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Hot melt emulsification shear method for solid lipid-based suspension: from laboratory-scale to pilot-scale production

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Abstract

The clinical application of solid lipid particles (SLPs) is hampered due to the need for advanced nano/micro-suspension production technology. This research aims to establish a pilot-scale production line employing high-speed shears as emulsification equipment. The primary purpose is to manufacture nano/micro-suspensions using solid lipid particles (SLPs). The study also exhaustively introduces and analyzes the regulatory schemes for process parameters and formulations at various stages of production. The process and formulation endured optimization through orthogonal or single-factor tests at various production steps: laboratory research, small-scale trial production, and pilot production. Quality standards for the product were determined, and key parameters were obtained at each stage. The laboratory research demonstrated that the optimal SLPs comprised 15 mL 3% polyvinyl alcohol (PVA) per 1.0 g tilmicosin and 2.5 g carnauba wax (WAX). During small-scale production, modifications were made to the volume of the aqueous phase, emulsifier concentration, and emulsification strength, setting them to 16 mL, 5%, and 2200 r/min, respectively. In the pilot production stage, the shear time was considered optimal at eight min. The impurity, content, polydispersion coefficient (PDI), and size of the pilot product were < 3%, 5%, 0.385 and 2.64 μm, respectively. Among the several parameters studied, heating temperature, drug-lipid ratio, and emulsifier concentration were identified as the main factors affecting product quality, and they were regulated at 100℃, 1:3, and 5%, respectively. A novel hot melt emulsification shear method aided the development of a new solid lipid-based suspension from its preliminary stages in the laboratory to pilot production. This innovation is expected to enhance solid lipid-based suspensions' industrial evolution extensively.

Keywords Laboratory research, Small-scale production, Pilot production, Quality evaluation, Solid lipid particles

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Introduction

Development of a new formulation typically progresses through four stages: laboratory research, small-scale trial production, pilot production, and industrial production. Various methods are designed to produce small sample quantities in the laboratory research stage. The small-scale trial production stage involves an inclusive and systematic improvement of the original preparation method to adapt it for larger-scale production in the laboratory. Based on these improvements, a suitable production process for pilot production is proposed through data accumulation. Pilot production

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bridges small-scale and full-scale industrial production to establish the necessary equipment and conditions to produce products meeting predetermined quality standards (Shin and Bae 2018). However, Small-scale trial and pilot production differ not only in the amount of feed and the equipment size (Touzet et al. 2018) but also in the completion of different tasks during various stages of product development (Fret et al. 2020). These stages serve different intents in the process of developing the product. Pilot-scale production is a fundamental step from drug research to full-scale production, offering a compelling indication to mitigate the hazards of industrialization. This procedure allows for recognizing variations in the formulation preparation at a particular scale and resolving issues that may have been oversaw or unsolved during laboratory research, providing solid grounds for industrial production. Despite its significance, there are restricted systematic and inclusive reports on the pilot process of new preparations like nanoparticles and microparticle inventions, hindering the industrialization of these novel formulations. Therefore, developing a production route, from laboratory technique screening and extending to pilot production, is vital to guide the successful development of innovative preparations.

Nano/micro-suspensions based on SLPs are acquiring significant devotion as drug delivery systems for lipophilic and hydrophilic drugs. This devotion is attributed to their tremendous biocompatibility and physiological compatibility, assisted by the lipid matrix they exploit. Their adaptability allows for administration through several routes, and the lipid excipients are generally recognized as safe (GRAS), further encouraging their approval in pharmaceutical applications (Severino et al. 2012; Neupane et al. 2013). Previous reports have significantly employed SLPs for numerous applications involving controlled release systems (Reitz and Kleinebudde 2007), protecting water-sensitive drugs from degradation (Windbergs et al. 2009; Schulze and Winter 2009), overcoming the bitter taste of drugs (Krause et al. 2009), and increasing drug absorption. Remarkably, the dermal deposition of optimum tetracycline-loaded solid lipid microparticles exhibited a sevenfold enhancement compared to the control formulations (Rahimpour et al. 2016). Recent studies revealed that the liposomes increased the dissolution of carvedilol in vitro and in vivo (Alskär et al. 2019). In our previous work, solid lipid nanoparticles could significantly increase the absorption and sustained release of praziquantel, tilmicosin, and enrofloxacin by different administration routes (Jiang et al. 2017; Tao et al. 2019). The enteric granules containing enrofloxacin-loaded SLPs significantly improved the palatability and achieved sustained release (Li et al. 2019).

Even though SLPs were widely studied in the laboratory, few reports on the pilot scale product are available. From 1973 to 2015, the FDA received 359 applications for nano-preparations, whereas these applications have gradually increased over the last 20 years. It was worth noting that the proportion of applications for liposome drugs remained consistently stable through the analysis of pharmaceutical declarations from 2010 to 2015. Liposomes account for 70% of the nanomedicine applications submitted to the FDA, indicating that these formulations have been widely recognized as safe and effective. However, no approved solid lipid-based suspension formulation is available in the market. The need for effective pilot production technologies might be one of the main obstacles. Currently, the microemulsion method (Kotmakçı et al. 2017) and the high-pressure homogenization method (Zhou et al. 2018) are two techniques commonly used in laboratory research, among which the microemulsion method was unsuitable for large-scale production because of the long preparation time and insufficient production equipment. The high-pressure homogenizer was mainly used as the laboratory and small-scale trial production equipment for lipid-based suspension formulation (Hu et al. 2016). The existing equipment for producing lipid-based suspension endured low production productivity and a complicated process, making it inappropriate for industrial production. Currently, there are only a few reported studies on this production line. Developing industrial technology is crucial to promote large-scale production of lipid-based suspension. In the early stages, our group introduced the hot melt emulsion and ultrasonic method, which proved highly influential on a laboratory scale. In this study, we aim to innovatively establish a pilot production line utilizing high-speed shears as emulsification equipment.

Tilmicosin has a well-known application in veterinary clinics, particularly for treating bacterial and mycoplasma infections (Arsic et al. 2018). However, its water insolubility, strong bitter taste, and variable bioavailability pose challenges (Li et al. 2017; Chen et al. 2014). In our previous study, tilmicosin-loaded solid lipid nanoparticles significantly improved the absorption and bioavailability of tilmicosin (Zhou et al. 2020). The current study employs this success as a model to establish a pilot-scale production process for solid lipid-based suspension. Our approach involves formulation screening and gradual optimization of process parameters, from laboratory research to pilot production. Throughout this process, we analyzed the effects of equipment conversion, heating temperature, emulsifier concentration, and emulsification strength on the product's particle size, properties, and stability as we transitioned from small-scale to pilot-scale production. This article proposes the pioneering

development of a pilot production line equipped with high-speed shears for fabricating nano/micro-suspensions based on SLPs. The detailed journey from laboratory research to pilot production is a valuable reference for researchers developing other new drugs.

Results

Preliminary determination of formula in laboratory research

During the formulation exploration stage, the primary task was to determine the raw materials and excipients preliminarily. The maximum solubility of tilmicosin in WAX, hydrogenated castor oil (HCO), hydrogenated soybean oil (HSO), stearic acid (SA), and behenic acid (BA) was shown in Fig. 1, where WAX exhibited the best solubility, with an optimal drug-to-wax ratio of 1:2.5. Hence, WAX was selected as the lipid matrix.

The thermal stability of tilmicosin was assessed to prevent drug degradation during preparation (Fig. 2). It was observed that tilmicosin remained stable at 100℃ and 115℃ heating temperatures for 10–30 min. However, at 120℃, the deprivation rate of the drug increased with extended heating time. The degradation proportions of tilmicosin were 3.1%, 15.26% and 27.4% over 10 to 30 min, respectively. Based on the thermal stability test and the drug's solubility in the oil phase, a temperature of 115℃ was chosen for the oil phase.

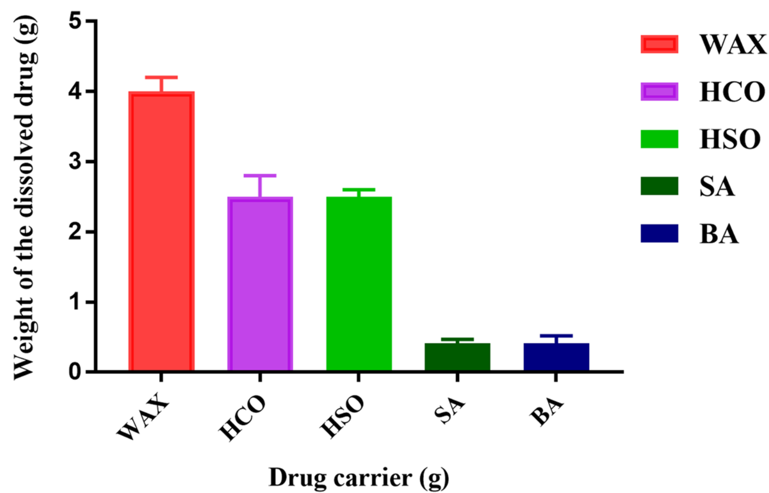

Fig. 1 Solubility of tilmicosin in various carriers. A certain number of tilmicosin was added to each excipient (10.0 g) to measure the maximum solubility. Abbreviations: WAX: carnauba wax; HCO: hydrogenated castor oil; HSO: hydrogenated soybean oil; SA: stearic acid; BA: behenic acid

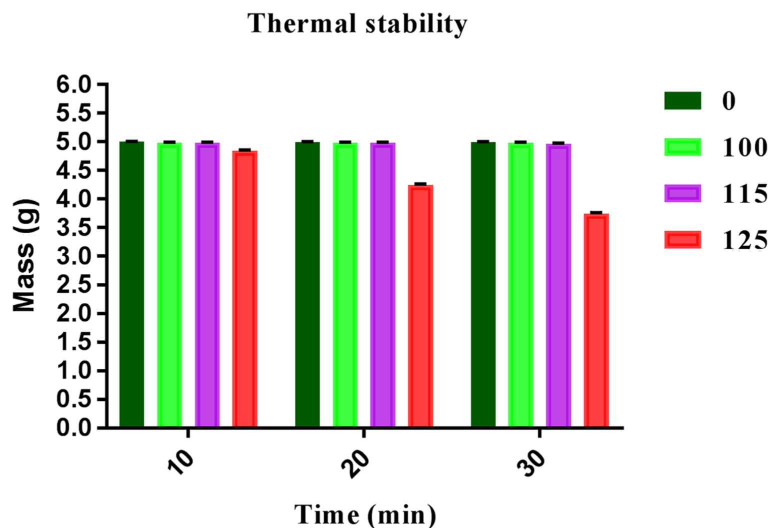

Fig. 2 Stability test of tilmicosin in oil at different heating times. The drug-to-lipid ratio was carried out at a ratio of 1: 2.5

Admiring the orthogonal test results, the size of SLNs fell within the nanosize range when using PVA as the emulsifier. Samples 1, 7 and 8 revealed PDI values of 0.401 ± 0.018 and 0.296 ± 0.028, while the other samples surpassed 0.5. Considering the final content and properties of the product, the LC was identified as the most important factor, followed by the size. The factors influencing the LC and size were in the orders: emulsifier concentration > type > volume. The final optimal aqueous phase is 15 mL 3% PVA per 1.0 g tilmicosin and 2.5 g WAX (Table 1). Combining previous studies, the emulsion process involved sonication using 6 mm microprobes at 95% amplitude (VCX 130 Vibra-CellTM, Sonics & Materials, Inc., Newtown, CT, USA) for 4 min to form a hot O/W emulsion.

Optimization of the formula in small-scale trial production

In the small-scale trial manufacturing, the emulsification equipment was adjusted to replicate the pilot conditions carefully. The output was amplified 50 times (1 L) compared to the laboratory research (20 mL). The primary markers for adjusting the formulation and preparation parameters during the small test were the prepared product's content, characteristics, and size. The preparation process is depicted in Fig. 3a.

Table 1 The optimization of emulsifier by orthogonal experiment (L_9 3^4)

Sample	Type (A)	Concentration (B)	Volume (C)	LC (%)	Size (μm)	PDI
1	2	2	3	14.99 ± 0.55	1.421 ± 0.022	> 0.5
2	3	1	3	16.42 ± 0.51	1.668 ± 0.012	> 0.5
3	2	3	1	18.03 ± 2.31	6.021 ± 0.064	> 0.5
4	1	3	3	19.80 ± 0.65	0.417 ± 0.007	0.401 ± 0.018
5	3	3	2	17.77 ± 0.45	5.449 ± 0.046	> 0.5
6	3	2	1	16.84 ± 2.67	2.385 ± 0.003	> 0.5
7	1	1	1	17.05 ± 0.78	0.490 ± 0.013	0.437 ± 0.026
8	1	2	2	15.57 ± 0.98	0.312 ± 0.006	0.296 ± 0.028
9	2	1	2	16.02 ± 0.53	1.501 ± 0.012	> 0.5
k1	17.47	16.50	17.30			
k2	16.34	15.80	16.45			
k3	17.01	18.53	17.07			
R	1.13	2.73	0.85			
Optimum	A1	B3	C1			

K_1, K_2, and K_3 are the average grades for 3 levels in each factor; R is the different value between the max and mix of K1, K2, and K3 in each level

Abbreviations: LC load capacity, *PDI* poly dispersion index

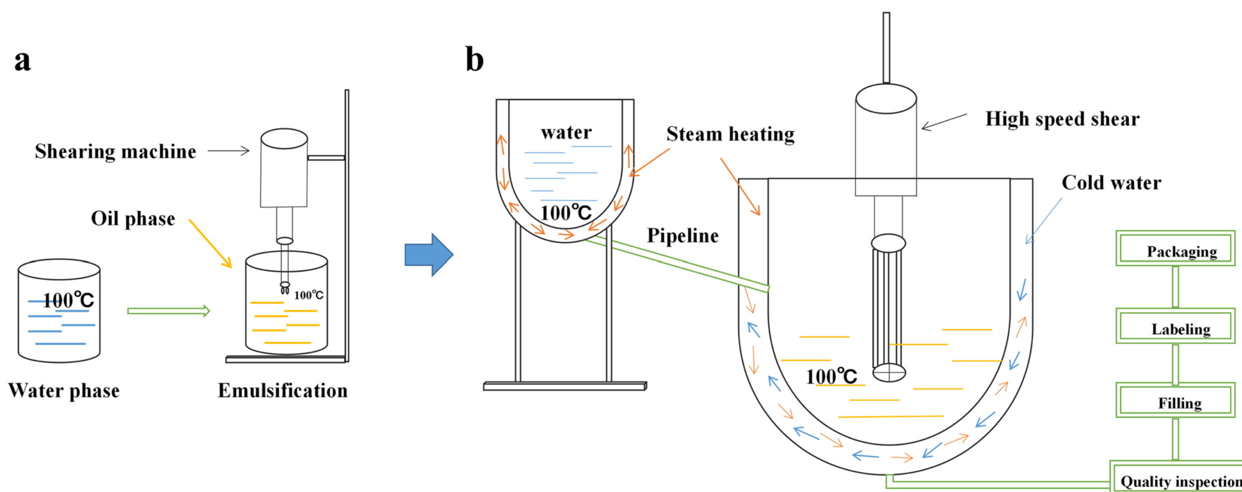

Fig. 3 Schematic view of the (**a**) small-scale trial production and (**b**) pilot production line

With the regulation of the emulsification equipment, the volume of the aqueous phase was amended from 15 mL in the laboratory formulation to 16 mL. After spotting the product's appearance (light yellow milky), a shear velocity of 2200 r/min was specified. Further results show that the shear time did not notably impact the product between 4–8 min. As a result, the shear velocity was set at 2200 r/min for 4 min. During the small-scale trial production, emulsifier concentrations were further adjusted using single-factor tests at 3%, 4% and 5%. Figure 4 illustrates that with an increase in emulsifier concentration from 3 to 5%, the LC consecutively increased to 17.98%, 19.04% and 20.05%, respectively. Simultaneously, the size of the particles was decreased with an increase in the emulsifier concentration. At the concentration of 5%, the D_{50} of the particle was 2.28 μm, and the D95 was below 3.14 μm (Fig. 4). Therefore, the concentration of the emulsifier was considered to be 5%.

In the small-scale amplification study, the temperature of the aqueous phase was determined to be 100℃ (boiling state) due to equipment adjustment (emulsification shear). To avoid possible drug degradation at high temperatures, the temperature of the oil phase is strictly controlled at about 100℃ in small batch production based on the above thermal stability of tilmicosin. As the temperature of the oil phase was decreased, the solubility of the drug in the oil was also reduced. Thus, the drug-to-solid lipid ratio was determined to be 1:3.

Pilot production

Pilot-scale production is decisive in verifying, evaluating, and refining the production process determined during the laboratory stage. It ensures its maturity, rationality, and alignment with full-scale production's required economic and technical indicators. The pilot-scale production setup is relatively simple, containing two main sections: shear emulsification and steam heating (Fig. 3b).

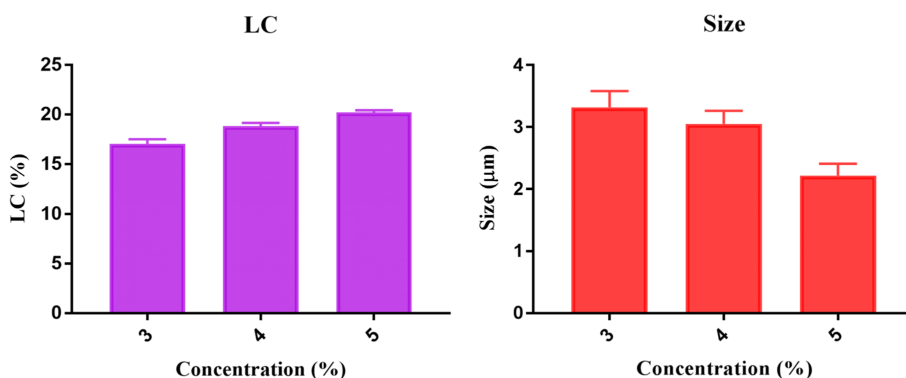

Fig. 4 The LC and size of SLNs. D_{50}: Refers to the average particle size or the corresponding particle size when the cumulative particle size of the sample reaches 50%

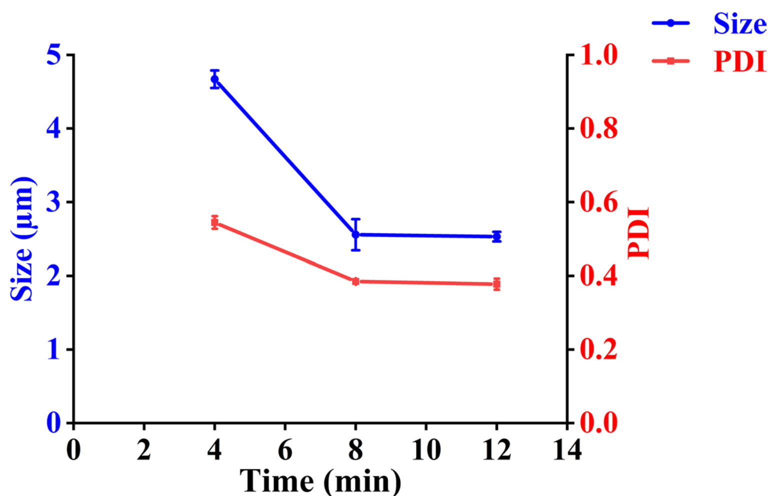

Fig. 5 Determination of size and PDI at different emulsification times in pilot production

During this stage, the emulsification equipment was adjusted to a high-speed shear, considering the increased yield of the pilot-scale production to 400 L. The shear velocity of the high-speed shear was tested at 2200 r/min for 4, 8 and 12 min (Fig. 5). The results indicated that when the emulsification time was 4 min, the particle size (D50) and PDI measured by sampling were 4.67 μm and 0.545, respectively. At 8 min, the particle size and PDI were 2.56 μm and 0.385; at 12 min, they were 2.53 μm and 0.379, respectively. It was observed that little change in particle size and PDI occurred with emulsification times exceeding 8 min. Therefore, the optimal shear velocity and time were determined to be 2200 r/min for 8 min. The temperature conditions of the water and oil phases were maintained as per the small-scale amplification.

Upon the preparation of the semi-product, its content, characteristics, and pH were analyzed, revealing a light yellow emulsion with a pH of 6.67, an average content of 4.96%, and a size of 2.56 μm. The quality of the semi-products met the required standards, enabling further packaging.

Quality evaluation of tilmicosin suspensions at different stages

At the laboratory stage, the particle size of the oral suspension was in the nanoscale (Fig. 6a), averaging 0.732 μm, using phacoemulsification equipment. As we progressed toward industrial production, a shearing machine was employed. At both the small-scale amplification and pilot production stages, the D50 of the particle size was 2.87 μm and 2.64 μm, respectively (Table 2). The size of the small-scale amplification (Fig. 6b) did not substantially differ from that of the pilot product (Fig. 6c).

Throughout the three stages, the appearance of the prepared products was regularly a pale yellow milky suspension. At the laboratory research stage, the average content of the formula was 5.34% due to the lower ratio of water phase to oil (Table 2). In the subsequent stages, the average drug content for the small-scale amplification and pilot production were 5.14% and 4.95%, respectively, as the proportion of the water phase was increased to meet final product requirements.

The sedimentation coefficient of the suspension prepared from the small-scale amplification to the pilot production stage was more significant than 0.9, and the dispersibility of the suspension was excellent.

Related substance determination

Following the ICH guidelines, the detection method for related substances was verified through a series of destructive tests on the formulation involving high temperature, intense light, strong oxidation, strong acid, and strong alkali conditions. The results of these tests demonstrated the suitability of the established HPLC method

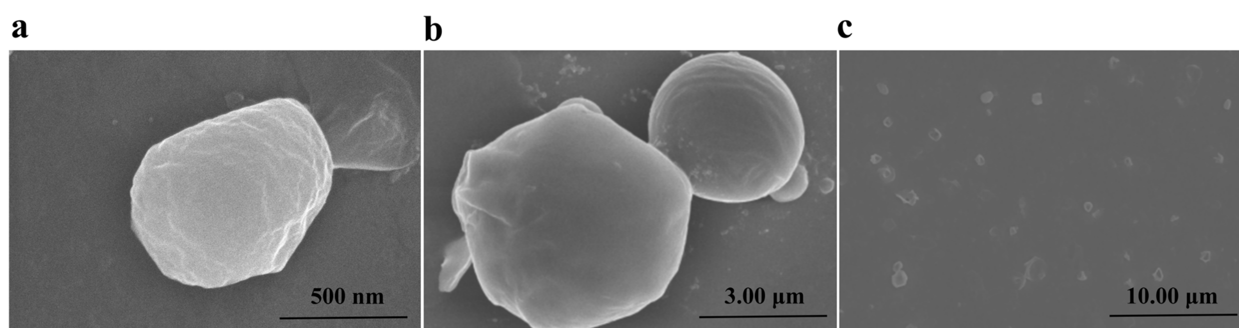

Fig. 6 Scanning electron microscopy photographs of tilmicosin suspension. **a** Electron micrograph of products prepared in the laboratory research stage; **b** Electron micrograph of a small test product; **c** Electron micrograph of pilot product. The product was measured by photon correlation spectroscopy using Zetasizer ZX3600 (Malvern Instruments, UK) at 25℃

Table 2 Properties of tilmicosin suspension at different stages (Mean ± SD, $N = 3$)

Evaluation index	Laboratory research	Small-scale production	Pilot production
Size (μm)	0.73 ± 0.21	2.87 ± 0.71	2.64 ± 0.55
Appearance	Light yellow milky	Light yellow milky	Light yellow milky
Content (%)	5.34 ± 0.12	5.14 ± 0.18	4.95 ± 0.31
Sedimentation coefficient	0.97 ± 0.08	0.95 ± 0.03	0.94 ± 0.05
Dispersibility	Excellent	Excellent	Excellent
Related substance (%)	< 3%	< 3%	< 3%

Dispersibility was excellent, which means the sediment disappeared completely within 2 min

for determining related substances. Under high-temperature conditions (80℃ for 96 h), only 9.6% of tilmicosin in the suspension was degraded, with a recovery rate of 97.55% compared to the reference drug peak. Similarly, when subjected to intense light exposure (8400 lx, 30℃) for 96 h, only 6.54% of tilmicosin was degraded, with a recovery rate of 97.34%. In the presence of high concentrations of hydrogen peroxide (1% and 30%), the degradation rate exceeded 50% within 4 h. However, adding 0.1% hydrogen peroxide degraded the drug by only 5.7% within 4 h, and the recovery rate was 97.05%. Furthermore, when 1 mol/L and 2 mol/L hydrochloric acid were added to the suspension, the drug remained relatively stable over 96 h, showing minimal degradation. However, when the concentration of hydrochloric acid was adjusted to 4 mol/L, the drug was degraded by 11.7% over 96 h, with a recovery rate of 99.1%. In the substantial degradation test using 1 mol/L sodium hydroxide solution, 12% of the drug was degraded within 4 h, and the recovery rate was 96.1% (Table 3, Supplementary Figs. 1–6). Based on the successful establishment of the HPLC method through these vigorous degradation tests, the related substances of suspensions at different stages were measured, and the results indicated that the related substance content remained below 3%, with no new impurities produced in different stages, aligning with the standards set by the veterinary pharmacopeia.

Stability of the suspension

The results of the influence factor test of tilmicosin suspension, including high temperature, high humidity, and strong light, are shown in Table 4. The influencing factors test found that more than 20% tilmicosin in the suspension was degraded on the 5th d under high temperature, high humidity, and strong light conditions without stabilizers. The suspension was adjusted to pH = 7.0 to stabilize the drug by adding 0.55 g of citric acid per 100 mL to the tilmicosin suspension. The commonly used propyl gallate was added to avoid the tilimicosin degradation further.

After adding stabilizer and antioxidant, the suspension prepared at different stages kept the light yellow emulsion liquid under the influencing factor test. Under high-temperature conditions of 60℃, the content of the small test product and the pilot product at 0 d and 10 d were 5.16%, 5.07%, 5.07% and 4.95%, respectively, indicating that the drug in the suspension was minor affected while the content of drug in products are within the range of the indicated amount. Under high humidity and strong light conditions, the content of the product remained stable.

The particle size of the small test product and the pilot product had a slight aggregation under high-temperature conditions. Under high humidity and strong light conditions, the change could be clearer. The sedimentation coefficient, redistribution, and impurity did not change significantly, meeting the 2015 veterinary pharmacopeia requirements.

In vitro release

In vitro release profiles (Fig. 7) of the small test product and the pilot product were observed under simulated gastric juice (SGF, pH = 2.0) and intestinal fluid conditions (SIF, pH = 8.0). The native tilmicosin was almost completely released in SGF and SIF within 30 min, with total release percentages of 97% and 90.12%, respectively. In contrast, the tilmicosin suspension exhibited a slower release in SGF and SIF than the native tilmicosin. In the SGF medium, the sustained release time of the suspension can last for 36 h, while it could reach up to 48 h in the SIF medium. It is worth noting that the small test product released 45.79% of the drug, and the pilot product released 39.04% in SGF within 30 min. The relatively large burst release might be attributed to the encapsulated and absorbable tilmicosin. Similarly, there was a biphasic release with a 33.15% and 34.54% burst release within 30 min for the small test and the pilot suspension in the SIF medium, respectively. The suspensions prepared in both stages released about half of the drug during pigs' average stomach emptying time (4 h), indicating that the tilmicosin suspension has a specific delayed release effect.

Table 3 Strong degradation test of tilmicosin suspension

Factors	Degradation rate (%)	Recovery rate (%)	Degradation condition
High temperature	9.6 ± 0.04	97.55 ± 0.03	80℃, 96 h
Strong light	6.54 ± 0.02	97.34 ± 0.06	8400 lx, 96 h
Strong oxidation	5.7 ± 0.02	97.05 ± 0.04	0.1%, 4 h
Strong acid	11.7 ± 0.02	99.1 ± 0.02	4 mol/L, 60 h
Strong alkali	12 ± 0.03	96.1 ± 0.02	1 mol/L, 4 h

Degradation conditions mean that the drug will have certain degradation and recovery rates under this condition

Table 4 The influence factor test of tilmicosin suspension (Mean ± SD, *n* = 3)

Factors	Evaluation index	No	Stabiliter	Small-test	Product		Polit	Product	
		0 d	5 d	0 d	5 d	10 d	0 d	5 d	10 d
High temperature	Properties	-	-	LYM	LYM	LYM	LYM	LYM	LYM
	Content (%)	5.21 ± 0.14	4.03 ± 0.15	5.16 ± 0.07	5.10 ± 0.15	5.07 ± 0.04	5.06 ± 0.21	5.00 ± 0.14	4.95 ± 0.23
	Size (μm)	-	-	2.01 ± 0.05	2.68 ± 0.06	2.63 ± 0.10	2.75 ± 0.05	2.57 ± 0.07	2.64 ± 0.15
	SC	-	-	1	1	1	1	1	1
	Dispersibility	-	-	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent
	RS (%)	> 3%	> 3%	< 3%	< 3%	< 3%	< 3%	< 3%	< 3%
High humility	Properties	-	-	LYM	LYM	LYM	LYM	LYM	LYM
	Content (%)	5.21 ± 0.14	4.08 ± 0.64	5.16 ± 0.07	5.17 ± 0.1	5.12 ± 0.13	5.06 ± 0.21	5.04 ± 0.17	5.04 ± 0.34
	Size (μm)	-	-	2.03 ± 0.02	1.86 ± 0.11	1.96 ± 0.01	2.91 ± 0.01	3.05 ± 0.13	3.10 ± 0.09
	SC	-	-	1	1	1	1	1	1
	Dispersibility	-	-	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent
	RS (%)	> 3%	> 3%	< 3%	< 3%	< 3%	< 3%	< 3%	< 3%
High light	Properties	-	-	LYM	LYM	LYM	LYM	LYM	LYM
	Content (%)	5.21 ± 0.14	4.14 ± 0.41	5.16 ± 0.07	5.12 ± 0.12	5.13 ± 0.37	5.06 ± 0.21	4.99 ± 0.14	5.00 ± 0.17
	Size (μm)	-	-	2.00 ± 0.03	2.29 ± 0.08	1.96 ± 0.10	2.70 ± 0.03	2.91 ± 0.23	3.04 ± 0.21
	SC	-	-	1	1	1	1	1	1
	Dispersibility	-	-	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent
	RS (%)	> 3%	> 3%	< 3%	< 3%	< 3%	< 3%	< 3%	< 3%

No stabilizer indicates that no stabilizer is added to the product. Three batches of samples were taken at 0 days, and the content was tested only once (in triplicate), so the initial content was consistent. The samples in the table were tested in triplicate. High temperature: The temperature is 60℃

Abbreviations: *LYM* light yellow milky, *SC* sedimentation coefficient, *RS* related substance

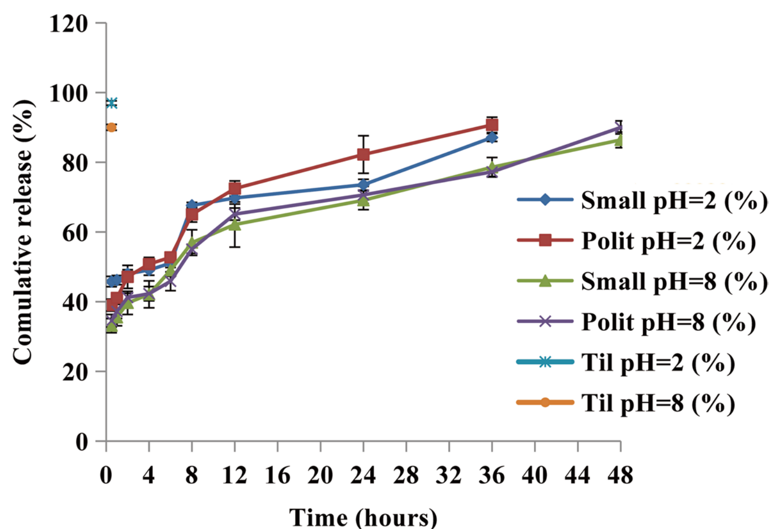

Fig. 7 The accumulative release profiles of native tilmicosin and suspensions in the simulated SGF (pH = 2.0) and SIF (pH = 8.0) (*n* = 3). Small and pilot represent the small test products and pilot scale products, respectively. Abbreviations: Til: tilmicosin

Discussion

The introduction of solid lipid-based suspensions into the market faces barriers due to the need for pilot production. The tilmicosin-loaded solid lipid-based suspension was used as a model for exploring production technologies to progress the industrialization of lipid-based suspensions.

During the laboratory stage, a preliminary formula and technology were established. Lipid screening was based on the drug's maximum solubility in melted

lipids. Among the options (HCO, HSO, SA, BA), WAX exhibited excellent solubility and was chosen as the lipid matrix to achieve higher drug LC to meet clinical requirements. An orthogonal experimental design optimized the emulsifier type, concentration, and volume. The best formulation with 15 mL of 3% PVA was selected based on LC, size, and PDI.

During stress testing, it was encountered that the drug degraded rigorously due to the suspension's high pH (9.0). Previous reports have indicated that Tilmicosin is prone to degradation when exposed to strong basic and oxidizing conditions (Ramesh and Mandal 2019). To mitigate this issue, we incorporated Citric acid as a pH adjuster and included propyl gallate as an antioxidant to boost the stability of the formulation. The suspension's pH was adjusted to 7.0. Citric acid was chosen because it is solid, easily measured industrially, and acts as a complexing agent to prevent pollution with minimal animal irritation. The selection of propyl gallate as the antioxidant considered factors such as price and source convenience of the excipients. Two doses were tested (0.1% and 0.01% as per the pharmaceutical excipient manual (Rowe et al. 2006)), and the formulation with 0.01% propyl gallate displayed no drug degradation. Thus, it was selected for the final formulation.

In the previous study, temperature emerged as a crucial factor governing drug degradation and emulsification. Extensive compatibility tests were conducted on raw and auxiliary materials and high-temperature tests on the raw materials. Notably, the simple excipients used did not cause any degradation of tilmicosin. At a temperature of 100℃, the drug remained stable within a short timeframe. However, beyond 120℃, the drug started to melt and undergo degradation. The solubility of tilmicosin in the oil phase (WAX) increased with higher temperatures despite the WAX melting at around 85℃. The optimal drug-to-WAX ratio of 1:2.5 was achieved at approximately 115℃.

On the other hand, the temperature of the water phase, consisting of water and PVA, could only reach boiling (100℃) or close to boiling (around 95℃). This temperature range did not influence the stability of tilmicosin. The temperature of the oil phase played a critical role in determining the solubility of tilmicosin in the melted WAX, the LC of SLNs, and the drug content of the suspensions. Based on these findings, it is determined that the oil phase (WAX) should be optimized at 115℃. In comparison, the water phase (PVA solution) should be set at 95℃ to achieve the desired product with ideal stability and emulsification.

The ultimate content of the prepared suspension was 5%. In the small test amplification stage, to avoid possible degradation of the drug at high temperatures, the temperature of the oil phase was lowered to 100℃, the solubility of the tilimicosin in the melted WAX was decreased, and their proportion was adjusted from 1:2.5 to 1:3. The temperature of the aqueous phase was determined to be 100℃ (boiling state) due to equipment adjustment (emulsification shear). Once the temperature of the water phase was lower than 100℃ (not boiling), the emulsification effect was poor. Simultaneously, the concentration and volume of the emulsifier were further optimized to ensure the emulsification effect of the product. The concentration of the emulsifier was screened by a single factor. When the concentration of the PVA was 3%, the upper layer of the suspension floated with a significant aggregate of the oil phase, and the LC was 17.98%. The suspension did not have significant mass but presented a small amount of accumulation when the concentration was increased to 4%, and the concentration was still increased to 5%, and it was excellently emulsified. The D50 size was 2.28 μm, and the emulsifier concentration was 5%. The emulsification effect was poor when the shear velocity was below 2200 r/min. The sheer time did not have a major impact on the product. Therefore, the shear velocity and time were set at 2200 r/min for 4 min.

Due to adjustments in the oil–water ratio, equipment, and yield, the particle size of the suspension transitioned from the nanoscale observed in the initial laboratory research to a microscale product during slight test amplification. A shearing machine was used as emulsification equipment for small-scale and pilot productions. Despite the increased output during pilot production, the formulation and main process parameters affecting the product were maintained, resulting in no significant differences in particle size, content, and characteristics between the small and pilot products. The final D_{50} of the pilot product was 2.64 μm. Considering whether the increase in particle size affects product quality, the stability of the suspension was measured. The small test and pilot products did not degrade on the 10th d under the stress testing. The sedimentation coefficient, dispersibility, and the in vitro release did not differ significantly. These results demonstrated that the increase in particle size of the pilot products did not affect the quality of the product.

The output of pilot products was enlarged to 400 L. Compared with high-pressure homogenizers as emulsification equipment, the output of high-speed shearing machines was more and less economical production costs. The heating device and emulsification instrument were adjusted during the pilot production to sandwich the steam heating pot and high-speed shearer according to the output demand. The shear emulsification time was adjusted from 4 to 8 min, and the other process parameters were unchanged. It is necessary to control the

temperature at 100℃to ensure the emulsification effect strictly. Each batch of output can reach 400 L products and even more. Of course, the disadvantage of this set of equipment was that the temperature needed to be monitored by a thermometer. In this case, the temperature may have a slight deviation regarding the oil phase temperature control and the water temperature setting combined with the data of small test amplification, which only needs to heat the water phase to boiling.

In the pilot production process, it was necessary to inspect the semi-product to ensure the qualification of the final product. The semi-product mainly monitored several key parameters, such as content, pH, particle size, and character, especially the product content and pH value. The results revealed that the measured average content and pH were 4.96% and 6.67, respectively. This result was acceptable and also conformed to expectations. The particle size of the products was 2.58 μm, and the appearance was a yellowish emulsion that also met the quality requirements of the product set in advance.

Stringent quality control was maintained at each stage of the production process, ensuring the product's overall quality also helped to validate the preparation process parameters. Identifying crucial factors such as temperature, emulsifier concentration, and shear strength was vital in achieving successful solid lipid-based suspension production. These parameters were paramount, as they directly influenced the product's success. Moreover, the product's pH value and oxidizing ability were also significant considerations. By promptly addressing these aspects by adding specific excipients, potential issues could be swiftly resolved, making them secondary but equally essential conditions in the production process. A robust quality control approach was integral to producing a successful and high-quality solid lipid-based suspension.

Conclusions

A new solid lipid-based suspension was developed through a series of well-defined stages, from initial laboratory research to pilot production. The early laboratory study prepared tilmicosin-loaded solid lipid-based suspensions using a hot melt homogenization with an ultrasonic emulsification method. The optimal SLP formulation consisted of 15 mL of 3% PVA per 1 g of tilmicosin and 2.5 g of WAX. The pH adjuster and antioxidant were optimized through stress testing, leading to the selection of citric acid and propyl gallate. The emulsification equipment was adjusted to accommodate shear and high-speed shear in the subsequent slight test amplification and pilot production stage. As a result, the best SLP formulation was 16 mL of 5% PVA per 1.0 g of tilmicosin and 3.0 g of WAX. In conclusion, the pilot production of a new solid lipid-based suspension was successfully developed based on a novel hot melt emulsification shear method, boosting the industrial development of solid lipid-based suspension.

Methods

Materials

Tilmicosin standard (content: 80.7%) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Native tilmicosin (content: > 96%) was obtained from Jinan Xinbao Star Animal Pharmaceutical CO., Ltd (Jinan, China). Polyvinyl alcohol (PVA) was bought from Sigma (St. Louis, MO, USA). Carnauba wax (WAX) and citric acid anhydrous were bought from Shanghai Changwei Medical Accessories Technology Co., Ltd (Shanghai, China). Propyl gallate was obtained from Xi'an Tianzheng Pharmaceutical Accessories Co., Ltd (Xi'an, China). Others were food grade.

Laboratory research

The suspensions of tilmicosin-loaded SLPs were produced by a hot melt homogenization with ultrasonic emulsification method. A specific amount of tilmicosin was dissolved in the melted WAX under stirring by a magnetic stirrer (9001, Shanghai Huxi Analysis Instrument Factory Co., Ltd, China) at different temperatures. After complete dissolution, a certain amount of volume and concentration of preheated emulsifier solution was swiftly poured into the above-melted lipid solution. The type, concentration, and volume of emulsifier were optimized by orthogonal experiment using drug loading capacity (LC), particle size, and PDI as assessment index, and the corresponding levels were PVA, polyvinylpyrrolidone (PVP), poloxamer 188; 1%, 2%, 3%; 10 mL, 15 mL, 20 mL, respectively (Table 5). The orthogonal test design was analyzed using SPSS software (version 20, IBM). The hot oil/water (O/W) emulsion was cooled at room temperature to form a solid lipid particle suspension.

Following the preparation of the suspension, a specific amount of stabilizer, composed of citric acid (pH regulator) and propyl gallate (antioxidant), was added to prevent tilmicosin degradation. The stability of a

Table 5 Factors and levels of the L9 (3^4) orthogonal design

Variable	Level		
	1	2	3
Type (A)	PVA	PVP	Poloxamer188
Concentration (B)	1%	2%	3%
Volume (C)	15 mL	20 mL	25 mL

drug in the suspension with and without stabilizer was evaluated by the influencing factor test. The samples under high temperature, high humidity, and strong light conditions were taken on 5 and 10 days to determine the suspension's drug content and particle size.

Small-scale trial production

A definite quantity of the drug was weighed and introduced into the melted lipid phase. Once the drug was fully dissolved, it was merged with the preheated aqueous phase, subjected to shear emulsification using a shearer (HR500, Shanghai Huxi Industry Co., Ltd, China), and finally cooled with cold water.

Pilot production

Based on a small-scale trial production, the emulsification equipment and heating device were converted into a high-speed shearer (101, Laizhou Wankai Machinery Co., Ltd, China) and a sandwich steam heating pot (Zhucheng Haopeng Machinery Technology Co., Ltd, China), respectively. Primarily, a specific amount of PVA was accurately weighed into a reaction kettle and then prepared into a 5% solution with hot distilled water. Simultaneously, a certain amount of WAX and tilmicosin were accurately weighed and placed in the sandwich steam heating pot. The heating was stopped and rapidly added into the boiling PVA solution when the tilmicosin was completely dissolved in WAX. The mixture of soil lipid phase and PVA solution was sheared using a high-speed shear (shear time: 4, 8 and 12 min, sampling at different times to observe particle size and dispersion index, power: 2500 r/min) to form a tilmicosin suspension of O/W emulsion.

Upon completing the emulsification process, the steam infusion was immediately stopped by closing the valve, and the cold water system valve was opened to cool the product using cold water rapidly. The stabilizer was added once the product was cooled and the semi-product was finalized. The leading indicators for assessing the semi-products were content characteristics and pH. After validating that the semi-products met the desired quality standards, they were filled, labeled, and packed for further use.

Quality evaluation

The detection of active content and loading capacity (LC)

A 1.02 g (volume:1 ml) sample was accurately weighed into an Erlenmeyer flask (250 mL), and 200 mL of acetonitrile–water (*V*/*V* = 1: 1) was added, weighed, and recorded. Then, it was extracted by reflux in a water bath (boiling for 30 min), and the evaporated liquid was replenished according to the weight. A 5 mL sample was diluted to 50 mL with phosphoric acid solution. Then, it was passed through a 0.22 μm filter, and a 20 μL sample was injected into high-performance liquid chromatography (HPLC) for content determination. Simultaneously, tilmicosin-related substances were determined by the same method.

The LC determination of SLPs was described in our previous work (Li et al. 2019). Briefly, the suspension was collected by centrifugation at 14,000 r/min (Hitachi Centrifugation CR21G; Hitachi Koki Co., Ltd., Japan) for 60 min at 4℃. The precipitated SLPs were re-suspended in distilled water and lyophilized for 48 h (Freeze Dry System; Labconco, America) to determine LC. After freeze-drying, 10.0 mg dried particles were added to a 15 mL tube containing 10 mL acetonitrile/water solution (*V*/*V* = 1: 1) and put in a boiling water bath to destroy the particles. The SLPs, after heating, were added to the volume of 10 mL and centrifuged at 8,000 r/min for 10 min. The supernatant, after filtration, was injected into HPLC for analysis. The assay was repeated in triplicate using different samples from independent preparations. The LC was defined as follows:

$$\text{LC (\%)} = [(\text{Weight of tilmicosin in SLPs})/(\text{Weight of dried SLPs})] \times 100\%$$

Particle size and pH determination

The suspensions' size, PDI, and zeta potential were measured using Zetasizer ZX3600 (Malvern Instruments, UK) and Laser particle size analyzer BT-9300S (Better, China) at 25°C. The samples were diluted in distilled water to ensure that the concentration was 2.7 mg/mL for the tests of size and PDI and 0.3 mg/mL for the zeta potential measurement to get the optimum kilo counts per second of 20–400. All determinations were repeated in triplicate by using independent preparations. The pH of the sample was determined by a pH meter (PHS-25, INESA Scientific Instrument Co., Ltd, China) at room temperature.

Determination of sedimentation coefficient, dispersibility, and observation of traits

The sample of 50 mL was placed into the graduated cylinder, and the initial height was H_0. After standing for 3 h, the height H_1 after a settlement was recorded, and the sedimentation coefficient was calculated using H_1/H_0.

The tilmicosin suspension of 20 mL was placed in a 100 mL graduated cylinder at room temperature and allowed to stand for 7 d. Then, the graduated cylinder was rotated at a rate of 20 r/min. The situation disappearance of the sediment within 2 min was observed and evaluated by excellent, good, general, and poor. The suspension of 5 mL was taken to observe the appearance.

Related substance testing

Chromatographic conditions and gradient elution methods

Column: Agilent SB C18 (250 mm × 4.6 mm × 5 um); UV detector; wavelength: 280 nm; Column temperature: 25℃; Sample volume: 10 µL; Velocity: 1 mL/min; Mobile phase: Phosphate water (975 mL aqueous phase add 25 mL phosphate buffer (1000 mL of water containing 5.71 g of phosphoric acid and the pH was adjusted to 2.5 ± 0.1 with 12.5 mol/L sodium hydroxide solution): acetonitrile. Gradient elution is shown in Table 6.

Strong degradation test

According to the ICH guidelines, the drug and blank excipients were destroyed by high temperature, oxidation, strong alkali, strong acid, and strong light, respectively. The specificity of the method was examined by injecting the above chromatographic condition and detecting it with a UV detector. The specific operations were as follows:

High-temperature test: 1 mL tilmicosin suspension and the blank suspension were accurately weighed into a test tube (10 mL) in triplicate. The samples were placed in a high-temperature incubator (80℃) and sampled at 24, 48, 72 and 96 h for chromatographic analysis.

Oxidation experiment: 1 mL tilmicosin suspension and the blank suspension were accurately weighed into a centrifuge tube (10 mL) in triplicate. 30%, 1% and 0.1% hydrogen peroxide solution (2 mL) were added, and samples were taken at 4, 12, 24 and 48 h for chromatographic analysis.

Strong base test: 1 mL tilmicosin suspension and the blank suspension were accurately weighed into a centrifuge tube (10 mL) in triplicate. 1 mol/L sodium hydroxide solution (2 mL) was added, and samples were taken at 4, 12, 24 and 48 h for neutralization with the same concentration of hydrochloric acid for chromatographic analysis.

Strong acid test: 1 mL tilmicosin suspension and the blank suspension were accurately weighed into a centrifuge tube (10 mL) in triplicate. 1 mol/L, 2 mol/L and 4 mol/L hydrochloric acid solution (2 mL) were added separately, and samples were taken at 4, 12, 24 and 48 h for neutralization with the same concentration of sodium hydroxide for chromatographic analysis.

Table 6 Linear gradient elution of tilmicosin-related substances

Time (min)	Mobile phase A	Mobile phase B
0	86	14
4	86	14
20	71	29
23	86	14
25	86	14

Strong light test: 1 mL of the tilmicosin suspension and the blank suspension were accurately weighed into a centrifuge tube (10 mL) in triplicate. The sample was placed in a light incubator (8400 lx, 30℃) and sampled at 24, 48, 72 and 96 h for chromatographic analysis.

Stability

The stability of tilmicosin suspension was evaluated by influencing factor experiments, including high temperature, high humidity, and strong light. The suspension was placed in a container and then placed in an environment of 60°C (high-temperature test), 25°C, and 90% ± 5% (humidity test) or 4500 ± 500 lx (illumination) for 10 d. Samples were taken on the 5th and 10th d to assess their appearance change, drug content, particle size, settlement coefficient, and relative substance.

In vitro release

In vitro release of the tilmicosin suspension was measured in simulated gastric fluid (SGF, pH = 2, 1000 mL contained 2.0 g NaCl and 3.2 g pepsin, and then HCl was used to adjust the pH to 2.0), and simulated intestine fluid (SIF, pH = 8.0, 1000 mL SIF contained 6.8 g KH_2PO_3 and 10 g trypsin and then NaOH solution were used to adjust the pH to 8) of pigs by using Dissolution tester RC806 (Tianjing Tiandatianfa Co., Ltd. China). Briefly, 10 mL suspension ($n = 3$) was placed in a dissolution cup containing 1 L buffer at 38°C under a rotating propeller stirring at 60 r/min. 1 mL sample was periodically collected from the dissolution cup at fixed times to measure the released drug, and the same volume of fresh SGF or SIF was added after each sampling to keep a constant volume. The drug concentration in the release media was determined by HPLC.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44149-023-00098-4.

Additional file 1: Figure 1. Chromatographic peak Blank nanosupension(Left); Chromatographic peak of tilmicosin suspension (Right). **Figure 2.** Chromatographic peaks of blank nanosuspensions under strong base conditions (1mol/L, 4h Left); Chromatographic peaks of suspensions under strong base conditions (1mol/L, 4h Right). **Figure 3.** Chromatographic peaks of blank nanosuspension under strong acid conditions (4mol/L, 60h, Left); Chromatographic peaks of suspension under strong acid conditions (4mol/L, 60h, Right). **Figure 4.** Chromatographic peaks of blank nanosuspension under strong oxidation conditions (0.1%, 4h, Left); Chromatographic peaks of suspension under strong oxidation conditions (0.1%, 4h, Right). **Figure 5.** Chromatographic peaks of blank nanosuspension under high temperature conditions (80℃, 96h, Left); Chromatographic peaks of suspension under high temperature conditions (80℃, 96h, Right). **Figure 6.** Chromatographic peaks of blank nanosuspension under Strong light conditions (8400lx, 96h, Left); Chromatographic peaks of suspension under Strong light conditions (8400lx, 96h, Right).

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Authors' contributions
Design of the study and acquisition of funding: Shuyu Xie, Dongmei Chen; performing the experiments and data analyses: Chao Li, Wenqing Xie, Liwen Yuan; drafting of the manuscript: Chao Li; revision of the article: Shuyu Xie, Dongmei Chen, Mubbashar Abbas.

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Availability of data and materials
All data are included in the manuscript and supplementary materials.

Declarations

Ethics approval and consent to participate
Not applicable.

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All authors confirmed the final version of the manuscript for publication.

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Author Shuyu Xie was not involved in the journal's review or decisions related to this manuscript. All authors have no competing interests to declare.

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