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Review

# Potentials of natural polymers as nanomaterials for pharmaceutical drug delivery

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## ABSTRACT

During the last decades, pharmaceutical technology has taken advantage of the advent of nano-technology for its application in four broad areas of the pharmaceutical industry: drug delivery, diagnostic products, biomarker discovery and product packaging. Of all the potential pharmaceutical applications of nanotechnology, drug delivery is currently the most developed and seems to be the most promising for the long-term. Of great advantage is the concept and ability to manipulate molecules and supramolecular structures to produce drug delivery devices with great potential for improving the efficacy of drug delivery systems. Polymeric nanoparticles are colloidal carriers which usually consist of synthetic, semi-synthetic or natural polymers, and depending on the materials used and their manufacturing methods, nanoparticles can adopt diverse shapes and sizes with distinct properties. Natural polymers such as gelatin, albumin, alginate and chitosan have great potentials because of their inherent properties such as biocompatibility, non-immunogenicity, non-toxicity and biodegradability. In addition, they can be subjected to physical and chemical modifications to alter their physico-chemical properties resulting in a wide range of functional properties that may permit their application as polymers for the formulation of nanoparticles. Moreover, they have been found to be more readily available, flexible to regulatory issues and relatively inexpensive when compared to the synthetic polymers. This paper is a review of some natural polymers that have shown promise as biodegradable polymers for the formulation of nanoparticulate drug delivery systems.

**KEYWORDS:** natural polymers, nanoparticles, nanomaterials, drug delivery

## INTRODUCTION

Over the past decades, there has been considerable research interest in nanotechnology by the utilization of nanomaterials as carriers for small and large molecules. The word ‘nano’ is derived from Latin, which means “dwarf” and refers to one thousand millionth of a particular unit. Nanoparticles could therefore be defined as particulates, dispersions or solid particles with a size in the range of 10-1000 nm [1]. Nanotechnology is not in itself a single emerging scientific discipline but rather a meeting point of traditional sciences such as chemistry, physics, material science and biology, to bring together the required collective expertise needed to develop these novel technologies [2]. Although nanotechnology has been most commonly used in the fields of science such as electronics, physics and engineering for many decades, the pharmaceutical fields has not been fully explored until the last decade [3].

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Pharmaceutical technology has taken advantage of the advent of nanotechnology for its application in four broad areas of the pharmaceutical/health industry: drug delivery, diagnostic products, biomarker discovery and product packaging. Of all the potential pharmaceutical applications of nanotechnology, drug delivery is currently the most developed and seems to be the most promising for the long-term [4]. Research and development of innovative drug delivery systems are increasing at a rapid pace and it is likely that this trend will intensify in future, as public health expenses demand lower costs and increased efficiency for new therapies. Due to their small size, nanosized drug delivery systems are promising tools in therapeutic approaches such as selective or targeted drug delivery towards a specific tissue or organ, enhanced drug transport across biological barriers and intracellular drug delivery which is of interest in gene and cancer therapy. Nanoparticles offer the following advantages in drug delivery systems [1]:

i. Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.

ii. They control and sustain release of the drug during transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.

iii. Particle degradation characteristics can be readily modulated by the choice of matrix constituent.

iv. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction which is an important factor for preserving the drug activity.

v. Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.

vi. Nanoparticles can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc.

In spite of these advantages, nanoparticles have some limitations. For example, their small size and large surface area can lead to particle-particle aggregation making physical handling of nanoparticles difficult in liquid and dry forms. In addition, small particle size and large surface area readily result in limited drug loading and burst release which is unsuitable for controlled drug delivery systems. These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available [1].

The constituents of nanoparticles for biomedical application are required to be physiologically compatible (biocompatible) and disintegrate easily in the physiological environment (biodegradable). Nanoparticles have been used as carriers for conventional drugs as well as proteins and peptides, enzymes, vaccines and/or antigens. Nanoparticles, nanospheres or nanocapsules can be obtained, depending on the process used for the preparation. Nanospheres or nanoparticles are homogenous matrix systems in which the drug is dispersed throughout the system, whereas nanocapsules are vesicular systems in which the drug is confined to a cavity surrounded by a polymeric membrane [1]. Based on their manufacturing methods and materials used, these particles can adopt diverse shapes and sizes with distinct properties.

Nanoparticles are prepared most often by three methods: dispersion of preformed polymers, polymerization of monomers and ionic gelation or coacervation of hydrophilic polymers. However, other methods such as supercritical fluid technology [5] and particle replication in non-wetting template [6] have been described in the literature for the production of nanoparticles. The latter was claimed to have absolute control of particle size, shape and composition, which could set an example for the future mass production of nanoparticles in the industry. Many types of nanoparticles are under various stages of development as drug delivery systems, including liposomes and other lipid-based carriers (such as lipid emulsions and lipid-drug complexes), polymer-drug conjugates, polymer microspheres, micelles, and various ligand-targeted products such as immuno-conjugates [7-9].

Polymeric nanoparticles consist of synthetic, semi-synthetic and natural polymers which serve as the encapsulating system for the drug. Synthetic polymers are less advantageous due to their

limited solubility in physiologically compatible solvents. They are often soluble only in organic solvents and, depending on their structure, they are highly lipophilic and require additional excipients such as surfactants to form stable nanoparticle dispersions [10]. On the other hand, natural polymers such as gelatin, albumin, alginate and chitosan, have inherent properties such as biocompatibility, non-immunogenicity, non-toxicity and biodegradability making them suitable for wider application [11-13]. Their flexibility to regulatory issues and relatively inexpensive cost when compared to synthetic polymers makes them suitable candidate for nanoparticulate systems [13]. In addition, they can be subjected to physical and chemical modifications to alter their physicochemical properties, conferring a wide range of functional properties that may permit their application as polymers for the formulation of nanoparticles in drug delivery.

With the availability of a wide variety of natural polymers, great successes have been achieved in developing promising drug delivery systems which provide effective therapy for prolonged periods of time [14]. Most of the natural polymers are water-soluble, but can be transformed into nanoparticles by means of denaturation, leading to cross-linking and thus reducing its water-solubility. In addition, the use of oppositely charged counter ions in cases of materials with charged groups leads to the formation of particles by electrostatic neutralization also known as coacervation [15].

The following are some of the natural polymers that have found application as polymer for the preparation of nanoparticulate systems for pharmaceutical drug delivery.

## 1. Albumin

Albumin, being established as a protein substitute for human use is a versatile protein carrier for drug delivery that has been shown to be nontoxic, non-immunogenic, biocompatible and biodegradable making it an ideal material for the preparation of nanoparticles for drug delivery [16]. Albumin nanoparticles have gained considerable attention owing to their high binding capacity to various drugs and they are well tolerated without any serious side-effects even at high amounts. Its surface-active properties make it well suitable for the stabilization of polymeric nanoparticles [17]. Albumin can form layers on drug nanoparticles, which can be introduced by cross-linking agents such as aldehydes, or by shear forces as they are applied during processes like emulsion-evaporation. In a study, albumin has been characterized and the *in vitro* drug release properties of three different formulations of ganciclovir-loaded albumin nanoparticles have been evaluated [18]. These carriers were prepared by coacervation method and chemical cross-linking with glutaraldehyde and depending on the step where the drug and/or cross-linking agent were added three different formulations were obtained. In all cases, the size of the different nanoparticulate formulations was between 200 and 400 nm while the yield ranged from 50 to 65%. The *in vitro* release profiles of the nanoparticles showed a biphasic pattern, with an initial burst and rapid drug release, followed by a slower step for up to 5 days. The release of the drug was increased in acidic or basic mediums due to the disruption of the covalent bonding between ganciclovir and the protein matrix via glutaraldehyde. Thus, albumin carriers were proven to be capable of sustained release of ganciclovir for up to 5 days [18].

In another study, aspirin loaded albumin nanoparticles have been prepared by coacervation method [19]. By varying the aspirin to albumin ratios from 0.06 to 1.0, stable nanoparticles of sizes of 46.8 nm to 190.8 nm with low polydispersibility were obtained. The drug encapsulation varied from 30% to 80% w/w for different ratios of aspirin to albumin. *In vitro* release study showed that in contrast to simple drug solutions whose concentration peaks within 1 hour, nanoparticle formulations containing albumin released aspirin at a sustained rate for prolonged duration ($t_{50}$ = 20 hrs, $t_{90}$ = 72 hrs). The authors concluded that coacervation method was well suited for the formulation of albumin nanoparticles which could be applied for intra-articular therapy in arthritis [19].

Albumin nanoparticles encapsulating curcumin prepared by desolvation technique have been shown to enhance the dissolution rate and aqueous solubility of curcumin [20]. *In vivo* studies in rats showed that albumin nanoparticles demonstrated

sustained drug release, higher bioavailability, improved pharmacokinetic properties, and enhanced tissue targetability of the drug with nanoparticulate curcumin showing better antiproliferative activity in tumor cells than the free drug [20]. Nanoparticles prepared of human serum albumin (HSA) using combination of methanol and ethanol as desolvating agent produced very small spherical HSA nanoparticles in a size range between 50 and 80 nm which could be promising carriers for drug delivery [21]. Curcumin-loaded human serum albumin (HSA) nanoparticles (CCM-HSA-NPs) for intravenous administration produced using albumin bound technology has revealed that CCM-HSA-NPs (10 or 20 mg/kg) had greater therapeutic effect (50% or 66% tumor growth inhibition vs. PBS-treated controls) than curcumin (18% inhibition vs. controls) in tumor xenograft HCT116 models without inducing toxicity [22]. HSA nanoparticles modified by adding an outer coating of the polyethylenimine (PEI) have also been shown to improve the therapeutic index of doxorubicin against MCF-7 breast cancer cells over longer time duration [23].

Albumin nanoparticles (ANPs) have been designed to penetrate deeper into solid tumor matrices using collagenase decoration on a three-dimensional multicellular melanoma tumor spheroid model [24]. Collagenase modified ANPs exhibited greater tumor penetration than unmodified ANPs into the spheroid mass after 96 hours, and showed preferential uptake into individual cancer cells for smaller sized ANPs (<100 nm). Collagenase coated ANPs modified with two therapeutic agents, curcumin and riluzole, were also found to significantly induce more cell death within a 3-D tumor model than the unmodified, dual drug loaded ANP particles. The kinetics of cytotoxicity was further influenced by the ANP size. Thus, multifunctional nanoparticles can be imbued with complementary size and protease activity features that allow them to penetrate solid tumors and deliver combinatorial therapeutic payload with enhanced cancer cytotoxicity but minimal collateral damage to healthy primary cells [24].

## 2. Gelatin

Gelatin is the product of the partial hydrolysis of collagen. It is widely used in pharmaceutical preparations in the manufacture of suppositories, coating of tablets and manufacturing of soft and hard shell capsules [25]. Gelatin based delivery systems are biocompatible and biodegradable without toxic degradation products and they are known for their high physiological tolerance and low immunogenicity [25-27].

Gelatin has been used for embedding or depositing drug on the surface of nanoparticles. Nanosized particles of gelatin have been prepared via water-in-oil microemulsion system for drug and gene delivery applications [28]. In the study, cross-linked gelatin nanoparticles encapsulating a fluorescent marker molecule fluorescein isothiocyanate-dextran (FITC-Dex, Mol. Wt. 19.3 kDa) were prepared in aqueous cores of sodium bis (2-ethylhexyl) sulfosuccinate (AOT)/ n-hexane reverse micelles and characterized, and their influence on human fibroblasts was assessed in terms of cell adhesion, cytotoxicity, and observation of cytoskeleton organization. Transmission electron microscopy image showed that the particles were spherical in shape with diameter of 0.84 nm. The release of FITC-Dex from the nanoparticles in phosphate buffer saline (pH 7.4) was found to increase with time and about 80% of the encapsulated dye was released in 6 h. Cell adhesion studies with human fibroblasts showed that gelatin nanoparticles do not affect the number of cells adhered to glass as compared to control cells with no particles. The result showed that gelatin nanoparticles prepared by water-in-oil microemulsion systems were endocytosed by the fibroblasts without being toxic to cells even at high concentration of nanoparticles [28].

Nanoparticulate formulations have been prepared based on gelatin and its admixtures with other polyelectrolytes by nanoprecipitation conditions for the delivery of proteins and peptide drugs [29]. The authors indicated that smooth and spherical particles with a unimodal distribution and high drug loading were obtained for the macromolecules. Bio-imaging using fluorescence microscopy also demonstrated uptake and internalization of the nanoparticles into the nucleus and the cytoplasm by Caco-2 cells [29]. Gelatin nanoparticles prepared by a two-step desolvation technique and subsequent chemical cross-linking maintained their non-toxic properties while featuring a higher

storage stability than liposomal liquid formulations and good *in vivo* stability upon administration [30, 31]. The undesired effects observed *in vitro* or *in vivo* ascribed to the gelatin nanoparticles were attributed to the toxicity of the starting material, gelatin and the chemical cross-linking agent, glutaraldehyde. Nanoparticles prepared by a two-step desolvation process using two types of gelatin, have also been shown to yield nanoparticles with different particle sizes with narrow size distribution which were found to be taken up by osteosarcoma cancer cells, which could lead to an improvement in the clinical effectiveness of anti-cancer treatments [32].

Gelatin nanoparticle-polylactic-*co*-glycolic acid (PLGA) microsphere composites have also been prepared by encapsulating protein-loaded gelatin nanoparticles by phase separation method in PLGA microspheres [33]. The gelatin nanoparticle-PLGA microsphere composites with size between 160 and 175 μm had a protein loading efficiency of 93.2%. The nanoparticle-microsphere composite system possessed sustained release characteristics and the capability of preventing the denaturation of protein drugs.

## 3. Chitosan

Chitosan is a natural polymer from renewable resources, obtained from the shell of shellfish, and the wastes of the seafood industry. Chitosan ((1-4)-2amino-2-deoxy-β-D-glucan) is a linear polysaccharide consisting of glucosamine and N-acetyl glucosamine units. It is biocompatible, biodegradable, and nontoxic in the application of peroral delivery of drugs and transmucosal absorption enhancement [34]. Janes *et al*. [35] evaluated the potential of chitosan nanoparticles as carrier for the anthracycline drug, doxorubicin (DOX). Doxorubicin, a cationic hydrophilic molecule, has been encapsulated in positively charged polysaccharide chitosan by ionic gelation. The positive charge of DOX was masked by complexation with the polyanion, dextran sulfate. This modification doubled the encapsulation efficiency of DOX relative to controls and enabled real loading up to 4.0% w/w. The possibility of forming a complex between chitosan and DOX prior to the formation of the particles was achieved and despite the low complexation efficiency, no dissociation of the complex was observed upon formation of the nanoparticles [35].

In another study, insulin-loaded chitosan nanoparticles prepared by ionotropic gelation of chitosan with tripolyphosphate anions with particle size range of 250-400 nm and polydispersity index of less than 0.1 has been found to show *in vitro* release with high initial burst with a pH-sensitivity property [36]. Chitosan nanoparticles enhanced the intestinal absorption of insulin to a greater extent than the aqueous solution of chitosan *in vivo*, and the hypoglycemic effect of the nanoparticles was prolonged for over 15 h and the average pharmacological bioavailability relative to subcutaneous injection of insulin solution was up to 14.9% indicating the ability of nanoparticles to improve the intestinal absorption of insulin [36].

The *in vitro* and *in vivo* interaction of chitosan nanoparticles (CSNPs) with epithelial cells on the ocular surface has been demonstrated by de Salamanca *et al*. [37]. Chitosan nanoparticles labeled with fluorescein isothiocyanate-bovine serum albumin which were produced by ionotropic gelation were introduced to human conjunctival epithelial cells (IOBA-NHC) for different time intervals. The results showed that cell survival and viability of CSNP-exposed cells were equivalent to that of the control. Uptake of CSNPs was temperature dependent and continuous for the 2-hour duration of the experiments and metabolic inhibition by sodium azide had no effect on CSNP uptake. The *in vivo* uptake and acute tolerance of the ocular surface to CSNPs in rabbits showed no signs of inflammation or alteration after CSNP exposure compared with the control. *In vivo* uptake by conjunctival and corneal epithelia was confirmed by fluorescence microscopy of the rabbit eyeball and lid sections. The chitosan nanoparticles were internalized by IOBA-NHC cells by an active transport mechanism that did not compromise cell viability. Moreover, these nanoparticles were well tolerated by the ocular surface tissues [37].

Chitosan nanoparticles were also found to be an efficient vehicle for the enhancement of the therapeutic index of clinically challenging drugs such as cyclosporin A [38]. *In vivo* experiments showed that topical instillation of cyclosporin A-loaded chitosan nanoparticles into the eye of

rabbits achieved therapeutic concentrations in the external ocular tissues (i.e., cornea and conjunctiva) in 48 h while maintaining negligible or undetectable cyclosporin A levels in inner ocular structures (i.e., iris/ciliary body and aqueous humour), blood and plasma. Encapsulation of insulin in chitosan nanoparticles has also been found to lead to an improvement in the systemic absorption of insulin following nasal instillation, by facilitating the transport of insulin through the nasal mucosa [39].

## 4. Alginate

Alginic acid and its derivatives are natural hydrophilic polysaccharides derived from seaweed. They consist of 1→4 linked D-mannuronic acid and L-glucuronic acid residues and their use as biopolymers have attracted more attention due to their unique properties such as biocompatibility, biodegradability and viscosity. Alginates have the ability to form gel easily in presence of multivalent cations such as calcium, zinc and barium ions [40]. The gelation or crosslinking is due to the stacking of the glucuronic acid blocks of alginate chains. These polymers can be described as linear binary copolymers of 1–4-linked M and G residues arranged with homopolymeric regions of α-L-guluronic acid residues (G-blocks) and a homopolymeric region of β-D-mannuronic acid sequences (M-blocks) interspersed by regions in which the two groups coexist in a strictly alternating sequence (MG-blocks) [41]. Alginic acid and its carboxylic salts are biopolymers that have found different applications in drug delivery.

Alginic acid nanoparticles have been successfully prepared by non-solvent-aided counterion complexation between anionic alginic acid and cationic 2,2′-(ethylenedioxy) diethylamine in aqueous solution followed by cross-linking the alginic acid moiety using calcium ion [42]. Alginic acid nanoparticles were spherical in shape with a diameter of 100 nm and zeta potential of −30 mV. The negatively charged alginic acid nanoparticles loaded with doxorubicin were taken up by cancer cells through an endocytosis mechanism and *in vivo* near-infrared (NIR) fluorescence imaging, and biodistribution examinations showed that the alginic acid nanoparticles were well-accumulated in the tumor site due to their enhanced permeability and retention effect. *In vivo* antitumor examination showed that the drug-loaded nanoparticles have superior efficacy in impeding tumor growth and prolonging the lifetime of H22 tumor-bearing mice than the free drug [42].

Sodium alginate has been used to prepare microbeads which have been shown to swell in presence of dissolution media and act as controlled release systems [43, 44]. Different enteric as well as sustained release polymers have also been applied as coat on calcium alginate beads. Shun and Ayres [45] prepared a system by coating calcium alginate beads with Aquacoat® a pH-independent polymer, followed by 2% w/w coating of Eudragit L-[30D]. Eudragit® being an enteric polymer resisted the release of drug in acidic media, and drug release was triggered at alkaline pH and controlled by the thickness of Aquacoat®. When the drug-loaded calcium alginate beads swelled sufficiently to exceed the strength of outer sustained released coat, the film bursts to release the drug [45].

Calcium alginate nanoparticles with mean particle size 400-500 nm have been synthesized as encapsulation agents for amphotericin B for the treatment of systemic candidiasis [46]. Sodium homopolymannuronate, one of the ingredients of alginate polymer which was synthesized and purified by partial acid hydrolysis of sodium alginate, was found to produce nanoparticles with lower mean particle size and better distribution than nanoparticles produced with sodium alginate. This could be due to the ionic interaction of calcium crosslinker ions with regular homo-polymeric chains of homopolymannuronate compared to the absence of regular chains of alginate polymer [47].

Nanoparticles with particle size of 350 nm have been prepared using microemulsion-based reactors using aqueous sodium alginate, aqueous calcium chloride, dioctyl sodium sulfosuccinate, and isopropyl myristate [48]. The nanoparticles incorporated with bovine serum albumin were characterized by an initial burst release followed by a sustained-release phase and the protein did not suffer covalent aggregation or degradation via fragmentation [48].

Venom derived peptides (ICD-85) loaded nanoparticles with spherical shapes and size of

approximately 200 nm have also been prepared by ionic gelation method using sodium alginate as biopolymer [49]. The nanoparticles were found to exhibit sustained release patterns with an initial burst release, followed by a subsequent slower release. Cytotoxicity assays showed that ICD-85 loaded nanoparticles is more potent than free ICD-85 in suppressing proliferation of human larynx carcinoma cell line (HEp-2) indicating that the nanoparticles could be a beneficial agent against human carcinoma [49].

A combination of natural polymers have also been used for the delivery of certain drugs [50, 51]. In a study, chitosan-calcium alginate microparticles have been prepared for the delivery of 5-aminosalicylic acid (5-ASA) to the colon after oral administration. The microparticles were prepared by spray-drying technique of 5-ASA/ sodium alginate aqueous solution to obtain spherical particles with a mean diameter less than 10 μm, and then the microparticles were cross-linked and coated with solution of calcium chloride and chitosan to obtain stable microsystem [51]. The spherical but flattened, disk-shaped particles with smooth surface and low porosity showed dominant localization of chitosan in the particle wall, while a homogeneous distribution of alginate was observed throughout the particle, giving the particles a negative charge [51]. Chitosan-alginate nanoparticles prepared by ionotropic pre-gelation of an alginate core followed by chitosan polyelectrolyte complexation using nifedipine as the model drug has yielded nanoparticles with size 20-50 nm, suitable for uptake within the gastrointestinal tract due to their nanosize range and mucoadhesive properties [52]. The release of nifedipine from the nanoparticles was pH-responsive with fast release occurring in simulated intestinal fluid (SIF, pH 6.8) and phosphate buffer solution (pH 7.4), while the release was slow in simulated gastric fluid (SGF, pH 1.5). The release profile was characterized by an initial burst release in three media, followed by a continuous and controlled release phase, which is by Fickian diffusion [52].

## 5. Hyaluronic acid

Hyaluronic acid (HA) is a naturally occurring biopolymer, which serves important biological functions in bacteria and higher animals including humans. It is found in the vitreous humors of the eye and in the synovial fluid of articular joints [53]. Since its discovery in human tissue, hyaluronic acid and its derivatives has been largely studied and applied in the biomedical arena. Its high level of biocompatibility has accentuated the appeal of this polymer and has been used in visco-surgery to allow surgeons to safely create space between tissues. Hyaluronic acid is comprised of linear, unbranching, polyanionic disaccharide units consisting of glucuronic acid (GlcUA), an N-acetyl glucosamine (GlcNAc), joined alternately by β-1-3 and β-1-4 glycosidic bonds. It is a member of the glycosaminoglycan family which includes chondroitin sulphate, dermatin sulphate and heparan sulphate. Unlike other members of this family, it is not found covalently bound to proteins. When incorporated into a neutral aqueous solution, hydrogen bond formation occurs between water molecules and adjacent carboxyl and N-acetyl groups. This imparts a conformational stiffness to the polymer, which limits its flexibility. The hydrogen bond formation results in the unique water-binding and retention capacity of the polymer (up to six litres of water per gram of hyaluronic acid). The water binding capacity was shown to be directly related to the molecular weight of the molecule. The viscoelastic property of hyaluronic acid solutions, which is important in its use as a biomaterial is controlled by the concentration and molecular weight of the hyaluronic acid chains [54].

Nano-sized drug delivery system for cancer therapy has been developed using amphiphilic hyaluronic acid conjugates synthesized by chemical conjugation of hydrophobic 5-β-cholanic acid to the backbone of hyaluronic acid [55]. The HA nanoparticles were spherical in shape and their sizes ranged from 350 to 400 nm, depending on the degree of substitution of 5-β-cholanic acid. Cellular experiment using Cy5.5-labeled HA nanoparticles demonstrated that the nanoparticles were efficiently taken up by the cancer cells. When the Cy5.5-labeled HA nanoparticles were systemically administrated into the tail vein of tumor-bearing mice, most of the nanoparticles were found in tumor and liver sites with the

intensity of nanoparticles at the tumor site four times higher than that of pure HA polymer probably due to prolonged circulation in blood and high affinity to tumor cells. These results reveal the promising potential of HA nanoparticles as a stable and effective nano-sized drug delivery system for cancer treatment [55, 56].

The antitumor efficacy of doxorubicin-loaded nanoparticles prepared from poly(ethylene glycol)-conjugated hyaluronic acid nanoparticles (PEG-HANPs) through controlled deposition of inorganic calcium and phosphate ions on the nanoparticular shell via a sequential addition method has been evaluated after systemic administration into tumor-bearing mice ([57]. The most effective antitumor efficacy was observed for DOX-loaded mineralized PEG-HANPs than the bare PEG-HANPs. Thus, mineralization (M-PEG-HANPs) allowed the formation of compact nanoparticles that facilitated the release of doxorubicin in a controlled manner making M-PEG-HANPs a promising carrier for biostable nanoparticles with high tumor targetability for anticancer drugs [57].

Hyaluronic acid and chitosan (CS) has been used for the preparation of nanoparticles intended for the delivery of genes to the cornea and conjunctiva [58]. HA-CS nanoparticles provided high transfection levels (up to 15% of cells transfected), without affecting cell viability and the nanoparticles were internalized by fluid endocytosis and the endocytic process was mediated by the hyaluronan receptor CD44.

## 6. Curdlan

Curdlan is a linear beta-1,3-glucan, a high-molecular-weight polymer of glucose. Curdlan consists of β-(1,3)-linked glucose residues and forms elastic gels upon heating in aqueous suspension. It is produced by non-pathogenic bacteria such as *Agrobacterium biobar* [59]. The unusual rheological properties of curdlan compared to other natural and synthetic polymers is the underlying reason for its use as a thickening and gelling agent in foods. Apart from being tasteless, colourless and odourless, the main advantages are that in contrast to cold-set gels and heat-set gels, the heating process alone produces different forms of curdlan gel with different textural qualities, physical stabilities and water-holding capacities. Gels of variable strength are formed depending on the heating temperature, time of heat-treatment and curdlan concentration. The safety of curdlan has been assessed in animal studies and *in vitro* tests and it is approved in food use in Korea, Taiwan and Japan as an inert dietary fibre. It is registered in the USA as a food additive [59].

Curdlan has been modified and used for the formulation of nanoparticles for drug targeting. Nanoparticles made of cholesterol-conjugated carboxymethyl curdlan (CCMC) entrapping epirubicin (EPB) have been prepared and characterized, and their *in vitro* and *in vivo* potentials established [60]. The *in vitro* drug release profiles revealed that epirubicin release from the nanoparticles was sensitive to pH as well as the drug loading contents. The cellular cytotoxicity and cellular uptake accessed by using human cervical carcinoma cells showed that the EPB-loaded CCMC nanoparticles were more cytotoxic with broader distribution within the cells than free EPB. The *in vivo* pharmacokinetics and biodistribution after intravenous injection in rats showed that the drug level was significantly increased in liver but decreased in the heart compared with the free EPB. The *in vivo* anti-tumor study indicated that the EPB-loaded CCMC self-assembled nanoparticles showed greater anti-tumor efficacy than the free EPB indicating its potential application as anti-cancer drug carriers.

Self-assembled hydrogel nanoparticles have also been synthesized from carboxymethylated (CM)-curdlan, substituted with a sulfonylurea (SU) as a hydrophobic moiety for self-assembly, for anti-cancer drug release and interaction with hepatoma cell line [61]. The all-*trans* retinoic acid (ATRA) loading efficiencies and release from the CM-curdlan/SU nanoparticles increased as the degree of SU substitution increased. The lactobionic acid (galactose moiety) CM-curdlan/SU hydrogel nanoparticles were found to be a useful drug carrier for the treatment of liver cancer due to the potential immunological enhancement activities of CM-curdlan in the body, the ligand-receptor mediated specific interactions, and the controlled release of the anti-cancer drug [61].

In another study, the *in vitro* and *in vivo* anti-tumor potentials of nanoparticles made of

cholesterol-conjugated carboxymethyl curdlan entrapping epirubicin (EPB) have shown that the nanoparticles were more cytotoxic and have broader distribution within the cells than free EPB [60]. *In vivo* pharmacokinetics and biodistribution after intravenous injection in rats indicate a four-fold increase in the mean residence time (MRT) and half-life with significantly increased drug level in the liver for EPB-loaded CCMC-self-assembled nanoparticles compared to the free EPB [60].

Deoxycholic acid hydrophobically modified-carboxymethylated-curdlan (DCMC) conjugate has been developed as a novel carrier for the delivery of epirubicin [62]. Epirubicin was loaded into DCMC self-assembled nanoparticles and the *in vitro* release studies showed sustained drug release of EPB which was dependent on the pH of release media and drug loading content. The EPB-loaded DCMC nanoparticles showed higher cytotoxic activity compared with free drug which was attributed to the enhanced cellular uptake. *In vivo* toxicity study indicated that DCMC conjugate did not induce unexpected side effects. Tissue biodistribution study performed in tumor-bearing mice showed that DCMC increased the uptake of EPB in the tumor but decreased the uptake in kidney and heart [62].

## 7. Starch

Starch is a natural, renewable, and biodegradable polymer produced by many plants as a source of stored energy. It is the second most abundant biomass material in nature [63]. Native starches are nontoxic, cheap and renewable raw materials that are hydrophilic in nature, which have limited their use in the development of starch-based nanoparticles [64]. However, native starches have been modified by physical and chemical methods to improve their physicochemical properties to make them more suitable for the formulation of nanoparticles. Nanoparticles have been prepared using modified starches by water-in-oil emulsions and cross-linking, and processing of granular pregelatinised or hydrolysed starches by mechanical treatment such as extrusion at elevated temperature under conditions of high shear and simultaneous cross-linking [65].

Insulin loaded starch nanoparticles prepared by different methods indicated that microparticles prepared by emulsion cross linking were smaller in size compared to those produced by the gel method, and size was further reduced when epichlorohydrin was used as cross-linking agent compared to phosphoryl chloride [66]. Nano-particles prepared by emulsion method using different cross-linking agents were optimized and a size dependent first order diffusion controlled release of insulin with an initial burst release was obtained. Nanoparticle formulation prepared with sodium glycocholate as permeation enhancer showed a superior hypoglycemic action compared to other nanoparticle formulations containing epichlorohydrin and lysophosphatidylcholine as permeation enhancers. Starch nanoparticles combined with permeation enhancers were found to be an efficient trans-nasal mucoadhesive carrier of insulin [66].

Hydrophobic starch has been prepared by grafting of hydrophobic side chains to the hydrophilic starch backbone and used as nanoparticulate drug carriers [67-69]. Propyl-starch derivatives have been prepared by the inclusion of propyl groups with low degree of substitution, and good solubility in low hazardous organic solvents such as ethyl acetate [70], using two different propyl-starch derivatives with high degrees of substitution, by a simple o/w emulsion diffusion technique, avoiding the use of hazardous solvents such as dichloromethane or dimethyl sulfoxide. The starch nanoparticles showed high encapsulation efficiency for the three tested drugs, flufenamic acid, testosterone and caffeine, with a close to linear release profile devoid of initial burst effect for hydrophobic drugs. The skin permeation data for the three drugs indicated the potential use of these nanoparticles for transdermal drug delivery applications [70].

## 8. Pectins

Pectins are non-starch linear polysaccharides that consist of α-1, 4 D-galacturonic acid and 1, 2 D-rhamnose with D-galactose and D-arabinose side chains having average molecular weights between 50,000 to 150,000. Depending on the plant source and the method of preparation, they contain varying degrees of methyl ester substituent [71]. Pectin is highly soluble in water and swells when it comes in contact with aqueous fluids of gastrointestinal tract (GIT) leading to the release

of the entrapped drug by the diffusion. This effect has been manipulated by the choice of pectin type or the presence of additives or by the use of hydrophobic polymers, e.g. ethylcellulose, which restricts the entry of water and the consequent swelling of the polymer [54]. Moreover, a better shielding effect has been obtained by reducing the solubility of pectin by forming its calcium salt i.e. calcium pectinate. Insulin-loaded calcium pectinate nanoparticles have been prepared as a potential colonic delivery system by ionotropic gelation with calcium ions [72].

The effects of the molecular weight of pectin and formulation pH on the characteristics of the nanoparticles prepared using different grades of pectin have been evaluated. Commercial pectins, LM101 and LM104, with respective degrees of esterification of 36% and 28% were depolymerized by mechanical milling to give Mv ranging from 89 to 5.6 kDa. Milled pectins did not yield nanoparticles with significantly different mean diameter and insulin association efficiency compared to nanoparticles of unmilled pectins [73]. Formulation pH significantly influenced the association efficiency and stability of the nanoparticles. Increasing the pH from 2 to 3 enhanced the association efficiency by three-fold and this increase in association efficiency was correlated to the charge density on the pectin molecules as a function of pH. Subsequent release of associated insulin from the nanoparticles was dependent on the extent of dilution of the nanoparticle dispersion and the pH of the dissolution medium.

Pectinate micro/nanoparticles produced by ionotropic gelation using magnesium chloride and calcium chloride as chelating agents have been shown to be effective for gene delivery. The transfection efficiency of both calcium pectinate and magnesium pectinate nanoparticles yielded relatively low levels of green fluorescent protein expression and low cytotoxicity in Huh7 cells indicating its potential usefulness as safe gene delivery carriers [74]. In another study, nanoparticles prepared by ionotropic gelation of low-methoxylated (LM) and amidated low-methoxylated (AM) pectin with zinc chloride have been found to be promising as a potential drug delivery system for certain drugs [73].

## 9. Guar gum

Guar gum, obtained from the ground endosperms of *Cyamposis tetragonolobus*, mainly consists of high-molecular weight hydrocolloidal poly-saccharides, composed of galactan and mannan units combined through glycosidic linkages. The structure of guar gum consists of a linear chain of β-D-mannopyranosyl units linked (1→4) with single member α-D-galactopyranosyl units occurring as side branches [75]. It contains about 80% galactomannan, 12% water, 5% protein, 2% acid soluble ash, and 0.7% fat. This galactomannan is soluble in cold water, hydrating quickly to produce viscous pseudoplastic solutions, although it undergoes shear-thinning and generally has greater low-shear viscosity than other hydrocolloids. Guar gum has a molecular weight of approximately 1 million, giving it a high viscosity in solution [76]. This gelling property retards the release of the drug from the dosage form, and it is susceptible to degradation in the colonic environment.

Guar gum has been used for the preparation of nanoparticles using nanoprecipitation and cross-linking methods [77]. It has been found that the formation of nanoparticles depended upon the molecular mass of the galactomannan, solvent, surfactant, cross-linker and agitation. Galactomannan nanoparticles functionalized with lipase and crystal violet as model drugs were spherical in shape with a size of approximately 20-50 nm and polydispersity index in the range of 0.1 to 0.4. Guar gum nanospheres have been prepared and characterized as carrier for targeted delivery of tamoxifen citrate, a non-steroidal drug used in the treatment of breast cancer [78]. The polymer coated nanoparticles were prepared by single step emulsion *in situ* by polymer-crosslinking technique of the citrate salt of the trans-isomer, tamoxifen citrate. During preparation, four-different drug loading solvents were tried and dichloromethane provided the best drug loading results. Drug load was confirmed by Fourier Transform Infra Red (FT-IR) spectroscopy and quantitated by high performance liquid chromatography (HPLC). Nanoparticles were further characterized for particle size and morphology. Particle size between 200 and 300 nm were obtained. It was observed that the concentration of the polymer and stabilizer determined the size of nanoparticles.

### 10. Glucomannan

Konjac glucomannan (KGM) is a high-molecular weight water-soluble non-ionic glucomannan extracted from tubers of the *Amorphophallus konjac* plant. KGM is a linear random copolymer of (1→4) linked β-D mannose and β-D-glucose. It has mannose and glucose units in a molar ratio of 1.6:1 with a low degree of acetyl groups (approximately 1 acetyl group per 17 residues) at the C-6 position [79]. The degree of solubility is controlled by the presence of acetyl groups. Konjac glucomannan has the ability to lower blood cholesterol and sugar levels, help in weight loss, and promote intestinal activity and immune function. Various derivatives of glucomannan have been prepared due to its good biocompatibility and biodegradability [79].

Chitosan and glucomannan (GM) have been used for the formulation of nanoparticles for the delivery of proteins and peptide drugs [80]. Two different types of glucomannan non-phosphorylated Konjac GM and phosphorylated GM have been used to prepare nanoparticles using two different approaches. These procedures involved the interaction of CS and GM in the presence or absence of sodium tripolyphosphate, which acted as an ionic cross-linking agent for CS. Nanoparticles with size in the range of 200 to 700 nm and a variable zeta potential (from −2 to +39 mV) were obtained depending on the formulation conditions. Despite the mild forces involved in their formation, it was possible to obtain nanoparticles that remained stable upon dilution with phosphate buffer saline. The nanoparticles exhibited a great capacity for the association of the model peptide, insulin, and immunomodulatory protein P1, reaching association efficiency values of 89%. Moreover, the release of the peptide/protein could be modulated by varying the composition of the system [80]. Thus, chitosan-glucomannan nanoparticles are promising carriers for the oral administration of peptides and proteins.

## CONCLUSION

Natural polymers appear to be promising nanomaterials for pharmaceutical drug delivery systems, which are considerably appealing for use in site-specific delivery of commercially available drugs. This is because of their non-toxic and biodegradable nature and wide adaptability to different processes and handling. The challenges in future will be to optimize the performance of these natural polymers for the formulation of nanoparticles to encourage their application in the treatment of different diseases.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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