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Multi-site detection in flow analysis

Part 3. Periodate tubular electrode with low inner volume as a relocatable detector

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Abstract

Periodate oxidation was exploited for the determination of sucrose and glycerol with potentiometric monitoring of the remaining periodate. For this task, a tubular electrode with a small inner volume and a fast response time was used in a flow-injection procedure with multi-site detection. With parallel monitoring, two almost identical systems shared the same detection unit. After peak maximum measurement, the detector was displaced to the other analytical channel, and then washing time was no longer a limiting factor with respect to sampling rate. The approach was applied to the determination of glycerol in soaps, detergents and lixivia samples. The sampling rate was improved by 85% relative to a comparable conventional flow-injection system without impairing other favourable analytical characteristics. The reagent consumption was only 190 μg of $NaIO_4$ per determination. With serial monitoring, measurements were made at two manifold sites under different sample processing conditions. In this way, sucrose was determined in sugar-cane juice and syrups by monitoring the sample zone prior to and after in-line acidic hydrolysis. The proposed system is very stable, no baseline drift being observed during 4-h operation periods. It provides 50 measurements per hour and yields precise results (R.S.D. usually $< 2\%$) in agreement with liquid chromatography. Clarification of the sample is not required.

Keywords: Flow injection; Potentiometry; Glycerol; Multi-site detection; Periodate tubular electrode; Sucrose; Waters

In flow-based methodologies with multi-site detection [1], after the measurement the detector is moved from its original site to another position. Although potentially beneficial, the approach may be limited by the inner volume of the detector, as was stressed in previous papers [1,2], where expected improvements in sampling rates were not so pronounced owing to the large inner volume of the spectrophotometric flow cell plus accessories. As tubular potentiometric detectors without an inner reference solution [3] can be constructed with very small inner volumes, they become attractive as relocatable detectors.

Oxidation by periodate is a widely used reaction in carbohydrate chemistry, and proceeds at room temperature under mild conditions [4]. It has often been used for the determination of reducing sugars and glycerol [5–7], but in flow

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analysis the relatively slow oxidation has often been the limiting factor with respect to sampling rate. This drawback is minimized by taking advantage of multi-site detection [1], especially when a flow-through detector with a small inner volume and fast response time is available.

The aim of this work was to investigate the use of a small inner-volume periodate-selective electrode [8] as a movable detector in flow systems. Two situations were considered, namely those involving parallel and those involving serial periodate monitoring.

In the former approach, two almost identical systems sharing the detector are proposed for the analysis of lixivia and waste waters with high glycerol contents in the soap industry. With serial monitoring, a flow system was developed for sucrose determination in sugar-cane juice and syrups. The sample zone was monitored at two manifold sites prior to and after partial and reproducible in-line sucrose hydrolysis.

EXPERIMENTAL

Apparatus

The system consisted of an MLW DP 2-2 peristaltic pump (Veb Labortechnik, Ilmenau, Germany), a B352 electronically operated commutator (Micronal, São Paulo, Brazil), a B375 digital potentiometer (Micronal), an REC61 strip-chart recorder (Radiometer, Copenhagen, Denmark), polyethylene tubing (0.7 mm i.d.) and accessories. The Reactor B_2 (see Fig. 2) was built up by coiling thin polyethylene tubing (6 m × 0.3 mm i.d., wall thickness 0.2 mm) around a glass cylinder (0.5 cm o.d.) which was immersed in a model 102/1 thermostated water-bath (Fanem, São Paulo, Brazil). With this long and thin B_2 coil, the temperature of the water-bath could be raised to about 60°C without the formation of air bubbles.

Working and reference (Ag/AgCl) electrodes were assembled in a Perspex device [3] which was connected to the sliding bar of the commutator. The inner volume of the potentiometric cell plus accessories was determined as 45 μl according to a procedure used previously [1].

The periodate-selective electrode was prepared as described [8]. The sensor system consisted of ca. 10% tetraoctylammonium periodate in *o*-nitrophenyl octyl ether solution which was further immobilized in PVC. The final membrane composition was ca. 30% (w/w) PVC and 70% (w/w) of the sensor solution. It should be stressed that use of *o*-nitrophenyl octyl ether as the mediator solvent resulted in improved selectivity and fast response times relative to the previously used dibutyl phthalate [8].

Solutions

All solutions were prepared with distilled, deionized water and analytical-reagent grade chemicals.

A 0.1 M $NaIO_4$ stock solution was prepared by dissolving 10.69 g of $NaIO_4$ in 500 ml of water and was kept in an amber-coloured bottle.

For the determination of glycerol, a stock standard solution [10.00% (w/v) glycerol] was standardized iodimetrically [9]. Working standard solutions containing 0.05–0.20% (w/v) glycerol were freshly prepared by dilution of the stock standard solution with water. The combined reagent for the determination of glycerol (C′ and C″, see Fig. 1) was 0.003 M $NaIO_4$–0.5 M Na_2SO_4–0.1 M acetic acid–0.1 M sodium acetate.

Bottom lixivia, industrial brine, washing solutions and waters from evaporators and condensers were collected at a soap production plant. The samples were heated (50–60°C for about 10 min) and the resulting clear solutions were manually diluted (100 g of sample plus 500 g of water). Immediately before injection into the flow system, the samples were further diluted with water.

For sucrose determination, working standard solutions [0.10–0.50% (w/v)] were prepared daily in water. A 0.50% (w/v) invert sucrose standard prepared as in previous work [6] was used to check hydrolysis. The combined reagent (R_1 in Fig. 2) was 0.002 M $NaIO_4$–0.5 M Na_2SO_4–0.01 M Na_2HPO_4–0.01 M NaH_2PO_4. C, R_2 and R_3 were water, 2.0 M H_2SO_4 and 1.9 M NaOH–0.1 M Na_3PO_4, respectively. The NaOH concentration should be accurately adjusted if the pH of the discarded stream (W, Fig. 2) lies outside the range 6.5–7.2.

Procedure

The system for the determination of glycerol (Fig. 1) consisted of two almost independent channels sharing the detection unit. It was designed according to the single-line system proposed for the analysis of residues relevant to soap production [8]. A small sample plug was intercalated into the carrier stream C′ flowing through reactor B′ towards detection site a. During sample transportation, glycerol was oxidized by periodate at pH ≈ 4.7. The baseline potential was related to the periodate concentration, and sample passage through the detector resulted in a transient signal proportional to the periodate consumed, reflecting the glycerol content in the sample. The commutator was switched a few seconds after the peak maximum was reached, intercalating the next sample into the stream C″ and displacing the movable detector to monitor at site b. During sample processing inside B″, the trailing edge of the previous sample still flowing inside B′ was discarded without flowing through the detector. In this way, the washing time was not a limiting factor with respect to the sampling rate. With the system in Fig. 1, effects of flow rate and commutation timing were studied.

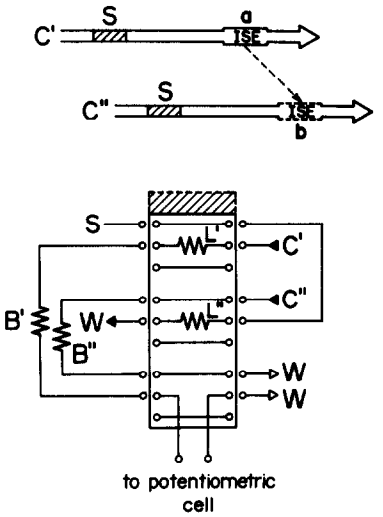

Fig. 1. Schematic representation and flow diagram of the system with parallel monitoring proposed for glycerol determination. S, sample (2.0 ml min^{-1}); L′ and L″, 6-cm (30-μl) sampling loops; C′ and C″, combined reagent (0.5 ml min^{-1}); B′ and B″, coiled reactors (100 cm); ISE, relocatable ion-selective electrode; a and b, monitoring sites; W, waste; black arrows, sites where pumping is applied; hatched area, movement of the commutator; interrupted lines, detector displacement.

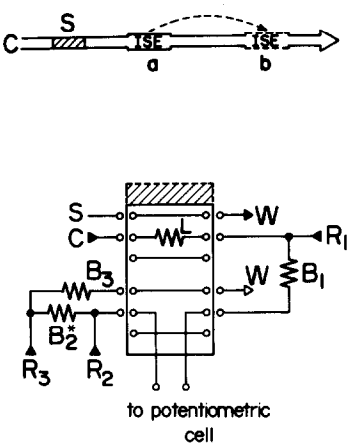

Fig. 2. Schematic representation and flow diagram of the system with serial monitoring proposed for sucrose determination. S, sample (1.0 ml min^{-1}); L, 50-cm (250-μl) sampling loop; C, water (0.4 ml min^{-1}); R_1, R_2 and R_3, reagents (0.4, 0.4 and 0.8 ml min^{-1}); B_1 and B_3, reactors (100 and 300 cm); B_2^*, heated coil (600 cm); temperature of the water-bath in which this coil and transmission line (200 cm) for R_2 addition are immersed, 60°C; other symbols as in Fig. 1.

The system for the determination of sucrose (Fig. 2) exploited serial monitoring to permit sample quantification prior to and after acidic hydrolysis. The sample was introduced into carrier stream C, reagent R_1 was added and periodate oxidation was initiated inside reactor B_1 under neutral conditions. The peak related to site a reflected mainly the concentrations of chemical species readily oxidized by periodate and reducing sugars occurring at higher concentrations such as fructose and glucose [4]. Thereafter, warm sulphuric acid solution (R_2) was added, promoting partial and reproducible sucrose hydrolysis inside the heated coil B_2. When most of the sample was flowing inside B_2, the commutator was switched to the loading position and the detector displaced downstream (site b). After neutralization of the sample zone by reagent R_3, further periodate oxidation proceeded inside coil B_3. Then, the recorded peak height related to site b referred also to the periodate consumed by the

hydrolysis products. The sucrose concentration in the sample was then evaluated after subtraction of signals related to both monitoring sites. After measurement of the peak maximum, the commutator was switched back to the position specified in Fig. 2 and the trailing edge of the dispersed zone was directly discarded. With this system, the effects of periodate concentration, flow-rates, temperature, acidity for hydrolysis and timing were studied.

RESULTS AND DISCUSSION

Determination of glycerol with parallel monitoring

The speed of the peristaltic pump is an important parameter to be considered in system design [8] as it is proportional to the washing time and to the mean available time for glycerol oxidation.

With the flow system outlined in Fig. 1, the peak heights observed for 0.20% (w/v) glycerol were 48.1, 75.0 and 119.5 mV when the flow-rate of C′ (or C″) was 1.0, 0.5 and 0.2 ml/min, respectively, which corresponded to about 30, 60 and 180 s for glycerol oxidation. Higher glycerol concentrations reaching the electrode should be avoided to prevent membrane deterioration, which was probably due to reduction of periodate ions in the membrane [8]. Therefore, the flow-rates of the sample carrier streams were set at 0.5 ml/min and the system was designed to allow a large dispersion (dispersion coefficient ca. 0.07). As a consequence, no pronounced modifications of slope, baseline stability or electrode response time were observed during extended working periods (4 h) and the electrode performance remained unchanged after at least 5 weeks. In addition, undesirable effects due to the establishment of concentration gradients along the sample zone, inherent to the single-line configuration, were not pronounced. The signal corresponding to 0.00% (w/v) glycerol was only 4.6 mV (Fig. 3). It should be stressed that the difference in peak heights for the same glycerol concentration (Fig. 3) which are not relevant in terms of the final results are due to the practical impossibility of designing identical sub-systems.

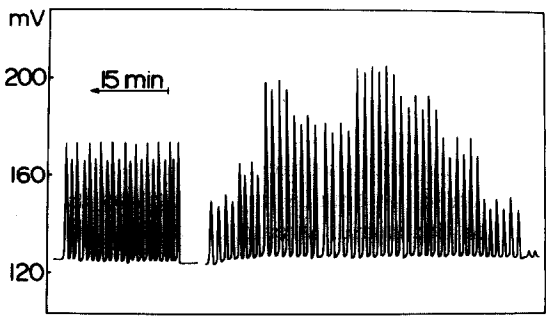

Fig. 3. Recorder tracing related to glycerol determination. The results relate to the system in Fig. 1 operated manually. From the left, recorded peaks refer to a lixivia sample processed ten times, five samples in duplicate, four standard solutions [0.20, 0.15, 0.10 and 0.05% (w/v) glycerol] in triplicate, and a blank (water) processed once. Higher signals correspond to site b.

Without detector relocation, the washing time was 55 s for a 1% carryover level (Fig. 4). This means a sampling rate of about 65 h^{-1} and a periodate consumption of 280 μg of $NaIO_4$ per determination. A remarkable improvement in sampling rate (85%) was achieved by relocating the detector immediately after measurement of the peak maximum, which reduced washing time to about 36 s. In this situation, 100 samples could be analysed per hour with a periodate consumption of 190 μg of $NaIO_4$ per determination, provided that the detector was able to monitor at the

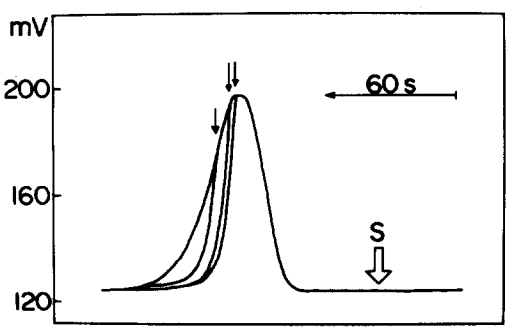

Fig. 4. Effect of detector relocation. Record tracings refer to a 0.20% (w/v) glycerol solution injected into the system in Fig. 1. All signals refer to site a. S, instant of sample injection. The broader peak corresponds to the situation without detector relocation. Instants of detector relocation/sample injection are specified by black arrows.

other channel. Studies on multi-site detection in n-channel systems with parallel monitoring are in progress.

The time for restoration of the baseline was almost independent of the instant of moving the detector (Fig. 4) and much shorter than those reported for spectrophotometric systems with multi-site detection [1,2]. This emphasizes the beneficial effect of the small inner volume of the potentiometric cell with accessories.

The proposed system does not require rigid temperature control; negligible variations in peak heights (< 2 mV) were noted after raising the ambient temperature from 25 to 30°C. It is suitable for on-line analysis related to industrial processes. A noteworthy feature of the proposed procedure is the linearity of the calibration equation ($r = 0.9982$, $n = 6$), which is a consequence of the combination of the Nerstian response of the detector and oxidation kinetics [4,6]. Reproducible results (S.D. < 0.5 mV) and a stable baseline were always observed and slope was > 58 mV per concentration decade. The accuracy can be assessed from Table 1.

Determination of sucrose with serial monitoring

The system for the determination of sucrose (Fig. 2) was based on that proposed previously for the spectrophotometric analysis of sugar-cane juice and molasses [6], but the coil volumes and flow-rates were lower in view of the small inner volume of the relocatable detector.

The flow-rate for R_3 was selected as relatively high. In this way, the need for a too concentrated R_3 solution was diminished, dilution at the last confluence became more pronounced and the ionic strength inside coil B_3 and the detector was decreased. The lengths of coils B_1 and B_3 were selected to permit similar available times for periodate reduction. Better heating conditions were attained by using a heated stream R_2 and a coil B_2 built up with thin-walled and small inner diameter tubing. The temperature of the water-bath was fixed at 60°C; increasing this temperature increased the possibility of the formation of air bubbles which impaired detection. The length of B_2 was a compromise between the available time for hydrolysis and hydrodynamic pressure. With $B_2 = 600$ cm, the mean sample residence time inside the tube was about 20 s and 32% hydrolysis was attained for sodium periodate and sulphuric acid concentrations of 0.002 and 2.0 M, respectively.

Hydrochloric acid has been widely used for sucrose hydrolysis and was also used when sucrose inversion was incorporated in flow-injection analysis [6]. However, this acid was not used here considering the need for high concentrations and the potentiometric selectivity towards chloride ($\log K^{pot} \approx -3.5$ [8]). For higher sucrose concentrations, lower periodate contents should be monitored, and the electrode response was more susceptible to the high chloride level. As a consequence, bending of the calibration graph became more pronounced, and injected solutions with sucrose concentrations $> 0.2\%$ (w/v) could not be analysed. As the potentiometric selectivity coefficient towards sulphate was more favourable ($\log K^{pot} < -5$), this drawback was circumvented by using sulphuric acid in R_2 and increasing the periodate concentration in reagent R_1.

The effects of acid concentration and temperature for hydrolysis were analogous to those already reported [6]. Lower sulphuric acid concentrations were not used; when it was < 1.0 M, the extent of hydrolysis was always $< 5\%$ On the other hand, the acid concentration could not be increased at will to avoid the need for special pumping tubes, bending of the calibration graph, high ionic strength and difficulties of pH adjustment inside coil B_3. R_2 was then chosen as 2.0 M H_2SO_4.

TABLE 1

Glycerol contents [% (w/w)] in lixivia and waters from a condenser as determined by the proposed procedure and by iodimetric titration [9].

Sample	Proposed procedure [a]	Titration [b]
Lixivia 1	32.5 (0.010)	30.0
Lixivia 2	32.7 (0.013)	32.5
Lixivia 3	35.6 (< 0.005)	35.4
Wash liquor 1	0.86 (0.007)	0.81
Wash liquor 2	0.54 (0.021)	0.59

[a] Numbers in parentheses are estimates of relative standard deviations (%), based on triplicate measurements. [b] Relative standard deviation of results < 0.01%.

The periodate concentration also could not be modified at will. Preliminary tests confirmed the pronounced decrease in sensitivity [8] for concentrations > 0.01 M. A noisy baseline, slow response time and unacceptable calibration characteristics were observed for concentrations < 0.001 M. The periodate concentration in R_1 reagent was consequently selected as 0.002 M.

The HPO_4^{2-}–$H_2PO_4^-$ buffer system was chosen mainly because under neutral conditions the oxidation of fructose was faster than for other reducing agents present in the samples at significant concentrations [6,10]. Thus, the measurement reflected efficiently the amount of inverted sucrose. An increase in sensitivity could be achieved by selecting a more alkaline medium, as the oxidation of fructose would be accelerated and glucose would become relevant. At pH ≈ 10 (ammonium–ammonia buffer), the signal almost doubled but the procedure became more susceptible to the presence of other sugars. Moreover, an increase in the signal related to site a impaired the precision of the results owing to error propagation effects. The concentration of buffer components in reagent R_1 was kept low to minimize the consumption of sulphuric acid prior to hydrolysis. The phosphate concentration in R_3 was selected as a compromise between easy adjustment of pH inside coil B_3, ionic strength and solubility.

The proposed system (Fig. 2) is remarkably stable and no baseline drift is observed during extended working periods, which in turn confirms the stability of the periodate reagent. The recording tracings show two baselines (Fig. 5) corresponding to measuring sites a and b which are related to different periodate concentrations and temperatures. The difference in baseline could be minimized by adding a suitable amount of sodium periodate to R_3 and minimizing the differences in temperature between sites a and b. Experiments involving periodate addition to R_3 reagent and immersion of coil B_3 in cold water were carried out, but no pronounced improvement in results or electrode lifetime were noted. When R_3 was immersed in an ice-bath, tubing clogging due to precipitation effects was observed. Therefore, and in order to retain simplicity, the set-up outlined in Fig. 2 was maintained.

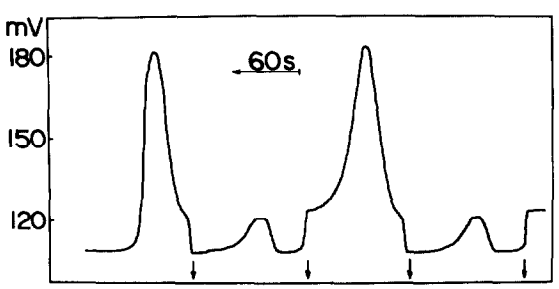

Fig. 5. Recorder tracing related to serial monitoring. The results relate to a diluted syrup sample (420 mg l^{-1} sucrose in the injected solution) processed with the system in Fig. 2. Lower and higher peaks correspond to monitoring sites a and b, reflecting situations prior to and after hydrolysis. Arrows indicate instants of detector relocation/sample injection.

The commutator can be operated any time after the peak reaches a maximum at site a provided that the baseline is restored before the sample arrives at site b. Next, commutation should be done immediately after reaching the peak maximum at site b so that most of the trailing portion of the sample zone is directly discarded. In this way, about 50 measurements can be performed per hour (Fig. 5).

The sensitivity could be improved by decreasing the flow-rates. This aspect was not exploited here in view of the high sucrose contents in the samples. As the system was designed with large dispersion, caramelization was not observed and the samples could be run without prior clarification.

The proposed procedure is potentially applicable to industrial process monitoring associated with sugar and ethanol production, as it is not susceptible to interferences by ethanol at concentrations up to 10% (v/v). As partial sucrose hydrolysis is involved, a precisely controlled water-bath should be available. Here, a 10% increase in the slope of the calibration equation was observed after a 1°C temperature increase. Analogously to the system of Fig. 1, good linearity of the calibration graph was attained.

Metal interferences are also negligible. For 10 mg l^{-1} of Mn(II), Mn(VI), Cu(II) and Cr(VI) in the injected solution, no measurable influence was observed on either the blank or the analytical

signals. Fe(III) concentrations higher than 5 mg l^{-1} cannot be tolerated. However, such levels are unlikely to be found in the assayed samples.

Results are obtained by expressing peak heights related to sites a and b as "apparent sucrose concentrations" and subtracting them. For this, both sucrose and inverted sucrose standards are needed. For most of the assayed samples, the signal related to site a was < 10% relative to that recorded at site b (see also Fig. 5). Therefore, a decrease in accuracy due to the presence of other species reacting with periodate and to error propagation effects is usually not relevant.

For a typical sugar-cane juice, the relative standard deviation (R.S.D.) of the results was < 2% for seven replicate determinations. A better precision (R.S.D. < 0.01%) was found for syrup analysis. After running ten juice samples already analysed by LC [11], no statistical differences between the two methods at the 95% confidence level were found.

Conclusions

Small inner volume detectors with fast response times are excellent as relocatable detectors, especially for parallel monitoring. With regard to this point, the system shown in Fig. 1 has been used in large-scale analyses without any major problems. The detector can be moved any instant after the peak maximum has been reached. Manual operation of the system is preferred.

Regarding serial monitoring, the beneficial effects of detector relocation are more worthwhile at site b (Fig. 2). As the trailing edge of the sample zone is not directly discarded after measurement at site a, the washing time of the first peak is not so relevant (see also Fig. 5). The system requires strict timing control, and in this case manual operation is not feasible.

Both systems can also be designed with computer-controlled three-way valves in order to improve versatility. Studies on this aspect are in progress.

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