REVIEW ARTICLE

Nanobiocomposite: A New Approach to Drug Delivery System

Shailesh L. Patwekar[1], Prasad Jamkhande[1], Surendra G. Gattani[1], Santosh A. Payghan[2]

[1]Department of Pharmaceutics, School of Pharmacy, Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, India, [2]Department of Pharmaceutics, Tatyasaheb Kore College of Pharmacy, Warananagar, Kolhapur, Maharashtra, India

Abstract

This article provides an overview on nanobiocomposite, their benefits, properties, methods of preparation, characterization, and application. The term nanobiocomposite combines benefits of nano size polymer, biodegradable polymer, and composite of polymers which provides the best platform for drug delivery. Nanobiocomposite contains two-phase system, of which, one has at least dimension lower than 100 nm. Nano size provides large surface area for interaction which results in beneficial properties. Benefits of nanobiocomposite include increase nanoparticle stability, increase water retention property, increase cellular uptake of drug, decrease inflammatory reaction, decrease hemolytic activity, and decrease degradation rate. The general method used for nanoparticle synthesis can be modified to prepare nanobiocomposite. Common methods of preparation are emulsion/solvent evaporation, emulsification solvent diffusion, emulsification solvent diffusion, solution intercalation, melt intercalation, double emulsion solvent evaporation, electrospinning, and ultrasonication. Sustained release, control release, antianemic, anticancer, antibacterial, and antifungal formulation can be prepared on nanobiocomposite platform. The objective of review is to enhance the use of nanobiocomposite as drug delivery system.

Key words: Biodegradable polymer, drug delivery, nanobiocomposite, nanocomposite

INTRODUCTION

Nanoparticles are becoming attractive drug delivery system because of their beneficial properties such as small size, high surface to volume ratio, and increased tumor penetration.[1] Properties of nanosized material such as conductivity, mechanical, and optical properties are different than bulk material although composition of material is same.[2]

Synthetic polymers are prepared from petrochemicals take very long time to degrade and hence toxic. This drawback can be overcome using biodegradable polymer. These polymers degraded by microorganism and enzyme.[3] Biodegradable polymer may be natural or synthetic origin. Natural biopolymers include chitosan, collagen, silk, and protein. Synthetic biopolymers are polylactic acid (PLA), polyglycolic acid, poly-l-lactic acid, and poly e-caprolactone.[4,5] In last few years, use of synthetic degradable polymer as drug delivery system has increased.[6] Recently, use of biopolymers such as polysaccharides, proteins, lipids, and their blends has been increased as packaging materials because of their biodegradability, sustainability, and availability. Biopolymer provides transparent film and coating that provide oxygen barrier and mechanical properties.[7] Biodegradable polymers are used in various fields such as drug delivery, gene therapy, tissue engineering, and plastic industry.[3,4]

However, the biopolymer possesses some disadvantages in comparison to synthetic polymer in terms of mechanical stiffness, transparency, and thermal stability. These limitations can be overcome by combining nanosize fillers in the biopolymer matrix to prepare nanobiocomposite.[8]

Address for correspondence:
Shailesh L. Patwekar, Department of Pharmaceutics, School of Pharmacy, Swami Ramanand Teerth Marathwada University, Vishnupuri, Nanded - 431 606, Maharashtra, India.
Phone: +91-9890763538. Fax: +91-2462-229242.
E-mail: shaileshpatwekar@rediffmail.com

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Agglomeration of nanoparticles can be avoided by dispersing in polymer matrix to prepare nanocomposite.[9] Biodegradability of nanocomposite increases after making composite with nanosized clays.[7] Nanobiocomposite contains two-phase system, of which, one has at least dimension lower than 100 nm. Word bio in nanobiocomposite indicates the use of biodegradable materials.[10] The first nanocomposite using silicon dioxide as the substrate for adsorption of drug but drug showed the problem of recrystallization due to high accumulation of drug on inorganic surfaces.[11] Bergese *et al*. The approach of three dimension inert matrixes such as cyclodextrin and crospovidone instead of inorganic surfaces that pose microstructure for drug entrapment to prevent drug crystallization.[12] Inorganic materials such as clays, hydroxyapatite, silica, and carbon nanotube are mostly used in the preparation of composite. Synergistic effect and strong interfacial interaction of biopolymer and inorganic material could improve the swelling properties, mechanical properties, drug loading capacity, and controlled rate of activity.[13] Nanobiocomposite can be prepared with polysaccharides such as hyaluronan and methylcellulose nanobiocomposite with poly (D,L-lactic-co-glycolic acid) (PLGA).[14] However, in nanobiocomposite, the interaction between filler-matrix and between filler-filler molecules is important for successful preparation.[15] Benefits of nanobiocomposite are increased tensile strength, increases water/moisture sensitivity, solubility, dissolution, solid state stability, and thermal stability.[9,16,17] However, sometimes, thermal stability of nanobiocomposite might reduce.[18] Nanobiocomposite can be prepared by method such as emulsion/solvent evaporation, emulsification solvent diffusion, emulsification solvent diffusion, solution intercalation, melt intercalation, double emulsion solvent evaporation, electrospinning, and ultrasonication. Characterization of nanobiocomposite involves techniques such as Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), differential scanning colorimetry (DSC), thermogravimetric analysis (TGA), differential thermal analysis, superconducting quantum interference device (SQUID), nuclear magnetic resonance, and X-ray diffraction (XRD).[16,19-23]

Nanobiocomposite hydrogel is prepared by addition of nanoclay, which results in increased in water retention in gel and provides a platform for hydrogel, which is used in drug delivery and contact lenses.[24] The combination of biopolymer and hydroxyapatite are mainly used in the lung drug delivery.[13] The various investigations show that nanobiocomposite proved to be beneficial as a drug delivery system such as enhanced antiemetic activity,[25] intrathecal delivery in gene therapy,[26] colon cancer treatment,[27] antibacterial and antifungal activity,[28] control release delivery of anticancer drug paclitaxel,[4] drug delivery injectable cell scaffold in tissue engineering,[29] and remote control drug release.[30]

BENEFITS

Key property of nanobiocomposite is large surface area due to nanosized polymer which results in improved interaction between filler and matrix.[8] It was found that tensile strength of composite increases as concentration of nanofiller increases up to some extent and then remain constant such as addition of nanocrystalline cellulose (up to 5% w/w), in the chitosan matrix increases tensile strength of composite, beyond this concentration, tensile strength of composite remain constant.[31] Nanoparticles, which are prepared by polysaccharides nanocrystals, showed strong interactions in the form of hydrogen bonding between them. It leads to agglomerates of nanoparticles. In nanocomposite, these interactions are beneficial to create filler network within matrix. Chitosan is biodegradable polymer and mostly used in drug delivery, but problem with chitosan is it is water sensitive. The chitosan cellulose nanocrystal composite showed increase tensile strength up to 150% and decrease hydrophilicity compared to neat chitosan.[15]

Magnetic nanoparticles are sensitive to external alternating magnetic field. It is used frequently in nanocomposite to control drug release.[30] Nanocomposite hydrogel has problem of undesired dehydration during its use in drug delivery. However, the study showed that addition of montmorillonite (MMT) nanoclay in the polyvinyl alcohol (PVA) hydrogel increases its water retention property.[24] Nanobiocomposite of PLGA-MMT with paclitaxel drug shows enhanced cellular uptake of paclitaxel in cancerous cell and better interaction of nanoparticle with gastrointestinal tract (GIT).[19] Chitosan/silk fibroin nanobiocomposite blend film shows improved tensile strength and enhanced water/moisture sensitivity.[17]

Poly(ester-amide) functionalized ZnO nanobiocomposite shows enhanced thermal stability.[20] PLGA alone shows inflammatory response in the spinal cord, but nanobiocomposite of PLGA with hyaluronan and methylcellulose shows much reduced inflammatory response.[14] PLA posses limitation such as low cell adhesion and inflammatory reaction, this can be overcome by making composite with calcium phosphate. PLA/hydroxyapatite composite shows decreased degradation rate compared to PLA alone.[21] The drug celecoxib is used as adjuvant for treatment of colon cancer. Hemocompatibility studies showed that free celecoxib revealed hemolysis but nanocomposite of hydroxyapatite-chitosan loaded with celecoxib showed decrease hemolytic activity. This is due to the rigid structure of composite which did not attach to red blood cell membrane.[27]

Other important properties of nanobiocomposite include good electrical conductivity, electrostatical stability, thermal stability, better barrier properties, better chemical resistance, and increased modulus.[32] Nanobiocomposite increases the residence time of drug in the GIT and oral delivery of drug.[19] Biodegradability and biocompatibility are principle

properties of nanobiocomposite which allow their used in the various types of drug delivery system.[33]

METHOD FOR PREPARATION OF NANOBIOCOMPOSITE

Methods which are used for the preparation of nanoparticles can be used to prepare nanobiocomposite with some modification. Various methods used to synthesize nanobiocomposite are listed in Table 1 with their starting material, solvent used, apparatus used, and characterization. However, some general methods are discussed here.

Emulsion/solvent evaporation

It is based on the formation of emulsion and then evaporation of solvent. Evaporation of solvent and high force stirring results in precipitate formation in nanoform. It is suitable for hydrophobic drugs. Both drug and polymer are dissolved in common organic solvent to make oil phase. Water phase is made up with water soluble polymer. Oil phase is then dispersed in aqueous phase with continuous stirring or sonication to form oil in water emulsion. Then, solvent is allowed to evaporate to form drug-loaded nanocomposite particles. In oil in oil emulsion, both phases are oil. In this method, oil phase and aqueous phase are selected depending on solubility of drug and polymer.

Paclitaxel-loaded PLGA/MMT nanocomposite by this method using dichloromethane (DCM) as solvent. 5 mg paclitaxel and 110 mg PLGA were dissolved in DCM to prepare clear solution of oil phase. Aqueous solution is prepared with 2% w/v PVA and various amounts of MMT (0, 0.046%, and 0.092% w/v). Oil phase is then emulsified in the aqueous phase with sonication for 120 s. The formed emulsion was allowed to evaporate overnight at room temperature to harden the particles.[22,34]

Emulsification solvent diffusion

This method is based on emulsification then diffusion of solvent to outer phase to form nanocomposite particle precipitate. Diffusion of solvent is due to its solubility in outer phase. Diffusion of solvent and high force stirring results in precipitate formation in nanoform. Polymers are dissolved in various solvents based on solubility and swelling nature of polymers. Then, internal organic oil phase is emulsified in outer aqueous phase with continues stirring or homogenization to form nanocomposite particle.

The prepared PLA/MMT nanobiocomposite emulsion by emulsification solvent diffusion method. PLA solution and MMT dispersion were separately prepared in ethyl acetate solvent. The PLA solution, clay dispersion, and lauryl alcohol were then mixed and used as an oil phase. The aqueous phase is prepared with surfactants and PVA in distilled water. Oil phase is dispersed in the aqueous phase with homogenization and then magnetic stirring.[35,36]

Solution intercalation

This method is mostly used for layered silicates as nanofiller which are to be intercalated in the polymer matrix. Principle involves diffusion of the polymer chain in the galleries between silicate layers. In this method, solvent is selected such that polymer is soluble in solvent while inorganic nanofiller just swells. Polymer is dissolved in solvent, and then, inorganic nanofiller is added in solution with stirring. Usually, fillers are allowed to swell before addition in polymer matrix. This leads to intercalation of polymer into silicate to form nanobiocomposite.[23,37]

Melt intercalation

This method operates on the same principle as that of solution intercalation, but here, heat is used instead of solvent for intercalation of polymers into the silicate. In this method, the mixture of polymer and layered silicate are heated till the softening point of polymer achieved. Then, it is mixed with high shear rate. This leads to intercalation of matrix into silicate layered. Instruments such as single screw extruder and double screw extruder are used for melt intercalation. It is beneficial over solution intercalation in terms of the absence of organic solvents and ease of industrial processes. Polymer chain diffuses in the galleries between silicate layers. Depending on degree of penetration of polymer chain into the silicate, nanobiocomposite may be of two types, i.e., intercalated and exfoliated.

Poly (butylene adipate-co-terephthalate) - MMT nanobiocomposite by melt intercalation method using interbatch mixer, counter rotating mixer at 160°C for 15 min for 50 rpm then 120°C for 20 min for 100 rpm.[23,39]

Double emulsion solvent evaporation

In this method, two polymers selected are dissolved in oil phase and aqueous phase depending on their solubility. Then, water in oil emulsion is prepared with stirring. The resulting emulsion is then added to external phase which is aqueous phase with stabilizer like PVA; then, system is stirred to evaporate solvent at room temperature.

The author[40] prepared calcium phosphate (Cap)/ poly(hydroxyl butyrate-co-hydroxyl valarate) (PHBV) nanobiocomposite by solid in oil in water (solid-in-oil-in-water [s/o/w]) emulsion using solvent evaporation method. W/O emulsion was made by aqueous solution of bovine serum albumin (BSA) and organic solution of PHBV in chloroform using homogenizer. The resulting emulsion was added in PVA aqueous solution to form w/o/w emulsion. Then, mixture

Table 1: Preparation of nanobiocomposite

Methods	Materials	Solvent	Equipment	Characterization	References
Covalent linkage	Chitosan, eucalyptus wood pulp, MAC	Sulfuric acid,acetone, toluene,triethylamine, acetic acid	Mechanical stirrer, centrifuge machine, ultrasonicator, freeze drier	TEM, FTIR, TGA, DTG, water uptake	[15]
In situ sol-gel process	Sodium alginate, hydroxyapatite, diclofenac sodium	Distilled water, ammonium hydroxide, Ca $(NO_3)_2$ $4H_2O$, $(NH4)_2$ HPO_4	Stirrer, oven, hypodermic syringe	FTIR, XRD, SEM, swelling behavior, drug release	[13]
Film casting	Chitosan, silk fibroin, methoxy poly (ethylene glycol)-b-poly (D, l-lactide) diblock polymer	Acetic acid, distilled water,acetone/ ethanol	Stirrer (600 rpm), dialysis chamber,Petri dish, dryer	FTIR, SEM, water contact angle, moisture uptake	[17]
Emulsion/ solvent evaporation method	PLGA, PVA, paclitaxel, sodium montmorillonite, coumarin-6	Distilled water, dichloromethane	Centrifuge machine, sonicator, freeze dryer	SEM, AFM, zeta potential analysis, drug encapsulation efficiency	[27]
	Chitosan polylactide, paclitaxel, acetic acid, sodium hydroxide, montmorillonite, NaH_2Po_4	Chloroform, dichloromethane	Sonicator, homogenizer, stirrer	XRD, SEM, FTIR, *in vitro* drug release, swelling studies	[4]
Freezing-thawing cyclic method	PVA, Cloisite Na^+	Double distilled water	Heating stirrer, freeze	XRD, TEM, dehydration kinetics at various temperature	[19]
Ultraviolet photo polymerization	N-isopropylacrylamide, poly (ethylene glycol) 400, dimethyl acrylate, 2, 2-dimethoxy-2-phenyl acetophenone	Deionized water, ethanol	Probe sonicator, ultrasonic bath	Swelling studies, penetration of remote control delivery by pyrocatechol violet dye	[25]
Ultrasonic irradiation	Polyesterimide, N, N-dimethylacetamide, N-methyl-2-pyrolidone, Pyromellitic dianhydride, 5-aminoisopthalic acid, TiO_2	N, N-dimethyl formamide, triethylamine	Viscometer, polarimeter, ultrasonicator	XRD, FTIR, TGA, FE-SEM	[31]
	S-tyrosine, Triethylamine, Isophthaloyl chloride, Zinc oxide nanoparticle, gamma methacryloxypropyltrimethoxysilane, HCL, ethanol	N-methyl-2-pyrolidone, distilled water, methanol	Ultrasonic liquid processor, ultrasonicator, stirrer	XRD, FTIRFE-SEM, AFM, TEM, UV/visible spectra	[28]
Electrospinning	SBF, PLA pellets, carbonated calcium-deficient hydroxyapatite	Chloroform, dimethyl formamide	Ultrasonicator, XP105 delta range analysis balance	XRD, SEM, TEM, EDX, FTIR, *in vitro* degradation test, bioactivity test	[29]
Solution casting method	Organically modified MMT, methyl tallow bis-2-hydroxyethyl quaternary ammonium glycerol, organoclay	Distilled water	Magnetic stirrer, Bath type ultrasound sonicator	Film thickness, tensile properties, water vapor permeability, antibacterial activity	[7]

(Contd...)

Table 1: *(Continued)*

Methods	Materials	Solvent	Equipment	Characterization	References
	Chitosan, Nanocrystalline cellulose	Distilled water	Homogenizer, High shear mixer, sonicator, magnetic stirrer	XRD, SEM, TGA, FTIR, Mechanical properties, water vapor permeability	[26]
	PLA, polycaprolactone, MMT	Chloroform	Ultrasonic bath	XRD, SEM, FTIR, TGA, swelling studies, *in vitro* drug release	[6]
Double emulsion solvent evaporation method	Calcium phosphate nanoparticle, PHBV, PVA, BSA, 3-hydroxy valerate	Chloroform, distilled water	Magnetic stirrer, homogenizer, ultrasonication	FE-SEM, *in vitro* drug release	[40]
Precipitation	Chitosan, goat blood, gelatin, $Fecl_2$ solution, ammonium hydroxide	Distilled water, acetic acid	Vortex mixture, centrifuge machine, muffle furnace	XRD, TEM, FTIR, VSM, MRI	[56]
In situ chemical reduction	Silver nitrate, sodium salt of polyacrylic acid, D (+)-maltose monohydrate, α-FeOOH nanoparticles	Distilled water	Ultrasound bath	XRD, TEM, SQUID, antimicrobial testing, cytotoxicity assay	[23]
Emulsion solvent diffusion	Salmon calcitonin, coumarin-6, PLGA, PVA 403, Chitosan, insoluble lactose	Acetone, methanol	Agglomaster, lyophilizer, mechanofusion	Zeta potential, LDSA, *in vitro* evaluation	[35]
	PLA, Sodium MMT, Cloisite 30B , PVA, SLS, Lauryl alcohol	Ethyl acetate	Homogenizer	DTA, TGA, zeta potential, particle size, morphology	[16]
Co-precipitation	Calcium chloride, rhodamine phalloidin, tartaric acid, chitosan, ammonia, flurosin isothiocyanate, propidium iodide, 4,6-Diamidino-2-phenylindole, RNase (Ribonuclease), 3-(4,5-dimethyl thiazole-2-yl)-2,5-diphenyl tetrazolium bromide, celecoxib, glutamine, penicillin, streptomycin	Dimethyl formamide	Stirrer	Drug release, cell proliferation assay, morphological analysis, cytoskeleton nuclear analysis, cell cycle analysis, tumor inhibition in human colon cancer xenograft mouse model	[22]
Ionic interaction	Polyethylene amine, alginic acid, DNA, 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide, agarose tris, YoYo – lethidium bromide xylene cyanol, tetramethylrhodamine isothiocyanate, 4,6-diamidino-2-phenylindole dilactate bromophenol, superfect, fugene, cell culture product	Distilled water	Stirrer, membrane filter (22 μ)	Zeta potential, DNA retardation assay, *in vitro* cell transfection, cytotoxicity assay, fluorescence confocal microscopy, DNA protection assay	[58]
Ultrasonic atomization	PLGA 85, PLDL 70, methyl-poly (ethylene glycol)-PLGA, PVA 403, coumarin-6, thrombin receptor, activator peptide-6	Acetone, dichloromethane	Sonicator	Particle size, zeta potential, *in vitro* drug release, *in vitro* cell adhesion	[24]

(Contd...)

Table 1: *(Continued)*

Methods	Materials	Solvent	Equipment	Characterization	References
In situ free radical polymerization	N-isopropylacrylamide laponite, hectorite, MMT, ammonium persulfate, tetramethyl ethylene diamine	Deionized water	Nitrogen flow cell	XRD, SEM, DSC, swelling kinetics	[53]
Solvent intercalation and melt intercalation	PBAT, MMT	Chloroform	Interbatch mixer, counter rating mixer	XRD, TEM, DSC, TGA, mechanical test	[39]
Solvent evaporation	PLGA, ammonium hydroxide, HCL, Ferrous chloride, span 80	Acetonitrile	Overhead mixer operated at 7000 rpm with specially designed high shear impeller	SEM, TEM, SQUID, DLLS	[32]
Microwave-induced diffusion	Glipizide drug, acacia gum, ghatti gum, cassia gum, gelatin	Distilled water	Microwave oven	Solubility study, dissolution studies, XRD, DSC, FTIR, SEM, TEM, *in vivo* evaluation	[59]

MAC: Methyl adipoyl chloride, XRD: X-ray diffraction, DSC: Differential scanning colorometry, FTIR: Fourier transform infrared spectroscopy, SEM: Scanning electron microscopy, TEM: Transmission electron microscopy, SQUID: Superconducting quantum interference device, TGA: Thermo gravimetric analysis, LDSA: Laser diffraction particle size analyzer, SBF: Simulated body fluid, EDX: Energy dispersive X-ray spectroscopy, PHBV: Poly (hydroxybutyrate-co-hydroxyvalerate), BSA: Bovine serum albumin, SLS: Sodium lauryl sulfate, PABT: Poly (butylene adipate-co-terephthalate), MMT: Montmorillonite, PLGA: Poly (D, L-lactic-co-glycolic acid), PVA: Polyvinyl alcohol, AFM: Atomic force microscopy, VSM: Vibrating sample magnetometer

was magnetically stirred to evaporate solvent. PHBV-BSA microspheres were filtered, freeze-dried. Modified s/o/w emulsion solvent evaporation method was used to produce BSA-loaded Ca-P/PHBV nanocomposite microspheres. Ca-P nanoparticles were dispersed in the PHBV-chloroform solution using ultrasonication and homogenization to form a s/o nanosuspension and it is dispersed in the inner water phase (the aqueous BSA solution), followed by the same procedure for PHBV-BSA microsphere preparation.[22,41]

Electrospinning

This technique is used to prepare nanobiocomposite fiber. The apparatus consist of flat tip needle, high voltage power supply, pump, and conducting collector plate. Mixture of polymer is prepared in organic solvents such as dimethyl formamide (DMF) and chloroform. Then, it is loaded on electrospun needle and the high voltage applied to form composite fiber.

The author[21] prepared PLA/carbonated calcium-deficient hydroxyapatite (CDHA) bionanocomposites fibers by this method. In brief, PLA pellets were dissolved in chloroform; CDHA precipitate was added to PLA solution to form mixture followed by DMF addition with continuous stirring for 4 h. This mixture is then loaded into the electrospun apparatus and injected through the needle to form fibers. Fibers are then dried in fume hood.[42-44]

Ultrasonication

Here, conversion of material into nanosize is due to high-frequency ultrasound waves. Usually, in this method, two polymers are added in solvent (usually ethanol), and the mixture is then ultrasonicated to obtain nanobiocomposite. The remaining solvent is removed. The frequency of irradiation, time for irradiation, and power supply are variable which controls size and morphology of nanobiocomposite.

The author.[20] prepared poly(ester-imide)s (PEA) ZnO nanobiocomposite by ultrasonication method. They used PEA as a matrix and modified ZnO nanoparticles (modification by the silane coupling agent, i.e., γ-methacryloxypropyltri-methoxysilane). PEA dispersion is made in ethanol using ice-water ultrasonic bath. Followed by addition of different proportion of modified ZnO nanoparticle in PEA suspension and mixture was ultrasonicated for 4 h. Then, solvent was removed, and nanobiocomposite was dried.[33,45]

Characterization of nanobiocomposite

Morphological characterization

Following techniques are used for morphological characterization.

XRD techniques

XRD interpretation operates on principle of constructive interference formed by X-ray (monochromatic) and crystalline sample. Bragg's law is used to explain constructive interference. Here, XRD is used to determine shape, crystalline, and amorphous nature of nanofiller, drug, and polymer. It is also used to determine phase separation of nanofiller and polymer. Intercalation of layered silicate with polymer can be identified that is exfoliated or intercalated.[46-50]

FTIR

Each functional group shows some fixed resonance frequency during infrared irradiation which is to detect this functional group. It is used to determine changes in nanobiocomposite in terms of functional group. Chemical changes occurred during composite preparation by different polymers and drug can be easily detected. It also identifies unknown metal in sample, quality, and consistency of sample and amount of component in mixture. It is used to determine chemical composition of intermediate and obtained particle.[4,49,51]

TEM

Here, electrons are transmitted through an ultrathin sample, these electrons interact with the sample during passing. An image is formed from the interaction of the electrons during transmission which is detected and magnified. TEM is used to detect quality about internal structure, various defects, and space distribution of different phases. It provides data about state of dispersion of nanofiller in polymer matrix. Nature of intercalation of layered silicate with polymer can be identified, i.e., exfoliated or intercalated.[46,47,52,53]

Atomic force microscopy

Images are formed by measuring the physical interaction between a sharp AFM tip and the sample. It provides three dimensional images of a particle and group of particles. Surface morphologies such as surface roughness, surface forces, and size range of the nanoparticles are determined. Information such as mechanical, chemical, and adhesive properties of surface can be obtained.[19,29,54]

SEM

Accelerated electrons are allowed to incident on sample, three-dimensional images are formed by secondary electrons and backscattered electrons. It provides data about morphology of single polymer, drug, and nanobiocomposite. It provides data about state of dispersion of nanofiller in polymer matrix. Surface fracture and aggregation of particles in nanobiocomposite can be easily detected.[46-48,55,56]

Thermal analysis

TGA

It is used to measure change in weight of sample as temperature or time changes. Change in weight loss between single polymer and composite can be compared. It suggests physical changes such as melting which do not involves weight loss as well as chemical changes such as combustion which involves weight loss. The weight of the sample is plotted against time or temperature which suggests thermal changes in the material such as loss of solvent, water of hydration in inorganic materials, and finally decomposition of the material.[16,20,57]

DSC

This technique is used to detect nature of crystallization, exothermic, and endothermic reaction. In endothermic reactions, e.g., solid sample melts to a liquid; it requires more heat flowing to the sample to increase its temperature at the same rate as the reference because sample absorbs heat to convert into liquid state, hence more heat is require to raise temperature of sample as compared to reference. Reverse is the case with endothermic reaction which occurs during crystallization. It provides data about thermal stability of pure polymer and nanobiocomposite by melting point.[29,46-48,52,57]

Magnetization

It is specially used in characterization of magnetic nanobiocomposite. It gives information about magnetic power of nanobiocomposite, i.e., what are the changes occurred in magnetic property of material after making composite. It tests the response of external magnetic field on nanobiocomposite. It also suggest about effect of temperature on magnetic property. The techniques used are vibrating sample magnetometer (VSM) and SQUID. VSM operates on Faraday's law of induction, i.e., changing magnetic field produce electric current which can be measured. Initially, sample is placed in constant magnetic field to induced magnetization. Magnetic field is created around sample by magnetic dipole moment then sample is vibrated. This creates change in magnetic field and, in turn, changes electric field. It indicates magnetic behavior and magnetic strength of materials.[58,59]

In vitro drug release

In this study, different types of apparatus and method are used depending on formulation. Wang *et al.* prepared sodium alginate hydroxyapatite nanobiocomposite. They used intelligent dissolution apparatus stirred at 37°C ± 0.5°C temperature. The release of magnetic hydrogel nanobiocomposite drug release by pyrocatechol violet dye as model drug. Nanda *et al*.[4] used dissolution tester equipped with 6 paddles at 100 rpm for the release of anticancer drug paclitaxel. Feng *et al*.[19] used 10 ml phosphate buffer solution in capped centrifuge tube for drug release from paclitaxel-loaded PLGA-MMT nanobiocomposite.

Swelling property

Swelling study is mostly performed in nanobiocomposite hydrogel using hydrogel disc. Temperature, solvent, and time of study varies according to formulation. Solvent used are

distilled water, hydrochloric acid, sodium hydroxide, etc. Swelling ratio can be determined by following formula.[30,60,61]

Swelling ratio = weight of swollen particle/weight of dry particle.

Application in drug delivery

Control release

The nanocomposite of hydrogel with magnetic particles can be used in pulsatile drug delivery system. Remote control release of drug is designed by magnetic nanocomposite of N-isopropylacrylamide (NIPAAm). Iron oxide is used as remote heating device, and NIPAAm is a temperature sensitive hydrogel. Alternating high-frequency magnetic field leads to heat generation in nanocomposite which forces the swelling transition of the hydrogel. It was found that drug release decreases with increase in temperature.[30] Nanobiocomposite of sodium alginate/hydroxyapatite demonstrates control release of diclofenac drug. Prepared nanocomposite beads could be used in the production of oral pharmaceutical formulations. Nanocomposite prolonged the release of diclofenac drug for 8 h more compared with the neat sodium alginate hydrogel beads.[13] Control release of drug observed in glycolic acid-g-chitosan-gold-nanoflower nanocomposite. The nanohybrid scaffolds were found to be stable towards the pH of the medium. The prepared nanohybrid scaffolds are biocompatible. This nanocomposite showed control drug release rate in the buffer solution (pH 7.4). Therefore, gold nanoflowers are the viable additive for the glycolic acid grafted chitosan-based system, for drug delivery.[59,62-65]

Sustained release

A preparation for spinal cord injury prepared successfully in the form of sustained release nanobiocomposite containing hyaluronan and methylcellulose hydrogel with PLGA nanoparticle, it was found to be safe and biocompatible. It was demonstrated that this preparation is well tolerated in intrathecal space of injured rats for 28 days and showing no increase in inflammation, scarring, or cavity volume relative to controls and no effect on locomotor functions.[14]

Anticancer

Nanobiocomposite prepared with PLGA-MMT with paclitaxel drug for oral delivery of anticancer. Paclitaxel is anticancer drug cannot be given orally because of low absorption through GIT and first-pass effect. PLGA-MMT nanocomposite demonstrated increased GIT absorption and increase cellular uptake by CaCo-2 and HT-29 cells. The drug release study showed an initial burst followed by a slow, sustained release, which was not significantly affected by the MMT component.[19] The study shows that nanobiocomposite of hydroxyapatite-chitosan with celecoxib drug is effective and safe vehicle for colon cancer drug delivery. It was found that nanocomposite particles overcome side effect shown by free celecoxib and also nanocomposite showed more potent anticancer activity than free celecoxib.[27]

Antianemic

Ferroarabinogalactan (A nanobiocomposite) prepared from nanodispersion of iron oxide in arabinogalactan matrix showed enhance antianemic activity. Arabinogalactan is obtained from Siberian larch (*Larix sibirica*) posses antianemic activity. It has been shown that this composite produces a hemopoiesis stimulator and iron stabilizing effect; this is due to synergistic effect of iron nanoparticle and arabinogalactan.[25]

Antibacterial and antifungal

The potent and safe targeted antibacterial and antifungal drug delivery can be achieved by magnetic nanocomposite of iron oxide and silver nanoparticles. At the observed minimum inhibition concentrations, nanocomposite did not exhibit acute cytotoxicity against mice embryonal fibroblasts. The synergistic effect of magnetic properties of iron oxide nanoparticles and antimicrobial property of silver nanoparticle thus demonstrate these nanocomposites to be used in antibacterial and antifungal applications as a targeted drug delivery.[28]

Research patent reported on nanobiocomposite

W. Wan, L. Millon patented (Pub. No. US 2009/0028927 A1) "poly vinyl alcohol-bacterial cellulose nanocomposite." They prepared polyvinyl alcohol-bacterial cellulose nanocomposite which is applicable for tissue engineering and control release of bioactive agent. A. Guillermo, R. W. Antonio patented (Pub. No. US 2007/0071790 A1) "Biodegradable nanocomposite with enhance mechanical properties for soft tissue." They described method and composition for making and using nanocomposite. J. Bako, S. Marta, J. V. Andrienn, C. Csaba, Z. M. Borbely, H. Csaba, J. Borbely (Pub. No. US 2007/0212419 A1) patented "synthesis of biocompatible nanocomposite hydrogels as a local drug delivery system." They prepared nanocomposite hydrogel as drug delivery for periodontal infection. K Makino, H. Terada, T. Nakajima, K. Tomoda (Pub. No. US 2009/0169637 A1) patented "nanocomposite particles." They prepared PLGA-trehalose nanocomposite for drug delivery. This showed good solubility and good redispersibility when brought in contact with water.

CONCLUSION

In nanobiocomposite, biodegradable polymer is used and one of polymer should be in nanosize. Benefits and properties include enhanced thermal stability, increased tensile strength, decreased side effect, increased potency and increased targeting, increased water retention to gel. Nanobiocomposite can be prepared by various methods.

Common methods of preparation are emulsion/solvent evaporation, emulsification solvent diffusion, emulsification solvent diffusion, solution intercalation, melt intercalation, double emulsion solvent evaporation, electrospinning, and ultrasonication. Characterization techniques include FTIR, XRD, SEM, TEM, DSC, TGA, VSM, SQUID, and *in vitro* drug release. It can show potential application in drug delivery. They show potency and safety in various cancer treatments compared to conventional drug delivery. They can be also used for tissue engineering, gene therapy, and antimicrobial application. Magnetic nanobiocomposite can be used in controlled drug release and targeted drug delivery system. However, nanobiocomposite preparation as a drug delivery is under investigation. Cost to benefit ratio is important as nanotechnology requires expensive equipment for preparation and characterization. In future, further development in nanobiocomposite technology may prove potent, safe, controlled, and targeted drug delivery.

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