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Preparation and evaluation of alendronate sodium solid lipid nanoparticles with high oral bioavailability in rats

Ruijin Geng[1#], Le Wu [1,2#], Haipeng Wang[1], Shuilin Sun[1], Huiming Huang[1], Yuanzhen Xiong[1] and Baogang Xie[1,2*]

[1]School of Pharmaceutical Science, Nanchang University, Nanchang, China
[2]Department of Pharmaceutics, School of Medicine, Jiaxing University, Jiaxing, China

Abstract: Low oral bioavailability of alendronate sodium (ALE) significantly limits its clinical application. However, few studies focus on preparing ALE solid lipid nanoparticles (ALE-SLNs) and investigating its oral bioavailability *in vivo* due to highly hydrophilic property of ALE. In this study, ALE-SLNs were prepared through high-speed shearing combined with ultrasonic treatment method. ALE-SLNs were evaluated by average particle size, electric potential, encapsulation efficiency (EE), and drug loading (DL). Our results showed that the average EE and DL reached 62.56±0.94% and 6.26±0.09% (n=3), respectively. 120.27±1.17nm, 0.29±0.13 and -19.1±0.27 mV (n=3) were obtained in the average particle size, polydispersity index and zeta potential, respectively. The stability test showed that ALE-SLNs remained stable for more than 2 months at 4°C. After oral administration of ALE-SLNs (4.5mg/kg), the bioavailability was 2.17 times higher than that of ALE solution (86.82±3.6 vs 40.1±1.3μg) in rats. Our results indicate that high-speed shearing combined with the ultrasound method is simple and rapid to prepare ALE-SLNs. SLNs can improve the oral delivery of ALE in rats, which may exert beneficial effects in clinical applications.

Keywords: Alendronate sodium, solid lipid nanoparticles, preparation, oral bioavailability, urinary excretion of drug.

INTRODUCTION

Alendronate sodium (ALE, fig. 1) is a third-generation amino bisphosphonate inhibitor of bone resorption. ALE can reduce the number of osteoclast cells and inhibit osteoclast cell activity to inhibit bone transformation and increase bone density. Tablets of ALE are mainly used for treating osteoporosis and hypercalcemia in postmenopausal women after oral administration (Luo *et al.,* 2014; La-Beck *et al.,* 2019), which is mainly absorbed by the small intestine. However, the high polarity of ALE due to the P-C-P bonds in the molecular structure results in very low oral bioavailability. The absolute bioavailability of oral ALE is reported to be lower than 1.0% (Hosny 2016a; Nakaya *et al.,* 2016).

The plasma concentrations of ALE are commonly low because of the low oral bioavailability (below 1%). ALE is mostly excreted in urine by prototypes, thus, ALE bioavailability is usually evaluated by cumulative excretion of the drug in urine (Kline and Matuszewski 1992; Nakhla *et al.,* 2011). Considerable work has been conducted to improve the oral bioavailability of ALE. Studies have shown that oral permeability enhancer, such as the ethylene diamine tetraacetic acid (Janner *et al.,* 1991), lauryl sodium sulfate (Boulenc *et al.,* 1995), sodium caprate (Maher *et al.,* 2009), and palm acyl carnitine chloride (Raiman *et al.,* 2003), can improve the bioavailability of ALE. The mechanism of mainly involves enhancing the permeability of the gastrointestinal cellular membrane; this effect changes membrane fluidity or promotes the paracrine pathway to boost the transdermal absorption capacity of bisphosphonates. However, the majority of oral permeability enhancer, especially oral absorption enhancers such as surfactants, can promote the absorption and transport of drugs and affect the cell membrane barrier to cause cell toxicity or adverse reactions. Hence, these methods are only limited to the research stage. Ezra A *et al.,* (Ezra *et al.,* 2000) amidated ALE and proline phenylalanine to modify a small-intestine oligopeptide transporter-mediated prodrug and the results showed that ALE concentrations in the bone and urine were 3.3 and 1.9 times higher, respectively, than those of ALE. Although this method can improve the oral bioavailability of ALE, the preparation process is complex.

Solid lipid nanoparticles (SLNs) are a new kind of nanodrug delivery system. SLNs have received remarkable research attention owing to their good biocompatibility, stability, targeting and sustained-release function (Li *et al.,* 2019; Partridge *et al.,* 2019; Ban *et al.,* 2020). To date, very few reports have examined the SLNs of ALE. Here, we used high-speed shearing combined with the ultrasonic treatment method to prepare ALE-SLNs. Particle size, zeta potential, encapsulation efficiency (EE), and drug loading (DL) were as the evaluation indexes for ALE-SLNs. The urinary pharmacokinetic parameters were determined and compared in rats after oral administration of ALE-SLNs and ALE solution.

**Corresponding author:* e-mail: xiebaogang49@zjxu.edu.cn

[#]Equal contributions to this work

MATERIALS AND METHODS

Chemicals and instruments

ALE, Poloxamer 188 and Behenic acid glycerides were provided by Aladdin Reagent Co. Ltd. Soybean lecithin was purchased from the Sigma-Aldrich (United States). Triethylamine was purchased from Agilent Reagents Co. Ltd. Phenylisothiocyanate was purchased from Da Mao Reagents Co. Ltd.

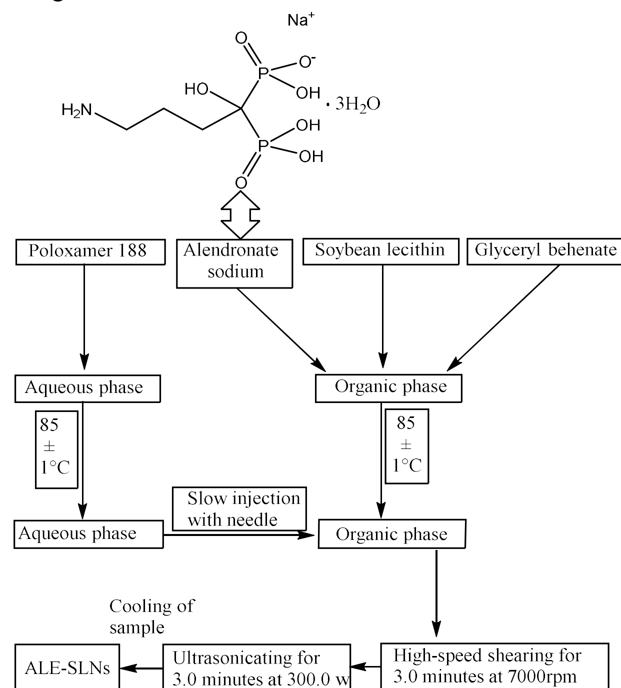

Fig. 1: Schematic of ALE-SLNs preparation.

The Agilent 1260 infinity liquid chromatograph used in the study was purchased from the Agilent Co (United States). An SCIENTZ-II Ultrasonic cell grinder was obtained from Andy Company (United States). V dispersion machine (ULTRA-TURRAX basic T18) was purchased from Ningbo Scientz Co. Ltd.

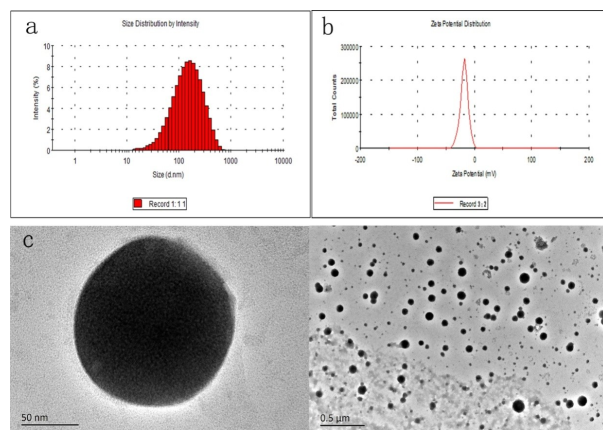

Fig. 2: Characterization of ALE-SLNs. (a) Size distribution of ALE-SLNs. (b) Zeta potential distribution of ALE-SLNs. (c) TEM photograph of ALE-SLNs.

Preparation of ALE-SLNs

ALE-SLNs were prepared by high-speed shearing combined with the ultrasonic treatment method. Fig. 1 shows the preparation process of ALE-SLNs. In brief, accurately weighed ALE was dissolved in 0.4mL anhydrous ethanol and then added to melted glyceryl behenate and soybean lecithin mixture, heated at 85°C in water bath and stirred to form the organic phase. A prescribed amount of Poloxamer 188 was dissolved in 10.0mL distilled water (85°C) to form the aqueous phase. The water phase was slowly injected into the oil phase, continuously sheared in 7000.0 rpm for 3.0min and disrupted for 3.0min by 300.0W ultrasound. After cooling the suspension at room temperature, ALE-SLNs were obtained.

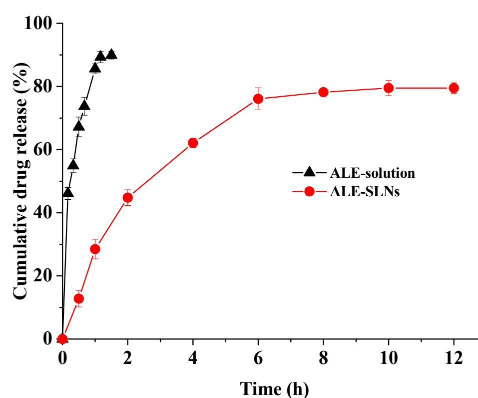

Fig. 3: *In vitro* ALE release profile (n=3)

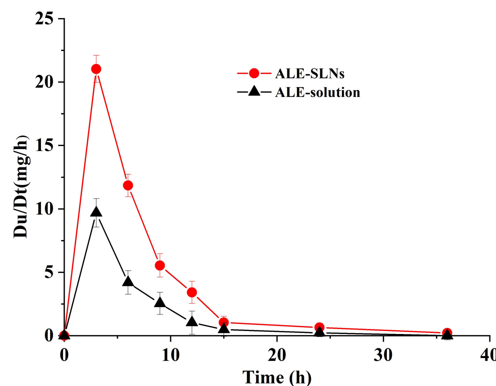

Fig. 4: Urinary alendronate sodium concentration–time profiles after the oral administration of ALE-SLNs and ALE-solution in rats (n=6).

Particle size, zeta potential and particle shape

Newly prepared ALE-SLNs were diluted with distilled water to the appropriate concentration. The particle suspension was then dripped into a special copper network and dried with 2% phosphor-tungstic acid. After drying, the surface morphology of ALE-SLNs was observed under a transmission electron microscope (JEM-2100, JEOL, Tokyo, Japan).

The particle size and zeta potential were determined by

dynamic light scattering 156 at a scattering angle of 90° at 25°C using a Malvern Mastersizer apparatus (PSA NANO2590, Malvern Instruments, Malvern, UK) and 3 measurements were carried out for each sample.

Determination of ALE concentration in vitro samples

The concentration of ALE *in vitro* was determined by HPLC-UV method. Conditions of HPLC Analysis: Venusil AA analytical column (4.6 × 250mm, 5μm); mobile phase: Acetate sodium-acetonitrile solution (10 mM, pH 6.5, solvent A) -80% acetonitrile (solvent B); gradient elution: 0-10min, 100% A; 10.1-12 min, 70% A; 12.1-15min, 50% A; 15.1-18 min, 0% A; 18.1-20 min, 100% A; 20.1-25 min, 100% A. Norleucine was used as internal standard (IS).The flow rate was 0.8mL/min, the column temperature was 40°C, the injection volume was 10μL and the detection wavelength was set at 270 nm.

Calibration curve: 1.0mg/mL ALE control solution was prepared and was diluted to serial concentrations (5.0, 25.0, 50.0, 100.0, 200.0 and 250.0μg/mL) by distilled water. 200μL of a series of alendronate sodium solution was taken and derivatized according to our previous reported method (Wang *et al.,* 2017). 20μL of sample was injected for HPLC analysis.

Determination of EE and DL

EE refers to the ratio of the amount of drug in the nanoparticles to the total drug amount. DL denotes the ratio of the amount of drug in the nanoparticles to carrier dosage. Higher EE and DL values result in better nanoparticle quality. In this study, ultra filtration (Magenheim *et al.,* 1993; Schwarz and Mehnert 1999) was adopted to determine the EE and DL of ALE-SLNs. An accurate quantity of 1.0mL ALE-SLNs was placed in a super filter with a molecular weight cut-off of 10000 Da, centrifuged at 3000.0 rpm for 10.0min, eluted thrice with distilled water and combined with the eluent. Then the concentration of ALE was determined by the HPLC method and calculated the free ALE content. EE and DL of ALE-SLNs were respectively calculated as follows:

$EE = (W_{total} - W_{free}) / W_{total} \times 100\%$;

$DL = (W_{total} - W_{free}) / W_{lipid} \times 100\%$,

where W_{total} refers to the total amount of ALE, W_{free} denotes the free ALE and W_{lipid} represents the lipid dosage.

Recovery rate was achieved as follows. ALE solutions (1.0mL) with concentrations of 50.0, 100.0 and 200.0 μg/mL were separately loaded onto the super filter with a molecular weight cut-off of 10000 Da. The samples were centrifuged for 10 min at 3000 rpm, eluted thrice with distilled water and examined for ALE levels to calculate for recovery rate.

ALE release from ALE-SLNs in vitro

Initially, 5.0mL of ALE-SLNs and the same concentration of ALE solution were placed in a pretreated dialysate bag (MW cut-off of 12 kDa), with both ends of the truss tied in the release medium (100.0mL 0.9% sodium chloride solution), and mixed at 37°C with gentle agitation (100 rpm/min). At predetermined time intervals (0.5, 1, 2, 4, 6, 8, 10, 12, 14, 24, 36, 48 and 60h), 5.0mL of each sample was collected and immediately added with the same volume of the new release mediums. After derivatization, ALE content was measured by HPLC. Cumulative release rate (Q) was calculated on the basis of released dosage and the total amount of ALE; the *in vitro* release curve was drawn.

Study of the physical stability of ALE-SLNs

Newly prepared ALE-SLNs were separately placed in ampule bottles and kept in a dark place at 4°C and 25°C. On days 0, 30, 45, 60 and 90, particle appearance was observed and particle size and EE were measured.

Pharmacokinetic study in vivo

Twelve healthy male Sprague–Dawley rats were randomly divided into two groups. After fasting for 12 h before treatment, the animals were orally administered with ALE solution and ALE-SLNs at 4.5mg/kg each. Urine was collected after 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 24.0, 36.0, 48.0 and 60.0 h and centrifuged at 5000 rpm for 5.0 min. The volume of supernatant was recorded, and the supernatant was stored at -20°C. Pre-processing, derivatization of urine samples and ALE measurement by HPLCwere performed as described in our previous studies (Wang *et al.,* 2017). This animal study was approved by Ethics Committee of the First Affiliated Hospital of Nanchang University (No. 063-2017).

STATISTICAL ANALYSIS

The data obtained was analyzed by Analysis of variance (ANOVA) technique or independent sample t test using SPSS version 19 statistical computer software at 5% probability. After a single oral administration of ALE solution and ALE-SLNs, a urinary excretion pharmacokinetic parameters of ALE were estimated as follows: Logarithmic transformation ($Log\Delta u/\Delta t$) is plotted against time with the amount of drug excreted per unit time and the linear regression of the elimination phase is performed to obtain the linear slope K. $T1/2 = 0.693/K$ is used to calculate the alendronate sodium *in vivo*.

RESULTS

Formulation optimization

To obtain the best quality of ALE-SLNs, we investigated the dosage of behenic acid glycerides, soybean lecithin, Poloxamer 188, and ALE on the basis of particle size and EE (table 1). Single-factor analysis was carried out at high-speed shearing rate, shearing time, ultrasonic power.

Table 1: The optimization of ALE-SLNs formulation (Mean ± SD, n=3)

Formulation code	Glyceryl behenate (mg)	ALE (mg)	Soybean lecithin (mg)	poloxamer 188 (mg)	EE (%)	Particle size (nm)
F1	10	1	10	20	32.70±3.14	262.31±9.65
F2	10	3	20	30	23.70±4.33	198.22±9.13
F3	10	5	25	50	34.53±2.61	230.64±7.57
F4	20	1	30	30	36.97±3.42	138.54±5.23
F5	20	3	20	50	21.45±5.08	130.97±4.96
F6	20	5	10	20	43.81±3.53	142.43±6.61
F7	25	1	25	20	26.97±2.71	108.72±3.48
F8	25	3	50	30	60.73±6.14	102.62±4.85
F9	25	5	30	50	59.57±3.76	98.78±4.16
F10	30	1	25	20	51.45±5.82	118.74±2.05
F11	30	3	20	50	62.56±2.82	120.27±1.17
F12	30	5	50	30	50.34±7.13	125.70±2.01
F13	50	1	30	20	53.45±1.97	136.49±3.71
F14	50	3	50	50	58.77±6.56	127.90±3.13
F15	50	5	25	30	51.16±4.43	131.11±3.38

Table 2: The optimization of ALE-SLNs preparation process (Mean ± SD, n=3)

		Particle size (nm)	PDI
Shearing rate (rpm)	4000	134.54±3.05	0.32±0.24
	7000	120.27±1.17	0.29±0.13
	10000	109.98±1.96	0.30±0.11
Shearing time (min)	3	120.27±1.17	0.29±0.13
	5	117.86±0.32	0.29±0.17
	8	123.18±1.61	0.29±0.60
Ultrasonic power (w)	300	120.27±1.17	0.29±0.13
	500	120.31±0.92	0.29±0.26
	800	119.24±1.13	0.31±0.37
Ultrasonic time (min)	3	120.27±1.17	0.29±0.13
	5	118.91±1.12	0.30±0.10
	8	118.53±3.61	0.30±0.25

Table 3: The recovery of ultrafiltration method (n=3)

No	50.0 (μg/ml)	100.0 (μg/ml)	200.0 (μg/ml)
1	79.56	81.27	80.95
2	80.34	80.29	79.31
3	79.77	81.31	80.86
Mean ± SD	79.89 ± 0.40	80.96 ± 0.58	80.37 ± 0.92

Table 4: Stability of ALE-SLNs at 4°C and 25°C (Mean ± SD, n=3)

Time (day)	4 °C			25 °C		
	Appearance	Particle size(nm)	EE (%)	Appearance	Particle size (nm)	EE (%)
0	Blue opalescent, clarified	120.27±2.11	62.56±1.12	Blue opalescent, clarified	120.27±1.89	62.56±1.34
30	Blue opalescent, clarified	120.59±2.56	62.15±1.73	Blue opalescent, clarified	126.31±2.87	58.27±1.67
45	Blue opalescent, clarified	122.71±2.63	61.62±1.98	Small amount of flocculation	180.19±2.60	34.74±2.58
60	Blue opalescent, clarified	123.89±2.02	60.34±2.05	Precipitation	265.98±3.75	23.45±1.07
90	Small amount of flocculation	129.56±2.86	59.93±1.76	-	-	-

Table 5: The urinary pharmacokinetic parameters after oral administration of ALE-Solution or ALE-SLNs in rats (4.5 mg/kg, n=6)

Parameter	ALE-Solution	ALE-SLNs
$\Delta u/\Delta t_{max}$ ($\mu g \cdot h^{-1}$)	22.4 ± 1.8	*49.62±0.58
T_{max} (h)	2.34	2.63
$T_{1/2}$ (h)	5.1 ± 0.22	4.87±0.26
Total drug amount excreted into urine after 36 hours (μg)	40.1 ± 1.3	*86.82±3.6

Note: $\Delta u/\Delta t_{max}$, the maximum excretion peak concentration. T_{max}, the time to reach the maximum excretion peak concentration. $T_{1/2}$, elimination half-time.
*Indicating a significant statistical difference between ALE-SLNs and ALE-Solution in rats ($p<0.05$, independent sample t test)

Particle size and polydispersity index were used as evaluation indexes (table 2). Optimization results achieved the following final prescription: 30.0mg behenic acid glycerides, 20.0mg soybean lecithin, 50.0mg Poloxamer 188 and 3.0mg ALE. Shearing rate, shearing time, ultrasonic power, and ultrasonic time measured 7000rpm, 3min, 300W and 3 min, respectively.

Particle size, zeta potential and particle morphology

According to the optimum formulation and process, three batches of ALE-SLNs were prepared. Average particle size, polydispersity index, and zeta potential reached 120.27±1.17 nm, 0.29±0.13 and -19.1±0.27 mV (n = 3), respectively. Fig. 2 show the average particle size and zeta potential value of the prepared ALE-SLNs, respectively. The morphology of ALE-SLNs was observed by transmission electron microscopy (fig. 2) and results showed the spherical shape of the prepared ALE-SLNs.

Determination of ALE concentration in vitro

Determination of ALE was conducted by HPLC-UV method previous reported (Wang *et al.,* 2017). The chromatograms of ALE The retention times of ALE and IS were 9.9 and 14.8 min, respectively. The calibration curve of ALE was linear over low (5.0μg/mL) and high concentration (250.0μg/mL) ranges. Calibration curve equation: $y = 0.0032x - 0.0164$, $R^2 = 0.999$.

EE and DL

In this study, the EE and DL of the nanoparticles were measured by ultrafiltration. The experimental results of recovery rate (table 3) showed that the ultrafiltration membrane showed no ALE adsorption and can be used for obtaining the EE and DL of the ALE-SLNs. Under the optimum formulation, average EE of the batch samples totaled 62.56±0.94% and DL was 6.26±0.09%.

Study of drug release in vitro

The *in vitro* release profiles of ALE from the ALE-SLNs and ALE solution were studied by the method mentioned above. The cumulative release rate of the drug in the solution group reached 90% after 1 h (fig. 3), whereas that in the nanoparticle group was 27%. The drug was incompletely released until 12h, indicating that ALE-SLNs exhibit a good sustained release effect *in vitro*.

Study of the physical stability of ALE-SLNs

Table 4 provides the results on ALE-SLN stability. Temperature significantly affected the stability of the nanoparticles. ALE-SLNs can be stabilized for more than 2 months at 4°C but can only be preserved for about 1 month at 25°C. The results showed that the nanoparticles were more stable at low temperature than at high temperature, the sameas those described in previous studies (Dolatabadi *et al.,* 2014; Hosny 2016b). The ALE-SLNs prepared by Dolatabadi *et al.,* (Dolatabadi *et al.,* 2014) remained stable for 4 weeks at 4°C, whereas those prepared in this study can stabilize for 2 months at 4°C. Therefore, ALE-SLNs preparation by high-speed shearing combined with the ultrasound method holds the potential to magnify the production of the drug.

Pharmacokinetic study in rats

After a single oral administration of ALE solution and ALE-SLNs, a pharmacokinetic curve was drawn with the amount of drug excretion per unit time ($\Delta u/\Delta t$) as the longitudinal coordinates and time as the abscissa (fig. 4). table 5 shows the pharmacokinetic parameters. ALE was absorbed rapidly in the rats, and maximum excretion peak was reached at 2h after administration. The maximum excretion concentration (49.62±0.58μg/h) in the nanoparticle group was 2.22 times that of the solution group (22.4±1.8μg/h). ALE solution was eliminated faster than ALE-SLNs in the rats. The half-life of the solution group was 5.1±0.22 h, and cumulative excretion in urine after 36 h was 40.1±1.3μg, accounting for 4.5% of the dosage and is higher than that reported in literature(Yang *et al.,* 2002; Yun and Kwon 2006; Men *et al.,* 2010). The results may be related to the low dosage and intake at an empty stomach. The half-life of nanoparticles was 4.87± 0.26h and cumulative excretion in urine after 36 h reached 86.82±3.6μg, which accounted for 12.1% of the dosage. Under the same concentration of the ALE solution, the oral ALE-SLNs increased in bioavailability by 2.17 times.

DISCUSSION

In this study, different surfactants (Tween 20, 60, and 80 and Poloxamer188) and lipid materials (glycerol monostearate, stearic acid, and behenic acid glycerides) to prepare ALE-SLNs were selected and optimized. Results

showed that Poloxamer 188 as surfactant, behenic acid glyceride as lipid material, soya bean lecithin and ALE were the optimum raw materials for preparing ALE-SLNs. Particle size is a key factor affecting absorption by the gastrointestinal tract. A previous study (Xiao *et al.,* 2013) showed that in rats that orally received radio-labeled gold particles, 0.05±0.01% of the 5 nm particles, 0.03±0.01% of the 80 nm particles, and 0.01±0.00% of the 200 nm particles entered circulation 24h after administration. In this study, we prepared ALE-SLNs with a particle size of about 120nm by high-speed shearing combined with the ultrasound method. The size is 114 nm smaller than that of the particles prepared by double emulsification/solvent evaporation (Posadowska *et al.,* 2015), indicating improved levels in circulation might be achieved.

Pharmacokinetic experiments were conducted to evaluate the oral bioavailability of ALE-SLNs. Given that ALE exhibits strong polarity and difficultly penetrates the cell membrane, its blood concentration is very low after oral intake. Bioavailability is usually evaluated by measuring the cumulative excretion of drugs in urine(Kline and Matuszewski 1992). After treatment of ALE-SLNs (4.5mg/kg) in rats, the urinary cumulative excretion of 36 h was 2.17 times higher than that of ALE solution in this study.

In previous studies (Hosny 2016b), the bioavailability of ALE enteric-coated SLNs in male rabbits administered by intravenous injection was 7.4 times higher than that of oral ALE tablets; however, no bioavailability data were compared with those of the solution formulations. Han, H.K *et al*.(Han *et al.,* 2012) fabricated chitosan liposome to improve oral ALE absorption and observed that the bioavailability of ALE with chitosan liposome increased by 2.6 times relative to that under the same concentration of nonmodified ALE. The value of bioavailability is close to those found in our study. However,, their preparation processes were complex and time-consuming and hence not conducive for large-scale production. Preparing ALE-SLNs by high-speed shearing combined with the ultrasound method is simple and more rapid than the above-mentioned techniques. Moreover, the proposed method requires no special instrument and equipment and thus remarkably reduces operational costs.

CONCLUSION

ALE-SLNs were prepared by simple high-speed shearing combined with the ultrasonic method and evaluated by their particle size, potential, EE, and DL as indexes. Our results showed that the ALE-SLNs remained stable for more than 2 months at 4 °C. The oral bioavailability of ALE-SLNs was significantly higher than that of ALE solution in rats. Our study indicated that SLNs are an effective means for ALE delivery *in vivo*.

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