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Chemical Stability of Teniposide in Aqueous and Parenteral Lipid Emulsions

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The purpose of this study was to investigate the degradation kinetics of teniposide in lipid emulsion and aqueous solution. The chemical stability of teniposide in lipid emulsion and aqueous solution at various pH values and temperatures was monitored by high-performance liquid chromatography. In addition, the viscosities of emulsion at different temperatures were investigated. The degradation of teniposide both in emulsion and in aqueous solution was shown to follow pseudo-first-order degradation kinetics. The $t_{1/2}$ values of teniposide lipid emulsion (TLE) and the aqueous solution were 80 and 2.6 days at 10°C, respectively. Under the most stable pH range of 6.0–6.5, stability of teniposide in the emulsion increased more than 30-fold compared with that in aqueous solution. Furthermore, there was a difference between the shelf life of TLE actually measured (29 days) at 10°C and the one deduced (15 days) from the degradation data of high temperatures by Arrhenius equation. It could be hypothesized that the difference was due to a slower diffusion of teniposide from oil phase to aqueous phase at the lower temperatures, which would be a speed-limited process in the degradation of TLE. The results of viscosity test confirmed the presumption.

Keywords teniposide; emulsion; chemical stability; degradation kinetics; viscosity

INTRODUCTION

Teniposide (VM-26), a semisynthetic glycosidic epipodophyllotoxin derivative, is a cytotoxic drug with antineoplastic activity, which targets topoisomeraseII. Teniposide is included in a wide variety of cancer chemotherapy protocols, such as lymphoblastic and acute lymphocytic leukemia and other experimentally induced leukemias, infantile non-Hodgkin lymphoblastic lymphoma, multiple myeloma, ascitic tumors, malignant brain tumors, colorectal and refractory or recurrent testicular carcinomas, and small-cell and nonsmall-cell lung cancer (Beijnen, Beijnen-Bandhoe, Dubbelman, Van Gijn, & Underberg, 1991; Gordaliza, Castro, Miguel del Corral, & San Feliciano, 2000; Kenneth, 1998; Minotti et al., 1998; Nagai1, Shikiia, Miharaa, Ogataa, & Sasakib, 1998). The chemical structure of teniposide is shown in Figure 1.

Because of its poor water solubility, teniposide is currently administered in the form of a nonaqueous formulation (Vumon®). A Vumon® (teniposide) ampoule has been reported to have several disadvantages, which contains 50 mg teniposide, 300 mg *N*,*N*′-dimethylacetamide, and 2.5 g polyethoxylated castor oil (purified). Meanwhile, maleic acid is added to adjust the pH to 5.0, and absolute ethanol is added to make up the volume to 5 mL. The main problem associated with Vumon® is the precipitation of teniposide during transfusion.

Furthermore, this formulation can cause several severe side effects such as hypersensitivity, hypertension, and cardiovascular collapse. These are most probably caused by the vehicle that contains the surfactant Cremophor, and organic solvents in the formulation, rather than the drug itself (Hayat & Kyounghee, 1992; Holthuis, 1988). Since, no special oral preparation of teniposide is currently commercially available, a more acceptable aqueous intravenous injection formulation is desirable (Hayat & Kyounghee, 1992).

Lipid emulsions (LEs) have been used as drug carriers for 30 years. The first prototype formulation was described as Intralipid™. It was well known as a source of calories and essential fatty acids for patients (Floyd, 1999). The oil-in-water submicron LEs have been rapidly developed as drug carriers because they have many advantages. They are biocompatible, biodegradable, physically stable, and relatively easy to produce on a large scale using proven technology. Because of its subcellular and submicron size, LE is expected to penetrate deep into tissues through fine capillaries and even cross the fenestrations present in the epithelial lining in the liver. This allows efficient delivery of therapeutic agents to target sites in the body (Tamilvanan, 2004). Meanwhile, LEs can also enhance the solubility or stability of the incorporated drugs resulting in low toxicity, sustained release, and efficient targeting (Dan, 2005; Floyd, 1999; Tamilvanan, 2004; Venkateswarlu & Patlolla, 2001). LEs have been studied as parenteral drug

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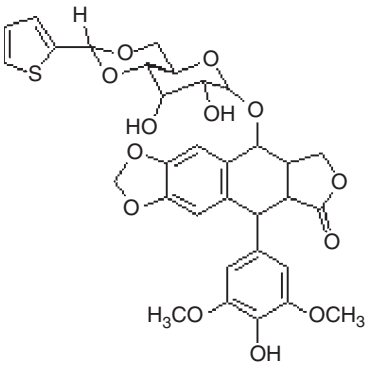

FIGURE 1. Structure of teniposide.

carriers for sustained release and organ targeting. By using the LEs, direct contact of the drug with body fluids and tissues can also be avoided, thereby minimizing any possible side effects (Wang, Sung, Hu, Yeh, & Fang, 2006).

In recent years, as a very useful drug delivery system (DDS), LEs have attracted a great deal of attention for cancer chemotherapy. So far, several antitumor drug-loaded LEs have been reported to be less toxic with enhanced activity and better stability, such as 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) Les (Mitsuko, 1996) and antitumor prostaglandins LEs (Fukushima, Nakano, Takeuchi, & Fukushima, 1997). Therefore, lipid microspheres appear to be ideal drug carriers. Considering the drug loading capacity, storage stability, and biocompatibility, LEs were chosen as a suitable DDS for teniposide.

The main problems of teniposide nonaqueous formulations are precipitation and irritation (Strong & Morris, 1990). In our study, these problems would be resolved by loading teniposide into a LE. High-pressure homogenization was applied in the preparation of a teniposide-loaded parenteral LE. It has been reported that this method can be successfully used for drugs with poor water and oil solubility in the oil-in-water emulsion preparation process (Akkar & Müller, 2003; Müller et al., 2004). In teniposide emulsion, the drug was in an aqueous system, and the main problem was that of drug degradation. Therefore, the degradation of teniposide both in LE and in aqueous solution was studied under forced conditions by changing the pH and the temperature. The entrapment efficiency (EE) and kinetic data were combined to describe the drug degradation behavior in the teniposide-loaded LE.

MATERIALS AND METHOD

Materials

Teniposide was purchased from Jangsu Yabang Technology Ltd. (Changzhou, China). Egg lecithin (EPIKURON170, PC72%) was purchased from Degussa Food Ingredients (Shanghai, China). Soybean oil was obtained from Tieling Beiya Foods Ltd. (Tieling, China), medium-chain triglycerides (MCT) was purchased from Lipoid KG (Ludwigshafen, Germany), Poloxamer 188 (F-68) was obtained from BASF AG (Ludwigshafen, Germany), and Tween-80 from Shenyu Medicine and Chemical Industry Ltd. Co. (Shanghai, China). All other chemicals and reagents used were of analytical or chromatographic grade.

Method

Preparation of Lipid Emulsion Containing VM-26

Teniposide (0.05 g) and egg lecithin (2.0 g) were dispersed in 10 mL absolute alcohol, and the mixture was refluxed at 80°C for 1 h. Then the solvent was evaporated and the mixture was vacuum-desiccated overnight. The oil phase was prepared by dispersing the mixture in soybean oil (10 g) and MCT (10 g), and then the system was heated to 80°C on a thermostatically controlled water bath while stirring. The aqueous phase consisting of 2.5% (wt/vol) glycerol, 0.2% (wt/vol) F-68, 0.2% (wt/vol) Tween-80, and sufficient double-distilled water was also heated in the water bath until it was uniformly dispersed at the same temperature. Then the coarse emulsion was prepared by high-shear mixing (ULTRA TURRAX® T18 basic, IKA® WORKS, Guangzhou, Germany) by adding the water phase to the oil phase rapidly at 10,000 rpm over a period of 5 min. The final emulsion was obtained by high-pressure homogenization using a Niro Soavi NS10012k homogenizer (Via M. da Erba, 29/A-43100 PARMA, Italy), applying a pressure of 700 bar and eight homogenization cycles. The temperature of emulsion during homogenization was controlled at 40°C using an ice bath. Then the volume was adjusted to 100 mL with double-distilled water and the pH was adjusted to 6–7 with 0.1 mol/L NaOH. Finally, the emulsion was transferred to vials under nitrogen gas and sterilized in a boiling water bath for 30 min (Table 1).

Particle Sizing and Zeta Potential Measurement

The particle size distribution of the teniposide emulsion was measured by photon correlation spectroscopy (PCS; dynamic light scattering, DLS) using a Nicomp™ 380 Particle Sizing System (Zeta Potential/Particle Sizer Nicomp™ 380ZLS; PSS.NICOMP PARTICLE SIZING SYSTEMS, Santa Barbara,

TABLE 1
Formulation of Teniposide Lipid Emulsion

Teniposide	Egg Lecithin	Soybean Oil	MCT	Glycerol	Tween-80	F-68	Double-Distilled Water
0.05 g	2.0 g	10 g	10 g	2.5 g	0.2 g	0.2 g	100 mL

CA, USA). The measuring range of the PCS covered was from 5 nm to approximately 3 μm. Prior to the measurements, the emulsion sample was diluted 1:5,000 with double-distilled water, which had been passed through a 0.22-μm micropore membrane. The equipment exhibited the highest accuracy and reproducibility at the dilution ratio chosen. Dilution had no significant effect on the particle size distribution as shown in an earlier validation. The zeta potential is very useful for evaluating the stability of any colloidal system. The Nicomp™ 380 system was also used to determine the zeta potential by the electrophoretic light scattering technique.

Light Microscopy Studies

A Motic DMBA450 microscope was used to investigate the emulsion sample. The emulsion was analyzed without being diluted using an oil immersion with 1,000-fold magnification; typically, 20 microscopic fields were analyzed for observation of the residual drug crystals in the emulsions.

Determination of Entrapment Efficiency

The EE of the system was determined by measuring the concentration of free teniposide in the aqueous phase. Centrifugation was carried out on a HITACHI ultracentrifugation apparatus at 46,000 rpm (approximately 107,000 × *g*) for 4 h. The sample temperature was preset to 10°C. Polyallomer tubes were used and their bottoms were pricked after centrifugation with a syringe needle (Wang, 2007). The amount of teniposide in the separated aqueous phase was measured by high-performance liquid chromatography (HPLC). Then, the EE of the drug was calculated by dividing the drug concentration in the dispersed phase by that in the whole emulsion.

High-Performance Liquid Chromatographic Analysis

The teniposide content of the LE was determined by HPLC. An HiQ sil C18 column (5 μm, 4.6 × 250 mm^2; KYA TECH Corporation, Japan) was employed. The mobile phase was acetonitrile–water (35:65, vol/vol) containing 0.3% sodium acetate (pH 4.0). The flow rate was 1.0 mL/min, the UV detector was set at 280 nm and the injection volume was 10 μL. The emulsion or buffer solution was diluted with methanol to the appropriate concentration and passed through a 0.22-μm micropore membrane filter before injection into the HPLC system.

Stability Study

Physical Stability Study. The physical stability of 0.5 mg/mL teniposide lipid emulsion (TLE) in 0.9% sodium chloride and 5% dextrose injection was investigated. The particle size distribution was determined 1, 2, 4, 8, 12, and 24 h after dilution at room temperature. The stability of TLE following storage at 10 and 20°C was studied by measuring the particle size distribution, zeta potential, and EE at different time points over a period of 1 month. The physical appearance of the emulsion was also examined.

Effect of pH on the Stability of Teniposide. A series of 0.5 mg/mL TLE solutions with different pH values were prepared by adjusting the emulsion with 0.1 mol/L HCl or NaOH to pH 4.0–8.0. Then 0.05 mg/mL teniposide solutions with a pH of 4.0–8.0 were prepared by adding the teniposide stock solution to 0.06 mol/L phosphate buffer solutions. They were stored separately at 80, 60, and 40°C in water baths, and HPLC was used to analyze the samples that were removed at intervals.

Effect of Temperature on the Stability of Teniposide. TLE sample with a concentration of 0.5 mg/mL and teniposide buffer solution with a concentration of 0.05 mg/mL at pH 6.0 were kept at 40, 60, 80, and 100°C in thermostatically controlled water baths in the dark. The degradation of teniposide in TLE at 10 and 20°C was also investigated. Samples were withdrawn at appropriate intervals. Then the concentration of teniposide was analyzed by HPLC according to the method above, and analyses were carried out in triplicate. The physical appearance, particle size, zeta potential, and EE were also investigated. In addition, the oil phase-loaded drug was heated in the 60°C water bath for 3 days.

Effect of Temperature on Viscosity. The viscosity of the emulsion was measured using an NDJ-7 rotary viscosimeter (Shenzhen SanLi Chemical. Ltd. Co., ShenZhen, China) from 4 to 90°C. The viscosity of the aqueous phase and pure water was also measured.

RESULT AND DISCUSSION

Physical Stability

Sodium chloride injection solution (0.9%) and dextrose (5%) were chosen as the dilution solvent, because they are both very commonly used infusion fluids to prevent blood vessel irritation. It has been reported that the precipitation of teniposide occurred during infusion after the commercial teniposide formulation (Vumon®) was diluted with 0.9% sodium chloride for injection (Strong & Morris, 1990). In our study, the precipitation was observed within 1 h when Vumon® was diluted to a teniposide concentration of 0.5 mg/mL. After the TLE was diluted with 0.9% sodium chloride for injection and 5% dextrose, the mean diameter was unaffected. The mean diameter ± *SD* changed slightly from 126.9 ± 39.97 to 127.2 ± 41.84 and 129.2 ± 37.66 to 131.1 ± 42.21. After a 1-month storage period at 4 and 20°C, the mean diameter ± *SD* were 128.2 ± 34.61 and 130.2 ± 40.09 nm, respectively, whereas the zeta potentials were 30.8 and 27.6 mV. Furthermore, the physical appearance of the 0.5 mg/mL TLE was excellent.

Degradation Kinetics

Effect of pH on the Stability of Teniposide

The disappearance of teniposide both in buffered media and in TLE followed pseudo-first-order kinetics over several half-lives at 80°C. This was indicated by the linearity of plots of the

natural logarithm of the residual teniposide concentration against time at 80°C over a range of pH values (4.0–8.0) (Hammad & Müller, 1998). The observed rate constants (k_{obs}) for the overall degradation, which were obtained from the slopes of these plots, were used to determine the half-lives at various pH values at 80°C (Table 2). It was observed that the TLE reached a maximum stability over the range of pH 6.0–6.5. The degradation of TLE at pH > 8.0 was extremely rapid with a half-life of less than 0.2 h. In the buffer solution, the most stable pH value was also in the range of 6.00–6.50. The buffer solutions were used to keep the pH constant; the observed rate constant (k_{aq}) for degradation of teniposide in buffer is as follows:

$$k_{aq} = k_0 + k_H[H^+] + k_{OH}[OH^-] + k_{buffer}[\text{buffer}], \quad (1)$$

where k_0 is the first-order rate constant of degradation only in water, and k_H and k_{OH} are second-order rate constants for proton- and hydroxyl-catalyzed degradation, respectively. The k_{buffer}[buffer] represents the degradation catalyzed by each of the buffer components multiplied by its concentration (Beijnen et al., 1988). However, the aqueous phase in the TLE was buffer free. The pseudo-first-order rate constant for [buffer] = 0 ($k'_{obs} = k_0 + k_H [H^+] + k_{OH}[OH^-]$) was calculated as the intercept from the linear part of the plot of k_{obs} versus [buffer] at a fixed pH. The degradation constants of TLE at various pH values were plotted in Figure 2, along with the calculated pH rate profile of buffer-free material. An interesting result was obtained by comparison of the data between the TLE and the aqueous solution. There was an overall change in stability by loading the drug in LE with the pH 4.0–8.0. Combined with the results of the EE of TLE with different pH values shown in Table 3, it appeared that the improvement in the stability was the result of the high EE. The data were processed in the style of one-way analysis of variance by using SPSS. We get the conclusion that the pH values of the emulsion had no significant effect on the EE ($p > .05$). Approximately, 91% of the teniposide was incorporated into the oil phase while the drug in the dispersing phase was less than 9%. These results explain the improvement in the stability of TLE.

TABLE 2
First-Order Degradation Half-Life ($t_{1/2}$) of 0.5 mg/mL TLE and Buffers with Various pH Values at 80°C

pH	$t_{1/2}$ (h) in TLE	$t_{1/2}$(h) in Buffer Solution
5.0	6.521	3.698
6.0	21.66	8.361
6.5	18.43	4.891
7.0	3.870	0.199
8.0	0.704	0.138

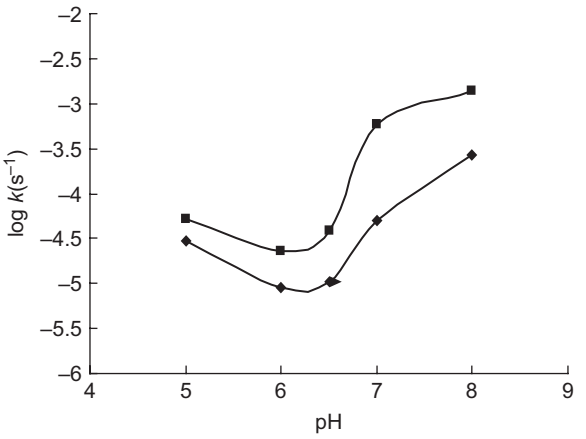

FIGURE 2. The log k_{obs} pH profile (—♦—) for the degradation of TLE and log k'_{obs} pH profile (—■—) of aqueous solutions without buffer at 80°C.

TABLE 3
The Entrapment Efficiency of TEL at Different pH Values (5–7)

pH	5.0	6.0	6.5	7.0
EE (%)	91.09 ± 0.26	91.42 ± 0.36	91.93 ± 0.86	91.67 ± 0.47

Effect of Temperature on the Stability of Teniposide

The influence of temperature on the degradation of teniposide was investigated in 0.025 mol/L buffer solutions and LE in the temperature range of 20–80°C at pH 6.0. The kinetic data were used to calculate the k_{obs}. The Arrhenius equation was used to describe the relationship between the natural logarithm of k_{obs} and the reciprocal of the absolute temperature

$$\ln k_{obs} = \frac{\ln A - E_a}{RT}, \quad (2)$$

where A is the frequency factor and a constant, E_a is the energy of activation, R is the universal gas constant, and T is the absolute temperature. The k_{obs} values were fitted using the equation as shown in Figure 3. The relationship between ln k_{obs} and $1/T$ was determined by linear regression and the linearity coefficient of the regression was 0.9895. The E_a and A of TLE were calculated to be 57 kJ/mol and 3.6×10^3/s, respectively. The shelf life ($T_{0.9}$) is the period a product can be stored without loss of potency. It is described as the time taken for 90% of the active component to remain at a given temperature. To determine the shelf life of teniposide at 20, 10, and 4°C, the following equation was used:

$$T_{0.9} = \frac{\ln(1/0.9)}{k_{obs}}. \quad (3)$$

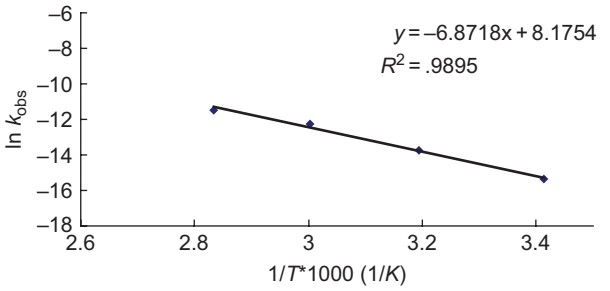

FIGURE 3. Arrhenius plot of teniposide in lipid emulsion.

The ln k_{obs} of teniposide in emulsion at 20, 10, and 4°C was determined to be –15.28, –16.11, and 16.67, respectively. The shelf life of TLE at 20, 10, and 4°C was calculated to be 6, 12, and 22 days, respectively. The shelf life of the aqueous solution was also calculated in the same manner as the TLE. The results were 7, 10, and 13 h at 20, 10, and 4°C, respectively.

Obviously, the chemical stability of TLE compared with aqueous solutions was improved significantly. To explain this phenomenon, the oil phase-loaded drug was heated in the 60°C water bath for 3 days. The k_{obs} (8.3×10^{-8}) of the oil phase was much less than the k_{obs} (3.4×10^{-6}) of the TLE at 60°C. Therefore, it appears that the degradation of drug took place mostly in the water phase. After loading in the LE, the concentration of teniposide in the aqueous phase was reduced, which was demonstrated by the EE result (91.4%) of TLE. However, there is an equilibrium between the oil phase and the aqueous phase. As the amount of drug in the aqueous phase is reduced, the teniposide in the oil phase will diffuse through the interfacial layer to the water phase. So, protection from degradation in the oil phase will never be complete because of the equilibrium. The effect of the aqueous phase on chemical degradation can merely be reduced, not abolished (Krickau, Mueller, & Thomsen, 2007). The observed reaction rate constant of TLE (k_0') was the result of the coordination of both the aqueous phase (k_0) and the oil phase ($k_{0(oil)}$). The degradation of teniposide in the oil phase was much slower than in the aqueous phase, as mentioned above, and so it concluded that $k'_0 \leq k_0$.

The temperature had an important effect on the stability of TLE. The ratio of drug degradation was reduced as temperature decreased. An interesting phenomenon was found—namely, the shelf life of TLE measured at 10°C was much longer than the one calculated by the Arrhenius equation. Also, there was no significant difference between the two shelf lives at 100°C, which are listed in Table 4 which indicates that the Arrhenius equation was much more suitable for higher temperatures than low temperatures. It has been reported that the interfacial layer was the influence factor of drug release in emulsion (Bjerregaard, Söderberg, Vermehrena, & Frokjaer, 1999; Santos Magalhaes, Cave, Seiller, & Benita, 1991). The permeability of the interfacial layer for teniposide might be the reason for this phenomenon. It has been reported that the viscosity of an emulsion is determined by the nature of the emulsifier (Jumaa & Müller, 1998). With the reduction in temperature, the viscosity of the emulsion increased (Jafari, He, & Bhandari, 2007). Thus, it was assumed that the fluidity of the lecithin in the interfacial layer was reduced. Therefore, there was a slowing down of the diffusion rate of the drug from the oil phase to the aqueous phase through the interfacial layer. With the reduction in temperature, the viscosity of the TLE increased. In our study, the change in the viscosity of the emulsion was at a constant rate in the range of 40–90°C. However, the change was much more marked when the temperature was lower than 40°C, especially in the range of 4–20°C. (Figure 4) To investigate whether this phenomenon was correlated with the change in the aqueous phase, the viscosity of the aqueous phase and pure water was also detected. In Figure 5, it could be observed that the viscosity of aqueous phase and pure water only changed slightly over the entire temperature range. However, the viscosity profile of TLE was quite different from that of the former two, especially at the lower temperatures. This indicated that the change was not caused by the aqueous phase and, so, this is a further proof of the correlation between the viscosity and the diffusion rate. This result may explain the difference between the calculated shelf life and that measured at 10°C. Because of the change in the interfacial layer with temperature, the E_a of TLE might increase at lower temperatures. Therefore, the real shelf life of TLE would be longer than the one calculated from the Arrhenius equation at 4°C.

TABLE 4
Comparison Between the Shelf Life Calculated and Measured at 10 and 100°C

	10°C	100°C
Calculated	12 days	50 min
Detected	29 days	49 min

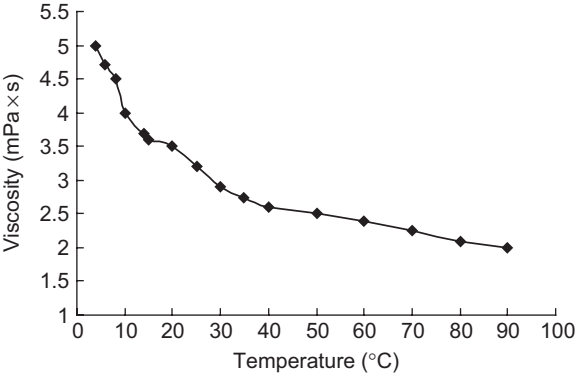

FIGURE 4. Effect of temperature on the viscosity of TLE at different temperatures.

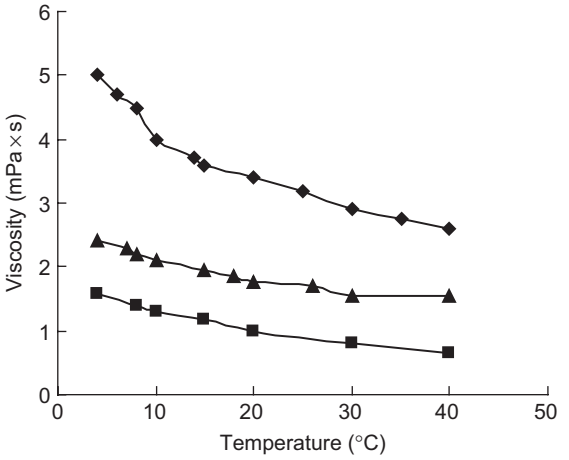

FIGURE 5. Comparison of the viscosity of TLE (—♦—), aqueous phase (—▲—), and pure water (—■—) at different temperatures.

CONCLUSIONS

The problems associated with the poor solubility of teniposide were overcome by loading teniposide into LE. In our study, the degradation pattern of teniposide in LE and aqueous solution followed pseudo-first-order kinetics. It was shown that teniposide both in LE and in aqueous solution was more stable at a pH of 6.0–6.5. Compared with the aqueous solution, the stability of teniposide was enhanced 30-folds in the emulsion. The change of viscosity resulting from the change of temperature (4–10°C) proved that the diffusion rate of teniposide from the oil phase to aqueous phase in TLE leaded to the degradation differences between the calculated value and the actual measurement at 10°C. However, the stability of TLE does not meet the clinical requirements and further studies will focus on enhancing the stability of teniposide emulsions.

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