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Preparation of tilmicosin-resin complex microsphere and its Pharmacokinetics study in rat

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Abstract: The objective of this study is to mask the extremely bitter taste of tilmicosin, and the tilmicosin-resin complex (DRC) microsphere were prepared by entrapping tilmicosin into resins (Tulsion® 339 and Eudragit® RS/ RL 100) for further pharmacokinetics study in rat. The DRC was characterized by FTIR and X-ray diffraction, and the microsphere containing DRC and Eudragit® RS/RL 100 were characterized by scanning electron microscopy (SEM). The rats were orally administrated with tilmicosin phosphate (10 mg/kg) and the microsphere containing the same dose of tilmicosin, respectively. These microspheres do not taste bitter and the kinetics study suggests that the drug released from microsphere meet the first order kinetics (r = 0.9911). The experimental results showed that $T_{½}$ and T_{max} of microsphere were much longer than tilmicosin phosphate, which indicates that the oral microsphere can be a promising long-active formulation for taste masking of tilmicosin.

Keywords: FTIR, X-ray diffraction, scanning electron microscopy, oral sustained-release microsphere

INTRODUCTION

Tilmicosin is a semisynthetic macrolide antibiotic which has been reported to have potent and broad-spectrum antimicrobial activity, especially against *Mycoplasma*, *Actinobacillus* and *Pasteurella multocida* (Hoflack *et al.*, 2001). Currently, solvable tilmicosin phosphate is used in clinics with its injectable form and Tilmicosin phosphate premix is used in veterinary medicine for the treatment of infections in poultry and livestock at a dose of 10 mg/kg injected subcutaneously (Clark *et al.,* 2008). Tilmicosin has a long serum half-life, large distribution volume and rapid accumulation in bovine macrophages and mammary gland epithelial cells. After subcutaneous administration, tilmicosin had a long elimination half-life and high concentration in milk (Ramadan, 1997). However, the drug has a narrow therapeutic index and rapidly achieves the maximum serum concentration in vivo and it may be fatal to swine and horses by intramuscular injection and intravenous administration at 10mg/kg subcutaneously (Yazar *et al.*, 2002). It is found that bovine tolerate dosages as high as 50mg/kg and swine show toxic reactions at 10mg/kg through subcutaneous injection (Van *et al.,* 2000). Furthermore, subcutaneous administration of tilmicosin resulted in severe reactions at the injection site. However, no adverse reactions were found when tilmicosin is orally administrated at 15-18mg/kg in swine. Tilmicosin phosphate premix could influence feed palatability, and thus affect the growth of animals because of its extremely bitter taste. So sustained-release formulation of tilmicosin was designed to achieve and maintain concentration in the target tissue minimum concentration inhibition. The hydrogenated castor oil (HCO)-solid lipid nanoparticle (SLN) system is tilmicosin carrier, which was developed for sustained release and the controlled release of tilmicosin from silica nanoparticles, but oil is only rarely a solubilizing medium to dissolve the required dose in a proper quantity of carrier and they are still an injection (Han *et al.*, 2008; Song *et al.*, 2011).

Ion exchange resin (a copolymer of vinyl, divinyl benzene and polystyrene) has been used for oral drug delivery systems and not absorbed by the body (Venkatesh *et al.*, 2013; Chaudhry *et al.*, 1956). The drug release rate from DRC can be further controlled by coating it using a variety of coating processes to achieve microsphere (Yin *et al.*, 2015; Sun *et al.*, 2013). Currently, the most mature system was Pennkinetic® systems (Raghunathan, 1980) developed from the United States Pennwalt company. Eudragit® RS100 (RS) and Eudragit® RL100 (RL) (a copolymer of ethyl acrylate, methyl methacrylate) are another type of resins for coating oral drug formulations to achieve a time-sustained delivery among the most used materials (Pawar *et al.*, 2012; Momoh *et al.*, 2014). They contain a certain amount of quaternary ammonium groups between 4.5-6.8% and 8.8-12% for RS and RL, respectively. So the drug with RL coating was released faster than the drug with RS coating under the same conditions. We herein selected the RS and RL to coat the DRC containing tilmicosin to form microspheres for a good taste masking, a significantly longer $T_{½}$ and greater bioavailability.

MATERIALS AND METHODS

Drugs and reagents

The tilmicosin phosphate (98.75%, w/w) was purchased

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from Ningxia Tairui Pharmaceutical Co., Ltd (Jinan, Ningxia Hui Autonomous Region, China). Tulsion® 339 (Thermax India Ltd., Pune), Eudragit® RS 100 and Eudragit® RL 100 (Rhm Pharma, Darmstadt, Germany) were purchased from Shanghai Chine way Pharmaceutical Tech. Co., Ltd. All other chemicals and solvents were of analytical grade.

Experimental animals

Ten Sprague-Dawley rats (male, 240±20g) were purchased from the Experimental Animal Center of Chongqing Medical University, Chongqing, China (SYXK (YU) 2012-0001). The present study conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85-23, revised in 1996). The animals were kept in a conditioned environment (22±1°C, 12h light/darkness cycle, free access to water and food). During the experiment, the rats drank tap water, but they were not fed any diet.

Determination of drug concentration

The chromatographic system consisted of a LC-20AB solvent delivery pump (Shimadzu) equipped with a 20-Al loop, Inert Sustain® C18 (5μm, 4.6 × 150 mm) analytical column, and SPD-20A UV-Visible detector (Shimadzu). The mobile phase was made up of 11.5% acetonitrile, 5.5% tetrahydrofuran, 2.5% 1M Dibutylamine phosphate buffer (DBAP) and 80.5% water. The processed sample solution was filtered with a 0.22μm syringe filter before injected into the HPLC system and detected at 280 nm.

Preparation of drug-resin complex (DRC)

Resin pretreatment

Approximately 20 g Tulsion® 339 was allowed to swell in 500 mL deionized water for 12h, followed by the activation by 0.1 N HCl. The obtained resin was then washed by deionized water until the pH of the eluent maintained unchanged, dried at 40°C for 24h, passed through a 75±4.1 μm stainless sieve, stored in an airtight glass bottle and kept in a desiccator.

Drug loading

DRC was prepared by batch process (Kim *et al*., 2013). The resin was allowed to swell in 2mL of deionized water for 30 min. To 200mL tilmicosin phosphate solution in deionized water at 1mg/mL, 2.5mg/mL, 3mg/mL and 4 mg/mL (C_0), was added the activated resin (600mg) at 25°C, respectively. The concentration of the obtained tilmicosin phosphate (C_t) at 0.25, 0.5, 1.5, 1, 2, 3, 4, 5, 6 and 7 h was determined by HPLC. Q_t was calculated according to the equation Ⅰ, the equilibrium time was the time when Q_t was almost not changed. The initial concentration of tilmicosin phosphate solution is selected for preparing DRC when resins were equipped with the maximum of drugs at the equilibrium time. DRC was prepared with drug/resin ratio (w/w) of 0.5: 1, 1: 1, 1: 2, and 1: 3, at 15, 25, 35, 45 and 55°C, respectively. The concentration of tilmicosin phosphate was initially optimized concentration. Q_t and E were calculated according to the equation and equation α at the equilibration time and the optimization of ratio and temperature were calculated when Q_t and E both reached maximum values.

$$Q_t = \frac{V}{W_R}(C_0 - C_t) \quad \text{I}$$

$$E = \frac{C_0 - C_t}{C_0} \times 100 \quad \text{II}$$

Here, Q_t is the mass of the drugs equipped with per gram of resin (g/g) at t h, V is the volume of the media, W_R is the mass of the resin, C_o is the initial concentration of tilmicosin phosphate solution, C_t is the concentration of tilmicosin phosphate at t h and E is the drug available.

Evaluation of drug-resin complex

Fourier Transform Infrared Spectroscopy (FI-IR)

FT-IR spectrum of tilmicosin phosphate, Tulsion® 339, their commensurable physical mixtures and DRC were recorded on (IR Prestige-21, Shimadzu, Japan) at the wave number range 4000-400 cm^{-1} (Tawakkul *et al.*, 2009).

X-Ray diffraction of complex

Powder X-ray diffraction (PXRD) patterns were collected for the drug, resin, physical mixtures. The powder X-ray diffraction patterns were measured by a Shimadzu XRD-7000 scanner with voltage 40 kV filter, Cu Kα radiation and a current of 20 mA. The scanning rate employed was 3 °/min over the 20-80° diffraction angle (2θ) range. The PXRD operation, data collection, and data analysis were achieved through Diffrac. Suite (V2.2).

Preparation of drug-resin complex microsphere

The microspheres were prepared by the O/O non-aqueous solvent evaporation method. Liquid paraffin was mixed with span 80 as the continuous phase. DRC (swelled in 10% PEG 4000 solution for 30min) was added to RS/RL (4:1 g/g) with PEG 400 and DEP respectively, was dissolved in acetone to form dispersed phase. The ratio of the liquid paraffin, span 80 and acetone was determined by ternary phase diagram. The dispersed phase was slowly added to the continuous phase to form emulsified state with stirring constantly. And then the acetone was vaporized at 40°C till the solid micro sphere formed from the emulsion. After 3h of agitation, the solidified microspheres were filtered and washed with petroleum ether. The micro spheres were dried for 12 h at 40±0.5°C. The factors that influence microencapsulated include the concentration of the RS/RL as well as the amount of PEG 400 or DEP used. The optimum conditions were optimized by L_9 (3^4) orthogonal experiment. The results of orthogonal experiment were analyzed with weighted grading method as follow.

Table 1: Analysis of orthogonal experiment.

NO.	A RS100/RL100(A) (g/100mL)	B PEG400(B) (%)	C DEP(C) (%)	E	f_i
1	1(2)	1(0)	1(10)	1	55.6
2	1(2)	2(5)	2(15)	2	47.4
3	1(2)	3(10)	3(20)	3	50.6
4	2(3)	1(0)	2(15)	3	23.4
5	2(3)	2(5)	3(20)	1	7.4
6	2(3)	3(10)	1(10)	2	16.2
7	3(4)	1(0)	3(20)	2	29.8
8	3(4)	2(5)	1(10)	3	23.6
9	3(4)	3(10)	2(15)	1	26.7
I	153.5	108.7	95.3	87.6	
II	46.9	78.3	97.4	93.3	
III	80.0	93.4	87.8	97.5	
SS	1965.9	135.3	4.5	126.4	
F	190.5	14.8	1.7		
P 0.05 >	$p > 0.01$	$p > 0.01$	$p > 0.01$		

I, II, III, respectively, type1, type 2, type 3 errors; SS, deviation; F, significance testing; *p*, probability

Table 2: Pharmacokinetic(PK) parameters of tilmicosin and microspheres after oral administration in rat

PK parameter	unit	tilmicosin	microspheres
A	μg/mL	1.896	1.112
α	h^{-1}	0.381	0.110
B	μg/mL	0.077	0.780
β	h^{-1}	0.069	0.028
k_{01}	1/h	2.612	0.335
k_{10}	1/h	2.183	2.715
k_{12}	1/h	0.049	0.025
k_{21}	1/h	0.083	0.068
$T_{½}α$	h	1.819	6.300
$T_{½}β$	h	1.004	24.75
$T_{½k01}$	h	0.265	2.069
T_{max}	h	0.877	6.479
C_{max}	μg/mL	1.230	0.982
$AUC_{0-∞}$	h*μg/mL	5.336	32.68
MRT	h	2.061	33.44

A, second chamber model disposal of intercept curve on the y axis; B, blood drug concentration of intercept curve on the y axis; α, drug distribution rate constant in vivo; β, drug elimination rate constant in vivo; k_{01}, the central chamber absorption rate constant; k_{10}, the central chamber elimination rate constant; k_{12}, rate constant of drugs from the room to the outer room level transfer; k_{21}, rate constant of drugs from peripheral room to the central chamber level transfer; $T_{½α}$, distribution half-life; $T_{½β}$, elimination half-life; $T_{½k01}$,absorption half-life; T_{max}, the time to maximum drug concentration; C_{max}, the high mean peak concentration; AUC, the mean area under the curve; MRT, mean residence time

STATISTICAL ANALYSIS

Data were expressed as mean±S.D. and were analysed with a two-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls post-test was used for comparisons of data between groups. P-values below 0.05 ($p< 0.05$) were considered indicative of statistical significance.

$f_i = |L_1\text{-}30\%| \times 100 \times 1 + |L_2\text{-}60\%| \times 100 \times 1 + |L_3\text{-}80\%| \times 100 \times 1$

Here, weighting factor is 1. L_1 represents the cumulative release for 3 h. L_2 represents the cumulative release for 6 h. L_3 represents the cumulative release for 8 h.

Evaluation of drug-resin complex microsphere

Scanning electron microscopy

The morphological examination of the particles was investigated by SEM (JSM-6510LV; JOEL, Tokyo, Japan). Before visualization, the samples were coated with palladium using a sputter coater (JEC-3000 FC).

Dissolution studies and curve fitting

Dissolution studies (RCZ-6C; Huanghai Medicine &

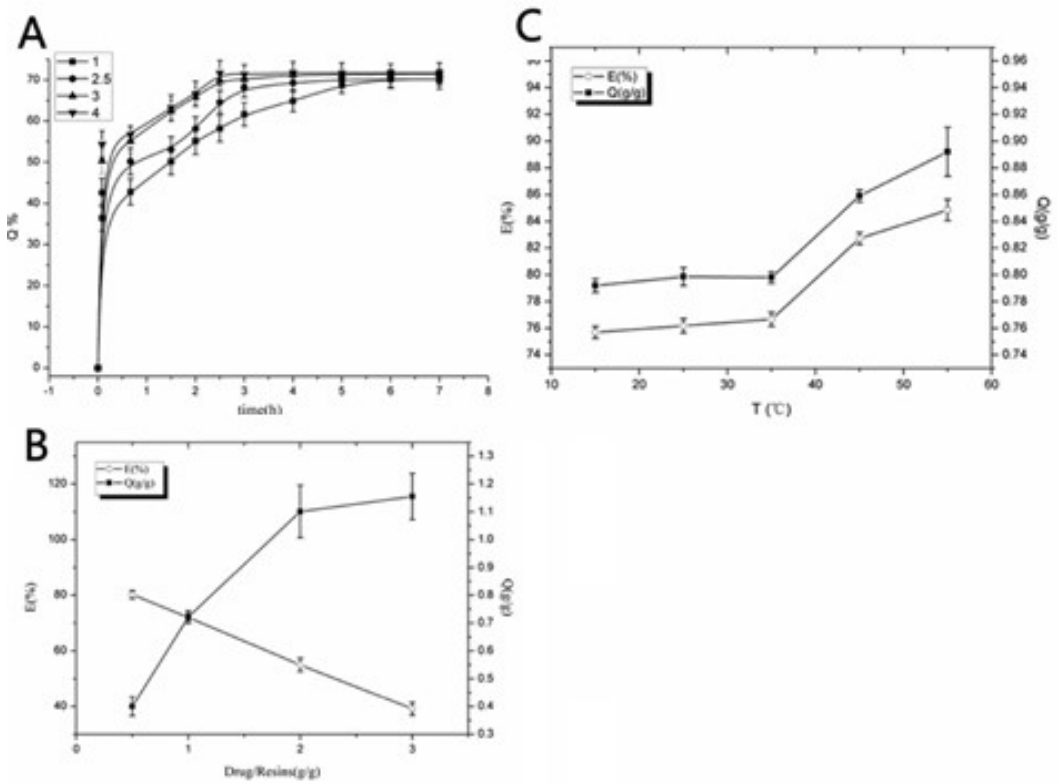

Fig. 1: (A) Q% with different initial concentration of 1, 2.5, 3, and 4 mg/mL of tilmicosin phosphate at different time (B) Plots of availability [E] and loading [Q] at the drug-resin ratios of 0.5: 1, 1: 1, 1: 2, and 1: 3 (C) Plots of availability [E] and loading[Q] at 15, 25, 35, 45, and 55°C

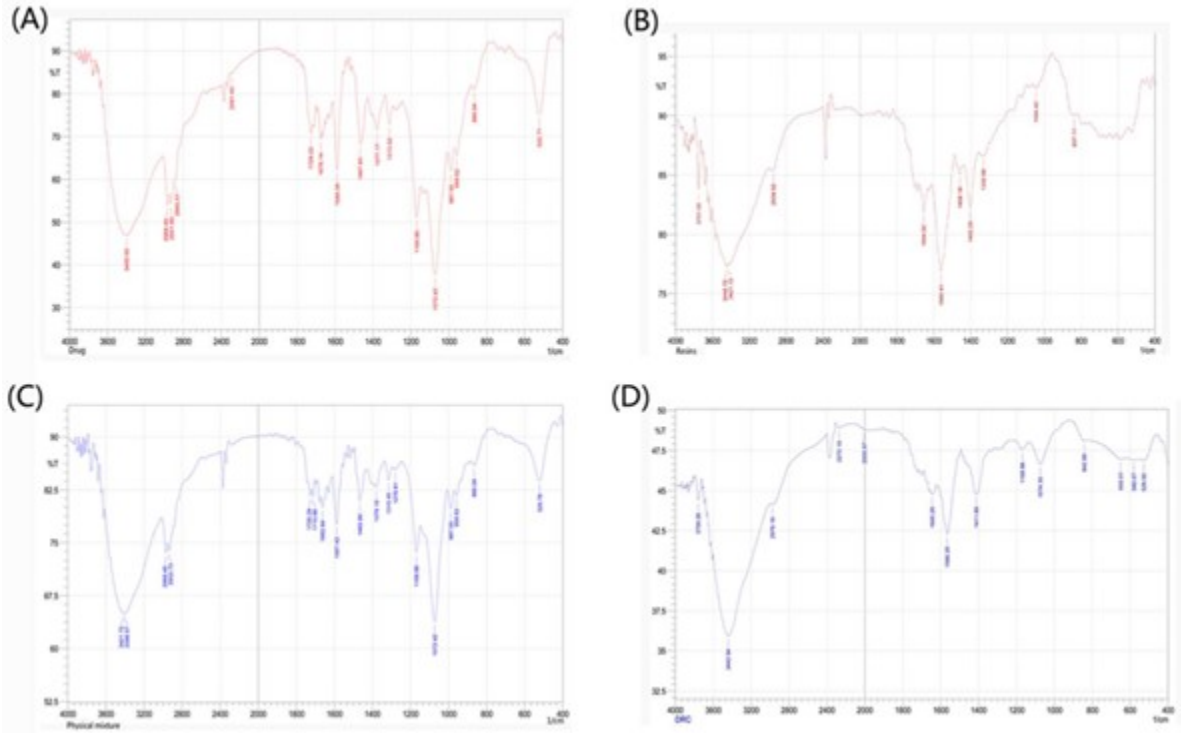

Fig. 2: The fourier transform infrared spectroscopy [FTIR] of the drug, resins, physical mixtures and DRC. (A) The FTIR of the tilmicosin phosphate; (B)The FTIR of the tulsion339 resins; (C)The FTIR of the drug resin 1:1 (g: g) physical mixture; (D) The FTIR of the drug resin compound [DRC]

Drug Testing Instruments) that simulate conditions of the stomach and the bowel were performed in dissolution medium, 900mL 0.1 N HCl for stomach and 0.1 M PBS at pH 6.8 for bowel, respectively. 25 mg microsphere was dissolved in the dissolution medium (n = 3), stirred at 50 rpm at 37±0.5°C. Samples of 0.5mL were withdrawn from the dissolution medium at 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14 and 24 h and filtered with 0.22 μm filters. The filtrates were analyzed for tilmicosin by using HPLC. This study was performed in triplicate for each sample,and the average values for respective microspheres were reported. The study was performed for three times. The drug content of the samples was calculated the at the equilibrium time. Then the release curve was fitted with the next three models by DDSolver (Kevadiya *et al.*, 2011).

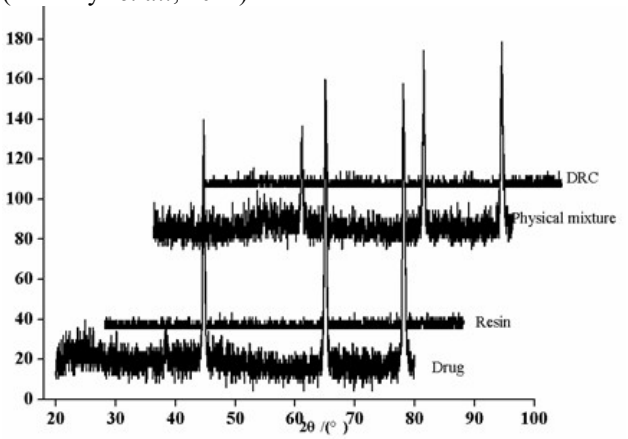

Fig. 3: The XRD of the drug, resins, physical mixtures and DRC

The zero order: $F = k_0 t$

The first order: $F = 100^*(1 - Exp(-k_1^*))$

The Higuchi order: $F = k_H t^{1/2}$

Here, F represents the degree of drug released (%).

Pharmacokinetic studies in vivo

Ten rats were divided into two groups, and each group was subjected to a single oral dose at 10 mg/kg of body weight of tilmicosin phosphate and the microsphere, respectively. Blood samples (0.5 mL) were collected at 0, 0.25, 0.5, 1, 4, 6, 8, 10, 12, 24, 36, 48, 72 and 96 h after drug administration, stored at 4°C for 24 h to clot. Serum was obtained after 10 min centrifugation at 3500 rpm and stored at -20°C (Han *et al.*, 2008). Tilmicosin was then extracted from the serum with 4mL acetonitrile, put under vortex movement for 30s and then centrifuged for 15 min at 12000rpm. The supernatant was collected and evaporated under a nitrogen stream at 30°C. The obtained solid was dissolved in the mobile phase, filtered with a 0.22 μm syringe filter and then injected into the HPLC system. The relationship between the serum concentrations and the time was analyzed by the Phoenix WinNonlin 6.1 (Pharsight, USA).

RESULTS

I had divided the result sections into two parts of results and discussion. And the changes were marked in blue.

Drug loading

As presented in Fig. 1A, the equilibrium time was 5 h when Q_t was constant. The Q_t and E with different drug/resin ratio (w/w) and the temperature for the preparation of DRC were shown in Fig. 1B and Fig. 1C. The Q_5 and E_5 at 15, 25, 35, 45 and 55°C of attach process were 0.7919±0.005 and 75.70%±0.446%, 0.07986±0.005 and 76.19%±5.367%, 0.7986±0.0043 and 76.67%±5.233%, 0.8591±0.0046 and 82.71%±4.5%, and 0.892 ± 0.0183 and 84.85%±7.9%. The Q_5 and E_5 for the drug-resin ratios of 1: 2, 1: 1, 2: 1, and 3: 1 were 0.4005± 0.034 and 80.21%±1.45%, 0.7212±0.023 and 72.05%± 1.29%, 1.1013±0.094 and 54.98%±2.37% and 1.1553± 0.083 and 39.15%±2.33%.

Evaluation of drug-resin complex

The results of FT-IR spectroscopy as shown in Fig2, the characteristic peaks at 3492.79, 2966.52–2885.51, and 1724.36, 1591.27 and 1462.04 cm^{-1} are assigned to -OH, -CH_3, -CH_2- and carboxylate C=O, conjugated double bonds C=O, methyl CH (Fig. 2A), respectively (Manuel *et a1*., 1989). These peaks were identical with those of tilmicosin alone, and present in all the physical mixtures but absent in the complex. FTIR spectrum of the pure drug showed a prominent (obvious) peak at 2967 cm^{-1}. However, there was no sharp peak around 2967 cm^{-1} in DRC. These results confirmed the intactness of drug in complexes. We did not find the overlap of the spectra of DRC (Fig. 2B) and the physical mixtures (Fig. 2C) at lower wave numbers between 1000 and 1400 cm^{-1}, and 644 cm^{-1}, and a peak of resins at 1654.92cm^{-1} are assigned to –COOK but not in DRC.

The results of PXDR of the drug, resins, physical mixtures and DRC are given in Fig. 3. XRD of the drug showed three sharp peaks at 44.85°, 65.22° and 78.34°.

Preparation of drug resin-complex microsphere

The liquid paraffin, span 80 and acetone at the ratio of 8: 1: 3 was used for the preparation according to the three ternary phase diagram. Other conditions were optimized by orthogonal experiment and the results were shown in Table 1. 3% RS/RL, 5% PEG 400 and 20% DEP were used after optimization. The cumulative release of drug from the optimum microsphere at 2, 6 and 8 h were 31.4%, 59.86% and 85.9%, respectively. The ratio of DRC and RS100/RL 100 was 7: 3 and microspheres weight gain was 3%. The variance analysis showed that factor A had statistically significant effect on the release of the drug, and B, C did not significantly affect drug release. The best coating formulation is $A_2B_2C_1$.

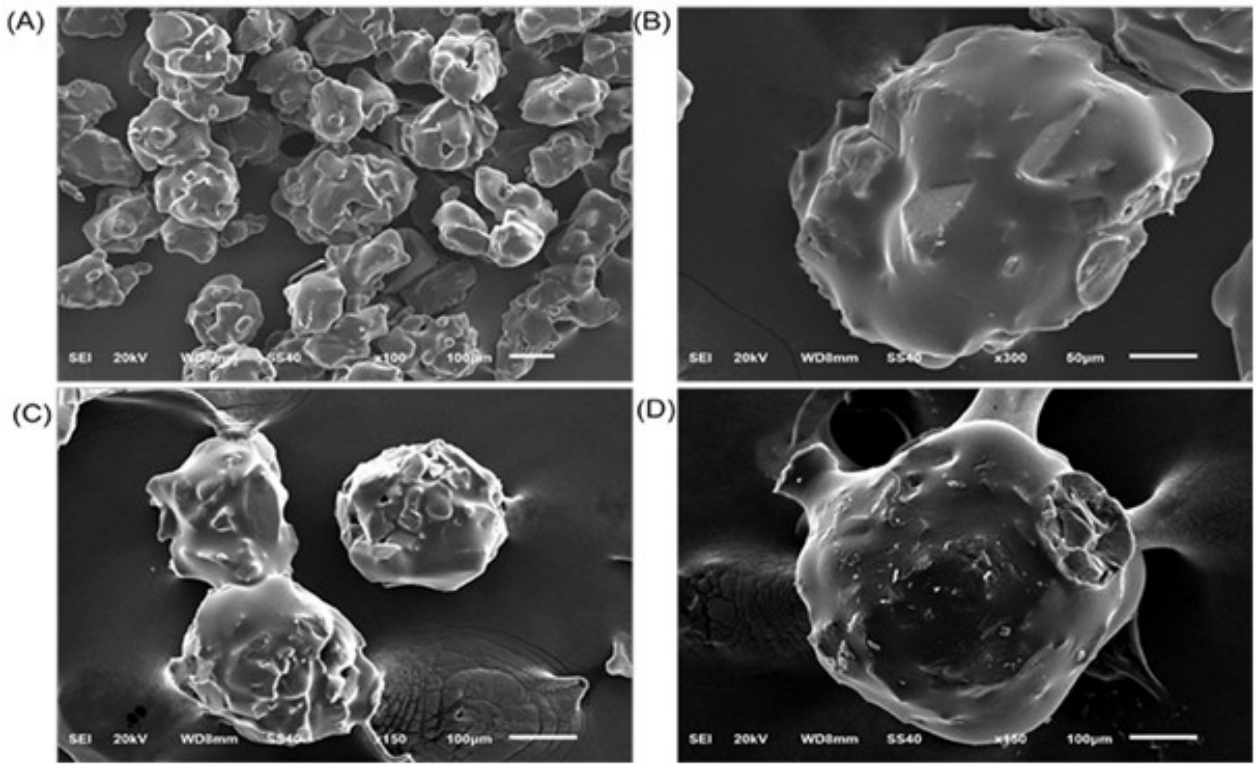

Fig. 4: The microspheres of the DRCs after impregnated with PEG 4000 were included to microspheres (A); The microspheres like (B) soaked in water for 24 h and dried (b); The microspheres of the DRCs were included directly to form microsphere (C); The microspheres like (C) soaked in water for 24 h and dried (D).

Evaluation of drug resin-complex microsphere

Fig. 4 was the Scanning electron microscopy results. In fig. 4A, the DRCs were included to microspheres after impregnated with PEG 4000 and these microspheres were coarse and not uniform. As shown in fig. 4B, the surface of microspheres were rough and the microspheres were not broken even after soaked in water for 24 h and dried. The DRCs were included directly to form microspheres, and their shape was more like a sphere in fig. 4C. And fig. 4D showed that the microsphere was ruptured after soaked in water for 24 h.

Drug release and content in vitro

The results of drug release in 0.1 N HCl and 0.1 M PBS at pH 6.8 were shown in fig. 5. The cumulative release of the microsphere was 30.53%±0.67%, 60.1%±1.32% and 81.23%±1.88%. In the PBS at pH 6.8, the cumulative releases were 33.93%±0.78%, 61.86%±1.22% and 85.23%±1.43% in the 900 mL HCl (0.1 N) solution at 2, 6 and 8 h. The release of the drug from microsphere in 0.1 N HCl showed similar trends to those in the phosphate buffer at pH 6.8, but the overall release was slower. The drug content of microsphere is 36.687%±1.236%.

Pharmacokinetic studies in vivo

Pharmacokinetic parameters (table 2), and the serum concentration-time curve (fig. 6) indicated a two compartment open model, the theoretical equation of tilmicosin phosphate and microspheres are $C= 1.896e^{-0.381t} + 0.077e^{-0.069t} - 1.973e^{-2.612t}$ and $C= 1.112e^{-0.11t} + 0.78e^{-0.028t} - 1.892e^{-0.335t}$. By oral administration of tilmicosin phosphate, the serum drug concentration rose dramatically with an average 1.23μg/mL at 0.877 h, and then declined rapidly. On the contrary, the serum drug concentrations for the microspheres increased more slowly and reached maximum concentration of 0.982 μg/mL at 6.479h.

DISCUSSION

Ion exchange reaction take place during the complex formation between the ion exchange resin and drug, and the loading of tilmicosin may be due to breaking of the van der Waals forces in the drug molecules (Rajesh *et al*., 2015). It has been reported that the resin is made of numerous layers, and each layer has the same number of exchangeable ions. Ion exchange firstly occurred in the first layer when the drug exchanged with free ions in the resin, and then it occurred to a second layer, and the rest layer can be deduced from this. Therefore, the phosphate radical just helped each layer exchangeable ions to reach saturation when the solution maintained a certain amount of the tilmicosin phosphate, and the drug concentration reached the optimal value (Yao and Tien, 1993). As can be seen from fig. 1a, the rate and degree of drug loading increased with the increase of initial drug concentration in the solution till 3mg/mL and have no significant change at

the concentration of 4mg/mL. Because that most of the ionic binding sites were still unoccupied at lower concentrations, but till 3mg/mL and all of the ion-exchange groups had been already occupied from 3mg/mL to 4mg/mL (Malinovskaja *et al*., 2013). The exchange process of drug and the resin is regarded as a series of reaction and mass transfer that similar to a reversible reaction (Woodworth *et al*., 1992). Qt and E increased with the temperature increase from 15 to 55°C. So the reaction was considered as an endothermic reaction. Tilmicosin decomposed over 55°C, due to its structure of macrolide antibiotic. In general, to achieve relatively high Qt and E, the ratio of 1: 1, 45°C and 3mg/mL (initial concentration) were selected as the optimum further microsphere studies.

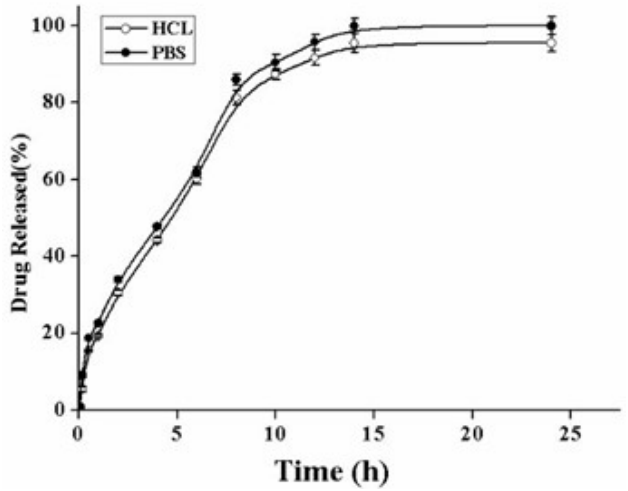

Fig. 5: Cumulative percent drug release from microspheres in 0.1N HCl (HCl) and 0.1M phosphate buffer pH 6.8 (PBS) (n=3).

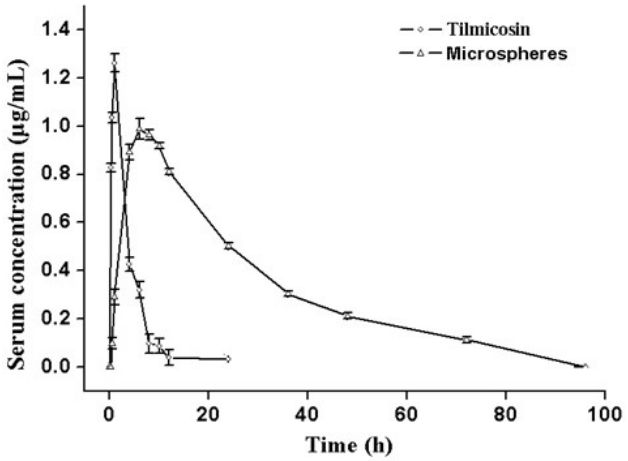

Fig. 6: Concentration-time profiles of tilmicosin phosphate and the microspheres following oral administration. Values are mean ± SD (n = 5)

We had evaluated the drug-resin complex through FT-IR spectroscopy and X-Ray Diffraction, which indicated that the drug was embedded into the resin (Sradhanjali *et al*., 2010). Tulsion® 339 give diffused peak due to their amorphous state. It was noted that, the diffractogram of the physical mixtures were practically constituted by the superposition of the single components, with the crystalline peaks of the drug emerging on the diffuse background of the resin, indicating that no new structure formed. However, the molecular state of the drug prepared as DRC gave diffused peak due to their amorphous state and the absence of drug peaks compared to the drug alone and it is suggested that the DRC was changed from the crystalline to the amorphous state (Akhtar *et al*., 2013). We assume that drug-resin interaction prevented the drug crystallization. This finding confirmed that the entrapped (embedded) drug was dispersed monomolecularly in the resin bead. AS for physical mixture of drug and resins, drug molecules are outside the resin bead. Similar results have been found in PXRD studies between an ion exchange resin and another drug as previously reported. (Tawakkul *et al*., 2009).

Next, we had prepared the drug resin-complex microsphere with the best coating formulation of A2B2C1 and evaluated the microsphere by scanning electron microscopy and drug release and content in vitro. We assume that the resin has a certain degree of expansion and the resin-impregnated surface becomes coarse, and the surface has a poor ability to form the membrane. The microspheres were evenly distributed and the diameter was in the range of 50-250µm. The median particle size is between 125µm and 150µm. In the drug release process, the DRC is pretreated with RS/RL 100 to maintain the geometry. The pretreated DRC are then coated with water-insoluble polymer, and the water-insoluble polymer helps in controlling the rate of swelling of the resin matrix in water (Vikas *et al*., 2001). To investigate the mechanism of drug release from microspheres, data were obtained from the drug-release studies and analyzed according to equations of the zero order model (k0 =6.332, r=0.8815), the first order model (k1=0.205, r=0.9911) and the Higuchi model (kH =25.183, r = 0.9718). The correlation (r) was used as the best fitting index for considering each model. It suggests that the first order kinetics model performed best among the models, indicating that the results are consistent with the commonly used description of a membrane-controlled process.

Finally, we had conducted pharmacokinetic studies in vivo, which results revealed that T½α, T½β and MRT of the suspensions were much longer than those of tilmicosin phosphate. The prominently greater AUC shows that the microspheres had much better bioavailability than tilmicosin phosphate in rat. Tilmicosin is semisynthetic macrolide antibiotic, with a 16-membered ring. The short half-life of 2.06 h following oral dosing makes it an ideal candidate for a modified release multiple-unit tilmicosin preparation and it belongs to a time-dependent on the

initial effect (Perumal, 2001). So the best way of taking into is controlled release formulations. It is easily broken its structure and stimulated the stomach at the acidic environment due to alkaline. Therefore, Cmax of tilmicosin only reached 1.23 μg/mL by oral administration. It might have affected the absorption of tilmicosin and caused the erratic absorption pattern, which may partially explain the phenomenon that the bioavailability of tilmicosin was much lower than microspheres. Eudragit RS/RL100 is the water-insoluble polymer, and the water-insoluble polymer helps in isolating gastric juice to avoid damage the drugs (Fritze *et al*., 2006). At the result, the microspheres reached Cmax of 0.982μg/mL at 6h. Another advantage of the microspheres is that the tilmicosin release was adequately controlled in spite of the rapid initial release rate (during 6h), so that the serum concentrations did not reach clinically obvious toxic levels (Olbrich *et al*., 2002). Finally, AUC of drug following administration of microsphere formulation is six times as much as that of the drug tilmicosin. It has been reported the minimum plasma concentration of tilmicosin is 0.312μg/mL (Guo *et al*., 2014). So the results from the fig 8 can be conclusion that the range is 1–42 h over the minimum concentration.

CONCLUSION

In the present study, the antibiotic tilmicosin was loaded onto ion exchange resin for the preparation of DRC microsphere by the emulsion solvent diffusion technique. The microsphere gave better taste mask than DRC. The in vivo studies in rats indicated that the bioactivity of tilmicosin maintained during the preparation procedure. The release of tilmicosin from microspheres is well-controlled by the adjustment of the DRC weight gain from RS/RL 100. The results indicate that tilmicosin sustained-release microsphere has eliminated slowly with long half-life in serum by oral administration the sustained-release, and the effect of the sustained-release microspheres is obvious.

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