Acetylsalicylic Acid Determination in Remedies Using a Gas-Diffusion / Flow Injection Analysis

Marta M. D. C. Vila, Matthieu Tubino, Francisco A. A. Matias and Alvino Rodrigues Jr.*

Instituto de Química, UNICAMP, CP 6154, 13083-970 Campinas, SP, Brazil

Abstract

A "Flow Injection Analysis" procedure is proposed for the quantitative analysis of acetylsalicylic acid in remedies. The method is based on the alkaline hydrolysis of the acetylsalicylic acid, producing acetate. The hydrolysed and diluted sample was directly introduced in the system where it was mixed with a carrier containing 0.2 mol l^{-1} sulphuric acid, forming acetic acid, that is pervaporated through a PTFE membrane into a carrier with an acid-base indicator at pH=7.0 that is conducted to a spectrophotometer. The transmittance changes monitored in 580 nm correlate with the original quantity of acetylsalicylic acid in the sample. Nine real samples of remedies were analysed. The results obtained with the proposed procedure were compared with those obtained with the method recommended by Pharmacopoeia, using the statistical Student's *t*-test procedure. Complete agreement was achieved between the two methods. The sampling frequency is relatively high as about 100 determinations can be performed per hour.

Keywords: flow injection analysis; acetylsalicylic acid; spectrophotometric detection

1. Introduction

The analytical technique known as "Flow Injection Analysis", FIA , was developed during seventies by Ruzicka and Hansen [1] and have been widely employed in quantitative analytical procedures. When proposed, this technique had great repercussion in the analytical community and now is one of the most used flow methods in automated analysis [2]. A variety of detectors can be associated to the FIA systems, including spectrophotometric detectors, maybe the most used, due to their high flexibility, availability and low cost [3]. Besides these factors, the versatility of the FIA systems can be amplified by the possibilities that are open with the incorporation of various usual analytical procedures. For example, can be incorporated to FIA systems: solvent extraction, distillation, ion-exchange, separation of volatile compound through membrane pervaporation, and so on [4]. The technique that uses pervaporation with membranes is usually employed for the separation and/or pre-concentration of a variety of inorganic and organic volatile substances as, for instance, ammonia, carbonic gas, hydrogen sulphide, phenols, aldehydes, ketones, organic acids, alcohols, and son on, present in liquid or gaseous samples [5]. The use of membranes of gaseous diffusion allows the selectivity increase as some species are sufficiently volatile at room temperature [6]. The development of new polymers relating structure, permeability and selectivity has motivated the employ of diffusion/permeation membranes in several areas of the chemical analysis [7].

The quality control of medicines in Brazil is a field that has received special attention due to the great number of fakes [8].Therefore, there is a great interest in the development of simple, low cost and rapid analytical techniques for the dosage of active substances in medicines, including acetylsalicylic acid.

The acetylsalicylic acid is used as analgesic, anti-inflammatory and antipyretic, with relatively low collateral effects. It had also been used in the treatment of cardiac and circulatory problems with great success due to its anti-plaquetary action. Its consumption in the United States reaches 20,000 tons per year [9].

A variety of methods is described in the literature for the analysis of the acetylsalicylic acid. Some methods are based on the reaction of the salicylate with iron III, that is the fundament of the Trinder reaction [10], associated to flow systems [11-13]. Other spectrofotometric techniques using ultraviolet [14], fluorescence [15] and infrared [16] are described. The use of high efficiency liquid chromatography is also proposed [17] as well as atomic absorption [18,19]. Ions sensitive electrodes have been also developed [20-24]. Other suggested procedures use biosensors where the enzyme salicylate hydroxylase is immobilised on a support, like for instance, glass beads [25-28]. Despite the great specificity of the methods using enzyme, its use, associated with also expensive purified co-factors, as NADH, increases the cost of the analysis.

The analytical method recommended by Pharmacopoeia [29] consists in the hydrolysis of the acetylsalicylic acid with a sodium hydroxide solution, following a titration with a standard hydrochloric acid solution, using phenolphthalein as indicator. In this hydrolysis is formed acetate. Therefore, using a membrane separator, for the pervaporation of

* e-mail:tubino@iqm.unicamp.br

the acetic acid formed by the action of a strong acid on the acetate, a flow injection system for the acetylsalicylic acid analysis can be proposed.

2.Experimental

2.2. Material

All the chemicals used were of analytical grade. The solutions were prepared with deionized water obtained with a MilliQ-Plus deionizer loaded with water distilled in a glass distiller. The remedies were purchased in the local market.

Solutions

Bromocresol purple, sodium salt (BCP) 0.27 g (4.8×10^{-4} mol) of the indicator were dissolved in 20 ml of ethanol and the volume completed to 500 ml with water, obtaining a solution 9.6×10^{-4} mol l^{-1}. From this stock solution, by dilution with water, was prepared the work solution (1.0×10^{-5} mol l^{-1}). The pH was adjusted to 7.0 by carefully dripping 0.2 mol l^{-1} H_2SO_4 or 0.5 mol l^{-1} NaOH solutions.

Sodium hydroxide 0.5 mol l^{-1} – This solution was daily prepared. 20 g of NaOH were dissolved in 1.0 l of water recently boiled and carefully cooled.

Sulphuric acid solution 0.2 mol l^{-1} – 20 g (11 ml) of 98% sulphuric acid were dissolved in water to complete 1.0 litre.

Acetylsalicylic acid standard solutions – 0.0900 g, 0.1801 g, 0.2702 g, 0.4504 g and 0.7206 g of the acid were dissolved in 50.0 ml of the 0.5 mol l^{-1} sodium hydroxide solution in order to obtain the respective concentrations 0.0100, 0.0200, 0.0300, 0.0500 and 0.0800 mol l^{-1}. These solutions were used to obtain the calibration curve.

Sample treatment – the tablets were triturated and dissolved in about 20 ml of the 0.5 mol l^{-1} NaOH solution at room temperature. The volume was completed to 50.0 or 100.0 ml volumetric flasks according to the desired final concentration. The final obtained concentrations were 0.011, 0.022, 0.036, 0.055 mol l^{-1} correspondent to the tablets containing respectively 100, 200, 650 and 500 mg of acetylsalicylic acid.

2.3. Measurements

The spectrophotometric measurements were performed in transmittance. The 100% was adjusted with the BCP solution at 580 nm (λ_{max}). Considering that the transmission of such solution is approximately 0.25 (25%), the used procedure allows an amplification of the signal in about four times.

2.4. Apparatus

Peristaltic pump: Ismatec mp13 GJ4.

Spectrophotometer: Single-beam Carl Zeiss Model PM2D.

Chart recorder: Cole Parmer Series 8375.

Flow cuvette: 1.00 cm path length quartz cuvette.

Sampling valve: This sampling valve has been described previously in detail [30,31].

Gas diffusion cell: Similar to the cell that has been described by van der Linden [32].

Pumping tubes: Ismatec two-stop tubes, blue-blue, Tygon®, internal diameter 1.65 mm.

Conducting tubes: Polyethylene tubes, internal diameter 1 mm.

Membrane: commercial polytetrafluoroethylene, PTFE, tape.

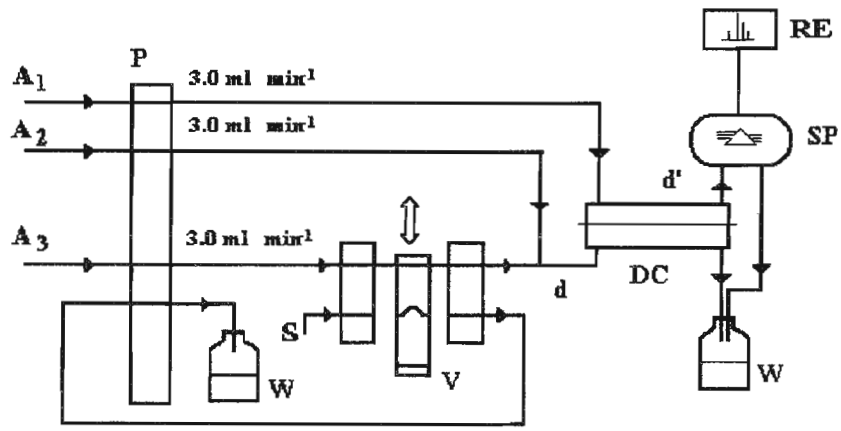

Fig.1 Scheme showing the main parts of the flow injection system used in this work. $\mathbf{A_1}$, BCP solution. $\mathbf{A_2}$, 0.2 mol l^{-1} H_2SO_4 solution. $\mathbf{A_3}$, deionized water carrier. **P**, peristaltic pump. **S**, sample inlet. **V**, sample introducing valve. **DC**, diffusion cell. **SP**, spectrophotometer. **RE**, chart recorder. **W**, waste. **d**, distance between the merging of the sample and sulphuric acid solution. **d'**, distance between the outlet of the **DC** and the spectrophotometric cell. Pumping tubes, Ismatec two-stop tubes, blue-blue, Tygon®, internal diameter 1.65 mm. Conducting tubes, polyethylene, 1 mm internal diameter.

2.5. Flow-injection system

The scheme of the flow injection system used is shown in Fig. 1. The alkaline sample solution of the remedy (**S**) is introduced, through the introduction valve (**V**), into the carrier stream ($\mathbf{A_3}$) consisting of deionized water, which is pumped by the peristaltic pump (**P**) at a flow rate of 3.0 ml min^{-1}. In sequence, the solution of the sample is mixed with a 0.2 mol l^{-1} sulfuric acid stream ($\mathbf{A_2}$). The acetic acid formed passes through the PTFE membrane in the diffusion cell (**DC**) and is carried out by BCP solution 1.0×10^{-5} mol l^{-1} in pH=7.0 ($\mathbf{A_1}$) to the spectrophotometer (**SP**) and registered on the chart recorder (**RE**).

3. Results and discussion

The dosage of the acetylsalicylic acid is performed through the determination of the acetic acid that permeates the PTFE membrane in the diffusion cell. The acid acetic is formed by the reaction of the acetate, that results in the alkaline hydrolysis of the acetylsalicylic acid, with the 0.2 mol l^{-1} sulphuric acid solution. As the acetic acid permeates the membrane it causes an increase in the protonic concentration of the solution containing BCP where the pH was originally adjusted to 7.0. Consequently, with the pH dropping, the absorption at 580 nm (BCP-purple) decreases, as the indicator is transformed in its acid form in which the maximum absorption occurs at 420 nm (BCP-yellow). As it was informed in the experimental section, the signal was monitored as transmittance. The 1.000 transmittance (100.0%) of the spectrophotometer was adjusted with the BCP 1.0×10^{-5} mol l^{-1} solution at 580 nm. Therefore, with the decreasing of the concentration of the purple form of the indicator, caused by the pH decrease, an increase in the transmittance is observed. As a consequence, the registered signal is the transmittance that exceeds 1.000 . This procedure gives an amplification of the analytical signal in about four times.

For the optimum performance of the system, some analytical parameters were studied, using the flow system shown in Fig. 1. The variables studied were the flow rate of the system, the sample volume and the sulphuric acid concentration in the flow A_2.

The rates of the flows $\mathbf{A_1}$, $\mathbf{A_2}$ and $\mathbf{A_3}$ were maintained the same. The effect of the flow rate on the acetylsalicylic acid determination was examined in the range from 1.4 to 3.0 ml min^{-1}. As it can be seen in Fig.2, the analytical signal decreases with the increase of the flow rate. From the rate 1.4 ml min^{-1} to the rate 3.0 ml min^{-1} for each independent flow, the total rate being 9.0 ml min^{-1}, a decrease of 2.5 times is observed. Nevertheless, as a high analytical rate was desired, the 3.0 ml min^{-1} flow rate was chosen for the work. This choice was based on the fact that, despite the high flow rate, under the proposed conditions, the analytical signal is very high, as it can be seen in Fig.2, and the sampling frequency can easily reach 100 determinations per hour. Indeed, higher flow rates can be used if higher analytical frequency is desired, depending on the analyte concentration. In the case of remedies, usually the concentration of the acetylsalicylic acid is relatively high. This characteristic implies that there is not necessary to apply a method with low detection limit.

The decrease of the signal, as shown in Fig.2, can be attributed to the decrease in the diffusion of the acetic acid through the PTFE membrane with the increase of the rate.

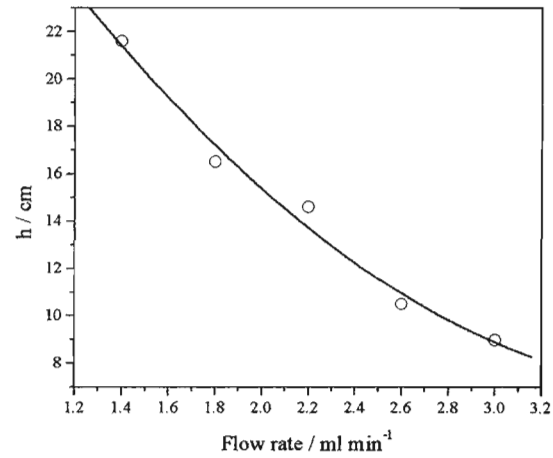

Fig.2. Dependence of the peak height on flow rate. $[H_2SO_4]$ = 0.2 mol l^{-1}; [acetylsalicylic acid] = 0.05 mol l^{-1}; sample volume = 150 µl. Signal height, 1.0 cm = 0.040 transmittance units. Bromocresol purple solution, 1.0×10^{-5} mol l^{-1}, pH = 7.0.

Fig.3 shows the influence of the concentration of the H_2SO_4 solution in the flow $\mathbf{A_2}$. As it can be observed, the signal height rapidly increases from 0 to 0.2 mol l^{-1} of the sulfuric acid concentration. A smaller increase is observed beyond this concentration up to 2.0 mol l^{-1}. The signal corresponding to 0.2 mol l^{-1} of sulphuric acid was considered satisfactory and was adopted as this choice also means less spend of acid.

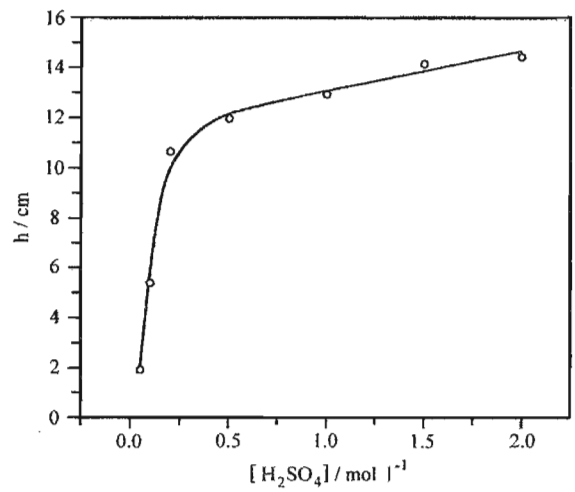

Fig.3 Dependence of the peak height on sulphuric acid concentration. Flow rate = 3.0 ml min^{-1}; [acetylsalicylic acid] = 0.05 mol l^{-1}; sample volume =150 µl. Signal height, 1.0 cm = 0.040 transmittance units. Bromocresol purple solution, 1.0×10^{-5} mol l^{-1}, pH = 7.0.

The influence of the sample volume was studied between 50 and 200 µl and the results can be seen in Fig.4. The relation between the signal height and the introduced volume is linear over the range studied. The volume of 150 µl was chosen for the posterior work as it implies in good signal height associated to better sampling frequency than higher sample volumes.

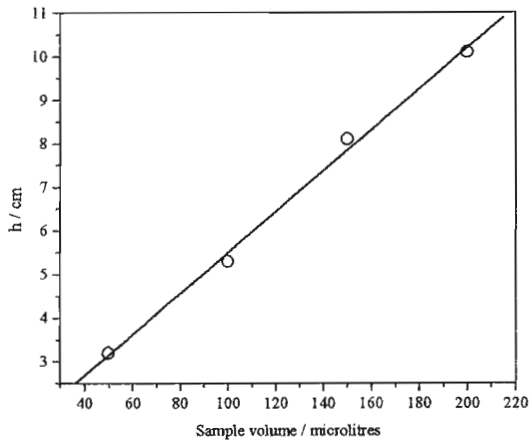

Fig.4. Dependence of the peak height on sample volume. $[H_2SO_4]$ = 0.2 mol l^{-1}; [acetylsalicylic acid] = 0.05 mol l^{-1}; flow rate = 3.0 ml min^{-1}; Signal height, 1.0 cm = 0.040 transmittance units. Bromocresol purple solution, 1.0 × 10^{-5} mol l^{-1}, pH = 7.0.

The distance from the merging of the sample and the sulphuric acid solution, **d**, was 20 cm and the distance, **d'**, between the outlet of the diffusing cell, **DC**, and the spectrophotometric cell was 50 cm.

Obeying the above established conditions, *i.e.*, flow rate of 3.0 ml min^{-1}, sulphuric acid concentration of 0.2 mol l^{-1} and sample volume of 150 µl, the calibration curve was obtained in the range from 0 to 0.08 mol l^{-1}. This curve is described by a simple straight line equation, **h**= -0.243 + 317 **C** (R=0.993), where **h** is the signal height in centimetres (1.0 cm = 0.040 transmittance units), representing the transmittance exceeding 1.000, and **C** is the acetylsalicylic acid concentration in the sample introduced in the flow system, in mol l^{-1}. This curve was applied to the analysis of the acetylsalicylic acid contained in nine different solid remedies. A typical experimental recording graph concerning to the calibration curve and to remedies analysis is shown in Fig.5. Table 1 summarises the results for the remedies that are compared, through *t*-test [33], with the concentrations found using Pharmacopoeia [29] procedure. It can be observed a complete agreement between the two methods under the degree of freedom ν=4 (n_1=n_2=3; $\nu = n_1 + n_2 - 2 = 4$) and at the confidence coefficient (1-α) = 0.95 (95% confidence level). Therefore it can be admitted that the results obtained with the proposed method are statistically identical to those resulted using the titrimetric procedure established by Pharmacopoeia [29].

The simplicity of the proposed method becomes clear when it is compared with that recommended by Pharmacopoeia, that uses alkaline hydrolysis of the acetylsalicylic acid during 30 minutes under heating, followed by titration of the excess of the added hydroxide with an HCl standard solution.

Table 1. Comparison using the statistical Student's *t*-test between the results obtained by the FIA proposed method and the titulometric analysis recommended by Pharmacopoeia [29]. Tabled t = 2.776 for degree of freedom ν = 4, ($n_1 + n_2 - 2 = 4$), and confidence coefficient (1-α) = 0.95 (95% confidence level) [33]; $n_1 = n_2 = 3$ in this instance.

Sample	Nominal content / (mg)	FIA method / (mg) ± SD	Pharmacopoeia method / (mg) ± SD	Calculated *Student's t* values
1	500	501.7 ± 2.9	498,5± 1.8	1.328
2[a]	100	98.9 ± 1.6	100.1 ± 1.2	0.849
3	500	503.9 ± 1.6	504.5 ± 1.5	0.387
4[b]	100	96.1 ± 1.7	97.3 ± 1.6	0.849
5	500	506.7 ± 1.7	506.0 ± 0.9	0.686
6[b]	100	98.9 ± 1.6	99.1 ± 1.4	0.133
7[c]	500	502.5 ± 3.3	499.0 ± 1.5	1.368
8[d]	200	202.2 ± 1.5	199.8 ± 1.7	1.497
9[e]	650	653.0 ± 3.5	650.6 ± 1.8	0.864

Other components besides starch: [a] yellow dye and flavour; [b] red dye and flavour; [c] caffeine 30 mg and red dye; [d] paracetamol 150 mg, caffeine 50 mg, yellow dye; [e] caffeine 65 mg.

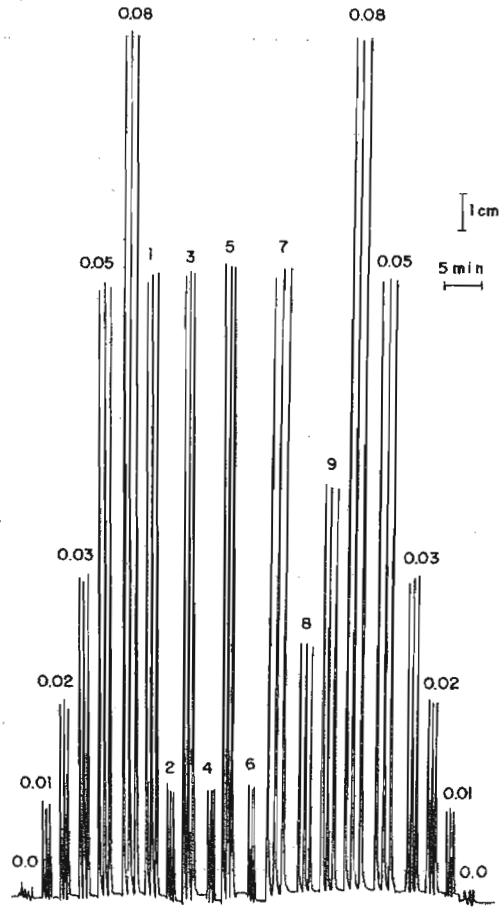

Fig.5 Typical recording graph for the calibration curve and for the determination of acetylsalicylic acid in real samples. Triplicate signals for standard solutions: 0.01, 0.02, 0.03, 0.05, 0.08 mol l^{-1}. Triplicate signals for determination of acetylsalicylic acid in real samples (numbered from 1 to 9). Flow rate = 3.0 ml min^{-1} for each carrier; sample volume = 150 µl; $[H_2SO_4]$ = 0.02 mol l^{-1}. Signal height, 1.0 cm = 0.040 transmittance units. Bromocresol purple solution, 1.0×10^{-5} mol l^{-1}, pH = 7.0. Calibration equation, **h** = -0.243 + 317 **C**.

4.Conclusions

To the operational simplicity must be, certainly, added the high sampling frequency as, under the proposed conditions, about 100 determinations can be performed per hour. The detection limit is about 1.0×10^{-3} mol l^{-1} (3 × SD). However, in the analysis of remedies, it is not necessary a very low detection limit as the matrixes usually present high concentration of the analyte.

Considering the performance, simplicity and low cost of the above proposed method, it can be recommended for the routine analysis of acetylsalicylic in remedies.

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