Anal. Chem. **1994**, *66*, 1965–1970

Measurement of Carbonyl Compounds as the 2,4-Dinitrophenylhydrazonate Anion. Reaction Mechanism and an Automated Measurement System

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The standard procedure for determining carbonyl compounds involves the addition of strong caustic alkali to 2,4-dinitrophenylhydrazones formed upon the reaction of carbonyl compounds with 2,4-dinitrophenylhydrazine (DNPH).[1] In strong base, the hydrazones form a dark red anion of quinonoid structure,[2] while the corresponding product from the unreacted DNPH decomposes rapidly, resulting in a low blank. The mechanism of this decomposition has never been clearly understood. In this work, we describe a photometric flow injection analyzer for the automated measurement of the carbonyl content of alcohol ethoxylate samples. A detailed study of the mechanism of the alkaline decomposition is reported. Parameters affecting the performance of the automated system were studied. The final system attained nearly equivalent responses of C_2–C_9 aliphatic carbonyl compounds (within ±12%), a sample throughput of 20 h^{-1}, a limit of detection of 0.5 mg/L >C=O, and response linearity over 2 orders of magnitude. The method was applied to industrial samples, and the results compared well with those from the manual standard method. It should be applicable for the measurement of the carbonyl content in a variety of other organic matrices.

Quantitative determination of the carbonyl functionality is an important measurement in a variety of sample types. The particular concern with the carbonyl content of alcohol ethoxylates, an important class of chemicals used in a multitude of consumer products, is related to the considerable reactivity of the >C=O group. Undesirable reactions that involve the carbonyl groups lead to conjugated structures contributing to off-color final products; manufacturers typically strive to attain a specification[3] of ≤10 mg/L >C=O. For the determination of trace quantities of carbonyl compounds, the use of 2,4-dinitrophenylhydrazine (DNPH) is the most popular. A photometric method was described by Mathewson as early as 1920; the hydrazone formed was dissolved in CCl_4 for photometry.[4] The basis of the current standard method[1] is the work of Lappin and Clark;[2] the dinitrophenylhydrazone initially formed is subjected to very strongly alkaline conditions whence a strongly absorbing anion of quinonoidal structure

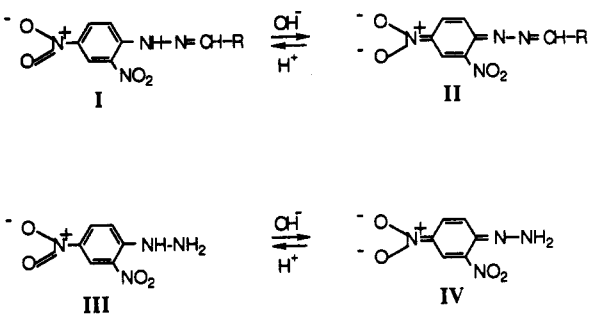

Figure 1. Dinitrophenylhyrazones (I) and dinitrophenylhydrazine (III) form the corresponding intensely colored anions (II and IV) in very strongly basic solutions.

is formed.[5,6] The recommended procedure involves two separate steps and takes 30–40 min.[1] Although the original work[2] suggested that incubation at higher temperatures considerably reduces the necessary reaction time, this was not found necessary in the standard method. It is known that the rate of formation of the hydrazone is very dependent on the acidity of the reaction medium.[7] In initial efforts to automate this determination, reproducible results could be obtained with individual carbonyl compounds using a packed bed reactor (80 °C, residence time ~2 min). However, there were large differences in the response of individual carbonyl compounds. The responses of dodecanaldehyde (DDA) and methyl isobutyl ketone (MIBK), for example, differed by as much as a factor of 2.5.[3] These compounds respond identically in the standard manual method on an equimolar basis.[1]

The measurement principle is based on the ionization of the dinitrophenylhyradazone (I) in strongly alkaline solutions to a wine-red quinonoid anion (II) (Figure 1). Excess DNPH (III) also reacts in a similar manner. The resulting color due to the anion IV is so intense in a typical assay procedure (the unreacted reagent is in large excess) that the solution at first turns essentially black. However, anion IV decomposes rapidly to nearly colorless products. In contrast, compound II does not decompose substantially in a time scale of minutes. Thus, if a suitable period of time is allowed for such decomposition to occur, the decolorization of anion IV will be essentially complete, allowing the measurement of II. The pathway of this decomposition has never been clearly elucidated.

We undertook the present study to (a) understand the nature of the decomposition reaction, (b) identify the products

(1) American Society of Testing and Materials. *Standard Test Method for Trace Quantities of Carbonyl Compounds With 2,4-dinitrophenylhydrazine.* Designation E411-70 (Reapproved 1986); ASTM: Philadelphia, PA, 1986.
(2) Lappin, G. R.; Clark, L. C. *Anal. Chem.* **1951**, 23, 541–542.
(3) Spino, L. A., Westhollow Research Center, Shell Development Co., Houston, TX. Personal Communication, 1991.
(4) Mathewson, W. E. *J. Am. Chem. Soc.* **1920**, *42*, 1277.
(5) Toren, P. E.; Heinrich B. *J. Anal. Chem.* **1955**, *27*, 1986–1988.
(6) Jordan, D. E.; Veatch, F. C. *Anal. Chem.* **1964**, *36*, 120–124.
(7) Jordan, D. E. *Anal. Chim. Acta* **1967**, *37*, 379–390.

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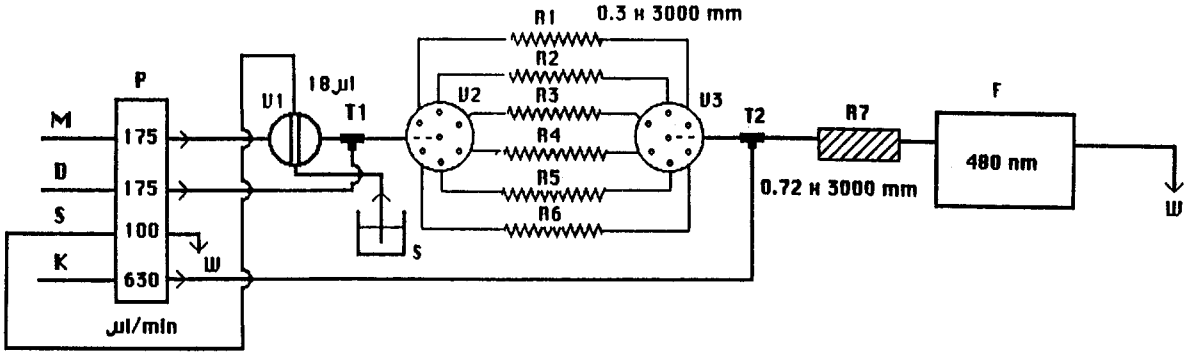

Figure 2. System schematic: P, peristaltic pump; M, methanol; D, DNPH reagent; S, sample; A, alkali reagent; W, waste; V1, sample injection valve; V2 and V3, simultaneously operated six-way selector valves connecting reactors R1–R6 individually at a time; T1 and T2, tees; R7, second-stage reactor; F, flow-through absorbance detector.

formed, and (c) identify the parameters that cause differing sensitivities among different carbonyl compounds in an automated procedure. On the basis of this understanding, an optimized instrument was designed for the measurement of carbonyl compounds in alcohol ethoxylate samples.

EXPERIMENTAL SECTION

Reagents. Methanol was initially distilled from a solution containing DNPH as suggested in the ASTM procedure.[1] However, spectroscopic grade methanol (SpectraR, Mallinckrodt) was subsequently found to produce comparably low blank values and was thence used without further purification. For the DNPH reagent, 0.2 g of the solid was dissolved in 50 mL of methanol, 4 mL of concentrated HCl was added, and the solution was diluted to 100 mL with distilled deionized water. The alkali reagent consisted of 50 g of KOH dissolved in 100 mL of water and was subsequently diluted to 500 mL with methanol. Test carbonyl compounds consisted of formaldehyde (37%, Mallinckrodt), acetaldehyde (99%, Aldrich), propionaldehyde (97%, Aldrich), isovaleraldehyde (98%, Aldrich), DDA (92%, Aldrich), benzaldehyde (Fisher), *p*-(dimethylamino)benzaldehyde (Fisher), glyoxal (Fisher), acetone (pure, Baker), MIBK (Fisher), benzoylacetone (99%, Aldrich), 2,3-butanedione (Kodak), and 2,4-pentanedione (Lancaster Synthesis). All of the above compounds were used without further purification. Stock solutions (10 mM) were prepared by weighing the compounds and dissolving them in pure methanol.

Flow Injection Analyzer. The system is schematically shown in Figure 2. Peristaltic pump P (Gilson Minipuls 2) is equipped with a four-channel head and Viton pump tubing. Methanol (M) is pumped as carrier through an electromechanically actuated six-port loop injector valve V1 (type HVXL 6–6, Hamilton). The valve was equipped with a narrow bore loop (0.3 × 180 mm); the injection volume includes the internal volume and was measured to be 18 µL. Following V1, the carrier stream is merged with a flow of the DNPH reagent (D) at tee T1 and then enters one of six mixing/incubation reactors R1–R6 as controlled by a pair of electropneumatically switched simultaneously operated six-way selector valves V2 and V3 (type 5703P, Rheodyne Inc.). The reactors and valves are configured so that flow occurs through one reactor at a time. Following the injection of one sample, the sample/reagent mixture is allowed to flow into one of the reactors. Flow rates, sample size, and reactor volumes are so chosen that essentially the entire injected sample can be contained within one of the reactor loops. Thus, when one sample is "parked" in one of the reactors, a new sample is injected and the V2/V3 pair is simultaneously switched to select a new reactor. Reactors R1 through R6 consist of 0.9-mm-o.d., 0.3- × 3000-mm PTFE tubes, each individually woven in a Serpentine-II design[8] on 1000-µm mesh 4- × 10-cm poly(propylene) screens (Small Parts Inc., Miami Lakes, FL). The woven design shows significantly reduced dispersion. The R1–R6 reactors were maintained (at 60 °C, except as stated) in a thermostated enclosure, interleaved by silicone encapsulated strip heaters (Watlow Inc., St. Louis, MO). Following valve V3, the flow stream is made strongly alkaline by introducing alkaline reagent A at tee T2. The necessary second-step reaction time is provided by a 0.72- × 3000-mm PTFE knotted reactor R7, also kept in the same thermostated enclosure as above. Very strongly alkaline conditions are needed for this step. The overall flow rate after introduction of the alkaline reagent is ~1 mL min^{-1}. Even at a temperature of 60 °C, the reaction time necessary for the decolorization of the black product formed from the excess DNPH reagent is at least 90 s. The large residence volume necessary for R7 thus necessitates a larger bore reactor. Finally, the product absorbance is detected by a flow-through absorbance detector F (model SF 757, heat exchanger removed to reduce backpressure, Applied Biosystems Inc.) set at 480 nm. A substantially simpler, light emitting diode (LED) based small path length radial path detectorm, utilizing a 470-nm SiC emitter according to the design reported in Figure 7 of Dasgupta et al.,[9] was also successfully used, with comparable performance.

In the final configuration of the system, V1 was operated with a 2-min load 1-min inject cycle. The switching of V2/V3 was made every 3 min, synchronized with the period of V1. This results in the following: when a fresh cycle begins, during the first 1 min the carrier propels the contents (e.g., the previous sample) of the reactor (R1–R6) to T2, during the second 1 min the reactor is washed with fresh carrier, and then, as the sample is injected, the reactor is occupied by a new sample during the third 1 min. After 3 min, the flow is switched to the next reactor and the whole cycle begins anew.

(8) Curtis, M. A.; Shahwan, G. J. *LC-GC* **1988**, *6*, 158–164.
(9) Dasgupta, P. K.; Bellamy, H. S.; Liu, H.; Lopez, J. L.; Loree, E. L.; Morris, K.; Petersen, K.; Mir, K. A. *Talanta* **1993**, *40*, 53–74.

Since there are six reactors, each sample is subjected to 18 min of reaction following mixing with DNPH.

Mechanistic Studies. NMR measurements were conducted on an IBM AF-300 instrument; dimethyl-d_6 sulfoxide (DMSO) was used in all cases as the solvent. The following studies were conducted.

Base-Catalyzed Decomposition of 2,4-Dinitrophenylhydrazine. DNPH (0.5 g) was added all at once to a mixture of 5 mL of 2.5 N NaOH and 5 mL of dimethoxyethane at 0 °C with stirring. After 15 min (at which time the solution had decolorized to a yellow color), 10% HCl was added to adjust the pH to 5. The precipitate was collected and dried to give 0.12 g of crude 3,3′-dinitroazoxybenzene. This was recrystallized from ethanol, mp 144–146°. Proton NMR: δ 9.04, dd, J = 2.0, 2.0 Hz (H-2); 8.99, dd, J = 2.0, 2.0 Hz (H-2′); 8.75, ddd, J = 8.2, 2.0, 0.8 Hz (H-4′); 8.58, ddd, J = 8.2, 2.0, 0.8 Hz (H-6′); 8.40, ddd, J = 8.2, 2.0, 0.8 Hz (H-4); 8.36, ddd, J = 8.2, 2.0, 0.8 Hz (H-6), 7.97, dd, J = 8.2, 8.2 (H-5′); 7.88, dd, J = 8.2, 8.2 (H-5). Carbon NMR (75 MHz): δ 147.9, 147.7, 147.6 (C-1, C-3, C-3′), 143.3 (C-1′), 132.1 (C-4), 131.7 (C-5′), 131.2 (C-5), 128.4 (C-4′), 127.2 (C-6′), 124.6 (C-6), 118.9 (C-2), 117.6 (C-2′). All assignments were confirmed by proton–carbon and proton–proton correlation spectroscopy.

The mother liquor from the above reaction was acidified to pH 2 and allowed to stand overnight, upon which crystals of 6-nitro-1-hydroxy-1,2,3-benzotriazole were deposited, 0.14 g, mp 201–203°. Proton NMR: δ 8.65, dd, J = 2.0, 0.8, (H-7); 8.25, dd, J = 11.0, 0.8 (H-5); 8.20, dd, J = 11.0, 2.0.

Extraction of the mother liquor from the above reaction with ethyl acetate gave *m*-dinitrobenzene, ca. 0.06 g, slightly contaminated with the other products. Proton NMR: δ 8.84, dd, J = 2.2, 2.2 (C-2); 8.66, 2H, dd, J = 8.2, 2.2 (C-4, C-6); 7.97, dd, J = 8.2, 8.2 (C-5).

Another run under the same conditions, but with immediate acidification to pH 3 and ethyl acetate extraction, gave all three of the above compounds, in the ratio 1.7:1:1, as judged by NMR integration of the signals at δ 8.16–8.28 vs 8.82–8.85 vs 8.98–9.05.

Deuteration Studies. A sample of 120 mg of DNPH was stirred in a mixture of 1.0 mL of D_2O and 1.0 mL of dimethoxyethane for 0.5 h; then the solvent was removed by evaporation. To a second 1-mL sample of D_2O was added 0.08 g of Na under N_2. After dissolution was complete, 1.0 mL of dimethoxyethane was added, the deuterium-exchanged dinitrophenylhydrazine was added, stirring was continued for 15 min, and then the reaction mixture was acidified to pH 4 and extracted well with ethyl acetate.

The proton NMR spectrum of the extract (after evaporation and reconstitution in DMSO-d_6) showed signals for 3,3′-dinitroazoxybenzene as follows: δ 9.04, d, J = 2.0 Hz (H-2); 8.99, d, J = 2.0 (H-2′); 8.75, 0.5 H, dd, J = 8.2, 2.0 Hz (H-4′); 8.58, 0.5 H, dd, J = 8.2, 2.0 Hz (H-6′); 8.40, 0.5 H, dd, J = 8.2, 2.0 Hz (H-4); 8.36, 0.5 H, dd, J = 8.2, 2.0, Hz (H-6), 7.97, d, J = 8.2 (H-5′); 7.88, d, J = 8.2 (H-5). This spectrum verified that deuterium is scrambled between the 4 and 6 positions and the 4′ and 6′ positions. The proton NMR signals for *m*-dinitrobenzene were as follows: δ 8.84, d, J = 2.2 (C-2); 8.66, 1H, d, J = 8.2, 2.2 (C-4 or C-6); 7.97, d, J = 8.2 (C-5). The signals for the triazole were unchanged.

RESULTS AND DISCUSSION

Flow Injection System Design. It was found in pilot studies[10] that a significant reaction time with the DNPH reagent is necessary to assure a reasonably uniform response from different carbonyl compounds (the pair typically studied was DDA vs MIBK), regardless of reagent acidity and reaction temperature. If response uniformity for different carbonyl compounds is not important, e.g., with an on-line instrument dedicated to a given process where the nature of the carbonyl compound does not vary, the system can be calibrated with that specific compound and a long reaction time is not essential. For a laboratory based analyzer that must encounter samples with different types of carbonyl impurities, the multiple "parking loop" concept, permitting long reaction times, was adapted. It was also found that reproducible timing is extraordinarily critical for the second reaction stage. The decolorization of the excess reagent is acutely time-dependent. Further, at high sensitivities, some degradation of the product formed from the analyte is also noticeable. In initial attempts, a stopped-flow approach[11] showed poor reproducibility; interpretation of the detector output was complicated by the rapid and continuous change in the absorbance registered by the detector during the stopped-flow period. The adapted approach therefore utilized continuous flow through the detector. It was also found necessary to use a relatively small sample volume. Unless the sample is significantly dispersed, the upper limit of sample concentration that can be introduced in the system is severely limited. Especially for long-chain carbonyl compounds such as DDA, the dinitrophenylhydrazone derivative readily precipitates in the reaction conduits at higher sample concentrations and leads to major problems. Aside from limiting the sample volume, precipitation is also deterred by operating at a high temperature.

The timing, reactor dimensions, and the pump flow rate necessary to place the injected sample in the desired position inside R1–R6 were adjusted with the help of 0.1% ethanolic methylene blue as the injected sample and visual monitoring of the bolus.

Effects of Reagent Composition and Reaction Conditions. *DNPH Concentration and Acidity of Reagent.* Different concentrations of acid and DNPH in the derivatization reagent have been previously recommended. Using 1 mM DDA and MIBK, respectively, as test samples, we determined the effect of varying the HCl concentration in the DNPH reagent (0.1% DNPH in 50:50 methanol:water) using the system described in the Experimental Section. It is important to point out that the injection of the solvent blank causes a measurable response in this system. Since the same solvent is used as the carrier, this signal is not due to the residual carbonyl content of the solvent. Rather, it is an artifact arising from flow interruptions caused by the switching of the valves. While the net DDA signals were not significantly dependent on the acidity, the less reactive MIBK showed a pronounced dependence on reagent acidity in the range of 1–4% HCl, reaching a plateau only at acid concentrations ≥3%. With acid concentrations substantially higher than 4%, problems were encountered with the precipitation of KCl in the conduit after neutralization.

(10) Dunn, C. D. Automated Determination of Carbonyl Compounds in Organic Solvents. M. S. Thesis, Texas Tech University, 1992.
(11) Ruzicka, J.; Hansen, E. H. *Flow Injection Analysis*, 2nd ed.; Wiley: New York, 1988.

The relative responses of the two analytes are very dependent on the DNPH concentration. At a DNPH concentration of 0.1%, the DDA response is nearly twice that of MIBK. The two responses become virtually equivalent as the DNPH concentration approaches 0.4%. However, at such concentrations, the DNPH hydrochloride salt slowly crystallizes out of solution, causing problems. A DNPH concentration of 0.2% was therefore chosen as a compromise. If additional pumping channels are simple to provide, it is preferable to add acid to a more concentrated DNPH solution in-line to further improve the uniformity of response; the hydrochloride salt precipitates only slowly.

Reaction Temperature and First-Stage Reaction Time. The effect of the first-stage reaction temperature was studied with 1 mM DDA and MIBK as the test samples with other conditions as described in the Experimental Section. The background absorbance decreases from 0.023 to 0.008 au in going from 24 to 46 °C and does not change significantly at higher temperatures. The net response from DDA does not vary markedly in the range 20–80 °C, but that for MIBK decreases linearly from 24 to 65 °C by 27%. While the responses from MIBK and DDA are experimentally indistinguishable at room temperature (as suggested in the ASTM method[1]), the response from MIBK is ~25% lower at 60 °C.

While it may seem at first sight that operation at room temperature should be the preferred mode from the standpoint of response equivalency, other problems are encountered. Without heating, the second reaction step is slow and detection limits deteriorate due to the high background absorbance. Further, although response equivalency is observed for DDA and MIBK at room temperature, this may not necessarily be the case for other test solutes. Very high temperatures also cause problems. Even with some back pressure applied to the detector exit, it is difficult to routinely operate at a temperature of 80 °C (the cited temperature is that of the enclosure; because of the insulating properties of PTFE, the actual liquid temperature is significantly lower) in a methanolic medium because of bubble formation in the detector. The operating temperature of 60 °C was chosen as a compromise; around this temperature, the temperature dependence of the response was also relatively small.

The system response shows little dependence on the reaction time in the range 10–60 min. Less than a 10% increase in the response is observed between reaction times of 10 and 60 min. Our analytical needs did not involve very fast sample throughput; on the basis of these considerations, we chose a reaction time of 18 min. It was found possible to run the system with a reaction time of 9 min with changes in sample volume, reactor dimensions, and flow rate. This can be adapted if sample throughput becomes a consideration.

Second-Stage Reaction. Although the Lappin–Clark procedure[2] involving the formation of the anionic dinitrophenylhydrazonate has been in use for more than four decades now, few details of the reaction have previously been reported. The color of the dark black, almost opaque solution initially formed upon making a solution of DNPH strongly alkaline fades at a rate that was found to increase with increasing base concentration. A pale yellow solution eventually remains. As measured with a diode array spectrophotometer, the reaction is essentially complete within 90 s under these conditions; very little change takes place thereafter.

When a carbonyl compound is present in the sample and the hydrazone is present along with excess DNPH, the dinitrophenylhydrazonate anion exhibits a wine-red color that becomes apparent only after the colored product from the excess reagent is largely decomposed. While the dinitrophenylhydrazonate anion is more stable than the corresponding dark product formed from DNPH itself, it also does decompose, at a rate very dependent on the parent carbonyl compound. In general, the ease of this decomposition parallels the ease of the initial formation of the dinitrophenylhydrazone. The decay of the MIBK derivative is very slow, while the decay of the DDA derivative is photometrically perceptible even after 20 min. The second-stage reaction time was therefore limited to 90 s.

Spectrally, both the DNPH reaction product and the hydrazone reaction products exhibit one absorption band centered at ca. 425 nm. Measurement at 425 nm is not advantageous, however, due to the large background absorption from the excess DNPH reaction product. According to Jordan and Veatch,[6] acceptable response linearity for individual carbonyl compounds in the Lappin–Clark procedure is observed over the entire wavelength range 400–500 nm. Under our specific experimental conditions, we have found that the difference in response between individual carbonyl compounds is minimum in the wavelength range 460–500 nm. The monitoring wavelength of 480 nm (where background absorption from the DNPH product is low) was thus chosen.

The background absorbance is acutely dependent on the KOH concentration: it decreases exponentially from 0.25 to 0.05 au from 1 to 10% KOH. The net analyte signal is also low at low analyte concentration. A reagent composition containing 10% KOH was therefore chosen.

System Performance. *Response Equivalence, Linearity, and Limits of Detection.* Calibration experiments were conducted with a large number of carbonyl compounds (see Experimental Section) in the 0–2.5 mM concentration range. Glyoxal forms a derivative that precipitates in the system and could not be determined. With the calibration slope of formaldehyde arbitrarily set as unity, the calibration slopes for acetaldehyde, propionaldehyde, butyraldehyde, isovaleraldehyde, dodecanaldehyde, acetone, butanedione, and MIBK are respectively 1.64, 1.47, 1.38, 1.79, 1.50, 1.33, 1.61, and 1.26 (mean 1.46, rsd 11.6%). A reasonably equivalent response can therefore be expected for such aliphatic carbonyl compounds. Lohman[12] has previously reported the molar absorptivities of the dinitrophenylhydrazones derived from a number of carbonyl compounds. In general, the sensitivities observed in our experiments closely parallel the ϵ_{340} values for the dinitrophenylhydrazones reported by Lohman; this is shown in Figure 3. Formaldehyde behaves uniquely, which is often the case for the first member of a homologous series. It should also be noted that, although butanedione is a dicarbonyl compound, on the basis of the observed response, it reacts as a monofunctional compound. Steric reasons may preclude derivatization of both carbonyl groups to a significant extent. 2,4-Pentanedione or benzoylacetone do not undergo any

(12) Lohman, F. H. *Anal. Chem.* **1958**, *30*, 972–974.

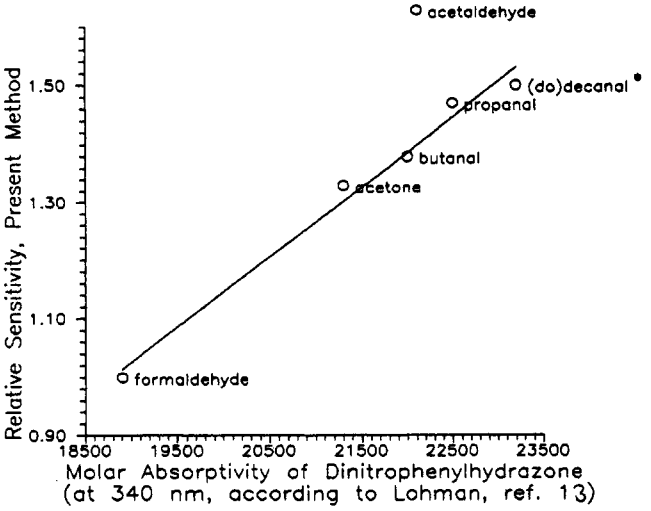

Figure 3. Plot of molar absorptivity versus relative sensitivity. Sensitivity of the present method appears to be directly related to the molar absorptivity of the dinitrophenylhydrazone derivatives reported by Lohman. The ϵ_{340} value for the DDA derivative is estimated from the data for straight chain aldehydes up to decanal given by Lohman.[13]

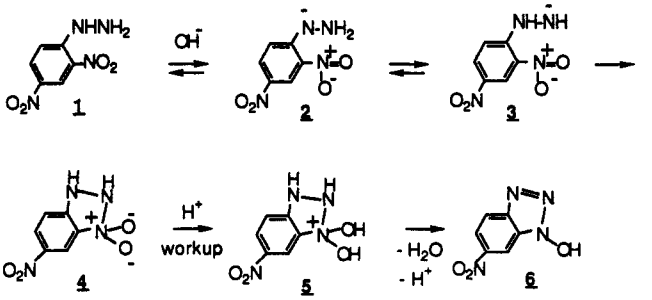

Figure 4. Reaction mechanism leading to the formation of 6-nitro-1-hydroxy-1,2,3-benzotriazole (**6**).

significant reaction at all. For 2,4-pentanedione, it is well-known that when a $-CH_2$ group is attached to two carbonyl groups, the compound exists largely in the enol tautomeric form.[13] For benzoylacetone,the reaction is likely inhibited due to steric reasons. For reactive aromatic carbonyl compounds, the aromatic ring may interact with the chromogenic structure and greatly increase the overall sensitivity. The relative calibration slopes of *p*-(dimethylamino)benzaldehyde and benzaldehyde are 5.35 and 3.58, respectively.

The limit of detection, based on the criterion of three times the standard deviation of the blank, was computed for both DDA and MIBK to be 0.5 mg/L. The system responses for DDA and MIBK over the 0–2.5 mM concentration range with data points acquired with a 0.5 mM interval are described by the following linear equations, respectively:

$$\text{signal (mau)} = 46.1 \pm 1.0\ (\text{C, mM}) + 3.62 \pm 0.38, \qquad r^2 = 0.000,$$

and

$$\text{signal (mau)} = 40.8 \pm 0.25\ (\text{C, mM}) + 3.62 \pm 0.38, \qquad r^2 = 0.999$$

Variations in Individual Reactors. The rsd of analyte response for continuous analysis of the same sample over a day long period at the 1 mM level was <5%. However, detailed analysis of the results showed that, if the results are considered for individual reactors R_1–R_6, the rsd was lower and ranged from 2.5 to 3.6%. Although the reactors were made from the same length of tubing, the tolerance in the diameter of such tubes, the manually woven nature of the reactors, and the difficulty in assuring identical temperatures for all the reactors suggests that the reactors were not likely to be completely equivalent. Analysis of variance (ANOVA) showed that the difference between the reactors was significant at the p = 0.01 level (the reactor with the maximum analyte response exhibited a response 6% greater relative to that of the reactor with the minimum response). A multiple *t*-test further indicated that two of the reactors in particular were different from the rest at a probability level of 99.9%. To improve the accuracy of individual analyses, it was therefore deemed advisable to have the data processing system assign individual calibration factors to the individual reactor loops.

Long-Term Stability. Analysis of Alcohol Ethoxylates. Long-term stability and reproducibility of the system were investigated for potential use in process analysis over a continuous period of 28 days. Quality control samples of the same concentration were analyzed each day. The results consistently fell within 2 standard deviations of the mean value, indicating acceptable system stability.

A blind determination of the carbonyl content of a number of C_9–C_{15} alcohol ethoxylate samples (preanalyzed at Shell Development, Houston, TX, by the manual standard method) was performed. The two sets of results are well correlated (r = 0.9960), and the standard error of the estimates in the 0–100 mg L^{-1} range was 3.5 mg L^{-1}.

Although the instrument described here was studied in detail only with alcoholic solutions of alcohol ethoxylates, we believe that the approach will be equally successful with a variety of other sample types. This technique makes possible facile automated assay of carbonyl compounds in samples where direct infrared monitoring of the >C=O absorption is inapplicable.

Mechanism of the Base-Catalyzed Decomposition of DNPH. Very early studies[14,15] suggested that 6-nitro-1-hydroxy-1,2,3-benzotriazole (**6**, Figure 4), *m*-dinitrobenzene (**12**, Figure 5), and 3,3′-dinitroazoxybenzene (**15**, Figure 5) may be products of alkaline decomposition of DNPH. As stated in the Experimental Section, all three products were unambiguously identified by NMR spectroscopy after workup of base-decomposed DNPH solutions. Compound **6** is doubtless formed as a simple condensation product upon the attack of the hydrazinyl anion **3** on the adjacent nitro group as shown in Figure 4. Note that for the hydrazone, this decomposition pathway is not available in as much as an analog to **3** cannot be generated. The formation of the azoxybenzene **15** requires a reduction of two nitro groups by a total of six electrons from DNPH molecules. The intramolecular hydride shift shown in Figure 5 involves a two-electron reduction of the adjacent nitro group per DNPH molecule, thus accounting for four of the electrons from two DNPH molecules. The postulated mechanism thus predicts that 3 mol of intermediate

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(14) Curtius, T.; Mayer, M. *J. Prakt. Chem.* **1907**, *76*, 369–400.
(15) Macbeth, A. K.; Price, J. R. *J. Chem. Soc.* **1934**, 1637–1639.

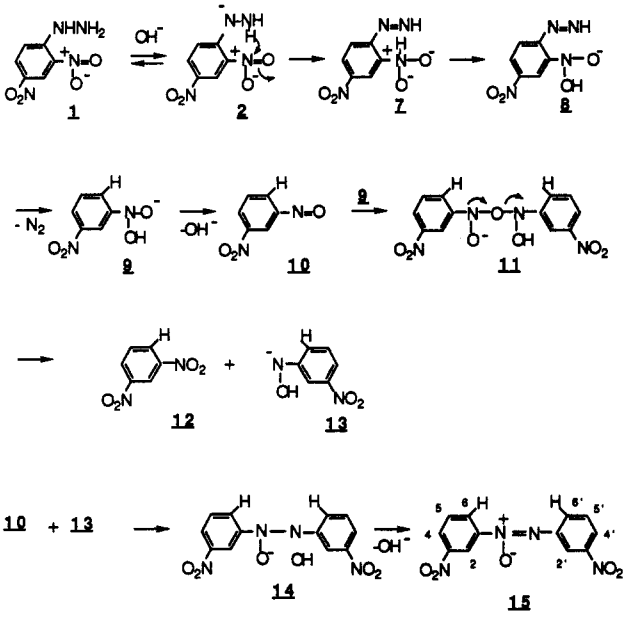

Figure 5. Reaction mechanism leading to the formation of *m*-dinitrobenzene (**12**) and 3,3′-dinitroazoxybenzene (**15**).

m-nitrosonitrobenzene (**10**) are formed per 1 mol of azoxybenzene **15**. Two moles of **10** go on to **15**, and one is oxidized to *m*-dinitrobenzene (**12**). The mechanism shown in Figure 5 thus postulates that *m*-dinitrobenzene (**12**) and the azoxybenzene **15** will be formed in a 1:1 ratio. This is in accord with the experimental facts, as judged from proton NMR data. Note that an intermediate such as **2** and a subsequent hydride shift reaction cannot occur for the hydrazone. This would also serve to explain the enhanced stability of the hydrazone vs DNPH in strongly alkaline solutions.

This mechanism postulates further that, if the reaction were carried out in deuterated solvent, deuterium would appear in position 4 in *m*-dinitrobenzene (the position marked with H in **9**-**12**) and in positions 6 and 6′ in the azoxybenzene **15**. In fact, carrying out the decomposition in D_2O/NaOD gave nondeuterated 6-nitro-1-hydroxy-1,2,3-benzotriazole (**6**) and 4-deuterio-1,3-dinitrobenzene (**12**-*d*) (Figure 6), as expected, but gave deuteration 50% each at the 4 and 6 positions and the 4′ and 6′ positions in the dinitroazoxybenzene, producing **15**-d_2 as a mixture of isomers. This indicates that some further subtleties are involved in addition to what is presented in Figure 5.

Consider also that, when the reaction was carried out in D_2O without *preexchange* of the hydrazinyl hydrogens for deuterium, the products were undeuterated. This fact suggests that the rate of exchange of NH for ND is slower than the decomposition reaction and that the decomposition of the intermediate diimide occurs via a concerted reaction (**8** → **9**), as shown in Figure 5, rather than via an aromatic carbanion.

The scrambling of the deuterium between the positions 4 and 6 and 4′ and 6′, i.e., adjacent to both the azoxy bridge

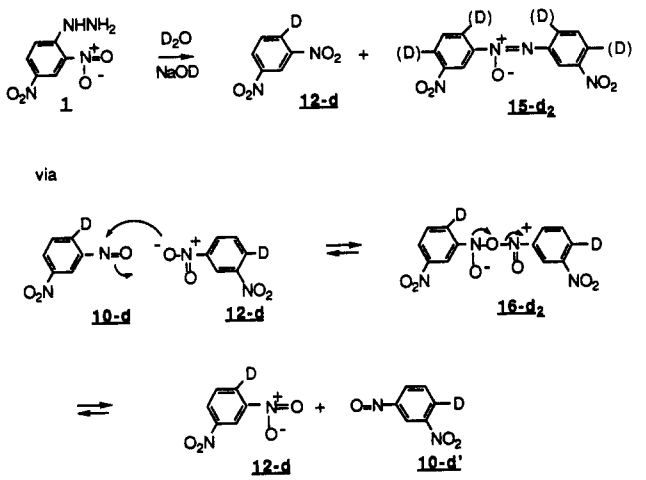

Figure 6. Reaction mechanism elucidating the scrambling of deuterium between the 4 and 6 and 4′ and 6′ positions in 3,3′-dinitroazoxybenzene.

and the nitro group, is not sufficiently straightforward to rationalize. The possibility that the formation of a symmetrical intermediate is involved is ruled out: experiments show that *m*-dinitrobenzene itself is stable to the reaction conditions; the formation of *m*-dinitrosobenzene or any other symmetrical intermediate appears to be mechanistically unreasonable.

The possibility that the initial reduction of the nitro group by hydride transfer from the hydrazinyl side chain occurs intermolecularly instead of intramolecularly, so that either nitro group of DNPH is reduced, was considered. This, however, would require *exactly* the same rate of hydride transfer on the *o*-nitro group as on the *p*-nitro group, since the scrambling of deuterium is *exactly* 50:50 within NMR accuracy. This appears to be highly unlikely both because the intramolecular reaction should be highly favored by entropy considerations and because the reactivity of the two nitro groups should not be the same for steric reasons.

We suggest that the only logical possibility is that a rapid equilibration occurs between the nitro and the nitroso groups as shown in Figure 6. Thus, the apparent scrambling of deuterium would actually be due to an interconversion of the nitro and nitroso groups. Such a mechanism readily explains the result of the deuteration studies. Unambiguous confirmation of this requires trapping of the intermediate **10**-*d*, which has not proved feasible.

ACKNOWLEDGMENT

The support for this research was provided by an unrestricted grant from Shell Development Co., Houston, TX. The continued help and assistance of Larry A. Spino is gratefully acknowledged.

Received for review December 17, 1993. Accepted April 6, 1994.[⊛]

[⊛] Abstract published in *Advance ACS Abstracts*, May 15, 1994.