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Review

The potential of flow-through optosensors in pharmaceutical analysis

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Abstract

The fundamental principles of flow-through optosensors, together with the most significant procedures and alternatives developed in the applications of flow-through optosensors to pharmaceutical analysis are reviewed. The most frequently used solid sensing zones, flow-through cells, manifolds, regeneration step and types of detection are considered. Single parameter, biparameter, and multiparameter sensors developed are reviewed and their most relevant features presented. Finally, a critical comparison with other analytical methodologies/techniques is performed and the potential of flow-through optosensors in pharmaceutical analysis and their future trends discussed.

Keywords: Flow-through optosensors; Pharmaceutical analysis; Flow injection-solid phase spectroscopy

1. Introduction

In 1976 K. Yoshimura [1] proposed the combined use of an active solid support to preconcentrate the analyte (or its reaction product with a chromogenic reagent) with the direct measurement of the light absorption of the species of interest sorbed on the solid phase. Since then, a lot of attention has been paid to this methodology, firstly called ion-exchanger colorimetry [1] (as the first supports used were ion exchangers) which more appropriately should be called solid phase spectrophotometry, and, from a more generic point of view, solid phase spectroscopy (SPS). Later, spectrofluorimetric detection was also used to direct determination of the species sorbed on the solid support [2] so broadening the possibilities of SPS to solid phase spectrofluorimetry. Based in the use of SPS, a lot of elements and compounds could be determined [1,3–7].

Sensitivity and selectivity are the two most remarkable analytical features of this methodology. This is due to the separation of the sample component from the matrix and its preconcentration (usually with an especially high preconcentration factor) on the support, that is, in the zone itself where it will be measured with a non-destructive molecular spectroscopic detector.

A few years later, solid phase retention was implemented with on line spectroscopic detection

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[8] so originating Flow Injection-Solid Phase Spectroscopy (FI-SPS) methodology. In this way, the advantages of SPS (mainly sensitivity and selectivity) were added to those intrinsic ones from FIA (rapidity, commodity, automation, less consumption of reagents and solid supports, etc). In these FI-SPS systems the separation and retention of the species of interest on the solid phase takes place in the detection area itself and simultaneously with it. So, these systems integrate (not only in the space, but also in the time) several analytical processes, as can be said: a) separation and detection; b) reaction and detection; and c) reaction, separation and detection [9].

The (micro)zone of the solid phase where the signal is continuously monitored is integrated in the detector and it is surrounded by a continuous stream flowing through it. The sample plug is inserted in this stream and so, the radiation directly interacts with the solid surface integrated in the detection area. This detection principle, based in the interaction of the radiation with the solid surface integrated in the detector is called a flow-through optosensor [10] or a flow-through spectroscopic sensor [11]. This miniature analytical device which (ideally) responds to the species of interest is brought in direct contact with the (untreated) sample providing a reversible, rapid and continuous response [11] which is transduced via a non destructive (molecular) spectroscopic detector; thus, a (near) real time information is obtained on site.

The main applications of FI-SPS systems were focused firstly towards inorganic analytes [8,12–14] (as well as the first applications of solid phase spectroscopic procedures in batch mode [1,15]). Then, organic analytes were also analysed by means of SPS combined with FIA. During the last few years, a number of FIA-SPS procedures have been developed for some organic compounds used as active principles in pharmaceutical preparations [16–33]. Also SPS in batch mode has also been used for pharmaceutical analysis [6,7,34,35].

The simplest flow-through optosensors developed in pharmaceutical analysis are those based in the measurement of an intrinsic analytical property of the analyte (e.g. absorbance, fluorescence) so avoiding any derivation reaction.

Today research efforts devoted to flow-through optosensors in pharmaceutical analysis are a very promising research area which produces very simple and inexpensive analytical procedures with remarkable analytical features, mainly sensitivity and selectivity compared with the respective conventional spectroscopic procedures.

In this paper the fundamental principles of SPS combined with FIA (that is, of the flow-through optosensors) together with the most significant procedures and alternatives developed in pharmaceutical analysis are reviewed. Finally a critical comparison with other analytical alternative procedures/techniques is performed and the potential of flow-through optosensors in pharmaceutical analysis discussed.

2. Fundamentals

In flow-through sensors the measurement of the analytical signal is directly relationed to the concentration of the analyte in the sample injected.

In the case of spectrophotometric sensors, absorbance (really attenuation) on the solid support at the working wavelength, A, consists of several components [36]:

$$A = A_A + A_R + A_S$$

where A_A is the absorbance of the analyte (or analytical derivative) sorbed on the resin, A_R is the absorbance of the background (solid support plus reagent, if it is the case), and A_S, that of the interstitial solution between the resin beads (which can be neglected as compared with the other terms). The packing of the resin beads in the flow cell affects the values of A_A and A_R. However, when the system is flowing for a few seconds, the packing keeps constant and so the baseline shows a constant value equal to $A_R + A_S \approx A_R$. Therefore, the analytical peak, A_A, corresponds to the difference between A and $A_R + A_S$. In this way, in the flow-through spectrophotometric system, the analytical signal can be obtained directly by measuring at only one wavelength and successive measurements are performed on the same resin packing. However, in SPS batch mode each measurement is performed on a different resin batch and the

packing is also different in each case, so rendering no reproducible measurements when they are performed at only one wavelength. For this reason, measurements at two different wavelengths are needed in SPS in batch mode (one at the absorption maximum of the species of interest, and another at the wavelength where only the resin absorbs light) [6,7,34,36].

The net intrinsic absorbance of the analyte sorbed, A_A, is given by $A_A = \varepsilon_R l_R C_R$, [37] where ε_R is the apparent molar absorptivity of the analyte in the ion exchanger phase as observed in the flow-through system (kg mol^{-1} cm^{-1}), l_R the mean light path length through the resin layer which can be supposed equal to 0.1 cm (although it usually will not be exactly this value) and, C_R, the analyte concentration in the solid phase (mol kg^{-1}). When V l of a sample at concentration C_o (mol l^{-1}) of analyte is injected in the system, supposing a high value of the distribution ratio (as it is usual), the concentration on the solid phase (C_R, mol kg^{-1}) will be [38]:

$$C_R = C_o\, 1000\ \frac{V}{m_r} \quad (1)$$

where m_r is the mass of resin (g) in which the analyte is retained. Therefore:

$$A_A = \varepsilon_R l_R V \frac{C_o}{m_r} \quad (2)$$

where m_r is expressed in kg. So, keeping V constant, there is a linear relationship between the analytical signal and the initial concentration, C_o, of the analyte in the injected solution, being $\varepsilon_R\ l_R\ V/m_r$ the slope of the calibration line.

An important feature of these flow-through sensing devices is derived from Eq. (2): keeping C_o constant, A_A increases as V increases. So, a linear relationship between A_A and the injected sample volume can be expected, that is, sensitivity is proportional to the sample volume used for analysis and this can easily be increased just by increasing the injection volume.

In fluorimetric sensors, a similar relationship between the analytical signal and the initial concentration of analyte in solution can be established. The fluorescence signal of the analyte sorbed on the solid support, I, is given by the following expression:

$$I = \phi_F I_o \varepsilon_R l_R C_R \quad (3)$$

where ϕ_F is the fluorescence efficiency in the solid phase, I_o is the intensity of the excitation light beam and, ε_R, l_R and C_R are as above described. From Eqs. (1) and (3) it follows:

$$I = \phi_F I_o \varepsilon_R l_R V \frac{C_o}{m_r}$$

Similar linear relations between the analytical signal and C_o can also be written for phosphorescence and chemiluminescence (CL) sensors.

3. Solid supports

Solid supports used (e.g. ion-exchange beads, C_{18} bonded phase silica beads and polymers without exchanging groups) are packed in the flow-cell(s) of a conventional non-destructive optical detector (e.g. a photometer or a fluorimeter). The analytes or their reaction products are immobilised on them temporarily for sensing, so integrating retention and detection, and sometimes reaction.

Sometimes, solid supports packing microcolumns coupled on-line to the flow injection system have also been used. The employ of these microcolumns has two main purposes: (a) the interference removal by use of a support suited to the sorption of the species to be removed [39]; and/or (b) to get a multi-determination. The multi-determination can be accomplished by retaining selectively one of the analytes in the microcolumn (placed just before the flow cell) and eluting it later (after developing the signal of another one) with an appropriate reagent [39].

It should be taken into account that the solid support required for these flow-through sensors should meet some requirements: (a) the particle size should be large enough to avoid overpressure in the system; (b) to warrant reproducibility in the sensor response, it should be mechanically resistant to the continuous flow; (c) it should be chemically inert to the components of the solutions constituting the flow; (d) it should be compatible with the detection system used (e.g. the background of the supports used in spectrophotometric sensors should be low enough to allow the absorbance measurements);

and, (e) the retention/elution process should be quick enough.

The most frequently used solid supports in flow-through sensors for pharmaceutical analysis can be divided into three groups: (a) ion-exchanging polymers [21–33,39,41–43,46], (b) non-ionic polymeric adsorbents or neutral resins [18,20] and, (c) non-polar sorbents [16,17,19,40,44,45].

(a) Usually, s*tyrene polymers*, constituted by a hydrophobic aromatic matrix, are discarded when working in UV region because of their very high background in this region. *Dextran polymers*, such as Sephadex absorbents, have been the most frequently used (e.g. Sephadex QAE A-25, Sephadex CM C-25 and Sephadex SP C-25).

(b) Between these resins, also known like *macroporous polymers*, Amberlite XAD resins are the most used. One of the drawbacks of this type of supports is their high background in the UV and Visible regions so, they have usually been used in luminescence (LU) sensors.

(c) The use of adsorptive hydrophobic materials located in the flow-cell, such as C_{18} bonded silica beds, has allowed multi analyte determinations [44,45]. They are achieved by using a diode array spectrophotometer for monitoring simultaneously absorbances at different wavelengths. The joint use of this principle and classical deconvolution chemometric approaches allows the development of multiparameter sensors. An important drawback of these materials, compared with (a)-type ones, is their low selectivity because of the adsorptive nature of the retention process.

4. Flow-through cells

Two main aims must be pursued when a suitable cell for the design of a flow-through sensor is being selected: (1) the concentration of the monitored product on the support in an area as small as possible of it; and, (2) the incident light beam must be focused to this area without loss of light to the surrounding zone. The best results are provided by cells with short path lengths (1–1.5 mm), which ensure compatibility between the system and the detector and prevent the species from lying outside the irradiated area.

The most appropriate commercial flow-through cell for spectrophotometric measurements in UV region is the Hellma 138-QS cell (1-mm light path, 50 μl inner volume) (Fig. 1a). The cell is blocked in the outlet with glass wool to prevent particle displacement by the carrier stream. The solid support, as a slurry suspension in water or in methanol (if C_{18} bonded silica beds are used), is loaded with the aid of a syringe and the inlet is kept free. The level of the packing material into the cell is a very important variable. It should be the necessary one to fill it up to a sufficient height, thus permitting the light beam to pass completely through the solid layer. Higher levels would imply that the support

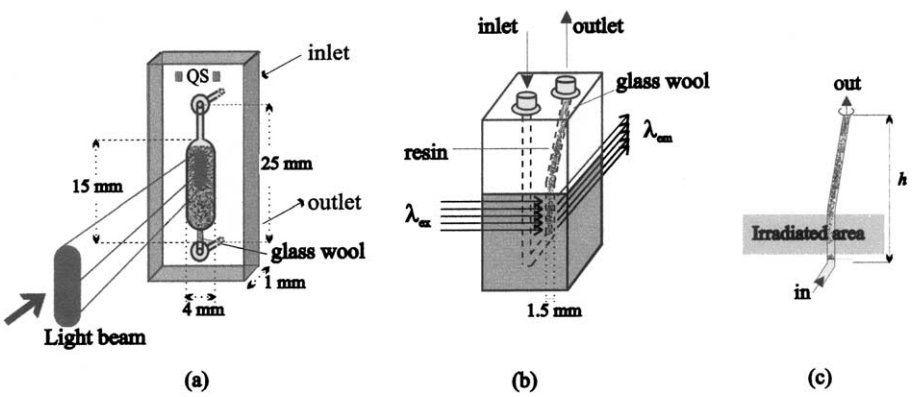

Fig. 1. Flow cells for pharmaceutical flow-through sensors: (a) PH sensors (Hellma 138-QS); (b) LU sensors (Hellma 176.52); (c) detail of the Hellma 176.52 cell.

zone where the species of interest is sorbed would fall outside the detection area and so, a lower and wider signal would be obtained; with lower levels, the light beam would pass through the solution completely or partially and, consequently, a decrease in the signal would be obtained. So, the top of the resin is kept as close as possible to the light beam, this latter being completely covered by the resin.

For fluorescence and phosphorescence measurements, the Hellma Model 176.52 flow-through cell (25 μl inner volume) with a light path of 1.5 mm has been found to be the most suitable cell (Fig. 1b). In this case, in order to secure that the species of interest retained by the packing solid material is in the light path, the solid support level is maintained just some millimetres beyond the cell window (Fig. 1c). Obviously, in every case the optimum level of solid support in the cell depends on the shape and height of the light beam and so, it depends of the instrument used for measurements.

It is necessary to take into account that, when an eluting solution other that carrier one is used to desorb the species of interest from the solid support, ion-exchange resins suffer alternately swelling and compaction, so altering the level of the support in the cell and the baseline. This is due to the different chemical nature and concentration of the carrier and the eluting solutions. So, in this case, and for preventing compactions lowering the support level below the light beam, the flow cell must be filled up passing the eluting solution through it. In every case, it is necessary the conditioning of the solid support by passing the carrier solution through it for a few minutes.

5. Regeneration step

The implementation of the regeneration step is an important and key requirement. It can be achieved in several ways (see Fig. 2):

(a) The carrier solution itself acts as the eluting agent: the regeneration process starts just as the tail end of the sample plug reaches the solid support in the flow-cell. In this case, a transitory signal will be developed. This is the simplest procedure to regenerate the solid sensing zone, and in turn, it allows high sampling frequencies (see A,D,E,F,G and I in Fig. 2).

(b) The eluting solution is transported to the active solid zone after the maximum signal is developed via injection (using a definite loop) or a selecting valve (see B,C,H and J in Fig. 2). This regeneration procedure is used when the carrier solution cannot act as eluting agent. It offers a lower sampling frequency although it shows a higher sensitivity because the carrier solution does not elute the analyte from the (micro)zone. Also, it shortens the lifetime of the sensor when an ion exchanger is used as solid support, due to the successive swelling and compactation of the resin beads. The regeneration of the sensing (micro)zone makes the sensor remain ready for the next determination, so allowing it to be reusable.

6. Manifolds

One of the most important features of SPS is its compatibility with continuos-flow systems, largely demonstrated over the last few years. Fig. 2 shows the most usual manifolds in pharmaceutical flow-through sensors.

(a) The simplest is a monochannel manifold in which the carrier solution acts also as eluting solution: as the sample plug tail reaches the sensing zone, the analyte is eluted from it, so originating a transient signal (see (A) in Fig. 2).
(b) The use of two different appropriate alternative carrier/eluting solutions by means of a selecting valve makes possible to perform the sequential determination of two analytes with a slight modification in the single channel manifold above described (see (D) in Fig. 2).
(c) When the species is retained on the solid support so strongly than the carrier cannot elute it, an additional eluting solution has to be used. An additional injection or selection valve is then used (usually placed as near to the sensing (micro)zone as possible) to allow the eluting solution to act (see (B) and (C) in Fig. 2). This manifold usually shortens the lifetime of the sensor when an ion exchanger is used as support.
(d) A double synchronised injection of both sam-

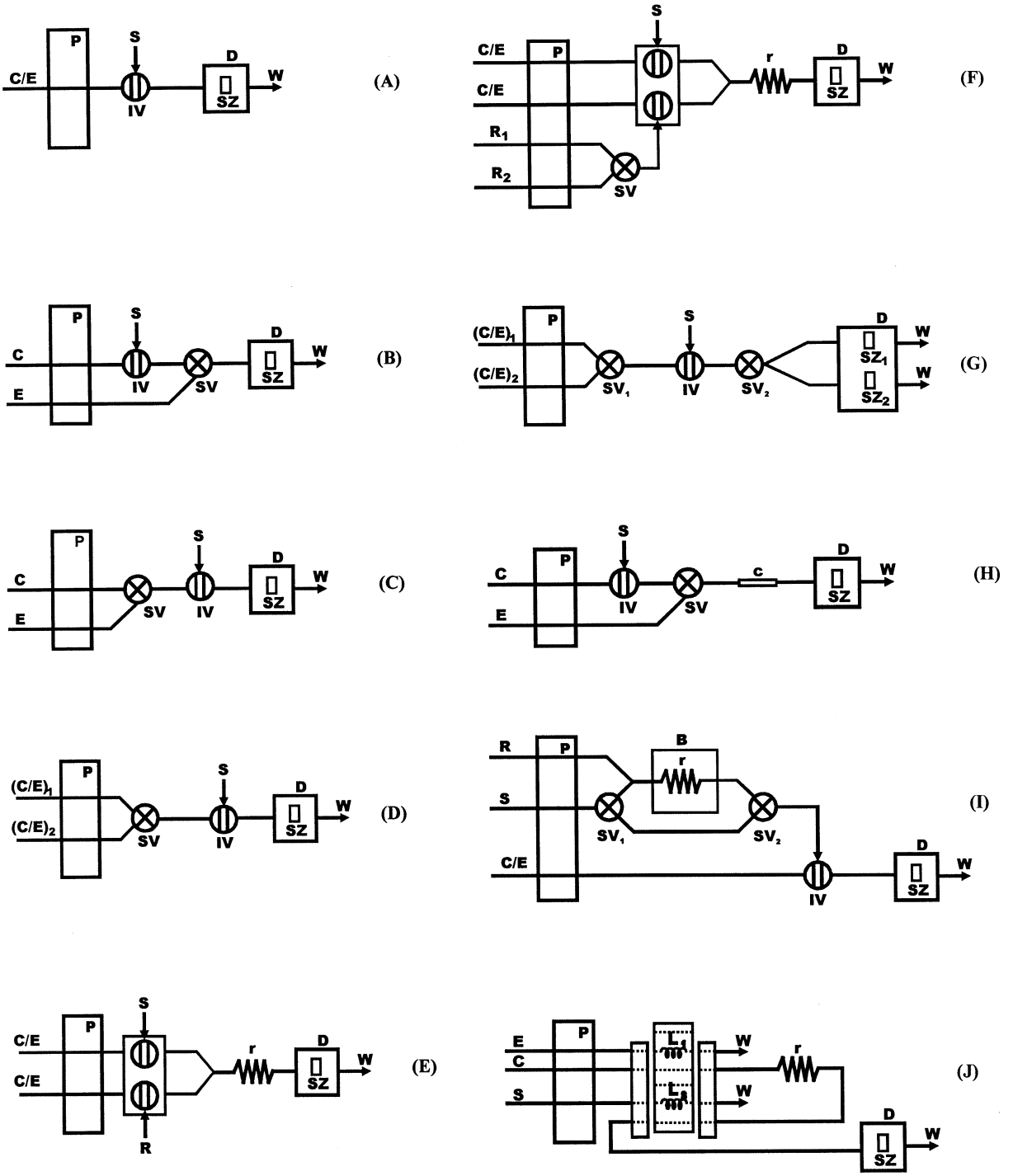

Fig. 2. Manifolds used in flow-through sensors developed for pharmaceutical analysis. S: sample; P: peristaltic pump; C: carrier solution; E: eluting solution; C/E: carrier/eluting solution; R: reagent solution; IV: injection valve; SV: selection valve; L: loop; B: thermostatic bath; r: reactor; c: microcolumn; SZ: sensing zone; D: detector; W: waste.

ple and reagent is performed in those sensors in which a previous derivation of the analyte(s) is performed due to the analytical signal does not correspond to an intrinsic property of the analyte(s). A reaction coil is then used in order to allow the reaction takes place in it before the sample plug reaches the cell (see (E) in Fig. 2). A modification of this manifold has also been used in order to perform the determination of several analytes. The use of a previous selecting valve before injecting the reagent in the carrier stream (see (F) in Fig. 2) allows to select alternatively the reagent to be injected in a synchronised way with the sample.

(e) The combination of the manifold (D) with the use of two different sensing (micro)zones and a double beam spectrophotometer is showed in (G), Fig. 2. An injection valve is operated twice successively to inject the sample plug which is carried out alternatively each time to the appropriate sensing (micro)zone just actuating on the selecting valve SV_2. The appropriate carrier stream is selected each time by means of the selecting valve SV_1. This is a dual sensor for the determination of two analytes based in the measurement of their intrinsic UV absorbances.

(f) A monochannel manifold including a minicolumn before the sensing zone broads the potentiality of the single channel manifolds. The simultaneous determination of more of one analyte can be achieved by the retention on line of one (or more) of them in the minicolumn while another one passes to the sensing zone. The sequential elution of (an)other analyte(s) by the appropriate eluting solution(s) allows to determine simultaneously two [39] and even three (non published) analytes by simply getting a temporary sequential arrival of them to the detection zone (see (H) in Fig. 2).

(g) Manifold (I), Fig. 2, has been used for the direct determination of an analyte or the indirect determination of another one. By actuating the selection valves SV_1 and SV_2 it is possible either to insert directly the sample stream into the carrier solution by means of the injection valve IV (direct determination of the first analyte) or previously to merge with a reagent stream (R) (to favour the hydrolysis of the second analyte, so rendering the first one) along the reactor (r), which is immersed in a thermostatic bath (B) (indirect determination).

(h) Finally, in manifold (J), Fig. 2, the loops L_1 and L_2 are filled with eluting and sample solutions, respectively. By sliding the commutator central bar, sample and eluent aliquots are simultaneously inserted into the carrier solution. The reactor (r) provides a proper time delay to avoid excessive carryover between sample and eluent zones. So, the elution is always performed by using just a definite volume of regenerating solution, in opposition to manifolds (B), (C) and (H) in Fig. 2.

7. Types of detection

In flow-through optical sensors developed for pharmaceutical analysis the transducer used to convert the chemical information into optical signal and the sensing microzone are integrated in a single element. The selectivity inherent in the transduction step in optical sensors is far higher than in any type of sensor and it is due basically to the resonant nature of the interaction between light and the species absorbing [47].

Molecular absorption spectroscopy is no doubt the most frequently used detection technique in analytical laboratories due to its high flexibility for adaptation to a wide variety of analytical problems. The literature abounds with references to spectrophotometric determinations of pharmaceuticals. Few of the applications, however, rely on absorbance measurements of pharmaceuticals themselves as their intrinsic light absorption occurs at the UV region, where other compounds accompanying the analyte(s) usually also absorb, so making impossible the determination. So, UV region usually does not show selectivity enough for conventional spectrophotometric analysis. However, by using spectrophotometric flow-through sensors, the active solid support strongly enhances selectivity excluding from the detection area all those species that cannot be retained in the experimental conditions and so it allows working in the UV region. In fact, all the photometric flow-through sensors

developed for organic active principles work in this spectral region [21,22,24,25,27–32,39,41,42,44,45].

Phosphorescence and fluorescence are both processes in which radiation is emitted by a molecule following an initial excitation by an electromagnetic radiation. Both forms of photoluminescence are the basis of analytical methods of great utility. In luminescence (LU) processes, including chemoluminescence (CL), it is found a linear relationship between the analytical signal and the analyte concentration. In solid phase this relationship can also be established between the analytical signal and the initial concentration of the analyte in solution, as above explained.

The most salient analytical feature of fluorimetry is its high selectivity, which is accompanied by low detection limits. The added economy and simplicity, and the relative flexibility of fluorimetry, have turned it into the officially recommended choice for determining the purity of many drugs on their contents in pharmaceuticals, forensic and biomedical samples, among others. The most serious shortcoming of fluorimetric (F) techniques is the small number of substances that exhibit native fluorescence. So, determination of dipyridamole [33] and vitamins such as riboflavin [19] and pyridoxine [23], has been made by direct measurement of the native fluorescence. However, a few straightforward reactions have enormously expanded the range of available fluorophores. A sensor of pyridoxal was developed by formation of a fluorescent complex between the analyte and beryllium, in an ammonia buffer [16]. Another sensor has also been developed, based on derivative synchronous fluorescence measurements for the multidetermination of B_6 vitamers (pyridoxal, pyridoxal-5-phosphate and pyridoxic acid) [17] using also beryllium as reagent.

Methods based on phosphorescence measurements are particularly versatile and extremely sensitive. Some applications have appeared using room-temperature phosphorescence (RTP), where heavy-atom materials such as lanthanides [Eu (III), for example] have been used to enhance RTP. In aqueous solution, these free ions do not luminesce efficiently, since any excited-state species are relaxed by vibrionic energy transfer to the aqueous solvent shell. Unlike molecular phosphorescence, sensitised lanthanide LU occurs readily in liquid solution at room temperature. Since energy transfer from the organic triplet state of the ligand to the emitting level of the lanthanide ion is an intramolecular process, the LU is not quenched by oxygen. Based on this idea, new sensors have been developed. They are the result of the combined use of FIA and solid substrate room temperature phosphorescence (SS-RTP) optosensing detection. Examples of these sensors are those applied to tetracyclines [18] and anthracyclines [20] determination. They are based on the transient immobilisation of the analyte-Eu(III) chelate, which exhibits RTP, on a solid support getting a high sensitivity in both cases.

Chemiluminescence (CL) is a phenomenon involving the emission of light (usually in the visible or infrared region) as a result of a chemical reaction. Essentially, the process by which LU is produced is identical to that for photoluminescence except that no excitation light source is necessary. The analytical interest of CL arises from the ability to produce fluorescent molecules with no prior irradiation, thereby avoiding various problems derived from light scatter, unselective excitation or light source instability. A short number of pharmaceuticals have been determined by using CL sensors. An example of these is the sensor developed for the determination of analgin [26], which is based on the auto-oxidation of the analyte in presence of Tween 80 sensitised by rhodamine 6G immobilised on a cation exchange support.

8. Active principles

8.1. Single-parameter sensors

Most of flow-through optosensors devoted to the determination of active principles in pharmaceutical analysis are single-parameter sensors, that is the (micro)sensing zone responds only to an active principle in the sample. Photometric (PH) and LU detection have been used. In turn, three different modes of LU detection have been used: fluorimetric (F), phosphorimetric (room temperature phosphorescence, RTP) and CL detection have been used (Table 1).

8.1.1. Photometric sensors

All PH flow-through optosensors developed for pharmaceutical analysis of only one (organic) active principle are based in the measurement of the intrinsic UV absorbance of the analyte, so no derivation reactions are used.

The first sensor of this group described for the determination of an anionic active principle was for ascorbic acid [22]. An acetate buffer solution is used as carrier/self-eluting solution and an anion exchanger gel as sensing solid support. Three different calibration ranges are obtained from three different sample injection volumes.

The determination of paracetamol [28] is based in the same principle but using NaCl/NaOH (pH 11.0) as carrier/self-eluting solution, because the analyte needs a higher pH value than ascorbic acid to get deprotonation and retention on an anion exchanger gel. The sensor was calibrated for only one sample injection volume.

Active principles showing a cationic nature have also been determined using a monochannel manifold, the carrier also acting as eluting solution: Vitamins B_1 (thiamine) and B_6 (pyridoxine) could be determined using both monochannel manifolds and a cation exchanger gel. Thiamine was determined in the presence of other vitamins B in the 0.6–33.0 μg ml^{-1} range using an acetate buffer (pH 4.8) and a Sephadex cation exchanger gel [21]. Three calibration lines were established from various loops. Pyridoxine could be determined using NaCl/HCl (pH 2.0) as carrier/self-eluting solution with a R.S.D. $< 1\%$ in the 1.0–10 or 2.0–20 μg ml^{-1} ranges [29].

A sensor operating in a similar way was proposed for minoxidil (NaCl/HCl at pH 2 as carrier/self-eluting agent). The method was developed for 600, 1000 and 2000 μl of sample, the concentrations ranging from 0.05 to 7.0 μg ml^{-1} and the detection limits from 0.006 to 0.06 μg ml^{-1} [24].

Three PH sensors have been proposed to determine anionic active principles, all of them using Sephadex QAE A-25 as sensing solid phase and needing an additional eluting agent via a selection valve: diclofenac sodium, amoxycillin and adrenaline. In all of them, the eluting solution has the same nature than the carrier one, but with a higher concentration in order to elute the analyte that is strongly retained on the anion exchanger. Diclofenac sodium could be analysed from 0.5 to 40 μg ml^{-1} with detection limits ranging from 0.13 to 0.44 (three sample injection volumes can be used) [27]. Also amoxycillin can be analysed using three different injection volumes [25]. A relatively high pH value had to be used in the carrier-conditioning stream in order to get the analyte could be retained on the solid phase (corresponding recorded signal is shown in Fig. 3a). Something similar can be said of the sensor for adrenaline [30]. In both cases, it was necessary a relatively high saline concentration in the eluent to desorb the analyte from the detection zone.

A single-parameter sensor responding to four tetracyclines, one in a time, has been developed for determination of tetracycline, doxycycline, oxycycline and chlortetracycline [31]. The response to the three former tetracyclines is very similar (linear dynamic ranges from 0.5 to 12 μg ml^{-1} and detection limits near to 0.07 μg ml^{-1}). Sensitivity for the determination of chlortetracycline is lower.

Finally, salicylic acid was determined by monitoring of its intrinsic absorbance at 297 nm sorbed on Sephadex QAE A-25 resin. The sensor also allows the indirect determination of acetylsalicylic acid previous on-line hydrolysis to salicylic acid [32].

Only one inorganic active principle, Zn, has been determined with a flow-through sensor. The sensor uses a derivative reaction with spectrophotometric transduction in the visible region (590 nm). It is based in the chromogenic reagent immobilisation (1-(2-tiazolylazo)-2-naphtol) (TAN) on the support (C_{18} bonded silica) loaded into a homemade flow-cell with 1 mm optical pathlength. Analyte reaction with TAN (apparent molar absorptivity $\approx 2.02 \times 10^5$ l mol^{-1} cm^{-1}), retention and detection were performed simultaneously on the support and the elution was achieved with 0.5 M hydrochloric acid which eluted Zn without removing the immobilised chromogenic reagent [40].

8.1.2. Fluorimetric sensors

Three F single-parameter sensors have been developed related to vitamins B (pyridoxine,

Table 1
Single-parameter flow-through optosensors

Active principle	Manifold/ carrier [pH]	Additional eluent [pH]	V_i (μl)	LDR (μg ml^{-1})	Sensitivity (ml μg^{-1})	R.S.D. (%)	DL (μg ml^{-1})	Support/cell[a]	Transduction[b] /wavelength (nm)	Sampling frequency	Reference
Thiamine	A/ NaAc/HAc 0.15M [4.8]	–	300	2.0–33.0	0.031	0.75	0.42	S.CM C-25/I	PH/247	18	[21]
			600	1.0–20.0	0.054	0.89	0.25			16	
			1000	0.6–12.0	0.078	1.77	0.16			14	
Ascorbic acid	A/ NaAc/HAc 0.04M [5.6]	–	300	1.0–20.0	0.056	0.87	0.04	S.QAE A-25/I	PH/267	28	[22]
			600	0.5–10.0	0.116	1.08	0.03			24	
			1000	0.2–6.0	0.187	0.90	0.02			21	
Diclofenac Sodium	C/ NaAc/HAc 0.1M [4.8]	NaAc/HAc 0.4M[4.8]	300	2.0–40.0	0.021	1.05	0.44	S. QAE A-25/I	PH/281	12	[27]
			600	1.0–22.0	0.038	1.16	0.24			11	
			1000	0.5–14.0	0.061	1.53	0.13			9	
Minoxidil	A/ NaCl (0.05M)/HCl (0.01M) [2.0]	–	600	0.2–7.0	0.143	0.38	0.060	S. SP C-25/I	PH/282	26	[24]
			1000	0.1–4.0	0.229	1.06	0.033			22	
			2000	0.05–2.0	0.430	2.63	0.006			16	
Amoxycillin	B/ NaCl (0.1M)/NaOH (0.032M) [12.5]	NaCl(0.5M) /NaOH (0.032M) [12]	300	2.0–50.0	0.020	0.87	0.22	S. QAE A-25/I	PH/248	17	[25]
			600	1.0–25.0	0.040	1.01	0.16			13	
			1200	0.5–15.0	0.066	0.99	0.12			10	
Pyridoxine	A/ NaCl (0.08M)/HCl (0.01M) [2.0]	–	600	2.0–20.0	0.047	0.65	0.08	S. SP C-25/I	PH/290	44	[29]
			1250	1.0–10.0	0.091	0.84	0.02			32	

Table 1 (*Continued*)

Active principle	Manifold/ carrier [pH]	Additional eluent [pH]	V_i (μl)	LDR (μg ml^{-1})	Sensitivity (ml μg^{-1})	R.S.D. (%)	DL (μg ml^{-1})	Support/cell[a]	Transduction[b]/ wavelength (nm)	Sampling frequency	Reference
Paracetamol	A/ NaCl (0.08M)/NaOH (0.001M) [11.0]	–	600	0.5–8.0	0.106	1.24	0.022	S. QAE A-25/I	PH/264	40	[28]
Adrenaline	B/ NaCl (0.05M)/NaOH (0.01M) [12.0]	NaCl(0.7M) /NaOH(0.01M) [12]	600	1.0–12.0	0.032	1.36	0.17	S. QAE A-25/I	PH/287	12	[30]
Tetracyclines:	A/	–	1000					S. QAE			[31]
Tetrac.	Na_2CO_3/$HNaCO_3$ 0.1M [10.5]			0.5–12.0	0.082	0.66	0.069	A-25/I	PH/380	16	
Doxic.				0.5–12.0	0.083	1.21	0.081		PH/380	16	
Oxitetrac.				0.5–12.0	0.081	0.83	0.069		PH/380	16	
Chlortetrac				1.0–20.0	0.050	0.92	0.121		PH/387	16	
Pyridoxine	A/NaCl (0.03M)/HCl (0.001M) [3.0]	–	200	0.050–1.800	547	0.45	0.00547	S. SP C-25/II	LU(F)/295(ex) 385(em)	40	[23]
			1000	0.010–0.400	2284	0.70	0.00067			32	
			2000	0.005–0.200	4729	1.31	0.00033			26	
Pyridoxal*	E/H_2O	–	--	0.033–2.674	58.29	0.79	--	C_{18}(60–100 μM)/II	LU(F)/360(ex) 450(em)	6	[16]
Riboflavin	C/KH_2PO_4/$K_2HPO_4$0.2M [6.0]	Methanol/water 65:35 (v:v), 2 ml	2000	0.002–0.092	2500	1.1	0.0004	C_{18}(10 μm) /P.E. L225, 1247	LU(F)/466(ex) 515(em)	25	[19]
Tetracyclines**	B/TEMED[c]	0.5M HCl,	1000					Amberlite	LU (RTP)/390	10–12	[18]
Tetrac.	(0.1M)/	2 ml		–	–	1.0	0.00025	XAD-2/ II	(ex)622 (em)		
Oxitetrac.	HCl/NaCl [7.5]			–	–	1.0	0.00040				
Chlortetrac.	($\mu = 0.25$M)			–	–	0.9	0.00030				

Table 1 (*Continued*)

Active principle	Manifold/ carrier [pH]	Additional eluent [pH]	V_i (μl)	LDR (μg ml^{-1})	Sensitivity (ml μg^{-1})	R.S.D. (%)	DL (μg ml^{-1})	Support/cell[a]	Transduction[b] /wavelength (nm)	Sampling frequency	Reference
Antracy-clines**:	B/TEMED (0.1M)/HCl/	0.5M HCl, 2 ml	1000					Amberlite XAD-2/ II	LU (RTP)/393 (ex)615 (em)	10–12	[20]
Daunorubicin	NaCl [7.5] ($\mu = 0.25$M)			3.10^{-8} –8.10^{-7} M	2.1×10^{7} M^{-1}	1.3	1.6×10^{-8}				
Doxorubicin				2.10^{-8} –8.10^{-7} M	3.0×10^{7} M^{-1}	0.9	1.0×10^{-8}M				
Epirubicin				2.10^{-8} –8.10^{-7} M	3.0×10^{7} M^{-1}	0.9	1.0×10^{-8}M				
Analgin	A/ reverse sample	–	200	0.4–10	27.07	3.5	0.15	Cation exchanger 732-type (Nankai)	LU(CL)/550 (em)	60	[26]
Acetylsalicylic acid or Salicylic acid	I/NaCl (0.08M)/NaOH (10^{-4}M) [10.0]	–	300	5–120	8.1×10^{-3}	0.38	0.321	S. QAE A-25/I	PH/297	25	[32]
			300	2–40	0.022	0.38	0.135			25	
			600	1.5–2.8	0.035	0.47	0.083			22	
			1000	1–20	0.051	0.52	0.064			18	
Dipyridamole	B/NaCl (0.1M)/NaOH (10^{-4}M) [10.0]	KH_2PO_4/NaOH 0.05M [6.0]	300	0.010–0.500	980	0.82	0.00094	S. QAE A-25/II	LU (F)/ 305 (ex)490 (em)	22	[33]
Zn***	J/H_2O	0.5M HCl	625	0.04–4.0	0.310	3.3	0.010	C_{18} (60–100 μm)/ Homemade	PH/ 590 nm	45	[40]

*, Formation of a fluorescent complex with beryllium in ammonia buffer. **, Formation of a LU complex with Eu (III). ***, Formation of a complex with 1-(2-tiazolylazo)-2-naphtol previously immobilised on the support (0.026 mmol g^{-1} C_{18}).

[a] Types of cells. I: Hellma 138-QS; II: Hellma 176.52.

[b] Transduction. PH: photometry; LU: Luminescence [RTP: room temperature phosphorescence; F: fluorimetry; CL: chemiluminescence].

[c] TEMED: *N*,*N*,*N*',*N*'-tetramethylendiamine.

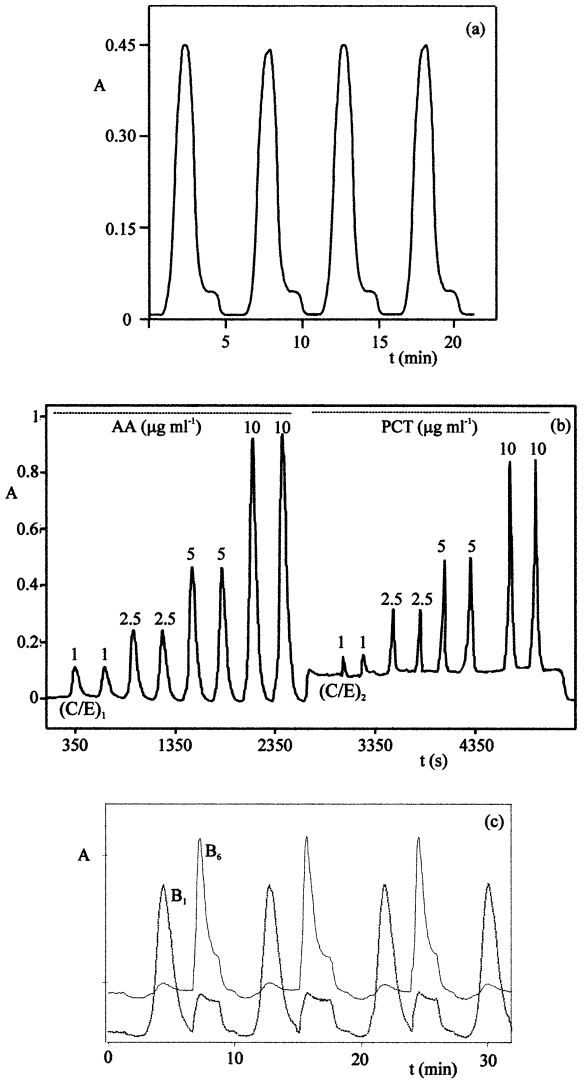

Fig. 3. Recorded signals for determination of: (a) *amoxycillin* [25], 10 μg ml^{-1}; (b) *ascorbic* (AA) and *paracetamol* (PCT) [41]; (c) *thiamine* (B_1) and *pyridoxine* (B_6) [39].

riboflavin, and pyridoxal) and another sensor has been developed for dipyridamole. Two of the former are based in the measurement of the native fluorescence of the analyte (pyridoxine and riboflavin) and another one (pyridoxal) uses a derivative reagent. Pyridoxine was determined by transitory retention on Sephadex SP C-25 using NaCl/HCl (pH 3) as carrier/self-eluting agent. Three calibration lines were constructed. Detection limits as low as 0.00033 μg ml^{-1} were reported [23]. Riboflavin was determined by measuring its native fluorescence at 515 nm ($\lambda_{ex} = 466$ nm) retained on a hydrophobic solid support (Silica-C_{18}, 10 μm) and transported by a phosphate buffer (pH 6.0) solution [19]. The analyte was eluted by injecting 2 ml of a mixture of methanol/water (65:35 w/w).

On the other hand, pyridoxal was determined [16] via formation of a fluorescent complex with beryllium in ammonia buffer and measurement of the fluorescence of the derivative product at 450 nm ($\lambda_{ex} = 360$ nm).

Finally, dypyridamole could be determined using NaOH (10^{-4} mol l^{-1})/NaCl (0.1 mol l^{-1}) as carrier and measuring its native fluorescence at 490 nm ($\lambda_{ex} = 305$ nm). The signal was developed as the sample plug reached the sensing solid phase (Sephadex QAE A-25 cation exchanger gel) and the analyte was then desorbed by an eluting solution (KH_2PO_4/NaOH, $c_T = 0.05$ mol l^{-1}, pH 6.0) after reaching the maximum signal. The sensor was applied to the determination of dipyridamole in pharmaceuticals and in human plasma, too [33].

8.1.3. Phosphorimetric sensors

Two room temperature phosphorimetric flow-through sensors have been developed: one of them responds individually to one of the three following tetracyclines: tetracycline, chlortetracycline or oxytetracycline. The other one responds, also individually, to one of the three following anthracyclines: daunorubicin, doxorubicin or epirubicin. Both sensors are based in the retention of the Eu(III)-tetracycline [18] or Eu(III)-anthracycline [20] complexes on a non-ionic resin (Amberlite XAD-2) following by the elution of the respective complex by injecting 2 ml of 0.5 M HCl after reaching the maximum signal. Therefore, the basis of the analytical signal ($\lambda_{em} = 622$ and 615 nm, respectively) is the sensitised lanthanide LU ($\lambda_{ex} =$ 390 and 393 nm, respectively) which occurs at room temperature without quenching by oxygen.

8.1.4. Chemiluminescence sensors

A flow-through sensor has been described for the determination of analgin based in the CL reaction of analgin and dissolved oxygen sensitised by Rhodamine 6G ($\lambda = 550$ nm) in the presence of

acidic Tween 80 [26]. Rodamine 6G was immobilised on a cation-exchanger column placed in front of the detector.

8.2. Biparameter sensors

Flow-through optosensors responding to more of one active principle from the same sample have been described. They can operate either in sequential or in simultaneous determination mode (Table 2).

In the former type (more usual) two successive sample injections have to be performed, the sensor responding (a) to only one analyte each time [17,39,41] or (b) firstly to one analyte and then to the other [42,43]. In all cases the detector directly measures an intrinsic property (absorbance or fluorescence emission). The first (a)-type sensor described was for the sequential UV spectrophotometric determination of ascorbic acid and paracetamol [41]. The sensing zone (Sephadex QAE A-25 ion exchanger gel) responds selectively to only one of the analytes when two alternate carriers are successively used. Ascorbic acid is determined when acetate buffer solution at pH 5.6 is used as carrier; at this pH value paracetamol is not sorbed on the solid support (because it is not ionised), so the signal is only due to ascorbic acid. Then, using a NaCl solution at pH 12.5 and a second sample injection, paracetamol is selectively sorbed on the sensing zone developing its analytical signal (working wavelength: 264 nm) (corresponding recorded signal is shown in Fig. 3b). The sensor is calibrated for three different injection volumes (300, 600 and 1000 μl) and shows R.S.D.s $< 1.5\%$.

A sensor has been proposed for the determination of three B_6 vitamers: pyridoxal, pyridoxal-5 phosphate and pyridoxic acid [17]. It is based on derivative synchronous F measurements and involves the formation of a fluorescent complex between the analytes and beryllium in ammonia buffers. The sample solution is injected twice simultaneously with a beryllium buffer solution of pH 9.9 or 7.9. In the first injection, (pH 9.9) pyridoxic acid and pyridoxal are determined simultaneously. In the second one (pH 7.9), pyridoxal-5 phosphate can be measured. Therefore, the use combined of synchronous fluorimetry with first derivative technique and the aid in discrimination performed by a change in the carrier pH allowed the determination of these three B_6 vitamers. This sensor works, therefore, as a biparameter one (simultaneous determination of pyridoxic acid and pyridoxal) and, in turn, as a single-parameter one, too (determination of pyridoxal-5 phosphate).

Recently, a sequential biparameter sensor has been developed by our research group for the UV absorptiometric determination of ascorbic acid and thiamine [46]. The sensor is based in the use of a double beam spectrophotometer and two different sensing zones (each one in a beam): an anion exchanger gel (Sephadex QAE A-25) for the determination of ascorbic acid and a cation exchanger gel (Sephadex SP C-25) for that of thiamine (manifold (G) in Fig. 1). Two injections are performed, each in an appropriate carrier-conditioning stream: ascorbic acid is transitorily and selectively sorbed and monitored on Sephadex QAE A-25 with citric acid/K_2HPO_4 buffer (pH 7.0); then, selecting the second carrier solution (acetate buffer, pH 4.8) and the second sensing zone, thiamine develops selectively a transitory (negative) signal. This type of sensors can be considered to be a combination of two appropriate single-parameter sensors.

Two sequential flow-through (b)-type sensors have been developed. One of them is devoted to the sequential photometric determination of paracetamol and salicylamide, by using their intrinsic UV absorbance as analytical signal [42]. With acetate buffer (pH 5.0) salicylamide is selectively sorbed on the sensing zone and eluted by the carrier itself, so developing a transient signal. With the second carrier (NaCl/NaOH at pH 12.0) a transient signal is developed, this time due to salicylamide and paracetamol together. The sensor has to be calibrated for salicylamide (alone) at pH 5.0 and pH 12.0 and for paracetamol (alone) at pH 12.0. Salicylamide is determined in the binary mixture with the first carrier (pH 5.0) and paracetamol indirectly from the analytical signal using the second carrier (pH 12.0).

In a similar way, the mixture salicylamide and salicylic acid could be resolved with a F biparameter sensor operating sequentially: with the first carrier (pH 2.0) salicylic acid was selectively and transitorily sorbed and salicylamide indirectly de-

Table 2
Biparameter flow-through optosensors

Active principle	Manifold/ carrier (pH)	V_i (μl)	LDR (μg ml^{-1})	Sensitivity (ml μg^{-1}) $\times 10^2$	R.S.D. (%)	DL (μg ml^{-1})	Support/Cell	Transduction[a]/ Wavelength (nm)	Sampling frequency	Reference
Ascorbic acid and	D/NaAc/HAc 0.04M [5.6]	300	1.0–20.0	5.58	1.06	0.045	S. QAE A-25/ I	PH/ 264	23	[41]
Paracetamol	D/NaCl (0.05M)	600	0.5–10.0	9.79	1.23	0.040			20	
	/NaOH	1000	0.3–6.0	15.60	0.88	0.018			18	
	(0.032M)	300	1.0–25.0	3.91	1.23	0.075	S. QAE	PH/264	30	
	[12.5]	600	0.5–12.0	8.14	1.41	0.045	A-25/ I		26	
		1000	0.4–10.0	9.95	1.09	0.025			24	
Paracetamol and	D/NaCl (0.1M)	600	2.5–40.0	2.57	0.60	0.10	S. QAE A-25/I	PH/300	36	[42]
Salicylamide	/NaOH (0.01M) [12.0]	600	5.0–80.0	1.11	0.36	0.24			36	
Salicylamide (alone)	D/NaAc/HAc 0.04M [5.0]	600	10.0–100.0	0.623	0.61	0.35	S. QAE A-25/ I	PH/300	36	[42]
Salicylamide and	D/NaCl (0.05M)/	1000	0.01–0.32	261400	1.13	0.0009	S. QAE A-25/I	LU (F)/260 (ex)415 (em)	35	[43]
Salicylic acid	NaOH (0.001M) [11.0]	1000	0.02–0.64	115500	0.86	0.002			28	
Salicylic acid (alone)	D/NaCl (0.025M)/HCl (0.01M) [2.0]	1000	0.04–1.00	69400	0.94	0.0045	S. SP C-25/ II	LU (F)/260 (ex)415 (em)	45	[43]
Pyridoxal* and	F/H_2O	50	0.05–15.00[c]	0.902[d]	2.3	–	C_{18} (60–100μm)/	LU (F)[b]/$\Delta\lambda = 50$	6	[17]
Pyridoxic acid			0.05–10.00[c]	1.513[d]	3.1	–	II	nm		

Table 2 (*Continued*)

Active principle	Manifold/ carrier (pH)	V_i (μl)	LDR (μg ml^{-1})	Sensitivity (ml μg^{-1}) $\times 10^2$	R.S.D. (%)	DL (μg ml^{-1})	Support/Cell	Transduction[a]/ Wavelength (nm)	Sampling frequency	Reference
Pyridoxal-5 phosphate**	F/H_2O	50	0.05–10.00[c]	0.641[d]	2.8	–		LU (F)[b]/$\Delta\lambda = 50$ nm	6	[17]
Thiamine and Pyridoxine	H/NaAc/HAc 0.4M [4.8]	40	7–130	0.74	2.1	0.40	S. SP C-25	PH/255	12	[39]
		1000	2–30	3.53	2.4	0.10			9	
	H/Sodium citrate/citric acid 0.1M [3.0]	40	5–110	0.91	2.7	0.21	S. SP C-25/ I	PH/293	12	
		1000	2–30	3.39	3.0	0.084			9	

*, Formation of a fluorescent complex with beryllium in ammonia medium, pH 9.9. **, Formation of a fluorescent complex with beryllium in ammonia medium, pH 7.9.

[a] Transduction. PH: photometry; LU: luminescence [F: fluorimetry].

[b] First derivative synchronous fluorescence (range 320–500 nm).

[c] μM.

[d] μM^{-1}.

termined [43]. The sensor could be used for determining mixtures of salicylamide and (indirectly) acetylsalicylic acid previous alkaline hydrolysis of the latter.

Up until today only a biparameter sensor has been described for the simultaneous determination of two active principles (thiamine and pyridoxine). In this case an only sample injection is needed [39]. This sensor is based in the use of a minicolumn placed on line just before the detector (Fig. 1 (H)). Sephadex SP C-25 cation exchanger gel is used both in the flow cell (sensing zone) and in the column (separating zone). The sample plug containing the two cationic analytes is transported by the carrier (citric acid/sodium citrate, pH 3.0) to the detector passing through the minicolumn in which thiamine is strongly retained while pyridoxine passes through it, reaching the detector and developing a transient absorbance signal. Then, by acting a selecting valve, thiamine is eluted from the minicolumn by an acetate buffer solution (pH 4.8), so developing its transient absorbance signal when it reaches the sensing zone. The absorbance signals are monitored with a diode array detector tuned at 293 (pyridoxine) and 255 nm (thiamine) (recorded signals are shown in Fig. 3c). Therefore, the selectivity stated by the solid support placed in the flow cell is now enhanced combining it with a previous on line separation of the two active principles by ion exchange. This separation is based in the different kinetic behaviour in the retention–elution process, which takes place in the column for the two analytes. So, the arrival of the analytes to the sensing zone is discriminated in the time.

8.3. Multiparameter sensors

Only two flow-through multioptosensing devices responding simultaneously to more than two active principles have been proposed (Table 3). They are based in the use of C_{18} bonded phase beads (55–105 μm) packed in a quartz flow cell to retain simultaneously three analytes in the detection area of a diode array spectrophotometer. Their intrinsic UV full spectra are acquired at definite time intervals (4 or 8 s) during the retention process (4 or 2.5 min). One of them [44] determines caffeine, dimenhydrinate and acetaminophen (paracetamol). The extensively overlapped spectra (acquired in the 245–310 nm region) were resolved by partial lest squares multivariate calibration (PLS-1). The other one resolves the mixture paracetamol, caffeine and acetylsalicylic acid in a similar way using the 240–350 nm region [45]. These two sensors exploit not only the spectral features of the analytes on the solid sensing zone by using a multicalibration chemometric approach, but also the different kinetic behaviour in their retention–elution process on the solid phase (paracetamol passes through the detection area quicker than the other respective analytes). So, selecting those spectra at two appropriate time values, the resolution of the ternary mixture can be successfully achieved, without any pretreatment of the sample other than its dissolution, in spite of the very different relative concentration values of these analytes in commercial pharmaceutical preparations.

9. Sensitivity

One of the most remarkable features of the flow-through optosensors is the increase in sensitivity with respect to the same procedure without using de solid support. It is a consequence of the concentration of the species of interest in the detection area itself on the solid support in which the measurement of the signal is performed without needing a previous elution from the solid phase, that obviously will cause an undesirable dilution. As above it was said, if the distribution ratio is high enough, the signal increases linearly with the sample volume injected. As the amount of support, m_r, in Eq. (1) is kept constant, the graphical representation of the experimental signal as a function of the sample volume injected is linear in a wide range of volumes [22,23], this is, sensitivity becomes linearly dependent on the sample volume. This feature can be favourably exploited when the active principle has to be determined in biological fluids such as urine [22] or when a strong matrix effect is present, so that it can be reduced by previously diluting conveniently the sample before injecting.

Table 3
Multiparameter flow-through optosensors

Multivariate calibration (PLS-1)										
Active principle	Manifold/ carrier	No factors	R^{2b}	RMSD[c]	REP[d]	Support/cell	Calibration matrix conc. ranges (μg ml^{-1})	Transduction/spectral range (nm)[a]	Time[e]	Reference
Caffeine	B/$HClO_4$ 0.5%	7	0.9964	0.1007	5.98	C_{18} (55–105μm)/ II	0.5–5.0	PH / 245–310	2.50 min	[44]
Dimenhydrinate		7	0.9962	0.1403	5.51		1–6		2.50 min	
Paracetamol		9	0.9916	1.506	10.5		5–40		1.92 min	
Caffeine	B/$HClO_4$ 0.7%	7	0.9711	0.503	7.3	C_{18} (55–105μm)/ II	1–15	PH / 240–350	59 s	[45]
Acetylsalicylic acid		5	0.9901	2.578	6.1		5–100		59 s	
Paracetamol		10	0.9648	3.168	11.1		5–70		25 s	

[a] Transduction PH: photometry.
[b] Regression coefficient (square of the correlation coefficient).
[c] Root mean square difference.
[d] Relative error of prediction.
[e] Spectral data acquisition time after injection.

10. Selectivity

Selectivity is another important feature of flow through optosensors that must be emphasised. The sorption of the species of interest on the solid sensing zone also states high selectivity conditions itself. Those chemical species that cannot be retained pass among the solid particles with the interstitial solution through the cell. The packing of the solid sorbent in the flow cell offers an extraordinarily low effective path length; therefore, the potential interfering species can exhibit a high concentration in the sample plug without producing serious errors. Thus, when ion exchangers are used as solid supports, all species with electric charge different from the counter-ion (and, consequently from the species to be measured) will be excluded on line from the sensing zone in the detection area itself. So, the maximum interference/analyte ratio tolerated is substantially increased with respect to the use of the same procedure without sensing support. Several examples of this are: a) vitamin B_1 can be analysed in the presence of vitamin C and folic acid at interference /vitamin B_1 ratios (w/w) of 20 and 50, respectively [21]; (b) ascorbic acid in the presence of vitamins B_1 and B_6, at ratios (w/w) >20 (maximum ratio tested) [22]; and (c) pyridoxine in the presence of ascorbic acid and diclofenac sodium at ratios (w/w) of 100 and 1, respectively [29]. This implies that, in some cases, tolerance ratios (w/w) achieved using flow-through sensors are up to 1000 times higher than the respective ratios in homogeneous solution [29].

Even though the potential interference has the same electric nature than that of the species being analysed, the amount tolerated can also be increased if the working conditions are not the optimum ones for its sorption on it. It is the case, for example, of the determination of vitamin B_1 in the presence of vitamin B_6 [21] and the determination of vitamin B_6 in the presence of vitamin B_1 [29], in which tolerance ratios interference/analyte up to 75 and 125 times higher than in homogeneous solution were achieved, respectively.

In some cases, the appropriate control of the working conditions allowed a same solid support to respond to the intrinsic signal of two different species, so acting as a dual sensing zone in such conditions that they are obviously impossible to reach in conventional spectrophotometry [41].

11. Comparison with other analytical methodologies

Flow-through optosensors applied to pharmaceutical analysis show a series of advantages as compared with the respective solution only FIA systems. Selectivity, as well as sensitivity are the two most remarkable differences.

The discrimination stated from the solid support in the flow cell, in combination with the use of an appropriate carrier/conditioning/eluting solution(s), usually allows to base the determination of the active principle in the measurement of an intrinsic property (absorbance or LU signal), so avoiding the use of any derivative reagent or previous separation. Thus, UV detection can be used in a lot of cases in such conditions that they are impossible to use in conventional UV flow systems. Up to three active principles [44,45] have been determined by means of UV optosensors using a single sensing support with a relatively high throughput and a very low cost per analysis. Chromatographic techniques such as High Performance Liquid Chromatography can offer a higher potential when determining simultaneously a high number of active principles. Nevertheless, flow through optosensors show both a much lower cost per analysis and a very much higher sampling rate than the former. This is another important advantage of flow-through optosensors.

We can conclude that flow-through optosensors in pharmaceutical analysis compare very favourably with conventional spectroscopic methods in terms of sensitivity, rapidity, economy and potentiality.

Finally, compared with SPS in batch mode, flow-through optosensors are advantageous in terms of rapidity, economy, versatility, potentiality, commodity and throughput. Selectivity is similar to the former and though sensitivity is higher in batch mode, it is high enough in flow-through optosensors for analysing pharmaceutical preparations and biological fluids. Finally, methods

based in flow-through optosensors require minor human intervention and are much easier to use.

12. Conclusions and future trends

Development and applications of chemical and biochemical flow-through sensors has shown fast growth over the past two decades. Of all of them, important attention has been paid to flow-through optosensors. The growing number of published papers in last few years clearly indicates the vitality of these ones.

The analytical features shown by flow-through optosensors in pharmaceutical analysis as well as their rapidity, simplicity, and the cost of the instrumentation required make it the analytical methods in pharmaceutical analysis based in these ones to be a highly attractive and promising approach.

One of the most promising key areas in recent studies in this field is that related to the research efforts devoted to the development of flow-through optosensors for simultaneous determination of several active principles in pharmaceuticals (multioptosensors). A very simple approach to achieve the simultaneous determination of two active principles is the use on-line of a microcolumn filled with the appropriate active solid support in order to discriminate in time the arrival of the analytes to the sensing zone [39]. Thus, our group could develop recently an UV multiparameter flow-through optosensor to determine simultaneously paracetamol, caffeine and propylphenazone using silica gel C_{18} as support in both, the microcolumn and the sensing microzone. An appropriate carrier, conveniently selected, allows the arrival of the first analyte to the detection area giving a transitory signal, while the other two analytes are strongly retained on the previous microcolumn. The sequential use of two appropriate eluting solutions allows transporting the other two analytes to the sensing zone. So, by using on-line microcolumns combined with flow-through optosensors their potential can easily be substantially enhanced.

New strategies exploiting the possibilities of the sensing detection can also be used. For example, the interstitial solution between the beads of an ion exchanger used as sensing zone in the flow cell can be used to determine an analyte that cannot be retained by an ion-exchanger process and which is found in a high concentration in the sample, and simultaneously to determine another analyte which is found together with the former in a much lower concentration and which can be retained in the sensing support. So, we could determine simultaneously thiamine and ascorbic acid, as well as acetylsalicylic acid and thiamine with an UV optosensor of this type using a cation exchanger as sensing support.

Other alternative strategies can still be exploited. The use of derivative reagents (an aspect scarcely exploited in sensors devoted to the pharmaceutical analysis field) is interesting in order to achieve a higher selectivity in both, PH and F flow-through optosensors. Used with non-intrinsic fluorophore analytes, fluorogenic reagents substantially will broad the applications of F flow-through optosensors. The reagent should allow on one hand to be immobilised on the sensing support, so giving the reaction product as the analyte reaches the cell and, on the other hand, its regeneration by eluting only the analyte with an appropriate regenerating solution. In other instances, the reaction product should be transitory retained and eluted from the sensing support with a regenerating solution.

The use combined of a PH optosensor with a second detection in conventional solution would also be another strategy that could be extensively exploited.

All this offers, from the point of view of the authors, a very attractive, fruitful and promising research field on the development and applications of flow-through optosensors to pharmaceutical analysis. The development reached on these ones nowadays has allowed applying them to real analytical problems in pharmaceutical analysis, so responding, as envisaged by the authors to the challenge stated in SAC'92 [11] for flow-through chemical sensors in this field.

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