



US 20040180091A1

(19) **United States**

(12) **Patent Application Publication**
Lin

(10) **Pub. No.: US 2004/0180091 A1**

(43) **Pub. Date: Sep. 16, 2004**

(54) **CARBONATED HYDROXYAPATITE-BASED
MICROSPHERICAL COMPOSITES FOR
BIOMEDICAL USES**

(76) Inventor: **Chang-Yi Lin**, Burnaby (CA)

Correspondence Address:

CHANG-YI LIN

5970 NELSON AVENUE.

BURNABY, BC VEH3H9 (CA)

(21) Appl. No.: **10/386,546**

(22) Filed: **Mar. 13, 2003**

Publication Classification

(51) **Int. Cl.⁷ A61K 9/14**

(52) **U.S. Cl. 424/489**

(57) **ABSTRACT**

A composite material comprising of carbonated hydroxyapatite and biopolymer was synthesized in the form of microsphere for biomedical applications via a novel colloidal technique. Novel colloidal suspensions were prepared by mixing phosphate salts, calcium salts and carbonate precursors with size ranging from nanometer to submicrometer, together with sufficient amount of biocompatible polymers, following by spherorization to form microspheres of various sizes. Nanostructure of the constituents allows final apatitic phase to be developed upon moisture exposure. Biologically or therapeutically active drugs can be encapsulated directly into the microspherical composites upon synthesis for biomedical applications such as orthopedics, dentistry, and drug delivery.

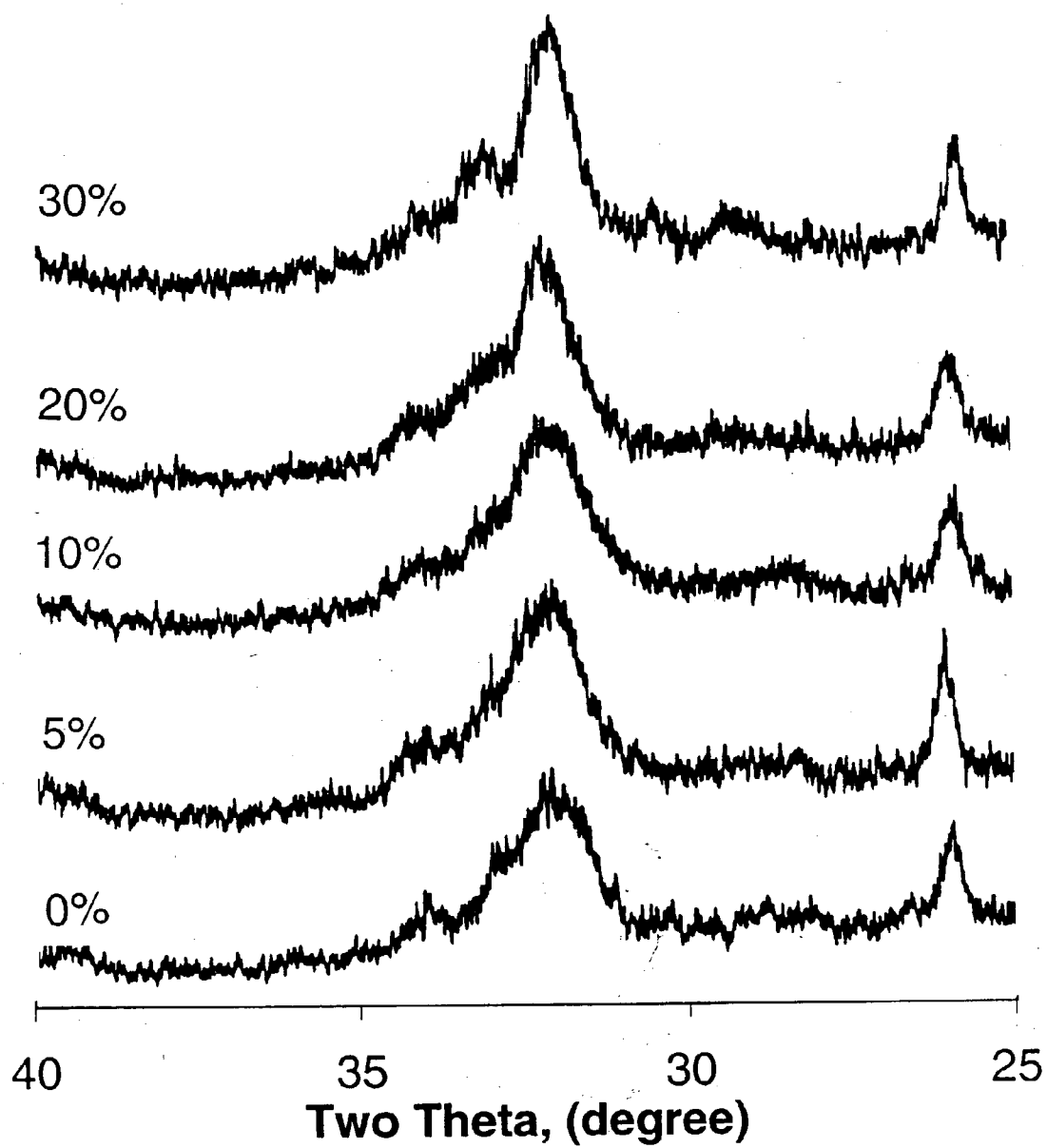


Figure 1

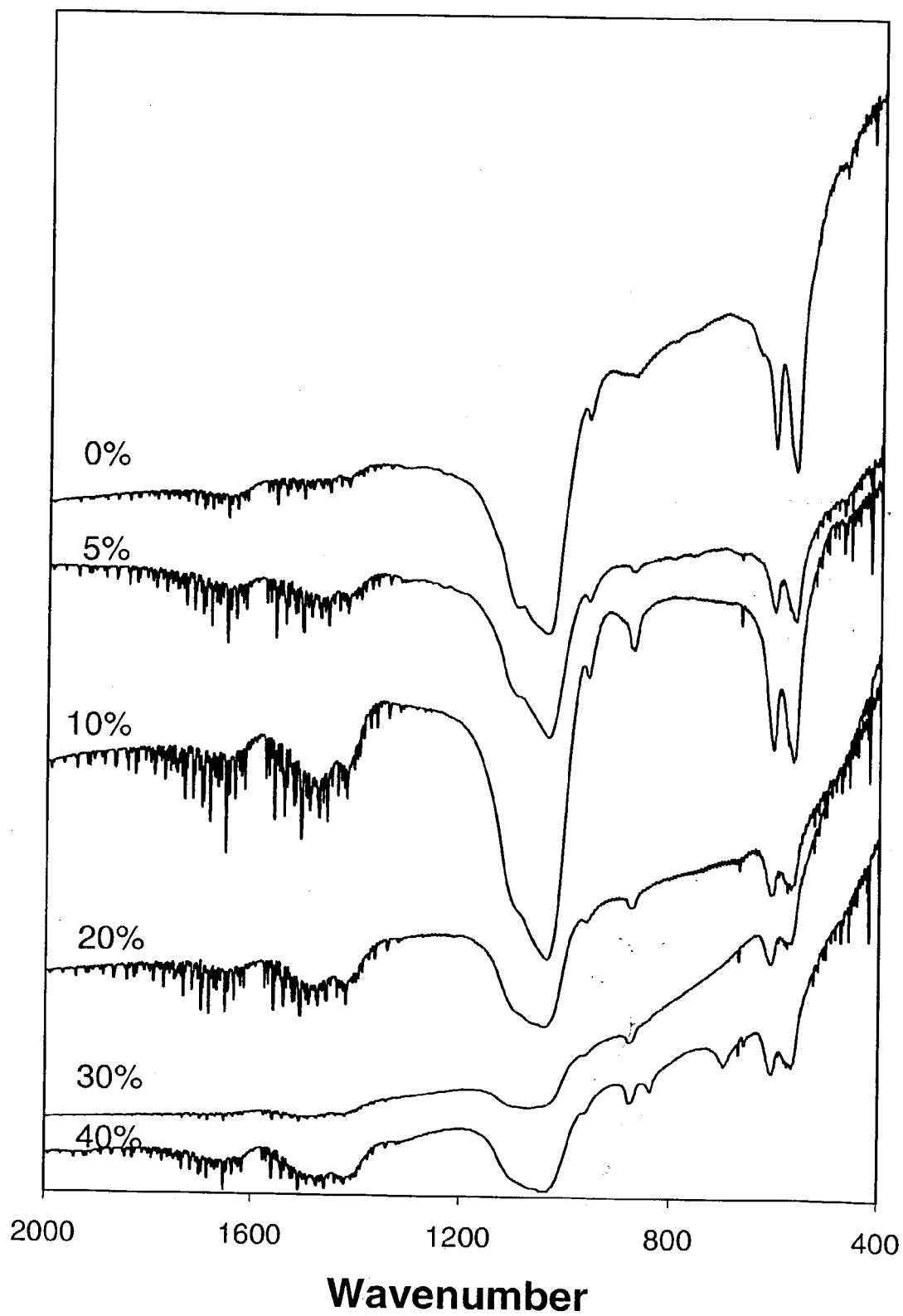


Figure 2

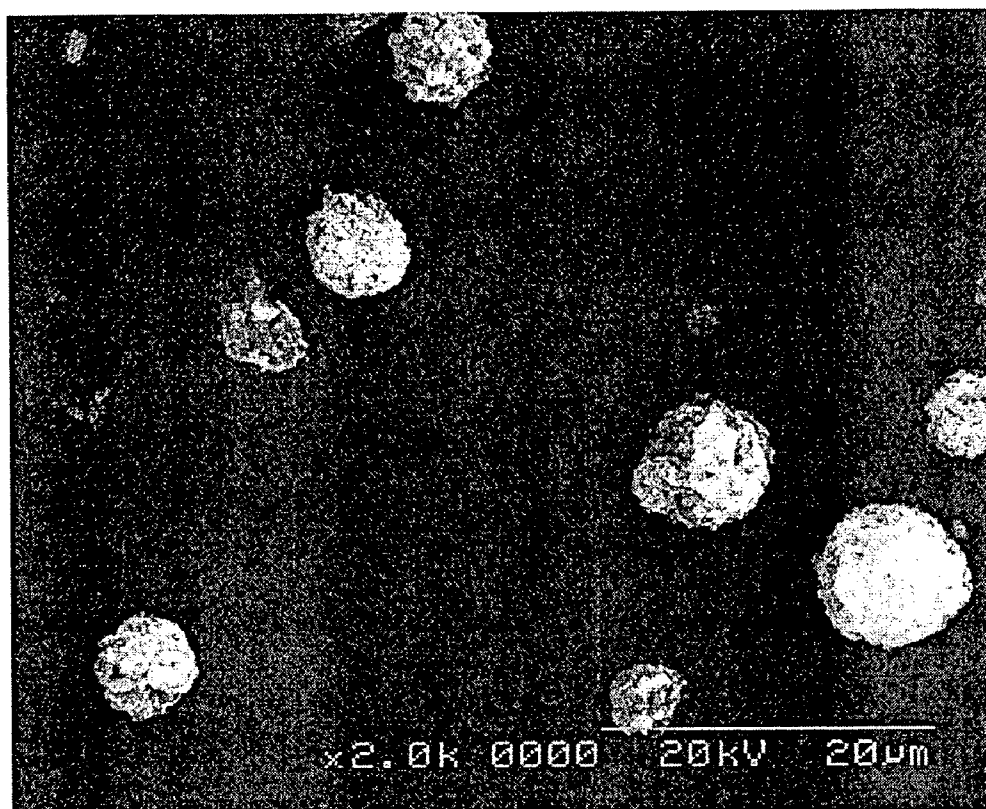


Figure 3

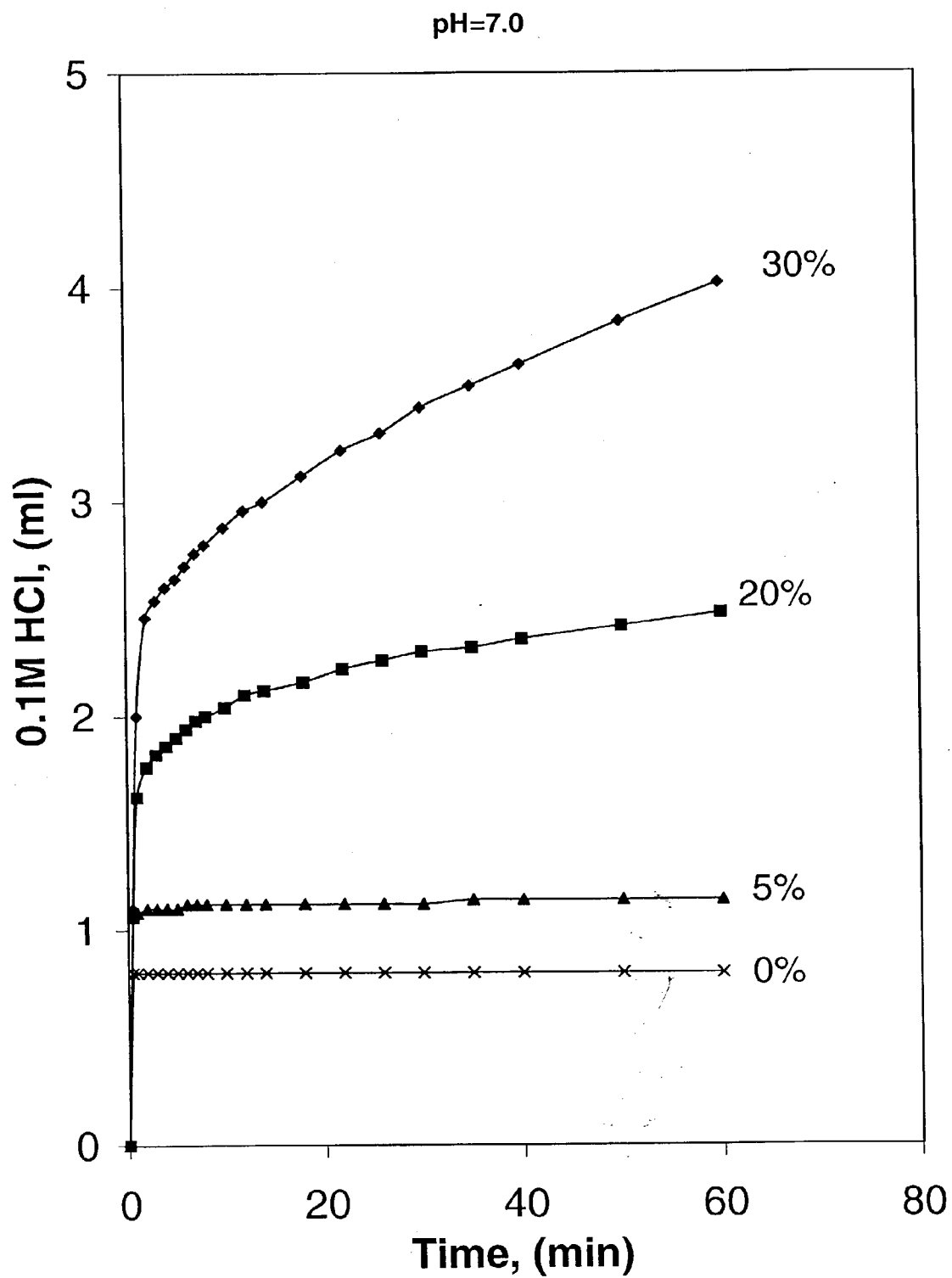


Figure 4

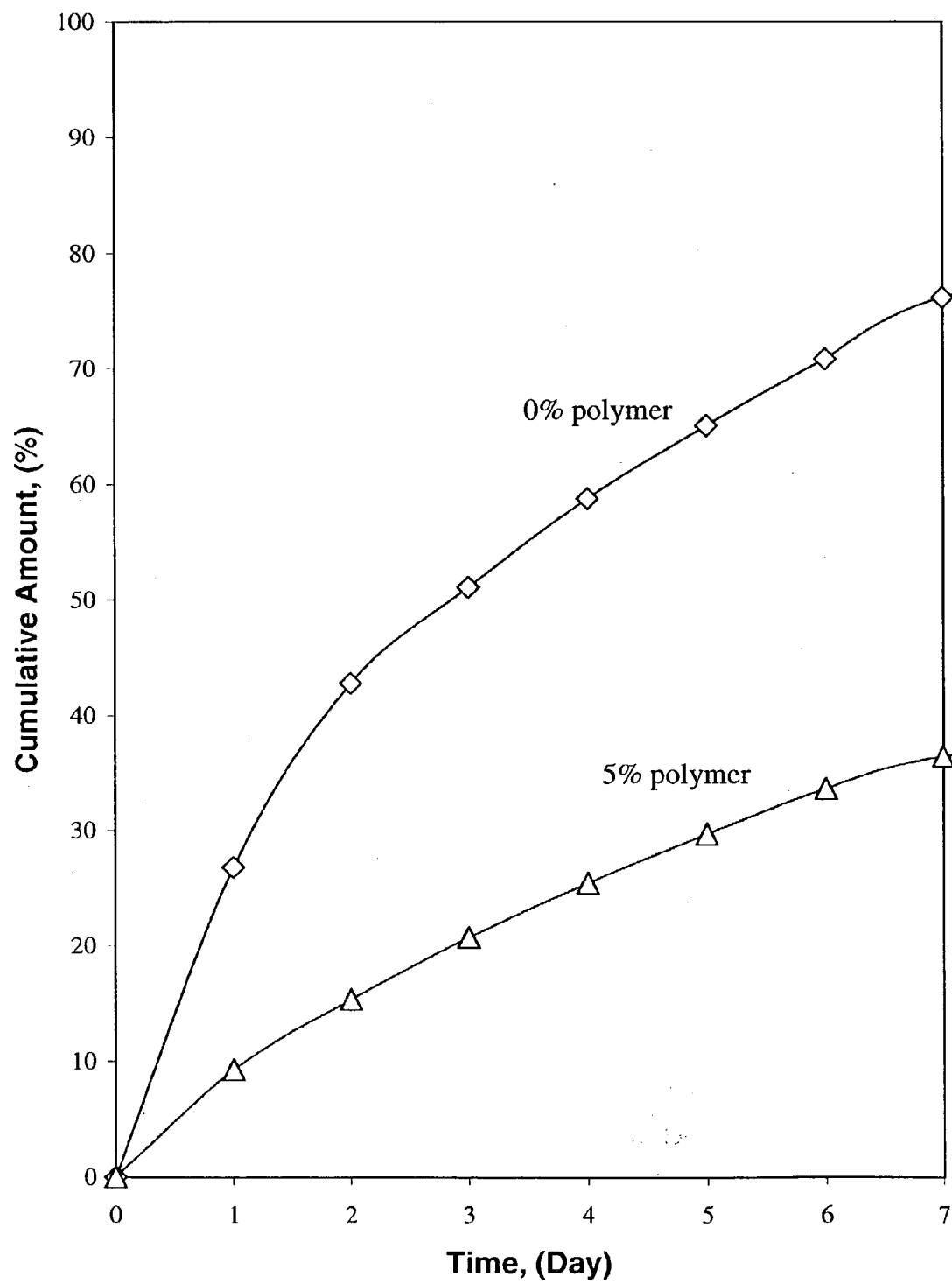


Figure 5

CARBONATED HYDROXYAPATITE-BASED MICROSPHERICAL COMPOSITES FOR BIOMEDICAL USES

BACKGROUND OF THE INVENTION

[0001] Calcium phosphate ceramics, particularly for those with Ca/P ratios between 1.5 and 1.67, standing for tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) and stoichiometric calcium hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), respectively, have long been used as prime candidate biomaterials to restore, reconstruct, and replace human bone tissues because of their close chemical similarity to that found in calcified tissues, such as bone, cartilage, tooth enamel and dentine, in human and vertebrates. Calcium hydroxyapatite has been the most widely studied materials over the past few decades since it demonstrated excellent biological affinity and activity to host tissue by forming an intimate chemical bonding at the material-tissue interface after implanted. However, bone mineral is essentially non-stoichiometric and contains HPO_4^{2-} and especially sufficient amount of CO_3^{2-} groups that replace the PO_4 and OH positions in the apatite lattice. Thus, naturally occurring apatite is essentially a carbonate-containing apatite and has a nanometered-size, poorly crystalline feature where metabolic activity with respect to the surrounding physiological environment to be more active than conventional synthetic calcium apatite. For instance, a carbonate content of as high as 11% by weight was detected in human dentine apatite [J. C. Elliott, D. W. Holcomb, and R. A. Young, *Calcif. Tissue Int.*, 37, 372-375, 1985] and 4-6% by weight in bone apatite [H A Lowenstam and S Weiner, *Biominerization*, Oxford University Press, Oxford, pp. 144-162, 1989]. A recent report [Jntema et al. *Int. J. Pharm.*, 112, 215, 1994] also indicated significant immune reaction, such as inflammation and tissue necrosis, that have been observed when well-crystalline apatite was implanted intramuscularly in animals. Prudhommeaux et al. [Arthritis and Rheumatism, 39, 1319-26, 1996] observed a lesser extent of white blood cell induced for a given specific surface area of calcium phosphate crystals when carbonated apatite crystals were injected in animal. This suggests in a given surface area, a lesser extent of interaction between inflammatory cell and carbonated apatite crystals can be expected. Besides, they also found a lower Ca/P ratio, for instance, below 1.5, or more preferably, 1.3, of the fine calcium phosphate particles providing the least inflammatory response after injection into the animal body. Such immune reactions can be largely reduced or avoided by the use of amorphous or poorly crystalline carbonated apatite, where an excellent biocompatibility to soft tissue and blood plasma has also been reported.

[0002] However, most synthetic calcium phosphate ceramics were thermally processed where a well-crystalline structure is expected. Such an improved crystallinity is adversely to that expects from a bioresorption point of view, and is essentially non-bioresorbable for highly crystalline calcium phosphate ceramics. Clinically, an ideal implanted material should be the one that is degradable and completely replaced by the host tissue. Therefore, it is more desirable to simulate the balance between resorption (by osteoclasts) and growth (by osteoblasts) activity of natural bone tissues. To this point, a controllable degradation behavior of synthetic calcium phosphate ceramics would be crucial to maintain a delicate metabolic balance between the cell-driven activities. Therefore, a poorly-crystalline and nanometer-sized

calcium phosphate ceramic having an apatitic structure together with the incorporation of carbonate would be more favorable for both hard and soft tissues substitution. Moreover, it is more advantageous for the synthetic nanocrystalline carbonated apatite over the allografts and autografts where the availability, risk of disease/viral transmission, host body rejection, etc. can be significant concerns. More critically, with the advancement of therapeutic concept, drug delivery has received great attention in recent decades [A K Jain et al., *Int. J. Pharm.*, 206, 1-12, 2000]. For instance, calcium phosphate ceramics have been successfully used as vehicles for delivery of anti-cancer and anti-inflammatory agents [M. Itokazu et al *Biomaterials*, 19,817-819,1998; F. Minguez et al *Drugs Exp. Clin. Res.*, 16, 231-235,1990; W. Paul and C. P. Sharma, *J. Mater. Sci. Mater. Med.*, 10, 383-388,1999]. This has further augmented by the rapid progress in the human genome project, where diseases that could not be treated before can be curable in near future.

[0003] However, conventional techniques for the incorporation of therapeutically active agents into the calcium hydroxyapatite are cost-ineffective and time-consuming, where sintered porous hydroxyapatite blocks or granules, frequently, soaked into drug-containing solution for sufficient time period (take for at least 24 h) in order for drug molecules to diffuse and anchor (adsorb) on the internal surface of the body [K. Yamamura et al, *J. Biomed. Mater. Res.*, 26, 1053-64,1992]. The drug release is accomplished through desorption and leaching of the drug to the surrounding tissue after exposure to physiological fluid. Unfortunately, most of the adsorbed drug molecules release from such system in a relatively short period of time. Impregnation of drug material into porous sintered calcium phosphate microspheres has been reported in patent literature. Starling et al. claimed in WO 98/43558 that hollow microspheres are sintered and impregnated with drugs for slow release. Sumiaki et al in U.S. Pat. No. 5,055,307 disclosed slow release drug delivery granules comprising porous granules of a calcium phosphate compound having a Ca/P of 1.3 to 1.8, porosity from 0.1 to 70%, and a specific surface area from 0.1 to 50 m^2/g and a pore size from 1 nm to 10 μm . The granules were fired at a temperature of 200 to 1400° C., and a drug component impregnated in pores of the granules. Porous calcium phosphate ceramics were impregnated with bone marrow cells [E. Kon et al, *J. Biomed. Mat. Res.* 49 328-337, 2000] and with human bone morphogenetic protein [I. Alam et al *J. Biomed. Mat. Res.* 52, 2000]. The amount and distribution of the drugs or proteins throughout the apatite body are difficult to control in a precise and uniform manner. This is especially critical for implant devices where a relatively high dosage of anti-inflammatory agents is needed during the earlier period after implantation. A non-uniform release of the adsorbed drugs into the surrounding physiological environment may cause adverse effect or reduce the therapeutic efficacy. Therefore, it is desirable to load drugs of interest in a given entity at low temperature, most preferably, at ambient temperature.

[0004] Then, an in-situ drug encapsulation into hydroxyapatite matrix becomes possible if a pertinent colloidal solution containing desirable reactants can be properly prepared, which may further ensure the uniformity and various concentrations of drug to be physically constrained within the matrix. It is more attractive if the matrix apatite can be synthesized at ambient temperature and has a nanometer-size, poorly-crystalline, carbonate-containing structure,

enabling biodegradation to be easily tailored. On this basis, drug release pattern can be controllable via pore diffusion or matrix resorption, or a combination of both in a desirable manner as clinically needed.

[0005] Calcium phosphate cements (CPCs) offer attractive synthesis methodology to incorporate drugs. The self-setting CPCs form apatitic phase as an end product led to promising biomedical applications involved orthopedics, dentistry, and also drug delivery [M. Dairra, et al. *Biomaterials*, 19, 1523-1527, 1998; M. Otsuka, et al. *J. of Controlled Release* 43, 115-122, 1997; Y. Tabata, *PSTT*, 3 [3], 80-89, 2000; M. Otsuka, et al. *J. Pharm. Sci.*, 83, 255-258, 259-263, 611-615, 1565-1568, 1569-1573, 1994]. CPC is typically formulated as a mixture of solid and liquid components in pertinent proportions, which react to form the apatite. The physico-chemical reactions that occur upon mixing of the solid and liquid components are complex, but dissolution and precipitation are the primarily mechanisms responsible for the final apatite formation [C. Hamanish et al. *J. Biomed. Mat. Res.*, 32, 383-389, 1996; E. Fernandez et al. *Materials in Med.*, 10, 223-230, 1999].

[0006] Otsuka et al. conducted a series of investigations of drug encapsulation in self-setting calcium phosphate cements derived from tetracalcium phosphate and dicalcium phosphate [J. *Contr. Rel.* 43, 115-122, 1997; J. *Contr. Rel.*, 52, 281-289 1998; J. *Pharm. Sci.*, 83, 255-258, 259-263, 611-615, 1565-1568, 1569-1573, 1994]. The cement was shaped with in-situ drug encapsulation, into 15 mm diameter pellets and drug (indomethacin) release sustained up to 3 weeks period. Lee et al. in WO98/16209 claimed that poorly crystalline apatite may simultaneously encapsulate drug material for slow release. It has been suggested to use porous, composite apatite as a carrier for encapsulating gentamicin sulfate, an antibiotic to treat bacterial infections at infected osseous sites [J. M. Rogers-Foy et al, *J. Inv. Surgery* 12, 263-275, 1997]. S. Gogolewski in WO00/23123 used hardenable hydraulic cement comprising a drug to be delivered to the human or animal body upon degradation or dissolution of the cement. However, conversion of CPC to phase-pure hydroxyapatite needed as long as 40 hours. L. Chow et al in U.S. Pat. No. 5,525,148 disclosed calcium phosphate cements, which self-harden substantially to hydroxyapatite at ambient temperature when in contact with an aqueous medium. More specifically the cements comprise a combination of calcium phosphates other than TTCP with a concentrated (>0.2 M) aqueous phosphate-containing solution having a pH equal to or greater than 12.5. However, high pH (>12.5) may cause denaturation of most biologically active molecules such as proteins, enzymes, DNA, etc., as well as adverse effect to the surrounding host tissue after implantation, so the process may not be as good as their earlier formulation [L. C. Chow et al. *J. Dent Res.*, 63, 200, 1984] and is not suitable for drug encapsulation vehicles.

[0007] The advantage of calcium hydroxyapatite for drug delivery is that side effects have seldom been a major concern for the materials [Y. Shinto et al, *J. Bone Jt. Surg.*, 74B4, 600-4, 1992]. Calcium phosphate—biodegradable polymer blends were also investigated as possible vehicles for drug delivery [I. Soriano and C. Evora, *J. Contr. Rel.*, 68, 121-134 2000]. Prolonged drug release (up to 10 weeks) was obtained for the composites coated with layers of polymeric materials. Very recently, Q. Qiu et al [J. *Biomed. Mat. Res.*

52, 66-76, 2000] processed polymer-bioactive glass-ceramic composite microspheres for drug delivery.

[0008] In the synthesis of carbonated hydroxyapatite (cHA), recently, Constantz et al. [*Science*, 267, pp.1796-9, 1995] used monocalcium phosphate monohydrate (MCPM), $\square$ -tricalcium phosphate ($\square$ -TCP), $\square$ -tricalcium phosphate ($\square$ -TCP), and calcium carbonate (CC) to form cHA with reasonably better crystallinity than bone apatite in an aqueous phosphate solution. Miyamoto et al. [*J. Biomed. Mater. Res.*, 54, 311-319, 2001] used tetracalcium phosphate (TTCP), dicalcium phosphate (DCP), and sodium bicarbonate in aqueous phosphate solution to form cHA; however, incomplete reaction between TTCP and DCP was detected when sodium bicarbonate exceeded 6% by weight, leaving a mixture of carbonate apatite, TTCP, and DCP. Dissolution of their carbonate apatite at solution of pH 5.5 is increased with carbonate content. However, its mechanical property decreased with carbonate concentration. Their finding provides evidence that cHA is prone to dissolve or resorb faster than carbonate-free hydroxyapatite (HA), where some studies on long-term tests on the HA showing that HA resorption starts gradually after 4-5 years [E. G. Nordstrom and K. H. Karlsson, *Materials in Medicine*, 1, 182-184, 1990], and the resorption rate is expected to increase with carbonate concentration. Otsuka et al. [*Pharmaceutical Res.* 14, 444-449, 1997] incorporated drug for control release study in a similar TTCP-DCP system and found that pore size in their cement system increased with carbonate content and resulted in a faster drug release to the surrounding physiological solution than others with lower carbonate concentration. To our viewpoint, both the pore diffusion and matrix dissolution may response for the resulting drug release rate. Therefore, it is important if an apatite system can be developed which allows incorporation of any desirable amount of carbonate (where a controllable bioresorption rate can be attained) and a further structural modification for drug release control and better mechanical compatibility to the host tissue, particularly the soft tissues, can approach through the incorporation of biopolymeric candidates.

[0009] Granulated form of apatite ceramics provides the most potential applications, compared to other physical forms, e.g., blocks, films, fibers, especially in the area of biomedicine, biochemistry, catalysis, etc. Apatite granules facilitate not only surgical operations but also benefit the tissue growth after implantation by creating large intergranular pores allowing ingrowth of the host tissue. Therefore, it is most interesting to develop carbonate apatite microspheres. A further in-situ incorporation of therapeutically active agents or other functional (bio)molecules into the microspheres during the synthesis process offers a greater potential for a variety of practical and clinical applications.

[0010] Controlled release of drugs from a spherical matrix has received considerable attention since it provides a more efficient manner to deliver drugs that enhance the therapeutic efficacy for a given dosage in comparison with conventional manner [DD Breimer, *J. Controlled Release*, 62, 3-6, 1999, J L Cleland et al., *Current Opinion in Biotechnology*, 12, 212-219, 2001]. This is particularly interesting for those patients that needed daily single or multiple administration, where patient's compliance can be largely improved through a sustained days-long-lasting or weeks-long-lasting therapy. Polymeric materials have been used for drug delivery con-

trol and enjoyed substantial clinical success for certain drug systems [N A Peppas et al. *Europ. J. Pharm. Biopharm.*, 50, 27-46, 2000, C Schmidt et al. *J. Controlled Release*, 57, 115-125, 1999, L Brannon-Peppas, *Int. J. Pharm.*, 116, 1-9, 1995].

[0011] We hereby disclose a composite composition through which carbonate-containing apatite (cHA)—biopolymer microspheres can be engineered to function as an efficient vehicle for a number of biomedicine, chemistry, cosmetics, agriculture, catalysis applications. Controlled drug delivery will be a critical alternative function to this invention. The method relates to the cHA microspheres capable of encapsulating any type of drugs or proteins or other functional (bio)molecules. Further optimization of the physical, chemical, surface and mechanical properties to meet a variety of applications of the cHA can be achieved with the combination of biopolymer candidates. The method starts with a colloidal suspension containing inorganic ingredients, which are suitable to form cHA and sufficient amount of biopolymers. Spherorization can be easily achieved through conventional routes, such as spray dry, freeze dry, drip casting, emulsification. Biologically or therapeutically active drugs, proteins, vaccines, or other functional macromolecules can be encapsulated into the resulting carbonated apatite-based microspherical composites and subsequent controlled release of the therapeutically active or functional agents from the microspheres can be achieved. These microspherical composites can also be consolidated into desirable geometry by compression to form desirable geometry, or by injection for different biomedical purposes.

DEFINITIONS

[0012] Colloidal Particles—refers to small particles that are substantially in suspension in a liquid.

[0013] Encapsulation or entrapment—used interchangeably, related to those biologically or therapeutically active agents, drugs, vaccines, proteins, etc. described in this invention that are physically constrained into voids of comparable dimensions within a matrix phase.

[0014] Matrix—refers to a host which, in this invention, is a composite structure comprising both inorganic phase and organic polymeric phase.

[0015] Nano-Structure—refers to the composite matrix, which exhibits a microstructure, such as grain size, pore size, having at least in one of dimensions that is less than 100 nm in scale.

[0016] Poorly Crystalline Structure—refers to a material with distinguishable but poor resolution of X-ray diffraction patterns or infrared spectrum, for instance, for the poorly crystalline apatite, two broad reflection peaks in the x-ray diffraction pattern at about 26° and 32° 2θ were detected.

[0017] Biocompatible—refers to materials that do not elicit a significant detrimental or adverse response when injected or implanted in a biological host, such as human, vertebrates, or other animals. Hydroxyapatite has well-known as a biocompatible materials, however, an improved biocompatibility can be reached by lowering the amorphousness of the crystalline phase by incorporation, such as disclosed in present invention, of carbonate ions. For instance, a lesser extent of the immune response, i.e., inflammation or tissue necrosis, was observed in an animal

model when carbonated hydroxyapatite crystals were injected intramuscularly [Arthritis and Rheumatism, 39, 1319-26, 1996].

[0018] Dissolution—refers to a material that is able to dissolve into a liquid medium in vitro, which in this invention is phosphate-buffered saline or simulated body fluid, at a rate that is essentially controllable. In this invention, for instance, increase of the carbonate concentration promotes or accelerates the dissolution rate over a constant time frame.

[0019] Bioresorption—refers to the ability of a material to be resorbed in vivo, where the process included cell-driven and mediate-driven mechanisms. In general, the in-vitro dissolution behavior may be related to that of in-vivo bioresorption behavior [C Ohtsuki, et al., *J. Non-Crystalline Solids*, 143, 84-92, 1992].

[0020] Setting—refers to a process by which the hydrated inorganic precursor is transformed into a rigid, non-flowable or non-injectable or non-formable entity. In this invention, the set carbonated hydroxyapatite showed certain strength and required some minimal compression to deform the entity.

[0021] BET sorption—a standard technique used to measure the surface area of a porous or powdered substance. BET are initials of the inventors of this technique (Brunaur, Emmett, Teller). It is conducted using commercial BET instrument. Nitrogen is the most common gas for this measurement. The principle rests on the fact that gas molecules tend to adsorb to the surface of a solid. The amount of gas adsorbed depends on the size of the pores of the solid and on the partial pressure of the gas relative to its saturation pressure. At higher partial pressure, hysteresis of adsorption/desorption curve can frequently be obtained, which can be further converted into pore size distribution via a Kelvin equation.

BRIEF DESCRIPTION OF THE FIGURES

[0022] FIG. 1. X-ray diffraction patterns of cHA containing different amounts of carbonate ranging from 0% to 30% by weight.

[0023] FIG. 2. Fourier transform infrared spectra of the cHA containing different amounts of carbonate ranging from 0% to 30% by weight. The absorption bands at 561 cm^{-1} and 600 cm^{-1} , together with a broad band over the region of $1100\text{-}1000\text{ cm}^{-1}$, assigned to be a typical apatitic structure. The bands at 871 cm^{-1} and 1430 cm^{-1} indicate the presence of CO_3 groups in the apatitic structure. Both CO_3 bands suggest that the apatite obtained in this composition is AB-type carbonated apatite.

[0024] FIG. 3. Morphology of the microspherical composites, having a size of about 2-5 μm in diameter, containing 5% by weight of poly(DL-lactic-co-glycolic) acid.

[0025] FIG. 4. In-vitro dissolution behavior of the microspheres containing various amounts of carbonate and 5% by weight of polyethylene glycol, where an abrupt increase in the volume of HCl used at first few minutes of contact for all the compositions was observed, suggesting an abrupt change in chemical potential between the microspheres and surrounding liquid medium. Fast dissolution of the microspheres is required to reach a chemical equilibrium of the

system. However, a continuous addition of HCl solution is needed in order to maintain the pH level for those microspheres with carbonate concentration greater than 10%.

[0026] FIG. 5. Release behavior of a model drug (5% by weight of fluorescein dye) from pellets into a phosphate-buffered saline at 37° C. A sustain release for over 2 months was observed for pellets containing 5% PLGA; however, for those without incorporating polymers, approximately 20 days of release was observed.

SUMMARY OF THE INVENTION

[0027] The present invention, therefore, meets the particular need in the art by providing a composition to form carbonated apatite-biopolymer microspherical composites capable of in-situ encapsulating therapeutically or biologically active substances. This invention is essentially cost-effective and of particular importance is providing wider processing parameters for the synthesis of biocompatible and bioactive composite microspheres. The composite composition discloses in this invention can be achieved by preparing a colloidal suspension comprising relatively fine calcium phosphate particles, such as monocalcium phosphate anhydrate, dicalcium phosphate, sodium phosphate, calcium hydroxide, carbonate precursors such as calcium bicarbonate or sodium bicarbonate or potassium bicarbonate, and polymers. A suitable solvent system was adopted which needed to completely dissolve the polymer and at the same time to prevent phase transformation to occur during synthesis. Following a granulation process included, but not limited to, spray dry, freeze dry, drip casting, emulsification, and combination thereof, to form microspherical intermediate products. Final microspherical products were then obtained by exposure of the intermediate product to small amount of liquid water or moisture for a time period of 4-16 hr, which is much shorter than other literature reports. For encapsulating therapeutically active drugs of different degrees of hydrophobicity or hydrophilicity, the process can be easily adjusted as desired. For instance, for those hydrophilic active agents, an oil-in-water emulsification step can be employed. Following the same spherulization process, microspheres of varying diameters ranging from 1 to 1,000 μm can be easily produced. These microspherical composites (with or without the therapeutically active agents) allows to form different geometry included, but not limited to monoliths, pellets, tablets, bulks, thin or thick sheets, by simply consolidating the microspheres. The temperature increases to a maximum of 35° C. upon the phase transformation, which is close to body temperature, and is expected to cause little or no adverse effect to host. The types of polymers that can be incorporated in these microspherical composites are essentially unlimited, and can be tailored as clinically needed.

[0028] The present invention provides a combined formulation of the mixed inorganic powders that required just distilled water (or commercial phosphate buffered saline, Hank's solution, if therapeutically active materials were employed) or even moisture to trigger phase conversion in a short time period of incubation. This indeed offers a major advantage over those conventional CPCs, where either acidified or highly basic, concentrated phosphate solution is required to trigger the transformation reaction over a sufficient time period of incubation. The rate for apatite formation in the art has been found to be polymer concentration

dependent and the pertinent concentration of the biopolymer used is less than 40%, or more preferably, ranging from 1%-30% by weight. For those with less concentration, for instance, 5-10% by weight, incubation time span of 8-16 h was observed. One key feature of this invention is the use of relatively fine inorganic powders, which were obtained by either mechanical means to reduce the particle size or self-synthesis of some component, e.g., DCP, to form reactive mixture powders with size of 0.02 μm -2 μm , or more preferably, ranging from 0.05 μm to 0.8 μm . Such fine powder mixture allows a complete phase conversion to achieve in an exceptional faster rate, i.e., few hours, than conventional CPC formulations. More critically, the resulting microspherical composites show an average pore size ranging from 1 nm to 1,000 nm as determined from the BET method and mercury porosimetry. The small pores allow physical constraint of a number of bioactive molecules of different molecular sizes. These pores also provide as conduits for a subsequent diffusion of the therapeutically active molecules towards surrounding environment.

[0029] This invention does provide a new class of hybrid biocomposites comprising carbonated apatite and biopolymer as matrix, allowing a direct incorporating biologically or therapeutically active drugs, proteins, vaccines or other functional materials. They are able to play some or significant role in drug delivery, human repair process, agriculture, bioremediation, and environmental process.

[0030] The polymers employed in this invention to form microspheres comprise a polymeric material, which selected from the group consisting of either biodegradable or non-biodegradable polymers, such as, but not limited to, polylactic acid, poly (DL-lactide-co-glycolide) polymer, polyglycolic acid, polypolyanhydrides, polyester, polyorthoester, polyamino acid, poly(alkyl cyanoacrylates), aspartate, polyethylene glycol, polyethylene oxide, poly(butylenes terephthalate), polyacrylates, polymethacrylates, dextran, polysaccharides, polylactate, polyglycolates, polystyrene, hyaluronic acid, polyphosphazenes, polyvinyls and derivatives, copolymers and mixture thereof.

[0031] Therapeutically active agents or drugs, which do not restrict the present invention are selected from the group of anti-cancer drugs; hormones and hormone antagonists; heavy metals and heavy metal antagonists; cytostatic agents for cancer treatment, drugs for treatment of trachea or stroke; antacids and anti-inflammatory drugs; chemotherapeutic agents for parasitic infections and microbial diseases, immunosuppressive agents; antibiotics; antiparasitics; antinauseants; relaxants; stimulants; anti-hypertensives; minerals and nutritional agents, anti-obesity drugs, anabolics, anti-asthmatics, and combinations thereof.

[0032] Those above-mentioned therapeutically active agents or drugs include, but not only restrict to, alkylating agents and antimetabolites; nitrogene mustards, ethylenimines; alkylsulfonates; folic acid analogs; pyrimidine analogs; purine analogs; vinca alkaloids; antibiotics drugs such as phenylbutazone, indomethacin, naproxen, ibuprofen, flubiprofen, diclofenac, dexamethasone, prednisone and prednisolone; cerebral vasodilators such as solocetidilum, vinamine, naftidrofuryl oxalate, codegergine mesylate, cyclandelate, papaverine, nicotinic acid; anti-infective drugs such as erythromycin stearate and cephalixin; antacids and anti-inflammatory drugs such as histamine, bradykinin, kal-

lidin; immunosuppressive drugs such as FK506, chemotherapeutic agents for parasitic infections and microbial diseases; anticholinesterase agents, catecholamine and sympathomimetic drugs such as epinephrine, norepinephrine and dopamine, adrenergic agonists, transmitters such as GABA, glycine, glutamate, acetylcholine, dopamine, 5-hydroxytryptamine, and histamine; analgesic and anesthetics such as opioid analgesics and antagonists.

[0033] The proteins or enzymes that can be incorporated include, but not limited to, active proteins, active fragments of DNA, RNA, and proteins. The protein or active fragments is a member of groups of enzymes or any active compounds including, but not limited to, growth factors (such as bone morphogenetic proteins), amylase, catalase, cellulase, kinase, lipase, oxygenase, peptidase, phosphatase, cholesterol, trypsin, enzymes, chymotrypsin, immunoglobulin, DNase, RNase, nuclease, dextranase, carboxylase, carboxypeptidase, and mixture thereof.

[0034] The solvents used in this invention included, but not limited to, water, methanol, ethanol, acetone, and those of water-insoluble or partially water-soluble such as methylene chloride, ethyl acetate, ethyl vinyl ether, dichloromethane, acetaldehyde dimethyl acetal, dioxane, hexane, toluene, and combination thereof.

[0035] The present invention also provides a method to produce carbonate-containing calcium phosphate microspheres with carbonate concentration ranging from 0.1% to 50% by weight (base on total weight of the powder mixture), or more preferably, ranging from 3% to 40%. The carbonate components used in this invention included, but not limited to, calcium carbonate, calcium hydrogen carbonate, sodium bicarbonate, and combination thereof. The carbonate salts can be used either in solid form or more preferably, in a liquid form.

[0036] The phosphates used in this invention included, but not limited to, monocalcium phosphate anhydrate, self-synthesized ultrafine dicalcium phosphate, sodium phosphate, and preferably, a combination thereof.

[0037] The present invention provides a method to produce carbonated hydroxyapatite—biopolymer composite microspheres, which are capable of encapsulating and delivering, but not limited to, therapeutically or biologically active agents or drugs, proteins, vaccines, polypeptides, or other functional substances for a number of applications, involved, biomedicine, biochemistry, agriculture, catalysis, cosmetics, environmental processes.

[0038] The invention will be more fully understood from the following description of the preferred embodiments, drawings, and examples, all of which are used for illustrative purposes and not intended in any way to limit the invention.

DETAILED DESCRIPTION OF THE INVENTION

[0039] The present invention is developed to synthesize microspherical biocomposites comprising a composite matrix, i.e., carbonated hydroxyapatite (cHA) phase and a secondary biopolymeric phase, for use in a number of areas, included biomedicine such as orthopedics, dentistry, and drug delivery, biochemistry, bioremediation, cosmetics, agriculture. The compositions used for cHA synthesis comprise monocalcium phosphate anhydrate, dicalcium phos-

phate, sodium phosphate, calcium hydroxide, together with calcium bicarbonate or potassium carbonate or sodium bicarbonate, together with a small amount of pure water or just moisture. The cHA has a total Ca/P ratio in the final composition ranging from 1.10 to 1.65, in a preferred embodiment, 1.20-1.55, which is essentially a calcium-deficient, carbonated apatite. Here the terms “hydroxyapatite” and “apatite” are interchangeable.

[0040] Calcium-deficient carbonate apatite (cHA) has essentially a chemical formulation closer resemblance that of natural bone apatite than the stoichiometric apatite, $\text{Ca/P} = 1.67$. Higher dissolution or bioresorption rate has been verified in vitro and in vivo for the cHA than stoichiometric HA. For the case of biological microcrystallites, i.e., non-stoichiometric carbonated apatite having a chemical formula like $\text{Ca}_{9-x}(\text{PO}_4)_{6-x-y}(\text{CO}_3)_y(\text{HPO}_4)_x(\text{OH})_{1-y/3}$, the poorly-crystalline structure, associated with nanometer dimensions provides the highest biological or metabolic activity to reach subtle balance to the surrounding environment. More importantly, natural bone tissue is essentially a composite structure comprising 60-70% of non-stoichiometric apatite, with less quantity of polymers, i.e., collagen, and trace amount of ionic species. Therefore, it is most desirable to simulate such a composite structure for biomedical uses. Furthermore, synthetic cHA was found to be more biologically compatible than the stoichiometric HA by a less inflammatory response, as well as a lesser extent of white blood cell population when contacted with soft tissue through an intramuscularly injection.

[0041] The chemical formulation described above allows the cHA to be developed at a temperature below 60° C., or more preferably, at ambient temperature or with sufficient strength after the composition forms a rigid solid entity, similar to those of conventional calcium phosphate bone cements. It may then be applied as bone filler for orthopedics and dentistry. However, compressive strength is decreased with increased involvement of carbonate concentration. The reaction of phase transformation of the inorganic powder ingredients towards final cHA may be complex, but accordingly, is a result of dissolution and precipitation to form fine apatitic crystals. The carbonate ions, from say calcium bicarbonate or sodium bicarbonate precursor, should be involved into the apatitic lattice by certain degree of replacement of OH and PO_4 groups upon phase development. The use of these carbonate precursors facilitates a quantitative control of the carbonate concentration involvement in the final product, rather than those arbitrarily taken from atmospheric CO_2 . A proper control of carbonate content allows a precise design of final cHA in a desirable manner, either structurally or chemically, to meet different biomedical needs to be highly feasible. This is particularly critical when the subject of bioresorption or biodegradation is a significant concern.

[0042] Dissolution behavior in-vitro for biological calcium phosphate materials can generally be related to bioresorption or biodegradation behavior in vivo. A number of in-vitro studies have been disclosed higher in-vitro dissolution rate for carbonate-containing poorly-crystalline apatite than those of stoichiometric or near-stoichiometric crystalline apatite, although quantitative estimate of the kinetic parameters for different kinds of apatite was not being compared. A resorbable implant is more feasible to that of non-resorbable one, since resorbable implanted devices allow

entire replacement by the host tissue achievable after sufficient time period of implantation. A comparable rate between resorption of the implanted materials and corresponding ingrowth of the host tissue becomes a crucial criterion for material design. For instance, Daculsi et al. [Biomaterials, 19, 1473-1478, 1998] employed biphasic calcium phosphate composites comprising hydroxyapatite and tricalcium phosphate (TCP) and a delicate balance of the resorption behavior, associated with the non-resorbable HA and resorbable TCP, equilibrium to the surrounding physiological environment can be obtained. Therefore, it is critical for a biomaterial to be resorbable, or more preferably, a controllable resorption behavior, under physiological environment.

[0043] In the present invention, a composition is provided to form cHA with controllable concentration of carbonate, ranging from 0.5% to 50%, or in a preferred embodiment, 3%-40% by weight. The cHA showed phase-pure and poorly-crystalline structure as evidenced from an X-ray diffraction analysis (XRD, FIG. 1), whereas no residual impurity phases, such as carbonate or calcium phosphate precursors were detectable under the resolution of the XRD. Fourier transformed infrared spectrum analysis (FTIR, FIG. 2) shows two absorption bands at 562 cm^{-1} and 600 cm^{-1} , together with a broad band in the region of $1,100\text{--}1,000\text{ cm}^{-1}$, indicating a typical apatitic structure. Bands at 871 cm^{-1} and 1430 cm^{-1} indicate the presence of CO_3 groups in the apatitic structure. Both CO_3 bands suggest that the apatite obtained in this composition is AB-type carbonated apatite. Increase in carbonate concentration suggests sufficient amount of the carbonate ions being incorporated into the apatitic lattice. Such carbonate incorporation produces a wide spectrum of in-vitro dissolution rate, as illustrated in Example 2, where the dissolution of the cHA (in term of the volume of diluted HCl solution that added in order to reach the pH level of a simulated body fluid, SBF, containing 142 mM of Na^+ , 5 mM of K^+ , 1.5 mM of Mg^{2+} , 147.8 mM of Cl^- , 0.63 mM of Ca^{2+} , 4.2 mM of HCO_3^- , 0.5 mM of SO_4^{2-} , and 1.0 mM of HPO_4^{2-} , mimicking that of human blood plasma) increases with carbonate concentration. The use of dilute acidic solution is to bring the pH value of the SBF, where the pH value is increased as cHA was added due to dissolution, down to physiological level. The pH increment due to cHA addition increases with carbonate content, where an abrupt increased pH was found for those with higher carbonate content, during earlier time period of the test. A gradual reduction of the acidic solution is followed until plateau region is observed. The plateau region suggests an equilibration of ionic concentration between the cHA and SBF. Commercial HA was also employed for comparison, where little or no change in the volume of the acidic solution used was observed over the same time frame of the measurement, indicating little or no dissolution of the HA crystals under physiological solution. By contrast, this observation further indicates that by proper control of carbonate content, from low (less resorbable in physiological environment) to high concentration (easily resorbable), the dissolution behavior of the final cHA allows to be finely tuned for application-oriented customization.

[0044] This invention also provides a method to prepare nano-structured cHA-biopolymer nanocomposite spheres, which can be achieved through the use of fine (nanometer-to-submicrometer) starting ingredients. Nano-structured cHA offers great advantage for encapsulating therapeutically

active agents, drugs, functional micro or macromolecules, etc., as well as for their controlled release for a number of biomedical, biochemical, cosmetic, agricultural applications. In this invention, dicalcium phosphate (DCP), CaHPO_4 , was synthesized through a simple co-precipitation method. A combination of commercially available calcium hydroxide and monocalcium phosphate monohydrate, or ammonium hydrogen phosphate, or phosphoric acid allows nanometer-size DCP particles to be synthesized by first adding small amount, say 0.1%-10%, or more preferably, 0.5%-5% by weight, of water-soluble surfactants included citric acid and/or polyacrylic acid into aqueous calcium hydroxide solution. The fine dicalcium phosphate crystals precipitate immediately upon an acidic phosphate solution was added via titration. The precipitates were separately right after the completion of the titration through a filter paper. To remove the surfactant, the filtered powder cake was further rinsed several times with large quantity of distilled water, following oven drying at a temperature of $150\text{--}180^\circ\text{C}$. The resulting powders, which show a diffraction pattern exactly the same as the DCP powder indexed by the Joint Committee Powder Diffraction Standard card, showed a uni-modal particle size distribution with an average particle size between 0.02 μm to 2 μm , depending upon the concentration of the surfactant used.

[0045] The other inorganic ingredients such as monocalcium phosphate anhydrate, sodium phosphate, calcium hydroxide, calcium bicarbonate or sodium bicarbonate are used as commercially available. However, a vigorously milling by, but not limited to, ball miller, attrition miller, rotary miller, was used for reduction of the particles to a size ranging from 0.05-2 μm , or more preferably, 0.05-1 μm . In a preferred embodiment, the carbonate precursors were first dissolved into water, where a liquid phase precursor was used directly instead of solid-phase carbonate precursor. Liquid-phase carbonate precursors further ensure better chemical homogeneity during the synthesis of cHA. In addition, the nanometer-to-submicrometer particle size of the inorganic powders promotes phase transformation towards final apatitic structure, accompanied with hardening of the cHA. However, measurement of the hardening time (ISO1566) was found to increase with carbonate concentration, from 5-20 min for <10% carbonate, to as high as 120-150 min for $\geq 30\%$ carbonate. Therefore, the amount of carbonate can be used as practically needed, for the case of typical bone filler or bone substitutes, lower carbonate may be suggested, and since a rapid phase conversion and hardening may benefit subsequent physiological response and facilitate clinical practices.

[0046] In this invention, the starting inorganic precursors were mixed and granulated through a conventional techniques included, but not limited to, spray dry, freeze dry, drip casting, emulsion-solvent evaporation, and combination thereof, to form spheres of diameters ranging from 0.5 μm to 1,000 μm , or more preferably, ranging from 0.5 μm to 500 μm , or most preferably, ranging from 0.5 μm to 300 μm . Biodegradable or non-degradable polymeric materials included, but not limited to, polylactic acid, poly (DL-lactide-co-glycolide) polymer, polyglycolic acid, polyanhydrides, polyester, polyorthoester, polyamino acid, poly(alkyl cyanoacrylates), aspartate, polyethylene glycol, polyethylene oxide, poly(butenes terephthalate), polyacrylates, polymethacrylates, dextran, polysaccharides, polylactate, polyglycolates, hyaluronic acid, polyphosphazenes, polystyrene,

polyvinyls and derivatives, copolymers and mixture thereof, was employed as a organic counterpart of the microspheres. In addition to the biological advantage, the use of the biocompatible polymers provides several advantages included facilitation of specific granulation process such as the case of emulsification, improvement of the mechanical integrity of the green microspheres, prevention of the loss (for instance, leaching) of therapeutically active agents during a subsequent phase conversion process, and providing an alternative phase, other than the nano-structured inorganic phase, to encapsulate those active agents. Moreover, mechanical compatibility of the resulting composite with respect to the host tissue, particularly for the soft tissues, can be tailored to a certain extend by modifying its hardness, ductility, viscoelastic responses, to improve mechanical compliance between the implanted composites and surrounding issues.

[0047] Such a combination of the inorganic cHA phase and organic polymeric phase allows a widest spectrum of therapeutically active agents to be encapsulated directly, which include, but not limited to, anti-AIDS agents, anti-cancer drugs; hormones and hormone antagonists; heavy metals and heavy metal antagonists; cytostatic agents for cancer treatment, drugs for treatment of trauma or stroke; antacoids and anti-inflammatory drugs; chemotherapeutic agents for parasitic infections and microbial diseases, immunosuppressive agents; immunoactivators, vaccines, antibiotics; antiparasitics; antinauseants; relaxants; stimulants; anti-hypertensives; minerals and nutritional agents, anti-obesity drugs, anabolics, anti-asthmatics, anti-histamines, anti-hypertensives, anti-Parkinson agents, imaging agents, opioids, anti-parasites, anti-convulsants, muscle relaxants, and combinations thereof. Those above-mentioned therapeutically active agents or drugs include, but not only restrict to, alkylating agents and antimetabolites; nitrogen mustards, ethylenimines; alkylsulfonates; folic acid analogs; pyrimidine analogs; purine analogs; vinca alkaloids; antibiotics drugs such as phenylbutazone, indomethacin, naproxen, ibuprofen, flubiprofen, diclofenac, dexamethasone, prednisone and prednisolone; cerebral vasodilators such as soloctidilum, vincamine, naftidrofuryl oxalate, cordergocrine mesylate, cyclandelate, papaverine, nicotinic acid; anti-infective drugs such as erythromycin stearate and cephalixin; antacoids and anti-inflammatory drugs such as histamine, bradykinin, kallidin; immunosuppressive drugs such as FK506, chemotherapeutic agents for parasitic infections and microbial diseases; anticholinesterase agents, catecholamine and sympathomimetic drugs such as epinephrine, norepinephrine and dopamine, adrenergic agonists, transmitters such as GABA, glycine, glutamate, acetylcholine, dopamine, 5-hydroxytryptamine, and histamine; analgesic and anesthetics such as opioid analgesics and antagonists.

[0048] The proteins or enzymes that can be incorporated include, but not restricted to, active proteins, active fragments of DNA, RNA, and proteins. The protein or active fragments is a member of groups of enzymes or any active compounds including, but not restrict to, bone growth factors, amylase, catalase, cellulase, kinase, lipase, oxygenase, peptidase, phosphatase, cholesterol, trypsin, enzymes, enzyme inhibitors, chymotrysin, immunoglobulin, DNase, RNase, nuclease, dextranase, carboxylase, carboxypeptidase, and combination therefore.

[0049] Furthermore, composite structure of the microspheres also facilitates control of drug delivery in a desirable release pattern to meet clinical requirements. However, incorporation of the polymeric phase retards phase conversion of the green microspheres to cHA spheres, and the time to obtain phase-pure cHA is increased with polymer concentration, from, for instance, several hours to 20-30 h, when polymer is increased from <5% to >15% by weight. This is because the polymeric phase may be developed as a barrier separating the powder ingredients to a certain extend, inhibiting a direct interaction among the inorganic powder mixture necessary to form final apatitic phase. A diffusion effect among the inorganic powders, together with the liquid water or moisture that needs to trigger the reaction, may thus take place that further retards the phase conversion rate. Therefore, in this invention, the amount of the polymeric phase was selected ranging from 0.5% to 30%, or in a preferred embodiment, from 3% to 20% by weight was used for composite synthesis.

[0050] The use of liquid water or vapor water as a reacting medium to form final composites provides advantages over conventional formulation where either acidic phosphate or basic phosphate solution is necessary to trigger the reaction, and in the use of basic phosphate solution (for instance a solution of sodium phosphate or sodium hydrogen phosphate), a somewhat high concentration solution, for instance, equal to or greater than 0.2 M, is needed for optimization purpose. In this invention, vapor water, i.e., moisture, was used where the green spherical composites can be stored in an incubator with 80-100% humidity at a pre-set temperature below 60° C. The smaller diameter of the green spheres, the faster the phase conversion results, for instance, a few hours are observed to obtain final micro-spherical cHA-biopolymer products with diameters generally below 100 μ m.

[0051] This invention also provides a method to prepare green microspheres, which may be applied as bone filler, for instance, by direct compaction into a bone cavity, or through injection with syringes of different gauges of needles, depending upon the size of the green microspheres, as observed in conventional calcium phosphate cement systems. Upon such application, the green microspheres were mixed with water or phosphate-buffered solution to form a flowable or injectable paste. The compact microspheres allow setting in a reasonable time frame, depending on the concentration of carbonate and/or the amount of the polymeric phase added, as described above. One advantage of the compact structure of the microspheres is providing interstitial voids of controllable sizes, depending on the size of microspheres; the greater size of the microspheres, the larger the interstitial voids develops, permitting invasion of surrounding tissues, which is particularly important for earlier fixation of the implanted devices.

[0052] In this invention, the preparation of a colloidal suspension is important, the starting fine powders are dispersed in a liquid phase which has a ability to dissolve the polymeric materials of interest. For instance, methylene chloride or ethyl acetate was utilized for preparing a poly(DL-lactide-co-glicolide) polymer solution. Therefore, the liquid phases, which are and can be used in this invention included, but not limited to, methanol, ethanol, acetone, and those of water-insoluble or partially water-soluble such as methylene chloride, ethyl acetate, dioxane, hexane, toluene,

and combination thereof. Dry inorganic powders were dispersed in the polymer solution, via, but not limited to, ball miller, attrition miller, rotary miller, for 10-24 h. Spherorization of the colloidal suspension can be performed through, but not limited to, spray dry, freeze dry, emulsion-solvent evaporation, drip casting, and combination thereof, resulting in a final microspheres with a size ranging from 0.5 μm to 1,000 μm , or more preferably, ranging from 0.5 μm to 500 μm , or most preferably, ranging from 0.5 μm to 300 μm .

[0053] With the incorporation of therapeutically active agents, particularly for those hydrophilic molecules, included, but not restricted to, proteins, enzymes, where an intimate contact with non-aqueous solvents may cause denaturation, resulting in loss of biological or therapeutical activity, a water-in-oil emulsification method may be employed. Water-soluble agents can be first dissolved in an aqueous solution, following emulsification of the aqueous phase in water-insoluble or partially water-soluble solvents. Spherorization can subsequently be achieved via, for instance, spray dry or emulsion-solvent evaporation. However, once the emulsified phase was utilized, either with drugs or without drugs, the resulting green microspheres needs no further water addition (liquid water) for phase conversion purpose. The obtained water-containing green microspheres can be stored in the incubator readily for practical uses. However, for those lipophilic active agents, a direct addition to the solvent-based colloidal suspensions is feasible, following granulation to form green microspheres. The obtained green microspheres are stored directly into an incubator with relative humidity ranging from 80% to 100% for phase conversion process and are ready for use.

[0054] The therapeutically active agents were encapsulated into the composite structure, distributed in both secondary polymer phase and inorganic cHA phase. Microstructural investigation showed a spherical-like or mostly cauliflower-like grain morphology, where the grain size is about 10-1,000 nm in diameter over the entire concentration range of carbonate and polymer, which is essentially a nano-structured microspherical composite. Increase of the carbonate concentration causes an increase in the grain size, for instance, about 10-50 nm for carbonate concentration less than 10% by weight, whilst for carbonate concentration greater than 15%, grains coarsened and up to >600 nm was detected. However, polymer concentration showed little or no effect on the resulting cHA grain morphology, included size and shape. In this invention, a higher polymer concentration was recommended to use once a higher concentration of the carbonate concentration was used to synthesize cHA. Such combination in fact provides a better mechanical integrity for the final composites, because higher carbonate content weakens the strength of the final cHA, the use of higher polymer concentration can just implement the mechanical deficiency, which makes the resulting composites with sufficient mechanical property suitable for practical uses.

[0055] In the encapsulation of those therapeutically active agents, porosity as well as pore size of the resulting composites becomes critical, since it determines the ability of the encapsulation and most importantly, release behavior of those substances. Large pores can hardly effectively entrap the molecules, a subsequent release of the entrapped molecules by leaching, rather than by slow diffusion, is highly feasible. Instead, small pores allow the substances to be

physically entrapped in confined spaces where the molecular conformation can be preserved to a considerable extent [D M Liu and I W Chen, *Acta Materialia*, 47, 4535-4544, 1999, L M Ellerby et al. *Science*, 255, 1113, 1992, J M Miller, J. Non-Cryst. Solids, 202, 279, 1996] and the biological or therapeutic activity of the encapsulated substances after release may reach a desirable level. The BET pore analysis showed the pore size of the resulting microspheric composites having a pore size ranging from 1 nm to 1,000 nm, or in a preferred embodiment, ranging from 1 nm to 500 nm, which is rather suitable for encapsulating many biological or therapeutic substances. Such small pores offer conduits for the encapsulated substances to diffuse from the internal region of the microspheres towards surrounding environment. The resulting release pattern may then be dominated by either diffusion or matrix phase dissolution, or a combination of both. A release from the decomposition of the secondary polymeric phase can also contribute to the overall release behavior. Since the polymers used in this invention are preferably biodegradable, a subsequent resorption by, for instance, hydrolysis in physiological solution, degrades the polymer structure, where, in the mean time, the molecular substances that entrapped by the polymer may release to the environment. Therefore, the resulting release behavior from the microspheric composites is essentially controlled by diffusion and dissolution of both matrix cHA and secondary polymer phase.

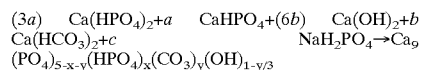
[0056] The specific surface area (SSA) of the resulting microspheres has a value ranging from 0.5 m^2/g to 70 m^2/g , or in a preferred embodiment, ranging from 10 to 55 m^2/g , depending on the size, porosity of the microspheres, and polymer concentration. Higher polymer concentration reduces the SSA by most probably forming a thin layer of barrier covering some of the nano-porosity, resulting in a lower value of specific surface area. The relative density of the resulting microspheres was measured by mercury porosimetry (AutoPore, model 9200), ranging from 40% to 95% of theoretical density of the composites. Higher polymer concentration gives rise to an increased relative density of the final microspherical composites. It should be noticed that this invention provides a method to produce microspheres with relative density that is generally higher than those disclosed in research papers or patents. What is more important is that a combination of pore size and porosity ensures a suitable encapsulation ability of the composite microspheres and particularly for a subsequent release control of those encapsulated biologically or therapeutically active substances.

EXAMPLE 1

Microsphere Synthesis

[0057] Colloidal suspensions containing monocalcium phosphate anhydrate or synthesized ultrafine dicalcium phosphate powder, sodium phosphate, calcium hydroxide, calcium bicarbonate, and 12% by weight of polylactic acid (PLA) were prepared via ball milling. The Ca/P ratio of the starting inorganic powder is fixed at 1.5. The resulting microspheres were developed via a simple spray dry or emulsification route, where the microspheres with a size ranging from 0.5 to 1,000 μm diameter, as shown in **FIG. 3**, where a phase-pure, poorly crystalline, calcium-deficient carbonated apatite (cHA)—polymer composite develops

successfully after 8 h exposure with moisture. Both the colloidal nature and the presence of polymers provide strong bond to retain mechanical and structural integrity of the green products during later handling. The inorganic component shows a nano-structured morphology, with a grain size well below 100 nm, which is essentially chemically and structurally similar to that of biological apatite. The polymer component, which is also biodegradable, is simulating that of organic content in the bone tissues in human and vertebrates. The pore analysis of the microspheric composites, as determined by the BET method, shows a pore size distribution ranging dominantly in the range of 1 nm to 200 nm. The mineralization process during synthesis under humid atmosphere, i.e., vapor water, may be grossly expressed as a result of interaction among the starting inorganic powder mixture as employed in this example,



[0058] where a has a value ranging from 0 to 1.6, and b has a value ranging from 0 to 6, and c, from 0.1 to 0.4. The carbonate ions can be replaced either OH or PO₄ groups, or both in the apatitic lattice, resulting in, as the case of present composition, an AB-type carbonated apatite.

EXAMPLE 2

In-Vitro Dissolution

[0059] Microspherical composites of varying amounts of carbonate concentration are prepared. The polymer content in the spheres is fixed at 5% by volume. One hundred and fifty mg of the microspheres were added into a 150 ml phosphate-buffered solution (PBS) at a pH value of 7. Diluted (0.1M) HCl solution was prepared for retaining a constant pH level of the PBS during microsphere dissolution. The volume of HCl that is used to maintain a constant solution pH during the test, at 37° C., can be used as an indication of the dissolution behavior. Accordingly, higher volume of the HCl consumed to maintain the constant pH level as a result of microsphere dissolution indicates a higher rate of dissolution, and may be applied as a corresponding in-vivo dissolution behavior. FIG. 4 shows the resulting curves, where an abrupt increase in the volume of HCl used at first few minutes of contact for all the compositions was observed, suggesting an abrupt change in chemical potential between the microspheres and surrounding liquid medium. Fast dissolution of the microspheres is required to reach a chemical equilibrium of the system. However, a continuous addition of HCl solution is needed in order to maintain the pH level for those microspheres with carbonate concentration greater than 10%. However, a lesser quantity of addition is observed for those ≤10% carbonate concentration, whereas a plateau is virtually reached after 40 min test, indicating a saturation between the microspheres and surrounding solution, where the dissolution rate of the microspheres becomes too small to be detectable. A lesser extent of the in-vitro dissolution kinetic was detected for the microspheres containing higher polymer concentration. This in-vitro test implies that the bioresorption behavior of the microspheres may be tailored in term of carbonate concentration and polymer concentration for different practical needs.

EXAMPLE 3

Consolidation of the Microspheres

[0060] Green microspherical composites (with 10% of potassium carbonate concentration) containing 20% by weight of polyethylene glycol (FCC grade, Union Carbide, USA) were prepared via an emulsification process. The green microspheres with approximately 0.5 g were compacted into a stainless steel die of 10 mm in diameter, following a uni-axial compression to 1 MPa. Thin pellets were developed and the relative density of the pellets is about 54-56%. The pellets were stored in an incubator with 100% relative humidity at 37° C. and showed soft feature when the needle (which is used for setting time measurement) was indented to the pellet, however, the pellets hardened after 20-30 minutes in the incubator.

EXAMPLE 4

Drug Release Study

[0061] Green microspheres containing 5% by weight fluorescein dye (sodium derivatives, JT Baker Chemicals, Co. USA) as model drug (an imaging agent) and 5% by weight of PLGA were prepared through spray dry. Pellets were prepared with the method described in Example 3. The pellets having a diameter of 10 mm and thickness of 0.5 mm, are subject to a release study by immersion into a phosphate-buffered saline (PBS at pH7.4) at constant weight (of the pellet)/volume (of the PBS) ratio of 0.5 mg/ml. Entire liquid samples were taken out and refilled with the same amount in a periodical manner. The concentration of the model drug in the supernatant was determined via a UV-Visible spectroscopy. The release kinetics is illustrated in FIG. 5 for the first 14 days; however, a sustain release over 2 months was detected. In addition, release behavior, for the first 14 days, was also measured for the pellets containing no polymer. A sustain release for a shorter time period, approximately 20 days, than the one with polymer was observed. The initial burst effect, which is detrimental to some medical application, can be reduced (or adjustable) to a considerable extent when the polymer phase was incorporated, which suggests to be a membrane effect that inhibits the initial fast release of the encapsulated model drug.

EXAMPLE 5

Drug Encapsulation and Release Study

[0062] Microspheres, prepared according to the procedure described in Example 1, containing 5% amethopterin (Sigma, USA) as model drug encapsulated were prepared via an emulsification process, where 10% polyethylene glycol was incorporated. The resulting microspheres are separated through a paper filter after vacuum evaporation, and stored in an incubator of 80-100% relative humidity at 40° C. for 16 hours. The amethopterin—contained microspheres of 50-200 um in diameter are collected and subject to drug release study with a procedure as described in Example 4. A sustain release with a behavior similar to the one (with polymer) in FIG. 5 over a time period of 2 weeks was detected.

EXAMPLE 6

Protein Encapsulation and Activity

[0063] Microspheres, prepared according to Example 1, containing 5% bovine serum albumin (BSA, Sigma, USA)

as a water-soluble model protein were prepared via an emulsification process. The microspheres contain 5% PLGA (85/15). After a 24-h release into phosphate-buffered saline at pH 7.4, the resulting supernatant was withdrawn and examined with UV-Visible spectroscopy at an absorbance peak of 220 nm. Little or no considerable difference in the UV-Visible spectra between the encapsulated-released BSA and those prepared in PBS was observed, suggesting conformational retention of BSA. This, according to the literature, further suggests sufficient retention of protein activity.

What I claim as my invention is:

1. A composite matrix consisted of a poorly-crystalline, calcium-deficient carbonated apatite (cHA) and biocompatible polymeric materials, and biologically or therapeutically active agents.

2. The composite of claim 1 wherein the cHA is prepared by thoroughly mixing a calcium phosphate system, sodium phosphate, calcium hydroxide, and carbonate precursors, in a mixture of non-aqueous liquid, to form a suspension.

3. The calcium phosphate system of claim 2 is monocalcium phosphate anhydrate.

4. The calcium phosphate system of claim 2 is a synthesized ultrafine dicalcium phosphate or more specifically, a surface-modified dicalcium phosphate powder.

5. The carbonate precursors of claim 2 are selected from a group of potassium bicarbonate and calcium bicarbonate.

6. The composite of claim 1 wherein the polymeric ingredients are selected from polylactic acid, polyglycolic acid, poly (DL-lactide-co-glycolide) copolymer, polyanhydrides, polyethylene glycol, polyethylene oxide, polyacrylates, polymethacrylates, dextran, polysaccharides, hyaluronic acid, and mixture thereof.

7. The amount of polymeric materials selected from claim 6 to form a composite entity of claim 1 is ranging from 0.5% to 30% by weight, or more preferably, ranging from 3% to 20% by weight.

8. The polymeric ingredients of claim 6 are mixed with the colloidal suspension prepared in claim 2 to form a polymer-powder suspension.

9. The polymer-powder suspension of claim 8 is prepared using ball miller and attrition miller.

10. The composites of claim 1 can be shaped into spherical geometry through a spherization procedure by spray drying, freeze drying, drip casting, emulsification evaporation, and combination thereof.

11. The microspheres of claim 10 have a size ranging from 0.1 μm to 1,000 μm in diameter.

12. The ultra-fine dicalcium phosphate of claim 4 was prepared by

- (a) dissolving calcium hydroxide into an aqueous solution containing small amount, 0.1%-10% by weight, of surfactants
- (b) preparing an aqueous phosphate solution from ammonia hydrogen phosphate or phosphoric acid.
- (c) mixing the aqueous phosphate solution and aqueous calcium solution via titration, whereas a white precipitate forms.
- (d) separating the precipitate via a cellulose paper filter
- (e) drying the precipitates at temperature of 150-180° C. for 2 h.

13. The dicalcium phosphate powder of claim 12 has a particle size of 20 nm-2,000 nm in size.

14. The surfactants of claim 12 are a mixture of citric acid and polyacrylic acid

15. The liquid phase of claim 2 is selected from water, methanol, ethanol, acetone, methylene chloride, ethyl acetate, ethyl vinyl ether, dichloromethane, acetaldehyde dimethyl acetal, dioxane, hexane, toluene, and preferably, a combination thereof.

16. The particle size of the starting inorganic powder mixture of claim 2 is ranging from 50 nm to 2,000 nm, or more preferably, ranging from 50 nm to 1,000 nm.

17. The amount of carbonate precursors used of claim 5 is ranging from 0.1% to 50% by weight, or more preferably, ranging from 3% to 40% by weight, or most preferably, ranging from 5% to 30% by weight.

18. The composites of claim 1 with various amounts of carbonate as in claim 17 allows dissolution behavior adjustable from weeks to months.

19. The cHA of claim 1 has an AB-type carbonated apatitic structure.

20. The microspheres prepared according to claim 10 can be transformed directly into apatitic phase under exposure of water or high moisture environment for a short time period from 4 to 16 h.

21. The composites of claim 1 have a poorly crystalline apatitic structure with X-ray diffraction of two broad peaks at 26° and 32° 2 θ over the range of 20°-40° 2 θ .

22. The microspheres of claim 20 have a porosity ranging from 5% to 70% by volume.

23. The microspheres of claim 20 have a specific surface area ranging from 0.5 m²/g to 70 m²/g.

24. The microspheres of claim 20 have a pore size ranging from 1 nm to 1,000 nm.

25. The microspheres of claim 20 have a grain size ranging from 10 nm to 1,000 nm.

26. The microspheres of claim 20 have a mole ratio of Ca/P ranging from 1.10 to 1.65, or more preferably, ranging from 1.20 to 1.55.

27. The microspheres of claim 10 can be further shaped into monoliths, pellets, tablets, bulks, thin or thick sheets, by simply uni-axial compression or other means.

28. Apatitic structure can be well developed in the composites according to claim 27 by mixing with small amount of water or exposure to moisture at a temperature below 60° C.

29. The microspheres of claim 10 form a deformable paste by mixing with water, ethylene glycol, or Hank's solution, which can be used for a variety of clinical situations.

30. The therapeutically or biologically active agents of claim 1 are antibiotics, anti-cancer drugs, peptides, bone growth factors, proteins, and DNA.

31. The therapeutically or biologically active agents of claim 30 are added into the suspension of claim 2 following a direct encapsulation process through spherization according to claim 10.

32. The biologically or therapeutically active agents of claim 30 can be physically encapsulated within the composites of claim 1 comprising an inorganic cHA phase and an organic polymeric phase.

33. The encapsulated biologically or therapeutically active agents of claim 30 can be released into a physiological solution over a time period from days to months.