A kind of lonidamine reverse micelle and the preparation method thereof

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Abstract. This experiment synthesized a new type of lonidamine reverse micelle, which uses the chemotherapy drug lonidamine (LND) as the hydrophobic end and polyethylene glycol (PEG) macromolecules as the hydrophilic chain. CASOH, with a cinnamaldehyde (Cin) structure, designed a surfactant for the middle chain link and used this surfactant to prepare polymeric nano reverse micelles without the need for additional co-surfactants. This experiment verified the feasibility of this kind of lonidamine reverse micelles for the first time. This kind of self-assembled nano-reverse micelles has a simple preparation process and low cost and greatly improves the delivery efficiency of the chemotherapy drug lonidamine.

Keywords: Drug delivery; reverse micelles; self-assembly.

1. Introduction

Polymer micelles are essentially surfactants. Surfactants are amphiphilic molecules. In an aqueous solution, when the surfactant reaches a certain concentration, the lipophilic part of the amphiphilic polymer points to the center of the micelle, and the hydrophilic part points to the outside, thus forming micelles through self-assembly. The mechanism of micelle formation is that after the surfactant with adsorption capacity reaches saturation, micelles are spontaneously constructed, driven by intermolecular hydrogen bonds, electrostatic interactions, van der Waals forces, etc., and are stably dispersed in the aqueous solution [1, 2].

The core and shell of the polymeric micelle endow the micelle with multiple functions and play a key role in the delivery system. Its functional structure is suitable for use in drug delivery systems. The properties of the drug and the size of the polymer chains determine the location of the drug molecules within the polymer micelles. In addition to non-polar drugs on the core and polar drugs on the shell, neutral drugs are also contained between the core and the shell. In copolymers, hydrophobic interactions between drugs and hydrophobic units play a key role in both reducing the rate of drug release into the external solution and making the drug soluble in polymer micelles.

With the changes in people's living environment and the increase in life pressure, the incidence and mortality of tumors are increasing year by year. Malignant tumors have complex structures and components that are different from normal tissues. They are prone to recurrence, metastasis, and drug resistance, making them one of the main factors threatening human health. Traditional cancer treatment methods include surgery, chemotherapy, radiotherapy (RT), and comprehensive treatment. Although remarkable results have been reported with the above therapeutic approaches, some limitations are still inevitable, such as severe toxicity, limited efficacy, and cancer drug resistance. Among them, nanoscale-targeted drug delivery systems, including polymer micelles, liposomes, carbon nanotubes, and dendrimers [3], can increase drug solubility, change drug distribution in the body, and improve drug targeting [4], thereby improving the therapeutic effect and reducing the incidence of adverse reactions.

2. Synthetic materials and methods

2.1. Materials

Table 1. Reagent Source.

Reagent name	CAS	Reagent source
Cinnamic aldehyde	104-55-2	Energy Chemical
2-Mercaptoethanol-1-ol	60-24-2	Energy Chemical
Anhydrous zirconium chloride	10026-11-6	Energy Chemical
1-Hydroxybenzotriazole	123333-53-9	Energy Chemical
lonidamine	50264-69-2	Energy Chemical
Triethylamine	121-44-8	Energy Chemical
1-ethyl-(3-dimethylaminopropyl)carbodiimide	1892-57-5	Energy Chemical
$mPEG_{2000}$-COOH		Chongqing Yusi Pharmaceutical Technology Co., Ltd

2.2. Synthesis steps

This experiment synthesized a new type of lonidamine reverse micelle, which effectively solved the problems of poor solubility and low bioavailability of chemotherapy drugs and provided new ideas for the delivery method of lonidamine.

In this experiment, the surfactant LND-CASOH-PEG was first synthesized, and then the appropriate LND-CASOH-PEG ethanol solution was slowly stirred and dropped into the water, stirred until the ethanol completely evaporated, and further freeze-dried to obtain white nanoreverse micelles. The specific steps are as follows:

2.2.1. Preparation of CASOH:

Under solvent-free conditions, add the catalyst anhydrous zirconium chloride to the mixture of cinnamaldehyde and 2-mercaptoethanol-1-ol, and stir at room temperature for 5–30 minutes. The mass concentration of anhydrous zirconium chloride is 0.6 wt%. Stop when the reaction system gradually transforms from a liquid to a white solid. Add 10 mL of water to the above system, and then add 10 mL of methylene chloride for extraction. Keep the organic phase and use an ultrasonic cleaner to sonicate for 5–10 minutes during extraction. Then dry the organic phase with anhydrous sodium sulfate, and finally use a rotary evaporator to concentrate and dry to obtain a white solid powder, which is CASOH (yields 99%). The 1H NMR spectrum is shown in Figure 1. The synthetic route of CASOH is shown in Figure 1:

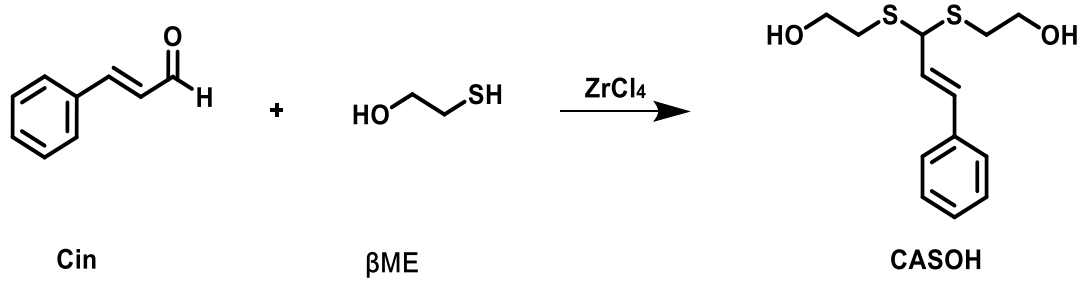

Figure 1. Schematic diagram of CASOH synthesis.

2.2.2. Preparation of LND-CASOH:

First, add the dichloromethane solution of lonidamine to 1-hydroxybenzotriazole and triethylamine for activation for 30 min-1 h, then add the CASOH and 1-ethyl-(3-dimethylamino) obtained in step 1 (propyl) carbodiimide hydrochloride. React at room temperature for 12 hours [5-7] . The mass concentration of 1-hydroxybenzotriazole is 2 wt%–3 wt%, the mass concentration of 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride is 3 wt%–4 wt%, and triethylamine The mass concentration is 10 wt%. After the reaction is completed, it is purified by column

chromatography separation technology, finally concentrated using a rotary evaporator, and dried to obtain a light gray transparent oil, which is LND-CASOH (yield 50%). The 1H NMR spectrum is shown in Figure 2. The synthesis route of LND-CASOH is shown in Figure 2:

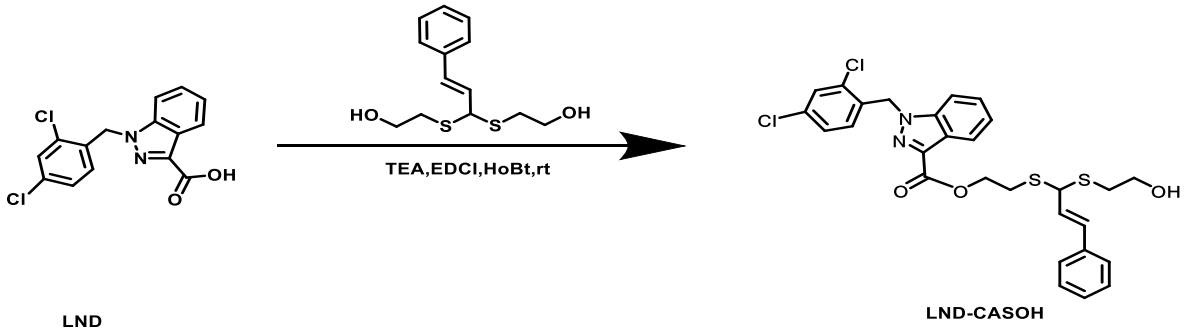

Figure 2. Schematic diagram of LNA-CASOH synthesis.

2.2.3. Preparation of LND-CASOH-PEG:

First add 1-hydroxybenzotriazole and triethylamine to the methylene chloride solution in step 2 for activation for 30 min-1h, then add methoxypolyethylene glycol-carboxylic acid and react at room temperature for 48 h. The mass concentration of 1-hydroxybenzotriazole is 2 wt%–3 wt%, the mass concentration of 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride is 3 wt%–4 wt%, and triethylamine The mass concentration is 10 wt%. After the reaction is completed, it is purified by column chromatography separation technology, finally concentrated using a rotary evaporator, and dried to obtain a light gray transparent oil, which is LND-CASOH-PEG (yield 20%). The 1H NMR spectrum is shown in Figure 3. The synthesis route of LND-CASOH-PEG is shown in the figure 3:

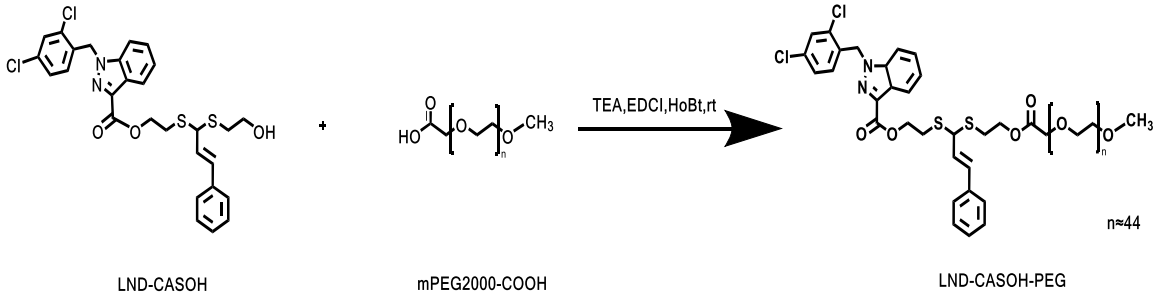

Figure 3. Schematic diagram of LNA-CASOH-PEG synthesis.

4) Dissolve the surfactant LND-CASOH-PEG in ethanol to form a first solution of 50 mg/mL.

5) Add the first solution drop by drop into the water, stirring until the solution turns milky white, to obtain lonidamine reverse micelles.

3. Conclusion and outlook

3.1. Results

Through scanning electron microscopy (SEM) observation, it was found that the surfactant LND-CASOH-PEG can self-assemble in water to form spherical micelles with a particle size between 120nm and 150nm.The SEM image (Supplementary Figure 4) demonstrates the morphology and size distribution of these spherical micelles. Each micelle is like a delicate nanoscale sphere. They exist stably in water and form a unique microstructure. The formation of this structure is of great significance for the function and application of surfactants.

3.2. Discussion

As a new formulation technology, reverse micelles have the advantages of simple operation and low cost, which have attracted widespread attention in the medical field. In this study, we successfully

prepared lonidamine reverse micelles. This innovative formulation technology not only significantly improved the water solubility of lonidamine [8], but also significantly enhanced its biocompatibility. This achievement has opened up a new path for the research and development of lonidamine pharmaceutical preparations and is expected to bring significant breakthroughs to the pharmaceutical field.

It is worth mentioning that LND-CASOH, the intermediate for the synthesis of LND-CASOH-PEG, has a unique chemical structure and contains an easily reactive OH group at its end. This characteristic gives LND-CASOH great potential in pharmaceutical research and development. Based on this structure, we can design and synthesize a variety of prodrugs and new micelle models to meet different drug delivery and release needs.

4. Attached pictures

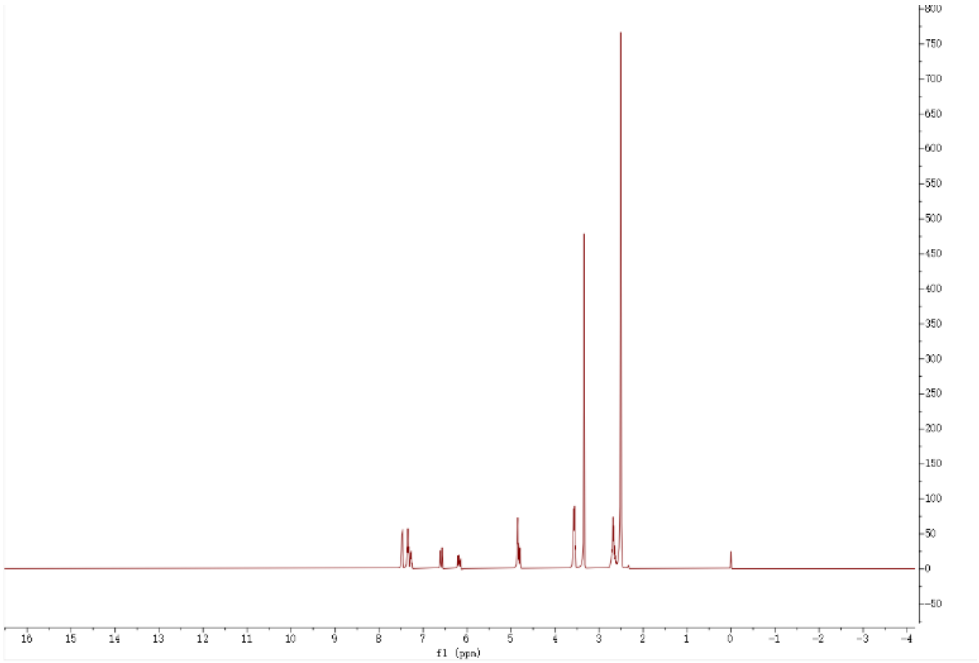

Figure 4. CASOH H NMR spectrum.

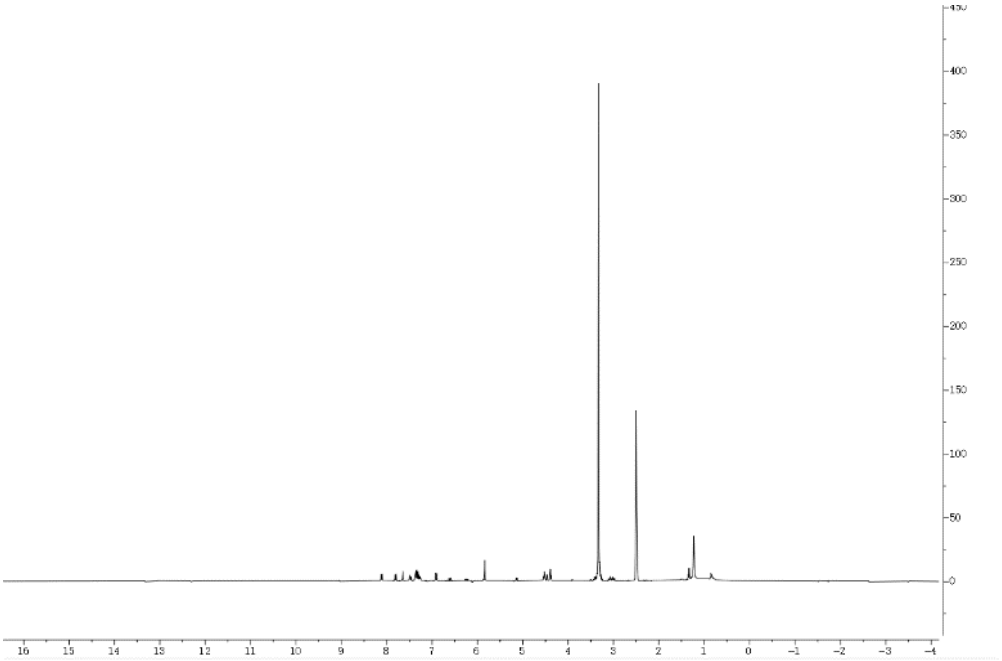

Figure 5. LNA-CASOH H NMR spectrum.

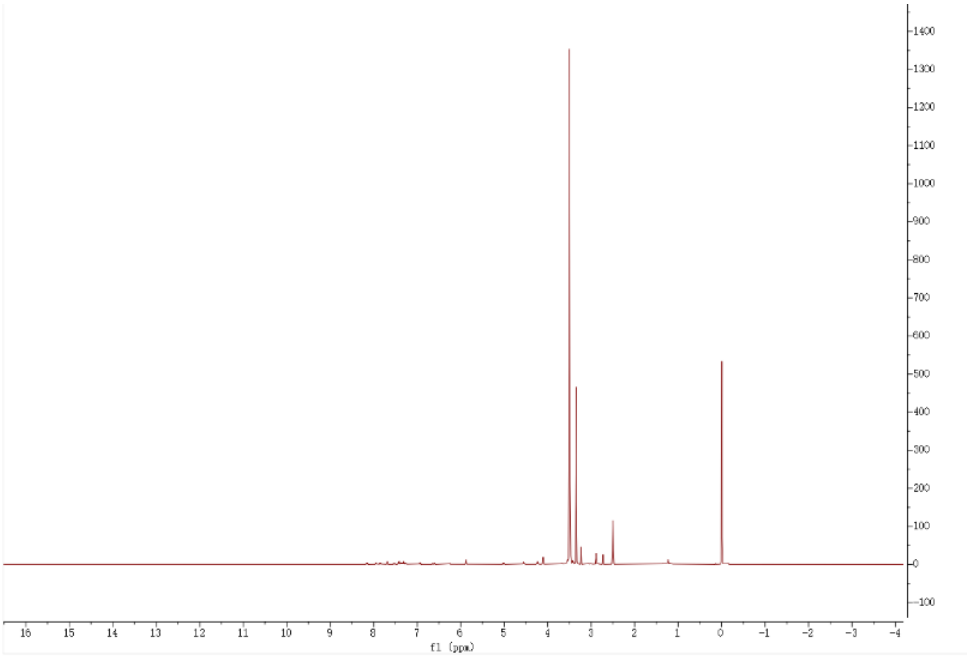

Figure 6. LNA-CASOH-PEG H NMR spectrum.

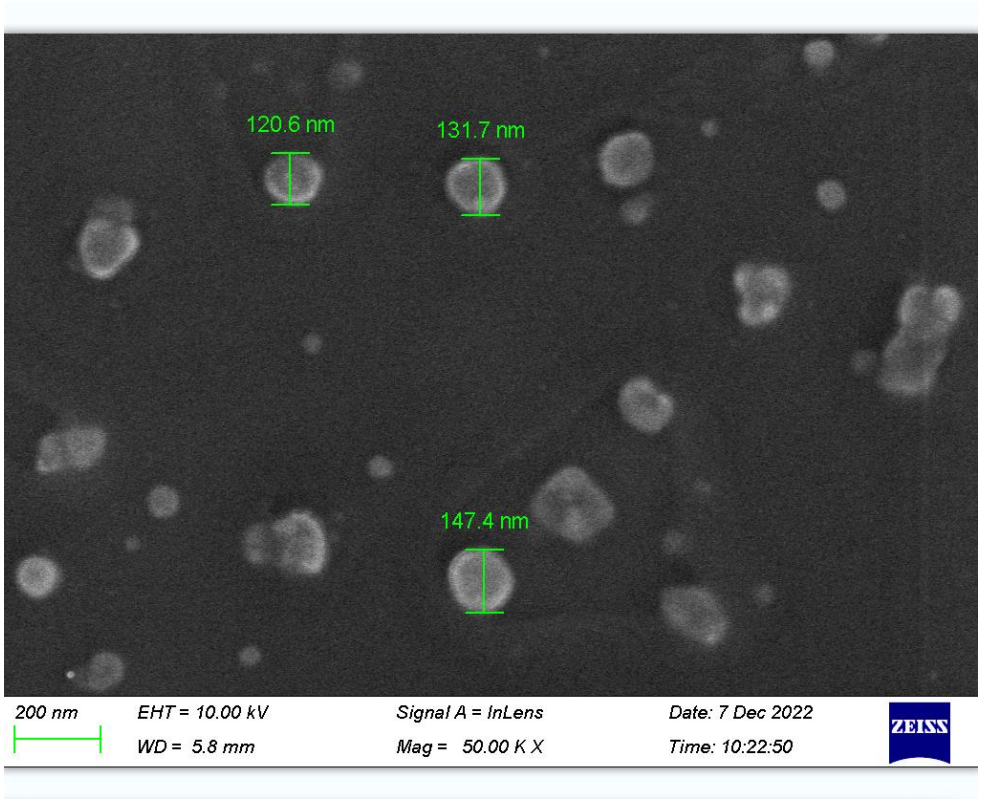

Figure 7. SEM of LNA-CASOH-PEG self-assembled nanoparticles.

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