Estuarine, Coastal and Shelf Science (1998) **47,** 415–418
Article No. ec980357

A Room Temperature Procedure for the Manual Determination of Urea in Seawater

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Received 10 July 1997 and accepted in revised form 16 March 1998

Several earlier studies underpin the important role of dissolved organic matter and more particularly urea in phytoplanktonic nitrogen uptake fluxes. Generally, the determination of urea concentrations relies on the formation of an imidazolone-thiosemicarbizide complex, a complexation which requires very accurate temperature control when carried out at high temperature. It is also possible, however, to obtain reliable results with a room temperature procedure. The measured abundances for both complexation at high temperature (85 °C, 20 min) and at ambient temperature (22 °C, 72 h) are closely comparable. Lower values are observed for temperatures <10 °C though. Moreover, a comparison of both techniques reveals similar precision (coefficient of variation: 2%), sensitivity (slope of calibration line: 0·2) and detection limit (0·14 mM). The room temperature alternative to the earlier described method is therefore a handy tool for urea analyses, when a strict temperature control is difficult or impossible. © 1998 Academic Press

Keywords: urea; colorimetric analysis

In their ' classic ' study Dugdale and Goering (1967) introduced the concepts of new and regenerated production, a distinction between two types of primary production which is related to differences in nitrogen source. New production is based on uptake of allochthonous nutrients, mainly supplied by upwelling, atmospheric deposition or riverine input. Regenerated production, on the contrary, consumes autochthonous nutrients, mainly reduced nitrogen such as ammonium and urea. Originally, the model of Dugdale and Goering was restricted to nitrate and ammonium fluxes. However, omission of organic substrates can introduce erratic interpretations of the ecosystem's dynamics; a shortcoming already recognized by Dugdale and Goering (1967). The elucidation of the complex nitrogen cycle requires the qualification of both the dissolved inorganic nitrogen (DIN) and the dissolved organic nitrogen (DON) fluxes. By-products of the metabolism of plankton have long been recognized as important nutrient sources for primary producers in aquatic ecosystems. The contribution of regenerated inorganic nitrogen and phosphorus to oceanic plankton growth, in particular, has been studied extensively (Harrison, 1980, 1993). Moreover, the role of organic metabolic substrates in the uptake regime of phytoplankton received considerable attention too (Bronk *et al.*, 1994).

The importance of urea has not gone unnoted (Remsen *et al.*, 1974; McCarthy, 1980): different studies have illustrated that it is found in significant concentrations in the surface layer of near-shore as well as offshore regions and have emphasized its importance in the nutritional requirements of phytoplankton. It was shown that urea is taken up in preference to DIN species such as nitrate and ammonium, even when the latter nutrients are in excess (McCarthy *et al.*, 1977; Kaufman *et al.*, 1983; Kristiansen, 1983; Probyn & Painting, 1985). Currently, either the indirect urease method or the direct diacetylmonoxime method is used for urea concentration measurements. The urease method (McCarthy, 1970) involves enzymatic hydrolysis of urea, with the released ammonium being assayed by an additional procedure. However, it was demonstrated that the enzymatic method underestimated dissolved urea concentrations as a result of urease inhibition (Price & Harrison, 1987). The non-enzymatic method relies basically on the formation of an imidazolone, which gives rise to the formation of a red complex with thiosemicarbazide at high temperature (Newell *et al.*, 1967; Mulvenna & Savidge, 1992). Its absorbance is measured at 520 nm. This paper describes an adaptation of the modified manual method introduced by Mulvenna and Savidge (1992). The proposed method allows for precise analyses of large numbers of samples when a strict control of thermostatization and cooling, an essential part of the Mulvena and Savidge technique, is not possible.

Mulvenna and Savidge (1992) described a modified manual diacetylmonoxime method with

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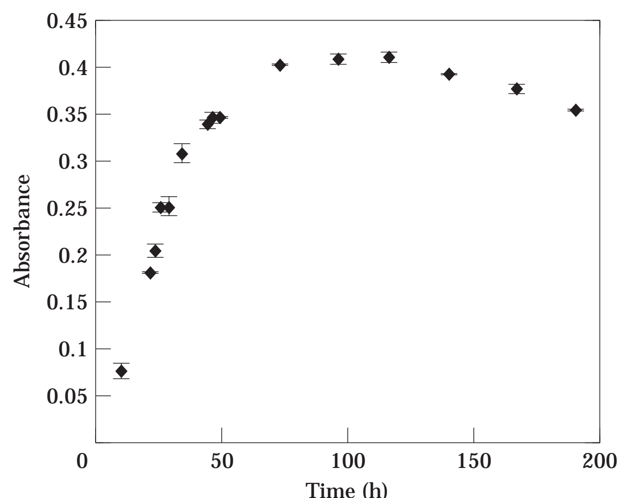

FIGURE 1. Absorbance *vs* time for a 2·0 μM standard solution; absorbances measured with 10 cm optical cells at 520 nm; maximal absorbances obtained after 72 and 120 h are not significantly different (99·9% confidence interval), absorbance values are without correction for the blanks.

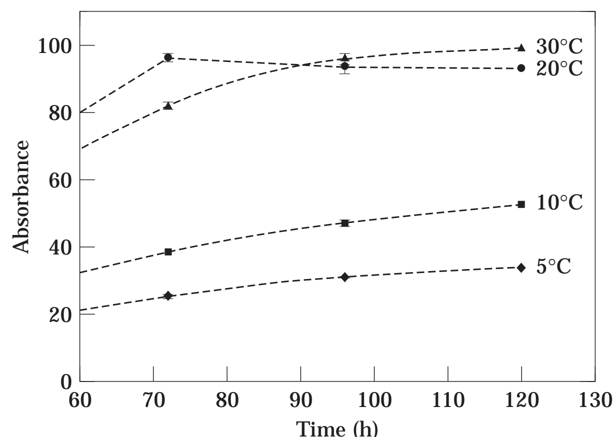

FIGURE 2. Comparison of colour development at different temperatures for 2 μM standard solutions; triplicate measurements of absorbances after 72, 96 and 120 h, absorbance values are without correction for the blanks.

close attention to specific points of the developed procedure. Basically, reagent A (diacetylmonoxime and thiosemicarbazide) and reagent B (sulphuric acid and ferric chloride) are separately added to the samples. Subsequently the bottles are covered tightly in aluminium foil and kept in a water-bath at 85 °C for 20 min. Following the incubation, the solutions are cooled in cold tap water (2 × 5 min) and their absorbances are read at 520 nm. The authors state the need for a very strict standardization of both the heating and cooling times in order to maintain adequate precision of the determination. The earlier observation that heating at temperatures >70 °C accelerates the destruction of the red colour and the contention that complexation at ambient temperature yields a complex which is stable for at least 3 days (Newell *et al.*, 1967) led us to investigate the urea analysis at room temperature in a similar way as is done for the manual determination of ammonium (Koroleff, 1969). The chemical principles of the imidazolone formation remain unchanged. The authors followed the method of Mulvenna and Savidge (1992), but omitted the thermostatization at 85 °C after addition of the reagents. Instead, the samples were stored at ambient temperature (22 °C ± 1) in the dark and their absorbances were measured 3 days later.

The absorbance *vs* time graph (Figure 1) has a curvilinear shape, showing that optimal colour development occurs after 72 h and remains constant for an additional 48 h at ambient temperature (22 °C ± 1). The mean absorbance value amounted to 0·444 (SD = 0·003, N = 9) after 72 h and to 0·450 (SD = 0·004, N = 10) after 120 h, respectively. For dark stored samples the authors did not notice any degradation of the coloured complex during 48 h; after that time the absorbance values decreased, which is in contrast to the earlier observations of Newell *et al.* (1967). Very strict standardizations of the time period between complex formation (addition of the reagents) and absorbance measurement is therefore redundant. However, a word of caution must be added here, since temperature effects on complex formation and colour stability cannot be disregarded completely. A comparison of temperature dependence of absorbances observed for 2 μM standard solutions demonstrates significantly ‘ uncomplete ’ colour development for reaction temperatures <10 °C and ‘ complete ’ colour development for ambient temperature and 30 °C (Figure 2). Normalized absorbance values (percentages of the maximal value, Figure 1) amount respectively to <30 and <50% for standard solutions stored at 5 °C (refrigerator) and 10 °C (thermostated water-bath). On the other hand, standards stored at ambient temperature (~20 °C) in the laboratory and at 30 °C (thermostated oven) gave near maximal absorbance between 72 and 120 h. The absorbance values of the standards stored for 72 h at ambient temperature did not exceed 82%, which must be explained by a temperature reduction for reasons of university economy during weekends. Moreover, the absorbance pattern for standards stored at 30 °C exhibits a weakly decreasing trend, which seems to confirm the earlier observation that heating induces colour destruction (Newell *et al.*, 1967). The latter variability can possibly induce reduced accuracy due to poor sensitivity (at very low temperatures or under conditions of high temperature and prolonged time intervals), but is

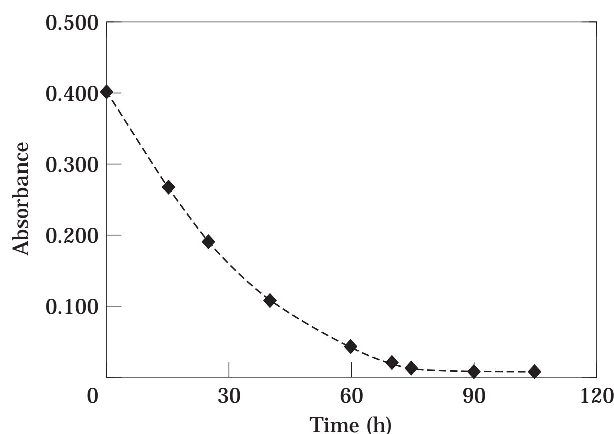

FIGURE 3. Decrease in absorbance due to light exposure; absorbance of a 2·0 μM standard solution measured in 10 cm optical cells at 520 nm.

generally corrected for by concurrent measurement of a standard series.

Additionally, very fast decreases of the absorbance read-out occurred at ambient light after complex formation was complete (Figure 3). The half-life time of the urea complex amounts to <25 min when kept at constant light intensity and 27 °C. This drastic complex decomposition is not accompanied by the formation of other absorbing compounds (Figure 4).

A comparison of the high temperature procedure (HTP) and room temperature procedure (RTP) revealed similar absorbance values, reproducibilities and detection limits (Table 1). Mean absorbances of a 2 μM standard solution, measured with 10 cm cells at 520 nm, amounted to 0·41 (SD=0·01) for HTP and 0·408 (SD=0·005) for RTP. This indicates that the RTP values reach 100% of the absorbance measured with the HTP. Reproducibilities and detection limits of both procedures compare well also. The coefficients of variation (N=12) for a 2 μM standard amount to 2·0 and 1·6% for HTP and RTP, respectively, emphasizing that both analytical procedures prove very similar in reproducibility. Detection limits are calculated as the minimal detectable urea concentrations, using the following formulae:

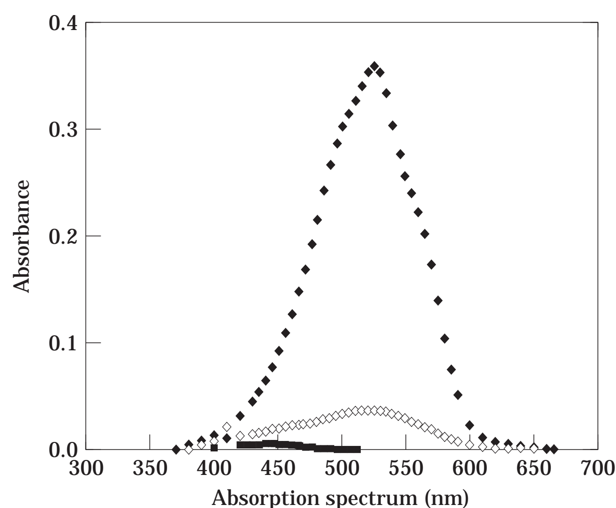

FIGURE 4. Decrease in absorbance due to light exposure shown by the variability of the absorption spectrum from 360 to 670 nm; absorbance of a 30 μM standard solution measured immediately (◆), after 1 h (◇) and 2 h (■) in 1 cm optical cells.

$$\Delta x_{\min} = \bar{x}_1 - \bar{x}_2 > t s_b \sqrt{\frac{N_1 + N_2}{N_1 N_2}} \quad (1)$$

$$S_b = \sqrt{\frac{x_b^2 - [\Sigma x_b]^2 / N_2}{N_2 - 1}} \quad (2)$$

where $\Delta x_{\min}$=minimal detectable absorbance value, x_b=mean absorbance value of the blanks, s_b=standard deviation of the blanks, t=t-value for the 99% confidence limit, N_1=number of analyses (2), N_2=number of analysed blanks (13).

Calculated detection limits are 0·14 μM for the HTP and 0·10 μM for the RTP, which is in perfect agreement with the value of 0·13 μM given by Mulvenna and Savidge (1992).

External standardization following the HTP shows good linear obeyance to Beer's law within a concentration range of 0–5 μM. Examination of higher concentrations is not warranted here since oceanic waters generally exhibit concentrations ranging from 0 to 3 μM. The correlation coefficient and slope are 0·995

TABLE 1. Comparison between high temperature (duplicate measurements in 10 cm optical cells) and room temperature procedures (10 measurements in 10 cm optical cells)

	HTP	RTP
Absorbance (2·0 μM)	0·41 (SD=0·01)	0·408 (SD=0·005)
Reproducibility (2·0 μM)	2·0%	1·6%
Detection limit	0·14 μM	0·10 μM

HTP, high temperature procedure; RTP, room temperature procedure.

TABLE 2. Comparison of calibration regressions for high temperature procedure (HTP) and low temperature procedure (LTP) (external standardization method; duplicate measurements in 10 cm optical cells)

	Slope	Standard error of slope	Correlation Coefficient	*P*-value
HTP	0·21	0·01	0·995	0·00001
RTP	0·19	0·01	0·992	0·0002

and 0·21 (SE=0·01). The corresponding molar extinction coefficient is $2{\cdot}1 = 10^4\ \mathrm{M}^{-1}\ \mathrm{cm}^{-1}$. Application of the RTP evidences a similarly good linear regression for the concentration range of 0–10 μM. The correlation coefficient and slope amount to 0·992 and 0·19 (SE=0·01), respectively. The observed linear response is comparable with data obtained by Mulvenna and Savidge (1992), who found that Beer's law was obeyed within the range from 0–15 μM. DeManche *et al.* (1973) as well as Aminot and Kerouel (1982) found a good linear obeyance to the Beer's law in this range for their automated high temperature method. Descriptive statistics are summarized in Table 2.

Generally, the diacetylmonoxime method does not require complex blanking correction (Aminot & Kerouel, 1982) and a comprehensive examination of the method's specificity revealed negligible interference of numerous inorganic and organic compounds with the exception of citrulline (DeManche *et al.*, 1973; Aminot & Kerouel, 1982; Price & Harrison, 1987). The accuracy of the RTP was compared to results of external standardization and standard addition methods. The authors analysed seawater samples taken in the Southern Bight of the North Sea and kept in 60 l containers in the laboratory without addition of any preservation reagent. The correlation coefficient and slope for standard addition method were 0·9998 and 0·212 ± 0·002, respectively, which is in good agreement with 0·992 and 0·19 ± 0·01 for external standardization. Fitted values for concentrations obtained by external standardization and standard addition methods amounted to 0·23 and 0·22 μM, respectively.

This comparative study shows that the RTP for the manual determination of dissolved urea is highly reproducible and sensitive. The maximal absorbance values are well comparable with those for HTP. Moreover, values determined with external standardization and standard addition procedures revealed that the method does not require laborious blank correction, nor does it suffer from any significant interference. This modest revision to an already existing urea analysis method might be inconvenient though, when scientists performing incubation experiments require a ' quick ' answer. On the other hand, it is very a suitable and cheap analytical tool for field studies (e.g. on board research vessels), when a strict control of time interval and reaction temperatures is difficult or even impossible.

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