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# Development and characterization of a new polyampholyte–surfactant complex applied to the solid phase extraction of bisphenol-A

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ABSTRACT

This paper presents a material that has both hydrophilic and hydrophobic domains, obtained by combination of a polyampholyte with a surfactant. This material was fully characterized by different spectroscopic techniques and microscopy.

Bisphenol-A (BPA) was chosen as a model molecule to study the interaction with compounds of intermediate polarity. We explored the kinetics and equilibrium of BPA on the surface of the polyampholyte–surfactant complex and found a significantly high loading capacity (2.02 mmol $g^{-1}$) and complete binding from solutions at concentration levels below 100 μmol $L^{-1}$.

The complex was encapsulated in agarose gel to be used as solid phase for extraction of BPA from food simulants in contact with polycarbonate bottles under different treatments. Bisphenol was preconcentrated, extracted and analysed by liquid chromatography with an amperometric detector. The instrumental detection limit of the technique was 10 μg $L^{-1}$, which was lowered to 0.14 μg $L^{-1}$ by the preconcentration step. The BPA released from baby bottles was 2.1 ng $cm^{-2}$ ($\sigma_{n-1}$: 0.1) in the first use with distilled water.



## 1. Introduction

Bisphenol-A (BPA), 2,2-bis(4-hydroxyphenyl) propane, is a chemical substance used mainly in the production of polycarbonate and epoxy resins [1]. A great variety of materials (storage containers, milk bottles, infant feeding bottles, microwave ovenware) derived from polycarbonate cause environmental contamination when they are in contact with water or food due to the leakage of non-polymerized monomers or residues from hydrolysis.

It has been reported that BPA has a weak affinity for estrogen receptors [2], has potential for disrupting thyroid hormone action [3], affects the proliferation of human prostate cancer cells [4] and blocks testosterone synthesis [5] at ng $L^{-1}$ doses.

Due to these toxicological properties, many analytical methodologies, including sample preparation, separation and detection of BPA, have been developed [1].

In 2004, the EU Commission established a specific migration limit (SML) of BPA from food plastic materials of 600 ng $g^{-1}$ [6]. Migration testing is usually carried out with food simulants instead of food, because its analysis usually presents some drawbacks. Simulants have been selected to model different categories of food (aqueous, acidic, alcoholic and fatty) to simplify the testing and evaluate whether a plastic is suitable for a given application [7].

Separation, identification and quantitation of BPA are reliably carried out with mass spectrometric methods, namely liquid chromatography–tandem mass spectrometry [8] and gas chromatography–tandem mass spectrometry [9]. Other methods such as liquid chromatography coupled to fluorescence or electrochemical detection and immunochemical methods have also been reported [1]. When food simulants are used, the sample preparation consists of a preconcentration step by solvent evaporation, liquid–liquid extraction, solid phase extraction (SPE), solid phase microextraction or stir bar sorptive extraction [1].

SPE is by far the most widely used technique for the extraction of BPA-containing liquids [9–12]. The technique uses a copolymer formed with the hydrophilic *N*-vinylpyrrolidone, which acts as a hydrogen acceptor, and the hydrophobic divinylbenzene, which provides reversed-phase retention (Oasis HLB).

A number of different approaches have been used to prepare new sorbents in order to improve particular features such as selectivity or capacity. Synthetic strategies such as the molecularly imprinting technique or the construction of immunoaffinity monoliths have been applied to generate selective binding sites in bulk or surfaces of polymeric matrixes [13–15].

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This paper presents a material that combines a hydrophilic network with hydrophobic regions, and exhibits a notably high loading capacity for BPA. The network is a polyampholyte obtained by reaction of methacrylic acid, ethylene glycol, diglycidyl ether and 2-methylimidazole (poly(EGDE–MAA–2MI)) [16–18], following a new synthetic procedure. The acid form of this polymeric material binds dodecyl sulfate on the surface. The interactions between surfactants and polyelectrolytes have been well reviewed [19–21] and the dominating factor that influences binding is the electrostatic charged neutralization of the polyion by oppositely charged surfactants. The electrostatic binding is an endothermic process driven by entropy. The positive entropy is attributed to the recovery of translational entropy of released counterions by the bound surfactant.

The new material was fully characterized by spectroscopy ($^{13}$C Nuclear Magnetic Resonance in the solid-state, Fourier Transform-Infrared), thermogravimetry, elemental analysis, nitrogen adsorption and scanning electron microscopy.

The kinetics and sorption equilibrium of BPA on the surface were also studied. The material was also encapsulated in agarose beads and tested as sorbent for the BPA released from baby bottles into food simulants, under different treatments. The analysis was completed by liquid chromatography with electrochemical detection (HPLC-ED), and the performance of the method is discussed.

## 2. Materials and methods

### 2.1. Reagents

2-Methylimidazole (2MI; 99 wt%; p$K_a$: 7.86), bisphenol-A (BPA 99 wt%, MW: 228.29), methacrylic acid (MAA; 99 wt%; p$K_a$: 4.66), ethylene glycol diglycidyl ether (EGDE; 50 wt% in ethylene glycol dimethyl ether), phenol (99 wt%), methylparaben (99 wt%), propylparaben (99 wt%) and diethyl phthalate (99 wt%) were purchased from Sigma–Aldrich, and benzoyl peroxide from Fluka. Sodium dodecylsulfate (SDS; MW: 288.38; solubility at 20 °C: 10 g in 100 g of water; critical micelle concentration (CMC): 8.2 mmol L$^{-1}$) and dodecylbenzene sulfonic acid sodium salt (SBDS; MW 348.5; solubility at 20 °C: 32.8 g in 100 g of water; CMC: 1.2 mmol L$^{-1}$) were purchased from ICN Biomedical.

Baby bottles (250 cm$^3$) from one brand made of polycarbonate were washed twice with distilled water before use; no further treatment was performed.

Acetonitrile, isopropyl alcohol and methanol from Baxter were HPLC grade. BPA-free distilled water was used for all aqueous solutions. Spectroscopic grade potassium bromide was used for infrared pellets. Hydrochloric acid, sodium chloride, sodium hydroxide, disodium hydrogen phosphate and potassium hydrogen phosphate were of analytical grade.

### 2.2. Synthesis of the polyampholyte modified with the surfactant

The polyampholyte was synthesized according to previous reports [16–18], from methacrylic acid, ethylene glycol diglycidyl ether and 2-methylimidazole. Then it was washed with water, dried at 60 °C for 24 h and milled in particles with an average diameter of 200 μm. The particles were treated with 0.1 mol L$^{-1}$ HCl to obtain the acid form of the polyampholyte (poly(EGDE–MAA–2MIH$^+$)), and 50 mg were put in contact with three aliquots of 4 mL of 20 mmol L$^{-1}$ sodium dodecyl sulfate (SDS) solution. The adsorption of dodecyl sulfate (DS) on the surface of the poly(EGDE–MAA–2MIH$^+$) was expected to take place under these conditions (Scheme 1). The complex poly(EGDE–MAA–2MIH$^+$)–DS was rinsed with distilled water, filtered, and let to dry at room temperature. Sodium dodecylbenzene sulfonate (SDBS) was also used for the formation of the complex since it absorbs radiation at 273 nm and can be monitored spectrophotometrically.

### 2.3. Characterization of the poly(EGDE–MAA–2MIH$^+$)–DS complex

The Fourier Transform-Infrared (FTIR) spectra of poly(EGDE–MAA–2MIH$^+$)–DS and polyampholyte were recorded on a Spectrum 1000 PerkinElmer spectrometer using KBr pellets. The material was dried and placed in a desiccator at 20 °C prior to pellet preparation.

High-resolution $^{13}$C Nuclear Magnetic Resonance (NMR) solid-state spectra were recorded using the ramp $\{^1H\} \rightarrow \{^{13}C\}$ CP-MAS (cross-polarization and magic angle spinning) sequence with proton decoupling during acquisition. All the solid-state NMR experiments were performed at room temperature in a Bruker Avance II-300 spectrometer equipped with a 4-mm MAS probe. The operating frequency for carbons was 75.46 MHz and the spinning rate was 10 kHz. Samples of two batches of each material were studied.

Thermogravimetric measurements were carried out with a TA Instrument SDT Q600, under nitrogen flux over a temperature range from 30 to 400 °C with a heating rate of 10 °C min$^{-1}$. The average sample size was 10 mg and the measurements were carried out in triplicate.

The surface of two batches of each polymer was characterized using a scanning electron microscope Field Emission SEM (Zeiss Gemini DSM 982) operated at a 0.3 kV acceleration voltage.

Elemental analysis was performed with a CE440 Elemental Analyser device (Exeter Analytical).

The nitrogen adsorption isotherm was collected at 77 K on a Micromeritics Gemini 2360 system. Specific surface area was calculated using the Brunauer–Emmett–Teller equation (BET).

### 2.4. Surfactant adsorption isotherms

For binding studies, SDS was replaced with SDBS, whose concentration was determined by UV measurements.

Kinetic experiments were performed with a 4 mmol L$^{-1}$ SDBS standard solution under forced convection.

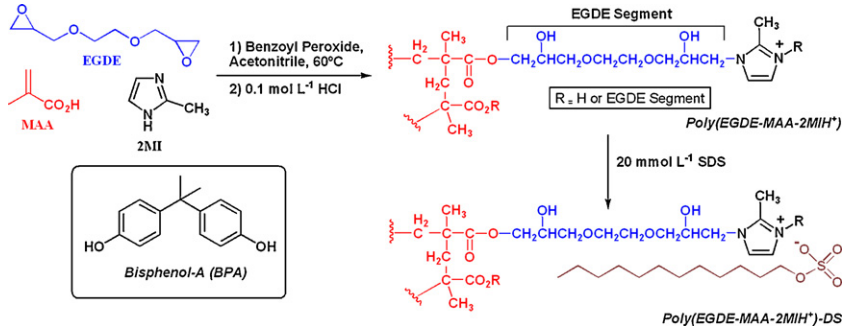
The adsorption isotherm was performed with aliquots of 80 mg of acid polyampholyte dispersed in 10.0 mL of SDBS standard solutions in a concentration range between 0.20 and 8.0 mmol L$^{-1}$. The suspensions were mixed and thermostated at 20.0 ± 0.5 °C for 16 h to reach equilibrium. The SDBS concentration in the supernatant and standard solutions was analysed spectrophotometrically at 273 nm.

### 2.5. Bisphenol-A (BPA) uptake

BPA in solution was analysed by liquid chromatography with electrochemical detection (HPLC-ED). The chromatographic system consisted of an HPLC SpectraSYSTEM Isocratic Pump P100, a Rheodyne injection valve (Model 7125) with a 20-μL sample loop, a guard column, and a C-18 bonded column (Hewlett-Packard) 5 μm, 200 mm × 4.6 mm. The mobile phase was methanol:aqueous solution containing 10 mmol L$^{-1}$ $KNO_3$ and 0.25 mmol L$^{-1}$ $H_2SO_4$ as supporting electrolyte in a proportion of 55:45, at a flow rate of 0.8 mL min$^{-1}$.

Amperometric detection was performed with a microprocessor-controlled electrochemical analyser. A thin-layer cell (7 μL volume), equipped with a glassy carbon working electrode (BAS, 7 mm$^2$ area) and a stainless steel block as auxiliary electrode, operated at +1 V vs. Ag/AgCl.

Stainless steel, PTFE and PEEK were used for the flow system connections.




**Scheme 1.** Chemical structure of bisphenol-A (BPA) and synthesis of the poly(EGDE–MAA–2MIH$^+$) and poly(EGDE–MAA–2MIH$^+$)–DS complex.

The linear range for BPA was verified up to 800 μg L$^{-1}$. All the samples were filtered and properly diluted for HPLC analysis.

Kinetic experiments of BPA sorption on the complex were carried out with 50 mg of poly(EGDE–MAA–2MIH$^+$)–DS in contact with 125 mL of 500 μmol L$^{-1}$ BPA (114 mg L$^{-1}$) dissolved in 10:90 methanol:water, under forced convection.

The sorption isotherm experiments were performed with aliquots of 40 mg of poly(EGDE–MAA–2MIH$^+$)–DS dispersed in 200 mL of BPA standard solutions in a concentration range between 75 and 800 μmol L$^{-1}$ (17 and 183 mg L$^{-1}$), in the same medium. The suspensions were mixed and thermostated at 20.0 ± 0.5 °C for 1 h.

For the adsorption isotherm of BPA on the polyampholyte without surfactant, 200 mg of polymer and 25 mL of BPA aqueous solution in the range between 3.7 and 216 μmol L$^{-1}$ were used.

### 2.6. Preconcentration and analysis of BPA released from baby bottles

The linear concentration range of the HPLC-ED analytical technique was examined injecting BPA solutions prepared in 60:40 isopropanol:methanol, with five concentration levels (0.050, 0.100, 0.200, 0.300 and 0.500 mg L$^{-1}$), in triplicate.

The detection and the quantification limits (LoD and LoQ) were calculated as 3 and 10 times the standard deviation of five replicate runs of the procedure blank, respectively.

The extraction process of BPA from baby bottles consisted of a migration assay by using food-simulating liquids, and a preconcentration step prior to detection. A new extraction sorbent was prepared by including poly(EGDE–MAA–2MIH$^+$)–DS in agarose gel. Sorbent beads were obtained by mixing 200 μL of 1% agarose solution at 60 °C with 50 mg of poly(EGDE–MAA–2MIH$^+$)–DS in 4 mm × 4 mm cavities, and cooling at room temperature.

In a first assay (treatment A1), the extraction process was performed following the MERCOSUR Regulations [22], which indicate that distilled water should be used as food-simulating liquid for plastic containers to be used with milk. The regulation also indicates that the samples tested have to complete a final contact surface of 600 cm$^2$. For this reason, three new baby bottles filled up to 250 mL were used in each experiment. They were filled with boiling distilled water and kept in contact at 100 °C at atmospheric pressure for 30 min. The extract was cooled at room temperature, completed to volume with distilled water (for loss due to evaporation), and then a 300-mL aliquot was mixed with 2.0 mL of 30:70 methanol:water. Four sorbent beads were put in contact with this mixture in a stainless steel basket under convection for 1 h. The beads were then dried with tissue paper and put in contact with 4.0 mL of 60:40 isopropanol:methanol in a tube with cap under agitation at 30 °C in a thermostated bath to extract BPA for HPLC analysis. For recovery experiments the water extract was spiked with BPA standard by replacing 2.0 mL of 30:70 methanol:water by 2.0 mL of 0.400 mg L$^{-1}$ BPA standard in the same solvent and thus making a 2.65 μg L$^{-1}$ BPA spike (final concentration of 0.200 mg L$^{-1}$ in the alcoholic extract). The analysis of the sample with and without the BPA spike was carried out in triplicate.

A blank was carried out with 300 mL of distilled water instead of the extract.

The beads were washed three times with 4.0 mL of 60:40 isopropanol:methanol for reutilization.

A comparison of the retention behaviour of BPA was made with Oasis HLB cartridges of 200 mg (60 μm particle size, purchased from Waters) after pretreatment with methanol and pure water. A 300-mL aliquot of the extract and another sample of distilled water spiked with 1.67 μg L$^{-1}$ of BPA were, respectively loaded on the solid phase column at 3 mL min$^{-1}$. Each sample was eluted with 4.0 mL of 60:40 isopropanol:methanol. The analyses of both the extract and the water spiked with BPA were carried out in triplicate.

Then three used baby bottles were rinsed with distilled water and the extraction procedure was repeated (treatment A2). They were then subjected to 10 cycles of simulated use by boiling for 10 min in tap water and washing with a brush, after which the migration assay was repeated (treatment A3).

In order to validate the method, other treatments and liquids were used on new baby bottles. Tap water 1 from the laboratory with pH 6.9 and 59 mg L$^{-1}$ of total alkalinity was kept in contact with the bottles for 10 min at 100 °C in treatment B. Tap water 2 with pH 7.6 and 499 mg L$^{-1}$ of total alkalinity was kept in contact with the bottles for 10 min at 100 °C in treatment C, and the sample was diluted 5-fold with recently distilled water (pH 7.0) for preconcentration. A blank was carried out with 300 mL of each tap water instead of the extract, and BPA was not detected.

In treatment D, distilled water was kept in contact with new bottles for 30 min at 100 °C and was then spiked with 1.2 μg L$^{-1}$ (8.0 nmol L$^{-1}$) methylparaben, 1.2 μg L$^{-1}$ (6.9 nmol L$^{-1}$) propylparaben, 5.3 μg L$^{-1}$ (57 nmol L$^{-1}$) phenol and 0.2 μg L$^{-1}$ (1 nmol L$^{-1}$) diethyl phthalate.

In treatments B, C and D, the analysis of each sample was carried out in triplicate. The recovery figures for a 2.65 μg L$^{-1}$ BPA spike were obtained in triplicate in treatments C and D.

## 3. Results and discussion

### 3.1. Characterization of the poly(EGDE–MAA–2MIH$^+$)–DS complex

Fig. 1 exhibits the FTIR spectra for the polyampholyte, SDS and the polyampholyte–DS complex. The results show a clear evidence of dodecyl sulfate (DS) adsorption on the surface of the poly(EGDE–MAA–2MIH$^+$). The polymer bands previously reported [16] were: 1730 cm$^{-1}$ (carbonyl symmetric stretching); 1570 cm$^{-1}$ (carboxylate asymmetric stretching); 1640 (C=C and/or C=N stretching vibrations), 750 and 670 cm$^{-1}$ (2-methylimidazole ring stretching); 1450 cm$^{-1}$ (C=C and/or C=N stretching vibrations

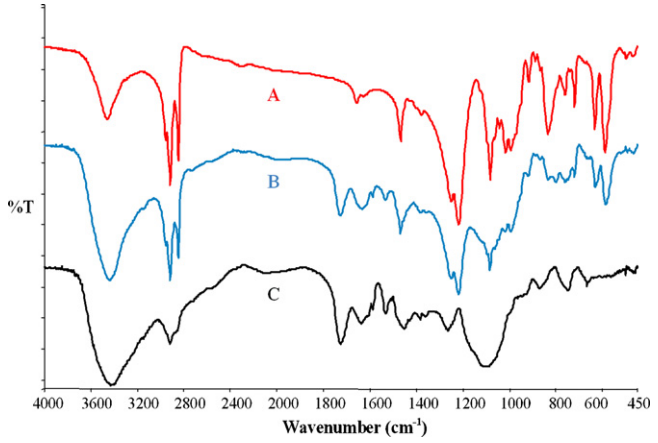



**Fig. 1.** FTIR spectra for SDS (A), the poly(EGDE–MAA–2MIH$^+$)–DS complex (B) and poly(EGDE–MAA–2MIH$^+$) (C).

and ether groups); 1100 cm$^{-1}$ (ether groups); 2930 cm$^{-1}$ (C–H) and 3435 cm$^{-1}$ (O–H). The main signals for SDS were: 1469 and 1220 cm$^{-1}$ ($SO_2$ stretching); 1253 and 1185 cm$^{-1}$ (asymmetric and symmetric stretching of $ROSO_3Na$); 837 and 760 cm$^{-1}$ (asymmetric and symmetric stretching of S–O–C); 635 and 591 cm$^{-1}$ ($SO_3$ flexion).

In the FTIR spectrum of the polyampholyte–DS complex strong bands related to DS appeared at 2950, 1230, 635 and 591 cm$^{-1}$, and the bands associated with the polyampholyte network were weaker.

The $^{13}$C CP-MAS signals for SDS were assigned tentatively according to the results reported by Ferruglio and co-workers in solution [23], and those for the polyampholyte were assigned as previously reported [18] (Fig. 2). The solid-state results indicate that important changes in the chemical environment occurred upon sorption with the DS surfactant. Fig. 3 clearly shows the effects on the chemical shifts associated with the carbons 3–9 of the DS molecule and for the signals of the carbons near the positive sites of the disubstituted imidazole ring in the polymer (C3, C4 and C6).

The thermogravimetric curves (TG and DTG) for the polymer and the polyampholyte–DS complex are shown in Fig. 4. The thermal

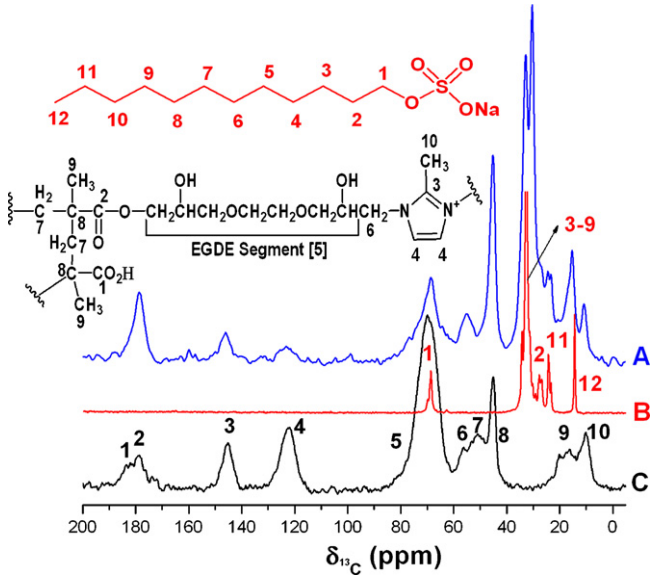



**Fig. 2.** Solid-state $^{13}$C spectra for the poly(EGDE–MAA–2MIH$^+$)–DS complex (A), SDS (B) and poly(EGDE–MAA–2MI) (C). Operating frequency: 75.46 MHz. Spinning rate: 10 kHz.

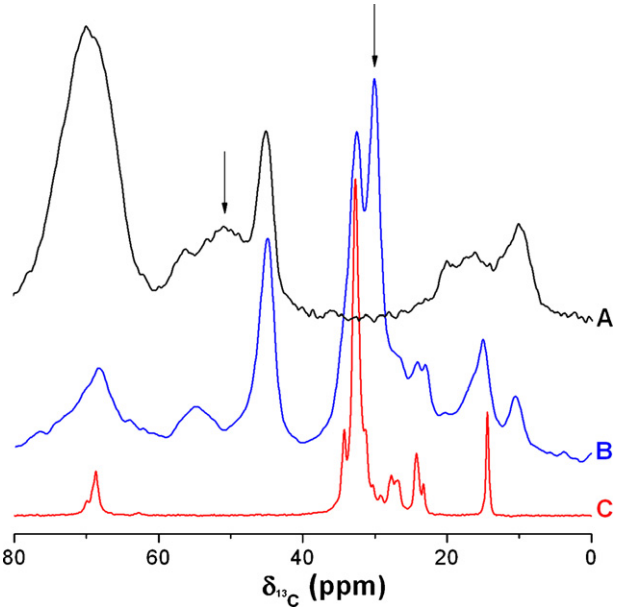



**Fig. 3.** Changes in the $^{13}$C chemical shift for poly(EGDE–MAA–2MI) (A), the poly(EGDE–MAA–2MIH$^+$)–DS complex (B) and SDS (C).

behaviour of this kind of polymer matrixes has been previously studied [18]. The results showed an initial loss of weight due to the evaporation of water in the complex. Also, the anionic surfactant interacted electrostatically and hydrophobically through the $ROSO_3^-$ group and the hydrocarbon chain with the polymer backbone, respectively. The most important interaction was located between the positive charges of the imidazolium units and the disubstituted imidazole ring of the matrix with the negative domain of the DS [18,24]. That association produced the low thermal stability of the complex against the polyampholyte matrix and partial degradation of the DS molecules, as a consequence of the disruption in the electronic density in the polymer backbone with the concomitant weakness of the chemical bonds [18].

The scanning electron microscopy (SEM) images exhibit the modifications produced by the DS adsorption on the polyampholyte particles. This coverage had left rows of macropores with an average diameter of 85 nm, sharing the same direction over a restricted region (Fig. 5).

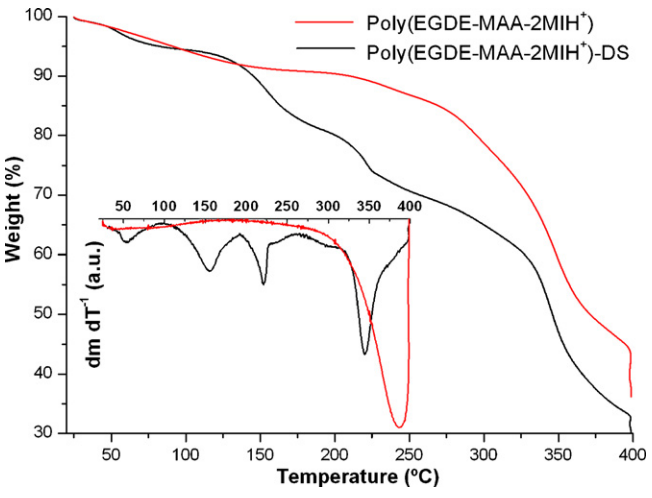



**Fig. 4.** TG and DTG curves for poly(EGDE–MAA–2MI) and the poly(EGDE–MAA–2MIH$^+$)–DS complex. Heating rate: 10 °C min$^{-1}$. Operating under nitrogen flux. Sample size: 10 mg.

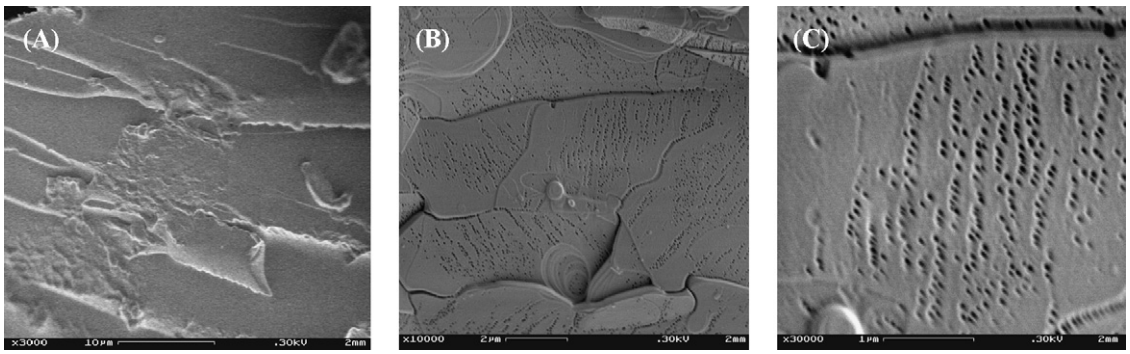



**Fig. 5.** SEM images for poly(EGDE–MAA–2MIH$^+$) (A), the poly(EGDE–MAA–2MIH$^+$)–DS complex (B) and the poly(EGDE–MAA–2MIH$^+$)–DS complex with magnification (C). Acceleration voltage: 0.3 kV.

The average nanopore width and the equivalent specific surface area obtained from the nitrogen adsorption isotherm were 36.8 Å and 3.04 $m^2\,g^{-1}$, respectively (the value for the polymer without DS is 0.6 $m^2\,g^{-1}$). These results were consistent with an increase in the specific surface area as a consequence of the increase in the hydrophobic segments from the interaction with DS.

### 3.2. Surfactant uptake

Kinetics and equilibrium studies were carried out with SDBS instead of SDS.

The results of the kinetics studies indicated that the binding equilibrium was reached in 5 min under forced convection at 20 °C.

The binding isotherm of dodecyl benzenesulfonate (DBS) to poly(EGDE–MAA–2MIH$^+$) is exhibited in Fig. 6. The experimental data fitted properly with Langmuir model with a regression coefficient ($R$) of 0.9911, a maximum loading capacity estimated in $1.5 \pm 0.2$ mmol $g^{-1}$ ($0.54 \pm 0.07$ g DBS $g^{-1}$) and a dissociation constant of $2.1 \pm 0.5 \times 10^{-3}$. This well-known model is based on the interaction of a ligand and a finite number of active sites available on the sorbent surface. In this case the nature of the interaction is mainly electrostatic: the ligand provides a negative charge and the polyampholyte presents regions of positive charge in protoned 2-methylimidazole terminal residues together with fixed positive charges in disubstituted 2-methylimidazole [16,17]. A hydrophobic interaction between the hydrocarbon chain of DS and the polymer backbone may take place to a lesser extent.

Even if the initial concentration of SDBS in the experiment in most of the experimental points of the isotherm was above the CMC, the interaction with the polymer was well described by Langmuir model, involving the uptake of one DBS molecule per site.

Then, a second binding experiment was performed as described in Section 2.2, using high amounts of SDS and SBDS. The elemental analysis of poly(EGDE–MAA–2MIH$^+$)–DS gave 4.992% of sulfur, equivalent to 1.557 mmol DS per gram of complex and 2.653 mmol DS per gram of polyampholyte. In the same way, 0.66 g of DBS (1.9 mmol) per gram of polyampholyte were adsorbed in this experiment, an amount higher than the binding predicted by Langmuir model. These results led us to conclude that some degree of aggregation took place, as expected for the interaction of polymers with surfactants [21]. Here, the concentration of the surfactant was far above the CMC, so an equilibrium between free micelles and the micelles bound to the polyampholyte must be taken into account.

### 3.3. Studies of interaction between the complex and bisphenol-A (BPA)

Poly(EGDE–MAA–2MIH$^+$)–DS was prepared following the procedure described in Section 2.2. Under that treatment, the polyampholyte was assumed to adsorb individual DS molecules to a larger extent and DS micelles.

The results of the kinetic experiments of BPA uptake are presented in Fig. 7. These results fit well with a first-order empiric model ($R$, 0.9962):

$$[\mathrm{BPA}] = [\mathrm{BPA}]_\infty + [\mathrm{BPA}]_b \times e^{-k \times t} \quad (1)$$

where $[\mathrm{BPA}]_\infty$ is the free amount in equilibrium, $[\mathrm{BPA}]_b$ the total amount of BPA withdrawn from the solution by sorption

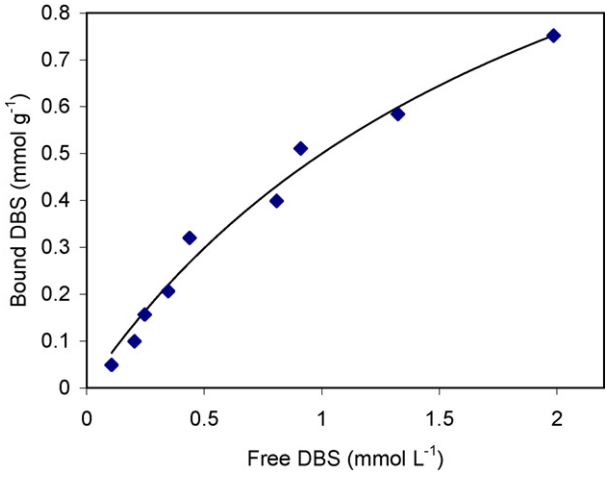



**Fig. 6.** Adsorption isotherm of DBS to poly(EGDE–MAA–2MIH$^+$). DBS concentration range: 0.2–8.0 mmol $L^{-1}$. Temperature: $20.0 \pm 0.5$ °C.

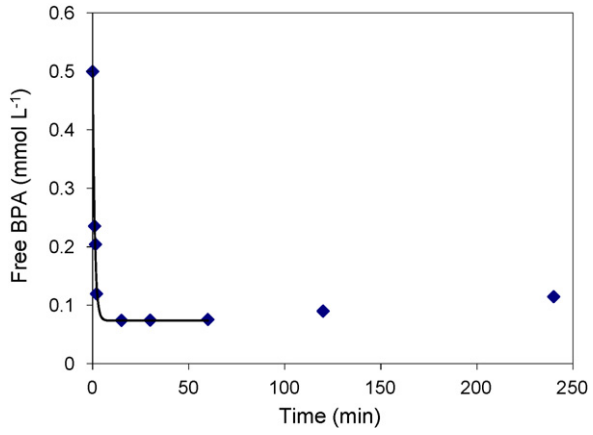



**Fig. 7.** Concentration of BPA for the solution in contact with poly(EGDE–MAA–2MIH$^+$)–DS as a function of time. Weight of the complex: 50 mg. Initial BPA concentration: 114 mg $L^{-1}$ (500 μmol $L^{-1}$); solution volume: 125 mL; medium: 10:90 methanol:water.

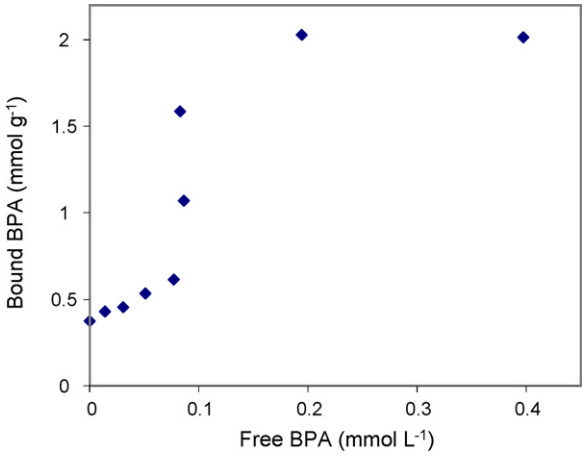



**Fig. 8.** Sorption isotherm of BPA to poly(EGDE–MAA–2MIH$^+$)–DS. Weight of the complex: 40 mg. BPA concentration range: 75–800 μmol $L^{-1}$; sample volume: 200 mL; medium: 10:90 methanol:water. Temperature: 20.0 ± 0.5 °C.

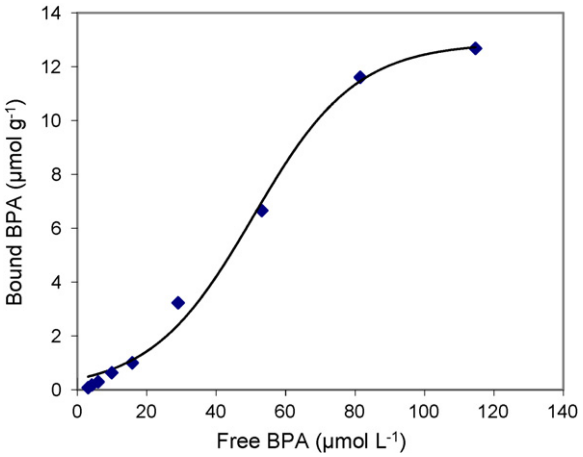



**Fig. 9.** Adsorption isotherm of BPA to poly(EGDE–MAA–2MIH$^+$). Polymer weight: 200 mg. BPA concentration range: 3.7–216 μmol $L^{-1}$; aqueous sample volume: 25 mL. Temperature: 20.0 ± 0.5 °C.

to the matrix, and $k$ the pseudo first-order kinetic constant for the decrease in BPA in solution. The estimated parameters were: $[BPA]_\infty$: $7.38 \times 10^{-5}$ mol $L^{-1}$ ($\sigma_{n-1}$: $9.4 \times 10^{-6}$); $[BPA]_b$: $4.26 \times 10^{-4}$ mol $L^{-1}$ ($\sigma_{n-1}$: $1.9 \times 10^{-5}$); $k$: 0.929 $min^{-1}$ ($\sigma_{n-1}$: 0.081).

This model properly describes the behaviour of this system for the first hour of contact between the matrix and the BPA solution. After this, a slow increase in BPA in solution was observed and attributed to the desorption of DS micelles from the polyampholyte surface. The contact time for BPA preconcentration on the polyampholyte–DS complex surface was then set in 60 min.

The results for BPA binding equilibrium gave a type-H4 isotherm (Fig. 8) in the classification of Giles et al. [25]. In this case the solute has such high affinity for the matrix that it was completely bound or there was no free measurable amount in equilibrium in diluted solutions (below 0.1 mmol $L^{-1}$). The first plateau corresponds to the formation of the first "monolayer". One possible mechanism for solute–sorbent interaction is hydrogen bonding between hydroxyl groups from BPA and the hydroxyl and carboxylic groups on the polyampholyte surface, forming isolated clusters of BPA molecules on the most active sites. Another possible mechanism of sorption is the partition of BPA into the hydrophobic environment of the hydrocarbon chains electrostatically bound to the polyampholyte. These simultaneous mechanisms would account for the complete solute uptake at low concentration levels. Electrostatic attraction is discarded under this experimental condition since BPA is expected to be neutral.

At total BPA concentrations above 0.2 mmol $L^{-1}$ there was a sharp increase in sorption, indicating a complete saturation of the active surface of the polymer. In this second step, the solute molecules could be partitioned in the DS micelles sorbed on the polymer, and hydrogen bonding between BPA molecules on the sorbent could also be considered. The amount of BPA bound in the first step was 0.450 mmol $g^{-1}$ (103 mg), and that in the second step 2.02 mmol $g^{-1}$ (461 mg), representing a significantly high loading capacity for this new matrix as compared with other sorbents [14,15,26]. Then, in conditions of saturation, 2.02 mmol BPA can be bound to a gram of complex that contains 2.653 mmol of DS.

These results were compared with the isotherm for BPA adsorption on the polyampholyte without DS (Fig. 9). In this case, a sigmoidal or S-type isotherm was obtained with an $R$ of 0.9975 for non-linear regression. This indicates cooperative adsorption, meaning that at low concentrations of BPA the interaction is weak but the adsorption becomes easier as the concentration of BPA increases. This behaviour is expected when the solute molecule is adsorbed as a single unit (not a micelle), has a moderate degree of intermolecular attraction by π–π interaction (which determines a vertical orientation in a regular arrangement), and presents strong competition with water for active sites on the surface of the polyampholyte [25]. In this case, BPA probably interacts by hydrogen bonding with carboxylic groups and the hydroxyl groups on the network resulting from the epoxide opening. Hydrogen bonding between BPA molecules could contribute to sorption at high solute concentrations.

The modification of the polyampholyte surface with DS significantly improved the uptake of BPA. The maximum binding capacity of the complex (2.02 mmol $g^{-1}$) was 160 times higher than the value observed for the polyampholyte (12.9 ± 0.5 μmol $g^{-1}$). When the concentration of BPA was lower than 100 μmol $L^{-1}$, a complete uptake of BPA on the complex was observed.

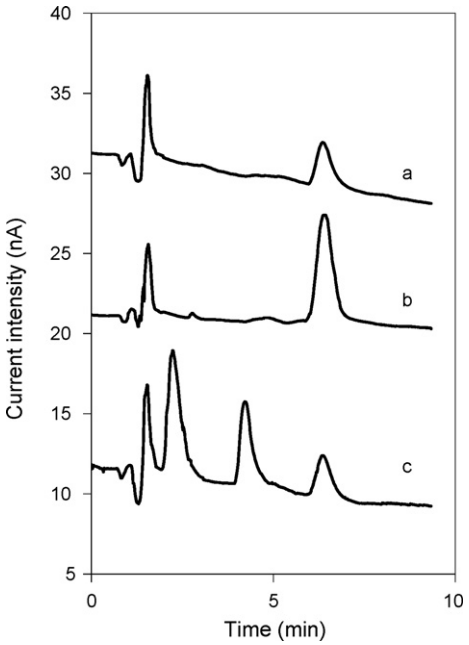



**Fig. 10.** Chromatograms of the sample in treatment A1 (a), the sample in treatment A1 with spiking of 0.200 mg $L^{-1}$ BPA (final concentration in alcoholic extract) (b) and the sample in treatment D (c). Mobile phase: 55:45 methanol:aqueous solution of 10 mmol $L^{-1}$ $KNO_3$ and 0.25 mmol $L^{-1}$ $H_2SO_4$. Injection volume: 20 μL. Flow rate: 0.8 mL $min^{-1}$. Electrode potential: +1 V vs. Ag/AgCl.

**Table 1**
BPA concentration in extracts from baby bottles.

| Sample treatment for extraction of BPA | BPA in extracts ($\mu g\,L^{-1}$) | Recovery[a] (%) |
|---|---|---|
| (A1) Distilled water at 100 °C for 30 min | 1.70 ($\sigma_{n-1}$: 0.09) | 96 ($\sigma_{n-1}$: 4) |
| (A2) Second sequence (after rinsing) | 0.52 | – |
| (A3) Third sequence (after 10 cycles of boiling for 10 min with tap water 1 and brushing) | 3.6 | – |
| (B) Tap water 1 at 100 °C for 10 min | 3.7 ($\sigma_{n-1}$: 0.1) | – |
| (C) Tap water 2 at 100 °C for 10 min | 9.1 ($\sigma_{n-1}$: 0.2) | 95 ($\sigma_{n-1}$: 5) |
| (D) Distilled water at 100 °C for 30 min, and then spiking with other pollutants | 1.7 ($\sigma_{n-1}$: 0.1) | 98 ($\sigma_{n-1}$: 5) |

[a] Recovery from a 2.65 $\mu g\,L^{-1}$ BPA spike.

### 3.4. Preconcentration and analysis of BPA released from baby bottles

The HPLC-ED system presented linearity for BPA tested in a concentration range up to 0.500 mg $L^{-1}$ with a sensitivity of 667 nCoulombs L $mg^{-1}$ ($\sigma_{n-1}$: 10) and a correlation coefficient of 0.9986. The repeatability of the technique was also good as the relative standard deviation for the standard BPA replicates of each concentration level was less than 4%.

The instrumental detection and quantification limits of the technique were 10 and 35 $\mu g\,L^{-1}$, respectively, and the preconcentration step allowed lowering the limits of the method to 0.14 and to 0.46 $\mu g\,L^{-1}$, respectively.

The new material was used as solid phase for preconcentration of BPA released by polycarbonate baby bottles to food-simulating liquids. The extraction sorbent was prepared by including poly(EGDE–MAA–2MIH$^+$)–DS in agarose gel as described in Section 2.6. Several sample treatments were carried out.

The first migration assay was performed following the MERCOSUR Regulations [22], using distilled water at 100 °C for 30 min as food simulant for milk (treatment A1). The BPA in the simulant was preconcentrated on the new sorbent and released to the alcoholic mixture for analysis by HPLC-ED. Fig. 10 shows the chromatogram of the sample (a) and the sample spiked with BPA in a final concentration of 0.200 mg $L^{-1}$ (b). The retention time for BPA was 5.6 min and the released BPA was 2.1 ng $cm^{-2}$ ($\sigma_{n-1}$: 0.1) using the new phase, and 2.3 ng $cm^{-2}$ ($\sigma_{n-1}$: 0.1) using Oasis HLB phase for preconcentration. The amounts were in the same order as the results obtained by evaporation of the simulant liquid as strategy for preconcentration [27], and lower than the SML established by the Mercosur (4.8 $\mu g\,cm^{-2}$) [22] or the SML established by the EU Commission (750 ng $cm^{-2}$).

Table 1 presents the concentrations of BPA found in the food simulants and the recoveries for the 2.65 $\mu g\,L^{-1}$ BPA spike (final concentration of 0.200 mg $L^{-1}$ in the alcoholic extract). The recovery of BPA with treatment A1 was 96% ($\sigma_{n-1}$: 4) with the new sorbent for a 2.65 $\mu g\,L^{-1}$ BPA spike, and 97% ($\sigma_{n-1}$: 4) with Oasis HLB for a 1.67 $\mu g\,L^{-1}$ BPA spike, indicating that the performance of the new matrix is good in comparison with the best sorbent [1,12].

The particularly high loading capacity of this new phase allowed the detection of released BPA by an amperometric technique with a relatively high LoD (10 $\mu g\,L^{-1}$) [1].

The sorbent beads were reused twice with new baby bottles and the relative difference between each replicate and the mean value found for BPA was lower than 7%.

The release of BPA into hot distilled water diminished in a successive test [28] as observed with treatment A2 (Table 1). Then, with treatment A3, the same polycarbonate baby bottles were subjected to simulated use by boiling in tap water and brushing in order to create scratches on the inner surface and increase the wettability of the wall. This fact could have contributed to the deposit of residual limescale which further dissolved in the food stimulant and promoted the degradation of the polymer [29,30]. Table 1 exhibits the release of 3.6 $\mu g\,L^{-1}$ BPA from these aged baby bottles into distilled water kept at 100 °C for 30 min.

Biedermann-Brem and Grob [30] found that hot water at high pH degraded the polycarbonate causing an increase in the amount of the released BPA. In treatments B and C we used tap water of different pH and alkalinity as food simulants. Tap water 1 was kept boiling in the bottle for 10 min, the pH rose from 6.9 to 7.3 and 3.7 $\mu g\,L^{-1}$ of BPA were released after 10 min. With tap water 2, the pH rose from 7.6 to 8.1 by loss of carbon dioxide causing the hydrolysis of polycarbonate and the release of 9.1 $\mu g\,L^{-1}$ of BPA, as expected [30].

In treatment D, we repeated the migration test on new bottles with distilled water at 100 °C for 30 min, and then four organic pollutants (phenol, methylparaben, propylparaben and diethyl phthalate) were added to the water extract prior to the preconcentration step. Parabens are a homologous series of hydroxybenzoic acid esterified at the C-4 position used as an antimicrobial in foods, beverages, pharmaceuticals and cosmetics [31]. Phenol is usually present in polluted water samples. The phenolic compounds were uptaken together with BPA on the new phase and later detected electrochemically. Fig. 10 exhibits their signals in chromatogram c; phenol had a retention time of 1.4 min and parabens coeluted at 3.4 min. Diethyl phthalate is a plasticizer designed to increase the durability and flexibility of plastics. Since plasticizers are not chemically bound to the polymer they can leach from the plastic into the environment [32]. Even if diethyl phthalate was expected to be preconcentrated on the new adsorbent, it is not electroactive and was not detected at +1 V vs. Ag/AgCl. The preconcentration of BPA on the sorbents tested was not affected by the presence of other structurally similar compounds in the matrix at the same concentration level. The concentration of BPA released to the simulant in treatment D did not differ from treatment A1 and the recovery figures were optimal.

## 4. Conclusions

The surface of a synthetic polyampholyte was modified with a surfactant in order to change its chemical and physical properties. The new material, which combines hydrophobic domains with hydrophilic functionality, was exhaustively characterized by spectroscopic and microscopic techniques.

BPA was chosen as a model molecule of intermediate polarity to evaluate the efficiency of this matrix as a solid phase for extraction. The sorption of this analyte was significantly improved by the presence of a surfactant and was complete at low concentration levels. The particularly high loading capacity of this new phase allowed the detection of released BPA by an amperometric technique with a relatively high LoD. The new complex was tested with different food simulants (distilled water, tap water and a model solution containing phenolic pollutants and a plasticizer) and the recovery figures were optimal. The use of this encapsulated sorbent was simple, not expensive and adequate for this application.

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