sample equivalent to 200.00 mg paracetamol into 100-mL volumetric, dissolved in and diluted to the volume with water.

Dl methionine (2.00 mg/mL)

It was prepared by accurately weighing pure sample equivalent to 200.00 mg DL methionine into 100-mL volumetric, dissolved in and diluted to the volume with water.

2.3. Procedure

A potentiometric titration procedure using Mettler Toledo automatic compact titrator is established by immersing a reference electrode and a highly folded platinum wire as an indicator electrode which are connected to the titrator in the titration vessel where ten mL of the solution of drug under study is added to 20 mL diluted sulfuric acid, the titrant used is 0.025 N potassium permanganate.

2.3.1. Method validation

2.3.1.1. Accuracy

Ten milliliters of different working solutions (0.40-2.00 mg/mL) of Paracetamol and dl methionine separately, were titrated against standard potassium permanganate (0.025 N), in triplicates, the specified conditions were set. After obtaining the end-point for each one, the average concentrations were calculated using the law of concentration and the percentage recoveries were calculated.

2.3.1.2. Precision

2.3.1.2.a. Repeatability

Three samples of concentrations 0.40, 0.60, 0.80 mg/mL of pure Paracetamol and dl methionine separately, were titrated against standard potassium permanganate, in triplicates under the specified conditions, within the same day, In order to evaluate the degree of method repeatability, the mean percentage recoveries and the relative standard deviations were then calculated.

2.3.1.2.b. Intermediate Precision

The interday variation was evaluated on above mentioned concentrations of each drug for three successive days, and then the mean percentage recoveries and the relative standard deviations were calculated.

2.3.1.3. Robustness

Robustness was studied by applying small deliberate changes in temperature (30^0C ± 5) during the method developing for concentration (0.4 mg/ mL) for each drug, separately. The % RSD was then calculated.

2.3.2. Application to Pharmaceutical formulations

The suggested procedure was applied for the analysis of Paracetamol in Abimol® tablets, where the contents of ten tablets were weighed, powdered and mixed. An amount of the powder equivalent to 100 mg of Paracetamol was transferred to 250 ml beaker. The powder was extracted with 50 ml water and filtered into clean 100 ml volumetric flasks, washed and then completed to mark to prepare 1mg.ml^{-1} Paracetamol. The method stated under accuracy is then repeated. Standard addition technique was applied to assess the accuracy of the proposed method by spiking different known concentrations of pure Paracetamol to the pharmaceutical preparation. The procedure described under the assay of Abimol® tablets was followed. Concentrations were calculated; the mean percentage recoveries and the relative standard deviations were then calculated.

3.Results and Discussion

3.1. Proposed reaction products

The power of certain antioxidants is associated with their reducing power[17].The proposed potentiometric titration procedure is based on redox reaction between the studied antioxidant drugs and standard potassium permanganate in acidic medium. For Paracetamol, the stoichiometry of the reaction of Paracetamol with permanganate in sulphuric acid proved to be 1:2, respectively[18] and it's apparent from previous equation that each mole of potassium permanganate needs five electrons to be reduced in acidic medium so Paracetamol will produce ten electrons for the redox reaction to be settled. For DL methionine, it had been reported that dl methionine in acidic medium ($C_5H_{12}NO_4S^+$) is oxidized by oxidizing agent leading to the formation of methionine sulfoxide ($C_5H_{11}NO_3S$) species[19]. The following equation represents the proposed half equation reaction:

$C_5H_{12}NO_2S^+ + H_2O \longrightarrow C_5H_{12}NO_3S^+ + 2H^+ + 2e$

The equivalent factor is calculated as follows[20]:
F = [Equivalent weight of sample drug X Normality of the standard] /1000
Where Equivalent weight of sample drug = Molecular weight of sample / n
Where n is number of electrons gained or lost by one molecule.
We can conclude that:
$F_{Paracetamol}$= [(151.19/ 10) X 0.025] / 1000=0.0003779g.
$F_{DL\ methionine}$ = [(149.2/ 2) X 0.025] / 1000= 0.001865g.

3.2.Method validation

3.2.1.Accuracy

The accuracy calculated as percentage recovery was in the range of 99.76± 1.12 for Paracetamol and 99.91 ± 1.336 for dl methionine. The low values of % RSD demonstrating an excellent accuracy of the method (tables1, 2). Titration curves are shown in (Figures 1, 2). Values of equivalence points were obtained by getting first and second derivatives of the titration curves.

3.2.2.Precision

The % RSD for repeatability and intermediate precision were found to be 0.62and 1.71% for Paracetamol and 0.62 and 1.60%for dl methionine. These low RSD% values suggesting an excellent precision of the method (Tables 3, 4)

3.2.3.Robustness

There were no significant changes in the end point readings of the studied drugs solutions when small deliberate changes in temperature (30^0C ± 5) were introduced during the method developing. The low % RSD (1.38 for Paracetamol and 0.79 for DL methionine) indicated the robustness of the method.

3.3. Analysis of pharmaceutical formulation

The proposed method was successfully applied for the determination of Paracetamol in pharmaceutical formulation and the validity of the method was further assessed by applying standard addition technique (Table 5).

The results obtained by applying the proposed method were statistically compared with those obtained by applying the reported methods for Paracetamol and dl methionine (Table 6). These results show that on using probability of 95%, the calculated t and F values are less than the theoretical ones indicating that there is no significant difference between the proposed potentiometric method and the reported methods with respect to accuracy and precision.

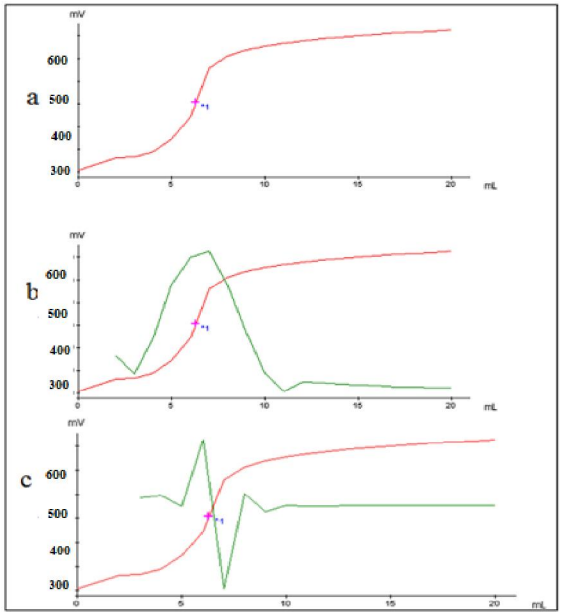

Figure 1: Titration curves of titrating 0.4 mg/mL Paracetamol with 0.025 N $KMnO_4$, a: zero order curve, b: first derivative curve and c: second derivative curve.

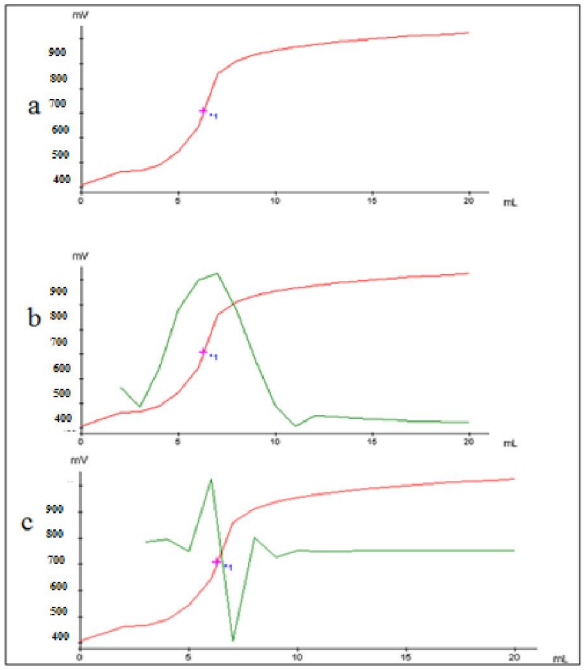

Figure 2: Titration curves of titrating 0.6 mg/mL DL methionine with 0.025 N $KMnO_4$, a: zero order curve, b: first derivative curve and c: second derivative curve.

Table 1: Accuracy results for the determination of Paracetamol in bulk powder by the proposed method.

Taken mg/mL	Found mg/mL	Recovery %
0.10	0.101	101.00
0.20	0.2003	100.15
0.40	0.402	100.50
0.50	0.494	98.80
0.60	0.605	100.83
0.80	0.79	98.75
1.00	0.983	98.30
Mean ± RSD %		99.76± 1.12

Table 2: Accuracy results for the determination of DL methionine in bulk powder by the proposed method.

Taken mg/mL	Found mg/mL	Recovery %
0.10	0.099	99.00
0.20	0.198	99.00
0.40	0.41	102.50
0.50	0.504	100.80
0.60	0.597	99.50
0.80	0.79	98.75
1.00	0.998	99.80
Mean ± RSD %		99.91± 1.34

Table 3: Repeatability and intermediate precision results for the determination of Paracetamol in bulk powder by the proposed method.

	Taken µg/spot	Found* µg/spot		Recovery %
Repeatability	0.40	0.41		102.50
	0.60	0.608		101.33
	0.80	0.812		101.50
	Mean ± RSD %			101.78±0.62
Intermediate precision	0.40	1st day	0.41	102.50
		2nd day	0.394	98.50
		3rd day	0.402	100.50
	0.60	1st day	0.609	101.50
		2nd day	0.59	98.33
		3rd day	0.611	101.833
	0.80	1st day	0.808	101.00
		2nd day	0.814	101.75
		3rd day	0.784	98.00
	Mean ± RSD %			100.43± 1.71

*Mean of three determinations

Table 4: Repeatability and intermediate precision results for the determination of DL methionine in bulk powder by the proposed method.

	Taken µg/spot	Found* µg/spot		Recovery %
Repeatability	0.40	0.405		101.25
	0.60	0.61		101.67
	0.80	0.82		102.50
	Mean ± RSD %			101.81±0.62
Intermediate precision	0.40	1st day	0.41	102.50
		2nd day	0.398	99.50
		3rd day	0.405	101.25
	0.60	1st day	0.607	101.17
		2nd day	0.585	97.50
		3rd day	0.61.	101.67
	0.80	1st day	0.81	101.25
		2nd day	0.811	101.38
		3rd day	0.79	98.75
	Mean ± RSD %			100.55± 1.60

*Mean of three determinations

Table 5: Application of standard addition technique on Abimol® tablets by the proposed method.

Taken (mg/mL)	Found (mg/mL)	Recovery %	Standard addition technique		
			Added standard (mg/mL)	Found (mg/mL)	Recovery %
0.2	0.205	102.50	0.20	0.199	99.50
	0.201	100.50	0.30	0.304	101.33
	0.203	101.50	0.40	0.401	100.25
Mean ± RSD %		101.50 ± 0.985	Mean ± RSD %		100.36± 0.92

Table 6: Statistical comparison of the results obtained by applying the proposed method and the published methods** for the analysis of Paracetamol and DL methionine.

Parameter	Paracetamol		DL methionine	
	Proposed method	Reported method**[a]	proposed method	Reported method** [b]
Mean	99.76	100.02	99.91	100.126
SD	1.115	0.9654	1.334	0.95
Variance	1.24	0.932	1.78	0.89
n	7	5	7	5
t(2.23)*	0.43	--	0.33	--
F(6.16)*	1.33	--	2.00	--

*The values between parentheses are the corresponding theortical values of **t** and **F** at the 95% confidence level.

**[a]Hplc method for Paracetamol using acetonitrile:water (25:75 v/v) pH 2.5[21]

**[b] Spectroscopic study of chloranil charge-transfer complex of methionine.[22]

4. Conclusion

One of the most widely used applications of electrochemistry is for determining the endpoint of titrations. Electrochemical methods are recognized as the best tool for investigation of electron transfer processes in solution. Advantages of electrochemical methods over other methods (e.g., visual methods) include greater sensitivity, as well as increased accuracy and precision, especially those depend on automatic techniques. The platinum indicator electrode serves only as an endpoint indicator and the analytical concentrations of the drugs are determined on the basis of stoichiometric chemical reaction. Platinum electrode, a noble metal is commonly used as inert electrode on which the half-cell reaction of interest takes place. The proposed method is accurate, precise and considered a desirable method due to permanganate's low cost, availability and ease of handling.

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5/12/2013