BAOJ Pharmaceutical Sciences

Yongheng Zhao, et al., BAOJ Pharm Sci 2019, 4: 1.
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Research

Preparation Technology of Nano-micelles of Alantoactone with Poloxamer F-127

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Abstract

Purpose

To prepare alantolactone /Poloxamer F-127 copolymer micelles

Method

The emulsion-solvent evaporation method was used to prepare alantolactone/Poloxamer F-127 copolymer micelles. Waters C_{18} chromatographic column was used to measure the alantolactone content in the copolymer micelles, comprising of water and acetonitrile as the mobile phases, at a column temperature of 25^0 C and test wavelength of 220 nm. A single-factor test was conducted to examine the amount of water, pH, feed ratio, hydration temperature and time. Orthogonal test was done to determine the optimal process of preparing alantolactone nanoparticles.

Results

The result showed that the encapsulation efficiency of alantolactone was the highest when the feed ratio was 1:15, the pH value was 5-6 and the hydration temperature was 40^0 C.

Keywords: Alantolactone; Nanoparticle Preparation; Poloxamer F-127

Introduction

Alantolactone was found in many medicinal plants of the composite family, such as Inula racemosa, L. Inula helenium, Aucklandia lappa, Inula japonica and Radix inulae [1]. Various pharmacological activities of Alantolactone have been reported, including against glioblastoma cells [2], triple-negative breast cancer MDA-MB-231 cells [3] and lung squamous cancer SK-MES-1 cells [4]. However, Alantolactone is a poorly water-soluble drug, which affects its dissolution rate and bioavailability.

Nano-particulates were primarily developed to overcome challenges related to poor solubility of drugs. Pluronic F-127 is an FDA-approved biocompatible and thermo reversible block copolymer, which widely use in the medical field as vehicle for delivery of drugs through the rectal, ophthalmic, and nasal mucosa because of its low toxicity, as while as controlling release of drugs [5]. Thus, in order to enhancing the dissolution of alantolactone and improving its bioavailability, accordingly, nanosization of alantolactone was studied with Pluronic F-127.

Materials and Methods

Reference substances for measuring the alantolactone content (Shanghai Baoman Biotechnology Co., Ltd., 546-43-0); Poloxamer F-127 (Shanghai Baoman Biotechnology Co., Ltd., 9003-11-6); Reversed phase C_{18} column; column temperature: 25^0 C; test wavelength: 220 nm; flow rate: 0.4 mL/min; sample size: 2μL; acetonitrile/water system

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Sub Date: March 13th, 2019, **Acc Date:** March 22nd, 2019, **Pub Date:** March 22nd, 2019

Citation: Yongheng Zhao, Jing Chen (2019) Preparation Technology of Nano-micelles of Alantoactone with Poloxamer F-127 BAOJ Pharm Sci 4: 51.

elution gradient determined according to previous literature [6] in the manner shown in Table 1. Acetonitrile and methanol (Darmstadt, Germany), all other chemicals used were of analytical grade, deionized water was obtained from a Milli Q Plus purification system (Millipore, Bedford, MA, USA).

Table 1: Gradient elution

Time/min	A (acetonitrile)	B (water)
0	53	47
4	53	47
5	100	0
7	53	47

Preparation of Stock

Precisely 3.0 mg of the alantolactone was weighed and mixed with 6 ml of methanol. The mixture was dissolved ultrasonically and shaken to yield a 0.5 mg/mL solution, which was then kept in a refrigerator (4^0 C)for further use.

Preparing Standard Curves

Precisely 1.2 mg of the standard alantolactone was weighed and placed in a volumetric flask. And then dissolved with 10 mL methanol as the standard solution. Next, 1 mL of the solution was placed in a 10 mL volumetric flask. It was then diluted with methanol to the graduation level and used as the reference solution (0.012 mg/mL). Precisely 2 μL, 4 μL, 6 μL, 8 μL, and 10 μL of the reference solution were injected respectively into the chromatograph for measurement. The sample size X (μg) was linearly regressed against peak area Y, thus yielding the regression equation for alantolactone as Y = 143737X-126000. The peak area became linear to the sample size within the 0.012–0.03 mg/mL interval.

Preparation of Alantolactone Copolymer Micelles

In producing drug-carrying polymer micelles, the drug can be entrapped through physical or chemical bonding or static processes [7]. Chemical bonding is not readily applicable as it requires active groups that are reactive. Therefore, physical approaches including direction dissolution, dialysis, emulsion-solvent evaporation, film-solvent evaporation, cosolvent-solvent evaporation, and self-assembling solvent evaporation, are more frequently used. After due consideration, Poloxamer F-127 was used since it is one of the new synthetic excipients-a macromolecular, nonionic surfactant that is soluble in water and many organic solvents.

Alantolactone/Poloxamer F-127 copolymer micelles were prepared using film-solvent evaporation process. A moderate amount of alantolactone and Poloxamer F-127 were weighed and placed in a 50 mL round-bottomed flask and mixed with of ethanol. The mixture was then dissolved ultrasonically for 5 min and fully reacted for 1 h using a water bath at 40^0 C. The organic solvent in the mixture was fully removed using a rotary evaporator for 20 min in a 40^0 C water bath, before the mixture was placed in a drying oven to evaporate the remaining solvent. The resultant drug/polymer gel was dissolved in ultrapure water and then hydrated for 40 min in a water bath at 40^0 C, to yield alantolactone micelles aqueous solution. Finally, the alantolactone micelles were got by freeze-drying method.

Measuring the Encapsulation Efficiency

Based on previous studies [8,9], the load capacity and encapsulation efficiency of the drug were measured using ultrahigh pressure liquid chromatography (LC-20AT, Shimadzu-GL). A moderate amount of the lyophilized powder was first dissolved into ultrapure water to yield alantolactone micelles, which were fully hydrated in 40^0 C water bath and centrifuged at 3,600 rpm for 15 min. The supernatant content was used to yield the nanoparticles. The supernatant content was dried with a nitrogen blowing instrument before it was dissolved, by adding 1 mL methanol and then filtered through a Millipore film. The encapsulated amount of drug was measured with HPLC. The encapsulation efficiency and drug loading capacity were calculated according to the formula below [10]: Drug encapsulation efficiency (%) = Mass of the elecaminolactone in the micelles/Total mass of the elecaminolactone micelles/feed ratio; Drug loading capacity (%) = Mass of the elecaminolactone in the micelles/Total mass of the elecaminolactone micelles.

Results and Discussion

Precision Test

0.5 mg/mL of the reference alantolactone solution were injected at 2 μL six times, to measure the peak area and calculate the alantolactone content. The relative standard deviation (RSD) was 1.07% (n = 6), suggesting that the instrument provided a high level of precision.

Stability Test

The reference solution (2 μL) at 0, 2, 4, 6, and 8 h to measure the peak area and calculate the alantolactone content. The RSD was 1.18% (n = 5), suggesting that the test solution had a high level of stability within 8 h.

Repeatability Test

Six fractions of the reference solution were individually injected at 2 μL to measure the peak area and calculate the alantolactone content.

The RSD was 2.11% (n = 6), suggesting that the sample preparation method had a high level of repeatability.

Single-factor Examination

As the pre-test had revealed, several influencing factors for the test such as the effects of the selected solvent, water pH, feed ratio, hydration time, and hydration temperature were examined through single-factor test, as shown in Table 2. As can be seen from the result of the single-factor test, the solvent, water pH, feed ratio, and hydration time selected for the test had a great impact on the drug encapsulation efficiency.

Table 2: Single-factor examination

Test ID	Solvent	pH	Feed Ratio	Hydration Time	Temp.	Encapsulation Efficiency
1	Ethanol	5-6	1:15	1	40	82
	Ethanol	5-6	1:15	1	50	81.4
	Ethanol	5-6	1:15	1	60	70.4
	Ethanol	5-6	1:15	1	70	67.34
2	Ethanol	5-6	1:15	1	40	80.84
	Acetone	5-6	1:15	1	40	50.05
	Acetone+Ethanol	5-6	1:15	1	40	69.51
3	Ethanol	2	1:14	1	40	75.83
	Ethanol	3	1:14	1	40	77.44
	Ethanol	5-6	1:14	1	40	85.29
	Ethanol	7	1:14	1	40	63.68
	Ethanol	8-9	1:14	1	40	63.84
4	Ethanol	5-6	1:10	1	40	64.33
	Ethanol	5 _6	1:14	1	40	65.29
	Ethanol	5-6	1:15	1	40	81.96
	Ethanol	5-6	1:17	1	40	72.68
	Ethanol	5-6	1:20	1	40	64.43
	Ethanol	5-6	1：30	1	40	68.67
	Ethanol	5-6	1：50	1	40	74.69
5	Ethanol	5-6	1：15	0.5	40	76.4
	Ethanol	5-6	1：15	0.75	40	79.8
	Ethanol	5-6	1：15	1	40	81.96

Optimizing the Preparation Process through orthogonal Test

After single-factor examination, an orthogonal test was conducted on how the solvent (A), pH (B), and hydration temperature (C) selected for the test, affected the encapsulation efficiency. The optimal levels of these factors were selected against the alantolactone content as the indicator.

$F_{0.05}(2, 6) = 5.14$

According to R, the effects of these factors on the yield are prioritized as A > B > C. Variance analysis showed that among the three factors, A and B were significant whereas C wasn't. Orthogonal test results showed optimal conditions for alantolactone nanoparticle preparation is A1B1C1, that means the feed ratio of alantolactone and Poloxamer F-127 is 1:15, pH is 5–6 and the hydration temperature is 40^0 C.

Table 3: Factors for orthogonal test

Factor			
Level	**Feed Ratio (A)**	**pH (B)**	**Hydration Temp. (C)**
1	1:15	5–6	40
2	1:5	2–3	50
3	1:30	8–9	70

Table 4 Results of orthogonal test

SN	A	B	C	D	Encapsulation Efficiency (%)
1	1	1	1	1	80.58
2	1	2	2	2	75.05
3	1	3	3	3	68.68
4	2	1	2	3	63.89
5	2	2	3	1	56.37
6	2	3	1	2	43.84
7	3	1	3	2	48.36
8	3	2	1	3	50.65
9	3	3	2	1	51.18
∑K1	224.31	192.83	175.07	188.13	
∑K2	164.1	182.04	190.12	47.25	
∑K3	150.19	163.7	173.41	63.22	
K1	74.77	64.28	58.36	62.71	
K2	54.7	60.68	63.37	55.75	
K3	50.06	54.57	57.80	61.07	
R	24.71	9.71	5.57	6.96	

Table 5: Variance Analysis of orthogonal test

Factor	DevSq	Freedom Degree	F Ratio	Significance
A	1034.723	2	18.314	$F>F_{0.05}(2,6)$
B	144.643	2	2.560	$F>F_{0.05}(2,6)$
C	56.498	2	1.000	$F<F_{0.05}(2,6)$
Error	56.50	2		

Conclusion

Finally, three fractions of the standard alantolactone (2 mg) were prepared into alantolactone nanoparticles under optimal processing conditions A1B1C1. The alantolactone encapsulation efficiencies were 79.8%, 81.96%, and 80.85%, respectively with an average of 80.87%. These are much higher than the presently reported encapsulation efficiencies, suggesting that our optimized preparation process is useful and feasible.

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