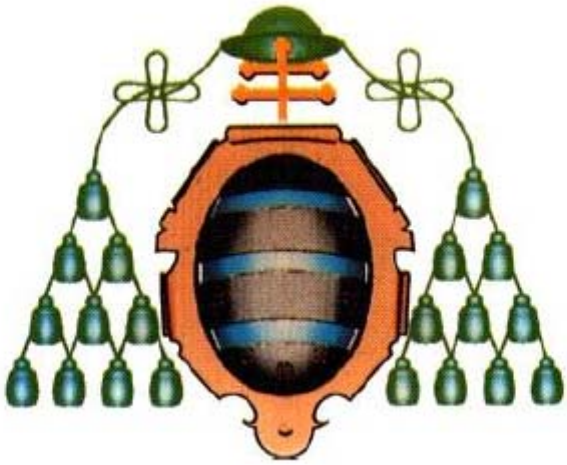

MASTER'S DEGREE IN ANALYTICAL AND BIOANALYTICAL SCIENCE

UNIVERSITY OF OVIEDO

Master's Dissertation

DETERMINATION OF IODINE BY ICP-AES

JAVIER GÓMEZ GARCÍA

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hereby certify,

that the present master thesis, entitled "DETERMINATION OF IODINE BY ICP-AES", has been written by the student Javier Gómez García as part of his research work, undertaken at the Institute of Analytical Chemistry of Graz University of Technology under the supervision of Helmar Wiltsche, with Austrian national ID number P3461748 (passport ID), Senior Lecturer at the Institute of Analytical Chemistry and Food Chemistry / Graz University of Technology, and is the student's Master's thesis, with our consent.

Oviedo, July 15th, 2013

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1. INTRODUCTION

Iodine is an essential trace element that plays an important role on the growth, development and well-being of human beings and mammals. It is found to be part of different molecules in living organisms. The most important among them are thyroxin (T4) and iodothyronine (T3), two thyroid hormones that regulate the cellular metabolism. A lack or an excess of iodine concentration changes the normal levels of these hormones and causes diseases like, for example, goiter (1). In addition, it has been shown that the hormone T4 play a very important role on the early brain development of babys (2). More specifically, the proper brain development of a baby depends on the proper functioning of the mother's thyroid gland, especially during pregnancy and lactation period, because the mother is then the only source of these hormones. Women unable to increase their production of T4 in the early pregnancy (hypothyroxinemia) can get a baby with neurological disabilities and therefore with learning problems. As mild- moderate iodine deficiency is the most usual cause of hypothyroxinemia in Western societies, the birth of many children with learning disabilities can be prevented by taking iodine supplements.

The concentration of iodine in human fluids and tissues depends directly on its intake with water and foodstuffs. Therefore, low concentrations of iodine in water or food result in a lack of iodine in the organism and the possible appearance of diseases. Preventive measures against these types of diseases involve the analysis in human fluids, particularly urine. If there is a deficiency on iodine detected then an adaption of the diet with foodstuffs with high iodine content like seafood is necessary. In some countries iodine supplemented salt is used for cooking.

It is also important to know that an increased intake of some elements, like bromine, can reduce iodine accumulation in the thyroid and mammary glands as well as increasing its elimination through the kidneys (3).

For these reasons, highly efficient and reliable analytical techniques are necessary for iodine determination in foodstuff, animal feed and biological tissues and fluids.

In biological tissues and fluids iodine is present in inorganic form as iodide and covalently bound to organic compounds. For the determination of the total iodine content a mineralization prior to the analysis is necessary. The low iodine content of food, the complexity of the organic matrix, chemical interferences, the possibility of contamination, and possibly losses by volatilization during sample pretreatment make this step the most critical of the whole process. In addition, the reagents used for the digestion should not interfere with the subsequent determination.

It is important to note that elemental iodine can easily get lost by volatilization, by adsorption on the surface of the vessel material and by reaction with organic compounds. To avoid losses iodine has to be transformed into iodide or iodate during the digestion step.

Suitable methods to carry out sample decomposition without losses of iodine are dry ashing in a muffle oven with alkaline ashing, aids to keep iodine in the state of iodide, combustion with oxygen in closed systems with subsequent absorption of the volatile iodine in alkaline solutioms, wet digestion in open systems with acids with high oxidation potential (sulfuric acid - perchloric acid – nitric acid or chloric acid – nitric acid) to bring iodine fast to iodate and last but not least wet digestion in closed pressurized vessels with nitric acid and a little perchloric acid (4-8).

1.1. Techniques to perform the analysis

There is a great variety of techniques which can be used to perform the analysis of iodine in biological samples. Some examples are shown in the following table 1.

Table 1: Different methodologies to perform this analysis

Methods		Measured property
Catalytic	Method of Sandell and Kolthoff	Photometric measurement
Non catalytic	Ion selective electrodes	Electric potential
	Cathodic stripping voltammetry	Electric current
	IC-AD	Current intensity
	HPLC-IC-MS	Relationship m/z
	CG-MS	Relationship m/z
	ICP-MS	Relationship m/z
	NAAS	Radioactive emissions
	ICP-AES	Radiation emission

1.1.1. Catalytic method for the determination of nano quantities of iodine

This methodology (9-11) is based on the catalytic effect of iodine on the redox reaction between the arsenious acid and ceric (IV) sulfate in sulfuric acid solution from Sandell and Kolthoff (10):

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

In the absence of iodide, the kinetics of the reaction is very slow, while small iodide amounts increase the rate of the reaction remarkably. The reaction rate at constant temperature is proportional to the iodide concentration. The iodine catalytic reaction corresponds to a first-order reaction. Mathematically it may be expressed as:

$$-\frac{d\,[Ce^{4+}]t}{dt} = k[Ce^{4+}]t\,.\,[J]$$

This gives:

$$\ln[Ce^{4+}]_t = \ln[Ce^{4+}]_{t=0} - kt[J]$$

$$[J] = \frac{\ln[Ce^{4+}]_{t=0} - \ln[Ce^{4+}]_t}{kt} = \frac{\ln E_{t=0} - \ln E_t}{kt} = \frac{\Delta \ln E}{kt}$$

$$E = extinction$$

$$[J] = iodine\ concentration$$

According to the equation 3, using a constant reaction time, the iodine concentration is proportional to ΔlnE. In addition, according to the Arrhenious equation, the rate of the reaction depends on the temperature, that is why it is important that the temperature remains constant during the analysis.

One of the advantages of this method is that it is a rather selective methodology, because osmium and ruthenium are the only elements with comparably catalytic efficiency and they are normally not existent in biological matrices. Alkali chlorides and bromides have a comparably slight catalytic effect, which does not interfere the iodine analysis of biological samples. Another advantage of the catalytic iodine determination is the possibility of automation.

It should be noted that this methodology was long considered as "state of the art" for determinations of iodine in biological samples.

1.1.2. Cathodic stripping voltammetry

A possible voltammetric method (12) to perform this analysis consists in the oxidation of the analyte, in form of iodide, by applying a potential of +0.95 V (vs Ag/AgCl). The iodine formed reacts with Br-, a complexing agent, to form I_2Br^-, which is associated with Zephiramine, an ionic associating agent. This reaction forms an insoluble complex, which deposits on the working electrode:

$$2I^- \rightarrow I_2 + 2e^-$$
$$I_2 + Br^- \rightarrow I_2Br^-$$
$$I_2Br^- + Zeph^+ \rightarrow ZephI_2Br\downarrow$$
$$ZephI_2Br + 2e^- \rightarrow 2I^- + Br^- + Zeph^+$$

The cathodic stripping voltammetry can easily be interfered by organic compounds and needs iodide as analyte. Therefore combustion of the organic sample in an oxygen flask is useful because most of the organic compounds will be oxidized and iodine remains as iodide in the alkaline absorption solution.

The ions that may affect the analysis are Hg^{2+}, S^{2-}, $S_2O_3^{2-}$ and SCN^-. Hg^{2+} is absent in ordinary cases and S^{2-},$S_2O_3^{2-}$ and SCN^- can be oxidized to convert into SO_3^{2-}, and thus the effect is eliminated.

Cathodic voltammetry is selective and highly sensitive and allows therefore the determinations of iodine in biological samples in the ppb range..

1.1.3. Chromatography techniques (HPLC & GC)

Chromatographic techniques are presented to carry out iodine determination in the ppb range. CG-MS (13, 14) stand out as representative technique in the field of gas chromatography, while HPLC-ICP-MS (15) does the same within the field of liquid chromatography. Both techniques combine the resolution of chromatographic analysis with the sensibility of mass spectrometry. Thus, they are two techniques with very good qualities to perform the determination of iodine in biological samples. In addition, chromatographic techniques allow speciation analysis, which is of increasing importance. However, the cost of the equipment and its maintenance is very high and not all laboratories can afford it. In addition highly qualified employees are necessary to run these complicated instrumentation.

Therefore, an attractive alternative could be ion chromatography with amperometric detection (IC-AD) (16). In this much cheaper methodology, iodine, in the form of iodide, is separated by ion-chromatography and detected amperometrically using a coal-based silver paste electrode, which is polarized to +0.080 V versus Ag / AgCl. The presence of iodide in solution considerably facilitates metallic silver oxidation, with response currents directly related to iodide concentration.

As it is necessary that the iodine in the sample solution is present as iodide, the different species of iodine in the sample have to be reduced to iodide. In the case of organic samples, oxygen flask combustion is used for that purpose (9). This technique allows the determination of iodine in the ppb level. The selectivity of ion chromatography can still be improved by adding small amounts of EDTA to the stationary phase.

1.1.4. Neutron activation analysis (NNA)

The neutron activation analysis (17) is based on a process known as neutron activation. Neutron activation takes place when an atomic nucleus captures free neutrons to become a heavier nucleus. The aim of this process is to obtain radioactive elements which release fast the excess of energy by emitting protons, neutrons or alpha particles. The radioactive emissions and

radioactive decay paths for each element are well known. Therefore it is possible to study emission spectra of the radioactive sample, and determine the concentrations of the elements.

This technique has an excellent intrinsic sensitivity for iodine. It is one of the most sensitive techniques to determine iodine. Also, it is a non-destructive technique. However, it is very expensive, since it requires a neutron source. Therefore, there are not many laboratories, which have access to this technique.

1.1.5. Inductively coupled plasma mass spectrometry (ICP-MS)

The ICP-MS (18) is another technique with very good analytical characteristics to determine iodine, especially in combination with isotope dilution. This is a very sensitive technique, with high precision and accuracy. Nevertheless, the acquisition of the instrumentation and its maintenance is extremely expensive and requires high skilled personnel.

1.1.6. Inductively coupled plasma atomic- emission spectrometry (ICP-AES)

The ICP-AES is one of the most widely used techniques to perform elemental analysis. It presents a wide linear dynamic range, high analytical sensitivity, and high sample throughput. However, its application to the analysis of non-metals, especially halogens, is quite limited because their most intense emission lines have very high energies compared to the other lines, which are not very intense. For this reason, the analysis of halogens by AES, iodine in this case, is performed by microwave induced helium plasma atomic-emission spectrometry (MIP-AES), which allows reaching much higher temperatures. On the other hand, the absence of commercial instruments using helium-MIP as an excitation source limits its application. As previously mentioned, among the group of analytical techniques that present good features, some are too expensive (i.e. mass spectrometry), their detection limits are not low enough (i.e. ion selective electrodes), the equipment is only affordable for a few laboratories (i.e. NAA) and /or require long, complicated and expensive treatments (i.e. voltammetry), which implies either way that they are only available in certain laboratories. Therefore it seems worthwhile to develop a procedure for iodine determination in biological samples and foodstuffs by ICP-AES, since that way the pretreatment would be the cheapest and simplest available. This pretreatment could consist in the oxidation of the iodine compounds to IO_3^- and then the reduction of the IO_3^- to molecular iodine. Iodine compounds oxidation can be performed by microwave assisted acid digestion of samples in closed systems.

1.2. Increase of the sensitivity of the ICP-AES

There are two ways to increase the sensitivity of atomic emission spectroscopy:

- Increase the amount of analyte introduced into the ICP.
- Working with a more intense emission line. Thus the signal / noise ratio increases and with it the sensitivity.

Increase the amount of analyte introduced into the ICP

In ICP-AES, the nebulization efficiency of pneumatic nebulizer systems is about 1%. To increase this efficiency by the application of ultrasonic nebulizers is combined with the drawback that the high amounts of liquid introduced into the plasma strongly interfere with the measurement. The best way to increase the amount of analyte in the plasma without increasing the matrix elements is the volatilization of the analyte and introduction into the plasma via the gasphase. This technique of gas phase introduction of the analyte into the plasma is state of the art for Hg (cold vapor technique) and for the hydride forming elements. Halogens can also be transferred into the volatile elemental form and swept into the plasma via a gas phase separator.

Study of the spectral lines of iodine

The most sensitive spectral lines of the halogens are found between 96 and 180 nm; however, in this range of wavelengths the light is absorbed by some components of the air, such as oxygen or water vapour. For this reason, the optical system of the spectrometer has to be filled in with an inert gas such as argon. This gas flows continuously through a filter that removes the molecules which absorb the radiation. In addition, the use of argon ensures a good optical transmission and protects the optical system from pollution.

The strongest lines of the iodine appear at 178.276 and 183.038 nm. However, the first one appears frequently overlapped with lines S (I) 178.226 nm and P (I) 178.287 nm, while the lines at 183.038 nm overlap for the N, S or Sc. N, S and P, elements which are often found in biological samples and therefore it is important to avoid their interference in the analysis.

In theory, the use of a gas-liquid separator only allows the entrance in the plasma of substances which have been transformed into volatile species during the generation of I_2 and an imperceptible amount of aerosol. Therefore, the use of the separator should eliminate the majority of the mentioned interferences, because none of these elements are volatile and only N_2 is a gas. The latter is eliminated by the argon flow, which protects the equipment.

1.3. Volatilization of I_2 using hydrogen peroxide

To transform IO_3^- into I_2, the reaction of Bray and Liebhofsky (24-28) may be used. It is an oscillating reaction in which hydrogen peroxide reduces and oxidizes alternately the IO_3^- and I_2, according to the following reaction scheme:

$$2IO_3^- + 5H_2O_2 + 2H^+ \rightarrow I_2 + 5O_2 + 6H_2O \qquad \text{(reaction 1)}$$

$$I_2 + 11\,H_2O_2 \rightarrow 2IO_3^- + 3O_2 + 2H^+ + 10H_2O \qquad \text{(reaction 2)}$$

$$16\,H_2O_2 \rightarrow 8O_2 + 16H_2O + 3061\,KJ$$

The difficulty of using this reaction is that iodate is first reduced to iodine by the hydrogen peroxide, but later this iodine is again oxidized to iodate by the hydrogen peroxide. Because of this dual role of the hydrogen peroxide in the reaction, it is difficult to obtain reproducible measurements and ensuring the results of the analysis performed. To show it, a sample of 10 mg/L I dissolved in 2.0 M HNO_3 was introduced in the volatilization manifold using the conditions reported in table 2. The results can be observed in the Figure 1:

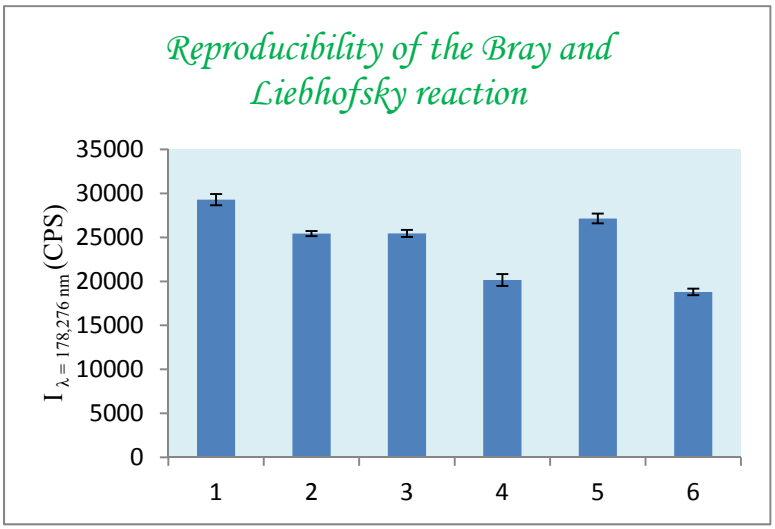


Figure 1: Reproducibility of the Bray and Liebhofsky reaction: Six consecutive measurement of the same sample solution

From this experiment evident that this reaction needs to be controlled to allow reproducible measurements.

1.4. Principle of operation

The principle of operation is shown in Figure 2: Acidified aqueous sample and reducing agent, H_2O_2, are mixed in the mixing coil, where they are in contact for a while before reaching the separator. When the sample-reducing mixture arrives in the separator, the iodine formed is transfer to the gas phase by a current of argon, which moves the iodine to the plasma and the sample-reducing mixture is eliminated to the waste. This separation increases the selectivity of the methodology because only substances, which have been transformed into volatile species during the generation of I_2 and imperceptible amount of aerosol can arrive to the plasma. In addition, the low solubility of molecular iodine in water contributes to the transfer of iodine into the gas phase and therefore to the increase of the sensibility because more analyte is injected into the plasma.

To avoid memory effects, the manifold is cleaned with 2.0 M sulfuric acid between two consecutive measures. A third, fast running peristaltic pump is switched on additionally in this step. That allows cleaning the separator faster but it is also necessary to use more sulfuric acid.

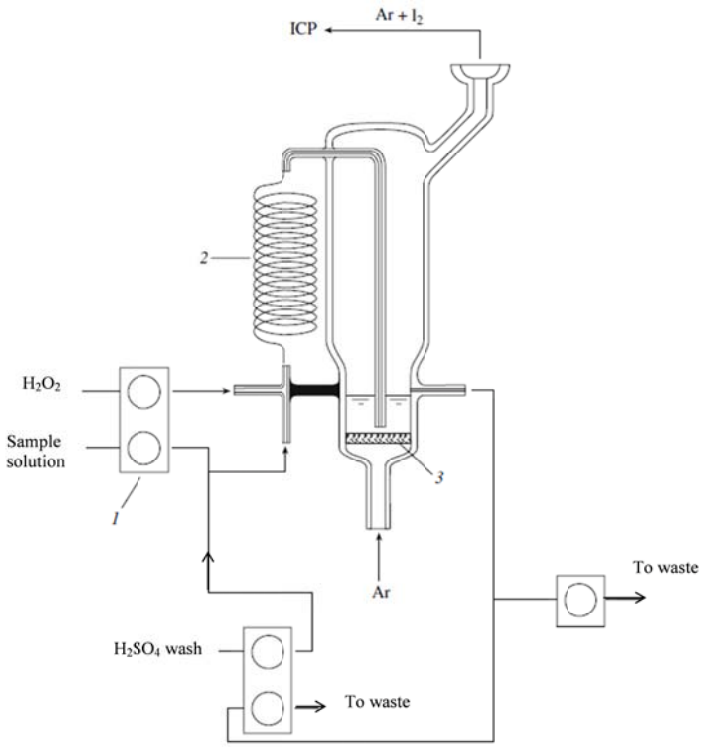


Figure 2: Principle of the iodine vapor generation manifold. Designation of the components: 1 peristaltic pumps, 2 mixing coil, 3 glass frit

2. OBJECTIVE

The goal of this Master Thesis is to find a way to control the Bray-Liebhofsky reaction, in order to make it more reliable and suitable for the analysis of iodine in biological samples by ICP-AES. This implies that the transformation of iodate into iodine (reaction 1) has to be the dominant reaction.

To this aim, it was first considered the inhibition of reaction 2 (iodine to iodate) as the best option. However, according to the bibliographical research carried out, this is not possible, but it was found a way to delay the onset of the oscillations.

The present work is focused on the study of the parameters that allow the delay of the oscillations and other parameters which contribute to the loss of iodine in gas phase. A chemometric study was performed in order to evaluate the influence of each parameter in the reaction and to determine the best analytical conditions to achieve the highest possible sensitivity.

Finally the suitability of the method to analyze iodine will be studied with certified reference materials.

3. EXPERIMENTAL

3.1. Materials and Reagents

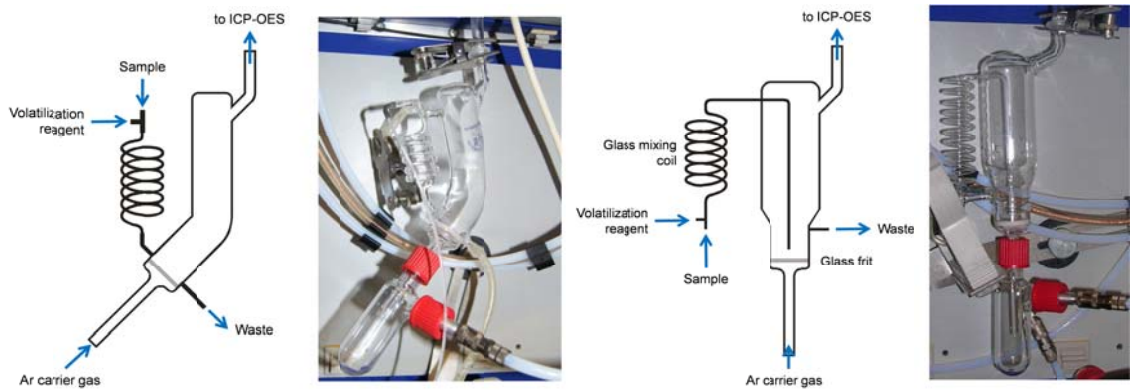
Iodine is prone to losses and contamination (from previous experiments) when using polymer sample containers. Therefore, glassware was used whenever possible. A stock solution of 1000 mg L^{-1} iodine was prepared from potassium iodate (dried at 105 °C for 6 h and stored in a dessicator). Calibration solutions where prepared daily from this stock by dilution. High purity water (MΩ cm^{-1}, Barnstead Nanopur, Thermo Fisher Scientific, USA) and analytical grade reagents were used throughout. Details are reported in the appendix of this work.

3.2. Instrumentation

An axially viewed ICP-OES (CIROS Vision EOP, Spectro, Germany) was used. The operating conditions are reported in Table 2. Elemental iodine was generated from samples containing iodate with hydrogen peroxide in the vapor generation manifold. As shown in Figure 3 two different manifolds were used in the various stages of the investigation. Both were made of glass to avoid ad- and absorption of elemental iodine.

Table 2: Optimized working parameters of the ICP and the volatilization manifold

Parameter	Value
Analytical lines	178.276 nm and 183.038 nm
Power, W	1450
Nebulizing flow, L/min	0.6
Auxiliary flow, L/min	0.8
Cooling flow, L/min	14
Sample flow rate, mL/min	4.4
Flow rate of H_2O_2 (30 %) mL/min	1.2
High speed peristaltic pump: Flow rate of additional H_2SO_4 during the washing step, ml/min	8
High speed peristaltic pump: flow capability of the secondary waste stream, ml/min	15



a) b)

Figure 3: Scheme of the employed vapor generation manifold. a) "fast washout manifold " and b) "high sensitivity manifold"

The "fast washout" vapor generation manifold had a short mixing coil and a tilted glass frit that acted as gas- liquid separator. On the other hand, the manifold b) was designed to retain the sample inside the separator for a determined period of time. This time is adjusted by varying the rate of the peristaltic pump of the waste.

3.3. Sample digestion

For a preliminary validation of the developed analytical procedure a certified reference material (CR) was used (BCR 151, skimmed milk powder). Due to the selected volatilization pathway the iodine in the sample had to be converted into iodate in the sample digestion step. It is well known that this can be accomplished by a microwave assisted pressurized acid sample digestion step using both, nitric and perchloric acid. About 0.25 g of the CRM were digested with 2 mL conc. HNO_3, 3 mL H_2O and 0.2 mL conc. $HClO_4$ in PTFE vessels at a maximum pressure of 40 bar by means of a commercial microwave digestion system (Multiwave 3000, Anton Paar, Graz, Austria) and made up to a final volume of 25 mL. The decomposition program is reported in Table 3.

Table 3: Parameters of microwave digestion

Stage	Power, W	Ramp, min	Hold, min
1	1400	10	15
2	0		15

4. RESULTS AND DISCUSSION

4.1. Effect of the studied parameters in the Bray-Liebhofsky reaction

Based on the reaction mechanisms proposed in literature the parameters shown in the Figure 4 were selected for further investigation.

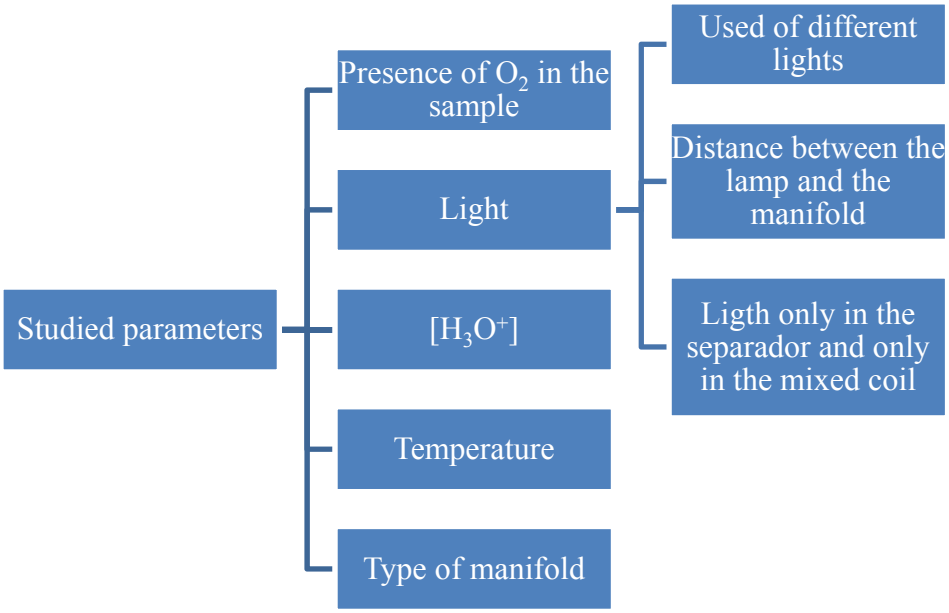


Figure 4: Studied parameters to control the Bray-Liebhofsky reaction

All parameters were studied first separately. Thereafter, combined effects were investigated using chemometric techniques.

4.1.1. Influence of dissolved oxygen

Numerical simulations based on a simplified model (27) suggest that dissolved oxygen might affect the formation of I_2. According to this simulations this effect can be explain by the next reactions:

$$I^- + H^+ + {}^1/_2\, O_2 \rightarrow HIO \qquad \text{(reaction 3)}$$

$$HIO + I^- + H^+ \rightarrow I_2 + H_2O \qquad \text{(reaction 4)}$$

It seems surprising that the initial presence of oxygen in the sample solution is reported to have a significant effect on the formation of I_2 when considering the large amount of oxygen produced during the cause of the reaction (see reaction 3). However, as shown in Figure 5, low concentration of dissolved oxygen in the sample is reported to alter the frequency of the Bray and Liebhofsky reaction:

Figure 5: Effect of oxygen on the amplitude and frequency of the oscillations. 1: low oxygen concentration. 2: high oxygen concentration (from 27)

As a change in the oscillation frequency of the iodine formation reaction might play a role in the context of this work, a comparison between solutions purged with nitrogen or oxygen and unpurged solution was conducted.

Three samples of 10 mg/L I dissolved in 2.0 M HNO_3 were prepared. One of them was purged with O_2 for ten minutes to saturate the solution with O_2. Another sample was purged ten minutes with N_2 and the third sample was not purged. This experiment was only performed with the "fast washout manifold". The operating parameters of the ICP source are shown in the table 2. Between two consecutive measures a blank solution (2 M HNO_3) was introduced into the separator for 240 s to circumvent potential analyte carry-over. Every sample was measured tree times with 5 replicates each.

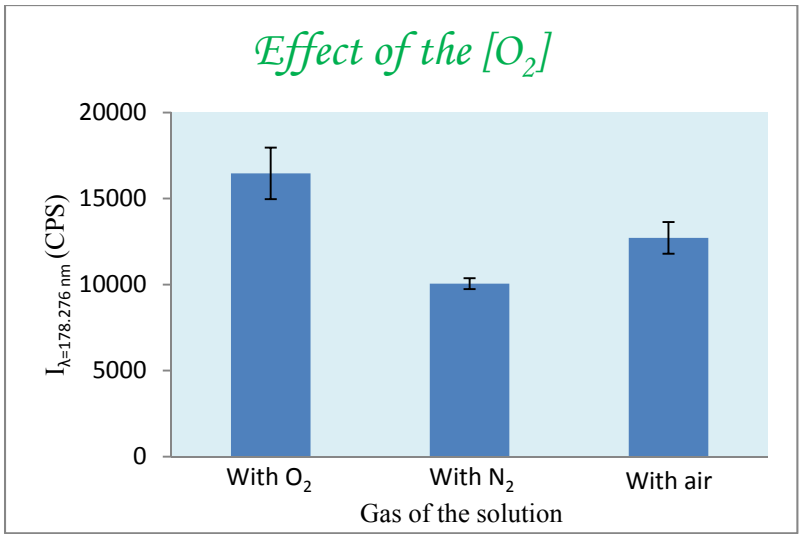


Figure 6: Effect of the O_2 concentration

Table 4: Numeric results of the experiment. λ = 178.276 nm

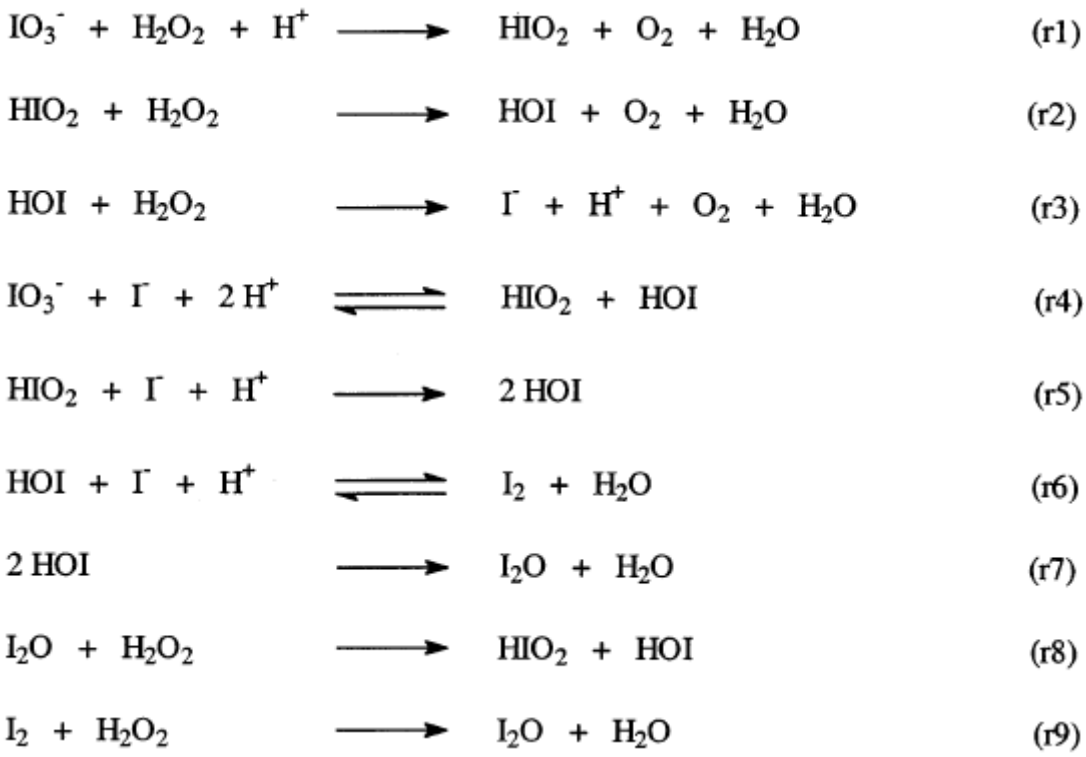
	Solution with O_2	Solution with N_2	Solution with air
Intensity (CPS)	16461 ± 1497	10055 ± 315	12714 ± 922
RDS (%)	9.09	3.13	7.25

From this experiment it can be concluded that the dissolved oxygen concentration affects the intensity of the signal. Using a student t-test there is a statistically significant difference between the nitrogen purged and the not purged samples as well as between the oxygen purged and the not purged solution on the 95% level. The increase of the dissolved oxygen concentration resulted in a signal increase of 30%. However, the oxygen also affects the precision of the measures. On the other hand, the precision improved, when the solution was purged with N_2.

From this experiment it can be concluded, that although purging the solution with oxygen increases the iodine signal slightly, the loss in precision outweighs this advantage. Similarly, the better precision obtained when purging the solutions with nitrogen does not justify this additional, time-consuming step.

4.1.2. Influence of the proton concentration and of the light

The influence of the proton concentration can be understood with the help of the model proposed by Sándor (28), which is based on the model proposed by Schmitz:



Scheme 1: Proposed Model for the Bray and Liebhafsky Reaction (from 28)

The rate constants used for modeling the Bray-Liebhofsky reaction are shown in Table 5:

Table 5: Rate Constants Used for Modeling the Bray-Liebhafsky Reaction

Rate constants	Value
k_1	1×10^{-6} M^{-2} s^{-1}
k_2	0.05 M^{-1} s^{-1}
k_3	0.25 M^{-1} s^{-1}
k_4	4×10^{4} M^{-3} s^{-1}
k_4	3000 M^{-1} s^{-1}
k_5	4×10^{10} M^{-2} s^{-1}
k_6	1×10^{12} M^{-2} s^{-1}
k_6	1000 s^{-1}
k_7	700 $M^{-1}s^{-1}$
k_8	1×10^{7} $M^{-1}s^{-1}$
k_9'	1×10^{-4} M^{-1} s^{-1}
a	6.6×10^{-6} $M^{-1}s^{-1}lux^{-1}$

Of the five reactions involved in the reaction scheme, four use H_3O^+ as reactant. This clearly indicates the importance of this parameter.

According to the proposed model by Sándor (23) light has also an important effect in the Bray-Liebhofsky reaction. I^- is involved in several steps and can be generated by homolytic cleavage of I_2 by light:

$$I_2 \xrightarrow{h\nu} 2I$$

More specifically, this model assumes that light can affect the kinetic of reaction r9 according to:

$$k_9 = k_9' + k_9'' \qquad \text{(equation 1)}$$

$$k_9'' = a \cdot I \qquad \text{(equation 2)}$$

$$k_9' = the\ rate\ constant\ of\ r9\ in\ the\ absence\ of\ light$$

$$k_9'' = the\ rate\ constant\ of\ r9\ in\ the\ presence\ of\ light$$

$$I = light\ intensity$$

$$a = proportionality\ factor$$

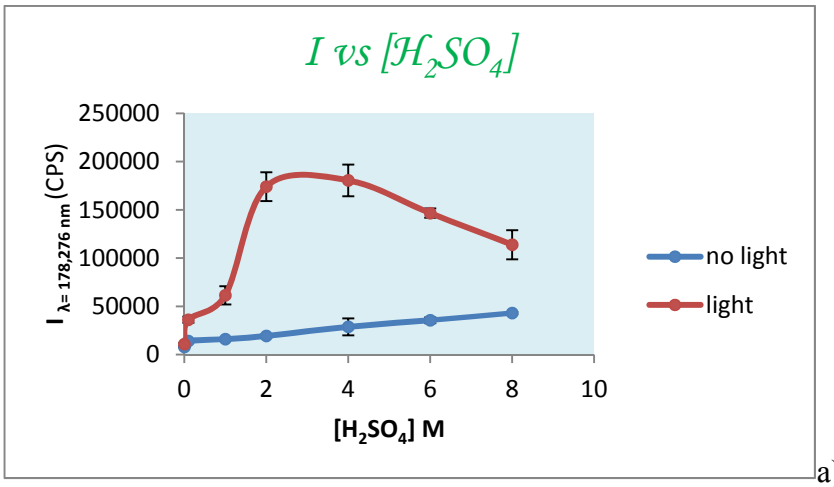
So, the equation 1 can be rewritten as:

$$k_9 = k_9' + a \cdot I \qquad \text{(equation 3)}$$

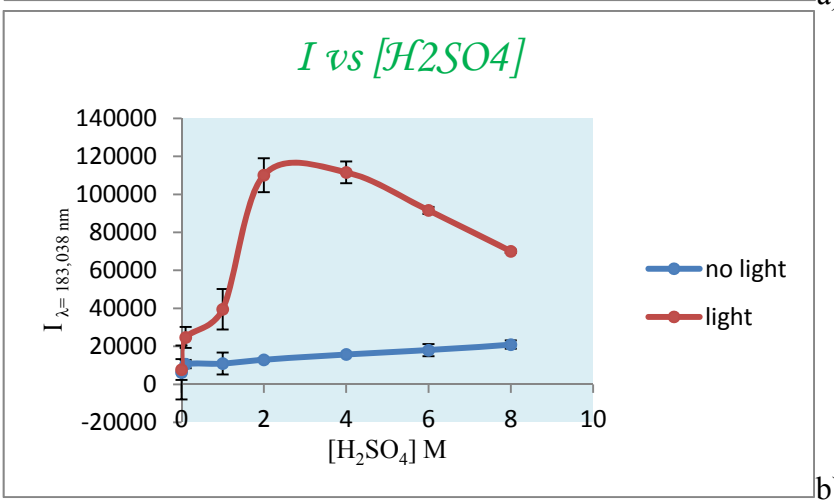
The light increases the rate of r9. This reaction forms IO_2, a very unstable chemical specie, which reacts quickly with H_2O_2 to form HIO_2 and HOI. The latter reacts with iodide to form molecular iodine and the first reacts with iodide to form HIO, which reacts in turn with iodide to form iodine. These reactions are faster than the oxidation of iodine to iodate and therefore the period of oscillation decreases.

The difficult mechanism of the Bray-Liebhofsky reaction makes a closer investigation under the specific conditions used for iodine volatilization necessary. The proposed reaction mechanism suggests that there might be an interrelated effect between proton concentration and light. Consequently, both effect where studied in a combined manner.

Samples of 10 mg/L iodine in different concentrations of sulfuric acid were analyzed in presence and absence of light using the "fast washout manifold" operated under the conditions reported in table 2. The sulfuric acid concentration ranged from 0 to 8 M: 0, 0.1, 1, 2, 4, 6, 8 M. A tungsten lamp of 400 W was used as light source. Between the lamp and the vaporization manifold a distance of about 30 cm was maintained to avoid significant heating of the sample. The samples were not purged with either O_2 or N_2.



a)



b)

Figure 7: a) Influence of the proton concentration and of the light. An interference of the sulfur is observed b) Influence of the proton concentration and of the light. No interference of the sulfur is observed

In the absence of light a nearly liner increase of the iodine signal with the concentration of sulfuric acid was encountered as shown in figure 7. From 0 M to 8 M sulfuric acid the signal increased about four-fold.

In the presence of light from the tungsten-lamp the iodine signals of all acidified samples were strongly enhanced when compared to the previous measurements. At 2 M sulfuric acid the signal of iodine was 9 times higher than the signal obtained at the same acid concentration but without additional irradiation. Compared to the not acidified sample the iodine signal at 2 M sulfuric acid was 16-fold enhanced and at 4M the an enhancement of a factor of 17 was encountered. Increasing the concentration of sulfuric acid above 4 M caused a steady reduction in the iodine emission signal.

It is interesting to speculate, that the increase of the iodine signal between 0 and 4 M is probably caused by a shifting of the reaction equilibrium of reactions r1, r4, r5, and r6. On the

other hand, the reduction of the iodine signal might be explained by an inhibition of r3 at very high H_3O^+ activity.

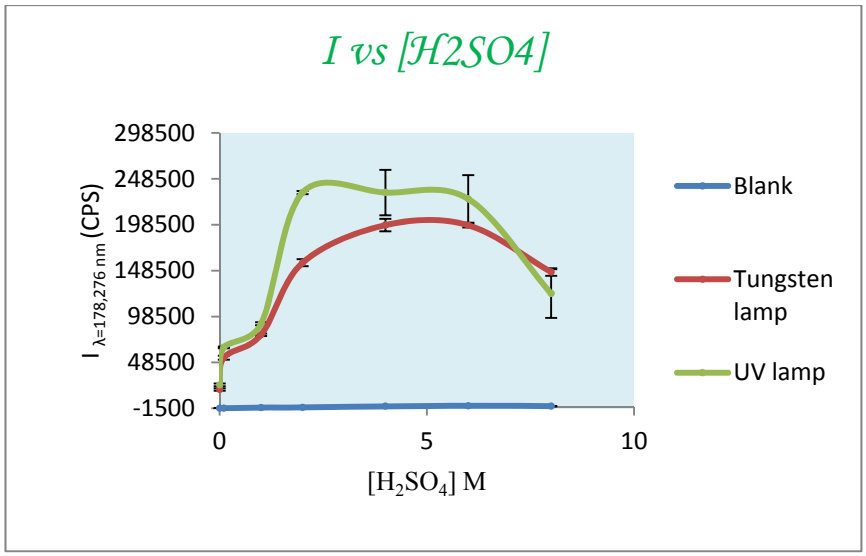
Clearly, the irradiation of the volatilization manifold caused a strong signal enhancement of iodine on all investigated iodine emission lines. Still, the mechanism is not entirely clear – mainly because the Bray-Liebhofsky reaction itself is, 8 decades after being first described, not completely understood.

4.1.3. Influence of the type of light: tungsten lamp vs ultraviolet lamp.

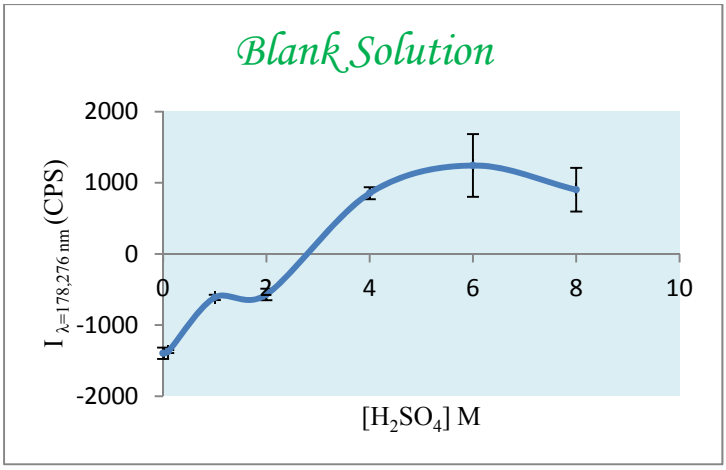
Though, a mechanistic study was beyond the scope of this work it was felt that the wavelength of the light, that caused signal enhancement, should be characterized broadly into UV, VIS or IR. The tungsten lamp emits dominantly VIS and IR. Therefore, experiments with UV irradiation and heated samples were conducted: As noted earlier, during the experiments with the tungsten lamp a significant heating of the sample solution in the vapor generation manifold was avoided. As a VIS-free IR light source was not available, it seemed reasonable to conduct experiments on the effect of IR radiation by simply heating the sample solution to 40°C.

Samples of 10 mg/L iodine in different concentrations of sulfuric acid were analyzed in presence and absence of light using the "fast washout" manifold operated under the conditions reported in table 2. The sulfuric acid concentration ranged from 0 to 8 M: 0, 0.1, 1, 2, 4, 6, 8 M. A tungsten lamp of 400 W and a commercial UV lamp for resin curing (JLV205, 4 x 9 W UV lamps) were used as light sources. Between the tungsten lamp and the vaporization manifold a distance of about 30 cm was maintained to avoid significant heating of the sample. No heating was encountered when using the UV lamp and the distance between lamp and volatilization manifold was about 10 cm.

In order to study the influence of the distance between tungsten lamp and the vaporization manifold an additional experiment was conducted wherein the distance was increased from 30 cm to 200 cm.



a)



b)

Figure 8: Influence of the type of light a, b) blank solution: apparent signal of iodine caused by a spectral interference with S 178.226 nm

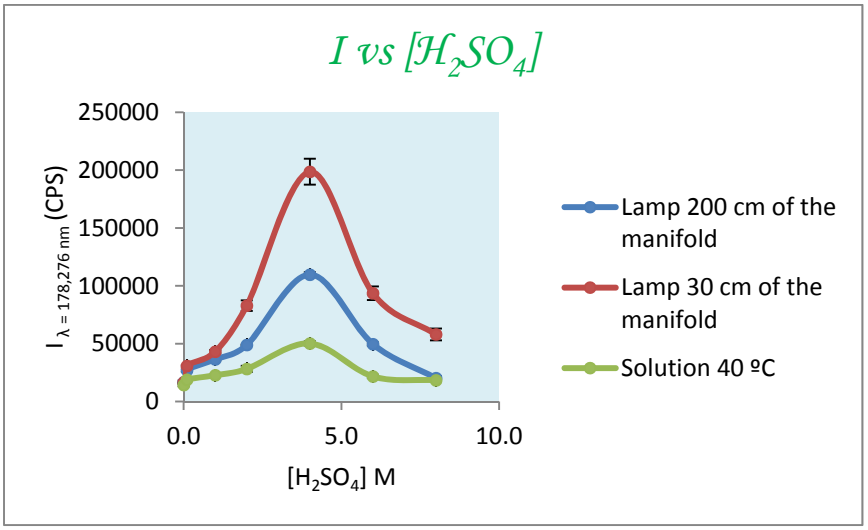


Figure 9: Effect of light or sample temperature on the iodine signal obtained by volatilization. Blue: effect of the light in the Bray and Liebhofsky reaction when the lamp is 200 cm away from the manifold; Red: effect of the light when the lamp is 30 cm away from the manifold; Green: Role of the temperature in the Bray and Liebhofsky reaction

The results presented in figure 8 clearly indicate that in vapor generation higher signal intensities can be obtained by using an UV light source than by using a tungsten lamp. Moreover, a stable signal plateau was reached at lower acidity in the sample (2 M) and remained stable up to 6 M. The highest iodine signal encountered by illumination with the tungsten lamp was obtained in 4 M sulfuric acid.

Though the iodine signals obtained by UV irradiation were higher than the ones obtained by tungsten lamp illumination they were less stable and higher RSD's were encountered. It is interesting to note, that for most samples an increasing signal trend was observed for the five replicates measured, indicating instabilities in the vapor generation process than could also not be circumvented by increasing the time between the autosampler needle dips into the sample and start of the detectors readout. Though the reason for this is unknown it seems to be inherent to both vapor generation manifolds. Consequently, UV irradiation was not perused further.

Heating the sample had a small but significant effect on the iodine emission signal, as shown in figure 9. With increasing sulfuric acid concentration the iodine signal first increased up to a maximum at 4 M and dropped thereafter. This behavior followed the same pattern than the illumination with the tungsten lamp. Nevertheless, the iodine signal at 4 M sulfuric acid is 4 times lower when heating the sample compared to illumination the same sample with the tungsten lamp. This is also evident from the iodine signal obtained at illumination from a distance of 200 cm rather than 30 cm. When the distance of the lamp was increased by a factor of 6.7 the iodine signal dropped by a factor of 2. It is important to note, that by increasing the distance between lamp and vapor generation manifold by a factor of 6.7 the radiation density decreases by a factor of 44. Clearly, the illumination of the acidified sample and hydrogen peroxide increases the reaction rate but this increase is not a linear function with illumination intensity.

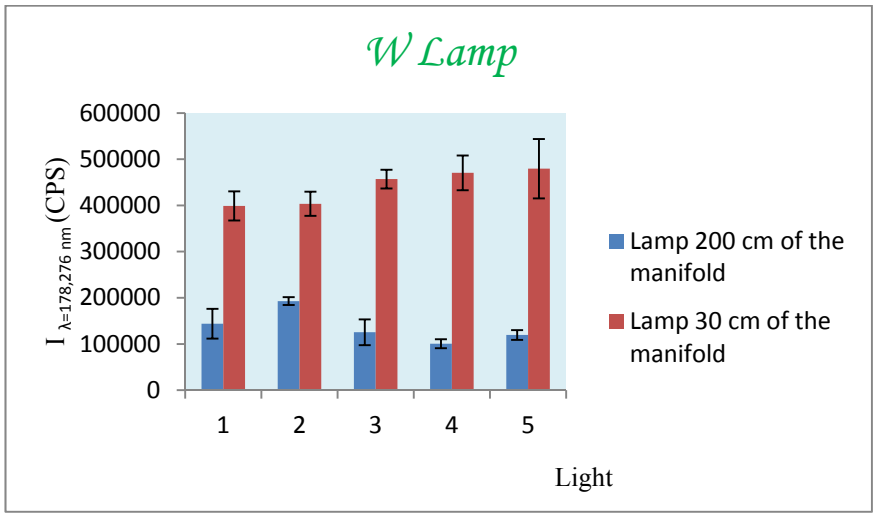
The use of a tungsten lamp has an additional advantage: there are fewer molecules, which react with the light of a tungsten lamp than with the light of an ultraviolet lamp. That is especially important in the analysis of real samples because there are a much bigger number of species and the ultraviolet light could produce some reaction, which interfere with the analysis.

The signals obtained using the tungsten lamp were different when compared with figure 7. This can be related to the sloped glass frit: It was observed, that the sample follows different paths on the frit for each sample before being eliminated through the waste. Therefore, iodine has sometimes more time to reach the gas phase. That may affect the reproducibility of the measurements.

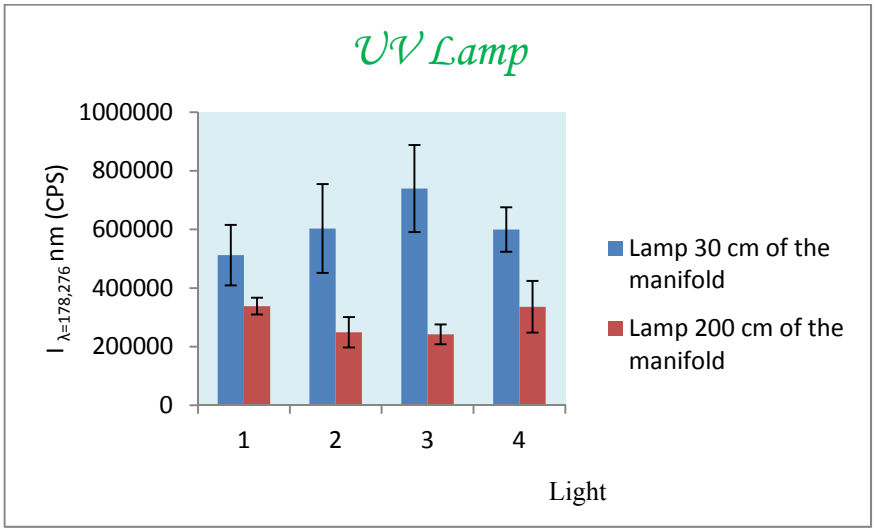
4.1.4. Influence of the volatilization manifold

From the previous experiment it was evident that some of the instabilities encountered might be related to the different flow-patterns of the sample on the tilted glass frit. As already shown in figure 3 a second volatilization manifold was available for evaluation – the "high sensitivity" manifold.

In order to clarify whether there is a manifold-induced source of instability, the effect of light from a tungsten / an UV lamp was repeated using the "high sensitivity" manifold. Samples of 10 mg/L in 4.0 M sulfuric acid were measured. The rate of the waste removal peristaltic pump was adjusted to work very fast, quickly removing the reaction products. Each experiment was repeated five times to assess the variability within different analysis.



a)



b)

Figure 10: Role of the visible und ultraviolet radiation in the Bray- Liebhofsky reaction and study of its influence with the distance; a) Influence of the visible radiation; b) Influence of the ultraviolet radiation

The results shown in figure 10 indicate as expected, that the high sensitivity manifold permits to obtain higher signals than the "fast washout" system. For the same iodine concentration the signal in the "high sensitivity" manifold is about a factor of 2.3 higher than in the "fast washout" manifold. This suggests that the intensity of the signal can be related to the residence time of the sample in the separator.

Consistently with previous experiments the highest signals, but inferior precision was obtained with UV irradiation. Consequently, illumination with the tungsten lamp was considered the best approach.

Though the inter-sample precision was smaller when illuminating the reaction mixture with the tungsten lamp when compared with the UV-lamp data, it was realized, that the first of fife individual replicates per sample was significantly smaller. This could be due to the fact that the preflush time might have been not long enough to attain stable reaction conditions. As shown in figure 11, by increasing the sample preflush time from 120 to 180 s the inter-sample precision could be significantly improved (from 8.05% to 3.03%).

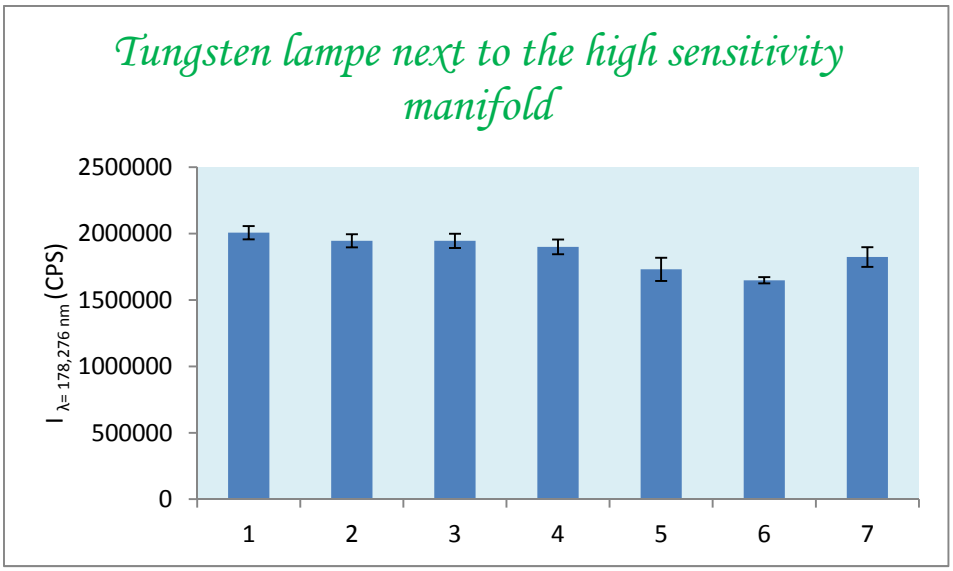


Figure 11: Seven consecutively measured samples of 10 mg/L iodine; preflush time: 180 s

Consequently, a sample preflush time of 180 s was maintained for further experiments.

4.1.5. Influence of the mixing coil on the Bray-Liebhofsky reaction

In the previous experiments a mixing coil was used to pre-react the acidified sample and the hydrogen peroxide. It was considered of importance to investigate whether the Bray-Liebhofsky reaction is momentary or if it takes some time to fully develop.

Samples of 10 mg/L iodine in 4.0 M H_2SO_4 were measured. The samples were mixed with the hydrogen peroxide either – similarly to the previous experiments – in the mixing coil or only in the separator (no mixing coil). The high sensitivity manifold illuminated with the light form the tungsten lamp was used for this experiment.

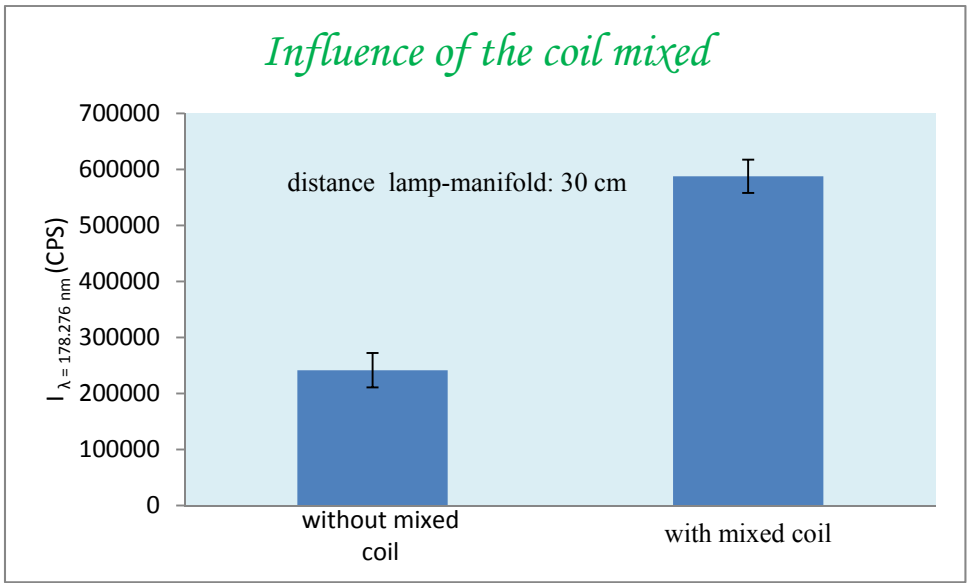


Figure 12: Influence of the mixing coil on the Bray- Liebhofsky reaction

From figure 12 it can be deduced, that the iodine signals are more intense when the reducing agent and the sample are mixed in the mixing coil. That suggests that the kinetic of the reaction is not very fast. . Taking into account all data up to now, it seems that this reaction time increases in absence of light. For this reason, the previous mixture of the reductant and the sample in the mixing coil is a crucial factor, because the sensitivity of the analysis depends strongly on the mixing time of the reducing agent and the sample. Though it was not attempted to modify the length of the mixing coil, this seems to be an important parameter for further investigations.

4.1.6. Study of the role of the light in the (mixing) coil and in the separator

The previous experiments revealed the crucial role of light to the formation of I_2 during the reaction of iodate with hydrogen peroxide. The previous experiment highlighted the importance of the mixing coil and the reaction mechanism suggests that the illumination should have highest effect if performed on the mixing coil. To foster this theory, an experiment was carried out wherein either the entire manifold was illuminated, or the mixing coil only, or the

gas/liquid separator only. Samples of 10 mg/L iodine in 4.0 M H_2SO_4 were introduced into the "high sensitivity" vapor generation manifold.

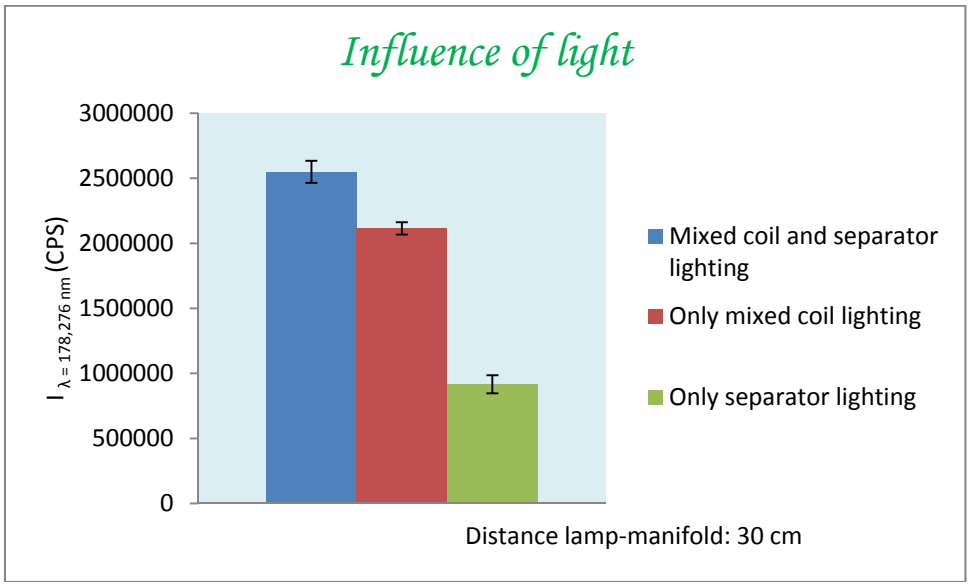


Figure 13: Influence of illumination of different parts of the volatilization manifold with a tungsten lamp: blue) illumination of the complete manifold, red) illumination of the mixing coil only, green) illumination of the gas/liquid separator only

The most intense iodine signals were obtained when the light illuminated both - the gas/liquid separator and the mixing coil. There is a small, but significant difference between the signals when the separator and the mixing coil are illuminated and when only the last one is illuminated.

4.1.7. Optimization of the volatilization conditions

In the previous experiments only one or two parameters were changed at a time. Though this simplex optimization provides insights into the reaction it does not fully cover the different interactions between the parameters. Based on the previous experiments the parameters listed in table 5 were selected for further investigation and a chemometric program was used for modeling a tree parameter center star design. The studied factors are light, proton concentration (M) and oxygen concentration in the solution. The source of light, a tungsten lamp, was positioned 30 cm away from the manifold.

Table 6: Different parameters to optimize the conditions to perform the analysis

N_2	$[H_2SO_4]$ M	Light
Purged (Yes)	1.0	With light (Yes)
	4.0	Without light (No)
Without purged (No)	6.0	Light only in the of the vapor generation manifold (LVM)

Table 7: Ranom order to study of all possible experimental combinations

DESIGN OF EXPERIMENTS							
Block	N_2	H_2SO_4 M	Light	Block	N_2	H_2SO_4 M	Light
1	Yes	6.0	LVM	2	No	6.0	No
	Yes	4.0	LVM		No	1.0	LVM
	No	6.0	LVM		Yes	4.0	Yes
	No	1.0	LVM		Yes	4.0	No
	No	4.0	No		No	6.0	LVM
	Yes	6.0	No		Yes	1.0	LVM
	No	6.0	No		Yes	4.0	LVM
	No	4.0	Yes		No	1.0	Yes
	No	1.0	Yes		Yes	1.0	Yes
	Yes	1.0	LVM		No	4.0	Yes
	Yes	4.0	Yes		Yes	6.0	Yes
	No	6.0	Yes		Yes	1.0	No
	No	1.0	No		Yes	6.0	No
	Yes	1.0	No		No	1.0	No
	Yes	6.0	Yes		No	6.0	Yes
	No	4.0	LVM		No	4.0	No
	Yes	4.0	No		No	4.0	LVM
	Yes	1.0	Yes		Yes	6.0	LMV

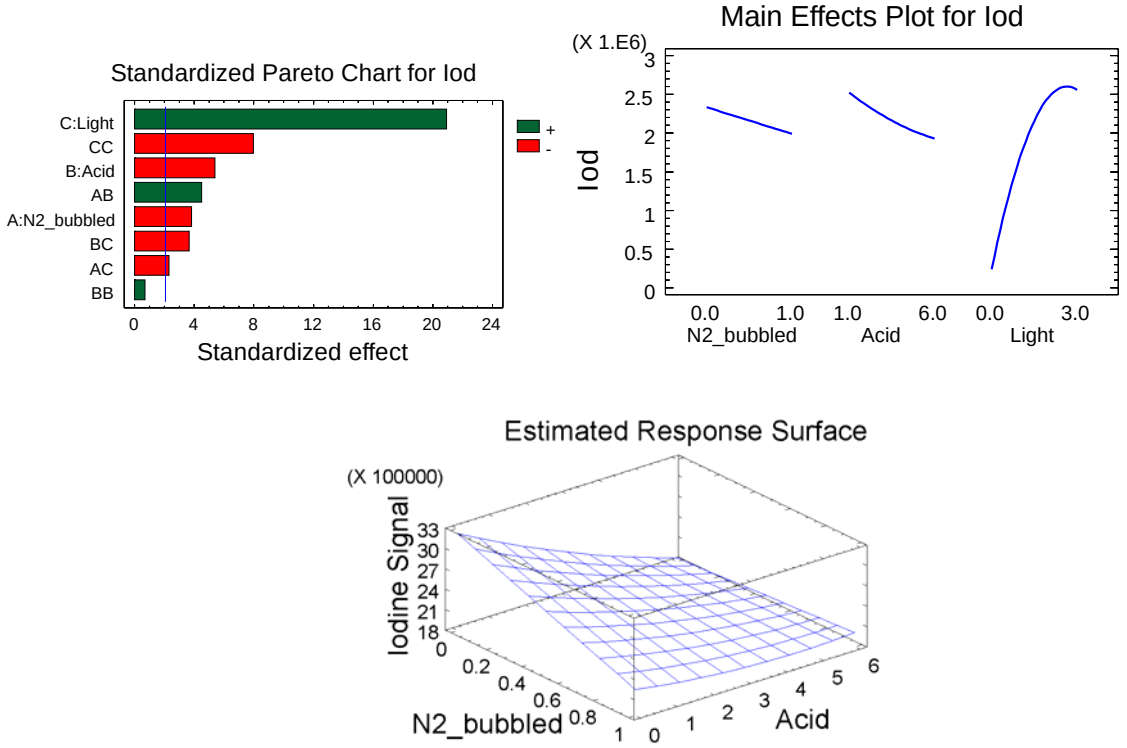


Figure 14: Standarized pareto chart for iodine (above left), main effects plots for iodine (above right), estimated response surface (down)

From the graphs in figure 14 can be concluded that the light was the dominating factor for maximizing the emission signal intensity of iodine. Sample acid concentration had also a significant but less dominant effect. It is interesting to note, that the statistical model used suggested that with increasing proton concentration the iodine signal decreased. This is in clear disagreement with the previous experiments. It is important to realize, that the starting parameters of every statistical model are of great importance. In this experiment, the sample acidity was selected between 1 and 6 M. Previous experiments showed that the ideal acid concentration for maximum signal is between 2 and 4 M – depending on the vaporization manifold used. It might have been, that the statistical model was not able to exactly determine the optimal acid concentration for maximal iodine signal as the selected starting parameters were not ideal. Nitrogen purging was found to have only a small effect on the iodine signal. The highest iodine signal was predicted by the statistical model for the following parameters:

$[H_2SO_4]$	1.0 M
Light	Light close of the vapor generation manifold
N_2	Without purge

The optimized conditions are in good agreement with the previous experiments with exception of the concentration of the sulfuric acid. It is important to note, that the initial experiments were conducted with the "fast washout" manifold whereas in this experiment the "high sensitivity" manifold was used.

4.1.8. Optimization of the sulfuric acid concentration with the "high sensitivity" manifold

Samples of 10 mg/L iodine in diluted sulfuric acid were measured in presence of light of the tungsten lamp (30 cm between lamp and manifold). The sulfuric concentration was modified from 0 to 8 M: 0, 0.1, 1, 2, 4, 6, 8 M. The samples were not purged. This experiment was performed with the "high sensitivity" manifold under the conditions reported in Table 2.

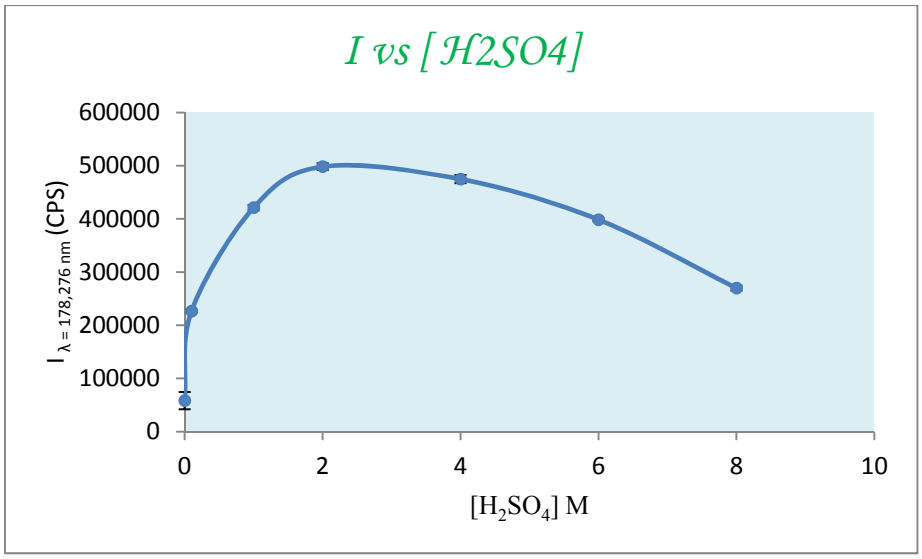


Figure 15. Influence of the sulfuric acid concentration with the high sensitivity manifold. It is not possible to see error bars because they are very small

The pattern shown in figure 12 follows the one also observed in figure 7 and 8. The main difference is that the maximum is slightly shifted from 4 M in case of the "fast washout" manifold to 2 M sulfuric acid in this manifold. This new lower proton concentration maximum presents two advantages:

1) Less sulfuric acid is required.
2) Lower heating patterns by mixing water and sulfuric acid, reduced cooling time and, therefore, less time spent in the preparation.

4.1.9. Effect of the H_2O_2 concentration and the presence of the stabilizers in the H_2O_2 on the volatilization of iodine

Hydrogen peroxide is commonly sold as 30 % solution. Depending on the purity and the intended use stabilizers are added. This is especially true for the 50 % solution that is also commercially available. The mechanism of the Bray-Liebhofsky reaction is already a highly complex. Therefore, any additional parameter that affects this reaction certainly alters the volatilization of iodine.

Two stabilizing reagents are dominantly used in commercial hydrogen peroxide: Na_2SnO_3 or a mixture of H_3PO_4 and $Na_2H_2P_2O_7$ (about 0.01 % each).

Consequently the effect of different concentrations of hydrogen peroxide (30 % vs. 50%) and the presence of stabilizers was investigated. It is important to note, that from previous experience it is known, that lower hydrogen peroxide concentrations than 30 % result in a

suppression of the iodine formation. At the time these experiments were conducted a 50 % solution of hydrogen peroxide was not available.

Samples of 10 mg/L iodine in 2.0 M sulfuric acid were measured in presence of light from a tungsten lamp (30 cm between lamp and volatilization manifold) and different qualities of hydrogen peroxide (see table 6) were employed.

Table 8: Different types of H_2O_2 employed

Nr.	Name	Number	Vendor	Concentration	Stabilizers
1	Hydrogen peroxide 30%	K44176709 315 1.07209.1000	Merck	30%	No
2	H_2O_2 30%	K42254197 119 1.08597.1000	Merck	30%	Yes 0.015%$NaH_2P_2O_7$, 0.01%H_3PO_4, 0.006% NH_4NO_3
3	H_2O_2 solution	101093886 516813-500MML	Sigma-Aldrich	50%	Yes Na_2SnO_3
4	H_2O_2 30 % for synthesis	231-765-0	Carl Roth	30%	Yes unknown stabilizer

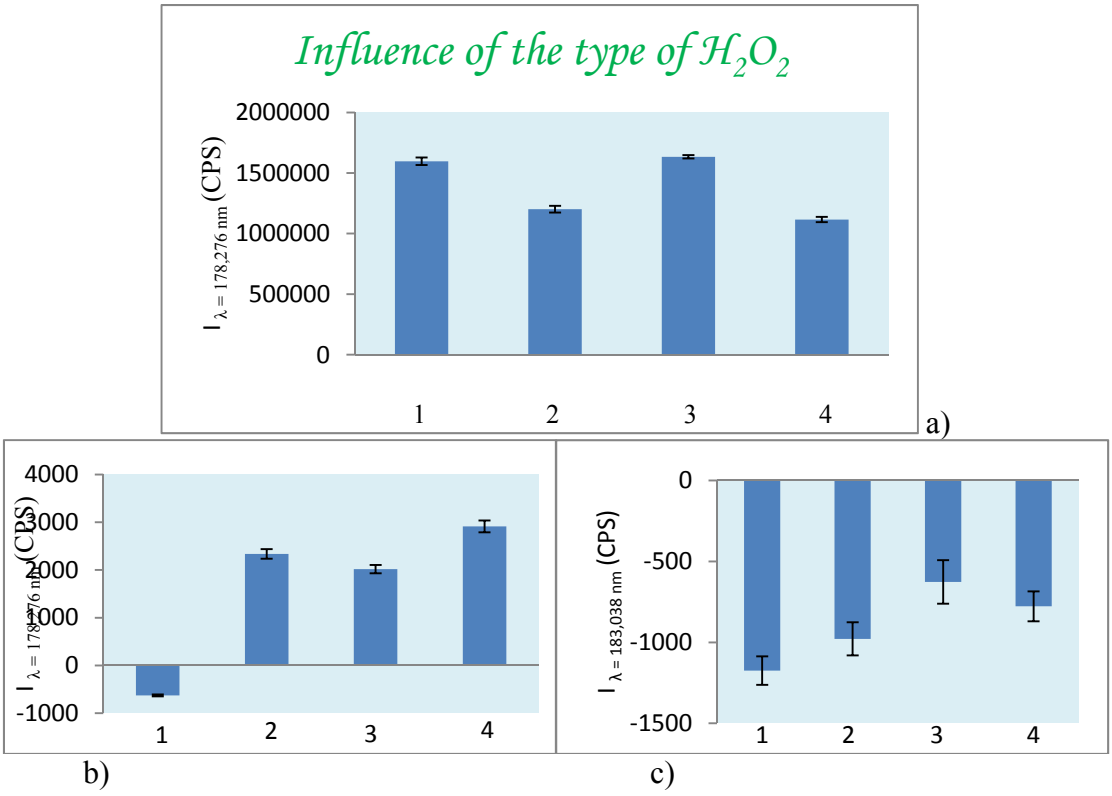


Figure 16: Iodine signal obtained from a 10 mg/L iodine solution using the "high sensitivity" manifold and different brands of hydrogen peroxide. Sample designation refers to table 8. a) iodine signal of the 10 mg/L sample for different brands of hydrogen peroxide, b) iodine signal of a blank solution on the 178.216 nm emission line, c) iodine signal of a blank solution on the 183.038 nm emission line

The results presented in figure 13 show that increasing the concentration of hydrogen peroxide above 30 % had no statistically significant effect on the iodine emission signal. On the other hand the presence of stabilization reagents in the 30 % hydrogen peroxide resulted in a signal loss of about 32 %. It is interesting to note, that the 50 % hydrogen peroxide is stabilized as well. Clearly, the presence of this stabilizing reagent has been compensated by the higher concentration.

Another interesting point is the behavior of the iodine signal of the blank solution due to the potential spectral interferences from phosphorous based stabilizing reagents. As shown in figure 16b the apparent iodine signal on the 178 nm emission line is significantly higher if stabilized hydrogen peroxide is used. This is not the case for the 183 nm emission line, where all 30 % H_2O_2 solutions had nearly similar signal level. This indicates, that there is indeed some spectral interference from P on the 178 nm emission line, though this effect is small and only of significance when very low concentrations of iodine should be quantified.

Clearly, unstabilized hydrogen peroxide with a concentration of 30 % is the most appropriate reagent for the volatilization of iodine.

4.2. Memory effects in the volatilization manifolds

The main reason for developing the “fast washout” manifold was the need for a high sample throughput that could not be fulfilled with the “high sensitivity” manifold due to the relatively large holdup volume in the gas/liquid separator. From the previous discussion it is evident, that the “high sensitivity” manifold is superior to the “fast washout” manifold in terms of inter-sample signal stability. Consequently, the “high sensitivity” manifold had to be improved to overcome the required long washing steps between two consecutive samples while not degrading its advantages.

Samples of 10 mg/L iodine in 2.0 M sulfuric acid were introduced into the “high sensitivity” manifold under optimized conditions (tungsten lamp in 30 cm distance to the volatilization manifold, 30 % hydrogen peroxide). After recording the iodine signal of the sample, the autosampler needle was moved to the rinsing solution and the iodine signal was constantly recorded over time. Two methods of rinsing were evaluated: In the first method only the standard peristaltic pump for removing the waste was used (the pump on the far right in figure 2). In the second mode a high speed peristaltic pump (Ismatec Reglo; pump speed 99; flow capability of 15 ml/min) was used to quickly drain the gas/liquid separator, while – using the same pump and a slightly smaller inner diameter pump tube (flow: 8 ml/min) was used to

flush the glass mixing coil and the gas/liquid separator with additional diluted sulfuric acid (see figure 2). This second mode of operation – also referred to as the high speed rinsing mode in this text - was expected to result in a fast dilution of the remaining reaction mixture in the volatilization manifold.

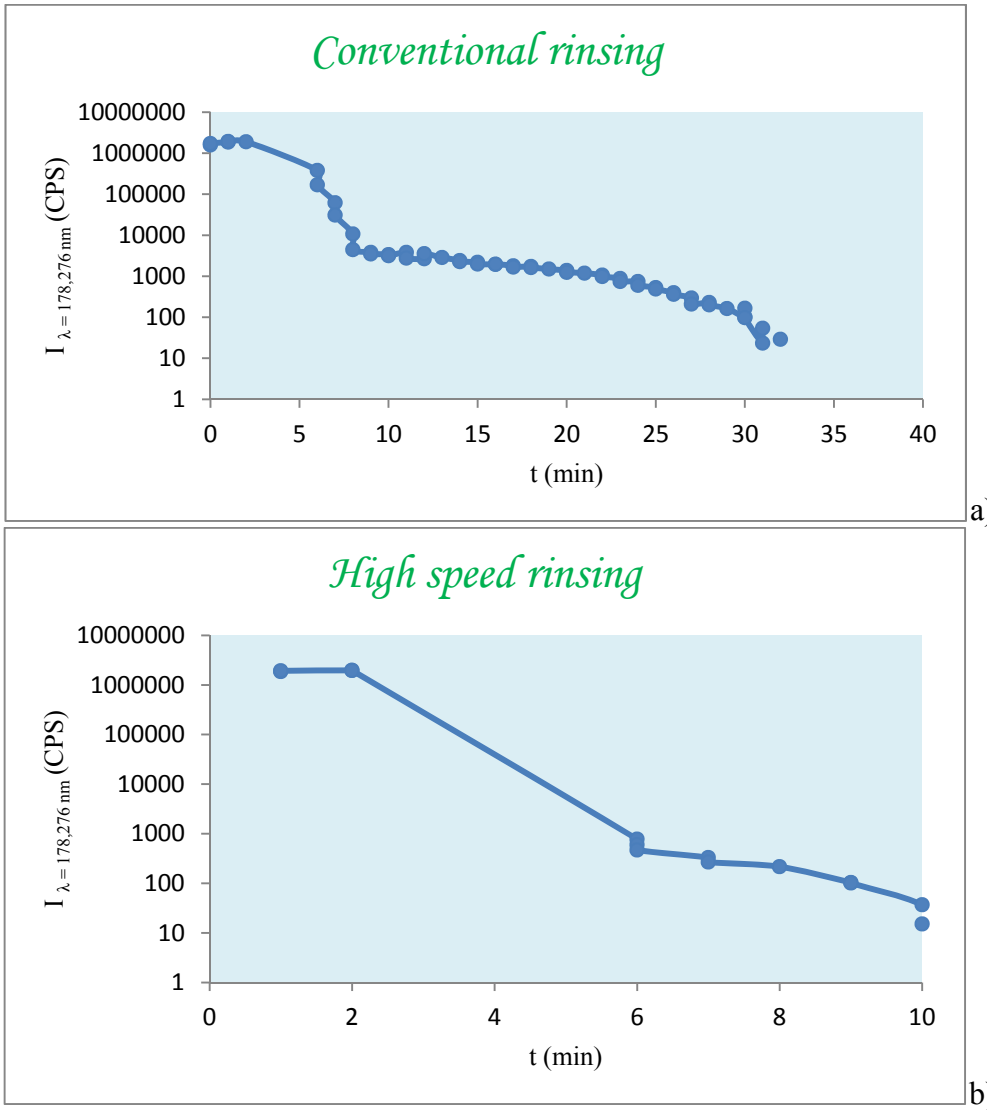


Figure 17: Necessary time to clean the system between two consecutive measures. a) conventional rinsing with only one peristaltic pump; b) high speed rinsing with an additional high speed peristaltic pump.

Not surprisingly, the time required for cleaning the separator decreased drastically when the high speed rinsing mode was used. To get the iodine signal after introducing a concentrated sample back to background level (below 10 cps) took 30 minutes in the standard rinsing mode but less than 10 minutes in the high speed mode. On the contrary, the amount of sulfuric acid increases significantly in the high speed rinsing mode due to the flow rate of 8 ml/min.

4.3. Sample level in the separator

During the chemical volatilization of the iodine in the manifold the liquid level was found to be slightly above (about 2 mm) the waste fluid exit tube. The reason for this is lies in the fundamental operating principle of the manifold: The flushing of the liquid by argon through the glass frit causes the formation of instable foam. Near the frit this foam was observed to contain more liquid than near the top of the "liquid" level. The waste pump always removed not only the liquid phase but also some of the gaseous phase. Thereby, an equilibrium between liquid and gas phase was formed in the vaporization manifold and the holdup volume was slightly larger than the one dictated by the manifolds geometry (see figure 18).

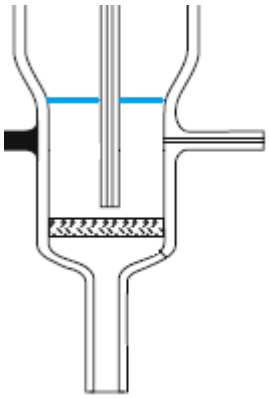


Figure 18: Level of the "liquid phase" in the volatilization manifold. Note, that due to the bubbling with argon always a mixture of liquid and gas is present above the glass frit.

The used volatilization manifold was equipped with two waste fluid ports: one 11 mm above the glass frit and one 19 mm above the glass frit. The initial setup used the waste fluid port that allowed a higher liquid level in the gas/liquid separator.

Preliminary experiments showed that for the same sample there was no significant difference in the iodine emission signal between the two waste fluid ports. In both cases – as described – the foam level was slightly above the waste fluid port. Only when the foam level was forced to the same height as the lower waste fluid port the signal dropped slightly but significantly.

As the lower waste fluid port also resulted in less holdup volume in the gas/liquid separator a reduced rinsing time was expected from this arrangement. The experiment described in section 4.2 was repeated und the same conditions but using the waste exit port at 11 mm above the glass frit.

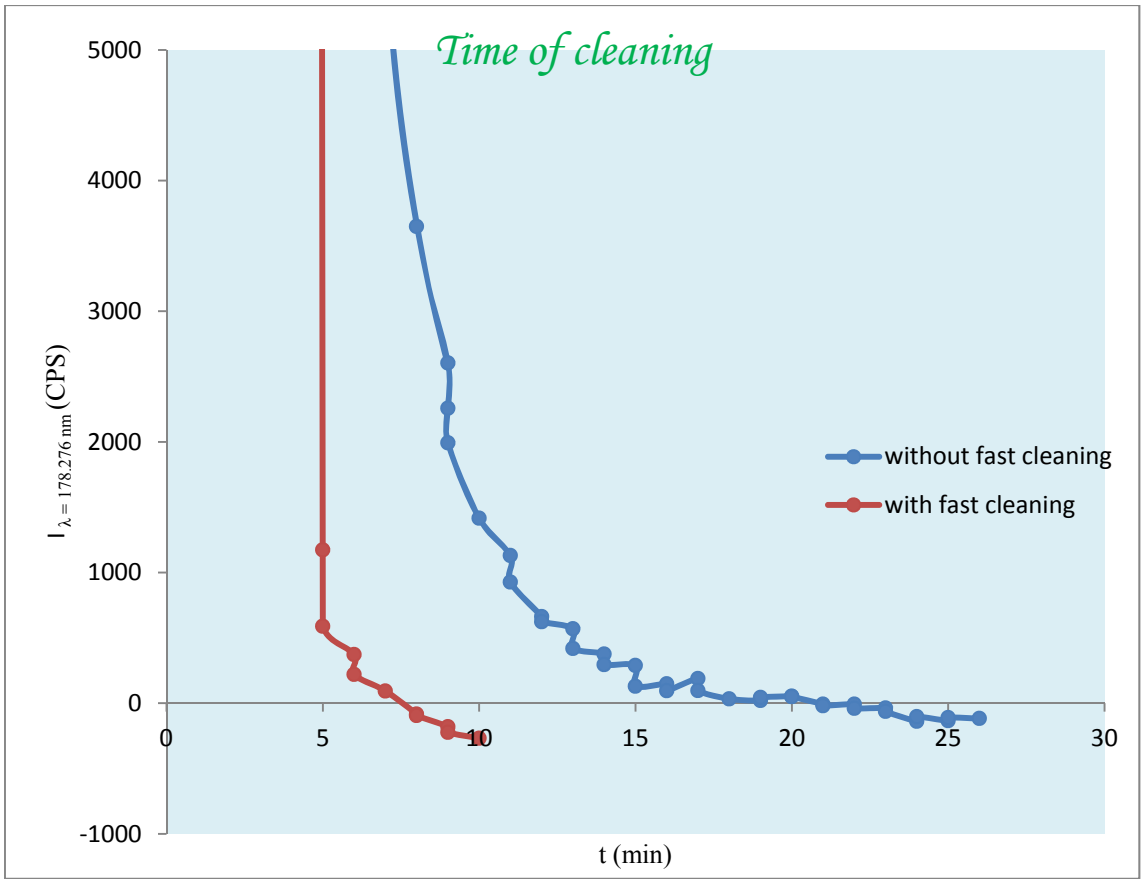


Figure 19: Necessary time to clean the system between two consecutive measures when using less holdup volume in the gas/liquid separator than in figure 17

From the results shown in figure 19 it is evident, that using the lower waste fluid port also reduced the washing time of the manifold between samples significantly. Blank level (below 10 cps) was reached after 20 minutes in standard rinsing mode and after 7 minutes in the high speed mode.

Consequently, the lower waste fluid port (11 mm above the glass frit) was used further on.

4.4. Optimized method

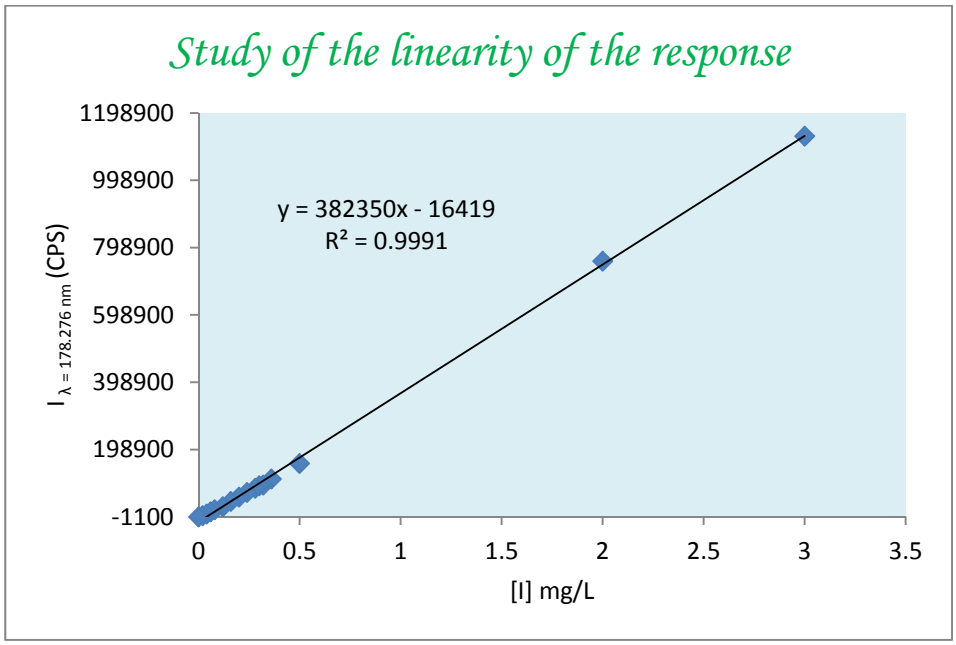
In conclusion, the following conditions were considered as being the optimum the iodine determination:

Tabelle 9: Optimized condition for the volatilization of iodine

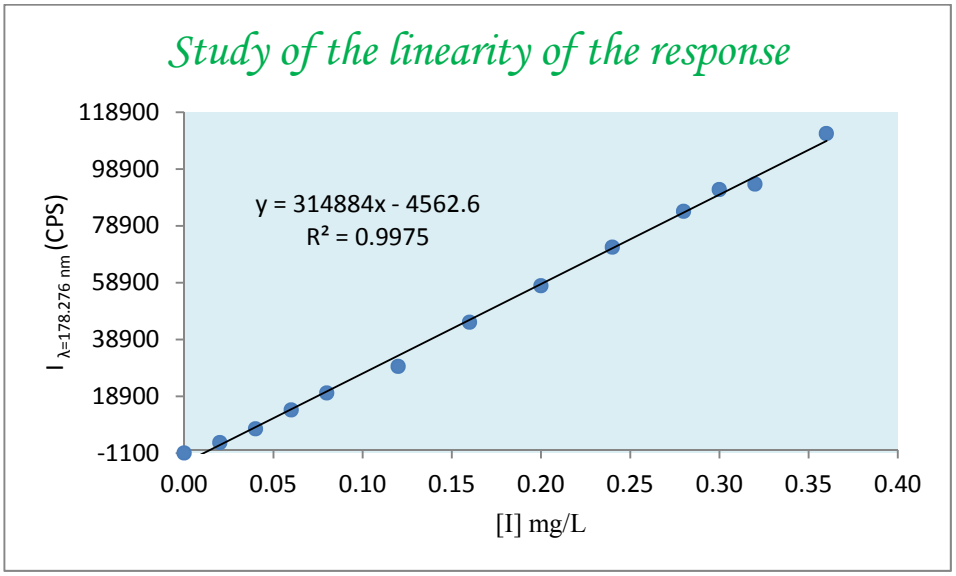
Parameter	Value
Presence the dissolved oxygen in the sample	No purging necessary
Concentration of the H_2SO_4	2 M
Source of light	Tungsten lamp
Manifold employed	The high sensitivity vapor generation manifold
Distance between the source of light and the manifold	30 cm
Temperature	Room temperature
Parameters of the plasma and rates of the peristaltic pumps	(see Table. 2)
Waste exit port (height above the glass frit), mm	11
Time of the preflush	180 s
Washing of the separator	Always between two consecutives measures
Time of the washing of the separator between two consecutives measures	7 min (30 s for calibration standards)
Level of the sample in the separator (measured from the bottom of the glass frit)	5 cm

4.5. Method validation: Linearity of the calibration function

To study the lineal range a calibration of iodine in 2.0 M H_2SO_4 was prepared (0.00, 0.02, 0.04, 0.06, 0.08, 0.12, 0.16, 0.20, 0.24, 0.28, 0.30, 0.32, 0.36, 0.5, 1.0, 2.0, 3.0 mg/L I). The iodine additions to the final calibration solutions were made with electronic pipette. This experiment was carried out using the optimized conditions reported in paragraph 4.4.



a)



b)

Figure 20: Iodine calibration function; a) Linearity of the response from 0.00 to 3.00 mg/L I; b) an enlargement of the calibration function from 0.00 to 0.36 mg/L I. Error bars are smaller than the dots in the graph.

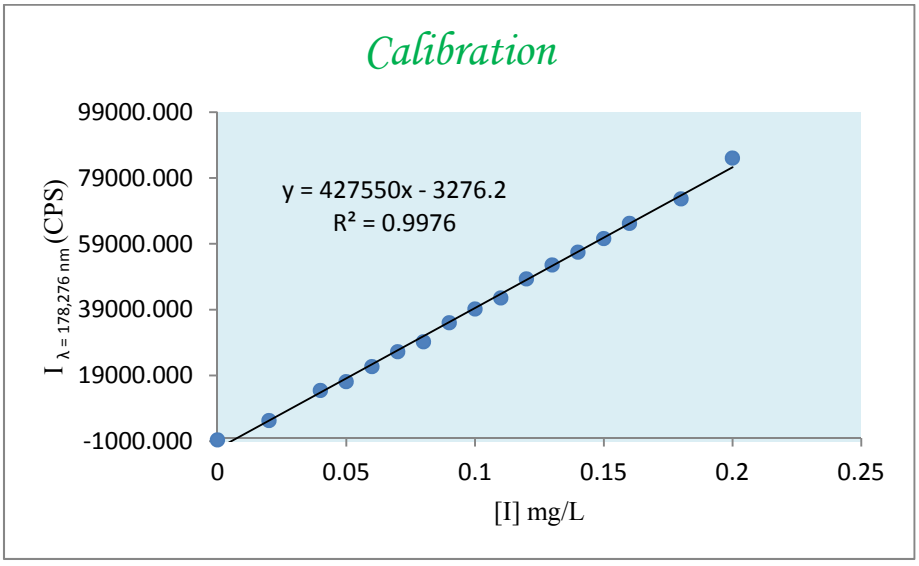
As shown in fugure 17 a linear response between iodine concentration in the calibration solution and the measured iodine emission signal was obtained up to 3 mg/L (highest tested concentration).

The 1 mg/L standard was suspected to be an outliner caused by erroneous preparation of the standard. The residual standard deviation of the calibration function with and without this potential outliner was calculated. By comparing these two values by means of an F-test the 1 mg/L standard was identified as outliner on the 95 % confidence level. Consequently the value was removed.

It seems interesting to note, that the signal of the blank solution was significantly higher than the calculated intercept of the calibration function (-1070 ± 201). The reason for this is unclear but it is interesting to speculate on the potential reasons: There might be a constant contamination of all samples that caused this bias in the blank solution. This contamination could only come from the sulfuric acid used in these experiments.

4.6. Method validation: Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated from a calibration function of the following standards: 0.00, 0.02, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.18, 0.2 mg/L iodine in 2 M sulfuric acid. The iodine additions were made with electronic pipette. This experiment was carried out using the optimized conditions reported in paragraph 4.4.



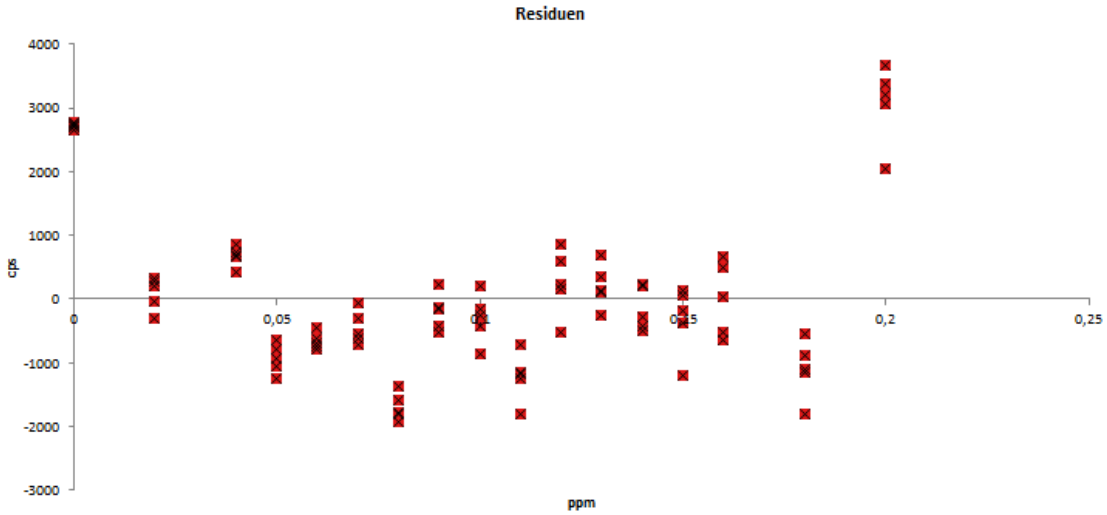


Figure 21: Calibration graph for iodine and the corresponding residuals. . It is not possible to see error bars because they are very small

Table 10: Detection limit and quantification limit in aqueous solution

Emission line	Limit detection	Limit quantification	Units
178.276 nm	0.0025	0.0087	mg/L
183.038 nm	0.0086	0.017	mg/L

The LOD was calculated using the blank method and the LOQ using the calibration method. The values listed in table 9 are much lower than the values obtained by pneumatic nebulization of 40 ppm - 280 ppm (29).

4.7. Method validation: Analysis of a certified reference material

A certified reference material BCR 151 (milk powder) was analyzed to assess accuracy and precision of the proposed iodine volatilization method. The sample digestion using microwave assisted acid digestion has been described in detail in section 3.3. A calibration function ranging from 0.00 to 0.18 mg/L iodine in 2 M sulfuric acid was used. The individual standard concentrations were 0.00, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18 mg/L iodine. In preliminary experiments an intense foaming in the gas/liquid separator was observed once the nitric acid concentration exceeds 0.8 M. Consequently, 6 mL HNO_3 were added to each 100 ml volumetric flask used for preparing the calibration standards prior making up to volume.

The iodine was added with an electronic pipette. This experiment was carried out using the optimized conditions reported in paragraph 4.4.

It seems interesting to note, that to the best of our knowledge the increased foaming of the reaction mixture in the cause of the Bray-Liebhofsky reaction has not been reported in literature. Therefore the mechanism remains unclear and should be subject to further investigations.

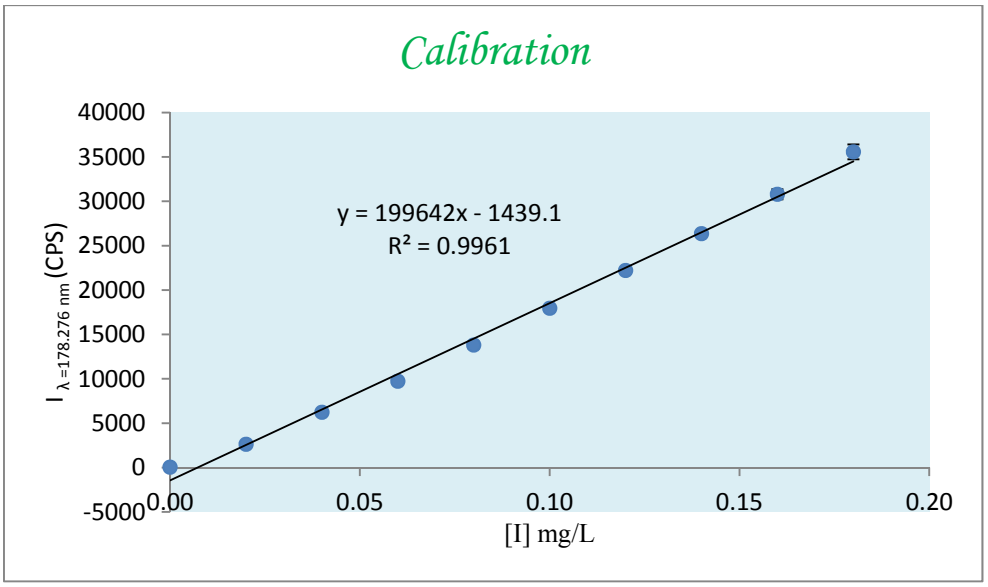


Figure 22: Calibration graph for iodine

The result for BCR151 shows that on the 95 % confidence level there is no statistically significant difference between the certified and the determined iodine value. Nevertheless the precision of the analysis is not satisfying. Further insights into the influence of nitric acid on the Bray-Liebhofsky reaction are needed to further improve this method.

Table 11: Determination of iodine in milk powder, mg/kg

Certified reference material	Measured concentration	Certified value
BCR 151	7±2 mg/kg	5.35 ± 0.14 mg/kg

5. CONSLUSIONS

From this work it can be concluded that the Bray-Liebhofsky reaction indeed can be used for chemical volatilization of iodine. The problems associated with this oscillating reaction can be overcome by illuminating the reaction mixture with light in the visible region of the spectrum. Moreover, a careful selection of the used reagents is of great importance: The hydrogen peroxide should not be stabilized and samples solutions should not contain more than about 1 M nitric acid. Not following the second guideline results in uncontrolled reaction conditions and extensive foaming in the gas liquid separator.

The obtained LOQ of 9 µg/L iodine for the more sensitive 178.276 nm emission line clearly shows the great potential of this method.

Method validation reveals that the linearity is maintained up to a concentration, where pneumatic nebulization is conveniently capable of quantifying the iodine level in the sample. It is important to note, that it was not attempted to investigate higher iodine concentrations than 3 mg/L.

By analyzing a certified reference material it was realized, that though the accuracy of the method is acceptable the precision needs to be improved and further work is necessary to understand and control the effects of additional reagents like nitric acid.

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7. APPENDIX

Materials

- Glass volumetric flask of 25, 50 y 100 mL
- Beaker 100 mL
- Erlenmeyer flask 1000 mL
- Pipettes
- Electronic pipette
- Tungsten lamp of 400 W
- Ultraviolet lamp
- Peristaltic pumps
- PTFE vessels

Reagents

- KIO_3 salt, Merck, Germany
- H_2O_2, 1.07209.1000, without stabilizers, 30%, Merck, Germany
- H_2O_2, 1.08597.1000, with stabilizers, 30%, Merck, Germany
- H_2O_2, for synthesis, 30 %, Carl Roth, German
- H_2O_2, with stabilizers, 50 %, Sigma-Aldrich, E.E.U.U.
- HNO_3, purified by subboiling in a quartz still
- $HClO_4$, pa, Merck, Germany
- Deionized water obtained on a Milli-Q system (18 M Ω Millipore system)
- Skim Milk Powder BCR 151