Method No. G-171-96 Rev. 14 (Multitest MT19)

Ammonia in Water and Seawater

Ranges: 0 - 3 to 0 - 27 µmol/L (0 - 42 to 0 - 380 µg/L as N)

and 0- 25 to 0- 300 µmol/L (0-0.35 to 4.2 mg/L as N)

Description

This method uses the Berthelot reaction, in which a blue-green coloured complex is formed which is measured at 660 nm. A complexing agent is used to prevent the precipitation of calcium and magnesium hydroxides. Sodium nitroprusside is used to enhance the sensitivity.
Alternative reagents are given for reaction with salicylate and phenate.

Hardware: 37°C heating bath (5.37 mL) **Pump tubes:** 6 + 2 air + sampler wash

Multitest: aluminium, ammonia, colour, copper, chloride, iron, manganese, nitrate, total N in persulphate digests, nitrogen (total Kjeldahl), phosphate, total phosphorus (Kjeldahl), silicate, sulphide and zinc.

Performance data using synthetic seawater standards and AA3 colorimeter

Test conditions: range: 0 - 10 µmol/L ð yel/yel sample tube, AA3 colorimeter, 10 mm flowcell and lamp
Reagents: **Salicylate**

	Sample A 0-10 µmol	**Sample B 0-300 µmol**
Sample tube	yel/yel	orn/grn
Sampling rate	60/h	60/h
Sample : wash ratio	4:1	4:1
Sensitivity at 10 / 300 µmol/L	0.04-0.07	0.15-0.19
Reagent absorbance	0.02-0.04	0.02-0.04
Coefficient of Variation	0.3%	
Pooled standard deviation 25 at 5 levels	0.05 µmol/L	
Correlation Coefficient (5 points, linear)	0.999	
Detection limit (determined according to EPA procedure pt. 136, app. B)	0.040 µmol/L (0.56 µg/L as N)	
Detection Limit (EPA pt. 136, app B) Range 0-3 µmol/L (0-40 µg/L N)	0.034 µmol/L (0.48 µg/L as N)	

Note: The above performance specifications were obtained with the exclusive use of genuine SEAL Analytical parts and consumables.

SEAL Analytical

REAGENTS

Unless otherwise stated all chemicals should be of Analytical Reagent grade or equivalent (e.g. ACS grade, Analar, Pro Analysi).

LIST OF RAW MATERIALS

	safety classification
Ammonium sulfate, $(NH_4)_2SO_4$	--
Brij-35, 30% solution	--
Dichloroisocyanuric acid sodium salt dihydrate, $C_3Cl_2N_3NaO_3.2H_2O$	oxidizing, harmful
or: Sodium hypochlorite solution, NaOCl (see Note 16)	harmful
Ethylenediamine tetra-acetic acid disodium, $C_{10}H_{14}N_2Na_2O_8.2H_2O$	--
Hydrochloric acid 36-38%, HCl	corrosive
Sodium hydroxide, NaOH	corrosive
Sodium nitroprusside, $Na_2[Fe(CN)_5NO].2H_2O$	toxic
Sodium salicylate, $C_7H_5NaO_3$ (for salycilate chemistry only)	harmful
tri-Sodium citrate dihydrate, $C_6H_5Na_3O_7.2H_2O$	--
Sodium chloride, NaCl (for artificial seawater)	--
Sodium hydrogen carbonate, $NaHCO_3$ (for artificial seawater)	--
Phenol, C_6H_5OH (for phenol chemistry only)	toxic
Low-nutrient seawater: See note 2	

REAGENT MAKE UP

Prepare reagents with distilled water or deionized water. Vacuum filter reagents through a filter with pore size 0.5 µm or less for best results. Be sure to avoid ammonia contamination: check the reagent absorbance before starting the analysis.

SAMPLER WASH SOLUTION = ARTIFICIAL SEAWATER (only for Seawater Analysis)

(see operating note 1)

Sodium chloride	35 g
Sodium hydrogencarbonate	0.5 g
DI water	to 1000 mL

Dissolve 35 g of sodium chloride and sodium hydrogen carbonate in about 900 mL of DI water. Dilute to 1000 mL with DI water and mix thoroughly.

SYSTEM WASH SOLUTION

Use DI Water containing 2 mL/L Brij-35, 30% solution.

SPECIAL WASH SOLUTION

Use a 1 N HCl solution (about 83 mL /L conc. hydrochloric acid). This should be pumped through all reagent tubes once a week for 10 minutes.

COMPLEXING REAGENT
see operating note 4
for salicylate and phenate chemistry

EDTA	30 g
tri-Sodium citrate dihydrate	120 g
Sodium nitroprusside	0.5 g
DI water	to 1000 mL
Brij-35	3 mL

Dissolve 30 g of EDTA, 120 g of tri-sodium citrate dihydrate and 0.5 g of sodium nitroprusside in about 800 mL of DI water. Dilute to 1000 mL with DI water. Add 3 mL of Brij-35. Store in an amber bottle. Prepare fresh every 2 weeks.

SALICYLATE Reagents

DICHLORO ISOCYANURIC ACID (DCI)
only for salicylate chemistry

Dichloroisocyanuric acid sodium salt dihydrate	0.2 g
Sodium hydroxide	3.5 g
DI water	to 100 mL

Dissolve (completely !) 3.5 g of sodium hydroxide (see operating note 4) in about 80 mL of DI water. Add 0.2g (see Note 13) of dichloroisocyanuric acid sodium salt dihydrate and dilute to 100 mL with DI water and mix thoroughly. Prepare fresh every day. Store in a glass bottle. See also Note 14.

SALICYLATE
only for salicylate chemistry

Sodium salicylate	300 g
DI water	to 1000 mL

Dissolve 300 g of sodium salicylate in about 800 mL of DI water. Dilute to 1000 mL with DI water. Store in an amber bottle. Prepare fresh every two weeks.

PHENATE Reagents
(see operating note 5)

DICHLORO ISOCYANURIC ACID (DCI)
only for phenate chemistry

Dichloroisocyanuric acid sodium salt dihydrate	1 g
DI water	to 100 mL

Dissolve 1 g (see Note 13) of dichloroisocyanuric acid sodium salt dihydrate in about 80 mL of DI water. Dilute to 100 mL with DI water and mix thoroughly. Prepare fresh every day. Store in a glass bottle. See also Note 14.

PHENOL
only for phenate chemistry

Phenol	50 g
Sodium hydroxide	36 g
DI water,	to 1000 mL

Dissolve 36 g of sodium hydroxide (see operating note 4) and 50 g of phenol in about 800 mL of DI water. Dilute to 1000 mL with DI water. Store in an amber bottle. Prepare fresh when the solution becomes brown.

STANDARDS
(see operating note 2)

STOCK STANDARD, 100 mg/L as N

Ammonium sulfate	0.4717 g
DI water	to 1000 mL

Dissolve 0.4717 g of ammonium sulfate in about 600 mL of DI water. Dilute to 1000 mL with DI water and mix thoroughly.

Prepare working standards as required.

OPERATING NOTES

1. For seawater analysis use artificial seawater. For water and wastewater analysis use DI water as sampler wash solution. See also operating note 2.

2. The diluent for the standards should have the same matrix as the samples. Therefore, use artificial seawater or low-nutrient seawater for seawater analysis. To avoid errors from ammonia content in the inorganic salts used for artificial seawater, we recommend using a zero calibration standard of low-nutrient seawater of known low concentration. This is obtainable from Ocean Scientific International, Station Road, Petersfield, Hampshire, England GU32 3ET. Fax +44 1730 265011.

3. Recommended procedures for best performance when analyzing low concentrations

- § Pure water may be double distilled (DD) water or deionized (DI) water. In the case of DDW, the analyst must be careful to avoid contamination with silicic acid from dissolution of glass.
- § For accurate low-level work, all glassware used for making reagents should be rinsed with 10% hydrochloric acid followed by thorough rinsing with DI water two or more times. Store flasks “shaken dry” and capped. Regular cleaning of storage containers reduces variances in analytical results. Do not wash the glassware in a washer or with any kind of detergent.
- § Sample cups must be perfectly clean. For low-level work, fill sample cups with 10% hydrochloric acid and leave standing for at least 15 min. Then rinse the sample cups twice with DI water followed by two rinses with sample or standard solution.
- § Sample storage or transport containers may be made of any of several plastics. High density polyethylene or polypropylene bottles are very acceptable. Glass containers of any kind are not acceptable. Any glass contaminates the samples with silicic acid. Sample containers must be rinsed at least twice with sample before filling.
- § Skin contact must be avoided with anything which will touch the reagents and samples. Ammonia contamination of the air must be avoided (e.g. by smoking, farmyard, industrial smoke or vapour, other reagents).
- § The laboratory temperature should be reasonably stable, with no strong air currents around analyzer. Run the system with the manifold cover in place.
- § All chemicals should be of very high purity. Old and/or contaminated SDS will cause carryover, drift and noise. Final working standards are best prepared using natural artificial seawater of low nutrient content (see operating note 1 and 2).
- § The prepared reagents should be degassed by vacuum membrane filtration for best performance. Filter with a pore size of 0.5 µm or less should be used. The reagents, pure water and standards should be protected from atmospheric contamination.
- § Samples should be measured as soon as possible after sampling.
- § Rinse the manifold according to operating note 8. Rinse wash receptacle each day by pumping baseline reagents for 15 minutes before starting a run. Clean the wash receptacle once a month with hypochlorite solution.
- § The volume between the air valve and the injection fitting should be minimal, using 0.015" polyethylene tubing cut as short as possible. The joints between glass parts must be perfect without gaps.

§ A regular bubble pattern is necessary for low noise. If the bubble pattern is irregular, check that all plastic tubing is correctly wetted (bubble shape round at front and back. After replacing the pump tubes or parts of the manifold, pump 1M NaOH through all tubes for 15 minutes. (see also operating note 15).

4. If running only in the lowest range, connect the colour reagent to the line for sample B and tie off the line for the colour reagent. The baseline noise, and thus the detection limit, can then be further reduced by diluting the reagents by a factor of 2 or even 5. The linearity of the used range must be checked. The high amount of EDTA in the complexing reagent is only necessary for seawater samples containing high concentrations of calcium and magnesium ions and when using the yel/yel sample pump tube. At lower calcium ($\leq$ 0.005 mol/L) and magnesium ($\leq$ 0.04 mol/L) concentrations, or when using the orn/grn sample pump tube, the EDTA concentration can be reduced to 15 g/L.

5. **Final pH**
   For optimum results the pH of the final reaction solution must lie within certain limits. Collect the solution from the flowcell waste line to check the pH.
   Final pH (with salicylate reagents) pH 12.8 - 13.1
   Final pH (with phenate reagents) pH 11.5 - 11.9
   If the final pH is too high, reduce the sodium hydroxide concentration . If the pH is too low, increase the sodium hydroxide concentration.

6. **WARNING for Seawater Analysis** Without salicylate and phenol the hydroxides of magnesium and calcium precipitate after the addition of DCI. Therefore add salicylate and phenol first or remove it last when starting up or shutting down the system. During the day it may occur that a slight precipitation occurs in the 5-turn coil after the DCI addition. This does not affect performance. If it occurs, during the shutdown procedure clean the system with 1 N HCl. Be sure that the salicylate has been washed out first, otherwise there will be a precipitate of salicylic acid, which will block the tubing.

7. Ammonia is a common contaminant in the atmosphere and the general environment. Take extra precautions to avoid contamination of the reagents. Do not touch any surfaces which will be in contact with reagents or samples. Check the reagent absorbance each time fresh reagents are made: if it is too high, the detection limit will be increased. Rinse sample cups with sample before filling them.

8. **Manifold cleaning procedure:**
   Every day: use system wash solution (2 mL/L Brij-35, 30% solution)
   Once a week, or when a precipitate is visible in the coils, pump for 10 min. special wash solution (1 N HCl) through the system and the sample line, then 30 min. system wash solution

9. If dual-range operation is not needed, remove sample line B and tie off or remove the T-piece.

10. If dual-range operation is needed, pump DI water through the sample line which is not connected to the sample probe.

11. **Wavelength Filter**
    Salicylate reagent: 660 nm
    Phenol reagent 630 nm

12. If nitrate is not used on this multitest cartridge, remove the polyethylene tubing between the C3 and the A10 fitting and connect them directly using a sleeve made of 116-0536-16 tubing.

13. **Concentration of free chlorine reagent**. The concentration of free chlorine in the reaction mixture is critical to correct sensitivity and linearity. As neither the source chemical nor the solution are stable, the concentration should be adjusted by experiment if the method becomes non-linear or the sensitivity is low. Baseline noise and drift will be optimized by using the lowest concentration which gives acceptable results.

14. If DIC is not readily available, sodium hypochlorite can be used as an alternative chlorine source. In most cases, diluting the stock solution 10:1 with water will produce an acceptable working reagent whose concentration should then be optimised by experiment. Reagent optimisation should be repeated every few months or when a new bottle of chemical is opened. If using NaOCl, which is alkaline, check the pH after optimising the concentration of the NaOCl.

15. Even flow and regular air/liquid distribution in the transmission tube from the debubbler after the first mixing coil to the pump is critical to correct method performance. Check for correct flow and that the tubing is wetted (the trailing edge of the bubbles must be rounded, not straight). If necessary, especially for new tubing, increase the concentration of surfactant to achieve correct wetting. See also (17).

16. LED Photometer
    By the operation of the AA3 on research vessels it is recommend to use the LED photometer. The noise of the signal caused by vibration and movement of the ship is reduced compared to the lamp photometer. The special filter for the LED must be used. The filter from the lamp photometer can not be used for the LED. LED Assy is available with 660 nm and therefore the salicylate chemistry should be run with the LED photometer. The performance data may change slightly by the use of the LED photometer.

17. If the bubble pattern out of the heating bath becomes irregular, it may help to change the second air tube from blk/blk to orn/orn.

REVISIONS

Revision 1, February 1999
Added AA3 data; integrated flow diagram

Revision 2, May 2000
Added sodium hypochlorite as alternative chlorine source. Revised Notes and artificial seawater formula.

Revision 3, March 2001
Added operating note 18.

Revision 4, April 2001
Added glassware inside AA3 colorimeter, redrawn flowchart

Revision 5, August 2001
Added 4-way valve in flowchart, redrawn flowchart, retyped operating notes, removed AAII performance data.

Revision 6, February 2002
Added LED photometer, expanded notes

Revision 7, October 2002
Removed Ca and Mg salts and bicarbonate from list of chemicals.
AAII flowcell and pull-through pump tube removed.

Revision 8, December 2002
First air tube changed from orn/wht to blk/blk.

Revision 9, February 2005
Revised details on DIC reagent and Note 13. Changed air bar tubing.

Revision 10, March 2005
Added Note 17.

Revision 11, May 2008
Changed logo

Revision 12, August 2008
Mg and Ca salts removed from raw materials, artificial seawater updated.

Revision 13, July 2009
Air supply and de-/rebubbling optimized, flowchart and consumables list updated

Revision 14, September 2009
Flowchart corrected

CONSUMABLES

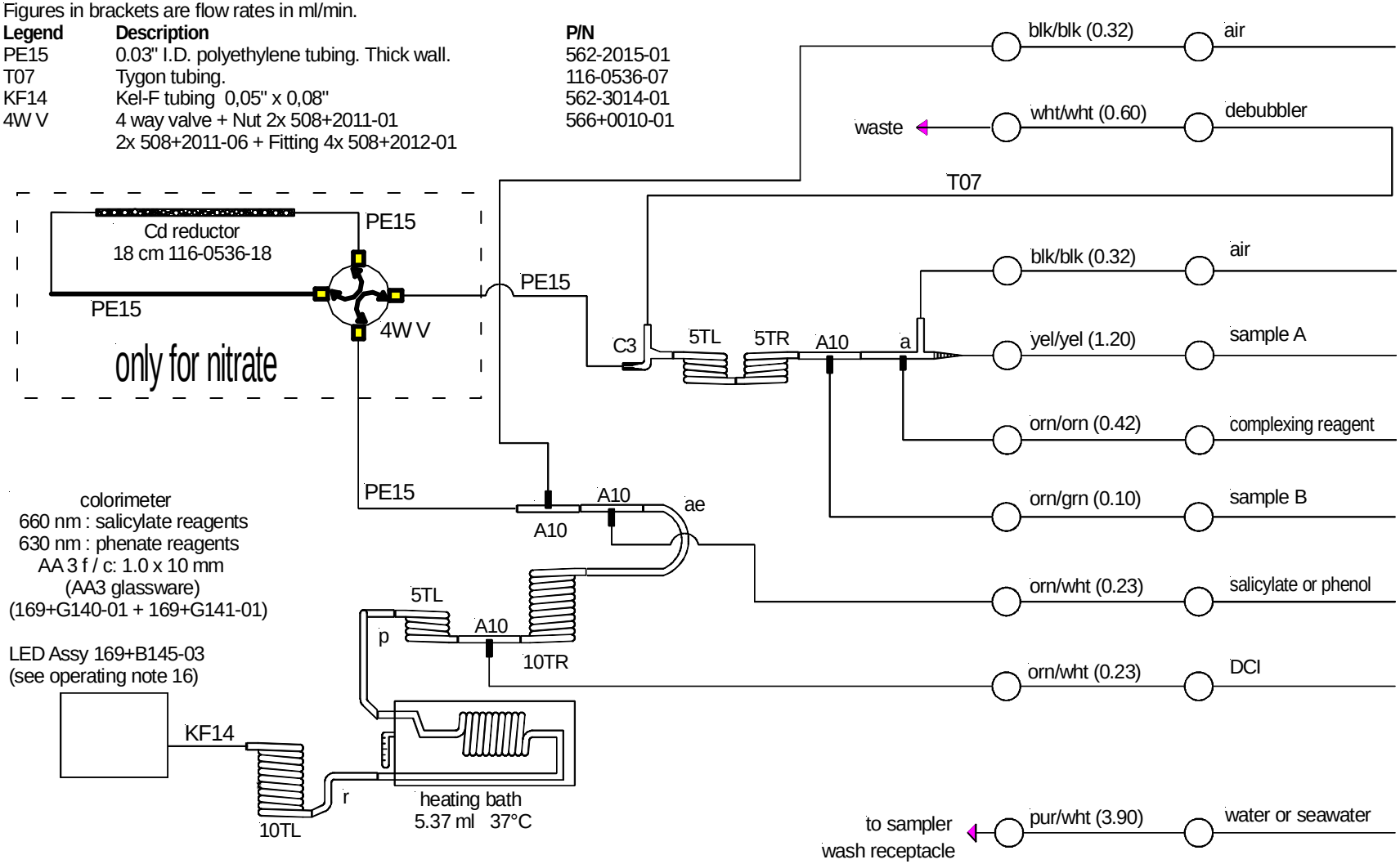
The following estimated annual consumption rates are based on system operation 8 hours/day, 250 days/year.

ORN/GRN, 0.10 mL/min		116-0549-04	1 pkg./12
ORN/WHT, 0.23 mL/min		116-0549-06	2 pkg./12
BLK/BLK, 0.32 mL/min		116-0549-07	2 pkg./12
ORN/ORN, 0.42 mL/min		116-0548-08	1 pkg./12
WHT/WHT, 0.60 mL/min		116-0549-09	1 pkg./12
YEL/YEL, 1.20 mL/min		116-0549-12	1 pkg./12
PUR/WHT, 3.90 mL/min		116-0549-18	1 pkg./12
Air valve tubing, Pharmed		117+0539-07	1 m
Polyethylene tubing 0.015” ID	PE15	562-2002-01	1 m
Polyethylene tubing 0.03" ID		562-2015-01	1 m
Tygon tubing	T07	116-0536-07	2 m
Tygon tubing		116-0536-11	1 m
Tygon tubing		116-0536-16	1 m
Tygon tubing (for nitrate)		116-0536-18	1 m
Kel-F tubing 0.050" x 0.08"	KF14	562-3014-01	1 m
Sample cups, plastic, 5 mL		171-0354-01	1 pkg./1000
Sample cups, plastic, 4 mL		127-0018-01	1 pkg./1000
Sample tubes, plastic, 8 mL		168-1000-01	1 pkg./2000
Sample tubes, plastic, 11 mL		168-1001-01	1 pkg./1600
Tubes for standards, 15.5 mL (XY2/3)		168-1004-01	1 pkg./100

SPARES			Recommended holding
AA3 Flowcell, 1.0 x 10 mm		169+B045-10	1 pc.
AA3 Filter Assy, 630 nm		165+B044-63	1 pc.
AA3 Filter Assy, 660 nm		165+B044-66	1 pc.
AA3 Colorimeter lamp		169+B143-01	1 pc.
AA3 colorimeter glassware		169+G140-01	1 pc.
AA3 colorimeter glassware		169+G141-01	1 pc.
Injection fitting, 3 pt.	a	116-0489-01	1 pc.
Glass coil, 5 turns right	5TR	170-0103-01	1 pc.
Glass coil, 5 turns left	5TL	170-0426-01	2 pcs.
Glass coil, 10 turns right	10TR	157-0251-01	1 pc.
Glass coil, 10 turns left	10TL	157-0226-01	1 pc.
Glass tubing	p	170-0187-01	1 pc.
Glass tubing	r	170-0193-01	1 pc.
Glass tubing U	ae	116-0223-48	1 pc.
Connector T	A10	116-B034-01	4 pcs.
Connector T	C3	116-0202-03	1 pc.
AA3 Heater assembly		169+B410-01	1 pc.
AA3 Coil, 5.37 mL		169+B442-01	1 pc.
AA3 Controller, 115V/230V		169+B430-01/02	1 pc.
Thermometer 32-42°C		157-0283-01	1 pc.
4 way valve (for nitrate)	4WV	566+0010-01	1 pc.
Nut		508+2011-01	2 pcs.
Nut		508-2011-06	2 pcs.
Fitting		508+2012-01	4 pcs.

NOTES :
Figures in brackets are flow rates in ml/min.

Legend	Description	P/N
PE15	0.03" I.D. polyethylene tubing. Thick wall.	562-2015-01
T07	Tygon tubing.	116-0536-07
KF14	Kel-F tubing 0,05" x 0,08"	562-3014-01
4W V	4 way valve + Nut 2x 508+2011-01 2x 508+2011-06 + Fitting 4x 508+2012-01	566+0010-01



DRAWN	S.Giedigkeit	01.03.99	SYSTEM	AA 3	PARAMETER	Ammonia	SEAL Analytical
CHANGED	S.Giedigkeit	07.07.2009	METHOD NO. (10)	G-171-96	MATRIX	Water and Seawater	
RELEASED	U. Grummisch	07.07.2009	REMARK	Multitest MT19	RANGE	Sample A: 0-3.0 to 0-27 µmol/l Sample B: 0-25 to 0-300 µmol/l	