Drug Development and Industrial Pharmacy, 2011; 37(7): 765–774

ISSN 0363-9045 print/ISSN 1520-5762 online
DOI: 10.3109/03639045.2010.541261

informa
healthcare

RESEARCH ARTICLE

Preparation and characterization of β-elemene-loaded microemulsion

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Abstract

Intravenously injectable emulsion of β-elemene was studied in detail. Both blank and β-elemene-loaded microemulsions were prepared using a simple water titration method. The pseudoternary phase diagram was constructed for the optimization of microemulsion. The loading capacity test, dilutability test, and especially the influence of antioxidants were conducted for further optimization of β-elemene-loaded microemulsion. Transmission electron microscope showed intact and spherical microemulsion droplets. Conductivity and viscosity measurements were used to study the phase behaviors of β-elemene-loaded microemulsions, providing convincing explanation. *In vitro* release study showed that β-elemene was steadily released until 12 h, which most fitted the first order.

Keywords: β-Elemene, *microemulsion*, *physicochemical properties*, *antioxidant*

Introduction

Drugs from the plant resource are especially popular these days. β-Elemene, the main effective monomer of natural sesquiterpene extracted from the roots and stems of Curcuma wenyujin[1] (see Figure 1 for its chemical structure), is a broad-spectrum antitumor agent[2]. The major advantages of β-elemene as an anticancer drug are as follows: (i) it has a broad-spectrum antitumor effect in many types of cancer, including drug-resistant tumors; (ii) it has no direct multidrug resistance activity and can reverse the resistance to other drugs; and (iii) it has low toxicity and is therefore well tolerated and accepted by cancer patients[3–5].

Formulation of this sesquiterpene until now has been realized by means of injectable emulsion of β-elemene[6], β-elemene Calcium Alginate-Chitosan Microcapsules[7], β-elemene Long-Circulating Liposome[8,9], and β-elemene Solid Lipid Nanoparticle[3–5]. Among them, after being demonstrated to use in clinic by the State Administration of Pharmacy of China and the Ministry of Health of People's Republic of China, injectable emulsion of β-elemene has been developed and found in clinical use on the therapy of cancers of lung, brain, and ascites or hydrothorax caused by cancer[10] and commercially available in Chinese market. Injectable emulsion of β-elemene has strong action of killing tumor cells and inhibiting the growth of tumor cells. However, it can cause phlebitis in some population after intravenous (i.v.) administration and gives a relatively low bioavailability after oral administration, which had severely limited the broad use of it[8,9]. Hence, other types of carriers would be more appealing as parenteral delivery systems of β-elemene, which are of submicron size and higher drug-loading capacity. Such carriers can be more physically stable and more compliant with cancer patients. The development of this kind of pharmaceutical formulations for the i.v. administration of β-elemene might therefore be desirable.

Microemulsion (ME) is a drug delivery system that has been widely investigated during the past decades. It is composed of oil (O), surfactants (S), and cosurfactants (C), and has typically a droplet diameter of approximately 100 nm or less[11,12]. ME is thermodynamically stable, low viscous, transparent, and has optical isotropic with a dynamic microstructure that forms spontaneously. It is

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(Received 30 June 2010; revised 05 November 2010; accepted 10 November 2010)

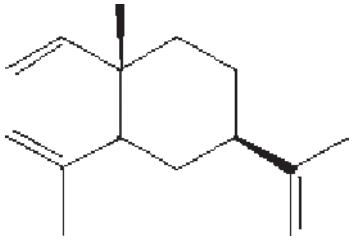

Figure 1. Structure of β-elemene.

easy to prepare with small and uniform particle size in the range of 10–100 nm. ME is of certain outstanding physical characteristics when compared to other colloidal systems such as liposomes and solid lipid nanoparticles (SLNs). It makes ME a promising candidate for drug delivery systems. Furthermore, the oily core allows highly efficient incorporation of lipophilic drugs. Multilayer liposomes are not stable and easy to aggregate because of their bigger particle diameter compared to ME. Meanwhile, the drug-loaded matrix used in SLN is single solid lipid, which is easy to form lipid crystal that could transform to perfect crystal gradually. The transformation may lead the extrusion of drugs out of crystal lattice to make SLN have problems of low drug-loaded capacity and easy to expose[13,14]. These years, phospholipids-based ME has attracted a great deal of interest as a pharmaceutically acceptable ME.

In spite of extensive scientific research in the past decade, the majority of the commercial use reported in the scientific literature concerning ME systems is about oral delivery vehicles. However, i.v. routes are also of great potential to which the ME technique can be applied. O/W MEs can be used for parenteral drug delivery[15,16].

Although some ME products are commercially available in the market, their problems should be noticed. First of all, high concentration surfactants and cosurfactants are used in ME, most of which have chronic side effects. So one of the focal points should be considered is how to depress or eliminate the toxicity and irritation of the formulation. High-performance and low-toxicity surfactants and cosurfactants should be found and their concentration should be modified to the minimum. For example, the surface-active formulation ingredient Solutol HS 15, the main component of which is the polyethylene glycol 660 ester of 12-hydroxystearic acid, shows the potential to increase the solubility of a number of sparingly water-soluble and highly lipophilic compounds. Solutol HS 15 has been approved in a parenteral phytonadion formulation for human use in Canadian market[17]. Another focal point is investigating the condition of forming ME by reforming pseudoternary phase diagram. Then, the localization of application in ME comes from the ability to dilute infinitely. ME is diluted by large amount of blood after intravenously injected. In this condition, how to maintain the morphous and stable characteristic of ME should be studied. In this way, the transformation when loading drug should be investigated carefully by utilizing water dilution. At the same time, the discontinuity of dilution line should be avoided.

The main objectives of this study were as follows: (1) to study the impacts of various factors on the ME; (2) to predict the *in vivo* behaviors of β-elemene ME; (3) to study the physicochemical properties of β-elemene ME through conductivity and viscosity measurements; and (4) to test the *in vitro* release of β-elemene ME.

Materials and methods

Materials

Standard β-elemene was purchased from National Institute for the Control of Pharmaceutical and Biological Products. β-Elemene was kindly supplied by Curcuma Oil Research Institute of Leqing Wente. Labrafac cc was a kind gift from Shanghai Gaowei Industry Co., Ltd. Sodium sulfite (SS), Natrium bisulfurosum (NB), sodium metabisulfite (SM), and propylene glycol were purchased from Yuwang Science and Technology Co., Ltd. Phosphatidylcholine (SbPC) (purity >96%) was obtained from Shanghai Taiwei Pharmaceutical Company Limited. 12-HAS-EO15, (Solutol or HS15), was from BASF with the batch number: 67056. All the other chemicals were of analytical reagent grade and used without any further purification.

ME preparation

Construction of pseudoternary phase diagram

The pseudoternary phase diagrams of oil, surfactant, cosurfactant and water were constructed according to the method described previously[18], which was based on the simple water titration method[19]. Briefly, Labrafac cc, the oil (O), phosphatidylcholines (SbPC), and HS 15 (1:1, w/w), the mixing surfactants (S), propylene glycol, the cosurfactant(C) were weighed in the same screw-capped dark-brown glass vial with tight closures. The mixture was magnetically stirred in order to reach the equilibrium quickly. And then it was stored overnight at room temperature. At Km 1:1 mixtures of oil phase (O), surfactant/cosurfactant were prepared, where ratio of oil and S/C in the mixtures was varied from 1:9 to 9:1. During the titration, samples were stirred to allow equilibration. In case that monophasic, clear, and transparent mixtures were visualized after stirring, the samples were indicated as points. The phase boundary was determined by observing the changes of the sample appearance from turbid to transparent or from transparent to turbid. The area covered by these points was considered as the ME region of existence. After being equilibrated, the mixtures were assessed visually for phase separation and transparency. MEs were prepared 24 h before the experiments and maintained to allow the system to reach complete equilibrium.

Selection of ME formulations for detailed studies

Based on the preliminary study and the chemicophysical properties of the oil phase, certain formulations were selected for further studies. Indeed, from the constructed pseudoternary phase diagram, one initial Labrafac cc/SbPC/HS 15/propylene glycol mixture was selected.

Furthermore, five potential MEs (referred to as A–E in Table 1) different from each other by the ratio of O/SC were selected and prepared at Km 1:1. For the preparation of the ME vehicles, appropriate quantities of Labrafac cc, SbPC, and HS 15 (1:1, w/w), propylene glycol, and aqueous phase were weighed into the screw-capped glass vial. The mixtures were stirred with a magnetic bar at room temperature. Transparent, single-phase formulations were formed in a few seconds.

Preparation of β-elemene-loaded ME

β-Elemene-loaded ME was prepared by dissolving β-elemene into the oil-S/C mixture. Since β-elemene is soluble in Labrafac cc, the amount of drug should not be ignored. So the whole oil's weight should be the weight of drug and Labrafac cc, namely, taking the place of some sums of Labrafac cc with the same amount of β-elemene. Other preparation procedures were as 2.2.1. The final mixture was stayed up for 24 h for balancing, and the concentration of β-elemene in the sample was determined by high-performance liquid chromatography (HPLC).

Characterization of ME

Optical transparency

The homogeneity and optical isotropy of pure and drug-loaded ME was examined by a cross polarizer (Aus Jena polarizing microscope, Carl Zeiss, Oberkochen, Germany) and visual examination at room temperature. Samples were analyzed for any precipitation or phase separation in presence of drug.

Determination of particle size

The mean droplet size of the MEs with or without drug was determined at 25°C by dynamic light scattering (DLS) measurements (Nicomp TM380, Santa Barbara, UAS PSS). Measurements were obtained at an angle of 90 degrees on MEs after filtration through a microporous filter with 0.45 μm pore diameter (Millipore®) and repeated three times for each sample. The average size was reported by the intensity distribution for each measurement.

Centrifugation

In order to eliminate metastable systems, the selected ME vehicles as well as drug-loaded MEs were centrifuged (Sigma 2–16 centrifuge, Sigma Laborzentrifugen GmbH, Osterode, Germany) at 13,000/rpm for 30 min[20].

Electrical conductivity measurements

The electric conductivity (σ) was measured using a DDS-11A digital conductivity meter (SPSIC-REX Instrument Ltd., Co.) operating at 50 Hz. The error limit of conductance measurements was ± 3%. The temperature was kept at 25°C and maintained by a LB801-2 thermostat (Liaoyang Thermostat Instrument Corporation, China). Conductivity measurements were carried out by titration of oil and surfactant/cosurfactant mixture with buffer. Readings were taken after the conductivity stabilized for at least 5 min. The data was expressed in terms of weight fraction Φ (%, wt) of aqueous component, which is defined as Φ (%, wt) = (wt of buffer/total wt of ME) ×100. It should be noted that the samples remained clear, and there were no observable changes in the phase diagram.

Table 1. Compositions of the improved microemulsion formulations (w/w, %).

Component	ME A	ME B	ME C	ME D	ME E
Oil phase (O)	8	10	12	14	16
Surfactant/ cosurfactant (S/C)	32	30	28	26	24
Aqueous phase	60	60	60	60	60

Rheological measurements

The rheological properties of β-elemene-unloaded and β-elemene-loaded ME (1% wt drug) were determined using Ubbelodhe Viscosimeter (Schott, SCHOTT ASTM). All rheological determinations were carried out in triplicate for all samples at 25.0 ± 0.1°C.

pH

The pH values of the samples were measured using a pH meter at 20 ± 1°C (model HI 8417, Hanna Instruments Inc, Woonsocket, RI, USA).

Transmission electron microscope

The shape and surface morphology of the ME droplets was observed using a JEM-1200EX TEC (JEOL Company, Japan) in China Medical University. A carbon-coated 200-mesh copper specimen grid (Ted Pella Inc., Redding, CA, USA) was put on a wax board. Approximately 2 μl of β-elemene ME was deposited on the grid and left to stand for 1.5 min, and all excess fluid was removed with filter paper. The grid was then stained with one drop of 2% phosphotungstic acid (pH = 7.4) for 15 min. The grid was allowed to dry at room temperature for an additional 30–60 min before being examined.

Determination of β-elemene

The solubility of β-elemene in various excipients, and the amounts of β-elemene in each formulation were measured by HPLC, and each measurement was repeated three times. The HPLC apparatus consisted of a pump (Shimadzu SPD-10A) and a 20 μl loop (Rhenodyne model 7725i). A Diamonsil TM C18 column (200 mm × 4.6 mm, 5 μm, Dikma Technologies) and a Phenomenex C18 security guard (4 mm × 3.0 mm, 5 μm, Torrance) were utilized for drug separation, using a mixture of acetonitrile:water (92:8, v/v) as mobile phase. The flow rate of 1.0 ml/min led to a retention time of 10.1 min. A 20 μl volume was injected into the column and the UV detector was set at λ = 210 nm. The assay was linear (r^2 = 0.9996) in the concentration range 3–223 μg/ml. The method was validated with respect to accuracy and inter-and intra-day precision and the relative standard deviation was less than 2% in both cases. Measurements were obtained after filtration through a microporous filter with 0.22 μm pore diameter.

In vitro release of β-elemene from ME

The study of *in vitro* release of β-elemene from ME was repeated five times. The *in vitro* release profile of β-elemene from ME was assessed by determination of the residual amount of β-elemene in ME[3–5]. Briefly, 5 ml of β-elemene ME (10 mg/ml) was placed in 10 dialysis bags (MW cutoff 12,000–14,000 Da), respectively, ligated, and immersed in the release media of 0.5% HS 15 (2500 ml) stirred by magnetic force at 25°C. Samples were taken out at predetermined time intervals of 0, 1, 2, 3, 4, 6, 8, 10, 12, 24, and 36 h. Aqueous dispersion in the dialysis bag was transferred into a 5 ml volumetric flask, and the remains in the bag were washed into the same volumetric flask with a little amount of release media. Then, 1 ml acetic ether was added into the flask to extract drug dissolved in release media and release media was added to the scale. The mixture was vortexed for 5 min and then centrifuged at 6000 rpm for 10 min. The supernatants were filtered through 0.22 μm microporous membranes and subjected to HPLC analysis for the determination of β-elemene.

Results and discussion

Selection of blank ME formulation

As the i.v. administration, O/W ME should be diluted by a large amount of water, decreasing the percentage of S/Co will make the ME safer when injected. So formulation in the region of unlimited dilution in the pseudoternary diagram should be selected for further comparison. Based on the facts which affect the forming of ME below, the phase diagram was investigated with Labrafac cc as oil phase, SbPC:HS 15 = 1:1 as mix-surfactant, propylene glycol as cosurfactant at the fixed Km of 1:1 at 25°C. The formulations with the different ratio of O/SC chosen (Table 1) were all well diluted with aqueous solution. As was seen in Figure 2, formulation of ME A, B, C, and D correspondingly had the particle diameters of 68 ± 7.6 nm, 69 ± 8.5 nm, 71 ± 8.7 nm, and 72 ± 8.9 nm, which were not significantly different. And ME E had the particle size of 85 ± 10.9 nm, which is bigger than the other four. From a formulation viewpoint, the increased oil content in ME may provide a greater opportunity for the solubilization of poorly water-soluble drugs. Formulation with larger percentage of oil and smaller particle size should be selected for a bigger drug-loaded capability and safety. So formulation of ME D, whose O/SC was 0.538, was selected for further investigation.

Optimization of β-elemene-loaded ME

Capacity of β-elemene loading in β-elemene ME

Different MEs of increasing concentration of β-elemene and different ratios of β-elemene and Labrafac cc of 1:7, 1:6, 1:5, 1:4, and 1:3 were prepared with the total amount of oil phase fixed. After being diluted by aqueous phase in 5 ml volumetric flask, the particle sizes of samples were determined by DLS. Their appearance and the results of particle sizes were shown in Table 2.

Figure 3 shows that it is a curious phenomenon that the particle size of blank ME is 72.0 ± 8.9 nm, which is larger than that of β-elemene-loaded ME with β-elemene/Labrafac cc ratio of 1:7 and 1:6, whose particle size is 45.8 ± 4.3 nm and 61.0 ± 9.7 nm, respectively. Namely, when β-elemene was added at different drug/oil ratio of 1:7 and 1:6, the ME size decreased compared to blank ME, but increased slightly with increasing β-elemene concentration. When drug loading increased to the ratio of 1:5, particle size was larger than that of blank ME, reaching 83.7 ± 15.4 nm. When β-elemene content further increased to the ratio of 1:3, particle size increased dramatically with turbid appearance and no more presentation of the properties of ME.

Currently, it is not clear by which mechanism droplet size decreases when β-elemene/Labrafac cc ratio is 1:7 and 1:6 compared to blank ME. However, on the one hand, the two following possibilities can be considered. First, a certain portion of undissolved drug could act as an emulsifying agent by the deposition of drug particles at the interface of ME[21]. Second, by the deposition of drug at the interface of ME, the reduced mobility of surfactant is thought to decrease the particle size of drug-loaded ME.

On the other hand, the incline of ME size with increasing the β-elemene content further may be related to the formation of drug aggregating on the surface of oil droplets due to the excess amount of drug undissolved. It indicated that maybe the excess amount of drug that existed in the interface of the oil–surfactant mixture at preparation was released to the aqueous phase and increased the precipitate of β-elemene in the course of time.

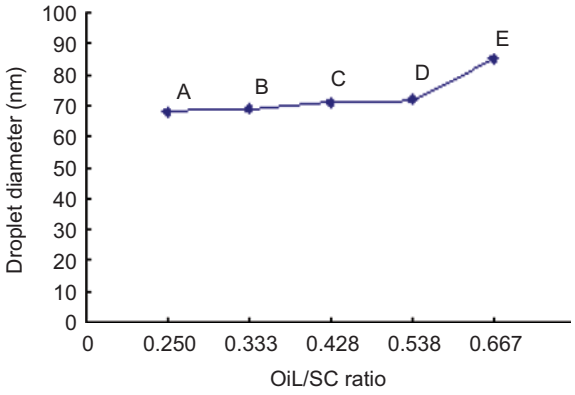

Figure 2. Determination of droplet size of the MEs by light scattering.

Table 2. The effect of drug capacity on the appearance of microemulsions.

M (β-elemene):m (Labrafac cc((w/w)	Drug content in oil phase(%)	Appearance
0:8	0	Translucent
1:7	14.28	Clear
1:6	16.67	Clear
1:5	20.00	Translucent
1:4	25.00	Translucent
1:3	33.00	Cloudy

Effect of dilutability on β-elemene ME

The dilutability of the MEs was assessed to investigate whether these systems could be diluted with the aqueous phase with separation or not. The ability of ME dilution was identified by determining the mean particle size of MEs of different diluted folds. For this purpose, β-elemene-loaded ME with drug/oil ratio of 1:7, which was of the smallest particle size in the drug-loaded MEs was selected, as well as the blank ME. They were diluted with aqueous phase to vary multiple and their transparency was assessed visually. The particle size measurements (at quantitative dilution) were conducted using DLS.

As the results showed in Table 3, after diluting samples by 5, 10, 25, 50, and 100 multiples, the particle size of blank ME and β-elemene-loaded ME increased slightly, but still in the range of ME particle size. The appearance was all clear with blue opalescence.

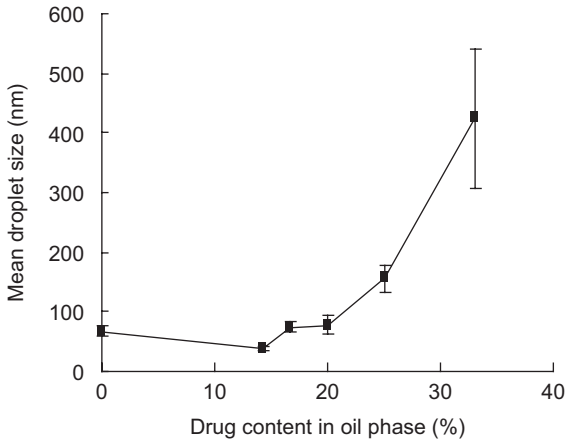

Figure 3. Particle sizes distribution of microemulsions as a function of β-elemene content (%, w/w) of the oily phase ($n=3$, mean).

Table 3. The effect of diluted fold on mean particle size of blank microemulsion and β-elemene-loaded microemulsion ($n=3$, mean).

Diluted fold					
	5	10	25	50	100
Blank	67.2±8.9	67.5±5.0	68.0±3.6	68.2±7.5	69.4±7.3
Drug-loaded	50.5±5.9	54.7±3.4	55.3±5.6	57.9±6.6	59.0±6.7

Influence of additives on the stability of β-elemene ME

Being an unsaturated fatty acid, β-elemene is susceptible to be oxidized. So, adequate amount of antioxidants should be added in the aqueous solution of the ME. Sodium sulfite (SS), Natrium bisulfurosum (NB), and sodium metabisulfite (SM) were selected as antioxidants in this study. As is known, the reaction rate of the three of them is fast in the surrounding of alkalescence. Namely, they have strong antioxidation capability. On the contrary, when put in acidity and neutral solution, their slow reaction rate and weak antioxidation capability are showed. According to what is concluded in a previous study[18,22], the optimal pH value of aqueous phase in this formulation is 7.4, which is corresponding to the surrounding of alkalescence exactly, so 0.1% antioxidizing agents were added in the aqueous phase. The best antioxidizing agent was chosen by investigating the long-term stability under 25°C and 4°C.

As Tables 4 and 5 showed, Natrium bisulfurosum has the best capability of antioxidation at both 25°C and 4°C. The oxidation resistance effect of NaHSO3 was better than the others. It might be known[23] that in the area of pharmaceutical preparation, added antioxidation and protected drug react competitively with oxygen, and the capability of antioxidation for protecting drug depends mainly on the velocity of oxidizing reaction. According to the rate of reaction, which determines preferential sequence of competitive reaction, the greater the rate of reaction is, the better antioxidizing effect will be. In the condition

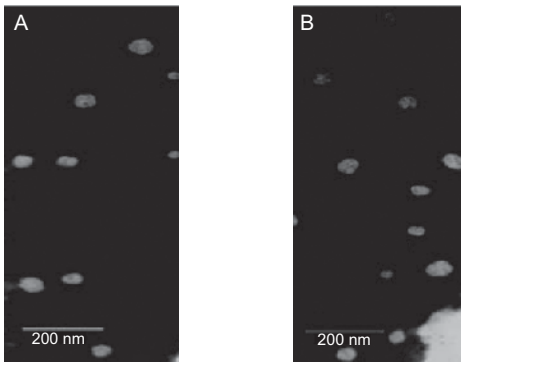

Figure 4. Transmission electron micrograph of the microemulsions. (A) Black microemulsion; (B) β-elemene-loaded microemulsion.

Table 4. Effect of different antioxidant agents on the stability of β-elemene ME at 25°C ($n=3$, mean).

	SS				NB			SM	
Monitoring period	pH	App.	Con.%	Ph	App.	Con.%	pH	App.	Con.%
0 d	8.02	–	100.7	7.66	–	100.5	7.74	–	100.7
3 d	8.02	–	100.5	7.96	–	100.4	8.04	–	100.6
6 d	7.98	–	100.1	7.98	–	100.4	8.06	–	100.6
15 d	7.90	–	100.0	8.09	–	100.5	8.17	+	99.9
30 d	7.78	–	99.5	8.51	–	100.3	8.59	+	98.4
60 d	7.49	–	99.4	8.60	–	99.8	8.70	++	89.0
90 d	7.30	+	93.2	8.74	+	97.4	8.84	+++	56.4

Note: '–' clear, blue, '+' translucent, blue I '++' translucent, blue II, and '+++' cloudy, yellow.

Table 5. Effect of different antioxidant agents on the stability of β-elemene ME at 4 °C (n = 3, mean).

	SS			NB			SM	
Monitoring period	pH	App.	Con.%	Ph	App.	Con.%	pH	App.	Con.%
0d	8.02	–	100.3	7.66	–	100.5	7.74	–	100.7
3d	8.02	–	100.1	7.68	–	100.4	7.78	–	100.6
6d	8.00	–	100.1	7.73	–	100.4	7.82	–	100.6
15d	7.98	–	100.0	7.86	–	100.4	8.06	–	100.0
30d	7.89	–	100.0	8.24	–	100.2	8.32	+	99.4
60d	7.86	–	99.9	8.33	–	100.0	8.70	+	97.3
90d	7.60	+	97.6	8.49	–	99.0	8.89	+++	66.4

Note: '–' clear, blue, '+' translucent, blue I '++' translucent, blue II, '+ + +' cloudy, Yellow

of weak alkalescence, the rate of reaction was ranked in the order of Natrium bisulfurosum > Sodium sulfite > sodium metabisulfite. Moreover, the above agreed with the conclusion we have drawn. That is, $NaHSO_3$ is the best antioxidizing agent in the formulation. With regard to the choice for the storage temperature, compared with 25°C, the preparation was more stable at 4°C. The reason was that increased temperature raises the rate of oxidizing reaction, so it is reasonable to choose the relatively low temperature to store samples.

TEM imaging

The cryo-TEM images provide complementary information on the size and shapes of different ME structures. The shapes of the diluted (70–95 wt%) ME O/W droplets appeared to be spherical. β-Elemene-loaded systems (B) with the same water concentration showed smaller droplets than the empty systems (A). These results are in good agreement with the DLS results—β-elemene shrinks the droplets.

The particle size determined by TEM micrograph was tending to be smaller (65nm for blank ME and 39nm for β-elemene-loaded ME, respectively) than that when measured using the Particle Sizer (Figure 4). This is understandable because the photon correlation spectroscopic particle Sizer determines the size of the particles by measuring the movement of the particles due to Brownian motion[24]. Therefore, the particle size determined using the particles Sizer was in fact the size of the particles with their surrounding aqueous boundary layer, which moved together with the particles. In contrast, the particle size derived from the TEM micrograph was the size of the particles alone.

Conductivity measurements

The physiological environment in the human body is hydrophilic and the β-elemene should be incorporated into the O/W ME to favor the applications in future. Therefore, it is important to investigate the phase transition in the ME. Electrical conductivity is a structure-sensitive property. In this study, the electrical conductivities of the ME were measured, and the results indicated that this method could be used for this system. Whether it is the systems of nonionic surfactants or ionic surfactants, their electrical conductivity follows Percolation threshold[21].

The electrical conductivity was measured as a function of the composition of the system. At first, when being lower than Percolation threshold (Φw≈15%(w/w)), the conductivity remains low up to a certain weight fraction of water, Φc. Surfactant solution, which is electric conduction, dispersed in continuous oil phase as droplets when the water concentration was low. The electric conduction channel had not been connected, which resulted in low conductivity of the systems. Systems with a low conductivity were therefore designated as W/O droplet MEs[25].

However, when the water content is raised to a certain value above Φc, along with the increase in the number of microparticle, which was electric conducting, the distance between the microparticles became reduced and connected each other. This Φ was designated as Φc, namely, Percolation threshold. When Φ was between 15.53% and 52.11%, the value of conductivity increased linearly, but steeply over the concentration of Φb. This suggested that the droplets will be 'sticky droplet collisions,' when the concentration of the W/O droplets was high enough. The direct results of this 'sticky droplet collisions' were forming lots of narrow and small channels in oil continuous phase[26]. When the water content is increasing, the narrow channels will extend rapidly or connect each other making the whole system like network overlapping with each other. W/O and O/W MEs are existing together in nonspherical shape. With the increasing of water content, the solution would become a middle transition in which the oil and water were partly continuity. It means it is transforming from W/O to O/W ME. The characteristic of the electrical conductivity curve of ME was often used to determine bicontinuous structure, namely the microstructure in which the oil and water were partly continuity (Figure 5).

When Φ was in water content between 52.11% and 60.46%, the value of conductivity increased nonlinearly and reached the maximum. Then, on the contrary, the electrical conductivity of the system decreased. The reason for the digression was that the concentration of the bicontinuous ME was decreased by increase in water content. Bicontinuous ME had transferred to the state that continuous phase is aqueous phase in which small oil droplets dispersed in water continuous phase. Meanwhile, the concentration of oil droplets in solution decreased because of the increase in water content. Equivalently speaking, this is dilution and the system is O/W type, hence, the electrical conductivity decreased.

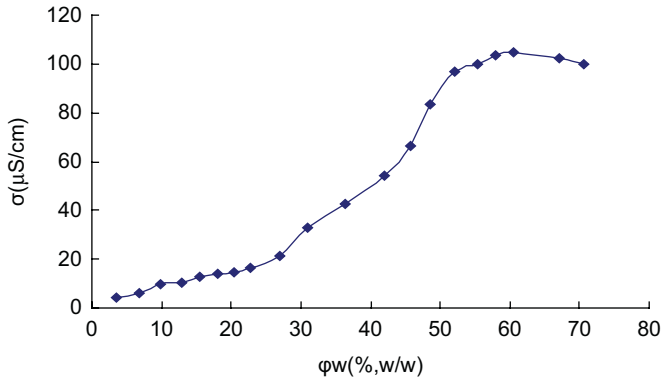

Figure 5. Electrical conductivity of unload microemulsion ($n=3$, mean).

Based on the combination of the results of the electrical conductivity, different ME regions could be divided as follows: a water-in-oil region in water content of Φ<15.53 % (wt), an oil-in-water region in water content of Φ>62.64% (wt), and a bicontinuous region in water content between 15.53% and 62.64% (wt).

However, the conductivity of ME system increased significantly with the addition of drugs. As is shown, the conductivity of drug-loaded ME increased with the incline of water content and reached the maximum value of 161.4 μS/cm, when Φ was 62.64%. Conductivity values for drug-loaded ME were increased by about a factor 1.5 to 2 folds in comparison with MEs without drugs (Figure 6).

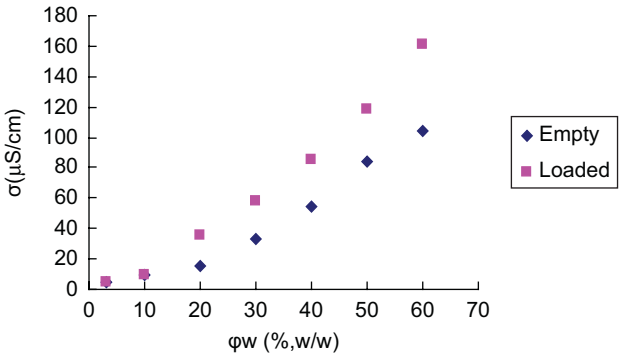

Figure 6. Electrical conductivity (σ) of empty and drug-loaded microemulsions ($n=3$, mean).

Viscosity measurements

The correlation between the measurements of phase behavior and electrical conductivity provided a solid foundation for further investigation on ME structures in rheological terms. In addition to the electrical conductivity, viscosity was also determined. The viscosity of the ME with an S/C ratio of 1 and an O/SC mix ratio of 0.538, titrated with water phase, was measured as a function of the solubilized water content and the obtained profile is presented in Figure 7. The empty samples were compared to samples that were loaded with β-elemene.

The η'–Φw curve shows that η' increases from 62 to 131 mPa·s when Φw increased from 0 to 14%. It is well known that raising the volume fraction of dispersed phase in ME brings increase in viscosity[27], and it could be expected that viscosity changes reflect a transformation of system microstructure in the change of Φw. Primal increase of viscosity with raising of Φw is possibly the consequence of attractive interaction and aggregation of droplets of aqueous phase including molecular recombination on the interface of oil and water[20]. Meanwhile, the hydrophobic hydrocarbon tails of the surfactants mixture of PC and HS-15 dangle out of the interface, resulting in great entanglements[28], and, therefore, enhanced viscosity.

Determination of ME viscosity by the increasing of Φw. Bicontinuous structure of liquid MEs is more likely to be linked with lower value of viscosity[29]. Measured values of η' of this sample decreased from 131 to 10 mPa s with increase in water phase concentration from 14 to 50% (w/w). Upon further dilution, the water becomes the continuous phase, and the viscosities are sharply reduced. The PC that is located at the interface has no effect on the viscosity since its hydrophilic head is dipped in the water and the tails are in the droplet core.

The β-elemene-loaded systems display slightly different viscosity profiles. At low water content (0–14 wt %), the viscosity of the β-elemene-loaded system is considerably greater than the viscosity of the empty system, indicating that the β-elemene molecules are also located (similarly to PC) at the interface and the hydrophobic tails dangle out into the continuous phase, thus bridging between the droplets. In 14–26 wt% water content, the effect is not seen, and the empty and loaded systems have similar viscosities. The viscosity decreased slowly in water content of 14–26%. In this water content, structural transformation from W/O to bicontinuous phase occurs, thus the water phase gradually becomes a continuous phase and the concentration of the β-elemene molecules that are associated with the phospholipid molecules decreases.

Upon further dilution, inversion into O/W takes place and the tail's interdigitation effect disappears. So when water content is about 26%, the viscosity is decreasing significantly with the increasing of water content. At this dilution point, the charged head groups of the PC, HS-15, and β-elemene are facing the continuous aqueous phase and no attraction forces exist. The transformation thus

leads to a sharp decrease in the viscosity. The viscosity of the β-elemene-loaded system in this region is low and is similar to the viscosity of the empty system.

Little change is seen in the viscosity of the system when the water content is above 50%. The data of electrical conductivity and viscosity measurements makes it feasible that quantitatively identify bicontinuous structure from droplet ME structures. Moreover, there have been tested optical texture and stability and measured pH of selected vehicles. Physicochemical characterizations were performed on both empty and drug-loaded MEs. The systems were isotropic, transparent dispersions, and no phase separation would be observed after centrifugation. pH values of ME vehicles were 7.42 and 7.66 (Table 6). The enveloped drug did not affect the optical texture of the ME formulations and did not significantly influence pH values of the vehicles (Table 6). β-Elemene strongly affected electrical conductivity of ME vehicles. And the influence of the drug on the η′ of the ME formulations is presented in Table 6 and Figure 7. The η′ of the drug-loaded ME were higher than that of empty ME vehicles.

In vitro release of β-elemene from ME

Phosphate-buffered saline (PBS) pH 7.4 solution containing 0.5% HS 15 was chosen as the dialysis medium for *in vitro* release of β-elemene from ME. The solubility of β-elemene in release media is 4.6 mg/ml, which can fulfill the sink condition without obviously changing the physical state of the ME. Percentage of release was determined by measuring the remaining drug in the dialysis bag because of the instability of the drug in the release medium. Figure 8 showed the release percentage from ME at different time points. Almost 80% of β-elemene was released in 12 h, and then the release became extremely slow. Drug concentration profile for ME was nonlinear. The nonlinear trend could be attributable to the more complex distribution of β-elemene between water, oil, and amphiphile phases including stronger drug–vehicle interactions. In general, experimental data suggests that nonspherical microstructure slightly hampers the drug release, probably due to drug–vehicle interactions. The obtained release data was fitted into First-order, Higuchi, and Weibull equations, and the results showed that β-elemene release from ME fits the first-order equation best (Table 7). Drug release profile can reflect the distribution of the drug in the vehicle. The release profile of β-elemene from ME was steady during the experimental time from 1 to 12 h, which suggested that β-elemene might be homogeneously dispersed in the oily phase. However, the rate of drug release profile was getting smaller, which indicated that the rate of

Table 6. Physicochemical property of unloaded vehicles and the vehicles loaded with β-elemene ($n=5$).

Parameter	Empty vehicles	Drug-loaded vehicles
pH	7.42 ± 0.02	7.66 ± 0.01
Viscosity/($mm^2 \cdot s^{-1}$)	5.76 ± 0.13	5.56 ± 0.11
Conductivity/($\mu s \cdot cm^{-1}$)	104.7 ± 0.88	161.4 ± 0.79
Average diameter/nm	72.0 ± 8.9	45.8 ± 4.3

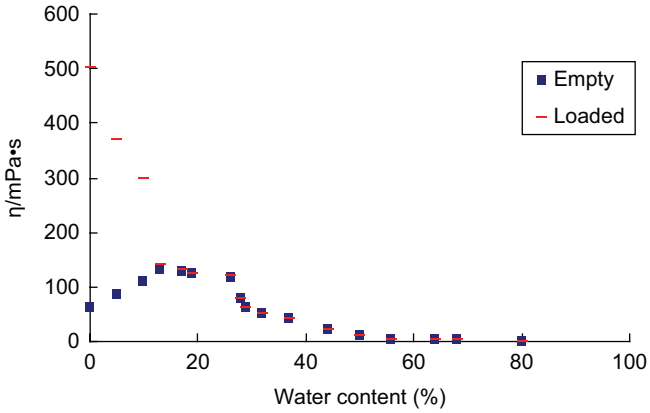

Figure 7. Apparent viscosity (η′) of empty and β-elemene-loaded microemulsions as a function of water volume fraction (Φw) in the system with O/SC 0.538 and Km 1:1. ($n=3$, mean).

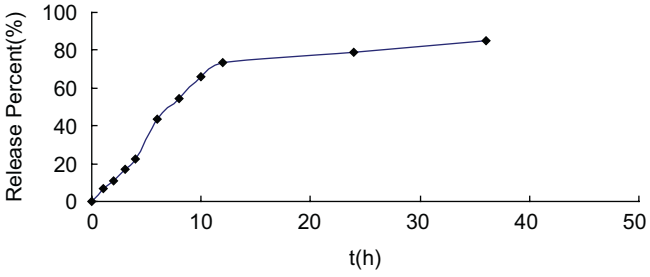

Figure 8. *In vitro* release of β-elemene from ME containing 10 mg/ml β-elemene.

Table 7. Nonlinear fits of β-elemene release from ME.

Equation type	Equation	R^2
First order, In(Q∞-Q)vs.t	$y = 0.1864x + 4.7511$	0.9900
Weibull In In{1/(1-Q/ Q∞)}vs lnt	$y = 1.2382x - 2.6756$	0.9656
Higuchi, Q vs. t	$y = 29.655x - 30.077$	0.9732

Note: Q∞ = maximum cumulate release percentage (%).
Q = cumulate release percentage after t (%). t = release time (h).

drug entering back into the dialysis bag slowed down. Consequently, drug concentration in dialysis medium increased gradually. It showed the slow release of drug-loaded ME in 36 h. This result was mainly attributed to the high compatibility of the drug and the selective oily phase.

Conclusion

Based on the results above, all the antioxidant agents have no significant impact on the stability of β-elemene ME. Besides, β-elemene itself has no influence on the ME. The dilutability of β-elemene ME is suitable for the *in vivo* absorption. Both the conductivity and the viscosity measurements offer insights into the properties of MEs, indicating a valuable way to investigate on ME. At last, the *in vitro* release study demonstrated that the prepared β-elemene ME can release drug steadily.

This study proved the utilization of the relatively simple experimental procedure for screening of the different MEs with the specific microstructures that are of great interest for their drug delivery potential. Phase behavior investigations of the system water/SbPC/HS 15/propylene glycol Labrafac cc demonstrated the suitable approach to reduce the surfactant content by varying oil-to-surfactant/cosurfactant mass ratio. Conductivity and viscosity data confirmed the continuous structural transitions during the increasing of water phase volume fraction in the selected oil/surfactant/cosurfactant mixture. Also, this study demonstrates the slow and steady release of β-elemene from constructed ME system. Furthermore, β-elemene, due to its hydrophobic properties, influenced significantly the microstructure of the investigated ME vehicles[30].

Acknowledgements

We thank Prof. Daweichen for his help and supervision of our research. The help of China Medical University would also be greatly appreciated.

Declaration of interest

The authors report no declaration of interest.

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