Facile synthesis of Titania-Zirconia monodisperse microspheres and application for phosphopeptides enrichment

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Experimental Section

Materials

Titanium dioxide was purchased from GL Sciences. α – casein, ammonium bicarbonate were obtained from Sigma - Aldrich. Trypsin was from Promega. Reversed phase C_{18} particles were from Sunchrom. Ammonium hydroxide was purchased from Fluka. Water used in MS was prepared with Millipore.

Preparation of zirconium acetyl acetate. 6.4 g of zirconyl chloride ($ZrOCl_2 \cdot 8H_2O$) and 8 g of acetonyl acetone were dissolved in 80 mL distilled water and sodium carbonate saturated solution was added with stirring vigorously, then the white precipitations were filtered until the solution was adjusted to pH 7. The product was recrystallized in acetone.

Preparation of titania-zirconia mixed microspheres. $Ti(OBu)_4$ (8 g), certain amount of $Zr(acac)_4$， acetonyl acetone and dodecylamine (2 g) were stirred vigorously in anhydrous ethanol (200 mL). Certain amount of water was slowly added to the mixture and stirred for another 4 minutes until the solution became cloudy. 50mL anhydrous ethanol was added to the turbid solution. The mixture was aged about 2 h at 35 ℃. Then the sediment was filtered and washed with anhydrous ethanol and dried at room temperature for 12 h. The obtained xerogel (2 g), urea (0.1 g), anhydrous ethanol (21 mL) and deionized water (14 mL) were sealed in a PTFE-lining stainless-steel autoclave and heated at 135 □ for 24 h. The particles were separated and washed with acetone and methanol for several times. Then the micropheres were calcinated at 200 □ (at a

heating rate of 5□ min^{-1}) keeping for 3 h and at 400 □ for 6 h to remove the residuary organic compound. To investigate the effect of temperature, 25□, 35□and 45□ were chose to optimize the uniformity of sphere size. Also to investigate the effect of varying amounts of zirconia in the composite, molar ratio of titanium butoxide /zirconium acetyl acetate was fixed at (A) 20 / 1 (B) 10 / 1 (C) 5 / 1 . Water added in the mixture for hydrolysis changed with corresponding molar ratio of start materials at (A) 7 mL (B) 8.5mL (C) 8 mL. Acetonyl acetone added as helping chelating agent changed at (A) 0.5mL (B) 0.5mL (C) 0mL.

Enrichment of phosphopeptides from *α*-casein. *α*-casein were dissolved in 1 mL of ammonium bicarbonate (50mM, pH 8.0) and digest in trypsin for 18 h at 37℃ with a 1 : 40 (w / w) enzyme – to – protein ratio. 1mg of TiO_2-ZrO_2 beads were packed in GELoader tips 2*μ*l (80pmol) of *α*-casein digested solution was loaded on the TiO_2-ZrO_2 tip. After successive washing with 40 *μ*l 0.1 % FA/50% CH_3CN and 20 *μ*l 0.1%FA, the bound peptides were eluted with 20*μ*l 2% ammonium hydroxide. The eluted solution was acidified with 10%FA and desalted with C18 microcolumns before Q-TOF analysis.

Characterization

The morphologies and elemental analysis of particles were studied using scanning electron microscopy (Jeol JSM-6360LV, Jeol Ltd., Japan) equipped with EDAX energy-dispersive spectrometer. Nitrogen physisorption isotherms were measured at 77 K using an ASAP 2010 analyzer instrument. The isotherms were analyzed by nonlocal density functional theory (NLDFT) method to evaluate pore sizes and micropore volumes of the samples using the kernel of NLDFT equilibrium capillary condensation isotherms of nitrogen at 77 K on silica (adsorption branch, assuming cylindrical pore geometry). The BET surface areas were calculated from the adsorption branches in the relative pressure range of 0.05-0.20, and the total pore volumes were evaluated at a relative pressure of 0.95. Powder X-ray diffraction (PXRD) patterns were obtained using on an X-ray diffractometer model D/MAX 2550 (Rigaku, Japan) using Cu-Kα radiation. High resolution transmission electron microscope images and elemental mapping were performed on a TECNI F30 field emission microscope with acceleration voltage of 300KV equipped with an EDAX energy-dispersive spectrometer.

Figures

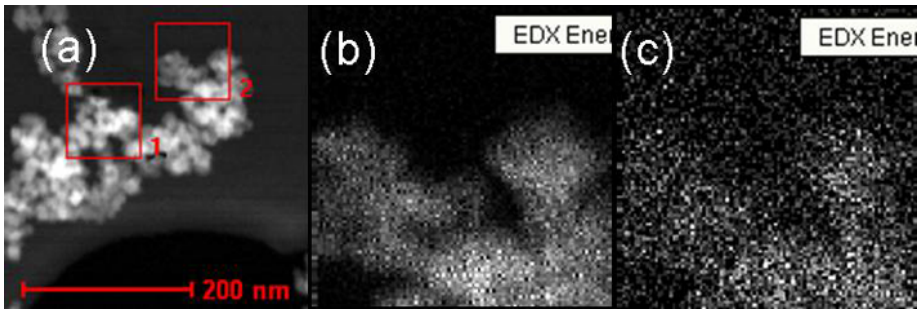

Figure 1 Elemental mapping of TZ-1 by HRTEM-EDX (a) map of TEM (b) map of titanium (c) map of zirconium

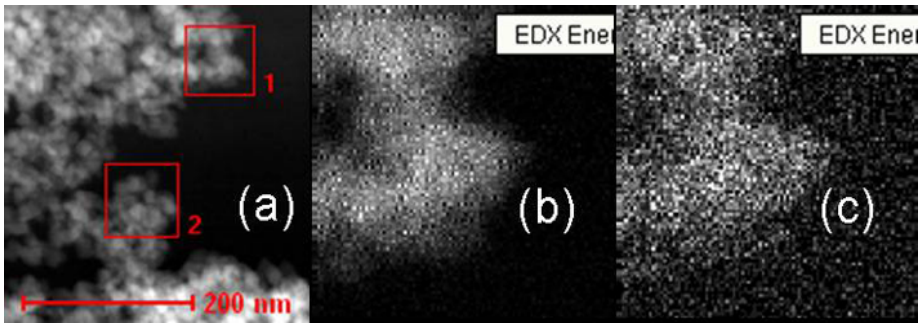

Figure 2 Elemental mapping of TZ-2 by HRTEM-EDX (a) map of TEM (b) map of titanium (c) map of zirconium

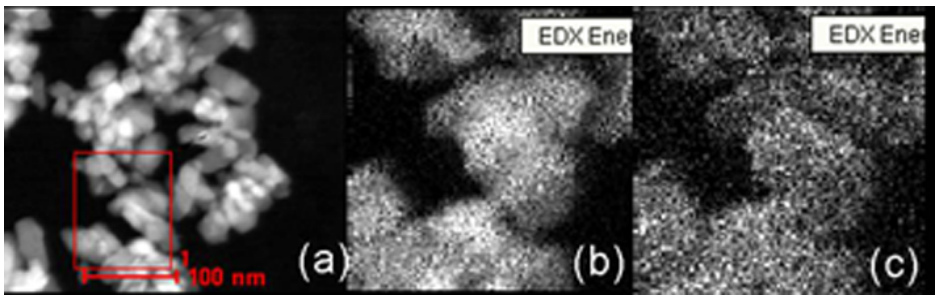

Figure 3 Elemental mapping of TZ-3 by HRTEM-EDX (a) map of TEM (b) map of titanium (c) map of zirconium