ไฮโดรเจลของแคปปา-คาราจีแนนที่ถูกหุ้มด้วยโซเดียมอัลจิเนต สำหรับระบบนำส่งยาที่ตอบสนองต่อการเปลี่ยนแปลงค่าพีเอช

Kappa-Carrageenan/Sodium Alginate Based Core-Shell Hydrogels for pH Sensitive Drug Delivery Systems

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บทคัดย่อ

เม็ดไฮโดรเจลของ **K**-คาราจีแนน/โซเดียมอัลจิเนตที่มีลักษณะเป็นแกน-เปลือกถูกเตรียมโดยทำให้เกิดเจลสองขั้นสำหรับใช้เป็นระบบนำส่งยา ภาพถ่าย SEM ยืนยันว่า **K**-คาราจีแนนถูกหุ้มด้วยเปลือกของโซเดียมอัลจิเนต จากการศึกษาพฤติกรรมการบวมตัวในสภาวะคล้ายระบบทางเดินอาหารพบว่าไฮโดรเจลมีสมบัติตอบสนองต่อการเปลี่ยนแปลงพีเอช โดยบวมตัวได้น้อยในสภาวะกรดและการบวมตัวเพิ่มขึ้นในสภาวะเบส ผลการศึกษาพฤติกรรมการปลดปล่อยยาโดยใช้เมทิลีนบลูเป็นยาต้นแบบพบว่ามีหลายปัจจัยที่ส่งผลต่อการปลดปล่อยยา ได้แก่ ความหนาแน่นของการเชื่อมโยงระหว่างโมเลกุล ความหนาของเปลือกอัลจิเนต และลักษณะรูพรุนในแกนของไฮโดรเจล พบว่าการหุ้มไฮโดรเจลด้วยอัลจิเนตสามารถลดการปลดปล่อยยาในสภาวะคล้ายกระเพาะอาหาร และการปลดปล่อยยาในสภาวะคล้ายลำไส้มีประสิทธิภาพดีขึ้น โดยกลไกการปลดปล่อยสอดคล้องกับแบบจำลอง Hixson-Crowell จากผลการวิจัยแสดงให้เห็นว่าไฮโดรเจลที่เตรียมขึ้นมีศักยภาพสำหรับนำไปพัฒนาและประยุกต์ใช้เป็นวัสดุนำส่งยาที่มีประสิทธิภาพได้

คำสำคัญ : คาราจีแนน โซเดียมอัลจิเนต ระบบนำส่งยา ไฮโดรเจลแบบแกน-เปลือก

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Abstract

The κ-carrageenan/sodium alginate core-shell beads were prepared by 2-step ionic gelation method for drug delivery system. SEM micrographs confirmed the successful encapsulation of κ-carrageenan core with sodium alginate shell. The pH sensitive behavior of core-shell beads was evident in simulated gastrointestinal tract condition. Swelling of the beads increased when pH of the environment changed from acidic (pH 1.2) to alkaline (pH 7.4). The hydrogel beads were loaded with a model drug, methylene blue, and their release was studied. The release profiles were effected with different parameters in the preparation step, such as, crosslink density, thickness of shell and porosity of the core. It was observed that the encapsulation of κ-carrageenan with sodium alginate shell reduced the premature release of drug in the stomach mimicked condition and released the drug more specifically to the colon mimicked condition. The release mechanism was investigated and best fitted with Hixson-Crowell model. These results suggest that core-shell beads could be further developed as effective drug delivery system with pH sensitive drug release ability.

Keywords : carrageenan, sodium alginate, drug delivery, core-shell hydrogel

Introduction

Biopolymers and their derivatives have drawn increasing attention in medical field as carriers for controlled drug delivery due to their non-toxicity, renewability and biocompatibility (Zare-Akbari *et al,* 2016). The challenging task in development of drug delivery carriers is that the system should protect drug from acidic environment of the stomach and release drug more specifically to the colon. The carrageenans (CG) are naturally ionic polymers of linear polysaccharides extracted from red seaweed. Within the carrageenan family, Kappa-carrageenan (κ-CG) is extensively used as gelling-stabilizing and thickening agent in the food industry (Xu *et al*, 2014). It has one sulfate group per a disaccharide repeating unit and can interact with oppositely charged polymers or drug by ionic interaction (Navikaite *et al*, 2016). An advantage of κ-CG is ability to form hydrogel with green condition. However, its high swelling leads to burst initial drug release. This deficiency can be solved by blending or coating the κ-CG with other polymers with lower swelling characteristic such as sodium alginate. Sodium alginate (SA) is an anionic linear polysaccharide. SA gel is pH-sensitive due to the presence of carboxylic group along the backbone. SA based core-shell structured polymers are particularly attractive in pharmaceutical and encapsulation application. (Yue *et al*, 2016 and Lin *et al*, 2016). To the best of our knowledge, no work has been reported on drug delivery device based on κ-CG core and SA shell structure. In this study, a novel core-shell biopolymeric hydrogels as a drug delivery device in simulated gastrointestinal system (SGI) were investigated. In this work, κ-CG/SA blend hydrogel was prepared and investigated as a core-shell drug carrier for the controlled release of methylene blue, a model drug.

Materials and methods

Materials

κ-CG (product number C1804 having viscosity > 10.0 mPa.S, 0.3% in H_2O at 25°C) was received from Tokyo Chemical Industry. SA (food grade CAS No. 9005.38-3) was purchased from Loba Chemie. Other chemicals were reagent grade and used without purification.

Preparation of core-shell beads

2.5% **κ**-CG solution was prepared in distilled water with continuous stirring at 80 °C. Methylene blue (MB) was added into this solution if drug loaded beads were required. The solution was transferred to a syringe and added drop-wise into a stirred salt solution, KCl/$CaCl_2$. To complete the gelation, the spherical beads were maintained in the salt solution for 10 min, washed with distilled water, followed by drying at 80 °C for 2 h.

In order to prepared core-shell structure beads, the obtained core **κ**-CG beads were immersed in a SA solution (0.5 g SA in 100 mL distilled water), filtered then kept in $CaCl_2$ solution for 10 min, with a constant stirring to crosslink the SA shell. The core-shell hydrogel beads were washed and dried at 50 °C. The preparation procedure can be seen in Fig.1. Experimental conditions such as concentration of salt solutions and gelation time in SA solution were varied as shown in Table 1.

Table 1 Formulations of sample preparation

Sample	**κ**-CG (%w/v)	KCl/$CaCl_2$ [a] (M)	SA (%w/v)	$CaCl_2$ [b] (M)	Time [c] (min)	MB (mg)
C	2.5	0.7	-	-	-	-
S	-	-	2.5	0.3	-	-
SC	2.5	0.7	0.5	0.3	5	-
CMB	2.5	0.7	-	-	-	10
SCMB1	2.5	0.5	0.5	0.3	5	10
SCMB2	2.5	0.7	0.5	0.3	5	10
SCMB3	2.5	1	0.5	0.3	5	10
SCMB4	2.5	0.7	0.5	0.5	5	10
SCMB5	2.5	0.7	0.5	0.3	10	10
SCMB6	2.5	0.7	0.5	0.3	20	10

a: crosslinking solution for CG core b: crosslinking solution for SA shell c: Immersing time of the bead in SA solution

Characterization

Surface morphology of the beads was observed by scanning electron microscope (SEM) model LEO 1450 VP. The samples were adhered onto the stubs with conductive tapes, and coated with gold under high vacuum condition before SEM investigation.

Swelling behavior of the hydrogels was evaluated at pH 1.2 and pH 7.4. The samples were accurately weighed and placed in 20 mL of buffer solution. After the specific time interval, the swollen hydrogels were taken out, removed the excess solution and weighed. The swelling ratio was calculated by the following equation:

$$\text{Swelling ratio (\%)} = \left(\frac{W_s - W_d}{W_d}\right) \times 100 \quad (1)$$

where W_s and W_d are the weights of swollen and dry hydrogels, respectively.

In vitro drug release and kinetics modeling study

In vitro MB release profiles of the core **κ**-CG and core-shell hydrogels were studied both in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). At different time intervals, the release medium was withdrawn, and the same volume of fresh medium was replaced to maintain the constant volume. The amount of MB released was analyzed using a UV–visible spectrophotometer at 665 nm wavelength after suitable dilutions. From this, cumulative percentage drug release was calculated:

$$\text{Release drug (\%)} = \frac{R_t}{R\infty} \times 100 \quad (2)$$

where R_t and $R\infty$ represent the weights of released drug at time t (mg) and the total weight of drug release (mg), respectively.

To study the release mechanism, the experimental data obtained from in vitro MB release were fitted with 3 kinetic models, the Higuchi model (Fan *et al,* 2016), Korsmeyer–Peppas model (Korsmeyer *et al*, 1983) and Hixson–Crowell cube root law (Hixson & Crowell, 1931).

Results and Discussion

Morphology of core-shell hydrogel beads

SEM analysis was used to analyze morphology of the SC hydrogel and their cross-section images are shown in Fig.1. SEM micrographs present in general a spherical bead with smooth surface. The average beads size was in range of 1.5-2.2 mm. The **κ**-CG core is completely encapsulated with the SA shell with a distinct phase separation. The core-shell structure as a result of two-step gelation procedure was confirmed.

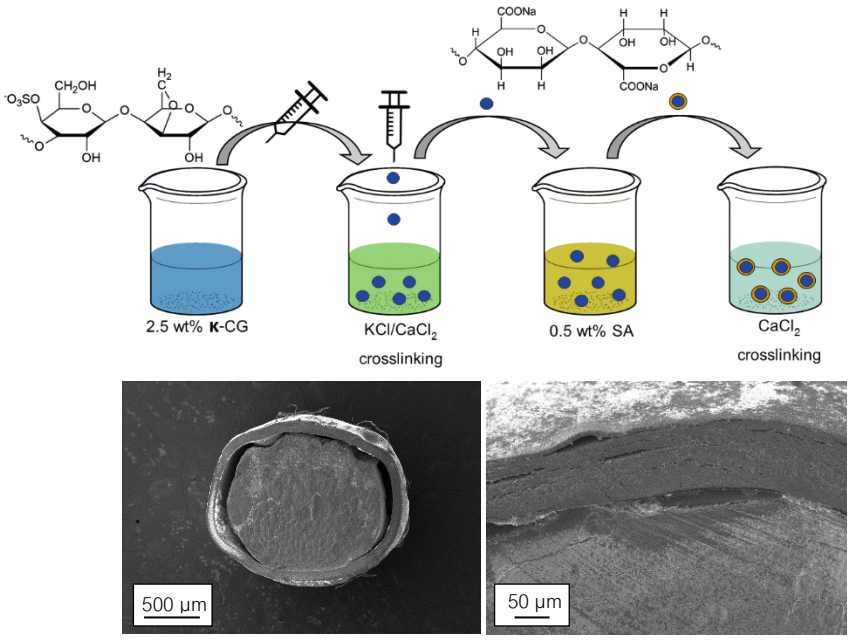

Figure 1 The preparation diagram and SEM micrographs of core-shell hydrogel beads

The swelling behavior

Swelling behavior of the prepared hydrogel was studied in order to gain a better understanding of the pH-sensitive core-shell carrier. This property is an important factor for drug delivery system, as it takes part in controlling the diffusion and release of drug from the device. The equilibrium swelling study of the **κ**-CG and SA beads was carried out in buffer solutions, pH 1.2 and pH 7.4. The results are shown in Fig. 2. As it can be seen, the swelling increased with time and pH. This pH-dependent swelling behavior was due to the carboxylate and sulfate groups of SA and **κ**-CG, respectively. In acidic solution (Fig. 2(A), the anionic charges along the polymeric chains were protonated. The gel structure was devoid of the charge resulting in the low swelling of hydrogels. In basic solution (Fig. 2(B), the carboxylate and sulfate groups became ionized, and the electrostatic repulsion caused the hydrogels to significantly swell. We also observed that SA hydrogel prepared in this work was more sensitive to the change of pH than the **κ**-CG. It was used as a shell matrix to protect the core **κ**-CG from releasing the drug in SGF and to enhance the release by swelling in SIF.

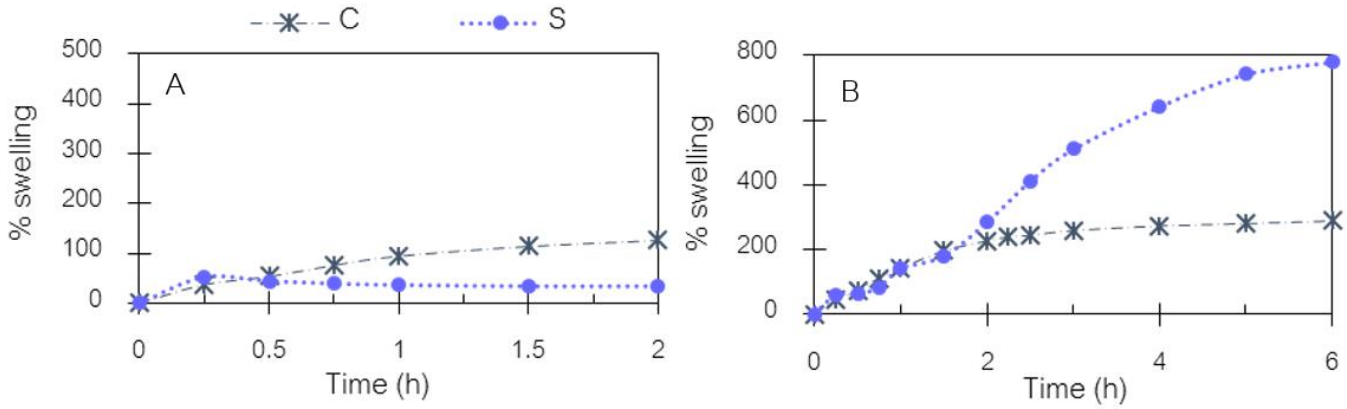

Figure 2 Swelling profiles of the beads in buffer solution pH 1.2 (A) and pH 7.4 (B), (C: κ-carrageenan and S. sodium alginate)

In vitro MB release study

The MB entrapment efficiency of κ-CG was measured before it was used for core-shell bead preparation and the value was 97.12%. To track the release behavior in response to simulated fluids, the release was studied in vitro at different pH environments: SGF, pH 1.2 for 2 h. and SIF, pH 7.4 for 6 h, respectively. The effect of crosslink density in core matrix on release characteristic was examined and the results are shown in Fig. 3. Release of MB from CMB, the κ-CG microgels without SA shell, occurred with a high rate. On the other hand, extended release profiles in simulated gastric condition were observed from core-shell samples, the SCMB2 and SCMB3, indicating that the release could be suppressed in stomach using a proper core-shell hydrogel matrix.

The effect of crosslink density in SA shell matrix on MB release profile was also investigated and the results are displayed in Fig.4. The SCMB4 prepared in higher concentration of crosslinking medium exhibited a slower and extended release comparing to the SCMB2, as higher crosslinking reduced the swelling and hindered the transport of MB through the shell matrix. In order to gain a better understanding on release behavior, the release experiments were conducted on the beads prepared from different periods of SA gelling time. We found that the immersing time strongly affected the initial release in simulated intestinal condition. The beads which were exposed to SA solution for 20 min released the drug significantly slower than those exposed for 5 and 10 min. Only 7.15% of MB was detected in the system after 5 h of the experiment, this could be explained in terms of slow penetration of fluid and delayed diffusion of MB from a dense shell network of SA. Digital photographs of the beads in SGF medium taken at 5 h of the test are shown as insets in Fig.5. The shells of SCMB2 were swollen and separated from the cores whereas the shells of SCMB5 and SCMB6 remained intact and encapsulated the core. This correlated well with the release profile of the samples. Comparing the beads with and without SA shell, SCMB2 and CMB2, it was evident that the core-shell structure benefited the

release behavior in that it helped to protect the drug from disintegration by acid and diminished the irritation in digestive tract.

For oral administration, the delivery matrix goes to the stomach and resides there for a certain period of time, then it passes along intestinal tract. The delivery matrix should inhibit the release of drug during 2 h incubated in stimulated gastric fluid and start releasing after the transfer to simulated intestinal medium. In the case of SCMB2, the beads only started drug releasing after 1.5 h transferring into the simulated intestinal medium, indicating that the drug was not effectively discharged to the target site.

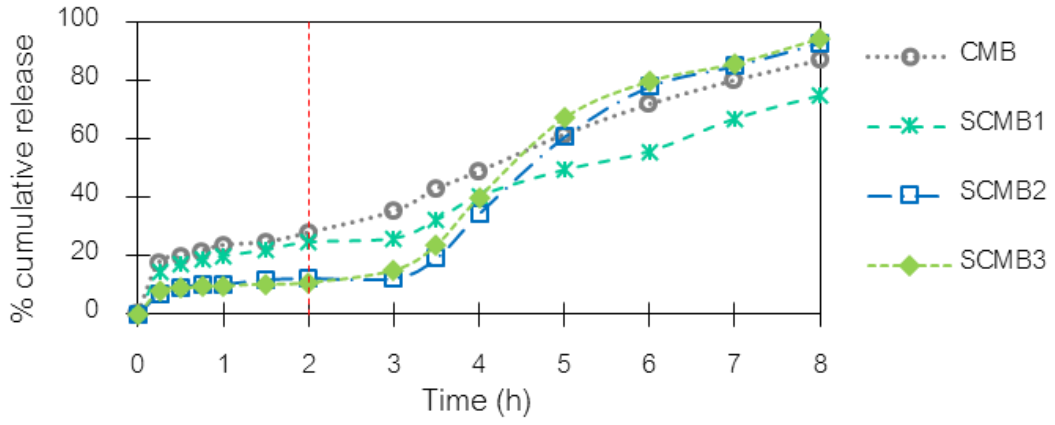

Figure 3 The effect of crosslink density of core matrix on MB release from the core carrageenan (CMB) and core-shell beads (SCMB1, SCMB2 and SCMB3).

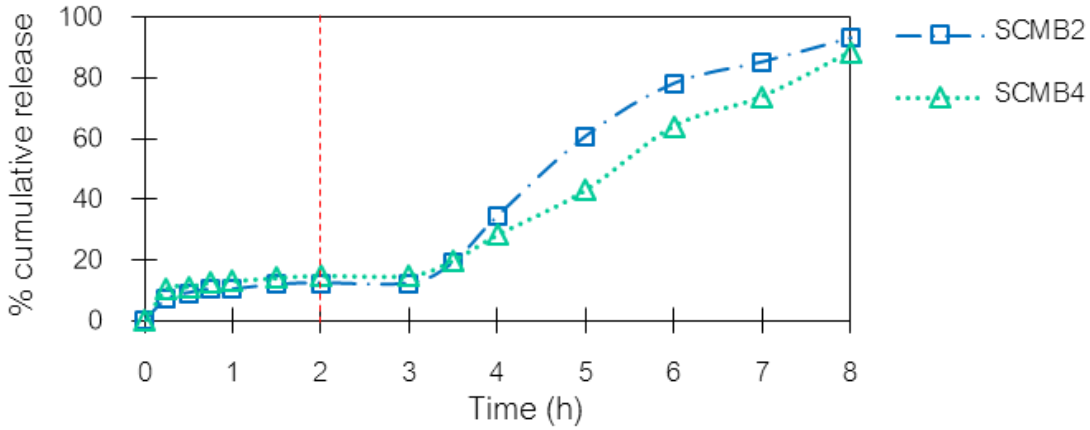

Figure 4 The effect of crosslink density of shell matrix on MB release profile.

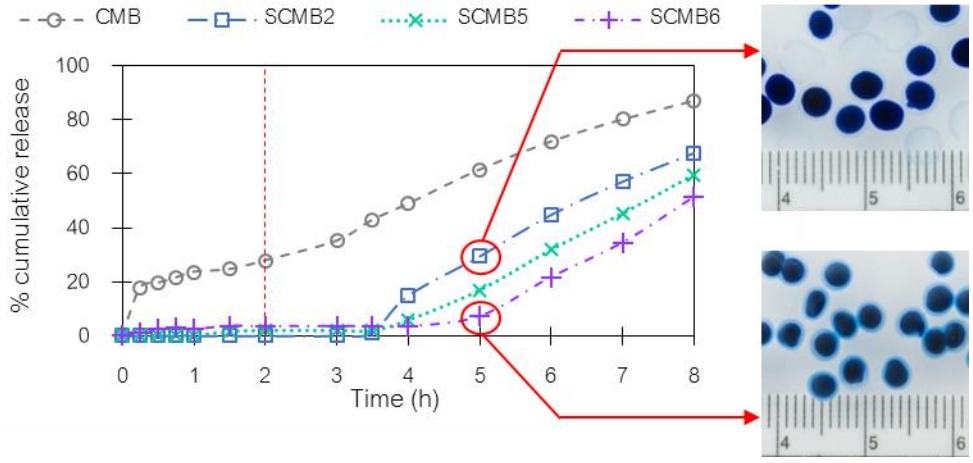

Figure 5 The effect of SA shell forming time on MB release profile.

To optimize the release behavior, the porous SCMB2 was also prepared using a freeze dried κ-CG core and the drug release was evaluated. The porous SCMB2 had higher ability to delivery drug to small intestine comparing with its nonporous matrix. In this system, only 7.5% of drug was detected in SGF with the remaining of 92.5% to be constantly released in SIF condition (Fig. 6). From this result, the porous core-shell was acceptable for oral delivery of drug (Zeitoun et al, 2003). To understanding the mechanism of MB release, different mathematical models were applied and the results are presented in Table 2. The experiment data were well fitted with Hixson-Crowell model, $Q_0^{1/3} - Q_t^{1/3} = k_{HC} \times t$, where Q_t is cumulative amount of drug released at the time t, Q_0 is the initial amount of the drug in the device, and k_{HC} is the rate constant for the Hixson–Crowell rate. According to this model, the release correlated with the erosion and dissolution of the hydrogel beads resulting in a change in surface area and diameter of hydrogels.

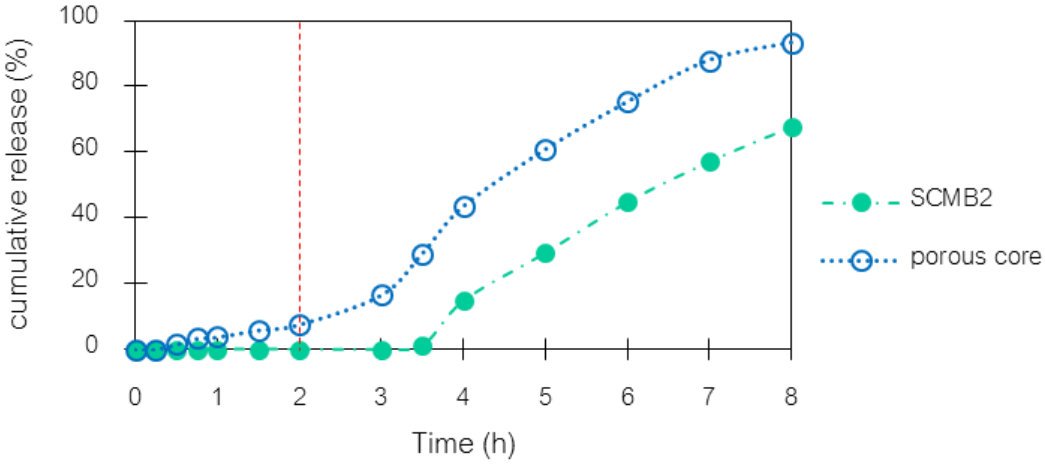

Figure 6 MB release profile of non-porous and porous core SCMB2 beads in simulated gastrointestinal system

Table 2 Kinetic release of porous SCMB2 beads

Model	Equation	R^2
Higuchi	$Q_t = k_H \times t^{1/2}$	0.9814
Korsmeyer-Peppas	$\frac{M_t}{M_\infty} = k_{KP} \times t^n$	0.9212
Hixson-Crowell	$Q_0^{\frac{1}{3}} - Q_t^{\frac{1}{3}} = k_{HC} \times t$	0.9981

Conclusions

Novel core-shell hydrogel beads of carrageenan/ sodium alginate were prepared as a pH-sensitive drug carrier for colon-targeted drug delivery. SEM micrographs showed that the core-shell structure carrier was approximately 1.4 mm in diameter and spherical in shape. Methylene blue was used as a model drug and loaded to the κ-CG core. The loaded core was encapsulated with SA shell to reduce the premature release of drug in the stomach and effectively release in the colon. The core-shell hydrogel showed pH-dependent swelling and drug release. It was observed that the release profile could be controlled by adjusting prepared conditions such as porous structure, crosslink density and thickness of the SA shell. The porous core-shell beads with an optimize crosslinking condition were suitable for the sustained release of drug in simulated gastrointestinal condition. According to these results, the prepared core-shell beads were considered as a strong potential material for an application of oral drug delivery.

Acknowledgments

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