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A multicommuted flow system for fast screening/sequential spectrophotometric determination of dichromate, salicylic acid, hydrogen peroxide and starch in milk samples

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ABSTRACT

In this work a multicommuted flow system for the sequential screening/determination of dichromate, salicylic acid, hydrogen peroxide and starch in milk samples was developed. The concept of multicommutation in flow injection analysis was chosen, resulting in an environmentally friendly system with minimal consumption of reagents and waste generation. The proposed approach is based on a simple binary DETECT or NO-DETECT response, thereby making it possible to determine analytes quickly, with high performance and easy operation. For dichromate determination, the proposed method was based on the reaction between Cr(VI) and 1,5-diphenylcarbazide, enabling a linear working range response, between 1.0 and 10.4 mg L^{-1}, ($R = 0.999$). In order to determine salicylic acid, the proposed method was based on a complexation reaction of Fe(III) and salicylic acid, with the linear working range response from 103.6 to 414.3 mg L^{-1} (7.5×10^{-4}–3.0×10^{-3} mol L^{-1}) ($R = 0.999$). The hydrogen peroxide determination was based on the oxidation reaction of hydrogen peroxide with vanadium oxide (V) in an acid environment, with a linear working range of 10.0–200.0 mg L^{-1} ($R = 0.996$). Starch determination was based on the complex reaction of starch and triiodide, with a linear working range of 12.5–150.0 mg L^{-1} ($R = 0.999$). The mean sampling rate for the four species was 83 determinations per hour. Performance curves were used to verify the quantity of false positives and false negatives. Addition and recovery tests were used for validation of the proposed procedures, resulting in variation between 90.1 and 108.7% for three different samples.

1. Introduction

Milk is a food consumed worldwide due to its widely known nutritional value and health benefits (Fox & McSweeney, 2009, chap. 4). Apart from being a source of nutrients essential for growth, development and maintenance of health, milk is a major source of protein in the diet of young animals and humans of all ages (Hurley, Coleman, Ireland, & Williams, 2006).

Recently, the occurrence of adulterations and frauds in liquid milk has increased. These adulterations have been practiced in a number of different ways, by the addition of an adultering agent to increase economic yield (Das, Sivaramakrishna, Biswas, & Goswami, 2011).

The addition of potassium dichromate, salicylic acid, hydrogen peroxide and starch in milk is not allowed in Brazil, in any concentration. Because of this, milk containing any of these substances is considered adulterated (Brasil, 2006). The adulteration of liquid milk by adding starch occurs with the intention of masking the extra addition of water. Adulteration using preservative substances such as dichromate, salicylic acid and hydrogen peroxide to milk have the objective of inhibiting or delaying the appearance of microorganisms in the liquid milk (Barbano, Wojciechowski, & Lynch, 2010; Borin, Ferrão, Mello, Maretto, & Poppi, 2006; Cerdán, Peris-Tortajada, & Maquieira, 1992; Souza et al., 2011).

Adulterations have increased because the consumption of milk has become more extensive and, in addition, the detection methods

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are difficult to implement in local production and packaging. The official procedures are time consuming, expensive and can provide false positive results, limiting their application in routine control (Kartheek, Smith, Muthu, & Manavalan, 2011).

Different analytical procedures have been proposed to detect dichromate in milk, such as titration with silver chloride (Brasil, 2006), and use of mid-infrared spectroscopy and molecular absorption spectrophotometry (Barbano et al., 2010; Cerdán et al., 1992).

The official methodology for salicylic acid determination in milk used in Brazil is based on the reaction of Fe^{3+} and salicylic acid in an acid medium (Brasil, 2006). Another analytical method based on amperometric biosensory methods has been reported for salicylic acid determination in milk (Zavar, Heydari, & Rounaghi, 2013).

Different methodologies for hydrogen peroxide determination in milk have been developed using colorimetric (Brasil, 2006) and electrochemical methods (Abbas, Luo, Zhu, Zou, & Tang, 2010; Campuzano, Pedrero, & Pingarrón, 2005; Shamsipur, Asgari, Maragheh, & Moosavi-Movahedi, 2012; Silva, Montes, Richter, & Munoz, 2012). One of the main methods for starch determination in milk involves the use of iodine titration with potentiometric or amperometric detection (Banks, Greenwood, & Muir, 1971). Another methodology was developed for starch determination in milk, using a spectroscopy technique such as Near-infrared (Borin et al., 2006). A complexation reaction of iodine with starch has been the official methodology used to determine starch in milk (Brasil, 2006).

It is important to develop analytical methodologies that are fast, less expensive and accurate for screening or quantification of adulterants in milk. The search for new analytical methodologies that reduce or replace materials harmful to human health and the environment, is becoming a key parameter in green chemistry research (Anastas, 1999; Vieira, Crispino, Perdigão, & Reis, 2013). Flow systems are excellent tools for dealing with solutions in wet chemical analysis (Rocha et al., 2002). The use micro-pumping in flow system analysis is a successful strategy, for the carrying, inserting and mixing of solutions in a flow analysis system (Lapa, Lima, Reis, Santos, & Zagatto, 2002). Another aspect of solenoid micro-pumping is that it allows implementation of a versatile and inexpensive flow system (Morales-Rubio, de la Guardia, & Reis, 2009). The multicommutation flow systems network, when designed by associating a solenoid valve, a solenoid pinch valve, and solenoid micro-pumps together with the concept of binary sampling is straightforward in operation, very versatile, robust, enables the development of green analytical methodologies and enables the use of automation (Lavorante, Pires, & Reis, 2006; Melchert, Reis, & Rocha, 2012).

Multicomponent flow analysis systems are efficient for the determination of multiple analytes. Most flow analysis systems deal with two analytes (c.a. 72%), three analytes (18% c.a.), four or five (c.a. 4%) analytes or six analytes (c.a. 2%) (Trojanwicz, 2008, chap. 1). In the literature there are some works that approach multidetermination employing different types of detectors. (Calvo, Durán, & Del Valle, 2007; Lavorante, Morales-Rubio, Guardia, & Reis, 2007; Rocha, Fatibello Filho, & Reis, 2003; Rocha, Martelli, & Reis, 2001). The development of these systems depends on a number of experimental circumstances, such as the number of components, the presence of interferences and the complexity of the sample matrix (Rocha, Reis, & Rohwedder, 2001; Silva, Nogueira, Souza, & Zagatto, 1998).

The Association of Official Analytical Chemists (AOAC) defines screening or qualitative methodologies of analysis as when the response is either the presence or the absence of the analyte detected in a specified test portion; a binary response may also be available to the analyst (Feldsine, Abeyta, & Andrews, 2002). Screening methodologies when associated with flow systems offer a fast answer when compared with quantitative methodologies (Amador-Hernández, Fernández-Romero, & Luque de Castro, 2001; Lima et al., 2004; Sainz-Gonzalo, Fernandez-Sanchez, & Fernandez-Gutierrez, 2011; Valcárcel, Cárdenas, & Gallego, 2002; Valcárcel, Gallego, Cárdenas, & Gambart, 1998).

This paper proposes a flow analysis screening system which is fast, simple and reliable for the sequential qualitative detection and determination of dichromate, salicylic acid, hydrogen peroxide and starch in milk. The methodology presented is based on the use of a multicommutation flow system based on binary sampling, with the use of pinch solenoid valves and solenoid micro-pumps. The methodology has been validated and applied to samples of liquid milk.

2. Experimental

2.1. Reagents, solutions and samples

All solutions were prepared with analytical grade chemicals. Purified water presenting conductivity less than 0.10 μS cm^{-1} was used throughout.

All stock solutions were stored away from light at 4 °C and remained for at least 15 days. A solution of 1381 mg L^{-1} (0.010 mol L^{-1}) salicylic acid (Vetec) was prepared by dissolving 0.1381 g in 10 mL of ethanol and subsequently, diluted to volume of 100 mL with deionized water. A solution of 1471 mg L^{-1} (0.010 mol L^{-1}) potassium dichromate (Vetec) was prepared by dissolving 0.1471 g in 100 mL deionized water. A solution of 3600 mg L^{-1} (0.106 mol L^{-1}) hydrogen peroxide (Vetec) was prepared by dissolving 658 μL in 100 mL deionized water. A solution of starch (Vetec) 400 mg L^{-1} was prepared by dissolving appropriate amounts of reagent to make up a volume of 100 mL.

Working reference solutions were prepared daily, by diluting stocks solutions with interval concentrations: 103.60–414.40 mg L^{-1} salicylic acid; 1.0–10.4 mg L^{-1} potassium dichromate; 10.0–200.0 mg L^{-1} hydrogen peroxide; and 12.5–150.0 mg L^{-1} starch.

The chromogenic reagents were prepared daily. For determination of salicylic acid, a solution concentration of 0.006 mol L^{-1} was prepared by diluting stock solution of 0.01 mol L^{-1}. A solution of $Fe_2(SO_4)_3$ 19.94 g L^{-1} (0.10 mol L^{-1}) (Vetec) was prepared by dissolving 1.9940 g in a 100 mL buffer HCl/KCl, pH 1.7, and another solution of $Fe_2(SO_4)_3$ with the same concentration in 100 mL buffer acetic acid/sodium acetate, pH 3.5. For potassium dichromate two solutions of 1.8×10^{-3} mol L^{-1} of 1,5-diphenylcarbazide (1,5-DFC), $C_{13}H_{14}N_4O$ (Sigma-Aldrich) were prepared by dissolving 0.0436 g in a 100 mL buffer HCl/KCl pH 1.2 and another 1,5-DFC solution at 100 mL buffer HCl/KCl pH 2.2. To determine of hydrogen peroxide, solutions of vanadium oxide, V_2O_5 (Sigma Aldrich) 1.8188 mg L^{-1} were prepared by dissolving 0.4547 g in 12% HCl (v/v) and 100 mL of V_2O_5 in same concentration dissolved in 6% HCl (v/v). For determination of starch, the reagent solution Lugol's iodine was prepared with 0.10 g of iodine (Vetec) that was first dissolved in 5 mL of ethanol P.A. (Vetec) then mixed with 0.20 g of potassium iodide KI dissolved in 250 mL of distilled water.

2.2. Apparatus

The following equipment was used: a multichannel spectrometer with linear array of diodes (USB4000, Ocean Optics), a flow cell with an optical path length of 10 mm (Hellma, Plainview, NY, USA) with an 80 μL internal volume. For the radiation source, a tungsten-halogen lamp (Ocean Optics, LS-1) was used to carry out spectrometric measurement. Fiber optic cable with 0.20 mm of diameter was used for transporting the radiation to the detection point.

The flow set up comprised six pinch solenoid valves normally closed (Ref. Nresearch 161P011) and two solenoid micro-pumps (Ref. Biochem 090SPSP12-8 12VDC), with a nominal volume of 10 μL per stroke. An Intel Pentium laptop connected with an interface (USB – 6009, National instruments) and a homemade electronic interface using one ULN 2803A integrated circuit (TOSHIBA) was used for data acquisition and control. The software to control the pinch solenoid valves and solenoid micro-pumps and for data acquisition was written in LAB VIEW 8.5.

2.3. Sample preparation

The milk samples were acquired from different supermarkets in Recife (Pernambuco – Brazil) and stored in amber glass flasks at a temperature of 4 °C. Three sample types were choose: UHT skimmed cow's milk, raw cow's milk and skimmed goat's milk. For dichromate, salicylic acid and hydrogen peroxide determination, one aliquot of each sample was diluted 20-fold with water. For the detection of starch, a sample aliquot was diluted 100-fold with water.

2.4. Proposed procedure

The physical chemistry characteristics of some reagents and their incompatibility with the carrier solution affected the measurements by refractive index gradients (Schlieren effect) (Rocha & Nóbrega, 1996, 1997). This was because the reactions with salicylic acid, dichromate and hydrogen peroxide were carried out in a strongly acidic environment. This effect was compensated by employing a strategy of dual-wavelength spectrophotometry (Rocha & Nóbrega, 1996).

The products resulting from chemical reactions for salicylic acid, dichromate, hydrogen peroxide and starch determination were monitored at 541, 514, 441, and 600 nm, respectively. Besides this, the products of the reactions with salicylic acid, dichromate and hydrogen peroxide were also monitored at 800 nm.

A flow system based on a multicommutation concept and binary sampling (Rocha et al., 2002) whose design is shown in Fig. 1 was used after optimization. In this configuration all the valves and micro-pumps are switched OFF and no solution is flowing along the analytical path. In order to obtain maximum sensitivity, the chemical and hydrodynamic variables of the system were optimized, including reagents pH and volume, and number of sampling cycles.

The pumping flow rate of sample and reagents solutions was controlled by the pump pulsation frequency maintained at 5 Hz in equal time intervals (0.1 s) to maintain each one switched either ON/OFF. The activation sequences of the valves and micro-pumps are summarized in Table 1.

The pinch valves V_1, V_3, V_4, V_5, V_6 and micro-pump P_1, as shown in Table 1, were employed to insert the sample (S) and chromogenic reagents (R_1, R_2, R_3, R_4) steps 1, 3, 5, 7, respectively. The micro-pump P_2 was employed for filling the flow lines, cleaning the system and transporting the sample zones up to the detector (steps A, B, C, D, E, F) (steps 2, 4, 6, 8). Two micro-pumps were employed to avoid the warming of the micro-pumps that occurs when one works continuously.

For salicylic acid determination, the pinch valves V_1, V_3 and micro-pumping P_1 were switched ON/OFF sequentially several times to allow the mixture of the slugs sample solution (S), with the slugs reagent solution of Fe^{3+} (R_1), to insert the sample zone into the reaction coil B in a tandem process. After this, the pinch valve (V_2) was switched ON simultaneously and the micro-pump P_2 was switched ON/OFF several times to displace the sample zone to the waste (W) (steps 1–2, Table 1).

In dichromate, hydrogen peroxide and starch determination, a similar strategy for salicylic acid determination was used. The determination of dichromate was carried out by switching the pinch valves V_1 and V_4 ON/OFF, to insert sample and reagent solution of 1,5-DFC (R_2), respectively. At the same time, the micro-pump P_1 was switched ON/OFF several times. Subsequently the pinch valve V_2 was switched ON for a period of time and the micro-pump P_2 was switched ON/OFF sequentially several times (steps 3–4, Table 1). After step 4 was finished, steps five and six (steps 5–6, Table 1) were carried out to determine hydrogen peroxide with pinch valves V_1 and V_5 switched between ON/OFF, simultaneously with micro-pump P_1 switched ON/OFF sequentially several times. These pinch valves contained the sample solution and a reagent solution of vanadium oxide (V_2O_5) (R_3), respectively, (Fig. 1). Afterwards pinch valve V_2 was switched ON for a period of time, at the same time as the micro-pump P_2 was switched ON/OFF sequentially several times (steps 5–6, Table 1). When steps 5–6 were finished, steps 7–8 (Table 1) for starch determination were started. The pinch valves V_1 (sample solution) and V_6 (Lugol's iodine solution) were switched ON/OFF while at the same time the micro-pump P_1 was switched ON/OFF sequentially several times, subsequently pinch valve V_2 was switched ON simultaneously and the micro-pump P_2 was switched ON/OFF sequentially several times (steps 7–8, Table 1).

The software was developed to study systematically the number of cycle samplings, the volumetric fractions and the pH reaction which worked best for each of the analytes. The best results were obtained with the parameters shown in Table 1.

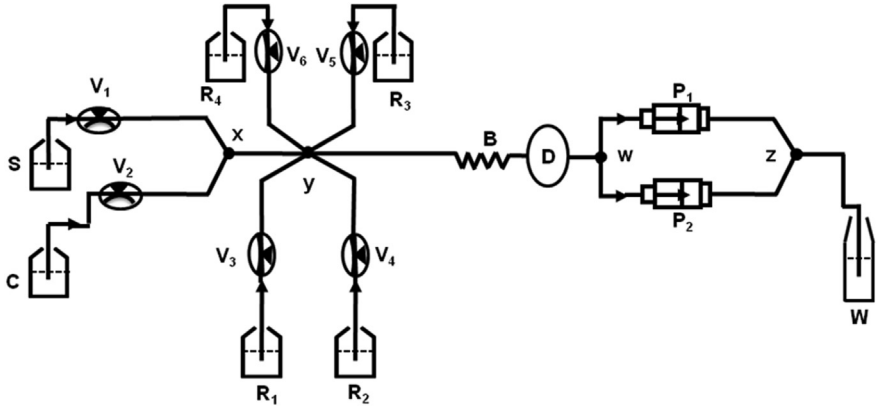

Fig. 1. Diagram of the flow system. V_1 V_2 V_3 V_4 V_5 and V_6 = pinch solenoid valve normally closed: P_1 and P_2 = solenoid micro-pumps; S = sample or standard solutions; R_1 = Fe (III) solution; R_2 = 1,5-diphenylcarbazide; R_3 = V_2O_5 solution; R_4 = Iodine solution; C = carrier solution, water; x, y, z e w = joint device; B_1 = reaction coil (120 cm); D = Detector; W = waste.

Table 1
Operational sequence of the screening system in flow multicommuted to sequential determination of salicylic acid, dichromate, hydrogen peroxide and starch.

Analite	Step	Event	V_1	V_2	V_3	V_4	V_5	V_6	P_1	P_2	Pulse	Time(s)	Cycles
	A	Flow lines filling	1[a]	0[a]	0	0	0	0	0	1/0[a]	15	3	–
	B		0	0	1	0	0	0	0	1/0	15	3	–
	C		0	0	0	1	0	0	0	1/0	15	3	–
	D		0	0	0	0	1	0	0	1/0	15	3	–
	E		0	0	0	0	0	1	0	1/0	15	3	–
	F	Washing	0	1	0	0	0	0	0	1/0	150	30	–
Salicylic acid	1	Inserting sample (S) and regent solution R_1	1	0	0	0	0	0	1/0	0	1	0.2	15
			0	0	1	0	0	0	1/0	0	5	1	
	2	Signal reading and Washing	0	1	0	0	0	0	0	1/0	150	30	–
Dichromate	3	Inserting sample (S) and regent solution R_2	1	0	0	0	0	0	1/0	0	1	0.2	15
			0	0	0	1	0	0	1/0	0	4	0.8	
	4	Signal reading and Washing	0	1	0	0	0	0	0	1/0	150	30	–
Hydrogen peroxide	5	Inserting sample (S) and regent solution R_3	1	0	0	0	0	0	1/0	0	2	0.4	15
			0	0	0	0	1	0	1/0	0	1	0.2	
	6	Signal reading and Washing	0	1	0	0	0	0	0	1/0	150	30	–
Starch	7	Inserting sample (S) and regent solution R_4	1	0	0	0	0	0	1/0	0	5	1	10
			0	0	0	0	0	1	1/0	1	1	0.2	
	8	Signal reading and Washing	0	1	0	0	0	0	0	1/0	150	30	–

[a] Note: 0 indicates that the corresponding pinch valve was switched OFF while 1 means that the valve or micro-pump was switched ON.

2.5. Optimization

The Job's method was employed to study the stoichiometric ratio of complexes (Ravichandran, Rajendran, & Devapiriam, 2014), between Cr(VI)/1,5-diphenylcarbazide and Fe(III)/salicylic acid. In this method, the total molar concentration of two reactants is kept constant while their molar concentration ratios are continuously varied throughout the series of samples. The solutions were prepared by mixing the solutions of both components, Cr(VI), 1,5-diphenylcarbazide, Fe(III) and salicylic acid, with an equal molar concentration in a ratio varying from 1:9 to 9:1, for both reactions.

Initially the reaction for dichromate determination was based on the reaction between Cr(VI) and 1,5-diphenylcarbazide in medium acid where the Cr(VI) oxidizes the diphenylcarbazide leading to the formation of difenilcarbazona and Cr (III) (Gardner & Comber, 2002).

The salicylic acid determination was based on the complexation reaction between salicylic acid and Fe(III) in an acid medium (Koupparis & Anagnostopoulou, 1988). For the aforementioned reactions, a study was made using lab bench analysis, to provide the exact stoichiometry and sensitivity for the reactions above.

The reaction of oxidation between hydrogen peroxide and vanadium (V) oxide in an acid medium resulting in an orange solution was used for hydrogen peroxide determination (Alonso & Livage, 1999).

Starch determination was based on the reaction of complexation of starch with triiodide. The reaction stops when the blue color caused by the triiodide–starch complex appears (Junior, 2003).

Firstly the stoichiometry of the complex formed between Cr (VI) and 1,5-Diphenylcarbazide and salicylic acid and Fe (III) was established through the method of continuous variation (Hill & MacCarthy, 1986), which was implemented by maintaining constant the sum of the concentrations of Cr (VI) and metal ion ligand, 1,5-DFC and volume of the solutions, while the ratio Cr (VI)/1,5-DFC was varied. In this study, solutions were prepared with Cr (VI) and colorimetric reagent 1,5-DFC between 0.001 mol L^{-1} up to 0.009 mol L^{-1}. The pH 1.2 was maintained constant during the study of the stoichiometric reactions. The complexation reaction between salicylic acid and Fe(III) was studied. The solutions were prepared from salicylic acid and colorimetric reagent 1,5-DFC from 0.01 mol L^{-1} up to 0.09 mol L^{-1}. The pH 5.0 was maintained constant.

Optimization of the reaction conditions for the determination of each analyte was carried out with the system outlined in Fig. 1. In all studies of optimization of the multicommuted flow system, a reaction coil of 120 cm and 15 sampling cycles was employed.

A 2^3 factorial design with two-levels was used to investigate three factors in the system of salicylic acid and dichromate determination. The following factors were studied pinch valves switching times ON (V_1, sample solution; V_3, Fe(III) solution) and the levels of pH (1.7 and 3.5). According to literature this reaction is very dependent on the pH of the reaction medium (Koupparis & Anagnostopoulou, 1988). The pinch valve switching times ON was investigated for the determination of dichromate (V_1, sample solution; V_4, 1,5-DFC solution). The levels of pH studied were 1.2 and 2.2, because in preliminary studies it had been observed that the pH reaction influenced the analytical signal. The runs were carried out in random order and in duplicate. To identify the best means for the determination of hydrogen peroxide, the following factor were studied for the V_1 and V_5 pinch valves: the switching time ON and HCl concentration, this latter factor, because it is quite significant (Alonso & Livage, 1999). In the starch determination the factors involving switching time ON of valves V_1 and V_6 were chosen employing a 2^2 factorial design. The better response was the absorbance measurement of height, i.e., greater height and shape of the analytical signal. The design matrix (Tables 2–5) lists the runs in the standard order together with experimental results.

After the factorial design was established, assays were made with variations in the sampling cycles. After setting the appropriate values of the flow system analysis, the milk samples were analyzed.

3. Results and discussion

3.1. General aspects

In this paper, the attention was focused on the development of a multicommuted automatic system for the analysis of sequential screening. The flow system depicted in Fig. 1 was designed to allow sequential determinations of dichromate, salicylic acid, hydrogen peroxide and starch in milk, which could be carried out sharing the same analytical path and flow cell. The employment of pinch solenoid valves associated with micro-pumps allowed the use of these devices in the pumping-pull mode. The solutions were sucked through the analytical path towards the micro-pumps. The employment of this type of assembly produced a significant reduction in the cost of implementation of this methodology (Lavorante et al., 2007).

Table 2
Factors, levels and response signal values for the 2^3 factorial design in reaction among salicylic acid and Fe(III).

Factors				(−)	(+)
1 – Pinch valve switching time ON V_1 (t_{1AS})				0.2	0.8
2 – Pinch valve switching time ON V_3 (t_{2AS})				0.4	1.0
3 – pH				1.7	3.5
Runs	1	2	3		Absorbance
1	−1	−1	−1		0.61
2	−1	−1	+1		0.77
3	−1	+1	−1		0.59
4	−1	+1	+1		0.98
5	+1	−1	−1		0.48
6	+1	−1	+1		0.36
7	+1	+1	−1		0.69
8	+1	+1	+1		0.61
9	−1	−1	−1		0.62
10	−1	−1	+1		0.84
11	−1	+1	−1		0.54
12	−1	+1	+1		0.97
13	+1	−1	−1		0.55
14	+1	−1	+1		0.51
15	+1	+1	−1		0.75
16	+1	+1	+1		0.56

Table 4
Factors, levels and response signal values for the 2^3 factorial design in reaction among hydrogen peroxide and V_2O_5.

Factors				(−)	(+)
1 – Pinch valve switching time ON (V_1) t_{1HP}				0.4	0.8
2 – Pinch valve switching time ON (V_5) t_{2HP}				0.2	1.0
3 – HCl (%)				6	12
Runs	1	2	3		Absorbance
1	−1	−1	−1		0.86
2	−1	−1	+1		1.05
3	+1	−1	−1		0.85
4	+1	−1	+1		0.64
5	−1	+1	−1		0.50
6	−1	+1	+1		0.54
7	+1	+1	−1		0.81
8	+1	+1	+1		0.64
9	−1	−1	−1		0.88
10	−1	−1	+1		1.05
11	+1	−1	−1		0.75
12	+1	−1	+1		0.83
13	−1	+1	−1		0.52
14	−1	+1	+1		0.56
15	+1	+1	−1		0.79
16	+1	+1	+1		0.70

3.2. Establishment of stoichiometric ratio of the reactions

Job's method was used to determine the stoichiometric ratio. The best stoichiometric ratio was 1:9, the result of the method of continuous variations obtained for the reaction between dichromate and 1,5-DFC. The same analysis was used to set the stoichiometric ratio of the complexation reaction between the salicylic acid and Fe(III) where the best stoichiometric ratio was 2:3. For other analytes, it was not necessary to study the stoichiometry, because it has already been established according to the literature (Odair Zenebon, Pascuet, & Tiglea, 2008, chap. 26).

3.3. Experimental design

The calculated effects for the two-level three-factor factorial are listed in Tables 6–8, and a two-level two-factor in Table 9 with experimental errors. The calculations of the effects were carried out according to standard methods (Barros, Scarminio, & Bruns, 2006, chap. 4). Employing the method of confidence intervals which uses the Student distribution, the statistically significant factors or interactions that is greater in magnitude than the point distribution of the Student product of the standard error, Equation (1), were considered significant, at 95% confidence, where $t_{(x)}$ is the Student's value to eight or four degrees of freedom and s value the standard error.

$$t_{(x,0.95)} \times s \tag{1}$$

Applying this equation, when the effects the absolute values are higher than 0.059995 (Table 6), 0.035899 (Table 7), 0.066941 (Table 8), and 0.270127 (Table 9) are considered significative. These results are shown in Tables 6–9, where the results highlighted in bold are not significant.

Table 6 shows the interaction effects for salicylic acid $t_{1SA} \times$ pH (1×3) and $t_{1SA} \times t_{2SA}$ (1×2), which were not statistically significant, indicating no interaction. The effects of first order interaction t_{1SA}, t_{2SA} e pH, $t_{2SA} \times$ pH e $t_{1SA} \times t_{2SA} \times$ pH are all significant. The top responses in terms of absorbance were 2, 4 and 7 experimental runs (Table 2). Condition 4 was chosen, because it has a higher sensitivity in terms of absorbance. This condition is equivalent to a pH 3.5; t_{1SA} 1.0 s; t_{2SA} 0.2 s. For dichromate on Table 7, the

Table 3
Factors, levels and response signal values for the 2^3 factorial design in reaction among dichromate and 1,5-DFC.

Factors				(−)	(+)
1 – Pinch valve switching time ON V_1 (t_{1C})				0.8	1.8
2 – Pinch valve switching time ON V_4 (t_{2C})				0.2	0.4
3 – pH				1.2	2.2
Runs	1	2	3		Absorbance
1	−1	−1	−1		1.00
2	−1	−1	+1		0.079
3	+1	−1	−1		0.85
4	+1	−1	+1		0.072
5	−1	+1	−1		0.28
6	−1	+1	+1		0.052
7	+1	+1	−1		0.50
8	−1	−1	−1		1.06
9	+1	+1	+1		0.011
10	−1	−1	+1		0.061
11	+1	−1	−1		0.83
12	+1	−1	+1		0.024
13	−1	+1	−1		0.20
14	−1	+1	+1		0.035
15	+1	+1	+1		0.063
16	+1	+1	−1		0.49

Table 5
Factors, levels and response signal values for the 2^2 factorial design in reaction among in starch and triiodide.

Factors				(−)	(+)
1 – Pinch valve switching time ON V_1 (t_{S1})				0.4	1.0
2 – Pinch valve switching time ON V_6 (t_{S2})				0.2	1.8
Runs	1	2			Absorbance
1	−1	−1			0.59
2	+1	−1			0.94
3	−1	+1			0.24
4	+1	+1			0.38
5	−1	−1			0.81
6	+1	−1			1.02
7	−1	+1			0.38
8	+1	+1			0.66

Table 6
Calculated effects for the 2^3 factorial design of in reaction among salicylic acid and Fe(III).

		Effects	Standard errors
Average		0.656413	0.013009
Main effects	$1 - t_{1SA}$	0.119267	0.026017
	$2 - t_{2SA}$	−0.176989	0.026017
	3 − pH	0.096584	0.026017
Two − factor Interactions	1 × 2	**0.055890**	0.026017
	1 × 3	**0.042533**	0.026017
	2 × 3	−0.201653	0.026017
Three − factor Interactions	1 × 2 × 3	−0.070497	0.026017

Interval with 95% confidence: $t_{(8,0,95)} \times s = 2.306 \times 0.026017 = 0.059995$.

Table 8
Calculated effects for the 2^3 factorial design of in reaction among in hydrogen peroxide and V_2O_5.

		Effects	Standard errors
Average		0.753037	0.014514
Main effects	$1 - t_{1HP}$	**0.007391**	0.029029
	$2 - t_{2HP}$	**0.008956**	0.029029
	3 − HCl	−0.231148	0.029029
Two − factor Interactions	1 × 2	−0.103587	0.029029
	1 × 3	**−0.053223**	0.029029
	2 × 3	0.199631	0.029029
Three − factor Interactions	1 × 2 × 3	**0.020105**	0.029029

Interval with 95% confidence: $t_{(8,0,95)} \times s = 2.303 \times 0.029029 = 0.066941$.

interaction effects $t_{1c} \times$ pH and $t_{1c} \times t_{2c}$ were not statistically significant, indicating that they do not interact. The best responses in terms of absorbance were with 1, 3 and 7 experimental runs (Table 3). The first condition 1 was chosen, because it has a higher sensitivity in terms of absorbance. This condition is equivalent to a pH 1.2, V_1 pinch valve switching time ON at 0.8 s and V_4 pinch valve switching time ON at 0.2 s. For hydrogen peroxide (Table 8) the best responses in terms of absorbance were 1, 2 and 3 (Table 4). The second condition was selected, because it has a higher sensitivity in terms of absorbance and is the condition that consumes less sample and produces less effluents. This condition is equivalent to a HCl concentration of 12% (v/v); $t_{1HP} = 0.4$ s; $t_{2HP} = 0.2$ s. For starch (Table 9) the same analysis may be made, where $t_1 = 1.0$ s; $t_2 = 0.2$ s was achieved.

3.4. Effect of sampling cycles

The volume of the sample zone can affect the sensitivity, thus, the number of sampling cycles was varied from 2 to 35 ($n = 3$), as shown in Fig. 2. As can be seen in Fig. 2, the better results for sensitivity were achieved when the sampling cycles were 15 (900 μL) for salicylic acid (Fig. 2A), and 15 (750 μL) for dichromate (Fig. 2B). In Fig. 2C shows that the signal increased up to 20 sampling cycles. But at 20 sampling cycles there was poorer repeatability and the Schlieren effect was pronounced. Thus, 15 sampling cycles (400 μL) were used for hydrogen peroxide. For starch, 10 sampling cycles (Fig. 2D) were employed.

3.5. Figures of merit and recovery

The analytical curve for determination of salicylic acid, dichromate, hydrogen peroxide and starch presenting typical equations A = (0.00321 ± 0.00002) C + (−0.03655 ± 0.00432), A = (0.08976 ± 0.00039) C + (−0.07139 ± 0.00337), A = (0.00346 ± 0.00005) C + (0.13824 ± 0.00419), A = (0.00696 ± 0.00017) C + (−0.05829 ± 0.01461), respectively where A is the analytical signal and C is the concentration. Fig. 3 shows the analytical signals for the determination of salicylic acid, dichromate, hydrogen peroxide, and starch. As illustrated, the multicommuted system has satisfactory repeatability and a stable baseline.

After the optimized conditions were identified, the system showed a satisfactory repeatability of analytical signals with the relative standard deviation of the recorded peak heights estimated ($n = 40$) as 2.16%, 2.33%, 1.12% and 0.85%, for salicylic acid, dichromate, hydrogen peroxide and starch, respectively, such as shown in a Table 10. The data as fitted by standard least-squares treatment (univariate method) can be seen in this table. The detection limit was estimated by an analysis of known solution concentrations of the analyte and decreasing them down to the smallest level differentiated by the signal blank (Beatriz, Bottoli, Collins, Sales, & Jardim, 2004). The multicommuted procedure showed other eco-friendly features, such as a low generation of waste for each determination: 3.25 mL for salicylic acid; 3.40 mL for potassium dichromate; 2.95 mL for hydrogen peroxide; and 3.10 mL for starch. The medium volume of waste generated was of 3.18 mL per determination. The sampling throughput was also evaluated, achieving the following values: 75 h^{-1} (salicylic acid); 80 h^{-1} (dichromate); 92 h^{-1} (hydrogen peroxide); and 86 h^{-1} (starch).

In order to demonstrate the accuracy of the proposed method, a recovery study was made. Three milk samples were chosen: UHT skimmed cow's milk (sample 1), raw cow's milk (sample 2) and skimmed goat's milk (sample 3). The samples were spiked with two levels of concentration for each type of analyte, as seen in Table 11. Recoveries which varied from 90.1 to 108.7% were obtained for the spiked milk samples indicating that the procedure is free from matrix effects.

Seven different milk samples were analyzed using the proposed method, but the analytes were not detected in the samples analyzed, in with the limits of detection established by method developed. Considering the advantages presented by a multicommutation approach employing binary sampling, with the use of pinch solenoid valves and micro-pumps solenoids, we concluded that the procedure is a sustainable alternative to the batch method in terms of reagent consumption and operator-handled solutions.

3.6. Screening method in flow multicommuted

The reliability of the method was measured by using of the performance curve approach (Lima et al., 2004; Song, Schlecht, &

Table 7
Calculated effects for the 2^3 factorial design of in reaction among dichromate and 1,5-DFC.

		Effects	Standard errors
Average		0.351811	0.007784
Main effects	$1 - t_{1C}$	**0.010162**	0.015568
	$2 - t_{2C}$	−0.293477	0.015568
	3 − pH	−0.603792	0.015568
Two − factor Interactions	1 × 2	0.114460	0.015568
	1 × 3	**−0.024807**	0.015568
	2 × 3	0.274648	0.015568
Three − factor Interactions	1 × 2 × 3	−0.106672	0.015568

Interval with 95% confidence: $t_{(8,0,95)} \times s = 2.306 \times 0.015568 = 0.035899$.

Table 9
Calculated effects for the 2^2 factorial design of in reaction among starch and triiodide.

		Effects	Standard errors
Average		0.633472	0.048654
Main effects	1	**0.242590**	0.097308
	2	−0.420793	0.097308
Two − factor Interactions	1 × 2	**−0.036041**	0.097308

Interval with 95% confidence: $t_{(4,0,95)} \times s = 2.776 \times 0.097308 = 0.270127$.

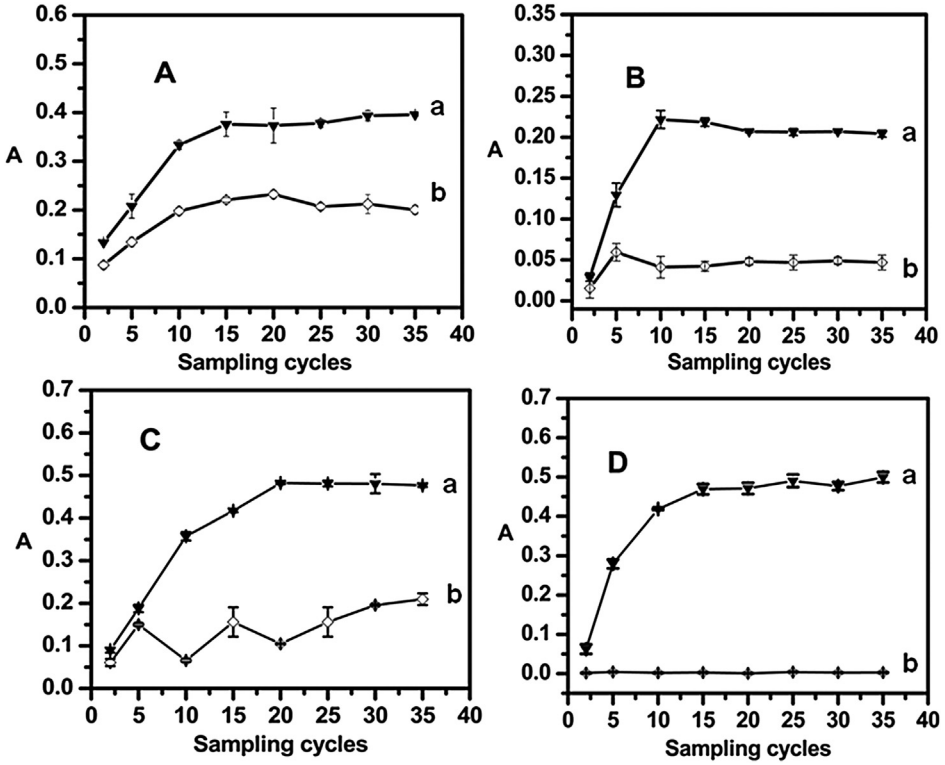

Fig. 2. Study of the effect of the number of sampling cycles on the analytical signal (a) and blank signals (b) for salicylic acid (A), dichromate (B), hydrogen peroxide (C) and starch (D). Employing the following concentrations: 3.90 mg L^{-1} dichromate, 155.4 mg L^{-1} salicylic acid, 100 mg L^{-1} hydrogen peroxide, 50 mg L^{-1} starch. ($n = 3$).

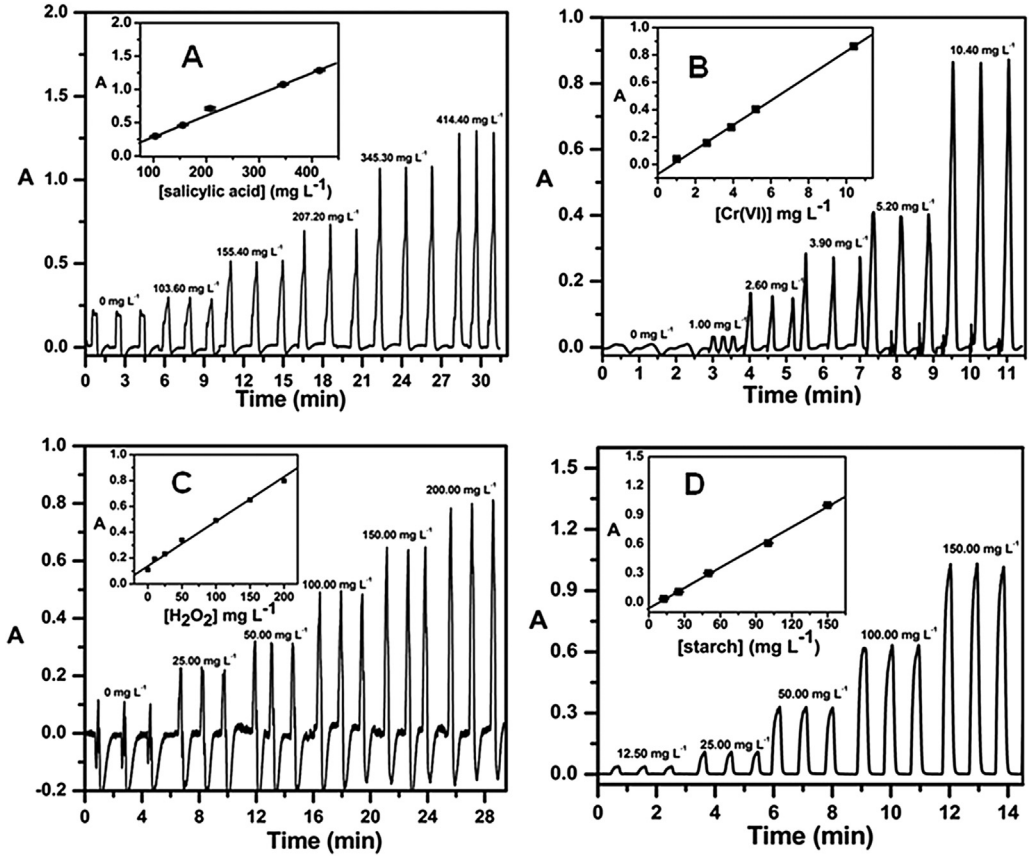

Fig. 3. Transient signals for reference solutions (A) salicylic acid, (B) dichromate, (C) hydrogen peroxide and (D) starch. In the inside on the graphs are shown the analytical curves, respectively. Concentrations are in mg L^{-1}.

Table 10
Analytical features of different procedures for determination of the analytes.

Parameters/Analyte	Dichromate	Salicylic acid	Hydrogen peroxide	Starch
Linear range (mg L^{-1})	1–10.4	103.6–414.4	10.0–200.0	12.5–150.0
LOD (mg L^{-1})	0.12	2.58	6.14	0.29
RSD (%)	2.33	2.16	1.12	0.85
Consumption of sample (mL)[a]	0.150	0.150	0.300	0.500
Consumption reagent (mL)[a]	0.750	0.600	0.150	0.100
Waste generation (mL)[a]	3.40	3.25	2.95	3.10
Sampling throughput (h^{-1})	75	80	92	86

[a] Per determination.

Table 11
Recoveries of different procedures for determination of the analytes.

Samples	Dichromate			Salicylic acid		
	Added (mg L^{-1})	Found (mg L^{-1})	Recovery (%)	Added (mg L^{-1})	Found (mg L^{-1})	Recovery (%)
Sample 1	2.6	2.82	108.7 ± 2.8	103.6	109.20	105.4 ± 0.5
	3.9	3.60	92.3 ± 4.0	155.4	167.15	107.5 ± 1.4
Sample 2	2.6	2.46	94.7 ± 3.9	103.6	98.42	95.0 ± 3.9
	3.9	3.51	90.1 ± 2.5	155.4	166.60	107.0 ± 1.4
Sample 3	2.6	2.73	105.0 ± 3.9	103.6	106.94	103.2 ± 4.8
	3.9	4.15	106.0 ± 4.3	155.4	154.69	99.5 ± 3.9
	Hydrogen peroxide			Starch		
	Added (mg L^{-1})	Found (mg L^{-1})	Recovery (%)	Added (mg L^{-1})	Found (mg L^{-1})	Recovery (%)
Sample 1	50.0	49.29	98.5 ± 2.8	25.0	27.07	108.2 ± 2.1
	100.0	107.45	107.4 ± 1.7	50.0	54.23	108.4 ± 2.9
Sample 2	50.0	46.17	92.30 ± 2.6	25.0	27.04	108.1 ± 2.9
	100.0	100.90	100.80 ± 2.2	50.00	52.66	105.3 ± 1.3
Sample 3	50.0	45.14	90.20 ± 1.4	25.0	23.10	92.4 ± 3.6
	100.0	98.50	98.40 ± 3.4	50.0	50.07	100.0 ± 3.2

Ashley, 2001). Fig. 4 shows the corresponding performance curve at 10.0 mg L^{-1}, 1.0 mg L^{-1}, 10.0 mg L^{-1}, and 6.3 mg L^{-1} for salicylic acid Fig. 4(A), dichromate Fig. 4(B), hydrogen peroxide Fig. 4(C) and starch Fig. 4(D), respectively.

The number of false positives and false negatives were measured using real milk samples that were prepared by same procedure describe in Section 2.3 and after addition at seven different concentration levels of the analytes (above and below concentration cut-off), for salicylic acid 5–15 mg L^{-1}, dichromate between 0.25 and 1.75 mg L^{-1}, hydrogen peroxide 5–15 mg L^{-1} and starch 3–9 mg L^{-1}. These studies are summarized in Fig. 4. As can be seen in the graph through of application of methodology of curve performance, uncertainty regions can be identified at the concentration range between 0.53 and 1.22 mg L^{-1} for dichromate, 8.75–10.96 mg L^{-1} for salicylic acid, 7.13–10.72 mg L^{-1} for hydrogen peroxide and 4.29–6.85 mg L^{-1} for starch. This assumes that the probability of a decision error is fixed at 5% for false positive and false negative responses. Thus, a concentration cut-off point of 1.0 mg L, cutting-1, 10.0 mg L^{-1}, 10.0 mg L^{-1} and 6.3 mg L^{-1} for dichromate, salicylic acid, hydrogen peroxide and starch, respectively, was established.

4. Conclusions

The methodology reported here could be employed as powerful tool to make timely decisions. For all methodologies developed, the use of the performance curve is able to establish reliable cut-off values with low probability of a false positive occurring for each analyte determinate. The procedures developed the concept of employing multicommutation and binary sampling, using solenoid mini-pumps to propel the solutions, which was shown to be efficient and appropriate to determine dichromate, salicylic acid, hydrogen peroxide and starch in UHT milk samples. The system developed is easy to operate and robust. Other types of adulterants

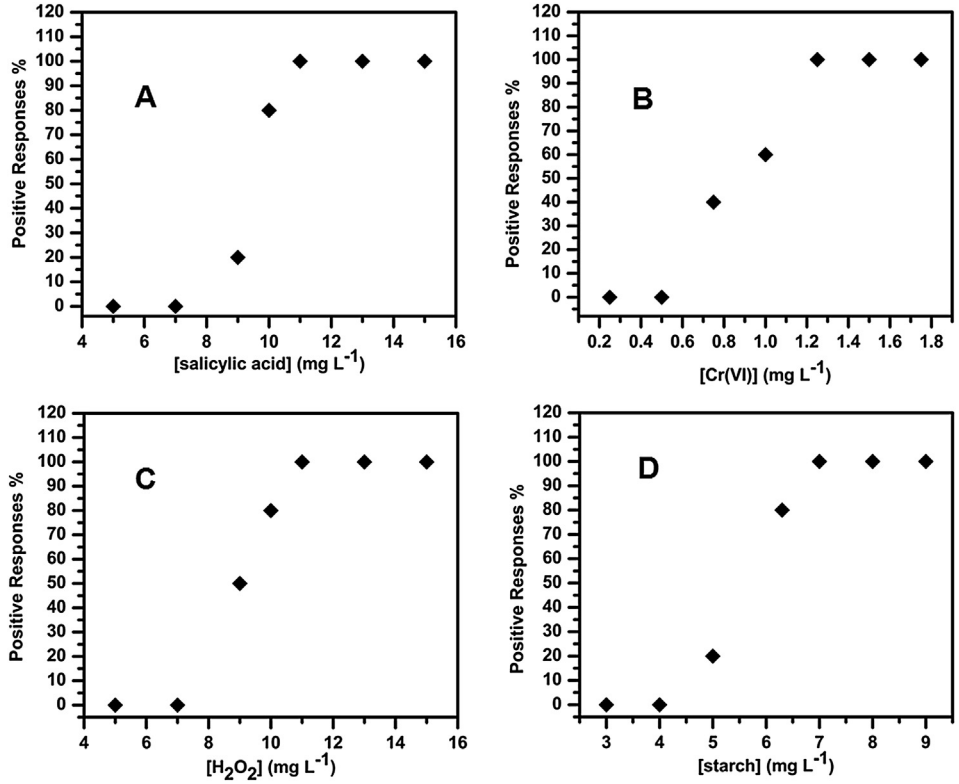

Fig. 4. Performance curves for salicylic acid (A), dichromate (B), hydrogen peroxide (C) and starch (D).

could be determined by changing the reagents. The strategy is that of reagent addition using the concept of binary sampling enabling the implementation of a sequential detection procedure without changing the system configuration. The proposed system has enabled the rational use of reagent, minimizing the consumption of reagents and samples.

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