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Biomaterials 23 (2002) 3465–3471

Biomaterials

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Effect of tricaprin on the physical characteristics and in vitro release of etoposide from PLGA microspheres

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Received 20 June 2001; accepted 6 February 2002

Abstract

The purpose of this article is to examine the effects of tricaprin on the physical characteristics and in vitro release of etoposide from poly (lactic-co-glycolic acid) microspheres. The microspheres were synthesized through the use of a single-emulsion solvent-extraction procedure. Samples from each batch of microspheres were then analyzed for size distribution, drug loading efficiency, surface characteristics, in vitro release, and in vitro degradation of microspheres. Microsphere batches were synthesized using three different etoposide concentrations (15%, 10%, and 5% w/w) with tricaprin concentrations of 25% and 50%. The incorporation of 50% tricaprin significantly increased ($p<0.05$) the size of the microspheres for all three etoposide concentrations in comparison to microspheres prepared without tricaprin (control). The percentage of tricaprin used did not significantly affect the drug loading efficiency of the microspheres. The addition of tricaprin was shown to significantly increase ($p<0.05$) the in vitro release of etoposide from the microspheres prepared with all three concentrations of etoposide and the two different tricaprin percentages. Examination of the surface characteristics of the tricaprin loaded microspheres showed a dimpled surface with what appeared to be pockets of tricaprin dispersed throughout. In the in vitro degradation study, the tricaprin microspheres grew very porous as the degradation time increased, but they still retained a recognizable structure even after 30 days of degradation. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Etoposide; PLGA microspheres; Tricaprin; Surface characteristics; In vitro release; In vitro degradation

1. Introduction

Targeting of chemotherapeutic agents to different organs of the body by employing colloidal particles is an area of active research. Based on the size of the colloidal particles and the route of administration, specific drugs can be passively targeted to areas of the body [1]. This selective targeting of chemotherapeutic agents has two advantages. It allows for the maximum fraction of the delivered drug molecules to react with the cancer cells without having any harmful effect on normal cells, and it increases the distribution of the drug to the cancer cells [2]. The end result of targeted chemotherapy is an increase in effectiveness of the chemotherapeutic agent and, due to lower systemic concentrations, decreased incidence of side effects.

Microparticles have been used for the purpose of drug delivery and targeting [3–7]. Passive targeting of microspheres can be achieved by exploiting the fact that particles reasonably large in size (more than 7 μm) are retained after intravenous administration by a simple process of mechanical entrapment in the capillary beds of the lung [8,9]. This process has been employed for many years in the area of diagnostic imaging [10]. It also has been utilized to treat respiratory diseases and for cancer treatment [11]. The drug-loaded biodegradable microspheres lodge within the capillary networks of the lung and release their bioactive agent into neighboring tissue by a process of diffusion and biodegradation [12].

Etoposide is effective in the treatment of acute myeloid leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, lung cancer (small cell and non-small cell), AIDS related Kaposi's sarcoma, gastric cancer, breast cancer, and ovarian cancer [13,14]. Research has shown that chemotherapy regimens that utilize etoposide are more effective when the drug is given over an extended period of time [13,15]. For the past decade,

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0142-9612/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0142-9612(02)00053-4

poly (lactic-co-glycolic acid) (PLGA) has been used extensively for controlled drug delivery systems [16–21]. The release of an active compound from the PLGA matrix of the microspheres can occur by one or all of three mechanisms: diffusion through the polymer matrix, dissolution after solubilization in connected channels through the microparticle, and release after matrix degradation [22]. The mechanism and the rate of release are highly dependent on the physico-chemical properties of the polymer and drug, and on the properties of the microspheres such as size, drug content, and porosity. The incorporation of additives in the microspheres can be used to modify the release profile of the active component. The literature reports several instances of the use of some plasticizers (tributyl citrate and glycerin) and fatty substances (isopropyl myristic acid ester and tricaprin) to increase the rate of release of drugs from microspheres [23–26]. In this study, we examined the effect of tricaprin on the size, drug loading, surface characteristics, and in vitro release of etoposide from PLGA microspheres.

2. Materials and methods

2.1. Materials

PLGA 50:50 (IV = 0.61) was obtained from Birmingham Polymers Inc. of Birmingham, AL and etoposide from ICN Pharmaceuticals of Aurora, OH. The poly vinyl alcohol, methylene chloride, and tricaprin were obtained from Sigma Chemical Company, St. Louis, MO. The isopropyl alcohol, Tween-80, methanol, acetonitrile, and acetic acid were obtained from Fischer Chemical Company, Fair Lawn, NJ.

2.2. Synthesis of etoposide-loaded PLGA 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, UK) and maintained at 60°C for 10 min to evaporate off the solvent. After 10 min, the heat was removed and stirring continued at a reduced rate of 500 rpm for 50 min to further evaporate the solvent. Residual methylene chloride was removed by a wash of 10% isopropyl alcohol (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 prepared with tricaprin were manufactured in a similar manner with the added step of the addition of the tricaprin to the initial drug-polymer solution.

2.3. Microsphere size determination and photography

A sample from each microsphere batch was dry mounted on a microscope slide with cover slip. A Cole-Parmer video caliper (Model 49910–20, Cole-Parmer, Chicago, IL) under a 10× lens (Meiji Microscope, Osaka, Japan) was used to size a representative number of microspheres ($n = 100$). The video caliper is a microprocessor controlled video based reticle generator that is projected onto a standard video monitor (CT-2086YD, Panasonic, Sacaucus, NJ). The caliper system was calibrated and utilized to size microspheres projected onto the video monitor through the video microscope system. Microspheres were photographed using a Polaroid Microcam (331 film).

2.4. Scanning electron microscopy

The shape and surface morphology of the microspheres were examined with a scanning electron microscope (JOEL SEM, Peabody, MA). Dry microspheres were mounted on an adhesive stub and then coated with gold-palladium under vacuum using an ion-coater. The coated specimen was then examined under the

Table 1
The effect of tricaprin on the drug loading and size of etoposide-PLGA microspheres prepared with 15%, 10%, and 5% drug concentrations ($n = 3$).

Batch designation	Polymer (mg)	Drug (mg)	Drug (% w/w)	Tricaprin (% w/w)	Drug loading efficiency[a] (mean ± s.d.)	Size (μm) (mean ± s.d.)
A1	200	30	15.0	0	70.31 ± 0.41	19.62 ± 0.72
A2	200	30	15.0	25	72.79 ± 5.60	21.95 ± 0.33*
A3	200	30	15.0	50	74.34 ± 4.11	24.08 ± 2.37*
B1	200	20	10.0	0	57.80 ± 3.36	18.01 ± 0.75
B2	200	20	10.0	25	61.33 ± 6.10	21.05 ± 1.67*
B3	200	20	10.0	50	61.95 ± 4.11*	21.63 ± 0.90*
C1	400	20	5.0	0	74.74 ± 4.11	25.50 ± 4.01
C2	400	20	5.0	25	74.22 ± 4.76	28.86 ± 2.07
C3	400	20	5.0	50	78.72 ± 3.53	32.70 ± 4.22*

[a] Drug loading efficiency = drug loading$_{actual}$/drug loading$_{theoretical}$.
*Indicates significant difference ($p < 0.05$) from microspheres with no tricaprin.

microscope at 10 kV and photographed. The degradation of microspheres during in vitro release studies was also examined. The microspheres were sampled at 10, 20, and 30 days during an in vitro release test and surface characteristics were studied by SEM as described above.

2.5. Drug loading content

A measured amount (≈ 5 mg) of microspheres was 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, Newtown, CT) for 10 min. The supernatant was then collected and analyzed by HPLC.

2.6. In vitro release

Two milligrams of microspheres were added to a standard screw top test tube. Ten milliliters of phosphate buffered saline (pH 7.4) containing 0.1% Tween-80 was added to each tube. Tween-80 was added to the dissolution fluid in order to maintain sink condition for etoposide during in vitro release. The samples were incubated in a water bath (Model 50 Reciprocal Shaking Water Bath, Precision Scientific, Winchester, VA) at 37°C, shaken at 100 rpm and sampled at selected time intervals (1, 5, 10, 15, 20, 25, and 30 days). The samples were then centrifuged at 2000 rpm for 1 min, and the supernatant was removed. Next, the remaining microspheres were washed in 5 ml of distilled water, centrifuged at 2000 rpm for 1 min, and the supernatant removed. Finally, the samples were frozen at −60°C and lyophilized overnight. The microspheres were then analyzed for residual drug content by HPLC. The amount of etoposide released was then calculated by subtracting the residual content from the initial amount present in the microspheres.

2.7. HPLC method for determination of etoposide content

The method of Chow et al. [27] 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 UV detector, SCL-10A system controller, SIL-10A auto-injector, dual LC-10AD pumps, and a CR-501 integrator. A mobile phase of 70% water:acetic acid (100:1)-30% acetonitrile passing 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 vs. 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 is 10 ng of etoposide on the column.

2.8. Data analysis

Statistical comparisons were made using Student's *t*-test and analysis of variance (ANOVA). The level of significance was taken as $p < 0.05$.

3. Results and discussion

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 release of etoposide from the PLGA microspheres. We incorporated an additive, tricaprin, during the synthesis phase in order to improve the in vitro release profile of the etoposide.

The effect of tricaprin concentrations, on the size and drug loading efficiency of etoposide PLGA microspheres prepared with 15, 10, and 5% etoposide is shown in Table 1. The percentage of tricaprin added to the 15% etoposide-loaded microspheres had a significant effect ($p < 0.05$) on the size (Table 1). Both the 25% (batch A2) and the 50% (batch A3) tricaprin containing microspheres were significantly larger ($p < 0.05$) than microspheres prepared without tricaprin (batch A1). The drug loading efficiency was not significantly affected ($p > 0.05$) by the tricaprin percentage for the 15% etoposide-loaded microspheres (Table 1). The tricaprin significantly increased ($p < 0.05$) the burst effect in the in vitro release studies. The plain etoposide loaded microspheres released 65.52 ± 1.05% of the etoposide in the first 24 h, as compared to the 84.81 ± 0.58 and 89.25 ± 1.39 for 25% (batch A2) and 50% (batch A3), respectively. The amount of etoposide released from the microspheres in batches A2 and A3 was significantly different ($p < 0.05$) from the amount released by batch A1 at every time point (Fig. 1).

The percentage of tricaprin incorporated in the 10% etoposide-loaded microspheres had a significant effect on the size of microspheres (Table 1). The batches containing 25% (batch B2) and 50% (batch B3) of tricaprin were significantly larger ($p < 0.05$) than the plain etoposide-loaded PLGA microspheres, but there was no significant size difference ($p > 0.05$) between batches B2 and B3. The 50% tricaprin containing microspheres had a significantly higher ($p < 0.05$) drug loading percentage than the plain etoposide-loaded microspheres (Table 1). In vitro release of etoposide from the microspheres was significantly affected by the

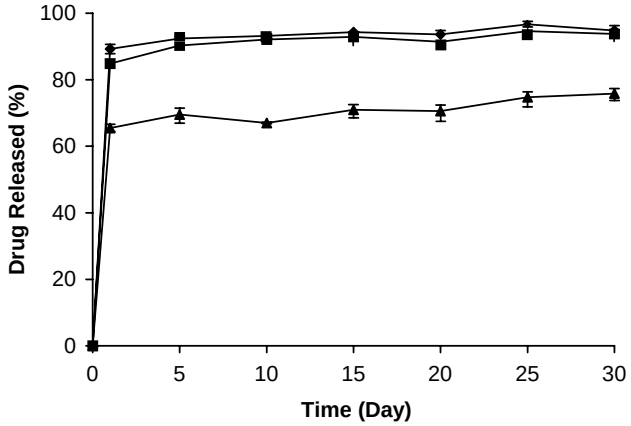

Fig. 1. The effect of tricaprin on the release of etoposide from 15% etoposide microspheres. Keys: ♦, no tricaprin; ■, 25% tricaprin; and ▲, 50% tricaprin.

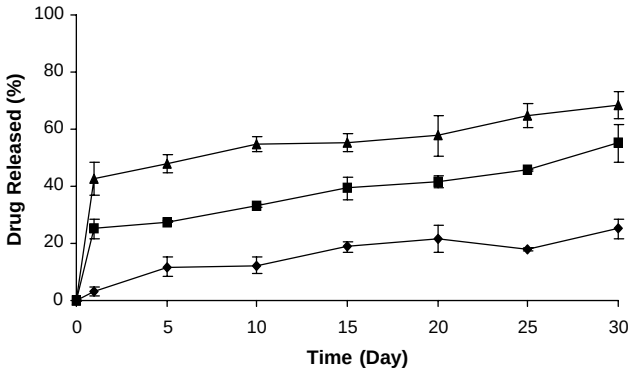

Fig. 2. The effect of tricaprin on the release of etoposide from 10% etoposide microspheres. Keys: ♦, no tricaprin; ■, 25% tricaprin; and ▲, 50% tricaprin.

percentage of tricaprin added (Fig. 2). The 25% (batch B2) and 50% (batch B3) tricaprin containing microspheres showed greater burst effect and an increase in total amount released as compared to the plain etoposide-loaded microspheres (batch B1).

The effect of tricaprin incorporation in the 5% etoposide-loaded microspheres is shown in Table 1. The 50% tricaprin (batch C3) significantly increased ($p<0.05$) the size of the microspheres compared to the plain etoposide-loaded microspheres (batch C1) (Table 1). The tricaprin percentage had no effect on the drug loading efficiency of the PLGA microspheres. The added tricaprin to the 5% etoposide microspheres significantly enhanced the in vitro release of etoposide in comparison to plain etoposide-loaded PLGA microspheres (Fig. 3). The burst effect was significantly enhanced ($p<0.05$) in the microspheres prepared with 25% and 50% tricaprin over the plain etoposide microspheres. The burst effect was significantly different

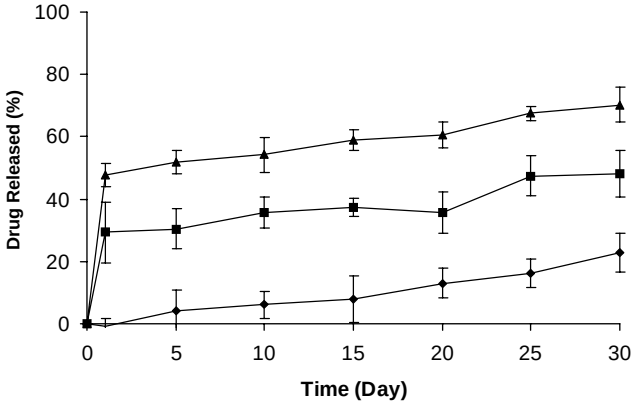

Fig. 3. The effect of tricaprin on the release of etoposide from 5% etoposide microspheres. Keys: ♦, no tricaprin; ■, 25% tricaprin; and ▲, 50% tricaprin.

($p<0.05$) between the 25% and the 50% tricaprin microspheres.

The addition of tricaprin to the microspheres generally increased the average size of the microspheres. However, the drug loading efficiency of the tricaprin microspheres indicates that the percentage of tricaprin incorporated into the microspheres has no effect on the drug loading efficiency of the microspheres. This finding is in accordance with published results [25,26]. The addition of tricaprin significantly increased ($p<0.05$) the in vitro release of etoposide from PLGA microspheres. A significant increase ($p<0.05$) in the release of etoposide from the 25% and 50% tricaprin microspheres in comparison to plain microspheres was observed. Juni et al. [25] had also observed similar results where tricaprin increased the in vitro release of aclarabicin from PLGA microspheres.

Fig. 4 shows the difference in surface morphology between the plain etoposide-loaded microspheres and those prepared with tricaprin. The plain etoposide-loaded microspheres (Fig. 4a) are smooth with a uniform matrix, however, the tricaprin microspheres (Fig. 4b) have tricaprin pockets dispersed throughout, as evidenced by what appears to be a dimpled surface. The pockets increase in size with the increasing tricaprin percentage. The result of the in vitro degradation studies with 5% etoposide-loaded microspheres without tricaprin, and 5% etoposide-loaded microspheres with 25% tricaprin, is also shown in Fig. 4. Initially, the 5% etoposide-loaded microspheres show a smooth, non-porous surface and the microspheres with tricaprin show a dimpled, non-porous surface (Figs. 4a and b). After 10 days of in vitro release studies (Figs. 4c and d), both microsphere batches show no evidence of polymer degradation. The first signs of degradation appear in the 20-day samples collected during in vitro release studies (Figs. 4e and f). The 5% etoposide-loaded microspheres show a smooth surface broken up by

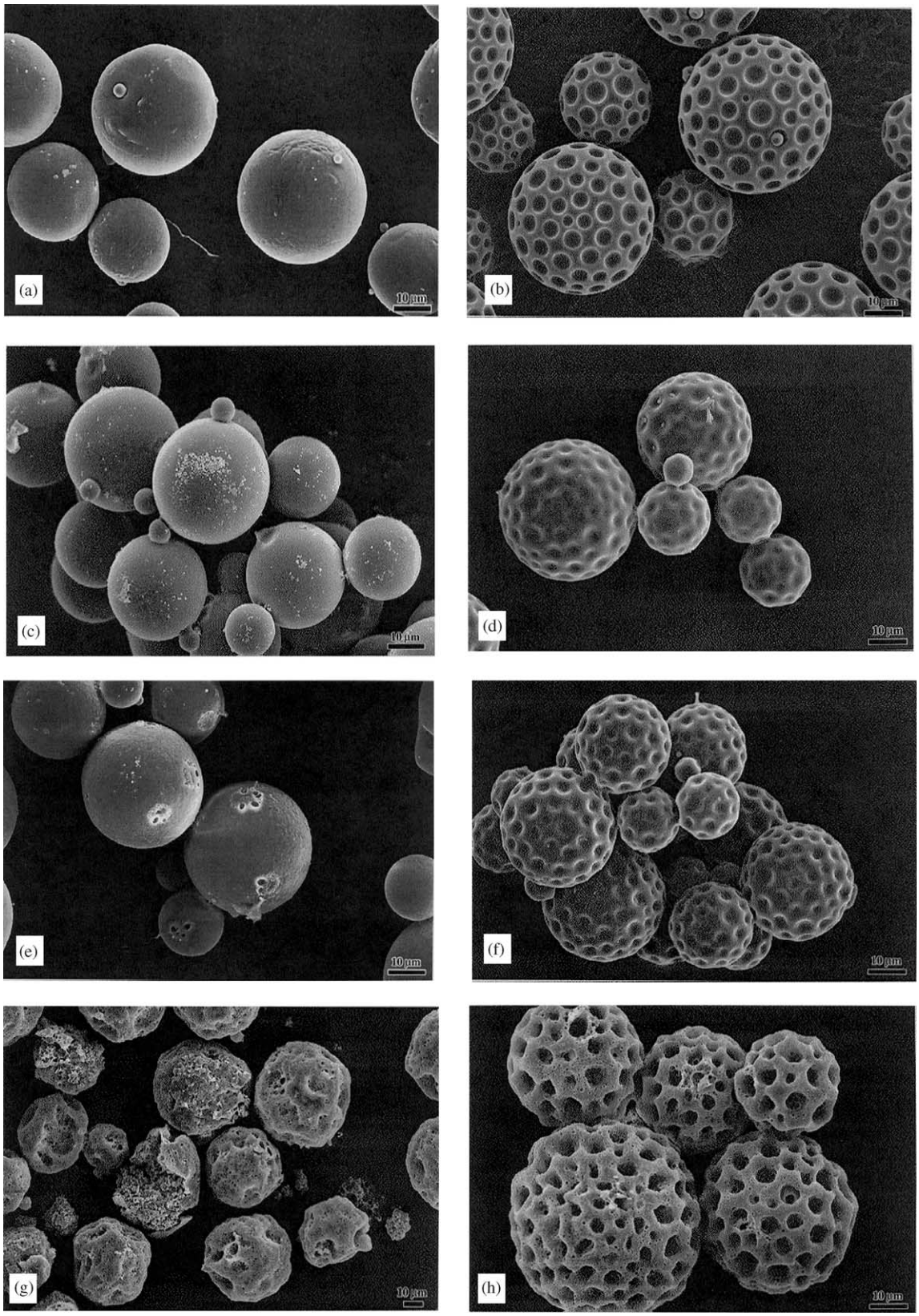

Fig. 4. The effect of in vitro degradation on microsphere surface morphology. Five percent etoposide microspheres (C1); (b) 5% etoposide microspheres containing 25% tricaprin (C2); (c) surface characteristics of microspheres (C1) after 10 days of in vitro release; (d) surface characteristics of microspheres (C2) after 10 days of in vitro release; (e) surface characteristics of microspheres (C1) after 20 days of in vitro release; (f) surface characteristics of microspheres (C2) after 20 days of in vitro release; (g) surface characteristics of microspheres (C1) after 30 days of in vitro release; (h) surface characteristics of microspheres (C2) after 30 days of in vitro release. Calibration bar = 10 μm.

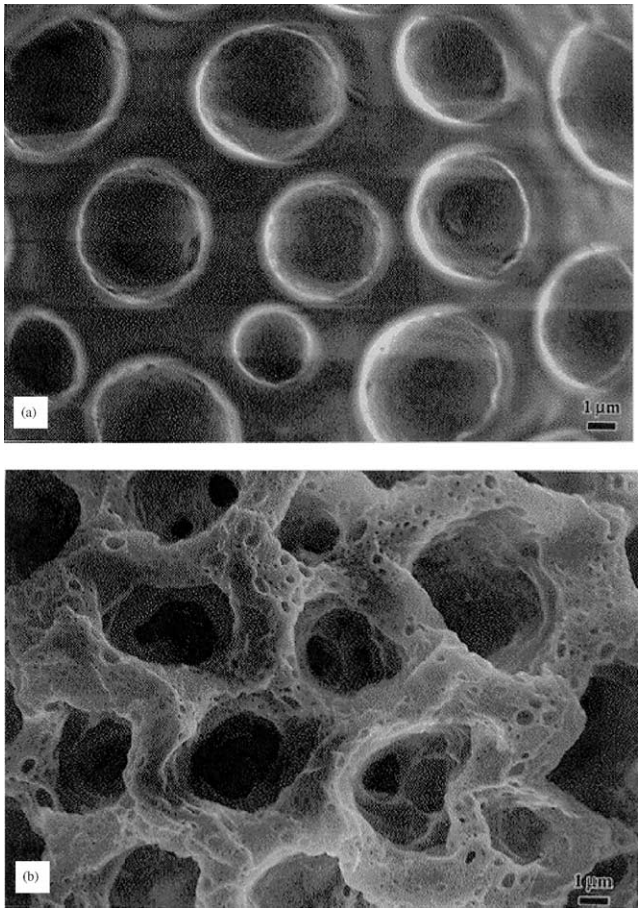

Fig. 5. Characterization of surface morphology of dimples associated with 5% etoposide microspheres containing 25% tricaprin (a) before in vitro release, and (b) after 30 days of in vitro release. Calibration bar = 1 μm.

occasional pores formed by polymer degradation (Fig. 4e). The tricaprin microspheres show pores in the dimpled surface formed by the degradation of the polymer matrix (Fig. 4f). The microspheres following 30 days of degradation during the in vitro release studies are shown in Figs. 4g and h. The polymer matrix of both samples has significantly degraded. The 5% etoposide-loaded microspheres surface has broken down, and the microspheres have lost much of their structural integrity (Fig. 4g). The tricaprin containing microspheres are also significantly degraded but retain their structural integrity (Fig. 4h).

We further characterized the dimples located on the surface of microspheres by SEM (Fig. 5). These dimples are spherical in shape and arranged over the surface in a remarkable regular manner. We assume that the formation of dimples is the result of tricaprin leaving the internal phase (dispersed phase) during solvent evaporation and micro-particles formation. We determined the diameter of microspheres and dimples through SEM. The total surface area of dimples is $A = n\pi d^2$ where n is the number of dimples and d the diameter. We found that the fraction of surface area occupied by dimples relative to the total surface area of the microsphere was 0.53. Fig. 5a shows the surface of dimples on the 5% etoposide microspheres containing 25% tricaprin. Fig. 5b shows the dimples after 30 days of in vitro release. We clearly observe that dimples have been turned into deeper holes. Such deeper holes have provided greater surface area for drug diffusion from microspheres in comparison to 5% etoposide microspheres without tricaprin.

An increase in release of etoposide from the microspheres can be explained by the effect that the addition of tricaprin has on the polymer matrix and surface morphology of the microspheres. As evidenced by SEM, the addition of tricaprin to the microspheres disrupts the polymer matrix of the microspheres. The result of this disruption is the formation of a dimpled microsphere surface that increases the release of the drug by improving the dissolution of the drug from the polymer matrix. As the microspheres dissolve, these dimples become larger and pores begin to form through the degradation of the polymer. The microspheres do not have the added tricaprin degraded by bulk erosion. The end result is that the plain microspheres release less etoposide over a given time frame. It is also important to note that the microspheres produced with tricaprin maintained their structural integrity, while the plain microspheres did not. This structural integrity is an important consideration given the desire to target these microspheres to the lung and to entrap them for long periods of time based on their size.

4. Conclusions

Tricaprin containing microspheres were significantly larger ($p < 0.05$) in size than the plain microspheres. However, tricaprin did not affect the drug loading efficiency of the microspheres. The addition of tricaprin at both 25% and 50% significantly increased ($p < 0.05$) the in vitro release of etoposide over the plain microspheres. The addition of tricaprin to microspheres changed their surface characteristics from smooth and non-porous to dimpled and non-porous. The surface morphology and its effect on the degradation of the microspheres explains the difference in the release of etoposide from the plain and the tricaprin containing microspheres.

References

[1] Burger JJ, Tomlinson E, Mulder EMA, McVie JG. Albumin microspheres for intra-arterial tumor targeting. I. Pharmaceutical aspects. Int J Pharm 1985;23:333–44.

[2] Chasin M, Langer R, editors. Biodegradable polymers as drug delivery systems. New York: Marcel Dekker Inc., 1990. p. 1–41.
[3] Schaefer M, Singh J. Etoposide microspheres for targeting to the lung. Proc Int Symp Control Rel Bioact Mater 1998;25:441–2.
[4] Singh J, Robinson DH. Controlled release captopril microcapsules: effect of ethyl cellulose viscosity grades on the in-vitro dissolution from microcapsules and tableted microcapsules. J Microencapsulation 1990;7:67–76.
[5] Karunakar S, Singh J. Preparation, characterization and in-vitro release kinetics of salbutamol sulfate loaded albumin microspheres. Drug Develop Ind Pharm 1994;20:1377–99.
[6] Singh J, Singh RP, Karunakar S, Tripathi KP. Preparation, characterization and in-vitro release kinetics of aminophylline loaded albumin microspheres. Pharm Ind 1994;56:1076–80.
[7] Tripathi KP, Singh J. Aminophylline targeting to lung: optimization of the size and drug loading of albumin microspheres. J Microencapsulation 1992;9:229–35.
[8] Kanke M, Simmons GH, Weiss DL, Bivins BA, DeLuca PP. Clearance of 141 Ce-labelled microspheres in specific organs following intravenous and intra-arterial administration in beagle dogs. J Pharm Sci 1980;69:755–62.
[9] Illum L, David SS. Targeting of colloidal particles to the bone marrow. Life Sci 1987;40:1553–60.
[10] Guiot P, Couvreur M, editors. Polymeric nanoparticles and microspheres. Boca Raton, FL: CRC Press, 1986. p. 175.
[11] David SS, Hunneyball IM, Illum L, Ratcliffe JH, Smith A, Wilson CG. Recent advances in the use of microspheres for targeted therapy. Drugs Expl Clin Res 1985;9:633–40.
[12] David SS, Illum L, McVie JG, Tomlinson E, editors. Microspheres and drug therapy. New York: Elsevier, 1984. p. 205–16.
[13] Hande KR. Etoposide: four decades of development of a topoisomerase II inhibitor. Eur J Cancer 1998;34:1514–21.
[14] O'Dwyer P, Leyland-Jones B, Alonso MT, Marsoni S, Wittes RE. Etoposide: current status of an active anticancer drug. N Engl J Med 1985;312:692–8.
[15] Wolf SN, Grosh WW, Prater K, et al. In vitro pharmacodynamic evaluation of VP-16213 and implications for chemotherapy. Cancer Chemother Pharmacol 1987;19:246–9.
[16] Lewis DD. Controlled release of bioactive agents from lactide/glycolide polymers. In: Chasin M, Langer R, editors. Biodegradable polymers as drug delivery system. New York, NY: Marcel Dekker, 1990. p. 1–41.
[17] Cohen S, Yoshika T, Lucarelli M, Hwang LH, Langer R. Controlled delivery systems for proteins based on poly (lactic/glycolic acid) microspheres. Pharm Res 1991;60:713–20.
[18] Kostanski JW, DeLuca PP. A novel in vitro release technique for peptide-containing biodegradable microspheres. AAPS PharmSciTech 2000;1(1). Available at http://www.pharmscitech.-com [article 4].
[19] Ravivarapu HB, Moyer KL, Dunn RL. Sustained activity and release of leuprolid eacetate from an in situ forming polymeric implant. AAPS PharmSciTech 2000;1(1). Available at http://www.pharmscitech.com [article 1].
[20] Kostanaski JW, Dani BA, Reynolds G-A, Bowers CY, DeLuca PP. Evaluation of orntide microspheres in rat animal model and correlation to in vitro release profiles. AAPS PharmSciTech 2000;1(4). http://www.pharmscitech.com [article 27].
[21] Gebrekidan S, Woo BH, DeLuca PP. Formulation and in vitro transfection efficiency of poly (d, l- lactide-co-glycolide) microspheres containing plasmid DNA for gene delivery. AAPS PharmSciTech 2000;1(4). http://www.pharmscitech.com [article 28].
[22] Jain R, Navnit HS, Mailick AW, Rhodes CT. Controlled delivery by biodegradable poly(ester) devices: different preparative approaches. Drug Develop Ind Pharm 1998;24:703–27.
[23] Sato H, Wang YM, Adachi I, Horikoshi I. Pharmacokinetic study of taxol- loaded poly(lactic-co-glycolic acid) microspheres containing isopropyl myristate after targeted delivery to the lung in mice. Biol Pharm Bull 1996;19:1596–601.
[24] Wang YW, Sato H, Adachi I, Horikoshi I. Preparation and characterization of poly(lactic-co-glycolic acid) microspheres for targeted delivery of a novel anticancer agent. Taxol, Chem Pharm Bull 1996;44:1935–40.
[25] Juni K, Ogata J, Matsui N, Kubota M, Nakano M. Modification of the release rate of aclarabicin from polylactic acid microspheres. Chem Pharm Bull 1985;33:1734–8.
[26] Sansdrap P, Moes J. Influence of additives on the release profile of nifedipine from poly(DL-lactide-co-glycolide) microspheres. J Microencapsulation 1998;15:545–53.
[27] Chow D, Shah J. Stability-indicating high performance liquid chromatography of etoposide at various pH conditions using a reversed-phase octyl column. J Chromatogr 1987;396:217–23.