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Preparation of Lipid Nanoparticles based on Phase Behaviors of Hot Microemulsions

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Abstract. The solid lipid nanoparticles (SLNs) were prepared based on the phase behaviors of hot microemulsions which were quite different from normal microemulisons for the solid lipid as oil phase. The pseudoternary phase diagrams for the system Brij78/deoxycholic acid sodium (DAS)/ glycerol monostearate (GMS)/water with and without the addition of retinoic acid (RA) were obtained at 60℃ using home-made apparatus with temperature control. O/W and W/O region were all found in the phase diagram. The introduction of RA has increased the O/W microemulsion region greatly. GMS-SLN and RA-GMS-SLN were prepared by direct cooling of hot O/W microemulsion obtained according to the phase behaviors results. The mean particle size of GMS-SLN and RA-GMS-SLN investigated by PCS is about 10 nm. TEM images indicated that both SLNs were spherical particles with diameter about 10 nm. PCS results showed that the particle dimension was kept almost the same after three months, proving good stability of both SLNs. The study on the phase behaviors of drug-loading hot microemulsions has been proved to be very significative for the controllable preparation of SLNs which could be used as nanoscale drug delivery system (DDS) for water insoluble drugs.

Introduction

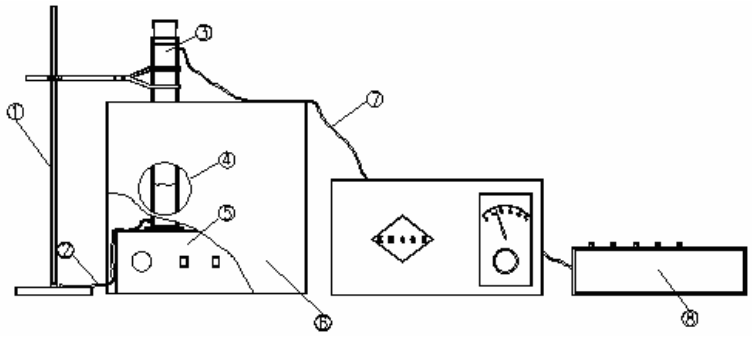
Nanoscale drug carriers, such as microemulsion[1,2], nanoparticles[3], liposome[4] and solid lipid nanoparticles (SLNs)[5,6], have attracted much attention due to their unique properties for the preparation of drug delivery system(DDS). SLN has been proved to be a very promiseful DDS for the water-insoluble drugs since 1990s. The lipids with good physiology compatibility were chosen as carrier materials in SLN to avoid the toxicity problem. The most valuable aspects of SLN also include the good stability induced from the solid character of carrier materials in DDS at room temperature and feasibility of large-scale production.

Hot drug-loading oil-in-water (O/W) microemulsions were the key intermediate product for the final preparation of many SLNs. To obtain a hot O/W microemulsion for the controllable preparation of SLN, it was very meaningful to have the knowledge about the phase behaviors of these special microemulsions, which were quite different from normal microemulsion for the melt lipid as oil phase. Because the lipid is solid at room temperature, so the phase diagrams must be obtained at the temperature at least 10 °C higher than the melting points of the lipids. Many researches have focused on the phase behaviors of microemulsions[7,8], including the drug-loading microemulsions[9]. However, routine titration method is not suitable for the study of such special microemulsion. So a home-made apparatus was used for the detection of phase states of hot microemulsions.

Experimental Section

Materials. Brij78 and DAS were purchased from Sigma. GMS was obtained from Shanghai Hengxin Chemistry Reagent Company. RA was bought from Shanghai No. Six Pharmaceutical Factory. MilliQ water was used in all the experiments.

Apparatus. In order to satisfy the higher temperature needed, we have made a microemulsion phase diagram detection instrument with accurate temperature control (Fig.1). It consists of sample cell, detection unit, thermostatic unit and control unit. The sample cell was a double-walled glass tube with the inlet and outlet on the bottom and top of the outer wall of glass tube, respectively. The temperature of sample cell was adjusted by means of thermostatic unit. The control units directed all the operations.



①-hob ②-infall pipe ③- sample tube ④-polarizer
⑤-stirrer ⑥-box ⑦-outfall pipe ⑧-controller

Fig.1 The schematic diagram of phase diagram apparatus of microemulsions

Methods. The mean size of SLN was determined by photon correlation spectroscopy (PCS) on a N4 PLUS submicron analyzer (Beckmann-Coulter, USA) at a scattering angle of 90°. Each experimental value was gained by PCS results from three independent experiments, each performed in triplicate. Zeta potential measurements were performed on a Delsa 440SX (Beckmann-Coulter, USA). TEM measurements were conducted on a TEM (JEM-2000EX, Japan Electron Optics Laboratory Corporation, Japan). The samples was negative stained by 2% phosphotungstic acid solution before TEM measurements due to the poor conductivity of organic samples.

Results and Discussion

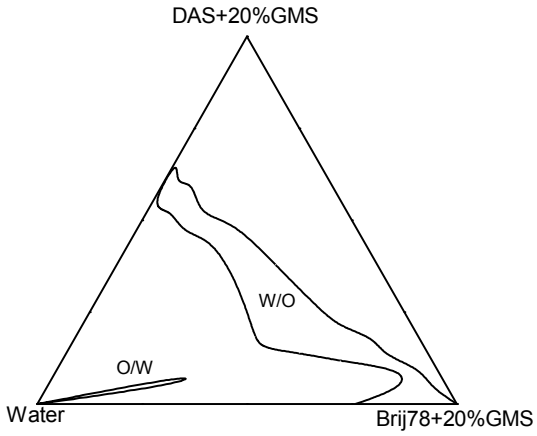


Fig.2 Pseudoternary phase diagram of Brij78/DAS/GMS/Water system at 60 °C

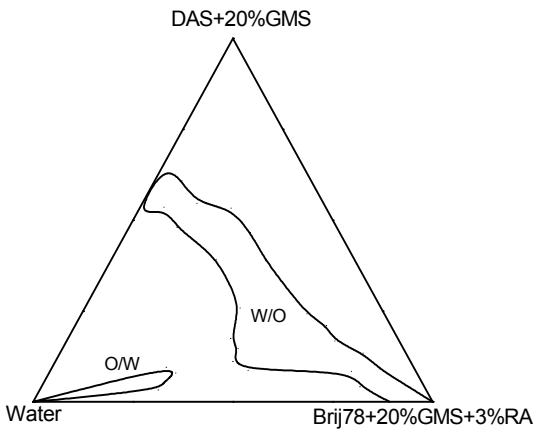


Fig.3 Pseudoternary phase diagram of Brij78/DAS/GMS/RA/Water system at 60 °C

Phase Bahaviors Study. It is well known that microemulsion is a kind of homogenous liquid-liquid dispersion system with good thermodynamic stability. So it is impossible to obtain the O/W

microemulison for GMS/Water mixing system at room temperature due to the solid character of GMS. It is necessary to reach higher enough temperature to made GMS melt and become liquid. The accurate temperature control made it possible to study the phase behavior of such special microemulsion with the aid of the home-made phase diagram apparatus.

Fig. 2 showed the pseudoternary phase diagram of Brij78/DAS/GMS/Water at 60 °C, the temperature at which GMS was melt. There existed two phase regions in the phase diagram: a large W/O microemulsion region in the middle and a relatively small O/W region near the water point. It meant that it was possible to obtain an O/W microemulsion with certain proportion of components. And also, it maybe suitable for the preparation of the corresponding GMS-SLNs using the formulation data obtained from the O/W microemulsion region.

The addition of RA had a great influence on the phase behavior of Brij78/DAS/GMS/Water system (Fig. 3). The O/W microemulsion region was enlarged obviously while W/O microemulsion was only enlarged slightly. It is valuable for the increase of O/W microemulsion to have much selectivity for its application in the preparation of SLNs. The operation precedure was the same as the system without RA except the solubilization of RA in melt GMS.

Preparation of SLN based on Phase Behavior Results. Both the GMS-SLN and GMS-SLN loaded with RA (RA-GMS-SLN) were prepared based on the formulation data obtained from the O/W microemulsion in phase diagram. The SLNs were obtained by direct cooling of hot microemulsion of Brij78/DAS/GMS/Water with and without addition of RA. The obtained SLN aqueous dispersion were very clear, homogenous and transparent. The study on the RA load capacity showed that the RA concentration could reach 1.0 mg/ml while keeping the same stability.

Characterization of SLNs. The mean size of GMS-SLNs and RA-GMS-SLNs were all in the range of 11.2 ± 1.2 nm according to the PCS results. And the zeta potential of all the SLNs were in the range of –34.1 ± 2.3 mV. Fig. 4 gave the TEM image of RA-GMS-SLN. The RA-GMS-SLNs were all the irregular spherical particles with the diameter of about 10 nm, corresponding to the PCS results.

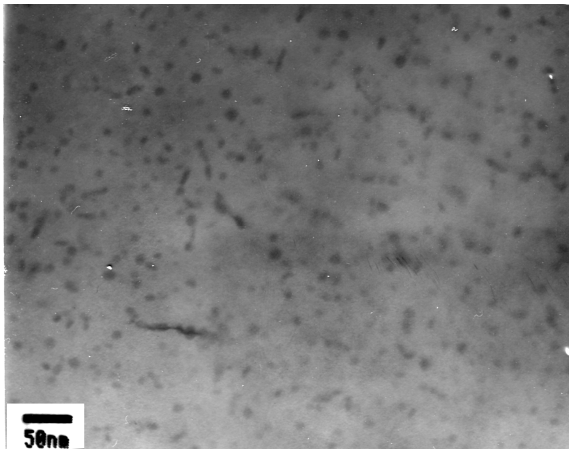


Fig. 4 TEM image of RA-GMS-SLN

Stability Studies. The influence of storage temperature, storage time and high speed centrifugation on the SLNs was investigated by means of PCS.

The SLN dispersions were put into the glass tubes with sealed lids and stored at the temperature of 5, 15, 25 and 37 °C for one month. There was no any change found. The SLN dispersion kept the same clear and transparent state as their original state at all observation times in one month.

Table 1 gave the PCS results of GMS-SLN at different storage times after preparation. The storage temperature was 25 °C. It could be seen from Table 1 that the GMS-SLN showed very good stability according to the PCS results. The mean size of GMS-SLN was kept almost the same in three months.

Table 1 PCS size results of GMS-SLN at different storage times

Time [days]	0	1	7	14	30	90
Mean Size [nm]	10.0	10.0	10.0	10.0	10.0	10.1

Table 2 showed the influence of storage time on three kinds of RA-GMS-SLNs with the RA concentration of 0.195, 0.390, 0.780 mg/ml, respectively. The storage temperature was 25 °C, too. It could be concluded that the RA-GMS-SLNs had relatively good stability due to the fact that the PCS results changed little during 3 months.

Table 2 PCS size results of RA-GMS-SLN at different storage times

Time [days]		0	1	7	14	30	90
Mean Size [nm]	RA [0.195 mg/ml]	10.3	11.7	11.5	10.0	10.8	11.2
	RA [0.390 mg/ml]	12.3	11.1	10.0	10.2	10.0	10.0
	RA [0.780 mg/ml]	11.3	10.7	10.0	10.2	10.4	10.0

The mean size of RA-GMS-SLNs after high speed centrifugation (12000 r/min) with different times were 10.0 nm (0 min), 10.0 nm (30 min), 11.6 nm (60 min) and 10.0 nm (120 min), respectively, also showing the good stability.

Summary. The phase behaviors of a kind of special hot microemulsion were studied and successfully used in the preparation of drug-free and drug-loading solid lipid nanoparticles.

Acknowledgement

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