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Molecularly imprinted solid-phase extraction of glutathione from urine samples

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ABSTRACT

Molecularly imprinted polymer (MIP) particles for glutathione were synthesized through iniferter-controlled living radical precipitation polymerization (IRPP) under ultraviolet radiation at ambient temperature. Static adsorption, solid-phase extraction, and high-performance liquid chromatography were carried out to evaluate the adsorption properties and selective recognition characteristics of the polymers for glutathione and its structural analogs. The obtained IRPP-MIP particles exhibited a regularly spherical shape, rapid binding kinetics, high imprinting factor, and high selectivity compared with the MIP particles prepared using traditional free-radical precipitation polymerization. The selective separation and enrichment of glutathione from the mixture of glycyl-glycine and glutathione disulfide could be achieved on the IRPP-MIP cartridge. The recoveries of glutathione, glycyl-glycine, and glutathione disulfide were 95.6% ± 3.65%, 29.5% ± 1.26%, and 49.9% ± 1.71%, respectively. The detection limit (S/N = 3) of glutathione was 0.5 mg·L^{-1}. The relative standard deviations (RSDs) for 10 replicate detections of 50 mg·L^{-1} of glutathione were 5.76%, and the linear range of the calibration curve was 0.5 mg·L^{-1} to 200 mg·L^{-1} under optimized conditions. The proposed approach was successfully applied to determine glutathione in spiked human urine samples with recoveries of 90.24% to 96.20% and RSDs of 0.48% to 5.67%.

1. Introduction

Glutathione (GSH, 5-L-glutamyl-L-cysteinylglycine) is a tripeptide with three amino acid residues [1–3]. Several methods, including spectrofluorometry [4], capillary electrophoresis [5], and chromatography [1] have been presented to analyze GSH in biological matrices. Although such methods offer more sensitive approaches to GSH analysis, relatively expensive instruments, which are not yet available in all laboratories, are required. In addition, most of the reported determination methods have difficulty in direct separation and enrichment of GSH from complex matrices through sample pretreatment. Generally, GSH is present in complex samples at low concentrations; thus, developing a novel, simple, and fast determination method appropriate for GSH is necessary.

Molecularly imprinted polymers (MIPs) are synthetic polymers with specific cavities designed for template molecules [6,7]. MIP applications have attracted significant attention in solid-phase extraction (SPE) as sorbent for generating three-dimensional cross-linked polymeric materials with a "memory" for the shape and functional group positions of template molecules [8]. Molecularly imprinted SPE (MISPE) is a simple and feasible alternative to multi-step SPE method for the preconcentration of target molecules in complex samples, for example, extraction of pollutants from river water and various biomolecules from food or vegetables [9–12].

Dinçer et al. [13] synthesized monolith MIPs for GSH via traditional bulk polymerization by using 1-vinyl imidazole as functional monomer. The monolith GSH-imprinted polymers were used as SPE material for detection of GSH in biological samples. A similar method was used by Tong et al. [14]. However, the monolith MIPs prepared through traditional radical polymerization have disadvantages, including broad binding site heterogeneity, relatively low binding affinity, slow kinetics, and low selectivity [15]; such disadvantages are attributed to the uncontrollable chain propagation and termination of the monolith MIPs. Thus, the propagation and termination steps of the reaction should be controlled. The introduction of controlled living radical polymerization (CRP) into imprinted polymerization systems can considerably improve the homogeneous network structures of MIPs and elucidate their structure–property relationship.

CRP is a very effective tool for controlling cross-linked polymer structures. Thus far, many different polymer networks with homogeneous structures have been prepared using CRP. Iniferter is an ideal candidate for CRP because of its versatility and simplicity. Iniferter, an initiator-chain transfer molecule, was first discovered by Otzu et al. [16] in 1982. Since then, iniferter has been used to produce linear polymer chains with low polydispersities, specific block copolymers, graft polymers, and cross-linked polymer systems on surfaces [17–22]. Several successful molecular imprinting applications have been reported.

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For example, Shufang et al. [23] prepared sulfamethazine-imprinted core-shell particles by using surface-initiated iniferter-controlled living radical polymerization (IRP) on silica particles. The resultant core-shell MIPs showed high affinity toward the template. A similar method was used by Barahona et al. [24]. Recently, Zhang et al. [25] reported that IRP combined with precipitation polymerization can effectively improve the binding and structural characteristics of MIP particles. Nevertheless, these studies mainly focused on the formation of MIPs on surfaces by using iniferter. By contrast, few studies have been conducted on the direct preparation of functional MIP particles, particularly imprinting of biomolecules, using IRP in the presence of the target molecule.

In this study, iniferter-controlled living radical precipitation polymerization (IRPP) method was applied to obtain MIP particles. The apparent morphology, composition, kinetics, adsorption isotherm, and selectivity of the IRPP-MIP particles were characterized with GSH as the model system. The adsorption speed, adsorption capacity, and selectivity of the IRPP-MIP particles improved remarkably because of their uniform distribution and homogenous binding sites compared with the MIP particles prepared through traditional free-radical precipitation polymerization (TRPP). In addition, the obtained IRPP-MIP particles were applied as SPE sorbent material for the selective separation and enrichment of GSH from its structural analogs or human urine samples.

2. Material and methods

2.1. Reagents and chemicals

GSH, glutathione disulfide (GSSG), and glycyl-glycine (Gly-Gly) were purchased from Shanghai Jingchun Ke Fine Chemical Corporation (Shanghai, China). Azobisisobutyronitrile (AIBN) was purchased from Tianjin Guangfu Chemical Industry (Tianjin, China). Ethylene glycol dimethacrylate (EGDMA) and 4-vinyl pyridine (4-VP) were purchased from Alfa (Shanghai, China). Tetramethylammonium hydroxide, phenylisothiocyanate (PITC), high-performance liquid chromatography (HPLC)-grade acetonitrile, and triethylamine were provided by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All chemicals were of analytical grade and used without further purification. Benzyl *N*,*N*-diethyldithiocarbamate (BDC) was prepared following the literature procedure [26].

2.2. Synthesis of imprinted polymer particles

IRPP-MIP particles were synthesized according to the method of Zhang et al. [25]. In a typical synthesis, a mixture of GSH (51.2 mg, 0.167 mmol), 4-VP (0.11 mL, 1 mmol), EGDMA (0.75 mL, 4 mmol), and BDC (35.85 mg, 0.15 mmol) was dissolved in an appropriate porogenic solvent (acetonitrile/water = 9/1, v/v). The mixed solution was sequentially added into a four-neck round-bottom quartz flask (100 mL). After sealing, mixing, and sparging the mixture with nitrogen for 30 min, polymerization was initiated using ultraviolet radiation from a high-pressure mercury lamp (300 W, 365 nm) with stirring at ambient temperature for 12 h. After polymerization, the resulting polymer was extracted with a mixture of ethanol, water, and acetic acid (70/28/2, v/v) by using a Soxhlet apparatus for 48 h. The obtained polymer was washed thrice with ethanol and water and then dried in a vacuum at 40 °C for 36 h.

AIBN (17.20 mg, 0.104 mmol) was used as initiator instead of BDC for the MIP particles prepared using TRPP. All other conditions were similar to the preparation of IRPP-MIP. The corresponding non-imprinted polymer (NIP) particles were prepared and purified under the same conditions but without template addition.

2.3. Characterization of chemical structure and morphology

The chemical structures of the obtained polymer were identified with a Fourier-transform infrared spectrometer (FT-IR; Shimadzu, WQF-310) by using KBr compressed pellet method. The surface morphology of the obtained polymer was determined through scanning electron microscopy (SEM, FEI, Quanta 600FEG).

2.4. Static adsorption experiments

Static adsorption experiments were carried out to evaluate the adsorption kinetics and adsorption isotherms at 25 °C. In a typical procedure, 10 mg of MIP/NIP was incubated with 10 mL of 0.5 $mg \cdot mL^{-1}$ GSH solution (pH 7.0) for different times. After filtration using an acetate membrane (0.45 μm pore size), the residual concentration of GSH was determined through HPLC, and the adsorption capacity Q ($mg \cdot g^{-1}$) was calculated according to the following formula (1):

$$Q_e = \frac{(C_0 - C_e)V}{m} \tag{1}$$

where C_0 ($mg \cdot mL^{-1}$) and C_e ($mg \cdot mL^{-1}$) represent the initial and residual concentrations, respectively. V (mL) is the volume of adsorption solution, and m (g) is the amount of adsorbent.

Equilibrium adsorption experiments were performed by incubating MIP/NIP with different concentrations of GSH solution for 8 h. Q is calculated using Eq. (1), and the imprinting factor (α) is obtained according to the following Eq. (2):

$$\alpha = \frac{Q_{MIP}}{Q_{NIP}} \tag{2}$$

where Q_{MIP} and Q_{NIP} are the adsorption capacities of MIP and NIP, respectively.

The effect of initial pH on the recognition property of MIP/NIP toward GSH was determined using batch equilibrium adsorption experiments. The adsorption experiments were performed by incubating MIP/NIP with different pH of the GSH solution for 8 h.

2.5. Selectivity experiments

In a typical procedure, 10 mg of MIP/NIP was incubated with 10 mL of GSH and 0.2 $mg \cdot mL^{-1}$ of the similar structural compounds (pH 7.0) at 25 °C for 8 h. The adsorption capacities of GSH or similar structural analogs were obtained by measuring the residual concentration similar to the batch experiments. The selectivity recognition factor (β) of the MIP was calculated according to the following Eq. (3):

$$\beta = \frac{\alpha_{Tem}}{\alpha_{Ana}} \tag{3}$$

where α_{Tem} and α_{Ana} are the imprinting factors of MIP for GSH and its structural analogs, respectively.

2.6. SPE experiments

The obtained IRPP-MIP particles (200 mg) were packed into an empty SPE cartridge with two polypropylene upper and lower frits at each end. The entire MISPE cartridge was carefully washed with sufficient methanol and preconditioned with a mixture of acetonitrile and water (7/3, v/v) at a flow rate of 0.5 $mL \cdot min^{-1}$ prior to use. Subsequently, 50 mL of the sample solution containing 50 $\mu g \cdot L^{-1}$ GSH, GSSG, or Gly-Gly was passed through the preconditioned MISPE cartridge at a specific flow rate. After loading, the MISPE cartridge was first washed with 3.0 mL of a mixture of acetonitrile and water (7/3, v/v) to remove the sample molecules retained by non-specific adsorption from the cartridge. The adsorbed analytes on the MISPE cartridge were eluted using 3.0 mL of a mixture of ethanol, water, and acetic acid (70/28/2, v/v). Finally, the eluate was analyzed through HPLC by using pre-column derivation with PITC at a flow rate of 0.5 $mL \cdot min^{-1}$