The Determination of Urea in Seawater[1]

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A clinical method for the determination of urea in blood and urine (Beale and Croft 1961[3]) has been modified for the determination of smaller quantities of urea (1–20 μg N/l) in seawater.

Reagents. (A) Dissolve 85 g $NaH_2PO_4 \cdot 2H_2O$ in 1 liter of concentrated H_2SO_4. (B) Dissolve 5 g di-acetyl-monoxime in 100 ml water. Warm to assist solution. Cool, and add 0.06 g semicarbazide hydrochloride. (C) Dissolve 200 g $MnCl_2 \cdot 4H_2O$ and 4 g KNO_3 in 500 ml water.

Procedure. (i) Place a 30-ml sample in a 50-ml test tube containing approximately 5 g solid NaCl. (ii) Add, from a burette, 4 ml Reagent (A). Mix by swirling. (iii) Mix equal volumes of reagents (B) and (C). Add 1 ml to the sample and mix. (iv) Immerse the test tube in a stirred waterbath set at 70°C. (v) After 90 minutes, remove the tube and cool rapidly in running tapwater. (vi) measure the absorbance of the contents in a 10-cm cell at 5200 Å.

A calibration factor should be determined in 20% NaCl solution. A stock urea solution containing 50 μg N/ml is stable for several weeks and may be diluted 100 times just before use. Volumes of 0.5, 1.0, 1.5, and 2.0 ml of this dilute standard in 30 ml of 20% NaCl solution will cover the range of urea so far encountered in seawater. Reagent blanks are also determined in 20% NaCl solution.

The effect of heating is important. Irregular heating (or cooling) produces erratic results. Above 70°C, destruction of the pink color is accelerated, and low yields are obtained. Below 70°C, color formation is slow. Once initiated by warming, color formation continues even at room temperature, but only very slowly, taking up to 48 hours to reach maximum. Once formed, however, the color is stable for at least three days. There is no interference by

1. Accepted for publication and submitted to press 25 January 1967.
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3. Beale, R. N., and D. Croft, 1961, A sensitive method for the colorimetric determination of urea. J. clin. Path., *14*: 418–424.

ammonia, leucine, tyrosine, cystine, arginine, lysine, histidine, or taurine. The reagents are stable for at least three weeks.

The molar (as urea) absorption coefficient of the method is about 18,000. Precision is fair, the coefficient of variation for 10 replicate samples (at a 33 μg N/l level) being $\pm 4.3\%$.

The method has been successfully applied in this laboratory to the determination of urea in samples of seawater collected from the English Channel, and to the measurements of urea excretion by *Calanus helgolandicus* and *Scyliorhinus caniculus*. The results of these investigations will be described elsewhere.