



Short communication

A flow-batch analyzer using a low cost aquarium pump for classification of citrus juice with respect to brand

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ABSTRACT

This paper proposes a novel flow-batch analyzer (FBA), which employs a compact, low-cost aquarium air pump as an alternative to a peristaltic pump. The feasibility of using this simple propulsion device is demonstrated in a case study involving the classification of citrus juice samples with respect to brand. For this purpose, UV–vis spectra and SIMCA (soft independent modelling of class analogies), PLS-DA (Partial Least Squares for Discriminant Analysis) and SPA-LDA (Linear Discriminant Analysis with variables selected by the Successive Projections Algorithm) are employed. Good classification results were obtained, thus indicating that the proposed FBA system is a viable alternative to the use of more costly peristaltic pumps. In addition, the smaller size and weight of the aquarium pump are useful features for the construction of portable FBAs to be deployed in field applications.

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1. Introduction

Automatic methods are widely used in several areas, such as clinical, environmental, pharmaceutical and food analysis. Such methods can be implemented in an efficient manner through the use of flow injection analysis systems, which facilitate sample pre-treatment, management of reagents, monitoring of the analytical signal, reduction of human effort, more precise analysis and increase in the sample rate [1,2]. Nevertheless, this type of system has some disadvantages, such as the need for frequent recalibration and manual adjustments, low sensitivity due to sample dispersion and inefficient homogenization, as well as the generation of a large volume of residues due to the use of carrier fluid [3]. These inconveniences motivated the development of flow-batch analyzers (FBA) [4].

FBA is an automated system that uses an instantaneous stop chamber and integrates batch and flow methods by means of programmed multi-commutation [5]. The main component is the mixing chamber where the whole analytical process, including fluids addition, sample pretreatment, homogenization, precipitation, extraction, preparation of standard solutions, and detection,

takes place under total control of the software [6]. The sample is processed seamlessly with less manipulation, consumption of reagents and samples, waste generation and chance for human error [3].

Most FBA systems described so far employ peristaltic pumps for fluid propulsion [3,7–9]. Such pumps have multiple channels and can be used for either propulsion or aspiration, without direct contact of the fluid with mechanical components. However, these propulsion devices are relatively costly (> US\$ 4000). Moreover, their size and weight hinder the deployment of portable FBA systems for field use. To circumvent these inconveniences, other propulsion alternatives for FBA have been exploited, such as piston propulsion [10,11] and solenoid micro-pumps [12,13].

Within this context, the present work proposes a new flow-batch analyzer, which employs a compact, low-cost propulsion system driven by an aquarium air pump (~US\$ 15). The proposed analyzer is applied to the screening analysis of citrus juice samples with respect to brand by using UV–vis spectrometry and chemometrics techniques. More specifically, SIMCA (Soft Independent Modelling of Class Analogies) [14], PLS-DA (Partial Least Squares for Discriminant Analysis) [15] and SPA-LDA (Linear Discriminant Analysis with variables selected by the Successive Projections Algorithm) [16,17] are employed to discriminate the UV–vis spectra of the juice samples.

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2. Experimental

2.1. Samples

This work involved 150 samples of six commercial brands of processed citrus juice (denoted by I1–I6) and 20 samples of fresh citrus juice (denoted by N), which were acquired in the city of João Pessoa (PB, Brazil). The samples for each brand were taken from different lots.

Due to the strong absorption of the citrus juices in the UV–vis range, all samples were diluted by addition of water in the proportion 1:70 (v/v). Water was distilled and deionized by using a Milli-Q Plus system (Millipore).

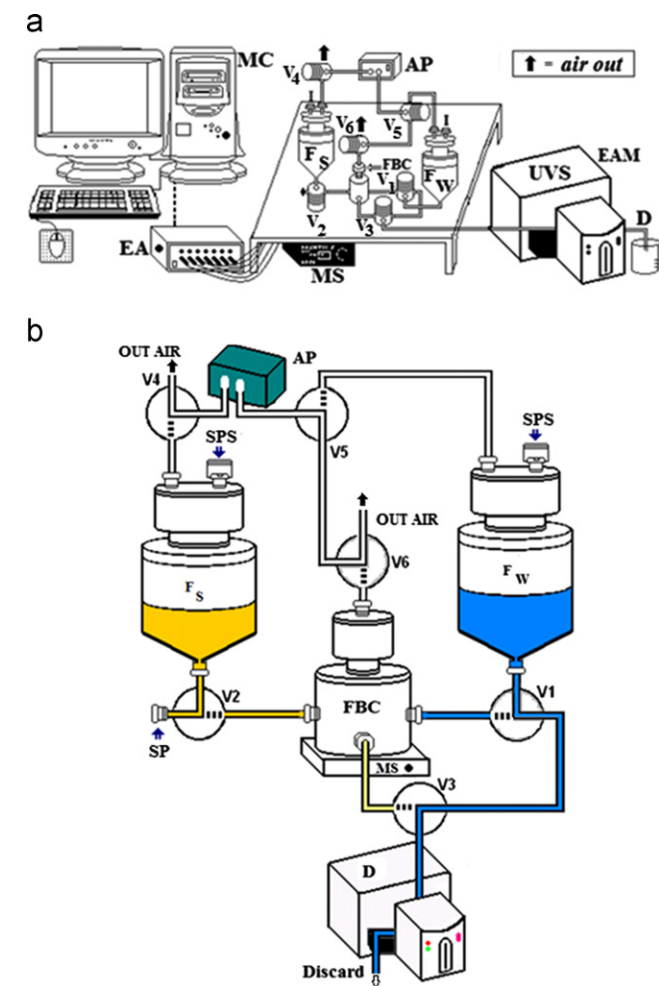


Fig. 1. (a) Proposed flow-batch analyzer: EA=electronic actuator, FBC=flow-batch chamber, MS=magnetic stirrer, AP=aquarium pump, V1–V6=solenoid valves, F_S and F_W =sample and water flasks, SP=screw plug, SPS=screw plug with septum, MC=microcomputer, D=UV–vis spectrophotometer. (b) Simplified diagram of the flow-batch analyzer with indication of the two possible valve configurations (ON and OFF).

2.2. Apparatus

The proposed FBA system is depicted in Fig. 1a. The system comprises six three-way solenoid valves (Cole-Parmer), an aquarium pump (Boyu S-2000A Duplo), hermetically sealed flasks for the sample and diluent, a PTFE (Teflon) flow-batch chamber (FBC) with a magnetic bar in its interior, a magnetic stirrer (Hanna Instruments, model HI 190M) and a quartz flow cell (Hellma) with optical path of 1.0 cm. The UV–vis spectra are acquired by using a Hewlett Packard 8453 spectrophotometer with photodiode array in the range 190–1100 nm with a resolution of 1 nm.

A microcomputer is employed for data acquisition and control of the flow-batch analyzer with software developed in the Lab-View 5.1 platform. An electronic actuator is used to switch the solenoid valves and to activate/deactivate the aquarium pump. Fig. 1b presents a simplified diagram of the analyzer. In this diagram, all valves are shown in the OFF position. When a valve is switched to the ON position, its output is moved to the dotted line.

2.3. Procedure

Initially, the sample flask F_S is washed with the sample under analysis. For this purpose, 10 ml of the sample are introduced through the septon of the screw plug at the top of the flask by using a syringe. The sample is then drained by removing the screw plug at the bottom. This procedure is always repeated three times for each new sample to be analyzed. After this cleaning step, another 10 ml of the sample are introduced for analysis. The water flask F_W is also filled for use in the subsequent cleaning, dilution and measurement steps, as summarized in Table 1. Since the volumes added to the flow-batch chamber FBC are proportional to the valve opening times, the analytical procedure is described in terms of time rather than volume.

In Step 1, valves V1, V2, V4 and V5 are simultaneously switched ON for 5.0 s in order to fill the channels between valves V1, V2 and the FBC. Step 2 consists of the cleaning and draining of the FBC. For this purpose, valves V1 and V5 are switched on during 5.0 s to introduce water into the FBC and then valves V3 and V6 are switched on for 10.0 s to discard the FBC content. This cleaning and draining procedure is carried out in triplicate. Steps 1 and 2 are repeated in the analysis of every new sample.

In Step 3, valve V5 is switched on for 10.0 s in order to fill the UV–vis spectrophotometric flow cell with water for the blank signal measurement.

In Step 4, valves V1, V4 and V5 are switched on for 6.9 s and valve V2 is simultaneously switched on for 0.1 s in order to dilute the sample in the proportion 1:70 v/v. In Step 5, valves V3 and V6 are switched on for 10.0 s in order to fill the flow cell for the sample signal measurement.

Prior to the analysis of a new sample, the screw plugs at the top and bottom of the sample flask F_S are removed and the flask is washed three times with water. The channel between F_S and FBC is also drained and cleaned by reinserting the screw plugs, filling

Table 1

Sequence of steps in the operation of the flow-batch analyzer, with indication of the switch-on times (in seconds) of valves V1–V6.

Step	Description	V1	V2	V3	V4	V5	V6
1	Channelfilling	5.0	5.0	–	5.0	5.0	–
2	Cleaning and draining of the flow-batch chamber	5.0	–	–	–	5.0	–
3	Blank signal measurement	–	–	10.0	–	–	10.0
4	Sample dilution	6.9	0.1	–	6.9	6.9	–
5	Sample signal measurement	–	–	10.0	–	–	10.0

FS with water and activating the aquarium pump for 10.0 s with valves V2, V3, V4, V6 switched on.

2.4. Software and data analysis

The 170 UV–vis spectra were smoothed by using a Savitzky–Golay filter with 17-point window and 2nd-order polynomial. The Kennard–Stone algorithm [18] was employed to divide the resulting spectra into training, validation and test sets, as indicated in Table 2. Principal Component Analysis (PCA) was employed in a preliminary assessment of the separation among the seven classes involved in the study (I1–I6, N). SIMCA, PLS-DA and SPA-LDA models were then built in order to discriminate the samples according to their class. The validation set was employed to optimize the number of latent variables in SIMCA and PLS-DA, as well as to guide the selection of variables in SPA-LDA. The test samples were used as an external set for the final performance assessment of the classifiers.

Savitzky–Golay, PCA, SIMCA and PLS-DA calculations were carried out by using The Unscrambler® 9.7 (CAMO AS). KS and SPA-LDA calculations were carried out in Matlab® 2010b (Mathworks).

3. Results and discussion

3.1. Optimization of the operation parameters of the flow-batch analyzer

The operation parameters of the FBA were optimized in order to achieve a suitable compromise among low sample consumption, high analytical frequency and good sensitivity and reproducibility of the spectral measurements. The errors associated to the solenoid valve activation depend mainly on its response time (approximately 20 ms according to the manufacturer's data sheet). The uncertainty on the fluid supply provided by each valve was evaluated by using a water flow rate of 3.0 ml min⁻¹. The water was collected in a volumetric flask at time intervals of 0.5–20 s and the mass was measured in an analytical balance. The relative standard deviation for 10 repeated measurements at each time interval was always smaller than 1.0%.

3.2. UV–vis spectra

Fig. 2a presents the smoothed spectra of the 170 diluted juice samples. As can be seen, the absorption in the visible range is substantially smaller as compared to the UV region. Therefore, only the UV region was adopted in the present study. Moreover, by excluding the saturation region at lower wavelengths, the working range was set to 232–358 nm, as shown in Fig. 2b.

Table 2

Division of the samples into training, validation and test sets.

Class	Set		
	Training	Validation	Test
N	10	5	5
I1	13	5	5
I2	16	5	5
I3	14	5	5
I4	16	5	5
I5	16	5	5
I6	15	5	5
Total	100	35	35

3.3. Principal component analysis

Fig. 3 presents the score plot resulting from a principal component analysis of the 170 diluted juice spectra in the working range. As can be seen, the spectra convey information that can

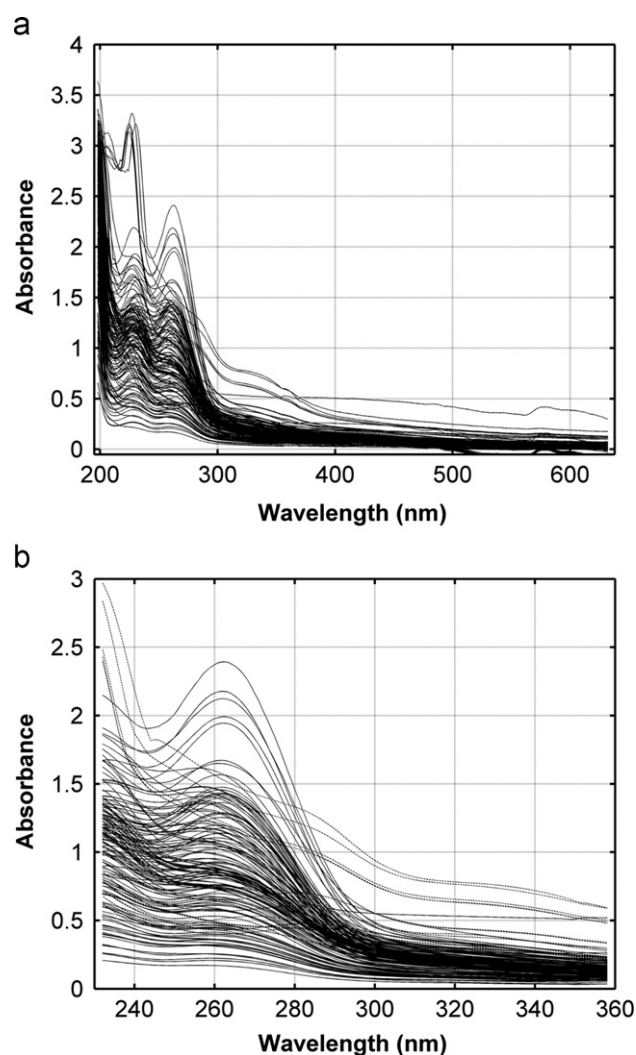


Fig. 2. (a) Smoothed UV–vis spectra of the 170 diluted juice samples. The horizontal axis was upper-limited to 630 nm for better visualization. (b) Spectra in the working range adopted in the study (232–358 nm).

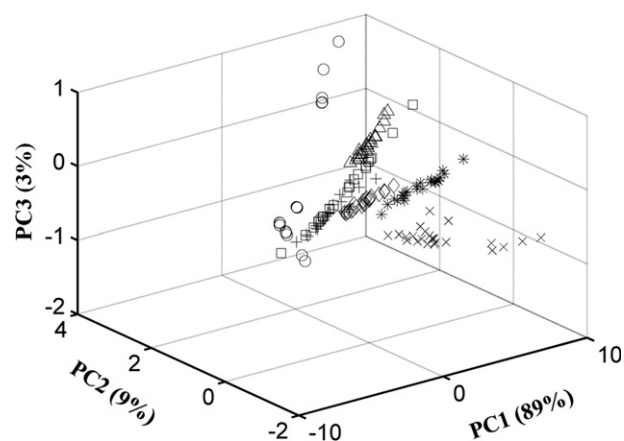


Fig. 3. PC score plot of the 170 diluted juice samples: N (o), I1 (◇), I2 (*), I3 (+), I4 (X), I5 (Δ), I6 (□).

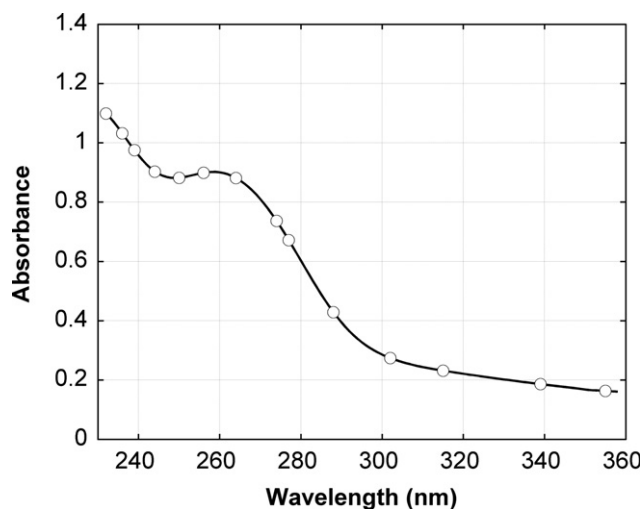


Fig. 4. Average spectrum of the diluted juice samples with indication of the wavelengths selected by SPA-LDA.

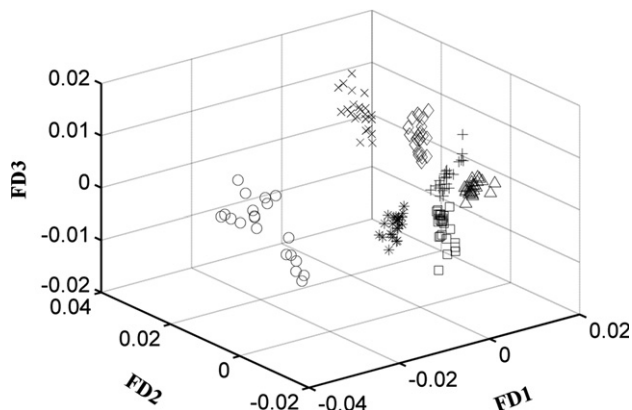


Fig. 5. Fisher discriminant scores of the 170 diluted juice samples obtained by using the wavelengths selected by SPA-LDA: N (o), I1 (◇), I2 (*), I3 (+), I4 (X), I5 (Δ), I6 (□).

be used to discriminate the classes. However, some overlapping is observed, which motivates the use of supervised classification techniques, as reported below.

3.4. Classification results

The SIMCA, PLS-DA and SPA-LDA models were built by using the default settings of the chemometrics computational routines. The performance of the resulting classifiers was then assessed over the test set of 35 samples.

By using SIMCA, all samples in the test set were correctly included in their own class and only five samples were also included in a different class. Good results were also obtained by PLS-DA, which correctly classified all samples. These findings confirm the discriminatory power of the spectral data with respect to the classification problem under consideration.

The final study involving SPA-LDA was aimed at investigating whether good classification results could be achieved by using a subset of spectral variables, instead of the entire working range. As a result, 14 wavelengths were selected, as indicated in Fig. 4. The LDA model thus obtained correctly classified all the samples in the test set, which indicates that the selected wavelengths do convey enough information to discriminate the classes. For illustration, Fig. 5 shows the plot of Fisher discriminant scores obtained by using the wavelengths selected by SPA-LDA. As can

be seen, the separation between the classes is more apparent as compared to the PC score plot presented in Fig. 3.

4. Conclusions

This paper proposed a novel flow-batch analyzer (FBA), which employs a compact, low-cost aquarium air pump as an alternative to the peristaltic pump usually employed in such systems. The feasibility of using this simple propulsion device was demonstrated in a case study involving the classification of citrus juice samples with respect to brand. For this purpose, UV-vis spectra and SIMCA, PLS-DA and SPA-LDA models were employed. The good classification results thus obtained indicate that the proposed FBA system is a viable alternative to the use of more costly peristaltic pumps. In addition, the smaller size and weight of the aquarium pump are useful features for the construction of portable FBAs to be deployed in field applications. Such portable systems could be built by using dedicated photometers to monitor the wavelengths selected by SPA-LDA. Alternatively, small fiber optics spectrometers such as Ocean Optics instruments [19] could be employed.

It should be noted that alternative designs could be conceived to reduce the number of solenoid valves involved in the proposed system. For example, additional aquarium pumps with on/off computer control could be used to replace some of the solenoid valves. Moreover, the level of automation of the proposed system could still be improved to avoid the need for manual changing of the sample. For this purpose, hybrid designs comprising more aquarium pumps together with solenoid valves and/or micro-pumps could be exploited without a significant increase in the overall cost.

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