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

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Introduction

Phosphorylation plays a vital role in regulating biological functions.

It helps to maintain the metabolic pathway. (ADP ⟶ ATP)
citric acid cycle or Krebs cycle

Effective characterization of phosphoproteins from complex samples is necessary for clarifying the regulatory mechanisms of biological systems.

MS analysis provides essential information.

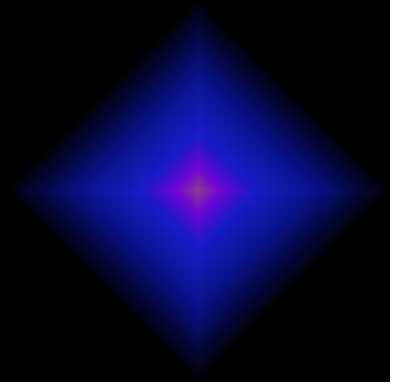
In presence of other non-phosphorylated peptides in the digestion product often suppresses the ion signal of phosphorylated peptides in MS

How to enrich the phosphopeptides present in complex samples?

- ✓ **Before MS analysis some treatment needed to be done to enrich phosphopeptides**

- ✓ **Immobilized metal-ion affinity chromatography (IMAC) technique helps to the selective enrichment of phosphopeptides and phosphoproteins**

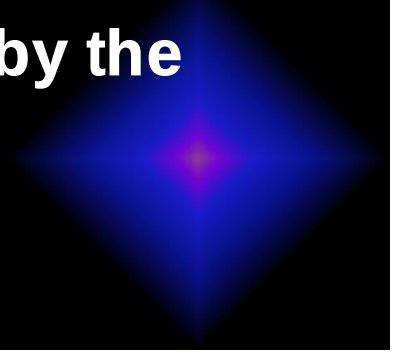
- ✓ **Metal oxides such as zirconia (ZrO_2) and titania (TiO_2) can also be used to specifically separate phosphopeptides from complex samples**

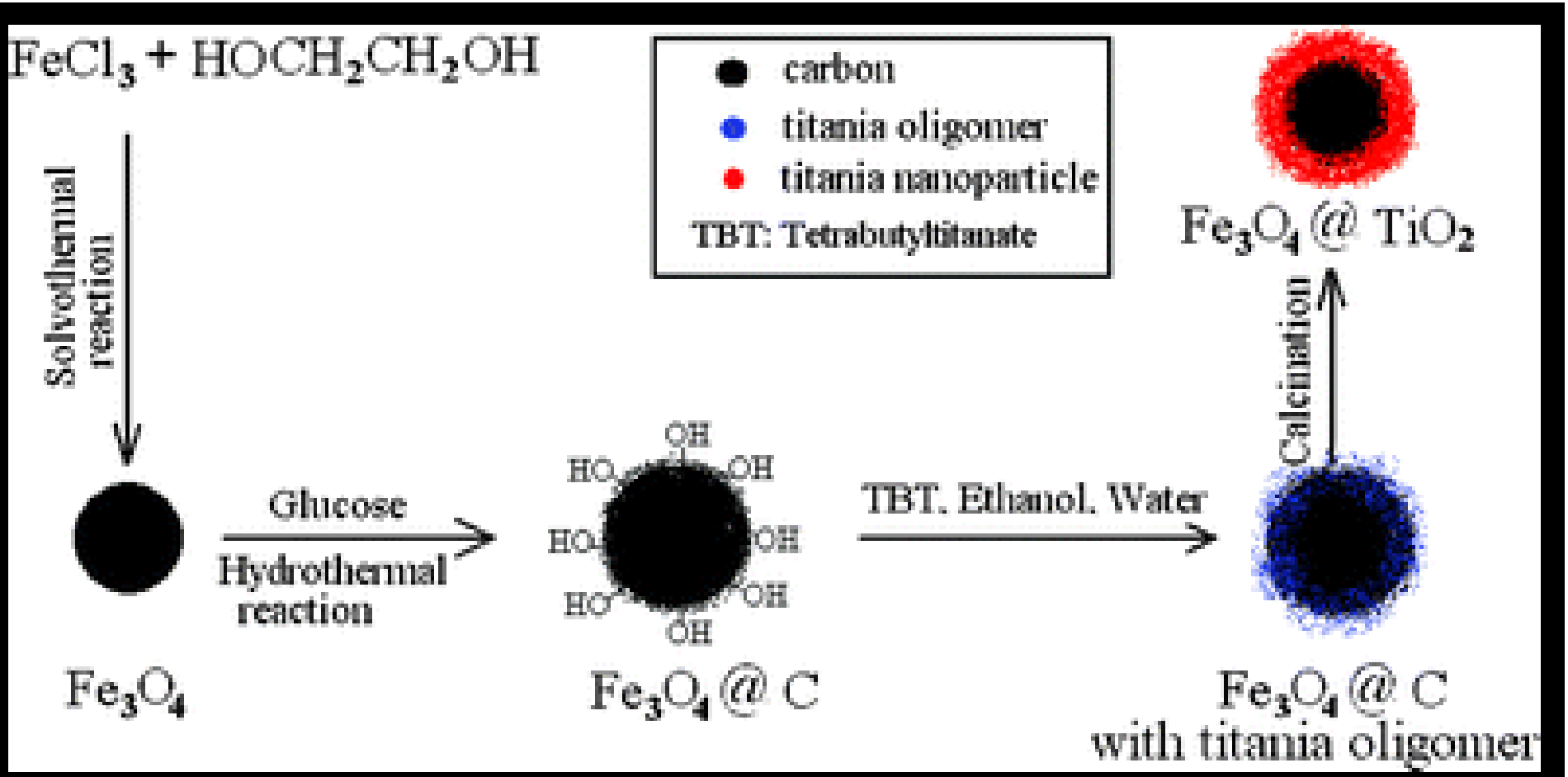


Chen *et al* attempted to synthesize magnetic titania particles by sol-gel method with an aim of combining the magnetic properties of magnetite particles and the affinity of TiO_2 towards phosphopeptides for the enrichment of phosphopeptides.

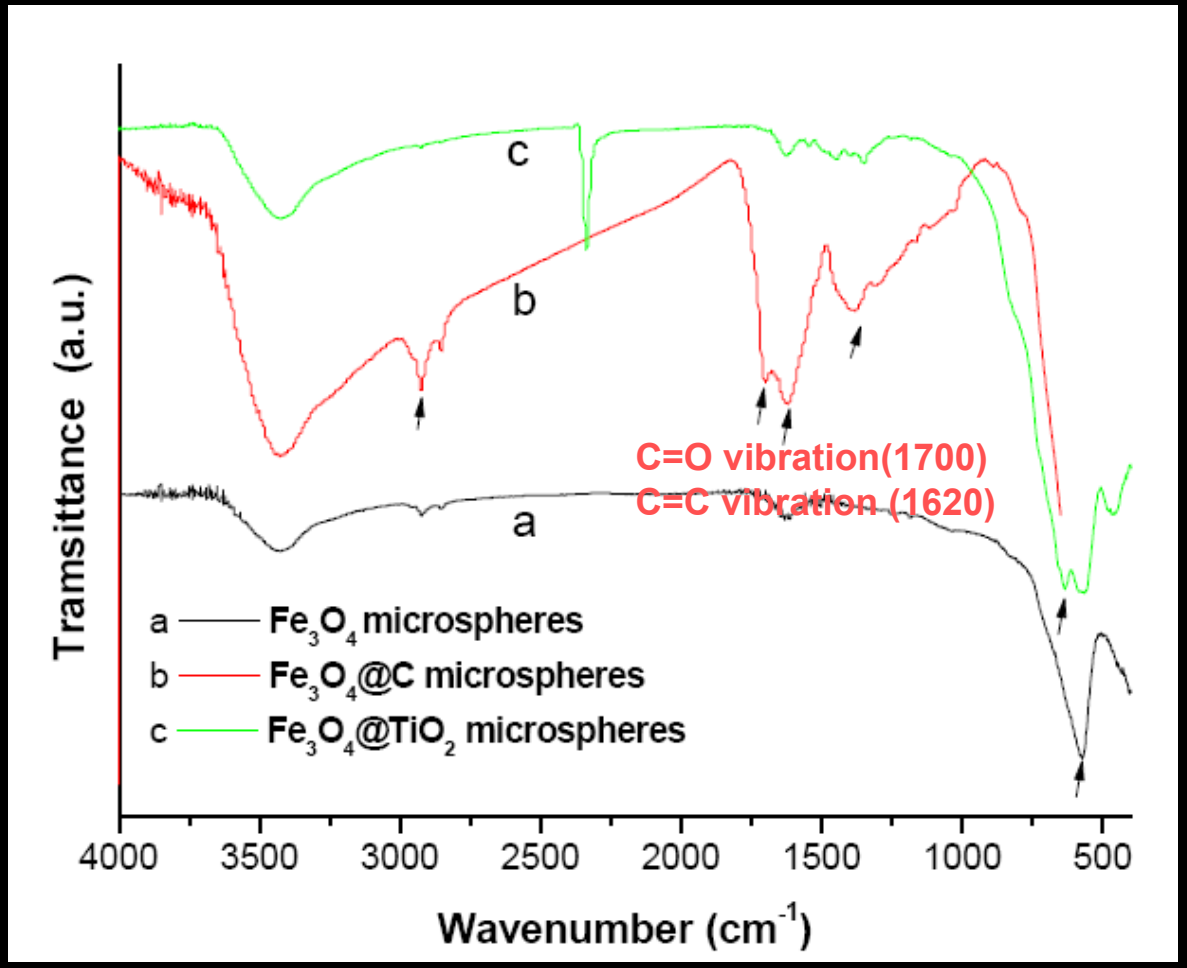
Unfortunately, they failed to obtain it, and ended up in product merely a composite particles of Fe_3O_4, SiO_2 and TiO_2 of ill-defined structure.

In this work **Yan Li *et al*** developed a novel synthesis route for the preparation of Fe_3O_4@TiO_2 microspheres by the scheme given here

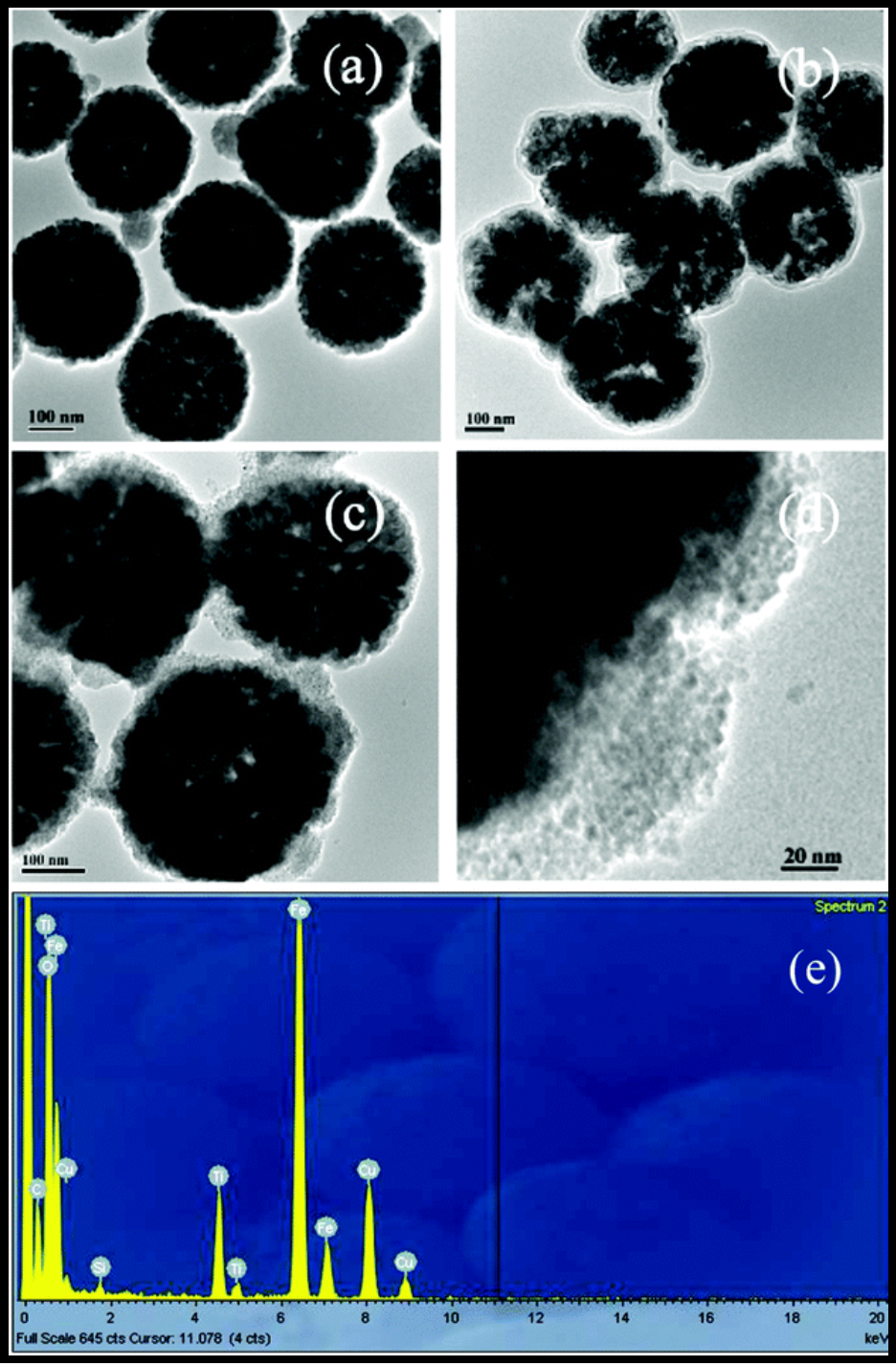




The synthetic route to Fe_3O_4@TiO_2 core–shell microspheres.



The FTIR spectra of (a) as-synthesized Fe_3O_4 microspheres (b)Fe_3O_4@C microspheres and (c) Fe_3O_4@TiO2 core-shell microspheres



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.

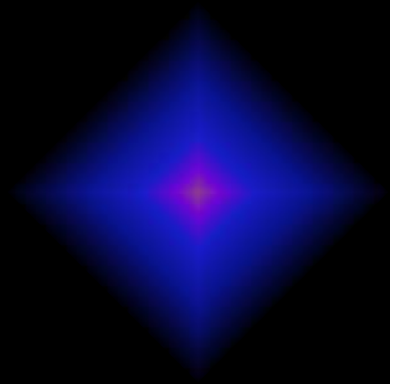
Titania possesses unique optical, electronic and chemical properties.

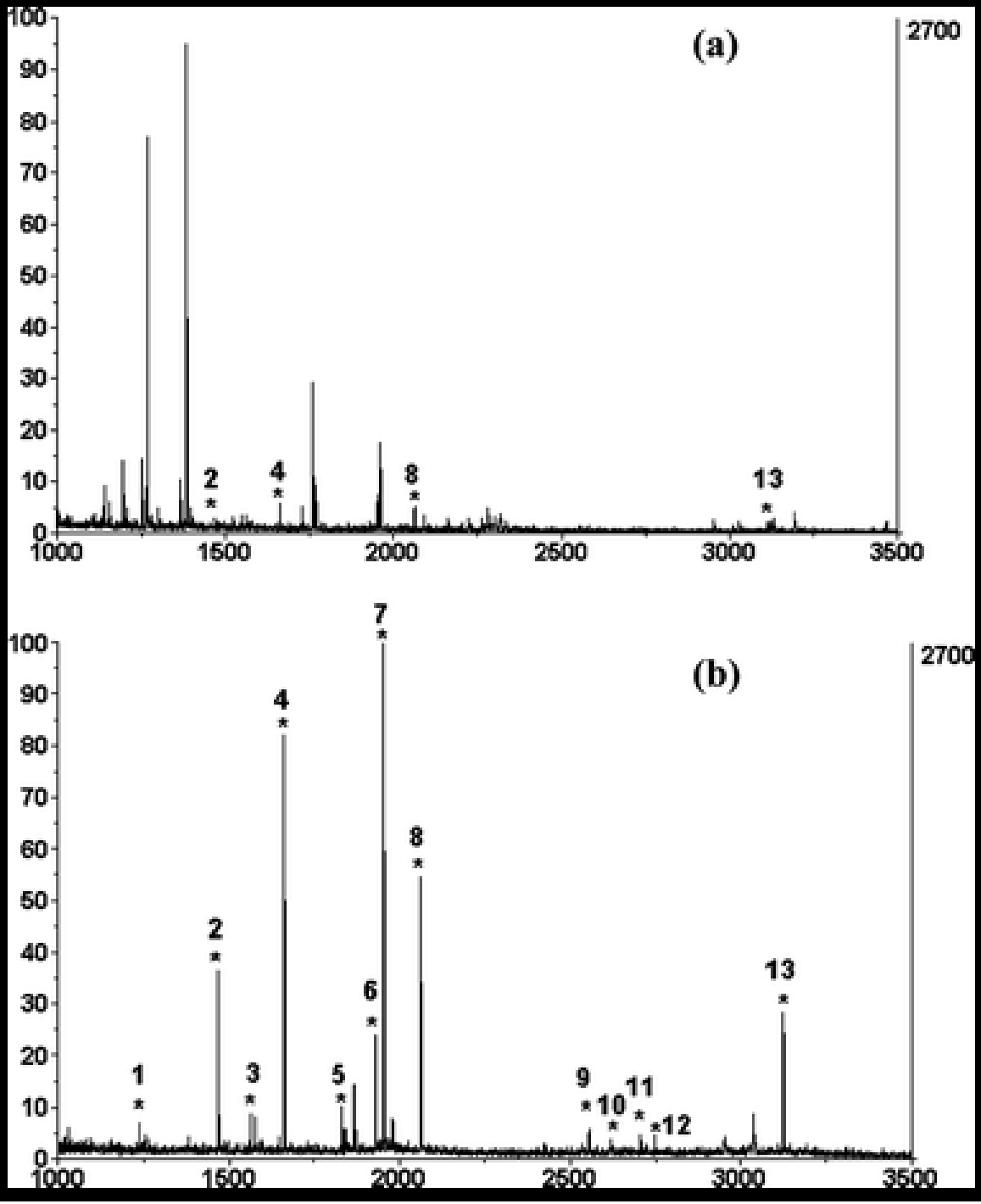
Coating magnetite microspheres with titania could endow the microspheres with many additional useful surface properties and functionalities (photocatalysis, magnetically-assisted separation and enrichment).

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. This enable the $Fe_3O_4@TiO_2$ microspheres to rapidly respond to an applied magnetic field, thus allowing a fast enrichment procedure and 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.

Tryptic digest of casein (composed of α-S1 and α -S2 units, and β-casein) at a low concentration (5 ng μL–1) was taken for study.

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.





MALDI mass spectra of (a) without any pre-treatment,

(b) After treatment

Peaks marked with 4, 5, 6, 7 and 11 (α-S1-casein), 1, 2, 3, 10 and 12 (α-S2-casein) and the remaining peaks, marked with the numbers 8, 9 and 13, are derived from β-casein.

Summary and conclusion:

An innovational pathway is proposed

The synthesized Fe_3O_4@TiO_2 core–shell microspheres were successfully applied to the enrichment and identification of phosphopeptides

The process of enrichment is very facile, efficient and highly selective.

These results are expected to open up a new possibility for the enrichment of phosphopeptides.

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.