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Simultaneous Determination of 4-Nitroanisole, 4-Nitrophenol, and 4-Nitrocatechol by Phase-Sensitive ac Polarography

H. BURGSCHAT and K. J. NETTER x

Abstract □ Phase-sensitive ac polarography was applied to the simultaneous quantitative determination of 4-nitroanisole, 4-nitrophenol, and 4-nitrocatechol in alkaline solutions. Certain experimental precautions are necessary to determine each compound in the presence of the other two. Thus, 4-nitrocatechol is determined indirectly by forming a yellow ratio chelate with cupric ions, whereas 4-nitroanisole is determined directly by the reduction waves of the nitro group. For the determination of 4-nitrophenol, the interference by the simultaneously present 4-nitrocatechol must be eliminated by masking it by the addition of magnesium ions. The method described permits a qualitative and quantitative analysis of all three compounds in one solution since linear calibration curves are obtained.

Keyphrases ■ 4-Nitroanisole—polarographic analysis in presence of 4-nitrophenol and 4-nitrocatechol, alkaline solutions ■ 4-Nitrophenol—polarographic analysis in presence of 4-nitroanisole and 4-nitrocatechol, alkaline solutions ■ 4-Nitrocatechol—polarographic analysis in presence of 4-nitroanisole and 4-nitrocatechol, alkaline solutions □ Polarography—simultaneous analyses, 4-nitrophenol, 4-nitroanisole, and 4-nitrocatechol, alkaline solutions

Polarography is a common procedure in the analysis of aromatic nitro compounds of different character (1–3), because these compounds are easily reduced at the dropping mercury electrode. The mechanism of the electrode reaction was studied in aqueous solutions (4), and the mechanism of nitro derivative reduction was investigated in aprotic solvents (5, 6). More detailed studies with nitrophenol derivatives also were reported (7, 8).

From these studies, it follows that the nitro compounds can be quantitatively determined when analyzed independently. However, conditions become more complex when several nitro compounds are present. As expected (9), the peak potentials of such compounds are so close to each other that their peaks often overlap, and it is difficult to obtain qualitative and quantitative results without prior separation. Because of the inaccuracy of the results obtained by dc polarography (9), use of phase-sensitive ac polarography was suggested (10). This method allows a better interpretation of neighboring peaks. The logarithmic analysis for overlapping waves (11) is not feasible routinely.

In drug metabolism studies, 4-nitroanisole (I) was used as a model drug for demonstrating oxidative *O*-demethylation activity (12). More recently, the production of 4-nitrocatechol (III) was observed in microsomal suspensions after incubations of I or its main metabolic product, 4-nitrophenol (II) (13, 14). Compound III has an absorption maximum slightly different from that of II, the formation of which is usually recorded spectrophotometrically for 5–10 min at pH 7.85 as a routine kinetic method (15) to measure the initial velocity of the oxidative *O*-demethylation of I to II. Thus, III might interfere with the spectral recording of II formation during prolonged incubations[1].

Pharmacologically, it is desirable to determine these three compounds with the greatest possible simplicity and rapidity. The described experiments resulted in the development of a rapid ac polarographic method, allowing the simultaneous determination of these compounds[2]. In this connection, special emphasis is placed on the effects of pH variation in the polarographic base solution.

EXPERIMENTAL

Measurements were carried out using a phase-sensitive ac polarograph, as described previously (16), with an additional phase-sensitive rectifier.

An internal low frequency oscillator creates a frequency of 12 Hz, which is superimposed on the polarographic cell potential. The variable phase of the phase-sensitive rectifier was adjusted so that the capacitance current was largely eliminated. To avoid beat phenomena, a synchronization of the increase in drop size with the alternating current was necessary. Therefore, the internal low frequency was applied to the drop controller in the electrode stand[3]. The amplitude of the alternating current was 30 mv for all experiments.

The polarograms were recorded with an *xy*-recorder[4] after bubbling purified nitrogen through the solution for 2.5 min. The flow rate of mercury dropping into 0.1 *N* NaOH at a potential of −1.0 v was 3.24 mg/sec, and the drop time was 2.72 sec. With automatic drop control, it amounted to 0.69 sec at a reservoir height of 67 cm. All measurements were recorded *versus* a normal calomel electrode at 21 ± 1°. A commercially available pH meter[5] with a glass electrode was used after calibration

[1] P. Bergheim and K. J. Netter, unpublished results.
[2] The application of the polarographic determination to kinetic analyses in biological samples will be described later.
[3] Metrohm E 354, Herisau, Switzerland.
[4] Metrawatt AG, Nürnberg, Germany.
[5] Knick, Berlin, Germany.

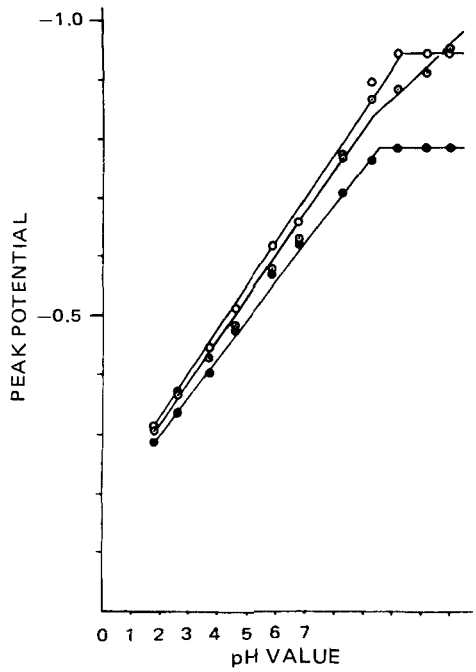

Figure 1—*The pH dependency of the peak potentials of I (●), II (○), and III (⊙). Concentration = 20 μM in Britton–Robinson buffer.*

with standard buffers[6]. All solvents and substances were of analytical grade and were not further purified.

RESULTS AND DISCUSSION

pH Dependency—Figure 1 shows the dependence of the peak potentials of I–III in Britton–Robinson buffer (17). The peak potentials exhibited a linear shift to more negative values with increasing pH; but at pH 9, the peak potentials for I and II became independent of pH. Similar results were obtained previously for nitroaniline (4). For III, however, the pH dependency persisted above pH 9 but showed a smaller increase. The shifts for the peak potentials per pH unit for I and II were 62 and 72 mv, respectively; for III, values of 68 mv below pH 9 and of 46 mv above pH 9 were found.

Further alkalinization above pH 12 by using 0.1 *N* NaOH instead of the buffer led to a further increase of the peak potential of III while the other two compounds showed a plateau. The values at pH 13 were −780 mv for I, −940 mv for II, and −1000 mv for III, showing that the second part of the curve for III in Fig. 1 was continued by 45 mv/pH unit. Therefore, 0.1 *N* NaOH (pH 13) was selected as a suitable medium for the quantitative and qualitative analyses of all three compounds.

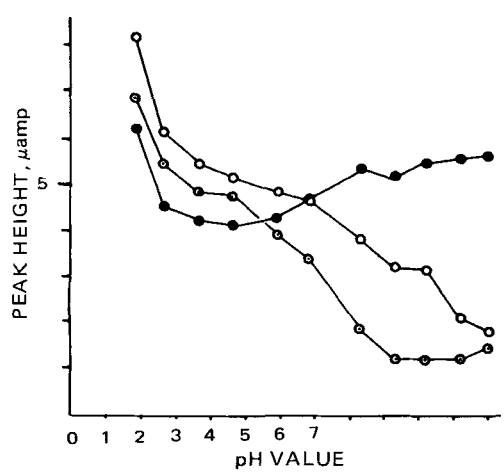

Figure 2—*The pH dependency of the peak heights of I (●), II (○), and III (⊙). Conditions were as in Fig. 1.*

[6] Merck, Darmstadt, Germany.

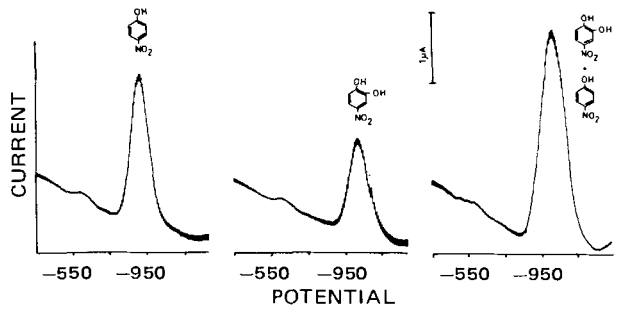

Figure 3—*Polarograms of II and III in 0.1 N NaOH. Key: left, polarogram of II (20 μM); middle, polarogram of III (20 μM); and right, polarogram of both phenols (each 20 μM).*

Not only the position of the peak potentials but also peak heights were influenced by an increase in pH. As shown in Fig. 2, the peak heights of II and III decreased with increasing pH. After an initial decrease, the peak height of I showed a slight increase above pH 4.5, which continued roughly until pH 12. In this region, it again reached the starting peak height of about pH 2.

The data in Fig. 1 show that, in the alkaline solution, I had a peak potential sufficiently different (about 160 mv) to allow a separate determination. The two phenols, however, did not possess peak potentials sufficiently apart from each other (Fig. 3), a fact that necessitated other means of determination. The left and middle polarograms of Fig. 3 represent the peaks of II and III in the base solution; the right one shows the effect of mixing both II and III. The result was a clear superposition of both peaks. The peak potentials and peak heights shown in the figures represent only the first observed reduction step of the nitro groups on the electrode surface. For II and III, this step was followed in alkaline solutions by a second reduction step in a more negative potential region located closely to the reduction of the sodium ion.

The second peaks (about −1.5 v) could not be quantitatively evaluated because of a lack of proportionality with concentration. This separation into two peaks, however, points to a different electrode mechanism for II and III in contrast to I, which did not show a reduction in peak height in alkaline solutions. This behavior is interpreted as representing two or four consecutive electron-transferring steps in II and III reduction (8, 18); in I, the transfer of all six electrons occurs in one step. Consequently, there is no second peak for I. For pH values below 7, however, all three substances seemed to be reduced by the same or a very similar mechanism.

Determination of II and III—*Indirect Determination of III in Presence of II*—In addition to the direct reduction of the nitro group, III can also indirectly be determined by utilizing the neighboring hydroxyl groups by the formation of a metal chelate (19–21).

Studies with a number of cations, *e.g.*, iron, copper, zinc, tin, cadmium, germanium, boron, aluminum, and magnesium, showed that only cupric and magnesium ions provided usable results in alkaline solution. On addition of cupric ions to the base solution containing III, the red color of III changed to yellow and a new peak at −540 mv appeared in the polarogram (Fig. 4, right). It was not obtained with cupric ions alone in the

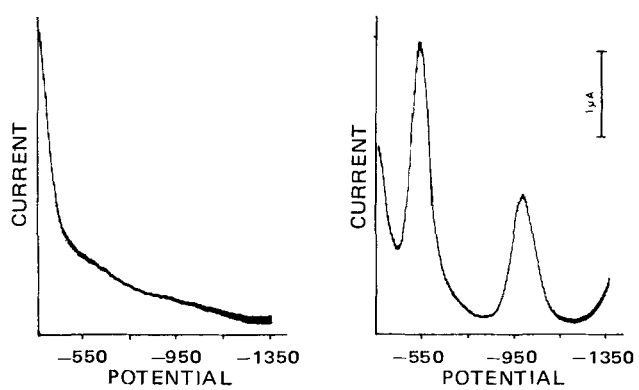

Figure 4—*Polarograms of III. Key: left, base current in 0.1 N NaOH in the presence of 5 μl of 1% $CuSO_4$ (final concentration of 40 μM); and right, polarogram of III (20 μM) under the same conditions. The peak at −540 mv represents chelate reduction, and the peak at −940 mv represents the first reduction step of the nitro group.*

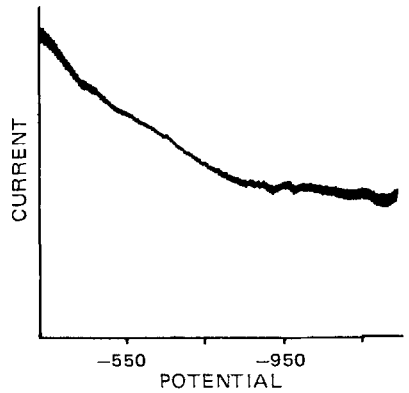

Figure 5—*Masking of III; polarogram after injection of 200 μl of 0.1 M $MgCl_2$ into the base solution containing III (20 μM).*

absence of III (Fig. 4, left). The reduction peaks of the nitro group appeared at the same potentials as mentioned previously.

Further experimentation revealed that only 0.5 mmole of cupric ions was necessary for the determination of 1 mmole of III, indicating that a 2:1 chelate had been formed. An increase in the cupric-ion concentration above this ratio had no effect on the peak height of III. Since it seemed desirable to have an excess of cupric ions, 5 μl of 1% $CuSO_4$ was added to 5 ml of the base solution (final concentration of 40 μ*M*). The relationship between the concentration of III and the respective peak heights of the "chelate peak" at −540 mv under these conditions was linear. The calibration curve was not influenced by the addition of I and II. A similar chelate with catechol and cupric ions was used for an indirect determination of catechol by atomic absorption spectrophotometry (21).

Determination of II in Presence of III—Magnesium ions showed an effect with III opposite to that of cupric ions. The addition of magnesium ions to the solution containing only III (Fig. 4, right) abolished the peaks of the chelate and of the nitro group reduction (Fig. 5). At the same time, the red color of III reappeared. In this case, the magnesium ion displaced the cupric ion in the chelate to form a more stable complex, which gave no polarographic signal in 0.1 *N* NaOH. The behavior of II in the presence of III is shown in Fig. 6. In the left polarogram, both phenols were present in a concentration of 20 μ*M*, each in 5 ml of base solution with 5 μl of 1% $CuSO_4$. The second peak at about −960 mv constitutes an overlapping mixture of the currents of two peaks as in Fig. 3 (right).

After adding 200 μl of 0.1 *M* $MgCl_2$ (Fig. 6, right), only the peak of II at −940 mv remained; the contribution by III disappeared. Experiments made with II alone under the same conditions in a volume of 5.2 ml (5.0 ml of 0.1 *N* NaOH and 0.2 ml of 0.1 *M* $MgCl_2$) gave a linear relationship between concentration and peak height. It is possible to determine II after "masking" III by the addition of magnesium ions.

Determination of I—As can be seen from Fig. 1, the peak potential of I in alkali was sufficiently different from those of the two phenols when measured in the absence of cupric ions. In their presence, there was no

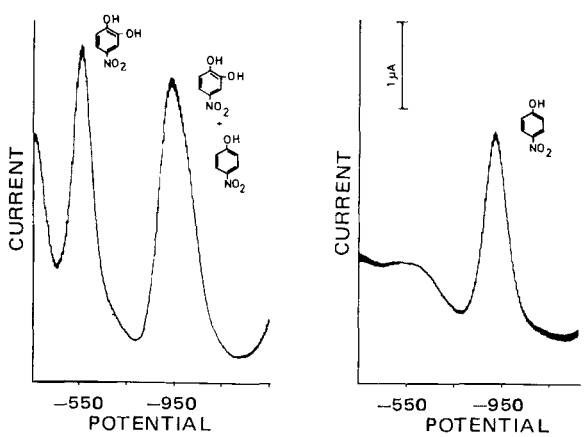

Figure 6—*Masking of III in the presence of II. Key: left, polarogram of both phenols (20 μM) in 0.1 N NaOH in the presence of 40 μM $CuSO_4$; and right, repeat polarogram of the same solution after addition of 200 μl of 0.1 M $MgCl_2$ (final volume of 5.2 ml). Only the peak of II remains in the right polarogram.*

Table I—Simultaneous Determination of I–III

Compound	Concentration Calculated, *M*	Concentration Found, *M*	Recovery, %
Concentration of I Was Varied and Concentrations of II and III Remained Constant			
I	2×10^{-6}	2.1×10^{-6}	105.0
II	2×10^{-5}	2×10^{-5}	100.0
III	2×10^{-5}	1.94×10^{-5}	97.0
I	4×10^{-6}	3.8×10^{-6}	95.0
II	2×10^{-5}	2×10^{-5}	100.0
III	2×10^{-5}	1.96×10^{-5}	98.0
I	6×10^{-6}	5.9×10^{-6}	98.3
II	2×10^{-5}	2×10^{-5}	100.0
III	2×10^{-5}	2.03×10^{-5}	101.5
I	8×10^{-6}	8.2×10^{-6}	102.5
II	2×10^{-5}	2.06×10^{-5}	103.0
III	2×10^{-5}	1.97×10^{-5}	98.5
I	1×10^{-6}	0.98×10^{-6}	98.0
II	2×10^{-5}	2.04×10^{-5}	102.0
III	2×10^{-5}	1.96×10^{-5}	98.0
Concentration of II Was Varied and Concentrations of I and III Remained Constant			
I	1×10^{-5}	0.97×10^{-5}	97.0
II	4×10^{-6}	4×10^{-6}	100.0
III	2×10^{-5}	1.94×10^{-5}	97.0
I	1×10^{-5}	0.98×10^{-5}	98.0
II	8×10^{-6}	7.8×10^{-6}	97.5
III	2×10^{-5}	2.02×10^{-5}	101.0
I	1×10^{-5}	0.97×10^{-5}	97.0
II	1.2×10^{-5}	1.18×10^{-5}	98.3
III	2×10^{-5}	1.96×10^{-5}	98.0
I	1×10^{-5}	0.98×10^{-5}	98.0
II	1.6×10^{-5}	1.6×10^{-5}	100.0
III	2×10^{-5}	1.98×10^{-5}	99.0
I	1×10^{-5}	0.99×10^{-5}	99.0
II	2×10^{-5}	1.95×10^{-5}	97.5
III	2×10^{-5}	2×10^{-5}	100.0
Concentration of III Was Varied and Concentrations of I and II Remained Constant			
I	1×10^{-5}	1.04×10^{-5}	104.0
II	2×10^{-5}	2.0×10^{-5}	100.0
III	4×10^{-6}	3.9×10^{-6}	97.5
I	1×10^{-5}	0.98×10^{-5}	98.0
II	2×10^{-5}	1.97×10^{-5}	98.5
III	8×10^{-6}	8.0×10^{-6}	100.0
I	1×10^{-5}	1×10^{-5}	100.0
II	2×10^{-5}	1.95×10^{-5}	97.5
III	1.2×10^{-5}	1.2×10^{-5}	100.0
I	1×10^{-5}	0.96×10^{-5}	96.4
II	2×10^{-5}	2.06×10^{-5}	103.0
III	1.6×10^{-5}	1.62×10^{-5}	101.25
I	1×10^{-5}	0.96×10^{-5}	96.4
II	2×10^{-5}	2.06×10^{-5}	103.0
III	2×10^{-5}	2.08×10^{-5}	104.0

interference from the newly formed chelate peak at −540 mv. The potential differences at pH 13 were 240 and 160 mv, as illustrated (in the presence of cupric ions) in Fig. 7 (left). The addition of magnesium ions did not affect the peak of I (Fig. 7, right), thereby allowing the quantitative evaluation of I in the presence of both II and III. Cupric ions, which are necessary for the routine estimation of III, had no influence on the peak height of I. Also, the subsequent addition of magnesium ions had only a negligible effect on the peak height of I.

Simultaneous Determination of I–III—In summary, the procedure for the polarographic estimation of the three nitro compounds is as follows. The compounds (0–250 nmoles) must be dissolved in 5 ml of 0.1 *N* NaOH in the polarographic vessel. Then 5 μl of 1% $CuSO_4$ in water is added by micropipet, and the vessel is gassed with purified nitrogen for 2.5 min. Recording of the polarogram yields curves of the type shown in Fig. 7 (left). The peak heights of these curves over the base current in

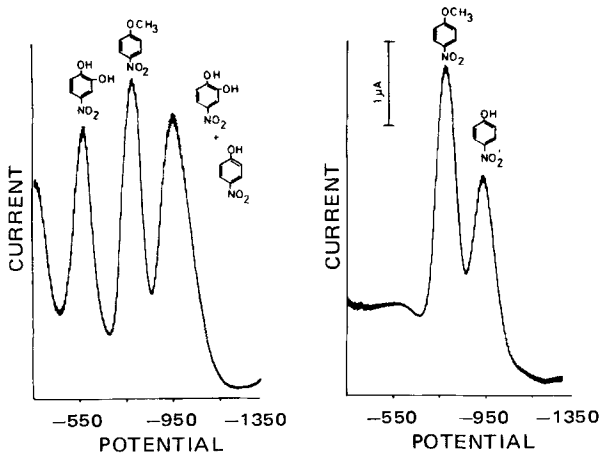

Figure 7—*Polarogram of I (10 μM), II (20 μM), and III (20 μM) in the presence of 40 μM $CuSO_4$. Key: left, before addition of magnesium ions; and right, after addition of magnesium ions.*

conjunction with calibration curves give direct measurements of III and I.

To determine II also, 200 μl of 0.1 *M* $MgCl_2$ is added to the same solution. Then this solution is bubbled again with nitrogen to achieve proper mixing and removal of the oxygen carried in during the manipulation. The newly taken polarogram (Fig. 7, right) shows peaks only for I and II, from which the concentration of II can be obtained by subtracting the base current. Theoretically, a second determination of I is possible from this second polarogram.

The accuracy of this method of determination for each substance in the presence of varying concentrations of the others is shown by the homogeneous and practically complete recovery rates given in Table I.

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Sustained-Release Applications of Montmorillonite Interaction with Amphetamine Sulfate

JAMES W. McGINITY [x] and JOHN L. LACH

Abstract □ Urinary recovery studies showed that montmorillonite significantly affects the initial therapeutic levels of amphetamine sulfate. The combination of a 1:20 drug–montmorillonite complex with pure drug in a 1:1 ratio, based on amphetamine content, resulted in recovery profiles resembling those obtained from prolonged-release dosage forms. The 1:20 complex, pure drug, and combination formulations showed comparable bioavailability after 48 hr.

Keyphrases □ Montmorillonite—effect on bioavailability of amphetamine sulfate, urinary recovery studies, applied to sustained-release dosage form □ Amphetamine sulfate—bioavailability, effect of montmorillonite, urinary recovery studies, applied to sustained-release dosage forms □ Urinary recovery studies—effect of montmorillonite on bioavailability of amphetamine sulfate, applied to sustained-release dosage form □ Dosage forms—sustained-release drug–clay complex, effect of montmorillonite on bioavailability of amphetamine sulfate □ Clays—montmorillonite, complex with amphetamine sulfate, effect on bioavailability □ Stimulants—amphetamine sulfate, effect of complexing with montmorillonite on bioavailability

The availability of a drug from a dosage form is dependent on both the rate and the completeness with which the drug is delivered to the absorption site in an absorbable form. In a recent review (1), excipients in solid dosage forms were shown to interact with various medicinals. Such drug–excipient interactions generally influence both the rate of drug absorption and the amount delivered unchanged to the general circulation.

An earlier publication (2) reported that cationic salts