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SHORT COMMUNICATION

One-pot synthesis, characterization, and drug loading of polysaccharide-based nanoparticles with carboxy functional groups

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Abstract Herein, various polysaccharide-based nanoparticles were synthesized from dextran, hydroxypropyl cellulose, and hydroxyethyl cellulose, respectively, by a self-assembly assisted approach. This approach enables us to prepare stable polysaccharide-based nanoparticles with carboxy functional groups directly from monomers without using any surfactant and organic solvent. The existence of abundant carboxyls in these polysaccharide-based nanoparticles provides them obvious pH sensitivity as verified by ^{1}H nuclear magnetic resonance as well as the potential in loading cationic drug.

Keywords Nanoparticle · Polysaccharide · Self-assembly · Carboxyl

Introduction

Polymeric nanoparticles stabilized with covalent linkage, especially those with functional groups simultaneously, have attracted extensive attention in recent years owing to their potential applications in large scope of fields [1–6]. The conventional fabrication of stable polymeric nanoparticles normally was performed by self-assembly approach [5, 7–11]. However, the complicated fabrication and comparatively low efficiency of this approach hindered its extensive application. Aimed at improving the efficiency of fabricating polymeric nanoparticles, many new approaches to stable nanoparticles have been explored [2, 3, 12]. However, the synthesis of block copolymers is often inevitable in these cases, as a preparatory step to fabricate nanoparticles.

Water-soluble polysaccharide, as one kind of biocompatible natural macromolecule, has been used in many biomedical applications [13, 14]. We proposed a facile approach to fabricate dextran-based nanoparticles (DANP) in previous studies [15, 16]. In this paper, we will apply this self-assembly approach to another kind of polysaccharide—water-soluble cellulose ether—and characterize these polysaccharide-based nanoparticles using various instruments. Besides, considering carboxylic acid group has been used as an effective functional group to load many cationic target feeds [17, 18], we will choose DANP as a typical example to study the potential of these polysaccharide-based nanoparticles in drug loading.

Experimental

Materials

Dextran (Shanghai Sinopharm Chemical Reagent, weight-average molecular weight, M_w=1.15e4 g/mol), hydroxypropyl cellulose (HPC; Aldrich, M_w=8.00e4 g/mol), and hydroxyethyl cellulose (HEC; Aldrich, M_w=9.00e4 g/mol) were used directly. Acrylic acid was vacuum distilled before use. *N*,*N*′-methylene bisacrylamide (MBA; Fluka) was recrystallized from methanol. Cerium (IV) ammonium nitrate (CAN; Shanghai Sinopharm Chemical Reagent) was recrystallized from dilute nitric acid containing appropriate ammonium nitrate.

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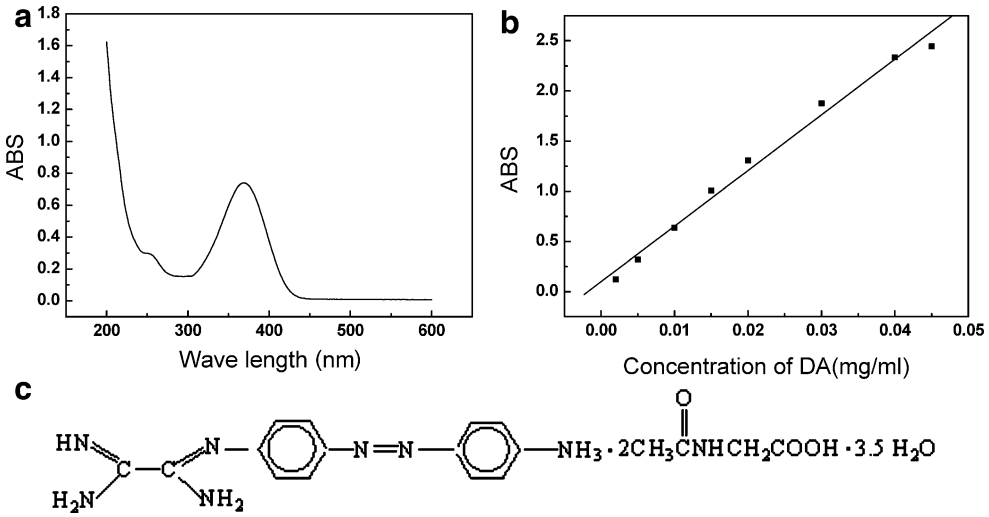


Scheme 1 The ultraviolet spectrum of Diminazene Aceturate (DA; **a**), the standard curve of DA at 370 nm (**b**), and the chemical structure of DA (**c**)

Synthesis of polysaccharide-based nanoparticles

Three kinds of polysaccharide-based nanoparticles were synthesized by using dextran, HPC, and HEC, respectively. The detailed synthesis is similar to that of DANP [15, 16]. The number of carboxyl groups per particle (N) can be calculated from the weight-percent molar mass ($M_{w(nanoparticle)}$) of nanoparticles, the weight percent of acrylic acid (AA) in nanoparticles, and the M_w of AA ($M_{w(AA)}$):

$$N = \left(M_{w(nanoparticle)} \times \text{the weight percent of acrylic acid}\right) / M_{w(AA)}$$

For example, as reported in our previous publication [15, 16], for the nanoparticle synthesized at M_{AGU}:M_{AA}:$M_{initiator}$:$M_{crosslinker}$=1:1:0.14:0.1, the $M_{w(nanoparticle)}$ and the weight percent of AA are 2.822×10^7 [16] and 37.6% [15] as determined by static light scattering and calculated for weight-calculated approach, therefore, the number of carboxyl groups can be calculated as 1.47×10^5.

The preparation of HPC-based nanoparticles (denoted as HANP particles) is also quite straightforward. An aqueous solution of CAN in nitric acid and AA was successively added to 150 ml of 1.2% (Wt) aqueous solution of HPC ($M_w\approx$80,000 g mol^{-1}) under gentle stirring and nitrogen bubbling, the concentration of nitric acid was maintained at ca. 0.0025 mol l^{-1}. Thirty minutes later, MBA was added, and the reaction was kept at 30 °C and pH=1–2 for 4 h, followed by adjusting the pH to 7.0 with 1 mol l^{-1} NaOH. The preparation of HEC-based nanoparticles (denoted as HENP particles) is similar to that of HPNP nanoparticles.

Dynamic light scattering

Malvern Autosizer 4700 Laser Light Scattering spectrometer was used. Dynamic light scattering (DLS) measurements were performed at a fixed scattering angle (θ) of 90°.

Morphology study

Transmission electron microscopy (TEM; PHILIPS CM 120 BioTwin) and atomic force microscopy (AFM; SEKO SPI 3800N Probe Station) were used to observe the morphology of nanoparticles. For TEM observations, the aqueous solutions of nanoparticles were placed onto carbon-coated copper grid and negative stained using phosphotungstic acid (5% *w*/*w*), and then dried at room temperature for 72 h.

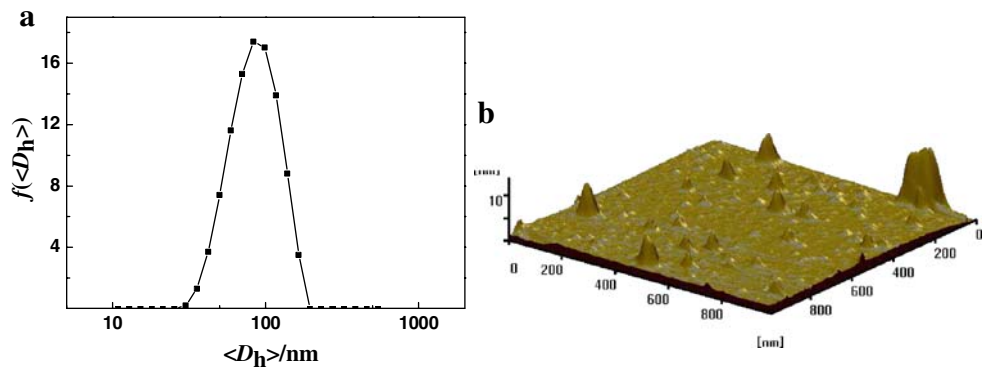


Fig. 1 Hydrodynamic diameter distribution of DANP2 in water (**a**) and AFM image of DANP2 (**b**)

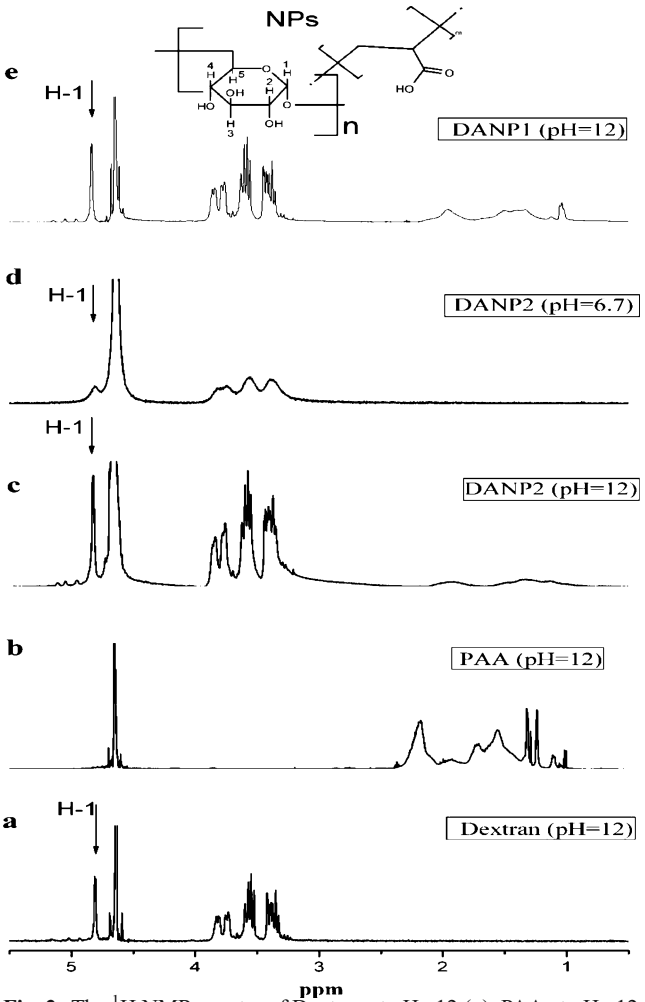


Fig. 2 The ^{1}H NMR spectra of Dextran at pH=12 (**a**), PAA at pH=12 (**b**), DANP2 at pH=12 (**c**), DANP2 at pH=6.7 (**d**), and DANP1 at pH=12 (**e**)

^{1}H nuclear magnetic resonance

^{1}H nuclear magnetic resonance (^{1}H NMR) measurements were carried out by Mercury Plus-400, Varian. The nanoparticles were solved in D_2O before measurement, and the pH value was adjusted by adding appropriate NaOD into the solution.

Diminazene aceturate loading of dextran-based nanoparticles

The aqueous solution of diminazene aceturate (DA; Sigma Chemical) was mixed directly with the aqueous solutions of DANP, and the final concentrations of DA and DANP were 1.5 and 3 mg/ml, respectively, the water used is Milli-Q water (pH=6.7). The time of exposure of the mixture before centrifugation is 10 h. DA-loaded nanoparticles were separated from aqueous suspension medium by ultracentrifugation of 28,000 rpm at 4 °C for 30 min. The amount of free DA in the clear upper solution was measured by UV (Unico UV-2102 PC) absorbency at 370 nm using a calibration curve at the same wavelength. The UV spectrum, calibration curve at 370 nm, and the chemical structure of DA are shown in Scheme 1. DA encapsulation efficiency (EE) and loading efficiency (LE) were calculated according to Eq. 1 and 2, respectively:

$$\mathrm{EE} = (\text{Total amount DA} - \text{Free amount DA})/\text{Total amount DA} \quad (1)$$

$$\mathrm{LE} = (\text{Total amount DA} - \text{Free amount DA})/\text{Total amount nanoparticles}. \quad (2)$$

Results and discussion

Fabrication and characterization of dextran-based nanoparticles

The hydrodynamic diameter distribution (<D_h>) and AFM image of DANP2 (synthesized at M_{AGU}:M_{AA}:M_{Ce}:M_{MBA}= 2:1:0.14:0.1) are shown in Fig. 1. DLS measurement reveals that DANP2 nanoparticles have an average hydrodynamic diameter of Ca. 100 nm (as shown in Fig. 1a). The

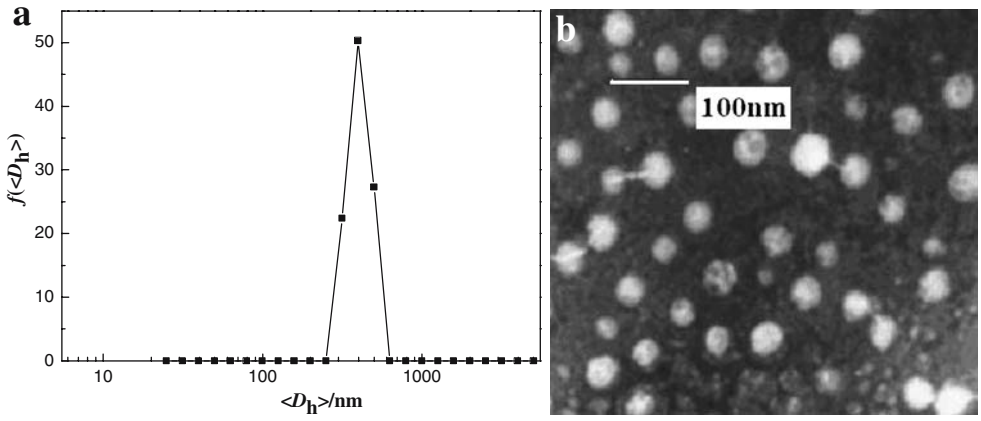
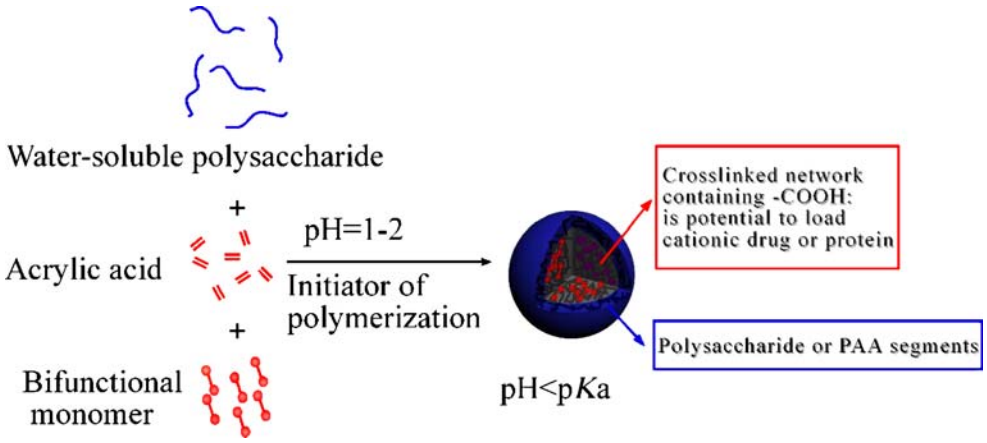


Fig. 3 Hydrodynamic diameter distribution (**a**) and TEM image (**b**) of HPC-based nanoparticles



Scheme 2 A schematic illustration of the one-pot synthesis to the polysaccharide-based nanoparticles containing carboxyls

typical AFM image of DANP2 nanoparticles is presented in Fig. 1b. The nanoparticles possess spherical morphology with a dimension of 50–100 nm. The diameter of nanoparticles observed by AFM is slightly smaller than what was determined by DLS; this decrease in size should be caused by the shrinkage of nanoparticles during water evaporation in the sample preparation, similar phenomenon was also observed in the morphology observation of other nanoparticles [19].

As described in our previous reports [15, 16], both dextran and polyacrylic acid (PAA) are water-soluble macromolecules, and it has been confirmed that many hydrophilic polysaccharides, including dextran, can form interpolymer hydrophobic complexes with polyacrylic acid (PAA) in acid medium owing to hydrogen bond interaction of carboxyl groups in PAA and proton acceptors in glucose units. Thus, the hydrophobic driving force for the formation of nanoparticles should be attributed to the complexation of PAA grafts and dextran segments. In addition, the copolymerization of AA and MBA in water normally results in macro-gel. However, in our approach, by simply initiating the graft polymerization of AA and MBA in acidic aqueous solution of polysaccharide, nanometer-sized particles, but no macro-gel, formed. From the results, the presence of polysaccharide should play a crucial role in our one-pot synthesis to nanoparticles. After initiation of graft copolymerization of AA from polysaccharide, the "micelle"-like nanoaggregates form attributed to the complexation between polysaccharide and PAA. As no macroscopic precipitation takes place in this process, we suggest that the polysaccharide chains with less-complexed segments may still keep solvated and thus stabilize the complex aggregates. Subsequently, participation of bifunctional monomer MBA leads to further fixation of the structure. Thanks to the shielding effect of the polysaccharide segments in periphery, the interparticle cross-linking was prevented, and the gelation does not occur.

Figure 2 shows the ^{1}H NMR spectra of dextran, PAA, DANP2 at pH=7 and pH=12, DANP1 (synthesized at M_{AGU}:M_{AA}:M_{Ce}:M_{MBA}=1:1:0.14:0.1) at pH=12, respectively. The signals around 4.8 ppm in the spectrum of Fig. 2a,c,d, and e should be attributed to the 1H of dextran [20], and the signal from 1.0 to 2.2 ppm in the spectrum of Fig. 2b and c can be mainly ascribed to the PAA grafts in NPs. In addition, By comparing Fig. 2c with d, it is obvious that the signal corresponding to carboxyls at 1.0–2.2 ppm can only be detected in acidic medium, indicating that the carboxyls were mostly embedded in the interior of the nanoparticles because of their complexation with polysaccharide chains.

Fabrication of nanoparticles from water-soluble cellulose ether

Considering similar complexation of PAA and other polysaccharides, this proposed approach is applicable to other water-soluble polysaccharides, such as HPC and HEC, the synthesis of nanoparticles from HPC and HEC are similar to that of DANP. The <D_h> and negative-stained TEM images of the nanoparticles synthesized from HPC and PAA (synthesized at M_{AGU}:M_{AA}:M_{Ce}:M_{MBA}= 0.2:1:0.022:0.0125) were shown in Fig. 3. The TEM

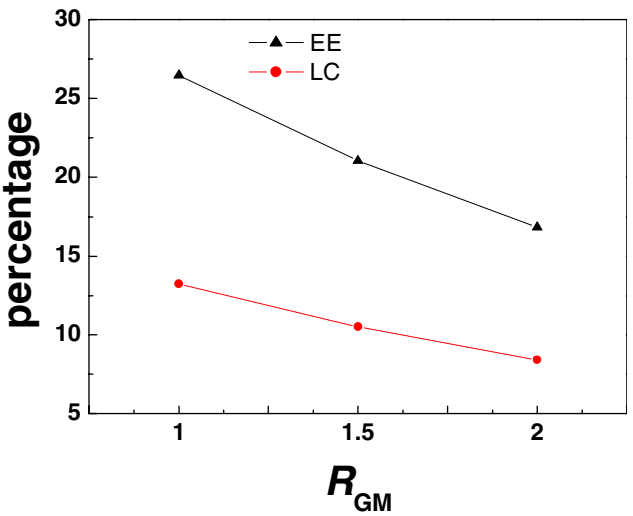


Fig. 4 The variation of encapsulation efficiency (*EE*) and loading content (*LC*) values of DANP with R_{GM} in water

images displays that the nanoparticles present the spherical morphology with a dimension of 50–90 nm. The diameter of nanoparticles observed by TEM is slightly smaller than what was determined by DLS, this decrease in size should also be caused by the shrinkage of nanoparticles during water evaporation in the sample preparation [19].

The mechanism of this synthesis of polysaccharide-based nanoparticles could be proposed as illustrated in Scheme 2. After the initiation of graft copolymerization of AA from polysaccharide, the "micelle"-like nanoaggregates form attributed to the complexation between polysaccharide and PAA. As no macroscopic precipitation takes place in this process, we suggest that the polysaccharide chains with less-complexed segments may still keep solvated and thus stabilize the complex aggregates. Subsequently, participation of cross-linker MBA leads to further fixation of the structure. Thanks to the shielding effect of superfluous polysaccharide or PAA segments in periphery, the interparticle cross-linking was prevented.

Diminazene aceturate loading of dextran-based nanoparticles

The application of nanoparticles in drug loading has attracted wide interests [21]. Hydrophilic drugs, such as protein, nucleic acid, etc., are more difficult to be delivered into their target compared to hydrophobic ones, because of their water solubility. Therefore, the nanocarriers for hydrophilic drugs has significant foreground. Polyelectrolyte interaction is an important driving force for the association of hydrophilic drug and nanocarrier [22]. Recently, Govender et al. [23] reported the interaction between Poly(aspartic acid)-poly(ethylene glycol) (Pasp-PEG) with DA, a small molecular weight cationic drug. In the present study, there is also a large amount of anionic groups in our polysaccharide-based nanoparticles, therefore, it is anticipated that they can also be used in the loading of cationic drugs, herein, DA was chosen as a model drug.

As shown in Fig. 4, the LE and EE of DANP was found to have a great relationship with the molar ratio of glucose units to AA (R_{GM}), keeping other conditions constant (M_{AA}:M_{Ce}:M_{MBA}=10:7:1). The increase in R_{GM} from 1 to 2 leads to the decrease in EE as well as LC. It is reasonable that the nanoparticles with higher R_{GM} have much higher encapsulation capability than those with lower R_{GM}, as the former can attract more cationic DA with more carboxyls in interior.

Upon drug loading, the zeta potential increases from a negative value to near zero, the size of nanoparticles also increase according to the characterization of DLS. And moreover, the drug-loaded nanoparticles is not as stable as that before drug loading in water, we suggest it is because some carboxyl groups extend to the periphery of nanoparticles and induce the possible aggregation upon drug loading.

Conclusions

The fabrication of nanoparticles from cellulose ether as well as dextran indicates that the proposed approach is applicable to different water-soluble polysaccharides. As determined by various characterizations, these polysaccharide-based nanoparticles have controllable nanosized diameter and obvious pH sensitivity. Moreover, the drug loading studies demonstrate that these nanoparticles have potential in loading cationic drug because there are abundant carboxyls within the nanoparticles.

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