# Preparation of Covalently Crosslinked Sodium Alginate/Hydroxypropyl Methylcellulose pH-Sensitive Microspheres for Controlled Drug Release

Pingping Song, Yuying Wu,* Xueming Zhang,* Zhongya Yan, Meng Wang, and Feng Xu

Due to their biocompatible and eco-friendly properties, natural polymers have attracted significant attention as controlled drug delivery carriers. In this paper, a facile method is presented for preparing pH-sensitive microspheres *via* acidic esterification between sodium alginate (SA) and hydroxypropyl methylcellulose (HPMC). The resulting microspheres were characterized by scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FT-IR), and thermogravimetric analysis (TGA). To evaluate the potential applications of microspheres as a drug carrier, the controlled release of a model drug—diclofenac—was examined. The SEM analysis revealed that the microbeads were spherical with a relatively smooth outer surface and porous inner network structure. The drug release experiments indicated that the cumulative release of diclofenac was less than 1% in an acidic medium, whereas it was close to 100% within 3 h in a pH 6.8 phosphate buffer saline solution. These results demonstrated that the SA/HPMC microspheres are promising candidates as pH-sensitive drug delivery system.

*Keywords: Covalently crosslinked; pH-sensitive; Methylcellulose; Esterification reaction; Drug release*

*Contact information: Beijing Key Laboratory of Lignocellulosic Chemistry, Beijing Forestry University, Beijing 100083, China; *Corresponding author: wuyuying-1980@163.com, xm_zhang@bjfu.edu.cn*

## INTRODUCTION

In the pharmaceutical industry there is a significant demand for new materials with high safety, effectiveness, and innovative formulations. Consequently, much research effort has been directed towards the development of new functional drug carriers using natural polymers, which have high biocompatibility and biodegradability. The most widely used biopolymers are pectin, starch, guar gum, chitosan, sodium alginate (SA), and hydroxypropyl methylcellulose (HPMC) (Kulkarni *et al.* 2001; Sriamornsak *et al.* 2004). These polymers can be exploited in various ways according to their different functional groups, wide range of molecular weights, and chemical compositions.

Among these polymers, sodium alginate and hydroxypropyl methylcellulose are considered suitable for drug delivery systems. Sodium alginate is an unbranched, linear polysaccharide, which contains varying amounts of 1,4-*β*-L-guluronic acid (G) and 1,4-*α*-D-mannuronic acid (M) (Ma *et al.* 2013). Due to the non-regular block pattern of carboxyl groups with the sodium alginate polysaccharide chains (Shu and Zhu 2002; Xu *et al.* 2007), the chains are prone to crosslink with divalent cations, such as $Ca^{2+}$ or $Zn^{2+}$, forming a reticular hydrogel structure (Bajpai and Sharma 2004; Hua *et al.* 2010; Liu *et al.* 2013). Hence, sodium alginate hydrogels have been extensively investigated in biotechnological systems and in drug delivery applications (Kulkarni *et al.* 2000; Rahman

*et al.* 2006), such as controlled delivery of proteins, encapsulated cell systems, and scaffolding for tissue (Rasmussen *et al.* 2003; Reyes *et al.* 2006). Hydroxypropyl methylcellulose is a non-toxic polymer that is also used for drug delivery (Liu *et al.* 2006). As a nonionic cellulose ether, HPMC does not react with metal salts or organic compounds in aqueous media, ensuring that no byproducts are generated in the preparation of drug carriers. Moreover, the high swelling capacity and surface activity of HPMC are beneficial for improving the drug load (Won *et al.* 2005). However, sodium alginate and hydroxypropyl methylcellulose are hydrophilic polymers with high water solubility. This means that a drug carrier formed by them alone will be soluble in water and not able to perform the function of controlling drug release. Therefore, modifications or crosslinking of these highly water-soluble biopolymers are the key for fabricating drug carrier systems with high loading and with sustained released.

Different strategies for fabricating controlled and modified drug release systems and devices have been developed using reservoir devices, matrix systems, pendents, enteric films, osmotically controlled devices, electrically stimulated devices, and hydrogels (Verma *et al.* 2002; Akhlaq *et al.* 2011). Matrix systems have been extensively investigated because they are easy to manufacture on a commercial scale. In addition, application of the hydrogels, mainly derived from biocompatible and biodegradable polymers, can achieve the prescribed goals of controlling the release of drugs, thereby reducing the number of times a patient takes the drug. Recently, covalently cross-linked polymeric hydrogels were prepared *via* a free radical polymerization process by Malana and Zohra (2013). The polymer matrix maintains its structure in the acidic medium of the stomach, and it also resists the peristaltic movements of the digestive tract, thus preventing drug release until it reaches its intended target. However, simple and efficient methods for production of a pH responsive drug delivery carrier are still challenging. So far, there has been little research on the synthesis of covalently cross-linked sodium alginate with hydroxypropyl methylcellulose to fabricate polymeric matrix. Hence, a facile and novel method for preparing water-insoluble SA/HPMC microspheres *via* cross-linking has been established in the present work.

Through esterification in a mildly acidic medium, a pH-sensitive sodium alginate/hydroxypropyl methylcellulose hydrogels was fabricated. These chemically hydrophilic hydrogels were used as drug carrier, capable of retaining both water as well as model drug. Moreover, in order to prepare more stable microspheres with enhanced encapsulation efficiency of the drug, bentonite clay was incorporated into the carrier as filler material because the clay has higher specific surface area, porosity, and cationic exchange capacity than magnesium oxide or calcium carbonate (Bhattacharyya *et al.* 2008; Doulia *et al.* 2009).

In the present work, a synthesized SA/HPMC/bentonite drug carrier with a porous network structure was characterized using scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FT-IR), and thermogravimetric analysis (TGA). In addition, the controlled drug release experiments were carried out under the simulated gastric fluid (pH=1.2) and intestinal fluid (pH=6.8) conditions by choosing diclofenac sodium ($C_{14}H_{10}Cl_2NNaO_2$) as the model drug. Meanwhile, various mathematical models are proposed in this work to describe the kinetics of the drug release for the purpose of precisely evaluating the drug release properties and mechanism. It was found that the biocompatible microspheres possessed extra high selectivity for drug release, in which they prevented release at simulated gastric fluid (pH=1.2) but allowed full release of a model drug at the high pH conditions of the intestines, meaning that the materials are safe

as a drug carriers for controlled drug release. The type of chemistry is suitable for cost-effective manufacture, leading to an expectation that the approach may be suitable for scale-up to industrial production.

## EXPERIMENTAL

### Materials

Sodium alginate was kindly supplied by Qing Dao Bright Moon Seaweed Group Co., Ltd. (Shandong Province, China). Hydroxypropyl methylcellulose was purchased from Alfa Aesar Chemical Co., Ltd. (Shanghai, China). Diclofenac sodium was purchased from Aladdin Industrial Corp. (Shanghai, China). Anhydrous calcium chloride was supplied by Beijing Chemical Plant. All reagents were analytical grade and used as received without further purification.

### Preparation of Sodium Alginate/Hydroxypropyl-Methylcellulose/Bentonite Microspheres

*Esterification of sodium alginate with hydroxypropyl-methylcellulose*

Sodium alginate and hydroxypropyl methylcellulose were separately dissolved in distilled water by magnetic stirring. After dissolution, the two solutions were mixed together, and the pH of the mixture was adjusted to 3 with diluted hydrochloric acid. The esterification reaction was performed for 6 h at 40 °C with a magnetic stirring. A scheme for sodium alginate esterification with hydroxypropyl-methylcellulose, as well as the preparation of microspheres, is shown in Fig. 1.

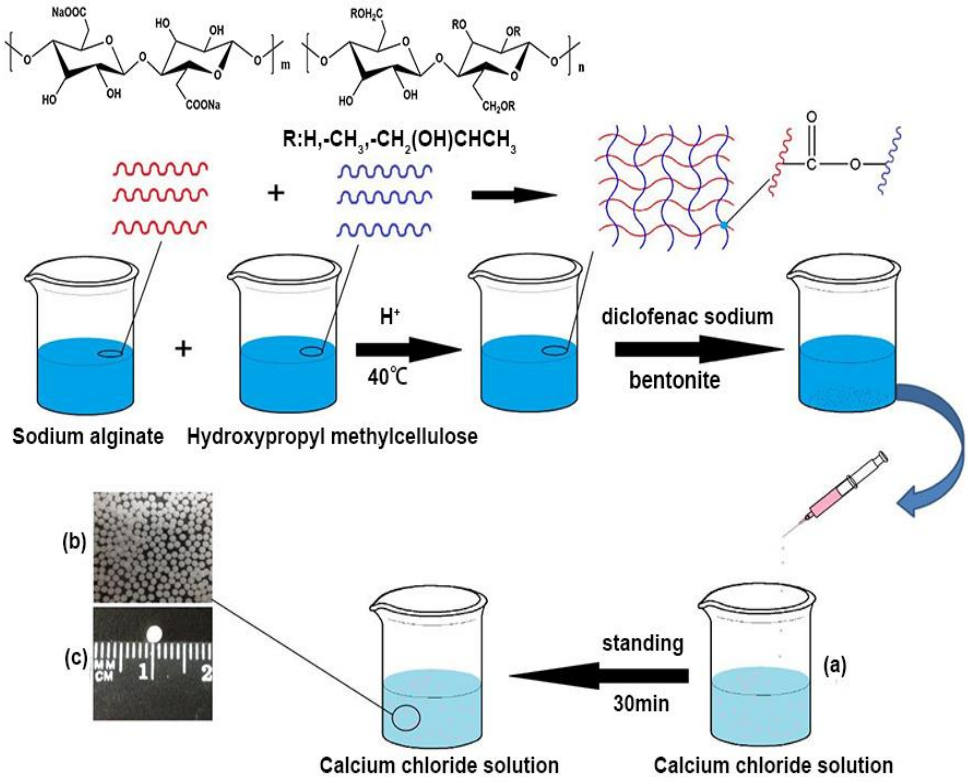


**Fig. 1.** Esterification of sodium alginate with hydroxypropyl methylcellulose and the formation procedure. of microspheres (a), and photographs of formed microspheres (b and c)

*Preparation of microspheres*

To prepare microspheres, 0.2 g of diclofenac sodium (model drug) and 2 mL of bentonite suspension (5% wt.) were added to the esterified mixture of sodium alginate and hydroxypropyl methylcellulose (20 mL). The resulting uniform suspension was transferred dropwise with a syringe into a solution of calcium chloride (5% w/v) and mixed with a magnetic stirrer; the syringe was held 10 cm above the calcium chloride solution. Microspheres were formed immediately and allowed to stand in the solution for 30 min. Microspheres were filtered, washed thoroughly with distilled water to remove residual calcium ions and reagents, and freeze-dried. A controlled trial was carried out in the absence of calcium by transferring the resulting uniform suspension into water with the same other conditions, but it showed that the microspheres could not be formed, even over a longer period. To compare the drug release and adsorption properties, three different formulations of microspheres were synthesized using sodium alginate-to-hydroxypropyl methylcellulose weight ratios of 2:1, 1:1, and 1:2. The corresponding SA/HPMC/bentonite microspheres were coded as SH-1, SH-2 and SH-3, respectively, as shown in Table 1.

**Table 1.** Formula for Preparation of SA/HPMC/Bentonite Microspheres

| Sample Name | Sodium alginate (g) | Hydroxypropyl methylcellulose (g) | Diclofenac sodium (g) | Bentonite (mL) |
|---|---|---|---|---|
| SH-1 | 2 | 1 | 0.2 | 2 |
| SH-2 | 1.5 | 1.5 | 0.2 | 2 |
| SH-3 | 1 | 2 | 0.2 | 2 |

## Characterization

*Morphological characterization of microspheres*

Samples were sputter-coated with gold under a vacuum prior to observation with a scanning electron microscope (JSM-6460LV, JEOL, Tokyo, Japan) at an acceleration voltages of 10 kV.

*FT-IR spectra*

Infrared spectra were recorded using a TENSOR 27 FTIR spectrometer (Bruker, Horiba, Oberursel, Germany). Scans were recorded over 400 to 4000 $cm^{-1}$ wavenumbers with a spectral resolution 2 $cm^{-1}$.

*Thermogravimetric analysis (TGA)*

The thermal stability of the microspheres was examined with a thermogravimetric analyzer (TGA2050, TA Instruments, New Castle, USA). The samples were heated from room temperature to 600 °C with a heating rate of 10 °C/min under a nitrogen atmosphere. The mass of the samples used for each TGA test was 3 to 5 mg.

*X-ray diffraction (XRD)*

Diclofenac sodium, SA/HPMC/bentonite microspheres, and loaded drugs were analyzed using an X-ray diffractometer (D8, Bruker, Germany) in the $2\theta$ range 5 to 60º with a scanning speed of 2° $min^{-1}$ . Operating conditions included CuKα radiation at 60 kV and 20 mA.

*In vitro drug release study*

To simulate the release of diclofenac salts in the human stomach and intestines, *in vitro* drug release experiments were carried out under acidic (pH=1.2) and near neutral (pH=6.8) conditions, respectively. Microsphere samples were accurately weighed into a conical flask containing 250 mL of buffer solution.

The flasks were sealed with plastic film and incubated in a rotary shaker at 100 rpm and 37 °C. For the first 2 h, the acidic medium was maintained at pH=1.2 (HCl/NaCl). Afterwards, the pH was adjusted to 6.8 with phosphate buffer ($Na_2HPO_4/NaH_2PO_4$/NaCl).

The buffer solution containing released diclofenac was sampled at different time intervals, and then the removed volume for sampling purposes was replaced by a fresh solution with the same volume. The collected samples were filtered using 0.22 μm filters, and the UV/Vis absorbance of resulting supernant was recorded using a UV spectrophotometer (UV/Visible-1601, Shimadzu, Tokyo, Japan) operating at 275 nm. Finally, the diclofenac concentration was calculated from the 275 nm absorption of the supernatant using a standardized calibration curve, which was generated with diclofenac solution of known concentration.

*Release kinetics study*

Diclofenac release from the microspheres was analyzed with zero-order kinetic, first-order kinetic, Higuchi diffusion, and Peppas kinetic models. Drug release from a carrier following a zero-order kinetic expression indicates that the amount of drug released is not depended upon the amount of drug that is embedded in the carrier. The zero-order model is given as follows,

$$M_t/M_\infty = kt \quad (1)$$

where $M_t/M_\infty$ is the fractional solute release, $t$ is the release time, and $k$ is the zero-order release constant for drug/polymer system. A first-order kinetic expression indicates that the amount of drug released is depended on the drug concentration within the polymeric carrier. The first-order model is given as follows,

$$Ln(1 - M_t/M_\infty) = -kt \quad (2)$$

where $k$ is the first-order release constant. The simplified Higuchi model expression relates the drug release from the matrix to the square root of time; the model is based on Fickian diffusion. The simplified Higuchi model is given in Eq. 3,

$$M_t/M_\infty = kt^{1/2} \quad (3)$$

where $k$ is the Higuchi dissolution constant, which is dependent upon the internal structure and the shape of the carrier matrix, as well as the solubility and the concentration of the drug. When a plot of fractional solute release versus $t^{1/2}$ is made, a straight line will be observed if the particular system can be modeled with the simplified Higuchi expression. When the drug release is strongly influenced by both diffusion and dissolution, a simple and semi-empirical model (Siepmann and Peppas 2001) is given as follows,

$$M_t/M_\infty = kt^n \quad (4)$$

where $k$ is a release rate constant that is characteristic of the drug/polymer system and "$n$" is a parameter that depended on the drug release mechanism. According to the value of $n$,

the drug release can be characterized by a Fickian, non-Fickian, or super case II transport mechanism. If the value of *n* is less than or equal to 0.43, then the release mechanism is considered as Fickian. If the value of *n* is equal to 0.89, then the release mechanism is considered as zero-order (time-independent). If the value of *n* is between 0.43 and 0.89, then the release mechanism is characterized as non-Fickian. If the value of *n* is above 0.89, then the release mechanism is considered as super case II transport.

## RESULTS AND DISCUSSION

### Formation Mechanism of Microspheres

The polymer matrices can be either chemically cross-linked through covalent or hydrogen bonds; the polymer cross-linking is dependent on the monomers used, the polymerization methods employed, and the mode of application (Hoffman 2012). In order to prepare pH-sensitive microspheres with highly stable structures and relatively narrow distribution size, a covalent cross-linked SA/HPMC matrix was fabricated *via* an esterification reaction between SA and HPMC.

The microspheres were prepared by extruding the reaction mixture into a coagulation bath as illustrated in Fig. 1. When extruding the reaction mixture through the syringe, a droplet was formed due to the combined forces of gravity and syringe pressure (Fig. 1a). Once the droplet fell into the calcium chloride solution of coagulation bath, a microspherical hydrogel immediately formed due to the formation of calcium alginate on the surface of the droplet (Fig. 1b).

The average diameters of the microspheres were 2 mm (standard deviation=0.48, Fig. 1c). Moreover, the shape of the microspheres was not perfectly spherical, which was due to the mechanical strain that the droplets encountered when hitting the surface of the coagulation bath solution.

### Morphology of Microspheres

The morphology and the internal structure of the microspheres were investigated by SEM images of the surface and cross-section, as shown in Fig. 2. Figure 2a indicated that the microspheres were somewhat spherical in shape with grainy and wrinkled surfaces, which might be ascribed to the formation of called "egg-box" structures crosslinked by G-guluronic acid in the SA molecule and $Ca^{2+}$ ions.

As the mass ratio of SA/HPMC was decreased from 2:1 to 1:2, the surface areas of the microspheres became much smoother (Figs. 2b and 2c), which was mainly caused by lower amounts of reticular gel structure being formed, which was due to the decreased amount of SA.

Notably, the diameters of the microspheres gradually increased with increasing HPMC mass fraction in the gel. As can be seen in the cross-sections images (Figs. 2d to 2f), the inner morphology was homogeneous with a porous network structure. Interestingly, the structure and arrangement of pores from Figs. 2d and 2e were quite similar. In comparison, higher amounts of macropores were observed in Fig. 2f (sample SH-3), which was presumably caused by the decreased of crosslinking of SA acid groups with $Ca^{2+}$ ions.

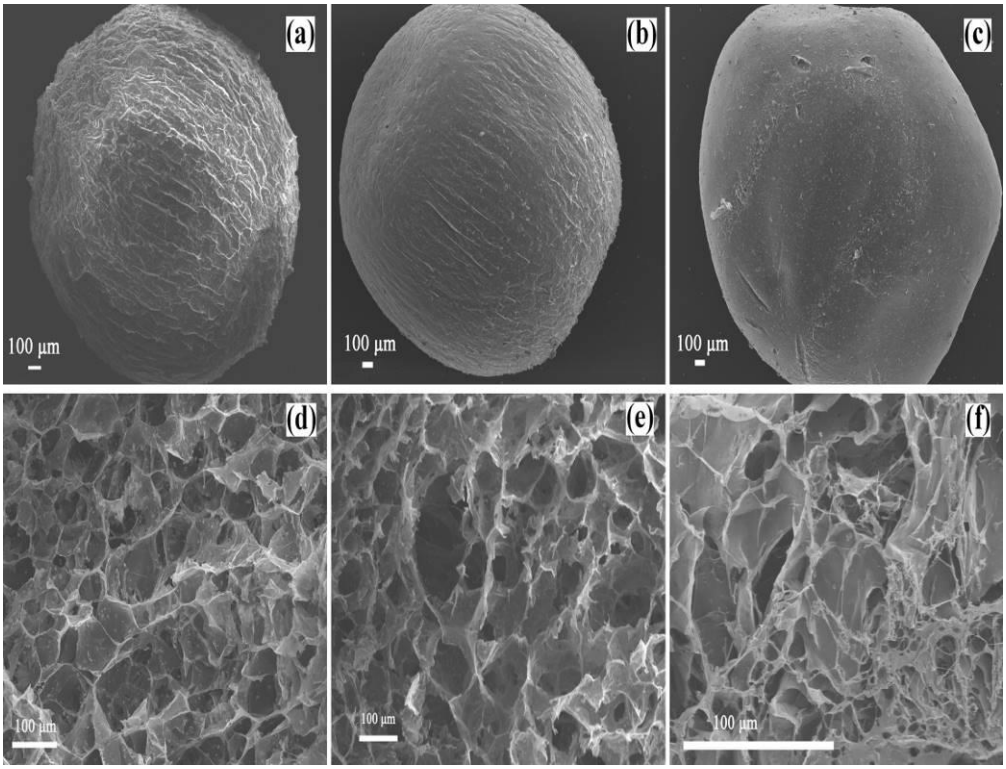


**Fig. 2.** SEM images of the surfaces (a–c) and cross-sections (d–f) of different microspheres samples: SH-1 (a and d); SH-2 (b and e); and SH-3 (c and f).

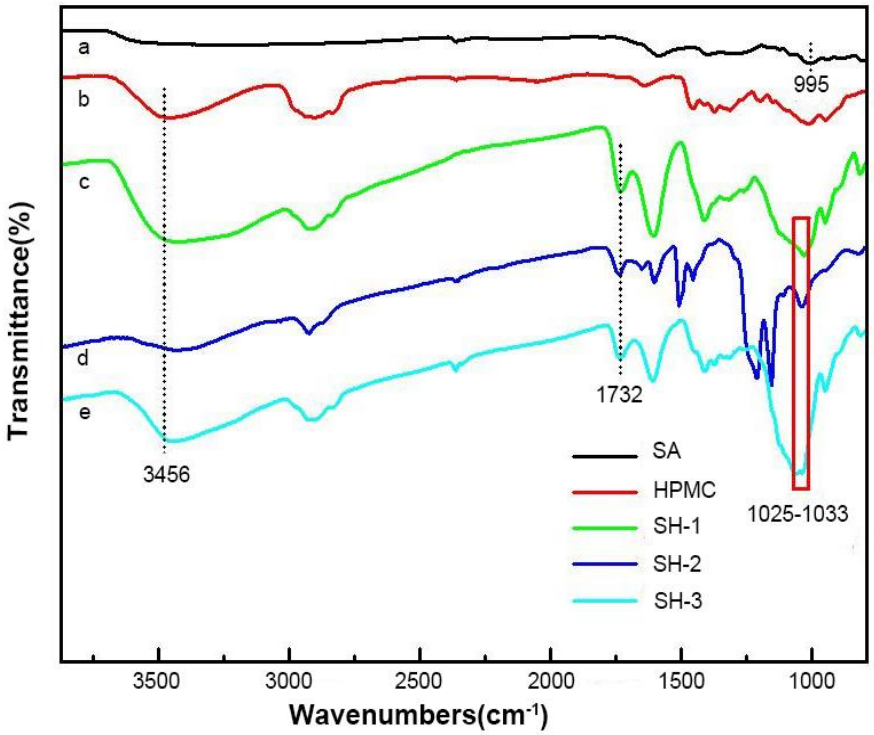


**Fig. 3.** FT-IR spectra of different samples: (a) pure SA; (b) pure HPMC; (c) SH-1; (d) SH-2; and (e) SH-3.

## FT-IR Spectra

The FT-IR spectra of the pure sodium alginate (Fig. 3a), hydroxypropyl methylcellulose (Fig. 3b), and esterified samples (Figs. 3c, 3d, and 3e) were recorded. The band at 3456 $cm^{-1}$ is attributed to hydroxyl stretching in the HMPC and microsphere samples (Figs. 3c, 3d and 3e). Also notable was a distinguishable peak at 1732 $cm^{-1}$ (C=O stretching), which was detected in the modified microsphere samples (Figs. 3c, 3d, and 3e); this observation indicated that the acidic esterification reaction between sodium alginate and hydroxypropyl methylcellulose had occurred. In addition, the cross-linking of sodium alginate carboxylate groups with $Ca^{2+}$ ions caused an obvious shift to higher wavenumbers (1025 to 1033 $cm^{-1}$) (Figs. 3c, 3d and 3e), which was attributed to ionic bonding between $Ca^{2+}$ ions and carboxylic group in SA.

## Thermogravimetric Analysis

As illustrated in Fig. 4, thermogravimetric analysis was applied to investigate the thermal stability and thermal degradation kinetics of the microspheres. The microspheres exhibited three distinct thermal degradation phases, which occurred in the temperature ranges of 27 to 100 °C, 200 to 350 °C, and 350 to 600 °C. The mass loss in the first phase was attributed to the presence of water. The maximum rate of weight loss was observed at the second stage, in which over 42% weight was pyrolyzed. The mass loss was mainly attributed to hydrothermal degradation of organic functional groups and cleavage of covalent bonds. According to the bond energy, we predicted that the decomposition of esterified polymers, such as that covalently cross-linking SA and HMPC, was the main reasons for the mass loss. Meanwhile, the degradation of sodium alginate and cellulose would also contribute to the mass loss. However, the mass loss at third phase tended to be balance, which meant that the main left composition in these microspheres was bentonite. In comparison with pyrolysis curve of SA, the amounts of bentonite was speculated at round 10%.

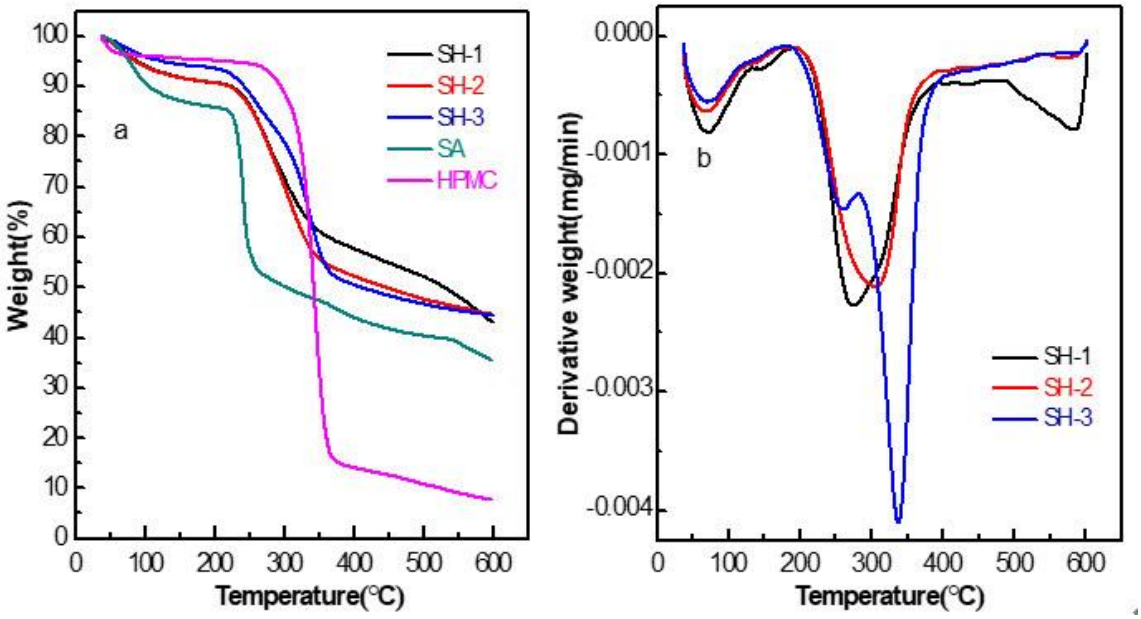


**Fig. 4.** Thermograms of microsphere samples: (a) TGA; and (b) differential thermal gravimetric (DTG) analysis

As shown in Fig. 4b, the maximum rate of mass loss occurred at 276 °C, 309 °C, and 337 °C for SH-1, SH-2, and SH-3, respectively. These results demonstrated that the thermal stability of the microspheres increased from SH-1 to SH-2 to SH-3. This trend was attributed to the higher proportion of HMPC in the microspheres because pure HMPC has higher thermal stability as compared with pure SA (Siepmann *et al.* 2001). After 600 °C, only 45% solid residue remained, which was mainly due to the presence of bentonite clay and the formation of char derived from SA.

## X-ray diffraction (XRD)

Figure 5 shows the XRD pattern of DS, SA/HPMC/bentonite microspheres after encapsulation, and controlled microspheres without loading drugs. The XRD of diclofenac showed sharp peaks in the region 10-30°, with characteristic of peaks observed at 10.72°, 13.45°, 15.33°, 18.78°, 20.50°, 23.71°, 23.51°, 27.14° and 27.91°, indicating the crystalline nature of the drug. These peaks also appeared in the diffractogram of the microspheres with DS loaded, which illustrated that the DS was successfully encapsulated into the SA/HPMC/bentonite microspheres and there was no chemical interaction between DS and microspheres.

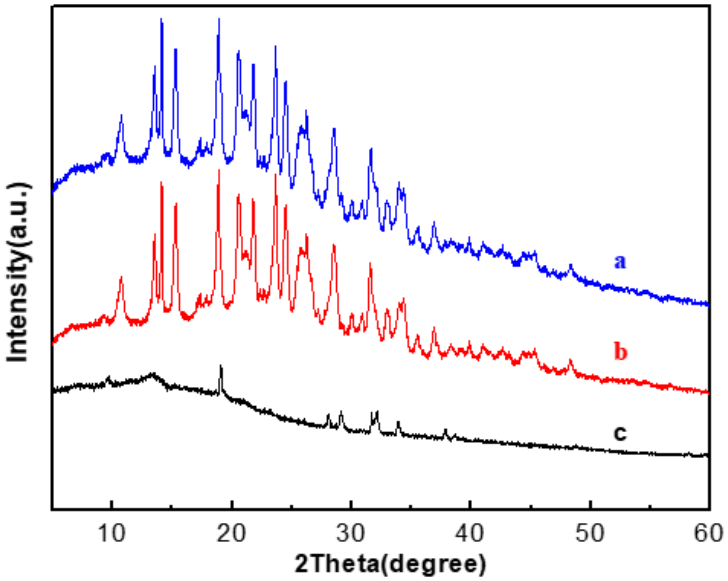


**Fig. 5.** X-ray diffraction of (a) diclofenac sodium, (b) microspheres after encapsulation (c) controlled microspheres

## Drug Release Study

To investigate the potential of microspheres as drug delivery carriers, the controlled release behaviors of the hydrogel microspheres were studied under acidic (pH=1.2, with HCl/NaCl) and neutral (pH=6.8, with phosphate-buffered saline) media. In the beginning, the encapsulation efficiency of the drug in the spheres using UV spectrophotometry before the drug release study were firstly investigated. The results showed that the encapsulation efficiency of SH-1, SH-2, and SH-3 microspheres was 76.36%, 88.18%, and 96.06%, respectively. These results indicated that DS was loaded into the microspheres successfully and encapsulation efficiency increased with the increasing of HPMC concentration. Figure 6 shows the cumulative release profiles of diclofenac from the sodium alginate/hydroxypropyl methylcellulose microspheres under

different pH conditions. The cumulative release of diclofenac was just 1% in the first 2 h, indicating the microspheres were nearly unswollen and dissolved in acidic medium, which was beneficial for the protection of the acid-sensitive drugs. In contrast, the release of diclofenac from the three microsphere formulations increased sharply until the cumulative release was close to 100% after 3 h at pH=6.8. Based on the above observations, the microspheres formed by the esterification between sodium alginate and hydroxypropyl methylcellulose demonstrated pH-responsive properties, which led to selective and sustainable drug release.

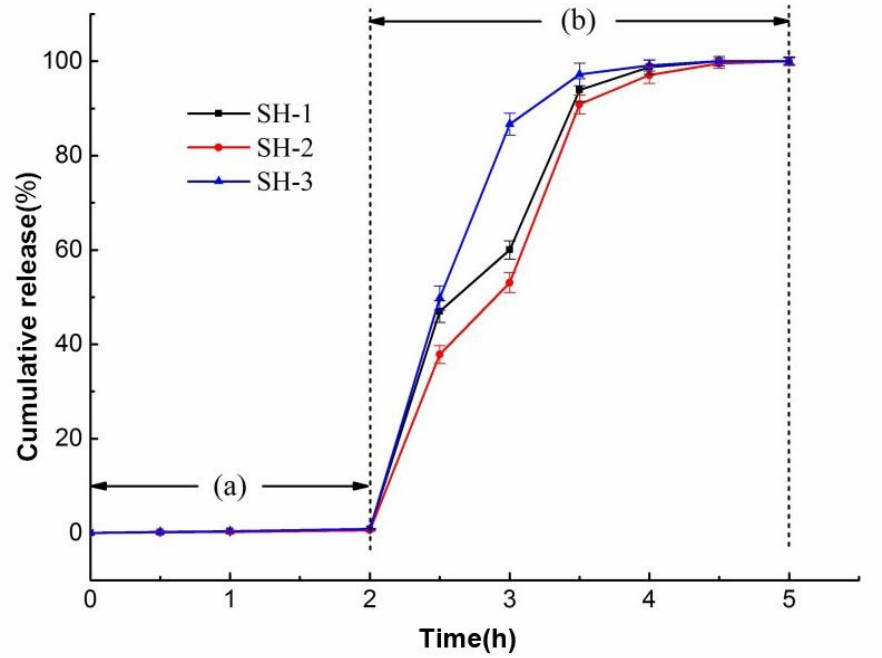


**Fig. 6.** *In vitro* release profile of diclofenac from microspheres at (a) pH=1.2 and (b) pH=6.8

## Release Kinetics Study

To precisely evaluate the drug release properties and mechanism, various mathematical models were proposed to describe the kinetics of the drug release in the phosphate buffer. Based on a literature review (Li *et al.* 2006; Sriamornsak *et al.* 2006; Nochos *et al.* 2008), the drug release behaviors of the microsphere samples were obtained by fitting the drug release profiles to the various models. The curve fitting of the various mathematical models are listed in Fig. 7, along with the corresponding coefficient of correlation for each model by statistical regression (Table S1; see Supplementary Information). The coefficient of determination ($R^2$) was used to evaluate which model best fitted the observed drug release data. The $R^2$ values (0.7301, 0.7572, and 0.5341) obtained from the zero-order model were lower than those from first-order model (0.9067, 0.9110, and 0.9454). This observation indicated that the drug release behavior was better modeled by a first-order expression. To clarify the release mechanism, the Higuchi and Peppas models were also fitted to the data. As seen in Table S1, the values obtained from fitting the data to Higuchi and to Peppas model were close to each other except those from SH-3. The values of "*n*" determined for the microbeads were in the range from 0.30 to 0.51. These results indicated that the drug release mechanism of SH-1 and SH-3 were Fickian diffusion. Therefore, the drug release mechanism of microspheres (SH-1 and SH-3) mainly depended on the drug concentration in the polymeric networks.

The drug release of SH-2 followed an anomalous transport mechanism (*i.e.* non-Fickian diffusion mechanism), whereby the drug delivery was governed by both diffusion release and dissolution of the polymeric network.

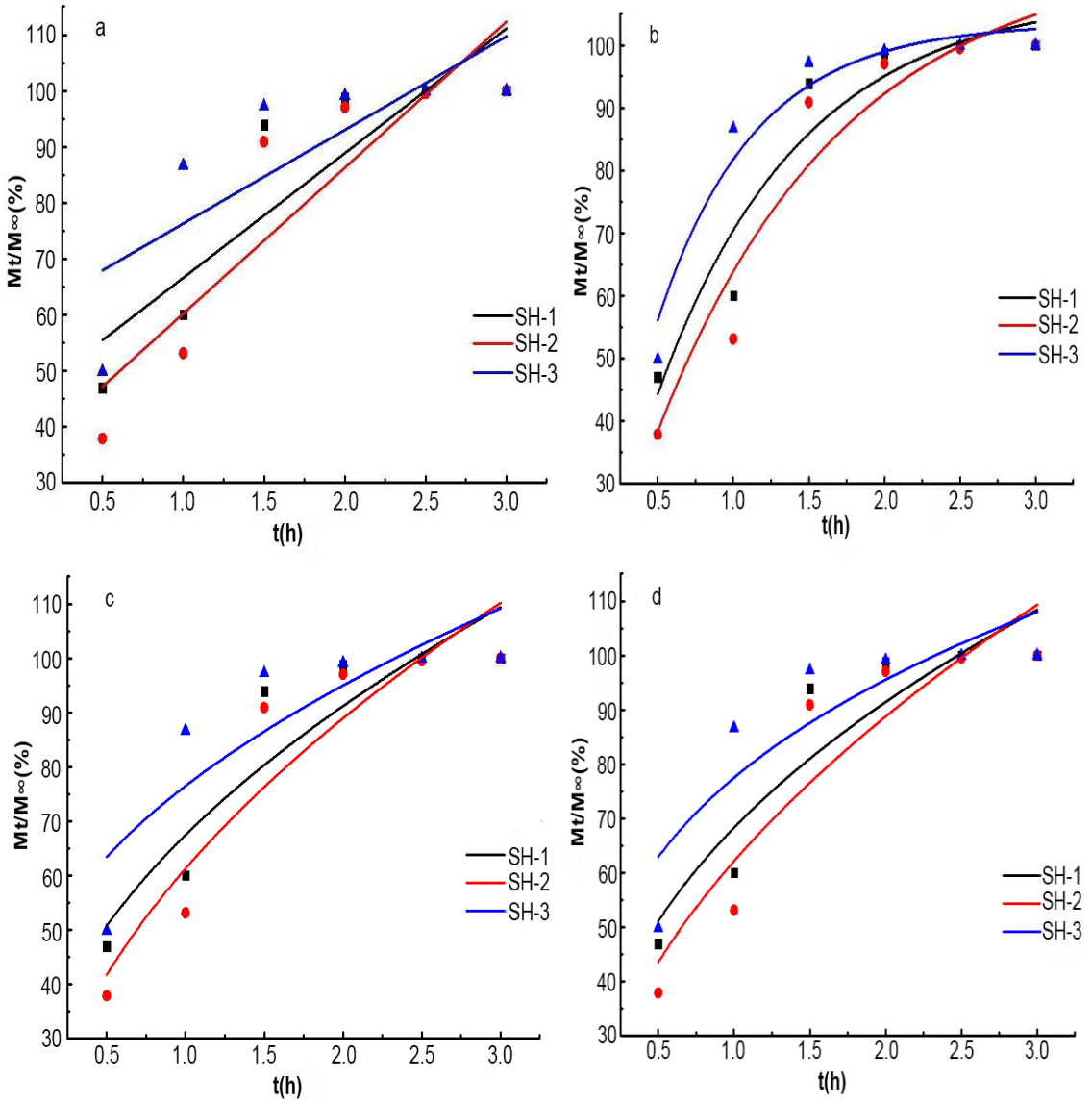


**Fig. 7.** Curve fitting of various models to the drug release data of the microspheres: (a) zero-order model; (b) first-order model; (c) Higuchi model; and (d) Peppas model

## CONCLUSIONS

1. Covalently cross-linked alginate/hydroxypropyl methylcellulose microspheres were successfully fabricated *via* acidic esterification.
2. SEM images of the microspheres revealed that the beads were approximately spherical with a homogeneous and porous network structure.
3. The drug release of diclofenac with the microsphere samples was extremely low in an

acidic medium, whereas it increased sharply in a neutral medium. This indicated that the microspheres could be used as a pH-sensitive drug delivery system. Therefore, the SA/HPMC/bentonite microspheres have desirable attributes required for a drug delivery carrier.

4. The drug release behavior of diclofenac was better modeled by a first-order mathematical models.

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**Table S1.** Fitting Results and Relative Parameters of *in vitro* Release of Diclofenac Sodium from Different Microspheres

| Fit method | Equation | | | Relative parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | n | | | k | | | $R^2$ | | |
| | SH-1 | SH-2 | SH-3 | SH-1 | SH-2 | SH-3 | SH-1 | SH-2 | SH-3 | SH-1 | SH-2 | SH-3 |
| Zero-order | M=22.28t+44.27 | M=26.07t+34.09 | M=16.73t+59.52 | - | - | - | 22.28 | 26.07 | 16.73 | 0.7301 | 0.7572 | 0.5341 |
| First-order | M=108.29(1-exp(-1.05t)) | M=115.01(1-exp(-0.81t)) | M=103.53(1-exp(-1.56t)) | - | - | - | 1.05 | 0.81 | 1.56 | 0.9067 | 0.9110 | 0.9454 |
| Higuchi | M=57.28(x^(1/2))+10.14 | M=66.83(x^(1/2))-5.59 | M=44.67(x^(1/2))+31.77 | - | - | - | 57.28 | 66.83 | 44.67 | 0.8200 | 0.8428 | 0.6734 |
| Peppas | M=68.28(x^(0.42)) | M=62.12(x^(0.51)) | M=77.48(x^(0.3)) | 0.42 | 0.51 | 0.3 | 68.28 | 62.12 | 77.48 | 0.8303 | 0.8391 | 0.7257 |