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Hybrid imprinted membranes for selective recognition of quercetin

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ABSTRACT

Hybrid imprinted membranes exhibiting specific recognition properties towards the quercetin (the template) were developed. In this perspective, an imprinted copolymer of acrylonitrile, using acrylamide as functional co-monomer was synthesized. For comparison, a non-imprinted polymer was also prepared. Various membranes were produced by dispersing different copolymer amounts in a poly(ether ether ketone) (PEEK-WC) matrix. Scanning Electron Microscopy, and Fourier Transform Infrared analysis confirmed the presence of the polymer into the hybrid system. Membrane permeability was in the ultrafiltration range (70–180 l/h m^2 bar). The addition of polymer to the support membrane determined a slight decrease of the membrane hydrophobicity. The solvent uptake degree was very low (between 1.2% and 1.32%), suggesting a weak interaction between the polymer and the binding solvent. No significant change in the mechanical properties were observed.

The membranes recognized the quercetin in different extend. The highest imprinting factor (3.6) and binding capacity (12.0 mol/$g_{memb.}$) was exhibited by the membranes containing the 25 wt.% of the imprinted polymer (PEEK-WC/25% MIP). This membrane was also selective *vs* chrysin and naringin, (structural homologs of quercetin). The selectivity factor quercetin/naringin was 4.5 and that one of the couple quercetin/chrysin was 1.8.

The Langmuir adsorption model was suitable for correlation of equilibrium data. The kinetic of quercetin adsorption of PEEK-WC 25% MIP membrane was efficiently predicted by the second-order model.

1. Introduction

In view of preventing the highly oxidizing environment in which we live, the antioxidant activity has attracted a great attention. Meanwhile, it presents an important property for life. In fact, it is the source of several biological functions such as anti-mutagenicity, anti-carcinogenicity, and anti-aging [1,2]. Owing to these efficacies, the interest in natural antioxidants significantly increased [1,3,4]. Natural antioxidants are chemical substances, which are able to inhibit the oxidation of other compounds. Their beneficial effect is reflected in their capacity to protect the human body against the harmful action of the free radicals, thus helping in the safeguard of the human health. In fact, they have the capacity to reduce the concentration of the free radicals by trapping and neutralize them, as they act as electron donors [5,6]. Among the powerful natural antioxidants, we find the group of flavonoids, which are a class of polyphenol compounds and secondary metabolites of plants [7]. They are the pigments responsible for the varied color of flowers and fruit. As well, they represent an important source of antioxidants in our diets [8–10]. They are also found in various food, beverages and complex matrices, like wine, soy milk, fruit pulps and herbs [8–10]. The high antioxidant property of flavonoids is due to the presence of the hydroxyl groups attached to the aromatic rings, along with the electronic delocalization through the entire structure [8]. Thanks to this feature, the flavonoids have important physiological activities such as cancer [11] and osteoporosis [12] prevention, anti-inflammatory [13] and cardio protective action [8].

An important member of the wide family of bioflavonoids is the Quercetin. It is a polyphenol aglycone, which particularly attracted the attention of the scientists owing to its strong antioxidant nature, widespread health benefits and its relative abundance in foods. Recent studies demonstrated that in addition to the traditional beneficial effects of flavonoids, quercetin also increases brain and muscle mitochondrial biogenesis [14] and stabilizes cell membranes [15]. Quercetin is present in many plants like grapes, tea and ginkgo leaves, onion, olives, and citrus [15–17]. It is of great interest for food, medical herb and pharmaceutical industry. In fact, it is added to the composition of many biologically active

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supplements and medical formulations. For this reason, is very important to develop reliable and efficient enrichments procedures for its extraction and purification from raw sources. Secondly, a pre-concentration step is necessary for determining quercetin in natural complex multicomponent like juices and wine. Up to date, different methods for extracting quercetin from natural matrices of for separating it from flavonoids mixtures and real extracts were developed. They include the employment of silica gels [15], ionic liquids-based monolithic cartridges [18], high performance liquid chromatography [19], capillary electrophoresis [20]. However, all these methods have some drawbacks in terms of selectivity and inefficiency. The employment of the molecular imprinting technique (MIT) can help to overcome these problems, owing its simplicity and accuracy, which permit the extraction/separation of quercetin on the base of its shape, size and chemical functionality. In this perspective, different scientists produced some molecularly imprinted polymers for the detection and separation of quercetin [11,16,21–25].

The principle of molecular imprinting consists in synthesizing polymeric materials in the presence of a compound of interest named "target molecule" or "template". The process allows obtaining a molecularly imprinted polymer (MIP) having specific molecular recognition sites towards the target analyte [26–28].

These materials are obtained by polymerization of a mixture of a cross-linker agent, the solvent, the template and a functional monomer, which is complementary to the template. The synthesis process starts by the pre-arrangement of the polymerizable monomers around the target molecule by chemical interactions. Then, the polymerization occurs in the presence of a cross-linking agent. The process ends by extracting the target molecule from the obtained polymer matrix. This last step leads to the creation of specific binding sites in the polymer, which are able of selectively recognizing the target molecule [28,29]. MIP technology is now widely applied in different areas [30,31]. As example, MIPs were used as sensors for detection of various compounds [32], in drug delivery [33], in solid-phase extraction of flavonoids [34], in chiral separations [35], for the detection of environmental contaminants [36], etc.

The imprinting technology was exploited for developing molecularly imprinted membranes (MIMs), permitting them to distinguish between a target molecule and its similar compounds, owing to its better selectivity with respect to a traditional membrane. MIMs can be used in aqueous [28] and organic environment [37]. Furthermore, in comparison with MIPs, MIMs can work in a continuous operation mode, combining at the same time the features of membrane technology and imprinting technique [28].

A commonly used method for the preparation of a MIM is the phase inversion technique, which allows obtaining a thin and flexible membrane from a viscous solution of a membrane-forming polymer. This process consists in a polymer transition from a liquid state to a solid phase, which represents the final membrane [29]. Interestingly, this technique was employed for producing hybrid imprinted membranes (HIMs) *via* the so-called "hybrid molecular imprinting" approach, which is based on the dispersion of the MIPs into a typically used polymer matrix and the successive formation of the membrane *via* phase inversion [38].

Up to date, imprinted adsorbent materials for application in different fields were developed [38–41]. HIMs were also produced as adsorbers [40,42,43], for food processing [39] enantio-separation [41] and for application in biomedical field [44]. The application of other adsorbent materials for the removal of safranin [45] and other dyes [46] and toxic compounds from effluents was also reported [47]. Furthermore, composite systems for the removal of lead (II) [48], Chromium [49]. Carbon nanotubes and composite systems were also produced for waste-water recycling [50] and for application in Nanoscience [51].

In this work, the hybrid molecular imprinting was used to firstly prepare novel hybrid membranes made of poly(ether ether ketone (PEEK-WC) with a polymer imprinted with the flavonoid quercetin.

The prepared membranes were tested for investigating their specific recognition properties and selectivity towards the template with respect to its structural homologs. For comparison, non-imprinted membranes were also prepared and tested.

2. Materials and methods

2.1. Materials

Acrylamide (AA), Acrylonitrile (AN), quercetin (QCT), naringin (NR), chrysin (CRY), potassium persulfate, ethylene glycol dimethacrylate (EGDMA), iron (II) sulfate, sodium metabisulfite, N-methyl-2-pyrrolidone (NMP), dimethylacetamide (DMA), dimethyl sulfoxide (DMSO) acetic acid, acetonitrile (ACN), and methanol were purchased from Sigma (Italy) and used as received. PEEK-WC was supplied by Chanchung Institute of Applied Chemistry, Academia Sinica.

2.2. HPLC analysis

Quercetin, naringin and chrysin concentration was determined by a LaChrom HPLC system (Hitachi) equipped with a UV detector. Analysis was carried out using the column Luna C18, 5 μm, 250 × 4.6 mm. The mobile phase was acetonitrile/orthophosphoric acid 0.1%, pH 3.5 (50/50, v/v). The operating conditions were: flow rate of 1.1 mL/min; temperature of 25 °C, pressure of 106 bars and wavelength of 254 nm.

2.3. Polymer synthesis and characterization

Imprinted polymer (MIP) was obtained from the water phase precipitation polymerization method, according to our previous paper but with slight modifications [38]. Briefly, 0.07 mol of acrylamide and 0.47 mol of acrylonitrile were mixed, and then 3.3×10^{-3} mol of QCT and 0.3 mol of EGDMA were added to the mixture. The amount of total monomer was 90 g. The solvent was 500 mL of water/dimethyl sulfoxide (4:1) mixture. The solvent/total monomer weight ratio was 5:1. The synthesis was carried out at 50 °C for 4 h, under nitrogen atmosphere and stirring conditions. The couple $K_2S_2O_8$/$NaHSO_3$ was used as initiator in the presence of Fe^{2+} (1 ppm of the total monomer). $K_2S_2O_8$ and $NaHSO_3$ were 1.55×10^{-3} mol and 10^{-2} mol, respectively. High monomer/template and cross-linker/monomer ratio were used for obtaining polymers with good mechanical stability and recognition performance. This choice was made considering that in the non-covalent imprinting (the case of this work), the interactions template-functional monomer are governed by an equilibrium process. Therefore, an excess of the functional monomer is required for moving the equilibrium *vs* the formation of the template-monomer complexes [26,52]. In addition, high cross-linker/monomer ratio (about 80%) are generally necessary for obtaining polymers with sufficient rigidity and stabilise the recognition sites [28,53,54].

However, an excessive amount of the cross-linker will result in an extreme polymer rigidity, which adversely affects the interactions between the polymeric matrix and the template [55,56].

At the end of the reaction, the synthesized polymer was rinsed several times with de-ionized water to remove the residual catalyst and un-reacted monomers. Then, by washing the polymer with dimethylacetamide (DMA), followed by a mixture of methanol/acetic acid (9:1, v/v), methanol, water and acetone, the template was extracted. The procedure was carried out until no QCT was