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Hydrogel nanoparticles in drug delivery

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ABSTRACT

Hydrogel nanoparticles have gained considerable attention in recent years as one of the most promising nanoparticulate drug delivery systems owing to their unique potentials via combining the characteristics of a hydrogel system (e.g., hydrophilicity and extremely high water content) with a nanoparticle (e.g., very small size). Several polymeric hydrogel nanoparticulate systems have been prepared and characterized in recent years, based on both natural and synthetic polymers, each with its own advantages and drawbacks. Among the natural polymers, chitosan and alginate have been studied extensively for preparation of hydrogel nanoparticles and from synthetic group, hydrogel nanoparticles based on poly (vinyl alcohol), poly (ethylene oxide), poly (ethyleneimine), poly (vinyl pyrrolidone), and poly-*N*-isopropylacrylamide have been reported with different characteristics and features with respect to drug delivery. Regardless of the type of polymer used, the release mechanism of the loaded agent from hydrogel nanoparticles is complex, while resulting from three main vectors, i.e., drug diffusion, hydrogel matrix swelling, and chemical reactivity of the drug/matrix. Several crosslinking methods have been used in the way to form the hydrogel matix structures, which can be classified in two major groups of chemically- and physically-induced crosslinking.

Contents

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1. Introduction: Nanoparticles in drug delivery

In recent years, significant efforts have been devoted to use the potentials of nanotechnology in drug delivery since it offers a suitable means of site-specific and/or time-controlled delivery of small or large molecular weight drugs and other bioactive agents [1–9]. Pharmaceutical nanotechnology focuses on formulating therapeutically active agents in biocompatible nanoforms such as nanoparticles, nanocapsules, micellar systems, and conjugates. These systems offer many advantages in drug delivery, mainly focusing on improved safety and efficacy of the drugs, e.g. providing targeted delivery of drugs, improving bioavailability, extending drug or gene effect in target tissue, and improving the stability of therapeutic agents against chemical/enzymatic degradation [3]. The nanoscale size of these delivery systems is the basis for all these advantages [10].

By a general definition, nanoparticles vary in size from 10 to 1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix and depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are vesicular systems in which the drug is confined to a cavity surrounded by a boundary structure, e.g., polymeric, while nanospheres are matrix spherical systems in which the drug is physically and uniformly dispersed [11] (Fig. 1).

Several types of nanoparticulate systems have been attempted as potential drug delivery systems, including biodegradable polymeric nanoparticles, polymeric micelles, solid nanoparticles, lipid-based nanoparticles, e.g., Solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and lipid drug conjugate (LDC), nanoliposomes, inorganic nanoparticles, dendrimers, magnetic nanoparticles, Ferrofluids, and quantum dots.

2. Hydrogels: A brief overview

Originally, Wichterle and Lim [12] introduced a type of hydrophobic gel for biological uses in the early 1960s. Later on toward the present, a huge sum of efforts and studies has been devoted to advancing and extending the potentials attributed to hydrogels [13–21]. Ever-growing hydrogel technology has led to dramatic advances in pharmaceutical and biomedical era [22–25]. By definition, hydrogels are polymeric networks with three-dimensional configuration capable of imbibing high amounts of water or biological fluids [26–28]. Their affinity to absorb water is attributed to the presence of hydrophilic groups such as –OH, –CONH–, –CONH2–, and –SO3H in polymers forming hydrogel structures [29]. Due to the contribution of these groups and domains in the network, the polymer is thus hydrated to different degrees (sometimes, more than 90%wt.), depending on the nature of the aqueous environment and polymer composition [30–33]. In contrast, polymeric networks of hydrophobic characteristics (e.g., poly(lactic acid)(PLA) or poly(lactide-co-glycolide)(PLGA)) have limited water absorbing capacities (<5–10%). While the water content of a hydrogel determines its unique physicochemical characteristics, these structures have some common physical properties resembling that of the living tissues, than any other class of synthetic biomaterials, which is attributed to their high water content, their soft and robbery consistency, and low interfacial tension with water or biological fluids [34,35]. Despite their high water absorbing affinity, hydrogels show a swelling behavior instead of being dissolved in the aqueous surrounding environment as a consequence of the critical crosslinks present in the hydrogel structure. These crosslinks are from two main categories including: i) physical (entanglements or crystallites), and ii) chemical (tie-points and junctions) [36–41]. The crosslinks in the polymer network are provided by covalent bonds, hydrogen binding, van der Waals interactions, or physical entanglements [42,43].

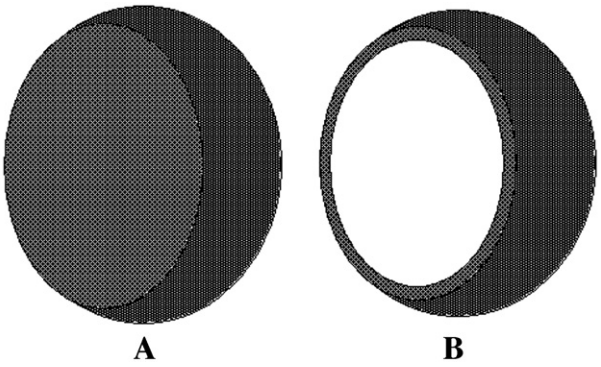

Fig. 1. Schematic representation of a nanosphere (A) and a nanocapsules (B). In nanospheres, the whole particle consists of a continuous polymer network. Nanocapsules present a core-shell structure with a liquid core surrounded by a polymer shell.

Table 1
Hydrophilic polymers used in preparation of hydrogels

Natural polymers and their derivatives
Anionic polymers: HA, alginic acid, pectin, carrageenan, chondroitin sulfate, dextran sulfate
Cationic polymers: chitosan, polylysine
Amphipathic polymers: collagen (and gelatin), carboxymethyl chitin, fibrin
Neutral polymers: dextran, agarose, pullulan
Synthetic polymers
Polyesters: PEG–PLA–PEG, PEG–PLGA–PEG, PEG–PCL–PEG, PLA–PEG–PLA, PHB, P(PF-co-EG)6acrylate end groups, P(PEG/PBO terephthalate)
Other polymers: PEG-bis-(PLA-acrylate), PEG6CDs, PEG-g-P(AAm-co-Vamine), PAAm, P(NIPAAm-co-AAc), P(NIPAAm-co-EMA), PVAc/PVA, PNVP, P(MMA-co-HEMA), P(AN-co-allyl sulfonate), P(biscarboxy-phenoxy-phosphazene), P(GEMA-sulfate)
Combinations of natural and synthetic polymers
P(PEG-co-peptides), alginate-g-(PEO–PPO–PEO), P(PLGA-co-serine), collagen-acrylate, alginate-acrylate, P(HPMA-g-peptide), P(HEMA/Matrigel®), HA-g-NIPAAm

Abbreviations: HA, hyaluronic acid; PEG, poly (ethylene glycol); PLA, poly(lactic acid); PLGA, poly(lactic-co-glycolic acid); PCL, polycaprolactone; PHB, poly(hydroxy butyrate); PF, propylene fumarate; EG, ethylene glycol; PBO, poly(butylene oxide); CD, cyclodextrin; PAAm, polyacrylamide PNIPAAm, poly(*N*-isopropyl acrylamide); PVA, poly(vinyl alcohol); PVamine, poly(vinyl amine) PVAc, poly(vinyl acetate); PNVP, poly(*N*-vinyl pyrrolidone); PAAc, poly(acrylic acid); HEMA, hydroxyethyl methacrylate; PAN, polyacrylonitrile; PGEMA, poly(glucosylethyl methacrylate); PEO, poly(ethylene oxide); PPO, poly(propyleneoxide); PHPMA, poly(hydroxypropyl methacrylamide); PEMA, poly(ethyl methacrylate); PAN, polyacrylonitrile; PMMA, poly(methyl methacrylate).

2.1. Hydrogel classifications

To achieve a hydrogel system with predetermined and well-defined physicochemical parameters and release profiles, a knowledge of polymer network synthesis and chemistry, quantitative and modelistic features of materials, interaction parameters, disintegration/release kinetic, and transport phenomena seems to be playing fundamentally important roles. In a general view, hydrogels can be classified based on a variety of characteristics, including the nature of side groups (neutral or ionic), mechanical and structural features (affine or phantom), method of preparation (homo- or co-polymer), physical structure (amorphous, semicrystalline, hydrogen bonded, supermolecular, and hydrocollodial), and responsiveness to physiologic environment stimuli (pH, ionic strength, temperature, electromagnetic radiation, etc.) [26,27,33,36–41,44–49]. The polymers commonly used in preparation of hydrogels with pharmaceutical and biological applications are from natural or synthetic origins [23,49–53]. Typical examples of natural, synthetic and combinational, i.e., semisynthetic polymers used in hydrogel preparations are summarized in Table 1. Although hydrogels of natural origin may show mechanically sub-optimal characteristics and may exert immunogenicity or evoke inflammatory responses due to the presence

of immunogen/pathogen moieties, they do offer various advantageous properties such as being usually non-toxic, biocompatibility, and showing a number of remarkable physicochemical properties that make them suitable for different applications in drug delivery systems [49,51]. In comparison, the well-defined structure of synthetic polymers may lead to hydrogels with well-defined and fine-tunnable degradation kinetic as well as mechanical properties.

As mentioned, water content plays an important role in determining the overall characteristic of a polymeric network. Accordingly, hydrophilic hydrogels with high amounts of water in their structures show distinctive properties compared to hydrophobic polymeric networks. Furthermore, hydrogels have significantly milder conditions for preparation with gel formation occurring at ambient temperatures and organic solvents are rarely required [51]. Hydrogels, particularly those intended for applications in drug delivery and biomedical purposes, are required to have acceptable biodegradability and biocompatibility which necessitates the development of novel synthesis and crosslinking methods to design the desired products. In this way, a great variety of crosslinking approaches have been developed to prepare desired hydrogels for each particular application [54]. These crosslinkling methods routinely used for preparation of hydrogels are listed in Fig. 2. Moreover, the characteristics and potential applications of hydrogels of different structures, rely not only on the preparation methods but also on the monomers used in the synthesis of hydrogel polymeric networks. A summary of monomers most commonly used in the fabrication of hydrogel structures of pharmaceutical interest is shown in Table 2 [53].

Table 2
Monomers commonly used in synthesis of synthetic hydrogels for pharmaceutical application

Monomer chemical name	Monomer abbreviation
Hydroxyethyl methacrylate	HEMA
Hydroxyethoxyethyl methacrylate	HEEMA
Hydroxydiethoxyethyl methacrylate	HDEEMA
Methoxyethyl methacrylate	MEMA
Methoxyethoxyethyl methacrylate	MEEMA
Methoxydiethoxyethyl methacrylate	MDEEMA
Ethylene glycol dimethacrylate	EGDMA
N-vinyl-2-pyrrolidone	NVP
N-isopropyl Aam	NIPAAm
Vinyl acetate	VAc
Aacrylic acid	AA
Methacrylic acid	MAA
N-(2-hydroxypropyl) methacrylamide	HPMA
EG	Ethylene glycol
PEG acrylate	PEGA
PEG methacrylate	PEGMA
PEG diacrylate	PEGDA
PEG dimethacrylate	PEGDMA

2.2. Release mechanism from hydrogel matrices

Since the most common mechanism of drug release from hydrogels is passive diffusion, molecules of different sizes and characteristics would freely diffuse into/out of hydrogel matrix during the loading and storage periods. The hydrophilic nature of a hydrogel makes it highly different from non-hydrophilic polymer matrices with respect to the release behavior of the incorporated agents. From various modelistic studies on the possible release mechanisms of an active compound from a hydrogel device, focused on the rate-limiting step of the release phenomena, drug release mechanisms from hydrogels can be categorized as: i) diffusion-controlled, ii) swelling-controlled, and iii) chemically-controlled. According to Fick's first law of diffusion (with constant or variable diffusion coefficients), the diffusion-controlled behavior is the most dominantly applicable mechanism to describe the drug release from hydrogels [55]. The drug diffusion out of a hydrogel matrix is primarily dependent on the mesh sizes within the matrix of the gel [56], which, in turn, is affected by several parameters, including, mainly, the degree of crosslinking, chemical structure of the composing monomers, and, when applicable, type as well as intensity of the external stimuli. Meanwhile, mechanical strength, degradability, diffusivity, and other physical properties of a hydrogel network are greatly dependent on its mesh size [55–57]. Typical mesh sizes reported for biomedical hydrogels range from 5 to 100nm (in their swollen state) [57,58], which are much larger than most small-molecule drugs. As a result, diffusion of these drugs is not considerably retarded in swollen state, whereas macromolecules like oligonucleotides, peptides, and proteins, due to their hydrodynamic radii, will have a sustained release unless the structure and mesh size of the swollen hydrogels are designed appropriately to obtain desired rates of macromolecular diffusion [59]. In the case of the swelling-controlled mechanism, when diffusion of a drug is significantly

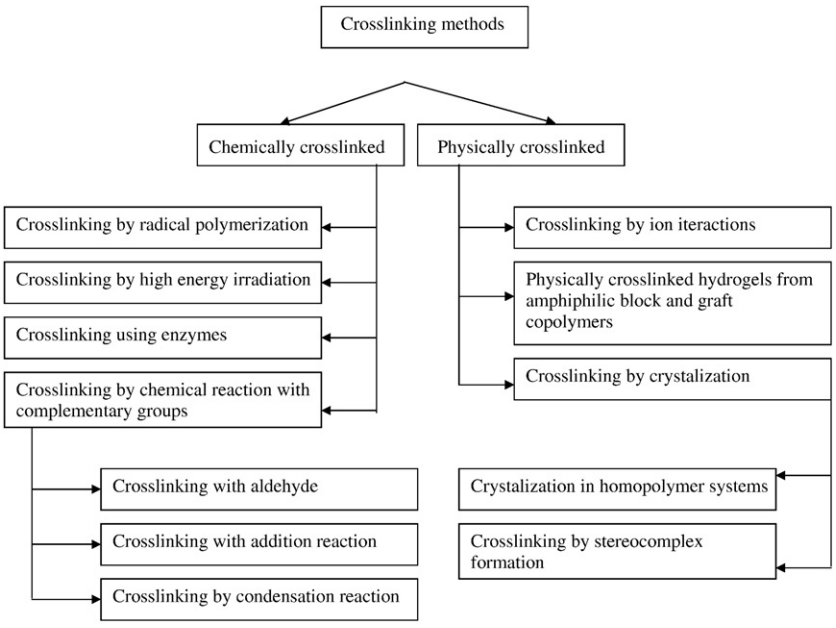

Fig. 2. Novel crosslinking methods used in hydrogels.

faster than hydrogel distention, swelling is considered to be controlling for the release behavior [60,61]. Finally, chemically-controlled release is determined by chemical reactions occurring within the gel matrix. These reactions include polymeric chain cleavage via hydrolytic or enzymatic degradation, or reversible/irreversible reactions occurring between the polymer network and the releasing drug. In addition to the above-mentioned release mechanisms, under certain circumstances, surface or bulk erosion of hydrogels or the binding equilibrium among the drug-binding moieties incorporated within the hydrogels, are two different mechanisms reported as controlling the rate of drug release. [53,55,62].

2.3. Controlled-release hydrogel systems

Controlled-release or controlled-delivery systems are intended to provide the drug or compound of interest at a specific predetermined temporal and/or spatial manner within the body to fulfill the specific therapeutic needs. Hydrogels, among the different controlled-release systems exploited so far, have particular properties which make them to be potentially considered as one of the ideal future controlled-release systems. The hydrogel-based delivery systems are of two major categories: i) time-controlled systems and ii) stimuli-induced release systems [51,63]. The latter, stimuli-induced release systems, are also referred to as 'stimuli-sensitive', 'stimuli-responsive', 'environment-sensitive', 'environment-responsive', or 'responsive' hydrogel systems. Responsive hydrogel systems are developed to deliver their content(s) in response to a fluctuating condition in a way that desirably coincides with the physiological requirements at the right time and proper place [51]. Despite the huge attraction centered towards the novel drug delivery systems based on the environment-sensitive hydrogels in the past and current times, these systems have disadvantages of their own. The most considerable drawback of stimuli-sensitive hydrogels is their significantly slow response time, with the easiest way to achieve fast-acting responsiveness being to develop thinner and smaller hydrogels which, in turn, bring about fragility and loss of mechanical strength in the polymer network and the hydrogel device itself [64].

Dependent on changes in the nature of the external environment, responsive hydrogels undergo drastic alterations in their structure/behavior [33,63,65]. The environment (stimuli)-sensitive hydrogel systems which are also famous as 'intelligent' or 'smart' systems, can be further sub-classified to:

i) Physically-induced release systems;
ii) Chemically-induced release systems; and
iii) Other stimuli-induced release systems.

Temperature, electricity, light, pressure, sound, and magnetic field are among the physical stimuli of interest in this context, while pH, solvent composition, ions, and specific molecular recognition events are chemical stimuli reported so far [63–65]. Temperature-sensitive (thermoresponsive) hydrogels have gained considerable attention due to their ability for repeated swelling–deswelling conversion in response to the environmental temperature changes [66,67]. A series of studies on application of these hydrogels in the pharmaceutical field has shown promising results [68–76]. On the other hand, chemical-responsive hydrogel systems propose several classes of hydrogels which can trigger drug release from a depot with respect to changes in the concentration of a specific molecule or bioactive compound in the surrounding media [63–65,77–84]. Furthermore, the challenge of potential need for chronotherapy has currently resulted in the development of electrically assisted release technologies using hydrogels, as well [85–90]. These technologies include iontophoresis, infusion pumps, and sonophoresis [85,190,191]. pH-responsive hydrogel systems are of great importance due to their unique pH dependant swelling–deswelling behavior [33,73,74,72,92–97]. Several environmental stimuli are being exploited extensively in drug delivery researches. As typical examples, physical stimuli such as light [98], magnetic field [99], electric current [100,101], and ultrasound [102] as well as chemical stimuli such as ionic species [103,104] can be listed among others. Finally, a series of studies on development of novel infection-responsive drug release systems has been performed by Suzuki et al. [105–108].

2.4. Hydrogels for pharmaceutical applications

Hydrogels have been attempted extensively to achieve ideal drug delivery systems with desirable therapeutic features [109]. The unique attractive physicochemical and biological characteristics of hydrogels, along with their huge diversity, collectively, have led to considerable attention to these polymeric materials as excellent candidates for delivery systems of therapeutic agents [19–21,110]. Pharmaceutical hydrogels have been categorized according to a variety of criteria mainly including, rout of administration [111–115], type of material being delivered [22,51,53], release kinetics [23,63–65], etc. Therefore, a common classification system for the therapeutic hydrogel formulations might not be found within the literature. Nonetheless, a classification based on the route of administration of the hydrogel drug delivery systems, seems to include the vast area of these therapeutic materials. Accordingly, the pharmaceutical hydrogels can be classified as: i) oral hydrogel systems [116–120], ii) transdermal and implantable hydrogel systems [35,121–124], iii) topical and transdermal hydrogel systems [125–129], iv) hydrogel devices for gastrointestinal (GI) drug delivery [130–136], and v) hydrogel-based ocular delivery systems [137–139]. Furthermore, hydrogel-based formulations applied via other routs are also noteworthy. In this regard, novel approaches to improve bioavailability through nasal [140,141] and vaginal [142,143] routes using hydrogels have been presented.

Valuable articles reviewing different aspects of hydrogel polymeric materials, their classifications and applications are available in the literature [49–51,53,54,91,92,94,95,63–65].

3. Hydrogel nanoparticles (Nanogels™)

As a family of nanoscale particulate materials, hydrogel nanoparticles (NPs) (recently referred to as nanogels[1]) have been the point of convergence of considerable amount of efforts devoted to the study of these systems dealing with drug delivery approaches. Interestingly, hydrogel nanoparticulate materials would demonstrate the features and characteristics hydrogels and NPs separately posses, at the same time. Therefore, it seems that the pharmacy world will benefit from both the hydrophilicity, flexibility, versatility, high water absorptivity, and biocompatibility of these particles and all the advantages of the NPs, mainly long life span in circulation and the possibility of being actively or passively targeted to the desired biophase, e.g. tumor sites. Different methods have been adopted to prepare NPs of hydrogel consistency. Besides the commonly used synthetic polymers, active research is focused on the preparation of NPs using naturally occurring hydrophilic polymers. The remainder of this text presents various types of nanogels prepared and characterized, using a classification based on the type of polymeric materials used in preparation of the NPs. Although this review covers the literature up-to-date in the field significantly, the reader is referred to the original literature in order to get more technical detail.

3.1. Chitosan-based hydrogel nanoparticles

Chitosan, α(1-4)-2-amino-2-deoxy β-D-glucan, is a deacetylated form of chitin, an abundant polysaccharide present in crustacean shells. Even though the discovery of chitosan dates back from 19th

[1] A registered trademark of Superateck Pharma Inc. (Montreal, Canada).

century, it has only been over the last two decades that this polymer has received attention as a material for biomedical and drug delivery applications. The accumulated information about the physicochemical and biological properties of chitosan led to the recognition of this polymer as a promising material for drug delivery and, more specifically for the delivery of macromolecules [144–150]. From a technical point of view, it is extremely important that chitosan is hydro-soluble and positively charged. These properties enable this polymer to interact with negatively charged polymers, macromolecules, and even with certain polyanions upon contact in aqueous environment. These interactive forces and the resulting sol–gel transition stages have been exploited for nano-encapsulation purposes [5,151–153]. On the other hand, chitosan has the special possibility of adhering to the mucosal surfaces within the body, a property leading to the attention to this polymer in mucosal drug delivery [148,150,154]. The potential of chitosan for this specific application, has been further enforced by the demonstrated capacity of chitosan to open tight junctions between epithelial cells though well organized epithelia [155–160]. The interesting biopharmaceutical characteristics of this polymer are accompanied by its well documented biocompatibility and low toxicity [161–164]. Many articles on the potential of chitosan for pharmaceutical applications have already been published [145,165,166]. Therefore, our purpose is to focus on the specific features and applications of the chitosan-based nanoparticulate systems prepared and characterized to date for delivery of macromolecular compounds such as peptides, proteins, antigens, oligonucleotides, and genes.

3.1.1. Chitosan-based nanoparticles with covalent crosslinks

The earliest works on chitosan-based nanostructures predominantly involved chemical crosslinking within polymer chain. Watzke and Dieschbourg [167] formed chitosan/silica nanocomposites by reacting tetramethoxysilan with hydroxyl groups on the chitosan monomers. However, it was not attempted to associate pharmaceutically active agents to the prepared polymer network. Ohya et al. was the first to present data involving chitosan nanospheres for drug delivery applications [168]. Using a water-in-oil (w/o) emulsion method followed by glutaraldehyde crosslinking of the chitosan amino groups, the group produced nanospheres loaded by 5-fluorouracil (5-FU), an anticancer drug. Since 5-FU derivatives in formulations also contained a terminal amine, glutaraldehyde addition indiscriminately bound the active agent to the polymer as it did between chitosan chains, causing drug immobilization rather than encapsulation. These studies demonstrated the feasibility of synthesizing stable, reproducible nanosized chitosan particles which could entrap and deliver drugs [155].

3.1.2. Chitosan-based nanoparticles with ionic crosslinks

As mentioned, the cationic nature of chitosan has been conveniently exploited for the development of particulate drug delivery systems. Aside from its complexation with negatively charged polymers, an interesting property of chitosan is its ability to gel upon contact with special polyanions, a process referred to as 'ionotropic gelation'. This gelation process is due to the formation of inter and intra crosslinkages between/within polymer chains, mediated by the polyanions. More recently, chitosan NPs have been developed based on the ionotropic gelation of chitosan with tripolyphosphate (TPP), for drug encapsulation [169–174]. This simple and straightforward technique involves the addition of an alkaline phase (pH = 7–9) containing TPP into an acidic phase (pH = 4–6) containing chitosan. NPs are formed immediately upon mixing of the two phases through inter and intra molecular linkages created between TPP phosphates and chitosan amino groups.

Insulin-loaded chitosan NPs have been prepared by mixing insulin with TPP solution and then adding the mixture to chitosan solution under constant stirring [175]. Chitosan NPs thus obtained were within size range of 300–400nm with a positive surface charge ranging from + 54 to + 25mV. Using this method, insulin loading was optimized reaching the loading efficiency of up to 55%. There are many ongoing investigations, which demonstrate the improved oral bioavailability of peptide and proteins upon undergoing this loading procedure. In these studies, it is claimed that the bioadhesion property of chitosan NPs further enhance the intestinal absorption of the drug. Pan et al. [176] prepared insulin-loaded chitosan NPs by ionotropic gelation of chitosan with TPP anions. The ability of chitosan NPs to enhance the intestinal absorption of insulin and the relative bioavailability of insulin was investigated by monitoring the plasma glucose level in alloxan-induced diabetic rats after oral administration of various doses of insulin-loaded chitosan NPs. The positively charged, stable chitosan NPs showed particle sizes within the range of 250–400nm with insulin association ratio of up to 80%. The *in vitro* release experiments indicated an initial burst phase which was pH-sensitive. The chitosan NPs enhanced the intestinal absorption of insulin to a greater extent than the aqueous solution of chitosan *in vivo*. After administration of 21.1IU/kg insulin loaded in the chitosan NPs, hypoglycemia was prolonged over 15h. The average bioavailability relative to the subcutaneous injection of free insulin solution was up to 14.9%.

Xu et al. [177] have studied different formulations of chitosan NPs produced by the ionic gelation of TPP and chitosan. Transmission electronic microscopy (TEM) indicated particle diameters ranging between 20 and 200nm with spherical shapes.

3.1.3. Chitosan-based nanoparticles prepared by desolvation method

The use of desolvating agents for the synthesis of chitosan particles originally emerged from the microencapsulation studies. Berthold et al. first proposed the use of sodium sulfate as a precipitating agent to form chitosan particles. Dropwise addition of sodium sulfate into a solution of chitosan and polysorbate 80 (used as a stabilizer for the suspension) under both stirring and ultrasonication, desolvated chitosan in a particulate form. Although the investigators called the resulting suspensions microspheres, the precipitated particles were at micro/nano interface (900±200 nm). Drug encapsulation was not reported, but the group demonstrated that by virtue of the positive charge on the particle surface, they were able to absorb significant amounts (up to 30% loading) of the hydrophilic anionic corticosteroid, prednisolone sodium phosphate to the particle surface [178]. A variation of this technique was later employed for the controlled release of antineoplastic proteoglycans for immunostimulation [179]. Following glutaraldehyde crosslinking of the nanoparticles, stable particles between 600 and 700nm were obtained. Unfortunately, the necessity for glutaraldehyde forbids the application of this formulation toward the delivery of therapeutically active macromolecules. Chitosan-DNA NPs have been prepared using the complex coacervation technique [165,180]. At the amino-to-phosphate groups' ratio between 3 and 8 and the chitosan concentration of 100mcg/ml, the particle size was optimized to 100–250nm range with a narrow distribution. The chitosan-DNA NPs could partially protect the encapsulated plasmid DNA from nuclease degradation.

3.1.4. Chitosan-based nanoparticles prepared by emulsion-droplet coalescence method

Emulsion-droplet coalescence method, introduced by Tokumitsu et al. [181], utilizes the principles of both emulsion crosslinking and precipitation. In this method, instead of crosslinking the stable droplets, precipitation is induced by allowing coalescence of chitosan droplets with NaOH droplets. A stable emulsion containing aqueous solution of chitosan along with the drug to be loaded is produced in liquid paraffin. At the same time, another stable emulsion containing chitosan aqueous solution containing NaOH is produced in the same manner. When, finally, both emulsions are mixed under high speed stirring, droplets of each emulsion would collide at random and

coalesce, thereby precipitating chitosan droplets to give small solid particles. In this study, Tokumitsu et al. prepared gadopentetic acid-loaded chitosan NPs by this method using 100% deacetylated chitosan, with the mean particle size of 452nm and drug loading efficiency of 45%.

3.1.5. Chitosan-based nanoparticles prepared by reverse micellar method

Reverse micelles are thermodynamically stable liquid mixtures of water, oil, and surfactant. Microscopically, they are homogenous and isotropic structures consisting of aqueous-in-oil droplets separated by surfactant-rich films. NPs prepared by conventional emulsion polymerization methods are not only large (>200 nm), but also have a broad size range. Preparation of ultrafine polymeric NPs with narrow size distribution could be achieved by using reverse micellar medium [182]. Aqueous core of the reverse micellar droplets can be used as a 'nanoreactor' to prepare such particles. Since the size of this highly monodispersed and narrow size range reverse micellar droplets usually lies between 1 and 10nm [183], they are among the promising NPs interested in drug delivery studies. Since micellar droplets are in Brownian motion in liquid medium, they undergo continuous coalescence followed by re-separation on a time scale that varies between milliseconds and microseconds [184]. The size, polydispersity and thermodynamic stability of these droplets are maintained in the system by a rapid dynamic equilibrium.

In this method, the surfactant is dissolved in an organic solvent to prepare reverse micelles. To this, aqueous solutions of chitosan and drug are added gradually with constant vortexing to avoid any turbidity. The aqueous phase is regulated in such a way as to keep the entire mixture in an optically transparent microemulsion phase. Additional amount of water may be added to obtain NPs of large sizes. To this transparent solution, a crosslinking agent is added with constant stirring overnight. The maximum amount of drug that can be dissolved in reverse micelles varies from drug to drug and has to be determined by gradually increasing the amount of drug until the clear dispersion is transformed into a translucent solution. The organic solvent is, then, evaporated to obtain the micellar transparent drug mass. The remaining material is dispersed in water and then, by adding a suitable salt, the surfactant precipitates out. The mixture is, then, subjected to centrifugation. The supernatant solution is decanted, which contains the drug-loaded NPs. The aqueous dispersion is immediately dialyzed through dialysis membrane for about 1h and the liquid is lyophilized to drug powder.

Mitra et al. [185] have encapsulated doxorubicin-dextran conjugate in chitosan NPs, using this method.

3.1.6. Chitosan-based nanoparticles prepared by self-assembly via chemical modification

The self-assembly of chemically modified chitosan into NPs has been investigated for the delivery of macromolecules [186–191]. Fractional conjugation of polyethylene glycol, PEG, via an amide linkage to soluble chitosan was shown to yield self-aggregation at basic pH [188]. These aggregates could trap insulin following incubation in phosphate buffer saline (PBS), likely due to the electrostatic interactions between the unconjugated chitosan monomers and the anionic residues of the protein. Depending on the degree of PEGylation, aggregate sizes between 5 and 150nm can be obtained. The degree of PEGylation also influences the release rate, as more extensively PEGylated aggregates release insulin more rapidly. However, it is difficult to draw conclusions based upon this data, as loading levels for the respective PEG formulations were not reported. An interesting approach leading to the formation of chitosan vesicles has been developed by Uchegbu et al. [189]. They linked palmitic acid to modified glycol chitosan chains, thus producing an amphiphilic polymer, which, upon mixing with cholesterol, formed nanovesicles approximately 300–600nm in size. These vesicles demonstrated good biocompatibility, hemocompatibility, and stability in serum and bile salt. Moreover, the vesicles were able to encapsulate bleomycin, a chemotherapeutic agent. The loading process was performed via an ammonium sulfate gradient which drove the peptide into the vesicles.

Lee et al. [190] have investigated the effects of conjugating chitosan with deoxycholic acid in their attempts to design a new carrier for DNA delivery. Attachment of this hydrophobic moiety to soluble chitosan was found to have substantial effects on its aqueous stability, and the resulting amphiphilic macromolecule formed self-assemblies of self-aggregates upon sonication. The group has reported the ability of these self-aggregates to associate with DNA and transfect *in vitro*. Further work is currently underway aiming at gaining a better understanding of the arrangement of the deoxycholic microdomains imbedded within the chitosan aggregates [191].

3.2. Alginate-based hydrogel nanoparticles

Alginic acid is an anionic biopolymer consisting of linear chains of α-L-glucuronic acid and β-D-mannuronic acid with properties such as a high degree of aqueous solubility, a tendency for gelation in proper condition with high porosity of the resulting gels, biocompatibility, and non-toxicity [192]. Generally speaking, Sequential crosslinking and formation of polymeric networks, results in hydrogel structured drug delivery carriers such as micro- and nanoparticles upon the addition of counter-ions to alginate. Any possible cationic species can initiate the reaction sequence, but calcium chloride is favorably utilized by most researchers. The methods of preparation are usually determined with the aim to control the gelification phenomenon, which leads to desired size ranges depending on various factors including alginate concentration/viscosity, counter-ion concentration, the speed of adding counter-ion solution onto the alginate solution, etc.

In 1993, Rajaonarivony et al. proposed a new drug carrier made up of sodium alginate [193]. They represented alginate NPs with a wide range of particle sizes (250–850nm), formed within a sodium alginate solution following the addition of calcium chloride followed by poly-L-lysine. In this study, the concentrations of both polymer and counter-ion solutions were lower than those regularly used for gel formation. Additionally, with doxorubicin as the model drug, they reported that loading capacity could be reached at more than 50mg of drug per 100mg of alginate.

Since the end of 1990s until now, the number of studies involving alginate-based NPs is increasing [193–195], using the therapeutic agents such as insulin [196–198], antitubercular and antifungal drugs [199–202], and even it has shown promising remarks in the field of gene delivery [203].

While the size range of alginate NPs is greatly dependent on the order of addition of counter-ion to the alginate solution, some people claim benefit from the addition of a polyelectrolyte complexation step in this procedure [197]. Sarmento et al. prepared insulin-loaded NPs by alginate ionotropic pre-gelation followed by chitosan polyelecterolyte complexation. In their effort, particles in nanometer size range were obtained under optimized condition with a loading capacity of 14.3%. In another study using dextran polysaccharide as the complexing agent, again, insulin was loaded in alginate-dextran nanospheres via nanoemulsion dispersion followed by triggered *in situ* gelation [198]. The resulting particles ranged in size from 267nm to 2.76μm. Particles prepared demonstrated a unimodal size distribution and insulin encapsulation efficiency was reached to 82.5%.

The failure of antitubercular chemotherapy is mainly attributed to the patient non-compliance to frequent long-term multidrug regimens. Interestingly, the application of modified-release drug delivery systems provides a novel and sound prospective for the treatment of mycobacterial infections [200]. In a study designed to evaluate the pharmacokinetic and tissue distribution of free and NP-encapsulated antitubercular drugs in different doses, alginate NPs containing isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB) were orally administered to mice [199]. The average size of NPs

was 235.5 with the drug encapsulation efficiencies of 70–90%, 80–90%, and 88–95% for INH, RIF, and EMB, respectively. The bioavailability of all drugs encapsulated in alginate NPs were significantly higher than those with free drugs. Moreover, local administration of inhalable alginate-based NPs bearing the same drugs except for EMB has been attempted by Ahmad et al. [202] with both loading capacity and sizes comparable to the previous study. Recently, another study has been published by the same research group, dealing with the chemotherapeutic evaluation of alginate NP-encapsulated azol antifungal and antitubercular drugs against murine tuberculosis [201]. A series of other studies involving NPs of alginate origin is currently available in the literature [204–206].

3.3. Poly (vinyl alcohol)-based hydrogel nanoparticles

Poly (vinyl alcohol), PVA, is the product of free radical polymerization of vinyl acetate with subsequent hydrolysis of acetate groups to hydroxyl moieties resulting in a wide molecular weight distribution. The molecular weight distribution is an important characteristic due to its roles in determining polymer properties including crystallizability, adhesion, mechanical strength, and diffusivity. PVA is among the most promising polymer candidates for hydrogel studies. Crosslinking of PVA polymeric chains is carried out using chemical (e.g., crosslinking agents, electron beam, γ-irradiation) as well as physical (e.g., freezing/thawing) methods, with the crosslinks being critical for PVA in order to be useful for various applications in medical and pharmaceutical fields [207].

In late 1990s, PVA NPs were prepared with the aim of protein/peptide drug delivery using a water-in-oil emulsion/cyclic freezing–thawing procedure [208]. In this study, the emulsion was kept frozen at − 20°C followed by a thawing phase at ambient temperature and no emulsifier involved. The average diameter of PVA NPs obtained was 675.5 ± 42.7 nm with a skewed or log-normalized size distribution. Bovine serum albumin, BSA, was loaded in this study in nanogels with a notable loading efficiency of 96.2 ± 3.8% and a diffusion-controlled release trend. In another study, three separate production methods, including salting-out, emulsification diffusion, and nanoprecipitation, have been used by Galindo-Rodriguez et al. as a comparative scale-up production evaluation to reach PVA-based NPs loaded with ibuprofen [209]. The pilot-scale stirring rates of 790–2000rpm led to mean sizes ranging from 174 to 557nm for salting-out and from 230 to 565nm for emulsification diffusion.

Heterogeneously structured composites involving PVA have been interested in the field of hydrogel nanoparticles. Biodegradable polyesters consisting of short poly(lactone) chains grafted to PVA or charge-modified sulfobutyl-PVA (SB-PVA) were prepared and used as a novel class of water soluble comb-like polyesters. These polymers undergo spontaneous self-assembling to produce NPs, which form stable complexes with a number of proteins such as human serum albumin, tetanous toxoid and cytochrom C [210]. However, the development of NPs from such polymers does not require the use of solvents or surfactants [211–213].

Preparation of PVA-based NPs encapsulated by poly (lactide-co-glycolic acid) (PLGA) microspheres [214], preparation and release kinetic evaluation of poly (*N*-vinyl caprolactone) NPs loaded by nandolo, propranolol, and tacrine [215], attempts to aerosol therapy using the biodegradable NPs prepared by branched polyesters diethylaminopropyl amine-poly (vinyl alcohol)-grafted-poly(lactide-co-glycolide) (DEAPA-PVA-g-PLGA) [216], DNA nanocarriers formed by a modified solvent displacement method [217], and the study on local delivery of paclitaxel via drug-loaded PVA-g-PLGA NPs for the treatment of restenosis [218] have all been reported in recent years using PVA or its derivatives as a basis for hydrogel formation.

3.4. Poly (ethylene oxide) and poly (ethyleneimine)-based hydrogel nanoparticles

A new family of nanoscale materials on the basis of dispersed networks of crosslinked poly (ethylene oxide) (PEO) and poly (ethyleneimine) (PEI), PEO-cl-PEI, has been developed [219]. Interaction of anionic/amphiphilic molecules or oligonucleotides with PEO-cl-PEI results in formation of nanocomposite materials in which the hydrophobic regions from polyion complex are joined by the hydrophilic PEO chain [220]. Formation of polyion complex leads to the collapse of the dispersed gel particles. However, the complexes form stable aqueous dispersions due to the stabilizing effect of the PEO chain. These systems allow for immobilization of negatively charged biologically active compounds such as retinoic acid, indomethacin [221], and oligonucleotides (bound to polycation chains) or hydrophobic molecules (incorporated into nonpolar regions of polyion–surfactant complexes) [219]. The nanogel particles carrying biologically active compounds have been modified with polypeptide ligands to enhance receptor-mediated delivery. Efficient cellular uptake and intracellular release of oligonucleotide immobilized in PEO-cl-PEI nanogel have been demonstrated [222]. Antisense activity of an oligonucleotide in a cell model was enhanced as a result of formation of oligonucleotide-nanogel association. This delivery system has a potential of enhancing oral [220] and brain [223–225] bioavailability of oligonucleotides as demonstrated using polarized epithelial and brain mircrovessel endothelial cell monolayers. PEO-cl-PEI nanogels were synthesized by crosslinking of branched PEI with bis-activated PEO molecules [220].When conducted in a homogenous aqueous solution, the reaction between amino groups of PEI and imidazolylcarbonyl ends of activated PEO proceeded very rapidly, resulting in formation of transparent hydrogels in only 3–5min. These bulk hydrogels retained large quantities of water reaching approximately 50-fold by weight, compared to the dried substance. Rigid hydrogels could be produced at the minimal PEO/PEI molar ratio of 6 or higher. To obtain fine disperse systems, the crosslinking reaction was performed by a modified solvent emulsification/evaporation method [226]. According to this method, activated PEO solution in dichloromethane was emulsified in the aqueous solution of PEI by sonication. The organic solvent was removed from the mixture *in vacuo* resulting in formation of a clear suspension. Most of the nanogel particles have had a very low density and could not be fractioned by ultracentrifugation. Therefore, crude suspension of nanogel particles was partitioned using gel-permeation chromatography. Several fractions could be separated by particle size from 300 to 400nm, with a major fraction having average particle diameters between 150 and 240nm.

3.5. Poly (vinyl pyrrolidone)-based hydrogel nanoparticles

Poly (vinyl pyrrolidone), PVP, is a hydrophilic polymer generally known and approved by FDA as a biocompatible and non-antigenic compound [227] and is therefore safe for biological experiments. Baharali et al. have described a procedure for preparation PVP-based hydrogel NPs with final diameter less than 100nm, using the aqueous cores of reverse micellar droplets as nanoreactors [228]. Since the reverse micellar droplets are highly monodispersed and the droplet sizes can be well-controlled, the NPs prepared using a reverse micellar medium are ideally monodispersed with narrow size distribution. Moreover, their size can be modulated by controlling the size of the reverse micellar droplets [229].

Guowie et al. [230] have synthesized and characterized a magnetic micromolecular delivery system based on PVP hydrogel with PVA as crosslinker. The PVP hydrogel magnetic nanospheres exhibited passive drug release that could be exploited to enhance therapeutic efficacy. The results indicated that hydrogel PVP-based magnetic nanospheres have the potential as drug carriers in magnetically guided chemotherapeutic drug delivery.

3.6. Poly-N-isopropylacrylamide-based hydrogel nanoparticles

Poly-*N*-isopropylacrylamide (PNIPAM) is perhaps the most well known member of the class of responsive polymers. Free chains of

PNIPAM in water, exhibit a low critical solution temperature. This very sharp transition is attributed to the disruption of hydrogen bonding of water molecules around the amide group of the side polymer chains.

Hydrogel NP networks containing dextran have been developed by G. Huang et al. [231]. In their study, PNIPAM-co-allylamine NP networks and PNIPAM-co-acrylic acid NP networks are formed by covalently crosslinking. Also, Gan and Lyon [232] have synthesized thermoresponsive core-shell PNIPAM NPs via seeding and feeding precipitation polymerization method. The influence of chemical differentiation between the core and the shell polymers on the phase transition kinetic and thermodynamic behavior, has been examined in their study.

3.7. Hydrogel nanoparticles of other origins

As noted in the hydrogel section, responsive hydrogel systems have devoted a great contribution to the drug delivery field. Sahoo et al. [233] have prepared pH- and temperature-sensitive hydrogel NPs from copolymers including vinylpyrrolidone (VP) and acrylic acid (AA), crosslinked by *N*,*N* methylene bis acrylamide (MBA), with particle sizes up to 50nm in diameter loaded with a marker compound FITC-dextran. The release of FITC-dextran was slow in acid solution, but it increased considerably as the pH of the medium was increased. The release rate also rose with the increment of temperature.

Moreover, magnetically responsive hydrogel networks based on composites of magnetic nanoparticles and temperature responsive hydrogels were developed [234]. These systems show great promise as active components of microscale and nanoscale devices and are expected to have a wide applicability in various biomedical applications. In this context, nanocomposite hydrogel systems based on the temperature-sensitive *N*-isopropylacrylamide hydrogels crosslinked with ethylene glycol dimethacrylate, tetraethylene glycol dimethacrylate, and poly (ethylene glycol) 400 dimethacrylate (PEG400DMA) were synthesized and characterized. The composite systems were synthesized by UV free radical polymerization. Iron oxide magnetic nanoparticles were incorporated into the hydrogel systems by polymerizing mixtures of the nanoparticles and monomer solutions. The swelling response of these composite systems to different crosslinking molecular weights, temperature, and the effect of the presence of the magnetic nanoparticles were examined.

Pullulan-based hydrogel NPs have been prepared as a drug delivery carrier. In a study dealing with self-assembled hydrogel NPs of cholesterol-bearing pullulan which led to the production of 20–30nm NPs, Kazunari et al. evaluated the complexation and stabilization of insulin [235]. They demonstrated that spontaneous dissociation of insulin from the complex and thermal denaturation/aggregation, were effectively suppressed upon complexation. In another study, Gupta et al. [236] provided a method for enhancing the delivery of nucleic acid molecules to cells by encapsulating them within the hydrogel pullulan NPs. In this work, pullulan NPs bearing plasmids were entrapped inside the aqueous droplets of a w/o microemulsion. Transmission electron microscopy (TEM) images showed spherical particles with diameter of 45±0.80 nm.

Poly (methacrylic acid-grafted-poly (ethylene glycol)) (P(MA-g-PEG)) hydrogel NPs were prepared by a thermally-initiated free radical polymerization method [237]. These hydrogel NPs show pH-sensitive swelling behavior, which is strongly influenced by the crosslinker dosage.

Self-assembled nanogels composed of dextran and PEG macromers prepared by Kim et al. [238] from glycidyl methacrylate dextran (GMD) and dimethyl methacrylate poly (ethylene glycol) (DMP) via radical polymerization has been exploited as a drug delivery system. Moreover, preparation of stable polymeric NPs composed of PEG and poloxamer 407 (Pluronic® F127) through inverse emulsion photopolymerization resulted in successful encapsulation of doxorubicin (loading efficiency = 8.7%) [239].

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