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Grafting of molecularly imprinted polymers from the surface of silica gel particles via reversible addition-fragmentation chain transfer polymerization: A selective sorbent for theophylline

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ABSTRACT

Molecularly imprinted polymers (MIPs) were grafted successfully from the surface of silica gel particles via surface initiated reversible addition-fragmentation chain transfer (RAFT) polymerization using RAFT agent functionalized silica gel as the chain transfer agent. The intrinsic characteristics of the controlled/living polymerization mechanism of RAFT allowed for the effective control of the grafting process. Thus the grafting copolymerization of methacrylic acid and divinyl benzene in the presence of template theophylline led to thin MIP film coating silica gel (MIP-Silica). The thickness of MIP film prepared in this study is about 1.98 nm, which was calculated from the nitrogen sorption analysis results. Measured binding kinetics for theophylline to the MIP-Silica and MIPs prepared by conventional bulk polymerization demonstrated that MIP-Silica had improved mass-transfer properties. In addition, the theophylline-imprinted MIP-Silica was used as the sorbent in solid-phase extraction to determine theophylline in blood serum with satisfactory recovery higher than 90%. Nonspecific adsorption of interfering compounds can be eliminated by a simple elution with acetonitrile, without sacrificing the selective binding of theophylline.

1. Introduction

Molecularly imprinted polymers (MIPs), first introduced by Mosbach and co-workers [1] and Wulff [2], are widely reported materials that can be used as biomimetic molecular recognition elements. The synthesis of MIPs involves the formation of a complex of a target molecule (template) with one or more functional monomers though either covalent or noncovalent bonds followed by a polymerization reaction with excess cross-linking agent. Upon removal of the template, the binding sits are produced that are complementary to the template in shape, size, and the position of the functional groups. The stability, ease of preparation and low cost of these MIPs make them particularly attractive [3–5]. However, the conventional techniques used to prepare MIPs most often result in materials exhibiting high affinity and selectivity but poor site accessibility for the target molecule [4–6].

Recently, a new molecular imprinting technique called "grafting from" technique has emerged to overcome this drawback [7–11]. In the "grafting from" technique, the initiating groups were immobilized on the surface of a solid support. During the course of polymerization, the grafted MIPs were propagated from the surface of the solid supports. Using the "grafting from" technique, MIP film with high graft density and nanometer thickness can be prepared on the surface of a solid support. The resulting MIP composites have the advantages of more accessible binding sites and faster mass transfer compared to the MIPs prepared by conventional bulk polymerization techniques. Recently, the "grafting from" technique has been used by several research groups to produce imprinted polymer films on various substrates, including silica gel [7–10] and silica nanoparticle [11].

In recent years, the use of surface initiated controlled/living radical polymerization (CRP) has proven to be the most versatile approach for producing a wide range of polymer chains on solid surfaces [12,13]. In CRP, the life-time of the growing radical can be controlled, resulting in the synthesis of polymer chains with predefined molar mass, low polydispersity, controlled composition, and functionality. In general surface initiated CRP can be achieved by stable free-radical polymerization, *e.g.* nitroxide-mediated processes (NMP), metal catalyzed atom transfer radical polymerization (ATRP) and degenerative transfer, *e.g.* reversible

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addition-fragmentation chain transfer (RAFT). Recently, significant interest has been shown in the use of surface initiated CRP for the preparation of MIP composites [14–18].

This paper describes the use of surface initiated RAFT polymerization technique to functionalize silica gel with molecularly imprinted polymer films. RAFT has recently emerged as a promising CRP technique due to its versatility and simplicity, and the product polymer is free from the contamination of metal catalyst [19]. The RAFT technique is compatible with almost all of the conventional radical polymerization monomers. In our approach, the RAFT agents are directly immobilized on the surface of silica gel. The resulting thin MIP film coated silica can be applied in solid-phase extraction, HPLC as well as in capillary electrochromatography.

Theophylline has long been used to treat asthmatic symptoms in children and adults as well as apnea in premature infants. This drug is effective, however, over a narrow concentration range. At high concentrations, it is known to cause permanent neurological damage or death. Current measurement of theophylline levels are done by GC or LC method and by immunoassays. However, these approaches can be seriously disadvantaged in the presence of a complex sample matrix containing much interference such as blood serum. Thus using molecularly imprinted polymers to selectively extract theophylline from serum is very attractive [20,21].

2. Experimental

2.1. Reagents

4-(Chloromethyl) phenyltrimethoxysilane and phenylmagnesium bromide (PMB, 3 M in ether) were purchased from Alfa. Theophylline, theobromine and caffeine were purchased from Sigma. Methacrylic acid (MAA) was purchased from Alfa and distilled to remove the polymerization inhibitor before use. Divinylbenzene (DVB) containing 20% ethylvinylbenzene was purchased from Fluka and treated with basic alumina immediately prior to use to remove the polymerization inhibitor. Silica gel (particle size 35–70 μm, pore size 15 nm, pore volume 1.15 cm^3/g, surface area 300 m^2/g) was purchased from Aldrich.

2.2. Synthesis of chloromethyl-phenyl functionalized silica gel (Cl-Silica)

The silica gel was pretreated in order to eliminate any surface contaminants and to activate the surface silanol groups for silanization. In a typical experiment, silica gel were pretreated by reflux in 10% HCl solution for 8 h, rinsed by deionic water, and then dried in vacuum at 120 °C before use.

Dried silica gel (500 mg), 4-(chloromethyl)phenyltrimethoxysilane (2 mmol) and 10 mL absolutely dry toluene were introduced into a conical flask under the atmosphere of nitrogen. Keeping under constant temperature of 90 °C and with continuous stirring, the reaction was allowed to proceed for 24 h. The particles were then separated from the mixture via centrifugation. The product was washed with toluene for five times and then washed with methanol for five times in order to remove excess 4-(chloromethyl)phenyltrimethoxysilane. At the end, the obtained Cl-Silica was dried under vacuum at room temperature.

2.3. Synthesis of SC(S)Ph-Silica

PhC(S)SMgBr was prepared using phenylmagnesium bromide and carbon disulfide. The phenylmagnesium bromide solution (0.6 M in dry tetrahydrofuran) was warmed to 50 °C and carbon disulfide was added over 10 min, then the reaction mixture was kept at 50 °C for 1 h. To the resultant brown mixture was added Cl-Silica (500 mg) and the reaction temperature was kept at 50 °C for 24 h. Ice hydrochloric acid (1 M, 50 mL) was then added. The product was washed with distilled water for five times and then washed with ether for five times. The RAFT agent functionalized silica gel (SC(S)Ph-Silica) obtained was dried under vacuum at room temperature.

2.4. Grafting of MIP film on silica gel (MIP-Silica)

The grafting was performed in a flask containing 300 mg of SC(S)Ph-Silica suspended in a polymerization mixture consisting of 1 mmol of theophylline, 12 mmol of MAA, 60 mmol of DVB and 4 mg of azobis (isobutyronitrile) (AIBN) dissolved in 20 mL of chloroform. After sealing, mixing, and sparging the mixture with nitrogen for 10 min, the flask was placed in a thermostated oil bath at 55 °C for 24 h. After polymerization, the product was extracted with ethanol containing 20% acetic acid using a Soxhlet apparatus for 24 h. Then the product was washed with ethanol for three times and dried under vacuum at room temperature. A similar procedure with no added template was used to prepare non-imprinted polymer composites (NIP-Silica) as control.

2.5. Synthesis of conventional MIP particles using conventional bulk technique (MIP)

To compare the binding ability of MIP-Silica materials to conventional MIP materials, we prepared the latter using the bulk synthesis technique. 1 mmol of theophylline was dissolved in a mixture of 12 mmol of MAA, 60 mmol of DVB, 0.6 mmol of AIBN and 10 mL of chloroform. The solution was sonicated for 5 min, sparged with nitrogen for 15 min, and then polymerized at 60 °C for 24 h. Following polymerization, the bulk polymer was dried overnight under vacuum at 50 °C. Next, it was ground with a mortar and pestle and sieved between 200 and 400 mesh screens to give particles with size dimensions between 38 and 75 μm. Fines were removed by sedimentation from ethanol. The product was extracted with ethanol containing 20% acetic acid using a Soxhlet apparatus for 24 h. Then the product was washed with ethanol for three times and dried under vacuum at room temperature.

2.6. Nitrogen sorption analysis

Nitrogen sorption measurements were performed on a Tristar3000 (Micrometritics Co., USA). Prior to measurements, the samples were heated at 60 °C under high vacuum for at least 12 h. The average pore diameter (dp) was evaluated using the BJH theory.

2.7. Elemental analysis

Elemental analysis was performed on Vario EL III universal CHNOS elemental analyzer (ELEMENTAR, Hanau, Germany).

2.8. Steady-state binding studies

5 mg of MIP-Silica, NIP-Silica, MIP, or NIP particles were contacted with 5 mL of theophylline solutions in acetonitrile with initial concentrations ranging from 0.005 to 0.1 mmol/L. The samples were placed in a constant temperature, reciprocating shaker bath at 35 °C and 80 rpm. Samples were taken at regular time intervals during incubation in order to measure the time needed to reach equilibrium. Initial and final theophylline concentrations were determined by HPLC using UV detection at 270 nm.

2.9. MIP-Silica SPE

Commercial SPE cartridges were emptied from their packing material. Next the cartridge tube and frits were thoroughly cleaned