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Note

Effect of pH on the reaction of 2,4-dinitrophenylhydrazine with formaldehyde and acetaldehyde*

MERLIN K. L. BICKING*** and W. MARCUS COOKE

Battelle Columbus Division, 505 King Avenue, Columbus, OH 43201 (U.S.A.)

and

FRED K. KAWAHARA and JAMES E. LONGBOTTOM

U.S. Environmental Protection Agency, 26 West St. Clair Street, Cincinnati, OH 45268 (U.S.A.)

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The acid-catalyzed condensation reaction of a molecule of 2,4-dinitrophenylhydrazine (DNPH) with a carbonyl compound is a well known reaction for characterizing aldehydes and ketones^{1,2}. The DNPH derivatives are used to identify qualitatively the parent carbonyl compound by melting point determination. Gram quantities of the derivatives are easily prepared by well-established procedures. The derivatization is also useful as a quantitative analytical procedure. Numerous applications have been reported using either gas chromatography (GC) with flame ionization^{3–6} or electron-capture⁷ detection, or high-performance liquid chromatography (HPLC) with absorbance^{8–13} or electrochemical^{14,15} detection. Detection limits are usually in the low $\mu\text{g/l}$ range for the more sensitive detectors. The GC methods have not found widespread acceptance because of the low volatility of the derivative, the relative insensitivity of the flame ionization detector, and the response variations of the electron capture detector. Similar response variations are common for the electrochemical detector.

The reaction is known to proceed by a multi-step mechanism^{16–18}, where the rate-limiting step involves the addition of reagent to the protonated carbonyl moiety. Rapid dehydration occurs to yield the hydrazone. The rate of reaction has been shown to be a function of several experimental variables: reaction pH, the concentration of acid used as catalyst, carbonyl compound concentration, and reagent concentration. At low pH, the carbonyl group is activated toward nucleophilic addition by the acid, but the amount of free nucleophile present is reduced because of its basicity. At higher pH values, typically in basic solution, more free reagent is available but the carbonyl group is less reactive. Because of these competing effects, the rate passes through a maximum at a pH which is characteristic of the basicity of the nucleophilic reagent¹⁶.

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** Present address: Twin City Testing Co., 662 Cromwell Avenue, St. Paul, MN 55114, U.S.A.

Under the conditions commonly employed in conventional analytical derivatization reactions, the reagent and buffer are present in at least ten-fold molar excess. The rate should then be a function only of acidity and carbonyl compound concentration. However, there are no reports in the literature to document these trends under conditions of interest to analytical chemists. Furthermore, the resulting yield of the reaction as a function of pH has not been reported. Based on published procedures^{8-10,19}. It appears that most analytical derivatizations with DNPH are performed at a pH in the range 0-2.

The effect of pH on reaction yield was studied here to ascertain whether the derivatization could be performed under less acidic conditions. Derivatizations of environmental samples should be performed under mild acid conditions to minimize generation of formaldehyde from matrix precursors, since numerous environmentally occurring species have been shown to generate formaldehyde under a variety of conditions²⁰. In general, mild acid conditions are preferred or a false positive formaldehyde result may be obtained.

The objective of this study was to determine if a quantitative derivatization could be performed at a reasonable rate under mild conditions which minimized the possibility of a matrix false positive. The results of this study indicate that the derivatization can indeed be performed in high yield at a pH as high as 5.

EXPERIMENTAL

Reagents and solutions

The following reagents were reagent grade and used as received: 37% formaldehyde (Baker); 2,4-dinitrophenylhydrazine (Aldrich), containing approximately 30% (w/w) water; acetaldehyde (Aldrich); phosphoric acid (Baker); glacial acetic acid (Baker); and sodium hydroxide (Baker). All solvents were distilled-in-glass or equivalent: methanol, ethanol, methylene chloride, and reagent water. DNPH derivatives of formaldehyde and acetaldehyde were prepared in gram quantities according to published procedures².

A 1000 mg/l stock solution of formaldehyde and acetaldehyde in reagent water was prepared from formalin and neat acetaldehyde. The formaldehyde concentration was determined by standard titrimetric procedures using sodium sulfite and hydrochloric acid. Individual 1 mg/l solutions of formaldehyde and acetaldehyde were prepared by dilution of this stock solution with reagent water. A 5 M phosphoric acid solution in reagent water was prepared from the concentrated acid. The DNPH reagent was prepared by dissolving 143 mg of the 70% (w/w) solid in absolute ethanol. Gentle warming was required to dissolve the solid. A 5 M sodium hydroxide solution was prepared in reagent water from the solid. Solutions of synthesized derivatives were prepared in methanol.

Equipment

Laboratory glassware included 500-ml separatory funnels equipped with PTFE stopcocks, 500-ml erlenmeyer flasks equipped with standard taper joints, and a Kuderna-Danish apparatus, including a 25-ml concentrator tube, evaporating flask, three-ball macro-Snyder column, and a two-ball micro-Snyder column. Volumetric flasks were 25 ml. The pH meter used (Orion Research) was capable of measuring solution pH in increments of 0.02 units.

Chromatographic apparatus and operating conditions

The LC system consisted of an Altex 110A pump, a 250 × 4.6 mm Zorbax ODS column, and an LDC Spectromonitor III detector. The mobile phase was methanol-water (75:25), injection volume was 20 μ l, and the detector was run at 360 nm.

Procedure

A 250-ml aliquot of reagent water spiked with 1 mg/l of formaldehyde and acetaldehyde was placed in a 500-ml flask and 5 ml of 5 M phosphoric acid was added. This resulted in a solution pH of approximately 1.7. Reaction yields were obtained at solution pH values of 1.7, 3, 4, 5, and 7. Higher pH values were obtained by addition of 5 M sodium hydroxide prior to addition of the derivatizing reagent. The method was as follows: a 10-ml aliquot of a DNPH solution (1 mg/ml) was added to the solution. The flask was capped and allowed to react one hour at room temperature on a wrist-action shaker. The solution was placed in a 500-ml separatory funnel and extracted manually with three 20-ml portions of methylene chloride. The combined organic layers were concentrated to 10 ml using the Kuderna-Danish apparatus. A solvent exchange to methanol was performed near the end of the concentration step. The resulting methanol solution was diluted to 25 ml using a volumetric flask. An aliquot of this solution was analyzed by LC with absorbance detection at 360 nm. Yields were calculated based on injection of independently synthesized derivative standards². All values reported were corrected for the presence of a non-zero laboratory blank.

RESULTS AND DISCUSSION

The yield of the reaction between formaldehyde and DNPH as a function of pH is shown in Fig. 1. The reaction yield data for reaction of formaldehyde and acetaldehyde in reagent water are summarized in Table I. Over the pH range of 1.7 to 7.0, a smooth relationship was observed with a maximum at a solution pH of approximately 4. However, reaction yields did not change significantly over the pH range from three to five. The observed yields followed the same trend as the rate of reaction, with the maximum under mild acid conditions and a sharp decrease at either extreme. The excellent yield under mild acid conditions is surprising, considering that most literature procedures used high acid conditions. However, the results reported here clearly indicated that milder conditions provided excellent yield and would be preferred when derivatizing an acid-sensitive matrix.

Yields were generally about 90% under the conditions employed. Standard deviations were less than 5% in each case. At pH 5, the yield appeared to be independent of formaldehyde concentration.

As illustrated in Table I, the yield did not appear to be a function of type of buffer. The value for acetate at pH 5 was not statistically different from the phosphate value. This is important since the buffering capacity of acetate is high in this region and pH 5 acetate buffers are used for extraction of environmental samples²¹.

The observed yields for acetaldehyde were significantly lower. This was expected due to steric hindrance of the carbonyl moiety. Yields could be increased by performing the derivatization under more rigorous conditions (*i.e.*, higher temperatures or longer times) but this was not attempted during the present investigation since the primary objective was to evaluate the effect of pH only. Acetaldehyde did not

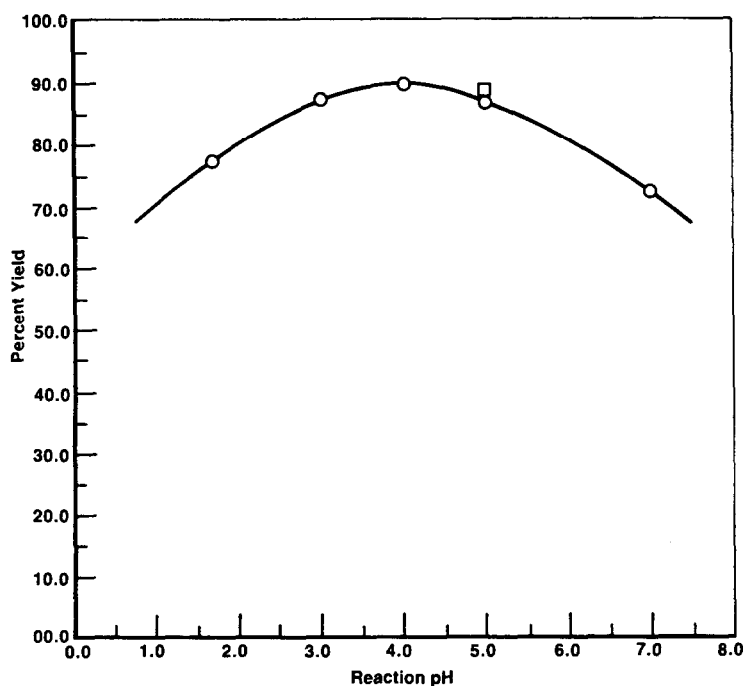


Fig. 1. Effect of reaction pH on percent yield of formaldehyde-DNPH derivative. Symbols: circles, phosphate buffer; square, acetate buffer.

show the same trend in yield as a function of pH, but was relatively constant at 60% in the pH range from 3 to 5. This was probably due to the presence of additional resonance structures in acetaldehyde, which affected the concentration of the reactive form. These additional structures were not present in formaldehyde. Other carbonyl compounds are likely to follow the same trend as acetaldehyde.

TABLE I

RECOVERY OF DNPH DERIVATIVES AS A FUNCTION OF pH

pH	Recovery (%) [*]	
	Formaldehyde	Acetaldehyde
1.7	77.3 (4.1)	64.2 (1.9)
3	87.3 (4.1)	60.0 (7.5)
4	89.6 (1.6)	58.9 (1.4)
5	86.8 (1.3)	57.1 (1.6)
5 ^{**}	88.8 (0.23)	63.0 (0.30)
7	72.0 (8.0)	48.1 (16)

^{*} Spiking levels: 1.02 mg/l formaldehyde, 1.00 mg/l acetaldehyde. Standard deviation ($N=3$) is given in parenthesis.

^{**} Acetate buffer, all other experiments used a phosphate buffer.

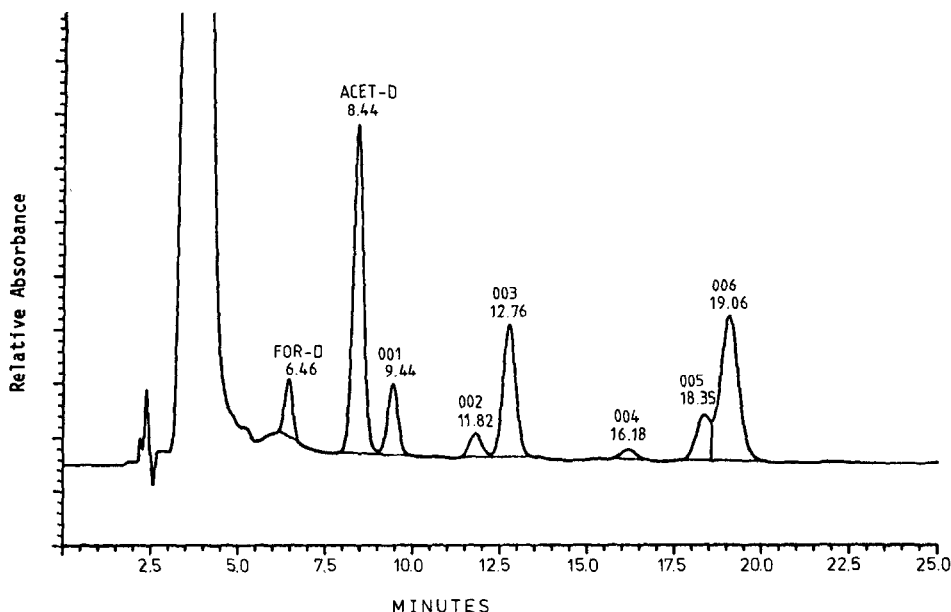


Fig. 2. Representative chromatogram from derivatization of landfill leachate sample using procedure described in Experimental section. Peak identity: FOR-D, formaldehyde derivative; ACET-D, acetaldehyde derivative. Both *syn*- and *anti*-forms are present for acetaldehyde but only the major product is quantified.

This general procedure is readily applicable to direct derivatization and analysis of environmental samples. The result of direct derivatization of an industrial landfill leachate is demonstrated in Fig. 2. A 250 ml aliquot was derivatized using the procedures described in the experimental section, employing a pH 5 acetate buffer in this case. For this matrix, no modifications of the original procedure were necessary. The formaldehyde and acetaldehyde derivatives were readily identified in the chromatogram, based on a comparison of retention times with independently synthesized standards. The identity of the derivatives of formaldehyde and acetaldehyde was confirmed by gas chromatography-mass spectrometry. The formaldehyde derivative peak (6.46 minutes) represented approximately 50 $\mu\text{g/l}$ formaldehyde in the original solution; the larger acetaldehyde derivative peak (8.44 min) represented approximately 120 $\mu\text{g/l}$ acetaldehyde in solution. There were no interferences observed and the derivatives were well separated from the excess DNPH reagent (3.5 min), which eluted near the void volume. Note that two peaks (8.44 min and 9.44 min) were observed for acetaldehyde. These peaks represented two possible isomers, *syn*- and *anti*-, for the derivative product. Geometric isomers were not possible for symmetrical carbonyl compounds, such as formaldehyde, and only a single peak was observed. The remaining peaks in the chromatogram represented low levels of other DNPH derivatives.

CONCLUSIONS

It is possible to achieve high recovery of DNPH derivatives of formaldehyde and acetaldehyde at trace levels under mild derivatization conditions. An acetate buffer at

pH 5 is convenient for this purpose. These data suggest that derivatization of other compounds will also be possible at similar pH values, but some modification of reaction conditions (time, temperature) will be necessary. The combination of solvent extraction followed by absorbance detection at 360 nm provides sufficient selectivity so that no interferences are observed in the chromatogram, even when complex environmental samples are derivatized directly. Additional data regarding the derivatization of other sample types over a range of formaldehyde concentrations will be the subject of a forthcoming manuscript. Subsequent issues to be discussed include the source of non-zero laboratory blanks, emulsion formation, the kinetics of derivatization, and an evaluation of ability of this method to avoid generation of false positives.

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