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FLOW INJECTION POTENTIOMETRIC DETERMINATION OF SACCHARIN IN DIETARY PRODUCTS WITH RELOCATION OF FILTRATION UNIT

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Summary—A flow injection potentiometric procedure for saccharin determination in dietary products is proposed. Saccharin is precipitated as mercurous saccharinate and the excess of the mercurous cation is potentiometrically measured using a silver wire coated with a mercury film as the working electrode. A filter unit is used to avoid contact between the precipitate and the electrode surface. With relocation in the flow manifold, the accumulated precipitate is removed on-line. Sucrose, glucose, aspartame, sodium cyclamate and sodium benzoate do not interfere when present in amounts similar to those observed in commercial products. Results are comparable with those obtained by UV-spectrophotometry and the correlation coefficient between methods is equal to 0.9930. A linear relationship between ΔE (mV) and the logarithm of saccharin concentration was obtained in the saccharin concentration range 2×10^{-3}–$1 \times 10^{-2} M$. The sampling frequency is 60/hour and only 0.76 mg of Hg_2^{2+} is consumed in each determination.

Saccharin is one of the most-consumed artificial sweeteners in some countries. In others, its use in foods and medicals is prohibited considering possible carcinogenic effects. So, due to medical and legal aspects, the determination of saccharin and other non-fattening artificials sweeteners in dietary products have an economical and social relevance. Flow injection analysis could be applied to this analytical task. However, this technique has been only applied to the determination of sugars in a variety of samples.[1–4] Alternatively, Luque de Castro *et al.*[5] emphasized that "new trends point to the start of a new age in which the emphasis will be placed on solving analytical problems in various fields of social interest".

Recently a simple and inexpensive manual method for determining saccharin based on the low solubility of mercurous saccharinate was proposed.[6] A nitrate mercurous solution was used as titrant and the remaining Hg_2^{2+} was monitored by a silver electrode coated with a metallic mercury film. This procedure seems suitable for flow injection application. To do this, the main difficulty was the presence of a precipitate which could adsorb on tube walls and the electrode surface. To avoid these undesirable effects, a relocatable filter unit was placed before the flow-through potentiometric cell and a surfactant was added to the carrier solution. The different positions occupied by the filter in the manifold, depending on the commutation state, are analogous to the relocation of the spectrophotometric detector recently proposed.[7,8]

Thus, using a homemade flow-through potentiometric cell and a precipitation reaction, a fast and inexpensive procedure was developed for determining saccharin in dietary products.

EXPERIMENTAL

Apparatus

An Ismatec peristaltic pump model 7618-40 (Switzerland) supplied with Tygon pump tubings was used for the propulsion of the fluids.

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The manifolds were constructed with polyethylene tubing (0.8 mm i.d.). Sample injection was done by using a three-piece manual commutator.[9] The filtration unit (Fig. 1) was constructed in PTFE.

The homemade flow-through potentiometric cell is shown in Fig. 2. This cell made of acrylic was previously used for amperometric determination of cyanide employing a stainless steel tube as counter electrode.[10] The reference electrode was an Ag/AgCl, NaCl 0.1*M*. The indicator electrode was a silver wire (Aldrich, 99.9%) of 1-mm diameter and 15-mm length coated with a metallic mercury film. The mercury film was renewed daily.

All potentiometric measurements were made with Micronal pH meter—model B374—(Brazil) with precision of 1 mV. The pH meter was connected to a two-channel strip-chart recorder (Cole Parmer, model 12020000—USA).

Flow diagram

The flow system was projected by linking the inlet and outlet tubes of the filter unit to manifold sites, which permit the relocation of this unit (Fig. 1). The filter can be washed on-line which avoids the increase of hydrodynamic pressure caused by precipitate accumulation.

Reagents and sample preparation

All solutions were prepared using distilled, deionized water. The saccharin stock solution (100 ml) was prepared by dissolution of 2.4363 g of sodium saccharinate (Synth, 99%) in water. This solution is stable for at least three months when stored at 5°C. The reference saccharin solutions from 2 to 15m*M* were prepared by suitable dilutions of the stock solution with water. The mercurous nitrate (Aldrich, 99%) solutions (2.5, 5.0 and 10.0m*M*) were prepared by dissolving the salt in a 1m*M* nitric acid solution. The ionic strength of these solutions was adjusted to 0.2*M* with sodium nitrate.

A 0.2*M* sodium nitrate solution in 1m*M* nitric acid was used as the carrier solution. The acidic medium avoids hydrolysis of the mercurous cation and the sodium salt adjusts the ionic strength. Some experiments were made adding 0.05% w/v polyvinyl alcohol to the carrier solution for avoiding precipitate accumulation on tube walls.

Saccharin was determined in four commercial samples of concentrated sweeteners: Dietil, Sucaryl and Assugrin (concentrated liquid sweeteners), and Doce Menor (soluble solid sweetener). The liquid samples were prepared by dilution of 1.0 ml of the concentrated samples in a 50.0-ml volumetric flask with water. In the case of the solid sample, an amount, exactly weighed, of about 1.3 g was dissolved and diluted to 50.0 ml with water.

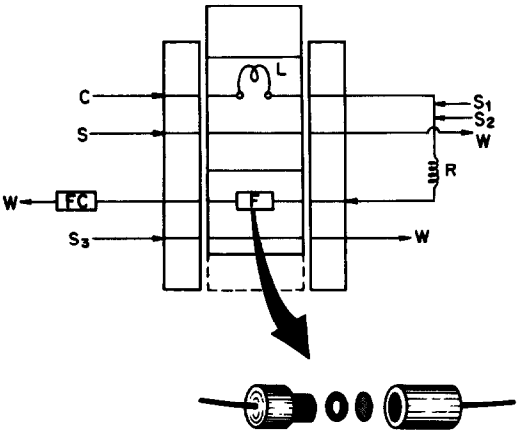

Fig. 1. Flow diagram of the proposed flow-injection system with a relocatable filter. The peristaltic pump is not shown and the dashed line indicates the position of the sliding central part after commutation. C: carrier solution, 0.2M sodium nitrate in 1m*M* nitric acid, flowing at 3.4 ml/min; S1 and S2: mercurous nitrate and 1m*M* nitric acid, both flowing at 0.76 ml/min; L: sample loop (20 cm, 100 μl); S: sample or reference solution aspirated at 3.4 ml/min; R: tubular helicoidal reactor (100 cm); F: filter; FC: flow-through potentiometric cell; W: waste. S3 is a solution identical to that used as carrier, flowing at 3.4 ml/min.

RESULTS AND DISCUSSION

Preliminary measurements carried out without a filter unit were troublesome because the adsorption of the mercurous saccharinate on the indicator electrode caused a continuous elevation of the baseline and loss of reproducibility. This effect was also observed in a batch potentiometric procedure where the electrode must be washed with distilled water after 10

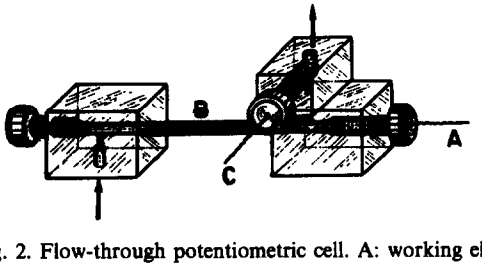

Fig. 2. Flow-through potentiometric cell. A: working electrode, a silver wire coated with a mercury film (length: 15 mm; diameter: 1 mm); B: stainless steel tube (i.d. 3 mm); C: reference electrode. The arrows indicate the flow direction.

titrations to dislodge the precipitate.[6] Then, a filter unit was put on before the flow-through potentiometric cell, but the precipitate accumulation on the filter caused an increase in the hydrodynamic pressure leading to solution leakage. Additionally, when using a filter unit, the choice of the filter paper porosity is critical. Large pores did not retain fine precipitate particles and did not avoid the salt adsorption on the electrode surface. On the other hand, very small pores retained all the precipitate particles, but caused an increase in the hydrodynamic pressure. To keep the long-term efficiency of the filter unit, the retained precipitate should be removed. Therefore, a flow injection system with a relocatable filtration unit was projected as shown in Fig. 1. During the sample injection, the filter was positioned before the flow-through detector and retained the mercurous saccharinate formed. After commutation, the relocatable filter is washed on-line by a counter-flow of a solution identical to that used as carrier. The best compromise between filtration efficiency and hydrodynamic pressure was attained using a paper filter suitable for retention of moderately fine crystals such as Whatman 40. However, a continuous increase in the baseline was again observed due to accumulation of fine particles of mercurous saccharinate on the electrode surface.

In fact, after 30 injections of 4–10mM saccharin solutions the reproducibility was not good (rsd > 10%, $n = 4$) and a gradual increase of the baseline was observed. To minimize precipitate adsorption on the electrode surface, a surfactant was added to the carrier solution. The addition of 0.05% w/v of polyvinyl alcohol to the carrier solution led to a better stability of the baseline.

Using the system outlined in Fig. 1, the effects of the mercurous cation concentration and the presence of foreign substances were evaluated. The flow rate of the carrier solution was adjusted considering the precipitation reaction and detector response. The dilution at the confluence points was the decisive factor to adjust the flow rates of mercurous nitrate and sodium nitrate solutions. The flow rate of the filter flushing solution was not critical, but it had to be adjusted to counter-flow through the unit filter.

The mercurous cation concentration influenced the precipitate formation and the magnitude of the electrode response. The potential measured at the working electrode is a function of the mercurous cation concentration in solution and this is dependent on the concentration of saccharin. The cation concentration measured at the electrode surface is inversely proportional to the analyte concentration due to the precipitation of the mercurous saccharinate, formed in a 1:2 stoichiometric proportion.[6] Thus, for application of the proposed procedure, the concentration of saccharin in the sample should be smaller than the mercurous concentration in the carrier solution. Except where mentioned, the results showed here were obtained using a 5mM mercurous nitrate solution. Employing a 8mM saccharin solution, it was observed that a two-fold increase in the concentration of the mercurous solution (*i.e.*, 10mM) caused a reduction of the potential (ΔE) from 51.4 to 33.3 mV. This effect can be explained considering that for a 10mM mercurous solution the concentration of mercury in excess is higher and, consequently, the electrode measured a lower variation in the signal background, *i.e.*, the signal in the absence of saccharin. Thus, depending on the analyte concentration, the mercurous concentration could be reduced. For saccharin solutions containing from 4 to 10mM, good results were also obtained when a 2.5mM mercurous solution was used in the continuous flow procedure. If both mercurous and saccharin concentrations were increased, more precipitate would be formed and the filter performance could be impaired.

The study of foreign substances was previously reported for a batch potentiometric method using mercurous nitrate as titrant.[6] Several organic substances which would be present in dietary products, such as sucrose, fructose, glucose, aspartame, sodium cyclamate, sorbitol and benzoic acid, did not exert a strong influence on saccharin determination by mercurous titration. Since the reaction involved in the proposed flow injection procedure is the same, a similar behavior should be expected. To confirm this hypothesis, solutions of a concentration of 8mM saccharin and from 8 to 100mM sodium benzoate, aspartame, sucrose, glucose or sodium cyclamate were prepared. No interferences were caused by aspartame, sucrose or glucose even when their concentrations were 12.5 times higher than that of saccharin. Sodium benzoate and sodium cyclamate, both in concentrations above 50mM, caused a 10% error on 8mM saccharin determination. The sodium benzoate positive error was probably caused by insoluble mercurous salts formation. Sodium cyclamate caused a negative error and this effect

Table 1. Saccharin concentrations in dietary products as determined by FIA-potentiometry and UV-spectrophotometry

	Saccharin concentration (*mg/ml* or *mg/g**)	
Sample	FIA-potentiometry	UV-spectrophotometry
Assugrin	61.40 ± 0.49	58.70 ± 2.10
Dietil	51.14 ± 1.13	52.10 ± 2.80
Sucaryl	58.18 ± 2.56	59.70 ± 1.30
Doce Menor*	26.34 ± 1.45	26.20 ± 1.90

was not completely understood. It is not clear if the cyclamate effect was caused either by interference on the solubility equilibrium of mercurous saccharinate or influence on the electrode response. However, the concentration of benzoate or cyclamate in saccharin dietetic products is not so high. The interference due to chloride or phosphate ions was not evaluated, but, as previously indicated,[6] these anions could be eliminated by previous solvent extraction of the sweetener from aqueous solution with ethyl acetate.

Results obtained for saccharin determination in four liquid or powder sweeteners are presented in Table 1 and the analytical curve and quadruplicate sample signals are shown in Fig. 3, where signal reproducibility and baseline stability are clearly demonstrated. The linear equation $\Delta E = 270.2 - 104.7$ log[saccharin] with $r = 0.9980$ was found in the saccharin concentration range of 2×10^{-3}–$10^{-2}M$. After a 4-hr working period, baseline drift was not observed and only slight variations in the calibration equation (<5%) were found. Irregularities observed on the rise of peak profiles are due to solution contained in the unit filter which is inserted in the analytical path during relocation of this device which affects the detector response. This effect was minimized by reduction of the filter unit volume and the reproducibility was not damaged.

For 4mM saccharin solution, the relative standard deviation was 2.78% for $n = 8$. Table 1 also presents the results obtained with UV spectrophotometry reported as one of the most frequently employed methods for saccharin determination.[11] The correlation coefficient between these methods was equal to 0.9930, which is a good indication of the accuracy of the proposed flow injection procedure. By applying the inexpensive FIA-potentiometric procedure, the content of saccharin could be determined in 60 samples in one hour. Only 0.76 mg of Hg_2^{2+} is consumed in each determination. This method can be easily implemented in routine laboratories.

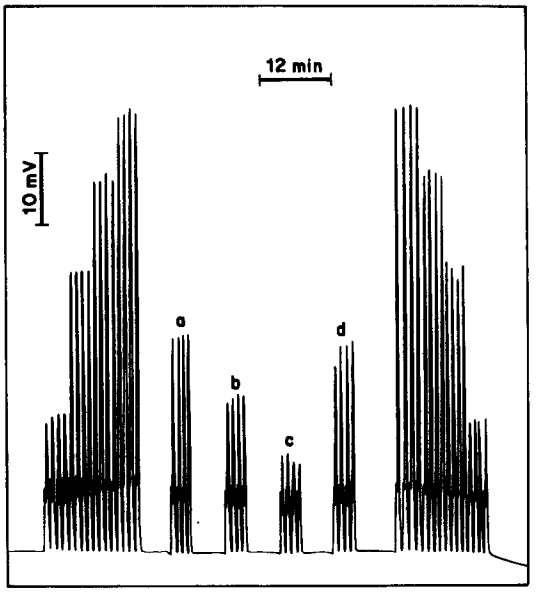

Fig. 3. From left to right: quadruplicate signals for 4.0; 6.0; 8.0 and 10.0mM reference solutions of saccharin; four commercial sweeteners samples (a: Assugrin; b: Dietil; c: Doce Menor; d: Sucaryl); and the reference solutions again.

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REFERENCES

1. J. Michalowski, A. Kojlo, M. Trojanowicz, B. Szostek and E. A. G. Zagatto, *Anal. Chim. Acta*, 1993, **271**, 239.
2. I. L. Mattos, E. A. G. Zagatto and A. O. Jacintho, *ibid.*, 1988, **214**, 247.
3. J. Abdul Hamid, G. J. Moody and J. D. R. Thomas, *Analyst*, 1988, **113**, 81.
4. K. Matsumoto, H. Kamikado, H. Matsubara and Y. Osajima, *Anal. Chem.*, 1988, **60**, 147.
5. M. D. Luque de Castro, M. Valcárcel and D. Chen, *Analyst*, 1991, **116**, 1095.
6. O. Fatibello-Filho and A. J. M. Guaritá-Santos, *Talanta*, 1993, **40**, 737.
7. E. A. G. Zagatto, H. Bergamin-Filho, S. M. B. Brienza, M. A. Z. Arruda, A. R. A. Nogueira and J. L. F. C. Lima, *Anal. Chim. Acta*, 1992, **261**, 59.
8. A. R. A. Nogueira, S. M. B. Brienza, E. A. G. Zagatto, J. L. F. C. Lima and A. N. Araújo, *ibid.*, 1993, **276**, 121.
9. H. Bergamin-Filho, E. A. G. Zagatto, F. J. Krug and B. F. Reis, *ibid.*, 1978, **101**, 17.
10. R. Gama and M. D. Capelato, Personal Communication.
11. M. M. Hussein, H. Jacin and F. B. Rodriguez, *J. Agric. Food Chem.*, 1976, **24**, 36.