MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016



DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY®
Vol. 29, No. 7, pp. 745–756, 2003

RESEARCH PAPER

# Evaluation of PLGA Microspheres as Delivery System for Antitumor Agent-Camptothecin

**Wenkai Tong,[1] Lejun Wang,[2] and Martin J. D'Souza[1,*]**

[1]Department of Pharmaceutical Sciences, Southern School of Pharmacy, Mercer University, Atlanta, Georgia, USA
[2]School of Materials Science and Engineering, Georgia Institute of Technology, Atlanta, Georgia, USA

## ABSTRACT

Camptothecin (CPT) and its analogues are a new class of anticancer agents that have been identified over the past several years. Camptothecin exists in two forms depending on the pH: An active lactone form at pH below 5 and an inactive carboxylate form at basic or physiological neutral pH. Poly(lactide-co-glycolide) (PLGA) microspheres have been considered good delivery vehicles for CPT because of acidic microenvironment formed through PLGA degradation. The objective of this study is to investigate antitumor activity of CPT after it is encapsulated in PLGA microspheres. In this study, PLGA microspheres containing various CPT loadings were prepared and characterized. Cytotoxicity of these microspheres to B16 melanoma cells was then evaluated, and uptake of microspheres by B16 cells was also studied. Analysis of drug stability revealed that CPT is released from the microspheres in its active lactone form over the entire release duration. It was also found that there was no interaction between CPT and PLGA matrix within microspheres through Differential Scanning Calorimetry (DSC) and Fourien Transform Infrared Spectroscopy (FT-IR) and hign performance liquid chromatography (HPLC) studies. Cytotoxicity assay showed that CPT encapsulated in PLGA microspheres still retained its antitumor potency. Uptake study revealed quick uptake of the microspheres by B16 cells, which was desirable. It was concluded that PLGA microspheres were suitable delivery vehicles to stabilize and deliver CPT for the treatment of cancer.

*Key Words:* Microspheres; Camptothecin; Poly(lactide-co-glycolide); Melanoma; Drug delivery.

*Correspondence: Dr. Martin J. D'Souza, Department of Pharmaceutical Sciences, Southern School of Pharmacy, Mercer University, 3001 Mercer University Drive, Atlanta, GA 30341, USA; E-mail: dsouza_mj@mercer.edu.



DOI: 10.1081/DDC-120021774
0363-9045 (Print); 1520-5762 (Online)

www.dekker.com

## INTRODUCTION

Camptothecin (CPT) is a naturally occurring quinoline alkaloid extracted from the bark and wood of *Camptotheca acuminata*, known as the "happy tree," which is native to China. An effusion, injectable, and powder prepared from the tree have been used in traditional Chinese medicine in the treatment of various illnesses including tumors.[1] In the West, since its isolation in the mid 1960s, CPT and its related analogues have been studied as potential anticancer drugs. The Δ-hydroxylactone in ring E of CPT exists in a pH-dependent equilibrium with an open carboxylate form as shown in Fig. 1. At physiological pH, more than 80% of the drug exists as the carboxylate at equilibrium, whereas at pH below 5, essentially all drug is in lactone form.

In the early 1970s, CPT was introduced into clinical trials as the water-soluble sodium carboxylate salt. But due to the high toxicity and low activity of this carboxylate form of the drug, clinical trials were discontinued in 1972. It has since been shown that DNA topoisomerase I (Topo I) is the molecular target of CPT, and that an intact lactone ring (E) is necessary for biological activity, including passive diffusion of the drug into cancer cells and successful interaction with the DNA topoisomerase I target. Following these discoveries, interest in CPT and its analogues has been rekindled in the late 1980s.[2]

To date, most of the studies on CPT are focused on biology and biochemistry of the DNA topoisomerases as well as considerable progress in syntheses of CPT derivatives. Recently, researchers began to realize that the delivery of the active lactone form of CPT is quite challenging, since the lactone exists in a pH-dependent equilibrium with an open carboxylate form (Fig. 1). Thus, the development of a controlled-release formulation of CPT that could continuously deliver the active lactone form of the drug appears to be an attractive goal to pursue.

Poly(lactide-co-glycolide) (PLGA) is the copolymer of polylactide and polyglycolide. It is typically made by ring-opening polymerization of their cyclic diester dimers. Figure 2 shows the structure and synthesis of this copolymer. It is commonly known in surgery as a resorbable suture material.[3]

Because of its good biodegradability and biocompatibility, PLGA has been used as a delivery vehicle for many types of drugs. It is degraded into acidic oligomers and monomers, which elicits a low microenvironmental pH within large specimens of PLGA.[4]

PLGA microspheres have been studied as delivery vehicles for CPT and its analogues.[5] Shenderova et al. investigated the potential of using PLGA microspheres to stabilize and deliver 10-hydroxycamptothecin[6] and identified the acidic microenvironment as the main source of stabilization of the active lactone form of this drug.[7] However, the ultimate goal of a drug-delivery system is to retain or enhance the bioactivity of that drug, thus the

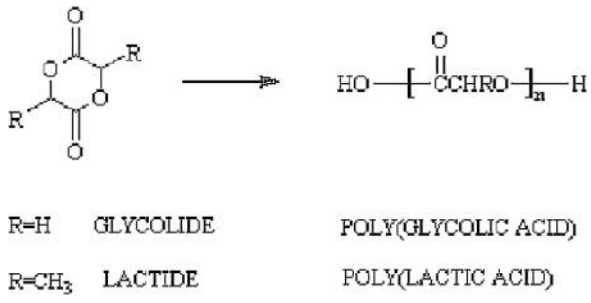


***Figure 2.*** Polymerization of polyglycolide, polylactide, and their copolymers.

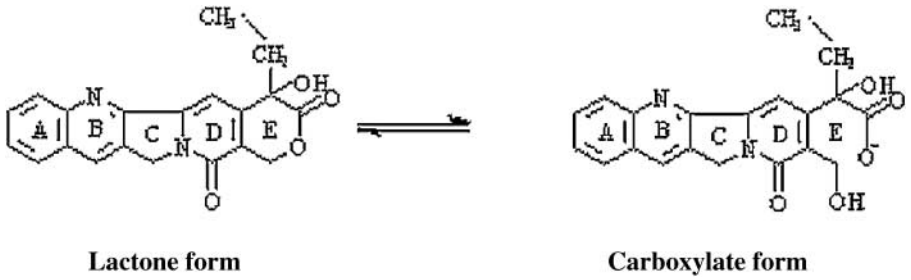


***Figure 1.*** Structure of lactone form and carboxylate form of camptothecin (CPT). The closed lactone E ring (lactone form) can undergo a rapid, pH-dependent, nonenzymatic hydrolysis to form this open-ring hydroxyl carboxylic acid (carboxylate form). At basic or physiological neutral pH, the equilibrium favors the formation of carboxylate form. While at acidic condition, pH below than 5, essentially all molecules are in lactone form.

antitumor activity of 10-hydroxycamptothecin after it was encapsulated in the PLGA microspheres was very important. But the authors did not study it. Furthermore, PLGA microspheres can stabilize the lactone form of CPT only when it is encapsulated. Once CPT is released from the microspheres, conversion from the lactone form to the carboxylate form takes place. A possible way to address this issue is to enhance the uptake of the microspheres by the cells so that most of CPT molecules can be released inside the cells, which is close to the molecular target of CPT.

The objectives of this study were to investigate the antitumor activity of CPT after CPT was encapsulated in PLGA microspheres, and uptake of the microspheres by tumor cells. In this study, PLGA microspheres containing various CPT loadings were prepared and characterized. Cytotoxicity of these microspheres to B16 melanoma cells was then evaluated, and uptake of microspheres by B16 cells was also studied. The mean particle size of the microspheres in this study is 1.3 μm, which is different from~30 μm used by Shenderova et al. The main reason for choosing this particle size is to enhance the antitumor activity of CPT through improving the microsphere uptake by cells.[8]

## MATERIALS

### Chemicals

20S-Camptothecin (95% purity), Poly(D,L-lactide-co-glycolide) (PLGA) was purchased from Sigma (St. Louis, MO). The type of PLGA used was Lactel BP-0100 (lactide/glycolide ratio 50:50, MW 40,000–75,000). Fluoresbrite yellow-green carboxylate microspheres with 1.75 μm particle size were obtained from Polysciences, Inc. (Warrington, PA). MTS (Owen's reagent) was purchased from Promega (Madison, WI). All other chemicals were of analytical grade or purer and purchased from commercial suppliers.

### Cell Culture

Murine B16 melanoma cells were maintained in RPMI 1640 medium (Sigma) supplemented with 2 mM of glutamine, 2.2 g/L of sodium bicarbonate, 10,000 units/L of penicillin, and 10% fetal calf serum (GIBCO, Carlsbad, CA, USA) at 37°C in a humidified $CO_2$ atmosphere.

## METHODS

### High-Performance Liquid Chromatography Analysis of Camptothecin

The amounts of lactone form and carboxylate form of CPT were determined using the high-performance liquid chromatography (HPLC) method.[9,10] The HPLC system included: a Hewlett Packard series 1100 system, equipped with a pump, a manual injector fitted with a 20 μL loop, and a UV/VIS detector with the wavelength set at 378 nm. Separations were carried out at ambient temperature using a Supelcosil LC-18 5 μm particle size reversed-phase 150 υ 4.6 mm I.D. column. The mobile phase consisted of 23% acetonitrile and 77% triethylamine acetate (TEAA) buffer (1% (v/v)triethylamine in water, adjusted to pH 5.5 with glacial acetic acid). A flow rate of 1.0 mL/min was employed. UV/VIS output signal was monitored and integrated using Hewlett Packard Chromatography Manager software. Linear calibration curves were performed from the peak areas of lactone and carboxylate form of CPT, respectively, by spiking drug-free buffer (PBS pH 3.0, and PBS pH 10.0) with standard solutions of CPT (final concentration ranging from 0 to 20 μm). All samples were analyzed in triplicate.

### Preparation of Microspheres

Poly(lactide-co-glycolide) microspheres containing CPT were prepared by a standard oil-in-water emulsion-solvent evaporation method.[11,12] The PLGA polymer (200 mg) was dissolved in 4 mL methylene chloride. Different amounts of CPT (5, 10, or 20 mg) were suspended in the polymer solution. The microspheres were named by the ratio of CPT to PLGA. Thus, these were called 1:40 MS, 1:20 MS, and 1:10 MS, accordingly. The suspension was dropwise added into 8 mL of aqueous polyvinyl alcohol (PVA) solution (2% w/v) kept in an ice bath. The two immisible solutions were first homogenized at high speed for 5 min using a biohomogenizer and sonicated for 60 s using a sonic dismembrator to produce the o/w emulsion. Then the emulsion was poured into 150 mL aqueous PVA solution (0.1% w/v) and kept stirring for 3 h at room temperature. After evaporation of the organic solvent, the hardened microspheres were washed three times with distilled water by centrifugation at 6000 rpm for 10 min. After the final spin, the microspheres were resuspended in

water and desiccated in a vacuum oven overnight. The final products were stored at −20°C until further use. Blank PLGA microspheres (called Blank MS) were prepared in a similar way and used as the control in the characterization studies.

## Characterization of the Microspheres

### Drug Loading

A known weight (~2 mg) of PLGA microspheres containing CPT was immersed in 1 mL dimethyl sulfoxide (DMSO) and vigorously vortexed. The undissolved PLGA polymer was spun down by brief centrifugation. An aliquot of the supernatant containing CPT was removed and properly diluted with PBS (pH = 3.0), then analyzed using the HPLC assay as described above. Drug loading was calculated from the ratio of the mass of drug in the microspheres to the mass of the microspheres, and encapsulation efficiency was calculated from the experimented loading divided by the theoretical loading.

### Particle Size

Over 100 particles for each preparation were sized by sight under a scanning electron microscope equipped with a sizing scale bar.

### In Vitro Release

About 6 mg of microspheres for each preparation were placed in 30 mL of phosphate-buffered saline (PBS, pH = 7.4) or colorless RPMI 1640 cell culture medium in a 50 mL beaker with a stirrer rotated at 100 rpm. The microspheres in the medium were incubated at 37°C. At appropriate time intervals, samples were withdrawn ($n = 6$) and replaced by an equal volume of fresh medium. The CPT released was analyzed using HPLC.

### Surface Morphology

The appearance of the microspheres before and after release study was evaluated by scanning electron microscopy (SEM). Microspheres were mounted onto a metal sample stub with a double-sided conducting tape and coated with a thin layer of gold using a gold sputter.

### Differential Scanning Calorimetry Study

A modulated differential scanning calorimetry instrument (DSC) (by TA Instruments, Model 2920, New Castle, DE, USA) was used to measure glass transition temperature ($T_g$) of PLGA and melting point ($T_m$) of CPT, and to obtain a thermograph of CPT-PLGA microspheres. About 10 mg of each sample was sealed in a hermetic aluminum pan and placed into the DSC cell. The sample was then heated at 5°C/min from 25°C to 300°C under modulated mode to obtain the thermograph.

### Fourier Transform Infrared Spectroscopy (FT-IR) Study

FT-IR spectra of CPT, PLGA, and CPT-PLGA microspheres (1:10 MS) were obtained using a Nicolet Model Magna 560 spectrometer (Thermo Nicolet, Madison, WI, USA). Each sample was measured in pellet form diluted with KBr powder.

### Stability of Lactone Form of CPT in PLGA Microspheres

The extent of each form of CPT that was actually delivered from the microspheres during release was determined according to the method described by Shenderova et al.[6] Briefly, 2 mg of 1:20 MS was incubated in 10 mL PBS buffer (pH = 7.4) at 37°C for different periods of time (0, 4, 8 hours, 1, 2, 5, 7 days). Released CPT was removed by cleaning with distilled water three times. Then the washed microspheres were exposed to drug-free PBS (pH = 6.5) for 10 min at 37°C. Aliquots containing freshly released CPT were withdrawn and analyzed by HPLC immediately. The pH = 6.5 was chosen because there was no significant conversion taking place between the lactone and the carboxylate form within 10 min of CPT exposure to pH = 6.5 release media as proven by Shenderova et al.[6]

For comparison with the stability results of CPT in PLGA microspheres, hydrolysis kinetics of the free drug in PBS pH = 7.4 was also evaluated. Reactions were initiated by adding 5 μL of CPT stock solution (2 mM CPT in DMSO) to 500 μ PBS pH = 7.4 to produce initial CPT concentration of around 20 μM. The solutions were incubated at 37°C.

At different time intervals, aliquots of the reaction solutions were withdrawn, injected directly onto the HPLC column, and analyzed for the amount of lactone form. The stability of lactone form of CPT was represented by the percentage of the lactone form, which was calculated from the peak area of the lactone of the each time interval compared with that of the initial time.

### Evaluation of Cytotoxicity of Microspheres

B16 melanoma cells were plated in 96 well microplates as $2\upsilon10^3$ cells in 180 μ RPMI 1640 medium per well and allowed to adhere in an incubator at 37°C and 5% $CO_2$. Twenty-four hours later, 20 μ of different CPT-PLGA microspheres suspension (blank MS, 1:40 MS, 1:20 MS, and 1:10 MS) was added to the wells to obtain 1 μg/mL of the final concentration of the microspheres. Then the plates were incubated for another 96 hours. The percentage of cell survival was measured with the MTS assay.[13]

### Evaluation of Uptake of Microspheres by B16 Melanoma Cells

A fluorescence microscopy study was developed to evaluate the uptake kinetics of fluorescent microspheres (1.75 μm) by B16 melanoma cells.[14] In this study, uptake was studied with B16 melanoma cells according to 5:1 microsphere/cell ratio and as a function of incubation time (0, 15, 30, 45, 60, 120, 240 min, 24 hours). The cell concentration was adjusted at $1.5\,\upsilon\,10^6$ cells/ml of RPMI 1640 medium containing 10% fetal calf serum. Thus, 2 mL of this cell suspension was seeded into each Petri dish ($35\upsilon10$ mm, Fisher) and incubated for 24 hours at 37°C and 5% $CO_2$. After incubation, the medium was discarded and each dish was washed three times with fresh medium to remove nonadherent cells. Fluorescent microspheres were suspended in RPMI 1640 medium. Two mL of the microspheres suspension at the desired concentration was added into each dish. Then, the plates were incubated at 37°C and 5% $CO_2$. At each incubation time, dishes were washed with fresh medium to eliminate nonengulfed microspheres and observed using a fluorescent optical microscope. Uptake intensity (%) was expressed as the percentage of B16 melanoma cells that have taken up at least one microsphere.

### Statistical Analysis of Data

The obtained data was statistically analyzed using one-way analysis of variance (ANOVA). The confidence level was fixed at 95%.

## RESULTS AND DISCUSSIONS

### Characterization of the Microspheres

#### CPT-Loading and Particle Size of PLGA Microspheres

Drug loading results of the prepared CPT-PLGA microspheres were tabulated in Table 1. It was found that CPT-loading was 2.4%, 4.7%, and 8.6% for 1:40 MS, 1:20 MS, and 1:10 MS, respectively, and the encapsulation efficiency of each of the three types of microspheres was close to 100%. This high encapsulation efficiency was caused by the extremely low water solubility of CPT, which minimized its loss to aqueous solution during the microsphere preparation.

As shown in Table 1, the mean diameter of the PLGA microspheres was 1.3 μm, and there was no significant difference in the particle size for these four types of microspheres. This particle size was

***Table 1.*** Characterization of CPT-PLGA microspheres.

| Microsphere type | CPT amount (mg) | PLGA amount (mg) | CPT loading (%) | Encapsulation efficiency (%) | Size of microsphere (μm) |
|---|---|---|---|---|---|
| Blank MS | 0 | 200 | | | $1.3\,\rho\,0.3$ |
| 1:40 MS | 5 | 200 | 2.4 | 99.9 | $1.3\,\rho\,0.3$ |
| 1:20 MS | 10 | 200 | 4.7 | 99.3 | $1.3\,\rho\,0.3$ |
| 1:10 MS | 20 | 200 | 8.6 | 94.1 | $1.3\,\rho\,0.4$ |

desirable in this study for the following reasons:

1. PLGA microspheres were selected as the delivery system for CPT because they could stabilize the active lactone form of CPT due to its acidic property.
2. Stabilization of PLGA microspheres to CPT diminished once CPT was released from the microspheres. Therefore, the release site of CPT is crucial to its antitumor activity; the closer it is to its target, the better.
3. The molecular target of CPT, DNA topoisomerase I, is a nuclear enzyme.[15] Maximum antitumor activity of CPT can be achieved when it is released from the microspheres inside the cells.
4. The size of the prepared microspheres should be optimized so that they can be easily taken up by the cells.
5. Optimal particle size of the microspheres for easy cell uptake is 1–2 μm for several types of cells such as macrophage, leukocyte, etc.[8]

## In Vitro Release

The release profile of CPT from PLGA microspheres in RPMI 1640 cell culture medium is shown in Fig. 3. The release pattern in this medium was biphasic, comprising an initial first burst effect followed by a sustained, continuous phase. The first burst effect was attributed to the immediate dissolution and release of the portion of the drug located at or near the surface of the microspheres. A similar profile was obtained for CPT released from microspheres in PBS, pH 7.4.

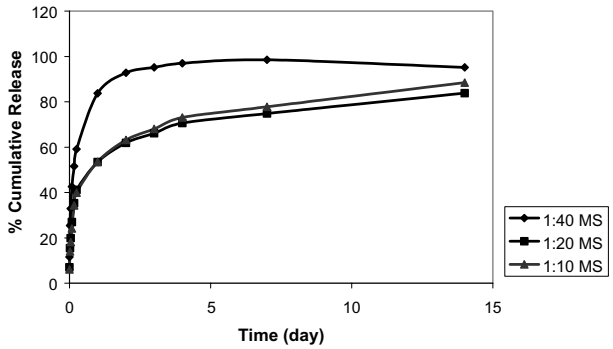


***Figure 3.*** In vitro release profile of CPT-PLGA microspheres in colorless RPMI 1640 cell culture medium.

## Surface Morphology

Images of the microspheres with different drug loading are presented in Fig. 4. Figure 5 shows the images of these microspheres after 20 days of in vitro erosion in RPMI 1640 medium.

It is shown in Fig. 4 that all preparations were spherical in shape. The surface of the low drug loading preparation (blank MS, 1:40 MS, 1:20 MS) was smooth, while the surface of high drug loading preparations (1:10 MS) was slightly rough. In Fig. 5, it was observed that the shape of all these preparations was altered significantly after incubation. The microspheres degraded with formation of numerous pores at the particle surface.

## DSC Study

The $T_g$ of a sample was determined by the midpoint of its thermograph slope shift. The $T_m$ of a sample was determined by the initial point of endothermic transition in its thermograph. The thermographs for PLGA, CPT, and CPT-PLGA microspheres are shown in Fig. 6, and the obtained $T_g$ and $T_m$ results are tabulated in Table 2.

It can be seen that all three types of CPT-PLGA microspheres had $T_g$ similar to that of PLGA and $T_m$ similar to that of CPT. Moreover, no additional thermal transition appeared in the microspheres.

C. Dubernet[16] discussed the possible interactions between a drug and its polymer microsphere matrix, and their effects on thermal properties of the microspheres. During the microsphere preparation procedure, if a drug is initially dispensed and remains in this form during the process, then it is physically suspended in the polymer matrix. Therefore, $T_g$ of the polymer and $T_m$ of the drug would be conserved. If the drug is initially dissolved, three opportunities may occur: solid solution, metastable molecular dispersion, and crystalline dispersion. Solid solution would lead to a lower $T_g$ of polymer and absence of drug $T_m$. Metastable molecular dispersion leads to conserved polymer $T_g$ and initially, to the absence of drug $T_m$. Crystalline dispersion retains both polymer $T_g$ and drug $T_m$. In this study, CPT was initially dispersed in PLGA solution, therefore, CPT should be physically suspended in PLGA matrix, and their $T_m$ or $T_g$ value should not be changed. This was proved by the DSC results in this study, indicating that there was no interaction between CPT and PLGA in the microspheres.

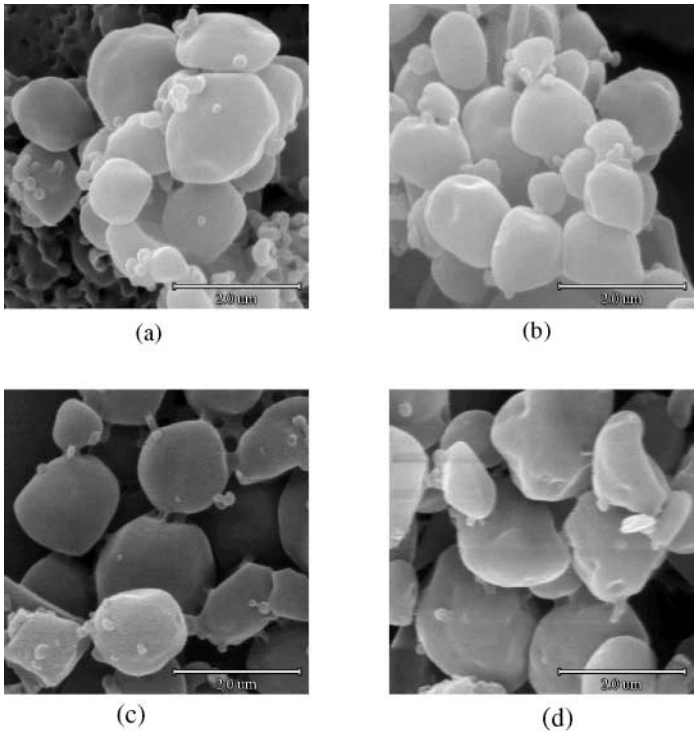


***Figure 4.*** SEM images obtained from unincubated CPT-PLGA microspheres: (a) Blank MS, (b) 1:40 MS, (c) 1:20 MS, (d) 1:10 MS.

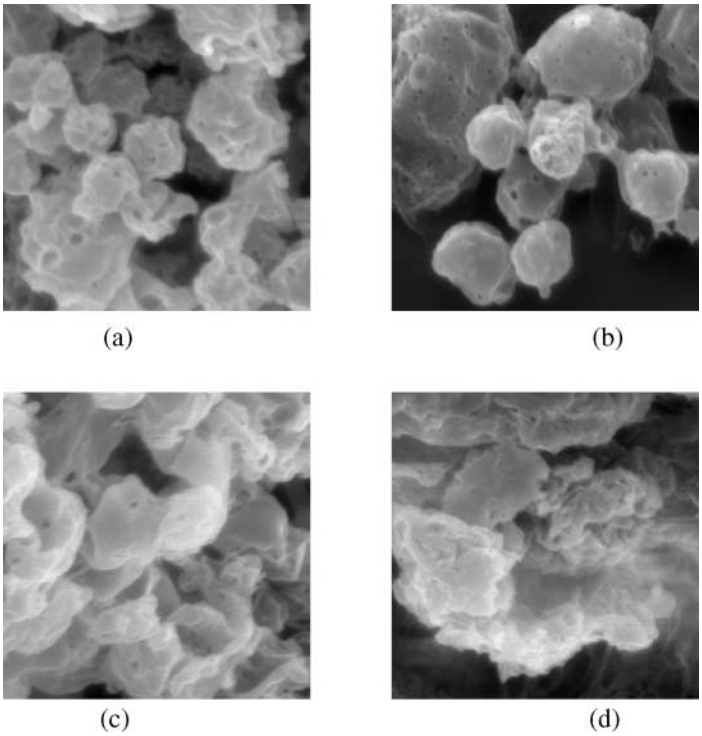


***Figure 5.*** SEM images obtained after 20 days of in vitro incubation of CPT-PLGA microspheres: (a) Blank MS, (b) 1:40 MS, (c) 1:20 MS, (d) 1:10 MS.

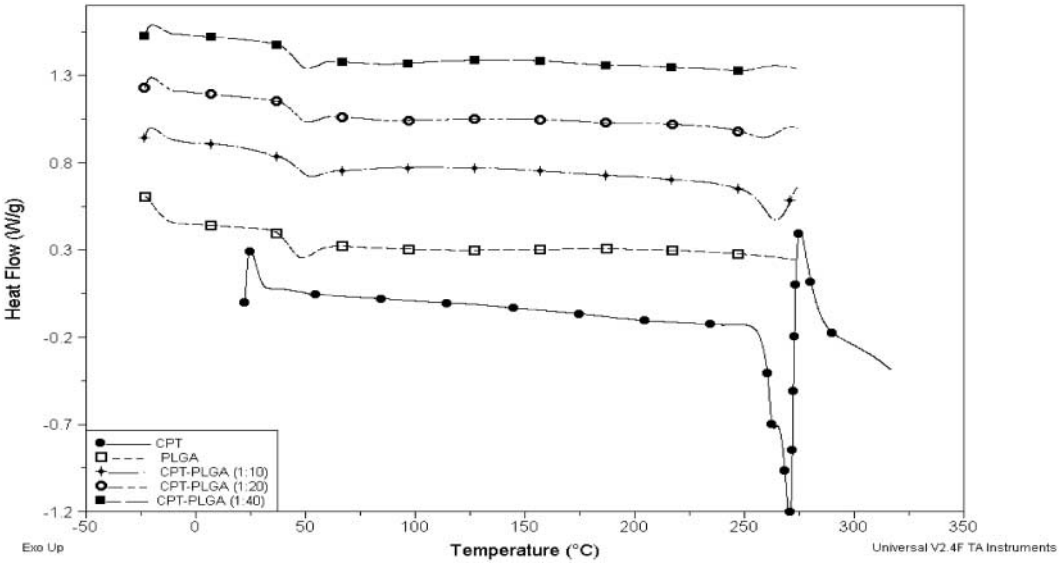


***Figure 6.*** Thermographs of CPT, PLGA, and CPT-PLGA microspheres (from bottom to top: CPT, PLGA, 1:10 MS, 1:20 MS, and 1:40 MS, respectively).

***Table 2.*** DSC results of CPT-PLGA microspheres.

| Sample | $T_g$ (°C) | $T_m$ (°C) |
|---|---|---|
| CPT | — | 255 |
| PLGA | 43 | — |
| 1:10 MS | 46 | 253 |
| 1:20 MS | 46 | 252 |
| 1:40 MS | 46 | —[a] |

$T_g$—glass transition temperature.
$T_m$—melting point.
[a]Signal is too weak.

Camptothecin-PLGA microspheres prepared by another procedure were also studied by DSC in order to further investigate the possible interactions between CPT and PLGA. In this procedure CPT was initially dissolved in the PLGA matrix using DMSO as a cosolvent. DSC results of these microspheres still showed conserved thermal properties for both CPT and PLGA, indicating the occurrence of CPT as crystalline dispersion in PLGA matrix, and no interaction between them (data not shown).

## FT-IR Study

Figure 7 shows obtained IR spectra for PLGA, CPT, and 1:10 MS. The major peaks of each spectrum are tabulated in Table 3.

It can be seen that the major peaks of 1:10 MS were either from CPT or PLGA. No new peak appeared in the spectrum of the microspheres sample. Also in this spectrum, none of the peaks of CPT or PLGA disappeared. This indicates that there was no interaction between CPT and PLGA in the microspheres, which was in good accordance with DSC results.

## Stability of Lactone Form of CPT in PLGA Microspheres

Camptothecin stability results for the PLGA microspheres (1:20 MS) are presented in Fig. 8. In PBS (pH 7.4, 37°C) most of the parent drug (80%) converted to the carboxylate form after only 6 hours. In contrast, after preparation the entire drug fraction (100%) remained in its active lactone form in the PLGA microspheres. Furthermore, the predominance of lactone form persisted for the entire duration of the release study, higher than 95% after 7 days. Thus, it was indicated that the encapsulated drug was released from the PLGA microspheres in the lactone form. But the inactivation mechanism after dissolution of the release drug remained unaltered.

Since PLGA is composed of lactic and glycolic acids linked together by ester bonds, its degradation proceeds with the formation of free carboxylic end groups. It has been reported that PLGA microspheres

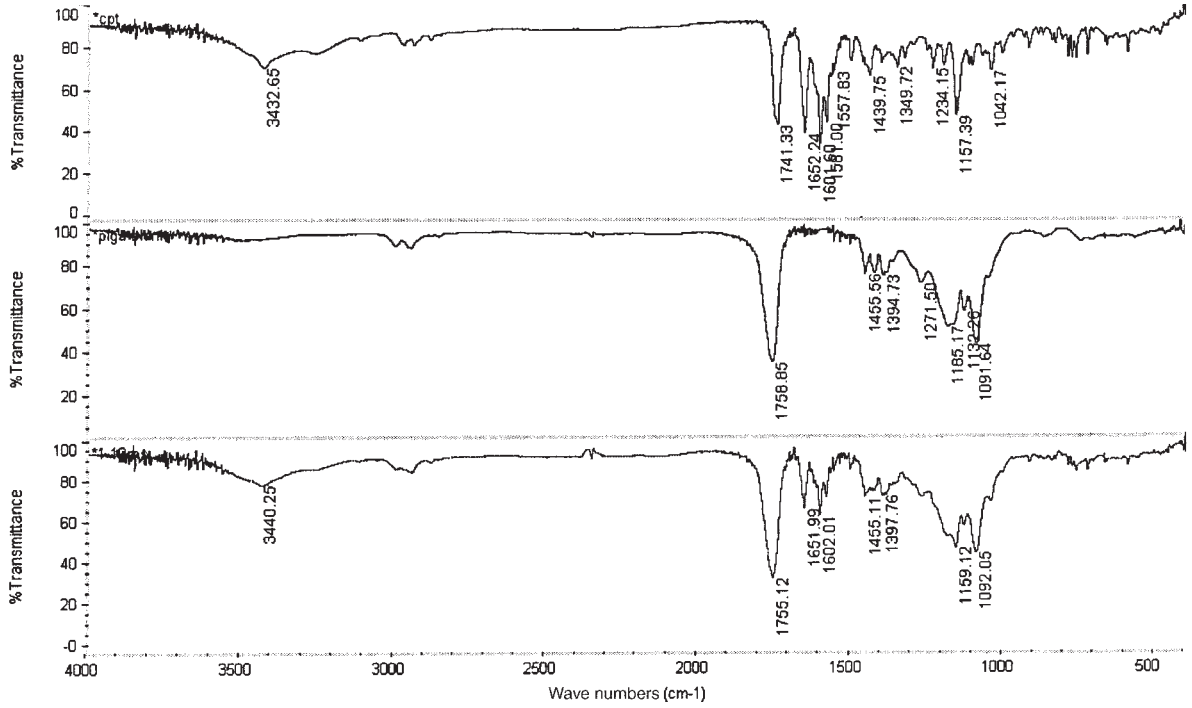


*Figure 7.* Spectra of FT-IR of CPT (top), PLGA (middle), and 1:10 MS (bottom).

*Table 3.* Major peaks of FT-IR spectra of CPT, PLGA, and 1:10 MS.

| Peak # | Wave number ($cm^{-1}$) CPT | PLGA | 1:10 MS |
|---|---|---|---|
| 1 | — | 1,759 | 1,755 |
| 2 | 1,652 | — | 1,652 |
| 3 | 1,602 | — | 1,602 |
| 4 | — | 1,455 | 1,455 |
| 5 | — | 1,395 | 1,398 |
| 6 | 1,157 | — | 1,159 |
| 7 | — | 1,092 | 1,092 |

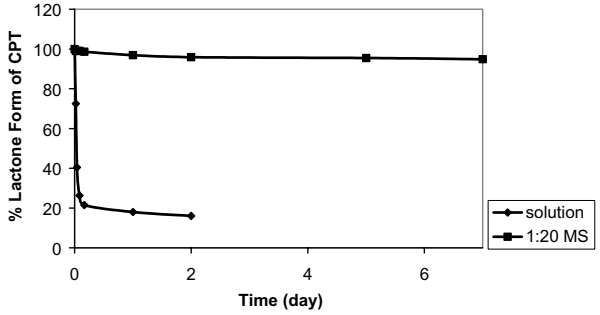


*Figure 8.* Stability profile of lactone form of CPT in PLGA microspheres and in free form incubated in PBS (pH 7.4, 37°C).

prepared by the standard solvent evaporation technique develop an acidic microclimate in the microspheres as proved by Fu et al.[17] This property favors the stabilization of CPT in its active lactone form. Since there is no interaction between CPT and PLGA, this acidic microclimate in the microspheres should therefore be the main reason for stabilizing CPT.

## Evaluation of Cytotoxicity of Microspheres

Figure 9 shows cytotoxicity of 1 μg/mL PLGA microspheres containing different amount of CPT to B16 melanoma cells. After 96 h of treatment, percentage of B16 survival was 100.4%, 23.3%, 9.1%, and 8.2% for blank MS, 1:40 MS, 1:20 MS, and 1:10 MS, respectively.

This figure clearly shows that the cytotoxic activity of microspheres increased with the increasing loading of CPT, and no influence of Blank MS on the growth of B16 cells, revealing that the growth inhibition of B16 melanoma cells was mainly due to the encapsulated CPT, not PLGA microspheres themselves. It was indicated that CPT still retained its antitumor activity when encapsulated inside the PLGA microspheres.

## Evaluation of Uptake of Microspheres by B16 Melanoma Cells

Figure 10 shows fluorescence microscopy photographs of 1.75 μm polycarboxylate microspheres taken up by B16 cells at eight different incubation times. It was clearly shown that more microspheres were taken up by B16 cells as incubation time prolonged. The plot of uptake intensity (%) vs. incubation time is shown in Fig. 11. It can be seen that about 80% of B16 cells engulfed the microspheres within 1 h of incubation, indicating quick uptaking of the microspheres by B16 cells.

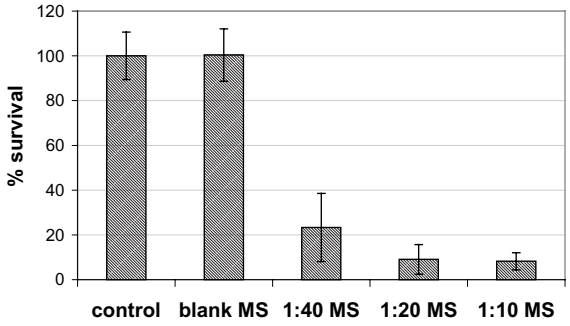


***Figure 9.*** Percent survival of B16 melanoma cells exposed to PLGA microspheres with different CPT loading.

It has been found that the main factors for influencing phagocytosis of microspheres are their particle size, surface charge, and surface hydrophobicity.[8] Fluorescent polycarboxylate microspheres used in this uptake study are polystyrenes that have carboxylate groups on their surfaces, while PLGA is a type of polyester with a carboxylic group on one end. Because of similarity in their functional groups, one can assume that PLGA microspheres should have similar surface properties as these fluorescent microspheres. Moreover, both types of microspheres had similar particle size.

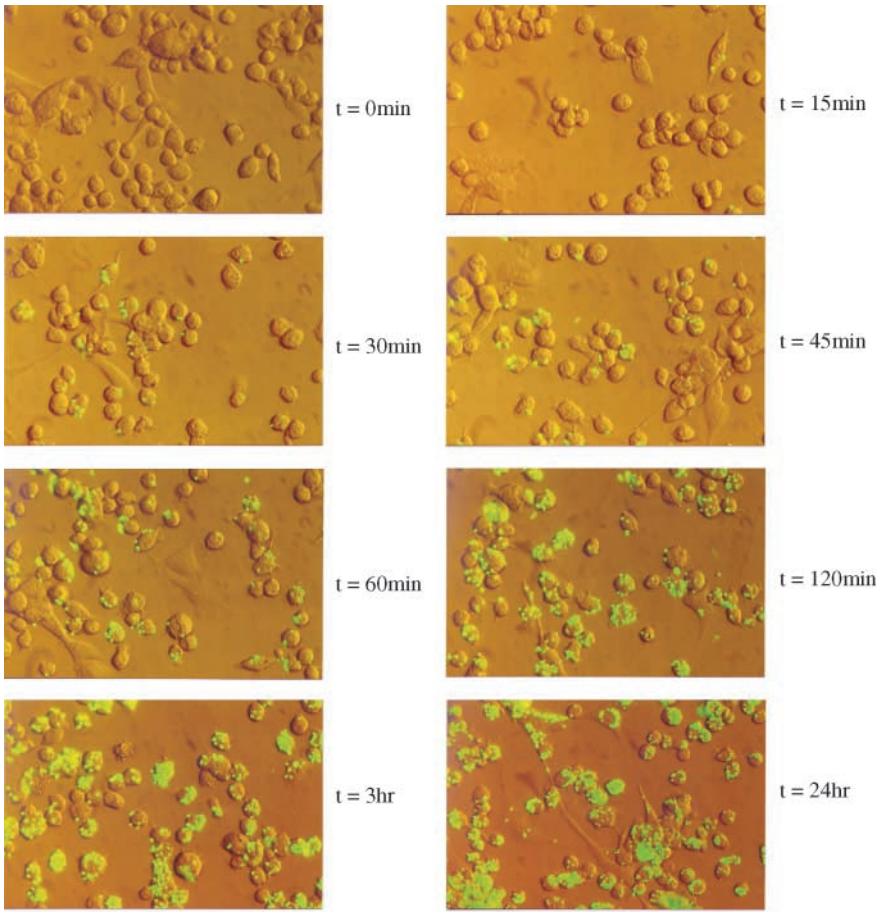


***Figure 10.*** Fluorescence microscopy photographs of 1.75 μm polycarboxylate microspheres taken up by B16 cells at eight different incubation times (B16 cells: Large gray sphere: microspheres: small bright spot).

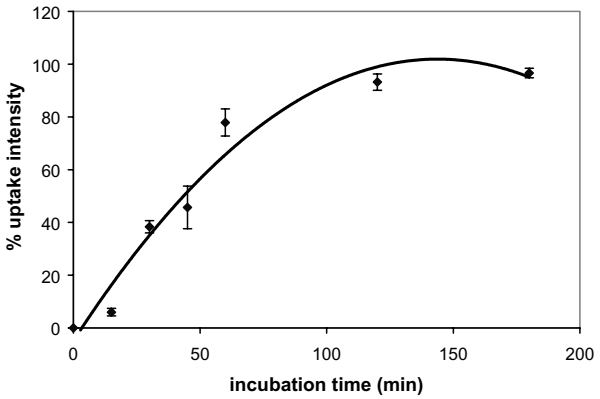


*Figure 11.* Uptake intensity of 1.75 μm polycarboxylate microspheres by B16 melanoma cells at different incubation times.

Therefore, it is assumed that the above uptake results of fluorescent polycarboxylate microspheres are representative of those of CPT-PLGA microspheres.

## CONCLUSIONS

Camptothecin-PLGA microspheres of approximately 1.3 μm in diameter, smooth surface for low drug-loading microspheres (blank MS, 1:40 MS, 1:20 MS), and slightly rough surface for high-loading microspheres (1:10 MS) were obtained. The drug CPT was released from the microspheres slowly and continuously over a 2-week period. Analysis of drug stability reveals that CPT was released from the microspheres in its active lactone form over the entire release duration. It is also found that there is no interaction between CPT and PLGA matrix within microspheres through DSC and FT-IR studies. Cytotoxicity assay shows that CPT encapsulated in PLGA microspheres still retains its antitumor potency. The uptake study reveals quick uptaking of the microspheres by B16 cells which is desirable.

It is concluded that PLGA microspheres are suitable delivery vehicles to stabilize and deliver CPT for the treatment of cancer.

## REFERENCES

1. Huang, S.-Y., Ed. *Seven Hundred Herbal Prescriptions for Cancer Medicine Treatment* (*in Chinese*); Bada Educational and Cultural Publishers: Taipei, Chinese Rep., 1986.
2. Potmesil, M.; Pinedo, H. Introduction. In *Camptothecins: New Anticancer Agents*; Potmesil, M., Pinedo, H., Eds.; CRC Press, 1995; 1–4.
3. Perrin, D.E.; English, J.P. Polyglycolide and Polylactide. In *Handbook of Biodegradable Polymers*; Domb, A.J., Kost, J., Wiseman, D.M., Eds.; Harwood Academic Publishers, 3–27.
4. Schwendeman, S.P.; Cardamone, M.; Klibanov, A.; Langer, R. Stability of proteins and their delivery from biodegradable polymer microspheres. In *Microparticulate Systems for the Delivery of Proteins and Vaccines*; Cohen, S., Bernstein, H., Eds.; Marcel Dekker: New York, 1996; 1–49.
5. Ertl, B.; Platzer, P.; Wirth, M.; Gabor, F. Poly(D,L-lactic-co-glycolic acid) microspheres for sustained delivery and stabilization of camptothecin. Journal of Controlled Release **1999**, *61*, 305.
6. Shenderova, A.; Burke, T.G.; Schwendeman, S.P. Stabilization of 10-hydroxycamptothecin in poly(lactide-co-glycolide) microsphere delivery vehicles. Pharmaceutical Research **1997**, *14* (10), 1406.
7. Shenderova, A.; Burke, T.G.; Schwendeman, S.P. The acidic microclimate in poly(lactide-co-glycolide) microspheres stabilizes camptothecin. Pharmaceutical Research **1999**, *16* (2), 241.
8. Tabata, Y.; Ikada, Y. Phagocytosis of polymeric microspheres. In *High Performance Biomaterials*; Szycher, M., Ed.; Technomic: Lancaster, PA, 1991; 621–646.
9. Warner, D.L.; Burke, T.G. Simple and versatile high-performance liquid chromatographic method for the simultaneous quantitation of the lactone and carboxylate forms of camptothecin anticancer drugs. Journal of Chromatography B **1997**, *691*, 161–171.
10. Fassberg, J.; Stella, V.J. A kinetic and mechanistic study of the hydrolysis of camptothecin and some analogues. Journal of Pharmaceutical Sciences **1992**, *81* (7), 676–684.
11. O'Donnell, P.B.; McGinity, J.W. Preparation of microspheres by the solvent evaporation technique. Advanced Drug Delivery Reviews **1997**, *28*, 25–42.
12. Conti, B.; Genta, I.; Modena, T.; Pavanetto, F. Investigation on process parameters involved in polylactide-co-glycolide microspheres preparation. Drug Development and Industrial Pharmacy **1995**, *21* (5), 615–622.

13. *CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay*, Technical Bulletin, Part# TB169, Promega: Madison, WI, 1996.
14. Torche, A.; Albina, E.; Corre, P.L.; Jestin, A.; Verge, R.L. Flow cytometric and optical microscopic evaluation of poly(D,L-lactide-co-glycolide) microspheres. phagocytosis by pig alveolar macrophages. Journal of Controlled Release **1999**, *58*, 289–301.
15. Liu, L.F. Biochemistry of camptothecin. In *Camptothecins*: *New Anticancer Agents*; Potmesil, M., Pinedo, H., Eds.; CRC press, 1995; 9–20.
16. Dubernet, C. Thermoanalysis of microspheres. Thermochimica Acta **1995**, *248*, 259–269.
17. Fu, K.; Pack, D.W.; Klibanov, A.M.; Langer, R. Visual evidence of acidic environment within degrading poly(lactic-co-glycolic acid) (PLGA) microspheres. Pharmaceutical Research **2000**, *17* (1), 100–106.