Solid State Phenomena Vols. 121-123 (2007) pp. 705-708
online at http://www.scientific.net

Preparation of tea polyphenols-loaded solid lipid nanoparticles based on the phase behaviors of hot microemulsions

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Keywords: microemulsion; tea polyphenols; solid lipid nanoparticles; percutaneous absorption

Abstract Solid lipid nanoparticles(SLN) appear a promising approach as a drug system for topical application. The solid lipid matrices allow protection of incorporated active ingredients against chemical and physical degradation. This paper deals with controllable preparation of solid lipid nanoparticles based on the phase behaviors of hot microemulsions. The pseudoternary phase diagram for the system of glyceryl monostearate(GMS)/ mixed surfactants (polyoxyethylene(40) stearate(S-40) and poloxamer 188 (F-68))/ water was obtained at 60 ℃ using self-made apparatus with temperature control. The mass ratios of glyceryl monostearate(GMS), mixed surfactants and water were determined according to the region of w/o hot microemulsion. It was shown that the size of TP-SLN increased slightly with the increasing of concentration of TP, polydispersity index was approximately 0.5, encapsulation efficiency decreased distinctly. The percutaneous absorption experiment of tea polyphenols loaded SLN through the rabbit skin were conducted using self-made Franz diffusion cell in vitro. The transdermal penetration was sustained.

1. Introduction

Green tea is consumed as a popular beverage worldwide because of its characteristic aroma, flavor and health benefits, which are associated with the presence of tea polyphenols. Tea polyphenols (TP) are promising antioxidant, anti-inflammatory and anti-carcinogenic agents, which has been investigated in the chemoprevention and treatment of skin photodamage [1-2]. The skin photoprotection of TP have been attributed to their antioxidant properties as scavengers of reactive oxygen species generated by solar ultraviolet radiation [3]. The chemical unstability of TP can be a major drawback for its clinical application. When TP are exposed on many different factors such light, heat and oxidants, tea polyphenols are rapidly oxidized. It is desirable to get a chemically stable dosage form of TP for the quality control of drug products.

Incorporation of drug in solid lipid-based carriers such as solid lipid nanoparticles (SLN) can overcome the chemical unstability of drugs [4-5]. SLN are generally produced by homogenization or microemulsion technique [6]. The hot homogenization is the most frequently applied technique. However for hydrophilic drugs, the cold homogenization is recommended. The manufacturing processes of the cold homogenization technique are too complicated to apply infrequently.

Microemulsion is clear, thermodynamically stable system being composed of lipid, surfactant, co-surfactant and water. The co-surfactants are selected from low molecular weight alcohols[7], such as butanol is less favourable with respect to regulatory aspects. In this study, an improved microemulsion method of preparing tea polyphenols-loaded SLN (TP-SLN) is presented. This method is suitable for preparation of SLN loaded with water soluble drugs. Polyoxyethylene(40) stearate(S-40) and poloxamer 188 (F-68) were selected as surfactant and co-surfactant avoiding the

use of organic solvent. Controllable preparation of TP-SLN was performed based on the w/o microemulsion region which determined by the studying of the phase behaviors of hot microemulsions. The percutaneous absorption experiment of TP-SLN through the rabbit skin was conducted using self-made Franz diffusion cell in vitro.

2. Materials and Methods

2.1 Materials

Tea polyphenols were purchased from Wuxi Biotechnology and Pharmaceutics company, glyceryl monostearate were obtained from Shanghai Chemical Reagent Corporation, The surfactants polyoxyethylene (40) stearate (S-40) and poloxamer 188 (F-68) were obtained from Nanjing Weier Laboratory. Rabbits were obtained from the Animals Centre of the First Affiliated Hospital of Nanjing medical University. Methanol was chromatography reagents. All other reagents were analytical reagents and used without further purification. The water used for all experiments was purified water obtained from a MilliQ Plus (Millipore, Schwalbach, Germany).

2.2 Methods

The pseudoternary phase diagrams for the system polyoxyethylene (40) stearate (S-40)/ poloxamer 188 blockcopolymer (F-68)/ Glyceryl monostearate (GMS)/water was obtained at 60 ℃ using self-made apparatus with temperature regulator[8]. Briefly, GMS, S-40 and F-68 were accurately weighted and put in the sample tube. The mixture were melted surrounding flowing 60℃ water (the variety of temperature was less than 0.1 ℃) with electromagnetic stirring apparatus until the mixture appeared clear. Pure water of the same temperature was accurately added into the sample tube and the phase transformations were recorded for plotting the phase diagram according to the literature [9].

Controllability preparation of TP-SLN was performed based on the w/o microemulsion region of the pseudoternary phase diagram. The optimization mass ratio of GMS and mixed surfactants was 1:2, meanwhile the optimization mass ratio of S-40 and F-68 was 7:3. The lipid and mixture of surfactants were heated to 60℃ until the mixture appeared transparent. TP solution was added into the clear oily phase under mild whirling and vibrating. A thermodynamically stable w/o microemulsion was formed. This microemulsion was then dispersed in a cold 50 ml 0.8% S-40 aqueous solution (2-4 ℃) by ultrasonication for 2h resulting the TP-SLN suspension.

The particle size of TP-SLN was measured by photon correlation spectroscopy (PCS) on a N4 plus submicron particle size analysis instrument (Beckmann-Coulter, USA) in triplicate.

The encapsulation efficiency was calculated from the concentration of TP in TP-SLN dispersion and in the TP-SLN congeries fraction assayed by HPLC (Perkin Elmer 200, USA) after dilution with methanol. The TP-SLN congeries were obtained using a freeze-centrifuge(Sorvall Biofuge Stratos, USA) at 13000 rpm for 30 min by adding strong electrolyte (2 mol/l $MgSO_4$), then the water was completely removed.

The in vitro percutaneous absorption experiments were performed on rabbit skin with self-made Franz diffusion cells (Fig.1). After removing the subcutaneous fat and surface rabbit hair, skin was mounted on cells with a surface area of 7.065 cm^2 and a receiver compartment (100 ml) filled with 50 ml sodium chloride(9 g/l). The receiver fluid was continuously stirred and maintained at 37 ℃. The dosing formulation was applied to the epidermal surface. The receiver fluid was removed 3 ml at 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h and supplied with the same volume fresh receiver solution. The concentrations of tea polyphenols in receiver fluid samples were determined using a UV spectroscopy (Spectrumlab 752S, Lingguang analysis instrument Co., China) at 275 nm with the help of the corresponding calibration curve.

3. Results and Discussion

3.1 The pseudoternary phase diagram of the system GMS/S-40/F-68/H_2O

Fig.2 shows the pseudoternary phase diagram of the system GMS/S-40/F-68/H_2O at 60℃. The w/o microemulsion is adapted to preparing SLN loaded with hydrophilic drugs such as TP. According to the phase diagram, the correct ratio for w/o microemulsion formation can be easily obtained. The w/o microemulsion region of this system is so large that it is produced in any ratio of lipid to mixed surfactants. The introduction of TP has little influence on the phase behaviors of drug-loaded hot microemulsion. This drug-loaded microemulsion was then dispersed in a cold aqueous medium under ultrasonication to produce the TP-SLN dispersion. However, if the ratio of GMS/surfactants(S-40/F-68) is less than 1:6, the microemulsion containing a more quantities of surfactant and a smaller quantityes of lipid is unfavorable to the entrapment of TP in lipid materials. Therefore, the ratio was chosen more than 1:6.

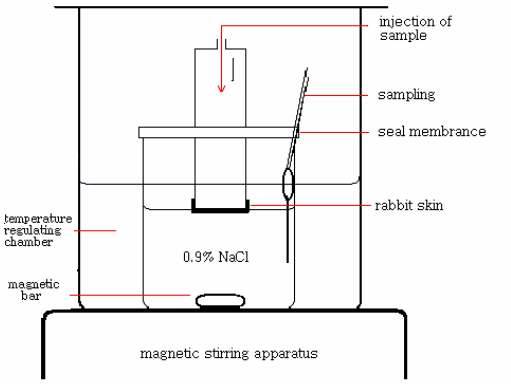

Fig. 1 Self-made France diffusion cell

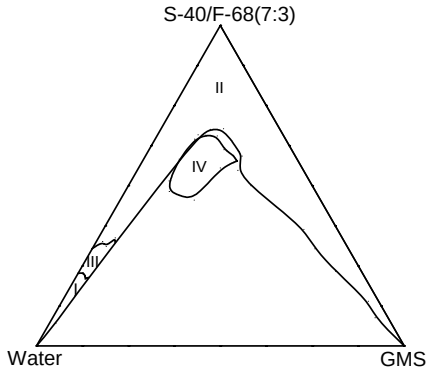

Fig.2 Pseudoternary phase diagram of GMS/S-40/F-68 /H_2O system(60 ℃)

I: o/w microemulsion region II: w/o microemulsion region

III: Bicontinuous structure region IV: Liquid crystal region

Table 1 gives the detailed appearances of TP-SLN dispersion produced from different ratios of GMS and surfactants. We were able to produce physicochemically stable TP-SLN dispersion when the ratio was 1:2. Results indicated that TP can be protected by the solid lipid avoiding the TP oxidization.

Table 1. Effect of the ratio of GMS and surfactants on the preparation of TP-SLN

GMS:S-40/F-68 (7:3)	Appearance of TP-SLN	Appearance of TP-SLN after 10 d	Appearance of TP-SLN after 30 d
1:6	buff emulision	yellow emulision	serious phase separation, brown water phase
1:4	buff emulision	buff emulision	partly phase separation
1:2	white emulsion	white emulsion	stable white emulsion

3.2 Effect of TP concentration on characterizations of TP-SLN

Table 2 shows the effect of TP concentration on the characterizations of TP-SLN. As the concentration of TP increased, the mean particle size of TP-SLN increased slightly, the polydispersity index did not change significantly, and the encapsulation efficiency decreased obviously.

Table 2. Effect of TP concentration on characterizations of TP-SLN

GMS:S-40/F-	Concentration of TP	Particle size	Polydispersity	Encapsulation

68(7:3)	[μg·ml^{-1}]	[nm]	index PI	efficiency[%]
1:2	245.0	146.2	0.463	47.91
1:2	163.8	141.5	0.518	67.65
1:2	81.9	134.4	0.520	71.24

3.3 Percutaneous absorption of TP-SLN

In this primary study, the penetration of TP from TP-SLN dispersion into sodium chloride (9 g/l) through rabbit skin was investigated over 24 h. Each sample was analyzed in triplicate. Fig.3 shows the penetration profile of TP-SLN suspension. According to Fig.3, the process of percutaneous absorption is sustained. There is a linear relationship between accumulation penetration count and time, the permeation coefficient is 3.22 μg·cm^{-2}·h^{-1}.

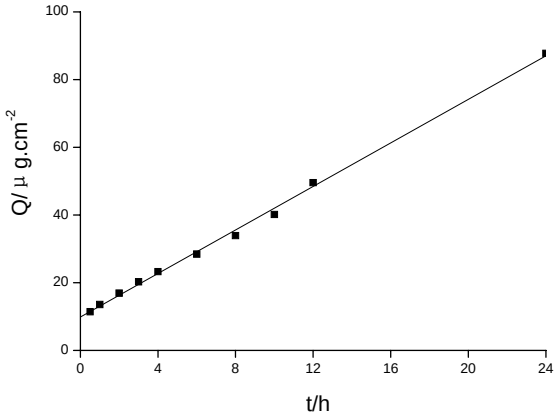

Fig.3 Percutaneous absorption of TP-SLN

4. Conclusion

Our studies show clearly that controllability preparing of TP-SLN is facilitated performed by improving hot microemulsion technique based on the pseudoternary phase diagram of the system GMS/S-40/F-68/H_2O. The mean particle size of TP-SLN prepared under optimum conditions is less than 150 nm with ploydispersity index of approximate 0.5. It is shown that the encapsulation efficiency is strongly dependent upon the concentration of TP. The transdermal penetration is sustained and the permeation coefficient is 3.22 μg·cm^{-2}·h^{-1}. The results of this percutaneous absorption preliminary study demonstrates that TP-SLN have promising potential as a topical treatment.

Acknowledgment This research was supported by National Natural Science Foundation of China (No. 60371027, No. 60171005), the Medical Key (Project 135) Foundation of Jiangsu (China) and Natural Science Foundation of Jiangsu (China) (No. BK 2002404).

References

[1] S.K. Katiyar, F. Afaq, K. Azizuddin, et al: Toxicology and Appl. Pharm. Vol. 176 (2001), p. 110

[2] M. Soriani, C. Rice-Evans, R.M. Tyrrelll: FEBS Letters. Vol. 439 (1998), p. 253

[3] R. Yu, J.J. Jiao, J.L. Duh, et al: Carcinogenesis. Vol. 18 (1997), p. 451

[4] R.H. Muller, W. Mehnert, J.S. Lucks, et al: Eur. J. Pharm. Biopharm. Vol. 41 (1995), p. 62

[5] R.H. Muller and C.M. Keck: J. Biotech Vol. 113 (2004), p. 151

[6] R.H. Muller, K. Mader and S. Gohla: Eur. J. Pharm. Biopharm. Vol. 50 (2000), p. 161

[7] M.R. Gasco: US Patent 5 250 236(1993)

[8] Y.Y. Lu, Q. Xia, Y. Xia, et al: Phy. Chem. Acta(Chinese) Vol. 21 (2005), p. 98

[9] G.Z. Li and S.E. Friberg:J. Am. Oil Chem. Soc Vol. 59 (1982), p. 569