Multi-Stimuli-Responsive Microcapsules for Adjustable Controlled-Release

Jie Wei, Xiao-Jie Ju, Xiao-Yi Zou, Rui Xie, Wei Wang, Ying-Mei Liu, and Liang-Yin Chu**

Novel multi-stimuli-responsive microcapsules with adjustable controlled-release characteristics are prepared by a microfluidic technique. The proposed microcapsules are composed of crosslinked chitosan acting as pH-responsive capsule membrane, embedded magnetic nanoparticles to realize "site-specific targeting", and embedded temperature-responsive sub-microspheres serving as "micro-valves". By applying an external magnetic field, the prepared smart microcapsules can achieve targeting aggregation at specific sites. Due to acid-induced swelling of the capsule membranes, the microcapsules exhibit higher release rate at specific acidic sites compared to that at normal sites with physiological pH. More importantly, through controlling the hydrodynamic size of sub-microsphere "micro-valves" by regulating the environment temperature, the release rate of drug molecules from the microcapsules can be flexibly adjusted. This kind of multi-stimuli-responsive microcapsules with site-specific targeting and adjustable controlled-release characteristics provides a new mode for designing "intelligent" controlled-release systems and is expected to realize more rational drug administration.

1. Introduction

Smart microcapsules, which can control the release of their encapsulated contents according to various environmental stimuli, have attracted great interests from various fields in recent years. Because of their relatively faster response rate and other advantages such as small size, large inner volume, huge total surface area and stable capsule membrane, these environmental stimuli-responsive microcapsules are considered to be the most ideally intelligent drug delivery systems. By encapsulated inside these microcapsules, drugs or chemicals can be released at a desired rate only when and/or where the release is needed.[1] A considerable amount of researches have been carried out on temperature-responsive,[2–12] pH-responsive,[13–27] magnetic-responsive,[28–37] and other stimuli-responsive microcapsules in recent years. However, in many cases, different environmental changes may occur at the same time, thus single stimulus-responsive microcapsules are insufficient for practical applications. Therefore, it is much more favorable that microcapsules possess multiple stimuli-responsive properties simultaneously.[38–41] More importantly, the patients' conditions are usually complex and diverse. To achieve best effects and reduce side effects of drugs, it is necessary to regulate the release dosage and the release rate in time according to patients' individual differences. However, the design and preparation of multi-stimuli-responsive microcapsules with adjustable controlled-release have not been reported yet.

Up to now, the stimuli-responsive microcapsules used as drug delivery systems are mainly developed based on the "on-off" mechanism, which can be divided into two categories. The first one is prepared by grafting stimuli-responsive materials into the pores of microcapsules,[1,2,5,11,12,37,38,42] or incorporating stimuli-responsive particles into the microcapsule membranes.[4,43] The microcapsule substrates have no environmental response and the controlled-release properties only depend on the abrupt deswelling/swelling properties of the graft chains or the embedded particles, which can regulate the trans-membrane diffusional permeation of drug molecules. Another one is fabricated by directly utilizing the stimuli-responsive materials as the microcapsule membrane. The release mechanisms of these microcapsules mainly rely on the deswelling/swelling properties of the capsule membrane themselves,[7–10,13–15,19,22–24,26,28,35,39–41] or the squeezing action from sudden shrinking of the capsule membranes,[36,44,45] or the decomposition of capsule membranes.[16,17,20,46,47] Generally, due to the "on-off" state of microcapsules, the drug controlled-release is often in an extreme "on" or "off" state. Their drug release rate can not be flexibly adjusted according to patients' individual differences; therefore, smart microcapsules with adjustable controlled-release rate are more rational for drug administration. Besides, pH is an important stimulus for stimuli-responsive controlled-release systems, because pH differentiation exists at many specific and pathological sites in human body. Temperature is also an important factor that can be easily controlled, and the site-specific targeting drug delivery can be achieved easily by

J. Wei, Prof. X.-J. Ju, X.-Y. Zou, Prof. R. Xie,
Dr. W. Wang, Y.-M. Liu, Prof. L.-Y. Chu
School of Chemical Engineering
Sichuan University
Chengdu, Sichuan 610065, P. R. China
E-mail: chuly@scu.edu.cn; juxiaojie@scu.edu.cn
Prof. L.-Y. Chu
State Key Laboratory of Polymer Materials Engineering
and Collaborative Innovation Center for
Biomaterials Science and Technology
Sichuan University
Chengdu, Sichuan 610065, P. R. China

DOI: 10.1002/adfm.201303844

magnetic targeting. Consequently, the design and preparation of microcapsules with pH-, temperature- and magnetic-responsive synergistic effects and adjustable controlled-release rate are of great potential in drug delivery.

In this study, we report on a novel multi-stimuli-responsive microcapsule with adjustable controlled-release by embedding temperature-responsive sub-microspheres as "micro-valves" into the magnetic- and pH-responsive microcapsule membrane. The proposed microcapsules can simultaneously achieve targeted delivery by applying an external magnetic field, self-regulated drug release according to pH difference at pathological sites, and adjustable controlled-release relying on temperature regulation. That is, this kind of novel microcapsule can achieve targeting aggregation at specific pathological sites and effectively adjustable controlled-release according to patients' individual differences, which is of great importance for realizing more rational drug administration.

2. Results and Discussion

2.1. Strategy for Preparation of Microcapsules

The fabrication procedure and the controlled-release mechanism of the proposed microcapsules are schematically illustrated in **Figure 1**. The microcapsule is composed of biocompatible and pH-responsive chitosan capsule membrane embedded with magnetic nanoparticles and temperature-responsive sub-microspheres. For drug delivery systems, microcapsules with narrow size distribution are preferable, since the drug loading levels and release kinetics are directly affected by the size distribution of microcapsules. Microfluidic techniques, which have been developed for generating highly monodisperse emulsions in recent years,[48,49] are used for preparing microcapsules with uniform size. As shown in Figure 1A, the chitosan aqueous solution containing magnetic-responsive

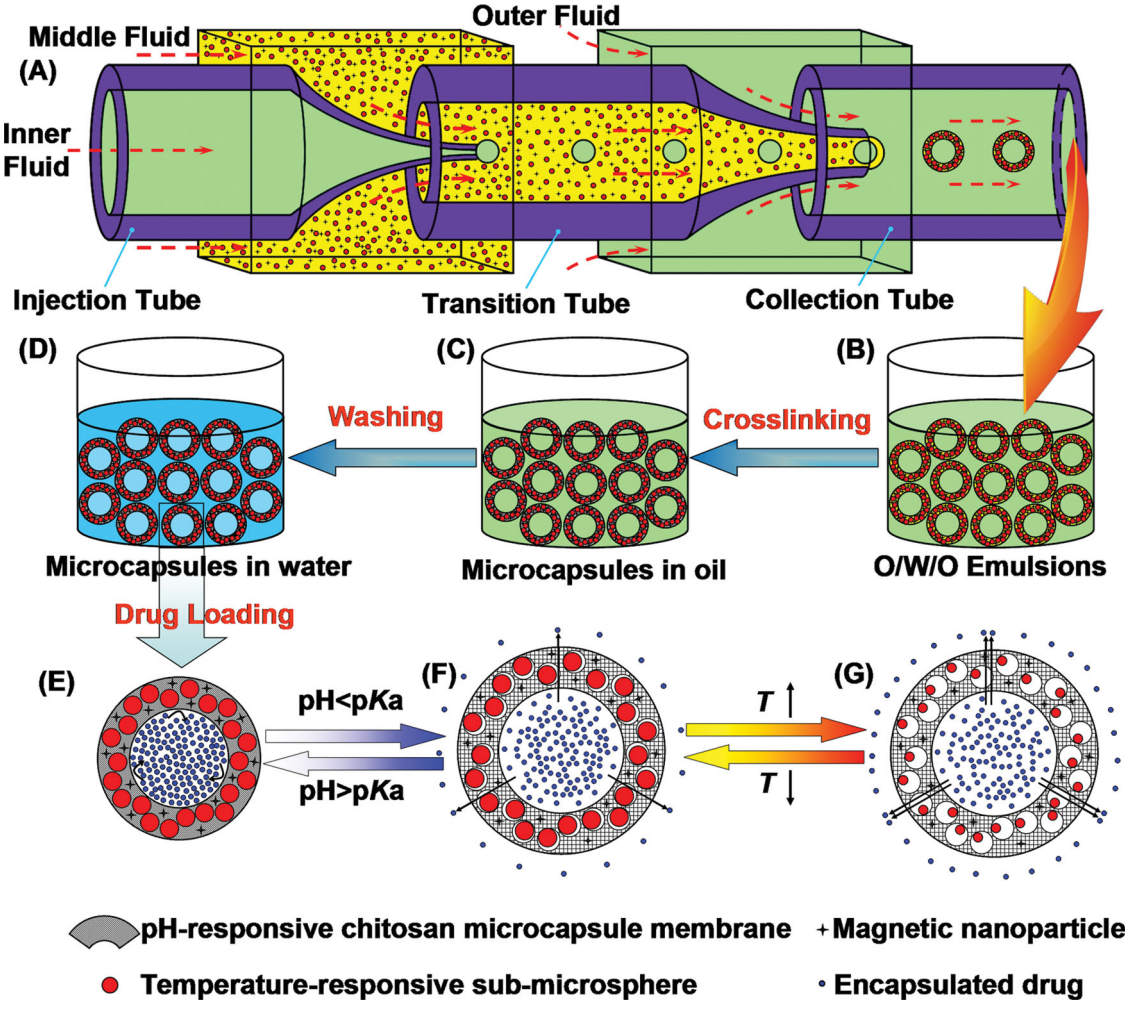

Figure 1. Schematic illustration of fabrication process (A–D) and controlled-release mechanism (E–G) of the proposed multi-stimuli-responsive microcapsules with adjustable controlled-release. The capillary microfluidic device (A) is used for generating O/W/O double emulsions (B), and microcapsules are prepared by using these emulsions as templates via crosslinking reaction (C,D). When pH > p*Ka*, the microcapsule is in a shrunken state, therefore the release rate is low (E); while a high release rate is resulted due to the swollen state of the microcapsule when pH < p*Ka* (F). By increasing environmental temperature, the interspace size between the capsule membrane and sub-microspheres becomes larger due to the shrinkage of sub-microspheres, so the drug release rate is further increased (G). The pH-/thermo-responsive behaviors are reversible.

nanoparticles and temperature-responsive sub-microspheres is used as the middle fluid (MF), while the oil phase containing crosslinker glutaraldehyde (GA) is used as the inner fluid (IF) and outer fluid (OF). The obtained oil-in-water-in-oil (O/W/O) emulsions (Figure 1B) are used as templates for the preparation of multi-stimuli-responsive microcapsules (Figure 1C,D).

By embedding magnetic nanoparticles into the microcapsule membrane, site-specific targeting can be achieved under an external magnetic field. Crosslinked chitosan have typical cationic pH-responsive properties. At specific sites where the environmental pH is higher than the p*K*a value of chitosan (about 6.2–7.0),[16] such as in the normal tissue (the body physiological pH is about 7.4), the microcapsule membrane is in the compact state due to the pH-responsive shrinking, which results in a low release rate of drugs from the microcapsule (Figure 1E). On the other hand, when the environmental pH is lower than the p*K*a value of chitosan, such as at some chronic wound sites whose pH values are as low as 5.45,[50] the microcapsule membrane is in the loose state due to the pH-responsive swelling, which leads to a high release rate (Figure 1F). That is, the prepared smart microcapsules can realize self-regulated drug release according to pH difference at pathological sites. More importantly, through the addition of temperature-responsive sub-microspheres as "micro-valves", drug release rate can be effectively adjusted through regulating external temperature via local heating/cooling. The interspace size between the microcapsule membrane and sub-microspheres can be flexibly regulated through the temperature-dependent volume change of sub-microspheres, which results in adjustable controlled-release properties. When ambient temperature is increased, the interspaces become lager due to the shrinking of sub-microspheres, and therefore drug release rate is higher (Figure 1G); on the contrary, when the temperature is decreased, the interspaces become smaller due to the swelling of sub-microspheres, and then a lower release rate is resulted (Figure 1F).

2.2. Characterization of Magnetic Nanoparticles and Temperature-Responsive Sub-Microspheres

The morphology of the magnetic nanoparticles is observed by transmission electron microscopy (TEM). As shown in **Figure 2**A, the size of prepared magnetic nanoparticles is around 20 nm and there is no obvious aggregation, which indicates that the nanoparticles are well dispersed in water. Magnetic property of the nanoparticles is measured with a vibrating sample magnetometer (VSM). As shown in Figure 2B, the saturation magnetization (*M*s) of the magnetic nanoparticles is 72.32 emu g^{-1}, and hysteresis and coercivity are almost undetectable, which suggests that the superparamagnetic property of the prepared nanoparticles is satisfactory.

The temperature-responsive sub-microspheres are labeled with red fluorescence dye to make them easy to be observed. Moreover, in order to make the temperature-responsive sub-microspheres have obvious volume phase transition near human body temperature (37 °C), acrylamide (AAm) is used as a hydrophilic comonomer to modulate the thermo-sensitivity of the sub-microspheres. Figure 2C shows the confocal laser scanning microscope (CLSM) image of obtained poly(*N*-isopropylacrylamide-*co*-acrylamide) (P(NIPAM-*co*-AAm)) sub-microspheres in water at room temperature on the red fluorescent channel excited at 543 nm. The sub-microspheres show red fluorescence and exhibit good monodispersity, and are well-dispersed in water. The equilibrium deswelling ratio ($D_T/D_{25°C}$) of P(NIPAM-*co*-AAm) sub-microspheres as a function of temperature is shown in Figure 2D. The results show that these temperature-responsive sub-microspheres deswell gradually with increasing temperature and the equilibrium deswelling ratio shows significant change near body temperature (37 °C). This kind of temperature-dependent volume phase transition provides feasibility for subsequent adjustable controlled-release near body temperature.

2.3. Morphologies of Emulsions and Microcapsules

The optical and fluorescent microscope images of O/W/O double emulsions generated by the microfluidic method are shown in **Figure 3**. The emulsions, which act as templates for fabricating microcapsules via crosslinking reaction, are highly monodisperse and quite stable (Figure 3A′–C′). Because only temperature-responsive sub-microspheres are labeled with red fluorescence, emulsions with only chitosan in middle water phase (Figure 3A) or with both chitosan and magnetic nanoparticles in middle water phase (Figure 3B) exhibit no fluorescence; while emulsions containing chitosan, magnetic nanoparticles and sub-microspheres (Figure 3C) show clearly red fluorescence.

Figure 4 shows CLSM images and optical microscope images of different microcapsules crosslinked from different double emulsion templates. The microcapsules are dispersed in buffer solution of pH 7.4 at 37 °C, which simulate the body physiological pH and temperature. Under the same condition, only chitosan microcapsules containing both magnetic nanoparticles and temperature-responsive sub-microspheres (CS-M-T, Figure 4C) show red fluorescent, while the chitosan microcapsules (CS, Figure 4A) and the magnetic nanoparticles embedded chitosan microcapsules (CS-M, Figure 4B) show no fluorescence. The red fluorescence from the capsule membranes of CS-M-T microcapsules indicates the successfully embedding of temperature-responsive sub-microspheres into the microcapsules. The optical microscope images of different microcapsules (Figure 4A′–C′) look almost the same, which all show that the obtained microcapsules are with good sphericity and monodispersity.

2.4. Influences of Preparation Conditions on pH-Sensitivities of Microcapsules

Microcapsules with pH-sensitivity are studied widely, because pH difference exists at many physiological, biological and/or chemical systems. Chronic wounds have been reported to have pH values between 8.65 and 5.45,[50] and cancer tissue is also reported to be acidic extracellularly.[51,52] The cationic pH-responsive microcapsules, which have acid-responsive swelling property due to protonation at acidic conditions, are suitable for rate-controlled release and sustained drug release in acidic conditions via self-regulated adjustment of molecular diffusion permeation. Chitosan is a well-known cationic polysaccharide with

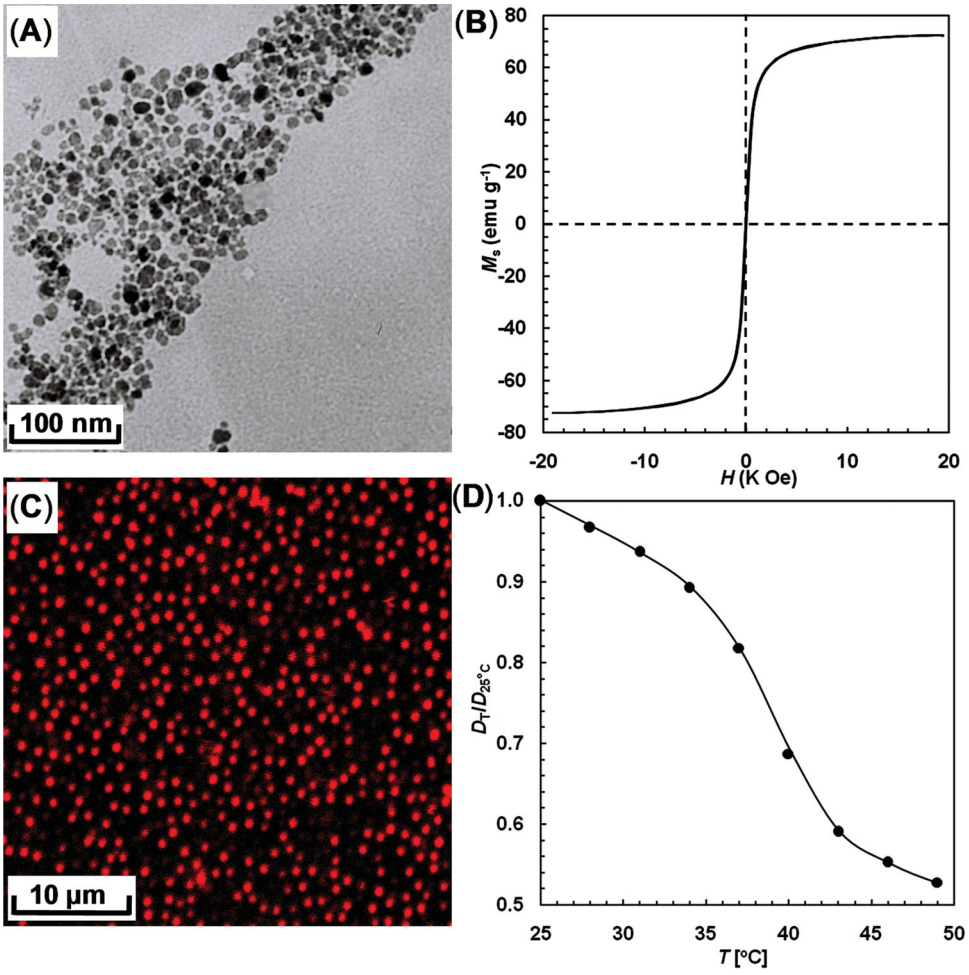

Figure 2. A) TEM image of the prepared magnetic nanoparticles, B) magnetization curve of magnetic nanoparticles at room temperature, C) CLSM image of the temperature-responsive sub-microspheres in water at room temperature on red fluorescent channel, and D) temperature-responsive property of sub-microspheres.

excellent biological activity, good biocompatibility and biodegradability, so microcapsules based on chitosan are attracting more and more interests in various applications. Microcapsules made from crosslinked chitosan exhibit typical cationic pH-responsive characteristics. When the environmental pH is lower than the p*K*a value of chitosan, the microcapsules swell due to the protonation of amino groups, and when the pH is higher than its p*K*a value, the deprotonation of amino groups results the deswell of microcapsules. Swelling ratios ($OD_{pH}/OD_{pH7.4}$) of microcapsule outer diameter at certain pH to that at body physiological pH 7.4 are used to characterize the acid-induced swelling change of these prepared microcapsules.

The influences of the preparation conditions on the pH-sensitivities of microcapsules are investigated and the recipes of different fluids are listed in **Table 1**. As shown in **Figure 5**, all microcapsules exhibit good pH-sensitivity, and the swelling ratios increase with decrease of external pH values. From Figure 5A it can be seen that, with the same IF and MF, the chitosan microcapsules prepared with different GA concentrations in OF show different swelling extents. The chitosan microcapsules prepared with lower GA concentration exhibit higher swelling ratio compared with that prepared with higher GA concentration. The similar results are also observed from microcapsules prepared with different GA concentrations in IF, as shown in Figure 5B. GA is the crosslinker for chitosan microcapsules. With the decrease of GA concentration, the crosslinking density of microcapsules decreases, which results the increase in swelling ratios of microcapsules. Microcapsules prepared with too low crosslinking density are easy to be broken, so GA concentrations of IF and OF are chosen to be 1/50 (v/v) in the subsequent studies.

For the preparation of chitosan microcapsules, hydroxyethyl cellulose (HEC) is added into MF to increase fluid viscosity. The chitosan microcapsules prepared with HEC addition are much stable than those prepared without HEC addition. The pH-responsive behaviors of microcapsules prepared with and without addition of HEC are also studied. Microcapsules prepared without HEC addition show slightly larger swelling ratio than that prepared with HEC addition at a same pH condition (Figure 5C). The reason is that HEC is a macromolecular compound, which has long polymer chains. The entanglements between HEC and chitosan molecular chains restrict the swelling of microcapsules, so the lower swelling ratio of microcapsules prepared with HEC addition is resulted. Considering

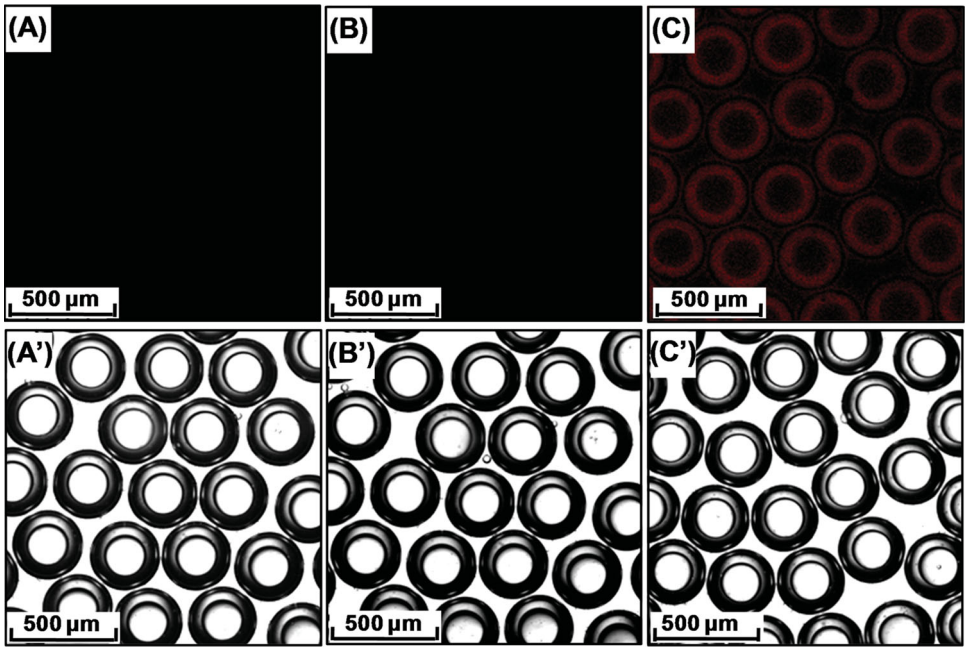

Figure 3. A–C) CLSM images of different O/W/O double emulsion templates on red fluorescent channel; and A′–C′) optical microscope images of different emulsion templates. (A,A') represent emulsions prepared with only chitosan in MF (4#), (B,B′) represent emulsions prepared with both chitosan and magnetic nanoparticles in MF (5#), C and C' represent emulsions prepared with chitosan, magnetic nanoparticles and temperature-responsive sub-microspheres in MF (6#). Scale bars are all 500 μm.

the addition of magnetic nanoparticles and sub-microspheres could also increase the viscosity of MF, and to make particles well dispersed in MF, the chitosan solution without addition of HEC is used in the subsequent studies.

By adding magnetic nanoparticles into MF, chitosan microcapsules with magnetic-sensitivity (CS-M) are obtained. With the addition of magnetic nanoparticles and thermo-sensitive sub-microspheres into MF, the generated chitosan microcapsules possess both magnetic-sensitivity and thermo-sensitivity (CS-M-T). The pH-sensitivities of these microcapsules are also investigated, as shown in Figure 5D. The addition of magnetic nanoparticles have almost no influence on the pH-sensitivity of

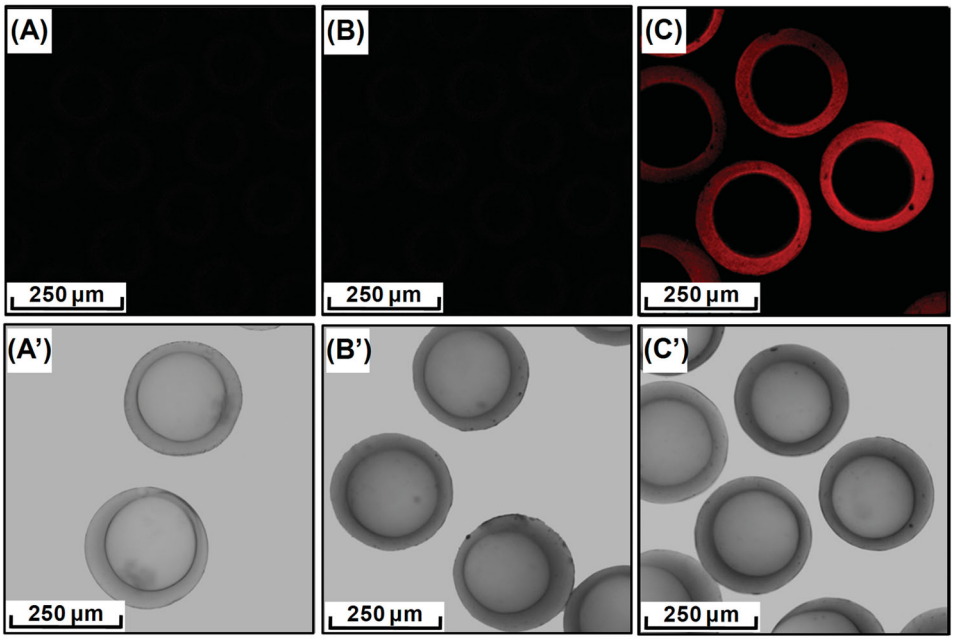

Figure 4. A–C) CLSM images of different microcapsules on red fluorescent channel; and A′–C′) optical microscope images of different microcapsules. (A,A′) represent CS microcapsules, (B,B′) represent CS-M microcapsules, (C,C′) represent CS-M-T microcapsules. The microcapsules are dispersed in buffer solution of pH 7.4 at 37 °C. Scale bars are all 250 μm.

Table 1. The recipes of different fluids.

Code	IF (Inner Fluid)	MF (Middle Fluid)	OF (Outer Fluid)
1#	Oil($V_{GA\text{-}S\text{-}BB}$:$V_{SO}$ = 1:20) +3%w/v PGPR	Pure water+4%w/v CS+0.25%w/v HEC+0.5%w/v F127	Oil($V_{GA\text{-}S\text{-}BB}$:$V_{SO}$ = 1:50) +8%w/v PGPR
2#	Oil($V_{GA\text{-}S\text{-}BB}$:$V_{SO}$ = 1:20) +3%w/v PGPR	Pure water+4%w/v CS+0.25%w/v HEC+0.5%w/v F127	Oil($V_{GA\text{-}S\text{-}BB}$:$V_{SO}$ = 1:75) +8%w/v PGPR
3#	Oil($V_{GA\text{-}S\text{-}BB}$:$V_{SO}$ = 1:50) +3%w/v PGPR	Pure water+4%w/v CS+0.25%w/v HEC+0.5%w/v F127	Oil($V_{GA\text{-}S\text{-}BB}$:$V_{SO}$ = 1:50) +8%w/v PGPR
4#	Oil($V_{GA\text{-}S\text{-}BB}$:$V_{SO}$ = 1:50) +3%w/v PGPR	Pure water+4%w/v CS+0.5%w/v F127	Oil($V_{GA\text{-}S\text{-}BB}$:$V_{SO}$ = 1:50) +8%w/v PGPR
5#	Oil($V_{GA\text{-}S\text{-}BB}$:$V_{SO}$ = 1:50) +3%w/v PGPR	Pure water+4%w/v CS+0.5%w/v F127+0.15%w/v magnetic nanoparticles	Oil($V_{GA\text{-}S\text{-}BB}$:$V_{SO}$ = 1:50) +8%w/v PGPR
6#	Oil($V_{GA\text{-}S\text{-}BB}$:$V_{SO}$ = 1:50) +3%w/v PGPR	Pure water+4%w/v CS+0.5%w/v F127+0.15%w/v magnetic nanoparticles+1%w/v sub-microspheres	Oil($V_{GA\text{-}S\text{-}BB}$:$V_{SO}$ = 1:50) +8%w/v PGPR

(Note: "$V_{GA\text{-}S\text{-}BB}$" represents the volume of glutaraldehyde-saturated benzyl benzoate; "V_{SO}" represents the volume of soybean oil.)

chitosan microcapsules, but the addition of sub-microspheres have slight influence on the pH-sensitivity of microcapsules. The magnetic nanoparticles are very small (about 20 nm) and the amount of nanoparticles added into the microcapsules is relatively low (0.15% w/v), so the pH-sensitivity of microcapsules is nearly not influenced by the addition of magnetic nanoparticles. However, the temperature-responsive sub-microspheres are relatively large (about 1 μm in water at room temperature) and the content of sub-microspheres in the microcapsules is relatively high (1%w/v). So, CS-M-T microcapsules exhibit slightly lower pH-responsive swelling ratios than CS and CS-M microcapsules at the same condition.

2.5. Magnetic Properties of Microcapsules

Drug release at specific pathological sites can effectively reduce the side effects. Incorporation of superparamagnetic nanoparticles into the chitosan capsule membrane enables the microcapsules to realize magnetic-guided targeting delivery. The CS-M (**Figure 6**A,A′,A″) and CS-M-T (Figure 6B,B′,B″) microcapsules both exhibit satisfactory magnetic properties. Without the external magnetic field, the microcapsules are randomly dispersed in water (Figure 6A,B); while after applying the external magnetic field, the microcapsules are aggregated at the sites where the magnet is placed (Figure 6A′,B′). A VSM is also

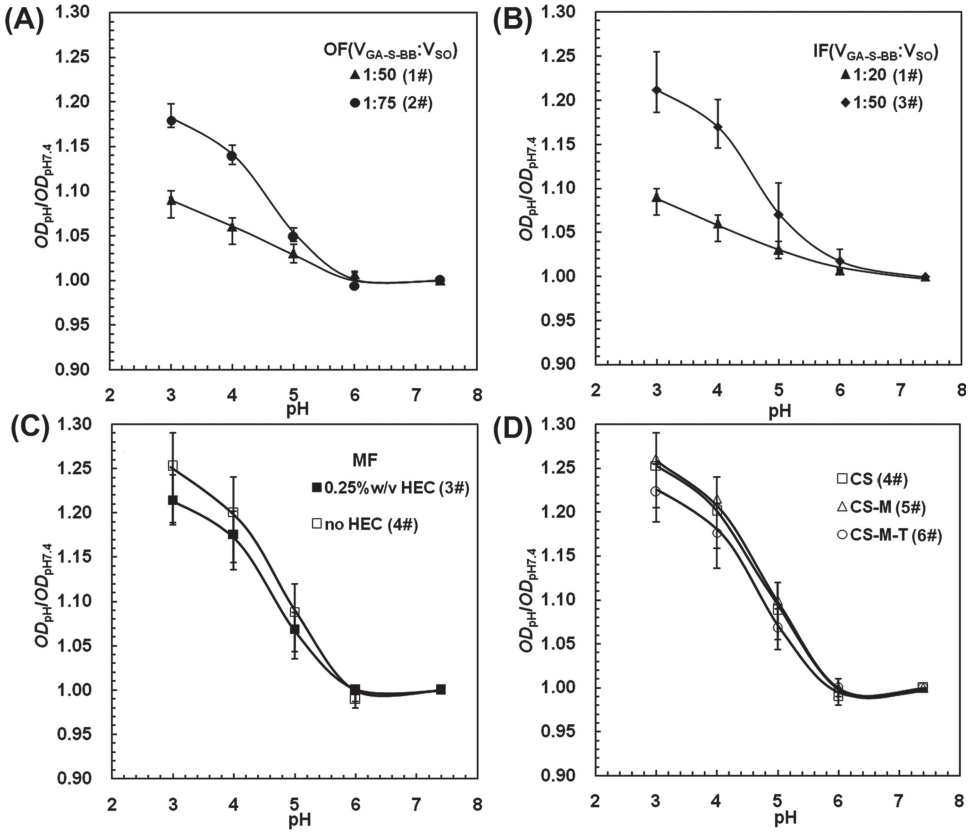

Figure 5. pH-responsive swelling ratios of CS microcapsules prepared A) with different concentrations of GA in OF, B) with different concentrations of GA in IF, C) with and without HEC addition in MF, and D) pH-responsive swelling ratios of CS, CS-M, and CS-M-T microcapsules. T = 37 °C.

used to measure the magnetic properties of CS-M and CS-M-T microcapsules and their magnetization curves are displayed in Figure 6A″ and Figure 6B″, respectively. Their hysteresis and coercivity are almost undetectable, which suggests that the CS-M and CS-M-T microcapsules also have superparamagnetic properties. The *M*s of CS-M and CS-M-T microcapsules are 4.47 emu g^{-1} and 4.11 emu g^{-1} respectively, which are much lower than that of pure magnetic nanoparticles (72.32 emu g^{-1}). This is attributed to the low content of nanoparticles in the microcapsule membrane. To confirm the nanoparticles can be stably kept inside the capsule membranes, the magnetic properties of nanoparticle-embedded microcapsules before and after repeatedly swelling/shrinking for 20 times are compared. The results show that *M*s values of CS-M-T microcapsules before and after repeatedly swelling/shrinking for 20 times are almost the same (Figure 6B″), which indicates that the nanoparticles embedded in the capsule membranes do not diffuse out even when the membranes undergo repeated swelling. Before being added in the chitosan solution, the nanoparticles are negatively charged because they have been modified with trisodium citrate. While the chitosan is positively charged at acidic conditions (pH < p*K*a of chitosan). Although the capsule membranes are swollen in the acidic conditions, the nanoparticles can be kept stably inside the membrane due to electrostatic attraction between the negatively charged nanoparticles and the positively charged chitosan networks.

2.6. Controlled-Release Characteristics of Multi-Stimuli-Responsive Microcapsules

The pH-responsive controlled-release behaviors of Vitamin B12 (VB12) as model drug from CS-M-T microcapsules are shown in **Figure 7**. The permeability coefficient of VB12 molecules (P_{VB12}) from microcapsules exhibits obvious pH-dependent characteristics. When the environmental pH is lower than the body physiological value (pH 7.4), the value of P_{VB12} increases with decreasing the pH value in external circumstances. The capsule membrane is in the loose state in acidic conditions due to the microcapsule swelling and then a high release rate is resulted. Another parameter defined as $P_{pH}/P_{pH7.4}$ is also introduced to characterize the permeability change degree compared to that at the body physiological pH. It can be seen that the $P_{pH}/P_{pH7.4}$ value also increases with decreasing pH value. As expected, the pH-responsive controlled-release behaviors of the CS-M-T microcapsules are consistent with their pH-dependent swelling results.

The adjustable drug controlled-release of microcapsules is achieved by regulating the interspace size between the microcapsule membrane and sub-microspheres, while the interspace size is determined by the size change of temperature-responsive sub-microspheres embedded in the capsule membrane. To estimate the adjustable controlled-release behaviors induced by temperature change, the pH value of external circumstances

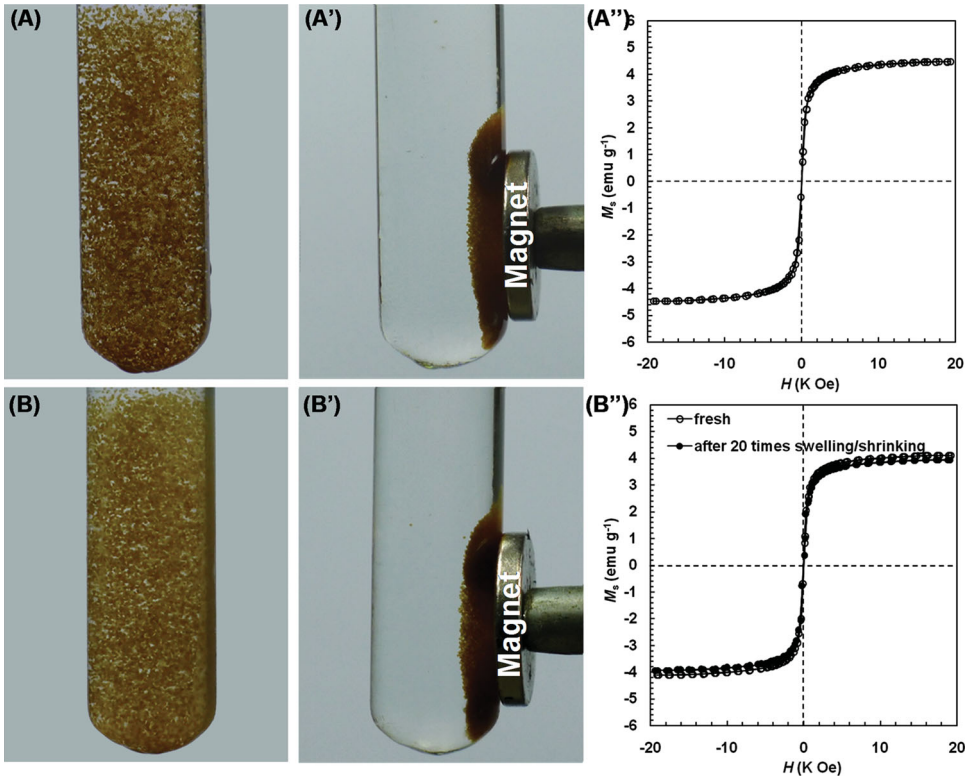

Figure 6. Magnetic-responsive property of CS-M and CS-M-T microcapsules. Microcapsules in buffer solution of pH 7.4 at room temperature A,B) without magnet and A′,B′)with an external magnet, and A",B") magnetization curves of microcapsules at room temperature. (A,A′,A″) represent CS-M microcapsules, and (B,B′,B″) represent CS-M-T microcapsules.

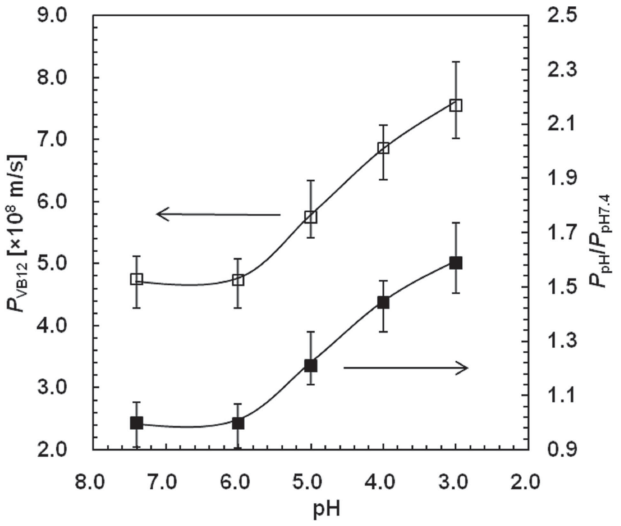

Figure 7. pH-responsive release of model drug VB12 from CS-M-T microcapsules at 37 °C.

should be fixed. Very low colonic pH values have been found in some severe active ulcerative colitis (the lowest pH values are 2.3–3.4) and some chronic wounds have been reported to have pH value as low as 5.45.[50,53] Herein, to reflect the adjustable controlled-release under pathologically acidic conditions, two pH values (pH 3 and pH 5) are chosen in this work.

The adjustable controlled-release of VB12 from CS-M-T microcapsules at pH 3 in response to temperature is exhibited in **Figure 8**A,B. The CS-M microcapsules are used as the control group. It is desirable that the P_{VB12} value of CS-M-T microcapsules increases with increasing of temperature from 25 °C to 49 °C (Figure 8A). In particular, the P_{VB12} value of CS-M-T microcapsules increases significantly in the temperature range of 34–43 °C, while it increases relatively slowly in the temperature ranges of 25–34 °C and 43–49 °C. The temperature-dependent P_{VB12} change of CS-M-T microcapsules corresponds well with the temperature-sensitivity of the sub-microspheres shown in Figure 2D. The temperature-responsive sub-microspheres are used as the "micro-valves" to adjust the release rate of microcapsules. Interspace size between the capsule membrane and sub-microspheres, which determines the VB12 release rate from the microcapsules, can be adjusted by temperature-dependent volume change of sub-microspheres. Therefore, the changing trend of VB12 permeability coefficients is consistent well with the temperature-responsive volume change of sub-microspheres. When the temperature is increased, the sub-microspheres embedded into the capsule membrane shrink, which causes the interspaces between the capsule membrane and sub-microspheres for VB12 passing through become larger. As a result, the value of P_{VB12} becomes higher with increasing temperature. While when the temperature is decreased, the interspaces become smaller due to the swelling of sub-microspheres, therefore resulting in a lower value of P_{VB12}. In contrast, the permeability coefficients of VB12 from CS-M microcapsules changes slightly in the whole temperature range (25–49 °C), and there is no obviously change in the range of 34–43 °C under the same condition. This slight change of P_{VB12} is due to the accelerated diffusivity of VB12 with increasing temperature. From practical application point of view, the adjustable controlled-release should be operated near body temperature (37 °C). A parameter defined as $P_T/P_{37\,°C}$ is introduced to evaluate the adjustable controlled-release characteristics with respect to VB12 release near body temperature, in which P_T is the permeability coefficient of VB12 from microcapsules at certain temperature T, while P_{37} °C is the permeability coefficient of VB12 at 37 °C. From Figure 8B it can be seen that the CS-M-T microcapsules can achieve a better and more effective adjustment of drug release in the temperature range near 37 °C than CS-M microcapsules through controlling the temperature due to the embedded temperature-responsive sub-microspheres.

The adjustable release characteristics of VB12 at pH 5 are also studied, as shown in Figure 8C,D. Just like the release behaviors at pH 3, the permeability coefficient of VB12 from CS-M-T microcapsules increases significantly with the increase of temperature in the range of 34–40 °C, while the P_{VB12} of CS-M microcapsules changes slightly under the same circumstances. P_T/P_{37} °C, as a function of temperature, also reflects the effective adjustment of drug controlled-release for CS-M-T microcapsules at pH 5 in the temperature range near 37 °C. That is, the CS-M-T microcapsules can also achieve an effective adjustable drug delivery than CS-M microcapsules at pH 5.

The temperature-controlled release behaviors of microcapsules at normal body physiological pH (about 7.4) are also studied. At pH 7.4, which is higher than the p*K*a value of chitosan, the chitosan membranes are in the compacted state. Here, FITC-dextran is selected as the model drug. The permeability of the capsule membrane can also be modulated by changing the temperature even when chitosan is in the compacted state at pH 7.4 (**Figure 9**). The release rate of FITC-dextran from CS-M-T microcapsules also increases significantly with the increase of temperature. In contrast, there is no obvious change in the release rate of FITC-dextran from CS-M microcapsules when the temperature is varied in the same range. That is, even when chitosan membranes are in the compacted state at pH 7.4, the embedded temperature-responsive sub-microspheres are still efficient as "micro-valves".

The influences of the added amount of sub-microspheres into the capsule membrane on the controlled-release behaviors are also investigated. The controlled-release characteristics of VB12 from CS-M-T microcapsules containing different contents of sub-microspheres at temperature of 37 °C and pH of 3 are shown in **Figure 10**. With increasing the added amount of sub-microspheres, the value of P_{VB12} increases obviously. The number of interspaces between the capsule membrane and sub-microspheres is directly determined by the added amount of sub-microspheres. Consequently, with the increase of sub-microspheres amount, the value of P_{VB12} increases due to the increase of the number of interspaces for VB12 passing through. When the added amount of sub-microspheres is low, the sub-microspheres are well dispersed in the capsule membrane and thus they do not form percolated networks. However, when a large amount of sub-microspheres are added in the capsule membrane, the sub-microspheres may be arrayed closely one by one although they are well dispersed, so that some connected porous networks may be formed through the shrinkage of sub-microspheres at 37 °C. Therefore, when a large amount

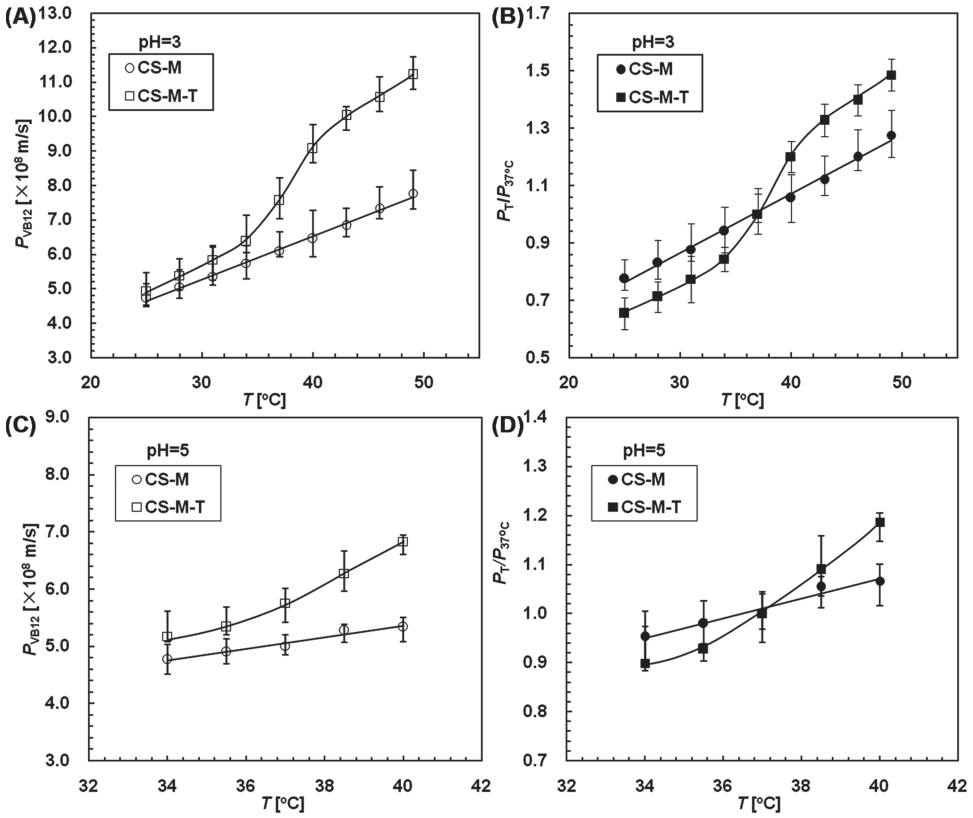

Figure 8. Adjustable controlled-release characteristics of model drug VB12 from CS-M and CS-M-T microcapsules in buffer solutions with different pH values and at different temperatures.

of sub-microspheres are added, the permeability of CS-M-T microcapsules at 37 °C increases a lot. A parameter defined as P_C/P_0 is also used to reflect the influence degree of the addition amount of sub-microspheres on the VB12 controlled-release, in which P_C is the permeability coefficient of VB12 from CS-M-T microcapsules prepared with certain concentration C of sub-microspheres and P_0 is that from chitosan microcapsules prepared without sub-microspheres. With increasing the amount of embedded sub-microspheres in the capsule membrane, the influence degree on the VB12 controlled-release also increases.

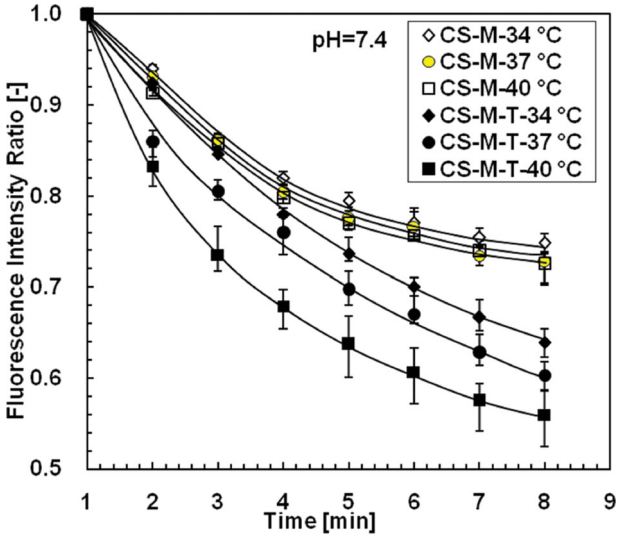

Figure 9. Release behaviors of FITC-dextran from CS-M and CS-M-T microcapsules in buffer solution of pH 7.4 at different temperatures. The fluorescence intensity is measured in the region that covers the microcapsules.

3. Conclusions

In summary, by embedding magnetic nanoparticles and temperature-responsive sub-microspheres into the capsule membrane of a pH-responsive microcapsule, a novel multi-stimuli-responsive microcapsule with adjustable controlled-release characteristics is successfully prepared in this work. By introduction of hydrophilic AAm units, the temperature-responsive sub-microspheres working as "micro-valves" exhibit significant volume phase transition near human body temperature. Due to the superparamagnetic property of magnetic nanoparticles, the prepared multi-stimuli-responsive microcapsules can achieve targeting aggregation at specific sites by applying an external magnetic field. Furthermore, these smart microcapsules exhibit cationic pH-responsive controlled-release properties, which are well consistent with their acid-induce volume swelling behaviors. More importantly, the interspace size between the capsule membrane and sub-microspheres, which controls the VB12 release rate from the microcapsules, can be tuned through the temperature-responsive volume change of the sub-microspheres. As a result, the smart microcapsules

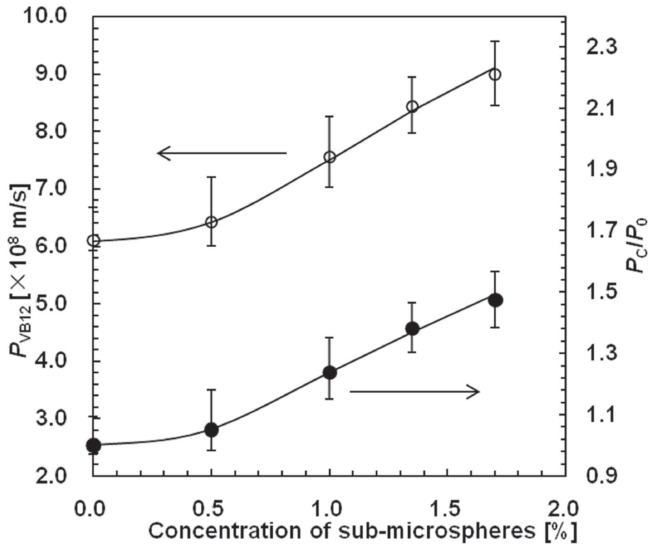

Figure 10. Controlled-release characteristics of model drug VB12 from CS-M-T microcapsules prepared with different concentrations of temperature-responsive sub-microspheres in buffer solution of pH 3 at 37 °C.

exhibit temperature-dependently adjustable controlled-release characteristics. Such multi-stimuli-responsive microcapsules are promising to achieve a more rational drug delivery and controlled-release according to patients' individual differences.

4. Experimental Section

Materials: Water-soluble chitosan (M_w = 5000, degree of deacetylation = 85%) is purchased from Ji'nan Haidebei Marine Bioengineering Co., Ltd. *N*-isopropylacrylamide (NIPAM), purchased from Sigma-Aldrich, is purified by recrystallization with a hexane/acetone mixture. GA, HEC (Mw ≥ 300 000), AAm, *N*,*N*′-methylene-bis-acrylamide (MBA), potassium persulfate ($K_2S_2O_8$), iron (III) chloride ($FeCl_3{\cdot}6H_2O$) and iron (II) chloride ($FeCl_2{\cdot}4H_2O$) are all purchased from Chengdu Kelong Chemical Reagents Co., Ltd. Pluronic F-127 (F127) and FITC-dextran (M_w = 4000) are purchased from Sigma-Aldrich. Benzyl benzoate is obtained from Sinopharm Chemical Reagent Co., Ltd. Soybean oil is provided by Kerry Oils & Grains Co., Ltd. Methacryloxy thiocarbonyl rhodamine B (Polyfluor 570) is purchased from Polysciences. All other chemicals are of analytical grade and used as received. Pure water (18.2 MΩ at 25 °C) from a Milli-Q Plus water purification system (Millipore) is used throughout the experiments.

Synthesis and Characterization of Magnetic-Responsive Nanoparticles and Temperature-Responsive Sub-Microspheres: Magnetic-responsive nanoparticles are prepared by the chemical coprecipitation method as reported.[36,54] Briefly, hydrochloric acid (4 mL) and $FeCl_2{\cdot}4H_2O$ (7.2 g) are dissolved into pure water (20 mL). The resultant solution is mixed with $FeCl_3{\cdot}6H_2O$ aqueous solution (27 wt%, 29 mL). Afterward, pure water is added to make the aqueous solution up to 100 mL. By adding ammonia (40 mL), the solution turns to dark indicating that magnetite nanoparticles are synthesized. To make the nanoparticles well dispersed in water, the obtained nanoparticles are further modified with trisodium citrate. After adding nitric acid (14.4 mL) to acidification, a boiling solution of ferric nitrate (20 wt%, 50 mL) is added into the above magnetite solution. After magnetic decantation, the precipitate is dispersed in a solution of trisodium citrate salt (14 wt%, 50 mL). The mixture is heated at 80 °C for half an hour and then the magnetic nanoparticles are precipitated by addition of acetone. The precipitated nanoparticles are isolated and then suspended in pure water leading to a stable colloidal dispersion.[55,56] The morphology of the prepared magnetic-responsive nanoparticles is observed by TEM (JEM-100CX, JEOL). The magnetic property of the nanoparticles is measured by a VSM (7400, Lakeshore) at room temperature.

Temperature-responsive sub-microspheres are prepared by precipitation polymerization.[57,58] First, NIPAM (1.92 g), AAm (0.21 g), MBA (0.154 g), and a small amount of fluorescence dye (Polyfluor 570) are dissolved in pure water (320 mL). Then, the solution is heated to 70 °C and bubbled with nitrogen gas to remove dissolved oxygen in the system. Approximately 10 min later, the $K_2S_2O_8$ initiator solution (1.6 wt%, 5 ml) is added into the reactor to initiate the polymerization, and the reaction is maintained at 70 °C for 4 h under stirring. The resultant fluorescence-labeled P(NIPAM-*co*-AAm) sub-microspheres are purified by centrifugation (Biofuge Primo R, Sorvall) to remove the unreacted monomers, crosslinker and initiator. The fluorescence and dispersity of the sub-microspheres in water are observed at room temperature using a CLSM (SP5 II, Leica) and the red fluorescent channel is excited at 543 nm. The hydrodynamic diameter of the sub-microspheres is measured by dynamic light scattering (DLS, Zetasizer Nano, ZEN 3690, Malvern). The temperature-sensitivity of P(NIPAM-*co*-AAm) sub-microspheres is studied by estimating their size change at different temperatures in the range of 25 °C to 49 °C.

Preparation and Characterization of Microcapsules: Chitosan microcapsules (CS), chitosan microcapsules with magnetic-sensitivity (CS-M), and chitosan microcapsules with both magnetic- and thermo-sensitivity (CS-M-T) are all prepared using O/W/O double emulsions as crosslinking templates by a microfluidic technique. The capillary microfluidic device is assembled according to previous work.[48,49] The compositions of different fluids are listed in Table 1. The generated O/W/O emulsions are collected in a container, and then left to stand for 24 h to make sure the chitosan in the water phase is completely crosslinked. The obtained microcapsules are washed using a mixture of ethyl acetate and isopropanol (1/5, v/v) to remove the inner and outer oil solutions, and finally the microcapsules are dispersed into water for further characterization.

The morphologies and fluorescence of obtained emulsions and microcapsules are observed using the CLSM. The pH-sensitivities of different microcapsules are studied by evaluating their pH-responsive swelling behaviors in acidic conditions. Here, 0.01 M citric acid and 0.01 M disodium are used to adjust pH values of buffer solutions ranging from 3.0 to 7.4, and the ionic strength of pH buffer solutions is adjusted to 0.1 M. The microcapsules are immersed in a series of buffers for 24 h before measurement. The pH-dependent size changes of these microcapsules at 37 °C are measured according to their corresponding micrographs taken by an optical microscope (BX61, Olympus) equipped with a CCD camera and a thermostatic stage system (TS62, Instec). A VSM is also used to study the magnetic properties of microcapsules.

Controlled-Release Experiment: The controlled-release experiments of VB12 are carried out with a previous published method.[1,11,12,37,42,59] The prepared microcapsules are dialyzed against VB12 aqueous solution with a known concentration for more than three days to load VB12 inside the microcapsules. The dialysis is carried out at a certain pH value at 37 °C when the pH-dependent controlled-release is investigated; while the dialysis is carried out at a certain temperature at pH 3 or pH 5 when temperature-depended adjustable controlled-release is studied. After mixing a known volume of microcapsule dispersion with the same volume of pure water, the permeability of VB12 across the capsule membrane is measured by determining the increase of VB12 concentration in the surrounding medium with time through an on-line monitoring UV-vis Spectrometer (UV-1700, Shimadzu) at a wavelength of 361 nm. The permeability coefficient of VB12 across the capsule membrane, P_{VB12}, can be calculated using the following equation derived from Fick's first law of diffusion:[11,12,37]

$$P_{VB12} = \frac{V_s V_m}{A(V_s + V_m)t} \ln \frac{C_f - C_i}{C_f - C_t} \quad (1)$$

where C_i, C_t, and C_f are the initial, intermediate (at time t) and final concentrations of VB12 in the surrounding medium respectively, V_m

and V_s are the total volume of microcapsules and the volume of the surrounding medium respectively, and A is the total surface area of microcapsules.

To load the FITC-dextran, the microcapsules are dialyzed against FITC-dextran aqueous solution (1 gL^{-1}) for more than three days. In the controlled-release experiments, the microcapsules are first equilibrated in buffer solution of pH 7.4 containing FITC-dextran (1 gL^{-1}) at a certain temperature, meanwhile, the buffer solution of pH 7.4 without FITC-dextran is also kept at the same temperature. After reaching equilibrium state, the microcapsules loaded with FITC-dextran are quickly transferred into the buffer solution of pH 7.4 without FITC-dextran. The time-dependent release behaviors of FITC-dextran from the microcapsules at test temperatures are observed and recorded by the CLSM. To quantitatively analyze the release behaviors, average fluorescence intensity of a certain region that reflects the release of the fluorescent FITC-dextran is estimated by Leica Analysis Software mounted on the CLSM. The decrease of average fluorescence intensity of a region that covers a typical microcapsule is estimated for monitoring the release behavior. The decrease of average fluorescence intensity is characterized by the ratio of fluorescence intensity at time t to that at the beginning. A thermostatic stage that mounted on the CLSM is used for temperature control throughout the experiments.

Acknowledgements

The authors gratefully acknowledge support from the National Natural Science Foundation of China (21136006, 21276002, 21322605), the Program for Changjiang Scholars and Innovative Research Team in University (IRT1163), the Program for New Century Excellent Talents in University (NCET-11-0352), and the Foundation for the Author of National Excellent Doctoral Dissertation of China (201163).

Received: November 13, 2013
Revised: December 10, 2013
Published online: February 12, 2014

[1] L. Y. Chu, T. Yamaguchi, S. Nakao, *Adv. Mater.* **2002**, *14*, 386.
[2] F. M. Chen, H. Lu, L. A. Wu, L. N. Gao, Y. An, J. Zhang, *Biomaterials* **2013**, *34*, 6515.
[3] A. R. Patel, C. Remijin, P. C. Heussen, R. den Adel, K. P. Velikov, *ChemPhysChem* **2013**, *14*, 305.
[4] Y. L. Yu, M. J. Zhang, R. Xie, X. J. Ju, J. Y. Wang, S. W. Pi, L. Y. Chu, *J. Colloid Interface Sci.* **2012**, *376*, 97.
[5] M. M. Rahman, A. Elaissari, *J. Mater. Chem.* **2012**, *22*, 1173.
[6] T. Trongsatitkul, B. M. Budhlall, *Langmuir* **2011**, *27*, 13468.
[7] C. H. Choi, J. H. Jung, D. W. Kim, Y. M. Chung, C. S. Lee, *Lab Chip* **2008**, *8*, 1544.
[8] G. L. Li, X. Y. Yang, B. Wang, J. Y. Wang, X. L. Yang, *Polymer* **2008**, *49*, 3436.
[9] Q. H. Sun, Y. L. Deng, *J. Am. Chem. Soc.* **2005**, *127*, 8274.
[10] X. C. Xiao, L. Y. Chu, W. M. Chen, S. Wang, Y. Li, *Adv. Funct. Mater.* **2003**, *13*, 847.
[11] L. Y. Chu, S. H. Park, T. Yamaguchi, S. Nakao, *Langmuir* **2002**, *18*, 1856.
[12] L. Y. Chu, S. H. Park, T. Yamaguchi, S. Nakao, *J. Membrane Sci.* **2001**, *192*, 27.
[13] H. S. Lim, E. Kwon, M. Lee, Y. M. Lee, K. D. Suh, *Macromol. Rapid Commun.* **2013**, *34*, 1243.
[14] R. Han, F. Wang, T. Ren, *J. Appl. Polym. Sci.* **2013**, *129*, 3601.
[15] J. Wei, X. J. Ju, R. Xie, C. L. Mou, X. Lin, L. Y. Chu, *J. Colloid Interface Sci.* **2011**, *357*, 101.
[16] L. Liu, J. P. Yang, X. J. Ju, R. Xie, Y. M. Liu, W. Wang, J. J. Zhang, C. H. Niu, L. Y. Chu, *Soft Matter* **2011**, *7*, 4821.
[17] L. Cao, G. F. Luo, H. Y. Wang, J. Zhang, Y. H. Gong, S. X. Cheng, R. X. Zhuo, X. Z. Zheng, *J. Phys. Chem. C* **2011**, *115*, 17651.
[18] D. Samanta, R. M. Sankar, S. N. Jaisankar, M. S. Alam, A. B. Mandal, *Chem. Commun.* **2011**, *47*, 11975.
[19] Y. Jia, J. B. Fei, Y. Cui, Y. Yang, L. Gao, J. B. Li, *Chem. Commun.* **2011**, *47*, 1175.
[20] K. E. Broaders, S. J. Pastine, S. Grandhe, J. M. J. Fréchet, *Chem. Commun.* **2011**, *47*, 665.
[21] V. Kozlovskaya, E. Kharlampieva, S. Chang, R. Muhlbauer, V. V. Tsukruk, *Chem. Mater.* **2009**, *21*, 2158.
[22] J. Yun, H. I. Kim, *J. Ind. Eng. Chem.* **2009**, *15*, 902.
[23] W. J. Tong, C. Y. Gao, H. Möhwald, *Colloid Polym. Sci.* **2008**, *286*, 1103.
[24] G. L. Li, G. Liu, E. T. Kang, K. G. Neoh, X. L. Yang, *Langmuir* **2008**, *24*, 9050.
[25] T. Mauser, C. Déjugnat, H. Möhwald, G. B. Sukhorukov, *Langmuir* **2006**, *22*, 5888.
[26] Y. F. Zhu, J. L. Shi, W. H. Shen, X. P. Dong, J. W. Feng, M. L. Ruan, Y. S. Li, *Angew. Chem. Int. Ed.* **2005**, *117*, 5213.
[27] D. G. Shchukin, G. B. Sukhorukov, H. Möhwald, *Angew. Chem. Int. Ed.* **2003**, *42*, 4472.
[28] S. Zhang, Y. F. Zhou, W. Y. Nie, L. Y. Song, J. N. Li, B. B. Yang, *J. Mater. Chem. B* **2013**, *1*, 4331.
[29] X. J. Cui, Z. F. Li, S. L. Zhong, B. N. Wang, Y. S. Han, H. Y. Wang, H. Möhwald, *Chem. Eur. J.* **2013**, *19*, 9485.
[30] J. N. Zhang, S. L. Qiu, Y. L. Zhu, Z. Q. Huang, B. B. Yang, W. L. Yang, M. Y. Wu, Q. Y. Wu, J. J. Yang, *Polym. Chem.* **2013**, *4*, 1459.
[31] S. Zhang, Y. F. Zhou, W. Y. Nie, L. Y. Song, T. Zhang, *Ind. Eng. Chem. Res.* **2012**, *51*, 14099.
[32] T. Miyazaki, A. Miyaoka, E. Ishida, Z. X. Li, M. Kawashita, M. Hiraoka, *Mater. Sci. Eng. C* **2012**, *32*, 692.
[33] X. P. Xiong, Y. Wang, W. W. Zou, J. J. Duan, Y. Chen, *J. Chem.* **2013**, *2013*, 1.
[34] Z. J. Wei, C. Y. Wang, S. W. Zou, H. Liu, Z. Tong, *Colloid Surf., A* **2011**, *392*, 116.
[35] P. V. Finotelli, D. Da Silva, M. Sola-Penna, A. M. Rossi, M. Farina, L. R. Andrade, A. Y. Takeuchi, M. H. Rocha-Leao, *Colloids Surf., B* **2010**, *81*, 206.
[36] W. Wang, L. Liu, X. J. Ju, D. Zerrouki, R. Xie, L. H. Yang, L. Y. Chu, *ChemPhysChem* **2009**, *10*, 2405.
[37] W. C. Yang, R. Xie, W. Q. Pang, X. J. Ju, L. Y. Chu, *J. Membrane Sci.* **2008**, *321*, 324.
[38] Z. Y. Ma, X. Jia, J. M. Hu, G. X. Zhang, F. Zhou, Z. Y. Liu, H. Y. Wang, *Langmuir* **2013**, *29*, 5631.
[39] J. Shi, C. Du, J. Shi, Y. M. Wang, S. K. Cao, *Macromol. Biosci.* **2013**, *13*, 494.
[40] Z. M. Xing, C. L. Wang, J. Yan, L. Zhang, L. Li, L. S. Zha, *Colloid Polym. Sci.* **2010**, *288*, 1723.
[41] J. Yun, J. S. Im, Y. S. Lee, T. S. Bae, Y. M. Lim, H. I. Kim, *Colloid Surf., A* **2010**, *368*, 23.
[42] L. Y. Chu, Y. J. Liang, W. M. Chen, X. J. Ju, H. D. Wang, *Colloids Surf., B* **2004**, *37*, 9.
[43] H. Ichikawa, Y. Fukumori, *J. Controlled Release* **2000**, *63*, 107.
[44] Z. Liu, L. Liu, X. J. Ju, R. Xie, B. Zhang, L. Y. Chu, *Chem. Commun.* **2011**, *47*, 12283.
[45] L. Liu, W. Wang, X. J. Ju, R. Xie, L. Y. Chu, *Soft Matter* **2010**, *6*, 3759.
[46] A. M. DiLauro, A. Abbaspourrad, D. A. Weitz, S. T. Phillips, *Macromolecules* **2013**, *46*, 3309.
[47] A. P. Esser-Kahn, N. R. Sottos, S. R. White, J. S. Moore, *J. Am. Chem. Soc.* **2010**, *132*, 10266.
[48] A. S. Utada, E. Lorenceau, D. R. Link, P. D. Kaplan, H. A. Stone, D. A. Weitz, *Science* **2005**, *308*, 537.
[49] A. S. Utada, L. Y. Chu, A. Fernandez-Nieves, D. R. Link, C. Holtze, D. A. Weitz, *MRS Bull.* **2007**, *32*, 702.

[50] J. Dissemond, M. Witthoff, T. C. Brauns, D. Harberer, M. Gros, *Hautarzt* **2003**, *54*, 959.
[51] P. Vaupel, F. Kallinowski, P. Okunieff, *Cancer Res.* **1989**, *49*, 6449.
[52] E. K. Rofstad, B. Mathiesen, K. Kindern, K. Galappathi, *Cancer Res.* **2006**, *66*, 6699.
[53] J. Fallingborg, L. A. Christensen, B. A. Jacobsen, S. N. Rasmussen, *Dig. Dis. Sci.* **1993**, *38*, 1989.
[54] R. Massart, *IEEE Trans. Magn.* **1981**, *17*, 1247.
[55] A. Bee, R. Massart, S. Neveu, *J. Magn. Magn. Mater.* **1995**, *149*, 6.
[56] C. Ménager, O. Sandre, J. Mangili, V. Cabuil, *Polymer* **2004**, *45*, 2475.
[57] Q. Wang, Y. B. Zhao, H. B. Xu, X. L. Yang, Y. J. Yang, *J. Appl. Polym. Sci.* **2009**, *113*, 321.
[58] E. C. Cho, J. W. Kim, A. Fernández-Nieves, D. A. Weitz, *Nano Lett.* **2008**, *8*, 168.
[59] L. Y. Chu, T. Niitsuma, T. Yamaguchi, S. Nakao, *AIChE J.* **2003**, *49*, 896.