The Spectroscopic Quinoidal Ion Method for the Analysis of Carbonyl Compounds

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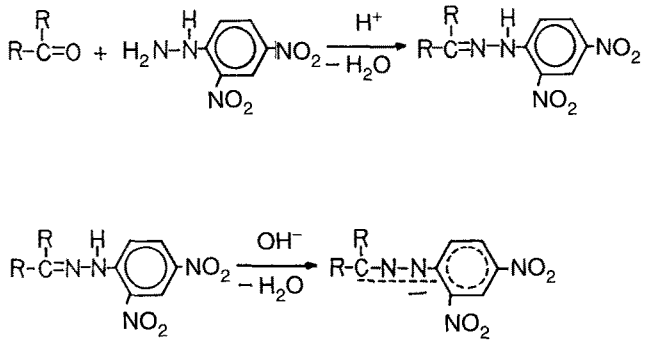
ABSTRACT: The spectroscopic quinoidal ion method for carbonyl compound analysis has been evaluated. Conjugated aldehydes and saturated ketones are rather stable after an initial time period. Saturated aldehydes are unstable and decompose rapidly with time. The stability of aldehydes is directly related to the size of the alkyl group attached to the aldehyde function. The larger the group, the slower it decomposes.
JAOCS 72, 385–387 (1995).

KEY WORDS: Absorbance, aldehyde, carbonyl, 2,4-dinitrophenylhydrazine, quinoidal ion, spectroscopic analysis.

The formation of carbonyl compounds from lipid oxidation is of great concern for many lipid scientists. Because of this concern, there is a need for accurate and reliable methods for carbonyl analysis (1). One of the more reliable methods for total carbonyl analysis is based on the absorbance of the quinoidal ion, a derivative of aldehydes and ketones. This ion is formed from the reaction of 2,4-dinitrophenylhydrazine (2,4-DNPH) with an aldehyde or ketone, followed by the reaction of the resulting hydrazone with alkali as shown in Scheme 1. Many variations of this spectroscopic method have been reported (2–6). Each method offers an alternative solvent, wavelength, or workup in which to analyze the quinoidal ion.



SCHEME 1

Of particular interest are the reports that the wine-red color of the quinoidal ion fades with time (2–4). A literature search has shown that there has been no study of the relationship between carbonyl type and rate of color fade or loss of absorbance. This communication presents the relationship between carbonyl type and rate of absorbance loss of this important analytical procedure.

EXPERIMENTAL PROCEDURE

Materials. Hexanal, *trans*-2-hexenal, 3-hexanone, and 2,4-DNPH were purchased from Aldrich Chemical Company (Milwaukee, WI). Purities of aldehydes were checked by gas–liquid chromatography (GLC). Methanol and ethanol were purchased from Curtin Matheson Scientific, Inc. (Florence, KY). The methanol needed no purification. The ethanol was purified as described by Lappin and Clark (3). A 50:50% (w/w) sodium hydroxide solution was purchased from Fisher (Fair Lawn, NJ). Potassium hydroxide was purchased from Mallinckrodt, Inc. (Paris, KY). Concentrated hydrochloric acid was purchased from Curtin Matheson Scientific, Inc. (Houston, TX).

Apparatus. The absorption spectrum was determined with a Milton Roy (Rochester, NY) Spectronic 21 MV spectrophotometer.

Determination of carbonyl compounds in methanol at 480 nm. This is the procedure of Lappin and Clark (3). Into a 50-mL graduated cylinder was pipetted 1.0 mL containing 80 μg of carbonyl group in methanol. A 1.0-mL aliquot of saturated, 2,4-DNPH/MeOH was then added and followed by two drops of concentrated HCL.

The cylinder was stoppered and heated in a waterbath for 30 min at 60°C. Afterwards, the reaction was cooled to room temperature. A 5-mL aliquot of 20% NaOH/70% MeOH/10% H_2O is added and immediately diluted to 50 mL with methanol. When the alkali is added, time zero is marked. Measurements are made at the wavelength of 480 nm.

Determination of carbonyl compounds in ethanol at 425 nm. This procedure essentially follows Yukawa, Takamura, and Matoba's method (6). A 1.0-mL aliquot containing 80 μg of carbonyl group in ethanol was pipetted into a 50-mL graduated cylinder. Afterward, 1.0 mL of saturated 2,4-DNPH/EtOH was added, followed by 1 drop of concentrated

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HCl (350 µL). The cylinder was stoppered and heated in a waterbath for 30 min at 50°C. Afterward, the reaction was cooled to room temperature. A 5-mL aliquot of 10% KOH/80%/10% H_2O EtOH is added and immediately diluted to 50 mL with ethanol. Then it is mixed and filtered through a 0.2-µm polytetrafluoroethylene filter (Gelman Acrodisc CR PTFE 0.2 µm; Gelman Sciences, Ann Arbor, MI). When the alkali is added, time zero is marked. Measurements are made at the wavelength of 425 nm.

RESULTS AND DISCUSSION

The analytical method described in this communication basically involves the reaction of carbonyl compounds (aldehydes and ketones) with 2,4-DNPH to form a hydrazone. The hydrazone is then allowed to react with a hydroxide to form a colored quinoidal ion, which is then analyzed spectroscopically at a given wavelength. Two established procedures were analyzed for quinoidal ion color fade. These procedures were the methods of Lappin and Clark (3) (solvent: methanol; wavelength: 480 nm) and of Yukawa, Takamura, and Matoba (6) (solvent: ethanol; wavelength: 425 nm).

Figures 1 and 2 show that the absorbance from the saturated aldehyde, hexanal, decreases with time. However, both the conjugated aldehyde, *trans*-2-hexanal and the saturated ketone, 3-hexanone, remain constant over the given time period in methanol (Fig. 1) and after about 5 min in ethanol (Fig. 2). Time was marked immediately after alkali addition. In all the cases studied, the net absorbance is directly related to the molar absorption coefficients because all aldehydes and ketones were analyzed on an equal carbonyl function concentration basis (80 µg C=O/mL).

Note that the molar absorption coefficients of the saturated aldehyde and ketone quinoidal ion are equal after approximately 5 min in methanol at 480 nm (Fig. 1). Also note that the conjugated aldehyde gives a molar absorption coefficient more than three times that of the saturated aldehyde, as previously reported (6).

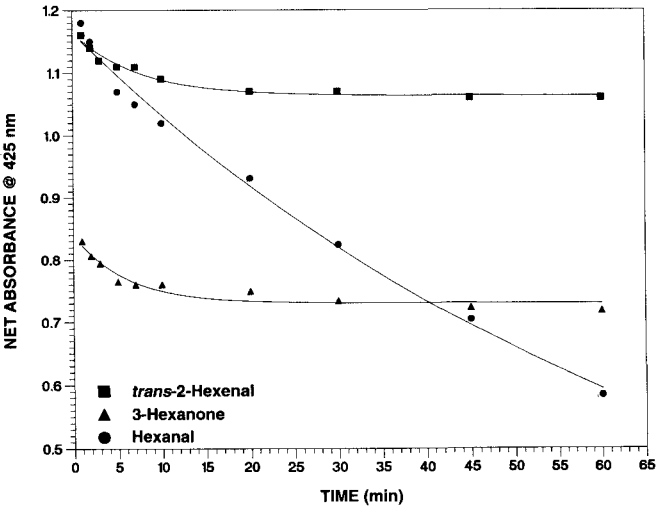

FIG. 2. Method of Yukawa, Takamura, and Matoba (Ref. 6). Concentration 80 µg in ethanol at 425 nm in 1.0-cm cell.

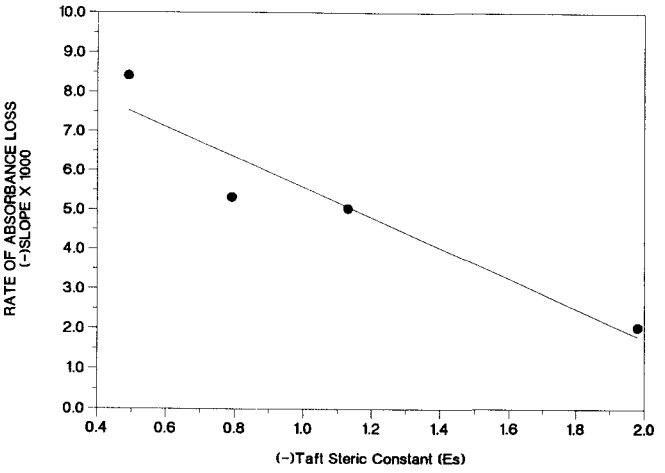

FIG. 3. Empirical slope of absorbance loss vs. size of the alkyl group (Es) on an aldehyde.

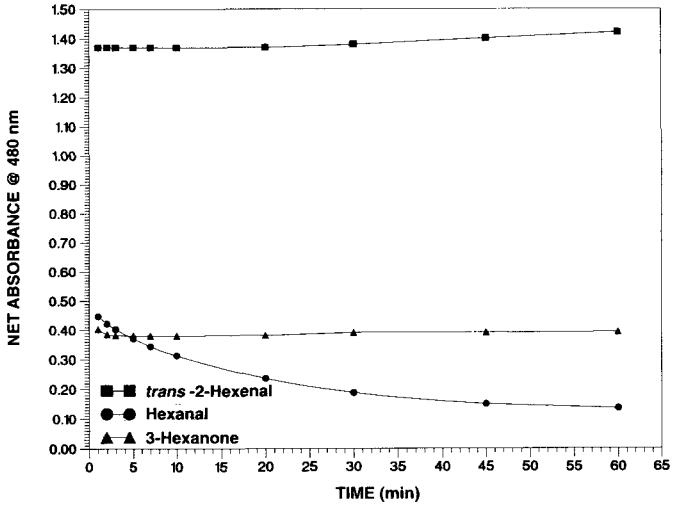

FIG. 1. Method of Lappin and Clark (Ref. 3). Concentration 80 µg in methanol at 480 nm in 1.0-cm cell.

Figure 2 shows that the molar absorption coefficient of the quinoidal ions from the saturated and conjugated aldehydes remain equal within 3 min in ethanol at 425 nm. However, they diverge markedly after this time period. The ketone quinoidal ion in this system has a much lower absorption coefficient and equals the falling molar absorption coefficient of the saturated aldehyde after a 42-min time period.

The drop in absorbance of the quinoidal ion from hexanal is not unique to that saturated aldehyde. The quinoidal ions from hexanal, nonanal, 2-methylbutyraldehyde, 2-ethylbutyraldehyde, and cyclohexanecarboxaldehyde all display a loss in absorbance with time. However, the larger the steric bulk of the alkyl group attached to the carboxaldehyde, the slower the drop in absorbance.

This can be seen in Figure 3 where the rate of absorbance loss (or loss in molar absorption coefficient) is plotted vs. the

size of the alkyl group (7). The larger the steric bulk surrounding the derivatized carbonyl carbon, the slower the reaction that causes absorbance loss. Apparently, the loss in absorbance of the quinoidal ion is the result of a reaction at the derivatized carbonyl carbon. In the case of the conjugated aldehyde, the negative charge is diminished through resonance over two more carbons, making it more stable and less susceptible to reaction at the derivatized carbonyl carbon.

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[Received March 28, 1994; accepted November 26, 1994]