Formation and Characterization of Cisplatin-Loaded Poly(benzyl l-glutamate) Microspheres for Chemoembolization

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Chemoembolization using microspheres of 100- to 200-μm is a useful way to treat primary and secondary hepatic tumors. In a search for a better embolic material, we described in detail the preparation and characterization of a poly(benzyl l-glutamate) (PBLG) microspheres containing cisplatin (CDDP). We determined the optimal experimental conditions to produce spherical free-flowing microspheres that were able to release drug content (44% [w/w] CDDP) in a sustained manner. We found that solvent viscosity played a key role in determining the resulting microsphere characteristics. Microscopic studies showed that increasing the polymer concentration (to 10% [w/v]) and the viscosity of the organic phase produced microspheres with uniform drug distribution. Increasing polymer concentration also markedly improved drug incorporation efficiency. *In vitro* release studies revealed that the release of CDDP was a function of drug loading; microspheres with a higher amount of entrapped CDDP had a slower release rate. This observation and the fact that CDDP/PBLG microspheres did not show "burst effect" at higher loading is ascribed to the formation of uniformly distributed drug crystal networks within the polymer matrix. The favorable properties of the CDDP/PBLG system warrants its further evaluation on experimental animal models for the treatment of hepatic tumors.

KEY WORDS: microspheres; chemoembolization; cisplatin; poly(benzyl l-glutamate).

INTRODUCTION

Cisplatin (CDDP) is one of the most potent anticancer agents known and is frequently used to treat primary and secondary liver tumors (1–3). However, administration of this drug can lead to a number of side effects, including nephrotoxicity and auditory disturbance (4). A promising way of optimizing its action is to target its delivery via selective arterial catheterization. Hepatic arterial embolization with various anticancer drugs has been considered an effective method for the treatment of unresectable primary and secondary hepatic neoplasms (5) because of the synergistic effect of embolic occlusion of the blood supply to the neoplasm and local retention of the infused chemotherapeutic agents. Although chemoembolization has been investigated experimentally and clinically using a variety of antitumor drugs with both natural and synthetic polymers as particulate emboli (5–10), little work has been done on the use of cisplatin microspheres (11).

The development of emboli containing CDDP is impeded by the restricted choice of suitable materials. The ethylcellulose/CDDP system is not favored due to its irregular shape and "burst effect." The only CDDP-containing microspheres that have been extensively investigated are biodegradable poly(dl-lactide) (PLA) microspheres (12,13). Thus, it is highly desirable that a range of CDDP-containing microspheres with defined material properties, including degradability, hydrophilicity, mechanical properties and drug release characteristics be developed. Correlation of such material properties with tumor response is a prerequisite for any rational design process. Based on these considerations, we selected poly(benzyl l-glutamate) (PBLG), a polyamino acid, as a carrier for CDDP to produce chemoembolic microspheres. Synthetic polyamino acids have been investigated for possible use in a variety of biomedical applications because of their biocompatibility (14–18). Degradability of PBLG may be manipulated by introducing hydrophilic moieties either to the side chain or to the backbone of the polymer (19,20a,b). The possibility of such modification makes PBLG a suitable model for the investigation of structure-property relationships in chemoembolization applications.

In this paper, we describe the formation and characterization of spherical CDDP-loaded PBLG microspheres. Attempts have been made to correlate CDDP loading and release characteristics to microsphere morphology.

MATERIALS AND METHODS

Materials

PBLG used in this study had a molecular weight (vis) of 58K and was obtained from Sigma Chemical Company (St. Louis, MO). CDDP and polyvinyl alcohol (PVA) (molecular weight 30–70K) were also purchased from Sigma. Methylene chloride was purchased from Curtin Matheson Scientific (Houston, TX). N,N-dimethylformamide (DMF) was supplied by Fisher Scientific (Fair Lawn, NJ).

Microspheres Preparation

The CDDP crystals were ground to fine powder with a mortar and pestle. CDDP-loaded microspheres were prepared by a solvent evaporation procedure. Various amounts of ground CDDP powder were suspended in a solution of PBLG in methylene chloride and sonicated. Concentration of the polymers in the solvent ranged from 2 to 10% (w/v), and the ratio of CDDP to PBLG varied from 1:1 to 2:1. The resulting organic phase was then slowly added to an aqueous solution of PVA (2%, w/v) which was stirred at 300 rpm. To ensure complete evaporation of methylene chloride, the resulting emulsion was stirred for an additional 5 hrs at room temperature (25°C) in an open beaker. Microspheres were collected by vacuum filtration, washed with 250 ml of distilled water and air dried. The microspheres were fractionated using a mechanical sieve and the 106- to 212-μm fraction was collected.

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Determination of Cisplatin Content

Ten milligrams of microspheres (106- to 212-μm fraction) from each batch were dissolved in 5 ml of DMF. The amount of CDDP in the resulting solution was determined spectrophotometrically using a Perkin Elmer model 55 UV spectrophotometer (Oak Brook, IL) at 310 nm. A standard curve was produced by dissolving a known amount of CDDP in DMF. The experiment was performed in triplicate. The drug content was calculated as a percentage of the total weight of the microspheres.

Particle Size Distribution

Dry microspheres (0.5 g) were sieved mechanically using a vibratory sieve shaker (CSC Scientific Co., Fairfax, VA). Four standard sieves having apertures ranging from 355 to 38 μm were mechanically shaken for 2 min. Size distribution curves were obtained by weighing the materials retained in each sieve.

In Vitro Release Studies

Thirty milligrams of microspheres (106- to 212-μm fraction) from each batch were incubated in 5 ml of phosphate-buffered saline (PBS, pH 7.4) at 37°C. At various times up to 22 days, tubes were centrifuged at 2500 rpm for 5 min and the supernatant drawn for UV analysis. Fresh PBS was added into the tubes after each measurement. Efforts were made to ensure that the incubation medium was not saturated by CDDP at each sampling point. The measurements were run in triplicate, and the amount of CDDP released was expressed as a percentage of accumulated drug in relation to the total drug loaded.

In order to examine the possible effect of various enzymes present in the blood on the CDDP release profiles, CDDP/PBLG microspheres were also incubated in dog plasma at 37°C. Two groups of microspheres were used in this study. Group 1 was microspheres prepared from high polymer concentration (10%, w/v) with 41% final drug load. Group 2 was microspheres prepared from low polymer concentration (2.5%, w/v) with 26% final drug load. The experimental procedures followed those performed in PBS solution. Since plasma samples could not be analyzed by UV measurement, the concentrations of released CDDP were determined by Atomic Absorption Spectrophotometer (AAS, Varian AA-1475 with Graphite Tube Atomizer, Palo Alto, CA). For sample preparation, the analyses were digested with hyamine hydroxide overnight at 60°C. Digested samples were then diluted 1:10 in 0.1 N HCl and analyzed for platinum content in a flameless AAS. A platinum standard series prepared in dog plasma was used to generate a standard curve.

Microscopy Examination

Surface characteristics of the microspheres were evaluated using a scanning electron microscope (Hitachi Model S520). For sample preparation, microspheres were placed on a 0.1 μm Nucleopore membrane, mounted onto stubs and sputter-coated with 200 Å gold-palladium (80:20) in a Hummer VI (Technics, Springfield, VA). To obtain 1 μm cross-section, the microspheres were embedded in EPON, cast in BEEM Embedding Capsules (Tedpella, Redding, CA) and cut on a microtome. Pictures of the cross-sections were taken on an Axiovert 405M inverted photomicroscope (Zeiss, Germany) equipped with a long distance condenser for differential-interference contrast.

RESULTS AND DISCUSSION

Formation of Plain PBLG Microspheres

For microspheres to be suitable for endovascular occlusion, they must have satisfactory morphologic properties, such as sphericity and individualization. To choose optimal processing conditions, plain PBLG microspheres were first prepared. PBLG is one of a few polyamino acids that are soluble in methylene chloride, making it particularly suitable for the formation of microspheres by the solvent evaporation technique. Although the concept of solvent evaporation is simple, characteristics of the final product are influenced by many variables. In this study, the effect of emulsifier and solvent removal rate on the properties of resulting product was examined. A number of stabilizing systems were tested, including Tween 80, polyvinylpyrrolidone and polyvinyl alcohol. In all cases, free-flowing powder was obtained after careful washing and subsequent drying. The rate of solvent removal, however, was found to have a significant effect on the morphologic appearance of the resulting microspheres. Microspheres prepared in an open beaker in a laminar hood were spherical and had smooth surfaces as revealed by scanning electron microscopy (Fig. 1a), but when the rate of methylene chloride removal was reduced by covering the beaker, the microspheres prepared were distorted and had pinholes on the surfaces (Fig. 1b). The embolization effectiveness of spherical and irregularly shaped particles has been compared by Wright *et al.* (21), who injected crosslinked dextran microspheres and polyvinyl alcohol particles into the splenic artery of dogs. A more homogeneous peripheral embolization was observed with the dextran microspheres, demonstrating the superiority of spherical particles. Based on the above observations, all experiments with CDDP loading were conducted in an open system using PVA (2%) as emulsifier.

Size Distribution Analysis

Microspheres ranging from 100- to 200-μm are considered to be optimal for tumor embolization. In order to control the particle size, the size distribution of CDDP-loaded PBLG microspheres was determined by differential sieving. Experiments illustrated in Figure 2a were all carried out with the same drug and polymer concentration (0.5 g CDDP, 0.5 g PBLG, 10 ml methylene chloride). When polymers of the same molecular weight were used (58K), increasing the stirring rate from 300 to 350 rpm decreased the fraction of 106- to 212-μm particles with a shift toward the recovery of more of the smaller-sized microspheres (38- to 106-μm). Decreasing polymer molecular weight from 58K to 20K while keeping the stirring rate constant (300 rpm) increased the recovery of the 106- to 212-μm fraction, mainly due to a more narrowed particle distribution (Fig. 2a). Nevertheless, changing polymer molecular weight and agitation rate did not appear to affect microsphere size distribution to a great

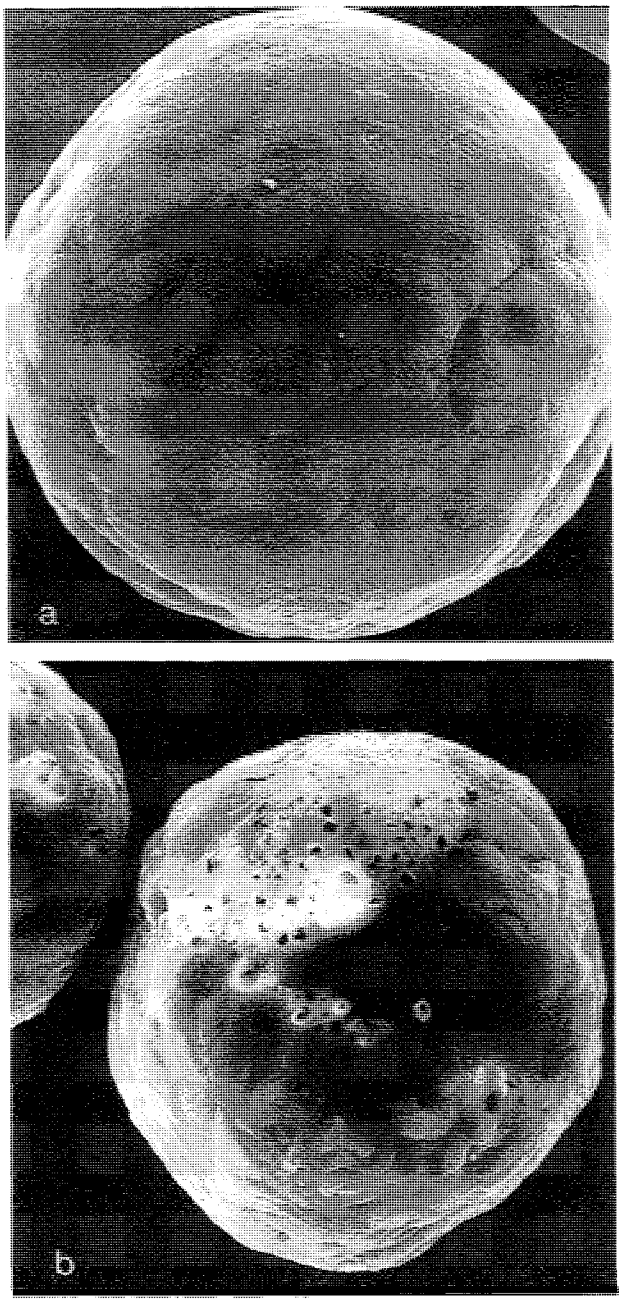
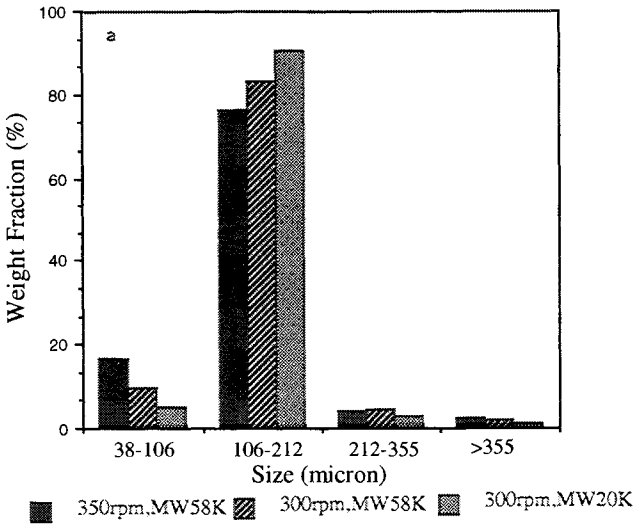


Fig. 1. Scanning electron micrograph of plain PBLG microspheres prepared, 1a: in an open system; 1b: in a closed system using 2% PVA as emulsifier (×1000).



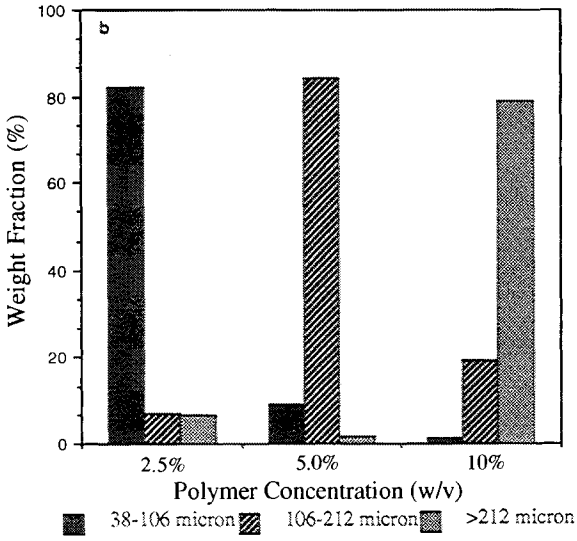


Fig. 2. Effect of agitation rate and polymer molecular weight on the size distribution of CDDP/PBLG microspheres (2a). Effect of polymer concentration on the size distribution of PBLG microspheres obtained at 300 rpm, with 58K polymer (2b).

degree. In all cases, 77% to 91% of the particles recovered were 106- to 212-μm. In contrast, varying the polymer concentration by changing the volume of methylene chloride used influenced the distribution pattern considerably. The major fraction recovered shifted from 38- to 106-μm to 106- to 212-μm to over 212 μm when the polymer concentration increased from 2.5% to 5% to 10% respectively (Fig. 2b). Thus, more concentrated polymer solutions produced microspheres with a larger average size. These results are consistent with another observation made on the CDDP/PLA system in which the difference in size distribution was explained by an increase in solvent viscosity (12).

Morphology of CDDP-Loaded Microspheres

To study the effect of polymer concentration on microsphere surface morphology, CDDP-loaded PBLG microspheres were prepared by increasing the methylene chloride volume and were examined by scanning electron microscope. Figure 3a shows a 44% loaded microsphere obtained by using a polymer concentration of 10% (w/v). The introduction of methylene chloride-insoluble CDDP crystals resulted in rough surfaces, although the microspheres remained spherical. No drug crystals were present on the microsphere surfaces; they were totally embedded in the polymer matrix (Fig. 3a). When the viscosity of the organic phase was reduced by decreasing the polymer concentration, a dramatic change in surface morphology was observed. As shown in Figure 3b, for microspheres prepared

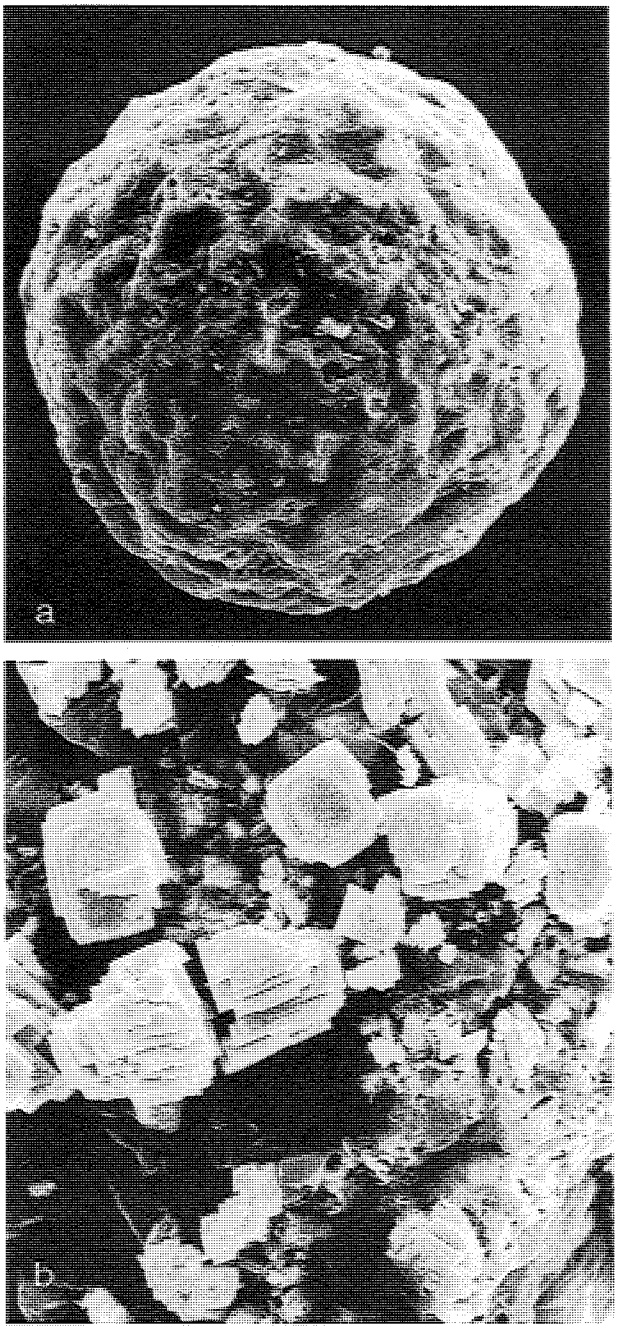


Fig. 3. Scanning electron microscope pictures of CDDP-loaded PBLG microspheres obtained with 0.5 g CDDP and 0.5 g PBLG, 3a: in 5 ml methylene chloride (×300); 3b: in 20 ml methylene chloride (×2000).

using a 2.5% (w/v) polymer concentration, CDDP crystals were found at the surface without polymer coverage.

The morphologic differences due to the different polymer concentration also corresponded to different patterns of drug distribution within the microspheres, as shown in cross-sections of CDDP/PBLG microspheres (Fig. 4). For microspheres prepared with the same initial drug to polymer ratio (0.5 g to 0.5 g), a high polymer concentration (10%, w/v) resulted in aggregation of drug crystals evenly distributed in the microsphere matrix (Fig. 4a); microspheres prepared with a lower polymer concentration (2.5%, w/v) had crystals of drug clustered near the outer surface (Fig. 4c). Indeed, the microspheres shown in Figure 4c are actually depleted of any drug crystals in the center of the matrix. In all cases, most of the crystals aggregated and formed channels in the microspheres, as clearly shown in Figure 4b.

The differences in surface morphology and drug distribution pattern with different polymer concentration could be ascribed to viscosity effects. During the stirring process, the CDDP crystals began to centrifuge out to the periphery of the microspheres. The use of higher polymer concentration and thus more viscous solution caused more resistance to the outward centrifugation force before evaporation and hardening, preventing the crystals from migrating further. Similar phenomena have been observed by Spenlehauer *et al.* in their studies on CDDP/PLA microspheres (13).

Because microspheres prepared at higher polymer concentrations did not have CDDP crystals present on the surfaces, they are favored for chemoembolization applications for the following reasons. First, CDDP crystals present on surface could potentially lead to a "burst effect" of CDDP release, which would cause a higher concentration of in the plasma and, subsequently, could lead to harmful side effects. Secondly, CDDP in direct contact with blood vessels could produce severe damage to the surrounding arterial wall. Removal of surface-associated CDDP would make CDDP/PBLG microspheres less likely to produce undesired side effects.

Drug Loading

Processing conditions and experimental loading yields are given in Table 1. The efficiency of drug loading is influenced by the viscosity of the organic phase. A slight increase of polymer concentration from 2.0% to 2.7% (w/v) resulted in an increase of incorporation efficiency from 65% to 87% (Table 1, batches 1 and 2; amount of drug 0.8 g). This trend is also noted in the next series of microspheres in which CDDP to PBLG ratio was 1:1 (Table 1, batches 3 to 6). The final drug load increased from 21.5% to 44.0% (w/w). Clearly, the effect of viscosity, which was responsible for the morphologic differences, was the major cause of the observed differences in drug loading. With lower viscosity of the organic phase, more crystals would migrate toward the outer surfaces, facilitating the dissolution of CDDP in the surrounding aqueous medium.

Initial drug load and final drug load are defined as the weight percentage of drug in the total weight of drug and polymer mixture. Incorporation efficiency is defined as percentage of final drug load to initial drug load, which is a quantity measuring the drug yield during microsphere preparation. It should be pointed out that the final drug load depends on both initial drug load and incorporation efficiency. In our study, microspheres containing up to 57.7% of CDDP (w/w) could be easily obtained with a CDDP to PBLG ratio of 2:1 at a polymer concentration of 2.7% (w/v) (Table 1, batch 2).

Drug Release Studies

The kinetics of drug release from the emboli is of major importance, because it determines the bioavailability of the

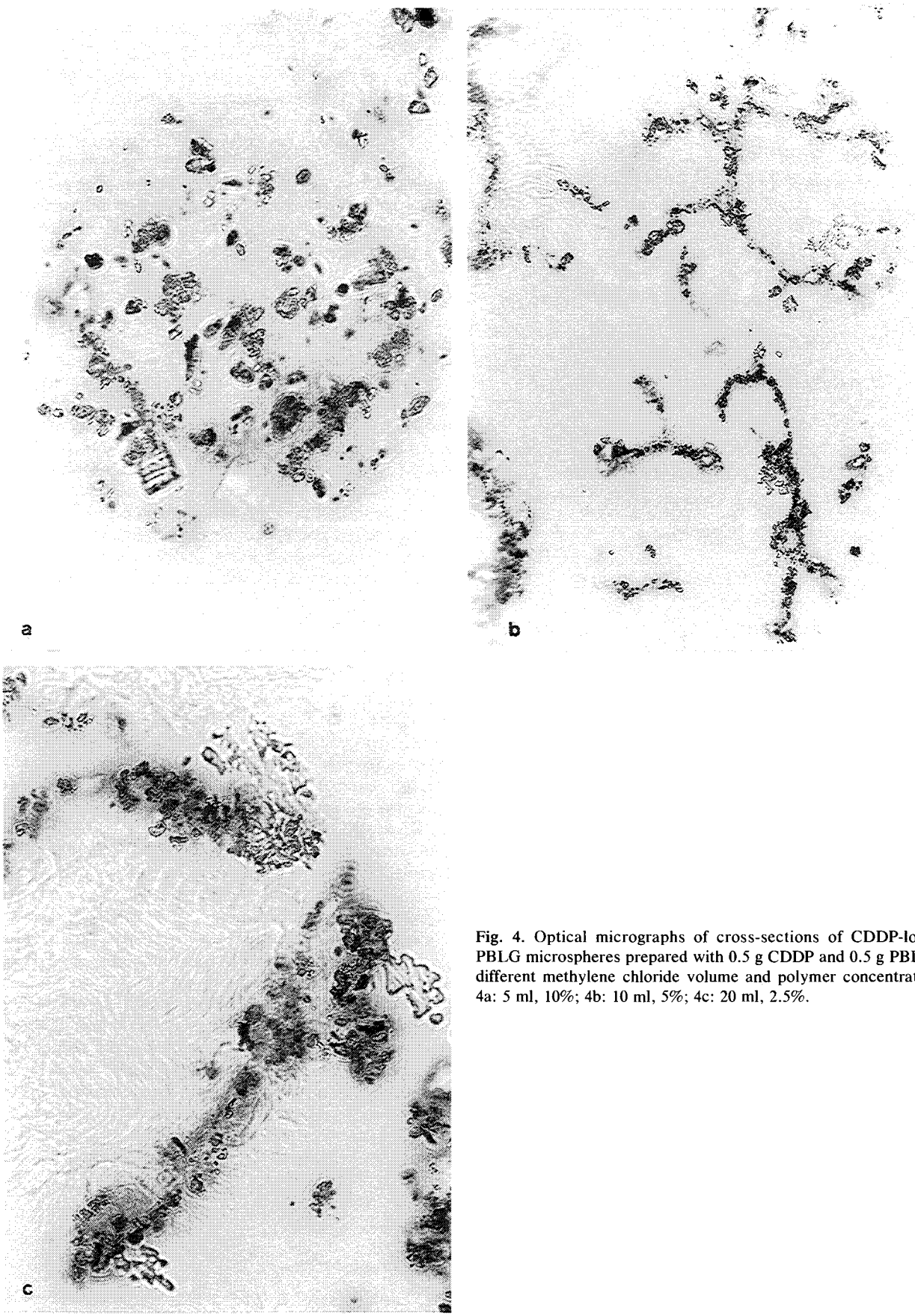


Fig. 4. Optical micrographs of cross-sections of CDDP-loaded PBLG microspheres prepared with 0.5 g CDDP and 0.5 g PBLG at different methylene chloride volume and polymer concentrations. 4a: 5 ml, 10%; 4b: 10 ml, 5%; 4c: 20 ml, 2.5%.

Table I. Processing Conditions and Experimental Loading Yields

Batch no.	Amount of CDDP (g)	Amount of PBLG (g)	CDDP: PBLG ratio	Amount of $MeCl_2$ (ml)	Polymer concentration (%, w/v)	Initial drug load (%, w/w)	Final drug load (%, w/w)	Incorporation efficiency (%)
1	0.8	0.4	2:1	20	2	66.7	43.4	65
2	0.8	0.4	2:1	15	2.7	66.7	57.7	87
3	0.5	0.5	1:1	20	2.5	50	21.5	43
4	0.5	0.5	1:1	15	3.3	50	32.5	65
5	0.5	0.5	1:1	10	5	50	34.3	69
6	0.5	0.5	1:1	5	10	50	44.0	88
7	0.33	0.5	1:1.5	10	5	40	29.2	73

drug and its antitumor effect. In this study, the release of CDDP from PBLG microspheres with various loading was documented. Figure 5 indicates that higher loading due to increased viscosity of the organic phase caused slower drug release. Microspheres with a 21.5% drug load (polymer concentration 2.5% [w/v]) exhibited a rapid, progressive release and quickly reached a plateau at 24 hrs. At 44.0% loading (polymer concentration, 10%), the microspheres released CDDP in a slower fashion and did not plateau until after 96 hrs (Fig. 5). The difference between the two release rates is especially striking during the first three hours. The less-loaded microspheres (21.5%) released 46.3% of their load within the first three hours of being introduced to the PBS, whereas those bearing 44.0% load lost only 9.8% of their drug load under the same conditions. This observation could be related to the distribution pattern of the CDDP crystals within the polymer matrix. At lower drug loading, the CDDP crystals were present in the outer periphery of the microspheres (Fig. 4c). Thus a large amount of drug is in close contact with the aqueous medium which contributes to the rapid release of CDDP.

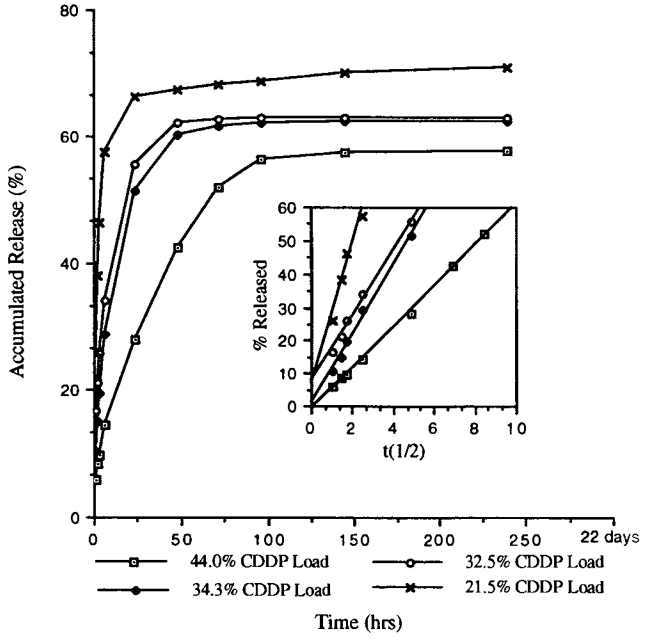
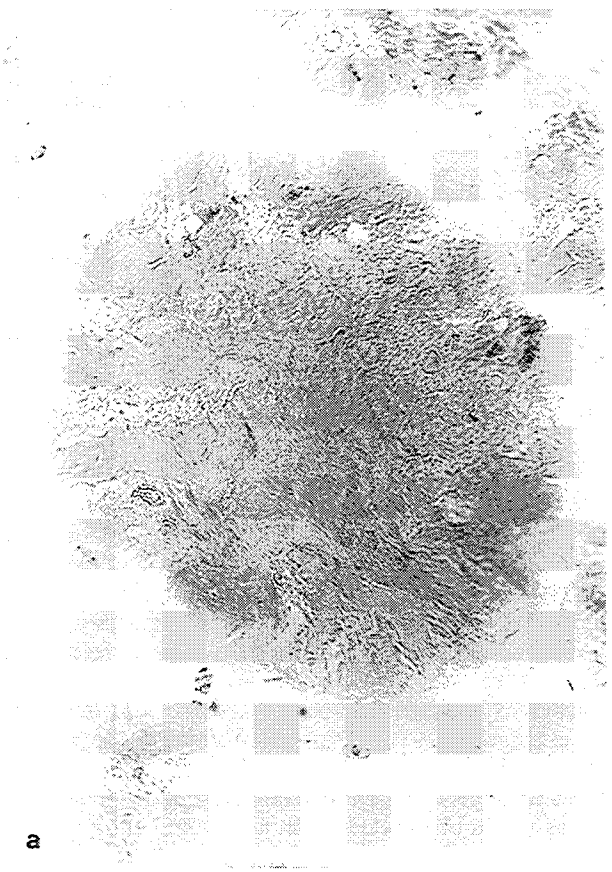


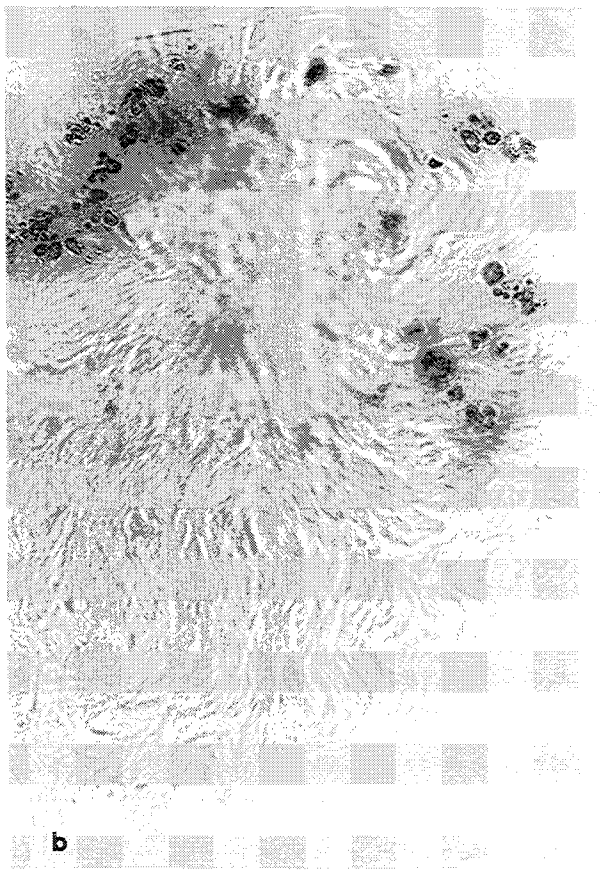
Fig. 5. Effect of drug loading after viscosity changes in the organic phase on the CDDP release from CDDP/PBLG microspheres. CDDP:PBLG = 0.5 g:0.5 g. Insert: % Release as a function of square-root-time.

In a study conducted on CDDP/PLA microspheres prepared by a comparable solvent evaporation technique, Spenlehauer *et al.* showed a very different result (13). For microspheres with an 18% drug load, less than 15% of the CDDP was released after 48 hrs, whereas microspheres with 41% loading released all their drug within the first day. In this case, higher drug loading corresponded to faster release. Although that result contradicts our result, both can be readily explained by the morphologic characteristics of the microspheres. Since the CDDP/PLA microspheres were prepared in an extra CDDP-containing-continuous phase, they had various amounts of CDDP crystals present at the surfaces. Thus as would be expected with higher drug loading, more crystal networks were formed and connected to the crystals located at the surfaces of the microspheres. These networks allow penetration of the dissolution medium from one crystal site to the other without any limitation by a polymer barrier, leading to more rapid release (13). In contrast, CDDP/PBLG microspheres were prepared in a CDDP-free-continuous phase; the amount of crystals present at the surfaces was solely a function of the processing conditions, i.e. polymer concentration. As mentioned before, higher loading translated to fewer crystals in the outer part of the microspheres, resulting in a slower release rate.

Cross-sections of microspheres with 21.5% loading at the end of the release studies (22 days) showed no CDDP crystals in the polymer matrix, confirming the complete release of the encapsulated drugs (Fig. 6a). Microspheres with higher drug loads still had a few crystals present in the matrix after the same time period (Fig. 6b). The fact that the microspheres with higher loads plateaued at a lower percentage of accumulated release (Fig. 5) can thus be explained by the presence of these remaining crystals, which are apparently isolated from other crystal networks. Leaching out of these crystals via a diffusion process is practically very slow. For microspheres with 21.5% loading, the percentage release only reached 70% at the end of the study although complete release of CDDP had been indicated. This result could be attributed to the analytical method used. UV measurement seemed to underestimate the CDDP concentration, since in a parallel release study conducted in plasma, over 90% CDDP released were recovered in a period of 5 days using atomic absorption method (see below).

The release profiles shown in Figure 5 appeared to fit a





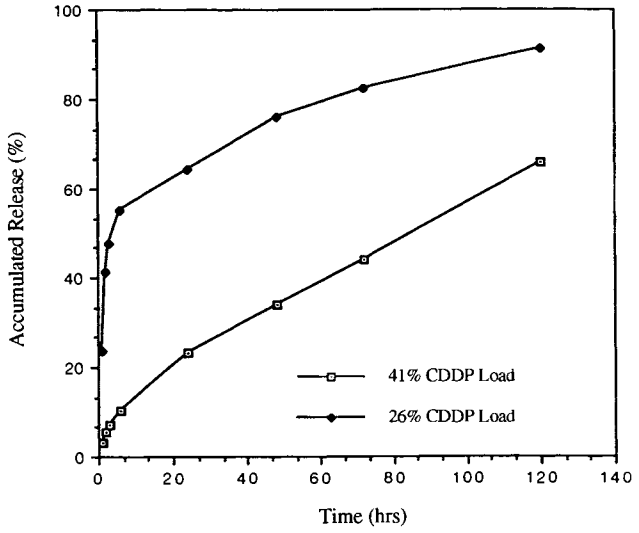


Fig. 7. Release of CDDP from CDDP/PBLG microspheres in dog plasma at 37°C.

$t^{1/2}$ expression in the first 60% of the total released drug (Fig. 5, insert). This result disagreed with a simple diffusion controlled release mechanism in which the fractional drug released can be described by equation $Mt/M \infty = kt^n$ where n has the value of 0.43 for release from spheres (22). The release of CDDP from PBLG, however, can be described by a matrix system in which the drug crystals form continuous channels (23).

CDDP release from CDDP/PBLG system was also studied in dog plasma. Microspheres of low drug load (prepared at low polymer concentration, 2.5% [w/v]) released 47.8% CDDP within the first 3 hrs. In contrast, microspheres of high drug load (prepared at high polymer concentration, 10% [w/v]) only released 7.2% CDDP in the same time period (Fig. 7). These results were comparable with those obtained in PBS solution, confirming previous finding that microspheres with higher drug load did not show "burst effect" and released CDDP in a sustained manner. They also suggested that the presence of various enzymes did not have significant effect on the degradation of PBLG polymer and CDDP release profile.

An initial fast efflux of drug is usually associated with high drug loading. Such a 'burst effect' should be avoided in dosage formulation because of possible side effects caused by higher drug concentration in the plasma. When a biodegradable polymer such as PLA is used, a massive release of CDDP due to the sudden collapsing of the polymer matrix could potentially impose the same problem (14). In the CDDP/PBLG system, an initial fast release process is suppressed at higher loading, resulting in a smoother and more sustained release of CDDP. In addition, the CDDP release profile is more predictable with CDDP/PBLG microspheres because there is no complication caused by degradation controlled release. Since the effectiveness of any chemotherapy

Fig. 6. Optical micrographs of cross-sections of CDDP-loaded PBLG microspheres retrieved at the end of release studies (22 days). 6a: microspheres with 21.5% CDDP load; 6b: microspheres with 32.5% CDDP load.

is mainly determined by a combination of the duration of drug action and the local drug concentration, these characteristics of the CDDP/PBLG system make it a potential candidate for use in chemoembolization.

CONCLUSION

Poly(benzyl l-glutamate) microspheres containing cisplatin suitable for chemoembolization applications were prepared and characterized. It was found that the polymer concentration of organic phase played a key role in determining the drug incorporation efficiency, drug distribution within polymer and *in vitro* release profiles. Prolonged release of cisplatin was achieved at high polymer concentration (10% [w/v]). Work on the evaluation of these microspheres for their effectiveness in treating hepatic tumors in tumor-bearing animal model is in progress.

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REFERENCES

1. B. Rosenberg. Fundamental studies with cisplatin. *Cancer.* 55:2303–2316 (1985).
2. S. Wallace, C. H. Carrasco, C. Charnsangavej, W. R. Richli, K. Wright, and C. Gianturco. Hepatic artery infusion and chemoembolization in the management of liver metastasis. *Cardiovasc. Intervent. Radiol.* 13:153–160 (1990).
3. Y. Sasaki, S. Imaoka, H. Kasugai, M. Fujita, S. Kawamoto, S. Ishiguro, J. Kojima, O. Ishikawa, H. Ohigashi, H. Furukawa, H. Koyama, and T. Iwanaga. A new approach to chemoembolization therapy for hepatoma using ethiodized oil, cisplatin, and gelatin sponge. *Cancer.* 60:1194–1203 (1987).
4. F. M. Balis, J. S. Hoicenberg, and W. A. Bleyer. Clinical pharmacokinetics of commonly used anticancer drugs. *Clinical Pharmacokinetics.* 8:202–232 (1983).
5. T. Kato, R. Nemoto, H. Mori, M. Takahashi, and M. Harada. Arterial chemoembolization with mitomycin C microcapsules in the treatment of primary and secondary carcinoma of the kidney, liver, bone and intrapelvic organs. *Cancer.* 48:674–680 (1981).
6. S. Fujimoto, M. Miyazaki, F. Endoh, O. Takahashi, K. Okui, and Y. Morimoto. Biodegradable mitomycin C microspheres given intra-arterially for inoperable hepatic cancer: with particular reference to a comparison with continuous infusion of mitomycin C and 5-fluorouracil. *Cancer.* 56:2404–2410 (1985).
7. M. Anderson, K. F. Aronsen, C. Balch, L. Domellof, S. Eksborg, L. O. Hafstrom, S. B. Howell, R. Karesen, J. Midander, and K. Teder. Pharmacokinetics of intra-arterial mitomycin C with or without degradable starch microspheres (DSM) in the treatment of non-resectable liver cancer. *Acta Oncologica.* 28:219–222 (1989).
8. T. Ichihara, K. Sakamoto, K. Mori, and M. Akagi. Transcatheter arterial chemoembolization therapy for hepatocellular carcinoma using polylactic acid microspheres containing aclarubicin hydrochloride. *Cancer Research.* 49:4357–4362 (1989).
9. D. J. Kerr, N. Willmott, J. H. McKillop, J. Cummings, H. J. Lewi, and C. S. McArdle. Target organ disposition and plasma pharmacokinetics of doxorubicin incorporated into albumin microspheres after intrarenal arterial administration. *Cancer.* 62:878–883 (1988).
10. S. Benita, O. Zouai, and J. P. Benoit. 5-Fluorouracil: carnauba wax microspheres for chemoembolization: an in vitro evaluation. *J. Pharm. Sci.* 75:847–851 (1986).
11. Y. Okamoto, A. Konno, K. Togawa, T. Kato, and Y. Amano. Microcapsule chemoembolization for head and neck cancer. *Arch. Otorhinolaryngol.* 242:105–111 (1985).
12. G. Spenlehauer, M. Veillard, and J. P. Benoit. Formation and characterization of cisplatin loaded poly(d,l-lactide microspheres for chemoembolization. *J. Pharm. Sci.* 75:750–755 (1986).
13. G. Spenlehauer, M. Vert, J. P. Benoit, F. Chabot, and M. Veillard. Biodegradable cisplatin microspheres prepared by the solvent evaporation method: morphology and release characteristics. *J. Controlled Release.* 7:217–229 (1988).
14. J. M. Anderson, D. F. Gibbons, R. L. Martin, A. Hiltner, and R. Woods. The potential for poly-α-amino acids as biomaterials. *J. Biomed. Mater. Res. Sym.* 5(1):197–207 (1974).
15. K. R. Sidman, W. D. Steber, A. D. Schwope, and G. R. Schnaper. Controlled release of macromolecules and pharmaceuticals from synthetic polypeptides based on glutamic acid. *Biopolymers.* 22:547–556 (1983).
16. C. Li, D. J. Yang, L. R. Kuang, and S. Wallace. Polyamino acid microspheres: preparation and distribution after intravenous injection in rats. *Int. J. Pharm.* 94:143–152 (1993).
17. R. Eloy, A. Brack, N. Dorme, and A. M. Cornillac. Physical and biological properties of a new synthetic amino acid copolymer used as wound dressing. *J. Biomed. Mater. Res.* 26:695–712 (1992).
18. F. Lescure, R. Gurny, E. Doelker, and J. M. Anderson. Long-term histopathological study of new polypeptidic biomaterials. *Biomaterials.* 13:1009–1011 (1992).
19. H. R. Dickinson and A. Hiltner. Biodegradation of a poly-a-amino acid hydrogel. II. in vitro. *J. Biomed. Mater. Res.* 15:591–603 (1981).
20a. C. S. Cho and S. U. Kim. In vitro degradation of poly(γ-benzyl l-glutamate)/poly(ethylene glycol) copolymers. *J. Controlled Release.* 7:283–286 (1988).
20b. H. Block. *Poly(g-benzyl l-glutamate) and other Glutamic Acid Containing Polymers.* Gordon & Breach, New York, 1983.
21. K. C. Wright, J. H. Anderson, C. Gianturco, S. Wallace, and V. P. Chuang. Partial splenic embolization using polyvinyl alcohol foam, dextran, polystyrene or silicone. *Radiology.* 142:351–354 (1982).
22. P. L. Ritger and N. A. Peppas. A simple equation for description of solute release. 1. Fickian and non-fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. *J. Controlled Release.* 5:23–36 (1987).
23. R. W. Baker. *Controlled Release of Biologically Active Agents.* John Wiley & Sons, New York, 1987, pp. 56–73.