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Altering Associations of Doxorubicin-Loaded Alginate Esters Micelles in Presence of β-Cyclodextrin

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ABSTRACT: The aim of this article was to evaluate the effects of β-cyclodextrin (β-CD) on doxorubicin (DOX)-loaded alginate esters (SA-C_{16}) micelles (DOX/SA-C_{16}) in aqueous solution. DOX was physically loaded into SA-C_{16} micelles by an o/w emulsion method with a substantial encapsulation efficiency (EE) level (36.12%), and DOX/SA-C_{16} was distributed in size diameters of approximately 254 nm. SA-C_{16} as carriers for the DOX can lead to the formation of associative networks in aqueous solutions between the hydrophobic tails of SA-C_{16} and DOX, and the dried morphology of DOX/SA-C_{16} aggregate was spherical shape. Addition of β-CD to the system of DOX/SA-C_{16} facilitated decoupling of these associations via inclusion complex formation between β-CD cavities and the polymer hydrophobic tails that produced the release of DOX immediately, and the EE level was dropped to 0.08%, and at the same time the size distribution of aggregate was increased to about 413 nm, moreover, the aggregate was relatively large and becoming irregular spherical shape. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40702.

KEYWORDS: biocompatibility; biomaterials; functionalization of polymers

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INTRODUCTION

Over the past decades, researchers have made a lot of progress in hydrophobically modified polysaccharides which are frequently used in pharmaceutical field, cosmetics industry, and textile industry.[1–3] Generally, the hydrophobically modified polymer exhibits viscosity values in several orders of magnitude higher than their precursors. In aqueous solution, the intramolecular and/or intermolecular hydrophobic associations caused the formation of three dimensional networks and/or aggregated structures which have shown great potential for the entrapment of therapeutic active molecules.[4,5]

Doxorubicin (DOX) and its bioactive derivers as anticancer drugs are widely used in chemotherapy treatment.[6] Some drug-loaded particles are too large to be endocytosed by most cells in the bloodstream, and thence DOX needs the suitable drug carrier to solubilization to give full play to its effectiveness. Owing to polymer micelle own advantages that were formed mainly by the selfassembly, in which the hydrophilic moieties form the outer shell, the hydrophobic moieties form the inner core, therefore it is widely used as a hydrophobic drug delivery carrier, meanwhile, in the aspect of medication tumor targeting polymer micelle has good application prospect.[7,8] β-cyclodextrin (β-CD) as a significant kind of natural molecule has attracted widespread attention for its extraordinary hydrophobic cavity, but β-CD due to its too small internal cavity sizes (6–7 Å) that could not include DOX.[9,10] β-CD can form inclusion complex with polymers in that the hydrophobic moieties of polymers can be induced to enter nanoscale cavity of β-CD, and thence the tendency to form hydrophobic associations of the polymers is decreased. This feature makes β-CD that has widely used in drug delivery,[11] food,[12,13] pharmaceutical,[14,15] and ecological environment engineering.[16]

In a previous study, we have reported that the nanoscale of amphiphilic alginate esters with different degree of substitution (DS) and hydrophobic alkyl chain were prepared by sodium alginate reacting with aliphatic alcohols, and it could selfassembly of regular nanosphere morphology in distilled water.[17] In this work, we first use hexadecanol-modified alginate (SA-C_{16}) form hydrophobic associations with DOX (DOX/SA-C_{16}) by an o/w emulsion method, and then addition of β-CD to the system of DOX/SA-C_{16} provides decoupling of associations via inclusion complex formation between β-CD cavities and the polymer hydrophobic tails. The findings provide us with significant information about the changes of association strength during the process of inclusion complex.

EXPERIMENTAL

Materials

Sodium alginate (Alg, $M/G = 0.18$), β-CD, chloroform, triethylamine (TEA), hexadecanol, *p*-toluenesulfonic acid (*P*-TSA),

formamide (FA), dimethyl formamide (DMF), 4-(*N,N*-dimethylamino) pyridine (DMAP), absolute ethanol, and sodium carbonate were bought from Sinopharm Chemical Reagent (Shanghai, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)·HCI) was purchased from Sangon Biotech (Shanghai, China). DOX·HCI was obtained from Huafeng United Technology (Beijing, China). Distilled water was used for the preparation of all solutions.

Synthesis and Characterization of SA-C_{16}

The hydrophobically modified SA-C_{16} was synthesized from the parent Alg partially protonated by utilizing the FA/DMF (10/9, v/v) containing some water-free *P*-TSA, then adding DMAP, EDC-HCI, and hexadecanol. Subsequently, the esterification reaction between the hydroxyl of hexadecanol and the carboxylic acid groups of protonated Alg were performed. The synthesis alginate esters (SA-C_{16}) method has been described elsewhere,[18,19] and the reaction product is denoted as *x*SA-C_n, where *x* represented DS and *n* represented the length of alkyl chain, and the characteristic and the critical micelle concentration of the hydrophobically modified alginate esters have been reported recently.[20] In this article, the synthesis product was 9.92% SA-C_{16}, and to get stable SA-C_{16} micelles, the pH of solution was adjusted to about 5.8.

Preparation of DOX-Loaded SA-C_{16} (DOX/SA-C_{16})

DOX HCI was dissolved in chloroform in the presence of TEA (3 times molar quantity of DOX) to form a DOX-containing solution, and the chloroform solution of DOX was added to the stirred SA-C_{16} aqueous solution. The chloroform was removed by evaporation, and then the remaining solution was dialyzed against distilled water and freeze dried to obtain DOX/SA-C_{16}. The encapsulation efficiency (EE) of DOX/SA-C_{16} was calculated through eq. (1). DOX-loaded SA-C_{16} (DOX/SA-C_{16}) micelles was done by the o/w emulsion method as previously reported in detail.[21,22]

$$\mathrm{EE} = \frac{\text{weight of drug in micelles}}{\text{weight of drug fed initially}} \times 100\% \qquad (1)$$

Evaluation of DOX Release in Addition of β-CD

DOX/SA-C_{16} solutions with a fixed concentration of 0.1 wt %, and then DOX/SA-C_{16} solution was added dropwise to β-CD (0.5 wt %, 10.0 mL) aqueous solution under stirring. Subsequently, the precipitated product was separated by centrifugation, and the supernatant was reserved for standby.

UV-Vis Spectrophotometric, Transmission Electron Microscopy, Dynamic Light Scattering

Determination the standard curve of DOX by UV-Vis method: a stock solution of DOX was prepared by dissolving 10.0 mg of DOX in 10 mL of distilled water. From the stock solution, various dilutions were made to obtain solutions of 0.01, 0.03, 0.05, 0.10, 0.30, and 0.50 mg mL^{-1}, and spectra were recorded from 600 to 400 nm using a UV-Vis spectrophotometer (UV-2501, Shimadzu).

The dried morphology of aggregate was characterized by transmission electron microscopy (TEM) with a Tecnai 12 (Philips Instruments, Holland). Samples were prepared by drop-casting onto a 400 mesh carbon-coated copper grids, then a drop of phosphotungstic acid was placed on copper grids, soon after suck up excess phosphotungstic acid.

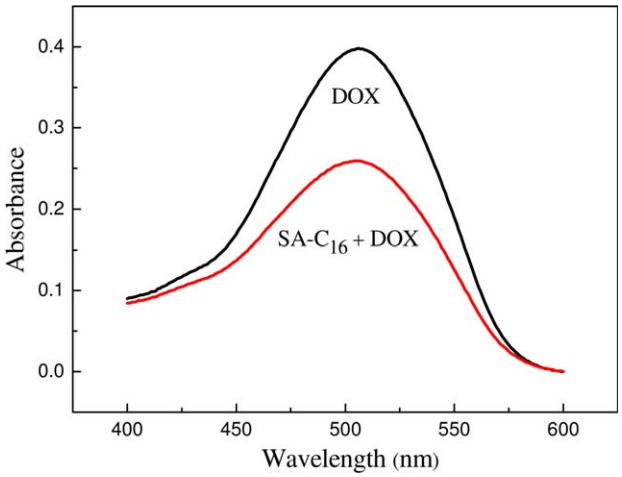

Figure 1. UV-Vis spectra of DOX solution and SA-C_{16} + DOX solution. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

To measure the nanoparticle size distribution of aggregate, dynamic light scattering (DLS) measurements were performed using the ZetaSizer Nano Series (Malvern Instruments, U.K.), which equipped with a standard 633 nm laser. A clear disposable capillary cell was used for measurements, and the cell was loaded with 1.0 mL of sample solution.

RESULTS AND DISCUSSION

UV-Vis Spectrophotometric Analysis

DOX solution at around 506 nm has a specific UV-Vis absorption, and therefore UV-Vis spectrophotometer can be used to measure EE of DOX/SA-C_{16}. It was found from absorbance standard curve of DOX solution that the absorbance intensity of DOX has a linear relationship with a certain concentration range of DOX, and the regression equation was $A = 3.8606C + 0.0123$, correlation coefficient ($R = 0.9999$), wherein A represented the absorbance, C represented the concentration of DOX, the units of C was mg mL^{-1}. The correlation coefficient values obtained were highly significant for the method.

To character the association interaction between DOX and SA-C_{16} solution, we performed UV-Vis spectroscopy measurements, and the results are shown in Figure 1. Obviously, the DOX peak at 506 nm was shrinking in presence of SA-C_{16}. This difference in absorbance should be attributed to the hydrophobic associations between polymers and DOX and less free DOX were detected in the solution. Therefore, DOX has been loaded successfully into associative regions of SA-C_{16}, moreover, EE of DOX/SA-C_{16} through eq. (1), with value of about 36.12%.

Effects of β-CD on the solution of DOX/SA-C_{16} are depicted in Figure 2, and in the presence of β-CD, the absorbance of DOX/SA-C_{16} solution has significant change. This finding suggests a gradual breakdown of the hydrophobic associations between SA-C_{16} and DOX through the formation of inclusion complexes between the hydrophobic tails of SA-C_{16} and β-CD cavities.

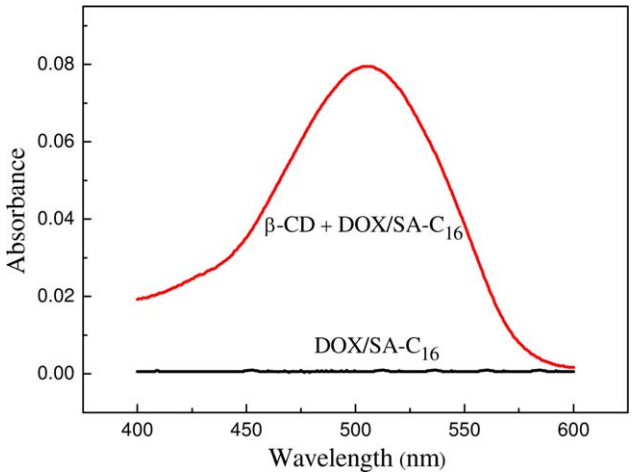

Figure 2. UV-Vis spectra of DOX/SA-C_{16} solution and β-CD + DOX/SA-C_{16} solution. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

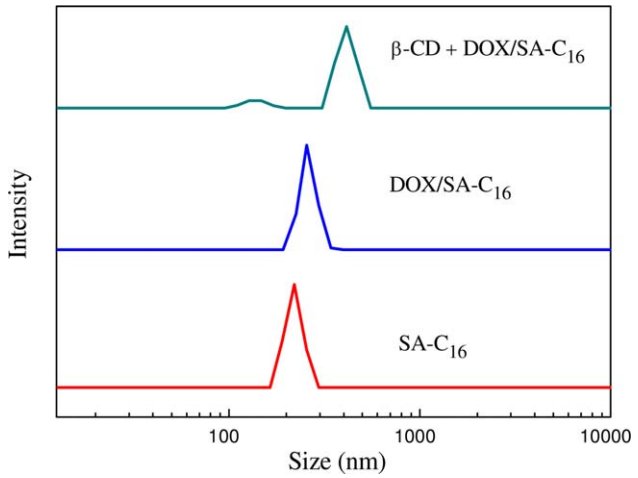

Figure 4. DLS measurements of SA-C_{16}, DOX/SA-C_{16}, and β-CD + DOX/SA-C_{16}. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Hence, in the presence of β-CD, the absorbance of DOX/SA-C_{16} solution increases. When β-CD was added into DOX/SA-C_{16} solution, it provided decoupling of associations via inclusion complex formation between β-CD cavities and the polymer hydrophobic tails, and therefore, it leads to the release of DOX immediately, and the rest of the DOX in SA-C16 micelles was about 0.08% through eq. (1).

TEM Results

TEM measurements were performed to gain insights into sample morphology change due to the β-CD added. The results are shown in Figure 3. Compared with TEM images of different systems, we can notice a significant change in morphology, and thence we infer that this is attributed to the inclusion interaction between β-CD and SA-C_{16}.

In absence of β-CD, the dried morphology of DOX/SA-C_{16} aggregates observed by TEM was almost regular spherical shape and proved that the hydrophobic alkyl chain of SA-C_{16} could form selfaggregates with DOX. Obviously, in the case of β-CD, the aggregates are relatively large and becoming irregular spherical shape, and therefore the hydrophobic alkyl chain of SA-C_{16} is imbedded into hydrophobic cavity of β-CD that leads to the release of DOX. The specific size of the particles diameter will be introduced in the following.

DLS Results

To measure the nanoparticles diameter change of DOX/SA-C_{16} in presence of β-CD, the dispersed nanoparticles was further analyzed by DLS in Figure 4. DLS measurement results slightly larger than TEM, because of TEM only provided the dried morphology of aggregates, and the samples must be dehydrated and immobilized on a solid support, and this can lead to structural distortions compared to the solvent-swollen state.

In absence of DOX HCI and β-CD, the self-assembled nanoparticles size of SA-C_{16} was distributed in size diameters of approximately 219 nm. In the case of DOX HCI, the size distribution of aggregates has increased to about 254 nm, and this is due to the association interaction between hydrophobic alkyl chain of

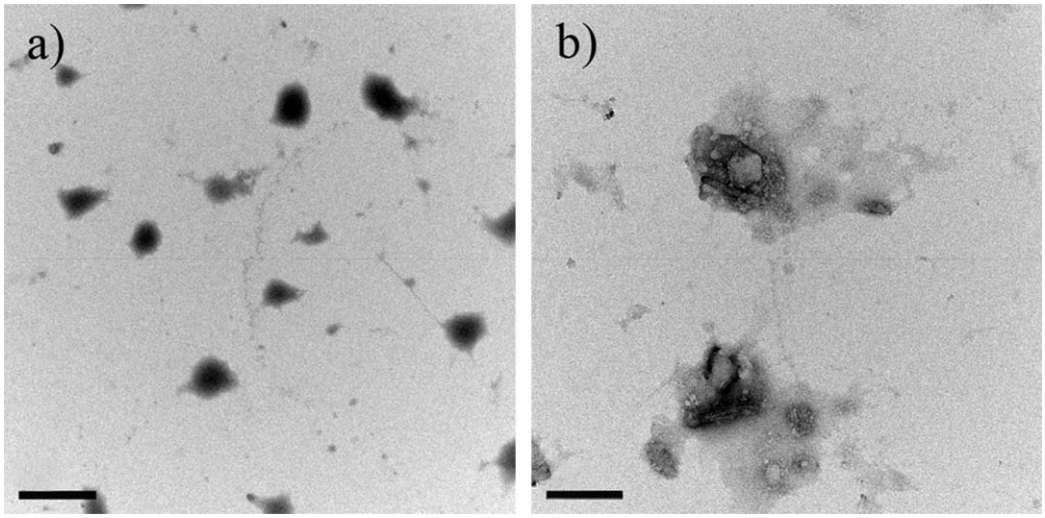

Figure 3. TEM images (a) DOX/SA-C_{16} and (b) β-CD + DOX/SA-C_{16} (scale bar is 200 nm)

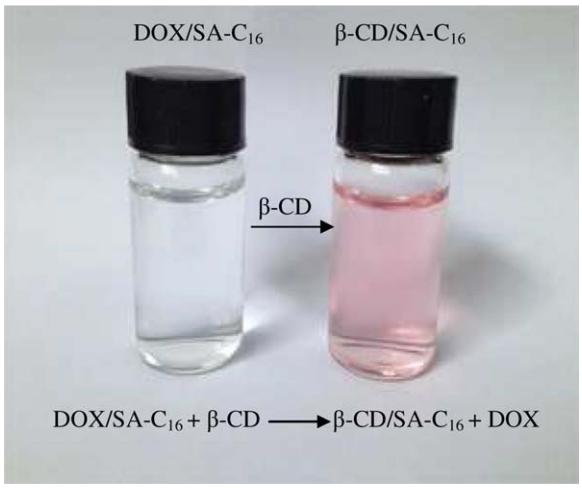

Figure 5. The color change of DOX/SA-C_{16} solution in presence of β-CD. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

SA-C_{16} and DOX can lead to the formation of associative regions. Addition of β-CD to the system of DOX/SA-C_{16} makes size distribution of aggregates has increased to about 413 nm, and the variation of size distribution should be attributed to the inclusion interaction between hydrophobic chain of β-CD and SA-C_{16}, therefore β-CD can facilitate decoupling of associations between DOX and the polymer hydrophobic tails, and it leads to the release of DOX immediately. Besides, when the β-CD was added to DOX/SA-C_{16} solution, the size distribution curve of DOX/SA-C_{16} presents a new single peak at about 150 nm which belongs to the characteristic peak of β-CD aggregates.[23]

The effects of β-CD on DOX/SA-C_{16} in aqueous solution can intuitively reflected the color changes of different systems in Figure 5. DOX/SA-C_{16} was obtained under room temperature by the o/w emulsion method. With the addition of β-CD to DOX/SA-C_{16} solution, the mixed solution became red and turbid, which indicated that by damaging hydrophobic associations between polymers and DOX make DOX release from micelles that form soluble salt in an acidic solution.

CONCLUSIONS

In the present study, we have examined the altering associations of DOX-loaded alginate esters micelles (DOX/SA-C_{16}) in presence of β-CD. By an o/w emulsion method, we first accomplished the preparation of DOX/SA-C_{16}, and DOX can lead to the formation of associative regions in aqueous solution with the hydrophobic tails of SA-C_{16}. Adding β-CD to the system of DOX/SA-C_{16} solution facilitates decoupling of associations via inclusion complex formation between SA-C_{16} hydrophobic tails and β-CD cavities, and therefore, it leads to the release of DOX immediately. In conclusion, the findings from above showed that β-CD addition can efficiently reduce the hydrophobic association effect of DOX/SA-C_{16} in aqueous solution, and this specific property may be useful to improve the control release of therapeutic molecules that can be entrapped in the polymer system.

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