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DETERMINATION OF PYRUVIC AND GLYOXYLIC ACIDS
IN THE PRESENCE OF ACETALDEHYDE

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July 1976

In the radiolysis of alanine and its derivatives pyruvic acid, glyoxylic acid and acetaldehyde are often formed. These compounds are often measured by trace colorimetric analytical methods.

In one such procedure, carbonyl groups are reacted with 2, 4 dinitrophenyl hydrazine (DNPH) under aqueous acidic conditions. If the solution is then made alkaline, a hydrazone derivative (quinoid) is formed. This chromophore has a characteristic maximum absorption at 450 nm. which follows Beer's law. DNPH concentrations of 0.1 to 1% in dilute aqueous HCl or methanol are usually used. These concentrations give complete reaction of carbonyl groups with DNPH, however, high concentrations of DNPH reagent cause other problems. Some compounds are oxidized by DNPH. For example, amino-carbonyl compounds are oxidized to dicarbonyls.¹ We have oxidized amino acetaldehyde to glyoxal in this way. Further, the instability of the methanolic DNPH reagent solution, which is used by Lappin and Clark,² is probably an indication of the formation

of oxidative products. Another reason for avoiding high concentrations of DNPH is that organic acids and derivatives form hydrazides with DNPH³ which interfere with carbonyl determinations by depleting reagent and forming hydrazide chromophores. In addition, DNPH in alkaline solutions gives undesirable high reagent backgrounds. Finally, if chloroform extracts are used in paper chromatography for carbonyl detection the DNPH reagent is also extracted. The resulting DNPH-streaks on paper obscure the carbonyl spots. Identification of the spots is thus made much more difficult.

Efforts have been made in this laboratory to reduce DNPH concentrations after reactions with carbonyl compounds. Dialysis was used for proteins but this, of course, is useless with low molecular weight compounds. Solvent extractions were tried, but separation of reagent from derivative was poor. Recently we found that much lower DNPH concentrations for the assay of pyruvic and glyoxylic acids could be used than earlier believed to be possible. If the DNPH reagent solution were kept at 0.012% rather than 0.1 - 1% Beer's law was followed for DNPH/carbonyl ratios of 3 to 1 and greater. Calibration curves for glyoxylic and pyruvic acid in this low reagent procedure were essentially identical. The molar extinction coefficient was 10,500 for each. Under these conditions acetaldehyde reacts only slightly. Actually, the DNPH and NaOH concentrations and aqueous media favor fading and instability of the acetaldehyde

derivative.⁴⁻⁹ It's molar extinction coefficient here is 1,500. (Formaldehyde does not interfere because it reacts very little with DNPH reagent at these low concentrations and its' alkaline chromophore fades very rapidly.) In practice the acetaldehyde content of sample can be determined by the method of Johnson and Scholes in which pyruvic and glyoxylic acids do not interfere⁹ and a correction for acetaldehyde can then be made.

If identification of the carbonyl - DNPH derivatives is desired the extractions with chloroform are done in the usual way, but in this case the excess reagent contamination will be greatly reduced.

A typical procedure for the determination of pyruvic and glyoxylic acids in a sample containing acetaldehyde and using 0.012% DNPH reagent follows:

To a 1 ml. sample containing 0.03 - 0.2 micromoles of pyruvic acid and acetaldehyde in 2 N HCl, and to a 1 ml. carbonyl-free sample blank in 2 N HCl add 1 ml. of 0.012% (6×10^{-4} M) DNPH in 2 N HCl. Let the reaction mixture set for 3 hours at room temperature and then add 6 ml of 10% aqueous KOH. Measure the sample absorption versus the sample blank at 450 nm. five minutes after the alkali addition.

On a separate aliquot of the sample measure the acetaldehyde content of the sample by the Johnson-Scholes method.⁹ Calculate the corresponding absorbance by the present low reagent procedure and subtract this from the total obtained

by this same procedure. The resultant absorbance is used with a calibration curve of pyruvic and glyoxylic acids. The presence of pyruvic and/or glyoxylic acid can be confirmed by paper chromatography of the hydrazones.

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