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Solid-phase extraction approach for phospholipids profiling by titania-coated silica microspheres prior to reversed-phase liquid chromatography–evaporative light scattering detection and tandem mass spectrometry analysis

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ABSTRACT

A novel strategy for selectively adsorbing phospholipids (PLs) on titania-coated silica core–shell microspheres (TiO_2/SiO_2) was developed. The TiO_2/SiO_2 microspheres were prepared through water-vapor-induced internal hydrolysis and then characterized by SEM, UV–vis spectroscopy, X-ray diffraction, and measurements of Brunauer–Emmett–Teller surface area. Analyses showed that the titania layer was uniformly distributed onto the surface of silica particles. The TiO_2/SiO_2 microspheres were employed as sorbent in solid-phase extraction (SPE), and their absorptive ability was investigated by reversed-phase liquid chromatography–evaporative light scattering detection (RPLC–ELSD). Important factors that affect the extraction, such as loading buffer, eluting buffer, and elution volume, were investigated in detail and optimized by using standard samples. Results reveal that the developed SPE approach had higher recoveries for PLs than that based on pure TiO_2 particles. The proposed SPE method was used for extraction of PLs from serum and showed great potential for identifying more kinds of endogenous PL metabolites by ultra performance liquid chromatography with quadrupole time-of-flight mass spectrometry (UPLC–QTOF MS). The proposed SPE method with the composite sorbent was used to screen PLs from a biological matrix with high selectivity and efficiency. This approach is a promising method for selective extraction of PLs in lipidomics or phospholipidomics.

1. Introduction

Phospholipids (PLs), the main constituents of biological membranes, have structural and functional properties. In particular, some PLs participate in many biological processes such as cell proliferation, differentiation, apoptosis, and oxidative stress [1]. In recent years, an increasing amount of research has demonstrated that metabolic disorders involving PLs and related metabolites and enzymes play an important role in many malignant diseases, including cancer of the breast, endometrium, colon, and kidney, as well as acute leukemia, malignant lymphomas, and multiple myeloma [2].

PLs comprise a polar head group with a phosphate moiety and various fatty acids that are attached to the glycerol backbone. According to differences in their polar head groups, PLs may be divided into classes such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), sphingomyelin (SM), and lysophosphatidylcholine [3]. Each class of PLs is composed of a mixture of molecular species. This structural diversity and complexity poses challenges in the analysis of PLs.

Selective enrichment of PLs from complex biological samples greatly helps in studying the change of PLs, especially some low-abundance species. It is critical to discover more PL signaling molecules, which have profound significance in lipidomics and phospholipidomics. Liquid–liquid extraction (LLE) and solid-phase extraction (SPE) protocols are commonly used to isolate PLs from total lipid extracts. The former is mainly based on the Bligh and Dyer extraction method [4,5], which often uses two organic solvents (methanol and chloroform) and involves phase separation [6]. The latter is a technique designed for rapid, selective sample preparation and purification prior to chromatographic analysis. It is used in sample cleanup, recovery, and concentration necessary for accurate quantitative analysis.

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Commonly used solid materials for SPE include porous silica or silica modified with octadecyl, cyanopropyl, aminopropyl, and 2,3-dihydroxypropoxypropyl groups [7]. However, the selectivity and efficiency of these materials for PL extraction is not very high. Metal oxides (e.g., ZrO_2, TiO_2) have higher selectivity and binding affinity for phosphate groups [8,9]. They have, therefore, been used in the enrichment of phosphopeptides for proteomics studies or of PLs from eggs and dairy products for quality control [10–13].

However, the small surface area, small pore volume, and lack of porous structure restrict the application of pure TiO_2. On the other hand, the interfacial electronic structure and interactions between the metal oxide and silica support lead to the porous structure and physicochemical properties of metal oxide layer. Thus, metal oxide-coated mesoporous silica may be an alternative to metal oxides for highly efficient and selective extraction of phosphates [14]. This material can be used as separation, adsorption, and host material [15,16]. Because of the strong affinity of coordinated Ti^{IV} for the phosphate group, highly dispersed Ti^{IV} preferentially enriches typical phosphate-containing substances such as phosphopeptides on the silica surface [17].

We want to establish a novel strategy for phospholipids pretreatment in order to discover more low-abundance phospholipids and improve their response intensity. In the present study, silica core–shell microspheres coated with a titania layer were synthesized through water-vapor-induced internal hydrolysis [18] and then characterized systematically. The material was employed as a sorbent in a SPE column to enrich PL standard compounds by reversed-phase liquid chromatography–evaporative light scattering detection (RPLC–ELSD). It was also used for comprehensive PL metabolic profiling of human serum followed by ultra performance liquid chromatography with quadrupole time-of-flight mass spectrometry (UPLC–QTOF MS). Such an approach featured the high selectivity of silica microspheres coated with metal oxide in adsorbing PLs, and showed promise in applications for pretreatment of biological samples in lipidomics.

2. Experiments

2.1. Chemicals and reagents

HPLC-grade acetonitrile (ACN), methanol (MeOH), isopropanol (IPA) and *n*-hexane were obtained from CNW Technologies GmbH (Germany). Ammonium formate and ammonia solution (7 mol/L in MeOH) were purchased from Sigma-Aldrich (St. Louis, USA). Chloroform ($CHCl_3$), ammonium acetate (AA), and formic acid (FA) were obtained from Sinopharm Chemical Reagent Company (Shanghai, China). Tetrabutyl titanate ($Ti(OC_4H_9)_4$), NH_3/H_2O (28%), titanium dioxide, and silica microspheres (20 μm–120) were purchased from Aladdin (Shanghai, China), Pinghu Chemical Reagent Company (Jiaxin, China), ZhongLan DingHui Material Company (Guangzhou, China), and Suzhou Global Chromatography Company (Suzhou, China), respectively. The water used was MilliQ grade (Millipore, Bedford, MA, USA). All chemicals used in preparing buffer solutions were analytical-grade reagents.

The PL standard solution consisted of 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LysoPC (16:0)), 1-stearoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LysoPC (18:0)), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (PC (14:0/14:0), DMPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (PC (16:0/18:1), POPC), and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (PE (16:0/18:1), POPE) (Avanti Polar Lipids, Alabaster AL, USA). The purity of all PLs standards is greater than 99%. It was stored at −20 °C and further diluted with MeOH before use.

2.2. Preparation of titania-coated silica composite microspheres

Synthesis of TiO_2/SiO_2 was done through a modification of the water-vapor-induced internal hydrolysis method. Tetrabutyl titanate, an equivalent of 10% (w/w) silica-support, was dissolved in ethanol at a ratio of 1:4 (v/v). The mixture was sealed and then vortexed for 5 min. The ethanol solution was wet-impregnated into the pores of silica under stirring at 60 °C until dryness. The silica was stored at 100 °C for 6 h. Afterward, 1 g of titania-precursor-loaded silica was placed in an open glass vial and kept inside an autoclave containing 20 mL water. There was no direct contact between the solid and water. The sample was heated at 60 °C for 5 h. Subsequently, the silica was removed and dried completely at room temperature. After the desiccated sample was calcined under air at 500 °C for 5 h, 10 wt% TiO_2/SiO_2 microspheres were obtained. Through similar procedures, 7.5 and 5 wt% TiO_2/SiO_2 microspheres could be obtained as well. Pure titanium dioxide was used as the reference material.

2.3. Characterization of TiO_2/SiO_2 composite microspheres

Scanning electron microscopy (SEM) images were obtained on an S-4800 field emission scanning electron microscope (Hitachi, Japan) with an accelerating voltage of 25 kV. Powder X-ray diffraction (XRD) was performed on a D/max-2600PC X-ray diffractometer (Rigaku Corporation, Japan) using Cu Kα radiation ($\lambda = 0.1542$ nm) and operating at 20 kV and 50 mA. N_2 adsorption–desorption experiments were undertaken at 77 K on an automatic ASAP 2020M Micromeritics apparatus. Before adsorption, the materials were degassed at 573 K for 10 h. Brunauer–Emmett–Teller (BET) surface areas were calculated from data on adsorption at relative pressure range of 0.05–0.25, and pore size distributions were calculated from the adsorption branches of the isotherms through the Barrett–Joyner–Halenda (BJH) method. Sorption experiments were performed on an ASAP 2020M surface area and pore size analyzer (Micromeritics Inc., USA). Ultraviolet–visible diffuse reflectance spectroscopy (UV–vis DRS) was performed on a Lambda 950 UV–vis spectrophotometer (Perkin-Elmer Inc., USA).

2.4. Serum sample collection and lipid extraction

Human serum was obtained from healthy volunteers at Ruijin Hospital (affiliated to Shanghai Jiao Tong University). The serum samples were prepared by pooling and mixing the same volume of each sample from 15 healthy volunteers. The total lipids fraction was obtained by LLE according to a modification of the Bligh and Dyer method. In this method, 30 μL of water followed by 150 μL of MeOH was added to 30 μL of human serum. The resulting solution was vortexed for 60 s. Afterward, 300 μL of $CHCl_3$ was added, and the solution was vortexed for another 60 s. Subsequently, 150 μL of water was added to the solution and thoroughly mixed to form a two-phase system. After centrifugation at 10,000 rpm for 10 min, the lower organic phase was collected. The remaining solution was then extracted again with $CHCl_3$. The two lipid extracts were combined and dried under vacuum, and the residue was redissolved in 180 μL of IPA/hexane (8/2, v/v) solution with 1% FA.

2.5. PLs extraction with the TiO_2/SiO_2 SPE cartridge

2.5.1. The recovery of PLs standard with TiO_2/SiO_2 cartridge

The synthesized TiO_2/SiO_2 microspheres (30 mg) were packed into 1 mL SPE columns with top and bottom frits. Columns were prewashed with MeOH and equilibrated by IPA/hexane (8/2, v/v) solution. Lipid extract or standard mixture (150 μL) combined with 300 μL of IPA/hexane (8/2, v/v) solution with 1% FA was added to the TiO_2/SiO_2 SPE columns, and effluent (loading solution, SPE–LS)