Template directed synthesis of nano-structured bioceramics

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TEMPLATE DIRECTED SYNTHESIS OF NANO-STRUCTURED BIO-CERAMICS

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Abstract

In the past few years, mesoporous structures have gained great attention for their applications such as catalyst, electronic materials and sensors etc. However, it is noted that the formation and control of pore size distribution remain to be a challenging area to date. Among the many approaches to form various meso-structures, tremendous interest has been placed on surfactant-based templating synthesis. A particular direction templating of meso-structures through the use of surfactants self-assembly, as exemplified through the development of meso-structured silica and their variants was synthesized.

In the present work, novel hexagonal-ordered ($P6mm$) mesoporous bioactive glasses have been successfully synthesized using block copolymer Pluronic F127 and P123 as surfactant. Mesoporous hydroxyapatite-silica composite materials with different Si:Ca:P ratio, as well as mesoporous hydroxyapatite have been synthesized for the first time, using block copolymer Pluronic P123 as surfactant. To explain the microbehaviour during the formation of mesoporous structure, the probable mechanisms have been proposed. Synchrotron in situ small angle x-ray diffraction study has been carried out for the first time on the mesoporous bioactive glasses and experimentally verified the proposed mechanism, which indicated that the systems go through a complicated phase transformation, from a disordered to a finely ordered hexagonal structure during the self-evaporation process. In vitro drug delivery property of the synthesized mesoporous materials has been systematically studied using a model drug metoclopramide. The P123 templated mesoporous bioactive glasses present significantly higher loading and better sustained release compared to other samples, which could be attributed to their higher pore volume and surface area.

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Chapter 1

Introduction

1.1 Background

Nanostructured systems exhibit unusual size-dependent properties and play important roles in various fields of science and technology, and have hence attracted great research efforts in the understanding and development of these materials since last decades. Nanostructured materials can be categorized as nanocrystalline and nanoporous systems. Nanocrystalline systems include quantum wells, quantum wires and quantum dots, which exhibit quantum confinement in one, two and three dimension, respectively. Nanoparticles of 1-100nm in size belong to the family of nanocrystalline systems. Nanoporous materials can also be thought as assemblies of nanometer-sized cavities or holes.

In general porous materials are commonly used as adsorbents, catalysts and catalyst supports owing to their high surface areas. According to the IUPAC definition [1], porous materials are divided into three classes: microporous ($< 2\text{nm}$), mesoporous ($2\text{--}50\text{nm}$) and macroporous ($> 50\text{nm}$). Well-known members of the microporous class are the zeolites, which provide excellent catalytic properties by virtue of their crystalline aluminosilicate network. However, their additional applications are limited by the relatively small pore openings, therefore, pore enlargement was one of the main aspects in zeolite chemistry. On the other hand, siliceous mesoporous materials synthesized through supramolecular

templating was reported by mobil scientist in 1992 [2]. Since then, mesoporous materials attracted huge attention. In a very short period of time, a large number of potential applications of these materials have been developed in the area of catalysis, separation, and advanced materials. Non-siliceous mesoporous materials were subsequently studied following similar pathway with the siliceous materials. Transition-metal oxides and related compositions constitute a very important branch of mesoporous materials.

Such materials are important both for the systematic fundamental study of structure-property relations and for their technological promise in extensive applications. The significantly high volume fraction of surface/interface atoms in meso-structured materials made them a competent candidate as catalysis; the unique pore structure and high surface areas provide mesoporous materials promising potential in environmental applications; rather low dielectric constant (k) and refractive index endow them with great electronic and optic property; meso-structured materials could also be used as high performance battery electrodes as well as gas storage. Biomedical applications especially the drug delivery property of mesoporous materials have also become an essential topic in recent years.

One of the major applications of porous materials is as target delivering vectors in biomedical applications. Target delivery includes controlled release of bioactive molecules, which will present at desired therapeutic levels for pre-determined periods of time, with limited (and ideally no) side effects [3]. At the same time, modern materials science has reached today a high degree of sophistication. As a result of continuous progress in synthesizing and controlling materials on the submicron and nanometer scales, novel advanced materials with tailored properties that cannot be extrapolated boundary between materials science and biology have become a fertile ground for new scientific and technological developments [4]. Fine-developed meso-structured biomaterials could facilitate further enhancement in target delivery technology for their relatively high

penetration ability and good release characterisations. Therefore, the synthesis and tailoring of the meso-structured biomaterials, as well as the exploration of the mechanisms involved, which is the main focus of the present work, are viewed to be of huge potential.

Several mechanisms have been proposed, in which one of the most popular mechanisms is the cooperative templating mechanism [5-7]. In this mechanism, both ionic templating and neutral templating models were proposed. It can successfully explain some phenomena, however, it seemed not to be able to suite all systems. Till now, limited effort was put into the corresponding experiments to verify these proposed theories, and few sound proofs have been provided to support it as a general principle. In this case, further focus is still needed on finding out the most convincing mechanism, to produce the most appropriate model for the synthesis of meso-structured materials.

1.2 Objectives

Mesoporous bioceramics are believed as the most promising materials as drug delivery vector, implant materials etc., with their proven biocompatibility. However it is only in the recent two years, there are several reports on the mesoporous bioactive glasses available. In the present work, the systematic study of mesoporous bioceramics including bioactive glasses, hydroxyapatite-silica composite materials, and hydroxyapatite is one of our main objectives.

Although enormous efforts have been put to understand the formation mechanism of mesoporous silica, it is noted that more studies are required to fully establish the the mechanism of meso-structure templating. Another objective of this study is to investigate the meso-structure formation mechanism of the synthesized materials, experimental verification of the proposed mechanism will also be carried out for the first time.

To date, only limited reports are available on the application of mesoporous silica in bioengineering field, and they have to face great challenge of the biocompatibility problem. The study of bioactivity and the bioapplication potential of the synthesized materials is also one of our objectives.

1.3 Scope

The thesis is composed of three main parts of work:

In the first part, a systematic study of mesoporous bioactive glasses is presented. The characterization of the synthesized sample is discussed specifically. Following which, the in vitro bioactivity and drug delivery property are discussed. Based on the experimental results and existing theories, the corresponding mechanisms on templating material and inorganic precursor are proposed and experimentally verified using synchrotron in situ small angle X-ray diffraction.

Study of mesoporous hydroxyapatite-silica composite materials makes the other part of the work. Both synthesis process and characterization of the HA-silica samples are described. Subsequently the in vitro drug delivery property for HA-silica composite material is also investigated.

Finally an investigation in mesoporous hydroxyapatite is discussed. The synthesis process and characterization of the HA-silica samples are described. The corresponding formation mechanism is also proposed.

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Chapter 2

Literature Review

2.1 Mesoporous Materials

2.1.1 Development of Meso-structured Materials

2.1.1.1 Siliceous Meso-structured Materials

(1) M41S-Type Silica Mesophases

Mesoporous silicate MCM-41 was firstly reported by Mobil scientist in 1992 [1]. Since then the Mobil scientists developed a broad family of mesoporous silica molecular sieves which is denoted as M41S. Three members of this family of materials were distinguished:

Figure 2-1 Phases of M41S [2].

hexagonal MCM-41, cubic MCM-48, and a lamellar MCM-50 silica phase as shown in **Figure 2-1**. The distinction between these materials is based on the mechanisms involved in

the synthesis, the pH conditions, the templates used, the topology, and the pore structure. The pore size of M41S materials can be tuned from 2 to 10 nm by changing the chain length of surfactants or adding expander molecules or thermal restructuring. Among all these mesophases, MCM-41 has been most widely studied, due to its structural simplicity and ease in preparation with negligible pore-networking and pore-blocking effects.

(2) SBA-n Structures

Stucky et al. [3, 4] have synthesized a series of acid-prepared meso-structured silicas, termed SBA-n ($n = 1-3, 8, 11, 12, 14-16$) structures, using cationic surfactant and halogen acids through balanced coulombic, hydrogen-bonding, and Van der Waals interactions. Zhao et al. [5] synthesized well-ordered hexagonal mesoporous SBA-15 with tunable large uniform pore sizes from 50 to 300 angstroms by use of amphiphilic block copolymers as organic structure-directing agents. **Figure 2-2** shows the TEM images of mesoporous SBA-15 with different pore sizes.

Figure 2-2 TEM images of calcined hexagonal SBA-15 mesoporous silica with different average pore sizes: (A) 60Å (B) 89Å (C) 200Å [5].

(3) FSM-16

Inagaki et al. [6] prepared mesoporous silica designated as FSM-16 independently, where they intercalated a layered silicate (kanemite) by cetyltrimethylammonium cations via ion-

exchange with sodium ions. The structure of FSM-16 is similar to MCM-41, however the adsorption properties and surface chemistry of these two materials are slightly different. Because of its highly ordered mesoporous structure with unidimensional and hexagonal arrays like MCM-41, FSM-16 is promising for the application such as catalytic reaction and adsorbent of large size molecules [7].

2.1.1.2 Non-siliceous Meso-structured Materials

It was suggested in 1993 that it should be possible to synthesize non-siliceous mesoporous materials following similar pathway with the siliceous materials [8]. However following that the first non-siliceous meso-structured example reported in 1994 [9], however, for these materials it was not possible to remove the template and thus no mesoporous materials could be obtained. The first mesoporous non-siliceous frameworks were reported in 1995/1996 [10, 11], after which much work has been done on the non-siliceous mesoporous materials.

(1) Aluminum Phosphate

The first stable mesostructured silicon aluminum phosphate was reported by Charkraborty et al. [12] in 1997, however, the pore sizes obtained from sorption experiments and the lattice parameters are not compatible. Perez et al. [13] prepared mesoporous aluminum phosphate but noted some collapse of the structure after the surfactant was removed. On the other hand, Zhao et al. [14] have reported the synthesis of a series of aluminum phosphates and silicon aluminum phosphates using cetyltrimethyl-ammonium at pH 9.5 that were stable upon calcination.

(2) Alumina

Alumina is widely used as a catalyst support, which is superior to silica for its higher hydrolytic stability, thus easier to load it with different metal species. Hence lots of attempts

have been made to synthesize ordered mesoporous alumina MCM-41 was synthesized. Bagshaw and Pinnavaia [15] have successfully derived well-defined mesoporous alumina. Cabrera et al. [16], on the other hand, developed “worm-hole” mesoporous alumina using a cationic surfactant. Very high surface area mesoporous alumina ($>700 \text{ m}^2/\text{g}$) was announced by Vaudry et al. [17] through liquid-crystal templating mechanism. However, the long-range order for these materials was rather low, although the pore size distributions were typically narrow.

(3) Transition-Metal Oxides and Related Compositions

With their redox activity and possible use in catalysis, transition metal oxides seemed to be among the most attractive targets for meso-structures. Stucky and Schüth [9, 11] have made the first exploration in synthesizing the meso-structured or even mesoporous transition metal oxides. A variety of meso-structured metals (Fe, W, Pb, Sb, Zr) have been prepared, however, there was no case that can avoid the collapse after the removal of surfactants. Later in 1996, Antonelli and Ying [10] developed a ligand-assisted templating approach that established a strong chemical bond between the inorganic precursor and the surfactant head group throughout the self-assembly process, and mesoporous TiO_2 was then synthesized successfully. The synthesis of meso-structured SnO_2 was done by Ulagappan and Rao [18], where they used SnCl_4 as the inorganic precursor and sodium di (2-ethylhexyl) sulfosuccinate (AOT) as the anionic surfactant, and obtaining hexagonal structure.

Other mesoporous transition metal oxides, such as vanadium oxide [19], zirconium based materials [20], manganese oxide [21], have also been synthesized. A remarkable structural perfection can be seen from zirconium-based meso-structured materials. Schüth [22] summarized the synthesized metal oxides with mesoporous structures listed in **Table 2-1**.

Table 2-1 Ordered mesoporous materials based on transition or rare earth metals composition structure references [22]

Composition	Structure
TiO ₂	Cubic hexagonal disordered lamellar
Ti-oxophosphate	Hexagonal disordered
ZrO ₂	Cubic hexagonal disordered lamellar
ZrP _x O _y	Disordered
Zr-oxophosphate	Hexagonal
HfO ₂	Disordered
V ₂ O ₅	Hexagonal disordered lamellar
V-P-oxide	Cubic hexagonal lamellar
Nb ₂ O ₅	Hexagonal disordered
Ta ₂ O ₅	Hexagonal
MoO ₃	Lamellar
WO ₃	Hexagonal
Mn-oxide	Hexagonal lamellar
ReO ₂	Disordered
Fe ₂ O ₃	Lamellar
CoO	Lamellar
NiO	Lamellar
ZnO	Lamellar
HZnPO ₄	Lamellar
Y ₂ O ₃	Lamellar, hexagonal lamellar
Rare earth metal	Hexagonal, oxides disordered
SnO ₂	Hexagonal

(4) Carbons and Metals

Through a different approach, metals and carbons can also form mesoporous structures. Attard et al. [23] have synthesized hexagonally ordered noble metals in the early 1990s. Ryoo et al. [24] produced ordered mesoporous carbons using MCM-48 and SBA-15 as a mold respectively, through the process of “nanocasting”. Later, Hyeon et al. [25, 26] used

the similar approach and reported the well ordered mesoporous carbon materials designated SNU-x. More recently, novel large-pore cage-type mesoporous carbon molecular sieves, carbon nanocage, with various pore diameters have been reported by Vinu et al. [27]. However, the methods for synthesizing these materials are not commonly applied for their limitation in the moving away of templates.

2.1.2 Applications of Meso-structured Materials

With high surface area, well-defined regular pore shape, narrow pore size distribution, large pore volume and tunable pore size, meso-structured materials find their application in many fields, such as adsorption and separation, ion exchange, catalysis, and molecular hosts etc. These applications will be discussed in the following sections.

2.1.2.1 Catalyst and Catalyst Supports

In catalysis, the active species are typically the surface and edge atoms, which are often associated with surface unsaturated bonds and a lower coordination number than in the bulk crystalline state. The high volume fraction of surface/interface atoms in meso-structured materials made them a competent candidate as catalysis.

Among the synthesized mesoporous materials, MCM-41 is considered promising for a variety of catalytic applications including photocatalysis and enzyme catalysis [28]. The incorporation of trivalent aluminium and tetravalent titanium in the silicate framework opens new possibilities for producing functionalized catalysts. On the other hand, spheres, hollow spheres, giroids, toroids, discoids shaped SBA-15 type silica have also been reported for these purposes.

2.1.2.2 Environmental Applications

The unique pore structure and high surface areas provide mesoporous materials promising potential in environmental applications. Periodic mesoporous silicas were purposely modified to be used as absorbents for pollutants in wastewaters. Thiol-functionalized large pore MCM-41 can remove the metals such as actinides and methylmercury for its remarkable adsorption capacity of 600 Hg/g [29]; more recently, the thiolated SBA-15 was found exhibiting high complexation affinity to mercury cations, while aminated SBA-15 showed a high binding ability to copper, zinc, chromium, and nickel cations [30].

In addition to metallic cations and anions, modified mesoporous materials were tested for the adsorption of some organic pollutants. One can use octylsilane-grafted hexagonal mesoporous silica (HMS) to selectively adsorb low concentrations of nonylphenol.

2.1.2.3 Sensors

Mesoporous materials are ideal for use in sensing applications. This kind of materials permits the confinement of optically or electrochemically active molecules within a matrix that can be constructed to have a variety of electrical or optical properties.

Walcarius et al. [31] modified the carbon paste electrodes with amine-functionalized MCM-41, for the reason of the enhanced surface area provided by the mesoporosity of silica. The sensitivity is found to be significantly improved. Li and Kawi [32] produced templated tin oxide electrodes by making the meso-structure itself out of an electrochemically active material, and found that the sensitivity of the material to hydrogen gas was directly proportional to the surface area.

The principle of optical sensing generally lies in the change in fluorescence or coloration of a dye in the presence to be detected, or the change in the absorption spectrum of an evanescent wave of infrared light. Typically, these sensors are operated by measuring the

transmission, emission or lifetime of a complex or an organic dye embedded in a silicate matrix. Taking advantage of the large-pore mesoporous materials, Wirnsberger et al. [33] developed an optical thin film pH sensor with a fast response time using a die carrying mesoporous thin film. The open pores enable fast diffusion of the solution towards the dye molecules, which resulted in the fast response.

2.1.2.4 Electronic and Optical Materials

(1) Electronic Materials

The rapid development of microelectronics produces high demanding in the material such as good mechanical strength, large break-down voltage, small leakage current, as well as low dielectric constant k (preferentially < 2). The low- k materials can be achieved by introducing “holes” to the dielectric layer. In a simple picture, the mesoporous SiO_2 consists of a regular array of air holes surrounded by a “ SiO_2 skin”. Baskaran et al. [34] had demonstrated the production of disordered templated mesoporous silica with k as low as 1.8. It is also believed that the meso-structured materials may be useful in the production of the next generation of electronic devices [35].

To date, the production of nanowires of ruthenium oxide, platinum, silver and gold within mesoporous hosts has been demonstrated. At the same time, the preparation of mesostructured materials for magnetic data storage has also been exploited. All these attempts should be regarded as important steps advanced electronic applications.

(2) Optical Materials

Similar to the production of the low dielectric constant k , the refractive index of the meso-structured materials is also very low ($n \sim 1.15$ -1.3). The low refractive index materials are

useful supporting layers for optical materials. Mesoporous silica fibres can function effectively as waveguides or as mirrorless lasers by inclusion of a suitable dye [36].

Non-linear optical behaviour has been observed by incorporating para-introaniline (*p*NA) into the one-dimensional mesoscopic pores of MCM-41. By co-assembly of silica/ block-copolymer dye, it is possible to construct new optical materials using the meso-structure architecture. Wirnsberger et al. [33] had successfully applied the meso-structured silica/ block-copolymer composites in lasing. The rather high disperse ability of the meso-structure can lower the amount of dimmers of the final solid, which is favourable for obtaining a low-threshold laser material.

Meso-structured materials can be also used as host of photochromic dyes, which change their color upon light illumination. Winsberger et al. [33] doped meso-structured block-copolymer/silica composites with spirooxazine molecules, the thin films exhibit colorless and ~100% transmission throughout the visible spectrum, while turn to blue when illuminated with 355 nm light from an UV lamp.

2.1.2.5 Bio-applications

Biomedical applications, especially the drug delivery property of mesoporous materials, become another hot topic in mesoporous field in recent years. Usually, drug delivery systems consist of a polymeric matrix from which the drug is released under appropriate conditions.[37] A wide number of materials have been employed, including mixtures of polymers and polymer based composites with different material such as bioactive glasses or ceramics [38, 39]. These methods have in general the disadvantage of heterogeneity of samples due to the difficulty of ensuring homogeneous distribution of the drug through the matrix, which can affect the release rate between different samples. Therefore, much improvement in this field would be expected if chemically homogeneous materials

possessing well-defined porosity to accept organic guest molecules would be available. Clearly, ordered mesoporous materials fulfilled these conditions. In vivo studies using the base matrix implant material SBA-15 has been reported by A. Díaz et al.[40] The results showed excellent biocompatibility with brain tissue (see **Figure 2-3**).

Figure 2-3 SBA-15 implant material in the temporal lobe of the rat [40].

The demand for applications in the encapsulation and separation of proteins, where biomolecules with large molecular weights are involved, drove the development of ordered mesoporous silica towards very large pore sizes of near 30 nm [41-43]. Aimin et al. [44] using the mesoporous silica (MS) particles have prepared enzyme-loaded biocompatible microcapsules which could find applications such as delivery vehicles, enzyme microreactors, or for bio-sensing.

2.1.2.6 Other applications

To store hydrogen gas safely and conveniently has been a challenge for long time. Recently, carbon nanotubes have been demonstrated to be able to store hydrogen at room temperature effectively [45]. Meso-structured materials may also be used as high performance battery electrodes. Attard et al. [46] had made surfactant-templated films of tin that show surface areas as much as 35 times as large as a rolled tin foil, which enable these materials to uptake much larger amount of Li^+ , and used for negative electrodes in lithium batteries.

2.2 Surfactants and Meso-structure Formation Mechanism

2.2.1 Surfactants

2.2.1.1 Chemistry Structure of Surfactants

Surfactants, also known as surface active materials, are compounds which, when present in relatively low concentrations, will preferentially adsorb at available interfaces, replacing the higher energy bulk phase molecules and resulting in a net reduction in the free energy of the system as a whole. These kinds of materials possess a characteristic chemical structure that consists of (1) molecular components that will have little attraction for the solvent or bulk material, normally called the lyophobic (hydrophobic) group, and (2) chemical units that will have a strong attraction for the solvent or bulk phase, called the lyophilic (hydrophilic) group (**Figure 2-4 A**). The basic property of a surfactant is its 'optimal curvature' which results from opposing forces operating at both sides of the interface: attractions between hydrophobic tails on the hydrophobic side and repulsions between hydrophilic heads on the water side. The 'optimal curvature' is the interfacial curvature that minimizes the free energy of the interface.

The solubilising groups of modern surfactants fall into two general categories: those that ionize in aqueous solution (or highly polar solvents) and those that do not. The functionality of ionizing hydrophiles derives from a strongly acidic or basic character, which permits the formation of true, highly ionizing salts upon neutralization. The nonionizing, or nonionic hydrophilic groups, on the other hand, have functionalities or element groups that are individually rather weak hydrophiles but have an additive effect so that increasing their number in a molecule increases the magnitude of their solubilising effect. **Figure 2-4 B** shows the different molecular architectures of surfactants.

Figure 2-4 (A) The basic chemical nature of surfactant; (B) Different molecular architectures of surfactants [47].

2.2.1.2 Micellization of Surfactants in Solution

The primary mechanism for energy reduction in most cases is adsorption at various interfaces. There is significant tendency of surfactants to concentrate at interfaces thereby to reduce the free energy of the system. This phenomenon can be seen as a result of the chemical structure of surfactant molecules, which have both lyophilic and lyophobic groups. However, when all available interfaces are saturated, the overall energy reduction may continue through other mechanisms as illustrated in **Figure 2-5**. The physical manifestation of such one mechanism is the crystallization or precipitation of the solute from solution, i.e., bulk phase separation. An alternative is the formation of molecular aggregates or micelles

that remain in “solution” as thermodynamically stable, dispersed species with properties distinct from those of the monomeric solution.

Figure 2-5 Modes of surfactant action for the reduction of surface and interfacial energies [48].

2.2.1.3 Micellar Growth and Phase Behaviour of Surfactants

Phase separation is observed as the concentration of surfactant systems is varied. For a relatively short-chain surfactant, for instance C_8 or C_{10} , phase separation usually appears at very high concentration, say 10~40%; while, for longer-chain surfactants, say C_{14} or above, the separation usually takes place in relatively lower concentration. The micelles grow with increasing concentration, at first the short prolates or cylinders then form long cylindrical or

thread-like micelles. For other types of surfactants there are different characteristics of micellar growth. As to the polyoxyethylene type non-ionic surfactants with shorter polar group, there is growth with increased of concentration; while for longer polar group, the surfactants show a micellar growth at higher temperature. Both ionic and non-ionic surfactants experience some phase structure evolvments as the surfactant concentration increases, including the characteristic phases: unimers, spherical and rod-like micelles, and cubic, hexagonal and lamellar mesophases. **Figure 2-6** shows the illustration of these phases.

Figure 2-6 Illustration of phase development [49].

Figure 2-7 Phase diagram of EO₂₅PO₄₀EO₂₅ in D₂O [50].

As to the block copolymer (PEO-PPO-PEO), things are more complicated. Aqueous solution of poly (propylene oxide), PPO exhibits dramatic temperature dependence. Below approximately 15°C at ambient pressure, water is a good solvent for PPO, whereas PPO at higher temperatures segregates. Poly (ethylene oxide) (PEO), on the other hand, is dominantly hydrophilic within the temperature range from 0 to 100 °C. With blocks of PEO and blocks of PPO combined into single-polymer chains, one therefore obtains molecules with amphiphilic characteristics which depends critically on both molecular architecture (i.e. total polymerization and relative block size and block sequence), and thermodynamic parameters like temperature and pressure. This leads to self-assembly into a variety of structures including spherical, rod-like, and pancake-shaped micelles, and complex-structured fluids like the microemulsion. **Figure 2-7** shows the phase development according to temperature and concentration of block copolymer (EO₂₅PO₄₀EO₂₅). Qualitatively similar phase behaviors are found for the other Pluronics such as F127 etc. However, the phase transitions are also affected by various factors such as temperature, counterions, added salts, and cosolvents etc. Large amount of groups are focusing on this field and try to make clear the phase transition mechanism.

2.2.2 Formation Mechanism of Meso-structured Materials

2.2.2.1 Beginning Models of the Formation Mechanisms

As mentioned previously, in the early 1990s, the Mobil scientists employed the concept of self-assembled molecular aggregate or supramolecular assembly, of surfactant and successfully synthesized the M41S family of mesoporous materials. Since then, extensive investigations were devoted to the mechanism of formation of these materials [51-55]. The liquid crystal templating (LCT) mechanism in which surfactant liquid crystal mesophases are

assumed to act as organic templates was first proposed [51, 54]. The originally proposed mechanism is melted to MCM-41, illustrated in **Figure 2-8**. In pathway (1), it is believed that the surfactant aggregates to hexagonal liquid-mesophase before the addition of the reagents, the silicate anions then migrate and polymerize to form the MCM-41 structure; in pathway (2), it is believed that the self-assembly of the liquid-crystal-like structures result from the mutual interactions between the silicate anion and the surfactant cations in the solution, i.e. the ordering of the surfactant micelles is influenced by the silica species.

Following this, Huo et al. [53] brought forward a more detailed analysis on the formation of these mesophases (pathway (3) in **Figure 2-9**), in this mechanism, there should be a layered silica-surfactant phase upon mixing the reactants; previous to the MCM-41 structure is formed. A similar mechanism (pathway (2) in **Figure 2-9**) was proposed by Davis and Burkett, in which, the formation of MCM-41 occurs via a disordered or lamellar structure depending on the reaction temperature [56].

However, until now there is no consistent opinion on these pathways yet, all above seems can be verified by certain experiment results.

Figure 2-8 Possible mechanistic pathways for the formation of MCM-41: (1) liquid-crystal-phase initiated and (2) silicate-anion initiated [1].

Figure 2-9 Proposed formation mechanistic pathways by (1) stacking of silicate surfactant rods, (2) lamellar intermediate and (3) silicate bilayer [56].

2.2.2.2 Cooperative Templating Mechanism

Ionic Models

Further studies on the formation mechanism were also carried out by the researchers including above groups. Stucky et al. [52, 57] first proposed the so-called cooperative templating mechanism in 1994, which made promising contributions towards the elucidation of the formation mechanism of surfactant templated periodic mesoporous materials.

In this mechanism, four different modes were suggested, S^+I^- (cationic surfactant S^+ /soluble anionic inorganic species I^-), S^-I^+ (anionic surfactant S^- /soluble cationic inorganic species I^+), $S^+X^-I^+$ (cationic surfactant S^+ /intermediate anion X^- /soluble cationic inorganic species I^+), $S^-X^+I^-$ (anionic surfactant S^- /intermediate cation X^+ /soluble anionic inorganic species I^-). All these four models are based on the electrostatic interactions between the

oppositely charged inorganic ions and the surfactant ions. **Figure 2-10** illustrates the mechanism schematically through a system with cationic surfactant (S^+) and inorganic silicate anions (I^-). Originally, the surfactant is in a dynamic equilibrium between spherical or cylindrical micelles and single molecules.

Figure 2-10 Cooperative templating mechanism [58].

Neutral Templating Models

In recent years, with the incorporation of the nonionic surfactants, the fifth model S^0I^0 , neutral templating mechanism was introduced by Pinnavaia et al. [59, 60]. This neutral S^0I^0 templating route produces meso-structures with larger wall thickness, small scattering domain sizes, and complementary textural mesoporosities relative to the ionic routes, as well as easier to remove the template. The thicker pore walls improve the thermal and hydrothermal stability of the mesopore framework; the small crystallite domain size introduces textural mesoporosity, which facilitates the accessing of the framework-confined pores.

During the early stage, the neutral surfactants are focused on the amines. It is supposed in this model that the meso-structure is produced through the organization of the surfactant molecules to neutral rod-like micelles. The nonionic inorganic species form hydrogen-bonding with the surfactant head groups, through hydrolysis, further hydrolysis and condensation result in the micelles and framework wall formation.

More recently, the amphiphilic block copolymer (BC) systems have been used to derive silicates with ultra-large pores. Upon self-assembly, these systems give rise to larger-size supramolecular aggregates than surfactant micelles, thus achieving uniform mesopores ≥ 6 nm [61]. **Figure 2-11 (A)** shows typical block copolymers used as templates, i.e. □. Oligomeric alkyl-poly (ethylene oxide) $C_m(PEO)_nOH$; □. triblock copolymer poly (ethylene oxide) – poly (propylene oxide) – poly (ethylene oxide) $(PEO)_n(PPO)_m(PEO)_n$, EO = $-CH_2CH_2O-$, PO = $-CH(CH_3)CH_2O-$; □. $(PS)_n(PAA)_m$; □. $(PI)_m(PEO)_n$.

Pinnavaia et al. [62, 63] firstly synthesized worm-like materials in near-neutral pH conditions following the model of S^0I^0 . Addition of anions (model $(S^0H^+)(X^{1-})$) for instance F^- (known as condensation catalysts) can help to obtain well-defined meso-structures by

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controlling both hydrolysis and condensation, alternatively, fluoride ions could compete for the water molecules or exert a “salting out” effect on PEO blocks. On the other hand, cations addition also seems to affect the organization. Zhang et al.[64, 65] created highly ordered silica by adding Mn^{+} (Li^{+} , Co^{2+} , Ni^{2+} , ...) to the reaction media, which can be depicted as $(S^0M^{+})I^0$; Bagshaw [15] later proposed a more complete model $(S^0M^{+})(XI^0)$, in which cations and anions play different roles.

Figure 2-11 (A) Typical block copolymer used as template (B) Schematic view of the interaction ways: $(S^0H^+)(XI^-)$, S^0I^0 , and $(S^0M^+)(XI^0)$ [63].

2.2.2.3 Some Extended Models

In 1996, Ying and Antonelli [66] introduced the ligand-assisted route and successfully synthesized mesoporous TiO_2 . In this technique, the inorganic species are linked to a long-chain hydrocarbon ligand in a tighter way, so that a surfactant-inorganic entity is used as the precursor for the mesophase assembly. Later, Attard et al. [67] introduced the so-called true liquid-crystal templating route, where the surfactant is used in high concentration, and the liquid crystal forms. Colloidal templating methods were also developed, in which either emulsions [68-70] or polymer latex spheres [71, 72] are utilized as templates.

2.2.2.4 Special Formation Pathways for Non-siliceous Materials

Routes of synthesizing mesoporous silicas could be adapted to that for non-siliceous materials. In addition, there are also some “shortcuts” for the formation of non-siliceous mesoporous materials.

“Nanocasting” is a recently discovered pathway for the formation of mesoporous materials. It uses the ordered mesoporous silica as molds for the other materials, to form other materials, such as polymers, carbons, and metals [73]. However, till now only MCM-48, MSU-1 and SBA-15 have been successfully used as the mold, MCM-41, on the other hand, is proven to be less suitable for the production of porous carbons or metals. And the generality of these pathways are also unclear. It is clear, though, that they will only be suitable for framework compositions which are stable in relatively high concentration of NaOH or HF, which is used to dissolve the mold.

Very recently, it was discovered that mesoporous silicon imido nitride can be synthesized by ammonolysis of silicon halides in aprotic solvents [74]. This is so-called crystal templating, in which a pore is templated by the hydrolysis product NH_4X ($\text{X} = \text{Cl}, \text{Br}, \text{I}$),

released upon heat treatment. The pore size is corresponding to the size of previous ammonium halide nanocrystals.

Figure 2-12 Schematic representation of general pathways leading to meso-structured and mesoporous non-siliceous materials [75].

Figure 2-12 shows the four general pathways in synthesizing the non-siliceous mesoporous materials: path (a) and (b) are the direct application of the corresponding system, (c) the “nanocasting” route, (d) the nanocrystal templating route.

2.2.2.5 Experimental Verification of Mechanism

Time resolved in situ techniques are applied to mesoporous research in recent years to provide experimental verification on the above suppositions. Changes in the micelle environment during the synthesis can be detected by using electron paramagnetic resonance (EPR) [76-79]. Time-resolved fluorescence quenching permits the determination of the surfactant aggregation numbers in these aggregates [80], as well as to study the exchange between two different counterions, and gives detailed information about the micellar interface region and has been shown to be a very useful tool for studying these systems [77]. Raoul et al. [81] investigated the mechanism of a strongly alkaline surfactant/ sodium silicate systems using time-resolved fluorescence, the result revealed that the introduction of hydroxyl and silicate anions into micellar solutions of CTAB resulted only in the exchange of a small fraction of micelle-bound bromide ions by the added ions. Also, additions of NaOH and sodium silicate did not bring about a significant change of micelle size or shape.

In situ synchrotron small-angle X-ray diffraction probes the appearance of long-range order in the system and has allowed the kinetics of mesophase formation [82-86]. More recently Katarina et al. [87] explained the phase transformation of non-ionic templating SBA-15 mesoporous silica through combination of SAXRD and SAXS (**Figure 2-13**). A five-step formation of the 2D hexagonal phase was proposed in agreement with the references [88-91]: (a) hydrolysis of the silicon alkoxide, (b) adsorption of hydrolyzed silicate species to the PEO units of spherical P123 surfactant micelles, (c) clustering of poorly condensed silica-surfactant hybrid micelles into flocs, (d) elongation of the surfactant micelles and parallel formation of domains exhibiting hexagonal order, and (e) growth of the domains.

Figure 2-13 Time-resolved SAXS pattern of the (a) P123-TMOS and (b) P123-TEOS systems. See text for details. The sudden jump in intensity of the micellar scattering reflection in part a originates from a 3 min break in the data collection [87].

Cryogenic transmission electron microscopy (cryo-TEM) is also an invaluable tool for the study of the structures present in solution during the early stages of the process, before long-range order can be observed [92, 93]. Katarina et al. [94], revealed the kinetics of mesoporous silica particle formation through a time resolved

TEM and NMR study. **Figure 2-14** shows TEM micrographs of the phase evolution during the

Figure 2-14 Transmission electron micrographs following the time evolution of the synthesis: (a) 25 min after TMOS addition; (b) 40 min after TMOS addition; (c) 55 min after TMOS addition; (d) 100 minutes after TMOS addition [94].

synthesis, which gives the detail of the phase transition from spherical micelle to a hexagonal structure. It is suggested that, for the block copolymer templated mesoporous silica system, the copolymer works as a structure determinants, while the polymerization of the silica leads to the introduction of an attractive force between micelles due to a bridging

mechanism. This attraction leads to the formation of flocs of micelles, and these flocs eventually reach a size where they precipitate. There is a continuing structural change within these clusters involving the coalescence of micelles that tend to form cylindrical aggregates, which order in a two-dimensional hexagonal pattern. It is furthermore important that the kinetics of the silica polymerization matches the kinetics of the micelle aggregation process in such a way that one forms large flocs of finite micelles before the flocs mature to their final equilibrium structure.

2.3 Bioceramics

2.3.1 Introduction of Biomaterials

There are roughly one million cases of skeletal defects a year that require bone-graft procedures to achieve union [95]. The use of certain materials as constituents of surgical implants is not new. Substitutions of bone parts or repairing seriously damaged portions of the human body have been reported since pre-Christian era, where either bronze or copper were mainly utilized, in the circumstances requiring the assembly of fractured bone parts. In 1880, Gluck applied an ivory prosthesis by using colophony-based cement for anchorage, which was a symbol of the research in the materials with biocompatibility with human body [96]. Today surgical interventions are possible in cases of highly degenerative situations, tumours, or minute fractures, with the aim of replacing skeletal parts.

Biocompatibility is the prerequisite for any synthetic material to be implanted to the human body. That is the material should not produce any inflammatory, or in a more general sense, does not result in any adverse tissue reaction according to the officially approved definition (Williams, 1987). Meanwhile, the biomaterials are expected to withstand any applied physiological load, resist any degradative or corrosive attack by physiological fluids, and not

promote any blood trauma, blood clotting, or denaturing of plasma proteins etc. Biomaterials can be classified as metallic alloys, polymeric substances, and ceramics according to the property of materials. **Figure 2-15** presents the classification and the application of the biomaterials.

In recent years, biocompatible alloys have been intensively studied with a strong emphasis on their corrosion behavior, surface properties and biocompatibility in implants [97, 98]. Current metallic biomaterials include stainless steels, cobalt-based alloys and titanium-based alloys [99]. Titanium alloys such as Ti-Nb-Zr-Ta, Ti-Mo-Zr-Fe, Ti-6Al-4V, Ti-Zr, Ti-Mo alloys have been the mostly used materials of choice for medical implants [100-104].

Figure 2-15 Biomaterials and their fields of applications; each field is coded by a number in the left pye that is recalled for each biomaterial inside the second pye [96].

Biopolymers were chiefly used to perform some specific functions in association with products such as fibres, films, or coatings at the beginning. In 1990s some polymers replaced aluminium and a number of other structural metals in certain applications. Today, biopolymers such as poly(methylmethacrylate) (PMMA), polysulfone (PSU), poly(etheretherketone) PEEK or Epoxy resins are considered the best materials for soft-tissue

implantation in the cardiovascular, respiratory, digestive, genitourinary, and nerve systems, playing a more important part in bioengineering field [105]. High-density polyethylenes (HDPE) with very high molecular weight fractions such as ultra high molecular weight polyethylene (UHMWPE) have found application as a load bearing material in joint endoprostheses [106-108]. Urethane dimethacrylate (UDMA) and 2-hydroxyethyl methacrylate (HEMA) serve a variety of applications in medicine and dentistry [109].

Another interesting alternative is represented by polymeric carbons with either ceramic or fibrous structure, used in a variety of biomedical applications. Carbon nanotubes (CNT) are unique, one-dimensional macromolecules, whose outstanding properties have sparked an abundance of research since their discovery in 1991 [110]. Many applications for CNT have been proposed including biosensors, drug and vaccine delivery vehicles and novel biomaterials [111]. They can also be used to create electrically conductive polymers and tissue engineering constructs with the capacity to provide controlled electrical stimulation [112-114].

Ceramic materials including alumina, glass, hydroxyapatite, pyrocarbon etc, are useful in both orthopedics and odontostomatology (which are among the major implantation sectors) in either competition or co-operation with metals. Ceramics has the following advantages compared to other types of biomaterials, which make bioceramics a promising material in bioengineering area.

1. Resistance to heat, wear, corrosion;
2. Good magnetic permeability;
3. Great hardness;
4. High mechanical strength at high temperatures;

Bioceramics can normally find their usages in the following field:

1. Skeletal system: artificial load-bearing applications: hip joint, knee joint, fixation devices;
2. Muscle/bone bonding: ligaments, tendons;
3. Maxillofacial system: ear, nose and throat (ENT), teeth, craniofacial system such as flat cranium bones, mandible;
4. Cardiovascular system: heart valves;
5. Stimulator devices: electrodes, electronic ceramics, piezoelectric ceramics, porous drug dispensers;
6. Others: fillers, larynx, trachea composites, percutaneous access devices, coatings for tissue ingrowth, genitor-urinary devices

2.3.2 Hydroxyapatite (HA)

2.3.2.1 Non-stoichiometric Hydroxyapatite

Bone is mainly composed of non-stoichiometric hydroxyapatite (HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) and collagen [115]. Natural bone is approximately 70% by weight and 50% by volume of HA [116]. Hydroxyapatite is considered as one of the most promising ceramic materials for artificial implant due to its chemical similarity with bone hard tissues and excellent bioactivity with skin and muscle tissues [117]. The chemical nature of hydroxyapatite lends itself to substitution, meaning that it is common for non-stoichiometric hydroxyapatite to exist. The most common substitutions involve carbonate, fluoride and chloride substitutions for hydroxyl groups while defects can also exist resulting in deficient hydroxyapatites. Biological apatites are calcium deficient. Bone apatites contain carbonate, while teeth contain substantial amounts of fluoride as well. In certain applications, for example, in the preparation of dense HA ceramics, stoichiometric rather than Ca-deficient HA (Ca-d HA,

Ca/P < 1.667) is desirable. The reason is that segregation of α - or β -tricalcium phosphate (β -TCP) occurs during sintering. And the presence of TCP phase increases slow crack growth susceptibility and biodegradability of the HA ceramic. Stoichiometric hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, has a Ca/P ratio of 1.67, while calcium-deficient hydroxyapatite, $\text{Ca}_{(10-x)}(\text{HPO}_4)_x(\text{PO}_4)_{(6-x)}\text{OH}_{(2x)}$, may have a Ca/P as low as 1.5. In the latter instance, x is equal to unity. **Figure 2-**

Figure 2-16 Solubility curves for DCP, HAP, and TetCP [118].

16 shows plots of the solubility curves for dicalcium phosphate (DCP), CaHPO_4 , tetracalcium phosphate (TetCP), $\text{Ca}_4(\text{PO}_4)_2\text{O}$, and HAP. Reactions between the acidic and basic calcium phosphates, DCP and TetCP, respectively, leading to the formation of stoichiometric and calcium-deficient HAP have been identified [119]:

$$\text{Ca} : \text{P} = 1.67 \quad (1)$$

$$\text{Ca} : \text{P} = 1.5 \quad (2)$$

As mentioned earlier, the chief characteristic of HA is its bioactivity. HA has the ability to elicit a specific biological response at the interface of the material, which results in the formation of a bond between the tissues and the material. Therefore, it is able to integrate into bone structures and support bone in-growth, without breaking down or dissolving. Bioactive materials develop an adherent interface with tissues that resist substantial

mechanical forces. In many cases the interfacial strength of adhesion is equivalent to or greater than the cohesive strength of bone. The interfacial strength of a bioactive implant bonded to bone is 15~40 times greater than the interfacial adherence of non-bioactive materials (such as Al_2O_3), tested in the same animal model.

Hydroxyapatite is a thermally unstable compound, decomposing at temperature from about 800~1200°C depending on its stoichiometry. Generally speaking dense hydroxyapatite does not have enough mechanical strength (in particular fatigue properties) to enable it to succeed in long-term load bearing applications.

2.3.2.2 Synthetic HA

It was first identified as being the mineral component of bone in 1926. However, it is not until about 30 years ago that synthetic HA is accepted as a potential biomaterial for use in orthopedics, bone grafts and dentistry. It is one of a limited number of materials that will form strong chemical bonds with bone in vivo, while remaining stable under the harsh conditions encountered in the human body. HA can exist in its amorphous or crystalline state and even other structural form depending on the processes it undergoes. Amorphous HA is undesirable because it resorbs in the biological system with time and thus degrades the mechanical properties of the fixation. Thermal spraying most commonly produces this type of structure during the spheroidisation of HA. However, biologically stable crystalline HA can still be achieved via some post heat treatment processes after thermal spraying.

The precipitation of calcium phosphates has attracted the interest of many researchers because of its importance in, such as biomineralization processes, industrial water system scale formation (e.g., heat exchangers, cooling towers, boilers, etc.), water treatment processes, and catalysis as supporting material and agriculture as fertilizers [120-123]. Depending on the supersaturation level and the solution pH, a number of calcium phosphates

may be formed at ambient temperatures and pressure. In aquatic solutions of $\text{pH} > 4$, the order of increasing solubility is as follows: hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3\text{OH}$, HAP), tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_3$, TCP), octacalcium phosphate ($\text{Ca}_8\text{H}(\text{PO}_4)_6 \cdot 2.5\text{H}_2\text{O}$, OCP), dicalcium phosphate dihydrate ($\text{Ca}(\text{HPO}_4)_2 \cdot \text{H}_2\text{O}$, DCPD).

HA powders and coatings can be synthesized using a number of methods including sol-gel processing [124], coprecipitation [125], emulsion techniques [126,127], batch hydrothermal processes [128-130] mechano-chemical methods [131] and chemical vapour deposition [132]. Biomimetic process is one of the promising method used to prepare apatite-metal and

Figure 2-17 Crystal structure of calcium hydroxyapatite powders synthesized in SBF at 37°C (Hexagonal, $P63/m$, $a = 9.4125$, $c = 6.8765\text{\AA}$) [133].

apatite-polymer composites. A variety of metals and organic polymers incorporated surface functional groups such as Si-OH , Ti-OH or Ta-OH to induce formation of a biologically active bonelike apatite by chemical treatment or physical adsorption. Subsequent immersion in a simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma or 1.5 SBF will lead to the formation of a dense and uniform bonelike apatite layer on the surface. The crystal structure of the “SBF-synthesized (at 37°C and $\text{pH} = 7.4$) calcium hydroxyapatite powders” is shown in **Figure 2-17**.

For the past few decades, researchers have intensively studied the structural behaviour of the synthetic HA. Brown et al. [134] figured out the kinetics of hydroxyapatite formation at low temperature, from reaction between acidic and basic calcium phosphates. The rate of hydroxyapatite formation during an initial period was revealed to be controlled by interfacial processes.

2.3.2.3 Applications

The most common application of HA and bioactive glasses are as coatings for prostheses made from a different material. Hydroxyapatite can also be employed in forms such as powders, porous blocks or beads to fill bone defects or voids. These may arise when large sections of bone have had to be removed (e.g. bone cancers) or when bone augmentations are required (e.g. maxillofacial reconstructions or dental applications). The bone filler will provide a scaffold and encourage the rapid filling of the void by naturally forming bone and provide an alternative to bone grafts. It will also become part of the bone structure and will reduce healing times compared to the situation, if no bone filler is used. Apart from their original use as a tissue replacement, they have increasingly been applied as carriers for drugs and cells [135-137] in recent years.

Among the many different kinds of hydroxyapatite (HA) implantation materials, porous forms of HA are especially interesting. Their strength is lower than that of dense bodies, but due to the possibility of being penetrated with bone tissue, they are considered to have potential as bone substitutes [138-140]. The architecture of bone varies widely depending on the type and function. Therefore porosity in the HA structure is important since it allows for the growth of tissue and bone around a supporting framework and allows passage of nutrients. The porosity in the human bone structure also varies widely with the type and

function. Much hope is now put on the application of bioceramics derived from calcium phosphates, mainly HA and TCP, as ceramic drug carriers [141].

In the past decade, researchers had also discovered other engineering uses of HA such as catalysts. When used with water and ammonium salt, hydroxyapatite is an excellent solid support for the various heterogeneous condensations such as Claisen-Schmidt et al. [142]. Taking into account environmental and economical considerations, the handling of hydroxyapatite used presents many advantages such as easier separation and recovery from the reaction mixture, enhancing recycling possibilities, which are now well established in fine organic synthesis.

2.3.3 Bioactive Glasses (BGs)

2.3.3.1 Properties

A number of studies have been carried out since 1950 with the aim of identifying the range of utilizable compositions. In 1971, Hench et al. [143] described a new formulation of glass composed of SiO_2 , CaO , Na_2O , and P_2O_5 formed through melt. It was characterized by its ability to bond to bone and called as Bioactive glasses. After that, sol-gel derived bioactive glasses attracted more attention. Li et al. [144] showed that $\text{CaO-P}_2\text{O}_5\text{-SiO}_2$ glass powders produced by sol-gel technique are more

Figure 2-18 Compositional ranges of bioactive glasses and glass-ceramics [96].

bioactive than the melt-derived glasses of the same composition. Furthermore, sol-gel glass

exhibits high specific area and porous structures in the microporous or mesoporous range, which can promote cell adhesion, adsorption of biologic metabolites, and resorbability at controlled rates to match that of tissue repair [145,146].

During the last 30 years, extensive research on bioactive glasses and glass–ceramics has been conducted. The most widely studied bioactive glasses are those with compositions containing SiO_2 , CaO , and P_2O_5 , including Cerabone A/W glass ceramics [147-149] 58S bioactive glasses [150-152], modified 45S5 glass compositions [153] etc. The ternary graph of **Figure 2-18** illustrates the compositional range that appears useful for the constitution of biological glasses. To improve melting conditions and directed crystallization of glasses as well as their properties, the glass composition was modified by adding sodium (Na), magnesium (Mg), aluminum (Al), boron (B), fluorine (F), and other elements.

2.3.3.2 Applications

Bioactive glasses can also be used as coatings for prostheses, for instance the metallic implants to alter the surface properties [154]. More recently, Takail et al. [155] used bioactive glass–mesoporous silica coatings to enhance the bioactivity of Ti6Al4V. In this manner, the body sees hydroxyapatite-type material that it is happy to accept. Without the coating the body would see a foreign body and work in such a way as to isolate it from surrounding tissues.

Bioactive glasses were attracting much attention for their excellent bone conductivity. In fact 45S5 Bioglass® has successful applications for more than 10 years. Recent research showed that there is genetic control of the cellular response of osteoblasts to bioactive glasses [156]. The structures with a maximum porosity of 5% by volume with pores measuring about $1\mu\text{m}$ consisting of crystal sizes exceeding $2000\text{\AA}$ are applied in certain clinical applications such as repair of bony defects in dental and orthopaedic applications,

guided tissue regeneration, percutaneous devices, middle ear reconstruction and alveolar ridge augmentation [157]. Structures with larger porosity ranging up to hundreds of microns in pore size are used in clinical applications including bone regeneration, maxillofacial surgery and orthopedic surgery. For medical applications, not only total and open porosity of porous materials is of importance but also pore size distribution, pore texture and shape [158]. Macroporous bioactive glasses prepared by sol–gel method would be an ideal scaffold material. 3D ordered macroporous sol–gel bioactive glasses (3DOMBGs) have been reported in recent years [159-161]. These materials have a good combination of bioactivity and degradability. On soaking in SBF at body temperature, 3DOMBG in the $\text{CaO-P}_2\text{O}_5\text{-SiO}_2$ system forms apatite and completely degrades. Therefore, the apatite is independent of the host bioactive material and easily investigated [162].

2.4 Concluding Remarks

As can be seen from above, the formation mechanism of meso-structured materials has been widely reported in recent years. However, so far there is no universal opinion about the mechanism especially on some complex non-siliceous system. More mesoporous material systems still remain undeveloped. Great efforts are still required for further development on science and technology in this area.

On the other hand, significant effort has been devoted to develop biocompatible materials such as hydroxyapatite and bioactive glasses. Side applications of these materials for instance implanting, tissue engineering, or as target delivery vector have been investigated. Further improvement on the properties of these materials is another attractive area. Fine-developed meso-structured biomaterials could facilitate further enhancement in target delivery technology for their relatively high penetration ability and good release characteristics.

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Chapter 3

Mesoporous Bioactive glasses: Synthesis, Mechanism Study and Drug Delivery Application

3.1 Introduction

As discussed in **Chapter 2**, after the first successful synthesis of meso-structured materials by Mobil Oil R&D Corporation through supramolecular templating, mesoporous materials have gone through a stage of very rapid development. To date, these materials have been well accepted in electronic, optical, as well as in biomedical applications, due to their regularly arranged 3-dimensional pores. In recent years, drug release properties of MCM 41 system have been studied. However the biocompatibility of these silicate materials remains a great challenge. Bioactive glasses, widely applied as bone spacers, fillers, artificial vertebrae, intervertebral discs as well as iliac crests, owing to its superior bioactivity and excellent bone-bonding ability. It is possible to synthesize $\text{SiO}_2\text{-CaO-P}_2\text{O}_5$ based mesoporous bioactive glasses (MBGs), and thus apply the MBGs into drug delivery field. Such drug delivery systems could be in terms of injectable drug-loaded micro/nano particles or even as localized drug-loaded implants for its impressive bioactivity and unit mesoporous structure. More recently, hexagonal ordered mesoporous $\text{CaO-SiO}_2\text{-P}_2\text{O}_5$ -based bioactive glasses (MBGs) using P123 as template, and worm-like mesostructure using F127 as template, have been sol-gel derived by Yan et al. [1].

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Ionic surfactants were widely used in synthesis of mesoporous materials in the last decade. However, two main limitations exist for the ionic route: (a) typical wall thickness of 8–13 Å, which is a serious limitation regarding stability; (b) limited pore size offered by molecular surfactants [2]. With the incorporation of non-ionic surfactants, neutral templating mechanism shows great potential in this area [3, 4]. Amphiphilic block copolymer, widely used in detergency, emulsifying, coating, thickening, etc, becomes one of the most popular candidates as neutral template recently. The self-assembly characteristics of these block copolymers permit to control the superstructure, as well as to vary the typical length scales and to add specific functions.

In this chapter, two groups of mesoporous bioactive glasses were synthesized using a sol-gel method, with block copolymer Pluronic F127 and P123 as template respectively. The meso-structure formation mechanism was studied through synchrotron in situ small angle x-ray diffraction during the self-evaporation process. And their potential in drug delivery application was investigated.

3.2 Preparation of Mesoporous Bioactive glasses (MBGs)

The same composition ratio with traditional bioactive glasses reported by Perriera et al. [5] is selected in the present work. The synthesis method was a modified one from the sol-gel process of traditional bioactive glasses reported by Laczka [6]. The MBGs were synthesized using two different block copolymers, EO₉₉PO₆₅EO₉₉ (F127) and EO₂₀PO₇₀EO₂₀ (P123) (where EO is poly (ethylene oxide), PO is poly (propylene oxide)), as templates. For the first group of MBG (F127-MBG), taking 80S-F127 as an example, 6g of F127 (Sigma Aldrich), 8.9g of tetraethyl orthosilicate (TEOS) (Fluka, ≥ 99.0%) was added to 65g of ethanol (Absolute L260, Comak) and stirred till the solution became clear. 5g of 1 M hydrochloric acid (HCl, 37 %) (VLSI Puranal) was then added with mixing for 15 minutes. Finally 1.89g

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of calcium nitrate (Lancaster) and 0.73g of triethyl phosphite (TEP) (Alfa Aesar) was added and mixed for 45 minutes. The obtained mixture was stirred at 30 °C for 24 hours, and then 2ml of which was taken out for in situ study. The rest sol was then introduced into a Petri dish to undergo an evaporation-induced self-assembly (EISA) process at room temperature. The dried gel was calcined at 600 °C for 5 hours to remove the organic composition.

An identical preparation technique was conducted for the second group of MBG (P123-MBG), with P123 (BASF) replacing F127 as the template. Normal bioactive glasses (BG) were synthesized using traditional sol-gel method without surfactant added. **Figure 3-1** gives the flowchart of the present process. The calcination schedule is demonstrated in **Figure 3-2**. For each group, three compositions with different SiO₂:CaO:P₂O₅ ratio were prepared, for the convenience of analysis, the samples were coded as 60S, 70S, 80S shown in **Table 3-1**.

Figure 3-1 Flowchart of the synthesis of mesoporous bioactive glasses.

Figure 3-2 Sample calcination schedule.

Table 3-1 Sample code and description

Sample code	Surfactant	Sample description (molar ratio)
F127-60S	F127	SiO ₂ :CaO:P ₂ O ₅ = 60:36:4
P123-60S	P123	
F127-70S	F127	SiO ₂ :CaO:P ₂ O ₅ = 70:26:4
P123-70S	P123	
F127-80S	F127	SiO ₂ :CaO:P ₂ O ₅ = 80:16:4
P123-80S	P123	

3.3 Characterization

The samples produced were then subjected to various characterizations to verify the mesoporous structure as well as to examine the phase and composition of the products. The characterization techniques and the facilities used are listed in **Table 3-2**.

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Table 3-2 Characterization methods and corresponding facilities used

Methods	Characterization	Facilities
Differential Thermo-Thermo Gravimetric Analysis (DT- TGA)	Thermal analysis	NETZSCH STA 449C
Energy Dispersive X-ray (EDX)	Element analysis	JEOL JSM-6340F
Field Emission Scanning Electron Microscope (FESEM)	Particle morphology analysis	JEOL JSM-6340F
Transmission Electron Microscopy (TEM)	Mesoporous structure verification	JEOL JEM-2010
Small Angle X-ray Diffraction (SAXRD)	Mesoporous structure verification	Bruker AXS GADDS
Nitrogen adsorption analysis	Isotherm, BET surface area, and BJH pore size analysis	ASAP2000 Surface area Analyzer Micromeritics
Fourier Transform Infrared (FTIR) spectra	Composition verification	Perkin Elmer System 2000

3.3.1 Differential Thermo -Thermo Gravimetric Analysis (DT-TGA)

Differential Thermo Analysis (DTA) measures the temperature difference between a reference material and the sample during a heat up or cooling down process. The temperature difference is an indication of the type of event that is occurring in the sample, and its magnitude. It is a "fingerprinting" technique that provides information on the chemical reactions, phase transformations, and structural changes that occur in a sample during a heat-up or a cool-down cycle. The DTA measures the differences in energies released or absorbed, and the changes in heat capacity of materials as a function of temperature.

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Thermogravimetry (TGA) is used to determine changes in sample weight, which may result from chemical or physical transformations, as a function of temperature or time. A thermogram (TG) is obtained, displaying the weight loss as a function of time at a constant temperature. The data obtained provides information concerning the thermal stability, composition and decomposition behavior of the original sample.

The DT-TGA analysis followed such procedures: around 40mg of sample is placed into a special shape cup so the test material surrounds a thermocouple bead. An identical cup (the reference cup) is filled with an inert material (powdered, high purity alumina) and is placed immediately beside the sample cup. The “DTA signal” is the difference in temperature between these two thermocouple beads while the sample cups are heated, and is constantly saved on the computer along with the temperature inside the reference cup and the elapsed time. At the same time, the TGA plot is produced.

The purpose of calcination is to burn off the surfactant template and achieve porous powder of HA. However, to ensure that the final MBGs did not contain any surfactant after a calcination process at 600°C, a decomposition profile is determined using the Thermo Gravimetric Analyzer (TGA).

To make sure the removal of organic groups of the block copolymer surfactant Pluronics F127/P123 after the calcination of samples at 600°C, the decomposition profile of F127 and calcium P123 are determined using DT-TGA thermoanalysis as shown in **Figure 3-3 (A)** and **(B)**. It can be seen that the main weight loss of both F127 and P123 appeared before the temperature rose to 500°C, which suggested the decomposition reaction taking place at our calcination environment. The main weight loss of 80S-F127 and 80S-P123 was also observed at 500°C (**Figure 3-3 (C)** and **(D)**), indicating the polymer surfactant has been completely removed at our calcination environment. The downward direction peaks in the

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DTA curves indicate the exothermal reaction. From **Figure 3-3 (A) and (B)**, the main decomposition of bare surfactant occurred around 350°C, however this peak shifts to 450°C at the DTA curves of MBGs samples (**Figure 3-3 (C) and (D)**) which was indicated with a perpendicular dashed line. This peak shifting phenomenon can be attributed to the chemistry binding formed during the synthesis process between the MBGs and organic templates.

Figure 3-3 DT-TGA plots for (A) bare F127 surfactant, (B) bare P123 surfactant, (C) 80S-F127, (D) 80S-P123.

3.3.2 Nitrogen Adsorption Analysis

The calcined sample was firstly dried in oven to remove the vapour prior to the BJH measurement. Then the sample was weighed and put into the glass tubal holder of ASAP2000 surface area analyzer (Micromeritics, USA) for nitrogen adsorption analysis.

3.3.2.1 Nitrogen Adsorption Isotherm

Mesoporous adsorbents and catalysts are commonly characterized by nitrogen adsorption isotherm data. Many of these materials have complex pore systems and desorption from capillary condensed liquid filled pores occurs at a lower relative pressure P/P_0 than adsorption. There are several explanations for hysteresis in complex pore systems. On one hand, molecular simulations, density functional theory, and recent experiments suggest that hysteresis is due to multiple metastable states which are induced by the disorder of the solid matrix [7, 8]. On the other hand, there are experiments shown that drainage of n-hexane from Vycor occurs via percolation [9, 10]. The latter is assumed to be caused by pore blocking: smaller pores retard the drainage of larger pores if the larger pores are connected to the sample surface by the smaller ones only. At a certain critical pressure drainage proceeds within a narrow pressure range sequentially along the path of least resistance, and a sample spanning cluster of vapor filled pores appears at the knee of the desorption isotherm. Drainage may also be described by the invasion of the vapor into the liquid filled pore system [11]. The adsorption isotherm shows hysteresis because capillary condensation proceeds without restraint.

Figure 3-4 (A) and (B) show the nitrogen adsorption isotherm plots for F127-MBG and P123-MBG at 77 K respectively. Type IV isotherm plots were achieved for all the MBGs samples. Typical hysteresis loop of mesoporous materials with a well-defined step in the adsorption curve between partial pressures P/P_0 of 0.4 and 0.8 were observed. The sharp increase in the adsorption volume at P/P_0 is related to the change in its average pore diameter. The adsorption volume increases as the molar percentage of SiO_2 increases, implying the increase of mesopore volume inside the sample. **Table 3-3** listed the porosity and BET surface area of all the MBGs. It can be read that, for both F127-MBG and P123-

MBG, 80S presents the biggest porosity and BET surface area. Meanwhile, P123-MBG presents larger adsorption volume for all 60S, 70S and 80S samples, as compared to F127-MBG. The largest BET surface area was obtained from sample 80S-P123 with a value of 478.2 m²/g.

Figure 3-4 Nitrogen adsorption isotherm plots: (A) F127-MBG (B) P123-MBG.

3.3.2.2 Pore Size Distribution Measurement with BJH Method

A traditional way to determine the pore size distribution is to via gas sorption techniques of Barrett, Joyner and Halenda (BJH). The mechanism of this method is based on the isotherm of gas adsorption and desorption.

The physical adsorption of gases by solids increases with decreasing temperature and increasing pressure. The pore size can be calculated simply according to the amount of gas taken up or released, through the developed Kelvin equation.

$$V_{pn} = \left(\frac{r_{pn}}{r_{Kn} + \Delta t_n / 2} \right)^2 \left(V_n - \Delta t_n \sum_{j=1}^{n-1} A c_j \right) \tag{Equation 3-1}$$

V_{pn} : pore volume of pore n

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r_{pn} : pore radius of pore n

r_{Kn} : inner capillary radius of pore n

V_n : emptying pore of its condensate or reduction in the thickness of its physically adsorbed layer by an amount of Δt_n .

$\Delta t_n/2$: average change in thickness

A_{cj} : area exposed by the previously emptied pores from which the physically adsorbed gas is desorbed.

$$c = \frac{\overline{r_c}}{\overline{r_p}} = \frac{\overline{r_p} - \overline{t_r}}{\overline{r_p}}$$

$\overline{r_p}$: average radius calculated from the Kelvin equation radii

$\overline{r_c}$: the average capillary (core) radius

$\overline{t_r}$: the thickness of the adsorbed layer at the average radius in the interval in the current decrement

Figure 3-5 shows the distribution graph for samples. The y-axis represents the pore volumes of desorption for unit weight length. Sharp peaks appear at around 77Å for 80S-F127 and 69Å for 80S-P123 were observed. This gives the information that most of the pore size is distributed around the peak value. The peak values for all samples are listed in **Table 3-3**. It can be seen that both P123 templated and F127 templated 60S samples contain smaller pore size compare to 70S and 80S. On the other hand, corresponding to isotherm result, the pore volume decreases as the percentage of SiO₂ composition decreases, and 80S-P123 presents the biggest pore volume with a value of 0.82cm³/g. All the surfactant templated mesoporous bioactive glasses have presented much larger surface area and porosity as compared to the normal bioactive glasses synthesized without surfactant [12]. The reason why the decreases

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of SiO₂ composition resulted in the falling down of pore volume and BET surface area will be discussed in section 3.4.

Figure 3-5 BJH pore size distribution: (A) F127-MBG (B) P123-MBG.

Table 3-3 Porosity and surface area of MBGs

Sample	BJH Pore size (nm)	BET Pore volume (cm ³ /g)	BET Surface area (m ² /g)
80S-F127	7.71	0.58	309.7
70S-F127	6.90	0.56	300.2
60S-F127	5.05	0.53	298.3
80S-P123	6.90	0.82	478.2
70S-P123	7.38	0.58	353.4
60S-P123	5.01	0.54	263.6

3.3.3 Pore Structure Determination with Small Angle X-ray Diffraction

Small Angle X-ray Diffraction (SAXRD) is used to verify the existence of long-range ordered structure inside the sample. The peaks within small angle range ($2\theta \sim 1^\circ$ - 10°) provide information on the microstructure of the pore. Moreover, the pore diameter can be estimated according to the d spacing values. The SAXRD detection was carried with monochromatic Cu-K α radiation with wave length (λ) of 1.5418Å, under 50kV and 20mA.

Figure 3-6 shows the SAXRD pattern of the samples. Sharp peaks were observed for both F127-MBG and P123-MBG, which suggests the existence of ordered mesoporous structure inside the samples. The peak intensity decreases as the percentage of SiO₂ composition decreases, i.e. the amount of P₂O₅ composition increases, which implies the regularity of the meso-structure tends to be poorer when less SiO₂ was incorporated. The d spacing can be calculated using the Bragg's equation (Equation 3-2). **Table 3-4** compares the evaluated results. Bragg's Law was used to calculate the d spacing as follows:

$$N \cdot \lambda = 2 d \cdot \sin \theta$$

$$d = \frac{\lambda}{2 \sin \theta} \quad (\text{Equation 3-2})$$

where n = number of order (assumed n = 1 for 1st order)

λ = wavelength of the X-ray used (i.e. CuK α = 1.5418 Å),

θ = angle of diffraction (deg).

As the 60S and 70S sample did not show clear peaks, which represent non-long range ordered structure existed inside these samples. And the peak values of F127-80S and P123-80S were calculated and indexed according to the spacing ratio in between the peaks as shown in **Figure 3-6 (C) and (D)**.

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Figure 3-6 Small angle XRD patterns for samples: (A) F127-MBG, (B) P123-MBG, (C) indexed F127-80S, (D) indexed P123-80S.

In the case of 80S-P123, peaks at $2\theta = 1.04^\circ$, 1.80° , 2.11° were observed, the combination of which with a sharp $1:\sqrt{3}:2$ could be ascribed to the first three peaks of a hexagonal structure (100), (110), (200). While for 80S-F127, one peak at $2\theta = 0.86^\circ$ and one hump at 2θ from 1.3° to 2.1° were observed. This hump could be attributed to the secondary phase of the meso-structure, which could be split into two peaks at 1.48° and 1.7° as indicated in **Figure 3-6 (C)**. These peaks can also be indexed to the (100), (110), (200) reflections of the hexagonal phase with a $1:\sqrt{3}:2$ spacing ratio.

3.3.4 Microstructural Analysis using Transmission Electron Microscopy (TEM)

3.3.4.1 TEM Sample Preparation

TEM observation was also performed to confirm the microstructure. Agglomerated fine powders were well dispersed and distributed on the surface of a carbon coated copper grid before characterization under TEM. About 5mg of the powder sample was ground in an agate mortar with 5ml ethanol. Then the material was ultrasonically dispersed into 100ml ethanol in a beaker. After left settled for 5 minutes, one to two drops of the clear solution were dripped onto a carbon coated copper grid. The sample on the copper grid was then dried in a dry keeper for 24 hours before characterizing under TEM instrument.

3.3.4.2 TEM Observation

High resolution TEM observation was carried out to verify the existence of mesoporous structure, as well as to determine the pore size and structure (**Figure 3-7**). It can be seen that the regularity of meso-structure tends to enhanced from 60S to 80S for both F127 and P123 templated bioactive glasses, indicating with the increase of silica content in the samples, the mesoporous structure becomes more regular. This is consistent with the results of SAXRD. It has been discussed that no long range ordered structure is revealed from the SAXRD patterns of 60S and 70S, while no fine-ordered meso-structure was revealed from their TEM micrographs. On the contrary, both microimages for 80S-F127 and 80S-P123 present well ordered mesoporous structure. From literature, only worm-like meso-structure was achieved by Yan et al. [1] using F127 as template, however in our case, F127 templated 80S samples also presents fine-ordered meso-structure. Diffraction pattern for these two samples were

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Figure 3-7 TEM micrographs of MBGs: (A) F127-60S, (B) P123-60S, (C) F127-70S, (D) P123-70S, (E) F127-80S, (F) P123-80S.

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also taken, the diffraction rings reveal the fine arrangement of the mesopores inside the samples. This phenomenon will be interpreted in the next section with our proposed mechanism.

3.4 Meso-structure Formation Mechanism Study and the Experimental Verification

As reviewed in **Chapter 2**, since the first report on supramolecular templated mesoporous materials was published, enormous efforts have been put to understand the formation mechanisms of these materials, especially mesoporous silica. Several suggestions had already been made with regards to the initial step of self-assembly process, such as the coating of rod-like micelles, or initial formation of a gel phase in analogy to the formation of polyelectrolyte-surfactant complexes [13-16]. Time resolved in-situ study with fluorescence probing, SAXS/SAXRD/SANS, NMR, TEM, are applied to mesoporous field in recent years to provide experimental verification on the above suppositions [17-19]. Flodström et al. [20] explained the phase transformation of P123-TEOS through analysis of SAXRD reflection. Synchrotron SAXRD is therefore identified as an essential technique to obtain time-resolved information of the structural changes during mesophase formation. However, as most of these studies focused on mesoporous silica, there are relatively fewer reports on the formation mechanism of other mesoporous materials, for example bioactive glasses. And the in situ study of the formation mechanism of mesoporous bioactive glasses was firstly carried out in the current work.

3.4.1 In Situ Synchrotron Small Angle XRD Experiment

The mesoporous formation process was inspected with synchrotron SAXRD in Singapore Synchrotron Light Source (SSLS). The reactant solution after 24 hours of stirring at 30°C

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was dropped to a shallow glass holder to undergo a rather quick evaporation process. The diffractometer used was a Huber 4-circle system 90000-0216/0 with high-precision 0.0001° step size for the ω and 2θ circles. The storage ring, Helios 2, was running at 700MeV with a stored electron beam current of typically 300mA. The X-ray beam was set to the photon energy of 8.048 keV or wavelength of $1.54056\text{\AA}$ by means of a Si (111) channel-cut monochromator (CCM) and a collimating mirror. The beam spot size was about $0.9\times 3\text{ mm}^2$ in vertical and horizontal directions respectively by a slit system. This set-up yielded an X-ray beam with about 0.005° of vertical divergence and the scans were taken during this process at a range of $0.25\text{--}4^\circ$ (2θ), with duration of 2 minutes for each scan.

3.4.2 SAXRD Analysis and Proposed Mechanism

The in situ synchrotron SAXRD patterns measured during the gelation of MBGs are shown in **Figure 3-8**. 80S samples were selected as an example for a systematic discussion. Here we still use the code MBG instead of 80S in discussion. At the very beginning before the gelation started, no peak appeared in the SAXRD patterns of either F127-MBG (**Figure 3-8 (A)**) or P123-MBG (**Figure 3-8 (B)**). In the case of P123-MBG, small peak $2\theta = 0.8^\circ$ show up after 8 minutes, which were originated from the of P123 phase, surrounded by the polymerized silica network. The intensity of the peaks increases with time, due to the further polymerization of silica, or the surfactant phase transformation caused by the condensation of P123 when more solvent was evaporated. As time increased, the intensity of this peak becomes even stronger, and finally reaches a constant value in around 20 minutes, indicating a relatively stable mesoporous phase coming into being. Meanwhile, two peaks at $2\theta = 1.75^\circ$ and 2.65° were observed which implies the further growth of the meso-structure. The intensity of these two peaks kept increasing during the whole evaporation process. The peak

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Figure 3-8 In-situ synchrotron SAXRD studies (A): Time resolved SAXRD of F127-MBG (B): Time-resolved SAXRD of P123-MBG.

spacing ratio for P123-MBG is $1:\sqrt{3}:\sqrt{7}$ corresponding to the index (100), (110) (210) diffractions for a two-dimensional hexagonal ($P6mm$) structure. The peak intensity

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especially for the secondary phase was enhanced after calcination at 600°C (refer to previous **Figure 3-6 (C)**, and **Figure 3-6 (D)**), the combination of the space ratio $1 : \sqrt{3} : 2$, could be ascribed to the first three peaks of a hexagonal structure (100), (110), (200). Similar phase transformation behavior was also observed from in situ SAXRD pattern of F127-MBG (**Figure 3-8 (A)**).

The phase transition behavior from disordered to fine ordered hexagonal arrangement is clearly shown from the SAXRD pattern during the sol-gel self-evaporation process. Goltner-Spickermann et al. [21] suggested that in the case of sol-gel derived mesoporous silica, it is the unspecific demixing of silica-PEO hybrid from bulk PEO by forming crystalline PEO before or during solidification that gives rise to the mesoporous structure. Based on our study, the case of sol-gel derived bioactive glasses is more complicated. Firstly, with the mixing of inorganic precursor and block copolymer, the hydrolysis of TEOS in the acidic environment resulted in the interaction between the EO corona of (PEO-PPO-PEO) micelle and Si-O tetrahedron. Ca^{2+} ion and P-O tetrahedron will then participate by forming a complex with Si-O-micelle group. With the polymerization of silica, the micelle-Si-O-Ca-P-O complex was then made into an increasingly firm network. Meanwhile, the polymerization of silica enhanced the phase transformation of the copolymer (F127/P123) from disordered micelle to liquid crystal with hexagonal structure, which then induced the ordered arrangement of the silica network. In the case of normal bioactive glasses (BGs), the Ca^{2+} ion and P-O tetrahedron would break the Si-O network, and result in some porous structure. However in MBGs, with the existence of surfactant-Si-O, the Si-O network tends to be more rigid, and the ordered arrangement can thus avoid to be disrupted by Ca^{2+} and P-O group. Finally, the interaction between the polymerization of silica and amphiphile block copolymer (F127/P123) achieved a highly ordered meso-structure framework. The

Figure 3-9 Schematic diagram of phase transformation from sol-gel to calcined mesoporous samples.

calcination provided further energy which enabled the full phase transformation during the heating up period before the organic template was removed, resulting in a fine ordered hexagonal mesoporous bioactive glasses. The whole process was illustrated schematically in **Figure 3-9**.

3.5 Evaluation of In Vitro Bioactivity

The basis of the bone-bonding property of bioactive glasses is the chemical reactivity of the glass in the presence of body fluids. The surface reactions lead to the formation of an apatite layer and hence bonding of implant to tissue.

Actually, bioactive glasses bond to bone through the formation of a new apatite-like phase at the surface [22]. This process is the result of an ionic exchange between the glass and the environment when the implant gets in contact with physiological fluids. Afterward, the glass

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develops a silica rich layer at the surface that facilitates the nucleation of an apatite phase taking Ca^{2+} and phosphates from the fluids. [23] In the current work the in vitro bioactivity of MBGs were tested by the Biomimetic Growth of HA in simulated body fluid (SBF).

3.5.1 Biomimetic Growth of HA

The calcined gels for both F127-MBG and P123-MBG samples were ground with agate mortar into particles of micron-size before immersing them in SBF (See **Appendix-II** for the preparation of SBF) for the in vitro bioactivity test. The ratio of MBGs powders weight to SBF solution volume is 1.5 mg/ml. Samples were put into a polyethylene bottle covered with a tight lid. The bottles were placed in a water bath at 37.5°C for 6 hours, 12 hours, 1 day and 3 days, without refreshing the SBF solution. After soaking, samples were removed from the SBF and washed with ethanol. In this section, we also take 80S for further analysis, and F127-MBG, and P123-MBG in this section also refer to 80S samples.

3.5.2 Phase Analysis using X-ray Diffractometer (XRD)

The calcined samples were subjected to phase analysis. Phases present in the samples were determined through XRD analysis. The sample was placed on the cavity of a glass plate and the surface of the powder was smoothed. The thickness of the sample together with the glass plate was measured and the height of the machine holder was adjusted accordingly. Monochromatic $\text{CuK}\alpha$ radiation with wave length (λ) of 1.5418Å was used under 50kV and 20mA. The XRD data were collected over the 2θ range of 10°~80° at a scanning speed of 2°/min. The determination of the 2θ values and peak heights, as well as the phase analysis of the collected XRD data was achieved by using the built-in computer software.

Figure 3-10 shows the wide angle XRD patterns of the samples after soaking in SBF. From the XRD patterns, it can be observed that after 6 hours of biomimetic growth, HA phase can

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be identified from the characteristic peak of HA phase at 2θ of 31.7° and 45.3° , which correspond to the (002) and (203) plane respectively. With increasing soaking time, the peak intensity tends to be stronger, and one day later, the (004), (413) and (513) diffractions were observed from the XRD patterns of both F127-MBG and P123-MBG. Interestingly, XRD patterns indicates a similar bioactivity for F127-MBG and P123-MBG, despite the XRD pattern of F127-MBG shows a slightly higher intensity after 12 hours soaking in SBF.

Figure 3-10 XRD patterns of F127/P123-MBG and that after soaking in SBF.

3.5.3 Morphology Analysis using Field Emission Scanning Electron Microscopy (FESEM)

The morphology of the final white precipitate obtained before and after calcination was observed using the FESEM. Element analysis was also carried out using EDX spectra.

3.5.3.1 FESEM Sample Preparation

A speck of powdered sample was well dispersed in small amount of ethanol. The mixture of sample and ethanol was then dropped onto a small piece of microscope glass. After that, by using the SPI-Module Sputter Coater machine, the well-dispersed powders samples were coated with platinum by ion beam sputtering. The sample was then subjected to FESEM observation.

3.5.3.2 FESEM Observation

The prepared sample was observed by using FESEM, under accelerated voltage of 5 KV, at different magnifications (from 1000 X to 100000 X). The nanosized particle images then can be observed at relatively high magnification. The dimensions of the particles can be also estimated from the FESEM images.

Figure 3-11 shows the micrographs of F127-MBG and P123-MBG of 80S with biomimetically grown HA on their surfaces. Needle shaped HA particles were clearly observed on the surface of the MBG samples especially after 3 days of soaking in SBF. The bioactivity of MBGs is proven to be much better as compared to that of the normal non-mesoporous bioactive glasses (BGs) from literature [24, 25]. EDX spectra attached to each FESEM micrograph display the rising P: Si ratio with increasing soaking time, which is consistent with the XRD patterns. For a better understanding of the phenomenon, the

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Figure 3-11 FESEM micrographs and EDX spectra of: (A) F127-MBG, (B) F127-MBG 1day soaking in SBF, (C) F127-MBG 3days soaking in SBF, (D) F127-MBG 6h soaking in SBF, (E) F127-MBG 12h soaking in SBF, (F) P123-MBG 1day soaking in SBF, (G) P123-MBG 3 days soaking in SBF, (H) P123-MBG 12h soaking in SBF, (I) P123-MBG 1 day soaking in SBF, (J) P123-MBG 3 days soaking in SBF.

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Figure 3-11 FESEM micrographs and EDX spectra of: (A) F127-MBG, (B) F127-MBG 6h soaking in SBF, (C) F127-MBG 12h soaking in SBF; (D) F127-MBG 1day soaking in SBF, (E) F127-MBG 3days soaking in SBF, (F) P123-MBG; (G) P123-MBG 6h soaking in SBF, (H) P123-MBG 12h soaking in SBF (I) P123-MBG 1 day soaking in SBF(J) P123-MBG 3 days soaking in SBF.

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properties of MBGs and BG obtained from various characterization (some of which will be discussed later) were listed in **Table 3-4**.

Table 3-4 Main parameters of MBGs and BG samples

Sample	BJH Pore Size(nm)	SXARD Pore Size(nm)	BET Pore Volume (cm ³ /g)	BET Surface Area(m ² /g)
F127-MBG	7.71	10.26	0.58	309.07
6h	7.79	/	0.56	300.95
12h	8.16	/	0.53	271.96
1d	8.51	/	0.51	240.68
3d	9.96	/	0.36	143.73
Drug loaded	7.80	/	0.48	280.55
P123-MBG	6.90	8.49	0.82	478.20
6h	7.32	/	0.81	473.45
12h	7.59	/	0.78	415.24
1d	8.13	/	0.56	357.61
3d	9.88	/	0.38	139.29
Drug loaded	7.94	/	0.64	321.60
BG	4.60	/	0.05	57.99

3.5.4 Apatite Growth Mechanism

It is observed that P123-MBG owns a larger surface area and pore volume compared to F127-MBG, which should result in better bioactivity. Hench and West [26] proposed a complex process for the formation of apatite layer involving five reaction stages. Kokubo et al. [27, 28] studied the growth of apatite layers on different compositional glasses and glass ceramics in the presence of simulated body fluid (SBF). The reaction mechanism was interpreted as follows:

- (1) The calcium ion dissolves from the surface of the glasses and glass ceramics and increases the ion activity product of the apatite in the surrounding body fluid, whereas the disrupted silica network on the surface forms into a sediment of hydrated silica, which provides favorable sites for apatite nucleation.

- (2) The apatite nuclei then grow spontaneously, consuming calcium and phosphate ions from the surrounding body fluid. This mechanism is illustrated in **Figure 3-12**.

Figure 3-12 Schematic representation of the mechanism of apatite formation on the bioactive surfaces [26].

To give a clear interpretation, we illustrated the apatite formation on MBGs based on the apatite formation mechanism proposed by Hench et al. [26] and Kokubo et al. [27, 28]. The calcium ion dissolves from the surface of the glasses and increases the ion activity product of the apatite in the surrounding body fluid, whereas the disrupted silica network on the pore wall/surface forms a sediment of hydrated silica (Si-OH) as follows:

This end group provides favorable sites for apatite nucleation, and hence apatite then grows spontaneously, consuming calcium and phosphate ions from the surrounding body fluid. Block copolymer F127 ($\text{EO}_{99}\text{PO}_{65}\text{EO}_{99}$) has more EO portion in its molecular chain than P123 ($\text{EO}_{20}\text{PO}_{70}\text{EO}_{20}$), which results in a much larger EO corona in the micelle structure during the condensation process. This large EO corona leads to the bigger pore size later on. On the other hand, this “giant” EO corona is not easy to be “squeezed” into a perfect “ball” as compared to a smaller corona, which may result in lots of defects (illustrated in **Figure 3-**

13 with circles in dashed line) in the pore wall when the template is removed. When the MBGs are immersed in SBF, the defects could act as nucleation sites for apatite formation.

Figure 3-13 Illustrated apatite formation on MBGs in simulated body fluid (SBF).

3.5.5 Nitrogen Adsorption Analysis

Analysis of N₂ adsorption at 77K was conducted on all the MBGs and their respective isotherm plots are shown in **Figure 3-14**. Type IV (see **Appendix I**) isotherm plots were achieved for all the samples, which confirms the presence of mesoporous materials. The absorption volume of P123-MBG decreases as soaking time in SBF increases, which is however not obvious for F127-MBG. It implies that for the same weight ratio of surfactant/inorganic precursor, the template structure of P123 is more stable than that of

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F127, in other words the meso-structure could be more easily destroyed for F127-MBG, as compared to P123-MBG. This is attributed to the higher amount of defects on the pore wall

Figure 3-14 Isotherm absorption plots: (A) F127-MBG (B) P123-MBG.

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(C)

(D)

Figure 3-15 Isotherm absorption and BJH pore size distribution plots: (A) F127-MBG isotherm plot (B) P123-MBG isotherm plot (C) F127-MBG BJH plot (D) P123-MBG BJH plot.

caused by the larger EO corona as mentioned earlier. **Figure 3-15** plots the distribution of pore diameters according to the BJH nitrogen adsorption. It can be seen that, for F127-MBG

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the pore size distribution of MBGs after soaking in SBF becomes much wider than original, and the average pore size increases (see **Table 3-4**), due to collapse of the mesoporous structure with the nucleation and growth of HA phase onto the original meso-structure of the bioactive glasses. In the case of P123-MBG, the pore size almost remains at the first 12 hours of immersion in SBF, with a slight decrease in pore volume and surface area. As soaking time increases, more $O \cdots Si \cdots O$ bonds are activated, and the biomimetic growth becomes faster, so that the surface area and pore volume of P123-MBG dropped down dramatically after one day of soaking. Finally three days later the surface is even smaller than that of F127-MBG. The maximum adsorption volume of which ($37\text{cm}^3/\text{g}$) is much smaller than our MBGs samples ($305\text{cm}^3/\text{g}$ for F127-MBG, $510\text{cm}^3/\text{g}$ for P123-MBG). BJH plot for BG sample doesn't present single peak distribution as that of MBGs.

3.6 In Vitro Study of Drug Incorporation and Release

3.6.1 Experiment

Metoclopramide is selected as the model drug for drug delivery evaluation systems, which is recommended for use as a potent antiemetic agent in various types of vomiting with high aqueous solubility, because of its pharmacokinetic properties of variable bioavailability (50%), short plasma half-life, and therapeutic interest [29]. The drug release measurements were carried out by means of UV spectroscopy as proposed by Sampath and Robinson [30]. The spectra absorbance value was measured at a characteristic wavelength ($\lambda = 309.4\text{ nm}$) using UV-Vis spectrophotometer (UV-1700, Shimadzu, Japan), at which the metoclopramide shows an absorbance maximum.

0.3 g of MBG powder was conformed into disk by uniaxial (2.75MPa) and isostatic (3MPa) pressure. The disk was then soaked in 30 ml of metoclopramide solution (10mg/ml), the vial was sealed to prevent evaporation and contamination of solvent. The amount of metoclopramide adsorbed onto MBGs is determined by depletion method.

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Drug release process was carried out after the disk was soaked in metoclopramide solution for 24 hours. The drug-loaded MBG disk was quickly washed with ethanol to remove the excess metoclopramide molecules coating at the outer surface, and then dried at 50°C. Successively the MBG disk with drug was placed into 10ml SBF solution, and agitated in a horizontally shaking water-bath maintained at 37.5°C. FTIR spectra (Perkin Elmer System 2000) were obtained at a resolution of 4cm^{-1} over the frequency range of $4000\text{-}400\text{cm}^{-1}$. The drug incorporation/release property of mesoporous MBGs was studied with metoclopramide chosen as the model drug.

3.6.2 Results and Discussion

Figure 3-16 (A) shows the percentage of metoclopramide loading as a function of time for the MBGs and BG samples. It was observed that both the loading and release were very fast on the first day. The metoclopramide loading in BG reached a maximum value of 10% at the fourth day, then remained constant for the subsequent days. For F127-MBG, the loading percentage rose to 16.6% at the 7th day. The speed of loading became very slow then, and only 18% was loaded after 3 weeks. In the case of P123-MBG, things are quite different. The loading rate was a bit slower than BG and F127-MBG on the first day; however after that the rate remained consistent till the 14th day reading a maximum value of 47.3% after 3 weeks. It is noticeable that, both F127 templated MBG and P123 templated MBG presents good drug loading potential, and the loading amount in 3 weeks of P123-MBG is 4.5 times of that for the normal BG (1.8 times for F127-MBG/BG).

The amount released of drugs over time was also different between the MBGs and BG, as observed from **Figure 3-16 (B)**. After 3 days, 87%, 70% and 53% of the drug were released from BG, F127-MBG and P123-MBG respectively. The only similarity was the release profile, where an initial burst release was observed for all three samples. This burst effect

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was probably due to the massive diffusion of drugs from the samples, during the initial stages. However, the rate of metoclopramide release started to decrease after 3 days and a more consistent release was subsequently observed. All the drugs in BG were released by the 14th day, whereas drugs were still remaining in the MBGs even after 24 days. The mesopores

Figure 3-16 Metoclopramide loading and release curve of MBGs and BG: (A) Loading curve; (B) Release curve.

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in the MBGs were therefore also responsible for the drug release profile of drugs up to 24 days. Although the drug release was probably diffusion-controlled, the mesopores were shown to be able to sustain this release consistently. From the drug loading and drug release results, it can be deduced that P123-MBG has the highest drug loading capacity and the longest controlled-release time. Based on the data from **Table 3-4**, the superior absorbability of P123-MBG than F127-MBG could be attributed to its relatively larger BET surface area, which leads to a high absorbance percentage as well as a slow release rate. However the relatively small pore size has limited the initial adsorbing speed. The BJH pore size distribution of drug-loaded MBGs is also characterized and shown earlier in **Figure 3- 15**. Xia et al. [31] has also studied the drug delivery property of mesoporous bioactive glasses templated by P123 with the composition of 77S and 58S through a co-precipitation method. However the drug release property of our P123-80S is more desirable than their P123-77S. For example, on the fourth days of the experiment, around 65% of the drug was released from their drug loaded P123-77S, while only 47% of the drug was released from our P123-80S. This indicates that as the composition is similar, our sol-gel derived MBGs present more desirable released property than their samples synthesized through a co-precipitation way, which could be attributed to that the sol-gel derived meso-structure is more uniformed as compared to the co-precipitation derived meso-structure.

The FTIR result further provides a direct qualification of metoclopramide loaded of the MBGs sample (**Figure 3-17 (A)**) Two characteristic adsorption peaks at 1634cm^{-1} and 1597cm^{-1} ($\text{C}=\text{C}$ stretching) have appeared in neat metoclopramide (see **Figure 3-17 (B)**)for molecular structure), due to the vinyl unsaturation present in metoclopramide formula. The absorption at 3406cm^{-1} can be ascribed to the asymmetry stretch vibration of NH_2 . And these peaks were observed in the P123-MBG and F127-MBG sample hosted with metoclopramide. On the other hand, metoclopramide was not expected to affect the

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bioactivity of the materials [32], so the bioactivity of the samples is believed to remain after the drug loaded.

(A)

(B)

Figure 3-17 (A) FTIR spectra of F127-MBG and P123-MBG and Drug-loaded MBGs and (B) Structural formula of Metoclopramide.

3.7 Properties Comparison between 60S, 70S and 80S

The characteristic of this optimum 80S samples were discussed in the earlier section. To give an overall comparison, the characteristic of 60S and 70S samples were also discussed in this section.

3.7.1 In Vitro Bioactivity Comparison

It is confirmed that the bioactivity of 60S and 70S is poorer than 80S. **Figure 3-18** shows the XRD patterns of the samples after soaking in SBF for 3 days. Same weight amount of HAS samples was applied for the XRD test, and stronger intensities of XRD patterns indicate there are more HA phase was biomimetically formed, which implies better in vitro bioactivity of the samples. From the intensity of the HA characteristic peak, it is obvious that the in vitro bioactivity of MBGs tends to be poorer as Si% decreases. For both F127-MBG and P123-MBG, the bioactivity behaves in the following sequence: 80S>70S>60S. For 60S and 70S, F127 templated MBGs also present better bioactivity than P123 templated MBGs, this is consistent with the findings of the 80S samples earlier. The FESEM micrographs and EDX spectra for 60S and 70S after three days soaking in SBF are shown in **Figure 3-19**. Compare the morphology and EDX spectra of 60S, 70S, and 80S (**Figure 3-11**) after three days of SBF soaking, the same trend of bioactivity as that from XRD can be seen, that is 80S>70S>60S, which is consistent with Yan et al's work [33]. The bioactivity should be related to not only the density of surface silanol groups ($\equiv\text{SiOH}$), but also the nature of the microstructure of the samples [34]. The bioactivity/composition correlation is contrast to that of the traditional sol-gel derived bioactive glasses, which shows higher calcium concentration results in higher bioactivity, which indicating that the MBGs present unique bone-forming bioactivities due to their unique mesoporous structure.

Figure 3-18 Bioactivity comparison between 60S, 70S and 80S with 3 days biomimetic growth of HA.

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Figure 3-19 FESEM micrographs and EDX spectra for 60S and 70S after 3 days soaking in SBF: (A) 60S-F127, (B) 70S-F127, (C) 60S-P123, (D) 70S-P123.

3.7.2 Comparison of In Vitro Drug Delivery Potential between 60S, 70S and 80S

To compare the in vitro drug delivery potential between the MBGs with different composition, drug delivery potential for 60S and 70S was also tested using metoclopramide as the model drug.

Figure 3-20 (A) shows the percentage of metoclopramide loading as a function of time for all 60S, 70S and 80S, while **Figure 3-20 (B)** shows the percentage of metoclopramide release as a function of time. It can be seen that, as the Si% increases, both the drug incorporation and release property for either P123-MBGs or F127-MBGs, the drug release property of 60S and 70S is much poorer than 80S. This should be attributed to the higher BET surface area and pore volume of the MBGs increase as Si% increases. On the other hand, among all the samples, 80S-P123 presents the largest drug loading amount, as well as the best drug release property, which is consistent to the result that 80S-P123 has the largest BET surface area and volume of the pore.

Figure 3-20 Metoclopramide loading and release curves of 60S, 70S and 80S: (A) Loading curves and (B) Release curves.

3.8 Concluding Remarks

Two systems of ordered mesoporous bioactive glasses were sol-gel derived using Pluronic F127 and P123 as template respectively. The phase transformation behaviour of these systems was in situ studied using synchrotron SAXRD. The corresponding meso-structure

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formation mechanism was proposed, which suggests that the surfactant not only provides a fine ordered template for mesoporous structure, but also reinforces the rigidity of Si-O network. The ordered Si-O arrangement can thus avoid the disruption by Ca^{2+} and P-O groups. F127 templated mesoporous bioactive glasses display better bioactivity during the biomimetic process than that templated by P123, due to the existence of more defects on the pore wall. The bioactivity of the samples behaves in the following sequence: 80S>70S>60S, which could be attributed to their unique mesoporous structure.

Fine drug delivery property of these two systems was proven by study of incorporation and release behavior of metoclopramide drug. Both F127 and P123 templated mesoporous bioactive glasses present good drug loading potential, with loading amount 4.5 times of the normal BG for P123-MBG, and 1.8 times for F127-MBG. A much better drug release property of MBGs due to their much higher surface area and pore volume was also obtained compared to normal BG. For small drug particles such as metoclopramide, P123-MBG shows some advantage as drug delivery vehicle for its high loading amount and low release rate, while F127-MBG maybe used for some drug with larger particle size, for its bigger pore size and better activity as it displayed in HA growth. The drug release property of 60S and 70S is much poorer than 80S which could be attributed to the unique structure of mesoporous bioactive glasses.

3.9 References

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Chapter 4

Co-synthesis of Mesoporous Hydroxyapatite-silica Composite Materials and Their Properties

4.1 Introduction

There has been immense interest in the biomedical field and the use of biomaterials in various biomedical applications. Mesoporous biomaterials have been identified to be a potential controlled drug delivery system due to their high specific surface area, well-ordered pores and large pore volumes [1-5]. More recently, Villacampa et al. [6] synthesized hydroxyapatite-silica composite material using a sol-gel process at different pH environments. The combination of hydroxyapatite (HA) with mesoporous bioactive glasses, would therefore have, as composites, added advantages in terms of their combined properties and enhanced drug delivery capabilities. The use of mesoporous bioactive glasses with hydroxyapatite also provides improved bioactivity, since supramolecular templated mesoporous bioactive glasses have been shown to have superior bioactivity in vitro compared to normal bioactive glasses as discussed in **Chapter 3**. However, their biocompatibility is not so good as compared to that of hydroxyapatite [7]. Synthetic hydroxyapatite has been proven to show an excellent biocompatibility and a certain amount of bioactivity, but its use is limited because its mechanical properties differ from those of natural bone. The concept of hydroxyapatite-silica composite is hence brought up [8-12]. As

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there is relatively few work conducted for this hydroxyapatite-silica material composite, it would therefore be of interest to study the synthesis and drug delivery properties of this composite. In this chapter, mesoporous hydroxyapatite-silica (HA-silica) composites would be investigated through a sol-gel method for the first time. Then, the drug delivery property of this system was studied, using a model drug metoclopramide.

4.2 Synthesis of mesoporous HA-silica

The mesoporous HA-silica gels were synthesized using nonionic block copolymer P123 as the structure-directing agent. The synthesis method is similar with the synthesis of MBG as stated above. 5g of P123 and corresponding amount of TEOS, calcium nitrate, TEP were added according to the ratio as described in **Table 4-1** (refer to HAS1-HAS3: molar ratio of Ca/P is kept at 1.67, which corresponds to that of HA) and these were all dissolved in 50g of ethanol. 0.5ml of 1 M HCl was then added and the mixture was stirred at room temperature for 1 day. The pH value was then adjusted to 7.6 for the condition required for HA crystallization. This was followed by the EISA process and the dried gel was calcined at 600°C for 5 hours to obtain the final product. The flowchart of synthesis is illustrated in **Figure 4-1**.

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Figure 4-1 Flowchart of the synthesis of mesoporous bioglasses.

Table 4-1 Sample description

Samples	Description (molar ratio)
HAS0	Si : Ca : P = 100:20:15
HAS1	Si : Ca : P = 64:25:15
HAS2	Si : Ca : P = 32:25:15
HAS3	Si : Ca : P = 24:25:15

4.3 Characterization

4.3.1 Phase Analysis using X-ray Diffractometer (XRD)

The phases presented in the samples were determined using X-ray diffraction (Standard attachment XRD6000, Shimadzu, Tokyo, Japan). Monochromatic $\text{CuK}\alpha$ radiation with wave length (λ) of $1.5418\text{\AA}$ was used under 50kV and 20mA.

Figure 4-2 XRD patterns of HAS0, HAS1, HAS2 and HAS3 samples.

Figure 4-2 plots the XRD patterns of the HA-silica samples after calcination. It can be seen from the XRD pattern of sample HAS0 that the sample is amorphous and hence no sharp crystalline HA peaks were observed. The XRD peak intensities increase with increasing ratio of Ca/Si, and the peaks were consistent with standard XRD patterns for HA i.e. P63/m. The characteristic peak of HA crystal at 2θ of 31.7° was observed from the pattern of sample HAS1. The XRD peaks become more defined for samples HAS2 and HAS3. In the pattern of sample HAS2, for instance, five peaks corresponding to 2θ at 22.9° , 25.9° , 31.7° , 46.7° and 49.5° were observed, corresponding to the (111), (002), (203), (222) and (213) of

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hydroxyapatite planes respectively. That is, with the increase of Ca/Si ratio more crystallized HA is formed.

4.3.2 Morphology Analysis using Field Emission Scanning Electron Microscopy (FESEM)

The morphology of the samples was observed using FESEM (JEOL JSM-6340F), working at 5kV, 12 μ A. The FESEM samples were prepared in the same way as in **Chapter 3**. The FESEM micrographs for HAS0 through HAS3 are shown in **Figure 4-3**. It is observed that

Figure 4-3 FESEM micrographs for HA-silica samples: (A) HAS0, (B) HAS1, (C) HAS2, (D) HAS3.

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the surface of HAS0 is smooth, whereas more spherically shaped particles of HA were observed for samples HAS1, HAS2 and HAS3. With the increasing Ca/Si ratio, the particle sizes of HA increases from 30-50nm for HAS1 to 80-100nm for HAS3. This suggests that the increase in Ca/Si ratio results in an environment more favorable for the nucleation and growth of HA crystals. HA composition inside HAS samples hence increases with the rising of Ca/Si ratio, which is in good agreement with the XRD results.

4.3.3 Nitrogen Adsorption Analysis

Nitrogen adsorption at 77K, was conducted for HA-silica samples using ASAP2020 Surface Area Analyzer (Micromeritics, USA). **Figure 4-4 (A), (B), (C), (D)** show the nitrogen adsorption isotherm plots at 77K for the HAS0, HAS1, HAS2, HAS3 samples

Figure 4-4 Isotherm plots for HAS samples: (A) HAS0, (B) HAS1, (C) HAS2, (D) HAS3.

Chapter 4. Co-synthesis of Mesoporous HA-Bioglass (HAS) Composite Materials and Their Application respectively. Type IV isotherm plots (see **Appendix I**) were achieved for all the HA-silica samples. Typical hysteresis loop of mesoporous materials with a well-defined step in the adsorption curve between partial pressures P/P_0 of 0.4 and 0.8, were observed for sample HAS0, HAS1 and HAS2. The rising speed of HAS3 adsorption curve in between partial pressures P/P_0 of 0.4 and 0.8 is much smaller than the former three samples, indicating a much lower volume of mesopores for HAS3. The pore size distribution achieved from the BJH method is plotted in **Figure 4-5**, and the peak values from BJH plot as well as the porosity and surface area of all the samples are summarized in **Table 4-2**. From both the shapes of the isotherm plot (**Figure 4-4**) and their respective peak values of pore size distribution plot calculated by the BJH method (**Table 4-2**), it was observed that the pore diameter increased with increasing ratio of Ca/Si, from HAS0 to HAS2. This indicates the destruction of the meso-structure due to the growth of HA crystals within the samples. Correspondingly, from **Table 4-2**, it was also found that the porosity and surface area of HA-silica samples decreases from sample HAS0 to HAS2. This implies that the decrease of Si% reduces the Si-O-micelle group, therefore resulting in the decrease of porosity as well as the pore regularity. The decrease in pore volume was also observed for HAS3, in comparison to HAS0, because of the high percentage of HA composition in HAS3 that restricts the formation of mesoporous HA-silica. Interestingly, the surface area for HAS3 was observed to be larger than that for HAS1 and HAS2. This increase in surface area could be attributed to the formation of HA nano-crystals, in which the nano-crystals of HA protrude from the amorphous silica, which can be observed from the SEM micrographs (**Figure 4-3**). From these results, it is hence proposed that there exist two mechanisms operating concurrently in the formation of mesoporous HA-silica composite materials. In the proposed mechanism, both the mesoporous structure and the nano-crystals contribute to the increase in surface area of the composite. When the amount of nano-crystals is low, the

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Figure 4-5 BJH pore size distribution plot for HAS samples.

Figure 4-6 SAXRD patterns for HAS samples.

Table 4-2 Porosity and surface area of HA-silica samples

Sample	BJH Pore size (nm)	BET Pore volume (cm ³ /g)	BET Surface area (m ² /g)
HAS0	3.46	0.55	323.46
HAS1	3.53	0.45	136.22
HAS2	3.85	0.36	117.52
HAS3	3.46, 15.19	0.30	171.15

contribution of meso-structure to the surface area is predominant, resulting in a lower surface area of HAS1 as compared to HAS0. On the other hand, when the amount of nano-crystal increases, the contribution of nano-crystals becomes predominant, hence HAS3 presents a higher surface area than HAS2, despite of its lower porosity. The proposed formation mechanism will be further discussed in the later part of this chapter.

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4.3.4 SAXRD Analysis of Mesoporous Structure

Small Angle X-ray Scattering (Bruker Nanostar, PW3830 X-ray generator, Anton Paar) were carried out to verify mesoporous structure inside the HA-silica samples. Monochromatic Cu K α radiation with wave length (λ) of 1.5418Å was used at 20KV and 50mA. **Figure 4-6** shows the SAXRD patterns of the samples. Close observation shows that only samples HAS0 and HAS1 contained a small broad peak, which indicates that a fraction of long-range ordered structure exists in these samples. On the other hand, the absence of similar peaks proves that there is no long-range ordered structure for samples HAS2 and HAS3. This is in agreement to that reported by Galo et al. [13], where ordered mesoporous structure is difficult to form in crystallized materials, such as HA.

4.3.5 TEM Observation of Mesoporous Structure

High resolution Transmission Electron Microscope (200kV, TEM, JEOL JEM-2010) micrographs of samples were shown in **Figure 4-7**. Relatively ordered mesostructure was observed for sample HAS0 and HAS1, wormlike mesoporous structure was observed for sample HAS2. No mesoporous structure for HAS3 sample was observed under TEM (**Figure 4-7 (D)**). The micrographs indeed show that the mesoporous structure of HA-silica tends to be disordered with increasing Ca/Si ratio. On one hand, the decrease of Si% composition reduces the regularity of mesoporous structure, on the other hand, the increase in Ca% has resulted in the increase of the amount of crystallized HA phase, and brings more difficulty in the forming of ordered mesoporous structure. This is also consistent with the SAXRD results. To understand the effect of composition to the regularity of the mesoporous structure, the formation mechanism of formation is discussed in the following section.

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Figure 4-7 TEM images of: (A) HAS0, (B) HAS1, (C) HAS2 (D) HAS3.

4.4 Meso-structure Formation Mechanism

The meso-structure formation mechanism is proposed to be similar with that of MBGs as discussed in **Chapter 3**.

Firstly, with the mixing of inorganic precursor and block copolymer, the hydrolysis of TEOS in the acidic environment resulted in the interaction between the EO corona of (PEO-PPO-PEO) micelle and Si-O tetrahedron. Ca^{2+} ion and P-O tetrahedron will then participate in by forming a complex with Si-O-micelle group. While the pH was adjusted to 7.6 after 24 hours of mixing, nucleation of hydroxyapatite occurred. At this time, the self evaporation process started, which resulted in the polymerization of silica. The micelle-Si-O-Ca-P-O

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Figure 4-8 Illustration of phase transformation from sol-gel to calced mesoporous samples.

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complex was then assembled into an increasingly firm network. Meanwhile, the polymerization of silica enhanced the phase transformation of the copolymer P123 from disordered micelle to ordered liquid crystal structure, which tends to induce the ordered arrangement of the silica network. However, different from the MBGs, the existence and growth of hydroxyapatite crystals tend to break the ordered arrangement of surfactant-Si-O network, and lead to the disordered meso-structure. This disordered tendency resulted from the crystallization of HA and the regularity created by the surfactant liquid crystal interacts with each other, and finally reaches a relevant equilibrium. For sample HAS1 instance, relatively ordered meso-structure was formed because the regularity of surfactant was in the ascendant. Wormlike mesoporous structure were observed from sample HAS2 because the amount of HA crystal increased. The whole process was illustrated schematically in **Figure 4-8**.

4.5 In Vitro Drug Incorporation and Release Study

4.5.1 Experiment

The in vitro drug incorporation and release study was carried out in the same way with MBGs in **Chapter 3**. Metoclopramide is also selected as the model drug for drug delivery evaluation. 0.3g of HA-silica powder was conformed into disk by uniaxial (2.75 MPa) and isostatic (3 MPa) pressure for drug incorporation and release test. The detailed experimental procedure has been described in section 3.6.1. FTIR spectra were also taken from the samples after 24 hours emerging in metoclopramide solution, to verify the loading of metoclopramide into the disks. The SEM micrographs of the discs were taken to show the macroporosity of the samples.

4.5.2 Results and Discussion

The macroporosity of the HA-silica discs for drug incorporation was calculated through the Equation 4-1:

Macroporosity=1 - (D_{dense} - D_{porous}) / D_{dense} (Equation 4-1)

The density of porous HA-silica (D_{porous}) was measured by applying 3 MPa of stress to 0.3 g of mesoporous HA-silica samples, and the density of dense mesoporous HA-silica samples (D_{dense}) was measured by applying 500 MPa of stress to the same amount of samples. The macroporosity was calculated and listed in Table 4-3, from which it can be seen there is a trend of the decrease of the porosity from sample HAS0 to sample HAS3. However the difference between the porosity of the samples is quite small, and the contribution from the macroporosity to the drug loading / release properties can thus be neglected. In this chapter, we will focus on the influence of mesoporosity of the samples to the drug loading / release properties. Figure 4-9 shows the SEM micrographs of the HA-silica discs for drug incorporation, however from which, which can give a direct sense of the macroporous structure inside the discs.

Table 4-3 Macroporosity of HAS samples

Description	Porosity (%)
HAS0	72.56
HAS1	71.14
HAS2	70.51
HAS3	68.97

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Figure 4-9 SEM micrographs of the discs: A: HAS0, B: HAS1, C: HAS2, D: HAS3

Figure 4-10 show the percentage of metoclopramide loading and release as a function of time for the HA-silica samples. It was observed that both the loading and release were very fast on the first day. The metoclopramide loading in HAS2 reached a maximum value of 6.12% on the fourth day. On the seventh day, the loading percentage of HAS1 and HAS3 reached their maximum value of 10% and 12% respectively. Among all the samples, HAS0 presented the largest drug loading amount, i.e. 16% after 10 days incorporation test. The amount of metoclopramide loading to the disks nearly remains constant after that. The whole experiment lasted 3 weeks, and no further drug incorporation was observed.

Figure 4-10 (B) shows the percentage of metoclopramide release as a function of time for HA-silica samples. It is interesting to find that, HAS2, which presented the poorest incorporation property, also displayed a rather fast release rate. However, HAS0 and HAS1 displayed the most desirable drug release property among all the samples. This phenomenon can be interpreted from their high surface area and ordered meso-structure,

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Figure 4-10 Metoclopramide loading and release curves of HA-silica samples: (A) loading curves, (B) release curves.

which leads to the uniform drug release distribution in the process. On the other hand, the existence of crystallized phase of HA, which enhances adsorption, also contributed to the

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release control, but has a lower influence compared to that of the mesoporous structure. This explains the slower release speed of HAS1 than HAS0, and HAS3 than HAS2.

It can be seen, either for mesoporous bioactive glasses (as discussed in **Chapter 3**) or meoporous HA-silica composite, the drug incorporation and release potential was determined by the regularity of the mesoporous structure as well as the amount of the surface area. Among all the samples of HA-silica composite and MBGs, P123-80S present the most ordered structure and the largest surface area, which gives it the best drug release property. However HAS2 and F127-60S present the poorest drug release property attributing to their poor mesoporous structure and the relatively low surface area.

Figure 4-11 shows the FTIR spectra of metoclopramide loaded HA-silica samples. Same adsorption characteristics of metoclopramide at 1634cm^{-1} and 1597cm^{-1} ($\text{C}=\text{C}$ stretching)

Figure 4-11 FTIR spectra of metoclopramide loaded HA-silica samples.

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were also observed in neat metoclopramide, due to the vinyl unsaturation present in metoclopramide formula. The absorption at 3406cm^{-1} can be ascribed to the asymmetry stretch vibration of NH_2 . These peaks were also observed from the spectra of HA-silica samples hosted with metoclopramide. This gives the evidence that the metoclopramide has been successfully loaded in HA-silica samples.

4.6 Concluding Remarks

Interesting results were obtained for HA-silica samples, which were concluded as following:

1. Mesoporous HA-silica composite with different Si:Ca:P ratio was successfully synthesized using block copolymer Pluronic P123 as template.
2. The mesoporous structure tends to become irregular as silica content decreases. The BET surface area and pore volume decreases from HAS0 to HAS2, however, HAS3 presents larger BET surface area compared to HAS2 due to the formation of nano-crystallized hydroxyapatite.
3. The pore size tends to be larger as more HA formed, indicating that the nucleation and growth of HA destroyed the meso-structure with increasing Ca/Si ratio.
4. The formation mechanism of mesoporous structure of HA-silica composite materials were proposed, the interaction between the crystallization of hydroxyapatite and the surfactant liquid crystal determines the regularity of the meso-structure.
5. In vitro drug release property evaluation shows that HAS1 presents a relatively better drug release property because the rather large surface area, and relatively ordered meso-structure.

4.7 References

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Chapter 5

Synthesis and Mechanism Study of Mesoporous Hydroxyapatite

5.1 Introduction

As discussed in **Chapter 2**, synthetic hydroxyapatite has long been recognized as one of the most important bone substitute materials in orthopaedics, dentistry and artificial implant over past few decades, which is attributed to its chemical and biological similarity to the mineral phase of human bone [1-4]. In recent years, potential use of HA as a protein delivery agent was examined [5]. Other engineering applications such as catalysts [6] have also been reported. Other potential uses for HA include drug/gene delivery agent, such as anti-tumor agents and antibodies for excision of necrotic tissue and irrigation of the wound [5].

Mesoporous bioactive glasses and mesoporous HA-silica composite materials have been studied in the earlier two chapters. However the biocompatibility of hydroxyapatite is believed much better than bioactive glasses [7]. In this chapter, block copolymer Pluronic F127 ($\text{EO}_{99}\text{PO}_{65}\text{EO}_{99}$) was used as template to synthesize mesoporous hydroxyapatite through co-precipitation. Various characterization techniques were introduced to determine and verify the phase and microstructure presented inside the product. The probable mechanism was also proposed to explain the microbehaviour during the formation of the mesoporous structure.

5.2 Synthesis of Hydroxyapatite

The mesoporous HA powder was produced in the process of co-precipitation. Block copolymer Pluronic F127 ($\text{EO}_{99}\text{PO}_{65}\text{EO}_{99}$) was selected as organic template. Two concentrations of F127 were used in the present work.

For the first group of samples, where rod-like configuration is targeted, relatively high surfactant concentration and high surfactant/ inorganic precursor ratio was introduced. 10g of F127 (Sigma) and 9.89g of calcium D-pantothenate monohydrate (Lancaster) (0.02 mole) was dissolved in 100g of distilled water and stirred vigorously to get clear micellar solution. 2.74g of potassium hydrogen phosphate trihydrate ($\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, Aldrich) (0.012 mole) was dissolved in 60g of distilled water as the phosphate ion source. Subsequently, the pH of the solution was adjusted to 12 with ammonia ($\text{NH}_3\text{H}_2\text{O}$, Fluka). The $\text{F127}/\text{Ca}^{2+}$ solution obtained was then slowly titrated with PO_4^{3-} solution and stirred homogenously. The mixture was then heated up to 90°C under reflux for 24 hours.

For the second group of samples, 3g of F127 (Sigma) and 19.78g of calcium D-pantothenate monohydrate (Lancaster) (0.04 mole) was used this time. Stoichiometrically 5.48g of $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ (Aldrich) (0.024 mole) was added as the source of phosphate ion. The same procedure carried out as described earlier.

The mixtures received from both F127 concentrations were filtered after 24 hours heating and stirring, the white precipitate produced was subsequently dried in an oven at 100°C for 24 hours. The dried white precipitate was then calcined in a furnace at 550°C for 6 hours to remove the block copolymer template. **Figure 5-1** illustrated the whole process of the sample preparation. For further convenience, the samples were termed according to the preparation methods shown in **Table 5-1**.

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Figure 5-1 Flowchart of the synthesis of HA powder with F127 as template.

Table 5-1 Sample description

Sample		Sample description
HA001	A	10g F127 templated sample, before calcination
	B	10g F127 templated, after 6 hours of calcination at 550°C
HA002	A	3g F127 templated sample, before calcination
	B	3g F127 templated sample, after 6 hours of calcination at 550°C

5.3 Characterization

5.3.1 Experimental

The identical characterization techniques with those used in **Chapter 3** were introduced in this chapter. The as-synthesized and calcined samples were subjected to phase analysis. Phases present in the samples were determined through X-ray diffraction (Standard attachment XRD 6000, Shimadzu, Tokyo, Japan) characterization. The morphology and dimensions of the final white precipitate obtained before and after calcination were observed using the FESEM (JEOL JSM-6340F). Energy dispersive X-ray (EDX) element analysis was carried on the JEOL JSM-5310 scanning microscope to obtain the elemental information of the samples. Transmission Electron Microscope (TEM, JEOL JEM-2010) observation was employed for microstructural analysis. Both the particle morphology and the pore structure and dimension could be examined from TEM images. High resolution observation was carried out to verify the existence of mesopore, as well as to determine the pore size and structure. Small Angle X-ray diffraction (Bruker AXS GADDS) was used to verify the existence of long-range ordered structure inside the sample. The peaks within low angle range ($2\theta \sim 1^\circ - 10^\circ$) can indicate the microstructure of the pores. Monochromatic $\text{CuK}\alpha$ radiation with wave length (λ) of $1.5418\text{\AA}$ was used under 50kV and 20mA. A traditional

way to determine the pore size distribution i.e. BJH (Barrett, Joyner and Halenda) gas sorption was also applied.

5.3.2 Differential Thermo -Thermo Gravimetric Analysis (DT-TGA)

To make sure the complete transition to hydroxyapatite, as well as the removal of organic groups of the calcium D-pantothenate monohydrate after the calcination of samples at 550°C, Thermo gravimetric analysis of the surfactant Pluronic F127 has been discussed in **Chapter 3**, that F127 decomposed completely below 500 °C. **Figure 5-2 (A)** shows the DT-TGA of the calcium precursor: calcium D-pantothenate monohydrate. It can be seen that the main weight loss calcium D-pantothenate also appeared before the temperature rose to 500°C, which suggested the organic composition on the Calcium D-pantothenate molecular chains can be completely burned out at our calcination environment.

Figure 5-2 (B) and **(C)** indicate the corresponding curves of sample HA001 and HA002. From the plots, we can find the main weight loss of the samples happened at the temperature lower than 400°C, a little bit of weight loss was detected after 800°C. This can be attributed to the dehydroxylation of hydroxyapatite. In fact, HA is a hydrated calcium phosphate material, it begins to dehydroxylate at about 800°C to form oxyhydroxyapatite [8], or $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_{2-2x}\text{O}_x\Box_x$, where $\Box$ =vacancy. This process is gradual and takes place over a range of temperatures. But this property is not possessed by all the samples, the synthesis method may have great effect on it. Under ambient sintering conditions decomposition has been reported to begin in the range 1050-1100°C, which is also shown in sample HA001 and HA002.

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Figure 5-2 DT-TGA plots of templates and samples: (A) Calcium D-pantothenate (B) HA001 (C) HA002.

Two mechanisms have been proposed for the decomposition as follows [9]:

where the former is the more accepted mechanism in the literatures. No weight loss was found at 600°C. The above results confirmed the removal of copolymer and organic groups from calcium D-pantothenate in the HA at the calcination temperature of 550°C. The structure observed under TEM hence expected to be porous.

5.3.3 Phase and Element Analysis

The patterns of synthesized samples and those after calcination are shown in **Figure 5-3**. The samples after calcination were noted to produce sharper peaks than those samples before calcination, which indicates better crystallinity. It is also revealed that standard hexagonal HA crystal was formed both at synthesis and remained stable after calcination. The result also shows that the existence of organic template did not induce the crystallization of other phases, as the XRD pattern indicates this has not occurred since the lattice dimensions are in exact agreement with reference data, i.e. $a \sim b \sim 0.9418$ nm, $c \sim 0.6884$ nm (space group P63/m). We observed consistency between the data from our sample and that obtained from the standard database, and confirmed that intermediates (e.g. tricalcium phosphate) are not present in the sample.

(A)

(B)

Figure 5-3 XRD patterns of samples (A) HA001, (B) HA002.

5.3.4 Particle Morphology Analysis

The particle morphology was observed under the field emission SEM. **Figure 5-4 (A), (B)** and **(C), (D)** show the pictures of sample HA001 and sample HA002 before and after calcination respectively. It can be seen, HA002 particles possess spherical morphology with

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diameter about 100nm, while HA001 particles are rod-like, of which the length is 100 to 300nm and diameter around 40 to 80nm. It is also noted that the calcination process does not show significant effect on the particle shape. The difference between the shapes of sample HA001 and HA002 are mainly attributed to the surfactant concentrations used during synthesis. As higher surfactant concentration (10% weight percent) was used for HA001, and rod-like micelles were expected to form; while for HA002, the concentration of surfactant (3% weight percent) was much lower, the spherical micelles formed during the synthesis and served as templates. The micelle which provide nucleation sites for target materials may induce the orientational growth of the HA particle. The mechanism for this phenomenon will be discussed specifically later.

Figure 5-4 FESEM images of samples: (A) HA001A (before calcination), (B) HA001B (after calcination), (C) HA002A (before calcination), (D) HA002B (after calcination).

5.3.5 Analysis of Mesoporous Structure

The distribution of pore diameters was plotted according to the BJH nitrogen desorption. **Figure 5-5** shows the distribution curves. Sharp peak appears at 58Å in the pattern of sample HA001; meanwhile a very weak peak around 24Å was also observed from this curve. That means the pore diameters around 5.8nm are in the majority, well relatively less of the pore diameters are distributed around 2.4nm for sample HA001. Two peaks at 25.3Å

Figure 5-5 BJH desorption pore size distribution: (A) HA001, (B) HA002.

Figure 5-6 Nitrogen adsorption isotherm plots: (A) HA001, (B) HA002.

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and 30 Å were observed from sample HA002, which indicates a relatively broader pore size distribution than HA001 as well as smaller pore diameters. On the other hand, it also shows that comparative amount of micropores (pore diameter less than 20 Å) around 11~12 Å presented in sample HA002. The BET surface area for HA001 was measured as 265 m²/g and 247 m²/g for HA002, and the pore volume for HA001 is 0.72 cm³/g and 0.68 cm³/g for HA002.

Figure 5-6 (A) and (B) show the nitrogen adsorption isotherm plots for HA001 and HA002 at 77 K respectively. Type IV isotherm plots (see **Appendix I**) were achieved for both samples. The rising speed of adsorption curve in between partial pressures P/P_0 of 0.4 and 0.8 for both samples is not as high as which in between 0.8 and 1. This could be attributed to that there should be a large amount of porous structure in between the HA particles, which contributed to the rise up in between 0.8 and 1 on the isotherm plot.

Figure 5-7 TEM image of samples after calcination produced from nonionic route: (A) Irregular mesopore structure of sample HA001, (B) Irregular mesopore structure of sample HA002.

High resolution TEM observation was carried out to verify the existence of mesoporous structure, as well as to determine the pore size. The TEM micrographs of sample HA001 are

shown in **Figure 5-7 (A)**. Irregular mesoporous structure was confirmed to exist in this sample, based on which, an average pore size value of 6 nm was estimated. **Figure 5-7 (B)** shows the TEM picture of sample HA002. Irregular mesopores similar to that of sample HA001 are also observed, and the pore size is measured to be 4nm.

5.4 Proposed Mechanism

Both poly-(ethylene oxide), PEO, and poly-(propylene oxide), PPO, are water-soluble macromolecules. However, the PPO polymers show much more hydrophobic property than the PEO polymers in solution. The properties of aqueous solutions of PEO and PPO have recently been examined as functions of both temperature and pressure [10], and varying solvent quality of water for PEO and PPO is noted, which leads to complex phase behaviour. Aqueous solutions of PEO/PPO block copolymers will therefore offer an important model system for phenomena such as aggregation of micelle, and micellar transformation.

At low temperature and low concentration, both PPO and PEO are hydrophilic. The block copolymers then appear as independent polymer chains or unimers. The poly (propylene oxide) block becomes more hydrophobic upon increased of temperature, and spherical micelles could then formed, which composed of a core dominated by propylene oxide blocks and surrounded by a corona of hydrated ethylene oxide subchains. The direct images of the F127 micelles using cryogenic temperature electron microscopy (cryo-TEM) captured by Kell [11] have also confirmed the formation of spherical micelles. It is also reported that, for F127, $R_h = 80\text{\AA}$ at ambient temperature.

For higher temperature and higher concentration, the behaviour is noted to be rather different. The spherical micelles formed at low-temperature would transform via prolate ellipsoids into rod or worm-like micelles within a small region at higher temperatures. For

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Pluronic F127, at low polymer concentrations the sphere-to-rod transition only appears at $T \approx 90^\circ\text{C}$. On increasing concentration, the transition temperature is reduced.

Liquid crystals are observed first at weight fractions exceeding 20%. The typical behaviour of the soluble, micelle-forming amphiphiles can be attributed to two phenomena. First, the aggregate-aggregate interaction is repulsive. There has to be a short-range repulsion for amphiphilic behaviour in general, and normally this is accompanied by repulsion or zero interaction at a longer range, although there are cases with an attractive part of the interaction curve. The second circumstance is that aggregates can favourably grow in size at most one direction so that aggregates either remain spherical or elongate into a more cylindrical shape. Even for a system where there is an intrinsic energetic preference for the cylindrical shape. Smaller more spherical micelles can still be the most common aggregate type because there is an entropic preference for forming many small aggregates rather than a few large ones. For block copolymers of the Pluronic family, the effectively repulsive interaction, between head groups and between aggregates, arises from the entropically unfavorable overlap of EO groups. In contrast to the electrical double-layer force, the interaction goes to zero when there is no more direct molecular contact. Large number of EO groups favour spherical aggregates, and at higher concentrations, the first liquid crystalline phase to emerge is formed by finite micelles packed in a cubic array. If one, on the other hand, makes the PO part large, a lamellar phase is the only stable liquid crystal. Between these extremes, Pluronic such as F127 show a delicate balance between spheres and hexagonal phases at high concentrations. Increasing the temperature should further stabilize the hexagonal structure and so will the addition of normal electrolytes. At the conditions at the start of the synthesis, the solution contains spherical micelles but it only requires a weak perturbation to make the cylindrical micelles the more stable form.

5.4.1 Interaction between Block Copolymer Template and Inorganic Precursors

Ion-exchange is one of the most popular mechanisms in explaining the microbehaviour during the meso-structure formation process. Electrostatic was supposed as the initiating force for the ionic templating route. However, the electrostatic effect doesn't play the key role in aggregation of Pluronics block copolymers; anyway another type of possible attraction must be responsible.

Three ways of interaction have been schematized: $(S^0H^+)(XT^-)$, S^0I^0 , and $(S^0M^+)(XT^-)$. In our case, the model of S^0I^0 was selected to explain the interaction mechanism, and hydrogen bonding was assumed to be the main attractive inter-aggregate force.

Figure 5-8 illustrates this process schematically. At first, the block copolymer F127 molecular chains go through a self-aggregate progress, and form micelles with the hydrophilic block (EO) outside as the coronal and hydrophobic block (PO) inside as the core. The shape and sizes of the micelles produced may either be spherical (in the case of HA001) or rod-like (in the case of HA002) depending on the different polymer concentrations. At the same time, the HO- group in the inorganic Ca^{2+} precursor made up hydrogen bonding with EO block, and the Ca^{2+} precursor is attracted to the F127 micelles, which provide multiple positions for HA nucleation. As the pH value is adjusted to 12, and K_2HPO_4 solution was added, hydroxyl ions and phosphate ions then interact with the

Figure 5-8 Schematic of interaction process.

Figure 5-9 Illustration of HA precipitation to organic micelles.

calcium ions, as the reactants are added in the ratio of $1.667 \text{ Ca}^{2+} : \text{PO}_4^{3-}$, the corresponding reaction to produce hydroxyapatite is expected, hence the precipitation of HA to the organic micelles starts. **Figure 5-9** shows the illustration of the way HA precipitated onto the micelle surface.

5.4.2 Formation of Mesoporous Hydroxyapatite

Attractive force and interaction mechanism are proposed based on the understanding of amphiphilic block copolymer and experimental results. **Figure 5-10** gives two different routes for spherical and rod-like HA particles respectively. The whole process from the precipitation of hydroxyapatite to the single micelles to the final formation of mesoporous structure is revealed in this illustration. Because the exact pore structure and the arrangement way of sample HA001 and HA002 are not clear, here we show the possible paths for the

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Figure 5-10 Formation of mesoporous HA powders through nonionic routes: (A) spherical particle, (B) rod-like particle.

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formation of the irregular pore structure. The spherical micelles were supposed to induce spherical particle preferentially, while the rod-like micelles prefer to form rod-like particle. This deduction is corresponded to the theory of the tendance to optimum geometrical morphology to get the most stable state. The template was finally removed through calcinations, the mesoporous HA particle then came into being.

5.5 Concluding Remarks

Mesoporous hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ or HA) was synthesized using non-ionic block co-polymer Pluronic F127 ($\text{EO}_{99}\text{PO}_{65}\text{EO}_{99}$) as template. Two groups of HA samples were prepared by co-precipitation method with different concentrations of the surfactant, which were characterized by means of X-ray diffraction (XRD), electron microscopes and BJH gas absorption method. It was found that the as-synthesized and calcined samples of the two groups had a typical HA crystal phase, but the particle morphologies and the pore size distribution were significantly different, depending on the surfactant concentration used. When a high surfactant concentration (10g F127/ 100g distilled water) was used, the sample obtained consisted of rod like particles ($\sim 100\text{--}300\text{nm}$ in length and $\sim 40\text{--}80\text{nm}$ in diameter), with a maximum distribution of pore size $58\text{\AA}$. However, when the F127 concentration was 3g/ 100g distilled water, the sample with spherical particles ($\sim 100\text{nm}$ in diameter) could be obtained, and a bimodal pore size distribution at $25.3\text{\AA}$ and $30\text{\AA}$ were also observed in this sample. A possible mechanism was proposed to interpret the formation procedure of mesoporous structure.

5.6 References

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Chapter 6

Conclusions

The self-assembly of surfactants to create hydrophobic and hydrophilic structured domains creates an opportunity to use these systems as media for the synthesis of materials with unique properties. Following this principle, a systematic investigation was carried out on the development of mesoporous bioceramics. Mesoporous bioactive glasses and HA-silica composite, as well as mesoporous hydroxyapatite, are successfully synthesized for the first time through sol-gel and coprecipitation method using nonionic block copolymer (PEO-PPO-PEO) as templates. The probable mechanisms as well as the in vitro drug delivery property for each system are also discussed.

The research work up-to-date had led to the following conclusions:

- Hexagonal-ordered ($P6mm$) mesoporous bioactive glasses were synthesized using block copolymer Pluronic F127 and P123 as surfactant. F127 templated mesoporous bioactive glasses shows up similar bioactivity with the P123 templated one despite the the P123 templated bioactive glasses have larger surface area.
- Synchrotron in situ small angle x-ray diffraction study was carried out for the first time to investigate the mesophase transformation of the mesoporous bioactive glasses. The corresponding mechanism was firstly proposed, which supposes that the surfactant not only provides a fine ordered template for mesoporous structure, but also reinforces the rigidity of Si-O network, and the ordered Si-O arrangement can thus avoid the disruption by Ca^{2+} ions and P-O group.

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- The phenomenon that P123 templated mesoporous bioactive glasses present more desirable drug release property compared to F127 templated mesoporous bioactive glasses was reported for the first time. Both F127 and P123 templated mesoporous bioactive glasses present good drug loading potential, with loading amount 4.5 times of the normal densified bioactive glasses for P123 templated bioactive glasses, and 1.8 times for F127 templated bioactive glasses. A much better drug release property of mesoporous bioactive glasses due to their much higher surface area and pore volume was also obtained compared to normal bioactive glasses.
- Pore regularity and drug release property of mesoporous bioactive glasses with lower content of silica is much poorer than that with higher content.
- Mesoporous HA-silica composite materials with different Si: Ca: P ratio was firstly synthesized using block copolymer Pluronic P123 as surfactant through a sol-gel process. The mesoporous structure tends to become disordered with increasing Ca/Si ratio. The mesoporous HA-silica composite materials showed great drug release property, and the sample with Si:Ca:P= 64:25:15 presents a relatively better drug incorporation and release properties in the range of our discussion.
- The formation mechanism of the meso-structure for HA-silica composite materials is proposed for the first time, the interaction between the crystallization of hydroxyapatite and the surfactant liquid crystal was believed to determine the regularity of the meso-structure.
- Mesoporous hydroxyapatite was firstly synthesized using block copolymer F127 as template. Rod-like and spherical particles were achieved by adjusting the concentration of surfactant. The possible mechanism was proposed, and S⁰I⁰ type of mechanism was introduced to interpret the interaction took place in the synthesis

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process. Hydrogen-bonding was proposed to be the main force attracting the inorganic Ca^{2+} precursor to the micelle surface.

- To compare the microstructure of mesoporous bioactive glasses (MBGs), mesoporous HA-silica composite (HAS), and mesoporous hydroxyapatite (HA), it was found hexagonally ordered MBGs, relatively ordered mesoporous HAS, and absolutely disordered mesoporous HA have been produced, hence the regularity of the meoporous structure displays in such sequence: MBGs > HAS > HA.

Chapter 7

Suggestion for Future Work

Despite the successful synthesis mesoporous bioactive glasses, HA-silica composite material, as well as mesoporous HA, a lot of work still can be done regarding this study.

7.1 Tailoring of Pore Size and Pore Morphology

Tailoring of the porous structure is an important issue in the design and application of mesoporous materials. Such features as pore size, pore size uniformity, interparticle porosity, and stability (thermal and hydrothermal) of these mesoporous materials were shown to be controlled by a proper choice of synthesis conditions.

7.1.1 Surfactant Selection

The chain length of the PO and EO of block copolymer $\text{PEO}_m\text{PPO}_n\text{PEO}_m$ greatly affect the phase behaviour of the surfactant. For this fact, surfactants with different EO and PO fraction, for instance Pluronic P85 ($\text{EO}_{25}\text{-PO}_{40}\text{-EO}_{25}$) or the combination of ionic surfactant and nonionic surfactant could be imported to tailor the pore structure. Influence of cosurfactant and cosolvents can also be examined for the tailoring purpose.

Anionic surfactant with phosphate group on the chain will be another interest especially for tailoring mesoporous hydroxyapatite. Better combination between surfactant and HA will be expected.

7.1.2 Hierarchical Structure

Practical applications require mesoporous materials having hierarchical pore structures at different length scales in order to achieve highly organized functions, since the limited diffusibility of substrates through confined nano-channels can be a problem. The introduction of secondary larger pores has been demonstrated to improve remarkably the activity of mesoporous catalysts due to the enhanced diffusion of reactants and products [1, 2].

Figure 7-1 SEM images of silica template: (a) silica nanoparticles of 12nm forming the wall of macropores of 330nm (b) resulting carbon replica composed of macropores of 317nm connected to mesopores of 10nm [3].

Hierarchically ordered mesoporous–macroporous materials can be prepared by using a self-assembling surfactant in conjunction with macro-templates such as colloidal crystals, polymer foams, bio-celluloses, emulsions etc. Bimodal mesoporous silicas having small and large mesopores have been reported [4, 5]. Ordered macroporous carbon with mesoporous

walls has also been produced by template replication of aggregates of small silica particles, which were themselves templated by a self-assembled lattice of larger mono-disperse polystyrene spheres shown in **Figure 7-1**.

Based on our current work, it is reasonable to synthesize hierarchically ordered meso-macroporous bioactive glasses through proper meso-template and macro-template. These materials can thus be used as a host for loading biomolecules such as enzymes, which are expected to be suitable for various enzyme applications such as bioremediation, biosensors, and bioconversion.

7.2 System and Mechanism Development

We have proposed the probable mechanism for either mesoporous bioactive glasses or mesoporous HA at present, moreover the mechanism has been experimentally verified through synchrotron in situ SAXRD. However due to the limitation of facilities, the experimental verification for the mechanism of mesoporous HA was not carried out. In order to have deeper understanding the meso-structure formation process inside the HA sample, therefore enhancing the porosity, further mechanism study is suggested.

7.3 Properties Study

The mechanical properties and structural integrity of the synthesized materials would be investigated in the future work. The comparison of bioactivity, drug delivery property, as well as the biocompatibility between mesoporous bioactive glasses, hydroxyapatite-silica composites and hydroxyapatite would also be studied.

7.4 Functionalization

Incorporation of metal centers in the silicate framework is well developed in recent years to provide more catalysis sites in the meso-structure. Several successful examples of transition-metal-incorporated mesoporous silicates had been demonstrated with metals such as Al, Ti, Ga, Mn, Cr, and Fe [6]. Transition-metal incorporated MCM-41 materials were used to study oxidation of organics in liquid or gas-phase reaction with or without oxidants [7]. Transition-metal-incorporated MCM-41 materials fulfill the requirements for catalyzing the reactions of large organic molecules while retaining a uniform pore size.

In the future study, we could explore a practicable way on the preparation of metal-loaded mesoporous biomaterials. Therefore, the drug “vehicle” can be detected by outside equipment when the meso-structured materials are applied in drug delivery. Functionalization of mesoporous thin film will be carried out as well.

7.5 In Vitro Cell Culture Study

Osteoblast cells could be seeded on the synthesized mesoporous materials to study the biocompatibility. Currently osteosarcoma cells have been successfully seeded to MBGs and mesoporous HAS, **Figure 7-2** shows the SEM microimages of the mesoporous HAS and MBGs after 5 days growth of osteosarcoma cells.

On the other hand, antibiotic drug Gentamicin and bone growth drug Alendronate would be selected for the further study of controlled drug release property of mesoporous bioceramics. And osteoblast cells will be seeded to the drug loaded mesoporous materials to study the effect of drug on the cell culture.

Figure 7-2 SEM microimages of the mesoporous HAS and MBGs after 5 days growth of osteosarcoma cells.

7.6 References

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Appendix I

Classification of Adsorption Isotherms

As easy as it might seem, it is the deviation from the normal adsorption behavior that will allow the observer to draw conclusions on the texture or porosity of the surface. And this is also the point where the theory starts to become complicated. This will not be discussed at full detail in this Entry.

In general there are six types of adsorption/desorption isotherms (**Figure A-1**). The conventional adsorption/desorption isotherm plot shows the volume of adsorbed molecules against pressure, instead of plain volume against pressure. All adsorption isotherms should fit at least one, or at least a combination of two or more, of the six recognized types classified by Brunauer, Deming, Deming and Teller (B.D.D.T. system). The figure above shows the possible shapes and information which may be drawn from them is outlined below:

Type I Isotherm - these are typical of adsorbents with a predominantly microporous structure, as the majority of micropore filling will occur at relative pressures below 0.1. The adsorption process is usually complete at a partial pressure of ~ 0.5 . Examples include the adsorption of nitrogen on carbon at 77K and ammonia on charcoal at 273K.

Type II Isotherm - physical adsorption of gases by non-porous solids is typified by this class of isotherm. Monolayer coverage is followed by multilayering at high relative pressures. Carbons with mixed micro- and meso-porosity produce Type II isotherms.

Appendix I. Classification of Adsorption Isotherms

Type III Isotherm - the plot obtained is convex to the relative pressure axis. This class of isotherm is characteristic of weak adsorbate-adsorbent interactions and is most commonly associated with both non-porous and microporous adsorbents. The weak interactions between the adsorbate and the adsorbent lead to low uptakes at low relative pressures. However, once a molecule has become adsorbed at a primary adsorption site, the adsorbate-adsorbate interaction, which is much stronger, becomes the driving force of the adsorption process, resulting in accelerated uptakes at higher relative pressure. This co-operative type of adsorption at high partial pressures is known as cluster theory and examples include the adsorption of water molecules on carbon where the primary adsorption sites are oxygen based.

Figure A-1 Diagrammatic representation of isotherm classification.

Appendix I. Classification of Adsorption Isotherms

Type IV Isotherm - a hysteresis loop, which is commonly associated with the presence of mesoporosity, is a common feature of Type IV isotherms, the shape of which is unique to each adsorption system. Capillary condensation gives rise to a hysteresis loop and these isotherms also exhibit a limited uptake at high relative pressures.

Type V Isotherm - these isotherms are convex to the relative pressure axis and are characteristic of weak adsorbate-adsorbent interactions. These isotherms are indicative of microporous or mesoporous solids. The reasons behind the shape of this class of isotherm are the same as those for Type III and again water adsorption on carbon may exhibit a Type V isotherm.

Type VI Isotherm - introduced primarily as a hypothetical isotherm, the shape is due to the complete formation of monomolecular layers before progression to a subsequent layer. It has been proposed by Halsey, that the isotherms arise from adsorption on extremely homogeneous, non-porous surfaces where the monolayer capacity corresponds to the step height.

Appendix II

Preparation of Simulated Body Fluid (SBF)

1. Wash all the bottles and wares with 1N-HCl solution, neutral detergent, and ion-exchanged and distilled water, and then dry them.
2. Put 500ml of ion-exchanged and distilled water into one liter polyethylene bottle, and cover the bottle with a watch glass.
3. Stir the water in the bottle with a magnetic stirrer, and dissolve the reagents one by one in the order as given in Table A-1 (One after the former reagent was completely dissolved).
4. Adjust the temperature of the solution in the bottle at 36.5°C with a water bath, and adjust pH of the solution at pH 7.40 by stirring the solution and titrating 1N-HCl solution. (When the pH electrode is removed from the solution, add the water used for washing the electrode to the solution).
5. Transfer the solution from the polyethylene bottle to a volumetric glass flask. Add the water used for washing the polyethylene bottle to the solution in the flask.
6. Adjust the total volume of the solution to one liter by adding ion-exchanged and distilled water and shaking the flask at 20°C.
7. Transfer the solution from the flask to a polyethylene or polystyrene bottle, and store the bottle in a refrigerator at 5-10°C (If some substance is precipitated in the solution during the storage, do not use this solution as SBF and its container again).

Table A-1 Composition of Simulated Body Fluid (SBF)

Order	Reagent	Amount	Discription
1	NaCL	7.996g	
2	NaHCO ₃	0.350g	
3	KCl	0.224g	
4	K ₂ HPO ₄ .3H ₂ O	0.228g	
5	MgCl ₂ .6H ₂ O	0.305g	
6	1N-HCl	40ml	About 90% of total amount of HCl to be added)
7	CaCl ₂	0.278g	
8	Na ₂ SO ₄	0.071g	
9	NH ₂ C(CH ₂ OH) ₃	6.057g	

Appendix III

Publications during PhD Period

1. **Y.F. Zhao**, J. Ma, *Triblock Copolymer Templating Synthesis of Meso-structured Hydroxyapatite*, Microporous and Mesoporous Materials; Vol. 87, 2005, 2, pp 110-117.
2. **Y.F. Zhao**, J. Ma, *Synthesis of Mesoporous Hydroxyapatite through Neutral Templating*, Special Issue in International Journal of Nanoscience (IJN); Vol. 5 2006.
3. **Y.F. Zhao**, Loo Say Chye Joachim, Y.Z. Chen, F.C.Y. Boey, J. Ma, *In Situ SAXRD Study of Sol-gel Induced Well-ordered Mesoporous Bioactive glasses For Drug Delivery*, Journal of Biomedical Materials Research, Part A, (In processing).
4. **Y.F. Zhao**, Loo Say Chye Joachim, Geoffrey Tan Eng Beng, J. Ma, *Co-synthesis and Drug Delivery Properties of Mesoporous Hydroxyapatite-Silica Composites*, Journal of Nanoscience and Nanotechnology (Submitted).
5. Y.Z. Chen, J. Ma, **Y.F. Zhao**, E. W. M. Fong, *Synthesis of Mesoporous Hydroxy-apatite (HA) Using Surfactant Templating*; Journal of Materials Research (Submitted).
6. **Yufeng Zhao**, Geoffrey Tan Eng Beng, Yunzhong Chen, Jan Ma, *Synthesis and Characterization of Meso-structure Hydroxyapatite Using Different Surfactant Systems*; 13th Int'l Conference on Processing and Fabrication of Advanced Materials (PFAM XIII), 2004, Singapore.
7. **Y.F. Zhao**, J. Ma, *Apatite Formation on Mesoporous Bioactive Glass CaO-P₂O₅-SiO₂*, 2nd-MRS-S Conference on Advanced Materials, 2006, Singapore.
8. **Y.F. Zhao**, Y.Z. Chen, Geoffrey Tan Eng Beng, J. Ma; *Ionic Templating of Meso-structured Hydroxyapatite and the Mechanism Study*; ACS national spring meeting 2006, Atlanta, GA, USA.