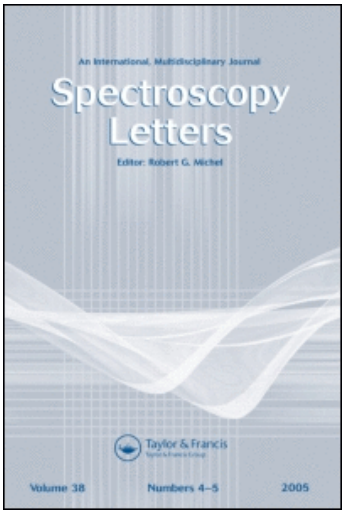

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Flow-Through Solid-Phase Spectroscopy: A Contribution to Green Analytical Chemistry

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Online publication date: 01 December 2009

To cite this Article García-Reyes, J. F. , Gilbert-López, B. and Molina-Díaz, A.(2009) 'Flow-Through Solid-Phase Spectroscopy: A Contribution to Green Analytical Chemistry', Spectroscopy Letters, 42: 6, 383 — 393
To link to this Article: DOI: 10.1080/00387010903187336
URL: http://dx.doi.org/10.1080/00387010903187336

Spectroscopy Letters, 42:383–393, 2009

ISSN: 0038-7010 print/1532-2289 online
DOI: 10.1080/00387010903187336

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Flow-Through Solid-Phase Spectroscopy: A Contribution to Green Analytical Chemistry

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ABSTRACT Spectroscopy and flow methodologies are an interesting and fruitful marriage in the field of Green Analytical Chemistry (GAC). Automation, immobilization of analyte, reagent(s), reaction product, or catalyst on active solid surfaces and miniaturization are highlighted as alternatives for waste decrease. Flow-Through Solid-Phase Spectroscopy (FSPS) can be envisaged as a variant of the use of immobilized reagents. It is implemented by placing the solid support inside the flow cell in order to perform the analyte (or its reaction product) retention and detection simultaneously. FSPS is an interesting spectroscopy approach that offers simple and rapid procedures that fulfil satisfactorily the requirements demanded by GAC. This article is focused on the contribution of FSPS methodologies to the GAC concept. The different strategies and types of FSPS developed will be presented and discussed in detail under the Green Spectroscopy point of view.

KEYWORDS Flow-Through Solid-Phase spectroscopy, Green Analytical Chemistry, Green Spectroscopy, optosensors

INTRODUCTION

In recent years, the need for improving analytical methodologies by reducing consumption of both solvent and reagents has become evident and this fact has brought the concept of the so-called clean analytical or environmentally-friendly analytical methods. Thus, attention is being paid to the environmental (and human) impact of analytical methods in order to drastically reduce their side effect: paradoxical situations such as the chemicals used for analysis showing higher toxicity than the analytes to be determined should be avoided. In this sense, Green Analytical Chemistry (GAC) is now an important trend in analytical chemistry in modern society and an emerging area of increasing importance in Green Chemistry, as some of the principles of the latter are strongly related to analytical chemistry (i.e., minimizing reagent consumption and waste generation; use of safer solvents and auxiliaries; minimizing the potential of chemical accidents by means of a safer chemistry). Nowadays, spectroscopic methods dominate the area of GAC and, on the other hand, it must be assumed that automated flow-based

An invited paper submitted to a special issue on Green Spectroscopy and Analytical Techniques, organized by Professor Miguel de la Guardia, of the Department of Chemistry, University of Valencia, Spain, and Professor Arabinda Kumar Das, of the Department of Chemistry, University of Burdwan, West Bengal, India.

Received 6 October 2008; accepted 14 November 2008.

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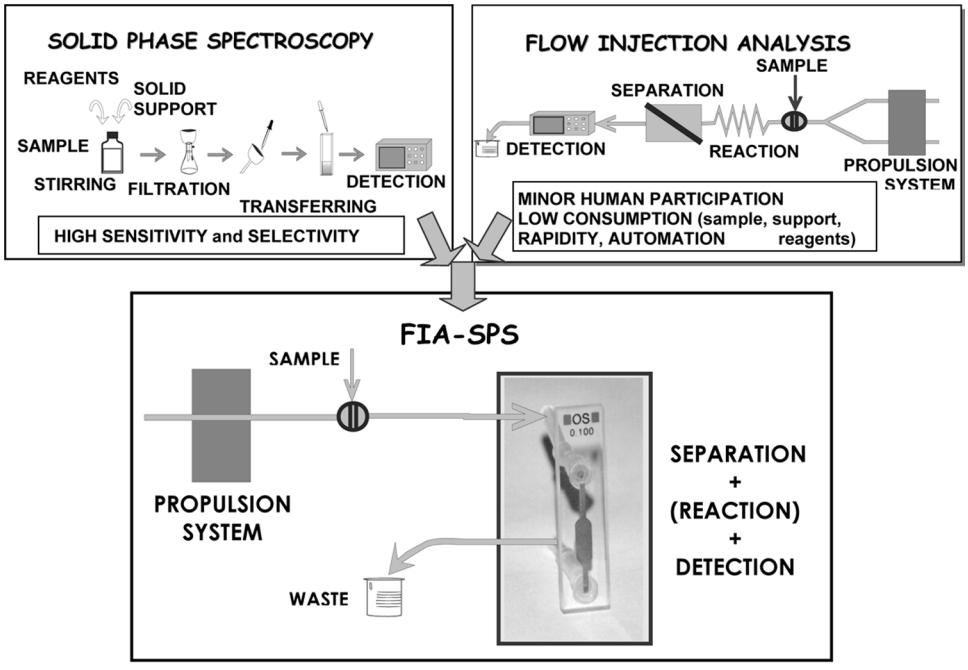
methodologies have an inherent potentiality to develop greener analytical procedures. Nevertheless, although the progress of flow methodologies has contributed to a greener analytical chemistry, its potentialities can, no doubt, still be exploited in this sense. Although, in general, changing reagents without affecting the analytical performance is not an easy task, general alternatives to minimize the reagent consumption are readily available. In this sense, flow-based methodologies combined with a conscientious use of reagents may produce positive results.[1] Therefore, to replace toxic reagents, to miniaturize and to automate analytical methods are the main approaches to make analytical methodologies go "greener,"[2] making it possible to reduce significantly the amounts of reagents consumed and wastes generated, so using analytical chemistry for pollution prevention, that is, to operate under the "Green Analytical Chemistry" concept.

There are three ways to reduce the environmental impact of analytical methodologies[2]: (1) reduction of the amount of solvents required in sample pretreatment; (2) reduction of the amount and toxicity of solvents and reagents used in the measurement step, mainly by automation and miniaturization; and (3) development of alternative direct analytical methodologies not requiring solvents or reagents. In this article, the combined use of automated continuous flow systems with solid-phase spectroscopy (SPS) will be described in detail as an automated strategy for reducing the amount of solvents, reagents, and waste generation for the analytical laboratory.

The immobilization of the species of interest (either the analyte, or its derivative product) on an appropriate solid support (usually microbeads from either a polymeric or a non polar material) and the direct measurement of its analytical property (typically absorbance or luminescence) on the solid support was firstly described by Yoshimura and Walki.[3] When this principle is implemented with flow methods by placing the solid microbeads inside the flow cell, so performing successive measurements on these microbeads (with appropriate regeneration after each measurement) the retention, preconcentration, and detection processes are all performed simultaneously and, moreover, in the same place of the flow system (in the flow cell itself). Thus, noticeably increasing in both sensitivity and selectivity are reached, together with a drastic decrease in reagent (including microbeads material) consumption. This implementation (FSPS) is called an optosensor or a flow-through optosensor[4] or, simply, a sensor. Fig. 1 illustrates the characteristics



Integration of SPS and Flow Injection Analysis: the optosensing concept

FIGURE 1 Characteristics of FSPS methodologies from those of SPS batch methods and conventional solution FIA methods.

of FSPS methodologies compared with SPS batch methods and conventional solutions flow methods. In many cases, it is possible to choose a carrier solution able to perform, moreover, the function of the eluting solution, so achieving a reproducible and reversible retention of the analyte on the solid support, resulting, thus, in a substantial decrease in reagent consumption. However, when the monitored species is so strongly retained on the solid sensing beads that the regeneration step is extraordinarily difficult to get, drastically reducing the sensor life time, flow injection renewable surface methodology[5] is recommended, in which a single-use solid sensing surface is employed, being automatically discarded after each determination, so the use of an eluting solution is not needed. Sequential Injection Analysis (SIA) is an alternative to FIA that has also been implemented with solid phase detection[6] providing strong reduction of both reagents and waste. Finally, the recent implementation of multicommutation principle[7] (which uses discrete commutation devices, usually solenoid valves) with SPS detection[8] has become another significant contribution to the GAC concept in the field of optosensors. In multicommutated optosensors, each reagent solution is managed independently, so introducing in the system only the required volume for each sample analysis, thus providing drastic minimization of both reagent solutions and waste generation.

An ideal green analytical procedure should be implemented without reagents or, in a more realistic approach, by employing only non-toxic chemicals. This is the case of FIA–SPS methodologies combined with the use of universal spectroscopic molecular detectors (such as UV spectrophotometers or spectrofluorimeters) to determine target species by reagentless procedures. Therefore, FSPS is an interesting spectroscopy approach that offers single and rapid procedures that fulfill satisfactorily the requirements demanded by GAC. This article is focused on the contribution of FSPS methodologies to the GAC concept. The different strategies and types of optosensors developed will be envisaged and commented on under the Green Spectroscopy point of view and presented according to the flow analysis approach (FIA, SIA, and Multicomutation) in which they are based.

FLOW THROUGH SOLID PHASE SPECTROSCOPIC METHODOLOGIES

FIA–SPS Methodologies (with No Derivatizing Reaction)

Spectroscopic sensing devices which do not use derivatizing reagents are the simplest FIA–SPS methods and they represent a realistic approach to the ideal green analytical procedure without reagents other than the carrier (typically a non-toxic buffer solution). It is convenient to design the system to achieve reversible retention of the analyte, as in this way a substantial decrease in reagent consumption (no carrier solution is needed) and a higher lifetime of the sensing phase is achieved (more stability in the base line is obtained). Fig. 2(a)–(f) shows some of the more relevant configurations designed in these types of FIA–SPS methodologies.

Monoparameter Sensors

The simplest FIA optosensor without derivatization of the analyte uses a monochannel manifold in which the carrier solution acts also as eluent, and at the same time, an intrinsic analytical property of the analyte is the basis of the determination (Fig. 2(a)). These are sensors reducing and avoiding side effects of analytical methods. In these systems, sensitivity is increased compared to the same flow method in homogeneous solution (that is) without using sensing support due to the concentration of the analyte in a small volume of the solid phase. Typically several orders of magnitude (2–3) higher can be achieved. They show several interesting features in relation to GAC:

1. Reutilization of the sensing zone (solid support) drastically saving a considerable amount of solid phase compared to the same procedure in batch mode: usually, the lifetime of the sensing support is about 300 (or more) determinations, so a 300-fold saving of it is achieved, even more, taking into account that the amount used in each batch measurement is higher than that used in the sensor.
2. The amount of sample required is drastically decreased: from ca. 100–1000 ml in batch mode to ca. 100–1000 μl (1000 times lower).
3. The only reagent required is the carrier solution (usually a buffer one) that, in turn, acts as an

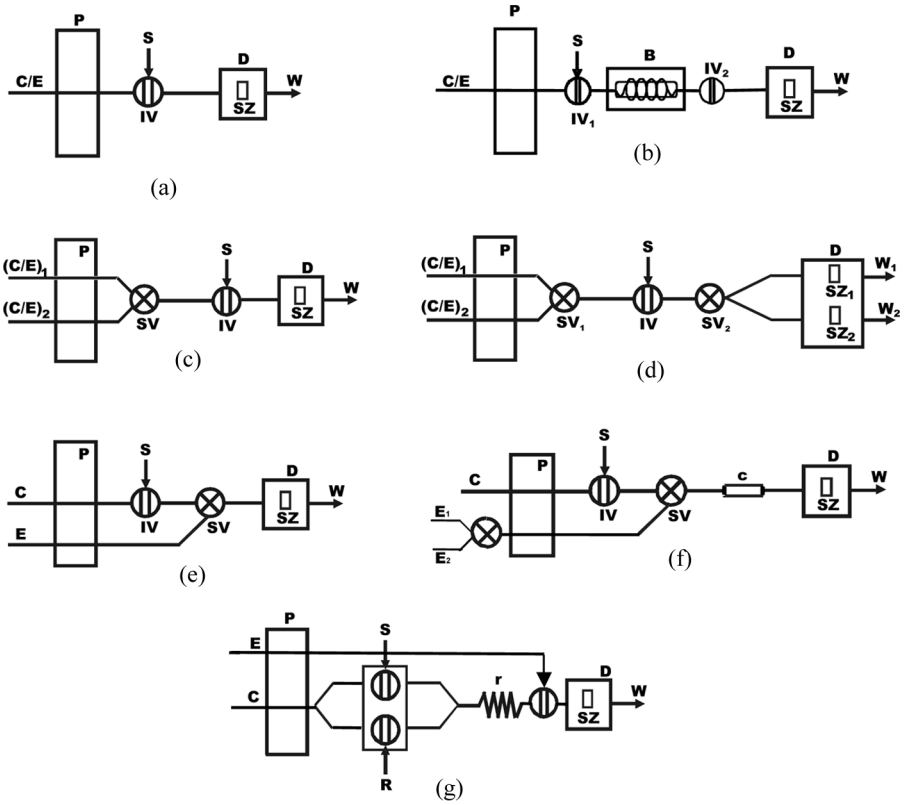


FIGURE 2 **Designed manifolds for FIA-SPS methods: (a)–(f): Without using reagents other than carrier. (g) With derivative (chromogenic or fluorogenic) reagent.**

eluting solution, which is able to make the sensor reversible, allowing one to perform many determinations on a small amount of it.

4. Absence of derivatizing reagent (the selectivity of sensors that do not use derivatization of the analyte are based on the online selective analyte retention just in the detection area).

Recently, our research group has implemented flow-through solid-phase spectroscopic transduction with Photochemically Induced Fluorescence (PIF) and has developed several types of sensors based on this principle.[9,10] The concept of PIF is based on the conversion upon UV irradiation of non-fluorescent analytes into strongly fluorescent photoproduct. The use of photons as the "derivatizing reagent" has the advantage of being easily "added" to and "removed" from the reaction system, and therefore problems caused by undesirable or dangerous reagents such as quenching or environmental pollution are avoided. Several monoparameters sensors based on this principle have been proposed[9,10] and they work similarly to those earlier described (Fig. 2(b)). These sensors are another example of green spectroscopic procedures development.

Multiparameter Sensors

The described characteristics are even more enhanced in those sensors based on the same principle described earlier, which are able to respond sequentially to two different analytes in the same sample (biparameter sensors). They just use two different appropriate alternative carrier/self-eluting solutions (by means of a selecting valve) and only a sensing zone, as usual (see Fig. 2(c)).[11] In this case two successive sample injections are needed (one for each analyte).

A similar situation is that in which the sensor works just as that described earlier does, but using two different sensing zones (one for each analyte) (Fig. 2(d)).[12]

When the species is strongly retained on the solid support in such a way that the carrier cannot elute it, an additional eluting solution has to be used. It is introduced in the system by either an injection valve or a selection one. However, the analytical

advantage these sensors show is a higher sensitivity, although they exhibit lower both sampling frequency and lifetime[13] (Fig. 2(e)).

Another strategy for a multiparameter sensor based on transitory measurements of an intrinsic analytical property (absorbance or luminescence) is to retain on-line one of the analytes in a minicolumn, while the (an)other one(s) passes to the sensing zone. The subsequent elution of the (an)other analyte(s) from the minicolumn by the appropriate eluting solution allows simultaneous multicomponent analysis (typically two or three analytes) in the same sample (temporary sequentiation of the arrival to the sensing microbeads, provided by the separation in the minicolumn), Fig. 2(f).[14] This set-up can be simplified by suppressing the minicolumn and substituting it by an additional amount of sensing microbeads inside the flow cell above the detection area. This strategy performs the separation of the analytes in a similar way to that from the on-line minicolumn, but the saving of solid phase is increased, and the manifold simplified, with subsequent advantages.[15]

Finally, another interesting strategy in biparameter sensors with no derivative reagents are those based on the two following aspects.[16,17]: (1) the measurement of one of the two analytes (which is not retained on the solid support) when it passes through the interstices among the sensing microbeads, and (2) the transitory measurement of the other analyte on the solid support as it passes thorough the sensing zone, being eluted by the carrier itself. These biparamenter sensors are used when the concentration of the first analyte is much higher than the second one. Therefore, it is a sensor with a dual functioning of the sensing area: (1) detection in homogeneous solution with a low effective pathlength (first analyte) and (2) retention (preconcentration)/elution/detection on the solid microbeads. The manifold used is the same shown in Fig. 2(f). With only a sample injection, two analytes can be determined in the sample by means of a minicolumn that retains on-line an analyte while the other is determined in the interstitial solution. Then, the second analyte is eluted from the minicolumn and sensed on the solid support. On the other hand, the sensor can be used to determine solely each analyte in different separate samples (no minicolumn needed). FIA–PIF–SPS has also been used to develop biparameter sensors.[18]

Simultaneous determination of three analytes can be easily achieved with a very simple manifold without performing previous separation: the spectral features of the analytes on the solid sensing zone are exploited by using a multicalibration chemometric approach (partial least squares calibration, PLS-1) together with the different kinetic behavior in their retention-elution process as they pass through the sensing zone.[19–21] In all these spectroscopic sensing devices, the contribution to Green Spectroscopy is evident and compatible with their application to real samples without using derivative reactions while sensitivity and selectivity are increased with respect to conventional FIA. An important decrease in both sample and reagents are achieved as compared to the use of SPS in batch mode.

FIA Sensors with Derivatizing Reactions

There are two possibilities in the development of these systems: (1) to develop the reaction on-line, before the sample plug reaches the sensing area or (2) to immobilize the reagent on the sensing support, so it is successively reutilized.

On-Line Reaction Development

This option involves the need of an additional channel for reagent. Reagent is injected in order to perform the reaction on-line before the species is sensed[22]: double synchronized injections of both sample and reagent with merging zones configuration are typically used, so contributing to a drastic saving of reagent. Fig. 2(g) shows the typical manifold used in a sensor of this type for determination of vitamin B_6 vitamers,[22] vanadium,[23] and vitamin B_6.[24]

Immobilization of the Reagent on the Sensing Support

The permanent immobilization of the derivatizing reagent on the sensing microbeads simplifies the manifold and, on the other hand, saves more reagent than the former, as the little amount of reagent immobilized is reutilized many times by choosing an appropriate eluent to only elute the analyte, so each time remaining the reagent untouched on the solid sensing phase for the next determination

(generally, the reactions described are chelating ones). In this option, the reaction, preconcentration (retention), and detection take place at the same time and in the same place of the system (just in the detection area). So, the minimization of reagents (included the solid support) and wastes, together with a simpler manifold are self-evident, so from the point of view of GAC this option is preferred to that in which an on-line reaction is performed. Two relevant examples are the sensors devoted to determine nickel[25] or zinc.[26] The reagent consumption is minimized in a factor of, at least, the number of determinations that can be performed according to the lifetime of the sensor (200 or more). A variant of this type of sensors is that in which the reagent is placed as a film in the wall of the cell.[27]

Sensors Based on Bead Injection Spectroscopy (BIS)

In all flow sensors described earlier, the solid phase is always viewed as a permanent component of the system. After measurement, the solid support beads have to be regenerated, so the sensing zone remaining ready for the next sample, thus making the sensor reusable. This is a key aspect of these systems, which have to fulfill the necessary compatibility requirements of the eluting agent to use. When the carrier solution is able to elute the retained species after the sample plug tail reaches the detection zone (just when the analytical signal reaches its maximum value), the base line shows a high stability and both sampling frequency and lifetime of the sensing beads are higher.[28] Nevertheless, in the necessary regeneration steps of the bead surface several problems can arise: (1) the surface deactivation after sensing a certain number of measurements always occurs, so limiting the expected requirements of longevity and repetitive use of the system and (2) when the monitored species is strongly retained (i.e., multicharged species on ion exchange beads), the solid surface regeneration becomes extraordinarily difficult to get and the lifetime of the sensing surface is drastically reduced. The drawbacks mentioned can be avoided by using the so-called flow injection-renewable surface sensing methodology.[29] This, methodology, based on the concept of bead injection spectroscopy (BIS), can be envisaged as the third generation of FI microanalytical techniques.[30] An exact volume of a homogeneous bead suspension is injected in the flow system. The beads are trapped in the cell. The analytical signal (molecular spectroscopy detection) is developed by passing appropriate reagents and the sample plug through the beads. At the end of the analysis, beads are automatically discarded from the flow cell by reversing the flow and transported out of the system. In this sense, these types of flow sensors based on the implementation of bead injection spectroscopy (BIS) with FIA (or SIA) work in a similar way to SPS in batch mode, using the beads for only a measurement each time. Nevertheless, the automation of the procedure guarantees the natural advantages of SPS–FIA (or SPS–SIA) against manual (batch) mode and both the sample and derivative reagent saving. So, the determination of ascorbic acid, for example, involves the use of 10 ml of reagent solutions (Ferrozine +Fe(III)), 10 ml of buffer solution and up to 80 ml of sample in manual mode,[31] versus a total of 4.8 ml (Ferrozine + Fe(III) + buffer solutions) and 0.1 ml of sample using the BIS–FIA sensor shown in Fig. 3,[6] although the amount of solid support is the same in both cases.

Sensors Based on Sequential Injection Analysis (SIA)

Sequential injection analysis is a robust alternative to flow injection that allows implementation of different flow methodologies without modification of the manifold. A selection valve is employed to manage all solutions and system operation can be designed to achieve an analytical performance comparable to that attained by usual flow injection systems.

The implementation of SIA with SPS is another recent alternative methodology in the field of optosensors that also contributes to the GAC concept.[6] Monoparameter[32] and multiparameter sensors have recently been described based on this implementation, the latter using dual spectroscopic detection (luminescence and chemiluminescence).[6] The SIA concept is thus combined with that of the SPS in such a way that a remarkable saving of reagents and waste is achieved with this implementation, together with a higher automation level.

Sensors Based on Multicommutation

Multicommutation consists in the employment of discrete commutation devices (typically three-way

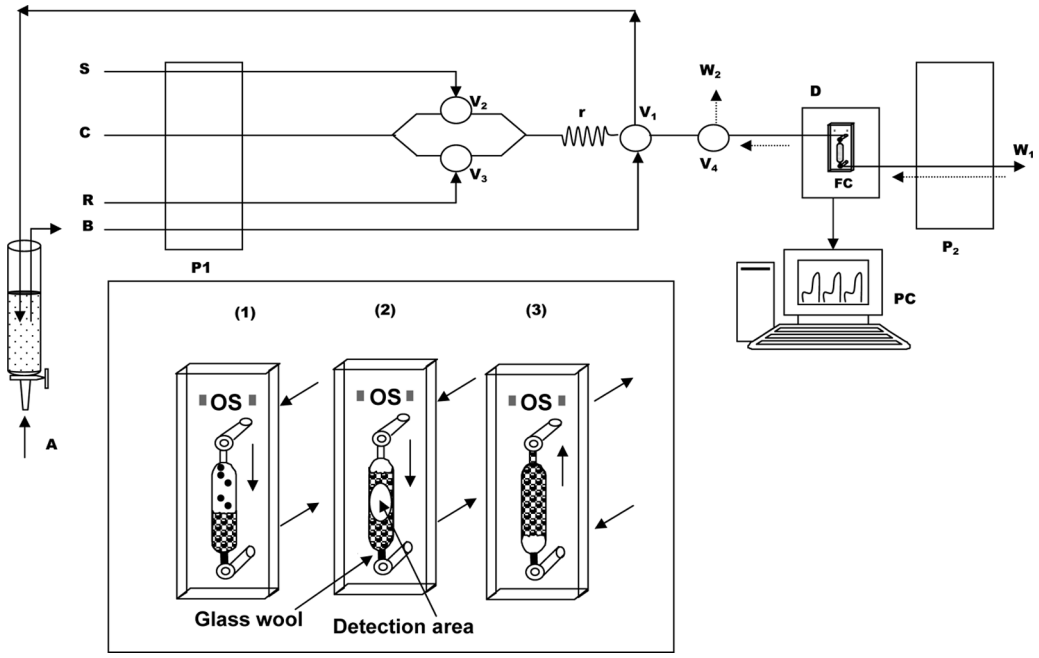


FIGURE 3 **FSPS manifold for determination of ascorbic acid (BIS-FIA optosensor) A: air; S: sample; P_1, P_2: peristaltic pumps; C: carrier solution; R: reagent solution; B: bead suspension; V_1, V_2, V_3: injection valves; V_4: selection valve; r: reaction coil; D: detector; FC: flow cell; W_1, W_2: waste; PC: computer. Inset: detail of flow cell during the different processes occurring in it: (1): loading the sensing microbeads; (2): measuring; (3): discarding the microbeads.**

solenoid valves) to build up dynamic manifolds that can easily be reconfigured by means of software.[7] This approach greatly increases the versatility of the flow systems because each analytical step can be independently implemented. Usually, one commutation device is employed to manage each solution and thus only the necessary reagent volume is introduced for sample analysis. Micro-volumes of samples and reagents are sequentially inserted into the reaction coil of a single line manifold, providing a simple system, suitable mixing conditions, and easy optimization of the sample/reagent ratio, avoiding excessive use of reagents. Multicommutation has the advantages of minimizing both reagent consumption and waste generation.

Recently, the combination of multicommutation with SPS has become a very fruitful implementation in the optosensor field[8] and both mono- and multi-parameter optosensors have been described based on the multicommutation–SPS coupling.[33–35]

The two flow configurations used in the described multicommutated multioptosensors are shown in Fig. 4. In the first one (Fig. 4(a)) separation takes place on-line on a minicolumn located before the sample reaches the detector and filled with the same type of support used in the flow cell. While one of the analytes passes through it and reaches the detector, originating a transitory signal, the another one (or other two) analyte(s) is (are) retained in the precolumn. Later, they are eluted (successively) from it with one (or two) eluent(s), that transport(s) separately the respective analyte, which is transitorily retained in the sensing zone, so developing its signal. In each case, both the carrier and eluent solution(s) act, regenerating the sensing solid phase. In the second configuration (Fig. 4(b)) no precolumn is used, but the flow cell is filled up with an additional amount of sensing support above the upper limit of the irradiated zone. Two processes take place in the cell: the separation and the detection. The separation of the analytes takes place one is strongly retained above the detection zone, while the other originates a transitory signal when it passes through the detector. Then, the second analyte is appropriately eluted, developing its analytical transitory signal. This second configuration requires that the two analytes show a very different behavior as far as their interaction with the solid support (they show a greatly different polarity). Based on the first configuration, a few photometric and fluorimetric optosensors have been developed that are able to respond to two or three analytes in the same sample

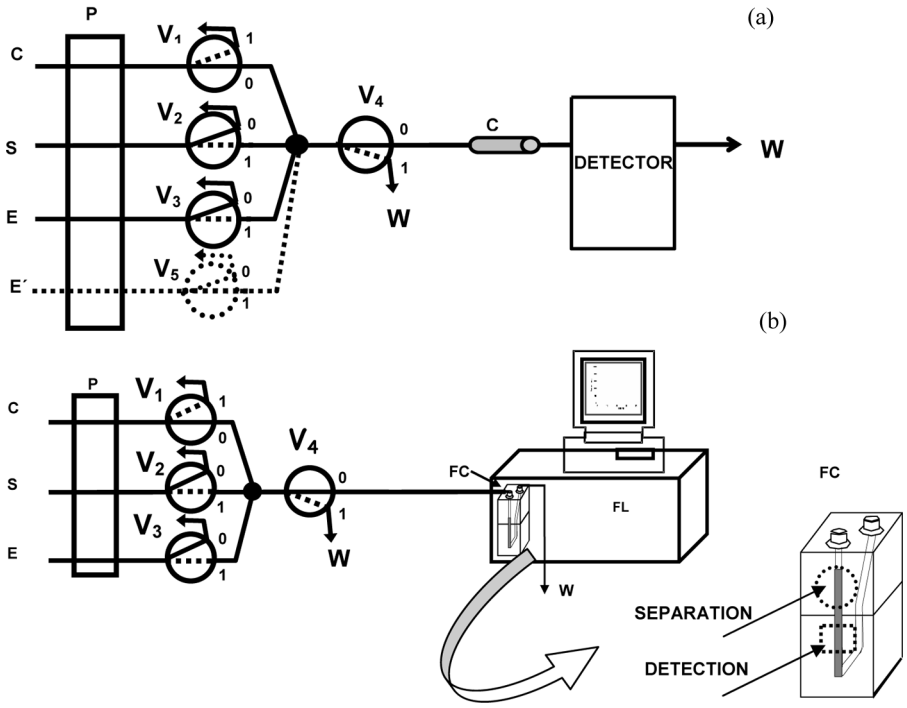


FIGURE 4 **Manifolds described for multicommutated multioptosensors. (a): on-line separations in a minicolumn filled with microbeads of the same solid support used in the cell. Photometric or fluorimetric detection. (b): separations performed inside the flow cell, above the detection zone, by using an additional extra amount of microbeads. Fluorimetric detection. P: peristaltic pump. Vi: three way solenoid valves: position 1 indicates activate valve; position 0 indicates deactivated valve. C: carrier solution; S: sample; E: eluent solution. C: minicolumn. FC: flow cell. FL: spectrofluorimeter. W: waste.**

with only a sample injection.[34] Based on the second configuration two fluorimetric biparameter sensors have been described.[33]

These multicommutated multioptosensors exhibit interesting analytical properties compatible with green spectroscopy. Thus, minimization of both sample and reagents consumption (inherent to multicommutation principle) is achieved by re-cycling them to their respective vessels while they are not being used in the flow system, no derivatizing reagent is used, a single injection allows multicomponent determination, and enhanced sensitivity and selectivity are provided by the solid phase in the cell.

FLOW SPS VERSUS BATCH SPS FROM A GAC POINT OF VIEW

FSPS methods are greener chemistry methodologies than batch SPS. As a representative example, Table 1 shows the amount of reagents and sample for the determination of ascorbic acid by means of different SPS methodologies, namely spectroscopic batch methods in UV and Vis regions: UV–SPS,[36] Vis–SPS,[31] and UV–FSPS;[11,37–39] Chemiluminescence-SPS (CL–SPS)[7]; and BI-SPS in Vis region.[6,40] As can be seen, reagent(s) and sample volumes are much higher in SPS batch mode (in both, UV and visible detections). The higher volume of reagent necessary in sensors is only that of the carrier solution. It should be emphasized that the drastic reduction of solid support per sample determination in all sensors (up to 400 times lower) is due to its reutilization. This is a remarkable characteristic of FSPS: in some cases, about 300 or even more determinations can be performed on the same amount of microbeads. As regarding the waste volumes, in some cases reductions by a factor of about 60 can be estimated from Table 1. Similar situations of sample and reagent(s) saving can be found as comparing the determination of a concrete analyte by batch SPS and by means of the respective FSPS (optosensors) schemes, that is, for the determination of iron with ferrozine,[4,41] vitamin B_1 (intrinsic UV absorption),[42,43] amiloride (UV absorption and native fluorescence),[44,45] vitamin B_6 (native fluorescence)[46,47]; or a conventional FIA method in homogeneous solution (without using solid phase)[48] with the respective sensor.[4]

TABLE 1 **Comparison of Different Methods for Ascorbic Acid Determination Based on the Use of SPS (Batch and Sensors Methodologies) in Ultraviolet (UV) and Visible (Vis) Regions**

Procedure	Reagent(s) volume (ml)	Sample volume (ml)	Amount solid support (mg/sample)	Sampling frequency (h^{-1})	Lifetime (estimated determinations)	Ref.
UV-SPS batch mode	1	10	40	—	—	36
Vis-SPS batch mode	20	80	30	—	—	31
UV-FSPS (Sensor)	2*	0.3–1	0.1**	21–28	300	37
UV-FSPS (Sensor)	2.6*	0.3–1	0.1**	18–23	300	11
UV-FSPS (Sensor)	3.8*	0.3–1	0.1**	22	300	38
UV-FSPS (Sensor)	2.5*	0.6	0.1**	24	300	39
CL-FSPS (Sensor***)	0.6	0.4	0.1**	8	300	7
BI-SPS (Sensor, Vis)	5.8* + 1.5	0.8	10	13	—	40
BI-SPS (Sensor, Vis)	4.7* + 0.1	0.1–1	30	13–16	—	6

*Volume of carrier (a buffer solution).
**Taking into account the reutilization of the solid phase according to lifetime.
***SIA sensor.

CONCLUDING REMARKS

FSPS using conventional FIA, BIS–FIA, SIA, or multicommutation allow one to develop very simple and reliable methodologies that contribute to a greener analytical chemistry by saving sample, reagents, and waste generation. In addition, they exhibit remarkable analytical features in terms of sensitivity and selectivity provided by the *in situ* preconcentration of analyte(s) on the solid sensing support. Moreover, the possibility of multicomponent determination under different operational designs, together with the potentiality offered by the easy reconfiguration of the systems by means of software in the case of multicommutation, makes the FSPS concept an attractive, cheap, and interesting analytical tool under a green spectroscopic point of view, applicable to solve real analytical problems. In spite of flow methodologies that have contributed to a GAC in the last decades, its potentialities can still be exploited in a deeper fashion. In this sense, the coupling of SPS with flow analysis is a fruitful area under a double point of view: the progress of flow methodologies by themselves and the contribution to a greener analytical spectroscopy or "Sustainable Analytical Procedures," as an alternative denomination proposed by Armenta et al.[2]

ACKNOWLEDGMENTS

The authors acknowledge funding support from Junta de Andalucía (Regional Government of Andalusia (Spain)) (Research Group FQM323). B. Gilbert-López also acknowledges a scholarship from Junta de Andalucia (Project reference FQM-01463).

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