

## SYNTHESIS OF $^{15}\text{N}$ -LABELLED UREA AND METHYLENEDIUREA

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### SUMMARY

A new technique was developed for the large-scale synthesis of  $^{15}\text{N}$ -labelled urea at low enrichment levels. The synthesis is based on nucleophilic displacement of the phenoxide ion from phenyl carbonate and uses anhydrous ammonia as the nucleophile. In previous reports a copper catalyst was used; however, in this study it was found that the copper resulted in product decomposition and tar formation, which makes product purification difficult. A novel set of reaction conditions was developed: no catalyst was used, and no product decomposition or tar formation occurred. The reaction product was easily purified, and consistently high yields of  $^{15}\text{N}$ -labelled urea were obtained.

$^{15}\text{N}$ -labelled methylenediurea was prepared by the dilute solution reaction of formalin with  $^{15}\text{N}$ -labelled urea. The methodology developed for the reclamation of unreacted urea resulted in minimum loss of labelled urea.

High performance liquid chromatography has been used to determine the chemical purity of both urea and methylenediurea.

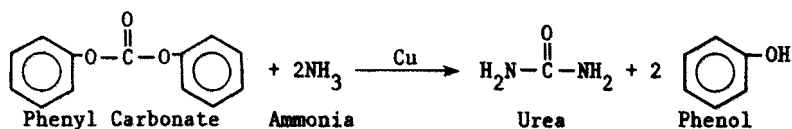
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### INTRODUCTION

The use of  $^{15}\text{N}$ -labelled fertilizer materials in agricultural research is well documented in the literature. A recent review, "Nitrogen--Isotope-Ratio Analysis," by Hauck (1) states that more than 3,000 papers have been published on the use of stable  $^{14}\text{N}$  and  $^{15}\text{N}$  isotopes in agricultural research. In general this research requires large quantities of  $^{15}\text{N}$ -labelled fertilizer materials with an  $^{15}\text{N}$  content of 5-10 atom percent. The rapidly increasing use of urea as a

primary nitrogen source in fertilizers throughout the world necessitates the availability of adequate supplies of  $^{15}\text{N}$ -labelled urea for research.

Rose and Dekker (2) described the use of Wohler's original carbamate route to urea as a preparative technique for the synthesis of  $^{15}\text{N}$ -labelled urea although the yields were poor. The most widely used technique for the synthesis of small amounts of labelled urea has been the phenyl carbonate route originally pioneered by Hentschel (3) and shown in the reaction below.

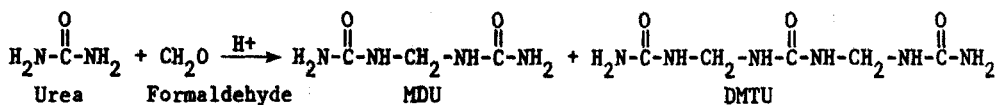


Cavalieri (4) used this reaction with labelled ammonia in the presence of a copper catalyst to prepare labelled urea with a yield of 57% based on starting ammonia. Leitch and Davidson (5) used the same reaction in a sealed glass tube to prepare small quantities of labelled urea with 87% yield.

In a different approach to the synthesis of urea, reported by Applegath et al. (6), carbon monoxide and sulfur were used to prepare carbonyl sulfide in a pressure reactor. Ammonia was added to the reactor to produce isocyanic acid, which reacted with more ammonia to produce urea. This method was investigated (7) as a potential route to labelled urea and showed promising results, but difficulties were encountered in complete removal of sulfur compounds from the product.

Although  $^{15}\text{N}$ -labelled urea has been synthesized, the need remains for a simple, efficient method for producing kilogram quantities of  $^{15}\text{N}$ -labelled urea suitable for agricultural research. There is also a need for  $^{15}\text{N}$ -labelled urea-related compounds such as methylenediurea (MDU), which is a common reaction product of urea and formaldehyde. Previous studies (8) have shown that when fertilizer-grade urea is conditioned with small amounts of formaldehyde (0.2%-0.4%), the main product of the reaction is MDU. It is also well documented that MDU is a major component of ureaform slow-release fertilizers.

The literature contains many reports on the synthesis of MDU but none on the synthesis of the <sup>15</sup>N-labelled compound. Syntheses of MDU from formalin solution and urea have also been reported (9, 10) as shown in the reaction below. The synthesis of MDU is usually accompanied by formation of small amounts of dimethylenetriurea (DMTU).



This paper reports the synthesis of <sup>15</sup>N-labelled urea and MDU by methods that are applicable to all levels of enrichment. It also provides high performance liquid chromatography (HPLC) analyses for the chemical purity of both compounds.

#### MATERIALS AND METHODS

Labelled ammonia (17.1 atom percent <sup>15</sup>N) was supplied by Monsanto Research Corporation, and ultra high purity ammonia (0.366 atom percent <sup>15</sup>N) was supplied by Matheson. Phenyl carbonate was 99% and came from Aldrich Chemical Company. Solvents and other reagents were supplied by Fisher Scientific and were ACS Certified. Labelled urea was synthesized in a 2-liter stirred Parr model 4542 reactor that was equipped with cooling bath and a special gas inlet system as shown in Figure 1. The special inlet system consists of a valve-isolated 500-ml stainless steel Matheson sampling cylinder equipped with a Dewar flask for heating and cooling. A Matheson model 8240 mass flow controller coupled with a model 8122 totalizer for monitoring and controlling the flow of labelled and natural abundance ammonia was installed between the sampling cylinder and a gas inlet manifold. This equipment measured the ammonia flow in liters per minute and the total volume in liters at 21°C and 760 mm of Hg. All tubing connections were 316 stainless steel. Standard laboratory glassware was used for the synthesis of <sup>15</sup>N-labelled MDU, and no special equipment was required.

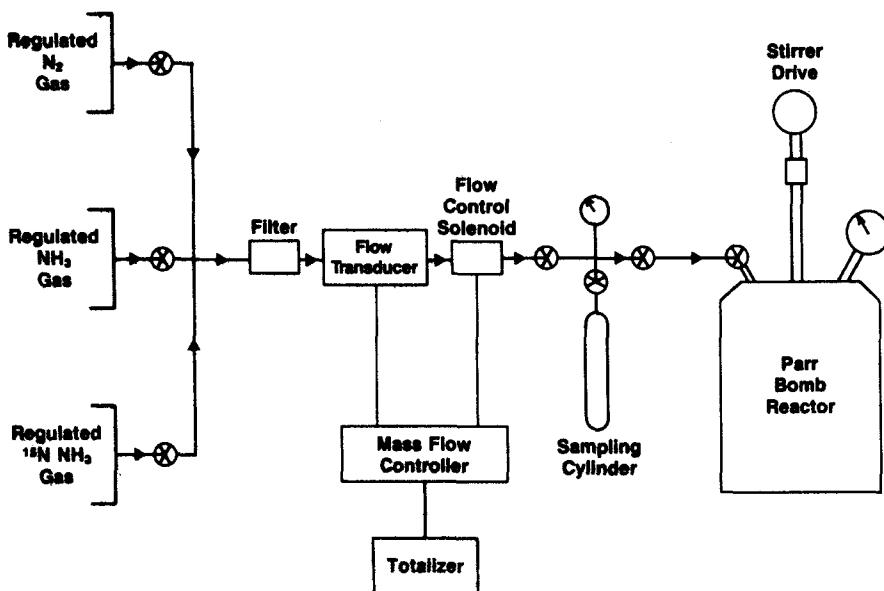


Figure 1. Schematic Drawing of the  $^{15}\text{N}$  Urea Synthesis Process Equipment.

Table I. Parameters for HPLC Analyses

<u>Compound</u>	<u>Retention Time</u> <sup>a</sup> (Minutes)	<u>Sensitivity Factor</u> ( $\text{mg ml}^{-1} \mu\text{V}^{-1} \text{sec}^{-1}$ )	<u>% RSD</u> <sup>b</sup>
Urea	2.03	$9.60 \times 10^{-5}$	1.08
MDU	2.47	$1.05 \times 10^{-5}$	0.19
Biuret	2.66	$2.38 \times 10^{-6}$	0.29
DMTU	3.33	$4.36 \times 10^{-6}$	1.40
Phenol	3.91 <sup>c</sup>	$1.06 \times 10^{-6}$	0.9

a. Flow rate of 1.5 ml/minute.

b. Relative standard deviation in peak area from multiple injections of a single sample used to obtain the sensitivity factor.

c. Eluent for phenol analysis was 50% methanol in water. All other analyses were done with 5% methanol in water.

Chemical purity was determined by using HPLC techniques previously reported (8). Detection was at 200 nM, and a Nelson Analytical Chromatographic Data System was used with an IBM personal computer. The solvent system was 5% methanol in water for analysis of urea, MDU, biuret, and DMTU, whereas 50% methanol in water was used for the analysis of phenol. Sensitivity factors for all analyses appear in Table I.

Isotope analyses were done on a model 622 VG Micromass Isotope Mass Spectrometer according to previously published techniques (11).

## EXPERIMENTAL

### Synthesis of $^{15}\text{N}$ -Labelled Urea

In a typical experiment 3.7 moles (786 g) of phenyl carbonate was melted on a hot plate in a 2-liter glass liner for the Parr reactor. Once molten, the liner was inserted into the reactor and sealed. The system was purged three times with nitrogen introduced through the manifold system, and the reactor was pressure tested. With the system at ambient pressure, the sample cylinder was isolated from the Parr reactor and cooled in liquid nitrogen to transfer the required amount of labelled ammonia into the cylinder. Depending upon the enrichment level desired in the final product, unlabelled ammonia could be added to the labelled ammonia at this time to afford a total of 7.4 moles of ammonia. The desired amounts of labelled and unlabelled ammonia were measured with an ammonia flow controller and totalizer. In a typical experiment 45.2 liters of labelled ammonia at 17.1 atom percent  $^{15}\text{N}$  was mixed with 124.4 liters of natural abundance ammonia to obtain ammonia with an enrichment of approximately 5.0 atom percent  $^{15}\text{N}$ . When the ammonia charge was in place, the sample cylinder was isolated from the inlet manifold and the ammonia was warmed to room temperature and introduced into the molten stirred phenyl carbonate. Since the reaction was exothermic, the reactor was cooled by using an external ice bath. The temperature inside the reactor was not allowed to exceed  $110^{\circ}\text{C}$ . The transfer of the ammonia from the sample cylinder to the reactor was facilitated by

heating the sample cylinder with warm water until a system pressure of 150 psig was obtained. A system pressure of 100 psig was maintained by controlling the ammonia feed rate until all the ammonia was used and the system pressure dropped to ambient. The reaction was terminated when the reaction temperature decreased to 70°C. The system was purged three times with nitrogen before the reactor was disassembled. The purge gas was sparged through 1.0 N H<sub>2</sub>SO<sub>4</sub> to trap any unreacted NH<sub>3</sub> gas.

The liner contents, a solution of urea and phenol, were transferred while molten to a 4-liter stainless steel beaker and stirred until the urea began to crystallize. One liter of ethyl ether was then added to the phenol-urea mixture, and the crystal slurry was cooled in an ice bath and filtered. The filter cake was washed with another liter of ether and air-dried. The filtrate and ether wash were saved for later treatment. The filter cake was placed in a vacuum oven at a temperature of 50°C and a pressure of 1.0 mm for 16 hours. This vacuum-oven treatment removed the bulk of the contaminating phenol. After the crude urea was vacuum dried, it was recrystallized from methanol with 100 g of methanol used for each 60 g of crude urea. An initial mass of crystals was collected by filtration, and the filtrate was concentrated and processed to produce additional crystalline urea. Ether was added to the methanol concentrate to facilitate maximum recovery of the urea. The concentration and crystallization process was repeated four times and yielded a total of 183 g of urea. The HPLC analysis (8) of the combined crystalline urea gave the following: urea, >99%; biuret, <0.05%; and phenol, <0.05%.

Additional urea could be obtained from the original filtrate by transferring the ether-phenol-urea mixture to a 2-liter separatory funnel and extracting the mixture with four 200-ml portions of water. The water extracts were concentrated on a steam bath to one-third the original volume. Five grams of decolorizing carbon was added to the hot solution, and the mixture was filtered. Cooling and the addition of 200 ml of ether yielded urea crystals. The concentration, cooling,

addition of ether, and filtration steps were repeated to produce an additional 14 g of urea which could then be recrystallized from methanol as previously described.

The overall yield for the synthesis was 90% (197 g) based on the starting ammonia. Mass spectral analysis of the product showed the urea to have an enrichment of 4.71 atom percent <sup>15</sup>N.

During production of <sup>15</sup>N urea, the vacuum-dried crude urea from each batch was combined until as much as 5 kg of material was obtained. This amount of material was then recrystallized as described above. Similarly, the ether washings from the many batches were combined and processed. By use of this procedure, three batches of crude urea (approximately 200 g per batch) can be produced per day. A week's production, approximately 3 kg of crude urea, can be processed into labelled urea (>99% pure) in 2 days.

#### Synthesis of <sup>15</sup>N-Labelled Methylenediurea

The procedure of Ludlam (9) was used with modifications as the basis for the synthesis of <sup>15</sup>N-labelled MDU. A urea solution was prepared by dissolving 1,000 g of 5 atom percent <sup>15</sup>N urea in 750 ml of stirred warm water. Five grams of 85% phosphoric acid was added. Over a 1-hour period, 62.5 g of 37% formalin solution was added dropwise to the stirred urea solution. The resulting mixture was stirred for 24 hours at room temperature before being refrigerated an additional 24 hours at 4°C. The resulting crystalline mass was broken and filtered to produce 568 g of urea-MDU mixture. The filtrate (A) was set aside for further processing.

The filter cake was treated with 500 ml of ethanol, heated to 50°C, and filtered hot. This filtrate was combined with filtrate A. The filter cake was slurried with 1,500 ml of ethanol, brought to boiling, and filtered hot to give 33 g of MDU. The filtrate was reduced to a volume of 800 ml and cooled to 0°C. The crystals that formed were filtered to give an additional 22 g of MDU and a filtrate labeled B. The total MDU sample (55 g, mp 196-198°C) was

recrystallized from the ethanol-water mixture to give 11.5 g of chromatographically pure MDU (mp 206-208°C). The remaining filtrate from recrystallization was combined with B.

Additional MDU and unreacted urea were reclaimed by processing filtrates A and B separately. In the first step the volatile alcohol was evaporated under reduced pressure. The samples were then freeze-dried. On the basis of the solubility data for urea and MDU in methanol at 23°C (urea 0.20 g/g and MDU 0.006 g/g), the two samples were stirred for 24 hours at 23°C with sufficient methanol to dissolve the urea. The samples then were filtered to obtain MDU, and the filtrates were evaporated to obtain urea. The MDU and urea separated by processing filtrates A and B, individually, were combined to give a total of 60.5 g of MDU and 882 g of reclaimed urea. The HPLC analyses for these products are given in Table II.

Table II. HPLC Analysis of Products From the Synthesis of  $^{15}\text{N}$ -Labelled MDU

<u>Compound</u>	<u>Weight</u> (g)	<u>% Urea</u>	<u>% MDU</u>	<u>% DMTU</u>
MDU <sup>a</sup>	11.5	0.0	>99.5	0.0
MDU <sup>b</sup>	60.5	0.0	96.2	2.3
Urea	882.5	96.6	2.5	0.3

a. Originally isolated sample.

b. Combined sample after separation from filtrates A and B.

## RESULTS AND DISCUSSION

The urea synthesis described above has been used to prepare over 20 kg of urea with enrichment levels ranging from 4 to 17 atom percent  $^{15}\text{N}$ . The average yield for all preparations to date has been 90%. The experimental procedure described in this paper does not require the use of a catalyst as indicated in the work of Cavalieri (4). When a copper catalyst is used, the induction



period for the start of the reaction is very short, but the reaction product, which is mainly a mixture of urea and phenol, is difficult to purify because of tars and black resins which form during the course of the reaction. When no catalyst is used, as reported in this paper, the reaction product is a clear, amber liquid that is easy to process into a phenol-free product. The HPLC methodology for chemical analysis of the product is very useful in tracking the purity of the product during the purification steps.

The synthesis of labelled MDU makes this important constituent of ureaform fertilizers available for agricultural research. It will be valuable for studying the mineralization rate of MDU in soils, the nitrogen uptake efficiency of MDU in plants, and the nitrogen loss mechanism of MDU.

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