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Development and optimization of chemically stable lipid microspheres containing flunarizine

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Abstract

Aim: The purpose of this study is to develop an appropriate dispersion system containing flunarizine, and most of all, to improve the chemical stability of flunarizine. *Method*: In this study, a higher incubation temperature (60°C), to induce a faster chemical degradation, was adopted to optimize a better vehicle, an appropriate pH value, and an effective antioxidant system for flunarizine. *Results*: The chemical stability of flunarizine was improved significantly in lipid microspheres (LMs) compared with the aqueous solution. The optimal formulation of LMs for flunarizine at pH 8.0 is composed of (w/v): flunarizine 0.1%, dl-α-tocopherol 0.1%, medium-chain triglyceride 5%, long-chain triglyceride 5%, soybean lecithin 1.8%, poloxamer 188 0.4 %, Tween-80 0.2%, glycerol 2.5% and L-cysteine 0.05%, Na_2SO_3 0.15%, and EDTA 0.01%. *Conclusions*: The long-term stability investigation, stored at 10 ± 2°C and 25 ± 2°C for 6 months, witnessed the better chemical stability of flunarizine in LMs. An intravenous delivery system of LMs for flunarizine focusing on a better chemical stability of flunarizine has been successfully developed and optimized.

Key words: *Antioxidation; aqueous dispersion system; chemical stability; flunarizine; lipid microspheres*

Introduction

Flunarizine (FZ), (E)-1-[bis(4-fluorophenyl)methyl]-4-(3-phenyl-2-propenyl) piperazine (Figure 1), a difluorinated derivative of cinnarizine, is a selective calcium entry blocker[1]. It has been found to be very effective for cardiovascular and neurological diseases, such as post-cerebrovascular disorders, cerebral infarction, and cerebral hemorrhage[2]. In addition, FZ possesses anticonvulsant properties not only in humans but also in animals, and it is at least as effective as pizotifen in migraine prophylaxis[2,3]. However, the commercially marketable dosage forms are just oral dosage forms involving tablets and capsules. However, these products are not suitable for treating patients who are dangerously ill. Even worse, it has been reported that the water solubility of pure FZ is 0.0165 g/L, which is slightly soluble in water[4]. Thus, when being directly administered intravenously as aqueous solution, the following two factors should be taken into consideration: one is the large amount of organic solvent that must be added because of the poor water solubility of FZ, which may cause severe irritation and higher toxicity in patients; the other is the very labile chemical stability of FZ in aqueous solution[5]. So, it is not an optimal drug delivery system for FZ. In view of the favorable pharmacological action but limitations of the present preparation, it is necessary to develop a proper delivery system for FZ by intravenous administration, to improve its therapeutic effects, to reduce its side effects, and to improve its overall stability.

Lipid microspheres (LMs), as far as current parenteral nutrients are concerned, are now the most attractive drug delivery carrier[6,7]. Over the last 30 years, drug-loaded LMs have been rapidly developed for their unique properties, in terms of the reduction in irritation or toxicity of the incorporated drug and the possibility of sustained release and targeted delivery of the drug to a number of organs[8]; they have also been found to be a suitable drug delivery system for improving the chemical stability of drugs in aqueous media[9,10]. In addition, LMs, as potential carriers or controlled delivery systems, are especially suitable for poorly water-soluble and insoluble drugs[11,12]. FZ,

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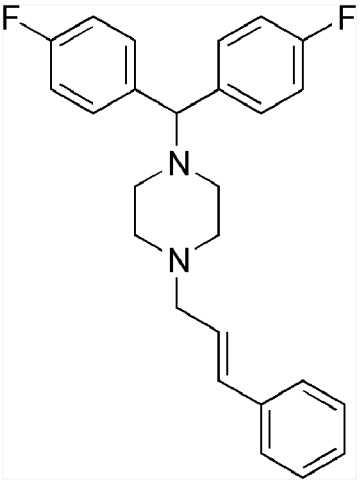

Figure 1. Structure of flunarizine.

because of its poor aqueous solubility[4], has the just promising attempt can be incorporated into LMs.

However, when drugs are incorporated into LMs, a new parameter that must be studied is the chemical stability of the drug. The issue is quite complicated, as the drug may be present in three different microenvironments in the LMs: the oil phase, the aqueous phase, and the oil–water interface[13]. The chemical properties of these environments are so different that it is reasonable to expect that the rate of degradation in each phase will be different. Furthermore, FZ is very unstable in aqueous solution. Considering the molecular structure of FZ, oxidation may be a very potential instability factor on the aspect of the chemical stability of FZ-LMs[5].

The pathways mainly used to inhibit or retard oxidation process are chelation of transition metals, singlet oxygen deactivation, free radical scavenging, and chain-breaking antioxidants[14]. In addition, it should be kept in mind that antioxidants usually act via mixed mechanisms that combine different types of antioxidants. Nevertheless, nonlinear synergistic and antagonistic effects may arise when the antioxidants are mixed[15]. Therefore, the rigorous selection of an effective antioxidant system is necessary to maintain the chemical stability of FZ-LMs.

In this study, to improve the chemical stability of FZ, a higher incubation temperature 60 ± 2°C accelerating the degradation of drug was used in the investigation of chemical stability of FZ following changes in the drug vehicle, pH value, and antioxidant system. Long-term stability investigation of FZ-LMs under practical storage conditions, 10 ± 2°C and 25 ± 2°C, which seemed to be more convincing, also have been established to optimize FZ-LMs owing better chemical stability.

Experimental

Materials

The materials were obtained from the following sources: FZ (Zhengzhou Ruikang Pharmaceutical Limited Co., Zhengzhou, China), soybean lecithin (EPIKURON 170, PC72%, Degussa Food Ingredients, Ludwigshafen, Germany), medium-chain triglyceride (MCT) (Lipoid G, Ludwigshafen, Germany), long-chain triglyceride (LCT) (TieLing BeiYa Pharmaceutical Co., Tieling, China), Poloxamer 188 (Pluronic F68®) (BASF AG, Ludwigshafen, Germany), Tween-80 for parenteral use (Shenyu Medicine and Chemical Industry Ltd. Co., Shanghai, China), dl-α-tocopherol (Zhejiang Medicine Ltd. Co., Zhejiang, China), L-cysteine, Na_2SO_3, and EDTA (Tianjin Chemical Agents Co., Tianjin, China), and glycerol (Zhejiang Suichang glycerol Plant, Zhejiang, China). In addition, all the other chemicals and reagents used were of analytical or chromatographic grade.

Methods

Preparation of FZ-LMs

Soybean lecithin, LCT, MCT, and dl-α-tocopherol were codissolved in anhydrous ethanol, and drug powder was added when the lecithin was dissolved and then anhydrous ethanol was volatilized completely under a stream of nitrogen. The dispersion was stirred at 80°C to prepare the oil phase, whereas Pluronic F68®, Tween-80, oleate sodium, glycerol, EDTA, Na_2SO_3, and L-cysteine were dispersed in injectable water stirring at 80°C to obtain the aqueous phase. The oil phase was added to the aqueous phase gradually and mixed for 10 minutes, using a high-shear mixer at 8000 rpm to prepare a coarse emulsion. After adjusting the pH to 8.0 with 0.1 mol/l HCl or NaOH solution, the primary emulsion was passed through a high-pressure homogenizer (Niro Soavi NS10012k, Niro Soavi S.p.A., Via M. Da Erba, Italy), at 700 bar for eight cycles. The temperature of the entire homogenization process was controlled at 40°C using ice–water bath. Finally, the LM was transferred to vials under nitrogen gas condition and sterilized in a 100°C rotating water bath for 45 minutes.

The final formulation contained (% w/w) FZ 0.1%, LCT 5%, MCT 5%, soybean lecithin 1.8%, Poloxamer 188 0.4%, Tween-80 0.2%, sodium oleate 0.03%, and glycerol 2.5% as the prototype formulations. In addition, dl-α-tocopherol 0.1%, EDTA 0.01%, and Na_2SO_3 0.15% were also involved as the antioxidant system.

Drug analysis

A high-performance liquid chromatography (HPLC) system (HITACHI D-7000) along with a C_{18} (5 μm, 4.6 × 250 mm^2) (Diamonsil) consisted of an autosampler (L-7200), two pumps (L-7100), and a UV detector (L-7420), all interfaced with D-7000 HSM software. For the analysis of FZ and according to ChP(II) 2005, the mobile phase is composed by a mixture of a 0.01 M aqueous solution of KH_2PO_4 (4 mL triethylamine added and then the pH was adjusted to 3.5 with H_3PO_4) and methanol (25:75, v/v); the flow rate was 1 mL/min, and the detection was at a wavelength of 253 nm. The FZ powder, FZ solution, or FZ-LMs was diluted with methanol, which prefiltered through 0.22-μm membrane filter. The filter was subjected to analysis at the injection volume of 20 μL.

Chemical stability studies

Effect of drug vehicle (LM and aqueous solution) on the degradation of FZ

Two series of samples (1 mg/mL FZ-LM and 0.01 mg/mL FZ in 0.01 M phosphate-buffer) were prepared by adjusting pH values with 0.1 mol/L HCl or NaOH from 5.0 to 9.0. Furthermore, the two series contained no antioxidants. All the samples were stored in 10-mL screw-capped glass vials.

Degradation of drug in different environments of FZ-LMs

The rate of degradation of FZ in the oil solution was carried out in the mixture of oil (LCT and MCT at a ratio of 5:5). The solution was saturated with injectable water and adjusted to pH 8.0 with 0.1 mol/L NaOH by mixing for 24 hours in an air bath agitator HZQ-C (Dongming Medicine Device Factory, Haerbin, China) at 37°C. The mixture was centrifuged at 1410 × *g* for 10 minutes to separate the excessive water. Drug powder was then added to the water-saturated oil solution and mixed for 24 hours in a HZQ-C air bath agitator at 37°C then centrifuged again at 1410 × *g* for 10 minutes to separate the excessive drug powder. Finally, the samples were stored in 10-mL screw-capped glass vials.

To study the kinetics of degradation of FZ in the aqueous solution, we dispersed 0.2% Tween-80 in water for injection stirring at 80°C until it was homogeneous. The pH value was then adjusted to 8.0 with 0.1 mol/L NaOH, and the samples were stored in 10-mL screw-capped glass vials.

To study the overall rate of degradation of FZ in the LMs, we prepared FZ-LMs without any antioxidants at a pH of 8.0. The samples were stored in 10-mL screw-capped glass vials.

Percentages of FZ assigned to the three potential environments in FZ-LM suspension were also investigated. Centrifugation was carried out on a HITACHI ultracentrifugation apparatus at 126,000 × *g* for 1.5 hours. The sample temperature was 4°C. Polyallomer tubes were used and their bottoms were pricked after centrifugation with a syringe needle to collect the aqueous phase, then creamed oil could be collected without further washing steps[16,17]. The content of FZ in the different phases was measured by HPLC.

Effect of antioxidants on the chemical stability of FZ-LMs

A series of 1 mg/mL FZ-LM samples were prepared with different antioxidants to evaluate the effect of different antioxidant systems. The candidate antioxidants in this study were dl-α-tocopherol, L-cysteine, EDTA, or the mixture of all the three.

To accelerate the degradation process, we conducted the chemical stability studies of FZ in all the above systems in an oven 101-2A (Taisite Instrument Co. Ltd., Tianjin, China) at 60 ± 2°C. At time intervals of 0, 1, 3, 5, 7, and 10 days, triplicate samples were removed from storage and allowed to cool to room temperature 25 ± 2°C. The chemical stability of the samples was determined by HPLC.

Long-term stability investigation on the chemical stability of FZ-LMs

According to the relative advanced formulations tested in the chemical stability studies, new batches of FZ-LMs were prepared and stored at 10 ± 2°C and 25 ± 2°C, which were much closer to the practical storage condition. At the predetermined time intervals, samples stored at 10 ± 2°C were removed and allowed to warm to room temperature 25 ± 2°C before evaluation. FZ-LMs stored at 10 ± 2°C and 25 ± 2°C were examined in triplicate at the specified time intervals for 6 months and the drug remaining was monitored as the main standard parameters by HPLC.

Results and discussion

Chemical stability studies

Effect of drug vehicle (LM and aqueous solution) on the degradation of FZ

The degradation of FZ in LM and phosphate-buffered solutions at 60 ± 2°C over a range of pH values (5.0–9.0) was monitored by HPLC. The LMs and solution plots of ln *C* (concentration) versus time were linear at all pH values indicating pseudo first-order degradation kinetics. The first-order rate constants for degradation of FZ-LMs at different pH values were plotted in Figure 2, along

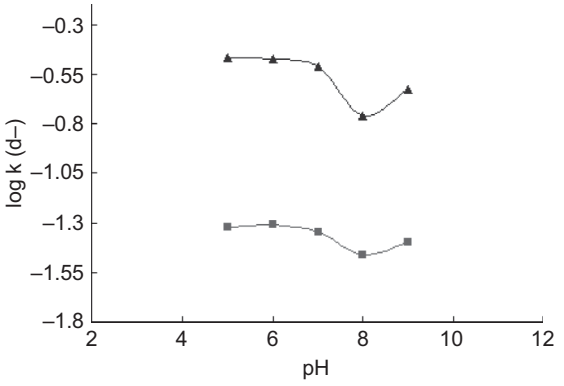

Figure 2. The log k–pH profile for the degradation of FZ-LMs and the log k′–pH profile of aqueous solutions at 60°C (closed squares, LMs rate constants; closed triangles, aqueous rate constants).

with the calculated pH-rate profile in 0.01 mol/L phosphate-buffer.

Evidently, in the FZ-LMs at all clinically used pH values (5.0–9.0) of intravenous administration, on the aspect of stability, FZ-LMs were superior to the FZ aqueous solution, although there was a similar degradation profile between the FZ-LMs and the aqueous solution. This phenomenon may be mainly due to the following two factors: one was the high entrapment efficiency of FZ-LMs at different pH values (over 90%)—illustrating that most of the drug was incorporated in the interfacial layer and oil phase, while very little amount of FZ remained in the aqueous phase—and the other was the different rate of degradation in the three phases of FZ-LMs. As studied in this article, the rate of degradation of FZ in different microenvironments was found to decrease in the following order: aqueous phase, oil–water interface, and the oil phase. Thus, as shown in Figure 2, at pH values ranging from 5.0 to 9.0, the chemical stability of FZ in the FZ-LMs was improved significantly compared with the stability of which in the aqueous solution.

As shown in Figure 2, the log k–pH profile of FZ in FZ-LMs at different pH values was much slowly compared with that in aqueous solution. This tendency suggested that, as a multiphase system with a high entrapment efficiency, FZ-LMs were less sensitive to the pH values of the microenvironments compared with the monophasic system of the aqueous solution.

This study suggested that the pH values significantly affect the chemical stability of FZ both in the LMs and in the aqueous solution. In addition, from a comparison of the FZ-LMs data with the stability data in aqueous solution, the most interesting finding was the overall improvement in the former at all clinically used pH values (5.0–9.0). Thus, LMs was selected as the final drug vehicle and the value of pH was chosen at 8.0.

Degradation of drug in different environments of FZ-LMs

The chemical degradation of the drug is complicated, for the drug may be present in three different microenvironments in the LMs: the oil phase; the water phase; and the oil–water interface[13]. The chemical properties of these environments are so different that it is reasonable to expect that the rate of degradation in each phase will be different and, consequently, this will influence the chemical stability of FZ-LMs. Here, it should be noted that the true degradation kinetics of the drug in the oil–water interface cannot be determined directly, which can be explained as follows: Firstly, the three phases in LMs—oil phase; oil–water interface; and the water phase—were continuous and relatively saturated by the other two adjacent phase. Secondly, the distribution of the three phases in LMs was a dynamic rather than static equilibrium. Thus, redistribution may occur till a new dynamic equilibrium arrived, if any of the phases changed. So, to better simulate the microenvironments in which drug is located in, oil phase was saturated with injectable water when the rate of degradation of FZ in the oil phase was studied.

In this article, it was assumed that the true value can be best estimated by Equation (1), where k_t, k_w, k_o, and k_i are the rate constants for the degradation of FZ in the LMs, the water phase, the oil phase, and the interface, respectively. In addition, f_t, f_w, f_o, and f_i are the concentration fractions of FZ in the corresponding phase:

$$k_t = k_w f_w + k_o f_o + k_i f_i. \quad (1)$$

In this study, the rate of degradation of FZ at pH 8.0 in the oil phase (k_o), in the water phase (k_w), and the overall rate of degradation of FZ in the LMs (k_t) was studied. Then the rate of degradation of FZ in the oil–water interface (k_i) at pH 8.0 can be calculated from Equation (1) indirectly.

In addition, most of the drug exhibits some surface activity because of polar or ionized groups[18,19], providing FZ its surface activity; hence, it tends to be adsorbed at the oil–water interface compared with the oil phase and the aqueous phase. This assumption can be confirmed by measuring the concentrations of FZ in the three phases by HPLC. It should be noted that the interfacial concentration of FZ cannot be calculated directly. In this study, the concentration of FZ at the interface can be calculated from Equation (2) indirectly, where C_i, C_o, C_w, and C_t, are the concentration of FZ at the interface, oil phase, aqueous phase, and total concentration in FZ-LMs, respectively. Furthermore, the

fraction of FZ in each phase of FZ-LMs can be calculated from Equations (2–5) below:

$$C_i = C_t - C_o - C_w \quad (2)$$

$$f_i = \frac{C_i}{C_t} \quad (3)$$

$$f_o = \frac{C_o}{C_t} \quad (4)$$

$$f_w = \frac{C_w}{C_t}. \quad (5)$$

On the basis of the mathematical relationship described above, the fractions of FZ in the FZ-LMs with a pH of 8.0 present in the aqueous phase, oil phase, and oil–water interface were finally calculated and the results are summarized in Table 1. The degradation of FZ in the LMs, aqueous phase, and oil phase followed pseudo first-order kinetics within the experimental time frame at 60 ± 2°C. Thus, the values of k_t, k_w, as well as k_o, could be directly obtained from the corresponding plots and the data are reported in Table 1.

As can be seen from Table 1, the rate of degradation of FZ at pH 8.0 in different microenvironments was found to decrease in the following order: water phase, LMs, oil–water interface, and the oil phase. Obviously, compared with that in the aqueous phase, the chemical stability of FZ in LMs was improved significantly. The explanation for this phenomenon was probably contributed by the high fraction in the oil phase, and at the interface, whereas only a very small fraction was present in the aqueous phase. However, the degradation of FZ in FZ-LMs was in the opposite direction. So the process of degradation was very possibly related to the aqueous phase in FZ-LMs.

The degradation of FZ was fastest in the water phase compared with oil–water interface, especially the oil phase, and the distribution of FZ in the FZ-LMs in the three phases was a dynamic equilibrium. It could be assumed that the FZ at the interface and even FZ in the LM core originally would simultaneously result in continuous migration to the aqueous phase, and then degradation accompanying the fast degradation of FZ in the water phase. In conclusion, the degradation of FZ in FZ-LMs occurred mainly in the aqueous phase compared with the oil phase and the interface. So, the aqueous phase appeared to be an important factor involved in the chemical instability of FZ in LMs.

Table 1. The effect of various microenvironments on the distribution and degradation of FZ in FZ-LMs at 60°C.

	Oil phase	Interface	LMs	Aqueous phase
f(%)	15.1	80.6	100	4.3
k (10^{-2} d^{-1})	1.89	2.98	3.44	17.5

f, fraction in various phases of FZ-LMs;
k, the rate of degradation of FZ in various phases of FZ-LMs.

Effect of different antioxidants on the chemical stability of FZ-LMs

During the long-term stability investigation of FZ-LMs, there was a severe reduction of the active substance of over 8% after just 1 month and 40% after 6 months when stored at 10 ± 2°C protected from light, and also the ratio of the degradation products increased accordingly. Considering aliphatic tertiary amine in the molecular structure of FZ, this represents a basic center with nonbonding electron as donors. Consequently, it can be assumed that the occurrence of degradation products was possibly induced by the oxidation of FZ involving the piperazine ring[5]. Therefore, it is necessary to optimize an effective antioxidant system for prototype formulations to maintain the FZ-LMs chemical stability.

However, there are many different antioxidation pathways for LMs because of the wide range of available oxidation initiators. Nevertheless, when the antioxidants are mixed together, nonlinear synergistic and antagonistic effects may arise[15]. Thus, only a single antioxidant was added to prototype formulations of FZ-LMs, one at a time, to obtain a relative effective antioxidant before using complex mixtures to obtain a more stable FZ-LM.

It can be observed from Figure 3 that there were no significant differences ($P > 0.05$) in any of the five O/W LMs when the drug remaining was analyzed immediately by HPLC after preparation. However, when the FZ concentration in FZ-LMs was measured at the predetermined time intervals over 10 days following incubation at 60 ± 2°C, the antioxidant-free ones exhibited a greater reduction in FZ whereas the samples in the presence of different antioxidants exhibited a better stability. However, the different antioxidant systems also showed a different percentage reduction in the active substance. As shown in Figure 3 and combined with Table 2, their antioxidant efficacy on FZ-LMs was found to decrease in the following order: L-cysteine, Na_2SO_3, dl-α-tocopherol, and EDTA.

It can be concluded that in the FZ-LMs, hydrosoluble rather than lipophilic antioxidants would ensure superior chemical stability, although FZ-LMs containing EDTA was greatly inferior to the antioxidant systems of L-cysteine, and Na_2SO_3. This was mainly because of the fact that hydrosoluble antioxidants were mainly absorbed at the interface membrane or dispersed uniformly in the continuous phase just around the disperse phase,

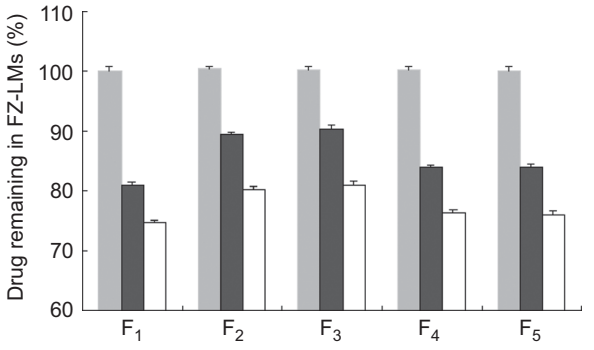

Figure 3. The effect of single antioxidant on the chemical stability of FZ in FZ-LMs (stored at 60°C grey squares 0 day; black squares 5 days; white squares 10 days).

Table 2. The effect of antioxidants on the chemical stability of FZ-LMs with constant amounts of FZ 0.1% (w/v), LCT 5% (w/v), MCT 5% (w/v), soybean lecithin 1.8% (w/v), F68 0.4% (w/v), Tween-80 0.2% (w/v), sodium oleate 0.03% (w/v), glycerol 2.5% (w/v), and the pH value fixed at 8.0.

	Formulations (w/v)							
Variables	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
L-cysteine (%)	—	0.05	—	—	—	—	0.05	0.05
Na_2SO_3 (%)	—	—	0.15	—	—	0.15	—	0.15
dl-α-tocopherol (%)	—	—	—	0.1	—	0.1	0.1	0.1
EDTA (%)	—	—	—	-	0.01	0.01	0.01	0.01

—: not added.

whereas lipophilic antioxidants of dl-α-tocopherol acting as a free radical scavenger were mainly buried in the hydrophobic core of LMs and just very little amount was located at the interface film. Furthermore, as has been explained in the earlier part of this article, the degradation of FZ in FZ-LMs occurred mostly in the water phase compared with the oil phase and the interface and possibly played an important role in the chemical instability of FZ in LMs.

The different antioxidant potencies of the three groups of hydrosoluble antioxidants may possibly result from the different antioxidation pathways. L-Cysteine was thought to act not only as a free radical scavenger by donating a hydrogen from its thiol group[20] but could also interact with lipid-derived hydroperoxides at the interface of the LMs[21]. Hydrosoluble Na_2SO_3, because of its reducible singlet oxygen, was usually added to formulations as an antioxidant[22], which could effectively maintain the chemical stability of FZ in the aqueous phase and the dynamic equilibrium in the different phases of FZ-LMs. Hydrosoluble EDTA, a transition metal chelator, has been reported to dramatically retard lipid oxidation in oil-in-water LMs by removing iron from the droplet surface[23], and it can also prevent interaction with peroxides[24] but only as far as iron is concerned. In this study, EDTA did not exhibit as good chemical stability as L-cysteine and Na_2SO_3 did. This suggested that the metallic ions present in the FZ-LMs may be a minor, but also an important factor as far as oxidation was concerned.

In summary, the different reduction profiles of FZ exhibited by different phases of FZ-LMs demonstrated that antioxidants did affect the stability of FZ-LMs, because a reduction in FZ occurred more slowly in the presence of antioxidant systems. To further improve the chemical stability of FZ-LMs, FZ-LMs combined with complex antioxidants were studied successively.

As found in the earlier part of this chapter, each phase in FZ-LMs contributed to the degradation of FZ in FZ-LMs, although at a different extent. Thus, both hydrosoluble and lipophilic antioxidants were necessary in the antioxidant system for FZ-LMs. Surprisingly, from Table 2 and as shown in Figure 4, F_6, F_7, and F_8 were much more stable compared with F_1 during the incubation. So, there was a nonlinear synergistic effect in the complex antioxidant system because of the properties of the different antioxidants. Particularly for F_8, which involving the advanced antioxidant system for FZ, embodied a superior chemical stability to all the other LMs. Additionally, F_7 was a little bit stabile to F_6, which can be properly explained by various ways to antioxidation between L-cysteine[20,21] and Na_2SO_3[22]. Therefore, through the course of the higher incubation of 60 ± 2°C 10 days, F_8 seemed to be the optimal formulation for improving the chemical stability of FZ-LMs.

Here, emphasis should be placed on the degradation of phospholipids and/or oils that are involved in oxidation and hydrolysis[25]. Oxidation can be inhibited by antioxidants; however, hydrolysis cannot be avoided. Furthermore, the hydrolysis of phospholipids was

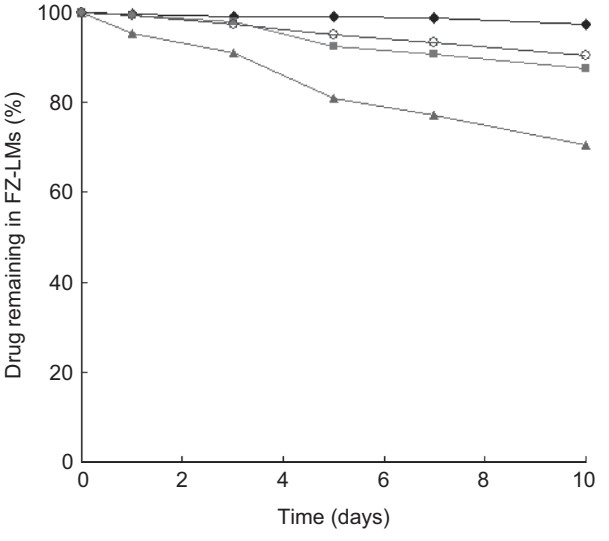

Figure 4. The effect of complex antioxidants on the chemical stabiity of FZ in FZ-LMs (stored at 60°C ▲F_1; ■ F_6; ○ F_7; ◆ F_8).

Table 3. Chemical stability of FZ in the various FZ-LMs during long-term stability investigations.

		10 ± 2°C			25 ± 2°C		
Formulation	After preparation	1 month	3 months	6 months	1 month	3 months	6 months
F_1	100.3 ± 0.20	91.6 ± 0.11	70.1 ± 0.25	56.3 ± 0.08	84.6 ± 0.07	63.2 ± 0.23	41.4 ± 0.14
F_6	100.1 ± 0.16	99.7 ± 0.13	98.4 ± 0.15	95.3 ± 0.10	98.8 ± 0.06	94.1 ± 0.11	86.6 ± 0.16
F_7	100.8 ± 0.07	100.5 ± 0.09	99.1 ± 0.31	97.5 ± 0.17	99.2 ± 0.25	96.3 ± 0.09	92.7 ± 0.11
F_8	100.5 ± 0.12	100.4 ± 0.21	100.1 ± 0.18	100.0 ± 0.06	100.2 ± 0.12	99.1 ± 0.31	98.7 ± 0.08

accompanied by some relative excess of FZ present at the interface film in that there are no longer enough lecithin molecules to incorporate FZ. Then, the relative excess of FZ would be exposed in the aqueous phase, which could rapidly quicken the degradation of FZ in FZ-LMs because FZ is rather unstable in the aqueous phase. So, hydrosoluble antioxidants instead of the lipophilic ones would play the main antioxidant role until a new dynamic equilibrium concentration of FZ was achieved for each phase in FZ-LMs.

Long-term stability investigation on the chemical stability of FZ-LMs

The long-term stability of FZ-LMs, which seemed to be of greatest commercial and scientific value, was monitored at time intervals over 6 months. The final results are summarized in Table 3.

As summarized in Table 3, the antioxidant-free one presented the highest reduction of drug remaining, whereas samples containing complex antioxidant system presented a much better stability profile for the active substance stored at 10 ± 2°C and 25 ± 2°C than the former. Between those complex antioxidant systems stored at 10 ± 2°C and 25 ± 2°C for 6 months, these data highlighted that the efficacy of sustaining the chemical stability was very consistent with the earlier study at an incubation of 60 ± 2°C in 10 days.

Different temperature conditions demonstrated that the degradation of the active substance occurred as a function of temperature and, presented a similar profile in all FZ-LMs, though, these profiles would be expressed at various cycles. This result was expected due to the molecular thermal motion in FZ-LMs. From 10 ± 2°C, 25 ± 2°C to 60 ± 2°C, the augment of the temperature was a determinant factor on intensifying the molecular thermal motion as well as the joint collision. Thus, it was also a determinant factor on the acceleration of drug degradation, confirmed by the study database.

Conclusion

The chemical stability of FZ in the LMs at all clinically used LMs pH values (5.0–9.0) was improved significantly compared with the aqueous dispersion system. The formulation of FZ-LMs was rather complex because of its labile chemical stability and the different chemical properties of the phases in which FZ may be present in FZ-LMs. The fraction of FZ in the aqueous phase was the lowest of all the phases of FZ-LMs but played a key role in the degradation of FZ-LMs because FZ was quite unstable in aqueous solution. These problems can be overcome by monitoring the drug distribution in FZ-LMs and selecting an optimal pH value and an effective antioxidant system for FZ-LMs. The optimal formulation of FZ-LMs at pH 8.0 is composed of (w/v) FZ 0.1%, dl-α-tocopherol 0.1%, MCT 5%, LCT 5%, soyabean lecithin 1.8%, Poloxamer 188 0.4%, Tween-80 0.2%, glycerol 2.5%, sodium oleate 0.03%, L-cysteine 0.05%, Na_2SO_3 0.15%, and EDTA 0.01%. Additionally, there were no significant change of drug remaining in LMs when stored at 10 ± 2°C and 25 ± 2°C for 6 months, which witnessed the excellent chemical stability of FZ in FZ-LMs. In conclusion, an intravenous delivery system of LMs for FZ focusing on a better chemical stability of FZ has been successfully developed and optimized.

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