Amphiphilic sodium alginate-vinyl acetate microparticles for drug delivery*

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Abstract To overcome the fast or burst release of hydrophilic drugs from hydrophilic alginate-based carriers, hydrophobic molecule (vinyl acetate, VAc) was grafted on alginate (Alg), which was further used to prepare drug carriers. Amphiphilic Alg-g-PVAc hydrogel beads were firstly prepared by emulsification/internal gelation technique for the loading of bovine serum albumin (BSA). Then, chitosan was coated on the surface of beads to form novel amphiphilic Alg-g-PVAc/chitosan (Alg-g-PVAc/CS) microcapsules. The BSA-loading amphiphilic Alg-g-PVAc/chitosan (Alg-g-PVAc/CS) microcapsules display similar morphology and size to the hydrophilic alginate/chitosan (AC) microcapsules. However, the drug loading and loading efficiency of BSA in Alg-g-PVAc/CS microcapsules are higher, and the release rate of BSA from Alg-g-PVAc/CS microcapsules is slower. The results demonstrate that the introduction of hydrophobic PVAc on alginate can effectively help retard the release of BSA, and the higher degree of substitution is, the slower the release rate is. In addition, the complex membrane can also be adjusted to delay the release of BSA. As a whole, amphiphilic sodium alginate-vinyl acetate/CS microparticles could be developed for macromolecular drug delivery.

Keyword: hydrophobic modification; sodium alginate-vinyl acetate; amphiphilic Alg-g-PVAc/chitosan microcapsules; drug delivery

1 INTRODUCTION

Alginate is a naturally derived polysaccharide extracted from brown seaweed. It is a kind of linear anionic polymer with the structural units of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues. They are randomly linked by 1,4-glycosidic bond to form homogenous blocks (MM and GG) and heterogeneous blocks (GM) along the polymer chains depending on the source. Most of the commercially available alginates are in the form of sodium alginate (Na-Alg). Specifically, sodium alginate solution can transform from sol to hydrogel state under biologically benign conditions (room temperature and near-neutral pH) when divalent cations such as Ca^{2+} are added to trigger the ionotropy effect between the divalent cations and Na^{+}. The formed calcium alginate (Ca-Alg) hydrogel has three dimensional (3D) network structure containing more than 90% of water, which is thought to be beneficial for the entrapment of bioactive substances. Together with its biocompatibility, low toxicity, and relatively low cost, alginate hydrogels have been extensively investigated and used for many biomedical applications including cell transplantation,

tissue regeneration and drug delivery (Liu et al., 2008; Lee and Mooney, 2012).

Generally, alginate hydrogels can be fabricated in the form of microparticles or microcapsules by various preparation methods such as electrostatic droplets, emulsification technique (Zhang et al., 2008; Song et al., 2013; Ching et al., 2017; Lopes et al., 2017), which have emerged as a competitive drug delivery platform due to above mentioned advantages. Both small chemical drugs and macromolecular proteins have been loaded in alginate particles (Liakos et al., 2013; Shin et al., 2013; Zhang et al., 2013; Du and Stenzel, 2014), and some alginate-based formulations can release drugs in a controlled manner according to the parameters optimization of materials and technical process. However, the 3D porous network and hydrophilicity of alginate hydrogels (pore size ~5 nm), always led to the rapid release of small molecules through the gel (Boontheekul et al., 2005). Moreover, macromolecular proteins also displayed 'burst release' behavior from chitosan coated alginate hydrogel microcapsules, which resulted in the reduction of drug bioavailability (Desai et al., 2010).

To resolve the problem of fast release, alginate modification via hydrophobic molecules or moieties has been proposed as a strategy to retard the release rate of hydrophilic drugs. The hydroxyl (-OH at C-2 and C-3) and carboxyl groups (-COOH at C-6) along the chain backbone of alginate can be modified for different purposes such as esterification, oxidation, and amidation (Yang et al., 2011; Pawar and Edgar, 2012). Acrylic, acrylamide, and vinyl acetate monomers have been grafted on alginate molecules or alginate hydrogel beads to form copolymers and used as drug carriers (Fang et al., 2005; Xu and Ni, 2012). However, the degree of substitution (DS) of the hydrophobic part on alginate molecules or gel beads was over 100% so that the hydrophobic part became the predominant structure of copolymers. This transformation suggested the solubility and biocompatibility of hydrophilic alginate were changed, which is against its application in drug delivery of bioactive molecules such as proteins. In our previous report, amphiphilic sodium alginate-vinyl acetate (Alg-g-PVAc) with a lower degree of substitution (<30%) was synthesized by homogeneous polymerization (Xu et al., 2013), which can keep the predominant hydrophilic nature of the product and be easily dissolved in the water for further manufacture and application.

With the purpose of retarding the release of macromolecular drugs from hydrophilic alginate-based carriers, hydrophobically modified alginate was synthesized to prepare drug carriers and tested for the release of macromolecular drug model. In detail, alginate modified via hydrophobic molecules (vinyl acetate) was used to firstly fabricate amphiphilic Alg-g-PVAc hydrogel beads by emulsification/internal gelation technique. Bovine serum albumin (BSA) was used as a model drug and entrapped into the amphiphilic hydrogel beads. Then another polysaccharide chitosan was assembled on the surface of beads to form novel BSA-loaded Alg-g-PVAc/chitosan (Alg-g-PVAc/CS) microcapsules. Finally, the physiochemical properties, drug loading and release behavior in simulated gastric fluid and simulated intestinal fluid from amphiphilic Alg-g-PVAc/CS microcapsule were evaluated.

2 MATERIAL AND METHOD

2.1 Material

Sodium alginate was purchased from the Qingdao Crystal Rock Biotechnological Development Co., Ltd. (Qingdao, China), whose viscosity is 0.81 Pa·s as labeled. The composition ratio of α-L-guluronic acid (G) residues and β-D-mannuronic acid (M) residues of alginate were characterized by ^{1}H NMR as G/M ratio of 34/66. Vinyl acetate was analytical reagent from Sinopharm Group. BSA (M_w=6.6 kDa), Tween 80 and Span 80 were purchased from Chemical Reagents Co. Ltd. (Sinopharm Group). Potassium persulfate, liquid paraffin, and absolute ethanol were analytical reagents from Tianjin Damao Chemical Reagent Factory. Chitosan (deacetylation degree of 96%, M_w 50, 150, and 250 kDa) was degraded from raw chitosan (Yuhuan Ocean Biomaterials Corporation, China) with gamma (γ) rays irradiation by Key Laboratory of Nuclear Analysis Techniques, Chinese Academy of Sciences. All other reagents and solvents were of reagent grade and were used without further purification.

2.2 Preparation of BSA-loaded amphiphilic Alg-g-PVAc/CS microcapsules

Amphiphilic sodium alginate-vinyl acetate (Alg-g-PVAc) synthesized by homogeneous polymerization was dissolved in deionized water in a concentration of 1.5% w/v. 0.1 g BSA and 0.1 g CaCO$_3$ were added under stir state to form a uniform suspension. Then,

the suspension was dispersed into liquid paraffin containing 0.5% (v/v) Span 80 for emulsification according to our established protocol (Song et al., 2013). After emulsification, the addition of glacial acetic acid dissociated $CaCO_3$ and released Ca^{2+} to trigger the gelation. The BSA-loaded calcium Alg-g-PVAc gel beads were collected and successively rinsed with 1% (v/v) Tween 80 solution quickly. At last, the BSA-loaded calcium Alg-g-PVAc gel beads were further immersed in 0.5% (w/v) chitosan solution (0.1 mol/L acetate buffer) at the beads/ solution ratio of 1:10 to form BSA-loaded Alg-g-PVAc/CS microcapsules. Meantime, hydrophilic alginate was used to prepare BSA-loaded alginate/ chitosan (AC) microcapsules with the same procedure as a control.

2.3 Morphology and size of BSA-loaded amphiphilic Alg-g-PVAc/CS microcapsules

Some droplets containing BSA-loaded Alg-g-PVAc/CS microcapsules were transferred on a watch glass and observed with an XDS-1C Inverted Research Microscope (Chongqing COIC instrument Co. Ltd., China) at a magnification of 100×. Meantime, the size of Alg-g-PVAc/CS microcapsules was determined with laser particle analyzer (LA-960, Horiba Group, Japan).

2.4 Determination of drug loading and loading efficiency

Firstly, the standard curve of absorbance (A)-BSA concentration (C) was obtained by UV-VIS spectrophotometer (UV-2600, Shimadzu Co. Japan) at the maximum absorption wavelength of 278.5 nm. Then, BSA-loaded microcapsules were added into sodium citrate solution (1:10 v/v), and the mixture was vibrated for sometimes to completely disrupt microcapsules. The absorbance of the supernatant was measured at 278.5 nm and the released BSA was calculated with a standard curve. Finally, the drug loading and loading efficiency were calculated according to the following equations.

For the drug loading (DL):

$$DL = \frac{Mass_{loadedBSA}}{Mass_{Alg\text{-}g\text{-}PVAc} + Mass_{loadedBSA}} \times 100\%. \qquad (1)$$

For the loading efficiency (LE):

$$LE = \frac{Mass_{loadedBSA}}{Mass_{addedBSA}} \times 100\%. \qquad (2)$$

2.5 In vitro release of BSA from amphiphilic Alg-g-PVAc/CS microcapsules

Simulated gastric fluid (SGF): the pepsin was dissolved in 0.2% NaCl (w/v) solution to reach a final concentration of 0.3 g/L, and then pH was adjusted to 1.0 with concentrated hydrochloric acid.

Simulated intestinal fluid (SIF): The trypsin and bile salt were dissolved in phosphate buffer (0.1 mol/L PBS, pH=7.4) to reach a final concentration of 1.0 g/L and 4.5 g/L, respectively.

An amount of 10-mL BSA-loaded amphiphilic Alg-g-PVAc/CS microcapsules was transferred into a beaker with 100 mL of SGF. Then, the beaker was placed in constant temperature incubator shaker (THZ-98 AB, Shanghai Yiheng Scientific Instrument Co., China) and shaken at 120 r/min (37°C). At fixed time interval (2 min, 5 min, 10 min, 15 min, 30 min, 1 h, and 2 h), 5 mL supernatant was taken out and analyzed by UV-VIS spectrophotometer (UV-2600, Shimadzu Co. Japan) at 278.5 nm. At each time, 5-mL fresh SGF was supplemented.

After 2-h release in SGF, BSA-loaded amphiphilic Alg-g-PVAc/CS microcapsules were separated and quickly rinsed with deionized water. Then they were put into a beaker with 100 mL of SIF, which was placed in constant temperature incubator shaker (THZ-98 AB, Shanghai Yiheng Scientific Instrument Co., China) and shaken at 120 r/min (37°C). At fixed time interval (2 min, 5 min, 10 min, 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 16 h, and 24 h), 5 mL supernatant was taken out and analyzed by UV-VIS spectrophotometer (UV-2600, Shimadzu Co. Japan) at 278.5 nm. At each time, 5 mL fresh SGF was supplemented.

3 RESULT AND DISCUSSION

3.1 Morphology and size of BSA-loaded amphiphilic Alg-g-PVAc/CS microcapsules

As shown in Fig.1, the negatively charged Alg-g-PVAc is mixed with BSA and prepared as BSA-loaded Alg-g-PVAc gel beads (Section 2.2). Then they are immersed in positively charged chitosan (CS) solution to form the microcapsule with polyelectrolyte complex membrane (as the arrows indicated in Fig.2). BSA-loaded amphiphilic Alg-g-PVAc/CS microcapsules were observed with an inverted microscope. The amphiphilic microcapsules show good sphericity and smooth surface (Fig.2). Both the optical photograph and the particle curve demonstrate the amphiphilic microcapsules have a relatively uniform size, and the
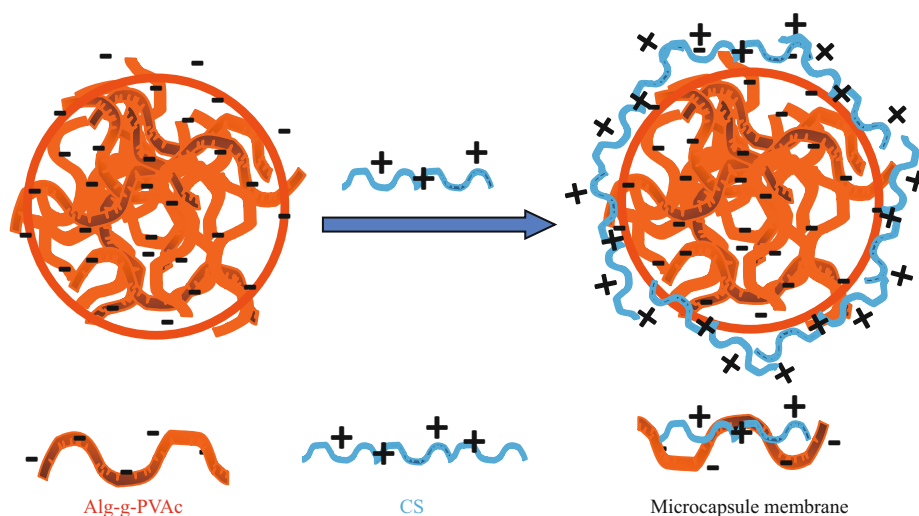
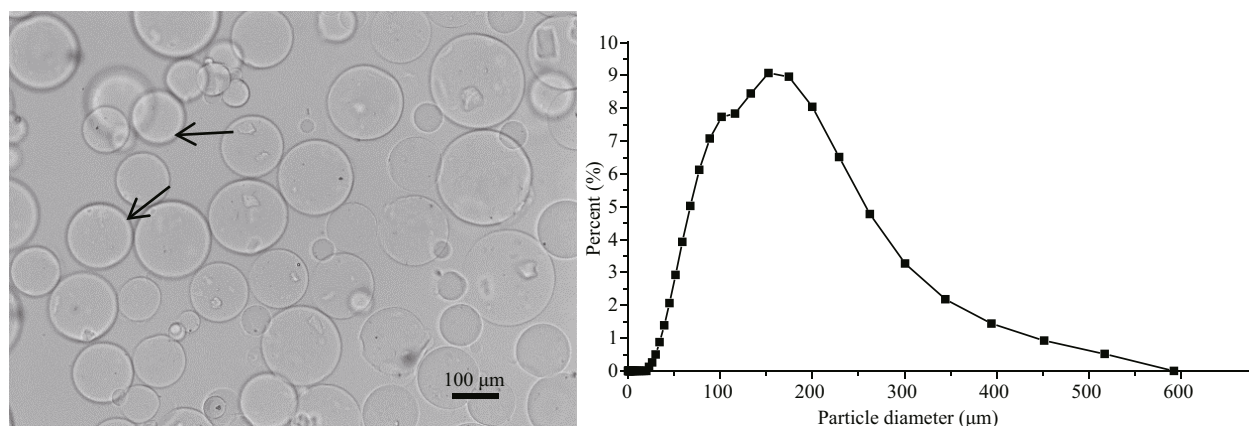**Fig.1 The illustration of the formation of Alg-g-PVAc/CS microcapsule**

Fig.2 The photograph (magnification 100×) and particle size distribution of BSA-loaded Alg-g-PVAc/CS microcapsules

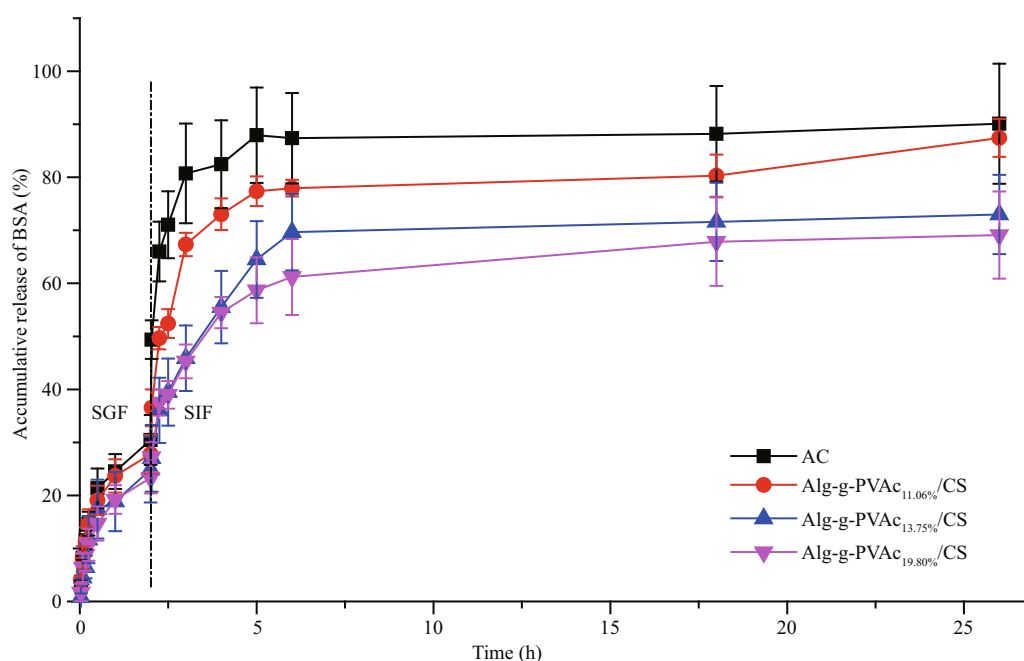The arrows indicate the microcapsule membrane.

average diameter is 141±32 μm calculated by the software of the particle analyzer. These results indicate the morphology of amphiphilic Alg-g-PVAc/CS microcapsules have no obvious difference to traditional hydrophilic alginate/chitosan microcapsules (Liu et al., 2008), suggesting that the moderately hydrophobic modification on alginate does not affect the gelation and membrane formation process.

3.2 Determination of drug loading and loading efficiency

By controlling the reaction conditions, amphiphilic sodium alginate-vinyl acetate (Alg-g-PVAc) with different degree of substitution (DS) of hydrophobic moieties was synthesized by homogeneous polymerization (Xu et al., 2013). Here, Alg-g-PVAc samples with DS of 11.06%, 13.75%, and 19.80% were used to prepare BSA-loaded Alg-g-PVAc/CS

microcapsules. The drug loading (DL) and loading efficiency (LE) of BSA in the amphiphilic microcapsules at different DS were measured and calculated as shown in Table 1. Meantime, BSA-loaded AC microcapsules were used as a control. DL and LE of BSA in Alg-g-PVAc/CS microcapsules are higher than those of BSA in traditional hydrophilic AC microcapsules. Moreover, DL and LE of BSA in Alg-g-PVAc/CS microcapsules slightly increase with the increase of DS. This can be attributed to the graft of polyvinyl acetate (PVAc) on alginate backbone, which increases the hydrophobicity of copolymer. The hydrophobic interaction may occur between the nonpolar chain segments of BSA and PVAc, and it is beneficial to limit more proteins in the amphiphilic microcapsules during preparation process compared to AC microcapsules. Therefore, both DL and LE of BSA increase.

Fig.3 The release of BSA from amphiphilic Alg-g-PVAc/CS microcapsules with different DS in simulated gastric fluid and simulated intestinal fluid

3.3 In vitro release of BSA from amphiphilic Alg-g-PVAc/CS microcapsules

3.3.1 Effect of DS of hydrophobic PVAc on BSA release

BSA-loaded Alg-g-PVAc/CS microcapsules were prepared with DS of 11.06%, 13.75%, and 19.80%, and they were put sequentially into SGF (2 h) and SIF (24 h) to evaluate BSA release behavior. In general, the accumulative release of BSA in SGF is lower than that in SIF (Fig.3). The release rate of BSA from hydrophilic AC microcapsules is faster than that from amphiphilic Alg-g-PVAc/CS microcapsules. In addition, DS of hydrophobic PVAc on alginate affects the release of BSA from Alg-g-PVAc/CS microcapsules. The accumulative release of BSA reaches about 90%, 80%, 70%, and 60% from AC, Alg-g-PVAc$_{11.06\%}$/CS, Alg-g-PVAc$_{13.75\%}$/CS, and Alg-g-PVAc$_{19.80\%}$/CS microcapsules, respectively. In other words, the higher the DS of PVAc is, the slower the release rate is.

It is well known that alginate hydrogel is a pH-responsive carrier (Mei et al., 2014), which can shrink in acidic solution but swell in neutral or alkaline medium. The shrinkage causes the decrease of pore size of alginate hydrogel and benefits for the retardation of substance diffusion. Therefore, the release of BSA is relatively slow in SGF. Moreover, the coating of chitosan on the surface of alginate
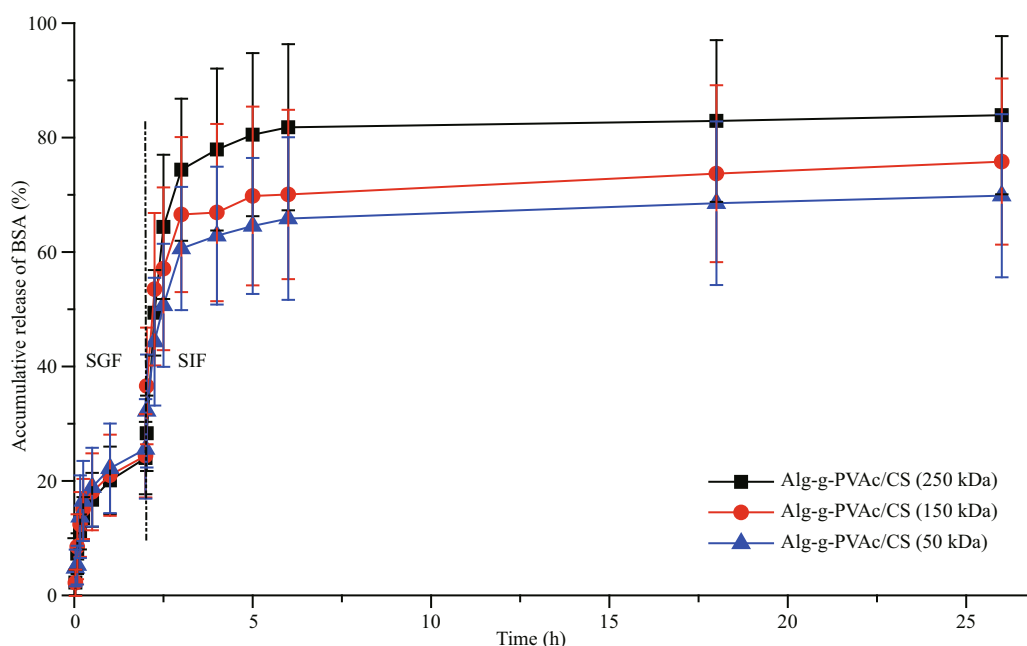
Table 1 DL and LE of BSA in the amphiphilic Alg-g-PVAc/CS microcapsules at different DS

Sample	DL (%)	LE (%)
AC	43.51	30.32
Alg-g-PVAc$_{11.06\%}$/CS	44.66	30.87
Alg-g-PVAc$_{13.75\%}$/CS	45.90	31.46
Alg-g-PVAc$_{19.80\%}$/CS	48.57	32.69

hydrogel beads forms the microcapsule membrane. In general, the thicker and denser membrane formed by regulating the parameters (such as M_w of chitosan and membrane formation time) can help reduce the pore size of the network and then decrease the release rate of BSA. On the other hand, more hydrophobic moieties (PVAc) grafted on alginate facilitates above-mentioned hydrophobic interaction to significantly retard the release of BSA from amphiphilic Alg-g-PVAc/CS microcapsules.

3.3.2 Effect of M_w of chitosan on BSA release

The purpose of coating chitosan on the surface of alginate hydrogel beads is to form a membrane and to regulate the release of drugs. Here, chitosan samples with different M_w (50 kDa, 150 kDa, and 250 kDa) were used to complex with Alg-g-PVAc (DS 13.75%) beads to form amphiphilic Alg-g-PVAc/CS microcapsules. The release of BSA in SGF and SIF also displays the different trends (Fig.4). The M_w of

Fig.4 The release of BSA from amphiphilic Alg-g-PVAc/CS microcapsules with different chitosan M_w in simulated gastric fluid and simulated intestinal fluid

chitosan hardly had an impact on the release of BSA in SGF probably due to the strong pH responsive effect of alginate hydrogel. In SIF, however, the accumulative release of BSA from Alg-g-PVAc/CS microcapsules reduces along the decrease of chitosan M_w. Because the smaller M_w of chitosan means the shorter molecular segments, which is easier to diffuse into the network of hydrogel beads, they can provide more positive amino groups to complex with negative carboxyl groups of alginate (Yu et al., 2010). As a result, thicker microcapsule membrane is formed, which effectively retards the outward diffusion rate of BSA. On the contrary, the longer molecular segments with bigger M_w are relatively hard to diffuse into the network of alginate hydrogel and only react with alginate molecules on the bead surface to form a thinner membrane. Therefore, the release of BSA is faster from amphiphilic Alg-g-PVAc/CS microcapsules with higher chitosan M_w.
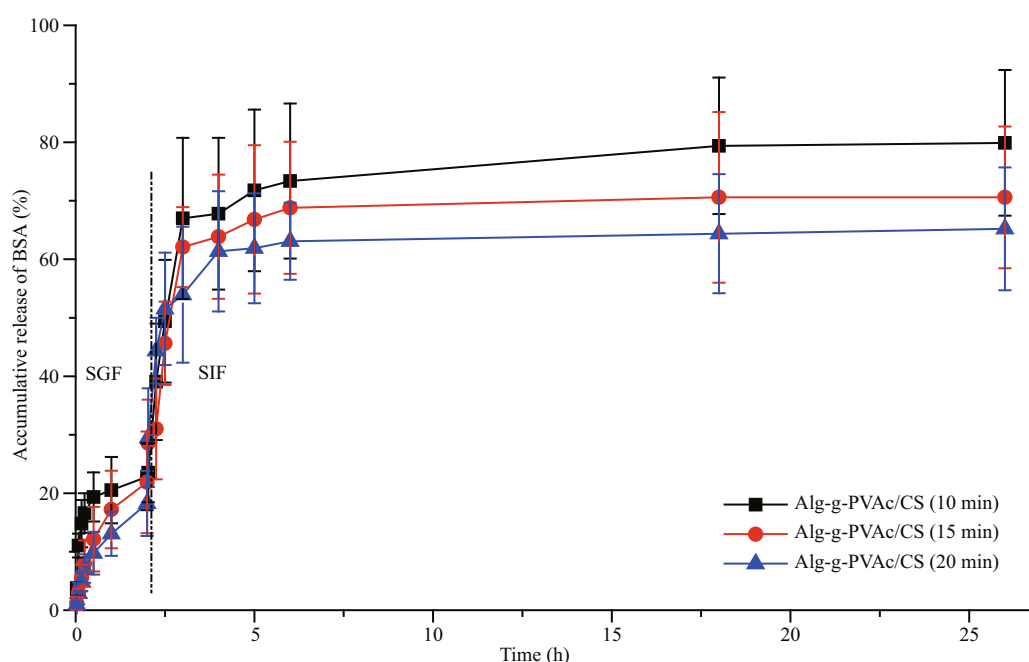
3.3.3 Effect of membrane formation time of chitosan on BSA release

Chitosan samples (M_w 150 kDa) were assembled on Alg-g-PVAc (DS 13.75%) beads to form amphiphilic Alg-g-PVAc/CS microcapsules at different membrane formation time (10 min, 15 min, and 20 min). The release of BSA in SGF and SIF is shown in Fig.5. Similarly, the release of BSA in SIF is higher than that in SGF. To prolong the membrane formation time, more chitosan molecules can diffuse

into the deeper network of alginate hydrogel beads, and then the complex reaction between chitosan and alginate under electrostatic interaction is sufficient to form a thick membrane. The resistance of the hydrogel network and membrane to BSA diffusion obviously increases, so that the accumulative release of BSA decreases.

4 CONCLUSION

In this paper, the hydrophobically modified alginate was used to prepare novel amphiphilic Alg-g-PVAc/CS microcapsules. The hydrophobic modification of alginate has no effect on the formation of hydrogel beads and microcapsules. After entrapping model protein drug (BSA), the amphiphilic Alg-g-PVAc/CS microcapsules displays good sphericity and relatively uniform size. Compared to traditional hydrophilic AC microcapsules, the drug loading (DL) and loading efficiency (LE) of BSA in Alg-g-PVAc/CS microcapsules are higher, and the release rate of BSA from Alg-g-PVAc/CS microcapsules is slower. Moreover, with the increase of degree of substitution of hydrophobic moieties (PVAc) from 11.06% to 19.80%, DL slightly increased by 5.89%, and LE increased by 8.75%, while the accumulative release of BSA from Alg-g-PVAc/CS microcapsules obviously reduces from about 80% (DS 11.06%) to 60% (DS 19.80%). These results demonstrate that the graft of hydrophobic PVAc on alginate molecules can effectively retard BSA release from microcapsules.

Fig.5 Release of BSA from amphiphilic Alg-g-PVAc/CS microcapsules at different membrane formation time in a simulated gastric fluid and simulated intestinal fluid

Furthermore, the regulation of parameters of chitosan (Mw and membrane formation time) can adjust the properties of the microcapsule membrane to postpone the release of BSA.

5 DATA AVAILABILITY STATEMENT

All data generated and/or analyzed during this study are included in this published article. The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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