Surface Properties of Lithospermum-Containing Multiple Phase Emulsion Systems

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ABSTRACT: In this study, we employed a one-step emulsion process—rather than the traditional, relatively difficult two-step process—to obtain multiple phase emulsion systems containing the Chinese herbal medicine lithospermum. We characterized these systems in terms of their contact angles, surface tensions, fluorescence, and stability and analyzed them using polarized microscopy, atomic force microscopy, and attenuated total reflection Fourier transform infrared spectroscopy. Our one-step emulsion process involved mixing lithospermum at various concentrations with three different emulsifiers to control the properties of the emulsion systems. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 117: 1041–1046, 2010

Key words: lithospermum; multiple phase emulsion systems; atomic force microscopy; fourier transform infrared spectroscopy; morphology; fluorescence; surfaces

INTRODUCTION

Lithospermun erythrorhizon Siebold et Zuccarini or *Macrotomia euchroma* (Royle) Paulsen is a Chinese herbal medicine prepared from the dried roots of the Boraginaceae family; it is known commonly as "lithospermum." The outside peel is purple and black and the inside core is white; a thick peel is better than a thinner one, which is commonly used to make a purple dye. Lithospermum is used as an anti-inflammatory agent, a promoter of granulation organization growth, an antibiotic, and an antioxidant.

Emulsion formation is a basic technology employed to make cosmetics; emulsion techniques have been expanded in recent years to include microemulsions,[1,2] multiple emulsions, nonfluid emulsions, phase inversion emulsions, D phase (detergent phase or surfactant phase) emulsions,[3] liquid crystal emulsions, and liquid membrane emulsions. A characteristic of W/O/W multiple phase emulsions is that the dispersed phase contains tiny scattered droplets. Of the three methods available to prepare multiple phase emulsions—mechanical stirring, phase changing, and two-step emulsion—the latter is most popular. Two-step emulsion involves separating internal and external aqueous solution droplets with an oil membrane, such that the released components can be wrapped up within the oil droplets. Under specific circumstances, the rate release of the materials can be controlled using various spreading and permeating methods or by breaking the membranes.

The multiple phase emulsification technique[4–8] was developed in 1965 and applied to regular insulin in 1968 to improve its absorption at the intestinal wall. In recent years, this technology has been applied, for example, to the manufacture of cosmetics, liquid film separators (to separate metal ions), devices for regular fermentation, systems for lengthening the release rates of drugs, and food research.

W/O/W-type multiple emulsification systems[9–11] can be divided into two classes: those prepared through one-step and two-step emulsification processes. Most multiple phase emulsification systems described in the technical literature have been prepared using two-step emulsification.[12–14] In this study, we developed a one-step emulsion strategy to prepare a multiple phase emulsion system. We employed three different emulsifiers—ceteareth-22/palmeth-2 Ceteareth-22 (INCI name) and palmeth-2 (INCI name) are polyethylene glycol ethers having the general formula $R(OCH_2CH_2)nOH$. Ceteareth-22

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is a cetearyl alcohol in which R represents a blend of alkyl groups derived from cetyl and stearyl alcohols, and n has an average value of 22. The R group of palmeth-2 is an alkyl group derived from palmityl alcohol; n has an average value of 2, polyglyceryl-2 diisosterate, and polyglyceryl-3 methylglucose distearate—that we combined together with added lithospermum and subjected the systems to specific experimental conditions (water phase temperature: 70°C; oil phase temperature: 40°C) for an appropriate mixing time to obtain the multiple phase emulsification systems. We characterized our multiple phase emulsification systems using polarized microscopy (2000×), contact angle measurements, transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), and attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy. We suspect that these systems might have relevance as future nanocosmetics and might expand the application of nanotechnology into people's everyday lives.

EXPERIMENTAL

Materials

Ceteareth-22/palmeth-2, polyglyceryl-2 diisostearate, and polyglyceryl-3 methylglucose distearate[1] (Kosfarm Co.) were used without further purification. Rhodamine B (Fig. 1) was supplied by Koch-Light Chemical Co.

Preparation of the lithospermum multiple phase

A W/O/W-type multiple phase emulsion features an oil phase containing a water-active compound that is then placed into another water phase. Aqueous phase B (Table I) was heated at 70°C to obtain an evenly dissolved mixture. Oil phase A (Table I) was heated at 40°C until uniformly dissolved. Aqueous phase C comprised lithospermum (0.1%). The two evenly slowly dissolved into the aqueous phase after the oil phase with stirring mixer over a suitable homogenization time to obtain the multiple phase emulsions. The homogenization time, the sequence

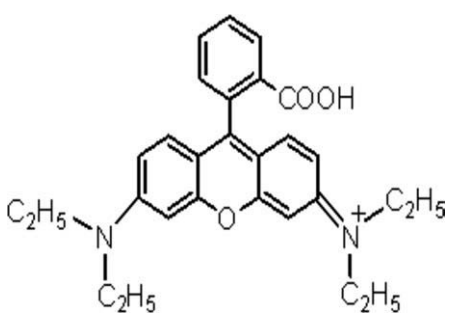

Figure 1 Structure of the fluorescence probe, rhodamine B.

TABLE I
Formulations of the lithospermum Multiple Phase Emulsion Creams

Phase	Material	Parts
A (oil phase)	ethylhexyl palmitate	7
	jojoba oil	5
	ceteareth-22, palmeth-2	2.5
	polyglyceryl-2, diisostearate	2
	$C_{12–15}$ alcohol benzoate	2
	polyglycery1–3 methyl glucose distearate	2
	stearyl alcohol (m.p. = 59 °C)	1
	cetyl esters	1
	phenoxyethanol and (Mp, Ep, Bp, PP)	0.5
B (water phase)	demineralized water	60.5
	polyacrylate and glycerine	10
	sodium hyaluronate	4
	Glycerine	2.5
C (water phase)	Lithospermum	0.1–0.5
Total		100

of addition of the additives, and the shear stress were the key variable affecting the multiple phase emulsification process. It was very easy to observe the structures of the multiple phase emulsification products using polarized microscopy because the active ingredient, lithospermum, is red.

Measurements

A Perkin–Elmer ATR-FTIR spectrometer (Cetus Instruments, Norwalk, CT) was used to monitor the spectral transmittance and absorbance changes of the lithospermum samples. Spectra were measured at a resolution of 4 cm^{-1}; 32 scans were recorded per sample.

Contact angles, i.e., the angles formed between planes tangent to the surfaces of the solid and the liquid at the wetting perimeter, were measured using a FACE CA-5 contact angle meter. A drop of water was placed on the sample using a syringe. The contact angle of the samples was measured at 25°C; the syringe size was 20 mm and the drop volume was 20 μL. After the tip of the needle was separated from the drop, the contact angle was measured. Each contact angle of a lithospermum sample was measured 10 times; average values are provided herein.

Surface tension was determined at room temperature using a Japan Kaimenkaguka CBVP-A3 surface tensiometer, which was calibrated with ultrapure water before use. The platinum plate was cleaned by flaming; glassware was rinsed with tap water and ultrapure water. A 2.0% (w/w) lithospermum solution was freshly prepared as a stock solution and then diluted to the desired concentration for each measurement. Surface tension was measured three

times for each concentration; an average error of less than 0.5 dyne/cm was obtained routinely.

The emission spectra of the solutions were measured using an Aminco–Bowman Series 2 luminescence spectrometer. The excitation wavelength was 350 nm; the emission was measured between 370 and 800 nm. Hydrophobicity was evaluated by monitoring the emission spectra of rhodamine B ($6 \times 10^{-5} M$ aqueous solutions). The probe solutions were prepared by dissolving rhodamine B in deionized water and adding lithospermum (20 g/L) to the solution.

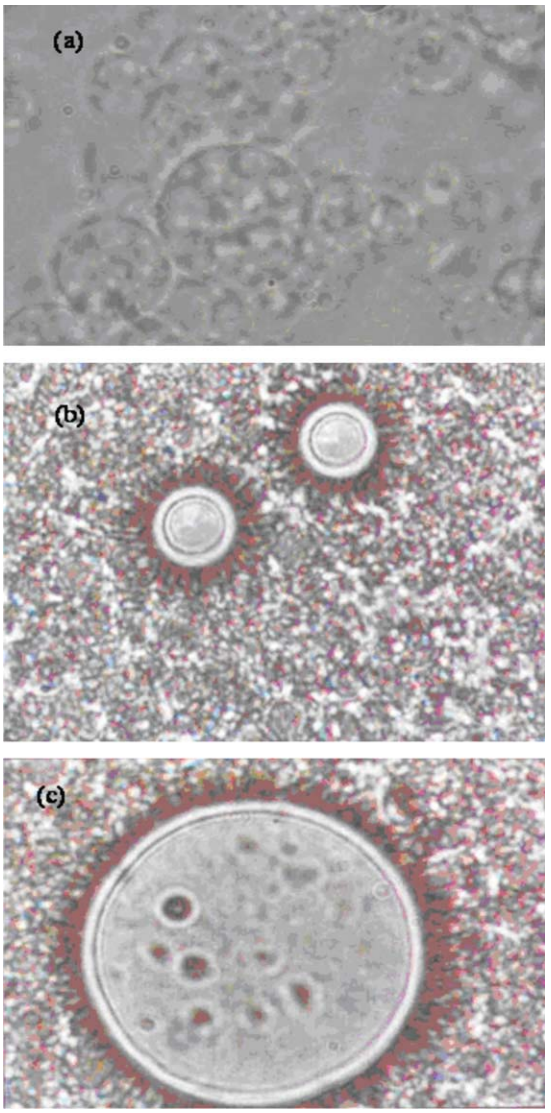

Figure 2 (a) Polarized microscopy image of the lithospermum multiple phase emulsion cream at 2000× amplification. (b) Enlarged image of the sample obtained after 5 min of homogenization, observed under a polarized microscope at 1200× amplification. (c) Enlarged image of the cream without lithospermum added, after 5 min of homogenization, observed under a polarized microscope at 2000× amplification. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE II
ATF-FTIR Spectroscopic Absorption Frequencies of Lithospermum

Bond	Frequency (cm^{-1})	Functional groups
O—H	3434	
C—H	2850–2926	
C=O	1720	aldehyde, ketones
C=C	1603	alkenes
C=C	1597	aromatic
RCO—OR′	1271	
RC—OH	1113	
R—H	1058	propyl, aldehyde, R—SO—R

AFM scans were performed using a Digital Instruments CP II apparatus operated in the tapping imaging mode, such that the oscillating probe cantilever would cause the tip to make only intermittent contact with the sample. With respect to the phase of the sine wave driving the cantilever, the phase of the tip oscillation was quite sensitive to the sample surface characteristics. Silicon nitride tip had a length of 200 mm and a scanning frequency of 0.5 Hz; the sample size was 10 mm × 10 mm. A mixture of the sample (2 g) in acetone (10 mL) was smeared on a glass slide and dried before AFM observation.

RESULTS AND DISCUSSION

Polarized microscopy

Polarized microscopy can be used to observe the properties of liquid crystals; pellet, needle, plate, and fiber phenomena; and the colors of minerals under single polarized light. For our multiple phase emulsion systems, Figure 2(a) reveals that the multiple phase structures were evident at amplifications greater than 2000×; the red region in the image represents lithospermum. Typically it is not easy to observe multiple phase emulsions using polarized microscopes. After 5 min of homogenization, Figure 2(b) reveals the distinct netted structure of the liquid crystal. Figure 2(c) presents the image of cream added with lithospermum; again, we observe a multiple phases emulsion under the polarized microscope set at an amplification of 2000×.

Tables II and III list the absorption wavelengths in ATR-FTIR spectra of lithospermum and the lithospermum multiple phase emulsion cream.[15–17] Figure 3 displays these ATR-FTIR spectra. This figure displayed bands at 3200–3550 (—OH, stretching), 2926 cm^{-1} (—CH_2, asymmetric), 2850 cm^{-1} (—CH_2, symmetric), 1640–1700 cm^{-1} (C=O, stretching), 1515–1650 cm^{-1} (N—H, bending) and 1450 cm^{-1} (C—N, stretching).

TABLE III
ATR-FTIR Spectroscopic Absorption Frequencies of the lithospermum Multiple Phase Emulsion Cream

Bond	Frequency (cm^{-1})	Functional groups
O—H	3345	
C—H	2853–2926	
C=O	1738	aldehyde, ketones
C=C	1637	alkenes
C=C	1597	aromatic
C—H	1469	alkanes
RCO—OR′	1260	acetates
R—H	1077	propyl, aldehyde, —SO—R

Contact angles

In general, surfactants not only reduce liquid surface tension and surface free energy but they also perform spreading, permeating, and wetting functions. The general moisture ability (moisturizing efficiency) can be determined through contact angle measurement. The smaller the contact angle, the better the moisturizing efficiency. According to the Figure 4, our lithospermum multiple phase emulsion cream exhibited superior moisture ability. Upon increasing the concentration, the contact angle decreased, reaching 10° when the concentration was less than 2%, implying superhydrophilicity and good wetting ability for cosmetic applications.

Surface tension

For a solution containing micelles, the surface tension of the solution decreases upon increasing the concentration of a surfactant, causing a clear break point that is considered to be the critical micelle concentration (CMC). Figure 5 displays the surface tensions of emulsions containing lithospermum (purple) at concentrations varying from 0 to 2%. We observe

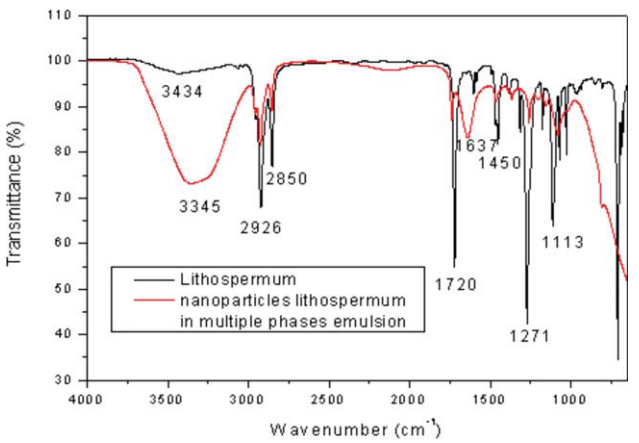

Figure 3 ATR-FTIR spectra of lithospermum and the lithospermum multiple phase emulsion cream. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

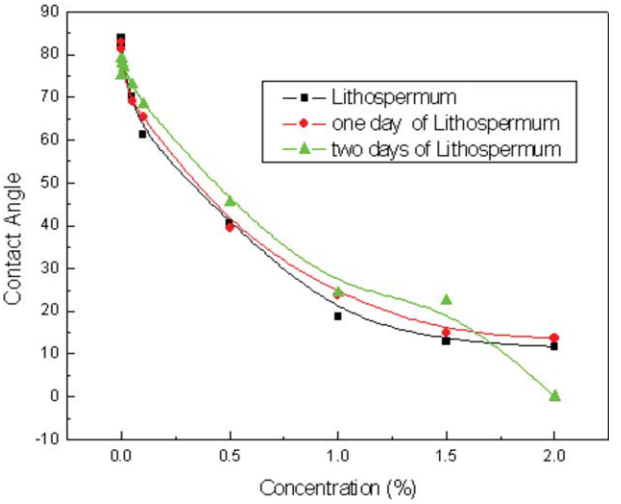

Figure 4 Contact angles of emulsions containing various concentrations of lithospermum. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

an obvious turning point when the lithospermum (purple root) concentration was in the range 0.0005–0.005%, suggesting the formation of micelles, with a CMC of ca. 0.01%; when the density increased to 1%, the surface tension tended toward stable conditions.

Fluorescence

We evaluated the fluorescence intensity of rhodamine B within the lithospermum samples as a probe for the polarities of the microenvironments experienced by rhodamine B, such as micelles.[18] Figure 6 reveals that the fluorescence intensity of the aqueous

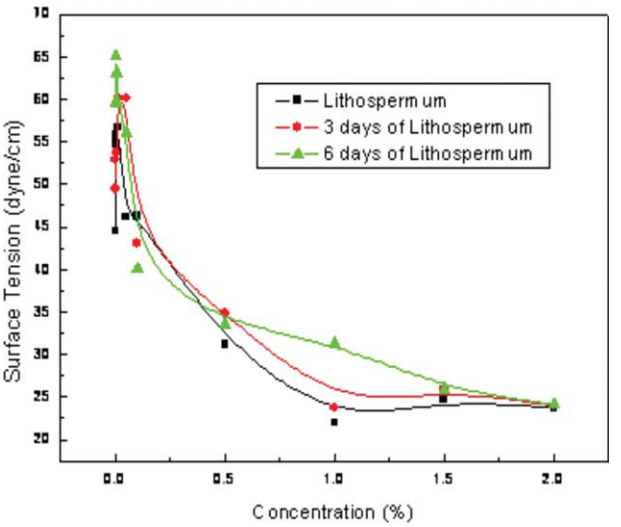

Figure 5 Surface tensions of emulsions containing various concentrations of lithospermum. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

solution increased upon increasing the amount of lithospermum in the sample. This phenomenon, similar to the surface tension, is consistent with an increase in the concentration of lithospermum at the liquid surface.

AFM

Phase imaging is a very effective means of mapping the submicron morphologies of multicomponent polymer composites based on the relative elasticity of individual components.[19] The red bulges in the AFM image in Figure 7(a) represent lithospermum micelles within the emulsion, over a scanning area of 20 μm × 20 μm. Figure 7(b) displays the height profile of the lithospermum micelles within a scanning range from 20 to 300 nm. The topographic image appears lumpy, while the phase image is filled with isolated small domains having irregular granular or channel-like shapes. In the phase image, the continuous brighter domain is the lithospermum phase.

CONCLUSIONS

Traditional multiple phase emulsification is typically a two-step emulsification process; in this study, we adopted a one-step multiple phase emulsification process, combined with nanotechnology, to add the Chinese herbal medicine lithospermum to a multiple phase emulsification system. The surface tension revealed that the system formed oil-in-water (O/W) micelles when the density increased to 1%, its contact surface activity better suits the increase to the cosmetics. Contact angle <90° expressions wettability worse, may from Contact Angle >90°, the data chart sees, the angle goes past the small wettability better, but when 1%, contact Angle goes past tends to 20°, its contact surface activity is better.

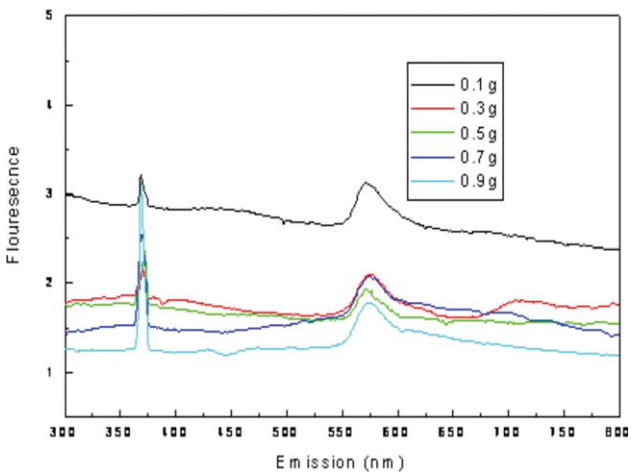

Figure 6 Fluorescence spectra of emulsion samples containing rhodamine B as a probe and various concentrations of lithospermum. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

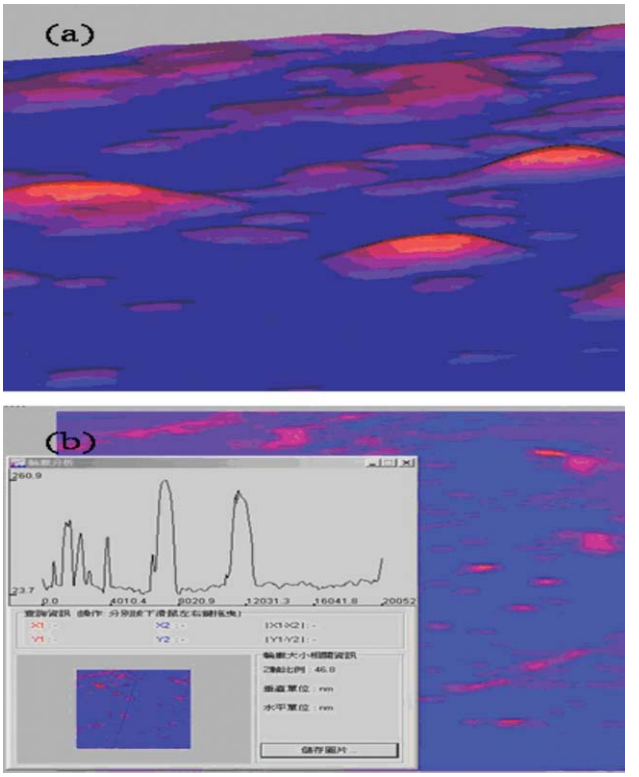

Figure 7 (a) AFM image of lithospermum micelles (scanning length: 20 μm; width: 20 μm; height: 4 μm). (b) AFM image and height profile (distribution: ca. 20–300 μm) of the lithospermum micelles. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The fluorescence intensity of rhodamine B increased strongly upon increasing the lithospermum (purple root) content above 0.1 g, suggesting that it was located in an environment of high polarity, providing superior contact surface activity.

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