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Poly(*N*-vinylimidazole/ethylene glycol dimethacrylate) for the purification and isolation of phenolic acids

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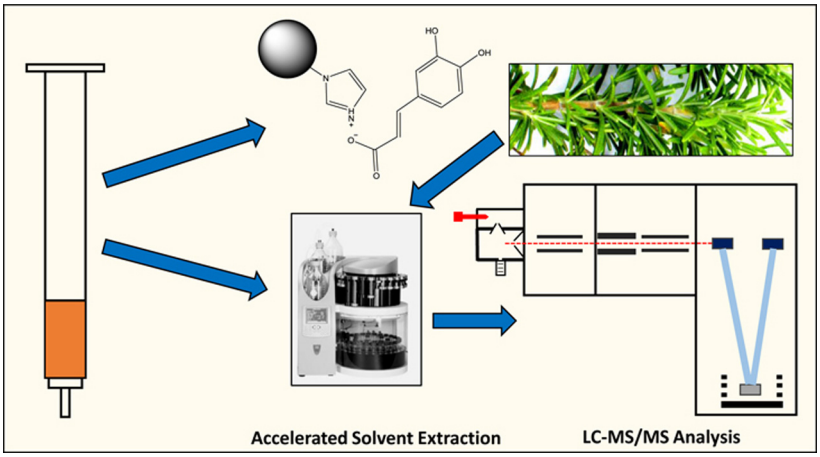
HIGHLIGHTS

- Free-radical polymerization of protonable vinylimidazole with EGMDA.
- Polymer-optimization by maximum loading capacity of phenolic acids.
- Performs better than SiO_2 and Al_2O_3 in normal phase mode using acetonitrile.
- Performs equal or even better in anion-exchange mode compared to Oasis-MAX.
- Efficient purification of phenolic compounds from crude extract.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study we report the novel polymeric resin poly(*N*-vinyl imidazole/ethylene glycol dimethacrylate) for the purification and isolation of phenolic acids. The monomer to crosslinker ratio and the porogen composition were optimized for isolating phenolic acids diluted in acetonitrile at normal phase chromatography conditions, first. Acetonitrile serves as polar, aprotic solvent, dissolving phenolic acids but not interrupting interactions with the stationary phase due to the approved Hansen solubility parameters. The optimized resin demonstrated high loading capacities and adsorption abilities particularly for phenolic acids in both, acetonitrile and aqueous solutions. The adsorption behavior of aqueous standards can be attributed to ion exchange effects due to electrostatic interactions between protonated imidazole residues and deprotonated phenolic acids. Furthermore, adsorption experiments and subsequent curve fittings provide information of maximum loading capacities of single standards according to the Langmuir adsorption model. Recovery studies of the optimized polymer in the normal-phase and ion-exchange mode illustrate the powerful isolation properties for phenolic acids and are

Abbreviations: SPE, solid phase extraction; SEM, scanning electron microscopy; AIBN, α,α-azoisobutyronitrile; ACN, acetonitrile; EGDMA, ethylen glycol dimethacrylate; NVI, *N*-vinyl imidazole; ASE, accelerated solvent extraction; DHHCA, 3,4-dihydroxyhydrocinnaminic acid; 2,3-DHB, 2,3-dihydroxy benzoic acid.

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comparable or even better than typical, commercially available solid phase extraction materials. In order to prove the applicability, a highly complex extract of rosemary leaves was purified by poly(*N*-vinyl imidazole/ethylene glycol dimethacrylate) and the isolated compounds were identified using UHPLC–qTOF-MS.

1. Introduction

Phenolic compounds are well known phytochemicals with increasing interest for dietary supplements or phytopharmaceuticals relating to their potent antioxidative properties [1,2]. The so-called "phenolics" comprise about 8000 naturally occurring compounds and include at least one phenol as the common structural characteristic [3]. Phenolic acids are a subclass of secondary plant metabolites containing two different structural frameworks, cinnamic acid and benzoic acid which in turn derive from chorismate, the final product of the shikimate pathway [4–6].

Phenolic compounds exist in nearly all parts of plants including leaves, stems, seeds or roots and serve there as protectors against pathogenic attacks of fungal, bacteria or high energy radiation such as permanent UV exposure [7–11]. Their antioxidative activities arise from the hydroxyl moiety of the phenol acting as a radical scavenger i.e., via hydrogen donation. Their stabilization by various substituents influence the impact of the scavenging ability [12,13]. According to the positive antioxidative effect on plants, the impact of phenolic compounds on the maintenance of health and disease prevention is respectively high. Hydroxyl radicals and superoxides as byproducts of cellular metabolism can react with surrounding water to form hydrogen peroxide which in turn damages DNA and other critical cell components and structures [14–16]. Apart from endogenous peroxidases, phenolics can serve as radical scavengers and quenchers in order to decrease the cellular degradation of the organism [1,17–19]. The literature provides a huge variety of information that correlates phenolics in fruits, vegetables and nutritional supplements with a positive effect on health protection and disease prevention.

Phenolic acids are a part of the large group of phenolics and represent a carboxylic functionality in the phenolic constitutive structure. Their isolation is highly desirable as they show positive effects on health maintenance and disease prevention similar to other phenolic compounds. Currently phenolic acids are isolated and characterized using different chromatographic methods including UHPLC and HPLC separations hyphenated to mass spectrometry, liquid–liquid extraction or solid-phase extraction [20–23]. However, the characterization of plant metabolites can be a complex procedure due to the presence of various compounds (sugars, chlorophyll, waxes, oils, etc.) which are able to damage or clog the analytical columns and influence the determination of target molecules [24]. Therefore, solid phase extraction (SPE) has been established as a pre-purification method which can further be combined with chromatographic separation [25–27]. Typical SPE sorbents for the isolation of phenolic acids include reversed-phase materials, ion-exchangers or mixed-mode materials whereby their retention mechanism is commonly based on π–π interactions, electrostatic ion–ion interactions, dipol–dipol interactions or hydrogen bonding. Michalkiewicz et al. [28] reported an extraction procedure for the determination of phenolic acids and flavonols in honey using commonly applied sorbents such as Bond Elut octadecyl C_{18}, Oasis HLB, Strata-X and Amberlite XAD-2. Cleanert-PEP SPE cartridges were used for the extraction of phenolic acids in root exudates of allelopathic rice showing reasonable and acceptable recoveries [29]. Apart from commonly available sorbents, molecularly imprinted anion-exchange polymers have been successfully used for the purification of phenolic acids and their derivatives in complex samples [30–32].

The aim of this study was the development of a new material for the purification and isolation of phenolic acids. Therefore, an imidazole-based resin was synthesized, optimized and applied in the normal-phase and ion-exchange mode. For characterization scanning electron microscope (SEM) images were recorded which show morphological differences in the porogen compositions. Moreover, single standards were isolated to study maximal loading capacities which were determined according to the Langmuir adsorption model. Apart from recovery studies, rosemary leaves were extracted by an accelerated solvent extractor and purified by the new polymeric resin.

2. Experimental

2.1. Chemicals

1-Vinylimidazole was purchased from Sigma–Aldrich (St. Louis, MO) and distilled under vacuum. α,α-Azoisobutyronitrile (AIBN) was purchased from Fluka (Buchs, Switzerland) and recrystallized from methanol before use. Ethylene glycol dimethacrylate (≤98%), activated aluminum oxide (58 Å), ferulic acid (≤98 %), tannic acid (ACS grade), gallic acid (97.5–102.5%), *trans*-cinnaminic acid (≤99%), 3,4-dihydroxyhydrocinnamic acid (98%), 2,3-dihydroxybenzoic acid (98%), 2,5-dihyroxybenzoic acid (≤98%), caffeic acid (98%), chlorogenic acid (≤95%), toluene (≤99,8%), cyclohexanol (99%), 1-propanol (≤99,9%), 1,4-butanediol (≤99%) and ethanol for ASE (≤99,8%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Rosmarinic acid (≤99%) was from Extrasynthese (Genay Cedex, France), PBS-buffer from Phynexus (San Jose, CA, USA), iso-octane (99.8%) from J.T.Baker, dodecanol (98%) and silica gel (60 Å) were bought from Fluka, acetic acid (100%) from Riedel-de-Haen (Seelze, Germany) ethanol (≤96%) from VWR (Darmstadt, Germany) and acetonitrile, methanol were purchased from Carl Roth (Karlsruhe, Germany). Water was used from a Milli-Q water purification system and *Rosmarini folium* (rosemary leaves) were used from Kottas Pharma GmbH (Vienna, Austria).

2.2. Instrumentation

For recovery experiments a Transgenomic HPLC System was used, equipped with an L-7100 pump module, L-7200 autosampler, L-7300 oven and an L-7400 UV-detector. A Hypersil OBS (3 μm, 4.6 × 250 mm, Knauer (Germany)) was applied as analytical column and the mobile phase was a composition of 0.1% trifluoroacetic acid (TFA) in eluent A and acetonitrile (ACN) in eluent B. A fast gradient system was set from 10 to 99% B within 10 min at a flowrate of 400 μL min^{-1}.

LC–MS/MS experiments were performed on a Thermo Fisher Ultimate 3000 (Dionex–Thermo Fisher, Germany), using a Zorbax Eclipse Plus C18 (1.8 μm; 2.1 × 100 mm, Agilent (Santa Clara, CA, USA)). Eluent A was 0.1% formic acid and eluent B was acetonitrile. A multistep gradient was set from 5 to 40% B in 2 min, 40 to 70% B in 3 min and 70 to 100% B in 10 min by applying a flowrate of 500 μL min^{-1}. Mass spectrometric detection was performed on a Maxis Impact (qTOF-MS, Bruker (Bremen, Germany)) in negative