pH-Controllable drug release using hydrogel encapsulated mesoporous silica†

S.-W. Song, K. Hidajat and S. Kawi*

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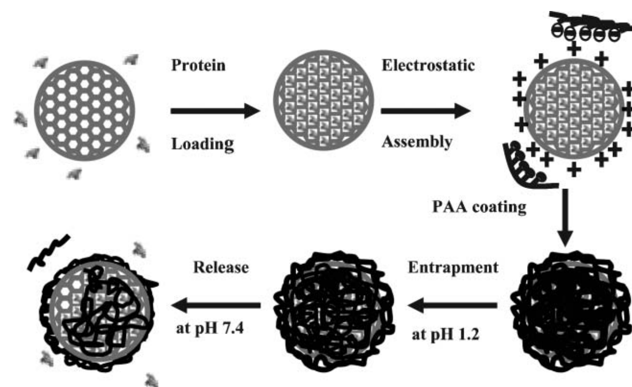
Amine-functionalized mesoporous SBA-15 silica loaded with bovine serum albumin (BSA) has been successfully encapsulated with a thin layer coating of poly(acrylic acid) PAA, with the entrapped BSA being released from the PAA-encapsulated SBA-15 at the higher pH value of 7.4 rather than at the lower pH value of 1.2. This novel drug delivery system has a potential application in the release of protein drug to the site of higher pH value, such as small intestine or colon.

The recent application of mesoporous silicas extended to controlled drug delivery has raised much interest due to their non-toxic nature, high surface area, large pore volume, tunable pore size and chemically modifiable surfaces, which allow them to be potential hosts for various drugs, such as ibuprofen, amoxicillin, gentamicin, captopril, or even large protein drug molecules.[1–6] Hydrogels – which belong to one category of widely employed polymer-drug matrices – are cross-linked macromolecular networks swollen in water or biological fluids. Due to their good biocompatibility and sensitivity in the physiological or biological environment, hydrogels have been found in numerous applications in the fields of biology and medicine, including ophthalmological devices, biosensors, scaffold, and controlled drug delivery systems.[7–11] This paper reports for the first time that we could successfully combine these two types of interesting materials together to prepare a pH-sensitive drug delivery system, which can hold a protein drug at a lower pH value (pH 1.2) and release it at a higher pH value (pH 7.4). This novel drug delivery system could have potential application for oral delivery of protein drugs, delivering the drug to the target site of higher pH value, such as small intestine or colon.

Scheme 1 shows the loading of protein into mesoporous silica, the encapsulation of mesoporous silica with hydrogel and the release of protein from mesoporous silica. The mesoporous silica selected in this study is SBA-15, as it has tunable pore size to host a large amount of the model protein drug – bovine serum albumin (BSA). In addition, SBA-15 can be easily chemically modified or functionalized, rendering its flexible chemical and physical properties to be coupled with various hydrogels. In this report, the mesopores of SBA-15 have been functionalized by amine groups and its surface properties have been finely tuned in order for the SBA-15 particles to be encapsulated with poly(acrylic acid) (PAA). PAA is used in this study to encapsulate the drug-loaded SBA-15 particles because it is a well-known bioadhesive hydrogel, which is often incorporated in drug formulation.[12] PAA has some beneficial properties which are critical for its usage in drug delivery. For instance, PAA can stick to the mucosal lining of the upper small intestine, allowing it to anchor drug-laden matrices to the intestinal wall and, during swelling, release therapeutic agents.[13] Additionally, PAA was found to be able to protect some protein drugs from degradation by inhibiting the hydrolytic activity of gastrointestinal enzymes.[14]

Ethanol-extracted unfunctionalized SBA-15 (ES-15), amine-SBA-15 functionalized by one-pot synthesis (OPS-15), and amine-SBA-15 functionalized by post-synthesis (PS-15) were prepared according to the procedure described in our previous study.[6] The N_2 adsorption–desorption analysis results and FTIR spectra are shown in Fig. S1 and S2 (ESI†). BSA was loaded onto SBA-15 samples by immersing SBA-15 powders into citrate–phosphate buffer solution of BSA at pH 4.7 for 24 h. (The samples loaded with BSA are named as BSA/ES-15, BSA/OPS-15, BSA/PS-15.) The powder samples were then separated from solution by centrifugation and freeze-dried. The loading amounts of BSA were determined by the change of BSA concentration in the buffer solution using Bradford assay and further confirmed by TGA analysis. The properties of the resulting SBA-15 materials and the loading amount of BSA are summarized in Table 1. Compared with ES-15, the loading amount of BSA on either OPS-15 or PS-15 has been increased, indicating that the introduction of surface amine groups can prompt the adsorption of BSA on SBA-15 due to the positive surface charge induced by functionalization with amine groups. It can be observed from ζ potential curve (Fig. S3 in ESI†) that the surface charge has been remarkably changed when SBA-15 was functionalized by amine groups.



Scheme 1 Schematic representation of protein loading, PAA encapsulation by ESA and drug release.

Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 119260. E-mail: chekawis@nus.edu.sg; Fax: +65-67791936; Tel: +65-65166312
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Table 1 Textural parameters of the samples and the loading amount of BSA

Sample	S_{BET}/m^{2}g	$D_{A,\text{BJH}}$/Å	V_{total}/ cm^3 g^{-1}	WP$_N$ [a] (w/w%)	WP$_{BSA}$ [b] (w/w%)
ES-15	446.8	109.0	1.27	0	9.1
OPS-15	405.0	106.1	1.03	0.58	20.2
PS-15	228.2	108.6	0.89	1.25	23.1

[a] Nitrogen elemental content [b] Loading amount of BSA

50 mg of BSA/ES-15, BSA/OPS-15 or BSA/PS-15 was then added to 25 ml of PAA solution (M_w = 130,000, 2 mg ml^{-1}, pH 5.35), and the mixture was vigorously shaken for about 10 min so that PAA could be assembled onto SBA-15 materials with appropriate surface properties. During this process, two important surface properties of SBA-15 are found to determine the encapsulation results: surface charge and hydrophilicity. Since PAA is a weak polyelectrolyte whose repeating units bear pendant carboxylic groups with pK_a values in the range of 4.5–5.0, PAA can be partially dissociated at pH 5.35, making it negatively charged. Hence, for hydrophilic unfunctionalized SBA-15 (ES-15) which is negatively charged above pH 2–3, it is difficult to encapsulate the negatively charged SBA-15 with PAA at pH 5.35 due to the electrostatic repulsion (see FESEM images in Fig. S4 in ESI†). In the case of amine-functionalized SBA-15 prepared by post-synthesis (PS-15), although it is positively charged at pH 4–6, however, due to its highly hydrophobic nature,[15] the powders of PS-15 sample tend to agglomerate together and float on the surface of PAA solution when they are in contact with an aqueous solution, making the encapsulation process unsuccessful. Therefore, these two kinds of SBA-15 cannot be encapsulated with PAA (ESI†). For amine-functionalized SBA-15 prepared by one-pot synthesis (OPS-15), since it is of hydrophilic nature and positively charged, within only 10 min, by electrostatic assembly (ESA), PAA could be assembled onto the outer surface of BSA/OPS-15. Therefore, TEM pictures (Fig. 1) and XPS spectra (ESI†) show the presence of PAA only for the BSA/OPS-15 sample. The final loading amount of BSA was 16.3% after BSA/OPS-15 was encapsulated with PAA.

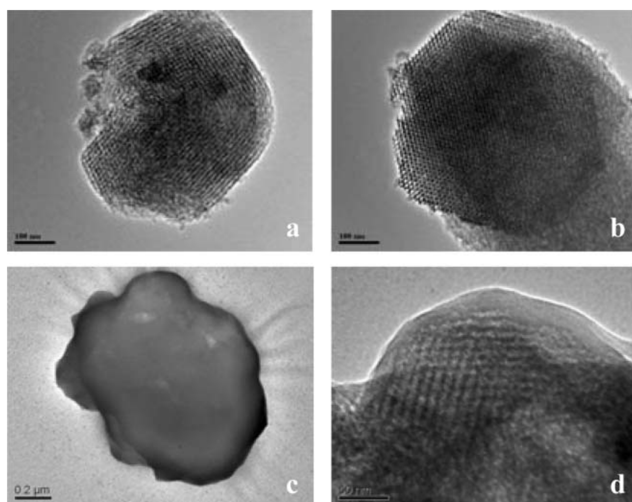

Fig. 1 TEM images of amine-functionalized SBA-15 without (a and b) and with PAA coatings (c and d).

Fig. 1 shows TEM images of BSA/OPS-15 before and after PAA encapsulation. It can be seen that, after PAA encapsulation, the parallel channels of OPS-15 are covered by PAA (Fig. 1c), and from a cross-sectional view, a thick layer of PAA (around 10 nm) can be observed on the outer surface of SBA-15 (Fig. 1d). As PAA is less charged at pH 5.35, the polymer chains tend to be adsorbed to form a thicker layer and more loopy type structures.[16]

The release studies of BSA from unencapsulated or PAA-encapsulated BSA/OPS-15 were conducted either in HCl–KCl buffer solution or in phosphate buffer solution. It is interesting to note from Fig. 2 that the release of BSA from PAA-encapsulated BSA/OPS-15 in HCl–KCl buffer solution (pH 1.2) is extremely slow as compared with that from unencapsulated BSA/OPS-15. In this acidic medium, BSA is hardly released from PAA-encapsulated BSA/OPS-15 samples within 5 h and only around 10% is released in 36 h. However, in phosphate buffer solution (pH 7.4), the release profile of BSA from PAA-encapsulated is similar to that from unencapsulated OPS-15 samples. These results indicate that after BSA/SBA-15 is encapsulated with PAA, BSA can only be released under certain pH conditions, *i.e.* at the higher pH value of 7.4.

In addition, TGA analysis also shows that the weight loss of PAA-encapsulated BSA/SBA-15 after the release testing at pH 1.2 is much higher than that of other samples (ESI†), indicating that there is still much more BSA remaining in the PAA-encapsulated SBA-15 after the release testing at pH 1.2. The TGA results confirm that BSA could be entrapped at lower pH conditions. In addition, it is also worth mentioning that the release rate of BSA from unencapsulated OPS-15 at pH 1.2 is higher than that at pH 7.4 due to the higher electrostatic repulsion between BSA and OPS-15. It can be seen from Fig. S3 (ESI†) that both OPS-15 and BSA are positively charged at pH 1.2; as a consequence, BSA could be easily released from the mesopores due to the electrostatic repulsion.

Since albumin has little enzymatic or immunological function,[17] it is unlikely we could measure the activity of the BSA after its release from the matrix. However, as the functions and structures of proteins are inherently correlated, the function of the released BSA can be estimated from its conformation. Hence, UV-CD is

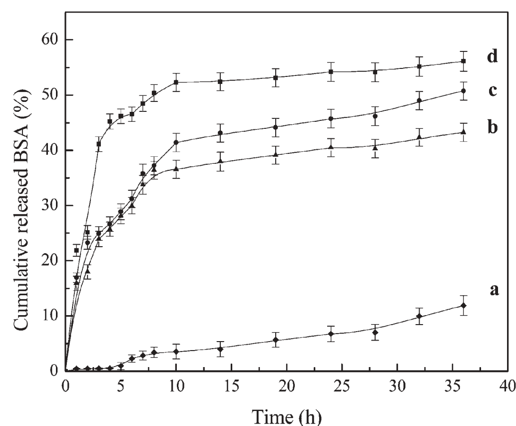

Fig. 2 Release profiles of BSA from: a) PAA-encapsulated OPS-15, released at pH 1.2; b) PAA-encapsulated OPS-15, released at pH 7.4; c) unencapsulated OPS-15, released at pH 7.4 and d) unencapsulated OPS-15, released at pH 1.2.

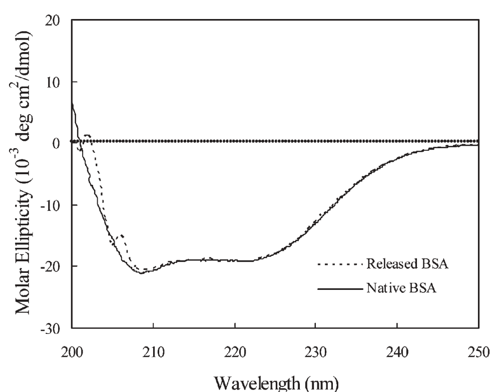

Fig. 3 CD spectra of released BSA from BSA/OPS-15 and native BSA.

used to estimate the secondary structure present in the released BSA. Fig. 3 shows the CD spectra of the native BSA and the released BSA from BSA/OPS-15. It shows that the CD spectrum of the released BSA is similar to that of the native BSA. In addition, the calculated percentage of α-helix in the native BSA and the released BSA are 67% and 65%, respectively, indicating that BSA conformation has not been severely or irreversibly altered by adsorption in the mesopores of functionalized SBA-15.

Furthermore, based on the MTT cytotoxicity studies (Fig. S7 in ESI†), this drug delivery system also has low toxicity, and can be safely used for oral drug administration.

The interesting pH-controllable release result obtained here is believed to be due to the pH-induced alteration of the configuration of the PAA coating on the surface of SBA-15. As mentioned earlier, PAA is a weak polyelectrolyte, and its swelling behavior depends on the nature of the ionizable side chains as well as the pH of aqueous medium. At the pH of 1.2 ($pH < pK_a$ 4.5), the side carboxylic groups cannot be dissociated and therefore PAA remains contracted or collapsed; hence, the encapsulated sample remains unchanged, resulting in the entrapment of BSA in SBA-15 channels. However, at the pH of 7.4 ($pH > pK_a$ 5.0), the carboxylic groups undergo dissociation and the charges on a linear PAA chain will repel each other, causing PAA to swell and finally dissolve into the release medium of higher pH value. PAA coating is then removed from the outer surface of SBA-15, hence releasing BSA from the mesopores of SBA-15. Zhu *et al.* and Yu *et al.* reported work using multilayers of polyelectrolyte pairs whereby drugs or proteins were released more at lower pH value.[18,19] However, for the present drug delivery system, the model protein drug could be held at lower pH value (pH 1.2) and released at higher pH value (pH 7.4). It is therefore believed that this pH-responsive drug delivery system could have a potential application for oral delivery of drugs, because on most occasions, it is desirable for drugs – especially protein drugs – to be protected in the stomach at pH 1–3 but released in the intestine at pH 6–8.[20]

In summary, using a simple and fast method, by suitably choosing the right combination of a pair of hydrogel and SBA-15, we have successfully encapsulated hydrophilic amine-functionalized SBA-15 (prepared by one-pot synthesis) with PAA through electrostatic assembly, and successfully prepared, for the first time, a smart pH-responsive protein delivery system. The entrapped protein can be released in a neutral medium rather than in an acidic medium. This novel drug delivery system could have potential application for the targeted oral delivery of therapeutic proteins, which could release drugs at sites like the small intestine or colon while protecting them from the acidic conditions present in the stomach.

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