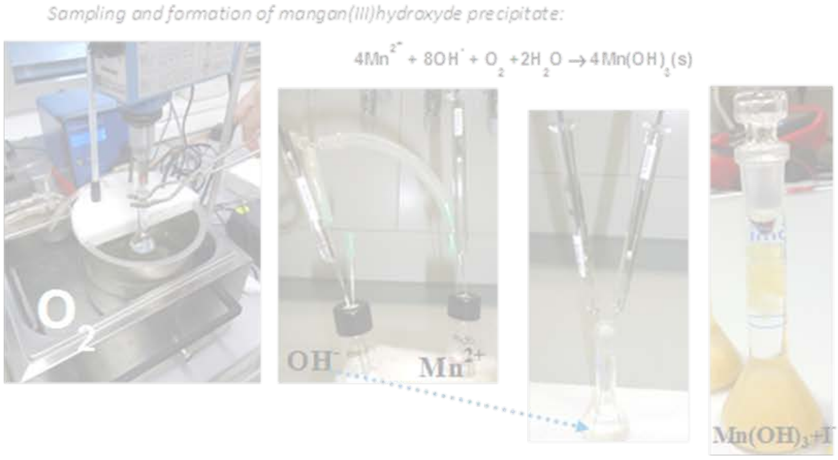
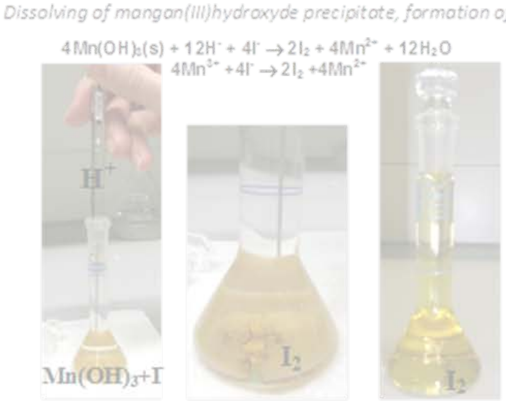


Report on method for improved, gravimetric Winkler titration



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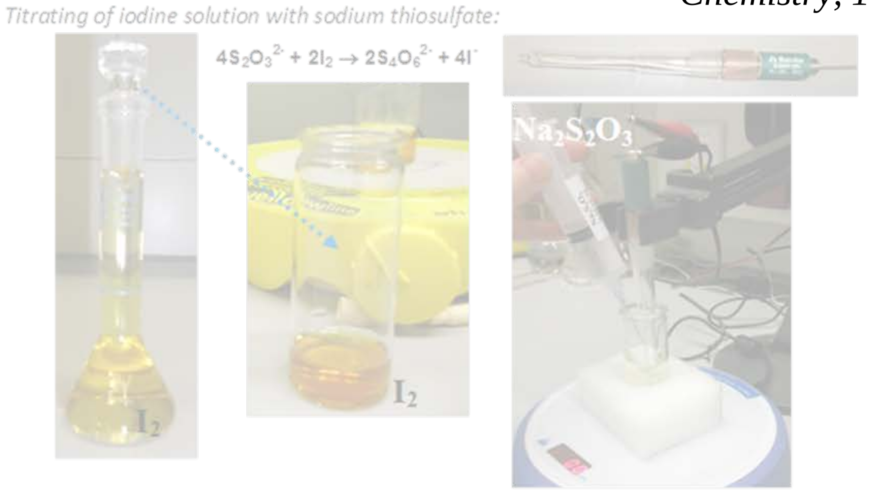

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Table of Contents

Abstract

This report presents a high-accuracy Winkler titration method that has been developed for determination of dissolved oxygen concentration. Careful analysis of uncertainty sources relevant to the Winkler method was carried out and the method was optimized for minimizing all uncertainty sources as far as practical. The most important improvements were: gravimetric measurement of all solutions, pre-titration to minimize the effect of iodine volatilization, accurate amperometric end point detection and careful accounting for dissolved oxygen in the reagents. As a result, the developed method is possibly the most accurate method of determination of dissolved oxygen available. Depending on measurement conditions and on the dissolved oxygen concentration the combined standard uncertainties of the method are in the range of 0.012 – 0.018 mg dm^{-3} corresponding to the $k = 2$ expanded uncertainty in the range of 0.023 – 0.035 mg dm^{-3} (0.27 – 0.38%, relative). This development enables more accurate calibration of electrochemical and optical dissolved oxygen sensors for routine analysis than has been possible before. Most of this report is based on the article I.Helm, L.Jalukse, I. Leito, Anal. Chim. Acta. 741 (2012) 21-31 (ref [1]).

Keywords

Dissolved oxygen; Winkler method; uncertainty; primary method.

1. Introduction

Dissolved oxygen (DO) is one of the most important dissolved gases in water. Sufficient concentration of DO is critical for the survival of most aquatic plants and animals [2] as well as in waste water treatment. DO concentration is a key parameter characterizing natural and wastewaters and for assessing the state of environment in general. Besides dissolved CO_2, DO concentration is an important parameter shaping our climate. It is increasingly evident that the concentration of DO in oceans is decreasing [3 - 6]. Even small changes in DO content can have serious consequences for many marine organisms, because DO concentration influences the cycling of nitrogen and other redox-sensitive elements [3]. Decrease of DO concentration leads to formation of hypoxic regions in coastal seas, in sediments, or in the open ocean, which are inhabitable for most marine organisms [3]. DO concentration is related to the changes in the ocean circulation and to the uptake of CO_2 (incl anthropogenic) by the ocean [7]. All these changes in turn have relation to the climate change.

Accurate measurements of DO concentration are very important for studying these processes, understanding their role and predicting climate changes. These processes are spread over the entire vast area of the world's oceans and at the same time are slow and need to be monitored over long periods of time. This invokes serious requirements for the measurement methods used to monitor DO. On one hand, the results obtained at different times need to be comparable to each other. This means that the sensors used for such measurements need to be highly stable and reproducible [8]. The performance of oxygen sensors – amperometric and (especially) optical – has dramatically improved in recent years [9].

On the other hand, measurements made in different locations of the oceans have to be comparable to each other. The latter requirement means that the sensors have to be rigorously calibrated so that the results produced with them are traceable to the SI (International System of Units). The sensors need to be calibrated with solutions of accurately known oxygen concentration in order to correct for sensor drift, temperature, salinity and pressure influences [10,11]. Oxygen is an unstable analyte thus significantly complicating sensor calibration.

It has been established that if every care is taken to achieve as accurate as possible results then the accuracy of DO measurements by amperometric sensors is limited by calibration [10] and specifically by the accuracy of the reference DO concentration(s) that can be obtained. This is similar with optical sensors: their lower intrinsic uncertainty may make the relative contribution of calibration reference values even larger.

The issues with sensors, among them issues with calibration, have caused a negative perception about the data using sensors in the oceanography community and because of this the recent issue of the World Ocean Atlas [12] was compiled with taking into account only DO concentrations obtained with chemical titration methods (first of all the Winkler method) and rejecting all sensor-based data. Similar decision was taken in a recent study of DO decline rates in coastal ocean [6]. It is nevertheless clear that there is need for large amounts of data, so that the slow and clumsy titration method cannot satisfy this need. It is necessary to be able to collect data automatically and in large amounts. It is thus expected that eventually sensors will be "back in business".

There are two ways to prepare DO calibration solutions with known concentrations: (1) saturating water with air at fixed temperature and air pressure and using the known saturation concentrations [13 - 15] and (2) preparing a DO solution and using some primary measurement method for measuring DO concentration. The premier method for this is the Winkler titration method (WM) [16]. WM was first described by Winkler [17] more than hundred years ago. Also gasometry is an old method for DO determinations, but it is a partly physical method requiring quite specific and complex experimental setup and is therefore not routinely used nowadays.

DO measurement practitioners currently almost exclusively use the saturation method for calibration of DO measurement instruments. This method gives quite accurate results when all assumptions made are correct. DO values obtained with the saturation method are also used in this work for comparison with the WM values. Nevertheless, it uses ambient air – a highly changing medium – as its reference, thereby relying on the assumption that the oxygen content of the Earth's atmosphere is constant, which is not entirely true [4]. The oxygen content of air depends on air humidity and CO_2 content, which both can change over a wide range of values. Also, this method needs careful accounting for air pressure, humidity and water temperature. It is customary to use published values of DO concentrations in air-saturated water at different temperatures. At the same time, different published values are in disagreement by up to 0.11 mg dm^{-3} at 20 ^{o}C at even up to 0.19 mg dm^{-3} at 40 ^{o}C [15]. Thus the saturation method has many factors that influence the results and it is difficult to realize it in a highly accurate way. An independent primary method, such as WM, would be free from these shortcomings.

Winkler method is known for a long time, it has been extensively studied and numerous modifications have been proposed. The data on accuracy of the Winkler methods described in the literature have been summarized in section 5.2. There have been very few studies that report combined uncertainties taking into account both random and systematic factors influencing the measurement. Usually repeatability and/or reproducibility data are presented that do not enable complete characterization of the accuracy of the methods and tend to leave too optimistic impression of the methods. Very illuminating in this respect are the results of an interlaboratory comparison study [18] where the between-lab reproducibility standard deviation is as large as 0.37 mg dm^{-3} [18]

From these data we conclude that there is a lot of room for improvement of the Winkler method. We present a realization of the Winkler method with the highest possible accuracy and present a careful analysis of the method for its uncertainty sources. To the best of our knowledge this is the most comprehensive uncertainty analysis of the Winkler method that has been published to date.

The wider aim of this investigation was to contribute to significant reduction of measurement uncertainty of measurement with sensors by proposing an improved calibration approach for sensors based on a high-accuracy Winkler titration procedure.

This work prepares the ground for putting the DO measurements as such onto a more reliable metrological basis enabling lower uncertainties and allowing detection of trends and relationships that may remain obscured with the current level of accuracy achievable for DO determination.

2. Experimental

In this section the developed method is described in detail, the calculation formulas are given, the sources of uncertainty and ways used for their estimation are listed.

2.1 Materials

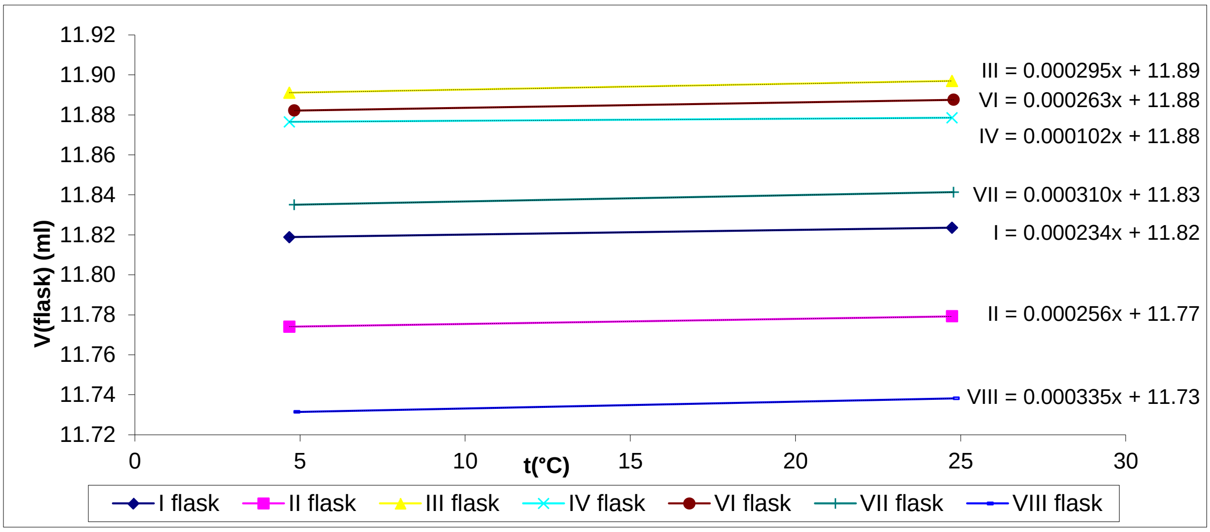
The water used for all operations was produced with a Millipore Milli-Q Advantage A10 setup (resistivity 18.2 MΩ cm). In present work the following reagents were used: Sodium thiosulfate solution, FIXANAL® (Fluka); Potassium iodate (declared purity: 99.997% on trace metals basis, Sigma-Aldrich); Sulphuric acid, puriss. p.a., reag. ISO, reag. Ph. Eur., 95-97% (Sigma-Aldrich); Manganese(II)sulfate monohydrate ($MnSO_4 \cdot H_2O$) puriss. p.a., ACS reagent, reag. Ph. Eur., 99.0-101.0%; potassium hydroxide (KOH) puriss. p.a., Reag. Ph. Eur., 85% (Sigma-Aldrich), potassium iodide (KI) puriss. p.a., reag. ISO, reag. Ph. Eur., 99.5% (Sigma-Aldrich). Influence of the reagents purity was checked by carrying out blank analysis according to ref 19.

2.2 Instrumentation and samples preparation

Weighing was done on a Precisa XR205SM-DR balance. The balance was regularly adjusted using the internal adjustment (calibration) weight. This adjustment was additionally checked using 5 independent reference weights in 9 different combinations resulting in masses ranging from 0.01 g to 200 g (and traceable to the SI via the Estonian national mass standard). The obtained differences of the readings from the masses of the weights were too small to justify correction, however they were taken into account in evaluation of mass measurement uncertainty. Air buoyancy correction was not taken into account because it essentially cancels out (for reasons explained in the Supplementary Material of ref 20.

Samples were taken and prepared in 10 cm^3 glass flasks with ground joint stoppers (standard ground glass stoppers). Flasks were calibrated before at different temperatures to account for the expansion/contraction of the flasks. This calibration was done at two different temperatures (5 °C and 25 °C) using water saturated with air. This approach makes it possible to have correct flask volume at every measured temperature of the sample, which means that the contraction and expansion of the flask due to temperature changes are accounted for. On Graph 1 are presented the calibration curves of all the used sample flasks. Water densities in this case were found as it has been described in section 2.2.1 of present document.

Graph 1 Calibration curves of sample flasks.



For adding reagent solutions into the sample solution previously calibrated glass syringes with tight plungers (250 µl, Hamilton) were used. In other cases plastic syringes were used. Titrations were carried out amperometrically, using a 20 cm^3 plastic syringe (Brown, needle external diameter 0.63 mm).

For titration endpoint determination an amperometric setup described in section 2.3.2 and Figure 2 was used.

2.2.1 Changes in water density due to dissolved gases

Dissolved nitrogen decreases the density of water. Oxygen and other gaseous components of air increase the density of water. The equivalent change of the density of water due to dissolved air is found as follows [21]:

$$\rho = \left(-5.252 + 0.1474 \cdot t - 3.0689 \cdot 10^{-3} \cdot t^2 + 4.0688 \cdot 10^{-5} \cdot t^3 - 2.3559 \cdot 10^{-7} \cdot t^4\right) \cdot 10^{-3} + \rho_0 \quad (1)$$

where ρ [kg dm^{-3}] is the density of the solution (air-saturated water), ρ_0 [kg dm^{-3}] is the pure water density (free of dissolved salts and gases), t is the temperature [°C].

Table 1. Corrections of water densities caused by dissolved air

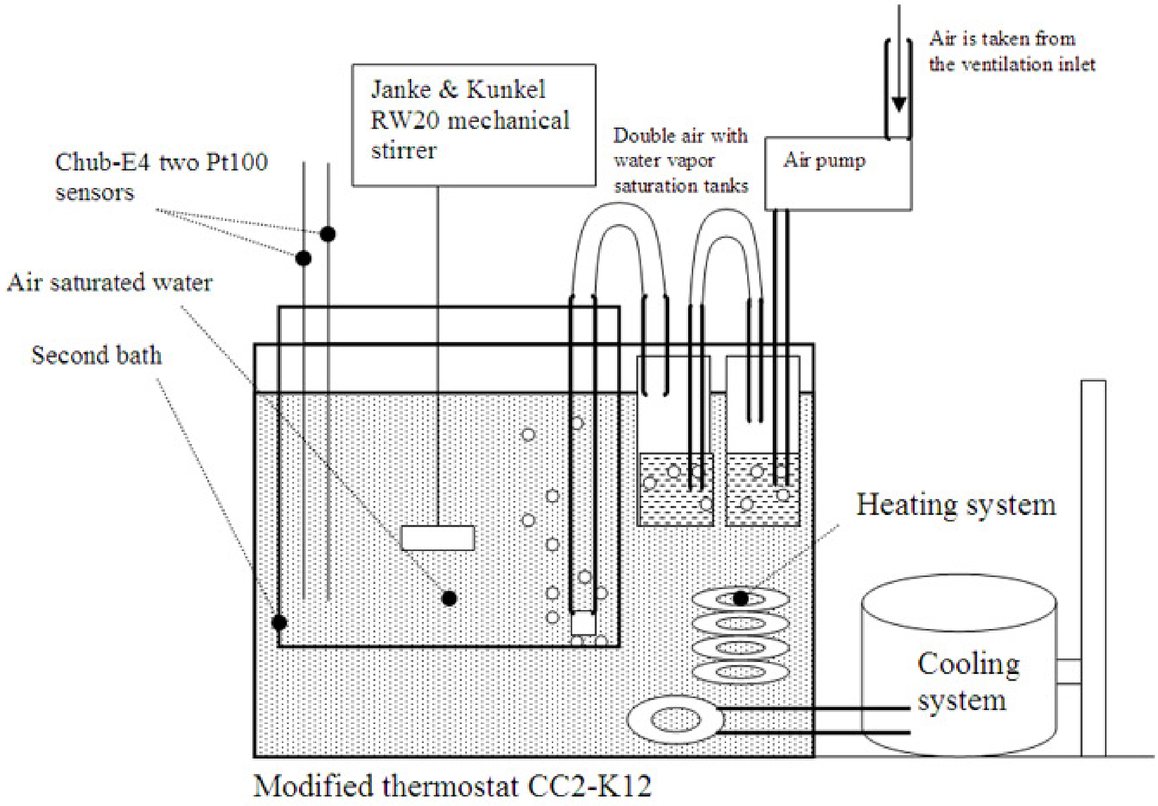
Temperature [°C]	**(ρ - ρ_0) [kg dm^{-3}]**	**ρ_0 [kg dm^{-3}]**[22]	**ρ [kg dm^{-3}]**
4	-0.00000471	0.9999764	0.9999717
5	-0.00000459	0.9999668	0.9999622
6	-0.00000447	0.9999424	0.9999379
7	-0.00000436	0.9999033	0.9998990
8	-0.00000425	0.9998501	0.9998458
9	-0.00000415	0.9997828	0.9997786
10	-0.00000405	0.9997018	0.9996978
11	-0.00000395	0.9996075	0.9996035
12	-0.00000386	0.9995001	0.9994962
13	-0.00000377	0.9993799	0.9993761
14	-0.00000369	0.9992472	0.9992435
15	-0.00000361	0.9991023	0.9990987
16	-0.00000353	0.9989456	0.9989421
17	-0.00000345	0.9987772	0.9987738
18	-0.00000338	0.9985976	0.9985942
19	-0.00000331	0.9984070	0.9984037
20	-0.00000324	0.9982057	0.9982024
21	-0.00000318	0.9979940	0.9979908
22	-0.00000312	0.9977722	0.9977691
23	-0.00000306	0.9975406	0.9975375
24	-0.00000300	0.9972995	0.9972965
25	-0.00000294	0.9970492	0.9970463

Dissolved air components under saturation concentrations decrease water density at temperatures 4 - 25 °C by approximately 0.0005 – 0.0003%.

2.2.2 Preparation of air-saturated water

Air-saturated fresh pure water (MilliQ) water (at constant humidity and temperature) was used as reference medium for validating the method. The water was aerated (and simultaneously stirred) until equilibrium was attained. The saturation medium was created in a modified (added a second bath) thermostat CC2-K12 (Peter Huber Kältemaschinenbau GmbH, Germany). See the Figure 1 and the photo series in section 2.5. The air used for saturation was taken from the air inlet situated on the roof of the building. The air flow velocity during calibration was around 1 dm^3 min^{-1}. The ordinary aquarium spray was used (at depth of 13 cm). The estimated (from photographs, see below) diameter of the bubbles was between 0.8 to 1.8 mm.

Figure 1 Thermostat CC2-K12 with additional bath, stirrer and thermometer.



The double-bath thermostat provides good temperature stability (see Table 2).

Table 2 Temperature stability test within 10 minutes before the sampling.

Temperature	30 °C	25 °C	20 °C	15 °C	10 °C	5 °C
	29.816	24.826	19.925	15.004	10.074	5.053
	29.810	24.824	19.926	15.002	10.073	5.054
	29.808	24.829	19.926	14.999	10.065	5.054
	29.808	24.826	19.925	14.999	10.066	5.057
	29.811	24.828	19.925	15.000	10.069	5.057
	29.812	24.832	19.923	14.998	10.065	5.053
	29.811	24.843	19.917	14.999	10.067	5.053
	29.809	24.840	19.923	14.997	10.067	5.048
	29.811	24.837	19.923	15.000	10.067	5.050
	29.816	24.837	19.921	15.004	10.068	5.054
St.dev	**0.0028**	**0.0065**	**0.0029**	**0.0023**	**0.0031**	**0.0027**

In this case the maximum standard deviation has been taken as the standard uncertainty ($u(T_{\mathrm{instab}})$=0.0065 K). Atmospheric pressure was measured by digital barometer PTB330 (Ser No G37300007, manufactured by Vaisala Oyj, Finland, calibrated by manufacturer 19.09.2011) with uncertainty $u(p_{\mathrm{cal}})$ = 7 Pa (k = 2). The air bubbled through the second bath was saturated with water vapor by passing it through two saturation bottles (both immersed in the same thermostat). The level of air humidity after the second saturation vessel was measured using digital hygrometer Almemo 2290-8 with sensor ALMEMO FH A646 E1C (manufacturer AHLBORN Mess- und

Regelungstechnik GmbH). The humidity of the air bubbled through the water in the second bath was never lower than 95 %RH. The uncertainties of all relative humidity measurements are ± 5 %RH (k = 2). The CO_2 content of the air was measured during calibration by Vaisala CARBOCAP® CO_2 Transmitter Series GMP 222 (SN: X0150001, manufactured by Vaisala, Finland). The evaluated uncertainty of the CO_2 concentration was ± 100 ppm (k = 2). The temperature of the measurement medium was measured by reference digital thermometer Chub-E4 (model nr 1529, serial No A44623, manufacturer Hart Scientific) with two Pt100 sensors. The uncertainties of all temperature measurements are ± 0.02 °C (k = 2 calibrated by the Estonian NMI, AS Metrosert on May 2011).

2.2.3 Concentration of oxygen in air-saturated water

$C_{\mathrm{O2\_saturation}}$ is the concentration of oxygen in air-saturated MilliQ water [mg dm^{-3}] at the measurement temperature. It is normally found using one of the various available empirical equations [29, 15]. We use the equation 2 from Benson and Krause [14]. This equation is considered one of the best available and has been adopted by the standard ISO 5814 [13].

$$C_{\mathrm{O2\_saturation}} = \exp\left(A_1 + \frac{A_2}{T+\Delta T_{\mathrm{instab}}} + \frac{A_3}{(T+\Delta T_{\mathrm{instab}})^2} + \frac{A_4}{(T+\Delta T_{\mathrm{instab}})^3} + \frac{A_5}{(T+\Delta T_{\mathrm{instab}})^4} \right) \cdot W + \Delta C_{\mathrm{O2\_saturation}} + \Delta C_{\mathrm{O2\_supersat}} \tag{2}$$

where T [K] is the temperature of the water and $\Delta T_{\mathrm{instab}}$ [K] is the term taking into account the instability of the temperature in the vessel; A_1, A_2, A_3, A_4 and A_5 are constants [14, 15]. W is the pressure correction factor [13]:

$$W = \frac{p - p_{\mathrm{H2O}} + \Delta p_{CO2}}{p_{\mathrm{n}} - p_{\mathrm{H2O\_100\%}}} \tag{3}$$

where p [Pa] is the atmospheric pressure at measurement conditions, Δp_{CO2} [Pa] is the uncertainty of carbon dioxide content in air, p_{n} [Pa] is the atmospheric pressure at standard conditions and $p_{\mathrm{H2O\_100\%}}$ [Pa] is the water vapor pressure at 100% relative humidity. It is found according to (4) [15].

$$p_{\mathrm{H2O\_100\%}} = p_{\mathrm{n}} \cdot \exp\left(B_1 + \frac{B_2}{T} + \frac{B_3}{(T)^2} \right) \tag{4}$$

where B_1, B_2 and B_3 are constants. The pressure p_{H2O} [Pa], the real content of H_2O in air, is found experimentally (during aeration at calibration conditions).

The $C_{\mathrm{O2\_saturation}}$ value was used as the reference value for comparing the DO concentrations found with the Winkler method: $C_{\mathrm{O2}}\mathrm{Ref} = C_{\mathrm{O2\_saturation}}$.

2.2.4 Uncertainty of DO concentration in the air saturated water

Uncertainty of $\Delta C_{\mathrm{O2\_saturation}}$. Numerous tables of saturated DO concentration values have been published [13, 14, 15, 23 - 19]. The differences between the data of different authors are generally in the order of 0.05 mg dm^{-3} [29]. It is assumed that these discrepancies come from the influence of two uncertainty sources:

(1) Uncertainty of the reference methods of determining the DO concentration [16] used for compiling the tables of published values of saturated oxygen concentrations [13,29].

(2) Uncertainty arising from the imperfect fit of the mathematical model of oxygen saturation concentrations to the data [13,29]. This uncertainty component can be regarded as one taking into account the uncertainties of the constants A_1 to A_5.

All these uncertainty sources are taken into account by the term $\Delta C_{\mathrm{O2\_saturation}}$. Its value is set to zero and based on the available data we estimate its uncertainty as ± 0.05 mg dm^{-3} ($k = 2$) that is $u(\Delta C_{\mathrm{O2\_saturation}}) = 0.025$ mg dm^{-3}.

Temperature *T*. This uncertainty source is caused by the limited accuracy of the thermometer used for temperature measurement and is taken into account as $u(T)$. In the case of the thermometer with uncertainty $u(T) = 0.01$ K was used.

Temperature instability of the calibration medium $\Delta T_{\mathrm{instab}}$. The uncertainty due to the non-ideal temperature stability of the thermostat is taken into account by the term $\Delta T_{\mathrm{instab}}$. Its value is set to zero and we estimate its uncertainty as follows: $u(\Delta T_{\mathrm{instab}}) =$ 0.0065 K.

Atmospheric pressure during calibration *p*. This uncertainty source is caused by the limited accuracy of the barometer used for measuring the atmospheric pressure and is taken into account as $u(p)$. In the case of the external barometer the standard uncertainty due to calibration is 3.5 Pa. Additionally drift and reading repeatability were taken into account and the following uncertainty estimate was obtained: $u(p) =$ 5.2 Pa.

Partial pressure of water vapor p_{H2O}. The partial water vapor pressure in air saturated with water (at minimum 95% relative humidity) was measured with uncertainty ± 5% (k=2) at our laboratory: $u(p_{\mathrm{H2O}}) = 111$ Pa (at temperature 20.0 °C).

Oxygen content in air Δp_{CO2}. The partial oxygen pressure in air saturated with water depends also on the content of carbon dioxide [29, 30, 31]. We found experimentally (during aeration, under calibration conditions) that the content of carbon dioxide in air varies in the range of 0.04% to 0.07%, the lowest end of this range being the standard content of CO_2 in air. The highest end of this range is possible only when the air is taken directly from the room where people are working, which is not the case with our measurements (air is taken from the ventilation inlet situated on the roof of the building). The effect of varying CO_2 content is small and thus we will not correct for it and will include it entirely in uncertainty. The value Δp_{CO2} is set to zero and its uncertainty $u(\Delta p_{\mathrm{CO2}})$ is conservatively estimated as 41 Pa (under the normal pressure 101325 Pa).

Supersaturation $\Delta C_{\mathrm{O2\_supersat}}$. This component takes into account the uncertainty originating from possible supersaturation (or undersaturation). In our case the used MilliQ water was pre-saturated at level of ca 70%. At least 2.5 hours were allowed for full saturation counting from the time when the temperature of the bath was stabilized. The saturation process was monitored by optical dissolved oxygen analyzer HACH 30d with a digital resolution of 0.01 mg dm^{-3}. The possible supersaturation depends on aeration speed (over-pressure generated by the pump), the intensity of mixing and the size of bubbles. The smaller are the bubbles the higher may be the supersaturation. Unfortunately, the exact saturation conditions, including the optimal size of the bubbles are not specified in the ISO 5814 standard [13] or in the original papers [14, 15]. In this work the size of the bubbles was in the range of 0.8 - 1.8 mm (estimated using a ruler immersed into the bath and comparing the bubble size to the ruler using photos). The standardized procedure of obtaining accurate dissolved oxygen concentrations in water from the former Soviet Union [32] contains detailed description of the saturation conditions and the bubble size according to that standard is 3 mm. The saturation values of ref [32] are in good agreement with the ISO 5814 standard [13]. The maximum difference in the temperature range 5-30 $^{\mathrm{o}}$C is ± 0.02 mg

dm^{-3}. Truesdale et al claim [31] that bubbles with the diameter of 0.1 mm lead to a supersaturation of ca 0.6%. If we tentatively assume that the extent of supersaturation is linearly related to bubble diameter then it means that when moving down from 3 mm bubbles then the supersaturation is ca 0.2% per 1 mm of bubble diameter. The smallest possible bubble diameter used in this work was 0.8 mm and this would mean ca 0.44% of supersaturation, which at 20 °C means ca 0.04 mg dm^{-3}. In order to verify this assumption a comparison between saturation conditions differing by bubble size was made using an optical dissolved oxygen analyzer HACH 30d. The difference of 0.03 mg dm^{-3} was found between the dissolved oxygen concentrations when saturation with 3 mm bubbles and 0.8 mm bubbles was compared. Thus the possible supersaturation might be as high as 0.03 mg dm^{-3}. Nevertheless we also cannot fully rule out undersaturation and therefore we assign to $\Delta C_{\mathrm{O2\_supersat}}$ the value of zero. Its standard uncertainty $u(\Delta C_{\mathrm{O2\_supersat}})$ is estimated from the maximum value 0.03 mg dm^{-3} (assuming rectangular distribution) as 0.017 mg dm^{-3}.

2.3 Procedures

All solutions where accurate concentration was important were prepared by weighing. The amounts of the solutions were measured by weighing.

All amounts of reagents, which directly influenced the result, were measured by weighing.

2.3.1 Preparing of standard working solutions of KIO_3

Standard solutions were prepared gravimetrically using the highest purity standard substance KIO_3 available (see section 2.1 and 3.3 for purity and its uncertainty). The working solution was made by consecutive dilutions. The first solution (KIO_3_I, c=36 g kg^{-1}) was made by weighing about 1.4 grams of solid KIO_3 and dissolving it in about 40 grams of water. The second solution (KIO_3_II, c=3 g kg^{-1}) was made by weighing about 3 grams of solution KIO_3_I and adding water to bring the volume to approximately 40 grams. The working solution (KIO_3_III, c=0.2 g kg^{-1} or 1 mmol kg^{-1}) was made by weighing about 4-6 grams of KIO_3_II and adding water to bring the volume up to approximately 100 grams. All these solutions were made into tightly capped bottles to avoid change of concentration of the solutions during and between the analyses.

2.3.2 Determination of the concentration of the $Na_2S_2O_3$ titrant

Concentration of the titrant was determined by titrating an iodine solution with known concentration. The iodine solution was prepared as follows. About 5 cm^3 of the standard KIO_3_III working solution (0.7 mmol kg^{-1}, see the previous paragraph) was transferred using a plastic syringe into a dried and weighed cylindrical wide-mouth 40 ml titration vessel (see photos in section 2.5). The vessel was weighed again. Using a one ml syringe approximately 0.2 cm^3 of solution containing KI (2.1 mol dm^{-3}) and KOH (8.7 mol dm^{-3}) (alkaline KI solution) was added. Using a third syringe approximately 0.2 cm^3 of H_2SO_4 solution (5.3 mol dm^{-3}) was added. Under acidic conditions iodine is formed quantitatively according to the following reaction:

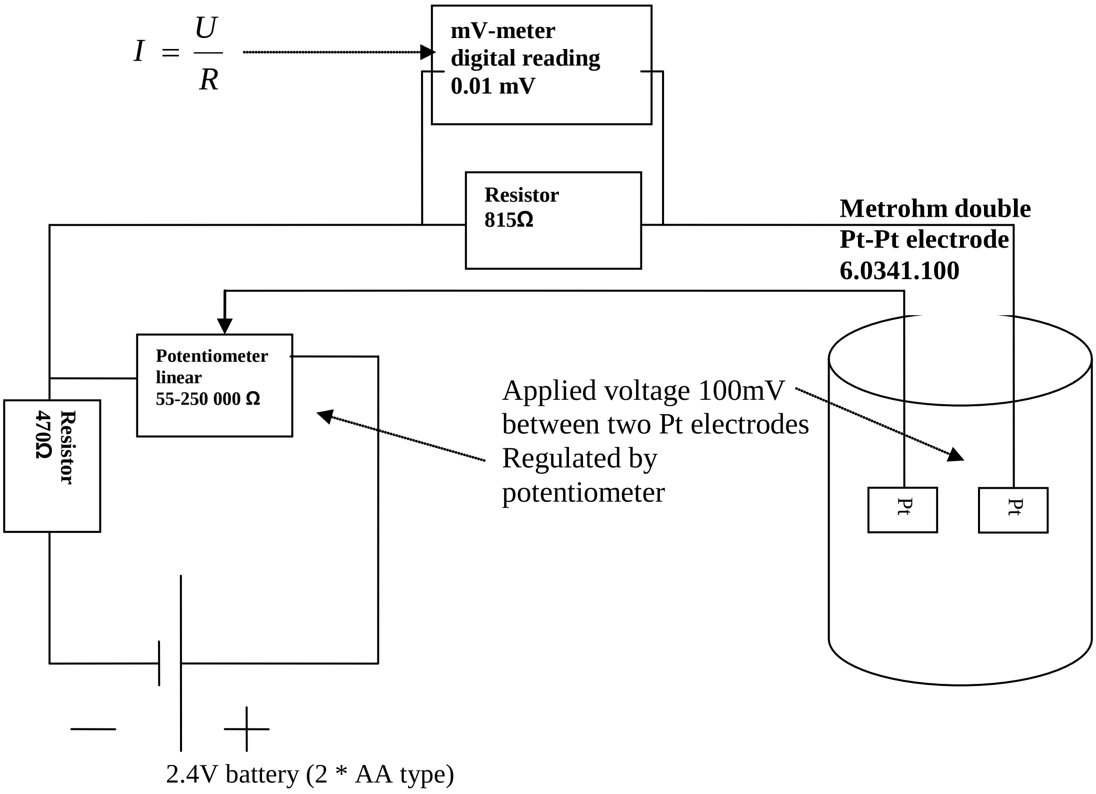
$$KIO_3 + 5KI + 3H_2SO_4 \rightarrow 3I_2 + 3K_2SO_4 + 3H_2O \qquad (5)$$

The iodine formed from KIO_3 was titrated with ca 0.0015 mol kg^{-1} $Na_2S_2O_3$ titrant as soon as the iodine was formed:

$$I_3^{-} + 2S_2O_3^{2-} \rightarrow 3I^{-} + S_4O_6^{2-} \qquad (6)$$

It is not possible to use pre-titration (see section 2.3.4) here in order to minimize iodine evaporation, because of the eq 13: until iodate (oxidizing agent) is in the solution we cannot add sodium thiosulphate (reducing agent) or else they react each other with a different stoichiometry [33]. Titration was carried out gravimetrically to decrease the uncertainty caused by volumetric operations [34]. It was done using a plastic syringe filled with titrant and weighed. The titration end point was determined amperometrically using two platinum electrodes (Metrohm Pt-Pt 6.0341.100). Titration equivalence point was determined amperometrically by using the system shown in Figure 2. The current was found from the voltage drop on a 815 Ω resistor.

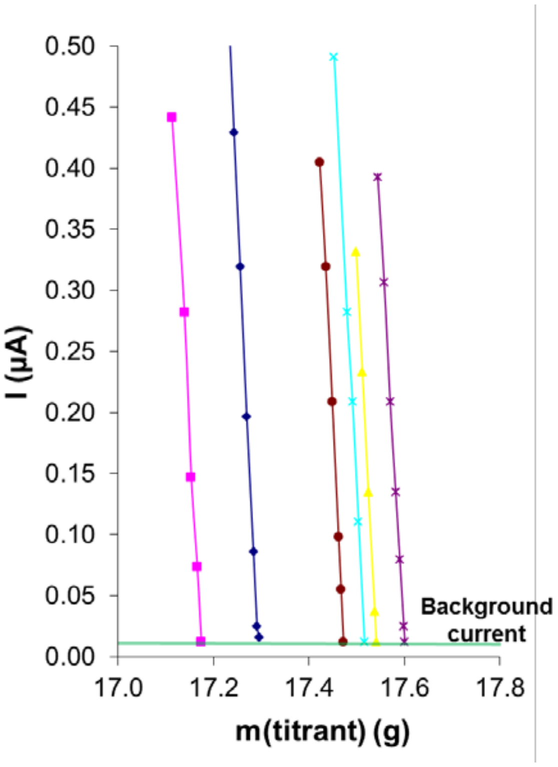
Figure 2 System for determination of equivalence point



A potential of 100 mV was applied between two platinum electrodes. As long as both iodine and iodide are present in solution there is non-zero current: on cathode iodine is reduced and on anode iodide is oxidized. When all the iodine has been converted to iodide the current will be equal to the background current. Near the equivalence point there is an excess of iodide in the solution and the current-limiting species is iodine. In this region the current is to a very good approximation linear with respect to the iodine concentration (see Graph 2 for illustration).

The background current value corresponding to the equivalence point was established every day before the titrations. The background current ranged on different days from 0.012 µA to 0.018 µA, but was essentially constant within a given day.

Graph 2. Iodine solution titration until the background current is reached.



One drop of the titrant from the syringe needle weighs about 0.0105 g. This drop is very small compared to the usual drop from a burette. There was also a possibility of adding just a fraction of a drop of the titrant for increasing the precision.
After titration the syringe was weighed again to determine the consumed titrant mass. Seven parallel measurements were carried out according to the described procedure and the average result was used as the titrant concentration.

2.3.3 Sampling and sample preparation

Seven parallel samples were taken as follows:

a) The flask was filled by submerging it under the water to be measured. Every care was taken to avoid air bubbles in the flask. The concentration of oxygen in air per volume unit is more than 30 times higher than in water saturated with air. Therefore avoiding air bubbles is extremely important.
b) 0.2 cm^3 $MnSO_4$ solution (2.1 mol dm^{-3}) and 0.2 cm^3 of the alkaline KI solution were added to the bottom of the glass flask simultaneously (an equal amount of water was forced out of the flask) by using syringes with long needles. Care was taken in order not to introduce air bubbles when adding those solutions.
c) The flask was stoppered with care to be sure no air was introduced. The contents of the flask were mixed by inverting several times. The presence of possible air bubbles was monitored. The sample was discarded if any air bubble was seen. A brownish-orange cloud of $Mn(OH)_3$ precipitate appeared. The precipitate was let to form until it was settled down according to eq 7

$$4Mn^{2+} + O_2 + 8OH^- + 2H_2O \rightarrow 4Mn(OH)_3 \downarrow \quad (7)$$

d) The solution was then acidified by adding 0.2 cm^3 of H_2SO_4 solution (5.3 mol dm^{-3}) with another glass syringe below the solution surface. It is very important that all the precipitate formed stays in the flask. Under acidic conditions Mn^{3+} ions oxidize iodide to iodine, which eventually forms I_3^- ions with the excess of KI [19, 35]:

$$2Mn(OH)_3\ (s) + 3H_2SO_4 \rightarrow 2Mn^{3+} + 3SO_4^{2-} + 3H_2O \quad (8)$$

$$2Mn^{3+} + 2I^- \rightarrow 2Mn^{2+} + I_2 \quad (9)$$

$$I_2 + I^- \rightarrow I_3^- \quad (10)$$

The flask was stoppered again and mixed until the precipitate was dissolved. At this stage the air bubbles do not interfere anymore.

2.3.4 Titration of the sample with the $Na_2S_2O_3$ titrant

Before the start of the actual titration about 80-90% of the supposed amount of the titrant ($Na_2S_2O_3$, 0.0015 mol kg^{-1}) is added to the titration vessel from a pre-weighed plastic syringe. The formed iodine solution is transferred quantitatively to the titration vessel (to minimize evaporation of iodine) and titrated to the end-point amperometrically as it has been discussed in section 2.3.2. This approach – so-called pre-titration (a kind of an initial titration) – allows ca 80% of the iodine to react immediately and is a powerful tool in helping to minimize the volatilization of iodine during titration. The remaining small extent of iodine volatilization is taken into account by a correction.

After reaching the end point the titration syringe was weighed again. The amount of the consumed titrant was determined from mass difference. Seven parallel titrations were carried out.

2.3.5 Determination of the correction for oxygen introduced from the reagents

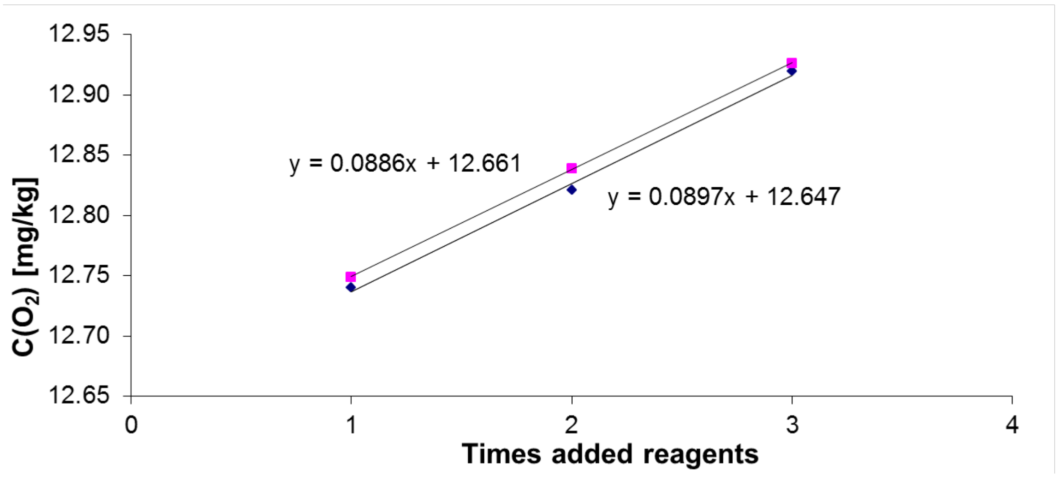
Due to the small sample volume the possible sources of parasitic oxygen have to be determined and their influence minimized. The two main sources of parasitic oxygen are the reagents and (with possible additional effect from the adding procedure) and invasion of oxygen through the junction between the stopper and the flask neck.

The concentration of DO in the reagents is low and the amount of the reagents is small. Nevertheless the amount of oxygen introduced by the reagents is on an average around 1 µg and thus has to be taken into account. In order to estimate the possible amount of oxygen carried into the sample by the reagents the latter were saturated with air for assuring their constant oxygen content. There are several ways for accounting for the effect caused by the reagents. In this work we have used addition experiments.

It is important to keep the oxygen content in the reagents under control and take it into account in calculations as a correction term in the model. There are two possible approaches for this: (1) use reagents where the oxygen content has been decreased to a minimum (deoxygenated reagents) or (2) use reagents saturated with air. In principle it would be desirable to use reagents with DO content as low as possible. Initially we took this approach. During the experiments we discovered that the oxygen content in the reagents was highly variable. This caused high uncertainty of the correction term (even though its magnitude was small). One of the reasons might be contamination of the reagents by atmospheric oxygen during transfer to the sample bottle. We then shifted to reagents saturated with air (which in turn was saturated with water vapor) and although the determined magnitude of the correction term was larger, its stability (spread of parallel measurements) and uncertainty were significantly better leading to ca two times lower combined standard uncertainties of determined oxygen content in samples. Air-saturated reagents are immune to contamination by air oxygen.

From the same sample different subsamples were collected at the same time and different amounts of the reagents were added to determine the amount of oxygen that is introduced with the reagents. Reagent solutions were added one to three times (different amounts) to consider not only the oxygen that was in the reagent solutions but also from the procedure itself (sample contamination). The concentration of DO found in the sample was plotted against times of added reagent solutions. The correction (CF_{O2}) was found from the slope of the resulting graph (Graph 3).

Graph 3. Curves from the adding tests (20.02.12).



Fourteen experiments were made for determination CF_{O2} (see the Table 3). Each determination was made with three points. The concentration of DO in reagents depends on atmospheric pressure. Therefore we converted all the obtained slope values to the normal (sea-level) pressure. Two of the resulting graphs were strongly non-linear (relative standard deviation of linear regression slope was above 20%, see the Table 3) and these were left out. The remaining 12 results (obtained on 7 different days) were evaluated for agreement with the Grubbs test [36] and no disagreeing results were found.

Table 3 Results of adding tests

Date	b_1[a]	b_0[a]	$s(b_1)$[a]	$s(b_0)$[a]	C_{O2}Ref[b]	Δ[c]	*P* (Pa)	St.dev.[d]	CF_{O2} (norm)
10.10.11	0.0862	9.0116	0.0074	0.0161	9.01	0.00	99896	9%	0.087
	0.0726	9.0467	0.0207	0.0447	9.01	-0.04	99896	**29%**	-
14.10.11	0.0984	9.1553	0.0361	0.0780	9.17	0.01	101677	**37%**	-
	0.0941	9.2034	0.0088	0.0189	9.17	-0.04	101677	9%	0.094
24.10.11	0.0983	9.2494	0.0048	0.0103	9.23	-0.02	102394	5%	0.097
	0.0897	9.2707	0.0046	0.0099	9.23	-0.04	102394	5%	0.089
28.11.11	0.1069	9.2171	0.0152	0.0327	9.20	-0.02	102054	14%	0.106
	0.1065	9.2131	0.0134	0.0289	9.20	-0.01	102054	13%	0.106
31.11.11	0.0912	9.0622	0.0077	0.0166	9.03	-0.03	100187	8%	0.092
	0.0920	9.0629	0.0061	0.0132	9.03	-0.03	100187	7%	0.093
23.01.12	0.0979	9.0105	0.0159	0.0344	9.00	-0.01	100057	16%	0.099
	0.0841	9.0305	0.0116	0.0250	9.00	-0.03	100057	14%	0.085
20.02.12	0.0897	12.6467	0.0052	0.0113	12.66	0.01	100677	6%	0.090
	0.0886	12.6608	0.0005	0.0010	12.66	0.00	100677	1%	0.089

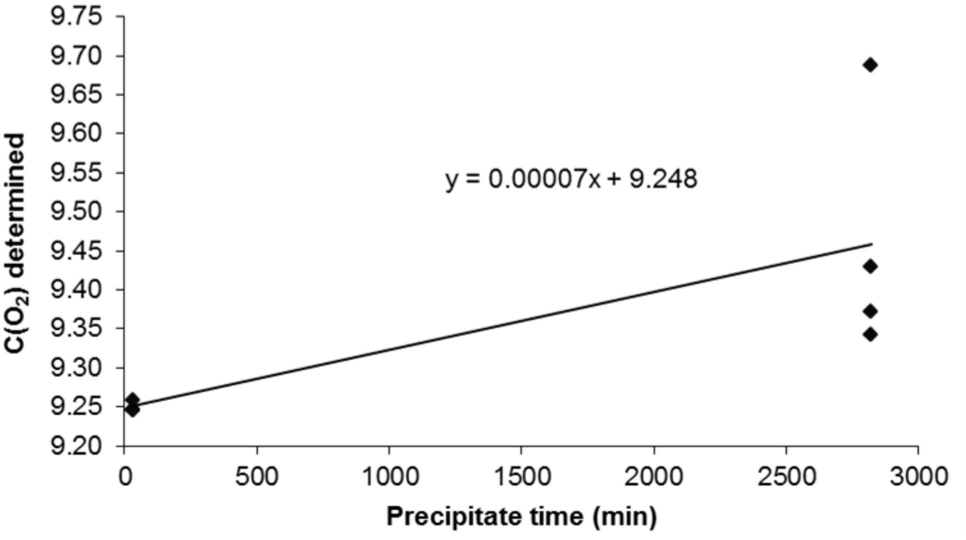
[a] Slope (b_1) and intercept (b_0) of the linear regression and their standard deviations.
[b] reference values of DO obtained from ref [13] (in mg kg^{-1}).
[c] difference between the calculated reference value and b_0.
[d] relative standard deviation of linear regression.

The results were averaged arriving at the averaged correction value of 0.0940 mg kg^{-1} (corresponding to the normal pressure) with standard deviation 0.0068 mg kg^{-1}. This standard deviation also accounts for the variability of the amount of added reagents. Each time the correction was used it was recalculated to the actual atmospheric pressure at the location of the measurement.

2.3.6 Determination of Parasitic Oxygen (Int_{O2})

In order to determine the amount of oxygen introduced to the sample through the junction between the stopper and the flask neck seven subsamples were collected at the same time and the reagents ($MnSO_4$ solution and alkaline KI solution) were added. Three of them were titrated on the same day. The remaining four were titrated two days later. The mass of DO found in the sample was plotted against the precipitation time. The mass of the introduced oxygen per minute was found as the slope of the graph (Graph 4). The amount of parasitic oxygen introduced was found as ca 0.00007 mg kg^{-1} min^{-1}. The precipitation time for the analysis is different and ranges from few tens of minutes to slightly more than an hour, so the content of intruded O_2 can be estimated to be in the range of 0.0015 to 0.0050 mg kg^{-1} during the precipitation time. This effect is small compared to the overall repeatability of the measurement, our understanding of the actual mechanism of this process is limited, the determination of this effect is very uncertain and the precipitation time also differs widely. Therefore we decided not to correct for this effect but take it entirely into account as an uncertainty, expressed as ±0.005 mg kg^{-1}.

Graph 4. Determination of *Int*$_{O2}$.



2.3.7 Iodine volatilization

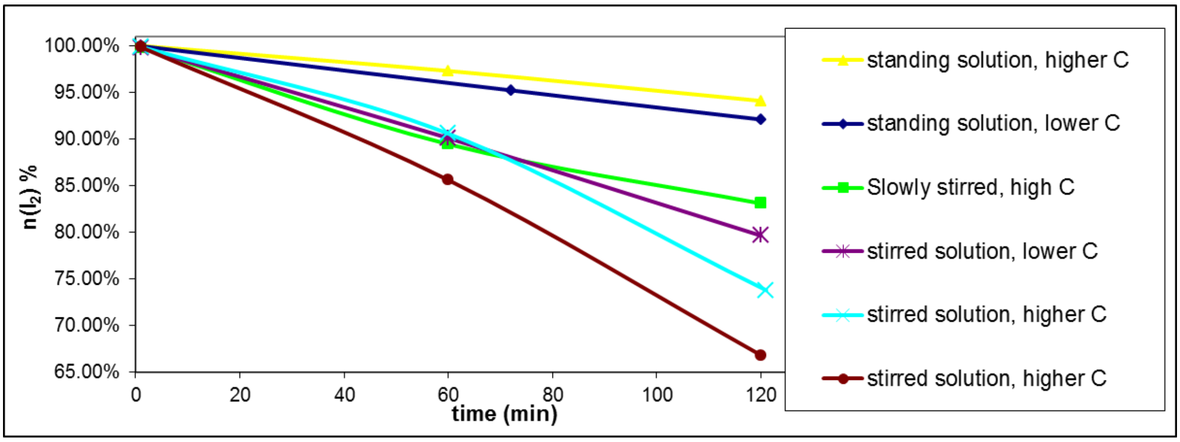
It has been stressed [19,37] that loss of iodine may be an important source of uncertainty in Winkler titration, however, concrete experimental data on the extent of this effect are rare. In the literature more sources of iodine-related errors have been described [38], such as hydrolysis of iodine by formation of oxyacid anions, which are not capable of oxidizing thiosulphate at the pH of the titration and iodine adsorption on glass surfaces. All these effectively lead to the loss of the iodine. At the same time under strongly acidic conditions additional iodine may form via light-induced oxidation of iodide by air oxygen [19,39]:

$$4I^{-} + 4H^{+} + O_2 \rightarrow 2I_2 + 2H_2O \qquad (11)$$

This process leads to the increase of iodine concentration. All these factors can have influence both during titration of the sample and during titrant standardization. There are also other influencing factors: pH, temperature and intensity of light in the laboratory. In our experiments these factors were found to have negligible influence.

We carried out some experiments to determine the iodine volatilization amount at different experimental conditions: stirred vs standing solution and high (ca 2.4 mmol kg^{-1}) vs low (ca 0.5 mmol kg^{-1}) concentration. As it is seen from the graph 5, the largest effect on iodine volatilization is stirring. Also the concentration of iodine in the solution influences volatilization, but this effect is not that large and it does not come out that clearly.

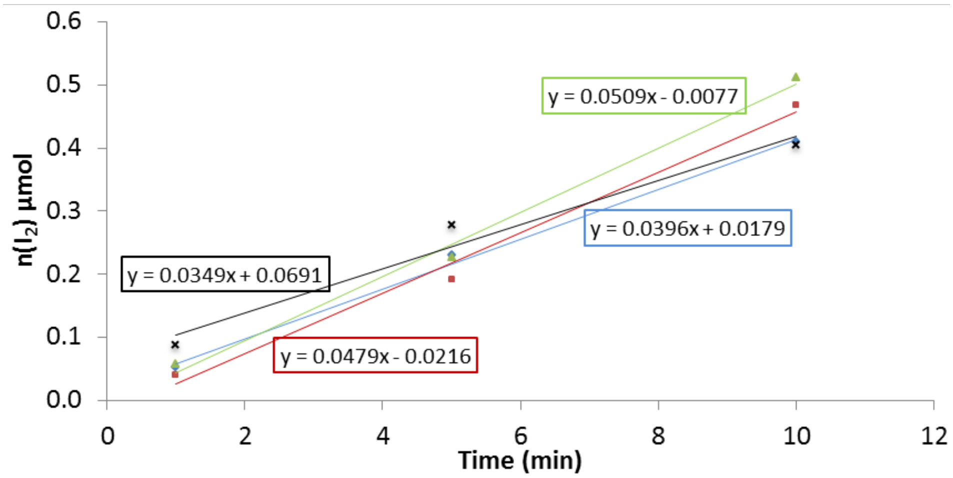
Graph 5. Stirring and concentration effect on iodine volatilization.



While stirring has a major effect, we decided not to use stirring until it is necessary. In our procedure in the case of titration of the sample it is possible to minimize the volatilization by adding about 80-90% of the expected titrant consumption into the titration vessel before transferring the iodine-containing sample solution to the titration vessel (so-called "pre-titration"). This way the main part of the iodine reacts immediately, significantly minimizing volatilization. At the same time, evaporation of iodine occurs during transfer of the iodine solution formed from the sample into the titration vessel and this has to be taken into account also. The pre-titration approach is not possible in the case of determination of the titrant concentration because there it is necessary to stir the solution containing iodate, iodide and sulphuric acid properly before starting the titration. If this is not done then thiosulphate can react directly with iodate, not with iodine and the reaction loses its stoichiometry. For both titrations the volatilization has been taken into account by introducing two corrections, $n_{I2\_vol\_s}$ and $n_{I2\_vol\_t}$, for titration of the sample and standardizing of the titrant, respectively.

For evaluating the effect of iodine volatilization on titrant standardization four parallel measurements of a solution of 5 ml with iodine concentration of 1.9 µmol g^{-1} were made by keeping them for different times, 1, 5 an 10 minutes while stirring at 800 rpm (PTFE stirrer bar: length 21 mm, diameter 6 mm). The results were plotted as iodine loss (in µmol) against time (see Graph 6) and the estimates for the loss of iodine in one minute were found as the slopes of the four graphs: 0.051, 0.040, 0.048, 0.035 µmol min^{-1}. The average iodine loss is thus 0.043 µmol min^{-1} with standard deviation of 0.007 µmol min^{-1}. The titration time during standardization ranges from 30 s to 60 s. The average time of 45 s was used as the estimate of titration time.

Graph 6. Determination of iodine volatilization.



For evaluating the effect of iodine volatilization on titration of the sample, we have done experiments on two different days, 7 replicates on both days. The data on determination of iodine volatilization during titration mimicking the titration of the sample are presented in Table 4.

Table 4. The absolute (in µmol) and relative (in %) losses of iodine during titration mimicking the titration of the sample.

Date	$\Delta n(I_2)$ µmol	$\Delta n(I_2)$ %
19.April.2012	0.01036	0.20%
	0.01098	0.22%
	0.01167	0.23%
	0.01074	0.22%
	0.01281	0.25%
	0.01079	0.21%
	0.01117	0.21%
27.April 2012	0.01365	0.23%
	0.01409	0.23%
	0.01134	0.20%
	0.01155	0.19%
	0.00866	0.14%
	0.01105	0.18%
	0.01284	0.21%
Average	0.0116	0.21%
St.dev	0.0014	0.026%

Every experiment consisted in titration of ca 10 ml of iodine solution (prepared from KIO_3) with concentration of 0.5 µmol g^{-1} (the concentration of iodine is in processed sample solutions is similar) in our usual sample bottle. This solution was transferred into the titration vessel in a similar way as was used for titration of the samples and was titrated (using pre-titration). The difference of the amounts of initially added iodine and iodine calculated from titration data gave the amount of volatilized iodine.

The average amount of volatilized iodine was found as 0.0116 µmol with the standard deviation of 0.0014 µmol. This amount is taken into account as $n_{I2\_vol\_s}$ – iodine volatilization by titrating the sample.

2.4 Measurement model of the gravimetric method

Potassium iodate (KIO_3) was used as the standard substance. The working solution concentration was found according to eq 12.

$$C_{KIO_3III} = \frac{m_{KIO_3\_s} \cdot 1000 \cdot 1000 \cdot m_{KIO_3\_I\_transf} \cdot m_{KIO_3\_II\_transf} \cdot P_{KIO_3}}{M_{KIO_3} \cdot m_{KIO_3\_I} \cdot m_{KIO_3\_II} \cdot m_{KIO_3\_III}} \qquad (12)$$

where $C_{\mathrm{KIO3\_III}}$ [mol kg^{-1}] is the concentration of the KIO_3 working solution, $m_{\mathrm{KIO3\_s}}$ [g] is the mass of the solid KIO_3, P_{KIO3} [-] is the purity (mass fraction) of KIO_3, M_{KIO3} [mg mol^{-1}] is molar mass of KIO_3, $m_{\mathrm{KIO3\_I}}$ [g], $m_{\mathrm{KIO3\_II}}$ [g] and $m_{\mathrm{KIO3\_III}}$ [g] are the masses of the prepared solutions, respectively, $m_{\mathrm{KIO3\_I\_transf}}$ [g] and $m_{\mathrm{KIO3\_II\_transf}}$ [g] are the masses of the transferred solutions for diluting the previous solution.

Concentration of the $Na_2S_2O_3$ titrant was found by titrating iodine liberated from the KIO_3 standard substance in acidic solution of KI. The titrant concentration was found according to eq 13.

$$C_{\mathrm{Na_2S_2O_3}} = 6 \cdot C_{\mathrm{KIO_3}} \cdot \mathit{\Gamma}^{m_{KIO3}}_{m_{Na2S2O3\_KIO3}} \cdot F_{m_{KIO3}} \cdot F_{m_{Na2S2O3\_KIO3}} \cdot F_{m_{KIO3\_endp}} - \frac{2 \cdot n_{I_2\_vol\_t}}{m_{Na_2S_2O_3\_KIO_3}} \qquad (13)$$

where C_{Na2S2O3} is the titrant concentration [mol kg^{-1}], m_{KIO3} [g] is the mass of the KIO_3 solution taken for titration, $m_{\mathrm{Na2S2O3\_KIO3}}$ [g] is the mass of the $Na_2S_2O_3$ titrant used for titrating the iodine liberated from KIO_3, $n_{\mathrm{I2\_vol\_t}}$ [mmol] evaporated iodine from the solution during the titration for determination of titrant concentration. Value of $\mathit{\Gamma}^{m_{KIO3}}_{m_{Na2S2O3\_KIO3}}$ is calculated by eq 14 and it is the average (from seven parallel determinations) ratio of the masses of KIO_3 and $Na_2S_2O_3$ solutions, used in the analysis.

$$\mathit{\Gamma}^{m_{KIO3}}_{m_{Na2S2O3\_KIO3}} = \frac{\sum_i \frac{m_{KIO_3}}{m_{Na_2S_2O_3\_KIO_3}}}{7} \qquad (14)$$

Such approach is needed (differently from volumetry), because it is impossible to take exactly the same mass of KIO_3 for titration in all parallel titrations. The uncertainty of $\mathit{\Gamma}^{m_{KIO3}}_{m_{Na2S2O3\_KIO3}}$ takes into account only the repeatability of titration. In order to account for the remaining uncertainty sources three factors F are introduced. F_{mKIO3} [-] and $F_{\mathrm{mNa2S2O3\_KIO3}}$ [-] are factors taking into account the uncertainties of weighing of these solutions. $F_{\mathrm{mKIO3\_endp}}$ [-] is the factor taking into account the uncertainty of determining the titration end-point. These factors have unity values and their uncertainties correspond to the respective relative uncertainty contributions to $\Gamma^{m_{KIO3}}_{m_{Na2S2O3\_KIO3}}$.

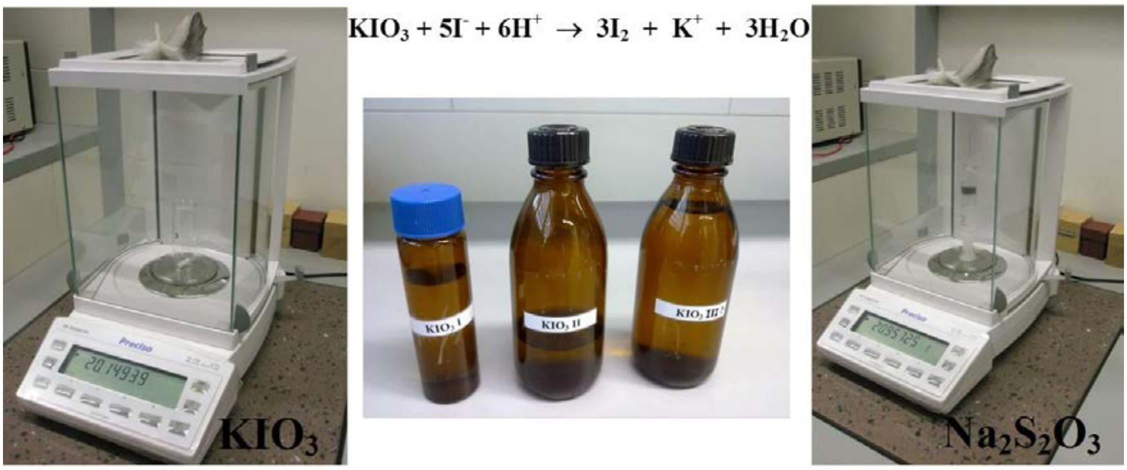
The DO concentration in the sample was found according to eq 15:

$$C_{O_2\_s} = \rho \cdot \left(M_{O_2} \cdot \frac{1}{4} \cdot \left(C_{Na_2S_2O_3} \cdot \Gamma_{m_s}^{m_{Na_2S_2O_3\_s}} \cdot F_{m_s} \cdot F_{m_{Na_2S_2O_3\_s}} \cdot F_{m_{s\_endp}} + \frac{2 \cdot n_{I_2\_vol\_s}}{\overline{m_s}} \right) + Int_{O_2} - CF_{O_2} \cdot \frac{p}{p_n} \right) \quad (15)$$

where C_{O2_s} [mg dm^{-3}] is the DO mass concentration in the sample, ρ [kg dm^{-3}] is the density of water saturated with air (see the section 2.2.1 for details), $\Gamma_{m_s}^{m_{Na_2S_2O_3\_s}}$ is the average (from seven parallel determinations) ratio of the masses of $Na_2S_2O_3$ and sample solutions, used in the analysis and is defined analogously to eq 14. As was explained above in this section, the uncertainty of $\Gamma_{m_s}^{m_{Na_2S_2O_3\_s}}$ takes into account only the repeatability of titration, $n_{I2\_vol\_s}$ [mmol] is the estimated amount evaporated iodine from the solution during the solution transfer from sample bottle to titration vessel and during the titration for determination of sample concentration, $\overline{m_s}$ is the average mass of the sample. In order to account for the remaining uncertainty sources three factors F are introduced. F_m [-] and $F_{mNa2S2O3\_s}$ [-] are factors taking into account the uncertainties of weighing of these solutions weighing. F_{m_endp} [-] is the factor taking into account the uncertainty of determining the titration end-point. These factors have unity values and uncertainties corresponding to the relative uncertainties of the effects they account for. Int_{O2} [mg kg^{-1}] is the correction taking into account the contamination of the sample by the parasitic oxygen introduced through the junction between the stopper and the flask neck. CF_{O2} [mg kg^{-1}] is the correction accounting for the parasitic oxygen introduced with reagent solutions. Both these effects lead to apparent increase of DO concentration in the sample (therefore the negative signs of the corrections). CF_{O2} is normalized to the sea-level pressure by multiplying it with the ratio of pressures p [Pa] and p_n [Pa], which are air pressures in the measurement location at the time of the measuring and the normal sea-level pressure, respectively.

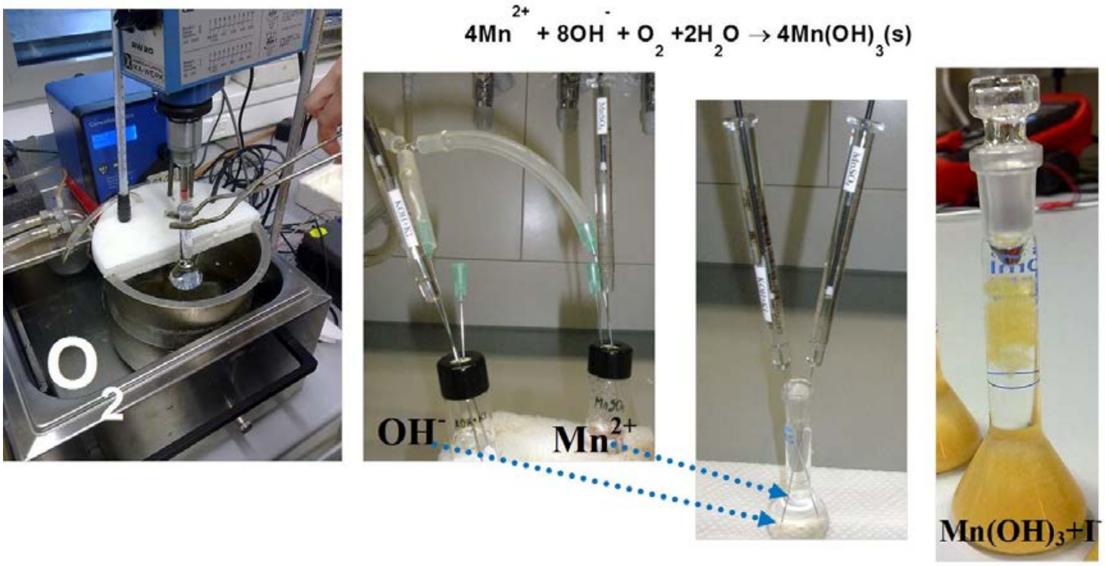
2.5 The photo series illustrating the gravimetric Winkler analysis

KIO_3 solutions preparation, forming the certain amount of iodine and titrations :



$$KIO_3 + 5I^- + 6H^+ \rightarrow 3I_2 + K^+ + 3H_2O$$

Sampling and formation of mangan(III)hydroxyde precipitate:



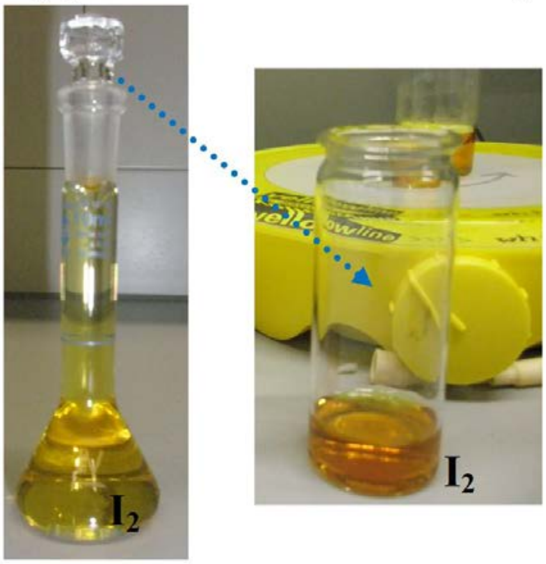
$$4Mn^{2+} + 8OH^- + O_2 + 2H_2O \rightarrow 4Mn(OH)_3(s)$$

Dissolving of mangan(III)hydroxyde precipitate, formation of iodine solution:

$$4Mn(OH)_3(s) + 12H^- + 4I^- \rightarrow 2I_2 + 4Mn^{2+} + 12H_2O$$

$$4Mn^{3+} + 4I^- \rightarrow 2I_2 + 4Mn^{2+}$$

Titrating of iodine solution with sodium thiosulfate:



3. Measurement Uncertainty

3.1 General Notes

Uncertainty estimation has been carried out according to the ISO GUM modelling approach [40] using the Kragten spreadsheet method [41]. The measurand is concentration of DO in the water sample (C_{O2_s}) expressed in mg dm^{-3}. The measurement model is presented in eqs 12-15. All uncertainties of molar masses are estimated as it has been done in ref 45. In all cases where uncertainty estimates are obtained as ±X without additional information on the probability distribution we assume rectangular distribution (the safest assumption) and convert such uncertainty estimates to the respective standard uncertainties by dividing with square root of three [40].

3.2 Uncertainty of weighing

Weighing was done on a Precisa XR205SM-DR balance. The balance has two measurement ranges: low: 0-92 g and high: 92-205 g with 4 and 5 decimal places, respectively. So, some of the components of weighing uncertainties have two different values – for higher and for lower range. Which one is used depends on the mass of the object together with tare. The uncertainty components of weighing are: repeatability, rounding of the digital reading, drift of the balance and calibration of the balance. The uncertainty contributions of all the components discussed below were found by taking into account that all masses were found as mass differences (weighed object with tare and empty tare). Repeatability estimates were found by weighing different masses in series of 10. These estimates are used for weighing of KIO_3 and its solutions. The repeatability of weighing during titration is accounted for by the Γ factors based on the actual parallel titrations data as detailed in section 2.4. Rounding of the digital reading is taken into account in the conventional way, as half of the last digit of the reading assuming rectangular distribution leading to standard uncertainty estimates u(rounding_low)=0.0000029 g and u(rounding_high)=0.000029 g. The effects of mass measurement accounted for as factors have no unit and they are relative quantities of the weighed masses. In the present work the linearity is not separately accounted for, because the tare and tare with the weighed object have comparable masses. Two additional uncertainty sources related to weighing were taken into account: possible partial evaporation of water and the "warm hand" effect (see below).

3.2.1 The uncertainty component for the repeatability of the balance

The analytical weighing instrument Precisa XR205SM-DR was used in this study. Experiments were carried out on two different days in order to estimate the uncertainty component of repeatability. Different weights or their combinations were weighed for ten times in the mass range that was also used for carrying out this study (100 mg – 200 g). Experimental results are presented in tables 5 and 6 and graph 7.

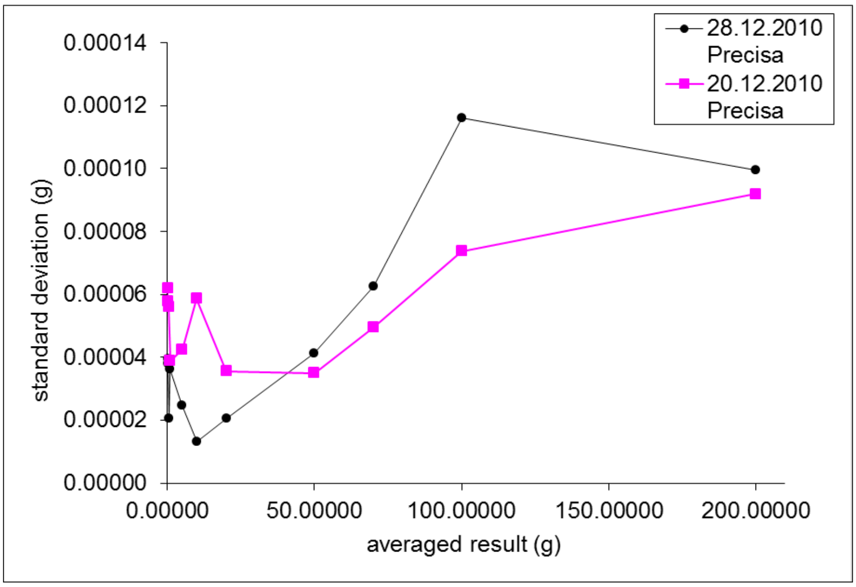
Table 5. Repeatability of the balance (20.12.2010)

Weights (g)	Average result (g)	St.dev (g)	Rel. st. dev
0.1	0.09987	0.000062	0.06191
0.2	0.19995	0.000058	0.02888
0.5	0.49987	0.000056	0.01124
1	0.99946	0.000039	0.00388
5	4.99938	0.000042	0.00085
10	9.99963	0.000059	0.00059
20	19.99900	0.000036	0.00018
50	49.99908	0.000035	0.00007
70	69.99912	0.000050	0.00007
100	99.9998	0.000074	0.00007
200	199.9999	0.000092	0.00005

Table 6. Repeatability of the balance (28.12.2010)

Weights (g)	Average result (g)	St.dev (g)	Rel. st. dev
0.1	0.09989	0.00004	0.03954
0.2	0.19996	0.00004	0.01904
0.5	0.49985	0.00002	0.00409
1	0.99944	0.00004	0.00362
5	4.99935	0.00002	0.00050
10	9.99962	0.00001	0.00013
20	19.99899	0.00002	0.00010
50	49.99906	0.00004	0.00008
70	69.99812	0.00006	0.00009
100	99.9998	0.00012	0.00012
200	200.0000	0.00010	0.00005

Graph 7. Repeatability dependence of mass.



It can be seen from the graph that the repeatability of weighing does not markedly depend on the mass weighed. However, this balance has two ranges (five decimals for weighing masses up to 79.99999 g and four decimals up to 205.0000 g). So we have two different repeatabilities: for lower and for higher range. The uncertainty of weighing repeatability has been calculated using the pooled standard deviation equation:

$$s_{pooled} = \sqrt{\frac{\sum s_i^{2}}{k}} \tag{S16}$$

where k is total number of different standard deviations of weighing and s_i are standard deviations of parallel weighings, all obtained with the same number of replicates. The value of the pooled standard deviation (the standard deviation of the repeatability of weighing) was estimated for lower range to be 0.000043 g and for higher range 0.000057 g.

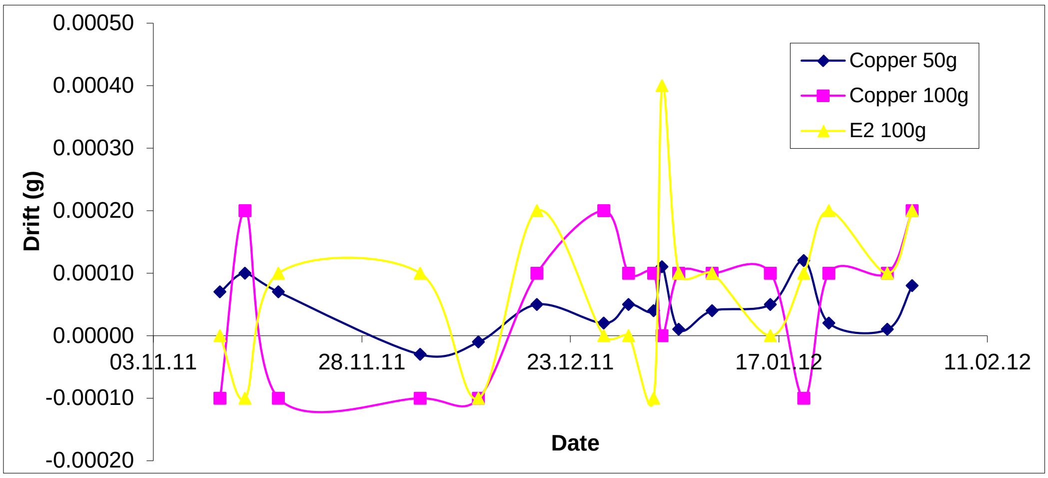
3.2.2 The drift uncertainty of the weighing instrument

Three weights (m_1 = 50 g, m_2 = 100 g and m_3 = 100 g) were weighed daily before and after making the Winkler titration. This experiment was carried out on 17 different days. The instrument was adjusted (internal calibration) on every morning before the start of the measurements. The average drift of the weighing instrument during one day was calculated using these data and the uncertainty component of weighing was estimated.

Table 7. Drift of Precisa XR205SM-DR during the time of measurement (6 hours).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**Drift (mg)**	**Drift (%) *10^{-3}**	**U_Drift (%) *10^{-3}**
Δm_1 (mg)	0.07	0.10	0.07	-0.03	-0.01	0.05	0.02	0.05	0.04	0.11	0.01	0.04	0.05	0.12	0.02	0.01	0.08	**0.062**	**0.124**	**0.072**
Δm_2 (mg)	-0.10	0.20	-0.10	-0.10	-0.10	0.10	0.20	0.10	0.10	0.00	0.10	0.10	0.10	-0.10	0.10	0.10	0.20	**0.121**	**0.121**	**0.070**
Δm_3 (mg)	0.00	-0.10	0.10	0.10	-0.10	0.20	0.00	0.00	-0.10	0.40	0.10	0.10	0.00	0.10	0.20	0.10	0.20	**0.148**	**0.148**	**0.085**
																			Average:	**0.076**

Graph 8 Drift of the Precisa XR205SM-DR during one measurement day.



The average (root mean) drift of the weighing instrument during an experiment day was 0.000052 g when weighing the 50 g weight and 0.00012 g when weighing the 100 g weights. This corresponds to ca 0.0001% of the mass of the weight. The second weighing was carried out at the end of the day, after the measurements so it was assumed that it gives the maximum possible deviation. These average drifts were divided by the square root of three in order to calculate the standard uncertainty estimates. So the drift uncertainty component of weighing was estimated to be 0.00008% relative to the weighed mass.

3.2.3 The calibration uncertainty of the balance

The weighing instrument has been adjusted every day with the internal adjustment (calibration) weights (80 g and 200 g). Additional experiment has been done by weighing reference weights. The biggest difference between mass of the reference weight and reading of the used scale was 0.0003 g and it was divided with the mass it was attained (120 g) to get a relative quantity and divided by the square root of three. This value was estimated as the uncertainty of the calibration of the balance.

3.2.4 The uncertainty component of the effects by weighing

Two additional uncertainty sources related to weighing were taken into account. For determining the possible partial evaporation of water from the KIO_3 solution was done an experiment with six measurements weighing solution mass in titration vessel and solution mass from syringe. This uncertainty component was estimated as root mean square of the six parallel measurements (u = 0.002 g). The "warm hand" effect is important when weighing the titrant syringe after titration. This uncertainty component was found weighing syringe at laboratory temperature and hand-warm syringe and the biggest difference between these two masses divided by the square root of three was accounted as uncertainty (u = 0.00046 g). The latter leads to lower mass of the syringe because it has been warmed by hand during titration and this causes ascending air flow in the balance compartment.

3.3 Uncertainty of purity of standard titrimetric substance KIO_3

The highest purity KIO_3 standard substance available was used. We consider its declared purity as too optimistic and use the following purity estimate: 100.0% ± 0.1%. The true content of KIO_3 in the substance was assumed to be rectangularly distributed in the range of 99.9% to 100.1%, leading to the standard uncertainty of purity 0.058%. KIO_3 is known for its negligible hygroscopicity [39]. We additionally tested this by drying the substance at 110 °C for 4 hours. It was not possible to detect a mass decrease.

3.4 Uncertainty of determination of the equivalence point

The random effects on the titration equivalence point are taken into account by the uncertainties of the Γ factors, as explained in section 2.4 and 3.2. The uncertainty contribution of the possible systematic effects was estimated as ± half of the drop of titrant (assuming rectangular distribution), whereby the drop mass is estimated as 0.0105 g of titrant. This leads to standard uncertainty estimate of 0.0029 g, which is a conservative estimate, because it is possible (and was used in the experiments) to dispense the titrant in amounts approximately equal to a tenth of a drop. The magnitude of this uncertainty estimate covers the human factor (deviation from the point where the operator considers that the equivalence has been reached), the possible uncertainty of the background current as well as the possible uncertainty of the reading of the amperometric device used for equivalence point determination. In calculations this uncertainty is divided by the respective titrant mass and is assigned as standard uncertainty to the respective F factors corresponding to the equivalence point uncertainty.

3.5 Uncertainty of the iodine volatilization

Determination of the effect of iodine volatilization has been explained in section 2.3.7. Based on the different ways of carrying out the experiments for determination of the titrant concentration and titration of the sample two different approaches of taking iodine volatilization into account were used.

In the case of determination of titrant concentration where pre-titration is not possible the volatilized iodine is accounted as $n_{\mathrm{I2\_vol\_t}}$ and it has two uncertainty components: repeatability (expressed as experimental standard deviation) of the iodine lost from the solution determined from three parallel measurements $u(\mathrm{rep}_{\mathrm{I2\_vol\_t}})$=0.0000074 mmol and possible variability of the duration of the first part of titration, estimated as ±15 seconds. The first part of titration is the one where the iodine concentration in the solution is still high and according to our experience it lasts until the current between the electrodes becomes around 3 µA. $n_{\mathrm{I2\_vol\_t}}$ corresponds to the amount of escaped iodine in one minute, so in fifteen seconds this amount is four times smaller. Assuming rectangular distribution u(time)=0.0000047 mmol.

In the case of sample titration pre-titration is possible, which strongly reduces the extent of iodine volatilization. Nevertheless, even under these conditions iodine evaporation is non-negligible and in order to achieve the lowest possible uncertainty this iodine volatilization has to be taken into account. The estimated loss of iodine is taken into account by the correction $n_{\mathrm{I2\_vol\_s}}$. This correction was estimated using iodine solutions prepared from KIO_3 and KI with accurately known iodine concentrations (resembling that in routine samples). These solutions were handled and titrated exactly the same way as routine samples. The value of $n_{\mathrm{I2\_vol\_s}}$ = 0.0116 µmol (with standard uncertainty 0.0014 μmol) was found from the difference of the prepared and found amounts of iodine. The experimental data are given in section 2.3.7.

3.6 Uncertainty of the correction for oxygen introduced from the reagents

Determination of the correction CF_{O2} has been explained in section 2.3.5. Although the mean value of CF_{O2} is used as correction the standard deviation of the single results (not the mean) is used as its uncertainty estimate, because this uncertainty takes into account the variability of CF_{O2} and is not averaged during the measurements in any way.

3.7 Uncertainty of the pressure

The corrections CF_{O2} depend on atmospheric pressure. Atmospheric pressure was measured by digital barometer PTB330 (Ser No G37300007, manufactured by Vaisala Oyj, Finland, calibrated by manufacturer 19.09.2011) with standard uncertainty of calibration $u(p_{cal})$ = 3.5 Pa. Including additionally the contributions from reading instability (u = 1.5 Pa) and possible drift (u = 3.5 Pa) leads to a combined standard uncertainty estimate of pressure $u_c(p_{cal})$ = 5.2 Pa.

3.8 Validation

Validation of the method includes a number of tests: determination of parasitic oxygen from different sources, iodine volatilization in two different cases, weighing tests and in addition the test for trueness. For evaluation of trueness water saturated with air (below termed as saturation conditions) under carefully controlled conditions (air source, temperature, air pressure, air humidity) was created in a double-bath thermostat. Freshly dispensed MilliQ water was saturated with air at 5 °C to 30 °C using the procedure described in section 2.2.2. Every trueness test consisted of taking seven samples, measuring their DO concentration with our method and comparing the obtained average DO concentration with the reference DO values evaluated according to the standard ISO 5814 [13] by an empirical formula originally published by Benson and Krause [14]. The uncertainties of the reference values were calculated as detailed in section 2.2.3. The agreement was quantified using the E_n number approach [42].

The repeatability of the method obtained as the pooled standard deviation [43] from 14 sets of seven parallel measurement results (made on 13 different days during six months) was found as 0.0094 mg dm^{-3} (0.108% relative). It is important to note that in no case were any of the parallel measurement results rejected.

4. Results

4.1 Validation of the method by comparison with independent reference values

The method was validated by measuring DO concentration in water saturated with air. The average results of 7 parallel measurements (as detailed in the experimental section) in comparison with the reference values from ref 13 are presented in Table 8.

Table 8 Results of the gravimetric Winkler method (C_{O2}GW) in comparison with the reference values (C_{O2}Ref) obtained from ref [13] (in mg dm^{-3}) under different experimental conditions

Date	**15.03.12**	**15.03.12**	**20.02.12**	**30.01.12**	**23.01.12**	**16.01.12**	**5.01.12**	**3.01.12**	**2.01.12**	**30.12.11**	**14.11.11**	**11.11.11**	**28.10.11**	**14.10.11**
Saturation conditions	**KCl 0.01M, 25°C**	**KCl 1M, 25°C**	**5°C**	**25°C**	**20°C**	**15°C**	**30°C**	**25°C**	**15°C**	**20°C**	**10°C**	**5°C**	**20°C**	**20°C**
C_{O2}GW	8.298	6.126	12.680	8.538	8.999	10.052	7.286	8.176	9.941	8.876	11.241	12.968	9.189	9.180
$U(C_{\mathrm{O2}}\mathrm{GW})$[a]	0.028	0.023	0.035	0.025	0.028	0.028	0.024	0.025	0.029	0.026	0.031	0.034	0.028	0.026
$U(C_{\mathrm{O2}}\mathrm{GW}_{\mathrm{rel}})$	0.34%	0.38%	0.28%	0.30%	0.31%	0.28%	0.34%	0.31%	0.29%	0.29%	0.28%	0.27%	0.31%	0.29%
C_{O2}Ref	8.310 [b]	6.197 [b]	12.698	8.534	9.001	10.052	7.289	8.168	9.944	8.868	11.219	12.948	9.189	9.166
$U(C_{\mathrm{O2}}\mathrm{Ref})$[a]	0.100 [c]	0.100 [c]	0.062	0.065	0.062	0.062	0.061	0.062	0.061	0.061	0.062	0.063	0.063	0.063
***t* (°C)**	24.616	24.597	5.032	24.901	19.957	15.019	29.785	24.861	15.053	19.915	10.044	4.968	19.852	19.853
***p* (Pa)**	101295	101282	100788	104289	100106	100963	97130	99734	99947	98545	100749	102595	101975	101731
C_{O2}GW (normP)[d]	8.300	6.175	12.748	8.295	9.109	10.088	7.601	8.307	10.078	9.126	11.305	12.807	9.131	9.143
C_{O2}Ref (normP)[d]	8.312	6.200	12.766	8.299	9.109	10.088	7.590	8.294	10.079	9.113	11.283	12.789	9.131	9.131
Δ[e]	-0.012	-0.025	-0.017	-0.004	0.000	0.000	0.011	0.012	-0.001	0.013	0.022	0.019	-0.001	0.013
En[f]	-0.1	-0.7	-0.3	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.3	0.3	0.0	0.2

[a] Expanded uncertainty $k = 2$ level.

[b] Reference value is calculated using values from ref 44.

[c] Uncertainty of the reference value is estimated to be larger due to different experimental conditions and due to using an additional function taking into account the salting out effect.

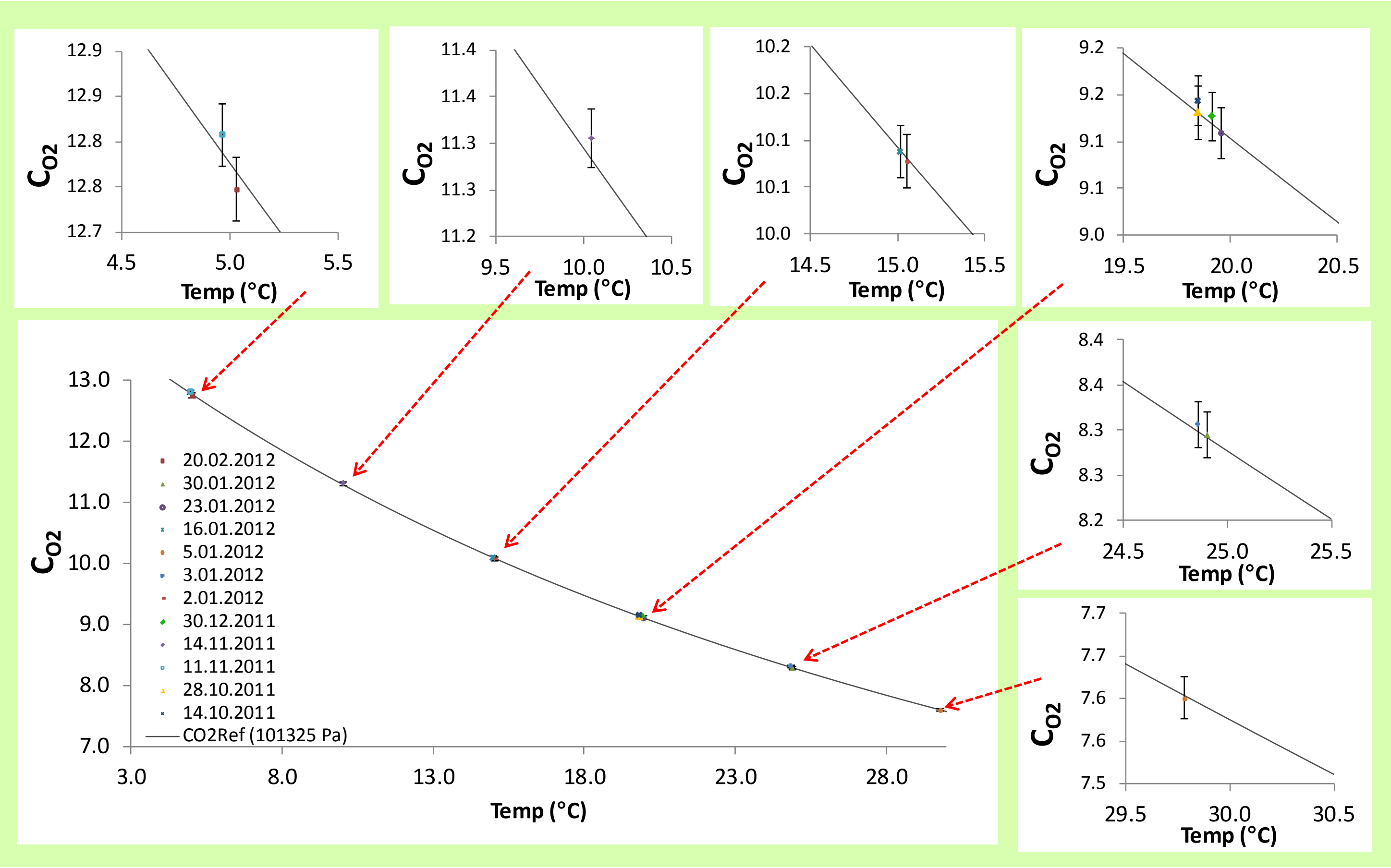
[d] Concentrations of DO recalculated to the normal pressure

[e] Difference between the measured and the reference value.

[f] The E_n score as defined in ref 42.

As it is seen from Table 8, the agreement between two methods in the concentration range from 7 to 13 mg dm^{-3} (temperature range 30 °C to 5 °C) is very good: the absolute values of E_n numbers [42] are below 1 in all cases. Because the uncertainties of the reference values in this case are higher than the uncertainties estimated by us for our method, we cannot use comparison with the reference values for confirming the uncertainties of our values. However, this comparison is usable for finding problems with our results: if the E_n values were larger than 1 then this would indicate underestimated uncertainty of our values. Our results confirm that the Benson-Krause function is appropriate for calculating DO concentration in water saturated with air. To demonstrate the fitness of our results with calculated values we converted the Winkler titration results to the normal pressure (see Table 8 for values). Compatibility is shown in Figure 3.

Figure 3. DO concentrations in normal pressure

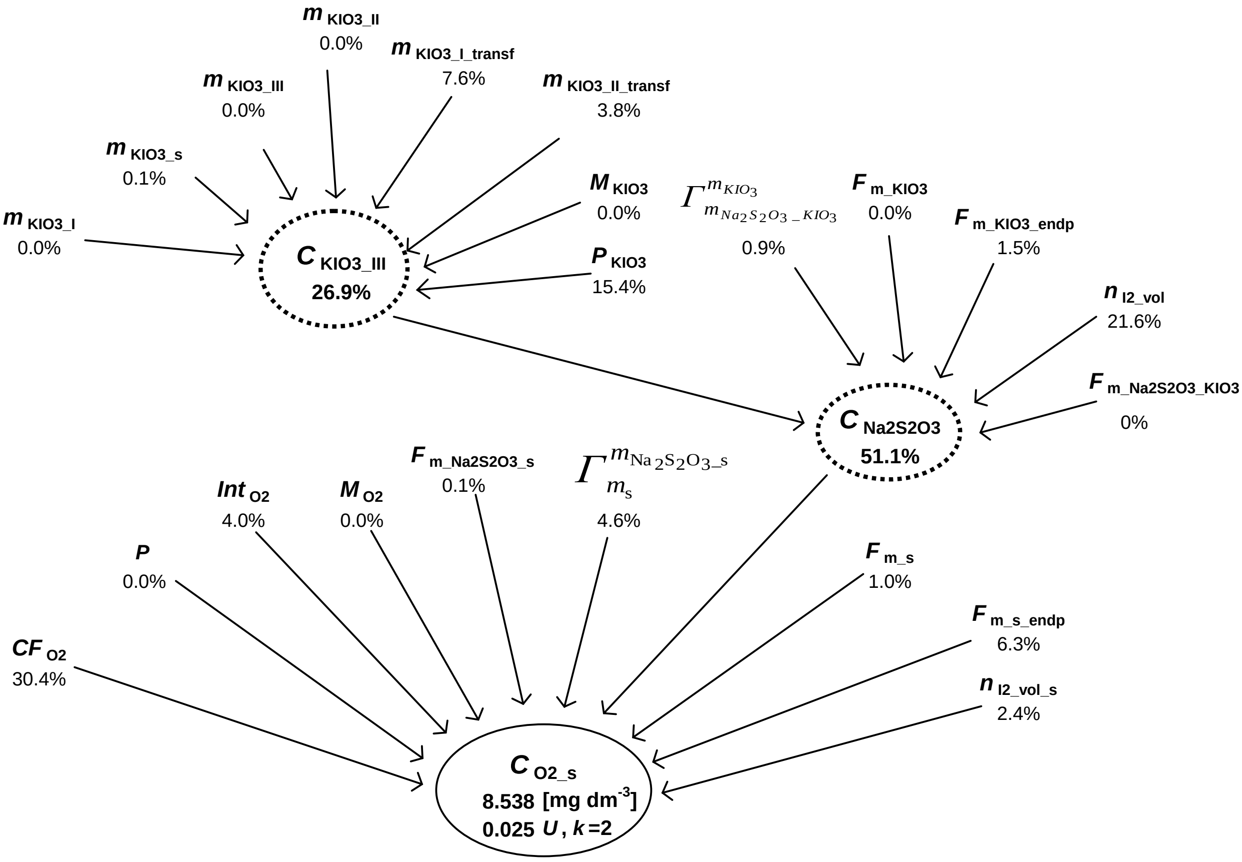


4.2 Measurement Uncertainty

Comprehensive uncertainty evaluation was made by taking into account the contributions of 22 individual uncertainty components having at least a measurable effect. Detailed uncertainty calculations as well as the full uncertainty budget are available in the attached material (see section 7). Depending on the exact measurement conditions the combined standard uncertainty of the method (at saturation conditions) varies in the range of 0.012 – 0.018 mg dm^{-3} corresponding to the k = 2 expanded uncertainty in the range of 0.023 – 0.035 mg dm^{-3} (0.27 – 0.38%, relative), see Table 8.

Figure 4 shows a sample cause and effect diagram with contributions of the uncertainty sources. The number of effective degrees of freedom as estimated using the Welch-Satterthwaite approach [40] ranges from 50 to 75 depending on the measurement conditions. The k = 2 coverage factor thus corresponds to roughly 95% confidence level.

Figure 4 Uncertainty budget of the gravimetric Winkler method on 30.01.2012



This low uncertainty is achieved mostly by using weighing instead of volumetry, amperometric end-point determination and using the purest reagents available. In this work also iodine volatilization and the effect of any kind of incoming oxygen is estimated and accounted for.

5. Discussion

5.1 Main uncertainty sources

Table 9 reveals that 41-75% of uncertainty originates from the concentration determination of the $Na_2S_2O_3$ solution.

This uncertainty in turn is mostly caused by the purity of KIO_3 (which is the biggest compound by the concentration of the standard substance KIO_3 solution), repeatability of titration of the standard iodine solutions prepared from KIO_3 and uncertainty due to partial volatilization of iodine from that solution. Iodine volatilization is under our conditions the most important effect causing the uncertainty of the $Na_2S_2O_3$ solution concentration. Furthermore, iodine volatilization has most probably an effect on the repeatability of titration (the $\Gamma^{m_{KIO_3}}_{m_{Na_2S_2O_3\_KIO_3}}$ value). Because of these effects a correction $n_{I2\_vol\_t}$ has been introduced and the uncertainty contribution $u(n_{I2\_vol\_t})$ is in fact the standard uncertainty of the correction.

Table 9 Contributions of the main uncertainty sources of the gravimetric Winkler method in the case of titration of different samples

	Uncertainty contributions (% from the overall uncertainty)[a]													
Condi-tions[b]	**$U(C_{O2})$ k=2 [mg dm^{-3}]**	**CF_{O2}**	**Int_{O2}**	$\Gamma^{m_{Na_2S_2O_3\_s}}_{m_s}$	**F_{m_s}**	**$F_{m\_s\_endp}$**	**$n_{I2\_vol\_s}$**	**$C_{Na2S2O3}$**	**Components of $C_{Na2S2O3}$**			**C_{KIO3}**	**Components of C_{KIO3}**		
									$n_{I2\_vol\_t}$	**$F_{m\_KIO3\_endp}$**	$\Gamma^{m_{KIO_3}}_{m_{Na_2S_2O_3\_KIO}}$		**P_{KIO3}**	**$m_{KIO3\_I\_transf}$**	**$m_{KIO3\_II\_transf}$**
0.01M KCl	0.028	24%	3%	8%	1%	5%	2%	57%	28%	2%	3%	22%	12%	6%	4%
1M KCl	0.023	34%	5%	5%	1%	8%	3%	44%	22%	2%	3%	18%	10%	5%	3%
5°C	0.035	15%	2%	2%	1%	3%	1%	75%	32%	2%	8%	33%	18%	9%	6%
25°C	0.025	30%	4%	5%	1%	6%	2%	51%	22%	1%	1%	27%	15%	8%	4%
15°C	0.028	24%	3%	3%	1%	5%	2%	62%	24%	2%	5%	30%	17%	9%	4%
20°C	0.028	24%	3%	12%	1%	5%	2%	53%	19%	1%	6%	25%	14%	7%	4%
30°C	0.024	28%	4%	16%	1%	7%	3%	41%	16%	1%	2%	22%	12%	7%	3%
25°C	0.025	28%	4%	9%	1%	7%	2%	48%	18%	1%	3%	26%	14%	8%	4%
15°C	0.029	22%	3%	4%	1%	5%	2%	62%	22%	2%	4%	34%	16%	9%	9%
20°C	0.026	26%	4%	3%	1%	6%	2%	57%	17%	1%	11%	27%	16%	9%	3%
10°C	0.031	19%	3%	2%	1%	4%	2%	69%	32%	2%	2%	32%	17%	10%	5%
5°C	0.034	16%	2%	2%	1%	3%	1%	74%	29%	2%	8%	34%	19%	11%	4%
20°C	0.028	23%	3%	21%	1%	5%	2%	45%	16%	1%	2%	25%	14%	8%	2%
20°C	0.026	27%	4%	5%	1%	6%	2%	55%	19%	1%	5%	29%	16%	9%	3%

[a] The input quantities are defined in section 2.4, presented in the same order as results in Table 8.
[b] The measurements were carried out under saturation conditions on 13 different days.

The next most important contribution is the uncertainty due to the oxygen introduced with the solutions of the Winkler reagents ($MnSO_4$ solution and alkaline KI solution) $u(CF_{O2})$ during titration of the sample. Due to its large effect this uncertainty also has been reduced by the use of a correction, so that $u(CF_{O2})$ in fact stands for the uncertainty of the correction.

Iodine volatilization effect has been minimized in the case of titration of the sample by the so-called "pre-titration" but it is still corrected (uncertainty of this correction is $u(n_{I2\_vol\_s})$). The resulting uncertainty contribution is similar to the one obtained for determination of $Na_2S_2O_3$ with correction.

The repeatability of titration of the sample solution expressed by $\Gamma^{m_{Na_2S_2O_3\_s}}_{m_s}$ is also an important uncertainty source, but its contribution differs greatly between samples, being dependent on the repeatability of the results of a particular sample. This effect is further strengthened by the non-rejection of any parallel titration data.

The remaining influential uncertainty sources are due to endpoint determination ($F_{m\_s\_endp}$) and oxygen diffusing into the sample solution where oxygen has been converted to $Mn(OH)_3$ through the junction between the flask neck and the stopper. The overall contribution of uncertainty sources related to mass measurement is between 8% and 15% as opposed to the more than 50% found in the case of our earlier syringe-based volumetric method [45].

In order to reduce the uncertainty of the method the most important uncertainty component that has to be reduced is the uncertainty of the correction for DO content in the reagents (CF_{O2}). However, its reduction is not easy. Our attempts to deoxygenize the reagents did not lead to a robust method. In our opinion the only viable way to reduce this uncertainty (and also some others) is to transfer the method (either wholly or in part – adding the reagents until the generation of iodine) into an inert-gas glovebox. This, however, would make the method very much more complex and clumsy to operate and would make it unusable by many routine analytical laboratories because of the lack of the an inert-gas glovebox.

5.2 Comparison with the uncertainties of other Winkler methods published in the literature

There are only few publications where the uncertainties of the Winkler method are presented and analyzed [45,46]. The reliability of the results is mostly discussed in terms of repeatability and agreement with other methods' data. Table 10 summarizes the available literature data.

Table 10 Accuracy information of DO from different sources. Accuracies estimated as repeatabilities are given in italic.[a]

Reference	Accuracy estimate (mg dm^{-3})	Remarks, the meaning of the accuracy estimate
Carritt et al. [19]	*0.07*	The precision or repeatability that can be achieved by a good analyst during the replication of certain standardization procedures.
Standard ISO 5813 [16]	*0.03 – 0.05*	Repeatability, 4 separate laboratories, batch standard deviation
Standard methods for wastewater [47]	*0.02*	Repeatability in distilled water. In wastewater the repeatability is around 0.06 mg dm^{-3}
Labasque et al. [48]	0.068	Within-lab reproducibility over ten consecutive days
Krogh [49]	0.27	Uncertainty (k=2), re-estimated by Jalukse et al. [46]
Fox et al. [50]	0.03 – 0.23	Uncertainty (k=2), re-estimated by Jalukse et al. [46]
Withney [51]	0.22	Uncertainty (k=2), re-estimated by Jalukse et al. [46]
Helm et al. [45]	0.08 – 0.14	Uncertainty (k=2), well documented uncertainty estimation information
Langdon [7]	*0.005*	0.15 μmol kg^{-1}, stated as precision. Calculations with data presented in the article gave average relative repeatability 0.35% that corresponds on DO concentration of 9 mg dm^{-3} to 0.03 mg dm^{-3}.
Horstkotte et al. [52]	*0.02 – 0.15*	Repeatabilities at 1.3 – 6.96 mg L^{-1} dissolved O_2 levels, in-line monitoring
Sahoo et al. [53]	*0.00014 – 0.11*	The repeatability in measurement at mg L$^{−1}$ levels is 0.11 mg L$^{−1}$ with RSD 1.9% and at 10 μg L$^{−1}$ level is 0.14 μg L$^{−1}$, RSD 1.4%
Carpenter et al. [37]	*0.004*	Repeatability recalculated to the saturated DO concentration at 20 °C

[a] All repeatability and reproducibility estimates are given as the respective standard deviations.

The last column of the table indicates the meaning of the accuracy estimate. There is a large variety of the ways how accuracy was estimated.

From the point of view of practical usage of the methods the most useful accuracy characteristic is the measurement uncertainty taking into account all important effects – both random and systematic – that influence the measurement results. A number of authors characterize their data by repeatability [40] estimates, which by definition do not take into account any systematic effects and may give a false impression of highly accurate method. Such estimates (presented in italics in Table 10) cannot be compared with measurement uncertainties and we leave them out of consideration.

As it is seen from Table 8 and Table 10 the gravimetric Winkler method described in the present work has the lowest uncertainty.

5.3 Comparison of the Gravimetric Winkler method with saturation method for calibration of DO sensors

Today calibration of electrochemical and optical sensors is generally done by using the saturation method. The reference values of DO saturation concentrations are usually found using the equation by Benson and Krause [13, 14, 15], see the section 2.2.3. For obtaining accurate results an accurate barometer, an accurate thermometer and a very stable thermostat are needed. Even with good equipment the saturation method is tricky to use and prone to errors. One of the main issues is the super- or undersaturation. The smaller are the bubbles used for saturation the faster the saturation conditions are achieved. At the same time small bubbles may lead to supersaturation [31]. Use of the larger bubbles avoids supersaturation, but makes the time necessary for saturation long. The result is that if the operator is not patient enough the solution is undersaturated. Furthermore, it is not documented in refs [13,14,15] what was the geometry of the nozzle and the bubble size. If uncertainty due to the possible super- or undersaturation is carefully taken into account then the resulting uncertainty is by 2-3 times higher than the uncertainty of our method.

When measuring DO concentration with optical or amperometric sensors in water with high salinity, e.g. seawater, then calibration should be carried out in water with similar salinity. This is very difficult to do rigorously with the saturation method because the available saturation values of DO concentration in seawater are significantly less accurate than the respective values in pure water. An alternative approach is to calibrate in water and apply a slainity correction, but this again introduces a substantial uncertainty from the correction. At the same time, the dissolved salts commonly found in seawater do not hinder usage of the Winkler method.

6. Conclusions

The key factors in achieving the low uncertainty of the presented Winkler titration method are (1) gravimetric (as opposed to volumetric) preparation of all solutions and measurement of the titrant amount by gravimetry, as well as the possibility of adding very small amounts of titrant; (2) careful investigation of the effect of iodine evaporation from the solutions and correcting the result for this evaporation; (3) using Winkler reagents with well-defined oxygen content and correcting for this content and (4) amperometric determination of the titration equivalence point. To the best of our knowledge the presented method is the most accurate published Winkler method available.

7. Attached Electronic Supplementary Material

Additional details of sample calculation worksheet of the result and measurement uncertainty of DO concentration determined with the gravimetric Winkler procedure (file U_GW_300112_25C.xls) and sample calculation worksheet of the reference DO concentration value and its uncertainty (file U_ref_300112_25C.xls) are attached to this report as attachments.

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