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NEW METHODS FOR IODINE DETERMINATION

Examination of sample digestion and determination

Master Thesis

for attainment of the academic degree of

Diplom-Ingenieur

Master studies Technical Chemistry

submitted to

Technical University of Graz

Supervisor

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Institute of Analytical Chemistry and Food Chemistry

Graz, August 2016

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ABSTRACT

Iodine was determined in biological samples with and without spiking with potassium and sodium iodide. Its stability during storage and digestion was examined, showing that both KI and NaI are stable during storage in the fridge (4 °C). It is important to note that flasks, designated for iodine analysis, have to be used. Other flasks may act on the stability of iodine since catalytically active impurities are present. In addition it was tested how KI standard had been affected by the solvent. Different sample digestion methods as well as various determination techniques were compared. The samples were digested using several wet digestion reagents, e.g. $HClO_3$, TMAH and a mixture of both $HClO_3$ and HNO_3. The digestion procedure concerning time and heating was developed. The interdependence of sample weight and acid volume was tested. In addition different digestion vessels were compared. Digestion tubes, glass vials, big and small eprouvettes were utilized. Digestion tubes were not suitable for digestion due to excessive splashing of the sample caused by superheating. Glass vials did not show any reproducible results due to their low height which led to rapid evaporation of the digestion acid and incomplete digestion. Big eprouvettes (for sample masses between 70 and 200 mg) and small eprouvettes (for sample masses < 70 mg) displayed reproducible results, showing no excessive splashing. 300 mg of sample mass was investigated too, showing harsh digestion conditions (foaming). Foaming was prevented by holding the samples at 95 °C at the beginning of the digestion; however recoveries were not higher than 80 %. The digested solutions were further analysed by means of ICPMS and UV-VIS photometry. The UV-VIS photometry was carried out by utilizing the catalytic reaction of Sandell and Kolthoff. A commercial UV-VIS spectrometer was compared with a dedicated “Mini Spectrometer” developed for iodine determination using the Sandell-Kolthoff method. The main features of this in-house spectrometer are the small size (100 by 50 mm footprint) and the use of a LED as light source. Measurements were carried out with different adjustments of temperature and pump velocity. Finally the method was validated using certified reference materials (BCR 151, skimmed milk powder), samples characterized previously in a round robin test (brown algae) and commercial milk powder.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 INTRODUCTION

Iodine is an essential trace element and therefore responsible for many metabolic processes in humans and animals. Their organisms have to be supplied with iodine over food. The most important natural source of iodine in the human diet is marine fish and other seafood products.[1] Within biological samples the iodine is usually present in the form of iodide[2] which can be easily absorbed by the gastric-intestinal tract. During metabolism the iodide is then further converted into various forms. The two major forms of iodine inside an organism are the two thyroid hormones thyroxin and triiodothyronine. Pre-stages and degradation products are present as well. If an organism is not appropriately supplied with iodine, various malfunctions can occur.[3] On the one hand deficiencies may lead to excessive sleepiness and cognitive disorders whereas a surplus results in increased activity of the cardio-vascular system and weight loss.

Since iodine influences the whole organism, methods had to be developed to measure the iodine content accurately. Today, ICPMS is commonly used for the determination of iodine in biological samples with a superior role to other methods due to its low detection limits. In this thesis ICPMS is compared to the catalytic method of Sandell and Kolthoff. Blood Plasma test kits are used to determine the thyroid status in the human body. The majority of these tests are based on immunochemical techniques that use antibodies as reagents and make us of the highly specific binding between antibodies and antigens.[4] There are two main methods of immunoassays: radioimmunoassay in which labelled antigen is used and immunoassay in which labelled antibodies are used. Neutron activation analysis in conjunction with anticoincidence gamma ray spectrometry had been developed for the determination of iodine by Zhang and Chatt[5]. Volatile organic iodine compounds had been determined by GC with MS or ECD used for detection[6].

Sandell and Kolthoff developed a catalytic method for determining trace iodine concentrations. This method relies on a redox reaction between arsenic and cerium with the reaction equation depicted here:[7]

$$2\ Ce^{4+} + As^{3+} \leftrightarrow 2\ Ce^{3+} + As^{5+}$$

Interestingly, the rate of reaction is rather low for this redox system. The reaction velocity is increased in the presence of iodide following this reaction scheme:

$$2\ Ce^{4+} + 2\ I^- \leftrightarrow 2\ Ce^{3+} + I_2$$

$$As^{3+}\ I_2 \leftrightarrow As^{5+} + 2\ I^-$$

The yellow Ce^{4+} gets reduced to the colourless Ce^{3+} and so the reaction can be monitored by using a spectrophotometer. The reaction proceeds first order with the iodine as an additional velocity depending step:

$$-\frac{d[Ce^{4+}]}{dt} = k * [Ce^{4+}] * [I]$$

Applying the natural logarithm gives:

$$\ln [Ce^{4+}]_t = \ln [Ce^{4+}]_{t=0} - k * [I] * t$$

If the reaction is recorded with a spectrophotometer the Lambert-Beer-law dictates:

$$\ln [E]_t = \ln [E]_{t=0} - k * [I] * t$$

The logarithm of the extinction is plotted against the time and now the iodine concentration is direct proportional to the slope.

The acidity of the reaction solution plays an important role for the sensitivity of the reaction itself. In order to understand the H^+-dependence of the reaction the following facts have to be taken into account:[8]

$2\ Ce^{4+} + I_2 \leftrightarrow 2\ Ce^{3+} + 2\ I^+$	velocity determining
$As^{3+}\ 2\ I^+ \leftrightarrow As^{5+} + I_2$	fast

I^+ is very unstable and reacts with water to form hypoiodite acid and a hydronium ion:

$$I^+ + H_2O \leftrightarrow HIO + H^+$$

It is clear to see that the equilibrium shifts to the left with increasing acidity. As a result the catalytic activity of I^+ is further increased.

Deman[8] studied the interferences on the Sandell-Kolthoff reaction and observed a dependence on the chloride concentration. At low iodine-concentration - in a high chloride containing matrix - the reaction of Ce^{4+} with As^{3+} is faster than expected since Cl^- is also catalytically reactive. Surprisingly, the reaction rate of high iodine concentration in the same matrix is inversely affected as the reaction is inhibited by inter-halogen compounds. Cyanide CN^- also affects the Sandell-Kolthoff reaction as I^+ reacts with cyanide to form the stable ICN as studied by Sandell and Kolthoff.[7]

The Sandell-Kolthoff reaction can be carried out in sulphuric or nitric acid medium, whereas at neutral pH the yellow colour of the reaction mixture is maintained for several days.

By adding HNO_3 or H_2SO_4 the reaction proceeds within minutes or seconds. As discussed in the previous section, the increased proton activity rises the content of the catalytic active I^+. HNO_3 accelerates the reaction by 20-fold in comparison to H_2SO_4.[9] The nitric medium increases the oxidational potential of Ce^{4+}. This reaction is monitored at a wavelength of 365 nm. This wavelength was also used in the determination of thyroid hormones.[10] A lower wavelength is not suitable since disruptive absorption of nitrate occurs in this region.

The reaction is also accelerated by increasing the acid concentration of H_2SO_4. By raising the H_2SO_4 concentration from 0.5 mol/l to 2.5 mol/l the sensitivity of the Sandell-Kolthoff reaction rises by a factor of 1.5. [11] However, above 2.5 mol/l the sensitivity decreases as Cerium tends to form complexes with H_2SO_4 that inhibit the catalytic measurement[12].

$$Ce^{4+} + HSO_4^- \leftrightarrow CeSO_4^{2+} + H^+$$

$$CeSO_4^{2+} + HSO_4^- \leftrightarrow Ce(SO_4)_2 + H^+$$

$$Ce(SO_4)_2 + HSO_4^- \leftrightarrow Ce(SO_4)_3^{2-} + H^+$$

In contrast to HNO_3 a wavelength of 317 nm can be used which is at the absorption maximum of Ce^{4+}. According to Thompson[13] a wavelength of 420 nm can be utilized as well, if the light is monochromatically enough.

Sample preparation is a critical step for the iodine determination. In biological samples the iodine is usually present as iodide. If iodide gets oxidized to volatile elemental iodine undesirable losses occur. Therefore iodide has to be oxidized to the stable form of iodate. Since an oxidation from state -1 to +5 is necessary, high oxidational power is needed. $HClO_3$ fulfils this requirement and the formed iodate is stable in acidic medium.

The highest oxidational power can be achieved by using a HPA-S (high pressure asher)[14]. A mixture of HNO_3 and small amounts of $HClO_4$ were used to completely decompose biological matrices. It is important to note, that most catalytic methods such as the Sandell-Kolthoff reaction require very low residual carbon concentration after the digestion. Only then the reaction is free of carbon-induced interferences. It is this requirement that dictates the use of very strong oxidizing reagents in the cause of the sample preparation for the Sandell-Kolthoff reaction.

Other analytical approaches are based on alkali extraction by either applying a mixture of TMAH/KOH or using TMAH at elevated temperature. Using TMAH for extracting, both iodides and iodates are stable.[3] This serves as an advantage compared to acidic media, since unwanted losses of iodine can occur during oxidation. Baumann used a 0.5 % ammonia solution for decomposing milk powder in a microwave oven.[15] However, as stated above, this procedure is only useful when using ICP-MS for iodine quantification, as these procedures "solubilize" the sample but do not appreciably reduce the total organic content of the digest.

Zak et al. used an acidic mixture of $HClO_3$ and Na_2CrO_4 to digest the iodide.[16] Two main disadvantages can be observed. First residues of the chromate influence the catalytic reaction in a way that the reaction does not behave like a first order reaction. Secondly some biological samples like serum tend to develop foam during the digestion. This problem can be overcome by using a mixture of $HClO_3$ and HNO_3.[17]

Microwave assisted sample digestion uses a mixture of 2 ml HNO_3 and 100-200 µl $HClO_4$ to achieve iodine recoveries of 94-96 %. By using different sample weights the recoveries do not decrease. However, the sole use of HNO_3 for digestion decreases the oxidational potential as more organic sample is used. As a result iodine losses occur.[3]

Schöninger combustion[18] is also used for digesting iodine in biological samples. The sample is placed in a glass flask, filled with oxygen and the ashing products are absorbed by a liquid alkaline absorbent. Difficulties arise with only small sample amounts to be used for combustion. To overcome this issues Mesko et al.[19] used microwave induced combustion with sample masses of up to 500 mg were completely combusted.

Today ICPMS is commonly used for determining iodine in biological samples. ICPMS is superior to all other methods due to detection limits in the ng-range.[3] Disadvantages can be seen in the high investment costs which means that Sandell-Kolthoff displays an attractive alternative for small laboratories.

ICPMS (inductively coupled plasma mass spectrometry)

The plasma used in an ICPMS is created by ionizing argon. Argon gas flows inside the concentric channels of the ICP torch. The RF load coil is connected to an RF-generator. This generator supplies power to the load coil. Inside of the torch oscillating electric and magnetic fields are established. Then a spark is applied to the argon that is flowing through the torch.

Argon ions are formed. These ions are caught in the oscillating fields and collide with other argon atoms, forming the argon plasma. The plasma itself has no net charge since the ions and free electrons are balancing each other.

Argon is favoured because it is abundant and cheaper than other noble gases. Argon has a higher first ionization potential than all other elements except He, F and Ne. Therefore the reaction of Ar^+ with an electron is energetically less favoured than the same reaction with the ionized sample.

The sample is introduced to the ICPMS by nebulization or laser ablation. The most common technique is nebulization with the liquid solution being converted into an aerosol. The aerosol is then swept into the plasma.
By using laser ablation a UV-laser is focussed onto the solid sample and creates ablated material that can be swept into the plasma.

Once the sample is introduced to the plasma, the sample is separated into its atoms. Afterwards the plasma ionizes the atoms.

[20]The ions from the plasma are extracted into the mass-spectrometer through a series of cones. First the ions pass through the first cone, which is the sampler cone. The sampler cone has an orifice diameter of 0.8-1.2 mm. Then the ions travel a short way to the skimmer cone which has a smaller diameter (0.4-0.8 mm) than the sampler cone. Ions emerge from the skimmer cone and are transported to the ions optics where the ion beam is focused. Finally the ions are guided into the mass analyser. This device is usually a quadrupole although TOF (time of flight) or sector field are used as well. The quadrupole consists of four cylindrical rods set parallel to each other. Each opposing rod pair is connected together electrically and a voltage is applied between one pair of rods and the other. The ions travel down the rods and are separated due to their mass to charge ratio. Only ions of a certain m/z-ratio are able to reach the detector. Ions of other ratios will collide with the rods and are not detected.

Since UV-VIS spectroscopy is used for carrying out the Sandell-Kolthoff reaction, light sources have to be discussed as well. As – though relatively broad – absorption bands have a finite width all photometers need a monochromator to filter off unwanted wavelengths. As LEDs are relatively monochromatic light sources there is no need for a monochromator. LEDS do not need a long warm up period, which allows higher throughput of samples.[21]

Another advantage is the minimization of the device itself. For other light sources, bulky power devices are needed that limit the working space available and increase the operating costs. With a device consisting of a LED, a flow through cuvette and a receiver diode it is possible to use the available space effectively. Such small mobile devices offer the possibility to reduce analysis time by bringing the device to the patient.

2 EXPERIMENTAL

2.1 Reagents

HNO_3 65 % (J.T. Baker, p.a. purified by subboiling distillation, 7697-37-2)
As_2O_3 (99.5 % metals basis, Alfa Aesar, p.a., 1327-53-3)
NaOH (Merck 6798 p.a.)
HCl 30 % (Merck, suprapure, 1.00318.1000)
$Ce(SO_4)_2$ * 4 H_2O (Merck, p.a., 10294-42-5)
H_2SO_4 95 % (VWR, normapure, 7664-93-9)
KIO_3 (Merck, p.a., 7758-05-6)
Te-standard (1000 mg/l); Te in 20 % HCl; Alfa Aesar; LOT: 08009085
KI (VWR, normapure, 7681-11-0)
$HClO_4$ 70-72 % (Merck, p.a., 1.00519.1000)
$KClO_3$ (Merck, p.a., 3811-04-9)
TMAH (25 w%); TAMA chemicals; Tamapure AA
High purity water (10 MΩ cm, Barnstaed Nanopur, Thermo Fisher Scientific)

2.2 Preparation of stock solutions

Arsenious acid reagent: 0.4 g As_2O_3 are dissolved in 4 ml 1 M NaOH (8 g NaOH dissolved in 200 ml H_2O) under heating. Then 0.2 ml HCl (36%) are added and the volume is made up to 100 ml with water.

Cer(IV) reagent: 0.2 g $Ce(SO_4)_2$ * 4 H_2O are dissolved in 10 ml H_2O and 1 ml H_2SO_4 (95–97%) while heating. After dissolution, the volume is made up to 100 ml with water.

Iodate stock solution (10 g I/l): 1.690 g KIO_3 is dissolved in 100 ml water.
Iodate stock solution (10 mg I/l): 0.1ml of iodate stock solution (10 g I/l) is made up to 100 ml with water.
Iodate standard solution (1000 µg I/l): 10 ml of iodate stock solution (10 mg I/l) is made up to 100 ml with water.
Iodate standard solution (100 µg I/l): 1 ml of iodate stock solution (10 mg I/l) is made up to 100 ml with water.

Iodide stock solution (10 g I/l): 1.3087 g KI is dissolved in 100 ml water.
Iodide standard solution (100 mg I/l): 1 ml of iodide stock solution (10 g I/l) is made up to 100 ml with water.

Tellurium standard solution (1 mg/l): 50 µl of tellurium stock solution (1000 mg/l) is made up to 50 ml with water

Chloric/perchloric acid-mixture: $KClO_3$ is trice recrystallized from water: 1100 g of $KClO_3$ are transferred to a 5 l Erlenmayer flask and dissolved in 1980 ml boiling water. After this solution has cooled to room temperature, the $KClO_3$ is crystalized by storing in a refrigerator overnight. $KClO_3$ is filtered off and recrystallized for a second and a third time. $KClO_3$ is filtered and dried at 60 °C. 100 g of the recrystallized $KClO_3$ is transferred to a 600 ml beaker with 180 ml water, heated and stirred until complete dissolution. 75 ml of 70 % $HClO_4$ is then added gradually, stirring well all the time, which gives a precipitate of $KClO_4$. After being cooled to room temperature, the mixture is placed in a refrigerator (–15 °C) overnight. The chloric/perchloric acid-mixture is then filtered off the following day.

TMAH (0.5 %): 2 ml of TMAH (25 %) is made up to 100 ml with water

ICPMS rinsing solution: 5 ml of HNO_3 conc. are made up to 1 l with water.

All reagents are stable for several months.

2.3 Instrumentation

ICPMS: Perkin Elmer Eland DRC+
UV-visible Spectrophotometer: Cary 50 Conc Varian: cuvettes with d = 0.5 cm and d = 1 cm,
Mini-spectrometer: temperature-controlled flow through cell (d = 0.5 or 1 cm), thermostat, peristaltic pump
Graphite heating block: Digiprep Jr; 410 W; SCP Science; CODE Jr09050267
Electronic pipetting system: EDOS 5222 Eppendorf

2.3.1 Mini-photometer

A temperature-controlled flow through cell (d = 0.5 or 1 cm) was used.

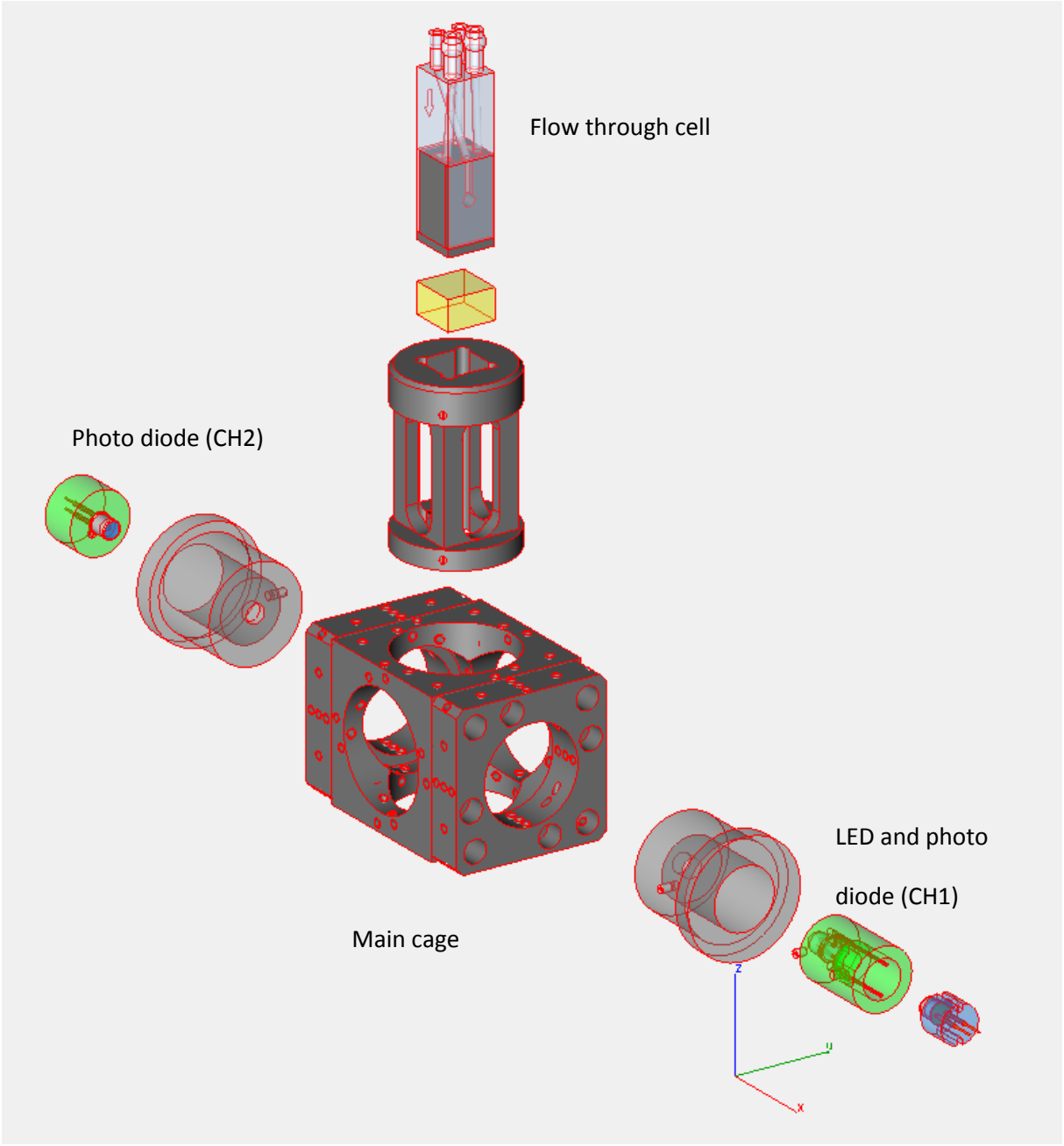

Figure 1. Explosion view of the mini photometer

The explosion view of the mini photometer can be observed in figure 1. The openings on the left and right of the main cage were light tight closed by two disks; Setup and rendering by Björn Ernecker

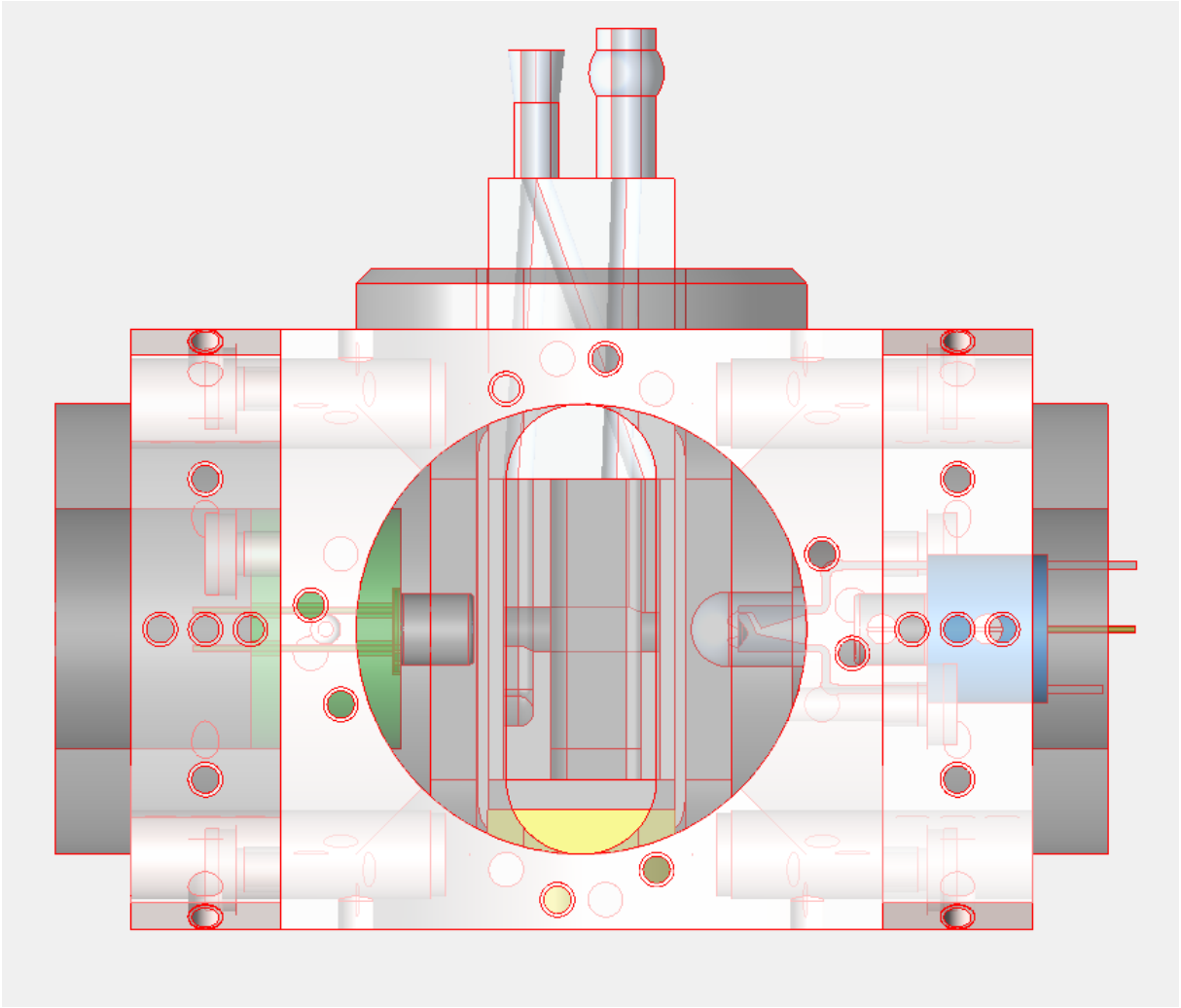

Figure 2. Optical arrangement showing the geometry of the mini photometer

The optical arrangement of the mini photometer is depicted in figure 2, showing its geometry. Note, that a band pass filter was inserted after the LED; Rendering by Björn Ernecker

2.4 Sample preparation

Materials used for the investigation of the sample digestion procedures:

- brown algae (interlaboratory test)
- BCR 151 (skimmed milk powder); IRMM
- Commercial milk powder (Aptamil)

Three different digestion procedures were used in this work:

- two step wet digestion using chloric acid
- two step wet digestion using chloric acid and nitric acid
- extraction with TMAH

2.4.1 Two step wet digestion in a graphite heating block using chloric acid

10-70 mg of organic sample was weight in a glass eprouvette (d_i = 1.4 cm, h = 10 cm). If higher sample amounts (70-300 mg) were needed, bigger glass vials had to be used (d_i = 2.8 cm, h = 20 cm). Then the sample was suspended in 0.5 ml of water. Appropriate amounts of chloric acid were added, depending on the sample amount. For instance 0.6 ml of $HClO_3$ was added for a sample weight of 10 mg and 4.2 ml of $HClO_3$ was used for 70 mg. The samples were put in a graphite heating block, which was held at a temperature of 90 °C. The following temperature/time program was used:

ramp from 90-140 °C within 30 minutes
hold at 140 °C until the solution turns colourless
add the same amount of chloric acid as before
ramp from 140-170 °C within 30 minutes
hold at 170 °C until the solution turned colourless and the sample volume was below 1 ml.

The complete removal of chloric acid and the efficient destruction of all organic matter are indicated by the occurrence of white percloric acid fumes.

The digestion residue was dissolved in water in order to yield 10 ml of sample solution. For samples with low iodine content (< 2 µg/g) the final volume was reduced to be 5 ml. The solution was mixed well and transferred to a PS vial. The vial was sealed with a push cap.

2.4.2 Two step wet digestion in a graphite heating block using $HClO_3$ and HNO_3

10-70 mg of organic sample was weight in a glass eprouvette (d_i = 1.4 cm, h = 10 cm). If higher sample amounts (70-300 mg) were needed, bigger glass vials had to be used (d_i = 2.8 cm, h = 20 cm). Then the sample was suspended in 0.5 ml of water. Appropriate amounts of chloric acid were added, depending on the sample amount. For instance 0.6 ml of $HClO_3$ was added for a sample weight of 10 mg and 10.8 ml of $HClO_3$ was used for 300 mg. The amount of HNO_3 (0.5 ml) remained constant for all sample amounts. The samples were put in a graphite heating block, which was held at a temperature of 90 °C.

The following temperature/time program was used:

ramp from 90-140 °C within 30 minutes
hold at 140 °C until the solution turns colourless
add the same amount of chloric acid as before
ramp from 140-170 °C within 30 minutes
hold at 170 °C until the solution turned colourless and the sample volume was below 1 ml

The solution should not evaporate to dryness, since the determination would not be reproducible anymore. Again, white fumes of perchloric acid indicated the completeness of the digestion step.

It is important to note that the digestion of 300 mg sample was rather harsh, with the formation of foam at a temperature of 95-100 °C. Consequently the sample was sticking to the glass wall and so it was not possible to fully digest the organic matrix and losses of iodine occurred. Therefore the samples were held at 90 °C for 10 minutes to avoid any losses of iodine.

The residual was dissolved in water in order to yield 10 ml of sample solution. The solution was mixed well and transferred to a PS vial that had been sealed with a push cap. 1 ml of this solution was used for the catalytic measurement.

2.4.3 Extraction using TMAH

250 mg of organic sample was weight in a 10 ml PP vial and dissolved in 5 ml water. Then 1 ml of TMAH (25 %) was added and the vial was sealed with a PE cap. The sample was put in a drying oven at 93 °C for 3 hours. Then the sample solution was transferred to a 50 ml PP vial and made up to 25 ml with water. 2 ml of the sample were filtered through a 0.45 µm syringe filter into a PS vial. After adding 0.04 ml of internal standard the solution was made up to 4 ml with water. This solution was ready for measuring by means of ICPMS.

2.5 Measurement

2.5.1 ICPMS

Calibration solutions were prepared by diluting the KIO_3 standard solution (100 µg I/l.). The iodine content of the calibration solutions was between 0.2 and 5 µg I/l. The required volume of KIO_3 standard solution was transferred to a PS vial and 0.1 ml of internal standard was added. The solution was made up to 10 ml with TMAH (0.5 w%). The PS vial was sealed with a push cap.

Table 1 shows the compositions of the standard solutions:

Table 1. ICPMS – TMAH digestion calibration solutions volumes

	KIO_3 [1,000 µg I/l]		Te [1 mg/l]	TMAH [0.5 w%]
	µg I/L	ml	ml	ml
Blank	0.0	0.00	0.01	9.90
Standard_1	0.2	0.02	0.01	9.88
Standard_2	0.5	0.05	0.01	9.85
Standard_3	1.0	0.10	0.01	9.80
Standard_4	2.0	0.20	0.01	9.70
Standard_5	5.0	0.50	0.01	9.40

Tellur was used as internal standard whereby m/z Te^{126} was selected. Te^{128} can be used as well if high purity argon is available – there is an isobaric interference with ^{128}Xe. H_2O was used as rinsing solution.

The same procedure was applied to samples from the acid digestion process. The only differences were in using H_2O instead of TMAH for diluting the solutions and extending the calibration range to 20 µg I/l.

Table 2 lists the composition of the standards:

Table 2. ICPMS – acid digestion calibration solutions volumes

	KIO_3 [1,000 µg I/l]			
	c [µg I/l]	V [ml]	Te [1 mg/l]	HNO_3 [5ml/l]
Blank	0	0.00	0.10	9.90
Std_1	2	0.02	0.10	9.88
Std_2	5	0.05	0.10	9.85
Std_3	10	0.10	0.10	9.80
Std_4	15	0.15	0.10	9.75
Std_5	20	0.20	0.10	9.70

2.5.2 UV-VIS spectroscopy by means of Sandell-Kolthoff - Cary spectrophotometer

Iodate calibration solutions were prepared by diluting the iodate standard solution. Their concentrations ranged from 2-20 µg I/l. A second calibration curve with 20-200 µg I/l was used if higher iodine concentrations had to be measured.

Calibration solutions were digested following the same procedure as described at wet digestion with chloric acid and nitric acid. It was important to digest the calibration solutions as well, since the acid matrix had a strong impact on the catalytic reaction, too.

Calibration solutions and samples for measurement were prepared according to the following scheme depicted in table 3:

Table 3. Agilent Cary calibration solutions volumes

	KIO_3 [1,000 µg I/l]					
	c [µg I/L]	Vol [ml] I stock solution	H_2O [ml]	H_2SO_4 [ml] conc.	As [ml] stock solution	Ce [ml] stock solution
Blank	0	0	6.95	1.3	1.0	0.75
Std_1	20	0.2	6.75	1.3	1.0	0.75
Std_2	50	0.5	6.45	1.3	1.0	0.75
Std_3	100	1.0	5.95	1.3	1.0	0.75
Std_4	150	1.5	5.45	1.3	1.0	0.75
Std_5	200	2.0	4.95	1.3	1.0	0.75

Water was transferred to a PS vial and 1.3 ml of H_2SO_4 conc. was added carefully. The vial was sealed with a push cap and the solution was mixed well. Until this step all the solutions can be prepared in advance. Since temperature has a huge influence on the catalytic reaction, the solutions had to be thermalized at 20 °C.

Next 1 ml of arsenious acid stock solution, 0.75 ml of cerium stock solution and 1 ml of calibration or sample solution were added. The PS vial was sealed with the push cap, shaken for three times and 2 ml of the solution were transferred to the cuvette (d = 0.5 cm). The samples were measured at a wavelength of 317 nm for 2 minutes.

To calculate the iodine concentration the logarithm is applied to the extinction exported from the instrument software and plotted versus the time. The resulting slope of the first minute is direct proportional to the iodine concentration.

2.5.3 UV-VIS spectroscopy by means of Sandell-Kolthoff - Mini photometer

The calibration and sample solutions were prepared according to the following scheme in table 4:

Table 4. Mini calibration solutions volumes

	KIO_3 [1,000 µg I/l]					
	c [µg I/L]	Vol [ml] I stock solution	H_2O [ml]	H_2SO_4 [ml] conc.	As [ml] stock solution	Ce [ml] stock solution
Blank	0	0	7.83	1.3	0.5	0.375
Std_1	20	0.2	7.63	1.3	0.5	0.375
Std_2	50	0.5	7.33	1.3	0.5	0.375
Std_3	100	1.0	6.83	1.3	0.5	0.375
Std_4	150	1.5	6.33	1.3	0.5	0.375
Std_5	200	2.0	5.83	1.3	0.5	0.375

After mixing, the samples were pumped into the temperature controlled flow through cell with an optical path length of d = 1 cm by a peristaltic pump. It was important to wait for 40 s from the point where the sample was pumped into the cuvette to starting the measurement. This was necessary in order to allow air bubbles to settle down. The reaction was monitored at a wavelength of 317 nm for 1 min. The system was rinsed with H_2O for 1 minute, between each sample. The values read out from the detector are transmission data. They have to be divided by a constant value, obtained by measuring H_2O. Next a double logarithm is applied to the inverse of the transmission and plotted against the time. The resulting slope of the first 30 seconds was used for calculation and therefore direct proportional to the iodine concentration. For iodine contents higher than 20 µg/l, the pumping time should not be longer than 40 seconds, because the reaction would be over before it can be monitored. The pump tube (Tygon R3607, color code red/red) used had an inner diameter of 1.14 mm and a wall diameter of 0.86 mm. The pumping speed has to be 30 rpm or lower, to avoid air bubbles inside the cuvette.

3 RESULTS AND DISCUSSION

3.1 Extraction using TMAH

For initial digestion experiments, the use of certified reference materials (CRMs) seems wasteful. Therefore, a commercial milk powder was bought in a shop in Graz and analysed for its iodine content using a well-established method. Following ÖNORM EN 15111 the milk powder was characterized by extracting it with TMAH and measurement by ICPMS. The extraction procedure was described in the section 'Experimental'.

The samples were measured by ICPMS and H_2O was used as rinsing solution.

Table 5. characterization milk powder, ICPMS

sample	I [µg/g]
1	0.93
2	1.13
3	1.02
4	0.94
5	1.11
average	1.03
SD	0.09

According to table 5 the milk powder showed iodine content of 1.03 ± 0.09 µg/g. This value was taken for all calculations concerning milk powder digestion. Blank values were in the range of 0.30 $\pm$ 0.21 µg/l on average.

3.2 $HClO_3$ one step digestion procedure

3.2.1 Initial experiments and stability of the iodide stock solution

In initial experiments conducted before the start of this thesis problems with the chloric acid digestion of organic matter were encountered: The results of Sandell-Kolthoff-based quantifications were unreproducible for digests but showed the expected linear correlation of iodine concentration with time for synthetic samples. Consequently, initial experiments on the optimization of the digestion procedure were deemed necessary. To avoid potential bias from the photometric quantification approach, ICP-MS was used for iodine quantification.

Milk powder and water were spiked with KI and digested. The aim was to examine if during the digestion process iodine had been lost.

digestion procedure:

milk powder_100mg_1-2: 0.1 g of milk powder was spiked with KI to yield 10 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std)

water_1-2: 0.5 ml H_2O was spiked with the same amount of KI

digestion vessel: digestion tube

digestion agent: 0.6 ml of $HClO_3$ added

T/t-programme: hold at 150 °C for 10 minutes

digestion residue made up to 10 ml with ICPMS rinsing solution

measurement technique: ICPMS, rinsing with ICPMS rinsing solution

standard concentrations: 0, 2, 5, 10, 15, 20 µg I/l prepared with ICPMS rinsing solution

samples: 0.1 ml made up to 10 ml with ICPMS rinsing solution

The calibration curve is displayed in figure 3. LOD was 0.09 µg/l.

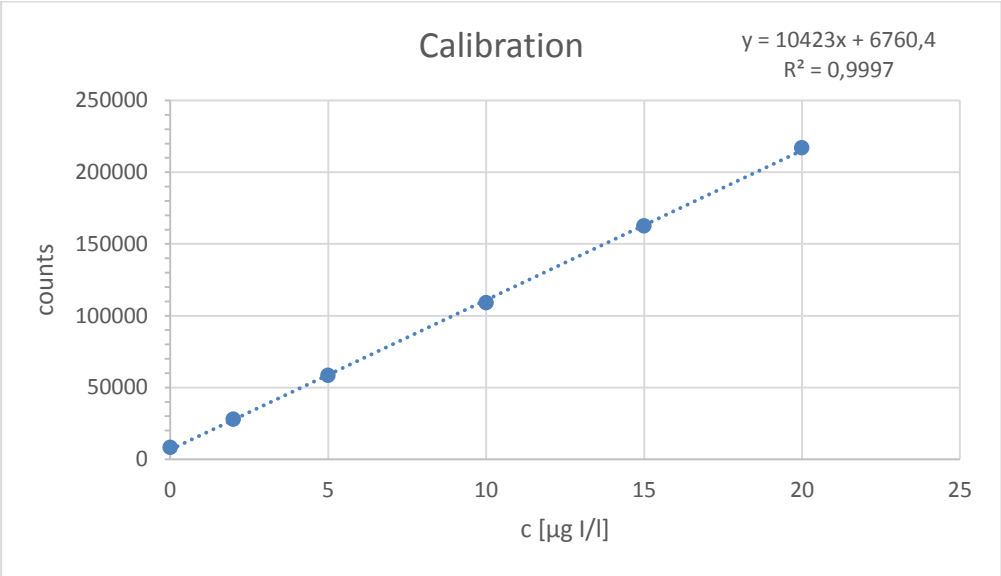

Figure 3. calibration curve iodine, ICPMS

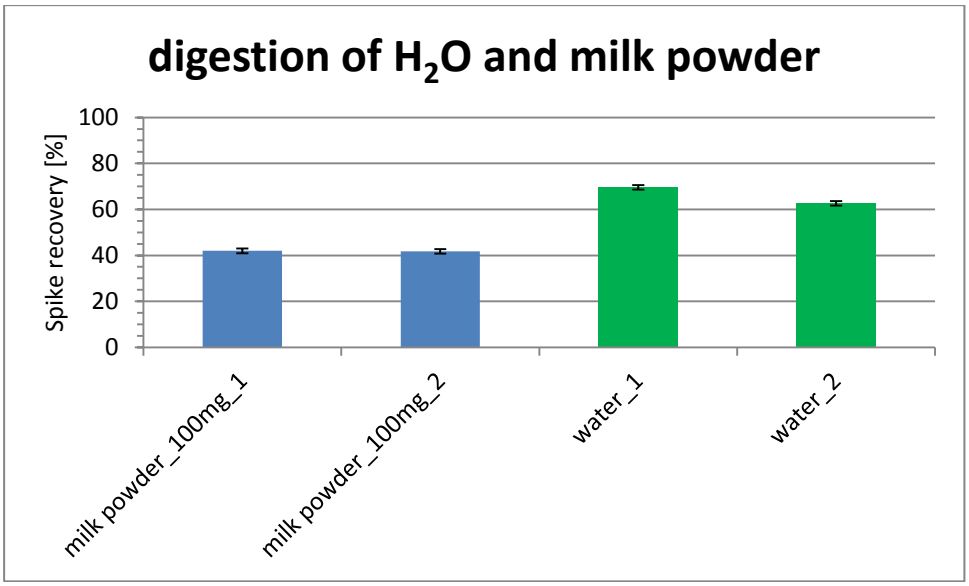

Figure 4. results digestion of milk powder and H_2O, ICPMS

As it can be seen in figure 4 the spiking experiment was not successful. The spiked milk powder samples showed a recovery of only 41 % whereas in pure water the spike recovery was higher with 62-69 %. Losses of iodine may have occurred due to incomplete digestion and the associated volatilization of iodine. Milk powder samples were not even clear and remaining particles had to be filtrated through a syringe filter.

The graphite block took about 30 minutes to heat up to 150 °C, so quick heating was not an option. The next experiment focussed on optimizing the heating procedure of the graphite block and the used digestion vessels.

Milk powder and water were again spiked with KI and iodine was again quantified by ICPMS after the digestion. Glass vials (VWR clean pack; 04123255; d_i = 0.9 cm, h = 4.4 cm) and digestion tubes (Schott Duran; d_i = 1.2 cm, h = 7.5 cm) were compared in order to examine the influence of the digestion vessel geometry.

digestion procedure:
Milk powder_tube_1-3: 0.05 g of milk powder was spiked with KI to yield 10 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: digestion tube; T/t-programme: ramp from 80-120 °C within 30 minutes
Milk powder_glass vial_4-6: 0.05 g of milk powder was spiked with KI to yield 10 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: glass vial; T/t-programme: ramp from 80-120 °C within 30 minutes and then from 120-140 °C in 30 minutes
water_1-4: 0.5 ml H_2O was spiked with the same amount of KI, digestion vessel: digestion tube; T/t-programme: ramp from 80-120 °C within 30 minutes

digestion agent: 0.6 ml of $HClO_3$ added for each sample

digestion residue made up to 10 ml with ICPMS rinsing solution

measurement technique: ICPMS, rinsing with ICPMS rinsing solution

standard concentrations: 0, 2, 5, 10, 15, 20 µg I/l prepared with ICPMS rinsing solution

samples: 0.1 ml made up to 10 ml with ICPMS rinsing solution

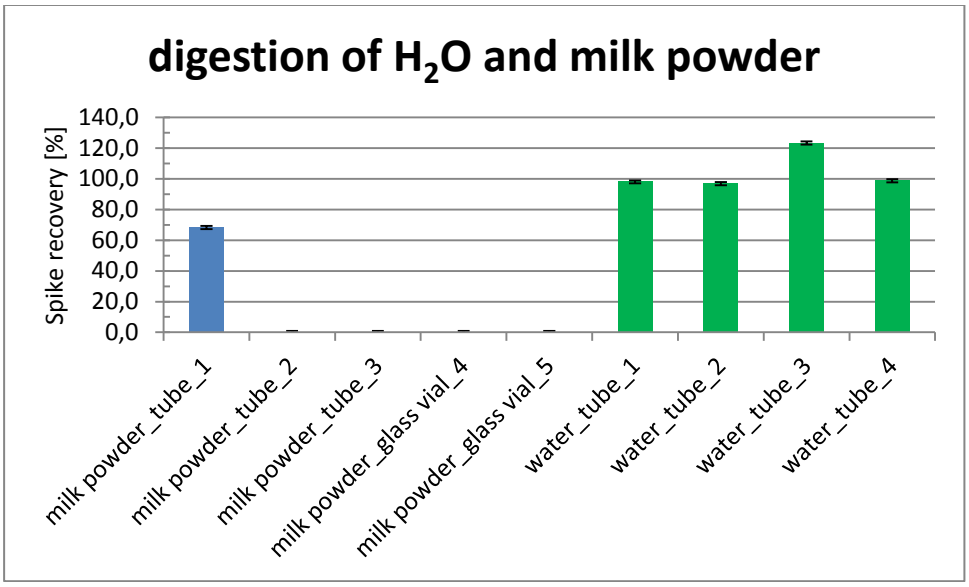

Figure 5. results digestion, digestion tube vs. glass vial, ICPMS, results of iodide were corrected for apparent losses of iodine during storage of the sample solution

As figure 5 shows the recoveries were good with 104.2 $\pm$ 12.8 % for the water samples. This is a clear indication of the deleterious effect of organic matter. LOD was 0.30 µg/l. For milk powder allow results were encountered. The samples milk powder_tube_1-3 were digested by using the digestion tubes which led to irreproducible results, though in one experiment the spike recovery was about 70 %. The other samples experienced excessive splashing due to superheating. By using the digestion tubes the digestion took longer since the acid condensates on the glass wall and flows back into the heated solution reservoir. This visually helped to better digest the samples without milk powder. Samples milk powder_glass vial_4-5 were digested by using glass vials. These vials were of low height and therefore the $HClO_3$ evaporated too fast to fully digest the samples. The iodide standard used for spiking was measured by ICP-MS as well to obtain the true concentration, as iodide is rather instable. Surprisingly, of the expected 10 µg/l only 4.5 µg/l were found. Further experiments focussed on choosing a suitable digestion vessel and evaluating the reason for the low concentration of the KI standard.

Consequently, a fresh KI standard was prepared and the above described experiment was repeated.

digestion procedure:

Milk powder_1-4: 0.05 g of milk powder was spiked with KI to yield 10 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 80-120 °C within 30 minutes

water_1-4: 0.5 ml H_2O was spiked with the same amount of KI; digestion vessel: eprouvette; T/t-programme: ramp from 80-120 °C within 30 minutes

digestion agent: 0.6 ml of $HClO_3$ added for each sample

digestion residue made up to 10 ml with ICPMS rinsing solution

measurement technique: ICPMS, rinsing with ICPMS rinsing solution

standard concentrations: 0, 2, 5, 10, 15, 20 µg I/l prepared with ICPMS rinsing solution

samples: 0.1 ml made up to 10 ml with ICPMS rinsing solution

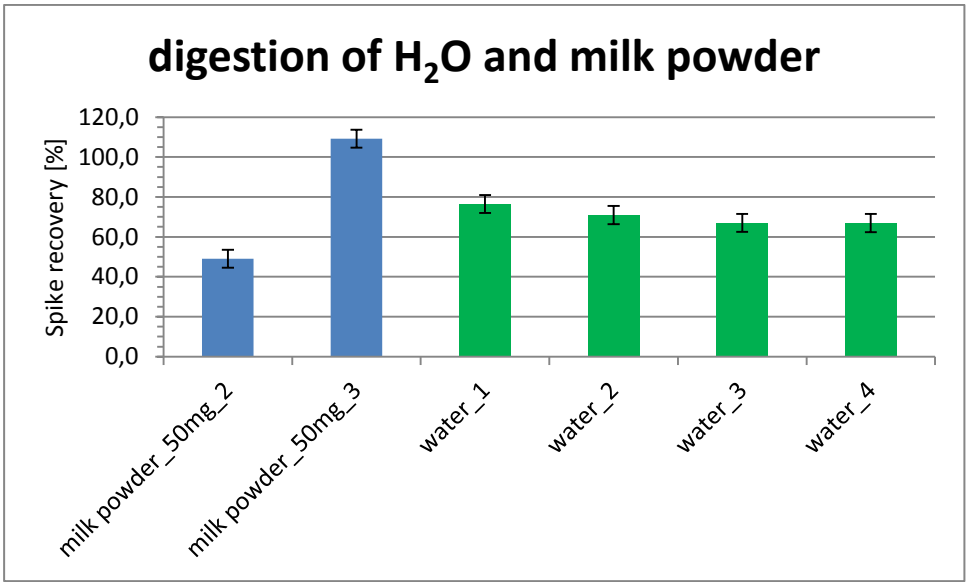

Figure 6. results digestion of milk powder and H_2O, ICPMS, results of iodide were corrected for apparent losses of iodine during storage of the sample solution

As it can be seen in figure 6 the recoveries improved, if eprouvettes had been used. The freshly prepared KI standard was found to contain 9.58 $\pm$ 0.23 µg/l of iodine which approached 10 µg/l. Additional attempts were made to stabilize the KI standard with ascorbic acid but this resulted in a concentration found by ICP-MS of 5 µg/l. This approach was not further pursued. Due to the fact that the recoveries were still 25-30 % below the target value, iodine losses may have occurred due to issues with matrix digestion. Further experiments focussed on examining the stability of the KI standard. It is important to note, that iodate was used for calibrating the ICP-MS.

H_2O was spiked with iodide or iodate, digested on the heating block and measured by ICPMS. In general, iodate is stable and should not be lost during digestion. By spiking with iodide, losses could have occurred if volatile iodine had been formed. The goal was to examine if iodine had been lost during the digestion.

digestion procedure:

Blanks_1-4: 0.2 ml H_2O was digested; digestion vessel: eprouvette;

T/t-programme: ramp from 80-120 °C within 30 minutes

Iodate_1-4: 0.2 ml H_2O was spiked with KIO_3 to yield 10 µg I/l (0.1 ml out of 100,000 µg I/l KIO3-Std); digestion vessel: eprouvette; T/t-programme: ramp from 80-120 °C within 30 minutes

Iodide_1-4: 0.2 ml H_2O was spiked with KI to yield 10 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 80-120 °C within 30 minutes

digestion agent: 0.6 ml of $HClO_3$ added for each sample

digestion residue made up to 10 ml with ICPMS rinsing solution

measurement technique: ICPMS, rinsing with ICPMS rinsing solution

standard concentrations: 0, 2, 5, 10, 15, 20 µg I/l prepared with ICPMS rinsing solution

samples: 0.1 ml made up to 10 ml with ICPMS rinsing solution

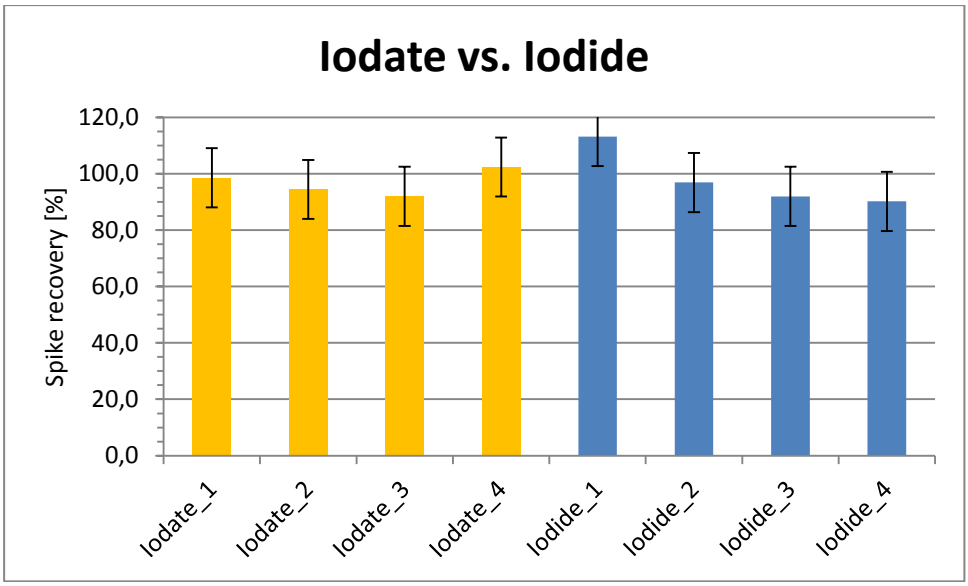

Figure 7. results digestion, iodate or iodide spiking, ICPMS; results of iodide were corrected for apparent losses of iodine during storage of the sample solution

Figure 7 shows that spiking with iodate gave spike recoveries of 96.8 $\pm$ 4.6 % and spiking with iodide gave 98.0 $\pm$ 10.5 %. This showed that the digestion worked and no iodine had been lost during the process. Since the KI standard showed only a concentration of 6.36 µg/l it was concluded, that the iodide standard is not stable during storage. Blanks displayed a value of 0.54 $\pm$ 0.14 µg/l on average. LOD was 0.59 µg/l.

Clearly, the iodide stock solution is not stable at room temperature and therefore this solution must be prepared freshly prior use. Cold storage in a fried was considered a possible approach for stabilizing the iodide solution. Iodate and iodide standards (10 µg I/l) were prepared in water and ICPMS rinsing solution (0.1 % v/v HNO_3). Furthermore one series was made freshly on the day of measurement and the other series was prepared the day before and put into the fridge overnight.

The goal was to examine if there were differences between storing in the fridge and preparing freshly. The second aim was to show if diluting with H_2O or HNO_3 had influenced the results. Iodate and iodine were compared as well.

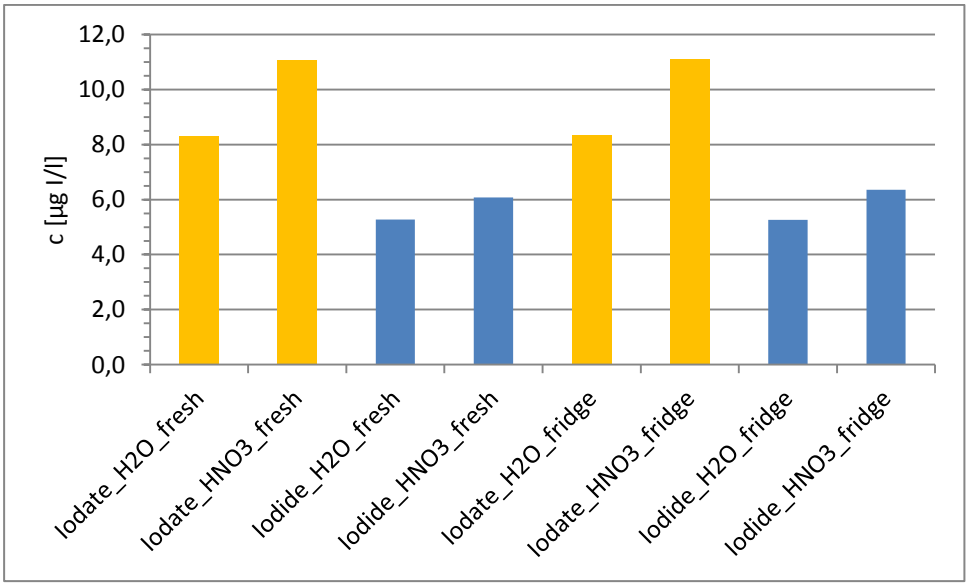

Figure 8. results standard preparation fresh vs. fridge and HNO_3 vs. H_2O, ICPMS, each bar represents only a single measurement

Figure 8 shows the results of the different preparation methods for iodate and iodide. First of all iodide standards had always a lower concentration than iodate. For iodide there was no difference between freshly prepared and storing in the fridge. Slightly better results could be achieved by preparing the standards in diluted HNO_3 (ICPMS rinsing solution) in comparison to H_2O. As it can be seen in figure 8 the iodate standards reached the target value when prepared with HNO_3. There was also no difference between storing in the fridge and preparing them freshly. Further experiments focussed on examining the stability of the KI standard.

From the foregoing experiments it can be concluded, that iodide stock solutions are rather instable, should be prepared freshly and the concentration of iodine has to be determined prior use. It is important to note, that iodide stock solutions are only needed for spiking experiments. Once the iodide has been converted to iodate by $HClO_3$, analyte stability is not troublesome anymore.

Based on these results, the digestion experiments could be further investigated. Water was spiked with iodide and digested afterwards. Iodine was quantified thereafter by ICPMS. The goal was to examine if iodine had been lost during the digestion.

digestion procedure:

Blanks_1-4: 0.2 ml H_2O was digested; digestion vessel: eprouvette;

T/t-programme: ramp from 80-120 °C within 30 minutes

Iodide_1-4: 0.2 ml H_2O was spiked with KI to yield 10 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 80-120 °C within 30 minutes

digestion agent: 0.6 ml of $HClO_3$ added for each sample

digestion residue made up to 10 ml with ICPMS rinsing solution

measurement technique: ICPMS, rinsing with ICPMS rinsing solution

standard concentrations: 0, 2, 5, 10, 15, 20 µg I/l prepared with ICPMS rinsing solution

samples: 0.1 ml made up to 10 ml with ICPMS rinsing solution

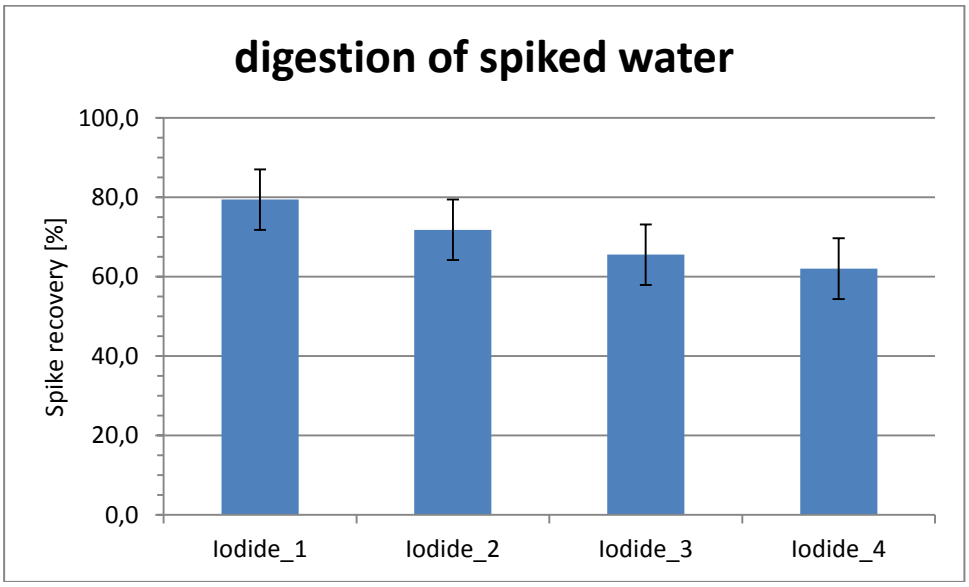

Figure 9. results digestion of spiked water using eprouvettes, ICPMS, results of iodide were corrected for apparent losses of iodine during storage of the sample solution

As it can be seen in figure 9 spiking with iodide gave recoveries of 69.7 $\pm$ 7.6 %. The difference to figure 5 lies in the use of eprouvettes as digestion vessels. This shows that iodine had been lost during the digestion. KI standard showed still a concentration of 6.5 µg/l. Blanks displayed a value of 0.59 $\pm$ 0.30 µg/l on average. LOD was 0.24 µg/l.

Another series of samples was prepared without digestion. The stock solution was diluted until the concentration of the measurement solutions was reached. The same was done for the second dilution. The old standard for spiking and a freshly prepared were measured as well. Iodide and Iodate standards were prepared again in water and HNO_3. Water and diluted HNO_3 where measured as blanks as well.

The first goal was to examine the stability of iodine during dilution. The second aim was to show if there are differences between old and freshly prepared standards.

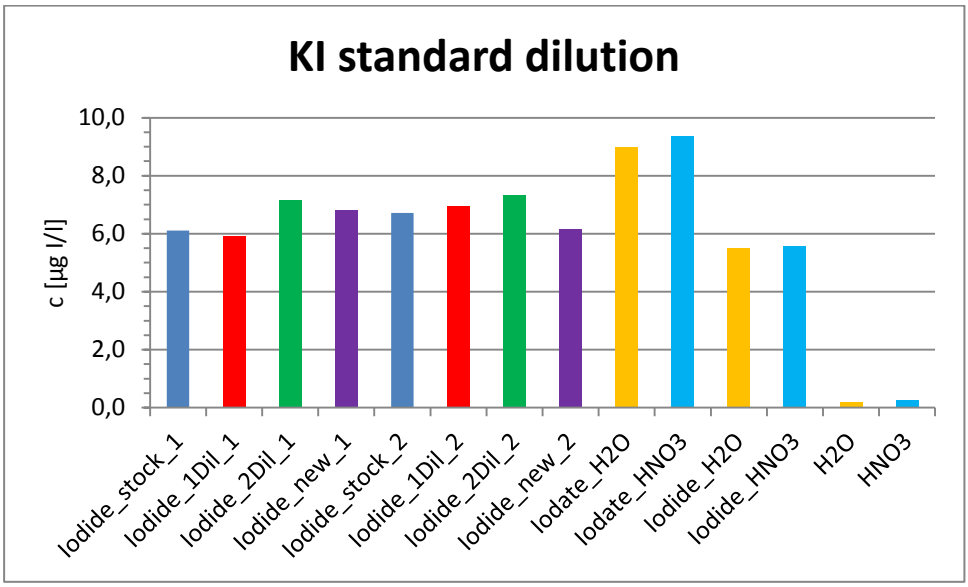

Figure 10. results standard dilution, ICPMS, each bar represents only a single measurement

Figure 10 shows that there was little difference between the iodine standards. All the standards displayed concentrations between 6 and 7 µg/l regardless of being prepared freshly or diluted from the old stock solution that was stored in a fridge for 10 days. Iodate showed higher concentrations (> 9 µg/l) than iodide when prepared in both H_2O and HNO_3. Blank values were low for H_2O and HNO_3. Neither for iodate nor for iodide a difference was encountered between preparing the standards in 0.1 % (v/v) HNO_3 and H_2O. Further experiments focussed on modifying the digestion method and examining the KI standard stability.

A new iodide standard was prepared from 1.3081 g of KI. Then 2 ml of TMAH were added and made up to 100 ml with water. The first and the second dilution were prepared as usual but also with adding 2 ml of TMAH before making up with water. Samples were measured by ICPMS.

digestion procedure:
Blanks_1-4: 0.2 ml H_2O was digested; digestion vessel: eprouvette;
T/t-programme: ramp from 80-120 °C within 30 minutes
Iodide_1-4: 0.2 ml H_2O was spiked with KI to yield 10 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 80-120 °C within 30 minutes
Iodide_1-4_HT: 0.2 ml H_2O was spiked with KI to yield 10 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 110-160 °C within 30 minutes
digestion agent: 0.6 ml of $HClO_3$ added for each sample
digestion residue made up to 10 ml with ICPMS rinsing solution

measurement technique: ICPMS, rinsing with ICPMS rinsing solution
standard concentrations: 0, 2, 5, 10, 15, 20 µg I/l prepared with ICPMS rinsing solution
samples: 0.1 ml made up to 10 ml with ICPMS rinsing solution

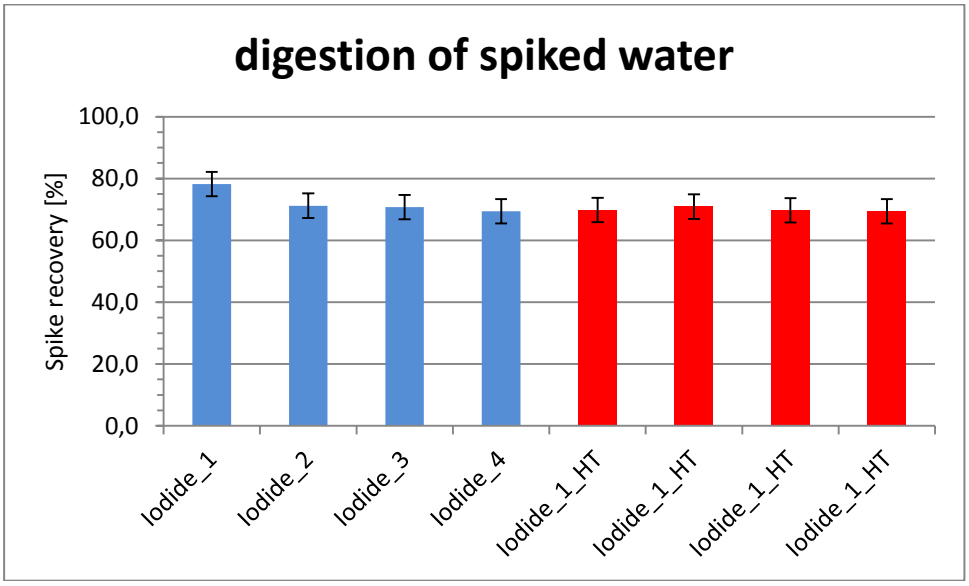

Figure 11. results digestion with TMAH stabilized standard, ICPMS; HT: heating to 160°C instead of 120°C

As figure 11 shows the recoveries were better than in figure 5. There was no difference between heating up to 120 or 160 °C. The concentration of the KI standard was still low with 6.2 + 0.5 µg/l. This showed that the attempt to stabilize the standard with TMAH was not successful. Blanks displayed a value of 0.53 $\pm$ 0.23 µg/l on average. Based on these results the preparation procedure of the iodide stock solution was questioned. As KI is hygroscopic, a bias might be introduced from water bound to the salt. Though it was considered unlikely that the 40 – 50 % deviation between expected iodine concentration and the determined one were solely based on insufficiently dried salt a comparison between NaI and KI was conducted.

A new iodide standard was prepared by from 1.3081 g of KI and making up with water. The solution was diluted as described in the experimental section. In addition a similar standard was prepared by weighing out 1.1817 g of NaI sodiumiodide. Samples were measured by ICPMS.

digestion procedure:
Blanks_1-4: 0.2 ml H_2O was digested; digestion vessel: eprouvette;
T/t-programme: ramp from 110-170 °C within 30 minutes
KI_1-4: 0.2 ml H_2O was spiked with KI to yield 10 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 110-170 °C within 30 minutes
NaI_1-4: 0.2 ml H_2O was spiked with KI to yield 10 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 110-170 °C within 30 minutes

digestion agent: 0.6 ml of $HClO_3$ added for each sample
digestion residue made up to 10 ml with ICPMS rinsing solution
measurement technique: ICPMS, rinsing with ICPMS rinsing solution
standard concentrations: 0, 2, 5, 10, 15, 20 µg I/l prepared with ICPMS rinsing solution
samples: 0.1 ml made up to 10 ml with ICPMS rinsing solution

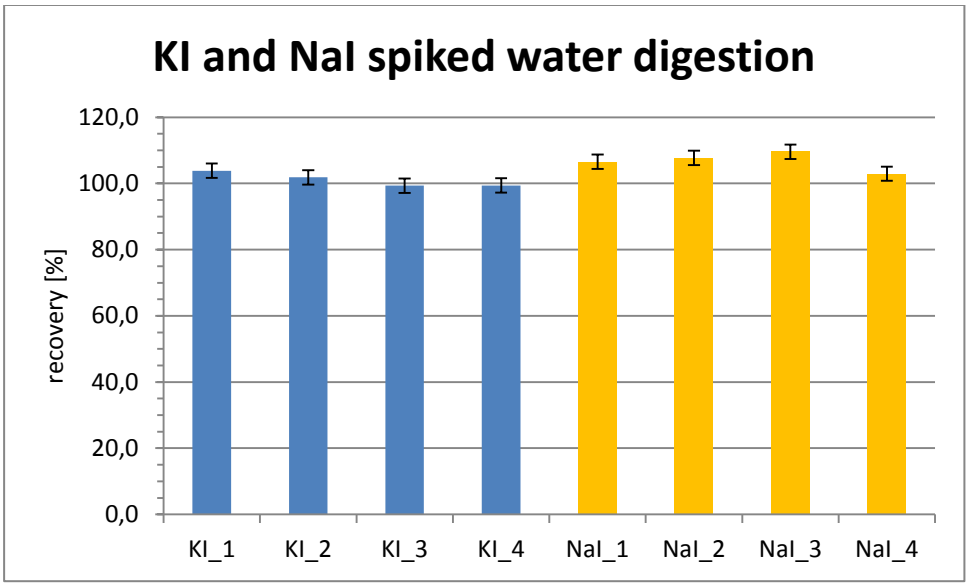

Figure 12. results digestion, KI vs. NaI spike, ICPMS

As it can be seen in figure 12 the recoveries of both, the KI and the NaI stock solution were about 100 % after digestion when corrected for the concentration of iodine determined in the respective stock solution as determined by ICP-MS. Nevertheless KI and NaI standards had an iodine concentration of 5.0 and 4.7 µg/l respectively, instead of the 10 µg/l. Again, this showed that both solutions were not stable and that an effect of water absorbed on the salts is unlikely. Blanks displayed a value of 0.41 $\pm$ 0.16 µg/l on average. LOD was 0.24 µg/l. Having established the low stability of iodide stock solutions, further experiments focussed on digesting milk powder.

A new iodide standard was prepared from 1.3081 g of KI and making up with water. The solution was diluted as before. Samples were measured by ICPMS. As in previous experiments, milk powder was digested with and without spiking with iodate. In order to exclude random findings, the experiment was conducted with 6 digestions in parallel.

digestion procedure:
Blanks_1-12: 0.2 ml H_2O was digested; digestion vessel: eprouvette;
T/t-programme: ramp from 110-170 °C within 30 minutes
Milk powder_50 mg_1-6: 0.05 g of milk powder, 0.2 ml of water added, digestion vessel: eprouvette;
T/t-programme: ramp from 80-120 °C within 30 minutes

MPI_1-6: 0.05 g of milk powder, 0.2 ml H_2O added, spiked with KI to yield 10 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 80-120 °C within 30 minutes

digestion agent: 0.6 ml of $HClO_3$ added for each sample

digestion residue made up to 10 ml with ICPMS rinsing solution

measurement technique: ICPMS, rinsing with ICPMS rinsing solution

standard concentrations: 0, 2, 5, 10, 15, 20 µg I/l prepared with ICPMS rinsing solution

samples: 0.1 ml made up to 10 ml with ICPMS rinsing solution

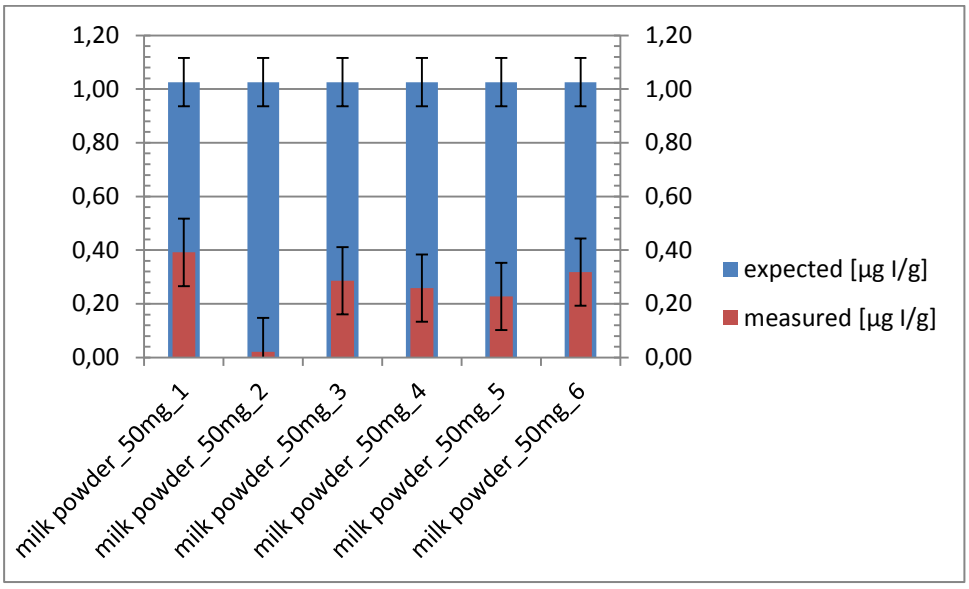

Figure 13. results digestion of milk powder, ICPMS

As it can be seen in figure 13 the digestion of milk powder showed 0.25 ± 0.12 µg I/g. That was lower than the expected value of 1.03 ± 0.09 µg I/g as examined in the TMAH experiment. It was not possible to fully digest the samples. There were no results for spiked milk powder, since only negative recoveries had been obtained. KI standard was measured without digestion and had a value of 5.4 ± 0.2 µg/l. Blanks displayed a value of 0.13 ± 0.03 µg/l on average. LOD was 0.28 µg/l. Due to the sill large scatter in the data, the ICP-MS method used for iodine quantification was re-evaluated. It is well known, that iodine can cause tailing in the sample introduction system. The sample tubes used during ICP-MS analysis were considered another source of potential quantification problems, as iodine might diffuse into the polymer.

Consequently, ten replications of a freshly prepared iodide solution were analysed in a row on the ICP-MS: The auto sampler took samples 10 times from the same vial and 10 times from 10 different vials.

A new iodide standard was prepared from 1.3087 g of KI and making up with water. The solution was diluted as always.

Blank_1-20: water prepared in *one* 50 ml PE-vial

Iodide_1-10: water, spiked with KI to yield 10 µg I/l (0.5 ml out of 1,000 µg I/l KI-Std), prepared in *one* 50 ml PE-vial, all 10 samples were measured from this vial

Iodide_1-10: water, spiked with KI to yield 10 µg I/l (0.1 ml out of 1,000 µg I/l KI-Std), prepared in 10 *single* PS-vials

measurement technique: ICPMS, rinsing with H_2O

standard concentrations: 0, 2, 5, 10, 15, 20 µg I/l prepared with H_2O

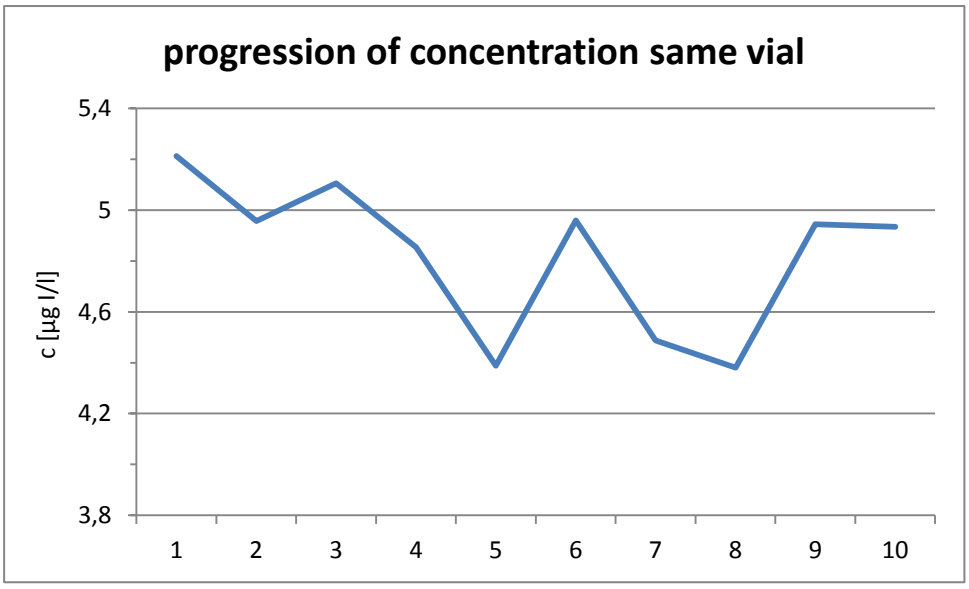

Figure 14. progression of concentration, same vial, ICPMS

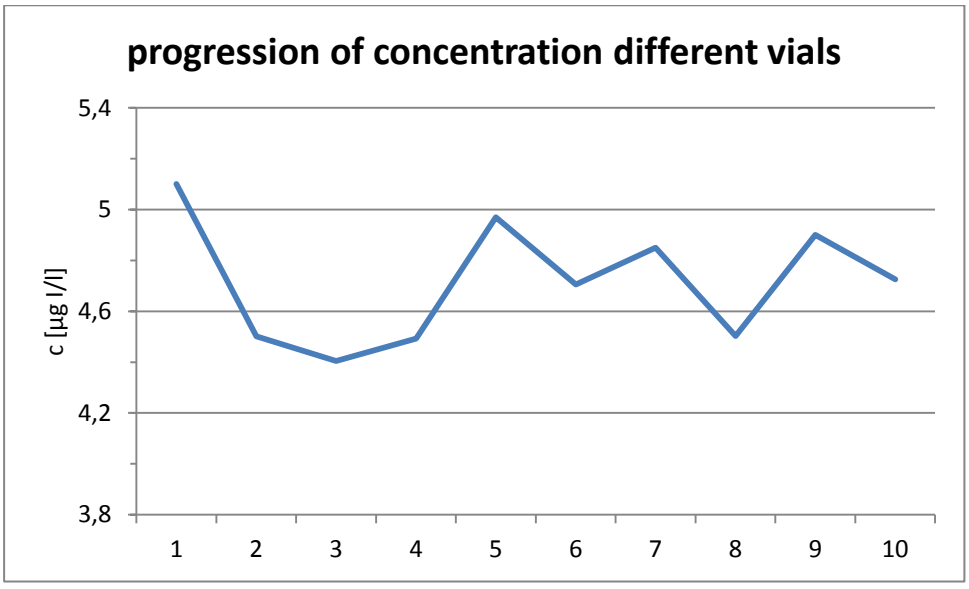

Figure 15. progression of concentration, different vials, ICPMS

As it can be seen in figure 14 and 15 there was no difference between measuring the samples from one vial or 10 different vials. This showed that the vial material is not the reason for the large deviations in the data of the previous experiments. However, the iodine concentrations were still too low with 4-5 µg/l instead of the target value of 10 µg/l. The repeatability of the iodine measurement was about 10 % for the 20 samples measured. This value was considered acceptable for this work and also doesn't explain the previously observed large deviations between individual measurements of digestion. Consequently, they must be attributed to the digestion procedure and the deleterious effect of the carbon based sample matrix. Blanks displayed a value of 0.32 $\pm$ 0.12 µg/l on average. LOD was 0.18 µg/l. Further experiments focussed on the digestion of the milk powder.

3.2.2 Effect of sample mass

Different sample weights were spiked with the same amount of KI standard. 0.6 ml of $HClO_3$ was used for all sample weights. Iodine quantification was performed by ICPMS. Calibration standards and samples were prepared in H_2O instead of ICPMS rinsing solution. Rinsing solution for ICPMS was also changed to H_2O. This was done in respect of the measurements with Sandell-Kolthoff where HNO_3 shows deleterious absorption in the used wavelength range.

digestion procedure:
Blanks_1-5: 0.2 ml H_2O was digested; digestion vessel: eprouvette;
T/t-programme: ramp from 90-140 °C within 30 minutes
1_1-5: 0.01 g of milk powder, 0.2 ml H_2O added, spiked with KI to yield 10 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes
20, 30, 40 and 50 mg were treated in the same way as 1_1-5.
digestion agent: 0.6 ml of $HClO_3$ added for each sample
digestion residue made up to 10 ml with H_2O
measurement technique: ICPMS, rinsing with H_2O
standard concentrations: 0, 2, 5, 10, 15, 20 µg I/l prepared with H_2O
samples: 0.1 ml made up to 10 ml with H_2O

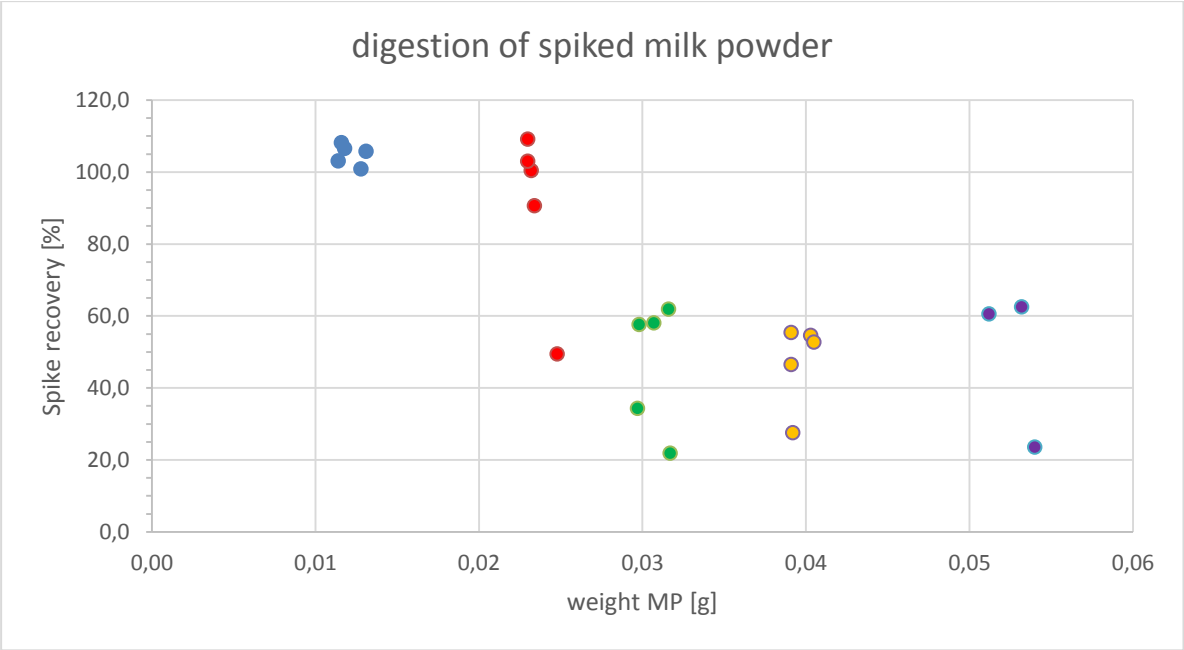

Figure 16. results digestion, $HClO_3$ constant, ICPMS

Figure 16 shows that the spike recoveries were about 100 % for small sample masses of 10 and 20 mg. For higher sample masses the recoveries were only at about 50 % and large SD's were encountered. The iodine concentration of the spiking solution was determined by ICP-MS, though surprisingly the KI standard had an iodine concentration of 8.98 $\pm$ 0.53 µg/l – much higher than previously encountered. Therefore, the volume of the digestion reagent must be adjusted to the sample mass used. Blanks displayed a value of 0.15 $\pm$ 0.11 µg/l on average. It is known that catalytically active impurities inside the glass flask act on the stability of iodide. Therefore only glass flasks designated for iodine analysis only were used for future experiments. LOD was 0.54 µg/l. The relationship between sample mass and acid volume was further investigated.

Milk powder was spiked with KI and the acid volume was adjusted to the sample weight. Iodine quantification was performed by ICPMS.

digestion procedure:
Blanks_1-5: 0.2 ml H_2O was digested; digestion vessel: eprouvette;
T/t-programme: ramp from 90-140 °C within 30 minutes
2_1-5: 0.02 g of milk powder, 0.2 ml H_2O added, spiked with KI to yield 10 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes
30 mg was treated the same way.

digestion agent: 1.2 ml of $HClO_3$ for 20 mg and 1.8 ml for 30 mg
digestion residue made up to 10 ml with H_2O
measurement technique: ICPMS, rinsing with H_2O
standard concentrations: 0, 2, 5, 10, 15, 20 µg I/l prepared with H_2O
samples: 0.1 ml made up to 10 ml with H_2O

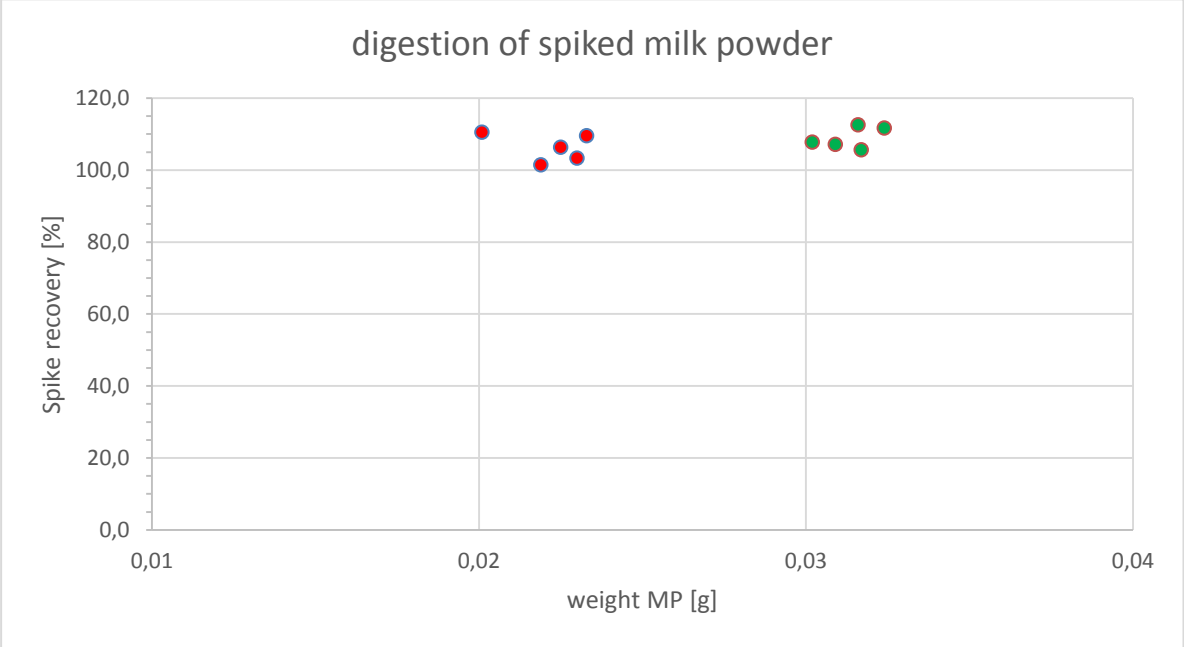

Figure 17. results digestion, $HClO_3$ adjusted, ICPMS

As figure 17 shows the recoveries were slightly higher than 100 % for 20 and 30 mg of sample weight. Clearly, the acid volume needs to be adjusted to the sample weight. Blanks displayed a value of 0.25 $\pm$ 0.20 µg/l on average. LOD was 0.32 µg/l. Further experiments focussed on the iodine determination following the method of Sandell and Kolthoff.

3.3 Initial experiments using the Sandell Kolthoff method

In a set of initial experiments, the Sandell Kolthoff reaction was used for the determination of iodine. Milk powder was spiked with KI and the acid volume was adjusted to the sample weight as noted above. Iodine concentration was determined spectrophotometrically using an Agilent Cary 50.

digestion procedure:

Blanks_1-5: 0.2 ml H_2O was digested; digestion vessel: eprouvette;

T/t-programme: ramp from 90-140 °C within 30 minutes

milk powder_20mg_1-5: 0.02 g of milk powder, 0.2 ml H_2O added, spiked with KI to yield 10 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes

digestion agent: 1.2 ml of $HClO_3$ for 20 mg

digestion residue made up to 10 ml with H_2O

measurement technique: Cary spectrophotometer, Sandell-Kolthoff

cuvette: d = 0.5 cm, t_{total} = 5 min, t_{scan} = 0.17 min

standard concentrations: 0, 2, 5, 10, 15, 20 µg I/l prepared with H_2O

samples: 1 ml made up to 10 ml with H_2O

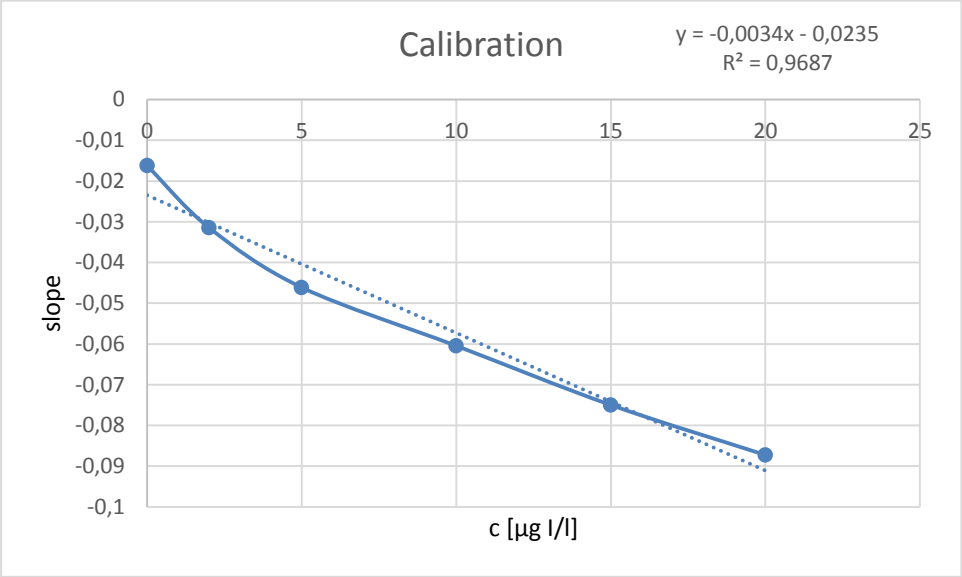

Figure 18. calibration curve iodine, 2-20 µg I/l, Cary

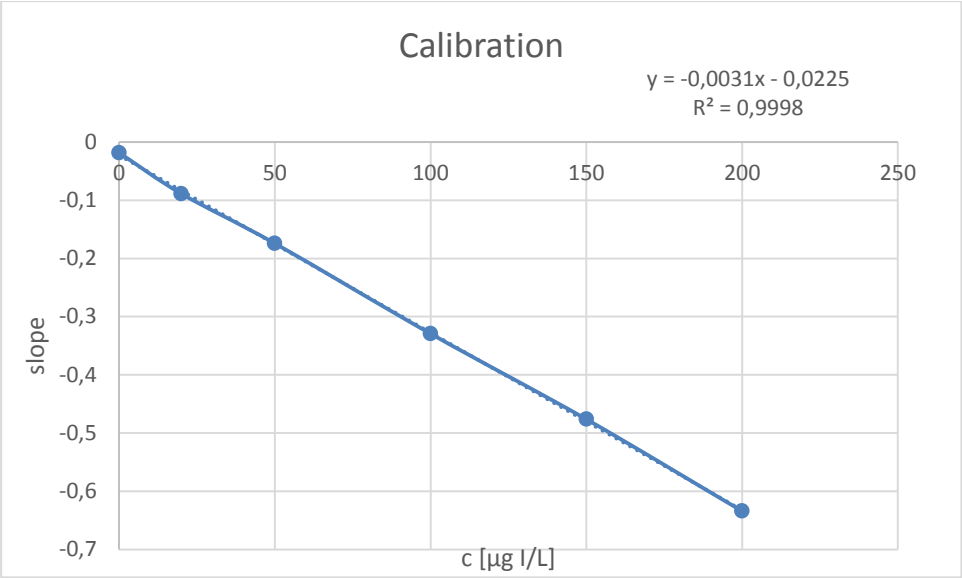

Figure 19. calibration curve iodine, 20-200 µg I/l, Agilent Cary

As shown in Figure 18 and 19 the calibration function is linear in the range of 20-200 µg/l for the instrument conditions used above. However, a significant curvature was recorded below 20 µg/l.

The iodine concentration in digested, spiked samples of milk powder was determined using a calibration function in the range of 20-200 µg/L. As shown in Figure 18, a time-dependent behaviour of the spike recoveries was encountered: While for the first sample of a series of fife digestions 100 % spike recovery was determined, the determined iodine concentration appeared to decrease from sample to sample, reaching only 66 % of the target value in sample milk powder_20mg_5.

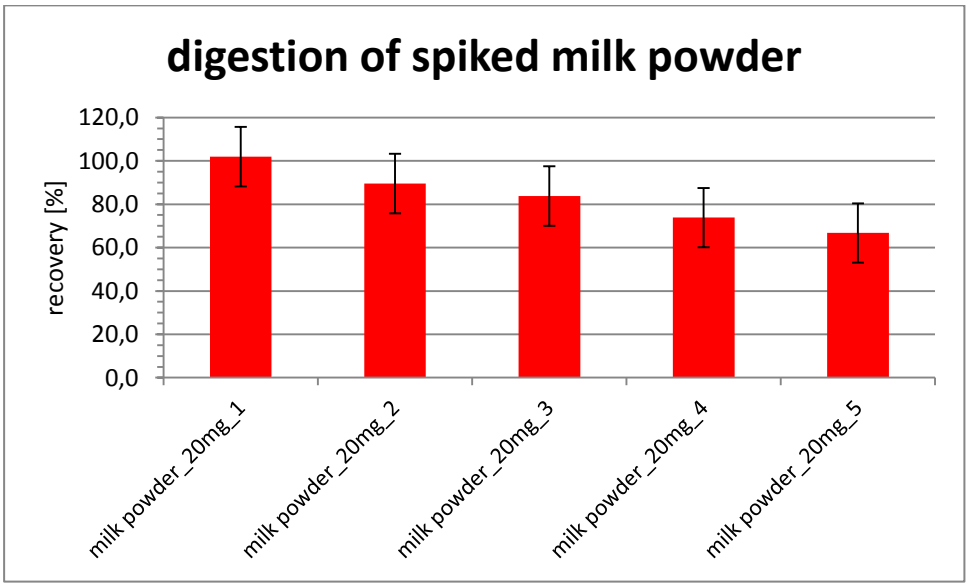

Figure 20. results digestion, no waiting between preparation and measurement, Agilent Cary; each bar represents a single measurement

This behaviour can be explained by the measurement procedure used: Since water was mixed with H_2SO_4 conc. in one of the first steps of the Sandell-Kolthoff procedure, the solution heats up due to the exothermic nature of this reaction. The samples were measured subsequently and so sample 5 had more time to cool down.

Blanks displayed a value of 0.42 $\pm$ 0.24 µg/l on average which was in the same range as ICPMS. Clearly, H_2O and H_2SO_4 need to be mixed in advance and all solutions need to cool down to room temperature as any reaction kinetic based analyte quantification method is affected by the temperature of the reaction mixture. Furthermore, thermostatization of the reaction mixture and the photometer can be expected to improve the reliability and robustness of the Sandell Kolthoff procedure. Though this is not possible on the Varian Cary instrument, the "Mini Photometer" discussed below is thermally stabilized.

In order to confirm the above made statement, the experiment was repeated and all solutions were allowed to reach room temperature after the addition of the sulphuric acid. Again, milk powder was spiked with KI and the $HClO_3$ volume was adjusted to the sample weight for digestion. H_2O and H_2SO_4 were mixed in advance and allowed to cool down to room temperature.

digestion procedure:
Blanks_1-5: 0.2 ml H_2O was digested; digestion vessel: eprouvette;
T/t-programme: ramp from 90-140 °C within 30 minutes
milk powder_20mg_1-5: 0.02 g of milk powder, 0.2 ml H_2O added, spiked with KI to yield 100 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes
digestion agent: 1.2 ml of $HClO_3$ for 20 mg
digestion residue made up to 10 ml with H_2O
measurement technique: Cary spectrophotometer, Sandell-Kolthoff
cuvette: d = 0.5 cm, t_{total} = 5 min, t_{scan} = 0.17 min
standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O
samples: 1 ml made up to 10 ml with H_2O

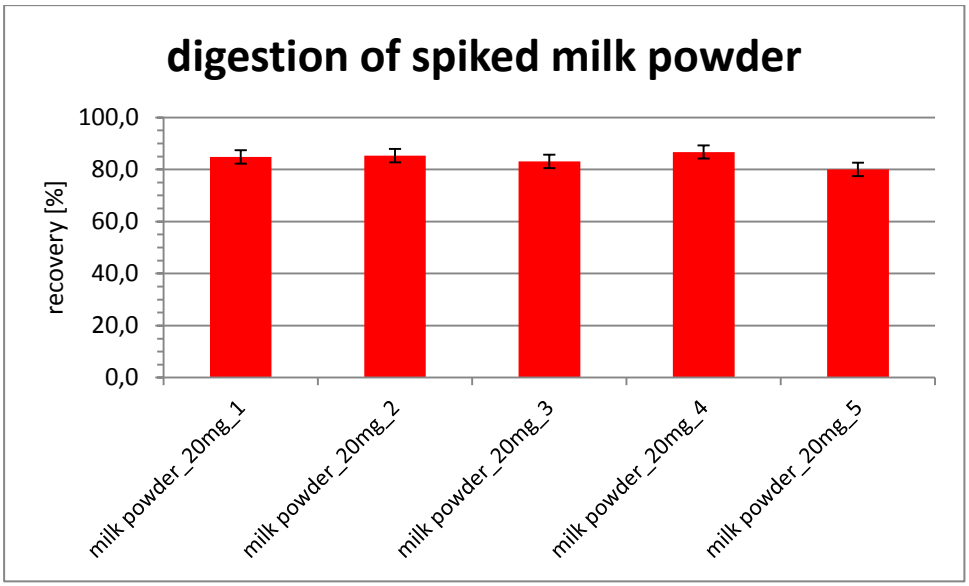

Figure 21. results for cooling time between preparation and measurement, Agilent Cary

As figure 21 shows the spike recoveries were only 84.0 ± 2.6 %, but no drift between the samples was encountered. The higher spike recovery of the first sample recorded in the previous experiment (see Figure 20) can be explained by the temperature-induced increase of the speed of the reaction, resulting in a higher "apparent" iodine concentration. The lower than expected spike recoveries of this experiment might be explained by the fact, that not the entire organic matrix had been digested. Though, elevated residual carbon levels don't cause problems with ICPMS, the Sandell-Kolthoff is well known for being prone to interferences from organic carbon that is able to influence the catalytic measurement. Blanks displayed a value of 3.23 ± 0.11 µg/l on average, which is above ICPMS by factor 10. Further experiments focussed on fully digesting the organic matrix.

Based on the previously described problems with the iodide stock solution, a test was performed to ensure, that there was no loss of iodine during the digestion procedure and that therefore the lower than expected recoveries of iodine by the Sandell-Kolthoff method only stems from this procedure. Different sample weights of milk powder or water were spiked with KI and the acid volume was adjusted to the sample weight. Measurement time on the Agilent Cary was reduced to 2 minutes since the previously used 5 minutes were found to be unnecessary long.

digestion procedure:
Blanks_1-5: 0.2 ml H_2O was digested; digestion vessel: eprouvette;
T/t-programme: ramp from 90-140 °C within 30 minutes
water_1-5: 0.2 ml H_2O added, spiked with KI to yield 100 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes
milk powder_10mg_1-5: 0.01 g of milk powder, 0.2 ml H_2O added, spiked with KI to yield 100 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes

digestion agent: 0.6 ml of $HClO_3$ for 10 mg sample weight, 1.2 ml of $HClO_3$ for 20 mg, etc.

digestion residue made up to 10 ml with H_2O

measurement technique: Cary spectrophotometer, Sandell-Kolthoff

cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min

standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O

samples: 1 ml made up to 10 ml with H_2O

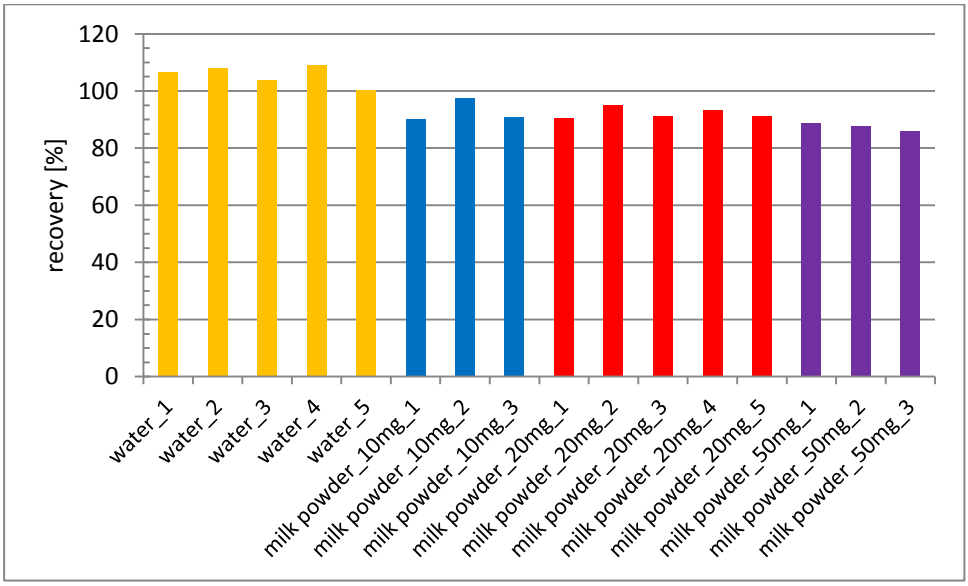

Figure 22. results digestion, $HClO_3$, acid volume adjusted to sample mass, Agilent Cary; each bar represents a single measurement

Figure 22 shows that the recoveries for iodide spiked water were all in the range of 90 – 110 %, which was deemed satisfactory for the purpose of this work. This also showed that no iodine had been lost during digestion. The results for the spiked milk powder samples improved and were then in the range of 90-100 %. All blanks displayed negative values. To conclude the wet digestion using $HClO_3$ was found to be successful and reliable. However, larger sample masses (> 0.2 g) were deemed dangerous to digest by $HClO_3$ only, as the explosive reaction of this acid with larger quantities of organic matter is well known.

3.4 $HClO_3$/HNO_3 one step digestion procedure

3.4.1 Initial experiments

Though not as potent in its oxidizing capabilities as $HClO_3$, HNO_3 is a widely used acid for digestions. In combination with $HClO_3$ it serves to pre-oxidize the organic matrix. Only after being boiled off, the temperature of the digestion increases towards the boiling point of $HClO_3$ unfolding this acid's oxidizing capabilities. Thereby the digestion with chloric acid can be made safer.

However, HNO_3 shows, as noted above strong UV absorption bands that cause spectral interferences when using the Sandell-Kolthoff reaction. Therefore, HNO_3 must be completely removed, too.
Different sample masses of milk powder or water were spiked with KI. A new digestion method was used, wherein HNO_3 was added to diminish the reaction conditions. The total acid volume was kept constant for all sample masses. ICPMS was used to verify that no iodine had been lost during digestion.

digestion procedure:
Blanks_1-5: 0.2 ml H_2O was digested; digestion vessel: eprouvette;
T/t-programme: ramp from 90-140 °C within 30 minutes
water_1-5: 0.2 ml H_2O added, spiked with KI to yield 10 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes
milk powder_10mg_1-5: 0.01 g of milk powder, 0.2 ml H_2O added, spiked with KI to yield 100 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes
20 and 50 mg of sample mass were treated alike
digestion agent: 1.5 ml of $HClO_3$ and 0.5 ml of HNO_3 conc. for all samples
digestion residue made up to 10 ml with H_2O
measurement technique: ICPMS, rinsing solution: H_2O
standard concentrations: 0, 2, 5, 10, 15, 20 µg I/l prepared with H_2O
samples: 0.1 ml made up to 10 ml with H_2O

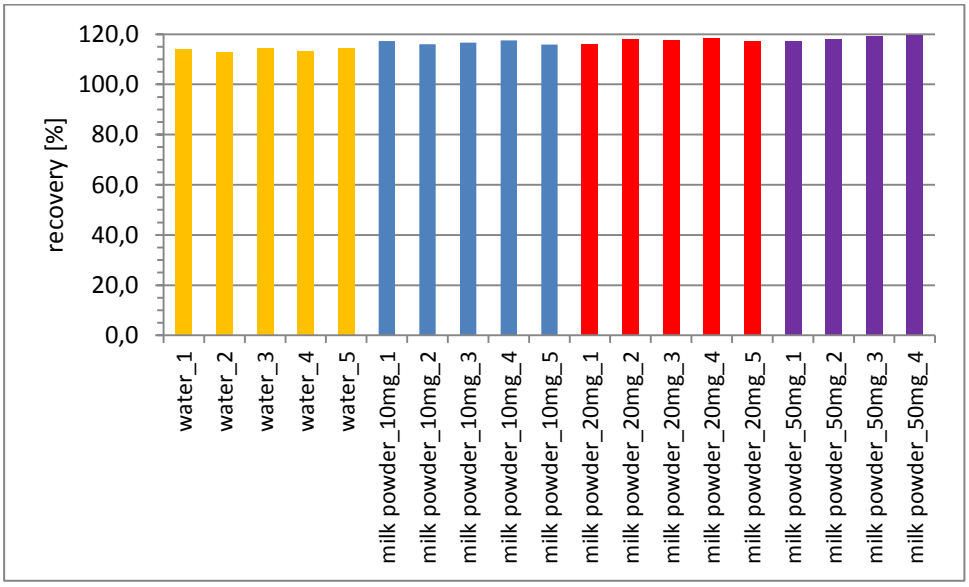

Figure 23. results digestion with $HClO_3$ and HNO_3, acid volume adjusted to sample mass, ICPMS; each bar represents a single measurement

As figure 23 shows the spike recoveries were all in the range of 100-110 %. Therefore no iodine had been lost during the digestion. The acid volume was sufficient enough for digestion and measurement by ICPMS. All blank values were below LOQ. LOD was 0.30 µg/l.

In the next step, iodine was quantified by the Sandell-Kolthoff method in the same samples.

measurement technique: Agilent Cary spectrophotometer, Sandell-Kolthoff

cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min

standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O

samples: 1 ml made up to 10 ml with H_2O

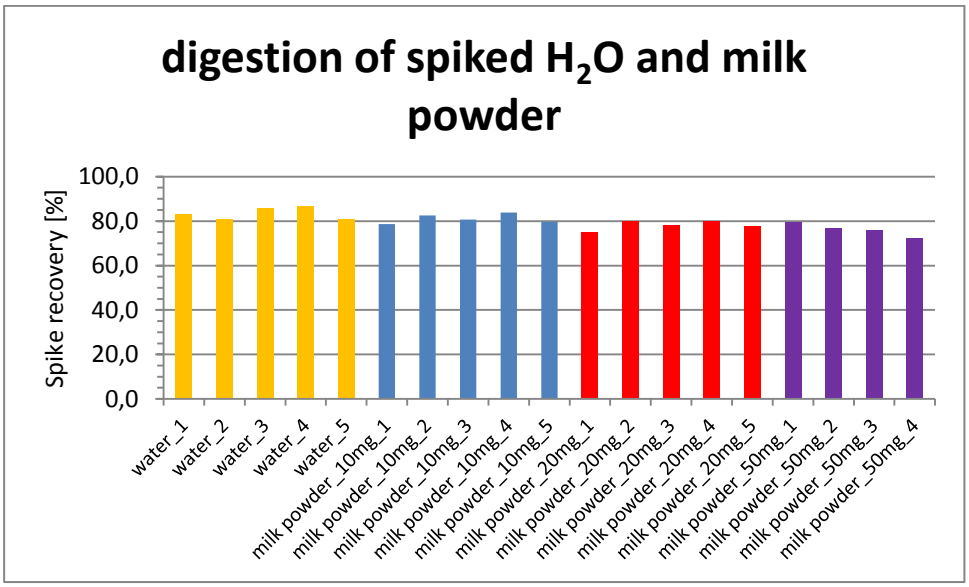

Figure 24. results digestion with $HClO_3$ and HNO_3, Agilent Cary

Figure 24 shows that the spike recoveries were lower than by ICPMS. All the recoveries were at about 80 % - consistent with the results presented in Figure 19. Again, this could be explained with residual organic carbon that influences the catalytic reaction. To overcome this issue more acid should be used in the future. Iodine was not lost since the ICPMS had shown very acceptable spike recoveries. Another influencing factor could be remaining HNO_3 because it is known to absorb at wavelengths below 365 nm. Digestion blanks displayed a value of 1.55 $\pm$ 0.12 µg/l on average which was five times higher than for ICPMS. The following experiment focussed on acquiring wavelength scans of the samples.

3.4.2 Spectral interferences

A wavelength scan on the Varian Cary was recorded for all samples of the previously discussed experiment in the range of 200 – 400 nm.

In addition two other samples were scanned:

1 ml KI-Standard (1,000 µg/l) + 0.5 ml $HClO_3$ made up with water to 10 ml

1 ml KI-Standard (1,000 µg/l)+ 0.5 ml $HClO_3$ + 0.5 ml HNO_3 conc. made up with water to 10 ml

Samples: 1 ml of sample made up to 10 ml with water

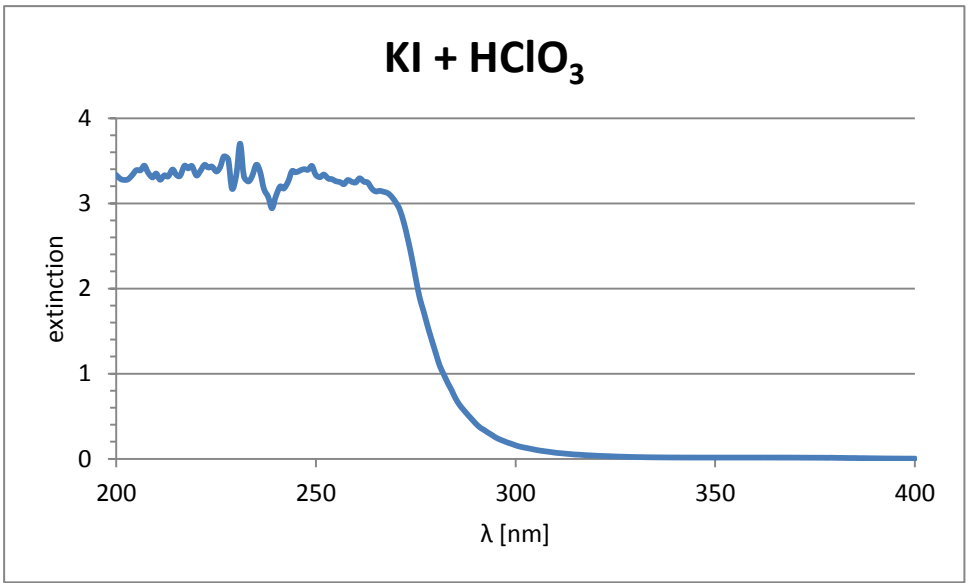

Figure 25. scan KI + $HClO_3$, Agilent Cary

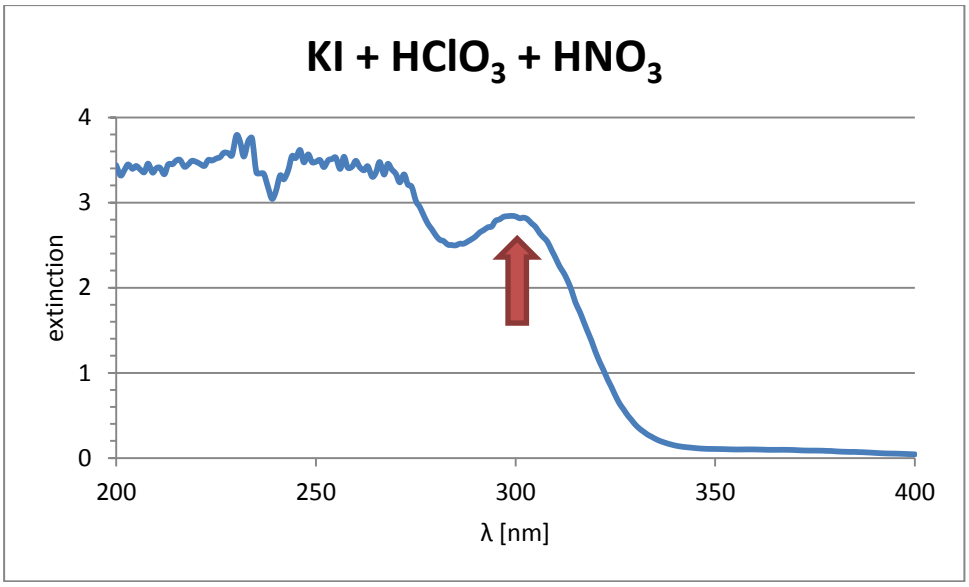

Figure 26. scan KI + $HClO_3$ + HNO_3, Agilent Cary

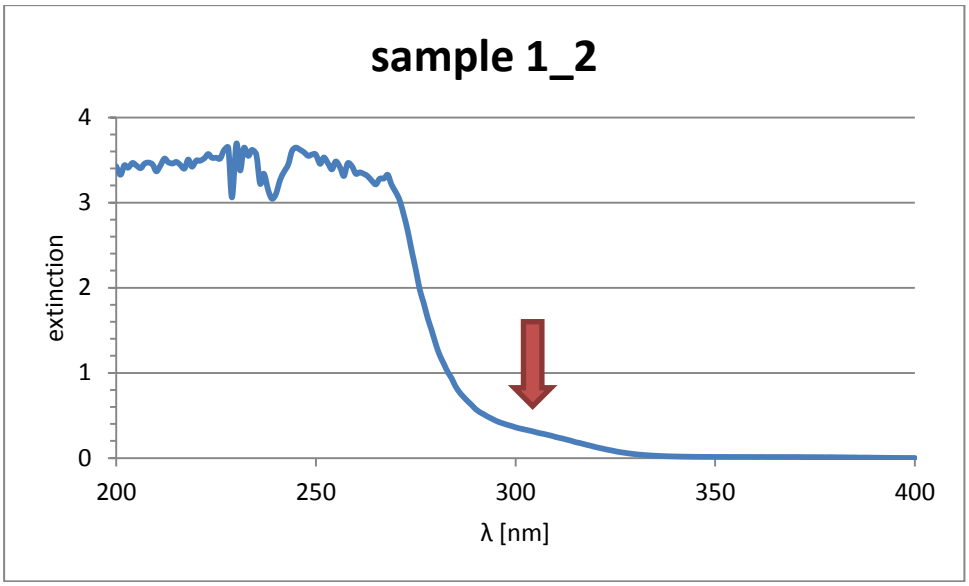

Figure 27. scan sample 1_2, Agilent Cary

Figure 26 (with HNO_3) displayed a shoulder (marked by a red arrow) at 317 nm whereas figure 25 (without HNO_3) did not. The same but slightly smaller shoulder can be seen in figure 27 (sample 1_2). The same was true for all other samples. It was obvious that HNO_3 had to be removed during digestion, as otherwise the catalytic reaction will be spectrally interfered. The following experiments focussed on modifying the digestion to fully remove the HNO_3.

3.5 $HClO_3$/HNO_3 two step digestion procedure

Due to the interferences by nitric acid, the digestion procedure had to be modified. Different sample masses of milk powder or water were spiked with KI. The digestion method was changed into a two-step procedure. After the first step, $HClO_3$ was added again. The solution was heated up to 170 °C to fully remove the HNO_3. The iodine concentration was determined spectrophotometrically using an Agilent Cary spectrophotometer.

digestion procedure:
Blanks_1-5: 0.2 ml H_2O was digested; digestion vessel: eprouvette;
T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
water_1-5: 0.2 ml H_2O added, spiked with KI to yield 10 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
milk powder_10mg_1-5: 0.01 g of milk powder, 0.2 ml H_2O added, spiked with KI to yield 100 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
20 and 50 mg of sample weight were treated alike

digestion agent: first step: 1.5 ml of $HClO_3$ and 0.5 ml of HNO_3 conc. for all samples;
second step: 0.6 ml of $HClO_3$ for all samples
digestion residue made up to 10 ml with H_2O
measurement technique: Agilent Cary spectrophotometer, Sandell-Kolthoff
cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min
standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O
samples: 1 ml made up to 10 ml with H_2O

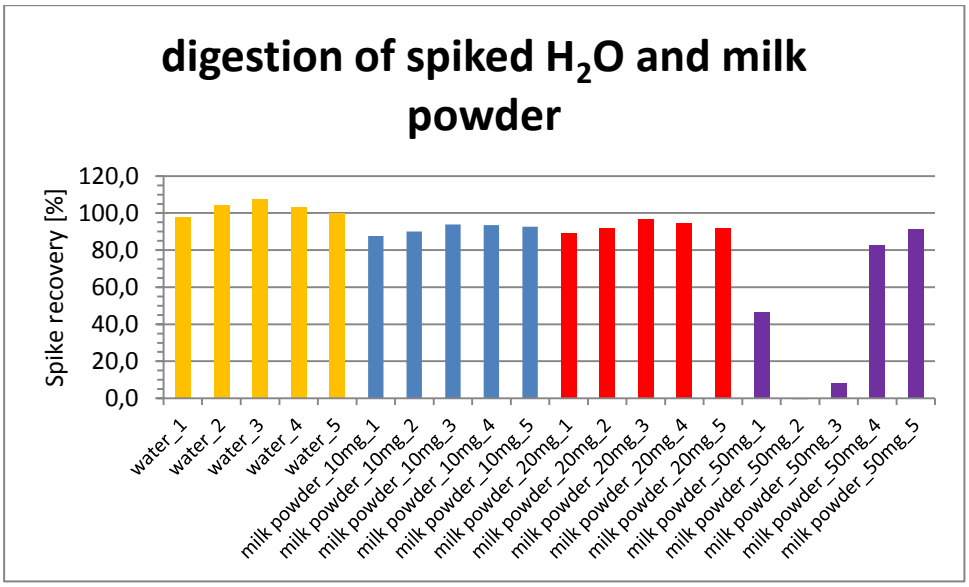

Figure 28. results two step wet digestion, Agilent Cary; each bar represents a single measurement

Figure 28 shows good recoveries (90-100 %) for spiked water and the milk powder samples with 10 and 20 mg sample mass. Samples of 50 mg resulted in rather irreproducible data, indicating an incomplete removal of carbon. However, the two-step digestion procedure is indeed suitable for removing HNO_3, though the acid volume has to be adjusted to the sample weight. All blanks were < LOQ.

The next stage of optimization was the adoption of the two-step digestion procedure to larger sample mass. 50 and 70 mg of milk powder or water were spiked with KI. The two step digestion procedure was retained. The acid volume within the second step was increased to 1.5 ml of $HClO_3$.

digestion procedure:
Blanks_1-5: 0.2 ml H_2O was digested; digestion vessel: eprouvette;
T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
water_1-5: 0.2 ml H_2O added, spiked with KI to yield 10 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes

milk powder_50mg_1-5: 0.05 g of milk powder, 0.2 ml H_2O added, spiked with KI to yield 100 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes

70 mg of sample mass was treated alike

digestion agent: first step: 1.5 ml of $HClO_3$ and 0.5 ml of HNO_3 conc. for all samples;

second step: 1.5 ml of $HClO_3$ for all samples

digestion residue made up to 10 ml with H_2O

measurement technique: Cary spectrophotometer, Sandell-Kolthoff

cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min

standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O

samples: 1 ml made up to 10 ml with H_2O

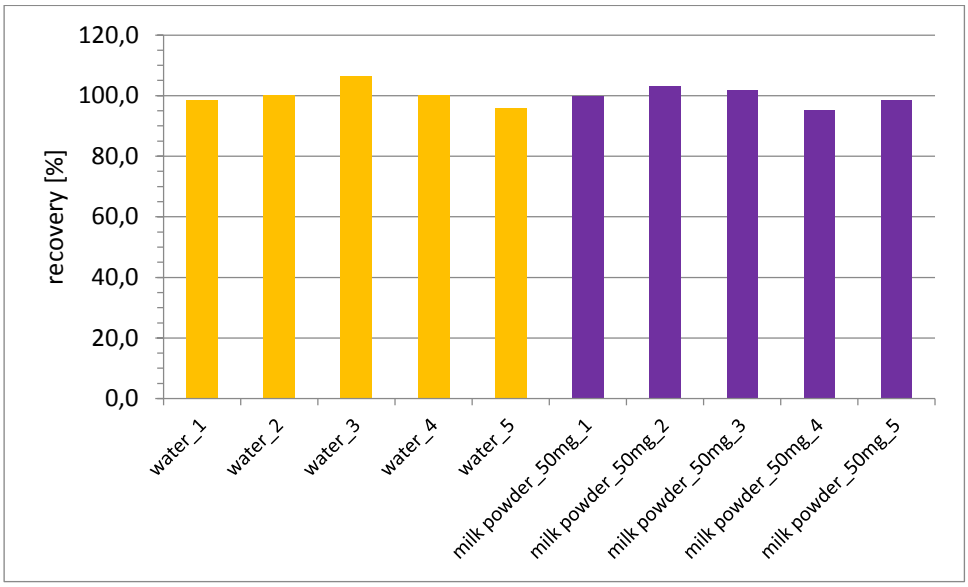

Figure 29. results digestion, $HClO_3$ adjusted, HNO_3 constant, Agilent Cary; each bar represents a single measurement

As it can be seen in figure 29 the recoveries were at 100 % for spiked water and 50 mg of milk powder. This indicates that during the second step the increased acid volume decomposes the entire organic matrix. Samples with 70 mg gave no recovery at all. Therefore acid volume had to be further increased for 70 mg. Blanks displayed a value of 2.23 $\pm$ 0.13 µg/l on average which was 8 times higher than for ICPMS. Though at this level indeed relevant, these blanks were investigated in a later stage.

The digestion of 70 mg sample mass was performed in a similar way as for the previous experiment, but the acid volume within first and second digestion step was increased to 2.5 ml of $HClO_3$ each.

digestion procedure:

Blanks_1-5: 0.2 ml H_2O was digested; digestion vessel: eprouvette;

T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes

milk powder_70mg_1-5: 0.07 g of milk powder, 0.2 ml H_2O added, spiked with KI to yield 100 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes

digestion agent: first step: 2.5 ml of $HClO_3$ and 0.5 ml of HNO_3 conc. for all samples;

second step: 2.5 ml of $HClO_3$ for all samples

digestion residue made up to 10 ml with H_2O

measurement technique: Cary spectrophotometer, Sandell-Kolthoff

cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min

standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O

samples: 1 ml made up to 10 ml with H_2O

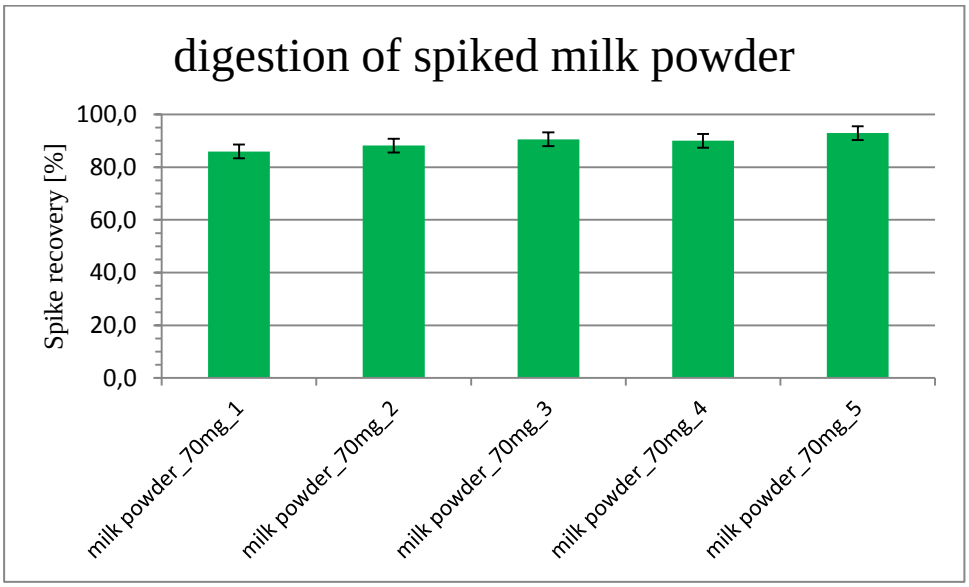

Figure 30. results digestion, $HClO_3$ adjusted, HNO_3 constant, Agilent Cary

As it can be seen in figure 30 the spike recoveries improved for sample weights of 70 mg by about 10 %, now reaching 89.5 $\pm$ 2.6 %. To conclude the digestion worked for higher sample masses if the acid volume had been adjusted. Blanks displayed a value of 2.97 $\pm$ 0.18 µg/l on average which was 10 times higher than for ICPMS, suggesting a slight contamination of the chloric acid with iodine.

3.6 Determination of iodine using the Sandell-Kolthoff reaction: Mini-photometer

All experiments listed up to now were performed with an Agilent Cary spectrophotometer and a cuvette with d = 0.5 cm. However for the Mini photometer there was only a flow through cuvette with d = 1 cm available, necessitating the use of a cuvette with d = 1 cm for comparison experiments on the Agilent Cary. Since the thickness of the cuvette was increased by factor of 2, the sample solutions were made up to a final volume of 25 ml instead of 10 ml. The goal was to examine if the measurement procedure also worked with a cuvette with d = 1 cm.

Calibration solutions were prepared according to table 6:

Table 6. pretest for Mini photometer by Agilent Cary calibration solutions volumes_1

	Vol [ml] I stock solution	H_2O [ml]	H_2SO_4 [ml] conc.	As [ml] stock solution	Ce [ml] stock solution
Blank	0	21.95	1.3	1.0	0.75
Std_1	0.50	21.45	1.3	1.0	0.75
Std_2	1.25	20.70	1.3	1.0	0.75
Std_3	2.50	19.45	1.3	1.0	0.75
Std_4	1.50	20.95	1.3	0.5	0.75
Std_5	2.00	20.325	1.3	1.0	0.375

It was not possible to record a slope for any of these standards. It was assumed that the acid concentration was too low to start the catalytic reaction. Therefore the experiment was repeated with more H_2SO_4.

Samples were prepared according to table 7:

Table 7. pretest for Mini photometer by Agilent Cary calibration solutions volumes_2

	Vol [ml] I stock solution	H_2O [ml]	H_2SO_4 [ml] conc.	As [ml] stock solution	Ce [ml] stock solution
Blank	0	20.0	3.25	1.0	0.75
Std_1	0.50	19.5	3.25	1.0	0.75
Std_2	1.25	18.75	3.25	1.0	0.75
Std_3	1.00	19.5	3.25	0.5	0.75
Std_4	1.50	19.375	3.25	0.5	0.375
Std_5	2.00	18.375	3.25	1.0	0.375

All experiments from table 7 gave reproducible slopes. It was decided to use 0.5 ml of As and 0.375 ml of Ce and to make it up to 10 ml with water. This showed the highest correspondence with the composition as used for the cuvette with d = 0.5 cm. The most important conclusion from this experiment was that the H_2SO_4 concentration had to be kept constant. The following experiment focussed on measuring the samples by the MINImini-photometer photometer.

Different masses of milk powder or water were spiked with KI. The samples were digested according to the two step wet digestion procedure described above. Then the samples were measured on the Mini photometer.

digestion procedure:
Blanks_1-5: 0.2 ml H_2O was digested; digestion vessel: eprouvette;
T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
milk powder_10mg_1-5: 0.01 g of milk powder, 0.2 ml H_2O added, spiked with KI to yield 100 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
digestion agent: first step: 1.5 ml of $HClO_3$ and 0.5 ml of HNO_3 conc. for all samples;
second step: 1.5 ml of $HClO_3$ for all samples
digestion residue made up to 10 ml with H_2O
measurement technique: mini photometer, Sandell-Kolthoff
cuvette: d = 1 cm, t_{total} = 1 min, t_{scan} = 0.5 seconds, light source = 250 AU, T = RT
standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O
samples: 1 ml made up to 10 ml with H_2O

The calibration curve is pictured in figure 31:

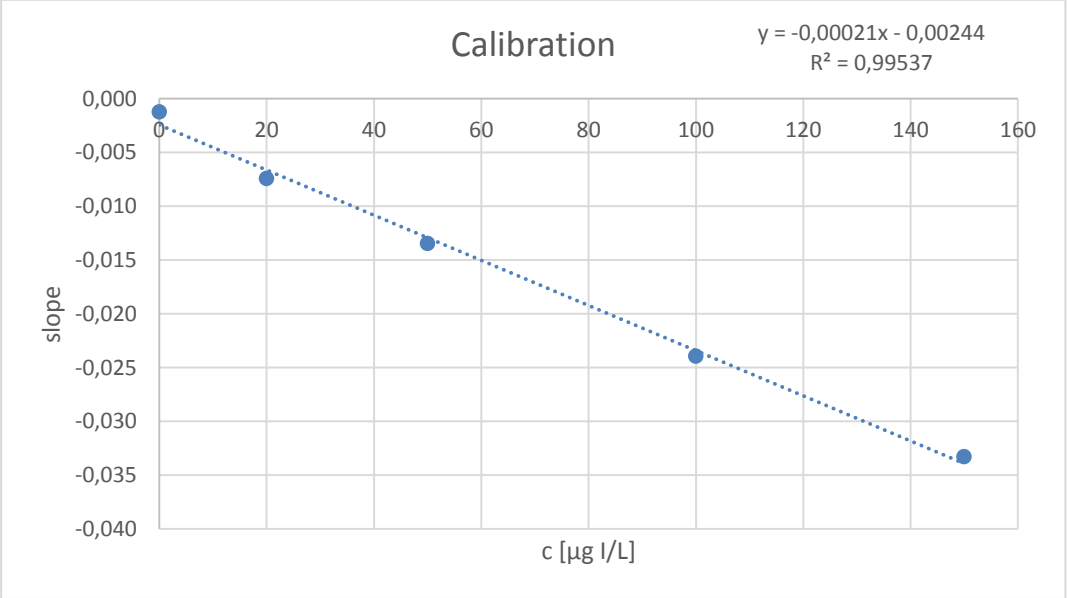

Figure 31. calibration curve iodine, mini photometer

As it can be seen in figure 31 a linear calibration function with a correlation coefficient of 0.9954 was attained.

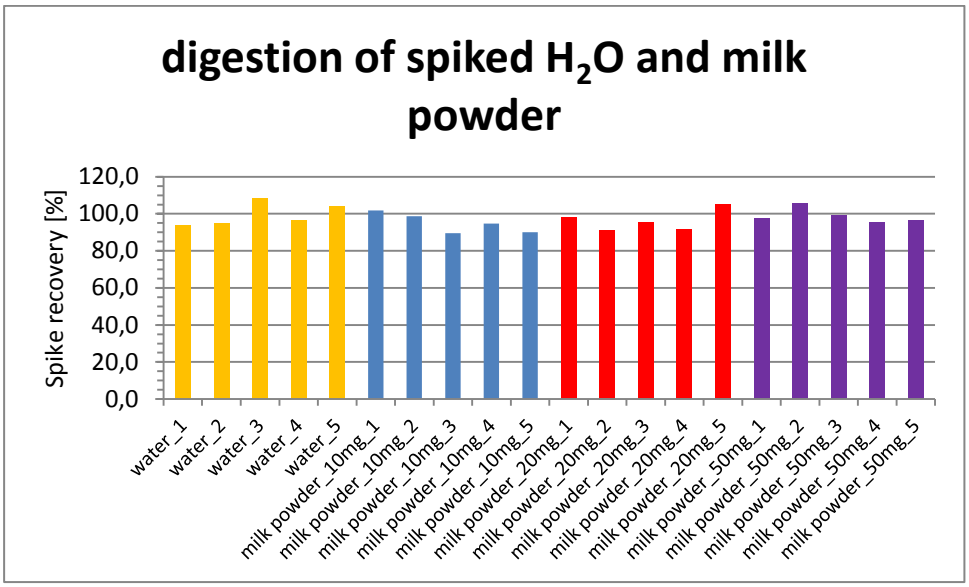

Figure 32. results digestion, $HClO_3$ adjusted, HNO_3 constant, mini photometer; each bar represents a single measurement

Figure 32 shows that the spike recoveries were in a very satisfactory range of 90-110 %. All blanks were < LOQ. From these data it can be concluded, that the mini photometer gave comparable results as the Agilent Cary spectrophotometer. The following experiment focussed on validating the method.

3.7 Factors that affect the robustness of the Sandell-Kolthoff reaction

3.7.1 Effect of the reaction temperature

As noted before, the Sandell-Kolthoff method is – as any analytical procedure that is based on kinetics – subject to reaction temperature variations. The magnitude of these variations was investigated in the temperature range of 10 – 30 °C.

A calibration was recorded by measuring 3 replicates per calibration standard. Standards were measured at room temperature by the Mini photometer. The power of the light source was increased to 300 AU in order to get higher signals.

measurement technique: Mini photometer, Sandell-Kolthoff

cuvette: d = 1 cm, t_{total} = 1 min, t_{scan} = 0.5 seconds, light source = 300 AU, T = RT

standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O

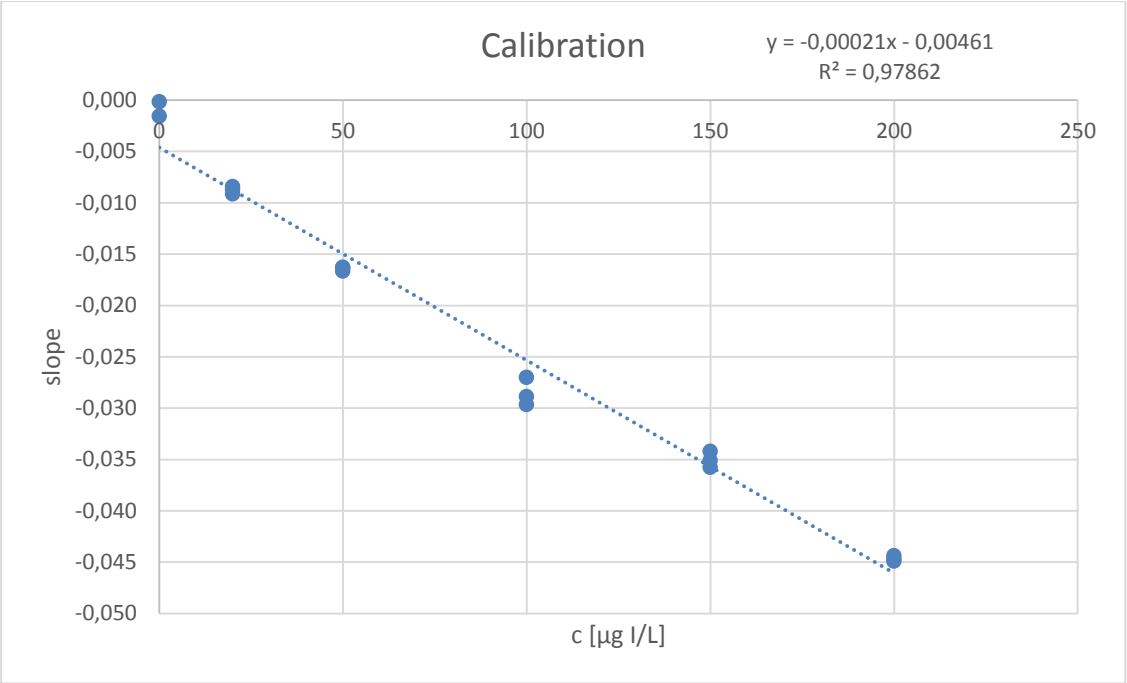

Figure 33. calibration curve iodine, RT, 300 AU, mini photometer

As it can be seen in figure 33 the calibration curve was not as linear as in the previous experiment as indicated by the correlation coefficient of 0.9786. It was suggested that 300 AU had been outside the linear range of the light source. Therefore the experiment was repeated by changing the light source's power to 250 AU.

measurement technique: Mini photometer, Sandell-Kolthoff

cuvette: d = 1 cm, t_{total} = 1 min, t_{scan} = 0.5 seconds, light source = 250 AU, T = RT

standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O

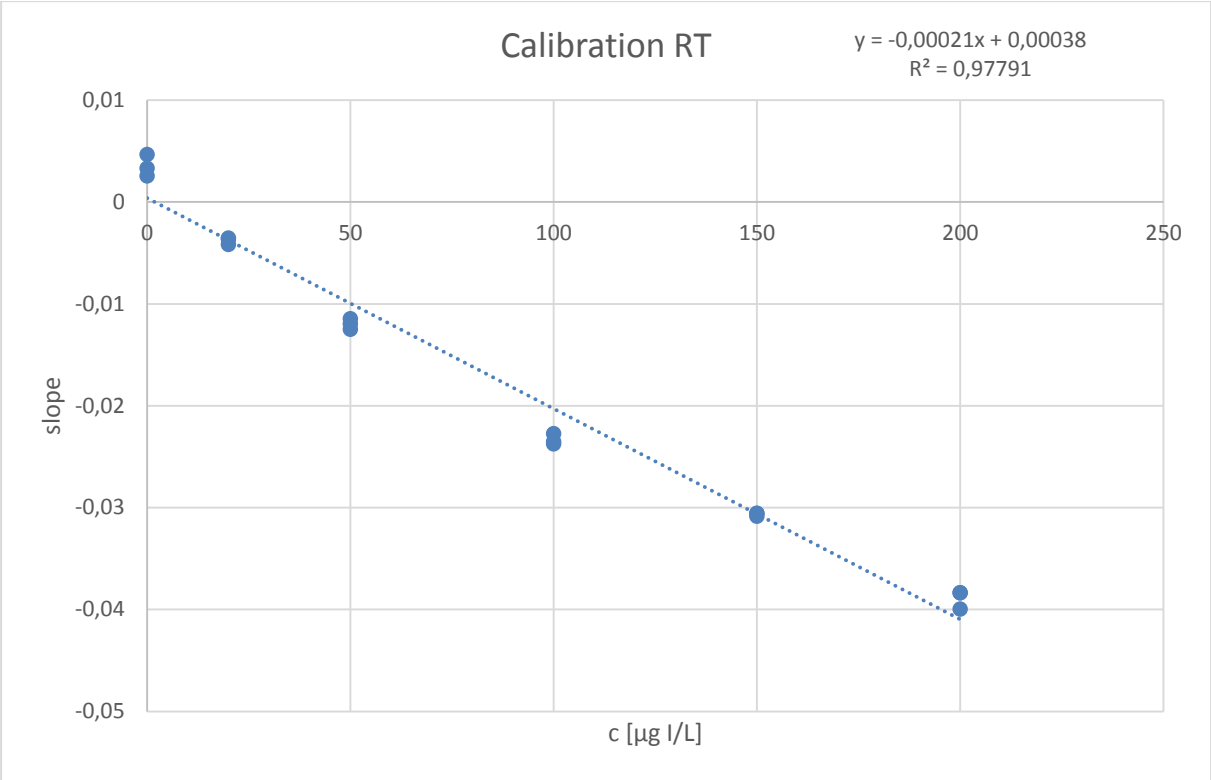

Figure 34. calibration curve iodine, RT, 250 AU, mini photometer

Figure 34 shows the calibration curve at room temperature. The correlation coefficient (0.9779) did not improve when decreasing the power of the light source. Next, it was assumed that the calibration solutions should be thermostated, as variations in the room temperature might have affected the results.

The experiment was repeated and the solutions and the flow through cuvette were cooled down to 15 °C. Samples were measured by the mini photometer. A calibration was recorded by measuring 3 replicates per calibration standard. The sample solution was soaked into the cuvette and a waiting time of 30 s was applied until the start of the measurement. This was done to allow the solution to reach the designated temperature.

measurement technique: Mini photometer, Sandell-Kolthoff
cuvette: d = 1 cm, t_{total} = 1 min, t_{scan} = 0.5 seconds, light source = 250 AU, T = 15 °C
waiting time: 30 s
pump: 80 rpm
standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O

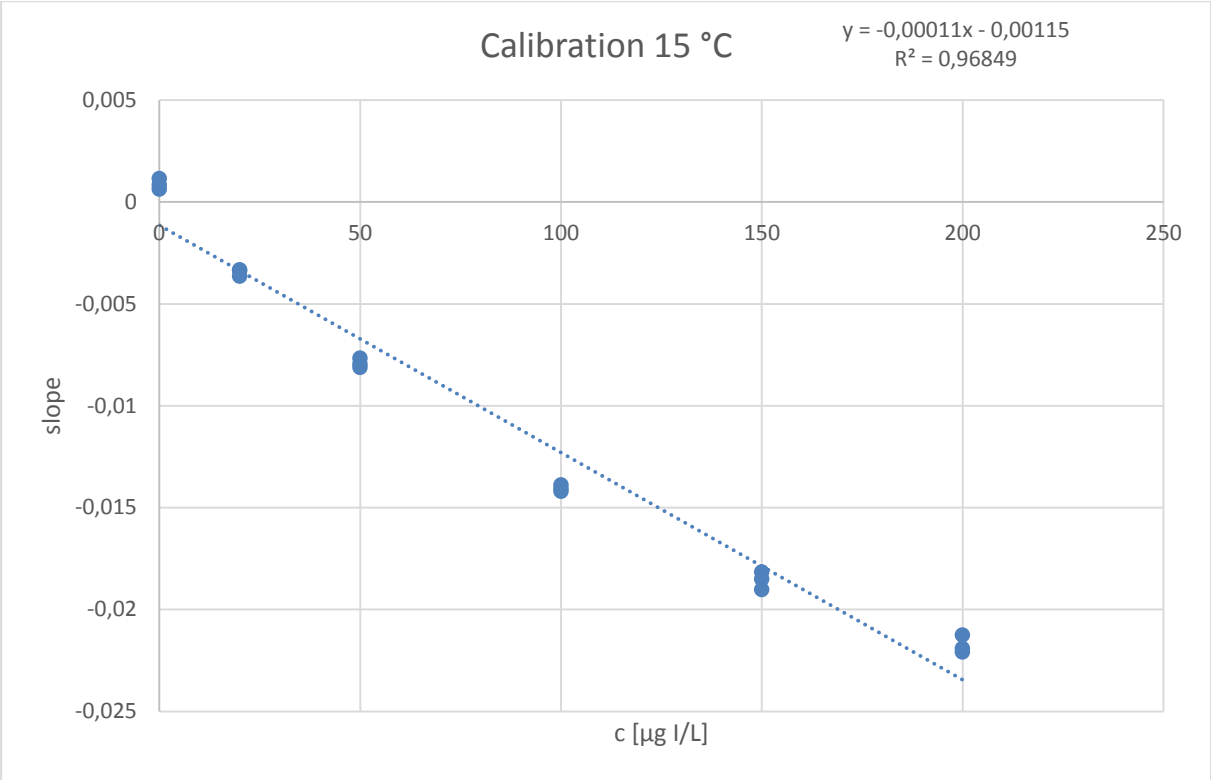

Figure 35. calibration curve iodine, 15 °C, mini photometer

As it can be seen in figure 35 the linearity did not improve. The correlation coefficient was only 0.9685. Apparently keeping the temperature constant was not the reason for the non-linear calibration function.

To exclude hardware-problems in the mini photometer creating the non-linear calibration function, a comparison with the Agilent Cary was conducted using the same set of solutions ant the same temperature (room temperature). A calibration was recorded by measuring 3 replicates per calibration standard.

measurement technique: Mini photometer, Sandell-Kolthoff
cuvette: d = 1 cm, t_{total} = 1 min, t_{scan} = 0.5 seconds, light source = 250 AU, T = RT
waiting time: 30 s
pump: 80 rpm
standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O

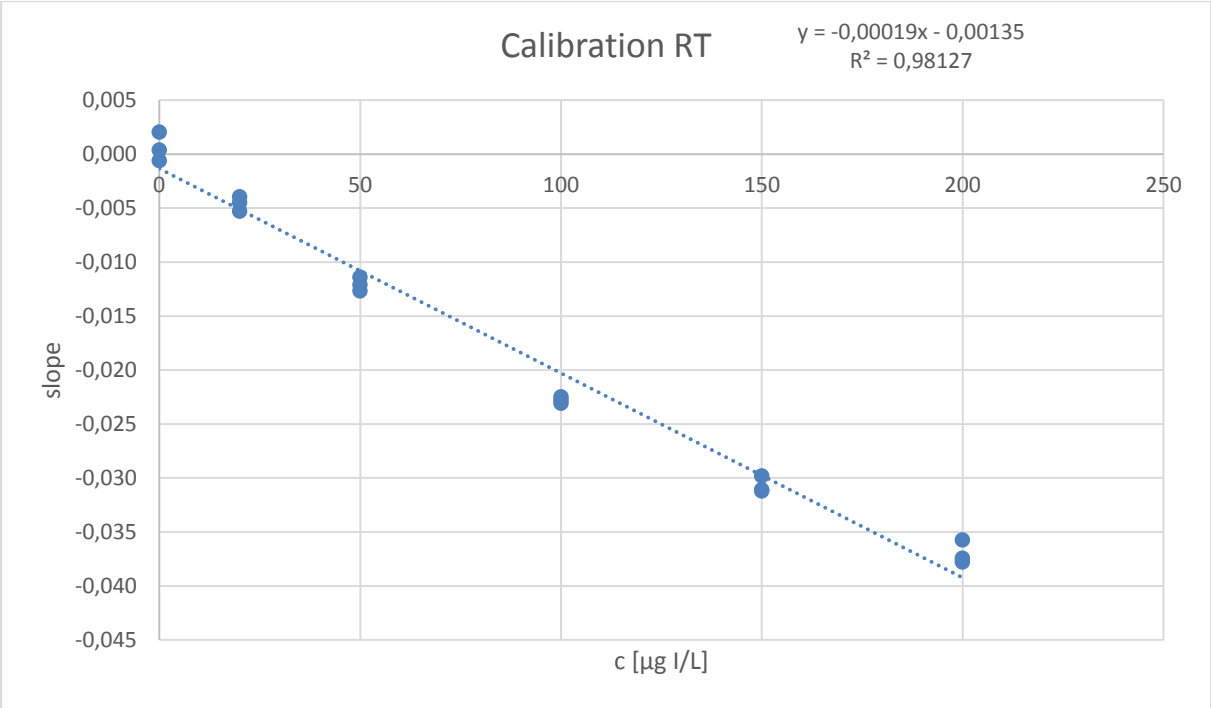

Figure 36. calibration curve iodine, RT, mini photometer

Figure 36 shows the calibration curve obtained by the Mini photometer. The calibration function was still non-linear.

measurement technique: Agilent Cary spectrophotometer, Sandell-Kolthoff

cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min

standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O

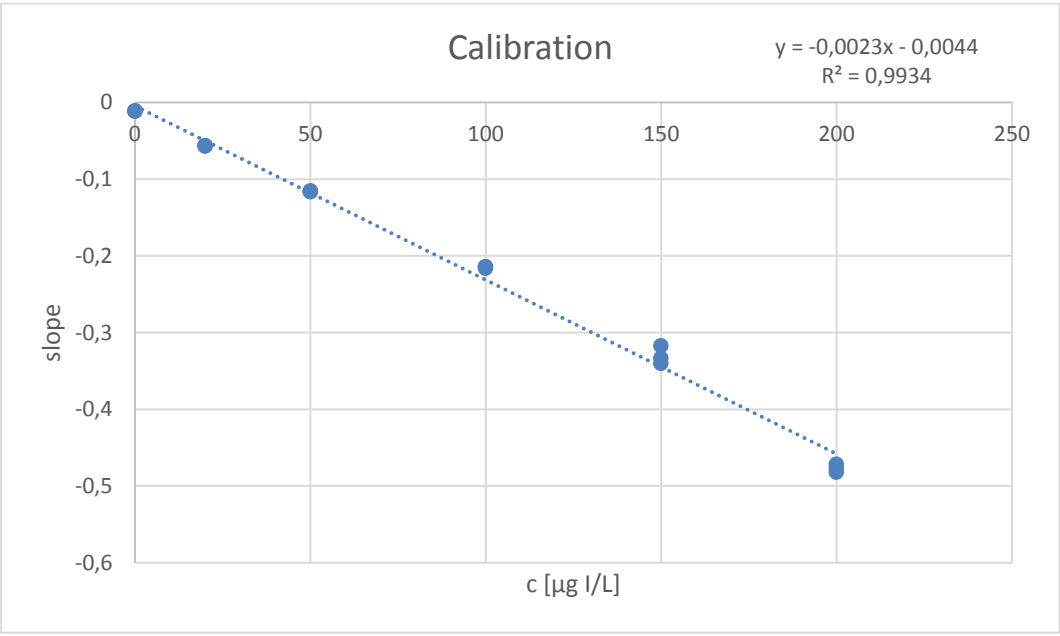

Figure 37. calibration curve iodine, Agilent Cary

Figure 37 shows the calibration curve obtained by the Agilent Cary. The linearity is much better with a correlation coefficient of 0.9934. Clearly, the mini photometer caused the observed non-linear calibration function.

Until this day the raw signal at canal 2 was determined to be I/I_0. For calculation the logarithm was applied to the inverse of I/I_0. After applying the second logarithm and plotting it versus the time, the slope was direct proportional to the iodine concentration.

It was assumed that the raw signal at canal 2 corresponds to I - the transmission. By dividing this value through a constant value it should be possible to achieve correct results. This constant value was obtained by recording the signal of pure water.

$$\frac{I}{I_0} = \frac{signal\ at\ canal\ 2\ from\ sample}{signal\ at\ canal\ 2\ from\ H_20} = \text{T (rel. transmission)}$$

The following calculation steps remained the same.

$$\text{slope} = \log\left(\log \frac{1}{T}\right) \text{ plotted against the time}$$

Reprocessed calibration is shown in figure 38:

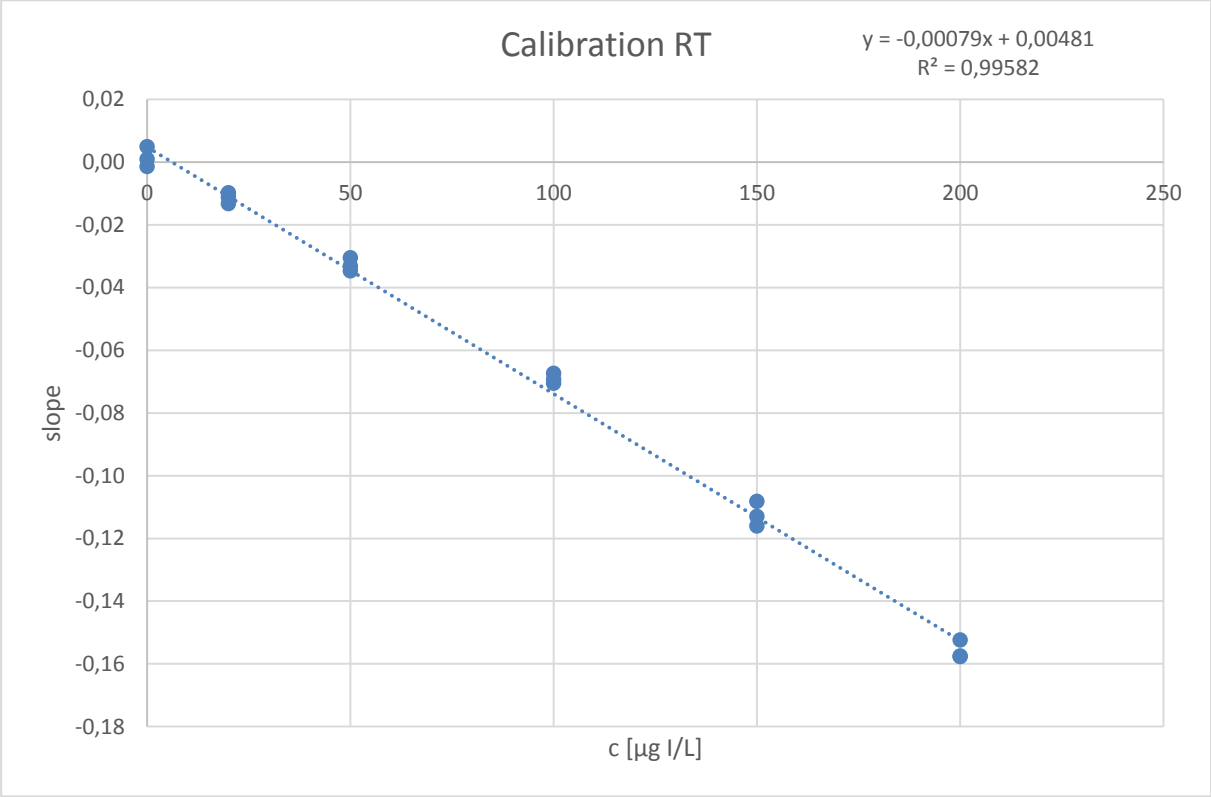

Figure 38. calibration curve iodine, reprocessed, RT, mini photometer

As it can be seen in figure 38 the calibration curve was linear with a correlation coefficient of 0.9958. To conclude the calculation algorithm had to be adopted. The good linearity shown in Figure 31 was probably the result of coincidence.

In a series of experiments a calibration was recorded (3 replicates per calibration standard) at different temperatures (10, 15, 20, 25, 30 °C). Each calibration standard was carefully thermostated prior measurement.

measurement technique: Mini photometer, Sandell-Kolthoff
cuvette: d = 1 cm, t_{total} = 1 min, t_{scan} = 0.5 seconds, light source = 250 AU, T = 10-30 °C
waiting time: 30 s
pump: 80 rpm
standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O

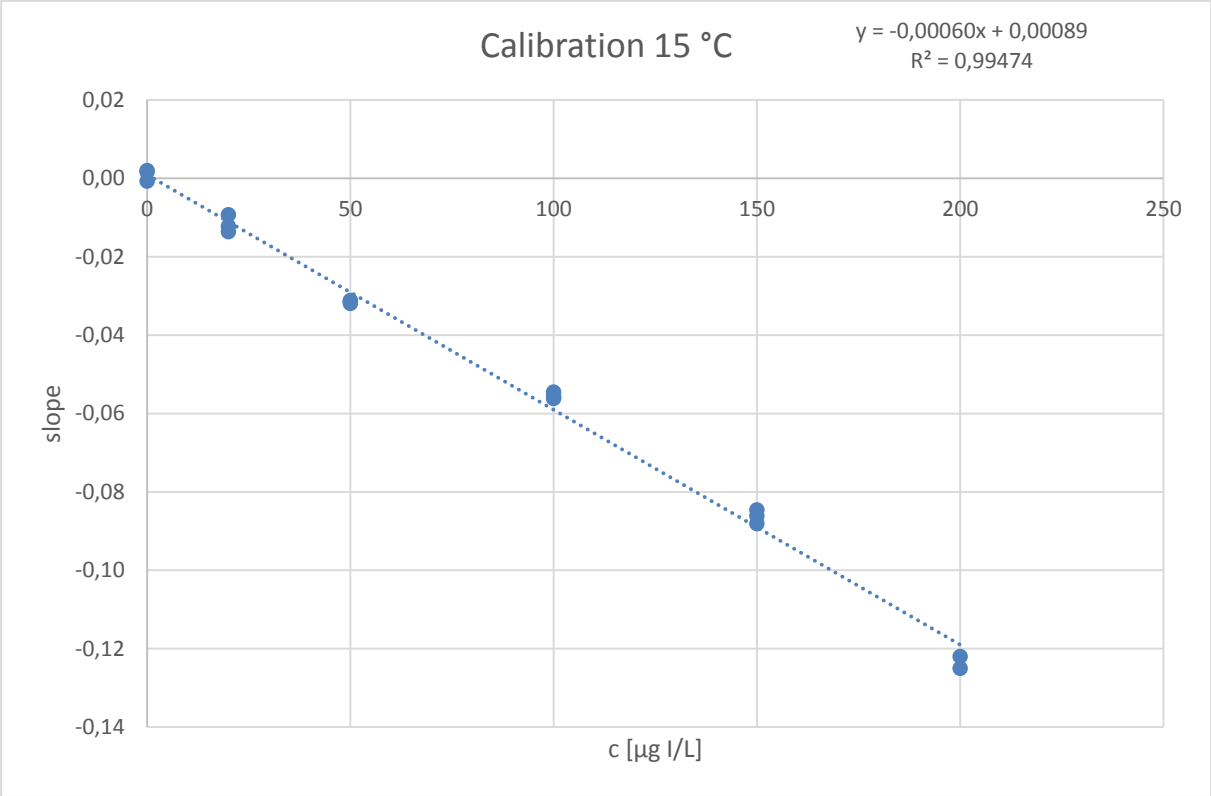

Figure 39. calibration curve iodine, 15 °C, mini photometer

As it can be seen in figure 39 the calibration functioned at 15 °C with a correlation coefficient of 0.9947. Slope was flatter than at room temperature. This is reasonable, since the reaction is slower at lower temperature

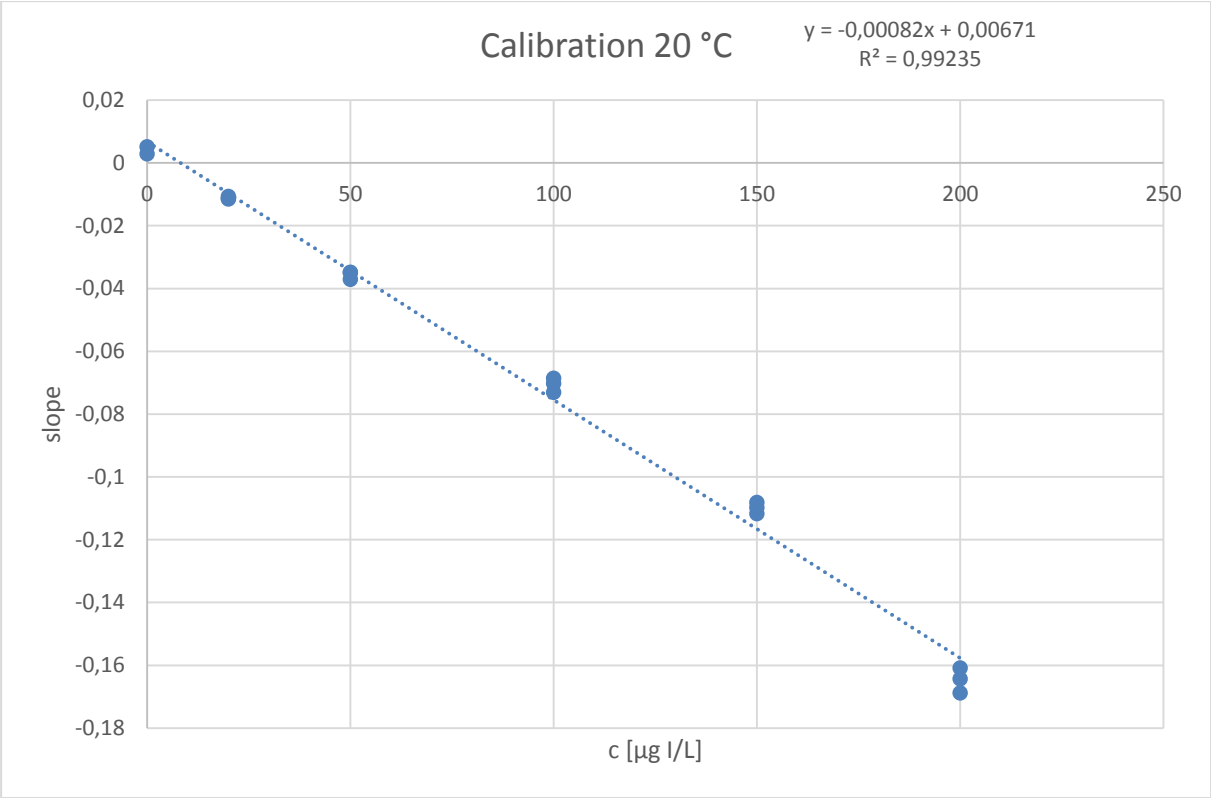

Figure 40. calibration curve iodine, 20 °C, mini photometer

Figure 40 shows the calibration curve at 20 °C. The experiment functioned with a correlation coefficient of 0.9924. However the three standards with 200 µg/l showed a higher slope than expected. The reaction proceeded faster at higher temperature and with higher iodine concentration.

The calibration curve where the 200 µg/l standards had been excluded is shown in figure 41. Correlation coefficient improved to the value of 0.9985. Slope was steeper than for 15 °C, but flatter than for room temperature.

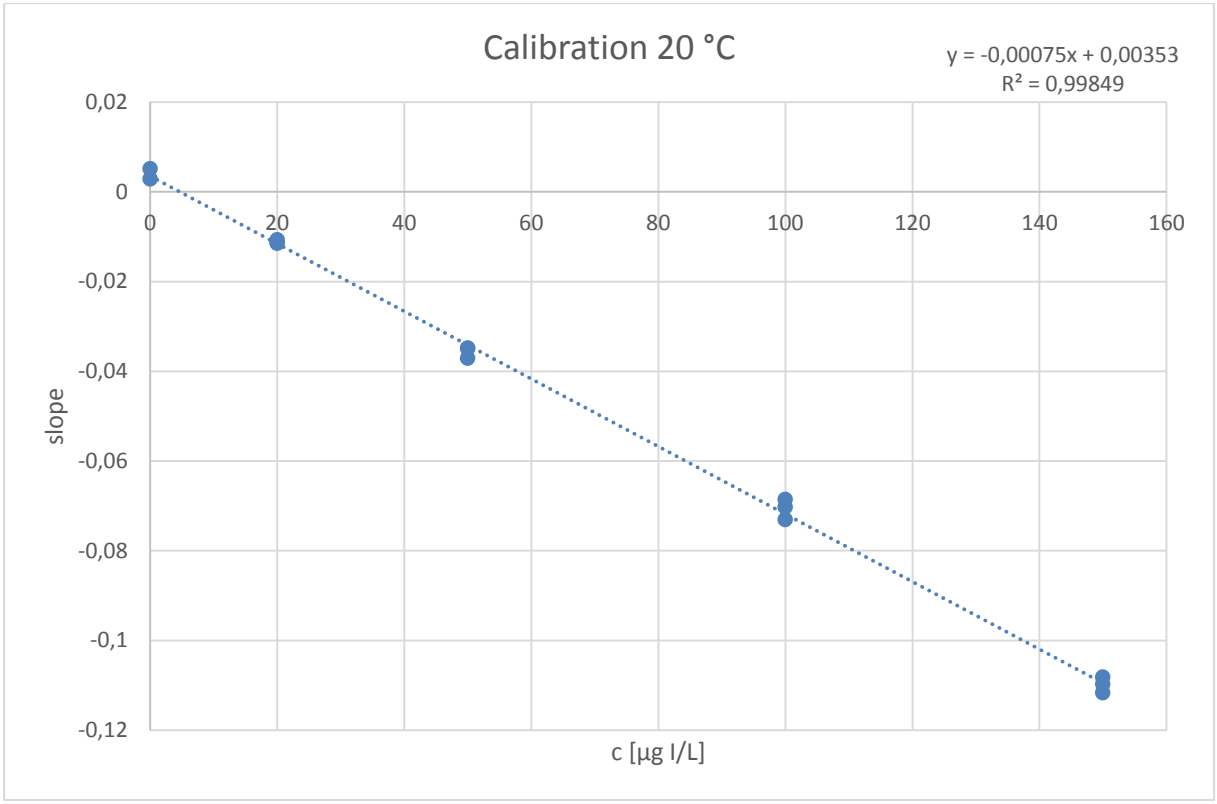

Figure 41. calibration curve iodine, 20 °C, mini photometer, without 200 µg I/l

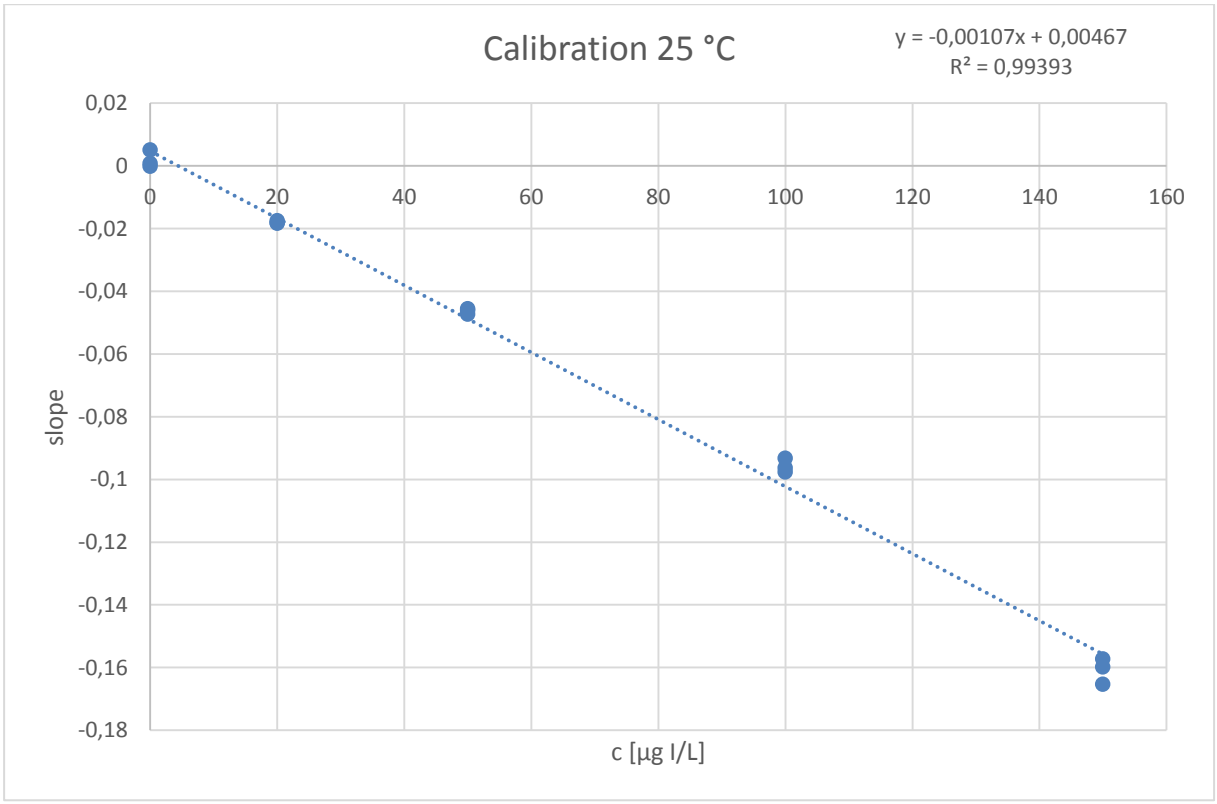

Figure 42. calibration curve iodine, 25 °C, mini photometer

Calibration curve at 25 °C is shown in figure 42. Linearity was achieved with a correlation coefficient of 0.9939. The slope was steeper than for 15 and 20 °C. Standards with a concentration of 200 µg/l had to be excluded since the slope was rather high. It is assumed, that the high concentration in combination with the elevated temperature resulted in a very fast reaction that could not be followed appropriately by the photometer.

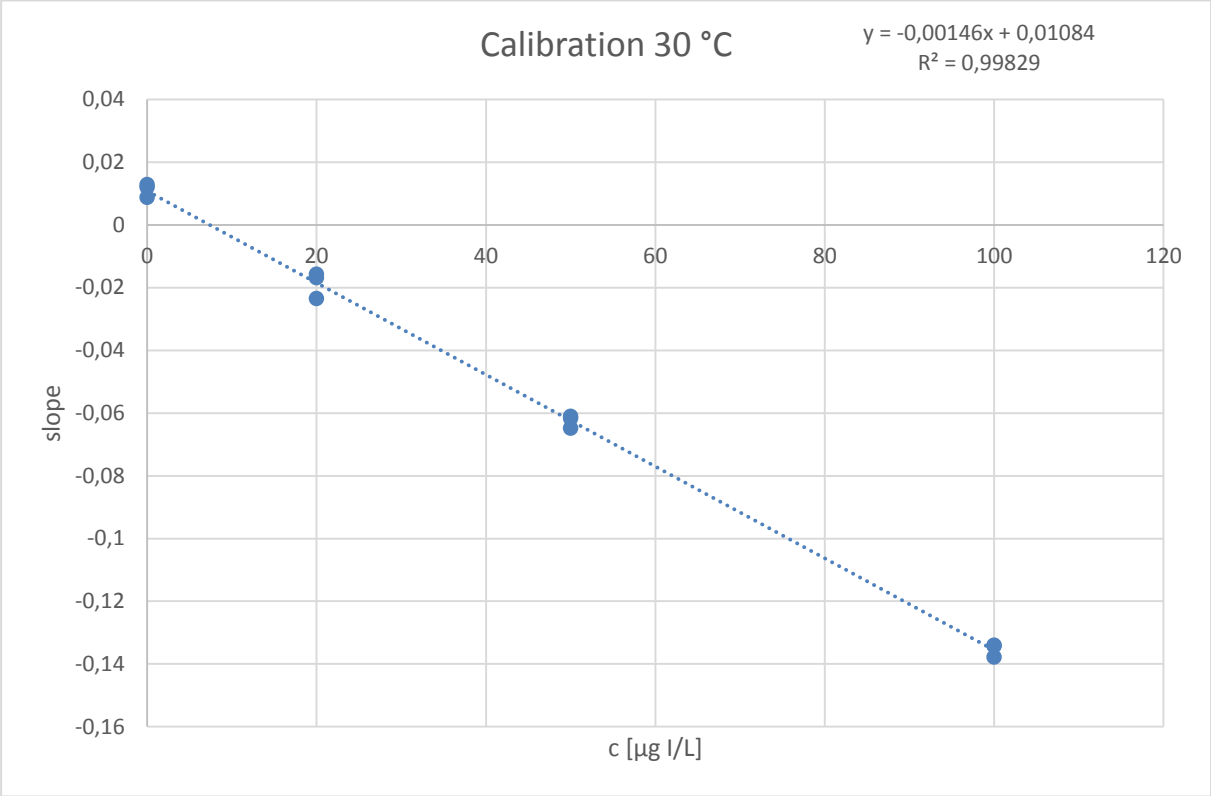

Figure 43. calibration curve iodine, 30 °C, mini photometer

Calibration curve at 30 °C is shown in figure 43. Linearity was achieved with a correlation coefficient of 0.9983. The slope was steeper than for 15, 20 and 25 °C. Standards with a concentration of 150 µg/l and 200 µg/l had to be excluded since the slopes were too high -as discussed above.

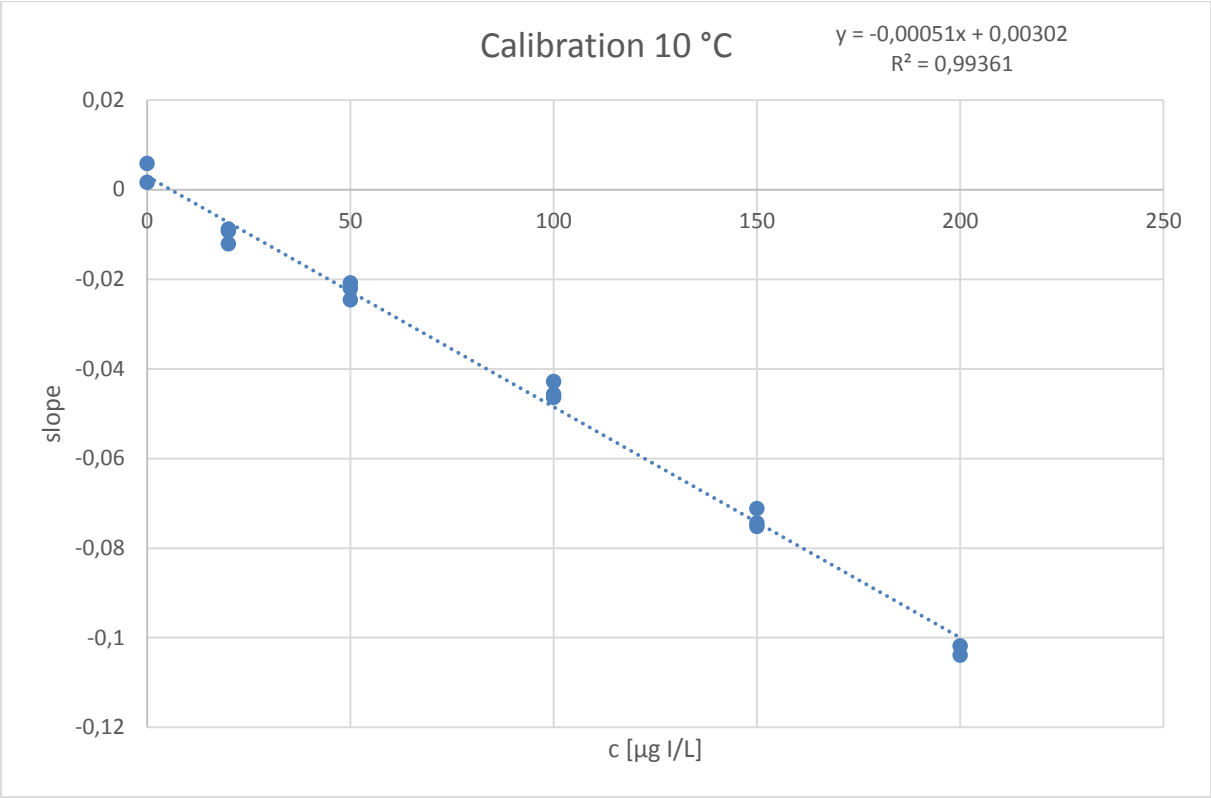

Figure 44. calibration curve iodine, 10 °C, mini photometer

Calibration curve at 30 °C is shown in figure 44. Linearity was achieved with a correlation coefficient of 0.9936. The slope was flatter than for all other temperatures. As expected, standards with 150 and 200 µg/l were measurable in contrast to 25 and 30 °C. This result was as expected since the reaction proceeds slower with decreased temperature.

All temperatures and slopes are compiled in table 8 and figure 45:

Table 8. slopes vs. temperatures mini photometer

T [°C]			slope
10	10	°C	-0.00051
15	15	°C	-0.00060
20	20	°C	-0.00075
23	RT		-0.00079
25	25	°C	-0.00107
30	30	°C	-0.00146

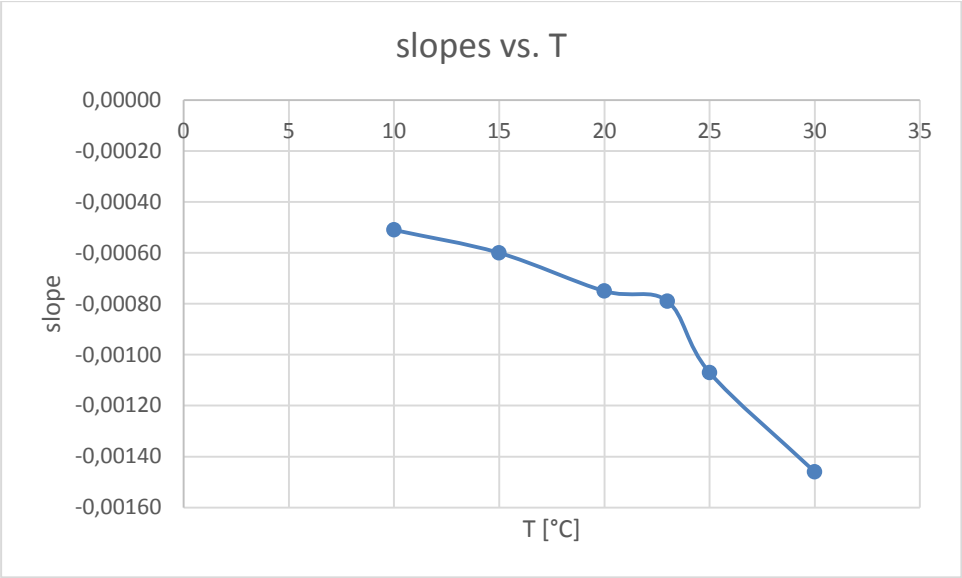

Figure 45. slope vs. temperature mini photometer

As it can be seen in figure 45 the slope increased with rising temperature. The reason for that was that the reaction proceeds faster with higher temperature. From these data it can be concluded, that a stable reaction temperature is of prime importance for the Sandell-Kolthoff reaction.

3.7.2 Stability of the acidified sample mixed with As-stock solution

Due to the importance of the temperature for the Sandell-Kolthoff reaction, the acidification of the sample with sulphuric acid must be followed by a cooling step. Though the reaction temperature has to be similar for all samples and calibration standards, the cooling of the sample is preferred, as at temperatures higher than RT the reaction progresses faster than the photometer can follow. From the experimental point of view the addition of the As-stock solution is desirable. Therefore, the stability of acidified samples containing already the appropriate volume of As-stock solution was investigated. Samples were measured where H_2O, H_2SO_4 and As were transferred to the PS-vial. Cerium and Iodate were added after 0, 1, 5 and 15 min. The samples were measured by the mini photometer.

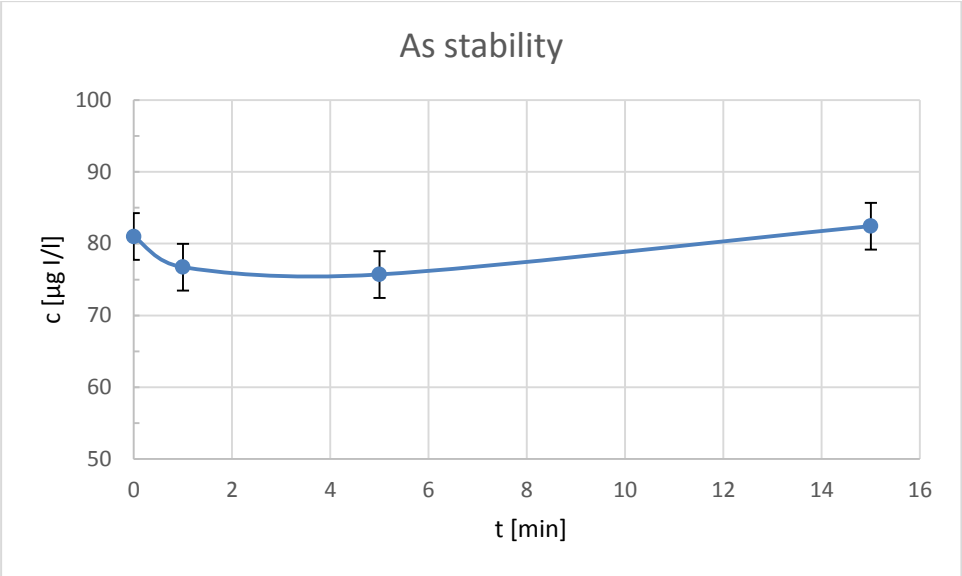

Figure 46. As stability in H_2SO_4 with time

As it can be seen in figure 46 the time of adding arsenic to the sulfuric medium did not have an influence on the measured concentration within the investigated period of 15 min.

3.7.3 Mixing of the reagents using a flow system

Up to now, the samples and calibration standards were mixed manually with the necessary reagents. For an automized setup, this mixing step must be conducted in a flow system.
A (100 µg/l stock solution was measured 4 times. A peristaltic pump with 4 inlets was used.to mix the reagents (H_2SO_4, As, Ce) and sample online.

measurement technique: Mini photometer, Sandell-Kolthoff
cuvette: d = 1 cm, t_{total} = 2 min, t_{scan} = 0.5 seconds, light source = 250 AU, T = 20 °C
pump: 10 rpm

Solutions had to be diluted in advance to achieve the same concentrations inside the cuvette as it had been for previous experiments. Total volume of the measurement solution was 10 ml and so 2.5 ml were assigned to each inlet. The preparation of the components is shown in table 9:

Table 9. 4 inlets, mini photometer, dilution of components

H_2SO_4	H_2O	As	H_2O	Ce	H_2O	Sample	H_2O
1.3 ml	1.2 ml	0.5 ml	2.0 ml	0.375 ml	2.125 ml	1 ml	1.5 ml

The experiment did not show any reproducible results pictured in figure 47:

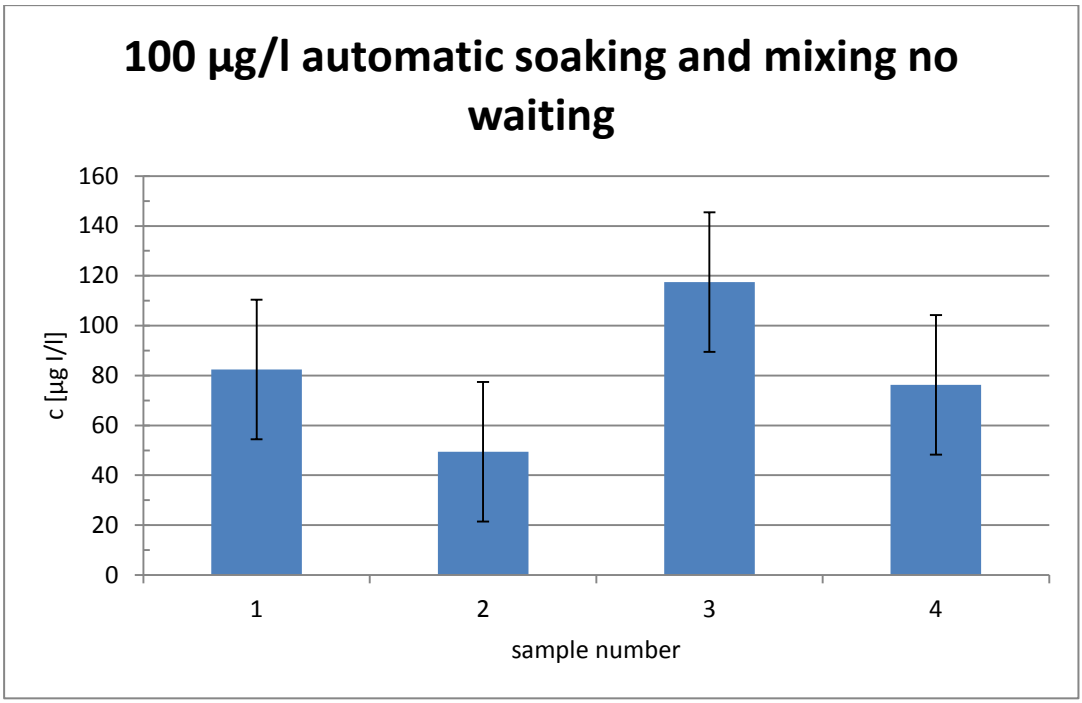

Figure 47. mini photometer automatic mixing, no waiting

Therefore a waiting time of 45 seconds was added between the point where the sample was pumped into the cuvette and the start of the measurement to allow the reaction mixture to stabilize the temperature. Results are shown below in figure 48:

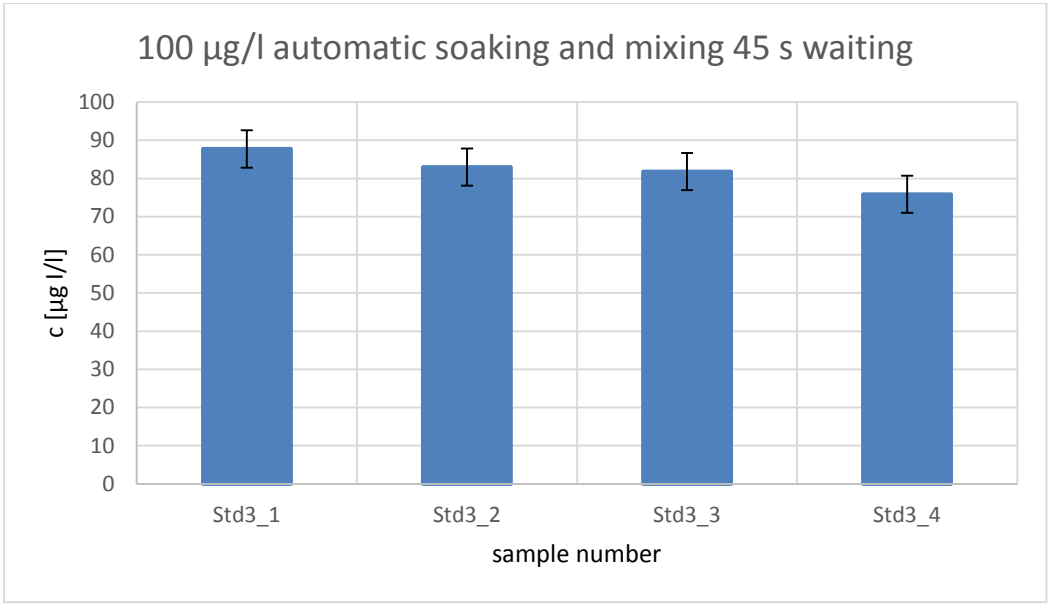

Figure 48. mini photometer automatic mixing, 45 s waiting

To conclude samples could be measured by automatic mixing if a waiting time of 45 seconds had been used. A waiting time was needed since as we discovered later on, air bubbles needed the time to settle down inside the cuvette. Otherwise these air bubbles were influencing the reaction negatively.

4 METHOD VALIDATION USING CERTIFIED REFERENCE MATERIALS

4.1 Optimization of the digestion for high sample mass

Reference materials should be used for method validation but the iodine content in biological samples is rather low (about 1 µg/g). Therefore a determination with the current sample masses was not possible. Based on the previous experiments it was concluded, that a two-step digestion procedure should be used in combination with greater volumes of chloric acid. The latter in turn required the use of larger eprouvettes for the digestion procedure - dimensions were doubled (d_{inner} = 2.8 cm, h = 20 cm).

Different masses of milk powder were spiked with KI. The samples were digested according to the two step wet digestion. Acid volume was adjusted to the sample weight. Then the samples were measured on the mini photometer with automatic mixing of the sample and the reagents.

digestion procedure:
Blanks_1-5: 0.5 ml H_2O was digested; digestion vessel: big eprouvette;
T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
milk powder_50mg_1-5: 0.05 g of milk powder, 0.5 ml H_2O added, spiked with KI to yield 100 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: big eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
70, 100, 150 and 200 mg were treated alike
digestion agent: first step: 1.8 ml of $HClO_3$ for 50 mg, 2.5 ml for 70 mg etc., 0.5 ml of HNO_3 conc. was retained for all samples; second step: same amount of $HClO_3$ as in step 1
digestion residue made up to 10 ml with H_2O
measurement technique: Mini photometer, Sandell-Kolthoff, 4 inlets
cuvette: d = 1 cm, t_{total} = 1 min, t_{scan} = 0.5 seconds, light source = 250 AU, T = 20 °C
pump: 10 rpm
waiting time: 45 seconds
standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O

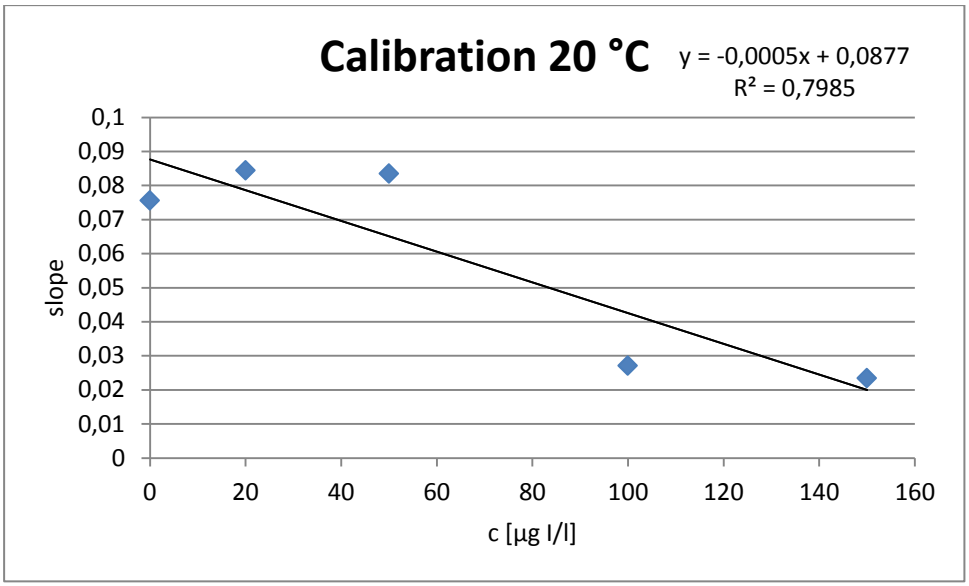

Figure 49. calibration curve iodine, 4 inlets, 20 °C, mini photometer

As it can be seen in figure 49 the calibration was not reproducible. The samples were not measured since there was no calibration available. Initially, the reason for this unexpected behaviour was unclear and the waiting time of 45 s prior start of the measurement was suspected to be too long.
Consequently the experiment was repeated and the waiting time was decreased to 30 seconds.

measurement technique: Mini photometer, Sandell-Kolthoff, 4 inlets
cuvette: d = 1 cm, t_{total} = 1 min, t_{scan} = 0.5 seconds, light source = 250 AU, T = 20 °C
pump: 10 rpm
waiting time: 30 seconds
standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O

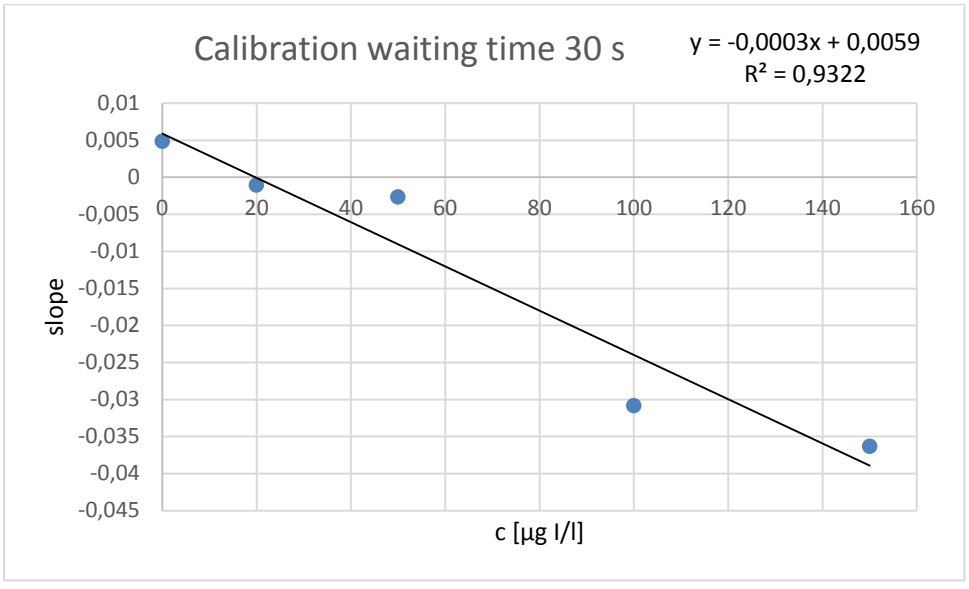

Figure 50. calibration curve iodine, 4 inlets, 20 °C, 30 s waiting time, mini photometer

Figure 50 shows the calibration with a waiting time of 30 seconds. Calibration was slightly better compared to the waiting time of 45 seconds. Nevertheless, it was far from linear and it remains questionable, if there is an improvement or just a statistical effect.
During the experiment tiny gas bubbles were observed in the tubing. They were accumulating right before the cuvette. The small gas bubbles accumulated to one big bubble that had been pumped into the cuvette causing unreproducible absorption conditions and light scattering. The occurrence of the bubbles rooted in the fact that the tubes had to be moved from a rinsing solution into a reagent or sample and then back again. Inevitably, each transfer created a small air bubble in the tube.
In order to avoid the accumulation of air bubbles in the cuvette, the pump speed was increased by a factor of four (to 40 rpm). Thereby small bubbles were pushed quickly through the system without accumulating in the cuvette.

measurement technique: Mini photometer, Sandell-Kolthoff, 4 inlets
cuvette: d = 1 cm, t_{total} = 1 min, t_{scan} = 0.5 seconds, light source = 250 AU, T = 20 °C
pump: 40 rpm
waiting time: 30 seconds
standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O
samples: 1 ml made up to 10 ml with H_2O

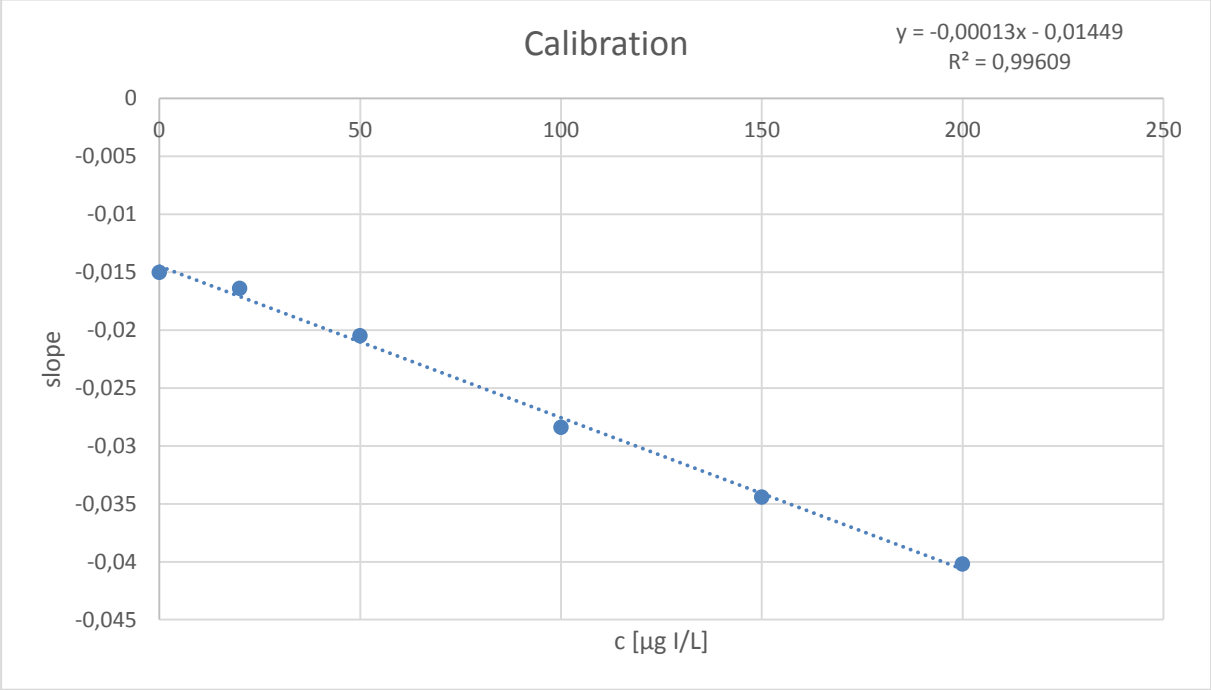

Figure 51. calibration curve iodine, 20 °C, 40 rpm, mini photometer

As it can be seen in figure 51 it was possible to record a calibration. It was very important to have a pump velocity of 40 rpm. This prevented small air bubbles from accumulating in front of the cuvette.

Nevertheless the blank values were 10 times higher compared to the experiments without the flow system (manual mixing). As the blank values were in the range of the spiked samples, no reasonable results could be obtained. In order to exclude hardware problems with the mini photometer, the experiment was repeated using the "reference method" on the Agilent Cary spectrophotometer.

measurement technique: Cary spectrophotometer, Sandell-Kolthoff
cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min
standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O
samples: 1 ml made up to 10 ml with H_2O

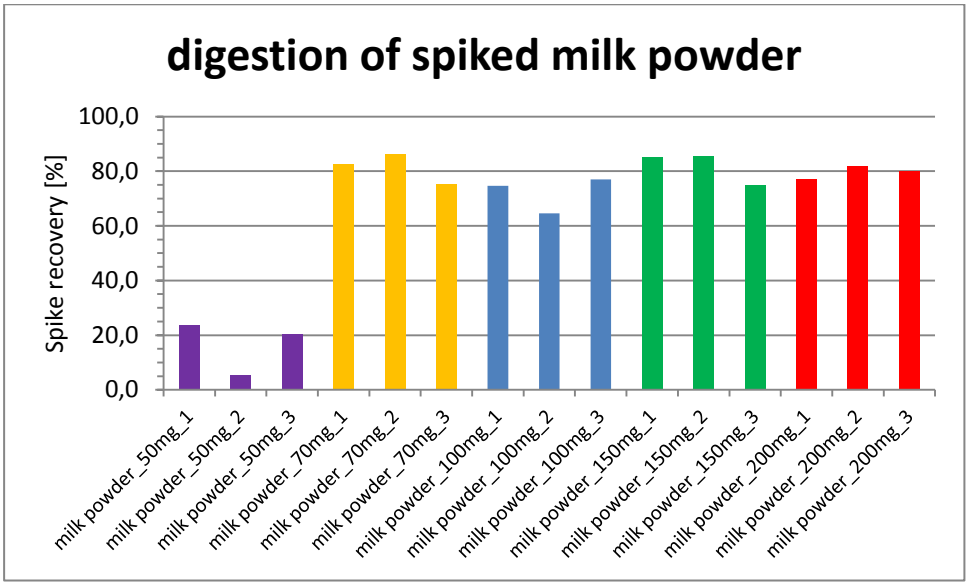

Figure 52. results digestion of spiked milk powder, Agilent Cary; each bar represents a single measurement

Figure 52 shows spike recoveries of approximately 80 % for all sample masses, except 50 mg. The reason for the low iodine recoveries for 50 mg are surprising. As always the end of the digestion had been indicated by the solution turning colourless. Samples with 50 mg had hardly any sample volume left when turning colourless, suggesting iodine losses at this point. To conclude sample weights until 50 mg need to be digested by using the smaller eprouvettes (d_{inner} = 2.8 cm, h = 10 cm).
The low recoveries for samples with 70-200 mg might be explained by residual HNO_3. It was suggested that HNO_3 condensed on the glass wall due to the eprouvettes being 10 cm higher. The residual HNO_3 could have led to unwanted absorption at 317 nm. Blanks displayed a value of 2.87 $\pm$ 0.19 µg/l on average.

To clarify these questions another set of experiments was conducted using the mini-photometer. Measurement solutions were prepared in three different ways: 1. Mixing of the four components in the classical flow system; 2. Manually mixing of the four components and 3. Pumping each of the four components into a vial and manually mix the components thereafter. The last experiment was conducted to identify potential deviations in the flow rate of each of the four channels.

measurement technique: Mini photometer, Sandell-Kolthoff
cuvette: d = 1 cm, t_{total} = 1 min, t_{scan} = 0.5 seconds, light source = 250 AU, T = 20 °C
pump: 30 rpm
waiting time: 30 s

sample 1: soaking in each component by 4 inlets into cuvette, mixing online, measurement
sample 2: measurement solution manually prepared, shaking, soaking in, measurement
sample 3: soaking in each component by 4 inlets into vial, shaking, soaking in, measurement

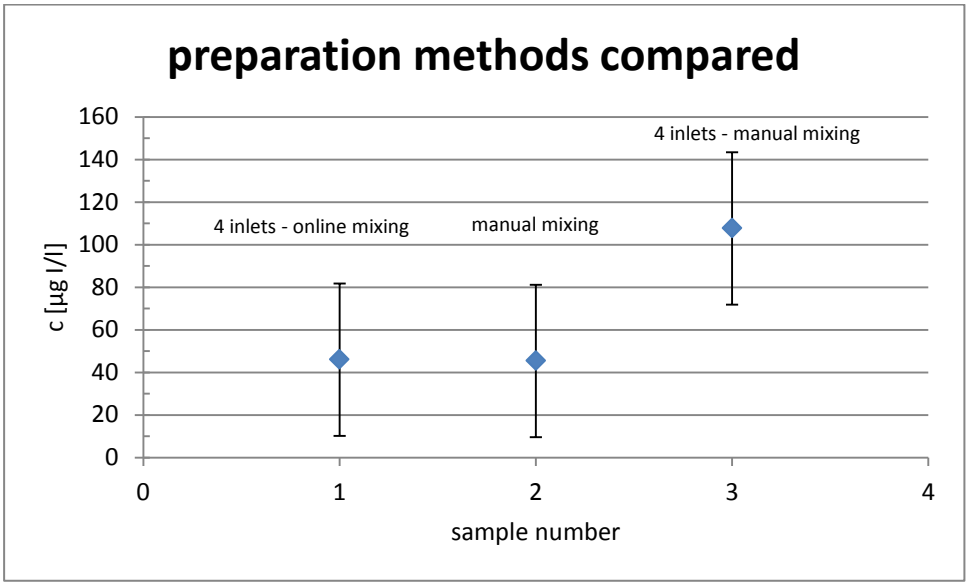

Figure 53. comparison preparation methods, mini photometer

As it can be seen in figure 53 the concentrations are the same for soaking in over 4 inlets with mixing online (45.9 µg/l) (1) and for manually preparing the solution (45.4 µg/l) (2). Although the time had been kept constant for all samples, method (3) showed a higher concentration with 107.6 µg/l. There was no difference between mixing online and manual mixing.

A wavelength scan was performed for all samples in order to identify residual HNO_3.

measurement technique: Cary spectrophotometer, Sandell-Kolthoff
cuvette: d = 0.5 cm, λ = 400-200 nm
samples: 1 ml made up to 10 ml with H_2O

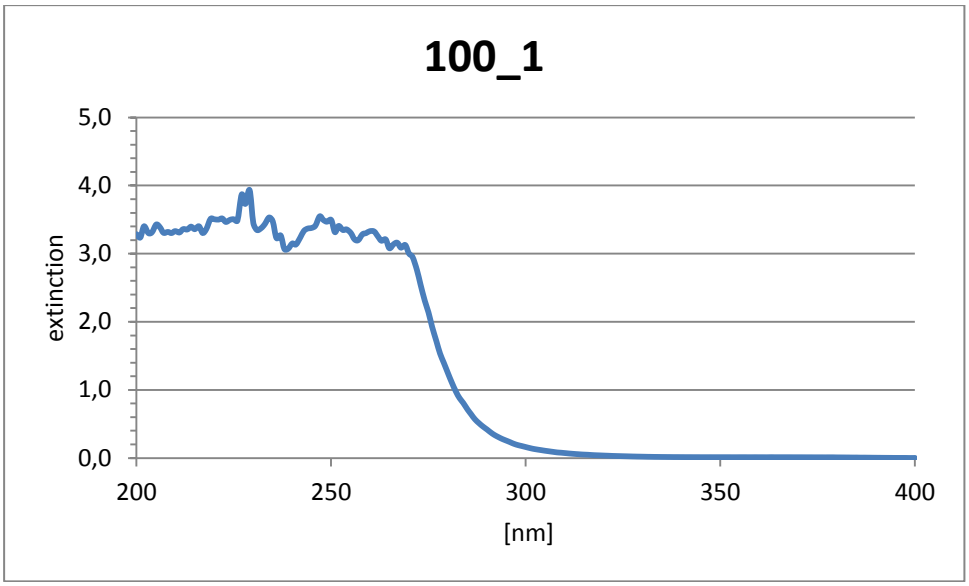

Figure 54. scan Agilent Cary 100_1

Figure 54 shows the wavelength scan of sample 100_1. There was no absorption from residual HNO_3 visible.

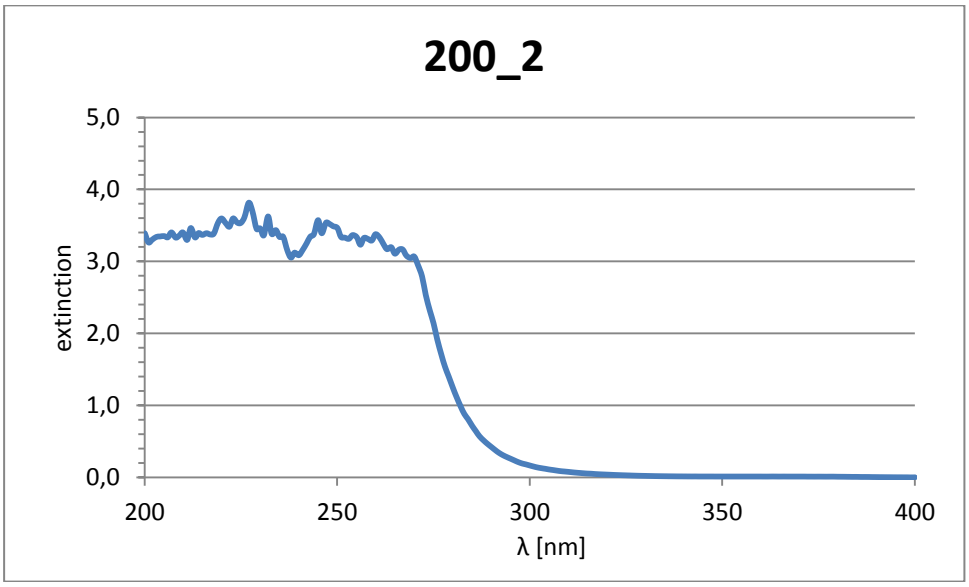

Figure 55. scan Agilent Cary 200_2

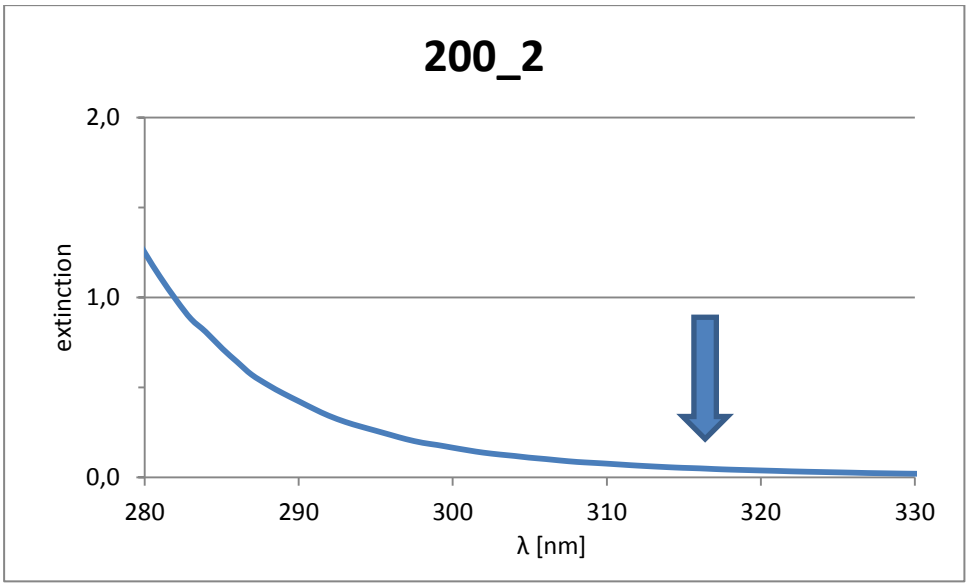

Figure 56. scan Agilent Cary 200_2, zoomed in

The same unwanted HNO_3 absorption was visible for sample 200_2 depicted in figure 56. Nevertheless these small absorptions could be responsible for the lack in recovery. As the wavelength scan revealed no clear signal of HNO_3 in the spectrum the concentration of this acid can be expected to be low. To unambiguously exclude problems with nitric acid, the digestion was repeated with eprouvettes of the same diameter but lower height.

Different masses of milk powder were spiked with KI. The samples were digested according to the two step wet digestion. Big eprouvettes were cut to make them 15 cm high. Thereby, HNO_3 can be expected to be evaporated without residues easier. Acid volume was adjusted to the sample weight. Then the samples were measured by the Agilent Cary spectrophotometer.

digestion procedure:

Blanks_1-3: 0.5 ml H_2O was digested; digestion vessel: big eprouvette, cut;

T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes

100_1-3: 0.1 g of milk powder, 0.5 ml H_2O added, spiked with KI to yield 100 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: big eprouvette, cut; T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes

200 mg were treated alike

digestion agent: first step: 3.6 ml of $HClO_3$ for 100 mg, 7.2 ml of $HClO_3$ for 200 mg etc., 0.5 ml of HNO_3 conc. was retained for all samples; second step: same amount of $HClO_3$ as in step 1

digestion residue made up to 10 ml with H_2O

measurement technique: Cary spectrophotometer, Sandell-Kolthoff

cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min

standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O

samples: 1 ml made up to 10 ml with H_2O

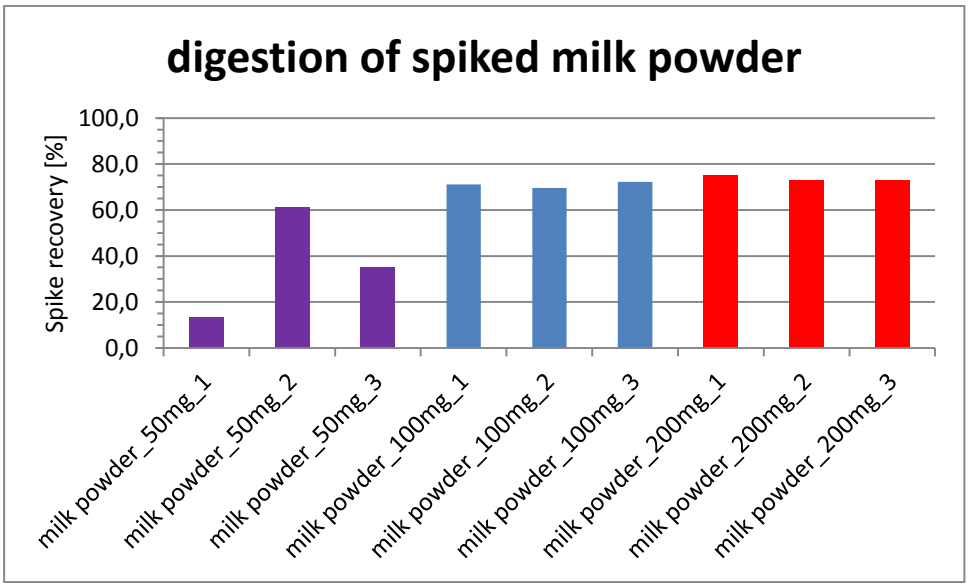

Figure 57. results digestion, eprouvettes with h = 15 cm, Agilent Cary

Figure 57 shows the recoveries for the digestion with lower eprouvettes (h = 15 cm). Obviously the recoveries did not improve. Initially, it was assumed that the HNO_3 would be easier to evaporate from shorter eprouvettes, but this was not the case. Blanks displayed a value of 1.48 $\pm$ 0.28 µg/l on average which was only half the value from the bigger eprouvettes.
Samples were measured using the mini-photometer, too

measurement technique: Mini photometer, Sandell-Kolthoff, 4 inlets
cuvette: d = 1 cm, t_{total} = 1 min, t_{scan} = 0.5 seconds, light source = 250 AU, T = 20 °C
pump: 30 rpm
waiting time: 30 s

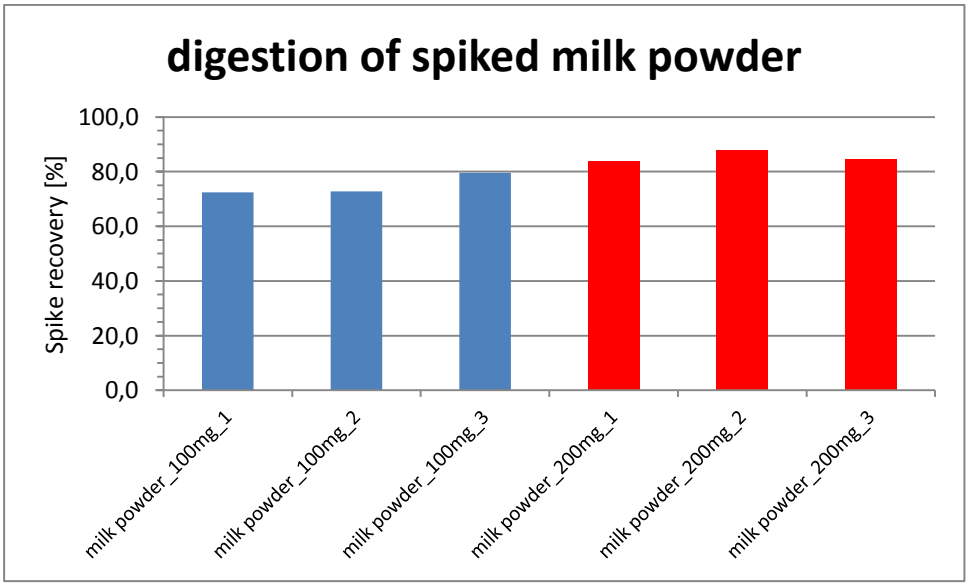

Figure 58. results digestion, eprouvettes with h = 15 cm, mini photometer; each bar represents a single measurement

The recoveries are shown in figure 58. Recoveries were slightly better than the ones obtained by the Agilent Cary. To conclude the measurements using the mini-photometer are as reliable as the ones obtained with the Agilent Cary spectrophotometer. However, the recoveries were still too low. All blanks were < LOQ. Based on the data obtained it remained unclear, if there was an advantage of the eprouvettes with reduced height over the ones with standard height. To clarify also this point another experiment was conducted.

100 mg of milk powder were spiked with KI. The samples were digested according to the two step wet digestion. Big eprouvettes (h = 20 cm) were compared to the cut eprouvettes (h = 15 cm). Cut eprouvettes were used to compare the influence of different HNO_3 volumes on the results. Then the samples were measured using the Agilent Cary spectrophotometer.

digestion procedure:
LA_1-3: 0.1 g of milk powder, 0.5 ml H_2O added, spiked with KI to yield 100 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: big eprouvette, cut; T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
KA_1-3: 0.1 g of milk powder, 0.5 ml H_2O added, spiked with KI to yield 100 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: big eprouvette, cut; T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
KB_1-3: 0.1 g of milk powder, 0.5 ml H_2O added, spiked with KI to yield 100 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: big eprouvette, cut; T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
digestion acid: 3.6 ml of $HClO_3$ for all samples within first and second step, 0.5 ml of HNO_3 for LA1-3 and KA1-3; 1.0 ml of HNO_3 for KB1-3
digestion residue made up to 10 ml with H_2O

measurement technique: Cary spectrophotometer, Sandell-Kolthoff
cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min
standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O
samples: 1 ml made up to 10 ml with H_2O

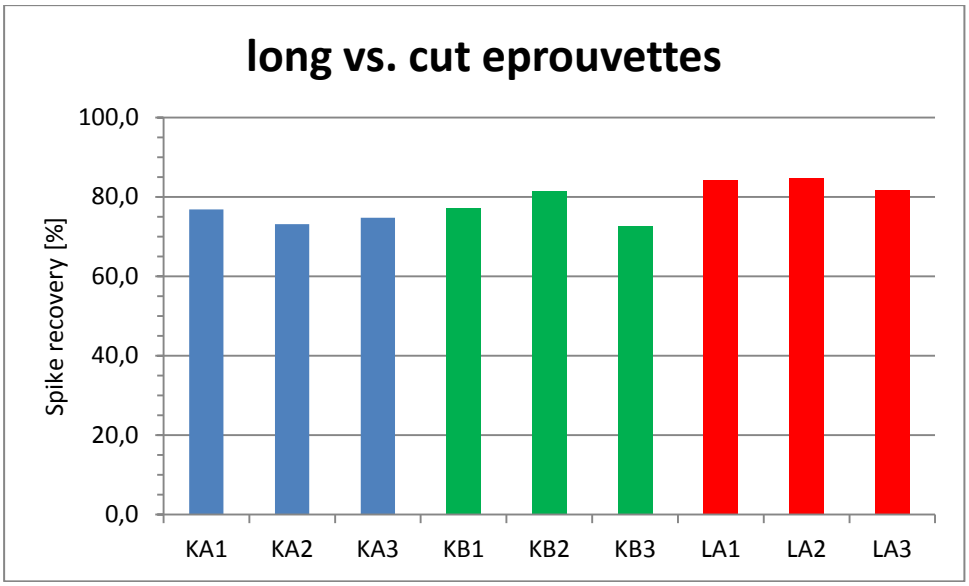

Figure 59. results digestion, eprouvettes with h = 15 and 20 cm, HNO_3 constant or varied, Agilent Cary; each bar represents a single measurement

Figure 59 shows that only small differences were encountered within the tested approaches. The best results were measured for the big eprouvettes (h = 20 cm) with 0.5 ml HNO_3 (LA), though the results were only marginally better than for the cut ones (h = 15 cm) with the same amount of HNO_3 (KA). The opposite had been expected since it should be easier to evaporate HNO_3 by using lower vessels. The vessels with higher HNO_3 volume (KB) were slightly better than the ones with lower volume (KA). That was also opposed to what had been expected. It should be easier to evaporate smaller volumes of HNO_3. Nevertheless HNO_3 should evaporate anyway at a temperature of 170 °C and especially by using lower eprouvettes. Samples were measured by ICPMS for verification purposes.

measurement technique: ICPMS, rinsing solution: H_2O
standard concentrations: 0, 2, 5, 10, 15, 20 µg I/l prepared with H_2O
samples: 0.1 ml made up to 10 ml with H_2O

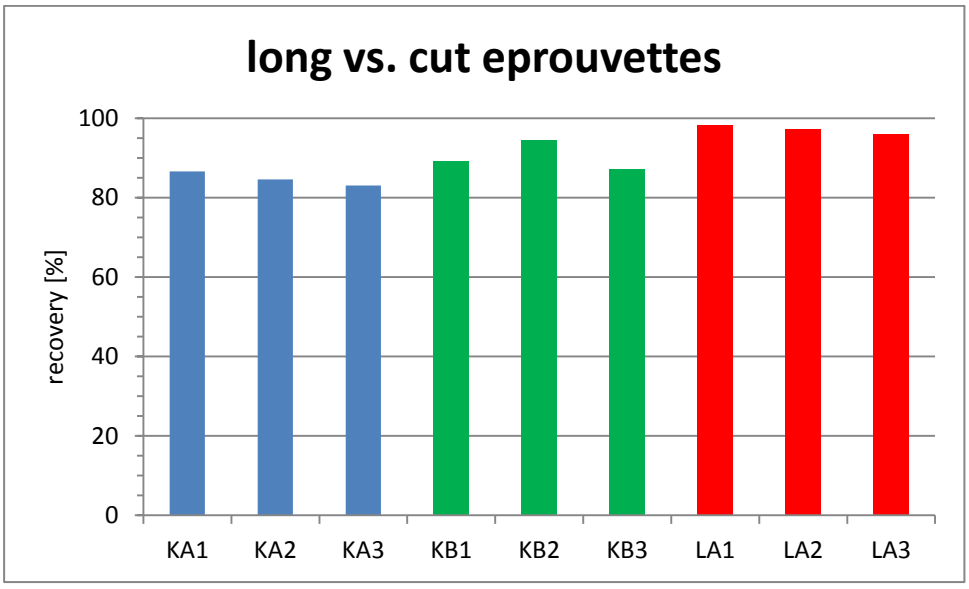

Figure 60. results digestion, eprouvettes with h = 15 and 20 cm, HNO_3 constant or varied, ICPMS; each bar represents a single measurement

Figure 60 shows the results obtained by ICPMS. LOD was 0.27 µg/l. In general the recoveries were better than the ones obtained by the Cary. This showed that there had been still unwanted HNO_3 absorption during the Cary measurement. ICPMS was not influenced by HNO_3. To summarize the big eprouvettes were used for all the following experiments. In addition the HNO_3 volume of 0.5 ml was retained.

In order to verify, that no experimental error obscured the results of the previous experiment it was repeated. 100 mg of milk powder were spiked with KI. The samples were digested according to the two step wet digestion. Big eprouvettes (h = 20 cm) were used. Then the samples were measured using the Agilent Cary spectrophotometer.

digestion procedure:
Blanks_1-3: 0.5 ml H_2O was digested; digestion vessel: big eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
milk powder_100mg_1-3: 0.1 g of milk powder, 0.5 ml H_2O added, spiked with KI to yield 100 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: big eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
digestion acid: 3.6 ml of $HClO_3$ for all samples within first and second step, 0.5 ml of HNO_3 for all samples only during first step
digestion residue made up to 10 ml with H_2O
measurement technique: Cary spectrophotometer, Sandell-Kolthoff
cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min
standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O
samples: 1 ml made up to 10 ml with H_2O

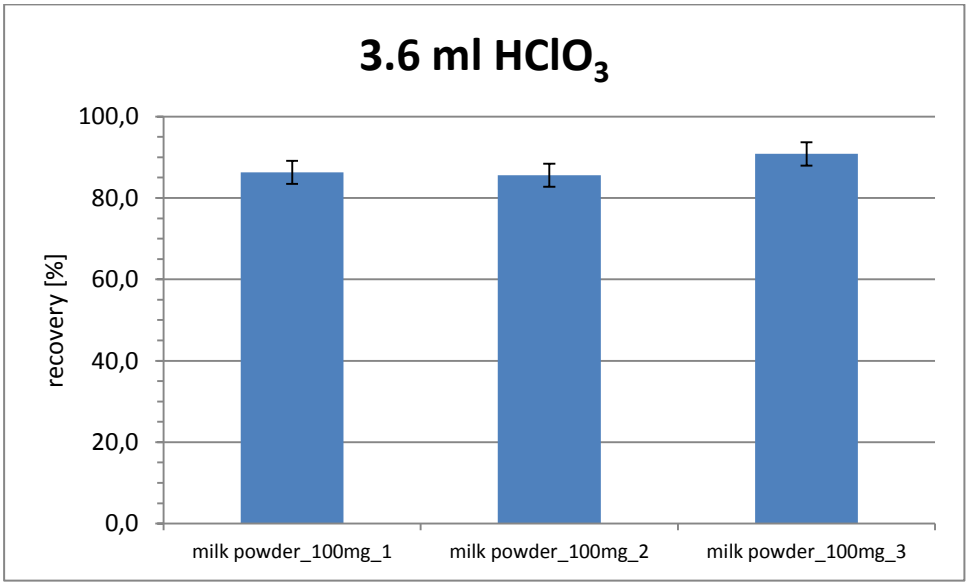

Figure 61. results digestion of 100 mg, 3.6 ml $HClO_3$, eprouvettes with h = 20 cm, Agilent Cary

As it can be seen in figure 61 the recoveries were at 87.6 ± 2.8 %. These values confirmed the results from the last experiment. It was decided to keep this method for further experiments. Blanks displayed a value of 2.02 ± 0.12 µg/l on average. The following experiments focussed on increasing the sample mass to 200 mg.

200 mg of milk powder were spiked with KI. The samples were digested according to the two step wet digestion. Big eprouvettes (h = 20 cm) were used. Then the samples were measured by the Agilent Cary spectrophotometer. The goal was to check if the recoveries for 200 mg were as good as for 100 mg.

digestion procedure:
200_1-3: 0.1 g of milk powder, 0.5 ml H_2O added, spiked with KI to yield 100 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: big eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
digestion acid: 7.2 ml of $HClO_3$ for all samples within first and second step, 0.5 ml of HNO_3 for all samples only during first step
digestion residue made up to 10 ml with H_2O
measurement technique: Cary spectrophotometer, Sandell-Kolthoff
cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min
standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O
samples: 1 ml made up to 10 ml with H_2O

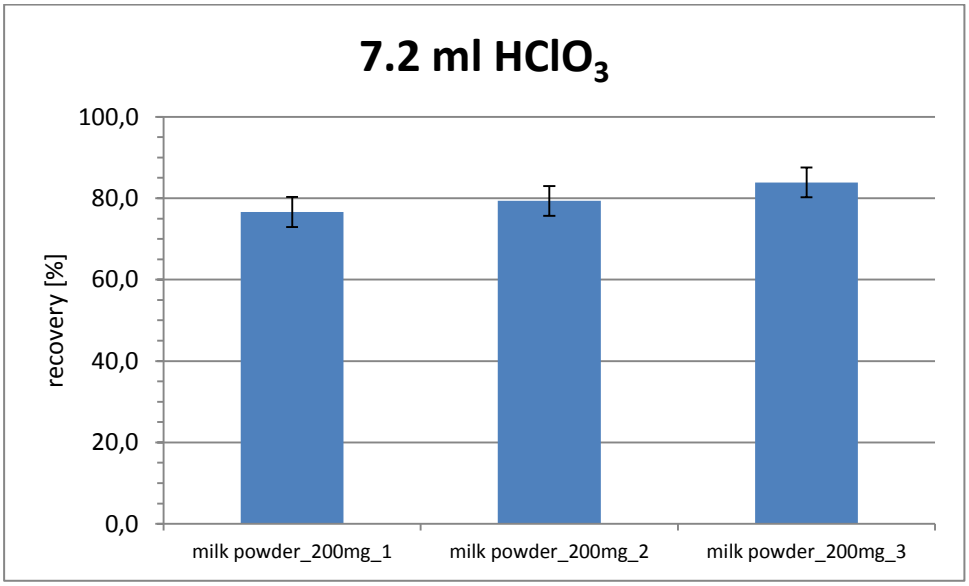

Figure 62. results digestion of 200 mg, 7.2 ml $HClO_3$, eprouvettes with h = 20 cm, Agilent Cary

Figure 62 shows the recoveries obtained for a sample weight of 200 mg. Recoveries were at 79.9 $\pm$ 3.7 %. Recoveries were 10 % lower than for 100 mg. This could be explained by residual organic carbon that influences the catalytic reaction. To confirm this hypothesis a TOC (total organic carbon) measurement was performed by ICP-OES. The higher the TOC value the lower the recovery can be expected.

measurement technique: ICPOES, rinsing solution: H_2O, internal standard: 0.1 ml of Scandium out of 1,000 mg/l stock
standard concentrations: 0, 10, 50, 100, and 200 mg/l TOC prepared with H_2O
samples: without dilution

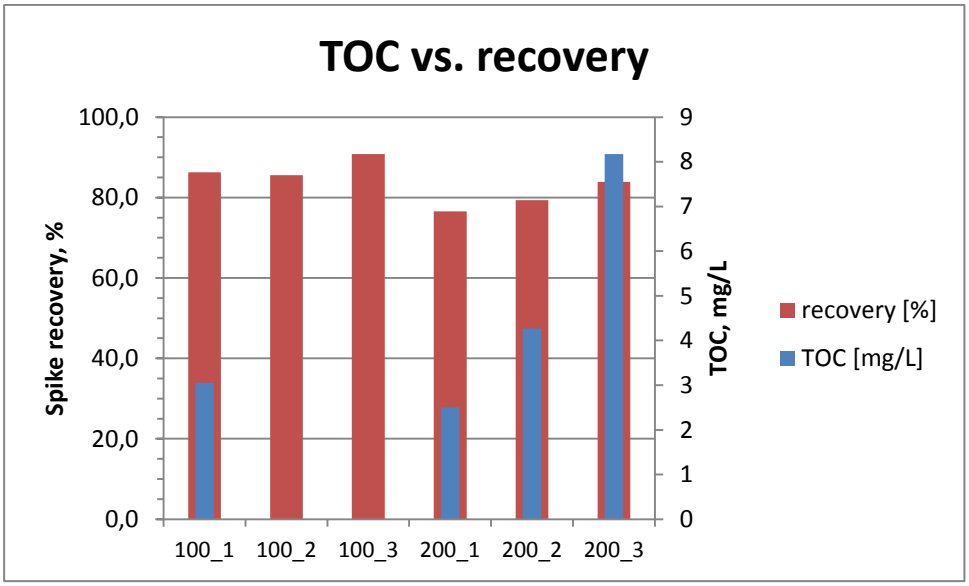

Figure 63. results TOC measurement, ICPOES

Figure 63 shows mixed results. Sample 100_3 showed the highest recovery and had no TOC at all. However sample 200_3 had the best recovery of all 200 mg samples but also had the highest TOC of all samples. However, it is interesting to note, that for the 200 mg sample mass in all tree samples investigated a TOC above the LOQ was determined. The following experiment focussed on repeating the digestion with 200 mg sample weight and further increasing the acid volume to 10 ml chloric acid as more acid can be expected to better decompose the organic matrix.

200 mg of milk powder were spiked with KI. The samples were digested according to the two step wet digestion. Big eprouvettes (h = 20 cm) were used and 10 ml $HClO_3$ were added in the first step. Then the samples were measured using the Agilent Cary spectrophotometer.

digestion procedure:

200_1-3: 0.1 g of milk powder, 0.5 ml H_2O added, spiked with KI to yield 100 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: big eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes

digestion acid: 10 ml of $HClO_3$ for all samples within first and second step, 0.5 ml of HNO_3 for all samples only during first step

digestion residue made up to 10 ml with H_2O

measurement technique: Cary spectrophotometer, Sandell-Kolthoff

cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min

standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O

samples: 1 ml made up to 10 ml with H_2O

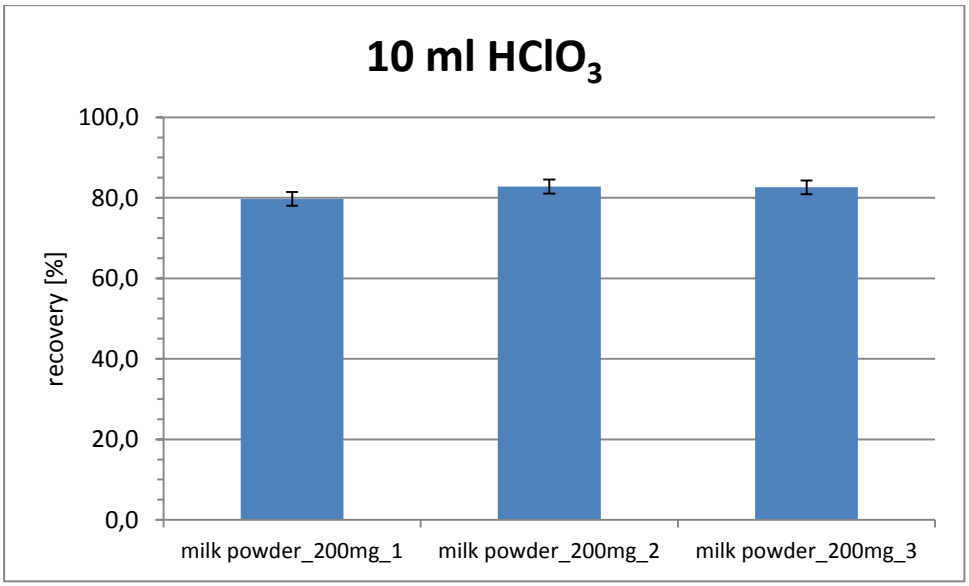

Figure 64. results digestion of 200 mg, 10 ml $HClO_3$, Agilent Cary

Figure 64 shows the recoveries for 10 ml of digestion acid. The results (81.7 $\pm$ 1.7 %) did not improve compared to 7.2 ml (79.9 $\pm$ 3.7 %). Therefore it was not necessary to further increase the acid volume. The following experiment focussed on measuring reference materials.

4.2 Reference materials

100 mg of different reference materials were digested and analysed using the Agilent Cary. After digestion the remaining liquid was made up to 5 ml with water (instead of 10 ml) due to low iodine content of the samples. The goal was to examine if biological materials could be digested by wet digestion and analysed by Sandell-Kolthoff.

digestion procedure:

Blanks_1-5: 0.5 ml H_2O was digested; digestion vessel: big eprouvette;

T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes

fish_1-5: 0.1 g of fish powder, 0.5 ml H_2O added, digestion vessel: big eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes

grass_1-5 (meadow grass), BCR151_1-5 (spiked milk powder), and algae_1-5 (brown algae) had been treated alike

digestion acid: 3.6 ml of $HClO_3$ for all samples within first and second step, 0.5 ml of HNO_3 for all samples only during first step

digestion residue made up to 5 ml with H_2O

measurement technique: Cary spectrophotometer, Sandell-Kolthoff

cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min

standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O

samples: 2 ml made up to 10 ml with H_2O (fish powder, milk powder and grass)

0.1ml made up to 10 ml with H_2O (brown algae)

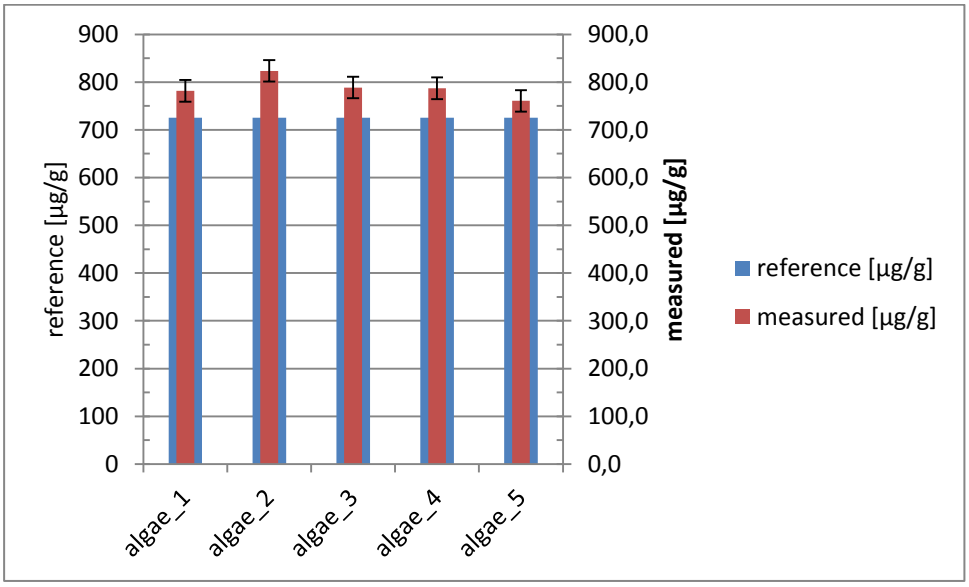

Figure 65. results digestion, reference material: algae, Agilent Cary

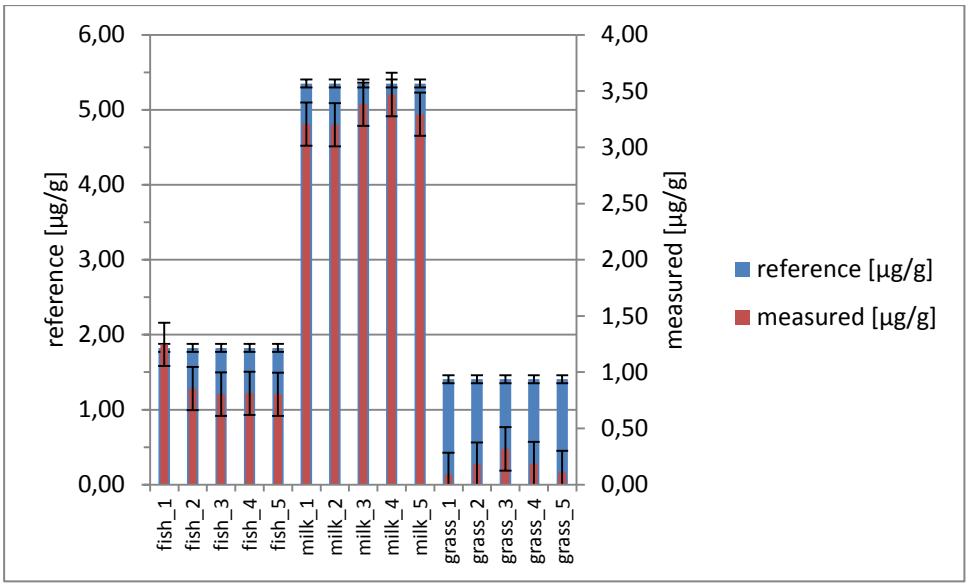

Figure 66. results digestion, reference materials, Agilent Cary

Figures 65 and 66 show the iodine contents of the reference materials together with their reference values. Brown algae achieved the best results with iodine contents of 788.3 ± 22.7 µg/g (reference 725.6 ± 15.8 µg/g) followed by milk powder with 3.31 ± 0.12 µg/g (reference 5.35 ± 0.14 µg/g).

Fish powder showed a content of 0.90 ± 0.19 µg/g (reference 1.823 ± 0.054 µg/g) and grass achieved 0.18 ± 0.09 µg/g (reference 1.407 ± 0.042 µg/g). Brown algae had the highest iodine content and the best correlation with the reference value whereas grass had the lowest iodine content and the worst correlation. Milk powder had a higher iodine content and higher reference value (5.35 µg/g) than fish powder. It is important to note, that the iodine concentration within the measurement solutions for fish, milk and grass were below the linear range of the calibration. Though the calibration was not linear in this range, the signals were reproducible and consequently not at or below the LOQ. Blanks displayed a value of 1.06 ± 0.31 µg/l on average. The following experiment focussed on increasing the sample mass for fish powder.

200 mg of fish powder were again digested and analysed as described for the previous experiment. The final volume after digestion and volume adjustment was 3 ml due to low iodine content of the fish powder. The milk powder samples from the previous experiment were measured again. The total volume of the measurement solution was decreased to 5 ml. The goal was to examine if the recoveries could be improved by increasing the iodine content within the measurement solution.

digestion procedure:
Fish_1-4: 0.2 g of fish powder, 0.5 ml H_2O added, digestion vessel: big eprouvette;
T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
digestion acid: 7.2 ml of $HClO_3$ for all samples within first and second step, 0.5 ml of HNO_3 for all samples only during first step

digestion residue made up to 3 ml with H_2O

measurement technique: Cary spectrophotometer, Sandell-Kolthoff

cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min

standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O

samples: 2 ml made up to 5 ml with H_2O (fish powder and milk powder)

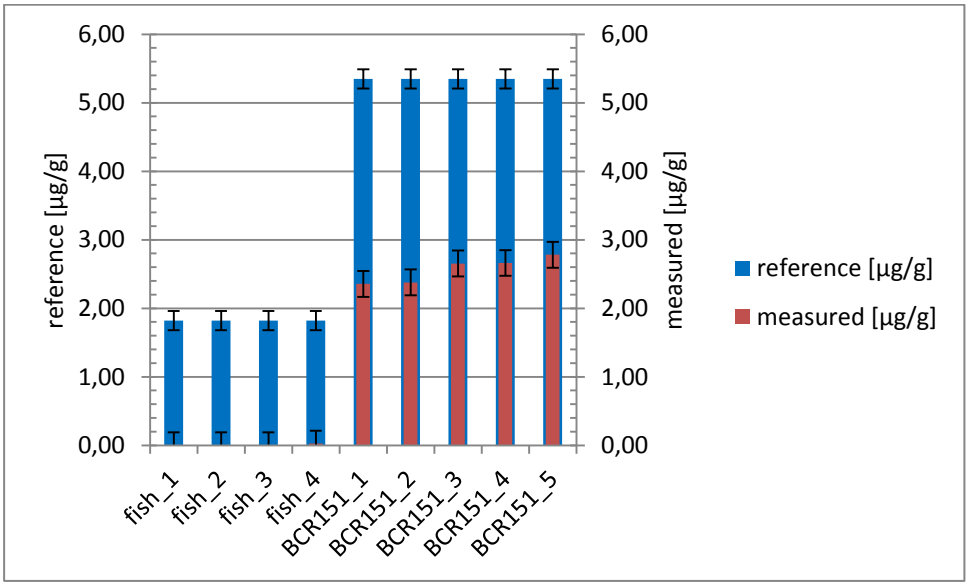

Figure 67. results digestion, fish powder (n = 1) and BCR 151

Figure 67 shows the iodine contents of fish (0.25 µg/g, n =1) with a reference value of 1.823 ± 0.054 µg/g and BCR 151 (2.57 ± 0.19 µg/g) with a reference value of 5.35 ± 0.14 µg/g. Reducing the total volume of the measurement solutions to 5 ml did not lead to better iodine contents. Much in contrary, the found concentration in the reference materials was even lower than in the previous experiment as strikingly illustrated by the fact, that making up to a final volume of only 3 ml after digestion resulted in iodine concentrations below the LOQ (negative iodine contents for all samples except fish_4). Clearly, there is an effect of the digestion process. Higher dilution of the digestion solutions eased these problems, but limited the applicability of this method for samples with low iodine concentrations. Further experiments focussed on digesting with only $HClO_3$ and increasing the iodine content within the measurement solution.

100 mg of milk powder and grass were digested and analysed using the Agilent Cary spectrophotometer. There was only $HClO_3$ used for digestion to apply harsher reaction conditions and avoid traces from nitric acid in the digested samples. The $HClO_3$ volume was increased to 5 ml. After digestion the remaining acid solution was made up to 3 ml with water due to low iodine content of the grass. The total volume of the measurement solution was retained at 5 ml for all samples. The goal was to examine if the recoveries could be improved by increasing the iodine content within the measurement solution while applying harsher reaction conditions.

digestion procedure:

BCR151_1-5: 0.1 g of milk powder, 0.5 ml H_2O added, digestion vessel: big eprouvette;

T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes

grass_1-5) was treated alike

digestion acid: 5 ml of $HClO_3$ for all samples within first and second step, no HNO_3 added

digestion residue made up to 3 ml with H_2O (grass), made up to 5 ml for milk powder

measurement technique: Cary spectrophotometer, Sandell-Kolthoff

cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min

standard concentrations: 0, 20, 50, 100, 150, 200 µg I/l prepared with H_2O

samples: 2 ml made up to 5 ml with H_2O (grass and milk powder)

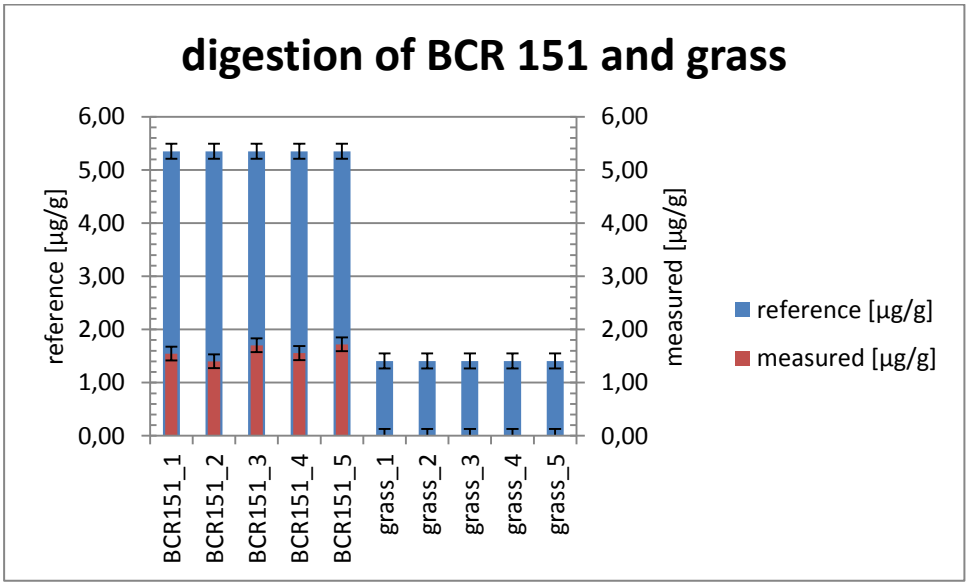

Figure 68. results digestion, BCR 151 and grass, Agilent Cary

Figure 68 shows the iodine contents for BCR 151 and grass. BCR 151 experienced an iodine content of 1.59 ± 0.13 µg/g which was very low compared to the reference (5.35 ± 0.14 µg/g). This was the lowest result compared to all other previous experiments with BCR 151. The iodine contents for grass were < LOQ with a reference value of 1.407 ± 0.042 µg/g. As it can be seen for BCR 151 the iodine contents decreased as it had been tried to increase the iodine content within the measurement solution by either larger sample mass or lower total dilution. The same pattern could be observed for grass: When the final volume after digestion and dilution of the grass sample was 5 ml and the total volume of the measurement solution was 10 ml about 10 % could of the certified iodine concentration in this reference material could be quantified (data listed in the previous experiment). In this experiment no iodine could be determined (< LOQ) as the volume of the diluted sample after digestion was only 3 ml and the total volume of the measurement solution 5 ml. Clearly, there was also no improvement from digesting only with $HClO_3$.or decreasing the volume of water used for diluting the remaining acid after digestion.

It was assumed, that degradation products of $HClO_3$ could have been present after digestion that interfered with the catalytic measurement. These issues got more pronounced the higher their concentration had been.

As the concentration of iodine in the grass reference material was very low a spiking experiment was conducted to identify potential matrix-induced problems during digestion.
Grass (grass_1) was spiked with KIO_3 to yield a concentration of 100 µg I/l and measured using the Agilent Cary. The spike recovery was only 80 %. As iodate cannot be lost during digestion it was concluded, that acid induced interferences affect Sandell-Kolthoff method and showed that the calibration and the samples were not compatible with each other. Moreover this was the explanation for the 80-90 % recoveries achieved by the spiking experiments. Clearly, the calibration solutions had to be digested and treated the same way as the samples.

To foster this theory, calibration solutions were digested in the same way as the samples. In addition 100 and 300 mg of conventional milk powder were spiked with KI, digested and then analysed using the Agilent Cary. The two step wet digestion procedure with $HClO_3$ and HNO_3 was used.

digestion procedure:
calibration solutions: 0.5 ml H_2O added, spiked with KIO_3 to yield 0, 20, 50, 100, 150 and 200 µg I/l (0, 20, 50, 100, 150 and 200 µl out of 100,000 µg I/l KIO_3-Std); digestion vessel: big eprouvette;
T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
100_1: 0.1 g of milk powder, 0.5 ml H_2O added, spiked with KI to yield 100 µg I/l (0.1 ml out of 100,000 µg I/l KI-Std); digestion vessel: big eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
300_1 was treated alike, except using 300 mg
digestion acid: 3.6 ml of $HClO_3$ for calibration solutions and 100 mg and 10.6 ml for 300 mg within first and second step, 0.5 ml of HNO_3 for calibration solutions and samples only during first step
digestion residue made up to 10 ml with H_2O (calibration solutions and samples)
measurement technique: Cary spectrophotometer, Sandell-Kolthoff
cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min
samples: 1 ml made up to 10 ml with H_2O (calibration solutions and samples)

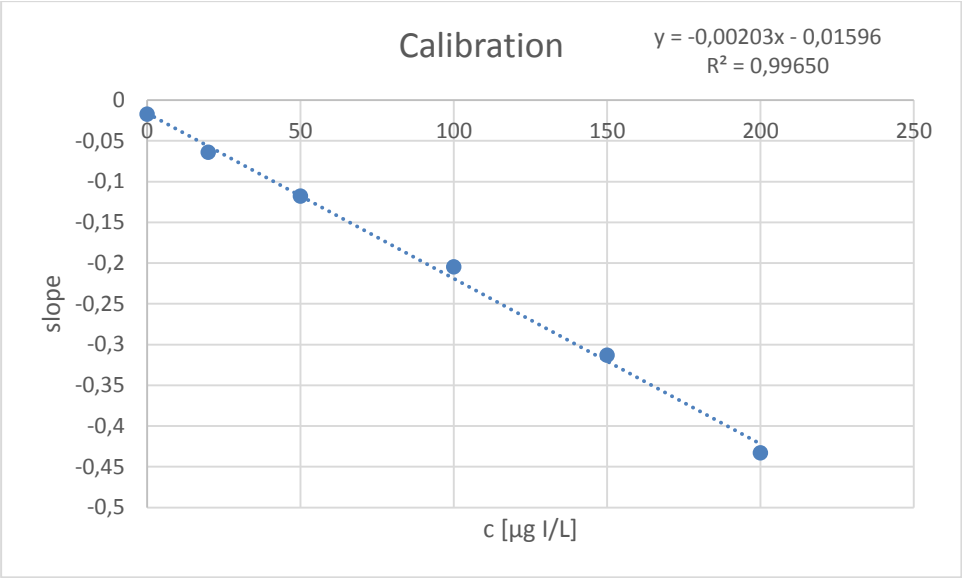

Figure 69. calibration curve iodine, digested calibration solutions, Agilent Cary

The calibration function of the digested calibration solutions is shown in figure 69. The calibration curve was linear with a correlation coefficient of 0.9965.

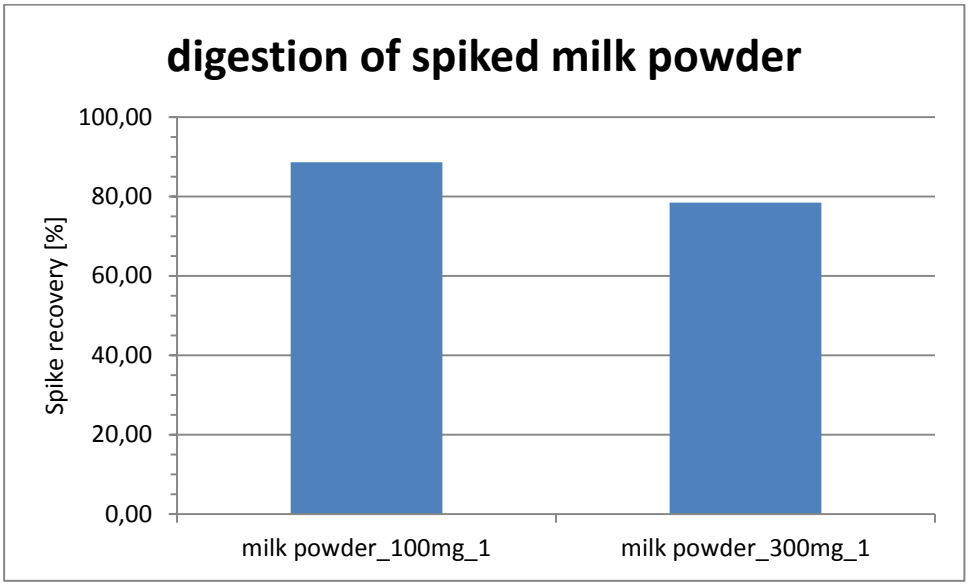

Figure 70. results digestion, spiked milk powder, n = 1 for both samples, Agilent Cary; each bar represents a single measurement

As it can be seen in figure 70 the recovery for 100 mg was 88.6 %. That was unexpectedly low. The recovery for 300 mg was even lower with approximately 78.4 %. The following experiment focussed on digesting the reference material BCR 151.

300 mg of BCR 151 were digested and then analysed using the Agilent Cary. The two step wet digestion procedure with $HClO_3$ and HNO_3 was used. The goal was to measure the iodine content by means of Sandell-Kolthoff.

digestion procedure:

Blanks_1-3: 0.5 ml H_2O was digested; digestion vessel: big eprouvette;

T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes

BCR151_1-3: 0.3 g of milk powder, 0.5 ml H_2O added, digestion vessel: big eprouvette; T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes

digestion acid: 10.6 ml of $HClO_3$ within first and second step for all samples, 0.5 ml of HNO_3 for all samples only during first step

digestion residue made up to 10 ml with H_2O

measurement technique: Cary spectrophotometer, Sandell-Kolthoff

cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min

standard concentrations: digested solutions (0, 20, 50, 100, 150, 200 µg I/l) from 10th of June 2016 were used again

samples: 2 ml made up to 10 ml with H_2O (blanks, M1 and M2)

1 ml made up to 10 ml with H_2O (M3)

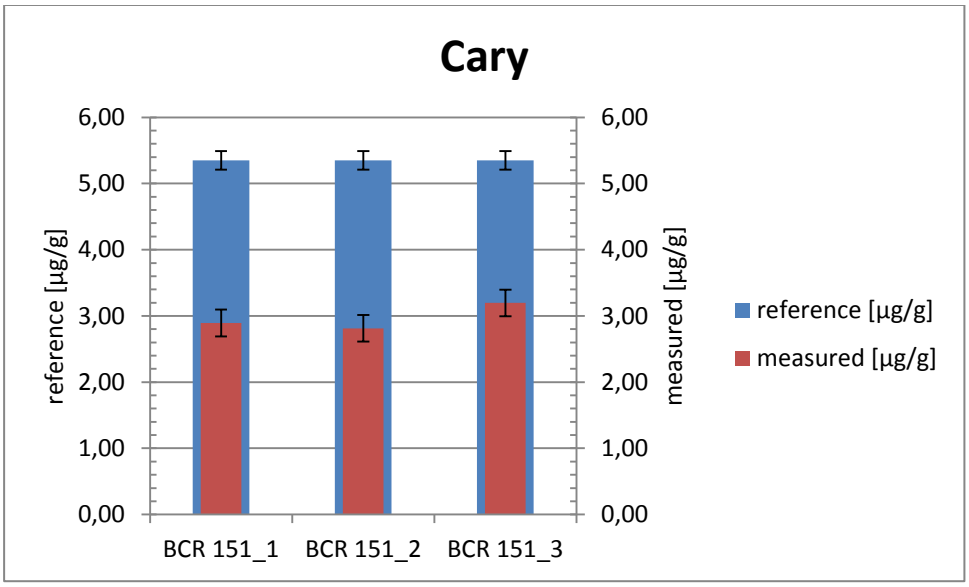

Figure 71. results digestion, BCR 151, Agilent Cary

Figure 71 shows the results for BCR 151. The concentrations obtained (2.97 $\pm$ 0.20 µg/g) were still lower than the certified value (5.35 + 0.14 µg/g) - similar results were obtained when using undigested calibration solutions. However, it is important to note that the digestion was not fully completed. The samples were foaming during digestion due to the high sample mass. The sample might not be fully digested as some of the material was sticking to the glass wall after being transported upwards by the foam. Clearly, the samples had to be hold at a certain temperature at the beginning of the digestion procedure to prevent the foaming. Blanks displayed a value of 1.29 $\pm$ 0.26 µg/l on average.

300 mg of BCR 151 were digested and then analysed using the Agilent Cary. The two step wet digestion procedure with $HClO_3$ and HNO_3 was used but the temperature program was modified: Samples were hold for 10 minutes at 95 °C at the beginning. A second pair of samples was hold at 85 °C at the beginning of the digestion. This should prevent the samples from foaming. The goal was to improve the recovery by making the digestion less harsh. BCR 151_2 from the previous experiment was measured a second time to check with the results from the previous day.

digestion procedure:
BCR151_1-2_95: 0.3 g of milk powder, 0.5 ml H_2O added, digestion vessel: big eprouvette; T/t-programme: hold for 10 minutes at 95 °C, ramp from 95-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
BCR151_1-2_85: samples were treated alike but with holding them for 10 minutes at 85 °C
digestion acid: 10.6 ml of $HClO_3$ within first and second step for all samples, 0.5 ml of HNO_3 for all samples only during first step
digestion residue made up to 10 ml with H_2O
measurement technique: Agilent Cary spectrophotometer, Sandell-Kolthoff
cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min
standard concentrations: digested solutions (0, 20, 50, 100, 150, 200 µg I/l) from 10th of June 2016 were used again
samples: 2 ml made up to 10 ml with H_2O (BCR151_1-2_95; BCR151_1-2_85)
1 ml made up to 10 ml with H_2O (BCR151_2)

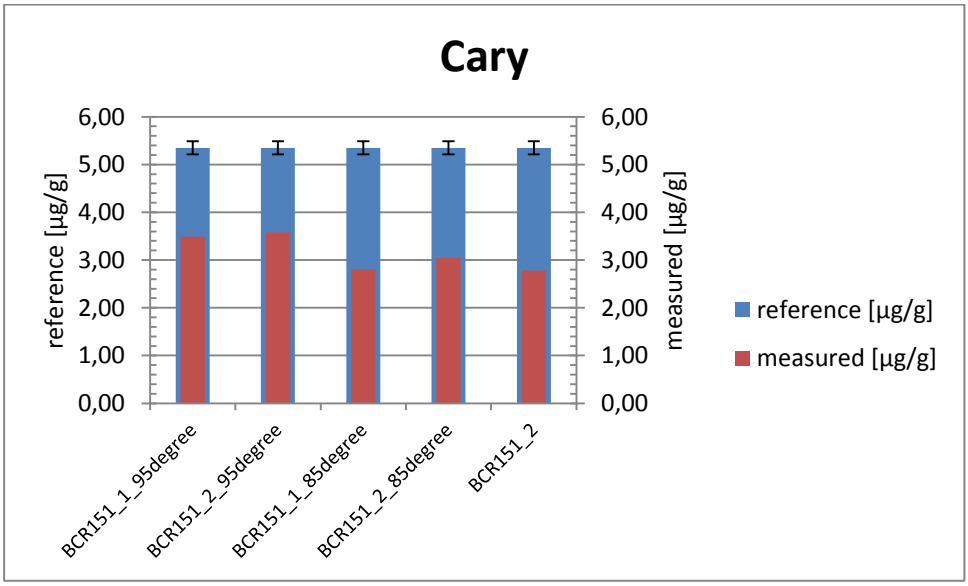

Figure 72. results digestion, BCR 151, Agilent Cary

The iodine contents for BCR 151 samples are shown in figure 72. The result for BCR151_2 was 2.78 µg/g which was the same as on the previous day (2.81 µg/g).

2 ml of digested solution were used the previous day and 1 ml was used for this experiment. The volume did not have any influence since the iodine contents did not improve. As stated before the reference value is 5.35 ± 0.14 µg/g, which none of the samples was able to achieve. Next the iodine contents could be improved with iodine contents of 3.49 µg/g and 3.58 µg/g by holding the samples at 95 °C at the beginning of the digestion. This was visible by the higher iodine contents of BCR151_1_95degree and BCR151_2_95degree compared to BCR151_2 (2.78 µg/g). On the other hand holding the samples at 85 °C barely improved the results (2.81 µg/g and 3.04 µg/g), in comparison with 2.78 µg/g of BCR151_2. 95 °C are needed to prevent foaming. To ensure, that the encountered results were not an artefact of the catalytic measurement, the iodine in all samples of this experiment was quantified by ICP-MS.

measurement technique: ICPMS, rinsing solution: H_2O
standard concentrations: 0, 2, 5, 10, 15, 20 µg I/l prepared with H_2O
samples: 0.1 ml made up to 10 ml with H_2O

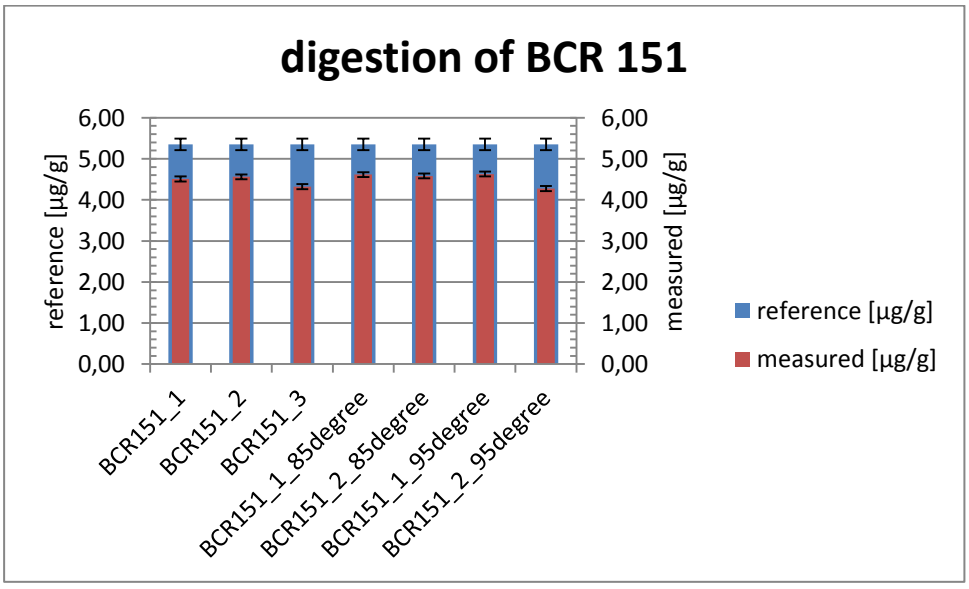

Figure 73. results digestion, BCR 151, ICPMS

Figure 73 shows that the results obtained by ICPMS were better than obtained by Sandell-Kolthoff. BCR 151_1-3 showed an iodine content of 4.46 ± 0.13 µg/g. As stated before the reference value is 5.35 ± 0.14 µg/g. Surprisingly – in contrast to Sandell-Kolthoff - there is no difference visible between the samples that were held at 85 °C (4.61 µg/g and 4.58 µg/g) and 95 °C (4.63 µg/g and 4.28 µg/g), respectively. By taking into account the uncertainty of the certified iodine value of BCR 151 the results were satisfying. Furthermore these results suggested that the matrix had a huge influence on the catalytic reaction. Therefore it was tried to measure the samples by applying standard addition.

BCR151_1_95degree was measured by the Agilent Cary but with applying standard addition according to table 10:

Table 10. Cary standard addition iodine

		KIO_3				
	sample [ml]	Vol. spike [ml]	H_2O [ml]	H_2SO_4 [ml] conc.	As [ml] stock	Ce [ml] stock
sample (2x)	1.00	0.00	6.83	1.3	0.5	0.375
sample + 50 µg/L	1.00	0.50	6.33	1.3	0.5	0.375
sample +100 µg/L	1.00	1.00	5.83	1.3	0.5	0.375
sample + 150 µg/L	1.00	1.50	5.33	1.3	0.5	0.375

The sample and three spiked solutions of the sample were measured.

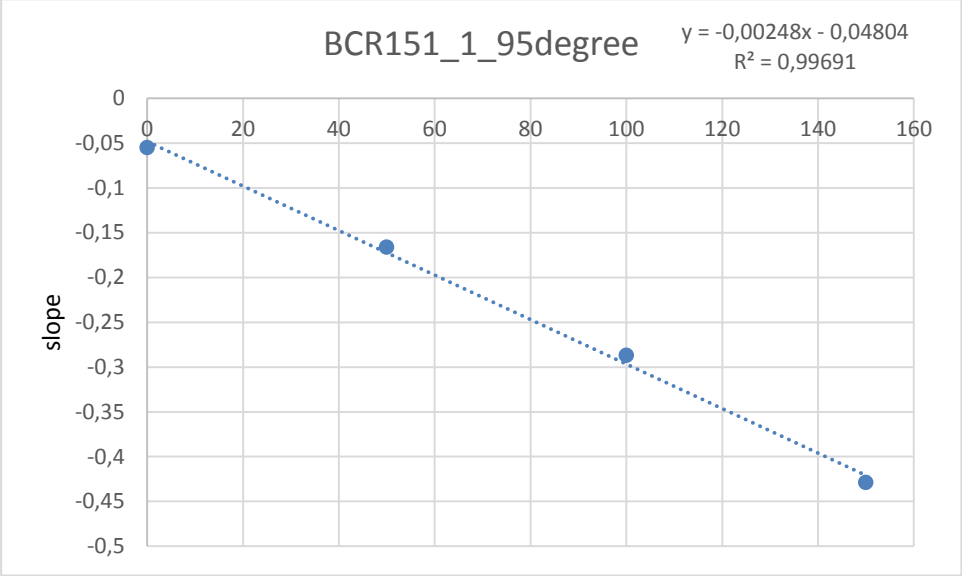

Figure 74. standard addition BCR151_1_95degree

Figure 74 shows the standard addition of sample BCR151_1_95degree. The calculation showed an iodine content of 4.34 µg/g which was almost the same result as obtained by ICPMS (4.63 µg/g). This showed that indeed a sample-matrix effect was present that caused interferences of the catalytic Sandell-Kolthoff method. As standard addition provided acceptable results the next experiment focussed on measuring the other samples by this technique.

Conventional milk powder was digested and measured using the Agilent Cary applying the method of standard addition. BCR 151 samples from the previous experiments were investigated too.

milk powder_1-4: 0.3 g of conventional milk powder, 0.5 ml H_2O added, digestion vessel: big eprouvette;
T/t-programme: hold for 10 minutes at 90 °C, ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes

digestion acid: 10.6 ml of $HClO_3$ within first and second step for all samples, 0.5 ml of HNO_3 for all samples only during first step

digestion residue made up to 5 ml with H_2O

measurement technique: Cary spectrophotometer, Sandell-Kolthoff, standard addition

cuvette: d = 1 cm, t_{total} = 2 min, t_{scan} = 0.17 min

samples: 1 ml made up to 10 ml with H_2O

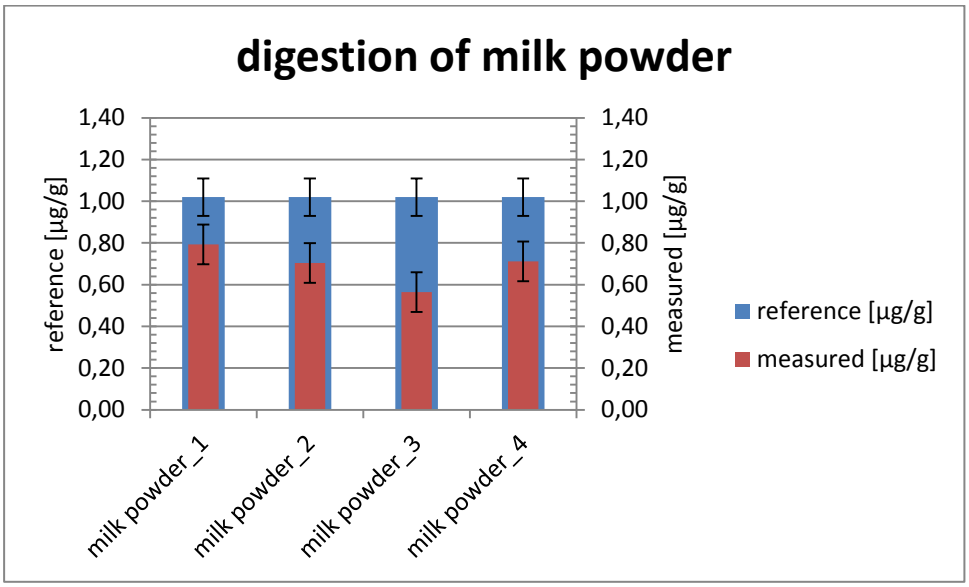

Figure 75. results digestion, milk powder, standard addition, Agilent Cary

Figure 75 shows the results obtained by standard addition for milk powder. Compared to the certified value (1.02 ± 0.09 µg/g), the iodine concentrations were low for samples milk powder_1-4 with 0.69 ± 0.10 µg/g. The samples were also measured again using the mini photometer.

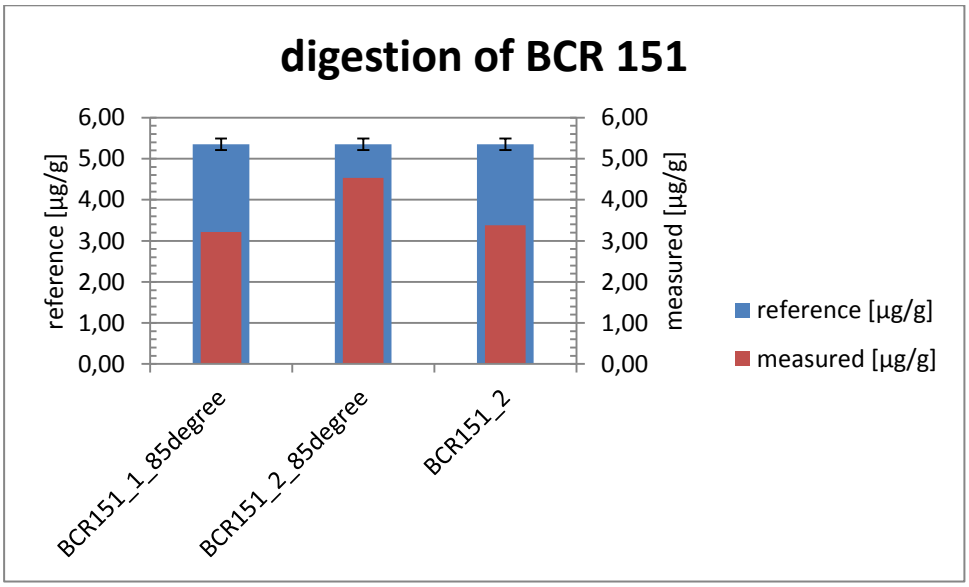

Figure 76. results digestion, BCR 151, standard addition, Agilent Cary

Figure 76 shows the results obtained by standard addition for BCR 151. Compared to the certified value (5.35 ± 0.14 µg/g), the iodine concentrations were low with 3.22 µg/g and 4.53 µg/g for the samples held at 85 °C at the beginning of the digestion. Sample BCR151_2 (no holding time at the beginning of digestion) gave 3.38 µg/g, which was also not satisfying. The samples were also measured again using the mini photometer.

Waiting time was set at 60 s since the iodine content was expected to be very low and the reaction needed time to start.

measurement technique: Mini photometer, Sandell-Kolthoff, standard addition
cuvette: d = 1 cm, t_{total} = 1 min, t_{scan} = 0.5 seconds, light source = 250 AU, T = 20 °C
pump: 30 rpm
waiting time: 60 s
samples: 1 ml made up to 10 ml with H_2O

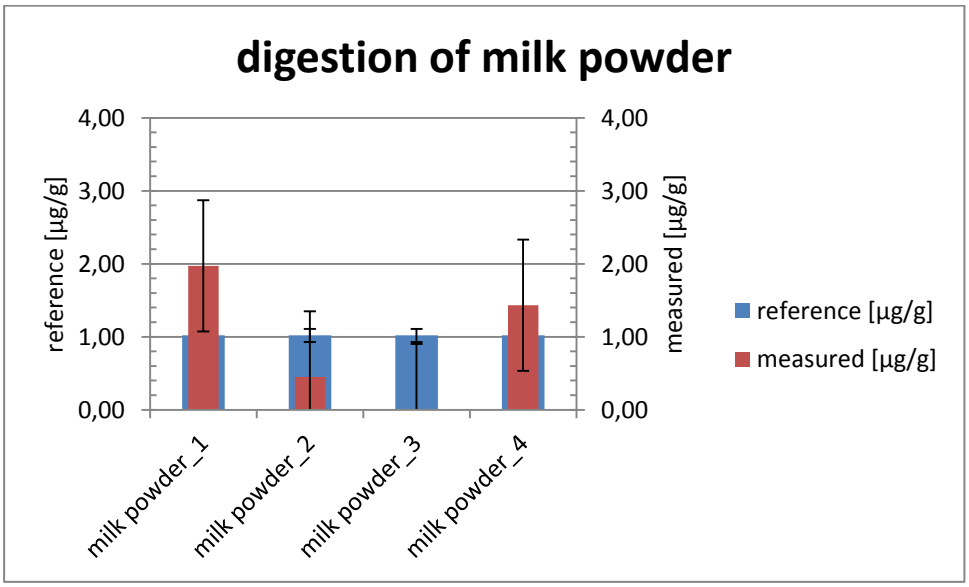

Figure 77. results digestion, BCR 151 and milk powder, standard addition, mini photometer

Figure 77 shows that none of the results (0.96 ± 0.90 µg/g) – showing huge deviation - corresponded to the certified value (1.02 + 0.09 µg/g) or the data obtained by the Agilent Cary (0.69 ± 0.10 µg/g).

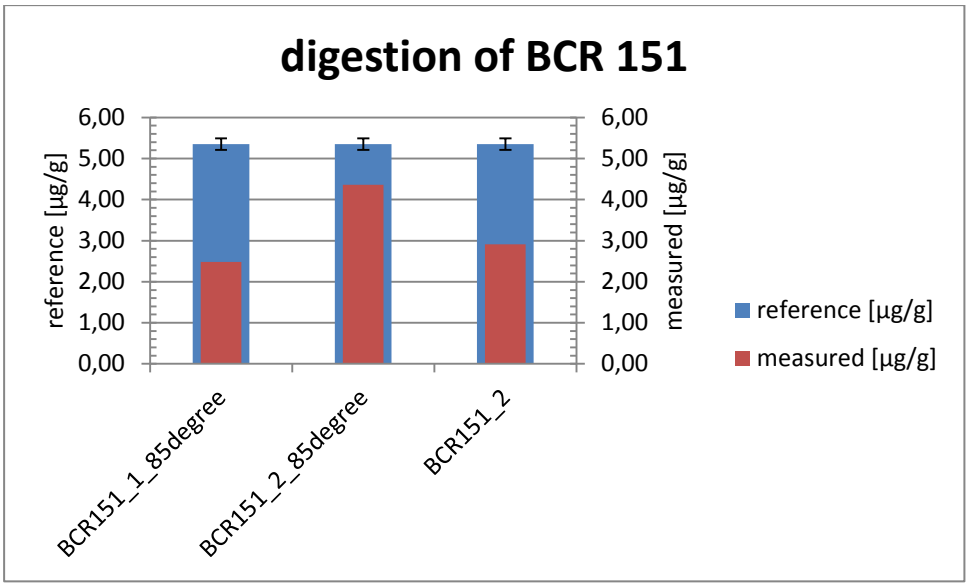

Figure 78. results digestion, BCR 151 and milk powder, standard addition, mini photometer

Figure 78 shows the results of BCR 151 obtained by standard addition on the mini photometer. Samples - held at 85 °C at the beginning of the digestion - showed iodine contents of 2.48 µg/g (Agilent Cary: 3.22 µg/g) and 4.36 µg/g (Agilent Cary: 4.53 µg/g). BCR 151_2 – no holding time – gave 2.91 µg/g (Agilent Cary: 3.38 µg/g). To conclude Agilent Cary and Mini results corresponded better for BCR 151 than for milk powder. Clearly, another issue caused these irreproducible results on the mini photometer. The following experiment focussed on retrying the standard addition with other samples.

BCR 151 was measured with standard addition using the Agilent Cary.

measurement technique: Cary spectrophotometer, Sandell-Kolthoff, standard addition

cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min

samples: 1 ml made up to 10 ml with H_2O

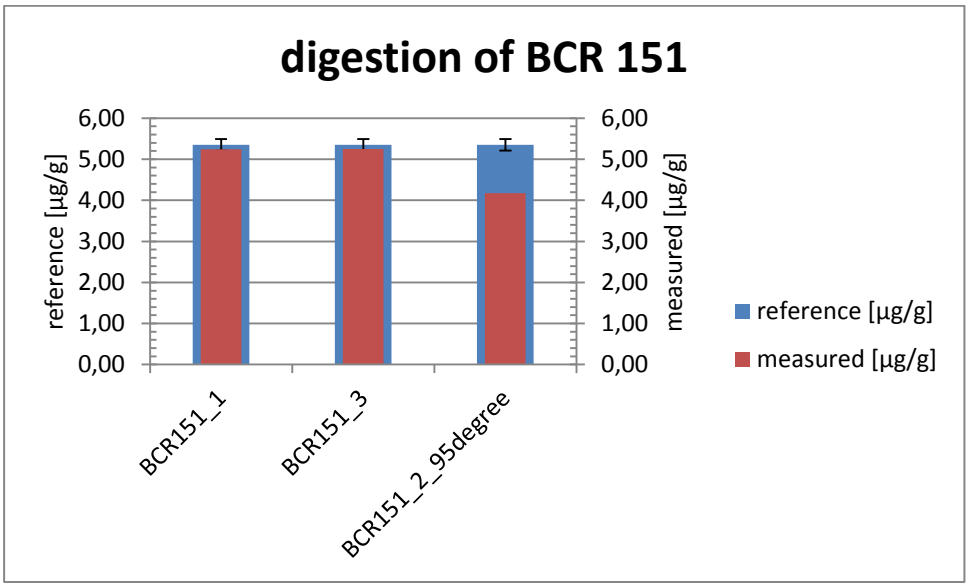

Figure 79. results digestion, BCR 151, standard addition Agilent Cary

As it can be observed in figure 79 the results obtained for BCR151_1 (5.24 µg/g) and BCR151_3 (5.25 µg/g) were in excellent agreement with the certified values (5.35 + 0.14 µg/g). The sample BCR151_2_95degree – which was held at 95 °C at the beginning of the digestion, to prevent the foaming - had a lower iodine content (4.17 µg/g) compared to the reference. It could be possible that the signals were out of the linear range of the calibration. Next a calibration curve with lower standards (0-20 µg/l) was recorded.

Calibration solutions with lower iodine contents (0-20 µg I/l) were digested in the same way as the samples. Then a calibration curve was recorded. In addition conventional milk powder was digested and then analysed using the Agilent Cary. Samples from previous experiments were measured again. The goal was to achieve better recoveries by recording a calibration curve with lower standards.

digestion procedure:
calibration solutions: 0.5 ml H_2O added, spiked with KIO_3 to yield 0, 2, 5, 10, 15 and 20 µg I/l (0, 200, 500, 1000, 1500 and 2000 µl out of 1,000 µg I/l KIO_3-Std); digestion vessel: big eprouvette;
T/t-programme: ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
Milk powder_1-3: 0.3 g of milk powder, 0.5 ml H_2O added, digestion vessel: big eprouvette; T/t-programme: hold for 10 minutes at 90 °C, ramp from 90-140 °C within 30 minutes, ramp from 140-170 °C within 30 minutes
digestion acid: 3.6 ml of $HClO_3$ for calibration solutions and milk powder_1-3 within first and second step, 0.5 ml of HNO_3 for calibration solutions and milk powder_1-3 only during first step
digestion residue made up to 10 ml with H_2O (calibration solutions), made up to 5 ml with H_2O (milk powder_1-3)

measurement technique: Cary spectrophotometer, Sandell-Kolthoff
cuvette: d = 0.5 cm, t_{total} = 2 min, t_{scan} = 0.17 min
samples: 1 ml made up to 10 ml with H_2O (calibration solutions and samples)

The calibration with the lower standards is shown in figure 80. The calibration curve was linear with a correlation coefficient of 0.9984.

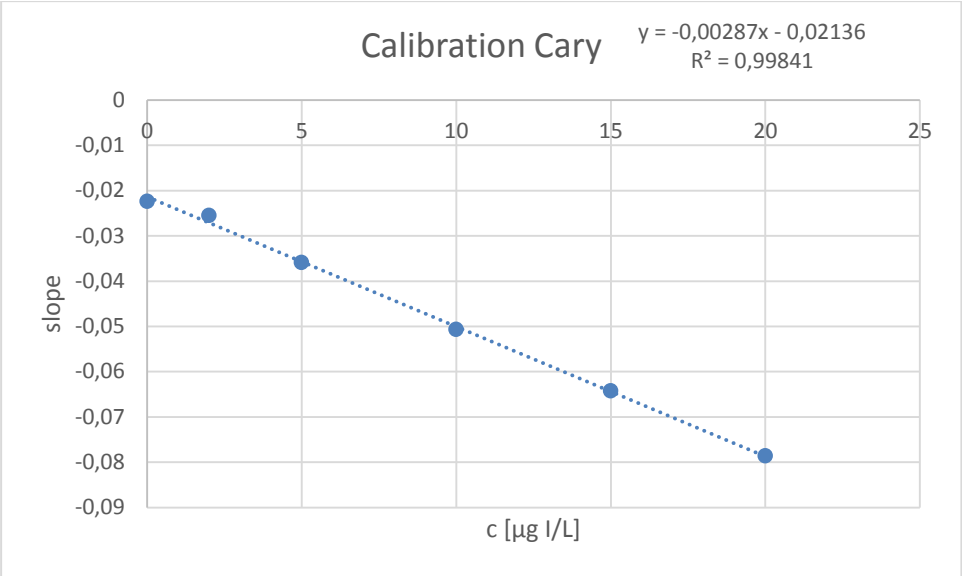

Figure 80. calibration curve iodine, digested, 2-20 µg I/l, Agilent Cary

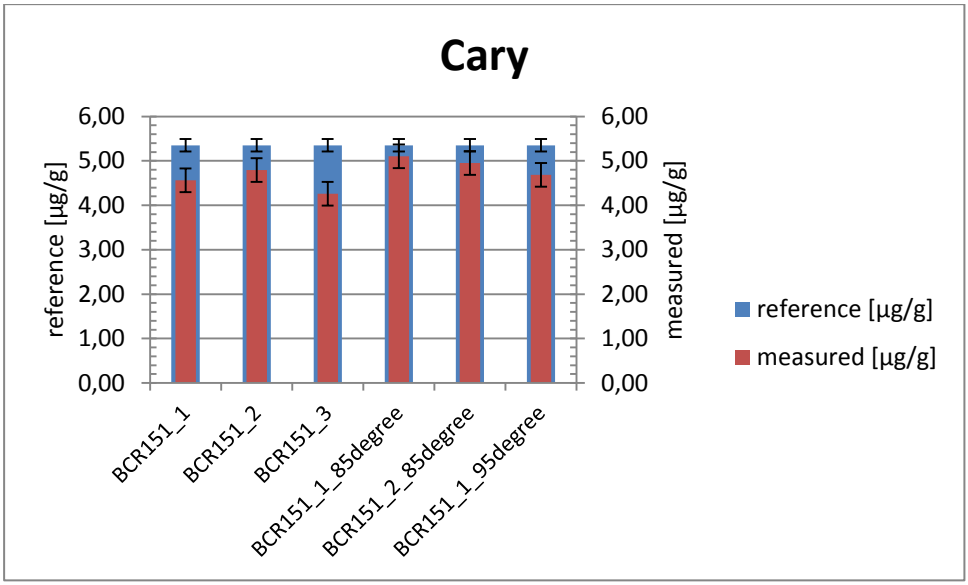

Figure 81. results digestion, BCR 151, Agilent Cary

As it can be seen in figure 81 the iodine contents for BCR 151 were all quite acceptable with good correspondence to the reference value (5.35 ± 0.14 µg/g). BCR151_1-3 showed an iodine content of 4.54 ± 0.27 µg/g. The samples BCR151_1_85degree and BCR151_2_85degree – where the temperature was held at 85 °C at the beginning of the digestion, to prevent foaming – showed iodine contents of 5.11 µg/g and 4.95 µg/g. The same is true for BCR151_1_95degree – temperature held at 95 °C – with an iodine content of 4.69 µg/g. This showed that for low iodine contents it had been necessary to record a calibration with lower standards.

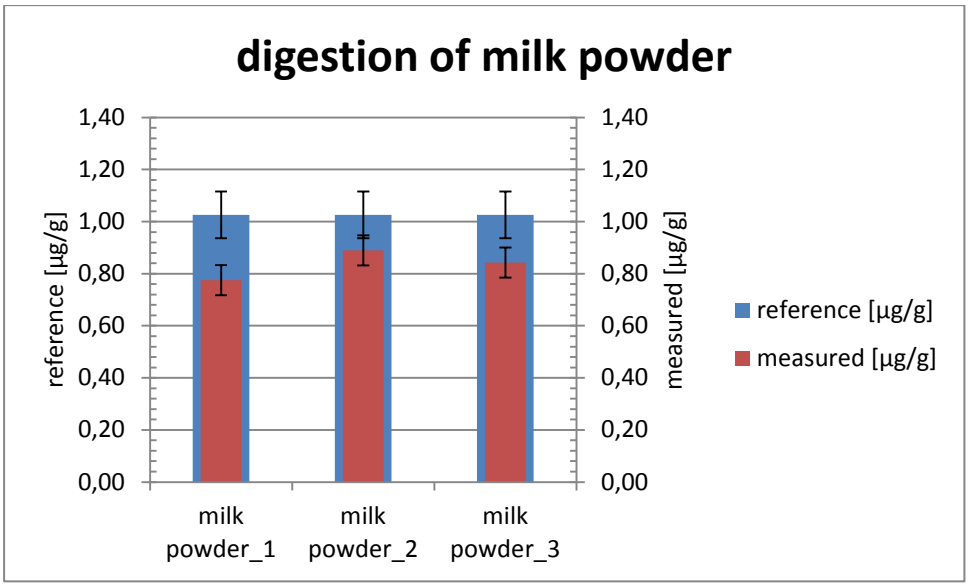

Figure 82. results digestion, milk powder, Agilent Cary

As it can be seen in figure 82 milk powder achieved good correlations between the measured iodine content (0.84 ± 0.06 µg/g) and the reference value (1.02 ± 0.09 µg/g). As discussed before a calibration curve with lower standards was needed to determine low concentrations.

Samples from the previous experiment were also measured using the mini photometer. Waiting time was set at 30 s since the iodine content was expected to be very low and the reaction needed time to start. A new flow through cuvette with d = 0.5 cm was used to correlate better with the Agilent Cary.

measurement technique: Mini photometer, Sandell-Kolthoff
cuvette: d = 0.5 cm, t_{total} = 1 min, t_{scan} = 0.5 seconds, light source = 250 AU, T = 20 °C
pump: 30 rpm
waiting time: 30 s
calibration solutions: 0, 2, 5, 10, 15 and 20 µg I/l digested
samples: 1 ml made up to 10 ml with H_2O (samples and calibration solutions)

It was not possible to record a calibration curve since there was no change in the signal. Therefore the experiment was repeated with a waiting time of 60 seconds.

A calibration curve with the lower standards was recorded again.

measurement technique: Mini photometer, Sandell-Kolthoff
cuvette: d = 0.5 cm, t_{total} = 1 min, t_{scan} = 0.5 seconds, light source = 250 AU, T = 20 °C
pump: 30 rpm
waiting time: 60 s
calibration solutions: 0, 2, 5, 10, 15 and 20 µg I/l digested
samples: 1 ml made up to 10 ml with H_2O (samples and calibration solutions)

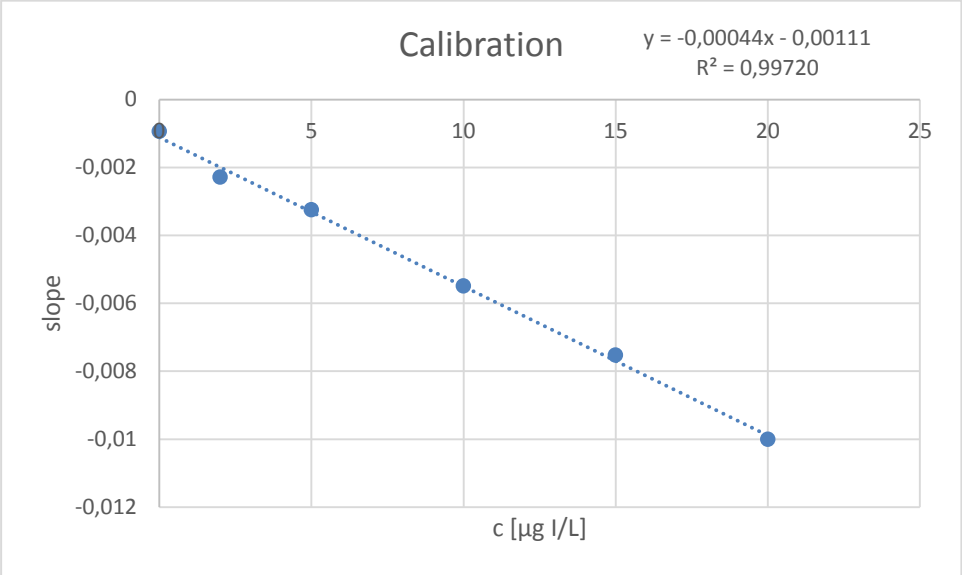

Figure 83. calibration curve iodine, 2-20 µg I/l, digested, mini photometer

Figure 83 shows the calibration curve with the lower standards (2-20 µg I/l). Curve was linear with a correlation coefficient of 0.9972. This showed that it had been possible to record a calibration curve with lower standards.

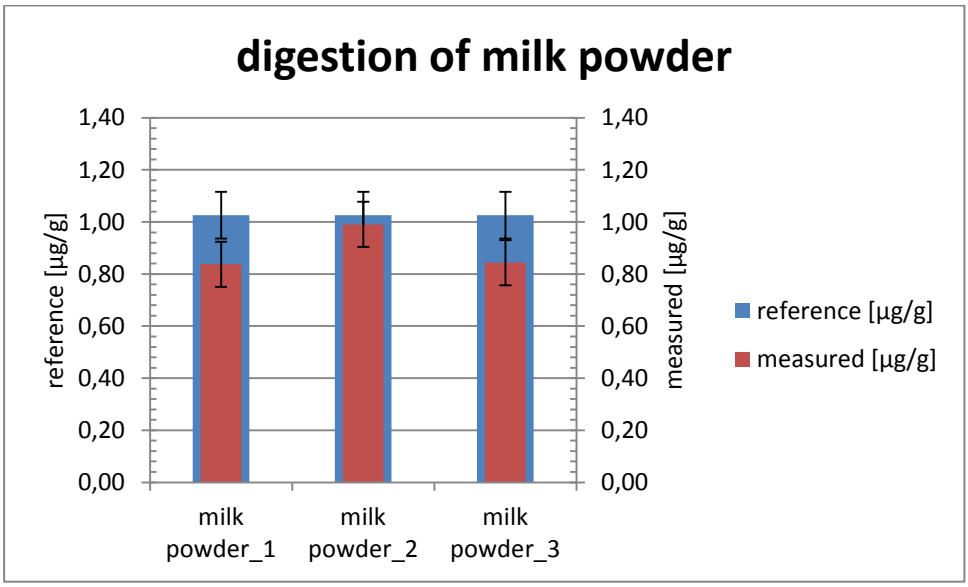

Figure 84. results digestion, milk powder, mini photometer

Figure 84 shows the iodine contents (0.89 ± 0.09 µg/g) for the conventional milk powder (milk powder_1-3) compared with the reference value (1.02 ± 0.09 µg/g) obtained by the mini photometer. The iodine content was matching the reference value and almost the same as for the Agilent Cary (0.84 ± 0.06 µg/g). It can be concluded that samples with low iodine content can be analysed also in the concentration range of 2-20 µg I/l if a 0.5 cm cuvette is used.

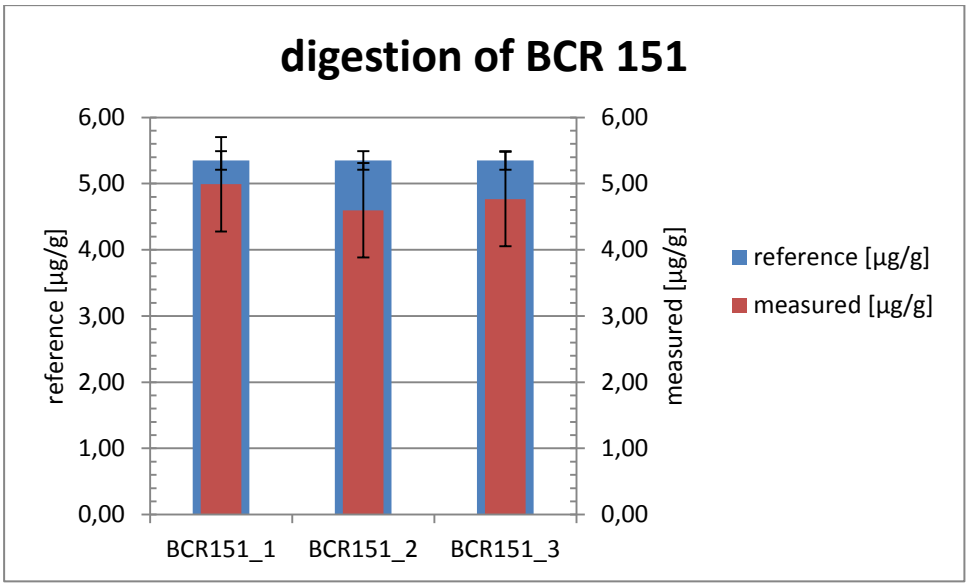

Figure 85. results digestion, BCR 151, mini photometer

Results for BCR 151 (4.50 ± 0.71 µg/g) shown in figure 85 were again in excellent agreement with the certified ones (5.35 ± 0.14 µg/g) and comparable to the results obtained using the Agilent Cary.

The experiment also clearly shows the importance of the pre-reaction time that was further investigated using the mini photometer.

Standard with 2 µg I/l was measured by the mini photometer to investigate the progress of the reaction beyond 1 minute.

measurement technique: Mini photometer, Sandell-Kolthoff
cuvette: d = 0.5 cm, t_{total} = 1 min, t_{scan} = 0.5 seconds, light source = 250 AU, T = 20 °C
pump: 30 rpm
waiting time: 60 s

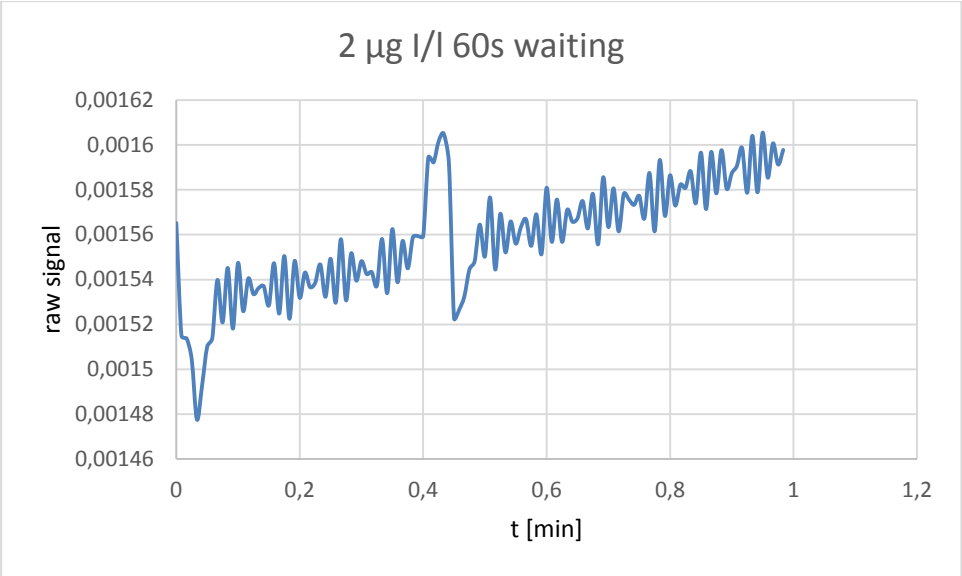

Figure 86. raw signal vs. time, 2 µg/l, 60 s waiting

Figure 86 shows the progress of the reaction with a waiting time of 60 seconds. There was much noise present.

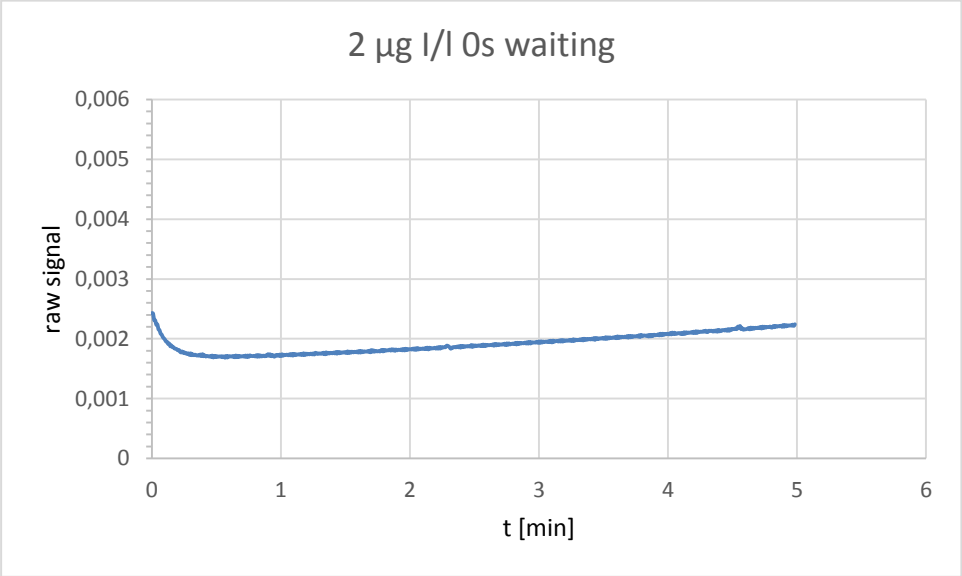

Figure 87. raw signal vs. time, 2 µg/l, 0 s waiting

Figure 87 shows the progress of the reaction without a waiting time. The onset of the reaction is clearly visible: After an initial decrease of the signal, a stabilization of the reaction can be observed after about 30 – to 40 s. The measurement should therefore maintain a waiting time of 60 s before the signal is recorded. Alternatively, the initial onset of the reaction must be removed prior further processing of the data.

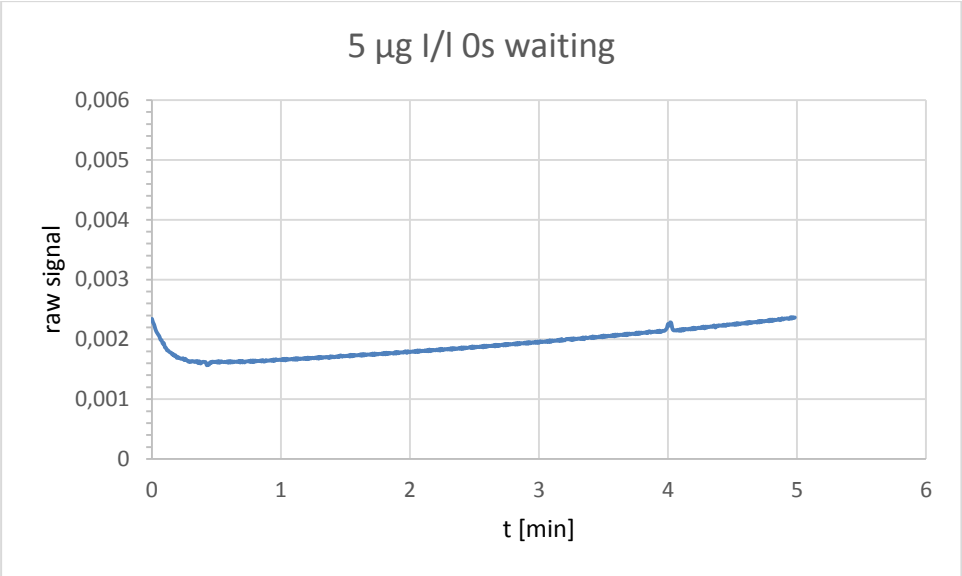

Figure 88. raw signal vs. time, 5 µg/l, 0 s waiting

The standard with 5 µg I/l showed the same behaviour as the Standard with 2 µg I/l. To conclude it was necessary to apply a waiting time in order to achieve reliable results. This artefact was only visible for measurements by the mini photometer. Therefore it was suggested that small air bubbles occurred due to the pump activity. Then within the 60 seconds waiting time the air bubbles were allowed to settle down. As a result the air bubbles did not occur for the standard with the waiting time. To verify this theory, the pump speed was altered as well as the waiting time. The goal was to examine possibilities to get rid of the artefact.

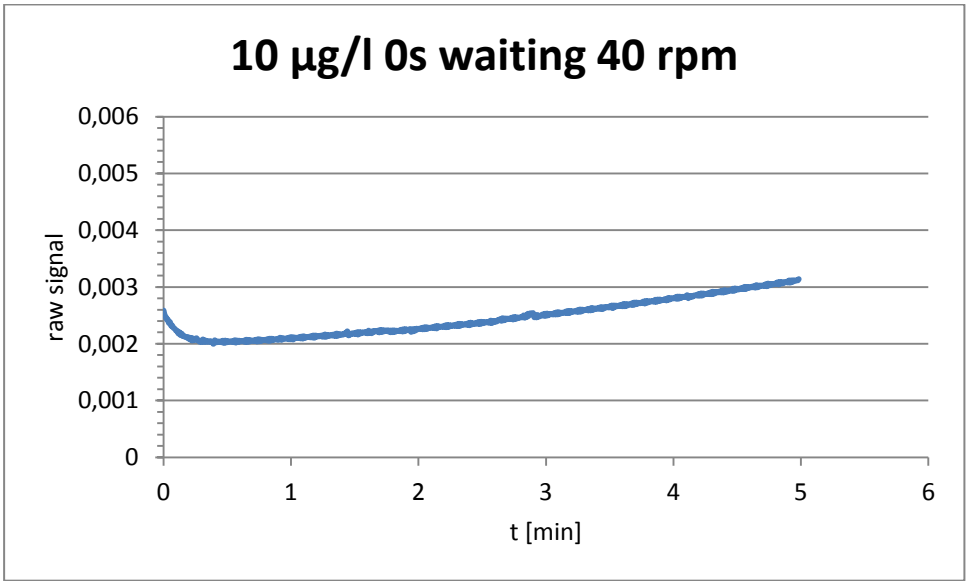

Figure 89. raw signal vs. time, 10 µg/l, 0 s waiting, 40 rpm

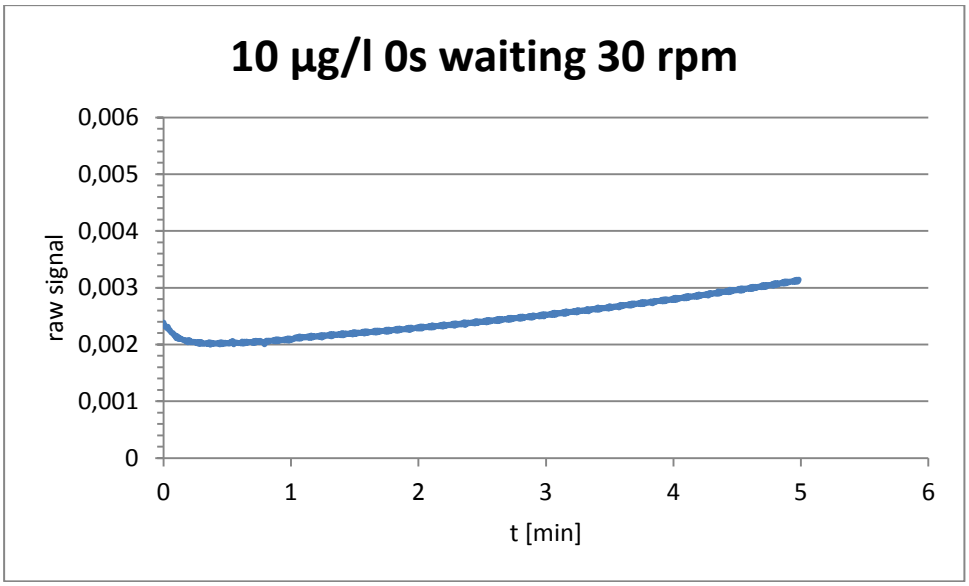

Figure 90. raw signal vs. time, 10 µg/l, 0 s waiting, 30 rpm

By comparing figure 89 and 90 it was observed that the artefact got less pronounced as the pump velocity had been decreased. This could be explained with less pump activity that produced less air bubbles.

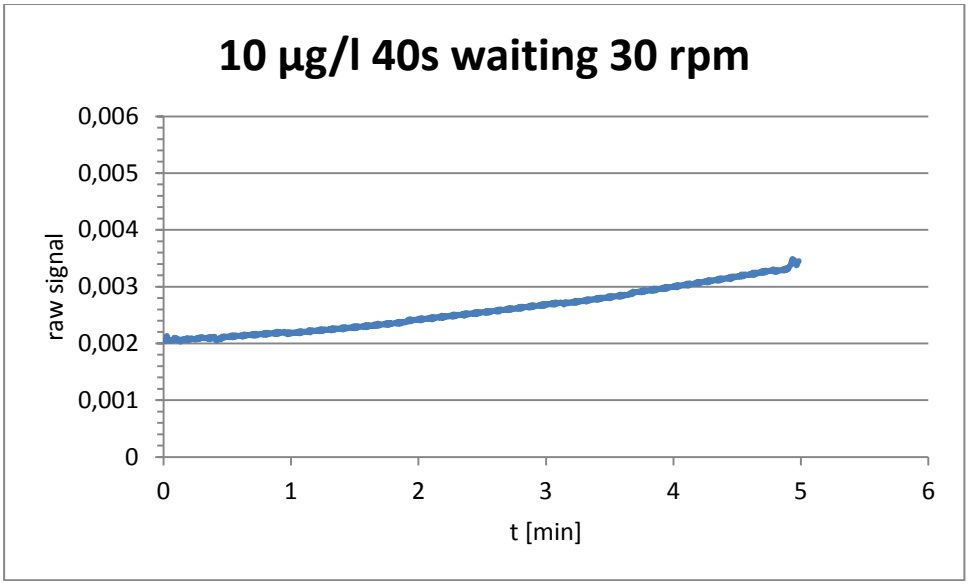

Figure 91. raw signal vs. time, 10 µg/l, 40 s waiting, 30 rpm

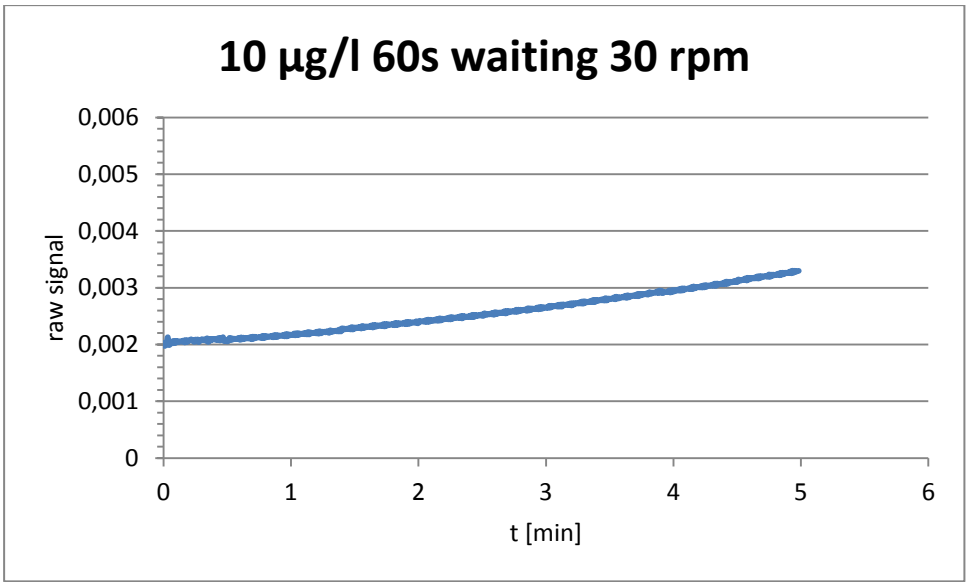

Figure 92. raw signal vs. time, 10 µg/l, 60 s waiting, 30 rpm

By comparing figure 91 and 92 to figure 90 it could be observed that the artefact became less pronounced by applying a waiting time and further decreased when increasing the waiting time from 40s to 60s. It was observed, that by increasing the waiting time the air bubbles were allowed to settle down. To conclude for samples with a concentration of 2-20 µg I/l a waiting time of 60 seconds should be applied.

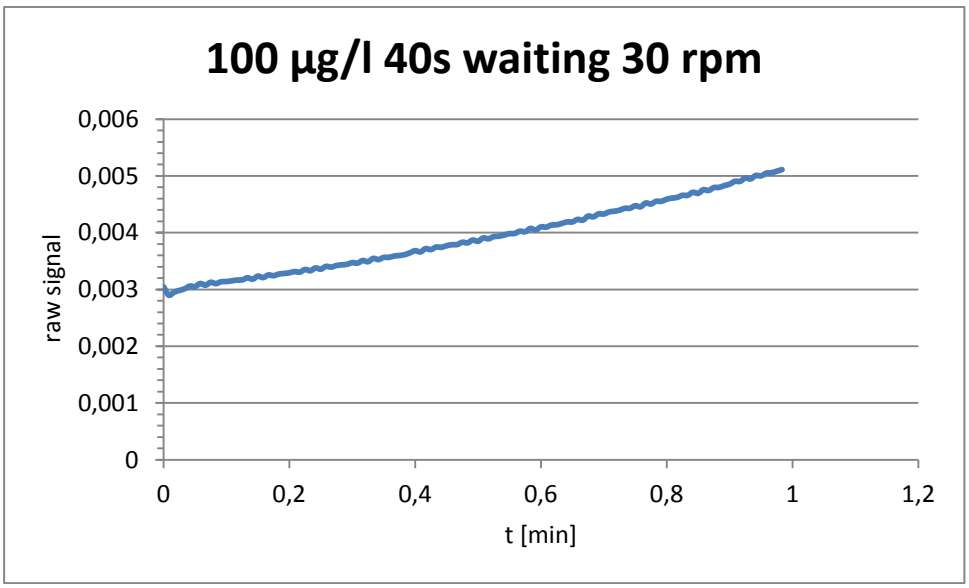

Figure 93. raw signal vs. time, 100 µg/l, 40 s waiting, 30 rpm

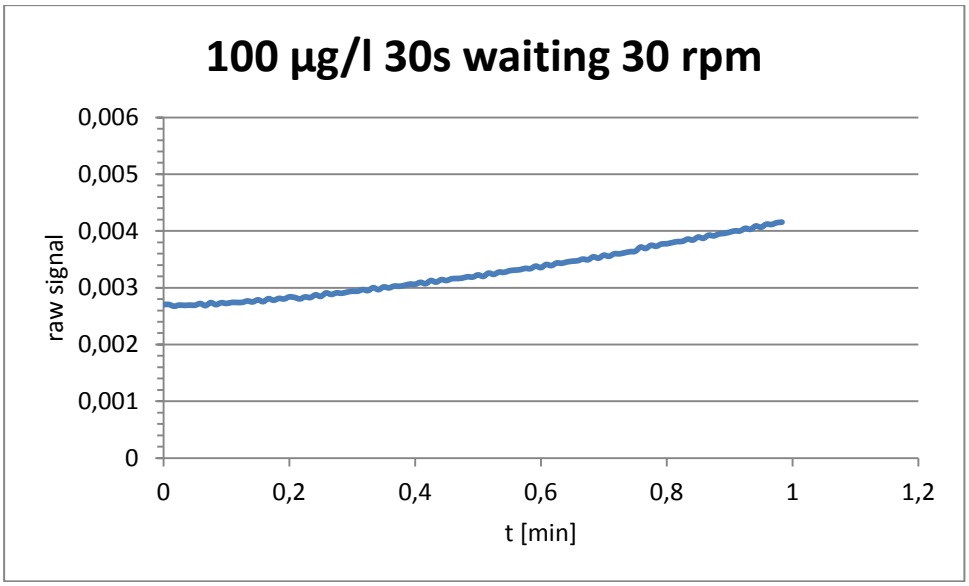

Figure 94. raw signal vs. time, 100 µg/l, 30 s waiting, 30 rpm

The standard with 100 µg I/l was measured with a waiting time of 40 s (figure 93) and 30 seconds (figure 94). The waiting time of 40 seconds gave the higher raw signal and the artefact was also less pronounced. To conclude for samples with a concentration of 20-200 µg I/l a waiting time of 40 seconds should be applied.

5 CONCLUSION

Biological samples were analysed by ICPMS and the Sandell-Kolthoff method. ICPMS was found to be superior because of the low detection limits, the freedom from digestion-induced interferences and the possibility to apply digestion procedures that do not need to aim at extremely low residual carbon. The catalytic method of Sandell-Kolthoff offers a cost-effective alternative but is limited in terms of sample preparation and LOD.

The stability of iodide stock solutions was found to be strongly dependant on the storage container and the temperature. A new glass flask, stored in the fried was found to provide best stability, whereas stabilization reagents such as ascorbic acid or TMAH were found ineffective.

Before determining iodine the organic matrix had to be completely decomposed and the iodine had to be converted to iodate for stability. Milk powder was chosen as organic matrix that was spiked with KI. Different digestion methods were investigated.

One method was alkaline extraction with TMAH and measuring by ICPMS. This method extracts the iodide from the organic matrix. The extraction was used to characterize the milk powder for its iodine content. Reliable results were obtained but it was difficult to filter the extraction solution through the syringe filter.

First wet digestion with $HClO_3$ was used. In biological samples the iodine is present in the form of iodide. If iodide gets oxidized to volatile iodine unwanted losses occur. Therefore high oxidational power is needed to convert iodide to iodate which is stable in acidic medium. $HClO_3$ fulfils this requirement. The digestion vessels used were digestion tubes, glass vials, eprouvettes (h = 10 cm) and big eprouvettes (h = 20 cm).
The digestion tubes were not practicable due to excessive splashing of the sample forced by superheating. The method cannot be considered as reliable if heating delays occur at any time. Heating delays were prevented by using glass vials. Unfortunately the digestion medium evaporated too fast from the glass vials. As a result the digestion was not fully completed. Eprouvettes (h = 10 cm) were successfully used for sample masses up to 70 mg. For higher sample masses it was necessary to use bigger eprouvettes (h = 20 cm) as larger volumes of acid were needed t. In addition it was not possible to digest sample masses below 70 mg with the big eprouvettes as the digestion acid evaporated too fast resulting in incomplete digestion.

Several digestion procedures were investigated: Initially, the digestion was performed as a one-step procedure. The samples were heated up from 90-140 °C within 30 minutes. However, this procedure resulted in unacceptable high levels of remaining organic components that cause interferences during the Sandell-Kolthoff method. Then a two-step digestion procedure was developed by adding $HClO_3$ again after the first step. During this second step the samples could be heated up to 170 °C to fully destroy the organic matrix. In addition white mist of perchloric acid could be used as indicator for the completeness of the digestion as this proofed that all of the chloric acid had been removed and an acidic medium was ensured. It was of prim importance to adjust the acid volume to the sample weight. Without adjusting the acid volume the organic matrix could not be fully digested. The procedure of adjusting acid volume to the sample weight led to excellent spike recoveries.

TOC (total organic carbon) was measured by ICPOES to analyse the influence on the recovery. As expected the sample with the highest recovery had no TOC at all.

The digestion by using $HClO_3$ was characterized by very harsh reaction conditions. Particularly for larger sample masses (> 100 mg) this was considered unsafe. In order to make the conditions less harsh HNO_3 were added. The $HClO_3$ volume had to be adjusted to the sample weight. The optimal volume of HNO_3 was found to be 0.5 ml, though higher volumes were found to cause no additional problems. Nevertheless HNO_3 had to be fully removed within the second step of the digestion otherwise unwanted absorptions occurred during Sandell-Kolthoff. Sandell-Kolthoff was measured at a wavelength of 317 nm and HNO_3 absorbs at wavelengths below 365 nm.

Calibration solutions had to be digested the same way as the samples. This procedure was necessary to prevent the $HClO_3$ from influencing the catalytic reaction. Standard addition was found to provide equally good results.

BCR 151 (skimmed milk powder), brown algae and conventional milk powder were used for method validation. Brown algae had a high (700 µg/g), BCR 151 an intermediate (5 µg/g) and conventional milk powder a low (1.0 µg/g) iodine content. All three iodine contents were accurately quantified by the Sandell-Kolthoff reaction.

Milk powder as well as BCR 151 were used to validate the mini-photometer that used a LED as a light source. The results were as good as the ones obtained by the Agilent Cary spectrophotometer. This mini-photometer proved to be a cost-effective alternative to the Agilent Cary spectrophotometer.
Experiments with different reaction temperatures were performed and it was concluded, that the effect dictates the use of a thermally stabilized measurement – that means, all solutions must have the same temperature and deviations should be less than 1 °C. The temperature inside the flow through cuvette of the mini-photometer was controlled by a thermostat.

The pump velocity of the sample and reagent into the mini-photometer was investigated as well. An artefact was present at the beginning of the reaction for a pump velocity of 40 rpm. This artefact got less pronounced as the pump velocity was decreased to 30 rpm. It was important that the pump velocity was not too low. By using 10 rpm small air bubbles accumulated to one big air bubble before or inside the cuvette resulting in unreproducible readings. By applying a waiting time between the start of the pump and the start of the measurement the artefact disappeared. It was suggested that the artefact also resulted from small air bubbles produced by the pump activity. By applying a waiting time the bubbles were allowed to settle down and so the artefact disappeared. Further experiments showed that iodine concentrations of 2-20 µg I/l needed a waiting time of 60 s. The best results for iodine concentrations of 20-200 µg I/l were obtained with a waiting time of 40 s. The reaction displayed a higher velocity at higher concentrations and so a shorter waiting time was needed to monitor the reaction.

Concerning the mini-photometer following scheme was used for data processing: The raw signal form canal 2 corresponded to the transmission trough the sample. This signal was divided by a constant value that was obtained by measuring H_2O as raw signal at canal 2. This operation corresponded to I/I_0 which is the relative transmission. Next the first logarithm was applied to the inverse of the relative transmission. After applying the second logarithm and plotting against the time, the slope was direct proportional to the iodine concentration.

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