Drug Development and Industrial Pharmacy, 27(4), 345–350 (2001)

RESEARCH PAPER

Effect of Additives on Stability of Etoposide in PLGA Microspheres

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ABSTRACT

The purpose of this article was to determine the shelf life of etoposide in poly(lactic-co-glycolic acid) (PLGA) microspheres prepared with and without additives (i.e., tricaprin and isopropyl myristic acid ester [IPM]). The microspheres were prepared by a single-emulsion solvent extraction technique with and without 25% w/w additive. The batches of microspheres were subjected to an accelerated stability study at two elevated temperatures (70°C and 80°C or 80°C and 90°C). Samples were taken at 7, 14, 21, 28, and 35 days for estimation of drug content by high-performance liquid chromatography (HPLC). The drug stability in the microspheres was determined by plotting the log percentage drug remaining versus time to obtain the degradation rate constant k *of etoposide at the measured temperature. This degradation rate constant was then used in the Arrhenius equation to obtain the activation energy of etoposide, which was utilized to determine the shelf life of the microspheres at room temperature. The results showed that all three microsphere formulations had good long-term stability at room temperature (6.62–8.86 years at 25°C). The plain microspheres were shown to possess a shelf life of 6.62 years, and the IPM and tricaprin were the most stable with shelf lives of 8.25 and 8.86 years, respectively.*

KEY WORDS: *Etoposide; HPLC; Microspheres; PLGA; Shelf life; Stability.*

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INTRODUCTION

The recent advancements in the area of biodegradable targeted delivery systems have opened a whole new avenue of therapeutic approach for many commonly used drugs. The most frequently encountered systems are those based on biodegradable polymers such as polylactic acid (PLA) or poly(lactic-*co*-glycolic acid) (PLGA). It is necessary for a long-term delivery system also to have long-term stability of the bioactive agent to be delivered. The attractiveness of biodegradable delivery systems lies in their ability to protect sensitive compounds from degradation.

Understanding the causes of instability is critical and requires the knowledge of the physicochemical environment to which the therapeutic agent is exposed (1–6). Recent research into this area has exposed that a highly acidic microenvironment is created as the polymer matrix is hydrolyzed. During the degradation process, the polymer backbone is degraded, and the carboxylic acid residues of the PLGA or PLA chain are exposed (1,7,8). The microacidic environment is the main cause of instability of bioactive agents in microsphere formulations and depends greatly on the extent to which water is able to penetrate the matrix.

Etoposide is used in the treatment of acute myeloid leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, lung cancer (small cell and non-small cell), Kaposi's sarcoma related to acquired immunodeficiency syndrome (AIDS), gastric cancer, breast cancer, and ovarian cancer (9–11). The chemotherapy regimens that utilized etoposide were more effective when the drug was given over an extended period of time (9,12). For the past decade, PLGA has been extensively utilized for controlled drug delivery systems (13,14). The mechanism and the rate of release of incorporated drug from PLGA microspheres were found to be highly dependent on the physicochemical properties of the polymer and drug and on the properties of the microspheres. The incorporation of additives in the microspheres is used to modify the release profile of the active component. Some plasticizers (tributyl citrate and glycerin) and fatty substances have been found to increase the rate of release of drugs from microspheres (15–17).

In the course of our studies, we found that the rate of release of etoposide from PLGA microspheres was slow. Two methods of attack were undertaken to increase the in vitro release of etoposide from the PLGA microspheres. We incorporated an additive, tricaprin, during the synthesis phase to improve the in vitro release profile of the etoposide. The goal of this research was to study the effect of IPM on the size, drug loading, surface characteristics, and in vitro release of etoposide from PLGA microspheres.

THEORY

The effect of temperature on the rate of degradation of a pharmaceutical agent can be exploited in an accelerated stability experiment to determine the shelf life of a product. The relationship of the temperature and the rate of reaction are evident in the Arrhenius equation

$$\log k = \log A - \frac{E_a}{2.303(RT)} \tag{1}$$

where k is a specific reaction rate, A is a constant known as the Arrhenius constant or frequency factor, E_a is the activation energy, R is the gas constant (1.987 cal/deg-mol), and T is absolute temperature in kelvin. The activation energy E_a can be calculated as discussed below.

Writing the Arrhenius equation for temperature T_2 as

$$\log k_2 = \log A - \frac{E_a}{2.303(RT_2)} \tag{2}$$

and for another temperature T_1 as

$$\log k_1 = \log A - \frac{E_a}{2.303(RT_1)} \tag{3}$$

where k_2 and k_1 are specific reaction rate constants at temperatures T_2 and T_1, respectively.

Subtracting Eq. 3 from Eq. 2 yields

$$\log \frac{k_2}{k_1} = \frac{E_a}{2.303R}\left(\frac{T_2 - T_1}{T_2T_1}\right) \tag{4}$$

The degradation rates at two higher temperatures can be determined through experimental methods. These values can then be inserted into Eq. 4 to determine E_a. Once E_a is determined, the degradation rate constant at 25°C can be obtained from Eq. 4, where T_1 would be 25°C (298 K) and k_1 the degradation rate constant at 25°C (298 K). The degradation rate constant at room temperature then can be used to find the time required for the sample to degrade to 90% efficacy through the use of the following equation:

$$t_{90\%} = \frac{2.303}{k_{298\ \mathrm{K}}}\left(\log \frac{C_o}{C}\right) \tag{5}$$

where $t_{90\%}$ is the time necessary to degrade 10% of the original amount of active ingredient, $k_{298\ \mathrm{K}}$ is the rate con-

stant at 298 K, C_o is the initial etoposide concentration, and C is the concentration equal to 90% of the original sample.

EXPERIMENTAL

Materials

PLGA 50:50 (intrinsic viscosity [IV] = 0.61) was obtained from Birmingham Polymers, Incorporated (Birmingham, AL), and the etoposide came from ICN Pharmaceuticals (Aurora, OH). The polyvinyl alcohol (PVA), methylene chloride, tricaprin, IPM, and phosphate buffered saline (pH 7.4) were obtained from Sigma Chemical Company (St. Louis, MO). The isopropyl alcohol (IPA), Tween 80, methanol, acetonitrile, and acetic acid were obtained from Fischer Chemical Company (Fair Lawn, NJ).

Synthesis of Etoposide-Loaded Poly(lactic-*co*-glycolic acid) Microspheres

A measured amount of etoposide and PLGA 50:50 (Table 1) was dissolved in 5 ml of methylene chloride. The solution containing drug and polymer was then dispersed in 10 ml of continuous phase (1% PVA) while being stirred at 1300 rpm (L4RT Homogenizer, Silverson Machine LTD, Chesham, U.K.) and maintained at 60°C for 10 min to evaporate the solvent. After 10 min, the heat was removed, and stirring continued at a reduced rate of 500 rpm for 50 min to evaporate the solvent further. Residual methylene chloride was removed by a wash of 10% IPA, and the microspheres were collected by suction filtration (Aspirator Pump, Cole-Parmer, Chicago, IL) and washed two more times with 10% IPA. The microspheres containing tricaprin or IPM were prepared in the same way, except that the selected additive was added to the drug/polymer solution at 25% w/w.

Accelerated Stability Studies

To each standard screw-top test tube, 2 mg of microspheres were added. The above samples were placed in the heating oven (DK-63 Constant Heating Oven, Scientific Products, West Chester, PA) at two temperatures (70°C and 80°C or 80°C and 90°C) and sampled at 7, 14, 21, 28, and 35 days for estimation of drug content by high-performance liquid chromatography (HPLC). The microspheres were dissolved in 1 ml of methylene chloride to which 5 ml of methanol was added to precipitate the polymer. The resultant solution was then centrifuged (RC-5 Superspeed Centrifuge, Sorvall, Newtown, CT) at 7000 rpm (SS-34 Rotor, Sorvall) for 10 min. The supernatant was then collected and analyzed by HPLC.

High-Performance Liquid Chromatography Method for Determination of Etoposide Content

The method of Chow and Shah (18) with slight modifications was used to analyze the content of etoposide in the sample. The samples were analyzed using a Shimadzu HPLC system (Shimadzu Scientific Instruments, Inc., Kyoto, Japan) consisting of an SPD-10AV ultraviolet detector, SCL-10A system controller, SIL-10A autoinjector, dual LC-10AD pumps, and a CR-501 integrator. A mobile phase of 70% H_2O:acetic acid (100:1) and 30% acetonitrile passed through a 5-μm phenyl column (Phase Sep, Deeside, UK) at a flow rate of 1.4 ml/min. The effluent of the column was then analyzed for etoposide at a detection wavelength of 239 nm. The amount of etoposide in the sample was determined from the peak area correlated with a standard curve. The standard curve was determined from a best-fit line of peak area versus amount ($r^2 = 0.9999$) of standard solutions (40, 20, 10, 5, 2.5, and 1.25 μg/ml) with a 10-μl injection. The detection limit of this method was 10 ng on the column.

Table 1

Batch Specification and Shelf Life of Etoposide in Poly(lactic-co-glycolic acid) (PLGA) Microspheres

Batch	Drug (mg)	Polymer (mg)	Additive	Degradation Rate (25°C)	Shelf Life (Years)
A	20	400	0	4.36×10^{-5}	6.62
B	20	400	100 mg IPM	3.50×10^{-5}	8.25
C	20	400	100 mg tricaprin	3.26×10^{-5}	8.86

IPM, isopropyl myristic acid ester.

Data Analysis

The stability of the drug in the microspheres was determined by plotting the log percentage drug remaining versus time. The data were plotted, and a best-fit line was determined to obtain the degradation rate k of etoposide at the measured temperature. The Arrhenius equation was used at two different temperatures for the determination of the activation energy of the degradation process. The calculated activation energy was then utilized to determine the degradation rate at 25°C and the shelf life of etoposide in the microsphere formulations at room temperature.

RESULTS AND DISCUSSION

The results of the accelerated stability study are shown in Table 1 and Figs. 1–3. The degradation rates at two different temperatures were determined from the slope of the plot of the log percentage remaining versus time. The degradation of etoposide in PLGA microspheres follows apparent first-order kinetics. The degradation rate constant for the plain microspheres was 4.36×10^{-5} at 25°C. The expected shelf life of the plain microspheres was calculated to be 6.62 years. The degradation rate constants for the microspheres containing IPM and tricaprin were 3.5×10^{-5} and 3.26×10^{-5}, respectively. The calculated values of the shelf lives for the IPM and tricaprin microspheres were 8.25 and 8.86 years, respectively.

On the basis of results of the accelerated stability studies, it can be concluded that etoposide-loaded microspheres are a relatively stable dosage form. This stability can probably be accounted for by two facts: the stability of etoposide in acidic conditions and the exclusion of water from the hydrophobic polymer matrix. It is now known that the internal microenvironment of the microsphere tends to be acidic due to the presence of glycolic and lactic acid residues. The acidic environment present in the microspheres helps to stabilize the etoposide as it is most stable in a pH range 3.05–7.3 (18). Etoposide is also sensitive to degradation in the presence of water, as in aqueous conditions it is stable for only 24–96 h (19). The hydrophobic nature of the polymer matrix tends to exclude water, which increases the stability of the etoposide. The differences in the stability of etoposide in the plain etoposide-loaded microspheres and the microspheres containing IPM and tricaprin are an interesting result. The increase in stability could be due to the lipophilic nature of the drug and the additive or to the combination of the hydrophobic polymer matrix and

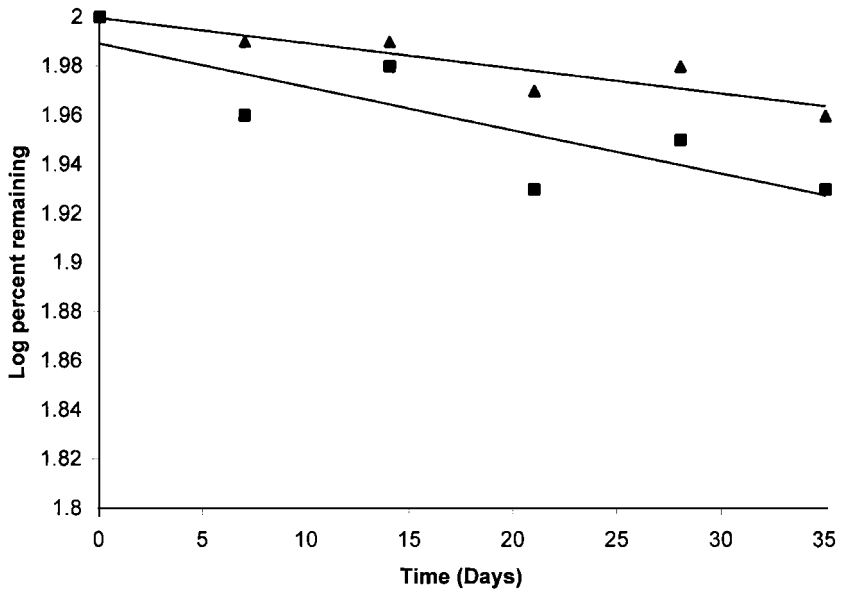

Figure 1. First-order plot of etoposide degradation from microspheres at elevated temperatures: ▲ 70°C; ■ 80°C.

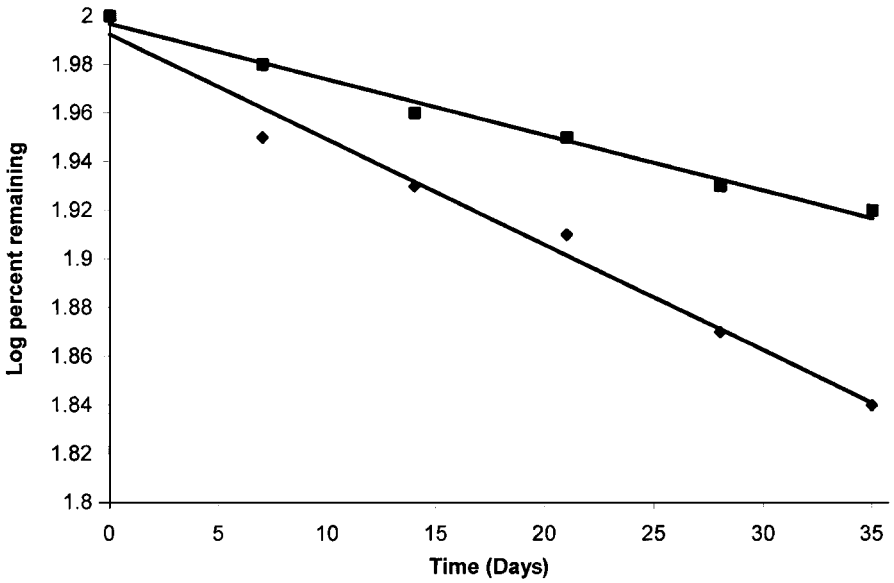

Figure 2. First-order plot of etoposide degradation from 25% IPM containing microspheres: ■ 80°C; ◆ 90°C.

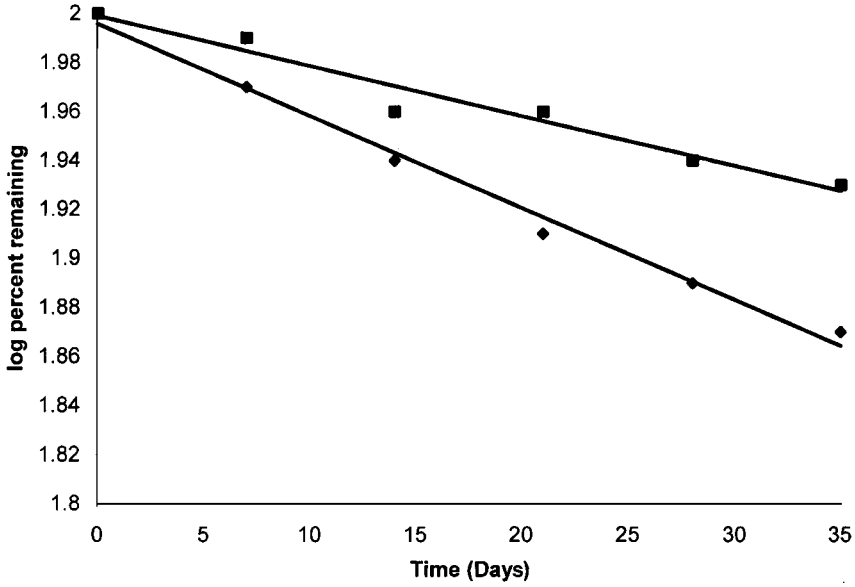

Figure 3. First-order plot of etoposide degradation from 25% tricaprin containing microspheres: ■ 80°C; ◆ 90°C.

the lipophilic additive further excluding water from the internal microenvironment. Thus, lipophilic additives such as IPM and tricaprin can be used to increase the shelf lives of etoposide in PLGA microspheres.

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