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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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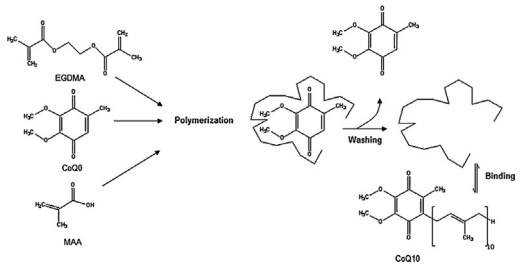
HIGHLIGHTS

- 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.

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