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APPLICATION NOTE 71461

Enzymatic analysis of urea in swimming pool water

Authors

Kirsti Nikkola,[1] Marita Jokinen,[2] Tuula Jumppanen,[1] Mari Klemm,[2] Juhani Airo,[1] and Annu Suoniemi-Kähärä[2]

[1]Metropolilab Oy, Helsinki, Finland

[2]Thermo Fisher Scientific, Vantaa, Finland

Keywords

Discrete Analyzer, Photometric Analyzer, Aquakem

Goal

To use an enzymatic method for determination of urea in the water of swimming pools

Introduction

Nitrogen-containing impurities such as urea, ammonia, amino-acids, creatinine, and uric acid introduced to swimming pool water by bathers react with free chlorine to form chlorine-containing compounds.[1] It is important to control the level of urea in swimming pool water because urea is a potential source of hazardous ammonia chloramines and a possible nutrient for bacteria and algae, all of which pose a hygienic risk.

In Finland, urea concentration in swimming pools is regulated by Valvira (National Supervisory Authority for Welfare and Health). Based on current quidelines, urea concentration must be less than 0.8 mg/L.

Urea is typically measured by the Koroleff method,[2] which is based on persulphate digestion. Urea can also be measured using an enzymatic method. In the SYKE (Finnish Environment Institute) swimming pool water report,[3] six of the proficiency test participants used the Koroleff method and three laboratories used the enzymatic method. Based on the study, the Koroleff method results were generally lower than results obtained from the enzymatic method. Results of the enzymatic method were closer to

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calculated results of the proficiency test samples. By switching from the Koroleff method to the enzymatic method, the result levels are expected to be more accurate and therefore higher than previously reported results.

This more accurate enzymatic method can be easily automated using the Thermo Scientific™ Gallery™ or Aquakem™ discrete analyzers with the capability of more than a hundred results typically reported in one hour.

Method Principles

The test is based on the enzymatic reaction of urease and glutamate dehydrogenase (GLDH) as illustrated below.

$$Urea + 2H_2O \xrightarrow{Urease} 2NH_4^+ + 2HCO_3^-$$

$$2\text{-}Oxoglutare + NH_4^+ + NADH \xrightarrow{GLDH} L\text{-}Glutamate + NAD^+ + H_2O$$

The method is performed at 37 °C using a 340 nm filter. The determined difference between ammonia with and without enzymatic conversion by urease indicates the value for urea. Reagents for this test are prepackaged and ready to use.

Results are calculated automatically by the analyzer using a calibration curve. Measured ammonia expressed as urea includes the amount of free ammonia plus the amount of ammonia after splitting urea with urease. The amount free ammonia should be measured in another analysis if that is the result desired. Urea is calculated by subtracting the content of free ammonia from total ammonia.

Experimental

Materials and methods

Sample preparation

Prior to analysis, sample dechlorination with sodium thiosulfate is recommended to remove chlorine interference. In the Gallery and Aquakem discrete analyzer swimming pool water applications, the dechlorinating reagent was added automatically to the test flow and required no further manual declorination steps. All samples were analyzed within 2 days.

Testflow for Aquakem discrete analyzer

Urea (Ammonia) test flow

The application consists of dispensing 80 µL of sample followed by adding 3 µL of Reagent R4 (dechlorination), then 40 µL of Reagent R1 followed by 10 µL of Reagent R2. The mixture is incubated for 300 seconds and then a blank measurement is taken to eliminate interference resulting from sample color. The reaction is completed by adding 10 µL of Reagent R3 and incubating for 900 seconds. Absorbance is measured at 340 nm.

The method is calibrated a using 6 mg/L stock solution, which is automatically diluted.

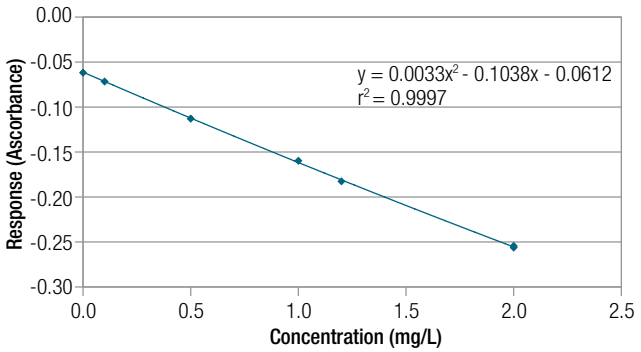

Figure 1. Calibration graph

Ammonia is measured separately by an application designed for low (500 µg/L) ammonia levels. This method is based on a salicylate and sodium nitroprusside reaction at an alkaline pH=8. The application consists of dispensing 100 µL sample, adding 15 µL of Reagent R1, blanking, adding 15 µL of Reagent R2, incubating for 540 seconds, and measuring at 660 nm.

Calibration is performed using a 2 mg/L stock which is automatically diluted. The calibration fitting is polynomial.

Calculated test for urea

This equation converts the ammonia result (µg N/L) to urea (mg/L) by subtracting it from urea (ammonia) (mg/L) results using the formula below.

$$Urea\ (mg/L) = Urea\ (Ammonia)\ (mg/L) - ((Ammonia\ as\ N\ (\mu g/L) \times 2.144)/1000)$$

Other methods

Samples were sent to an external laboratory for a separate enzymatic method analysis (referred to as the enzymatic reference method). The reference method was an enzymatic urea method performed using the Aquakem discrete analyzer at Aqualab Zuid in the Netherlands.

The Koroleff method is an accreditated method at Metropolilab in Finland.

Results and discussion

Method correlation studies

According to these method correlation studies, both enzymatic methods correlated well with each other (r^2 = 0.995) as shown in Table 1 and Figure 2. However, the Koroleff method correlated better with low level samples. Samples with high concentration seemed to have lower recoveries when compared to the enzymatic measurement.

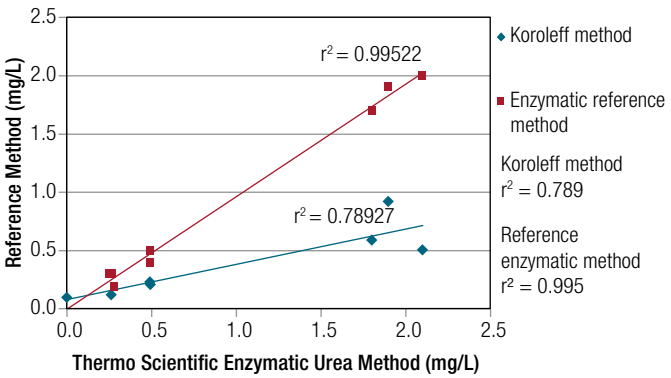

Figure 2. Correlation graph of the Thermo Scientific enzymatic urea method compared to the Koroleff and enzymatic reference methods

Analysis of errors

Determination limit

A control sample of 0.1 mg/L was measured 25 times and the changes between the urea results and theoretical urea values were reported. Results ranged from 0.080 to 0.130 mg/L and the calculated determination limit was set at 0.064 mg/L.

Systematic error analysis

Systematic error was determined using two concentrations, 1 and 0.2 mg/L. For the 1 mg/L concentration, the error rate calculated from the result and the theoretical value changed from 0 to 18%, at an average of 6.52%. Samples were analyzed over a 3 month period and represented multiple reagent lots and calibrations.

Similar analysis was performed using a 0.2 mg/L control sample. The average error from the calculated theoretical concentration was 18.13%. As described in the introduction, the enzymatic urea test provided results calculated from separate measurements of urea and ammonia. Calculated tests often create more errors because they are based on two independent chemistries. Chemical analysis of ammonia is also very sensitive to atmospheric contamination. These facts may explain the higher than average error in low concentration samples.

Table 1. Results of urea analysis using two enzymatic methods and the chemical Koroleff method

Sample	Thermo Scientific Enzymatic Urea Method (mg/L)	Enzymatic Reference Method (mg/L)	Koroleff Reference Method (mg/L)
1	0.00	<0.2	0.10
2	0.26	0.30	0.12
3	1.90	1.90	0.92
4	0.27	0.30	<0.1
5	1.80	1.70	0.59
6	0.28	0.20	<0.1
7	0.49	0.40	0.21
8	0.49	0.50	0.23
9	2.10	2.00	0.51

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Profiency test results

According to the profiency test shown in Table 2, the Thermo Scientific urea method correlated well with the samples tested. These results provided a higher level of confidence demonstrating that enzymatic methods are more accurate than the Koroleff method.

Conclusion

The Thermo Scientific urea enzymatic method is a more accurate and specific test for urea concentrations. The Koroleff method measured higher concentration samples with a lower recovery than enzymatic methods. Changing from the Koroleff method to the enzymatic method improved accuracy with high concentration samples. The two different enzymatic methods used in this study correlated well with one another. The Thermo Scientific method measured in a profiency test also demonstrated excellent results. This method is a calculated test based on individual urea and ammonia measurements and is repeatable at higher concentrations with a determination limit that can be set as low as 0.064 mg/L.

References

1. Wojtowicz, J. Cyanuric and Isocyanuric Acids. Kirk- Othmer Encyclopedia of Chemical Technology, John Wiley & Sons, 2000.
2. Koroleff, F. Determination of Urea. In Methods of Seawater Analysis, Grasshoff, K., Erhardt, M. & Kremling K., eds. Weinheim, *Verlag Chemie,* **1983,** pp. 158–162.
3. SYKE (Finnish Environment Institute) Swimming Pool Water Report, 2013.

Table 2. Results of the SYKE profiency test

Sample	Result (mg/L)	Expected Value (mg/L)	Profiency Test Result Status
A1U (Synthetic)	0.56	0.54	Excellent
U2U (Swimming Pool Water)	0.995	0.96	Excellent
U3U (Swimming Pool Water)	0.575	0.54	Excellent

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