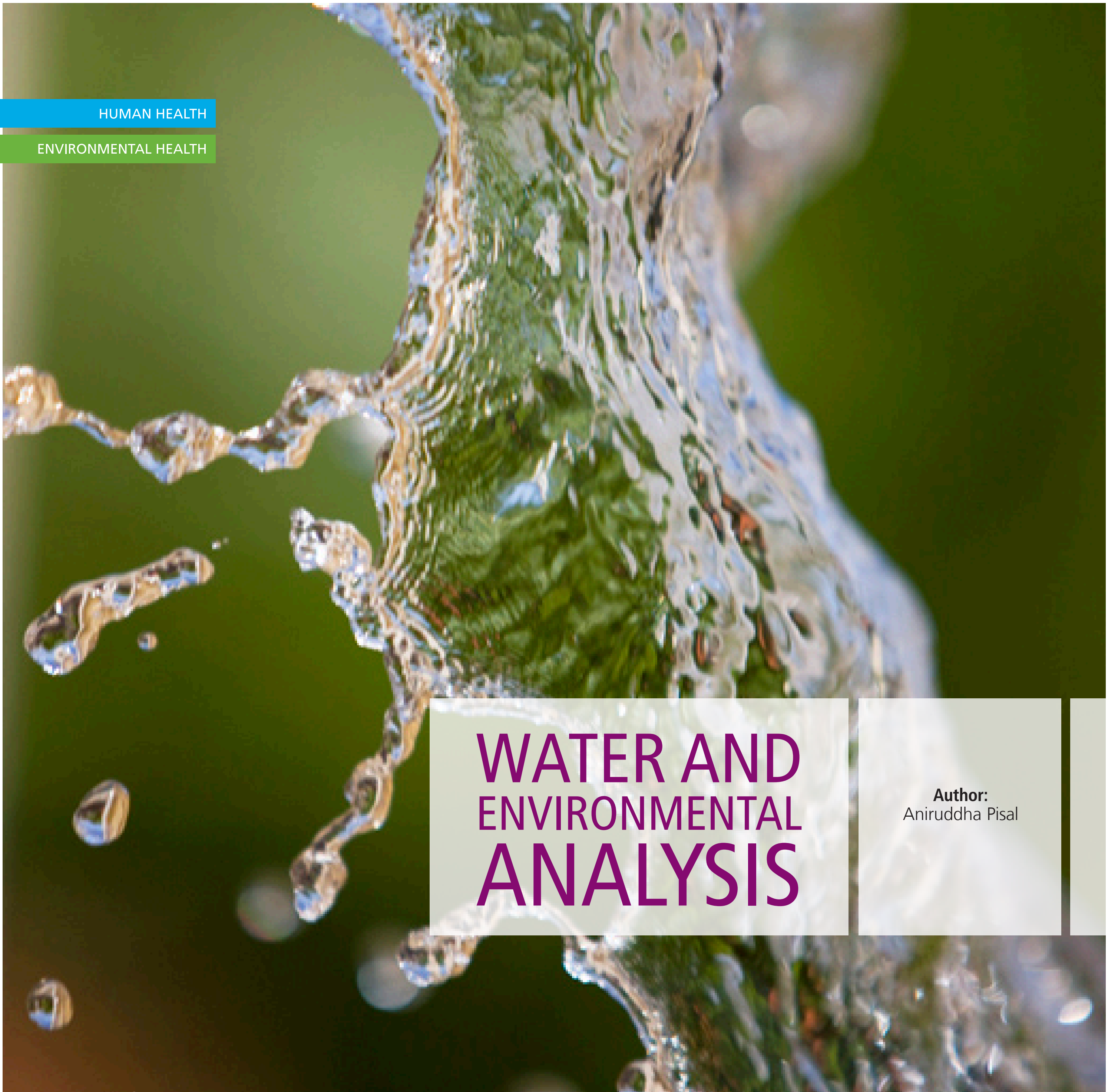
HUMAN HEALTH

ENVIRONMENTAL HEALTH



WATER AND ENVIRONMENTAL ANALYSIS

Author:
Aniruddha Pisal

LAMBDA XLS and XLS+
UV/Vis Spectrophotometers

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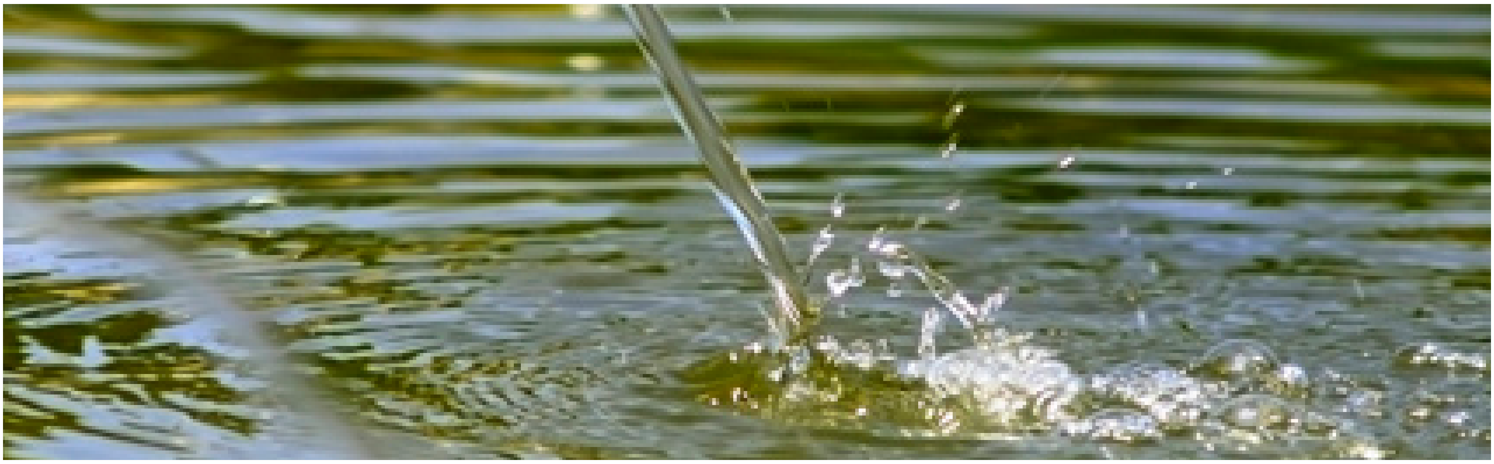

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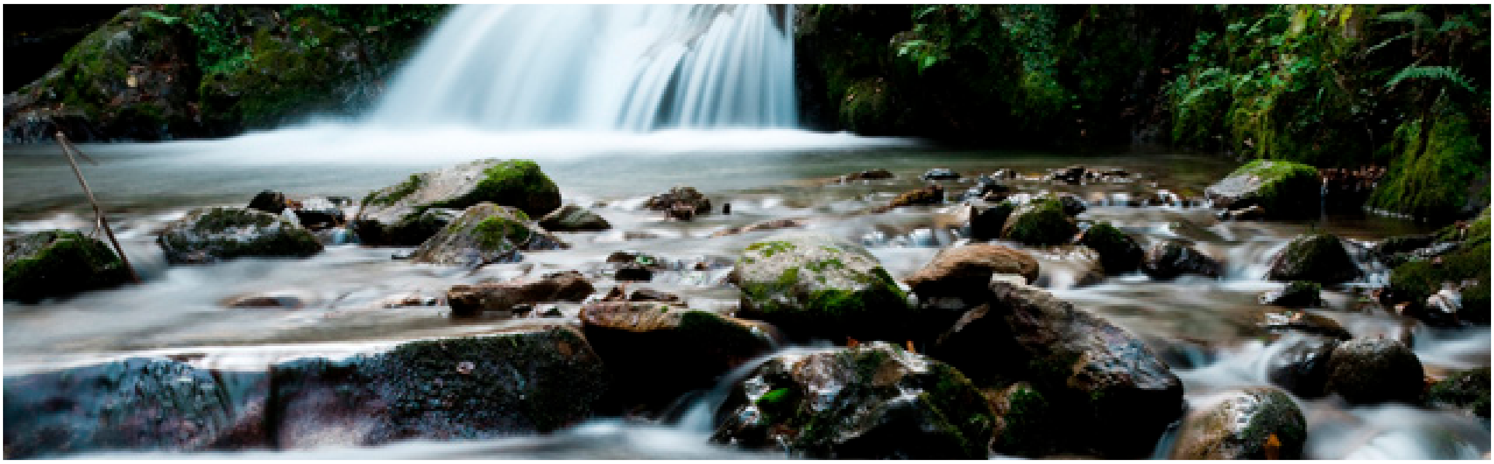
Acknowledgement:
Mr. Amit Patil and Mr. Brijeshkumar Yadav, students at Garware Institute of Career Education and Development, Mumbai University, who helped in data acquisition.



INTRODUCTION

UV/Vis spectroscopy is an important analytical technique in modern water analysis. Many cations and anions in water can be determined with high selectivity and sensitivity.

The common principle of water analysis is that the sample is mixed with reagents, which react specifically with the constituent to be analyzed. In most cases a colored compound or complex is formed. The intensity of the color is a measure for the concentration and can be quantified using UV/Vis Spectroscopy. Here the attenuation of radiation of a specific wavelength can be measured and a relation between absorbance and concentration is made by establishing a calibration curve.

The absorbance increases with the concentration of the analyte in the water. The Beer-Lambert law defines this relation between concentration and absorbance:

$$A = \varepsilon \cdot c \cdot d$$

where A is absorbance at a defined wavelength, ε is the molar absorption coefficient, c is the concentration and d is the pathlength (in cm). ε is a substance-specific constant which remains unaltered during a specific analysis. The same is true for the pathlength, so that the absorbance measured stands in linear correlation to the concentration [1, 2, 3].

The Beer-Lambert law is only valid in a limited absorbance or concentration range. The analyst must be aware of this and should know the limit. In practice, it is recommended that measurements be made in the linear range of the calibration curve, although in specific cases it might be useful to extend beyond the linear range.

Deviations from the Beer-Lambert law are caused by two different effects:

- Chemical influences, like changes of ionic strength or pH value, electrostatic interactions and associations, dissociations or polymerizations, may cause deviations from the Beer-Lambert law. Such influences can be controlled to a certain extent by modification of the reaction conditions.
- Instrumental influences like insufficient dispersion of the beam (stray radiation) cause a deviation of the Beer-Lambert law. Because stray radiation is independent of sample concentration its relative effect is greatest at high absorbance (and high concentration) levels [4, 5].

The measurement range in absorbance spectroscopy thus is limited by chemical and instrumental parameters. The use of a spectrophotometer with good stray radiation specifications helps to improve the measurement range compared to simple photometers.

Due to the Beer-Lambert law an additional increase in sensitivity can be achieved by using cells with longer pathlengths. Standard pathlength of cells is 10 mm; but there is a wide variety of cells with pathlengths between 1 mm and 100 mm. In water analysis, cells with 20, 50 and 100-mm pathlength play an important role because an up to 10-fold increase in sensitivity may be achieved.

Before performing quantitative analysis of water constituents, a calibration curve using reference solutions of known concentrations is established. When the Beer-Lambert law is valid this calibration curve is linear. Modern UV/Vis spectrophotometers are equipped with the possibilities to select nonlinear curve fit, so that measurements outside of the validity of the Beer-Lambert law can be done in a limited extended range.

In the present publication methods for the analysis of different cations, anions and some organic substances are presented. All analyses were performed with the LAMBDA™ Series UV/Vis spectrophotometers from PerkinElmer.

Literature

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[3] Perkampus H.H., UV/Vis-Spektroskopie und ihre Anwendungen. Springer Verlag, Berlin, Heidelberg, New York, Tokyo 1986.

[4] Müller HW., Horn K., Some Technical Aspects of a High Quality UV/Vis Spectrometer for Routine Analysis, Applied UV Spectroscopy 19D, Bodenseewerk Perkin-Elmer GmbH, Überlingen, 1990

[5] Demey, R.C., Sinclair R., Visible and Ultraviolet Spectroscopy, John Wiley and Sons, New York 1987.

THE LAMBDA XLS UV/VIS SPECTROPHOTOMETER

PerkinElmer® LAMBDA XLS and XLS+ UV/Vis spectrophotometers have been specially designed for academic and industrial laboratories that require small-footprint, dedicated instrumentation for a wide range of applications and offers complete and easy to use facilities for quantitative analysis.

The instrument includes built-in basic methods for spectra recording, wavelength programming, kinetics and quantitative analysis. These basic methods can be modified by the user according to specific analytical demands and can be stored as ready-to-use methods in the instrument. A total of 90 methods can be stored in the instrument. This principle is well suited for all kinds of routine measurement in the field of water and environmental UV/Vis analysis.

Basic methods in the instrument are 'Single Wavelength', 'Multi Wavelength', 'Spectrum', 'Concentration', 'Standard Curve', 'Kinetics' and 'Absorbance Ratio'.

In the field of water analysis the basic method 'Standard Curve' plays an important role. It comprises a program for quantitative photometric analysis including multistandard calibration and flexible curve fitting.

Each method allows the calibration with up to 9 references and selection of four curve fitting algorithms as Regression, Interpolation, Cubic Spline and Zero Regression.

For the LAMBDA XLS instrument it is easily possible to load ready-to-use, predefined methods into the spectrometer. No further programming or methods development is necessary. When these methods are stored in the instrument it is only necessary to call up the requested method, which sets all necessary instrument parameters and makes it ready for the respective analysis.

LAMBDA XLS+ UV/Vis Spectrophotometer

Performing Analyses:

Analytical methods including calibration curves, once defined, can be stored and are ready for use with one keystroke. With this method concept all photometric methods as recommended by national regulations can be performed reliably and easily.

The analytical protocols described in this booklet are instructions for the determination of different water constituents. These instructions are specifically targeted for use with the LAMBDA XLS/XLS+ UV/Vis spectrophotometers from PerkinElmer.

When a special water analysis is to be performed, first all the reagents and references must be prepared according to the protocol.

Note: It is necessary to set up a new calibration curve prior to the first analysis. The calibration curves in the present publication are examples – they are not meant to be taken without further evaluation.

To set up new calibration curve, select the basic method 'Standard Curve' and enter the parameters 'wavelength', 'number of reference standards', 'units', 'curve fit' and 'calibration type'. Then enter the concentration details for all reference standards. During analysis, the instrument automatically enters the absorbance value measured for the respective reference standards. From the reference standard concentration and the value measured, the instrument automatically calculated the calibration curve which is then printed out. When a calibration curve is found to be reproducible, use a calibration curve for further series of analyses.

It is usual practice to periodically check a stored calibration curve with references of known concentration of the analyte. In this case it is possible to decide prior to each series of analyses whether a calibration curve should be measured or not.

In the examples in the present publication, the calibration curve was measured in many cases with a large number of references. The reason was mainly because the linear range of the calibration curve was to be estimated.

In practice, calibration curves are often made up with fewer references than described here, although a high number of reference measurements improve the quality and reliability of the analytical results.

If in individual cases, fewer references are to be measured than described in the example, the parameter 'standards' must be changed and the corresponding concentration has to be entered in the parameter 'concentration'.

When a method created as described is started, the LAMBDA XLS spectrophotometer prompts the user to perform a background correction. After this, the user is asked to insert the particular reference and to press 'start'. When all references have been measured, the instrument calculates the calibration curve and prints it out together with statistical information.

The analyst now has the chance to evaluate the calibration and obtain an impression about the precision of the reference measurements. When necessary, it is possible to remeasure individual references.

After termination of the calibration, the spectrometer is ready to measure the first unknown sample. After the sample has been inserted into the sample compartment and the 'start' arrow key depressed, the LAMBDA XLS instrument measures the absorbance value together with the analyte concentration (in respective unit of measurement). Then the analyst may print the sample data by using the set-up keys present on the instrument.

A total of set parameters for a special analysis, including calibration curve, can be stored in the LAMBDA XLS/XLS+ spectrometer as a method. When required, the user only has to call up the desired method with its number and the spectrophotometer is ready to perform the respective analysis.

Regulatory Requirements:

UV/Vis spectroscopy is an important analytical technique used for the determination of many water constituents. National regulations in many countries prescribe the use of UV/Vis spectroscopy for the analysis of water constituents as listed in Table 1.

The present publication describes important analytical methods using UV/Vis spectroscopy. All methods are based on the U.S. EPA standard methods. Most of the limits and standard values listed in Table 1 can be measured using LAMBDA XLS+ UV/Vis spectroscopy with the methods described. The Method Detection Limit is determined as per the guideline given under 40 CFR Part 136 Appendix B – Definition and Procedure for the determination of Method Detection Limit as follows,

- Estimation of Method Detection Limit using the concentration equivalent of three times the standard deviation of replicate instrumental measurements of the analyte in reagent water.
- Preparation of laboratory standard at a concentration which is equal to or in the same concentration range as the estimated method detection limit (between 1 and 5 times the estimated MDL).
- Analysis of seven aliquots of the sample as per the entire analytical method.
- Calculation of MDL as follows,

MDL = t X s where,

t = students' t value for n-1 degrees of freedom at the 99% confidence limit (t = 3.143 for six degrees of freedom)

s = standard deviation of the replicate analyses.

Although the MDL is often misused, it can provide an indication of whether a method has sufficient detection capability when compared with other methods, for compliance purposes.

Table 1: Summary of Analytical Methods for U.S. Regulations for Water and Environmental Analysis along with Method Detection Limit obtained.

Method	Analytical Parameter	Reference Method	MDL	U.S. Limits for Drinking Water MCL [mg/L]$^{1)}$
1	Ammonia-Nitrogen	Nesselerization (APHA 4500)	0.030	---
2	Chromium(VI)	Diphenylcarbazide (APHA 3500)	0.003	0.1 $^{2)}$
3	Chemical Oxygen Demand (COD)	410.4	22.000	---
4	Iron	Phenanthroline	0.015	0.3 $^{3)}$
5	Nitrate/Nitrite-Nitrogen	Sulfanilamide/ethylenediamine with Cd reduction (353.3)	0.007	10 $^{4)}$
6	Nitrite-Nitrogen	Sulfanilamide/ethylenediamine (354.1)	0.002	1
7	Phosphorus	Molybdenum blue, single reagent (365.2)	0.005	---
8	Phosphorus	Molybdenum blue, two reagent (365.3)	0.008	---
9	Sulfate	Turbidimetric as barium sulfate (375.4)	0.400	250 $^{3)}$
10	Turbidity	Formazin, Nephelometric (180.1)	3.500	5 NTU

1) MCL = Maximum contaminant level in mg/L
2) Total Chromium
3) Secondary maximum contaminant level-not enforceable, only a guideline
4) The sum of Nitrate plus Nitrite

Literature

[1] Resource conservation and recovery act methods from S-846 – "Test Methods for Evaluating Solid Waste, Physical/Chemical Methods", 3rd ed., U.S. GPO, Washington, DC, 1987.

[2] "Methods for Chemical Analysis of Water and Waste". Environmental Monitoring Systems Laboratory (EMSL), Cincinnati, OH, 1983.

[3] "Standard Methods for the Examination of Water and Wastewater" 18th edition, American Public Health Association, 1992.

[4] 40 CFR Part 136 Appendix B - Definition and Procedure for the Determination of Method Detection Limit.

[5] "Water and Environmental Analysis According to U.S. EPA Regulations – PerkinElmer, Part Number 0993-5033, February 1995."

DETERMINATION OF AMMONIA-NITROGEN BY NESSLER REACTION - METHOD 4500-NH_3 A-C

Introduction:

Ammonia can occur in all kinds of surface waters, in some ground waters and in wastewaters from industry and households. Whether ammonia nitrogen is found as NH_4+ ion or as NH_4OH or NH_3 depends on the pH value of the water.

Applications:

This method is suitable for determining ammonia - nitrogen in drinking, surface and saline waters, domestic and industrial wastes in the range from 0.05 mg/L to 1.0 mg/L NH_3-N.

Principle of the Method:

The sample is buffered at a pH of 9.5 with a borate in order to decrease hydrolysis of cyanates and organic nitrogen compounds and is then distilled into a solution of boric acid. The ammonia in the distillate is determined colorimetrically by Nesslerization.

Ammonium forms with Nessler reagent $K_2[HgI_4]$ a yellow-brownish colored substituted ammonium salt. The amount of color is a function of the concentration of ammonium-nitrogen.

Interferences:

A number of aromatic and aliphatic amines as well as other compounds both organic and inorganic will cause turbidity upon the addition of Nessler reagent so direct Nesslerization has been discarded as an official method.

Cyanate which may be encountered in certain industrial effluents will hydrolyze to some extent even at the pH of 9.5 at which the distillation is carried out. Volatile alkaline compounds such as certain ketones, aldehydes, and alcohol may cause an off-color upon Nesslerization in the distillation method. Some of these such as formaldehyde may be eliminated by boiling off at a low pH (approximately 2 to 3) prior to distillation and Nesslerization.

Residual chlorine must also be removed by pretreatment of the sample with sodium thiosulfate before distillation.

Experimental

Instrumentation and operating condition:

The absorbance measurements were performed using the PerkinElmer LAMBDA XLS+ UV/Vis spectrophotometer with single beam sample compartment.

Operation parameters used for acquiring spectra were as,

Wavelength	425.0 nm
Measurement Mode	Absorbance
Cell	10-mm glass cell

Apparatus and Reagents:

pH Meter	Boric acid solution
Volumetric flasks, volume 50 mL	Sodium hydroxide solution 0.1 mol/L
Volumetric flasks, volume 100 mL	Sodium tetraborate solution 0.025 mol/L
Hot plate for distillation	Borate buffer, pH 9.5
Boiling chips	Nessler reagent
Erlenmeyer flasks, volume 100 mL	Ammonia-nitrogen stock solution (1 g/L NH_3-N)
Eppendorf® micropipettes	Ammonia-nitrogen working solution 0.01 g/L NH_3-N
Nessler tubes	Sodium thiosulfate (1/35 mol/L) or sodium arsenite
Distillation apparatus	Sodium hydroxide solution, 1 mol/L

Boric acid solution: Dissolve 20 g of boric acid, H_3BO_3, in distilled water and dilute up to the mark with distilled water in 1000-mL volumetric flask.

Sodium Hydroxide solution 0.1 mol/L: Dissolve 4 g sodium hydroxide, NaOH, in distilled water in a 1000-mL volumetric flask and dilute with distilled water up to the mark.

Sodium Hydroxide solution 1 mol/L: Dissolve 40 g sodium hydroxide, NaOH, in ammonia-free water in a 1000-mL volumetric flask and dilute with distilled water up to the mark.

Sodium Tetraborate solution 0.025 mol/L: Dissolve 5 g of anhydrous sodium tetraborate, $Na_2B_4O_7$ (or $Na_2B_4O_7.10H_2O$) in distilled water and dilute up to the mark with distilled water in 1000-mL volumetric flask.

Borate buffer, pH 9.5: Add 88 mL of 0.1 mol/L sodium hydroxide solution to 500 mL of 0.025 mol/L sodium tetraborate solution and dilute up to the mark with distilled water in a 1000-mL volumetric flask.

Nessler reagent: Dissolve 100 g mercuric iodide and 70 g of potassium iodide in small amount of water. Add this mixture to a cooled solution of 160 g NaOH in 500 mL of distilled water and dilute up to the mark with distilled water in a 1000-mL volumetric flask.

Ammonia-nitrogen stock solution: Dissolve 3.819 gm of ammonium chloride NH_4Cl in distilled water and dilute up to the mark with distilled water in a 1000-mL volumetric flask.

Ammonia-nitrogen working solution: Dilute 10 mL of ammonia-nitrogen stock solution to 1000 mL with distilled water in 1000-mL volumetric flask.

Dechlorinating reagents: Sodium thiosulfate (1/35 mol/L): Dissolve 3.5 g sodium thiosulfate in distilled water and dilute up to the mark with distilled water in a 1000-mL volumetric flask.

Introduction:

- Prepare a series of reference solutions, as shown in Table 1, by pipetting suitable volumes of the ammonia-nitrogen working solution in Nessler tubes with markings at 50 mL as follows,
- Make up to the mark with distilled water.

(The procedure was followed by assuming 50 mL of sample volume.)

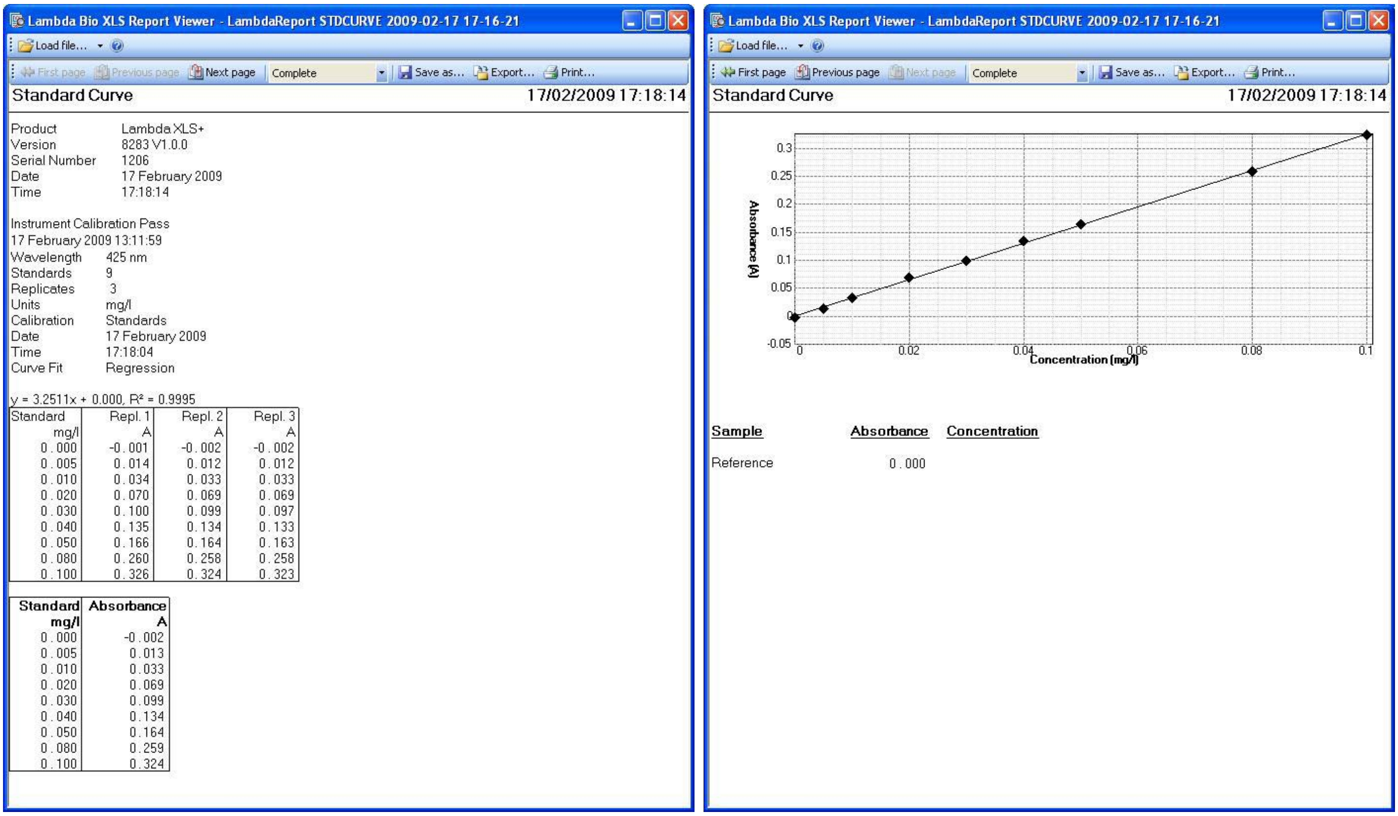
Table 1: Calibration reference solutions.

	Amount of Ammonia-nitrogen (NH_3-N) working solution in 50 mL	Concentration (mg/L) NH_3-N
Blank	---	0.0
Reference 1	0.5 mL	0.1
Reference 2	1.0 mL	0.2
Reference 3	2.0 mL	0.4
Reference 4	3.0 mL	0.5
Reference 5	4.0 mL	0.8
Reference 6	5.0 mL	1.0
Reference 7	8.0 mL	1.6
Reference 8	10.0 mL	2.0

- Add 2 mL of Nessler reagent to each of the flasks and mix thoroughly.
- Allow the solutions to sit for 20 minutes for color development.
- Perform background correction with a blank solution and measure the absorbance of the solutions at 425 nm using a 10-mm glass cuvette. Figure 1 shows the calibration curve and Figure 2 shows typical absorption spectrum of the color formed.

Sample Preparation:

- Add 400 mL of distilled water to a 500-mL Kjeldahl flask with previously treated boiling chips to avoid bumping. Steam distillate until it shows no positive reaction with Nessler reagent.
- Add dechlorinating agent (sodium thiosulfate (1/35 mol/L)) equivalent to the chlorine residue in the sample. Adjust pH of sample to 9.5 with 1 mol/L sodium hydroxide solution.
- Take 500 mL of samples (drinking mineral water and wastewater) in Kjeldahl flask and add 25 mL of borate buffer. Distill 150 mL of the sample into 25 mL boric acid solution in 250-mL Erlenmeyer flask. The tip of the condenser should be dipped below the level of boric acid solution.
- Dilute the distillate to 250 mL with distilled water.
- To 50 mL of the diluted distillate, add 2 mL Nessler reagent and mix well.
- Allow the solutions to sit for 20 minutes for color development.
- Perform background correction with blank solution followed by sample analyses.



Standard Curve 17/02/2009 17:18:14

Product	Lambda XLS+
Version	8283 V1.0.0
Serial Number	1206
Date	17 February 2009
Time	17:18:14

Instrument Calibration Pass
17 February 2009 13:11:59

Wavelength	425 nm
Standards	9
Replicates	3
Units	mg/l
Calibration	Standards
Date	17 February 2009
Time	17:18:04
Curve Fit	Regression

$y = 3.2511x + 0.000,\ R^2 = 0.9995$

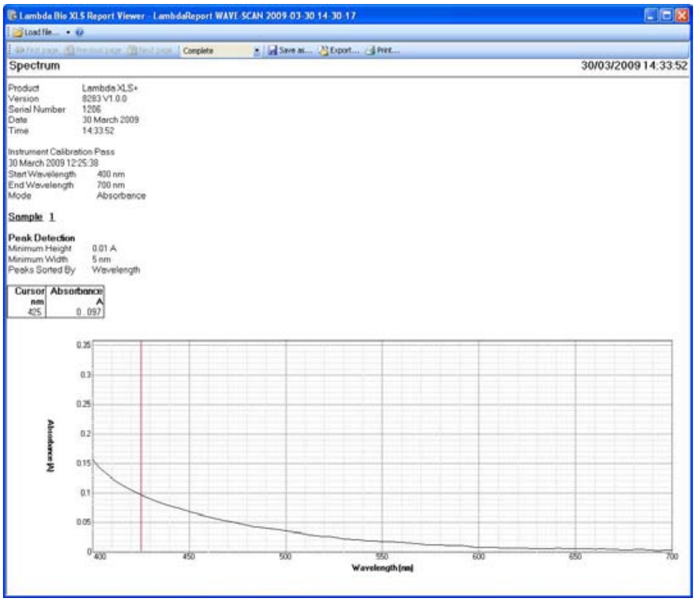
Standard mg/l	Repl. 1 A	Repl. 2 A	Repl. 3 A
0.000	-0.001	-0.002	-0.002
0.005	0.014	0.012	0.012
0.010	0.034	0.033	0.033
0.020	0.070	0.069	0.069
0.030	0.100	0.099	0.097
0.040	0.135	0.134	0.133
0.050	0.166	0.164	0.163
0.080	0.260	0.258	0.258
0.100	0.326	0.324	0.323

Standard mg/l	Absorbance A
0.000	-0.002
0.005	0.013
0.010	0.033
0.020	0.069
0.030	0.099
0.040	0.134
0.050	0.164
0.080	0.259
0.100	0.324

Standard Curve 17/02/2009 17:18:14

Sample	Absorbance	Concentration
Reference	0.000	

Figure 1: Calibration curve for the determination of ammonia-nitrogen.



Spectrum 30/03/2009 14:33:52

Product	Lambda XLS+
Version	8283 V1.0.0
Serial Number	1206
Date	30 March 2009
Time	14:33:52

Instrument Calibration Pass
30 March 2009 12:25:38

Start Wavelength	400 nm
End Wavelength	700 nm
Mode	Absorbance

Sample 1

Peak Detection

Minimum Height	0.01 A
Minimum Width	5 nm
Peaks Sorted By	Wavelength

Cursor nm	Absorbance A
425	0.097

Figure 2: Absorption spectrum of the color formed in the determination of ammonia-nitrogen with Nessler reagent.

Results and Discussion:

- Calibration linearity curve was generated using seven different levels of calibration standards in the range from 0.005 mg/50 mL (0.1 mg/L) to 0.1 mg/50 mL (2.0 mg/L) NH_3-N including the blank as first level. Results showed linearity with correlation coefficient of 0.9997
- Method Detection Limit is determined as per the procedure given under 40 CFR Part 136. MDL is found to be 0.030 mg/L.

Drinking mineral water and wastewater samples were prepared in duplicate and analyzed as per the procedure mentioned above. The results obtained are as below.

Name	Observed Concentration (mg/L)
Himalaya mineral water sample	0.183
Wastewater sample	0.307

Conclusion:

The LAMBDA XLS+ UV/Vis spectrophotometer can be utilized successfully for determination of Ammonia-Nitrogen in water samples within the range of 0.1 mg/L to 2.0 mg/L NH_3-N.

References:

- Method 4500- NH_3 A-C, APHA, Standard Methods for the Examination of Water and Wastewater.
- Methods for Chemical Analysis of Water and Waste, Method 350.2, Nitrogen Ammonia (EMSL).
- Standard Methods for the Examination of Water and Wastewater, Method 418A and 418B.
- 40 CFR Part 136 Appendix B – Definition and Procedure for the Determination of the Method Detection Limit.

DETERMINATION OF HEXAVALENT CHROMIUM - DIPHENYLCARBAZIDE METHOD 3500-CR

Introduction:

Chromium can occur as Cr(III) or Cr(VI) in dissolved form in different concentrations in all kinds of waters. Cr(VI) is more toxic than Cr(III) and is of specific interest.

Applications:

This method is suitable for determining hexavalent chromium in drinking and surface waters, domestic and industrial wastes in the range from 0.1 mg/L to 1.0 mg/L Cr.

Principle of the Method:

This procedure measures only hexavalent chromium. To determine total chromium, prior oxidation with potassium permanganate is necessary. Chromates combine with diphenylcarbazide in strong acid solution to produce a red-violet complex.

To determine total chromium, the sample is digested with a sulfuric-nitric acid mixture and then oxidized with potassium permanganate before reaction with diphenylcarbazide.

Interferences:

Heavy metals such as molybdenum, vanadium and mercury interfere.

Iron in concentrations greater than 1 mg/L may produce a yellow color but the ferric iron color is not strong and is not normally encountered if the absorbance is measured photometrically at the appropriate wavelength.

Interfering amounts of molybdenum, vanadium, iron and copper can be removed by extraction of the cupferrates form of these metals into chloroform. But do not use this unless necessary because residual cupferron and chloroform in the aqueous solution complicate the later oxidation. Therefore, follow the extraction by additional treatment with acid fuming to decompose these compounds.

Nitrite interferes in concentrations higher than 20 mg/L.

Chromium(III) and other interfering metals are precipitated from a phosphate buffered solution by addition of aluminum sulfate prior to determination. The precipitate is filtered off and the solution retained.

Experimental

Instrumentation and operating conditions:

The absorbance measurements were performed using the PerkinElmer LAMBDA XLS+ UV/Vis spectrophotometer with single beam sample compartment.

Operation parameters used for acquiring spectra were as,

Wavelength	540.0 nm
Measurement Mode	Absorbance
Cell	10-mm glass cell

Apparatus and Reagents:

Volumetric flasks, volume 100 mL	Sulfuric acid 0.2 N
Volumetric flasks, volume 50 mL	Diphenylcarbazide solution
Eppendorf® micropipettes	Potassium dichromate
Polyethylene bottle	Phosphoric acid
Erlenmeyer flask 250 mL	
pH meter	
Beaker	

*The reagents, chemicals, standards used were of ACS grade.

Potassium dichromate stock solution (500 mg/L): Dissolve 141.4 mg of dried potassium dichromate in distilled water and diluted up to the mark with distilled water in 100-mL volumetric flask.

Diphenylcarbazide solution: Dissolve 250 mg 1,5 Diphenylcarbazide in 50 mL acetone in a dark brown colored bottle.

Calibration:

- Prepare a series of reference solutions, as shown in Table 1, by pipetting suitable volumes of chromium stock solution into 100-mL volumetric flasks.
- Make volume up to 95 mL with distilled water.
- Adjust pH of the solution to 2.0 ±0.5 with 0.25 mL phosphoric acid and 0.2 N sulfuric acid, mix well and dilute up to the mark with distilled water.
- Add 2.0 mL of diphenylcarbazide solution to the solutions, mix well and allow the solutions to stand for 10 minutes for color development.

(The procedure was followed by assuming 100 mL of sample volume.)

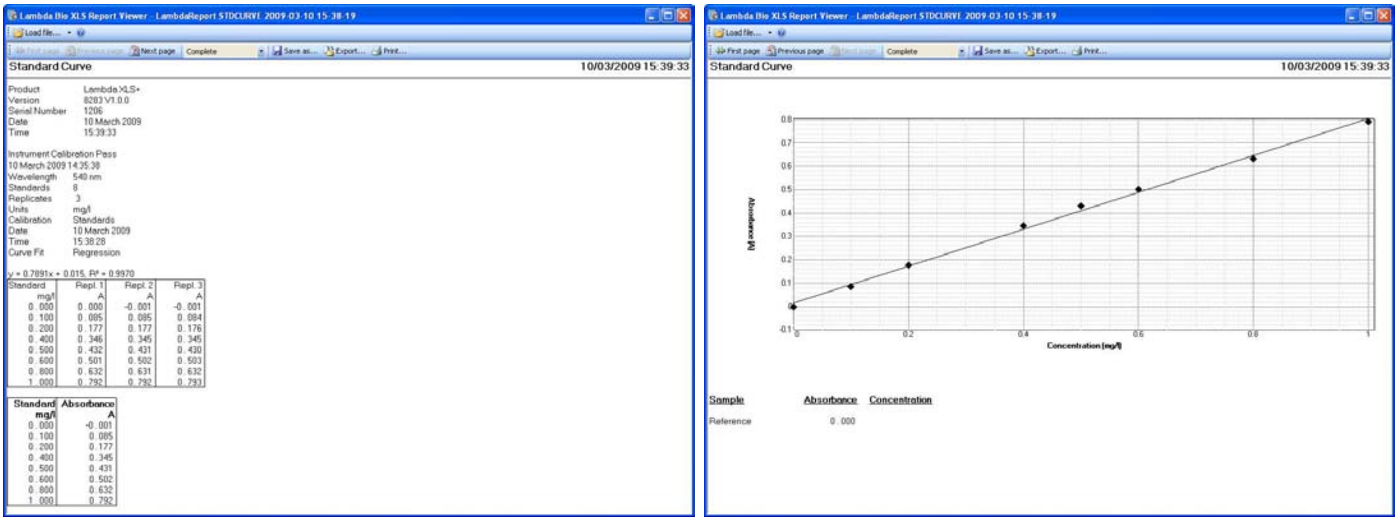
Table 1: Calibration reference solutions

	Amount of Chromium stock solution in 100 mL	Concentration (mg/L) Cr(VI)
Blank	---	0.00
Reference 1	0.02 mL	0.10
Reference 2	0.04 mL	0.20
Reference 3	0.08 mL	0.40
Reference 4	0.10 mL	0.50
Reference 5	0.12 mL	0.60
Reference 6	0.16 mL	0.80
Reference 7	0.20 mL	1.00

- Perform background correction with the blank solution and measure the absorbance of the solutions at 540 nm using a 10-mm glass cuvette. Figure 1 shows the calibration curve and Figure 2 shows typical absorption spectrum of the color formed.

Sample Preparation:

A drinking mineral water sample was used as received and a wastewater sample was filtered through 0.45 micron filter.



Standard Curve 10/03/2009 15:39:33

Product	Lambda XLS+
Version	8283 V1.0.0
Serial Number	1206
Date	10 March 2009
Time	15:39:33

Instrument Calibration Pass
10 March 2009 14:35:38

Wavelength	540 nm
Standards	8
Replicates	3
Units	mg/l
Calibration	Standards
Date	10 March 2009
Time	15:38:28
Curve Fit	Regression

y = 0.7891x + 0.015, R² = 0.9970

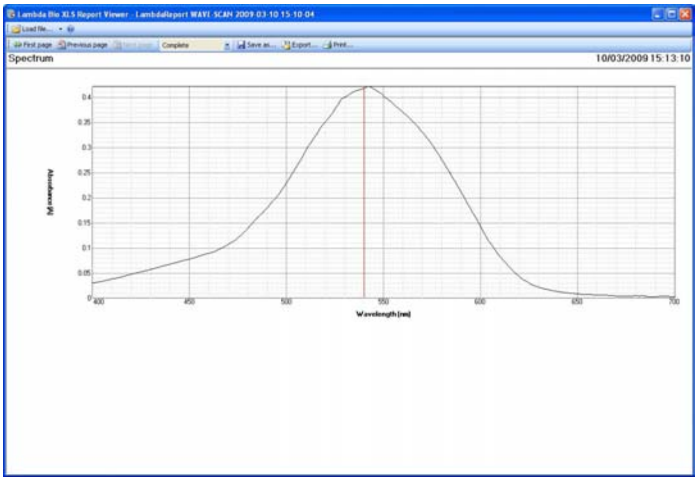
Standard mg/l	Repl. 1 A	Repl. 2 A	Repl. 3 A
0.000	0.000	-0.001	-0.001
0.100	0.085	0.085	0.084
0.200	0.177	0.177	0.176
0.400	0.346	0.345	0.345
0.500	0.432	0.431	0.430
0.600	0.501	0.502	0.503
0.800	0.632	0.631	0.632
1.000	0.792	0.792	0.793

Standard mg/l	Absorbance A
0.000	-0.001
0.100	0.085
0.200	0.177
0.400	0.345
0.500	0.431
0.600	0.502
0.800	0.632
1.000	0.792

Standard Curve 10/03/2009 15:39:33

Sample	Absorbance	Concentration
Reference	0.000	

Figure 1: Calibration curve for the determination of hexavalent chromium.



Spectrum 10/03/2009 15:13:10

Figure 2: Spectrum of the dye formed in the determination of hexavalent chromium.

Results and Discussion:

- Calibration linearity curve was generated using eight different levels of calibration standards in the range from 0.1 mg/L to 1.0 mg/L, including the blank as the first level. Results showed linearity with correlation coefficient of 0.9970.
- Method Detection Limit is determined as per the procedure given under 40 CFR Part 136. MDL is found to be 0.003 mg/L.
- Sample of drinking mineral water and wastewater were prepared in duplicate and analyzed as per the procedure mentioned above. The results are as below,

Sample	Observed Concentration (mg/L)
Drinking mineral water	< 0.003
Wastewater	0.04

Conclusion:

The LAMBDA XLS+ UV/Vis spectrophotometer can be utilized successfully for determination of hexavalent chromium in water samples within the range of 0.1 mg/L to 1.0 mg/L.

Reference:

- Method 3500-Cr A+D, APHA “Standard Methods for the Examination of Water and Wastewater”.
- Test Method for Evaluating Solid Waste, Physical/Chemical Methods. Method 7196.
- 40 CFR Part 136 Appendix B – Definition and Procedure for the Determination of the Method Detection Limit.

DETERMINATION OF CHEMICAL OXYGEN DEMAND - METHOD 410.4

Introduction:

The Chemical oxygen demand is an analytical parameter giving estimation about the overall pollution of a wastewater. With this method the sum of all oxidizable substances in water can be determined.

Applications:

This method is suitable for determining chemical oxygen demand in drinking, surface and saline waters, domestic and industrial wastes in the range from 3 mg/L to 900 mg/L COD.

Principle of the Method:

The sum of all oxidizable substances in water is determined. These are mainly organic constituents which are oxidized during a heating step of 2 hours at 150 °C by chromosulfuric acid with silver sulfate as a catalyst. The dichromate present in the reaction mixture is the oxidative agent. During the reaction the green chromate ion is formed from yellow dichromate. This increase in absorption at 600 nm is in relation to the amount of oxidizable substances in the sample.

It is also possible to follow this reaction at 446 nm where the decrease of dichromate can be monitored. The resulting calibration curve then has a negative slope.

Interferences:

Chlorides are quantitatively oxidized by dichromate and represent a positive interference. Mercuric sulfate is added to the digestion tubes to complex the chlorides.

Experimental

Instrumentation and operating condition:

The absorbance measurements were performed using the PerkinElmer LAMBDA XLS+ UV/Vis spectrophotometer with single beam sample compartment.

Operation parameters used for acquiring spectra were as,

Wavelength	600 nm
Measurement Mode	Absorbance
Cell	10-mm glass cell

Apparatus and Reagents:

Volumetric flasks, volume 50 mL	Digestion solution
Volumetric flasks, volume 100 mL	Catalyst solution
Volumetric flasks, volume 250 mL	Wash solution, H_2SO_4 20%
Eppendorf® micropipettes	Potassium hydrogen phthalate stock solution (1 g/L COD)
Culture tubes with screw caps, 25 X 100 mm	Block digestor or oven 150 °C

* The reagents, chemicals, standards should be of ACS grade.

Digestion solution: Add 10.2 g of potassium dichromate $K_2Cr_2O_7$, 167 mL of conc. sulfuric acid, H_2SO_4, and 33.3 g of mercury sulfate $HgSO_4$ to 500 mL of distilled water, cool and dilute to 1000 mL with distilled water in 1000-mL volumetric flask.

Catalyst solution: Add 22 g of silver sulfate, $AgSO_4$ to a 4.09 kg bottle of concentrated sulfuric acid, H_2SO_4 and let stand for 2 days until dissolved.

Potassium hydrogen phthalate stock solution: Dissolve 0.850 g of potassium hydrogen phthalate in 800 mL of distilled water in a 1000-mL volumetric flask and dilute up to the mark with distilled water.

Calibration:

- All culture tubes (25 X 100 mm) and screw caps should be washed with 20% H_2SO_4 before their first use to prevent contamination.
- Prepare a series of reference solutions, as shown in Table 1, by pipetting suitable volumes of the phthalate stock solution in 50-mL volumetric flasks.
- Make up to the mark with distilled water.

(The procedure was followed by assuming 50 mL of sample volume.)

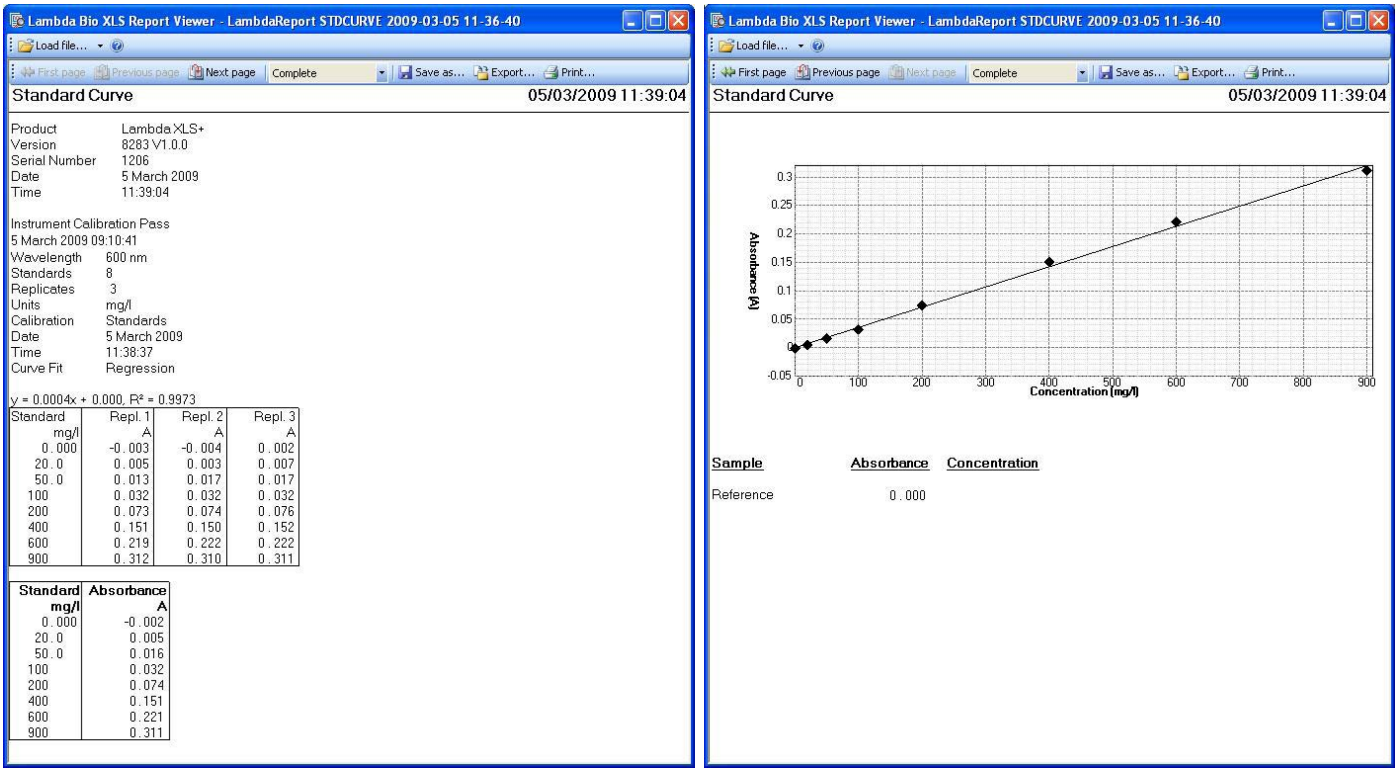
Table 1: Calibration reference solutions.

	Amount of phthalate stock solution in 50 mL	Concentration (mg/L) COD
Blank	---	0.0
Reference 1	1.0 mL	20.0
Reference 2	2.5 mL	50.0
Reference 3	5.0 mL	100.0
Reference 4	10.0 mL	200.0
Reference 5	20.0 mL	400.0
Reference 6	30.0 mL	600.0
Reference 7	45.0 mL	900.0

- Transfer 10 mL of the reference solutions to the culture tubes.
- Add 6 mL of digestion solution to each of the references and mix thoroughly.
- Add 14 mL of catalyst solution to each of the references, down the side of the culture tube.
- Cap tightly and shake to mix the layers (The mixture becomes hot).
- Place the culture tubes in an oven at 150 °C for 2 hours.
- Cool and allow any precipitate to settle.
- Perform background correction with the blank solution and measure the absorbance of the solutions at 600 nm using the 10-mm glass cuvette. Figure 1 shows the calibration curve and Figure 2 shows typical absorption spectrum of the color formed.

Sample Preparation:

Take 10 mL of the sample in to a culture tube and follow the same procedure as given under ‘Calibration’.



Standard Curve 05/03/2009 11:39:04

Product Lambda XLS+
Version 8283 V1.0.0
Serial Number 1206
Date 5 March 2009
Time 11:39:04

Instrument Calibration Pass
5 March 2009 09:10:41
Wavelength 600 nm
Standards 8
Replicates 3
Units mg/l
Calibration Standards
Date 5 March 2009
Time 11:38:37
Curve Fit Regression

$y = 0.0004x + 0.000$, $R^2 = 0.9973$

Standard mg/l	Repl. 1 A	Repl. 2 A	Repl. 3 A
0.000	-0.003	-0.004	0.002
20.0	0.005	0.003	0.007
50.0	0.013	0.017	0.017
100	0.032	0.032	0.032
200	0.073	0.074	0.076
400	0.151	0.150	0.152
600	0.219	0.222	0.222
900	0.312	0.310	0.311

Standard mg/l	**Absorbance A**
0.000	-0.002
20.0	0.005
50.0	0.016
100	0.032
200	0.074
400	0.151
600	0.221
900	0.311

Standard Curve 05/03/2009 11:39:04

Sample	Absorbance	Concentration
Reference	0.000	

Figure 1: Calibration curve for determination of COD.

Figure 2: Spectrum for dye formed in determination of COD.

Results and Discussion:

- Calibration linearity curve has been generated using eight different levels of calibration standards in the range from 20 mg/L to 900 mg/L including blank as first level. Results showed linearity with a correlation co-efficient of 0.9973.
- Method Detection Limit is determined as per the procedure given under 40 CFR Part 136. MDL is found to be 22 mg/L.
- Samples of drinking mineral water and wastewater were prepared in duplicate and analyzed as per the procedure mentioned above. The results are as below,

Name	Observed Concentration (mg/L)
Mineral water sample	< 22
Wastewater sample	28.9

Conclusion:

The LAMBDA XLS+ UV/Vis spectrophotometer can be utilized successfully for determination of Chemical Oxygen Demand in water samples within the range of 20 mg/L to 900 mg/L.

Reference:

- Methods for Chemical Analysis of Water and Waste, Method 410.4 Chemical Oxygen Demand (COD), Environmental Monitoring Systems Laboratory.
- Standard Method for the Examination of Water and Wastewater, 18th edition p. 5-6 Method 5220 A+B+D American Public Health Association.
- 40 CFR Part 136 Appendix B – Definition and Procedure for the Determination of the Method Detection Limit.

DETERMINATION OF
IRON - PHENANTHROLINE
METHOD 3500-FE D

Introduction:

Iron can occur as Fe(ll) or as Fe(lll) in different concentrations in all kinds of waters. It appears not only in true but also in colloidal solution, in organic compounds or as a complex compound.

Applications:

This method is suitable for determining Iron in drinking and surface waters, domestic and industrial wastes in the range from 0.5 mg/L to 5 mg/L Fe.

Principle of the Method:

1,10-Phenanthroline ($C_{12}H_8N_2$), combines in aqueous solution with Iron(II) ions to produce an orange-red complex: tri-o-phenanthroline Iron(II) ion.

The intensity of the color is independent of the pH between 3-9. A pH between 2.9 and 3.5 insures rapid color development in the presence of an excess of phenanthroline. Therefore the reaction is buffered.

Interferences:

Strong oxidizing agents, cyanide, nitrite and phosphates (polyphosphates more than orthophosphates) interfere. An initial boiling with acid converts polyphosphate to orthophosphate and removes cyanide and nitrite that otherwise would interfere. Adding excess hydroxylamine eliminates errors caused by excessive concentrations of strong oxidizing reagents.

Chromium and zinc in concentrations exceeding 10 times that of iron, cobalt and copper in excess of 5 mg/L, nickel in excess 2 mg/L cause interference.

In the presence of interfering metal ions, use a larger excess of phenanthroline to replace that complexed by the interfering metals. Where excessive concentrations of interfering metal ions are present, extraction by isopropyl ether can remove the interference.

If noticeable amounts of color or organic matter are present, it may be necessary to evaporate the sample, gently ash the residue and redissolve in acid. The ashing may be carried out in silica, porcelain or platinum crucibles that have been boiled for several hours in 1+1 HCl.

The presence of excessive amounts of organic matter may necessitate digestion by nitric acid/sulfuric acid or nitric acid/perchloric acid before use of the extraction procedure.

Experimental

Instrumentation and operating conditions:

The absorbance measurements were performed using the PerkinElmer LAMBDA XLS+ UV/Vis spectrophotometer with single beam sample compartment.

Operation parameters used for acquiring spectra were as,

Wavelength	510 nm
Measurement Mode	Absorbance
Cell	10-mm glass cell

Apparatus and Reagents:

Volumetric flasks, volume 25 mL	Concentrated sulfuric acid
Volumetric flasks, volume 50 mL	Hydroxylamine solution
Volumetric flasks, volume 100 mL	Ammonium acetate buffer solution
Erlenmeyer flasks, volume 100 mL	Phenanthroline
Eppendorf® micropipettes	Iron(II) ammonium sulfate
Concentrated Hydrochloric acid	Iron stock solution (200 mg/L Fe)

*The reagents, chemicals, standards should be of ACS grade.

Hydroxylamine solution: Dissolve 10 g of hydroxylamine hydrochloride, $NH_2OH.HCl$ in 100 mL distilled water.

Ammonium acetate buffer solution: Dissolve 250 g of ammonium acetate in 150 mL of distilled water and add 700 mL of concentrated (glacial) acetic acid.

Phenanthroline solution: Dissolve 100 mg of 1,10-phenanthroline monohydrate in 100 mL of distilled water by stirring and heating to 80 ˚C in water bath.

Iron stock solution: Slowly add 20 mL of concentrated sulfuric acid to 50 mL of water and dissolve 1.404 g of Iron(II) ammonium sulfate. Add 0.02 mL/L potassium permanganate solution drop wise until pink color persists. Dilute to 1000 mL with water and mix.

Calibration:

- Prepare a series of reference solutions by pipetting suitable volumes of iron stock solution, as shown in Table 1, into 50-mL volumetric flasks.
- Make up to the mark with distilled water.

(The procedure was followed by assuming a sample volume of 50 mL and an iron concentration between 0.5 and 5.0 mg/L Fe.)

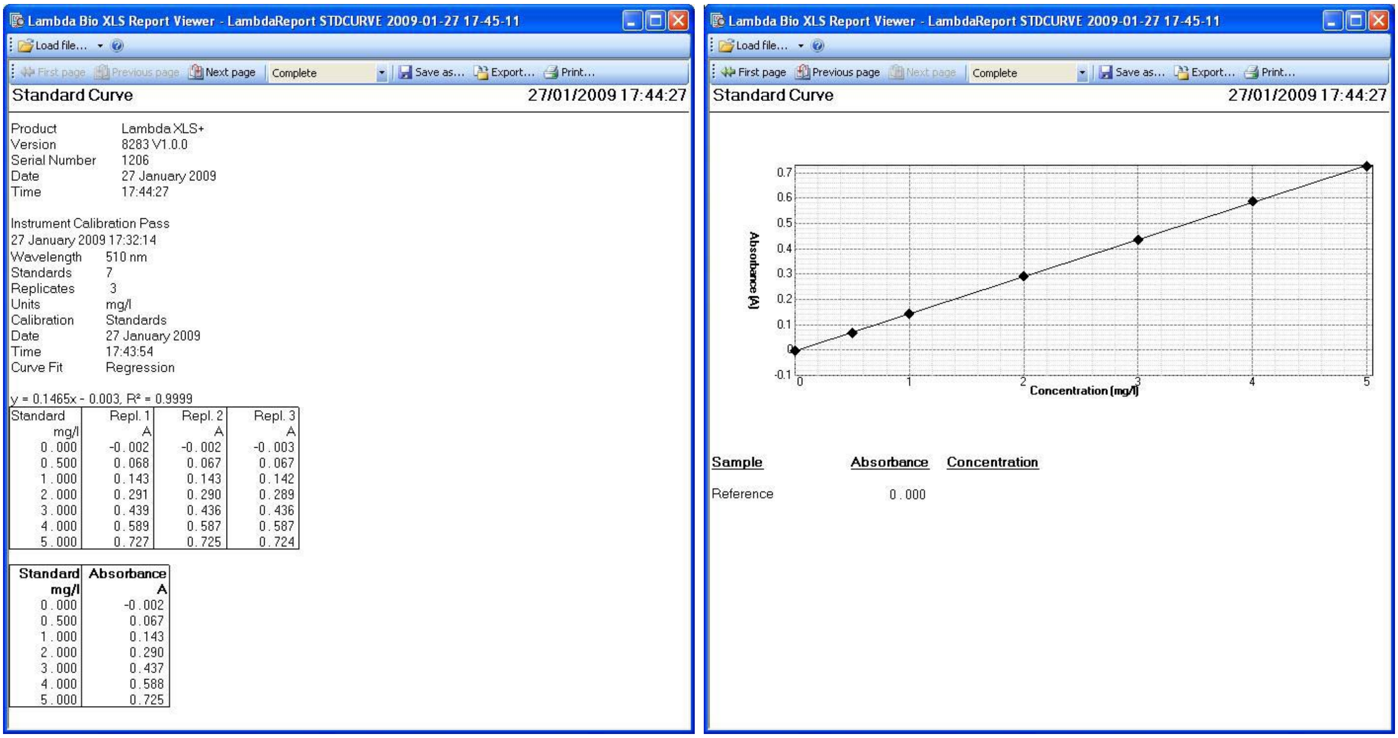
Table 1: Calibration reference solutions.

	Amount of iron stock solution in 50 mL	Concentration (mg/L) Fe
Blank	---	0.0
Reference 1	0.125 mL	0.5
Reference 2	0.250 mL	1.0
Reference 3	0.500 mL	2.0
Reference 4	0.750 mL	3.0
Reference 5	1.000 mL	4.0
Reference 6	1.250 mL	5.0

- Transfer the solutions into 125-mL Erlenmeyer flasks.
- Add 2 mL of concentrated hydrochloric acid and 1 mL of hydroxylamine solution.
- Add few glass beads and heat the flasks to boiling.
- Continue the boiling until volume is reduced to 15 to 20 mL.
- Cool the solutions to room temperature and transfer to 50-mL volumetric flasks.
- Add 10 mL of ammonium acetate buffer solution and 4 mL of phenanthroline solution.
- Dilute up to the mark, mix thoroughly and allow to sit for 10 to 15 minutes for color development.
- Perform background correction with the blank solution and measure the absorbance of reference solutions at 510 nm using a 10-mm glass cuvette. Figure 1 shows the calibration curve and Figure 2 shows typical absorption spectrum of the color formed.

Sample Preparation:

- Pipette 50 mL of sample into a 125-mL Erlenmeyer flask and follow the same procedure as given under ‘Calibration’.



Standard Curve 27/01/2009 17:44:27

Product	Lambda XLS+
Version	8283 V1.0.0
Serial Number	1206
Date	27 January 2009
Time	17:44:27

Instrument Calibration Pass
27 January 2009 17:32:14

Wavelength	510 nm
Standards	7
Replicates	3
Units	mg/l
Calibration	Standards
Date	27 January 2009
Time	17:43:54
Curve Fit	Regression

$y = 0.1465x - 0.003,\ R^2 = 0.9999$

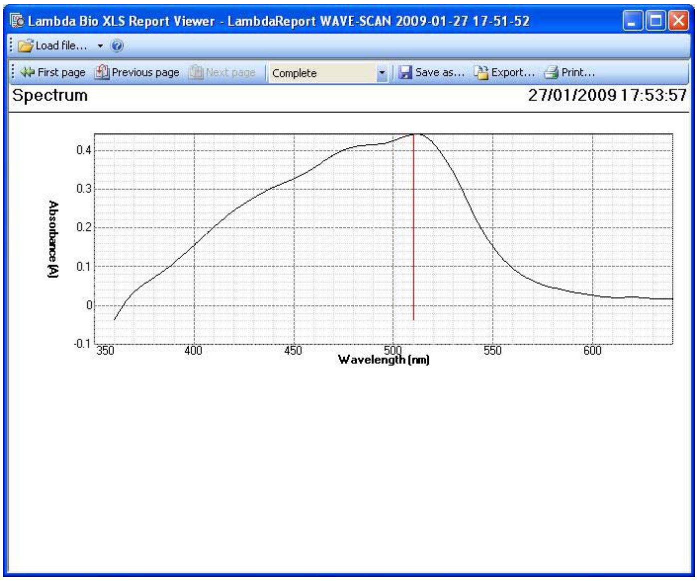
Standard mg/l	Repl. 1 A	Repl. 2 A	Repl. 3 A
0.000	-0.002	-0.002	-0.003
0.500	0.068	0.067	0.067
1.000	0.143	0.143	0.142
2.000	0.291	0.290	0.289
3.000	0.439	0.436	0.436
4.000	0.589	0.587	0.587
5.000	0.727	0.725	0.724

Standard mg/l	**Absorbance A**
0.000	-0.002
0.500	0.067
1.000	0.143
2.000	0.290
3.000	0.437
4.000	0.588
5.000	0.725

Standard Curve 27/01/2009 17:44:27

Sample	Absorbance	Concentration
Reference	0.000	

Figure 1: Calibration curve for the determination of iron.



Spectrum 27/01/2009 17:53:57

Figure 2: Spectrum of the dye formed in the determination of iron.

Results and Discussion:

- Calibration linearity curve was generated using seven different levels of calibration standards in the range from 0.5 mg/L to 5.0 mg/L including a blank as the first level. Results showed linearity with correlation coefficient of 0.9999.
- Method Detection Limit is determined as per the procedure given under 40 CFR Part 136. MDL is found to be 0.015 mg/L.
- Samples of drinking mineral water and wastewater samples were prepared in duplicate and analyzed as per the procedure mentioned above. The results are as below,

Name	Observed Concentration (mg/L)
Drinking mineral water	0.106
Wastewater sample	0.645

Conclusion:

The LAMBDA XLS+ UV/Vis spectrophotometer can be utilized successfully for determination of iron in water samples within the range of 0.5 mg/L to 5.0 mg/L.

Reference:

- Method 3500-Fe D, APHA "Standard Methods for the Examination of Water and Wastewater".
- 40 CFR Part 136 Appendix B – Definition and Procedure for the Determination of the Method Detection Limit.

DETERMINATION OF SUM OF NITRATE - AND NITRITE – NITROGEN BY AZO DYE METHOD WITH CADMIUM REDUCTION OF NITRATE – METHOD 353.3

Introduction:

Nitrate occurs in surface and ground waters in much higher concentrations than nitrite. Nitrite is formed during the biological nitrification process; higher nitrite concentrations indicate pollution by intensive fertilization in agriculture or by industrial wastewater.

Applications:

This method is suitable for determining sum of nitrate and nitrite in drinking, surface and saline waters, domestic and industrial wastes in the range from 0.01 mg/L to 1.0 mg/L nitrate/nitrite - nitrogen.

Principle of the Method:

A filtered sample is passed through the column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite (that originally present plus reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which is measured spectrophotometrically. Separate, rather than combined nitrate-nitrite values are readily obtained by carrying out the procedure first with and then without the Cu-Cd reduction step.

Interferences:

Build up of suspended matter in the reduction column will restrict sample flow. Since nitrate-nitrogen is found in a soluble state the sample may be pre-filtered through a glass fiber filter or membrane filter. Highly turbid samples may be pretreated with zinc sulfate before filtration to remove the bulk of particulate matter present in the sample.

Low results might be obtained for samples that contain high concentrations of iron, copper or other metals. EDTA is added to the samples to eliminate the interference.

Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This interference is eliminated by pre-extracting the sample with an organic solvent.

This procedure determines both nitrate and nitrite. If only nitrate is desired a separate determination must be made for nitrite and subsequent corrections made. The nitrite may be determined by the procedure below without reduction step.

Experimental

Instrumentation and operating conditions:

The absorbance measurements were performed using the PerkinElmer LAMBDA XLS+ UV/Vis spectrophotometer with single beam sample compartment.

Operation parameters used for acquiring spectra were as,

Wavelength	540.0 nm
Measurement Mode	Absorbance
Cell	10-mm glass cell

Apparatus and Reagents:

pH Meter	Dilute hydrochloric acid solution 6 mol/L
Volumetric flasks, volume 1 L	Copper sulfate solution 2%
Volumetric flasks, volume 100 mL	Ammonium chloride – EDTA solution
Erlenmeyer flasks, volume 100 mL	Granulated cadmium
Eppendorf® micropipettes	Nitrate stock solution (1 g/L NO_3-N)
Reduction column	Nitrate working solution (10 mg/L NO_3-N)

* The reagents, chemicals, standards used should be of ACS grade.

Dilute Hydrochloric acid solution 6 mol/L: Dilute 50 mL of concentrated hydrochloric acid to 100 mL with distilled water in 100-mL volumetric flask.

Copper Sulfate solution 2%: Dissolve 20 g of Copper Sulfate in 500 mL distilled water and dilute up to the mark in 1000-mL volumetric flask.

Ammonium Chloride – EDTA solution: Dissolve 13 g ammonium chloride and 1.7 g disodium ethylenediamine tetraacetate in 900 mL of distilled water and adjust pH to 8.5 with concentrated ammonium hydroxide or concentrated HCl and dilute to 1 L in a 1000-mL volumetric flask.

Color reagent: Dissolve 10 g of sulfanilamide and 1 g of N–(1-naphthyl)-ethylenediamine dihydrochloride in a mixture of 100 mL of concentrated phosphoric acid and 800 mL of distilled water and dilute up to the mark in 1000-mL volumetric flask.

Nitrate stock solution (1 g/L NO_3-N): Dissolve 7.218 g of potassium nitrate in distilled water and dilute to 1000 mL in a volumetric flask, preserve with 2 mL of chloroform.

Nitrate working solution (10 mg/L NO_3-N): Dilute 10 mL of nitrate stock solution to 1000 mL with distilled water in 1000-mL volumetric flask

Preparation of Reduction column: Wash cadmium granules of 40 – 60 mesh size with dilute hydrochloric acid, rinse with distilled water and copperize with 2% solution of copper sulfate for 5 min until blue color fades. Repeat procedure with fresh copper sulfate until a brown colloidal precipitate forms. Then wash copper – cadmium with distilled water to remove all the precipitated copper. The color of the treated cadmium is black.

A glass wool plug was inserted into the bottom of the reduction column and the column filled with distilled water. The treated copper–cadmium granules were poured in the column to produce 18.5 cm in length. A level of distilled water was maintained above the copper cadmium granules to eliminate entrapment of air. The column was washed with 200 mL of dilute ammonium chloride-EDTA solution. The column was activated by passing 100 mL of solution composed of 25 mL of 1.0 mg/L NO_3- N reference solution and 75 mL of ammonium chloride - EDTA solution through the column at a flow rate between 7 and 10 mL per minute.

Calibration:

- Prepare a series of reference solutions by pipetting suitable volumes of the nitrate working solution, as shown in Table 1, in 100-mL volumetric flasks.
- Add 75 mL of ammonium chloride - EDTA solution and mix.
- Make up to the mark with distilled water.

(The procedure was followed by assuming 50 mL of sample volume for color reaction and a nitrate/nitrite – nitrogen concentration between 0.01mg/L and 1.0 mg/L N)

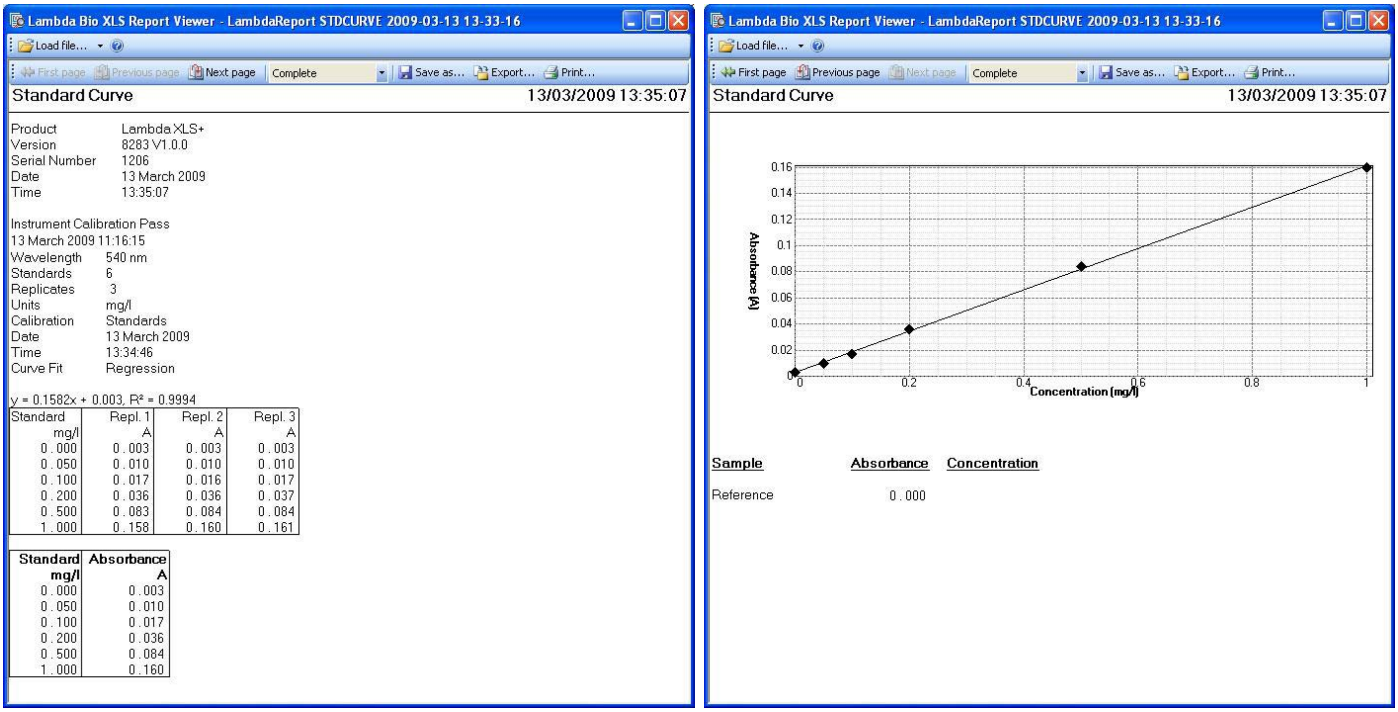
Table 1: Calibration reference solutions.

	Amount of Nitrate - N working solution in 100 mL	Concentration (mg/L) NO_3-N
Blank	---	0.0
Reference 1	0.5 mL	0.05
Reference 2	1.0 mL	0.1
Reference 3	2.0 mL	0.2
Reference 4	5.0 mL	0.5
Reference 5	10.0 mL	1.0

- Pour the reference solutions into the column for reduction of nitrate to nitrite. The first 25 mL is discarded and rest of the sample is collected in the original reference flask.
- Add 2 mL of color reagent to 50 mL of reduced reference in each of the flasks and mixed thoroughly. Color reagent should be added within 15 minutes of the reduced reference preparation.
- Allow the solutions to stand for 10 minutes for color development.
- Perform background correction with the blank solution and measure absorbance of solutions at 540 nm. Figure 1 shows the calibration curve and Figure 2 shows typical absorption spectrum of the color formed.

Sample Preparation:

- The pH of the sample should be adjusted between 5 to 9 with either concentrated hydrochloric acid or concentrated ammonium hydroxide solution and the same procedure followed as given under ‘Calibration’.



Standard Curve 13/03/2009 13:35:07

Product Lambda XLS+
Version 8283 V1.0.0
Serial Number 1206
Date 13 March 2009
Time 13:35:07

Instrument Calibration Pass
13 March 2009 11:16:15
Wavelength 540 nm
Standards 6
Replicates 3
Units mg/l
Calibration Standards
Date 13 March 2009
Time 13:34:46
Curve Fit Regression

$y = 0.1582x + 0.003,\ R^2 = 0.9994$

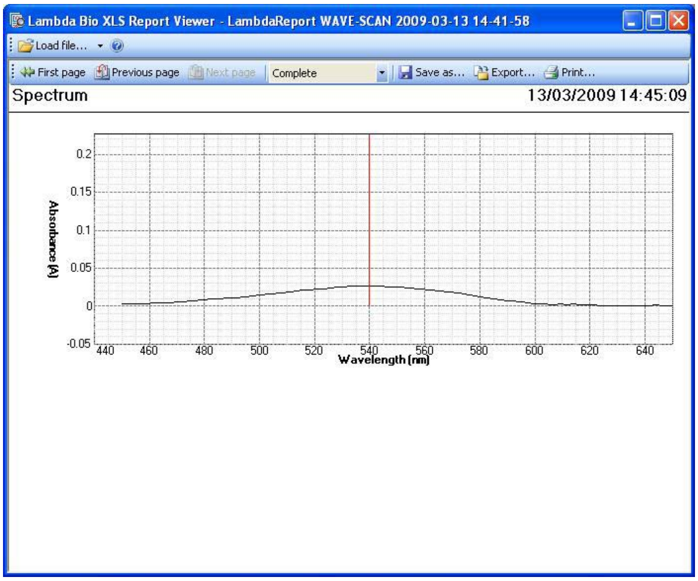
Standard mg/l	Repl. 1 A	Repl. 2 A	Repl. 3 A
0.000	0.003	0.003	0.003
0.050	0.010	0.010	0.010
0.100	0.017	0.016	0.017
0.200	0.036	0.036	0.037
0.500	0.083	0.084	0.084
1.000	0.158	0.160	0.161

Standard mg/l	**Absorbance A**
0.000	0.003
0.050	0.010
0.100	0.017
0.200	0.036
0.500	0.084
1.000	0.160

Standard Curve 13/03/2009 13:35:07

Sample	Absorbance	Concentration
Reference	0.000	

Figure 1: Calibration curve for the determination of nitrate/nitrite-nitrogen.



Spectrum 13/03/2009 14:45:09

Figure 2: Absorption spectrum of the color formed in the determination of nitrate/nitrite-nitrogen with color reagent.

Results and Discussion:

- Calibration linearity curve has been generated using six different levels of calibration standards in the range from 0.01 mg/L to 1.0 mg/L NO_3-N. Results showed linearity with correlation coefficient of 0.9994.
- Method Detection Limit is determined as per the procedure given under 40 CFR Part 136. MDL is found to be 0.007 mg/L.
- Drinking mineral water sample and wastewater sample were prepared in duplicate and analyzed as per the procedure mentioned above. The results are as below,

Name	Observed Concentration (mg/L)
Drinking mineral water	0.007
Wastewater sample	0.766

Conclusion:

The LAMBDA XLS+ UV/Vis spectrophotometer can be utilized successfully for determination of nitrate/nitrite – nitrogen in water samples within the range of 0.01 mg/L to 1.0 mg/L NO_3-N.

Reference:

- Method 4500- NO_3 E, APHA Standard Methods for the Examination of Water and Wastewater.
- Methods for Chemical Analysis of Water and Waste." Method 353.3, Nitrogen, Nitrate - Nitrite (EMSL).
- Standard Methods for the Examination of Water and Wastewater, Method 419C.
- 40 CFR Part 136 Appendix B – Definition and Procedure for the Determination of the Method Detection Limit.

DETERMINATION OF NITRITE – NITROGEN

- AZO DYE METHOD 354.1

Introduction:

Nitrite can occur in low concentrations in surface and ground waters. In specific wastewaters, nitrite may occur in higher concentrations. Nitrite is formed during the biological nitrification process and thus indicates pollution of drinking water.

Applications:

This method covers the determination of nitrite in drinking, surface and saline waters, domestic and industrial wastes in the range from 0.01 mg/L to 1.0 mg/L nitrite – nitrogen.

Principle of the Method:

The diazonium compound formed by diazotation of sulfanilamide by nitrite in water under acidic conditions is coupled with N- (1- naphthyl)- ethylenediamine dihydrochloride to produce a reddish-purple colored azo dye which is measured spectrophotometrically.

Interferences:

There are very few known interferences at concentrations less than 1000 times that of the nitrite; however the presence of strong oxidants or reductants in the samples will readily affect the nitrite concentrations.

High alkalinity (> 600mg/L) will give low results due to a shift in pH.

Experimental

Instrumentation and operating conditions:

The absorbance measurements were performed using the PerkinElmer LAMBDA XLS+ UV/Vis spectrophotometer with single beam sample compartment.

Operation parameters used for acquiring spectra were as,

Wavelength	540 nm
Measurement Mode	Absorbance
Cell	10-mm glass cell

Apparatus and Reagents:

Volumetric flasks, volume 100 mL	Buffer - color reagent
Volumetric flasks, volume 500 mL	Chloroform
Volumetric flasks, volume 50 mL	Nitrite stock solution, 100 mg/L
Eppendorf® micropipettes	Nitrite working solution, 1 mg/L
Erlenmeyer flask, 250 mL	Hydrochloric acid 1:3

* The reagents, chemicals, standards used should be of ACS grade.

Buffer Color reagent: Add 105 mL of concentrated hydrochloric acid (HCl), 5.0 g sulfanilamide and 0.5 g N- (1- naphthyl)- ethylenediamine dihydrochloride to 250 mL of distilled water, stir until dissolved and add 136 g of sodium acetate and stir again until it dissolves. Dilute the solution to 500 mL with distilled water and store in dark.

Nitrite stock solution 100 mg/L NO_2-N: Dissolve 0.1493 gm of dried anhydrous sodium nitrite in 500 mL of distilled water and dilute up to the mark with distilled water in 1000-mL volumetric flask. Preserve the solution with 2 mL of chloroform and keep under refrigeration.

Nitrite working solution 1 mg/L NO_2-N: Dilute 10 mL of nitrite stock solution to 1000 mL with distilled water.

Calibration:

- Prepare a series of reference solutions by pipetting suitable volumes of the nitrite working solution, as shown in Table 1, in 50-mL volumetric flasks.
- Make up to the mark with distilled water.

(The procedure was followed by assuming 50 mL of sample volume and concentration between 0.01 mg/L and 1.0 mg/L N.)

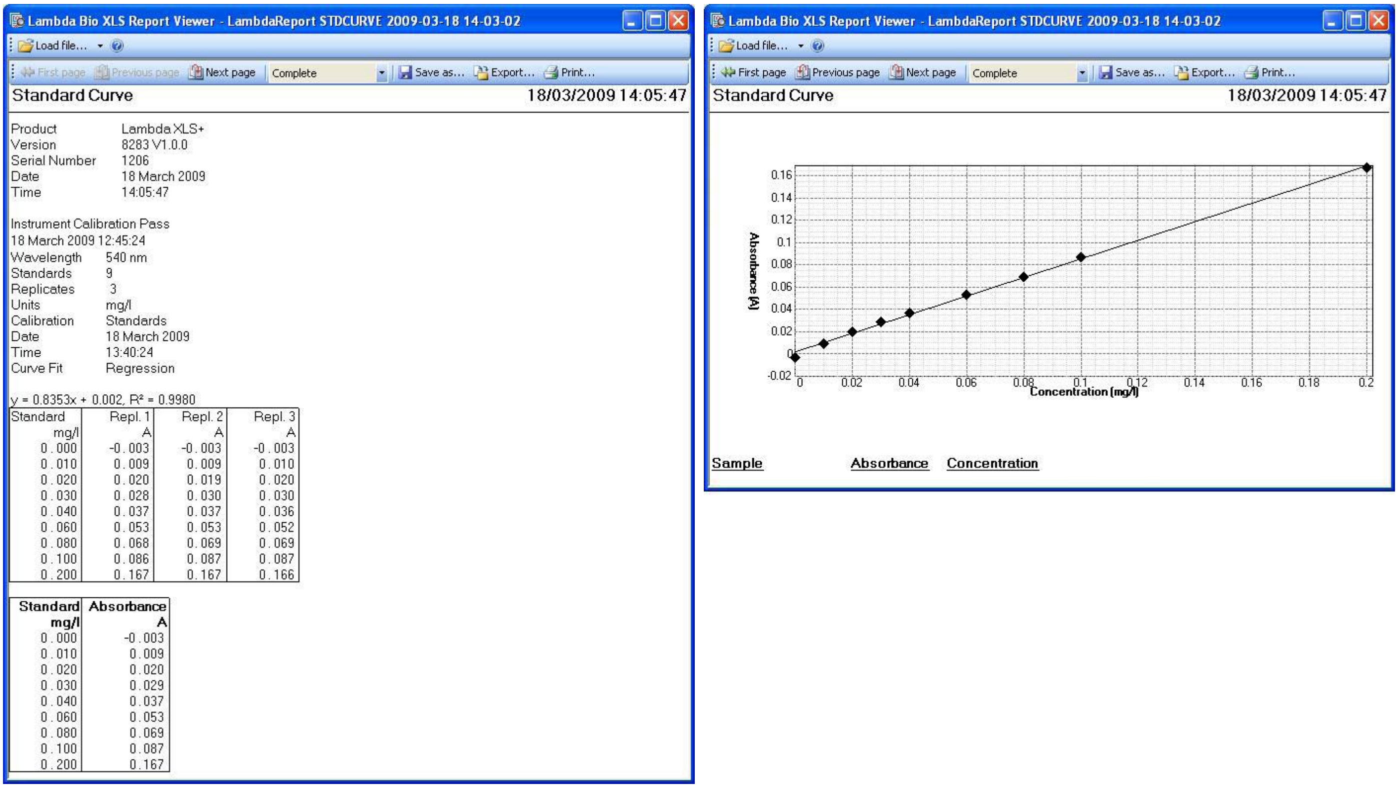
Table 1: Calibration reference solutions.

	Amount of nitrite working solution in 50 mL	Concentration (mg/L) NO_2N
Blank	---	0.0
Reference 1	0.5 mL	0.01
Reference 2	1.0 mL	0.02
Reference 3	1.5 mL	0.03
Reference 4	2.0 mL	0.04
Reference 5	3.0 mL	0.06
Reference 6	4.0 mL	0.08
Reference 7	5.0 mL	0.10
Reference 8	10.0 mL	0.20

- Add 2.0 mL of buffer – color reagent to each of the references, mix thoroughly and ensure the pH is between 1.5 and 2.
- Allow the solutions to sit for 15 minutes for color development.
- Perform background correction with a blank solution and measure the absorbance of the solutions at 540 nm. Record the readings within 2 hours. Figure 1 shows the calibration curve and Figure 2 shows typical absorption spectrum of the color formed.

Sample Preparation:

- Pipette 50 mL of the sample into a 100-mL Erlenmeyer flask. Check the pH of the solution for alkalinity. (Adjust the pH to 6 with 1:3 HCl if the pH of the sample is greater than 10).
- Filter the sample through a 0.45 micron filter paper and follow the same procedure as given under Calibration.



Product Lambda XLS+
Version 8283 V1.0.0
Serial Number 1206
Date 18 March 2009
Time 14:05:47

Instrument Calibration Pass
18 March 2009 12:45:24
Wavelength 540 nm
Standards 9
Replicates 3
Units mg/l
Calibration Standards
Date 18 March 2009
Time 13:40:24
Curve Fit Regression

y = 0.8353x + 0.002, R² = 0.9980

Standard mg/l	Repl. 1 A	Repl. 2 A	Repl. 3 A
0.000	-0.003	-0.003	-0.003
0.010	0.009	0.009	0.010
0.020	0.020	0.019	0.020
0.030	0.028	0.030	0.030
0.040	0.037	0.037	0.036
0.060	0.053	0.053	0.052
0.080	0.068	0.069	0.069
0.100	0.086	0.087	0.087
0.200	0.167	0.167	0.166

Standard mg/l	**Absorbance A**
0.000	-0.003
0.010	0.009
0.020	0.020
0.030	0.029
0.040	0.037
0.060	0.053
0.080	0.069
0.100	0.087
0.200	0.167

Figure 1: Calibration curve for the determination of nitrite-nitrogen.

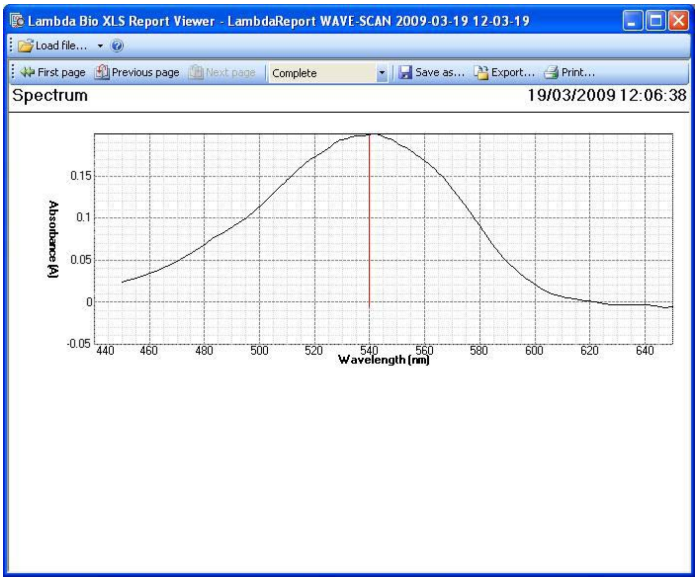


Figure 2: Absorption spectrum of the azo dye formed in the determination of nitrite-nitrogen.

Results and Discussion:

- Calibration linearity curve has been generated using nine different levels of calibration standards in the range from 0.01 mg/L to 1.0 mg/L including a blank as first level. Results showed linearity with correlation coefficient of 0.9980.
- Method Detection Limit is determined as per the procedure given under 40 CFR Part 136. The MDL is found to be 0.0017 mg/L.
- Drinking mineral water and wastewater samples were prepared in duplicate and analyzed as per the procedure mentioned above. The results are as below,

Name	Observed Concentration (mg/L)
Drinking water sample	<0.0017
Wastewater sample	0.179

Conclusion:

The LAMBDA XLS+ UV/Vis spectrophotometer can be utilized successfully for determination of nitrite-nitrogen in water samples within the range of 0.01 mg/L to 1.0 mg/L.

Reference:

- Methods for Chemical Analysis of Water and Waste - Method 354.1 Nitrogen, nitrite. Environmental Monitoring Systems Laboratory.
- Standard Method For the Examination of Water and Wastewater 14th edition p.434, Method 420.
- 40 CFR Part 136 Appendix B – Definition and Procedure for the Determination of the Method Detection Limit.

DETERMINATION OF PHOSPHORUS, ALL FORMS – MOLYBDATE/ASCORBIC ACID METHOD WITH SINGLE REAGENT, METHOD 365.2

Introduction:

Phosphorus can occur in different concentrations in all kinds of waters. It can occur in dissolved and undissolved forms. Depending on the sample pretreatment, the following forms of phosphorus can be determined by this method:

- Orthophosphate
- Hydrolyzable and orthophosphate
- Total phosphorus
- The dissolved forms of the above types.

Applications:

This method is suitable for determining phosphorus in drinking, surface and saline waters, domestic and industrial wastes in the range from 0.01 mg/L to 0.5 mg/L P.

Principle of the Method:

Ammonium molybdate and antimony potassium tartrate react in an acidic medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.

Only orthophosphate forms a blue color in this test. Polyphosphate (and some organic phosphorus compounds) may be converted to the orthophosphate form by sulfuric acid hydrolysis. Organic phosphorus compounds may be converted to the orthophosphate form by persulfate digestion.

Interferences:

No interference is caused by copper, iron or silicate in concentrations many times greater than their reported concentration in sea water. However, high iron concentrations can cause precipitation of and subsequent loss of phosphorus.

The salt error for samples ranging from 5 to 20% salt content was found to be less than 1%.

Arsenate is determined similarly to phosphorus and should be considered when present in concentrations higher than phosphorus. However, at concentrations found in sea water it does not interfere.

Experimental

Instrumentation and operating conditions:

The absorbance measurements were performed using the PerkinElmer LAMBDA XLS+ UV/Vis spectrophotometer with single beam sample compartment.

Operation parameters used for acquiring spectra were as,

Wavelength	880 nm
Measurement Mode	Absorbance
Cell	10-mm glass cell

Apparatus and Reagents:

pH Meter	Sulfuric acid solution, 2.5 mol/L
Volumetric flasks, volume 50 mL	Antimony potassium tartrate solution
Volumetric flasks, volume 100 mL	Ammonium molybdate solution
Volumetric flasks, volume 250 mL	Ascorbic acid, 0.1 mol/L
Volumetric flasks, volume 500 mL	Combined reagent
Erlenmeyer flasks, volume 100 mL	Phosphorus stock solution (50 mg/L P)
Eppendorf® micropipettes	Phosphorus working solution (0.5 mg/L P)

* The reagents, chemicals, standards used should be of ACS grade.

Sulfuric acid solution 2.5 mol/L: Dilute 70 mL of sulfuric acid, H_2SO_4, to 500 mL with distilled water.

Antimony potassium tartrate solution: Dissolve 1.3715 g of antimony potassium tartrate in 400 mL distilled water in a 500-mL volumetric flask and dilute with distilled water up to the mark and store at 40 °C in dark glass stoppered bottle.

Ammonium molybdate solution: Dissolve 20 g of ammonium molybdate in 500 mL distilled water and store in plastic bottle at 4 °C.

Ascorbic acid, 0.1 mol/L: Dissolve 1.76 g of ascorbic acid $C_6H_8O_6$ in 100 mL of distilled water.

Combined reagent: Above reagents are mixed in the following proportions for 100 mL, 50 mL, 2.5 mol/L of sulfuric acid solution + 5 mL of antimony potassium tartrate solution + 15 mL of ammonium molybdate solution + 30 mL of ascorbic acid, 0.1 mol/L.

All reagents were allowed to attain room temperature before mixing.

Sulfuric acid solution, 5.5 mol/L: Add 310 mL of sulfuric acid slowly to 600 mL of distilled water and dilute to 1 liter when cooled.

Phosphorus stock solution (50 mg/L): Dissolve 0.2197 g of potassium dihydrogen phosphate in a 1000-mL volumetric flask and dilute up to the mark with distilled water.

Phosphorus working solution (0.5 mg/L): Dilute 10 mL of phosphorus stock solution to 1000 mL with distilled water.

Sodium hydroxide 1 mol/L: Dissolve 40 g of NaOH in 600 mL of distilled water, cool and dilute to 1 liter.

Calibration:

- Prepare a series of reference solutions by pipetting suitable volumes of the phosphorus working solution, as shown in Table 1, in 50-mL volumetric flasks.
- Make up to the mark with distilled water.

(The procedure was followed by assuming 50 mL of sample volume.)

Table 1: Calibration reference solutions

	Amount of Phosphorus working solution (0.5 mg/L) in 50 mL	Concentration (mg/L) P
Blank	---	0.0
Reference 1	1.0 mL	0.01
Reference 2	3.0 mL	0.03
Reference 3	5.0 mL	0.05
Reference 4	10.0 mL	0.1
Reference 5	20.0 mL	0.2
Reference 6	30.0 mL	0.3
Reference 7	40.0 mL	0.4
Reference 8	50.0 mL	0.5

- Transfer the reference solutions to 100-mL Erlenmeyer flasks.
- Add 8 mL of combined reagent to each of the flasks and mix thoroughly.
- Allow the solutions to sit for 10 to 15 minutes for color development.
- Perform background correction with blank solution and measure the absorbance to 880 nm using 10-mm glass cuvette. Figure 1 shows the calibration curve and Figure 2 shows typical absorption spectrum of the color formed.

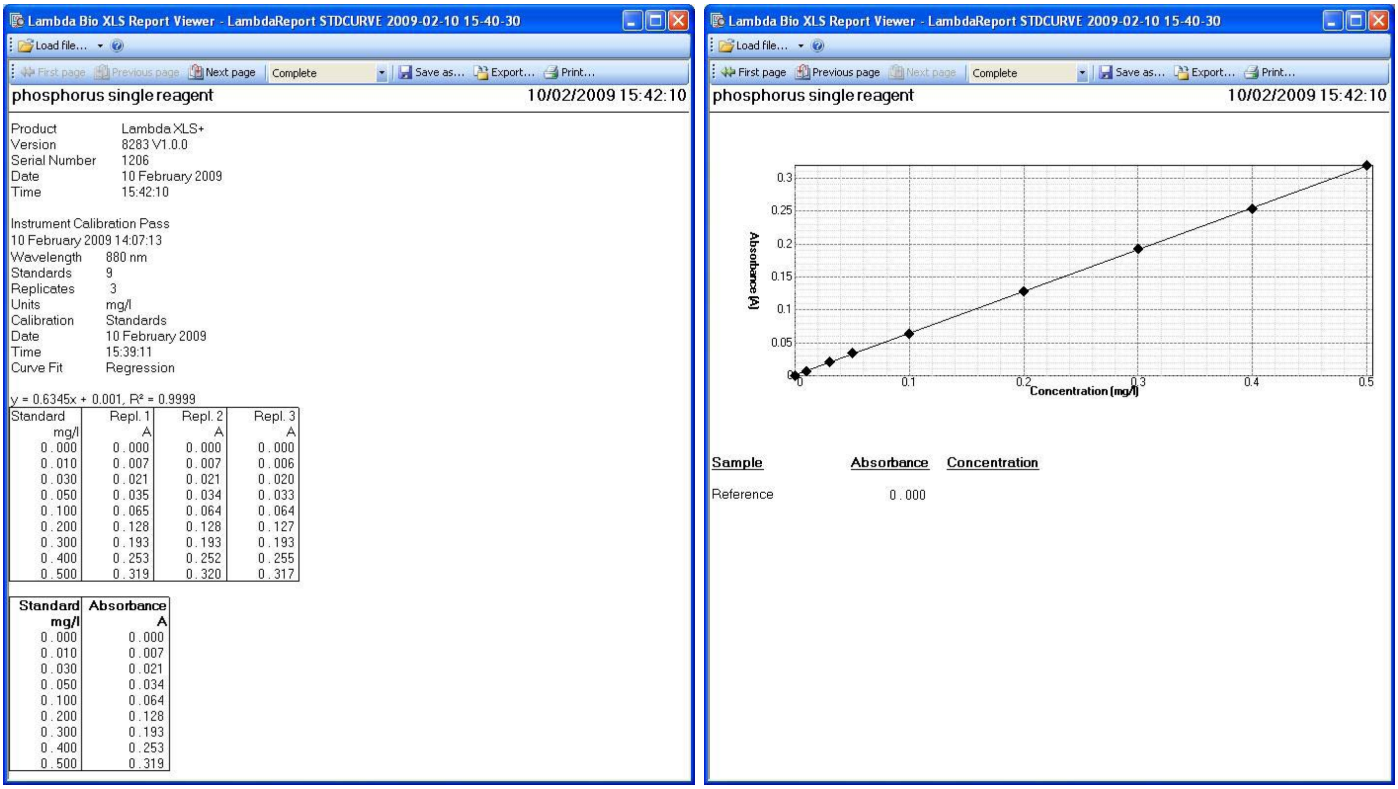
Sample Preparation:

Total phosphorus:

- 50 mL of sample was transferred to 125-mL Erlenmeyer flask.
- Added 1 mL of sulfuric acid solution and mixed.
- Added 0.4 gm of ammonium per sulfate.
- Boil gently on a preheated hot plate for approximately 30-40 min until a final volume of about 10 mL was reached.
- Cooled and diluted the sample to about 30 mL and adjusted the pH of the sample to 7.0 ±0.2 with 1 mol/L NaOH using a pH meter, diluted the sample to 50 mL.
- Added 8 mL of combined reagent to the sample and mixed thoroughly.
- Performed background correction with blank solution and followed sample absorbance measurements.

Hydrolyzable phosphorus

- Transfer 50 mL of sample into 125-mL Erlenmeyer flask.
- Add 1 mL of sulfuric acid solution and mix.
- Boil gently on a preheated hot plate for approximately 30-40 minutes until a final volume of about 10 mL is reached.
- Cool and dilute the sample to about 30 mL and adjust the pH of the sample to 7.0 ±0.2 with 1 mol/L NaOH using a pH meter. Dilute the sample to 50 mL.
- Add 8 mL of combined reagent to the sample and mix thoroughly.
- Allow to sit for 5 minutes for color development.
- Perform background correction with a blank solution and follow sample absorbance measurement procedures.



phosphorus single reagent 10/02/2009 15:42:10

Product Lambda XLS+
Version 8283 V1.0.0
Serial Number 1206
Date 10 February 2009
Time 15:42:10

Instrument Calibration Pass
10 February 2009 14:07:13
Wavelength 880 nm
Standards 9
Replicates 3
Units mg/l
Calibration Standards
Date 10 February 2009
Time 15:39:11
Curve Fit Regression

$y = 0.6345x + 0.001$, $R^2 = 0.9999$

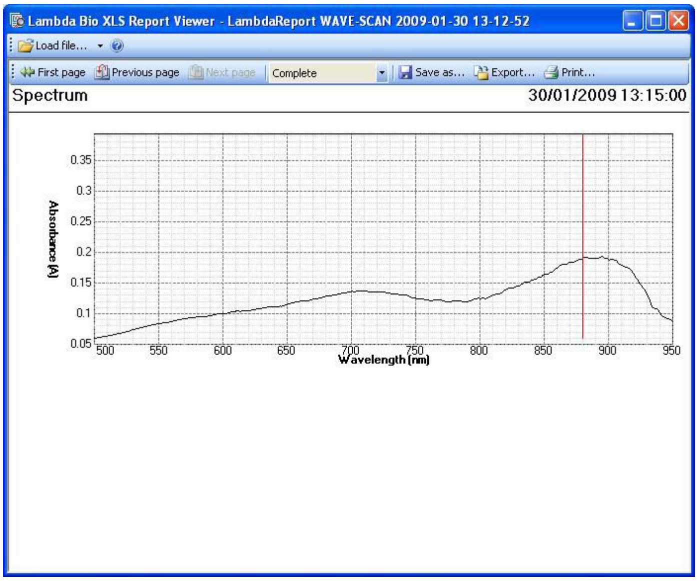
Standard mg/l	Repl. 1 A	Repl. 2 A	Repl. 3 A
0 . 000	0 . 000	0 . 000	0 . 000
0 . 010	0 . 007	0 . 007	0 . 006
0 . 030	0 . 021	0 . 021	0 . 020
0 . 050	0 . 035	0 . 034	0 . 033
0 . 100	0 . 065	0 . 064	0 . 064
0 . 200	0 . 128	0 . 128	0 . 127
0 . 300	0 . 193	0 . 193	0 . 193
0 . 400	0 . 253	0 . 252	0 . 255
0 . 500	0 . 319	0 . 320	0 . 317

Standard mg/l	**Absorbance A**
0 . 000	0 . 000
0 . 010	0 . 007
0 . 030	0 . 021
0 . 050	0 . 034
0 . 100	0 . 064
0 . 200	0 . 128
0 . 300	0 . 193
0 . 400	0 . 253
0 . 500	0 . 319

phosphorus single reagent 10/02/2009 15:42:10

Sample	**Absorbance**	**Concentration**
Reference	0 . 000	

Figure 1: Calibration curve for the determination of phosphorus.



Spectrum 30/01/2009 13:15:00

Figure 2: Spectrum of the dye formed in the determination of phosphorus.

Results and Discussion:

- Calibration linearity curve was generated using nine different levels of calibration standards in the range from 0.01 mg/L to 0.5 mg/L including a blank as first level. Results showed linearity with correlation co-efficient of 0.9999.
- Method Detection Limit is determined as per the procedure given under 40 CFR Part 136. MDL is found to be 0.0046 mg/L.
- A drinking mineral water sample and wastewater sample were prepared in duplicate and analyzed as per the procedure mentioned above. The results are as below,

Name	Observed Concentration (mg/L) Total phosphorus	Observed Concentration (mg/L) Hydrolyzable phosphorus
Drinking mineral water	0.016	< 0.0046
Wastewater sample sample	0.035	0.023

Conclusion:

The LAMBDA XLS+ UV/Vis spectrophotometer can be utilized successfully for determination of phosphorous in water samples within the range of 0.01 mg/L to 0.5 mg/L.

Reference:

- Method 4500- PE, APHA, Standard Methods for the Examination of Water and Wastewater.
- Method 365.2, Phosphorus, All forms, (EMSL), Methods for Chemical Analysis of Water and Waste.
- Standard Methods for the Examination of Water and Wastewater, 14th edition, p.476 and 481.
- Annual Book of ASTM® Standards Part 31, Water, Standard D515-72, Method A, p.389.
- 40 CFR Part 136 Appendix B – Definition and Procedure for the Determination of the Method Detection Limit.

DETERMINATION OF PHOSPHORUS, ALL FORMS – MOLYBDATE/ASCORBIC ACID METHOD WITH TWO REAGENTS, METHOD -365.3

Introduction:

Phosphorus can occur in different concentrations in all kinds of waters. They can occur in dissolved and undissolved forms. Depending on the sample pretreatment, the following forms of phosphorus can be determined by this method:

- Orthophosphate
- Hydrolyzable and orthophosphate
- Total phosphorus
- The dissolved forms of the above types.

Applications:

This method is suitable for determining phosphorus in drinking, surface and saline waters, domestic and industrial wastes in the range from 0.01 mg/L to 1.2 mg/L P.

Principle of the Method:

Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.

Only orthophosphate forms a blue color in this test. Polyphosphates (and some organic phosphorus compounds) may be converted to the orthophosphate form by sulfuric acid hydrolysis. Organic phosphorus compounds may be converted to the orthophosphate form by persulfate digestion.

Interferences:

Arsenate is determined similarly to phosphorus and should be considered when present. This interference may be eliminated by reducing the arsenic acid to arsenious acid with sodium bisulfate.

When high concentrations of iron are present, low recovery of phosphorus will be obtained because iron will use some of the reducing agent. The bisulfate treatment will also eliminate this interference.

Experimental

Instrumentation and operating conditions:

The absorbance measurements were performed using the PerkinElmer LAMBDA XLS+ UV/Vis spectrophotometer with single beam sample compartment.

Operation parameters used for acquiring spectra were as,

Wavelength	650 nm
Measurement Mode	Absorbance
Cell	10-mm glass cell

Apparatus and Reagents:

pH Meter	Sulfuric acid solution, 5.5 mol/L
Volumetric flasks, volume 50 mL	Ammonium molybdate-antimony potassium tartarate solution
Volumetric flasks, volume 100 mL	Sodium bisulfate
Volumetric flasks, volume 250 mL	Ascorbic acid solution
Volumetric flasks, volume 500 mL	Ammonium persulfate
Erlenmeyer flasks, volume 100 mL	Phosphorus stock solution (100 mg/L P)
Eppendorf® micropipettes	Phosphorus working solution (10 mg/L P)

*The reagents, chemicals, standards used were of ACS grade.

Sulfuric acid solution 5.5 mol/L: Add 310 mL of sulfuric acid, H_2SO_4, slowly to 600 mL of distilled water and dilute to 1 liter.

Ammonium molybdate-antimony potassium tartrate solution: Dissolve 8 g ammonium molybdate and 0.2 g of antimony potassium tartrate in 800 mL distilled water into a 1000-mL volumetric flask and dilute up to the mark with distilled water.

Ascorbic acid solution: Dissolve 60 g ascorbic acid in 1000 mL of distilled water and add 2 mL of acetone.

Sodium bisulfate: Dissolve 5.2 g of sodium bisulfate in 100 mL of 0.5 mol/L sulfuric acid.

Phosphorus stock solution (100 mg/L): Dissolve 4393 gm of potassium dihydrogen in a 1000-mL volumetric flask and dilute up to the mark with distilled water.

Phosphorus working solution (10 mg/L): Dilute 100 mL of phosphorus stock solution to 1000 mL with distilled water.

Calibration:

- Prepare a series of reference solutions by pipetting suitable volumes of the phosphorus working solution, as shown in Table 1, in 50-mL volumetric flasks.
- Make up to the mark with distilled water.

(The procedure was followed by assuming 50 mL of sample volume.)

Table 1: Calibration reference solutions.

	Amount of Phosphorus working solution (0.5 mg/L) in 50 mL	Concentration (mg/L) P
Blank	---	0.00
Reference 1	0.25 mL	0.05
Reference 2	0.50 mL	0.10
Reference 3	1.5 mL	0.30
Reference 4	2.5 mL	0.50
Reference 5	3.5 mL	0.70
Reference 6	4.0 mL	0.80
Reference 7	5.0 mL	1.00
Reference 8	6.0 mL	1.20

- Transfer the reference solutions to 100-mL Erlenmeyer flasks.
- Add 1 mL of 5.5 mol/L sulfuric acid and mix.
- Add 4 mL of ammonium molybdate antimony potassium tartrate solution and mix.
- Add 2 mL of ascorbic acid solution and mix.
- Allow the solutions to sit for 5 min for color development.
- Perform background correction with a blank solution and measure the absorbance at 650 nm. Figure 1 shows the calibration curve and Figure 2 shows typical absorption spectrum of the color formed.

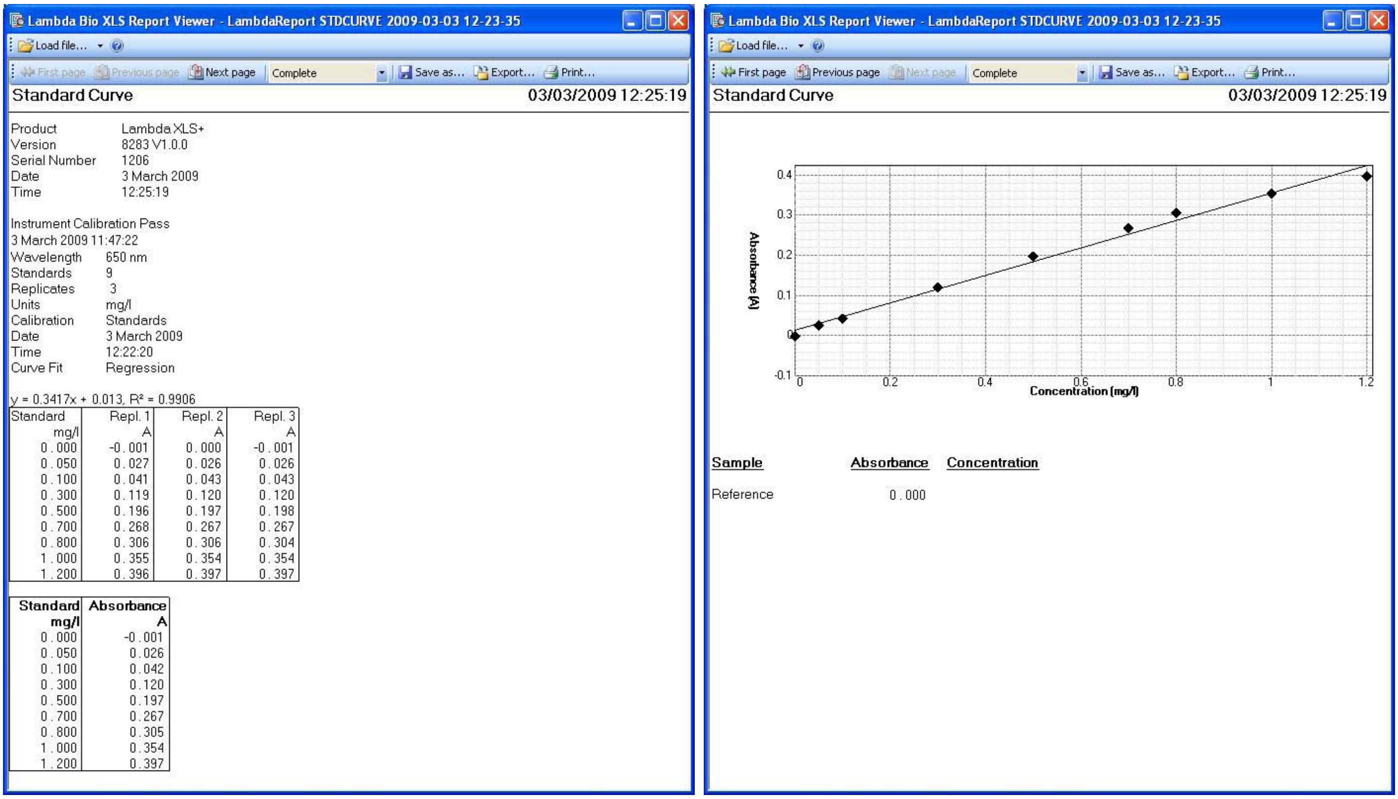
Sample Preparation:

Total phosphorus:

- Transfer 50 mL of the sample into 125-mL Erlenmeyer flask.
- Add 1 mL of 5.5 mol/L sulfuric acid solution and mix.
- Add 0.4 g of ammonium persulfate and mix.
- Boil gently on a preheated hot plate for approximately 30-40 minutes until a final volume of about 10 mL is reached.
- Cool and dilute the sample to about 40 mL and filter.
- Add 5 mL of sodium bisulfate; mix and place in a 95 ˚C water bath for 30 minutes. Cool and dilute to 50 mL.
- Add 4 mL of ammonium molybdate potassium tartrate solution and mix.
- Add 2 mL of ascorbic acid solution and mix.
- Allow to sit for 5 minutes for color development.
- Perform background correction with a blank solution followed by sample absorbance measurements.

Hydrolyzable phosphorus

- Transfer 50 mL of sample into 125-mL Erlenmeyer flask.
- Add 1 mL of 5.5 mol/L sulfuric acid solution and mix.
- Boil gently on a preheated hot plate for approximately 30-40 minutes until a final volume of about 10 mL is reached.
- Cool and dilute the sample to about 40 mL and filter.
- Add 5 mL of sodium bisulfate; mix and place in a 95 °C water bath for 30 minutes. Cool and dilute to 50 mL.
- Add 4 mL of ammonium molybdate potassium tartrate solution and mix.
- Add 2 mL of ascorbic acid solution and mix.
- Allow to sit for 5 minutes for color development.
- Perform background correction with a blank solution followed by sample absorbance measurements.



Standard Curve 03/03/2009 12:25:19

Product Lambda XLS+
Version 8283 V1.0.0
Serial Number 1206
Date 3 March 2009
Time 12:25:19

Instrument Calibration Pass
3 March 2009 11:47:22
Wavelength 650 nm
Standards 9
Replicates 3
Units mg/l
Calibration Standards
Date 3 March 2009
Time 12:22:20
Curve Fit Regression

$y = 0.3417x + 0.013, R^2 = 0.9906$

Standard mg/l	Repl. 1 A	Repl. 2 A	Repl. 3 A
0.000	-0.001	0.000	-0.001
0.050	0.027	0.026	0.026
0.100	0.041	0.043	0.043
0.300	0.119	0.120	0.120
0.500	0.196	0.197	0.198
0.700	0.268	0.267	0.267
0.800	0.306	0.306	0.304
1.000	0.355	0.354	0.354
1.200	0.396	0.397	0.397

Standard mg/l	**Absorbance A**
0.000	-0.001
0.050	0.026
0.100	0.042
0.300	0.120
0.500	0.197
0.700	0.267
0.800	0.305
1.000	0.354
1.200	0.397

Standard Curve 03/03/2009 12:25:19

Sample	Absorbance	Concentration
Reference	0.000	

Figure 1: Calibration curve for the determination of phosphorus.

Spectrum 03/03/2009 13:05:08

Figure 2: Spectrum of the dye formed in the determination of phosphorus.

Results and Discussion:

- Calibration linearity curve has been generated using nine different levels of calibration standards in the range from 0.05 mg/L to 1.2 mg/L including blank as first level. Results showed linearity with correlation coefficient of 0.9906.
- Method Detection Limit is determined as per the procedure given under 40 CFR Part 136. MDL is found to be 0.0077 mg/L.
- Sample of drinking mineral water and sewage (waste) water were prepared in duplicate and analyzed as per the procedure described. The results are as below,

Name	Observed Concentration (mg/L)
Mineral Water - Total phosphorus	< 0.0077
Mineral Water - Hydrolyzable phosphorus	< 0.0077
Wastewater - Total phosphorus	0.054
Wastewater - Hydrolyzable phosphorus	0.032

Conclusion:

The LAMBDA XLS+ UV/Vis spectrophotometer can be utilized successfully for determination of phosphorus in water samples within the range of 0.05 mg/L to 1.2 mg/L.

Reference:

- Methods for Chemical Analysis of Water and Waste, Method 365.3, phosphorus, All Forms. Environmental Monitoring Systems Laboratory (EMSL).
- 40 CFR Part 136 Appendix B – Definition and Procedure for the Determination of the Method Detection Limit

DETERMINATION OF SULFATE - TURBIDIMETRIC METHOD 375.4

Introduction:

Sulfates can occur in surface, ground and wastewaters in different concentrations. They may occur in dissolved and undissolved forms.

Applications:

This method is suitable for determining sulfate in drinking, surface and saline waters, domestic and industrial wastes in the range from 5 mg/L to 40 mg/L SO_4.

Principle of the Method:

Sulfate ion is converted to a barium sulfate suspension under controlled conditions. The resulting turbidity is determined spectrophotometrically at 420 nm with a 4 cm pathlength cell.

Interferences:

Turbidity increases the measured value.

Experimental

Instrumentation and operating conditions:

The absorbance measurements were performed using the PerkinElmer LAMBDA XLS+ UV/Vis spectrophotometer with single beam sample compartment.

Operation parameters used for acquiring spectra were as,

Wavelength	420 nm
Measurement Mode	Absorbance
Cell	4-cm glass cell

Apparatus and Reagents:

Volumetric flasks, volume 100 mL	Conditioning reagent
Volumetric flasks, volume 500 mL	Sulfate stock solution, 100 mg/L
Volumetric flasks, volume 1000 mL	Barium chloride crystals 20 - 30 mesh
Eppendorf® micropipettes	Concentrated hydrochloric acid
Erlenmeyer flask, 250 mL	Magnetic stirrer

*The reagents, chemicals, standards used should be of ACS grade.

Conditioning reagent: Add 30 mL of concentrated hydrochloric acid, HCl, 300 mL of distilled water, 100 mL of isopropanol and 75 g of sodium chloride to a container, followed by addition of 50 g of glycerol and mix well.

Barium chloride crystals of size 20 to 30 mesh

Sulfate stock solution 100 mg/L SO_4: Dissolve 147.9 mg of sodium sulfate, Na_2SO_4 in 800 mL of distilled water in a 1000-mL volumetric flask and dilute up to the mark with distilled water.

Calibration:

- Prepare a series of reference solutions by pipetting suitable volumes of the sulfate stock solution, as shown in Table 1, in 100-mL volumetric flasks.
- Make up to the mark with distilled water.

(The procedure was followed by assuming 100 mL of sample volume and concentration between 5 mg/L and 40 mg/L SO_4.)

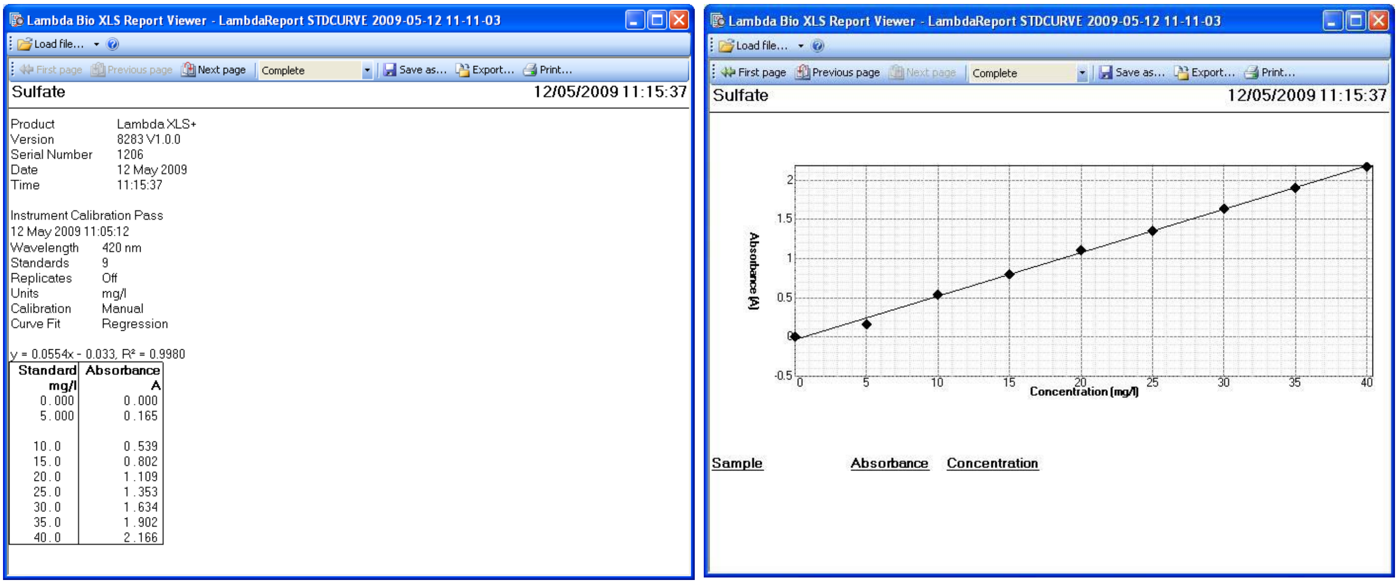
Table 1: Calibration reference solutions.

	Amount of sulfate stock solution in 100 mL	Concentration (mg/L) SO_4
Blank	---	0.0
Reference 1	5.0 mL	5.0
Reference 2	10.0 mL	10.0
Reference 3	15.0 mL	15.0
Reference 4	20.0 mL	20.0
Reference 5	25.0 mL	25.0
Reference 6	30.0 mL	30.0
Reference 7	35.0 mL	35.0
Reference 8	40.0 mL	40.0

- Transfer the contents to 250-mL Erlenmeyer flask.
- Add 5 mL of conditioning reagent to each of the reference solutions and mix in a stirring apparatus.
- While solution is being stirred, add a measuring spoonful of barium chloride crystals to the solution and stir at a constant speed for exactly 1 minute.
- Perform background correction with a blank solution and immediately after stirring; pour the reference solution in cell and measure the absorbance at 420 nm at 30 second interval for 4 minutes.
- Record the maximum absorbance reading obtained in the 4 minute period.
- Calibration curve was produced automatically by manually entering the concentration values and corresponding maximum absorbance values for all reference solutions. Figure 1 shows the calibration curve and Figure 2 shows typical absorption spectrum of the color formed.

Sample Preparation:

- Transfer 100 mL of sample to a 250-mL Erlenmeyer flask.
- Added 5 mL of conditioning reagent and mix in a stirring apparatus.
- While the solution was being stirred, a measuring spoonful of barium chloride crystals were added and stirred at constant speed for exactly 1 minute.
- Performed background correction with blank solution and immediately after stirring; pour the solution in a cell and measure the absorbance at 420 nm at 30 second intervals for 4 minutes.
- Record the maximum absorbance reading obtained in the 4 minute period.



Sulfate 12/05/2009 11:15:37

Product Lambda XLS+
Version 8283 V1.0.0
Serial Number 1206
Date 12 May 2009
Time 11:15:37

Instrument Calibration Pass
12 May 2009 11:05:12
Wavelength 420 nm
Standards 9
Replicates Off
Units mg/l
Calibration Manual
Curve Fit Regression

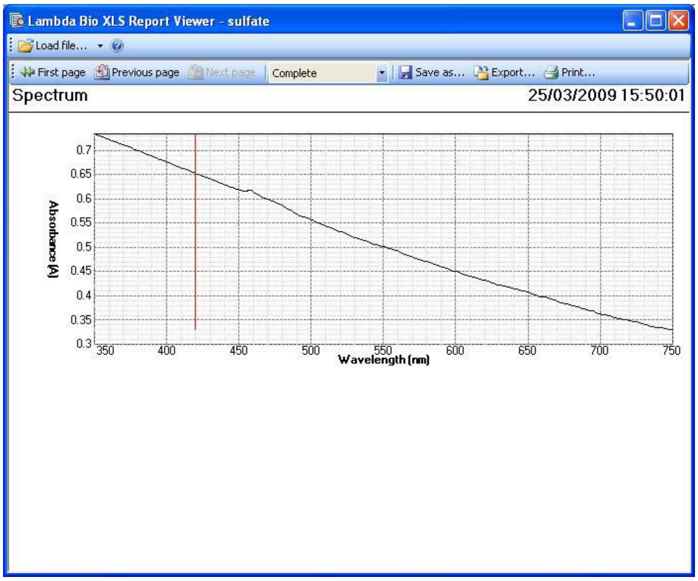
$y = 0.0554x - 0.033$, $R^2 = 0.9980$

Standard mg/l	Absorbance A
0.000	0.000
5.000	0.165
10.0	0.539
15.0	0.802
20.0	1.109
25.0	1.353
30.0	1.634
35.0	1.902
40.0	2.166

Sulfate 12/05/2009 11:15:37

Sample **Absorbance** **Concentration**

Figure 1: Calibration curve for the determination of sulfate.



Spectrum 25/03/2009 15:50:01

Figure 2: Spectrum of turbidity formed in the determination of sulfate.

To determine the concentration of samples; absorbance values obtained were read off from calibration graph which was prepared manually on graph paper.

Results and Discussion:

- Calibration linearity curve was generated using nine different levels of calibration standards in the range from 5 mg/L to 40 mg/L including a blank as first level. Results showed linearity with correlation coefficient of 0.9980.
- Method Detection Limit is determined as per the procedure given under 40 CFR Part 136. MDL is found to be 0.42 mg/L.
- Samples of drinking mineral water and sewage wastewater were prepared in duplicate and analyzed as per the procedure mentioned above. The results are as below,

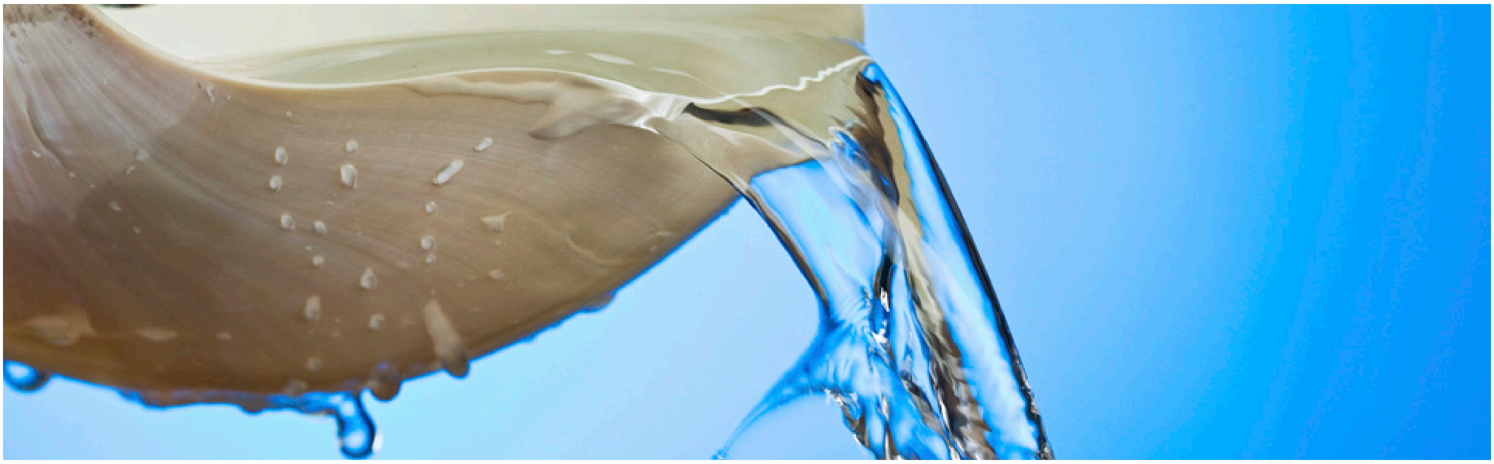
Name	Observed Concentration (mg/L)
Drinking mineral water	16.00
Sewage wastewater	32.75

Conclusion:

The LAMBDA XLS+ UV/Vis spectrophotometer can be utilized successfully for determination of sulfate in water samples within the range of 5 mg/L to 40 mg/L.

Reference:

- Methods for Chemical Analysis of Water and Waste, Method 375.4 Sulfate. Environmental Monitoring Systems Laboratory.
- Standard Methods for the Examination of Water and Wastewater" 14th edition p.496 Method 427C.
- Annual book of ASTM® Standards , Part 31, Water, Standard D516-68, Method B.
- 40 CFR Part 136 Appendix B – Definition and Procedure for the Determination of the Method Detection Limit.



DETERMINATION OF TURBIDITY
- FORMAZIN METHOD 180.1

Introduction:

Turbidity is formed by the presence of undissolved matter in a solution. This turbidity can be measured by the decrease in intensity of the radiation passing through the solution. The original EPA method uses a nephelometer for this determination. When no nephelometer is available, it is possible to perform this analysis using a spectrometer. The increase of absorbance at 860 nm can be taken as a measure for turbidity.

Applications:

This method is suitable for determining turbidity in drinking, surface and saline waters, domestic and industrial wastes in the range from 0.0 to 40 nephelometric turbidity units (NTU).

Principle of the Method:

A reference suspension of formazin, prepared under closely defined conditions, is used for calibration. The turbidity of the sample is measured spectrophotometrically using this calibration curve. The measurement wavelength is 860 nm.

Interferences:

The presence of floating debris and coarse sediments which settle out rapidly will give low readings.

Finely divided air bubbles will affect the results in a positive manner.

The presence of true color will cause turbidities to be low. True color is the color of water which is due to dissolved substances which absorb light. This effect is generally not significant with finished waters.

Experimental

Instrumentation and operating conditions:

The absorbance measurements were performed using the PerkinElmer LAMBDA XLS+ UV/Vis spectrophotometer with single beam sample compartment.

Operation parameters used for acquiring spectra were as,

Wavelength	860 nm
Measurement Mode	Absorbance
Cell	10-mm glass cell

Apparatus and Reagents:

Volumetric flasks, volume 10 mL	Hydrazine sulfate solution
Volumetric flasks, volume 25 mL	Hexamethylene tetramine solution

*The reagents, chemicals, standards used should be of ACS grade.

Hydrazine Sulfate solution: Dissolve 1 g of hydrazine sulfate, $(NH_2)2.H_2SO_4$, in distilled water and dilute up to the mark with distilled water in 100-mL volumetric flask.

Hexamethylene tetramine solution: Dissolve 10 g hexamethylene tetramine, $C_6H_{12}N_4$, in distilled water and dilute with distilled water up to the mark in a 100-mL volumetric flask.

Formazin stock turbidity suspension (400 NTU): In 100-mL volumetric flask, mix 5.0 mL hydrazine sulfate solution with 5.00 mL of hexamethylene tetramine solution and allow to stand for 24 hours at 25 ±30 ˚C and then dilute up to the mark with distilled water.

Formazin working turbidity suspension (40 NTU): Dilute 10 mL of formazin stock turbidity suspension to 100 mL with distilled water in 100-mL volumetric flask.

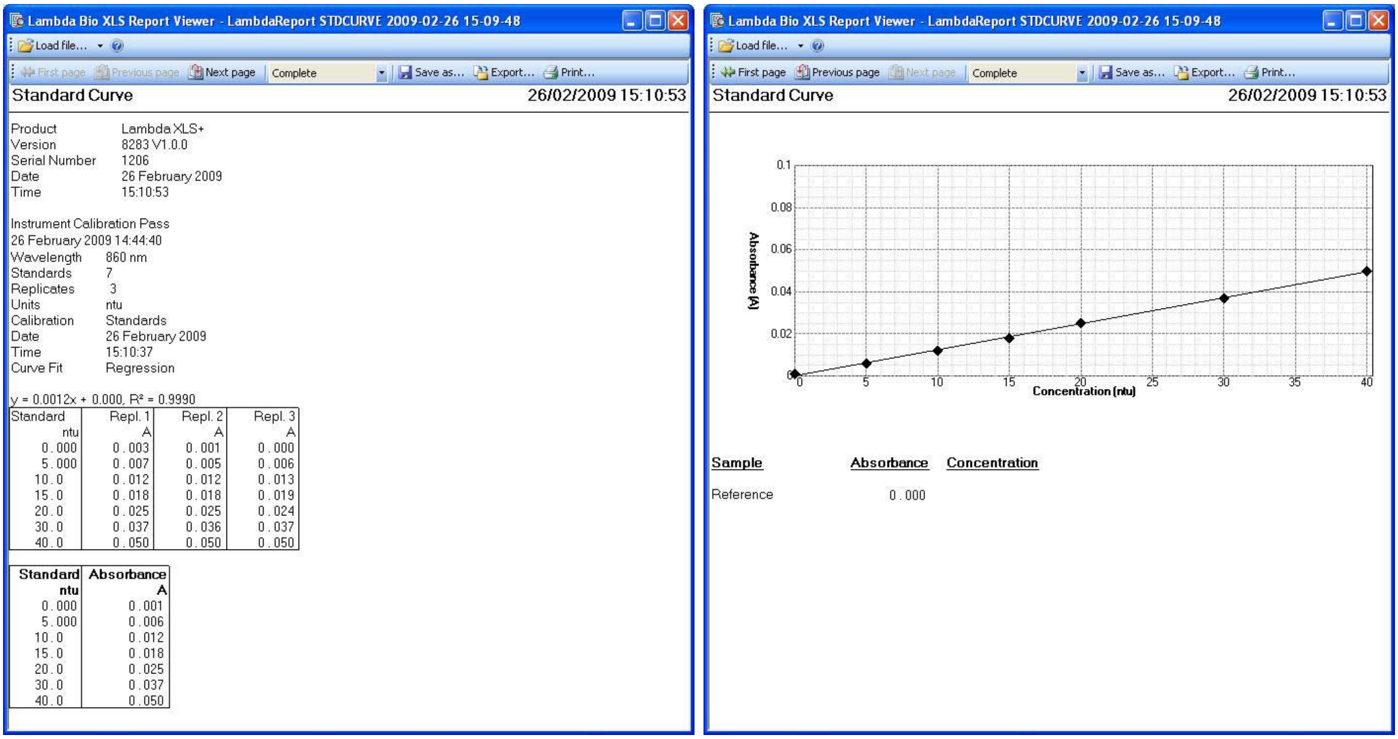
Calibration:

- Prepare a series of reference solutions by pipetting suitable volumes of the formazin working turbidity suspension, as shown in Table 1, in 50-mL volumetric flasks.
- Make up to the mark with distilled water
- The procedure described assumes a sample volume of 20 mL and turbidity between 0 NTU and 40 NTU.

Table 1: Calibration reference solutions.

	Amount of Formazin Working Turbidity Suspension (40 NTU)	Concentration NTU (Turbidity)
Blank	---	0
Reference 1	2.0 mL	5
Reference 2	5.0 mL	10
Reference 3	7.5 mL	15
Reference 4	10.0 mL	20
Reference 5	15.0 mL	30
Reference 6	20.0 mL	40

- Perform background correction with a blank solution and measure the absorbance of the reference suspensions at 860 nm using a 10-mm glass cell. Figure 1 shows the calibration curve and Figure 2 shows typical absorption spectrum.
- Samples were used as received.



Standard Curve 26/02/2009 15:10:53

Product Lambda XLS+
Version 8283 V1.0.0
Serial Number 1206
Date 26 February 2009
Time 15:10:53

Instrument Calibration Pass
26 February 2009 14:44:40
Wavelength 860 nm
Standards 7
Replicates 3
Units ntu
Calibration Standards
Date 26 February 2009
Time 15:10:37
Curve Fit Regression

$y = 0.0012x + 0.000, R^2 = 0.9990$

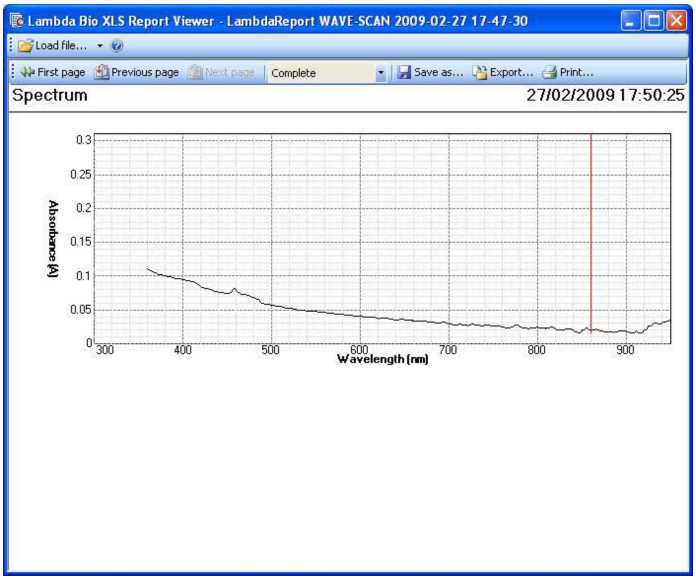
Standard ntu	Repl. 1 A	Repl. 2 A	Repl. 3 A
0.000	0.003	0.001	0.000
5.000	0.007	0.005	0.006
10.0	0.012	0.012	0.013
15.0	0.018	0.018	0.019
20.0	0.025	0.025	0.024
30.0	0.037	0.036	0.037
40.0	0.050	0.050	0.050

Standard **ntu**	**Absorbance** **A**
0.000	0.001
5.000	0.006
10.0	0.012
15.0	0.018
20.0	0.025
30.0	0.037
40.0	0.050

Standard Curve 26/02/2009 15:10:53

Sample	Absorbance	Concentration
Reference	0.000	

Figure 1: Calibration curve for the measurement of turbidity.



Spectrum 27/02/2009 17:50:25

Figure 2: Spectrum of the formazin reference suspension used for determination of turbidity.

Results and Discussion:

- Calibration linearity curve was generated using seven different levels of calibration standards in the range from 0 NTU to 40 NTU including a blank as the first level. Results showed linearity with correlation coefficient of 0.9990.
- Method Detection Limit is determined as per the procedure given under 40 CFR Part 136. MDL is found to be 3.506 mg/L.
- Sample of drinking mineral water and wastewater were prepared in duplicate and analyzed as per the procedure mentioned above. The results are as below,

Name	Observed Concentration (mg/L)
Drinking mineral water	3.64
Wastewater sample	5.03

Conclusion:

The LAMBDA XLS+ UV/Vis spectrophotometer can be utilized successfully for determination of turbidity in water samples within the range of 0 NTU to 40 NTU.

Reference:

- Method 214A, Standard Methods for the Examination of Water and Wastewater.
- Methods for Chemical Analysis of Water and Waste, Method 180.1 Turbidity. Environmental Monitoring Systems Laboratory (EMSL).
- International Norm ISO 7027: "Bestimmung der Trubung" DIN 38404 C2.
- Annual Book of ASTM® Standards. Part 31, Water, Standard D1889-71.
- 40 CFR Part 136 Appendix B – Definition and Procedure for the Determination of the Method Detection Limit.

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