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Research Article

Synthesis of uniform core–shell gelatin–alginate microparticles as intestine-released oral delivery drug carrier

A core–shell gelatin–alginate composite used for intestine-released oral delivery drug carrier was synthesized through microfluidic technique. At the fixed continuous phase flow rate, the size of the core–shell gelatin–alginate microparticles increases with the dispersed phase flow rate, and monodispersity can be retained (the variation coefficient for the diameter distribution can be kept less than 10%). The fabricated microparticles could remain intact in gastric juice for at least 3 h, indicating that the gelatin core could be well protected by alginate shell in acid environment. However, the alginate shell of the microparticles would swell or collapse in alkali environment in half an hour, assuring the controlled drug release in intestinal juice. The fabricated uniform core–shell gelatin–alginate microparticles were potential as pH-responsive drug carriers.

Keywords:
Alginate / Gelatin / Intestinal-released / Microfluidic / Oral delivery drug carrier
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1 Introduction

Core–shell structure particles with predefined diameter and shell thickness exhibit several advantages in drug delivery, and thus drug efficacy could be enhanced. For example, higher drug loads and better drug solubility may be achieved, prolonged, or pulsatile drug release can be scheduled, sequentially release could be realized through loading drug into the core or shell phase separately, and the embedded core can provide a favorable environment to stabilize fragile therapeutics such as proteins or DNA [1–4]. Various techniques for fabricating core–shell microparticles have been reported in the literatures [4–9]. Multiple emulsions, which allow the discontinuous phase of the primary emulsion to encapsulate and form the core, are facile manufactured through two-step bulk emulsification [10,11]. The limitations of the technology are that the core and shell material must be immiscible, and the architecture and size distribution are not easy to be well controlled [12–14]. The phase inversion method is another synthesis technology for core–shell structure, but has similar limitations [15]. Some researches focused on generating solid cores by coating preformed microparticles with a second polymer to form "microencapsulated microspheres" [16]. Another approach is to control the phase separation of two sphere-forming materials, such that a "double-wall" microparticles comprising polymer cores and shells can be fabricated [17].

For the drug delivery in the digestive tract, the encapsulation formulation plays an important role in the success of drug delivery. Alginate composites composed of D-mannuronic and L-guluronic acid residues joined linearly by (1–4)-glycosidic linkages are a smart material for drug delivery in the digestive tract. When placed in gastric juice with pH lower than the pKa values of D-mannuronic and L-guluronic acid (3.6 and 3.7, respectively), alginate becomes more stable and dense due to water loss. However, alginate becomes swelling in intestinal juice due to the repulsive negative charges of D-mannuronic and L-guluronic through the backbone. Therefore the embedded materials (i.e. cells, capsules, or drugs) could be well protected in gastric juice, but would release in intestinal juice.

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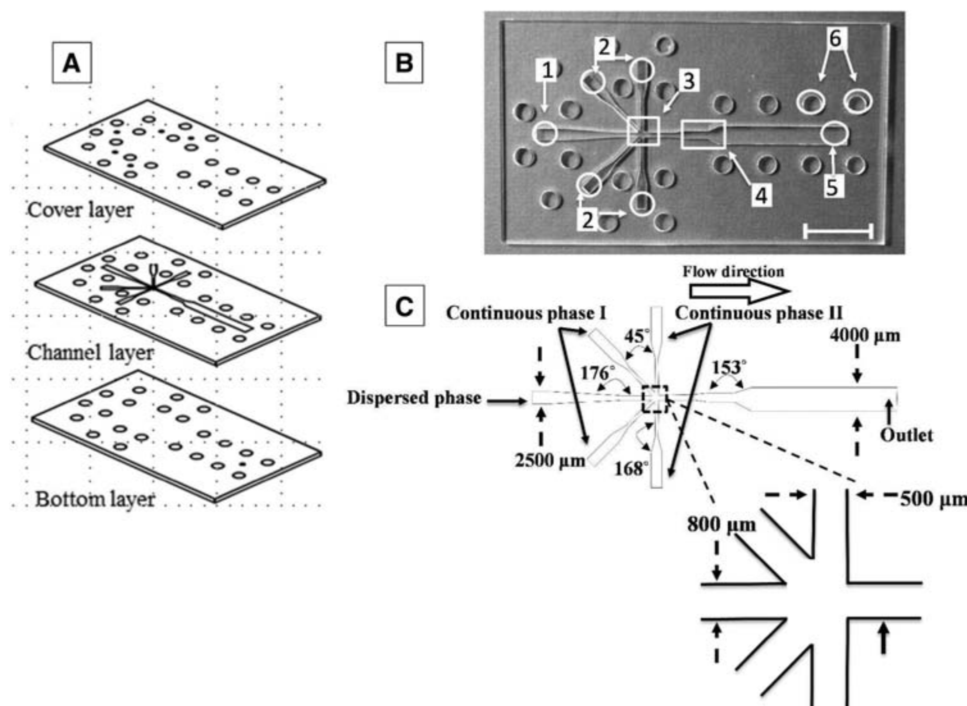

Figure 1. The exploded view and photograph of the microfluidic chip. (A) Exploded view of the microfluidic chip. (B) Photograph of the microfluidic chip: (1) sample inlet, (2) oil inlets, (3) cross-junction channel, (4) observation area, (5) outlet, and (6) screw orifices. The scale bar is 1 cm. (C) Design of the microfluidic chip.

Since alginate is stable in acidic environment but swelling in alkaline environment, gelatin capsules and microspheres-embedded alginate particles can be used as smart carrier to protect drugs from the acidity of gastric juice, but allowing controlled release in the intestinal fluids [18]. Composite gelatin and alginate materials have gained attention for biomedical applications [19–21]. The negative charges of alginate in aqueous solution also potentially allow poly-ion complexation with positively charged gelatin polymers for a production preparation. Alginate-coated gelatin capsules can be used to protect drugs from the acid reaction of gastric juice while allowing subsequent release in the intestinal juice for absorption. In literatures, gelatin–alginate composites fabricated from gelatin–alginate mixture were implemented, and their activity in various pH conditions was well studied [19–22]. However, little attention had been paid on the employment of microfluidic technology for generating uniform gelatin–alginate core–shell particles. To our knowledge, this study is the first report to synthesize the gelatin–alginate composites core–shell particles by using a multiple branches (combined cross-junction and Y-junction) microfluidic devices, which has several advantages such as high throughput, high monodispersity, and facile synthesis approach.

In this study, we propose a gelatin–alginate composite, intestinal-released oral drug carrier synthesized through microfluidic technique. The fabricated microparticles were uniform in size, anti-acid, and degradable in alkaline solution. The size, dispersity, and composition of the core–shell gelatin–alginate microparticles were characterized. Furthermore, the response of the core–shell gelatin–alginate microparticles in various pH environments was evaluated.

2 Materials and methods

2.1 Materials

Sodium alginate (cat. A2158) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Vitamin A gelatin capsule (part number: A16237–14) was purchased from Alfa Aesar (UK). Sunflower seed oil was obtained from Uni-President Enterprise Co. (Taiwan). Deionized water was prepared using a Milli-QH system (Millipore, Clifton, NJ, USA). All other reagents were commercially available and of analytical grade.

2.2 Microfluidic device

The design and implementation of the microfluidic device was designed based on our previous studies [23–25]. In brief, we employed a CO_2 laser machine (LaserPro Venus, GCC, Taiwan) construct a PMMA substrate based cross-junction channel microfluidic chip. Figure 1 shows a microfluidic device with multiple branches (combined with a cross-junction and a Y-junction). The use of one branch chip (only Y-junction or only cross-junction) is capable of adjusting the particle size by changing the ratio of the flow rate between the continuous and dispersed phases. In this study, we would like to explore the possibility in tuning the particle size with two branches simultaneously. By using multiple branches of microchannels, the shearing forces could be adjusted separately with different continuous flow rate in Y-junction and cross-junction respectively [26, 27]. This device was made up by PMMA and consisted of top floor (with three reagent inlets and 20 screw orifices for binding), middle floor (with

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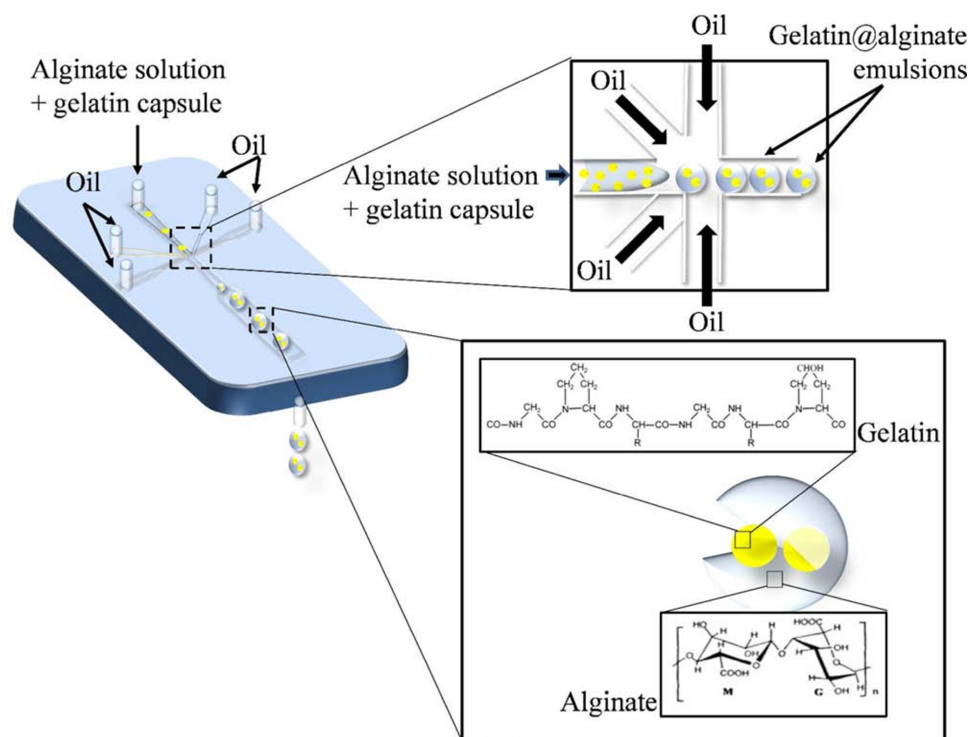

Figure 2. Schematic of microfluidic emulsification for the production of core–shell gelatin–alginate microparticles.

cross-junction channels and 20 screw orifices), and bottom floor (with an outlet and 20 screw orifices). The three layers were laminated together by 20 M4 screws (0.5 mm pitch, 4 mm in diameter) and tightened at 1.5∼2 Nm to ensure leak proof of the device.

2.3 Synthesis of the alginate microparticles

Sodium alginate solution (1 wt.%) used as a disperse phase was injected from the central inlet. Sunflower seed oil was used for the continuous phase I (which was kept at a constant flow rate of 0.5 mL/min) and continuous phase II (which was kept at a constant flow rate of 0.1 mL/min) simultaneously (Fig. 2). Syringe pumps (KDS Model 220 Series, Kd Scientific, USA) were used to simultaneously inject both sample flow (disperse phase) and sheath flow (continuous phase) through Teflon tubes into the microfluidic chip. Alginate droplets were then dripped into $CaCl_2$ solutions (20 wt.%) through the other Teflon tube connected to the distinct microchannel. After 10 min, alginate microparticles were observed, collected by centrifugation, and then washed twice with 30 mL dd-H_2O to remove any residue.

2.4 Synthesis of the vitamin A gelatin capsules encapsulated alginate microparticles

Vitamin A gelatin capsule mixed with sodium alginate solution (1 wt.%) used as a disperse phase was injected from the central inlet. Sunflower seed oil was used for the continuous phase I (which was kept at a constant flow rate of 0.5 mL/min) and continuous phase II (which was kept at a constant flow rate of 0.1 mL/min) simultaneously (Fig. 2). Syringe pumps (KDS Model 220 Series, Kd Scientific, USA) were used to simultaneously inject both sample flow (disperse phase) and sheath flow (continuous phase) through Teflon tubes into the microfluidic chip. Vitamin A gelatin capsule mixed alginate droplets were then dripped into $CaCl_2$ solutions (20 wt.%) through the other Teflon tube connected to the distinct microchannel. After 10 min, vitamin A gelatin capsule mixed alginate microparticles were observed, collected by centrifugation, and then washed twice with 30 mL dd-H_2O to remove any residue.

2.5 Characterization of synthesized microparticles

The collected solidified alginate microparticles with and without vitamin A gelatin capsule were characterized under the optical microscopy system (TE2000U, Nikon, USA). The diameter of the microparticles, expressed as mean ± SD, was obtained from the photomicrographs. A total of more than 50 particles were counted to ensure a statistical representation.

2.6 Response of vitamin A gelatin capsules encapsulated alginate microparticles in various pH conditions

Degradation of the core–shell gelatin capsule encapsulated alginate microparticles are observed by an optical imaging system in pancreatic juice (pH 7.7), gastric juice (pH 1.3),

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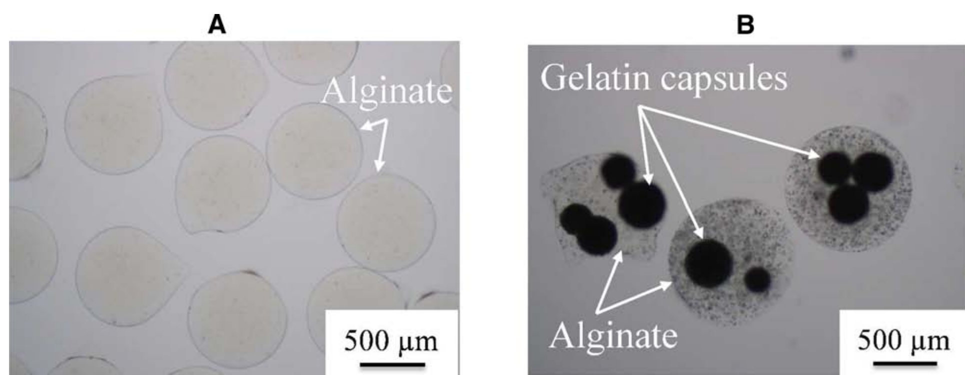

Figure 3. Optical microscope images of (A) alginate microparticles and (B) vitamin A gelatin capsule embedded alginate microparticles. The flow rate of disperse phase was in 0.1 mL/min (Y-junction) and 0.5 mL/min (cross-junction). The flow rate of disperse phase was in 0.4 mL/h.

and dd-H_2O (pH 7) respectively at room temperature for 3 h. The images were recorded through an imaging processing software.

3 Results and discussion

3.1 Morphology

Figure 3A and B shows the optical images of the alginate particles and vitamin A gelatin capsules embedded alginate microparticles, respectively. The particles were synthesized with the disperse flow rate of 0.1 mL/min (Y-junction) and 0.5 mL/min (cross-junction). The flow rate of disperse phase was in 0.4 mL/h. The synthesized alginate particles were transparent and spherical with diameters of 810 ± 11 μm (Fig. 3A), while the synthesized vitamin A capsule@alginate particles have black cores inside and spherical with diameters of 969 ± 19 μm (Fig. 3B), which was somewhat larger than alginate particles. The vitamin A gelatin capsules were commercial product and thus the size of particles was nonhomogeneous. Supporting Information Fig. 1 shows the particle size distribution of the vitamin A gelatin capsules (please see the Supporting Information). We found that most of the particles have sizes ranging from 40 to 100 μm. The average diameter is about 65 μm. Since the average size of the gelatin particles is much smaller than the synthesized alginate particles, we presume that the size of the gelatin particles did affect the synthesis significantly. Although the drug delivery behavior for individual capsule may be different due to various sizes, the total amount of capsules encapsulation might be predetermined by controlling the gelatin–alginate ratio in the syringe (1 mL gelatin solution contained 0.5 g gelatin capsules in our case).

3.2 Influences of synthesis parameters on the morphology of core–shell structures

Table 1 shows the alginate particle diameters variation according to the flow rates (including disperse phase, continuous phase I, and continuous phase II). It is found that increasing disperse phase flow rate resulted in larger particles. However, increasing both continuous phases phase-flow rates resulted in smaller particles. Table 2 shows the diameters variation for the alginate shell of vitamin A capsule@alginate particles according to the disperse phase flow rates under the condition of 0.5 mL/min (continuous phase I) and 0.1 mL/min (continuous phase II). It is found that the diameters of alginate shell increase with the dispersed phase flow rate. Diameters for the alginate shell of the synthesized microparticles could be controlled with a variation coefficient for the diameter distribution of less than 10%, indicating that the manufactured microparticles meet the typical criterion for monodispersity [28]. The relationships between flow rates and alginate particle sizes could be expressed as the following equations:

$$D = 95 \times DFR + 775, \qquad R^2 = 0.94, \tag{1}$$

$$D = -35 \times CFR_I + 811, \qquad R^2 = 0.94, \tag{2}$$

$$D = -281 \times CFR_II + 952, \qquad R^2 = 0.96, \tag{3}$$

where D indicates the diameter of synthesized alginate particles; DFR represents the flow rate of disperse phase; CFR_I is the flow rate of continuous phase I (i.e. the Y-junction); CFR_II is the flow rate of continuous phase II (i.e. the cross-junction). After comparing Eq. (3) with Eq. (2), we found that the absolute value of slope in Eq. (3) is much larger than that in Eq. (2), suggesting that the variation of flow rate of continuous phase II (cross-junction) has more influence on the particle size than that of flow rate of continuous phase I (Y-junction).

The relationship between flow rates and alginate particle sizes could be expressed as the following equation:

$$D = 61 \times DFR + 928, \qquad R^2 = 0.93, \tag{4}$$

where D indicates the diameter for the alginate shell of the synthesized alginate particles; DFR represents the flow rate of disperse phase. After comparing Eq. (1) with Eq. (4), we found that the slope of Eq. (1) is larger than that in Eq. (4), suggesting that the variation of flow rate of disperse phase has more influence on the alginate particle size than that of vitamin A capsulate@alginate particles. Supporting Information Fig. 2 shows the optical microscope images of the vitamin A gelatin capsule embedded alginate microparticles with

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Table 1. The relationships among the diameter of the particles, the flow rate of dispersed phase and the flow rate of continuous phase

Continuous phase (mL/min)		Dispersed phase (mL/h)	Average diameter (μm)	SD (μm)	RSD (%)
Phase II (cross-junction)	Phase I (Y-junction)				
0.5	0.1	0.4	810	11	1.39
		0.3	803	11	1.36
		0.2	800	14	1.72
		0.1	784	12	1.48
		0.05	781	19	2.37
		0.02	772	19	2.44
0.5	0.1	0.4	810	11	1.39
	0.2		803	18	2.19
	0.3		799	17	2.17
	0.4		797	16	2.07
	0.5		795	17	2.17
	0.6		753	15	1.95
0.4	0.1	0.4	852	11	2.43
0.5			810	11	1.39
0.6			771	15	1.96
0.7			750	15	1.94
0.8			720	12	1.62
0.9			714	15	2.13

Table 2. The relationships between the diameter for the alginate shell of the core–shell vitamin gelatin capsules@alginate particles and the flow rate of dispersed phase

Flow rate of continuous phase (mL/min)		Flow rate of dispersed phase (mL/h)	Average diameter (μm)	SD (μm)	RSD (%)
Phase II (cross-junction)	Phase I (Y-junction)				
0.5	0.1	0.2	946	16	1.67
		0.4	969	19	1.9
		1	985	16	1.65
		2	1009	72	7.11
		3	1146	37	3.2
		4	1167	106	9.05

various flow rate of disperse phase (please see the Supporting Information).

Corresponding to the theoretical prediction of classic literatures [29, 30], a higher dispersed phase flow rate brings about a larger accumulated volume before a droplet detaches itself from the dispersed phase flow. Under a dripping regime, which is an extremely low Reynolds numbers for the droplet formation, the droplet diameter is proportional to the dispersed flow rate. This observation entirely agrees with our previous conclusion in the cross-junction chip [23–25].

3.3 Response of the core–shell gelatin–alginate microparticles in various pH environments

Figure 4 shows the degradations of core–shell gelatin–alginate microparticles in gastric juice (Fig. 4A and B; pH 1.32, mimicking the acidic environment in the stomach), intestinal juice (Fig. 4C and D; pH 7.7, mimicking the alkaline environment of the small intestine), and dd-H_2O (Fig. 4E and F; mimicking the normal storage environment). The core–shell gelatin–alginate microparticles in Fig. 4A, C, and E was about 945 μm in diameters with one gelatin capsule embedded, (synthesized at the flow rates of 0.1 mL/min, 0.5 mL/min, and 0.2 mL/h for continuous phase I, continuous phase II, and dispersed phase, respectively), while that in Fig. 4B, D, and F was about 970 μm in diameters with multiple gelatin capsule embedded (synthesized at the flow rates of 0.1 mL/min, 0.5 mL/min, and 0.4 mL/h for continuous phase I, continuous phase II, and dispersed phase, respectively). In all cases the capsule@alginate microparticles could remain intact in gastric juice for at least 3 h (Fig. 4A and B), but the alginate shell of the microparticles totally degraded in alkali environment in half an hour (Fig. 4C and D), which would allow the subsequent drug release of the gelatin microparticles. Figure 4E and F shows that the morphology of core–shell gelatin–alginate microparticles did not change

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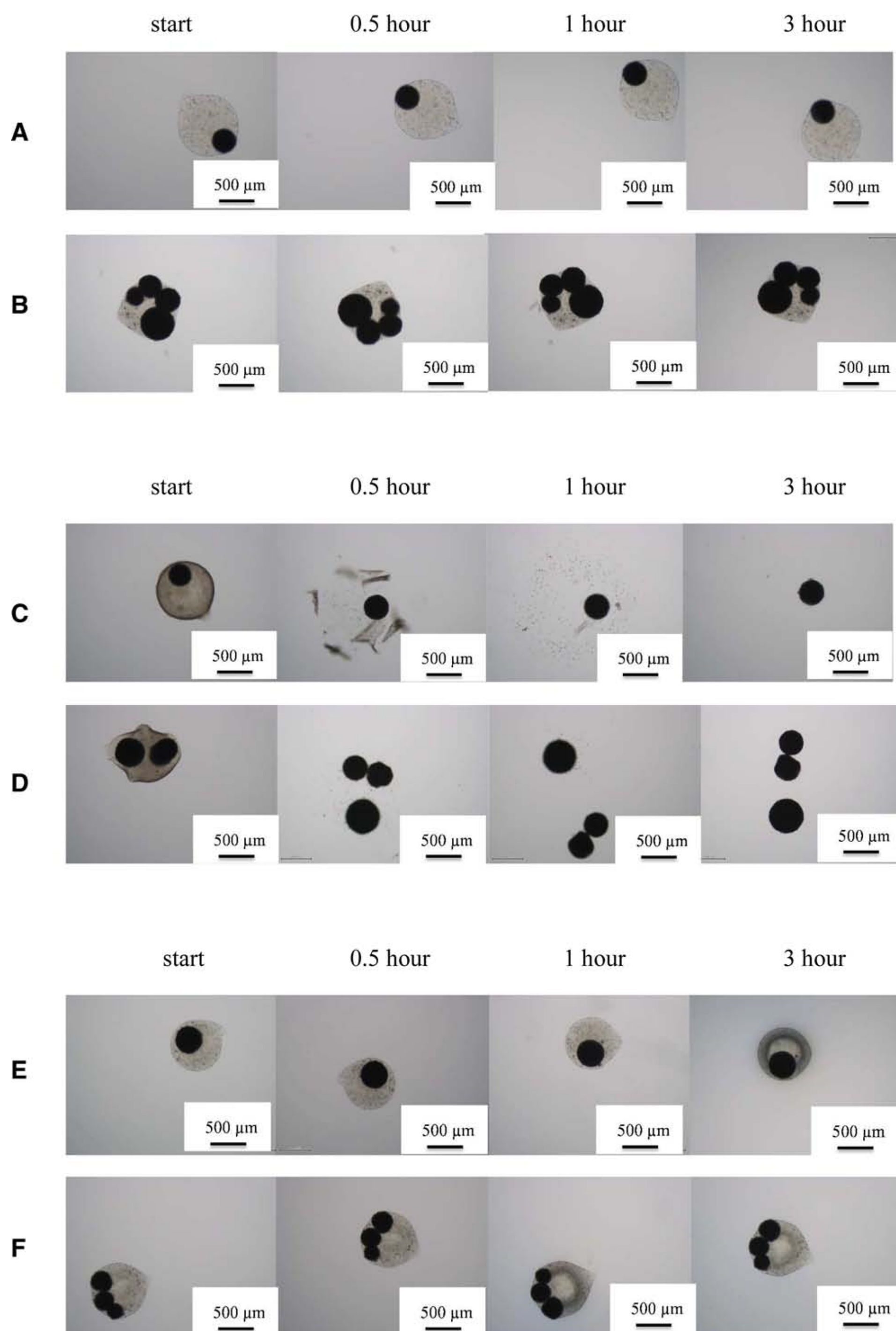

Figure 4. In vitro dissolution of tested capsules in gastric juice (Fig. 4A and B; pH 1.32, mimicking the acidic environment in the stomach), (B) intestinal juice (Fig. 4C and D; pH 7.7, mimicking the alkaline environment of the small intestine), and dd-H_2O (Fig. 4E and F; mimicking the normal storage environment).

in dd-H_2O, indicating that they can be well stored in water. For various diameters and number of embedded gelatin capsule, the gelatin core could be well protected by alginate shell in acid environment and be released in alkali environment.

Gelatin and alginates are two kinds of promising polymers that can be used as drug carriers, but suffer from hydrolysis of their glycosidic bonds in the gastrointestinal tract fluids. Thus, it is necessary to prevent them from being destroyed before achieving the destination. The pH-sensitive properties of the core–shell structure can be used to control the drug release process when the carriers passing through the gastrointestinal tract. In the literature, Rao et al. proposed a chitosan-based pH sensitive micronetworks for the controlled release of 5-fluorouracil [31]. Wang et al. presented a pH-sensitive magnetic alginate–chitosan beads for

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albendazole delivery [32]. In our study, the synthesized pH-responsive core–shell microparticles would be a good drug carrier for controlled delivery.

4 Concluding remarks

A vitamin A gelatin capsule composite intestinal-released oral delivery drug carrier was successfully obtained by microfluidic technique. The influence of dispersed phase flow rate on the size of the gelatin–chitosan core–shell particle was evaluated. In a pH-sensitivity test, we found that the fabricated particles could be well maintained in gastric juice, and the gelatin core could be protected. The particles would begin degradation in pancreatic juice in half an hour, indicating the possibility of drug release in alkali environment. The main advantages of this approach are: (i) an intestinal release for oral drug carrier could be obtained; (ii) uniform-sized gelatin–alginate core–shell particles could be fabricated; (iii) facile control of the particle diameters by varying the flow rates of disperse and continuous phase.

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The authors have declared no conflict of interest.

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