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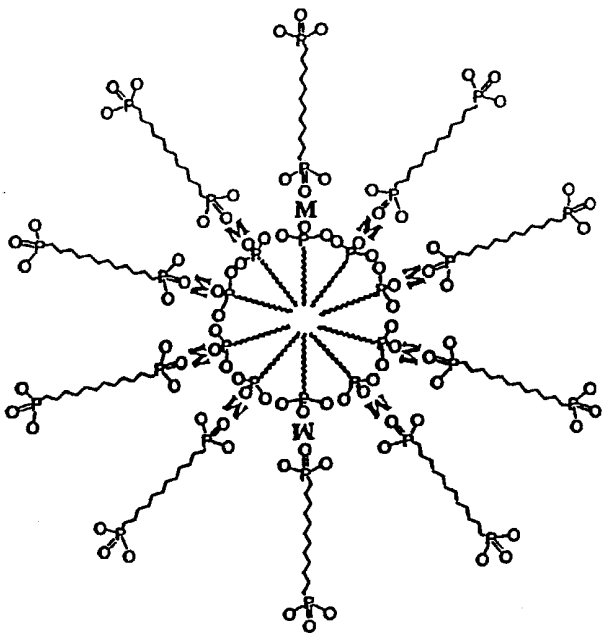

(57) Abstract: Metal encrusted micelles, optionally including targeting components are disclosed. These micelles may be used to advantage as drug delivery vehicles for the treatment of bone-related disorders.

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Complexes of Alkylphosphonic Acids

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FIELD OF THE INVENTION

This invention relates to the fields of multilayer thin film chemistry and delivery of therapeutic and diagnostic agents in vivo. Specifically, novel compositions are provided comprising supramolecular aggregates of alkylphosphonic acids which may be used to advantage as delivery vehicles for therapeutic and diagnostic agents useful in the treatment and/or diagnosis of a variety of pathological disorders.

BACKGROUND OF THE INVENTION

Several publications are referenced in this application in order to more fully describe the state of the art to which this invention pertains. The disclosure of each of these publications is incorporated by reference herein. A numerical listing of the referenced publications is provided at the end of this specification.

Rapid advances in the field of rational drug delivery have yielded a plethora of new and more efficient means for treating and diagnosing a wide range of pathological conditions, including various types of cancer (1-3). However, these therapeutic or diagnostic agents are often introduced into the body through the bloodstream, and only a small fraction of the active

agent actually reaches the affected area (1,4). The remaining quantities are taken up by other body tissues where they can have toxic side effects, or are scavenged from the bloodstream as waste. As a result, there is an increased need for strategic methods to target these agents directly to the disease site to increase their efficiency. Over the past two decades, methods for either incorporating therapeutic or diagnostic agents into vesicles (1, 4-6) or polymer micelles (6-9), or attaching them directly to molecular carrier structures that can serve as transporters have been investigated. Such carrier molecules can be specifically functionalized to target delivery of therapeutic or diagnostic agents to a specific organ or region of interest, thus reducing the amount of agent lost to other cells (10).

Micelles are formed when amphiphilic molecules are dissolved in water above some critical concentration (the critical micelle concentration, or CMC) (11-13). Amphiphilic molecules are molecules which have polar and nonpolar regions, such as a soap molecule which has a polar (usually charged) head group and a long hydrophobic hydrocarbon 'tail'. In an aqueous environment, the amphiphilic molecules self-aggregate to form structures in which the hydrophobic portions of the molecules are on the interior of the structure, and the hydrophilic polar head groups are exposed to the aqueous solution. When the amphiphiles form double-layer structures (akin to cell membranes), these structures are known as vesicles. Micelles are dynamic structures that are in equilibrium with the monomers from which they are composed.

Because of their dynamic nature, micelles have been previously studied as potential drug delivery systems (6, 7, 9). Most of this prior research has focused on

the use of block copolymers (which form micelles at low concentrations that are exceptionally stable) as vehicles for delivering anti-cancer drugs *in vivo* (7). These polymers are often composed of a hydrophobic chain of a specific length of a polyamino acid. A hydrophobic drug molecule can then be covalently linked to the amino acid backbone. Upon dialysis from an organic phase, the polymer chains quickly associate to form micelles to protect the hydrophobic amino acid-drug segment of the molecules. When the polymer-drug matrix is introduced *in vivo*, the drug can be cleaved from the amino acid backbone through hydrolysis and can then diffuse into the body.

Perhaps the biggest shortcoming of the systems described above is their inability to deliver the encapsulated drug to specific areas of the body. The use of antibodies and proteins as a means of targeting does not prevent the delivery vehicle from binding to receptor sites located in tissues other than those targeted by the antibody or protein. Also, reaction with serum proteins and other biocomponents could lead to the aggregation of the solubilized material within the bloodstream. As a result, such functionalization may lead to toxic side effects that limit the utility of these materials, especially for chemotherapeutic use.

SUMMARY OF THE INVENTION

In accordance with the present invention, a composition is provided which comprises a therapeutic or diagnostic agent, and and alkylphosphonic acids, with negatively charged acid head groups and hydrophilic tail groups, which undergo self-assembly in an aqueous environment to yield a supramolecular aggregate. The resulting aggregate has a non-polar core region comprising the hydrophobic tail groups and a polar

exterior region comprising the negatively charged acid head groups, to which biologically compatible metal ions are bound. Optionally, a targeting component may be bound to the metal ions on the exterior of the aggregate, the targeting component being effective to deliver the composition to a pathologic site *in vivo*. In a preferred embodiment, the targeting component is an α, ω-alkylbisphosphonic acid.

Futhermore, methods of making the compositions described above and of using the same in the treatment and diagnosis of various diseases are also within the scope of the present invention. Specifically, in accordance with the present invention, there is provided a method of making supramolecular aggregates comprising alkyl phosphonic acids, having negatively charged acid head groups and hydrophillic tail groups. The aggregates also have a polar exterior region comprising the negatively charged acid head groups and a non-polar quarter region comprising hydrophobic tail groups. A predetermined quantity of dodecanephosphonic acid is mixed with a sufficient amount of a biologically accetable buffer, the mixture is then heated and cooled thereby providing a mixture of aggregates of diverse size. The resulting mixture is then subjected to extrusion to produce a supramolecular aggregate of uniform size distribution.

The present invention also provides a method for the treatment of a pathological condition in a patient in need of said treatment comprising administering a therapeutic agent to said patient which is present in an amount having a therapeutic effect on the condition to be treated. Preferably the method is employed to treat a patient with cancer, e.g., bone cancer.

The present invention further provides a method for the diagnosis of pathological tissue in a patient in

need of said diagnosis. Specifically, the method comprises delivering a diagnostically effective amount of the composition of the invention to the pathological tissue site.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows an illustration of one embodiment of the drug delivery vehicle of the invention.

Figure 2 is a micrograph obtained by transmission electron microscopy (TEM) of a negatively stained image of n-dodecanephosphonic acid micelles.

Figures 3A-3C depict from left to right, TEM images of micelles after treating n-dodecanephosphonic acid micelles with $Fe(ClO_4)_3$, $Fe(NO_3)_3$, $FeCl_3$.

DETAILED DESCRIPTION OF THE INVENTION

A composition for delivering therapeutic and diagnostic agents *in vivo*, based on alkylphosphonic acid aggregates in the form of micelles or vesicles that have biologically compatible metal ions bound to the exterior thereof, is provided in accordance with the present invention. The metal component of the aggregate can be further functionalized with a targeting component to promote site-specific delivery of the therapeutic or diagnostic agent.

The supramolecular aggregate may comprise various alkylphosphonic acids having a carbon chain length of C_6 to C_{22},. In a preferred embodiment the carbon chain length is between C_8-C_{16}. These chemicals are commercially available from Oryza. Alternatively, they may be prepared according to the procedure of Schultz et al., (22). These long chain acids self-assemble into aggregate form when exposed to an aqueous environment

under conditions described by Schultz et al., supra. The biologically compatible metal ions are preferably selected from the group consisting of iron (III), cobalt (II), zinc (II), zirconium (IV), magnesium (II) and calcium (II). Of course other biologically compatible salts may be used, if desired. The metal ions are bound to the exterior of the above-described aggregate by direct injection of salt solution into the aggregate preparation, followed by dialysis of excess or unbound ions through dialysis tubing. Solutions were dialyzed for approximately one week.

In one embodiment of the invention, the therapeutic or diagnostic agent is hydrophobic and is incorporated in the non-polar core region of the aggregate.

In another embodiment, the hydrophobic tail group of at least one of the alkylphosphonic acids is modified to include a functional group, which is utilized to covalently bind the therapeutic or diagnostic agent to the aggregate. The covalently bound therapeutic or diagnostic agent may be either hydrophilic or hydrophobic. The chemical nature of the agent will dictate the nature of the functional group utilized for covalent binding thereof. For example, if the therapeutic agent is a compound having a free amine group ($-NH_2$), the alkylphosphonic acid may be modified to include a free carboxyl group (-COOH), preferably at the terminus of the hydrophobic tail, thereby allowing for covalent binding of the therapeutic agent to the aggregate via an amide bond formed between the amine group and the carboxyl group. Alternatively, the targeting component may be chemically modified in a similar manner allowing for binding of the therapeutic agent thereto.

In a particularly preferred embodiment of the invention, the composition further comprises a suitable

physiologically compatible buffer. Buffers suitable for this purpose are known in the art and include, but are not limited to, phosphate buffered saline (PBS), 3-[N-Morpholino]propanesulfonic acid (MOPS) and 3-[N-Morpholino]ethanesulfonic acid (MES). The compositions of the invention may be administered both systemically as well as injected directly at a disease site.

The composition of this invention can be prepared as briefly described above and characterized using a combination of TEM (24) and static light scattering measurements (27-29). By measuring the absolute intensity of the scattered light as a function of angle and concentration, the root mean square radius of gyration as well as the average molecular weight of the aggregate in solution can be determined using the following relationship (30-32):

$Kc / \Delta R_\theta = 1/ M_w P(\theta) + 2 A_2 c + \ldots$

where: K is an optical constant = $4\pi^2 n^2 / \lambda^4 N_A . (dn/dc)^2$

n = refractive index of solvent

c = concentration

N_A = Avogadro's number

λ = wavelength of incident light source in vacuo

c = concentration of scattering species

ΔR_θ = excess Rayliegh intensity

M_w = average molecular weight of the sample

$P(\theta)$ = shape factor

$A_2 c$ = second virial coefficient

The plot of $\Delta R_\theta / Kc$ versus $\sin^2(\theta)$ yields the root mean square of the radius of gyration which can be used to estimate the shape of the molecule in solution. One

important part of this equation is the change in refractive index with concentration, or (dn/dc). This value allows for the calculation of the molecular weight of the material which can then be converted to an average aggregate number. This technique allows monitoring of the growth and the physical changes of the aggregates in solution without altering their local environment. One drawback of light scattering is that it is an averaging technique and therefore is affected by the polydispersity of the sample. Nevertheless the average size, shape and molecular weight information thus obtained is extremely useful for further characterization of this system and for following changes associated with metallation or drug incorporation or release.

An alternative technique for assessing vesicle size distribution in these solutions is dynamic light scattering (31). These more sophisticated measurements rely on analysis of the scattering in terms of real-time diffusion of species in solution. For example, use of a Protein Solutions Dyna-Pro 99 Dynamic Light Scattering apparatus and the associated Dynals software allows direct determination of the hydrodynamic radius distribution of aggregate in solution.

A model aggregate has been prepared with a variety of iron (III) salts bound to the exterior thereof, as exemplified below. The salts used in this embodiment include $FeCl_3$, $Fe(NO_3)_3 \cdot 9H_2O$, $Fe(ClO_4)_3 \cdot H_2O$, and tris(2,2'-bipyridine)iron(III) hexafluorophosphate. The last-mentioned metal complex was chosen to cap the exterior of the aggregates to prevent further aggregation and to further elucidate the supramolecular structure of the aggregates. Other complexes using a range of biologically acceptable inorganic salts, e.g., Co(II) and Zn(II) metals can be similarly prepared. Model

aggregates fall within the range of 4-500 nm.

There are two principal means of incorporating therapeutic or diagnostic agents into the aggregates. The first involves incorporating a hydrophobic agent into the buried hydrophobic regions of the aggregate. This provides the most direct and general (i.e. a host of hydrophobic drugs, e.g., paclitaxel, could be used) way to incorporate therapeutic or diagnostic substances into the aggregate delivery system. To determine the effectiveness of this approach for a particular agent, measurements of the uptake and release rates of both the 'bare' aggregate and the metallated form containing such agents may be performed. Both encapsulation and release can be assessed using methodology well known in the art. For example, the extent to which a drug component enters the unmetallated and metallated versions of the aggregate can be assessed by introducing a candidate fluorescent, hydrophobic drug compound into aggregate solutions, and monitoring the uptake using fluorescence, UV-Vis and NMR (8,9). Release can be determined by assembling the aggregates in the presence of the drug prior to metallation so that the drug is fully encapsulated. Both the uptake and release characteristics of these compositions in solution can be measured spectroscopically through the use of UV-Vis and fluorescence experiments (8,9). For example, the ability of the aggregate to release a drug incorporated therein can be monitored by following shifts and changes in the intensity of the candidate drug molecule. Correlation of these results with the intensity of the UV-Vis absorption peak for the candidate drug in solution will allow monitoring of the release of the drug over time in order to calculate the release rate. Nuclear magnetic resonance experiments and static light scattering (to measure changes in size and molecular

weight of the aggregates) may also provide insight into the release characteristics of these materials (7).

In order to enhance release from these metallated materials, optionally, one may add small amounts of impurities, such as another surfactant, e.g., one with a sulfonate head group, which is unable to bind metal ions. This would reduce the number of sites available for metal binding on the exterior of the aggregate and should increase the fluidity of the outer shell and promote release. Suitable surfactants for this purpose are described in Liposomes: A Practical Approach, IRL Press, Oxford, 1997

The second principal means of incorporating a drug into the aggregates involves the use of a modified monomer phosphonic acid in which the drug is covalently attached to the tail of the amphiphile. This second approach has been used successfully to attach the hydrophobic anti-cancer drug adriamyacin (ADR) to the interior of several block copolymer micelles (8). For example, the tail of the hydrophobic portion of the alkylphosphonic acid monomer may be converted to a carboxylic acid, which allows for the formation of an amide linkage with the amine on ADR. Upon the breakdown of this linkage, the drug is released from the hydrophobic interior and can diffuse from the aggregate to enter the body. This monomer can be used in various ratios with unmodified phosphonic acid monomers to prepare aggregates.

The diagnostic agents that may be used in the practice of this invention include those having utility in imaging. Suitable therapeutic agents are those capable of acting on a cell, organ or organism to create a change in the functioning of the cell, organ or organism, including but not limited to pharmaceutical agents or drugs. Such agents include a wide variety of

substances that are used in therapy, immunization or otherwise are applied to combat human and animal disease. Such agents include but are not limited to analgesic agents, anti-inflamatory agents, antibacterial agents, antiviral agents, antifungal agents, antiparasitic agents, tumoricidal or anti-cancer agents, proteins, toxins, enzymes, hormones, neurotransmitters, glycoproteins, immunoglobulins, immunomodulators, dyes, radiolabels, radio-opaque compounds, fluorescent compounds, polysaccharides, cell receptor binding molecules, anti-inflammatories, anti-glaucomic agents, mydriatic compounds and local anesthetics.

Exemplary non-steroidal anti-inflamatories include, but are not limited to, indomethacin, salicylic acid acetate, ibuprofen, sulindac, piroxicam, and naproxen, antiglaucomic agents such as timolol or pilocarpine, neurotransmitters such as acetylcholine, anesthetics such as dibucaine, neuroleptics such as the phenothiazines (e.g., compazine, thorazine, promazine, chlorpromazine, acepromazine, aminopromazine, perazine, prochlorperazine, trifluoperazine, and thioproperazine), rauwolfia alkaloids (e.g., resperine and deserpine), thioxanthenes (e.g., chlorprothixene and tiotixene), butyrophenones (e.g., haloperidol, moperone, trifluoperidol, timiperone, and droperidol), diphenylbutylpiperidines (e.g., pimozde), and benzamides (e.g., sulpiride and tiapride); tranquilizers such as glycerol derivatives (e.g., mephenesin and methocarbamol), propanediols (e.g., meprobamate), diphenylmethane derivatives (e.g., orphenadrine, benzotrapine, and hydroxyzine), and benzodiazepines (e.g., chlordiazepoxide and diazepam); hypnotics (e.g., zolpdem and butoctamide); beta-blockers (e.g., propranolol, acebutonol, metoprolol, and pindolol); antidepressants such as dibenzazepines (e.g.,

imipramine), dibenzocycloheptenes (e.g., amtiriptyline), and the tetracyclics (e.g., mianserine); MAO inhibitors (e.g., phenelzine, iproniazid, and selegeline); psychostimulants such as phenylehtylamine derivatives (e.g., amphetamines, dexamphetamines, fenproporex, phentermine, amfeprramone, and pemoline) and dimethylaminoethanols (e.g., clofenciclan, cyprodenate, aminorex, and mazindol); GABA-mimetics (e.g., progabide); alkaloids (e.g., codergocrine, dihydroergocristine, and vincamine); anti-Parkinsonism agents (e.g., L-dopamine and selegeline); agents utilized in the treatment of Altzheimer's disease, cholinergics (e.g., citicoline and physostigmine); vasodilators (e.g., pentoxifyline); and cerebro active agents (e.g., pyritinol and meclofenoxate).

Anti-neoplastic agents can also be used advantageously as biological agents in the compositions of the invention. Representative examples include, but are not limited to paclitaxel, daunorubicin, doxorubicin, carminomycin, 4'-epiadriamycin, 4-demethoxy-daunomycin, 11-deoxydaunorubicin, 13-deoxydaunorubicin, adriamycin-14-benzoate, adriamycin-14-actanoate, adriamycin-14-naphthaleneacetate, vinblastine, vincristine, mitomycin C, N-methyl mitomycin C, bleomycin A_2, dideazatetrahydrofolic acid, aminopterin, methotrexate, cholchicine and cisplatin. Representative antibacterial agents are the aminoglycosides including gentamicin. Representative antiviral compounds are rifampicin, 3'-azido-3'-deoxythymidine (AZT), and acylovir. Representative antifungal agents are the azoles, including fluconazole, macrolides such as amphotericin B, and candicidin. Representative anti-parastic compounds are the antimonials. Suitable biological agents also include, without limitation vinca

alkaloids, such as vincristine and vinblastine, mitomycin-type antibiotics, such as mitomycin C and N-methyl mitomycin, bleomycin-type antibiotics such as bleomycin A_2, antifolates such as methotrexate, aminopterin, and dideaza-tetrahydrofolic acid, taxanes, anthracycline antibiotics and others.

The compositions of this invention also can utilize a variety of polypeptides, such as antibodies, toxins, such as diphtheria toxin, peptide hormones, such as colony stimulating factor, and tumor necrosis factors, neuropeptides, growth hormone, erythropoietin, and thyroid hormone, lipoproteins such as μ-lipoprotein, proteoglycans such as hyaluronic acid, glycoproteins such as gonadotropin hormone, immunomodulators or cytokines such as the interferons or interleukins, as well as hormone receptors such as the estrogen receptor.

The compositions also can comprise enzyme inhibiting agents such as reverse transcriptase inhibitors, protease inhibitors, angiotensin converting enzymes, 5μ-reductase, and the like. Typical of these agents are peptide and nonpeptide structures such as finasteride, quinapril, ramipril, lisinopril, saquinavir, ritonavir, indinavir, nelfinavir, zidovudine, zalcitabine, allophenylnorstatine, kynostatin, delaviridine, *bis*-tetrahydrofuran ligands (see, for example Ghosh et al., *J. Med. Chem.* 1996, 39: 3278), and didanosine. Such agents can be adminitered alone or in combination therapy; e.g., a combination therapy utilizing saquinavir, zalcitabine, and didanosine, zalcitabine, and zidovudine. See, for example, Collier et al., *Antiviral Res.* 1996, 29: 99.

The compositions described herein may also comprise nucleotides, such as thymine, nucleic acids, such as DNA or RNA, or synthetic oligonucleotides, which may be derivatized by covalently modifying the 5' or the

3' end of the polynucleic acid molecule with hydrophobic substituents to facilitate entry into cells (see for example, Kabanov et al., *FEBS Lett.* 1990, 259, 327; Kabanov and Alakhov, *J. Contr. Rel.* 1990, 28: 15). Additionally, the phosphate backbone of the polynucleotides may be modified to remove the negative charge (see, for example, Agris et al., *Biochemistry* 1968, 25:6268, Cazenave and Helene in *Antisense Nucleic Acids and Proteins: Fundamentals and Applications*, Mol and Van der Krol, Eds., p. 47 et seq., Marcel Dekker, New York, 1991), or the purine or pyrimidine bases may been modified, for example, to incorporate photo-induced crosslinking groups, alkylating groups, organometallic groups, intercalating groups, biotin, fluorescent and radioactive groups (see, for example, *Antisense Nucleic Acids and Proteins: Fundamentals and Applications*, Mol and Van der Krol, Eds., p. 47 et seq., Marcel Dekker, New York, 1991; Milligan et al., In *Gene Therapy for Neoplastic Diseases*, Huber and Laso, Eds. P. 228 et seq., New York Academy of Sciences, New York, 1994). Such nucleic acid molecules can be, among other things, antisense nucleic acid molecules, phosphodiester, oligonucleotide α-anomers, ethylphospotriester analogs, phosphorothioates, phosphorodithioates, phosphoroethyletriesters, methylphosphonates, and the like (see, e.g., Crooke, *Anti-Cancer Drug Design* 1991, 6: 609; De Mesmaeker et al, *Acc. Chem. Res.* 1995, 28: 366).

The compositions of the invention may also include antigene, ribozyme and aptamer nucleic acid drugs (see, for example, Stull and Szoka, *Pharm. Res.* 1995, 12: 465).

Other suitable biologically active agents include oxygen transporters (e.g. porphines, porphirines and

their complexes with metal ions), coenzymes and vitamins (e.g. NAD/NADH, vitamins B12, chlorophylls), and the like.

Suitable biologically active agents further include those used in diagnostic visualization methods, such as magnetic resonance imaging (e.g., gadolinium (III) diethylenetriamine pentaacetic acid), and may be a chelating group (e.g., diethylenetriamine pentaacetic acid, triethylenetriamine pentaacetic acid, ethylenediamine-tetraacetic acid, 1,2-diaminocyclo-hexane-N,N,N',N'-tetraaceticacid, N,N'-di(2-hydroxybenzyl) ethylene diamine), N-(2-hydroxyethyl) ethylene diamine triacetic acid and the like). Such agents may further include an alpha-, beta-, or gamma-emitting radionuclide (e.g., galliun 67, indium 111, technetium 99). Iodine-containing radiopaque molecules are also suitable diagnostic agents. The diagnostic agent may also include a paramagnetic or superparamagnetic element, or combination of paramagnetic element and radionuclide. The paramagnetic elements include but are not limited to gadolinium (III), dysporsium (III), holmium (III), europium (III) iron (III) or manganese (II).

The composition may further include a targeting group including but not limited to antibody, fragment of an antibody, protein ligand, polysaccharide, polynucleotide, polypeptide, low molecular mass organic molecule and the like. Such targeting group can be linked covalently to the surfactant, or can be non-covalently incorporated in the compositions, e.g., through hydrophobic, electrostatic interactions or hydrogen bonds.

In a preferred embodiment of the invention, a monolayer of α, ω bisphosphonic acid, such as decylbisphosphonic acid, geminal bisphosphonic acid (the

latter being characterized by a P-C-P bond) or tetrakisphosphonic acid (the latter containing two P-C-P bisphosphonate moieties connected by a carbon chain), is added to the metal-bound aggregate to serve as a targeting agent and complete the delivery system. Because DBPA is soluble in water, the addition of this monolayer to the aggregates can be carried out at room temperature (i.e. similar conditions to those used for preparing self-assembled multilayers with DBPA)(20). Size and molecular weight of the resulting delivery vehicle may be determined by static and dynamic light scattering (as previously described) and TEM acquired by negatively staining the samples. Determination of drug release rates from the resulting aggregates may be determined as described above, e.g., by UV-Visible spectroscopy, fluorescence and NMR techniques. Due to the large negatively charged acid head groups on the exterior of the resulting delivery vehicle system, it should have a long circulation time within the bloodstream and not be taken up by other organs in the body (16,17).

The compositions of the present invention allow diverse routes of administration, including but not limited to parenteral (such as intramuscular, subcutaneous, intraperitoneal, and intraveneous), oral, otic, topical, vaginal, pulmonary, and ocular. These compositions can take the form of aqueous solutions, suspensions, micelles, vesicles, emulsions and microemulsions.

Conventional pharmaceutical formulations may be employed. When aqueous suspensions are required for oral use, the composition can be combined with emulsifying and suspending agents. For parenteral administration, sterile solutions of the composition are usually prepared, and the pH of the solutions are suitably

adjusted and buffered. For intravenous use, the total concentration of solutes should be controlled to render the preparation isotonic. For ocular administration, ointments or droppable liquids may be delivered by well-known ocular delivery systems such as applicators or eyedroppers. Such compositions can include mucomimetics such as hyaluronic acid, chondroitin sulfate, hydroxypropyl methylcellulose or polyvinyl alcohol, preservatives such as sorbic acid, EDTA or benzylchronium chloride, and the usual quantities of diluents and/or carriers. For pulmonary administration, diluents and/or carriers will be selected to be appropriate to allow for formation of an aerosol.

The invention will be described hereinbelow with particular emphasis on the formulation of a chemotherapeutic composition, in accordance with the teachings herein, for the treatment of bone cancer. It should be understood, however, that the present invention has a substantially broader range of applications as indicated above.

It is known that phosphonic acid moieties bind specifically to bone tissue. For about the past two decades geminal bisphosphonic acids have been increasingly studied for use as therapeutic agents for osteoporosis (16,17). Osteoporosis is a condition in which the natural process of bone breakdown by osteoclastic cells (resorption) is dramatically increased without subsequent reformation of new tissue (16). Rapid breakdown of the bone matrix dramatically increases the amount of exposed Ca^{++} at the bone surface, and the strongly binding phosphonic acids are readily targeted to the affected area. Although the exact mechanism is still unknown, it is believed that the negatively charged acid headgroups bind tightly to exposed calcium ions thus blocking osteoclastic activity

(15,17). This binding "locks" the calcium in place strengthening the weakened tissue, while providing a protective coat over the surface to prevent further resorption (15,17). The disclosed compositions of the invention are, therefore, unique in that they enable targeted delivery of an encapsulated therapeutic or diagnostic agent to one specific site.

When a cancer cell metastasizes to the bone, it also triggers the release of chemical stimulators which increase bone breakdown (15). Using the knowledge that phosphonic acids will specifically target areas where resorption is occurring rapidly, a bisphosphonic acid is used to target drug delivery to areas where cancer has metastasized. This approach relies on the ability to bind an α,ω alkylbisphosphonic acid to the metallated aggregate described herein due to the strong binding affinity of the acid moiety for the metal ions which are then dispersed on the surface of the aggregate. The resulting functionalized aggregate has free acids extended out from the main vehicle assembly which serve to target drug delivery.

Bisphosphonic acids and their use in self-assembled multilayer thin films with divalent (18) and tetravalent transition metals (18-20) have been assessed. In these studies, the strong binding ability of the phosphonic acids to the metal ions provides stability and directional order within the layered films (20). Accordingly, α,ω alkylbisphosphonic acid should also bind strongly to the metal ions bound to the aggregate exterior.

The following examples are provided to illustrate certain embodiments of the present invention. These examples set forth the best mode presently contemplated for carrying out the invention described herein; they are not intended to limit the invention in

any way.

EXAMPLE I

A model aggregate was prepared using *n*-dodecanephosphonic acid. Specifically, we use (on average) a 3 x 10^{-4} molar solution of n-dodecanephosphonic acid prepared by adding 17 mg dodecanephosphonic acid to 250 mL of a 5 mM solution of morpholinoethanesulfonic acid (MES) buffer solution. This solution is heated in a boiling water bath for 40-60 minutes. The solution is then cooled and allowed to sit undisturbed for 3 days (following Shulz' procedure (21)). We have used this preparation to synthesize the desired micelles and have independently confirmed the reported critical micelle concentration (CMC) through measurement of the surface tension by means of a Wilhelmy Plate Balance (22). We have found that the aggregates formed through this preparation are sperical in shape with an average diameter of 20-120 nm as visualized through TEM. See Figure 2. It has also been determined that these aggregates are stable over the pH range of 4-6, with minimal structural changes.

The choice of biologically compatible metal ion used to bind to the micelle exterior directly influences the size and the shape of the resulting aggregates. Iron (III) salts are preferred because they are relatively inexpensive, readily available in several simple forms and known to be biologically compatible. However, other metal ions may be used if desired, including, without limitation, Zr (IV), Mg(II), Ca(II), Co(II) and Zn(II) salts. The TEM images in Figure 3 represent three aggregate samples from the same starting micelle solution that have been metallated with different iron salt sources; Fig. 3A:$Fe(ClO_4)_3$; Fig. 3B:$Fe(NO_3)_3$; Fig. 3C:$FeCl_3$.

The aggregates in each of these samples are similar, ranging in size from 25 nm to 275 nm in diameter with an average size of 110 nm. Aggregates of a relatively uniform size distribution can be obtained by multiple extrusions through a Nuclepore filter® (Whatman) prior to metallation. Specifically this entails multiple extrusions through a membrane filter to yeild aggregates that are more monodisperse in size, i.e., on the order of 25-75 nm.

The diversity of sizes of the metallated aggregates and the change in shape relative to the metal-free aggregates can only be explained if some type of reorganization is occurring upon addition of the metal. For the large spheres observed upon metallation with $Fe(ClO_4)_3$, the size of these aggregates indicates reorganization to form larger vesicular species. This type of reorganization phenomenon has been previously reported in vesicle systems with the addition of group II elements such as magnesium and calcium (23,24). In these systems it was found that the addition of metal ions to the exterior of the vesicle negated the surface charge of the exposed headgroups. This in turn allowed the aggregates to approach each other closely and fuse together to form larger more stable "super vesicles" (23,24).

EXAMPLE II

Bisphosphonate-Coated Aggregate Binding to Bone

Two types of experiments can be performed to confirm that the bisphosphonate-containing aggregates of this invention bind to bone tissue. In the first experiment, a silicon wafer functionalized with a zirconium-bisphosphonate monolayer is exposed to a suspension of micron-sized calcium hydroxyapatite (bone) particles. Following exposure, the sample is rinsed

thoroughly to remove any particles not bound to the surface. The thickness of the film is measured before and after the addition of particles using ellipsometry. An observed increase in thickness of the order of the hydroxyapatite particle size confirms binding of the hydroxyapatite to a surface of densely packed phosphonate moieties.

Alternatively, binding affinity between bone and the bisphosphonate-containing aggregates can be demonstrated by exposing a thin section of bone tissue to a solution of the functionalized aggregates. After washing the sample vigourously, the tissue is embedded in resin and thinly sectioned perpendicular to the tissue surface. The resulting sections are stained and visualized by TEM to confirm that the aggregates had bound to the surface. The surface of the aggregate-coated tissue can also be visualized by SEM to confirm binding.

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While certain preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present invention, as set forth in the following claims.

What is claimed is:

1. A composition which comprises a therapeutic or diagnostic agent and a supramolecular aggregates comprising alkylphosphonic acids, having negatively charged acid head groups and hydrophilic tail groups, said aggregates having a polar exterior region comprising said negatively charged acid head groups, a non-polar core region comprising said hydrophobic tail groups, and biologically compatible metal ions bound to said polar exterior region, and, optionally, a targeting component bound to said metal ions, said targeting component being effective to deliver said composition to a pathologic site *in vivo*.

2. A composition as claimed in claim 1 in the form of a micelle.

3. A composition as claimed in claim 1 in the form of a vesicle.

4. A composition as claimed in claim 1, which includes said targeting component, said targeting component being an α, ω-alkylbisphosphonic acid.

5. A composition as claimed in claim 1, which includes a therapeutic agent.

6. A composition as claimed in claim 5, wherein said therapeutic agent is hydrophobic and is incorporated in the non-polar core region of said aggregate.

7. A composition as claimed in claim 5, wherein said supramolecular aggregate comprises n-dodecanephosphonic acid, said biologically compatible metal ions are selected from the group consisting of iron (III), Zr(IV), Mg(II), Ca(II), cobalt (II) and zinc (II), and said therapeutic agent is adriamycin.

8. A composition as claimed in claim 7, which further includes said targeting component, said targeting component being selected from the group consisting of α,ω bisphosphonic acid and a geminal bisphosphonic acid.

9. A composition as claimed in claim 5, wherein the hydrophobic tail group of at least one of said alkylphosphonic acids is modified to include a functional group, and said therapeutic agent is covalently bound to said aggregate through said functional group.

10. A composition as claimed in claim 9, wherein said therapeutic agent is hydrophobic.

11. A composition as claimed in claim 10, wherein said therapeutic agent is a compound having a free amine group ($-NH_2$), said at least one alkylphosphonic acid is modified to include a free carboxyl group (-COOH) and said therapeutic agent is covalently bound to said aggregate by an amide bond formed between said amine group and said carboxyl group.

12. A composition as claimed in claim 1, wherein said aggregates further include a surfactant which does not bind to metal ions.

13. A composition as claimed in claim 12, wherein said surfactant has a sulfonate head group.

14. A drug delivery composition which comprises multiple micelles comprising the composition of claim 5, in a physiologically compatible carrier medium.

15. A drug delivery composition which comprises multiple vesicles comprising the composition of claim 5 in a physiologically compatible carrier medium.

16. A composition as claimed in claim 1, which includes a diagnostic agent.

17. A composition as claimed in claim 1, wherein said aggregates are of uniform size.

18. The method of making supramolecular aggregates comprising alkyl phosphonic acids, having negatively charged acid head groups and hydrophillic tail groups, said aggregates having a polar exterior region comprising said negatively charged acid head groups and a non-polar quarter region comprising said hydrophobic tail groups, said method comprising

a) mixing a predetermined quantity of dodecanephosphonic acid with a sufficient amount of a biologically accetable buffer, to form a mixture, heating said mixture said mixture to boiling, and cooling said mixture, thereby providing a mixture of aggregates of diverse size; and

b) subjecting the resulting mixture to extrusion to produce a supramolecular aggregate of uniform size distribution.

19. The method of claim 18, wherein said mixture is subjected to repeated extrusions.

20. A method for treatment of a pathological condition in a patient in need of said treatment, said method comprising administering to said patient a composition according to claim 5, wherein the therapeutic agent is present in an amount having a therapeutic effect on the condition to be treated.

21. The method of claim 20, wherein the composition comprises a targeting component which is effective to deliver said composition to the site of said pathological condition.

22. The method of claim 20, wherein said therapeutic agent is selected from the group consisting of analgesic agents, anti-inflamatory agents, antibacterial agents, antiviral agents, antifungal agents, antiparasitic agents, tumoricidal or anti-cancer agents, proteins, toxins, enzymes, hormones, neurotransmitters, glycoproteins, immunoglobulins, immunomodulators, dyes, radiolabels, radio-opaque compounds, fluorescent compounds, polysaccharides, cell receptor binding molecules, anti-inflammatories, anti-glaucomic agents, mydriatic compounds and local anesthetics.

23. A method of treating cancer in a patient in need of said treatment, said method comprising administering to said patient a therapeutically effective amount of the composition of claim 5, wherein said therapeutic agent is an anti-cancer drug.

24. The method of claim 23, wherein said anti-cancer drug is hydrophobic.

25. The method of claim 23, wherein said anti-cancer drug is selected from the group consisting of paclitaxel, daunorubicin, doxorubicin, carminomycin, 4'-epiadriamycin, 4-demethoxy-daunomycin, 11-deoxydaunorubicin, 13-deoxydaunorubicin, adriamycin-14-benzoate, adriamycin-14-actanoate, adriamycin-14-naphthaleneacetate, vinblastine, vincristine, mitomycin C, N-methyl mitomycin C, bleomycin A_2, dideazatetrahydrofolic acid, aminopterin, methotrexate, cholchicine and cisplatin.

26. The method of claim 23, wherein said composition includes a targeting component which is effective to deliver said composition to the site of said cancer.

27. The method of claim 26, wherein said targeting component is an α,ω-alkylbisphosphonic acid.

28. The method of claim 27, wherein said cancer is bone cancer.

29. A method for the diagnosis of pathological tissue in a patient in need of said diagnosis, said method comprising delivering to the site of said pathological tissue a composition of claim 1 comprising an effective amount of a diagnostic agent.

30. The method claim 29, wherein said composition includes a targeting component which is effective to deliver said composition to the site of said pathologic tissue.

31. The method of claim 29, wherein said diagnostic agent is an imaging agent.

32. The method of claim 29, wherein said diagnostic agent is selected from the group consisting of (i) a chelating agent comprising a gamma-emitting radionuclide, a paramagnetic element or a superparamagnetic element or (ii) an iodine-containing radiopaque molecule.

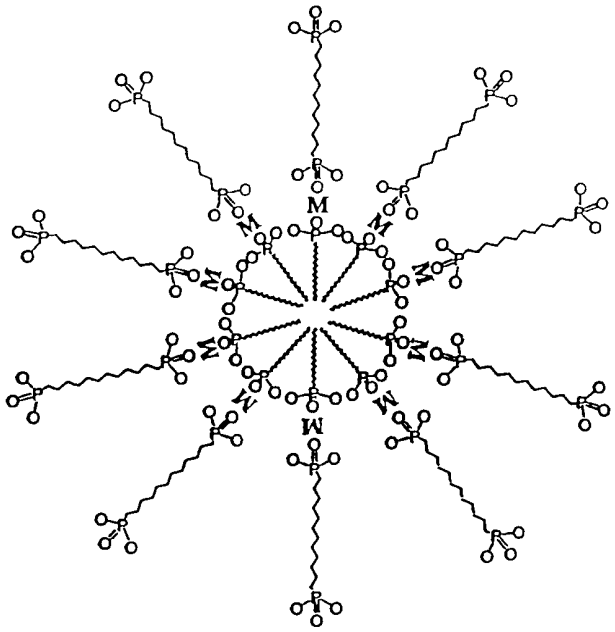


Figure 1

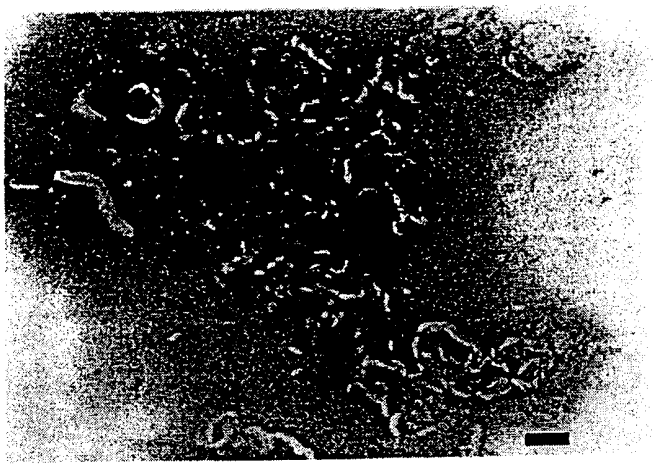


Figure 2

Fig. 3A

Fig. 3B

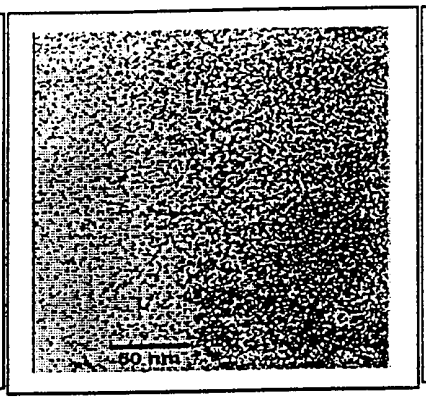
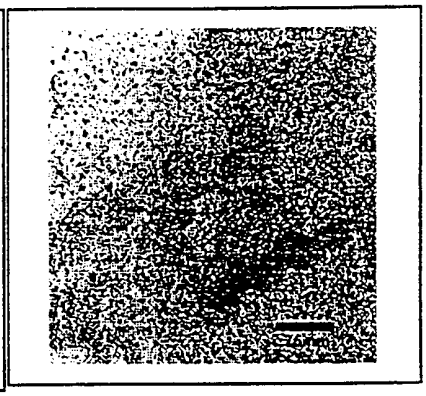


Fig. 3C



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/32057

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 51/00; A61B 5/055; A61K 9/127; A61K 9/14

US CL :424/1.29, 9.32, 9.4, 450, 489

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/1.29, 9.32, 9.4, 450, 489

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAPLUS, MEDLINE, EMBASE

search terms: phosphonic, vesicles, micelles, alkylphosphonic, bisphosphonic, diagnostic, contrast, therapeutic, metal.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,183,815 A (SAARI ET AL.) 02 February 1993, see columns 1-2.	1-32
Y	US 5,958,901 A (DWYER ET AL.) 28 September 1999, see columns 11-12 and column 13, lines 33-46.	1-32
Y	WALDE PETER. Preparation and Characterization of Vesicles from Mono-n-alkyl Phosphates and Phosphonates, J. Phys. Chem., vol. 101, 1997, pp. 7390-7397.	1-32
Y	MOSS ROBERT A. Metal Cation Micelle Mediated Hydrolysis of Phosphonic Acid Esters, Langmuir,Vol.15, 1999, pp. 107-110.	1-32

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

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