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Iodine-starch reaction renewed: determination of iodate in table salts *via* the lab-in-syringe technique

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A novel in-syringe automated method was developed for simple, sensitive, and green determination of iodate in table salts. It is based on iodine–starch complexation as an environmentally benign and economic approach. Triiodide is generated in the acidic buffered sample from iodide. Embedding it in the amylose helix resulted in a steel-blue-colored complex, which was spectrophotometrically measured at 600 nm. Step-by-step addition and mixing of a reductant, acid, and soluble starch to the sample were carried out in the void of an automated syringe pump. Essential experimental parameters were fine-tuned to optimize method sensitivity and linearity of response. A limit of quantification of 0.4 μmol L^{-1} (50 μg L^{-1}) iodate and a sampling frequency of 13 h^{-1} were achieved. Linearity of calibration was confirmed between 1.5 μmol L^{-1} to at least 10 μmol L^{-1} with possible straightforward working range extension by in-syringe sample dilution. The determination of iodate content in table salts was precise with relative standard deviation values of <1.8% and accurate with a mean analyte recovery of 98.4 ± 3.8% studied at 2 and 5 μmol L^{-1} levels. Noteworthy advantages are high method robustness, safe reagent manipulation, and a greenness score of 0.7 based on the AGREE metric tool, comparable to or surpassing those of formerly reported spectrophotometric methodologies (non-automated and flow-based). The developed method was a convincing, quick, and simple option to the existing protocols for iodate determination in commercial salts.

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1 Introduction

Iodine is a vital micronutrient that plays a crucial part in human growth and metabolism. The deficiency of iodine remains a global public health issue. It can lead to goiter, brain damage, intellectual disability, and stillbirth, while its overdose may lead to thyrotoxicosis.[1] The World Health Organization (WHO) advises consuming 90 μg of iodine per day for infants up to 5 years, 120 μg for school-age children (ages 6 to 12), 150 μg for adults, and 250 μg for pregnant and nursing mothers.[2] The use of iodized salts was proven effective in preventing iodine deficiency. Therefore, table salts are generally iodized preferably using potassium iodate (KIO_3) to ensure adequate iodine intake. Less frequently, potassium iodide (KI) is used for this purpose since iodide can be oxidized to volatile iodine due to humidity, atmospheric oxygen, heat, and sunlight and can also lead to a color change of the product.[3] The recommended iodization level is 50–60 mg kg^{-1} during production to achieve at least 15 mg kg^{-1} at the moment of consumption,[4] considering that losses during packaging, retail, and storage can occur.[2] In this picture, the determination of iodine/iodate concentrations in table salts during production is an important process indicator in terms of quality control to assure adequate iodine supplementation.[5] On the consumer side, urinary iodine concentration can be considered an impact indicator that reflects the actual state of iodine supply and covers the human variables influencing the iodine intake.[5]

Monitoring iodine and iodate concentrations is mostly based on photometric assays,[5] above all the Sandell–Kolthoff reaction.[6] It relies on the catalytic properties of inorganic iodine species (I_2, I^-, and IO_3^-) promoting the reduction of yellow-colored ceric ions by arsenic to the colorless cerous ions and following the decrease in color intensity photometrically.[7] However, even when opting for benign agents for sample digestion, the Sandell–Kolthoff reaction itself requires precise timing and handling and, due to the chemicals used, in particular the highly toxic arsenite, cannot be considered green.

Further methods have been proposed for the determination of iodine species based on a variety of instrumental techniques including potentiometry,[8] chemiluminescence,[9,10] capillary electrophoresis,[11] ion chromatography,[12,13] atomic emission spectrometry (AES),[14] and inductively coupled plasma mass spectrometry (ICP-MS).[15] Some of these methods may suffer from interference (methods based on redox reactions), or their robustness is disputable (chemiluminescence and capillary

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electrophoresis), or they require extensive operator training (ICP-AES and ICP-MS); moreover, the costly instrumentation needed is not universally available. Therefore, spectrophotometric methods, either manual or automated, *e.g.* in a flow manifold, represent a compromise between instrumental demands and reached sensitivity and selectivity. Various chromogenic approaches have been reported including bleaching of thionine or azure B,[16] methylene blue,[17] or methylene red,[18] reaction with *N*,*N*-diethyl-phenylenediamine (DPD) to yield the Wurster's dye,[19–21] azo-dye formation with[22] or without[18] sensitivity enhancement by homogeneous liquid–liquid extraction, or transformation into directly quantifiable triiodide.[23,24] While these methods are highly sensitive, they are also prone to interference, particularly in redox reactions, and thus have limited selectivity. In this sense, starch as an iodine-specific reagent remains a gold standard when determining iodate/iodine in table salts.

The iodine-starch reaction is used either in volumetric determination[25] or photometric assays.[26] However, issues with the final product precipitation, limited reactivity, or complex formation, *e.g.* due to sub-optimal pH, buffering, and system cleaning, might impair the determination regarding method linearity and sensitivity.[27] In this work, a photometric assay was fine-tuned and automated to achieve a user-friendly green method with improved analytical performance.

Whatever the chosen assay, reliability, throughput, and cost-efficiency of analytical methods can often be improved significantly by automation. It can considerably reduce the time for sample preparation, reduce errors in handling, and enable procedural downscaling with simultaneous reduction in waste and sample needs. Flow analysis techniques (FTs) are a group of versatile automation approaches for laboratory procedures comprising steps such as solution metering, mixing, dilution, matrix separation, colorimetric reaction or analyte extraction. Most often, sample and reagent zones are introduced and transported in a tubing system aided by a liquid carrier, and undergo some of the above listed unit operations. FTs are simple and cost-efficient alternatives to other automation approaches and ideally suited to miniaturize analytical assays based on chromogenic reactions.[28]

Classical FT approaches based on gradual mixing patterns have been successfully applied to the determination of iodine, iodate or periodate.[3,19,24,29,30] Flow injection analysis (FIA) was applied for iodine determination using several reagents, including starch,[29,30] prochlorperazine,[32] 3,5-Br2-PADAP,[33] and others, for spectrophotometric determination. Sequential injection analysis (SIA) was used to automate iodate determination as triiodide.[30] These methodologies require a certain effort for setup and optimization of the tubing manifold, more so when dealing with multistep procedures. Moreover, handling viscous solutions or efficient mixing with large volumetric ratios in SIA is tricky.

In this regard, flow-batch approaches present an interesting alternative. They combine solution handling in-flow with batch concepts, such as step-by-step addition of reagents in a mixing chamber. This way, they overcome disadvantages of classical FTs and represent an economic alternative to robotic systems and autosamplers. As a highly efficient flow-batch approach, Lab-In-Syringe (LIS), introduced by Maya *et al.* in 2012 (ref. 34 and 35) relies on the use of the void of an automatic, computer-controlled syringe pump as a steadily enclosed but size-adjustable reaction chamber. Homogeneous mixing can be achieved using a magnetic stir bar inside the syringe void. LIS has proven advantageous for the automation of diverse sample preparation techniques and colorimetric assays.[35]

Herein we present an automated method for the colorimetric determination of iodate in table salts based on the starch–triiodide complex as a green and automated, thus reliable, reproducible, and sensitive method using the LIS approach.

2 Experimental

2.1. Reagents and chemicals

All reagents were of analytical reagent grade and purchased from Sigma Aldrich (Prague, Czech Republic). Ultrapure water obtained from a Milli-Q purification system (Prague, Czech Republic) was used for preparation of all solutions. A stock solution of 0.1 mmol L^{-1} iodate was prepared by dissolving 0.01979 g $NaIO_3$ in 100 mL water. Working standard solutions were prepared daily by appropriate dilution with water. Iodide was used as the reducing reagent and prepared by dissolving 0.083 g of potassium iodide in 50 mL water, yielding a final concentration of 10 mmol L^{-1}.

For method optimization, 2.5 mol L^{-1} HSO_4^-/SO_4^{2-} buffer solutions were prepared from 96% (w/w) sulfuric acid and adjusted to the required pH 0.9 with 5 mol L^{-1} NaOH while preliminary experiments were carried out using 2.5 mol L^{-1} sulfuric acid for reaction mixture acidification.

As a selective and sensitive reagent for iodine, a 4% (w/v) aqueous starch solution was prepared by mixing 0.4 g of soluble starch (Sigma Aldrich, Czech Republic) with water. The suspension was boiled under constant magnetic stirring until a change in transparency was no longer observed, or for at least 10 min, then allowed to cool down, quantitatively transferred to a 100 mL volumetric flask, and filled up to the mark with water. The solution was stored overnight at 4 °C and freshly prepared every other day. In initial experiments, the signal response was compared with that of a starch solution saturated with 10 mg L^{-1} HgI_2 as recommended for stabilization.[36] After optimization of all variables, the starch solution was combined with the acidic buffer that also ensured stabilization against microbial decomposition and HgI_2 was omitted. Eventually, starch and acid buffer were combined. This led to prolonged stability of the starch for over one week.

Method applicability was studied by analyzing commercial table salts of different brands that were purchased at a local supermarket. To mimic the real sample matrices, iodate standard solutions were prepared in the presence of 40 g L^{-1} NaCl.

2.2. Apparatus

The lab-in-syringe system used is shown in Fig. 1, with tubing dimensions and solution positioning indicated. The LIS setup consisted of a Cavro XC9+ automatic syringe pump purchased

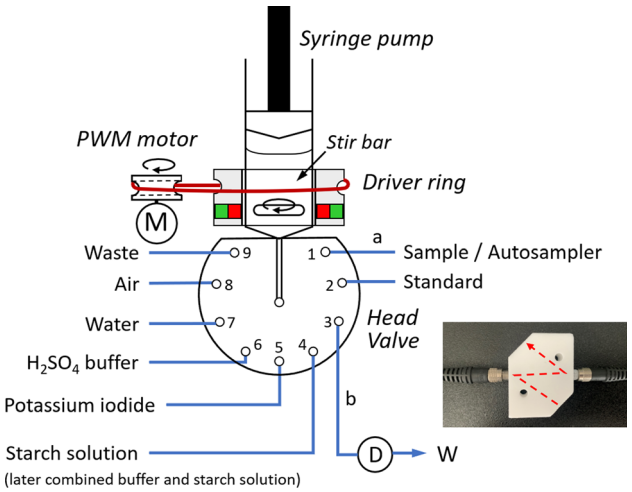

Fig. 1 Lab-in-syringe setup. Abbreviations: a – 20 cm FEP tube, 0.8 mm i.d., b – 60 cm FEP tube, 0.8 mm i.d., M – motor, D – detector, and W – waste. The 2 cm detection cell with mounted optical fibers is shown in the photograph with the flow direction indicated.

from Tecan Trading AG (Männedorf, Switzerland) featuring a 9-position rotary head valve. It was equipped with a 2.5 mL glass syringe (10.5 mm i.d. and 3 cm stroke length). It was positioned upside-down to enable complete content discharge during emptying. The head valve ports were connected *via* FEP tubing (0.8 mm i.d., *ca.* 30 cm) to reservoirs of sample (2), water (7), acidic buffer (6), potassium iodide (5), and starch solution (4). During method characterization and sample analyses, the combined starch-buffer solution was placed on port 5, and samples were aspirated from an AIM3200 autosampler (AIM Inc., Virginia, Queensland, Australia) connected to port 1 instead. Port 9 enabled solution discharge to waste, while port 8 allowed air aspiration.

For spectrophotometric measurements, a detection flow cell with a 2 cm path length and Z-shape channel geometry from FIAlab® Instrument Systems Inc. (Seattle, WA, USA) was connected to head valve port 3 *via* 20 cm FEP tubing (0.8 mm i.d.). A standard halogen lamp (Model LS-1, Ocean Optics Inc., Orlando, FL USA) was used as the light source in combination with a fiber optic spectrophotometer (USB 4000, Ocean Optics Inc.) and two optical light guides of a 0.8 mm quartz core (FIAlab). The absorbance of the triiodide–starch complex was monitored at 600 nm and referenced using the absorbance signal measured at a reference wavelength of 900 nm. Spectrum smoothing using a moving average of over 7 pixels was done and measurements were carried out with integration times over 20 × 2 ms. As the complex showed negligible absorption at 900 nm, alterations of the light intensity caused by microbubbles or changes in the refraction index at the expulsion of cleaning solution residues from the detection flow cell by the reaction mixture could be compensated for by using a reference wavelength.

For homogenization of the syringe content, a commercial PTFE-covered magnetic stir bar (10 mm in length and 3 mm in diameter) was placed inside the syringe. Stir bar rotation was forced using a driver ring that aligned the stir bar at the bottom position *via* two neodymium magnets, as previously described.[35] In the syringe, stirring was driven *via* a rubber belt from a brushless DC motor, featuring a pulse-width modulated PC fan. Motor activation *via* the control software was done *via* relay control and a simple analog circuit for speed adjustment.[37] System control, data acquisition, and evaluation were done with FIALab® software 5.11 (FIAlab®).

2.3. Procedure

The optimized automated procedure for iodate determination and for syringe cleaning is given in SI, Tables S-1 and S-2 respectively. In brief, the connecting tube to the sample (*e.g.*, located on the autosampler) was filled with the fresh sample by its aspiration into the syringe and after that discharged. Then, the syringe was cleaned with water by aspiration of 800 µL followed by 500 mL of air while stirring the syringe content and solution discharge with stirring stopped. Afterwards, water and the sample were used to clean the detection cell and perform a blank measurement, and finally, the syringe was cleaned again with water.

The actual analytical procedure started with the aspiration of air (200 µL) and the standard or sample (1000 µL). In this step, some of the sample could be replaced by water for straightforward in-syringe dilution. After activation of stirring, starch solution (100 µL), potassium iodide (KI, 200 µL), and acidic buffer (125 µL) were successively aspirated. A small volume of air (50 µL) was used to drive all remaining liquid from the head valve into the syringe void. For aspirating the more viscous starch solution, the flow rate was reduced. For the combined starch-acidic buffer solution, the respective volume was the sum of both single volumes (225 µL). To homogenize the syringe content before the aspiration of the subsequent solution and to assure volumetric accuracy, waiting times of 1–3 s were added after each aspiration step. The starch complex was allowed to form over 100 s while stirring. Thereafter, stirring was stopped, and the syringe content was emptied through the detection cell towards waste. Spectrophotometric measurements were conducted over 7 s in which the cell was filled completely with the homogeneous reaction solution and the average value was used as the analytical signal for data evaluation.

3 Results and discussion

3.1. Starch reagent

The starch complex formation assay offers the advantage of doubling the detection sensitivity compared to spectrophotometric determination of free triiodide plus an increase in method selectivity given the detection in the visible range.

Preliminary experiments were carried out with a 4% (w/v) starch reagent prepared with warm water and preserved against bacterial decomposition by the addition of 10 mg L^{-1} HgI_2. In comparison to this solution, both viscosity and transparency of the starch solution considerably increased when the starch solution was boiled; however, they did not, to the naked eye, increase further after 10 min. It was found that aqueous

solutions of the iodine–starch complex at a 10 mmol L^{-1} iodine concentration were blackish and showed certain turbidity and distinguishable particles when boiling was omitted and were transparent and steel-blue using boiled starch solution. On comparing boiled starch solutions at a 10 mmol L^{-1} iodine level with and without HgI_2 for stabilization, visually higher color intensity was obtained when HgI_2 addition was omitted. In consequence, we decided to use boiled starch solution prepared freshly every other day and to avoid stabilization with HgI_2.

3.2. System parameters

The syringe was positioned upside-down to enable complete emptying of the syringe content and therefore to reduce possible carry-over and to improve the efficiency of syringe cleaning. The stirring speed was fixed at 1500 rpm to ensure rapid homogenization of the syringe content but to avoid excessive bubble formation; however, the influence of this parameter on the signal was negligible. Concerning spectrophotometric detection, the use of a reference wavelength was found advantageous yielding stable and reproducible readings. Even though a 1.5 mL homogeneous reaction mixture was available to flush out any remaining cleaning solutions from the detection cell (enough to use a detection cell of increased optical path length) preliminary tests showed that due to the remaining turbidity of the starch solution, the use of a detection cell with a 10 cm path length and method application to correspondingly lower analyte concentrations were not feasible, and a 2 cm long detection cell was used instead.

3.3. Optimization of procedural parameters

Physicochemical parameters were studied using iodate standard solutions in a univariate manner. In acidic media, iodate is reduced by iodide to iodine which combines further with iodide added in surplus to form the better soluble triiodide. In preliminary experiments using 2.5 mol L^{-1} sulfuric acid, it was found that the volume of acid had a crucial influence on method sensitivity and, even more, on response linearity. In effect, the starch complex is destroyed in excessively strong acidic media, and the blue complex would form only at elevated iodate concentrations. This was observed as a right shift of the calibration curve and negative *y*-axis intercept. On the other hand, at low acidity, so much acid is consumed that the reaction cannot proceed with the same efficiency at high iodate concentrations as it does at low iodate concentrations, which was observed as a saturation-like signal behavior.

Under this premise, we decided to use a strongly acidic buffer based on the dissociation of HSO_4^-. Signal linearity and sensitivity were therefore studied using iodine solutions to evaluate the sole effect on the starch complex formation with acidic HSO_4^-/SO_4^{2-} buffers of pH values ranging from 0.7 to 1.5. The buffer concentration was high enough so that the iodine-producing reaction would not alter the reaction pH significantly. It was found that the highest sensitivity was obtained for a pH value of 0.9, which also yielded the highest signal for the smallest iodine concentration tested (data not shown). It is noteworthy that safe handling of the acidic buffer in the fully enclosed syringe prevented any hazard for the operator from unwanted contact.

3.3.1 Reaction time. Next, the effect of the reaction time on method sensitivity was studied between 5 and 300 s. The results and experimental conditions are given in Fig. 2A. The signal obtained with a 10 μmol L^{-1} iodate standard increased over the first 80 s and was stable for longer reaction times. In consequence, 100 s were chosen for all further work aiming for the highest method reliability and sensitivity. However, it would be possible to shorten the time of analysis by even 1.5 min taking into account that only 16% lower sensitivity was found for a reaction time of 10 s.

3.3.2 Iodide reagent concentration. The effect of the concentration and volume of the iodide solution was investigated. The results given in Fig. 2B show that the signal for a 10 μmol L^{-1} iodate standard followed a saturation behavior that leveled off for iodide concentrations higher than 10 mmol L^{-1}, which was therefore chosen in continuation. Fig. 2C shows that the method sensitivity increased by increasing the volume of the iodide solution up to 200 μL so this value was adopted for all further experiments.

3.3.3 Volume of starch solution. The effect of the starch solution volume on the method sensitivity was tested between 20 μL and 320 μL with the results and experimental conditions given in Fig. 2D. Signals increased up to 40 μL and slightly decreased for volumes larger than 80 μL due to the dilution effect. A volume of 100 μL was chosen for further experiments considering the method application to up to 18 μmol L^{-1} iodate expectedly corresponding to an absorbance of about 1.1 AU.

3.3.4 Volume of sulphate buffer. Finally, the effect of the acidic buffer volume on the method sensitivity was studied in the range of 25 μL to 200 μL. As for the other parameters, the signals followed saturation behavior (see Fig. 2E) with increasing sensitivity up to 125 μL, so this volume was adopted for future work.

3.3.5 Aspiration order of reagents. The effect of the aspiration order of the three reagents (starch – S, iodide – I, and acidic buffer – A) on the method sensitivity was evaluated. The results and experimental conditions are given in Fig. 2F. It was found that the aspiration of first starch, then iodide, and finally acid was superior to all other combinations, so this order was selected. These results were attributed to the need for starch molecules to disentangle but no further studies were performed to evaluate this theory. At the final step of optimization experiments, starch and acidic buffer solutions were combined to prevent degradation of the starch over time, *i.e.*, for stabilization. This pre-mixing of reagents did not decrease the analytical signal nor did RSD values increase significantly.

3.3.6 Flow system cleaning. Finally, detection cell and syringe cleaning protocols were compared regarding method sensitivity, measurement repeatability, and carry-over. The initial protocol (A) was cleaning the syringe twice and the detection cell once with water between each analysis, but the first of four consecutive measurements was typically by about 5–10% lower than the subsequent determinations, which showed RSD values of generally <3.5%. Therefore, further cleaning modes were tested: additional cleaning of the syringe and

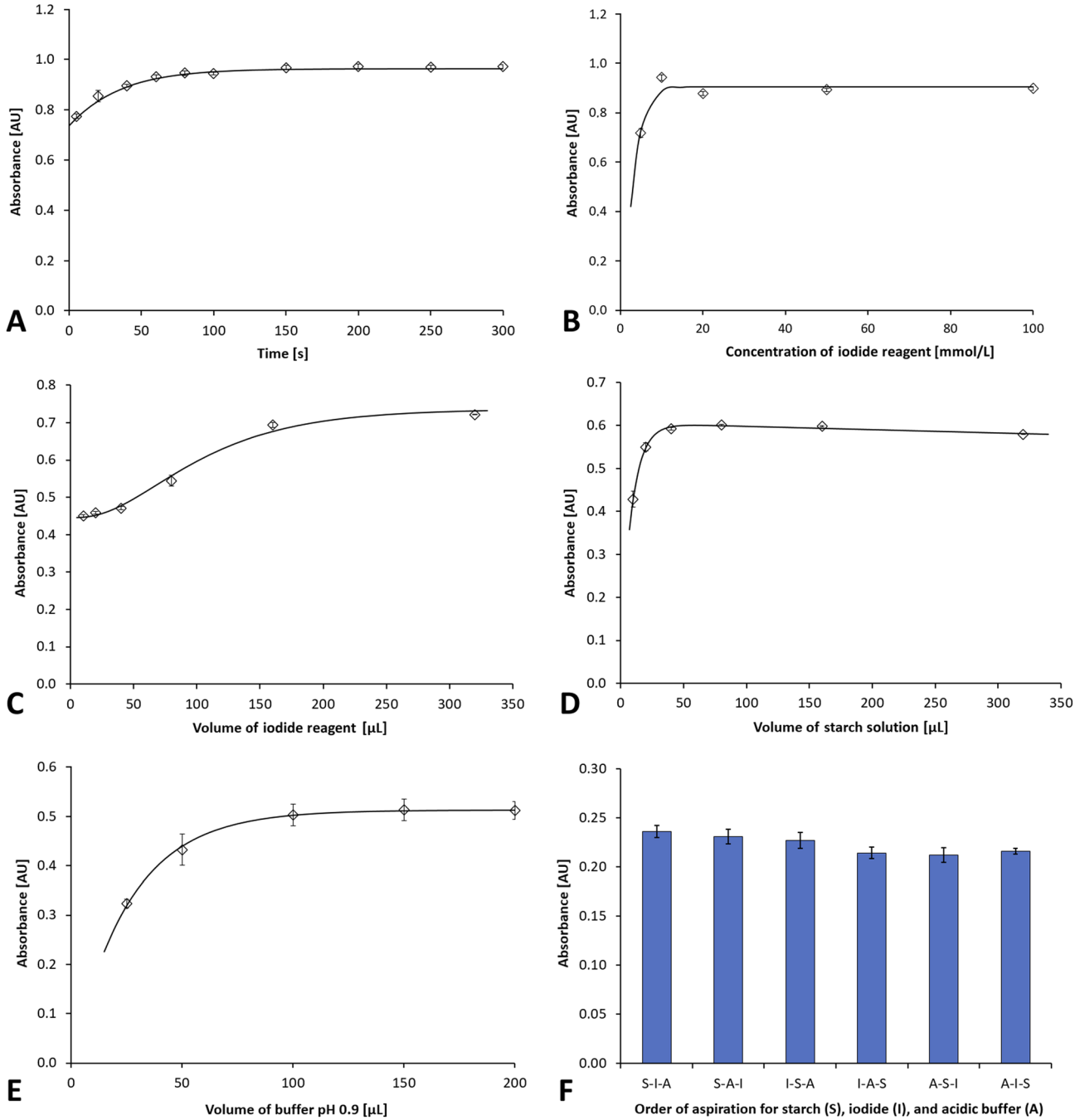

Fig. 2 (A) Effect of reaction time. Conditions: 1 mL of 10 µmol L^{-1} iodate standard, subsequent addition of 200 µL of 10 mmol L^{-1} iodide, 100 µL of 500 mmol L^{-1} acidic buffer of pH 0.9, and 100 µL of 0.4% (w/v) starch solution. (B) Effect of iodide concentration, under conditions as in 'A' with a reaction time of 100 s. (C) Effect of iodide solution volume, under conditions as in 'B' using a 10 mmol L^{-1} iodide solution and an iodate concentration of 8 µmol L^{-1}. (D) Effect of starch solution volume (0.4% (w/v)), under conditions as in 'C' but with the use of 200 µL iodide solution. (E) Effect of volume of acidic buffer, under conditions as in 'D' using 100 µL starch solution. (F) Effect of aspiration order, under conditions as in 'E' using 125 µL acidic buffer and 4 µmol L^{-1} iodate. A – acidic buffer, I – iodide, and S – starch solution.

detection cell with 25% (v/v) ethanol aspirated from head valve position 3 (B), one additional cleaning of the syringe with sample (C), one additional cleaning of the syringe and detection cell with water (D), and one additional cleaning of the syringe and detection cell with sample (E). The carry-over effect was evaluated by measuring a 2 µmol L^{-1} iodate standard in quadruplicate, then an 8 µmol L^{-1} standard, and then the 2 µmol L^{-1} standard in quadruplicate again. Protocols A and B were abandoned since protocol C was superior in terms of reproducibility and carry-over diminishment (see Fig. S-1). Protocols D and E were effective in eliminating carry-over but also decreased method sensitivity by about 10%. Protocol D was

Table 1 Effect of different salts/ions on method sensitivity in comparison with a 5 μmol L^{-1} iodate standard prepared with pure water

Tested solution	Concentrations of NaCl/potential interferents [g L^{-1}]	Effect on blank[a]	Effect on a 5 μmol L^{-1} iodate standard[a]
Water	—	75%	109%
NaCl	40	100%	100%
NaCl + NaF	40/1.0	97%	100%
NaCl + $FeCl_3$	40/0.035	91%	92%
NaCl + $CuSO_4 \cdot 5H_2O$	40/0.035	93%	92%
NaCl + $NaHCO_3$	40/5.0	75%	98%
NaCl + KBr	40/5.0	90%	95%

[a] Compared to solution or an equal iodate standard prepared with NaCl solution.

Table 2 Data from linearity experiments with c given in μmol L^{-1}

Parameter	Value
Linear fit	$y = a \times c + b$
Slope (a)	0.084 ± 0.001
Intercept (b)	−0.045 ± 0.006
Regression coefficient	0.9988 ± 0.0005
Power fit	$y = a \times c^b$
Factor (a)	0.0600 ± 0.006
Power (b)	1.132 ± 0.039
Regression coefficient	0.9992 ± 0.0005

finally adopted for showing a repeatability of <1.8% on average ($n = 4$) whereas protocol E yielded lower signal reproducibility. Using protocol D, single measurements per sample yielded reliable quantification results; however, for method characterization we continued measurements in triplicate.

3.4. Interference study

The effects of selected ions on the analytical response were studied considering those with likely presence in the sample, *e.g.* originating as contaminants from salt processing, and with possible action as reductants or precipitants, or acting as buffers. For each ion, a blank solution and a 5 μmol L^{-1} iodate standard, both prepared with 40 g L^{-1} NaCl, were prepared at the given concentration. The results were compared to those of pure NaCl standards (blank and with iodate addition) and those prepared with ultrapure water only (not salty). Ion concentrations and the results are given in Table 1 and are depicted in Fig. S-2.

It was observed that the ion strength had a significant influence on the signals of both blank and standard solutions prepared with 40 g L^{-1} NaCl. In effect, signals for the iodate standard without salt addition increased by about 9% while those for the blank solution decreased by about 25%. The interfering effects on the method sensitivity were negligible for bicarbonate and fluoride yet standard signals decreased in the presence of the tested concentrations of Fe^{3+}, Cu^{2+} and Br^- by 6–8%. Cu^{2+} and Fe^{3+} were tested as potential interferents as both can oxidize iodide to iodine; however, no such effect was observed, possibly related to the high salinity of the samples and formation of weaker oxidizing species $FeCl_4^-$ and $CuCl_4^{2-}$.

The blank signal decreased slightly, in particular in the presence of bicarbonate, but in terms of absolute absorbance values this decrease was insignificant (<5 mAU corresponding to <0.057 μmol L^{-1} iodate). Considering that the interferent concentrations exceeded by far real amounts found in table

Table 3 Method application to salt sample analysis. The salt concentration of standards (NaCl) and samples (product in question) was 40 g L^{-1}[a]

Sample	Added IO_3^- [μmol L^{-1}]	Found [μmol L^{-1}]	Recovery [%]	Comment	Content determined by the reference method[38]
Sample 1: sea salt declared content IO_3^-: 2 mg kg^{-1} iodine	—	5.26	100.5	*ca.* 6.6× of the declared value (13.4 mg kg^{-1})	13.8 mg kg^{-1}
	2	7.27	102.2		
	5	10.4			
Sample 2: stone salt declared content IO_3^-: 2 mg kg^{-1} iodine	—	3.52	99.1	*ca.* 4.5× of the declared value (8.96 mg kg^{-1})	10.5 mg kg^{-1}
	2	5.50	99.5		
	5	8.50			
Sample 3: Himalayan salt No IO_3^- content declared	—	<LOQ	89.5	Rosy hue of the sample material	<LOQ
	2	1.79	98.6		
	5	4.93			
Sample 4: stone salt declared content IO_3^-: 5–35 mg kg^{-1} calc. as KI	—	3.53	98.7	*ca.* 78% of the declared value (11.7 KI mg kg^{-1})	11.3
	2	5.51	99.3		
	5	8.50			

[a] Iodate contents in mg kg^{-1} refer to given iodate content in the product analyzed by the producer. The values given in μmol L^{-1} refer to the concentrations of iodate in the sample solutions containing 40 g L^{-1} of the commercial salt samples.

salts, the developed method can reasonably be considered selective. However, the salinity of standards had to be adapted to the concentration of NaCl in the samples as was done for method validation and analyses of commercial table salt samples.

3.5. Analytical figures of merit and method application

Calibration curves were obtained with iodate standards prepared in 4% (w/v) NaCl on subsequent days. Under optimized conditions, the method was proven linear from 0.5 to at least 10 μmol L^{-1} iodate corresponding to approximately 0.1 to 2 mg L^{-1} iodate. The relative standard deviation (RSD) at all calibration levels was 1.3 ± 0.9% on average and 1.75% for a 0.1 mg L^{-1} $NaIO_3$ solution.

The limitation of the starch-iodine assay for quantitative purposes is the linearity of response but it was greatly improved in this study by using an acidic buffer. Data from nine 5-point calibrations showed excellent inter-day repeatability/reproducibility and linearity of response with R^2 values generally exceeding 0.999 yet with a negative intercept. On adding the blank measurement (15 ± 2 mAU on average) to the calibration, R^2 values decreased to an average value of 0.995 and a power regression model was found ideal for concentrations below 1.5 mg L^{-1} yielding R^2 values of 0.9998 on average. The difference between the two models was insignificant with maximal deviations of 2.5% over the calibration range (5% for concentrations lower than 1.5 μmol L^{-1}). The results of the linearity studies are given in Table 2. Limits of detection (LOD) and quantification (LOQ) were calculated for the concentrations corresponding to 3 and 10 times the average standard deviation of repeated measurements of the lowest standard and blank values (0.002 AU), *i.e.* 0.14 and 0.41 μmol L^{-1}.

Iodate contents in the four commercial salt samples were quantified, and spiking experiments on these samples were performed to evaluate the accuracy and a possible matrix effect. 4 g of each sample was dissolved in 100 mL water yielding a 40 g L^{-1} salt concentration. Standards were prepared with 4 g L^{-1} NaCl solution. The results of sample analyses are shown in Table 3 and were compared to those of an earlier reported spectrophotometric method omitting starch.[38] Quantitative analyte recovery was found in the analysis of solutions of the salt samples spiked with iodate at two concentration levels. Only for sample 3, a lower recovery was found, which was presumably related to the rosy hue of the solid sample and related content of calcium. It was further found that all measured iodate concentrations deviated significantly from the declared values. This once more underlines the need for quality control of iodate addition to table salts. Finally, the measured data matched well with those of the reference method used for comparison.[38]

3.6. Comparison with previous iodate methods and final discussion

The importance of iodine in the diet has been known for many years now and it is reflected in the number of publications on iodine species determination in table salts, marine samples or

Table 4 Comparison of the earlier methods based on (spectro)photometry for the determination of iodate. Not automated methods

Approach	Sample	Reaction/reagent	Wavelength [nm]	Linear range [mg L^{-1}]	LOD [mg L^{-1}]	Sample [mL]	Reaction time [min]	Precision [RSD%]	Ref.
Dye bleaching by I_2	Table salt	Thionine	600	1–12	0.036	0.5	n.r.	n.r.	16
		Azure B	644	0.2–16	0.07	0.5			
Oxidation of the dye precursor by I_2	Veterinary drugs and table salt	DPD	550	0.7–7	1.16	0.5	n.r.	n.r.	21
Oxidation by IO_3^- following azo-coupling and HLLME	Table salt	Phenylhydrazine, 8-HQ or 2-naphthol, and 2-propanol	475/484	0.08–10	0.08	1	5 for oxidation and + 5 for coupling reactions	6.6–12.6	22
Dye bleaching by ICl_2^-	Table salt and pharmaceuticals	Methyl red bleaching by ICl_2^-	520	0–0.35	0.022	10	10	3.6	18
Detection as I_3^-	Table salts	H_3PO_4 and I^-	288/352	0.1–0.6	0.035	10	n.r.	3.3	23
Dye bleaching	Table salts & waters	Pyrogallol red	470	0.1–12	n.r.	0.020	5	n.r.	41
In situ formation of I_2, and extraction into *N,N′*-DMFA, HS-LLME	Natural water	I^-	295	0.008–0.175	0.001	10	7	4.2	39
Reduction to I^-, VALLE	Salt, seawater, and mineral water	TBA-I; amyl acetate	295	0.05–0.088	0.002	0.2[a]	10 + 2	5.1–5.9[a]	40

[a] For salt samples. Used abbreviations: 8-HQ – 8-hydroxyquinoline, DPD – *N,N*-dimethyl-*p*-phenylenediamine, *N,N′*-DMFA – *N,N′*-dimethylformamide, HLLME – homogenous liquid–liquid microextraction, HS-LLME – head-space liquid–liquid microextraction, TBA-I – tetrabutylammonium iodide, VALLE – vortex-assisted liquid–liquid extraction, n.r. – not reported.

Table 5 Comparison of the earlier methods based on (spectro-)photometry for the determination of iodate. Automated methods[a]

Approach	Automation approach	Sample	Reaction/reagent	Wavelength [nm]	Linear range [mg L^{-1}]	LOD [mg L^{-1}]	Sample [mL]	Throughput [h^{-1}]	Precision [RSD%]	Ref.
Oxidation of the dye precursor by I_2	Downscaled MCFIA	Table salts	DPD	520	0.1–3	0.017	0.045	117	0.9	19
Oxidation of the dye precursor by I_2	Downscaled flow-batch	Table salts	DPD	520	0.01–10	0.004	0.016	170	<1.5	20
Detection as I_3^-	Reverse FIA	Table salts	H_3PO_4 and I^-	351	0.02–3	0.008	0.150	102	0.9	24
Detection as I_3^-	SIA	Waters	Acetate buffer, I^-, and Na_2MoO_4 for IO_4^- masking	352	0.050–10	0.050	0.250	20	0.72	30
Chemiluminescence analyte was I^-	FIA with PV	Iodine & multivitamin tablets	Luminol	425	1–10	0.5	10	30	5.2	9
Formation of a colored product	FIA	Iodine tablets	PCP	525	0.2–6.4	0.018	0.300	80	0.1	32
Formation of a colored product	FIA	Table salts & laver	3,5-Br_2-PADAP and SCN^-	605	0.2–5.14	0.107	0.100	80	n.r.	33
Starch complex + gas diffusion analyte was I^-	FIA	Plant-based pharmaceuticals	Starch	590	50–300/6–10 × 10^3	1/200	0.050/0.500	30/20	1.27/1.44	31
Starch complex	FIA	Table salts	Starch and S_2O_3	590	1.1–8.6	0.088	0.250	65	0.66	29
Starch complex	LIS	Table salts	Starch	600	0.05–2	0.029	1	13	1.3	This method

[a] Used abbreviations: 3,5-Br_2-PADAP – 2-(3,5-dibromo-2-pyridylazo)-5-diethylamino-phenol, FIA – Flow Injection Analysis, MCFIA – multicommutated FIA, PCP – prochlorperazine, PV – pervaporation, SIA – sequential injection analysis, n.r. – not reported.

urine. Hence, a variety of methods have been proposed with constant improvements in terms of selectivity, robustness, or precision. For a critical evaluation, performance parameters of spectrophotometric methodologies, both non-automated and automated, are summarized in Tables 4 and 5 in juxtaposition with the figures of merit of the developed LIS-automated method.

Non-automated spectrophotometric methods rely mostly on dye formation or bleaching in the presence of iodate or reaction product iodine (Table 4). Pena Pereira *et al.* suggested a manual method using head-space extraction of the generated iodine.[39] They used a 2.5 μL drop of the acceptor medium. Although a high preconcentration factor was achieved, manipulation with such small volumes can be troublesome. Also, microwaving the sample was necessary to prevent interference. A vortex-assisted liquid–liquid extraction method was proposed by Zaruba *et al.*[40] Organic solvent and various reagents were used in their method, unlike in the present one, where organic solvents are omitted and only starch and a small amount of sulfuric acid are used as reagents, following the green chemistry rules. Harsh or harmful reagents might be needed to mask interference in other instances as well.[16,18] To increase method sensitivity, some methods used additional liquid–liquid extraction[22] or required a two-step reaction.[21] Despite these efforts, insufficient method selectivity was observed.[22] Carrying out the determination manually increases the chance of an error, inferior repeatability, and poor throughput.

Flow technique automated methods are listed in Table 5. These approaches generally achieve high repeatabilities and sample throughput. However, it should also be pointed out that sample differences in gas content (bubble formation) and viscosity can considerably alter the comparability between analyses and mixing solutions of large volumetric ratios is highly challenging in these approaches and that the reagent needed per microliter of sample is high. In this regard, the LIS technique enables straightforward in-system sample dilution and thus a working range in one step by a factor of 50 and thus widening of the working range if needed.

In contrast to the complex chemistry used in many methodologies listed in Tables 4 and 5, the LIS methodology is rugged regarding sample properties. A reasonable throughput of 13 h^{-1} was achieved, which could be further increased to about 20 h^{-1} (see 3.3). In terms of method sensitivity and precision, the developed method ranked satisfactorily high, yet not top, but stands out for its simplicity, selectivity, and greenness. Uraisin and Sun developed an FIA method with remarkable throughput and LOD.[32,33] However, a nonspecific reagent was used giving leeway to interference. Borges *et al.* used a miniaturized FIA system that is not commercially available and must be fabricated in laboratory.[19] Nacapricha *et al.* used a gas diffusion unit in their FIA system, adding an element of potentially cumbersome optimization, since bubbles cause troubles in a tubing system.[31] Ensafi and Chamjangali[30] developed a flow injection spectrophotometric system applied to determine iodate in iodized salts. Xie developed a simple method directly detecting triiodide but it required additional sample preparation to first convert iodide to iodate.[24] Another

FIA method, likewise, applying the starch reagent, required intermediate cleaning with thiosulfate.[29]

The lab-in-syringe method uses commercially available instrumentation and presents a straightforward, well-acceptable application given that the manual approach is emulated. Automation allowed improving the repeatability significantly and achieved assay linearity. LOD values and sample throughput are suitable for routine application for quality control. By selecting an iodine-specific reagent, interference from other possible sample constituents was low. Sensitivity was improved thanks to the optimization of the pH value. Using a sulphate buffer at low pH was troublesome given the small amounts needed and handling in the closed LIS system. While in-syringe sample dilution could widen the working range, the here-achieved linear range proved suitable for the intended determination of iodate in table salts.

The greenness of the developed method was evaluated using the AGREE metric tool reported by Pena-Perreira and coworkers[42] to be 0.7 (see Table S-3). This high value was due to omitting reagents of high toxicity such as arsenite and carrying out a simple yet automated procedure with a power consumption of a few Watts only. Instead, adopting the starch–iodine assay enabled the determination of iodate in table salts and excellent method precision and repeatability. However, even the small amounts of H_2SO_4 and KI used are rated highly critically with this tool.

The LIS technique shows the important advantage over tubing-based automation techniques and related methods of requiring a low number of procedural steps and straightforward optimization and the need for reagents and waste are minimized by avoiding continuous flow.

Adaptation of the methodology for iodide using nitrite as an oxidant and a further increase in method sensitivity for application to, *e.g.*, seawater analysis, could be achieved by additional analyte enrichment. While elongation of the optical light path was found impractical as the used starch reagent decreased the sample transparency, detection inside the syringe could be carried out with a photodiode as a detector and an LED as a light source, further simplifying the instrumental setup and decreasing the related costs.

4 Conclusions

The iodine-starch reaction is the reaction of choice in iodine species determination due to its specificity and simplicity. This method was re-optimized, and it benefits from complete automation in a lab-in-syringe system, short processing time, and high precision and repeatability. The developed method is easy and straightforward to carry out thanks to the step-by-step addition of the reagents in a completely closed, thus safe, flow-batch system. It does not require costly instrumentation or complex training of the operator; it is thus suitable for a wide range of laboratories. The method provides the desired sensitivity, selectivity, and precision for iodate determination in salts. The issue of linearity was handled *via* the correct pH setting using a buffer. The risk of handling a reagent of a very low pH was eliminated by working in a fully closed environment of the lab-in-syringe system. Straightforward on-line dilution can increase the linear range easily if required by the application. Simple programming of the cleaning step improves the precision. Samples of different viscosities might be handled with ease due to the stirring. The method was successfully applied to the analysis of iodine in four table salts.

Author contributions

C. Yıldız: investigation, data curation, writing – original draft, visualization. S. Yıldırım: supervision, validation, writing – review & editing. I. H. Šrámková: investigation, formal analysis, data curation, writing – review & editing. P. Solich: funding acquisition, project administration. B. Horstkotte: conceptualization, methodology, funding acquisition, supervision, visualization, writing – original draft.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

Data availability

Experimental data are shared *via* Zenodo database under the reference. Supplementary information (SI) is available. See DOI: https://doi.org/10.1039/d6ay00438e.

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