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Bionanocomposites: Green sustainable materials for the near future*

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Abstract: Bionanocomposites are a novel class of nanosized materials. They contain the constituent of biological origin and particles with at least one dimension in the range of 1–100 nm. There are similarities with nanocomposites but also fundamental differences in the methods of preparation, properties, functionalities, biodegradability, biocompatibility, and applications. The article includes two parts. Bionanocomposite definition and classification along with nanoparticles, biomaterials, and methods of their preparation are initially reviewed. Then, novel approaches developed by our team are presented. The first approach concerns the preparation of bionanocomposites from chitosan and nanoparticles. It is based on the regulated charging of polysaccharide by the gradual shift of solution pH. When charges appear, the biomacromolecules come into the electrostatic interactions with negatively charged nanoparticles that cause the jellification of solutions. It is also applied to form films. They have a nacre-like structure from stacked planar nanoparticles separated by aligned biomacromolecules. The second approach deals with the biomimicking mineralization of biopolymers by using a novel silica precursor. Its advantage over the current sol-gel processing is in the compatibility and regulation of processes and structure of generated silica. Another example of the mineralization is presented by titania. Syntheses are performed in anhydrous ethylene glycol. Processes and structure of bionanocomposites are regulated by water that is added in an amount to only hydrate functional groups in the carbohydrate macromolecule.

Keywords: biocomposites; biomaterials; biomimetic; bionanocomposites; chitosan; composite materials; nanoparticles; nanocomposites; polysaccharides; proteins; silica; sol-gel.

INTRODUCTION

Plastics on the basis of synthetic polymers are widely used to make various materials for daily life. They can meet any commercial and industrial market requirements, such as low cost, good performance, convenience, durability, high variability in mechanical and other properties, etc. [1,2]. A significant percentage (ca. 40 %) of plastics' consumption is used for packaging, which has grown rapidly from the last decade of the 20th century [3,4]. Because plastics are highly resistant to degradation, humankind is now being faced with a wealth of non-biodegradable waste, which increases every year. What is more, the synthetic plastics are manufactured from fossil resources that are under a contraction. As carbon-

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containing substances, they could be produced from CO_2, which exists in Earth's atmosphere, but an industrial technology for absorbing atmospheric CO_2 has not been developed yet. It can be done only by photoautotrophs—eukaryotic algae, higher green plants, and some bacteria that accumulate more than 10^{10} tons of carbon each year [5,6], producing only a terrestrial biomass of about 220 billion tons annually [7]. It proceeds in accordance with the well-known carbon cycle on Earth. Absorbed CO_2 is coupled with water under the action of sunlight, forming carbohydrates in the course of photosynthesis. The photosynthetically generated biomass transferred into fossil resources (petroleum, natural gas, coal) that took millions of years to form by very slow natural processes. At present, petroleum is the main source for most chemicals and plastics including packaging [1]. The problem is that the fossil resources are not renewable. Fuel, chemicals, and materials are used very quickly in comparison with fossil generation. Furthermore, these resources are exhaustible, being finite in quantity.

There is also another side of this problem. The usage of carbon-containing materials mainly ends up with CO_2 emission. Increasing consumption of fossil resources has reached such a level that a novel, artificial cycle is created. Its influence on global processes and Earth's environment becomes more obvious and nowadays is considered as a global environmental problem [3,8,9].

Petroleum serves as a comparatively recent source for chemicals and plastics. Their accelerated production began with the rapidly developing petroleum industry in the 1950s [1]. Until then, many of the industrially important chemicals had been manufactured from sugars by fermentation [10]. Plastics were fabricated from vegetable sources containing cellulose. Shellac, gutta percha, ebonite, and casein were used as well [1]. Henry Ford started by examining various plants to develop car parts in the 1910s, research that produced a prototype car by Ford Motor Co. in 1941 that contained plastics made from soybeans [4,11]. His developments were interrupted by World War II and then stopped when inexpensive petroleum-based plastics were coming into use. Nowadays there is a renewed interest in plastics on the basis of biopolymers, which is helped by rising oil prices. Furthermore, the direct use of photosynthetically generated biomass to produce the fuels, chemicals, and materials without recourse to fossils reduces fossil consumption.

Wide-range applications of polymers of biological origin are restricted by some inadequate properties, such as insufficient mechanical strength, high gas and water vapor permeability, low heat degradation temperature, etc. Therefore, the reinforcement and improvement of their properties is much needed and is frequently done through the addition of inorganic fillers. Henry Ford explored with composites when trying to develop biobased plastics. An appropriate material was created by reinforcing wheat gluten with asbestos fibers [11].

Interest in composites with nanoparticles, which are usually known as nanocomposites, was initiated by researchers from Toyota in the early 1990s. They found that montmorillonite properly exfoliated into individual nanoparticles could reinforce notably and improve the dimensional stability and water and gas barrier properties of naylon-6 by a few percentage points [12–14]. Although it was demonstrated well before their finding that the decrease of filler dimensions down to nanometers caused sharp reinforcement of rubber [15,16], this considerable study opened a new era of research and application of composite materials.

Nanocomposites containing biopolymers in place of synthetic, petroleum-derived polymers are separated into an individual class of materials sometimes called "bionanocomposites" because the exchange results in a significant change in the preparation methods, properties, and functionalities of the materials [17–21]. This is due to notable differences in the properties between synthetic polymers and biopolymers. Of greater importance is the solubility. The biopolymers are soluble in polar solvents, mainly in water, whereas most petroleum-derived polymers, in organic solvents. Furthermore, the majority of the former are not thermoplastic because of their decomposition before melting. As a result, methods like the extrusion widely applied in the case of petroleum-derived polymers to prepare nanocomposites are unusable for biopolymers. The difference between them is also in the biocompatibility and biodegradability, which determines distinctions in the areas of their applications. It is pertinent to note such a principal advantage of polymers of biological origin as renewable resources from

which they are derived and which are widely abundant and relatively low-cost. Surplus agricultural products and agricultural, food, industrial, and domestic wastes can serve as the source for their production.

The literature devoted to the bionanocomposites frequently points out mineralized tissues of living organisms, referred to as biominerals [20,22–26]. As examples, shellfish, crustaceans, sponges, corals, bones, teeth, etc. can be mentioned. They consist of inorganics and biopolymers. It is demonstrated that their combination in living cells gives materials with unique properties and functionalities. When comparing the biominerals with inorganics industrially manufactured or of geological origin, one will find great differences in their properties. A familiar example is calcium carbonate. It is difficult to crack the mollusk shells, whereas the chalk is very soft. Attention is drawn to the fact that biominerals are synthesized at ambient conditions, without using acids/alkali, high temperature, and pressure [20,23,26–29]. Biomineralization processes, which are responsible for the formation of mineralized tissues, are under considerable study to reveal their mechanisms. Furthermore, there are numerous attempts to biomimic these processes through the mineralization of biopolymers in vitro, preparing bionanocomposites in aqueous solutions at mild conditions by green chemistry methods [20,26,27,29–37].

This article is devoted to some aspects of the bionanocomposite materials, consisting of two parts. There is first a review of some literature concerning the emergent bionanocomposites. This is an area of much current research activity commenced recently. A universally accepted definition is still absent so a definition for the bionanocomposites and their classification is planned. Then nanoparticles and biomaterials used for their preparation as well as the preparation methods will be considered. The second part briefly discusses two novel general approaches to the making of bionanocomposites suggested recently by our team. One refers to chitosan with clay nanoparticles, another, mineralized biopolymers. Methods of their preparation, structure, some properties, and applications are considered.

DEFINITIONS OF COMPOSITE MATERIALS

Bionanocomposites are a new class of composite materials. The term has been introduced recently. According to the Web of Science, the term was used first in 2004 and the number of publications devoted to the bionanocomposites has grown exponentially, as shown in Fig. 1. In 2011, there were as much as 66 articles in which this term was used. In actuality, the number of articles has increased a few times over because the term has not been universally accepted yet. Furthermore, bionanocomposites

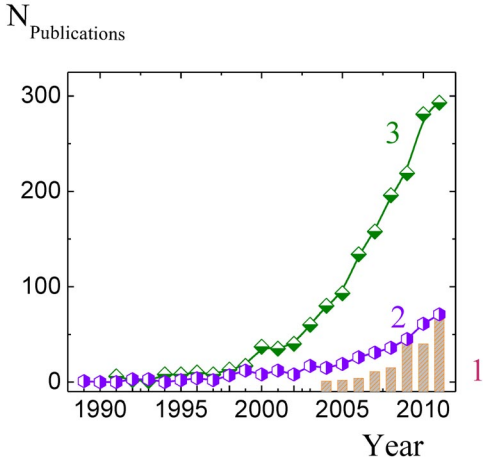


Fig. 1 Number of publications per year according to the ISI Web of Knowledge (January 2012). Keywords for search: 1 – bionanocomposites, 2 – bioplastics, 3 – biocomposites.

were being studied well before 2004. By way of example, Wagner applied silica nanoparticles with diameters of 10–100 nm to reinforce natural rubber in 1941 [15]. The influence of cellulose on a silica nanoparticle synthesis by sol-gel techniques and the formation of ordered sheet-like composites was observed in 1992 [38]. Hydrogel consisting of a mixture of starch with montmorillonite was developed in 1997 [39]. A large body of research with various biopolymers and nanoparticles can be found in the literature that presents a specialized aspect of the problem. Because the term is only coming into common use, bionanocomposites are sometimes called "nanocomposites", "nanobiocomposites", "biocomposites", "green composites", "biohybrids", or "biobased plastics", known shortly as "bioplastics". In any case, composite materials with biopolymers are a subject of much current interest. This follows from Fig. 1 in which one may see a number of publications devoted not only to bionanocomposites, but also to the biocomposites and bioplastics among which they can be frequently met. As seen, there is an exponential growth of articles every year, which is evidence of great interest in biobased materials.

Bionanocomposites may be considered as a variety of nanocomposites. Therefore, the generally accepted definition of nanomaterials can be applied. It accounts for the presence of dispersed particles of which at least one dimension is in the nanometer range. Nonetheless, the profound distinctions of biopolymers from synthetic, petroleum-based polymers does not allow identifying bionanocomposites with the nanocomposites. They differ in the solubility in water, thermal stability, biocompatibility, and biodegradability, which determine the methods of preparation, functionalities, and areas of applications of materials. If the foregoing is taken into account, the following definition for the bionanocomposites can be suggested. **The bionanocomposites are composite materials that contain constituent(s) of the biological origin and particles with at least one dimension in the range of 1–100 nm**.

It should be pointed out that the "constituent(s) of biological origin" is mentioned in the definition, but not biopolymers that are thought to be only in the bionanocomposites. There are composites that are made from variable biomaterials. It can include low-molecular weight substances, for example, lecithin, which is one of the main components of the lipid matrix of biological membranes [40], and living organisms like microorganisms. They are also mineralized to make composites that are usually called "hybrids". This term does not specify their origin and features, but reveals whether they should be considered bionanocomposites. This is because they include nanosized inorganics and components of biological origin, per the definition suggested above.

There are composites that are manufactured by combining synthetic and natural polymers [4,8,11,17,41–43]. Biopolymers can be reinforced with synthetic fibers, or synthetic plastics are impregnated with natural fibers to improve their biocompatibility and biodegradability. Inorganic additives (e.g., clay minerals and calcium carbonate) are also added because of their reinforcement effect, low cost, easy availability, and absence of negative impact on the environment. They are taken in non-nanoparticulate state. Therefore, these fillers reinforce the plastics in amounts of several tens of percent [14,41,44–47]. Composite materials of this kind are known as biocomposites and bioplastics. They are defined in [4] as "… composites made from both bioplastics and synthetic plastics impregnated with natural fibers or synthetic fibers or both". The definition can be used to specify this type of material.

The place of the bionanocomposite among the other composite materials in accordance with the above-discussed definitions is illustrated in Fig. 2. **Composites** present materials that are prepared by combining synthetic plastics and microsized inorganic additives such as layered silicates, talc, carbon black, calcium carbonate, etc. They are used as fillers in the rubber industry since the early 20th century when the reinforcing effect was discovered [1]. Where the natural rubber is taken, these composites are among the **biocomposites** or **bioplastics**. It is interesting that bioplastics were the main composite materials before the 1950s when the petroleum-based chemical industry, including the polymer production, started sharply developing [1]. **Nanocomposites** differ from the composite in inorganic additives that are taken in the nanosized state. The organic component(s) is presented by the petroleum-based polymer(s). If it is exchanged for the biopolymers, one has the **bionanocomposite**. This is not only the formal exchange. There is a notable or even drastic modification of materials, including the biocompatibility, biodegradability, preparation methods, properties, and functionalities [20,42,48–51].

Bionanocomposites differ also from the biocomposites that are made partially from biopolymers, but they do not have the nanosized additives. The discussed set of composite materials represents their main types, separating from one another in accordance with the constituents that determine the structure, properties, functionalities, and applications. This is illustrated by Fig. 2.

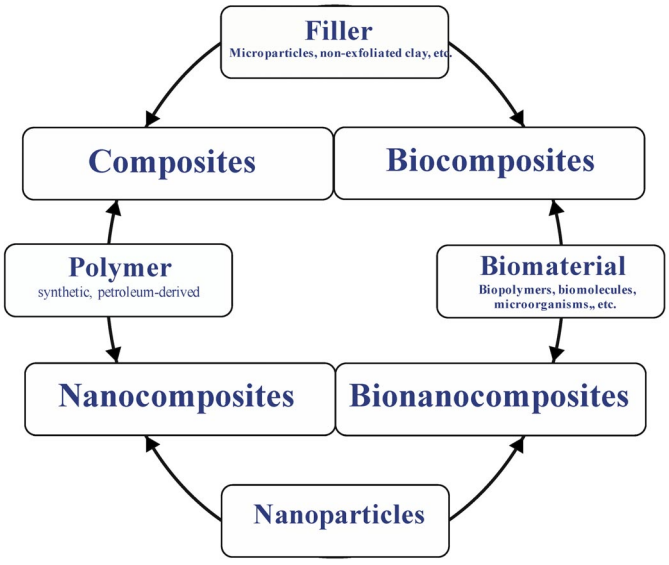


Fig. 2 Main types of composite materials and their constituents. **Composites** present commercial materials produced from synthetic, petroleum-based polymers and microfillers admixed in the amount of several tens wt %. When biopolymers are taken instead of polymers, one has **biocomposites**. They are also frequently made from synthetic polymers and biopolymers. **Nanocomposites** are formed by combining synthetic polymers and nanoparticles. The maximum effect of nanofillers on the material properties shows up at the concentration of 3–5 wt %. The exchange of polymers to biomaterials—biopolymers, low-molecular-weight biomolecules or microorganisms—gives **bionanocomposites**. They can be prepared by mixing with nanoparticles or mineralizing with the help of biomicking methods of sol-gel chemistry. See the text for further details.

NANOPARTICLES

Nanoparticles are entrapped into the biopolymer matrix with the intention, first and foremost, to reinforce a material, but there is an influence also on other physical properties. One can find a change in the color, optical, rheological, thermal, electrical, and magnetic properties and improvement of the dimensional stability, surface characteristics, and durability. The chemical reactivity, biodegradability, and processability are affected as well. These numerous modifications are determined by the interactions between nanoparticles and biomolecules.

Nanosized particulates differ by their dimensions and shape. Both these factors hold much significance for the formation and properties of bionanocomposites. The nanoparticles have a highly extended surface area that is increased with the decrease of dimensions. The surface area provides an abundance of contacts and effectiveness of interactions with biomolecules in the bionanocomposite matrix [52,53]. Consequently, a significant effect of nanoparticulate additives to the composite materials occurs at the small amount of a few percentage points.

Another controlling factor in the bionanocomposite formation and properties is a nanoparticle shape. When only the geometry is taken into account, three main categories of nanoparticulates are distinguished [44,45,47]. They are presented in Fig. 3. The classification is based on the number of dimensions that are in the nanometer range.

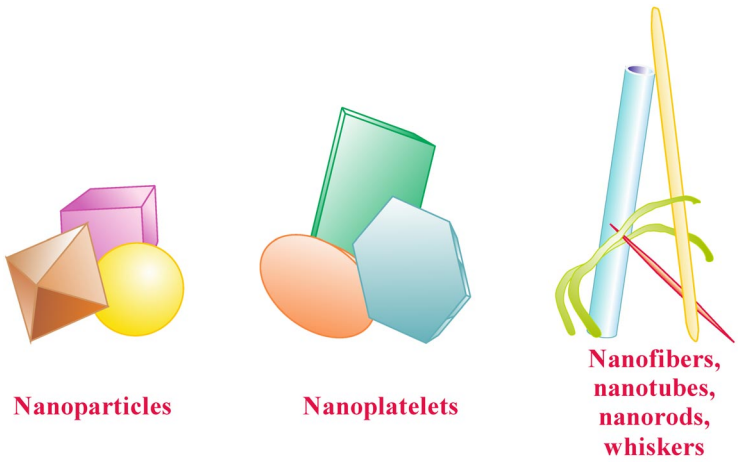


Fig. 3 Various types of nanoparticles used to prepare bionanocomposites. Details may be found in the text.

Nanoparticles

All three dimensions of the particles are of the order of nanometers. Although they are three-dimensional particulates, this category of nanosized particles is called "isodimensional". It includes spherical, cubic, and shapeless nanoparticles with a size up to 100 nm. Colloidal silica synthesized by the sol-gel methods [54,55], noble nanometals (gold, silver, etc.) [56–59], metal oxides [60,61], and semiconductor nanoclusters [62,63] constitute this category.

A special case is the hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). It is a major mineral component of bones (up to 70 wt %). Nanosized hydroxyapatite as the active component of scaffolds and implants is widely employed in orthopedic surgery and dentistry to repair the mineralized hard tissues in the living body owing to its excellent biocompatibility and osteogenic effect [34,64,65].

Nanofibers, nanotubes, nanorods, whiskers

Only two dimensions of nanoparticulates are in the nanometer scale range (Fig. 3). Carbon nanotubes consisting of a single sheet or more sheets of graphene are likely to be the best known example [20,52,66–68]. Their extraordinary stiffness, strength, and resilience provide a significant improvement of the mechanical properties of composite materials.

Nanofibrillar clays used in the bionanocomposite formulations are presented mainly by sepiolite [$Si_{12}O_{30}Mg_8(OH,F)_4(H_2O)_4{\cdot}8H_2O$] [20,69,70]. Their shape can be represented by a square cuboid with tunnels of ca. 0.4 × 1.1 nm^2 [71,72]. Pristine sepiolite consists of two-dimensional tetrahedral sheets that are built up from fibrils ranging from 10 to 30 nm wide and from 5 to 10 nm thick [72]. When it was treated with the help of a surfactant, nanofibrils about 20 nm across were obtained [73]. Their aspect ratio reached 40 though the fibrillar length can lie between 2 and 10 μm [71]. Ruiz-Hitsky with collaborators, who deal systematically with sepiolite-based materials, developed various bionanocomposites on its basis by combining it with different polysaccharides [20,70,74–76].

This category of nanoparticulates, along with inorganic carbon nanotubes, also includes nanosized fibers of biological origin that come from plants and animals. Plants like cotton, wood, soy, hemp, flax, jute, sisal, banana, and kenaf serve as a source of the cellulose and starch nanofibers [21,43,77–79]. Linear cellulose chains align parallel to each other in such a manner that numerous hydrogen bondings are formed between them. This results in the formation of crystalline fibrils including up to 100 macromolecules. Their diameter ranges from 2 to 20 nm, whereas the length can reach several tens of microns.

Animal nanofibrils are represented by chitin. It is also a linear polysaccharide forming crystalline fibrils like the cellulose [43,77,80]. Although cellulose has been widely used in the textile industry for

many decades, its application in nanofibrillar form as well as starch and chitin has recently attracted much attention for the reinforcement of composite materials, owing to unique mechanical properties that go together with biocompatibility and biodegradability. Polysaccharide nanofibrils are considered as a sustainable alternative for carbon nanotubes because of abundant renewable raw sources. They are low-cost materials as well. Among their advantages over carbon nanotubes are also their hydrophilic nature and well-developed synthetic methods of carbohydrate chemistry, which enable one to tailor the surface functionality.

The reinforcement of bionanocomposites by nanoparticulates of this category depends strongly on the aspect ratio (length to diameter). With increasing the ratio and providing the uniform orientation of nanofibrils in the matrix, one can reach, as expected, a reinforcing effect like in Kevlar [77].

Nanoplatelets

The thickness of nanoplatelets is only in the nanometer scale range (Fig. 3). This category includes phyllosilicates, silicic acid (magadiite), layered double hydroxides [$M_6A_{l2}(OH)_{16}CO_3{\cdot}nH_2O$; M = Mg, Zn], zirconium phosphates [$Zr(HPO_4{\cdot}2H_2O$], and di-chalcogenides [$(PbS)_{1.18}(TiS_2)_2$, MoS_2] [20,44,46,81]. The most popular nanoplatelets applied in bionanocomposite formulations are layered or plate-like clay minerals [18–21,50,51,80,82–84]. They can have a huge aspect ratio because of the thickness of ca. 1 nm and the width or diameter ranging from tens of nanometers up to a few micrometers. Therefore, the substantial reinforcement of composite materials occurs even at very low nanoplatelet content, which is attractive from the economic point of view. Such layered phyllosilicates of 2:1 type as montmorillonite [39,85–97], lapomite [98,99], hectorite [18], and saponite [100,101] have been applied. They were combined mainly with the chitosan but also with starch [18,21,39,50,80,83,92], some other polysaccharides [20,85], and proteins [18,83,85–87,94,100,102].

It should be pointed out that an overwhelming majority of nanosized materials, among them the natural clay minerals, are negatively charged. It can at times present a challenge to combine them with anionic biomacromolecules, which are the most part of biomaterial as well. In these instances, synthetic layered double hydroxides could be appropriate inorganics for bionanocomposite making [20,103,104]. In particular, the structure of the hydrotalcite family may be presented as $[M^{2+}_{1-x}M^{3+}_x(OH)_2]^{-}(A^{m-})_{x/m}{\cdot}nH_2O$ [81,103–105]. The positive charging is caused by isomorphous substitution of M^{2+} cations with M^{3+} ones. The interlayer charge-compensating anions A^{m-} are exchangeable for anionic biomolecules.

BIOPOLYMERS

Polymers of biological origin are the primary constituents for bionanocomposite preparation. They can be classified as: (i) polysaccharides, (ii) proteins, (iii) DNA, and (iv) poly(hydroxyalkanoates). The mainly used groups are polysaccharides and proteins.

Polysaccharides

Polysaccharides are known also as glycans, belonging to the carbohydrates. They are built from monosaccharides or sugars that are covalently linked together by the glycosidic bond (see Fig. 4), forming a linear or branched polymeric chain [5,6,106]. Carbohydrates are the most widely distributed and abundant organic compounds on Earth. **Cellulose**, of which the linear chains are built from D-glucose units (Fig. 4), heads the list first. Its main sources are plants that synthesize the carbohydrates from CO_2 and water in the course of photosynthesis under the sun's illumination. Its sustainable annual production accounts for 10^{11}–10^{12} tons [107].

Chitin (Fig. 4) is in the second place. Its renewable amount is rather close to the cellulose value, ranging up to 10^{11} tons [108]. Sources of this polysaccharide have mainly marine origin. Chitin is found in crustacean exoskeletons (shell) and mollusks, as well as in insects and fungi [108–110]. Although cellulose and chitin are the most abundant biopolymers, their application in bionanocomposite formulations is very restricted. This is because they are soluble only at harsh conditions or in toxic solvents

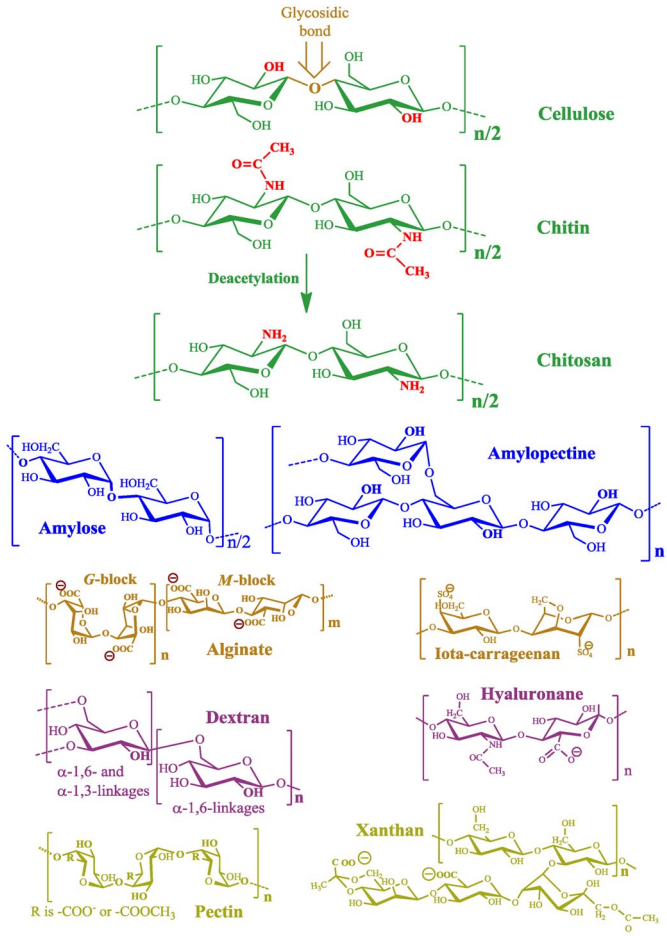


Fig. 4 Structural formulas of polysaccharides used for bionanocomposite preparations. **Cellulose** ((1 → 4)-β-D-glucan) and **chitin** ((1 → 4)-β-D-2-*N*-acetylamido-2-deoxyglucan) are the most abundant biopolymers on Earth. The former consists of glucose residues, the latter, acetylglucosamine ones in which hydroxyl groups at the second carbon atom of glucose is exchanged for the acetylated amino group. **Starch** is composed of glucose residues connected by (1 → 4)-α- and (1 → 6)-α-glycosidic linkages that gives **amylose** ((1 → 4)-α-D-glucan) and **amylopectin**, respectively. Amylose has a linear chain, being an isomer of cellulose. Amylopectin consists also of (1 → 4)-α-linked glucose residues but this is a branched polysaccharide because of (1 → 6)-α-linkages at every 20 to 25 glucose residues. **Alginate** consists of β-D-mannuronic (*M*) and α-L-guluronic (*G*) acid residues. They can form consecutive *G*-blocks and *M*-blocks or alternating *MG*-blocks and randomly organized blocks. **Carrageenans** are presented by *iota*-carrageenan. This linear anionic polysaccharide consists of alternating (1 → 4)-3,6-anhydro-α-D-galactose and (1 → 3)-β-D-galactose residues. There is also a varying proportion and position of sulfate groups in this group of polysaccharides. **Dextran** is a (1 → 6)-α-glucan. There are several glucose side-chains that are mainly bound to the backbone through (1 → 3)-linkages and randomly also through (1 → 4)- and (1 → 2)-ones. **Hyaluronic acid** or **hyaluronan** is a linear polysaccharide consisting of (1 → 4)-β-D-glucuronic acid-(1 → 3)-β-D-*N*-acetylglucosamine disaccharide units. **Pectin** is a mixed polysaccharide with a complicated structure. Its backbone is composed of ca. 65 % of (1 → 4)-α-D-galactopyranosyluronate residues. Side-chains have differently arranged building blocks of apiose, fucose, arabinose, and xylose residues. There is also a backbone chain from disaccharide (1 → 4)-α-D-galactopyranosyluronate-(1 → 2)-α-L-rhamnogalacturonan with its rhamnose residues linked by arabinan and galactan chains. **Xanthan** is a microbial polysaccharide of which the backbone is made from (1 → 4)-β-linked glucose molecules as in the cellulose. There is a side-chain of trisaccharide composed of one glucuronic acid and two mannoses that are attached covalently at the every second glucose residue at 0–3 position.

[108,111–113]. Furthermore, they are non-thermoplastic. Their nanofibrils, which are discussed above, find the usage for reinforcing the polymers. Some cellulose derivatives are applied but not so much [20]. They are employed predominantly to fabricate the biocomposites (Fig. 2) [4].

Chitin, which is a linear amino-containing polysaccharide composed of *N*-acetyl-D-glucosamine (2-acetamido-2-deoxy-D-glucose) (Fig. 4), can be easily deacetylated by means of hot alkali, transferring into a cationic form (D-glucosamine) known as **chitosan** (Fig. 4) [108,110,112,113]. This cationic polysaccharide is being increasingly applied, which is detailed below in the article.

Of all the polysaccharides, **starch** is mostly used to fabricate both the bionanocomposites and biocomposites [4,18,20,21,50,83,114,115]. It is a mixture of two glucans, amylose and amylopectin, shown in Fig. 4 [116,117]. The distinctive property of starch is that it can be converted into a thermoplastic material by introducing plasticizers [83]. "Thermoplastic starch" thus prepared is hot-worked at 90–180 °C by means of extrusion, injection-molding, and blow-molding techniques, similar to the synthetic thermoplastics. Its inadequate properties, such as mechanical ones, high water-sensitivity, and poor barrier properties need further profound improvement that is done with the help of nanosized additives [18,21,39,50,80,83,92].

One may see other polysaccharides in Fig. 4 that are also used in bionanocomposite formulations. These are **alginate**, **carrageenans**, **dextrans**, **hyaluronane**, **pectin**, and **xanthan**. These polysaccharides demonstrate unique properties and functionalities, which are further improved significantly after combining with nanosized inorganics (see, e.g., [76,85,118–124]), but they await wider applications, especially in biomedicine.

Proteins

A list of proteins is presented by soy, wheat gluten, corn zein, caseinate, whey, silk, collagen, and gelatin. They are mainly employed to fabricate both bionanocomposites and biocomposites (Fig. 2) [17,20,34,42,65,75,79,80,84,125–131]. Proteins such as soy, wheat gluten, corn zein, caseinate and whey are thermoplastics. They demonstrate the good film-forming properties that make them appropriate for manufacturing biodegradable packaging. Sometimes the proteins are combined along with polysaccharides, starch, chitosan, and cellulose to improve the processability and functional properties [11,17,21,80,83,84,131,132]. These proteins were used rather widely up to the 1950s before the chemical industry started manufacturing commercially petroleum-based polymers on a substantial scale [1]. Henry Ford pursued experiments with proteins since the 1910s, developing a prototype car with various parts made from soybean plastics in 1941 [4,11].

Silk proteins, also known as silk fibroins, are available in a fibrillar form from a number of natural sources including *Bombyx mori* silkworms in the first place and spiders [126,128]. In the case of silkworm fibers, the fibroin serves as a structural protein, forming nanofibrils consisting of β-sheets, the bundles of which are linked together to give microfilaments coated by glue-like glycoprotein sericin. There are two bound microfilaments in each fiber. This structural organization, together with oriented crystalline fibroin, provides the high tensile strength of fibrillar silk and the substantial reinforcement of bionanocomposites and biocomposites [11,126,128,131,133].

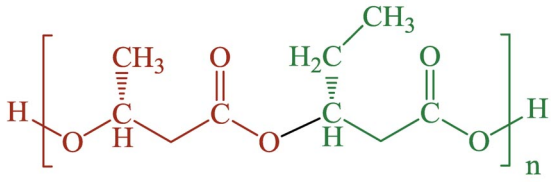
Collagens are also the fibrous proteins of which peptide chains are in the α-helical state [5,106]. They are the most abundant proteins in mammals, accounting for about 20–25 % of the total. They often have a protective, connective, or supportive role in the living organism. The most used and studied collagen is collagen I, which is present in the skin, tendons, and bones. Bovine Achilles tendons usually serve for its commercial production. The excellent cell adhesive, osteoinductive, and mechanical properties of collagen I determine its current applications in tissue engineering. Collagen-containing bionanocomposites are usually formulated with nanosized hydroxyapatite to make implants [20,65,79,127,130,134,135]. They are intended to biomimic bone tissues.

Thermal treatment of collagen results in its denaturation followed by the formation of **gelatin** [136–138]. This derivative is usually prepared through hydrolysis by treating with an acid or alkali. Gelatin can be applied instead of the collagen owing to the compositional and some structural resem-

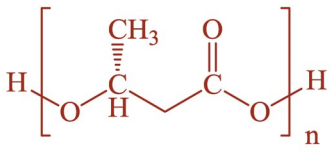
blance, but it is notably worse in the mechanical properties. The advantage gelatin has over collagen is its good solubility in water, which simplifies processability [136,138,139]. Bionanocomposites are frequently prepared through the mineralization of its macromolecule [140–145].

Polyhydroxyalkanoates

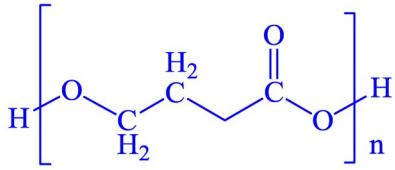
Polyhydroxyalkanoates (PHAs) are a linear polyester of biological origin. There are more than 300 both gram-positive and -negative bacteria that can synthesize the PHAs [17,146–151]. The biopolyesters serve as the carbon- and energy-storage compounds of which multiple syntheses are begun with sugar or vegetable oils. By now various PHAs were prepared by using about 150 types of monomers, but poly-3-hydroxybutyrate is still one of the main biopolyesters. This PHA and its copolymers with hydroxyvalerate as well as poly(4-hydroxybutyrate) are considered the best commercial biopolyesters. Their structural formulas are shown in Fig. 5. Of interest is that the PHAs are enantiomerically pure compounds. They are synthesized regioselectively only from (R) enantiomer of the hydroxyalkanoates [146,148,149,151].



Poly(3-hydroxybutyrate-co-3-hydroxyvalerate))



Poly(3-hydroxybutyrate)



Poly(4-hydroxybutyrate)

Fig. 5 Structural formulas of PHAs that are manufactured and applied for the bionanocomposite preparation.

Poly-3-hydroxybutyrate films and fibers have mechanical properties close to that of polypropylene [149] and poly-4-hydroxybutyrate, to polyethylene [151]. By varying the ratio of poly-3-hydroxybutyrate and poly-3-hydroxyvalerate or poly-4-hydroxybutyrate in their copolymers, one can prepare thermoplastic to elastomeric materials with the elongation at break ranging from 3 up to 1.000 %, whereas the melting temperature is decreased from 180 to 50 °C [146,147,149,151]. General polymer technologies, such as extrusion, thermoforming, injection-molding, and blown film are appropriate for this biopolyester family.

PHAs attracted attention rather recently, ca. 30 years ago, as a possible biological component of composite materials [146,149,151]. Their indubitable advantage is the biodegradability to harmless products in a wide range of environments, including marine and wetlands ecosystems [147–150,152]. They present an actual alternative to petroleum-made polymers, particularly in biomedicine and tissue engineering [131,147,150,151,153]. Their bionanocomposites made with hydroxyapatite nanoparticles had mechanical properties similar to that of bones [154,155]. PHAs were combined also with layered silicates, but this is only the beginning in the development of a new, promising generation of materials.

It is believed that PHA bionanocomposites can successfully compete with petroleum-based polymers [80,147,150–152].

Deoxyribonucleic acid

Deoxyribonucleic acid (DNA) presents a biopolymer of great biological significance. Its vitally important role consists in the genetic information carrier and the provision of synthesis of all substances that are arranged as an entire living organism [5,106]. Therefore, DNA is used above all in genetic engineering, but it can also serve as a building block for bionanocomposite materials. Their fabrication is based on an untypical combination of DNA features [156–162], which includes: single- and double-helical forms; double- and triple-crossover macromolecules; self-assembly into various planar and three-dimensional structures; high density charge of the polyphosphate backbone determining the electrostatic interactions and polyelectrolyte complex formation; and covalent attachment of metal nanoparticles and easy conjugation to the 3' and/or 5' ends of the oligonucleotide strand. Its self-assembly into supramolecular structures offers a great potential as the scaffolds for positioning substances and nanosized inorganics at the nanometer scale [58,156,157,159,161–165]. Metallized macromolecules, frequently by gold, are very attractive as conductive nanowires for electronic circuits in nanoelectronics.

Single- and double-stranded DNAs can solubilize the single-walled carbon nanotubes, winding around an individual nanotube that allows them to be dispersed in aqueous solutions [163,166–168]. These associates have high stability and behave together as a unit, which enables their separation by anion-exchange chromatography. DNA-carbon bionanocomposites are very promising for the broad spectrum of applications from materials science to pharmaceutics and biomedicine.

Negative charges bearing by macromolecule present difficulties for the formation of composite materials with the same charged nanoparticles, among them with natural clay minerals, but it can be formulated with synthetic, positively charged, layered double hydroxides [104,169]. The main objective in preparing bionanocomposites with DNA is to develop the means for biodiagnostic, biosensors, biochips, and nonviral gene delivery [104,156,170–173].

PREPARATION

It is feasible to make most of the unique properties and advantages of nanosized constituent of nanocomposites materials if, and only if, it is completely disaggregated and homogeneously distributed in formulation [14,44–46,103,174,175]. Therefore, the method of fabrication is of basic importance. Four different approaches to fabricating nanocomposites have been developed in nanotechnology.

Solution method

It is usual to mix the initial solutions of the biomaterial and dispersed nanoscale particles. The mixing results in the thickening, jellification, or precipitation that is determined by interactions between the components and their association. It should be taken into account that the precipitation or separation into two solutions can also be observed in a case of poor compatibility of the biomaterial with nanoparticles. A heterogeneous composite with no properly distributed nanosized particles is formed. A similar situation holds when the mixed components interact/aggregate strongly with one another. The approach is in common practice because it is easy to handle, but a really homogeneous bionanocomposite is prepared in exceptional cases. The interested reader can find more details in the recent review of Aimé and Coradin [176].

The precipitation of interacting bioorganics and nanosized inorganics is well used in the method known as layer-by-layer assembly [37,177–180]. It is a solution technique in which an initial layer of, for example, biopolymer is formed on a solid surface through adsorption and then a film is constructed by using alternate assembling of the oppositely charged nanoparticles and biomacromolecules in the course of the repeated sequential change of their solutions. Bionanocomposite coating, shells, films,

capsules, membranes, etc. with the tailored structure and properties can be prepared. There is a limitation on the preparation of bulk materials like hydrogels by the layer-by-layer technique.

It seems that the proper distribution of a nanoscale component in bulk bionanocomposites by means of simple mixing of solutions is physically impracticable. Vigorous stirring or ultrasonic treatment enables one to improve the homogeneity but not in full measure. As shown in [101], slow processing accompanied with a self-organization in the system allows one to better realize the opportunities of the solution method. Details will be considered in the section devoted to chitosan-clay bionanocomposites.

Melt mixing

A polymer is heated at the appropriate temperature to transfer it in the molten state and mixed directly with nanoparticles to distribute them properly in the polymer matrix. The preparation is usually performed in the extruder [20,21,41,42,44–47,50,82,83,103]. The melt-mixing technique can be applied only for thermoplastics. Proteins, such as soy, wheat gluten, corn zein, caseinate, whey, and gelatin, match the requirement. PHAs are also among the materials being worked by the extrusion. Most of the polysaccharides are thermally decomposed before the melting. The exception is starch, but in its native form the polysaccharide is not a true thermoplastic material. It is converted into a thermoplastic-like state by admixing plasticizers at 90–180 °C under the shear [83].

As the nanoscale particles, natural layered clay minerals are frequently used. The conditions of extrusion treatment make it possible to provide the good intercalation of macromolecules in the intergalleries of closely stacked silicate sheets and their exfoliation into individual nanoplatelets. It is convenient for the preparation of bionanocomposite on lab to commercial scales.

Template synthesis

Biomolecules, parts and whole cells, microorganisms serve as the template for inorganics that are generated from a precursor [20,23,35,41,48,127,156,181–183]. It can be synthesized as nanosized particles, coating or shell and mesoporous matrix in which templating bioorganics are entrapped. This technique is highly versatile, being adapted to many different bionanocomposite preparations. The inorganic constituent is often synthesized by sol-gel chemistry methods [24,26,35,36,48,118,182,184–187]. As an example of the ultimate in fabrication, the biomineralization processes of living cells are mentioned. Hierarchically structured biopolymer-inorganic nanocomposites such as bone, teeth, nacre, and silica diatoms, of which the properties are much superior to that prepared by material scientists, are synthesized in a highly controllable manner at ambient conditions [23,24,27,29,30,188–194]. The biomineralization processes proceed at low temperature (0–30 °C), in neutral media, no acids/alkalis, only in aqueous solutions that at present fall within the realms of "green" chemistry. The surprising thing is that the biosyntheses occur in diluted or highly diluted natural media. For example, the concentration of orthosilicic acid is 5 to 70 μM in sea and fresh waters, whereas its condensation can be observed at concentrations exceeding 2 mM [26,28,195]. It means that the biosilica of glass sponges and diatoms is synthesized in the environments inconvenient for the silica precipitation. However, more than 6700×10^6 tons of silica are generated annually in living nature, while only ca. 1×10^6 tons are manufactured by the chemical industry [26]. Although biosilicification occurs at highly undersaturated environmental conditions, it proceeds at a high rate [28,196]. For instance, it was demonstrated in [196] that the formation of diatom silica-based cell wall was accomplished within ca. 1 h.

There are rather numerous attempts to biomimic the biomineralization processes in a bionanocomposite preparation. Polysaccharides, proteins, DNA, and parts of cells and whole microorganisms were used as the template for metals, metal oxides, silica, calcium carbonate, and calcium phosphate (hydroxyapatite) (see, e.g., [20,26,27,31,32,35,127,135,184,185,193,198–201]). These studies have resulted in development of a variety of novel functional materials ranging from conducting nanowires and photocatalysts to biocatalyst and targeting drug-delivery systems.

There is a problem in the template synthesis of biomaterials because of their incompatibility with the common sol-gel processing [36,185,202–205], which restricts opportunities to biomimic the bio-

mineralization processes of living cells. Two novel approaches devoted to the improvement of compatibility have been suggested by our team. They are considered at the final section of the article.

In situ polymerization

Liquid monomer or monomer solution is used to first disperse the nanoparticles. The polymerization is performed thereafter [44–47,103,206]. The method is among the basic techniques in nanocomposite preparation, but at this time it is impossible to realize it in the case of biopolymers.

CHITOSAN-BASED BIONANOCOMPOSITES

Chitosan is a deacetylated derivative of chitin (Fig. 4) occurring mainly in crustaceans, mollusks, insects, and fungi [108–110,207,208]. This polysaccharide is at the second place among the abundant organic compounds on Earth after cellulose. As much as 100 billion tons of chitin are produced annually in living nature [108]. The surprising thing is that chitin is still almost unutilized in spite of the huge renewal resource of biomass [108,112,208–211]. It is agreed that this polysaccharide is an extraordinary underexploited biopolymer of significant versatility and promise, having a much higher potential for applications in many fields owing to its distinctive physico-chemical properties and antiseptic, wound-healing, and immune-stimulating activities [112,113,208,211–216]. The whole range of mentioned advantages is even superior to that of the cellulose.

Chitosan macromolecule consists primarily of $\beta(1 \rightarrow 4)$ linked D-glucosamine residues (Fig. 4). It is the only cationic polysaccharide, and therein lies its uniqueness. The surface of most nanoscale materials is negatively charged. Therefore, bionanocomposite construction can be performed via electrostatic interactions of oppositely charged counterparts. Chitosan has a great potential as a building block for making bionanocomposites. It seems to be a simple matter to mix them together but in actual practice it is not so easily realizable. Chitosan is very sensitive to the presence of anionic substances in its solutions [217–220]. Even their trace amount brings about the formation of a heterogeneous precipitate of aggregated counterparts.

A peculiarity of the chitosan is that it is a weak polyelectrolyte, which means that its charging is dependent on the solution pH [220]. By adding an acid/alkali, one can easily transfer chitosan to the charged/uncharged state in its aqueous solution (see Fig. 6). This property was used by our team in an approach developed recently to regulate its interactions with anionic substances and nanoparticles and thereby bionanocomposite formation [101,221,222].

Figure 7 shows how bionanocomposite formation is carried out. Clay or other nanoparticles are dispersed in water. Any technique may be applied to have them homogeneously distributed in the bulk. Preference was given to a synthetic saponite. It is easily dispersed in aqueous media into individual nanoparticles by using a magnetic stirrer, forming a stable dispersion. They are apt to be negatively charged.

At the second stage, ground fine particles of chitosan are introduced and also distributed homogeneously over the entire volume of initial dispersion of the nanoparticles. The pH of solution is a little higher than the pK value of chitosan (Fig. 6). Experimentally, it can be between 6.5 and 7.0. Polysaccharide is not soluble at these conditions. Furthermore, any visible interactions with nanoparticles are not obvious because the chitosan occurs in its uncharged state [101,221,222].

The solution of both homogeneously distributed components is acidified at the final stage. This is of fundamental importance how the pH is shifted. When admixing an acid, one will obtain a heterogeneous mixture. There has to be a gradual shift of the pH value. It was suggested in [101,221] to use a glucono-δ-lactone as the natural chemical acidulant. It starts hydrolyzing after the contact with water, forming gluconic acid (Fig. 7). Owing to the slow reaction, there is a progressive acidification [223].

The gradual shift of the solution pH provides a progressive charging of chitosan macromolecules. They come into the electrostatic interactions with a negatively charged surface of nanoparticles. The number of electrostatic linkages increases smoothly as the pH is shifted further into the acidic region.

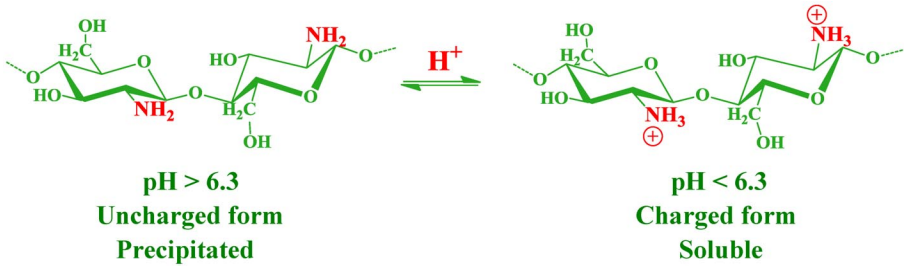


Fig. 6 Charged and uncharged forms of chitosan. Polysaccharide in the uncharged state is insoluble in water solutions. It precipitates at pH larger than 6. The acidification results in its charging and solubilization in aqueous solutions. By varying the pH around the p*K* value, one can regulate the charging and solubilization of chitosan.

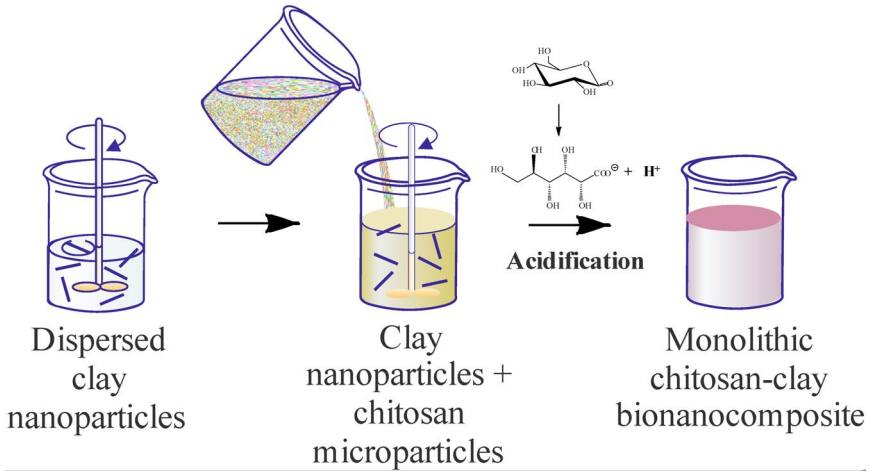


Fig. 7 Schematic presentation of the main stages of formation of monolithic bionanocomposite by regulated charging of chitosan dispersed as microparticles in solution of initially dispersed clay nanoparticles. Acidification of mixture is provided by gluconic acid formed in the course of hydrolysis of glucono-δ-lactone. The reaction is shown vertically at the stage of acidification.

A result of these physical cross-linkings is the formation of a three-dimensional network from aggregated carbohydrate macromolecules and nanoparticles that causes a jellification of solutions. Schematic drawings in Fig. 8 illustrate the processing and structural organization.

A morphological study on the bionanocomposites prepared from chitosan and synthetic saponite by means of scanning electron microscopy (SEM) revealed a three-dimensional network constituting cross-linked fibrils. A representative image may be seen in Fig. 8A. The fibrillar network is responsible for the formation of a mechanically strong hydrogel [101,224]. The jellification was observed even at small amounts of the chitosan added in a solution of the dispersed clay nanoparticles.

The method has been extended for film preparation (Fig. 8) [225]. A homogeneously distributed mixture of nanosized platelets and fine chitosan particles was cast for the solvent evaporation after the addition of glucono-δ-lactone. A preliminary study has shown that there is a stoichiometric ratio of the oppositely charged constituents at which the chitosan-saponite films were least swelling and mechanically strong at the most. They had a pronounced stratified layer (nacre-like) structure from stacked planar plates of high aspect ratio (Fig. 8B). The thickness was 20–40 nm, and the length run to a few micrometers. An examination by using small-angle X-ray scattering showed that the plates could consist of parallel saponite sheets separated by aligning chitosan macromolecules from each of the sides.

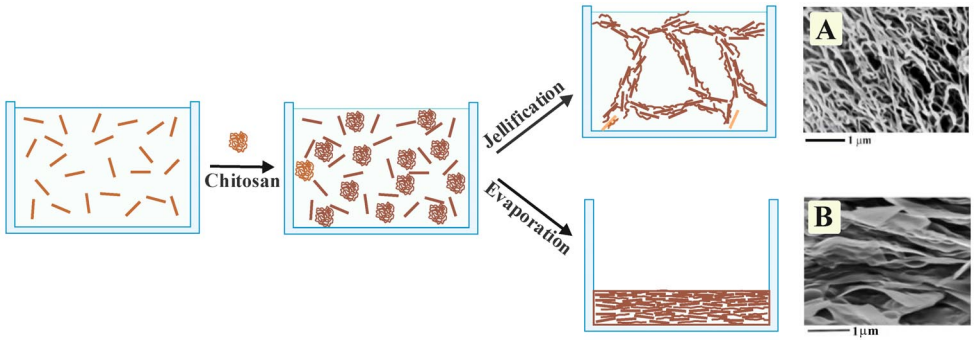


Fig. 8 Schematic representation of the three-dimensional fibrillar network formation and stratified film with nacre-like structure from chitosan and clay nanoparticulates. SEM pictures shows morphological features of hydrogel (A) and film (B) prepared by the gradual acidification of a solution containing saponite dispersion and chitosan microparticles. Composition of initial solutions: A – 0.75 wt % chitosan and 1.25 wt % saponite, B – 0.8 wt % chitosan and 1.2 wt % saponite.

The thickness of an individual stack is about 1.8 nm, and their number can vary from 10 to 20 [225]. This means that the films are hierarchically structured. The initial level is presented by nanosized plates made from clay nanoplatelets with chitosan macromolecules and another, by stratified film composing of these plates. The nacre structural organization at the nanoscale level, at which films start forming, is then transferred to the microscopic level in the course of film formation. This is an example of efficient bottom-up self-assembly discussed in more details in [225].

The well-defined structural organization of the bionanocomposite hydrogel and films, which were prepared through the gradual charging of chitosan, points out processes proceeding in the self-organization manner [224,225]. It is significant that they are regulated by the simple pH change. It is our belief that the developed approach is much promising for making chitosan bionanocomposites. This polysaccharide still presents a challenge for materials scientists.

BIONANOCOMPOSITES PREPARED VIA BIOMIMETIC MINERALIZATION

The mineralization of organic templates is actively engaged in sol-gel chemistry to synthesize inorganic materials with the required structure, porosity, properties, and functionalities (see, e.g., [226–232]). When biosubstances (parts or whole cells) are applied, one meets with the incompatibility of processing and/or precursors [36,186,202,203,233–235]. Biomineralization is considered as an exciting example of how the living nature could achieve highly efficient processing, complete integration of organics with inorganics, hierarchical structural organization from the molecular to the macroscopic level, and their strict regulations that are genetically predetermined and preserved across the generations [22,24,27,188,189,236,237]. The surprising thing is that the syntheses take place at ambient conditions in aqueous media with the very low concentration of inorganic substances, whereas the chemical industry is based on the use of acid or alkali, heating, and organic solvents. Furthermore, the mineralized tissues of the living organism are far in excess in the properties of similar geologically and commercially produced inorganic materials. These facts generate a great interest in the processes of living nature and their biomimicking. There are major breakthroughs in this area, but we still remain too far from really efficient biomimetic mineralization comparable to natural biomineralization. To have an insight into the nature of their differences, let us consider key points of the immobilization biomaterial by means of the sol-gel technique.

It is customary to use tetraethoxysilane, also called tetraethyl orthosilicate (TEOS), as the silica precursor (Fig. 9). It hydrolyzes after mixing with water in accordance with a reaction [230,238,239]

$$Si(–OCH_2–CH_2)_4 + nH_2O \rightarrow Si\ (–OH)_4 + 4C_2H_5OH \quad (1)$$

up to the orthosilicic acid that enters into a condensation reaction

$$(OH)_3Si–OH + HO–Si(OH)_3 \leftrightarrows (OH)_3Si–O–Si(OH)_3. + H_2O \quad (2)$$

Their sequence leads to a polysilisic acid called silica. It can be presented as $SiO_2 \cdot H_2O$ in a hydrated form.

These reactions have a set of special features that determine peculiarities of the sol-gel technique with regard to the biomaterial [228,236,237]. One is an inconvenient media. The hydrolysis of silica precursor has a very slow rate in neutral media, but it is fast in acidic or alkaline solutions. To accelerate it, an acid or alkali is added that serves as a catalyst.

This is the first disadvantage of the processing. An acid and alkali added causes protein denaturation. Such polysaccharides as alginate-bearing carboxylic groups and chitosan-containing amino groups precipitate in acidic and alkaline media, respectively.

Another great disadvantage of sol-gel chemistry is an organic solvent. Ethanol is issued after the hydrolysis (see reaction 1). It is sometimes also added to solubilize the poorly water-soluble TEOS [230,239]. Ethanol has a denaturating effect on the proteins and causes the precipitation of most of the polysaccharides.

A serious problem is brought about by the heating as well. The condensation reaction 2 is reversible. To shift it in the right-hand side and increase the processing rate, the reaction system is heated to 100 °C [230,239,240].

The heating has a considerable negative impact on the biomaterial. It mainly causes protein and DNA denaturation. If the temperature is decreased, the processing slows down and needs a long time.

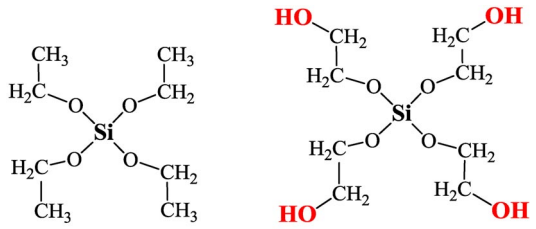


Fig. 9 Structural formulas of TEOS and THEOS. The difference between them is in ethylene glycol residues in THEOS instead of ethanol in TEOS. Additional hydroxyl groups in the THEOS molecule makes the precursor completely water-soluble.

The above-mentioned factors pose serious complications for the biomimetic opportunities of sol-gel chemistry. To minimize their detrimental effects, the immobilization of biomaterial is performed in a two-stage procedure shown schematically in Fig. 10. The first stage serves to produce a silica sol solution. TEOS is admixed with an acidified water. The fast hydrolysis (1) and slow condensation (2) reactions result in the formation of stable silica nanoparticles. This is a starting system for entrapping the biomaterial. It is made at the second stage. The solution pH is shifted into the neutral region in which the condensation reactions are accelerated. The introduced biomaterial is brought into the pores of the silica matrix that is formed via the cross-linking of sol particles [36,182,241–243].

The two-stage procedure eliminates the detrimental effect of acid or alkali, but not ethanol, which is separated after the precursor hydrolysis (eq. 1, Fig. 10). Its presence in the reaction media appears to

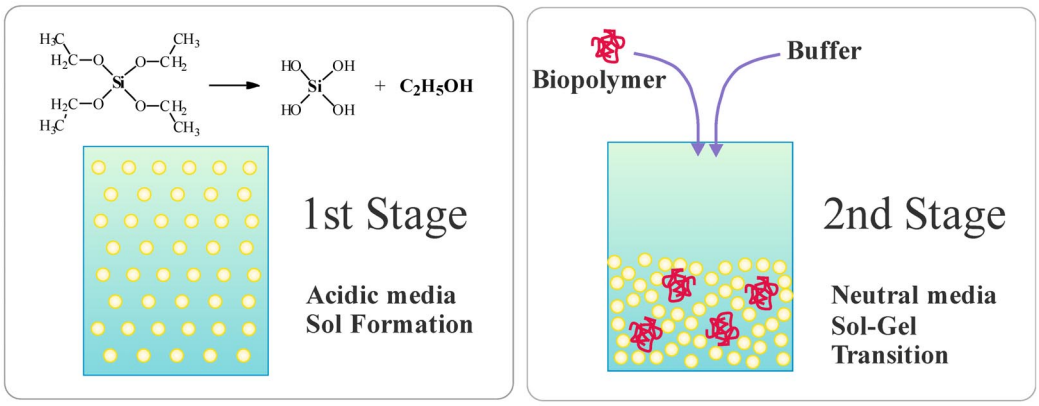


Fig. 10 Current approach used to entrap biomaterial in a silica matrix by common sol-gel chemistry. It is performed in two stages. The first stage serves to hydrolyze TEOS in an acidic or alkaline solution. Oligomeric silica is formed in the form of sol nanoparticles. The sol is homogeneously distributed in the solution bulk. Biomaterial is entrapped at the second stage. The pH is shifted into the neutral region with the help of a buffer solution. It triggers the cross-linking of sol nanoparticles into a three-dimensional network. Added biomaterial is brought into pores. Because the cross-linking can occur in case of tight contact of sol particles, the jellification is followed by the shrinkage of gel.

be the main factor deteriorating the biocompatibility of the sol-gel processing. There are attempts to improve it by the removal of alcohol from a sol solution at the first stage under vacuum (see, e.g., [203,204,244]) but it is not appropriate for the commercial scale.

Figure 11 summarizes the key features of sol-gel chemistry and biomineralization to make the differences between them obvious. The main disadvantage of the present-day commonly used biomimicking approach is in the poor biocompatibility of the precursor and processing. Biomaterial, which is entrapped into the silica matrix at the second stage (Fig. 10), has a minimal effect on its formation. Manipulation on the structure of inorganics is absent. It differs radically from biomineralization. All the processes are under the strict control of biopolymers that serve also as a template for the inorganics, regulating the structural organization from the molecular to the macroscopic level.

In summary, an efficient biomimicking procedure is not available at the present time. The application of sol-gel chemistry needs a change of the precursor and a modification of the processing. It is

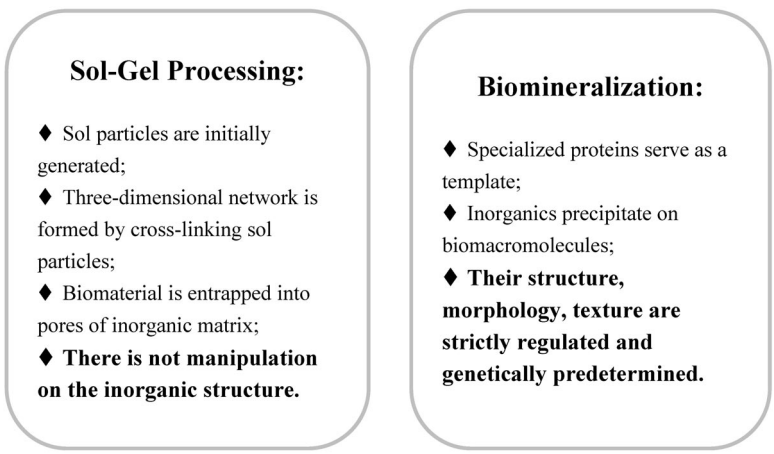


Fig. 11 Differences between the current sol-gel processing and biomineralization in living organisms.

required to improve the biocompatibility and provide the regulation of processes by the biomaterial as much as possible. Two approaches developed by our team are considered below.

Biocompatible silica precursor

It was suggested in [245] to apply tetrakis(2-hydroxyethyl) orthosilicate (THEOS) instead of TEOS (Fig. 9). Their difference is in the substitution of ethanol to the ethylene glycol. The precursor was synthesized first in 1967 [246] and used by Hoffmann with collaborators to mineralize a surfactant template in 1998 [244]. The exchange of ethanol to ethylene glycol is of fundamental importance for both the precursor biocompatibility and processing.

The first important issue is the solubility. THEOS is a hydrophilic substance owing to hydroxyl groups in its molecule (Fig. 9). It can be mixed at any ratio with the water without a phase separation. Therefore, an organic solvent does not need to be added.

The benefit of THEOS is its biocompatibility. The precursor hydrolysis results in ethylene glycol separation in accordance with reaction 1. This solvent does not cause the denaturation of proteins and the precipitation of polysaccharides at a rather large concentration in aqueous solutions. It means that THEOS has a good compatibility with the biomaterial. It was demonstrated first in [118,245,248,249] and then conformed by other authors [205,250–252].

The exchange of ethanol to the ethylene glycol in the precursor leads to a mechanism change. It was found [36,119,245,248,249] that there is an opposite dependence of the reaction rates for THEOS in comparison with that for TEOS. The hydrolysis proceeds quickly in the neutral region. The processing rates decrease sharply with transferring in a rather strong acidic and alkaline media. The silica formation in the neutral region can be accomplished within a few minutes, which depends on the precur-

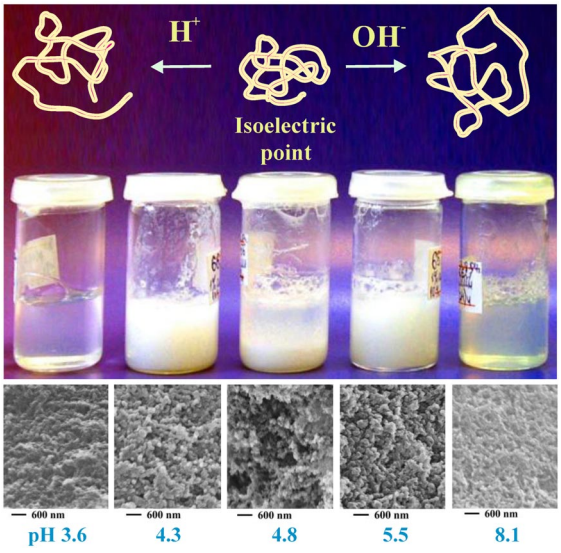


Fig. 12 Pictures of vials with bionanocomposites and images of their aerogels taken by SEM. Samples were prepared by adding 10 wt % THEOS into an aqueous solution containing 1 wt % albumin. Set of numbers at the bottom shows the pH values at which the syntheses were performed. The isoelectric point of albumin is at pH 4.8 at which the protein is in the most compact conformation. It is easily aggregated and precipitated when the precursor was added. A shift of the pH in the acidic or alkaline region from the isoelectric point leads to the expanding (unfolding) of macromolecules owing to their charging. They are not aggregated. Bionanocomposites prepared are transparent. The scale bars correspond to 600 nm.

sor concentration and the biopolymer. The change of pH-dependence of the reaction rates was assigned to a catalysis by biomacromolecules. It was shown that THEOS nucleates on them through the hydrogen bonds [36,253,254]. It is aided by hydroxyl groups in the ethylene glycol residues. Nucleated precursor enters easily into reactions of hydrolysis and condensation.

Reactions of nucleated THEOS provide the silica formation directly on biomacromolecules. An inorganic shell is generated, and biomacromolecules are encased into the silica matrix. As shown in a set of articles [36,245,248,253–256], its structure is regulated by biopolymers in full measure. Even a conformational change of protein macromolecules makes itself evident in the structural organization of silica matrix. By way of example, in Fig. 12 one may see pictures of albumin-silica bionanocomposites prepared at various solution pHs. Their morphology is regulated by the protein conformation that is changed with the pH variation. It has been concluded that the biopolymer mineralization by using THEOS bears close similarities to biomineralization in living organisms [36,118,253–255]. This approach can be reasonably considered as a successful biomimicking technique.

Biopolymer mineralization by metal oxides

The sol-gel chemistry of metal oxides differs very largely from that of silica [60,230,239]. There are the same hydrolysis and condensation reactions but they are too fast. When trying to control them, one fails to do it. A precursor enters into immediate reactions after the contact with water. Metal oxides formed prior to the precursor can approach the template. After that they can precipitate on the templating structures, but the real regulation of processing is impossible. Our attempts to modify precursors in much the same manner as the silica one have failed. Therefore, it was suggested to exchange an aqueous solution with the organic media [257].

The problem is that biopolymers—proteins and polysaccharides—are insoluble in organic solvents with only a few exceptions. Ethylene glycol is one of them. Its advantages have been mentioned previously, and it was used to solubilize a xanthan. This polysaccharide is of microbial origin [258]. Its backbone consists of (1 → 4)-linked glucose as in the cellulose. There is a side-chain of trisaccharide composing of two mannoses and one glucuronic acid residues that are attached to every second glucose residue at the 0–3 position. A structural formula of xanthan can be seen in Fig. 4. Most of the experiments have been performed with (titanium dioxide) preparation because of its commercial significance.

Schematic drawings in Fig. 13 serve to illustrate the principal details of the approach. The polysaccharide is dissolved in the anhydrous (water-free) ethylene glycol. Then an appropriate H_2O amount is added. It is linked mainly with xanthan functional groups because of the polysaccharide's high hygroscopicity. Of great importance is the water amount that should not be added more than it is necessary for the hydration of carbohydrate macromolecules. The H_2O should be absent in the solution bulk.

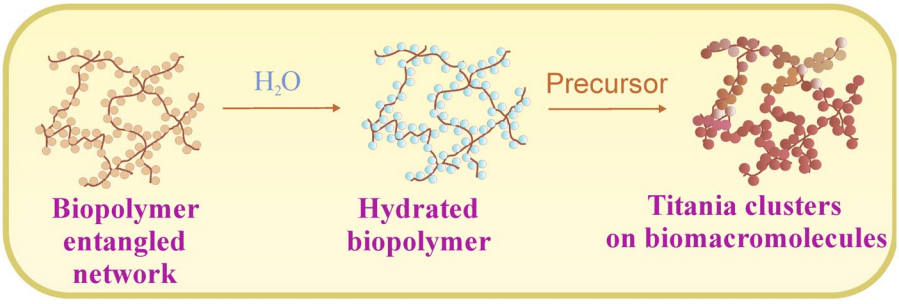


Fig. 13 Schematic representation of the biomimicking template synthesis of titania via the regulated hydration of carbohydrate macromolecules.

An introduced titania precursor enters into instant hydrolysis and condensation reaction similar to 1 and 2 when it comes into the contact with the water. Since the H_2O molecules locate only in the hydration shells of polysaccharide functional groups, TiO_2 is formed primarily on the carbohydrate macromolecules (Fig. 13).

Syntheses are performed in one stage, resulting in the formation of monolithic materials. When the ethylene glycol with the same amount of added water was used alone without the polysaccharide, the precursor addition was accomplished by the titania precipitation. Carbohydrate macromolecules form an entanglement network in the solution bulk. They serve as a template for the TiO_2 that is formed instantly after the precursor contact with the hydration water. The titania builds up a shell surrounding the carbohydrate macromolecules. Crossing points of the entangled chains serve to a junction of shells with the resulting formation of a three-dimensional inorganic network. Representative images of synthesized titania taken at two magnifications are given in Fig. 14.

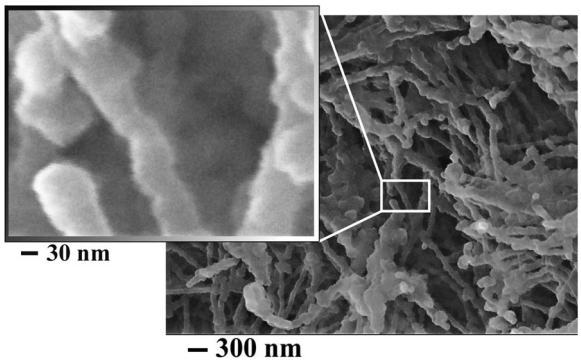


Fig. 14 Images of a titania aerogel taken by SEM at two magnifications. The aerogel for the observation was prepared from a monolithic bionanocomposite that was synthesized at the molar ratio of water to the precursor equal to 3.3. The xanthan concentration was 0.1 wt %, Ti(IV) isopropoxide, 3.1 wt %. A solution was prepared on the basis of anhydrous ethylene glycol. Fibrils present the titania with encased carbohydrate macromolecules that were mineralized in the course of biomimicking processing. The scale bars correspond to 300 (right image) and 30 (left image) nm.

This simple approach, in which the precursor is targeted at the hydrated biomacromolecules, provides full control of the titania synthesis. The approach bears similarities to the biomineralization processes in living cells. Therefore, it falls in the biomimicking methods. The concentrations of water, precursor, and polysaccharides as well as their ratio are factors that serve to regulate the structure. By varying them, one can have from fibrillar structure to particulate material and plate-like morphology [257]. The approach, as shown in [259], can also be applied to synthesize hybrid nanocomposites by using a hyperbranched polyglycerol as the template. This highly hygroscopic dendrimeric polymer absorbs the regulated amount of added water that results in the titania formation of various morphology on its macromolecules.

The biomimicking mineralization results in the making of bionanocomposites consisting of biomacromolecules that are encased into the TiO_2 matrix. The titania thus prepared is in an amorphous state. To transfer it in the crystalline forms, it is necessary to put TiO_2 through a thermal treatment. The transition to the anatase and rutile forms or their mixture occurred at decreased temperatures in comparison with the generally prepared titania. It was attributed to an effect of the biomacromolecule template on the inorganic structure [257].

CONCLUDING REMARKS: PERSPECTIVES

The aim of this overview was to discuss the current status of research on bionanocomposites. This is an exciting and quickly developing area. At present, humankind is on the threshold of large-scale technological application of biopolymers, which are considered as the only alternative to synthetic, petroleum-based polymers. Biopolymers are abundant, renewable resources that can provide a basis for sustainable economic development. Furthermore, they are biocompatible, biodegradable, and environmentally friendly. Their usage has a range of environmental benefits, including reduced reliance on fossil carbon, decreased greenhouse gas emissions, and biodegradation to harmless products by the action of microorganisms. It is believed that biopolymers will allow us to avoid problems caused by synthetic polymers.

There is a wide variety of sources of biological origin to make bionanocomposites. They are highly versatile, having a broad range of physical properties suitable for diverse applications. Nevertheless, biopolymers themselves have the restricted potential to replace petroleum-based polymers because their properties are not good enough for material fabrication. Biopolymers are generally not competitive with polymers in mechanical strength. They have high gas and water permeability, low heat degradation temperature, etc. Nanosized particles are introduced to improve the properties and functionalities of biopolymers.

The benefits of nanoparticles reveal themselves if and only if they are properly distributed in a polymer matrix. Owing to a very large contacting area, there is a plethora of interactions and linkages between the mixed components, influencing the mobility and relaxation behavior of macromolecules that is reflected in the first place in the mechanical and thermal properties of materials. The effect of homogeneously distributed nanoparticles peaks at the concentration of 3–5 wt %.

This is a real challenge to gain the homogeneous dispersion of nanosized additive in the biopolymer matrix. The main obstacle consists in the strong aggregation and pronounced tendency to the agglomeration of nanoparticles owing to their very high surface and surface energy. If they are poorly dispersed and not de-aggregated in the course of preparation or agglomerate with time because of insufficient stabilization, one has a common composite material with the filler. The latter modifies the properties at the concentration of a few tens of percentage. Materials composed of biopolymers and fillers do not demonstrate proper properties inherent to nanocomposites. They should be classified as biocomposites (see Fig. 2).

Bionanocomposites are still a loosely defined family of nanomaterials. Nowadays, this is a quickly developing area in the making. One may find a sharply increased number of publications devoted to composites on the basis of biopolymers with nanosized additives during the past decade (see Fig. 1) in which they are denoted in various manners. A universally accepted term is absent. This type of material is called nanocomposites, nanobiocomposites, biocomposites, green composites, biohybrids, biobased plastics, and bioplastics. In my opinion, the term "bionanocomposite" is best suited. It points out the similarity with nanocomposites, which are well known at present, and takes account of the difference in the polymer origin.

A wide range of available biopolymers and inorganic nanoparticles offers a means of developing a diversity of bionanocomposites with different structure, properties, functionalities, and applications. This is achieved by mixing them in various combinations. Biopolymers form the matrix, determining the shape, structural organization, and main functionalities of bionanocomposites. Dispersed nanoparticles modify the matrix. They allow tuning of the structure, properties, and, as a result, functionality. The nanoparticles can be also added for introducing a special functionality that is not provided by biopolymers themselves. A wide variety of both biopolymers and nanosized particles makes, in principle, possible materials for any desired application. As of now, only certain ones of them are put to fabricate the bionanocomposites. They await wider applications

An inestimable advantage of bionanocomposites over nanocomposites based on synthetic polymers is the biocompatibility. This makes them suitable for biomedical applications. Nowadays, this is

an area of great activity in which the bionanocomposites have dominant significance. They are used to prepare biomaterials like scaffolds and implants, drug-delivery systems, diagnostics, and biomedical devices. The biocompatibility makes them also appropriate for cosmetics and biotechnology. In principle, bionanocomposites will substitute for the current materials on the basis of petroleum-based polymers that are in contact with the living body.

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