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Study of glycol chitosan-carboxymethyl β-cyclodextrins as anticancer drugs carrier

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ABSTRACT

Efficient target delivery system for insoluble anticancer drugs to increase the intracellular drug concentration has become a focus in cancer therapy. Herein, glycol chitosan-carboxymethyl β-cyclodextrins (G-chitosan-CM-dextrins) was synthesized for delivering different hydrophobic anticancer drugs. Surface plasmon resonance and UV–vis spectroscopy results showed that all the three anticancer drugs (5-fluorouracil, doxorubicin, and vinblastine) could be successfully loaded into the cavities of the covalently linked CM-dextrins. Moreover, the free carboxymethyl groups could enhance the binding interactions between the covalently linked CM-dextrins and anticancer drugs. Release behaviors with pH changes of the three drugs were also explored, result showed different drugs would be released by different ways, as for doxorubicin, pH sensitive release has been realized. The obtained G-chitosan-CM-dextrins carrier has both mucoadhesive property of G-chitosan and hydrophobic cavities of β-cyclodextrins. Therefore, the new synthesized G-chitosan-CM-dextrins carrier exhibits a promising potential capability for anticancer drug delivery in tumor therapy.

1. Introduction

Currently, most chemotherapeutic drugs for treating cancer patients are poorly soluble in aqueous media (Yang et al., 2011). In this situation, a great challenge is faced for effective clinical application of these hydrophobic agents. For example, the insufficient drug doses at the lesion site after administration of these agents will cause very low bioavailability (Wei et al., 2010). To address the challenge, many attempts have been made to deliver hydrophobic anticancer agents to the targeting sites as much as possible. At present, the most popular approach to improve the bioavailability of insoluble anticancer drugs is to deliver via carriers, such as self-assembling peptide for ellipticine (Fung et al., 2009), magnetic iron oxide nanotubes for paclitaxel (Yue et al., 2011), and polymeric carrier for doxorubicin (Chen et al., 2011). Chitosan can interact with mucins and open the tight junctions between epithelial cells, which are beneficial to drug transport. The chitosan-based delivery systems have shown great potential to deliver nucleic acids, therapeutic proteins, vaccination and so on (Amidi, Mastrobattista, Jiskoot, & Hennink, 2010; Han, Shin, & Ha, 2012; Mao et al., 2001; Subbiah et al., 2012; Van der Lubben, Verhoef, Borchard, & Junginger, 2001; Zambito, Pedreschi, & Di Colo, 2012). Besides the evident advantages of chitosan, one important disadvantage as drug carrier is its hydrophilicity, which means its poor loading capacity for hydrophobic therapeutic agents (Wu, Zeng, Zhu, & Tong, 2005). Then, researchers have done a lot of work to improve the loading ability of chitosan for hydrophobic drugs (Hua et al., 2011; Zhou et al., 2010). Among them, chitosan modified by cyclodextrins has attracted great attention (Krauland & Alonso, 2007; Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Muzzarelli, 2010; Prabaharan & Mano, 2005; Trapani et al., 2010).

Cyclodextrins have special hydrophobic cavities, which can be used as pharmaceutical excipients to improve the solubility, stability and bioavailability of hydrophobic and biomolecular drugs (Uekama, Hirayama, & Irie, 1998). There were many reports about cyclodextrins combined with chitosan to form new drug carriers. For instance, Prabaharan and Mano synthesized hydroxypropyl chitosan-carboxymethyl β-cyclodextrins microparticles and studied their inclusion capability for ketoprofen. Compared with chitosan microparticles, the carrier had stronger inclusion ability, which was caused by the special hydrophobic cavity structure of β-cyclodextrins (Prabaharan & Mano, 2005). Krauland and Alonso (2007) utilized chitosan/carboxymethyl β-cyclodextrins nanoparticles for delivery of insulin and heparin and the nanoparticles exhibited a great capacity for loading the two selected

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macromolecular drugs. Trapani and co-workers also reported chitosan/sulfobutyl ether β-cyclodextrins nanoparticles for oral administration of small peptide glutathione. The results indicated that the nanoparticles could improve the loading of glutathione and promote its transport through the simulated intestinal media without enzymes (Trapani et al., 2010). In a word, chitosan-β-cyclodextrins derivatives are attractive carriers since they possess both the mucoadhesive property of chitosan and the hydrophobic cavities of β-cyclodextrins. The anticancer drug carriers with satisfactory loading and release ability are highly desirable on the way to applying insoluble anticancer drugs for the clinic.

In this study, we subtly designed and synthesized glycol chitosan-carboxymethyl β-cyclodextrins (G-chitosan-CM-dextrins) by a covalently linked reaction between amino of G-chitosan and carboxylic acid group of CM-dextrins. Using our home-built SPR instrument, a simple, rapid and sensitive SPR system was established to evaluate the delivery ability of G-chitosan-CM-dextrin for three different anticancer drugs: 5-fluorouracil (5-Fu), doxorubicin (DXR), and vinblastine (VBL). Their chemical structures are shown in Fig. S1. In addition, the enhancement affect of other free carboxylic acid group on the carrier's loading ability of was further investigated by UV–vis spectroscopy. The release behaviors with pH changes of the three drugs were also explored.

2. Materials and methods

2.1. Materials and reagents

Glycol chitosan (G-chitosan, degree of polymerization ≥400), N-hydroxysulfosuccinimide sodium salt (sulfo-NHS), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), and porcine gastric mucin (PGM, Type III) were purchased from Sigma–Aldrich (USA). β-Cyclodextrin (β-CD) was obtained from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). 1-Octanethiol (1-OT) was acquired from Acros Organics (Belgium). 5-Fluorouracil (5-Fu), DXR, and VBL were purchased from the National Institute for Food and Drug Control (NIFDC, Beijing, China). PGM was prepared with 10 mM Tris–HCl solution containing 0.15 M NaCl (pH 3.0). G-chitosan was dissolved in 10 mM phosphate buffer solution (PBS, pH 7.0). Tris(hydroxymethyl)aminomethane(Tris), hydrochloric acid (HCl) and all other reagents were purchased as analytical grade from Beijing Chemical Reagents Co. (Beijing, China). Millipore Milli Q (18 MΩ cm) water was used in all experiments.

CM-dextrins were synthesized as described in literature with some modifications (Furusaki, Ueno, Sakairi, Nishi, & Tokura, 1996). The obtained CM-dextrins were easily soluble in water and were characterized by TGA, MALDI-TOF MS, and HPLC/ESI-MS. Subsequently, the prepared CM-dextrins reacted with G-chitosan by adding sulfo-NHS/EDC cross-linking reagents according to the procedures described in literature with little modification (Prabaharan & Mano, 2005). The resulting solution was dialyzed against 10 mM PBS (pH 7.0) for 24 h using dialysis bags with a 3500 MW cutoff. The final solution concentration was about 510 μg/mL, and the obtained G-chitosan-CM-dextrins were identified by TGA and UV–vis spectroscopy.

2.2. Instruments and measurements

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, Autoflex III), high performance liquid chromatography (Agilent Technologies, Agilent 1100) coupled with electrospray ionization mass spectrometry (Thermo Finnigan, LCQ DECA XP) (HPLC/ESI-MS) were used to analyze the content of the synthesized samples. The thermogravimetric analysis (TGA, Shimadzu DTG-60H) was recorded from room temperature to 500 °C with a heating rate of 10 °C/min. UV–vis spectrophotometer (Shimadzu, UV-2550) was used to measure the samples' absorption.

The home-built FI-SPR instrument, equipped with a bi-cell detector for high-resolution measurements, has been described elsewhere (Luan, Zhou, Tan, Yang, & Yao, 2011). Briefly, the SPR setup recorded reflected light falling onto the two cells (A and B) of the bi-photodetector, then both the differential (A − B) and the sum (A + B) signals were recorded by a PCI-1731 interface card (Advantech, Taiwan) controlled by a Lab-view program. The ratio of the differential to sum signals, which is linearly proportional to the SPR angular shift, was obtained numerically by dividing (A − B) with (A + B). In order to convert the ratio of (A − B)/(A + B) to the SPR dip shift, an instrumental calibration experiment was performed by gradually adding predetermined amounts of ethanol to water and monitoring the corresponding SPR reflectivity changes (Kolomenskii, Gershon, & Schuessler, 1997). Herein, 5-Fu, DXR, and VBL were dissolved in 10 mM PBS (pH 6.0), respectively. In each experiment, the sample solution was introduced into the 10 mM PBS (pH 6.0) carrier solution via a 50 μL sample loop on a six-port rotary valve, and then was delivered into the sample cell at a constant flow rate of 1.0 mL/h by a syringe pump (KD Scientific Inc, KDS100).

2.3. Preparation of SPR sensor surface

BK7 glass cover slides (Fisher Scientific, Tustin, CA) were heated in a piranha solution (30% H_2O_2 and 70% concentrated H_2SO_4) at 80 °C for 30 min. (Note: This is strongly oxidizing solution and should be handed with extreme caution.) After cooling, the slides were thoroughly rinsed with water and stored in water. Before preparation of gold substrate, the slide was dried under a N_2 stream. The gold substrate was obtained by evaporating 50-nm-thick Au film on the slide which had been previously coated with a 2-nm Cr thin film using a sputtering coater (Kert J. Lester Inc., Model 108).

For each experiment, a self-assembled monolayer was affixed to the pretreated Au film through an overnight immersion at 4 °C in 1 mM 1-OT ethanol solution. This step was followed by rinsing with copious amounts of ethanol and water, and then dried by nitrogen. Afterward, 20 μL 100 μg/mL PGM was dropped onto the modified film and allowed to react for 1 h. The film was then washed with 10 mM Tris–HCl (pH 3.0) and water, later dried by nitrogen, 20 μL 100 μg/mL G-chitosan or G-chitosan-CM-dextrin was dropped onto the film and incubated for 30 min. Finally, the interface (G-chitosan or G-chitosan-CM-dextrin/PGM/1-OT/Au) thus formed was rinsed thoroughly with 10 mM PBS (pH 7.0) and water, dried with N_2, and then installed in flow cell for SPR experiment.

3. Results and discussion

3.1. Characterization of CM-dextrins and G-chitosan-CM-dextrin

The components of CM-dextrins were measured by MALDI-TOF MS (Fig. S2). According to the mass to charge ratios (m/z), there are seven components: from β-CD-CH_2COOH to β-CD-$(CH_2COOH)_7$. HPLC/ESI-MS was used to obtain the relative contents of these CM-dextrins components (Fig. S3). It can be seen that there are five main peaks (Fig. S3A), and the relative contents from β-CD-$(CH_2COOH)_3$ to β-CD-$(CH_2COOH)_7$ were calculated from Fig. S3B, they are 2.468%, 16.481%, 33.513%, 28.902%, and 18.266%, respectively. Among them, β-CD-$(CH_2COOH)_4$ to β-CD-$(CH_2COOH)_7$ are the main components. Except one carboxylic acid group of

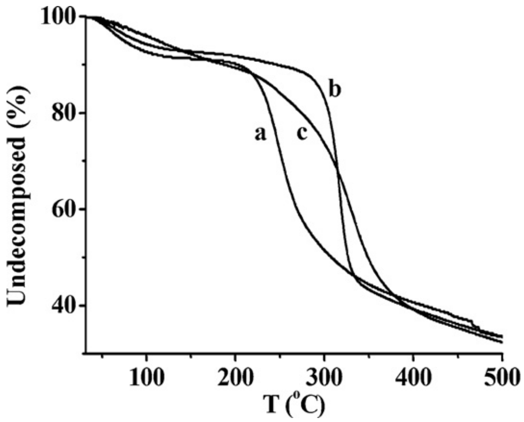

Fig. 1. TGA curves of (a) G-chitosan, (b) CM-dextrins, and (c) G-chitosan-CM-dextrins.

CM-dextrins reacting with the amino of G-chitosan, other carboxylic acid groups are free around the cavity of CM-dextrin.

TGA was used to prove the synthesis of G-chitosan-CM-dextrins. The results of G-chitosan, CM-dextrins, and G-chitosan-CM-dextrins were shown in Fig. 1. As can be seen, the initial decomposition temperature of G-chitosan-CM-dextrin is higher than that of G-chitosan but lower than that of CM-dextrins, suggesting CM-dextrins have been covalently linked onto G-chitosan. When the temperature exceeds 320 °C, the decomposition temperature of G-chitosan-CM-dextrin is higher than both of G-chitosan and CM-dextrins, indicating better thermal stability of the covalently linked polymer than that of the two matrixes at high temperature (Sharma & Mishra, 2010).

Further, the degree of substitution (Q) of CM-dextrins covalently linked onto G-chitosan was obtained by colorimetric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) (Fig. S4). And the calculated Q is about 223.05 μmol/g. That is to say, there is about one CM-dextrins covalently linked on average fifteen monosaccharide units of G-chitosan.

3.2. The principle of the SPR experiment

In order to evaluate the targeted delivery ability of G-chitosan-CM-dextrin for hydrophobic anticancer drugs, a label-free and simple SPR sensor was designed. Fig. 2 depicts the principle of this work: Firstly, a monolayer of 1-OT was immobilized on the Au film by self-assembly. As we know, mucins are aberrantly over expressed in various malignant tumors, and play unique roles in the pathogenesis of cancer (Bafna, Kaur, & Batra, 2010). G-chitosan has mucoadhesive property, which can interact with mucins, thereby leading to the targeted delivery for anticancer drugs to some extent. Here, PGM was chosen to be used as the model mucin and was affixed to the 1-OT modified Au film by hydrophobic interaction, because PGM (pI ≈ 3) exposed its hydrophobic segments in pH 3.0 environment (Chayed & Winnik, 2007). Subsequently, some negatively charged hydrophilic chains of PGM existed on the surface at pH 7.0, so the positively charged G-chitosan-CM-dextrin could immobilize onto the PGM modified film by electrostatic interaction, meaning its targeting delivery to some extent. Finally, when anticancer drugs were introduced into the flow cell, they will interact with the covalently linked CM-dextrins, resulting in the refractive index change. The refractive index change would cause the SPR dip shift, which can be recorded in real time.

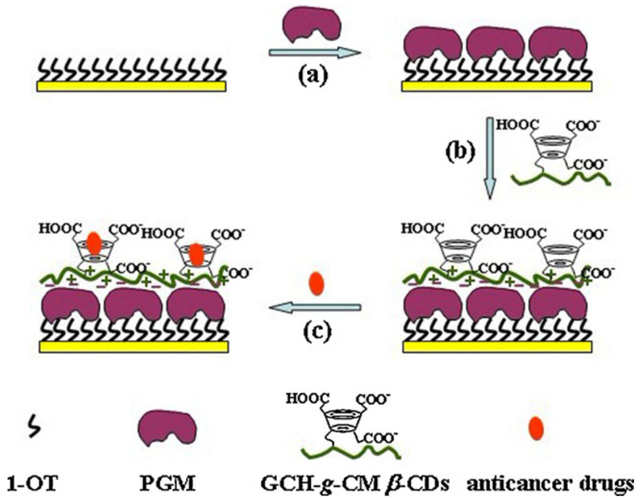

Fig. 2. Schematic illustration of the principle of SPR detection. For clarity, all of the molecules were not drawn to scale. The Au film is depicted as a rectangle.

3.3. Loading ability of G-chitosan-CM-dextrins

Fig. 3 shows the SPR dip shift curves acquired by respectively injecting the three anticancer drugs to the modified films. As can be seen from curve a to curve c, when anticancer drugs were introduced into the flow cells housing G-chitosan-CM-dextrins/PGM/1-OT/Au substrates, SPR signal changes of 0.0012° (curve a, DXR), 0.0022° (curve b, 5-Fu), and 0.0043° (curve c, VBL) could be observed. It is obvious that the SPR signal is different for the three drugs, for example, though the concentration of DXR is the highest, the SPR signal is the lowest. The different SPR signals may be due to the different sizes and structures of the three drugs, which result in the different binding forces and binding amount, and then the refractive index changes are different. There is just part of DXR enters into the cavity of CM-dextrins (Oda, Yanagisawa, Maruyama, Hattori, & Yamanoi, 2008), which may be causing the small refractive index change on the surface of Au film. The signals keep stable when the samples flow out of the flow cells, suggesting the binding interactions between the carrier and these anticancer drugs are stable and strong. Moreover, as shown in Fig. 3, there is no SPR signal change (curves d, e, and f) during injecting three drugs to the G-chitosan/PGM/1-OT/Au films. The results could be attributed to the hydrophilic property of G-chitosan, so it is not able to load hydrophobic therapeutic agents, and the introduction of CM-dextrins just makes up this limitation. That is to say, G-chitosan-CM-dextrins have the ability for loading the three anticancer drugs.

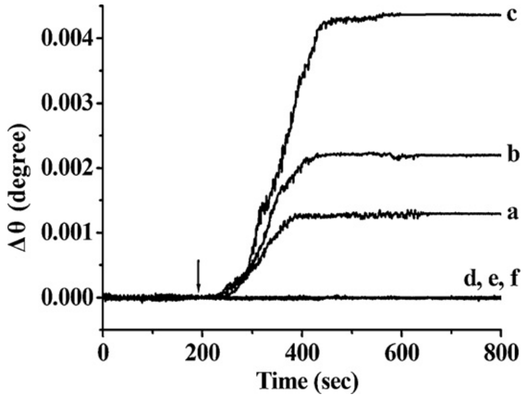

Fig. 3. SPR responses of injecting (a) 10 nM DXR, (b) 0.1 nM 5-Fu, and (c) 0.1 nM VBL for G-chitosan-CM-dextrins/PGM/1-OT/Au films; (d) 10 nM DXR, (e) 0.1 nM 5-Fu, and (f) 0.1 nM VBL for G-chitosan/PGM/1-OT/Au films. PBS (pH 6.0) was used as the carrier solution. Each curve was repeated for at least three times. The arrow indicated the time when the injections were performed.

On the other hand, the SPR signal changes result from the drugs' injection are also proved the successful design of the SPR detection principle. That was to say PGM adsorbed onto the 1-OT/Au film by hydrophobic interaction. Then, G-chitosan-CM dextrin was also immobilized by the electrostatic interaction between NH_3^+ of G-chitosan and either COO^-or SO_3^{2-}on the carbohydrate side chains of PGM. This result demonstrates the mucoadhesive property of G-chitosan-CM dextrin, which means G-chitosan-CM-dextrins could deliver the anticancer drugs to the target-site by recognizing mucins and increase their residence time at the absorbing location (Chayed & Winnik, 2007). Then the immobilized G-chitosan-CM dextrins could recognize the anticancer drugs and resulted in the SPR signal changes.

Taken together, it is proved that the binding interactions between the synthesized G-chitosan-CM-dextrins and anticancer drugs could be evaluated by SPR in real time and in situ, and as compared with the conventional methods such as the phase-solubility study and the continuous variation method (Kobayashi et al., 2011), SPR has advantages on the small amounts usage of the carrier and drugs and the short time for measurements. The results further demonstrate that the loading ability of G-chitosan-CM-dextrin is from the solubilizing ability of the covalently linked CM-dextrins and G-chitosan-CM-dextrins could be used to load different anticancer drugs.

In order to further clarify the formation of inclusion complexes, the drug/G-chitosan-CM-dextrins complexes were characterized by UV–vis spectroscopy. As shown in Fig. 4, at the beginning, the absorbance of each anticancer drug increased upon addition of G-chitosan-CM-dextrins, which indicates the covalently linked CM-dextrins have formed inclusion complexes with each drug (Stancanelli et al., 2008). Besides, as for 5-Fu (Fig. 4A), when the molar ratio between 5-Fu and CM-dextrin was 2:1, the absorbance approached the maximum. Added G-chitosan-CM-dextrins continually, the absorbance decreased, which indicates that the inclusion complex is most stable at the ratio of 2:1 for 5-Fu/CM-dextrin, thus 2:1 is the matching molar ratio. Similarly, as for DXR (Fig. 4B) and VBL (Fig. 4C), the matching molar ratio is both 1:1. Then as can be seen that the inclusion ratios for the three drugs are different, indicating further that the different binding way and amount for the three drugs. The reason for those results may be also due to the different structures and sizes of the three drugs and is consistent with the SPR results.

The above results further proved that CM-dextrins could solubilize hydrophobic anticancer drugs and form stable drug/CM-dextrins complexes. Therefore, based on the combined results of SPR and UV–vis experiments, it can be summarized that G-chitosan-CM-dextrins can encapsulate hydrophobic anticancer drugs into the cavities of covalently linked CM-dextrins, thereby possessing very good loading ability for anticancer drugs.

3.4. *The effect of free carboxymethyl groups*

As point out above, β-CD-$(CH_2COOH)_4$ to β-CD-$(CH_2COOH)_7$ are the main components for CM-dextrin, that is to say, with one carboxylic acid group of CM-dextrin reacting with the amino of G-chitosan, other carboxylic acid groups are free around the cavity of CM-dextrin. Then, in order to know the roles of these free carboxymethyl groups, the interaction between drugs and CM-dextrin and β-CD were studied by UV–vis. As shown in Fig. 5A, 5-Fu has a maximum absorption peak at 265 nm (curve a). After adding CM-dextrin and β-CD, the absorbance increased, which further demonstrates their binding ability. The absorbance of 5-Fu/CM-dextrin complex is higher than that of 5-Fu/β-CD, indicating that the binding interactions between CM-dextrin and 5-Fu are stronger than that of β-CD and 5-Fu. This is because except hydrophobic interaction, there is strong electrostatic interaction between CM-dextrin and 5-Fu, which is attributed that 5-Fu is positive charge ($pK_{a1} \approx 8.0$, $pK_{a2} \approx 13.0$) and the free carboxymethyl groups ($pK_a \approx 4.7$) (Badruddoza, Tay, Tan, Hidajat, & Uddin, 2011) around the cavity of β-CD are negative charge at pH 6.0. However, there is only hydrophobic interaction between β-CD and 5-Fu. And the results of the other two drugs are similar to that of 5-Fu, where the binding interactions between CM-dextrin and drugs are stronger than that of β-CD and drugs. For DXR ($pK_a \approx 8.2$) is also positive charged in the solution of pH 5.0, so there is also electrostatic interaction between CM-dextrin and DXR. Though the pKa of VBL ($pK_{a1} \approx 5.0$, $pK_{a2} \approx 7.4$) is some lower than that of 5-Fu and DXR, there is likely positive charged part in the molecular, so the electrostatic interaction can also play the role. Therefore, the existence of free carboxymethyl groups around the cavity of β-CD is beneficial for the binding interactions between CM-dextrins and drugs, thereby improving the loading ability of the carrier for anticancer drugs.

From the results, the binding process between the anticancer drugs and G-chitosan-CM-dextrins could be explained that the three drugs are positively charged and most carboxylic acid groups dissociate into carboxylates in pH 6.0 solutions, so there are strong electrostatic interaction between the drugs and CM-dextrin, which could attract more drugs near to the cavities of the covalently linked CM-dextrins. Then the drugs enter more easily into the CM-dextrin cavities by the hydrophobic interaction between them. Therefore, both the electrostatic and hydrophobic interactions between CM-dextrins and the drugs could enhance the loading ability of G-chitosan-CM-dextrins carrier, and result in the formation of the stable drugs/CM-dextrins inclusion complexes.

3.5. *The release behaviors of anticancer drugs from G-chitosan-CM-dextrins carrier*

The drug release investigation is of great importance in applying carriers to the practical drug delivery (Gu, Su, Li, He, & Shi, 2011). Since the above results have demonstrated the loading ability of G-chitosan-CM-dextrins carrier to anticancer drugs, the release ability of anticancer drugs from the carrier was also evaluated by SPR. As is known, the tumor extracellular environment is more acidic than the blood and normal tissues, and the intracellular pH values, such as endosome and lysosome, are even lower at about pH 5.0. If the carrier could release anticancer drugs in response to the acidic pH condition of tumor cells, it will promote drug accumulation at the tumor site and facilitate the cell drug release, thereby greatly enhancing the drug delivery efficiency (Ganta, Devalapally, Shahiwala, & Amiji, 2008). Thus, the drug release experiment of G-chitosan-CM-dextrins was performed in pH 5.0 environment.

To clearly observe the release process, the drug was firstly loaded by G-chitosan-CM-dextrins, then PBS (pH 5.0) was injected. Both the load and the release processes were recorded by SPR in real time. The three different types of drug release profiles can be clearly seen from their SPR signal changes as shown in Fig. 6. For 5-Fu, as displayed in Fig. 6A, there is an abrupt increase of SPR signal when PBS (pH 5.0) was injected, which is due to the solution refractive index change. Subsequently, SPR signal decreased slowly, and at last the signal did not return to the second baseline with a net SPR signal increase of 0.0013° (curve b). That is to say, instead of leading to the release of 5-Fu from the cavities of covalently linked CM-dextrins, the injection of PBS (pH 5.0) makes the interactions between 5-Fu and CM-dextrins become stronger, which is due to that there are more positive charges on 5-Fu molecule at pH 5.0 in comparison with that at pH 6.0, the conformational and electronic changes of the two 5-Fu molecules might tend to form well-matched interactions in the cavity of CM-dextrin, and the conformational and electronic changes would result in the increase

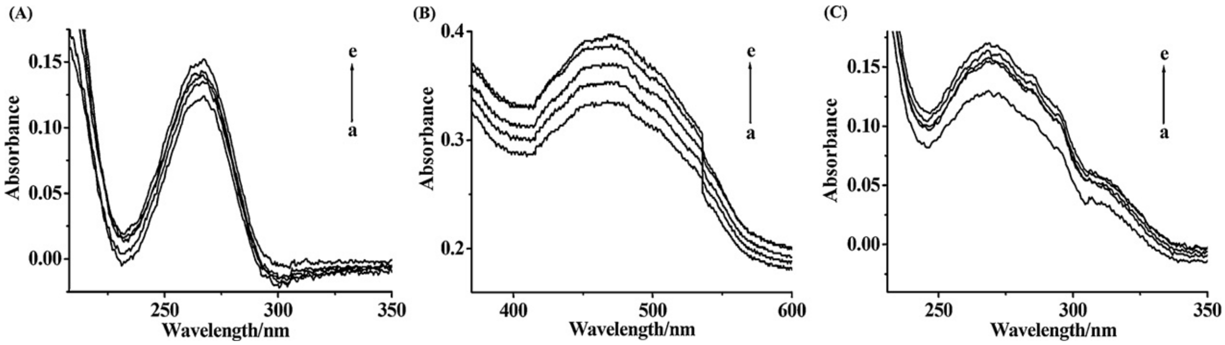

Fig. 4. UV–vis absorption spectra of (A) 5-Fu, the molar ratios between 5-Fu and CM-dextrin are (a) 1:0, (b) 2.5:1, (c) 1:1.25, (d) 1:1, and (e) 2:1; (B) DXR, the molar ratios between DXR and CM-dextrins are (a) 1:0, (b) 2:1, (c) 1:2, (d) 1:1.5, and (e) 1:1; (C) VBL, the molar ratios between VBL and CM-dextrins are (a) 1:0, (b) 2:1, (c) 1.5:1, (d) 1:1.5, and (e) 1:1. All experiments were carried out in PBS (pH 6.0).

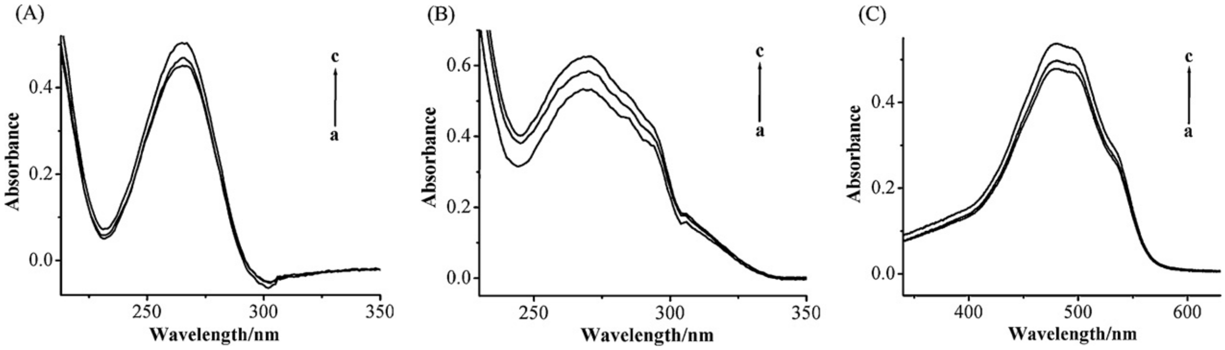

Fig. 5. UV–vis absorption spectra of (A): (a) 5-Fu, (b) 5-Fu/β-CD 2:1, and (c) 5-Fu/CM-dextrin 2:1; (B): (a) VBL, (b) VBL/β-CD 1:1, and (c) VBL/CM-dextrin 1:1; (C): (a) DXR, (b) DXR/β-CD 1:1, and (c) DXR/CM-dextrin 1:1. All experiments were carried out in PBS (pH 6.0).

of SPR signal (Boussaad, Pean, & Tao, 2000). As for VBL (Fig. 6B), it is noted that there is also an abrupt SPR signal increase after the injection of PBS (pH 5.0), but then the signal slowly returns to the second baseline (curve b). These phenomena demonstrate that PBS (pH 5.0) also does not work on the release of VBL. This result can be attributed to the high binding force between VBL and CM-dextrins, so the SPR signal changes after injecting PBS (pH 5.0) is just due to the solution refractive index change from pH 6.0 to pH 5.0. Since both 5-Fu and VBL could not be released from the cavities of covalently linked CM-dextrins via regulating pH value, their release could be achieved through the slow biodegradation of the G-chitosan-CM-dextrins carrier.

However, the release profiles are totally different for DXR. As can be observed from Fig. 6C, after PBS (pH 5.0) was injected, there is an abrupt increase of SPR signal, then a slow decrease, and at last the signal almost returns to the original baseline (curve a). The SPR signal increase is caused by the solution refractive index change from pH 6.0 to pH 5.0 (Yao et al., 2006), and the signal decrease is due to the release of DXR from the cavities of covalently linked CM-dextrins into the carrier solution. As mentioned above, except one carboxylic acid group reacts with the amino of G-chitosan, there are several free carboxylic acid groups around the cavity of β-CD. In pH 6.0 solutions, most free carboxylic acid groups dissociate into carboxylates while DXR are positive charge, thus, there are strong electrostatic interactions between DXR and CM-dextrins, which are favorable for the loading of the carrier as analyzed above. However, pH 5.0 is close to the pK_a of carboxylic acid groups, so carboxylic acid groups are the main existence form. That is to say,

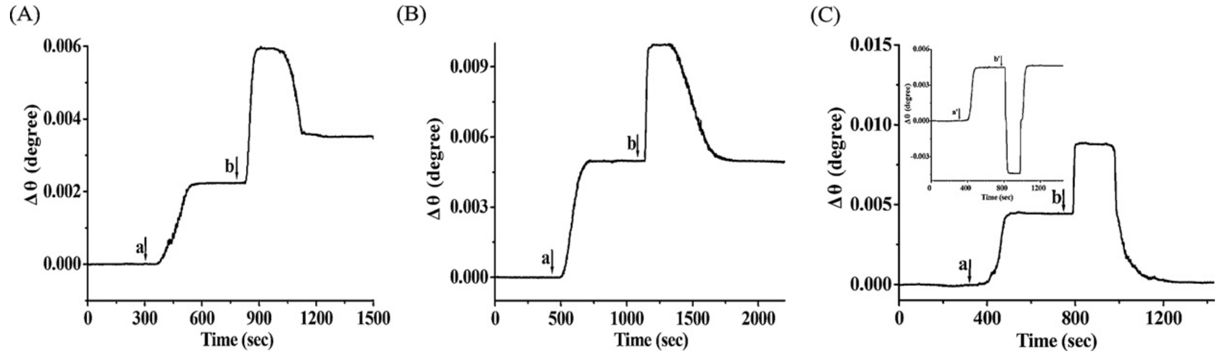

Fig. 6. SPR responses of G-chitosan-CM-dextrins/PGM/1-OT/Au films by consecutive injecting (a) different drugs (A): 0.1 nM 5-Fu, (B): 0.1 nM VBL, (C): 1 μM DXR, and (b) PBS (pH 5.0). The inset of C: SPR responses of G-chitosan-CM-dextrins/PGM/1-OT/Au films on injecting (a′) 1 μM DXR and (b′) PBS (pH 7.0). PBS (pH 6.0) was used as the carrier solution. Each curve was repeated at least three times. The arrows indicated the time when the injections were performed.

there is little electrostatic interaction between CM-dextrins and DXR, resulting in the release of DXR. Further, the drug release was also investigated at pH 7.0 (inset in Fig. 6C). After a drastic dip shift arises due to the solution refractive index change, the SPR signal maintains the level of loading DXR (curve b′), indicating that there is no DXR release at pH 7.0. Based on the above results, it can be summarized that the G-chitosan-CM dextrin carrier could release DXR in response to the acidic (pH 5.0) environment, not to neutral (pH 7.0) environment. Then, the newly synthesized pH-sensitive carrier could release DXR in tumor cells but not in the normal cells, which make the carrier appropriate for selective anticancer drug delivery. It can prevent the premature release during the transporting process in the physiological environment, thereby ensuring a sufficient drug release to effectively kill tumor cells (Chen et al., 2011).

On the basis of the combined results, it is concluded that the synthesized G-chitosan-CM-dextrins could load several anticancer drugs, but the release way of the anticancer drugs is different because of the different structures and properties of these drugs. For 5-Fu and VBL, it is possible that the binding interactions with CM-dextrins are strong, so they could not be released in response to pH changes. Maybe their release could be achieved through the slow biodegradation of the G-chitosan-CM-dextrins carrier. As for DXR, the electrostatic and hydrophobic interactions are appropriate, thus the carrier possesses pH-controlled release ability. It is worth mentioning that the pH-sensitive carrier is attractive for delivering anticancer drug, and has promising potential for application in tumor therapy. Moreover, the existence of free carboxymethyl groups around the cavity of β-CD makes the carrier have better loading ability for anticancer drugs. It can be speculate that if the amount of carboxymethyl groups around the cavity of β-CD can be adjusted, the interaction between the drugs and G-chitosan-CM-dextrin can also be regulated. Then there may be more anticancer drugs which can be released by pH controlled using the new obtained drug carrier.

4. Conclusions

This work has evaluated the capability of the newly synthesized G-chitosan-CM-dextrins as a carrier vehicle for hydrophobic anticancer drugs using UV–vis spectroscopy and a straightforward and efficient SPR sensor. Experiment results showed that all the three hydrophobic anticancer agents (5-Fu, DXR, and VBL) could be successfully loaded into the cavities of the covalently linked CM-dextrins. The free carboxymethyl groups could enhance the binding interactions between the covalently linked CM-dextrins and anticancer drugs. Due to the different properties of the anticancer drugs, the G-chitosan-CM dextrin carrier could release them via different ways, for DXR, the carrier could release them in weak acidic medium (pH 5.0) but not at pH 7.0, which is benefit for the anticancer drug delivery. The obtained G-chitosan-CM-dextrins carrier has both mucoadhesive property of G-chitosan and hydrophobic cavities of β-CDs, thus it is able to targeting deliver three different hydrophobic anticancer agents. Moreover, the existence of free carboxymethyl groups around the cavity of β-CD makes the carrier more possibilities to regulate the load and release for different anticancer drugs. Therefore, the G-chitosan-CM dextrin carrier has great potential for cancer therapy.

Supplementary information

Chemical structure of three drugs, MALDI-TOF MS spectra of CM-dextrins, total ion current chromatogram and the selected ion chromatograms of seven CM-dextrins components, the principle of colorimetric method.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.12.016.

References

Amidi, M., Mastrobattista, E., Jiskoot, W., & Hennink, W. E. (2010). Chitosan-based delivery systems for protein therapeutics and antigens. *Advanced Drug Delivery Reviews*, *62*(1), 59–82.

Badruddoza, A. Z. M., Tay, A. S. H., Tan, P. Y., Hidajat, K., & Uddin, M. S. (2011). Carboxymethyl-beta-cyclodextrin conjugated magnetic nanoparticles as nano-adsorbents for removal of copper ions: Synthesis and adsorption studies. *Journal of Hazardous Materials*, *185*(2–3), 1177–1186.

Bafna, S., Kaur, S., & Batra, S. K. (2010). Membrane-bound mucins: The mechanistic basis for alterations in the growth and survival of cancer cells. *Oncogene*, *29*(20), 2893–2904.

Boussaad, S., Pean, J., & Tao, N. J. (2000). High-resolution multiwavelength surface plasmon resonance spectroscopy for probing conformational and electronic changes in redox proteins. *Analytical Chemistry*, *72*(1), 222–226.

Chayed, S., & Winnik, F. M. (2007). In vitro evaluation of the mucoadhesive properties of polysaccharide-based nanoparticulate oral drug delivery systems. *European Journal of Pharmaceutics and Biopharmaceutics*, *65*(3), 363–370.

Chen, D. Y., Xia, X. W., Gu, H. W., Xu, Q. F., Ge, J. F., Li, Y. G., et al. (2011). pH-responsive polymeric carrier encapsulated magnetic nanoparticles for cancer targeted imaging and delivery. *Journal of Materials Chemistry*, *21*(34), 12682–12690.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*(3), 350–356.

Fung, S. Y., Yang, H., Bhola, P. T., Sadatmousavi, P., Muzar, E., Liu, M. Y., et al. (2009). Self-assembling peptide as a potential carrier for hydrophobic anticancer drug ellipticine: Complexation, release and in vitro delivery. *Advanced Functional Materials*, *19*(1), 74–83.

Furusaki, E., Ueno, Y., Sakairi, N., Nishi, N., & Tokura, S. (1996). Facile preparation and inclusion ability of a chitosan derivative bearing carboxymethyl-beta-cyclodextrin. *Carbohydrate Polymer*, *29*(1), 29–34.

Ganta, S., Devalapally, H., Shahiwala, A., & Amiji, M. (2008). A review of stimuli-responsive nanocarriers for drug and gene delivery. *Journal of Controlled Release*, *126*(3), 187–204.

Gu, J. L., Su, S. S., Li, Y. S., He, Q. J., & Shi, J. L. (2011). Hydrophilic mesoporous carbon nanoparticles as carriers for sustained release of hydrophobic anti-cancer drugs. *Chemical Communications*, *47*(7), 2101–2103.

Han, H. K., Shin, H. J., & Ha, D. H. (2012). Improved oral bioavailability of alendronate via the mucoadhesive liposomal delivery system. *European Journal of Pharmaceutical Sciences*, *46*(5), 500–507.

Hua, D. B., Jiang, J. L., Kuang, L. J., Jiang, J., Zheng, W., & Liang, H. J. (2011). Smart chitosan-based stimuli-responsive nanocarriers for the controlled delivery of hydrophobic pharmaceuticals. *Macromolecules*, *44*(6), 1298–1302.

Kobayashi, H., Endo, T., Ogawa, N., Nagase, H., Iwata, M., & Ueda, H. (2011). Evaluation of the interaction between beta-cyclodextrin and psychotropic drugs by surface plasmon resonance assay with a Biacore (R) system. *Journal of Pharmaceutical and Biomedical Analysis*, *54*(1), 258–263.

Kolomenskii, A. A., Gershon, P. D., & Schuessler, H. A. (1997). Sensitivity and detection limit of concentration and adsorption measurements by laser-induced surface-plasmon resonance. *Applied Optics*, *36*(25), 6539–6547.

Krauland, A. H., & Alonso, M. J. (2007). Chitosan/cyclodextrin nanoparticles as macromolecular drug delivery system. *International Journal of Pharmaceutics*, *340*(1–2), 134–142.

Kumar, M., Muzzarelli, R. A. A., Muzzarelli, C., Sashiwa, H., & Domb, A. J. (2004). Chitosan chemistry and pharmaceutical perspectives. *Chemical Reviews*, *104*(12), 6017–6084.

Luan, Q. F., Zhou, K. B., Tan, H. N., Yang, D., & Yao, X. (2011). Au-NPs enhanced SPR biosensor based on hairpin DNA without the effect of nonspecific adsorption. *Biosensors and Bioelectronics*, *26*(5), 2473–2477.

Mao, H. Q., Roy, K., Troung-Le, V. L., Janes, K. A., Lin, K. Y., Wang, Y., et al. (2001). Chitosan-DNA nanoparticles as gene carriers: Synthesis, characterization and transfection efficiency. *Journal of Controlled Release*, *70*(3), 399–421.

Muzzarelli, R. A. A. (2010). Chitins and chitosans as immunoadjuvants and non-allergenic drug carriers. *Marine Drugs*, *8*(2), 292–312.

Oda, Y., Yanagisawa, H., Maruyama, M., Hattori, K., & Yamanoi, T. (2008). Design, synthesis and evaluation of D-galactose-beta-cyclodextrin conjugates as drug-carrying molecules. *Bioorganic & Medicinal Chemistry*, *16*(19), 8830–8840.

Prabaharan, M., & Mano, J. F. (2005). Hydroxypropyl chitosan bearing beta-cyclodextrin cavities: Synthesis and slow release of its inclusion complex with a model hydrophobic drug. *Macromolecular Bioscience*, *5*(10), 965–973.

Sharma, A. K., & Mishra, A. K. (2010). Microwave induced beta-cyclodextrin modification of chitosan for lead sorption. *International Journal of Biological Macromolecules*, *47*(3), 410–419.

Stancanelli, R., Ficarra, R., Cannava, C., Guardo, M., Calabro, M. L., Ficarra, P., et al. (2008). UV–vis and FTIR-ATR characterization of 9-fluorenon-2-carboxyester/(2-hydroxypropyl)-beta-cyclodextrin inclusion complex. *Journal of Pharmaceutical and Biomedical Analysis*, *47*(4–5), 704–709.

Subbiah, R., Ramalingam, P., Ramasundaram, S., Kim, D. Y., Park, K., Ramasamy, M. K., et al. (2012). N,N,N-Trimethyl chitosan nanoparticles for controlled intranasal delivery of HBV surface antigen. *Carbohydrate Polymers*, *89*(4), 1289–1297.

Trapani, A., Lopedota, A., Franco, M., Cioffi, N., Ieva, E., Garcia-Fuentes, M., et al. (2010). A comparative study of chitosan and chitosan/cyclodextrin nanoparticles as potential carriers for the oral delivery of small peptides. *European Journal of Pharmaceutics and Biopharmaceutics*, *75*(1), 26–32.

Uekama, K., Hirayama, F., & Irie, T. (1998). Cyclodextrin drug carrier systems. *Chemical Reviews*, *98*(5), 2045–2076.

Van der Lubben, I. M., Verhoef, J. C., Borchard, G., & Junginger, H. E. (2001). Chitosan for mucosal vaccination. *Advanced Drug Delivery Reviews*, *52*(2), 139–144.

Wei, W., Yue, Z. G., Qu, J. B., Yue, H., Su, Z. G., & Ma, G. H. (2010). Galactosylated nanocrystallites of insoluble anticancer drug for liver-targeting therapy: An in vitro evaluation. *Nanomedicine*, *5*(4), 589–596.

Wu, S. Z., Zeng, F., Zhu, H. P., & Tong, Z. (2005). Energy and electron transfers in photosensitive chitosan. *Journal of the American Chemical Society*, *127*(7), 2048–2049.

Yang, X. Q., Hong, H., Grailer, J. J., Rowland, I. J., Javadi, A., Hurley, S. A., et al. (2011). cRGD-functionalized, DOX-conjugated, and (64)Cu-labeled superparamagnetic iron oxide nanoparticles for targeted anticancer drug delivery and PET/MR imaging. *Biomaterials*, *32*(17), 4151–4160.

Yao, X., Li, X., Toledo, F., Zurita-Lopez, C., Gutova, M., Momand, J., et al. (2006). Sub-attomole oligonucleotide and p53 cDNA determinations via a high-resolution surface plasmon resonance combined with oligonucleotide-capped gold nanoparticle signal amplification. *Analytical Biochemistry*, *354*(2), 220–228.

Yue, Z. G., Wei, W., You, Z. X., Yang, Q. Z., Yue, H., Su, Z. G., et al. (2011). Iron oxide nanotubes for magnetically guided delivery and pH-activated release of insoluble anticancer drugs. *Advanced Functional Materials*, *21*(18), 3446–3453.

Zambito, Y., Pedreschi, E., & Di Colo, G. (2012). Is dialysis a reliable method for studying drug release from nanoparticulate systems?—A case study. *International Journal of Pharmaceutics*, *434*(1–2), 28–34.

Zhou, H. F., Yu, W. T., Guo, X., Liu, X. D., Li, N., Zhang, Y., et al. (2010). Synthesis and characterization of amphiphilic glycidol-chitosan-deoxycholic acid nanoparticles as a drug carrier for doxorubicin. *Biomacromolecules*, *11*(12), 3480–3486.