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A multi-purpose flow manifold for the spectrophotometric determination of sulphide, sulphite and ethanol involving gas diffusion: Application to wine and molasses analysis

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ABSTRACT

A simple and rugged flow set up was designed for spectrophotometric determination of sulphide, sulphite and ethanol aiming at quality assessment of wines, control of industrial fermentation, and selection of yeast strain. The different assays involved gas diffusion through a Teflon planar membrane and were carried out after minor modifications in the manifold, namely reagent composition and total flow rate. Main figures of merit: linear analytical curves = 0.50–6.0 mg L^{-1} S^{2-}, 2.5–20.0 mg L^{-1} $SO_3^{=}$ and 5.0–25.0% (*v*/*v*) of ethanol; detection limits (3σ) = 0.035 mg L^{-1} S^{2-}, 0.2 mg L^{-1} $SO_3^{=}$ and 0.18% (*v*/*v*) of ethanol; peak height r.s.d. = 2.18% for 4.03 mg L^{-1} S^{2-} spiked molasses, 2.21% for a 9.82 mg L^{-1} $SO_3^{=}$ wine and 2.07% for a typical wine (12.53% *v*/*v* of ethanol), sampling rate = 15, 57 and 29 h^{-1}, reagent consumptions = 1.9 μmol of *N*,*N*-dimethyl-*p*-phenylenediamine, 1.68 μg of Malachite green and 0.68 mmol Cr(VI) per determination, respectively.

1. Introduction

A multi-purpose flow system is characterized by a flexible and simple manifold and can be used for different assays after slight modifications. In small laboratories it can be regarded as the main instrument, dedicated to different single-analyte determinations. It is also useful as a secondary instrument in large laboratories, as it can replace an instrument under maintenance, perform urgent analysis and/or assess the laboratory quality, this later aspect often demanding comparative analysis. This was already realized in 1975 when a single-channel segmented-flow analyzer was designed for biologic fluid assays [1]. Calcium, uric acid, glucose, phosphorus, chloride, urea nitrogen and carbon dioxide were determined without the need for manifold changes: only the chromogenic reagent and the monitoring wavelength were modified from one assay to another.

The approach is efficiently implemented in unsegmented flow systems, as a long time interval for restoration of the flow pattern after every manual reagent replacement is not needed. This was demonstrated when multi-purpose flow systems were designed for the flow injection analysis of plant digests [2,3]. A noteworthy feature is that sample dispersion was not significantly affected by the reagent replacement: in fact, sampling rate was maintained for the different assays. The approach was further applied to the spectrophotometric determination of copper, iron, manganese and zinc in animal feeds [4]. Development led to a single flow system for the determination of protein, phosphorus, calcium, chloride, copper, manganese, iron and zinc in animal feeds or premixes [5]. Alternatively, multi-purpose flow systems can be designed with reagent injection [6], as the reagents to be inserted are easily replaced by each other. This was confirmed in the determination of phosphate, phenolic compounds, nitrite, sulphide and total iron in natural waters [7].

The favourable characteristics of the multi-purpose flow systems and their acceptance have lead to several innovations. A logical evolution was to mechanically accomplish the reagent replacement, and this aspect led to the concept of random reagent access [8,9] and to the inception of sequential injection analysis [10] and derived modalities. Flow systems with mechanical reagent selection are more concerned with sequential/simultaneous determinations and with expert systems. Although these systems may accomplish multi-analysis with the same manifold, they cannot be regarded as typical multi-purpose systems. In fact, a multi-purpose flow system undergoes slight manual modifications in order to be applied to different yet independent assays; as a rule, simultaneous/sequential determinations are not aimed at.

Quality assessment of wines often requires the evaluation of the sulphite and ethanol concentrations [11–13]. Real-time

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sulphide monitoring in molasses under fermentation is relevant, as its excess during sugar fermentation by *Saccharomyces cerevisiae* aiming at industrial ethanol production, may alter the yeast metabolism thus affecting the process [12,14,15]. For an efficient industrial control, sulphide monitoring during sucrose fermentation is then needed.

To this end, portable, rugged and simple equipment is required and these characteristics are fulfilled by the multi-purpose systems. The aim of this work was therefore to propose such a flow system for spectrophotometric analysis of wines and molasses under fermentation. The determinations involved analyte release, gas diffusion through a Teflon planar membrane, analyte collection into the acceptor stream, derivatisation and detection. The S^{2-} reacted with *N*,*N*-dimethyl-*p*-phenylenediamine, in the presence of Fe(III), yielding methylene blue, monitored at 668 nm [16]. The HSO_3^- established into the buffered acceptor stream reacted with malachite green promoting a decolourization which was enhanced in the presence of the cetylpyridine chloride and monitored at 620 nm [17]. The collected ethanol reduced Cr(VI) under acidic conditions and the formed Cr(III) was monitored at 600 nm [18].

Although multi-analysis with the single manifold is not new, a typical multi-purpose flow system involving gas diffusion has not yet been proposed.

2. Experimental

2.1. Standards, reagents, samples

The solutions were prepared with chemicals of analytical grade quality and distilled-deionised water. The working standard solutions were daily prepared.

For sulphide determination in molasses, the stock standard solution (1000 mg L^{-1} S^{2-} in 0.025 mol L^{-1} NaOH) was based on $Na_2S \cdot 9H_2O$ and the working standard solutions (0.5–6.0 mg L^{-1} S^{2-}) were also 0.025 mol L^{-1} in NaOH. The chromogenic reagent R_3 (Fig. 1) was a 5.0 mmol L^{-1} *N*,*N*-dimethyl-*p*-phenylenediamine, DMPD, plus 1.0 mol L^{-1} HCl solution, and the R_4 reagent was a 50 mmol L^{-1} Fe^{3+} (based on $FeCl_3 \cdot 6H_2O$) plus 1.0 mol L^{-1} HCl solution. A 0.01 mol L^{-1} NaOH and a 0.5 mol L^{-1} HCl solutions were used as the acceptor (R_2) and sample conditioning (R_1) streams.

For sulphite determination in wines, the aqueous stock standard solution (1000 mgL^{-1} SO_3^-) was based on Na_2SO_3, and the working standard solutions covered the 2.5–20.0 mg L^{-1} SO_3^- concentration range were prepared with the wine matrix [19]. A 2.0 mmol L^{-1} malachite Green, MG, plus 6.0 mmol L^{-1} KH_2PO_4 solution was used as the R_3 and R_4 reagents. The R_2 stream was a 0.1 mol L^{-1} K_2HPO_4 plus 6.0 mmol L^{-1} cetylpyridine chloride, CPC (pH adjusted to 8.0 with 0.1 mol L^{-1} H_3PO_4) and the R_1 stream was a 1.0 mol L^{-1} HCl solution.

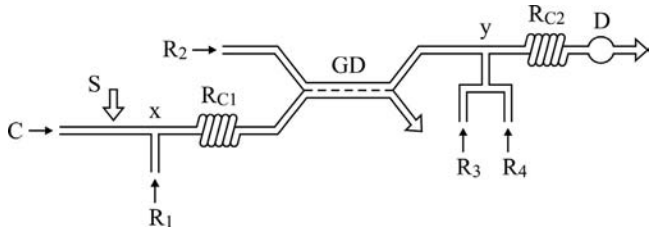

Fig. 1. Flow diagram of the multi-purpose flow system. *S*=sample; *C*=sample carrier stream; R_1=sample conditioning reagent; R_2=acceptor stream; R_3 and R_4=colour-forming reagents; R_{C1} and R_{C2}=coiled reactors; GD=gas diffusion unit; *D*=detector; *x* and *y*=confluence sites; black arrows=sites where pumping is applied; empty arrow=sample loop-based injection. For details, see text.

For ethanol determination in wines, 5.0–25.0% (*v*/*v*) working standard solutions prepared in the wine matrix [20] were used. A 0.3 mol L^{-1} $Cr_2K_2O_7$ in 4.0 mol L^{-1} H_2SO_4 solution was used as the R_3 and R_4 streams. Water and the same Cr(VI) acidic solution were placed as the R_1 and R_2 streams.

Wine samples were purchased from a local supermarket; the red wines underwent a 5-fold water dilution immediately before analysis whereas the white wines were analysed as received. The sugar-cane molasses (at different fermentation degrees) were provided by the Agronomical College from the University of S. Paulo. Prior to analysis, they underwent a 1:10 *w*/*w* water dilution (under stirring) and the alkalinity was adjusted to pH 11 with a 0.1 mol L^{-1} NaOH solution.

2.2. The flow system

The UV–vis spectrophotometer was a model 4000 USB from Ocean Optics, furnished with a Z-shaped flow-cell (optical path=10 mm, inner volume=18 μL); for data acquisition and treatment, the control software provided by the manufacturer was used. The peristaltic pump was a model 7618-40 from Ismatec, furnished with Tygon pumping tubes. The sliding bar injector-commuter was similar to that used in earlier work [13]. The manifold was built up with 0.8 mm i.d. polyethylene tubing. Perspex connectors and other accessories were used.

As a multi-purpose flow system was aimed at, the gas diffusion unit was the same for all analytes, namely the sandwich type Technicon AAII standard chamber (length=70 mm; channel deepness=1.0 mm; material=Perspex). The gas permeable membrane was a PTFE commercial tape (thickness 0.1 mm, pore size=0.22 μm).

The flow system (Fig. 1) was operated as follows. The selected sample aliquot was inserted into the C_S water carrier stream, originating a reproducible sample zone that was pushed forwards by this carrier stream. At the confluence point *x*, the R_1 conditioning reagent was added to promote the analyte conversion to a volatile chemical species (H_2S or SO_2) inside the following R_{C1} coiled reactor. As analyte conversion was not required for ethanol determination, water was pumped as the R_1 stream. Thereafter, the sample zone reached the gas diffusion unit, where the gaseous species permeated through the membrane towards the R_2 acceptor stream. Different solutions were used as the R_2 reagent, depending on the considered analyte (see Section 2.1). After collection, a secondary sample zone was established and directed towards detection. At the following *y* confluence, the R_3 and R_4 combined reagents were added, and the colour-forming reactions occurred inside the R_{C2} reactor. The passage of the sample through the flow cell caused a transient variation in the monitored absorbance that was recorded as a peak. In the present application, peak height constituted itself in the measurement basis.

3. Results and discussion

3.1. Initial experiments

Preliminary experiments revealed that the procedure for sulphide determination was more critical in relation to the other analytes. Design of the multi-purpose system relied then on this determination, and results from earlier work [13] were relevant in the context. Moreover, it was verified that the rotation speed of the peristaltic pump should be modified from one assay to another. For sulphide, sulphite and ethanol, these speeds were set as 46, 154 and 100% of the nominal speed.

The system was dimensioned to provide limited sample dispersion. To this end, a 100-cm sampling loop (*ca* 500 μL) was

Table 1
Investigated parameters for the determinations of sulphide, sulphite and ethanol.

Parameter	Sulphide		Sulphite		Ethanol	
	Range	Selected	Range	Selected	Range	Selected
Sampling loop (μL)	120–1000	500	120–1000	500	120–1000	500
C flow rate (mL min^{-1})	0.20–0.90	0.53	0.92–2.70	1.84	0.46–1.85	1.20
R_{C1} length (cm)	20–100	75	20–100	75	20–100	75
R_{C2} length (cm)	50–200	120	50–200	120	50–200	120
R_1 flow rate (mL min^{-1})	0.10–0.46	0.28	0.48–1.43	0.96	0.22–1.00	0.60
R_2 flow rate (mL min^{-1})	0.15–0.80	0.48	0.74–2.20	1.48	0.35–1.54	1.00
R_3–R_4 flow rates (mL min^{-1})	0.042–0.22	0.13	0.21–0.64	0.43	0.11–0.46	0.30
Pump rotation speed (% nominal speed)	15–77	46	77–230	154	38–154	100

selected and the ratio of flow rates at the x and y confluent sites were set as two, as a compromise between sample dilution and mixing conditions. Lengths of the R_{C1} and R_{C2} reactors were set as 70 and 120 cm. Under these conditions, the need for using excessively concentrated reagent solutions was avoided and suitable mixing conditions were attained, as a less noisy baseline (uncertainty < 0.01 absorbance) was recorded.

Other relevant parameters were optimized by the univariate method, aiming at the magnitude of the analytical signal, analytical precision and reagent consumption. The investigated ranges and selected values are listed in Table 1.

3.2. Influence of temperature

This parameter was investigated by immersing the gas diffusion unit, GD, in a thermostated water-bath and varying the temperature between 20 and 60 °C. After each temperature variation, a 5-min time interval was needed for attaining thermal equilibrium. This time interval was enough, as no modifications in recorded peak heights after 4-fold runs of sulphide, sulphite or ethanol standard solutions were noted after each temperature variation. Higher temperatures were not tested in order to avoid melting of the GD Perspex components.

The efficiency of mass transfer, thus sensitivity, increased with the temperature. Hopefully, this parameter was not critical in the system design. For sulphide determination, the efficiency was practically unaffected by temperature variations, whereas slightly improvement in sensitivity (*ca* 15%) was noted for sulphite when the temperature was raised from 20 to 40 °C. No improvements were noted after further temperature increase. Regarding ethanol, a 5% sensitivity improvement was noted when the temperature was modified from 20 to 45 °C, and a pronounced increase in mass transfer (49%) was observed within the 45–60 °C temperature range. This effect can be explained by keeping in mind that the ethanol boiling point was approached. As the influence of temperature variations around 20 °C was negligible for the three analytes, and the sensitivity for ethanol determination was not critical, the heating step was not implemented, providing that the polyvalent flow system was operated in an air-conditioning environment.

3.3. Reagent concentrations

Influence of the R_1 conditioning reagent acidity was studied between 0.1 and 2.0 mol L^{-1} HCl, for both sulphide and sulphite determinations. A sensitivity drop was verified for $R_1 < 0.4$ mol L^{-1} HCl, probably because the analyte conversion was not quantitative during passage of the sample zone through the R_{C1} and GD. On the other hand, no sensitivity enhancement was verified for $R_1 > 1.0$ mol L^{-1} HCl, and this result permits one to infer that increasing the ionic strength of the donor stream did not improve the mass transfer. The R_1 acidity was then selected as 0.5 mol L^{-1} for both sulphide and sulphite. With this high acidity, the different convertible sulphide species (e.g. MeSH, EtSH, DMS, DES, DMDS [21]) are converted to H_2S, and the analytical signal reflected the total sulphide content. Water was used as R_1 for the ethanol determination (see Section 2.2).

Different solutions were used as the acceptor stream, depending on the considered analyte. For sulphide and ethanol, the R_2 reagents (Section 2.1) were the same as used in earlier works [13,19]. Regarding sulphite determination, influence of pH of the acceptor stream was investigated by using different buffered phosphate or borate solutions ($6.7 < pH < 9.7$) as the R_2 reagent, and best sensitivity was attained for pH 8.0. The buffer capacity of the acceptor stream played a relevant role in the context. For too low a buffer capacity (< 0.05 mol L^{-1} phosphate, pH adjusted to 8.0), the linearity of the analytical curve deteriorated, assuming an asymptotical shape. This result can be explained by recalling that, for the more concentrated solutions, the analyte collection was impaired in view of the pH lowering involved. With a 0.1 mol L^{-1} phosphate, the selected concentration, suitable buffer capacity was established and a strictly linear analytical curve was attained. A sensitivity enhancement was attained by adding the CPC cationic surfactant at a concentration of 6.0×10^{-3} mol L^{-1}, higher than the critical micellar concentration inside the R_{C2} coil. Also, baseline became more stable.

Regarding the colour-forming reagent, the R_3 and R_4 reagent concentrations related to the sulphide determination were defined according to earlier work [13] by considering the involved volumetric fractions [22]. For sulphite determination, the MG concentration in the R_3 and R_4 streams was set as 2.0 mmol L^{-1} plus 6.0 mmol L^{-1} KH_2PO_4 which corresponded to a baseline of around 1.0 absorbance. Higher concentrations were not tested, as monitoring of too high absorbance values is not recommended. On the other hand, the dynamical concentration range would be narrowed by using lower MG concentrations. For ethanol, the system could be designed without the R_3 and R_4 streams. As a polyvalent system was aimed at, an acidic Cr(VI) solution was placed as the R_3 and R_4 streams, in order to speed up the relatively slow Cr(III) formation.

3.4. Evaluation of the matrix effect

For evaluation of the matrix effect in the wine analysis, the single-analyte standard and the blank solutions were prepared either in water or in a medium containing 2.5, 5.0 or 10.0 mg L^{-1} of ascorbic acid, acetic acid, tartaric acid, lactic acid, glucose and fructose. Each solution was processed in triplicate.

Lactic acid, glucose or fructose in any of the tested concentrations, or tartaric, ascorbic or acetic acids in concentrations lower or equal 5.0 mg L^{-1} did not cause any interfering effect. In

fact, alterations in recorded peak heights were noted only for >5 mg L^{-1} tartaric, ascorbic or acetic acids, as positive interferences (+8, +3 and +5%) were verified for the highest standard solutions (10 mg L^{-1} S^{2-}, 10 mg L^{-1} SO_3^{-} or 15% *v/v* ethanol). These interfering effects were less pronounced for lower analyte concentrations and were not noted for the blank, thus characterizing a typical matrix effect.

Additionally, nine standard solutions (2.5 mg L^{-1} S^{2-}, 10.0 mg L^{-1} SO_3^{-} or 15% *v/v* ethanol) and the blank solution were prepared in the presence of all potential interfering species (all of them at 2.5, 5.0 or 10.0 mg L^{-1}). Again, a positive interference (+5%) was noted when all potential interferents were present in concentrations of 10.0 mg L^{-1}. In order to apply the without concerns related to matrix effects, the wine matrix [19] was added to the working standard solutions.

For evaluation of the matrix effect in the molasses analysis, the single-analyte standard and the blank solutions were prepared to contain also 5.0, 10.0 and 15.0% m/V sucrose, glucose and fructose. For the sulphide determination, negative interferences (−16, −32 and −79%) were noted for sucrose, fructose and glucose, respectively. Although relatively high, these values are not restrictive for fermentation monitoring purposes, as molasses at different fermentation degrees are concerned. During the fermentative process, the concentrations of these carbohydrates undergo a pronounced lessening, whereas the concentrations of ethanol and eventually sulphide are increased [14]. In addition, a high variability in analyte concentrations is inherent to the fermentation monitoring. It should be stressed that monitoring the entire fermentative process is required for industrial control purposes, and the initial monitoring step is less relevant in the context.

3.5. Analytical figures of merit

The flow system in Fig. 1 is very stable, and baseline drift has not been noted during extended (8 h) operation periods. Minor modifications ($<10\%$) in reagent concentrations do not modify the main analytical figures of merit in a pronounced manner, and this is a favourable aspect towards system ruggedness. Regarding durability of the gas permeable membrane, no differences in analytical performance were noted after one week of continuous system operation, in spite of the different flow rates at both sides of the membrane.

The relative standard deviation for sulphide in a spiked molasses sample (4.03 mg L^{-1} S^{2-}) was estimated as 2.18% ($n=20$). A linear analytical curve is verified for the 0.50–6.0 mg L^{-1} S^{2-} ($r=0.9982$, $n=5$) range (Fig. 2). The detection and quantification limits (DL and QL), relying on 3σ and 10σ [23] were estimated as 0.035 and 0.15 mg L^{-1}. These later values are higher than those reported for a flow injection system specifically designed for the analysis of yeast culture and relying on the same method [16]. Anyhow, these figures of merit are suitable for fermentation industrial control, where the sulphide concentration is increased when the fermentation route is not properly followed [13]. Sampling rate is 15 h^{-1} meaning 1.90 μmol DMPD per determination. For wine analysis, this DL is not enough [21], thus a lower rotation speed of the peristaltic pump is recommended.

For sulphite determination in wines, the relative standard deviation of results for a typical sample (9.82 mg L^{-1} SO_3^{-}) was estimated as 2.21% ($n=10$) and detection and quantification limits, as 0.2 and 0.7 mg L^{-1} SO_3^{-}, respectively. These limits are similar to those reported by other authors [19,24,25]. Linearity of the analytical curve holds for the 2.5–20.0 mg L^{-1} range SO_3^{-} ($r=0.9974$, $n=5$). With a sampling rate of 57 h^{-1}, 1.68 μg MG is required per determination. This very low reagent consumption matches the tendency towards a clean analytical chemistry.

For ethanol determination, linearity of the analytical curve ($r=0.9972$, $n=5$) is noted between 5 and 25.0% (*v/v*). DL and QL are 0.18 and 0.6% (*v/v*) ethanol, r.s.d. associated to the analytical results for a typical table wine (12.53% *v/v* ethanol) was 2.07 ($n=20$), and sampling rate was 29 h^{-1}.

The mean available times for reaction development for sulphide, sulphite and ethanol are 17, 56 and 36 s, and these differences are mainly due to the peristaltic pump rotation speed involved.

Regarding accuracy, sulphite and ethanol were determined in different wine samples by the proposed and two reference methods [26,27] and results are presented in Table 2. The *t* values for these analytes were estimated as 1.269 and 1.327 and the tabulated value (95% confidence level) is 2.36. Analysis of these data did not reveal any significant differences between methods at the 95% confidence level. Red wine ♯1 and white wine ♯1 are anomalous samples with too low an ethanol content and this can perhaps explain the relative high errors. As the sulphide contents in the assayed molasses were $<$QL, accuracy assessment relied on recovery tests. These tests were performed on samples in the initial and final stages of fermentation. Recovery data ranged from 75 to 88% and from 91 to 100% for these groups, revealing that molasses analysis is less subjected to matrix effects relatively to partially fermented molasses. This result reflects the combined effects of lessening of the carbohydrate concentrations, increase in ethanol content and formation of sulphide during the fermentative process [12].

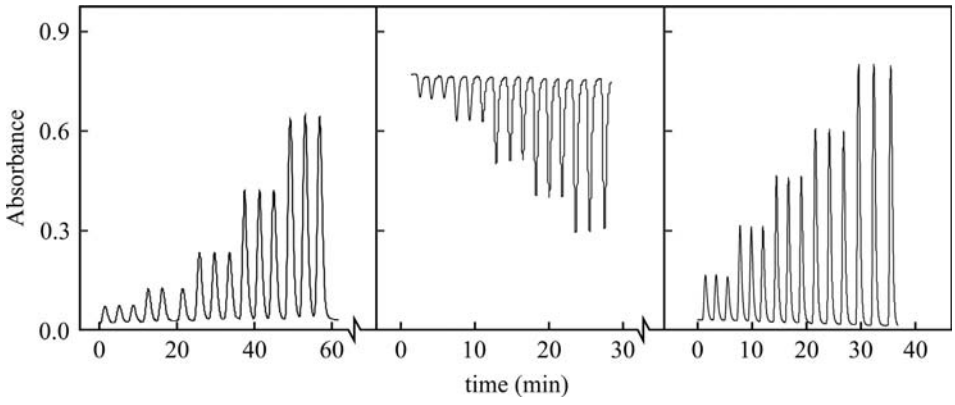

Fig. 2. Recorder tracings. Peaks recorded for sulphide (left), sulphite (centre) and ethanol (right) associated with the analytical curves. From left to right, peaks refer to 0.5, 1.0, 2.0, 4.0, 6.0 mg L^{-1} S^{2-}; 2.5, 5.0, 10.0, 15.0, 20.0 mg L^{-1} SO_3^{-} and 5.0, 10.0, 15.0, 20.0, 25.0% (*v/v*) ethanol.

Table 2
Sulphite and ethanol contents in wines as determined by the proposed and Ref. [26,27] procedures. Data refer to three replicates. The reference method was carried out at the Agricultural College of University of S. Paulo and uncertainties are not available.

Wine	% (*v*/*v*) ethanol		mg L^{-1} sulphite	
	Proposed	Reference	Proposed	Reference
Red wine no. 1	9.0 ± 0.1	10.7	25.5 ± 0.3	25.2
Red wine no. 2	13.3 ± 0.2	13.5	24.8 ± 0.2	24.3
Red wine no. 3	12.4 ± 0.4	12.5	30.8 ± 0.2	31.5
Red wine no. 4	12.3 ± 0.1	13.0	27.6 ± 0.1	28.5
Red wine no. 5	12.5 ± 0.1	12.5	31.4 ± 0.4	30.8
White wine no. 1	8.5 ± 0.0	10.8	6.7 ± 0.1	7.2
White wine no. 2	13.2 ± 0.7	12.0	8.0 ± 0.3	9.0
White wine no. 3	10.0 ± 0.1	10.1	10.7 ± 0.2	11.5

4. Conclusions

This pioneering implementation of gas diffusion in a multi-purpose flow system resulted in good figures of merit. A single gas-diffusion unit is placed into the manifold in order to permit the samples to be run without any prior treatment other then dilution. System versatility is high, as there is the possibility to modify the pump rotation speed. Variations in this parameter enable the sensitivity to be adjusted thus allowing other sample lots with different expected ranges in analyte concentrations to be assayed.

The system is an attractive alternative for routine analysis, since it permits a more efficient laboratory management and minimizes the operational costs. It is simple, versatile, portable, rugged, and suitable for wine and molasses analysis. The experimental conditions differ from the optimal ones for the determination of each species, as a compromise is unavoidable. If a single analyte determination is aimed at, these conditions can be set, and this is another favourable characteristic of the multi-purpose flow systems.

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