Preparation of orally disintegrated membranes from liquorice inclusion complexes

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Abstract: Objective: to prepare and evaluate orally disintegrating films of liquorice inclusion complexes. Methods: the active components of Chinese liquorice were extracted by pressurised extraction technique, and the inclusion complexes were prepared by antisolvent method, while the buccal disintegration films of liquorice inclusion complexes were prepared by solvent casting method, and the prepared buccal disintegration films were evaluated. Results: the home-made liquorice inclusion complexes orally disintegrated films formed better films, had good hardness and toughness, the thickness was around 0.1 mm and could disintegrate within 40 s, and the dissolution was rapid, the cumulative release reached 80.1% within 10 min, reaching the immediate release effect. Conclusion: the prepared buccal disintegrating membranes of liquorice inclusion complexes, which are expected to provide new ideas for the development and study of other drugs in the future, deserve further investigation.

1. INTRODUCTION

Licorice (Glycyrrhiza uralensis Fisch) is a common Chinese herbal medicine alias guoold, sweet grass, Ural Licorice, sweet root, genus legume, which has various medicinal effects, such as hepatoprotective, anticancer, antioxidant, anti-inflammatory, antiviral, immunomodulatory, antitussive and so on. Liquorice extracts mainly include liquorice saponins (also known as glycyrrhizic acid, also known as glycyrrhizin due to the sweet taste) and liquorice flavonoids, etc. [1]. However, its poor stability and low bioavailability as a terpenoid largely limit its clinical use [2]. While zein, as a natural amphiphilic polymeric material, prepared as inclusion complexes can improve the entrapment, loading and bioavailability of bioactive materials. So this test used liquorice extract with zein to make inclusion complexes, then added suitable hydrophilic polymers, plasticizers and so on to make inclusion complexes oral disintegration films, which may provide a new route for clinical application of liquorice.

2. EXPERIMENTAL METHODS

2.1 Pressure extraction process of licorice

2.1.1Analytical methods

The determination of saponins in Glycyrrhiza uralensis Fisch was carried out using ultraviolet/visible spectrophotometry, using standard glycyrrhizic acid. The method in Reference [3] draws a standard curve and determines the content of saponins. Using the least square method for linear regression, the standard curve of total glycyrrhizic saponins was obtained as y=2.154x+0.0866, (R2=0.9989). The linear relationship of glycyrrhizic acid control sample was good within the range of 0.1 mg · mL-1-0.3 mg · mL-1.

2.1.2Extraction method

Weigh 10g of licorice and crush it, place it in a pressurized extraction tank, add deionized water, seal the pressurized extraction tank, and perform pressurized extraction under conditions of pressure 0.1MPa, extraction temperature 130 ℃, extraction time 40min, and material liquid ratio 1:15 to obtain a pressurized extract of licorice. The extraction rate of total glycyrrhizic acid saponins is 4.29%, which meets the requirement that glycyrrhizic acid should not be less than 2.0% in the pharmacopoeia.

2.2 Preparation and Characterization of Inclusion Complexes

2.2.1Preparation method of inclusion complex [4]

Dissolve zein in 80% ethanol to obtain an ethanol solution of zein. Drop it into the licorice extract, stirring while dripping at a stirring rate of 1500 r/min. After mixing, continue stirring for 10 minutes, then rotate and evaporate

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to remove the ethanol, and bring to volume with distilled water to obtain the licorice inclusion complex solution.

2.2.2Determination of encapsulation efficiency

Accurately pipette 0.1 mL of the prepared 5 mL glycyrrhiza inclusion complex through 0.45 μ After filtering with a microporous membrane, measure the absorbance A at the wavelength of 589 nm according to the method in 2.1.1 Reference [3], and substitute it into the standard curve to calculate the dosage. After calculation, the encapsulation efficiency can be 57.76%.

The formula for calculating the encapsulation ratio is as follows:

Encapsulation rate (EE)%=(m_{total}- m_{free})/m_{total} × 100%

m_{total} is the total saponin content in the licorice extract

m_{free} is the free drug content in the glycyrrhiza uralensis inclusion complex

2.2.3Characterization of Glycyrrhiza Inclusion Complex

2.2.3.1 Particle size binding of glycyrrhiza uralensis inclusion complex observed by optical microscopy

Use a rubber tipped dropper to absorb an appropriate amount of licorice inclusion compound onto a slide, lay it flat and cover it with a cover glass, observe and take photos under an optical microscope to record the surface morphology of the inclusion compound. The results are shown in Figure 1.

Figure 1: Microscopic observation of licorice inclusion complex

2.2.3.2 Scanning electron microscope analysis

An appropriate amount of glycyrrhiza uralensis inclusion compound was taken for ion sputtering gold plating, and its surface morphology was observed and photographed under scanning electron microscopy. The results are shown in Figure 2. It can be seen that the glycyrrhiza inclusion complex is spherical in shape, and some drugs are wrapped with bumps by excipients. The glycyrrhiza inclusion complex has already formed.

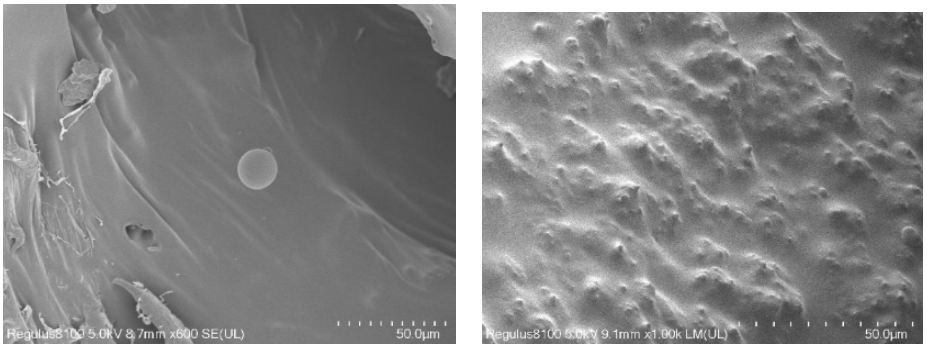

Figure 2: Scanning electron micrograph of licorice inclusion complex

2.3 Preparation of oral disintegrating film of glycyrrhiza uralensis inclusion complex

2.3.1Preparation method of oral disintegrating film

The solvent casting method was used to prepare oral disintegrating films. Add 2.4g of HPMC into 50mL of water in a stirred state, fully stir and dissolve in a water bath at 80 ℃, then add 1.2g of PEG400 and 0.1g of ALG-Na and stir evenly. Add the active ingredients of the drug and stir evenly to obtain a drug-containing solution, degass, evenly coat the solution on a stainless steel plate, heat and dry at 40~60 ℃, and cut into 2cm × 2cm, remove the film to obtain the oral disintegrating film.

2.3.2Prescription screening

2.3.2.1Screening of film forming materials

The film forming materials HPMC, PVA, and HPC were screened, and their appearance, film forming thickness, and disintegration time were compared.

2.3.2.2 Selection of plasticizers

For oral disintegrating films, adding suitable plasticizers can increase the toughness of the film, prevent membrane damage during film removal, and facilitate the use and storage of the film. Three commonly used plasticizers were selected: propylene glycol, glycerol, and PEG400.

2.3.2.3 Screening of disintegrants

The main role of disintegrants is to help the drug quickly disintegrate and disperse in the oral cavity, allowing the drug to quickly and fully exert its effects. Two commonly used disintegrants were selected: CMS-Na and ALG-Na.

2.3.2.4 Optimization of the formulation of licorice oral disintegrating film by orthogonal experimental design

According to the results of single factor investigation, the dosage of HPMC, PEG400, and ALG-Na were selected for a three factor and three level L_9 (3^4) orthogonal test design. The factor level is shown in Table 4, and the orthogonal test arrangement is shown in Table 5.

2.4 Preliminary evaluation methods for film agents

2.4.1Appearance

Observe the appearance, color, transparency, bubbles, integrity, and film formation of the film agent [5].

2.4.2Thickness

After cutting to 2.0cm × Three randomly selected points on a 2.0cm sized licorice oral disintegrating film were measured with an electronic digital micrometer and the average value was calculated.

2.4.3Disintegration time

The disintegration performance and solubility of the membrane were investigated by measuring the disintegration time of the membrane agent. Measure 10 mL of 37 ℃ distilled water into a watch glass, place the liquorice oral disintegrating film to be tested in water and start timing. Use a stopwatch to record the disintegrating time of the film agent, repeat the measurement three times, and calculate the average value [6].

2.4.4Mechanical property measurement

Take 2cm × For the finished film agent with a size of 2cm, use a jig to clamp both ends of the film agent for 0.5cm respectively, and make the tensile force straighten at a constant speed in a vertical state until the film agent breaks. The peak value of the tensile force reached during the recording process is the maximum load. Measure the distance between the two clamps when the film agent breaks, and calculate the elongation at break of the film agent according to the following formula.

Elongation at break=breaking length - original length/original length × 100%

2.4.5Content measurement and content uniformity measurement [7]

Cut the finished film into 2cm × A total of 5 tablets with a size of 2cm were placed in a 10ml volumetric flask and dissolved with pure water ultrasound. The content was determined by ultraviolet method after taking an appropriate amount. The results show that 2 cm × A 2cm oral instant film is dissolved in 10ml of pure water at a concentration of (25.14 ± 0.67) μ g/ml (n=5), i.e., 2cm per tablet × The 2 cm finished film contains about 5.03 mg, which meets the pharmaceutical dose requirements of the drug. In addition, the measurement results show that the RSD is 0.27% (n=5), indicating good content uniformity.

2.5 Study on dissolution in vitro

Using 20mL of pH 6.8 PBS buffer solution as the release medium, magnetically stir at a constant temperature of (37 ± 0.5) ℃ at 2cm × Start timing when the 2cm liquorice oral disintegrating membrane contacts the medium, and suck 3mL of release solution at fixed positions for 2, 4, 6, 8, 10, 15, 30, 45, 60, and 90 minutes, while replenishing 3mL of medium with the same temperature and volume. UV test the absorbance value A of each sample, and draw the dissolution curve as shown in Figure 4.

3. RESULT

3.1 Optimization of prescription of oral disintegrating film by single factor test

3.1.1Screening of film forming materials

Three film forming materials HPMC, PVA, and HPC were selected, as shown in Table 1. HPMC has good film forming property, smooth and transparent appearance, moderate thickness, and the shortest disintegration time. Therefore, HPMC is ultimately selected as the film forming material.

Table 1 Screening of film forming materials（n=3, $\bar{x}$±s)

Film forming materials	Appearance	Thickness（μm）	Disintegration time（s）
HPMC	The surface of the film agents was smooth and transparent, and the toughness strength was relatively good, which facilitated the film removal.	100	29
PVA	The surface of film agents is smooth and white, toughness strength is general, it is difficult to film.	130	32
	Film agents have smooth, translucent	132	36

HPC	surface, toughness strength in general, harder to film.

3.1.2 Selection of plasticizers

Three commonly used plasticizers: propylene glycol, glycerol, and PEG400 were screened, and the results are shown in Table 2. When PEG400 is the plasticizer, its disintegration time is the shortest and has the smallest impact on the original properties of the film agent. Finally, PEG400 is selected as the plasticizer.

Table 2 Selection of plasticizers（n=3, $\bar{x}\pm s$)

plasticizer	Appearance	Thickness（μm）	Disintegration time（s）
Propylene glycol	The film agents have smooth surfaces, which are not very transparent, brittle, and difficult to film.	131	30
glycerol	The surface of the film agent is smooth and transparent, and it is tough, making it convenient to film removal.	101	29
PEG400	The surface of the film agent is smooth and transparent, and it is tough, making it convenient to film removal.	100	26

3.1.3 Screening of disintegrants

The commonly used disintegrants include sodium alginate (ALG-Na), sodium carboxymethyl starch (CMS-Na), and cross-linked sodium carboxymethyl cellulose (CCNa). Two commonly used disintegrants were selected: CMS-Na and ALG-Na.The filtering results are shown in Table 3. It is known that when ALG-Na is a disintegrant, the resulting film has a shorter disintegration time. Therefore, ALG-Na was selected as the disintegrant.

Table 3 Screening of disintegrants（n=2, $\bar{x}\pm s$)

Disintegrants	Appearance	Thickness（μm）	Disintegration time（s）
ALG-Na	The surface of film agents is smooth, transparent, ductile, and convenient for film removal.	114	24
CMS-Na	The film agents exhibit smooth, translucent surface, toughness in general, and convenient for film removal.	125	27

3.2 Optimization of the formulation of liquorice oral disintegrating film by orthogonal test

When placed on the tongue, the oral disintegrating membrane immediately liquefies when saliva is encountered, rapidly disintegrates and/or dissolves, releasing active substances[8]. The thickness and disintegration time of the oral disintegrating film are required, and the pH range of the normal human oral cavity is 6.6 to 7.1 [9]. Due to the consideration of drug compliance, the pH of the film is more easily accepted by the human oral cavity. According to the pre test results, the dosage of HPMC (A), PEG400 (B), CMS-Na, and ALG-Na (C) were prepared using a three factor and three level orthogonal experiment. The optimal formulation was selected by comprehensively considering the three indicators of film thickness, disintegration time, and surface pH value. The factor levels are shown in the table, and the orthogonal test arrangement and results are shown in Tables 4 and 5.

Table 4 Orthogonal test factor level table of licorice oral disintegrating film

Level	Factor A(g)	Factor B(g)	Factor C(g)
1	1.9	0.7	0.1
2	2.4	1.2	0.15
3	2.9	1.7	0.2

Table 5 L_9 (3^4) orthogonal test results of licorice oral disintegrating film

Number	Plan				Result		
	A	B	C	Error terms	Thickness/μm	Disintegration time /s	Surface pH
1	1	1	1	1	0.20	36	7.7
2	1	2	2	2	0.17	38	7.5
3	1	3	3	3	0.19	39	7.2
4	2	1	2	3	0.16	36	6.7
5	2	2	3	1	0.15	35	6.6
6	2	3	1	2	0.15	36	6.7
7	3	1	3	2	0.18	38	7.2
8	3	2	1	3	0.16	37	7.4
9	3	3	2	1	0.18	39	7.1

3.3 Prescription Validation

Based on the above formulation optimization results, the optimal formulation of licorice oral disintegrating film was determined as follows: HPMC 2.4g, PEG400 1.2g, ALG-Na 0.15g. Prepare 3 batches of test sample films with the optimal formulation and verify the formulation. The results showed that the film prepared by this prescription had a smooth and smooth surface, uniform texture, and excellent mechanical properties. The disintegration times of three batches were 37, 36, and 38 seconds, respectively, with a good in vitro dissolution effect.

3.4 Quality evaluation of oral disintegrating film

3.4.1Appearance evaluation

The resulting finished film has a complete and smooth appearance, is uniform without bubbles, has a consistent thickness, is easy to peel off, and has good expected compliance. As shown in Figure 3.

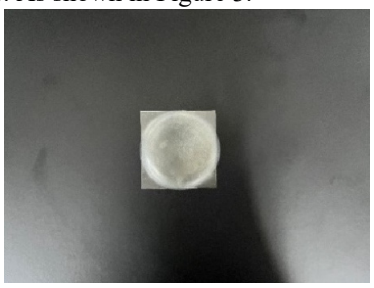

Fig.3 The appearance of the film

3.4.2Film performance inspection

The properties of three batches of film agent samples were investigated, and the results are shown in Table 6. The thickness of licorice oral disintegrating film is about 0.10 mm, the disintegrating time is within 40 seconds, and the average elongation at break is 9.11% (n=3), indicating good mechanical properties.

Table6 The properties of the film(n=3, $\bar{x} \pm s$)

Detection items	lot number		
	20221203	20221204	20221205
Thickness（mm）	0.16	0.15	0.15
Disintegration time（s）	37	36	38
Extensibility（%）	9.27	8.85	9.22

3.4.3Content measurement and content uniformity measurement

Cut the prepared licorice oral disintegrating film to a size of 2cm ×2cm square small membrane is made up of 3 pieces, and its mass is accurately weighed. Place the square small membrane into a 10mL volumetric flask and dissolve it with pure water and ultrasound to volume. Take an appropriate amount and determine the content by ultraviolet spectrophotometry. The results show that 2 cm ×2cm oral disintegrating film was placed in 10mL of pure water at a concentration of (21.45 ± 0.31) μ g/ml (n=3), that is, 2cm per piece × The content of the 2cm finished film is about 4.29mg, which meets the pharmaceutical dose requirements of the drug. In addition, the measurement results show that the RSD is 0.25% (n=3), indicating good content uniformity.

3.5 Study on dissolution in vitro

The dissolution curve of the film sample measured is shown in Figure 4. From the results, it can be seen that the release rate of the film agent is faster, and the cumulative release rate reaches 80.1% within 10 minutes. After 60 minutes, all the film agents are dissolved.

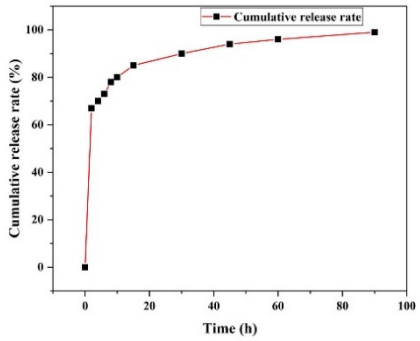

Fig4: In vitro dissolution curve of licorice oral disintegrating membrane

3.6 SEM observation

The blank film and the dosing film were respectively sprayed with gold by ion sputtering, and their surface morphology was observed under scanning electron microscopy and photographed. The results are shown in Figure 5. From the electron microscope photos, it can be seen that the surface of the blank film and the liquorice disintegrating film is smooth, uniform in texture, and free of cracks.

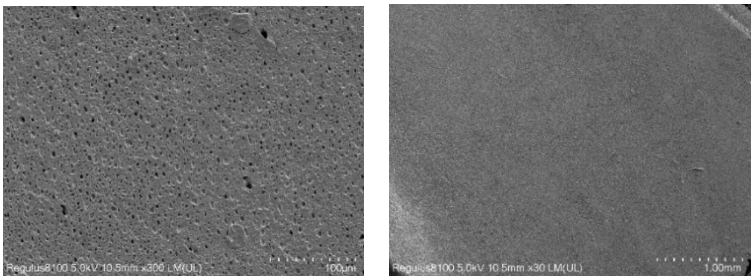

Fig5: Blank membrane; Dosing film

4. DISCUSSION

In recent years, new delivery systems specifically prepared using modern new technologies for patients with dysphagia and poor compliance, such as infants and the elderly, have emerged, and oral disintegrating membranes have shown significant market value [10].

Inclusion complexes, as a unique form of complex, can increase the dissolution of insoluble drugs, increase drug stability, and improve drug bioavailability. Some studies have shown that loading active substances with zein can effectively improve its stability and slow down the release rate of drugs [11]. The study found that liquorice and its extract have a variety of pharmacological effects. In particular, in 2020, novel coronavirus pneumonia (COVID-19) swept the world. Traditional Chinese medicine participated in the treatment of COVID-19 in the whole process. Glycyrrhiza has the highest frequency of use in all immune regulation antiviral traditional Chinese medicine and TCM clinical prescriptions.

5. CONCLUSION

Therefore, in this study, pressure extraction technology was used to extract the effective components of licorice, and anti solvent method was used to prepare licorice inclusion complexes, and solvent casting method was used to prepare licorice oral disintegrating films. The developed licorice oral disintegrating film has a smooth and smooth appearance, rapid disintegration time, good mechanical properties, stable quality, and rapid dissolution, which can provide some ideas for the research and development of new dosage forms of traditional Chinese medicine licorice, and also provide a certain reference for the preparation and development of licorice oral disintegrating film.

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