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Preparation and release of curcumin/silk fibroin/sodium alginate film

Yerong Yuan †, Jun'an Zheng †, Zunchao Liu †, Wei Li †*, Jiaqing Cao †*, Xiangrong Zhang †*

Faculty of Functional Food and Wine, Shenyang Pharmaceutical University, Shenyang 110016, China

Abstract The aim of this study was to prepare silk fibroin/sodium alginate composite film containing curcumin by casting method. Orthogonal test was used to optimize the formulation according to the values of tensile strength and elongation at break. The release of curcumin in the optimal film was studied in order to explore its application as wound dressing. The results showed that the optimum composition of curcumin/silk fibroin/sodium alginate composite film was as follows: Silk fibroin (70 mg/mL) 2.7 g, sodium alginate (24 mg/mL) 0.84 g, span 40 (5.0 mg/mL) 0.4 g, glycerol (3.75%, *V*/*V*) 3 mL, curcumin (0.2 mg/mL) 0.016 g. The optimum film showed the tensile strength and the elongation at break was (0.628 ± 0.032) MPa and (0.794 ± 0.046) %, respectively.

Keywords: curcumin; silk fibroin; sodium alginate; composite film

1 Introduction

Curcumin is a natural polyphenol from the rhizome of turmeric (*Curcuma longa* L.), which has many excellent properties including anti-inflammatory, antibacterial properties and pH-dependent solubility [1, 2]. The formula of curcumin is $C_{21}H_{20}O_6$ with molecular weight of 368.39. The chemical structure (Fig. 1) was composed of a 1, 7-diarylheptane with one β-diketone and two o-methylated phenols [3]. Under alkaline conditions, it is mainly an enol-type structure with red brown [4]. In acidic and neutral environments, keto structure is dominant and with bright yellow [5]. Curcumin has benefit on promoting wound healing, and it is often applied as external preparation for skin injury in clinical practice [6].

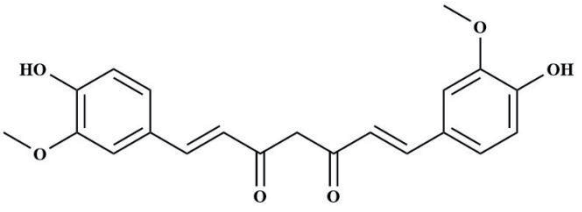

Fig. 1 Molecular structure of curcumin

Wound dressing is a medical material for mechanical skin injuries, burns, sores, etc., which can protect the wound, prevent bacterial infection and promote wound healing [7]. Traditional wound dressings are gauze dressings. However, the protection of gauze dressings for wound is limited and there are many shortcomings including bacteria breeding, poor coverage of wound surface, easy to fall off and secondary trauma. The new dressing is mainly prepared by collagen, alginate, chitosan, hydrogels and other biological materials. In addition, antibacterial agents, honey, essential oils, cationic peptides, aloe vera, plant extracts and some other natural

* Corresponding author: Wei Li (wei_li1981@163.com);
Jiaqing Cao (jiaqingcao@163.com);
Xiangrong Zhang (zhangxr@vip.sina.com).
† These authors contributed equally to this work;
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antibacterial, anti-inflammatory and wound healing promoting molecular materials can be loaded in the dressing [8]. There new biological dressings showed superior breathability, bacteriostasis, biocompatibility and cell adsorption capacity, which can reduce the risk of infection and promote wound healing [9]. The film is widely designed in the preparation of wound dressing due to its excellent adhesion on the wound surface, simple preparation process and convenience.

Silk fibroin (SF) is obtained through silk degumming. SF is a natural polymer material composed of glutamic acid, glycine, serine and other 18 kinds of amino acids. SF is a popular biomaterial for tissue engineering and regenerative medicine applications because of its low immunogenicity, good biocompatibility, modifiable biodegradability, and mechanical properties [10]. Liu, et al. [11] verified that SF film was non-toxic to cells and had no adverse effects on cell adhesion, growth and apoptosis, which could promote the healing of skin defect wounds. The films of SF were more brittle and had poor mechanical properties, so other materials can be incorporated to prepare composite film [9]. Alginate is a natural polysaccharide produced by brown algae and bacteria, widely used in the food and medical industries. Na^+ ions in the alginate macromolecular chain can be replaced by divalent cations (Ca^{2+}, Cu^{2+}, and Zn^{2+}). Then the macromolecular chain residues are chelated with divalent cations to form new alginate-based polymers [12]. Polymer-based antibacterial wound dressings prepared from sodium alginate (SA) have been reported to exhibit unique permeability, minimize bacterial infections, promote rapid re-epithelialization and granulation tissue formation, etc. [13]. Natarajan et al. reported SA and chitosan-based transdermal wound dressing with controlled drug release behavior [14]. The single SF film has the defect of brittleness. By blending SA with SF, the composite film can overcome the shortcoming of single film [15]. The chemical structures of silk fibroin and sodium alginate are shown in Fig. 2.

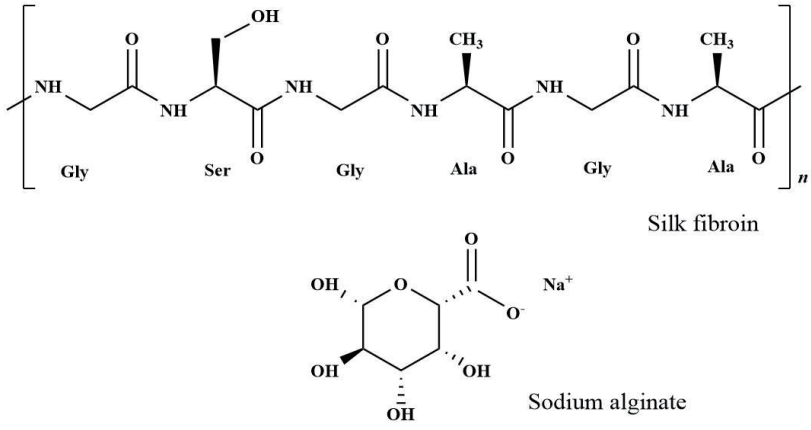

Fig. 2 Molecular structure of silk fibroin and sodium alginate

In the present work, SF and SA were used as the film carrier and curcumin as the active ingredient added in the film. The formulation of curcumin/silk fibroin/sodium alginate film was optimized by orthogonal test, and the preparation was obtained based on tensile strength and elongation at break. The release rate of curcumin in the composite film was evaluated to provide a basis for its application in wound dressing.

2 Materials and methods

2.1 Materials

Curcumin (95% purity, Shanxi Baichuan Biotechnology Co., LTD.), silk fibroin (Shandong Qilu Biotechnology Group), sodium alginate (Henan Homey wonbond Biotechnology Co., LTD.), span 40 (Tianjin Damao Chemical Reagent Factory),

propylene glycol (Tianjin Hengxing Chemical Reagent Co., LTD.), anhydrous ethanol (Shandong Yuwang Pharmaceutical Co., LTD.).

2.2 Preparation of SF/SA composite film

The SF and SA solution were mixed with 80 mL water, and placed in water bath at 60 °C. Glycerol and span 40 were dissolved in above solution and continued stirring in a magnetic stirrer (DF-101S, Gongyi Yuhua Instrument Co., LTD.) for 3 h. The film forming solution was obtained by ultrasound (KQ3200E, Kunshan Ultrasonic Instrument Co., LTD.) for 30 min. After that, 10 mL was cast in a glass plate and transferred to an electric thermostatic air blast drying oven (DHG-9053A, Shanghai Jinghong Experimental Equipment Co., LTD.) for 2.5 h. Films were peeled off the glass plate and placed inside desiccators adjusted to 50% RH.

2.3 Preparation of curcumin/SF/SA composite film

An appropriate amount of curcumin was dissolved in anhydrous alcohol to obtain 0.2 mg/mL curcumin alcohol solution. Then, it was added into the prepared SF and SA aqueous solution and placed in a water bath at 60 °C. Next, glycerol and span 40 was dissolved to in the above solution as additives and stirred with a magnetic stirrer at 60 °C for 3 h to obtain a film forming solution. The prepared film-solutions were degassed, poured into a glass plate and transferred to an electric thermostatic blast oven for drying. Finally, films were placed in the 50% RH dryer.

2.4 Thickness and mechanical properties

The thickness of the films was measured from ten random points with a thickness gauge (Epp Measuring Instrument Co., LTD.). The readings were accurate to 0.001mm. The tensile strength (TS) and elongation at break (E) were measured by the tensiometer (JHY-5000, Xiamen Jinheyuan Technology Co., LTD.) according to GB/T 104.3-2006 and adjusted appropriately. The composite film was cut into 70 mm × 10 mm strips and fixed to the clamp of the tensioner. The initial spacing was set as 50 mm and the running speed as 180 mm/min. Three parallel groups were made for each composite film to record the value of TS and E.

2.5 Single factor experimental design

SF concentration (30, 50, 70, 90, 110 mg/mL), SA concentration (16, 20, 24, 28, 32 mg/mL), glycerol content (2.75%, 3.25%, 3.75%, 4.25%, 5.75%, *V*/*V*), span 40 content (5.0, 10.0, 15.0, 20.0, 25.0, mg/mL) were as factors to explore the effects on the value of TS and E of the composite film, as shown in Table 1.

Table 1 Horizontal table of orthogonal test factors

Leve]	A. SF concentration (mg/mL)	B. SA concentration (mg/mL)	C. Span 40 (mg/mL)	D. Glycerol (*V*/*V*)
1	50.00	20.00	5.0	3.25
2	70.00	24.00	10.0	3.75
3	90.00	28.00	15.0	4.25

2.6 Orthogonal experimental optimization

In order to optimize the reaction conditions, the range of data collection was expanded in the orthogonal experimental design, and 3 levels were taken for each factor. The optimal combination of reaction conditions for film preparation was determined by $L_9(4)^3$ orthogonal test.

2.7 In vitro release test

2.7.1 Method for determination of curcumin content

The concentration of curcumin was determined

by ultraviolet-visible spectrophotometer (4802H, Shanghai Unique Company). The mass of 10 mg curcumin dissolved in 10 mL anhydrous ethanol solution to obtain curcumin solution. Absolute ethanol was used as a blank reference, and the wavelength was scanned in the range of 200−500 nm. The maximum absorption wavelength of curcumin was determined according to the map obtained by scanning. The results showed that maximum absorption at 425 nm, so the detection wavelength of curcumin was determined to be 425 nm.

2.7.2 Establishment of curcumin standard curve

The reserve solution of curcumin was diluted to standard solution of different standard concentrations (1.0, 2.0, 3.0, 4.0, 5.0 μg/mL). Different absorbance values of curcumin at the detection wavelength (425 nm) were measured, and the absorbance value was used to make linear regression on the curcumin concentration value to establish the standard curve. The equation of curcumin standard curve is A = 0.164 5 C − 0.011 9, and the linear correlation coefficient R^2 = 0.999 5.

2.7.3 Precision

The three concentrations of low (2 μg/mL), medium (4 μg/mL) and high (6 μg/mL) was prepared, and the absorbance was measured at 425 nm. On the same day, each different test sample was repeated for 6 times, and the intra-day precision was calculated. All different samples were tested once at the same time period on the 1st, 2nd, 3rd, 4th and 5th days respectively to calculate the daytime precision. The calculation results are shown in Table 2.

Table 2 The diurnal and intra-day precision of curcumin was determined by UV method

C (μg/mL)	Inter-day		Intra-day	
	Average (μg/mL)	RSD (%)	Average (μg/mL)	RSD (%)
2.00	1.996	0.99	1.994	1.02
4.00	3.984	0.56	3.987	0.44
6.00	5.876	0.60	5.878	0.58

2.7.4 In vitro release study

Three kinds of film with good appearance and uniform thickness were selected for release study. The samples were placed in a conical flask containing 30 mL ethanol /PBS (pH = 7.4) as the release medium (ethanol:VPBS = 20:80 or 60:40) placed in a 37 °C gas bath thermostatic oscillator (THZ-82A, Jintan Xicheng Xinrui Medical Instrument Factory). At a fixed time (10, 20, 30, 40, 50, 60, 120, 180, 240, 300, 360, 480 and 600 min), 5.0 mL of the release medium was precisely collected, replaced by adding 5.0 mL of fresh release medium. The absorbance of samples at 425 nm at each time point was determined. The cumulative release rate of each group was calculated according to the formula and the cumulative release curve *in vitro* was drawn.

$$Q_n\% = (C_n V_0 + V_s \sum_{i-1}^{n} C_{i-1}) / W \times 100\%$$

C_n is the drug concentration measured at the n sampling; V_0 is the total volume of the medium; V_s is the sampling volume; W is the mass of the drug contained.

3 Results and discussion

The response variables at different processing conditions (SF concentration, SA concentration, glycerol content, span 40 content) during film

formation are shown below.

3.1 Single factor test analysis

3.1.1 SF concentration

Under the conditions of SA concentration of 20 mg/mL, glycerol content of 3.75% and span 40 content of 15.0%, the effects of different SF concentrations on the properties of composite film are shown in Fig. 3. Within the range of 30−70 mg/mL SF supplemental levels, values of TS and E were increased with increasing SF levels. When the amount of SF was 70 mg/mL, the value of E was the maximum value. Afterwards, with the increase of the amount of SF, the E and TS in composite film decreased.

The G unit (α-L-guluronic) in SA forms a special "egg-box" structure with Ca^{2+}, and the network "egg-box" structure enhances the intermolecular force. However, with the increase of SF content, the distance between SA molecular chains gradually increases, the number of "egg-box" structures formed with Ca^{2+} decreases, and the networking structure decreases, so the fiber fracture strength will have a downward trend [16]. A bigger value E is beneficial to increase the extension of the wound dressing. That is, the SF content of 70 mg/mL was selected to prepare the composite film.

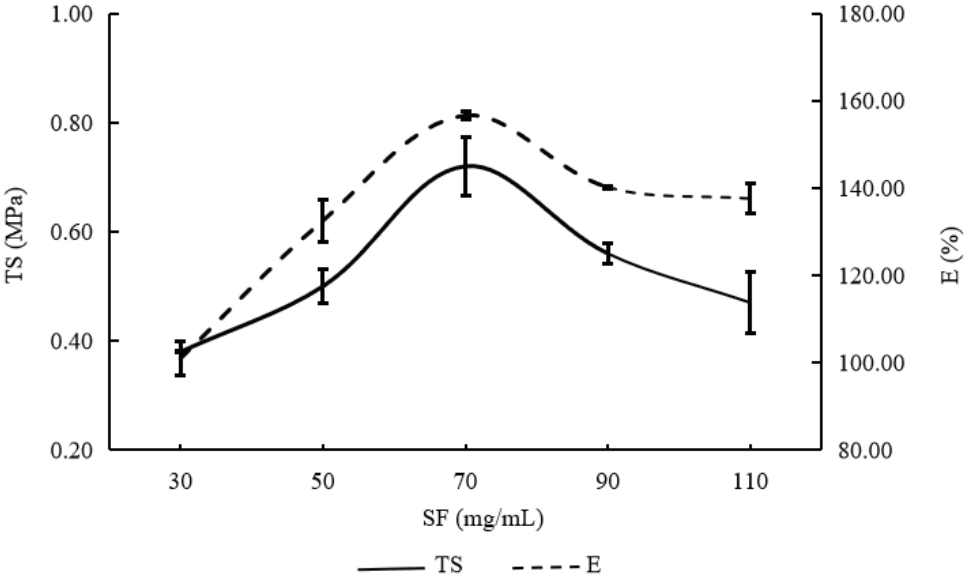

Fig. 3 Effect of silk fibroin content on mechanical properties

3.1.2 SA concentration

The effect of SA content on the mechanical properties of the composite film is shown in Fig. 4. As shown in the Fig. 4, with the increase of SA content, the value of TS and E of the composite films increased significantly. This indicated that the incorporation of SA could effectively improve the strength and resistance to distortion and deformation of the composite film. The composite films showed the highest TS and E when the concentration of SA was 24 mg/mL. Therefore, the optimal mechanical properties of the composite film can be regulated by adjusting the SA concentration.

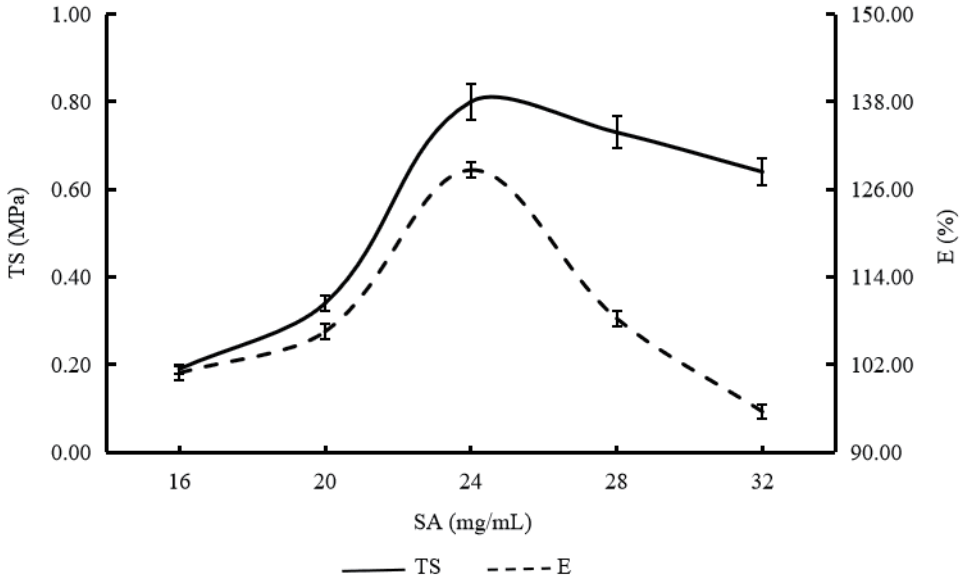

Fig. 4 Effect of sodium alginate content on mechanical properties

3.1.3 Amount of glycerol added

The influence of glycerin addition on the mechanical properties of the composite film is shown in Fig. 5. The TS first increased with the increase of glycerol content. Then the TS reached a maximum when the glycerol content reached 3.25%. After, with the content increase of glycerol, it showed a downward trend. E increased with increasing glycerol content. This was attributed to the addition of glycerol as a plasticizer, which increased the flexibility of the composite film [17]. Therefore, when the glycerol content is 3.25%, the film forming agent has an advantageous performance.

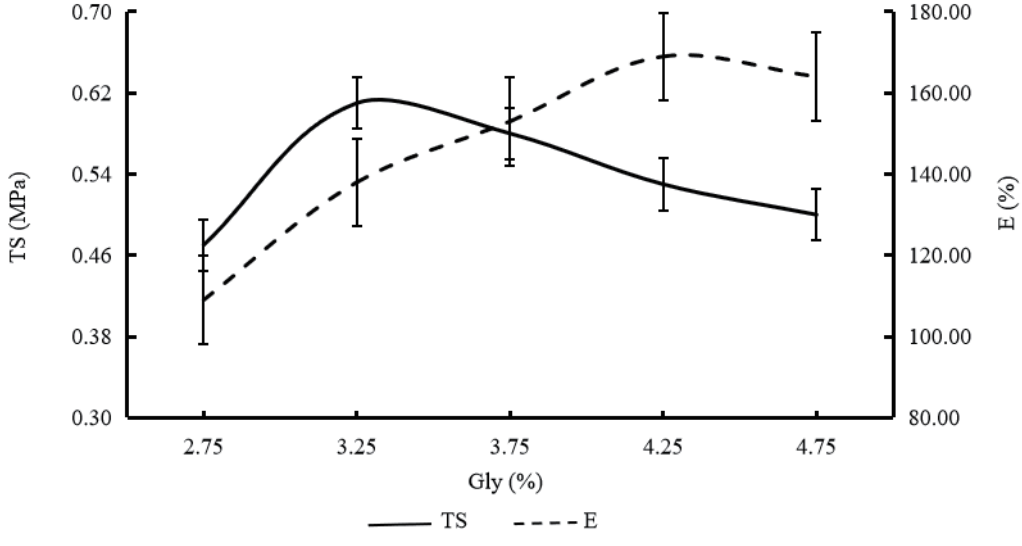

Fig. 5 Effect of glycerol content on mechanical properties

3.1.4 Amount of span 40

The effect of different span 40 contents on the composite film properties is shown in Fig. 6. It can be seen from the figure that the TS and E showed a trend of first increasing and then decreasing with the change of span 40 content. The reason may be that span 40 as a surfactant which can reduce the rate of water loss

of composite film, increase the wettability between composite film and food, and enhance the interaction between polymers [18]. TS and E was the highest when the span content was 10.0 mg/mL. Therefore, when the content of span 40 is 10.0 mg/mL, the composite film has good performance.

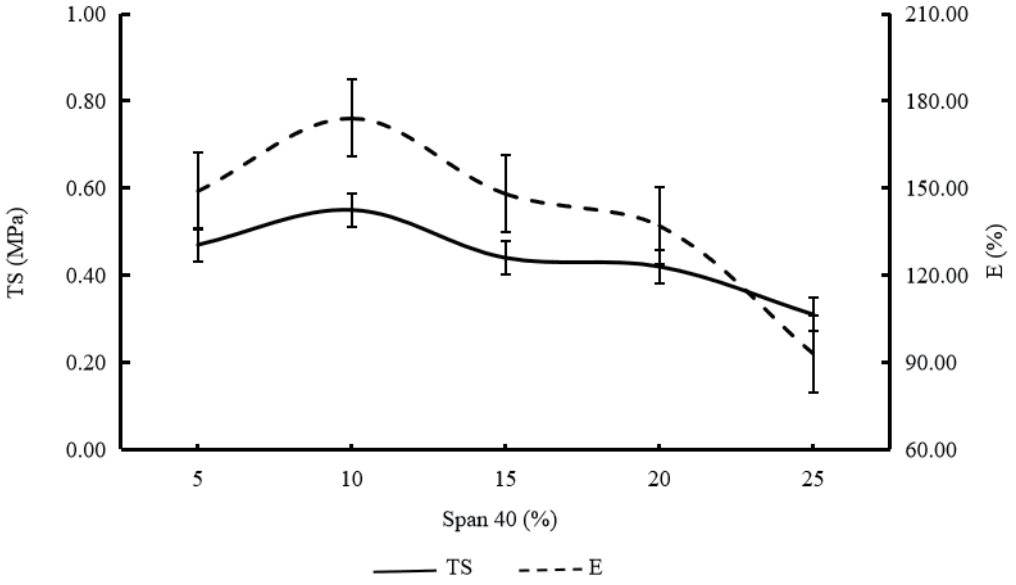

Fig. 6 Effect of span 40 content on mechanical properties

3.2 Orthogonal test analysis

The effects of SF concentration, SA concentration, glycerol content, and span 40 content were investigated using TS and E as response values, and the test data are shown in Table 3.

According to the results of range analysis in Table 4, the influencing factors on the mechanical properties of the composite film are: SF concentration, SA concentration, glycerin content, and span 40 content. Therefore, the formulation of the composite film with the optimal mechanical properties is $A_3B_3C_1D_2$. Yen, et al. [19] reported that topical curcumin could promote skin wound healing in mice, and when the concentration of curcumin was 0.2 mg/mL.

Table 3 Orthogonal test results

Number	SA	SF	Span 40	Gly	Tensile strength (MPa)	E (%)	E/100	Weighting
1	1	1	1	1	0.62 ± 0.04	110.89 ±1.91	1.11	0.86
2	1	2	2	2	0.86 ± 0.04	117.77 ± 10.21	1.18	1.02
3	1	3	3	3	0.78 ± 0.10	156.36 ± 8.47	1.56	1.17
4	2	1	2	3	0.45 ± 0.02	151.43 ±0.55	1.51	0.98
5	2	2	3	1	0.97 ± 0.03	121.24 ± 6.54	1.21	1.09
6	2	3	1	2	1.08 ± 0.10	154.37 ± 14.21	1.54	1.31
7	3	1	3	2	1.01 ± 0.06	165.10 ± 2.21	1.65	1.33
8	3	2	1	3	1.08 ± 0.03	184.10 ± 3.45	1.84	1.46
9	3	3	2	1	1.20 ± 0.10	135.70 ± 12.8	1.36	1.28

Table 4 Range analysis of orthogonal test

Index	Number	A	B	C	D
Tensile	Mean 1	0.75	0.69	0.93	0.93
Strength	Mean 2	0.83	0.97	0.84	0.98
	Mean 3	1.10	1.02	0.92	0.77
	Range	0.34	0.33	0.09	0.21
Elongation	Mean 1	1.28	1.42	1.50	1.23
at break	Mean 2	1.42	1.41	1.35	1.46
	Mean 3	1.62	1.49	1.47	1.64
	Range	0.33	0.08	0.15	0.41
Weighting	Mean 1	1.018	1.058	1.212	1.079
	Mean 2	1.127	1.190	1.094	1.220
	Mean 3	1.357	1.254	1.197	1.203
	Range	0.339	0.196	0.119	0.142

3.3 In vitro release

Fig. 7 shows the cumulative release curve of curcumin from the composite film in different media. The results showed that the release trend of curcumin in various media was slightly different. When the volume ratio of ethanol to PBS was 20:80, the release rate of curcumin was maintained at a low level, and its cumulative release rate did not exceed 30%, then displayed a slow increase trend. When the volume ratio of ethanol to PBS was 60:40, the burst release occurred during 0−50 min, and the cumulative release rate exceeded 50%. When the release time was 1 h, the cumulative release rate was 55.6%, and then maintained a low release rate. The cumulative release rate of curcumin in 60:40 release medium is higher than that in 80:20, which is due to the higher porosity of SF/SA and the larger surface area in contact with the solution in 60:40 medium, which is more conducive to the dissolution of curcumin in contact with the solution [20]. These results indicate that curcumin has a gradually release effect in different media and could be used as an antibacterial agent in wound dressings.

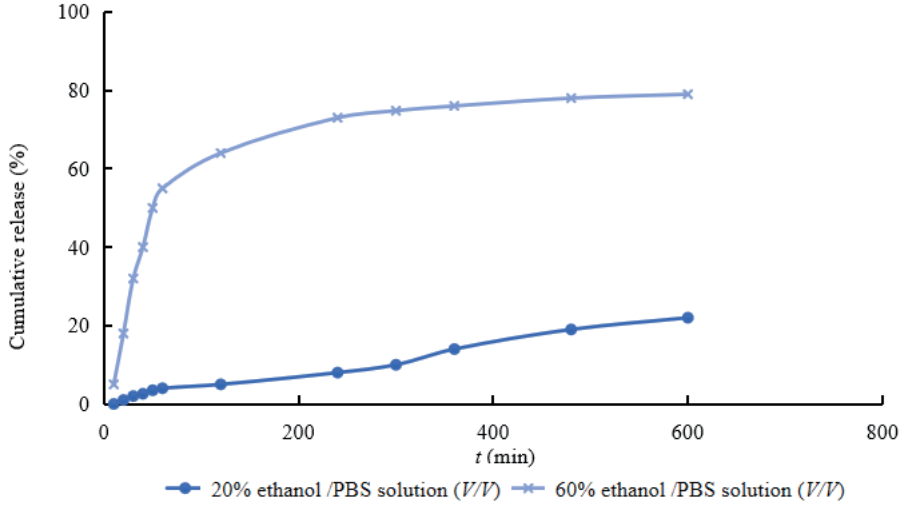

Fig. 7 Release curves of curcumin/SF/SA film agents in different media

4 Conclusion

In summary, based on the TS and E, the optimum preparation technology of the film was obtained by orthogonal test. The dosage of SF was 2.7 g, sodium alginate was 0.84 g, the content of span 40 was 0.4 g, and the content of glycerol was 3 mL. Under the proportion again, the tensile strength was (0.628 ± 0.032) MPa, and the elongation at break was (0.794 ± 0.046) %. At this point, curcumin was uniformly dispersed in the film forming solution. The quantitative curve of curcumin was obtained by spectrophotometry. *In vitro* release of curcumin, 79.8% of curcumin was released within 10 h, showing a good sustained release effect. Therefore, the film agent of curcumin/SF/SA prepared in this study has a predominant application potential in the field of medical wound dressing.

Acknowledgements

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