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Preparation and *in vitro* release of total alkaloids from alstonia scholaris leaf-loaded mPEG-PMA microspheres

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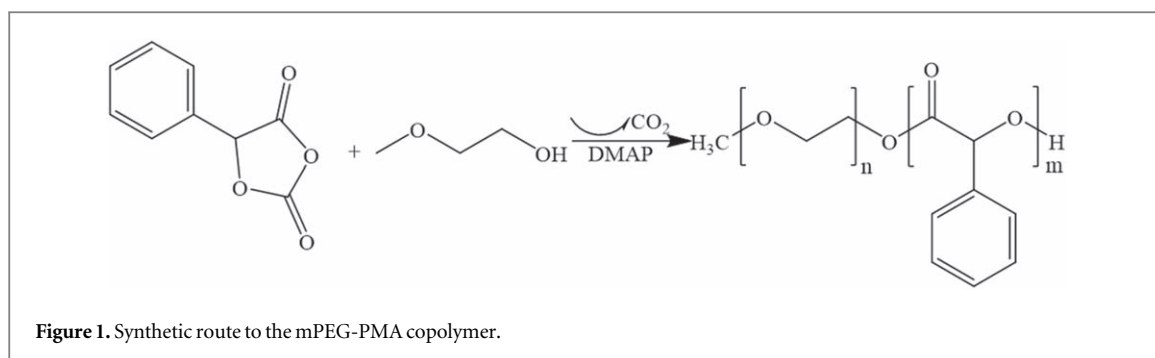
Abstract

The total alkaloids extracted from the leaves of *Alstonia scholaris* (ASAs) have been reported to reduce fever, remove phlegm, and relieve coughs. However, their drug half-lives are short. Thus, to obtain sustained-release preparations of total alkaloids from ASAs, mandelic acid oxanhydride (mandelic acid OCA) was synthesized by the reaction of L-mandelic acid (MA) with triphosgene, and subsequent copolymerization with polyethylene glycol monomethyl ether (mPEG) of different molecular weights yielded the corresponding mPEG poly-MA (mPEG-PMA) copolymers. ASAs-loaded microspheres were then prepared using the double emulsion method, and their *in vitro* release (15 d, 37 °C) and *in vitro* degradation behaviors were studied. The morphology, size, embedding efficiency, and drug loading efficiency were investigated for the prepared microspheres, and screening was carried out using the mPEG_{10K}-PMA drug-loaded microspheres to analyze their biological characteristics. Anti-inflammatory experiments using Kunming mice and Sprague Dawley rats showed that the microspheres exhibited good anti-inflammatory properties. Moreover, the ASAs-loaded microspheres exhibited a good biocompatibility, and the hemolysis rate was <5%.

1. Introduction

Alstonia scholaris (L.) R. Br. is a tree of the Chicken Bone Changshan genus of the Oleaceae, which is mainly distributed in the tropical regions of Asia and Africa [1]. Previously, Salim *et al* reported the isolation of indole alkaloids from *Alstonia Scholaris* leaves (ASAs) [2], while Luo discovered the most effective alkaloids present in *Alstonia Scholaris* leaves for the treatment of respiratory diseases [3–7]. However, the half-lives of the active compounds present in ASAs are short, thereby limiting their clinical application due to the requirement for multiple administrations.

Chitosan, sodium alginate, polylactic acid, and other biodegradable materials are widely used as drug carriers in drug delivery systems [8–14]. In addition, due to the mild conditions employed for the ring opening polymerization of oxanhydrides, which exhibit a favorable biocompatibility, their degradation rates can be controlled. These compounds are therefore expected to be applicable in controlled drug release or carrier therapies [15–19]. Furthermore, poly(mandelic acid) (PMA) and polylactic acid (PLA) are lipophilic polymers with good biodegradabilities and biocompatibilities, thereby permitting their use in drug delivery systems [20]. However, PMA exhibits a rather low solubility in water. In contrast, polyethylene glycol monomethyl ether (mPEG) has a good water solubility, and is currently employed in the preparation of amphiphilic block



polymers, drug-loaded micelles, and microspheres to improve the water solubilities of these lipophilic materials [21–27]. Based on previous studies, it is therefore expected that the problems associated with short drug half-lives and low drug utilization efficiencies in traditional drug delivery systems could be effectively improved by the use of sustained drug release preparations [28].

Thus, we here in report the use of mPEG-PMA as a carrier to prepare ASAs-loaded microspheres using the double emulsion method. The resulting mPEG-PMA copolymer is then characterized by proton nuclear magnetic resonance (^1H NMR) spectroscopy and gel permeation chromatography (GPC), and the *in vitro* release and degradation behaviors of the ASAs-loaded microspheres are studied. Finally, a series of analyses and biological experiments (e.g., hemolytic, anticoagulant, and cytotoxicity experiments) are carried out on the ASAs-loaded microspheres to confirm the suitability of mPEG-PMA for preparing ASAs-loaded sustained-release materials.

2. Method

2.1. Materials

L-Mandelic acid was provided by Chengdu McCarthy Chemical Co., Ltd. The ASAs were provided by the Kunming Institute of Botany, Chinese Academy of Sciences (batch number: 20180501). DMAP ($\text{C}_7\text{H}_{10}\text{N}_2$) was provided by GL Biochem Ltd (Shanghai, China). Deionized water is obtained by water machine (UPH-I-20T, Chengdu, China). HL-7702 normal liver cells were provided by the Shanghai Institute for Biological Sciences, Chinese Academy of Sciences. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was provided by the Kunming Cell Bank. Poly(ethylene glycol methyl ether) (Mw 2000, 5000, and 10000) and fetal bovine serum (FBS) were obtained from Adamas (Shanghai, China). An EDTA trypsin digestion solution and cyan streptomycin were provided by Solarbio. Kunming mice, Sprague-Dawley (SD) rats, and rabbits were purchased from Kunming Medical University. Shijiazhuang Kangli Pharmaceutical Co., Ltd. Provided the aspirin samples, while Yunnan Phytopharmaceutical Co., Ltd provided the Dengtaiye KeLi (DKL).

2.2. Preparation of the mPEG-PMA

Mandelic acid OCA (Mac-OCA) was synthesized as described in the literature [29, 30]. Dimethylaminopyridine (DMAP) was firstly employed as a catalyst to deprotonate the active proton of the terminal $-\text{OH}$ group of mPEG, which initiated the ring-opening polymerization of Mac-OCA. More specifically, mPEG (0.0002 mol), Mac-OCA (0.0202 mol), and DMAP (0.00007 mol) were added to a dry round-bottomed flask at a certain mass ratio along with a certain amount of dichloromethane (DCM), and nitrogen replacement of the environment was performed three times. The resulting mixture was then allowed to stir for >24 h at 25°C . After subsequent vacuum concentration, ethanol precipitation, and filtration, a pale-yellow viscous substance was obtained by vacuum drying over P_2O_5 for 24 h to give the mPEG-PMA copolymer. The synthetic process is shown in figure 1.

2.3. Characterization of mPEG-PMA

The ^1H NMR spectrum of mPEG-PMA was recorded at 400 MHz (Bruker 400 MHz, Germany) using CDCl_3 as the solvent. The molecular weight of the polymer was determined by GPC (Waters 2414, Waters Inc., Milford, MA, USA) using a polystyrene standard.

2.4. Preparation of the blank microspheres and the ASAs-loaded microspheres

All microspheres were synthesized as described in the literature [31].

Table 1. Particle sizes, embedding efficiencies, and loading efficiencies of the mPEG-PMA microspheres with different molecular weights.

Sample	Average diameter (μm)	EE (%)	LE (%)
mPEG _{2k} -PMA	1.593 $\pm$ 0.38	64.27 $\pm$ 2.21	3.01 $\pm$ 0.13
mPEG _{5k} -PMA	2.260 $\pm$ 0.42	69.39 $\pm$ 2.04	3.15 $\pm$ 0.17
mPEG _{10k} -PMA	3.290 $\pm$ 0.39	72.88 $\pm$ 1.87	4.37 $\pm$ 0.09

EE: encapsulation efficiency; LE: loading efficiency.

2.5. Microsphere morphology, particle size, and particle size distribution

The morphological characteristics of the mPEG-PMA microspheres and the blank microspheres were observed using scanning electron microscopy (SEM; NOVA NANOSEM-450, FEI, Hillsboro, OR, USA). A laser particle size analyzer (Mastersizer 2000, United Kingdom) was used to determine the particle size distributions of the microspheres. For this purpose, a small amount of the microsphere suspension was diluted in a 15 ml centrifuge tube to give an almost transparent solution.

2.6. Determination of the EE and LE values of the microspheres

The lyophilized microspheres (20 mg) were accurately weighed, dissolved in DCM (0.5 ml), and added to methanol (2 ml). Following centrifugation to collect the supernatant, filtration was carried out using a 0.45 μm microporous membrane, and an aliquot (20 μl) was injected into the chromatogram. The embedding efficiency (EE) and loading efficiency (LE) values were calculated according to the area normalization method, and all experiments were repeated in triplicate to obtain average values.

$$EE = (\text{weight1} / \text{weight2}) \times 100\%$$

where *weight 1* is the weight of the ASAs in the microspheres, and *weight 2* is the weight of the added ASAs.

$$LE = (\text{weight of ASAs in microspheres} / \text{total weight of microspheres}) \times 100\%$$

Table 1 shows the results for EE and LE.

2.7. In vitro release experiments of the ASAs-loaded microspheres

Initially, a sample (50 mg) of the lyophilized microspheres was placed in a centrifuge tube with PBS (5 ml), and oscillated at 37 °C. The centrifuge tube was removed at fixed time intervals. After centrifugation for 6 min, a sample (1 ml) was taken from the centrifuge tube and an aliquot (1 ml) of PBS was added prior to restarting oscillation. Determination of the ASAs present in the solution was then carried out by HPLC (Agilent-1220, California, America, C18 column, 5 μm , 250 mm $\times$ 4.6 mm; column temperature, 40 °C; mobile phase, 20% A (HCOOH:H₂O = 0.1:100) and 80%B (CH₃CN), 1 ml min⁻¹ flow rate), and the total release of ASAs was calculated according to the following formula. The release curve was obtained by plotting the drug release amount against time (d).

$$Q = C_n \times V_t + V_s \Sigma C_{n-1}$$

Q: total amount of ASAs released

C_n: ASAs concentration at time t

V_t: bulk volume of PBS

V_s: removal volume of PBS

2.8. In vitro degradation of the microspheres

As described in section 2.7, the supernatant was removed by centrifugation, and subsequently its pH value was determined. The microspheres were then rinsed with pure water and freeze-dried for 24 h at -50 °C. The weights of the microspheres were accurately determined using an analytical balance, and aliquots (3 mg) were subjected to GPC to determine their molecular weights. The microsphere weight and number average relative molecular weight Mn and pH value are then used to determine the degradation performance of the microspheres *in vitro* over time.

2.9. Hemolysis experiments and coagulation experiments of the microspheres

All trials were approved by the Laboratory Animals Ethics Committee of Yunnan Minzu University and were registered with the Kunming Science and Technology Bureau (SYXK (Yunnan) K2017-0001, January 16, 2017).

Sample preparation and application of the test methods for the hemolysis and coagulation experiments were carried out as described in the literature [31]

$$\text{Hemolysis rate(\%)} = (\text{Abs}_{\text{sample}} - \text{Abs}_{\text{negative control}}) / (\text{Abs}_{\text{positive control}} - \text{Abs}_{\text{negative control}}) \times 100\%$$

The anticoagulant properties of the samples were calculated using the following formula:

$$\text{BCI} = (I_0/I_w) \times 100\%$$

where I_0 is the relative absorbance after the desired contact time between the blood mixture, calcium chloride, and the sample, and I_w is the relative absorbance after mixing the blood with a desired quantity of deionized water.

2.10. Cytotoxicity of the microspheres

Pulmonary cell lines were inoculated into flasks and cultured in DMEM high-sugar medium. After incubation in an incubator containing 5% CO_2 at 37 °C, the cells showed monolayer adherence growth and were sub-cultured every 3–5 d. Finally, the cells were digested and sub-cultured with 0.25% trypsin.

HL-7702 cells (5×10^4 cells/ml), logarithmically grown at $180 \mu\text{g l}^{-1}$, were inoculated into 96 well plates. After overnight incubation, 20 μl samples (5 mg ml^{-1}) were added, and three concentration gradients were set up with three multiple holes at each concentration. An aliquot (20 μl) of 5 mg ml^{-1} MTT was added to each well after 24 h and incubation carried out for 4 h. After this time, the medium was removed, and DMSO (0.15 ml) was added to quench the reaction. The absorbance at 490 nm was measured using an enzyme label (Molecular Devices, Spectra Max iD3). The survival rate was calculated using the following formula [32, 33]:

$$\text{Survival rate} = (\text{OD}_{\text{specimen}}/\text{OD}_{\text{control}}) \times 100\%$$

2.11. Statistical analysis

Plots were drawn using GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA 92037, USA), and all data were analyzed using one-way ANOVA. The results are expressed as mean $\pm$ standard deviation ($\bar{X} \pm s$). When $*P < 0.05$, the difference between the groups is considered significant; where $**P < 0.01$, the difference between the groups is considered extremely significant [34].

2.12. Anti-inflammatory liveness test of the ASAs-loaded microspheres

2.12.1. Effect of p-xylene on auricle swelling in mice

According to their body weight and sex, 35 mice (20 ± 2 g) were divided into seven groups containing five mice. These groups were named Gm_1 (aspirin group), Gm_2 (control group), Gm_3 , Gm_4 , Gm_5 , Gm_6 , and Gm_7 . The Gm_1 group was administered aspirin once per day on the experimental day, while the other groups were administered aspirin once per day for three consecutive days. The Gm_2 group was administered 1% CMC-Na to a level of 20 ml kg^{-1} . After 30 min, xylene (0.05 ml) was evenly applied to the right ear of each mouse in each group. The left ear is not painted as a control. At a time of 1 h after inflammation, the animals were sacrificed for cervical spondylolisthesis. The same area of each ear was cut using a perforator measuring 10 mm in diameter, and the difference in weight between the ears was taken as the degree of swelling.

2.12.2. Effect of egg white on paw swelling in rats

Thirty-five SD rats (200 ± 20 g) were randomly divided into seven groups according to the previous grouping method and group assignments. The Gr_1 group was administered egg white by gavage only once on the experimental day, while the other groups were administered egg white once per day for three consecutive days. The Gr_2 group was administered an equal volume of 1% CMC Na by gavage, wherein the gavage volume of each group was 10 ml kg^{-1} . An aliquot (0.01 ml) of egg white was injected subcutaneously at the end of each test period. The foot volume of each mouse was measured before inflammation, and at times of 0.5, 1, 2, 3, 4, and 5 h after inflammation. The difference in the foot area before and after inflammation was taken as the degree of swelling, and the swelling rate was calculated using the following formula:

$$\text{Swell} = ((A_{t_1} - A_{t_0})/A_{t_0}) \times 100\%$$

A_{t_0} : foot area before injection

A_{t_1} : foot area after injection

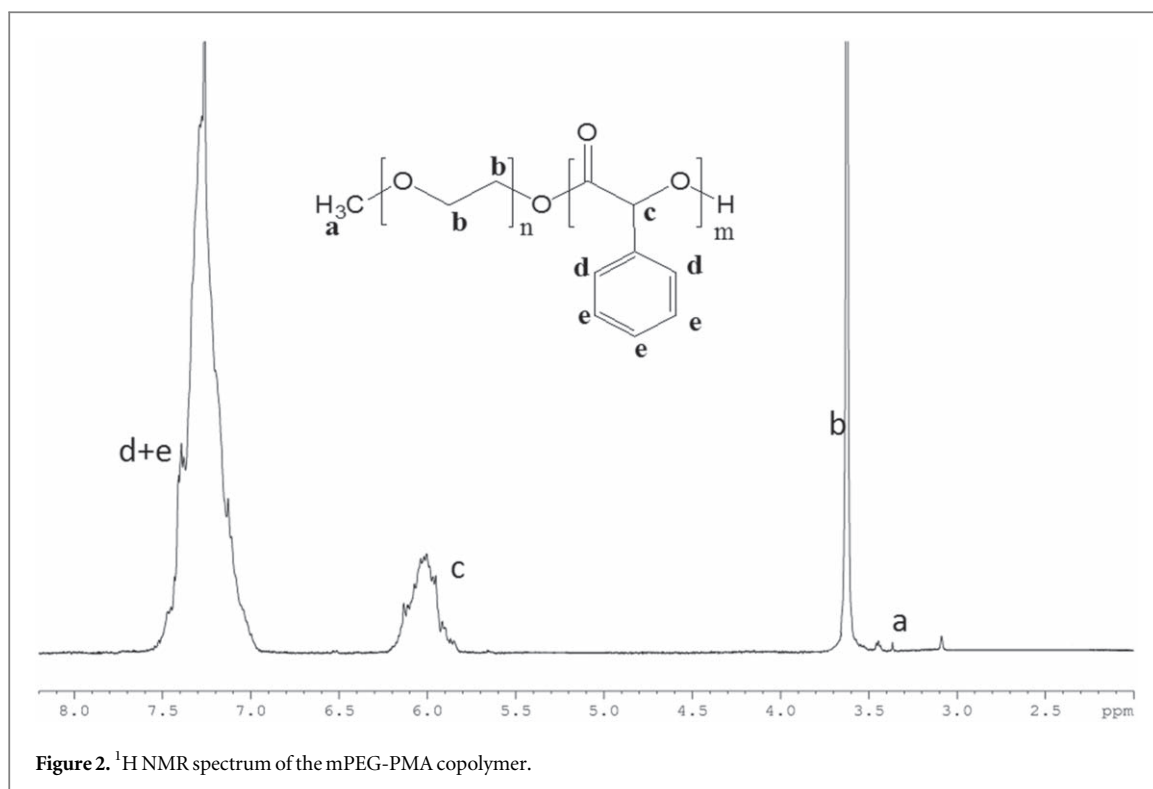


Table 2. Distribution of the Mn, Mw, and PDI of the prepared mPEG-PMA samples.

Sample	Mn ^a	Mn ^b	Mw ^a	PDI ^a
mPEG _{2k} -PMA	3321	3389	4019	1.21
mPEG _{5k} -PMA	5375	5465	6343	1.18
mPEG _{10k} -PMA	11706	12091	13345	1.14

^a Determined by GPC via a universal calibration curve and appropriate Mark-Houwink parameters for THF.

^b Calculated by ^1H NMR spectroscopy.

Mn: number-average molecular weight; Mw: weight-average molecular weight.

PDI: The polydispersity index of the molecular weight distribution.

3. Results and discussion

3.1. Characterization of the mPEG-PMA copolymer

As shown in figure 2, the ^1H NMR results were consistent with those of previous studies. The molecular weights (Mn and Mw) of the mPEG_{10k}-PMA copolymer determined by GPC (Waters 2414) are shown in table 2. More specifically, the Mw of the copolymer was 13345 Da, the molecular weight distribution was narrow, and the polydispersity index (PDI) was 1.14.

3.2. Morphological characterization and particle size distribution analysis of the microspheres

Figure 3 shows the SEM images of the ASAs-loaded microspheres, where it can be seen that the spherical shape of the mPEG_{2k}-PMA microspheres in figure 3(A) is superior, the surface is smooth, and the particle size is smaller, but there are also a small number of microspheres with a large particle size and reduced adhesion. Figure 3(B) shows the SEM images of the mPEG_{5k}-PMA microspheres, where a suitable size, smooth surface, and good adhesion can be observed. In addition, figure 3(C) shows the SEM images of the mPEG_{10k}-PMA microspheres, which exhibit relatively large particles of different sizes. In this case, it was expected that the presence of small amounts of residual organic solvents may have caused aggregation of the microspheres. As indicated in figure 4, the particle sizes of the mPEG_{2k}-PMA, mPEG_{5k}-PMA, and mPEG_{10k}-PMA microspheres were 1.593 ± 0.38 , 2.26 ± 0.42 , and 3.29 ± 0.39 μm , respectively. Although Widder *et al* [35] reported that the intravascular

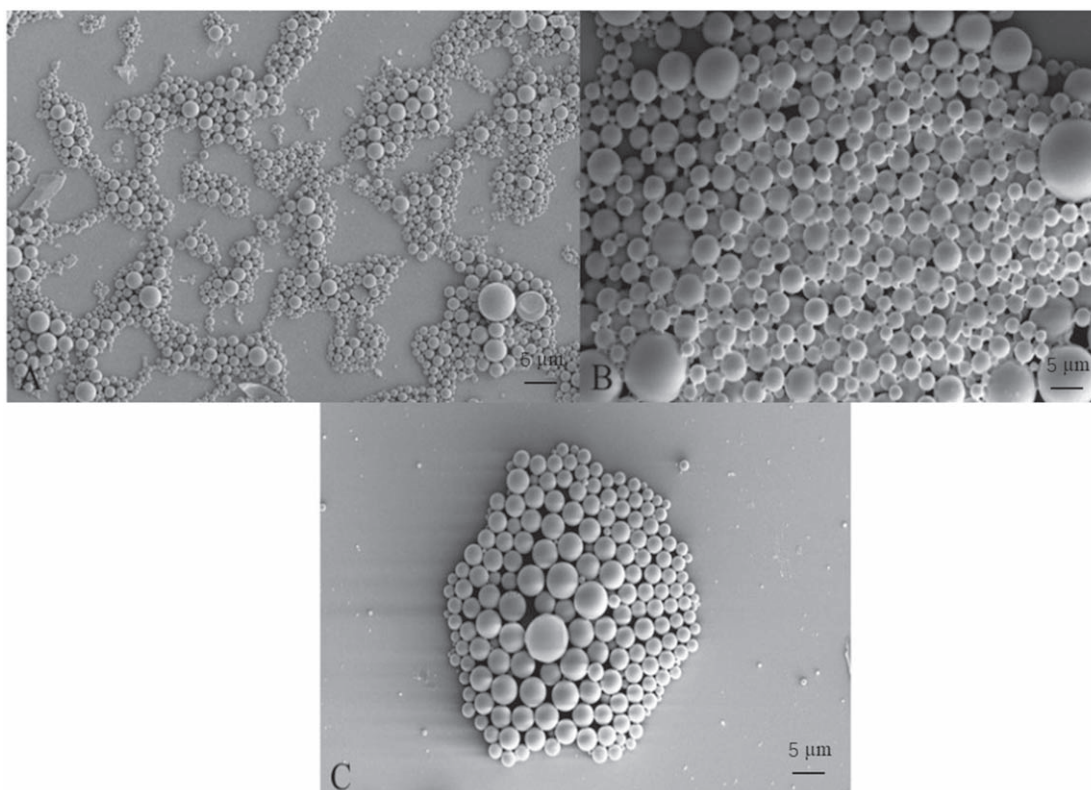


Figure 3. SEM images of the ASAs-loaded mPEG-PMA microspheres ((A): mPEG_{2k}-PMA microspheres; (B): mPEG_{5k}-PMA microspheres; (C): mPEG_{10k}-PMA microspheres).

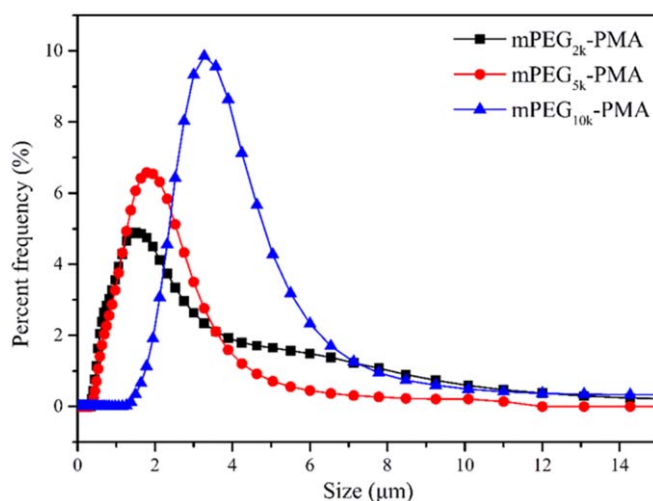


Figure 4. Size distributions of the ASAs-loaded mPEG-PMA microspheres.

injection of microspheres measuring $<1.4 \mu\text{m}$ in diameter did not result in pulmonary embolism, the diameters of the microspheres prepared herein were larger than $1.4 \mu\text{m}$, thereby indicating their potential suitability for use in non-injection drug delivery or oral administration [36].

3.3. Encapsulation rates and drug loading properties of the microspheres

The mPEG_{2k}-PMA, mPEG_{5k}-PMA, and mPEG_{10k}-PMA drug-loaded microspheres were prepared according to the double emulsion method [29] to give EEs of 64.27 ± 2.21 , 69.39 ± 2.04 , and $72.88 \pm 1.87\%$, in addition to LEs of 3.01 ± 0.13 , 3.15 ± 0.17 , and $4.37 \pm 0.09\%$, respectively. Furthermore, it was found that with an increase in the polymer molecular weight, chain length, diameter, and microsphere inner space, the EE and LE also increased, which is consistent with previous research results [31, 37].

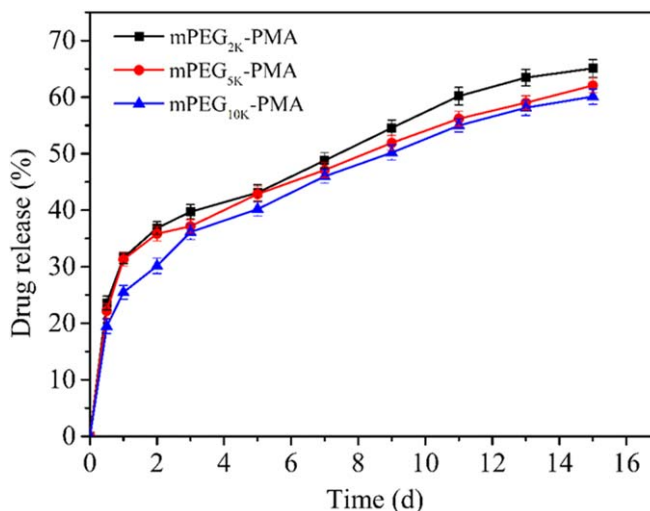


Figure 5. *In vitro* release curve of the ASAs-loaded mPEG-PMA microspheres.

3.4. *In vitro* release analysis of the ASAs-loaded microspheres

Figure 5 shows the *in vitro* release curves obtained for the ASAs-loaded microspheres. As indicated, cumulative release of the mPEG_{2k}-PMA microspheres reached $31.55 \pm 1.01\%$ within 24 h, and could be sustained at a stable level over 15 d. After this time, the total release reached $65.09 \pm 1.54\%$. In addition, over 24 h, the cumulative release of the mPEG_{5k}-PMA microspheres reached $31.28 \pm 1.16\%$, and this was sustained over 15 d, with the total release in this case reaching $62.07 \pm 1.29\%$. Furthermore, the cumulative release of the mPEG_{10k}-PMA microspheres reached $25.48\% \pm 1.21\%$ within 24 h and could be sustained at a stable level over 15 d. After this time, the total release reached $60.1 \pm 1.34\%$. Due to the fact that the drugs were embedded in the polymer materials via a physical method, and subsequent drug release was conducted through the degradation and dissolution of the polymer materials, it was found that upon increasing the size of the microspheres, the drug release rate initially decreased, and so this was attributed to the first degradation of the microspheres with a large specific surface area and a small drug loading [38]. Due to the slower rate of polymer degradation, the release rate subsequently slowed down.

3.5. Degradation analysis of the microspheres *in vitro*

Based on the drug loading, morphology, embedding efficiency, and *in vitro* release results, mPEG_{10k}-PMA was chosen as the optimal microsphere for this study. Thus, figure 6 shows the degradation process of the ASAs-loaded mPEG_{10k}-PMA microspheres, wherein the variation in the molecular number, pH value, dry mass with time and SEM image of mPEG_{10k}-PMA microspheres after 60 days of degradation are shown. More specifically, in PBS (pH = 7.40, 10 mM), the curve showed a downward trend over time. After 60 d, the pH value of the mPEG_{10k}-PMA microspheres decreased from 7.40 to 5.42, the dry weight loss of the microspheres was 63.61%, and the M_w decreased from 11706 to 7446 Da. During this time, the lipid bonds present in the polymer were hydrolyzed, resulting in a decrease in the molecular weight and the dry weight, in addition to the production of mandelic acid, which in turn reduced the pH. After 60 days of degradation, the surface of the microspheres is no longer smooth, and the microspheres are bonded.

3.6. Hemocompatibility of the microspheres *in vitro*

3.6.1. *In vitro* hemolysis

Figure 7 shows the hemolytic activities of the total alkaloids from the ASAs, the mPEG_{10k}-PMA microspheres, and the ASAs-loaded mPEG_{10k}-PMA microspheres. It can be seen from the figure that the hemolysis rates of the total alkaloids increased upon increasing the sample concentration. More specifically, the hemolysis rates of the mPEG_{10k}-PMA microspheres loaded with the total alkaloids were 1.33, 2.48, and 3.80% at concentrations of 0.4, 4, and 40 $\mu\text{g ml}^{-1}$, respectively. These results showed that erythrocyte destruction by the microspheres was low at these concentrations, and the obtained values are in accordance with the ISO hemolysis standard [39].

3.6.2. *In vitro* coagulation

Figure 8 shows the anticoagulant indices of the total alkaloids from the ASAs, the mPEG_{10k}-PMA microspheres, and the ASAs-loaded mPEG_{10k}-PMA microspheres. As shown, the blood clotting index (BCI) values of the mPEG_{10k}-PMA microspheres loaded with the total alkaloids at levels of 0.4, 4, and 40 $\mu\text{g ml}^{-1}$ were higher than

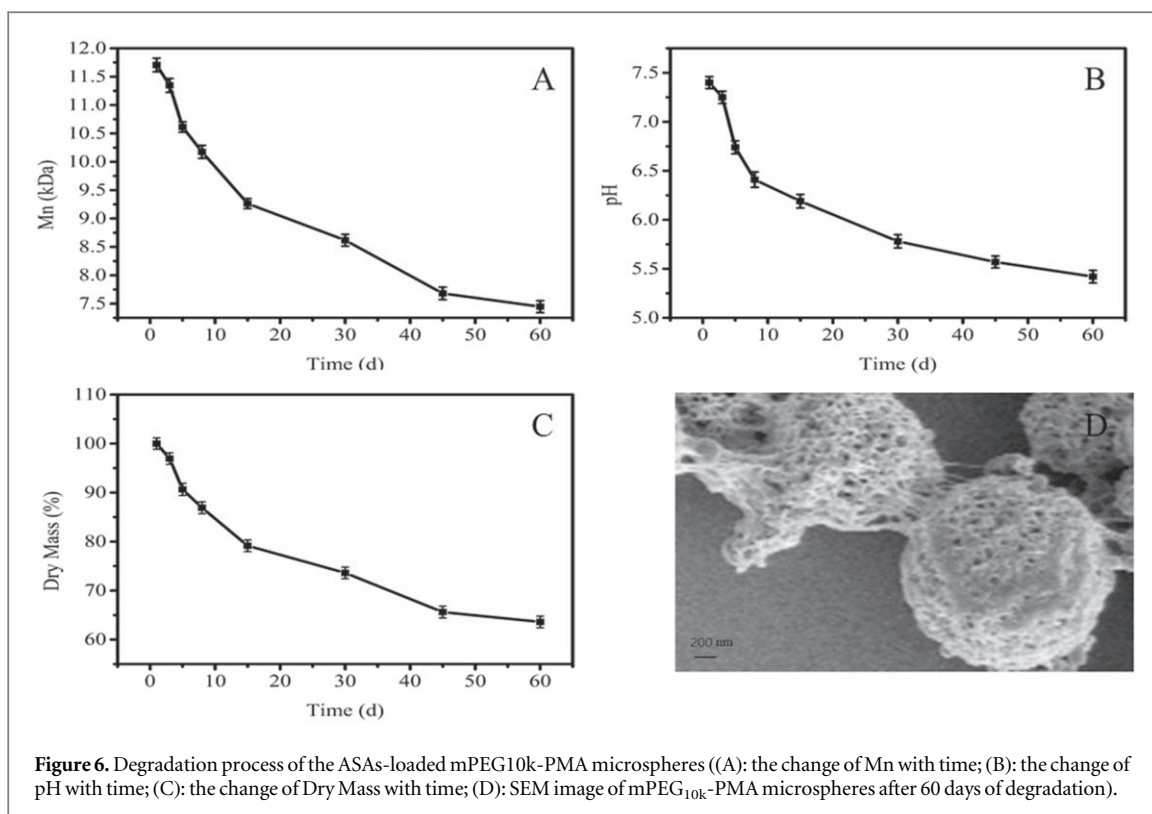


Figure 6. Degradation process of the ASAs-loaded mPEG10k-PMA microspheres ((A): the change of Mn with time; (B): the change of pH with time; (C): the change of Dry Mass with time; (D): SEM image of mPEG10k-PMA microspheres after 60 days of degradation).

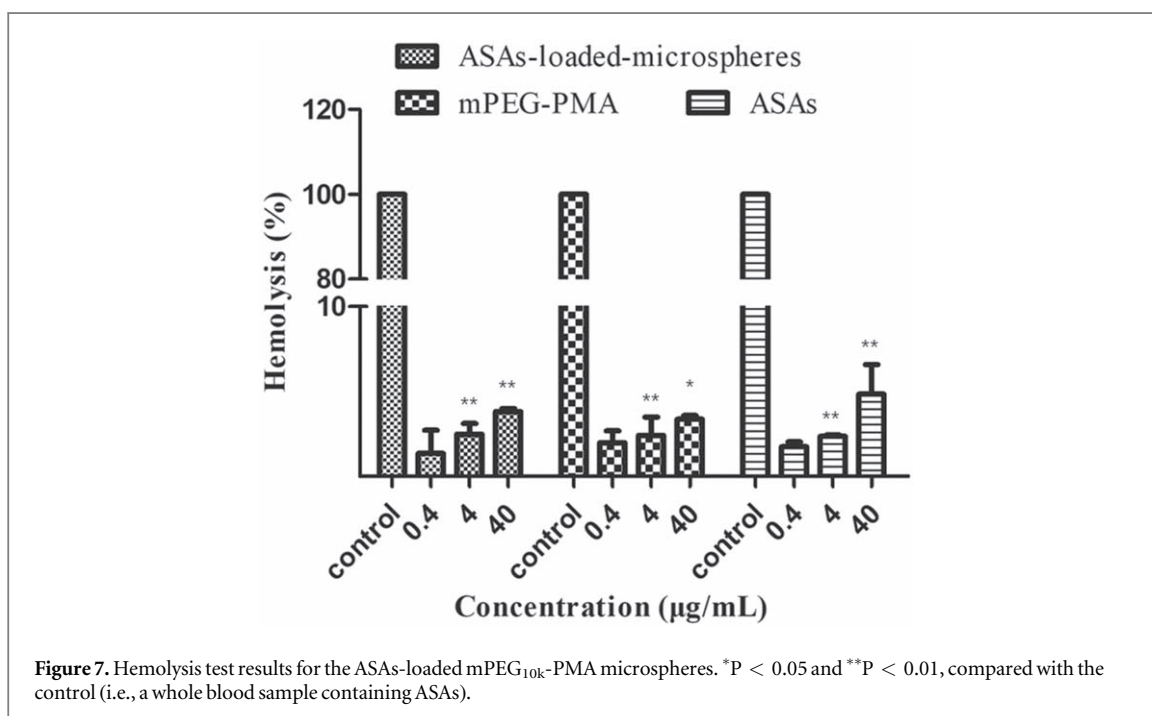


Figure 7. Hemolysis test results for the ASAs-loaded mPEG10k-PMA microspheres. *P < 0.05 and **P < 0.01, compared with the control (i.e., a whole blood sample containing ASAs).

those of the ASAs, while the anticoagulant capacities of the drug-loaded microspheres and the polymer materials increased upon increasing the concentration ($0.4\text{--}40\ \mu\text{g ml}^{-1}$). This result indicating that the drug-loaded microspheres do not affect the anticoagulant properties of ASAs itself.

3.7. Cytotoxicity of the microspheres *in vitro*

Figure 9 shows the results of cytotoxicity tests for the total alkaloids from the ASAs, the mPEG10k-PMA microspheres, and the ASAs-loaded mPEG10k-PMA microspheres. These tests were carried out on normal human liver cells (HL-7702). As can be seen, the ASAs do not exhibit any toxicity toward HL-7702 cells between concentrations of 0.4 and $40\ \mu\text{g ml}^{-1}$, and a similar result was observed for mPEG10k-PMA between

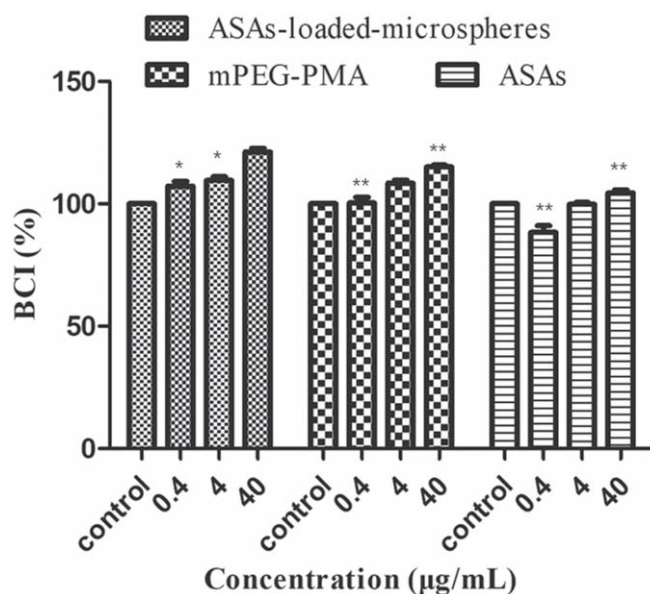


Figure 8. Anticoagulation test results for the ASAs-loaded microspheres. *P < 0.05 and **P < 0.01, compared with the control (i.e., a whole blood sample containing ASAs).

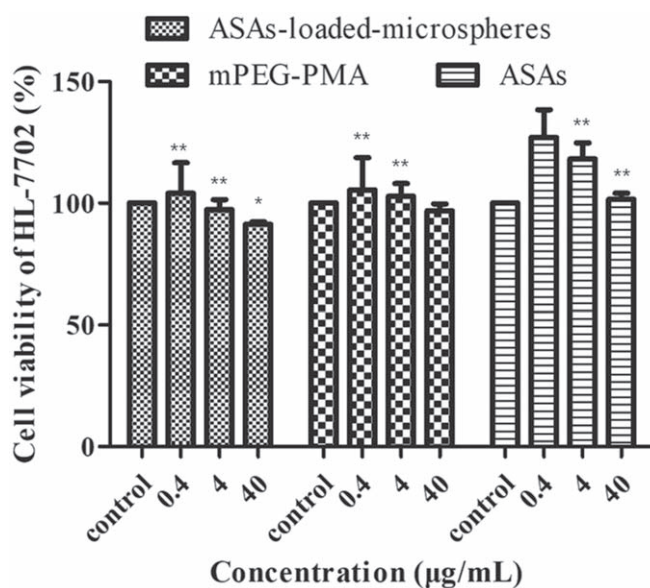


Figure 9. Cytotoxicity test results for the ASAs-loaded microspheres. *P < 0.05 and **P < 0.01, compared with the control (i.e., cells that grew in the same environment, but in the absence of ASAs).

concentrations of 0.4 and 4 $\mu\text{g ml}^{-1}$. However, upon increasing the concentration to 40 $\mu\text{g ml}^{-1}$, the survival rate of HL-7702 cells decreased to 96.83%. In addition, for the ASAs-loaded mPEG_{10k}-PMA microspheres, no toxicity was observed at 0.4 $\mu\text{g ml}^{-1}$, although at concentrations of 4 and 40 $\mu\text{g ml}^{-1}$, the cell survival rates decreased to 97.25 and 91.31%, respectively, thereby indicating a generally low toxicity overall [40].

3.8. Analysis of the anti-inflammatory effects of the microspheres

To determine the anti-inflammatory effects of the microspheres, the effects of *p*-xylene on ear swelling in mice under different treatment regimens were initially examined, and the results are shown in table 3. As indicated, the drug-loaded microspheres effectively inhibited auricle swelling in mice, with the inhibitory effect observed for 10 mg kg^{-1} of the ASAs-loaded microspheres being superior to that of 10 mg kg^{-1} aspirin, which was similar to that of 300 mg kg^{-1} DKL. Moreover, the inhibition rates achieved using 20 and 40 mg kg^{-1} of the microspheres alone were significantly higher than that achieved using 10 mg kg^{-1} aspirin.

Table 3. Anti-inflammatory activity of the ASAs on xylene-induced auricular swelling in mice.

Groups	Dose (/kg)	Auricular swelling ($\bar{x} \pm s$, mg)	Inhibition rate (%)
Control	—	50.85 $\pm$ 3.61	—
Aspirin	10 mg	34.65 $\pm$ 2.90	31.86 $\pm$ 1.90
DKL	0.3 g	37.65 $\pm$ 3.04	25.96 $\pm$ 1.51
ASAs	0.1 g	39.65 $\pm$ 0.78	22.03 $\pm$ 1.26
ASAs-Loaded microspheres	10 mg	33.55 $\pm$ 5.44	34.02 $\pm$ 2.24
	20 mg	27.10 $\pm$ 5.09	46.71 $\pm$ 3.86
	40 mg	21.25 $\pm$ 7.21	58.31 $\pm$ 4.14

Table 4. Effect of the ASAs on egg-white-induced pedal swelling in rats.

Groups	Dose (per kg)	Inhibitory rate of toe swelling (%)				
		1 h	2 h	3 h	4 h	5 h
Control	—	—	—	—	—	—
Aspirin	20 mg	66.77 $\pm$ 3.23	62.07 $\pm$ 3.04	57.02 $\pm$ 2.84	52.17 $\pm$ 2.56	49.55 $\pm$ 2.54
DKL	0.24 g	68.81 $\pm$ 3.30	65.86 $\pm$ 3.21	60.39 $\pm$ 2.90	54.31 $\pm$ 2.72	54.48 $\pm$ 2.74
ASAs	0.16 g	78.97 $\pm$ 4.12	69.99 $\pm$ 3.55	68.29 $\pm$ 3.12	60.14 $\pm$ 2.87	57.87 $\pm$ 2.86
ASAs-loaded microspheres	20 mg	47.64 $\pm$ 1.94	42.85 $\pm$ 1.87	38.21 $\pm$ 1.77	35.11 $\pm$ 1.61	32.87 $\pm$ 1.49
	40 mg	55.75 $\pm$ 2.85	49.01 $\pm$ 2.56	45.14 $\pm$ 2.47	40.63 $\pm$ 2.36	37.59 $\pm$ 1.69
	80 mg	64.09 $\pm$ 3.14	58.94 $\pm$ 2.88	55.49 $\pm$ 2.81	47.24 $\pm$ 2.52	42.18 $\pm$ 1.84

The effects of egg white on paw swelling in SD rats were also examined, and the results are shown in table 4. As indicated, the ASAs-loaded microspheres presented an inhibitory effect on the swelling of the toes. In addition, although the effect of aspirin at a dose of 20 mg kg⁻¹ was greater than that of DKL at a dose of 240 mg kg⁻¹, superior results were obtained for the ASAs-loaded microspheres at doses of 20, 40, and 80 mg kg⁻¹.

4. Conclusions

In this study, a series of biodegradable copolymers were prepared by the reaction between mandelic acid oxyanhydride (MA-OCA) and polyethylene glycol monomethyl ether (mPEG) with different molecular weights. These copolymers were used as carrier materials to prepare *Alstonia scholaris* leaf (ASAs)-loaded microspheres using the double emulsion method. The microspheres were characterized by their morphology, particle size, drug loading, encapsulation efficiency, and *in vitro* release performances. In addition, the *in vitro* degradation conditions and optimal drug loadings were determined for the mPEG_{10k}-PMA microspheres. *In vitro* release experiments showed that the microspheres could release the total alkaloids from the ASAs continuously for 15 d, and undergo complete degradation over ~60 d. Furthermore, coagulation, hemolysis, and anti-inflammation experiments demonstrated that the microspheres exhibited excellent anti-inflammatory activities and biocompatibilities. More specifically, anti-inflammatory experiments showed that the ASAs-loaded microspheres showed a superior performance than aspirin or Dengtaiye KeLi (DKL) under the same dosage conditions. The results presented herein therefore point to a new research direction for the development of sustained release formulations.

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Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

Conflicts of interest

The authors declare no conflict of interest.

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