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The use of coenzyme Q0 as a template in the development of a molecularly imprinted polymer for the selective recognition of coenzyme Q10

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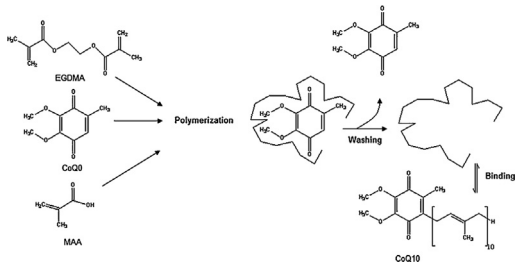
HIGHLIGHTS

- The first development of a coenzyme Q0 imprinted polymer used as a specific sorbent in CoQ10 analysis of biological matrices.
- The successful use of an analogue of the target analyte as template to avoid the interference due to template bleeding.
- The easy, low cost and high reproducibility in the polymer preparation.
- The better clean-up of the sample with respect to traditional methodologies.
- The application of the procedure to a real sample.

GRAPHICAL ABSTRACT

Scheme of the synthesis of CoQ10-MIP obtained by polymerisation of CoQ0, MAA and EGDMA.



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ABSTRACT

In this work, a novel molecularly imprinted polymer (MIP) for use as a solid phase extraction sorbent was developed for the determination of coenzyme Q10 (CoQ10) in liver extract. CoQ10 is an essential cofactor in mitochondrial oxidative phosphorylation and a powerful antioxidant agent found in low concentrations in biological samples. This fact and its high hydrophobicity make the analysis of CoQ10 technically challenging. Accordingly, a MIP was synthesised using coenzyme Q0 as the template, methacrylic acid as the functional monomer, acetonitrile as the porogen, ethylene glycol dimethacrylate as the crosslinker and benzoyl peroxide as the initiator. Various parameters affecting the polymer preparation and extraction efficiency were evaluated. Morphological characterisation of the MIP and its proper comparison with C18 as a sorbent in solid phase extraction were performed. The optimal conditions for the molecularly imprinted solid phase extraction (MISPE) consisted of 400 μL of sample mixed with 30 mg of MIP and 600 μL of water to reach the optimum solution loading. The loading was followed by a washing

Abbreviations: MIP, molecularly imprinted polymers; CoQ10, coenzyme Q10; MISPE, molecularly imprinted solid phase extraction; SPE, solid phase extraction; ATP, adenosine-triphosphate; HPLC, high performance liquid chromatography; ECD, electrochemical detection; MS, mass spectrometry; CoQ0, coenzyme Q0; UC, ubicromenol; MAA, methacrylic acid; EGDMA, ethylene glycoldimethacrylate; FEG-SEM, field emission gun scanning electron microscopy; NIP, non-imprinted polymer; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation; SEM, scanning electron microscopy; BET, Brunauer–Emmett–Teller; IPB, imprinting-induced promotion of binding; N, theoretical plates.

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step consisting of 1 mL of a 1-propanol solution (1-propanol:water, 30:70,v/v) and elution with 1 mL of 1-propanol. After clean-up, the CoQ10 in the samples was analysed by high performance liquid chromatography. The extraction recoveries were higher than 73.7% with good precision (3.6–8.3%). The limits of detection and quantification were 2.4 and 7.5 $\mu g\,g^{-1}$, respectively, and a linear range between 7.5 and 150 $\mu g\,g^{-1}$ of tissue was achieved. The new MISPE procedure provided a successful clean-up for the determination of CoQ10 in a complex matrix.

1. Introduction

The selective, precise and accurate determination of organic compounds at very low concentrations in complex matrices requires a special focus on the sample preparation as a critical step before the analysis. Solid phase extraction (SPE) using C8 and C18 silica sorbents has been extensively carried out for sample clean-up and preconcentration and has the advantages of simplicity, high reproducibility and high recovery. However, it often lacks the ability to extract target compounds selectively because these sorbents primarily retain the analytes by hydrophobic interactions. Moreover, as the detection of diluted samples needs to be achieved by applying large sample volumes, the interfering substances, which are also retained and coextracted, can impair the selectivity and analytical sensitivity [1]. Therefore, methods based on molecular recognition, such as the use of antibodies for high affinity and selective extraction, have been employed as alternatives [2]. Although immunological techniques are attractive because of their simplicity, speed and high sensitivity, the generation of antibodies presents many disadvantages such as time-consumption, expensive costs and high grade lot-to-lot variation [3,4]. This fact has prompted the development of synthetic antibodies, namely molecularly imprinted polymers (MIPs).

Molecular imprinting, which is included in the area of biomimetics, is the process where a molecule, the molecular template, can induce the formation of specific recognition sites within a synthetic polymer [5].

To date, MIPs have been used as sensors for chromatography, immunoassays, controlled drug delivery and catalysis; however, their principal application is in solid phase extraction. Molecularly imprinted solid phase extraction (MISPE) allows not only the analytes to be preconcentrated but also allows the matrix components to be eliminated.

The 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. The MIP possesses a molecular "memory", and thus, it is able to specifically recognise and bind the target molecule.

Typically, the target analyte and template are the same molecule. However, this can lead to template bleeding, where traces of the template can remain in the polymer even after exhaustive washing [6]. The leaking of this residual template from the polymer might cause erroneous results.

The best way to avoid template bleeding is to use an analogue of the target analyte as the template, which is called a pseudo-template molecule. Therefore, if the template bleeds, it will not interfere in the quantification of the target analyte as long as the template and target analyte can be discriminated between by the analytical method. At least some portion of this pseudo-template molecule has to be similar to the target analyte in terms of shape, size and functionality. Other good reasons to use a pseudo-template molecule are a decrease in the synthetic cost and the use of a more readily available molecule [7–9].

Coenzyme Q10 (CoQ10) is an essential cofactor in mitochondrial oxidative phosphorylation and is necessary for adenosine-triphosphate (ATP) production. CoQ10 is known as a powerful antioxidant agent and is able to protect circulating lipoproteins and cell membranes against oxidative damage [10,11].

Recent reports have suggested that endogenous CoQ10 levels may be lower in individuals with certain conditions such as cancer, Parkinson's, Alzheimer's, cardiovascular, mitochondrial, neurological and muscular diseases [12,13]. For these reasons, the determination of the CoQ10 level in biological samples and the study of the correlation of its levels with states of deficiency are very important for the diagnosis and therapeutic treatment of certain diseases [10,14].

However, the extremely low concentrations of CoQ10 in biological samples (0.4–2.0 $\mu g\,mL^{-1}$ in the plasma and on the order of $\mu g\,g^{-1}$ in various tissues), which can be even lower in individuals with pathological conditions, the complexity of these matrices and the two molecular properties necessary for the function of CoQ10 (its high hydrophobicity and its ability to be easily oxidised) make the analysis of CoQ10 technically challenging [13,15]. Many procedures have been reported to quantitate CoQ10 in biological matrices: high performance liquid chromatography (HPLC) with electrochemical detection (ECD) (limit of detection (LOD) = 1–10 $ng\,mL^{-1}$), mass spectrometry (MS) (LOD = 1 $ng\,mL^{-1}$), chemiluminescence (LOD = 26 $ng\,mL^{-1}$),and fluorimetric detection (LOD = 9–30 $ng\,mL^{-1}$). Although these methods have low LOD values, they are expensive, excessively time consuming, may require several steps during the operation of the equipment and need qualified operators, which makes them more difficult to implement for routine analyses. Although HPLC with UV detection is simple and frequently employed in clinical laboratories, it is less sensitive (LOD = 50 $ng\,mL^{-1}$) than the other methods. However, the HPLC-UV LOD decreases to approximately 15 $ng\,mL^{-1}$ when columns with reduced diameters are used [12,16].

CoQ10 determination is usually carried out by HPLC after liquid extraction from plasma or tissues, but solid phase extraction is also used [17,18]. In a previous work, we developed a simple and rapid miniaturised HPLC-UV system for the analysis of CoQ10, which was suitable for analysing samples of human plasma, platelets, and muscle [12,19]. However, in liver samples, a unclear baseline and some interferences were observed. In this sense, a MISPE might be applied to clean up the liver extract to obtain a cleaner baseline and to increase the selectivity for CoQ10. Another advantage, when a MISPE is used prior to HPLC-ECD, is the elimination of lipophilic components that could passivate the electrodes and considerably shorten their lifetime [20].

The aim of this study was to develop a non-covalent molecularly imprinted polymer using coenzyme Q0 (CoQ0) as the template to be used in a MISPE procedure prior to the analysis of CoQ10 in a real sample.

To our knowledge, this is the first work aimed to develop a CoQ0 imprinted polymer to be applied as a specific sorbent in the analysis of CoQ10 in biological matrices.

2. Materials and methods

2.1. Reagents and apparatus

CoQ0 and CoQ10 were purchased from Sigma (St. Louis, MO, USA). Ubicromenol (UC) was synthesised in our laboratory by the basic catalysis of CoQ10 with triethylamine using a method described elsewhere [21].

Methanol and 1-propanol were of HPLC grade while acetonitrile and acetic acid were of analytical grade, and they all were supplied by Sintorgan (Argentina). Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA) and benzoyl peroxide were purchased from Aldrich (Germany). Ultrapure water was obtained from an EASYpure™ RF equipment (Barnstead, Dudubuque, IA, USA).

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

The polymers were characterised by field emission gun scanning electron microscopy (FEG-SEM, Zeuss Supra 40 apparatus with a Gemini column, Germany) operated at an acceleration voltage of 3.0 kV.

All of the samples were coated with gold.

N_2 adsorption–desorption isotherms at −196 °C for MIP and non-imprinted polymer (NIP) were determined with an automatic Micromeritics ASAP-2020 HV volumetric sorption analyser. Prior to gas adsorption measurements, the samples were degassed at 60 °C overnight. The Brunauer–Emmett–Teller (BET) surface area was determined by the standard BET procedure.

Additional instrumentation including an Agilent 8452 diode array spectrophotometer (Santa Clara, California), an ultrasonic bath (Transsonic Digitals, ELMA, Kolpingstr) and a shaker (Minitherm-Shaker, Adolf Kuhner AG Schweiz) were used.

2.2. Experimental

2.2.1. Chromatographic analysis

The determinations of CoQ0, CoQ10 and UC were carried out using a miniaturised HPLC-UV method previously developed [12,19]. Briefly, the separation was performed using an Xterra microcolumn (Waters, Milford, Massachusetts, 50 mm × 2.1 mm i.d., 3.5 μm particle size).

For the determination of CoQ0, the isocratic mobile phase consisted of a mixture of methanol:water (30:70 v/v). For the analysis of CoQ10 and UC, the mobile phase consisted of 100% methanol. In both cases, the flow rate was set at 0.4 mL min^{-1} with an injection volume of 10 μL, and the UV-detection was performed at 275 nm.

2.2.2. Polymer preparation

Different imprinted polymers were prepared by bulk polymerisation according to the non-covalent approach by dissolving the template and functional monomer (MAA) in acetonitrile. The prepolymerisation mixture was incubated for an hour, and following this, the cross-linking monomer (EGDMA) and the initiator, benzoyl peroxide, were added. Then, the mixture was purged with nitrogen for 2 min, and afterwards, the vials were immediately closed. The polymerisation was induced by heat in a glycerin bath at 60 °C for 24 h. In all cases, benzoyl peroxide represented 1% of the total weight of monomer used, the ratio of the total monomers:porogen was 20:80 (w w^{-1}), and the template:cross-linker molar ratio was 1:25.

Removal of the template was carried out with methanol:acetic acid (9:1, v/v) by shaking the mixture in a vortex, and then, the supernatant was separated by centrifugation at 5000 rpm for 15 min. This operation was repeated 8 times until the template was not detected by HPLC-UV in the supernatant.

As a control, a NIP was simultaneously synthesised under the same conditions in the absence of the template molecule, and it was washed as described above.

2.2.3. Binding procedure

One millilitre of CoQ10 or UC standard was added to an appropriate amount of sorbent (MIP or NIP) 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 at room temperature for 10 min. After that, the supernatant (unbound analyte) was separated by centrifugation at 5000 rpm for 15 min and analysed by HPLC-UV. The adsorption quantity was calculated by subtracting the unbound analyte concentration from the initial concentration.

2.2.4. Analysis of the liver samples

Liver samples were stored at −80 °C until they were used. Pieces of bovine liver (200 mg) were accurately weighed and subsequently homogenised in a mortar on an ice bath with 3 mL of cold 1-propanol. The mixture was vortexed for 1 min, and then, it was sonicated and centrifuged at 5000 rpm for 15 min. The supernatant (liver extract) was loaded onto an optimised amount of polymer (30 mg) and placed in a 2.0 mL polypropylene tube as described in Section 2.2.3. The mixture was vortexed at 1600 rpm for 2 min, mechanically shaken at 120 rpm at room temperature for 10 min and centrifuged at 5000 rpm for 15 min. After removing the supernatant, the polymer was washed, eluted and injected into the HPLC-UV system. The optimised procedure is described in Section 3.8.

Using this procedure, the same amount of polymer was replaced by a commercially available C18 sorbent (Enviro Clean®, 40–63 μm particle size), which was placed into a 2.0 mL polypropylene tube. This traditional sorbent was used for comparing the extractions.

The recovery assay was carried out by spiking the liver extract with CoQ10 at three different levels (7.5, 30 and 60 μg g^{-1}) in triplicate. The LOD and limit of quantitation (LOQ) were determined at signal-to-noise ratios of 3:1 and 10:1, respectively. The test for linearity was performed using six calibration points.

3. Results and discussion

3.1. Template:functional monomer ratio

Five molar ratios between the template and functional monomer (1:1, 1:2, 1:4, 1:8, and 1:16) were tested by UV spectrometry. This approach was used to limit the number of polymers that needed to be synthesised to reduce the time consumption and cost [7,22].

To study the effect of increasing MAA concentrations on the spectrum of the CoQ0-MAA complex, the UV spectra of CoQ0 (80 μmol L^{-1}) were determined in the presence of various concentration of MAA (0, 80, 160, 320, 640 and 1280 μmol L^{-1}) while using the corresponding pure MAA solutions as blanks (Fig. 1).

To evaluate the best ratio between the template and the functional monomer, the difference of the absorbance between CoQ0-MAA and MAA was determined in the valley (Fig. 2). Fig. 2 shows a leap at a template:functional monomer ratio of 1:4 and an increase of absorbance at a 1:8 ratio, which was maintained until a ratio of 1:16. On the basis of these results, the 1:8 ratio was chosen to synthesise the final polymer for the subsequent assays, although the 1:4 ratio was also used for comparison.

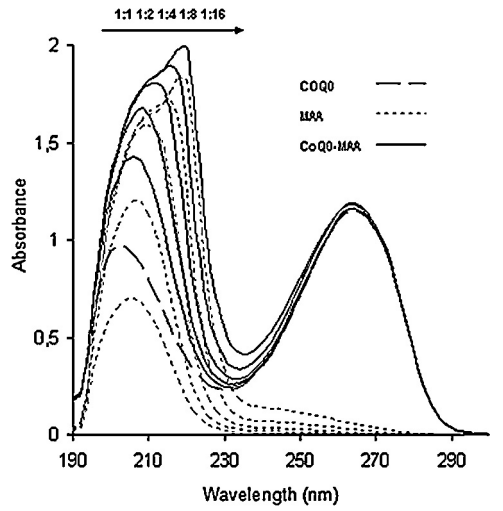

Fig. 1. The UV spectra of CoQ0 80 μmol L^{-1} (– –); MAA corresponding to 80, 160, 320, 640, and 1280 μmol L^{-1} (- - - -) and the CoQ0-MAA complex (—) corresponding to 1:1, 1:2, 1:4, 1:8, and 1:16 molar ratios.

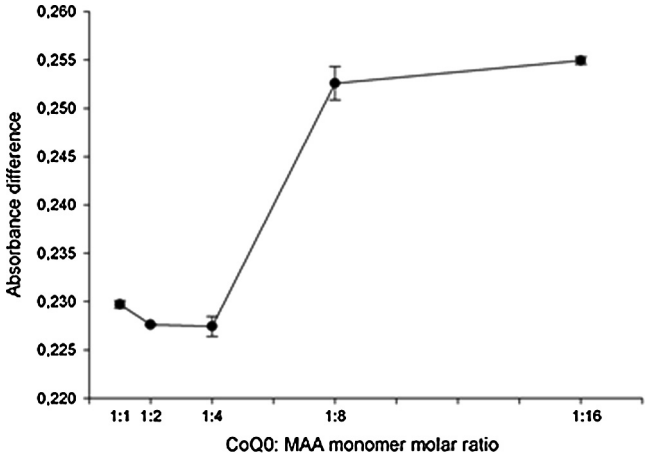

Fig. 2. Absorbance differences between CoQ0-MAA and MAA in the valley of the UV spectrum at different CoQ0:MAA molar ratios.

3.2. Evaluation of the selected template

Fig. 3 shows the structural difference between CoQ0 and CoQ10. CoQ0 shares the same quinone group with CoQ10 but not the reactive isoprene tail, which makes it ideal to be used as the template.

Two different polymers were synthesised using CoQ0 or CoQ10 as the template. It was observed that the percentage of template removed from the polymer after polymerisation when CoQ0 was used instead of CoQ10 was significantly higher (96.4 vs. 19.8, respectively).

In this sense, CoQ10, was shown to not to be a suitable template molecule. Another advantage of using CoQ0 as the template is that

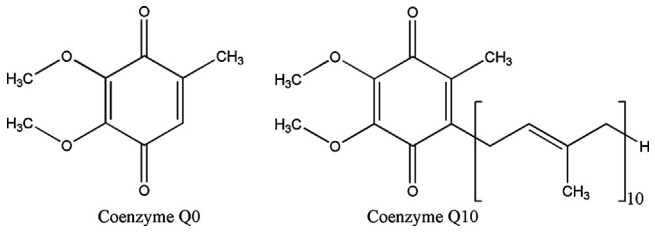

Fig. 3. Molecular structure of CoQ0 and CoQ10.

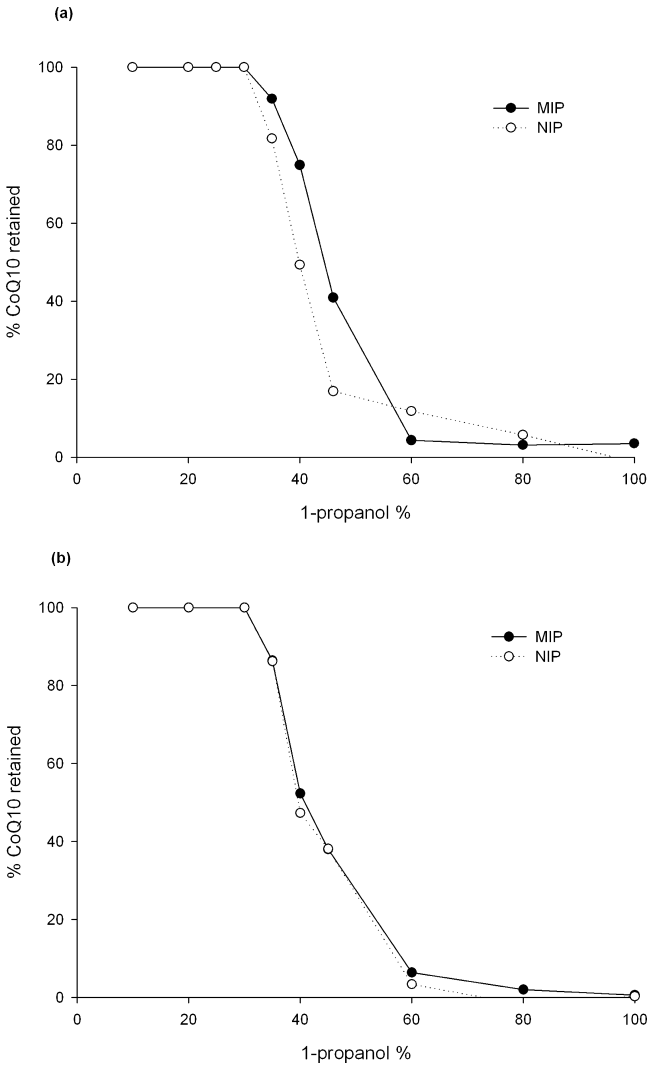

Fig. 4. Percentage of CoQ10 retained to MIP and its corresponding NIP using different 1-propanol percentages. (a) MIP polymer (1:8 template:monomer molar ratio). (b) MIP polymer (1:4 template:monomer molar ratio).

interference due to template bleeding during the chromatographic quantification of CoQ10 can be avoided.

3.3. Solvent effect in the CoQ10 binding efficiency assays

MIPs are not intrinsically selective. The selectivity is in the combination of a polymerisation process that produces selective cavities from the template along with the use of solvents with abilities to develop specific interactions between the analyte and the cavity formed both during the polymerisation and extraction processes [7]. In general, when the sample is dissolved in the solvent used for the MIP preparation, the greatest selectivity is obtained [7]. For this reason, acetonitrile was the first solvent chosen for use in the binding experiments. However, the binding of CoQ10 between MIP and NIP was found to be independent of the percentage of acetonitrile used, and no binding difference was observed.

1-Propanol, the most commonly used solvent in the extraction of CoQ10 from biological matrices [13,14], was finally selected as the organic solvent to be used in the binding assay.

Fig. 4 shows the percentage of CoQ10 retained using different proportions of 1-propanol and water. In this assay, 20 mg of MIP or NIP was added to 1 mL of a standard CoQ10 solution (10 μg mL^{-1}).

The mixture was vortexed for 2 min, and then, it was mechanically shaken for 1.5 h.

It was observed that while there was no difference between the MIP and NIP polymers at the 1:4 template:monomer rmolar ratio, there was a variation in the retained percentage of CoQ10 between the MIP and NIP polymers at the 1:8 template:monomer molar ratio within the range of 35:65–45:55 1-propanol:water proportion. When a high proportion of water was used, nonspecific physicochemical retention due to hydrophobic interactions was observed. When a high proportion of organic solvent was used, CoQ10 was not retained due to its elevated solubility in 1-propanol.

As the recognition of a target molecule on a polymer could be influenced by pH, the impact of this parameter (from 2.98 to 11.24) on the MIP and NIP binding assay was evaluated. While maintaining the optimised solvent ratio (1-propanol = 40%), the pH was varied without significant variations in the recoveries (relative standard deviation (RSD) = 3.0).

Hereafter, assays were performed with the optimised imprinted polymer prepared with CoQ0 as the template, with a CoQ0:MAA:EGDMA ratio of 1:8:25 and a 1-propanol:water proportion of 40:60 (v/v) as the selected binding solvent proportion.

3.4. Characterisation of the morphology

The morphology of the optimised polymer was investigated using BET N_2 adsorption–desorption analysis along with scanning electron microscopy (SEM). Comparing the imprinted and the non-imprinted polymer, it was observed that MIP showed an almost three times higher specific surface area than NIP (24.3730 vs. 8.5738 $m^2\ g^{-1}$, respectively), potentially indicating a significant influence of the template on the polymer structure. Polymer particles seemed to be composed of small and interconnected granules. The size of the granules in the MIP was determined to be in the range of 228–280 nm with an average size of 249 nm (Fig. 5a); the range in the NIP was from 354 to 514 nm with an average size of 414 nm (Fig. 5b).

3.5. Optimisation of the shaking time and amount of polymer

The kinetic adsorption profile of CoQ10 was investigated (Fig. 6a). For this assay, 20 mg of MIP or NIP was added to 1 mL of a CoQ10 standard (10 $\mu g\ mL^{-1}$). The mixture was vortexed for 2 min, and then, it was mechanically shaken for time periods of 10–360 min. The adsorption process was completed in a short time, and only 10 min was necessary to perform the procedure. Moreover, there was no improvement in the adsorption percentage beyond 10 min.

The amount of polymer needed to obtain the maximum absorption was evaluated by using different amounts of the polymer from 5 to 40 mg. The procedure was performed with an incubation time of 10 min (Fig. 6b). In this study, 30 mg were needed to reach the maximum adsorption percentage. Increasing the amount of polymer above 30 mg did not yield an improvement in the MIP binding.

In the next set of assays, 10 min of shaking and 30 mg of MIP or NIP were used for the study. Using these conditions, a high reproducibility of the CoQ10 retention percentage between polymer batches was obtained (RSD = 0.78, $n = 3$).

3.6. Evaluation of the polymer cavity specificity

To verify the existence of selective cavities complementary to the quinone nucleus of CoQ10, a binding assay was performed using CoQ10 and UC as analytes. UC is the principal CoQ10 related substance. Both compounds have similar lipophilicities (Xlog P: 19.4 vs 19.6 for Q10 and UC, respectively) [23] and the same molecular weight (863.34 $g\ mol^{-1}$). Although UC is not expected to be found in a biological matrix, it has a different head group but a similar tail compared to CoQ10 (Fig. 7), which makes UC suitable to be used in this assay. It is thought that non-specific UC interactions are similar to those of CoQ10 because of the isoprene tail, but its benzopyran structure suggests that the interactions with the cavities might be different and less noticeable compared to the interactions of CoQ10 with its quinone nucleus.

Recognition properties due to the molecular imprinting effect can be expressed in terms of the "imprinting-induced promotion of binding" (IPB), a parameter that allows the study of the efficiency of the imprinting effect more correctly than just studying the amount of the analyte that was bound by MIP (Amip). IPB is defined as:

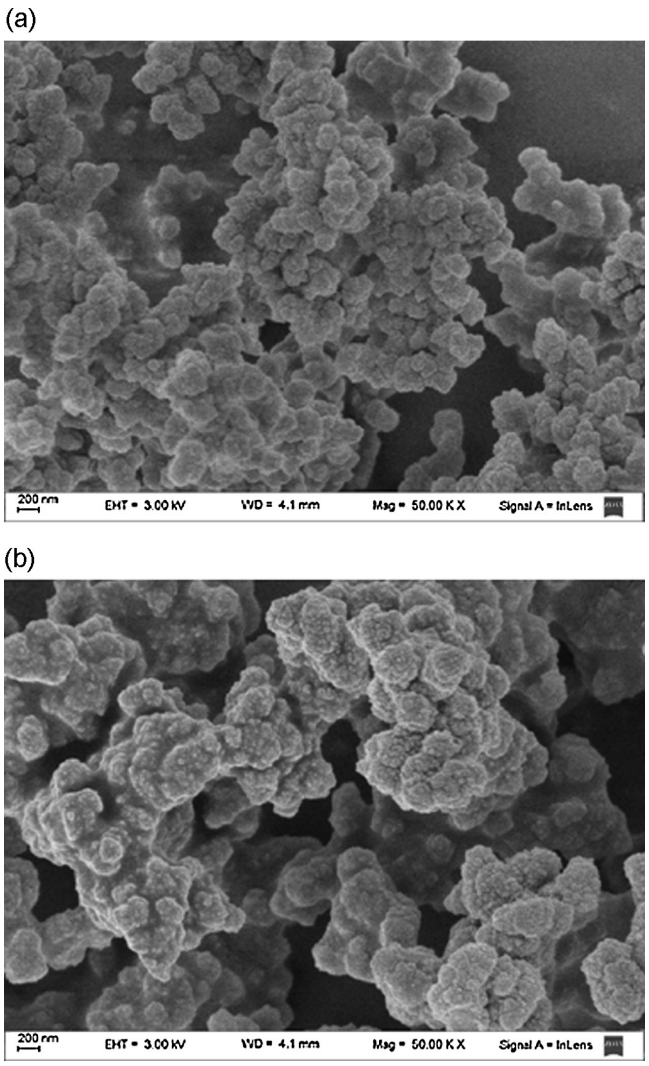

Fig. 5. Scanning electron microscopy (SEM) images of (a) MIP polymer (1:8:25 CoQ0:MAA:EGDMA molar ratio). (b) NIP polymer (0:8:25CoQ0:MAA:EGDMA molar ratio).

Table 1
Imprinting-induced promotion of binding (IPB) of CoQ10 and UC.

	CoQ10	UC
IPB	0.42	0.076

Results of three repeated adsorption experiments.

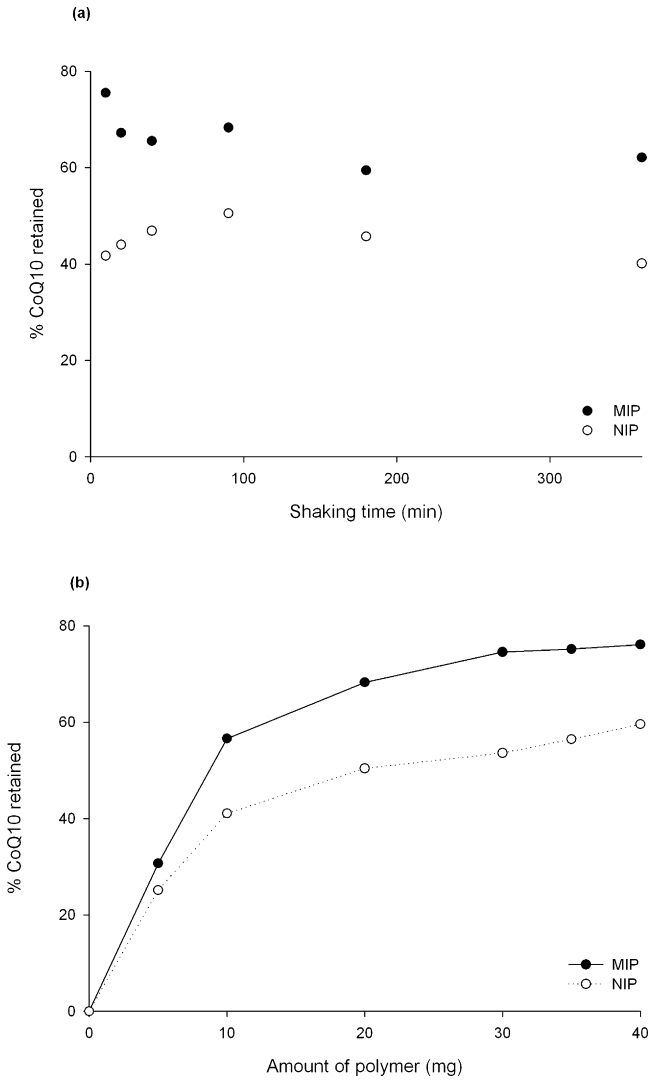

Fig. 6. (a) Percentage of CoQ10 retained by MIP or NIP using different shaking times. (b) Percentage of CoQ10 retained by MIP or NIP using different amounts of polymer.

IPB = (Amip − Anip)/Anip, where Anip is the analyte that was bound by NIP [24].

It is shown that CoQ10 showed a higher IPB than UC (Table 1). This could indicate the presence of selective cavities complementary to the quinone nucleus of CoQ10.

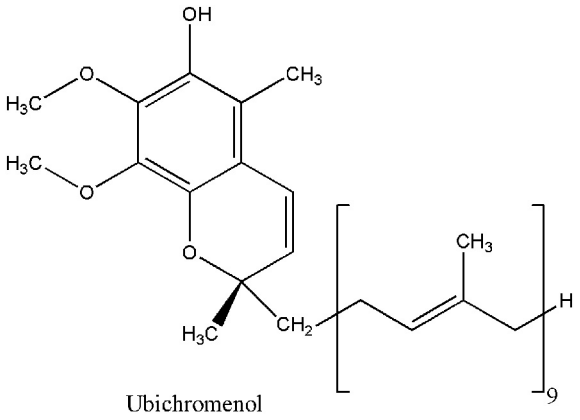

Fig. 7. Molecular structure of ubichromenol (UC).

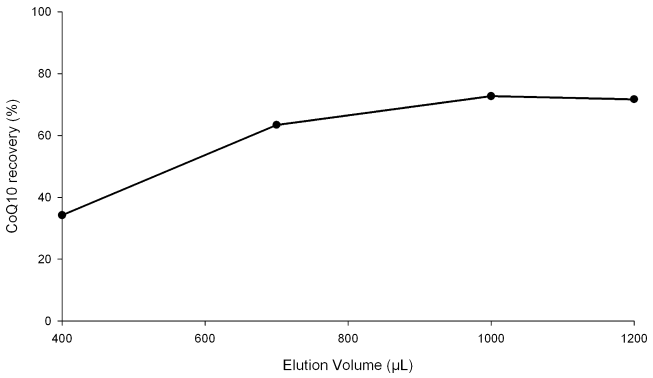

Fig. 8. CoQ10 recovery using different elution volumes.

3.7. Binding capacities at low concentrations of CoQ10

Some authors [25,26] have reported rather different binding capacities for MIP and NIP when the initial concentration of the analyte is high. As the aim of this work was to obtain a suitable polymer that would be able to be used in the clean-up of biological samples with low concentrations of CoQ10, it was important to examine whether the different retention capacities between MIP and NIP remained at low CoQ10 concentrations.

Binding capacity experiments were assayed by loading different CoQ10 concentrations ranging from 0.5 to 10 μg mL^{-1}, and differences in the retention percentages between MIP and NIP were obtained in the range of 14–26.

These results allowed us to conclude that, even at low CoQ10 concentrations, a different retention between MIP and NIP was still observed and indicates that the clean-up of biological samples could be achieved using the developed MIP.

3.8. MISPE procedure in a real sample

On the basis of the previous assays, the final MISPE procedure to determine the CoQ10 in liver samples was performed using 400 μL of 1-propanol liver extract mixed with 30 mg of MIP and 600 μL of water to reach the optimum solution loading. After that, it was washed with 1 mL of 1-propanol:water 30:70 (v/v) and showed a percentage of the total absorption (Fig. 4a). Therefore, in this step, the CoQ10 was not expected to be removed. The elution step was achieved using 400 μL of 1-propanol. Under these conditions, the original liver extract was neither concentrated nor diluted, and it could be compared to a direct injection of the extract into the HPLC system.

A cleaner baseline and a higher number of theoretical plates (N) corresponding to the endogenous CoQ10 peak were obtained when using the MISPE procedure instead of a direct injection of the liver extract (2656 vs. 1789). However, despite improvements in the chromatographic procedure, a low recovery was obtained. To improve the recovery, higher amounts of 1-propanol were used in the elution step. Fig. 8 shows that by using higher amounts of 1-propanol in the elution step, a remarkable increase in the recovery was achieved, and the maximum possible recovery was reached when 1 mL was used.

Table 2
Imprinting-induced promotion of binding (IPB) of CoQ10 and endogenous contaminants (X, Y, and Z) in real samples.

	CoQ10	X	Y	Z
IPB	0.44	0.12	0.13	0.19

Results of three repeated adsorption experiments.

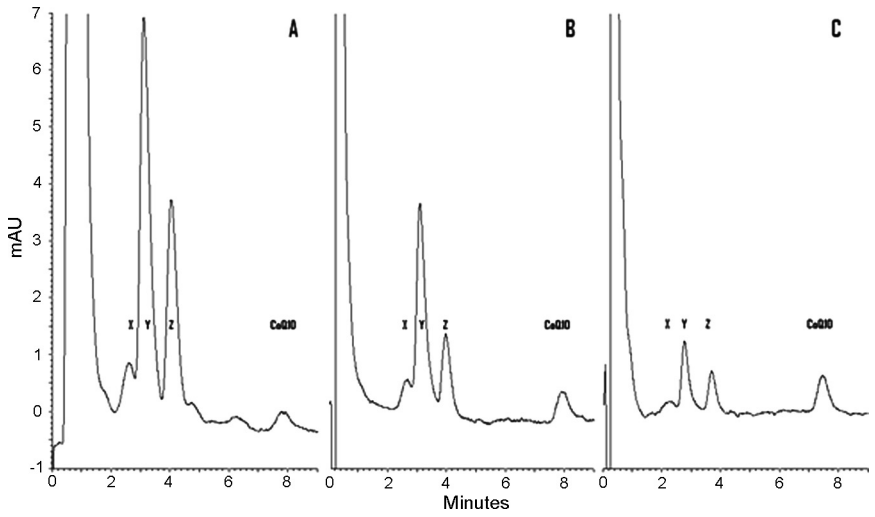

Fig. 9. CoQ10 determination in a unspiked real liver sample. (A) Liver extract, (B) extraction using C-18, (C) MISPE.CoQ10 sample concentration: 15.5 μg g^{-1} of tissue.

Table 3
CoQ10 relative peak areas and its purification factors using different sample preparations.

	Liver extract	C-18	MISPE
Relative CoQ10 peak area ± SD[a]	0.027 ± 0.005	0.113 ± 0.001	0.347 ± 0.003
Purification factor[b]	–	4.1	12.8

[a] CoQ10 area/total peak area (as CoQ10 plus *X*, *Y* and *Z*) with the standard deviation values (SD).
[b] Defined as the relative CoQ10 peak area from C-18 or MISPE/relative CoQ10 peak area from the liver extract.

Table 4
Recovery assay using the optimised MISPE procedure.

n	Spiked concentration (μg g^{-1})	Recovery (%)	RSD
3	7.5	80.8	8.3
3	30.0	73.7	3.6
3	60.0	74.2	4.3

A selectivity comparison between MIP and NIP was also performed using a real sample. Table 2 shows the IPB values of CoQ10 and unknown endogenous contaminants (*X*, *Y*, and *Z*) and shows the high selectivity for CoQ10.

The advantage of MISPE compared to a C18 sorbent was also shown (Fig. 9). Although, when C18 was used the endogenous contaminants (*X*, *Y* and *Z*) were reduced with respect to the liver extract, this reduction was more noticeable using MISPE. In addition, the peak shape of CoQ10 using MIP was also improved.

Fig. 9 CoQ10 determination in a unspiked real liver sample. (A) liver extract, (B) extraction using C-18, (C) MISPE.CoQ10 sample concentration: 15.5 μg g^{-1} of tissue.

Table 3 shows that the relative CoQ10 peak area and its purification factor were increased using the MISPE procedure with respect to the use of C18 extraction due to a decrease in the peak areas of the contaminants.

Table 4 exhibits the results of the recovery assays using the optimised MISPE procedure and shows good precision for biological samples.

Results from HPLC analyses showed that the calibration curve of CoQ10 in liver extract was linear over the range of 7.5–150 μg g^{-1} and r^2 = 0.9910. The LOD and LOQ were 2.4 and 7.5 μg g^{-1}, respectively. The normal levels of CoQ10 in bovine and human livers reported in literature are in the range of 37–50 μg g^{-1} of tissue [27–29], so this MISPE procedure could be used to evaluate up to a 10 times decrease of CoQ10.

4. Conclusion

A molecularly imprinted polymer (MIP) was developed using coenzyme Q0 as the template, methacrylic acid (MAA) as the monomer and ethylene glycol dimethacrylate (EDMA) as the crosslinker. The use of an analogue of the target analyte as the template has the unique advantage of avoiding interference due to template bleeding. In this work, morphological differences between MIP and NIP were shown using SEM and BET analyses, and the higher specific retention rate in the MIP was evaluated by the IPB factors for CoQ10 with respect to UC and endogenous interferences. This study has demonstrated that a successful clean-up of a liver sample was achieved using the developed MISPE procedure with respect to traditional solid phase extraction.

Conflict of interest

The authors declare no conflict of interest.

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