

## Polarographic Study of Meta-Hydroxyacetanilide and its Determination

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### Abstract

Present paper deals with polarographic study of effect of maxima suppressors and supporting electrolytes on anodic waves of meta-hydroxyacetanilide and polarographic determination of meta-hydroxyacetanilide under optimum concentration of maxima suppressors and supporting electrolytes.

The polarographic method has been developed to study qualitatively the effect of maxima suppressor (fuchsin) and supporting electrolyte (nitric acid) on oxidation wave of meta-hydroxyacetanilide. Polarograms of system were recorded between 500 to 1300 mV by using Rotating Platinum micro Electrode as anode and Saturated Calomel Electrode as cathode on D.C. Recording Polarograph using Omniscribe recorder.

It shows a similar behavior to that observed for paracetamol. It produces anodic wave at rotating platinum electrode. The oxidation yields the 3-N-acetylaminosemiquinone and represents a reversible reaction. Polarographically a value of 900 mV is found for decomposition potential of meta-hydroxyacetanilide. Wave analysis point to 1-electron step for each wave. Waves of meta-hydroxyacetanilide are only proportional to concentration at low concentrations.

**Keywords:** Fuchsin; Meta-hydroxyacetanilide; Nitric acid; Paracetamol

### Introduction

Acetaminophen (250 mg/kg) administered intraperitoneally to fasted, Phenobarbital-induced mice produced hepatotoxicity. No hepatotoxicity was observed after the administration of the regioisomer 3'-hydroxyacetanilide (600 mg/kg). Similar levels of covalent binding to liver homogenates occurred in mice receiving either acetaminophen or 3'-hydroxyacetanilide at these doses [1]. The administration of 3'-hydroxyacetanilide, a regioisomer of acetaminophen, to mice failed to produce hepatotoxicity even after the administration of diethyl maleate. In contrast, hepatotoxicity did occur when 3'-hydroxyacetanilide was administered to buthionine sulfoximine pretreated mice [2]. Acetaminophen (4'-hydroxyacetanilide), a widely used analgesic and antipyretic drug, is hepatotoxic in large doses, whereas the m-hydroxy isomer of acetaminophen, 3'-hydroxyacetanilide, is not hepatotoxic. Both are oxidised by mouse liver cytochromes P-450 to reactive metabolites that bind covalently to hepatic proteins [3]. Acetaminophen (APAP), a widely used analgesic and antipyretic that is considered to be relatively safe at recommended doses, is the leading cause of drug-induced liver failure in the United States. 3'-Hydroxyacetanilide (AMAP), a regioisomer of APAP, is useful as a comparative tool for studying APAP-induced toxicity because it is nontoxic relative to APAP. Transforming growth factor- $\alpha$  transgenic mouse hepatocytes were treated with both isomers to investigate mitogen-activated protein kinase (MAPK) cascades in order to differentiate their toxicological outcomes [4].

Acetaminophen (N-acetyl-p-aminophenol, APAP) is a widely used analgesic and antipyretic that is considered to be relatively safe at recommended doses. However, overdose cases are fairly common because of the widespread availability of APAP [5]. The majority of an APAP dose is metabolized to relatively nontoxic products via conjugation reactions, such as sulfation and glucuronidation [6]. The remaining dose is predominantly metabolized in the liver by cytochrome P450E1 to the presumed reactive intermediate, N-acetyl-p-benzoquinoneimine (NAPQI) [7,8]. At therapeutic doses, low levels of P450-generated NAPQI are cleared by glutathione and glutathione-S-transferases [9]. However, in APAP overdose situations, higher concentrations of NAPQI deplete cellular glutathione pools leading to arylation of intracellular nucleophiles by NAPQI. Modification of these nucleophilic residues within the cell can lead to oxidative/electrophilic stress resulting in hepatocellular injury [10]. A comparative tool

useful for studying APAP-induced toxicity is its regioisomer, 3'-hydroxyacetanilide (N-acetyl-m-aminophenol, AMAP) [11-15].

Present paper deals with polarographic study of effect of maxima suppressors and supporting electrolytes on anodic waves of meta-hydroxyacetanilide and polarographic determination of meta-hydroxyacetanilide under optimum concentration of maxima suppressors and supporting electrolytes.

### Methodology

All chemicals were of A.R. grade. Polarograms of all system were recorded on D.C. Recording Polarograph using Omniscribe recorder between 200 to 1300 mV using Rotating Platinum micro Electrode (RPE) as anode and Saturated Calomel Electrode (S.C.E.) as cathode. Detail procedure is given under each heading.

### Effect of maxima suppressors and supporting electrolytes on polarographic waves of meta-hydroxyacetanilide

The maxima suppressor capacity of fuchsin on the anodic wave of meta-hydroxyacetanilide in presence of 0.1 M  $\text{HNO}_3$  was studied by preparing three systems containing 0.6 ml of  $5 \times 10^{-3}$  M meta-hydroxyacetanilide, 1 ml 5 M  $\text{HNO}_3$  solution and different amount of fuchsin, viz., 0,  $2.5 \times 10^{-9}$ ,  $1.25 \times 10^{-5}$  g and diluted to 50 ml with distilled water. Polarograms of each system were recorded between 500 to 1300 mV by using Rotating Platinum micro Electrode (RPE) as anode and Saturated Calomel Electrode (S.C.E.) as cathode. Similar experiments were carried out using various concentrations of nitric acid.

### Polarographic determination of meta - hydroxyacetanilide (calibration method)

Polarograms of system containing varying concentrations of meta-

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hydroxyacetanilide in 0.1 M  $\text{HNO}_3$  were recorded on D.C. Recording Polarograph using Omniscrite recorder.

## Observations

### Effect of maxima suppressors and supporting electrolytes on polarographic waves of meta-hydroxyacetanilide

Effect of fuchsin concentration on the oxidation wave of meta-hydroxyacetanilide in 0.1 M  $\text{HNO}_3$  is given in Figure 1. Fuchsin suppresses the wave but does not improve the shape of wave thereby anodic wave is not well defined. Decomposition potential is found to be +950 mV. Similarly  $\text{HNO}_3$  concentration exerts pronounced effect on current-potential wave of meta-hydroxyacetanilide (Figure 2). As concentration of  $\text{HNO}_3$  varies from 0.1 M to 3 M initial current shifts to higher values for same applied potential, at the same time increase in wave height is observed.

### Polarographic determination of meta-hydroxyacetanilide (calibration method)

Polarograms obtained for different amount of meta-hydroxyacetanilide in 0.1 M  $\text{HNO}_3$  are shown in Figure 3.

## Results and Discussion

There is not much literature review on the approaches used for meta-hydroxyacetanilide detection. The advantages of the application of polarography in the analysis of metahydroxyacetanilide are speed, sensitivity, which enables trace analysis to be carried out, and to follow changes in the composition of the preparation, the small sample requirements and selectivity. It is possible to carry out a polarographic analysis even in the presence of colouring matters and comparable amounts of other ingredients.

### Effect of maxima suppressors and supporting electrolytes on polarographic waves of meta-hydroxyacetanilide

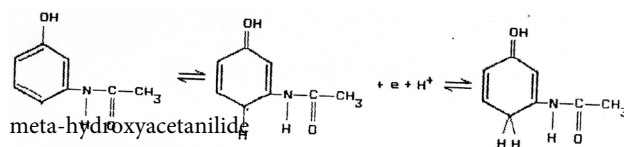
The polarographic method has been used to study qualitatively the effect of maxima suppressor (fuchsin) and supporting electrolyte (nitric acid) on oxidation wave of meta-hydroxyacetanilide. It shows a similar behavior to that observed for paracetamol [16-19]. Figures 1 and 2 represents effect of various concentrations of fuchsin (in 0.1 M  $\text{HNO}_3$ ) and nitric acid on the anodic wave of  $6.0 \times 10^{-5}$  M meta-hydroxyacetanilide. It was found that fuchsin suppressed the wave (Figure 1) while increasing concentrations of nitric acid was found to increase residual as well as diffusion current values at the same time making the limiting current plateau much defined (Figure 2).

### Polarographic determination of meta-hydroxyacetanilide (calibration method)

The log plots of the C-V curves of meta-hydroxyacetanilide in 0.1 and 3.0 M  $\text{HNO}_3$  is given in Figure 4. The polarographic method has been used to identify the products of oxidation of meta-hydroxyacetanilide. In this connection the half-wave potentials (1025 mV and 1090 mV vs S.C.E.) and values of  $n$  are determined in 0.1 M and 3.0 M nitric acid (Table 1) and is found to agree with the values obtained for the oxidized product. A good agreement between experimental and theoretical value is shown by the log plots of the C-V curves of meta-hydroxyacetanilide in 0.1 and 3.0 M  $\text{HNO}_3$  (Figure 4).

The experimental points of the log plots gave good straight lines with slopes of 0.0656 and 0.0770 V, in close agreement with the theoretical values which is 0.0591 V. Hence the value of  $n$ , the number of electrons taking part in the reversible reaction is found to be 0.9 and 0.8 (~1).

Thus meta-hydroxyacetanilide produces anodic wave at the rotating platinum electrode. The oxidation yields the 3-N-acetylaminosemiquinone and represents a reversible reaction. Polarographically a value of 900 mV is found for decomposition potential of meta-hydroxyacetanilide. Wave analysis point to a 1-electron step for each of the waves. Meta-hydroxyacetanilide is oxidized in the following manner:



3-N-acetylaminosemiquinone

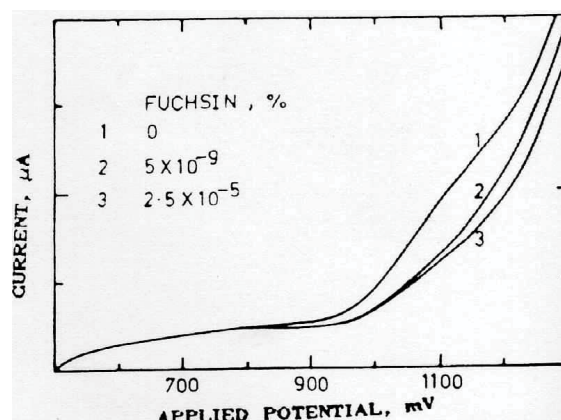


Figure 1: Effect of Fuchsin concentration on the anodic wave of  $6.0 \times 10^{-5}$  M meta-hydroxyacetanilide in 0.1 M  $\text{HNO}_3$ .

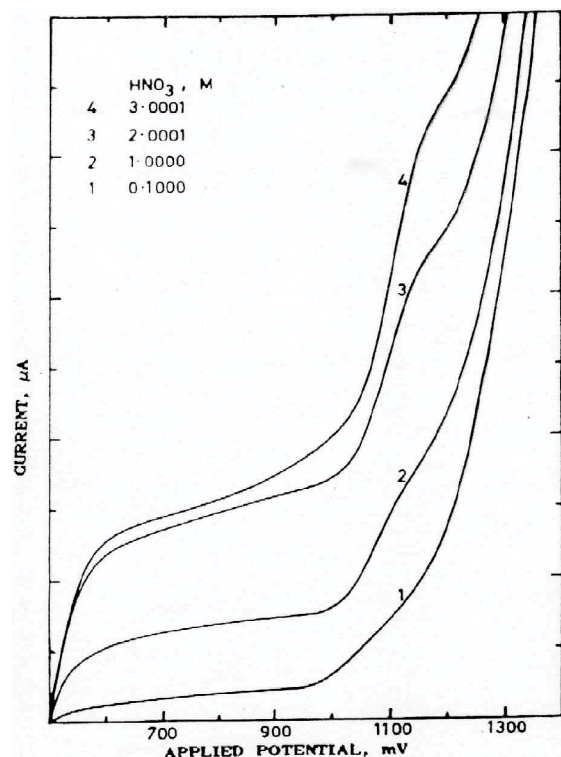


Figure 2: Effect of  $\text{HNO}_3$  concentration on the anodic wave of  $6.0 \times 10^{-5}$  M meta-hydroxyacetanilide.

HNO <sub>3</sub> M	iR	id		Slope, V		Value of n		E <sub>1/2</sub> mV
		Observed	Corrected	Theoretical	Experimental	Theoretical	Experimental	
0.1	1	1.95	0.85	0.06	0.07	1	0.9	1025
3	4	8.88	4.98	0.06	0.08	1	0.8	1090

Table 1: Data of log plots of the waves of meta-hydroxyacetanilide in 0.1 M and 3.0 M Nitric acid.

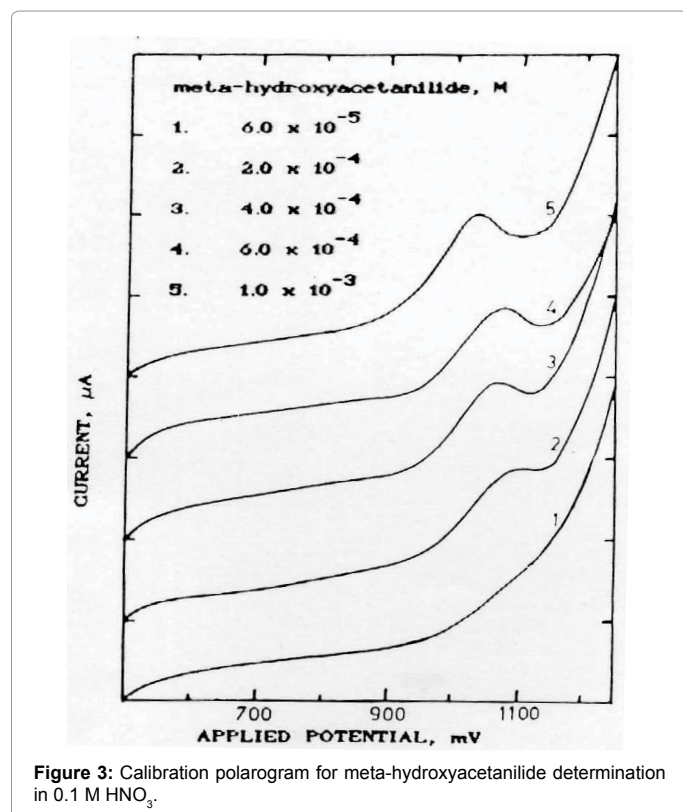


Figure 3: Calibration polarogram for meta-hydroxyacetanilide determination in 0.1 M HNO<sub>3</sub>.

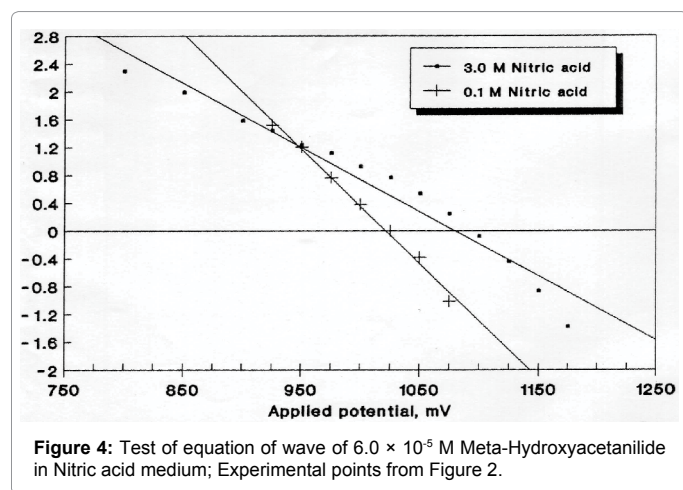


Figure 4: Test of equation of wave of  $6.0 \times 10^{-5}$  M Meta-Hydroxyacetanilide in Nitric acid medium; Experimental points from Figure 2.

While carrying out determination of meta-hydroxyacetanilide it is observed that waves of meta-hydroxyacetanilide are only proportional to concentration at low concentrations (below approximately  $2 \times 10^{-4}$  M). Moreover meta-hydroxyacetanilide gives no reproducible wave and the nature of it changes as concentration of meta-hydroxyacetanilide varies from  $6.0 \times 10^{-5}$  M to  $1.0 \times 10^{-3}$  M as shown in Figure 3. At  $6.0 \times 10^{-5}$  M meta-hydroxyacetanilide, limiting current plateau is not well defined; at  $2.0 \times 10^{-4}$  M meta-hydroxyacetanilide, limiting current region become well developed; at  $4.0 \times 10^{-4}$  M meta-hydroxyacetanilide

round streaming maxima appears which become more pronounced with further increase in its concentration.

## Conclusion

The polarographic method has been used to study qualitatively the effect of maxima suppressor (fuchsin) and supporting electrolyte (nitric acid) on oxidation wave of meta-hydroxyacetanilide an analogue of paracetamol. It shows a similar behavior to that observed for paracetamol. It produces anodic wave at the rotating platinum electrode. The oxidation yields the 3-N-acetylaminosemiquinone and represents a reversible reaction. Polarographically a value of 900 mV is found for decomposition potential of meta-hydroxyacetanilide. Wave analysis point to a 1-electron step for each of the waves. Waves of meta-hydroxyacetanilide are only proportional to concentration at low concentrations.

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