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Spectrophotometric Determination of Urea in Ammonium Nitrate Fertilizers

Margaret H. Wilding and E. Ladell Blanton*

A rapid spectrophotometric method is described for the determination of low concentrations of urea in ammonium nitrate fertilizer. Analyses require 30 min to process with numerous samples run sequentially. This method is based on the absorbance of the red color complex produced by thiosemicarbazide, diacetyl monooxime, and urea. The color is stable for at least 30 min, and the procedure follows Beer's law to 25 ppm (0.5 mg) when measured at 525 nm. Interferences have been noted with some amines which produce an orange color complex. Enhancement from ammonium ion is compensated for in the reference and standard. Due to this enhancement, the reference, standards, and samples must contain the same ammonium nitrate concentration.

Ammonium nitrate fertilizers sometimes are contaminated as a result of urea carry-over in ammonia/carbon dioxide off gas from urea synthesis to the neutralizer section of the ammonium nitrate process. Urea levels are normally very low in ammonium nitrate product, 1–20 ppm, but during process upsets may reach 200–400 ppm. Since urea contamination adversely affects ammonium nitrate quality, it is desirable to quickly and accurately determine the amount of urea present. Most colorimetric determinations for urea require lengthy reaction or color development times of 1 h or more and have low sensitivity. A time-saving and more sensitive method of determination would be of high practical importance, especially in quality control analysis.

Generally, spectrophotometric determination of urea falls into two main categories. The first category (indirect method) involves hydrolysis of urea to ammonia or an ammonium salt and carbon dioxide. The ammonia or ammonium ion then undergoes a color reaction. The most common hydrolysis procedure is catalysis with urease [AOAC Procedure 2.080 (Association of Official Analytical Chemists, 1980)].

The second category (direct method) involves the direct reaction of urea with another substance to produce a precipitate or color complex. One colorimetric method is based on the yellow-green color produced by p-(dimethylamino)benzaldehyde and urea in dilute hydrochloric acid solution. Interference from the ammonium ion is compensated for in the blank (Watt and Chrisp, 1954; Welcher, 1963). The concentration range of 50–240 ppm for this procedure fails to give satisfactory results with low urea concentrations as normally found in ammonium nitrate. The majority of work on the determination of low concentrations of urea (less than 50 ppm) has been done by clinical and diagnostic laboratories. Their work falls into four categories, depending on the amount of sensitizing agent, thiosemicarbazide, present. Fearon (1939) and Davidsohn-Wells (1963) discuss urea determination using diacetyl monooxime without thiosemicarbazide in

which a yellow color complex is formed. Coulombe and Favreau (1963) and Crocker (1967) use thiosemicarbazide as a sensitizing agent with diacetyl monooxime to produce a red color complex. Natelson (1971) also discusses thiosemicarbazide as increasing sensitivity but decreasing specificity.

This new procedure is an adaptation of a clinical test for urea nitrogen in urine and blood, which reduces color development time. Sample preparation does not involve ammonia evolution as in the AOAC method. However, ammonium ion concentration is a factor in color intensity and must therefore be consistent in the reference, standards, and samples. The purpose of this work is to develop a spectrophotometric technique for the fast and sensitive determination of urea at low concentrations in ammonium nitrate fertilizers.

MATERIALS AND METHODS

Apparatus. *Spectrophotometer:* double beam with concentration mode, Hitachi Model 100-60 (NSA/Hitachi, Mountainview, CA 94043). *Constant temperature bath:* 75 ± 1 °C, Tempstir 66540 (Precision Scientific Co., Chicago, IL 60647) with an aluminum water bath (29.85 × 22.86 × 19.05 cm). *Test tubes:* 25 × 150 mm (Corning Glass Works, Corning NY 14830). *Wire basket:* round; 12.7-cm diameter × 15.24 cm.

Reagents. *Working Reagent.* This solution is prepared by thoroughly mixing equal volumes of color and acid reagents listed below. This solution is stable for 8 h. Urea Nitrogen Rapid Stat Color Reagent (8883-479552, Lancer, Foster City, CA 94404). Urea Nitrogen Rapid Stat Acid Reagent (8883-479548, Lancer, Foster City, CA 94404).

Urea Standards. 1.0 mg/mL urea standard: dissolve 1.0000 g of urea (ACS grade) to 1 L with distilled water. 0.10 mg/mL urea standard: dilute a 10-mL aliquot of 1.0 mg/mL standard to 100 mL.

Ammonium Nitrate. ACS grade.

Procedure. *Solid Samples.* Weigh 20.00 g of uncoated ammonium nitrate fertilizer sample in a 50-mL volumetric flask and dissolve with distilled water. Allow to warm to room temperature and then dilute to volume. If the sample is coated, then filter to obtain clear filtrate. Prepare a reference sample in the same manner by using reag-

Mississippi Chemical Corporation, Yazoo City, Mississippi 39194.

ent-grade ammonium nitrate (AN). Also prepare a 15-ppm standard by using 20.00 g of reagent-grade AN plus 3 mL of 0.1 mg/mL urea standard solution and dilute to 50 mL. The 15-ppm (0.3-mg) urea standard is based on 20.00 g of ammonium nitrate weight and not on the final dilution concentration.

Place 5-mL aliquots of reference, standard, and samples in separate test tubes. To each test tube add 4 mL of working reagent. Mix well. Place test tubes in wire basket and immerse the basket in 75 °C water bath. After 20 min immediately transfer the basket to a cold-water bath (ice slush) for 5 min and then to a room temperature water bath for an additional 5 min. Remove test tubes from water bath and mix well to collect condensate from the inner test tube walls.

Absorbance is measured at 525 nm within 30 min. Set the concentration on the 15-ppm standard and read samples directly in ppm of urea. The limit to this method is 25 ppm of urea based on a 20.00-g AN sample. If a sample reads over this amount, then rerun the test by using a smaller sample size. A new reference and standard will have to be prepared by using the reduced AN concentration. Set the concentration mode on the 15-ppm standard. Read the sample. The calculation for samples over 25 ppm of urea is

ppm of urea (20.00 g basis) =
    ppm of urea readout × (20.00 g/sample weight) (1)

Aqueous Samples. For AN solutions, determine the percentage of AN in solution by formaldehyde titration [AOAC Procedure 2.066 Association of Official Analytical Chemists, 1980)]. Calculate the weight of the AN solution to use in the urea determination by

wt of AN solution =
    (20.00 g of AN/% AN in solution) × 100 (2)

Weigh the sample into a 50-mL volumetric flask. Dilute to volume with distilled water and continue with procedure as described under Solid Sample. The sample is read as ppm of urea on a 20.00-g AN basis. For ppm of urea in the original AN solution, use the calculation

ppm of urea = ppm of urea on 20.00 g basis ×
    (% AN of solution/100) (3)

RESULTS AND DISCUSSION

The intensity of the color complex developed is influenced by reaction temperature, reaction time, and AN concentration. As each of these factors increases, an increase in absorbance is noted. The reaction temperature originally was 100 °C. At this temperature samples periodically would develop color, and then a second reaction occurred in which the solution would effervesce and lose its color. The temperature was lowered with a reduction of color intensity but also a reduction in the frequency of color loss. A final temperature of 75 °C was selected.

Reaction times ranging from 8 to 43 min were next examined to determine their effect on color intensity and linearity. Linearity is excellent to 33 min with a gradual decrease in absorbance at longer exposure times. A reaction time of 20 min was selected because it gives the desired accuracy in a reasonable time period. Other series of tests were run in which the reference, standard, and samples were spiked with 1 and 5 ppm of urea. The purpose of this was to investigate whether slight differences in reaction time from each run of samples could be compensated for with urea in the reference. This would allow a calibration curve to be drawn and would eliminate the necessity of running standards with each test. Reaction times of 18 and 22 min in a 75 °C hot-water bath showed
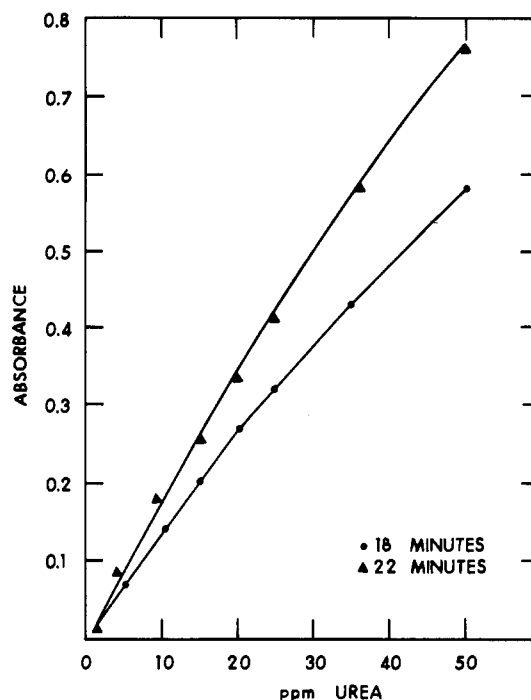

Figure 1. Absorbance at 525 nm of urea standards spiked with 5 ppm of urea at reaction times of 18 and 22 min.
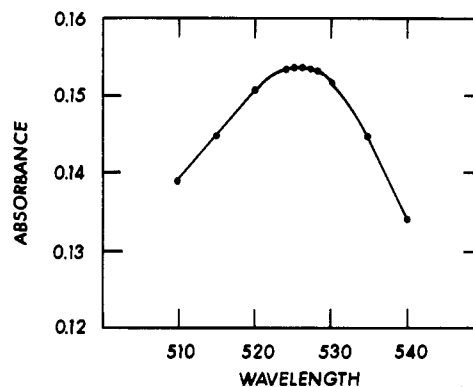

Figure 2. Effects of wavelength on absorbance of 10 ppm of urea in 20.0 g of AN.

a difference in the slope of the curves with an increase in urea concentration (Figure 1). The effect of reaction time is critical with this method. It is of utmost importance that the reference, standard, and sample test tubes be placed in and removed from the water baths at the same time. The use of a wire basket makes this possible.

Ammonium nitrate concentration enhances the color intensity. A 20.00 g of reagent-grade AN/50-mL sample with 0.5 mg of urea gave an absorbance of 0.500 compared to 0.244 with a 5.00 g of AN/50 mL with 0.5 mg of urea sample. It is imperative for the AN concentration to be the same for the reference, standards, and samples in each test. When AN solutions are run, the percentage AN is first determined. Then either the reference and 0.3 mg of urea standard are made up to contain the same AN concentration as the aliquot or an aliquot is used to give a final concentration of 20 g of AN/50 mL. We have found it more convenient to make up samples on a 20.00 g of AN/50 mL basis where the concentration of the AN solution allows.

Absorbance of the red color complex was measured from 510 to 540 nm at 5-nm intervals and from 520 to 530 nm at 1-nm intervals. The maximum absorbance was seen from 524 to 528 nm (Figure 2). The wavelength selected

for use was 525 nm due to the wavelength dial calibration on the Hatachi 100-60 spectrophotometer. The developed color was very stable for at least 30 min.

Physical interferences include coloration, turbidity, and excessive temperature, above 85 °C. Filtering samples of AN usually removes turbidity. Chemical interferences from some primary amines and monosodium dimethyl-naphthalenesulfonate have been noted. These compounds form an orange-colored complex.

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COMMUNICATIONS

Glutathione Conjugate of the Pyrethroid Tetramethrin

Tetramethrin and its cleavage product tetrahydrophthalimide readily undergo Michael addition with thiols. In the case of glutathione (GSH), the resulting tetramethrin–GSH conjugate is less stable than the mercapturic acid conjugates of tetramethrin and tetrahydrophthalimide. The tetramethrin–GSH conjugate is formed under physiological conditions in the presence of mouse liver and housefly abdomen homogenate fractions but probably as a nonenzymatic reaction. The mouse liver soluble thiol level is diminished by intraperitoneal (ip) administration of tetrahydrophthalimide. Mercapturic acid and GSH conjugates of tetramethrin are not evident in the bile or urine of ip-treated rats and mice. Although conjugation with GSH is not a significant factor in the metabolism of tetramethrin, it is interesting to speculate that reversible Michael addition with a critical thiol in the pyrethroid receptor site might contribute to the unique potency and transient character of the neuroactivity of tetramethrin.

Pyrethroid insecticides are biodegraded by hydrolysis and/or oxidation to form acids and alcohols undergoing conjugation (Casida and Ruzo, 1980). Tetramethrin (Figure 1) may be unusual since, by analogy with *N*-ethylmaleimide (NEM) (Kosower, 1976), it has the possibility of undergoing Michael addition with glutathione (GSH) to form **2A** (Figure 2). In addition, tetramethrin may form related conjugates (Figure 2) by cleavage to tetrahydrophthalimide (**1B**) (Miyamoto et al., 1968; Suzuki and Miyamoto, 1974), followed by GSH addition to give **2B** and by subsequent metabolism of GSH conjugates **2A** and **2B** to yield mercapturic acids **3A** and **3B** for urinary excretion. Tetramethrin is unique among the pyrethroids in its high knockdown activity (Kato et al., 1964) and exquisite potency and brief duration of action on cockroach cercal sensory nerves (Gammon et al., 1981). These observations together with the large number of polar, unidentified tetramethrin metabolites (Miyamoto et al., 1968) prompted the present study to evaluate the possible role of GSH and other sulfur nucleophiles in tetramethrin metabolism and action.

MATERIALS AND METHODS

Preparation of GSH and Mercapturic Acid Conjugates. **1A** or **1B** was mixed with an equimolar amount of either GSH or *N*-acetyl-L-cysteine in methanol containing triethylamine (10 equiv). After the mixture was stirred overnight at 25 °C, the solvent was removed under vacuum, and the requisite product (**2A**, **3A**, or **3B**) was isolated in 20–30% yield by TLC (MTBW, Table I). Methanol

Table I. Thin-Layer Chromatographic Properties of the Glutathione Conjugate of Tetrametrhin (2A) and Related Compounds

compd	$R_f{}^a$ BAW	$R_f{}^a$ MTBW
1A	0.71	0.97
2A	0.14	0.66
3A	0.51	0.69
3B	0.41	0.56
GSH	0.11	0.33
N-acetylcysteine	0.41	0.49

^{a} Silica gel F254 chromatoplates, 0.25-mm gel thickness, developed with 1-butanol–acetic acid–water (6:1:1) (BAW) or methanol–toluene–1-butanol–water (10:5:5:4) (MTBW).

solutions of **3A** and **3B** were methylated by addition of excess ethereal diazomethane at 0 °C to obtain **3A-Me** and a mixture of **3B-Me** and **3C-Me**. (Compound **2A** decomposed on attempted methylation.). Each compound was pure based on TLC (BAW and MTBW, Table I). Mass spectral data confirmed compound identities: chemical ionization with methane as the reagent gas, **3A-Me** 509 (16%, M + 1), 341, and 178, **3B-Me** 329 (100%, M + 1), 178, 176, and 152, and **3C-Me** 343 (36%, M + 1), 192 and 152; fast atom bombardment with sodium as the ionizing source [instrument configuration similar to that described by Barber et al. (1981)], **2A** 661 (M + Na)$^+$, 683 (MNa + Na)$^+$, and 705 (MNa$_2$ + Na)$^+$. ^{1}H NMR (CDCl$_3$, 90 MHz) for **3A-Me**, **3B-Me**, and **3C-Me** δ 3.77 (s, OCH$_3$), 3.19 (d,