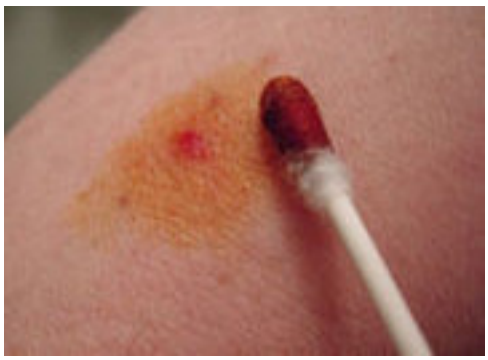
ANALYSING ANTISEPTICS
Comparing and validating analytical methods



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Languages available
English, Hungarian, Slovenian

Summary
You run an analytical laboratory controlling various types of over-the-counter (OTC) medicines. Whether the drugs in circulation are good or poor quality is your responsibility. It is vital that the laboratory uses reliable analytical methods. However, tedious and/or expensive procedures would bankrupt your business. You have to find the optimum process.

In this activity you have to deal with two problems:
1. The quality of an antiseptic solution has to be checked by a method that you have used for years.
2. You have heard about a new analytical process. Before you use this method instead of the 'old' one, you have to validate it. If it proves to be reliable you have to make a financial comparison between the old and new procedures. You can make the right decision only if you take both aspects into consideration.

Activity type **F**
Use of scientific knowledge and understanding to solve problems
Working in teams to solve problems
Resource/ budget management
Time and workload management

Techniques
Potentiometry
Titration

Field
Pharmaceuticals
Tests of significance

Time
Practical lessons: 360 minutes
Theory lessons: 45 minutes
Out of class time: 135 minutes

StandardBase procedures
None

StandardBase techniques
Redox titration
Potentiometry

Other resources
Gedeon Richter plc, Budapest, Gyömrői u. 19-21, Hungary; www.richter.hu
G. D. Christian: Analytical Chemistry, 4th Edition, John Wiley & Sons, 1986
D. A. Skoog, D. M. West, F. J. Holler: Fundamentals of Analytical Chemistry, 6th Edition, Saunders College Publishing, 1992
http://en.wikipedia.org/wiki/disinfection (with iodine)
http://www.google.co.uk/disinfection (using iodine)
http://www.google.co.uk/F (test)
http://en.wikipedia.org/wiki/validation
http://en.wikipedia.org/wiki/F-test
http://www.itl.nist.gov/div898/handbook/eda/section3/eda359.htm

Student's document

ANALYSING ANTISEPTICS: Comparing and validating new analytical methods

You work at an analytical laboratory controlling various types of over-the-counter (OTC) medicines. One of these is an antiseptic solution applied to living tissue/skin to reduce the possibility of infection (in Hungary its name is *Betadine*). The main ingredient of this medicine is iodine. Iodine has been used for a long time as an antiseptic. Lugol's iodine, also known as Lugol's solution, first made in 1829, is a solution of iodine named after the French physician J. G. A. Lugol. It consists of 5 g I_2 and 10 g KI in 100 cm^3 aqueous solution with a total iodine content of 130 mg/cm^3. Tincture of iodine is usually 100 mg/cm^3 elemental iodine in ethanol, although it is also available in 20 mg/cm^3, 30 mg/cm^3 and 70 mg/cm^3 mixtures. It is an essential component of any emergency survival kit, used both to disinfect wounds and to disinfect surface water for drinking. Novel iodine antiseptics containing povidone-iodine, a water-soluble polymer with I_3^- anions, are far better tolerated, don't affect wound healing negatively and leave a deposit of active iodine, creating the so-called 'remnant', or persistent, effect. The great advantage of iodine antiseptics is their wide scope of antimicrobial activity, killing all principal pathogens and, given enough time, even spores, which are considered to be the most difficult form of micro-organisms to be inactivated by disinfectants and antiseptics.
In the lab there is a standard method for the determination of the iodine content of this solution, but you have read about a new possibility which seems to be profitable.
First you have to test and validate this new procedure. Validation has several meanings: generally, validation is the process of checking if something satisfied a certain criterion. Validation implies one is able to testify that a solution or process is correct. The final step is to compare the costs and workload for the two methods.

Your brief

Your laboratory has had many years' experience, and good results, determining the iodine content of an antiseptic solution with iodometric titration using starch as indicator of the end point. The 'new' idea is a potentiometric measurement. You have to compare the results of the potentiometric titration with the results of the iodometric titration.

How can you tell if there is a significant difference between the two methods? The answer is to use an appropriate statistical evaluation, the F-test. If the result of the F-test shows that there is no significant difference in the precision of the two methods, then the new procedure can be accepted (as long as it is quicker and/or cheaper). Precision is mentioned in the last sentence: what does this mean? Precision is the degree of agreement between replicate measurements - the *reproducibility* of a result. Although a method may give a result with high *precision*, you have to check the agreement between the measured value and the 'true' value – this is the *accuracy* of the method.

The reason for differentiating between precision and accuracy is that there are two types of error: systematic (determinate) errors and statistical

(indeterminate) errors. For example, in the potentiometric titration, the origin of a systematic error can be the adsorption of the organic compound on the surface of the platinum electrode. This phenomenon can shift the end-point. If the adsorption is reproducible, then the result of the titration has *high precision*. But if we accept the analytical result of the titration using starch as indicator as 'true' value and the difference is large, then the potentiometric method has *low accuracy.*

In this investigation, if the potentiometric method gives a result close to the 'true' value, i.e. the accuracy of the two methods is similar, then the next step is to compare the precision of the two analytical procedures.

Statistical errors represent the experimental uncertainties. Every analytical method inherits statistical errors. This error can diminish by increasing the number of parallel measurements.

Finally, make a financial comparison. If the potentiometric method is favourable in both respects, then you can replace the 'old' starch method with potentiometry.

Your investigation
Work in a group of two.

Before you start the laboratory work, read about potentiometric techniques and iodometric titrations from StandardBase. To plan the experimental work, you should also find out what the F-test is and how many parallel titrations must be done. Take into account the amount of laboratory time you are allowed.

Find out what kind of iodine-containing antiseptic solution is on the market in your country. The solubility of iodine in water is very poor: 3.5 mg/cm^3 at room temperature. In days gone by, the iodine content of the antiseptic solutions was increased to 48-52 mg/cm^3 by adding 37-44 mg/cm^3 KI with ethanol as solvent. Nowadays, the addition of a complexing agent makes it possible to get a sufficiently high iodine content in aqueous solution. In *Betadine* the active iodine content is 10 mg/cm^3.

Tasks
After carefully reading the practical tasks, share out the work among the members of the group. You will have 360 minutes for the experimental work.

One student should prepare the standardised $Na_2S_2O_3$ solution, the other the starch and the KIO_3 solutions (see **Student sheet:** *Preparation of solutions*).Then one student titrates the sample with starch indicator, while the other makes the potentiometric measurements. Each student measures 5 parallels.

Task 1
Using **Student sheet***: Standardisation of the $Na_2S_2O_3$ titrant* determine the exact concentration of $Na_2S_2O_3$ solution. It has to be done every day.

Task 2
Using **Student sheet***: Titration of the sample using starch to indicate the end point* titrate the *Betadine* sample with the $Na_2S_2O_3$ titrant.

Task 3
Using **Student sheet***: Potentiometric titration of the sample* titrate the *Betadine* sample with the $Na_2S_2O_3$ titrant.

Measure 5-5 parallels to get realistic statistical data.

A risk assessment must be carried out before starting practical work.

Your findings
Evaluation of the experimental results
Using **Student sheet***: Evaluation of experimental results*, calculate
- the concentration of the titrant
- the iodine content separately for the two types of the measurements
- the standard deviations for the two methods
- a value for *F*.

Consider the difference in the precision of the two methods and decide whether or not there is significant difference between the 'old' and the 'new' methods.

Financial management
Calculate the cost of the chemicals. You can find prices in catalogues.
How much time have you spent to get the final analytical results? Try to obtain information about the average hourly wage of a technician in your country! Take this into account.
Compare the costs of the two methods.

Conclusion
What is your final decision? Will you use potentiometry instead of traditional iodometry using starch?

Student self assessment
This activity is about using scientific knowledge and understanding to solve problems, working in a team and managing resources and budgets.

In 50-100 words:
- say what scientific skills and knowledge you needed and how effectively you used them
- describe the advantages of working in a team
- describe how you managed your time and workload
- describe how you managed resources and budgets

Student sheet: Preparation of solutions

Prepare the following solutions

$Na_2S_2O_3$ titrant

Weigh 4.96 g $Na_2S_2O_3.5H_2O$. Dissolve it in distilled water, wash into a 1000 cm^3 volumetric flask and fill up to the mark. The concentration of this solution is about 0.02 mol/dm^3.

KIO_3 solution to standardise the titrant

Weigh 0.7133 g KIO_3 on an analytical balance. Dissolve it in distilled water, wash it quantitatively into a 1000 cm^3 volumetric flask and fill up to the mark. The concentration of this solution is 1/300 mol/dm^3. Therefore 1.00 cm^3 of this solution will measure 3.1622×10^{-3} g $Na_2S_2O_3$. This solution can be stored for a long time without any change in its concentration.

Starch indicator solution

Weigh 1.0 g water-soluble starch. Dissolve in 100 cm^3 distilled water, add 0.1 g salicylic acid and boil. Cool it down and pour into a bottle with a stopper. This can be stored for several weeks.

Student sheet: Standardisation of the $Na_2S_2O_3$ titrant

Pipette 10.00 cm^3 KIO_3 solution into a 100 cm^3 glass-stoppered Erlenmeyer flask. Add 30 cm^3 distilled water, 2 cm^3 20% HCl and 0.3 g KI. Wait for 1-2 minutes, then titrate with the $Na_2S_2O_3$ titrant. When the solution becomes light yellow, add 1 cm^3 starch. Titrate until the solution turns colourless.

Student sheet: Titration of the sample using starch to indicate the end point

Betadine contains 10 mg/cm^3 free iodine.

Pipette 5.00 cm^3 *Betadine* into a 100 cm^3 Erlenmeyer flask. Add 30 cm^3 distilled water and immediately titrate with the standardised $Na_2S_2O_3$ titrant. When the dark brown solution turns yellow, add 1 cm^3 starch indicator and continue the titration until the solution becomes colourless.

Student sheet: Potentiometric titration of the sample

Pipette 5.00 cm^3 *Betadine* into a 250 cm^3 beaker and add about 50 cm^3 distilled water.

Immerse a smooth platinum and a calomel electrode into the solution. Put the beaker on a magnetic stirrer and join the electrodes to a mV/pH meter. Stir the solution continuously during titration. While the colour of the sample is brown, add 1.00 − 1.00 cm^3 titrant; when the colour pales, decrease the portions to 0.2 − 0.2 cm^3.

Student sheet: Evaluation of experimental results

Calculating the concentration of the titrant

The amount of iodine formed from 10.00 cm^3 KIO_3 solution in the $IO_3^- + I^-$ reaction, requires 2 x 10^{-4} mole $Na_2S_2O_3$

$$IO_3^- + 5I^- + 6H^+ = 3I_2 + 3H_2O$$
$$I_2 + 2S_2O_3^{2-} = 2I^- + S_4O_6^{2-}$$

Therefore the concentration of the titrant is

$$c_{titrant} = \frac{1000 \text{ x } 2 \text{ x } 10^{-4} \text{ (mol)}}{V_{Na_2S_2O_3}\ (cm^3)} \qquad \frac{mol}{dm^3}$$

Calculate the iodine content of the sample from the 5-5 parallels of the two types of the measurements separately.

$$x = \frac{126.90 \left(\frac{g}{mol}\right) \text{ x } V_{Na_2S_2O_3}\ (cm^3) \text{ x } c_{Na_2S_2O_3} \left(\frac{mol}{cm^3}\right)}{V_{pipette}\ (cm^3)} \qquad \frac{g}{cm^3}$$

Calculating standard deviation

Determine the standard deviations for the two methods separately.

$$S_{starch} = \left[\frac{\sum \left(x_i - \overline{x}\right)^2}{(N-1)}\right]^{\frac{1}{2}}$$

$$S_{potentiometry} = \left[\frac{\sum \left(x_i - \overline{x}\right)^2}{(N-1)}\right]^{\frac{1}{2}}$$

where

x_i is the result of the individual measurements
$\overline{x}$ is the mean of the individual results
N is the number of titrations run parallel

Applying the F-test

Look for the F-test in an analytical chemistry textbook (e.g. G. D. Christian: Analytical Chemistry, 4th Edition, John Wiley & Sons, 1986). If you wish to get a deeper insight into the theoretical background of the F-test, visit the websites listed in *Other resources*. In the above-mentioned textbook there is an example, how the F-test can be used to compare a new analytical method with an accepted procedure.

From the calculated standard deviations for the two sets of analytical results, s_{starch} and $s_{potentiometry}$ F is defined as:

$$F = \frac{s_{starch}^2}{s_{potentiometry}^2}$$

if $s_{starch} > s_{potentiometry}$,
or

$$F = \frac{s_{potentiometry}^2}{s_{starch}^2}$$

if $s_{potentiometry} > s_{starch}$
F must always be greater than 1.

From the calculated standard deviations, the value of F can be determined.

In the statistical program packages, there are tables containing the F-values, e.g. at the 95% confidence level:

$(N-1)_{starch}$	2	3	4	5	6
$(N-1)_{potentiometry}$					
2	19.00	19.16	19.25	19.30	19.33
3	9.55	9.28	9.12	9.01	8.94
4	6.94	6.59	6.39	6.26	6.16
5	5.79	5.41	5.19	5.05	4.95
6	5.14	4.76	4.53	4.39	4.28

In this activity, the suggested number of the parallels is 5 in both experiments, therefore:

$$(N-1)_{starch} = (N-1)_{potentiometry} = 4$$

giving a tabulated F value of 6.39. If the calculated F value is less than 6.39, you can conclude that there is no significant difference in the precision of the two methods.

Thus you can decide whether or not there is significant difference between the 'old' and the 'new' methods.

Teacher's document

Overview

The aim of this activity is to show how a new analytical method can be validated. In the example there is an analytical procedure that the students can accept as a standard one. They have to compare another method with that procedure.

As well as the practical work, the team becomes acquainted with one of the statistical methods used by analytical chemists, to help them judge whether or not there is significant difference in the precision of two methods. However, accuracy and precision is not enough. There are two other factors which are important: time and the cost of an analytical method. The second part of the activity deals with these problems.

Developing students' skills

In this type of activity attention is focused on the

- time and workload management
- budget management.

Because they work in a team, students should learn

- how they can organise their work effectively
- how to co-operate with each other during the practical work.

They will deepen critical thinking skills through analysing data and errors. Call the students' attention to the importance of validation. They should carefully read the websites dealing with it (see *Other resources*). They will learn that the word validation has several uses and is important in many fields, not only in analytical chemistry.

If students have not learned about accuracy and precision previously, then this is a good opportunity to do so.

If you have an automatic titrator in the laboratory, you will immediately obtain the end point of the potentiometric titration and the result of the analysis. In this case, it is useful to show to the students what the computer has done. Students should realise how important it is to know the underlying chemistry in their experimental work.

Students will learn to make good decisions about economy. They should realise that the price of chemicals depends on the quality and the quantity of the reagents. Also it is useful for the team to find out (from the finance department of the school or a research laboratory), a rough estimate for the hourly wage of a technician. This is another important factor in a realistic financial appraisal.

Because the websites listed in *Other resources* have a lot of external links, a sharp eye should be kept on the clock!

Organising and managing the class

Suggested size of the team: 2

Teachers can help students to find the appropriate antiseptic solution in their country. If the iodine content differs from the Hungarian *Betadine*, then the

teacher should indicate the need to modify the volume or concentration of the sample used for the titration.

Session plan for teachers

Suggested timing:

- Introduction, checking prior knowledge and planning the work (45 minutes)
- Carrying out the practical work (360 minutes)
- Evaluation of the experimental data (45 minutes)
- Preparation of the financial information; drawing the final conclusion (90 minutes)

Technical notes

- **Equipment and materials**

Materials and their CAS numbers

	CAS number
potassium iodate	7758-05-6
potassium iodide	7681-11-0
sodium thiosulfate $5H_2O$	10102-07-7
hydrochloric acid	7647-01-0
starch	9005-25-8
salicylic acid	69-72-7

Equipment and glassware

- weighing bottle
- 1000 cm^3 volumetric flasks
- 100 cm^3 glass bottle with stopper
- 5.00 and 10.00 cm^3 pipettes
- 1 cm^3 pipette for starch
- 100 cm^3 glass-stoppered Erlenmeyer flasks
- burettes
- 250 cm^3 beakers
- 50 cm^3 measuring cylinder
- analytical balance
- magnetic stirrer
- mV/pH meter
- platinum electrode
- calomel electrode

Testing prior knowledge

Choose the correct answer.

1. What are the products of the thiosulfate ion + iodine reaction?
   a. sulfate ion + iodide ion
   b. elementary sulfur + iodide ion
   c. tetrathionate ion + iodide ion
   d. sulfide ion + iodate ion

2. Why does the dark blue solution turn colourless at the end of the titration of iodine with thiosulfate titrant when starch is the indicator?
   a. Because of the change of the redox potential
   b. Because the blue colour belongs to a complex formed between starch and iodine
   c. Because thiosulfate reacts with starch when iodine is consumed

3. 0.7133 g KIO_3 is dissolved in 1000 cm^3 distilled water. 10.00 cm^3 of this solution is titrated with $Na_2S_2O_3$ titrant. 10.17 cm^3 of the titrant is consumed. What is the concentration of the $Na_2S_2O_3$ solution?
   a. 0.0204 mol/dm^3
   b. 0.0211 mol/dm^3
   c. 0.0187 mol/dm^3
   d. 0.0197 mol/dm^3

4. Platinum and calomel electrodes are used for indicating the end point of the titration. Why does the mV-value change?
   a. Because the electrode potential of the calomel electrode changes
   b. Because the electrode potential of both electrodes changes
   c. Because the electrode potential of the platinum electrode changes

5. Why does the electrode potential of the platinum electrode change?
   a. Because the redox potential changes during titration
   b. Because the pH changes during titration
   c. Because the platinum electrode is sensitive to the concentration change of the tetrathionate ion

Note: answers are given in red. These need to be removed before test is used.

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