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(54) **Title**: ADHESIVE COMPLEX COACERVATES AND METHODS OF MAKING AND USING THEREOF

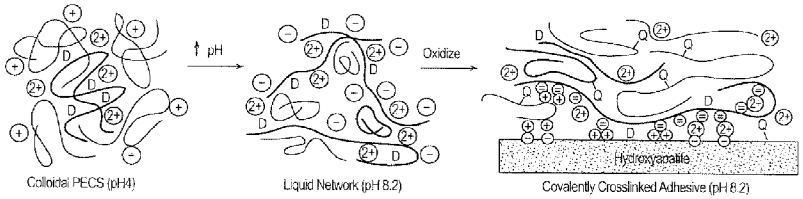

FIG. 1A FIG. 1B FIG. 1C

(57) **Abstract**: Adhesive complex coacervates are composed of a mixture of one or more polycations and one or more polyanions. The polycations andpolyanions in the adhesive complex coacervate are crosslinked with one another bycovalent bonds upon curing. The adhesive complex coacervates have several desirable features when compared to conventional bioadhesives, which are effective in water-based applications. The adhesive complex coacervates described herein exhibit good interfacial tension in water when applied to a substrate (i.e., they spread over the interface rather than being beaded up). Additionally, the ability of the complex coacervate to crosslink intermolecularly increases the cohesive strength of the adhesive complex coacervate. The adhesive complex coacervates have numerous biological applications as bioadhesives and drug delivery devices. In particular, the adhesive complex coacervates described herein are particularly useful in underwater applications and situations where water is present such as, for example, physiological conditions.

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ADHESIVE COMPLEX COACERVATES AND METHODS OF MAKING AND USING THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority upon U.S. nonprovisional application Serial No. 12/508,280, filed July 23, 2009. This application is hereby incorporated by reference in its entirety.

CROSS REFERENCE TO SEQUENCE LISTING

Proteins described herein are referred to by a sequence identifier number (SEQ ID NO). The SEQ ID NO corresponds numerically to the sequence identifiers <400>1, <400>2, etc. The Sequence Listing, in written computer readable format (CFR), is incorporated by reference in its entirety.

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BACKGROUND

Bone fractures are a serious health concern in society today. In addition to the fracture itself, a number of additional health risks are associated with the fracture. For example, intra-articular fractures are bony injuries that extend into a joint surface and fragment the cartilage surface. Fractures of the cartilage surface often lead to debilitating posttraumatic arthritis. The main determining factors in the development of posttraumatic arthritis are thought to be the amount of energy imparted at the time of injury, the patient's genetic predisposition (or lack thereof) to posttraumatic arthritis, and the accuracy and maintenance of reduction. Of the three prognostic factors, the only factor controllable by orthopedic caregivers is achievement and maintenance of reduction. Comminuted injuries of the articular surface (the cartilage) and the metaphysis (the portion of the bone immediately below the cartilage) are

particularly challenging to maintain in reduced (aligned) position. This relates to the quality and type of bone in this area. It also relates to the limitations of fixation with titanium or stainless steel implants.

Currently, stainless steel and titanium implants are the primary methods of fixation, but their size and the drilling necessary to place them frequently interfere with the exact manipulation and reduction of smaller pieces of bone and cartilage. A variety of bone adhesives have been tested as alternatives to mechanical fixation. These fall into four categories: polymethylmethacrylates (PMMA), fibrin-based glues, calcium phosphate (CP) cements, and CP resin composites. PMMA cements, which are used in the fixation of protheses, have well-known drawbacks, one of the most serious being that the heat generated from the exothermic setting reaction can kill adjacent bone tissue. Also, the poor bonding to bone leads to aseptic loosening, the major cause of PMMA cemented prothesis failure.

Fibrin glues, based on the blood clotting protein fibrinogen, have been tested for fixing bone grafts and repairing cartilage since the 1970s and yet have not been widely deployed. One of the drawbacks of fibrin glues is that they are manufactured from pooled human donor blood. As such, they carry risk of transmitting infections and could potentially be of limited supply.

CP cements are powders of one or more forms of CP, e.g., tetracalcium phosphate, dicalcium phosphate anhydride, and β-tricalcium phosphate. When the powder is mixed with water it forms a paste that sets up and hardens through the entanglement of one or more forms of CP crystals, including hydroxyapatite. Advantages of CP cements include isothermal set, proven biocompatibility, osteoconductivity, and they serve as a reservoir for Ca and PO_4 for hydroxyapatite formation during healing. The primary disadvantages are that CP cements are brittle, have low mechanical strength and are therefore not ideal for stable reduction of small articular segments. CP cements are used mostly as bone void fillers. The poor mechanical properties of CP cements have led to composite cements of CP particles and polymers. By varying the volume fractions of the particulate phase and the

polymer phase, the modulus and strength of the glue can be adjusted toward those of natural bone, an avenue that is also open to us.

Given the overall health impact associated with bone fractures and the imperfect state of current fixation methods, new fixation methods are needed.

SUMMARY

Described herein is the synthesis of biodegradable adhesive complex coacervates and their use thereof. The adhesive complex coacervates are composed of a mixture of one or more polycations and one or more polyanions. The polycations and polyanions are crosslinked with one another by covalent bonds upon curing. The adhesive complex coacervates have several desirable features when compared to conventional adhesives, which are effective in water-based applications. The adhesive complex coacervates described herein exhibit low interfacial tension in water when applied to a substrate (*i.e.*, they spread over the interface rather than being beaded up). Additionally, the ability of the complex coacervate to crosslink intermolecularly increases the cohesive strength of the adhesive complex coacervate. The adhesive complex coacervates have numerous biological applications as bioadhesives and drug delivery devices. In particular, the adhesive complex coacervates described herein are particularly useful in underwater applications and situations where water is present such as, for example, physiological conditions.

The advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the aspects described below. The advantages described below will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several aspects described below.

Figure 1 shows a model of pH dependent coacervate structure and adhesive mechanisms. (A) The polyphosphate (black) with low charge density paired with the polyamine (red) form nm-scale complexes. The complexes have a net positive charge. (B) Extended high charge density polyphosphates form a network connected by more compact lower charge density polyamines and when present divalent cations (green symbols). The net charge on the copolymers is negative. (C) Oxidation of 3,4-dihydroxyphenol (D) by O_2 or an added oxidant initiates crosslinking between the quinone (Q) and primary amine sidechains. The coacervate can adhere to the hydroxyapatite surface through electrostatic interactions, 3,4-dihydroxyphenol sidechains, and quinone-mediated covalent coupling to matrix proteins.

Figures 2-7 shows several protein sequences produced by *P. californica* that can be used as polycations and polyanions in the present invention as well as synthetic polycations and polyanions useful in the present invention.

Figure 8 shows different mechanisms of DOPA crosslinking.

Figure 9 shows dual syringe systems for applying small "spot welds" of complex coacervates described herein to repair fractures (A), small bone injuries (B), or bonding synthetic scaffolds to bony tissue (C).

Figure 10 shows the structure and UV/VIS characterization of mimetic copolymers. (A) The Pc3 analog, 1, contained 88.4 mol% phosphate, 9.7 mol% dopamide, and 0.1 mol% FITC sidechains. The Pc1 analog, 2, contained 8.1 mol% amine sidechains. The balance was acrylamide subunits in both cases. (B) A single peak at 280 nm characteristic of the catechol form of 3,4-dihydroxyphenol was present in the spectrum of 1. Following oxidation with $NaIO_4$ a peak at 395 nm corresponding to the quinone form appeared confirming the expected redox behavior of the 3,4-dihydroxyphenol containing polymer.

Figure 11 shows the pH dependent complex coacervation of mixed polyelectrolytes. (A) At low pH, a 50 mg/ml mixture of 1 and 2 having equal quantities of amine and phosphate sidechains formed stable colloidal PECs. As the pH increased the polymers condensed into a dense liquid complex coacervate phase. At pH 10 the copolymers went into solution and oxidatively crosslinked into a clear hydrogel. (B) The net charge of the copolymer sidechains as a function of pH calculated from the copolymer sidechain densities. (C) The diameter of the PECs (circles) increased nearly three-fold over the pH range 2-4. Above pH 4 the complexes flocculate and their size could not be measured. The zeta potential (squares) was zero near pH 3.6 in agreement with the calculated net charge.

Figure 12 shows the liquid character of an adhesive complex coacervate. The solution of 1 and 2 contained equal quantities of amine and phosphate sidechains, pH 7.4.

Figure 13 shows the phase diagram of polyelectrolytes and divalent cations. The amine to phosphate sidechain and phosphate sidechain to divalent cation ratios were varied at a fixed pH 8.2. The state of the solutions represented in a gray scale. The mass (mg) of the coacervate phase is indicated in the dark grey squares. The compositions indicated with an asterisk were used to test bond strength.

Figure 14 shows the bond strength, shear modulus, and dimensional stability of coacervate bonded bones. (A) Bond strength at failure increased ~50% and the stiffness doubled as the divalent cation ratio went from 0 to 0.4 relative to phosphate sidechains. Specimens wet bonded with a commercial cyanoacrylate adhesive were used as a reference. (n=6 for all conditions) (B) Bonds of adhered bone specimens fully submerged in PBS for four months (pH 7.2) did not swell appreciably.

Figure 15 shows UV-vis spectra of dopamine copolymers before and after oxidation (pH 7.2). A catechol peak present before oxidation was converted into the quinone form. Top left: p(DMA[8]-Aam[92]). Bottom left: p(AEMA[30]-DMA[8]). Right: Hydrogel formation by oxidative crosslinking of dopamine copolymers. (A) p(DMA[8]-Aam[92]). (B) p(EGMP[92]-DMA[8]). (C) p(DMA[8]-Aam[92]) mixed

with p(AEMA[30]-Aam[70]). (D) p(EGMP[92]-DMA[8]) mixed with p(AEMA[30]-Aam[70]). Bracketed numbers indicate mol% of sidechains. Arrows indicate direction spectra are changing over time.

Figure 16 shows pH dependence of dopamine oxidation in poly(EGMP[92]-DMA[8]). Arrows indicate direction spectra change with time. Top: pH 5.0, time course inset. Bottom: pH 6.0.

Figure 17 shows direct contact of (A) human foreskin fibroblasts, (B) human tracheal fibroblasts, and (C) rat primary astrocytes with adhesive (red auto-fluorescent chunks, white asterisks). Cell morphology, fibronectin secretion, and motility are indistinguishable from cells growing in the absence of glue. Green = intermediate filament proteins. Red = secreted fibronection. Blue = DAPI stained nuclei.

Figure 18 shows a multi-fragment rat calvarial defect model. (A) Generation of defect. (B) Fragmentation of bone cap. (C) Replacement of fragments in defect. (D) Application of bone glue. (E-F) Curing (darkening) of glue. Fragments are firmly fixed in E and F.

Figure 19 shows the effect of pH and normalized net charge with respect to forming adhesive complex coacervates.

Figure 20 provides the amino acid mole % of Pc1-Pc8.

Figure 21 shows a reaction scheme for producing amine-modified gelatin.

Figure 22 shows (A) an example of an adhesive complex coacervate in water (white arrow) and (B) the phase behavior of polyelectrolytes with setting and crosslinking mechanisms.

Figure 23 shows phase diagrams of polyphosphate-gelatin-divalent cation mixtures: (A) Ca^{2+} compositions, pH 5; (B) Ca^{2+} compositions, pH 7.4; (C) Mg^{2+} compositions, pH 5; (D) Mg^{2+} compositions, pH 7.4. The total concentration of copolymers in each mixture was 5 wt%. Soluble compositions are white, compositions that condensed into complex coacervates are light grey, compositions that formed gels or hard solid precipitates are darker grey. The numbers in the

squares represent the concentration (wt%) of the separated complex coacervate phase. Grey boxes without numbers contained complex coacervates but with volumes too low to allow accurate measurement of the concentration. The compositional space containing complex coacervates is higher with Mg^{2+} and increases with pH. The Mg^{2+} solid phases were softer and more gel-like than the hard Ca^{2+} precipitates.

Figure 24 shows the solidification temperature determined by dynamic oscillatory rheology. (A) Ca^{2+}/gelatin/polyphosphate rheology. The elastic modulus (G', black symbol) increased sigmoidally as the temperature was raised from 0 to 40 °C at Ca^{2+} ratios greater than 0.15. (Inset) The crossover of the elastic (G') and viscous (G", grey symbol) moduli, the solidification or gellation temperature, decreased with increasing Ca^{2+} ratio. The 0.25 Ca^{2+} ratio was excluded from the inset for clarity. (Symbols: ♦ 0.3/0.6, ■ 0.25/0.6, ▲ 0.2/0.6, ● 0.15/0.6 Ca^{2+} ratios). (B) Mg^{2+}/gelatin/polyphosphate rheology. (Symbols: ♦ 0.8/01.0, ■ 0.9/1.0, ▲ 1.0/01.0 Mg^{2+} ratios). The comparative measurements were made with constant strain of 0.1% and frequency of 1.0 hz.

Figure 25 shows the shear strength as a function of divalent cation ratio and temperature. (A) The Ca^{2+} ratio to phosphate was varied at a constant amine ratio. (B) The Mg^{2+} ratio was varied with a constant amine ratio. Tests were done with adherents fully submerged in a temperature-controlled water bath (pH 7.4). Dark bars represent shear tests done at 37 °C without oxidative crosslinking. White bars indicate shear tests done below the transition temperature without oxidative crosslinking. Cross hatched bars represent shear tests done at 37°C after oxidative crosslinking with $NaIO_4$ at a ratio of 1:2 relative to dopamide sidechains. The crosslinked bonds were cured (24 hr) and tested while fully submerged in a temperature-controlled water bath. The bars represent the average +/- s.d. (n=9 for all compositions).

Figure 26 shows the synthesis of polycations and polyanions with actinically crosslinkable groups and subsequent crosslinking of the polyacations and polyanions.

DETAILED DESCRIPTION

Before the present compounds, compositions, articles, devices, and/or methods are disclosed and described, it is to be understood that the aspects described below are not limited to specific compounds, synthetic methods, or uses as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting.

In this specification and in the claims that follow, reference will be made to a number of terms that shall be defined to have the following meanings:

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

"Optional" or "optionally" means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where the event or circumstance occurs and instances where it does not. For example, the phrase "optionally substituted lower alkyl" means that the lower alkyl group can or can not be substituted and that the description includes both unsubstituted lower alkyl and lower alkyl where there is substitution.

Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another aspect includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

References in the specification and concluding claims to parts by weight, of a particular element or component in a composition or article, denotes the weight relationship between the element or component and any other elements or

components in the composition or article for which a part by weight is expressed. Thus, in a compound containing 2 parts by weight of component X and 5 parts by weight component Y, X and Y are present at a weight ratio of 2:5, and are present in such ratio regardless of whether additional components are contained in the compound.

A weight percent of a component, unless specifically stated to the contrary, is based on the total weight of the formulation or composition in which the component is included.

Variables such as R^1, R^2, R^3, R^4, R^5, R^{13}-R^{22}, A, X, d, m, n, s, t, u, v, w, and x used throughout the application are the same variables as previously defined unless stated to the contrary.

The term "alkyl group" as used herein is a branched or unbranched saturated hydrocarbon group of 1 to 25 carbon atoms, such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *t*-butyl, pentyl, hexyl, heptyl, octyl, decyl, tetradecyl, hexadecyl, eicosyl, tetracosyl and the like. Examples of longer chain alkyl groups include, but are not limited to, a palmitate group. A "lower alkyl" group is an alkyl group containing from one to six carbon atoms.

Any of the compounds described herein can be the pharmaceutically-acceptable salt. In one aspect, pharmaceutically-acceptable salts are prepared by treating the free acid with an appropriate amount of a pharmaceutically- acceptable base. Representative pharmaceutically-acceptable bases are ammonium hydroxide, sodium hydroxide, potassium hydroxide, lithium hydroxide, calcium hydroxide, magnesium hydroxide, ferrous hydroxide, zinc hydroxide, copper hydroxide, aluminum hydroxide, ferric hydroxide, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, lysine, arginine, histidine, and the like. In one aspect, the reaction is conducted in water, alone or in combination with an inert, water-miscible organic solvent, at a temperature of from about 0 °C to about 100 °C such as at room temperature. In certain aspects where applicable, the molar ratio of the compounds described herein to base used are chosen to provide the ratio desired for any particular

salts. For preparing, for example, the ammonium salts of the free acid starting material, the starting material can be treated with approximately one equivalent of pharmaceutically-acceptable base to yield a neutral salt.

In another aspect, if the compound possesses a basic group, it can be protonated with an acid such as, for example, HCI, HBr, or H_2SO_4, to produce the cationic salt. In one aspect, the reaction of the compound with the acid or base is conducted in water, alone or in combination with an inert, water-miscible organic solvent, at a temperature of from about 0 °C to about 100 °C such as at room temperature. In certain aspects where applicable, the molar ratio of the compounds described herein to base used are chosen to provide the ratio desired for any particular salts. For preparing, for example, the ammonium salts of the free acid starting material, the starting material can be treated with approximately one equivalent of pharmaceutically-acceptable base to yield a neutral salt.

Described herein are biodegradable adhesive complex coacervates and their applications thereof. In general, the complexes are a mixture of cations and anions in balanced proportions to produce stable aqueous complexes at a desired pH. The adhesive complex coacervate comprises at least one polyacation and at least one polyanion, wherein at least one polycation and/or polyanion is a biodegradable, and the polycation and polyanion comprises at least one group capable of crosslinking with each other. Each component of the coacervate and methods for making the same are described below.

The adhesive complex coacervate is an associative liquid with a dynamic structure in which the individual polymer components diffuse throughout the entire phase. Complex coacervates behave rheologically like viscous particle dispersions rather than a viscoelastic polymer solution. As described above, the adhesive complex coacervates exhibit low interfacial tension in water when applied to substrates either under water or that are wet. In other words, the complex coacervate spreads evenly over the interface rather than beading up. Additionally, upon intermolecular crosslinking, the adhesive complex coacervate forms a strong, insoluble, cohesive material.

Conversely, polyeletrolyte complexes (PECs), which can be a precursor to the adhesive complex coacervates described herein, are small colloidal particles. For example, referring to Figure 11A, a solution of PECs at pH 3.1 and 4.2 exists as a milky solution of colloidal particles having a diameter of about 300 nm. Upon raising the pH to 7.2 and 8.1, the PEC condenses into a liquid phase of concentrated polymers (the coacervate phase) and a dilute equilibrium phase. In this aspect, the PEC can be converted to an adhesive complex coacervate described herein.

An exemplary model of the differences in phase behavior between the polyelectrolyte complex and the adhesive complex coacervate is presented in Figure 1. At low pH the oppositely charged polyelectrolytes associate electrostatically into nano-complexes with a net positive surface charge that stabilizes the suspension to produce PEC 1. With increasing pH the net charge of the complexes changes from positive to negative but remains near net neutrality. The PEC can form a loose precipitate phase, which can be converted to a complex coacervate 2 by raising the pH further (Figure 1). Thus, in certain aspects, the conversion of the PEC to complex coacervate can be "triggered" by adjusting the pH and/or the concentration of the multivalent cation. For example, the PEC can be produced at a pH of less than or equal to 4, and the pH of the PEC can be raised to greater than or equal to 7.0, from 7.0 to 9.0, or from 8.0 to 9.0 to convert the PEC to a complex coacervate. Subsequent crosslinking between the polycation and polyanions (*e.g.*, oxidation and covalent crosslinking as shown in Figure 1C) results in the formation of the adhesive complex coacervate described herein.

The polycations and polyanions contain groups that permit crosslinking between the two polymers upon curing to produce new covalent bonds and ultimately an adhesive. The mechanism of crosslinking can vary depending upon the selection of the crosslinking groups. In one aspect, the crosslinking groups can be electrophiles and nucleophiles. For example, the polyanion can have one or more electrohilic groups, and the polycations can have one or more nucleophilic groups capable of reacting with the electrophilic groups to produce new covalent bonds. Examples of

electrophilic groups include, but are not limited to, anhydride groups, esters, ketones, lactams (*e.g.*, maleimides and succinimides), lactones, epoxide groups, isocyanate groups, and aldehydes. Examples of nucleophilic groups are presented below. Alternatively, the polyanion can have one or more nucleophilic groups, and the polycation can have one or more electrophilic groups capable of reacting with the nucleophilic groups to produce new covalent bonds.

In another aspect, the polycation and polyanion each have an actinically crosslinkable group. As used herein, "actinically crosslinkable group" in reference to curing or polymerizing means that the crosslinking between the polycation and polyanion is performed by actinic irradiation, such as, for example, UV irradiation, visible light irradiation, ionized radiation (*e.g.* gamma ray or X-ray irradiation), microwave irradiation, and the like. Actinic curing methods are well-known to a person skilled in the art. The actinically crosslinkable group can be an unsaturated organic group such as, for example, an olefinic group. Examples of olefinic groups useful herein include, but are not limited to, an acrylate group, a methacrylate group, an acrylamide group, a methacrylamide group, an allyl group, a vinyl group, a vinylester group, or a styrenyl group. The use of polymerization initiators to facilitate crosslinking is described in detail below.

In another aspect, crosslinking can occur between the polycation and polyanion via light activated crosslinking through azido groups. Once again, new covalent bonds are formed during this type of crosslinking.

In another aspect, the crosslinkable group includes any group capable of undergoing oxidative crosslinking. The term "oxidative crosslinking" is defined as the ability of a group or moiety to undergo oxidation then subsequently react with another group in order to produce a new covalent bond. An example of a group capable of undergoing oxidative crosslinking includes a dihydroxyl-substituted aromatic group capable of undergoing oxidation in the presence of an oxidant. In one aspect, the dihydroxyl-substituted aromatic group is a dihydroxyphenol or halogenated dihydroxyphenol group such as, for example, DOPA and catechol (3,4

dihydroxyphenol). For example, in the case of DOPA, it can be oxidized to dopaquinone. Dopaquinone is an electrophilic group that is capable of either reacting with a neighboring DOPA group or another nucleophilic group. In the presence of an oxidant such as oxygen or other additives including, but not limited to, peroxides, periodates (*e.g.*, $NaIO_4$), persulfates, permanganates, dichromates, transition metal oxidants (*e.g.*, a Fe^{+3} compound, osmium tetroxide), or enzymes (*e.g.*, catechol oxidase), the dihydroxyl-substituted aromatic group can be oxidized.

In one aspect, the polyanion and/or polycation comprises at least one dihydroxyl aromatic group capable of undergoing oxidative crosslinking, wherein the dihydroxyl aromatic group is covalently attached to the polyanion or polyanion. In one aspect, both the polycation and polyanion comprise an ortho-dihydroxy aromatic group capable of undergoing oxidative crosslinking. In another aspect, the polycation comprises an ortho-dihydroxy aromatic group and the polyanion comprises a nucleophilic group capable of reacting with an oxidized form of the dihydroxyl aromatic group to form a covalent bond.

In certain aspects, the oxidant can be stabilized. For example, a compound that forms a complex with periodate that is not redox active can result in a stabilized oxidant. In other words, the periodate is stabilized in a non-oxidative form and cannot oxidize the dihydroxyl-substituted aromatic group while in the complex. The complex is reversible and even if it has a very high stability constant there is a small amount of uncomplexed periodate formed. The stable but reversible oxidant permits the slow release of oxidant in order to control the rate of oxidative crosslinking. The dihydroxyl-substituted aromatic group competes with the compound for the small amount of free periodate. As the free periodate is oxidized more is released from the complex because it is in equilibrium. In one aspect, sugars possessing a cis,cis-1,2,3-triol grouping on a six-membered ring can form competitive periodate complexes. An example of a specific compound that forms stable periodate complex is 1,2-O-isopropylidene-alpha-D-glucofuranose. The stabilized oxidant can control the rate of crosslinking. Not wishing to be bound by theory, the stabilized oxidant slows it down

the rate of oxidation so that there is time to add the oxidant and position the substrate before the adhesive hardens irreversibly.

The stability of the oxidized crosslinker can vary. For example, the phosphono containing polyanions described herein that contain oxidizable crosslinkers are stable in solution and do not crosslink with themselves. This permits nucleophilic groups present on the polycation to react with the oxidized crosslinker. This is a desirable feature of the invention, which permits the formation of intermolecular bonds and, ultimately, the formation of a strong adhesive. Examples of nucleophilic groups that are useful include, but are not limited to, hydroxyl, thiol, and nitrogen containing groups such as substituted or unsubstituted amino groups and imidazole groups. For example, residues of lysine, histidine, and/or cysteine can be incorporated into the polycation and introduce nucleophilic groups. An example of this is shown in Figure 8. DOPA residue 1 can be oxidized to form a dopaquinone residue 2. Dopaquinone is a reactive intermediate and can crosslink (*i.e.*, react) with a DOPA residue on another polymer or the same polymer to produce a di-DOPA group. Alternatively, the dopaquinone residue can react with nucleophiles such as, for example, amino, hydroxyl, or thiol groups via a Michael-type addition to form a new covalent bond. Referring to Figure 8, a lysyl group, cysteinyl group, and histidyl group react with the dopaquinone residue to produce new covalent bonds. Although DOPA is a suitable crosslinking group, other groups such as, for example, tyrosine can be used herein. The importance of crosslinking with respect to the use of the adhesive complex coacervates described herein will be discussed below.

In other aspects, the crosslinkers present on the polycation and/or polyanion can form coordination complexes with transition metal ions. For example, a transition metal ion can be added to a mixture of polycation and polyanion, where both polymers contain crosslinkers capable of coordinating with the transition metal ion. The rate of coordination and dissociation can be controlled by the selection of the crosslinker, the transition metal ion, and the pH. Thus, in addition to covalent crosslinking as described above, crosslinking can occur through electrostatic, ionic, or

other non-covalent bonding. Transition metal ions such as, for example, iron, copper, vanadium, zinc, and nickel can be used herein.

The polycation and polyanion are generally composed of a polymer backbone with a plurality of chargeable groups at a particular pH. The groups can be pendant to the polymer backbone and/or incorporated within the polymer backbone. In certain aspects, (*e.g.*, biomedical applications), the polycation is any biocompatible polymer possessing cationic groups or groups that can be readily converted to cationic groups by adjusting the pH. In one aspect, the polycation is a polyamine compound. The amino groups of the polyamine can be branched or part of the polymer backbone. The amino group can be a primary, secondary, or tertiary amino group that can be protonated to produce a cationic ammonium group at a selected pH. In general, the polyamine is a polymer with a large excess of positive charges relative to negative charges at the relevant pH, as reflected in its isoelectric point (pI), which is the pH at which the polymer has a net neutral charge. The number of amino groups present on the polycation ultimately determines the charge of the polycation at a particular pH. For example, the polycation can have from 10 to 90 mole %, 10 to 80 mole %, 10 to 70 mole %, 10 to 60 mole %, 10 to 50 mole %, 10 to 40 mole %, 10 to 30 mole %, or 10 to 20 mole % amino groups. In one aspect, the polyamine has an excess positive charge at a pH of about 7, with a pI significantly greater than 7. As will be discussed below, additional amino groups can be incorporated into the polymer in order to increase the pI value.

In one aspect, the amino group can be derived from a residue of lysine, histidine, or imidazole attached to the polycation. Any anionic counterions can be used in association with the cationic polymers. The counterions should be physically and chemically compatible with the essential components of the composition and do not otherwise unduly impair product performance, stability or aesthetics. Non-limiting examples of such counterions include halides (*e.g.*, chloride, fluoride, bromide, iodide), sulfate and methylsulfate.

In one aspect, when the polycation is naturally-occurring, the polycation can

be a positively-charged protein produced from a natural organism. For example, proteins produced by *P. californica* can be used as the polycation. Figures 2-6 show the protein sequences of several cement proteins produced by *P. californica* (Zhao *et al.* "Cement Proteins of the tube building polychaete Phragmatopoma californica" *J. Biol. Chem.* (2005) 280: 42938-42944). Figure 20 provides the amino acid mole % of each protein. Referring to Figures 2-5, Pc1, Pc2, Pc4-Pc18 (SEQ ID NOS 1, 2, 5-19, respectively) are polycations, where the polymers are cationic at neutral pH. The type and number of amino acids present in the protein can vary in order to achieve the desired solution properties. For example, referring to Figure 20, Pc1 is enriched with lysine (13.5 mole %) while Pc4 and Pc5 are enriched with histidine (12.6 and 11.3 mole %, respectively).

In another aspect, the polycation is a recombinant protein produced by artificial expression of a gene or a modified gene or a composite gene containing parts from several genes in a heterologous host such as, for example, bacteria, yeast, cows, goats, tobacco, and the like. In another aspect, the polycation can be a genetically modified protein.

In another aspect, the polycation can be a biodegradable polyamine. The biodegradable polyamine can be a synthetic polymer or naturally-occurring polymer. The mechanism by which the polyamine can degrade will vary depending upon the polyamine that is used. In the case of natural polymers, they are biodegradable because there are enzymes that can hydrolyze the polymers and break the polymer chain. For example, proteases can hydrolyze natural proteins like gelatin. In the case of synthetic biodegradable polyamines, they also possess chemically labile bonds. For example, β-aminoesters have hydrolyzable ester groups. In addition to the nature of the polyamine, other considerations such as the molecular weight of the polyamine and crosslink density of the adhesive can be varied in order to modify the degree of biodegradability.

In one aspect, the biodegradable polyamine includes a polysaccharide, a protein, or a synthetic polyamine. Polysaccharides bearing one or more amino groups

can be used herein. In one aspect, the polysaccharide is a natural polysaccharide such as chitosan. Similarly, the protein can be a synthetic or naturally-occurring compound. In another aspect, the biodegradable polyamine is a synthetic polyamine such as poly(β-aminoesters), polyester amines, poly(disulfide amines), mixed poly(ester and amide amines), and peptide crosslinked polyamines. It is desirable in certain aspects that the polycation as well as the polyanion be non-gelling and a low-endotoxin.

In the case when the polycation is a synthetic polymer, a variety of different polymers can be used; however, in certain applications such as, for example, biomedical applications, it is desirable that the polymer be biocompatible and non-toxic to cells and tissue. In one aspect, the biodegradable polyamine can be an amine-modified natural polymer. The term "amine modified natural polymer" is defined as any natural polymer that has been subsequently manipulated or processed to change the natural state of the polymer. For example, the natural polymer can be chemically modified using the techniques described herein. Alternatively, the natural polymer can be denatured or digested by an enzyme. In one aspect, the amine-modified natural polymer can be an amine-modified protein such as, for example, gelatin or collagen modified with one or more alkylamino groups, heteroaryl groups, or an aromatic group substituted with one or more amino groups. Examples of alkylamino groups are depicted in Formulae IV-VI

$$-NR^{13}(CH_2)_sNR^{14}R^{15} \qquad IV$$

$$-NR^{13}(\underset{\displaystyle R^{16}}{\underset{|}{C}}H_2)_tN(CH_2)_uNR^{17}R^{18} \qquad V$$

$$-NR^{13}(\underset{\displaystyle R^{19}}{\underset{|}{C}}H_2)_vN\text{-}\{(\underset{\displaystyle R^{20}}{\underset{|}{C}}H_2)_wN\}_A\text{-}(CH_2)_xNR^{21}R^{22} \qquad VI$$

wherein R^{13}-R^{22} are, independently, hydrogen, an alkyl group, or a nitrogen containing substituent;

s, t, u, v, w, and x are an integer from 1 to 10; and

A is an integer from 1 to 50,

where the alkylamino group is covalently attached to the natural polymer. In one aspect, if the natural polymer has a carboxyl group (*e.g.*, acid or ester), the carboxyl group can be reacted with a polyamine compound to produce an amide bond and incorporate the alkylamino group into the polymer. Thus, referring to formulae IV-VI, the amino group NR^{13} is covalently attached to the carbonyl group of the natural polymer.

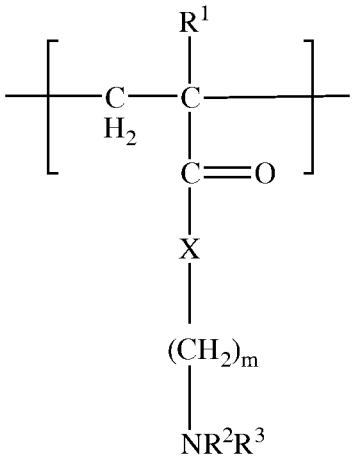
As shown in formula IV-VI, the number of amino groups can vary. In one aspect, the alkylamino group is $-NHCH_2NH_2$, $-NHCH_2CH_2NH_2$, $-NHCH_2CH_2CH_2NH_2$, $-NHCH_2CH_2CH_2CH_2NH_2$, $-NHCH_2CH_2CH_2CH_2CH_2NH_2$, $-NHCH_2NHCH_2CH_2CH_2NH_2$, $-NHCH_2CH_2NHCH_2CH_2CH_2NH_2$, $-NHCH_2CH_2CH_2NHCH_2CH_2CH_2CH_2NHCH_2CH_2CH_2NH_2$, $-NHCH_2CH_2NHCH_2CH_2CH_2CH_2NH_2$, $-NHCH_2CH_2NHCH_2CH_2CH_2NHCH_2CH_2CH_2NH_2$, or $-NHCH_2CH_2NH(CH_2CH_2NH)_dCH_2CH_2NH_2$, where d is from 0 to 50.

In one aspect, the amine-modified natural polymer can include an aryl group having one or more amino groups directly or indirectly attached to the aromatic group. Alternatively, the amino group can be incorporated in the aromatic ring. For example, the aromatic amino group is a pyrrole, an isopyrrole, a pyrazole, imidazole, a triazole, or an indole. In another aspect, the aromatic amino group includes the isoimidazole group present in histidine. In another aspect, the biodegradable polyamine can be gelatin modified with ethylenediamine.

In one aspect, the polycation includes a polyacrylate having one or more pendant amino groups. For example, the backbone can be a homopolymer or copolymer derived from the polymerization of acrylate monomers including, but not limited to, acrylates, methacrylates, acrylamides, and the like. In one aspect, the backbone of the polycation is polyacrylamide. In other aspects, the polycation is a block co-polymer, where segments or portions of the co-polymer possess cationic

groups depending upon the selection of the monomers used to produce the co-polymer.

In one aspect, the polycation is a polyamino compound. In another aspect, the polyamino compound has 10 to 90 mole % tertiary amino groups. In a further aspect, the polycation polymer has at least one fragment of the formula I



I

wherein R^1, R^2, and R^3 are, independently, hydrogen or an alkyl group, X is oxygen or NR^5, where R^5 is hydrogen or an alkyl group, and m is from 1 to 10, or the pharmaceutically-acceptable salt thereof. In another aspect, R^1, R^2, and R^3 are methyl and m is 2. Referring to formula I, the polymer backbone is composed of CH_2-CR^1 units with pendant -$C(O)X(CH_2)_mNR^2R^3$ units. In this aspect, the fragment having the formula I is a residue of an acrylate, methacrylate, acrylamide, or methacrylamide. Figure 3 (structures C and D) and Figure 6 (4 and 7) show examples of polycations having the fragment of formula I, where the polymer backbone is derived from acrylamide and methacrylate residues as discussed above. In one aspect, the polycation is the free radical polymerization product of a cationic tertiary amine monomer (2-dimethylamino-ethyl methacrylate) and acrylamide, where the molecular weight is from 10 to 20 kd and possesses tertiary monomer concentrations from 15 to 30 mol %. Figure 4 (structures E and F) and Figure 6 (5) provide examples of polycations useful herein, where imidazole groups are directly attached to the polymer backbone (structure F) or indirectly attached to the polymer backbone via a linker

(structure E via a methylene linker).

Similar to the polycation, the polyanion can be a synthetic polymer or naturally-occurring. In one aspect, when the polyanion is naturally-occurring, the polyanion is a negatively-charged protein produced from *P. californica.* Figures 2 and 7 show the sequences of two proteins (Pc3a and Pc3b) produced by *P. californica* (Zhao *et al.* "Cement Proteins of the tube building polychaete Phragmatopoma californica" *J. Biol. Chem.* (2005) 280: 42938-42944). Referring to Figure 20, Pc3a and Pc3b are essentially composed of polyphosphoserine, which is anionic at neutral pH. Examples of other naturally-occurring polyanions include glycosaminoglycans such as condroitin sulfate, heparin, heparin sulfate, dermatan sulfate, and hyaluronic acid.

When the polyanion is a synthetic polymer, it is generally any polymer possessing anionic groups or groups that can be readily converted to anionic groups by adjusting the pH. Examples of groups that can be converted to anionic groups include, but are not limited to, carboxylate, sulfonate, phosphonate, boronate, sulfate, borate, or phosphate. Any cationic counterions can be used in association with the anionic polymers if the considerations discussed above are met. Depending upon the selection of the anionic group, the group can be pendant to the polymer backbone and/or incorporated in the polymer backbone.

In one aspect, the polyanion is a polyphosphate. In another aspect, the polyanion is a polyphosphate compound having from 10 to 90 mole % phosphate groups. For example, the polyphosphate can be a naturally-occurring compound such as, for example, highly phosphorylated proteins like phosvitin (an egg protein), dentin (a natural tooth phosphoprotein), casein (a phosphorylated milk protein), bone proteins (e.g. osteopontin), or DNA. In another aspect, the polyphosphate is an inorganic polyphosphonate such as, for example, sodium polymetaphosphate (Graham's salt).

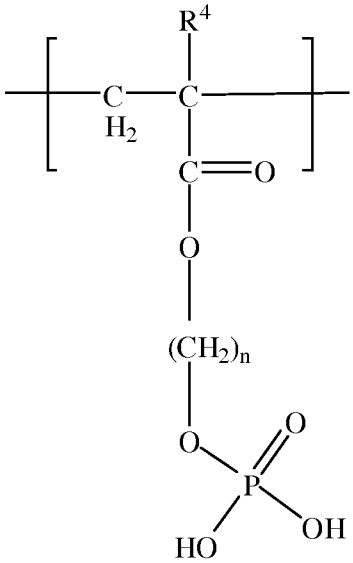
In other aspects, phosphorous containing polymers can be converted to polyanions. For example, a phospholipid or phosphosugar is not a polyanion but it

can be converted into a polyanion by creating a liposome or a micelle with it. Thus, in this aspect, the complex coacervate is a charged colloid. Alternatively, the colloid can be produced by any of the polyanions or polycations described herein.

In another aspect, the polyphosphate can be a synthetic compound. For example, the polyphosphate can be a polymer with pendant phosphate groups attached to the polymer backbone and/or present in the polymer backbone. (*e.g.*, a phosphodiester backbone). In one aspect, the polyphosphate can be produced by chemically or enzymatically phosphorylating a natural compound. In one aspect, a natural serine-rich protein can be phosphorylated to incorporate phosphonate groups into the protein. In another aspect, hydroxyl groups present on a polysaccharide can be phosphorylated to produce a polyanion useful herein.

In one aspect, the polyanion includes a polyacrylate having one or more pendant phosphate groups. For example, the backbone can be a homopolymer or copolymer derived from the polymerization of acrylate monomers including, but not limited to, acrylates, methacrylates, acrylamides, and the like. In one aspect, the backbone of the polyanion is derived from the polymerization of polyacrylamide. In other aspects, the polyanion is a block co-polymer, where segments or portions of the co-polymer possess anionic groups depending upon the selection of the monomers used to produce the co-polymer. In a further aspect, the polyanion can be heparin sulfate, hyaluronic acid, chitosan, and other biocompatible and biodegradable polymers typically used in the art.

In one aspect, the polyanion is a polyphosphate. In another aspect, the polyanion is a polymer having at least one fragment having the formula II

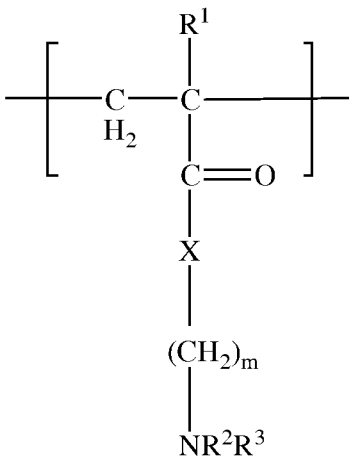


II

wherein R^4 is hydrogen or an alkyl group, and n is from 1 to 10, or the pharmaceutically-acceptable salt thereof. In another aspect, wherein R^4 is methyl and n is 2. Similar to formula I, the polymer backbone of formula II is composed of a residue of an acrylate or methacrylate. The remaining portion of formula II is the pendant phosphate group. Figure 7 (structure B), shows an example of a polyanion useful herein that has the fragment of formula II, where the polymer backbone is derived from acrylamide and methacrylate residues. In one aspect, the polyanion is the copolymerization product of ethylene glycol methacrylate phosphate and acrylamide, where the molecular weight is from 10,000 to 50,000, preferably 30,000, and has phosphate groups in the amount of 45 to 90 mol%.

As described above, the polycation and polyanion contain crosslinkable groups. In one aspect, the polycation and polyanion includes an actinically crosslinkable group defined herein. Any of the polymers described above (synthetic or naturally-occurring) that can be used as the polycation and polyanion can be modified to include the actinically crosslinkable group. For example, the polycation can be a polyacrylate having one or more pendant amino groups (*e.g.*, imidazole groups). In the case of the polyanion, in one aspect, a polyphosphate can be modified to include the actinically crosslinkable group(s). For example, wherein the polycation

and polyanion includes at least one fragment having the formula VII



VII

wherein R^1, R^2, and R^3 are, independently, hydrogen or an alkyl group, X is oxygen or NR^5, where R^5 is hydrogen or an alkyl group, and m is from 1 to 10, or the pharmaceutically-acceptable salt thereof, wherein at least one of R^2 or R^3 is an actinically crosslinkable group. In one aspect, referring to formula VII, R^1 is methyl, R^2 is hydrogen, R^3 is an acrylate or methacrylate group, X is NH, and m is 2.

In one aspect, the polyanion can include one or more groups that can undergo oxidative crosslinking as previously described, and the polycation contains on or more nucleophiles that can react with the oxidized crosslinker to produce new covalent bonds. In one aspect, the polyanion includes at least one dihydroxyl aromatic group capable of undergoing oxidation, wherein the dihydroxyl aromatic group is covalently attached to the polyanion. Examples of dihydroxyl aromatic groups include a DOPA residue or a catechol residue. Any of the polyanions described above can be modified to include one or more dihydroxyl aromatic residues. In one aspect, the polyanion is polymerization product between two or more monomers, where one of the monomers has a dihydroxyl aromatic group covalently attached to the monomer. For example, the monomer can have an unsaturated group capable of undergoing free-radical polymerization with the dihydroxyl aromatic group attached to the monomer. For example, the polyanion can be the polymerization product between (1) a phosphate

acrylate and/or phosphate methacrylate and (2) a second acrylate and/or second methacrylate having a dihydroxyl aromatic group covalently bonded to the second acrylate or second methacrylate. In another aspect, the polyanion is the polymerization product between monoacryloxyethyl phosphate and dopamine methacrylamide. Polymers 3 and 7 in Figure 6 provide examples of DOPA residues incorporated into a polyanion and polycation, respectively. In each of these polymers, an acrylate containing the pendant DOPA residue is polymerized with the appropriate monomers to produce the polyanion 3 and polycation 7 with pendant DOPA residues.

Not wishing to be bound by theory, the polyanion with the dihydroxyl aromatic group(s) are stable in that they react slowly with itself in solution. Thus, the polyanion reacts with the polycation primarily via intermolecular cross-linking (e.g., polycation has a nucleophilic group or a dihydroxyl aromatic group) to produce the complex coacervate. This provides numerous advantages with respect to the use and administration of the complex coacervate. For example, the polycation and polyanion can be premixed and administered to a subject instead of the sequential administration of the polymers. This greatly simplifies administration of the complex coacervate that is not an option with currently available bioadhesives.

It is contemplated that the polycation can be a naturally occurring compound (*e.g.*, protein from *P. californica*) and the polyanion is a synthetic compound. In another aspect, the polycation can be a synthetic compound and the polyanion is a naturally occurring compound (*e.g.*, protein from *P. californica*). In a further aspect, both the polyanion and polycation are synthetic compounds.

The adhesive complex coacervates can optionally contain one or more multivalent cations (*i.e.*, cations having a charge of +2 or greater). In one aspect, the multivalent cation can be a divalent cation composed of one or more alkaline earth metals. For example, the divalent cation can be Ca^{+2} and/or Mg^{+2}. In other aspects, transition metal ions with a charge of +2 or greater can be used as the multivalent cation. In addition to the pH, the concentration of the multivalent cations can determine the rate and extent of coacervate formation. Not wishing to be bound by

theory, weak cohesive forces between particles in the fluid may be mediated by multivalent cations bridging excess negative surface charges. The amount of multivalent cation used herein can vary. In one aspect, the amount is based upon the number of anionic groups and cationic groups present in the polyanion and polycation. For example, when the multivalent cation is a mixture of calcium and magnesium, the polycation is a polyamine, the polyanion is a polyphosphate, and the ratio of calcium to amine/phosphate groups can be from 0.1 to 0.3, and the ratio of magnesium to amine/phosphate groups can be from 0.8 to 1.0. In the Examples, the selection of the amount of multivalent cations with respect to producing adhesive complex coacervates and other physical states is addressed.

In certain aspects, the coacervate also includes one or more initiators. For example, a photoinitiator can be entrapped in the coacervate. Thus, when the photoinitiator is activated (e.g., exposed to light), crosslinking can occur between the polycation and polyanion when the crosslinkable groups are actinically crosslinkable groups. Examples of photoinitiators include, but are not limited to a phosphine oxide, a peroxide group, an azide group, an α-hydroxyketone, or an α-aminoketone. In one aspect, the photoinitiator includes, but is not limited to, camphorquinone, benzoin methyl ether, 1-hydroxycyclohexylphenyl ketone, or Darocure® or Irgacure® types, for example Darocure® 1173 or Irgacure® 2959. The photoinitiators disclosed in European Patent No. 0632329, which are incorporated by reference, can be used herein. In other aspects, the photoinitiator is a water-soluble photoinitiator including, but not limited to, riboflavin, eosin, eosin y, and rose Bengal.

In certain aspects, multiple initiators can be used to broaden the absorption profile of the initiator system in order to increase the initiation rate. For example, two different photoinitiators can be employed that are activated by different wavelengths of light. In another aspect, a chemical initiator can be used in combination with a photoinitiator. In another aspect, a co-initiator can be used in combination with any of the polymerization initiators described herein. In one aspect, the co-initiator is 2-(diethylamino)ethyl acrylate, 2-(dimethylamino)ethyl acrylate, 2-

(dimethylamino)ethyl benzoate, 2-(dimethylamino)ethyl methacrylate, 2-ethylhexyl 4-(dimethylamino)benzoate, 3-(dimethylamino)propyl acrylate, 4,4′-bis(diethylamino)benzophenone, or 4-(diethylamino)benzophenone.

In certain aspects, the photoinitiator and/or co-initiator are covalently attached to the polycation and/or polyanion. In another aspect, the initiators can be chemically grafted onto the backbone of the polycation and polyanion. Thus, in these aspects, the photoinitiator and/or co-initiator are covalently attached to the polymer and pendant to the polymer backbone. This approach will simply formulation and possibly enhance storage and stability.

The adhesive complex coacervate can be synthesized a number of different ways. In one aspect, the adhesive complex coacervate can be produced by the process comprising admixing at least one polyacation and at least one polyanion, wherein at least one polycation and/or polyanion is a biodegradable, and the polycation and polyanion comprises at least one group capable of crosslinking with each other.

In certain aspects, the pH of the admixture and/or the concentration of at least one multivalent cation, can be adjusted to produce the adhesive complex coacervate. Exemplary techniques for producing the coacervates with the polymerizable monomer are provided in the Examples.

The adhesive complex coacervates produced herein can undergo subsequent phase changes that ultimately lead to the formation of a water-insoluble adhesive. In one aspect, an adhesive is produced by crosslinking the polycation and polyanion in the adhesive complex coacervate. Any of the techniques and approaches previously described herein can be used to crosslink the polycation and polyanion. In another aspect, the adhesive can be produced by the process comprising:

(a) heating an adhesive complex coacervate described herein; and

(b) crosslinking the polycation and polyanion in the coacervate,

wherein step (a) can be performed prior to step (b), after step (b), or simultaneously with step (b) to produce the adhesive.

In this aspect, heating and crosslinking the adhesive complex coacervate converts the coacervate to an insoluble solid (*i.e.*, adhesive). The temperature can vary depending upon the nature of the coacervate (*i.e.*, selection of polycation, polyanion, multivalent cations, etc.). For example, at room temperature, a complex coacervate can be present. However, by injecting the coacervate into a subject where the temperature is 37 °C, the coacervate solidifies at body temperature. As will be discussed below, this has numerous applications in tissue/bone repair as well as for the delivery of drugs.

In other aspects, the adhesive is produced by the process comprising

(a) preparing an adhesive complex coacervate described herein;

(b) adjusting the pH of the adhesive complex coacervate; and

(c) crosslinking the polycation and polyanion in the coacervate,

wherein step (b) can be performed prior to step (c), after step (c), or simultaneously with step (c) to produce the adhesive.

In this aspect, the complex coacervate is converted to an adhesive by adjusting the pH. The adjustment of the pH can be accomplished by a number of techniques. For example, the pH can be actively changed by the delivery of a second component (*e.g.*, acid or base) in combination with the complex coacervate to convert the complex coacervate to an insoluble solid. Alternatively, the complex coacervate can be introduced into an environment having a pH that is different from that of the complex coacervate, where the change in pH can convert the complex coacervate to an insoluble solid. In one aspect, the pH is raised to a pH greater than or equal to 7.0, or up to a pH of 8.0.

The adhesive complex coacervates and adhesives produced therefrom described herein have numerous applications as biological glues and delivery devices. For example, the coacervates have low initial viscosity, specific gravity greater than one, and being mostly water by weight, low interfacial tension in an aqueous environment, all of which contribute to their ability to adhere to a wet surface. An

additional advantage with respect to the bonding mechanism (*i.e.*, crosslinking) of the adhesive complex coacervates includes low heat production during setting, which prevents damage to living tissue. The components can be pre-polymerized in order to avoid heat generation by *in situ* exothermic polymerization. This is due for the most part by the ability of the adhesive complex coacervates to crosslink intermolecularly under very mild conditions as described above.

The adhesive complex coacervates described herein can be applied to a number of different biological substrates. The substrate can be contacted *in vitro* or *in vivo*. Upon crosslinking the polycation and polyanion in the coacervate, a water-insoluble solid is produced, which yields a strong adhesive. The rate of crosslinking within the adhesive complex coacervate can be controlled by for example pH and the presence of an oxidant or other agents that facilitate crosslinking. One approach for applying the adhesive complex coacervate to the substrate can be found in Figure 9. The techniques depicted in Figure 9 are referred to herein as "spot welding," where the adhesive complex coacervate is applied at distinct and specific regions of the substrate. In one aspect, the adhesive complex coacervate can be produced *in situ*. Referring to Figure 9A, a pre-formed stable PEC solution 1 composed of polycations and polyanions at low pH (e.g., 5) is simultaneously applied to a substrate with a curing solution 2 composed of an oxidant at a higher pH (e.g., 10) with the use of syringes. Upon mixing, the curing solution simultaneously produces the adhesive complex coacervate by crosslinking the polymers on the surface of the substrate.

In another aspect, referring to Figure 9B, a solution of polyanions 3 and polycations 4 are applied simultaneously to the substrate. One of the solutions has a pH higher than the other in order to produce the adhesive complex coacervate. Referring to Figure 9B, polyanion 3 is at a lower pH than the polycation solution 4; however, it is also contemplated that the polyanion can be in solution having a higher pH than the polycation. The solution having the higher pH can include an oxidant in order to facilitate crosslinking.

Figure 9C depicts another aspect of spot welding. In this aspect, the substrate

is primed with polycation at a particular pH. Next, a solution of the polyanion at a higher pH is applied to the polycation in order to produce the adhesive complex coacervate *in situ*. It is also contemplated that the substrate can be primed with polyanion first followed by polycation. An oxidant can then be applied separately on the complex coacervate to facilitate crosslinking to produce the adhesive complex coacervate. Alternatively, the solution applied after the substrate has been primed can contain the oxidant so that the adhesive complex coacervate is formed and subsequently crosslinked *in situ*.

The properties of the adhesive complex coacervates described herein make them ideal for underwater applications such as the administration to a subject. For example, the adhesive complex coacervates and adhesives produced therefrom can be used to repair a number of different bone fractures and breaks. The coacervates adhere to bone (and other minerals) through several mechanisms (*see* Figure 1C). The surface of the bone's hydroxyapatite mineral phase ($Ca_5(PO_4)_3(OH)$) is an array of both positive and negative charges. The negative groups present on the polyanion (*e.g.*, phosphate groups) can interact directly with the positive surface charges or it can be bridged to the negative surface charges through the cationic groups on the polycation and/or multivalent cations. Likewise, direct interaction of the polycation with the negative surface charges would contribute to adhesion. Additionally, when the polycation and/or polyanion contain catechol moieties, they can facilitate the adhesion of the coacervate to readily wet hydroxyapatite. Other adhesion mechanisms include direct bonding of unoxidized crosslinker (*e.g.*, DOPA or other catechols) to hydroxyapatite. Alternatively, oxidized crosslinkers can couple to nucleophilic sidechains of bone matrix proteins.

Examples of such breaks include a complete fracture, an incomplete fracture, a linear fracture, a transverse fracture, an oblique fracture, a compression fracture, a spiral fracture, a comminuted fracture, a compacted fracture, or an open fracture. In one aspect, the fracture is an intra-articular fracture or a craniofacial bone fracture. Fractures such as intra-articular fractures are bony injuries that extend into and

fragment the cartilage surface. The adhesive complex coacervates may aid in the maintenance of the reduction of such fractures, allow less invasive surgery, reduce operating room time, reduce costs, and provide a better outcome by reducing the risk of post-traumatic arthritis.

In other aspects, the adhesive complex coacervates and adhesives produced therefrom can be used to join small fragments of highly comminuted fractures. In this aspect, small pieces of fractured bone can be adhered to an existing bone. For example, the coacervate can be applied to the fractured bone and/or the existing bone. It is especially challenging to maintain reduction of the small fragments by drilling them with mechanical fixators. The smaller and greater number of fragments the greater the problem. In one aspect, the adhesive complex coacervate or precursor thereof may be injected in small volumes to create spot welds as described above in order to fix the fracture rather than filling the entire crack. The small biocompatible spot welds would minimize interference with healing of the surrounding tissue and would not necessarily have to be biodegradable. In this respect it would be similar to permanently implanted hardware.

In other aspects, the adhesive complex coacervates and adhesives produced therefrom can be used to secure scaffolds to bone and other tissues such as, for example, cartilage, ligaments, tendons, soft tissues, organs, membranous tissues (e.g., vaginal, nasal, amniotic membrane) and synthetic derivatives of these materials. Using the complexes and spot welding techniques described herein, the adhesive complex coacervates and adhesives produced therefrom can be used to position biological scaffolds in a subject. The coacervate can be applied to the biological scaffold and/or the bone or tissue prior to securing the scaffold. Small adhesive tacks composed of the adhesive complex coacervates described herein would not interfere with migration of cells or transport of small molecules into or out of the scaffold. In certain aspects, the scaffold can contain one or more drugs that facilitate growth or repair of the bone and tissue. In other aspects, the scaffold can include drugs that prevent infection such as, for example, antibiotics. For example, the scaffold can be

coated with the drug or, in the alternative, the drug can be incorporated within the scaffold so that the drug elutes from the scaffold over time.

The adhesive complex coacervates and adhesives produced therefrom have numerous dental applications. Using the spot weld techniques described herein, the adhesive complex coacervates can be applied to specific points in the mouth (*e.g.*, jaw, sections of a tooth). For example, the coacervates can be used in the treatment of recession defects, increasing gingival tissue height and width, increase the amount of attached gingival tissue at the gingival margin, and increase the zone of attached gingival tissue. In oral surgery they could be used to improve soft tissue outcomes and grow new bone in guided bone regeneration procedures. Additionally, the coacervates can facilitate wound healing of gums after a periodontal procedure and help prevent or reduce bleeding. As will be discussed below, the coacervates can be used to deliver bioactive agents. Thus, the coacervates can be used to deliver bioactive agents to the gums and roots of teeth. In other aspects, the coacervates can be used to secure dental implants to teeth (*e.g.*, crowns, dentures). Alternatively, the coacervates can be used as a primer to prepare the dentin or enamel surface of a tooth to bond dental cements.

In other aspects, the adhesive complex coacervates and adhesives produced therefrom can adhere a substrate to bone. Examples of substrates include metal substrates (*e.g.*, plates, medical implants, etc.), fibers, foils, pieces of cloth, or any other materials that can be implanted within a subject. The coacervate can be applied to the substrate and/or bone prior to use. For example, implants made from titanium oxide, stainless steel, or other metals are commonly used to repair fractured bones. The adhesive complex coacervate or a precursor thereof can be applied to the metal substrate, the bone, or both prior to adhering the substrate to the bone. In certain aspects, the crosslinking group present on the polycation or polyanion can form a strong bond with titanium oxide. For example, it has been shown that DOPA can strongly bind to wet titanium oxide surfaces (Lee *et al.*, PNAS 103:12999 (2006)). Thus, in addition to bonding bone fragments, the adhesive complex coacervates

described herein can facilitate the bonding of metal substrates to bone, which can facilitate bone repair and recovery.

It is also contemplated that the adhesive complex coacervates and adhesives produced therefrom can include one or more bioactive agents. The bioactive agents can be any drug that will facilitate bone growth and repair when the complex is applied to the bone. The rate of release can be controlled by the selection of the materials used to prepare the complex as well as the charge of the bioactive agent if the agent is a salt. In certain aspects, when the adhesive complex coacervate is converted to an insoluble solid by a change in temperature and/or pH, the complex coacervate can be administered to a subject and produce an insoluble solid in situ. Thus, in this aspect, the insoluble solid can perform as a localized controlled drug release depot. It may be possible to simultaneously fix tissue and bones as well as deliver bioactive agents to provide greater patient comfort, accelerate bone healing, and/or prevent infections.

The adhesive complex coacervates and adhesives produced therefrom can be used in a variety of other surgical procedures. For example, adhesive complex coacervates and adhesives produced therefrom can be used to repair lacerations caused by trauma or by the surgical procedure itself. In one aspect, the adhesive complex coacervates and adhesives produced therefrom can be used to repair a corneal or conjunctival laceration in a subject. In other aspects, the adhesive complex coacervates and adhesives produced therefrom can be used to inhibit blood flow in a blood vessel of a subject. In general, the adhesive complex coacervate is injected into the vessel followed by crosslinking (*e.g.*, heating the complex coacervate or other crosslinking techniques described herein) in order to convert the coacervate to an insoluble solid and to partially or completely block the vessel. This method has numerous applications including hemostasis or the creation of an artificial embolism to inhibit blood flow to a tumor or aneurysm.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in

the art with a complete disclosure and description of how the compounds, compositions, and methods described and claimed herein are made and evaluated, and are intended to be purely exemplary and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.) but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric. There are numerous variations and combinations of reaction conditions, e.g., component concentrations, desired solvents, solvent mixtures, temperatures, pressures and other reaction ranges and conditions that can be used to optimize the product purity and yield obtained from the described process. Only reasonable and routine experimentation will be required to optimize such process conditions.

I. Synthesis and Characterization of Adhesive Complex Coacervates

Mimetic copolymer synthesis and characterization.

Pc3 analogs. The dopa analog monomer (dopamine methacrylamide, DMA) was prepared by slight modification of a published procedure. (Lee BP, Huang K, Nunalee FN, Shull KR, Messersmith PB. Synthesis of 3,4-dihydroxyphenylalanine (DOPA) containing monomers and their co-polymerization with PEG-diacrylate to form hydrogels. J Biomater Sci Polym Ed 2004;15(4):449-464). Briefly, a borate-dopamine complex was reacted at pH >9 with methacryloyl chloride. After disrupting the borate-catechol bond by acidification, the product was washed with ethyl acetate, recrystallized from hexane, and verified by ^{1}H NMR (400MHz, DMSO-TMS): d8.8-8.58 (2H, (O***H***)$_2$-Ar-), 7.92 (1H, -C(=O)-N***H***-), 6.64-6.57 (2H,C_6H***H_2***$(OH)_2$-), 6.42 (1H, C_6H_2***H***$(OH)_2$-), 5.61 (1H, -C(=O)-C(-CH_3)=C***H***H), 5.30 (1H, -C(=O)-C(-CH_3)=CH***H***), 3.21 (2H, $C_6H_3(OH)_2$-CH_2-CH_2(NH)-C(=O)-), 2.55 (2H, $C_6H_3(OH)_2$-CH_2-CH_2(NH)-C(=O)-), 1.84 (3H, -C(=O)-C(-CH_3)=CH_2).

Before polymerization monoacryloxyethyl phosphate (MAEP, Polysciences) was diluted in MeOH and extracted with hexane to remove dienes. Copolymer **1** was prepared by mixing 90 mol% MAEP, 8 mol% DMA, 2 mol% acrylamide (Aam,

Polysciences), and 0.1 mol% FITC-methacrylamide in MeOH at a final monomer concentration of 5 wt%. Free radical polymerization was initiated with azobisisobutyronitrile (AIBN) and proceeded at 60 °C for 24 hrs in sealed ampules. A similar procedure was used to make polymers **3-7** as shown in Figures 2-7.

Copolymer **1** (Figure 10) was recovered by size exclusion chromatography (SEC) in MeOH on a Sephadex LH-20 column (Sigma-Aldrich), concentrated by rotary evaporation, dissolved in DI water, and freeze dried.

The MW and polydispersity index (PDI) of **1** were determined by SEC in DMF on a PLgel column (Polymer Labs) connected to a small angle light scattering detector (Brookhaven BI-MWA) and refractive index monitor (Brookhaven BI-DNDC). The column was calibrated with polystyrene standards. The MW of **1** was 245 kda with a PDI of 1.9. The dopamine sidechain concentration and reactivity was verified by UV/VIS spectroscopy (e_{280} = 2600 $M^{-1}cm^{-1}$). The phosphate sidechain concentration were determined by titration with 0.005 M NaOH using an automated titrator (Brinkmann Titrando 808). The UV/vis spectrum of **1** contained a single absorption peak at 280 nm characteristic of the catechol form of dopamine (Figure 10B). Addition of a 1:1 molar ratio of $NaIO_4$ to **1** at pH 5.0 oxidized the dopa catechol to dopaquinone with an absorption peak near 395 nm as expected. The dopaquinone peak was stable for several hrs at pH <5.

Pc1 analogs. The lysine sidechains of Pc1 were mimicked with N-(3-aminopropyl) methacrylamide hydrochloride (APMA, Polysciences). Copolymer **2** (Figure 10) was synthesized by dissolving 10 mol% APMA and 90 mol% Aam in DI water, degassing with N_2 and initiating polymerization with 2 mol% ammonium persulfate (Polysciences). Polymerization proceeded at 50° C for 24 hrs in sealed ampules. Polymer was recovered by dialysis against water for 3 days, and then freeze dried. The primary amine sidechain mol% was determined by ^1H NMR (400MHz, DMSO-TMS) from the ratios of d 13.45 (3H, -C***H***3) and d 51.04 (1H, RC(=O)C***H***R2). The MW and PDI of **2** were determined by SEC in PBS (20 mM PO_4, 300 mM NaCl, pH 7.2) on a Superose 6 column (Pharmacia). The column was calibrated with poly-2-

hydroxypropyl methacrylate standards. The MW of **2** was 165 kd and PDI was 2.4.

Coacervate formation and characterization. A 5 wt% aqueous solution of **2** was added dropwise while stirring to a 5 wt% aqueous solution of **1** until reaching the target amine/phosphate ratio. Total copolymer concentration was 50 mg/ml. After mixing for 30 min the pH was adjusted with NaOH (6M). Compositions at pH (<4) conducive to polyelectrolyte complex (PEC) formation were diluted to 1 mg/ml in DI H_20 and the zeta potentials and size distribution of PECs were measured on a Zeta-Sizer 3000HS (Malvern Instruments). At higher pH, coacervated compositions were centrifuged at 2500 rpm in a microfuge (Eppendorf), at 25 °C for 2 min to collect the coacervate phase. The volume of both phases was measured. The coacervate phases were freeze dried and weighed to determine their mass and concentration.

The phase behavior of **1** and **2** mixed at a 1:1 molar ratio of phosphate to amine sidechains (50 mg/ml combined concentration) over the pH range 3-10 is shown in Figure 11A. The calculated net copolymer charge normalized to the total ionizable sidechain concentration is shown in figure 11B. Ascorbate, a reductant, was added at a 1:5 molar ratio to dopa to retard oxidation of dopa by O_2 and subsequent crosslinking at elevated pH. At low pH, the polyelectrolytes formed a stable milky solution of colloidal polyelectrolyte complexes (PECs). The mean diameter of the PECs at pH 2.1, determined by dynamic light scattering, was 360 nm with a narrow dispersity and increased to 1080 nm at pH 4.0 (Figure 11C). The crossover of the zeta potential from positive to negative at pH 3.6 fit well with the calculated pH dependent net charge of the complexes (Figure 11B). The particle size could not be measured accurately above pH 4 because the complexes flocculated. As the net charge increased due to the deprotonation of the phosphate sidechains, the copolymers condensed into a dense second phase. At pH 5.1 the separated phase had the character of a loose low density precipitate. At pH 7.2 and 8.3 the dense phase had the character of a cohesive liquid complex coacervate (Figure 12). The copolymers were concentrated about three-fold to 148 and 153 mg/ml, respectively, in the coacervated phases. At pH 9.5 the polyelectrolyte mixture formed a dense non-liquid

ionic gel. At pH 10 the copolymers went into solution and spontaneously crosslinked through the dopaquinone and amine sidechains into a clear hydrogel.

Extraction of divalent cations with the chelator EDTA resulted in a 50% decrease in compressive strength of *P. californica* tubes, a ten-fold decrease in adhesiveness, and collapse of the glues porous structure. The effect of divalent cations on the phase behavior of the mimetic polyelectrolytes was investigated by mixing **1** and **2** at amine to phosphate sidechain ratios ranging from 1:1 to 0:1 with divalent cation to phosphate sidechain ratios ranging from 0:1 to 1:1 to create a coacervate phase diagram (Figure 13). The pH was fixed at 8.2, the pH of seawater, and divalent cations were added as a 4:1 mixture of Mg^{2+} and Ca^{2+}, the approximate Mg^{2+}/Ca^{2+} ratio in the natural glue determined by elemental analysis. The highest mass of coacervate (dark gray squares) occurred in mixtures with higher amine to phosphate sidechain ratios and lower divalent cation to phosphate sidechain ratios. Mixtures with lower polyamine ratios were clear (clear squares) even at higher divalent cation/phosphate sidechain ratios. At higher amine/phosphate and divalent cation/phosphate ratios the solutions were turbid (light gray squares) with slight precipitates but much less turbid than solutions containing PECs (medium gray squares).

Mechanical bond testing. Bone test specimens, ~1 cm^3, were cut with a band saw from bovine femur cortical bone, obtained from a local grocery store, sanded with 320 grit sandpaper, and stored at -20 °C. $NaIO_4$ at a 1:2 molar ratio to dopa sidechains was evenly applied to one face each of two wet bone specimens. Forty ml, a volume sufficient to completely fill the space between 1 cm^2 bone interfaces, of the test coacervate solution was applied with a pipette, the bone specimens were pressed together squeezing out a small excess of adhesive, clamped, and immediately wrapped in PBS (20 mM PO_4, 150 mM NaCl, pH 7.4) soaked gauze. The applied coacervate contained ascorbate at a 1:5 molar ratio to dopa to prevent premature crosslinking. The bonded specimens were incubated at 37 °C for at least 24 hr in a sealed container containing soaked sponges to maintain 100% humidity. Reference

specimens were bonded with 40 ml Loctite 401 superglue in exactly the same manner. A commercial non-medical grade cyanoacrylate was used because there are no hard tissue medical adhesives available for comparison. Mechanical tests were performed on a custom built material testing system using a 1 kg load cell. The instrument was controlled and data aquired using LabView (National Instruments). One bone of a bonded pair was clamped laterally 1mm from the bond interface. The second bone was pressed with a cross-head speed of 0.02 mm/s against a dull blade positioned 1 mm lateral to the bond interface. Bond strength tests were performed at room temperature immediately after unwrapping the wet specimens to prevent drying. After testing, the bonds were examined for failure mode. The bonded area was measured by tracing an outline of the bone contact surface on paper, cutting out the trace, and determining its area from the weight of the paper cut-out. At least 6 specimens were tested for each condition.

The shear modulus and strength at failure were measured with bovine cortical bone specimens bonded while wet with the three coacervating compositions marked with an asterisk in Figure 13. The coacervate density in the three compositions increased with increasing divalent cation ratios (to 120, 125, and 130 mg/ml, respectively). Both the modulus and bond strength of the fully hydrated specimens increased with increasing divalent cation concentration, reaching 37% of the strength of wet bones bonded with a commercial cyanoacrylate adhesive (Figure 14A). The cyanoacrylate adhesive was used as a reference point because there are no bone adhesives in clinical use for comparison. The strength of the mimetic adhesive is also about 1/3 the strength of natural *P. californica* glue estimated to be 350 kPa and mussel byssal glue estimated to range from 320 to 750 kPa dependent on the season. In almost all cases the bonds failed cohesively leaving adhesive on both bone interfaces, which suggested the compositions formed strong interfacial bonds with hydroxyapatite. The bonds were dimensionally stable, neither shrinking nor swelling appreciably after complete submersion in PBS pH 7.2 for several months (Figure 14B). Dimensional stability during cure and long term exposure to water is an important requirement for a useful bone adhesive.

Dopamine-mediated copolymer crosslinking. Addition of $NaIO_4$ to solutions of **3** at a 1:1 molar ratio immediately and quantitatively oxidized DOPA (280 nm) to dopaquinone (392 nm). Within a few minutes the quinine peak decayed into broad general absorption as the reactive quinones formed covalent diDOPA crosslinks (Figure 15, top left). Crosslinking between the quinones and primary amines (Figure 15, bottom left) led to a broader general absorption than diDOPA crosslinking. Dopamine oxidation and crosslinking chemistry therefore behaved as expected in the dopamine copolymers. The dopamine copolymers rapidly formed hydrogels as a result of oxidative crosslinking (Figure 15, A&C). Oxidized phosphodopamine **3** did not gel by itself (Figure 15B) but when mixed with **4** it gelled rapidly (Figure 15D). Intermolecular diDOPA crosslinking between PO_4 copolymers was inhibited but not intermolecular DOPA-amine crosslinking. This provides a crosslinking control mechanism that may be useful for formulating and delivering a synthetic adhesive.

pH triggered DOPA-mediated crosslinking. To explore, the pH dependence and kinetics of DOPA oxidation, crosslinking of the dopamine copolymers were evaluated by UV-Vis spectroscopy. Results with p(EGMP[92]-DMA[8]) (**3**) are shown in Figure 16. UV-vis spectra were acquired at increasing time after addition of a stoichiometric amount of $NaIO_4$. At pH 5.0 (top), dopaquinone absorbance (398 nm) was maximal in ~15 min and remained stable for several hrs (inset). At pH 6.0, absorbance at 398 nm peaked in < 1 min and evolved into broad absorbance with peaks at 310 and 525 nm. The broad absorbance is not due to dopaquinone crosslinking since gels do not form (Figure 16). For comparison, **6** was oxidized at low pH crosslinked but at a significantly slower rate (not shown).

The results show that the dopaquinone is stable at low pH and diDOPA crosslinking was inhibited at higher pH in the phosphodopamine copolymers. In the presence of the polyamine, the covalent crosslinking was channeled toward intermolecular amine-DOPA bonds. This is an important observation because it lays out a path to controlled delivery and setting of the synthetic adhesive.

In vitro cytotoxicity. Solutions of **3** and **4**, 40 wt% each, were mixed at low pH to

form a polyelectrolyte complex. The solution was partially oxidized with $NaIO_4$ and basified with NaOH just before application to sterile glass coverslips. The adhesive-treated coverslips were placed in the bottom of culture plate wells and human foreskin fibroblasts, human tracheal fibroblasts, and rat primary astrocytes in serum containing media were added to separate wells at 30K cells/well (Figure 17). After 24 hr, the cells were fixed with 4% para-formaldehdye, then immunostained for the intermediate filament protein, vimentin, to visualize cell morphology (green, A-B), pericellular fibronectin to assess ECM secretion (red, B), glial fibrillary protein to visual primary astrocyte morphology (green, C), and DAPI to visualize nuclei (blue,C). The granular globs of adhesive auto-fluoresced orangish-red (A-C).

In the representative images (Figure 17), all cell types had morphologies indistinguishable from cells growing on glass without adhesive. The cells had normal motility and in several cases extended processes that directly contacted the adhesive. No toxicity was apparent.

Rat calvarial defect model. Production of the fragmented defect and repair with an adhesive complex coacervate is shown in Figures 18A-F. Male Sprague Dawley rats (256-290g) (Harlan) were anesthetized with a mixture of ketamine (65mg/kg), xylazine (7.5mg/kg), and acepromazine (0.5mg/kg). At full depth of anesthesia, the eyes were covered with ophthalmic ointment, the head shaved, and the scalp disinfected with isopropanol and butadiene. With the prepped rats in a stereotactic frame, a compressed air-driven drill operating at ~5000 RPM was lowered using a stereotactic fine toothed manipulator. Sterile saline or PBS was continuously applied at the craniotomy site while the custom made trephine tool was lowered 600 microns (previously determined as the skull thickness of rats the age of which were used in the experiment). The result is a round, accurate hole through the skull with little observable effect on the underlying dura or vasculature (Figure 18A-B). The bone plug was recovered with fine curved forceps and broken into fragments using a hemostat and fine rongeur (Figure 18B). The bone fragments were returned to the defect (Figure 18C) and 5 μl of test adhesive (**3** and **4** mixed immediately prior to the

application of the fracture) was applied with a micropipettor (Figure 18D). The low viscosity adhesive solution (pre-formed PECS mixed with curing solution just before delivery) readily and cleanly wicked into the fractures. Within 5 min the fragments were sufficiently fixed that they could be tapped sharply with the forceps without displacement. The adhesive continued to turn dark reddish brown as it cured (Figure 18E-F).

II. Adhesive Complex Coacervates Produced from an Amine-Modified Polymer

A. Materials and Methods

Materials. Low endotoxin, non-gelling, gelatin (MW 3.5 kDa) was provided by Gelita Inc. (Souix City,Iowa). 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) and ethylenediamine dihydrochloride were purchased from Thermo Scientific Inc.. Monoacryloxyethyl phosphate (MAEP), 2, 2'-azobisisobutyronitrile (AIBN) were purchased from Polysciences, Inc. Sodium periodate ($NaIO_4$), Sephadex LH-20, dopamine hydrochloride was obtained from Sigma-Aldrich.

Polyphosphodopamide synthesis. The polyphosphodopamide copolymer (poly($MAEP_{85}$-DMA_{15})) was synthesized by free radical polymerization of MAEP and dopamine methacrylamide (DMA) using azobisisobutyronitrile (AIBN) as initiator. The copolymer was recovered by size exclusion chromatography (SEC) in MeOH on a Sephadex LH-20 column (Sigma-Aldrich). MeOH was removed, the copolymer resuspended in water, lyophilized, and stored at -80 °C. The mol% dopamide side chains in the copolymers were determined by UV/vis spectroscopy: the catechol form of dopamide has an absorption peak at 279 nm (λ_{279} = 2600 $M^{-1}cm^{-1}$).

Gelatin modification. The general reaction scheme for producing amine-modified gelatin is provided in Figure 21. Gelatin (100 mg/ml) was mixed with ethylenediamine dihydrochloride (1:1 molar ratio to the gelatin carboxyl groups). The pH was adjusted to 5.2 with 6M HCl. EDC at 1.2:1 molar ratio to ethylenediamine

dihydrochloride was added to the reaction mixture while stirring. The reaction proceeded for 2 hrs at room temperature. The amine-modified gelatin was dialyzed against DI water for 3 days then lyophilized. The primary amine side chain concentration was determined by ninhydrin assay using glycine as a standard. Zeta potential measurements of gelatin (1 mg/ml in water) were determined by electrophoresis using a Malvern Zetasizer Nano-ZS ZEN 3600 (Malvern Instruments Ltd., Worcestershire, UK).

Gelatin coacervate formation. A 50 mg/ml aqueous solution of amine-modified gelatin (pH 5.0) was added dropwise while stirring to a 50 mg/ml aqueous solution (pH 5.0) of poly($MAEP_{85}$-$DOPA_{15}$) containing various ratios of divalent cation (Ca^{2+} or Mg^{2+}) until reaching the target amine/phosphate ratio. The pH of the mixture was raised to 7.4 with NaOH. The coacervate phase was allowed to settle for 24 hrs. The coacervate and equilibrium phases were separated and their volumes measured. The coacervate phases were lyophilized and weighed to determine their mass and concentration.

Dynamic rheology. The elastic (G') and storage (G") moduli were measured with a cone and plate configuration (20 mm diameter, 4°C cone) on a stress-controlled rheometer (TA Instruments, AR 500). To compare coacervate compositions the measurements were made with a constant frequency of 1Hz and dynamic strain of 0.1% as the temperature was ramped from 0°C to 40°C at a rate of 0.5°C /min.

Adhesive bond strength. Aluminum test adherends, 0.12×0.6×5 cm, were cut from 5052 aluminum sheet (0.050 in) with a water saw. The adherends were polished with 600 grit super fine sandpaper and then cleaned following the procedure of ASTM D2651. Briefly, the adherends were sonicated twice in MeOH, air-dried, dipped into a solution of sulfuric acid and nochromix for 15 mins, then rinsed thoroughly with DI water and stored in DI water until bonded. The adherends were bonded within 12 hr of cleaning. For each adhesive sample, 9 wet aluminum test specimens were bonded. $NaIO_4$ at 1:2 molar ratio to dopamide sidechains was evenly applied to the bond area of two aluminum adherends. The test coacervate solution (6 μl) was applied to wet

adherends with a pipette, which were then pressed together with an overlap of about 25 mm, clamped, and immediately submerged in water adjusted to pH 7.4 with NaOH. The bonded specimens cured fully submerged in water for ~24 hr at the specified temperature. Shear strengths were measured while the adherends where fully submerged in a temperature-controlled water bath mounted on an Instron 3342 materials testing system with a 100 N load cell. The instrument was controlled and data acquired using Bluehill Lite software (Instron, Inc.).

B. Results

An adhesive complex coacervate was created using a low MW (3-5 kda) non-gelling collagen hydrolysate as the polycation. As received the collagen hydrolysate did not form complex coacervates with the phosphodopa copolymer (poly(MAEP)$_{85}$-co-dopamide$_{15}$)) at physiological pH. Amination of carboxylic acid sidechains with ethylenediamine increased the amine concentration to ~16 mol% and shifted the pI from 5.5 to 10.4. The aminated collagen formed dense coacervates at 25 °C over a broad range of compositions. At pH 5, concentrated coacervates formed at amine to phosphate sidechain ratios from 0.5-1.0 and Ca^{2+} to phosphate ratios up to 0.8 (Figure 23A). None of the compositions precipitated. At pH 7.4, the coacervation space was more confined; at Ca^{2+} ratios higher than 0.2 the copolymers precipitated as hard solids, reflecting the decreased solubility of the mixed polyelectrolytes and Ca^{2+} with increasing pH (Figure 23B).

Investigation of the separate effect of Mg^{2+} on coacervation of the polyelectrolytes revealed significant differences compared with Ca^{2+}. At pH 5 the coacervated region was larger. At ratios up to 1:1 Mg^{2+} to phosphate none of the compositions precipitated (Figure 23C). With Mg^{2+} the copolymers condensed into more concentrated coacervates, in some cases >380 mg/ml, an almost 8-fold increase from the initial copolymer concentration. At pH 7.4 the coacervation range is broader and at high Mg^{2+} ratios compositions with mixed phases of fluid and solid occur due to decreased solubility with increased pH (Figure 23D). The expanded coacervation space at higher pH again illustrates the dense fluid coacervates are stably balanced

intermediates between soluble polyelectrolytes and insoluble solids. The physical nature of the solidified state at high Mg^{2+} ratios is non-fluid, but softer and more gel-like than the hard Ca^{2+} precipitates, reflecting perhaps an intermediate state of desolvation relative to fluid coacervates and solids. The distinct physical nature and solubility profile of the Mg^{2+} complexes are likely consequences of the smaller radius, higher charge density, and smaller coordination number of Mg^{2+} ions compared to Ca^{2+} ions. Mg^{2+} tends to coordinate single bulky ligands, like phosphate, because multiple ligands won't fit around the small ion. As a consequence much of its solvation sphere is retained. The larger Ca^{2+} ion, on the other hand, can accommodate several bulky ligands resulting in displacement of its solvation sphere and cross-link formation between ligands. Coacervates prepared with mixed Mg^{2+} and Ca^{2+} occupied space in between the coacervated regions of the individual cations.

The phase diagrams in Figure 23 illustrate empirically how the pH differential between secretory granules and seawater could trigger a phase change that drives the rapid but well-timed initial setting reaction of the natural adhesive. The condensed fluid complex coacervate phase is thermodynamically balanced between stable colloidal complexes and gelled or precipitated polymeric salts. The composition of the natural adhesive may be adapted to fall just inside the coacervation boundary within the secretory pathway, but to be outside of the coacervated region at the elevated pH of seawater. In other words, they are composed to undergo a pH dependent phase change upon secretion. For example, row 4 compositions (Figures 23A and B), with ratios of 0.4 Ca^{2+} and greater than 0.3 amine are coacervated at pH 5 but solid at pH 7.4 and higher.

At 0°C the coacervated region in Figure 23B was shifted approximately one row lower, while at 37 °C it is shifted one row higher (not shown). The temperature dependent phase transition of several compositions at pH 7.4 with increasing Ca^{2+} ratios and a fixed amine ratio of 0.6 were investigated in more detail by dynamic oscillatory rheology (Figure 24A). At low temperature the viscous shear moduli (G") were greater than the elastic moduli (G') consistent with the fluid character of the

complex coacervates. With increasing temperature G' rose sigmoidally in a Ca^{2+} ratio dependent manner. The crossover points at which G' = G" (Figure 24A, inset), taken as the transition temperature where the compositions begin to change from viscous fluids to load-bearing elastic solids, were 36, 21, 12, and 9°C, for Ca^{2+} ratios of 0.15, 0.20, 0.25, and 0.30, respectively. The Mg^{2+} containing coacervates demonstrated qualitatively similar behavior: there was no crossover of G' and G" at Mg^{2+} to phosphate ratios up to 0.8 at pH 7.4, at higher ratios the crossover temperature again decreased with increasing Mg^{2+} ratios. The elastic moduli at 37 °C were much lower with Mg^{2+} than with Ca^{2+} (Figure 24B), consistent with the more hydrated gel-like quality of the solidified Mg^{2+} coacervates.

Bonds formed with Ca^{2+} ratios ranging from 0 to 0.3 with an amine ratio fixed at 0.6 were tested with polished aluminum adherends fully submerged in a temperature controlled water bath at 37 °C, well above the transition temperatures of the compositions. The lap shear strength increased with increasing Ca^{2+} up to a ratio of 0.3 (Figure 25A, black bars). The 0.2 and 0.25 Ca^{2+}/0.6 amine compositions were also tested slightly below their respective transition temperatures at 10 and 20°C. In both cases, the bond strengths above the transition temperature were greater than below the transition temperature (Figure 25A, white bars). Under the conditions of the test set-up there is likely to be little covalent oxidative crosslinking between the dopamide sidechains of the polyphosphate and the amines of gelatin: the rate of dopa oxidation is much slower at pH 7.4 than 8.2, diffusion of dissolved O_2 into the narrow bond gap (62 μm) was restricted, and there was no evident browning of the adhesive indicative of dopa oxidation. Therefore the increase in bond strength above the transition temperature was predominantly due to the state change of the adhesive. Similar tests with the 1.0 Mg^{2+} ratio demonstrated a more dramatic increase, a more than six-fold increase in bond strength above the transition temperature than below (Figure 25B). As a practical matter, the results demonstrated that temperature differentials can be exploited as a convenient means to trigger the initial set of the synthetic adhesive and that the temperature trigger can be adjusted within a

physiologically relevant range by small changes in the divalent cation ratio.

Next, oxidative coupling between the polyphosphate dopamide sidechains and the gelatin amines was initiated by adding 0.5 equivalents $NaIO_4$ relative to the dopamide sidechains during the bonding procedure in order to investigate the contribution of covalent crosslinking to bond strength of the synthetic adhesive. The bonds were cured and tested at 37 °C while fully submerged in water adjusted to pH 7.4. The bonds strengths increased with increasing divalent cation ratio for both Ca^{2+} and Mg^{2+} (Figure 25, hatched bars). Maximum bond strengths with Mg^{2+} were double the bond strength of Ca^{2+}, reaching 765 kPa.

In conclusion, the adhesive complex coacervates were dense, partially water-immiscible fluids precariously balanced between soluble polymers and insoluble polymeric salts (*see* white arrow in Figure 22A). Referring to Figure 22B, the top row represents the phase behavior of the polyelectrolytes. The bottom row connects the features of the phase behavior to solving the several problems of creating an underwater glue. The change from fluid complex coacervate to insoluble solid, the initial setting reaction, is triggered by a change in the pH, temperature, or both. Covalent hardening occurs through oxidative coupling between catechol and primary amine sidechains.

III. Preparation of Photocrosslinkable Polymers

Synthesis of methacrylate-grafted polyphosphate (Figure 26). A mixture of N-(3-aminopropyl)methacrylamide hydrochloride (5 mol%), monomethacryloxyethyl phosphate (94.95 mol%) and FITC-methacrylamide (0.05 mol%) was dissolved in methanol (90 wt%). The initiator AIBN (2 mol%) was added and the solution was purged with argon for 30 min. Polymerization proceeded at 65 °C for 24h. To methacrylate the amine sidechains of the copolymer, a very small amount of t-octylpyrocatechin, 2.1 equivalents of triethylamine and 1 equivalent of methacryolyl chloride were added and the reaction was stirred for 30 min. The methacrylate-grafted copolymer was purified by size exclusion chromatography in MeOH on LH-20 sephadex. The copolymer was concentrated by rotoevaporation, then dissolved in

deionized water and freeze dried.

Synthesis of methacrylate-grafted polyamine (Figure 26). The protected monomer N-(t-BOC-aminopropyl)methacrylamide (10 mol%) was dissolved in a minimum amount of methanol and diluted with water. Monomers N-(3-aminopropyl) methacrylamide hydrochloride (5 mol%) and hydroxypropylmethacrylamide (85 mol%) and the initiator AIBN (2 mol%, in a minimum amount of methanol) were added. The total monomer concentration was 2 wt%. The solution was purged with argon for 30 min. then heated at 65 °C for 24 h. The terpolymer was purified by dialysis (12,000-14,000 MWCO) in deionized water for 3 days then freeze dried to obtain the polymer as a white solid.

The methacrylate terpolymers was dissolved in DMF then, relative to the free amine group, 2.1 equivalents of triethylamine followed by 1 equivalent of methacryloyl chloride was added. The reaction was stirred for 30 min. The t-BOC group was removed by adding 5 equivalents of TFA. The deprotected terpolymer was precipitated with diethyl ether, resuspended in DI water and lyophilized. The degree of methacrylolyl substitution was calculated by ^{1}H NMR using the ratio of the vinyl proton signals to ethyl and propyl proton signals.

Photocrosslinking (Figure 26). The photoinitiator IRGACURE 2959 (0.1 wt%) was added to a 5 wt% solution of the methacrylated copolymers in water. The solution was irradiated at 365 nm with a Novacure photocuring light source.

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the compounds, compositions and methods described herein.

Various modifications and variations can be made to the compounds, compositions and methods described herein. Other aspects of the compounds, compositions and methods described herein will be apparent from consideration of the specification and practice of the compounds, compositions and methods disclosed herein. It is intended that the specification and examples be considered as exemplary.

What is claimed:

1. A biodegradable adhesive complex coacervate comprising at least one polyacation and at least one polyanion, wherein at least one polycation and/or polyanion is a biodegradable, and the polycation and polyanion comprises at least one group capable of crosslinking with each other.

2. The coacervate of claim 1, wherein the polycation comprises a polysaccharide, a protein, or a synthetic polyamine.

3. The coacervate of claim 2, wherein the protein comprises a recombinant protein or a genetically modified protein.

4. The coacervate of claim 1, wherein the polycation comprises an amine-modified natural polymer.

5. The coacervate of claim 1, wherein the polycation comprises an amine-modified protein.

6. The coacervate of claim 4, wherein the amine-modified natural polymer comprises gelatin or collagen modified with one or more alkylamino groups, heteroaryl groups, or an aromatic group substituted with one or more amino groups.

7. The coacervate of claim 1, wherein the polycation comprises gelatin modified with ethylenediamine.

8. The coacervate of claim 1, wherein the polycation has a pI value greater than 7 at physiological pH.

9. The coacervate of claim 1, wherein the polyanion comprises one or more sulfate, sulfonate, carboxylate, borate, boronate, phosphonate, phosphate groups, or any combination thereof.

10. The coacervate of claim 1, wherein the polyanion comprises a polyphosphate compound.

11. The coacervate of claim 10, wherein the polyphosphate compound comprises a natural compound, a chemically modified natural compound, or a synthetic analog.

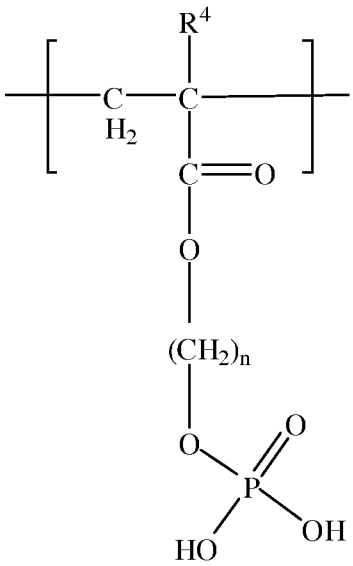
12. The coacervate of claim 11, wherein the natural compound comprises DNA, a cyclic polyphosphonate, or a protein.

13. The coacervate of claim 11, wherein the chemically modified natural compound comprises a phosphorylated protein or polysaccharide.

14. The coacervate of claim 10, wherein the polyphosphate compound comprises at least one phosphate group pendant to the polymer backbone and/or at least one phosphate group incorporated in the polymer backbone..

15. The coacervate of claim 1, wherein the polyanion comprises a polyacrylate comprising one or more pendant phosphate groups.

16. The coacervate of claim 1, wherein the polyanion comprises a polymer comprising at least one fragment comprising the formula II



II

wherein R^4 is hydrogen or an alkyl group, and n is from 1 to 10, or the pharmaceutically-acceptable salt thereof.

17. The coacervate of claim 16, wherein R^4 is methyl and n is 2.

18. The coacervate of claim 10, wherein the polyphosphate compound comprises from 10 to 90 mole % phosphate groups.

19. The coacervate of claim 1, wherein the polyanion and/or polycation comprises at least one dihydroxyl aromatic group capable of undergoing oxidation, wherein the dihydroxyl aromatic group is covalently attached to the polyanion.

20. The coacervate of claim 1, wherein the coacervate comprises at least one multivalent metal cation.

21. The coacervate of claim 1, wherein the multivalent cation comprises one or more divalent cations or one or more transition metal ions or rare earth metals.

22. The coacervate of claim 21, wherein the multivalent cation comprises Ca^{+2} and/or Mg^{+2}.

23. The coacervate of claim 1, wherein the composition further comprises one or more bioactive agents.

24. The coacervate of claim 1, wherein the coacervate further comprises a reversible oxidant complex.

25. The coacervate of claim 1, wherein the crosslinking group on the polycation comprises a nucleophilic group and the crosslinking group on the polyanion comprises an electrophilic group.

26. The coacervate of claim 1, wherein the crosslinking group on the polycation comprises an electrophilic group and the crosslinking group on the polyanion comprises a nucleophilic group.

27. The coacervate of claim 1, wherein the crosslinking group on the polycation and polyanion comprises an ortho-dihydroxy aromatic group capable of undergoing oxidative crosslinking.

28. The coacervate of claim 1, wherein the crosslinking group on the polyanion comprises an ortho-dihydroxy aromatic group and the polycation comprises a

nucleophilic group capable of reacting with the crosslinking group to form a covalent bond.

29. The coacervate of claim 1, wherein the crosslinking group on the polycation comprises an ortho-dihydroxy aromatic group and the polyanion comprises a nucleophilic group capable of reacting with the crosslinking group to form a covalent bond.

30. The coacervate of claim 1, wherein the crosslinking group on the polyanion and the polycation comprises an actinically crosslinkable group.

31. The coacervate of claim 30, wherein the actinically crosslinkable group comprises an olefinic group.

32. The coacervate of claim 31, wherein the olefinic group comprises an acrylate group, a methacrylate group, an acrylamide group, a methacrylamide group, an allyl group, a vinyl group, a vinylester group, or a styrenyl group.

33. The coacervate of claim 1, wherein the polycation comprises a polyacrylate comprising one or more pendant amino groups.

34. The coacervate of claim 1, wherein the polycation comprises a polyacrylate comprising one or more pendant imidazole groups.

35. The coacervate of claim 1, wherein the coacervate further comprises a polymerization initiator and optionally a co-initiator.

36. The coacervate of claim 35, wherein the polymerization initiator comprises (1) one or more of a radical initiator, a thermal initiator, or a photoinitiator, or (2) two or more radical initiators, thermal initiators, or a photoinitiators.

37. The coacervate of claim 36, wherein the photoinitiator and optionally a co-initiator are covalently attached to the polycation and/or polyanion.

38. The coacervate of claim 36, wherein the photoinitiator comprises a water-soluble initiator comprising riboflavin, eosin, eosin y, or rose Bengal.

39. The coacervate of claim 36, wherein the photoinitiator comprises a phosphine oxide, a peroxide, an azide compound, an α-hydroxyketone, or an α-aminoketone.

40. An adhesive produced by the process comprising

(a) heating the adhesive complex coacervate of claims 1-39; and

(b) crosslinking the polycation and polyanion in the coacervate,

wherein step (a) can be performed prior to step (b), after step (b), or simultaneously with step (b) to produce the adhesive.

41. The adhesive of claim 40, wherein step (b) comprises the use of an oxidant in order to facilitate the crosslinking between the polycation and polyanion.

42. The adhesive of claim 41, wherein the oxidant comprises O_2, $NaIO_4$, a peroxide, or a transition metal oxidant, or a reversible oxidant complex.

43. An adhesive produced by the process comprising

(a) preparing an adhesive complex coacervate of claims 1-39;

(b) adjusting the pH of the adhesive complex coacervate; and

(c) crosslinking the polycation and polyanion in the coacervate,

wherein step (b) can be performed prior to step (c), after step (c), or simultaneously with step (c) to produce the adhesive.

44. The adhesive of claim 43, wherein the multivalent cation is a calcium and/or magnesium, the polycation is a polyamine, the polyanion is a polyphosphate, and the ratio of calcium to amine/phosphate groups is from 0.1 to 0.3, and the ratio of magnesium to amine/phosphate groups is from 0.8 to 1.0.

45. The adhesive of claim 43, wherein step (b) comprises raising the pH of the adhesive complex coacervate to a pH greater than or equal to 7.0.

46. The adhesive of claim 45, wherein step (b) comprises raising the pH of the adhesive complex coacervate to a pH up to 8.0.

47. The adhesive of claim 43, wherein step (c) comprises the use of an oxidant in order to facilitate the crosslinking between the polycation and polyanion.

48. The adhesive of claim 47, wherein the oxidant comprises O_2, $NaIO_4$, a peroxide, a transition metal oxidant, or a reversible oxidant complex.

49. A compound comprising a polyanion or polycation comprising at least one dihydroxyl aromatic group capable of undergoing oxidative crosslinking, wherein the dihydroxyl aromatic group is covalently attached to the polyanion or polycation.

50. The compound of claim 49, wherein the polyanion comprises a polyphosphate.

51. The polyanion of claim 50, wherein the polyphosphate compound comprises a natural compound, a chemically modified natural compound, or a synthetic analog.

52. The compound of claim 50, wherein the polyphosphate compound comprises at least one phosphate group pendant to the polymer backbone and/or at least one phosphate group incorporated in the polymer backbone,.

53. The compound of claim 49, wherein the polyanion comprises a polyacrylate comprising one or more pendant phosphate groups.

54. The compound of claim 49, wherein the dihydroxyl aromatic group comprises a DOPA or a catechol moiety.

55. The compound of claim 49, wherein the polyanion is the polymerization product between (1) a phosphate acrylate and/or phosphate methacrylate and (2) a second acrylate and/or second methacrylate comprising a dihydroxyl aromatic group covalently bonded to the second acrylate or second methacrylate.

56. The compound of claim 49, wherein the polyanion is the polymerization product between monoacryloxyethyl phosphate and dopamine methacrylamide.

57. A method for repairing a bone fracture in a subject, comprising (a) contacting the fractured bone with the adhesive complex coacervate of claims 1-39 and (b) crosslinking the polycation and polyanion in the coacervate.

58. The method of claim 57, wherein the fracture comprises complete fracture, an incomplete fracture, a linear fracture, a transverse fracture, an oblique fracture, a compression fracture, a spiral fracture, a comminuted fracture, a compacted fracture, an open fracture, an intra-articular fracture, or a craniofacial bone fracture.

59. The method of claim 57, wherein the method comprises adhering a fractured piece of bone to an existing bone.

60. A method for adhering a substrate to a bone of a subject comprising (a) contacting the bone and/or substrate with the adhesive complex coacervate of claims 1-39; (b) applying the substrate to the bone; and (c) crosslinking the polycation and polyanion in the coacervate.

61. The method of claim 60, wherein the substrate comprises a metal substrate, a foil, a fiber, or a piece of cloth.

62. A method for adhering a bone-tissue scaffold to a bone of a subject comprising (a) contacting the bone and/or tissue with the adhesive complex coacervate of claims 1-39; (b) applying the bone-tissue scaffold to the bone and tissue; and (c) crosslinking the polycation and polyanion in the coacervate.

63. The method of claim 62, wherein the tissue comprises cartilage, a ligament, a tendon, a soft tissue, an organ, a membranous tissue, or synthetic derivative thereof.

64. The method of claim 62, wherein the scaffold comprises one or more drugs that facilitate growth or repair of the bone and tissue.

65. The use of the adhesive complex coacervate of claims 1-39 in a dental application.

66. The use of claim 65, wherein the use comprises treating a dental defect.

67. A method for securing a dental implant, comprising (a) applying to an oral substrate and/or dental implant the adhesive complex coacervate of claims 1-39; (b) attaching the dental implant to the substrate; and (c) crosslinking the polycation and polyanion in the coacervate.

68. A method for delivering one or more bioactive agents comprising administering the adhesive complex coacervate of claims 1-39 to a subject.

69. A method for repairing a corneal and/or conjunctival laceration in a subject, comprising (a) applying to the laceration the adhesive complex coacervate of claims 1-39 and (b) crosslinking the polycation and polyanion in the coacervate.

70. A method for inhibiting blood flow in a blood vessel of a subject comprising (a) introducing the adhesive complex coacervate of claims 1-39 into the vessel and (b) crosslinking the polycation and polyanion in the coacervate.

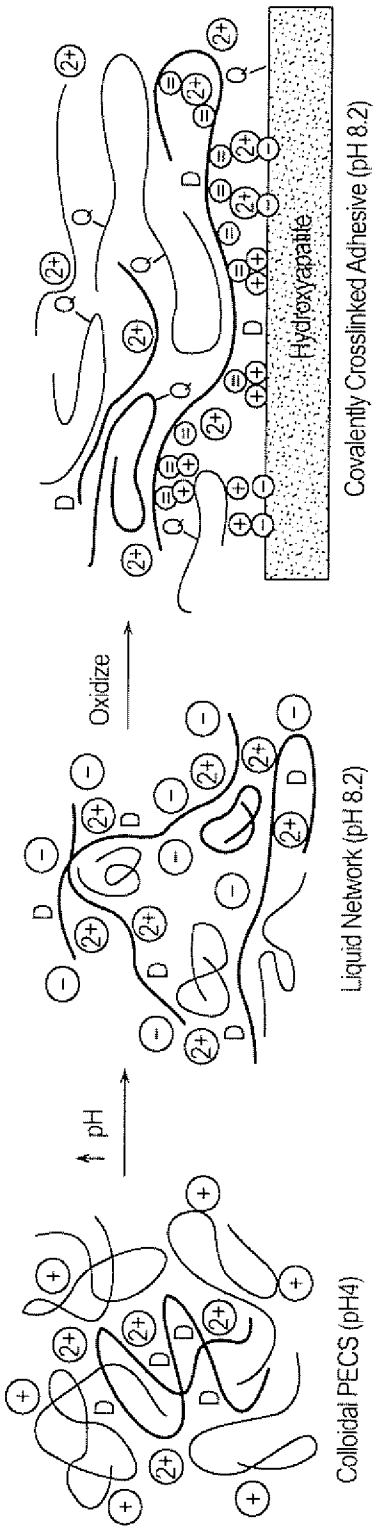


FIG. 1A FIG. 1B FIG. 1C

Pc-1:

MKVFIVLALVSAA YGCGVGIGC

AGGRCGGACG GKGYGYGG-K LGYGAYGKGG

IGGYGYGKGC VGGYGYGGLG AGK-------

LGGYGYGGSK CGGYGYGGQK LGGYGYGGKK

LGGYGYAAKK VGGYGYGAKK VGGYGYGAKK

VGGYGYGAKK VGGYGYGAKK VGGYGYGAKK

VGGYGYGAKK VGGYGYGVKK VGGYGYG---

MW = 18,240

pI = 9.74

K = 13.5 mol%
Y = 17.2 mol%
G = 41.7 mol%

FIG. 2

Pc-1:

MKVFIVLALVSAA YGCGVGIGC
AGGRCGGACG GKGYGYGG-K LGYGAYGKGG
IGGYGYGKGC VGGYGYGGLG AGK-------
LGGYGYGGSK CGGYGYGGQK LGGYGYGGKK
LGGYGYAAKK VGGYGYGAKK VGGYGYGAKK
VGGYGYGAKK VGGYGYGAKK VGGYGYGAKK
VGGYGYGAKK VGGYGYGVKK VGGYGYG---

MW = 18,240
pI = 9.74

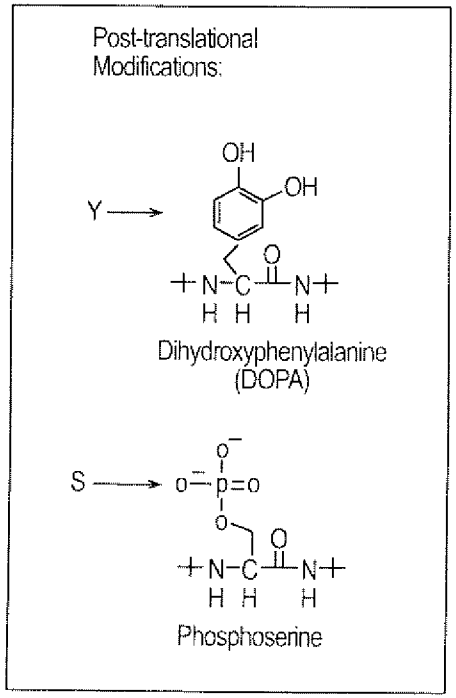


FIG. 3

Pc-2:
MKVLIFLATVAAVYG CGGGG WRSGSCGG
RWGHPAV----HKALGGYG-G
MGAHPAVHAAVHKALGGYGAGAYGAGA
WG-HPAV----HKALGGYGAGA
WG-HPAV----HKALGGYG-G
YGAHPAVHVAVHKALGGYGAGACGHKTGGYGG
YGAHP---VAV-KA--AY-NHGFNYGANNAIKSTKRFGG
YGAHP---V-VKKAFSRGLSHGAY-AG
SKAATGYGYGSGKAAGGYGY

MW = 21,116
pI = 9.91

Pc-3A:
MKLLSVFAIVVLAVYITHVEA
DSSSSSTSSSSSYSSSSSSSSSSSSSYSSSSSSYSSSSSS
SYSSSSSSYSSSSSYSSSSSYSSSSSYSSSSSILTSTS
SSDWKRKVPARRVLRTRRFLKCVTRCTLRCTLFRSAKT
CARKCSRRCLKRVF

MW = 13,979
pI = 2.5

polyphosphoserines

Pc-3H:
MKSFTIFAAILVALCYIQISEAG
CCKRYSSSSYSSSSSSSSSSYSSSSSSSSSYSSSSSSSS
SYSSSSSSSSSSYSSSSSSYSSSSSSSSYSSSSSSSSSS
YSSSSSSSYSSSSSSSSSYSSSSSSSSSSYSSSSSSSSS
YSSSSSSSYSSSSSSSSYSSSSSSSSSSSYSSSSSSSYSSSS
SSSSSSYSSSSSSSSSSYSSSSSSSSYSSSSSSSYSSSSSS
SSSSSYSSSSSSSSSSSYSSSSSSSSSSSSYSSSSS
SSSSSYSSSSSSSSSSSYSSSSSSSSSSSSSSYSSSS
SSSSSYSSSSSSSSSSSYSSSSSSSSSSSSSSSSYSS
SSSSSSSSSSSSYSSS

MW=30.525
pI=2.5

Pc-4:
MPTLYKKVGKLVILAIIVTVASVASA
GYPTYSPSGGTHSGYNGPHGNVVKK
TYRGPYGAGAAK
AWNGYHGAGYTSVHHGPASTSWHTS
WSNKKGGYGYGYGLK
---NK-GYGYGLKKVGY
-GVGL-------HAAGW
HGVGPYGAGY--HGAGW
NGLGYHGAGYGV
HGVGLHGAGYGL
HGVGLHGAGVGL
H = 12.6
Y = 10.6
G = 33.7
HGVGLHGAGYGL
HGVGLHGAGYGL
HGVGLHGAGYGL
HGVGLHGAGYGL
HGVGLHGAGYGL
HGVGLHGAGCGIHKTACY
-GVGLHG-----HY
MW = 24,330
pI = 9.49
E
histidinyl-glycine
F
vinylimidazole
acrylamide
pK = 6.0

FIG. 4

Pc-4: MW = 24,330 pI =9.49
MPTLYKKVGKLVILAIIVTVASVASA
GYPTYSPSGGTHSGYNGPHGNVVKK
TYRGPYGAGAAK
AWNGYHGAGYTSVHHGPASTSWHTS
WSNKKGGYGYGLK
---NK-GYGYGLKKVGY
-GVGL------HAAGW
HGVGPYGAGY--HGAGW
NGLGYHGAGYGV HGVGLHGAGYGL
HGVGLHGVGYGL HGVGLHGAGYGL
HGVGLHGVGYGL HGVGLHGAGYGI
HGVGLHGVGYGL HGVGLHGAGYGL
HGVGLHGVGYGL HGVGLHGAGCGIHKTACY
-GVGLHG-----HY

Pc-5: MW = 14,963 pI = 8.34
MKFLVLLALVASASA
YYPLMGGF
HGGWHAPMVHGGLY
HGGWHAPMVHGGLY
HGGWHAPIV
HGGWHAPVF
----HAPAPIHTVSHSVVN
----HVPMMPM
---WHHPAPAPAPAPRP
GRIIILGGGKYGPFGKYGGG
AGLLALGALGGNGGFWKRR

Pc-6: MW = 37,763 pI = 8.25
METLFYNANFVQKSWVLILLGLAAVVA
CSEYDKGLGGYGRPSYGGRRGYGGRRGLQYHGK
YQGRCEYDGLYFRDEKSFVYCSNRNSYIQPCAP
GTRNSPYTKYNRGSKYNYRDFCEFNLVDSGYVP
KPGYLPAPKKAYPTKVYDL
KVDYAP KVDYAP KVDYAP KVDYAP
KVDYAPAKASVVPPKASYVDPTPTYGYEAPFK
GGYDKPSYGKDVDTSYESKTTYTVEKTAD
KGYGKGYGDKEISAKKSYTLTEKRDYDT
GYDNSRSDEDSKEY
GYDNDRSESYERTESYTDERTDGYGTQK
VEYTQQSEYDRVTRRGIWLHKGTEVEHVLY

Pc-7: MW = 15,073 pI = 8.50
MNTFVVIAAIVAVAA
CSGGYDGRQYTYRGR
YNNKCGNDGLYFKDDKNFXFCSN
GNSYVQPCAPGTRNS
GYNNYKQGSIYNYRDFCDVNLVDE
GYGVGAKPGYNKGYNP
GYNPGYGGYNPGYSYT
GYGGYKAGPGPYW

Pc-8: MW = 16,772 pI = 10.29
MSNAFLXCQLCTKKLALLLLVAVCAAVAVNA
CGPLGCS GGYGGVLK
CGVGGCALGGYGGGYSAGIGGYGIK
RLGCRGGRCGLRRRVGCRGGRCGLRG
RLGCRGCROGLR KLGCRGGRCGLRG
RLGCRGGROGLRKRLGCRGGR
GRGGYGGGYGGVCSKGVCGGYPAYGK

FIG. 5

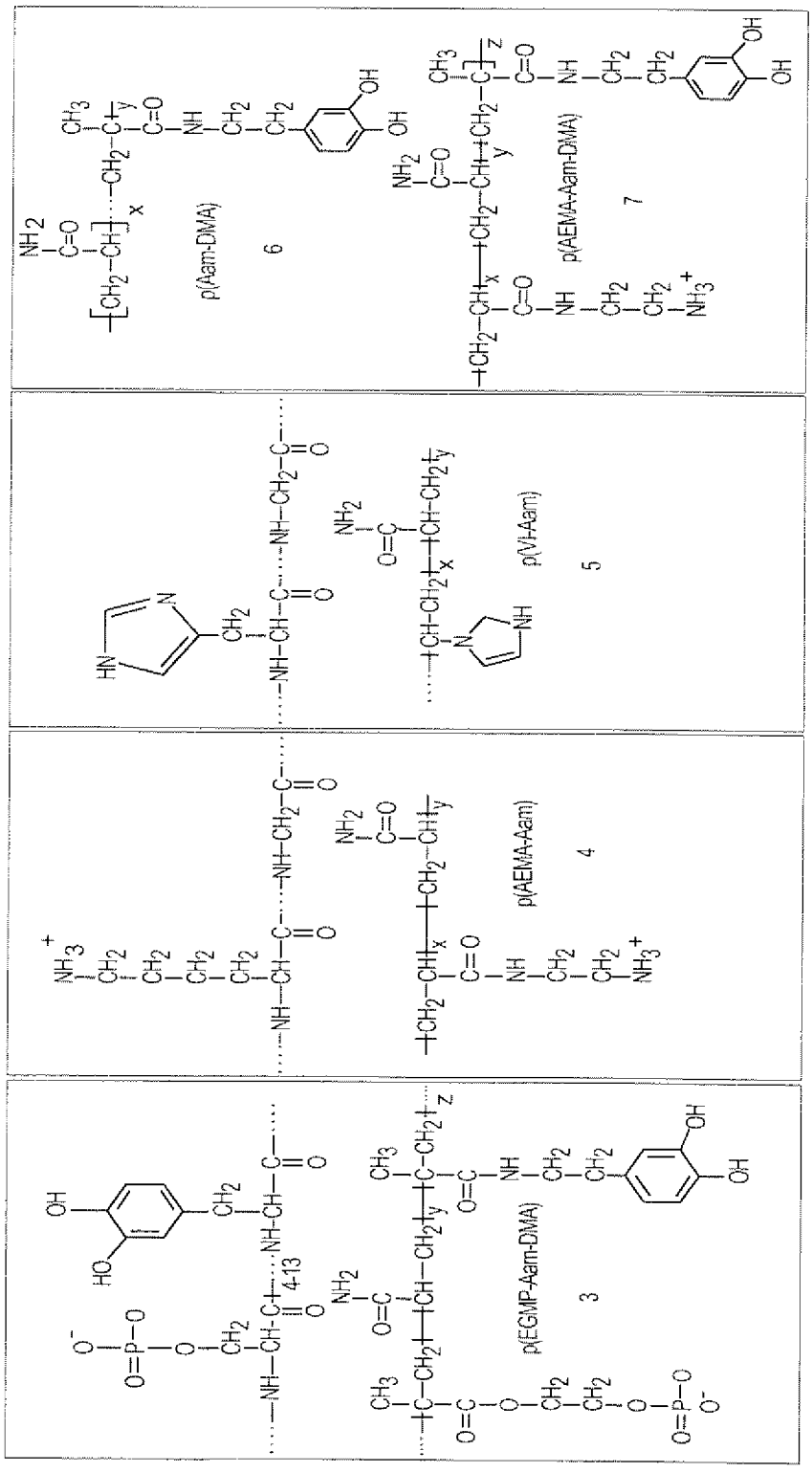
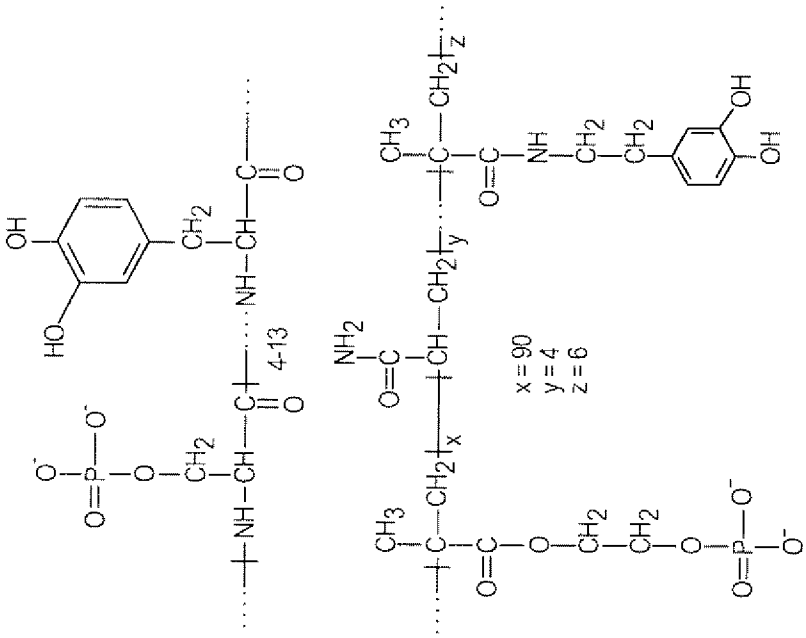


FIG. 6A FIG. 6B FIG. 6C FIG. 6D



Pc-3B:

MKSFTIFAALLVALCYIQISEAG

CCKRYSSSSYSSSSSSSSSSYSSSSSSSSSSS

SYSSSSSSSSSSYSSSSSSSSYSSSSSSSSSS

YSSSSSSYSSSSSSSSSSYSSSSSSSSSSS

YSSSSSSYSSSSSSSSSSSSSYSSSSSSYSSS

SSSSSYSSSSSSSSSSYSSSSSSSSSYSSSSS

SSSSYSSSSSSSSSSSYSSSSSSSSSYSSSS

SSSSYSSSSSSSSSSSYSSSSSSSSSSYSSS

SSSSYSSSSSSSSSSSYSSSSSSSSSSSYSS

SSSSSSSSSSSSSS

MW = 30,525

pI = 2.5

Post-translational Modividations:

Dihydroxyphenylalanine (DOPA)

Phosphoserine

FIG. 7

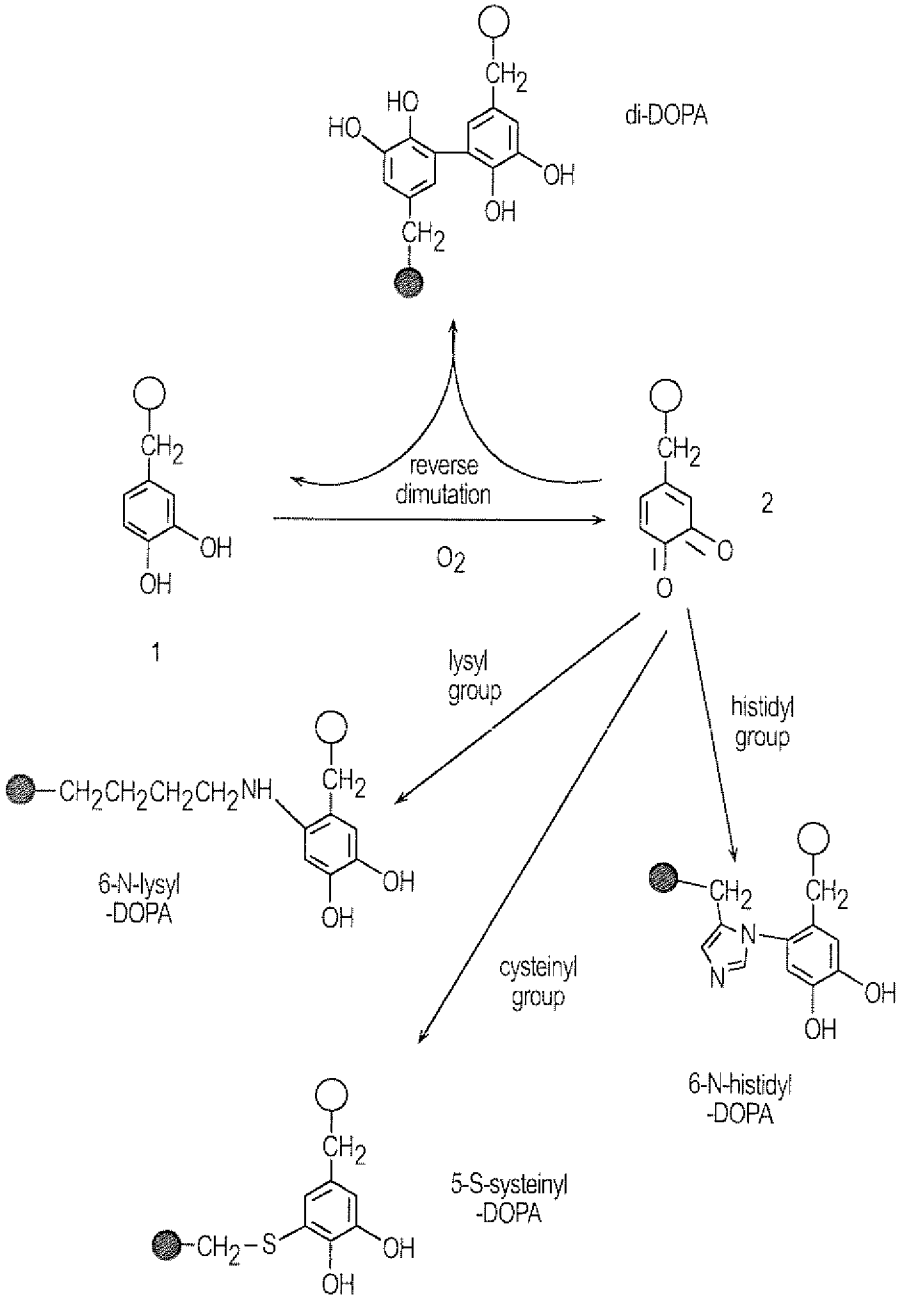

di-DOPA
reverse
dimutation
O_2
1
2
lysyl
group
histidyl
group
cysteinyl
group
6-N-lysyl
-DOPA
6-N-histidyl
-DOPA
5-S-systeinyl
-DOPA

FIG. 8

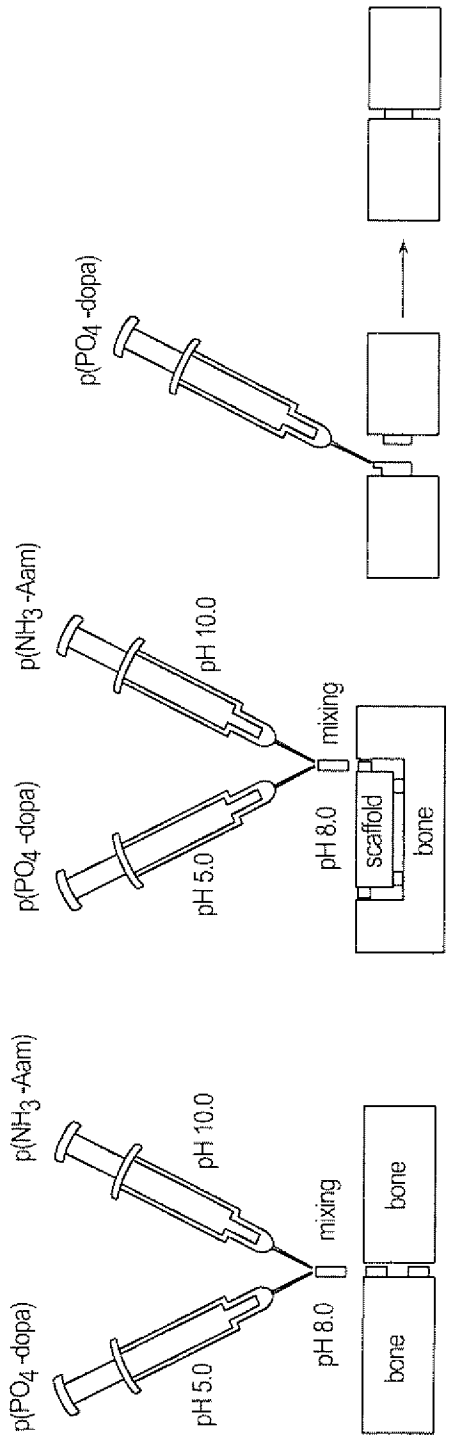


FIG. 9A

FIG. 9B

FIG. 9C

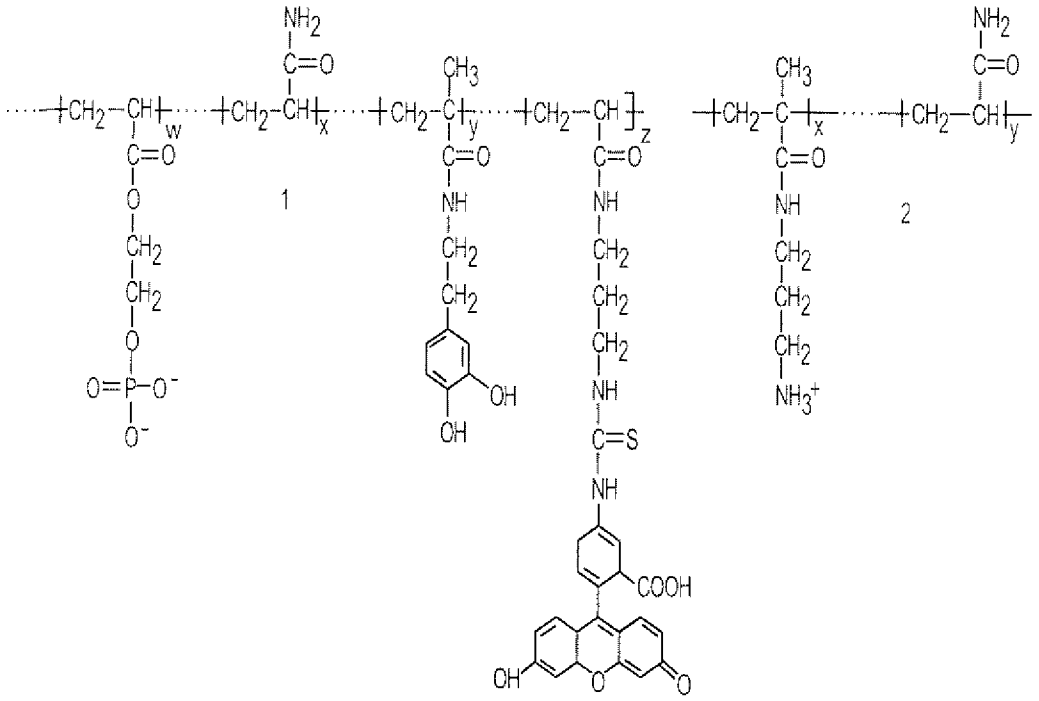


FIG. 10A

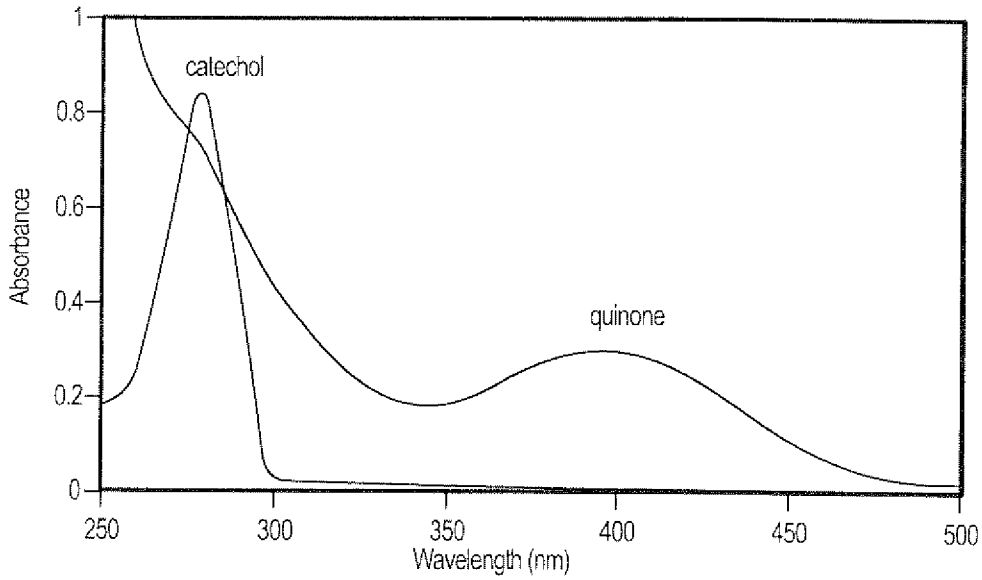


FIG. 10B

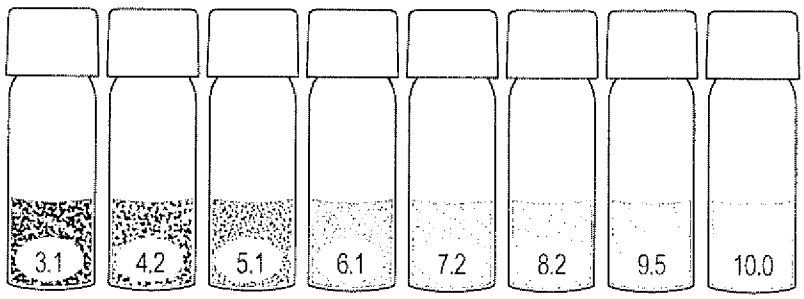


FIG. 11A

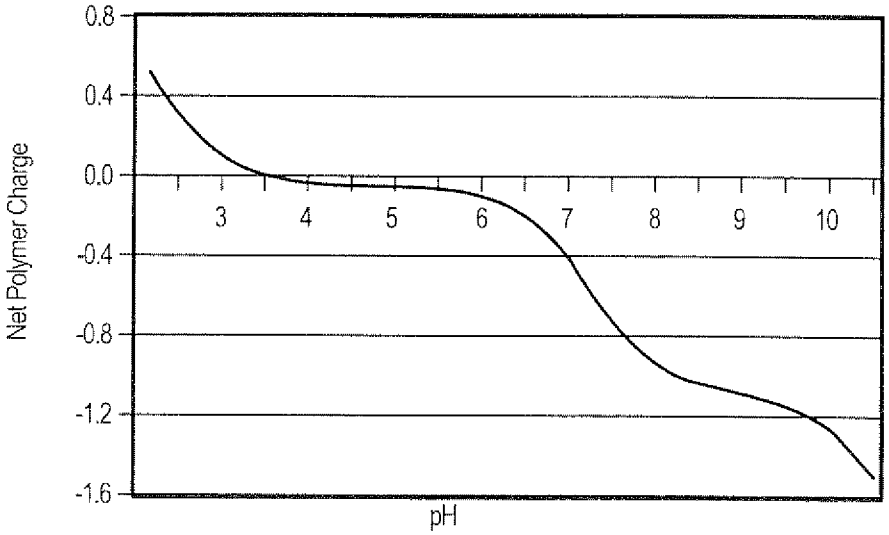


FIG. 11B

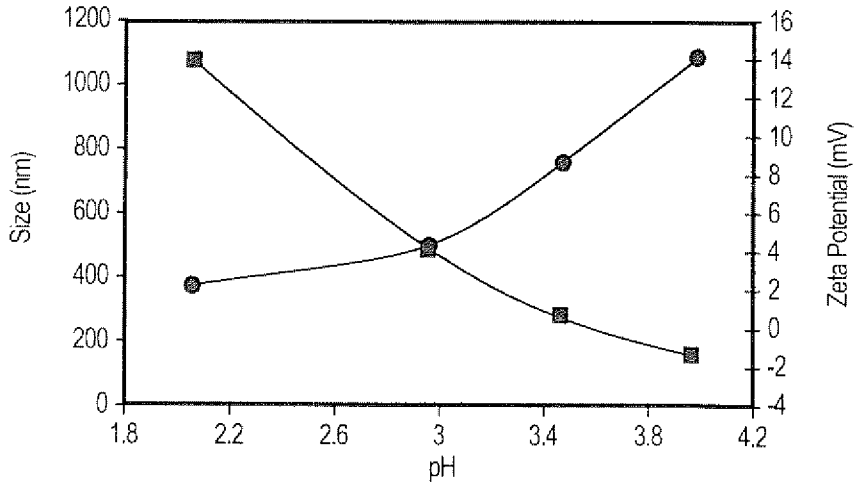


FIG. 11C

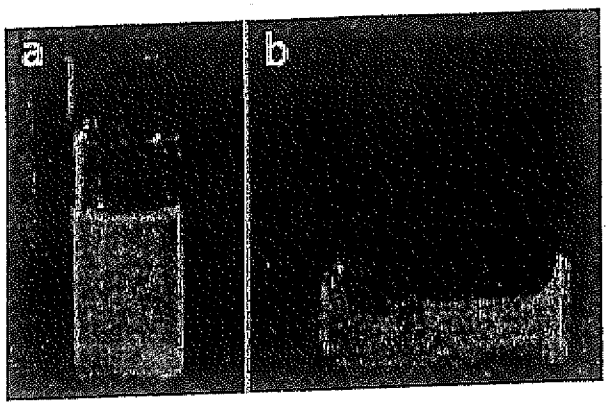


FIGURE 12

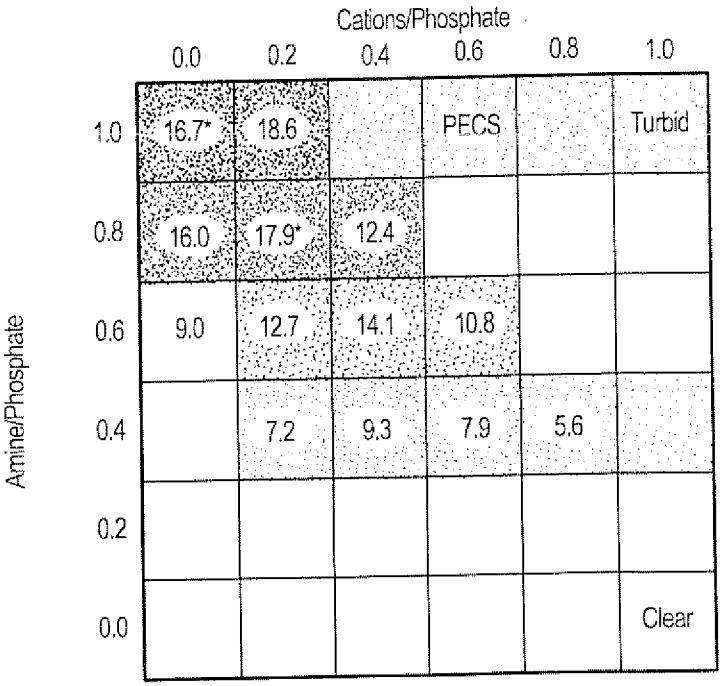


FIG. 13

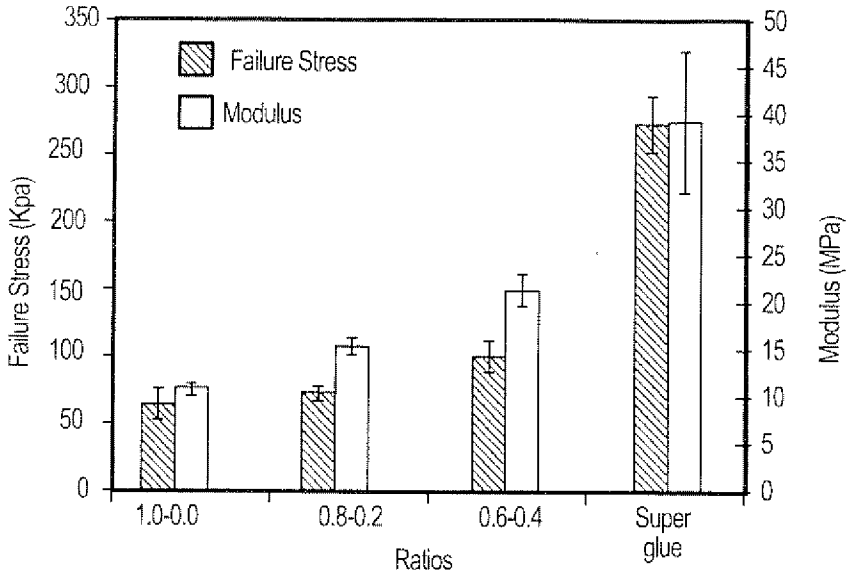


FIG. 14A

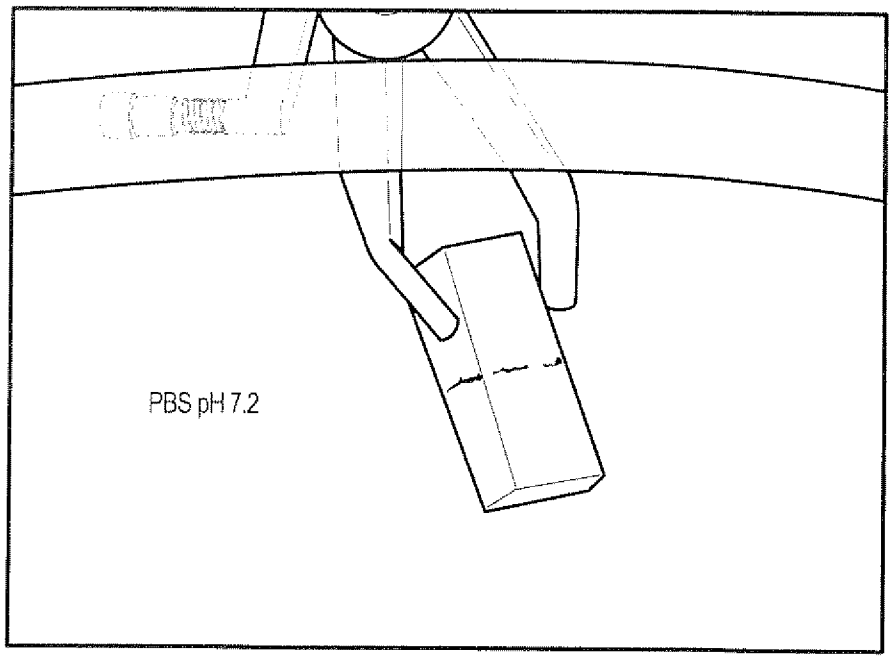


FIG. 14B

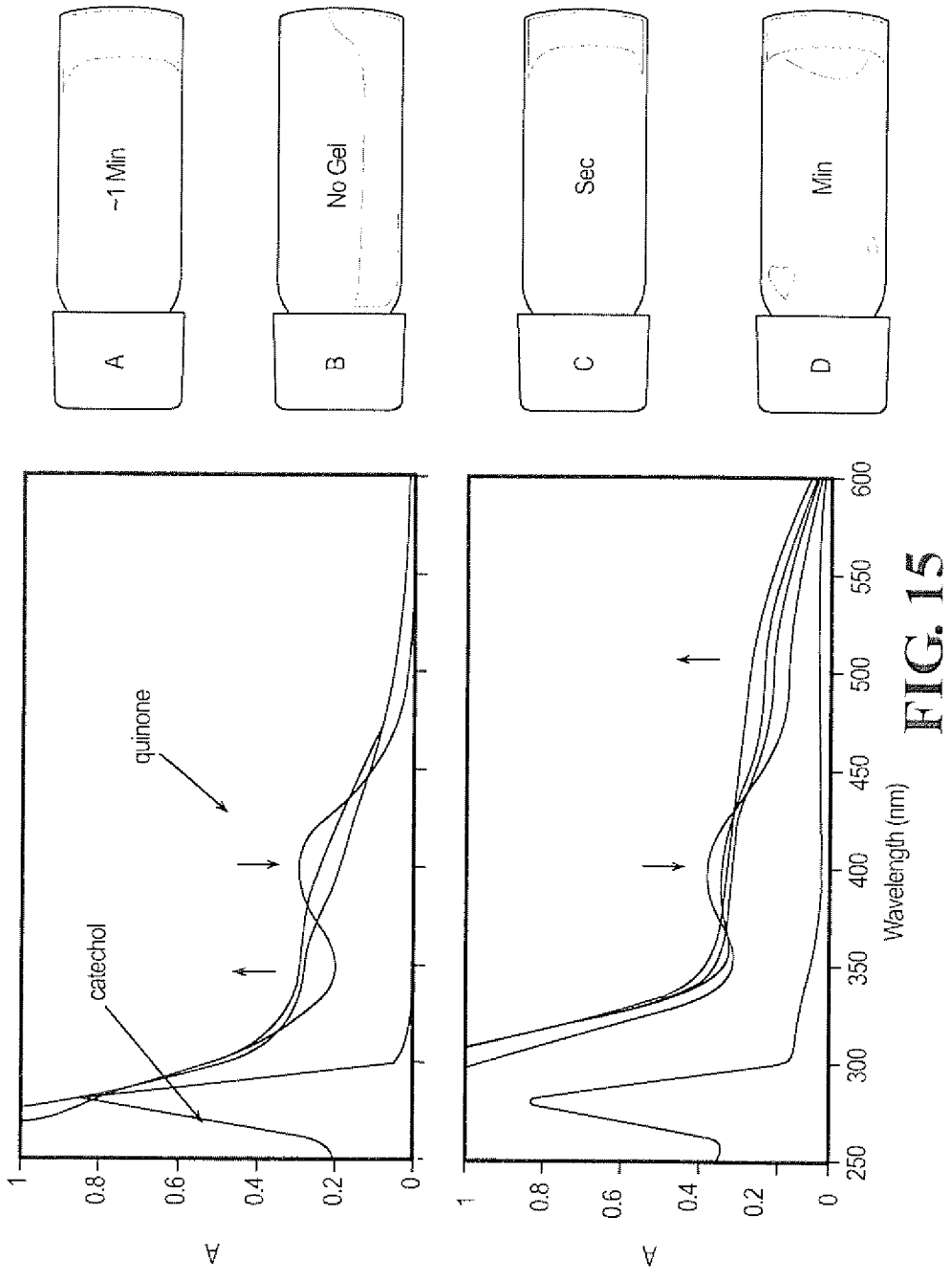

A
~1 Min
B
No Gel
C
Sec
D
Min
quinone
catechol
A
1
0.8
0.6
0.4
0.2
0
250
300
350
400
450
500
550
600
Wavelength (nm)

FIG. 15

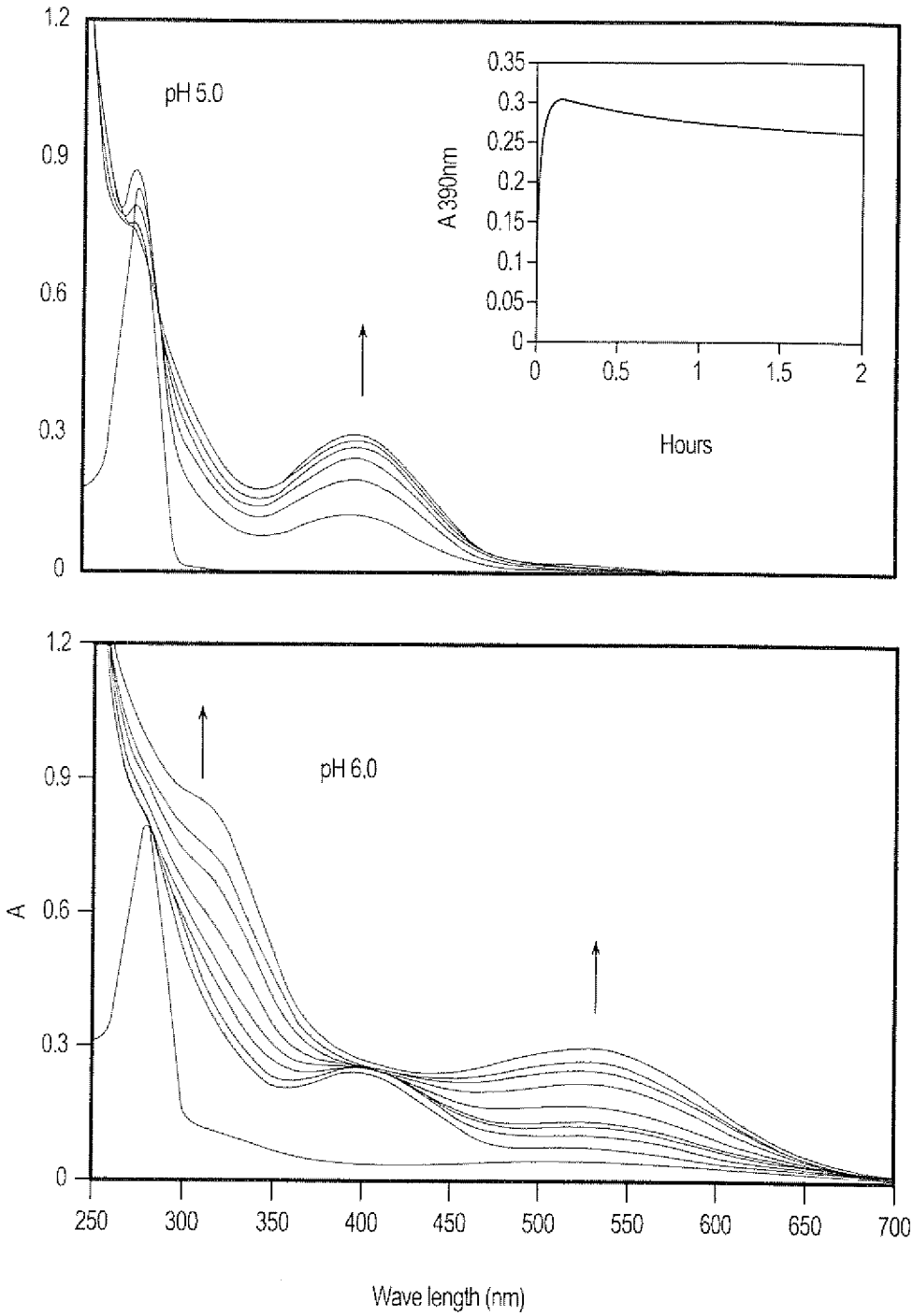


FIG. 16

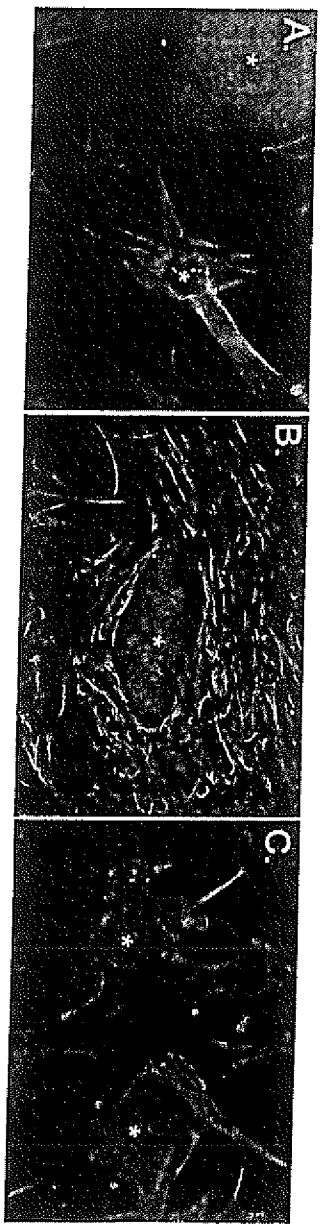


FIGURE 17

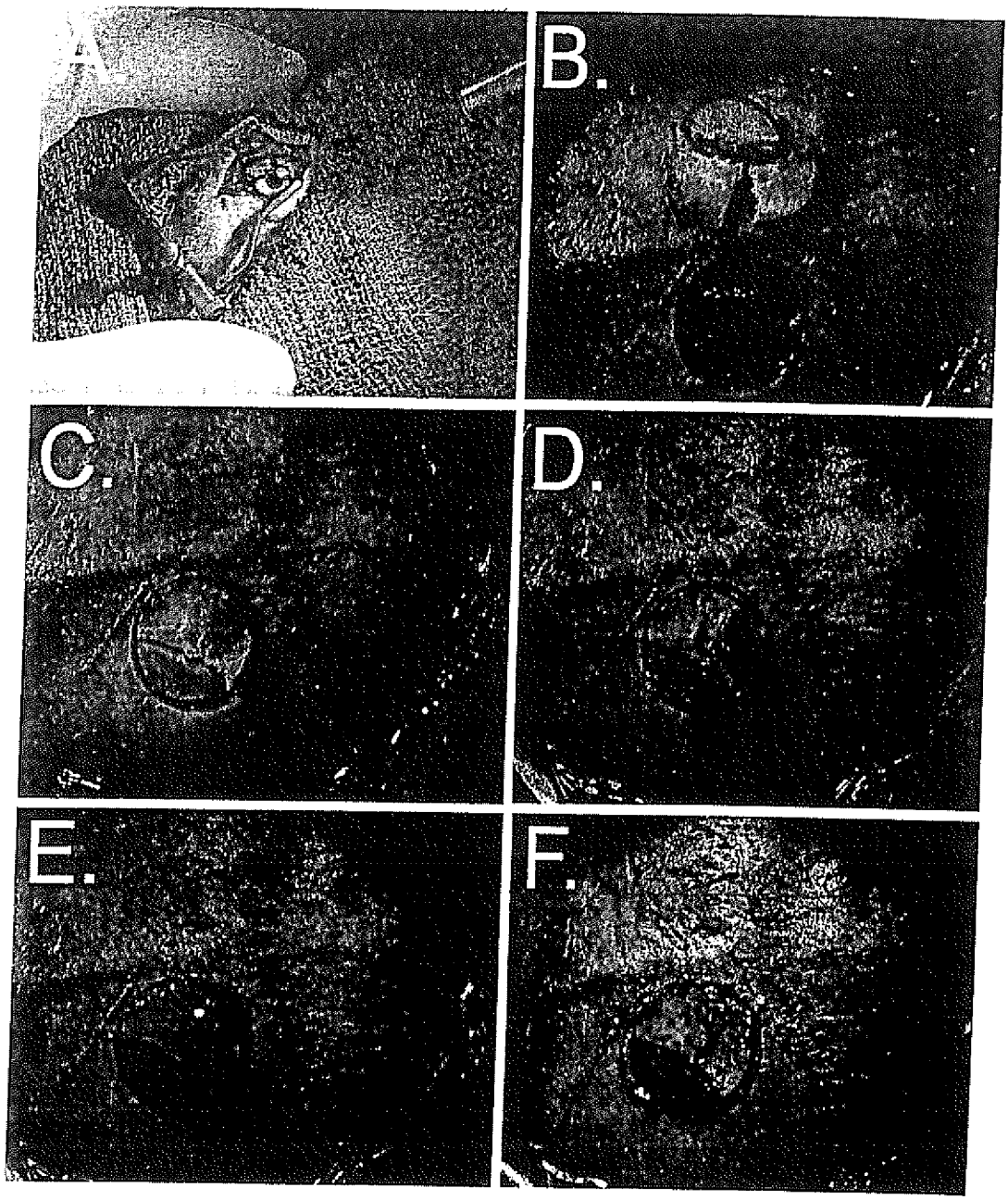


FIGURE 18

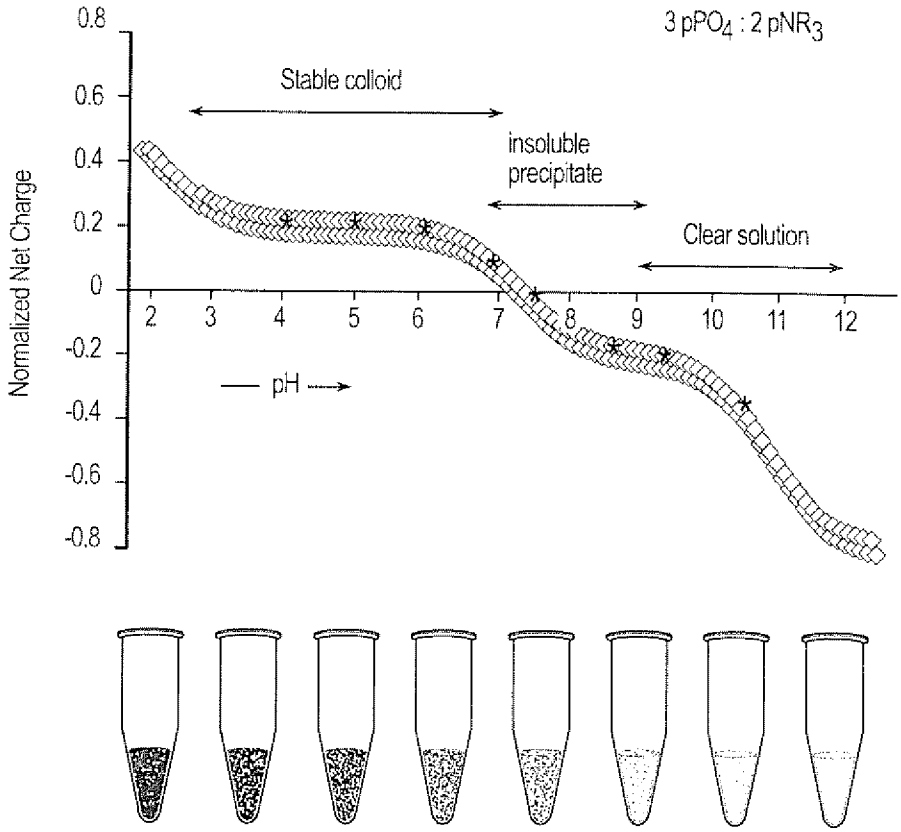
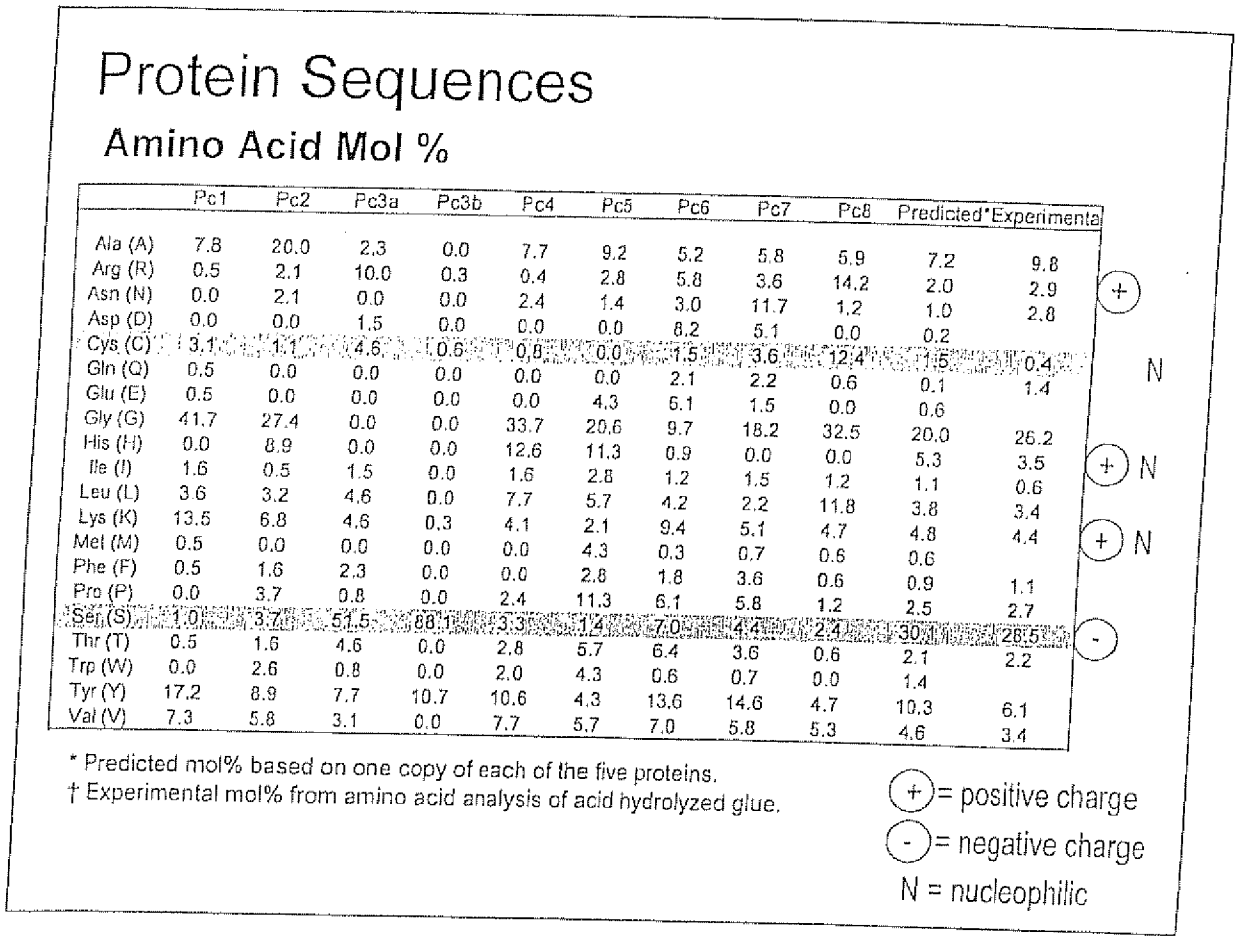

3 pPO4 : 2 pNR3
Stable colloid
insoluble precipitate
Clear solution
Normalized Net Charge
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
2
3
4
5
6
7
8
9
10
11
12
pH

FIG. 19



Protein Sequences

Amino Acid Mol %

	Pc1	Pc2	Pc3a	Pc3b	Pc4	Pc5	Pc6	Pc7	Pc8	Predicted*	Experimental†	
Ala (A)	7.8	20.0	2.3	0.0	7.7	9.2	5.2	5.8	5.9	7.2	9.8	
Arg (R)	0.5	2.1	10.0	0.3	0.4	2.8	5.8	3.6	14.2	2.0	2.9	⊕
Asn (N)	0.0	2.1	0.0	0.0	2.4	1.4	3.0	11.7	1.2	1.0	2.8	
Asp (D)	0.0	0.0	1.5	0.0	0.0	0.0	8.2	5.1	0.0	0.2		
Cys (C)	3.1	1.1	4.6	0.6	0.8	0.0	1.5	3.6	12.4	1.5	0.4	N
Gln (Q)	0.5	0.0	0.0	0.0	0.0	0.0	2.1	2.2	0.6	0.1	1.4	
Glu (E)	0.5	0.0	0.0	0.0	0.0	4.3	5.1	1.5	0.0	0.6		
Gly (G)	41.7	27.4	0.0	0.0	33.7	20.6	9.7	18.2	32.5	20.0	26.2	
His (H)	0.0	8.9	0.0	0.0	12.6	11.3	0.9	0.0	0.0	5.3	3.5	⊕ N
Ile (I)	1.6	0.5	1.5	0.0	1.6	2.8	1.2	1.5	1.2	1.1	0.6	
Leu (L)	3.6	3.2	4.6	0.0	7.7	5.7	4.2	2.2	11.8	3.8	3.4	
Lys (K)	13.5	6.8	4.6	0.3	4.1	2.1	9.4	5.1	4.7	4.8	4.4	⊕ N
Met (M)	0.5	0.0	0.0	0.0	0.0	4.3	0.3	0.7	0.6	0.6		
Phe (F)	0.5	1.6	2.3	0.0	0.0	2.8	1.8	3.6	0.6	0.9	1.1	
Pro (P)	0.0	3.7	0.8	0.0	2.4	11.3	6.1	5.8	1.2	2.5	2.7	
Ser (S)	1.0	3.7	51.5	88.1	3.3	1.4	7.0	4.4	2.4	30.1	28.5	⊖
Thr (T)	0.5	1.6	4.6	0.0	2.8	5.7	6.4	3.6	0.6	2.1	2.2	
Trp (W)	0.0	2.6	0.8	0.0	2.0	4.3	0.6	0.7	0.0	1.4		
Tyr (Y)	17.2	8.9	7.7	10.7	10.6	4.3	13.6	14.6	4.7	10.3	6.1	
Val (V)	7.3	5.8	3.1	0.0	7.7	5.7	7.0	5.8	5.3	4.6	3.4	

* Predicted mol% based on one copy of each of the five proteins.

† Experimental mol% from amino acid analysis of acid hydrolyzed glue.

⊕ = positive charge

⊖ = negative charge

N = nucleophilic

FIG. 20

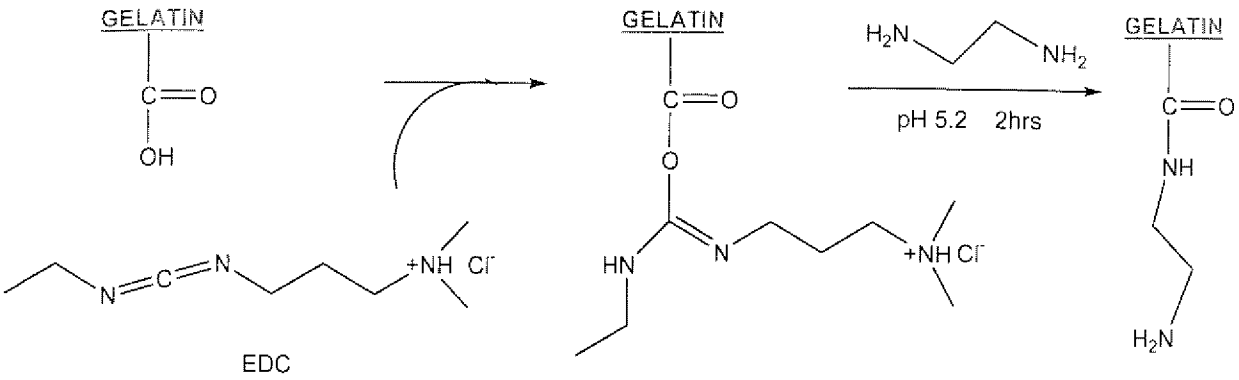

GELATIN
C=O
OH
N=C=N
+NH Cl⁻
EDC
GELATIN
C=O
O
HN
N
+NH Cl⁻
H₂N
NH₂
pH 5.2 2hrs
GELATIN
C=O
NH
H₂N

FIGURE 21

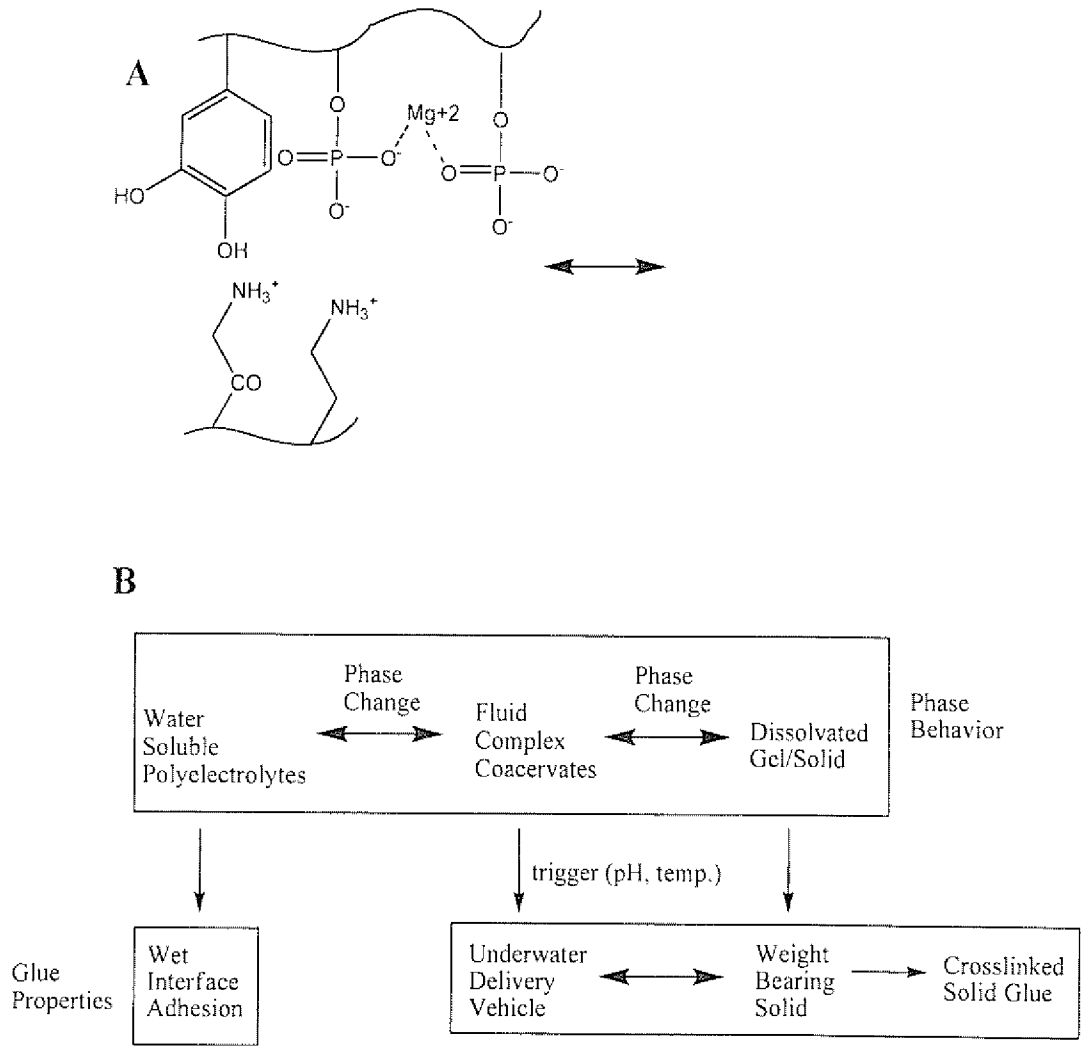

A
HO
OH
O
Mg+2
O
O
O
O
O
O
O
O
NH3+
NH3+
CO
B
Water Soluble Polyelectrolytes
Phase Change
Fluid Complex Coacervates
Phase Change
Dissolvated Gel/Solid
Phase Behavior
trigger (pH, temp.)
Glue Properties
Wet Interface Adhesion
Underwater Delivery Vehicle
Weight Bearing Solid
Crosslinked Solid Glue

FIGURE 22

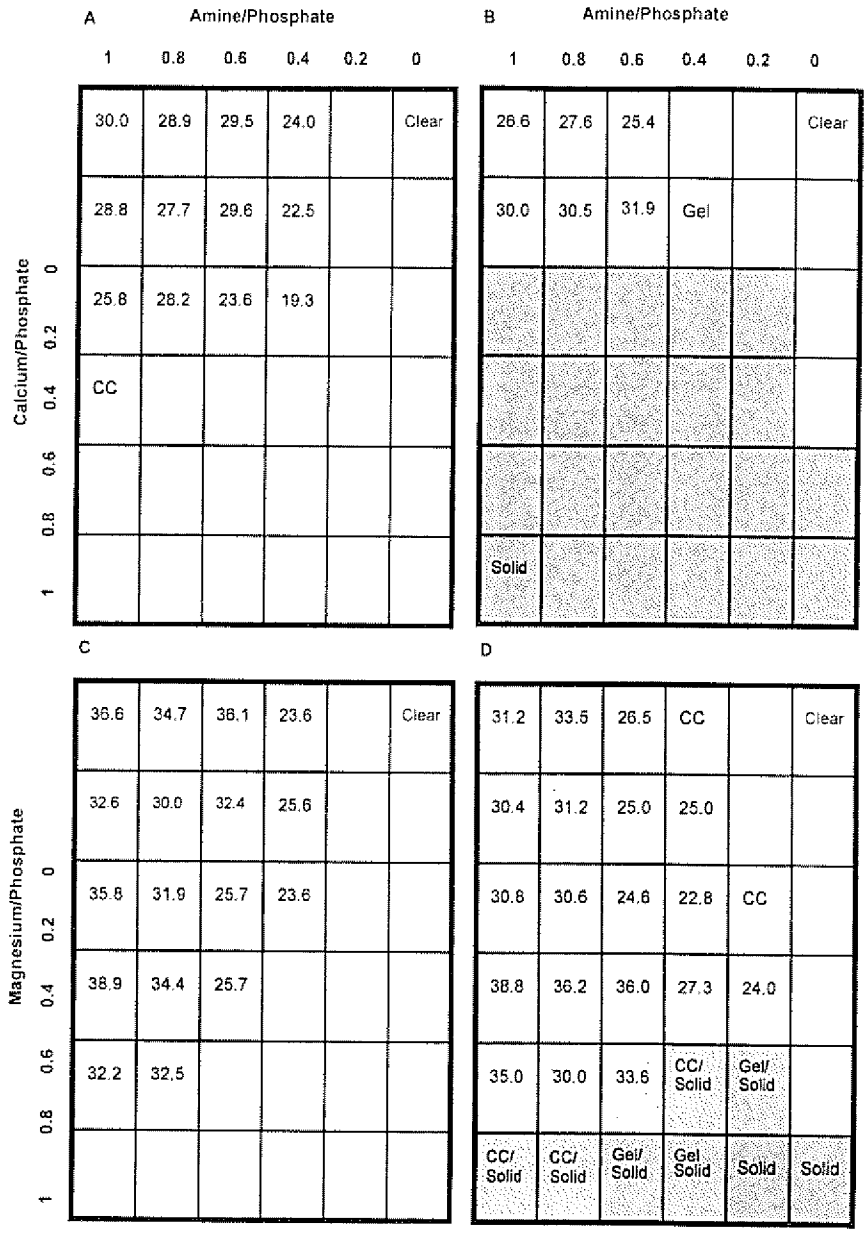

A
Amine/Phosphate
1 0.8 0.6 0.4 0.2 0
Calcium/Phosphate
0 0.2 0.4 0.6 0.8 1
30.0 28.9 29.5 24.0 Clear
28.8 27.7 29.6 22.5
25.8 28.2 23.6 19.3
CC
B
Amine/Phosphate
1 0.8 0.6 0.4 0.2 0
26.6 27.6 25.4 Clear
30.0 30.5 31.9 Gel
Solid
C
Magnesium/Phosphate
0 0.2 0.4 0.6 0.8 1
36.6 34.7 36.1 23.6 Clear
32.6 30.0 32.4 25.6
35.8 31.9 25.7 23.6
38.9 34.4 25.7
32.2 32.5
D
31.2 33.5 26.5 CC Clear
30.4 31.2 25.0 25.0
30.8 30.6 24.6 22.8 CC
38.8 36.2 36.0 27.3 24.0
35.0 30.0 33.6 CC/ Solid Gel/ Solid
CC/ Solid CC/ Solid Gel/ Solid Gel Solid Solid Solid

FIGURE 23

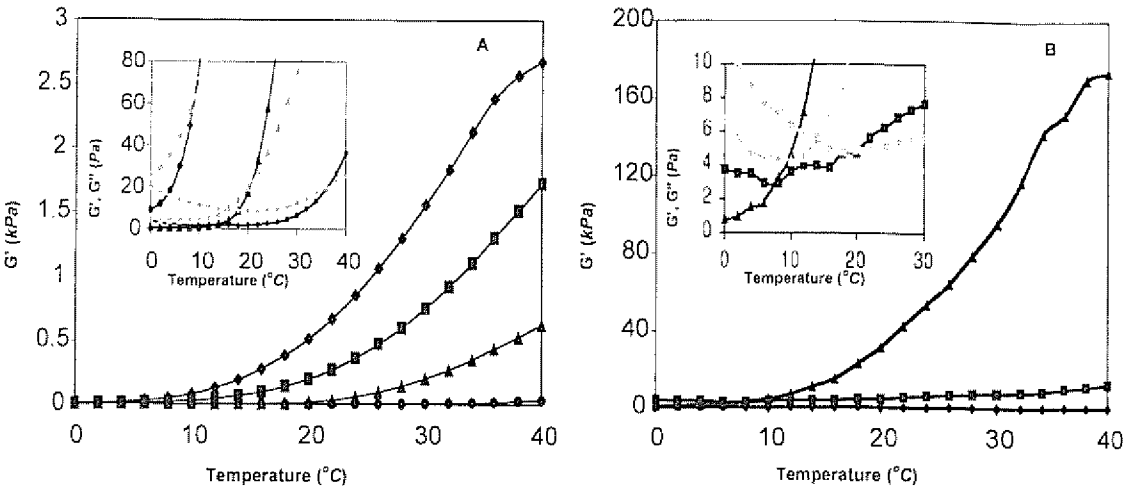


FIGURE 24

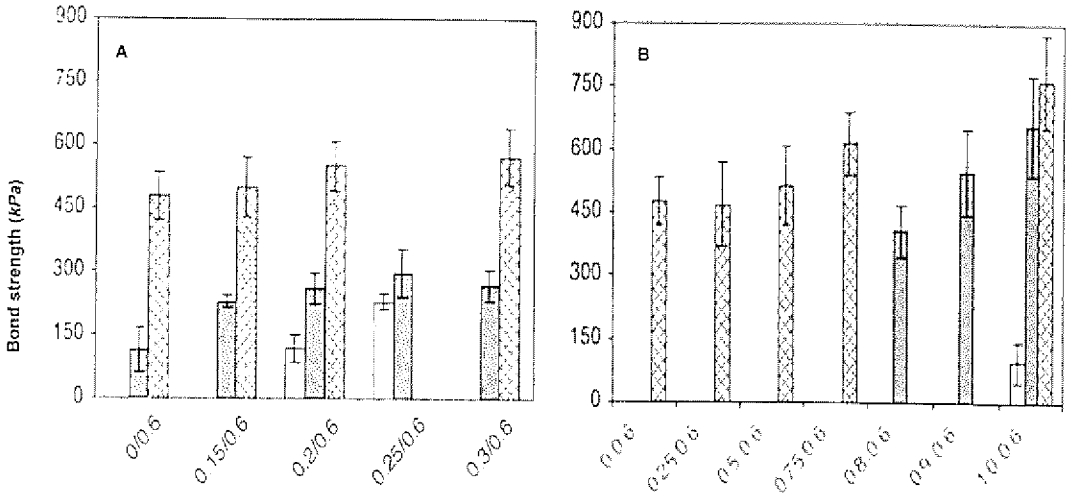

A
B
Bond strength (kPa)
900
750
600
450
300
150
0
0/0.6
0.15/0.6
0.2/0.6
0.25/0.6
0.3/0.6

FIGURE 25

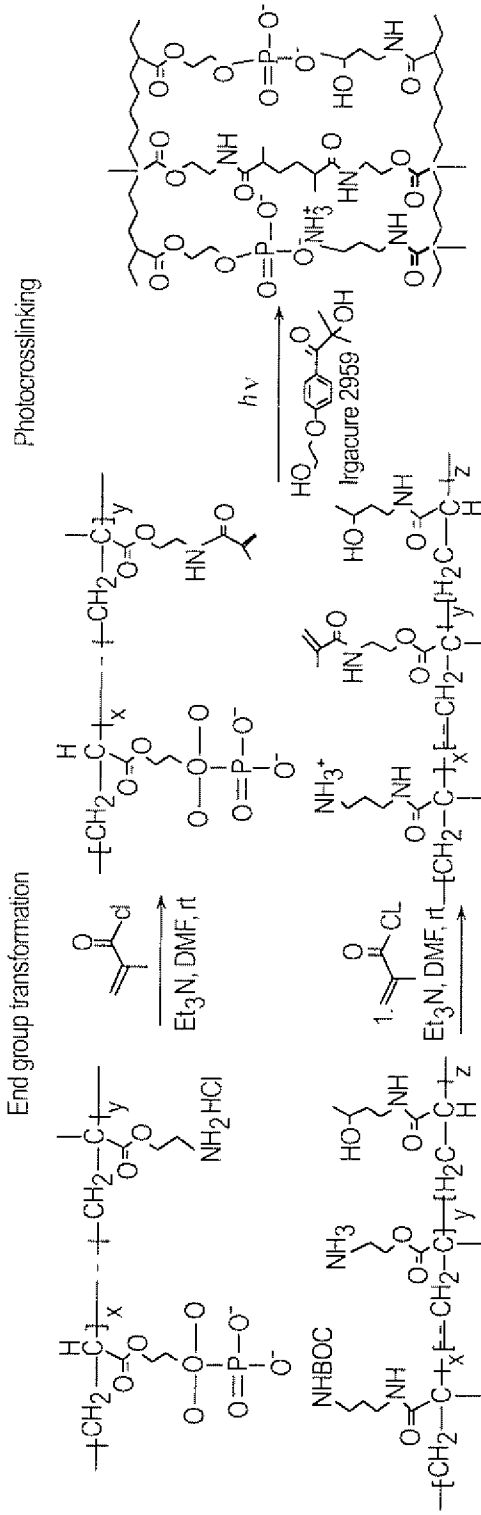

End group transformation
Et3N, DMF, rt
Et3N, DMF, rt
1.
2, Deprotect with TFA
Photocrosslinking
hν
Irgacure 2959

FIG. 26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/43009

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 47/30; A61P 19/08; C09J 123/26 (2010.01)
USPC - 514/772.1
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)
IPC- A61K 47/30; A61P 19/08; C09J 123/26 (2010.01);
USPC- 514/772.1

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest (US Pat, PgPub, EPO, JPO: classification, keyword), GoogleScholar; search terms: coacervate, anion, cation, polyanion, polycation, crosslink, adhesive, adhesion, adhere, amine, gelatin, ethylene diamine, phosphorylate, saccharide, polysaccharide, biodegrade, nucleophile, electrophile, initiate, actinic, riboflavin, eosin, bengal, bone, tiss

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2006/0122290 A1 (HUBBELL et al.) 08 June 2006 (08.06.2006), [0010], [0028], [0029], [0034], [0036], [0041]-[0047], [0050]-[0060], [0068], [0072], [0073], [0081], [0110], [0114]	1-48, 57-70
Y	US 2001/0056301 A1 (GOUPIL et al.) 27 December 2001 (27.12.2001), para [0002], [0011], [0022]-[0025], [0028], [0035], [0041], [0061], [0065], [0066], [0077], [0087], [0093], [0094], [0099], [0100], [0104], [0189], [0198]	1-48, 57-70
Y	WO 2007/030811 A2 (GUILAK et al.) 15 March 2007 (15.03.2007), pg 4, 5, 14, 51-53	57-67
Y	US 2007/0191273 A1 (AMBATI et al.) 16 August 2007 (16.08.2007), para [0006], [0108], [0131]	69
A, P	WO 2009/094060 A1 (STEWART et al.) 30 July 2009 (30.07.2009), entire document	1-48, 57-70
A	US 2009/0162407 A1 (BIGGS et al.) 25 June 2009 (25.06.2009), entire document	1-48, 57-70
A	US 2006/0183848 A1 (MAIER et al.) 17 August 2006 (17.08.2006), entire document	1-48, 57-70
A	US 2006/0007528 A1 (CAO et al.) 12 January 2006 (12.01.2006), entire document	1-48, 57-70
A	US 2005/0220751 A1 (CHARMOT et al.) 06 October 2005 (06.10.2005), entire document	1-48, 57-70
A	US 6,916,488 B1 (MEIER et al.) 12 July 2005 (12.07.2005), entire document	1-48, 57-70
A	US 2005/0147580 A1 (CONNOR et al.) 07 July 2005 (07.07.2005), entire document	1-48, 57-70

☒ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"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
"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
"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
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 November 2010 (08.11.2010)	22 NOV 2010
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/43009

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see Extra Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-48, 57-70

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/43009

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2002/0164364 A1 (QUONG) 07 November 2002 (07.11.2002), entire document	1-48, 57-70
A	US 3,950,296 A (KANGAS et al.) 13 April 1976 (13.04.1976), entire document	1-48, 57-70
A	US 3,947,396 A (KANGAS et al.) 30 March 1976 (30.03.1976), entire document	1-48, 57-70

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/43009

Box No. III, Observations where unity of invention is lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1-48 and 57-70: directed to a biodegradable adhesive complex coacervate comprising at least one polyacation and at least one polyanion, wherein at least one polycation and/or polyanion is a biodegradable, and the polycation and polyanion comprises at least one group capable of crosslinking with each other.

Group II: claims 49-56: directed to a compound comprising a polyanion or polycation comprising at least one dihydroxyl aromatic group capable of undergoing oxidative crosslinking, wherein the dihydroxyl aromatic group is covalently attached to the polyanion or polycation.

The inventions listed as Groups I - II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Group I does not include the inventive concept of a compound comprising a polyanion or polycation comprising at least one dihydroxyl aromatic group covalently attached to the polyanion or polycation, as required by Group II.

Group II does not include the inventive concept of a biodegradable adhesive complex coacervate comprising at least one polyacation and at least one polyanion, wherein at least one polycation and/or polyanion is a biodegradable, as required by Group I.

Groups I and II share the technical feature of a group capable of crosslinking with each other. However, this shared technical feature does not represent a contribution over the prior art of US2006/0183848 to Maier et al. (17 August 2006), which teaches an adhesive composition (para [0063]) that oxidatively crosslinks via dihydroxyl groups (para [0001], [0035]). As the above functionality was known, as evidenced by the teaching of Maier, this cannot be considered a special technical feature that would otherwise unify the groups.

Groups I and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.