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Preparation and characterization of galactosylated chitosan coated BSA microspheres containing 5-fluorouracil

Can Zhang [a], Yao Cheng [a], Guowei Qu [a], Xiaoli Wu [a], Ya Ding [a], Zhihong Cheng [b], Liangli Yu [b], Qineng Ping [a,*]

[a] College of Pharmacy, China Pharmaceutical University, Nanjing 210009, China
[b] Department of Nutrition and Food Science, University of Maryland, College Park, MD 20742, USA

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Abstract

In this paper, new sustained release microspheres with a surface coating of targeting moieties coating are presented. Bovine serum albumin (BSA) was used to prepare the microspheres loading with 5-fluorouracil (5-FU) by means of chemical crosslinking method and then the microspheres were coated with N-galactosylated chitosan by electrostatic interaction. The structure of coating layers on the surface of 5-FU-loaded BSA microspheres was characterized by attenuated total reflection Fourier (ATR-FTIR), electron spectroscopy for chemical analysis (ESCA), wide X-ray diffraction (WXRD) and transmission electron microscopy (TEM). The properties of the coated microspheres containing 5-FU were determined. The size was in the range of 0.60–0.65 μm, zeta potential was 16.6 mv, and the encapsulation efficiency, drug loading, and the content of galactosyl groups were 40.3% (w/w), 2.9% (w/w), and 5.4% (w/w), respectively. In comparison with uncoated microspheres, the coated microspheres showed delayed release and less burst release in vitro. All results suggested the BSA microspheres preparation with galactosyl chitosan coating could be a promising method for targeted delivery to the liver.

Keywords: Galactosylated chitosan; 5-Fluorouracil; Bovine serum albumin; Coating; Microspheres; Targeting delivery

1. Introduction

Targeted delivery of therapeutic agents has been well recognized for its potential advantages in enhancing drug therapeutic efficacy and reducing side effects. There are growing interests in developing delivery systems for drug targeting to liver cells because of the lack of other effective and practical pharmacological approaches. A number of previous studies have been performed to investigate possible carriers for selectively delivering drugs to the liver (Bhadra, Yadav, Bhadra S., & Jain, 2005; Liang et al., 2006; Managit, Kawakami, Nishikawa, Yamashita, & Hashida, 2003; Wang et al., 2006). These approaches include but were not limited to passive trapping of microparticles by reticuloendothelium (Ogawara et al., 1999) and active targeting based on hepatic receptor recognition (Akamatsu et al., 1998). It is well accepted that particle size is critical for passive targeting (Moghimi, Porter, Muir, Illum, & Davis, 1991), whereas active targeting systems require the need for the receptor to trap specific ligands. Liposomes (Kim & Han, 1995) and microspheres (Kim, Hwang, & Lee, 1993) are two examples of passive delivery systems for liver chemotherapeutic agents. Asialoglycoprotein receptors (ASGR) including the galactose receptor are a group of well known surface receptors present only in hepatocytes and several human hepatoma cell lines (Ashwell & Harford, 1982; Fallon & Schwartz, 1988). A ligand containing galactose moiety can be recognized by and bound to the liver-specific galactose receptor. The ligand-receptor complex is uptaken rapidly into the cells, and

* Corresponding author. Tel./fax: +86 25 83271098.
E-mail address: pingqn@cpu.edu.cn (Q. Ping).

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the receptor recycles back to the cellular surface (Ciechanover, Schwartz, & Lodish, 1983). In other words, the galactose receptor has a high binding capacity and leads to efficient cellular uptake of galactosylated ligands. The galactose receptor mediated endocytosis makes this receptor an ideal target for developing active targeting delivery systems for hepatocyte/liver and hepatoma cells (Goto et al., 1994; Nishikawa et al., 1993).

Chitosan is a naturally occurring polysaccharide with biodegradable and biocompatible characteristics. The material and its derivatives have been widely utilized in the development of drug delivery and biomedical engineering. A number of chitosan or chitosan derivatives' microspheres have been reported (Gupta & Jabrail, 2006; Szczubialka, Zomerska, Karewicz, & Nowakowska, 2006; Zhang, Ping, Ding, Cheng, & Shen, 2004). Because of good film forming properties, chitosan also was used to coat the microsphere composed of poly (lactic acid)–poly (caprolactone) blends. The microsphere showed good potential for the targeted delivery of antiproliferative agents to treat restenosis (Chandy, Wilson, Rao, & Das, 2002). Furthermore, a few of lactosaminated or galactosylated chitosan derivatives were chemically conjugated with drugs. For example, lactosaminated *N*-succinyl-chitosan was utilized in mice as drug-specific to the liver (Kato, Onishi, & Machida, 2001). Galactosylated chitosan-graft-dextran, galactosylated chitosan–graft–poly (ethylene glycol) and galactosylated chitosan-graft-PVP had excellent specificity to liver cells as hepatocyte-targeting DNA carrier (Park et al., 2000, 2001, 2003). In our previous research, the galactosylated chitosan microspheres were prepared and demonstrated for their utilization for active targeted drug delivery to liver (Zhang et al., 2004). However, not all galactose moieties in the galactosylated chitosan were bound to the galactose receptor and the efficacy of active targeting may be reduced because a part of the active moieties may be buried within the microspheres' matrix. This raises a possibility as to whether a coating of galactosylated chitosan on the surface of microspheres may create a new and more efficient liver targeted delivery system with sustained release properties.

Bovine serum albumin (BSA) is a natural biocompatibility, nontoxic, nonantigenic microspheres forming material (Gupta & Hung, 1989). We expect that the BSA microspheres with negative charges may strongly adsorb soluble galactosyl chitosan with positive charges so that the liver-targeting properties on its surface may be significantly changed. 5-Fluorouracil (5-FU), one of the primary drugs used for the liver cancer treatment, is selected as the model drug. The 5-FU loaded BSA micropheres with galactosyl chitosan coating may possess both passive and active targeting properties against liver cells, and potentially improve the safety and efficiency. The present study optimized the preparation procedure of 5-FU BSA micropheres and developed a lab-scale protocol for coating the micropheres by using galactosyl chitosan as coating material. The galactosyl chitosan coating layer was confirmed using ATR, ESCA, WAXD, and TEM techniques, while the release behaviors of the BSA micropheres with and without the galactosyl chitosan coating were compared and determined by HPLC analysis. The stability of the coating BSA microspheres as the lyophilized powder was also studied at 4 °C.

2. Experimental

2.1. Materials

5-FU was obtained from Nantong pharmaceutical company (China). Bovine serum albumin was purchased from Sigma Company (USA). Chitosan was provided by Nantong Suanglin Biochemical Co. Ltd. (China), which has a degree of deacetylation of 91.5% and viscosity average molecular weight of 25 KD. *N, N, N′, N′*-tetramethylethylenediamine (TEMED) and lactobionic acid (LA) were purchased from Acros (USA). Glutaraldehyde (25%) and *N, N′*-Dicyclohexylcarbodiimide (DCC) were obtained from Shanghai Chemical Regent Company (China). All other chemical solvents and reagents were used without further purification.

2.2. Methods

2.2.1. Synthesis of galactosylated chitosan

Galactosylated chitosan was synthesized following the procedure described previously (Zhang et al., 2004). Briefly, chitosan (1 g, 6.2 mM) was dissolved in 120 ml of 2% hydrochloric acid aqueous solution. Then the mixed solution containing 3.3 g (9.2 mM) LA in 5 ml water and 1.5 g (7.3 mM) of DCC in 5 ml of TEMED was dropped into the chitosan solution and stirred for 72 h at ambient temperature. The reaction solution was filtered and dialyzed (Molecular weight cut-off, MWCO 10000) against distilled water for 5 days and then lyophilized to obtain 0.3 g of galactosylated chitosan.

2.2.2. Preparation of 5-FU loaded BSA microsphere

BSA microspheres were prepared by an emulsion crosslinking method. Briefly, a known amount of bovine serum albumin (BSA) and 5-FU were dissolved in boric acid buffered solution (pH 9.0), and the solution was dispersed in 5-FU saturated castor oil with strong stirring to form water-in-oil (w/o) emulsion. After stirring for a selected time period, a certain amount of glutaraldehyde was slowly dropped into the emulsion, and stirred continuously. Ten minutes later, the rest of glutaraldehyde was added in, and the emulsion was stirred for crosslinking solidification of the dispersed particles. Then the whole dispersed system was centrifuged to separate microspheres, which were further washed several times with diethyl ether and then lyophilized, and stored at 4 °C.

5-FU concentration, volume ratio of w/o, the amount of glutaraldehyde, and time for crosslinking reaction were also employed to optimize the microspheres preparation

procedure for maximum entrapment efficiency and desired release properties.

2.2.3. Preparation of microspheres coated galactosylated chitosan

To prepare the galactosylated chitosan coated 5-FU BSA microspheres, lyophilized 5-FU loaded BSA microspheres were dispersed in acetic acid (2%, w/v) solutions containing galactosyl chitosan (2%, w/v). After incubating in thermostatic water bath for 5–10 min, the dispersed system was centrifuged, and the precipitates were washed with water for several times and lyophilized. The coating time, the concentration of galactosed chitosan, and acetic acid concentration were chosen to optimize the coating procedure.

2.2.4. Characterization of microspheres

Attenuated total reflection Fourier (ATR-FTIR) spectra were obtained using a Nicolet 170sx Fourier transform infrared spectrometer. The electron spectroscopy for chemical analysis (ESCA) spectra was obtained using a V.G. ESCALAB MK II spectrometer. Wide X-ray diffraction (WXRD) was performed with a XD-3A powder diffraction meter with CuKα radiation in the range of 5–40° (2θ) at 40 kV and 30 mA.

2.2.5. Morphological properties of microspheres

Ten milligram of microspheres was dispersed in 5 ml of deionized water containing Tween 80 (0.2%, w/v) and sonicated in an ultrasonic bath for 2 min. Transmission electron micrograph (TEM) observation was carried out at 75 kV with H-7000 (Hitachi, Japan). The micellar solution was negatively stained with 0.01% phosphotungstic acid and placed on a copper grid coated with former film.

2.2.6. Analysis of particle size and zeta potential

The particle size and zeta potential of BSA microspheres was determined by Zetasizer 3000HS instrument (Malvern Instruments, Malvern, UK) with 633 nm He-Ne lasers at 25 °C. Prior to size analysis, a small amount of the microspheres (10 mg) was dispersed in 5 ml of deionized water containing Tween 80 (0.2%, w/v) and sonicated in an ultrasonic bath for 2 min.

2.2.7. Drug loading and encapsulation efficiency

Drug loading and encapsulation efficiency were measured by HPLC analysis (Agilent 1100, Agilent, Germany). The mobile phase was potassium dihydrogen phosphate solution (0.013 mol/L), and the pH was adjusted to 4.3 with acetic acid. The column was Diamohsil^{TM} C18 (250 × 4.6 mm) with 10 μm particles. The flow rate was 1.0 ml/min, the detection wavelength was 266 nm (Agilent 8453, UV detector, Agilent, Japan), and the column temperature was maintained at 30 °C. Injected volume of the sample was 20 μl.

Ten milligram of 5-FU BSA lyophilized microspheres was placed in 10 ml of NaOH (1 M) solution and immersed in boiling water bath for 2 h, then cooled to room temperature. Two milliliter of the above solution was adjusted to pH 4.5 with 1 mol/l HCl and diluted with deionized water to a total volume of 10 ml. The drug content of the microspheres was determined with HPLC. Drug loading content and encapsulation efficiency were calculated by Eqs. (1) and (2).

$$\text{Drug loading content}\% = \frac{\text{weight of the drug in microspheres}}{\text{weight of the microspheres}} \times 100\% \quad (1)$$

$$\text{Encapsulation efficiency}\% = \frac{\text{weight of the drug in microspheres}}{\text{weight of the feeding drugs}} \times 100\% \quad (2)$$

Lyophilized powder of chitosan coated 5-FU BSA microspheres (10 mg) was placed in 5 ml of 1 M HCl and immersed in a boiling water bath for 2 h. After adding 5 ml of 2 mol/l NaOH, the resulting microspheres solution was kept in boiling water bath for another 2 h, then cooled to room temperature. Two milliliter of the mixture was transferred into a 10 ml content bottle and adjusted to pH 4.5 with 1 mol/l HCl, deionized water was added to make the volume up to 10 ml. The solution was analyzed by HPLC. The drug content of the microspheres was also determined by HPLC analysis. Drug loading content and encapsulation efficiency were calculated according to Eqs. (1) and (2), respectively.

The galactose moieties content on the microspheres surface was determined using the phenol-sulfuric acid method (Dubios, Gilles, Hamilton, Rebers, & Smith, 1956). Briefly, after coating, the microspheres were centrifuged and washed with 5 ml of water for three times. The supernatant and the elution were mixed and diluted with water to 50 ml (testing solution). One milliliter of 5% phenol solution was added to 2 ml of the testing solution, followed by 5 ml of concentrated sulfuric acid. The tube was kept at ambient temperature for 10 min, and at 30 °C for 15 min. The absorbance was measured at 490 nm and used to calculate galactose content using a standard curve prepared using pure galactose. Blanks were prepared by substituting distilled water for the galactose solution. All experiments were performed in triplicates. The concentration of galactose was obtained by Eq. (3).

$$\text{Galactose content of microspheres}\% = \frac{\text{total amount of feeded galactose} - \text{galactose content in solution}}{\text{weight of microspheres}} \times 100\% \quad (3)$$

2.2.8. In vitro release

In vitro drug release was examined using the dynamic dialysis method. In brief, known amounts of both microspheres were suspended in phosphate buffered saline (pH 7.4, PBS) in a dialysis tube, which was placed in a shaking basket. The basket was immersed in 250 ml of the PBS and kept at 37 °C with continuous agitation. At selected time intervals, a small amount of the release medium was taken and measured for 5-FU concentration, and an equal volume of PBS was added to the dissolution medium to maintain a constant volume. In addition, in vitro drug release behavior was compared with that of 5-FU.

2.2.9. Stability tests

The lyophilized powder (5.3%, w/w drug loading) of the coated BSA microspheres with 5-FU loaded was stored at 4 °C for a period. The entrapment efficiency, content of drug and the size of the microspheres were measured by HPLC analyses using a Zetasizer as described above.

3. Results and discussion

3.1. Preparation of 5-FU loaded BSA microsphere

5-FU loaded BSA microspheres were prepared under the optimal conditions, with the initial drug concentrations of 10 mg/ml and 20 mg/ml, respectively. And their in vitro release behavior was examined by a dynamic dialysis method. Fig. 1 shows the release of 5-FU from BSA microspheres as a function of time in pH 7.4 PBS. It was noted that a lower drug concentration (10 mg/ml) is associated with a slower initial drug release rate ($P < 0.05$). It was found that microspheres prepared with two different drug concentrations (different amounts of drug used in the preparation of microsphere) possess both similar drug release rate values and trends of change in drug release rates after 120 min. So we can conclude that the drug concentration (amount of drug used) in microsphere preparation did not have a great effect on drug release rate value and trend in in vitro release experiment after 120 min. These data suggested that the burst effect of BSA microspheres may be weakened by using a lower initial drug concentration.

To further investigate the effects of crosslinking degree on release behavior of the microspheres, 5-FU loaded BSA microspheres were prepared under optimal conditions, with the glutaraldehyde levels of 1.0, 2.0, and 3.0 ml/100 mg BSA, respectively. The in vitro release behavior of the 5-FU BSA microspheres was examined using the dynamic dialysis method. Fig. 2 demonstrated that glutaraldehyde concentration used in BSA microsphere preparation exhibited no influence on the release rate ($P > 0.05$). Furthermore, 1.0 ml glutaraldehyde per 100 mg BSA was adequate for albumin crosslinking and used in further investigation.

In addition, crosslinking time was evaluated for their effects on release behavior of 5-FU BSA microspheres. The microspheres were prepared with crosslinking times of 0.5, 2.0, 4.0, and 6.0 h under the optimal preparation conditions. The in vitro release behavior of these microspheres was evaluated using dynamic dialysis. The drug release rate was negatively associated with the crosslinking time for up to 4.0 h (Fig. 3). Further increase of the crosslinking time from 4 to 6 h did not significantly alter the release rate. In addition, the 5-FU BSA microspheres prepared with a crosslinking time of 6 h were able to achieve a 100% drug release after 8 h under the testing conditions, and those prepared with less crosslinking time might release all the loaded 5-FU over a shorter time period (Fig. 3). These data suggested a preferred crosslinking time may be crucial for the desired release behavior. Taking

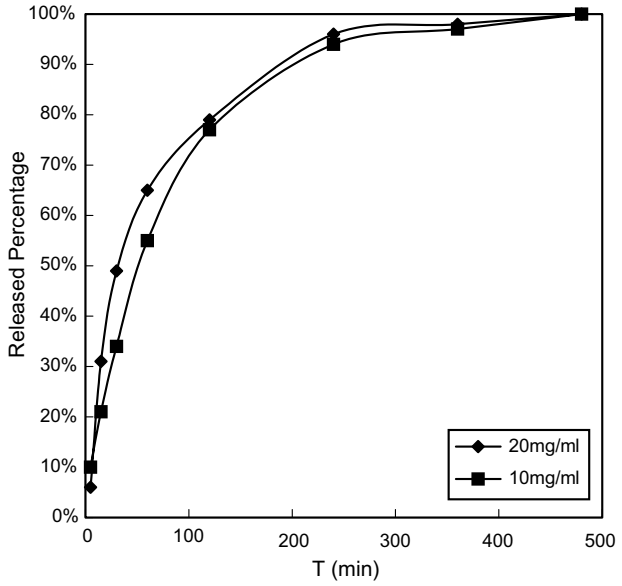

Fig. 1. Effect of 5-FU concentration on the release of 5-FU BSA microspheres in vitro.

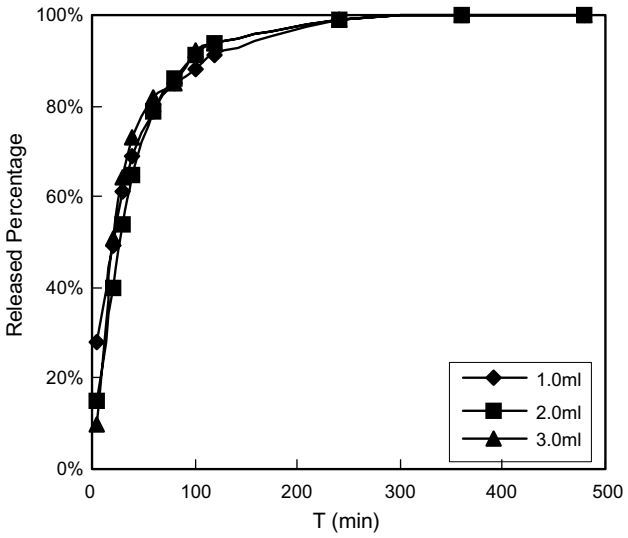

Fig. 2. Effect of glutaraldehyde on 5-FU BSA microspheres in vitro release.

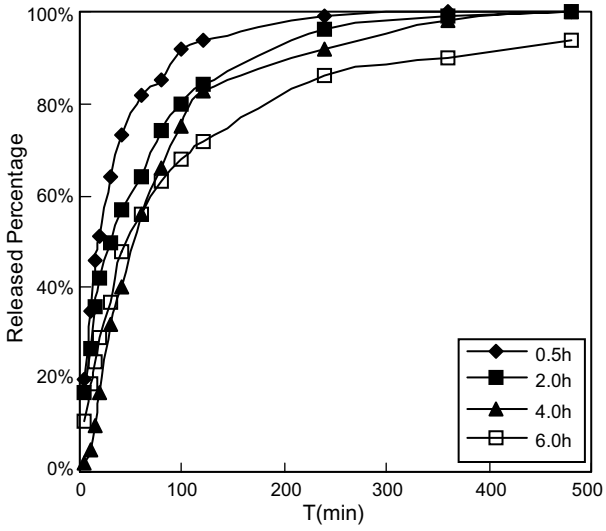

Fig. 3. Effect of crosslink time on 5-FU BSA microspheres in vitro release.

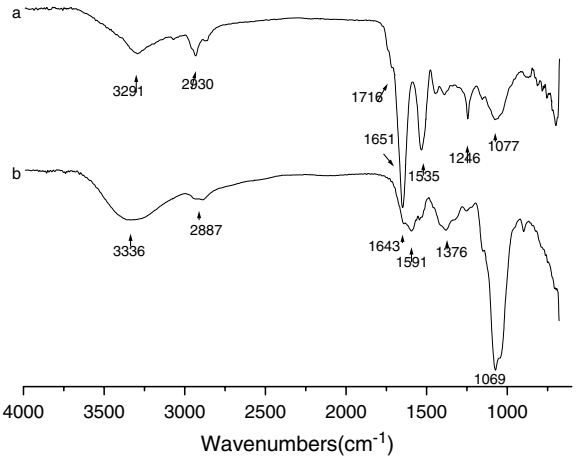

Fig. 4. ATR-FTIR spectra of (a) 5-FU BSA microspheres and (b) 5-FU BSA microspheres coated by chitosan derivative.

together, the optimal conditions for BSA microsphere preparation are 10 mg/ml for initial 5-FU concentration, 1/20 for volume ratio of w/o, 1.0 ml glutaraldehyde per 100 mg of BSA, and a crosslinking time of 4 h.

In the present study, 5-FU exhibited significant solubility in castor oil, and a higher o/w partition coefficient, although it was previously reported as poorly dissolving in lipid. This hydrophobic nature of 5-FU might be responsible to the reduced loading efficiency of the BSA microsphere preparation, as well as the presence of the drug on microsphere surface, the latter contributes to the burst release behavior for the uncoated microspheres. However, it is not necessary to remove the excess drug on microspheres surface since they will be removed during the coating step.

3.2. Coating of 5-FU loaded BSA microspheres

A preferred procedure and optimal conditions for galactosyl chitosan coated 5-FU BSA microspheres are obtained. The 5-FU loaded BSA microspheres preparation method was described above. The lyophilized 5-FU loaded BSA microspheres are dispersed in acetic acid solution, which contains 2% galactosed chitosan. After 10 min of coating, the dispersed system is subjected to centrifugation, followed by several water washes and lyophilization.

3.3. Characterization of 5-FU BSA microspheres with and without chitosan coating

The ATR-FTIR spectra of 5-FU BSA microspheres with and without the galactosylated chitosan coating are shown in Fig. 4. The characteristic absorption bands of crosslinked BSA were present in the ATR-FTIR spectrum at 1716 cm^{-1}, which was attributed to the carbonyl group of carboxylic acid. In the ATR-FTIR IR spectrum of galactosylated chitosan, coated microspheres had the characteristics signal of galactosylated chitosan. The peak at 1657 cm^{-1} was assigned to the acylamino group and the peak at 1590 cm^{-1} was attributed to the free amino groups. The intensity of the peak at 3400 cm^{-1} increased substantially. This increase was due to the increased number of the hydroxyl groups, and showed that the microspheres were coated with galactosylated chitosan.

ESCA is a widely accepted method for evaluating the surface properties of chemical materials. ESCA spectra were obtained for the microspheres and coated microspheres to further investigate the chitosan coating. The C 1s peak for 5-FU microspheres could be resolved into three components: a hydrocarbon (C–C–C) peak at 285.0 eV, an amine (C–C–NH_2) peak at 286.9 eV, and an ester (O=C–O) peak at 287.9 eV (Fig. 5). The C 1s peak for the coated microspheres is the combination peak: a hydrocarbon (C–C–C) peak at 284.88 eV (Fig. 6). The peaks of ether carbon (C–C–O), acylamide carbon (N–C=O), and ester carbon (O=C–O) are too small to obtain accurate peak resolution. The peak at 398.63 eV was the characteristic peak of amine (C–NH–C). The wider O 1s peak reflected a combination of the contributions from the ester oxygen (O=C–O–, O=C–O–), ether oxygen (C–O–C), hydroxyl oxygen (C–OH), and carbon oxygen (O–C–O) atoms. Furthermore, the difference of surface elemental composition between 5-FU microspheres and coated microspheres was obtained from ESCA and summarized in Table 1. ESCA data indicated the presence of galactosylated chitosan coating on the BSA microsphere surface.

The X-ray diffraction patterns of 5-FU, 5-FU BSA microspheres, and 5-FU loaded BSA microspheres with galactosylated chitosan coating are shown in Fig. 7. The graphs showed one sharp crystal peak at 2θ of 26∼31° and two small peaks at 2θ of 31.5° and 60° for 5-FU (Fig. 7a). The blank microspheres had one broad peak at 2θ of 22° contributed to the crosslinked BSA (Fig. 7c). When 5-FU and blank BSA microspheres were physically

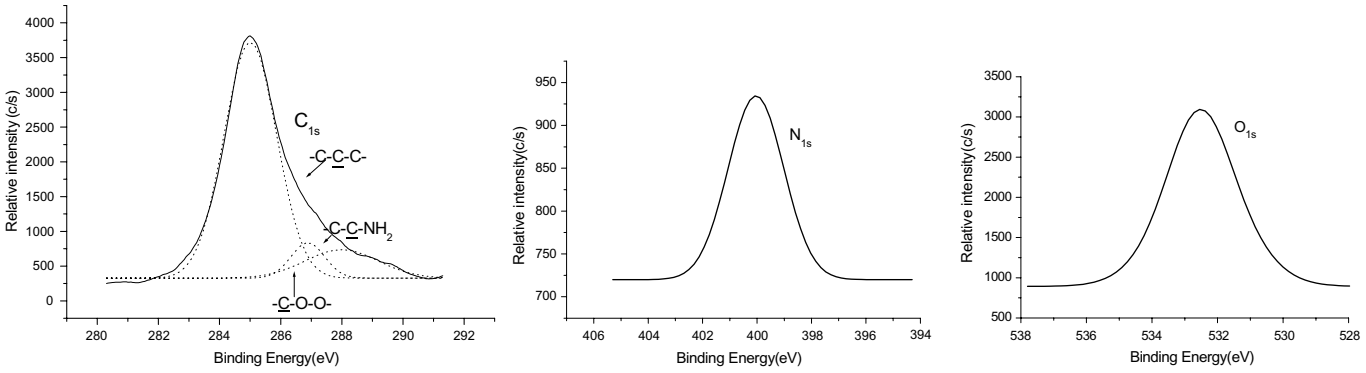

Fig. 5. ESCA spectrum of 5-FU BSA microspheres.

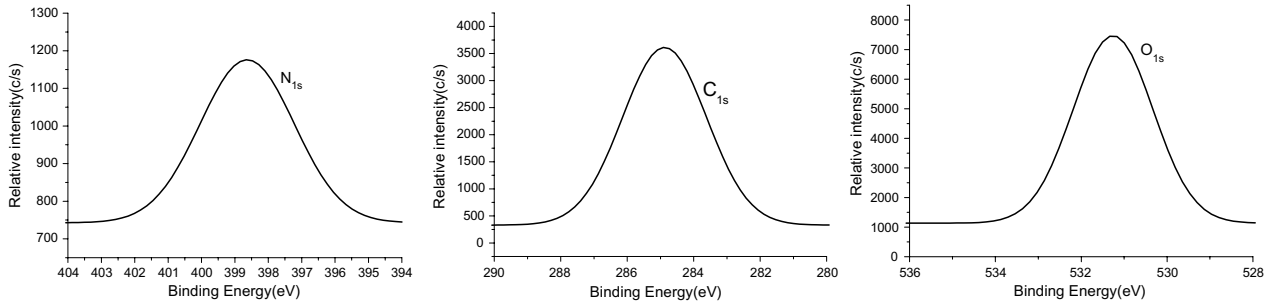

Fig. 6. ESCA spectrum of 5-FU BSA microspheres coated by chitosan derivative.

Table 1
Surface elemental composition (%) from ESCA

Sample	C_{1s}	O_{1s}	N_{1s}	O_{1s}/C_{1s}
a	77.33	19.39	3.28	0.25
b	60.55	33.73	5.72	0.56

a, 5-FU BSA microspheres.
b, 5-FU BSA microspheres coated by chitosan derivative.

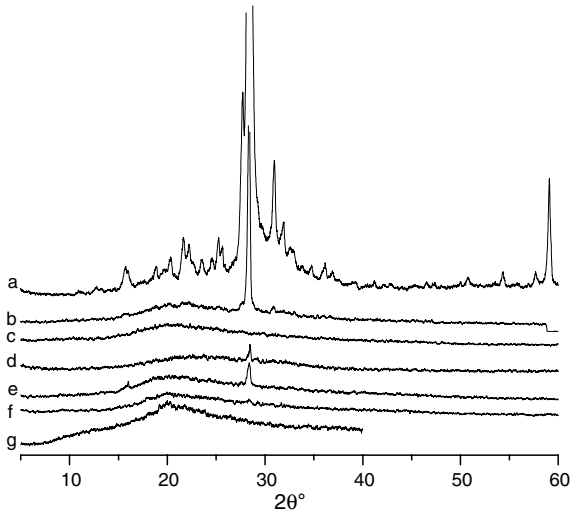

Fig. 7. WXRD patterns of (a) 5-FU, (b) mechanical mixture of blank BSA microspheres and 5-FU (10:1, w/w), (c) blank BSA microspheres, (d) 5-FU BSA microspheres, (e) mechanical mixture of 5-FU BSA microspheres and chitosan derivative (3:1, w/w), (f) 5-FU BSA microspheres coated by chitosan derivative, and (g) chitosan derivative.

mixed according to the ratio of 10:1 (w/w), a sharp peak for crystalline 5-FU and one broad peak at $2\theta = 22°$ for crosslinked BSA were observed (Fig. 7b). The graph for BSA microspheres with 5-FU loaded had one broad peak at $2\theta = 22°$ assigned to crosslinked BSA and peak at 2θ of 29° attributed to characteristic peak of 5-FU (Fig. 7d). These data indicated that the crystal state of 5-FU in microspheres remained the same. Galactosylated chitosan also showed one broad peak at $2\theta = 20°$ (Fig. 7g). The mixture of BSA microspheres with 5-FU loaded and galactosylated chitosan (3:1, w/w) had one sharp crystal peak of 5-FU and one broad peak at $2\theta = 20°$ for galactosylated chitosan (Fig. 7e). Interestingly, the 5-FU BSA microspheres with *N*-galactosylated chitosan coating had no 5-FU peak but only the broad peak at 2θ of 20° from the chitosan moiety (Fig. 7f). These data support the presence of chitosan coating on the BSA microsphere surface.

TEM was employed to further characterize and compare the 5-FU BSA microspheres with and without the galactosyl chitosan coating. The microspheres and chitosan coated microspheres appeared as smooth spheres with particle size of 0.60 and 0.65 μm, respectively (Fig. 8). The galactosylated chitosan coating thickness was measured as about 50 nm under the TEM assay conditions. In addition, there were no noticeable changes in the size after coating, 0.54 μm of 5-FU loaded BSA microspheres and 0.55 μm of the coated microspheres.

After coating, the zeta potential of the microspheres changed from -21.2 ± 1.6 mv to 16.6 ± 1.6 mv. The resid-

Fig. 8. Transmission electron micrograph of (a) 5-FU BSA microspheres and (b) microspheres coated by chitosan derivative.

ual amino groups of galactosylated chitosan would be responsible for the positive zeta potential interactions with anionic BSA microspheres. Coating might have attributed to static interaction between cationic galactosylated chitosan and anionic BSA microspheres. And the coated microspheres contained $5.40 \pm 1.50\%$ (w/w) galactose according to the determined galactose content. These data further supported that galactosylated chitosan had been coated on the surface of the microspheres.

3.4. Drug loading and encapsulation efficiency

HPLC analyses showed that the encapsulation efficiency and drug loading for the uncoated microspheres were $72.4 \pm 5.7\%$ ($n = 3$) and $5.3 \pm 2.2\%$ ($n = 3$), respectively, whereas after coating with galactosylated chitosan, the encapsulation efficiency and drug loading were $40.3 \pm 2.8\%$ ($n = 3$) and $2.9 \pm 0.6\%$ ($n = 3$), respectively. The results indicated that a considerable amount of drugs on the surface of microspheres was removed by the coating process including the latest washing procedure.

3.5. In vitro release

In vitro release tests of 5-FU BSA microspheres and 5-FU BSA microspheres coated with galactosylated chitosan indicated that the release of 5-FU from the microspheres, whether coated or not, was delayed in comparison to that of 5-FU (Fig. 9). A heavy burst was observed from uncoated 5-FU BSA microspheres. After coating, the residual drug from the surface was removed and led to the significant delayed-release of 5-FU during the initial phases. Furthermore, the delayed-release might be partially due to the presence of the encapsulated layer, which could act as a barrier.

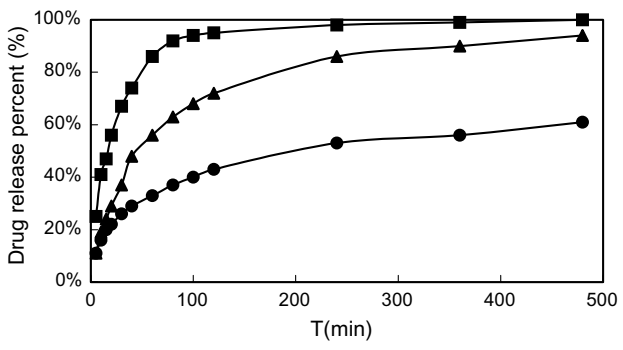

Fig. 9. In vitro release of (■) 5-FU, (▲) 5-FU BSA microspheres, and (●) 5-FU BSA microspheres coated by chitosan derivative.

3.6. Stability tests

According to a preliminary stability test, there appears no agglomeration in freeze-dried powder of 5-FU BSA microspheres, inspected by the naked eye, after 3-month storage at 4 °C. In addition, the powder could be well dispersed in aqueous media. The tests also found the encapsulation efficiency and drug loading to be $69.4 \pm 3.0\%$ ($n = 3$) and $5.2 \pm 2.1\%$ ($n = 3$), respectively. Comparing with that before the storage, the results suggested a good stability for the 5-FU loaded BSA microspheres coated with *N*-galactosylated chitosan.

4. Conclusions

A newly designed microspheres coated by targeting moieties were successfully prepared. More targeting moieties were exposed on the surface of the BSA microsphere so that the higher binding efficiency to the receptors may be reached through the general matrix microsphere, in which a part of targeting moieties may be buried in the core. In addition, it seems that the coating layer may affect the diffusion barrier to prevent the fast release and reduce the burst effect of the microspheres. The optimal conditions for preparing 5-FU loaded BSA microspheres coated with *N*-galactosylated chitosan were determined in the present studies. The conditions are mild, simple, and suitable for the preparation of microsphere materials and coating materials which have the opposite charges. The coating efficiency and the coating layer can be proved or characterized by several advanced analysis method, ATR-FTIR, ESCA, WXRD, and TEM are useful tools for the studies. In general, the BSA microspheres with galactosylated chitosan coating may serve as potential vehicle for both of the delayed release and targeting delivery. Additional study is required to further investigate the capacity of this novel delivery system for targeting the delivery of drugs to the liver in vivo.

Acknowledgements

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