

# Determination of Bisphenol A in Food-Simulating Liquids Using LCED with a Chemically Modified Electrode

Alejandra D'Antuono, Viviana Campo Dall'Orto, Alfredo Lo Balbo, Santiago Sobral, and Irene Rezzano\*

Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, Buenos Aires (1113), Argentina

Liquid chromatography with electrochemical detector (LC–ED), using a chemically modified electrode coated with a metalloporphyrin film, is reported for determination of bisphenol A (BPA) migration from polycarbonate baby bottles. The extraction process of the samples was performed according to regulations of the Southern Common Market (MERCOSUR), where certain food-simulating liquids [(A) distilled water, (B) acetic acid 3% V/V in distilled water, and (C) ethanol 15% V/V in distilled water] are defined along with controlled time and temperature conditions. The baseline obtained using the naked electrode showed a considerable drift which increased the detection limit. This effect was suppressed with the chemically modified electrode. A linear range up to 450 ppb along with a detection limit of 20 ppb for the amperometric detection technique was observed. The procedure described herein allowed lowering the detection limit of the method to 0.2 ppb. The value found for BPA in the food-simulating liquid is 1.2 ppb, which is below the tolerance limit for specific migration (4.8 ppm).

**Keywords:** *Bisphenol A; amperometric detector; chemically modified electrode; simulating liquids*

## INTRODUCTION

There are several reports in the literature documenting that bisphenol-A (BPA; 4,4'-isopropylidenediphenol) exhibits estrogenic activity (1, 2, 3). This finding is particularly important because BPA is the monomer used in the manufacture of polycarbonate, which is frequently employed in food containers. It is highly probable that certain residues of this material migrate to food. In fact, it has been reported that BPA has been released from polycarbonate flasks during autoclaving (1), BPA has migrated from epoxy-coated cans to infant formula concentrates (4), and BPA is present in some resin-based composites used in dentistry (3, 5).

Most of the studies report the use of gas chromatography–mass spectrometry (GC–MS) or HPLC equipped with a UV (5) or fluorescence detector (6).

The electrochemical detector (ED) has been widely used when polar mobile phases are required, with aromatic amines and especially phenolic compounds the most important analytes tested (7). The ED method is characterized by a remarkable sensitivity, wide linear range, fast response, and low cost. Such devices are frequently found in food industry and clinical laboratories for routine analysis. Considering the mentioned advantages and the widespread use of amperometric detectors we decided to explore this technique for BPA quantification.

Recently, HPLC–ED was used for the assay of BPA in serum samples (8). The oxidation of phenolic compounds on glassy carbon requires the use of high applied potentials (7), which in the case of BPA leads to an increase in the background current which lowers the sensitivity. Covering the electrode surface with the adequate coating (9) can prevent this unwanted effect.

In previous results, we have reported a chemically modified electrode (CME) prepared by electropolymerization of Ni–Protoporphyrin IX dimethyl ester [Ni–(PPIX)] (10) for efficient quantification of phenols via LC–ED.

The aim of this work was to develop a method for BPA analysis in food-simulating liquids under the regulations of the Southern Common Market (MERCOSUR) (11). In our chromatographic system the BPA is detected at +1.3 V, where a drift of the baseline is obtained. To solve this problem we explored the use of the chemically modified electrode already described (10).

## EXPERIMENTAL PROCEDURES

**Reagents.** Bisphenol A (analytical reagent) was purchased from Aldrich (Milwaukee, WI). Tetrabutylammonium perchlorate (TBAP) was obtained by precipitation of a saturated solution of the TBA hydroxide with perchloric acid, recrystallized twice from ethanol, and dried in a vacuum. **CAUTION:** This product is very explosive, it may easily detonate during preparation. It can be obtained commercially (Fluka).

All aqueous solutions were prepared with doubly distilled water. The porphyrin solutions were prepared with Cl<sub>2</sub>CH<sub>2</sub>, HPLC-grade. Methanol was chromatographic-grade, and the rest of the reagents (ethanol, H<sub>2</sub>SO<sub>4</sub>, and acetic acid) were analytical grade.

**Samples.** Four different brands of baby bottles (250 cm<sup>3</sup>) made of polycarbonate, manufactured in Argentina, were purchased from several markets in Buenos Aires. These samples were washed twice with distilled water before use; no further treatment was performed.

**Apparatus.** Cyclic voltammetries were performed with a homemade electrochemical analyzer, microprocessor controlled, with digital signal generator, for implementation of different electrochemical techniques. A glassy carbon working electrode, 0.2 cm<sup>2</sup> area, and a platinum-wire auxiliary electrode were used for voltammetric experiments. Liquid chromatography experiments were performed with a Perkin-Elmer Series

\* Corresponding author. E-mail: irezzano@ffyba.uba.ar.

**Table 1**

simulating liquid	temp. (°C)	contact time (min)	specific migration (ng BPA/cm <sup>2</sup> )	$\sigma$ (n-1)
A: distilled water	100	0.5	1.2	$\pm 0.1$
	80	2	1.1	$\pm 0.1$
B: 3% V/V acetic acid	80	2	2.2	$\pm 0.2$
C: 15% V/V ethanol	80	2	2.5	$\pm 0.3$

10 pump, a Rheodyne (Berkeley, CA) model 7125 injector with a 20- $\mu$ L sample loop, and the same homemade voltammetric analyzer as above but operated in the constant potential mode. A thin-layer cell (7  $\mu$ L volume) equipped with a dual glassy carbon (BAS, 7 mm<sup>2</sup> area) and stainless steel block as auxiliary electrode was used. All potentials were measured against Ag/AgCl reference electrodes (BAS).

The chromatographic separations utilized a C18 bonded column (Hewlett-Packard) 5  $\mu$ m, 200  $\times$  4.6 mm. The mobile phase was CH<sub>3</sub>OH/H<sub>2</sub>O, 60:40, containing 10 mM KNO<sub>3</sub> and 0.25 mM H<sub>2</sub>SO<sub>4</sub> as supporting electrolyte.

**Preparation of Metalloporphyrin Complexes.** (Protoporphyrin IX) dimethyl ester was prepared from hemin-a. Metalation of protoporphyrin IX dimethyl ester with Ni<sup>II</sup> was carried out by the method of Adler.

**Coating the Glassy Carbon Electrode with Poly[Ni-(protoporphyrin IX) dimethyl ester].** Prior to its coating, the electrode was polished with 0.3 and 0.05  $\mu$ m alumina particles and finally rinsed with deionized water. The metalloporphyrin films were formed by electrochemical cycling through the metalloporphyrin oxidation waves. Film deactivation by oxidation in mobile phase was carried out by electrochemical cycling between 0 and +1.5 V. The HPLC experiments were performed on different days; the variation of polymer thickness was 3%.

**Extraction Process of BPA.** The extraction process was performed following the MERCOSUR Regulations (11). In this protocol a series of food-simulating liquids are defined: (A) distilled water, (B) acetic acid 3% V/V in distilled water, and (C) ethanol 15% V/V in distilled water. Distilled water is indicated for plastic containers to be used with milk. We also surveyed the other two simulating liquids. The regulation also indicates that the number of samples tested has to complete a final contact surface of 600 cm<sup>2</sup>, for this reason three baby bottles have been used in each experiment.

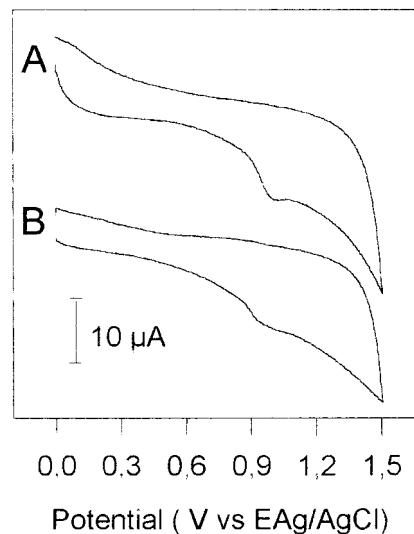
The extraction process consists of a migration assay by using three simulating liquids (Table 1), and a concentration step (reduction of volume between 2 and 20 mL).

## RESULTS AND DISCUSSION

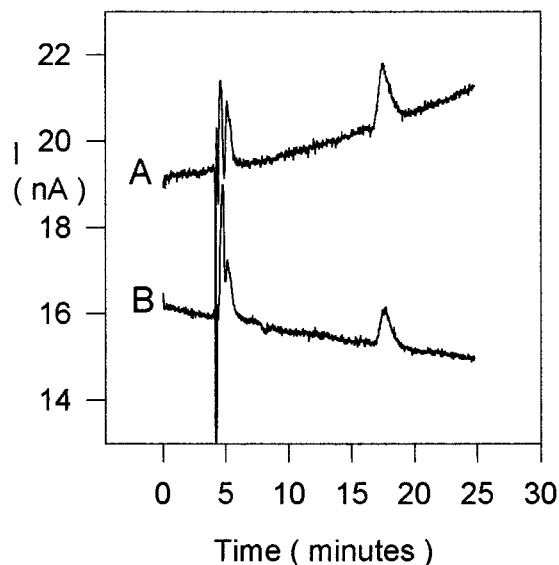
**Analytical Performance of the Chemically Modified Electrode (CME).** To evaluate the behavior of the amperometric detector for BPA analysis, the cyclic voltammograms of standard solutions of bisphenol-A at the CME and bare electrode were obtained. Figure 1 shows these results, indicating a slight increase of signal at the CME (Figure 1A) in comparison with that of the bare electrode (Figure 1B). This experiment was repeated with varying thicknesses of the film, showing similar response in the range of 50 to 200 monolayers.

Figure 2 compares the response of the two working electrodes in a flow system. The baseline obtained using a bare electrode showed a considerable drift (Figure 2A) with the consequent increase of BPA detection limit. This unwanted effect was suppressed in the measurements performed with the CME (Figure 2B). In this case, a detection limit for the amperometric technique of 20 ppb was achieved.

**Application to Food-Simulating Liquids.** We explored the migration of BPA toward the three types of simulating liquids.



**Figure 1.** Cyclic voltammograms of 0.04 mM BPA solution in mobile phase (CH<sub>3</sub>OH/H<sub>2</sub>O 60:40, 10 mM KNO<sub>3</sub>, and 0.25 mM H<sub>2</sub>SO<sub>4</sub> as supporting electrolyte), obtained with (A) poly[Ni-(protoporphyrin IX)] and (B) bare electrode. Geometric area of working electrode: 0.2 cm<sup>2</sup>. Reference electrode: AgCl/Ag.

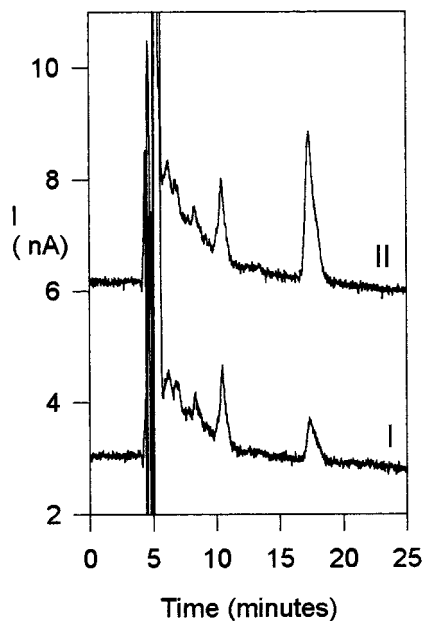


**Figure 2.** HPLC-ED chromatograms of 90 ng mL<sup>-1</sup> BPA solution obtained with (A) bare electrode and (B) poly[Ni-(protoporphyrin IX)] CME.

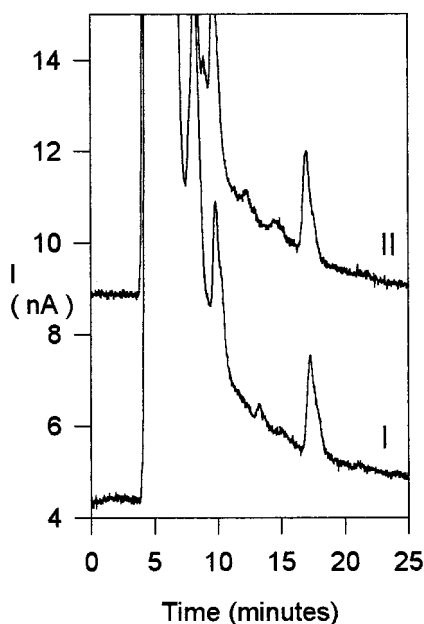
To validate the extraction process, a series of control experiments was performed. The recovery experiments consisted of adding 40 ppb of BPA to simulating liquid A before and after the migration step, in two independent experiments. The percent of recovery in both cases was above 95%, indicating no relevant matrix effect and small amount of BPA degradation.

The linear range was up to 450 ppb and the correlation coefficient was 0.9991. They were obtained by injecting standard solutions of BPA at different concentration levels. The detection limit of the technique at +1.3 V was 20 ppb. The chemically modified electrode along with the concentration step allow lowering the detection limit of the method to 0.2 ppb.

The chromatograms obtained with the CME amperometric detector are presented in Figures 3 and 4. The retention time for BPA is 16.7 min, confirmed by the increase of signal after the standard addition (Figure



**Figure 3.** (I) Simulating liquid B, 80 °C during 2 h. (II) Simulating liquid B and BPA to final concentration 120 ng mL<sup>-1</sup>, 80 °C during 2h.



**Figure 4.** (I) Simulating liquid A, 100 °C during 30 min. (II) Simulating liquid A, 80 °C during 2 h.

3). The quantification has been performed by standard addition to the sample.

The Figure 4 shows chromatograms obtained from releasing assays performed with simulating liquid A in different extractive conditions, as described in the MERCOSUR Regulations. The object of this experiment was to evaluate the variation of the released impurities and their effect on the chromatographic separation. In every analysis, blank tests using BPA-free simulating liquids were conducted.

According to the Argentine Food Regulations (Annex MERCOSUR; 11) the limit value for specific migration of BPA is 4.8  $\mu\text{g cm}^{-2}$  or 4.8 ppm relative to simulating liquid volume. Table 1 shows the results of specific migration for the three simulating liquids employed. The lowest value was obtained for distilled water, which

is recommended as the simulating liquid for milk; the highest value, 2.5 ng cm<sup>-2</sup> or 2.5 ppb in the initial simulating liquid volume, was obtained for simulating-liquid C. These values are both below the tolerance limit for specific migration in MERCOSUR Regulations (11).

It must be pointed out that there were no significant differences in the amount of BPA released from the different brands of baby bottles (results not shown).

The chromatograms obtained with the CME amperometric detector are presented in Figures 3 and 4. The retention time for BPA is 16.7 min, confirmed by the increase of signal after the standard addition (Figure 3). The quantification has been performed by standard addition to the sample.

## CONCLUSIONS

The use of HPLC with amperometric detection is an alternative procedure for BPA quantification. The successful measurement was achieved with metalloporphyrin films CME, where the drift of the baseline was avoided.

The linear range was up to 450 ppb; the detection limit of the technique at +1.3 V was 20 ppb within the specified conditions. The chemically modified electrode along with the concentration step allows lowering the detection limit of the method to 0.2 ppb. Consequently, the application of this method to migration assays was successfully performed.

## LITERATURE CITED

- (1) Krishnan, A.; Stathis, P.; Permuth, S.; Tokes, L.; Feldman, D. An estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology* **1993**, *132* (6), 2279–2286.
- (2) Brotons, J.; Olea-Serrano, M.; Villalobos, M.; Pedraza V.; Olea, N. Xenoestrogens released from lacquer coatings in food cans. *Environ. Health Perspect.* **1995**, *103* (6), 608–612.
- (3) Olea, N.; Pulgar, R.; Perez, P.; Olea-Serrano, F.; Rivas, A.; Novillo-Fertrell, A.; Pedraza, V.; Soto, A. M.; Sonnenschein, C. Estrogenicity of resin-based composites and sealants used in dentistry. *Environ. Health Perspect.* **1996**, *104* (2) 298–299.
- (4) Biles, J.; MacNeal, T.; Begley, T. Determination of Bisphenol A migrating from epoxy can coatings to infant formula liquid concentrates. *J. Agric. Food Chem.* **1997**, *45* (12), 4697–4700.
- (5) Pulgar, R.; Olea-Serrano, M. F.; Novillo-Fertrell, A.; Rivas A.; Pazos, P.; Pedraza, V.; Narvajás, J. M.; Olea, N. Determination of Bisphenol A and Related Aromatic Compounds Released from Bis-GMA-Based Composites and Sealants by High Performance Liquid Chromatography. *Environ. Health Perspect.* **2000**, *108* (1) 21–27.
- (6) Biles, J.; MacNeal, T.; Begley, T.; Hollifield, H. Determination of Bisphenol A in reusable polycarbonate food-contact plastics and migration to food-simulating liquids. *J. Agric. Food Chem.* **1997**, *45* (9), 3541–3544.
- (7) Puig, D.; Barceló, D. Comparative study of on-line solid-phase extraction followed by UV and electrochemical detection in liquid chromatography for the determination of priority phenols in river water samples. *Anal. Chim. Acta* **1995**, *311*, 63–69.
- (8) Saijiki, J.; Takahashi, K.; Yonekubo, J. Sensitive method for the determination of bisphenol A in serum using two systems of high-performance liquid chromatography. *J. Chromatogr. B*, **1999**, *736*, 255–261.
- (9) Murray, R.; Ewing, A.; Durst, R. Chemically Modified Electrodes. Molecular Design for Electroanalysis. *Anal. Chem.* **1987**, *59*, 379A–390A.

- (10) Campo Dall'Orto, V.; Danilowicz, C.; Sobral, S.; Lo Balbo, A.; Rezzano, I. Liquid Chromatography/electrochemical detection of phenols at a poly [Ni-(Protoporphyrin IX)] chemically modified electrode. *Anal. Chim. Acta* **1996**, *336*, 195–199.
- (11) MERCOSUR Regulations. Argentine Food Regulations. The Southern Common Market. De la Canal y Asoc. SRL. MERCOSUR-GMC-Res No. 030/92.

Received for review May 31, 2000. Revised manuscript received December 5, 2000. Accepted December 6, 2000. This work was supported by Grant TB67, UBACyT 98-2000 from Universidad de Buenos Aires and PIA no. 6562/96 from CONICET of Argentina.

JF000660N