An oxidative column for the flow injection analysis–spectrophotometric determination of paracetamol

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Abstract: A flow injection–spectrophotometric determination of paracetamol is reported. The procedure is based on the oxidation of the analyte with potassium hexacyanoferrate(III) previously retained in an anionic exchange column and the reaction of the *N*-(hydroxyphenyl)-*p*-benzoquinonimine so produced with phenol. The oxidation is carried out at room temperature and in aqueous ammoniacal solution. Concentrations of paracetamol in the 0.20–20 ppm range are determined with relative standard deviations (RSD) of 0.6% ($n = 40$) at an injection rate of 42 samples h^{-1}. The influence of foreign species on the assay and its application to the determination of paracetamol in several pharmaceutical formulations are reported.

Keywords: *FIA–spectrophotometry; oxidizing minicolumns; paracetamol assay.*

Introduction

Paracetamol or acetaminophen (*N*-acetyl-*p*-aminophenol), is often used as a replacement to aspirin in pharmaceutical preparations because of its analgesic and antipyretic activities. Overdose of paracetamol causes hepatic necrosis probably due to a metabolite, *N*-acetyl-*p*-benzoquinone. Blood concentrations over 300 ppm, several hours after ingestion, causes serious hepatic damage. The diagnosis must be quick, and rapid analytical methods for paracetamol are essential in such circumstances.

Data on the physical, chemical or biopharmaceutical properties of paracetamol metabolic routes, pharmacokinetics, etc. are readily available [1, 2]. A number of review articles have dealt with the analytical chemistry of paracetamol [3, 4]. Several types of analytical procedure have been proposed involving colorimetry based upon nitration, oxidation and hydrolysis reactions [5–7].

Ultraviolet spectrophotometric procedures have been adopted by the British Pharmacopoeia [8] and US National Formulary XI [9] for the determination of paracetamol in tablets. Most recent methods for paracetamol determination are chromatographic in nature [10–12]; however, two flow injection analysis (FIA) colorimetric procedures have been proposed [13, 14].

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The wide use of immobilized reagents in FIA is one of the recent attractive trends. The use of such reagents is of general interest because it results in simpler equipment, a reduction in reagent consumption and lower sample dispersion which leads to increased sensitivity and sample throughput. The present use of immobilized reagents is restricted mainly to enzymatic catalysis. Few papers can be found on the use of reductive minicolumns [15, 16] and others dealing with preconcentration methods [17, 18]. This paper presents the use of an immobilized strong oxidant and is devoted to the spectrophotometric determination of paracetamol with the aid of the FIA technique. The method is based on the oxidation of paracetamol with hexacyanoferrate(III), retained on an ionic exchange resin as an immobilized reagent. The oxidation product, *N*-acetyl-*p*-benzoquinonimine, reacts with phenol giving a blue product, *N*-(*p*-hydroxy-phenyl)-*p*-benzoquinonimine [19], which is monitored at 630 nm.

Experimental

Reagents and apparatus

Aqueous solutions of potassium hexacyanoferrate(III) (Panreac, PRS, Barcelona, Spain); phenol (Probus, QP, Barcelona, Spain); ascorbic acid (Panreac, PA, Barcelona, Spain); acetylsalycilic acid (Panreac, PRS, Barcelona, Spain); citric acid (Probus, AR, Barcelona, Spain); folcodine (donated by Lab. Semar, S.A.); phenilephrine hydrochloride and chlorphineramine maleate (donated by Europharma, Madrid, Spain), pheno-barbital acid (donated by Lasa, Madrid, Spain); Diazepam (donated by Roche, Madrid, Spain); paracetamol (Sterling Wintrop, Madrid, Spain); and, caffeine (Eastman Kodak) were used.

Preparation of the oxidative resin. The resin (Duolite A102D, Probus) is added to a saturated aqueous potassium hexacyanoferrate(III) solution and stirred slowly for 30 min. The separated resin is repeatedly washed with distilled water until the filtrates are colourless. The preselected size of resin particles was in the range $0.50–0.70 \pm 0.05$ mm. Oxidative minicolumns were prepared by introducing, under suction, the resin into Teflon coils of different internal diameter. The resin was stored in 0.40 M aqueous ammonia solution before its use as an immobilized oxidant.

Samples of the same resin were treated in a similar way using different oxidants such as MnO_4^- and $Cr_2O_7^{2-}$. Likewise a cationic resin (Duolite C20, Probus) was treated with cerium(IV) ions.

FIA assembly. This is illustrated in Fig. 1b and consists of a sample injector from Rheodyne a Model Minipuls 2 pump (Gilson, Villiers, France) and a CE 202 UV spectrometer (Cecil Instruments, Cambridge, UK) equipped with a 30 μl flow-cell (Hellma) and a D5000 Omniscribe recorder (Houston Instruments). Teflon tube coils of several internal diameters were used to construct the oxidation columns.

Procedures

Figure 1b illustrates the flow manifold used. An ammoniacal solution of paracetamol was injected into a carrier stream (ammoniacal aqueous solution) flowing at 1.2 ml min^{-1}, through the oxidative resin column (25 cm × 1.50 mm i.d., plus 225 cm × 0.80 mm i.d.) maintained at room temperature. The effluent was merged with a 0.16 M phenol solution flowing at 1.2 ml min^{-1}, and reacted at 80°C in a coil (length 300 cm). The exit stream was cooled in a coil of 150 cm at room temperature, followed by a 100-cm coil suspended in a continuous circulating tap water-bath (at between 15–20°C).
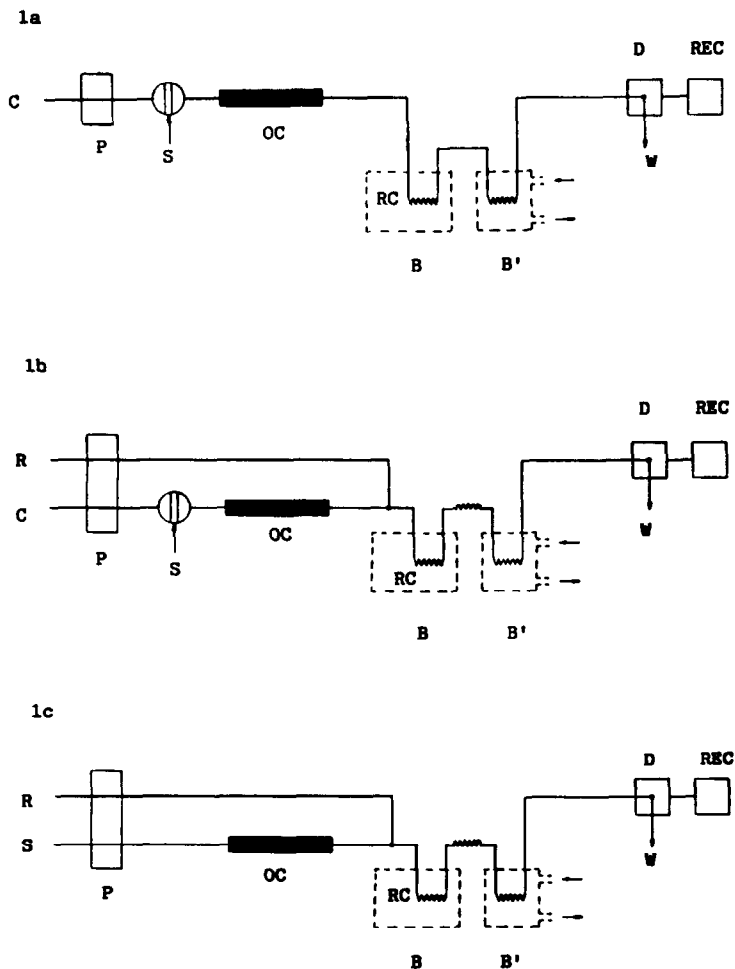
1a

1b

1c

Figure 1
Continuous flow assemblies: C, carrier; P, sample; OC, oxidative column; RC, reacting coil; B, water-bath at 80°C; B', continuous circulating tap water; D, detector; REC, recorder; and W, waste. (a) With phenol stream flowing through the oxidative column. (b) Proposed FIA assembly for paracetamol oxidative determination. (c) Flow assembly with continuous stream of paracetamol for testing column stability.

Determination of paracetamol in pharmaceutical samples (tablets). Powdered tablets were dissolved in distilled water, any residual insoluble matter was removed by filtration and the volume adjusted to 500 ml with distilled water. Different aliquots (according to formulations) of this solution (five as minimum) were taken and diluted to 100 ml; the resulting solution was made up to 0.40 M in ammonium hydroxide.

Results and Discussion

The previous work carried out under static conditions indicate that the hexacyano-ferrate(III) resin is the only reagent able to oxidize the paracetamol solution in the required basic medium. The oxidative reaction was checked by observing the colour

obtained on reaction with the phenol solution. The presence of ammonia was necessary for the paracetamol oxidation and it is important to control the concentration precisely, in order to get reliable results. The oxidation rate is slightly higher when the temperature is increased, however, the rate is sufficient at room temperature, to prevent the use of high temperatures which can cause problems when working with resins.

The preliminary tests on the FIA system were carried out using the assembly represented in Fig. 1a. These revealed that the oxidation resin retained, by adsorption, the blue derivatization product. From which it may be deduced that the FIA assembly should not allow the phenol stream to flow through the resin reactor. This and other chemical problems encountered with the particular assembly led to the adoption of the FIA assembly represented in Fig. 1b.

A 0.1 M ammonia concentration was used both in the sample and carrier stream solutions, in order to wash the column with the same solution as the sample. Other parameters were phenol concentration 5 g l^{-1}, reaction coil length 300 cm operated at 60.0°C and 100 cm length of coil in the continuous circulating tap water-bath. A flow-rate of 1.2 ml min^{-1} was used throughout and samples introduced as 110-μl volumes of 50 ppm paracetamol solution. The absorbance was monitored at 630 nm.

A tedious problem which arose from the formation of bubbles, may be due to the presence of ammonia, at high temperatures, was avoided by cooling the stream after the reaction with phenol.

The optimization of the chemical and FIA parameters was carried out by means of the univariate method with the aim of a compromise between sensitivity, sample throughput and reproducibility (RSD). The temperature for the oxidized paracetamol and phenol reaction was studied up to 80 ± 1°C. The results show a higher analytical signal with increased temperature. The quality FIA peaks obtained, led to the selection of 80°C for further work.

The use of a coil between the oxidative column and the merging point with the phenol stream, also was tested up to 100-cm lengths. No variation was observed in the average peak height but the reproducibility is best when the coil is not used.

The influence of the total amount of resin inside the reactor column was studied with columns of different length, all of them 0.80 ± 0.05 mm i.d. The range studied was between 50–250 cm. The signal was found to increase with the length up to 150 cm, which suggests that insufficient oxidant is available with" the shorter columns. The optimum length was shown to be 150 cm. Longer tubes resulted in slightly shorter peaks, due to zone dispersion. However, higher resin amounts would be expected to result in longer life-span of the column, accordingly, a 250 cm column length was chosen for further studies.

The influence of the sample volume was tested over the range 30–580 μl. The higher sample volumes tested required more resin for complete oxidation of the analyte and these points resulted in wider base-peaks leading to a lower effective injection rate. Therefore, the range 135–300 μl, was selected as a compromise.

The amount of resin required was studied by testing two parameters simultaneously, namely length and internal diameter of the Teflon column. The injected samples were 260 μl of a 10-ppm paracetamol solution.

The set of experiments carried out resulted in the following results and conclusions. The variation of the column length with a coil of 0.80 mm i.d., in which the resin is placed as a SBSR configuration, resulted in the range 200–225 cm being found to be the most suitable choice. Using three different internal diameters, 0.80, 1.50 and 2.20 mm,

all of them 100 cm long, two conclusions were drawn: (i) 2.20 mm was not suitable as the base-peak was too wide; and, (b) the analytical signal is significantly increased with the use of a 1.50 mm i.d. column, compared with that of 0.80 mm i.d. The next experiments were carried out with two diameters, 0.80 and 1.50 mm, a length of 225 cm and sample volumes varying from 135 to 305 µl. The results indicate that no clear selection could be made, the thinner column appears to be preferable because of the greater peak heights and sharper peaks and the lower amount of resin required. On the other hand, such a column would prevent the use of a larger sample (volume or concentration), and results in shorter column life. Accordingly, a compromise was made involving a column consisting of 25 cm of 1.50 mm i.d. tubing plus a 225 cm × 0.80 mm coil. The injected sample volume was maintained at 150 µl.

The effect of flow-rate was studied by varying, simultaneously, the rate for both streams from 0.6 to 2.1 ml min^{-1}. The value of 1.2 ml min^{-1} was selected.

The coil length for the reaction with phenol was tested up to 700 cm. Also, it was observed that an additional coil placed between both baths avoids bubbling and probably allows the reaction to run to completion. Because of this, a definitive FIA assembly is made by 300-cm coil lengths immersed in the water-bath at 80°C, followed by 150 cm outside the bath plus 100 cm inside the continuous circulating tap water-bath.

The influence of phenol concentration over the range 5–30 g l^{-1} showed 15 g l^{-1} as the most appropriate.

The effect upon performance of the temperature of the oxidative column was studied between ambient temperature and 50°C. A slight increase of peak height was observed up to 30°C, followed by a small decrease up to 50°C. An observation which indicates that working at room temperature would be acceptable.

The ammonia concentration is another important parameter to control because of its effect upon the chemistry of the paracetamol oxidation and the stability of the resin. A concentration over 0.6 M is unnecessary. The stability of the column was checked using a separate continuous flow assembly (similar to that represented in Fig. 1c) in order to submit the resin to a fast consumption; the resin is preconditioned during 6 min with an ammonia solution at the same concentration (studied range over 0.10–0.60 M; Fig. 2).

Those results were considered as orientative as the FIA assembly implies a washing step between injections; anyway the consumption of the resin is faster when increased ammonia concentrations are used; the preselected 0.40 M was checked by injecting a high number of samples using again the FIA assembly in Fig. 1b. Five sets of 36 injections were tested on five different days, always using the same column. Figure 3 shows some of the obtained peak heights, it can be seen as a slight decrease of the transient signal. The analytical signal was always slightly higher with newly prepared resin, than resin stored some time before use. This suggests that the useful life of the column depends on the slow hexacyanoferrate(III) evolution from the resin rather than its reduction to hexacyanoferrate(II).

The efficiency of the resin was also checked after several days storage containing (a) 0.40 M aqueous ammonia solution, and (b) distilled water. Both resins show a slight diminution in their efficiency, which suggests that the resin life is dependent more upon the slow loss of ferricyanide, than a redox reaction. However, there was no significant difference in the oxidation behaviour of resin when it was kept in either water or 0.40 M ammonium hydroxide.
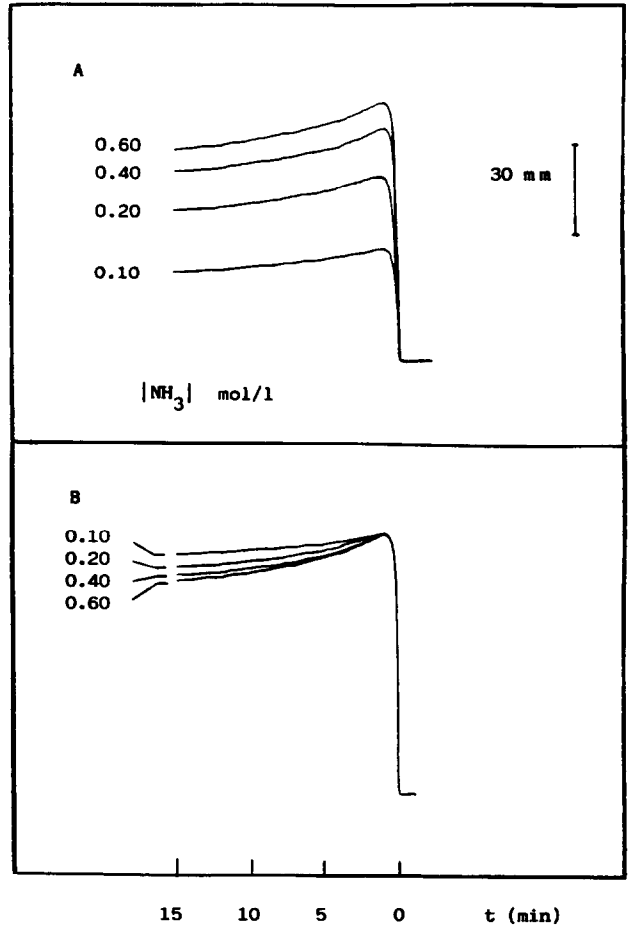
Figure 2
Study of the consumption of the oxidative strength of the column. (A) Obtained experimental curves. (B)
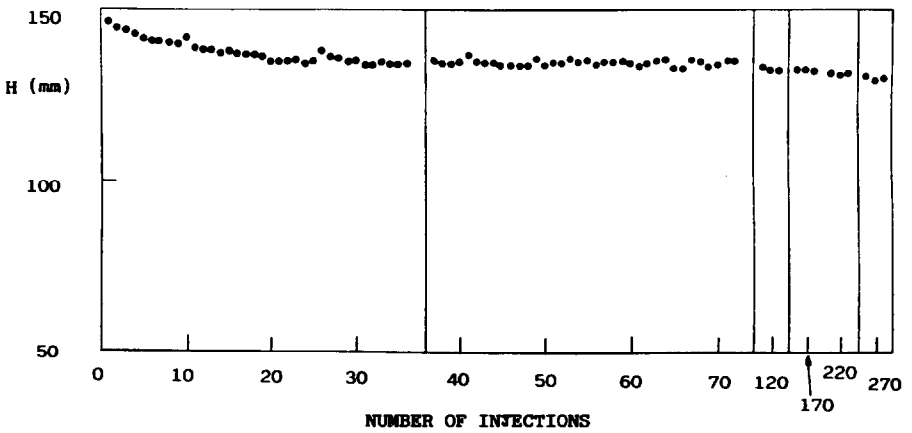Comparative slope of the obtained curves.

Figure 3
Constancy of peak height with column use. Sets of injections on different days.

Analytical application

The calibration graph is found to be linear in the range 0.20–20 ppm paracetamol (equation $y = 0.005 + 0.0331$, with a correlation coefficient 0.9998); and the minimum error in the range 5–15 ppm (see Fig. 4).

The reproducibility of the determination was tested by injecting into the 0.40 M ammonia carrier stream, 40 samples containing 10 ppm paracetamol. An RSD of 0.6% was obtained. A sample injection rate of 42 samples h^{-1} was achieved.

The tolerance of the method to interfering compounds, which commonly are found in typical paracetamol pharmaceutical formulations, was investigated. The results from these studies are summarized in Table 1. The interference due to ascorbic acid is decreased with aged solutions.

Paracetamol was determined in different pharmaceutical formulations, and the results compared with those supplied by the manufacturer. Typical results are shown in Table 2.
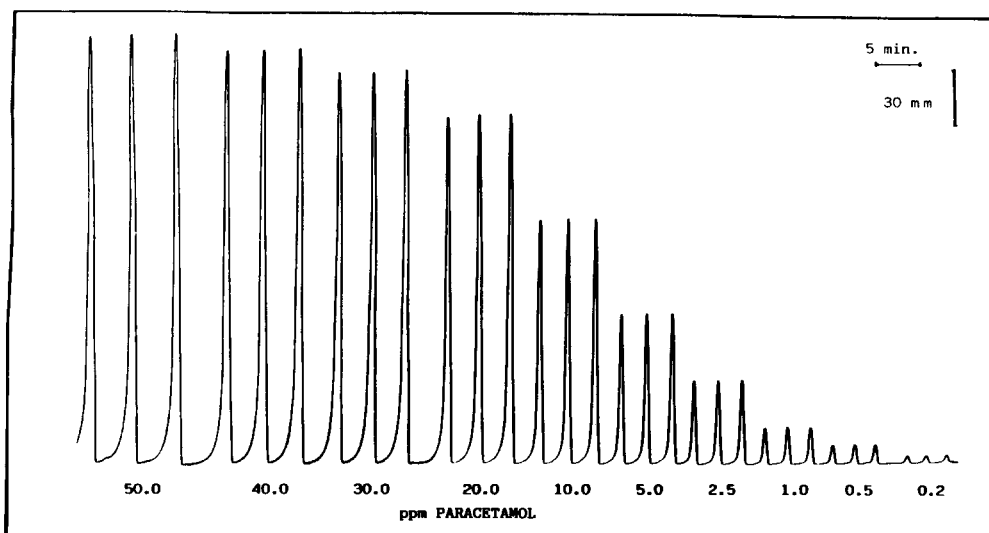

Figure 4
Calibration FIA peaks.

Table 1
Influence of active ingredients on the paracetamol determination

Interference	Amount (ppm)	Relative error (%)
Folcodine	125	1.7
Citric acid	125	2.0
Caffeine	250	1.3
Salycilic acid	250	0.3
Phenylephrine	125	−1.0
Phenobarbital	250	1.3
Diazepam	Sat.	−1.5
Acetylsalicilic acid	250	1.1
Ascorbic acid	100	−51.5 (Newly prep.)
Ascorbic acid	100	−36.5 (30 min)

Table 2
Amount of paracetamol in different pharmaceutical formulations

Formulation	Reported amount (g)	Obtained (g)	Relative error (%)
Effelgaran	0.330	0.3180	−3.6
Fluipiron	0.200	0.1988	−0.6
Actron	0.133	0.1395	4.9
Meridol	0.250	0.2561	2.4

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