Glucose-Responsive Supramolecular Vesicles Based on Water-Soluble Pillar[5]arene and Pyridylboronic Acid Derivatives for Controlled Insulin Delivery


Abstract: The stimuli-responsive behavior of supramolecular nanocarriers is crucial for their potential applications as smart drug delivery systems. We hereby constructed a glucose-responsive supramolecular drug delivery system based on the host–guest interaction between a water-soluble pillar[5]arene (WPS) and a pyridylboronic acid derivative (G) for insulin delivery and controlled release under physiological conditions. The approach represents the ideal treatment of diabetes mellitus. The drug loading and in vitro drug release experiments demonstrated that large molecular weight insulin could be encapsulated into the vesicles with high loading efficiency, which, to our knowledge, is the first example of small-size supramolecular vesicles with excellent encapsulation capacity of a large protein molecule. Moreover, FITC-labeled insulin was used to evaluate the release behavior of insulin, and it was demonstrated that high glucose concentration could facilitate the quick release of insulin, suggesting a smart drug delivery system for potential application in controlled insulin release only under hyperglycemic conditions. Finally, we demonstrated that these supramolecular nanocarriers have good cytocompatibility, which is essential for their further biomedical applications. The present study provides a novel strategy for the construction of glucose-responsive smart supramolecular drug delivery systems, which has potential applications for the treatment of diabetes mellitus.

Introduction

Diabetes mellitus is a metabolic disorder disease that is characterized by accumulating concentration of glucose in the plasma due to insufficient secretion of insulin from the pancreas.[1] Currently, the main treatment for diabetes is daily injections of insulin and regular monitoring of the blood glucose levels after diets, which can bring great discomfort and inconvenience to the patients. More importantly, for conventional treatment, the glucose sensing and drug therapy are not directly coupled, which does not tightly regulate glucose levels in patients. Therefore, lack of tight control of blood glucose levels is often associated with significant side-effects including fatal hypoglycemia, blindness, and kidney failure.[2] To improve the quality of life of diabetic patients, it is essential to design and develop efficient and smart drug delivery systems (DDSs) with glucose-responsive function, which only release insulin in response to hyperglycemic conditions.

Smart drug delivery systems or drug nanocarriers refer to DDSs with responsive behavior to the external stimuli such as pH,[3] temperature,[4] light,[5,6] enzyme,[7] or chemo-stimuli,[8] which can not only deliver drugs to the target disease sites, but also can release them in response to changes in the external environment, especially under the specific microenvironment of some organs, intracellular space, or pathological sites.[9] In recent years, with the rapid development of supramolecular chemistry, supramolecular amphiphiles that are based on noncovalent interactions, especially host–guest interactions between macrocyclic hosts and guest molecules have been extensively employed to constructed smart nanocarriers. Moreover, due to the dynamic nature of noncovalent interactions, supramolecular amphiphiles have the special ability to undergo reversible switching of structures, morphologies, and functions in response to certain external stimuli.[10] These systems have great potential for applications in areas such as controlled drug delivery and release. So far, inspired by the excellent binding reaction between boric acid and cis-diols, glucose-responsive materials have also been well developed, but there are only a few reports on the construction of functional drug delivery systems for the controlled delivery of insulin.[11]
Moreover, among various reported glucose-responsive DDSs, most of them have been focused on the construction of mesoporous silica nanoparticles or polymeric materials. Very little research work has focused on the construction of glucose-responsive supramolecular DDSs based on supra-amphiphiles, which could endow the obtained nanoparticles with excellent smart behavior. Recently, research on pillararene-based supramolecular materials has received increased attention and the field has advanced rapidly. Our group has also developed a series of supramolecular amphiphiles based on water-soluble pillararenes, which can further assemble to form higher-ordered aggregates such as vesicles, micelles, and nanoparticles to achieve controllable drug delivery. Based on the above research, it could be concluded that water-soluble pillararene, which contains a hydrophobic cavity and hydrophilic terminal groups distributed on both rims of the macrocycle, should be an ideal candidate for the construction of supramolecular amphiphiles because of their symmetrical pillar and unique rigid architectures, excellent properties in host–guest chemistry, as well as outstanding biocompatibility. On the other hand, it is well known that heterocyclic-containing boric acid derivatives can bind d-glucose under approximate neutral pH range due to the presence of an electron-rich hetero atom such as N, O or S, which can increase the binding affinity between the boric acid group and glucose under physiological conditions. Moreover, pyridinium salts have been confirmed to have strong binding affinity with water-soluble pillar[5]arene (WP5) to form stable inclusion complexes. Accordingly, a pyridylboronic acid derivative (G), containing a long alkyl chain as the hydrophobic tail, was synthesized for further assembling to form a supra-amphiphile with WP5. Based on the above design, herein, we report a novel glucose-responsive supramolecular drug delivery system based on the host–guest interaction between WP5 and a pyridylboronic acid derivative G, which can encapsulate large molecular weight insulin with high loading efficiency (Scheme 1). To our knowledge, this is the first example of small size supramolecular vesicles with excellent encapsulation capacity of a large protein molecule such as insulin. Moreover, it was demonstrated that high glucose concentration could facilitate the quick release of insulin from insulin-loaded supramolecular nanocarriers, suggesting a smart DDS with potential application in diabetic therapy.

Results and Discussion

Host–guest complexation studies in water

WP5 was synthesized according to previous reports and a well-designed guest molecule G, bearing a pyridylboronic acid motif, was synthesized by using pyridin-4-ylboronic acid as the starting material (Scheme 2). Since G exhibited poor water solubility and was inclined to self-aggregate in water, a model guest MG was synthesized (Scheme S2, see the Supporting Information) to investigate the host–guest complexation between WP5 and the guest molecule G based on 1H NMR spectroscopy in D2O. Figure 1 showed the 1H NMR spectra of MG in the absence and presence of WP5 (1.0 equiv), and it was found that after complexation the signals of phenyl protons (H1 and H2) of WP5 shifted downfield slightly. Whereas, the signals derived from pyridine protons (H3, H4, H5, and H6) of MG shifted upfield remarkably and showed clear broadening effects due to the shielding effect of the electron-rich cavities of pillar[5]arene, which suggested the inclusion of the alkyl chain and partial pyridine group of MG into the hydrophobic WP5 cavity and the formation of a WP5–MG inclusion complex.

The stoichiometry of complexation between WP5 and MG in water was further investigated by applying the Job’s plot

Scheme 1. Schematic illustration of the formation of supramolecular vesicles based on WP5–G supra-amphiphile and their glucose-responsive insulin release.
method based on UV/Vis spectroscopy, which confirmed the 1:1 binding stoichiometry between WP5 and MG (Figure S8, see the Supporting Information). The association constant ($K_a$) for WP5$\rightleftharpoons$MG was also determined to be $(1.55 \pm 0.33) \times 10^5$ M$^{-1}$ based on fluorescent titration experiments (Figure S9, see the Supporting Information). The binding affinity for such host–guest inclusion was mainly driven by cooperative electrostatic and hydrophobic interactions. Therefore, we deduced that WP5 and pyridylboronic acid derivative G could form a “tadpole-like” 1:1 amphiphilic supramolecular inclusion complex, as shown in Scheme 1.

Construction of supramolecular binary vesicles in water based on the WP5$\rightleftharpoons$G supra-amphiphiles

After achieving the above amphiphilic WP5$\rightleftharpoons$G supramolecular complex in water, the ability of such a supra-amphiphile to form higher-order aggregates in aqueous phase was further investigated. When a certain amount of WP5 solution was added to the transparent G solution, a light opalescence and clear Tyndall effect could be observed (Figure 2b inset), indicating the formation of microaggregates. The morphology and size of these aggregates were further investigated by transmission electron microscopy (TEM) and dynamic laser scattering (DLS) experiments. From the TEM images (Figure 2a), the obtained aggregates showed a hollow spherical morphology with an average diameter around 120 nm, which was in good agreement with the DLS result (average diameter of 132 nm, Figure 2b), indicating the formation of a vesicular structure. Subsequently, the best molar ratio between WP5 and G for the formation of supramolecular aggregates was determined to be 10:1 ([G]/[WP5]) based on the UV/Vis spectra[18] (Figure S10, see the Supporting Information). Based on this best molar ratio, the critical aggregation concentration (CAC) for WP5$\rightleftharpoons$G assembly was determined to be $1 \times 10^{-4}$ M (Figure S11, see the Supporting Information). Furthermore, $\zeta$-potential measurements were carried out to examine the stability of the obtained supramolecular vesicles with different WP5/G molar ratios. Finally, the molar ratio of [WP5]/[G] = 1:3 ($\zeta$-potential = $-38.8$ mV, Figure 2c) was used in the following study to further investigate the stimuli-responsive behaviors of the obtained vesicles, because under this condition the formed vesicles will not further aggregate due to the repulsive force-induced increasing stability. In contrast, after loading with cargos, a molar ratio of [WP5]/[G] = 1:1.5 was used for constructing the insulin-loaded vesicles, because much more stable nanocarriers with high insulin-loading efficiency could be obtained under these conditions.

Stimuli-responsiveness of the supramolecular vesicles

Stimuli-responsive properties are often incorporated into supramolecular nanocarrier systems, which is of great importance for achieving their controllable drug release. Therefore, before investigating the insulin-loading efficiency of the obtained WP5$\rightleftharpoons$G vesicles, we first studied their stimuli-responsive behaviors. It was found that the above WP5$\rightleftharpoons$G supramolecular vesicles exhibited the characteristic d-glucose-responsive behavior caused by the strong bonding affinity between the pyridylboronic acid motif and cis-diol-containing d-glucose, which was also supported by the $^1$H NMR and $^1$B NMR spectra of MG and WP5$\rightleftharpoons$MG complex in the presence and absence of d-glucose (Figure S6 and S7, see the Supporting Information). After adding 1.0 equiv of d-glucose to the

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**Scheme 2.** Synthesis of the pyridine-4-boronic acid derivatives G and MG.
MG solution (pD 7.4), the protons H_{1-3} of MG shifted upfield clearly, indicating the binding of MG with α-glucose. Whereas, when α-glucose (1.0 equiv) was added to the mixed solution containing WP5 and MG, protons H_{1-6} of MG shifted upfield slightly, which demonstrated that the deeper inclusion of the alkyl chain of MG into the cavity of WP5 resulted from the tensile force of α-glucose to MG. Moreover, 11BNMR experiments also proved the strong bonding between boric acid ligand and α-glucose for both MG and WP5:MG complex, from which clear chemical shift changes could be observed after the addition of α-glucose (Figure S7, see the Supporting Information). Furthermore, upon adding α-glucose to the WP5:G vesicular solution (pH 7.4), no vesicle could be observed from the TEM image (Figure S13a, see the Supporting Information) and this was accompanied by the disappearance of the Tyndall effect (Figure S13b, see the Supporting Information). Moreover, after adjusting the vesicular solution to pH 6.0, the Tyndall effect also disappeared and no vesicular structure could be found from the TEM image (Figure S13c and S13d, see the Supporting Information) because the generated pillar[5]arene in its acid form is water-insoluble and will precipitate from the acidic aqueous solution.  

A model hydrophobic fluorescent dye, doxorubicin (DOX), was also used to further confirm the stimuli-responsive behavior of the obtained WP5:G vesicles. After being desalted by TEA, DOX could be successfully loaded into the hydrophobic Stern layer of the vesicles, as confirmed by the TEM images (Figure 3a) and DLS results (Figure 3b). Moreover, the fluorescence spectra of DOX-loaded vesicular solution in the presence and absence of α-glucose were recorded (Figure 3c), from which the characteristic emission peaks of DOX became much stronger after the addition of α-glucose, especially with extended response time (Figure S14b, see the Supporting Information). The same phenomena could also be observed after adjusting the vesicular solution to pH 6.0 (Figure S14a, see the Supporting Information), indicating the collapse of vesicles after the external stimuli of α-glucose or an acidic environment. All the above results confirmed that such WP5:G supramolecular vesicles exhibited excellent α-glucose- and pH-responsiveness, which endowed them with the ability to encapsulate insulin under neutral conditions and release them in response to an environment with high α-glucose concentration or low pH value, making the obtained WP5:G supramolecular vesicles ideal candidates for controlled insulin release. 

Insulin encapsulation and in vitro release

In the following study, the insulin encapsulation efficiency of the above resulting supramolecular vesicles and their release behavior of the loaded insulin were evaluated. To prepare insulin-loaded vesicles, aqueous solution of human recombinant insulin was added quickly to a aqueous solution containing WP5 and G ([G]/[WP5] = 1.5:1, 1% EtOH was added to improve the solubility of G). After standing overnight, the unloaded insulin was removed by dialysis against deionized water. TEM images (Figure 4a) and DLS results (Figure 4b) showed that insulin-loaded vesicles were much larger in size (ca. 320 nm) than those of the unloaded vesicles (ca. 132 nm), and the insu-
Figure 3. a) TEM images of DOX-loaded vesicles formed by WP5 and G; b) DLS result of DOX-loaded vesicles. Inset: images of the Tyndall effect of DOX-loaded vesicles; c) florescence spectra of DOX-loaded vesicles (black line), DOX-loaded vesicles after the addition of d-glucose (5.5 mg mL⁻¹; red line), and DOX-loaded vesicles after the solution pH was adjusted to 6.0 (blue line); ([WP5] = 0.062 mM, [G] = 0.186 mM, [WP5]/[G] = 1:3, pH 7.4).

Figure 4. a) TEM images of insulin-loaded vesicles formed by WP5-G supra-amphiphile; b) DLS result of insulin-loaded vesicles formed by WP5-G supra-amphiphile. Inset: images of WP5-G vesicles (right: insulin-loaded vesicular solution, left: unloaded vesicular solution); c) the ζ-potential of insulin-loaded WP5-G vesicles ([WP5] = 0.118 mM, [G] = 0.177 mM, [WP5]/[G] = 1:1.5).
lin-loaded vesicles adopted an irregular morphology based on the TEM observations (Figure 4a inset), confirming that insulin molecules were successfully loaded into the cavities of the vesicles. More intuitively, the vesicular solution became remarkably cloudy and its turbidity increased significantly after insulin was successfully loaded (Figure 4b inset).

The HPLC results of the vesicular solution after adding Triton X-100, which was used to destroy the nanoparticles, also revealed that insulin was successfully encapsulated into the cavity of WP5\textsubscript{G} vesicles (Figure 5). Furthermore, the encapsulation efficiency of insulin was calculated to be 64% based on the peak area detected by HPLC at ca. 11 min, which is assigned to the pure insulin peak. Meanwhile, \( \zeta \)-potential measurements showed that such insulin-loaded supramolecular vesicles also had a negative \( \zeta \)-potential (–25.6 mV, Figure 4c) with good stability, ensuring their potential application for insulin delivery and controlled release.

The intrinsic properties of human recombinant insulin (without any UV or fluorescence signal) meant that it was difficult to monitor the release behavior of insulin. Therefore, fluorescein isothiocyanate (FITC) labeled insulin was used as a model fluorescent probe as well as an analogous drug to evaluate the release behavior of insulin in real time. Initially, FITC-insulin-loaded vesicles were successfully prepared by using a similar drug-loading process. After adding \( \alpha \)-glucose or Triton X-100 to the obtained drug loaded vesicular solution, the changes in fluorescence spectra (Figure 6a) indicated that FITC-labeled insulin was successfully encapsulated into the WP5\textsubscript{G} vesicles. Moreover, DLS results (Figure 6b) also confirmed that FITC-insulin molecules were successfully loaded into the WP5\textsubscript{G} vesicles, which could be concluded from the size changing after the loading of FITC-insulin. Meanwhile, the size of FITC-insulin-loaded vesicles was similar to that of human recombinant insulin loaded vesicles. All the above results indicated that FITC-insulin could be used as a fluorescent probe as well as an analogous drug to directly evaluate the release behavior of insulin in real time.

Subsequently, the release efficiency of FITC-insulin from the loaded vesicles upon \( \alpha \)-glucose stimulus was investigated. As shown in Figure 7a, almost no FITC-insulin release could be observed under physiological conditions (pH 7.4). The cumulative release amount of insulin was only about 20% within 60 minutes when the concentration of \( \alpha \)-glucose was in the normal blood-glucose level (1.0 mg mL\(^{-1}\)), indicating that unwanted insulin release would not occur under normal blood-glucose levels. However, the encapsulated FITC-insulin was released rapidly in significant amounts and the cumulative re-
lease amount of FITC-insulin was increased with increasing concentration of d-glucose. The cumulative release amount of FITC-labeled insulin was about 70% within 60 minutes when the concentration of d-glucose was increased to 5.5 mg mL⁻¹ (Figure S15, see the Supporting Information), indicating approximately 70% of the loaded FITC-insulin was released from the cavities of WP5>G vesicles. On the other hand, the pH value also played a vital role in the cargo release behavior. The FITC-insulin release profiles under neutral (pH 7.0) and physiological (pH 7.4) conditions were presented in Figure 7b, from which much less FITC-insulin release could be observed under neutral conditions ([d-glucose]=2.0 mg mL⁻¹), whereas the cumulative release of FITC-insulin reached 60% within 60 minutes when the pH value was increased to 7.4 ([d-glucose]=2.0 mg mL⁻¹). Given that the normal condition of human blood is weakly alkaline, the rapid FITC-insulin release could be efficiently achieved under physiological conditions when the blood glucose concentration becomes higher, enabling these insulin-loaded vesicles to function as ideal candidates for diabetes therapy.

Cytocompatibility of the drug nanocarriers

The cytocompatibility of the nanocarrier is also an important index for evaluating its biomedical application. Therefore, the cytotoxicity of these drug nanocarriers was further evaluated by using the MTT cell survival assay against MRC-5 normal cells. Figure 8 shows the cell viability after 24 h incubation with WP5, blank vesicles, and FITC-insulin-loaded vesicles at a concentration varying from 5 to 40 μM. Clearly, the cell viability remains above 70% after 24 h incubation even when the concentration of the examined vesicles was increased to 40 μM. Therefore, these supramolecular vesicles have good cytocompatibility and are suitable nanocarriers for insulin delivery, which is also essential for their further biomedical applications.

Conclusions

We have constructed a novel glucose-responsive supramolecular drug delivery system based on the host–guest amphiphilic inclusion complex formed between a water-soluble pillar[5]arene (WP5) and a pyridylboronic acid derivative (G) for insulin delivery and controlled release under physiological conditions. The insulin loading and in vitro drug release experiments demonstrated that insulin could be encapsulated into the vesicles with high loading efficiency (64%), which is the first example of small size supramolecular vesicles with excellent encapsulation capacity of a large protein molecule. Moreover, FITC-labeled insulin was used as a fluorescent probe as well as an analogous drug to directly evaluate the release behavior of insulin in real time. The results demonstrated that the insulin-loaded vesicles exhibit excellent glucose-responsiveness and quick release of insulin at high glucose concentration environment under physiological conditions, enabling them to be suitable for potential applications in controlled insulin release. Further cytotoxicity experiments indicated that these supramolecular vesicles exhibited good cytocompatibility and they are a suitable nanocarrier for insulin delivery. The present study opens a novel avenue for the construction of glucose-responsive smart supramolecular nanocarriers, which have great potential applications for the treatment of diabetes mellitus. The in vivo activity of the insulin-loaded supramolecular vesicles in the treatment of diabetes in a mouse model is in progress in our laboratory.

Experimental Section

Materials

Human recombinant insulin (Min: >27.5 units mL⁻¹) was provided by CCIS Reagent Co., Ltd. (Shanghai, China). FITC-insulin was provided by Sigma–Aldrich (Shanghai, China). Doxorubicin (DOX-HCl; >98%) was provided by Melone Pharmaceutical Co., Ltd. (Dalian, China). WP5, G, and MG were synthesized and purified according to previously reported procedures, and they were identified by ¹H and ¹³C NMR spectroscopy and by mass spectrometry.

Cell culture

MRC-5 normal human cells were cultivated in Dulbecco’s modified Eagle’s medium (DMEM) supplied with 10% fetal bovine serum (FBS) and antibiotics (50 μM penicillin and 50 μM streptomycin) at 37 °C in a humidified atmosphere containing 5% CO₂.

Synthetic procedures

All reactions were performed in open atmosphere unless noted and all yields were given as isolated yields. WP5[20] was synthesized according to a reported procedure. Detailed synthetic procedures for pyridine-4-boronic acid derivatives (G and MG) are shown in Scheme 2.

Synthesis of 1.[21] Pyridine-4-boronic acid (1.2 g, 10.0 mmol) and neopentyl glycol (1.1 g, 10.0 mmol) were dissolved in toluene (120 mL) and the mixture was stirred and heated at 130 °C for 12 h. The precipitation was collected by filtration and dried in vacuum. The target compound 1 (1.6 g, 8.38 mmol, 84%) was obtained as a white solid. ¹H NMR (400 MHz, CDCl₃, 298 K): δ = 8.61 (d, J = 5.6 Hz, 2H; PyH), 7.66 (d, J = 5.6 Hz, 2H; PyH), 7.38 (s, 4H; OCH₃), 1.03 ppm (s, 6H; CH₂).
acetonitrile (25 mL) and the mixture was heated to reflux at 80 °C for 72 h. The reaction mixture was cooled to RT, and extracted with petroleum ether (5 × 15 mL) to remove the unreacted n-hexadecyl bromide. After removing acetonitrile under vacuum, the residue was hydrolyzed in a mixed solvent of water (10 mL) and acetone (10 mL) at 60 °C for 24 h. The precipitate was collected, washed with water, and dissolved in methanol (1 mL), and the obtained solution was added dropwise to an excess of isopropyl ether (100 mL). The precipitates were collected by filtration, washed with diethyl ether, and dried in vacuum. The target compound G (500.0 mg, 1.17 mmol, 56%) was obtained as a white solid. M.p. 196–198 °C. 1H NMR (400 MHz, CD3OD, 298 K): δ = 8.83 (d, J = 5.9 Hz, 2H; Py), 8.20 (d, J = 5.9 Hz, 2H; Py), 4.58 (t, J = 7.5 Hz, 2H; PyCH2), 2.03–1.99 (m, 2H; PyCH2CH2), 1.38–1.28 (m, 26H; CH2), 0.90 ppm (t, J = 6.3 Hz, 3H; CH3); 13C NMR (100 MHz, CD3OD, 298 K): δ = 143.7, 133.3, 62.7, 33.2, 30.91, 30.89, 30.8, 30.7, 30.6, 30.2, 27.3, 23.9, 14.6 ppm; HRMS (ESI): m/z calcd for C19H14N4O2Br [M – Br]-: 304.2999; found 304.3000.

Synthesis of compound MG: Compound 1 (191.0 mg, 1.0 mmol) and n-butyl bromide (2.7 g, 20.0 mmol) were dissolved in acetonitrile (30 mL), and stirred at 60 °C for 48 h in the an Ar atmosphere. The solution was removed under vacuum and the residue was hydrolyzed in a mixed solvent of water (10 mL) and acetone (10 mL) for 48 h in an Ar atmosphere. After removing the acetonitrile under vacuum, the water solution was washed with n-butanol (2 × 20 mL), and the obtained aqueous solution was concentrated under vacuum to give the target compound MG (78.0 mg, 0.41 mmol, 41%) as a light-yellow solid. M.p. 73–75 °C; 1H NMR (400 MHz, D2O, 298 K): δ = 9.12 (s, 2H; OH), 9.04 (d, J = 6.4 Hz, 2H; Py), 8.31 (d, J = 6.4 Hz, 2H; Py), 4.59 (t, J = 7.3 Hz, 2H; PyCH2), 1.94–1.86 (m, 2H; PyCH2CH2), 1.30–1.24 (m, 2H; CH2CH2), 0.92 ppm (t, J = 7.4 Hz, 3H; CH3; CH3); 13C NMR (100 MHz, D2O, 298 K): δ = 143.2, 132.0, 95.5, 60.3, 32.6, 18.8, 13.3 ppm; HRMS (ESI): m/z calcd for C16H12N4O2Br [M – Br]-: 216.1120; found 216.1120.

DOX loading and release of WPS–G vesicles

DOX-loaded vesicles were prepared as follows: a defined amount of DOX (after being desalted by TEA) was added to a solution containing WPS and G (1% EtOH was added to improve the solubility of G). The ultimate concentrations of DOX, G, and WPS were 0.036, 0.186, and 0.062 mm, respectively. After standing overnight, the prepared DOX-loaded vesicles were purified by dialysis (molecular weight cutoff 10 000) in distilled water for several times until the water outside the dialysis tube exhibited negligible DOX fluorescence. The DOX encapsulation efficiency was calculated by using Equation (1):

\[
\text{Encapsulation efficiency} \ (% ) = \left( \frac{m_{\text{DOX-loaded}}}{m_{\text{DOX}}} \right) \times 100 \tag{1}
\]

where \( m_{\text{DOX-loaded}} \) and \( m_{\text{DOX}} \) are mass of DOX encapsulated into the vesicles and mass of DOX added, respectively. The mass of DOX was measured with a fluorescence spectrophotometer at 560 nm and calculated as relative to a standard calibration curve of concentrations from 0.5 to 7.0 μg mL⁻¹.

In a typical pH-induced release experiment, a trace amount of 0.01 M HCl solution was added into 10 mL of DOX-loaded vesicular solution, and the final pH of the vesicular solution was adjusted to 6.0. At selected time intervals, 4 mL of the release media was taken out for measuring the released DOX concentrations by using the fluorescence technique, and then was returned to the original release media. The concentration of DOX was determined by measuring of emission intensity at 560 nm by using a standard emission vs. concentration curve constructed for DOX in the corresponding release medium. A nearly 100% release of DOX from DOX-loaded vesicles could be obtained by adding Triton X-100 to the vesicular solution.

In a typical α-glucose-induced release experiment, a defined amount of α-glucose was added into 10 mL of DOX-loaded vesicular solution, the final concentration of α-glucose in the vesicular solution was 5.5 mg mL⁻¹, and the solution pH was adjusted to 7.4. At selected time intervals, 4 mL of the release media was taken out for measuring the released DOX concentrations by using the fluorescence technique, and then was returned to the original release media. The concentration of DOX was determined by measuring of emission intensity at 560 nm by using a standard emission vs. concentration curve constructed for DOX in the corresponding release medium. Nearly 100% release of DOX from DOX-loaded vesicles could be obtained by adding Triton X-100 to the vesicular solution.

**Insulin loading and release of WPS–G vesicles**

Insulin-loaded vesicles were prepared as follows: a defined amount of insulin was added to a solution containing WPS and G (1% EtOH was added to improve the solubility of G). The ultimate concentrations of insulin, G, and WPS were 0.0033, 0.177, and 0.118 mm, respectively. After standing overnight, the prepared insulin-loaded vesicles were purified by dialysis (molecular weight cutoff 10 000) in distilled water for several times until the water outside the dialysis tube exhibited negligible HPLC traces. The insulin encapsulation efficiency was calculated by using Equation (2):

\[
\text{Encapsulation efficiency} \ (% ) = \left( \frac{m_{\text{insulin-loaded}}}{m_{\text{insulin}}} \right) \times 100 \tag{2}
\]

where \( m_{\text{insulin-loaded}} \) and \( m_{\text{insulin}} \) are mass of insulin encapsulated into the vesicles and mass of insulin added, respectively. The mass of insulin was detected by HPLC at 214 nm and calculated as relative to a standard calibration curve of concentrations from 1.0 to 10.6 μg mL⁻¹.

In a complete release experiment, insulin-loaded vesicles prepared as mentioned above were treated with Triton X-100. After standing at 25 °C for 10 min, 0.5 mL of sample was withdrawn and analyzed by HPLC with an AKZONOBEL KR100-5C18 reverse-phase column (4.6 × 250 mm) with UV detection at 214 nm at 25 °C. An isocratic elution of acetonitrile-0.2 μM sodium sulfate buffer solution (26.74 v/v, phosphoric acid and ethanol amine were used to adjust pH to 2.3) was applied at a flow rate of 1 mL min⁻¹. During assays, 20 μL of sample was injected into the analytic column and the release of insulin was detected by detector at 214 nm. The encapsulated efficiency of insulin was determined based on the peak area that was detected at ca. 11 min, which is assigned to the pure insulin peak.
Conflict of interest

The authors declare no conflict of interest.

Keywords: diabetes • drug delivery • host-guest systems • supramolecular chemistry • vesicles


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