Preparation and in vitro evaluation of matrine nanoparticles

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Abstract. For improving the therapeutic effect as well as bioavailability exhibited by the matrine drugs, and meanwhile enhancing patients’ compliance, the emulsion evaporation-low temperature curing method was adopted to prepare matrine nanoparticles and the prepared matrine nanoparticles underwent lyophilization treatment. Transmission electron microscopy (TEM) and Scanning electron microscope (SEM) was carried out to determine the morphology of matrine nanoparticles, and characterized for particle size and in vitro release. The chemical stability of matrine nanoparticles were studied by Fourier transform infrared (FT-IR) and differential scanning calorimetry (DSC). The results show the obtained nanoparticles presented amorphous structure, with the particle size of prepared matrine nanoparticles averaged 164 nm and that of the lyophilized preparations averaged 259 nm following the reconstitution. As shown by the in vitro evaluation, both of them exhibited uniform size, spherical shape, and strong chemical and physical stability. Based on the in vitro release results, matrine nanoparticles could continuously release for as long as 48 hours and based on the release kinetics, matrine nanoparticles meet the first-order kinetic release and follow the Ritger-peppas equation.

1. Introduction
As nanoscale systems are potentially applied in the filed of pharmacy and biotechnology, researchers have paid increasing attention to related research and development recently. There are many forms of nanocarriers, including nanocapsules [1, 2], nanobubbles [3], nanoliposomes [4, 5], micelles [6, 7] and nanoparticles [8, 9]. Nanoparticle is a skeleton entity composed by high-molecular substances, which can dissolve or absorb drugs [10, 11]. For nanoparticles, the size is small, the specific surface area is large [12], the drug loading is high and the drug stability is better, making them extensively applied to tumor diagnosis [13], gene transfer [14], drug delivery [15].

As a traditional Chinese herb, sophora flavescens can clear heat and dampness as well as kill insects [16, 17]. Its main ingredient is matrine [18], which can be easily soluble in water, acetone, chloroform, methanol and other solvents [19]. Matrine enjoys a wide application in the clinical treatment of different diseases including cancer, itchy skin [20], eczema [21] and viral hepatitis [22], relying on its anticancer [23, 24], antiviral [25] and anti-inflammatory properties [26, 27] in pharmacology. However, external-use matrine has a relatively simple dosage form, mainly limited to mixture and lotion. Due to the short action time for external preparation, it needs to be repeatedly administrated. It tastes bitter and patients’ compliance is weak. Recently, researchers have developed different forms of nanocarriers for dealing with above mentioned disadvantages.

The paper focused on investigating the way to prepare matrine nanoparticles as well as their lyophilized preparations, and conducting in vitro evaluation, aiming at helping to prolong drug action
time, reduce administration number, reduce adverse effects as well as deal with the inconvenience and weak compliance regarding matrine.

2. Materials and methods

2.1. Materials
Matrines and mannitols were provided by the Nanjing Digel Pharmaceutical Biological Co., Ltd., and Qingdao Mingyue Seaweed Group, respectively. Poloxamer-188 came from the BASF, Germany. Glycerol monostearate together with lecithin were provided by the Sinopharm Group Chemical Reagent Co. Ltd. Other chemicals exhibited analytical grade.

2.2. Preparation of matrine nanoparticles and freeze-dried preparations
The emulsion evaporation-low temperature curing method was adopted to prepare drug-containing nanoparticles. Glycerol monostearate (GMS) was melted in a water bath 10 °C above the melting point. The drug as well as glyceryl monostearate were dissolved in proper amounts of ethanol. An organic phase was prepared in a water bath at 70 ± 2 °C.

We dissolved the Poloxamer-188 and lecithin in deionized water (20 mL) and maintained the organic phase temperature at 70 ± 2 °C. The organic phase was rapidly injected into the water phase in a water bath at 70 ± 2 °C, which was then magnetically stirred for fifty minutes for removing the organic solvent as well as forming a translucent nanoemulsion. The prepared nanoemulsion was poured into water (10 mL) at 4 °C, followed by two hour of magnetic stirring to prepare the matrine solid lipid nanoparticles. 5% mannitol was added into the prepared matrine nanoparticles being a freeze-drying protective additive, under the pre-freezing temperature of -80 °C for 18 hours. The freeze-dried preparation of matrine nanoparticle was obtained by the freeze-drying treatment.

2.3. Determination of particle size of matrine nanoparticles
The dynamic light scattering technique was employed to measure the particle size of the matrine nanoparticles as well as the prepared samples under freeze-drying treatment. Prior to measurement, we diluted samples by using distilled water in the ratio of 1:20 for obtaining the optimal signal density.

2.4. Scanning electron microscope (SEM)
Matrine nanoparticles together with their lyophilized preparations were taken as the study object. Scanning electron microscopy (SEM) assisted in observing the surface morphology.

2.5. Transmission electron microscope (TEM)
Transmission electron microscope (TEM) assisted in observing the morphology exhibited by matrine solid lipid nanoparticles. Blank drug-containing matrine nanoparticles were taken as the study object, and deionized water was used to accurately replace the freeze-dried samples. The drug was applied to copper mesh which had amorphous carbon film, followed by staining in 2% phosphotungstic acid and drying treatment in air. TEM helped to complete the sample inspection.

2.6. Differential scanning calorimetry (DSC)
DSC assisted in examining the formation of the prepared nanoparticles. Four samples were weighted in proper amount, including matrine, glyceryl monostearate, nanoparticle lyophilized preparation as well as the same proportion of physical mixture. We controlled the temperature at 0~350 °C in the program at 5 °C/min at flow rate of 20 mL/min, for investigating the impact of temperature.

2.7. Fourier infrared change map
Proper amounts of the above mentioned four samples received infrared scanning KBr tablet method).
2.8. Studies on the stability of matrine nanoparticles
Aiming at determining the stability exhibited by the prepared as well as lyophilized matrine nanoparticles, we stored them at various temperatures (4 °C in refrigerator, 25 °C for room temperature and 40 °C in oven) for one month, for observing their changes in terms of the particle size, appearance and other morphological characteristics.

2.9. Studies on drug release and kinetics regarding matrine nanoparticles in vitro
The prepared matrine nanoparticles (i.e. 2 mg of drug) were placed in a dialysis bag which was then immersed into the receiver chamber that contained 100 mL of dissolution medium (water, physiological saline, and PBS at pH 5.8), which were then stirred at 100 rpm at a stable temperature of 35 ± 0.5 °C. We took about 5 mL of samples from the receptor chamber, and made them pass through a filter membrane (0.45 μm). Fresh release medium at the same volume was formed simultaneously and the sampling time was fixed. The cumulative release percentage was calculated based on the formula below, and the change with time was plotted. The release model was fitted.

\[ Q = \frac{\sum_{i=1}^{n} C_i V_i}{M} \times 100\% \]

3. Results and discussion

3.1. Particle size characterization
It was observed from Fig. 1 (a, b) that, the average particle size of matrine nanoparticles was 164.2 ± 26 nm. In Fig. 1 (c, d), the particle size range measured after reconstitution of the lyophilized matrine nanoparticles was 259 ± 36 nm. Obviously, the particle size of matrine nanoparticles after lyophilization was slightly higher than that before, but it was still within the particle size range of nanoparticles, and the distribution was more uniform.

![Figure 1. Matrine nanoparticle size distribution. (a, b: matrine nanoparticle size distribution; c, d: matrine dry-freeze preparation particle size distribution)](image-url)
3.2. Analysis of SEM results
It was illustrated in Fig. 2 that, the surface of matrine nanoparticles was spherical and uniform in size, and the nanoparticles after lyophilization were also spherical. Such observation indicated that the freeze-drying process did not damage the nanoparticle structure, which was achieved by increasing the nanoparticle stability.

![Figure 2. Scanning electron microscopes. (a, b: Matrine nanoparticles; c, d: Matrine Dry-freeze nanoparticles)](image)

3.3. Analysis of TEM results
It was seen from Fig. 3 that, both the matrine nanoparticles and the lyophilized preparations exhibited a spherical-like structure.

![Figure 3. Transmission electron microscopes. (a, b: Matrine nanoparticles; c, d: Matrine Dry-freeze nanoparticles)](image)
Figure 3. Transmission electron micrograph. (a, b: Matrine nanoparticles; c, d: Matrine Dry-freeze nanoparticles)

3.4. Differential scanning calorimetry

As observed from Fig. 4, matrine produced the endothermic peaks at 61.2 °C and 84.7 °C, respectively, of which, the endothermic peak at 84.7 °C might be ascribed to the thermal decomposition of matrine. Glyceryl monostearate exhibited a peak at 63.7 °C. Meanwhile, the physical mixture of matrine and glyceryl monostearate generated the endothermic peaks at 61.5 °C and 85.0 °C, separately; of them, the endothermic peak at 85.0 °C was produced by thermal decomposition. The freeze-dried preparation of matrine nanoparticles showed an endothermic peak at 56.6 °C, with no other peak being observed, indicating that matrine was not decomposed in the nano-formulation. In this regard, the stability of matrine might be improved by preparing a nano-freeze-dried preparation.

Figure 4. Differential scanning calorimetry results. (a: Matrine; b: glyceryl monostearate; c: physical mixture; d: matrine nanoparticle freeze-dried preparation)
3.5. FT-IR

FT-IR spectroscopy allows to detect the conformation of lipid molecules in the matrix. The FT-IR spectra of matrine, glyceryl monostearate, physical mixture, and matrine nanoparticle freeze-dried preparation are displayed in Fig. 5. As shown in Fig. 5a, matrine showed obvious characteristic absorption within the range of 4000-1500 cm\(^{-1}\), including matrine that showed an absorption peak of \(\nu_{\text{C}=\text{O}}\) at 1624.85 cm\(^{-1}\). Besides, there was asymmetric stretching vibration of \(\nu_{-\text{CH}_2}\) at 2918.34 cm\(^{-1}\); while a peak of stretching vibration absorption of \(\nu_{\text{NH}}\) was detected at 3407.57 cm\(^{-1}\).

As observed from Fig. 5b, glyceryl monostearate exhibited obvious characteristic absorption within the range of 4000-1500 cm\(^{-1}\). Besides, the out-of-plane bending vibration of \(\delta_{\text{CH}_2}\) at 717 cm\(^{-1}\) indicated that the number of \(-\text{CH}_2-\) in the glyceryl acid ester of single stearin was greater than 4, whereas the in-plane bending vibration of \(\delta_{\text{CH}_2}\) was observed at 1471 cm\(^{-1}\). In the meantime, a strong absorption peak was seen at 1730 cm\(^{-1}\) (due to \(\nu_{\text{OC}=\text{O}}\) glyceryl monostearate), the stretching vibration of \(\nu_{\text{OH}}\) was observed at 3394 cm\(^{-1}\), and the stretching vibrations of \(\nu_{\text{CH}}\) were found at 2915 cm\(^{-1}\) and 2849 cm\(^{-1}\).

It was found from Fig. 5c that, the characteristic absorption peaks of glyceryl monostearate and matrine were clearly present in the physically modular IR spectrum.

According to Fig. 5d, the absorption peak of matrine was masked by glyceryl monostearate in the IR spectra of the nanoparticle lyophilized preparation. The absorption peaks of glyceryl monostearate only existed at 1730, 2915 and 2850 cm\(^{-1}\), and no new peak was observed in the formed nanoparticles. This indicated that there was no chemical reaction between the drug and the lipid material, and that the drug was encapsulated in lipid material.

![Figure 5](image-url)

**Figure 5.** Fourier transform infrared spectrum of matrine nanoparticle freeze-dried preparation. (a: Matrine; b: glyceryl monostearate; c: physical mixture; d: matrine nanoparticle freeze-dried preparation)

3.6. Analysis of the nanoparticle stability

Changes in the appearance and particle size of matrine nanoparticles and nanoparticle lyophilized preparations stored at different temperatures (4, 25, 40 °C) for 15, 30, and 45 days are exhibited in Table 1 and Fig. 6, respectively.
By testing the appearance and particle size of the prepared matrine nanoparticles and the lyophilized preparation, the stability was investigated. In the nanoparticle suspension part, the nanoparticle lyophilized preparation showed superior physical and chemical stability, which was stable at 4 °C under the room temperature of 25 °C. After 45 days of storage, it was still a loose white powder, its particle size increased from 295 nm to 350 nm after reconstitution, and such small increase in particle size revealed the good stability.

Table 1. Effect of storage temperature on the appearance of matrine nanoparticles and their freeze-dried preparations

| Time (d) | Matrine Nanoparticle Suspension | Matrine nanoparticle freeze-dried sample |
|---------|---------------------------------|----------------------------------------|
|         | 4 °C                            | 25 °C                                  | 40 °C                                  |
| 0       | evenly, milky white liquid      | porosity, white powder                 | porosity, white powder                 |
| 15      | evenly, milky white liquid      | porosity, white powder                 | porosity, white powder                 |
| 30      | evenly, milky white liquid      | porosity, white powder                 | porosity, white powder                 |
| 45      | evenly, milky white liquid      | porosity, white powder                 | porosity, white powder                 |

Figure 6. Effect of Storage Temperature on the Size of Matrine Nanoparticles and Their Lyophllized Formulations

3.7. Results and analysis of the matrine nanoparticle release behavior in vitro

3.7.1. Matrine nanoparticle release profile. The release behaviors of matrine nanoparticles in water, normal saline, and PBS at pH 5.8 are shown in Fig. 7.

As observed from Fig. 7, the matrine solution released nearly 100% drug within 6 h, showing a sudden release. In addition, the drug release pattern of matrine nanoparticles showed a biphasic release behavior, which consisted of the initial burst release followed by sustained release. The initial burst
release of the drug might be ascribed to the presence of the adsorbed drug on the nanoparticle surface, whereas the sustained release might be attributed to the impacts of both the increased distance and the obstruction of surrounding solid lipid shell. Matrine nanoparticles continued to release drugs for up to 48 h. The cumulative release in the medium at pH 5.8 was close to 80%, while that in water was about 78%, and that in normal saline was 70%.

![Cumulation drug release curves of nanoparticle in different medium](image)

**Figure 7.** Cumulation drug release curves of nanoparticle in different medium

3.7.2. Release kinetic results. Model fitting was performed on the matrine nanoparticles released in water, normal saline, and PBS at pH 5.8, respectively, as presented in Table 2.

| Dissolution medium | Release model | Fitting equation | $R^2$ | n |
|--------------------|---------------|------------------|-------|---|
| **pH5.8**          | Level 0       | $Q=0.317t+34.000$| 0.5525|   |
|                    | Level 1       | $Q=74.13(1-e^{-0.247t})$| 0.9716|   |
|                    | Higuchi       | $Q=11.0352t^{1/2}+17.2512$| 0.7764|   |
|                    | Ritger-peppas | $Q=27.556(1-e^{-0.305t})$| 0.8633| 0.3035|
|                    | Hixon-Crowell | $(1-Q)^{1/3}=-0.0359t-3.0464$| 0.4162|   |
|                    | Baker-Lonsdale| $3/2[1-(1-Q)^{2/3}]Q=-1.6202t+47.268$| 0.5716|   |
| **Water**          | Level 0       | $Q=1.424t+22.372$| 0.7795|   |
|                    | Level 1       | $Q=72.544(1-e^{-0.1302t})$| 0.9544|   |
|                    | Higuchi       | $Q=11.7489t^{1/2}+5.5326$| 0.9255|   |
|                    | Ritger-peppas | $Q=17.7232(1-e^{-0.409t})$| 0.9493| 0.4009|
|                    | Hixon-Crowell | $(1-Q)^{1/3}=-0.0468t-2.5657$| 0.4723|   |
|                    | Baker-Lonsdale| $3/2[1-(1-Q)^{2/3}]Q=-1.8536t-31.831$| 0.7513|   |
| **Normal saline**  | Level 0       | $Q=1.3733t+17.776$| 0.7842|   |
|                    | Level 1       | $Q=68.2779(1-e^{-0.1099t})$| 0.9902|   |
|                    | Higuchi       | $Q=84.9337(1-e^{-0.1454t})-76.0763$| 0.9884|   |
|                    | Ritger-peppas | $Q=14.0580(1-e^{-0.4404t})$| 0.9443| 0.4404|
|                    | Hixon-Crowell | $(1-Q)^{1/3}=-0.0476t-2.4046$| 0.5714|   |
|                    | Baker-Lonsdale| $3/2[1-(1-Q)^{2/3}]Q=-1.8039t-25.625$| 0.7629|   |
It was illustrated from Table 2 that, the release of matrine nanoparticles in the buffer solution at pH 5.8 conformed to the first-order kinetic release, with the fitting coefficient of $R^2=0.9716$, meanwhile, the fitting coefficient of the Ritger-peppas model release behavior was $R^2=0.8633$, and $n=0.3035 <0.45$. Such results indicated that the release of matrine nanoparticles in the buffer solution at pH 5.8 was mainly the Fick diffusion; in other words, the solvent entered the nanoparticle structure and dissolved the drug contained in the structure. Finally, due to the internal and external osmotic pressure, the drugdiffuses out.

On the other hand, the release of matrine nanoparticles in water primarily conformed to the first-order kinetic model, with the fitting coefficient of $R^2=0.9544$, followed by the Higuchi and Ritger-peppas models, with the fitting coefficients of $R^2=0.9255$ and $0.9493>0.9$, and the Ritger-peppas model, with the release factor of $n=0.4009<0.45$. It suggested that the release of matrine nanoparticles in water was dominated by Fick diffusion.

Additionally, the release of matrine nanoparticles in normal saline accorded with the first-order kinetic release, with the fitting coefficient of $R^2 = 0.9902$. Besides, it also accorded with the Higuchi equation with the fitting coefficient of $R^2 = 0.9884$. The fitting coefficients were all greater than 0.9, revealing that the equation showed a high degree of fitting to the nanoparticle release behavior. Moreover, the fitting coefficient of the Ritger-peppas model release behavior was $R^2 = 0.9443$ and $n = 0.4404$ close to 0.45, demonstrating that the release of matrine nanoparticles in normal saline was dominated by Fick diffusion, but there was also dissolution diffusion.

4. Conclusions

In summary, matrine nanoparticles and their lyophilized preparations are successfully prepared in this study, and their particle size, morphology, stability, and in vitro release behavior are studied. The results show that the average particle size of the spherical matrine nanoparticles is $164.2 \pm 26$ nm. The lyophilized preparations and reconstituted particles have a particle size range of $259 \pm 36$ nm, and both of them are spherical with good stability, which is conductive to storage. Furthermore, results of in vitro release studies indicate that, the matrine nanoparticles have a drug release time of up to 48 h, and the release kinetics conforms to the first-order kinetic process. This shows that the use of drug nanoparticles improves their bioavailability and prolongs the action time. All in all, results in this study lay a theoretical foundation for the development of new dosage forms of matrine.

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