Exploiting Pulsed Flows for Heating Improvement: Application to Determination of Total Reducing Sugars in Molasses and Sugar-Cane Juices

Evandro R. Alves¹, Mário A. Feres¹, Elias A.G. Zagatto¹ and José L.F.C. Lima²,*

¹Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, P.O. Box 96, Piracicaba SP 13400-970, Brazil;
²REQUIMTE, Departamento de Química-Física, Faculdade de Farmácia, Universidade do Porto, Rua Aníbal Cunha 164, Porto 4099-030, Portugal

Abstract: Laminar and pulsed flows typical of multi-commuted and multi-pumping flow systems, were evaluated in relation to analytical procedures carried out at high temperatures. As application, the spectrophotometric determination of total reducing sugars (TRS, hydrolyzed sucrose plus reducing sugars) in sugar-cane juice and molasses was selected. The method involves in-line hydrolysis of sucrose and alkaline degradation of the reducing sugars at about 98 °C. Better results were obtained with pulsed flows, due to the efficient radial mass transport inherent to the multi-pumping flow system.

The proposed system presents favorable characteristics of ruggedness, analytical precision (r.s.d. < 0.013 for typical samples), stability (no measurable baseline drift during 4-h working periods), linearity of the analytical curve (r > 0.992, n = 5, 0.05 - 0.50% w/v TRS) and sampling rate (65 h⁻¹). Results are in agreement with ion chromatography.

Keywords: Multi-pumping flow analysis, Pulsed flow, Laminar flow, Heat transference, Total reducing sugars, Sugar-cane juice and molasses, Spectrophotometry.

INTRODUCTION

Multi-pumping flow systems (MPFS) [1,2] utilize discretely-operated solenoid pumps positioned in the manifold, aiming a versatility enhancement. Driving solutions, improving mixing conditions, selecting sample and reagent aliquots, introducing these aliquots into the analytical path, establishing tandem streams, stopping the sample, and providing commuting facilities that are then efficiently accomplished [2].

The solenoid pumps deliver pulsed flows which are characterized by a very short GO period corresponding to the sudden delivery of the aqueous stroke, followed by a relatively long STOP period. During the GO period, turbulent mixing is noted, and the feature leads a fast homogenization of the involved solutions [3].

Pulsed flows have been exploited for attaining improved mixing conditions [4], enhanced radial mass transport thus lower sample broadening [5] and efficient management of solid reagents (fluidized beds) [6]. However, its usefulness in respect to heating of the reaction medium was not yet demonstrated.

The improved mixing conditions and efficient radial mass transport inherent to MPFS certainly plays a relevant role in the heating process, as a better temperature homogenization inside the flowing sample is attained. This might be a positive factor in analytical procedures requiring reaction development at temperatures higher than ambient.

The goal of this work was then to demonstrate the feasibility of MPFS for improving heating conditions in flow analysis. To this end, a critical comparison between laminar and pulsed flows was carried out.

As application, the spectrophotometric determination of TRS (reducing sugars plus hydrolyzed sucrose [7]) in sugar-cane juice and molasses, previously implemented in an ordinary flow-injection system with laminar flow regime [8] was selected. This determination is often required in the sugar production plant, as it provides information for evaluating the quality of the raw material and for improving the industrial control [9].

EXPERIMENTAL

Apparatus

A model Spectro-22RS LaboMed spectrophotometer with a tubular flow-cell (80-μL inner volume, ca 10-mm optical path), solenoid pumps (Bio-Chem Valve, Inc.) with 8.0 or 25.0-μL stroke volume, 161T031 three-way solenoid valves (NResearch), a model IPC-8R peristaltic pump (Ismatec), flow lines and reaction coils made from PTFE tubing (i.d. 0.8 mm), Perspex connectors, a heating device exploiting boiling water under reflux [8], a glass de-bubbler and accessories were also used.

For system control and data acquisition, a desktop microcomputer with a PCL-711B Advantech interface card and running software in QuickBasic 4.5 (Microsoft) was used. The solenoid pumps were operated through a drive based on a ULN 2803A integrated circuit [10].

Samples, Standards, Reagents

All solutions were prepared with chemicals of analytical grade quality and distilled water. The samples were collected
in a sugar production plant near Piracicaba (Brazil). Molasses were sampled directly from the fermentation media [11]; immediately before analysis, about 1.0 g was accurately weighed and diluted to 500 mL with water. The sugarcane juices underwent a 1:200 (v/v) manual water dilution.

The 10.0% (w/v) invert sugar stock standard solution was prepared as recommended [11], and the 0.01-2.00 (w/v) TRS working standards based on invert sugar were daily prepared. The 10.0% (w/v) glucose, fructose and sucrose standard solutions were prepared by water dilutions of the corresponding carbohydrates.

The reagent (R - Fig. (1)) was a 0.1 mol L\(^{-1}\) sodium potassium tartrate tetrahydrate plus 0.1 mol L\(^{-1}\) sodium hydroxide solution. For TRS determination with the system in Fig. (2), the reagent was prepared with the same composition but at different concentrations (0.05 mol L\(^{-1}\) NaKC\(_4\)H\(_4\)O\(_6\) + 0.5 mol L\(^{-1}\) NaOH) in order to provide similar concentrations inside the B\(_2\) reactor relatively to the flow systems in Fig. (1).

![Flow diagram](image)

**Fig. (1).** Flow diagrams of the multi-pumping (upper) and multi-commuted (lower) flow systems. S = sample; C = water carrier stream; R = reagent; B = 400-cm reactor; Pi = solenoid pumps; Vi = three-way solenoid valves; Db = de-bubbler; D = detector (420 nm). Boxed components are immersed in boiling water under reflux. Alternative Vi positions are specified by traced lines.

**Flow Diagrams**

For a comparative evaluation of influence of laminar and pulsed flows in the heat transference, the multi-pumping (MPFS) and the multi-commuted (MCFS) flow systems in Fig. (1) were used. They were dimensioned according to the earlier proposed flow-injection system [8] but without including the step of sucrose hydrolysis. Performance of both systems was experimentally verified by processing fructose and glucose standard solutions.

In the MPFS (Fig. (1), upper), the solenoid pumps were accountable for selection of sample and reagent volumes, as well as for their individual insertion into the analytical pathway, providing also facilities for timing setting, sequence of insertion and sample transportation towards detection [4]. In the MCFS (Fig. (1), lower), the involved solutions were pushed forwards by means of the peristaltic pump and their additions to the analytical path were controlled through the three-way solenoid valves. The multi-comutated flow systems (MCFS) was operated analogously to the multi-pumping flow systems (MPFS), but relied on laminar flow.

Proper operation of the pumps (MPFS) or valves (MCFS) allowed variations in the number of operation cycles (thus inserted volumes), and in the way how the sample and reagent plugs were put into physical contact.

Regarding MPFS, the analytical procedure started by operating only P\(_2\) pump (100 pulses, \(ca\) 2.5 mL) for propelling the reagent stream, thus washing the analytical path. For sample insertion as a single plug, P\(_2\) pump was switched OFF and P\(_1\) pump was operated during a pre-set time interval, proportional to the number of delivered pulses, which defined the sample inserted volume [12]. Thereafter, this pump was switched OFF and P\(_2\) pump was operated again (100 pulses) in order to push the sample introduced volume towards detection and proceed with system washing. For sample insertion as multiple plugs (binary sampling [13]), P\(_1\) and P\(_2\) pumps were switched in a fast and alternate manner, so that sample and reagent plugs were inserted in tandem. Multiple interfaces were established between them, therefore efficient mixing was attained. Alternatively, P\(_1\) and P\(_2\) pumps could be simultaneously switched ON and OFF, in a strategy analogous to the merging zones configuration [14] characterized by better mixing conditions.

Regarding MCFS, sample insertion was analogously performed, yet the pump operation was replaced by the valve operation providing that the peristaltic pump delivered a constant flow.

In both systems, the inserted sample and reagent plugs resulted in a sample zone that was directed towards the
heated reactor (B – Fig. (1)) immersed in boiling water under reflux. Carbohydrate degradation occurred then under high temperature and alkalinity, resulting in the formation of brownish chemical species [15,16]. Temperature of the boiling water was experimentally measured as 98 ± 1 °C. Thereafter, the sample passed successively through the de-bubbler for removal of the released air bubbles and the spectrophotometric flow cell. The transient increase in absorbance (420 nm) was recorded as a peak proportional to the analyte content in the sample. The sample was thereafter discarded.

The multi-pumping flow system in Fig. (2) included in-line sucrose hydrolysis, and was optimized for the determination of TRS. Plugs of the sample and the carrier stream solutions were inserted in tandem and the originated sample zone passed through the B1 heated reactor where hydrolysis took place under high temperature and acidic conditions. Thereafter, the sample zone merged with the R reagent. Further steps were similar to those above described.

RESULTS AND DISCUSSION

Formation and Degradation of the Monitored Species

Sucrose alkaline degradation was applied for quantitative analysis only recently [8], and details of the involved chemistry are not yet known. In order to gather information about kinetic of formation and degradation of the unstable brownish chemical species to be monitored, absorption spectra were obtained under dynamic conditions. To this end, either glucose or fructose standard solutions (0.05 % w/v) were used as the carrier stream of MPFS in Fig. (1), upper, thus establishing the “sample infinite volume” situation [17]. The spectra of the brownish solutions resulting from sugar degradation present two absorption maxima at ca 340 and 420 nm both for glucose (Fig. (3)) and for fructose. As fructose and glucose are the main hydrolysis products, and considering that the spectral region around 420 nm is very similar for both carbohydrates, wavelength was selected as 420 nm.

![Absorbance vs Wavelength](image)

**Fig. (3).** Absorption spectra. Figure refers to the flow system in Fig. (1), lower, and to a 0.05 % w/v glucose solution. Curves a, b and c refer to 1.6, 3.1 and 4.7 mL min⁻¹ total flow rates.

Spectra were affected by variations in total flow rate, as formation of the colored products is relatively slow and their lifetimes are short. This latter aspect was confirmed by injecting the glucose or fructose solution into the multi-pumping flow system in Fig. (1) and stopping the pump when the maximum signal was recorded: baseline was restored in about 70 s, corroborating earlier findings [8]. As two antagonist processes (formation / degradation) are involved, there is a total flow rate yielding maximum sensitivity, for which the formation rate of the products is matches their degradation rate. This is evident in Fig. (3) where neither the lowest nor the highest flow rate corresponds to the maximum analytical signal. Total flow rate was then set as 3.1 mL min⁻¹.

Influence of Temperature

For a comparative evaluation of the heating efficiency of the MPFS and MCFS systems, temperature of the reaction medium was studied by handling a 0.25 % (w/v) glucose standard solution. The B reactor (Fig. (1)) was immersed into a thermostatic water bath where the temperature ranged between 70 and 100 °C. After each temperature variation, a 5-min interval was provided for reaching thermal equilibrium.

Temperature proved to be an important parameter, as it affects rate of sugar alkaline degradation in a pronounced manner [18,19]. For < 75 °C water bath temperature, no analytical signals were noted both for MPFS or MCFS systems, as the sample mean residence time inside the heated reactor was not enough for measurable sugar degradation. For 80 °C, a low analytical signal (about 0.02 absorbance) was noted for the MPFS, but no signal was recorded for MCFS. Raising the water bath temperature up to 85 °C led to appearance of the analytical signal related to MCFS and to an increase in the signal related to MPFS to 0.05 absorbance. At this temperature however, the MCFS signal was only about 20 % of that related to MPFS. For 98 °C a pronounced increase in analytical signals was noted, but the MCFS signal was always lower as compared to MPFS. Peak heights of 0.355 and 0.285 absorbance were obtained for the MPFS and MCFS, respectively.

This is a remarkable result, as it demonstrates the superior heat transference in the MPFS that relies on pulsed flows. The effect is a consequence of the movement of the fluid elements during the GO period, which leads to a fast and efficient radial mass transport that can be regarded as a positive factor towards heat transference, thus reaction development. On the other hand, under the laminar flow regime inherent to the multi-commuted flow system, the stream lines close to the inner tubing walls are heated but transference of the heated fluid elements towards the central lines is less efficient. In fact, the central fluid lines travel with a linear speed about twice the mean linear speed [20], thus acting as a refrigerating agent.

In view of these results, and considering the easy implementation of boiling water under reflux in the industrial laboratories, the water-bath temperature was selected as 98 °C.

System Dimensioning

The ordinary flow-injection system proposed for ART determination [8] relying on laminar flows could be simpli-
fied by taking advantage of pulsed flows. Experiments were then carried out in order to optimize the main involved parameters (reagent concentration, total flow rate, sample inserted volume, reactor lengths). To this end, 0.00 – 0.20 % (w/v) glucose or fructose standard solutions were inserted in triplicate into the MPFS in Fig. (1).

Influence of the required of alkalinity was investigated by using 0.05 – 2.0 mol L\(^{-1}\) NaOH concentrations in the R reagent. For lower concentrations, a pronounced difference between analytical signals recorded for glucose or fructose solutions was noted, and this difference was lessened by increasing the alkalinity of the reaction medium. The differences in recorded peak heights are due mainly to the different rates of degradations involved, and did not manifest themselves for > 0.10 mol L\(^{-1}\) NaOH. This alkalinity is lower relatively to that inherent to the earlier proposed flow-injection system [8], probably due to the better heat transferance involved.

Furthermore, for a constant alkalinity (0.1 mol L\(^{-1}\) NaOH), the tartrate concentration was varied between 0.05 and 1.0 mol L\(^{-1}\). No pronounced difference in peak heights recorded for glucose or fructose and no particle settlement on the tubing inner walls were noted. The lower tartrate concentration value was then selected.

Influence of total flow rate was investigated by varying the pump frequency [2]. The 25-µL pump was used in order to provide 1.0 - 7.2 mL min\(^{-1}\) flow rates (0.7 – 4.8 Hz pulse frequency). The sample inserted volume, defined as number of delivered pulses during the sampling step [2], was investigated within 250 and 2000 µL (10 – 80 sample pulses).

Influence of total flow rate cannot be considered independently of the sample volume, as for larger volumes, the sample reaches the heated reactor during the sampling step. For a sample volume of 750 µL (30 pulses), slight variations in recorded peak heights were noted for both fructose and glucose solutions, and this result demonstrates again the faster sugar degradation under improved heat transference conditions.

The analytical signals obtained with the multi-pumping flow systems in Fig. (1) and in Fig. (2), both exploiting binary sampling (five sample / reagent plugs) underwent a 22 and 90 % increases by doubling total flow rate. This result supports the evidence of better and faster sample homogenization, thus sample / reagent interaction provided by the pulsed flows. The improvement in sensitivity gathered by inserting more than six sample / reagent plugs was too low to justify the related drop in sampling rate.

Regarding the MPFS in Fig. (2), the reagent concentration and alkalinity could be reduced to about 50 % or total flow rate could be increased in about 20 % without lessening the analytical signals. Moreover, the fructose and glucose signals remained similar to each other. This feature is a consequence of the over-dimensioned temperature, and can be regarded as positive factor in relation to robustness. It was then decided to reduce the B\(_1\) and B\(_2\) reactor lengths from 250 and 400 cm to 100 and 250 cm, respectively, and to maintain the other evaluated parameters. A reduction in this length was supported by the fact that the sample zone was previously heated (sucrose hydrolysis step) when reaching the B\(_2\) reactor. The shorter path was beneficial in terms of sampling rate, reagent consumption and hydrodynamic back pressure.

### TRS Determination

The proposed MPFS is very stable, yielding precise results. For typical sugar-cane juices (8 – 20 % w/v TRS) or molasses (40 – 60 % w/w TRS), relative standard deviations of peak heights were estimated as 0.8 - 1.5 % after ten successive sample handling. After six hours of continuous operation, baseline drift has not been observed, and baseline noise is < 0.04 absorbance.

The system handles 65 samples per hour and requires only 10.0 mg of sodium hydroxide plus 76 mg of sodium potassium tartrate tetrahydrate per determination. Lambert-Beer’s law is followed between 0.0 and 2.0% (w/v) TRS, and detection limit was estimated as 0.018 % w/v TRS based on the 3σ approach. A typical equation describing the analytical curve is:

### Table 1. Total Reducing Sugar Concentrations in Sugar-Cane Juices and Molasses Obtained by the Proposed Procedure and by Ion Chromatography [21]. Data Expressed in % (w/w) TRS or % (w/v) TRS for Sugar-Cane Juices or Molasses, Respectively. Uncertainties are Standard Deviations Estimated After Three Replications, and Expressed in the Same Unity as the Mean Concentration Values

<table>
<thead>
<tr>
<th>Sample</th>
<th>Proposed Procedure</th>
<th>Ion Chromatography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juice 1</td>
<td>21.6 ± 0.2</td>
<td>20.5 ± 0.3</td>
</tr>
<tr>
<td>Juice 2</td>
<td>20.4 ± 0.3</td>
<td>20.9 ± 0.2</td>
</tr>
<tr>
<td>Juice 3</td>
<td>21.1 ± 0.1</td>
<td>22.2 ± 0.2</td>
</tr>
<tr>
<td>Juice 4</td>
<td>21.5 ± 0.1</td>
<td>20.6 ± 0.1</td>
</tr>
<tr>
<td>Molasses 1</td>
<td>56.5 ± 0.2</td>
<td>57.2 ± 0.1</td>
</tr>
<tr>
<td>Molasses 2</td>
<td>54.8 ± 0.4</td>
<td>56.7 ± 0.5</td>
</tr>
<tr>
<td>Molasses 3</td>
<td>55.2 ± 0.2</td>
<td>56.8 ± 0.3</td>
</tr>
<tr>
<td>Molasses 4</td>
<td>56.8 ± 0.1</td>
<td>55.7 ± 0.1</td>
</tr>
</tbody>
</table>
Exploiting Pulsed Flows for Heating Improvement

h = 0.2661 TRS + 0.0045 (r = 0.9924; n = 10)
where h = peak height in absorbance; TRS = analyte concentration in % (w/v).

Accuracy of the proposed method was assessed against ion chromatography [21] (Table 1). After applying the t-test to the paired data, no significant differences between methods were found at the 95% confidence level.

CONCLUSIONS

Heat transfer towards and inside the flowing sample is more efficiently accomplished by taking advantage of pulsed flows rather than laminar flows, these later being inherent to ordinary unsegmented flow systems. This aspect reflects the superior radial mass transport and allows a simplification in system design.

The multi-pumping flow system becomes then attractive for implementing automated analytical procedures that require heating. Proper operation of the solenoid pumps permits an efficient management of the solutions yet improving mixing conditions and promoting a better heat distribution inside the flowing sample. These favorable features are worthwhile in relation to in-line sucrose hydrolysis and sugar alkaline degradation. The proposed multi-pumping flow system is recommended for the quality control in the sugar production plant.

ACKNOWLEDGEMENTS

Partial support from FAPESP and CNPq/GRICES is greatly appreciated. S. Joao sugar production plant is thanked for providing the samples.

REFERENCES