# Characterization of an Attenuated Total Reflection-Based Sensor for Integrated Solid-Phase Extraction and Infrared Detection

**4 AUTHORS,** INCLUDING:

Rafael Lucena
University of Cordoba (Spain)
**82** PUBLICATIONS **1,530** CITATIONS

SEE PROFILE

Miguel Valcárcel
University of Cordoba (Spain)
**296** PUBLICATIONS **5,601** CITATIONS

SEE PROFILE

# Characterization of an Attenuated Total Reflection-Based Sensor for Integrated Solid-Phase Extraction and Infrared Detection

**M. Carmen Alcudia-León, Rafael Lucena, Soledad Cárdenas, and Miguel Valcárcel***

*Department of Analytical Chemistry, Marie Curie Building (Annex), Campus de Rabanales, University of Cordoba, E-14071 Cordoba, Spain*

**A novel attenuated total reflection (ATR) sensor, which integrates solid-phase extraction (SPE) and infrared detection, is presented. The flow cell, which enables the on-line coupling with a sequential injection system, is the core of the proposed sensor since it allows the continuous delivery of different solutions through the ATR and also the continuous spectrum acquisition. A SPE sorbent material (LiChrolut EN) was located in the sensitive element of the ATR without using any external coating substance, increasing the versatility of the system. A marked sensitivity enhancement was obtained as the analyte was concentrated before detection. The new sensor was qualitatively and quantitatively validated using the determination of caffeine in soft drinks as the model analytical problem. Linearity, precision (RSD = 4%), and sensitivity (LOD = 7 $\mu$g/mL) levels have been established. Finally, the main advantages and limitations of the new proposal are presented and compared with existing alternatives.**

Infrared spectroscopy (IR) is an instrumental technique that provides qualitative information about the chemical groups and chemical structure of a given molecule.[1] It has been massively used for the characterization of new materials and substances since the infrared spectrum, particularly the 600−1500-cm$^{-1}$ region, can be considered as the fingerprint of a given molecule. Besides, infrared spectroscopy can provide valuable quantitative information, which clearly increases its practical applicability.

The successful coupling of flow processing devices (the usual alternative of choice for automated sample treatment), based either on flow or on sequential injection modes, and IR detection has been revealed as a powerful tool for the resolution of different analytical problems.[2−4] Within the sample treatment alternatives, solid-phase extraction (SPE) has been widely employed for sensitivity enhancement in IR determination,[5−7] which is the main drawback of the instrumental technique.

Attenuated total reflection (ATR) modality[8,9] has gained importance because of its simplicity and expeditiousness in IR spectrum acquisition, allowing also the measurement of analytes in complex samples, unbroachable by the normal transmission techniques.[10] ATR-FT-IR has been extensively used for the development of optical chemical sensors.[11] In the most common approach, a polymeric membrane is chemically coated to the internal reflection element (IRE) of the ATR module.[12−14] The polymeric membrane enhances the selectivity since only the analytes that can diffuse through it contribute to the final signal. A sensitivity improvement is also obtained thanks to the analyte preconcentration in the membrane following the principles of the solid-phase extraction. In recent years, the use of tailored films has introduced versatility in sensor design, allowing the use of organic and inorganic hybrid materials for chemical analysis.[15]

An alternative to these sensors based on the on-line coupling of a SIA manifold and a ATR-FT-IR instrument has been recently proposed. In this configuration, the polymeric membrane is not in close contact with the IRE, leaving a small gap (~25 $\mu$m) filled with a specific organic solvent layer.[16] In this case, the analytes diffuse through the membrane to the organic solvent where they are detected. A selectivity enhancement is obtained in this system, which follows the liquid−liquid extraction (LLE) principles. The LLE-based sensor has been applied to the determination of grease and surfactants in industrial alkaline degreasing baths.[17]

* Corresponding author: (tel/fax) +34-957-218-616; (e-mail) qa1meobj@uco.es.

(1) Lendl, B. In *Analytical Chemistry, a modern approach to analytical science*; Kellner, R., Mermet, J. M., Otto, M., Valcarcel, M., Widmer, H. M., Wiley: Weinheim, 2004; Chapter 25.2.

(2) Mazarevica, G.; Diewok, J.; Baena, J. R.; Rosenberg, E.; Lendl, B. *Appl. Spectrosc.* **2004,** *58,* 804.

(3) Schindler, R.; Lendl, B.; Kellner, R. *Anal. Chim. Acta* **1998,** 366, 35.

(4) Gallignani, M.; Brunetto, M. R. *Talanta* **2004,** *64,* 1127.

(5) LeThanh, H.; Lendl, B. *Anal. Chim. Acta* **2000,** *422,* 63.

(6) Daghbouche, Y.; Garrigues, S.; Vidal, M. T.; de la Guardia, M. *Anal. Chem.* **1997,** *69,* 1086.

(7) El Hattab, N.; Daghbouche, Y.; El Hattab, M.; Piovetti, L.; Garrigues, S.; de la Guardia, M. *Talanta* **2006,** *68,* 1230.

(8) Goormaghtigh, E.; Raussens, V.; Ruysschaert, J. M. *Biochim. Biophys. Acta* **1999,** *1422,* 105.

(9) Hind, A. R.; Bhargava, S. K.; McKinnon, A. *Adv. Colloid Interface Sci.* **2001,** *93,* 91.

(10) Settle, F. A. *Handbook of instrumental techniques for Analytical Chemistry*; Prentice Hall: Upper Saddle River, NJ, 1997.

(11) Vigano, C.; Ruysschaert, J. M.; Goormaghtigh, E. *Talanta* **2005,** *65,* 1132.

(12) Karlowatz, M.; Kraft, M.; Mizaikoff, B. *Anal. Chem.* **2004,** *76,* 2643.

(13) Regan, F.; Meaney, M.; Vos, J. G.; MacCraith, B. D.; Walsh, J. E. *Anal. Chim. Acta* **1996,** *334,* 85.

(14) Branan, N.; Wells, T. A. *Vib. Spectrosc.* **2007,** *44,* 192.

(15) Flavin, K.; Mullowney, J.; Murphy, B.; Owens, E.; Kirwan, P.; Murphy, K.; Hughes, H.; McLoughlin, P. *Analyst* **2007,** *132,* 224.

(16) Lucena, R.; Cárdenas, S.; Gallego, M.; Valcarcel, M. *Anal. Chem.* **2005,** *77,* 7472.

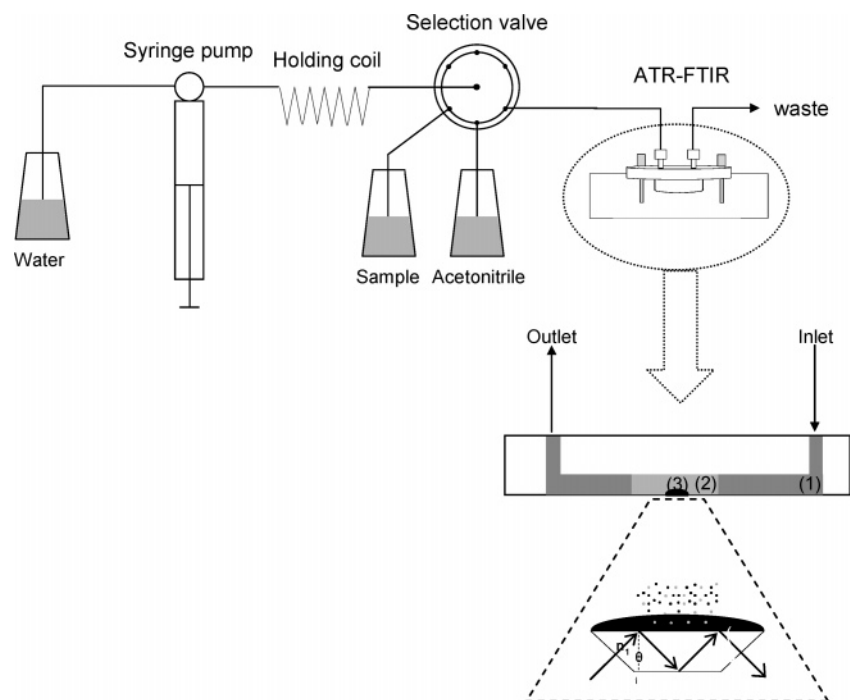(17) Lucena, R.; Cárdenas, S.; Gallego, M.; Valcarcel, M. *Analyst* **2006,** *131,* 415.

**Figure 1.** Schematic diagram of the sequential injection analysis manifold employed on-line coupled to the ATR-FT-IR spectrometer. A closer view of the flow cell is also given. (1) Microflow channel; (2) cotton frit; (3) LiChrolut EN sorbent material.

The ATR fundaments have also been applied in the so-called fiber evanescent wave sensors (FEWS).[18] In this case, a fiber optic is used to guide the infrared light to the sensing material where the evanescent wave interacts with the sample. The light is then conducted back to the detector. FEWS has been successfully applied for the determination of pollutants in wastewater,[19] chlorinated hydrocarbons in water,[20] and also in blood diagnostics.[21]

In this paper, a new ATR sensor based on the integration of SPE and infrared detection has been developed and characterized. The main goals of the present work are as follows: (a) to propose an alternative to the existing ATR-based sensors in which the polymeric membrane has been substituted by a conventional sorbent material; (b) to characterize the functioning of the proposed sensor in terms of linearity, precision, and reusability; and (c) to solve a specific analytical problem in order to demonstrate the potential of the present approach.

## EXPERIMENTAL SECTION

**Reagents and Samples.** All reagents were of analytical grade or better. Sucrose and caffeine were supplied by Sigma-Aldrich (Madrid, Spain). Acetonitrile (HPLC grade) and ammonium hydroxide were purchased from Scharlau (Barcelona, Spain). Class IV caramel powder was kindly supplied by D.D. Williamson (Quimidroga, S.A., Barcelona, Spain).

LiChrolut-EN from Merck (Darmstadt, Germany) was selected as sorbent material. It is a polystyrene−divinylbenzene-based

material with particle size in the range 40−60 $\mu$m and chemical stability in the pH interval 1−13.

Stock standard solutions of the analytes (200 g/L for sucrose, 5 g/L for class IV caramel, and 1 g/L for caffeine) were prepared separately by dissolving appropriate amount of these compounds in Milli-Q water (Millipore Corp., Madrid, Spain). Working solutions were prepared on a daily basis by rigorous dilution of the stocks in Milli-Q water.

Soft drinks samples were purchased from local markets and degasified in an ultrasonic bath (50 W, 60 Hz) for 10 min before the analysis. Finally, they were adjusted to pH 8 in order to avoid the presence of carbon dioxide bubbles.

**Apparatus.** The automated configuration used in this work is schematically shown in Figure 1. It consists of a SIA manifold on-line connected to an ATR-FT-IR spectrometer through an in-home-built flow-through PTFE accessory (obtained from the working group on Chemical Analysis and Vibrational Spectroscopy, Vienna University of Technology, Vienna, Austria), which enables pumping of the solutions over the ATR diamond surface with minimal dead-volume ($\sim$3 $\mu$L).

The SIA configuration comprises a Cavro XP 3000 syringe pump (Sunnyvale, CA) equipped with a 1-mL syringe, a Cavro six-port selection valve equipped with a microactuator, and a 4-mL holding coil. PTFE tubing of 0.5-mm i.d. and standard connectors are also employed. The setup is computer controlled by Sagittarius 3.0 software package (obtained from the working group on Chemical Analysis and Vibrational Spectroscopy, Vienna University of Technology, Vienna, Austria). This software also allows triggering the FT-IR spectrometer for exact timing of the collection of spectra.

The selection valve was connected via 15-cm-long PTFE tubing to a Bruker Tensor37 FT-IR spectrometer, equipped with a diamond ATR cell with a circular surface of 3-mm diameter and

(18) Mizaikoff, B. *Anal. Chem.* **2003**, *75*, 258A.
(19) Michel, K.; Bureau, B.; Boussard-Plédel, C.; Jouan, T.; Adam, J. L.; Staubmann, K.; Baumann, T. *Sens. Actuators, B: Chem.* **2004**, *101*, 252.
(20) Jakusch, M.; Mizaikoff, B.; Kellner, R.; Katzir, A. *Sens. Actuators, B: Chem.* **1997**, *38−39*, 83.
(21) Gotshal, Y.; Simhi, R.; Sela, B. A.; Katzir, A. *Sens. Actuators, B: Chem.* **1997**, *42*, 157.
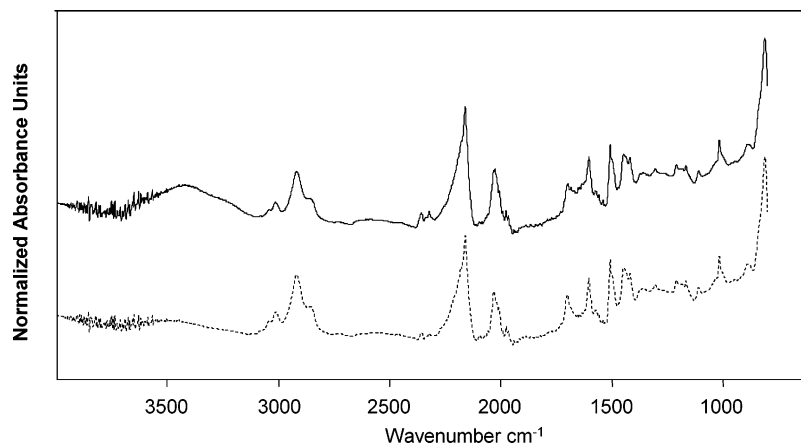
**Figure 2.** Spectra of the dry cell (solid line) and pure LiChrolut EN (dotted line).

three internal reflections. A liquid nitrogen-cooled mercury−cadmium−telluride detector was used for spectra acquisition. Spectra are collected between 4000 and 700 cm$^{-1}$ at a 4-cm$^{-1}$ resolution with 128 coadded scans each. Data collection and processing was made using OPUS software (Bruker, Ettligen, Germany).

**Sensor Description.** The ATR flow cell, described in Figure 1, can be considered as the core of the proposed sensor. The flow cell allows the continuous introduction of different streams through the sensing zone and also permits the constant monitoring of the sorbent material surface. The inlet and outlet of the PTFE accessory are connected via a microchannel (1.5 cm long) made in the lower part of the piece where a homemade cotton frit is situated. A small amount (∼5 mg) of LiChrolut EN is located between the sensitive zone of the ATR and the cotton frit. The whole accessory is then screwed on a stainless piece and thus tightly sealed to avoid any leakage. Under these conditions, the cotton frit makes pressure on the sorbent, allowing continuous surface monitoring. The absence of dead volume on the SPE flow cell and the reduced amount of sorbent minimize analyte dispersion, enhancing the sensitivity of the analytical signal.

The continuous nature of the ATR diffusion cell developed offers the inherent advantages of the automated systems as all the operations involved in the whole process, including regeneration of the sorbent, can be done without opening the cell. In addition, as the system is computer controlled it can work unattended.

**Experimental Procedure.** The proposed sensor operates in a sequential fashion comprising four basic steps: spectrum background acquisition, sample introduction, sample spectrum acquisition, and system cleanup/regeneration. At the beginning of the measurement process, 2 mL of water was pumped through the system; once the cell was completely filled with water, a reference background spectrum was acquired. After that, 3 mL of sample or standard solution containing the analyte was passed through the cell for caffeine retention. The sample matrix was washed with 2 mL of water, and the spectrum of the retained compounds was subsequently acquired. In the final step, the sorbent material was regenerated by flushing 0.25 mL of acetonitrile at which quantitatively eluted the caffeine. In all cases, the flow rate was maintained at 0.5 mL/min. The whole process is carried out without opening the cell, and every cycle took ∼14 min to be completed.

## RESULTS AND DISCUSSION

The low sensitivity can be considered the main shortcoming in infrared detection, being necessary for the implementation of a preconcentration step before measurement. Membrane-based sensors achieve this enhancement retaining the analytes on the membrane although they require a long equilibration-regeneration time and the types of membrane commercially available are not of high enough quality to deal with a wide variety of analytical problems. The inclusion of a sorbent material in a flow cell placed on the ATR module would increase the applicability of infrared detection, considering the great variety of available sorbents. It will overcome the low sensitivity, increasing also the selectivity and versatility of the technique. To be used in the proposed configuration, the sorbent must be chemically compatible with the target analytes and it should allow the interaction between the analyte and the radiation (i.e., it should not absorb all the photons coming from the interferometer). Moreover, the sorbent has to be capable of interacting with the analytes, allowing their retention on the surface.

The sensor proposed in this work uses a commercially available sorbent material, LiChrolut EN, in order to increase the analytical signal obtained. Figure 2 represents the spectrum of the dry ATR cell in which the characteristic bands of the sorbent are identified (solid line) by comparison with the spectrum of pure LiChrolut EN directly measured in the ATR (dotted line). The effect of the sorbent placed in the IRE of the proposed sensor on the analytical signal is described in Figure 3 by means of a selected example, i.e., the retention of caffeine on LiChrolut EN. As can be seen, the direct analysis of a 50 μg/mL standard solution of caffeine provides a straight line spectrum coincident with the blank signal. When the same standard solution is analyzed using the proposed sensor, an evident sensitivity enhancement is obtained as the characteristic bands of caffeine can be identified. This fact demonstrates the efficiency of the analyte preconcentration on the sorbent surface.

**Model Analytical Problem.** The determination of caffeine in soft drink samples was selected to characterize the response of the proposed sensor. Soft drinks consist of four main components: water; sweeteners, colorant, and caffeine. In order to
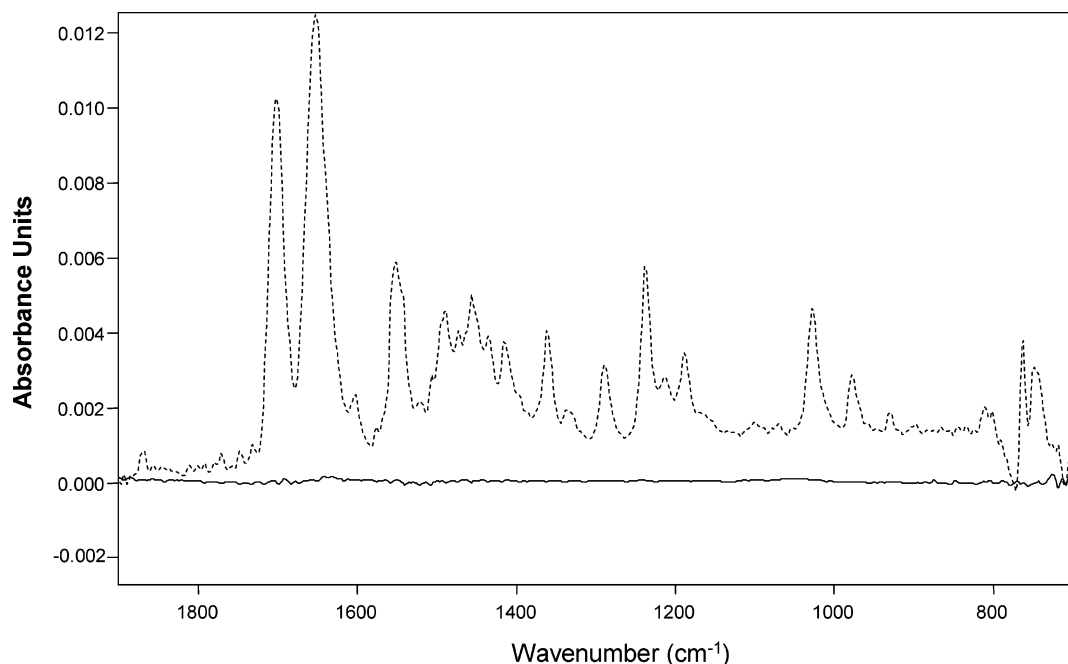
**Figure 3.** Effect of the sorbent in the IRE of the ATR on the sensitivity. Caffeine spectrum obtained after the SPE step (dotted line); Spectrum obtained without SPE step (continuous line).

facilitate the study, only regular soft drinks were taken into consideration. These beverages use sucrose (or a mixture of glucose and fructose) and class IV caramel as sweeteners and colorant, respectively, at concentration of ~100 g/L (sucrose) and ~0.8 g/L (class IV caramel). Caffeine is added to soft drinks as a part of the flavor profile as its bitter taste enhances other flavors. The U.S. FDA limits the maximum amount to 200 mg/L, and therefore, a sample treatment is required to improve the selectivity (avoiding the interferences of sucrose and caramel) and sensitivity (preconcentrating the analyte) of the determination.

**Sensor Characterization.** The operation principles of the new sensor were unequivocally proved by means of a simple study. In a first step, the retention of caffeine was monitored against the time passing through the system an accurate volume (3 mL) of a 50 $\mu$g/mL standard solution of caffeine. At the beginning, only the expected variation of the water band intensities (regions 1700−1600 and 900−800 cm$^{-1}$) is observed. Later on, the characteristic bands of a caffeine spectrum appear increasing their intensity gradually. The evolution of the spectrum area against time is presented in Figure 4. An obvious increase in sensitivity is obtained with the proposed system, which clearly demonstrates the excellent performance of the sensor.

In order to evaluate the potential interference of other compounds that can be present in the matrix of interest (e.g., soft drinks), a systematic study was carried out using sucrose and class IV caramel. In this way, a standard solution containing caffeine and sucrose at a final concentration of 50 $\mu$g/mL and 100 g/L, respectively, was prepared. The concentration for each analyte was selected taking into account the composition of real samples. The obtained results are summarized in Figure 5. After injection, a rapid increase in the absorption intensities is observed due to the presence of sucrose at a high concentration (spectrum I). Once the sample plug has completely passed through the cell, the intensity of the sucrose spectrum decreases and caffeine peaks appear (spectrum II). Finally, sucrose is completely removed from
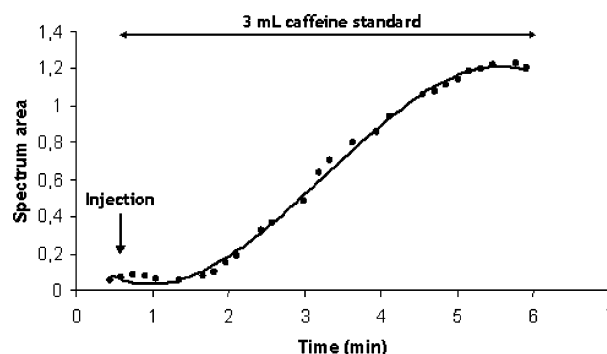


**Figure 4.** Evolution of caffeine spectrum area against time.

the system and only caffeine remains in the sensor (spectrum III). The presence of sucrose peaks in the recorded spectra can be explained taking into account the porosity of the sorbent, which allows the diffusion of the analytes together with water to the sensitive element of the instrument. The cleanup is therefore essential in order to determine only the adsorbed analytes.

A similar study was carried out using a standard solution containing caffeine and class IV caramel at a final concentration of 50 $\mu$g/mL and 0.8 g/L, respectively. The spectra obtained lead to the same conclusion as no interference from caramel was observed in caffeine determination.

Reusability is another valuable characteristic of a sensor and it was also evaluated in this configuration. For this purpose, once the analyte was retained under the previously described conditions, elution, cleanup, and conditioning steps were evaluated. Acetonitrile was selected for caffeine elution, and the eluent volume needed was studied between 0.1 and 1 mL (flow rate of 0.5 mL/min). It was demonstrated that 0.25 mL of acetonitrile was enough for both caffeine elution and sorbent conditioning. The sorbent reusability was calculated by the injection of 1 mL of a standard solution of caffeine by triplicate during 10 working days ($n = 30$). The reproducibility of the spectra was better than 5% in
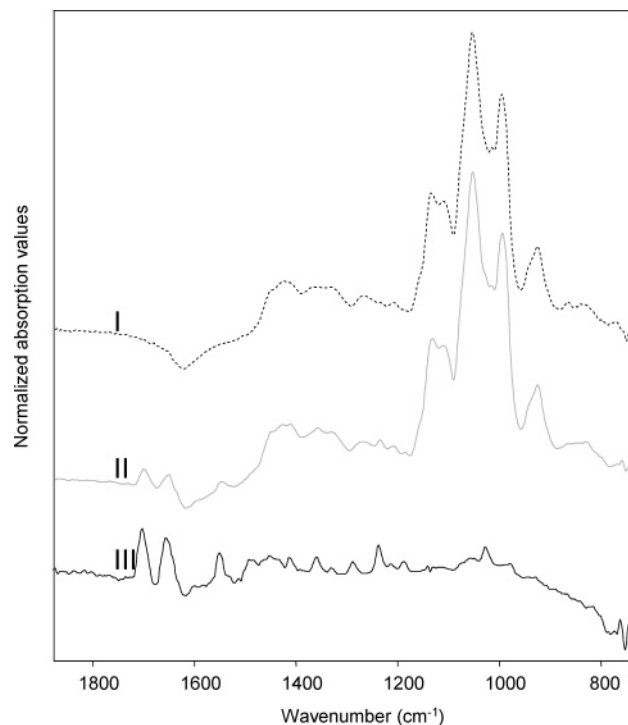
**Figure 5.** Evolution of the signal during the SPE procedure. (I) Spectrum when the sample plug is passing through the cell; (II) spectrum in the cleanup step; (III) after cleanup.

### Table 1. Statistical Data of the Regression[a]

| regression equation | $S_a$ | $S_b$ | $S_{yx}$ |
|---|---|---|---|
| $y = 0.0154x + 0.5382$ | 0.0314 | 0.0005 | 0.0365 |

[a] $S_a$, standard deviation of intercept; $S_b$, standard deviation of slope; $S_{yx}$, standard deviation of estimate.

### Table 2. Limits of Detection and Quantification

| method | equation[a] | LOD ($\mu$g/mL) | LOQ ($\mu$g/mL) |
|---|---|---|---|
| intercept standard deviation | $LOD = 3S_a/b$ | 6.1 | 20.3 |
| estimate standard deviation | $LOD = 3S_{yx}/b$ | 7.1 | 23.6 |
| method limit of detection | $MDL = t_{(n-1)}S_n$ | 5.2 | 17.3 |

[a] $S_a$, standard deviation of the intercept of the calibration curve. $b$, slope of the calibration curve. $S_{yx}$, standard deviation of the estimate of the calibration curve. $S_n$, standard deviation of n replicates of a sample in which the analyte is spiked at a concentration for 2−5 times the expected LOD.

### Table 3. Analysis of Real Samples with the Proposed Sensor

| sample | caffeine concn ($\mu$g/mL) $\pm SD^a$ ($n = 3$) |
|---|---|
| regular soft drink 1 | $93 \pm 2$ |
| regular soft drink 2 | $77 \pm 2$ |
| regular soft drink 3 | $65 \pm 2$ |
| caffeine-free soft drink 1 | $38 \pm 1$ |
| caffeine-free soft drink 2 | $35 \pm 1$ |

[a] SD, standard deviation.

terms of integrated area from 1800 to 1000 cm$^{-1}$. The results obtained demonstrated the efficient functioning of the proposed sensor since a quantitative preconcentration of the analyte is achieved in a simple way. Moreover, the potential interference of other compounds, even if they are present at higher concentrations is avoided.

Once the functioning of the sensor was characterized, two important aspects, the precision and the linearity of the response, were evaluated. Precision was calculated by seven sequential injections of 3 mL of the same caffeine standard solution (50 $\mu$g/mL) in the system. The relative standard deviation of the peak area integrated between 1800 and 1000 cm$^{-1}$ resulted to be 4%.

In order to be useful for quantitative determinations, the signal provided by the proposed sensor must respond to the retained analyte amount. For this purpose, different volumes (in the range 0.5−15 mL) of a 50 $\mu$g/mL caffeine standard solution were injected into the system, being the signal acquired after each milliliter. If the integrated areas of each spectrum (from 1800 to 800 cm$^{-1}$) are plotted versus the volume of the standard, a quadratic relationship ($R = 0.9996$) is obtained. It indicates that analyte retention becomes more difficult when higher volumes are passed through the system. The limited sorbent capacity, because of the small amount used, can be the reason for this behavior.

**Analytical Performance of the Method.** In light of the presented data, the proposed sensor seems to be an excellent tool for quantitative analysis, allowing the determination of caffeine in a rapid and simple way. Once the sensor was characterized, its analytical figures of merit were calculated. For this purpose, different standards containing the analyte in the range 20−100 $\mu$g/mL were injected by triplicate and the corresponding spectra acquired. The spectra were integrated in the range 1800−800 cm$^{-1}$, and the areas were used as analytical signal. The main statistical data of the regression are represented in Table 1.

For the calculation of the limits of detection and quantification, different criteria were employed. In a first approach, the standard deviation of the intercept and estimate of the obtained regression were used. In this case, the limits of detection (LOD) and quantification (LOQ) were calculated as 3 and 10 times, respectively, the standard deviation divided by the slope of the graph. Also, using the criterion adopted by the U.S. Environmental Protection Agency (U.S. EPA), which revolves around a parameter called the method detection limit (MDL), led to a similar value for the lowest detectable concentration. All the results are summarized in Table 2.

**Sample Analysis.** Once the calibration model was obtained, different soft drinks containing variable amounts of caffeine were analyzed. Commercial samples were purchased in local markets. The samples were degassed in an ultrasonic bath during 30 min and the pH was adjusted to 8 before analysis, in order to dissolve the remaining carbon dioxide in the form of bicarbonate, and 3 mL of the sample was injected in the SPE-based sensor. The potential interference of sweeteners (sucrose, glucose, fructose) was removed by using a water stream. Finally, the spectrum of the LiChrolut EN surface was acquired, monitoring the adsorbed caffeine. The recorded spectrum was integrated within the wavenumber range 1800−1000 cm$^{-1}$, and the obtained area was interpolated in the calibration model, providing the concentration of caffeine in soft drinks. Samples were analyzed in triplicate, and the resulting data are presented in Table 3. The results are in

good agreement with the National Soft Drink Association (NSDA) recommended values for these analytes in these types of samples.

## CONCLUSIONS

In the present work, a new sensor based on the integration of a solid-phase extraction procedure and infrared detection is reported for the first time. The new sensor is a good and useful alternative to the existing ones, allowing a rapid and simple measurement of a target analyte. The main advantage is the ability of sorbent surface monitoring reaching a marked enhancement in the analytical signal provided by the system. The solid-phase extraction step improves not only the sensitivity of the determinations (which is the main limitation of infrared detection) but also the selectivity since only the target analytes are monitored. The main shortcoming of the proposed configuration is probably the limited adsorption capacity of the sorbent taking into account the small amount employed.

Compared with the alternatives that use a polymeric membrane coating, the new sensor shares the main advantages of the solid-phase extraction procedure, i.e., sensitivity and selectivity enhancement. The new proposal permits the use of a conventional SPE sorbent, which may extend the scope of the technique.

Moreover, the proposal does not need a large equilibration or regeneration time. Although the efficiency of the solid-phase extraction procedure is comparable in both cases; it is a little bit higher in the membrane-based sensor.

Regarding the proposal approaches that are based on liquid−liquid extraction, the new sensor provides an evident improvement in the sensitivity level since a preconcentration takes place during measurement. The liquid−liquid-based sensor is restricted to applications in which the analyte can be found at high concentrations (at the g/L level). Moreover, the volume of required organic solvent is lower in the SPE-based sensor than in the LLE-based one.