Korean J. Chem. Eng., 36(5), 822-827 (2019)
DOI: 10.1007/s11814-019-0238-6

pISSN: 0256-1115
eISSN: 1975-7220

Preparation, characterization and in vitro release properties of pectin-based curcumin film

Jie Pan†, Duanlin Cao, Xuemei Ma, and Jing Yang

School of Chemical Engineering and Technology, North University of China, Taiyuan 030051, Shanxi Province, P. R. China
(*Received 26 October 2018 • accepted 30 January 2019*)

Abstract−Curcumin is a kind of natural polyphenol whose functions are anti-tumor, anti-oxidant, anti-inflammatory and anti-microbial with low side effects. Solubility of Cur in water is poor, which results in low bioavailability, rapid metabolism, instability and poor absorption in vivo. Based on the crosslinking mechanism of pectin-calcium, the curcumin-pectin film (PT-Curf) was prepared by casting method, where chitosan and sodium alginate were added to the film, forming a system for blending modification to improve the performance of PT-Curf. Furthermore, the release of the curcumin films in vitro was studied and the effects of natural polysaccharides on the drug delivery system were discussed. According to our results, the natural polysaccharides involving pectin, chitosan and sodium alginate were excellent carriers of the fat-soluble drug Cur, and Cur was uniformly dispersed in the composite films in different crystal forms. Out of different drug delivery media, the three films exhibited different release rate and sustained release effect in vitro. However, the pectin-sodium alginate curcumin film (PT-SA-Curf) exhibited the most sustained release effect, the pectin-chitosan curcumin film (PT-CS-Curf) was second.

Keywords: Pectin, Chitosan, Sodium Alginate, Curcumin, In Vitro Release

INTRODUCTION

Curcumin (Cur), as the main active ingredient of Chinese herbal medicine and turmeric zedoary, is associated with a large number of pharmacological properties. Thus, it is widely applied in the medical industry [1,2]. Curcumin is one of the acidic polyphenols isolated from the rhizome of zingiberaceae plants such as turmeric [3]. With Cur easily inactivated by hydrolysis and oxidation in vivo, even if multiple doses are administered frequently, the blood concentration of drug remains low [4]. The reason for this is as follows: Curcumin is easily decomposed, so it is difficult to be absorbed into blood. It also has low solubility in water, and only a quarter of the total dose can be absorbed, which seriously restricts its application [5,6].

As drug carriers, natural polymers are often used to encapsulate drugs to improve solubility [7,8]. Pectin is a natural acidic heteropolysaccharide mainly composed of galacturonic acid [9]. Due to its gel properties, pectin is used in pharmaceuticals and health treatment [10]. Chitosan is the only cationic polysaccharide in nature [11]. Its sensitivity to pH can be used as a pH-responsive drug carrier [12,13]. Chitosan also has good biocompatibility and antibacterial properties, which represents a broad prospect in many industries such as food, chemical engineering, pharmacy, and so on [14]. Sodium alginate is the most abundant polysaccharide in brown algae, which has antiviral, antitumor and anti-mutation advantages [15, 16]. These above-mentioned natural high molecular polysaccharides have excellent film forming properties and are often used as drug carriers.

The excellent characteristics of polysaccharide make it utilized to prepare an edible functional film without plasticizer or surfactant on which curcumin is loaded. Such film is not only edible, but also has the medical effect of general external film, generating a complementary effect. Small molecule drug curcumin can be embedded in the network structure formed by natural polymer polysaccharides, which can effectively protect its metabolism, remaining the original drug form in vivo. Meanwhile, by regulating the film network structure, it is easy to achieve the drug delayed release effect [17].

In the study, based on the pectin-calcium gel crosslinking mechanism, PT-Curf was first prepared by utilizing the film forming properties of natural polysaccharides. To eliminate the defect in the performance of pure pectin film [18], cationic polyelectrolyte chitosan and anionic polyelectrolyte sodium alginate were selected and separately blended with pectin in preparation of PT-CS-Curf and PT-SA-Curf. Specific reaction steps are illustrated in Fig. 1. Through the release behavior of the functional film in different pH release media, the effect of the network structure of polysaccharides on the drug release performance was investigated.

EXPERIMENTAL

1. Materials and Instruments

Low-ester pectin: purchased from Yantai Andre Pectin Co., Ltd.

Carboxylated chitosan: purchased from Shanghai Yuanye Biological Co., Ltd.

Curcumin, anhydrous calcium chloride, sodium alginate, pepsin (enzyme activity≥1,200.0 U/g), and pectinase (enzyme activity≥50.0 U/g): purchased from Sinopharm Chemical Reagent Co., Ltd.

†To whom correspondence should be addressed.
E-mail: panjienuc@163.com

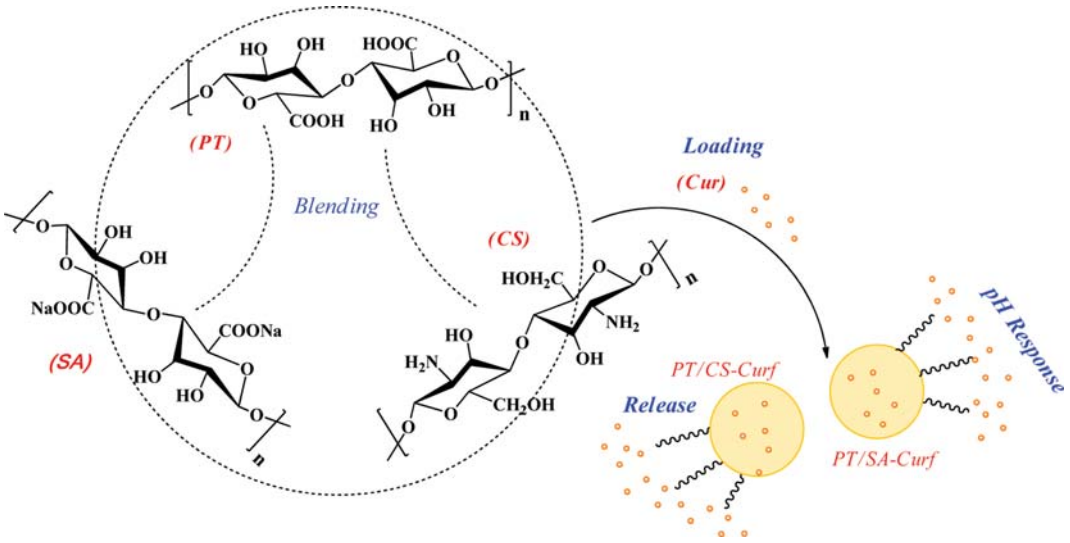

Fig. 1. Schematic description of film formation and drug delivery system.

Sodium dihydrogen phosphate (99.0%) and disodium hydrogen phosphate: purchased from Shanghai Aladdin Biochemical Technology Co., Ltd.

All the experimental drugs and reagents used were commercially available analytical reagents.

Electronic universal testing machine: (LDS-5, Xiamen Yi Shite Instrument Co., Ltd.);

X-ray Diffraction (D8 ADVANCE A25, Beijing Aikesi Rui Technology Co., Ltd.);

Scanning Electronic Microscope (JEM-2100F, JEOL Ltd.);

Three-dimensional mirror microscope (VHX-6000, Keyence China Ltd.);

Differential Scanning Calorimeter (Mettler Toledo, DSC-1);

UV-detector (752N UV-Vis, Shanghai Yidian Analytical Instrument Co., Ltd.);

All of the above were used as analytical instruments.

2. Film Preparation

2-1. Preparation of PT-Curf

A pectin solution (100 mL) with a concentration of 0.8% (W/V) was prepared and then transferred to a flask (250 mL) with continuous agitation at 55 °C, followed by slowly adding 10 mL of 0.03 mol/L $CaCl_2$ aqueous solution. The mixture was stirred for another 1.5 h. Then, 2 g/L of curcumin ethanol solution was added and stirred for 30 min. The resulting gel solution (25 mL) was poured onto leveled plates, defoamed, and dried at 37 °C, and the film PT-Curf was prepared. In the same operation procedure, the pure pectin film was prepared.

2-2. Preparation of PT-CS-Curf

A pectin-chitosan solution (100 mL) containing 0.9 g pectin and 0.15 g chitosan was prepared and then transferred to a flask (250 mL) with continuous agitation at 55 °C, followed by slowly adding 10 mL of 0.06 mol/L $CaCl_2$ aqueous solution. The mixture was stirred for another 1.5 h. Then, 2 g/L of curcumin ethanol solution was added and stirred for 30 min. The resulting gel solution (25 mL) was poured onto leveled plates, defoamed, and dried at 37 °C, and the film PT-CS-Curf was prepared.

2-3. Preparation of PT-SA-Cur Film

A pectin-sodium alginate solution (100 mL) containing 0.6 g pectin and 0.3 g sodium alginate was prepared and then transferred to a flask (250 mL) with continuous agitation at 55 °C, followed by slowly adding 10 mL of 0.02 mol/L $CaCl_2$ aqueous solution. The mixture was stirred for another 1.5 h. Then, 2 g/L of curcumin ethanol solution was added and stirred for 30 min. The resulting mixture was poured onto leveled plates, defoamed, and dried at 37 °C, and the film PT-SA-Curf was prepared.

The films PT-Curf, PT-CA-Curf and PT-SA-Curf were characterized by using SEM, XRD and DSC.

3. Characterization of the Film

3-1. Mechanical Properties of Film

The film thickness was measured using a spiral micrometer with an accuracy of 0.001 mm. The film was cut into a rectangular shape of a certain size (20 mm×50 mm), and a tensile test was performed. The clamping distance L_0 was 20 mm, the pulling rate was 10 mm/min. The standard deviation was calculated according to the average value of each film in the relative humidity of 50±3% at 25±2 °C. The tensile strength and elongation at break of the composite film were calculated by using Eq. (1) and Eq. (2).

$$\delta b = \frac{F}{A} \tag{1}$$

$$\varepsilon b\% = \frac{\Delta L}{L_0} \times 100 \tag{2}$$

where δb (MPa) is the tensile strength; F (N) is the tensile force; A (m^2) is the cross-sectional area; εb (%) is the elongation at break; ΔL (mm) is the displacement.

3-2. Moisture Sorption of Film

The composite film (10 mm×20 mm) was dried to constant weight (M_1) and placed in a volumetric flask filled with saturated NaCl aqueous solution, then placed in incubator with a humidity of 50±3% at 37 °C until the mass remained constant. The product was taken out and weighed as M_2. The moisture absorption rate

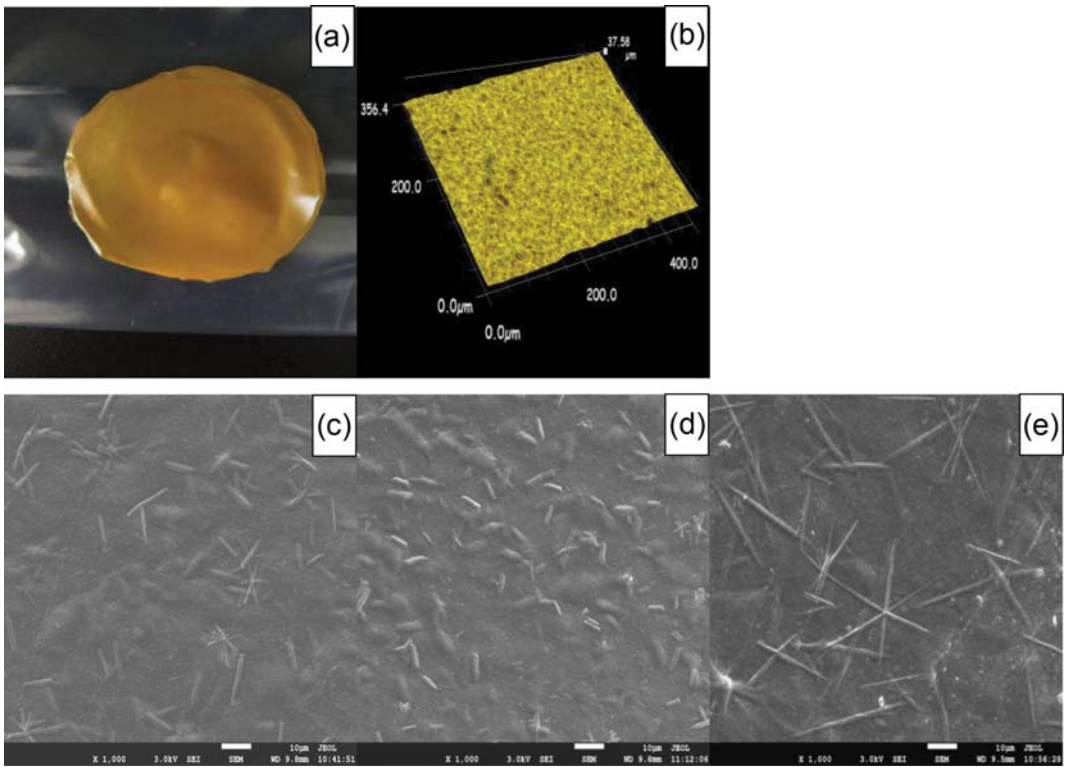

Fig. 2. Different forms of the edible curcumin films. (a) Photograph of PT-Curf; (b) Mirror microscope of PT-Curf; (c) SEM image of PT-Curf; (d) SEM image of PT-CS-Curf; (e) SEM image of PT-SA-Curf.

was calculated by using Eq. (3).

$$W\% = \frac{M_2 - M_1}{M_1} \times 100 \quad (3)$$

4. In Vitro Release Properties of Curcumin Film

4-1. Preparation of Artificial Body Fluid

The methods for preparing different artificial body fluids using phosphate buffer saline (PBS) were as follows: Simulated gastric juice was prepared with pH adjusted to 1.20 by using HCl solution and then 0.32 wt% pepsin was added. Simulated small intestinal juice was prepared by using PBS buffer solution of pH 6.86. Simulated colon juice was prepared by using PBS buffer solution of pH 7.40 and then 0.25 wt% pectinase was added.

4-2. Determination of In Vitro Release

Each film (20 mm×20 mm) was added to 30 mL of artificial gastric juice, small intestinal fluid, and colon fluid, respectively. The experiment was performed in an isothermal oscillator at 37 °C with shaking speed of 100 rpm. Samples of 3 mL each were withdrawn at definite time intervals and the absorbance was measured by using UV. The cumulative release rate of curcumin was calculated according to Eq. (4).

$$Re = \frac{c_n \times V_0 + V_i \sum_{i=1}^{n-1} c_i}{m} \times 100 \quad (4)$$

where R_e is the cumulative release; C_n is the drug concentration in the release medium after the nth sampling; V_0 is the volume of the release medium; V_i is the volume of each sample; C_i is the drug concentration in the release medium at the ith sampling replacement; m is the curcumin content in the film.

RESULTS AND DISCUSSION

1. Morphology of Film

The dry films were pasted with conductive adhesive on a sample table, and a scanning electron microscope was used to observe the morphology of the films. The films were placed under a mirror microscope to observe the dispersion and orientation.

The film PT-Curf was flat and the degree of orientation was high, and the curcumin was uniformly dispersed in the film (Fig. 2(a)). The orientation of the film was higher, with a distance difference of 37.58 μm (Fig. 2(b)). This implied that curcumin was highly dispersed in the film without obvious aggregation.

The crystal of curcumin in PT-Curf mainly existed in the form of needle and rod (Fig. 2(c)). Meanwhile, the curcumin crystal of PT-CS-Curf mainly existed in the form of rod (Fig. 2(d)). Chitosan is a polycation electrolyte, which will generate intermolecular force, coupling interaction force, and hydrogen bond force when mixed with the polyelectrolyte pectin [19]. This enhanced the strength and rigidity of the film, causing damage to the "pinpoint". The crystal of curcumin in PT-SA-Curf was mainly in the form of long needles (Fig. 2(f)). The addition of sodium alginate enhanced the flexibility of the film, and reduced the rigidity of the crosslinked network structure, making the "pinpoint" of curcumin exposed [20].

2. XRD Analysis of Film

The X-ray diffraction analysis of the films was performed using a D8 ADVANCE A25 diffractometer with monochromic Cu-Kα

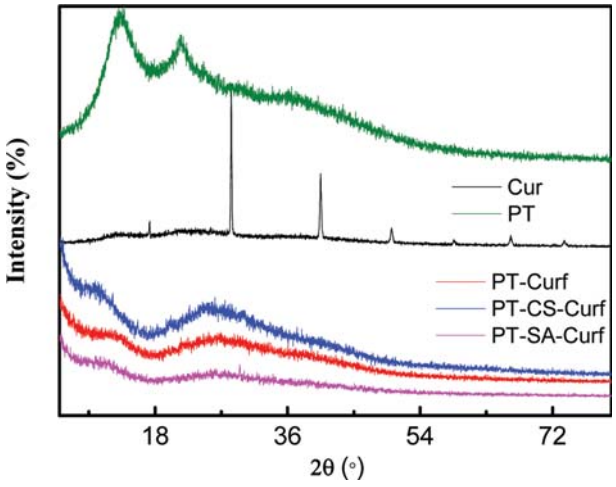

Fig. 3. XRD spectra of Cur, PT, PT-Curf, PT-CS-Curf and PT-SA-Curf.

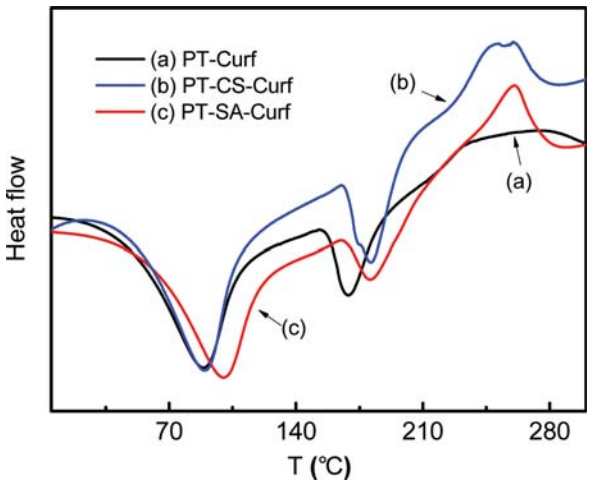

Fig. 4. Thermal behavior of the different films, (a) PT-Curf, (b) PT-CS-Curf and (c) PT-SA-Curf.

radiation. The tube pressure of XRD was 36 kV, and the 2θ scan range was from 5 to 80°, with a speed of 2°/min.

Curcumin has five distinct diffraction peaks at 18°, 28°, 40°, 50° and 66° (Fig. 3), with high intensity peak and narrow peak area, which indicated that curcumin existed as crystal. Two distinct diffraction peaks of pectin can be observed at 13° and 21°. The peak height, width and position of the three kinds of pectin-based film in XRD patterns, were changed. The diffraction peaks at 13° disappeared, and the diffraction peaks at 21° migrated to the right. Furthermore, the diffraction peaks of curcumin were not present in the three kinds of films, indicating that the polysaccharide network can be embedded in the small drug molecules, which was consistent with the SEM results.

Pectin-calcium is ionically crosslinked to form a stable dimer "egg-box" network structure [21]. The addition of CS and SA caused interaction and chimerism between the polysaccharide chains, thereby changing the structure and strength of the network. Meanwhile, curcumin can be embedded in the network and completely coated by the polysaccharide chains. Therefore, these polysaccharides can be used as excellent carriers for loading crystalline drugs. The network structure and strength formed by polysaccharide can be changed by adjusting the blending ratio, ionic strength, pH, and co-soluble substance [22], thereby improving the sustained release effect of curcumin.

3. DSC Analysis of Film

The thermal properties of various films were evaluated with a DSC after drying under vacuum. A sample of 3-5 mg was sealed in a perforated aluminum pan and an aluminum pan without the sample was used as a reference. The sample was heated from 0 to 300 °C, with a heating rate of 10 °C/min under nitrogen.

The DSC curves showed that all the three films had broad endothermic properties below 100 °C (Fig. 4), corresponding to the evaporation of water in the films. The PT-SA-Curf had strong water-blocking properties, whose evaporation temperature was higher than the other two films. The endothermic zone around 180 °C was caused by the degradation of curcumin. Due to the different carriers, the pyrolysis temperature of curcumin in the film varied differently and blending can increase the thermal stability. A tropical release was observed above 200 °C, which was caused by thermal degradation of the polysaccharide [23].

4. Mechanical Properties and Moisture Sorption

Many studies have shown that polysaccharide blending can improve certain properties of homopolymers. As shown in Table 1, the thickness results show that all of the three kinds of films are thin. The thickness of PT-Curf is the thinnest and that of PT-CS-Curf and PT-SA-Curf is similar. Compared to PT-Curf, the tensile strength of the film can be enhanced by addition of chitosan, and the elongation at break of the film can be increased by addition of sodium alginate. The main factors affecting the mechanical properties are the charge between macromolecules, the flexibility of polymer chains and their compatibility [24].

Hygroscopicity represents the water absorbing ability, reflecting the circumstance of hydrophilic group in the film [15]. The water absorption rate of the films is shown in Table 1. The PT-SA-Curf film has the strongest hygroscopicity, and the PT-CS-Curf film has the strongest performance of moisture resistance.

5. Determination of Curcumin Content

The curcumin content of the films was determined by using UV-

Table 1. Tensile strength, elongation at break, moisture absorption rate of films

Film	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)	Moisture absorption rate (%)
PT-Curf	0.15±0.03	2.04±0.12	6.12±0.91	28.18±1.16
PT-CS-Curf	0.11±0.02	5.40±1.02	8.63±1.77	13.98±1.55
PT-SA-Curf	0.20±0.03	1.80±0.72	10.10±3.95	45.71±2.28

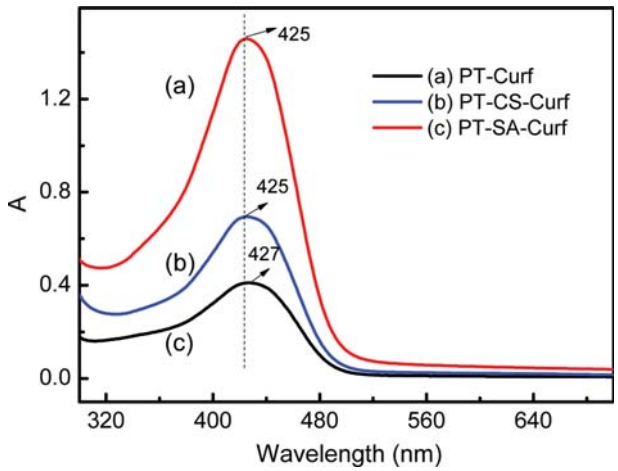

Fig. 5. UV-vis spectrum of different films, (a) PT-Curf, (b) PT-CS-Curf and (c) PT-SA-Curf.

vis. The maximum absorption peaks of PT-Curf, PT-CS-Curf and PT-SA-Curf were at 427 nm, 425 nm and 425 nm (Fig. 5), respectively. Curcumin dissolved in ethanol had a maximum absorption peak at 427 nm, which indicated that the polymer blending caused the migration of maximum absorption peak.

The curcumin contents in the film were as follows: 24.35±5.08 mg/g (PT-Curf), 26.58±4.42 mg/g (PT-CS-Curf), 21.43±4.17 mg/g (PT-SA-Curf). The average content of curcumin in the three films was 24.12±2.58 mg/g, and the difference in mass was less than 5%.

6. Effect of Release Medium on Properties of Drug Release

Samples were taken from different simulated body fluids at different drug release times, and the release concentration and cumulative release rate of curcumin were calculated. The release curves of curcumin in composite films were obtained. At the same time, a blank film release solution was used as a control under the same conditions.

6-1. Release of Curcumin from PT-Curf

The cumulative release increased with the rise of the medium pH (Fig. 6(a)). The release amount of curcumin in artificial gastric juice (pH 1.20) was lower than detection limit during the whole drug release process, which can be regarded as no release.

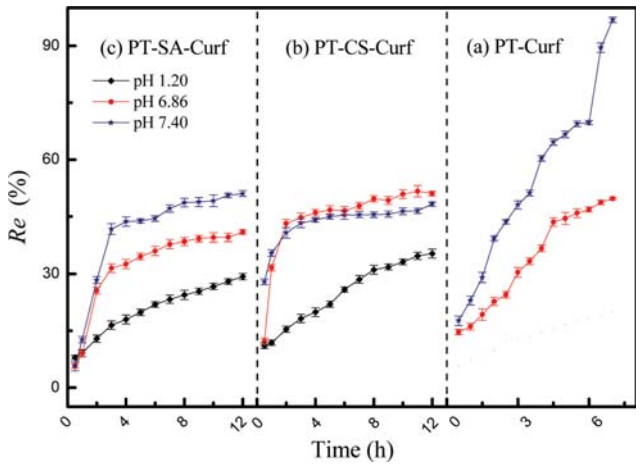

Fig. 6. Drug release profile of the edible films in the simulated digestive fluids, (a) PT-Curf, (b) PT-CS-Curf and (c) PT-SA-Curf.

The cumulative release was less than 50% at pH 6.86 in 6 h, showing a slow release effect. The cumulative release reached 70% at pH 7.40 in 6 h, and the burst behavior was shown at 7 h. Pectin is a polyelectrolyte. Under acidic conditions, the molecular chains exist in agglomerated state due to strong electrostatic action, resulting in a very low release of curcumin. As the pH increased, the degree of protonation of the carboxyl group weakened, and the molecular chain existed in stretched state. Meanwhile, the crosslinked network structure dissolved, and the release rate of curcumin increased. Otherwise, when the film reached the colon, under the action of various microorganisms, the pectin degraded and the curcumin was released [25].

6-2. Release of Curcumin from PT-CS-Curf

The cumulative release increased with the rise of pH during the same time (Fig. 6(b)). The cumulative release was less than 35% at pH 1.20 in 12 h, which witnessed a greater improvement compared to PT-Curf. Curcumin was released uniformly and slowly in the artificial intestinal juice, and the cumulative release rate was 50% in 12 h, which represented a significant effect of sustained release. The cumulative release at pH 7.40 was lower than that of pH 6.86 as the release time was prolonged. Under acidic conditions, the drug was released with the degradation of chitosan in PT-CS-Cur film. With the increase of pH, pectin will undergo a certain degree of degradation, resulting in increased release. Under alkaline conditions in the colon, the alkaline polysaccharide chitosan in the PT-CS-Cur can reduce the dissolution rate of the film skeleton, resulting in a lower release of curcumin than in the intestinal environment.

6-3. Release of Curcumin from PT-SA-Curf

The cumulative release of PT-SA-Curf in different media increased with the increase of pH during the same time (Fig. 6(c)). The cumulative release was 50%, 40%, and 30% in 12 h at pH 7.40, 6.86 and 1.20, respectively. The results showed that PT-SA-Curf had a significant sustained release effect, and the delayed release effect depended on pH value. Both pectin and sodium alginate are polyelectrolytes, and the polysaccharides chains exist in agglomerated state under low pH. The drug release in artificial gastric juice was caused by the enhancement of intermolecular repulsive force in the film structure [26].

Pectin and sodium alginate have similar gelling mechanisms, can form a coherent gel network [27,28], and have a competitive crosslinking effect with Ca^{2+}. Due to the intermolecular chimerism between the molecular chains, the strength of the network structure will intensify, forming a denser structure. Hence, the effect of sustained release was improved obviously, and the degradation time took longer.

6-4. Release behavior of Three Films in Simulated Gastrointestinal Environment

Referring to the actual residence time of drugs in the gastrointestinal fluid, the film was separately placed in release vials containing 30 mL of the same simulated body fluid for continuous shock release. Fig. 7 shows the release behavior of the drug-loaded film under simulated gastrointestinal conditions.

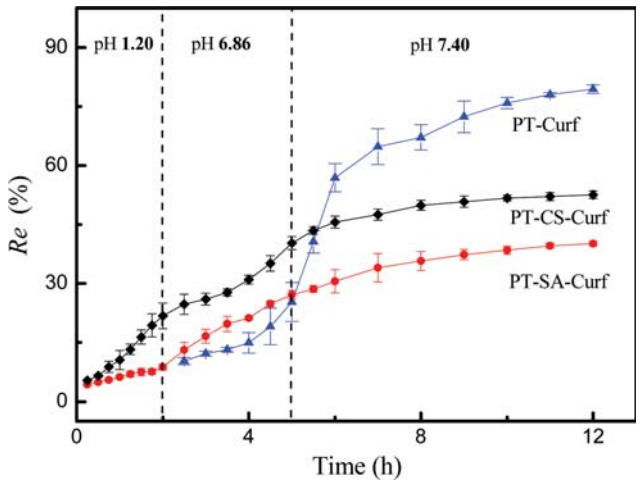

Fig. 7. Curcumin release from PT-Curf, PT-CS-Curf and PT-SA-Curf in simulated gastrointestinal fluid (Sustained release solutions for different periods of time: 0-2 h was the simulated gastric juice, 2-5 h was the simulated small intestinal juice, and 5-12 h was the simulated colon juice).

The cumulative release rates of the three films increased as the pH of the medium increased. For the first 2 h at pH 1.2, the curcumin release of PT-Curf was lower than detection limit, and the cumulative release rate of PT-CS-Curf was the highest. As the pH increased to 6.86, all of the three films were partially released. In the final release stage, the cumulative release rates of PT-SA-Curf and PT-CS-Curf continued to increase slowly and evenly in colon simulated fluid. However, the cumulative release rate of PT-Curf was almost 60% after 6 h. The addition of chitosan and sodium alginate made the molecular chain of the polysaccharide form an interpenetrating polymer network structure, which can have a slow and controlled release effect on the drug.

CONCLUSIONS

The resulting pectin-based polysaccharide films had complete appearance, good quality, as well as uniform thickness and color. The toughness of the film can be enhanced by addition of chitosan and sodium alginate, and blending can improve the thermal stability of curcumin. Due to the interaction and chimerism between polysaccharide chains and curcumin, which influenced the swelling, the addition of natural polysaccharides can slow the release of curcumin. All of the three films exhibited sustained release effects in different simulated digestive fluids, the PT-SA-Curf film exhibited the most sustained release effect, and the PT-CS-Curf film was second. To conclude, chitosan and sodium alginate used in curcumin films change not only the tautomeric curcumin forms and the physical state but also the curcumin solubility and the encapsulation efficiency.

REFERENCES

1. S. V. Kanagarajan and D. Thiyagarajan, *Mater. Res. Express.*, **6**, 016105 (2019).
2. K. Mahmood, K. M. Zia, M. Zuber, Z. Nazli, S. Rehman and F. Zia, *Korean J. Chem. Eng.*, **33**, 3316 (2016).
3. X. L. Li, Y. J. Hu, R. Mi, X. Y. Li, P. Q. Li and Y. Ouyang, *Mol. Biol. Rep.*, **40**, 4405 (2013).
4. R. Meena, S. Kumar, R. Kumar, U. S. Gaharwar and P. Rajamani, *Biomed. Pharmacother.*, **94**, 944 (2017).
5. A. Faralli, E. Shekarforoush, F. Ajalloueian and A. C. Mendes, *Carbohyd. Polym.*, **206**, 38 (2019).
6. F. Y. Shih, I. J. Su, L. L. Chu and X. J. Lin, *Int. J. Mol. Sci.*, **19**, 3625 (2018).
7. L. Y. Zhu, X. Q. Yan, H. M. Zhang, S. J. Yao and L. Jiang, *Korean J. Chem. Eng.*, **32**, 369 (2015).
8. A. Ahmed, J. Hearn, W. Abdelmagid and H. F Zhang, *J. Mater. Chem.*, **22**, 25027 (2012).
9. A. T.-B. Nguyen, P. Winckler, P. Loison, Y. Wache and O. Chambin, *Colloid Surface B*, **121**, 290 (2014).
10. S. Tripathi, G. K. Mehrotra and P. K. Dutta, *Carbohyd. Polym.*, **79**, 711 (2010).
11. R. Jayakumara, D. Menona, K. Manzoora, S. V. Naira and H. Tamura, *Carbohyd. Polym.*, **82**, 227 (2010).
12. M. Rinaudo, *Prog. Polym. Sci.*, **31**, 603 (2006).
13. V. K Mourya and N. N. Inamdar, *React. Funct. Polym.*, **68**, 1013 (2008).
14. M. Ul-Islam, N. Shah, J. H. Ha and J. K. Park, *Korean J. Chem. Eng.*, **28**, 1736 (2011).
15. B. K. S. Khanal, B. Bhandari, S. Prakash and D. S. Liu, *Food Hydrocolloid*, **83**, 97 (2018).
16. C. Jeon, J. O. Kim and S. M. Lee, *Korean J. Chem. Eng.*, **31**, 1219 (2014).
17. T. M. S. U. Gunathilake, Y. C. Ching, C. H. Chuah, H. A. Illias and K. Y. Ching, *Int. J. Biol. Macromol.*, **118**, 1055 (2018).
18. R. S. Francisco, S. R. Maria, G. Luana, R. A. Angelo and D. Felipe, *Food Hydrocolloid*, **70**, 181 (2017).
19. Y. Fang, B. Duan, A. Lu, M. Liu and H. Liu, *Biomacromolecules*, **16**, 1410 (2015).
20. M. Hartmann, O. B. Holm, G. A. B. Johansen, G. Skjåkbraek and B. T. Stokke, *Biopolymers*, **63**, 77 (2002).
21. J. T. Fu and M. A. Rao, *Food Hydrocolloid*, **15**, 93 (2001).
22. P. Shukla, A. K. Verma, J. Dewangan, S. K. Rath and P. R. Mishra, *RSC Adv.*, **5**, 57006 (2015).
23. E. V. Lopez, A. L. Alvarez and J. B. Mendez, *J. Drug. Deliv. Sci. Tec.*, **42**, 1 (2017).
24. H. Y. Pan, B. Jiang, J. Chen and Z. Jin, *Food Chem.*, **151**, 1 (2014).
25. B. Gómez, B. Gullón, C. Remoroza, H. A. Schols and J. C. Parajó, *J. Agr. Food Chem.*, **62**, 9769 (2014).
26. L. Chen and Y. Du, *J. Appl. Polym. Sci.*, **86**, 1838 (2002).
27. I. Braccini and S. Perez, *Biomacromolecules*, **2**, 1089 (2001).
28. D. A. Powell, E. R. Morris, M. J. Gidley and D. A. Rees, *J. Mol. Biol.*, **155**, 517 (1982).