Peak Voltammetry at Glassy Carbon Electrode of Acetaminophen Dosage Forms

C. M. SHEARER▲, K. CHRISTENSON, A. MUKHERJI, and G. J. PAPARIELLO

Abstract □ A new, rapid, stability-indicating assay for acetaminophen in both solid and liquid dosage forms was developed. The assay is based upon the oxidation of acetaminophen polarographically at a glassy carbon electrode, using an acetate system in methanol as the supporting electrolyte. The peak potential for acetaminophen is about 0.5 v. *versus* the saturated calomel electrode, while the peak potential for *p*-aminophenol, the major hydrolytic degradation product of acetaminophen, is about 0.2 v. This stability-indicating analysis can be performed without prior separation of acetaminophen from *p*-aminophenol. Most common inert solid dosage form and liquid preparation components do not interfere in this procedure. It was also established that most therapeutically active components used in combination with acetaminophen do not interfere in this procedure.

Keyphrases □ Acetaminophen dosage forms—stability-indicating polarographic analysis, glassy carbon electrode □ Polarography, glassy carbon electrode—stability-indicating analysis, acetaminophen dosage forms □ Voltammetry, glassy carbon electrode—stability-indicating analysis, acetaminophen dosage forms

Many assay methods for acetaminophen are reported in the literature. However, few of these claim to be stability indicating; those that do make this claim, with one exception, involve time-consuming, often troublesome, separation techniques. The one exception is a colorimetric method developed by Chafetz *et al.* (1), which will be discussed in detail shortly.

The major route of degradation of acetaminophen is hydrolysis to *p*-aminophenol and acetic acid. The kinetics of this hydrolytic reaction were studied by Koshy and Lach (2). In their study, they assayed for the intact material by separating it from the *p*-aminophenol by means of a strong cation-exchange resin. This ion-exchange separation was used by the same authors (3, 4) to analyze urine samples and commercial formulations containing acetaminophen. The concentration of the acetaminophen in the eluant was determined either by UV spectrophotometry or by a colorimetric reaction using the procedure of Greenberg and Lester (5). At a later date, Koshy (6) reported on the separation of acetaminophen from *p*-aminophenol as well as from aspirin, caffeine, and salicylic acid by using column partition chromatography. Recently, Prescott (7) published a GC procedure applicable to body fluid analysis, which separates the trimethylsilyl derivatives of acetaminophen and *p*-aminophenol.

Table I—Results of the Analysis of Known Mixtures of Acetaminophen and *p*-Aminophenol in Solution

Mole Fractions of Acetaminophen					
	Found				
Added	Run 1	Run 2	Run 3	Run 4	Average
0.92	0.91	0.92	0.91	0.93	0.92
0.80	0.80	0.78	0.78	0.79	0.79
0.68	0.68	0.70	0.68	0.67	0.68
0.60	0.58	0.55	0.58	0.57	0.57
0.40	0.41	0.32	0.31	0.35	0.35
0.20	0.26	0.18	0.28	0.17	0.22

The Chafetz *et al.* (1) colorimetric technique is based upon the reaction of acetaminophen with nitrous acid to form 2-nitro-4-acetamidophenol. These researchers found that the presence of *p*-aminophenol does not interfere with the measurement of this product by spectrophotometry. Unfortunately, in our hands the same selectivity in the nitrous acid colorimetric reaction was not obtained. A minimum of 25% contribution to the absorbance was observed when an equivalent molar concentration of *p*-aminophenol was tested.

Thus, when forced to seek an alternative method of analysis, we considered electrochemical techniques. The electrochemistry of *p*-aminophenol has been widely studied (8). *p*-Aminophenol undergoes electrochemical oxidation with the loss of two electrons and two protons

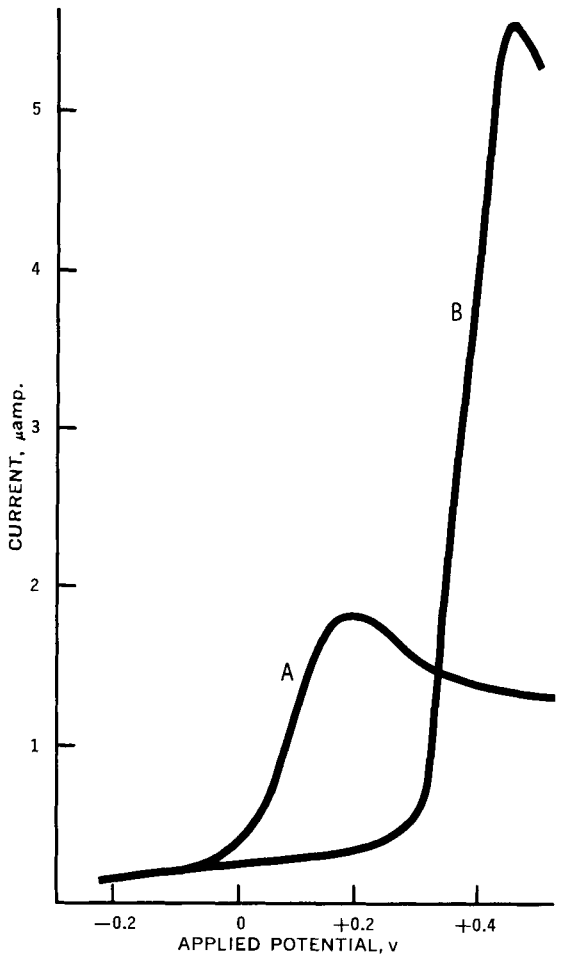

Figure 1—*Polarogram of: (A) p-aminophenol (concentration = 0.012 mg./ml.), and (B) acetaminophen (concentration = 0.1 mg./ml.), using a glassy carbon electrode.*

Table II—Recovery of Acetaminophen when in Combination with Other Drugs

Drug	Milligrams Drug	Percent Acetaminophen Recovered[a]
Glyceryl guaiacolate	15	100
Caffeine	20	96
Aspirin	50	102
Mephenesin	50	100
Codeine phosphate	10	100
Sodium barbital	10	102
Amphetamine sulfate	1.5	102
Salicylamide	75	100
Phenylephrine hydrochloride	2	100
Prednisolone	0.3	104
Pentobarbital	0.5	98
Ascorbic acid	20	>200

[a] 50 mg. of acetaminophen was present in each case.

forming quinoneimine. Several other aminophenol derivatives have been studied; however, no extensive work has been done on acetaminophen. The dropping mercury electrode, although useful for some studies, undergoes oxidative attack at about 0.4 v. *versus* the saturated calomel electrode in aqueous systems. To overcome this difficulty, the commonly used electrodes

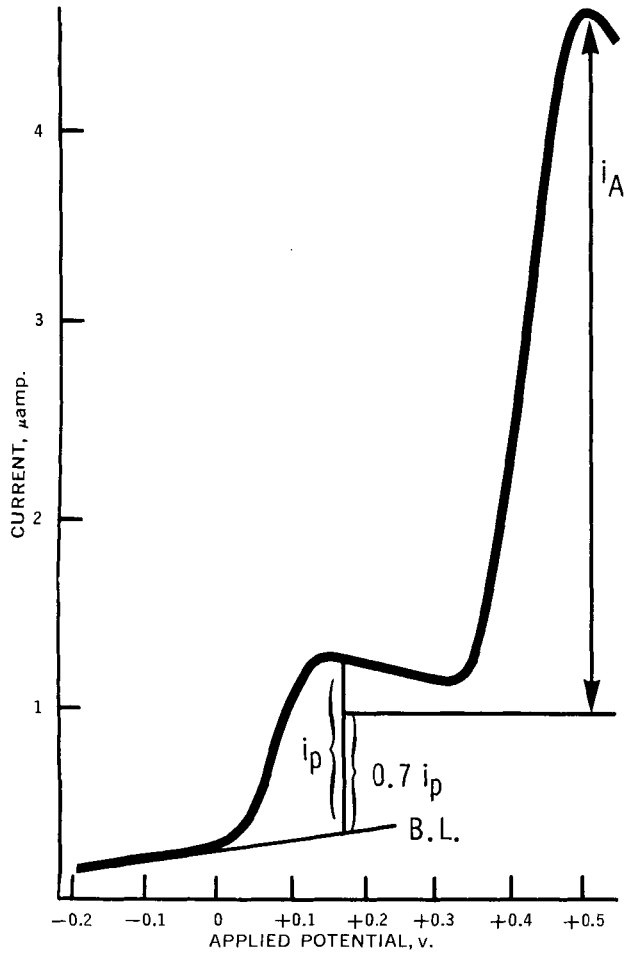

Figure 2—*Polarogram of mixture of 80% acetaminophen–20% p-aminophenol using a glassy carbon electrode. Key: B. L., baseline extended from −0.2 to 0.25 v.;* i_p, *current due to p-aminophenol at +0.18 v.; 0.7* i_p, *correction for current at 0.5 v. due to p-aminophenol and supporting electrolyte; and* i_A, *current due to acetaminophen.*

Table III—Recovery of Acetaminophen from Common Inert Solid Dosage Components

Component	Milligrams Component	Percent Acetaminophen Recovered[a]
Lactose	50	100
Microcrystalline cellulose	50	102
Starch	50	100
Talc	50	100
Stearic acid	0.5	100
Magnesium stearate	0.5	100

[a] 50 mg. of acetaminophen was present in each case.

for anodic voltammetry are made of platinum or various forms of carbon. Since the problems of poor reproducibility which might be encountered with the platinum electrode (9) were well known, a glassy carbon electrode was tried in this study. Glassy carbon[1], first introduced by Yamada and Sato (10), is electrical conducting, highly resistant to chemical attack, gas impermeable, and obtainable in a relatively pure state. Zittell and Miller (11) and Jennings *et al.* (12) reported that reproducible data could be obtained for analysis using a glassy carbon electrode in aqueous and alcoholic systems, respectively.

Presented here is a simple voltammetric technique utilizing a glassy carbon electrode for the analysis of acetaminophen in complex pharmaceutical dosage forms. The method is shown to be stability indicating with respect to hydrolytic degradation. Most common formulation inactives, as well as most therapeutically active materials, used in combination with acetaminophen were found not to interfere in this method.

EXPERIMENTAL

Apparatus—A recording polarograph[2] was used in all polarographic determinations.The analysis was run in an H-form electrolysis cell which contained a saturated calomel electrode (SCE) as the reference electrode. The sample compartment was connected to the SCE by means of an agar-saturated potassium nitrate bridge. The following instrumental parameters were used: sensitivity, 3×10^{-8} amp./mm.; damping, 2; voltage scan range, +1 to −1 v.; and polarity, reverse.

Both commercially available carbon electrodes[3] and those constructed by sealing the glassy carbon rod into a glass tube, by heating the glass so it flows around the rod to form a seal, were used in this study. In all instances, the end of the glassy carbon electrode was highly polished with metallographic cloth.

Experiments were carried out at ambient room temperature (about 23°).

Supporting Electrolyte—Dissolve 8.2 g. of sodium acetate, anhydrous, in 20 ml. of distilled water. Add 5.8 ml. of glacial acetic acid and dilute to 1 l. with methanol.

Acetaminophen Reference Standard Solution—Accurately weigh approximately 50 mg. of acetaminophen NF reference standard into a 250-ml. volumetric flask. Dissolve completely in methanol and then dilute to volume with methanol. Pipet 25.0 ml. of this solution into a 50-ml. volumetric flask, dilute to volume with the supporting electrolyte, and mix well.

Procedure—*Sample Preparation*—To a 250-ml. volumetric flask containing about 100 ml. of methanol, add an accurately weighed sample of powdered tablets or capsule contents or an accurately

[1] Glassy carbon is an exclusively owned preparation of the Tokai Electrode Manufacturing Co., Tameike-Cho, Minato-Ku, Tokyo, Japan.

[2] Sargent model XV.

[3] Chemtrix, Inc., Beaverton, Ore.

Table IV—Recovery of Acetaminophen from Common Liquid Dosage Components

Component	Component Quantity	Percent Acetaminophen Recovered[a]
Water	2.0 ml.	100
Chloroform	0.5 ml.	102
Propylene glycol	0.5 ml.	98
Corn syrup	2.0 ml.	100
Citric acid	2.0 mg.	102
Polyethylene glycol 350[b]	5.0 mg.	98
Ammonium chloride	5.0 mg.	100
Sodium benzoate	4.0 mg.	100
Sodium saccharin	0.5 mg.	100
Sodium propionate	5.0 mg.	102

[a] 50 mg. of acetaminophen was present in each case. [b] Carbowax 350.

pipeted sample of a liquid dosage form equivalent to about 50 mg. of acetaminophen. Shake the flask thoroughly to disperse the sample. Dilute to volume with methanol and thoroughly mix and filter, discarding the first 25 ml. of filtrate. Transfer 25.0 ml. of the filtrate to a 50-ml. volumetric flask, dilute to volume with the supporting electrolyte, and mix well.

Polarography—Transfer a portion of the sample or reference solution to the electrolysis cell, and insert the glassy carbon electrode. Deoxygenate with solvent-saturated nitrogen for 10 min., and obtain a polarogram from −0.2 to +0.6 v. *versus* SCE with nitrogen flowing on the surface of the solution only. If any *p*-aminophenol is present, it will be oxidized to give a peak current near +0.2 v. while acetaminophen will be oxidized giving a peak current near 0.5 v. (Fig. 1). Consequently, in mixtures of the two, the current at 0.5 v. will be the sum of the current due to the acetaminophen and *p*-aminophenol. Figure 2 gives a polarogram of such a mixture and illustrates how a correction can be made at the acetaminophen peak potential, near 0.5 v., for the current due to the *p*-aminophenol. A baseline is extrapolated from the residual current line (−0.2–0.0 v.) to 0.25 v. The current above the baseline at the peak potential due to *p*-aminophenol, at about 0.2 v., is determined. By using 70% of this value as the background current, due to supporting electrolyte and *p*-aminophenol, the current for the acetaminophen only can be obtained at its peak potential at about 0.5 v. This 0.7 correction factor must be redetermined for each new electrode and for instrumental parameters if they differ markedly from those prescribed. After determining the peak current for the sample in this manner and comparing it with that of the standard, the concentration of acetaminophen in the sample can be calculated.

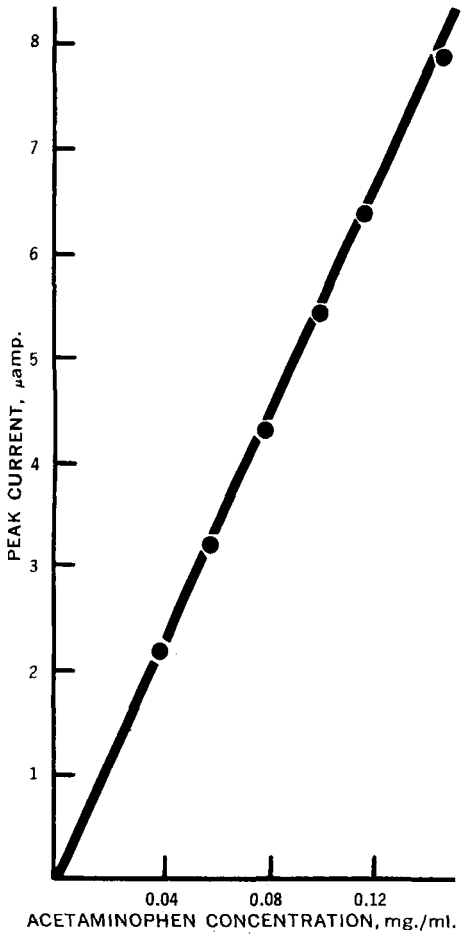

Figure 3—*Plot of acetaminophen concentration* versus *peak current.*

Table V—Comparison of Peak Polarographic Method and NF XIII Method on Commercial Dosage Forms

Dosage Form	Acetaminophen, mg./Dose—Claim	Found—Polarographic Method	Found—NF Method[a]	Other Labeled Ingredients
Tablet A	325	332	318	—
Tablet B	125	126	128	Aspirin, 230 mg. Caffeine, 30 mg.
Capsule A	175	178	179	Salicylamide, 150 mg. Caffeine, 15 mg. Phenylpropanolamine hydrochloride, 18 mg.
Elixir A	120	120	122	—
Elixir B	150	159	155	Phenylephrine hydrochloride, 2.5 mg. Dextromethorphan hydrobromide, 7.5 mg. Glyceryl guaiacolate, 50 mg.

[a] Assay from NF monograph for acetaminophen tablets or acetaminophen elixir, whichever is applicable, was used.

RESULTS AND DISCUSSION

A linear relationship between the peak current measured at the peak potential (approximately 0.5 v.) and the concentration of acetaminophen up to 0.15 mg./ml. was established (Fig. 3). Thus, it was concluded that this technique could be a useful quantitative measure of acetaminophen if it also was able to distinguish electrochemically between the intact drug and *p*-aminophenol, the major hydrolytic degradation product. Figure 1 illustrates the polarographic response obtained from acetaminophen and *p*-aminophenol. One can see that the peak potential of the *p*-aminophenol is well separated from that of acetaminophen, but that there would be a contribution to the current measured at the peak potential of acetaminophen if *p*-aminophenol were also present in the system. Since one is dealing here with a planar stationary electrode and an unstirred solution, one obtains a decay of the *p*-aminophenol current with time. This decay is, of course, diffusion controlled but, unfortunately, it was found that no simple theoretical relationship such as $i \propto 1/t^{1/2}$ was followed. Consequently, the basis for correcting for the current contributed by *p*-aminophenol at the peak potential for acetaminophen had to be developed empirically. By running different known concentrations of *p*-aminophenol and measuring the percent of current at the peak potential for acetaminophen near 0.5 v., it was determined that 0.7 times the peak current for *p*-aminophenol was contributed to the current at the peak potential for acetaminophen.

Table VI—Influence of Water on the Measured Peak Current for Acetaminophen

Percent Water in Supporting Electrolyte	Microamperes Measured
2	5.44
6	5.48
10	5.36
22	4.58

To check on the reliability of this correction factor, a number of solutions containing varying amounts of acetaminophen and *p*-aminophenol were run. The results of this experiment are summarized in Table I. It is seen that in those samples where acetaminophen is the predominant species, accurate values can be obtained. From a practical point of view, this is not a very limiting shortcoming of the method since one is almost always analyzing samples with less than 10% or no more than 20% degradation.

To determine the general applicability of the technique to dosage form analysis, studies were run on acetaminophen in combination with other drugs, common tablet excipients, and common components of liquid preparations. In these studies, 50 mg. of acetaminophen was combined separately with each component under study. The amount of each component was chosen to represent that level normally found in a formulation. Polarograms were obtained on these mixtures as well as on the component under investigation without any acetaminophen present. The results of this work are listed in Tables II–IV. Ascorbic acid was the only interfering component found.

The relative standard deviation of this technique for the analysis of solid dosage forms was found to be 0.5%; for liquid dosage forms it was 0.7%. This peak polarographic technique gives results that are in good agreement with those obtained by the NF XIII (13) method on commercial samples (Table V).

It should be noted that the amount of water present in the supporting electrolyte influences the magnitude of the peak current for acetaminophen. If the water content is increased over a certain level, it decreases the measured peak current for a given concentration of acetaminophen. This is demonstrated in Table VI. A maximum water concentration of 5% in the supporting electrolyte is recommended.

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Stability-Indicating Method for Analysis of Homatropine Methylbromide in Pharmaceutical Formulations

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Abstract □ A stability-indicating method of analysis for homatropine methylbromide in pharmaceutical formulations containing antacids was developed. The method is based upon the extraction of homatropine methylbromide with methanolic hydrochloric acid, followed by reaction with a modified Dragendorff reagent. The iodobismuthate complex is measured spectrophotometrically in a stabilized methanol–acetone solution at 382 nm. A homatropine methylbromide degradation product was isolated from these formulations and identified as tropine methylbromide. The Dragendorff method of analysis was found to be selective for homatropine methylbromide in that there was no interference from its major decomposition product.

Keyphrases □ Homatropine methylbromide formulations with antacids—extraction, iodobismuthate (modified Dragendorff reagent) complexation, UV analysis, compared to silver nitrate titration □ Dragendorff reagent, modified—determination of homatropine methylbromide in formulations with antacids □ UV spectrophotometry—analysis, homatropine methylbromide, Dragendorff reagent complex

A specific method was desired for analyzing homatropine methylbromide in two capsule formulations containing antacids along with other excipients. Because some problems had been encountered with a relatively nonspecific silver nitrate titration for samples stored under accelerated conditions, experimental work was initiated to develop an alternative method of analysis for homatropine methylbromide in these formulations.

The literature revealed that many researchers have investigated methods for analyzing mandelic acid esters of tropine compounds. Durick *et al.* (1) estimated some tropine alkaloids, including homatropine methylbromide, using an acid–dye procedure with bromcresol purple. Our investigations showed that the method yielded erratic results because it was very sensitive to even slight variations in the acid strength of the analytical solutions. The colorimetric determination with ammonium reineckate (2) and the UV estimations of the oxidation reaction products from cerimetric measurements (3, 4) had to be rejected due to interferences from the formulations. A colorimetric procedure was used successfully by Patel and Lemberger (5) to study the kinetics of the hydrolysis of homatropine methylbromide; however, their procedure could not be used to evaluate the stability of the alkaloid because of difficulties encountered in separating degradation products prior to color development. The phosphomolybdic acid reaction (6) has been used to analyze homatropine