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# Micelles/sodium-alginate composite gel beads: A new matrix for oral drug delivery of indomethacin

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ABSTRACT

Micelles/sodium-alginate composite gel beads (micelles/SA beads) were constructed from SA matrix and indomethacin (IND) loaded six-arm block copolymer poly($\varepsilon$-caprolactone)-*block*-(dimethylamino)ethyl methacrylate (S(PCL-*b*-PDMA)$_6$) micelles. The morphology, swelling property and drug loading level of the beads were investigated. The release behavior of IND from three matrices including micelles, SA beads and micelles/SA beads was evaluated at pH 1.2, 6.8 and 7.4, as well as in the simulated gastrointestinal (GI) tract. Significant differences in the release of IND from each matrix were observed in the acidic and near neutral pH fluids. The results suggested that IND could be protected by the micelles/SA beads from being released under acidic conditions in the stomach and would be mainly delivered to the small intestine and the colon. The release behavior was dependent on the initial concentrations of SA and calcium chloride ($CaCl_2$) due to the different crosslinking densities. The results demonstrated that these micelles/SA beads could represent a promising oral drug delivery system for drugs with poor water solubility.



## 1. Introduction

Oral administration is one of the most traditional methods because of its convenience and patient acceptance. Nevertheless, many disadvantages such as low absorption, negative effects on the stomach and low bioavailability can be encountered (Deng, Zhai, & Lin, 2008; Oostendorp, Beijnen, & Schellens, 2009; Varela, Guzman, & Molpeceres, 2001). Significant progress has been made in eliminating these adverse effects. Stimuli-responsive systems such as polymeric micelles or vesicles (Sant, Smith, & Leroux, 2005), hydrogel nanoparticles (Deng et al., 2008) and hydrogel films (He, Cui, & Yin, 2009) have been developed to enhance the oral bioavailability. However, there still remain challenges to improve the oral drug delivery systems.

Polymeric micelles can provide a unique core–shell structure in which water-insoluble drugs can be incorporated and thereby enhance the solubility of hydrophobic drugs (Feng, Li, Yang, & Hu, 2011; He, Zhang, & Gu, 2010; Rapoport, 2007; Zhang, Li, & Li, 2008). Moreover, such micellar systems are known to have advantages over many other carriers due to their good water solubility, excellent structural stability and strong ability to prolong drug retention time (Gaucher, Dufresne, & Sant, 2005). Until now polymeric micelles have been extensively investigated as a promising drug carrier by intravenous administration, primarily because they can reach the lesion sites by avoiding biological barriers in the human body such as limited gastrointestinal absorption and high hepatic first-pass effect (Rijcken, Soga, & Hennink, 2007). In recent years, polymeric micelles have also been studied as an oral drug delivery system (Huang, Gu, & Lu, 2008; Huang, Gu, & Lu, 2009; Kim, Kim, & Huh, 2008; Mathot, van Beijsterveldt, & Préat, 2006; Sant et al., 2005). In general, these micelles are required to be pH-dependent for the release at different stages in the gastrointestinal (GI) tract. For example, drug-loaded micelles can be stable at the low pH of the stomach and disaggregate at the near physiological pH of the intestine or the colon to release the drugs. Therefore, polymers for constructing an oral delivery system should be composed of pH-sensitive moieties, e.g., acrylic acid (AA) (Kim et al., 2008). However, not all synthetic polymers with pH-sensitive moieties are appropriate for oral delivery system. Many other factors such as non-toxicity, biodegradation and metabolism should be taken into account and thereby lead to the limitation of the diversity of polymers. To develop a safe and efficient drug carrier for oral delivery, natural polymers may be an appropriate option.

Sodium alginate (SA) is an excellent candidate to fulfill the abovementioned requirements due to its unique properties, such as good biocompatibility, good biodegradability, low immunogenicity, non-toxicity and environmental sensitivity (Becker, Kipke, & Brandon, 2001; Martins, Sarmento, & Souto, 2007; Rowley, Madlambayan, & Mooney, 1999; Yang, Zhang, & Wen, 2007). It is also a relatively inexpensive raw material which can be

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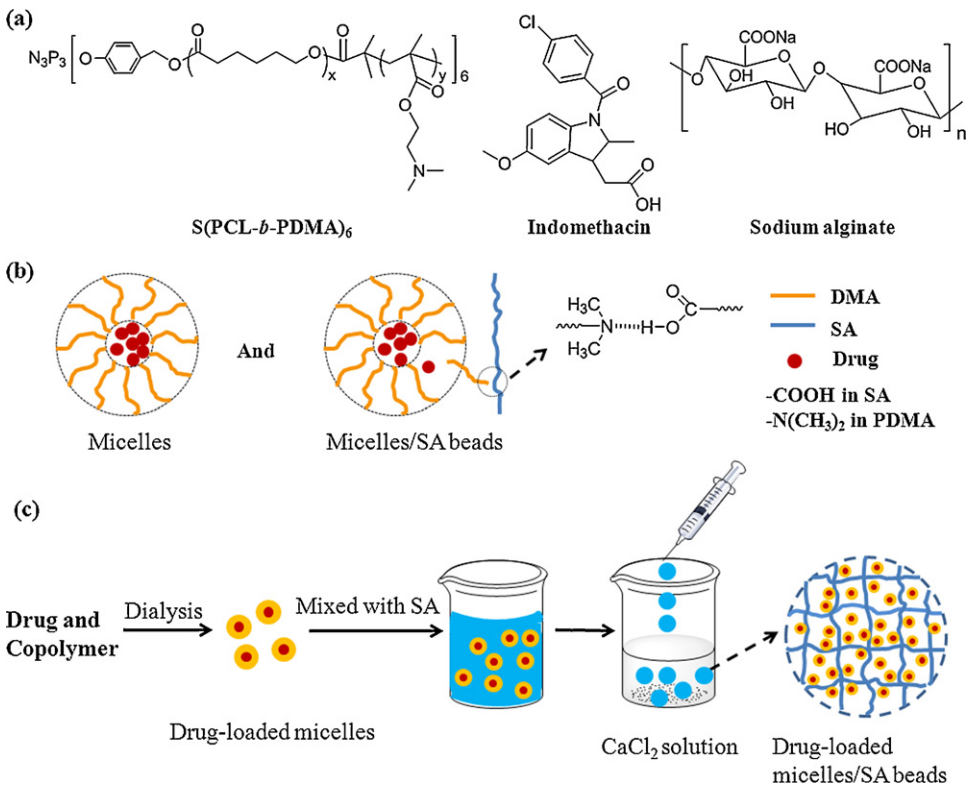


**Fig. 1.** (a) The chemical structures of six-arm block copolymer and indomethacin, (b) interaction of carboxyl groups and tertiary amine groups, and (c) schematic diagram of the fabrication of drug-loaded micelles/SA beads.

abundantly extracted from marine brown algae (Shilpa, Agrawal, & Ray, 2003). In addition, SA can be readily crosslinked by the addition of di- and trivalent cations in aqueous solution, such as $Ca^{2+}$, $Sr^{2+}$, $Ba^{2+}$ or $Fe^{3+}$, $Al^{3+}$ (Shilpa et al., 2003), to form a three-dimensional network. Among them, the calcium ion is widely used in the fabrication of sodium alginate gels by chelating calcium, forming the so called 'egg-box' structure (Ferreira Almeida & Almeida, 2004; Nochos, Douroumis, & Bouropoulos, 2008; Shilpa et al., 2003). The prominent feature of using such alginate gels as a drug carrier is that they can protect an acid-sensitive drug from gastric juices, and then release the drug in the intestine (Pongjanyakul & Rongthong, 2010; Shiraishi, Imai, & Iwaoka, 1991). From an economic and effective perspective, sodium alginate gels have great potential for oral administration.

In order to optimize the release property of the delivery system, the combination of two or more matrices to form a composite vehicle would be a more promising system which can overcome the drawbacks of using a single material. For example, Wei, Cai, and Lin (2009) have developed the poly(vinyl alcohol) (PVA) hydrogel/polypeptide micelle composites which showed the independent release behaviors of doxorubicin (DOX) and aspirin (Asp) due to the pH and temperature sensitivities. Zhang, Wang, and Wang (2010) have reported that the biopolymer/inorganic material composites could overcome the burst release of the drug. They also pointed out that incorporating vermiculite (VMT) into the chitosan-*graft*-poly(acrylic acid)/sodium alginate composite hydrogel substantially slowed down the release rate (Wang, Xie, & Zhang, 2010). Based on the above background, the combination of biopolymer and micelles could be a feasible way to develop a promising carrier system for oral delivery of unstable or insoluble drugs. In this study, micelles/SA composite gel beads were designed as a novel delivery system for oral formulations. The main concept was that the polymeric micelles were utilized to solubilize the water-insoluble drugs and then the drug-loaded micelles were encapsulated into the SA matrix. The morphology and swelling property of the gel beads were characterized by scanning electron microscopy (SEM) and a gravimetric method, respectively. The drug loading level and release behavior were systematically evaluated using indomethacin (IND) as a model drug.

## 2. Experimental

### 2.1. Materials

Indomethacin (IND) was supplied by Aladdin (Shanghai, China). Sodium alginate (SA, a viscosity of 0.02 Pa s in 1% aqueous solution at 20 °C) was purchased from the Beijing Chemical Reagents Co. (Beijing, China). The number average molecular weight of SA was $1.1 \times 10^5$, determined by the size exclusion chromatography (SEC). All other chemicals were purchased from Shanghai Chemical Reagents Co. (Shanghai, China) and used as received unless otherwise mentioned. The designed amphiphilic six-arm block copolymer poly($\varepsilon$-caprolactone)-*block*-poly(dimethylamino)ethyl methacrylate S(PCL-*b*-PDMA)$_6$ was synthesized by ring-opening polymerization (ROP) and atom transfer radical polymerization (ATRP) as described in an earlier publication (Huang, Xiao, & Lang, 2011). The structures of S(PCL-*b*-PDMA)$_6$, IND and SA are shown in Fig. 1(a). The number-average molecular weight molecular ($M_n$) and molecular weight distribution ($M_w/M_n$) of S(PCL-*b*-PDMA)$_6$ were 41 100 and 1.24, determined by $^1$H NMR and SEC, respectively.

### 2.2. Preparation of drug-loaded matrices

#### 2.2.1. Drug-loaded micelles

IND-loaded polymeric micelles were prepared by dialysis (Rapoport, 2007). Briefly, the copolymer S(PCL-*b*-PDMA)$_6$ and IND in the appropriate weight ratio were co-dissolved in *N*,*N*-dimethylformamide (DMF) with a final polymer concentration of

10 mg/mL, followed by the addition of ultrapure water (40 mL) for over 4 h under stirring. The solution was then placed into the dialysis membrane (MWCO: 3.5 kDa) and dialyzed against ultrapure water for 24 h at room temperature to remove the organic solvent and unloaded IND dissolved in aqueous solution. After filtering through a 0.45-μm microporous membrane to remove aggregated particles, a part of the solution was frozen and lyophilized to calculate the concentration and yield. The remaining part of the solution was stored before use.

#### 2.2.2. Drug-loaded micelles/SA beads

SA beads are traditionally prepared by extrusion through a syringe needle into a calcium chloride ($CaCl_2$) solution. The fabrication of drug-loaded micelles/SA beads is shown in Fig. 1(c). Typically, 1 g of SA was dissolved in the aforementioned drug-loaded micelle solution with agitation at a final concentration of 2% (w/v). The mixture solution (5 mL) was dropped into a $CaCl_2$ solution (20 mL, 6%, w/v) with gentle stirring. The gel beads were allowed to crosslink with $Ca^{2+}$ immediately. Subsequently, the beads in the solution were incubated for 8 min at room temperature. The resultant beads were rinsed twice with distilled water to remove the unreacted $Ca^{2+}$ on their surface and subsequently freeze-dried for two days. Several samples were prepared at different concentrations of SA and $CaCl_2$ and a constant micelle concentration of 1 mg/mL. The related data on the beads are summarized in Table 2.

#### 2.2.3. Drug-loaded SA beads

Drug-loaded SA beads were prepared by the suspension method. Typically, 10 mg of IND was directly suspended in 50 mL of SA solution (2%, w/v) and drastically stirred for 24 h to produce a well-dispersed mixture. The suspension was dropped into a $CaCl_2$ solution (6%, w/v), followed by rinsing and freeze drying as described above.

### 2.3. Swelling studies

Swelling behavior was studied by a gravimetric method. Dry samples were immersed in a 0.1 M HCl solution at 37 °C for 2 h, and subsequently transferred into a phosphate buffer solution (pH 6.8), simulating the gastrointestinal tract conditions. The swollen weight was measured for each sample at a given time after excess surface water was wiped off carefully with filter papers. All experiments were performed in triplicate. The swelling ratio ($Q_s$) was calculated according to the following expression:

$$Q_s = \frac{W_t - W_d}{W_d}$$

where $W_t$ and $W_d$ denoted the weight of the swollen at time $t$ and dry samples, respectively.

### 2.4. In vitro drug release studies

Drug-loaded micelles or beads were put in a dialysis bag, and then immersed in a vial containing 100 mL of three different media: (a) HCl solution and 10% (v/v) of ethanol, pH 1.2; (b) phosphate buffer solution (PBS), pH 6.8; and (c) PBS, pH 7.4. The vial was placed in a horizontally shaking incubator at 37 ± 0.5 °C. At predetermined intervals, a 5 mL aliquot of the test solution was withdrawn periodically and replaced with an equal volume of the fresh release medium. The drug concentration in the removed solution was determined by measuring the absorbance at 320 nm in a UV–vis spectrophotometer.

To mimic the drug release in the GI tract, the release behavior of the drug-loaded matrices (micelles, SA beads and micelles/SA beads) was carried out according to a previously reported method (Liu, Chen, & Xie, 2003). The sample was tested in 100 mL of 0.1 M HCl (pH 1.2, without ethanol) for 2 h, as the average gastric emptying time is about 2 h. Then the medium was replaced with 100 mL of PBS (pH 6.8) and tested for 3 h, as the average small intestinal transit time is about 3 h. Subsequently, 100 mL of PBS (pH 7.4) was used to test for 8 h. A 5 mL of the test solution was taken at suitable time intervals and an equal volume of fresh HCl or PBS was replaced into the release medium to maintain constant volume. The amount of IND released was analyzed as described above.

### 2.5. Determination of drug loading level

#### 2.5.1. Drug-loaded micelles

The amount of IND encapsulated in the micelles was measured by a UV–vis spectrophotometer (725, Shanghai). 4 mL of DMF was introduced into 0.1 mL of IND-loaded micelle solution and 0.9 mL of $H_2O$. The micelles were broken up by ultrasonic vibration for 10 min and IND was dissolved in the solution. The characteristic absorbance of IND at 320 nm was recorded and compared with a standard curve generated from a DMF/$H_2O$ (v/v = 4/1) mixture. The drug loading content (DLC) and entrapment efficiency (EE) were calculated using the following Eqs. (1) and (2).

$$\text{Drug loading content } (\%\text{DLC}) = \frac{\text{weight of drug in micelles}}{\text{weight of drug-loaded micelles}} \quad (1)$$

$$\text{Encapsulation efficiency } (\%\text{EE}) = \frac{\text{weight of drug in micelles}}{\text{weight of drug initially added}} \quad (2)$$

#### 2.5.2. Drug-loaded micelles/SA beads

A certain amount of the dry drug-loaded beads were placed in 2 mL of pH 7.4 buffer solution and sonicated for 5 min. 4 mL of DMF was added to the solution, which can facilitate the extraction of IND but not SA, and then sonicated again. The mixture was centrifuged to remove the undissolved substance and the supernatant solution was detected by a UV–vis spectrophotometer at 320 nm. The drug loading was calculated according to the standard curve obtained from IND in a DMF/$H_2O$ mixture. The drug loading content (DLC) was calculated using Eq. (1).

#### 2.5.3. Drug-loaded SA beads

The amount of IND loaded in the SA beads was analyzed using the same method as mentioned above.

### 2.6. Morphology of drug-loaded micelles/SA beads

The morphology of the drug-loaded micelles/SA beads was examined using scanning electron microscope (SEM, JSM-6360LV), equipped with a backscatter detector and a PCI digital imaging system at an accelerating voltage of 15 kV. Cross-sectioned samples were prepared by fracturing beads in liquid nitrogen. Samples were sputtered with gold under vacuum before observation.

## 3. Results and discussion

### 3.1. Preparation and characterization of drug-loaded micelles/SA beads

The beads were prepared by dropping an aqueous solution of SA into a $CaCl_2$ solution. This dripping method has been extensively applied because the process is convenient to handle and the

raw materials are relatively cheap. And more importantly, toxic solvent can be avoided. It has been reported that the properties of the beads, e.g., particle size, morphology, swelling, and release behavior, were dependent on the viscosity of SA, chemical compositions, $CaCl_2$ concentration, curing time and so on (Liew, Chan, & Ching, 2006; Shilpa et al., 2003; Liu et al., 2003). Here two factors, SA and $CaCl_2$ concentrations were mainly considered.

To prepare the drug-loaded micelles/SA beads, SA was dissolved in the IND-loaded micelle solution with a final concentration of 1.5% or 2% (w/v), and then the obtained solution was added dropwise into a $CaCl_2$ solution (3% or 6%, w/v) (Fig. 1(c)). Due to the rapid crosslinking, the gel beads were formed instantaneously once the SA contacted with $Ca^{2+}$ in the solution before their shape was distorted. Subsequently, the beads were incubated for 8 min at room temperature to promote crosslinking and to minimize the IND-loaded micelle loss by diffusion into the surrounding medium. In the experiment, it was observed that the beads obtained from 1.0% (w/v) SA were very fragile and sticky. This could be attributed to the fact that more porous structures were formed in the gel beads at a given $CaCl_2$ concentration as the number of macromolecules per unit solution volume decreased (Liu et al., 2003). On the other hand, a high SA concentration (>3%, w/v) was not feasible because the viscosity of SA solution was too high to extrude the solution through the needle. Thus, 1.5% and 2% SA were investigated in this study.

The morphology of drug-loaded micelles/SA beads was investigated through digital photographs and SEM. As seen in Fig. 2(a and b), the beads in the wet state exhibited a spherical shape and a smooth surface with a diameter of about 2.5 mm. After freeze drying, the beads were approximately spherical in shape (about 1.4 mm in diameter) and had a rough surface. SEM was further used to characterize the microstructure of the beads, as illustrated in Fig. 2(c and d). It can be noticed that the surface of the beads was highly wrinkled and many cavities were in the internal zones. This structure may be beneficial for the diffusion of drugs. This was because the porous structure could generate the capillary forces which facilitated the penetration of fluids into the beads and thereby the drug easily diffused into water (George & Abraham, 2007).

The wet beads were dried by the freeze-drying technique because of the following factors. Firstly, freeze-drying can preserve the initial morphology of the beads (Abubakr, Lin, & Chen, 2009). In the case of oven/air drying, sodium alginate shrinks and collapses severely and thus the beads lose their initial morphology. After freeze drying, the shrinkage of SA was greatly suppressed and the morphology was mostly maintained because SA was in the glassy state. Secondly, SA was protected from thermal decomposition which might be caused by the high temperature in the oven-drying process (Wong, Chan, & Kho, 2002). Furthermore, when water was quickly frozen, the drug-loaded micelles could be fixed in the SA beads rather than diffusing into an external medium. Thus, the freeze-drying technique was considered to be superior to the air-drying and oven-drying and was adopted in this study.

**Table 1a**
Characteristics of IND-loaded micelles.

| Copolymer/IND ratio (w/w) | Theoretical DLC (wt%) | Actual DLC (wt%) | EE (wt%) |
|---|---|---|---|
| 10/1 | 9.1 | 8.44 | 92.2 |
| 10/3 | 23.1 | 21.7 | 92.4 |
| 10/5 | 33.3 | 31.9 | 93.9 |
| 10/8 | 44.4 | 43.2 | 95.0 |
| 10/10 | 50.0 | 48.0 | 92.2 |
| 10/15 | 60.0 | 58.7 | 95.0 |

**Table 1b**
Characteristics of SA beads without micelles.

| SA/IND (w/w)[a] | DLC (wt%) | EE (wt%) | Beads diameter[b] (mm ±SD) |
|---|---|---|---|
| 200/10 | 1.23 | 47.2 | 1.36 ± 0.09 |
| 200/15 | 2.27 | 65.1 | 1.43 ± 0.12 |
| 200/25 | 5.05 | 78.2 | 1.52 ± 0.11 |
| 200/35 | 6.28 | 82.8 | 1.56 ± 0.14 |
| 200/45 | 8.29 | 82.0 | 1.49 ± 0.14 |

[a] The concentrations of SA and $CaCl_2$ were 2% (w/v) and 6% (w/v), respectively. The aperture of needle was 0.5 mm except for the SA/IND ratio of 200/10 (0.45 mm).
[b] The mean diameters of dry beads were determined by vernier caliper ($n = 20$).

### 3.2. Incorporation of drug into various matrices

IND-loaded micelles were prepared by dialysis. As summarized in Table 1a, with increasing amount of the drug in the feed, the actual drug loading content (DLC) increased accordingly. The actual value was close to the corresponding theoretical one. These results indicated that the incorporation capacity of the micelles was quite high. In addition, the entrapment efficiency (EE) was relatively high but no clear tendency could be observed. The obtained values of the actual DLC were calculated by assuming that there was no loss of the copolymer throughout the incorporation process. This assumption was based on the fact that the yield of drug-loaded micelles was higher than 97% and no precipitates or aggregates was observed in the dialysis process, suggesting that the micelles were stable enough to encapsulate the drug. The result showed that the EE of IND was higher than that of other IND-loaded micelles (Zhang et al., 2008), which could be attributed to the highly hydrophobic nature of the drug and the strong hydrophobic interactions between the micelles and the drugs. In this study, the weight ratio of copolymer to IND was selected as 10/15 for the subsequent tests due to the low copolymer content and the high DLC.

For comparison, the IND-loaded SA beads without polymeric micelles were also fabricated. As presented in Table 1b, the DLC increased as the ratio of SA/IND decreased and the EE was higher than 45%. However, the higher IND content in the feed, the larger the aperture of needle needed. This was because IND could not form a homogeneous solution with SA but could only be dispersed as fine particles in a SA solution due to its low solubility in water. Such a suspension would often block the needle during the preparation process and the pinhole should be changed to a larger one,

**Table 2**
Preparation condition, properties and release characteristics of drug-load micelles/SA beads.

| Sample[a] | SA (%, w/v) | $CaCl_2$ (%, w/v) | Beads diameter[b] (mm ±SD) | DLC (wt% ±SD) | EE (wt% ±SD) | pH 6.8[c] (%) | pH 7.4[d] (%) |
|---|---|---|---|---|---|---|---|
| 1 | 1.5 | 3 | 1.59 ± 0.07 | 2.041 ± 0.018 | 33.7 ± 0.3 | 63.2 | 26.7 |
| 2 | 1.5 | 6 | 1.49 ± 0.13 | 1.898 ± 0.019 | 41.1 ± 0.4 | 60.1 | 29.2 |
| 3 | 2 | 3 | 1.56 ± 0.09 | 2.127 ± 0.020 | 40.5 ± 0.4 | 47.3 | 41.5 |
| 4 | 2 | 6 | 1.43 ± 0.10 | 1.123 ± 0.003 | 27.4 ± 0.1 | 26.4 | 44.3 |

[a] The final concentration of micelle solution was 1 mg/mL. The aperture of needle was 0.45 mm.
[b] The mean diameters of dry beads were determined by vernier caliper ($n = 20$).
[c] The release amount of drug in the period of 2–5 h.
[d] The release amount of drug in the period of 5–13 h.

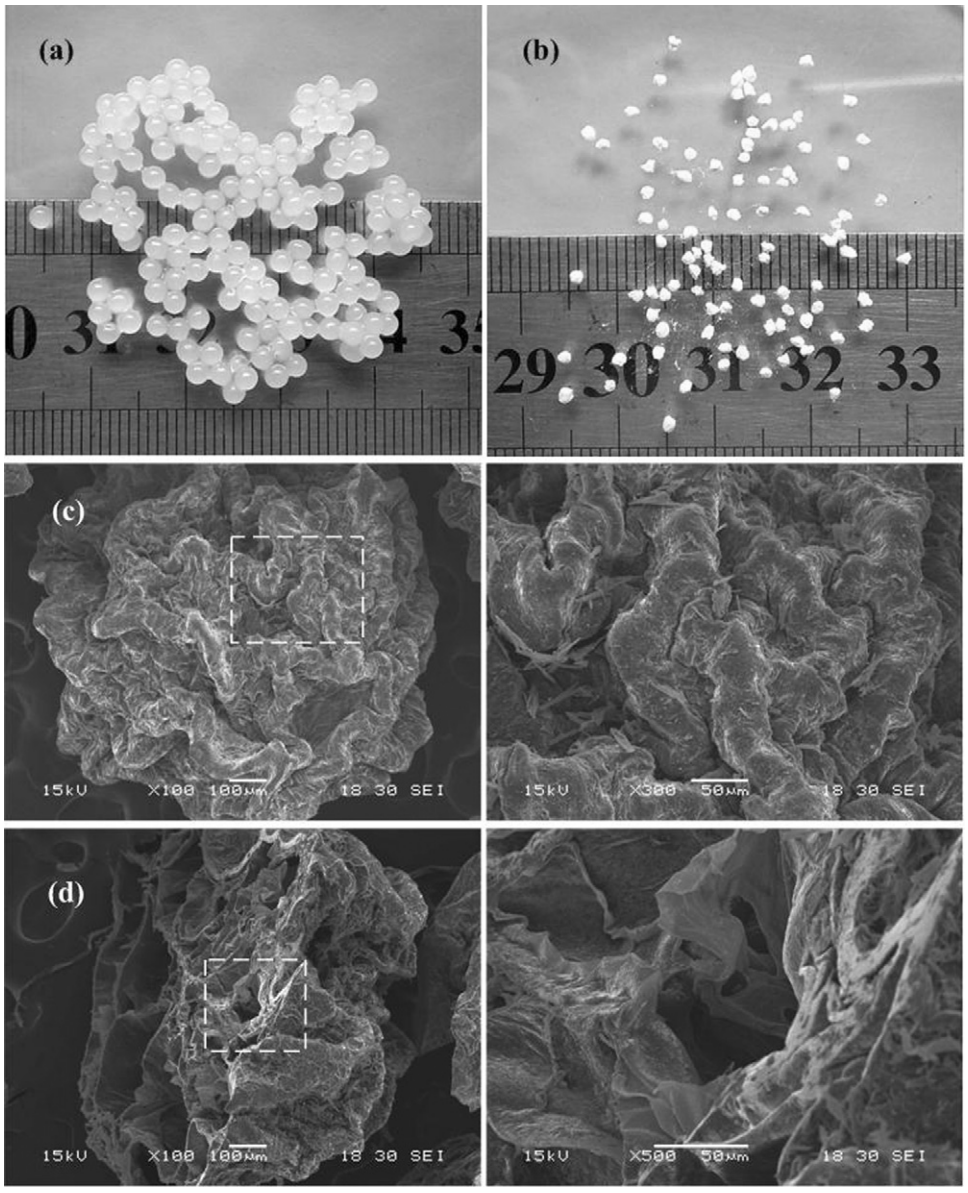


**Fig. 2.** The morphology of drug-loaded micelles/SA beads (2% SA, 3% $CaCl_2$). Digital photographs: (a) in wet state and (b) in dry state; SEM images: (c) surface, (d) cross section and the magnification of the area indicated in c and d.

which resulted in forming larger beads. Therefore it is impossible to fabricate IND-loaded SA beads in practice.

Different concentrations of SA and $CaCl_2$ were used to fabricate the drug-load micelles/SA beads. As shown in Table 2, the concentrations of SA and $CaCl_2$ had great effects on the properties and the release behavior (discussed later) of the drug-loaded micelles/SA beads. It was observed that for a given SA concentration, the higher the concentration of $CaCl_2$ was, the lower the DLC and the diameter became. This can be explained by the crosslinking density of the gel. For 1.5% or 2% SA, as the $CaCl_2$ concentration increased, the crosslinking density was increased accordingly, resulting in the shrinkage of the beads. The constricted structure would drive the drug-loaded micelles rapidly diffuse into the medium during the preparation process and result in more drug loss.

### 3.3. Swelling characteristics of SA beads

Swelling behavior is a very important property for drug delivery system because it has a great influence on the drug release behavior (Wang et al., 2010). Fig. 3 shows the swelling ratio (SR) as a function of time for blank SA beads in a simulated GI tract at 37 °C. The SA beads were fabricated at different concentrations of SA and $CaCl_2$ and immersed at pH 1.2 for 2 h followed by pH 6.8 for a predetermined time. It was found that the SR of the test beads at pH 1.2 was low and quickly reached the steady state within 30 min. The increase in SR was caused by the water penetration into the cavities in the beads. Since water did not swell the polymer network, the shape of the beads was not changed much. This may be beneficial for limiting the drug diffusion. At pH 6.8, the SR of the beads increased significantly within 15 min compared with that at pH 1.2. Subsequently, the SR reduced rapidly because the swelling of the beads occurred in parallel with the disintegration which decreased the weight of the beads. Similar observations have been reported by Dai, Li, and Zhang (2008).

Note that the SR of beads at pH 1.2 was low because the insoluble alginic acid was formed, caused by the protonated carboxyl groups (–COOH) in SA (Işıklan, İnal & Yiğitoğlu, 2008). At pH 6.8, the carboxyl groups in SA became ionized ($–COO^-$) and thus the intermolecular electrostatic repulsion forces were enhanced (Chen, Wu,

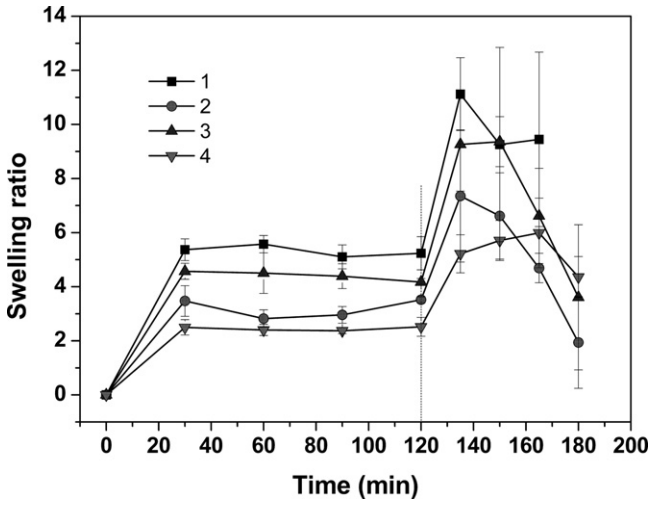


**Fig. 3.** The swelling behavior of dry SA beads in simulated GI tract at 37 °C ($n = 3$): 1–1.5% SA, 3% $CaCl_2$; 2–1.5% SA, 6% $CaCl_2$; 3–2% SA, 3% $CaCl_2$; 4–2% SA, 6% $CaCl_2$.

& Mi, 2004). More water could enter into the network, resulting in the increase of the SR.

The swelling property of SA beads at different pH values was beneficial for drugs which were acid sensitive. Due to the low SR at pH 1.2, the drugs could be fixed in the SA beads and thereby could be protected from the attack of the gastric juices. As pH increased to 6.8, the drug would be quickly released due to the rapid swelling and the disintegration of the beads. As shown in Fig. 3, the SR of the SA beads at pH 1.2 increased with decreasing $CaCl_2$ concentration. For a given $CaCl_2$ concentration, the higher SA content in the beads, the lower the SR value. This SR value varied with the crosslinking density of the network. When the crosslinking density was lower, the SA exhibited a more expanded conformation and more water entered into the polymeric networks. Moreover, it seemed difficult to find out the distinct varying trend on the SR at pH 6.8, because the swelling and the disintegration rates of SA beads were too fast to be detected precisely. Even so, the SR of the SA beads with time and pH may be beneficial to control the drug release.

### 3.4. In vitro release studies

#### 3.4.1. Drug release from various matrices at different pH

The pH-sensitivity of the oral carrier is a significant factor for controlling drug release behavior. We first examined the release behavior of IND from various matrices including micelles, SA beads and micelles/SA beads at pH 1.2, 6.8 and 7.4, respectively. 10% (v/v) of ethanol was added into the pH 1.2 medium to generate a sink condition due to the low solubility of IND at pH 1.2. Fig. 4(a) shows the release profiles of IND from the micelles in different media. In the acidic medium, the release was quite slow and only 20% of IND was released at the end of 12 h. However, the release rates at pH 6.8 and 7.4 were nearly identical but much quicker than that at pH 1.2. This significant difference of release behavior in acidic and near neutral solutions can be explained by several factors, including the low solubility of IND ($pK_a$ of IND is ~4.5 Zhang et al., 2008), the hydrogen bonding and the hydrophobic interactions between IND and PCL segments in the micelle core. Below $pK_a$, IND was molecularly distributed in the micelle cores so the carboxyl groups of IND associated with each other (as shown in Fig. 1(b)). Such interaction thereby limited the mobility of IND. At pH >4.5, however, the carboxyl groups of IND became ionized and thereby the interaction between IND and the micelles was weakened, which caused an easy escape of IND from the micelles. Notice that 100% of IND was released in 6–7 h at pH 7 nearby, which was shorter than

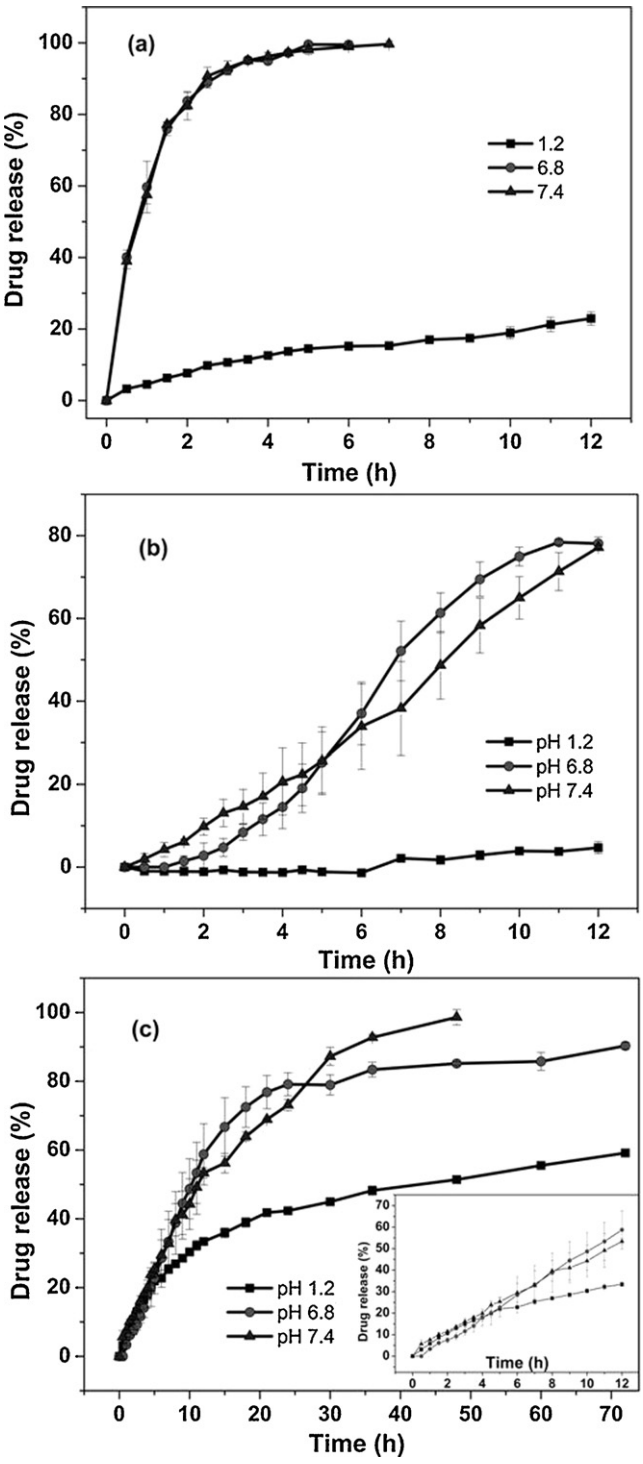


**Fig. 4.** Release profiles of IND from (a) micelles, (b) SA beads and (c) micelles/SA beads at different pH values (pH 1.2: HCl solution and 10% alcohol; pH 6.8 and pH 7.4: phosphate buffer solution) ($n = 3$).

the previously reported values for the release of IND (Michailova, Berlinova, & Iliev, 2010). This might be due to the higher amount of IND loaded in the micelles. On one hand, a high concentration gradient would accelerate the release. On the other hand, when the molar ratio of polymer to IND was reduced, IND passed through fewer channels and leaked from the micelles more easily.

It was observed that the release of IND from the SA beads was slower than that from the micelles in the same medium (Fig. 4(b)). At pH 1.2, less than 5% of IND could be released within 12 h, whereas

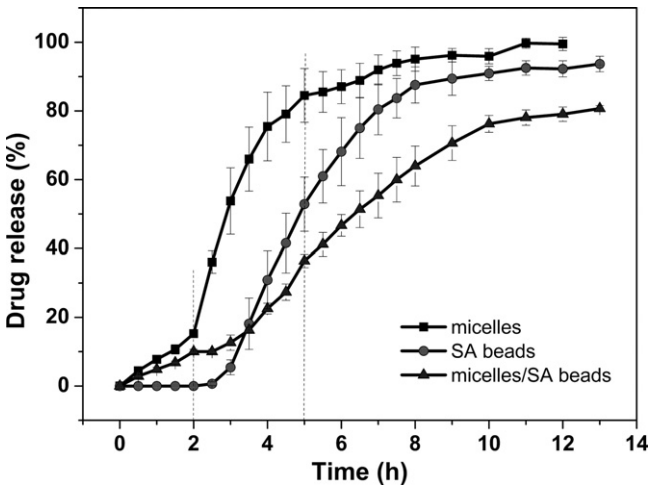


**Fig. 5.** Release profiles of IND from micelles, SA beads and micelles/SA beads in the simulated GI tract. Samples were tested at pH 1.2 for 2 h, followed by pH 6.8 for 3 h and then pH 7.4 for 8 h ($n = 3$).

at pH 6.8 or 7.4, about 80% was observed. The time taken for 50% release, $T_{0.5}$, was 7 h and 8 h at pH 6.8 and 7.4, respectively, which was much longer than that of the micelles. At pH 1.2, the release amount of IND was zero within 6 h. This could be attributed to the low swelling of the SA beads in the acidic medium which hampered the drug diffusion. At pH 6.8 and pH 7.4, a rapid release was observed due to the swelling of the beads and followed by disintegration. These results indicated that the drug could be protected from premature degradation in the gastric fluid by the SA beads and could be rapidly released in the small intestine and the colon.

The release behavior of IND from the micelles/SA beads was further examined. Fig. 4(c) illustrates the release profiles of IND from the micelles/SA beads in the release media. The inset in Fig. 4(c) shows the local enlarged release profiles within 12 h. The profiles revealed that IND had a sustained and long-term release for at least two days. $T_{0.5}$ was 36 h, 10.5 h and 11 h at pH 1.2, 6.8 and 7.4, respectively. The release rates at pH 6.8 and 7.4 were nearly the same within 12 h, afterward the rate at pH 7.4 was slower than that at pH 6.8 until 25 h. 100% of IND could be released at pH 7.4 within 48 h, whereas 90% came to be an equilibration at pH 6.8 within 72 h. The release amount of IND from the micelles, SA beads and micelles/SA beads at pH 1.2 was 20%, 5% and 30% within 12 h, respectively. It was found that IND release from the micelles at pH 1.2 was faster than that from the SA beads without micelles. The difference might be ascribed to the fact that IND was molecularly incorporated into the micelle cores, which made IND diffusion much easier (Carstens, de Jong, & van Nostrum, 2008). While IND was dispersed as particles in the SA matrix, it was more difficult to be released due to its insolubility in water. A comparison of IND release from the micelles/SA beads and micelles revealed that the former was faster. It was proposed that the strong interaction between the tertiary amine groups ($-N(CH_3)_2$) on the micelle shell and the carboxyl groups (–COOH) in the SA might destabilize the micelles and thus transform the system, which led to the easier diffusion of IND to the medium (as shown in Fig. 1(b)). Impressively, both the SA beads and the micelles/SA beads could prolong the release time and protect the stomach from the drug irritation, which implied that the two matrices might be suitable for drug delivery by oral administration.

### 3.4.2. Drug release from various matrices in the GI tract

It is known that the pH environment in the gastrointestinal tract varies from acidic to slightly alkaline. Fig. 5 represents the release of IND from different matrices including micelles, SA beads and micelles/SA beads in the simulated GI tract over 13 h. The SA beads and the micelles/SA beads were fabricated using 2% SA and 6% $CaCl_2$. Here the drug release behavior has been modified to simulate the *in vivo* condition with respect to both pH and time. The sample was maintained at pH 1.2 for 2 h, followed by pH 6.8 for 3 h and then pH 7.4 for 8 h. It showed that there was a significant difference in the release behavior. The quantity of IND in the micelles reached 84.5% in the initial 5 h, and only 15% of IND was leaked at pH 7.4. In contrast, the release could be significantly delayed by the introduction of SA, which might be related to the swelling behavior and the diffusion barrier of the beads. For the curve of SA beads, no release was observed in the acidic medium, but after the medium was turned into pH 6.8, it showed a steep initial release of about 52.8% in 5 h and sustained release to 93.7% at the end of 13 h. Therefore 52.8% and 40.9% of IND was utilized in the intestine and the colon, respectively. This fast release at pH 6.8 could be attributed to the swelling and the disintegration of the beads, as previously analyzed. For the curve of micelles/SA beads, it showed that only a small amount of IND (10%) was released at pH 1.2 and the cumulative release reached 36.4% within the first 5 h, and then gently went up to 80.7% at the end of 13 h. Thus 44.3% of IND was absorbed in the colon. Such matrix may be beneficial for the colonic treatment.

It is expected that drug release from any oral drug formulation is completed within 12–13 h to avoid the drug loss and maximize the bioavailability. As seen from the results, it showed that IND in the SA beads or the micelles/SA beads could be effectively delivered to the intestine and the colon and the side effect of IND in the stomach could be minimized, whereas IND in the micelles could not be used as an oral delivery system. From the view of the gastric damage, IND in the SA beads was minimal; from the view of the bioavailability, a large amount of IND in the micelles/SA beads was released in the colon and the release was gentle; from the view of fabrication, IND in the micelles/SA beads was more available because the IND-loaded micelles could be well dispersed in the SA matrix and would not clog the needles. Based on the above points, it demonstrated that the micelles/SA beads were more attractive for the oral drug delivery system.

### 3.4.3. Drug release from micelles/SA beads with different SA and $CaCl_2$ concentrations in the GI tract

We further investigated the release behavior of the drug-loaded micelle/SA beads in the simulated GI tract. The beads were maintained at pH 1.2 for 2 h, followed by pH 6.8 for 3 h and then pH 7.4 for 8 h. These beads were fabricated at different concentrations of SA and $CaCl_2$ as previously mentioned in Table 2. The concentration of IND in the simulated fluids was fixed at 11.2 mg/L. As illustrated in Fig. 6, the release of IND from each sample at pH 1.2 was less than 10%, afterward it significantly increased at pH 6.8 and then slowed down at pH 7.4. The results indicated that the release of IND was minimal in the stomach and maximal in the intestine and the colon, corresponding to the swelling property of the SA beads at low and high pH, respectively. For Samples 2 and 4 with a given $CaCl_2$ concentration, it was observed that the higher the amount of SA, the lower the release percentage of IND at high pH. This trend was because the higher amount of SA provided the stronger resistance for the drug diffusion. Similar trend was also observed for Samples 1 and 3. Moreover, for Samples 1 and 2 with the same SA concentration, it was found that the higher the $CaCl_2$ concentration, the lower the release rate of IND. This was attributed to the crosslinking density of the beads. The high crosslinking density would inhibit the penetration of water through the beads and thus the diffusion of IND from the beads was slowed down. The results for Samples 3 and 4 were similar. As shown in Fig. 6 and Table 2, the beads obtained from 2% SA could effectively transfer IND to the colon, while those obtained from 1.5% SA could deliver IND to the intestine. Therefore the release rate and the site-specific delivery

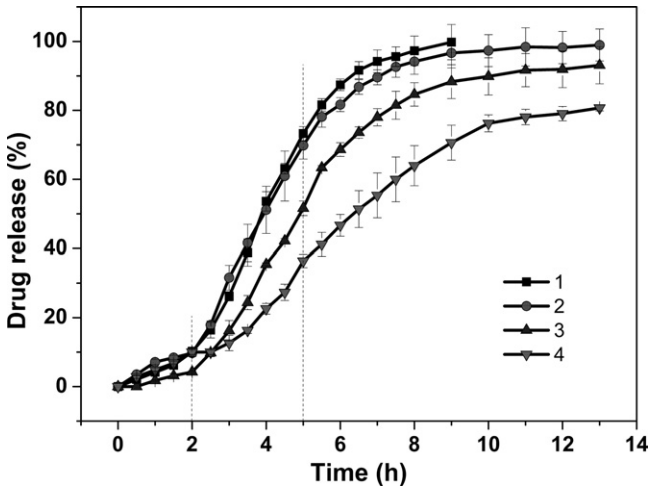


**Fig. 6.** Release profiles of IND-loaded micelle/SA beads in simulated gastrointestinal tract ($n = 3$). Samples: 1–1.5% SA, 3% $CaCl_2$; 2–1.5% SA, 6% $CaCl_2$; 3–2% SA, 3% $CaCl_2$; 4–2% SA, 6% $CaCl_2$. Samples were tested at pH 1.2 for 2 h, followed by pH 6.8 for 3 h and then pH 7.4 for 8 h.

of drugs can be controlled by altering the concentrations of SA and $CaCl_2$.

## 4. Conclusion

In this study, a new matrix for the oral delivery system based on the micelle/SA beads was successfully fabricated. For comparison, two other matrices (micelles and SA beads) were also prepared. The release of IND from each matrix in the acidic medium was much slower than that in the near neutral fluids, which might be due to the highly hydrophobic character of the drug and the enhanced interaction between the drug and the matrix in the acidic fluid. Moreover, the morphology and swelling behavior of SA beads were favorable for controlling the drug diffusion. The release behavior of the micelle/SA beads was investigated in the simulated GI tract. It was found that the release of IND was affected not only by the pH of the medium but also the concentrations of SA and $CaCl_2$. Importantly, the rate and the site-specific delivery of drugs could be controlled by altering the concentrations of SA and $CaCl_2$. The results suggested that the micelles/SA beads would be a good candidate for the oral drug delivery system.

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