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Department of Analytical and Bioinorganic Chemistry & Water Quality

WORKBOOK
in CHEMISTRY WITH THE FOUNDATIONS OF BIOGEOCHEMISTRY

of student_____

(Name, Surname)

_____

(group, year of study)

Branch of knowledge – 10 Natural Sciences

Speciality – 101 Ecology

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Робочий зошит призначено для виконання лабораторного практикуму з дисципліни Хімія з основами біогеохімії. Включає методики виконання експериментальних задач, форми протоколів аналізу, питання для самоперевірки.

Рекомендовано методичною радою факультету захисту рослин, біотехнологій та екології Національного університету біоресурсів і природокористування України для спеціальності 101 – Екологія, для яких викладання ряду дисциплін проводиться англійською мовою (протокол № ___ від _____ 2020 р.).

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The workbook is intended to train in lab course of Chemistry with the foundations of biogeochemistry. It contains the description of experimental strategies, lab techniques, the templates of lab reports, test questions for own training.

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INTRODUCTION

GENERAL CHEMISTRY SAFETY AND LABORATORY RULES

Chemistry laboratories can be hazardous if the rules are not followed. During a chemistry course a student may handle materials which are carcinogenic, poisonous, flammable, and explosive. Some of these materials and equipment may also cause severe burns, cuts, or bruises if handled improperly or carelessly. Most accidents that occur in the chemistry laboratory are a result of carelessness, impatience, improper or unauthorized experimentation, and disregard for safety rules or proper operating procedures. In order to minimize the chances of an accident in the laboratory certain rules and regulations must be obeyed at all times when one is working or observing in a chemical laboratory. Therefore, it is not advisable for anyone to work in a laboratory without proper knowledge of the dangers involved. Due to the inherent dangers present in a chemical laboratory exercise, it should be understood that the following rules must be obeyed to minimize the chance of an accident. The student is expected to exercise proper judgement and extreme caution at all times when working in the laboratory.

1. Do not perform unauthorized experiments or work in a laboratory alone.

2. Approved eye protection must be worn at all times in the laboratory. If you do get a chemical in your eye rinse immediately with large quantities of water using the eye-wash stations.

3. Long hair and loose clothing must be confined while in a laboratory.

4. Appropriate clothing must be worn at all times while in the laboratory. Your legs must be completely covered below the knee by your choice of clothing. If your clothing does not meet the requirement you may choose to wear an approved laboratory coat or apron which does cover your legs to your knees.

5. Closed shoes with socks must be worn.

6. Know the location and proper use of fire extinguishers, fire blankets, eye wash devices, and first aid kits.

7. Before obtaining any chemicals carefully read the label on the reagent bottles.

8. Eating, smoking, and drinking are not allowed in a chem laboratory.

9. Thoroughly wash your hands after leaving the laboratory.

10. Use the fume hoods when toxic or irritating vapours are involved.

11. Mouth suction is never used to fill a pipette.

13. Never direct the open end of test tube toward yourself or anyone else.

14. Never pour water into concentrated acid (**especially H_2SO_4**).

15. Learn the proper procedure for igniting and operating a laboratory burner. Always extinguish the flame when the burner is not being used. Make sure that all flammable reagents are well removed before lighting the burner.

16. Liquid and solid waste containers must be properly used at all times.

17. Never place chemicals directly on the balance pan. Always use a proper weighing container when using a balance to weigh a chemical. Never pour chemicals directly over the balance.

18. Never return unused chemicals to their original container (unless directed to do so by the instructor).

19. Securely replace lids, caps, and stoppers after removing reagents from containers.

20. Always wipe spatulas clean before and after inserting into reagent bottles.

21. Report any accident and/or injury, however minor, to your instructor immediately.

22. Never place anything that is not directly required for the experiment on laboratory desks; other items may interfere with the experiment.

23. All personal belongings should be placed in the bookcases as you enter the laboratory.

24. Clean up any spill immediately.

25. Before leaving the laboratory, make sure your work area is clean and dry.

26. Your instructor is available for any assistance you may need. Never hesitate to ask questions especially if there is any question concerning proper operating procedure. Be sure that you understand every instruction before proceeding.

1.2. LAB GLASSWEARE CLEANING RULES

In the analysis of samples the preparation of scrupulously clean glassware is mandatory. Lab glassware cleaning procedures must follow specific method requirements. If procedures are not listed then the method of cleaning should be adapted to both the substances that are to be removed, and the determinations (tests) to be performed. Recommendations for such cleaning procedures are listed below.

Analysis/Parameter	Cleaning Procedure (In order specified)
ORGANICS	

INORGANICS	
Trace Metals:	1-4, 9, 8 (optional), 4
Nutrients, Minerals:	1-4, 8, 4
Solids:	1-4, 11 (Volatile Solids 16)
Non-Metals, Physical Properties: (Cyanide)	1-4, 14
MICROBIOLOGY	1-4 (Sterilize per approved method)
BIOASSAY	
Freshwater:	18, 2, 3, 9 or 8, 4, 5, 4, 20
Marine & Estuarine:	19, 2, 3, 9 or 8, 4, 5, 4, 20
RADIONUCLIDES	17, 3, 8, 4

Cleaning Procedures:

1. Remove all labels using sponge or acetone.
2. Wash with hot tap water and a brush to scrub inside of glassware, stopcocks, and other small pieces, if possible, using a suitable laboratory-grade detergent. Organics - Liquinox, Alconox or equivalents. Inorganic anions - Liquinox or equivalent. Inorganic cations - Liquinox, Acationox, Micro or equivalents.
3. Rinse thoroughly with hot tap water.
4. Rinse thoroughly with distilled water (DW).
5. Rinse thoroughly with pesticide grade Acetone.
6. Rinse thoroughly with pesticide grade Methanol.
7. Rinse thoroughly with pesticide grade Hexane.
8. Rinse or soak with 1:1 HCl (Hydrochloric Acid).
9. Rinse or soak with >10% HNO_3 (Nitrate Acid).
10. Bake at 105 °C for 1 hour.
11. Bake at 180 °C (prior to use as per method).
12. Drain, then heat in muffle furnace for 30-60 minutes at 400 °C.
13. Clean, dry glassware should be sealed and stored in dust-free environment.
14. Soak in oxidizing agent (Chromic acid or equivalent); preferably hot (40-50 °C).
15. Last step (prior to use) should be a rinse with the solvent used in analysis.
16. Drain, then heat in muffle furnace for 1 hour at 550 °C.
17. Heat 1 hour in EDTA solution at 90-100 °C.
18. New glassware must be soaked overnight in 10% HNO_3 or HCl.
19. New glassware must be soaked overnight in seawater.
20. Rinse thoroughly with DW.

LAB WORK #1 QUALITATIVE METHODS OF THE ENVIRONMNETAL ANALYTICAL CHEMISTRY. STATISTIC TREATMENT OF ENVIRONMNETAL ANALYSIS DATA

The reliable measurement of environmental pollutants is essential in making decisions for waste management and public health protection. The measurement of these pollutants in multimedia is by no means a trivial task. Given that the physical and chemical properties of certain wastes are inherently complex, producing reliable data on the distribution and concentration of pollutants in the environment is an arduous task, often requiring specialized training. As a result, sampling and analysis can be the most expensive and time consuming aspects of the environmental monitoring.

To choice the analytical environmental methods, it is important to know their comparison characteristics (Tables 1 and 2).

Table 1 – Comparison of the main indexes of the analytical methods for environmental monitoring [1]

Index	Methods of analysis		
	Chemical	Physico-chemical	Physical
1. Sensitivity (minimum concentration), ppm (without concentration)	1,0 – 0,1	0,05 – 0,005	0,01 – 0,001
2. Accuracy, % (relative)	0,01 – 0,5	1 - 10	2 - 20
3. Selectivity	Satisfactory	High	Very high
4. Duration of analysis, min (without sample preparation)	30 - 200	15 - 60	10 - 30
5. Cost of measuring equipment, relative units	1	20 - 100	100 - 500
6. Possibility of rapid execution for mass analyses	Low	Middle	High
7. Necessity of service personnel	No needed	Desired	Required
8. Possibility of complete automation	Low	Middle	High

The comparative analysis of analytical methods shows that the main advantages of the chemical methods are their high accuracy and relative low cost of measuring equipment, but shortcoming are considerable duration and impossibility of complete automation.

Table 2 – Methods of analysis of pollutants [2]

Method	Aggregative state of samples	Equipment	Cost, standard units	Relative sensitivity	Duration, hours	Sencitivity	Accuracy, % (relative)	Remarks
1	2	3	4	5	6	7	8	9
Gravimetry	Solid – liquid – gas	Standard labware	200	Good	1–2	0,1 – 1 g 1-10 μg	0,005-0,01 0,1	Main components of the environment
Titrimetry	Solid – liquid – gas	Standard labware	200	Good	0,25-0,5*	10^{-2} 10^{-5} 10^{-5}-10^{-7} mol/L*	0,01 0,1 0,2-1,0	Main and semimicro-components
Visual spectroscopy	Solid – gas	Colorimeter; spectro-photometer	200 800 - 3000	Satisfactory	0,5 – 1,0*	10^{-3} – 10^{-2} $5 \cdot 10^{-7}$-10^{-5} mol/L*	1-5 5-10 (0,1)*	Semimicro- and micro-components (trace elements)
UV spectroscopy	Solid – liquid – gas	UV spectro-photometer	3000	Satisfactory	0,5 – 1,0*	10^{-3} – 10^{-2} $5 \cdot 10^{-7}$–10^{-5} mol/L*	1-5 5-10 (0,1)*	Semimicro- and micro-components (chromophores and organic components)

1	2	3	4	5	6	7	8	9
Flame emission spectroscopy (FES)	Solid – liquid	Flame photometer and spectroscope	800 3000-5000	Good	0,25–0,5*	10^{-5} – 10^{-2} $5\cdot10^{-7}$–10^{-5} mol/L*	0,5 – 3 5 –10	Micro-components of alkali, alkali-earth and a few d-elements
Atomic absorption spectroscopy (AAC)	Solid – liquid	AA-spectro-photometer	4000-10000	Excellent	0,25–0,5*	10^{-5} – 10^{-3} 10^{-7} – 10^{-5} mol/L*	0,5 – 3 5 – 10	Micro-components of some d-elements and semimicro-components
Gas chromato-graphy	Liquid –gas	Gas chroma-tograph	3500-5000	Excellent	0,25-0,5	Main component 1 –2 0,1 – 1 0,01 – 0,1 10^{-3} – 10^{-2} 10^{-3}	0,1 0,2 - 0,5 0,5 – 1,0 1 – 5 5 – 10 10	Main and micro-components; organic and metal-organic compounds
Voltammetry with a loose electrode	Liquid	Pulse polarograph DC	2500	Good	0,25-0,5	10^{-3} – 10^{-2} 10^{-5} – 10^{-3} 10^{-7} mol/L*	1 – 2 3 5	Micro-components (trace metals: Ag, Bi, Cd, Fe, In, Pb, Sb, Sn, Zn)

1	2	3	4	5	6	7	8	9
Spectro-fluorimetry	Solid-liquid	recording spectro-fluorimeter	7000	Good	0,5 – 1,0	$10^{-7} – 10^{-3}$	0,5 - 10	Inorganic and organic micro-components
X-ray fluorescence spectrometry	Solid-liquid	X-ray fluorescence spectrometer	50000	Good	0,25-0,5	$10^{-3} – 2 \cdot 10^{-2}$	1 - 2	Semimicro-components in solis
Liquid chromato-graphy	Solid-liquid	HPLC	6000-8000	Good	0,5 – 1,0	$10^{-7} – 10^{-4}$	2 – 20	Micro-components (as usual, organic substances)
Polarography	Liquid	Multi-functional polarograph	100000	Good	0,25-0,5	$10^{-3} – 10^{-2}$ $10^{-5} – 10^{-3}$ mol/L *	1 – 2 3	Semimicro- and micro-components: organic substances

* With using precise (differential) techniques

Nowadays the importance of the 3d group (spectroscopy, thermal methods et al) is strongly increasing due to high sensitivity, selectivity, rapidity and possibility of automation. They are widely used in test systems for field analysis (for example, electroconductivity, pH of water and soils, dust in the air and so on).

Statistical treatment of Quantitative Analysis Data

Figures often beguile me, particularly when I have the arranging of them myself; in which case the remark attributed to Disraeli would often apply with justice and force: 'There are three kinds of lies: lies, damned lies and statistics.'" - *Mark Twain*

No measurement is perfectly accurate or exact. Many instrumental, physical and human limitations cause measurements to deviate from the "true" values of the quantities being measured. These deviations are called "experimental uncertainties," but more commonly the shorter word "error" is used. It is impossible to make an exact measurement. Therefore, all experimental results are wrong. Just how wrong they are depends on the kinds of errors that were made in the experiment. Wrong result doesn't mean bad. We're using the word "wrong" to emphasize a point. All experimental data is imperfect. Scientists know that their results always contain errors. However, one of their goals is to minimize errors, and to be aware of what the errors may be. Significant digits are one way of keeping track of how much error there is in a measurement.

Results should only be reported to the proper number of significant digits, because the number of significant digits and associated error are indications of the precision of the analytical results. Correct handling of significant digits (and error) and retention of the available precision requires an understanding of the propagation of significance in calculations.

Generally, if not specified, the precision may be assumed to be ±1 in the last reported digit, which is termed the least significant digit. However, some values effectively have an infinite number of significant digits. For instance, 1 inch is defined as exactly 2,54 cm (2,54 with an infinite number of zeros following) and each value is infinitely precise for purposes of conversion. In addition, for practical purposes, many constants (speed of light, Planck's constant, etc.) are comparably precise and do not limit the precision of the results of calculations involving them.

The number of significant figures is defined as the quantity of digits in the number excluding leading or trailing zeros. For example, 3,142 has 4 significant figures; 23,459,000 has 5 significant figures; 0,31910 has 4 significant figures (the last zero does not count); and 0,0004086 has 4 significant figures (the zero between 4 and 8 is not a leading or trailing zero and so is counted). Trailing zeros are a main source of confusion, but use of scientific notation allows the writer to indicate the precision by only showing significant figures. Consider the number 2000 (which when written this way has 1 significant digit). The best way to indicate the number of significant digits is to use scientific notation:

$2x10^3$		1 significant digit;
$2,0x10^3$	2,00	2 significant digits;
$x\ 10^3$		3 significant digits.

One should retain all digits when performing calculations and when finished, round the result to the appropriate number of significant digits. For addition and subtraction, the result should have the same number of significant digits as the least precise number in the calculation. For example,

14,72 + 1,4331 + 0,00235 = 16,16.

In contrast, theoretically the only way to determine the correct number of significant digits for the results of calculations involving multiplication and division is to propagate significance as one would propagate error. Thus, the precision of the result cannot be better than the square root of the sum of the squares of the relative errors. For example, a measurement of 52,3 has an implied error of ± 0,1, corresponding to a relative error of 0,0019. If we wished to square this value, the relative error of the result is: $\sigma = 0{,}00268$.

Now, $52{,}3^2 = 2735{,}29$, so the relative error corresponds to an absolute error of: 2735,29 x 0,00268 = 7,3.

The limit on precision is thus 7,3 (rounding to 1 in the tens place), and the result should be presented as 2,74 x 101. In practice this procedure is cumbersome, and usually, unnecessary. Note that the result has the same number of significant digits as the two numbers, which were multiplied. Generally, one can simply follow the rule of rounding the result to the same number of significant figures as the least precise quantity used in the calculation. As examples, the limiting factors for some common calculations are given below:

Calculation	Limiting Factor
Molecular Weight, Weight Fraction, Energy from Wavelength, Wavelength from Energy	The smallest number of significant digits in atomic weights used and smallest number of significant digits in the conversion factor or known quantity
Spectrometer Position	The smallest number of significant digits in the 2d values or spectrometer position
Absorption Coefficient	The smallest number of significant digits in the elemental absorption coefficients

Finally, when it is necessary to reduce the number of digits in a result this should be accomplished by rounding. If the number after the last significant digit is greater than 5, one should round the final digit up; if less, round down. If the digit is exactly 5, round up if the digit preceding it is odd (and down if it is even) to average out the effects of rounding.

In the fields of science, engineering, industry and statistics, the **accuracy** of a measurement system is the degree of closeness of measurements of a quantity to that quantity's actual (true) value. The **precision** of a measurement system, also called reproducibility or repeatability, is the degree to which repeated measurements under unchanged conditions show the same results. Although the two words can be synonymous in colloquial use, they are deliberately contrasted in the context of the scientific method.

Accuracy indicates proximity of measurement results to the true value, precision to the repeatability or reproducibility of the measurement

A measurement system can be accurate but not precise, precise but not accurate, neither, or both. For example, if an experiment contains a systematic

error, then increasing the sample size generally increases precision but does not improve accuracy. The end result would be a consistent yet inaccurate string of results from the flawed experiment. Eliminating the systematic error improves accuracy but does not change precision.

A measurement system is designated *valid* if it is both *accurate* and *precise*. Related terms include *bias* (non-random or directed effects caused by a factor or factors unrelated to the independent variable) and *error* (random variability).

The terminology is also applied to indirect measurements - that is, values obtained by a computational procedure from observed data. In addition to accuracy and precision, measurements may also have a measurement resolution, which is the smallest change in the underlying physical quantity that produces a response in the measurement (ISO/TS 8000-130:2009 Data quality – Part 130: Master data: Exchange of characteristic data: Accuracy).

In the case of full reproducibility, such as when rounding a number to a representable floating point number, the word *precision* has a meaning not related to reproducibility. For example, in the IEEE 754-2008 standard it means the number of bits in the significant, so it is used as a measure for the relative accuracy with which an arbitrary number can be represented.

Relationships of accuracy and precisions are presented in Fig. 1.

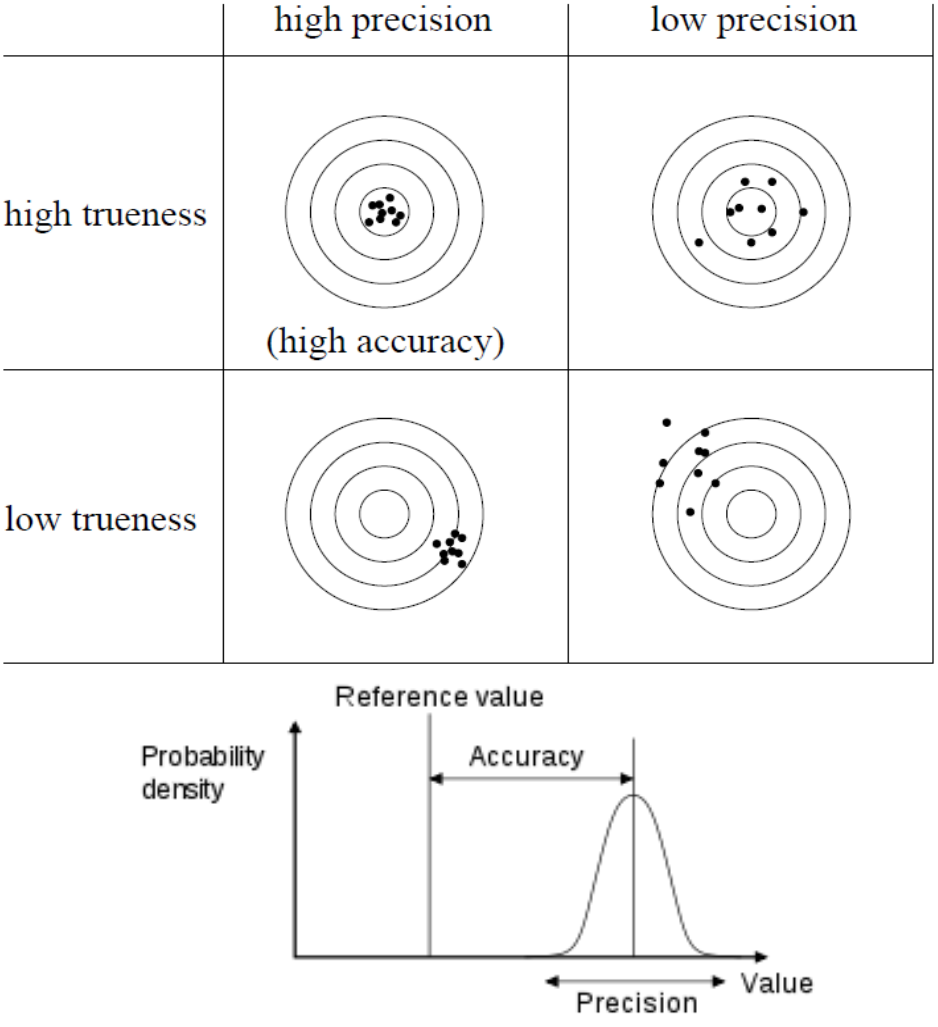


Figure 1 – Accuracy and precision: conceptual illustration

Accuracy may be defined as how close a measured value is to the "true" value. It is often difficult to establish a "true" value and thus the accuracy of an analysis based upon it. Accuracy is affected if the compositions of the standards are not well known whereas precision is not. Standard compositions are often determined by wet chemistry, but this process also analyzes tiny inclusions in the standard materials making it impossible to establish "true" composition.

Sometimes for the minor elements, microprobe analysis of inclusion free areas in a mineral is the best method for determining the true composition. One way to avoid potential problems with the standard compositions is to use mono-elemental (synthetic) standards. With these materials, one only has to look for impurities and make sure that they are insignificant, not determine their abundances. However, the matrix effects may be so significant that a multi-element standard (similar to the unknown) is required.

Precision refers to how well a given measurement or results can be reproduced. Values can be very precisely determined and still be very inaccurate. Conversely, a number of imprecise analyses may average to a very accurate value. Precision is effectively limited by counting statistics when dealing with X-ray analysis.

Many factors, many out of the control of the analyst, can affect both precision and accuracy. Among them are:

- Incorrect standard values, which affect accuracy and produce systematic errors (high values if standard compositions are higher than they should be, lower if low).
- In spectroscopy – focus problems, which can produce significant unsystematic errors and a loss of precision and accuracy. Defocusing the beam on the sample results in imperfect spectrometer optics and reduced count rates. This produces unsystematic errors unless one always misfocuses identically.
- Specimen tilt, which produces systematic errors by changing the take-off angle.
- Irregularities in the sample surface or volume also may produce unsystematic errors.
- Errors in matrix-correction factors, which can significantly reduce accuracy especially where correction coefficients are poorly known. The element F is a good example of this -F in apatite should be determined using a fluorapatite standard, while determining F in micas requires a F-rich mica. Errors in the matrix-correction coefficients will produce systematic errors during data reduction. Everyone worries about getting better (correct) data-reduction factors, but not many do anything about it.
- Errors in nominal accelerating voltage, which cause systematic errors.
- Electronic instability, which primarily reduces precision.

Basic Statistical Tools

A working knowledge of statistics is necessary to the understanding of the limitations of microprobe analysis and results. The sections below provide a very basic review of appropriate terms and statistical methods. The methodology

presented is applicable to all analytical data that involve counting or multiple measurements.

Systematic errors

Systematic errors arise from a flaw in the measurement scheme which is repeated each time a measurement is made. If you do the same thing wrong each time you make the measurement, your measurement will differ systematically (that is, in the same direction each time) from the correct result. Some sources of systematic error are:

- Errors in the calibration of the measuring instruments.
- Incorrect measuring technique: For example, one might make an incorrect scale reading because of parallax error.
- Bias of the experimenter. The experimenter might consistently read an instrument incorrectly, or might let knowledge of the expected value of a result influence the measurements.

It is clear that systematic errors do not average to zero if you average many measurements. If a systematic error is discovered, a correction can be made to the data for this error. If you measure a voltage with a meter that later turns out to have a 0,2 V offset, you can correct the originally determined voltages by this amount and eliminate the error. Although random errors can be handled more or less routinely, there is no prescribed way to find systematic errors. One must simply sit down and think about all of the possible sources of error in a given measurement, and then do small experiments to see if these sources are active. The goal of a good experiment is to reduce the systematic errors to a value smaller than the random errors. For example a meter stick should have been manufactured such that the millimeter markings are positioned much more accurately than one millimeter.

Random errors

Random errors arise from the fluctuations that are most easily observed by making multiple trials of a given measurement. For example, if you were to measure the period of a pendulum many times with a stop watch, you would find that your measurements were not always the same. The main source of these fluctuations would probably be the difficulty of judging exactly when the pendulum came to a given point in its motion, and in starting and stopping the stop watch at the time that you judge. Since you would not get the same value of the period each time that you try to measure it, your result is obviously uncertain. There are several common sources of such random uncertainties in the type of experiments that you are likely to perform:

- Uncontrollable fluctuations in initial conditions in the measurements. Such fluctuations are the main reason why, no matter how skilled the player, no individual can toss a basketball from the free throw line through the hoop each and every time, guaranteed. Small variations in launch conditions or air motion cause the trajectory to vary and the ball misses the hoop.
- Limitations imposed by the precision of your measuring apparatus, and the uncertainty in interpolating between the smallest divisions. The precision

simply means the smallest amount that can be measured directly. A typical meter stick is subdivided into millimeters and its precision is thus one millimeter.

- Lack of precise definition of the quantity being measured. The length of a table in the laboratory is not well defined after it has suffered years of use. You would find different lengths if you measured at different points on the table. Another possibility is that the quantity being measured also depends on an uncontrolled variable. (The temperature of the object for example).
- Sometimes the quantity you measure is well defined but is subject to inherent random fluctuations. Such fluctuations may be of a quantum nature or arise from the fact that the values of the quantity being measured are determined by the statistical behavior of a large number of particles. Another example is AC noise causing the needle of a voltmeter to fluctuate.

No matter what the source of the uncertainty, to be labeled "random" an uncertainty must have the property that the fluctuations from some "true" value are equally likely to be positive or negative. This fact gives us a key for understanding what to do about random errors. You could make a large number of measurements, and average the result.

If the uncertainties are really equally likely to be positive or negative, you would expect that the average of a large number of measurements would be very near to the correct value of the quantity measured, since positive and negative fluctuations would tend to cancel each other.

Assessing Precision

Mean

If x_i is an individual measurement and n measurements are made, then the mean value of the measurements is:

$$\bar{x} = \frac{x_1 + x_2 + x_3 + ... + x_n}{n} = \frac{\sum_{i=1}^{n} x_i}{n}, \qquad (1)$$

where *n* – quantity of parallel measurements.

Standard Deviation

The standard deviation (σ) from the mean of these measurements is defined as:

$$\sigma = \sqrt{\frac{\sum_{i}^{n} (x_i - \bar{x})^2}{n-1}} \qquad (2)$$

A population of measurements with *normal* or *Gaussian* distribution will have 68,3% of the population within ±1 σ , 95,4% within ±2 σ, 99,7% within ±3 σ, and 99,9% within ±4 σ (Fig. 2).

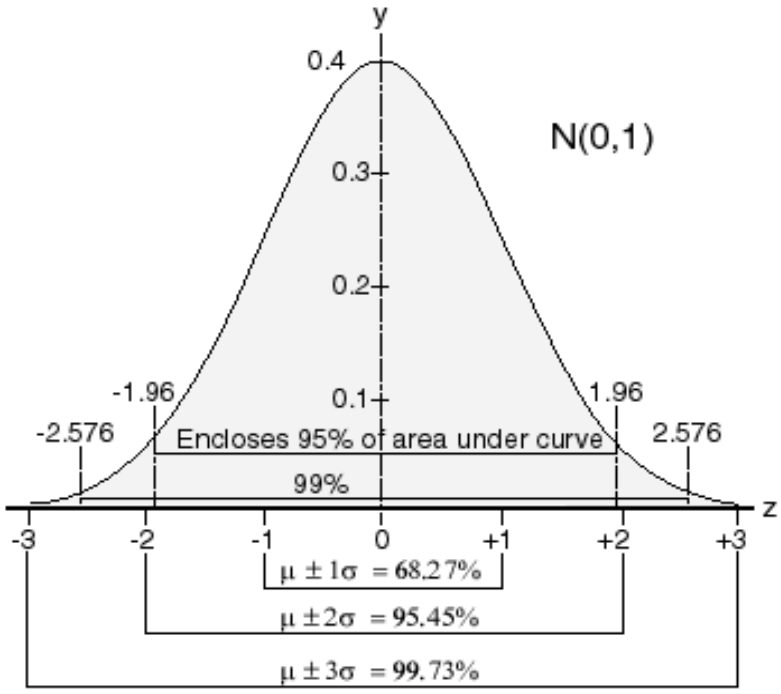


Figure 2 – The standardized normal distribution N (0,1) and its properties

The variance of the measurements may be defined as σ^2, and the coefficient of variation (also called relative error or relative standard deviation) is:

$$\varepsilon = \frac{\sigma}{\bar{x}} \qquad (3)$$

The relative error is often expressed as a percentage of the mean value.

Accuracy of analysis result is characterized by value of confidence Interval of the mean:

$$\Delta x = \frac{t(P) \cdot \sigma}{\sqrt{n}} \ , \qquad (4)$$

where t (P) – so-called t-table (Student's criterion).

Determine the critical value for t by using a t-table (see Table 3). The number of *degrees of freedom* is equal to one less than the number of data points in your set and the p-value is the confidence level. In the example, if you wanted a 97,5% confidence interval and you had seven degrees of freedom (eight data points), your critical value for t would be 2,365.

As usual, for the most analytical data in environmental measuring the P value is 95%.

Result of analysis in any units is presented as (note: remember, that quantity of significant figures are the same ones):

$$\bar{x} \pm \Delta\bar{x}.$$

Table 3 – Values of *t* at the different confidence levels p for various degrees of freedom

Degrees of freedom, n-1	p-value, %			
	90	95	97,5	99
1	3,077684	6,313752	12,70620	31,82052

2	1,885618	2,919986	4,30265	6,96456
3	1,637744	2,353363	3,18245	4,54070
4	1,533206	2,131847	2,77645	3,74695
5	1,475884	2,015048	2,57058	3,36493
6	1,439756	1,943180	2,44691	3,14267
7	1,414924	1,894579	2,36462	2,99795
8	1,396815	1,859548	2,30600	2,89646
9	1,383029	1,833113	2,26216	2,82144
10	1,372184	1,812461	2,22814	2,76377
11	1,363430	1,795885	2,20099	2,71808
12	1,356217	1,782288	2,17881	2,68100
13	1,350171	1,770933	2,16037	2,65031
14	1,345030	1,761310	2,14479	2,62449
15	1,340606	1,753050	2,13145	2,60248
16	1,336757	1,745884	2,11991	2,58349
17	1,333379	1,739607	2,10982	2,56693
18	1,330391	1,734064	2,10092	2,55238
19	1,327728	1,729133	2,09302	2,53948
20	1,325341	1,724718	2,08596	2,52798
21	1,323188	1,720743	2,07961	2,51765
22	1,321237	1,717144	2,07387	2,50832
23	1,319460	1,713872	2,06866	2,49987
24	1,317836	1,710882	2,06390	2,49216
25	1,316345	1,708141	2,05954	2,48511
26	1,314972	1,705618	2,05553	2,47863
27	1,313703	1,703288	2,05183	2,47266
28	1,312527	1,701131	2,04841	2,46714
29	1,311434	1,699127	2,04523	2,46202
30	1,310415	1,697261	2,04227	2,45726
Infinity	1,281552	1,644854	1,95996	2,32635

For example, pH of drinking water : 7,32 ± 0,05; content of nitrate : 54,2±1,8. Optimal quantity of parallel measuring is 3-4, because increasing of their quantity doesn't change t-value greatly and as a result accuracy of analysis. Basing on Egn. 2, it is clear, why single measurement is statistically unreliable: if n=1 that $\sigma \to \infty$ and $\Delta\bar{x}$ would not be determined.

Reporting Results

There are three ways in which the statistical information that accompanies a measurement (average, standard deviation, and confidence limit) can be stated. If, for example, five replicate measurements of a solid's density were made, and the average was 1.015 g/cm^3 with an estimated standard deviation of 0,006, then the results of this experiment could be reported in any of the following ways:

- The average density is 1,015 g/cm^3 with an estimated standard deviation of 0,006 g/cm^3;

- The density is 1,015(6) g/cm^3;
- The density is 1,015 ± 0,007 g/cm^3 at the 95% confidence limit.

Using Statistics to Identify Hidden Gross Error (Dixon's test)

I t is important to calculate the statistical reliability of the mean value $\bar{x}$, or detects the possible crude error in the process of parallel measurements. Gross errors are undetected mistakes that cause a measurement to be very much farther from the mean measurement than other measurements. Another way in which statistics can be used is in the evaluation of suspect data on gross errors by the Q-test. The Q-test is used to identify outlying ("bad") data points in a data set for which there is no obvious gross error. The Q-test involves applying statistics to examine the overall scatter of the data. This is accomplished by comparing the gap between the suspect point (*outlier*) and its nearest neighbor with the range, as shown in Eqn. 5. The calculated *Q* is then compared to the critical *Q* values, Q_c, at given confidence level, like those in Table 4. If the measured *Q* is greater than Q_c, then that data point can be excluded on the basis of the Q-test.

$$Q = \frac{|\text{suspect value} - \text{closest value}|}{\text{highest value} - \text{lowest value}} \qquad (5)$$

Table 4 – Critical Q (Q_c) values at the 90% and 95% confidence limit for a small number of data points, n.

n	3	4	5	6	7	8	9	10
	P = 90%							
Q_c	0,94	0,76	0,64	0,56	0,51	0,47	0,44	0,41
	P = 95%							
Q_c	0,97	0,83	0,71	0,63	0,57	0,53	0,49	0,47

For large data sets ($n > 10$) a data point that lies more than 2,6 times σ from the average may be excluded. Although for medium-sized data sets (between 11 and 15 data points), there is an alternative treatment that is usually sufficient. In these cases, we can use Q_c for $N = 10$, but in doing so, a higher criterion is placed on the data for exclusion of a point than is required by statistics. So, an outlying point that could have been discarded is retained and the precision is quoted as being less than it actually is. But again, it is better to err on the side of caution in our data treatment.

In any case, only one data point per data set may be excluded on the basis of the Q-test. More than one point may be tested, but only one may be discarded.

For example, you have measured the density of copper as 9,43; 8,95; 8,97; 8,96; and 8,93 g/cm^3. Can any of these points be excluded?

First, we must remember that the Q-test is only valid at the extremes, not in the middle of the data set. So before performing a Q-test, it is best to sort the data (as already been done with the data that we are considering). Now look at the extremes and see whether either of the points look odd. In this case, the low value (8,93 g/cm^3) is not that much different than the values in the middle of the set, while the high value (9,43 g/cm^3) looks to be suspect.

Having decided that the 9,43 g/cm^3 value is suspect, we can calculate Q using Eqn. 5, (suspect value = 9,43, closest value = 8,97, highest value = 9,43 and lowest value = 8,93). This gives Q = 0,92 for this point. Since this exceeds Q_c for five data points (for n = 5, Q_c = 0,64 in Table 4), this point may be excluded on the basis of the Q-test. The Q-test may not be repeated on the remaining data to exclude more points.

One last important thing about the Q-test is that it cannot be performed on identical data points. For example, if our data set had been 9,43, 9,43, 8,95, 8,97, 8,96 and 8,93 g/cm^3, we would not have been able to use the Q-test on the 9,43 g/cm^3 values.

Example 1. Measured results of calcium content in soil are the next: 2,87; 2,89; 2,90; 2,95. Estimate extreme result 2,95 using Q-test.

$$\bar{x} = \frac{2{,}87 + 2{,}89 + 2{,}90 + 2{,}95}{4} = 2{,}90;$$

R = highest value – lowest value = 2,95 - 2,87 = 0,08;

If P = 0,95; n = 4 (Table 4): Q_c (P, n) = 0,83;

$$Q = \frac{|2{,}95 - 2{,}90|}{0{,}08} = 0{,}63 < 0{,}83.$$

$Q < Q_c$ (P, n) – no gross error.

$S = 3{,}42 \cdot 10^{-2}$.

$$\Delta\bar{x} = \frac{3{,}18 \cdot 3{,}42 \cdot 10^{-2}}{\sqrt{4}} = 0{,}053 \approx 0{,}05.$$

% (Ca) = 2,90 ± 0,05.

Systematic errors are errors associated with a flaw in the equipment or in the design of the experiment. Systematic errors cannot be estimated by repeating the experiment with the same equipment. Consider again the example of measuring an oscillation period with a stopwatch. Suppose that the stopwatch is running slow. This will lead to underestimation of **all** our time results. Systematic errors, unlike random errors, shift the results always in one direction.

The fundamental limitation to accuracy in chemical analysis is systematic error. Unfortunately, systematic error - which comprises all nonrandom deviations of analytical results from the truth - is the rule in analytical chemistry.

Systematic error comes about whenever the actual nature of the analytical process differs from that assumed. It results from invalid sampling, operator or equipment instability and blunders, unrecognized sample loss or contamination, poor instrument calibration, inadequate physical (mathematical) or random error distribution models, and faulty reporting of data. These problems, which will be covered in some detail below, are not exceptional. It is only through exhaustive, quantitative evaluation of the individual and collective effects of such violations in assumption that the analyst can hope to provide meaningful bounds for systematic error.

Single analysis of the environmental object, especially water or air, doesn't determine reliably its chemical composition, because it varies considerably in space and time.

So, monitoring of the environmental components is organized by the way of repeated sampling in different locations. Averaging the results, the estimation of chemical or biological pollution is done.

It is clear, that discrepancy between the average results of many analyses will be much more than between results of simultaneous parallel measurements of a single example.

So, in this case it is recommended to calculate not only the standard deviation, but the statistically significant extreme results x_{max} and x_{min}. Usually this scenario is used when the quantity of data is from 10 till 1000.

The method of estimation includes the next steps:

1. The extreme points are excluded from data chart;
2. Calculate and σ as above described;
3. Statistically insignificant data are considered such ones that deviate from $\bar{x}$ more that 4 σ (see Fig. 2):
4. After excluding of statistically insignificant data the calculation of $\bar{x} \pm \Delta\bar{x}$ it is repeated and determine a new x_{max} and x_{min} (see Fig. 3).

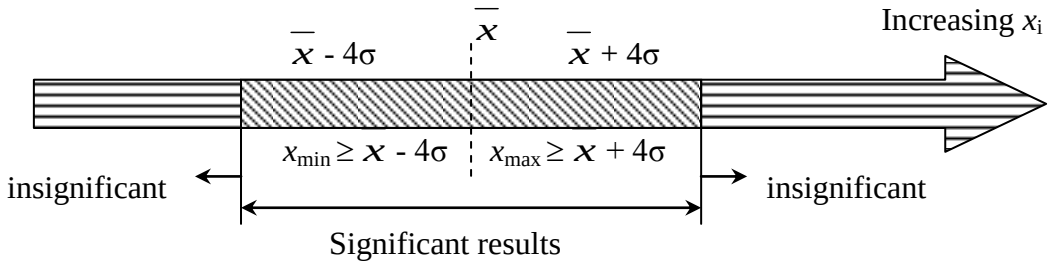


Figure 3 – Conditions of the statistically significant extreme results for mass data

Example 2. During one week the calcium content was determined in lake fresh water. The data chart is: 12,0; 10,1; 8,9; 8,6; 8,3; 8,0; 7,9; 7,8; 7,6 and 6,5 ppm. Determine statistically significant extreme results.

1. To do the statistic treatment as above described, excluded two points: *maximum* - 12,0 and *minimal* - 6,5.

$$\bar{x} = 8{,}4;\ \sigma = 0{,}81;\ \Delta\bar{x} = 0{,}68 \approx 0{,}7.$$

So, $x_{min} = 0{,}65 > \bar{x} - 4\sigma = 5{,}2$ and $x_{max} = 12{,}0 > \bar{x} + 4\sigma = 11{,}6$, The result x_{min} is statistically significant, while x_{max} – insignificant. For the next corrected calculations the nearest value will be used: $x_{max} = 10{,}1$.

In general, the average calcium concentration and its extreme values during time of monitoring are:

$C\ (Ca^{2+}) = 8{,}4 \pm 0{,}7$ ppm; $C\ (Ca^{2+})_{min} = 6{,}5$ ppm; $C\ (Ca^{2+})_{max} = 10{,}1$ ppm.

Recommended sources

1. Pradyot Patnaik. Handbook of environmental analysis: chemical pollutants in air, water, soil, and solid wastes / 2nd ed. – New York: CRC Press, 2010. - 729 pp.

2. Dunnivant F.M. Environmental laboratory exercises for instrument analysis and environmental chemistry / John Wiley & Sons, Inc., Hoboken, New Jersey, 2004. – 268 pp.
3. ISO/TS 8000-130:2009 Data quality - Part 130: Master data: Exchange of characteristic data: Accuracy
4. NIST/SEMATECH e-Handbook of Statistical Methods http://www.itl.nist.gov/div898/handbook/index.htm**.**
5. Statistics for Analytical Chemistry http://science.widener.edu/ svb/stats/header.html

Control questions

Question 1. Which of the following are characteristics of **random** errors? Check all that apply.

A. Doing several trials and finding the average will minimize them;
B. The observed results will usually be consistently too high, or too low;
C. Proper design of the experiment can eliminate them;
D. There is no way to know what they are.

Question 2. Which of the following are **systematic** errors in measuring the density of a liquid as described in this procedure? Check all that apply.

A. Your eye level will move a bit while reading the meniscus;
B. Some of the liquid will evaporate while it is being measured;
C. Air currents cause the balance to fluctuate;
D. The balance may not be properly calibrated.

Question 3. You should always make sure to include "human error" in your lab writeup?

True
False

Never report these things as "human error". They are mistakes that should not have happened.	• Spilling, or sloppiness, dropping the equipment, etc. • Bad calculations, doing math incorrectly, or using the wrong formula; • Reading a measuring device incorrectly (thermometer, balance, etc.) • Not cleaning the equipment • Using the wrong chemical • Not following the planned procedure

Question 4. Calculate sum (Hint: significant figures): 104,630 + 27,08362 + 0,61 =

Question 5. What is the average of 0,1707; 0,1713; 0,1720; 0,1704; and 0,1715?

Question 6. Calculate the sum of the squares of the deviations from the mean for the five numbers given in Question 5 above, in two different ways: (a) carrying all digits through all the calculations; (b) rounding all intermediate results

to 2 significant figures after subtracting the mean from each (on your way to calculating the sum of the squares about the mean). Compare the two results.

Question 7. The following 21 results were obtained by the colorimetric determination of iron in hard-water samples: 10,1; 10,0; 9.9; 10,4; 9,8; 10,2; 9,6; 10,1; 9,9; 9,4; 9,3; 10,6; 10,7; 9,8; 10,2; 10,0; 10,4; 10,0; 9,6; 10,1, and 9,9 ppm Fe.

Calculate:

- The mean;
- Absolute value of the standard deviation;
- The confidence limits for the mean at the 95% probability level.

Question 8. A certain analytical method should give a relative standard deviation of 5 ppt or better. A sample is first analyzed three times using this method to give the following set of results: 40,12%, 40,15%, and 40,55%. Because the 40,55% result appears questionable, two additional results are obtained as follows: 40,20%, and 40,39%. What is the standard deviation of the first set (n=3) and of the complete set (n=5)?

Question 9. Is the standard deviation always a better estimate of precision that the range, even if n=3? If so, why is the range (and the Q test) used?

Question 10. The absolute value of the range for an analysis for sodium carbonate is usually around 1,0% or less. An analyst obtains sodium carbonate percentages of 30,00%, 30,20%, and 32,00%. He then decides to obtain a fourth result. Why? Assuming his fourth result is 30,50%,calculate central tendency of his results.

LAB WORK 2 NITRATES IN DRINKING WATER

Background

Nitrates are present naturally in soils, waters, all plant materials, and in meats. They are also found in small concentrations (1-40 μg/m^3) in air as a result of air pollution. Levels in cultivated soils and thus, levels in water, (which normally do not exceed 10 mg/L) may be increased by the use of commercial nitrogenous fertilizers and by the return of wastes, derived from animal husbandry or other sources, to the soil.

Nitrate contents of crops are influenced by the plant species, by genetic and environmental factors, and by agricultural management practices. In certain crops the levels may be very high (1000 mg/kg or more).

Nitrites are formed in nature by the action of nitrifying bacteria as an intermediate stage in the formation of nitrates, but concentrations in plants and water are usually very low. However, microbiological conversion of nitrate to nitrite may occur during the storage of fresh vegetables, particularly at room temperature, when nitrite concentrations may rise to exceptionally high levels (about 3600 mg/kg dry weight). Both nitrates and nitrites are widely used in the production and preservation of cured meat products and of some fish. Such uses, which are controlled by law in many countries, are considered vital for the prevention of botulism caused by the growth of the toxin-producing strains of *Clostridium botulinum* that are sometimes present in raw meat and that may persist in cooked meats.

The weekly intake of nitrates by a member of the general population in England or in the USA has been roughly estimated to average about 400-500 mg but these figures cannot be applied generally because of variations in feeding habits and in the nitrate concentrations in food and water.

Metabolism and health risks of nitrates and nitrites

In normal healthy individuals, nitrates and nitrites are rapidly absorbed from the gastro-intestinal tract. Absorbed nitrite reacts with haemoglobin to form methaemoglobin which, in adults, is rapidly converted to oxyhaemoglobin by reducing systems such as NADHmethaemoglobin reductase. In infants up to three months old and in very young animals this enzyme system is not completely developed.

Under these conditions, the methaemoglobin formed may increase in the body resulting in a characteristic clinical condition (methaemoglobinaemia, syndrome “Blue baby”). Microorganisms present in the food and gastrointestinal tract of very young infants may convert nitrates to nitrites and thus exacerbate the problem in this age group. In healthy individuals, absorbed nitrates are rapidly excreted by the kidneys.

Adults do not appear to be harmed directly by exposure to the prevailing concentrations of nitrates and nitrites in the environment, although some recent studies have indicated that nitrate aerosols in the ambient air may act as respiratory irritants. However infants and very young children are particularly susceptible to the induction of methaemoglobinaemia by nitrates and nitrites, ingested in water

and food, and several cases of illness and death have been reported. In most cases of methaemoglobinaemia, well-water containing high concentrations of nitrates had been used in the reconstitution of infant dried milk preparations. Most instances have been associated with water containing more than 90 mg/L but a few cases of methaemoglobinaemia in infants have been associated with the consumption of water containing less than 50 mg/L. Cases of methaemoglobinaemia in babies fed with spinach purée or carrot juice (both of which may contain very high levels of nitrates) have been reported, but there are too few data to establish dose-response relationships.

With respect to the adverse effects of nitrates and nitrites on infants, there is a need to investigate the relationship between methaemoglobinaemia and sudden infant death and to make further studies on the role of gastroenteritis in increasing infant susceptibility to nitrate poisoning. The role of acidified milk preparations and *Lactobacilli* in protecting infants against methaemoglobinaemia, and the possible protective role of ascorbic acid fortification of infant milk preparations should also be elaborated.

Sources of nitrates and nitrites and levels in the environment

In the environment (e.g. surface waters, soil) both nitrite and nitrate ions can be formed from the ammonium ion (NH_4^+) in a two step biological oxidation (nitrification) process:

$$2\ NH_4^+ + 2OH^- + 3O_2 \rightleftharpoons 2\ NO_2^- + 2H^+ + 4H_2O \quad (1)$$

$$2\ NO_2^- + O_2 \rightleftharpoons 2\ NO_3^- \quad (2)$$

These two reactions are mediated by different microorganisms: reaction (1) by an aerobic chemolithotroph *Nitrosomonas;* reaction (2) by *Nitrobacter* which obtains almost all its energy from the oxidation of nitrites.

Higher plants assimilate nitrite from the soil by (a) reduction of nitrate to nitrite which is catalyzed by nitrate reductase (NADPH), and (b) reduction of nitrite to ammonia catalysed by nitrite reductase. Bacteria of many kinds can also reduce nitrate to nitrite. However, because nitrite is easily oxidized to nitrate the concentration of nitrites in environmental media such as surface waters is usually very low (about 1 mg/L) even when the nitrate concentration is high (50-100 mg/L). These biochemical reactions are a part of the nitrogen cycle.

Purification of Contaminated Water

While it may be technically possible to treat contaminated groundwater, it can be difficult, expensive and not totally effective. For this reason, prevention is the best way to ensure clean water. Water treatments include distillation, reverse osmosis, ion exchange or blending.

- **Distillation** boils the water, catches the resulting steam, and condenses the steam on a cold surface (a condenser). Nitrates and other minerals remain behind in the boiling tank.
- **Reverse osmosis** forces water under pressure through a membrane that filters out minerals and nitrate. One-half to two-thirds of the water remains behind the membrane as rejected water. Higher-yield systems use water pressures of 150 psi.

- **Ion-exchange** takes another substance, such as chloride, and trades places with nitrate. An ion exchange unit is filled with special resin beads that are charged with chloride. As water passes over the beads, the resin takes up nitrate in exchange for chloride. As more water passes over the resin, all the chloride is exchanged for nitrate. The resin is recharged by backwashing with sodium chloride solution. The backwash solution, which is high in nitrate, must be properly disposed of.
- **Blending** is another method to reduce nitrates in drinking water. Mix contaminated water with clean water from another source to lower overall nitrate concentration. Blended water is not safe for infants but is acceptable for livestock and healthy adults.

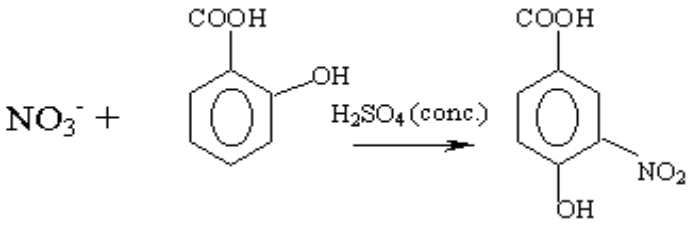
Charcoal filters and water softeners **do not adequately remove** nitrates from water. Boiling nitrate-contaminated water does not make it safe to drink and actually increases the concentration of nitrates. Drilling a new well to deeper water with less nitrate may be a feasible remedy in certain areas. In many cases, the most effective alternative is to use bottled water for drinking and cooking.

Principle of nitrates determination

Determination of nitrate (NO_3^-) is difficult because of the relatively complex procedure required, the high probability that interfering constituents will be present and the limited concentration ranges of the various techniques. It is proposed to use so-called colorimetric method with sodium salicylate.

Nitrate reacts with sodium salicylate in presence of sulfate acid and produces a nitrosalicylate salt colored in yellow color.

The color produced follows Beer's law and its intensity is proportional to the concentration of NO_3^- present in the sample. The chemical reaction involved in the method is given below:



Sensitivity of method is 0,1 mg NO_3-N/L.

Apparatus and equipment

1. Colorimeter or spectrophotometer having a range of 300-700 nm.
2. Water bath
3. Volume flacks, capacity 50 and 100 mL.
4. Graduated pipets, capacity 1 and 10 mL with graduation 0,01 and 0,1 mL correspondingly.
5. Beakers, capacity, 100 mL.
6. Porcelain evaporating dishes.
7. Matched color-comparison tubes.
8. Stirring rods.

Reagents and standards

1. *Sulfate acid*, concentrated (AR).
2. *Caustic soda* (AR).
3. *Cobaltous chloride* $CoCl_2 \cdot 6H_2O$ (AR).
4. *Potassium nitrate* KNO_3 (AR).
5. *Sodium salicylate* $C_7H_5NaO_3$ (AR).
6. *Chloroform* CH_3Cl (AR).
7. *Seignette's* salt or Rochelle salt (potassium sodium tartrate) $NaKC_4H_4O_6$ (AR).
8. *Distilled water*.

All reagents must be nitrate-free.

Preparation to analysis

1. *Basic standard potassium nitrate solution*: dissolve in distilled water 0,7218 g KNO_3, dried previously at (105±2°C), add 1 cm^3 chloroform, and dilute to 1000 mL. 1 cm^3 = 0,1 mg NO_3-N.

2. *Working standard potassium nitrate solution:* Dilute by distilled water 10 cm^3 of basic solution to 100 cm^3 in measuring flack 100 mL. 1 cm^3 = 0,01 mg NO_3-N. Use fresh-prepared solution.

3. *Potassium sodium tartrate solution*: Dissolve 30 g of salt in 70 cm^3 distilled water.

4. *0,5% Sodium salicylate solution:* Dissolve 0,5 g of salt in distilled water and dilute to 100 mL. Use fresh-prepared solution.

5. *10 N NaOH solution:* Dissolve 400 g caustic soda in distilled water and after cooling dilute to 1000 mL.

Procedure

The next additives in analyzing water are interfered to determination:

- Color, which is eliminated to add of alum suspension;
- Chlorides (more than 200 mg/L) – to precipitate by silver sulfate and filtrate of sediment;
- Nitrates (more than1-2 mg/L);
- Iron (more than 0,5 mg/L) , which is eliminated to add of 8-10 drops Seignette's salt before evaporating in porcelain dish.

Take 10 cm^3 (or less, if nitrate content is predicted high, for example, for well it is recommended 1 cm^3) of examining water sample in porcelain dish. Add 1 cm^3 0,5% sodium salicylate solution and evaporate to dryness in water bath. After cooling moisture dry residue by 1 cm^3 of concentrated sulfate acid, rub thoroughly by stirring rod, and wait 10 min.

After than add 5-10 cm^3 of distilled water and transfer quantitatively in 50 cm^3 volume flack. Add 7 cm^3 10 N NaOH solution and dilute to 50 mL by distilled water. Color must be stable 10 min. after caustic soda adding.

Determine optical density of prepared solution on photocolorimeter, using wavelength filter 440 nm (violet) and 10 mm cuvettes. Subtract the value of blank solution optical density and determine nitrates content using calibration curve.

Remember, that blank solution contains all the reagents and solvents used in the analysis, but no deliberately added analyte.

Calibration curves construction

Add 0,0; 0,5; 1,0; 2,0; 3,0; 4,0; 6,0 and 10 cm^3 working standard potassium nitrate solution to 10 mL-volume matched color-comparison tubes and dilute of distilled water. Content of nitrate nitrogen will be equal to 0,5; 1,0; 2,0; 3,0; 4,0; 6,0; and 10 mg/L responsibly. Transfer these solutions to evaporating dishes, add 1 cm^3 0,5% sodium salicylate solution and evaporate to dryness in water bath. Treat dry residues as described above for examining water sample. Measure optical density of prepared solutions on photocolorimeter, using wavelength filter 440 nm (violet) and 10 mm cuvettes. Subtract the value of blank solution optical density. Prepare calibration curve (example – Fig. 11), using Exel (type of approximation – linear, according to Beer's law).

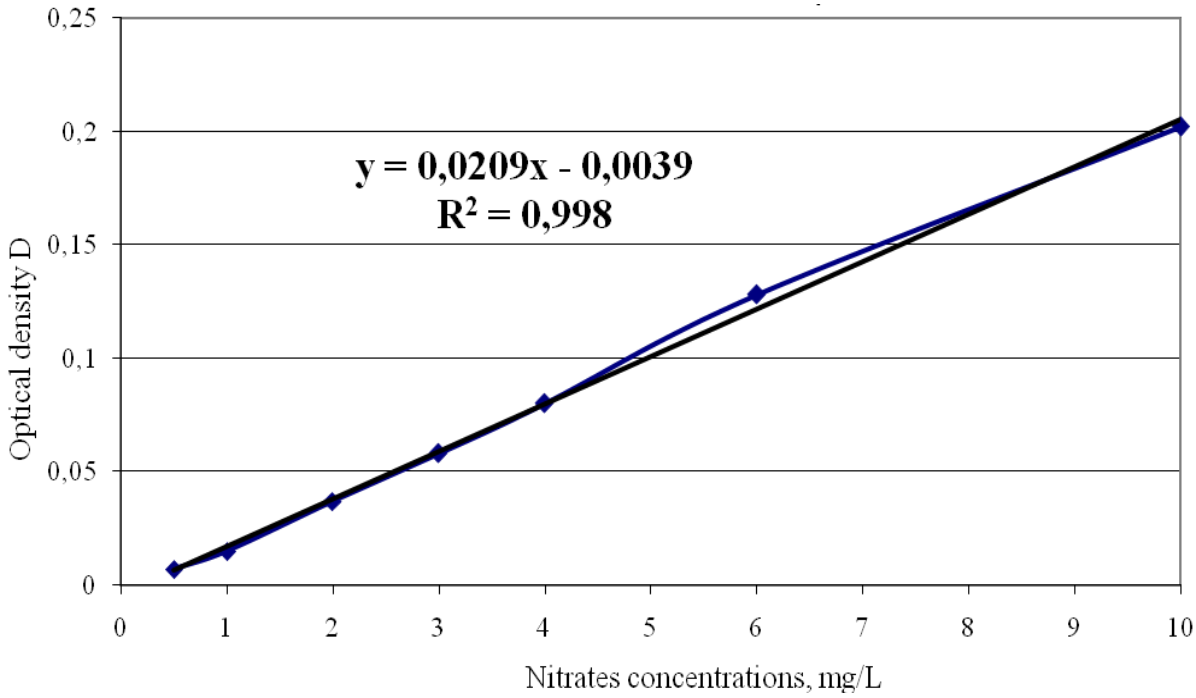


Figure 11 – Example of Calibration curve

Calculation

Nitrates content (X) is determined as $N\text{-}NO_3^-$ (mg/L), using calibration data:

$$X = \frac{C_{NO_3^-} \text{ of calibration curve} \times 10}{V \text{ water sample}} = XX,X \text{ mg/L}.$$

Nitrates concentration of calibration curve is calculated basing on regression equation, obtained from linear approximation.

For example, using calibration curve, presented above:

$$x = C_{NO_3 \text{ of calibration curve}} = \frac{y + 0{,}0039}{0{,}0209},$$

where y - measured optical density.

Example

Volume of well water sample – 2 cm^3; measured optical density – 0,378.

Calculation:

$$x = C_{NO_3 \text{ of calibration curve}} = \frac{y + 0{,}0039}{0{,}0209} = \frac{0{,}378 + 0{,}0039}{0{,}0209} =$$

= 18,27 mg/L.

$$X = \frac{C_{NO_3 \text{ of calibration curve}} \times 10}{V \text{ water sample}} = \frac{18{,}27 \times 10}{2} = 91{,}4 \text{ mg/L}.$$

Compare obtained experimental results with guidelines of different levels (see Table 16).

Table 16 – Guidelines of nitrates content NO_3^-, mg/L

Ukraine*	WHO [1]	EU [3]	US EPA [4]
Tap water – ≤ 50 Wells – ≤ 50 Bottled, buvets - ≤ 10	≤ 50	≤ 50	≤ 10 ($N\text{-}NO_3^-$) ≤ 44,3 (as NO_3^-)

* ДСанПіН 2.2.4-171-10 «Гігієнічні вимоги до води питної, призначеної для споживання людиною».

Recommended sources

1. Nitrate and nitrite in drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. – WHO, 2007. – 21 p. [http://www.who.int/entity/water_ sanitation_health/gdwqrevision/nitratesnitrite/en/index.html]

2. Protecting Groundwater for Health Managing the quality of Drinking-water sources

http://www.who.int/water_sanitation_health/publications/protecting_ground water/en/index.html

3. Council Directive 75/440/EEC of 16 June 1975 concerning the quality required of surface water intended for the abstraction of drinking water in the Member States (OJ L 194 25.07.1975 p. 26)

4. Drinking Water Contaminants | Drinking Water Contaminants | US EPA - http://water.epa.gov/drink/contaminants/index.cfm

Control questions

Question 1. Which contaminants found in some drinking water should young children avoid?

A. Lead;
B. Phosphates;
C. Nitrates;
D. Calcium.

Question 2. Organic nitrogen is converted back to inorganic nitrogen like ammonium through the process of:

A. Nitrogen fixation;
B. Nitrofication;
C. Decay;
D. None of the choices.

Question 3. Ammonium (NH_4^+) stays in soil, while nitrate (NO_3^-) is easily leached out. Why do they behave so differently?

A. NO_3^- is broken down by bacteria, so it is easily carried away;
B. NH_4^+ has a positive charge and sticks to soil particles;
C. NH_4^+ is unavailable to organisms, so it remains unchanged in the soil;
D. NO_3^- is only produced when water is flowing through soil.

Question 4. Circle the correct answer to each True or False statement:

A. If the Nitrosomonas bacteria are killed off, the Nitrobacter bacteria will continue working on the ammonia and you will have a jammed cycle with high levels of nitrite.

B. Nitrate reactions in fresh water can cause oxygen to increase.

Question 5. Note possible methods for nitrate removal from drinking water. What is the best from your opinion?