Novel approach for the synthesis of Fe_3O_4@TiO_2 core–shell microspheres and their application to the highly specific capture of phosphopeptides for MALDI-TOF MS analysis†

Yan Li,‡[a] Jinsong Wu,‡[b] Dawei Qi,[a] Xiuqing Xu,[a] Chunhui Deng,*[a] Pengyuan Yang[a] and Xiangmin Zhang*[a]

Received (in Cambridge, UK) 17th October 2007, Accepted 12th November 2007
First published as an Advance Article on the web 20th November 2007
DOI: 10.1039/b716055k

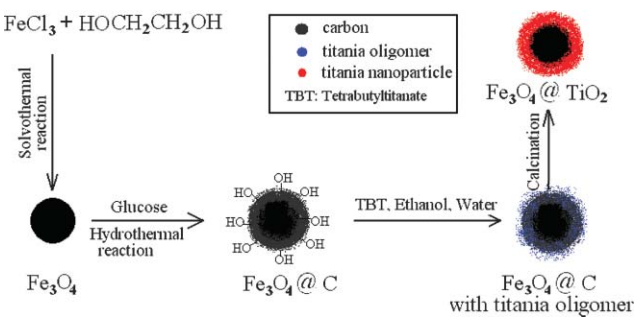
A novel approach is proposed to synthesize Fe_3O_4@TiO_2 microspheres with a well-defined core–shell structure, and the synthesized Fe_3O_4@TiO_2 core–shell microspheres were successfully applied for the simple and fast enrichment of phosphopeptides *via* direct MALDI-TOF mass spectrometry analysis.

Phosphorylation plays a vital role in regulating biological functions. Effective characterization of phosphoproteins from complex samples is necessary for clarifying the regulatory mechanisms of biological systems. However, the presence of non-phosphorylated peptides in the digestion product often suppresses the ion signal of phosphorylated peptides in mass spectra (MS). Therefore, enriching the phosphopeptides of complex samples becomes a required step prior to MS analysis. Immobilized metal-ion affinity chromatography (IMAC) has become one of the most widely applied techniques for the selective enrichment of phosphopeptides and phosphoproteins.[1] With rapid advances in the separation and identification of phosphopeptides, researchers have realized that many metal oxides, such as zirconia (ZrO_2) and titania (TiO_2), can also be used to specifically separate phosphopeptides from complex samples.[2–4] Metal oxides have been demonstrated to be effective in the enrichment of phosphopeptides, and their selectivity is found to be higher and the results more reproducible than IMAC.

Chen and Chen attempted to synthesize magnetic titania particles with an aim of combining the magnetic properties of magnetite particles and the affinity of TiO_2 towards phosphopeptides for the enrichment of phosphopeptides.[5] Unfortunately, they failed to obtain magnetite/titania core–shell particles, because they adopted a two-step sol–gel process, involving the hydrolysis and condensation of two precursors, *i.e.* tetraethyl orthosilicate and titanium butoxide. Because the hydrolysis and condensation of titanium butoxide are very difficult to control in a sol–gel process, as a result, the obtained product was merely composite particles of Fe_3O_4, SiO_2 and TiO_2 of ill-defined structure. In fact, as indicated by their results, these particles simultaneously trap other acidic peptide residues, suggesting that the particle surface is not pure TiO_2 phase.

In this study, we developed a novel synthesis route for the preparation of Fe_3O_4@TiO_2 microspheres (Scheme 1) that involves three steps. First, 280 nm-sized magnetite microspheres were synthesized *via* a solvothermal reaction, using $FeCl_3$ as the source of magnetite, and ethylene glycol as both the solvent and reductant. Then, the magnetite microspheres were coated with a thin layer of carbon by the polymerization and carbonization of glucose through a hydrothermal reaction, resulting in Fe_3O_4@C microspheres. Finally, tetrabutyltitanate was pre-hydrolyzed and absorbed onto the microspheres, and eventually converted into titania by calcination under nitrogen.

Fig. 1 shows the transmission electron microscopy (TEM) image of the as-synthesized Fe_3O_4 microspheres. From the image, it can be seen that the microspheres have a mean diameter of 280 nm with a narrow size distribution. Because titania possesses unique optical, electronic and chemical properties, coating magnetite microspheres with titania could endow the microspheres with many additional useful surface properties and functionalities, and thus greatly widen their utility in fields such as photocatalysis, magnetically-assisted separation and enrichment. A sol–gel approach has frequently been used to coat various particles with titania *via* the hydrolysis and condensation of titania precursors in a mixture of alcohol and water.[6] However, the coating process based on this approach is strongly influenced by conditions such as reactant concentration, pH, temperature, dispersibility of the seed particles, and the affinity between the seed particles and the coating species. Therefore, it often leads to the formation of irregular composite microspheres and the occurrence of bare seed particles and blank titania particles. In the present work, in order to coat titania uniformly onto single magnetite microspheres, the as-made Fe_3O_4 microspheres were first coated with hydrophilic



Scheme 1 The synthetic route to Fe_3O_4@TiO_2 core–shell microspheres.

[a]*Department of Chemistry, Fudan University, Shanghai 200433, China. E-mail: chdeng@fudan.edu.cn; xmzhang@fudan.edu.cn; Fax: +86 21-6564 1740*
[b]*Shanghai Neurosurgical Center, Department of Neurosurgery, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai 200400, China*

† Electronic supplementary information (ESI) available: Experimental details, FTIR spectra, TEM images of various metal oxide coated magnetite microspheres, and detailed information on the enrichment of β-casein and casein. See DOI: 10.1039/b716055k

‡ These authors contributed equally to the work.

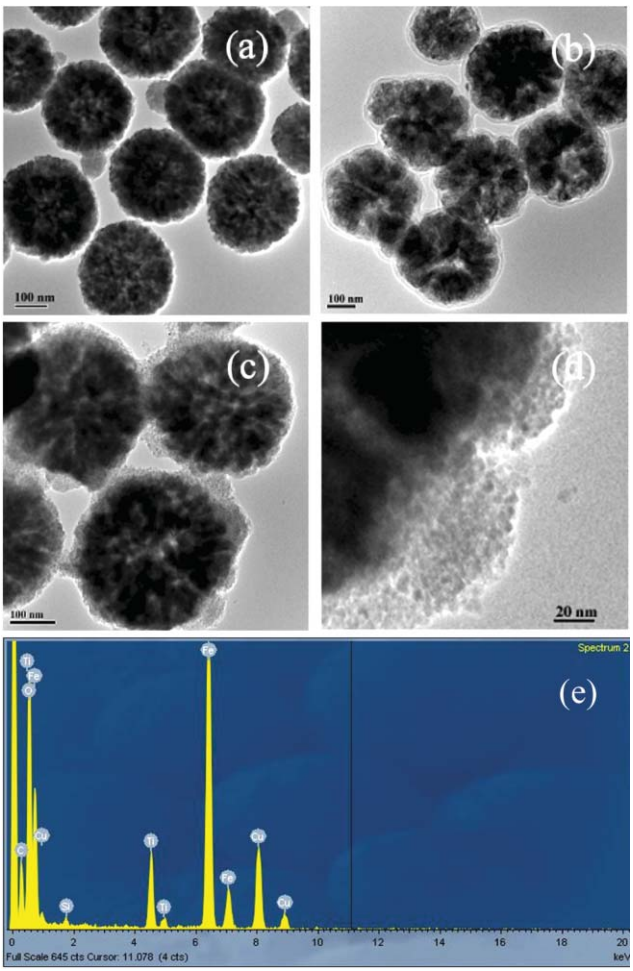


Fig. 1 TEM images of (a) the as-synthesized Fe_3O_4 microspheres and (b) the Fe_3O_4@C microspheres. (c,d) The Fe_3O_4@TiO_2 core–shell microspheres. (e) EDX spectrum data of the obtained Fe_3O_4@TiO_2 core–shell microspheres.

carbon by a hydrothermal reaction of glucose, which results in core–shell-structured Fe_3O_4@C microspheres covered in a uniform carbon layer of about 20 nm thickness (Fig. 1(b)). According to previous reports,[7] the carbonization of glucose during a hydrothermal treatment is due to the cross-linking of the intermolecular dehydration products of the glucose, oligosaccharides and/or other macromolecules derived from glucose. Fourier transform infrared (FTIR) spectra of the as-made Fe_3O_4, Fe_3O_4@C and Fe_3O_4@TiO_2 microspheres are shown in Fig. S1.† The Fe_3O_4@C microspheres show bands at 1700 and 1620 cm^{-1} associated with the C=O vibration and C=C vibration, respectively, indicating the carbonization of glucose during the hydrothermal reaction (Fig. S1b†). The peaks at 1000–1300 cm^{-1}, attributed to the C–OH stretching and O–H bending vibrations, suggest the presence of large amounts of hydrophilic groups. The presence of these hydrophilic groups not only endows the Fe_3O_4@C microspheres with better dispersibility and stability than those of the as-synthesized Fe_3O_4 microspheres, but also significantly enhances the affinity between the microspheres and the pre-hydrolyzed tetrabutyltitanate (*i.e.*, titania oligomers). Fig. 1(c) shows the TEM image of the Fe_3O_4@TiO_2 microspheres obtained by the calcination of Fe_3O_4@C microspheres with absorbed titania oligomers, in which the typical core–shell structure can clearly be observed, indicating the successful coating of Fe_3O_4 with TiO_2. Notably, as can be seen in the TEM image of high magnification (Fig. 1(d)), the outer shell of the obtained Fe_3O_4@TiO_2 core–shell microspheres consists of a larger amount of titania nanoparticles, which may provide a higher specific area and be beneficial for the specific trapping phosphopeptides. The energy-dispersive X-ray analysis (EDXA) (Fig. 1(e)) of the obtained Fe_3O_4@TiO_2 microspheres reveals the existence of Fe, Ti and O elements. Their FTIR spectrum shows characteristic bands at 500–800 cm^{-1} for titania, providing additional evidence of the successful synthesis of Fe_3O_4@TiO_2 microspheres (Fig. S1c†). The wide angle X-ray diffraction measurement result indicates that TiO_2 nanoparticles in the outer layer of the Fe_3O_4@TiO_2 microspheres are of anatase phase (JCPDS no. 211272) (Fig. S2†). The magnetic properties of the microspheres were investigated by a vibrating sample magnetometer at room temperature, and the saturation magnetization values of the obtained Fe_3O_4 and Fe_3O_4@TiO_2 microspheres were calculated to be about 80.5 and 71.2 emu g^{-1}, respectively. Such a high magnetite content (*ca.* 88 wt%) could enable the Fe_3O_4@TiO_2 microspheres to rapidly respond to an applied magnetic field, thus allowing a fast enrichment procedure. With a well-defined core–shell structure, these Fe_3O_4@TiO_2 microspheres not only have a shell of titanium oxide, giving them a high trapping capacity for phosphopeptides, but also their magnetic properties enable their easy isolation by the positioning of an external magnetic field. Based on the successful synthesis of core–shell Fe_3O_4@TiO_2 microspheres, our recent work further reveals that this novel synthesis approach could be extended to prepare various core–shell-structured metal oxide-coated magnetic microspheres, such as aluminium oxide and zirconium oxide (see ESI, Fig. S3†).

In order to investigate the selectivity and efficiency of the obtained Fe_3O_4@TiO_2 microspheres for the enrichment of phosphopeptides, bovine β-casein was first chosen as a model compound due to its well characterized phosphorylation sites. Even when the concentration of the sample was lowered to 2×10^{-9} M, as shown in Fig. S4a and S4b,† the efficient and specific enrichment of the three phosphorylated peptides (marked as 2061.70, 2555.82 and 3121.98) was observed in the mass spectrum (Fig. S4b†). The result indicates that within only 30 seconds, phosphopeptides in quantities sufficient for MALDI mass spectral analysis are enriched, indicating the excellent enrichment capacity of the Fe_3O_4@TiO_2 core–shell microspheres, even at a very low level.

The selectivity of these magnetic particles for phosphopeptides was demonstrated with a tryptic digest of a mixture of casein (composed of α-S1 and α-S2 units, and β-casein) at a low concentration (5 ng μL^{-1}). Fig. 2(a) presents the direct MALDI mass spectrum of the tryptic digest of casein (5 ng μL^{-1}) prior to enrichment. Among the peaks, only four phosphorylated peptide ions of low intensity were observed. After using the affinity probes to enrich the phosphopeptides, phosphopeptide residue ions started to appear in the MALDI mass spectrum (Fig. 2(b) and Table S1†). The peaks marked with the numbers 4, 5, 6, 7 and 11 are derived from α-S1-casein, whereas the peaks marked with the numbers 1, 2, 3, 10 and 12 are derived from α-S2-casein. The remaining peaks, marked with the numbers 8, 9 and 13, are derived from β-casein. The corresponding peptide sequences of

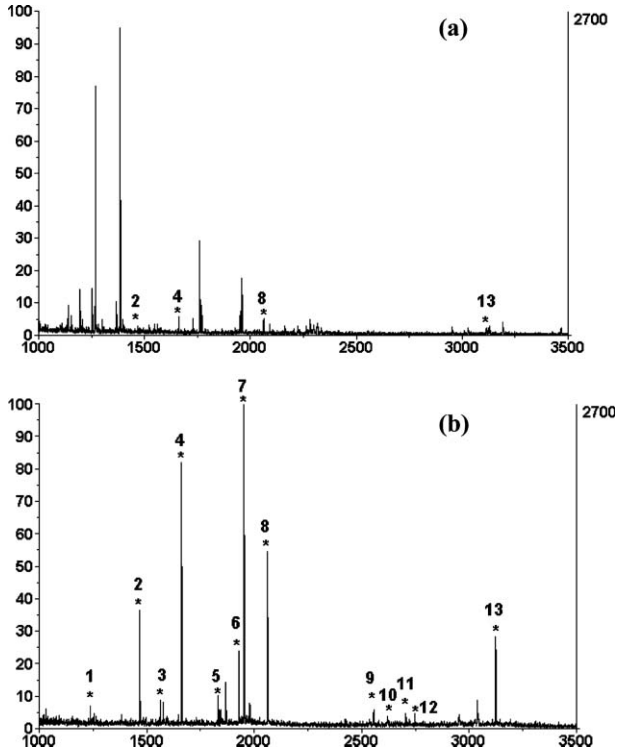


Fig. 2 MALDI mass spectra of (a) 5 ng μL^{-1} tryptic casein digest without any pre-treatment, (b) on-bead analysis of phosphopeptides obtained when using Fe_3O_4/TiO_2 microspheres to selectively trap target peptides from the 5 ng μL^{-1} tryptic digest product of casein (200 μL). The phosphopeptides are marked with asterisks.

these ions are listed in Table S1.† All of the ions revealed in the mass spectrum are phosphopeptide residues. The results demonstrate that the $Fe_3O_4@TiO_2$ microspheres are very effective in selectively trapping phosphopeptides from a complex sample. The high sensitivity and selectivity of the $Fe_3O_4@TiO_2$ microspheres towards phosphopeptides are ascribed to the typical core–shell structure and the high surface area arising from the loose and rough shells made of titanium oxide nanoparticles.

In summary, an innovational pathway is proposed to synthesize $Fe_3O_4@TiO_2$ microspheres with a well-defined core–shell structure, and the synthesized $Fe_3O_4@TiO_2$ core–shell microspheres were successfully applied to the enrichment and identification of phosphopeptides from a complex protein tryptic digest mixture *via* a direct MALDI-TOF mass spectrometry analysis. The process of enrichment is very facile, efficient and highly selective. The peptides adsorbed onto the $Fe_3O_4@TiO_2$ core–shell microspheres could be directly analyzed by MALDI-TOF MS analysis without elution from the $Fe_3O_4@TiO_2$ core–shell microspheres. These results are expected to open up a new possibility for the enrichment of phosphopeptides. Moreover, the new synthesis method presented in this study is versatile, low-cost and reproducible, and could be extended to synthesize magnetic microspheres with a core of magnetite and a shell of various metal oxides.

This work was supported by the National Basic Research Priorities Program (Project: 2007CB914100/3), the 863 Project (no. 2006AA02Z4C5) and the National Key Natural Science Foundation of China (Project: 20735005).

Notes and references

1 M. C. Posewitz and P. Tempst, *Anal. Chem.*, 1999, **71**, 2883; A. Stensballe, S. Andersen and O. N. Jensen, *Proteomics*, 2001, **1**, 207; C. S. Raska, C. E. Parker, A. Dominski, W. F. Marzluff, G. L. Glish, R. M. Pope and C. H. Borchers, *Anal. Chem.*, 2002, **74**, 3429; A. Stensballe and O. N. Jensen, *Rapid Commun. Mass Spectrom.*, 2004, **18**, 1721; H. Liu, J. Stupak, J. Zhang, B. O. Keller, B. J. Brix, L. Fliegel and L. Li, *Anal. Chem.*, 2004, **76**, 4223; D. Hirschberg, T. Jagerbrink, J. Samskog, M. Gustafsson, M. Stahlberg, G. Alveliusm, B. Husman, M. Carlquist, H. Jornvall and T. Bergman, *Anal. Chem.*, 2004, **76**, 5864.

2 J. P. Folkers, C. B. Gorman, P. E. Laibinis, S. Buchholz, G. M. Whitesides and R. G. Nuzzo, *Langmuir*, 1995, **11**, 813; W. Gao, L. Dickinson, C. Grozinger, F. G. Morin and L. Reven, *Langmuir*, 1996, **12**, 6429.

3 D. Brovelli, G. Hahner, L. Ruiz, R. Hofer, G. Kraus, A. Waldner, J. Schlosser, P. Oroszlan, M. Ehrat and N. D. Spencer, *Langmuir*, 1999, **15**, 4324; M. Textor, L. Ruiz, R. Hofer, A. Rossi, K. Feldman, G. Hahner and N. D. Spencer, *Langmuir*, 2000, **16**, 3257; N. D. Spencer and R. H. Textor, *Langmuir*, 2001, **17**, 4014.

4 M. H. Pinkse, P. M. Uitto, M. J. Hilhorst, B. Ooms and A. J. R. Heck, *Anal. Chem.*, 2004, **76**, 3935.

5 C. T. Chen and Y. C. Chen, *Anal. Chem.*, 2005, **77**, 5912.

6 X. C. Guo and P. Dong, *Langmuir*, 1999, **15**, 5535; A. Hanprasopwattana, S. Srinivasan, A. G. Sault and A. K. Datye, *Langmuir*, 1996, **12**, 3173; W. P. Hsu, R. Yu and E. J. Matijevic, *J. Colloid Interface Sci.*, 1993, **156**, 56; I. Haq and E. Matijevic, *Colloids Surf., A*, 1993, **81**, 153; M. Ocana, W. P. Hsu and E. Matijevic, *Langmuir*, 1991, **7**, 2911; R. C. Stephenson and R. E. Partch, *Mater. Res. Soc. Symp. Proc.*, 1997, **458**, 435; Z. Y. Zhong, Y. D. Yin, B. Gates and Y. N. Xia, *Adv. Mater.*, 2000, **12**, 206; A. R. Caruso, A. Susha and F. Caruso, *Chem. Mater.*, 2001, **13**, 400.

7 X. Sun and Y. Li, *Angew. Chem., Int. Ed.*, 2004, **43**, 597; Q. Peng, Y. J. Dong and Y. D. Li, *Angew. Chem., Int. Ed.*, 2003, **115**, 3135; Q. Peng, Y. J. Dong and Y. D. Li, *Angew. Chem., Int. Ed.*, 2003, **42**, 3027; T. Sakaki, M. Shibata, T. Miki, H. Hirosue and N. Hayashi, *Bioresour. Technol.*, 1996, **58**, 197; G. C. A. Luijkx, F. van Rantwijk, H. van Bekkum and M. J. Antal, *Carbohydr. Res.*, 1995, **272**, 191.

