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Fast determination of paracetamol by using a very simple photometric flow-through sensing device

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Abstract

A simple flow-through UV optosensing device was developed for the determination of paracetamol based on its transient retention and concentration on a suitable active solid support (Sephadex QAE A-25 anion-exchange resin) packed in the flow cell and the continuous monitoring of its native absorbance on the solid phase at 264 nm. The sample was injected into a 0.08-M NaCl carrier stream at pH 11.0 by using a simple monochannel FIA manifold. After developing the analytical signal, paracetamol was desorbed from the solid support by the carrier solution itself. A very good linear response was found in the concentration range 0.5–8.0 μg ml^{-1} with a RSD (%) of 1.24, a detection limit of 0.022 μg ml^{-1} and a sampling rate of 40 h^{-1}. A strong increase in sensitivity as well as a very much higher selectivity were achieved as compared with the conventional flow injection method as a consequence of the separation of the analyte from the sample plug and its retention on the active solid support placed in the detection area. Applicability of the proposed sensor to direct determination of paracetamol in pharmaceuticals (to solve the sample being the only treatment) was successfully demonstrated.

Keywords: Paracetamol; Solid phase spectroscopy-FIA; Flow-through optosensor; Pharmaceuticals

1. Introduction

Paracetamol (PCT) (acetaminophen, *N*-acetyl-*p*-aminophenol, 4-acetamidophenol) is an extensively employed antipyretic analgesic frequently prescribed solely or with other related drugs. With usual doses neither digestive upsets nor gastric erosions are observed as occurs with salicylates, and so, PCT has become a powerful alternative to the use of the latter.

Numerous methods have been reported for the analysis of PCT in pharmaceuticals [1] such as volumetric, spectrophotometric, electrochemical, fluorimetric and chromatographic methods.

The determination of paracetamol by direct UV spectrophotometry (both in batch and FIA modes) is usually not possible in the presence of compounds that are frequently found along with

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it, due to the interference caused by them because they also absorb light in the UV region. To avoid this problem arising from the non-specific absorption in this spectral region, derivative reactions have often been used in order to give coloured compounds followed by spectrophotometric determination in the visible region [2,3].

In order to solve the above indicated problem, another alternative to use can be a high resolving technique prior to the UV determination such as HPLC [4–9]. This involves more sophisticated instrumentation and a higher cost per analysis.

Another optical determinative technique, such as spectrofluorimetry, although more sensitive, also requires the use of derivative reactions [10] because PCT is not an intrinsic fluorophor and, in any case, the determination will be quicker and cheaper than that obtained by spectrofluorimetry if direct UV spectrophotometry is used.

Solid phase spectroscopy (SPS) has recently proved to be very appropriate for simple and direct UV spectrophotometric determination of organic compounds absorbing UV radiation in the presence of other species also absorbing light in this spectral region [11,12]. The use of an active solid support (usually an ionic exchanger) both to concentrate and separate the analyte from the matrix solution suggests by itself high selectivity conditions. In addition, the determination of the analyte concentrated on the solid support (or its reaction product, if it is the case) by direct solid phase light absorption measurements also intrinsically suggests high sensitivity conditions. Thus, by using SPS in the UV region several drugs could be selectively determined: vitamin B_1 in the presence of vitamins B_2, B_6 and B_{12} both in batch [11] and FIA [13] modes, diclofenac sodium in the presence of PCT and vitamins B_1, B_6 and B_{12} [12], ascorbic acid in the presence of codeine, caffeine, paracetamol and vitamins B_1, B_6 and B_{12} [14,15], and amoxicyllin in the presence of other penicillins and cephalosporins [16].

When SPS is implemented by FIA [17,18] two intrinsic advantages of SPS (namely, selectivity and sensitivity) are added to those from FIA such as speed, lower consumption of both reagents and solid support, higher reproducibility and minor human participation [13,15,16]. Such continuous flow systems with solid phase spectroscopic detection belong to the so-called flow through optosensors [19].

In this paper we propose a simple, speedy and inexpensive spectrophotometric continuous flow sensor for determination of PCT based on its transitory retention on an anion exchanger after the injection of the sample solution in an appropriate basic carrier stream. When the sample plug reaches the solid phase (located in the flow cell of the detector and continuously irradiated) PCT is retained on it because of the dissociation of its phenolic group and the signal corresponding to its intrinsic absorbance on the solid support is monitored at 264 nm. When the end of the tail of the sample plug reaches the cell, the arrival of the carrier quickly elutes the retained analyte and the recorder comes back to the base line, and so the active support remains ready for the next sample.

The sensor shows a high sampling frequency and a very good selectivity and it has been successfully applied to the determination of PCT in pharmaceuticals containing other accompanying species which are usually serious interferents in UV solution spectrophotometry.

2. Experimental

2.1. Reagents

All chemicals were of analytical grade. Deionized water was used throughout the work for the dilution of samples and reagents. All solutions, the same as deionized water, were filtered through a 0.45-μm Millipore membrane filter.

Standard aqueous solution of paracetamol of 1000 mg l^{-1} was used. It was prepared by dissolving 1000 mg of 4-acetamide-phenol (Fluka, Spain) in water.

Sephadex QAE A-25 (Aldrich, Spain) ion-exchange gel (40–120 μm; capacity 3 meq g^{-1}) in the Cl^- form was used as solid support placed with the aid of a syringe inside a 1-mm Hellma 138-QS quartz flow-through cell (50-μl inner volume) with glass wool in the outlet to keep resin beads from movement. The carrier/self-eluting solution used consisted of a 0.08-M NaCl solution at pH 11.0 (adjusted with NaOH solution).

2.2. Apparatus

All spectral measurements and real-time data acquisition of flow injection peaks were made with a Perkin Elmer Lambda-2 double-beam spectrophotometer controlled by means of a 386 personal computer, fitted with the software package PECSS 4.2 (Perkin Elmer, England) for data acquisition and processing. A Gilson Minipuls-3 (France) peristaltic pump with rate selector was used to generate the flow stream required for the system in the single manifold. Injections were carried out by using a Rheodyne Type 50 six-port rotary injection valve. The alternative selection of the carrier was also made by means of another Reodyne Type 50 rotary injection valve connected as a selecting valve.

Other apparatus consisted of a Selecta (Barcelona, Spain) Model Ultrasons ultrasonic bath and a digital Crison Model 2002 pH meter (Spain) fitted with a glass/saturated calomel electrode assembly and a temperature probe. Teflon tubing 0.8 mm. i.d. was also used.

2.3. Procedure

A monochannel manifold having a very low residence time was used. The length of the coil between the injection valve and the detector was the minimum required ($\sim$ 30 cm) as no derivative reactions were required.

A 0.08-M NaCl solution (at pH 11.0) stream was pumped through a Teflon tube at a flow rate of 1.08 ml min^{-1} and 600 μl of the sample containing PCT were injected into it. The absorbance was monitored at a wavelength of 264 nm in the personal computer, and sent to the printer. When the sample plug reached the detection area, the analytical signal was developed, and then the carrier itself eluted the analyte, so regenerating the ion-exchanger gel and the absorbance value returned to baseline.

2.4. Sample treatment

2.4.1. Tablets and granular packs

A total of ten tablets or granular packs were weighed and crushed. A suitable amount was then dissolved in water in an ultrasonic bath (10 min), filtered if necessary through a 0.45-μm pore size Millipore membrane filter and the filtrate and washing were made up to an appropriate volume.

2.4.2. 'Febrectal' (suppository)

The sample was dissolved in $CHCl_3$ (50 ml) and PCT was extracted by using four successive extractions with 25 ml of water each time at pH 8–9, and the extract was made up to a final volume of 1000 ml.

2.4.3. Paracetamol solutions

An appropriate volume of the pharmaceutical preparation 'Apiretal' was conveniently diluted to a known volume with deionized water without previous pretreatment.

In all cases, the final concentration of PCT in the solution to be injected was within the range 0.5–8.0 μg ml^{-1}.

3. Results and discussion

Preliminary experiments showed that PCT was sorbed on an anion exchanger only at pH values above 10. This fact can be explained by the dissociation of its phenolic group, it being fixed by means of an ion exchange process. Sephadex resins (QAE A-25 and DEAE A-25) were tested and it was found that the strongly basic resin QAE A-25 was more suitable because the fixation was faster (peak width of 135 s against 460 s with Sephadex DEAE) and the analytical signal higher (by a factor $\sim$2).

The spectrum of PCT in homogeneous solution at a 6.6×10^{-4} M concentration and a pH value of 11.0 obtained in a Hellma 138 QS cell (1 mm optical path length) showed a maximum absorbance value of 0.300 a.u. at 259 nm. When 600 μl of a 3.3×10^{-5} M solution were injected in the single channel manifold of the sensing device (by using a 0.08-M NaCl solution at pH 11.0 as carrier), a maximum absorbance (0.480 a.u.) was found at 264 nm. Thus, by using the sensor, both a slight bathochromic effect and a very high hyperchromic effect were observed with respect to similar conditions in homogeneous solution

phase: the analytical signal of PCT measured on the solid support was 32 times higher than in solution. This is a consequence of the concentration of the analyte in the detection area of the spectrophotometer due to its retention on the active solid support. A value of 264 nm was chosen as working wavelength.

3.1. Influence of experimental variables

3.1.1. Amount of solid support in the cell

The resin, as a slurry suspension in water, was introduced in the flow cell with the aid of a syringe. After conditioning it by passing the carrier solution for a few minutes, it was ready for use. Different amounts of resin (reaching different height levels in the cell) were tested and it was found that the maximum absorbance signal was obtained when the height of resin from the bottom of the cell was 17 mm because the sample plug was retained and concentrated on the irradiated zone of the cell in an optimized way. If the amount of solid support increased the signal decreased because a fraction of the sample plug was retained in an upper region, which was in an unirradiated zone. On the other hand if the height reached by the resin was smaller than 17 mm, the light beam irradiated the solution either partially or totally, and so the signal decreased drastically. The optimum level of resin corresponds to ~ 20 mg of it.

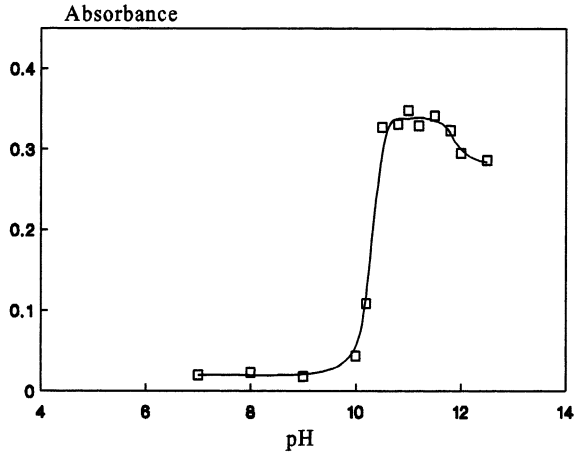

Fig. 1. Influence of the carrier pH on the analytical signal from 600 μl of a 3.3×10^{-5} M paracetamol solution. Flow rate: 1.35 ml min^{-1}.

Table 1
Influence of the nature of the carrier solution

Electrolyte	Absorbance	Peak width (s)
Potassium chloride	0.429	174
Sodium chloride	0.417	165
Sodium carbonate	0.281	91
Di-potassium hydrogen phosphate	0.288	135
Sodium tetraborate	0.362	122

3.1.2. Chemical variables

The influence of the pH of the carrier solution on the absorbance at 264 nm was studied by using a 0.05-M KCl solution and adjusting the pH value with a 0.1-M solution of KOH. This solution also eluted the analyte from the resin. The results obtained by injecting 600 μl of a 3.3×10^{-5} M sample solution of PCT are shown in Fig. 1. Below pH 10 PCT is not sorbed on the resin; from this pH value the retention strongly increases due to the dissociation of the phenolic group and fixation by ion-exchange. Above pH 10.5 the dissociation is complete and the signal is constant until pH 11.8. Above this pH value a slight decrease is observed probably due to the competition between the analyte and the free OH^- groups for the active sites of the ion exchanger. A working pH value of 11.0 was chosen.

Several 0.05-M salt solutions were tested as carrier/self-eluting solutions. The analytical signals and the corresponding peak widths are shown in Table 1. Na_2CO_3 shows the highest sampling frequency but also the lowest signal. NaCl was chosen as a compromise between a higher sensor response and a lower peak width. Several NaCl concentrations from 5×10^{-3} to 5×10^{-1} M at pH 11.0 were tested by injecting 600 μl of a 3.3×10^{-5} M PCT solution. It can be seen from Fig. 2 that the increase in the carrier concentration causes both the signal development time and eluting time to decrease, so allowing a higher sampling frequency but also decreasing the peak height due to the competition of the carrier

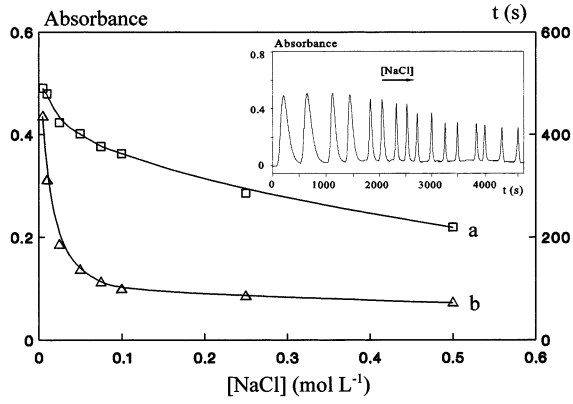

Fig. 2. Effect of the carrier/eluent solution concentration on (a) maximum absorbance and (b) peak width (time). Inset: signal development profiles (duplicate injections of each).

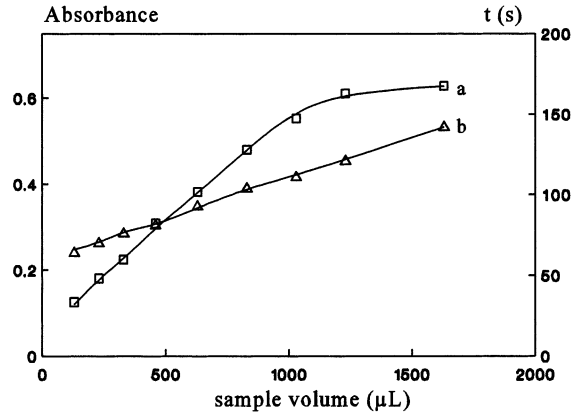

Fig. 3. Effect of the increase of sample volume injected on (a) analytical signal and (b) peak width (time).

ions for the active sites in the anion exchanger (as the ionic strength increases). A 0.08-M concentration was chosen as the most appropriate one.

The sample pH did not influence the analytical response in the range 3.0–13.0 and so there was no need for sample buffering. This can be explained because the surrounding alkaline environment of the resin beads provided by the carrier solution is suitable to allow the complete dissociation of the overall amount of PCT contained in the short sample plug reaching the sensing solid resin when its pH value is in the above range. pH values below 3 partially or totally neutralize the alkaline environment of the solid phase, so the signal drastically decreases because PCT (above all in the central zone of the sample plug) was not dissociated. Above pH 13, the free OH^- groups from the sample themselves compete with PCT for their fixation on the resin, also decreasing the analytical signal.

3.1.3. Flow system variables

A study of the influence of the flow system variables (flow rate and sample volume) was performed. It was observed that the increase in the flow rate from 0.3 to 1.5 ml min^{-1} caused the absorbance signal to decrease $\sim$30%. This can be explained by the fact that the retention of the analyte from the sample plug is not instantaneous and so, the contact time between the sample and the resin beads is too short at high speeds. A flow rate of 1.08 ml min^{-1}, that caused a signal decrease of only $\sim$15%, was selected because a very low flow rate becomes incompatible with a high sampling frequency.

The injection of increasing sample volumes with the same analyte concentration gave the results shown in Fig. 3. The sensor response increased linearly from 100- to 1000-µl sample volume because a larger amount of analyte was retained and concentrated in the same amount of solid support located in the sensing zone (from this sample volume value the signal does not increase much more because the pH value of the central zone of the sample plug does not allow all the analyte to be retained on the resin).

This is an interesting feature of this type of sensor because it allows working with a wide range of analyte concentrations by simply varying the sample volume used and carrying out a previous calibration for the selected volume. So, sensitivity can be increased by enlarging the sample volume due to the integrating nature of the sensor signal. Nevertheless, the higher the sample volume injected, the lower the sampling rate (Fig. 3). With 600 µl of sample, both a very good sensitivity and a very high sampling rate were obtained with this sensing device.

3.2. Analytical figures of merit of the optosensing device

The calibration of the sensor was carried out

for 600 μl of sample. A very good linear response was found within the concentration range 0.5–8.0 μg ml^{-1}. The linear regression equation obtained by least squares adjustment ($r = 0.9991$) was the following:

$$A = (0.004 \pm 0.006) + (0.106 \pm 0.002)C$$

where A is absorbance and C is PCT concentration in μg ml^{-1}.

The percent relative standard deviation (% RSD) was studied for a 4-μg ml^{-1} PCT solution. A value of 1.24 was obtained for ten independent sample injections, the detection limit (3σ criterion) being 22 ng ml^{-1} and the sampling frequency 40 h^{-1}. The extreme rapidity of the analysis should be noted for this flow-through sensing device with a regenerable solid phase as active zone. The sensing zone showed an operational life-time higher than 250 injections.

3.3. Interferences

The effect of the presence of foreign species in the determination of PCT by using the proposed optosensor was performed for an analyte concentration level of 5 μg ml^{-1}. The foreign species assayed were those usually found along with paracetamol either as active principles or as excipients. The highest concentration of potential interferents assayed was 1000 μg ml^{-1}. The tolerance level was considered to be the highest concentration of foreign species giving an error in the determination of PCT not exceeding $\pm$ 5%. Table 2 summarizes the tolerance levels found.

Table 2
Study of interferences

Foreign species	Tolerance level (μg ml^{-1})
Metocarbamol, lactose, saccharose, glucose	>100
Ephedrine, glutamic acid	50
Codeine, caffeine,	10
Salicylamide, ascorbic acid	2
Acetylsalicylic acid, saccharine	1

As can be seen, the method shows a very good tolerance level to most of the foreign species assayed, usually very much higher than the concentrations in which they are found along with the analyte in pharmaceuticals. The most serious interferences are from acetylsalicylic acid and saccharine (due to their competitive fixation on the anionic resin at the working pH and their spectral overlapping with the analyte). Nevertheless, the amounts of these species in pharmaceutical preparations allow PCT to be determined with the proposed sensor. On the other hand, it must be emphasized that the tolerance levels shown by the sensor are much higher than those in conventional homogeneous solution UV spectrophotometry. This is a consequence of the higher selectivity provided by the solid support in addition to its intrinsically higher sensitivity exhibited due to the concentration of the analyte from the sample plug in the solid phase.

3.4. Analytical applications

The proposed UV flow-through sensing device was applied to the determination of PCT in 14 different pharmaceuticals containing this active principle either alone or along with other compounds, including excipients. To check the accuracy of the proposed procedure, a recovery study was carried out by adding known amounts of the analyte (three different levels). Results are summarized in Table 3. Relative errors compared with the claimed amount were lower than 4% and the recovery study showed average values between 96 and 106% so indicating the utility of the proposed sensor for routine analytical control in pharmaceuticals.

4. Conclusions

The UV photometric flow-through sensing device here proposed allows rapid and continuous determination of PCT by simply injecting the sample in the on-line optosensor without any requirement other than the simple dissolution of

Table 3
Analytical applications[a]

Pharmaceutical	Amount added	Amount found $\pm \sigma$ (mg)	Recovery (%)	Pharmaceutical	Amount added	Amount found $\pm \sigma$ (mg)	Recovery (%)
Termalgin[b]	–	483 ± 2	–	Relaxibys[b]	–	479 ± 9	–
	100	582 ± 3	99		100	578 ± 2	99
	200	675 ± 4	96		200	682 ± 3	101
	400	895 ± 7	103		400	878 ± 5	100
Duorol[b]	–	489 ± 1	–	Cortafriol[b]	–	516 ± 4	–
	100	589 ± 3	100		100	622 ± 3	106
	200	689 ± 3	97		200	712 ± 6	98
	400	889 ± 6	101		400	900 ± 4	96
Robaxisal[b]	–	308 ± 9	–	Propalgina Lemon[c]	–	486 ± 8	–
	100	411 ± 6	104		100	582 ± 3	105
	200	513 ± 7	103		200	695 ± 8	99
	400	805 ± 5	99		300	786 ± 5	100
Apiretal[c]	–	97 ± 1	–	Frenadol[b]	–	648 ± 8	–
	50	147 ± 1	98		50	701 ± 6	106
	100	201 ± 2	104		100	752 ± 5	104
	200	300 ± 3	101		200	845 ± 8	98
Febrectal[b,d]	–	297 ± 3	–	Veganin[c]	–	248 ± 2	–
	100	402 ± 2	105		100	350 ± 3	102
	200	497 ± 4	99		200	446 ± 7	99
	300	396 ± 5	100		400	653 ± 6	101
Saldeva Forte[b]	–	482 ± 2	–	Algidol[b]	–	663 ± 9	–
	100	580 ± 2	98		50	712 ± 4	98
	200	687 ± 8	103		100	769 ± 5	106
	400	882 ± 6	104		200	866 ± 4	101
Analgilasa[b]	–	506 ± 3	–	Rinomicine[b]	–	399 ± 6	–
	100	607 ± 2	101		100	503 ± 2	104
	200	709 ± 6	101		200	595 ± 4	98
	400	908 ± 7	98		400	897 ± 5	102

[a] Amounts expressed as: [b]mg/unit; [c]mg/ml; [d]addition standard method.

the sample. The solid phase beads placed in the flow cell allow separation, retention and detection to take place simultaneously in the continuous flow cell and so, a drastic increase in the selectivity as compared with the conventional FIA UV system (without using the solid support) is reached in addition to a very significant increase in sensitivity. It shows excellent analytical features including a very high sampling rate (due to the carrier acting as self-eluting agent), high operational life-time and a very low cost per analysis, so being appropriate for pharmaceutical routine and quality control analysis.

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