Synthesis and characterization of mesoporous zirconia nanocomposite using self-assembled block copolymer template

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Synthesis and characterization of mesoporous zirconia nanocomposite using self-assembled block copolymer template

by

Qinwen Ge

A thesis submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Materials Science and Engineering

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Chapter 1. General Introduction

With the increasing attention of the world climate change and environmental issue, the solution for global warming and the exploring of clean energy have become the most important issues for people all over the world. Many steps have been taken to solve these issues, the widely use of methanol, produced by hydrogenation of carbon dioxide, as a cleaner alternative fuel in fuel cells, is one of the steps. At present, developing an effective catalyst with high activity and selectivity is a key factor to methanol production. Various catalysts have been reported for the synthesis of methanol, such as Cu–ZnO/Al₂O₃ ternary catalyst[¹], ZnO+Cu/ZrO₂ catalyst [²], Cu/Ga₂O₃/ZrO₂ and Cu/B₂O₃/ZrO₂ catalysts [³]. Zirconia, as a catalyst support in this process, which possesses the intrinsic advantages such as stable in hydrogenation environment and chemically resistant. But it has a significant shortage----the specific surface area of zirconia is usually much smaller compared with other similar catalysts, like alumina or silica. So increasing the specific surface area of zirconia particle becomes one important study direction for researchers.

Reduction of NOₓ with NH₃ is another example for solving the environmental issue and global warming problem, as we all know that NOₓ can be a major air pollutant or greenhouse gas when x changes to different values. Furthermore, catalyst also plays a vital role in this process. The commonly used catalyst for this reduction process is V₂O₅/TiO₂, but this traditional catalyst has nonnegligible drawbacks that drive researchers to continue their efforts to find new catalysts. Firstly, V₂O₅ is toxic and volatile at high temperature, which make this catalyst less favorable from the point of
environmental view, and also making it impossible to reuse which leads to high cost in production. Secondly, the catalytic activity of TiO₂ decreases dramatically at higher temperature, indicating this catalyst will not perform well at high temperature. Because of the main drawbacks mentioned above, zirconia becomes a favorable potential replacement for this catalytic application. Firstly, zirconia is nontoxic and less volatile, thus making it more environmentally friendly and easier to reuse. Secondly, zirconia is more stable at high temperature and reducing environment, which maintains its catalytic activity at high temperature. These all contribute to a better performance for catalytic reaction at high temperature, and drawing much attention from the research community. However, in order to have a comprehensive understanding of the features and mechanisms of zirconia catalyst, we should know the structure and properties of zirconium dioxide.

1.1 Zirconium dioxide

1.1.1 Crystal Structure

Zirconium dioxide (ZrO₂), which is also known to be zirconia, is a white powder with three different polymorphs: monoclinic, tetragonal, and cubic. Below 1170 °C, monoclinic phase is stable; between 1170 °C and 2370 °C, zirconia exists in tetragonal phase; while above 2370 °C, zirconia transforms to cubic phase. Figure 1-1 shows the crystal structures of these three polymorphs, tetragonal and cubic zirconia have an eightfold coordination while monoclinic zirconia has a sevenfold coordination.

A typical method to obtain zirconia is to precipitate hydrous zirconia from a zirconium salt solution, and calcine this hydrous zirconia to higher temperatures to get zirconia
crystals. The crystalline zirconia first forms tetragonal phase at around 450 °C, then transforms to monoclinic phase at a wide range of higher temperatures, and can turn into cubic phase at a considerably high temperature.\[8\]

![Crystal structures](image)

**Figure 1-1.** Crystal structures for (a) monoclinic, (b) tetragonal, and (c) cubic\[7\]

The tetragonal to monoclinic phase transformation, which is also known as the martensitic phase transformation, is of great importance due to its contribution to the toughening of ceramics.\[9\] This transformation is a stress induced phase transformation accompanied by a 3% to 5% volume expansion, and contributes to the increase of wear and corrosion resistance, thus making zirconia an important ceramic material.\[10-12\] A lot of works had been done with the purpose of explaining this transformation, Garvie first proposed the mechanism of bulk and surface free energy hypothesis.\[13\] According to his hypothesis, the tetragonal phase has a lower surface free energy than monoclinic phase, but a higher bulk free energy, so the tetragonal phase is stable below a critical particle size of about 30 nm. Since tetragonal zirconia has better catalytic property than monoclinic zirconia, many studies aimed at stabilizing the tetragonal phase.\[14\] So far,
there are two commonly known ways to stabilize the tetragonal phase, one is doping, and 
the other is limiting the zirconia crystal size.\textsuperscript{[13,15-17]} However, the doping agent in 
zirconia is detrimental for catalytic as well as other uses, thus there is an ongoing effort to 
replace this traditional method with new approaches of synthesis of nano-scale zirconia.

1.1.2 Physical Properties
Zirconia is an important ceramic material with attractive physical and mechanical 
properties. It has a melting point of 2715°C, and a boiling point of 4300°C. At room 
temperature, the density of zirconia is about 5.68 g/cm\textsuperscript{3}, and Moh's hardness is about 7. 
Beside the high temperature stability and hardness, zirconia also possesses relatively high 
toughness and strength. Another special property of zirconia is its low thermal 
conductivity, combined with the non-magnetic electrical insulator nature, making it a 
potential candidate for many thermal applications.\textsuperscript{[18,19]}

1.1.3 Chemical Properties
Zirconia is a chemically inert material; it has a good corrosion resistance in acids and 
alkalis. Zirconia is also an amphoteric material, which has both acidic and basic 
properties. Another outstanding property of zirconia is stability under reducing conditions, 
which makes it an important material in catalytic field.\textsuperscript{[7]}

1.1.4 Applications
Due to the variety of attractive properties mentioned above, zirconia is widely used in 
many fields as listed below\textsuperscript{[7,20-24]}:

Refractory materials: the high melting point makes zirconia a good refractory material, 
and it finds important utilization in high temperature applications. For instance, zirconia
can be used as thermal barrier coating in jet engines, making use of its low thermal conductivity and low thermal expansion coefficient combined with its refractory property. It can also be used as hot metal extrusion dies, due to its refractory nature as well as high hardness, high toughness and strength. Fuel cell and sensors: zirconia has a high ionic conductivity especially for oxygen ions, so it is often used in high temperature fuel cells and as oxygen sensors. Zirconia is an insulator at ambient temperatures, but will turn into super ionic conductor at very high temperature. Because of its good biocompatibility, high hardness and strength, zirconia often used as orthopedic implants, such as femoral head component in hip implants and dental implants. As discussed above, zirconia is chemically and thermally stable, combined with its unique amphoteric characteristic, makes it an ideal candidate for catalyst. For example, the hydrogenation of aromatic carboxylic acids, the decomposition of nitrous oxide, the isomerization of alkanes, and many other reactions.

Zirconia, as a well-known catalyst, possesses both the common advantages of transition metal compound catalysts and its own unique merits:\[5\]:

1) The incomplete d sub-shell provides a location for the electrons of reaction chemicals to sit in, in other words, the incomplete d sub-shell can serve as a clamp which can bind the reagents to the metal surface, thus making it a good catalyst.

2) The high melting point and hardness is a big advantage for zirconia as a catalyst, which allows it to perform in extreme environments such as high temperature catalysis.
3) The amphoteric property is considered to be another advantage, the relative acidic and basic sites present on the surface of metal oxides alter the catalytic properties.

4) Chemically inert, a significant requirement with respect of catalytic uses.

5) Stable under reducing conditions, making it possible in catalytic reduction reactions such as the reduction of NO\textsubscript{x} mentioned above.

6) Moreover, zirconia has many practical advantages such as nontoxic, easy to handle, able to reuse, and less expensive, thus making it an important acidic catalyst in chemical industry.

However, the problem of low surface area of zirconia (< 100 m\textsuperscript{2}/g) strongly limits its use especially in catalytic applications\textsuperscript{[6]}. It is known that a relatively high surface area catalyst can often provide more active sites thus giving higher catalytic activity\textsuperscript{[7]}. Also, a higher surface area catalyst with suitable pore structures is conducive to higher metal dispersion. Furthermore, higher surface area catalyst has significant advantage in catalyzing gas reactions due to high adsorption capacity. These factors are all contribute to the higher performance of a catalyst, thus making high surface area an important requirement in catalytic applications. So there is a significant interest for exploring synthesis methods for high surface area zirconia with suitable pore structures.

1.1.5 Synthesis Methods

As mentioned above, preparing high surface area zirconia becomes a main direction for researchers, and many synthesis methods have been reported to obtain high surface area tetragonal zirconia with suitable pore sizes.\textsuperscript{[25-40]}
• Sol-gel method: a soluble metal salt precursor acts as ‘sol’, and gradually evolves towards the formation of a gel-like network containing both a liquid phase and a solid phase; during which procedure undergoes various forms of hydrolysis and polycondensation reactions.

• Precipitation method: this method starts with a metal salt solution, after the addition of precipitating agent; the product condenses as a solid phase thus separate from the original liquid phase. For example, zirconia can be synthesized by simply adding NH₄OH to ZrCl₄ salt solution.

• Hydrothermal synthesis: in this method, the product crystallizes from high-temperature aqueous solutions at high pressures. For instance, the growth of zirconia nano-rods can form ZrO₂ powder and NaOH solution at about 200 °C.

• Solid-state reaction: this method involves a mixture of solid reactant materials. For example, ZrO₂ will form by reacting ZrCl₄ with LiOH.

• Microwave-assisted method: microwave irradiation can induce the crystallization of zirconia.

• Reverse micelle method: this procedure often takes place in reverse micellar solutions. For instance, tungsten-promoted zirconia (WO₃/ZrO₂) nanoparticles can be synthesized in reverse micelles of water/sodium bis (2-ethylhexyl) sulfosuccinate/isoctane.

• Hard and soft templating method: this procedure takes place in the presence of template or surfactant. For example, zirconia can be synthesized using Pluronic P123 block copolymer as the surfactant.
Among these methods, sol-gel method is the most studied, since zirconia synthesized using this method has advantages like high purity, homogeneity, reproducibility, and simple maneuverability since this method doesn’t require extreme environment or difficult inducing conditions. Moreover, sol-gel method is low temperature, which can reduce the loss of volatile components thus more environmentally friendly.

1.2 Template assisted sol-gel method

Template assisted sol-gel method is a combination of sol-gel method and templating method, it has the advantages of both sol-gel and templating methods, thus widely used and studied. The template used in this method works as a structure directing agent, and plays a vital role in the synthesis process.

1.2.1 Templates

Template is a material that can be used as a structure-directing agent for organizing different material networks in the synthesis procedure. Block copolymers, especially amphiphilic block copolymers, are often used as a template to organize mesostructured composites \[41-43\]. Much research has been reported using a variety of polymer templates. Yang et al. synthesized mesoporous metal oxides, with semicrystalline frameworks using Pluronic P-123 as template in organic solvents \[42, 43\]. Jiahe Liang et al. used polyvinyl alcohol (PVA) as a template in synthesis of stable t-ZrO$_2$ nanoparticles \[44\]. Eltejaei and et al. used PEG–PPG–PEG block copolymer to synthesize high surface area mesoporous zirconia powders, and found the addition of this template as well as a surfactant had a positive effect on the specific surface area and small nanocrystalline size \[45\]. Shukla and his coworkers employed hydroxypropyl cellulose (HPC) in the synthesis of nano-sized
zirconia and found that HPC can prevent the agglomeration tendency of nanosize zirconia particles [46]. Yanjun and et al. used cationic enzyme Lysozyme to synthesize zirconia nanocomposites with an enhanced thermal and pH stability in the immobilization process of yeast alcohol dehydrogenase [47]. Jingfang and et al. used agarose as a template to synthesize macroporous zirconia, the template also found to influence the morphology of the final zirconia product as well as increase the specific surface area [48].

The templates used in the present study are listed below.

- Pluronic F127: poly(ethyleneoxide)-poly(propyleneoxide)-poly(ethyleneoxide) (E\textsubscript{100} P\textsubscript{65} E\textsubscript{100}) is a nonionic triblock copolymer, the polyoxyethylene chain is hydrophilic and the polyoxypropylene is hydrophobic, the structure is shown below in Figure 1-2 [49].

![Figure 1-2 Chemical structure of Pluronic F127](image)

It is a thermoreversible gel, whose behavior is sensitive to temperature and concentration [50]. As shown schematically in Figure 1-3, at low concentration or temperature, the Pluronic F127 exists in unimer form; when the temperature or concentration is increased, the unimers form micelles with a hydrophobic central core and hydrophilic chains in the medium; while at high concentration or temperature, the micelles can aggregate to from gel [49, 50].
Lysozyme: Lysozyme (MW 14.7 kDa, 147 amino acid residues, pI=10.5) is a cationic enzyme obtained from chicken egg white, this protein can hydrolyze the glycosyl groups and has a relatively high decomposition temperature, which finds application in biomineralization [47]. The full sequence of amino acids for Lysozyme from egg white is listed below in Figure 1-4 [51], and the abbreviation of amino acids are listed in Table 1-1 [52].
Figure 1-4 Full sequence of amino acids for Lysozyme from egg white[51]
Table 1-1 Abbreviation for the amino acids\cite{52}

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Amino acid</th>
<th>Abbreviation</th>
<th>Amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>Alanine</td>
<td>Leu</td>
<td>Leucine</td>
</tr>
<tr>
<td>Arg</td>
<td>Arginine</td>
<td>Lys</td>
<td>Lysine</td>
</tr>
<tr>
<td>Asp</td>
<td>Aspartic acid</td>
<td>Met</td>
<td>Methionine</td>
</tr>
<tr>
<td>Asn</td>
<td>Asparagine</td>
<td>Phe</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Cys</td>
<td>Cysteine</td>
<td>Pro</td>
<td>Proline</td>
</tr>
<tr>
<td>Gln</td>
<td>Glutamine</td>
<td>Ser</td>
<td>Serine</td>
</tr>
<tr>
<td>Glu</td>
<td>Glutamic acid</td>
<td>Thr</td>
<td>Threonine</td>
</tr>
<tr>
<td>Gly</td>
<td>Glycine</td>
<td>Trp</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>His</td>
<td>Histidine</td>
<td>Tyr</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>Ile</td>
<td>Isoleucine</td>
<td>Val</td>
<td>Valine</td>
</tr>
</tbody>
</table>

- **Block copolymer-lysozyme conjugate:** This conjugate polymer is prepared by attaching the Lysozyme to the modified Pluronic F127 using an NHS-based coupling method \cite{53}. Firstly, the hydroxyl end groups of Pluronic F127 were converted to carboxyl groups by reacting it with succinic anhydride in pyridine. Then the carboxyl-terminated PF127 was activated by reaction with NHS at room temperature for 24 h. Finally, the NHS-activated PF127 was covalently bonded (conjugated) with lysozyme.

- **Penta-PDEAEM:** The cationic pentablock copolymer (PDEAEM-based) exhibits reversible pH and thermo-responsive behavior, and was synthesized by atom transfer radical polymerization (ATRP). Poly (diethyl amino ethyl methacrylate) (PDEAEM) blocks were grown to the ends of PF127 by ATRP as described earlier, and its structure is shown in Figure 1-5 \cite{54}.
These templates are all self-assembled copolymers. Self-assembly of block copolymers, surfactants, colloidal suspensions and proteins, which create nanostructures through versatile approaches, play a key role in directing the formation of organized porous structures.\[^{41, 55, 56}\]

1.2.2 Self-assembly

Self-assembly is a process of forming an organized structure or pattern from a disordered system of pre-existing components without external direction\[^{21, 41}\]. This method is often used in the synthesis of mesoporous materials, and the supramolecular assembly property of this kind of templates is often used to direct the formation of special structures.

1.2.3 Process parameters and their influences

There are several process parameters that have significant influence on the final properties of zirconia prepared using this sol-gel method, some of them are listed below.

- Zirconium salt: several soluble zirconium salts had been reported as precursors in this sol-gel method including zirconium (IV) oxynitrate hydrate (ZrO\(_2\)).
(NO₃)₂·xH₂O), zirconium nitrate hydrate (Zr(NO₃)₄·3H₂O), zirconyl chloride octahydrate (ZrOCl₂·8H₂O), zirconium perchlorate hydrate (ZrO(ClO₄)₂·xH₂O), potassium hexafluorozirconate (K₂ZrF₆), and zirconium(IV) butoxide (Zr(OC₃H₇)₄). Due to its low solubility, potassium hexafluorozirconate, is not widely used. Zirconium perchlorate hydrate leads to toxic by-products, zirconium (IV) butoxide will involve organic side products, thus are not widely used due to handling difficulty. The other three kinds of precursors are commonly used. However, zirconium (IV) oxynitrate hydrate is favored, since it does not leave any undesirable impurity upon heat treatment of the precursor.

- Precursor concentration: several studies were reported with respect to the zirconium solution concentration effect, the results showed that lower precursor concentration is conducive to obtaining thermally stable tetragonal zirconia nanoparticles with higher specific surface area as well as smaller particle sizes [8, 14, 45, 57, 58].

- Surfactant-to-zirconium molar ratio: Based on the studies reported so far, there is no direct correlation between surfactant-to-zirconium molar ratio on the properties of zirconia, but it is confirmed that the addition of surfactant leads to the increase in specific surface area as well as a decrease in particle size [39, 59].

- Precipitation pH: The effect of pH was found to be profound in sol-gel synthesis procedure. The solubility difference of hydrous zirconia under different pH was put forth to explain the crystallization path and properties of zirconia. At low pH, the solubility is high and the amorphous hydrous zirconia tends to form monoclinic phase; at intermediate pH, the solubility is low and the amorphous
hydrous zirconia tends to form tetragonal phase first; at high pH, though the solubility is high, the in situ crystallization of hydrous zirconia to tetragonal phase predominates over transformation of the monoclinic phase [7]. Rezaei and his coworkers also reported that at high pH, the increase of pH will lead to increase in specific surface area as well as smaller particle size and more stable tetragonal phase [59].

- Digestion time and temperature: digestion is very important for preparing high surface area zirconia, usually longer digestion time leads to higher surface area and more stable tetragonal phase, the digestion time reported is from 0 to about 200 hours [7, 57, 59, 60]. The digestion temperature often used is from room temperature to about 130°C, while 80°C was the most often used due to a phase transformation of the hydrous precursor at around 80°C [7, 57, 59, 60]. And these studies all indicated that digestion leads to a higher surface area powders.

- Calcination: most properties of zirconia vary as a function of calcination temperature. During calcination from room temperature to 1000°C, zirconia transform from amorphous to tetragonal to monoclinic accompanied by a growth of crystal; the specific surface area increase first and then decrease, the highest surface area can be obtained around 450°C to 650°C ranging from 50 to 400 m²/g for different systems [39, 57-59].

Based on these earlier studies, we have a basic understanding about template assisted sol-gel method and the parameters that can influence it. In our study, we still chose
this synthesis method due to its variety advantages mentioned above, and we investigated two brand-new templates (Pluronic-lysozyme conjugate, and penta-PDEAEM), studied their effects and reported them in chapter 2. Furthermore, we expanded this study to how different preparation conditions (calcination temperature, reaction pH and precursor concentration) influence the properties of the final zirconia product, including the particle size, the structure, morphology, and the specific surface area, this detailed study was based on one of our new templates----Pluronic-lysozyme conjugate, and the results are reported in chapter 3. Finally, we summarized our study and the conclusions we obtained in chapter 4.

References


Chapter 2. Bioinspired synthesis and characterization of mesoporous zirconia templated by cationic block copolymers in aqueous media

A paper submitted to Chemistry of Materials

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Abstract

Mesoporous zirconia has attracted great attention from the research community due to its unique properties such as high surface area, uniform pore size distribution, and large pore volume. Self-assembled structures have been used as directing agents to synthesize mesoporous zirconia. Here, we investigate the use of block copolymers conjugated to cationic biomolecules such as lysozyme, as well as cationic block copolymers as templates for synthesis of mesoporous zirconia in completely aqueous media. This is the first report for synthesis of mesoporous zirconia in completely aqueous media, thereby opening up opportunities for introducing biomolecules for zirconia synthesis.

Thermogravimetric analysis (TGA) was used to determine the inorganic content of the nanocomposite. X-ray diffraction (XRD), nitrogen adsorption, transmission electron microscopy (TEM) along with scanning electron microscopy (SEM), small angle neutron scattering (SANS), and solid state nuclear magnetic resonance (NMR) were employed to characterize the samples. The results showed zirconia crystals formed after 500 °C
calcination of the as-synthesized nanocomposite, and the crystals were larger after 900 °C calcination. The conjugate templated zirconia had a surface area of 174 m²/g after 500 °C calcination, and retained its tetragonal structure even after calcining at 900 °C for 3 hours. The cationic pentablock copolymer templated zirconia had the highest surface area 191 m²/g after 500 °C calcination, and also had improved thermal stability. This method could be easily scaled up and used for synthesis of other oxides.

1. Introduction

In recent years, zirconia has received much attention from the materials research community due to its attractive intrinsic properties such as hardness, shock wear, excellent acid and alkali resistance, low frictional resistance, and high melting temperature.¹ It has been widely used in applications, such as gas sensors, solid oxide fuel cells (SOFCs), catalysis, catalysis carriers, and orthopaedic implants.¹–⁴ However, bulk zirconia synthesized by the conventional methods usually have relatively low specific surface area (<100 m² g⁻¹) and microporosity, which limit some of its applications.⁴ Much research has been focused on increasing the specific surface area of zirconia. Synthesis of mesoporous zirconia has received significant attention, due to its high surface area, uniform pore size distribution, and large pore volume.⁵

A number of synthesis methods such as sol–gel synthesis, aqueous precipitation, thermal decomposition, and hydrothermal synthesis have been reported.¹ However, some of these methods require extremes of temperature or pressure, or are costly. A large number of researchers have focused on the preparation of porous zirconia with high specific surface area by using self-assembled structures as directing agents. The unique of the synthesis of
mesoporous materials is its focus on the use of supramolecular assembly of template molecules as structure-directing agents. Self-assembly of block copolymers, surfactants, colloidal suspensions and proteins, which create nanostructures through versatile approaches, plays a key role in directing the formation of organized porous structures.5–7

Block copolymers, especially amphiphilic block copolymers, have been increasingly used to organize mesostructured composites. For example, Pluronic block copolymers have been used as structure-directing agents for organizing different inorganic material networks.5,8–10 Yang et al. synthesized large-pore mesoporous metal oxides, with semicrystalline frameworks using Pluronic P-123 as template in organic solvents.

Previous research on templated zirconia synthesis was almost exclusively carried out in organic solvents.4–6,8,11–14 This is probably the reason that there are hardly any bioinspired methods for the synthesis of zirconia, although such approaches provide environmentally clean and energy-conserving processes, and have been widely used to synthesize other oxides such as silica.15 A few studies have recently reported biosynthesis of zirconia, but these methods suffer from several limitations.1,16 Bansal et al. synthesized zirconia nanoparticles using the fungus Fusarium oxysporum, and pointed out that a cationic protein in the fungus may play an important role in the biosynthesis.1 Although zirconia was successfully synthesized under mild conditions, this study was limited by the availability of the fungus, the difficulties in identification and separation of the protein, and the very low yield of zirconia. Jiang et al. reported zirconia synthesis catalyzed and templated by lysozyme.16 This protein (MW 14.7 kDa, 147 amino acid residues, pI=10.5) is one of the most prominent members in the class of cationic enzymes, which can
hydrolyze the glycosyl groups. Recent reports indicate the involvement of lysozyme in the biomineralization of silica, titania, and calcium carbonate, and heat-denatured lysozyme has also been implicated in the synthesis of bismuth sulfide, although the mechanism is unclear. However, the zirconia yield was very limited due to the low solubility of the precursor (K₂ZrF₆). In addition, the fluoride precursor is neither environmentally safe, nor inexpensive for large scale zirconia production. Moreover, neither of these were mesoporous zirconia.

Previously we have used bioinspired approaches involving Pluronic polymers conjugated to mineralization peptides to template calcium phosphate nanocomposites. Using a similar approach in this study, we have used Pluronic triblock as well as cationic pentablock copolymer templates conjugated with lysozyme as the mineralization protein to synthesize mesoporous zirconia with high surface area in completely aqueous media. Aqueous solutions of Pluronic F127 (PF127) and the cationic pentablock copolymers (poly(diethylaminoethylmethacrylate) (PDEAEM) blocks attached to PF127) self-assemble into spherical micelles at low temperature and concentration, and transform to viscous gels with increasing temperature (typically above 25 °C) or concentration. Since cationic groups have shown to be important for zirconia synthesis in aqueous solutions, the PDEAEM is responsive to pH changes and provides tertiary amine groups. In addition, at specific temperature and pH ranges (T>50 °C and pH of 8-11), PDEAEM-based pentablock copolymers can form cylindrical micelles in aqueous solutions. These various self-assembled polymer micelle structures were used as templates for the mesoporous zirconia formation. The synthesized zirconia was characterized and found to exhibit high specific surface area, porosity and thermal stability even at high
temperatures. This is the first time that mesoporous zirconia was reported to be synthesized in completely aqueous media, with a potential to be scaled up relatively easily.

2. Experimental Section

2.1 Chemicals and Materials

Triblock copolymer poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) Pluronic F127 (PF127) (PEO\textsubscript{100}PPO\textsubscript{65}EO\textsubscript{100}, $M_n = 12600$ g mol$^{-1}$), lysozyme from chicken egg white, zirconium(IV) oxynitrate hydrate (ZrO(NO\textsubscript{3})\textsubscript{2}) (99.99%), N,N-(diethyl amino)ethyl methacrylate (DEAEM), succinic anhydride, and N-hydroxy succinimide(NHS) were all purchased from Sigma-Aldrich. Ammonium hydroxide was purchased from Fisher scientific. All of the chemicals were used as received without further purification. A bicinchoninic acid (BCA) protein assay kit and dialysis cassettes were purchased from Thermo Scientific. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) kit was purchased from Bio-Rad.

2.2 Synthesis of Pluronic-lysozyme conjugate, and pentablock copolymers

The Pluronic was conjugated to the lysozyme using an NHS-based coupling method.\textsuperscript{18} The hydroxyl end groups of PF127 Pluronic were converted to carboxyl groups by reacting it with succinic anhydride in pyridine. The carboxyl-terminated PF127 was activated by reaction with NHS at room temperature for 24 h. The NHS-activated PF127 was then covalently bonded (conjugated) with lysozyme. After reacting at room temperature for 24 h in PBS buffer, the mixture was dialyzed against water, using a cellulose ester membrane with a molecular weight cut off of 25,000 (Thermo Scientific),
for 4 days at room temperature to remove the unreacted polymer and lysozyme. The mixture was then freeze dried to form the solid conjugate.

The cationic pentablock copolymer (PDEAEM-based) exhibits reversible pH and thermo-responsive behavior, and was synthesized by atom transfer radical polymerization (ATRP). Poly(diethyl amino ethyl methacrylate) (PDEAEM) blocks were grown to the ends of PF127 by ATRP as described earlier.\textsuperscript{21}

Scheme 1. Structure of the cationic pentablock copolymer (PDEAEM-based pentablock)

2.3 Characterization of the conjugate

The synthesized conjugate was characterized by fast protein liquid chromatography (FPLC) and SDS-PAGE to confirm covalent binding between the PF127 and lysozyme, and to estimate the molecular weight. A BCA assay was used to determine the percentage of lysozyme in the conjugate. Dynamic light scattering (DLS) was used to observe the micelle sizes of the conjugates formed in aqueous media.

**FPLC.** The size-exclusion chromatography studies were carried out using an AKTA FPLC system (GE healthcare) through a prepacked Superdex 75 column. The flow rate was 0.4 mL/min. The sample was the solution right after the conjugation reaction of Pluronic-NHS and lysozyme. The intensity was measured based on the absorbance of UV
light (A280) by the aromatic amino acids. The fractions of the FPLC were collected at 0.5 mL each, and selected to run SDS-PAGE to estimate the molecular weight of the fractions.

**DLS.** The diameter of the micelles formed by the PF127 and PF127-lysozyme conjugate in water was determined using quasi-elastic light scattering (Zetasizer Nano, Malvern Instruments Ltd.) with laser irradiation at 633 nm.

### 2.4 Synthesis of the zirconia nanocomposite and mesoporous zirconia

Since a sol-gel process was important for the polymer to template the zirconia formation, the amount of polymer template in each sample was chosen at a percentage that allows the polymer and precursor mixture to form a viscous solution at 4 °C and self-assemble to form a physical gel at room temperature. The conjugate amount was limited by its solubility in water; therefore, additional PF127 was added to help gel formation during the aging process.

In a typical synthesis, the zirconia precursor solution was prepared by dissolving 1.15 g ZrO(NO$_3$)$_2$ into 10 mL water. To this solution, 3.0 g PF127, 2.6 g PF127 and 0.4 g conjugate, or 2 g PDEAEM was added and dissolved at 4 °C. The solution was then brought up to room temperature, which resulted in formation of a gel within 30 minutes. After the gel formation, ammonium hydroxide (28-30%) was added with stirring until the pH was equal to 10. A few samples were prepared at pH=4. A white precipitate was formed within the gel when ammonium hydroxide was added. The precipitated gel was aged at room temperature or 60 °C for 3 days before washing three times with water to remove the ammonium nitrate, excess ammonium hydroxide, and free polymer. The
sample was then frozen in liquid nitrogen and lyophilized for two days to obtain polymer/zirconia nanocomposite powder. The composite was heated to 500 °C or 900 °C for 3 hours in air to pyrolyze the polymer template and form mesoporous zirconia for further characterization. Six representative samples are selected for detailed characterization as shown in Table 1:

Table 1. List of samples with their different polymer templates and aging conditions

<table>
<thead>
<tr>
<th>Template</th>
<th>No template</th>
<th>PF127</th>
<th>PF127 + Conjugate</th>
<th>Pentablock</th>
<th>Pentablock</th>
<th>Pentablock</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aging Temp., °C</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>pH</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

2.5 Characterization of the zirconia nanocomposite and mesoporous zirconia

Polymer/zirconia nanocomposite and mesoporous zirconia samples were characterized by thermogravimetric analysis (TGA), X-ray diffraction (XRD), nitrogen sorption, transmission electron microscopy (TEM), scanning electron microscopy (SEM), small angle neutron scattering (SANS), and solid state nuclear magnetic resonance (NMR) techniques.

TGA. The percentage of inorganic material in the nanocomposite was determined by TGA, which was performed on TGA 7 equipment (Perkin Elmer). Approximately 10 mg of the freeze dried nanocomposite sample was placed in a platinum pan, and was heated from room temperature to higher temperature at a rate of 10 °C/min under 20 mL/min air flow. Isothermal heating at 500 °C for 3 hours was applied to some samples to calculate the ZrO₂ yield of the process after template is completely removed.
**XRD.** The crystal structure of the calcined zirconia was studied by an X-ray diffractometer (X’Pert PRO, PANalytical Inc.). The diffractometer was operated at 45 kV and 40 mA. The monochromatic light was from CuKα radiation with a wavelength of 0.15418 nm. The scan rate was 0.021°/s with a step size of 0.017°, over the range of 20° ≤ 2θ ≤ 80°. X’pert Data collector software was used to collect the data.

**Nitrogen sorption.** Nitrogen adsorption and desorption runs were carried out using an AUTOSORB-1 QUANTACHROME Instrument (Quantachrome Corporation). The adsorption and desorption isotherms of the 500 °C calcined zirconia were obtained at 77 K. All samples were degassed under vacuum at 110 °C prior to adsorption. Surface area was calculated by applying the Brunauer-Emmett-Teller (BET) method and using the data in the 0.03< P/P₀< 0.3 range. The pore size distribution was calculated using the Barrett-Joyner-Halenda (BJH) method and the desorption branch of the isotherm. The total pore volume was calculated based on the volume of nitrogen adsorbed near saturation pressure.

**TEM.** Imaging of the samples was performed using a Tecnai G2 F20 Scanning Transmission Electron Microscope (STEM) (FEI Company, Hillsboro, OR) equipped with High Angle Annular Dark Field (HAADF) and Energy Dispersive X-ray Spectroscopy (EDS) detectors at an operating voltage of 200 kV. 20 µL of diluted zirconia powder suspension was deposited on a holey carbon-supported copper grid. Multiple areas of each sample were examined.

**SEM.** Morphological features of the synthesized mesoporous zirconia were studied by FEI quanta 250 SEM (FEI Company, Hillsboro, OR). 20 µg of zirconia powder sample
was dispersed on a carbon stub, and was sputter-coated with iridium for imaging. Multiple areas of each sample were examined.

**SANS.** This technique was used to highlight the structure of the block copolymer in the nanocomposite gels. In a deuterated solvent, the polymer phase has higher neutron-scattering contrast than the inorganic phase. A specific batch of samples using D$_2$O as solvent was prepared for the characterization. The SANS measurements were performed on the Low-Q Diffractometer (LQD) of the Lujan Center at Los Alamos National Laboratory (LANL). The nanocomposite samples during the aging stage were sealed in quartz benjo cells with a 2-mm path length. The scattering vector, $q$, was varied between $0.003 \, \text{Å}^{-1} < q < 0.3 \, \text{Å}^{-1}$, where $q = (4\pi/\lambda) \sin(\theta/2)$ with the neutron wavelength $\lambda$ and the scattering angle $\theta$. The scattered intensity $I(q)$ was placed on an absolute scale in the units of cm$^{-1}$. SANS data were reduced by software provided at the Lujan center and corrected for empty-cell and background scattering.

**NMR.** Solid-state NMR spectra were obtained using a Bruker Biospin DSX-400 spectrometer (Bruker-Biospin, Billerica) at 400 MHz for $^1$H and 100 MHz for $^{13}$C. A Bruker 7-mm double-resonance magic-angle-spinning (MAS) probe-head was used with a spinning frequency of 7 kHz. The 90° pulse length was 4.5 $\mu$s for $^1$H and 4 $\mu$s for $^{13}$C. A 150-s recycle delay was used in direct-polarization $^{13}$C NMR experiments, with two-pulse phase-modulation (TPPM) heteronuclear decoupling during detection.
3. Results and Discussion

3.1 Characterization of the Pluronic-lysozyme conjugate

The covalent attachment between PF127 and lysozyme was confirmed by FPLC and SDS-PAGE, shown in the supporting information. The conjugate had a molecular weight of about 30 kDa. Some larger molecular weight fractions were also detected, which may be due to the non-specific binding between the NHS-modified PF127 and the free amine groups in lysozyme. The BCA assay indicated that lysozyme constitutes 38 wt.% of the conjugate.

Figure 1 shows the diameter of the lysozyme molecules and of the micelles formed by PF127 and conjugate as determined by dynamic light scattering (DLS). The conjugate micelles were larger than the PF127 micelles, and the increase was probably due to the lysozyme end groups on the conjugate. A small fraction of larger micelles (around 13 nm) observed for the conjugate is attributable to and consistent with the formation of the larger molecular weight conjugates mentioned above.
Figure 1. DLS of lysozyme, conjugate and PF127 in water, molecule or micelle size distribution by number

3.2 Characterization of zirconia nanocomposite and mesoporous zirconia

TGA. A thermogravimetric analyzer was used to determine the decomposition temperature of the polymeric templates and the fraction of the inorganic phase in the composite. Figure 2 shows the TGA results obtained by heating the pure polymer templates. PF127 was totally burned off by 400 °C. Lysozyme was burned off once the temperature reached 700 °C. To mimic the annealing conditions, pure pentablock, lysozyme and the conjugate were heated to 500°C and held at 500 °C for 3 hours. After a 3-hour isothermal hold at 500 °C, almost all the organics were gone. Therefore, only inorganic zirconia was left after the freeze-dried nanocomposite had been calcined at 500 °C for 3 hours.
The inorganic content of each zirconia nanocomposite sample can be estimated from Figure 3. The sample prepared under identical conditions except without any organic template resulted in 77% zirconia, and the 23% weight loss corresponds to almost exactly the dehydroxylation of the Zr(OH)$_4$ precipitate according to:

$$\text{Zr(OH)}_4 \rightarrow \text{ZrO}_2 + 2\text{H}_2\text{O}$$

More organics remained in the conjugate templated sample than in the PF127-templated one (i.e. the zirconia content was lower in the conjugate than in PF127), indicating that the lysozyme helped enhance the interactions between the template and the precursor, which was also confirmed by solid state NMR as discussed later. Although the mechanism is still unknown, the cationic characteristics of lysozyme may play an important role.\textsuperscript{1,16} The fact that the cationic pentablock templated samples had an even higher fraction of polymer, about 55% to 65%, indicate its more extensive interactions with the zirconia precursor.
**Figure 2.** TGA of organic templates: PF127 and lysozyme were heated to 500 °C and 900 °C, respectively, with a ramp of 10 °C/min; pentablock, lysozyme and conjugate were heated to 500 °C with a ramp of 10 °C/min, and then kept at 500 °C for an isothermal step for 3 hours.
**Figure 3.** TGA for freeze dried zirconia nanocomposites with different templates shown in the legend. The aging conditions were 20°C and pH 10. All the samples were heated to 900 °C at a rate of 10 °C/min.

**13C NMR of the organic components in the composites.** Figure 4 shows 13C NMR spectra of the organic templates and the organic-inorganic composites. The spectra were obtained by direct polarization after long (150-s) recycle delays for full relaxation, which avoids the selective signal enhancement seen in standard cross polarization from 1H. The spectra of lysozyme and PF127 are clearly distinct, with characteristic C=O and N-CH peaks of lysozyme near 173 and 55 ppm, and OCH signals of PF127 between 70 and 75 ppm. This enables peak assignment in the spectra of materials containing both components. In PF127, the signal of crystalline PEO at 70 ppm is almost invisible, most likely due to line broadening by helical jumps that interfere with H-C dipolar decoupling. The signal of the CH₃ groups in the PPO block near 17 ppm is observed at the expected
intensity level. The spectrum of the conjugate template, see Fig. 4(c), is dominated by the signals of PF127, since the lysozyme signals, e.g. near 173 and 55 ppm, are broad and low.

Figure 4(d) shows the spectrum of the composites with PF127 template. The signal intensity is ca. 20 times smaller than in neat PF127, indicating that only a relatively small fraction of the polymer is strongly bound to zirconia. In addition to the regular PF127 signals, the spectrum shows unexpected peaks at 180 ppm and 23 ppm, which are also observed in the spectra of the other mineralized samples. The chemical shifts are consistent with acetyl groups, -OOC-CH₃, but their origin remains unclear.

Figures 4(e) and (f) compare the spectra of the composites templated by lysozyme-PF127 conjugate and by lysozyme and PF127 physical mixture. In these spectra, the signals of lysozyme are clearly visible and their area exceeds that of the PF127 peaks by an order of magnitude. In other words, the PF127:lysozyme ratio is reduced by a factor of 10 to 20 relative to the neat template in Figure 4(c). This demonstrates selective retention of lysozyme, which can be attributed to its stronger binding to zirconia. The spectrum from the conjugate exhibits a significantly larger total area than that of the physical mixture, which indicates stronger binding of the conjugate template to zirconia.
Figure 4. Direct-polarization $^{13}$C NMR spectra of the organic templates: (a) pure lysozyme; (b) pure PF127 triblock copolymer; (c) pure PF127-lysozyme conjugate template; and $^{13}$C NMR spectra of the freeze dried organic-inorganic composites with different organic templates: (d) PF127 template; (e) PF127-lysozyme conjugate template. The signal at 111 ppm is background from Teflon tape used to balance the magic-angle-spinning rotor. (f) Mineralized physically mixed PF127 and lysozyme. All spectra were scaled to account for differences in sample mass. Recycle delay: 150 s; magic-angle-spinning frequency: 7 kHz.

XRD. The XRD patterns shown in Figure 5 clearly indicate that the amorphous zirconia precursors were completely crystallized to tetragonal zirconia after calcination at 500 °C.2
The broad peaks indicate that the size of the crystals was in nanometer range and the calculated crystal sizes according to the Debye-Scherrer formula (using the peak at $2\theta = 30^\circ$) are listed in Table 2. The results are consistent with the TEM data shown in the following. The zirconia synthesized without any templates showed sharper and stronger peaks than any other samples, which demonstrated it had the largest crystal size. As shown in TEM micrographs and Table 2, polymer templation results in smaller crystallites and higher specific surface as will be discussed below. The benchmark sample prepared without polymer template shows a small peak at $2\theta = 28^\circ$ indicative of monoclinic phase. Apparently, polymer templates prevent formation of monoclinic phase at this temperature either by forming smaller crystals or directing organization of the precursors.

The zirconia templated by pentablock copolymers and aged at 60 °C had broader peaks than the other samples. This implied that it had the smallest crystal size and may have the largest surface area among the samples, which was confirmed by the TEM and BET surface area measurements, and could be attributed to the reversible thermal and pH responsive properties of cationic pentablocks. The pentablock solution exhibits micellar and gel phases in response to changes in both the temperature and pH by virtue of the lower critical solution temperature of the PPO and PDEAEM blocks and the polyelectrolyte character of the pendant PDEAEM blocks. PDEAEM has a pKa of 7.6, and at pH<pKa, the micelles are charged, and cannot be well packed due to electrostatic repulsion; at 8.1<pH<11, the micelles are deprotonated. At higher pH values (typically 8.1-11) and temperature (typically 37-75 °C) the progressive dehydration of the PPO blocks causes the formation of cylindrical micelles. Therefore, the tight packing of the
pentablock template micelles in the zirconia composite and aging at 60 °C, may be the reason for better templating and the resulting smaller crystal sizes.

The XRD patterns of 900 °C calcined zirconia are shown in Figure 6. Clearly, all the peaks sharpen with increasing temperature, indicating the growth of the zirconia crystal size as temperature increases. The zirconia synthesized without template, the zirconia templated by pentablocks and aged at a pH of 4, and templated by PF127 exhibit strong peaks associated with the monoclinic phase, whereas for the other samples, the tetragonal phase was more predominant. Especially in the zirconia templated by the conjugate, only a small peak was detected at 28 °, indicating that only a small fraction of the sample was converted to the monoclinic structure at this temperature. Retention of tetragonal phase may be due to the higher decomposition temperature of lysozyme, which allows the conjugate template remain in the composite to a higher temperature during the calcination, and inhibits pore collapse to form larger crystals and transition to monoclinic zirconia. And the smaller initial crystallite size also results in retardation of crystal growth during heat treatment, and hence delays transformation to the monoclinic phase. Zirconia formed at lower pH had a lower surface area and a lower thermal stability, and formed the monoclinic phase more easily than the one at higher pH. In summary, we conclude that the conjugate and pentablock copolymer templates were more effective at maintaining the tetragonal zirconia phase and forming smaller zirconia particles.
Figure 5. XRD of the 500 °C calcined zirconia. The legend stands for the polymer template for each sample.
Figure 6. XRD of the 900 °C calcined zirconia. The legend identifies the polymer template for each sample.

Specific surface area of the mesoporous zirconia. Figure 7 shows a typical isotherm plot for the 500 °C calcined zirconia sample. The existence of a hysteresis loop indicates the mesoporous structure of the 500 °C calcined zirconia. Table 2 compiles the specific surface area, average pore size, and total pore volume for the six as-prepared zirconia samples. Both the pentablock copolymer and the conjugate templated zirconia samples had higher surface areas than the PF127 templated and the no-template zirconia sample. Templating by the pentablock at 60 °C further increased the surface area of the zirconia samples compared to templating by the conjugate under the same conditions. The specific surface area was also related to the pore size and total pore volume. In this specific study, the surface area is larger when the pore size is smaller and the total pore volume is larger. As shown in Table 2, the conjugate-templated zirconia had a significantly smaller average pore size; the pentablock-60 °C templated zirconia had the largest total pore volume and relatively small average pore size, so these two samples had relatively higher surface areas compared to the other zirconia samples.

At pH=4, the pentablock micelles could not pack to form micellar aggregates, while the other two pentablock templated zirconia samples could form close packed templates that performed better with respect to increasing the template sample’s surface area.

The typical pore size distribution of the 500 °C calcined zirconia samples were studied by applying the Barrett-Joyner-Halenda (BJH) method to the N2 desorption branch of the isotherm plots, as shown in Figure 8. In Figure 8, all pores falls into a range of 3 nm and
50 nm (mesoporous). The conjugate templated zirconia sample exhibited a close to unimodal pore size distribution, with a nearly uniform pore size of about 3.6–3.7 nm. The no-template zirconia sample had a close to bimodal pore size distribution; the majority of pore sizes in this sample are either 3.5–3.7 nm or 45 nm. The pentablock-20 °C-pH4 templated sample exhibited a secondary peak in addition to the main peak, so the majority of pore sizes in this sample were between 3.8 nm and 5.1 nm. These mesopores can form either from removal of the polymer templates or dehydroxylation and crystallization of amorphous zirconia, and all contribute to the increase of the specific surface area of the mesoporous zirconia samples.

Figure 7. N₂-adsorption-desorption Isotherm plots for 500 °C calcined mesoporous zirconia
Figure 8. Pore size distributions for 500 °C calcined mesoporous zirconia samples.

Table 2. Surface area, average pore size, pore volume and estimated crystal size from the XRD results for the 500 °C calcined zirconia samples

<table>
<thead>
<tr>
<th></th>
<th>Surface area (m²/g)</th>
<th>Average pore size (nm)</th>
<th>Pore Volume (cm³/g)</th>
<th>Crystal Size* (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No template</td>
<td>52</td>
<td>10.0</td>
<td>0.13</td>
<td>26.0</td>
</tr>
<tr>
<td>PF127</td>
<td>126</td>
<td>8.7</td>
<td>0.27</td>
<td>22.4</td>
</tr>
<tr>
<td>Conjugate+PF127</td>
<td>174</td>
<td>4.5</td>
<td>0.20</td>
<td>11.7</td>
</tr>
<tr>
<td>Pentablock-60 °C</td>
<td>191</td>
<td>6.8</td>
<td>0.33</td>
<td>6.3</td>
</tr>
<tr>
<td>Pentablock-20 °C</td>
<td>140</td>
<td>8.2</td>
<td>0.29</td>
<td>6.8</td>
</tr>
<tr>
<td>Pentablock-20 °C-pH4</td>
<td>98</td>
<td>5.8</td>
<td>0.14</td>
<td>9.3</td>
</tr>
</tbody>
</table>

*Estimated from XRD line broadening.
**Morphology of the mesoporous zirconia.** The morphology of the mesoporous zirconia was viewed by TEM and SEM. Figure 9 shows TEM images of the zirconia formed after 500 °C calcination. Nano structure of zirconia particles is evident, the samples also exhibit pores in the 5-10 nm range which is consistent with the BJH results. The zirconia formed without template, shown in Figure 9-a, had much larger crystal sizes than the other samples. Also, it was a mixture of tetragonal and monoclinic phases, as shown in the inset (Figure 9-a), while all the other polymer templated samples showed mostly the tetragonal phase consistent with the XRD results.\textsuperscript{25,26} The conjugate template resulted in smaller crystal sizes than the PF127 template, which may be due to the higher decomposition temperature of the lysozyme in the conjugate, as mentioned above.

The pentablock templated samples (Figure 9-d, -e, and -f) had similar morphology. These samples exhibited more uniform structures than other samples. The slight difference among the three samples may be attributed to the different aging temperature and/or pH, since the pentablock template could respond to both temperature and pH changes. In a certain range, at higher temperatures and pH, cylindrical micelles could form. The zirconia templated by pentablock and aged at 60 °C (Figure 9-e) showed the smallest crystal size, about 5-10 nm, while the samples synthesized at the same pH but at lower aging temperature shows (Figure 9-d) slightly larger particle size, about 10 nm; Figure 9-f shows the sample templated with pentablock aged at room temperature and at pH=4, where the polymer could not pack as effectively because the cationic groups were
charged. These observations matched the BET surface area data and the crystal sizes in Table 2.

HAADF-STEM is sensitive to scattered electrons generated in proportion to the atomic number (Z) of the atoms in the specimen. It can be used for obtaining compositional and morphological information of a sample, with contrast varying roughly as a function of Z. An STEM image of the 500 °C calcined zirconia samples is shown in Figure 9-g. Here the bright areas correspond to the zirconium-rich areas of the mesoporous zirconia. Porous structure of this zirconia is clearly evident in the micrograph. Figure 10 shows TEM images of some of the zirconia composite samples heated to 900 °C. Clearly, the zirconia crystals grew larger with the increase in calcination temperature. The diffraction patterns of the zirconia showed that only the conjugate templated zirconia retained the tetragonal phase, which is consistent with the XRD data for the same heat treatment temperature as discussed above (Figure 6). The spotty pattern within the crystal seen in Figure 10-a is believed to have formed due to the volume change associated with the phase transformation.
Figure 9. TEM images and diffraction patterns of the 500 °C calcined zirconia templated by: a) no template; b) PF127; c) conjugate; d) pentablock; e) pentablock, aged at 60 °C; f) pentablock template, pH=4. The scale bars in image a) through f) are 20 nm. g) Dark field image of the zirconia templated by pentablock, aged at 60 °C; the scale bar is 50 nm.

A high resolution TEM image of this sample is shown in the supporting information. By contrast, the zirconia templated by conjugate in Figure 10-c exhibits no such pattern, as it has not gone through the tetragonal to monoclinic phase transformation.
Figure 10. TEM images and diffraction patterns of the 900 °C calcined zirconia templated by: a) no template; b) PF127; c) conjugate.

Although SEM does not have as high a resolution as TEM, it reveals the packing of the zirconia particles and the surface morphology. Figure 11 shows the SEM images of the zirconia calcined at 500 °C. We can see in the micrograph of Figure 11-f that the zirconia formed at pH=4 has a very different morphology, compared with the other zirconia samples, apparently due to aging the nanocomposite at low pH as discussed above. TEM images (Figure 10-f) also show similar features. The zirconia synthesized without template and with the PF127 template had loose packing compared to the other samples, and these two samples also had lower surface area, indicating that the smaller sized particles may r large surface area in this study.
Figure 11. SEM images of the 500 °C calcined zirconia templated by: a) no template; b) PF127; c) conjugate; d) pentablock; e) pentablock, aged at 60 °C; f) pentablock template, pH=4.

Templation. Small angle neutron scattering technique is a powerful tool in elucidating the phase behavior, organization in ordered arrays, and the morphological transitions of the self-assembled micelles and gels. Figure 12 shows the SANS plots of the composite gel during the aging process. The samples with PF127 and conjugate templates showed scattering peaks from PF127 micelles, and samples with pentablock templates exhibited peaks from the pentablock copolymer. The composite with PF127 template shows diffraction peaks with Q/Q* (Q* is the first order peak position) of √3: √4: √8 , indicating a FCC structure; while the composite with pentablock copolymer template
shows diffraction peaks with $Q/Q^* = 1: \sqrt{3}$, indicating a hexagonal closed packing.\textsuperscript{19}

Although we did not fit the data to a model in this study, literature reports on modeling of these two polymers are abundant.\textsuperscript{22,27} The data implied that the zirconia composite formed in a manner that did not interrupt the organization of the template micelles.

**Figure 12.** SANS plots of the composite gel after aging. The legend indicates the polymer template in each sample. Red arrows show the peak positions of PF127 templated composite, and green arrows show the peak positions of Pentablock templated composite.

In Figure 13, we propose a plausible mechanism for the templation process in this study. The zirconyl nitrate precursor solution is naturally acidic (pH<1). When dissolving the pentablock copolymer template into the precursor, a mixture of micelles, unimers, and precursors form. At high temperatures and pH, cylindrical micelle structures arrange in a hexagonal close packed form,\textsuperscript{19,22} creating a larger number of pores after template
removal. The sample aged at low temperature and pH would stay at the first stage in Figure 13, forming a large cluster of zirconia with fewer pores after calcination, as the TEM image in Figure 9-f and SEM image in Figure 11-f showed.

Figure 13. A schematic representation of a plausible mechanism for the formation of mesoporous zirconia templated by pentablock polymer.

The differences in packing between PF127 and pentablock templates, as shown in Figure 12, may be responsible for the surface area differences between their resultant templated zirconia. The presence of lysozyme in the conjugate polymer template potentially facilitates to binding to the precursor, creating smaller particle sizes after calcination, and maintaining the tetragonal phase.

4. Conclusions

In this study, we have developed a new bioinspired templation method for the synthesis of self-assembled mesoporous zirconia nanoparticles, with high surface area and a thermally stable tetragonal phase. This is also the first report of well-ordered mesoporous zirconia synthesized in completely aqueous media. We have also developed new templates for zirconia synthesis based on cationic self-assembling pentablock polymers,
since cationic groups have been shown to be important in zirconia formation. The XRD, TEM and the specific surface area results indicated that pentablock copolymer templates could create the smallest zirconia nanocrystals with the highest surface area. The lysozyme and PF127 conjugate template could also produce zirconia with much higher surface area, and maintain the tetragonal phase zirconia up to 900 °C. These templates could potentially be employed to synthesize other metal oxides, because the templation process is carried out in aqueous media, with facile treatment afterwards. More biological molecules could be introduced into the bioinspired synthesis, which will help to create milder synthesis conditions for the metal oxides.

Acknowledgments

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Chapter 3. Synthesis of mesoporous zirconia templated by block copolymer-lysozyme conjugate in aqueous media

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Abstract:

High surface area mesoporous zirconia/polymer nanocomposite was obtained using a block copolymer-lysozyme conjugate template and applying aqueous sol-gel method. Several parameters such as pH, zirconium ion concentration, and calcination temperature were studied. Samples were characterized by X-ray diffraction (XRD), nitrogen sorption, and transmission electron microscopy (TEM). The results showed that the tetragonal zirconia started to form above 300°C calcination and became fully crystallized after 500°C, grew larger at higher temperatures, and monoclinic phase formed above 900°C calcination. This study also found that a more dilute precursor solution would lead to more thermally stable and smaller particles with higher surface area. Likewise, higher pH, such as pH=10, is more conducive to obtain higher surface area thermally stable tetragonal zirconia with smaller particle sizes compared with pH=4, 6, 8. The aging at pH=4 formed ill-defined gels rather than distinct particles. The surface area of the as-synthesized zirconia sample increased up to 500°C reaching a maximum with a specific
surface area of 348 m$^2$/g using 0.08mol/L zirconium ion concentration and precipitating at pH=10.

1. Introduction

Synthesis of zirconium dioxide, a well-known structural ceramic, continues to receive high interest from the scientific community. The attractive properties such as hardness, shock resistance, chemical inertness, low frictional resistance, and high thermal stability, make zirconia a potential candidate for refractory materials, fuel cell membranes, sensors, catalysis or catalysis carriers, biomedical materials, and hot metal extrusion dies. $^{1-7}$ But some of these applications, especially catalytic applications, require high surface area and nano size, uniform pore structure. Therefore, many methods have been explored to obtain fine zirconia powders with high surface area and fine pore structure, such as sol-gel, aqueous precipitation, hydrothermal synthesis, microwave, hard and soft templating. $^{8-23}$ Among them, the surfactant assisted sol-gel method was given considerable attention on synthesis of nanostructure mesoporous zirconia powders with high surface area. Many surfactants, such as the block copolymer$^1$, the non-ionic surfactant PEG–PPG–PEG$^{24}$, the HPC polymer$^{25}$, the cationic protein lysozyme$^{26}$, and agarose$^{27}$ have been explored. In this paper, we have employed a Pluronic block copolymer-lysozyme conjugate as a template, since conjugate was found to have better performance on increasing the surface area as well as uniform size pores by our previous study. $^{28}$ A number of experimental variables may play a significant role in determining the characteristics of the zirconia formed by this process. Chuah and his coworkers reported the digestion time and temperature as the key factors for obtaining high surface area$^{29}$. Stefanic et al and others reported that the pH value has a significant influence on the phase transformation and
surface area. Rezaei and others reported that the surfactant-to-zirconium molar ratio is also an important parameter in this process. Deshmane and Adewuyi showed that the calcination temperature had a profound influence on the surface area and crystallization, particles grew larger and surface area decreased dramatically when samples heat treated at high temperature. Stichert and Schuth reported the influence of the precursor concentration; they found that lower precursor concentration would lead to smaller particle size, more stable tetragonal phase and higher surface area. In this paper, role of several experimental variables on the characteristics of the zirconia powder was investigated. In particular, the conditions that lead to high surface area mesoporous nanostructure zirconia powders were established.

2. Experimental

2.1 Chemicals and Materials

Triblock copolymer, poly (ethylene oxide)-b-poly (propylene oxide)-b-poly (ethylene oxide) Pluronic F127 (PF127) (PEO\textsubscript{100}PPO\textsubscript{65}PEO\textsubscript{100}, M\textsubscript{av} = 12600 g mol\textsuperscript{-1}), lysozyme from chicken egg white, zirconium (IV) oxynitrate hydrate (ZrO (NO\textsubscript{3})\textsubscript{2}•xH\textsubscript{2}O) (99.99%), succinic anhydride, and N-hydroxy succinimide (NHS) were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Ammonium hydroxide was purchased from Fisher Scientific (Pittsburgh, PA, USA). All of the chemicals were used without further treatment.

2.2 Synthesis of the Pluronic-lysozyme conjugate template

The method of synthesis of Pluronic-lysozyme conjugate was reported before. The hydroxyl end groups of PF127 Pluronic were converted to carboxyl groups by reacting it
with succinic anhydride in pyridine. The carboxyl-terminated PF127 was activated by reaction with NHS at room temperature for 24 h. The NHS-activated PF127 was then covalently bonded (conjugated) with lysozyme. After reacting at room temperature for 24 h in phosphate buffered saline buffer, the mixture was dialyzed against water using a cellulose ester membrane with a molecular weight cut off of 25,000 (Thermo Scientific), for 4 days at room temperature to remove the unreacted polymer and lysozyme. The mixture was then freeze-dried to form the solid conjugate.

2.3 Synthesis of the mesoporous zirconia nanocomposite

In a typical experiment, 0.231g zirconyl nitrate ZrO(NO\(_3\))\(_2\)\(\times\)H\(_2\)O was dissolved in 10 mL deionized water to make 0.08mol/L ZrO(NO\(_3\))\(_2\) solution. Similarly, 1.15 or 2.31 g zirconyl nitrate hydrate was used to prepare 0.4 or 0.8 mol/L solution. 2.6g PF127 and 0.4g Pluronic-Lysozyme conjugate were dissolved in the precursor solution. For each experiment, total polymer content was kept constant at 3 g, and was added at 4°C. The solution was then brought to room temperature, and diluted ammonium hydroxide (about 3%) was added dropwise with pipette and with continuous stirring, until it reaches a predetermined, desired pH value (4, 6, 8, and 10). A white precipitate was formed during this step. The precipitated gel was then aged at room temperature for 3 days, and rinsed with deionized water and centrifuged for 3 times (15 minutes at 4400 rpm) to remove the ammonium nitrate, excess ammonium hydroxide, and the free polymers. The composite was then calcined at 300, 500, 700 and 900 °C for 3 hours in air.
2.5 Characterization

The samples were characterized by X-ray diffraction (XRD), nitrogen sorption, and transmission electron microscopy (TEM).

**X-Ray Diffraction.** X-ray diffraction patterns were obtained using an X’Pert PRO X-ray diffractometer (PANalytical Inc., Westborough, MA, USA). Operational parameters of diffractometer were set at 45 kV and 40 mA, with a scan rate of 0.021°/s and step size of 0.017°, over the range of 20° ≤ 2θ ≤ 80°. CuKα radiation with a wavelength of λ=1.54059 Å was used for the monochromatic light, X’pert Data collector software was used to collect the data.

**Nitrogen sorption.** Nitrogen sorption isotherms were carried out using an AUTOSORB-1 Physisorption analyzer (Quantachrome Corporation, Boynton Beach, FL, USA) at 77 K, the Quantachrome Autosorb Software was used for collecting and processing the data. All samples were degassed under vacuum at 110 °C prior to adsorption. Surface area was calculated by applying Brunauer-Emmett-Teller (BET) method and using the sorption data in the 0.03<P/P₀<0.3 range. Pore size distribution was calculated using Barrett-Joyner-Halenda (BJH) method and the desorption branch of the isotherm. The total pore volume was estimated from the volume of nitrogen adsorbed near saturation pressure.

**TEM.** TEM images were taken using a Tecnai G2 F20 Scanning Transmission Electron Microscope (STEM) (FEI Company, Hillsboro, OR, USA) instrument at operating voltage of 200 kV with High Angle Annular Dark Field (HAADF) and Energy Dispersive X-ray Spectroscopy (EDS) detectors. About 20 μL of diluted zirconia powder suspension was placed on a mesh carbon-supported copper grid and left in ambient
atmosphere until the water evaporates completely before loading it to TEM. Multiple areas of each sample were examined.

3. Results and Discussion

3.1 The effect of calcination temperature

Crystallization, surface area and pore size distribution of the as synthesized zirconia sample were followed as a function of calcination temperature. Figure 1 shows the XRD patterns of the zirconia sample as a function of temperature. At room temperature, the sample is amorphous. At 300 °C, tetragonal zirconia starts to form and becomes fully crystallized at 500 °C. At 700 °C, the tetragonal zirconia crystal grew larger. At 900 °C, the sample is still tetragonal, but small monoclinic phase peaks (such as the small peak at 2θ=28°) also emerge.
Figure 1. XRD patterns for 0.4M-pH10 zirconia samples after calcination at indicated temperatures.

As shown in Table 1, the specific surface area of the sample increases from 41 m$^2$/g at room temperature to its maximum value of 174 m$^2$/g at 500 °C before decreasing progressively as the calcination temperature increases. At 900 °C, the sample exhibits a specific surface area of 31 m$^2$/g. Initial increase in surface area with temperature may be attributed to removal of polymeric template and evolution of structural water and hydroxide from zirconia precipitate while the decrease from 500 to 900 °C is due to the growth of the crystals and the collapse of large amount of pores at elevated temperatures.
Table 1. Nitrogen sorption isotherm data for 0.4M-pH10 zirconia samples at several calcination temperatures

<table>
<thead>
<tr>
<th>Calcination temperature, °C</th>
<th>Freeze-dried</th>
<th>300</th>
<th>500</th>
<th>700</th>
<th>900</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific Surface area, m²/g</td>
<td>41</td>
<td>143</td>
<td>174</td>
<td>86</td>
<td>31</td>
</tr>
<tr>
<td>Total pore volume, cc/g</td>
<td>0.10</td>
<td>0.16</td>
<td>0.20</td>
<td>0.13</td>
<td>0.08</td>
</tr>
<tr>
<td>Average pore size, nm</td>
<td>10</td>
<td>4.3</td>
<td>4.5</td>
<td>6.2</td>
<td>10</td>
</tr>
<tr>
<td>Crystal size calculated from T(101), nm</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>13.4</td>
<td>20</td>
</tr>
</tbody>
</table>

![Figure 2. Pore size distributions for the 0.4M-pH10 sample at different calcination temperatures](image-url)
Figure 2 shows that at the freeze-dried stage, the zirconia sample did not show a high porosity. When the polymer template is removed by calcination, it created a large amount of pores with the pore size of around 3-4 nm; after further heat treatment at 900 °C, these pores collapsed. This result is consistent with the surface area measurements.

**Figure 3.** TEM images and diffraction patterns of freeze-dried nanocomposite: a) Freeze-dried, and heat treated at: b) 300, c) 500, d) 700, and e) 900 °C for 3 h. All the samples were synthesized with 0.4 M ZrO(NO$_3$)$_2$ precursor and aged at pH 10.

Figure 3 shows the TEM images of the nanocomposite and the zirconia nanoparticles formed after calcination. Comparison of TEM image of nanocomposite to those that are heat treated indicates that the precipitated sample is amorphous as indicated by the diffuse rings in SAED inset, while the heat treated samples show electron diffraction patterns. Also, smaller and distinct particles are observed after heat treatment as low as
300 °C. Crystallite size is estimated to be less than 10 nm at this temperature, progressively increasing to about 50-100 nm at 900 °C in agreement with the XRD observations. The inset of Figure 3-e shows the diffraction pattern of the 900 °C calcined zirconia, which is consistent with that of tetragonal phase\textsuperscript{34-36}, indicating that the nanocrystalline zirconia templated by the lysozyme conjugate has excellent thermal stability.

3.2 The effect of precursor concentration

Crystallization, surface area and pore size distribution of the as synthesized zirconia sample were followed as a function of zirconium concentration. Figure 4 shows that after 500 °C calcination, the samples prepared using three different precursor concentrations (0.08, 0.4, and 0.8 mol/L) and at pH=10, all exhibited tetragonal phase, and the crystal size increased with increasing zirconium concentration (the calculated crystal sizes using T (101) are listed in Table 2), this result is consistent with the TEM observations.

Table 2 shows the specific surface area of the samples calcined at 500 °C samples prepared at pH=10 and using 0.08, 0.4, and 0.8 mol/L zirconium concentration. Highest specific surface area of 348 m\textsuperscript{2}/g was obtained at 0.08mol/L. Again, surface area correlates well with the XRD crystal size and particle sizes seen in TEM micrographs.
Figure 4. XRD patterns for 500 °C-pH10 samples prepared at several zirconium concentrations.

Table 2. Surface area, average pore size and pore volume for 500 °C-pH10 zirconia samples prepared at several zirconium concentrations.

<table>
<thead>
<tr>
<th>Zirconium concentration, mol/L</th>
<th>0.08</th>
<th>0.4</th>
<th>0.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific surface area, m$^2$/g</td>
<td>348</td>
<td>174</td>
<td>93</td>
</tr>
<tr>
<td>Total pore volume, cc/g</td>
<td>0.52</td>
<td>0.20</td>
<td>0.19</td>
</tr>
<tr>
<td>Average pore size, nm</td>
<td>6.0</td>
<td>4.5</td>
<td>8</td>
</tr>
<tr>
<td>Crystal size calculated from T(101), nm</td>
<td>5</td>
<td>12</td>
<td>20</td>
</tr>
</tbody>
</table>
Figure 5 shows the pore size distribution for three samples. All three samples exhibit a single peak centering around 3.6-3.8 nm, and the largest pore volume is observed for 0.08 mol/L. In addition, this sample shows larger size pores extending to about 11 nm while the higher concentration samples have little porosity above 4.2 nm. Pore volume data correlates well with the specific surface area measurements that higher the pore volume higher the specific surface area.
Increase in particle size with the zirconium ion concentration is also depicted in TEM micrographs as shown in Figure 6. The estimated particles sizes from the images were about 5 nm, 10 nm and 20 nm for the zirconia prepared from 0.08, 0.4 and 0.8 mol/L precursor, respectively. This is consistent with the BET surface area measurement, and explains why the zirconia with 0.08 M precursor had very high surface area. SAED patterns of the respective samples indicate that the zirconia synthesized with 0.8 mol/L precursor does not exhibit as neat a pattern as the other two perhaps some crystals already transformed to monoclinic structure already \(^{34-36}\) as indicated by the XRD patterns of the 900 °C calcined zirconia.

**Figure 6.** TEM images and SAED patterns of zirconia calcined at 500 °C for 3 h, with ZrO(NO\(_3\))\(_2\) concentration: a) 0.08, b) 0.4, and c) 0.8 mol/L. All the samples were aged at pH=10.
Figure 7. XRD patterns for samples after calcining at 900 °C prepared using 0.08, 0.4, and 0.8 mol/L zirconium concentration.

Figure 7 shows that after 900 °C calcination, the 0.08 mol/L sample still exhibits tetragonal phase while the 0.4 mol/L sample exhibits minor monoclinic phase in addition to the major tetragonal peaks, but the 0.8 mol/L sample is almost completely transformed to monoclinic phase. Apparently, small crystallite size retains metastable tetragonal phase to much higher temperatures than larger crystals. This result is in good agreement with previous studies of Stichert and Schüth who stated that large precursor particle size
will lead to monoclinic phase after calcination, while smaller precursor particle size will lead to tetragonal phase \(^{32}\).

The results above demonstrated that the concentration of the starting precursor had a significant influence on the physical characteristics of the final mesoporous zirconia. The first conclusion we can derive from results is that lower zirconium concentration will lead to smaller crystal size. Although one might surmise that lower zirconium concentration would lead to fewer nuclei hence forming larger particles, our result is consistent with earlier similar studies \(^{24-25, 31-32, 37}\). To understand this, we would like to draw attention to the work of Stichert and Schuth\(^{32}\), who proposed that the crystallization is a result of agglomeration of small precursor particles, so that the crystal size after calcination is determined by the precursor primary particle size. Thus, dilute precursor solution leads to smaller precursor primary particles which will further result in smaller crystal size after calcination and vice versa which is consistent with our result. These authors also pointed out that the precursor particle size has a significant influence on the crystal structure of zirconia. They stated that large precursor particle size will lead to monoclinic phase after calcination, while smaller precursor particle size will lead to tetragonal phase. This hypothesis is also in good agreement with our results. Shubhlakshmi and et al\(^{25, 38}\) in another study also reported that lower concentration precursor solution leads to smaller crystal size and fewer agglomerates after calcination. Later, Kongwudthiti et al\(^{37}\), Deshmane and Adewuyi\(^{31}\) reported similar results. A most recent study by Eltejaei and et al\(^{24}\) obtained similar results but offered an alternative explanation: when the precursor concentration increases, the ions from the salt will compete for water molecules combined with the surfactant’s hydrophilic part, makes the solubility of the surfactant
more difficult and leads to a decrease in specific surface area of the as-synthesized zirconia sample.

3.3 The effect of pH

The precipitation pH plays an important role in the sol-gel synthesis. Figure 8 shows that after 500 °C calcination, all samples exhibit tetragonal phase with sample precipitated at pH=10 has a broader peak compared with the other three samples indicating smaller crystal size. Crystal sizes calculated from X-ray line broadening also is in good agreement with specific surface area and TEM observations mentioned above.

**Figure 8.** XRD patterns of samples precipitated at pH 4, 6, 8, and 10, and calcined at 500°C
Table 3. Surface area, average pore size and pore volume for samples precipitated at several pH using 0.4 mol/L zirconium and calcined at 500 °C.

<table>
<thead>
<tr>
<th>Precipitate pH</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total pore volume, cc/g</td>
<td>0.12</td>
<td>0.16</td>
<td>0.15</td>
<td>0.20</td>
</tr>
<tr>
<td>Average pore size, nm</td>
<td>5.5</td>
<td>5.1</td>
<td>5.2</td>
<td>4.5</td>
</tr>
<tr>
<td>specific surface area, m²/g</td>
<td>86</td>
<td>129</td>
<td>125</td>
<td>174</td>
</tr>
<tr>
<td>Crystal size calculated from T(101), nm</td>
<td>18</td>
<td>19</td>
<td>19</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 3 shows that the average pore size decreases while the surface area increases with increasing pH. Samples precipitated at pH=4 had the smallest specific surface area among the four samples; the samples precipitated at pH=6 and 8 had similar surface area values while the sample precipitated at pH=10 had the largest specific surface area. These observations are in good agreement with earlier reports 29-31, 39-41.

Figure 9 shows that all samples exhibit a single major peak within the mesopore range, centering around 3.5-3.7 nm, largest pore volume is observed for pH=10. In addition, the sample precipitated at pH=4 showed much lower pore volume than the other three samples, which is also in agreement with the specific surface area results.
Figure 9. Pore size distributions for 500 °C-0.4M samples prepared at pH=4, 6, 8, and 10.

Figure 10 shows the TEM images and their diffraction pattern of the samples after 500 °C calcination. The diffraction patterns indicate that all samples exhibit tetragonal zirconia\textsuperscript{34-36}, which is consistent with the XRD results. Image 10-a shows no distinct particle features even after 500 °C, and this may be the reason that this sample had relatively low surface area. Zirconia aged at pH=6 and pH=8 are shown in Figure 10-b and 10-c, and they exhibit similar features. While the zirconia formed at pH=10 had the smallest particle size, and BET surface area showed that it had the largest surface area. These results were all self-consistent.
Figure 10. TEM images and electron diffraction patterns of 500 °C calcined zirconia precipitated at: a) pH=4, b) pH=6, c) pH=8, and d) pH=10. All samples were synthesized using 0.4 mol/L zirconium concentration.
Figure 11 shows that samples precipitated at pH=4~8 exhibited a coexistence of both tetragonal phase and monoclinic phase after 900 °C calcination. While the pH=10 sample is still tetragonal with only a small monoclinic peak emerged around 2θ=28°, indicating the beginning of formation of the monoclinic phase.

The results above demonstrated that the pH value during precipitation has an important influence on the properties of the final zirconia product. The point of zero charge (pzc) of hydrous zirconia is about 4-6 (also depend on other ions in the solution)\(^{41-45}\). So, the particles will assume a positive charge below the pzc\(^{46-49}\). Therefore, zirconia particles tend to aggregate around the pzc pH due to the lack of repulsive force. In our case the
pH=4 and pH=6 samples should exhibit slight positive and slight negative charge respectively. The sample prepared at pH=4 appears to form gel structure rather than distinct particles as shown in Figure 10, and thus leading to low surface area and poor porosity as shown in Table 3. Based on pzc argument only, the sample prepared at pH=6 should exhibit similar morphology but apparently surface charge and hence the flocculation is not the only factor in determining the particle morphology. This result is in good agreement with earlier study by Chuah and et al. who reported that digestion in acidic medium resulted in a lower surface area both for hydrous oxides and the calcined zirconia. When pH is increased to 8 and 10, the hydrous zirconia in the solution is negatively charged, so they react with the cationic surfactant which leads to a mesoporous structure as shown in Figure 9. All these three samples had high porosity after 500 °C calcination. While the pH=6 and pH=8 samples had similar surface areas because they are in the same neutral medium pH range, perhaps the low solubility of ZrO$_2$ in this pH range leads to a relatively low crystallization rate compared to the ones at high pH range. At pH=10, the solubility of ZrO$_2$ is much higher than in pH=6 and 8, thus leading to higher crystallization rate and more nuclei resulting in smaller particles as shown in Figure 10, and higher surface area as shown in Table 3.

4. Conclusions

In this study, we studied the effects of different preparation parameters such as calcination temperature, precursor concentration, and precipitation pH in the bioinspired synthesis of mesoporous zirconia templated by the Pluronic-lysozyme conjugate. This is also the first time report of the detailed preparation study of well-ordered mesoporous zirconia synthesized in completely aqueous media. The XRD showed that all samples
precipitated as amorphous hydrated zirconium hydroxide and converted to tetragonal zirconia with heat treatment as low as 300 °C. Depending on the preparation conditions, tetragonal phase is retained up to 900 °C. The specific surface area progressively increased, reaching its maximum value for all samples at 500 °C mostly due to the removal of the polymer template and dehydroxylation. The specific surface area decreased above 500 °C as a result of crystal growth and the collapse of mesopores. This study also showed that lower zirconium ion concentration will lead to smaller particle size as well as larger surface area, and more stable tetragonal phase The largest surface area, 348 m²/g, was obtained at pH=10 using 0.08mol/L zirconium concentration and after calcination at 500 °C. This study also indicated that the as synthesized mesoporous zirconia sample had larger surface area when precipitated in alkaline medium than those precipitated in acidic or neutral medium. Thus the sample precipitated at pH=10 resulted in smaller crystal size which leads to a higher surface area, and also more stable tetragonal phase.

Acknowledgments

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References:


Materials through the Self-Assembly of Functionalized Nanoparticles,


Chapter 4. General conclusions

Zirconia with high surface area and porous structure has important applications especially in catalytic field. In order to obtain zirconia with high surface area, a number of synthesis methods such as sol-gel, aqueous precipitation, hydrothermal synthesis, microwave induced, hard and soft templating have been reported. However, some of these methods require extremes of temperature or pressure, or are costly, so sol-gel method with a self-assembled template was given considerable attention due to the effective templation, reproducibility, and simple processing conditions.

For the study, we focused on this self-assembled template sol-gel method, using bioinspired approach, to synthesize high surface area mesoporous zirconia nanoparticles. The objective of this project was to study the effects of different polymer templates and preparation conditions on the properties of the final zirconia products. High surface area zirconia nanoparticles with well-ordered mesopores were synthesized with or without self-assembling copolymers as directing agents under variety of conditions, and characterized using several techniques.

In this work, we investigated the use of block copolymers conjugated to cationic biomolecules such as lysozyme, as well as cationic block copolymers as templates, and studied their effects. We also studied the effects of different preparation conditions including calcination temperature, reaction pH, and precursor concentration. Several technics were used to characterize the samples, such as TGA, XRD, TEM, N₂ sorption, and NMR. The results showed that the tetragonal zirconia started to form after 300 °C calcination and became fully crystallized after 500 °C, the crystals grew larger when
heated to higher temperatures, and began to form monoclinic phase after 900 °C calcination. The specific surface area progressively increased reaching its maximum value for all samples at 500 °C mostly due to the removal of the polymer template and dehydroxylation; and then decreased after 500 °C as a result of crystal growth and the collapse of mesopores. The specific surface areas of the as-synthesized zirconia samples after 500 °C calcination were 174 m²/g and 191 m²/g respectively for Pluronic-Lysozyme conjugate template and pentablock template.

Our results indicated that the presence of the self-assembling copolymer templates potentially helped to bind to the precursor, creating smaller particles as well as mesopores after calcination, and maintaining the tetragonal phase. The two novel bioinspired templates investigated using modified Pluronic F127 is conducive to obtaining mesoporous tetragonal nanoparticles with enhanced thermal stability and higher specific surface area. The Pluronic-Lysozyme conjugate template worked better in creating uniform pores after 500 °C and maintaining tetragonal phase even up to 900 °C; while the pentablock template worked better in increasing the surface area as well as decreasing the particle size. These templates could potentially be employed to synthesize other metal oxides, because the templation process is carried out in aqueous media, with facile treatment afterwards.

The results also demonstrated that low zirconium ion concentration and alkaline precipitating medium would lead to more thermally stable and smaller size tetragonal zirconia with higher surface area. So, the largest surface area in this study, 348 m²/g was obtained at pH=10 using 0.08mol/L zirconium concentration and after calcination at 500
\[ ^\circ \text{C} \]. The reason for precursor concentration influence may be due to the difference of the primary crystal precursor sizes, which lead to different crystal sizes after calcination.

Two plausible explanations may be offered for pH influence: one is the binding negatively charged zirconia precursor particles with positively charged template which creates the mesoporous structures; and the other is the relatively high solubility of ZrO\(_2\) at higher pH, which lead to a relatively high crystallization rate and smaller crystal size. As a result, the influence of these two preparation parameters can be boiled down to particle size effect.

However, there are still controversies about the particle size effect on zirconia phase transformation. One opinion is the critical particle size theory proposed by Garvie, this theory is based on the lowest energy principle. He pointed out that tetragonal zirconia has a lower surface free energy, while the monoclinic zirconia has a lower bulk free energy, so the tetragonal phase is stabilized below a critical size which is 30nm according to his theory. Another is the nucleation requirement. Many researchers reported that smaller primary crystal precursor form tetragonal phase nuclei and grow to tetragonal phase; while bigger primary crystal precursor form monoclinic phase nuclei and grow to monoclinic phase. So more discriminating research is still needed to elucidate the crystallization and phase transformation mechanism of zirconia.

Another work that needs to be accomplished is to find out the intrinsic reason of the template effect. In this study, we investigated Pluronic-Lysozyme conjugate and pentablock templates, and found that Pluronic-Lysozyme conjugate template is better in producing uniform pore size, while the pentablock template is better in decreasing the
crystal size as well as increasing the specific surface area, but further research is needed to explain this difference.

Finally, there is a practical demand to study the influence of preparation parameters on certain application performance, such as how will the catalytic activity and selectivity change using zirconia prepared under different conditions.
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