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Analysis of Bisphenol A in milk by using a multicommuted fluorimetric sensor

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ABSTRACT

Bisphenol A (BPA) is a polyphenol widely used in industry as an intermediate in the production of polycarbonate plastics and epoxy resins, which are applied to produce plastic food containers, inner surface coating of food and beverage cans. Hence, BPA can migrate from these containers and cans with epoxy coating into foods. It is dangerous taking into account that BPA is considered as a potential endocrine disruptor, which mimics the action of the hormone estrogen. The method here proposed for the determination of BPA involves the implementation of solid-phase spectroscopy (SPS) in an automatic flow system. With this purpose, the measurement of the native fluorescence of BPA, retained on C_{18} silica gel together with the implementation of multicommutation have been employed for its determination in different types of milk. The analytical measurements were made at 271/305 nm ($\lambda_{ex}/\lambda_{em}$) obtaining a detection limit (LOD) of 0.06 ng mL^{-1}. The pre-cleaning procedure and the posterior extraction with C_{18} applied to the samples allowed the removal of proteins and the extraction of BPA from the matrix, respectively. The method showed an RSD lower than 6.0% (n = 10). BPA was determined in powdered milk, infant formula and pure liquid milk samples, being found in five samples at levels lower than the maximum residue limit (MRL) established by the European Union. In addition, a recovery study has been carried out where values close to 100% were observed in all cases, so demonstrating that the proposed analytical method fulfills the requirements for its application in quality control analyses.

1. Introduction

Bisphenol A (2,2-bis(4-hydroxyphenyl)propane), known as BPA, is a chemical substance widely used in industry as a monomer in the production of epoxy resins and polycarbonate plastics (PC), and as an antioxidant in PVC plastics [1,2]. PC are used in food storage containers such as water bottles and baby bottles, while epoxy resins are used as inner surface coating of food and beverage containers and cans, in order to protect the food from the direct contact with metal. Therefore, BPA can inevitably migrate into foodstuffs and beverages from packing of product, and then humans may routinely ingest trace amounts of BPA. In fact, BPA residues have been detected in wine, mineral water and different foodstuffs. Health risks can result from exposure to doses [3] much lower than the limit of 5 μg kg^{-1} body weight day^{-1}. BPA can potentially interfere with the endocrine system of wildlife and human, increase the cancer rate, reduce immune function and impair reproduction [4]. Its estrogenic action has been shown both in vitro and in vivo experiments [5]. Hence, the potential effects of BPA in human health through beverage and food consumption have become a growing concern.

The presence of BPA in complex foodstuff samples, or environmental and biological samples, is normally in trace amounts. Levels found indicate very low concentrations in most of the cases, below MRL established by the European Union Legislation. For this reason, several preconcentration and/or clean-up techniques including electrophoresis [6], solid-phase extraction (SPE) [7], molecularly imprinted solid-phase extraction (MISPE) [8] and matrix solid phase dispersion (MSPD) [9] have been proposed for using prior to its determination. To date, analysis of BPA has mainly been accomplished by gas chromatography (GC) [10–13] and liquid chromatography (LC) [14–17] both coupled to mass spectrometry (MS). These chromatographic methods show high sensitivity but they are time-consuming and expensive. In addition, complicated instruments and skilled operators are required, which makes their popularization difficult. Other classic analytical techniques such as luminescence [18,19] and electrochemical [20,21] detection have also been employed for the analysis of BPA. Taking into account the important environmental and health problem originated by the presence of BPA in different matrices, the complexity of these latter and the low concentrations to be analyzed, it is of primary interest the development of analytical methods with quick response, cheap instrument, low consumption of reagents, simplified operation and time-saving. Therefore, there is need for researching and revising of existing methods in order to have reliable tools for risk assessment and control of human exposure to this compound.

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BPA manifests weak fluorescence property in aqueous solution because of its low fluorescent efficiency. With the purpose of overcoming this problem, the use of cyclodextrin complexes has been proposed [18]. In this paper, we propose a new alternative to that one previously reported, the use of solid-phase spectroscopy (SPS). In this technique, proposed by Yoshimura et al. [22], the combined use of an active solid support to retain the species of interest and the direct measurement of its emission once retained on the solid phase allows the improvement of both sensitivity and selectivity. Separation and preconcentration steps occur simultaneously with detection. Sensitivity is easily increased, when comparing with conventional fluorimetric methods in solution, due to the preconcentration of BPA on an appropriate solid support. In addition, the exclusion from this latter of other co-existing species of the matrix originates an improvement in selectivity. To date, different methodologies have been employed in SPS, but nowadays flow methodologies are the most widely used [23–25]. Several modifications to conventional flow injection analysis (FIA) [26] have been introduced in flow systems to obtain complete miniaturization, automation, high repeatability and low reagent consumption. One of these modifications has been multicommutation [27], which consists of the employment of discrete commutation devices to build up dynamic manifolds that can be easily reconfigured by software, being insertion volumes replaced with insertion times. Among the multicommuted flow techniques, multicommuted flow analysis (MCFIA) has been chosen for the development of the analytical method here proposed. MCFIA systems are typically constituted by a peristaltic pump and a set of three-way solenoid valves, automatically controlled by appropriate software, which can be arranged creating a flow network [28]. Each valve can adopt two positions, "On" and "Off", being the whole system assimilated to an electronic circuit with a variable number of active nodes. This approach increases the versatility of the system, allowing its complete reconfiguration without its physical alteration, by just changing the valve operation in the computer [29].

The aim of this work was to develop a sensitive, selective, automatic and reproducible analytical strategy for determining BPA contents in milk by using an automatic fluorimetric sensor. To the best of our knowledge, only chromatographic methods have been proposed to the determination of BPA in this matrix [9,12,15], being the method here proposed the first one based on the measurement of its native fluorescence. The optimization of the multicommuted system, coupled to SPS methodology, just as all the variables influencing the analytical signal are described. The developed method has been conveniently validated and applied to the determination of BPA in powdered milk, infant formula and pure liquid milk samples.

2. Experimental

2.1. Apparatus and instrumentation

A Varian Cary-Eclipse Luminescence Spectrometer (Varian Inc., Mulgrave, Australia) was used for recording spectra and making fluorescence measurements. It was controlled by a microprocessor fitted with a Cary-Eclipse (Varian) software package for data collection and treatment. The following instrumental parameters were employed: excitation and emission slit widths were set at 20 and 10 nm, respectively and photomultiplier voltage was 720 V. The excitation and emission wavelengths established were 271/305 nm, respectively. UV–visible spectra were recorded with a Varian Cary 50 Spectrophotometer (Madrid, Spain) controlled by means of a PC fitted with the Varian computerized spectroscopy software, WIN-UV. A 100-QS Hellma cell with a light path length of 10 mm was employed in this last instrument.

The multicommuted flow system is shown in Fig. 1. This was built with a four channel Gilson Minipuls-3 (Villiers Le Bell, France) peristaltic pump fitted with a rate selector and pump tubing type Solvflex (Elkay Products, Shrewsbury, MA, USA), three 161T031 NResearch three-way solenoid valves (Neptune Research, MA, USA) and an electronic interface based on ULN 2803 integrate circuits. The valves were operated at an electric potential of 12 V and a direct current of 100 mA. PTFE tubing (0.8 mm i.d.) and methacrylate connections were also used. The software for controlling the system was developed in Visual Basic 6.0 by our research group. A 176.752-QS Hellma flow cell (Müllheim, Baden, Germany) (inner volume, 25 μL; light path length, 1.5 mm) filled with C_{18} silica gel was used in the detection area. The solid support was loaded as methanol slurry just up to a height which enabled the light beam to pass completely through the solid phase and the outlet was locked with glass wool, to avoid the beads movement and allow the continuous flow. All the experiments were carried out at room temperature.

Other apparatus consisted of a vacuum system 12-port Visiprep SPE Vacuum Manifold (Supelco, Bellefonte, PA), a Crison Model 2002 pH-meter with a glass/saturated calomel combination electrode (Crison, Barcelona, Spain) and a Selecta Ultrasons ultrasonic bath (Barcelona, Spain).

2.2. Reagents and solutions

All reagents were of analytical reagent grade and ultrapure water obtained from a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout.

BPA was purchased from Sigma–Aldrich (St. Louis, MO, USA). The stock standard solution (120 mg L^{-1}) was prepared by dissolving the analyte in 40% (v/v) methanol solution. It was kept away from light and stored at 4 °C in a refrigerator, remaining stable for at least 4 weeks. Working standard solutions were prepared daily by taking an aliquot of the stock solution and diluting with 0.08 mol L^{-1} sodium acetate/acetic acid (NaAc/HAc) buffer solution, pH 6.0. The carrier solution, 60% (v/v) methanol solution, was prepared by dissolving the required volume of methanol in ultrapure water. C_{18} bonded phase silica gel beads (55–105 μm average particle size) (Waters, Milford, MA, USA) were used as active solid support in the detection area. Cation and anion exchangers (Sephadex SP C-25, Sephadex CM C-25, Sephadex QAE A-25 and Sephadex DEAE A-25, all of them having 40–120 μm average particle size) (Sigma, Alcobendas, Madrid, Spain) were also tested for the retention of BPA.

Methanol, NaAc, HAc and trichloroacetic acid (TCA) were obtained from Panreac (Barcelona, Spain). Octadecyl (C_{18}) Sep-Pak Vac 6 mL (500 mg) SPE cartridges (Waters, Ireland) and 0.20 μm nylon filters (Millipore Corporation, Bedford, MA) were also used for SPE procedure.

2.3. Samples treatment

Milk samples were purchased from local markets. Protection against light was kept during all the process, keeping the samples in darkness at 4 °C.

2.3.1. Pre-cleaning of the sample

A previous clean-up step was carried out in samples in order to improve the subsequent extraction of BPA. For this, 20 mL of liquid milk (milk/infant formula milk) or 1 g of powder (powdered milk/infant formula milk), this latter suspended in 10 mL of water, were spiked with an appropriate amount of BPA and proteins were removed from the matrix by adding 2.5% (v/v) TCA solution. Finally, after being shaken for 30 s, the samples were centrifuged for 3 min (4200 rpm) and the supernatant was collected.

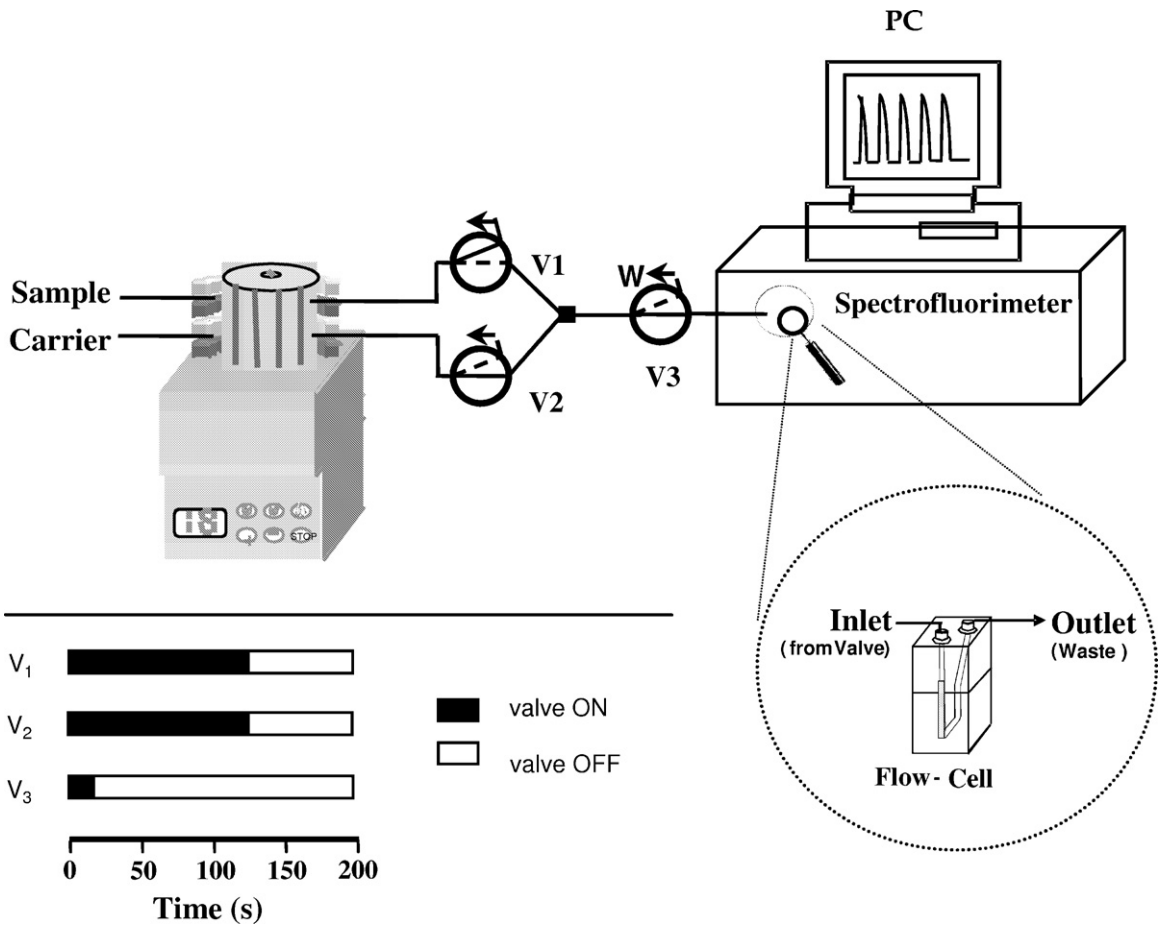

Fig. 1. Multicommuted flow-injection system. Carrier solution (60% (v/v) methanol solution); V_1, V_2 and V_3 are three-way solenoid valves; flow cell filled with C_{18} silica gel; flow rate, 1.7 mL min^{-1}; sampling time, 110 s. For each solenoid valve, the solid and dotted lines refer to "Off" and "On" positions, respectively. The scheme at the lower part shows the valve time program.

2.3.2. Extraction of BPA

C_{18} cartridges were previously conditioned by passing consecutively 5 mL of methanol and 5 mL of water and, subsequently, cleaned milk samples were loaded onto the column at a flow-rate of 3–4 mL min^{-1} under vacuum. Then, the cartridges were washed with 2 mL of 30% (v/v) methanol solution and vacuum dried for 1 min. Finally, BPA retained in the cartridges was eluted with 3 mL of 80% (v/v) methanol solution and the eluate was concentrated to dryness under a gentle stream of nitrogen. The final residue was reconstituted to 10 mL with 0.08 mol L^{-1} NaAc/HAc buffer solution (pH 6.0).

2.4. Procedure

The flow network is shown in Fig. 1. In the initial status, all valves were switched off and the carrier solution, 60% (v/v) methanol solution, flowed through the system while the sample solution was recycled to its vessel. In this way a stable baseline was recorded. Next, valves V_1, V_2 and V_3 were switched on for 125, 125 and 15 s, respectively. Thus the sample solution circulated through the system, whereas the carrier solution was recycled to its recipient. For the first 15 s of this step, the sample plug was directed toward the waste through V_3, so cleaning the tubing between V_1 and V_3 with the new sample solution. Over the next 110 s the sample plug was pumped toward the detection area. When BPA reached the detection area, it was temporarily retained on the solid support (C_{18} silica gel) and monitored (271/305 nm, $\lambda_{ex}/\lambda_{em}$). Then, the solid support was regenerated by the carrier solution itself and the system was prepared for the next insertion of sample. All experiments were carried out in triplicate, and the results are expressed as peak height mean values.

3. Results and discussion

3.1. Optimization of BPA extraction

In order to both to extract BPA from milk and recover high amounts of this latter, a previous clean-up step was necessary to remove proteins. This was carried out by following a procedure previously described by Ji et al. [30]. In this latter the proteins were precipitated with 2.5% (v/v) TCA and the precipitate was rinsed with methanol to avoid the adsorption of BPA onto it.

Next, the optimization of the SPE procedure developed for the extraction of BPA from milk samples is described. Solutions with different percentages of methanol (v/v) were tested for the rinsing and elution steps. With this purpose, the washout fractions were monitored with a UV-spectrophotometer from 250 to 425 nm. Firstly, aqueous solutions containing different methanol percentages (10, 20, 30, 40 and 50% (v/v)) were assayed for the removal of potential interfering more polar compounds from the cartridge. 2 mL of a 30% (v/v) methanol solution proved to be the optimal one for this purpose, providing an optimal elimination of other species of the matrix without the elution of BPA. Secondly, methanol solutions with concentrations ranging from 70 to 100% (v/v) were tested for the elution of BPA. An 80% (v/v) methanol solution (3 mL)

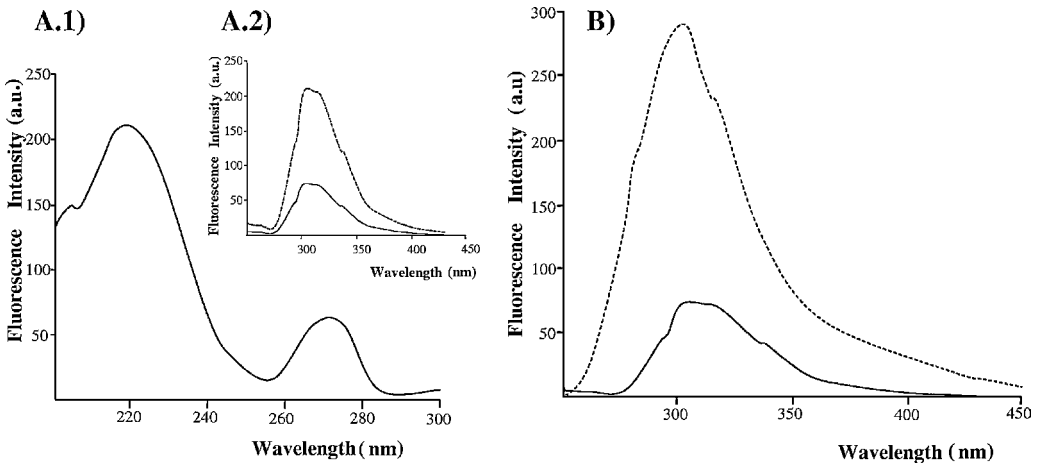

Fig. 2. (A.1) Excitation fluorescence spectra of 28 ng mL^{-1} BPA in homogeneous solution (λ_{em} = 305 nm). (A.2) Emission fluorescence spectra of 28 ng mL^{-1} BPA in homogeneous solution (λ_{ex} = 221 nm (dashed line) and λ_{ex} = 271 nm (continuous line)). (B) Emission fluorescence spectra of BPA in homogeneous solution (continuous line) and retained on C_{18} silica gel (dashed line). 28 ng mL^{-1} of BPA (homogeneous solution); 3.5 ng mL^{-1} of BPA (solid phase); λ_{ex} = 271 nm.

was selected for this purpose, since it provided a good recovery (90–100%) of the BPA amounts assayed.

3.2. Spectral characteristics and selection of the solid support

Spectra of BPA in aqueous solution and in solid phase were obtained in order to select the working wavelengths. The fluorescence spectra in aqueous solution show two maximum excitation wavelengths at 221 and 271 nm, and one maximum emission wavelength at 305 nm (Fig. 2A.1). The fluorescence signal obtained for 221 nm excitation wavelength was 3-fold higher than that obtained for 271 nm (Fig. 2A.2). With the purpose of improving both sensitivity and selectivity of the determination of BPA, several tests of sorption on different solid supports were carried out. The retention assays were performed at different pH values and with anion-exchangers (Sephadex QAE A-25, and Sephadex DEAE-25), cation-exchangers (Sephadex SP C-25 and Chelex 100) and non-ionic supports (C_{18} silica gel). In the case of cationic-exchangers, the analytical signal obtained was very low due to the weak retention of BPA, which was expected taking into account its structure. Due to the high value of its pk_a, 10.3, [31], BPA could ionize under conditions of high pH and to be retained on an anion-exchanger through its hydroxyl groups. Although this fact could be checked, the pH value necessary for the retention of BPA on the anionic resins assayed was very high and close to their limit working pH (around 11.0). Therefore, the use of this type of exchangers was discarded. C_{18} silica gel provided a strong retention of the analyte. Due to the BPA also could be eluted from this solid support, it was selected for retention purposes. The emission spectra of BPA retained on C_{18} silica gel (λ_{ex} = 221 nm or λ_{ex} = 271 nm) showed maxima at the same wavelengths than those in homogeneous solution and, in both cases, an important improvement in the fluorescence signal was obtained. However, for λ_{ex} = 221 nm the background fluorescence signal of the solid support was so high that it was not possible to carry out the analytical measurements. Therefore, 271/305 nm were finally chosen as excitation and emission wavelengths, respectively. As a result of the preconcentration process of BPA on the active sensing area, a 33-fold increase in the signal was obtained when SPS methodology was used (Fig. 2B).

The emission and excitation slits as well as filters ranges were established providing the best sensitivity. The selected excitation and emission slits were 20 and 10 nm, respectively. Filter ranges were set at 250–395 nm for both excitation and emission. These values also supplied the best ratio between analytical signal and background noise. With the same criterion, the voltage of the photomultiplier tube was set at 720 V.

3.3. Chemical variables

The chemical variables studied were the nature of the carrier, eluting and sample solutions.

Taking into account the non-polar nature of the C_{18} silica gel support, methanol solution was used for BPA elution purposes in the previously described tests of sorption. Due to the good results obtained with this solvent, the carrier solution was prepared with methanol in water and its concentration was tested in the range from 10 to 80% (v/v). The results obtained showed that concentrations of methanol lower than 60% (v/v) did not allow a complete and quick elution of BPA from the solid support. On the other hand, a slight decrease in the fluorescence signal was obtained with increasing methanol concentrations. For methanol concentrations ranging from 60 to 80% (v/v) the carrier solution itself quickly eluted BPA from the solid support. Therefore, 60% (v/v) methanol solution was selected as carrier solution, since provided both a high analytical signal and a quick and complete regeneration of the solid phase. Although the use of a carrier solution with methanol concentration lower than 60% (v/v) combined with the introduction in the flow system of an additional eluting solution was also tested, as an alternative to the use of a carrier/eluting solution, it did not provide a significant improvement in sensitivity or sample throughput.

The optimum pH of sample solution was also studied. In order to optimize this variable, standard solutions with a constant BPA concentration and different pH values (from 2.0 to 9.0), adjusted with nitric acid or sodium hydroxide solutions, were injected in the system and the corresponding analytical signals were recorded. No significant variations in the fluorescence signals were observed in the assayed pH range. Because the pH of milk is around 6.0, this value was chosen as working pH for further experiments. Several buffer solutions at pH 6.0 were tested in order to adjust the pH of the sample solution (citric acid/sodium hydroxide, sodium dihydrogen phosphate/sodium hydroxide and NaAc/HAc). The best results were achieved with NaAc/HAc buffer solution. The influence of its concentration on the analytical response was assayed in the range of 0.005–0.1 mol L^{-1}. Finally, a 0.08 mol L^{-1} NaAc/HAc solution was chosen for buffering the sample since it provided the highest analytical signal.

Table 1
Analytical parameters.

Parameter	Value
Linear dynamic range/ng mL^{-1}	0.2–5.0
Calibration graph	
Intercept	34.32 ± 0.03
Slope/mL ng^{-1}	72.73 ± 0.08
Correlation coefficient	0.9985
Detection limit/ng mL^{-1}	0.06
Quantification limit/ng mL^{-1}	0.2
RSD (%)[a]	
Intra-day	3.4[b]
Inter-day	5.7[b]
Sampling frequency/h^{-1}	30

[a] $n = 10$.
[b] [BPA] = 4 ng mL^{-1}.

3.4. Flow variables

The effect of both the flow-rate and the sampling time were the flow variables studied.

The influence of the flow-rate was investigated from 0.5 to 2 mL min^{-1}. A slight decrease in the fluorescence signal was obtained for increasing flow-rates, although the sampling frequency increased. On the other hand, flow-rates higher than 1.7 mL min^{-1} originated overpressure problems in the flow system. Finally, this flow-rate value was chosen as a compromise between sensitivity and throughput.

In multicommutation, sample volumes are replaced by times of insertion (known the flow-rate and the time of insertion, the volume can be easily calculated). When the time of insertion of sample was increased in the proposed system (20–120 s) the analytical signal increased, because of a larger amount of BPA was retained on the solid support. This feature allows both the enhancement of sensitivity by increasing the time of insertion and the reduction of strong matrix effects by means of a previous dilution of the sample before its insertion in the system. Fig. 3 shows the influence of this variable in the recorded signal. The increase of the fluorescence signal with the time of insertion was linear up to 110 s (3.1 mL). Finally, this time of insertion was selected for further experiments since higher values did not provide a significant increment in sensitivity and the sampling frequency decreased.

3.5. Figures of merits

Following the optimized working conditions above described, the calibration curve for BPA was obtained by standard addition calibration method, due to the existence of a considerable matrix effect. Analytical parameters are shown in Table 1. Quantification was carried out by using peak height as analytical signal. Data were fitted by standard least-squares treatment. The proposed methodology responds linearly in the concentration range 0.2–5.0 ng mL^{-1}. The standard deviations of intercept and slope were also calculated (average of three determinations). Detection limit (LOD) and quantification limit (LOQ) were estimated as the concentration that produced a fluorescence signal equal to three and ten times the standard deviation of background fluorescence, respectively [32]. A LOD as low as 0.06 ng mL^{-1} was obtained. Therefore, the method exhibits a LOD lower than those ones reported for HPLC methods with UV [30] or fluorescence detection [33,34].

The reproducibility and robustness of the method were also studied, using a commercial milk sample. The reproducibility was assessed by comparison of the intra- and inter-day assay results undertaken by two analysts. The RSD values (%) for intra- and inter-day assays did not exceed 4.0 and 6.0%, respectively. The robustness of the method was investigated under a variety of conditions such as small changes in the concentration of NaAc/HAc in the sample solution (0.05–0.1 mol L^{-1}) and in the flow rate (1.5–1.9 mL min^{-1}). In all the cases, the BPA recoveries were in the 94–105% range (considering 100% the recovery value obtained under the optimum conditions), so demonstrating the robustness of the proposed method.

Table 2
Determination of BPA in milk samples.

Sample	Type	State	Found ± σ[a] (ng mL^{-1})
1	A_1	Liquid	2.26 ± 0.01
2	A_2	Liquid	<LOD
3	A_3	Liquid	5.47 ± 0.04
4	A_4	Liquid	<LOD
5	A_5	Liquid	<LOD
6	A_6	Liquid	0.38 ± 0.06
7	B_1	Solid (powdered)	<LOD
8	B_2	Solid (powdered)	<LOD
9	B_3	Liquid	6.31 ± 0.03
10	B_4	Liquid	<LOD
11	B_5	Liquid	1.26 ± 0.03
12	C_1	Solid	<LOD
13	C_2	Solid	<LOD
14	C_3	Solid	<LOD

A, pure milk; B, infant formula; C, powdered milk.
[a] Average of three replicates.

3.6. Analytical applications

The developed sensor was applied to the determination of BPA in several milk samples. The pre-treatment and procedure described in Sections 2.3 and 2.4 were used in each instance. In spite of the removal of proteins and other species of the matrix carried out in the sample treatment, a positive matrix effect was noticed which was evaluated by comparing the slopes of aqueous standards and standard addition calibration curves for different milk samples ($m_{\text{standard addition}}/m_{\text{standard}} \approx 1.3$). The slope of the calibration curve obtained by spiking the final milk extracts with BPA was different to that obtained by spiking the original sample and both of them were different than that obtained by external calibration. This fact is due to the presence in the final extracts of interfering species and the incomplete recovery of BPA in the SPE procedure. Consequently, the calibration curves were constructed with matrix-matched standards, that is, the analysis was carried out by spiking different aliquots of a milk sample with increasing amounts of analyte.

Fourteen commercially available milk samples, with different presentations, were analyzed with the proposed method (Table 2). BPA was found in three of the six pure liquid milk samples (0.38, 2.26 and 5.47 ng mL^{-1}) and two of the liquid infant formula samples (6.31 and 1.26 ng mL^{-1}). Nevertheless, BPA was not detected in solid infant formula or powdered milk products. Migration of BPA from cans into solid products is unlikely because those usually are not coated and, even if there is a coating inside, migration will be extremely slow compared to that for the liquid products. BPA concentrations in all of the milk samples studied were in the range 0.38–6.31 ng mL^{-1} (about 0.37–6.13 μg kg^{-1}). These results are in agreement with previous studies that report BPA concentration between 0.28 and 2.92 ng g^{-1} [35] and 1.7 and 15.2 ng g^{-1} [15]. In all cases, the obtained concentrations were lower than the MRL established by the European Union for BPA content in milk (0.6 mg kg^{-1}) [36]. However, the low BPA concentrations found are not reassuring enough if we consider the long shelf-life of infant formula and powder milks as well as their lipid content (especially in infant formula), that increase the migration from packaging toward the sample. For this reason, determination of BPA in milk samples is essential, considering that its estrogenic activity at low levels is under discussion and that milk is the main nourishment of babies.

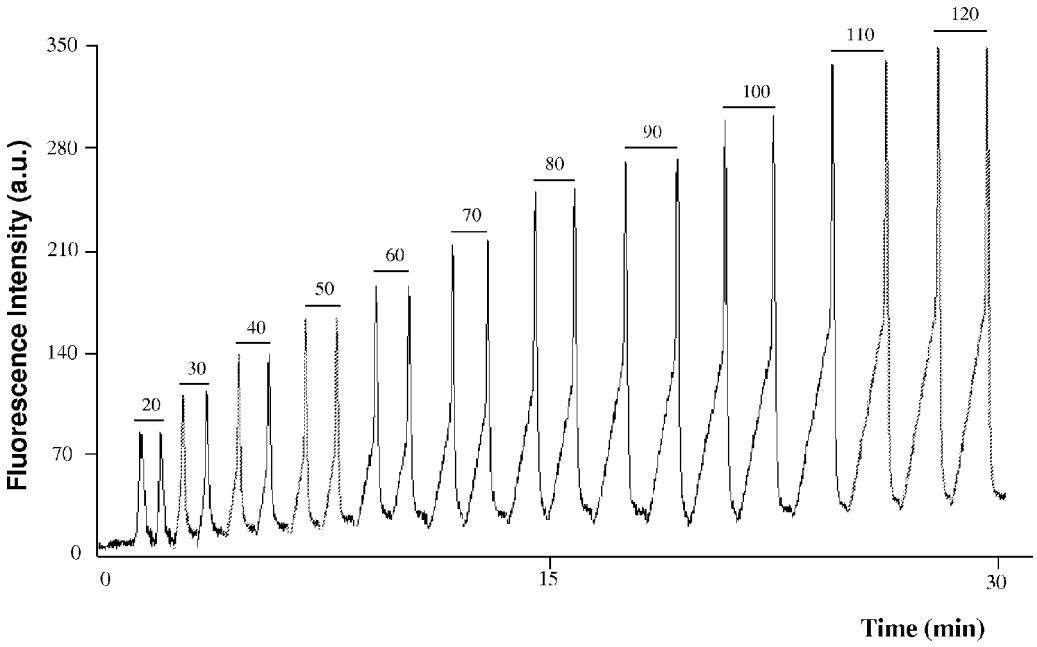

Fig. 3. Influence of the sampling time in the fluorescence intensity. 4 ng mL^{-1} BPA; pH 6.0; flow-rate, 1.7 mL min^{-1}. Sampling time ranging from 20 s to 120 s.

Table 3
Recovery study of BPA in milk samples.

Sample	Proposed method		Reference method	t_{calc}[b]	F_{calc}[c]
	Added (ng mL^{-1})	Found ± σ[a] (ng mL^{-1})	Found ± σ[a] (ng mL^{-1})		
A_6 (pure liquid milk)	–	0.38 ± 0.06	0.40 ± 0.03	0.55	4.00
	0.5	0.91 ± 0.08	0.90 ± 0.05	0.18	2.56
	2.0	2.21 ± 0.03	2.31 ± 0.08	2.03	0.14
	5.0	5.36 ± 0.05	5.26 ± 0.04	2.44	1.56
B_3 (infant formula)	–	6.31 ± 0.03	6.29 ± 0.06	0.51	0.25
	5.0	12.03 ± 0.06	11.75 ± 0.06	0.40	1.00
	10.0	16.36 ± 0.09	16.50 ± 0.04	2.44	5.06
	20.0	26.50 ± 0.07	26.38 ± 0.05	2.44	1.96
B_1 (infant formula)	–	<LOD	<LOD	–	–
	1.0	1.11 ± 0.08	1.10 ± 0.04	0.19	4.00
	2.5	2.48 ± 0.06	2.52 ± 0.02	1.10	2.25
	4.0	4.03 ± 0.07	3.97 ± 0.03	1.38	5.44
C_2 (powdered milk)	–	<LOD	<LOD	–	–
	1.0	1.04 ± 0.05	0.95 ± 0.03	2.67	2.77
	5.0	5.10 ± 0.05	4.99 ± 0.05	2.68	1.00
	10.0	9.98 ± 0.06	10.13 ± 0.08	2.61	0.56

[a] Average of three replicates.
[b] Theoretical value 2.772 (p = 0.05).
[c] Theoretical value 39.00 (p = 0.05).

In order to demonstrate the accuracy of the proposed method, a recovery study at three concentration levels on both two of the samples containing BPA and two of the samples no-containing this latter was also performed. The obtained results are summarized in Table 3. In all cases good recoveries, ranging from 93 to 106% were achieved. The applicability of the proposed method to the analysis of BPA in milk was demonstrated by comparison of the results obtained with those found with a reference method [15] based on liquid chromatography-tandem mass spectrometry. The statistical study of precision and accuracy of both the proposed and the reference method was performed from F criterion and the t test, respectively [37]. The results show that there is no significant statistical difference between the values obtained by both methods, so indicating the utility of the proposed method for BPA routine analytical control.

4. Conclusions

BPA, a known endocrine disruptor, can be found in milk and dairy products due to the contact with plastic materials or epoxy resins during food processing and storage. Therefore, there is a strong need for specific studies on the levels of migration of BPA in these samples and, consequently, for the development of sensitive and quick analytical methods for its determination. The multicommuted flow sensor here developed, based on the measurement of the fluorescence native of BPA, allows the determination of this compound in milk at concentration levels of 0.2 ng mL^{-1} (0.19 μg kg^{-1}) (LOQ), very lower than the MRL established by European Union (0.6 mg kg^{-1}). The application of multicommutation principles drastically reduces the consumption of reagents and human intervention compared to FIA or SIA methodologies. Taking

into account all these features and, in addition, the simplicity of the system used and the high throughput, the method here proposed can be considered as a suitable alternative to chromatographic methods, being appropriate for routine analysis in the control of BPA residues in milk.

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References

[1] European Directive 2002/72/EC, Amendment 2004/19/EC.
[2] Scientific Committee on Food, Opinion of the Scientific Committee on Food on Bisphenol A, SCF/CS/PM/3936, Brussel, 2002.
[3] F.S. Vom Saal, W.V. Welshons, Environ. Res. 100 (2006) 50–76.
[4] S.H. Safe, Environ. Health Perspect. 108 (2000) 487–493.
[5] N. Suzuki, A. Hattori, Life Sci. 73 (2003) 2237–2247.
[6] H. Gallart-Ayala, O. Nuñez, E. Moyano, M.T. Galceran, Electrophoresis 31 (2010) 1550–1559.
[7] T. Wu, W.Y. Wang, L.M. Jiang, Q.C. Chu, J. Delaire, J.N. Ye, Chromatographia 68 (2008) 339–344.
[8] S. Mei, D. Wu, M. Jiang, B. Lu, J.M. Lim, Y.K. Zhou, Y.I. Lee, Microchem. J. 98 (2011) 150–155.
[9] B. Shao, H. Han, X. Tu, L. Huang, J. Chromatogr. B 850 (2007) 412–416.
[10] S. Morales-Muñoz, J.L. Luque García, M.J. Ramos, A. Fernández-Alba, M.D. Luque de Castro, Anal. Chim. Acta 552 (2005) 50–59.
[11] X. Peng, Z. Wang, C. Yang, F. Chen, B. Mai, J. Chromatogr. A 1116 (2006) 51–56.
[12] H.W. Kuo, W.H. Ding, J. Chromatogr. A 1027 (2004) 67–74.
[13] T. Geens, H. Neels, A. Covaci, J. Chromatogr. B 877 (2009) 4042–4046.
[14] J.D. Stuart, C.P. Capulong, K.D. Launer, X. Pan, J. Chromatogr. A 1079 (2005) 136–145.
[15] N.C. Maragou, E.N. Lampi, N.S. Thomaidis, M.A. Koupparis, J. Chromatogr. A 1129 (2006) 165–173.
[16] Y. Watabe, T. Kondo, M. Morita, N. Tanaka, J. Haginaka, K. Hosoya, J. Chromatogr. A 1032 (2004) 45–49.
[17] X. Ye, Z. Kuklenyik, L.L. Needham, A.M. Calafat, J. Chromatogr. B 831 (2006) 110–115.
[18] X. Wang, H. Zeng, L. Zhao, J.M. Lin, Anal. Chim. Acta 556 (2006) 313–318.
[19] C. Lu, J. Li, Y. Yang, J.M. Lin, Talanta 82 (2010) 1576–1580.
[20] F. Wang, J. Yang, K. Wu, Anal. Chim. Acta 638 (2009) 23–28.
[21] J. Huang, X. Zhang, Q. Lin, X. He, X. Xing, H. Huai, W. Lian, H. Zhu, Food Control 22 (2011) 786–791.
[22] K. Yoshimura, H. Waki, S. Ohashi, Talanta 23 (1976) 449–454.
[23] M.J. Ruedas Rama, A. Ruiz Medina, A. Molina Díaz, Talanta 66 (2005) 1333–1339.
[24] L. Molina-García, E.J. Llorent-Martínez, M.L. Fernández-de Córdova, A. Ruiz-Medina, Talanta 79 (2009) 627–632.
[25] E.J. Llorent-Martínez, P. Ortega-Barrales, M.L. Fernández-de Córdova, A. Ruiz-Medina, Anal. Bioanal. Chem. 394 (2009) 845–853.
[26] J. Ruzicka, E.H. Hansen, Anal. Chim. Acta 78 (1975) 145–157.
[27] F.R.P. Rocha, B.F. Reis, E.A.G. Zagatto, J.L.F.C. Lima, R.A.S. Lapa, J.L.M. Santos, Anal. Chim. Acta 468 (2002) 119–131.
[28] E.J. Llorent-Martínez, P. Ortega-Barrales, M.L. Fernández-de Córdova, A. Ruiz-Medina, Curr. Pharm. Anal. 6 (2010) 53–65.
[29] M. Catalá Icardo, J.V. García Mateo, J. Martínez Calatayud, TrAC: Trends Anal. Chem. 21 (2002) 366–378.
[30] Y. Ji, J. Yin, Z. Xu, C. Zhao, H. Huang, H. Zhang, C. Wang, Anal. Bioanal. Chem. 395 (2009) 1125–1133.
[31] M. Clara, B. Strenn, E. Saracevic, N. Kreuzinger, Chemosphere 56 (2004) 843–851.
[32] D. MacDougall, W.B. Crummett, Anal. Chem. 52 (1980) 2242–2249.
[33] X. Liu, Y. Ji, H. Zang, M. Liu, Food Addit. Contam. 25 (2008) 772–778.
[34] Y. Sun, M. Irie, N. Kishikawa, M. Wada, N. Kuroda, K. Nakashima, Biomed. Chromatogr. 18 (2004) 501–507.
[35] N. Casajuana, S. Lacorte, J. Agric. Food Chem. 52 (2004) 3702–3707.
[36] Commission Directive 2004/19/EC, Off. J. Eur. Communities L71 (2004) 8–21.
[37] L. Saunders, R. Fleming, Mathematics and Statistics, 2nd ed., Pharmaceutical Press, London, 1971, p. 192.