Journal of Chromatography A, 1456 (2016) 1–9

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Synthesis and characterization of molecularly imprinted polymer nanoparticles for coenzyme Q10 dispersive micro solid phase extraction

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ARTICLE INFO

Article history:
Received 16 November 2015
Received in revised form 23 May 2016
Accepted 26 May 2016
Available online 27 May 2016

Keywords:
Coenzyme Q10
Molecularly imprinted polymer nanoparticles
Dispersive micro solid phase extraction

ABSTRACT

Molecularly imprinted polymer nanoparticles (MIPNPs) with the ability to recognize coenzyme Q10 (CoQ10) were synthesised in order to be employed as sorbent in a dispersive micro-solid phase extraction (DMSPE) for the determination of CoQ10 in a liver extract. CoQ10 is a redox-active, lipophilic substance integrated in the mitochondrial respiratory chain which acts as an electron carrier, shuttling electrons from complex I (NADH-ubiquinone oxidoreductase) and II (succinate-ubiquinone oxidoreductase) to complex III (ubiquinol-cytochrome *c* reductase), for the production of cellular energy. The MIPNPs were synthesised by precipitation polymerization using coenzyme Q0 as the dummy template, methacrylic acid as the functional monomer, an acetonitrile: water mixture as the porogen, ethylene glycol dimethacrylate as the crosslinker and potassium persulfate as initiator. The nanoparticles were characterized by microscopy, capillary electrophoresis, dynamic light scattering, N_2 adsorption–desorption isotherms, and infrared spectroscopy. The MIPNPs demonstrated the presence of selective cavities complementary to the quinone nucleus of CoQ10, leading to a specific recognition of CoQ10 compared with related compounds. In the liver extract the relative CoQ10 peak area (CoQ10 area/total peak area) increased from 4.6% to 25.4% after the DMSPE procedure. The recovery percentage of CoQ10 from the liver matrix was between 70.5% and 83.7% quantified against CoQ10 standard processed under the same conditions. The DMSPE procedure allows the elution of almost all the CoQ10 retained (99.4%) in a small volume (200 μL), allowing the sample to be concentrated 2.5 times (LOD: 1.1 $\mu g\,g^{-1}$ and LOQ: 3.7 $\mu g\,g^{-1}$ of tissue). The resulted clean up of the sample, the improvement in peak shape and baseline and the reduction of interferences, evidence that the MIPNPs could potentially be applied as sorbent in a DMSPE with satisfactory results and with a minimum amount of sorbent (1 mg).

1. Introduction

Coenzyme Q (2,3-dimethoxy-5-methyl-6-polyisoprene-1,4-benzoquinone) is a redox-active, lipophilic substance integrated in the mitochondrial respiratory chain which acts as an electron carrier, shuttling electrons from complex I (NADH-ubiquinone oxidoreductase) and II (succinate-ubiquinone oxidoreductase) to complex III (ubiquinol-cytochrome *c* reductase), for the production of cellular energy. The quinones of the coenzyme Q series, which are found in various biological species, differ in the number of isoprene units in the side chain being coenzyme Q10 (CoQ10), with 10 isoprene units, the predominant isoform found in humans [1,2]. Due to its principal role in the mitochondrial respiratory chain, CoQ10 is known to be involved in a number of aspects of cellular metabolism, especially for its antioxidant function considered one of the most important studied.

In a previous work, we developed the first molecularly imprinted polymer (MIP) able to selectively recognize CoQ10

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http://dx.doi.org/10.1016/j.chroma.2016.05.091
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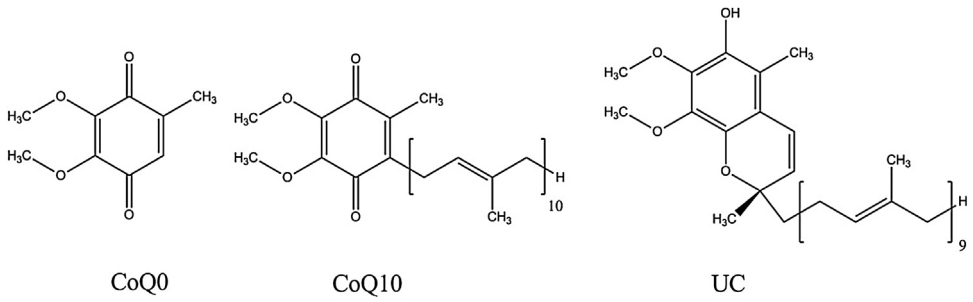

Fig. 1. Molecular structure of coenzyme Q0 (CoQ0), coenzyme Q10 (CoQ10) and ubichromenol (UC).

[3]. Molecular imprinting is a strategy to introduce a "molecular memory" in a polymeric system to obtain materials with specific recognition properties [4]. MIP is usually developed by mixing a template molecule with functional monomers, a cross-linker and an initiator. After polymerisation, the template molecules are removed making the binding sites and the cavities, which are complementary to the template in size, shape and functionality, accessible [3].

This reported CoQ10 MIP was synthesised by bulk polymerisation according to the non-covalent approach, employing coenzyme Q0 (CoQ0) (2,3-dimethoxy-5-methyl-1,4-benzoquinone) as dummy template because it shares the same quinone group with CoQ10 but not the reactive isoprene tail of the coenzyme [3].

In general, bulk procedure is time-consuming, the cavities of the MIPs may be destroyed and also particles obtained by this procedure have irregular shape. With respect to this polymerisation there are another techniques developed to enhance MIPs properties for new analytical applications and also new MIPs morphologies [5,6].

Different procedures like precipitation polymerization [7], suspension polymerization [8], core shell emulsion polymerization [9], and mini-emulsion polymerisation [10] have been employed to synthesise micro and nanoparticles (NPs) [11].

Although all those methods have a clear value, the precipitation polymerization procedure is in general more economical and labor-saving compared to bulk polymerisation and enables the recovery of MIPs with uniform shape and low disperse size distribution, promoting the collection of smaller particles and increasing surface area. In addition, precipitation polymerisation is a surfactant or stabilizer free method in contrast to suspension or emulsion polymerization making unnecessary to remove residual surfactant in the polymerisation media, which can potentially affect the selectivity of the final imprinted polymer [6].

The aim of this work was to synthesize and characterize molecularly imprinted polymer nanoparticles (MIPNPs) able to recognize CoQ10 maintaining the molar ratio between the template, functional monomer and the crosslinker previously optimized. The effect of the miniaturization of the polymer on the CoQ10 recognition was finally studied analyzing CoQ10 in a liver extract, a complex biological material of study, by dispersive micro-SPE (DMSPE).

2. Experimental

2.1. Reagents

CoQ0 (MW 182.2) and CoQ10 (MW 863.3, Xlog P: 19.4 [12]) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ubichromenol (UC) (MW 863.3, Xlog P: 19.6 [12]) was synthesised in our laboratory by the basic catalysis of CoQ10 with triethylamine using a method previously described [13]. The molecular structure of these compounds is shown in Fig. 1.

Methanol and 1-propanol were of HPLC grade while acetonitrile and acetic acid were of analytical grade, supplied by Sintorgan (Argentina). Methacrylic acid (MAA) (MW 86.1), ethylene glycol dimethacrylate (EGDMA) (MW 198.2), potassium persulfate (KPS) (MW 270.3), dimethyl sulfoxide (DMSO) (MW 78.1), sodium phosphate tribasic dodecahydrate (MW 380.1) and sodium phosphate monobasic monohydrate (MW 138.0) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water (conductivity of 0.055 μS/cm) was used.

2.2. Solution preparation

Standard solution of CoQ10 and UC were prepared in ethanol (1 mg mL^{-1}) and diluted properly before HPLC analysis.

pH 11.2 phosphate solution was prepared from sodium phosphate tribasic dodecahydrate and the pH was adjusted with a HCl solution, while pH 2.5 phosphate solution was prepared from sodium phosphate monobasic monohydrate and the pH was adjusted with a HCl solution. NaCl was added to the phosphate solution (pH 2.5) in order to ensure the same ionic strength in both solutions.

A monomer stock solution of EGDMA (0.4224 mmol mL^{-1}) and MAA (0.117 mmol mL^{-1}) was prepared in acetonitrile. A stock solution of CoQ0 (0.0145 mmol mL^{-1}) was also prepared in acetonitrile. KPS (3 mmol L^{-1}) was prepared in water.

2.3. Apparatus

Capillary electrophoresis (CE) analysis was carried out with a P/ACE™ MDQ capillary electrophoretic system, equipped with a DAD detector (Beckman, Fullerton, CA, USA). A 32 Karat software was used to control the instrumental parameters. Analysis was carried out with uncoated fused silica capillaries (Microsolv technology, Eatontown, NJ, USA) of 50 cm (39.5 cm length to the detector) × 75 μm i.d. In all cases the capillary temperature was maintained at 25 °C, and UV detection was set at 214 nm. Samples were injected under a pressure of 0.5 psi for 5 s and electrophoretic system was operated under a constant voltage of 20 kV.

The MIPNPs and non imprinted polymer nanoparticles (NIPNPs) electrophoretic mobility assay was carried out employing a 10 mmol L^{-1} pH 11.2 phosphate solution and the sample was injected at the anode site. A pH 2.5 phosphate buffer was also employed, and the samples were injected from the cathode (negative polarity). The ionic strength was the same in both cases (current intensity of ±37 μA). DMSO was used as electroendosmotic flow (EOF) marker.

HPLC analysis was performed using a Thermo Scientific HPLC (Waltham, Massachusetts, USA) equipped with a quaternary pump (P4000), a temperature control, a vacuum degasser (SCM 1000), a dual UV detector (UV2000), an automatic injector (AS3000) and Chrom Quest 5.0 software was used to control the instrumental parameters. All chromatographic determinations by HPLC were

Table 1
Synthesis of polymers.

Polymer	Monomer stock solution (mL)	Acetonitrile (mL)	CoQ0 solution (mL)	KPS solution (mL)	% monomers(p/p)
MIP A	1.0	8.0	1.0	20.0	0.3
MIP B	0.5	9.0	0.5	30.0	0.1
MIP C	0.5	9.0	0.5	20.0	0.1
MIP D	2.0	6.0	2.0	20.0	0.6
MIP E	1.0	8.0	1.0	30.0	0.2
NIP A	1.0	9.0	–	20.0	0.3
NIP B	0.5	9.5	–	30.0	0.1
NIP C	0.5	9.5	–	20.0	0.1
NIP D	2.0	8.0	–	20.0	0.6
NIP E	1.0	9.0	–	30.0	0.2

carried out employing a miniaturized HPLC-UV method. Briefly, the separation was performed using an Xterra microcolum (Waters, Milford, Massachusetts) with dimensions of 50 mm and 2.1 mm i.d., filled with particles of 3.5 μm size. CoQ0 determinations were performed using methanol: water (30:70 v/v) as mobile phase but for the analysis of CoQ10 and UC, 100% methanol was used instead. In both cases the flow rate was set at 0.4 mL min^{-1}, the injection volume of 10 μL, and the UV-detection was achieved at 275 nm of wavelength [14].

FT-IR spectra of polymers were recorded on a Nicolet 380 FT-IR Spectrometer, Thermo Scientific (Waltham, Massachusetts, USA). The number of scanning was 32 with a resolution of 4 cm^{-1} and an angle of incidence of 45, using KBr disks.

Textural characterization was performed by N_2 adsorption–desorption isotherms at −196 °C determined with an automatic Micromeritics ASAP-2020 HV volumetric sorption analyser (Micromeritics Instrument, Norcross, GA, USA). Prior to gas adsorption measurements, the samples were degassed at 60 °C overnight. The Brunauer–Emmett–Teller (BET) surface area was determined by applying the standard BET procedure. Pore size distributions were also calculated from N_2 adsorption isotherms data using the DFT plus software (Micromeritic Instrument Corp.) based on non local density functional theory.

The size, size distribution and Z potential were determined by dynamic light scattering (DLS) (Zetasizer Nano-Zs, Malvern Instruments, UK). Data were processed using CONTIN algorithms [15] (see Section 2.7). Samples were dispersed in water prior to analysis. Size results expressed as the hydrodynamic diameter (Dh), poly dispersion index (PDI) and Z potential were calculated from the average of six measurements. The instrument was previously calibrated with standard latex nanoparticles provided by Malvern Instruments.

Transmission electron microscopy (TEM) analysis was performed on a Philips CM-12 TEM (FEI company, Eindhoven Nederland) operated at an acceleration voltage of 50 kV. Before analysis, one drop of nanoparticle water suspension was placed in the sample holder and dried at room temperature.

Field emission gun scanning electron microscopy (FEG-SEM, Zeuss Supra 40 apparatus with a Gemini column, Germany) was operated at an acceleration voltage of 3.0 kV. Before analysis, one drop of nanoparticle water suspension was placed in the sample holder, dried at room temperature and coated with gold using a sputter coating method for 20 s. The atomic composition of polymers was also studied by energy dispersive X-ray spectroscopy (EDX, inca x-sight, Oxford Instrument, Abingdon, UK).

Polymers were lyophilized in a Freeze-dryer FIC-L05, FIC, liophilizer (Scientific Instrumental, Argentina). The temperatures of the freeze-dryer shelf and the condenser were −14° C and −40° C, respectively and the pressure was 0.03 mbar.

Additional instrumentation including an ultrasonic bath (Transsonic Digitals, ELMA, Kolpingstr), pH meter (UB–10 pH/mV meter, Denver Instrument, Bohemia, NY, USA) calibrated with three standard buffer solutions (pH 1.0, 7.0 and 10.0) provided by Sigma-Aldrich (St. Louis, MO, USA), a shaker (Minitherm-Shaker, Adolf Kuhner AG Schweiz), and a centrifuge (Minispin plus, Ependorff®, Hamburgo, Germany) were used. All centrifugation were performed at room temperature.

Ultrapure water was obtained from an EASY pure™ RF equipment (Barnstead, Dudubuque, IA, USA).

2.4. Material synthesis

The preparation of different polymers was performed by precipitation using CoQ0 as dummy template, MAA as functional monomer, EGDMA as the crosslinking monomer and KPS as initiator in a mixture of water and acetonitrile as solvents.

Table 1 shows the different polymers synthesised.

All polymers were prepared by a mixture of a CoQ0, MAA and EGDMA in a molar ratio of 1:8:25 respectively, previously optimized in order to enhance the CoQ10 selective binding [3].

The polymerisation was induced by heat in a glycerin bath at 70 °C for 4 h.

Removal of the template was carried out with a methanol: acetic acid mixture (9:1 v/v) by shaking in a vortex, and then separating the supernatant by centrifugation at 14500 rpm for 20 min. Three washes were necessary until the template was not detected in the supernatant by HPLC-UV. Finally, the polymers were resuspended in water, frozen at −80° C overnight, liophilized and stored at room temperature.

As a control, a non imprinted polymer (NIP) was simultaneously synthesised under the same conditions but in the absence of the template molecule.

2.5. Preparation of the polymer for analyte adsorption

Prior to use, the polymers were accurately weighed and suspended by sonication at 25° C in the interaction solvent (900 μL) during 15 min.

One hundred microliters of CoQ10 or UC standard (30 μg mL^{-1}) were added to the polymer (MIP or NIP) suspension in a 2.0 mL polypropylene tube (Eppendorf® Safe-Lock microcentrifuge tube). The mixture was vortexed at 1600 rpm for 2 min, and then, it was mechanically shaken at 120 rpm for 10 min at room temperature. Immediately the supernatant containing unbound analyte was separated by centrifugation at 14500 rpm for 20 min and analyzed by HPLC. The amount of analyte retained was calculated by subtracting the unbound analyte concentration from the initial concentration.

2.6. Pretreatment of liver samples

The liver extract was obtained as previously described [3]. Briefly, pieces of bovine liver (200 mg) were accurately weighed and subsequently homogenized in a mortar on an ice bath with 3 mL of cold 1-propanol, then it was sonicated for 5 min at 25° C. and centrifugated at 9000 rpm. The supernatant (liver extract) (500 μL) was

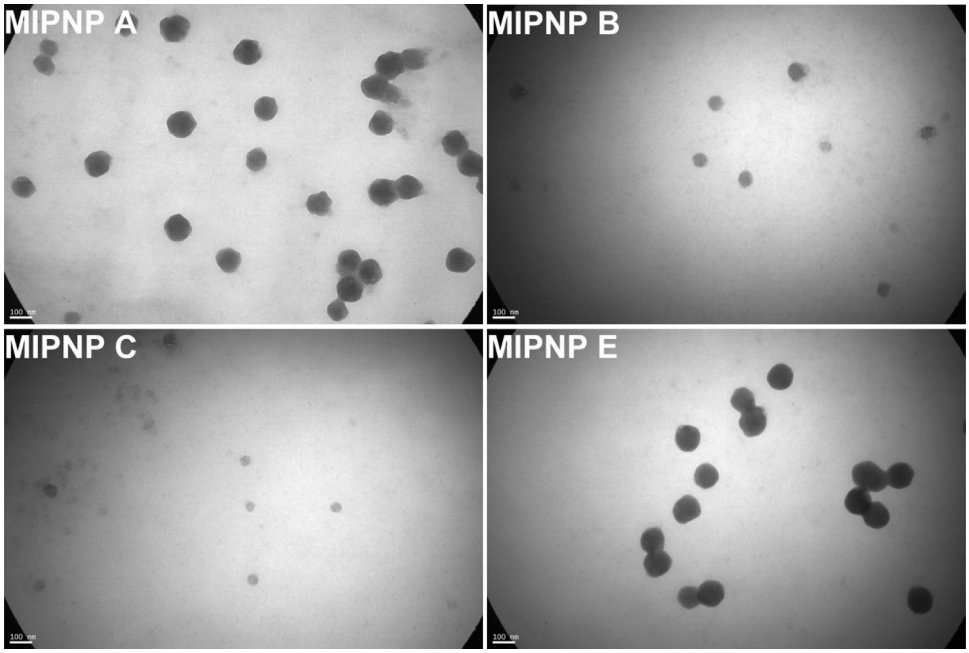

Fig. 2. Transmission electron microscopy (TEM) images of MIPNPs. The line represents 100 nm.

firstly diluted with 500 μL of water and then 1 mL was loaded onto 1 mg of MIPNPs in a 2.0 mL polypropylene tube. The interaction MIPNPs- CoQ10 was performed as described in Section 2.5.

After removing the supernatant, 200 μL of 1-propanol were added in order to elute the CoQ10 retained.

The recovery assay was carried out by spiking the liver extract with CoQ10 at two different levels (46 $\mu g\,g^{-1}$ and 15 $\mu g\,g^{-1}$ of tissue) in duplicate. The limit of detection (LOD) and limit of quantitation (LOQ) were determined at signal-to-noise ratios of 3:1 and 10:1 respectively.

2.7. Equations

The specific interaction due to the molecularly imprinted effect was expressed in terms of the "imprinting-induced promotion of binding" (IPB). IPB is defined as

$$\mathrm{IPB} = (\mathrm{AMIPNPs} - \mathrm{ANIPNPs})/\mathrm{ANIPNPs}$$

where ANIPNPs is the amount of analyte bound to NIPNPs and AMIPNPs is the amount of analyte bound to MIPNPs [16].

The intrinsic electrophoretic mobility (μ_i) was calculated according to the following equation:

$$\mu_i = \mu_{app} - \mu_{EOF}.$$

Being μ_{app} the apparent mobility, and μ_{EOF} the electroendosmotic flow mobility. Therefore, the apparent mobility results from the sum of the μ_i and μ_{EOF} [17].

CONTIN algorithm is base on the theory of the Browninan motion and the Stocks-Einstein equation:

$$D = k_B T/3\pi\eta Dh$$

where D is the diffusion coefficient, k_B is the Boltzman constant, T is the temperature and η is the solvent viscosity and Dh is the hydrodynamic diameter.

3. Results and discussion

3.1. Macroscopic results

After the polymerisation process, polymers A–C and E (MIP and NIP) formed a colloidal dispersion with different degrees of turbidity (see Supporting information: Fig. S1.). Macroscopically, polymers B and C were maintained as stable suspensions over time. However, polymers A and E only remained as a suspension for two days, afterwards, polymer precipitation was observed but it was easily dispersible. This could possibly be explained due to the polymer A and E larger particle size compared with polymer B and C. Polymer C was difficult to purify, probably due to the small size of the particles, which could lead to a low recovery.

In contrast, polymer D showed a completely different morphology; instead of a homogeneous suspension, the polymerisation resulted in interconnected macroscopic granules. Therefore, polymer D did not satisfy the objective of NP format.

3.2. Characterization

3.2.1. Electronic microscopy

The morphology of the different MIPNPs and NIPNPs were assessed by TEM (Fig. 2 and Fig. S2). This study established that each kind of NPs have a quasi spherical shape and a homogeneous size distribution.

Polymer D presented a total different morphology studied by SEM. Instead of individual NPs, beaded particles of interconnected granules were produced, resembling the end product of a bulk polymerization process (Fig. 3). For comparison, Fig. 3 shows the SEM image of MIPNP (polymer B, MIPNP B), where a quasi spherical, uneven surface nanoparticle is observed.

Sulfur was present in NPs composition, according to the EDX determinations, which likely comes from the initiator (Supporting information: Fig. S3).

3.2.2. Infrared spectroscopy

There were no appreciable differences between the spectra of polymer A to E, nor when they were compared to the polymer

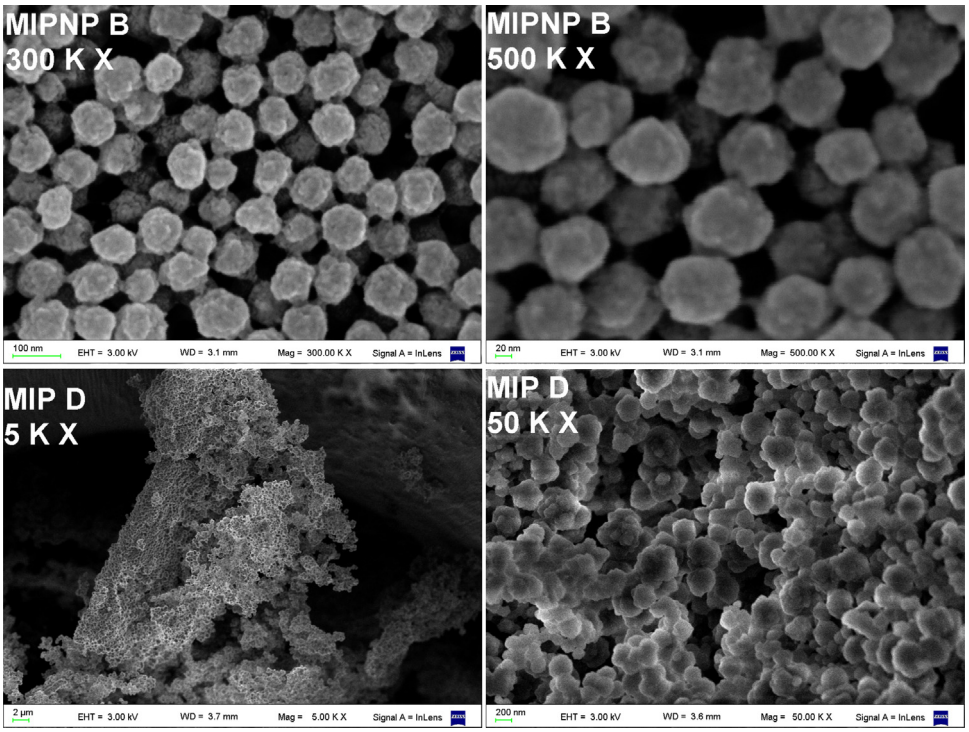

Fig. 3. Scanning electron microscopy (SEM) images of MIPNP B and MIP D.

previously reported by bulk polymerisation (F), in the range from 1000 to 4000 cm^{-1} (Supporting information: Fig. S4). There was no difference between MIP and NIP spectra either (data not shown).

The observed similarity in the MIP and NIP spectra as well as in different polymers could be explained on the basis of their similar internal structure which is in agreement with previous reports [18].

As seen in Fig. S4, the characteristic signal of the different polymers lies around 3445 cm^{-1} which corresponds to the O—H stretching and 2955 cm^{-1} due to the C—C vibration. The signals at 1730 cm^{-1} and 1160 cm^{-1} correspond to the C=O and C—O stretching, respectively.

MAA (functional monomer) and EGDMA (crosslinker) present a remarkable signal at 1632 cm^{-1} and 1637 cm^{-1} attributed to the C=C stretching of the polymerizable vinyl group. These signals practically disappear in the polymer due to the polymerisation process.

Finally, the signal of the carbonyl group suffers a shift from 1690 cm^{-1} (MAA) and 1716 cm^{-1} (EGDMA) to 1730 cm^{-1} in the polymer because of the lack of conjugation with the C=C group, which almost disappears in the polymer.

3.2.3. *Electrophoretic mobility shift assay*

This assay was only performed with the NPs (polymers A–C and E). Polymer D was discarded due to its morphology and wide size which is unsuitable for the CE analysis.

When the analysis is performed at pH 11.2 and the sample is injected from the anode (+), the electrophoretic mobility observed (μ_{app}) for an analyte with positive charge will be the sum of the intrinsic electrophoretic mobility (μ_i) and the electroendosmotic flow (μ_{EOF}) because both mobilities have the same direction.

Fig. 4A shows the analysis of MIPNPs at pH 11.2. It can be seen that the NPs have a longer migration time compared to the μ_{EOF}, so at this pH a negative charge of the NPs can be evidenced.

The results of the electrophoretic mobility shift assay are presented in Table 2.

Table 2
Results from the CE and DLS studies.

Polymer	Electrophoretic Mobility ($cm^2\ V^{-1}\ s^{-1}$) x 10^{-4}		Dh (nm) (sd)	PDI (sd)	Z potential (mV) (sd)
	pH 2.5	pH 11.2			
MIPNP A	−2.57	−4.24	126.2 (0.7)	0.03 (0.02)	−31 (2.4)
MIPNP B	−2.51	−4.13	79.2 (0.5)	0.05 (0.01)	−50 (2.1)
MIPNP C	−2.33	−3.84	74.9 (0.6)	0.07 (0.01)	−38 (1.5)
MIPNP E	−2.71	−4.40	170.2 (2.5)	0.08 (0.02)	−31 (2.1)
NIPNP A	−2.71	−4.26	138.4 (1.0)	0.03 (0.01)	−55 (0.6)
NIPNP B	−2.60	−4.24	90.0 (0.3)	0.03 (0.01)	−46 (0.8)
NIPNP C	−2.78	−3.99	74.1 (0.5)	0.08 (0.01)	−31 (1.1)
NIPNP E	−2.74	−4.57	155.6 (2.5)	0.03 (0.01)	−33 (0.6)

Intrinsic electrophoretic mobility at pH 2.5 is lower compared to μ_i at pH 11.2 indicating a reduction in the charge/mass ratio. It can be attributed to the carboxyl group from the MAA (pKa = 4.4) protonated at the pH of the assay. In order to support these results, MAA was analyzed in the same electrophoretic system confirming that it was protonated at pH 2.5 and with a negative charge at pH 11.2.

No appreciable difference in electrophoretic mobility was observed between MIP and NIP.

3.2.4. *Size and Z potential determinations*

Size and Z potential assays were also performed only for the MIPNPs and NIPNPs (Table 2). The values of zeta potentials were negative in all cases (below −30 mV). These results indicate that the NPs have a negative charge in water, which is in agreement with the mobility shift assay shown by capillary electrophoresis, and it could also explain the high stability of NPs.

The size and distribution of each type of NP presented an unimodal population and a low PDI (Fig. 4B). Nanoparticles C seems to be the smallest one, followed by B then A, whereas E was the largest. Those results were in agreement with the microscopy studies.

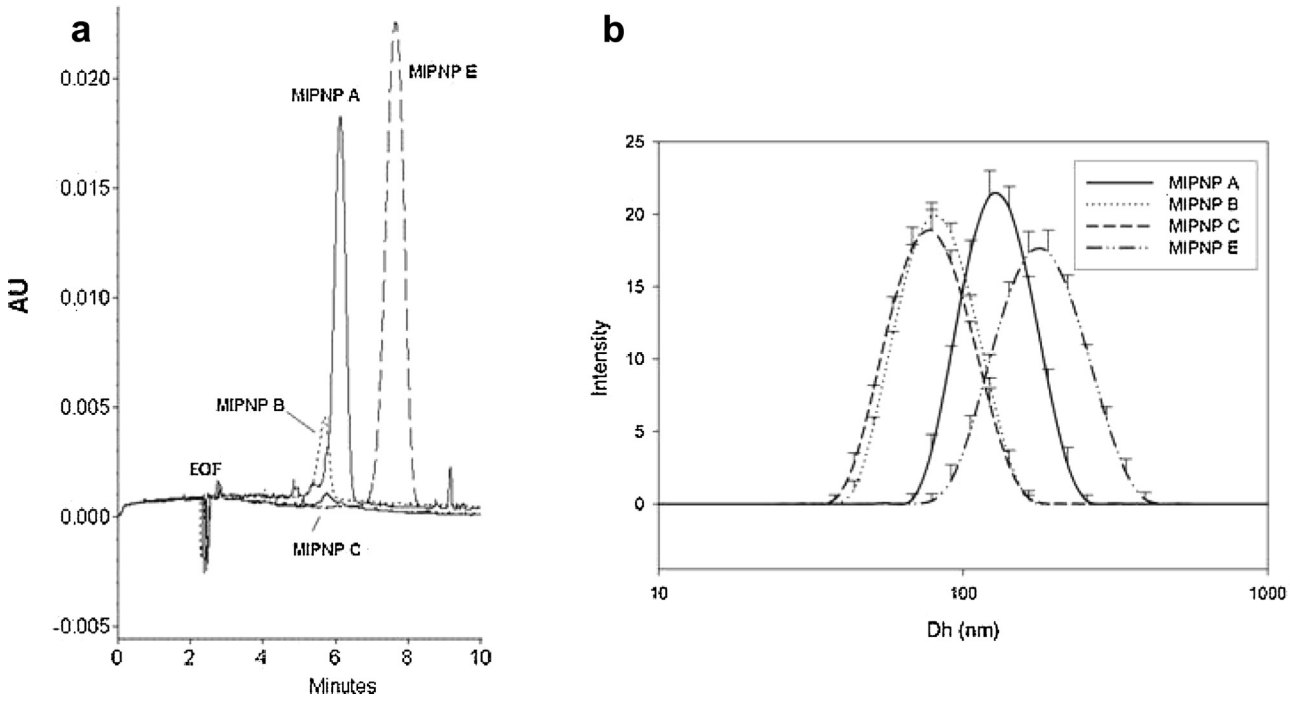

Fig. 4. (A) Analysis of NPs by CE at pH 11.20. Analysis was carried out with uncoated fused silica capillaries (50 cm × 75 μm i.d).Temperature 25° C, detection at 214 nm. Samples were injected under a pressure of 0.5 psi for 5 s and operating voltage of 20 kV. (B) Distribution of particle size for MIPNPs.

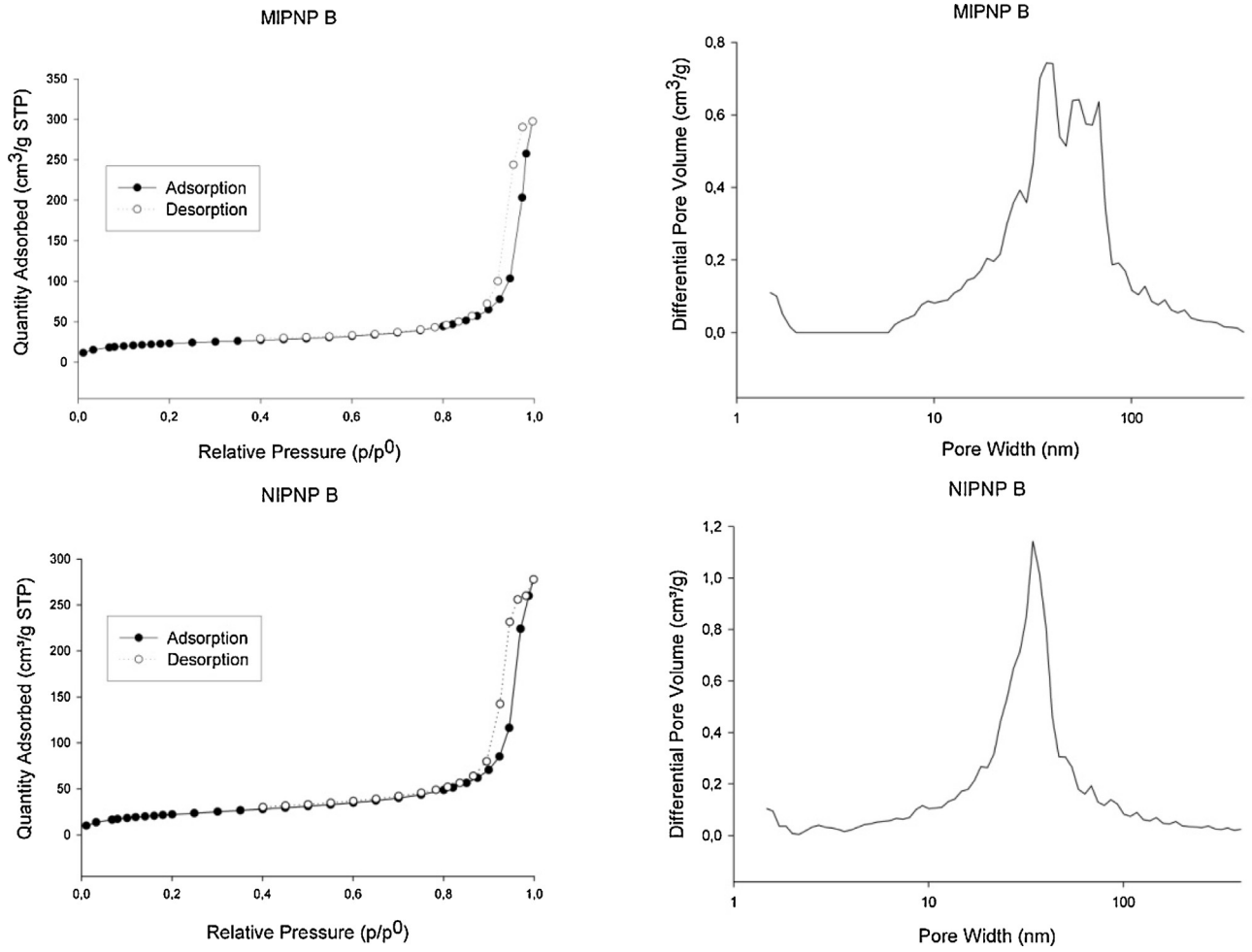

Fig. 5. N_2 adsorption–desorption isotherms, and pore size distribution for polymer B. Pore size distributions were also calculated from N_2 adsorption isotherms data.

3.2.5. Textural characterization

Polymers A, D and E presented a "type II" isotherm according to IUPAC classification, which is obtained for non-porous or macroporous samples. These polymers showed BET surfaces areas ranging between 36 and 88 $m^2\ g^{-1}$ with total pore volumes ranging between 0.06 $cm^3\ g^{-1}$ and 0.10 $cm^3\ g^{-1}$. Instead, NP B (MIPNP and NIPNP) showed a "type IV" isotherm, typically characteristic of mesoporous materials, with BET surfaces areas of 84 $m^2\ g^{-1}$ and 81 $m^2\ g^{-1}$ and total pore volumes of 0.16 $cm^3\ g^{-1}$ and 0.18 $cm^3\ g^{-1}$, respectively (Fig. 5) [19].

The pore size distribution plots obtained by density functional theory (DFT) for MIPNP B and NIPNP B showed a homogeneous distribution with a maximum centered at 30 nm (Fig. 5). There were no noticeable differences between MIP and NIP in any case.

3.3. Interaction studies

Taking into account the importance of the solvent effect during the process of MIP and the target analyte interaction, several proportions of 1-propanol and water were tested in order to optimize those interactions [3,20].

The interaction between each polymer and CoQ10 is strongly influenced by the 1-propanol percentage. The highest amount of CoQ10 retained was in the range of 30–50% of 1-propanol. Fig. 6 shows the difference between each MIP and NIP in the % of CoQ10 retained. The most appreciable difference between MIP and NIP in the retained amount of CoQ10 was found between MIPNP B and NIPNP B when 50% of 1-propanol was used. Accordingly, MIPNP B presented a higher specific capacity retention compared to the other polymers.

The interaction time was also optimized from 10 to 300 min. No improvement was found in the adsorption percentage beyond 10 min.

The percentage of CoQ10 retained with different amounts of MIPNP B and NIPNP B was studied. Only 1 mg was needed to reach the maximum adsorption percentage. The MIPNP B retains 1 $\mu g\ mg^{-1}$ which was 2.5 times higher than that of NIPNP B (0.4 $\mu g\ mg^{-1}$).

Hereafter, assays were performed with NPs B (MIP or NIP) and a 1-propanol: water proportion of 50:50 (v/v) as the binding solvent, 10 min interaction time and 1 mg of NPs. According to these results, the measured N_2 adsorption isotherms for MIPNP B and NIPNP B pointout to certain mesoporous development, which might enhance the specific CoQ10-polymer interaction. For polymers A, D and E, the surface areas would correspond to the external particle and interparticle surface areas; this fact could be related to the lower specific interaction of CoQ10-polymer observed for these samples.

3.4. Evaluation of the polymer cavity specificity

A binding assay under the optimized conditions was performed using CoQ10 and UC as analytes. Although the same molecular weight, a similar isoprene tail and a similar lipophilicity are shared by both compounds, UC presents a benzopyrane group instead of the quinone group in the molecule of CoQ10. This means that the interaction between the cavities complementary to the quinone ring in the MIPNP B and the UC head group will be weaker than the interaction between CoQ10 and MIPNP B, although the non-specific interactions due to the isoprene tail will be similar in UC and CoQ10.

The IPB of CoQ10 was higher than for UC (1.24 vs. −0.048), which is in agreement with the presence of selective cavities complementary to the quinone nucleus of CoQ10, and the interaction MIPNP–UC or NIPNP–UC is almost the same due to non-specific interactions. The IPB for NPs B compared to the previously IPB reported for polymer F [3] (obtained by bulk polymerisation), shows that the specific interaction of MIP B is remarkably higher (see Supporting information, Table S1).

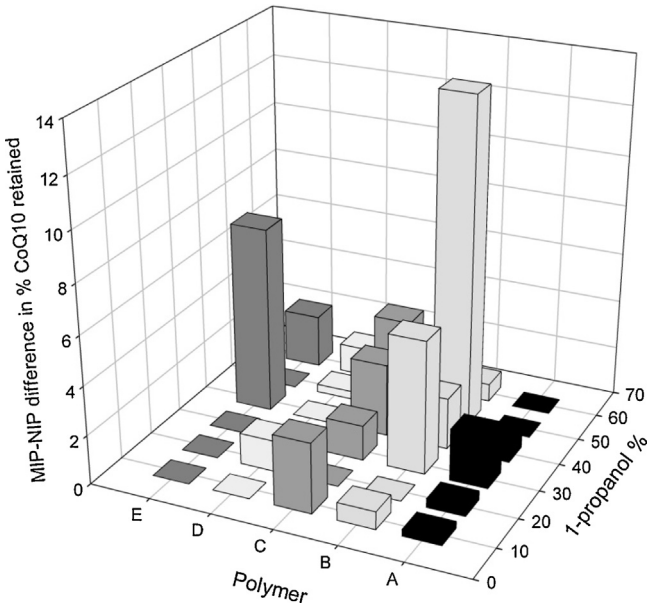

Fig. 6. Influence of the 1-propanol percentage in the CoQ10 retention. A 3 $\mu g\ mL^{-1}$ CoQ10 standard solution and 1 mg of polymer were employed in all cases.

3.5. Evaluation of the dispersive micro-SPE (DMSPE) procedure in a liver sample

The chromatographic analysis of CoQ10 by HPLC in liver samples is usually accompanied by an unclear baseline and some interference which is not observed in other matrices like muscle, plasma, cells or platelets [21]. Therefore; it was decided to evaluate the MIPNP B performance in DMSPE and analysis of CoQ10 from a liver extract.

Fig. 7 shows the comparison of CoQ10 determination in an unspiked liver extract before and after the DMSPE. A notorious cleaner baseline and a reduction in the peak areas of the endogenous contaminants were achieved after the micro extraction. As it can be seen, the size of CoQ10 peak was increased after DMSPE. However, the other peaks decreased indicating that the MIPNP B retains principally CoQ10 instead of other endogenous compounds.

The relative CoQ10 peak area (CoQ10 area/total peak area) increased from 4.6% in the liver extract up to 25.4% after DMSPE procedure.

The percentage of CoQ10 recovered from the liver matrix represents 83.7 and 70.5% (from 15 and 46 $\mu g\ g^{-1}$ of tissue, respectively) (RSD < 1.26) quantified against CoQ10 standard processed under the same conditions.

The DMSPE procedure allows the elution of almost all the CoQ10 retained (99.4%) in a small volume (200 μL), allowing the sample to be concentrated.

The LOD and LOQ were 1.1 $\mu g\ g^{-1}$ and 3.7 $\mu g\ g^{-1}$ of tissue for MIPNP B compared to LOD: 3.0 $\mu g\ g^{-1}$ and LOQ: 10.1 $\mu g\ g^{-1}$ without DMSPE.

A comparison between the previously reported method [3] and the present shows that although both methods employed a dispersive molecularly imprinted solid phase extraction to extract CoQ10, in the first case a polymer obtained by bulk polymerization (polymer F) was employed while in the second the extraction was performed using MIPNPs with a defined diameter. Both meth-

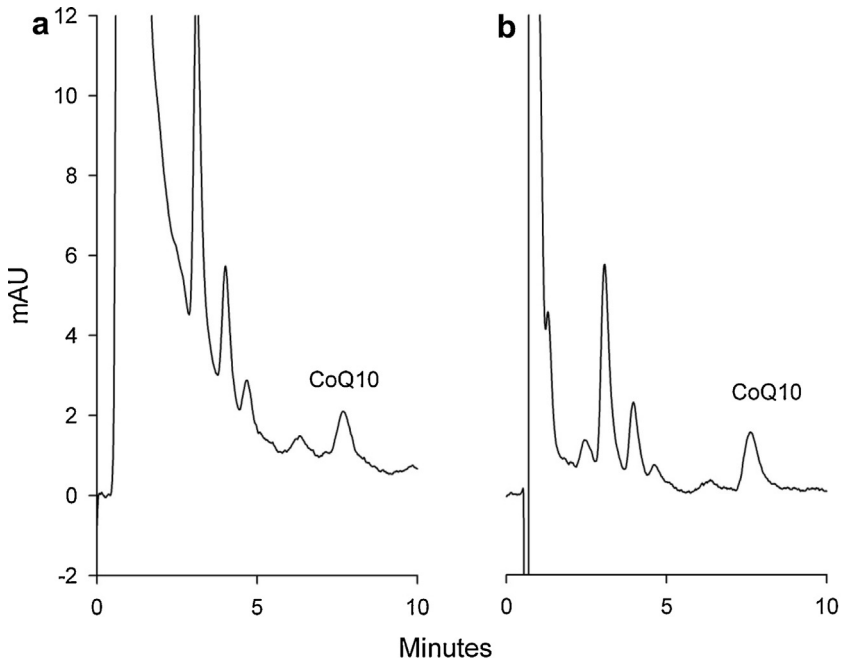

Fig. 7. CoQ10 determination in aunspiked real liver sample. (a) Liver extract, (b) extraction using DMSPE (39.4 μg g^{-1} of tissue). Separation was performed using an Xterra microcolum (50 mm × 2.1 mm i.d., 3.5 μm particle size). Methanol 100% as mobile phase, the flow rate was set at 0.4 mL min^{-1}, the injection volume of 10 μL, and detection at 275 nm.

ods were successful in the CoQ10 selective extraction, the clean-up of the sample and in the method efficiency improvement respect to traditional extraction methods, the decrease of LOD and LOQ and the reduced matrix interferences. However, with polymer F it was necessary to use 2.5 times the volume of loading for total desorption of CoQ10, diluting the sample. Nevertheless, using MIPNPs the sample can be concentrated at least 2.5 times. This fact results in a 5-fold increase in the concentration ability between polymers.

On the other hand, for a successful CoQ10 extraction at least 30 mg of the polymer obtained by bulk polymerization were employed. However, using MIPNPs, the extraction can be performed with 1 mg of the polymer, which greatly reduces sample processing costs.

Since the template is CoQ0, structure sheared by all isoforms of CoQ, it is very likely that all of them interact with the cavity of the synthesized polymeric nanoparticle. However, for each analyte the loading conditions should be optimized to ensure the maximum retention. Although this manuscript has focused in the analysis of CoQ10 in bovine liver samples, the extraction method could potentially be valid for other CoQ isoforms such as CoQ9 in rodents, CoQ6 in yeast or CoQ8 in bacteria.

4. Conclusion

In this work, the first development of molecularly imprinted polymer nanoparticles for the selective extraction of CoQ10, is shown.

Furthermore, these MIPNPs were applied in a DMSPE procedure before CoQ10 analysis by HPLC from a liver sample. The resulted clean up of the sample and the improvement in the chromatographic parameters, demonstrated that the MIPNPs could potentially be satisfactorily applied as sorbent in a miniaturized dispersive extraction employing a minimum amount of sorbent (1 mg).

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors thank University of Buenos Aires (UBACyT 20020110100030) and CONICET (PIP 11220110100375) for financial support and Dr. Cecilia Dobrecky, Dr. Jorge Muse and Dr. Nancy Garcia, for their assistance. M.C. received a post-doctoral scholarship from the CONICET.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2016.05.091.

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