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### Determination of Carbonyl 2,4-Dinitrophenylhydrazones by Liquid Chromatography/Electrochemistry

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DETERMINATION OF CARBONYL 2,4-DINITROPHENYLHYDRAZONES  
BY LIQUID CHROMATOGRAPHY/ELECTROCHEMISTRY

W. A. Jacobs and P. T. Kissinger

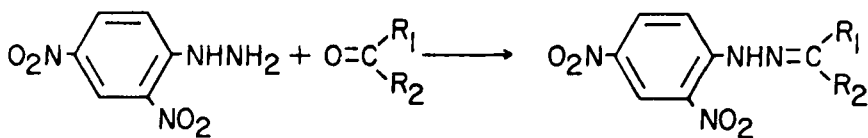
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ABSTRACT

Reductive mode liquid chromatography/electrochemistry was evaluated for use in quantitating 2,4-dinitrophenylhydrazine derivatives of carbonyls. Optimum performance was achieved at an operating potential of  $-0.75\text{V}$  vs  $\text{Ag}/\text{AgCl}$ . Detection limits for DNPH-formaldehyde, acetaldehyde, acetone, and acrolein were 54, 80, 76, and 99 pg respectively. These detection limits were approximately 20 times lower than obtainable with UV absorbance at 254 nm.

INTRODUCTION

The reaction of aldehydes and ketones with 2,4-dinitrophenylhydrazine (DNPH; equation 1) has long formed the basis of



colorimetric methods for determining carbonyl content (1). More recently the use of liquid chromatography to resolve the individual derivatives followed by absorbance detection has become very popular (2-8). This approach is particularly advantageous in allowing pre-chromatographic concentration of airborne carbonyls by trapping DNPH derivatives, thereby facilitating detection at low concentrations. Selectivity is also enhanced since the reaction is quite specific for the carbonyl functionality. A further benefit is that the derivatization stabilizes otherwise volatile and often reactive compounds.

The potential utility of nitroaromatics as derivatization labels for liquid chromatography/electrochemistry (LCEC) has been discussed previously (9,10). Such an approach has already been shown to be applicable to the determination of amino acids in brain tissue (11). The purpose of this communication is to demonstrate the utility of LCEC for carbonyl determinations using DNPH derivatization. The superior sensitivity of EC detection is expected to provide advantages, including decreased detection limits and/or less restrictive sampling requirements.

### EXPERIMENTAL

Chemicals: All chemicals were ACS Reagent grade or better. Tetrahydrofuran (stabilized) and n-propanol were from Mallinckrodt (St. Louis, MO). Acetonitrile was from J. T. Baker (Phillipsburg, NJ). Ammonium Acetate, perchloric acid and dimethylsulfoxide were from Fisher Scientific (Pittsburg, PA). Acetone, acrolein, acetaldehyde, and formaldehyde (37 wt. % solution) were all from Aldrich Chemical Co. (Milwaukee, WI).

Derivative Formation: Standard derivatives were prepared by precipitation from DNPH/Perchlorate (1.2g DNPH in 100 mL of 30% Perchloric acid) and purified by repeated recrystallization from 95% ethanol.

Liquid Chromatography: All separations were carried out using a Bioanalytical Systems (West Lafayette, IN) LC-304 liquid chromatograph modified to allow continuous deoxygenation of the mobile phase, and of samples prior to injection (12). The amperometric transducer was a model TL-5A glassy carbon cell (BAS). The system was equipped with a Biophase octyl column, 0.46 x 25 cm, 5  $\mu$ m particle size (BAS). The mobile phase consisted of (by volume) 20% acetonitrile, 20% tetrahydrofuran, 10% n-propanol, and 50% 0.1M ammonium acetate, pH 5.0. Derivatives were dissolved in DMSO for injection onto the chromatograph.

For LCUV experiments, an LC-6 254 nm fixed wavelength UV detector was used (BAS).

## RESULTS AND DISCUSSION

### Voltammetry of DNPH-Derivatives

Hydrodynamic voltammograms (HDV's) of DNPH-formaldehyde, acetaldehyde, and acetone are shown in Figure 1. The derivative

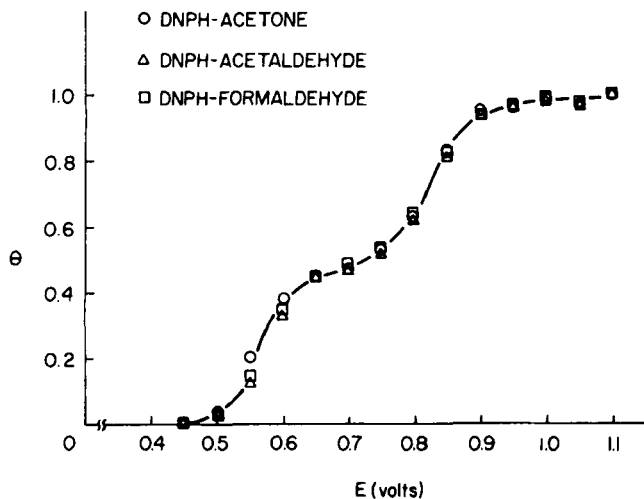


FIGURE 1. Hydrodynamic Voltammograms (HDV's) of Selected Carbonyl Derivatives.  $\theta$  is the normalized response,  $\theta_E = i_E/i_{E,1}$ .

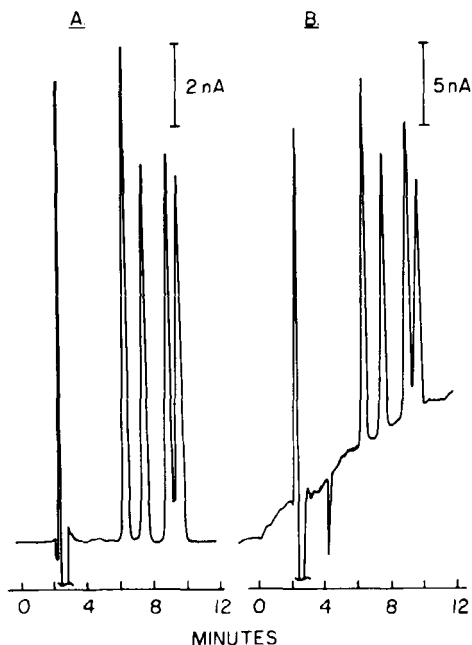
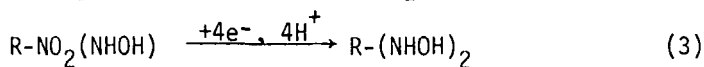
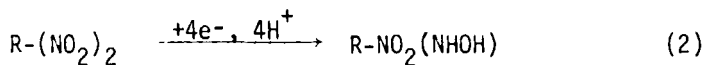


FIGURE 2. Chromatograms of Carbonyl Derivatives at A.  $-0.75V$  and B.  $-1.00V$  vs  $Ag/AgCl$ . Peaks: 1. DNPH-formaldehyde 2. Acetaldehyde 3. Acetone 4. Acrolein Approximately 2 ng of each derivative.

HDV's reflect the favorable electrochemical properties of these compounds. The voltammetric behavior of all derivatives is virtually identical, each exhibiting two equivalent reductive waves. The observed waves are presumed to be due to successive four electron reductions of the nitro groups to hydroxylamines (equations 2 and 3). This would be in agreement with the



classically observed electrochemistry of nitroaromatics (13).

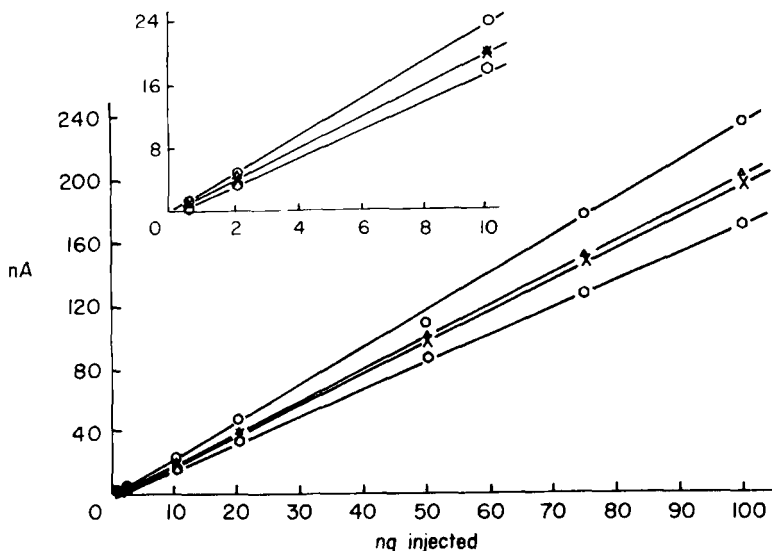


FIGURE 3. Calibration curves for Carbonyl Derivatives

- DNPH - Acrolein,  $r = 0.999$
- × DNPH - Acetaldehyde,  $r = 0.999$
- △ DNPH - Acetone,  $r = 0.999$
- DNPH - Formaldehyde,  $r = 1.000$

### Selection of Applied Potential for LCEC

In selecting the optimum detector potential, it is desirable to maximize current response to analytes of interest while minimizing background noise. In the case of the DNPH carbonyls an applied potential of  $-0.75$  volts (on wave 1,  $n = 4$ ) was found to be superior to  $-1.00$  volts (on wave 2,  $n = 8$ ) even though the absolute current response was smaller. Increased noise and baseline drift at the higher potential more than offset the gain in absolute response (Figure 2).

### Detector Linearity and Sensitivity

Typical calibration curves for DNPH-formaldehyde, acetaldehyde, acetone, and acrolein are shown in Figure 3.

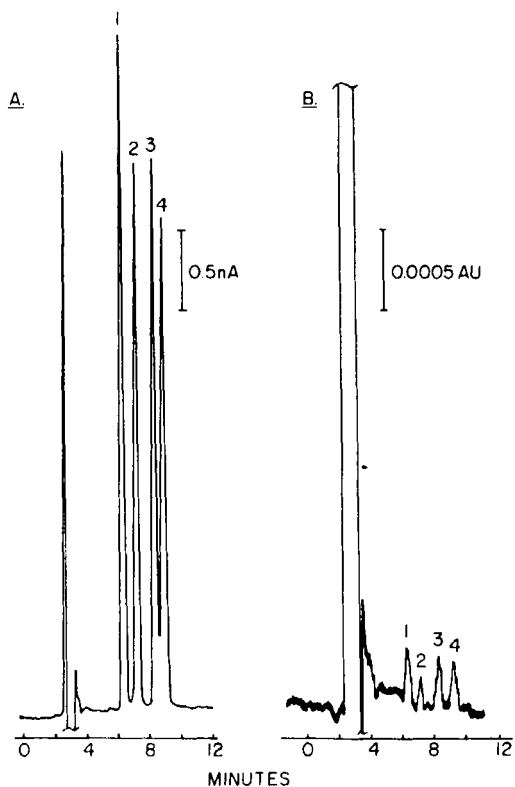


FIGURE 4. Chromatograms using A. LCEC at  $-0.75\text{V}$  and B. LCUV at  $254\text{ nm}$ .

TABLE 1

Detection Limits for Carbonyl 2,4-Dinitrophenylhydrazones

	LCEC*	LCUV <sup>†</sup>
Formaldehyde	54pg	10ng(5), 1.1ng(4), 1.3ng <sup>1</sup>
Acetaldehyde	80pg	10ng(5), 2.0ng(4), 2.0ng <sup>1</sup>
Acetone	76pg	10ng(5), 1.4ng <sup>1</sup>
Acrolein	88pg	10ng(5), 1.5ng <sup>1</sup>

\* S/N = 4

<sup>†</sup> references in parentheses

<sup>1</sup> this work S/N = 4

Excellent linearity was obtained in the range from 100 pg to 100 ng injected for each of the derivatives. Corresponding detection limits are given in Table 1. Detection limits reported by LCUV are also listed for comparison. Electrochemistry is at least 20-30 times more sensitive than absorbance in all cases. This is clearly illustrated in Figure 4 which shows chromatograms obtained in this laboratory using EC and UV detectors in series (UV first) for 2 ng of each derivative.

As shown here, LCEC provides a viable alternative to LCUV for quantitating carbonyl 2,4-dinitrophenylhydrazones. The significant gain in sensitivity using LCEC results in substantially lowered detection limits. This should allow improvement of existing methods which utilize DNPH derivatization. In particular, the use of electrochemical detection should be useful for determinations of low level airborne carbonyls where long sampling times (typ 1-2 hrs) are currently required.

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