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PH-sensitive nanoparticles of derivated dextran grafted with 1-(3-aminopropyl) imidazole

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Abstract. Dextran catches increasing attentions as a drug carrier because of its biocompatibility, biodegradability and ease of modification. In this study, we synthesized the derivated dextran by grafting with 1-(3-aminopropyl) imidazole. The dextran-based nanoparticles with sizes of < 200 nm were successfully prepared by nanoprecipitation, and its pH-sensitivities were investigated. The samples were fully characterized by FTIR, ^{1}H NMR, SEM, AFM and Zetasizer.

Introduction

In the past two decades, significant efforts have been devoted to develop novel polymeric carriers, including amphiphilic block copolymers[1], hydrophobically modified water-soluble polymers[2] and natural polysaccharides bearing hydrophobic twigs[3]. The polysaccharides (e.g. dextran, chitosan, pullulan, starch, etc.) are nontoxic, biocompatible, and have a high degree of OH moieties, which could be used to prepare amphiphilic polymers. The usual path is the partial derivatization of glucans with hydrophobic substituents, such as dextran introducing bile acids[4].

To overcome the solubility and target specificity limits of drugs, stimuli-sensitive polysaccharide derivatives have emerged as novel delivery systems, in which the release of drugs can be readily modulated by exerting an appropriate stimulus such as pH[5, 6], temperature[7, 8], magnetite[7, 8], etc. Considering the extracellular pH value (<7.0) of solid tumors is lower than that of surrounding tissues and blood (pH 7.5)[9], pH-sensitive polysaccharide derivatives have attracted considerable attention in anticancer drugs delivery. Although many pH-responsive nanoparticles have been reported, exploiting new biodegradable and non-polluting materials is still necessary.

Dextran has drawn increasing attention as a drug carrier because of its biocompatibility, biodegradability, wide availability and ease of modification[10]. In addition, it can be produced on a large scale and has wide applications in food and medical fields. 1-(3-aminopropyl) imidazole is a pH-sensitive moiety in aqueous media, with pH-sensitive aggregates behavior and buffering behavior between pH 7 and pH 5[11, 12].

In this study, we attempted to synthesis the derivated dextran grafted with 1-(3-aminopropyl) imidazole. New pH-sensitive dextran-based nanoparticles were prepared by nanoprecipitation, and their pH sensitive properties were evaluated.

Experimental Section

Materials. Dextran (*M*w ≈ 70 kDa), 5*β*-cholanic acid (5*β*-CHA) and 1-(3-aminopropyl) imidazole (API) were purchased from Sigma, USA. 4-dimethylaminopyridine (DMAP), succinic anhydride (SA) dicy-clohexyl carbodiimide (DCC), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were obtained from Aladdin-reagent Co. Ltd., Shanghai. Dimethyl sulfoxide (DMSO) was purchased from Guangfu Fine Chemical Research Institute, Tianjin. All other chemicals used were of reagent grade. Dialysis membrane of 12–14 kDa MWCO and 3500 MWCO was obtained from Lianxing Biotechnology Co., Ltd., Tianjin.

Modification of Dextran with 5β-cholanic Acid (DCA). 5*β*-cholanic acid (0.45 g, 1.25 mmol) were dissolved in DMSO, and ultrasounded until the solution was optically transparent. To activate carboxylic acid pertaining to 5*β*-cholanic acid, required amounts of DMAP and DCC were added into the polymer solution. The mixture was stirred at room temperature for 24 h, then precipitated in 100 mL ethanol, washed two times with 50 mL ethanol. The solid was dissolved in 50 mL water and dialyzed (MWCO: 14 kDa) for 3 d against deionized water and lyophilized to obtain DCA.

Succinoylation of DCA with Succinic Anhydride(coohDCA). DCA (0.1 g) was dissolved in 25 mL of DMSO and ultrasounded 10 min. Succinic anhydride (2.2 g) and DMAP (2.0 g) as catalyst were added. The mixture was then heated at 50 °C in water bath with stirring for 12 h. After reaction, the reactant mixture was dialyzed for 3 days and lyophilized to obtain coohDCA.

Grafting of Imidazole to CoohDCA(DCA/API). CoohDCA (0.1 g) was dissolved in MES solution (pH 5.5, 20 mL), and ultrasounded until the solution were optically transparent. Required amounts of EDC and NHS were added to the stirring solution to activate carboxylic acid pertaining to coohDCA. The mixture was stirred continuously for 1 h, and then 1-(3-aminopropyl) imidazole was added. The graft reaction was conducted for 14 h at room temperature. The solution was dialyzed against deionized water and lyophilized to obtain DCA/API.

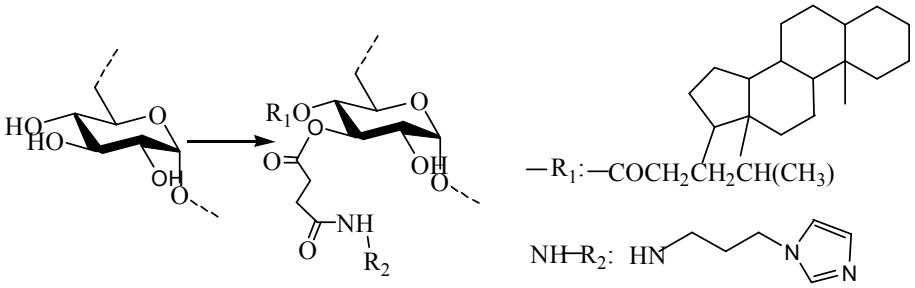
Nanoparticles Preparation. For dialysis method, 20 mg of dextran ester was dissolved in 20 mL DMSO and was dialyzed (MWCO: 3500) against 500 mL distilled water. The deionized water was exchanged 5 times in a period of three days.

For dropping technique, 20 mg of the dextran ester was dissolved in 20 mL DMSO. Distilled water (50 mL) was added dropwise to the polymer solution. The suspensions were dialyzed (MWCO: 3500) against deionized water until the DMSO was completely removed from the aqueous suspension.

Characterizations. The samples were characterized by FTIR (Thermo Nicolet Nexus FTIR), ^{1}H NMR (Varian Inova 500 MHz), scanning electron microscopy (SEM) (Hitachi S 4800), atomic force microscope (AFM) (CSPM 5000), Zetasizer (Malvern Instruments Ltd, Nano ZS) equipped with He–Ne laser at a wavelength of 633 nm.

Results and Discussion

Synthesis of Dextran Derivatives. Several methods have been previously described for the esterification of dextran with carboxylic groups containing compounds. Better results concerning the degree of substitution and integrity of polysaccharide chain have been obtained by carrying out the esterification in the presence of coupling agents such as DCC or EDC. The synthetic structure of DCA/API is represented in Scheme 1.



Sheme 1. The structure of highly functionalized amphiphilic DCA/API.

Firstly, the synthesis process was the conversion of dextran with 5*β*-cholanic acid, to produce amphiphilic polymer by using DCC as coupling agent and DMAP as catalyst. Formation of the ester linkage of DCA was confirmed by the increase in the band at 1735 cm^{-1} (C=O) and the band at 1018 cm^{-1} (C–O–C) of the FTIR spectra (Fig. 1b). Also, the presence of 5*β*-cholanic acid in DCA was demonstrated by the characteristic peaks of bile acid appearing at 0.6–2.7 ppm including 18 –CH_3 (0.61 ppm), 19 –CH_3 (0.93 ppm), 21 –CH_3 (0.96 ppm), and methylene methine envelope (1–2.7 ppm) in the ^{1}H NMR spectrum (Fig. 2b).

Secondly, the homogeneous reaction was carried out by DCA with succinic anhydride using DMAP as catalyst. Compared to the spectrum of DCA, the absorbances at 1732 cm^{-1} and 1567 cm^{-1} in FTIR provided the evidence of succinoylation (Fig. 1c). The band at 1567 cm^{-1} corresponded to the antisymmetric stretching of carboxylic anions[13]. Moreover, the intensity of the absorption band at 1158 cm^{-1} for C–O antisymmetric stretching and 1420 cm^{-1} (δ=CH_2) in ester groups increased. The ^{1}H NMR spectrum of DCA/API (Fig. 3b) showed peaks at 2.20 ppm, 2.27 ppm and 2.98 ppm, which were assigned to methylene and methine protons of the succinimide unit, respectively. Note that the stretches of –OH (4.50–5.01 ppm, Fig. 2a) was significantly reduced in DCA/API.

Finally, coohDCA reacted with 1-(3-aminopropyl) imidazole, with EDC as coupling agent and DMAP as catalyst. The characteristic absorption of DCA/API (Fig. 1d) at the band 1700 cm^{-1} (amide I), 1540 cm^{-1} (amide II), 1042 cm^{-1} and 1018 cm^{-1} (bands of azole C–H) and 1400 cm^{-1} (δ=CH_2) were emerged. The successful addition of imidazole groups was also confirmed by the ^{1}H NMR characteristic peaks at δ=7.60 (–N–CH=N– of imidazole ring), 7.16 (–N–CH=CH– of imidazole ring), 6.91 (–CH=*CH*–N– of imidazole ring), 3.90–4.07 (–*CH_2*–CH_2–*CH_2*–), 2.30 (–CH_2–*CH_2*–CH_2–) in Fig. 2b.

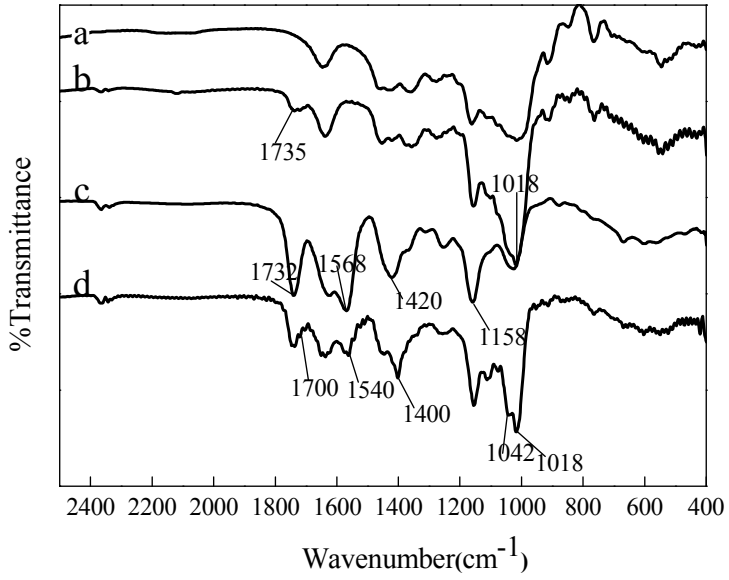


Fig. 1 The FTIR spectra of (a) Dextran; (b) DCA; (c) coohDCA;(d) DCA/API

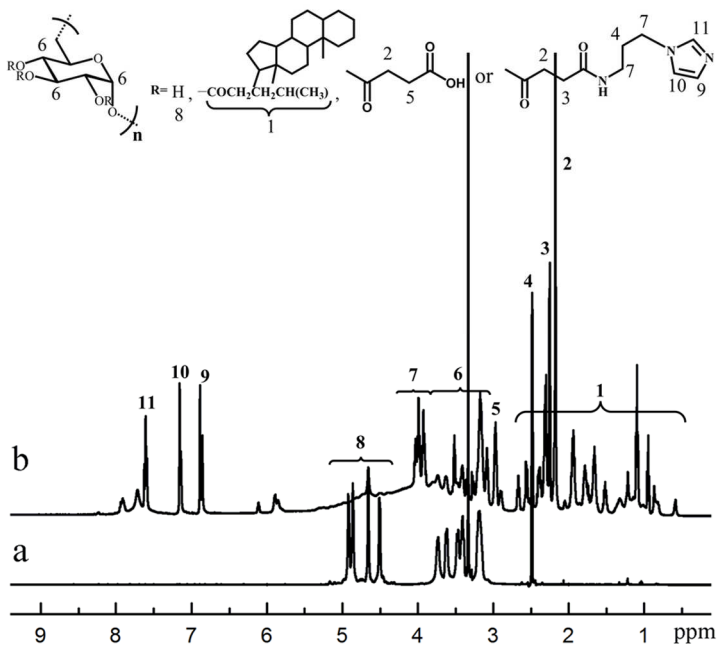


Fig. 2 The ^{1}H NMR spectra of dextran derivatives in DMSO-d6; (a) Dextran; (b) DCA/API. Note that the peaks at 2.50 ppm were assigned to DMSO-d6, the peaks at 3.30 ppm were assigned to H_2O contained in samples.

Nanoparticle Formation. Hydrophobic polymers may self-assemble into nanoparticles during precipitation in dilute solutions of water. A simple approach for the ideally complete exchange of the solvents against water is dialysis. The dialysis results in an exchange of the solvent against the non-solvent water. The size and shape of nanoparticles were analyzed by DLS, SEM and AFM. By applying nanoprecipitation via dialysis of a DMSO solution against water, the size of DCA^{a} nanoparticles was 135 nm according to Table 1.

Another technique of nanoprecipitation is the introduction of water into the polymer solution in droplets under continuously stirring. As summarized in Table 1 and Fig. 3, the dropwise addition of water to a DMSO solution resulted in particles in the same size range as obtained by dialysis at the same concentration, whereas the latter leaded to a relatively narrow particle size distribution and more stable for DCA^{b} contrast to DCA^{a}. These results suggest that the dropping technique is better for the dextran derivatives. We also proved this conclusion through preparing DCA/API nanoparticles by using the dropping technique.

The balance between the different functional groups was an important criterion for the formation of nanoparticles. The average size of DCA^{b} is 110 nm (Tab. 1). After grafted with imidazole, the size grew larger, indicating the importance of the polarity and the hydrophilic/hydrophobic balance of the polymer backbone for the particle size. Moreover, after grafted with imidazole, the nanoparticles changed from spherical to irregular, probably owing to the imidazole rings are hydrophilic at pH below its pKa value.

Table 1 z-Average mean diameter and polydispersity index (PDI) of nanoparticle suspensions of DCA and DCA/API prepared by different methods.

Sample	Appearance	DS	Size[nm][d]	PDI [d]	Zeta[mV] [d]
DCA[a]	transparent	0.49 [c-1]	135	0.320	-10.53
DCA[b]	transparent	0.49 [c-1]	110	0.146	-13.21
DCA/API[b]	transparent	0.93 [c-2]	121	0.114	-18.05

[a] Dialysis of the dissolved polymers (1 mg/mL) against water.
[b] Dropwise addition of water to polymer dissolved in DMSO (1 mg/mL).
[c-1] DS of 5β-cholanic acid per anhydroglucose unit of dextran; [c-2] DS of API per anhydroglucose unit of dextran (using NMR).
[d] Be measured by Zetasizer.

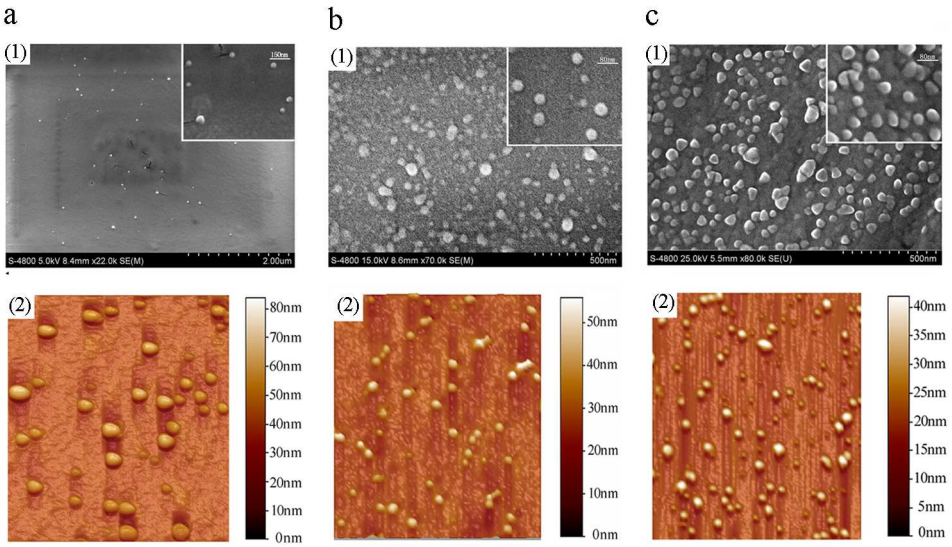


Fig. 3 Images of nanoparticles (a) DCA prepared by the dialysis process (b) DCA and (c) DCA/API prepared by the dropping method. (1) SEM and (2) AFM images.

Nanoparticle Characterization. The pH sensitivities of the hydrogel nanoparticles were also examined by the changes of the particle sizes and zeta potential values. As shown in Fig. 4a, the particle size of DCA/API nanoparticle drastically increased from 124 nm to 251 nm when the pH decreased to 5.5, indicating that the aggregation. This was attributed to the transition from hydrophilic to hydrophobic, arising from deprotonation of imidazole groups whose pKa value is 6.5. The pH sensitivity of the nanoparticles was also confirmed by the zeta potential measurement. A higher absolute value of zeta potential indicates a stronger electrostatic repulsion between the particles, and therefore a higher stability of the suspensions. The zeta potential values of the DCA/API nanoparticles increased with the decrease of pH. In particular, the absolute value of zeta potential of DCA/API nanoparticles changed sharply around pH 7.0 presenting in Fig. 4b.

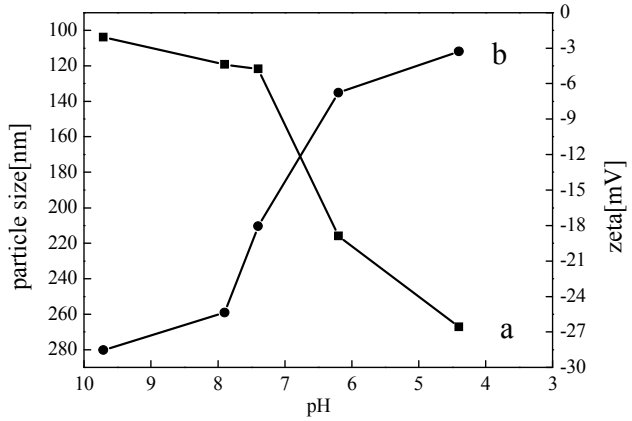


Fig. 4 Mean particle size (a) and zeta potential (b) curves of DCA/API nanoparticles as pH is varied.

Summary

In summary, we successfully synthesized a new dextran derivative by grafting with 1-(3-aminopropyl) imidazole. By comparisons, dropping technique was found to be the best nanoprecipitation strategy to prepare the DCA/API nanoparticles. The sizes of these dextran-based nanoparticles are < 200 nm, and its pH-sensitivities were fully demonstrated. The pH-sensitive nanoparticles have possible applications over a diverse range of drug delivery fields.

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