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Preparation of ionic liquid-mediated imprinted monolith for selective capture and purification of corilagin

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ABSTRACT

A method for solid-phase extraction (SPE) of corilagin from natural plant extracts based on molecularly imprinted polymers (MIPs) was developed. For the preparation of corilagin-MIP monoliths, 4-vinylpyridine was used as functional monomer, and ethylene glycol dimethacrylate was used as cross-linking monomer, using a mixture of 1-butyl-3-methylimidazoliumtetrafluoroborate (ionic liquid)-N,N-dimethylformamide-dimethyl sulfoxide as a porogen. A morphological characteristic of the corilagin imprinted monolith was further studied by scanning electron microscopy and nitrogen sorption method. The greatest imprinting factor of COR was up to 9. The MIPs were used as solid-phase extraction (SPE) sorbents for purification of COR and the mean recoveries of corilagin was 78.0% with COR purity of 98.0% from the crude extract of phyllanthus urinaria L. The resulting COR-imprinted polymer also displayed the good performance of fragment imprinting polymer for gallic acid with the mean recoveries of 94.0% and purity of 99.7%.

1. Introduction

Ellagitannins are polyphenols characterized by a complex chemical structure and bioavailability differs greatly from one polyphenol to another [1]. Because of the shortage of pure compounds, structure determination and measurement of various biological activities of ellagitannins have been limited to further study on the concept of tannins as active constituents of medicinal plants. However, to achieve highly purified ellagitannins from natural product is a primary challenge due to the great complexity of plants component. Corilagin is one of important ellagitannins which can be discovered in a variety of plants such as Terminalia chebula and Euphorbia longana [2]. Recent research showed corilagin had chemotherapeutic activity on some tumour cells and virus. In addition, corilagin possessed activities beneficial for cardiovascular diseases. Recently, some methods have been applied to the separation and purification of corilagin, such as high speed countercurrent chromatography [3], high pressure extraction [4], and microwave-assisted enzymatic extraction [5]. Nevertheless, effective isolation from interference is still a problem because of the low selectivity of these techniques.

Molecularly imprinted polymers (MIPs) are increasing materials of molecular recognition with predetermination selectivity [6–9]. These highly cross-linked polymers possess affinity complementary domains of the given molecule with good physicochemical stability. In recent years, attempts have been made to apply MIPs to solid phase extraction (SPE) for preconcentration and cleaning of samples [10–12]. Advantages of MIP-based solid phase extraction (MISPE) are relatively low cost, good mechanical properties and long life. To date, corilagin (COR) –imprinted polymers have been made using α-methacrylic acid and acrylamide as functional monomers but smaller imprinting effect was not enough to achieve desire result for SPE [13,14], since a polyphenol is likely to inhibit the polymerisation reaction. Thus, to achieve COR from natural product with high-purity is a primary challenge because of difficulty in effective imprinting of COR.

Ionic liquids (ILs) are molten salts with excellent solvation qualities that display ionic-covalent crystalline structures [15,16]. The use of ILs for porogen has been suggested to produce MIPs with highly specific selectivity [17], including the polymers contain-

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ing imprints with propranolol [18], naproxen [19], methyl gallate [20], ketoprofen [21], R-mandelic acid [22], and carprofen [23]. It is believed that when ILs as porogenic solvents in preparing MIPs, the discrete polar and non-polar microenvironments formed by ILs cause an enhanced interaction between the template and functional monomer and limit nonspecific binding [17]. In addition, ILs has been shown to be able to accelerate the polymerization and improve effect of imprinting [24,25], which might be due to low degree of polymer swelling.

In the present study, COR-imprinted monolith was prepared in ILs-based porogenic solvent using 4-vinylpyridine as functional monomer. In this investigation, 1-butyl-3-methylimidazoliumtetrafluoroborate ([BMIM]BF_4) was used as ILs porogen. The detailed properties of the resulting MIPs were examined by SEM, IR and N_2 adsorption–desorption. Separation of COR from natural plant extracts was performed to demonstrate the selectivity of the COR-imprinted material.

2. Experimental

2.1. Materials

Corilagin (COR, 98%), catechin (CA, 98%), epigallocatechin gallate (EGCG, 98%), methyl gallate (MG, 98%), procyanidin B2 (OPC-B2, 98%) and procyanidin B1 (OPC-B1, 98%) were obtained from Shifeng Biotechnology Co., Ltd. (Shanghai, China). Ethyleneglycol dimethacrylate (EDMA, 98%) and 4-vinylpyridine (4-VP, HPLC grade) were purchased from Sigma (St. Louis, MO, USA). *N′N*-Dimethylformamide (DMF, 99.6%) and 1-butyl-3-methylimidazoliumtetrafluoroborate ([BMIM]BF_4, AR) were from Jiecheng Chemical Co., Ltd (Shanghai, China). Azobisisobutyronitrile (AIBN, AR) were purchased from Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). Dimethyl sulfoxide (DMSO, HPLC grade) was from Tianjin Jiangtian Pharmachem Technology Co., Ltd. (Tianjin, China). Other regents were HPLC grade.

2.2. Instrumentation

Imprinted monolith was evaluated by HPLC system consisting of a quaternary gradient LPG-3400SD pump, a VWD-3100 detector, WPS-3000SL auto sampler, online degasser and reagent rack and four bottles (Thermofisher, USA) was used. Every mobile phase and sample was leached through membrane (0.22 μm) before use. The dead time was determined by injecting 20 μL acetone (0.1%, v/v) in the corresponding mobile phase. Imprinting factor (IF) was calculated by the following equation [19,23]:

$$\mathrm{IF} = k_{\mathrm{MIP}}/k_{\mathrm{NIP}} \quad (1)$$

k_{MIP} and k_{NIP} were retention factor of the imprinted monoliths and bank monoliths, respectively.

2.3. Preparation of corilagin imprinted monolith

The template molecule (COR, 56.78 mg), functional monomer (4-VP, 96 μL) and cross-linker (EDMA, 679.5 μL) were dissolved in ternary porogen ([BMIM]BF_4/DMF/DMSO, 1324 μL/30 μL/600 μL), and the initiator AIBN (10 mg) was also added. Then the pre-polymerization mixture was sonicated for 10 min to remove the air and poured into stainless steel column (100 mm × 4.6 mm). The monolith was then sealed and reacted in 60 °C water bath for 18 h. The unreacted reagents were rinsed with acetonitrile after polymerization and the template molecules were removed by methanol/acid (9:1, v/v) thoroughly. The blank column was prepared in similar way but without the template molecule.

2.4. Frontal analysis

The binding characters of the imprinted and non-imprinted monolith were explored by frontal analysis method. The column was equilibrated in the mobile phase in absence of the template at 280 nm with a flow rate of 1.0 mL min^{-1}. Then a series of gradient concentrations of COR (0.01, 0.02, 0.04, 0.06, 0.08 and 0.1 mg mL^{-1}) in the above mobile phase were introduced into column successively. The breakthrough curves and the retention times were obtained with half-height method and the concentrations of COR adsorbed on the monolith were calculated according to the equation [26]:

$$Q_i = [(C_i - C_{i-1})(V_{r,i} - V_0)/V_{sp}] + Q_{i-1} \quad (2)$$

where C_{i-1} is the analyte concentrations in the sample solutions at the beginning of frontal development. $V_{r,i}$ derived from the half-height method is the retention volume of the *i* frontal development. V_0 and V_{sp} are the hold-up volume of the monolith and the solid volume of the monolith, respectively.

2.5. Separation and purification of COR from extract of phyllanthus urinaria l

The resulting imprinted monolith was taken out from the stainless steel column and ground in a grinder. Then the mashy particles were sieved by 4.5 μm sieve and packed into a blank home-made SPE column (20 mm × 9 mm) after placing a cloud of cotton into the end of the column. The ratio of diameter to height of the filling was set at 1:4.

The aqueous crude extract (20.22 mg) of phyllanthus urinaria L. was dissolved in 1 mL of water and 0.7 mL of the solution sample was added into the packed MIP SPE column which was activated by 10 mL water. Then, all steps were taken in sequence according to Table S1. The content of COR in the elution and crude extract was determined by HPLC. Separation and quantitation were made on Waters SunFire TM C18 (250 mm × 4.6 mm, 5 μm). All significant variables of SPE were investigated.

3. Results and discussion

3.1. Imprinting effect of COR-imprinted monolith

The affinity of the resultant COR MIP monolith was evaluated in a mobile phase of methanol/sodium acetate buffer (pH 4.4) (95:5, v/v). Greater difference of retention factor for COR between the MIP (k = 44.72) and NIP (k = 5.23) can be observed (Fig. 1). The analogs of COR were less retained on the MIP, but more retained than on the NIP (Fig. 2). A group of drugs similar to COR in structure was utilized to evaluate the selectivity of the COR MIP. Separation factors (α) of COR for its analogs on the MIP were all increased compared with the NIP (Table 1). As shown in Fig. 2, on the NIP, procyanidin B1 and catechin eluted at almost identical retention times. Methyl gallate hardly bound to the MIP in terms of shape and disposition of its functional groups. The selectivity factor (S), defined by the ratio of the retention factor of the analogs of COR (catechin, EGCG, methyl gallate, procyanidin B1, procyanidin B2) between the imprinted and non-imprinted monolith, was 0.78, 1.19, 1.57, 1.09, and 1.07, respectively. In addition, methacrylic acid was made also used to prepare MIP monolith in a similar mode. However, no imprinting effect was found on the methacrylic acid type columns.

The morphology of the MIP and NIP monoliths was observed by scanning electron microscopy. As shown in Fig. 3, two polymers are composed of microglobules interconnected with each other, which were agglomerated to larger clusters through neighboring microglobules. The results indicated that the existence of COR had little effect on the size of the microglobules. The micrograph