Rev. Adv. Mater. Sci. 38 (2014) 138-147

NANOPOROUS HYDROXYAPATITE PREPARATION METHODS FOR DRUG DELIVERY APPLICATIONS

Nur Farahiyah Mohammad[1,2], Radzali Othman[1] and Fei Yee-Yeoh[1]

[1] School of Materials and Mineral Resources Engineering, Engineering Campus, Universiti Sains Malaysia, 14300 Nibong Tebal, Penang, Malaysia
[2] Programme of Biomedical Electronic Engineering, School of Mechatronic Engeneering, Pauh Putra Campus, Universiti Malaysia Perlis, 02600 Arau, Perlis, Malaysia

Received: January 15, 2014

Abstract. This review highlights the most common synthesis methods used in the preparation of mesoporous hydroxyapatite (HA); specifically using surfactants as a pore template. Basically, nanoporous hydroxyapatite is synthesized using wet precipitation methods that consist of chemical precipitation, hydrothermal, and emulsion. Each of these techniques uses either ionic or non-ionic surfactants as structure directing agents. Most studies initially utilize ionic surfactants e.g., cetyltrimethylammonium bromide (CTAB) during the synthesis process. However, due to a demand for more consistent pore sizes and higher surface areas, non-ionic surfactants e.g., F127 and P123 became more commonly used as the structure directing agent. Drug loading and release characteristics of nanoporous HA against different types of drugs, such as antibiotics and anti-cancer, are discussed in the second part of this report. Finally, a brief conclusion will describe the limitations of current techniques, and comment on the main aspects that need to be considered in the future for further improvements and better performances.

1. INTRODUCTION

Bioceramics can be defined as biomaterials of ceramic origin that are "specially designed and fabricated for the repair and reconstruction of diseased, damaged, missing or worn out parts of the body" [1]. Bioceramics are usually used as implants, coatings, or therapeutic agent carriers within the human body. Naturally, when a synthetic material is placed within the body, tissues react with the implanted material in different ways; depending on the type of material. In general, based on the tissues' response, biomaterials can be classified as bioinert, bioactive, or bioresorbable [1-3]. Therefore, bioceramics should also be expected to comply with these classifications. Bioinert bioceramics, such as alumina and zirconia, can be described as ceramics that have minimal interaction with the surrounding tissue, once they have been implanted within the human body [1-3]. Meanwhile, bioactive bioceramics refers to ceramics that interact and form a strong interface with the surrounding tissues [1-3]. Common examples of bioactive bioceramics include glasses (silica or phosphate based) and calcium phosphates that include hydroxyapatites (HA), beta-tricalcium phosphate (β-TCP), and biphasic calcium phosphate (BCP) [1-3]. Some bioceramics are also bioresorbable materials; which can be described as materials that will be dissolved and gradually replaced by natural tissue, once they are implanted within the human body [1-3]. Tricalcium phosphate (TCP), calcium carbonate, calcium oxide, and gypsum are all common examples of bioresorbable materials [1-3].

Bioactive and bioresorbable bioceramics are commonly utilised in treating diseases, repairs, or treatments related to bone. Most of these materials

Corresponding author: Fei Yee-Yeoh, e-male: feiyee@usm.my and feiyee@eng.usm.my

are used as bone scaffolds, coatings in orthopaedics and dental implants, or as the main construction of joint implants. Hydroxyapatite is widely recognised as the most popular material for these specific applications, mainly because HA is known as a mineral component of bone [4-6]. Human bones mainly contain ≈70 wt.% apatite, 20 wt.% collagen, and 10 wt.% water [4,5]. HA is a mineral of the apatite family, where $Ca_5(PO_4)_3OH$ is the chemical formula and $Ca_{10}(PO_4)_6OH_2$ is the formula of the unit cell. In human bone, HA is always impure and nonstoichiometric with the major impurity is consist of carbonate (CO_3^{2-}) ion (3-8 wt.%). Biological apatite is also known as carbonated apatite or carbonated hydroxyapatite (carbonated HA) [7]. Substitution of carbonate is known to weaken the apatite structure and make it more soluble [7]. Therefore, carbonatic HA is shown to improve bioactivity compared to pure HA. Carbonated HA is nonstoichiometric, with a chemical formula denoted as $Ca_{10-x/2}[(PO_4)_{6-x}CO_3)_x][(OH_{2-2y}(CO_3)_y]$, where *x* and *y* are numbers of CO_3^{2-} ions substituting for PO_4^{3-} and OH, respectively [8].

The use of HA as biomaterials began in a dense form, but later, porous forms were favoured since they were found to possess better bioactivity and bioresorption as bone implants and scaffolds. Since the discovery of Mobil Composition of Matter No. 41 (MCM-41) in 1992, porous materials have become major research topics [9]. Initially, no specific term was designated to specifically define pore size. In 1994, the International Union of Physical and Applied Chemistry (IUPAC) released the standard definition of pore size, where micropores are smaller than 2 nm in diameter, mesopores are 2 to 50 nm, and macropores are larger than 50 nm [10]. Since the emergence of nanotech in 2000, micro-, meso-, and macropores can also be described as nanopores, because their pore sizes are between 1 and 100 nm, which fall into the nanoscale range. Since the material's pores are suitable for loading substances, porous HA has been seen as an excellent drug carrier candidate. The main reason for using HA as a drug carrier is because its chemical composition is similar to human bone. Compared with other drug delivery carriers, HA has better biodegradability and biocompatibility. Bioactive properties of HA also could have an added value as a drug carrier for bone tissue. The degradation product of HA is less toxic than silica, quantum dots, carbon nanotubes, or magnetic particles [4]. Moreover, HA can adsorb different chemical species onto its surfaces, and due to its solubility, the chemicals (or drugs) can be released at specific targeted areas. The solubility of HA can be controlled through the substitution of different types of ions, such as carbonate, chloride, or fluoride. Moreover, HA is a pH dependent dissolution, compared to polymers. Because dissolution rate increases in low pH mediums, which is typically found in cancerous cells, this enables drugs to be delivered to specific targeted cancerous zones [11]. With HA, pore sizes are controllable to regulate the release rate of drugs [12]. In addition, any antibiotics can be placed in the porous HA, because the properties of the drug does not change during the loading and releasing process [13,14]. The preparation of nanoparticles HA is not a complicated task and has a low production cost. Furthermore, most precursors are easily and widely available on the market.

Previous studies have shown that drugs were carried either in a porous HA block or as nanoparticles (Table 1). Porous HA blocks, with antibiotic impregnations have been used as implantable drug delivery systems for the treatment of osteomyelitis [13,15-18]. Shinto et al., showed that macroporous HAP blocks with 40-150 μm pore sizes carrying gentamicin sodium, exhibited a continuous drug release for a period of *ca.* 90 days [13]. They also successfully demonstrated that HAP did not cause any thermal damage to the drug and stimulated the growth of new bone. Itokazu et al., studied the drug release profiles of two different drugs, arbekacin sulphate [15] and isepamicin sulphate [16], which were used to treat osteomyelitis. Arbekacin sulphate was loaded into macroporous block HAP, with 50-300 μm pore sizes using centrifugation. Meanwhile, isepamicin sulphate was loaded into macroporous block HAP with 2-2000 μm pore sizes using a vacuum method. Both studies proved that macroporous HAP block was able to maintain a slow-release of the drug; however, the release period was much shorter than that demonstrated by Shinto et al., which was up to 42 [15] and 18 [16] days only. Cefuroxime axetil was also impregnated within porous HAP and tricalcium phosphate (TCP) block, and was successfully used to treat osteomyelitis, with the porous block release of the highest concentration of the drug on day 20 of the implantation, which reduced marginally by day 42 [17].

Other than pore size, the porosity of HA graft also greatly influenced drug release kinetics [18-20]. Netz et al. [19] conducted *in vitro* test that showed that HA block, with a high porosity (i.e., 82.63%), exhibited an irregular release of the drug (cisplatin), because high porosity possibly caused an irregular structure, which may have affected the

Table 1. Form of HA used to carry different type of drugs.

Porous HA form	Drugs carried		
	Anti-cancer	Anti-biotic	Others
Block	Cis-diamminedichloroplatinum (II) [12] [19]	Gentamicin [13]	
	Methotrexate [42]	Cefoperazone sodium [13]	
		Flomoxef sodium [13]	
		Arbekacin sulphate [15]	
		Isepamicin sulphate [16]	
		Ibuprofen [21]	
		Hydrocortisone Na-succinate [21]	
		Cefuroxime axetil [17]	
		Ceftriaxone [18]	
Nanoparticles	Cis-diamminedichloroplatinum (II) [47]	Norfloxacin [43]	Carvedilol [45,46]
	Di(ethylenediamineplatinum) medronate [47]	Ibuprofen [14,44]	
	Biphosphonate alendronate [47]	Vancomycin [30]	
	Paclitaxel [48]		

drug's release. Therefore, they claimed that porous HA block would be useful as drug delivery systems; only if the porosity was below 78%. This study revealed the limitation of porous block HA as a drug delivery agent. However, a study conducted by Al-Sokanee et al., showed that the release rate of ceftriaxone decreased with the decreasing porosity of HA [18]. Furthermore, scaffolds with a porosity of 10% demonstrated a faster, higher, and more regular drug release than porosities of 2%, 4%, 6%, and 8%. Without targeting specific diseases, Palazzo et al., loaded ibuprofen and hydrocortisone into two HA grafts with different porosities (40% and 60%) [21]. These two studies confirmed the effects of porosity on drug release, where a lower porosity of HA showed a more significant initial burst release. Based on the investigations of these researchers, their results indicated that the porosity of HA was a key factor in achieving the desired drug release profile. It was believed that a higher porosity could provide more motion freedom of the molecules to be regularly carried out from the porous structure of HA by the solvent.

Therefore, in order to increase the porosity of the HA, pores were introduced into the HA structure at the nano level. The latest review on HA, by Sadat-Shojai and co-workers, focused on synthesis methods for nanosized hydroxyapatite with diverse structures [22]; however, they did not take a detailed look into the method of preparing nanoporous HA using surfactants as the structure directing agent using different synthesis techniques. Therefore, this article focuses specifically on the preparation methods of mesoporous HA using surfactants as a pore template. This review article is divided into two parts. The first part is devoted to the procedures of preparing mesoporous HA nanoparticles, reported between 2001 and 2013. The second part reviews the applications of mesoporous HA as drug carriers.

2. SYNTHESIS METHODS OF MESOPOROUS HA NANOPARTICLES

The study of mesoporous hydroxyapatite (HA) has been considered as a new and emerging scope of study within the field of porous bioceramics. Published works on these materials can only be traced back to the 21st century. So far, based on the published works, most preparation methods of mesoporous HA have involved wet chemical reactions. The advantages of using wet chemical reactions in the preparation of mesoporous HA is their ability to control the morphology and mean size of the powder, and the pore size of the nanoparticles [22]. Different types of wet methods are available; but to the best of our knowledge, until recently, mesoporous HA has been synthesized using chemical precipitation, hydrothermal, sol gel, and emulsion methods. These methods were promising nanocrystalline material synthesizing techniques

[23]; therefore, they were commonly used in most synthesis processes of mesoporous HA. The surfactant based template system is the most common technique used to introduce pores within the nanoparticles. This well-known technique is efficient for controlling the morphology, particle size, crystallinity degree, and pore characteristics of nanoparticles. Surfactants can be classified into four types, based on the composition of their head as non-ionic, cationic, anionic , and amphoteric. Surfactants are usually amphiphilic molecules with a hydrophobic tail and hydrophilic head, which are able to self-assemble to form micelle with a designated shape, as soon as their concentration exceeds the critical micelle concentration [22]. In the solution mixture of Ca^{2+} and PO_4^{3-}, at a certain concentration and pH, micelles with a specific shape are formed and act as a template for crystal growth [24,25]. In the following sections, we will describe the use of different types of surfactants and the characteristics of the nanoparticles obtained from each preparation method.

2.1. Chemical precipitation

The chemical precipitation method is the simplest route to synthesizing mesoporous HA, compared to all other methods. This surfactant based template system method involves several chemicals, consisting of calcium and phosphate containing reagents, surfactants and pH controller reagents. According to Table 2, calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$) is the most frequent Ca^{2+} source used in this method, compared to other reagents. From Table 2, it can also be seen that diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) and dipotassium hydrogen phosphate trihydrate ($K_2HPO_4.3H_2O$) were deemed more favourable for PO_4^{3-} sources than other phosphorus precursors. The basic common synthesis procedure involves the drop wise addition of one phosphate reagent to the calcium reagent under continuous stirring, while the molar ratio of element (Ca/P) is maintained at stoichiometry; according to the standard ratio in HA, which is 1.67. Next, the sample is aged in a temperature controlled bath, and finally, the white precipitate is washed, filtered (or centrifuged), dried, calcined, and ground into a powder [23,24,26-30].

Different types of surfactant are used in the chemical precipitation method to control particle size and shape [31], surface area, and pore characteristics at significantly lower temperatures and pHs. Cationic surfactants, which are commonly used for the soft templating of HA, are the members of the alkyl trimethyl ammonium bromide group i.e., CTAB. Yao and co-workers [25] synthesized HAP nanorods, 50-100 nm in diameter and 500-1000 nm in length, (determined by TEM), using surfactants of Cetyl Trimethyl Ammonium Bromide (CTAB). Nitrogen adsorption indicated that the pore size within the nanorod was approximately 3 nm, with a pore volume of 0.0113 cm^3g^{-1}. The pores within the sample were revealed through combustion at 550 °C for 6 hours. The results obtained indicate that CTAB can be used as a soft template to produce mesoporous HA. Recently, a number of works [24,32] have confirmed the ability of CTAB as a surfactant to induce pore formation within rod like nanoparticles. A study by Wang and his group demonstrated that irregular mesoporous HA was successfully synthesized with CTAB surfactant, with a pore size of approximately 40 nm and a specific surface area of 37.6 m^2g^{-1}. Furthermore, Tari and co-workers [23] synthesized different shapes of HA nanoparticle using a mixture of cationic CTAB and anionic Sodium Dodecyl Sulphate (SDS) as templates. The particles were rod shaped; when the concentration of anionic was higher than the cationic surfactant. Meanwhile, a sheet like structure was obtained when the cationic was higher than the anionic. The results demonstrate that by changing the concentration ratio of cationic to anionic surfactant in the mixture, the morphology of the nanoparticle HA can be controlled.

As the pore sizes of the mesoporous HA prepared using ionic surfactants were not consistent and the surface area was rather low, attempts were also made to control pore characteristics using non-ionic surfactants, including poly (ethylene oxide) triblock co-polymers i.e., F127, P123 [28,30,33], and Tween-16 [30]. Zhao and Ma [33] prepared different shapes of mesoporous HA i.e., spherical and rod, using the F127 surfactant. They suggested that different surfactant concentrations may produce different morphologies of HA nanoparticles. According to their results, when a higher surfactant concentration (0.1 g/ml of F127) was used, the sample obtained consisted of spherical nanoparticles, 100 nm in diameter, and 5.8 nm in pore size (maximum pore size of BJH). However, when a lower surfactant concentration was used (0.03 g/ml of F127), the rod like nanoparticles could be obtained with a diameter ranging between 40 to 50 nm and 100 to 300 nm long and a BJH pore size ranging between 2.5 and 3 nm. The pore size of HA nanoparticles synthesized with F127 was not as big as expected. Several years later, a study by Ye et al. [30], showed that a combination of P123 and Tween-16 as a soft template was able to produce hollow nanospheres

Table 2. Comparison of different methods for the preparation of nanoparous hydroxyapatite.

Synthesis Method	Precursors		Surfactants			Ref.
	calcium	phosphorus	cationic	anionic	Non-ionic	
Chemical Precipitation	calcium nitrate tetrahydrate $Ca(NO_3)_2.4H_2O$	diammonium hydrogen phosphate $(NH_4)_2HPO_4$	cetyltrimethyl ammonium bromide (CTAB)	-	-	[24]
	calcium nitrate tetrahydrate $Ca(NO_3)_2.4H_2O$	diammonium hydrogen phosphate $(NH_4)_2HPO_4$	cetrimide ($C_{17}H_{38}NBr$)	-	-	[27]
	calcium nitrate tetrahydrate $Ca(NO_3)_2.4H_2O$	phosphoric acid (H_3PO_4)	-	-	P123, Tween-16	[30]
	calcium nitrate tetrahydrate $Ca(NO_3)_2.4H_2O$	ammonium phosphate $NH_4H_2PO_4$	cetyltrimethyl ammonium bromide (CTAB)	-	-	[49]
	calcium nitrate tetrahydrate $Ca(NO_3)_2.4H_2O$	phosphoric acid (H_3PO_4)	-	monodedecyl sulphate	-	[31]
	calcium chloride ($CaCl_2$)	phosphoric acid (H_3PO_4)	cetyltrimethyl ammonium bromide (CTAB)	sodium dodecyl sulphate (SDS)	-	[23]
	calcium chloride ($CaCl_2$)	dipotassium hydrogen phosphate trihydrate ($K_2HPO_4.3H_2O$)	cetyltrimethyl ammonium bromide (CTAB)	-	-	[25]
	calcium D pantothenate monohydrate	dipotassium hydrogen phosphate trihydrate ($K_2HPO_4.3H_2O$)	-	-	P123, F127	[28]
	calcium D-pantothenate	dipotassium hydrogen phosphate trihydrate ($K_2HPO_4.3H_2O$)	-	-	F127	[33]
	calcium D-gluconate	potassium hydrogen phosphate (K_2HPO_4)	-	-	F127	[26]
	calcium chloride dehydrate ($CaCl_2.H_2O$)	diammonium hydrogen phosphate $(NH_4)_2HPO_4$	-	-	CMK-3	[35]
Hydrothermal	calcium chloride ($CaCl_2$)	dipotassium hydrogen phosphate trihydrate ($K_2HPO_4.3H_2O$)	cetyltrimethyl ammonium bromide (CTAB)	-	-	[32]
	calcium nitrate tetrahydrate $Ca(NO_3)_2.4H_2O$	diammonium hydrogen phosphate $(NH_4)_2HPO_4$	-	-	P123 F127	[34]

	calcium chloride ($CaCl_2$)	dipotassium hydrogen phosphate trihydrate ($K_2HPO_4.3H_2O$)	cetyltrimethyl ammonium bromide (CTAB)	-	-	[36]
	calcium chloride ($CaCl_2$)	phosphorous acid (H_3PO_4)	cetyltrimethyl ammonium bromide (CTAB)	-	-	[38]
	calcium nitrate	trisodium phosphate ($Na_3 PO_4$)	cetyltrimethyl ammonium bromide (CTAB)	-	-	[38]
	calcium chloride ($CaCl_2$)	dipotassium hydrogen phosphate trihydrate ($K_2HPO_4.3H_2O$)	-	-	P123 F127F87	[39]
Emulsion	calcium nitrate tetrahydrate $Ca(NO_3)_2.4H_2O$	orthophosphoric acid, H_3PO_4	-	-	AOTDP, NP5, NP6	[40]
	calcium chloride ($CaCl_2.H_2O$)	diammonium hydrogen phosphate $(NH_4)_2HPO_4$	-	-	Span 20, Tween 80	[41]

and nanorods with much higher pore sizes. The nanotubes HA were obtained by adding citric acid as a co-surfactant into the solution of P123 during synthesis. These HA nanospheres had an average diameter of 60 nm (determined by TEM) and BJH pore size of 36 nm, with a specific surface area of 66.11 m^2g^{-1} and pore volume of 0.47 cm^3g^{-1}. The nanorods were 35 nm in diameter, 50-250 nm in length, and had a BJH pore size of 15.6 nm. The specific surface area was higher than that of the nanospheres, which was 116.8 m^2g^{-1}. However, the pore volume showed a slightly lower value of 0.34 cm^3g^{-1}.

Conversely, Ng et al. [28] stated that there was no significant difference in specific surface area for samples synthesized with surfactant F127 or P123. However, pore diameters were consistently larger for F127 samples compared to P123. These results conflicted with the results of previous studies conducted by Zhao et al. [33]. The pore size of mesoporous HA synthesized with F127 by Zhao et al. was very small; most probably because the samples were aged at 90 °C for 24 hours, while Ng et al., samples were aged at 120 °C for 24 hours. The pure phase of HA only develops at 120 °C [34]. It is likely that at 90 °C, the samples were not totally converted to HA, and secondary phase of β-tricalcium phosphate occurred in the samples. Besides that, Ng and co-workers described that other synthesis parameters, such as synthesis temperature, washing solvents, and pH, may also have affected the pore characteristics of the HA nanoparticles. They claimed that the highest surface area of the mesoporous HA was synthesized at 25° and pH 12, with an 80 wt.% surfactant concentration.

Besides using soft templates i.e., ionic and non-ionic surfactants, several attempts have also been made to use hard templates in producing mesoporous HA. Xia et al. [35] used mesoporous carbon with a two-dimensional hexagonal structure and CMK-3 as the template, for the precipitation of HA. The nanoparticles produced were rod like structured, at 100 nm long and approximately 20 nm wide. Nitrogen adsorption analysis showed that pore size, surface area, and pore volume of the mesoporous HA were 2.73 nm, 42.43 m^2g^{-1}, and 0.12 cm^3g^{-1}, respectively. Other than that, microbial cells have also been used as a template to synthesize mesoporous HA nanoparticles. They managed to achieve a much higher BET surface area of 86 m^2g^{-1} and a BJH pore width within the range 2-4 nm. Even though these methods seemed to be promising, the results obtained did not showany significant improvement compared to those synthesized using the soft template method. For example, the method proposed by Xia et al. involved relatively more complicated procedures to remove the carbon template, which may have increased the number of experimental errors.

2.2. Hydrothermal

The hydrothermal method basically has a similar route as the chemical precipitation method in the synthesis of nanoporous bioceramics in the presence of surfactant. The only difference is the ageing step, which is conducted at a high temperature; typically above the boiling point of water and conducted inside an autoclave or pressure vessel [22]. The number of chemicals used in this method varies and the HAP nanoparticles obtained from hydrothermal conditions is relatively stoichiometric and highly crystalline [22]. CTAB and block co-polymers e.g., F87, P123, and F127 have been used as surfactants in the synthesis of HAP nanoparticles using the hydrothermal method [32,34,36-39]. Most of these methods successfully synthesized nanorod HA nanoparticles, with diameters ranging from 10 to 60 nm and lengths ranging between 75 nm and 1125 nm. The phase purity and Ca/P ratio of the HA particles were improved significantly by increasing the hydrothermal temperature. Li et al. [32] used the hydrothermal method to synthesize mesoporous HA, using CTAB as the pore template. Nanorod HA was synthesized at four different hydrothermal temperatures: 40 °C, 80 °C, 160 °C, and 120 °C. They were then calcined at four different calcination temperatures: 550 °C, 700 °C, 800 °C, and 1000 °C for six hours. For the sample calcined at the same temperature, the crystallinity was increased while the pore size decreased from ca. 5 nm to 1 nm, as the hydrothermal temperature was increased. This can be attributed to the tighter packing of HA at a high temperature, due to the improved crystallinity of the structure. Since the hydrothermal method promised the production of stoichiometric and highly crystalline HA nanoparticles, in the future, this method is seen as a promising method to synthesize carbonated HA. The pore size of HA nanoparticles can be modified by changing CTAB with non-ionic surfactants i.e., F127 or P123, to produce larger pore sizes and high surface areas.

2.3. Emulsion

The emulsion process is another common method for the preparation of mesoporous HA. Up to now, very few studies have directly focused on the emul-

sion synthesis of mesoporous HA nanoparticles. Kumar et al. [40] used calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$) and phosphoric acid, H_3PO_4 as the main precursor for Ca^{2+} and PO_4^{3-} sources. They managed to synthesize HA nanoparticles of different morphologies as spherical, needle shapes, or rod like, by adjusting the conditions of the emulsion system. The needle shaped elongated nanoparticles had BET specific surface areas as high as 121 m^2g^{-1}, which were obtained using NP_5 (poly(oxythylene)$_5$ nonylphenol ether as a non-ionic surfactant at a water/surfactant ratio of 1:5, pH 7 and calcined at 450 °C. Similarly, Somnuk and his co-workers [41] synthesized HA nanoparticles using mixtures of non-ionic surfactants, such as Spam 20 and Tween 80. Their work indicates that increasing reaction and calcination temperatures resulted in the reduction of surface area. Calcination at temperatures ranging from 450 to 750 °C leads to a decrease of the specific area from 227 to 58 m^2g^{-1}. Based on these two studies, it can be concluded that a high surface area can be obtained at lower reaction and calcination times; however, it was feared that some impurity may be present in the samples, or in other words, phase pure HA could not be obtained. Therefore, a higher temperature is likely to be chosen as an optimum temperature. However, based on the previous results, the secondary phase may occur at temperatures greater than 635 °C [41]. For this reason, 550 °C was found to be the optimum calcination temperature, because as already known, the polymeric surfactants basically decompose at around 300-400 °C [28].

3. POROUS HA NANOPARTICLES AS A DRUG CARIER

As indicated in Table 1, previous studies have shown that drugs were carried either in porous HA block [12,13,15-19,21,42] or nanoparticles [14,30,43-46]. This chapter reviews the application of porous HA nanoparticles as antibiotic and anticancer drug carriers. Most of the applications focused on using nanoparticles for treating diseases related to hard tissue; especially bone.

3.1. Porous HA nanoparticles as carriers for antibiotics

Norflaxacin is an antibiotic that has been used as a target drug in the study of macroporous HA nanoparticles; with an average pore size of 313 nm as a drug carrier [43]. This study shows that macroporous HA can adsorb and release antibiotics in vitro. Unfortunately, drug release can only be maintained for up to 6 hours; which is too short for a controlled release application. Ibuprofen was also impregnated within a nanostructured porous hollow ellipsoidal capsule of HA [14]. The nanostructured hollow ellipsoidal capsule could store approximately 459.5 mg g^{-1} of ibuprofen; indicating that the hollow ellipsoidal capsule of HA had a high drug loading capacity. The ibuprofen loaded HA hollow ellipsoidal capsule disk demonstrated a slow and sustained release of ibuprofen for 100 hours. Without using any surfactant or two-step procedure of HA nanocarriers synthesis, ibuprofen was introduced into a nanoparticle of HA [44]. The nanoparticles produced exhibited an extremely high drug loading capacity (up to 1.4 g g^{-1}) and a prolonged drug release of 336 hours. Vancomycin, a glycopeptide antibiotic used in the prophylaxis and to treat infections caused by Gram-positive bacteria, was also encapsulated within HA nanoparticles, and exhibited a high drug loading capacity and a sustained release characteristic [30]. In this study, Ye and co-workers [30] successfully loaded vancomycin into nanospheres and nanotubes mesoporous HA. The nanotubes HA, functionalized with citrate carboxyl-cationic poly-electrolyte gates, showed excellent pH responsivity, and the highest payload ratio of 35.83 wt.%.

3.2. HA nanoparticle as carriers for anticancer drugs

Besides their application as antibiotics carriers, HA nanoparticles have also been used as carriers for anticancer drugs. Palazzo et al. [47] investigated in vitro adsorption and desorption of cisplatin, di(ethylenediamineplatinum) medronate (DPM), and biphosphonate alendronate towards needle-shaped and plate-shaped porous HA nanoparticles. The specific properties of the drugs and the morphology of the HA nanoparticles effected the adsorption and desorption kinetics of the drugs. The negatively charged alendronate and the positively charged cisplatin were strongly adsorbed, while the neutral DPM complex showed a lower affinity towards the negative surface of the HA nanoparticles. Furthermore, cisplatin was adsorbed more by the needle-shaped HA surface, while alendronate was more favoured by the plate-shaped surface. Analysis showed that drug release was greater for neutral DPM than for the charged alendronate and cisplatin. Also, DPM was released faster from the needle-shaped surface than the plate-shaped surface. Meanwhile, both charged drugs demonstrated simi-

lar release rates from both types of HA nanoparticles. A study by Venkatasubbu et al. [48] confirmed the effectiveness of porous hydroxyapatite nanoparticles as a drug carrier. However, instead of being encapsulated inside the HA nanoparticles, the anticancer drug (paclitaxel), was attached within the porous surface of the nanoparticles. The porous surface was caused by the Polyethylene glycol (PEG) functionalization and folic acid modification on the surface of the nanoparticles. The drug release profile showed an initial burst release, followed by a sustained release that lasted 50 hours. The initial burst release was caused by the drug molecule being easily detached from the surface of the HA nanoparticles.

4. CONCLUSIONS

In this review, mesoporous HAP nanoparticles were synthesized using three main different wet chemical reactions, namely chemical precipitation, hydrothermal, and emulsion. All of these methods involved surfactant based templates to introduce pores within the nanoparticles, whilst controlling morphology and pore characteristics i.e., pore sizes, pore volumes, and specific surface areas of the HA nanoparticles. Basically, cationic, anionic, and nonionic surfactants are three common types of surfactants that are commonly used in the preparation of mesoporous HA nanoparticles. From the review, it can be concluded that more study is needed to improve the production of HA nanoparticles with high surface areas and preferred pore size distributions. Current techniques face a number of limitations, such as serious aggregation and agglomeration of particles, wide particle size distributions, and low specific surface areas. To address these issues, new or improved synthesis routes have to be established. In terms of application as a drug carrier, HA nanoparticles must be able to load large amounts of drug within their pores and must be able to control the release of that drug. Mesoporous HA nanoparticles were found to have good prospects in drug delivery applications and new improvements are essential to optimize their controlled sustained release properties.

ACKNOWLEDGEMENT

We would like to thank the Research University-Postgraduate Research Grant 1001/PBAHAN/8046030 (RU-PRGS), Universiti Sains Malaysia and Ministry of Education for supporting this work.

REFERENCES

[1] R.Z. LeGeros and G. Daculsi, In: *Musculoskeletal Tissue Regeneration:Biological Materials and Methods*, ed. by S.P. William (Humana Press: Totowa, USA, 2008), p. 153.
[2] B. Ben-Nissan, In: Learning from Nature How to Design New Implantable Biomaterialsis: From Biomineralization Fundamentals to Biomimetic Materials and Processing Routes, edț by R.L. Reis and S. Weiner (Kluwer Academic Publishers, Netherlands, 2005), p. 89.
[3] L.L. Hench // *J. Am. Ceram. Soc.* **81** (1998) 1705.
[4] S.V. Dorozhkin // *Biomaterials* **31** (2010) 1465.
[5] S.V. Dorozhkin and M. Epple // *Angew. Chem. Int. Ed*. **41** (2002) 3130.
[6] S. Weiner and H.D. Wagner // *Annu. Rev. Mater. Sci.* **28** (1998) 271.
[7] R.Z. LeGeros // *Chem. Rev.* **108** (2008) 4742.
[8] V. Uskoković and D.P. Uskoković // *J. Biomed. Mater. Res. B* **96B** (2011) 152.
[9] C.T. Kresge, M.E. Leonowicz, W.J. Roth, J.C. Vartuli and J.S. Beck // *Nature* **359** (1992) 710.
[10] J.Rouquerol, D. Avnir, C.W. Fairbridge, D.H. Everett, J.H. Haynes, N. Pernicone, J.D.F. Ramsay, K.S.W.Sing and K.K. Unger // *Pure Appl. Chem*. **66 (No. 8)** (1994) 1739.
[11] K. Gomi, B. Lowenberg, G. Shapiro and J.E. Davies // *Biomaterials* **14** (1993) 91.
[12] A. Uchida, Y. Shinto, N. Araki and K. Ono // *J. Orthop. Res.* **10** (1992) 440.
[13] Y. Shinto, A. Uchida, F. Korkusuz, N. Araki and K. Ono // *J Bone Joint Surg Br* **74-B** (1992) 600.
[14] M.-Y. Ma, Y.-J. Zhu, L. Li and S.-W. Cao // *J. Mater. Chem.* **18** (2008) 2722.
[15] M. Itokazu, T. Matsunaga, S. Kumazawa and Y. Wenyi // *J. Appl. Biomater.* **6** (1995) 167.
[16] M. Itokazu, W. Yang, T. Aoki, A. Ohara and N. Kato // *Biomaterials* **19** (1998) 817.
[17] S.K. Nandi, B. Kundu, S.K. Ghosh, T.K. Mandal, S. Datta, D.K. De and D. Basu // *Ceram. Int.* **35** (2009) 1367.
[18] Z. Al-Sokanee, A. Toabi, M. Al-Assadi and E.S. Alassadi // *AAPS PharmSciTech* **10** (2009) 772.
[19] D.J.A. Netz, P. Sepulveda, V.C. Pandolfelli, A.C.C. Spadaro, J.B. Alencastre, M.V.L.B. Bentley and J.M. Marchetti // *Int. J. Pharm*. **213** (2001) 117.

[20] F. Galbusera, L. Bertolazzi, R. Balossino and G. Dubini // *Biomech. Model. Mechanobiol.* **8** (2009) 209.
[21] B. Palazzo, M.C. Sidoti, N. Roveri, A. Tampieri, M. Sandri, L. Bertolazzi, F. Galbusera, G. Dubini, P. Vena and R. Contro // *Mater. Sci. Eng. C* **25** (2005) 207.
[22] M. Sadat-Shojai, M.-T. Khorasani, E. Dinpanah-Khoshdargi and A. Jamshidi // *Acta Biomater.* **9** (2013) 7591.
[23] N.E. Tari, M.M. Kashani Motlagh and B. Sohrabi // *Mater. Chem. Phys.* **131** (2011) 132.
[24] P.M.S.L. Shanthi, M. Ashok, T. Balasubramanian, A. Riyasdeen and M.A. Akbarsha // *Mater. Lett.* **63** (2009) 2123.
[25] J. Yao, W. Tjandra, Y.Z. Chen, K.C. Tam, J. Ma and B. Soh // *J. Mater. Chem.* **13** (2003) 3053.
[26] Y.-T. Huang, M. Imura, Y. Nemoto, C.-H. Cheng and Y. Yamauchi // *Sci Technology Adv Mat* **12** (2011) 045005.
[27] P.M.S.L. Shanthi, R.V. Mangalaraja, A.P. Uthirakumar, S. Velmathi, T. Balasubramanian and M. Ashok // *J. Colloid Interface Sci*. **350** (2010) 39.
[28] S. Ng, J. Guo, J. Ma and S.C.J. Loo // *Acta Biomater*. **6** (2010) 3772.
[29] W. He, Z. Li, Y. Wang, X. Chen, X. Zhang, H. Zhao, S. Yan and W. Zhou // *J. Mater. Sci.: Mater. Med*. **21** (2010) 155.
[30] F. Ye, H. Guo, H. Zhang and X. He // *Acta Biomater*. **6** (2010) 2212.
[31] Y. Wu and S. Bose // *Langmuir* **21** (2005) 3232.
[32] Y. Li, W. Tjandra and K.C. Tam // *Mater. Res. Bull*. **43** (2008) 2318.
[33] Y.F. Zhao and J. Ma // *Microporous Mesoporous Mater.* **87** (2005) 110.
[34] N.F. Mohammad, Y. Fei Yee and R. Othman // *Advanced Materials Research* **686** (2013) 33.
[35] Z. Xia, L. Liao and S. Zhao // *Mater. Res. Bull*. **44** (2009) 1626.
[36] Y. Wang, S. Zhang, K. Wei, N. Zhao, J. Chen and X. Wang // *Mater. Lett.* **60** (2006) 1484.
[37] Y. Wang, J. Chen, K. Wei, S. Zhang and X. Wang // *Mater. Lett.* **60** (2006) 3227.
[38] L. Yan, Y. Li, Z.-X. Deng, J. Zhuang and X. Sun // *Int. J. Inorg. Mater*. **3** (2001) 633.
[39] Y. Li, D. Li and Z. Xu // *J Mater Sci* **44** (2009) 1258.
[40] S.K. Saha, A. Banerjee, S. Banerjee and S. Bose // *Mater. Sci. Eng. C* **29** (2009) 2294.
[41] S. Jarudilokkul, W. Tanthapanichakoon and V. Boonamnuayvittaya // *Colloids Surf. A* **296** (2007) 149.
[42] M. Itokazu, T. Sugiyama, T. Ohno, E. Wada and Y. Katagiri // *J. Biomed. Mater. Res.* **39** (1998) 536.
[43] B.J. Melde and A. Stein // *Chem. Mater.* **14** (2002) 3326.
[44] Q.L. Tang, Y.J. Zhu, J. Wu, F. Chen and S.W. Cao // *Nanomed.* **7** (2011) 428.
[45] Q. Zhao, T. Wang, J. Wang, L. Zheng, T. Jiang, G. Cheng and S. Wang // *Appl. Surf. Sci.* **257** (2011) 10126.
[46] Q. Zhao, T. Wang, J. Wang, L. Zheng, T. Jiang, G. Cheng and S. Wang // *J. Non-Cryst. Solids* **358** (2012) 229.
[47] B. Palazzo, M. Iafisco, M. Laforgia, N. Margiotta, G. Natile, C.L. Bianchi, D. Walsh, S. Mann and N. Roveri // *Adv. Funct. Mater.* **17** (2007) 2180.
[48] G.D. Venkatasubbu, S. Ramasamy, G.S. Avadhani, V. Ramakrishnan and J. Kumar // *Powder Technol.* **235** (2013) 437.
[49] H. Wang, L. Zhai, Y. Li and T. Shi // *Mater. Res. Bull.* **43** (2008) 1607.