A SIA SYSTEM WITH A MIXING CHAMBER FOR HANDLING HIGH CONCENTRATED SOLUTIONS: SPECTROPHOTOMETRIC CATALYTIC DETERMINATION OF IODIDE IN NUTRITION SALTS

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ABSTRACT

A mixing chamber is incorporated to the manifold of a SIA system in order to expand the applicability range of sequential injection analysis. This is an efficient strategy when high concentrated solutions are involved. Different system configurations were studied in a system with spectrophotometric detection and that yielding the lowest Schlieren noise was elected. As application, the determination of iodide in nutrition salts was selected. The method was based on the catalytic effect of iodide on the oxidation of chlorpromazine by concentrated hydrogen peroxide under high acidity. A 500 μL sample aliquot and 100 μL of both reagents were sent to the mixing chamber: after a 60 s reaction period, 350 μL aliquots of the processed sample were withdrawn and directed towards the detector. Precise results (r.s.d. usually < 0.04) were attained within the 10-100 μg L^{-1} I^- range, and Schlieren noise was very reproducible. The detection limit was determined as 4 μg L^{-1} I^- and the sample throughput as 24 h^{-1}. Variations in temperature within 20 and 40 °C did not produce any observable modification in the reaction rate. Recovery data between 95.5 and 105.0 % were obtained.

Key words: iodized salt, sequential injection analysis, spectrophotometry, catalytic procedure, iodide determination.

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iodide on the chlorpromazine oxidation by hydrogen peroxide under high acidity which yields a colored species [16]. Among the favorable analytical characteristics of this method, selectivity, sensitivity and slow progress of the uncatalyzed reaction should be mentioned. However, concentrated sulfuric acid and hydrogen peroxide are required and sample dispersion should be kept low in view of the expected low analyte contents. In this way, the SIA system should be designed with capabilities to manage concentrated solutions under conditions of low sample dispersion.

EXPERIMENTAL

Reagents, standards, samples

For preparation of the solutions, doubly deionized water (specific conductivity < 0.1 $\mu S\ cm^{-1}$) and analytical grade chemicals were used.

A 4.5×10^{-3} mol L^{-1} chlorpromazine solution was daily prepared by dissolution of 15 mg of the compound in 10 mL of water, and kept in an amber bottle to protect from light. Hydrogen peroxide solution (6.0 mol L^{-1} H_2O_2) was prepared by water dilution of a 30 % v/v solution which was standardized against permanganate before use. Water was used as carrier stream (C - Fig.1).

The iodide stock solution, 100 $\mu g\ L^{-1}$ I^-, was prepared by dissolving 26.16 mg KI in about 80 mL of water and filling the volume up to 200 mL with water. Working standard solutions (10.0 - 100.0 $\mu g\ L^{-1}$ I^-, also 5.0 mol L^{-1} H_2SO_4) were daily prepared.

Prior to analysis, about 10 mg of iodized salts were accurately weighed and dissolved in 10 mL of a 5.0 mol L^{-1} sulfuric acid solution.

Apparatus

The sequential injection system (Fig. 1) comprised a Gilson Minipuls 3, bi-directional peristaltic pump equipped with a PVC pumping tube with a 1.42 mm i.d. of the same manufacturer and a Vici (Valco Instruments, Houston) model C15-

Procedure

Chlorpromazine is oxidized by hydrogen peroxide under acidic medium, yielding an unstable semiquinone radical with maximum absorption at 528 nm [16]; next step of this oxidation process results in the formation of a stable colorless compound. Formation of the colored species is catalyzed by iodide, but the uncatalyzed reaction occurs also in the absence of this catalyst. Therefore, it was decided to sandwich the sample plug between two plugs of chlorpromazine and peroxide solutions. The sequential aspiration of these solutions towards the SIA system would be a positive factor in the reaction development, even in the absence of iodide. The SIA system for spectrophotometric catalytic determination of iodide was then designed to accomplish this task. Moreover, with the inlet of solutions, mixing chamber and detector placed as specified in Fig. 1, the dead time for rotation of the valve (*ca* 4s per full revolution) was minimized.

Ten steps were required for proper operation of the system (Table 1). Initially, a 250-μL sample aliquot was aspirated (step 1) to fill the holding coil and rejected (step 2) through the waste line, in order to avoid carryover between the sample and the amount of previous sample stopped inside the aspirating tube. Thereafter, the hydrogen peroxide, the sample to be assayed and the chlorpromazine solutions were aspirated (steps 3 - 5) and the resulting mixed zone was directed towards the holding coil and then towards the mixing chamber (step 6). After a pre-defined time interval (step 7), an aliquot of the mixing chamber content was aspirated back to the holding coil (step 8) and then pushed towards detection and wasted (step 9). Washing of the mixing chamber was accomplished (step 10) by pushing its content towards waste. In this way, cross-contamination inside this device was avoided.

For replicate measurements, steps 3 - 10 were repeated, and for analysis of next sample, the initial (1 and 2) steps involving sample aspiration/discartion were needed.

Tab. 1. System operation. For details, see text.

Step	Event	Port	Time s	Flow direction	Flow rate mL min^{-1}	Volume µL
1	Sample aspiration	2	5	reversed	3.0	-
2	Sample propelling towards waste	3	10	forward	5.0	-
3	Reagent 1 aspiration	1	2	reversed	3.0	100
4	Sample aspiration	2	10	reversed	3.0	500
5	Reagent 2 aspiration	4	2	reversed	3.0	100
6	Propelling towards mixing chamber	5	15	reversed	3.0	750
7	STOP period	5	60	-	-	-
8	Aliquoting from mixing chamber to holding coil	5	7	reversed	3.0	350
9	Propelling towards detector	6	30	forward	5.0	-
10	Washing the mixing chamber	5	25	forward	5.0	-

RESULTS AND DISCUSSION

As concentrated solutions were involved, initial experiments were carried out to define the highest flow rates which could be pumped or aspirated. For < 3 mL min^{-1} aspiration or < 5 mL min^{-1} pumping rate, the established flow rates were not dependent on the physical characteristics of the flowing solutions. This was confirmed by weighing the aspirated/pumped volumes after varying the rotation speed of the peristaltic pump. The experiments were 10-fold repeated in order to assess the short term stability of the flow rates.

In these initial experiments, it was also verified that development of the indicator reaction was very dependent of the volume and acidity of the sample plug inserted between the reagents. In order to improve the reagent dispersion into the sample zone, the mixing chamber was initially placed between the rotary valve and the holding coil. With this design, background signal due to Schlieren effects

presented two maxima. The first one was more pronounced (*ca* 0.4 absorbance) in view of the shorter associated residence time. As seen in Fig. 2, increasing the sample aspirated volume within 100 and 500 μL improved the sensitivity in an almost linear fashion. However, the blank value underwent a pronounced increase in view of the increment of the Schlieren effects and in development of the uncatalyzed reaction. For iodide concentration higher than 50 μg L^{-1}, formation of the colored species resulted in a pronounced increase near the first recorded maximum which was higher than the Schlieren noise. As this noise was very reproducible, analytical signals could be properly measured within the entire iodide concentration range.

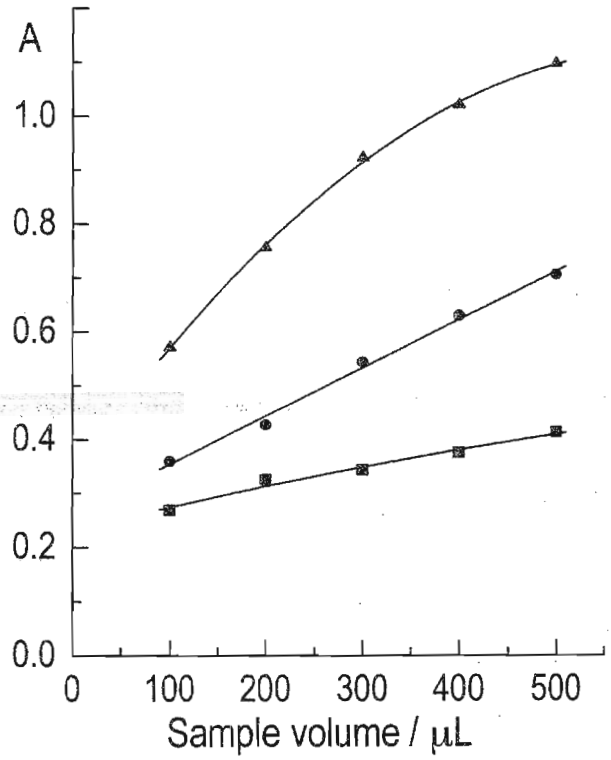

Fig. 2. Influence of sample volume. Figure refers to a blank (▪), a 50 μg L^{-1} I^- (●) and a 100 μg L^{-1} I^- (▲) solutions processed in the SIA system with the mixing chamber between the rotary valve and the holding coil. R_1 and R_2 volumes = 100 μL; reaction time = 60 s.

INTRODUCTION

Sequential Injection Analysis (SIA) was conceived as means to satisfy the requirements for mechanical simplicity in flow procedures [1]. Development pointed out the main potentialities of the approach such as cell-perfusion biological studies [2], renewable sensors surface technique [3], reaction stoichiometric studies [4], solvent extraction without segmentation [5], multiple reagent addition [6], on-line bioprocess control [7], etc. Moreover, the feasibility of a SIA system with abilities of autodesign was recently demonstrated [8,9].

The SIA analyzer comprises a single stream into which the sample and reagent solutions are sequentially intercalated. In view of this single-line fashion, the establishment of concentration gradients is unavoidable. This feature is not relevant for most applications, but may impair the signal-to-noise ratio especially in situations where highly concentrated reagents are concerned and suitable mixing conditions are not attained. The presence of pronounced undesired concentration gradients may lead to the appearance of Schlieren noise in spectrophotometry [10,11]. The drawback can be minimized by designing SIA systems where reagents are efficiently added to the sample under good mixing conditions without increasing the sample dispersion. This can be accomplished by taking advantage of mixing chambers associated with reversed flows.

The feasibility of this proposal is demonstrated in the present paper which discusses the potentialities and limitations of adding a mixing chamber to different sites of a SIA manifold. As application, the spectrophotometric determination of iodide in table salts and mineral supplements was elected. Use of iodized table salt is important to prevent the development of goiters in populations living away from sea air [12]. In several countries, the amount of iodide added to salt is ruled by legislation, so that iodide monitoring becomes important.

The official volumetric method for iodide determination [13] involves bromine water and is less suitable for automation. Other spectrophotometric methods were reported [14], and the method based on the Sandell-Kolthoff reaction [15] is of common use. The method selected here is based on the catalytic effect of

3118 E stream selecting rotary valve. These devices were controlled by means of a 386 IBM-PC compatible microcomputer with a home-made program written in QuickBasic.

The manifold was built up with 0.8 mm i.d. PTFE tubing; length of the holding coil was 350 cm. A home-made perspex mixing chamber with an inner volume of *ca* 400 μL (including a magnetic stirrer) was coupled to a lateral port of the rotary valve. In this way, its use was optional if other analytical procedures were to be implemented.

A Unicam SP6-350 spectrophotometer equipped with a 18-μL Helma 178.712Q flow cell, was used with the wavelength set at 528 nm. The monitored absorbance was recorded by a Kipp & Zonen BD111 flat bed strip-chart recorder.

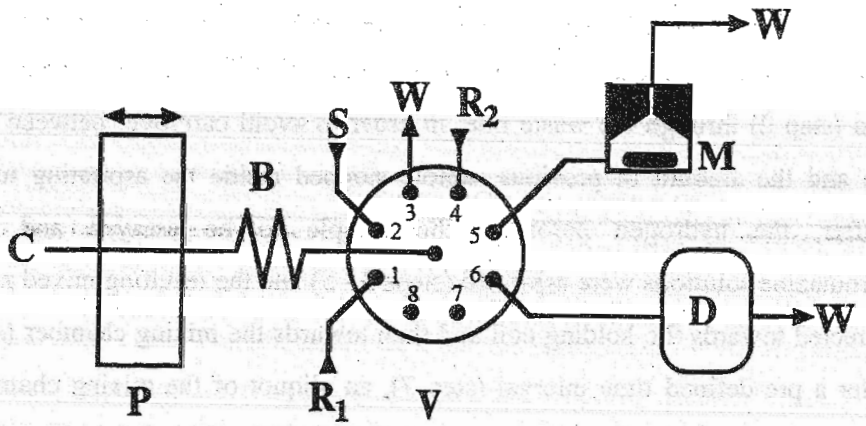

Fig. 1. The SIA system. C = carrier stream; P = peristaltic pump; B = holding coil; V = stream directing rotary valve (clockwise rotation); M = mixing chamber; D = detector; S = sample; R_1 = H_2O_2 solution; R_2 = chlorpromazine solution; W = waste. Numbers 1 to 8 = valve ports.

Thereafter, the mixing chamber was placed in an independent channel (Fig. 1) in order to permit the design of a more general SIA system able also to accommodate other analytical procedures. The sample aspirated volume was kept as 500 μL, and influence of the aliquot volume removed from the chamber after 60 s was evaluated. Both analytical and background signals underwent pronounced increases when the aliquot of processed sample removed from the mixing chamber to the holding coil increased from 100 to 350 μL (Fig. 3). This allows one to conclude that sensitivity is dependent on dispersion of the processed sample aliquot and the volumetric fractions of the involved solutions which are favored when the transferred aliquot of processed sample increases. Better sensitivity was verified for 250 - 350 μL. Beyond 350 μL, increasing the removed aliquot was not effective as means to improve sensitivity, due to the associated increase in dispersion.

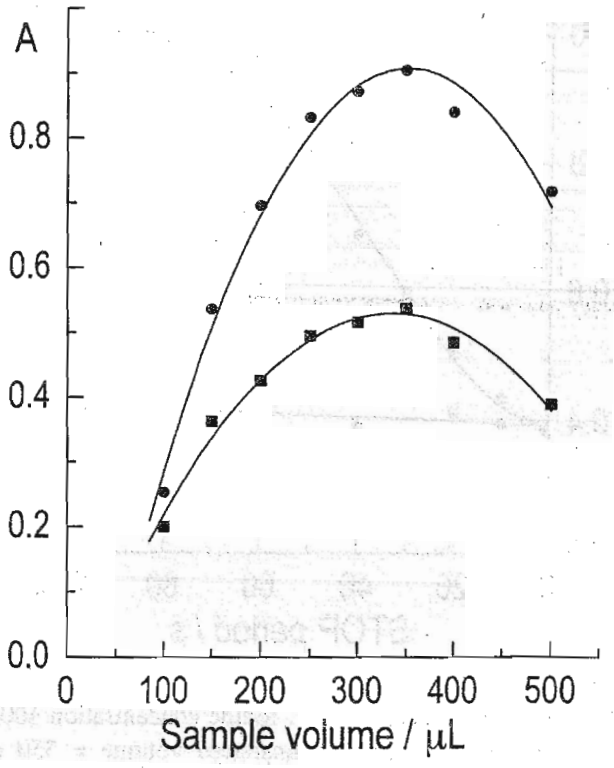

Fig. 3. Influence of the sample volume transferred from the mixing chamber to the holding coil. Figure refers to a blank (■) and a 100 μg L^{-1} I^- (●) solutions processed in the SIA system of Fig. 1, with a sample volume of 500 μL. Other conditions as in Fig. 2.

With the above defined operating conditions, effects of sample acidity and reagent concentrations were investigated, and sample acidity was found to be most relevant parameter. Increasing the sample acidity from 3.0 to 5.0 mol L^{-1} H_2SO_4 speeded up both catalyzed and uncatalyzed reactions, the effect being more pronounced for the former reaction. Sensitivity almost doubled, and the blank signal was increased from 0.23 to 0.48 absorbance. A similar but less pronounced effect was noted relatively to chlorpromazine: when its concentration was varied from 1.5 to 4.5 x 10^{-2} mol L^{-1}, the related sensitivities were determined as 0.004 and 0.008 A per μg L^{-1}. An analogous increase in blank value was also verified, due to the accelaration of the uncatalyzed reaction, as increase in chlorpromazine concentration did not cause any observable Schlieren noise. With regard to hydrogen peroxide concentration, its increase from 3.0 to 6.0 mol L^{-1} resulted in an almost linear improvement in the slope of the calibration plot with no further improvement being noted beyond 9.0 mol L^{-1}. For higher concentrations, a slight lessening of the blank signal was noted. It is important to stress that the procedure is not affected by temperature variations within 20 and 40 °C. This feature is in agreement with earlier work [16] and was confirmed by immersing the manifold to the SIA system into a thermostated water bath.

Performance of the SIA system was evaluated by successive processing of the blank and working standards, and the calibration equation was:

Peak height (absorbance) = 0.404 ± 0.009 (absorbance) + 0.0076 ± 0.0002 μg L^{-1} I^-

(r = 0.9995, n = 7)

In addition, detection limit was estimated [17] as 4 μg L^{-1} after 15-fold processing of the blank solution.

The proposed system permits about 24 samples to be run per hour and yields precise results (r.s.d. usually < 0.04). This means 150 μg chlorpromazine consumed per measurement. A noteworthy feature is the positioning of the analytical signals over the reproducible Schlieren signals. Presence of chloride in the samples up to 10 g L^{-1} did not alter the results. The procedure is not affected by the presence of other

Regarding R_1 and R_2 reagents, it was verified that within 100 and 200 μL, the reagent selected amounts had little influence in the extent of the indicator reaction. Therefore it was decided to use the lowest investigated volume.

Another relevant parameter in the system design is the STOP period of the processed sample inside the mixing chamber, which defines the available time for reaction development. A fast increase in the amount of formed semiquinone radical was observed when the sample resident time inside the chamber was varied within 1 and 60 s (Fig. 4). For longer STOP periods (60 - 120 s), almost no further increase was noted probably because the formation rate of the colorless sulfoxide was similar to the formation rate of the colored species. A 60-s STOP period was then chosen.

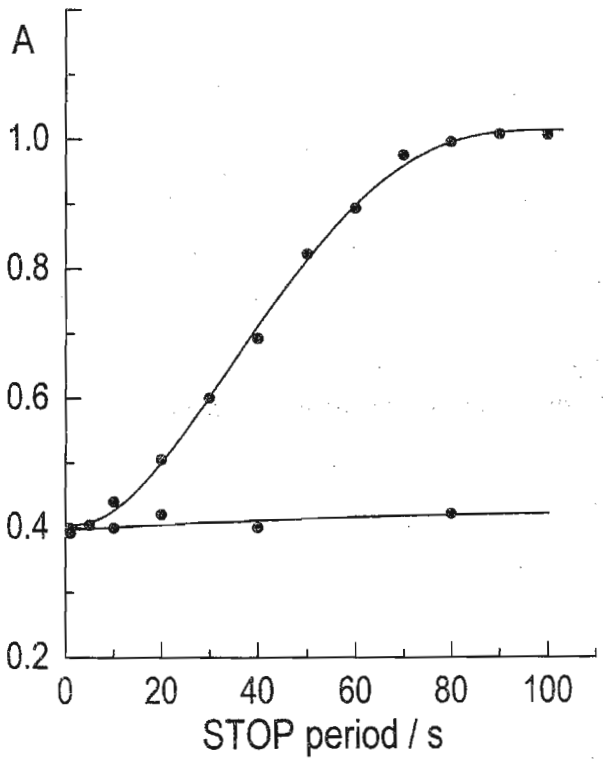

Fig. 4. Influence of the STOP period. Iodide concentration 100 μg L^{-1} I^-. Sample volume = 500 μL; sample transferred volume = 350 μL. Other conditions as in Fig. 2.

potential interfering species (Ca, Mg, Na, K, Al(III), Pb(II), phosphate, sulphate, silicate, carbonate, fluoride) in view of the high sample dilution prior to analysis and the low expected contents of potential interferants. Iodate can be tolarated in levels < 10 % relatively to I^-, and Fe(III) interfered when present in a 20-fold excess relatively to I^-, situations seldom oberved. It should be noted that selectivity data were similar to those already reported [16].

Table 2 shows the results of analysis of seven samples of table salts and one of iodized supplement for animal feeding. After two iodide spikings performed on the samples, recovery data within 95.5 and 105.0 % were calculated emphasizing the insusceptibility of the proposed procedure to matrix effects. For the supplement sample with 12.1 μg L^{-1} I^-, relative standard deviation of the results calculated after 10 replicated processing was estimated as 0.035. During a 4-h system operation, no baseline drift was observed.

Tab. 2. Analysis of iodized table salts.

Sample	Iodide concentration/μg L^{-1}			Recovery/%
	Initial	Added	Found*	
1	15.5	20.0	19.6	98.0
		60.0	61.5	102.5
2	37.4	20.0	19.1	95.5
		60.0	58.9	98.2
3	18.8	20.0	20.5	102.5
		60.0	61.5	102.5
4	52.3	20.0	20.3	101.5
		60.0	57.7	96.2
5	135	20.0	20.2	101.0
		60.0	61.3	102.3
6	14.1	20.0	19.6	98.0
		60.0	58.8	98.0
7	115	20.0	21.0	105.0
		60.0	62.1	103.5
8	25.5	20.0	20.5	102.5
		60.0	60.1	100.2

* Data include compensation of concentration increase due to the additions.

CONCLUSIONS

A SIA system with a mixing chamber and including spectrophotometric detection can efficiently handle concentrated solutions. Reproducible signals are obtained even in the presence of pronounced but reproducible Schlieren effects.

The proposed procedure for iodide determination involving the catalyzed chlorpromazine oxidation is robust, simple, low-cost and reliable. It is suitable for large scale analysis, as it is not affected by temperature variations and/or presence of potential interferants.

Placement of the mixing chamber in a dedicate port of the rotary valve permits an efficient control of the signal-to-noise ratio and an improvement in sensitivity mainly in view of the involved flow reversal towards the detection unit. Moreover, the system is able to be used for other determinations not requiring this device.

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