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Short communication

Determination of dicoumarol in *Melilotus officinalis* L. by using molecularly imprinted polymer solid-phase extraction coupled with high performance liquid chromatography☆

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ABSTRACT

In this work, a method for the extraction of dicoumarol from plant sample utilizing selective adsorbent based on molecularly imprinted polymer was developed. Molecularly imprinted polymer (MIP) was synthesized by bulk polymerization, and on the surface of magnetic particles using dicoumarol as a template, methacrylic acid as functional monomer, chloroform as a porogen, and ethyleneglycol dimethacrylate as a cross-linker. Prepared polymeric materials were evaluated on the base of capacity, selectivity, and morphology. The maximal specific adsoption capacities of the sorbents were 45 μg (for bulk MIP) and 36 μg (for MIP on the magnetic particles) of dicoumarol per 1 g of polymer. An efficiencies of sorption processes of dicoumarol on prepared sorbents were evaluated under various conditions (type of sample solvent, pH, types of washing and elution solvents). The MIP based sorbents were used for solid phase extraction of dicoumarol. Applicability of MIP-SPE coupled to HPLC-DAD was tested for the selective extraction of dicoumarol from sample of sweet clover. The method was linear over concentration range from 1 to 100 μg mL^{-1} (the correlation coefficient 0.9984) with limit of detection 0.2 μg mL^{-1}. Accuracy of the method was assessed for spiked sample at three concentration levels and recovery values were higher than 84% with relative standard deviation lower than 3.2%.

1. Introduction

Plants are a source of many biologically active compounds, including coumarins [1]. Dicoumarol (3,3'-methylenebis(4-hydroxycoumarin); Fig. 1), is an coumarin-like compound produced from coumarin upon spoilage by fungi in sweet clover (*Melilotus albus*) [2]. It is used as an oral anticoagulant and its presence in forage at high concentration is undesirable for grazing animals. Dicoumarol acts by inhibiting the hepatic synthesis of vitamin K-dependent coagulation factors (prothrombin and other). It is also used in biochemical experiments as an inhibitor of reductases [3].

Several analytical techniques are available for determination of dicoumarol, such as colorimetry, fluorimetry, and liquid chromatography with UV or MS detection, as is documented in Table 1.

Plant macerates are mixtures of many compounds and this type of samples require application of selective sample pretratment techniques for cleaning and/or preconcentration of analytes before HPLC analysis. Molecular imprinting technique allows preparation of polymeric recognition materials (molecularly imprinted polymers, MIPs), which are able to specifically rebind a target analyte in preference to other related compounds. MIP based sorbents provide higher selectivity than traditional sorbents (e.g. octadecyl silica or polymeric reversed-phase materials) and the great advantage of MIP is also a good mechanical and chemical stability, especially when compared to immunosorbents [11]. MIP materials have been used as adsorbents for extraction techniques, mainly for solid phase extraction (SPE) including solid phase microextraction (SPME), SPME with MIP-coated fibers, and magnetic solid phase extraction [12], mainly for increase of selectivity of extraction. SPE can be offline or online combine with separation technique [13,14]. Most common methods of preparation of MIP-SPE sorbents are the bulk or precipitation polymerizations, and molecule imprinting on the surface of particles [14]. They allow to obtain polymers of characteristic sorption and morphology properties. Several studies evaluated selectivity and extraction efficiency of

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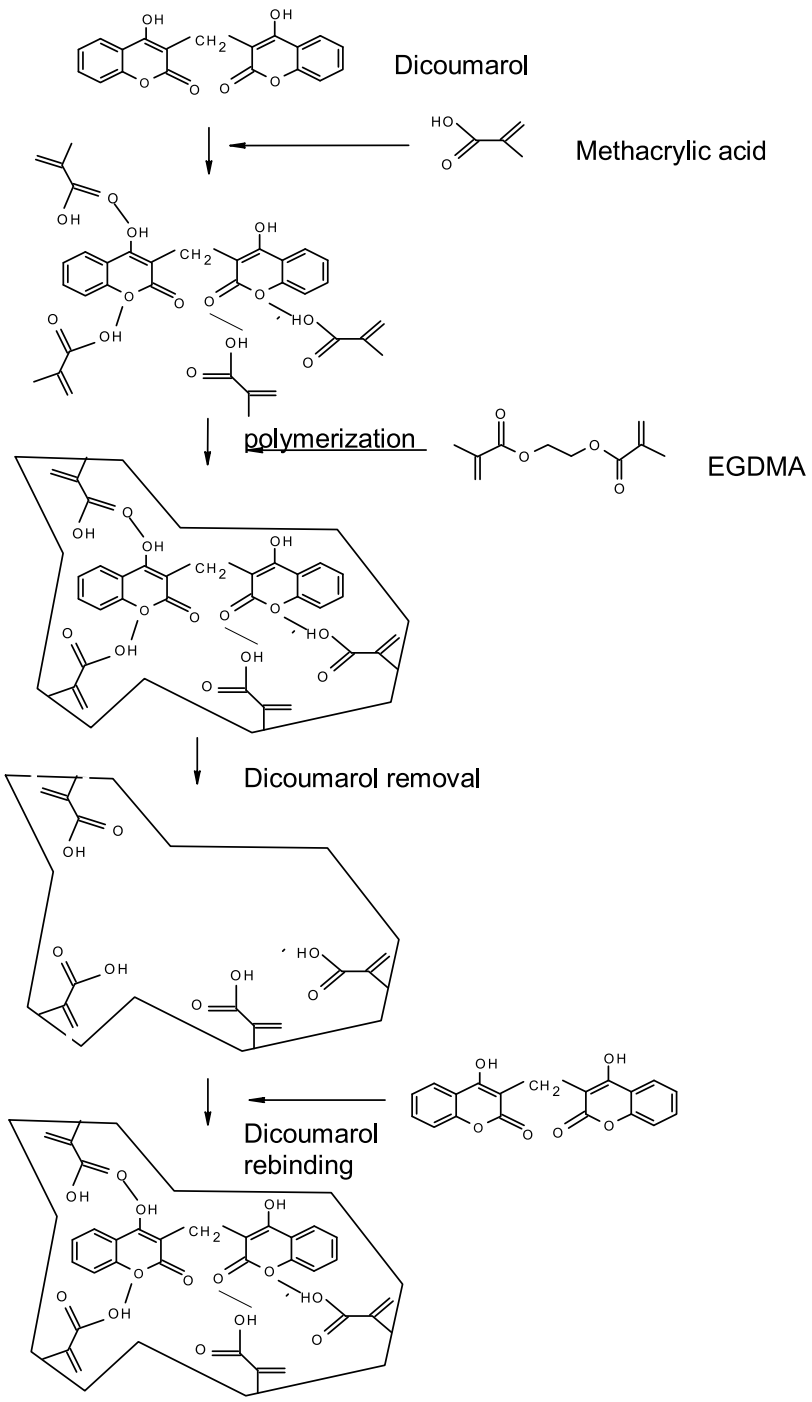

Fig. 1. Scheme of proposed interactions between template (dicoumarol) and monomer (methacrylic acid) in MIP preparation and rebinding process.

sorbents based on MIP-coumarins depending on polymerization or extraction conditions. References [15,16] refer about preparation of MIPs for coumarins by using of traditional polymerization strategy (bulk polymerization). Obtained MIP particles often displayed non-spherical shapes. Therefore, MIP coated on the surface of solid carrier (magnetite, silica, alumina) have been also employed [17,18]. These types of MIPs, especially those coated on magnetic particles, combine high selectivity and capacity with easy isolation of sorbent from liquid phase, which also lead to reduction of sorbent amount and solvent consumption [17].

The objective of this work was to develop fast and sensitive analytical method for dicoumarol determination. Lab-made MIP based sorbent prepared by bulk polymerization and on the surface of magnetic particles was used for solid phase extraction. The extraction process and HPLC separation were optimized and applied for plant macerate analysis.

2. Materials and methods

2.1. Chemicals and sample

The standards of dicoumarol (98 %), 7-hydroxycoumarin (99 %), 4-hydroxycoumarin (98 %), coumarin (99%), and warfarin (98 %) were purchased from Sigma-Aldrich (St. Louis, USA). Solvents, chloroform, ethanol, methanol, acetonitrile (HPLC grade) and reagents, acetic acid (99 %), acetone, 2,2'-azobisisobutyronitrile

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