FLOW INJECTION DETERMINATION OF ACETYLSALICYLIC ACID IN PHARMACEUTICAL PREPARATIONS WITH AN AMPEROMETRIC DETECTOR

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This paper reports on an automated method based on a flow injection analysis single stream system with amperometric detection for the determination of acetylsalicylic acid in pharmaceutical preparations. The amperometric detection is based on the measurement at 0.8 V *vs* Ag/AgCl of the current generated when the acetylsalicylic acid hydrolysis product, salicylic acid, is oxidised at a glassy carbon electrode. The current produced is directly related to the concentration of the electroactive specie and the salicylic acid content is proportional to the level of acetylsalicylic acid present in the sample, thus it is possible to determine the concentration of acetylsalicylic acid electrochemically. This system allows a sampling rate of 150 samples per hour without requiring any pre-treatment, apart from dissolving them in a NaOH/KCl buffer, pH 13.0. The results obtained were compared with those obtained using reference methods and demonstrated excellent agreement with relative deviation always lower than 2%.

Key words: Acetylsalicylic acid; FIA; Amperometric detection; Pharmaceutical preparations; Electrochemistry.

Acetylsalicylic acid (ASA), commonly known as aspirin, was introduced in medicine in 1899 and is still one of the most commonly used analgesic, antipyretic or anti-inflammatory drugs. Its determination in pharmaceutical preparations requires considerable work in control laboratories of the pharmaceutical industry.

The official analytical method, described in the Portuguese Pharmacopoeia[1] and the British Pharmacopoeia[2], for its determination is a back-titration method, that is simple and economical, but requires heating under

reflux for 10 min. The U.S. Pharmacopoeia[3] recommends a more specific method, HPLC, which allows simultaneous determination of ASA and salicylic acid (SA). Several methods have been developed for the determination of ASA in pharmaceutical preparations, *e.g.* UV-VIS spectrophotometric[4], fluorimetric[5], potentiometric[6] and HPLC (ref.[7]).

Some of these procedures can be considered neither simple nor rapid and some of them involve expensive and complex equipment, which is inappropriate for routine analysis. This situation justified the development of an alternative method that combines the advantages of amperometric detection with those of flow injection analysis (FIA). This system was used in the determination of ASA in pharmaceutical preparations available on the Portuguese market without subjecting the samples to any pre-treatment other than dissolution in a suitable electrolyte. The results obtained were compared with those obtained by reference methods[1,3]. The FIA system developed may also be used for the determination of SA, for which it is sufficient just to modify pH of the electrolyte and the detection potential[8].

EXPERIMENTAL

Apparatus

The FIA system developed (Fig. 1) consists of an Ismatec Mini S-840 peristaltic pump to propel the solutions, a Rheodyne 5020 valve to inject the solutions and, as the detector unit, an electrochemical system consisting of a VA 641 Metrohm detector and a 656 Metrohm electrochemical wall-jet cell. The cell consists of three electrodes: a Metrohm glassy carbon electrode as the working electrode, a Metrohm gold electrode as the auxiliary electrode and a Metrohm Ag/AgCl (3 M KCl) as the reference electrode. At the end of every day, the surface of the working electrode was manually cleaned and polished with an abrasive surface (Kemet, PSU 8 type) which had been previously sprayed with Kemet spray (1 μm) and kept dry until the next experiment. The electrode surface was then washed with water and stored dry until the next day. To link the various components of the FIA set up, Omnifit Teflon tubing (0.8 mm i.d.) and Gilson end fittings were used. Additional home-made dampers

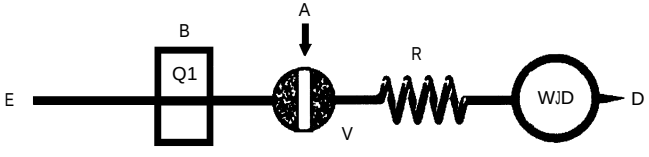

FIG. 1
Flow injection manifold used for acetylsalicylic acid determination: A sample, B peristaltic pump, E support electrolyte (NaOH/KCl buffer), Q1 flow rate (2.4 ml min^{-1}), V injection valve (80 μl), R mixing coil (40 cm), WJD wall-jet detector, D waste

were constructed as described elsewhere[9]. To record analytical signals, a Kipp & Zonen (model BD 112) data recorder was used.

The electrochemical behaviour of ASA and SA was investigated using a 663 VA Metrohm system containing a glassy carbon working electrode (Metrohm, d = 3.0 mm), a glassy carbon rod counter electrode (Metrohm) and a Ag/AgCl (3 M KCl) reference electrode (Metrohm) attached to a Autolab PSTAT 10 potentiostat/galvanostat running with model GPES version 3 software (EcoChimie, Netherlands). The measurements of pH were performed using a Metrohm E 520 pH-meter connected to a glass electrode of the same brand. In the determinations carried out by the HPLC reference method[3], a Sykan model A 1210 liquid chromatograph, equipped with a UV visible detector (λ = 280 nm) model 3200, was used. Separation of sample components was accomplished using a Nucleosil 100-10 C18 column (250 × 4 mm, 10 μm particle size, Macherey–Nagel, Germany). The separation was carried out at room temperature using a mixture of 850 ml of water, 150 ml of acetonitrile and 2 g of sodium heptane-1-sulfonate as mobile phase, adjusted with glacial acetic acid to pH 3.4.

Reagents and Solutions

Analytical grade chemicals were used without any additional purification. Deionised water with conductivity less than 0.1 μS cm^{-1} was used throughout. The support electrolyte (NaOH/KCl, pH 13.0 and I = 0.5 mol l^{-1}) was prepared by dilution to 100 ml of 66.0 ml of 0.5 M NaOH and 25.0 ml of 0.5 M KCl. This electrolyte was used as carrier. In the reference method (HPLC), all the solvents used were of HPLC grade. Prior to use, the solvents were filtered and the air removed with helium.

Standard and Sample Preparation

The acetylsalicylic acid (Riedel) stock solutions (10^{-3} mol l^{-1}) were prepared in a supporting electrolyte, by dissolution of weighed amounts of ASA (this compound is not readily soluble in water). More dilute solutions, between 5 and 50 μmol l^{-1}, were prepared by careful dilution of the stock solutions with the supporting electrolyte. These working solutions were used for calibrating the system. All these solutions were prepared daily.

The determination of acetylsalicylic acid was performed with commercial tablets available on the Portuguese market. Ten tablets were powdered and a quantity corresponding to 500 mg of ASA was carefully weighed. This sample was then diluted with 50.00 ml of supporting electrolyte (NaOH/KCl, pH 13.0). The sample was afterwards carefully diluted with the same support electrolyte in order to obtain a concentration within the calibration curve range.

RESULTS AND DISCUSSION

The electrochemical behaviour of ASA was initially studied over a large pH range, between 1.2 and 13.0, at a glassy carbon working electrode, using differential pulse voltammetry (Fig. 2). The appearance of an oxidation peak was only confirmed at strongly alkaline pH. A decrease in peak potential as the alkalinity increased was also observed. A more detailed study allowed us to conclude that the anodic peak obtained is due to the oxidation of the SA, formed by hydrolysis of ASA. In fact, the dominant degradation

reaction of ASA is the hydrolysis to SA and acetic acid[10]. In solution, the rate of decomposition of ASA to SA is dependent on pH. At pH 11–12, ASA is immediately hydrolysed; in neutral and acidic solutions (pH 4–8), the hydrolysis rate is slow, and the maximum stability is attained at pH 2–3 (ref.[11]). After hydrolysis, the SA content is proportional to the ASA concentration present in the sample allowing, therefore, the electrochemical determination of ASA concentration.

The initial design of the FIA manifold, which was afterwards gradually optimised, was set up with the objective of allowing the introduction of samples without pre-treatment. Hence, a single channel manifold (Fig. 1) was set up, and the solution responsible for preparation of the sample was used as a carrier to perform the electrochemical measurement.

The FIA system used in the determination of acetylsalicylic acid was optimised by the univariant method with the purpose of maximising both the sample rate and reproducibility. The parameters studied were pH and ionic strength of supporting electrolyte (E), the working electrode potential, the flow rate (Q1), the injection volume (A) and the length of the reactor (R). The study of the best working conditions of the FIA manifold was made using a 100 μM solution of acetylsalicylic acid.

First the influence of pH of supporting electrolyte on the rate of decomposition of ASA to SA, in the interval between 11.0 and 13.5, was studied at flow conditions. It was observed that at pH higher than 12.5, ASA was hy-

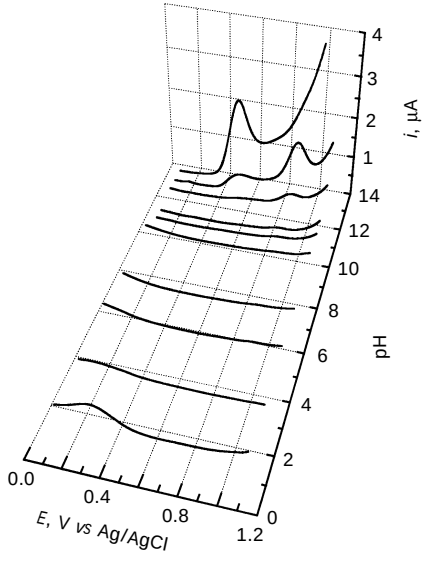

FIG. 2
3D plot of differential pulse voltammograms of a 100 μM acetylsalicylic acid solution in different buffer solutions. Pulse amplitude 50 mV, scan rate 5 mV s^{-1}

drolysed rapidly but only above pH 13.0 could it be considered instantaneous. Therefore to guarantee that there is no time dependence of peak heights, we have chosen to use a supporting electrolyte with pH 13.0 in all studies. Additionally, the optimal ionic strength of the buffer solution was studied over the interval of 0.1 to 1 mol l^{-1} by measuring the peak height. It was noted that there was a slight increase in peak height with ionic strength until it reached a maximum near ionic strength 0.5 mol l^{-1}. Thus we used a buffer solution of NaOH/KCl of pH 13.0 and ionic strength 0.5 mol l^{-1} as a supporting electrolyte in all subsequent studies.

To select a value of the working electrode potential, a study of the variation of the peak height with the potential applied between 0.5 and 1.1 V was carried out. It was found that the height of the analytical signal increased to a maximum at 0.8 V, and then it was virtually constant (Fig. 3). The value of 0.8 V was selected as the working potential since it showed the highest peak height and greater reproducibility.

Selection of the most appropriate flow rate was dependent on limitations of the wall-jet cell indicated by the manufacturer, who advised against the use of very high flow rates[12]. We have verified that flow rates higher than 2.4 ml min^{-1} are unsatisfactory since they produce high pressures within the system due to the mechanical characteristics of the electrochemical detector whose dead volume is about 1 μl (ref.[12]). These high pressures inside the system gave rise to irreproducible signals. Although lower values gave reproducible signals they compromised the sampling rate and therefore the value of 2.4 ml min^{-1} was chosen.

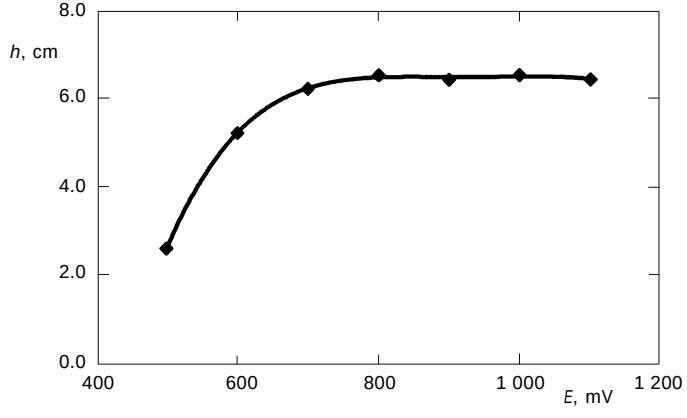

FIG. 3
Peak height *h* obtained for a 100 μM acetylsalicylic acid solution as function of the working electrode potential *E*

Having optimised the flow rate, the injection volume was investigated. For this purpose, loops were made of the same Teflon tubing that was used for other parts of the system (0.8 mm i.d.) with lengths between 8 and 20 cm. A 16 cm loop was selected since higher injection volumes lead to a decrease of sampling rates and lower injection volumes produced smaller peak heights and less reproducible analytical signals. The real volume that could actually be introduced in the system (including the internal volume of the injection valve) was accurately evaluated for a 16 cm loop by titration of the volume obtained from 10 replicate injections of a solution of known concentration[13] and this corresponded to 80 μl.

Finally, the way in which the variation in the reactor length (R), which comes before the amperometric detector and in which the mixture of the sample plug with the electrolyte takes place, affected the analytical signal was evaluated. We have tested reactors (made with Teflon tubing 0.8 mm i.d.) with lengths between 20 and 50 cm. As predicted, those systems in which the reactor length was short produced a less reproducible analytical signal due to insufficient mixing between the sample and electrolyte. Although the reproducibility of the recorder output was constant for reactor lengths greater than 40 cm, the amplitude of the analytical signal was unnecessarily diminished due to dilution and this lead to decreased sampling rate. Therefore a reactor length of 40 cm was selected.

With the chosen parameters, the system allows a detection limit, evaluated as the concentration corresponding to three times the standard deviation of the background signal[14] of 0.9 μmol l^{-1} and about 150 samples per hour could be determined.

Determination of Acetylsalicylic Acid in Pharmaceutical Preparations

In order to assess the quality of the results obtained with the single-channel manifold developed, determinations by FIA were performed for eight samples commercially available in Portugal. In five of these pharmaceutical products, only acetylsalicylic acid is present as active substance and the other components do not give a significant signal under the experimental conditions used in the determinations. The other three pharmaceutical preparations contain a second active substance, caffeine, at lower concentrations.

The FIA system was calibrated by three replicate injections of different acetylsalicylic acid standards in a concentration range from 5 to 50 μmol l^{-1}. Figure 4 shows the diagram corresponding to the injection in triplicate of

five standard solutions and four samples, obtained in the determination of ASA in pharmaceutical preparations.

The accuracy of the results provided by the developed FIA system (CF) was assessed by comparing them with the results obtained from the reference methods (CR), *i.e.*, titrimetry[1,2] and HPLC (ref.[3]). The relative standard deviation of the proposed methodology was always lower than 2%. Table I lists the mean results obtained for three replicate determinations of eight pharmaceutical formulations commonly available in Portugal.

With this set of values, applying an orthogonal least squares fitting, we can obtain in any case (comparison between CF and titrimetry or HPLC) a linear relationship $x_{CF} = (0 \pm 2) + (1.000 \pm 0.006)x_{CR}$. Considering these parameters we can conclude that there is a good agreement between the methodologies used. We have compared the experimentally determined calibration parameters (slope and intercept) with the theoretically expected values by means of Student's *t*-test and, for a confidence interval of 95%, obtained a value of *t* lower than the critical value[15]. Repeatability of the method was evaluated by performing ten consecutive injections of each of the solutions to be analysed. The relative standard deviations obtained were less than 2%.

We have also studied possible interference of other active substances, which are associated with acetylsalicylic acid in pharmaceutical preparations available on the Portuguese market. We have concluded that

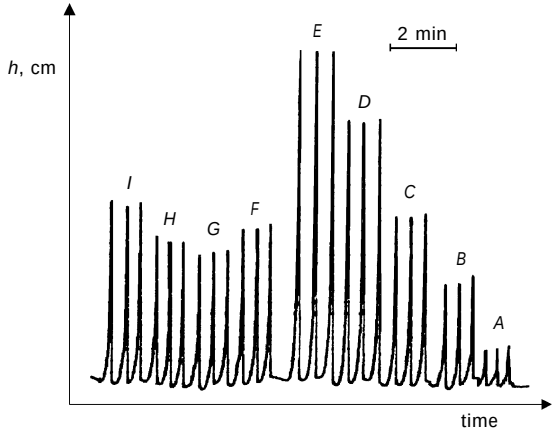

FIG. 4

Recorder output for a series of acetylsalicylic acid standards in μmol l^{-1} (*A* 5.0, *B* 8.0, *C* 10, *D* 30, *E* 50) and real samples (*F*, *G*, *H* and *I*) under the conditions proposed for the FIA technique

paracetamol and ascorbic acid interfere with ASA determination, as these compounds exhibit oxidation peaks at lower potentials than that of ASA. We have observed, however, that caffeine does not interfere with the determination of ASA by amperometry, as the former has an oxidation peak at a much higher potential (around 1.7 V *vs* Ag/AgCl). Recovery studies using three pharmaceutical formulations containing both ASA and caffeine were performed and the results obtained were between 98 and 101%.

CONCLUSIONS

The FIA system which has been developed allows the determination of acetylsalicylic acid in pharmaceutical preparations at a sampling rate of 150 samples per hour. The results obtained are in good agreement with those obtained by reference procedures. The proposed method is simple, easy to operate, inexpensive and rather economical taking into account the handling and use of reagents. Moreover, complex pre-treatment of the samples is unnecessary since the preparation of the samples for analysis only involves dilution with an appropriate buffer.

The results obtained suggest the feasibility of replacing time-consuming and costly procedures for the analysis of ASA by the proposed system because the same manifold may be used to determine SA in pharmaceutical

TABLE I
Determination of acetylsalicylic acid in commercial pharmaceutical preparations using the FIA manifold, titrimetry[1,2] and HPLC (ref.[3])

Preparation[a]	FIA[b], mg/tablet	Titrimetry[b], mg/tablet	HPLC[b], mg/tablet
AAS®	491.8 ± 6.0	492.1 ± 5.7	496.7 ± 3.5
Aspirina®	501.3 ± 6.9	497.4 ± 6.2	498.2 ± 4.1
Aspro®	315.8 ± 3.4	320.8 ± 5.3	314.7 ± 4.3
Melhoral Infantil®	102.7 ± 4.6	103.4 ± 4.4	100.8 ± 1.0
ASP Infantil®	101.2 ± 2.4	100.3 ± 3.5	100.9 ± 1.6
Cafiaspirina®[c]	495.7 ± 8.7	496.4 ± 6.9	499.5 ± 2.4
Asfeina®[c]	500.6 ± 3.5	498.9 ± 5.4	498.7 ± 3.7
Zimaina®[c]	501.6 ± 6.4	502.1 ± 8.6	501.4 ± 3.8

[a] Commercially available dosage forms with Portuguese commercial names. [b] Mean and standard deviations of 3 determinations for different samples. [c] Samples containing ASA and caffeine as active substances.

preparations simply by substituting the pH 13.0 buffer for one of pH 7 and adjusting the working electrode potential[8]. By coupling the FIA technique and amperometric detection, this method can be easily implemented in any routine analytical laboratory.

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