Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

A Novel Magnetic pH-sensitive *N*-Succinyl Chitosan /Alginate Hydrogel Bead for 5-Fluorouracil Delivery

XIAO-QIANG LI[1,2], PING LI[1,2*], FA-QIN WANG[1,2], YU-MIN LI[1,2]

[1]Lanzhou University Second Hospital, Lanzhou, 730030, China

[2]Key Laboratory of Digestive System Tumors, Gansu Province, Lanzhou, 730030, China

ARTICLE DETAILS

Article history:
Received on 22 January 2014
Modified on 20 February 2014
Accepted on 25 February 2014

Keywords:
5- Fluorouracil,
Magnetic/ pH-sensitivety,
Hydrogel,
N–succinyl chitosan,
Release

ABSTRACT

In this study, a novel magnetic pH-sensitive N-succinyl chitosan/alginate hydrogel bead was prepared by the single factors test design for controlled the delivery of 5-Fluorouracil (5-FU) and the bead was evaluated for pH sensitivity due to the drug release characteristics. The structure and surface morphology of the bead were characterized by FTIR, SEM, and VSM. The effects of six different factors influencing the swelling ability of the hydrogel bead were also investigated, such as the weight ratio of *N*-succinyl chitosan and alginate (X1), the weight ratio of drug to polymer (X2), $CaCl_2$ concentration (X3) ,the volume ratio of *N*-succinyl chitosan/alginate to $CaCl_2$ (X4), crosslinking time (X5), the weight ratio of Fe_3O_4 to polymer (X6). In addition, the delivery behavior of 5-FU from the hydrogel bead was studied. The amount of 5-FU released from the hydrogel bead at pH 1.5 was relatively low (47.93%), while this value approached 93.91% at pH 6.8. The results clearly suggested that the *N*-succinyl chitosan/alginate beads had pH-dependent swelling behaviors and a continuous release of 5-FU. From the magnetometer measurements data, the beads also had super paramagnetic property as well as fast magnetic response. So the magnetic pH-sensitive beads may be a potential polymeric carrier for drug delivery in the intestinal tract.

INTRODUCTION

5-FU is widely used in the treatment of many cancers including gastrointestinal carcinoma. But its half-life in plasma is short (15-20 min), continuous intravenous administration is needed to maintain the efficient concentration in plasma[1,2].However, the clinical dosage of 5-FU is very close to its toxic dosage when given intravenously administration, resulting in its strong toxicities to the gastric and intestinal mucosa and the bone marrow [3,4].Many studies have shown that prolonged continuous steady-state concentrations of 5-FU are superior to intermittent bolus injections in patients receiving adjuvant therapy[5]. Oral site-specific rate-controlled 5-FU delivery is expected to reduce systemic side-effects, and also, to provide an effective and safe therapy for gastrointestinal cancer with reduced dose and duration of therapy.

*Author for Correspondence:
Email: gsliping@163.com

There are several potential advantages to oral administration, including patient's convenience and reduced costs associated with drug preparation and administration. Although patients would prefer an oral agent rather than an intravenous one, 5-FU shows incomplete and unpredictable absorption due to its degradation in the gastrointestinal tract. We can design a 5-FU carrier system to release 5-FU just in situ of the cancer area. This may overcome the problem of oral administration of 5-FU in clinical applications [6, 7].

Local delivery of chemotherapeutic drugs is recognized as a potential method to deliver drug at the target site with minimal systemic exposure [8]. Because chemotherapeutic drugs in systemic administration can result in severe toxicity, the local delivery of these drugs in pathological tissue may create a great opportunity to improve both safety and efficacy of cancer chemotherapy. This is especially true in the case of inoperable peritoneal carcinomatosis, which often causes the accumulation of malignant ascites [9]. To avoid the systemic toxicity associated with

chemotherapeutic drugs and maintain their therapeutic concentrations in local region of tumors, one of the possible ways is incorporating these drugs into polymer carriers. Thus, a qualified delivery device is crucial for successful local chemotherapy.

Hydrogels can absorb and retain large amounts of aqueous fluids, and the absorbed water is hardly removable even under some pressure. Due to excellent properties compared with traditional water absorbing materials, hydrogels have drawn much attention in a wide variety of fields such as drug delivery system, wound dressings, gel actuators, artificial organs,medical pharmaceuticals and contact lenses [10–12]. pH-sensitive hydrogels have attracted increasing attention due to their unique properties. Swelling of such hydrogels in the stomach is minimal and thus the drug release is also minimal. Due to increase in pH, the swelling degree increases as the hydrogels pass down the intestinal tract.

A variety of synthetic or natural polymers with acidic or basic pendant groups have been employed to fabricate pH-sensitive hydrogels for getting the desired controlled release of drugs. The use of natural polymers in the design of pH sensitive hydrogels has received much attention because of their excellent biocompatibility and biodegradability. Among them, alginate, chitosan and its derivates are promising. They have excellent characteristics, such as biodegradability, biocompatibility, mild undesirable host reactions, enough tensility and three- dimensional and directional porous structures. Alginate can be ionically cross linked by the addition of divalent cations in aqueous solution. Theoretically, alginate shrinks at low pH and the encapsulated drugs cannot be released [13]. It was reported that the drugs can be retained in the calcium- crosslinked alginate encapsulation process [14]. Chitosan and its derivates have also been investigated as polymeric drug carriers for the optimization of drug delivery in the pharmaceutical field because of their biocompatibility and biodegradability. *N*-succinyl chitosan has unique characteristics such as biocompatibility, low toxicity and long-term retention in the body. So *N*-succinyl chitosan is valuable as a drug carrier to prepare its conjugates with many kinds of agents because of having -NH_2 and -COOH groups [15].In recent years, water-soluble derivatives of chitosan were used to blend with alginate to prepare Ca^{2+}-crosslinked hydrogel beads which generally have pH- sensitive and ionic-sensitive swelling and drug release properties [16–18]. So *N*-succinyl chitosan and alginate can be used in site-specific or controlled-release drug delivery systems.

Magnetically targeted beads can be used as drug carriers to provide targeted delivery and sustained release of chemotherapeutic agents to improve bioavailability. Drugs based on carriers of core-shell magnetic beads can be easily guided to arrive at the interest position in the body by means of physical force from magnetic field. Meanwhile, the outer shell (polymer layer) of the drug can effectively slow down the rate of release. Therefore, drug delivery system using polymer-coated magnetic beads is considered as an effective strategy for passive targeting, which can increase drug circulation and reduce pain in patients [19]. In this regard, super paramagnetic magnetite (Fe_3O_4) beads exhibiting higher magnetization and good biocompatibility have found increasing and very promising applications in the biomedical field, such as immobilization of enzymes[20], separation of biomolecules[21], magnetic resonance imaging (MRI) contrast enhancement[22], and, particularly, drug delivery[23].

In this study, novel *N*-succinyl chitosan/alginate hydrogel beads were prepared by dropping aqueous solution containing *N*-succinyl chitosan and alginate into a Ca^{2+} solution. Then, the beads were used as a pH-sensitive controlled release system for the delivery of 5-FU. The process is mild and simple. Factors influencing swelling characteristics of the hydrogels were investigated. Additionally, release properties of 5-FU from the hydrogel beads were studied in simulated gastric and intestinal fluid.

MATERIALS AND METHODS

Materials

N-succinyl chitosan (molecular weight is 3×10^5, degree of deacetylation is 67%) was supplied by Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences (Lanzhou, China). Sodium alginate (viscosity≥0.02 Pa·S in 1% aqueous solution at 20°C) was purchased from Shanghai Chemical Reagent Co., Ltd (Shanghai, China). Fe_3O_4 (average size is 20 nm) was purchased from Nanjing Emperor Nano Material Co., Ltd (Nanjing, China). Polyethylene glycol (PEG) 4000 was purchased from Tianjin Tiancheng pharmaceutical Co., Ltd (Tianjin, China). 5-Fluorouracil (5-FU) was purchased from Nantong Haiers Pharmaceutical Co., Ltd (Nantong, China). $CaCl_2{\cdot}2H_2O$ were purchased

from Tianjin Tanggu denzhong Chemical Factory (Tianjin, China). All other reagents were analytical grade and used directly without any purification. Deionized water was used for all the experiments.

Single factor design experiments

N-succinyl chitosan/alginate hydrogel beads were prepared based on the single factor design. The independent variables are the weight ratio of *N*-succinyl chitosan and alginate (X1), the weight ratio of drug to polymer (X2), $CaCl_2$ concentration (X3), the volume ratio of *N*-succinyl chitosan/alginate to $CaCl_2$ (X4), crosslinking time (X5), the weight ratio of Fe_3O_4 to polymer (X6). On the other hand, encapsulation efficiency and loading efficiency are the response parameters as the dependent variable.

Preparation of magnetic N-Succinyl Chitosan/Alginate hydrogel beads

PEG aqueous solution (5%, w/v) was prepared by expanding 2.5g of PEG in 50 mL of deionized water. A stock solution of Fe_3O_4 (10%, w/v) were prepared though adding 5g of Fe_3O_4 into 50mL PEG aqueous solution (5%, w/v) and was sonicated for 2 hours before the subsequent experiments.

A series of *N*-succinyl chitosan/alginate beads with different composition were prepared as shown in Table 1. The beads were prepared according to the following steps: 0.15g of *N*-succinyl chitosan and 0.15g sodium alginate was dissolved in 10 mL of deionized water. 0.52mL stock solution of Fe_3O_4 (10%, w/v) were mixed adequately and sonicated for 2 hours. Then the suspension was introduced into a 5mL syringe and then extruded through a needle with an internal diameter of 0.7mm into a 30mL of solution containing 2.5% (w/v) $CaCl_2$ at a dropping rate of 1.2 mL·min^{-1}. The beads were allowed to crosslink with Ca^{2+} in solution for 30 min with gentle stirring, the speed has been chosen to avoid the sedimentation. The distance between the edge of the needle and the surface of the solution was 5 cm. The calcium-crosslinked beads were rinsed with distilled water for several times to remove unreacted calcium chloride on surface and subsequently dried in air overnight at room temperature.

Preparation of 5-FU-loaded magnetic N-Succinyl Chitosan/Alginate hydrogel beads

0.10g of 5-FU was dispersed in 10ml solution containing *N*-succinyl chitosan and sodium alginate (1.5%, w/v), which under stirring until a uniform suspension was obtained. The other processes were the same as the preparation of magnetic *N*-succinyl chitosan/alginate blank beads (not adding 5-Fu).

Scanning electron microscopy

Micrographs of the samples were taken using a scanning electron microscope (SEM) (JEOL, JSM-6380LV, Akishima, Tokyo, Japan). Before the observation, all samples were mounted on aluminum stubs, using double-sided adhesive tape, and then beads samples were coated with gold, and scanned at an accelerating voltage of 15 kV.

Fourier transform infrared spectroscopy

Individual beads were crushed with pestle in an agate mortar and the crushed material was mixed with potassium bromide in 1:100 proportions. The mixture was compressed to a 1 mm semitransparent disk by applying a pressure of 20 MPa (FW-4A pelleter) for 5 minutes. The Fourier transform infrared (FTIR) spectra over the wavelength range 4000-400 cm^{-1} were recorded using an FTIR spectrometer (Thermo Nicolet Nexus, TM, Madison, WI, USA).

Swelling studies

Swelling characteristics of the beads were determined by immersing dried test beads in two aqueous media: simulated gastric fluid (SGF, pH 1.5) and phosphate buffer solutions (pH 6.8) prepared according to the Chinese Pharmacopoeia 2010 at 37℃. Accurately weighed amounts of beads (ranging from 100 to 110 mg) were immersed in 30 mL media solution and the beads were removed from the swelling medium at specific time intervals. They were blotted with filter paper to absorb water on the surface and then weighed using electronic microbalance (Model NE2155; Genius, Goettingen, Germany) immediately. Swelling ratio (SR) of the sample was calculated according to the following expression [24]:

$$SR = (W - W_0) / W_0 \qquad (1)$$

Where W is the weight of the swollen beads and W_0 is the initial weight of the beads.

Table 1.Formulation of the Magnetic pH-sensitive *N*-Succinyl Chitosan/Alginate Beads Utilizing Single Factor Design

Formulation	X1 [%,w/v]	X2 [w/w]	X3 [%,w/v]	X4 [v/v]	X5 [min]	X6 [w/w]	Encapsulation Efficiency [%]	Loading Efficiency [%]
A1	1.5:1.5	2:3	2.5	1:3	30	1:3	20.90	5.17
A2	2.0:2.0	2:3	2.5	1:3	30	1:3	26.20	7.23
A3	2.5:2.5	2:3	2.5	1:3	30	1:3	29.28	8.01
A4	3.0:3.0	2:3	2.5	1:3	30	1:3	28.73	9.11
A5	2.0:2.5	2:3	2.5	1:3	30	1:3	30.33	8.87
A6	2.5:2.0	2:3	2.5	1:3	30	1:3	23.90	5.88
A7	2.0:1.5	2:3	2.5	1:3	30	1:3	24.42	5.15
A8	1.5:2.0	2:3	2.5	1:3	30	1:3	27.36	7.55
B1	1.5:2.0	1:1	2.5	1:3	30	1:3	23.78	10.20
B2	1.5:2.0	4:3	2.5	1:3	30	1:3	27.01	14.49
B3	1.5:2.0	4:5	2.5	1:3	30	1:3	29.16	10.77
C1	1.5:2.0	4:5	1.5	1:3	30	1:3	25.01	9.92
C2	1.5:2.0	4:5	3.5	1:3	30	1:3	31.61	9.63
C3	1.5:2.0	4:5	5.0	1:3	30	1:3	22.22	6.29
C4	1.5:2.0	4:5	7.0	1:3	30	1:3	25.03	5.64
C5	1.5:2.0	4:5	9.0	1:3	30	1:3	26.60	5.51
D1	1.5:2.0	4:5	3.5	1:1.5	30	1:3	38.20	11.63
D2	1.5:2.0	4:5	3.5	1:2	30	1:3	40.34	11.44
D3	1.5:2.0	4:5	3.5	1:2.5	30	1:3	41.79	12.79
D4	1.5:2.0	4:5	3.5	1:3.5	30	1:3	29.17	9.10
D5	1.5:2.0	4:5	3.5	1:4	30	1:3	32.80	8.49
E1	1.5:2.0	4:5	3.5	1:3	20	1:3	25.26	7.45
E2	1.5:2.0	4:5	3.5	1:3	40	1:3	20.23	6.42
E3	1.5:2.0	4:5	3.5	1:3	50	1:3	26.34	7.63
E4	1.5:2.0	4:5	3.5	1:3	60	1:3	29.58	9.15
F1	1.5:2.0	4:5	3.5	1:3	30	1:2	25.71	6.73
F2	1.5:2.0	4:5	3.5	1:3	30	1:4	19.93	5.87
F3	1.5:2.0	4:5	3.5	1:3	30	1:5	23.11	7.43

The weight ratio of *N*-succinyl chitosan to alginate (X1), the weight ratio of drug to polymer (X2), $CaCl_2$ concentration (X3), the volume ratio of *N*-succinyl chitosan/ alginate to $CaCl_2$ (X4), crosslinking time (X5), the weight ratio of Fe_3O_4 to polymer (X6).

Determination of encapsulation efficiency and loading efficiency

The 5-FU loaded beads (100 mg) were incubated in 50 ml phosphate buffer (pH 6.8) at room temperature for 24h. Then the phosphate buffer was filtrated, the filtrate was assayed by a UV spectrophotometer at 265 nm (UV-2401PC, Shimadzu, Japan). All the experiments were carried out in triplicate. Encapsulation efficiency is the percentage of 5-FU contained within the bead in relation to the initial feed amount. The loading efficiency (%) is defined as the weight percentage of loaded drug based on the feed amount. All samples were analyzed in triplicate.

$$\text{Encapsulation efficiency (\%)} = W_a / W_t \times 100\% \quad (2)$$

$$\text{Loading efficiency (\%)} = W_a / W_b \times 100\% \quad (3)$$

Where W_a is the actual 5-FU content, W_t is the theoretical 5-FU content and W_b is the weight of the loaded drug beads.

Magnetic property

A vibrating sample magnetometer (VSM) (Lake Shore, 735 VSM, Model 7304, USA) was used at room temperature to characterize the magnetic properties of pure Fe_3O_4 nanoparticles and 5-FU loaded magnetic *N*-succinyl chitosan/alginate beads.

In vitro drug release

The in vitro 5-FU release properties from the beads were determined as follows: 1.0-1.1g 5-FU-loaded magnetic hydrogel beads were suspended in 500 mL solution and maintained at 37±0.5℃ under 100 rpm. The solutions were HCl solution (pH =1.5) and phosphate buffer solutions of various pH (2.5, 5.0, 6.8, 7.4, and 8.0). At predetermined time intervals, 2mL solution was withdrawn and the dissolution medium was supplied with 2mL fresh buffer solution to maintain the total volume. The drug release percent was determined as follows: the

sample was transferred into a 10mL volumetric flask, added deionized water to scale, and assayed spectrophotometrically at 265 nm (UV-2401PC, Shimadzu, Japan). The drug release percent was determined using equation (4). All samples were analyzed in triplicate.

$$\text{Drug release (\%)} = R_t / L \times 100\%, \quad (4)$$

Where L and R_t represent the initial amount of drug loaded and cumulative amount of drug released at time *t*.

Release kinetics

The mathematical models Korsmeyer-Peppas equation $M_t/M_\infty = kt^n$ or $\log (M_t/M_\infty) = \log k + n\log M_t/M_\infty$ were fitted to individual dissolution data with linear regression by SPSS 11.0 for Windows [25, 26]. The drug release mechanisms of hydrogel beads were described by a semi empirical equation.

Statistical analysis

Statistical analysis for the determination of differences in the swelling characteristics within groups was accomplished using one-way analysis of variance, performed with a statistical program (SPSS 11.0 for Windows). For all statistical calculations, the level of significance was set at 0.05.

RESULTS AND DISCUSSION

Morphology of the beads

Morphological study of *N*-succinyl chitosan/alginate beads was investigated in this section. It can be seen in Figure 1 (a, b), the shape of 5-FU loaded *N*-succinyl chitosan /alginate beads in the wet state was spherical and the surface was smooth, the diameter was about 2.0-3.0 mm. The color of the test beads appeared pitchy because of the presence of magnetite nanoparticles and the beads can be easily removed from the aqueous solution with a magnetic field (*N*-succinyl chitosan: alginate, 1.5%:2.0%). After drying in air, the test beads usually retained their spherical shape but showed a rather rough surface with large wrinkles. The diameter was found to be 1.0 mm approximately (Figure 1b).

The test beads had a spherical surface at a lower total composition of *N*-succinyl chitosan and alginate (1.5%:1.5%, 2.0%:2.0%, 1.5%:2.0% and 2.0%:1.5%). With the content of *N*-succinyl chitosan and alginate increased (2.5%:2.5%, 3.0%:3.0%, 2.0%:2.5% and 2.5%:2.0%), the shape of beads was irregular and it looks like tadpole having a tail. Therefore, the ratio of *N*-succinyl chitosan and alginate would influence the shape of the beads. The SEM pictures of beads were illustrated in Figure 1 (c, d). As can be seen, the surface morphology was regular. Detailed examination of the surface structure revealed cracks and wrinkles caused by partly collapsing the polymer network during dehydration[24].

FTIR spectroscopy

FTIR spectra of Fe_3O_4, *N*-succinyl chitosan,sodium alginate,magnetic *N*-succinyl chitosan/ alginate blank beads, 5-FU,magnetic *N*-succinyl chitosan/alginate beads containing 5-FU are shown in Figure 2. The FTIR spectrum of *N*-succinyl chitosan showed stretching vibration of-OH and -NH_2 at 3424 cm^{-1}, the weak band of-CH_2 stretching at 2924 cm^{-1}, the C = O stretching of amide I band at 1653 cm^{-1}, and the amide II band at 1571cm^{-1}[27]. The peak at 1423 cm^{-1} belongs to -COOH symmetric stretching vibration, the peaks observed at 1069 and 1030 cm^{-1} were the secondary hydroxyl group (characteristic peak of -CH-OH in cyclic alcohols, C-O stretching) and the primary hydroxyl group (characteristic peak of -CH_2-OH in primary alcohols, C–O stretching) [13]. Sodium alginate showed the following distinct peaks: (1) strong absorption bands at 1633 and 1424 cm^{-1} due to carboxyl anions (asymmetric and symmetric stretching vibrations); (2) the bridge oxygen (C-O-C, cyclic ether) stretching bands at 1032 cm^{-1}.For the magnetic *N*-succinyl chitosan/alginate blank beads, the peaks observed at 1638, 1432, 1076, and 861 cm^{-1} were the characteristic absorption band of *N*-succinyl chitosan and alginate; the absorption band at 1571 cm^{-1} of *N*-succinyl chitosan shifts to 1638 cm^{-1} after the reaction with sodium alginate, the stretching vibration of-OH and -NH_2 at 3424 cm^{-1} shifts to 3434 cm^{-1} and comes broad, indicating that the polyelectrolyte complexes are formed and enhanced between *N*-succinyl chitosan and sodium alginate. The characteristic absorption peak of magnetite Fe_3O_4 appears at around 557 cm^{-1}.

The FTIR spectra of 5-FU showed the characteristic absorption peak at 3135 cm^{-1} (stretching vibration), C=O and C=C stretching vibration at 1659 cm^{-1}, C-N stretching vibration at 1247cm^{-1}. CF=CH showed the following distinct peaks: C-H in-plane bending vibration at 1428cm^{-1}, C-H out-of-plane bending vibration at 880cm^{-1}, C-H out-of-plane deformation vibration at 813cm^{-1} and 751cm^{-1}.

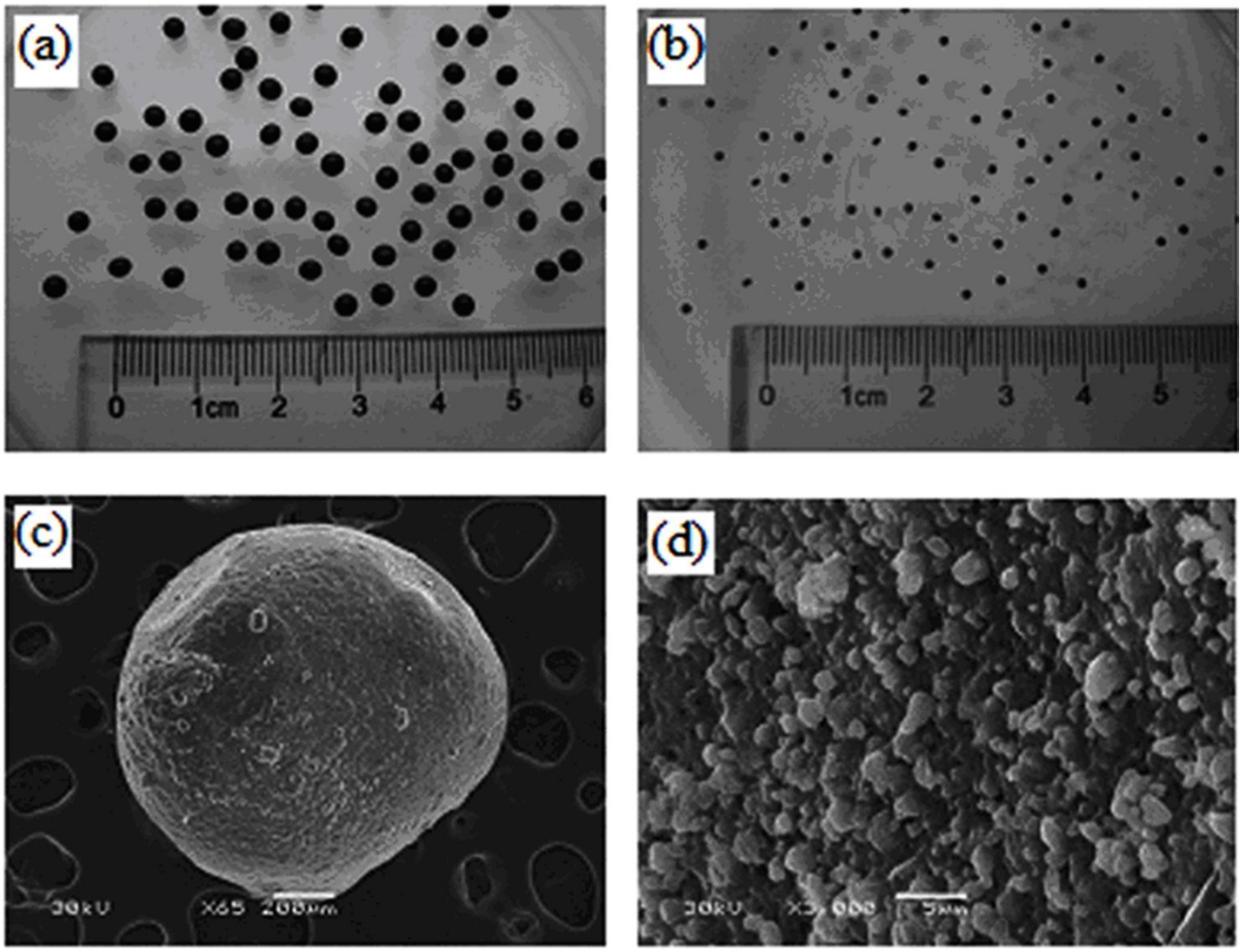

Figure 1: Photographs of magnetic pH-sensitive *N*-succinyl chitosan/alginate beads: wet beads (a); dried beads (b).SEM micrographs of the surface morphology of magnetic *N*-succinyl chitosan/alginate hydrogel beads: magnification(c×65), (d×3000).

The peaks at 3136, 1662, 1430, 881,815,753,1248 cm^{-1} were observed in the FTIR spectra of magnetic *N*-succinyl chitosan/alginate beads containing 5-FU as well, which indicated that the chemical stability of 5-FU was physically filled in the polymeric network.

Swelling characteristics of magnetic N-Succinyl Chitosan/Alginate beads

The swelling ratio of magnetic *N*-succinyl chitosan /alginate beads at pH 1.5 was low and had no distinct difference among all the factors investigated. So, the figures of the swelling ratio of test beads at pH 1.5 were not shown.

Figure 3 shows the swelling characteristics of magnetic *N*-succinyl chitosan /alginate beads at various weight ratio of *N*-succinyl chitosan to alginate. At pH 6.8, the swelling ratio of test beads increased significantly.By increasing the ratio of N-succinyl chitosan and alginate, the swelling ratio of the test beads decreased significantly, the test beads eroded becomed slowly. The high swelling degree may fail to retain drug at the lower end of the gastric pH range. By the weight ratio of *N*-succinyl chitosan to alginate increasing, the shape of beads becomes irregular. The lower the weight ratio of *N*-succinyl chitosan to alginate, the lower the loading efficiency and the encapsulation efficiency. It was found that the test bead (*N*-succinyl chitosan : alginate =1.5% : 2.0%) had a better swelling characteristic among all studied groups (0.64 at pH 1.5 and 27.48 at pH 6.8), more spherical shape and drug release was lower at pH 1.5 than others beads.

Figure 4 shows the effect of the weight ratio of drug to polymer on the swelling characteristic. Obviously, the weight ratio of drug to polymer is another important factor influencing the swelling characteristic. As can be seen, the swelling ratio of test beads with weight ratio of drug to polymer of 1/1 and 4/3 was higher and the beads eroded quickly.

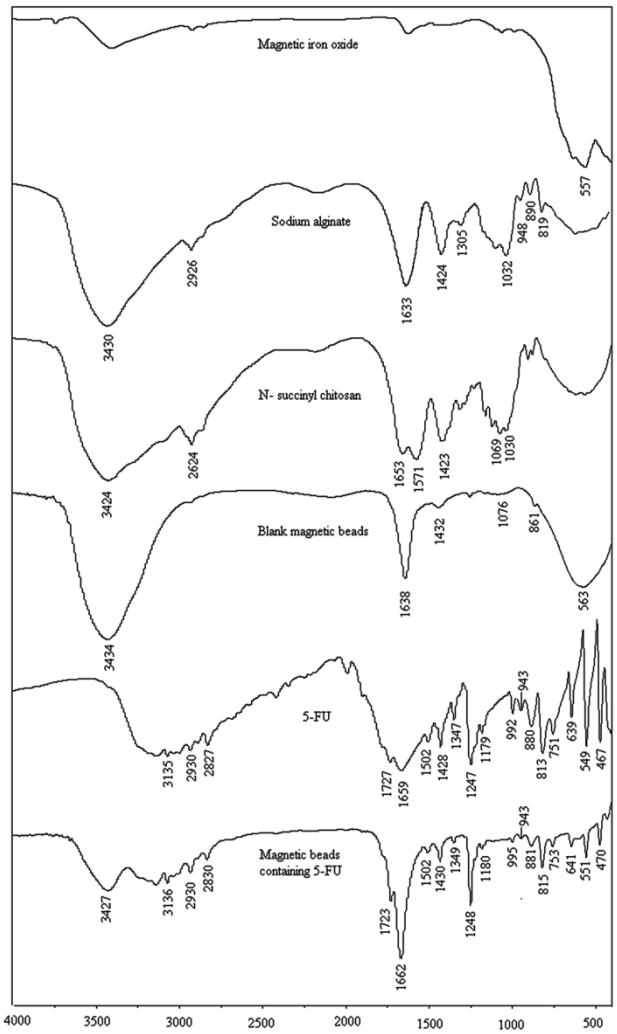

Figure 2: The FTIR spectra of Fe_3O_4, sodium alginate, *N*-succinyl chitosan, magnetic pH-sensitive *N*-succinyl chitosan/alginate blank beads, 5-FU, and magnetic pH-sensitive *N*-succinyl chitosan/ alginate beads containing 5-FU.

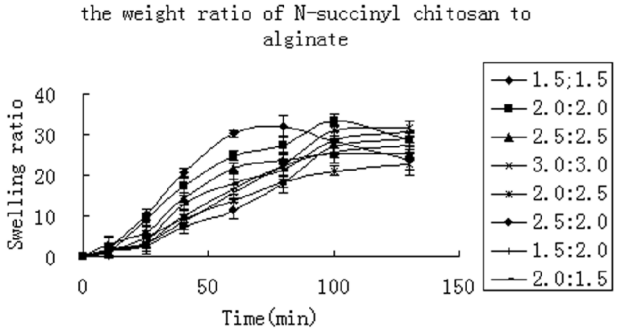

Figure 3: The influence of the weight ratio of *N*-succinyl chitosan to alginate on the swelling characteristic from magnetic *N*-succinyl chitosan /alginate beads (the weight ratio of drug to polymer,2/3;$CaCl_2$ concentration,2.5%; the volume ratio of *N*-succinyl chitosan/alginate to $CaCl_2$,1/3; crosslinking time,30min; the weight ratio of Fe_3O_4 to polymer,1/3).

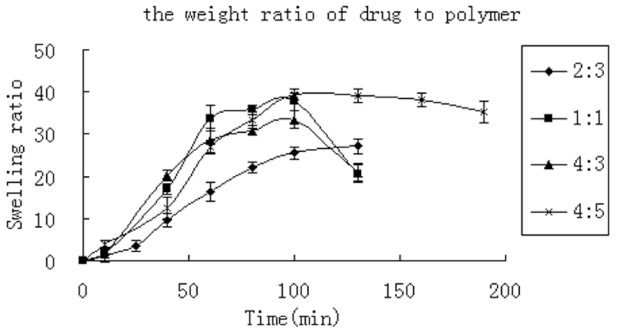

Figure 4: The influence of the weight ratio of drug to polymer on the swelling characteristic from magnetic *N*-succinyl chitosan/alginate beads(the weight ratio of *N*-succinyl chitosan to alginate, 1.5/2.0;$CaCl_2$ concentration,2.5%; the volume ratio of *N*-succinyl chitosan/alginate to $CaCl_2$,1/3; crosslinking time,30min; the weight ratio of Fe_3O_4 to polymer,1/3).

This phenomenon would result in a drug burst release at pH 6.8. So, the hydrogel beads could not become a potential polymer carrier for the controlled release of drug at pH 6.8. It was found that the test bead (the weight ratio of drug to polymer,4/5) had a better swelling characteristic(2.73 at pH 1.5 and 39.42 at pH 6.8) among all studied groups; the loading efficiency and the encapsulation efficiency was higher than others beads; drug release was lower at pH 1.5 than others beads.

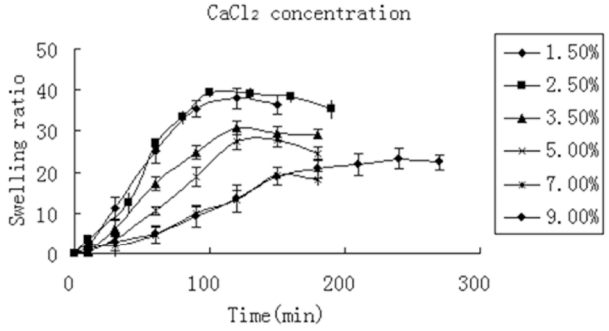

Figure 5: The influence of $CaCl_2$ concentration on the swelling characteristic from magnetic *N*-succinyl chitosan/alginate beads(the weight ratio of *N*-succinyl chitosan to alginate,1.5/2.0; weight ratio of drug to polymer,4/5; the volume ratio of *N*-succinyl chitosan/alginate to $CaCl_2$, 1/3; crosslinking time,30min; the weight ratio of Fe_3O_4 to polymer,1/3).

Figure 5 shows the effect of $CaCl_2$ concentration on the swelling characteristic. By increasing $CaCl_2$ concentration, the swelling ratio of the test beads decreased significantly, the test beads eroded becomed slowly. By increasing $CaCl_2$ concentration, the loading efficiency and the encapsulation efficiency was decreased significantly. At pH 1.5, the drug released from

the beads prepared with different concentration of $CaCl_2$ showed no distinct difference. It was found that the test bead ($CaCl_2$ concentration, 3.5%) had a higher loading efficiency and encapsulation efficiency among all studied groups.

Figure 6 shows the swelling characteristic of *N*-succinyl chitosan/alginate beads prepared at various volume ratio of *N*-succinyl chitosan/alginate to $CaCl_2$. By increasing the volume of $CaCl_2$, the swelling ratio of the test beads decreased significantly, the test beads eroded become slowly. But when the volume ratio of *N*-succinyl chitosan/alginate to $CaCl_2$ was larger than 1/3, the swelling ratio of the test beads became larger again. It was found that the test bead (the volume ratio of *N*-succinyl chitosan/alginate to $CaCl_2$, 1/3) had the lowest swelling ratio (1.12 at pH 1.5 and 30.84at pH 6.8).

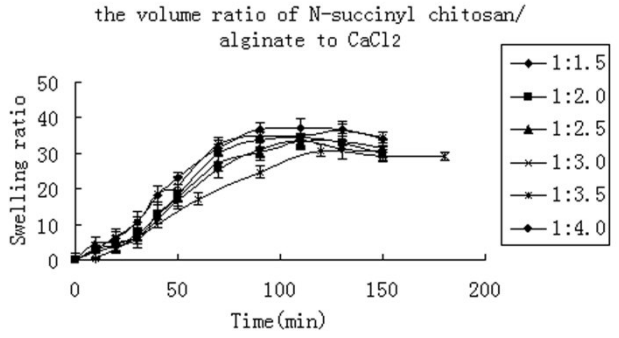

Figure 6: The influence of the volume ratio of *N*-succinyl chitosan/alginate to $CaCl_2$ on the swelling characteristic from magnetic *N*-succinyl chitosan/alginate beads(the weight ratio of *N*-succinyl chitosan to alginate,1.5/2.0; weight ratio of drug to polymer, 4/5; $CaCl_2$ concentration ,3.5%; crosslinking time,30min; the weight ratio of Fe_3O_4 to polymer,1/3).

Figure 7 shows the effect of crosslinking time on swelling characteristics of the *N*-succinyl chitosan/alginate beads. The swelling ratio of all test groups had no distinct difference at pH 1.5 and 6.8, respectively. The swelling ratio of the test group with a crosslinking time of 30 min was lower than the other groups at pH 6.8 and had a higher loading efficiency and encapsulation efficiency among all studied groups.

Figure 8 shows the effect of the weight ratio of Fe_3O_4 to polymer on the swelling characteristic. As shown, the swelling ratio of test beads with the weight ratio of Fe_3O_4 to polymer of 1:3 was lower than the other groups at pH 6.8 and 1.5. It was found that the test bead (the weight ratio of Fe_3O_4 to polymer, 1/3) had a higher loading efficiency and encapsulation efficiency among all studied groups, and the release of 5-FU was least at pH 1.5 than the other groups.

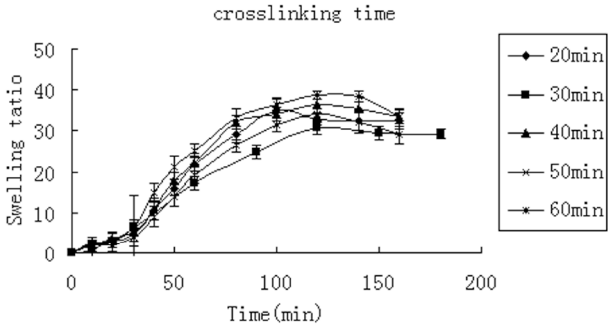

Figure 7: The influence of crosslinking time on the swelling characteristic from magnetic *N*-succinyl chitosan/alginate beads(the weight ratio of *N*-succinyl chitosan to alginate,1.5/2.0; weight ratio of drug to polymer,4/5; $CaCl_2$ concentration ,3.5%; the volume ratio of *N*-succinyl chitosan/alginate to $CaCl_2$,1/3; the weight ratio of Fe_3O_4 to polymer,1/3).

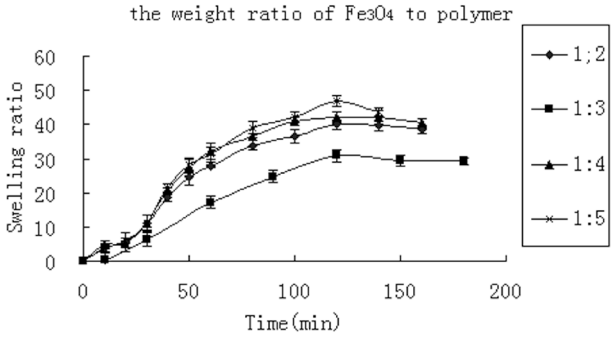

Figure 8: The influence of the weight ratio of Fe_3O_4 to polymer on the swelling characteristic from magnetic *N*-succinyl chitosan/alginate beads(the weight ratio of *N*-succinyl chitosan to alginate, 1.5/2.0; weight ratio of drug to polymer,4/5; $CaCl_2$ concentration,3.5%; the volume ratio of *N*-succinyl chitosan/alginate to $CaCl_2$,1/3; crosslinking time,30min).

Magnetic property

Figure 9 shows a magnetization curve of the *N*-succinyl chitosan/alginate beads. The saturation magnetization (σs) of the magnetic *N*-succinyl chitosan/alginate beads was 5.64 emu/g, which comparing the reference value for the pure magnetite nanoparticles of σs was 58.57 emu/g.

Magnetic response property of the beads was determined by dispersing dried test beads in 0.2% Tween-80 solution (w/v) adequately. A given magnetic field (MF) of about 3200 Oe was used to determine magnetic response time of the beads. When the weight ratio of magnetite nanoparticles to polymer increased, response

time of the beads to magnetic field decreased quickly. After increasing weight ratio of magnetite nanoparticles to polymer from 1:3 to 1:2, response time of the beads to MF was decreased from 18 to 11 seconds. Therefore, the higher the weight ratio of magnetite nanoparticles to polymer, the shorter the response time and the better magnetic response property.

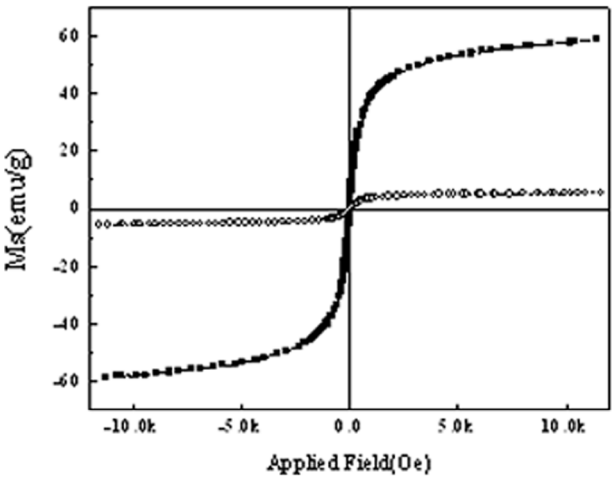

Figure 9: Magnetization curve of magnetic pH-sensitive *N*-succinyl chitosan/alginate beads: (magnetic *N*-succinyl chitosan/alginate beads containing 5-FU), (pure Fe_3O_4).

The superparamagnetic property of polymer magnetic beads is critical for their application in biomedical and bioengineering fields, which can prevent polymer magnetic beads from aggregating and make them to redisperse rapidly when the MF is removed. As could be seen from Figure 9, the hysteresis loop showed superparamagnetic property, which indicated the single-domain magnetic nanoparticles remained in these polymer nanoparticles.

In vitro release properties of 5-FU

Figure 10 shows the cumulative release curves of 5-FU from *N*-succinyl chitosan/ alginate beads at various pH at 37±0.5°C as a function of time. It can be seen that the percentage released increased with the pH of the medium. Within 1.5 hours, the amount of 5-FU released from the magnetic beads was 35.78% at pH 1.5, 39.35% at pH 2.5, 46.52% at pH 5.0, 61.03% at pH 6.8, 60.91% at pH 7.4 and 61.56% at pH 8.0, This suggests that the drug release properties of magnetic *N*-succinyl chitosan/alginate beads are pH sensitive. Two factors are related to the noticeably higher release rate of 5-FU at pH 6.8 than at pH 1.5. The first factor is the dissociation of the polyelectrolyte complex and the breakage of hydrogen bond. The second factor is the swelling of the hydrogel increased considerably due to ionization of carboxylic groups in test beads at pH 6.8.

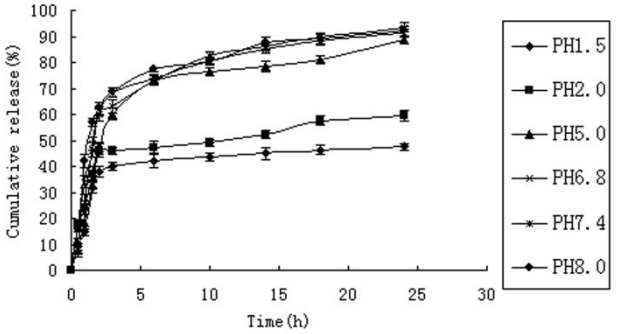

Figure 10: The cumulative release curves of 5-FU from magnetic pH-sensitive *N*-succinyl chitosan/alginate beads at various pH at 37±0.5°C.

Drug Release Kinetics

The Korsmeyer-Peppas equation (5) is often used to describe the drug release behavior from polymeric systems when the mechanism is not well known or when more than one type of release phenomena are involved. This equation was used to explain the drug release mechanism and to compare the release profiles; the drug released amount versus time was used.

$$M_t/M_\infty = kt^n \text{ or } \log(M_t/M_\infty) = \log k + n\log t, \quad (5)$$

Where M_t/M_∞ is the fractional release of the drug at time *t*, *n* is a diffusion exponent that can indicate the release mechanism, and *k* is a characteristic constant of the system. From the slope and intercept of the plot of log (M_t/M_∞) versus log *t*, kinetic parameters *n* and *k* were calculated. For spheres, values of *n* between 0.43 and 0.85 are an indication of both diffusion-controlled drug release and swelling-controlled drug release (anomalous transport). Values above 0.85 indicate case-II transport that relate to polymer relaxation during gel swelling. Values below 0.43 indicate that drug release from polymer was due to Fickian diffusion [25, 26].

5-FU release kinetics from *N*-succinyl chitosan/alginate beads at different pH was shown in Table 2. 5-FU release profile changes showed the drug undergoing immediate release within the first 1.5 hours. After 1.5 hours, the remaining drug is released almost linearly. At pH 5.0, the release was both diffusion and erosion mechanisms, whereas drug release mechanism at other pHs was Fickian diffusion.

Table 2: Estimated parameters and drug release mechanism of magnetic pH-sensitive N-succinyl chitosan/alginate beads at different pH

pH	*n*	*k*	*r*	Drug transport mechanism
1.5	0.3631	4.2727	0.8478	Fickian diffusion
2.0	0.2866	8.1677	0.9166	Fickian diffusion
5.0	0.5483	2.0701	0.9204	anomalous transport
6.8	0.3826	6.9103	0.9097	Fickian diffusion
7.4	0.4252	5.2638	0.8859	Fickian diffusion
8.0	0.4123	6.0007	0.8416	Fickian diffusion

CONCLUSION

Drug delivery system using polymer magnetic carriers is considered as an effective strategy for passive target. It can increase drug utilization and reduce the adverse reaction. Because the carriers are sensitive to physical stimuli (such as magnetic field and pH), the drug could be located to desire position. The magnetic pH-sensitive *N*-succinyl chitosan/alginate beads are produced by simple ionic gelation. The preparation procedure was modified by various factors to control the swelling behavior of the beads. The results showed that the beads prepared at the weight ratio of *N*-succinyl chitosan to alginate(1.5/2.0), weight ratio of drug to polymer (4/5),$CaCl_2$ concentration(3.5%), the volume ratio of *N*-succinyl chitosan/alginate to $CaCl_2$(1/3), crosslinking time(30min), the weight ratio of Fe_3O_4 to polymer(1/3) demonstrated the best swelling characteristic and pH sensitivity. The beads swelled slightly at pH 1.5 and the drug released is 47.93%, however, they swelled more at pH 6.8 and the drug released is 93.91%.In this study, *N*-succinyl chitosan/alginate beads containing magnetic nanoparticles and 5-FU were prepared to obtain magnetic pH-sensitive hydrogel. The results showed the beads possess pH sensitivity in swelling ratio and superparamagnetic property as well as fast magnetic response, which indicated the potential applications of these beads as a magnetic targeting system in the gastrointestinal tract.

ACKNOWLEDGEMENTS

This study was supported by the youth innovation research fund for interdisciplince of Lanzhou University, China (Grant No: LZUJC200926) and the pedestal plan fund for science and technology of Gansu Province, China (Grant No: 0708NKCA129).

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

[1] Schmidt Laugesen C, Steffansen B, Scherfig E, la Cour M. Pharmacokinetics of intravitreal 5-fluorouracil prodrugs in silicone oil: experimental studies in pigs. Acta Ophthalmol Scand 2005; 83: 184–190.

[2] Krishnaiah YSR, Satyanarayana V, Dinesh Kumar B, Karthikeyan R S , Bhaskar P. *In vivo* pharmacokinetics in human volunteers: oral administered guar gum-based colon-targeted 5-fluorouracil tablets. Eur J Pharm Sci 2003; 19: 355–362.

[3] Martinez J, Martin C, Chacon M, Korbenfeld E, Bella S, Senna S, Richardet E, Coppola F, Bas C, Hidalgo J, Escobar E, Reale M, Smilovich A, Wasserman E. Irinotecan, oxaliplatin plus bolus 5-fluorouracil and low dose folinic acid every 2 weeks: a feasibility study in metastatic colorectal cancer patients. Am J Clin Oncol 2006; 29: 45– 5.

[4] Mitsuhashi H, Suemaru K, Li BJ, Cui RJ, Araki H. Evaluation of topical external medicine for 5-fluorouracil-induced oral mucositis in hamsters. Eur J Pharmacol 2006; 551: 152–155.

[5] Yim E K, Lee S B, Lee K H, Kim C J, Park J S. Analysis of the in vitro synergistic effect of 5-fluorouracil and cisplatin on cervical carcinoma cells. Int J Gynecol Cancer 2006; 16: 1321– 1329.

[6] Haller DG. An overview of adjuvant therapy for colorectal. Eur J Cancer 1995; 31A (7/8): 1255–1263.

[7] Bajetta E, Di Bartolomeo M, Somma L, Del Vecchio M, Artale S, Zunino F, Bignami P, Magnani E, Buzzoni R.. Doxifluridine in colorectal cancer patients resistant to 5-Fluorouracil (5-FU) containing regimens. Eur J Cancer 1997; 33(4):687–690.

[8] Almond B A, Hadba A R, Freeman S T, Cuevas B J , York A M, Detrisac C J, Goldberg E P. Efficacy of mitoxantroneloaded albumin microspheres for intratumoral chemotherapy of breast cancer.J Control Release 2003;91(1–2):147–155.

[9] Tamura T, Fujita F, Tanimoto M, Koike M , Suzuki A, Fujita M, Horikiri Y, Sakamoto Y, Suzuki T, Yoshino H.Anti-tumor effect of intraperitoneal administration of cisplatin-loaded microspheres to human tumor xenografted nude mice, J Control Release 2002;80(1–3):295– 307.

[10] Chen LY, Tian ZG, Du YM. Synthesis and pH sensitivity of carboxymethyl chitosan-based polyampholyte hydrogels for protein carrier matrices. Biomaterials 2004;25: 3725–3732.

[11] Einerson NJ, Stevens KR, Kao WJ. Synthesis and physicochemical analysis of gelatin-based hydrogels fordrug carrier matrices. Biomaterials 2003; 24: 509–523.

[12] Zhang H, Mardyani S, Chan WC W, Kumacheva E.Design of biocompatible chitosan microgels for targeted pHmediated intracellular release of cancer therapeutics.
Biomacromolecules 2006; 7: 1568–1572.

[13] Chen SC, Wu YC, Mi FL, Lin YH, Yu LC, Sung HW.A novel pH-sensitive hydrogel composed of N,O-carboxymethyl chitosan and alginate crosslinked by genipin for protein drug delivery. J Control Rel 2004; 96: 285–300.

[14] Gray C J, Dowsett J.Retention of insulin in alginate gel beads. Biotech Bioeng 1988;31: 607–612.

[15] Kato Y, Onishi H, Machida Y.N-succinyl-chitosan as a drug carrier:water-insoluble and water-soluble conjugates. Biomaterials 2004; 25: 907–915.

[16] Chen LY, Tian ZG, Du YM. Synthesis and pH sensitivity of carboxymethyl chitosan-based polyampholyte hydrogels for protein carrier matrices. Biomaterials 2004; 25: 3725–3732.

[17] Liu ZH, Jiao YP, Zhang ZY. Calcium-carboxymethyl chitosan hydrogel beads for protein drug delivery system. J Appl Polymer Sci 2007; 103: 3164–3168.

[18] Yan LF, Qian F, Zhu QS. Interpolymer complex polyampholytic hydrogel of chitosan and carboxymethyl cellulose (CMC): synthesis and ion effect. Polym Int 2001; 50: 1370–1374.

[19] Liu TY, Hu SH, Liu KH, Liu DM, Chen SY.Study on controlled drug permeation of magnetic-sensitive ferrogels: Effect of Fe3O4 and PVA. J Control Release 2008; 126:228–236.

[20] Li GY, Huang KL, Jiang YR, Yang DL, Ding P. Preparation and characterization of Saccharomyces cerevisiae alcohol dehydrogenase immobilized on magnetic nanoparticles. Int J Biol Macromol 2008; 42: 405–412.

[21] Shamim N, Hong L, Hidajat K, Uddin MS.Thermosensitive polymer coated nanomagnetic particles for separation of bio-molecules.Sep Purif Technol 2007;53:164–170.

[22] Mulder W J M, Strijkers G J, Van Tilborg G A F, Griffioen A W, Nicolay K. Lipid-based nanoparticles for contrast- enhanced MRI and molecular imaging. NMR Biomed 2006;19:142– 164.

[23] Müeller-Schulte D, Schmitz-Rode T. Thermoresponsive magnetic polymer particles as contactless controllable drug carriers. J Magn Magn Mater 2006; 302: 267–271.

[24] Pasparakis G, Bouropoulos N.Swelling studies and in vitro release of verapamil from calcium alginate and calcium alginate-chitosan beads. Int J Pharm 2006; 323: 34–42.

[25] Takka S, Ocak O H, Acartürk F.Formulation and investigation of nicardipine HCl–alginate gel beads with factorial design-based studies. Eur J Pharm Biopharm 1998; 6: 241–246.

[26] Siepmann J, Peppas N A. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Adv Drug Deliv Rev 2001; 48: 139–157.

[27] Sankalia M G, Mashru RC, Sankalia JM, Sutariya VB. Reversed chitosan–alginate polyelectrolyte complex for stability improvement of alpha-amylase: optimization and physicochemical characterization. Eur J Pharm Biopharm 2007; 65: 215–232.