Dissolution, Stability, and Morphological Properties of Conventional and Multiphase Poly(DL-Lactic-Co-Glycolic Acid) Microspheres Containing Water-Soluble Compounds

Motokazu Iwata[1,2] and James W. McGinity[1,3]

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Multiphase microspheres of poly(DL-lactic-co-glycolic acid) (PLGA) containing water-soluble compounds were prepared by a multiple-emulsion solvent evaporation technique. These compounds were dissolved in the aqueous phase of a W/O emulsion with soybean oil as the oil phase. This emulsion was dispersed throughout the matrix of the microsphere. The morphological properties of the multiphase microspheres during *in vitro* dissolution studies were compared to those of conventional microspheres prepared from the same polymer. Drug release from the multiphase microspheres was characterized by an initial uniform release for the first 20 days followed by a more rapid phase of drug release. Chlorpheniramine maleate (CPM) and brilliant blue (BB) were the soluble model compounds investigated. The release rates of these agents from the multiphase microspheres were independent of the drug content in the microspheres. The release profiles from the conventional microspheres showed a lag time of 10 and 16 days for the CPM and BB, respectively. The dissolution rate of the model soluble compounds from the conventional microspheres increased as the loading in the microspheres increased. No differences in the degradation rate of the PLGA from the multiphase and the conventional microspheres were seen during the dissolution studies.

KEY WORDS: poly(lactic-co-glycolic acid); multiphase microspheres; water-soluble compounds; *in vitro* dissolution; morphology; biodegradable drug delivery systems.

INTRODUCTION

Poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) have been studied extensively as polymeric carriers for biodegradable microspheres. The properties of the drug-containing microspheres have been reviewed and studied by many authors (1–6). The solvent evaporation method using methylene chloride/water or similar immiscible solvents has been the method of choice to prepare these microspheres. For this method, the drug and polymer are dissolved in the organic solvent. Following the addition and dispersion of the organic solution in the aqueous phase, the evaporation of the solvent results in the formation of drug-containing microspheres. Because of partitioning effects, however, low drug loading efficiencies are generally obtained with water-soluble compounds (7). Bodmeier and McGinity (8) increased the loading efficiency of quinidine sulfate by increasing the pH of the external aqueous phase that was used in the solvent evaporation process.

An anhydrous emulsion system for the microencapsulation of barbiturates was reported by Jalil and Nixon using poly(L-lactic acid) as the polymeric carrier (9,10). Acetonitrile was used as the solvent ("W" phase) for the poly(L-lactic acid) and the drug, and light mineral oil was used as the continuous dispersion medium (O phase). In these microspheres, the drug particles were dispersed in the polymeric matrix. Recently, Cohen and co-workers emulsified an aqueous solution of water-soluble proteins in methylene chloride and PLGA prior to a second emulsification step in water (11). The structures of certain proteins may be disrupted in the presence of organic solvents (12). Atriopeptin III was recently reported by Johnson and co-workers to be denatured if placed in contact with PLA or PLGA (13).

In previous reports by the authors (14,15), a method to prepare multiphase microspheres for incorporating water-soluble compounds into biodegradable polymers was described. Drug loading efficiencies greater than 90% were obtained from these systems. For drugs or proteins that are unstable in aqueous media, the elimination of water from the system and dispersing the compound in the soybean oil has resulted in similar high levels of drug loading (15). The objectives of the present investigation were to study the dissolution, stability, and morphological properties of biodegradable microspheres containing chlorpheniramine maleate and brilliant blue as model soluble compounds. The properties of multiphase microspheres were compared to drug-containing conventional microspheres prepared from acetonitrile/mineral oil media.

MATERIALS AND METHODS

Materials

Poly(DL-lactic-co-glycolic acid) (MW 57,000; lactic acid/glycolic acid, 50/50) (Birmingham Polymers Inc., Birmingham, AL), purified gelatin (Fisher Scientific Co., Fair Lawn, NJ), brilliant blue (Allied Chemical Co., New York), DL-chlorpheniramine maleate (Sigma Chemical Co., St. Louis, MO), soybean oil (USP) and aluminum monostearate (USP/NF) (Spectrum Chemical Mfg. Co., Gardena, CA), and sorbitan monooleate (Span 80) and polyoxyethylene sorbitan monooleate (Tween 80) (ICI Americas Inc., Wilmington, DE) were used.

Preparation of Microspheres

Multiphase microspheres of PLGA containing brilliant blue (BB) and chlorpheniramine maleate (CPM) were prepared using a multiple emulsion solvent evaporation process as previously described by Iwata and McGinity (14,15). An aqueous drug solution was emulsified into soybean oil containing aluminum monostearate and Span 80. The resulting W/O emulsion was dispersed in a PLGA–acetonitrile solu-

[1] College of Pharmacy, The University of Texas at Austin, Austin, Texas 78712-1074.

[2] Present address: Department of Pharmaceutical Technology, Dainippon Pharmaceutical Co., Ltd. 1-5-51, Ebie, Fukushima-ku, Osaka 553, Japan.

[3] To whom correspondence should be addressed.

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tion to form a W/O emulsion-in-acetonitrile (W/O/"W") emulsion. The W/O/"W" emulsion was dispersed in light mineral oil to form a W/O/"W"/O multiple emulsion and agitated for 24 hr to evaporate and remove the acetonitrile. The hardened microspheres were filtered and washed with *n*-hexane and water. For further solvent removal, the microspheres were placed under reduced pressure for 48 hr. The conventional PLGA microspheres containing the drugs were prepared by an acetonitrile-in-oil ("W"/O) emulsion solvent evaporation technique which was described by Jalil and Nixon (9,10). The drugs and the PLGA were dissolved in the acetonitrile ("W" phase). Light mineral oil was used as the external medium (O phase). These conventional microspheres belong to the class of matrix-type drug delivery systems in which fine drug particles are homogeneously dispersed in the microspheres.

Determination of Drug Content in the Microspheres

The multiphase and the conventional microspheres were dissolved in methylene chloride and the brilliant blue and CPM were extracted from these microspheres with distilled water and 0.01 *N* HCl, respectively. After sufficient agitation and partitioning time, the aqueous solutions containing the extracted dye or drug were clarified by centrifugation at 2000 rpm for 20 min. The drug content of each sample was evaluated using UV-visible spectroscopy.

Scanning Electron Microscopy (SEM)

Samples of microspheres were coated with gold–palladium for 70 sec under an argon atmosphere using a cold sputter module in a high-vacuum evaporator equipped with an omni-rotary stage. Samples were examined with a JEOL Model 35 scanning electron microscope at 25 kV. Cross sections of the conventional and multiphase microspheres were obtained by imbedding the particles in a gelatin block. The microspheres were added to a warm 10% (w/v) gelatin solution containing 1% (w/v) glycerin as a plasticizer. The gelatin dispersion was dried under high vacuum for 24 hr, and strips of gelatin, of 1 mm thickness, containing the sliced microspheres were examined by SEM.

In Vitro Dissolution Studies

The multiphase and the conventional microspheres (100–300 mg) were placed in 25 mL of 0.01 *M* phosphate-buffered saline (PBS) (pH 7.4) contained in a 30-mL test tube. The microspheres in the PBS medium were agitated using a rotating-bottle apparatus (20 rpm rotating end-over-end) in a water bath maintained at 37°C. Samples (5 mL) were withdrawn at appropriate time intervals using a glass syringe fitted with a nylon screen (50 μm) held in a polyethylene housing. The supernatant of the dissolution medium was withdrawn following sedimentation of the microspheres in the dissolution vial after standing for 10 to 15 min. The dissolution medium (7 to 8 mL) was withdrawn with a glass syringe through the nylon screen. The excess sample volume (2 to 3 mL) was pushed back into the dissolution vial to adjust the sample volume to 5 mL. The screen was further flushed with replenishing medium (5 mL) into the dissolution vial. The loss of microspheres during the sampling procedure was found to be negligible.

PLGA Molecular Weight Determination by Gel Permeation Chromatography (GPC)

The molecular weight (M_w) of the PLGA was determined by GPC in tetrahydrofuran using a set of three Ultrastyragel columns (Waters) with nominal pore size of 10^3, 10^2, and 10 nm and a flow rate of 1 mL/min. The molecular weights were evaluated by elusion volume against polystyrene standards (Polysciences, Inc.).

RESULTS AND DISCUSSION

The *in vitro* dissolution properties of brilliant blue from conventional and multiphase microspheres were studied in 0.01 *M* phosphate buffered saline (PBS) medium at pH 7.4, and the profiles appear in Fig. 1. As can be seen in Fig. 1a, the conventional PLGA microspheres containing the dye demonstrated a lag time of approximately 16 days for the formulations examined. During this lag time period, the dissolution medium penetrated, hydrated, and plasticized the polymer prior to the release of the dye from the microspheres. Ikada and co-workers have recently demonstrated that the wetting phenomenon was completed during the early stages of the lag time (16). Therefore, polymer degradation and the formation of pores in the polymer matrix were necessary before drug release could be initiated. A rapid release of the dye from the microspheres followed during the next 14 days. For very low drug loadings, which would be typical for potent peptide or protein drugs, the rate of drug release from conventional matrix-type microspheres was found to be dependent on the content of agent in the microspheres. These results are in agreement with other published reports where release rates of drug dispersed in the microspheres increased as the ratio of drug:polymer increased (8,17,18).

A two-stage dissolution profile was also seen with the multiphase microspheres containing various loadings of brilliant blue (Fig. 1b). However, no lag time was evident for the dissolution process with these microspheres. Slow and relatively linear dissolution profiles were obtained during the first 20 days, at which time approximately 30% of the dye had been released. The second phase of these profiles was nonlinear and a more rapid release of the dye from the microspheres was seen. Similar results to those with the dye were obtained with both the conventional and the multiphase microspheres containing chlorpheniramine maleate (Fig. 2). The dissolution profiles in Figs. 1 and 2 represent the average of at least duplicate runs on the same batch of microspheres. The lag time for the conventional microspheres for the four formulations containing CPM was approximately 10 days. A second linear phase lasting from 7 to 10 days was present before the burst effect. The shorter lag time for the CPM microspheres was due to the higher level of drug loading than dye loading (Fig. 1A) into the conventional microspheres. The increase in dissolution rate after day 20 for both soluble compounds in the multiphase microspheres resulted from an increase in porosity and decrease in tortuosity in the matrix of the microspheres. The dissolution profiles of both model compounds from the multiphase microspheres was independent of the amount of drug dissolved in the aqueous

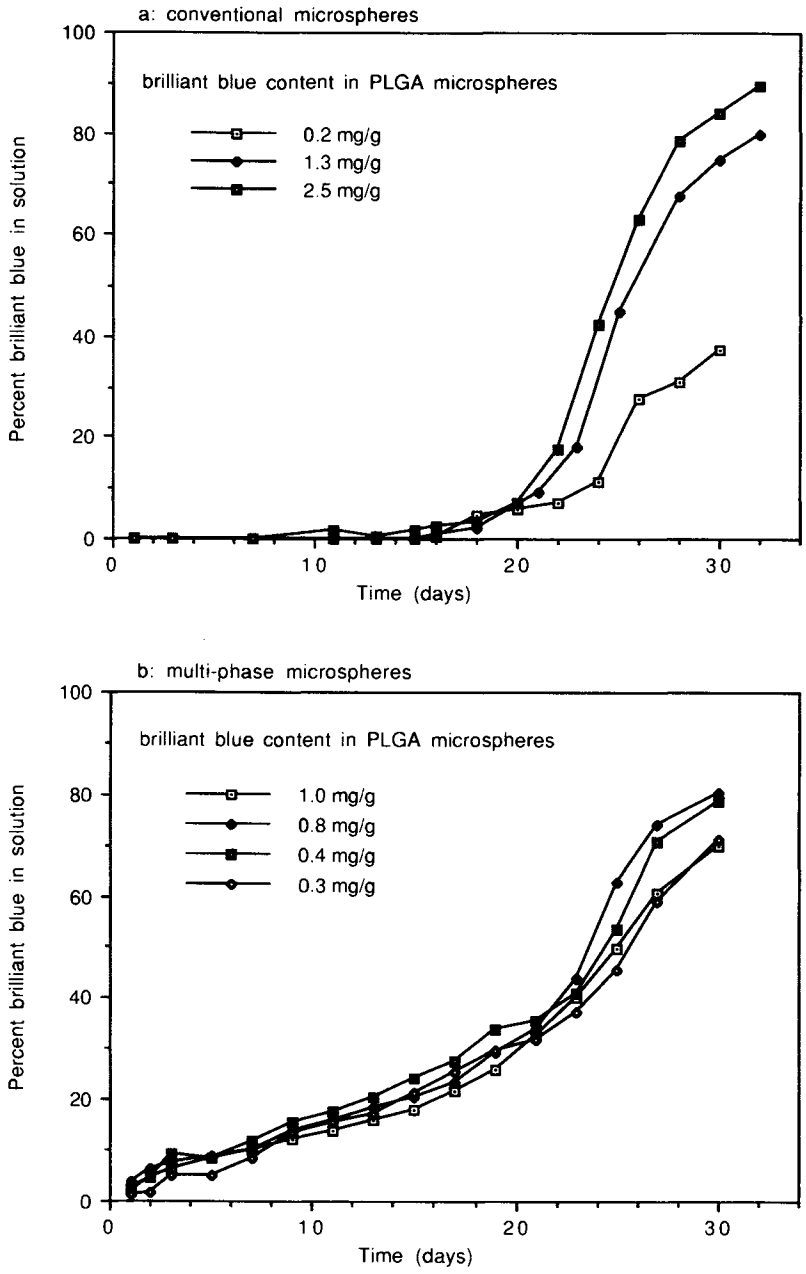

Fig. 1. *In vitro* dissolution profiles of brilliant blue from conventional (a) and multiphase (b) PLGA microspheres containing various amounts of the dye, in phosphate-buffered saline (pH 7.4), maintained at 37°C.

phase of the W/O emulsion that was dispersed in the multiphase microspheres. During the dissolution process, the emulsion droplets containing the model soluble agents gradually diffused through pores or cracks in the polymer following the degradation and hydrolysis of the polymer. Drug diffusion from the microspheres also occurred by water penetration into the emulsion droplets to dissolve the soluble compound and promote release through the porous matrix.

The conventional and multiphase microspheres were suspended in hardened gelatin and cross-sectional samples were prepared for SEM evaluation. The scanning electron micrographs in Fig. 3 represent cross sections of conventional microspheres containing brilliant blue after the microspheres were exposed to various time periods in the dissolution test media. Small pores and concentric lamella-like structures appeared in the microspheres after 1 week in the PBS medium (Fig. 3b). Erosion of the internal polymer wall had developed after 2 weeks, resulting in a porous sponge-like matix structure (Fig. 3c). Significant polymer degradation had occurred in the microspheres after 4 weeks as shown in Fig. 3d. This hydrolytic breakdown of the polymers and increase in the porosity of the microspheres resulted in over 90% of the dye being removed from the microspheres after 4 weeks.

Scanning electron micrographs of the multiphase microspheres before dissolution and following 1, 2, and 4 weeks of

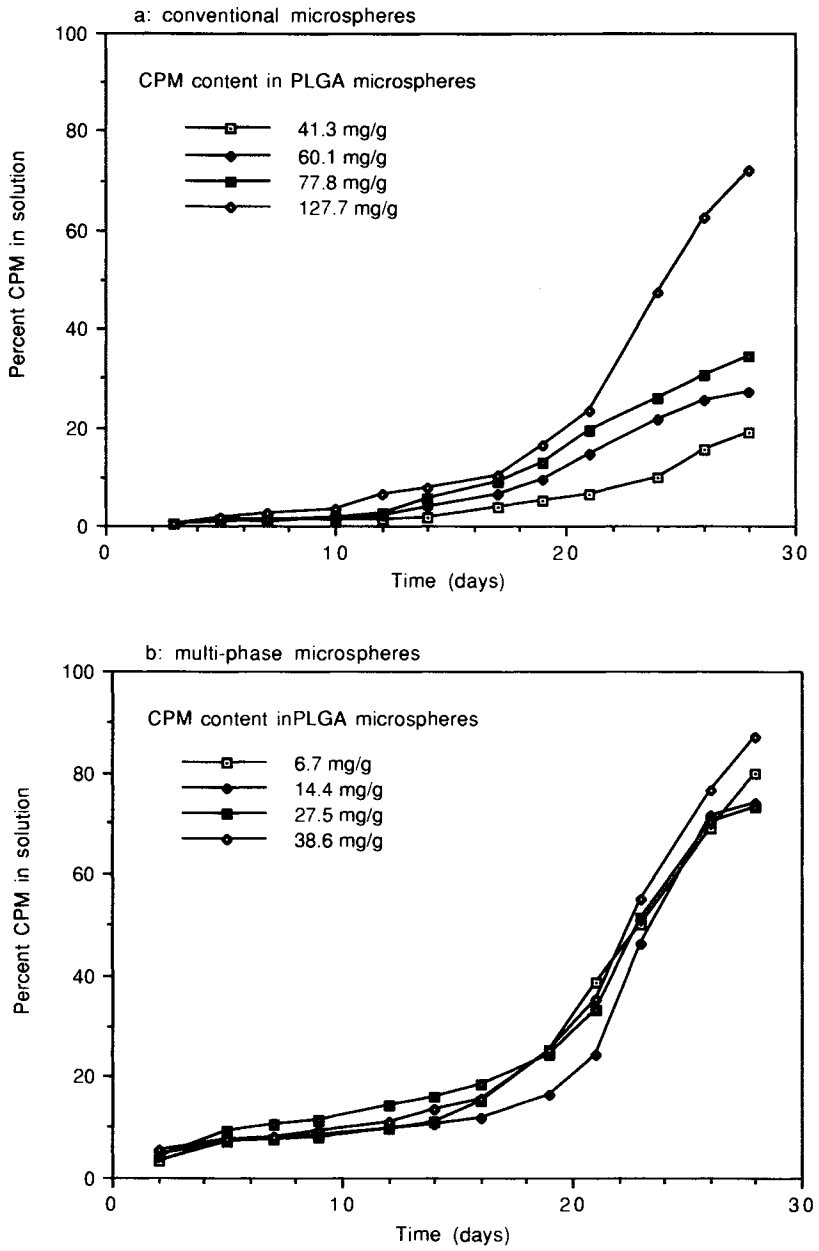

Fig. 2. *In vitro* dissolution profiles of chlorpheniramine maleate from conventional (a) and multiphase (b) PLGA microspheres containing various amounts of the drug in phosphate-buffered saline (pH 7.4), maintained at 37°C.

exposure to the media are shown in Fig. 4. The lamella structures that were found in the conventional microspheres were not visible. Fragmented gelatin in the cross sections are evident in Fig. 4a. Many pores appeared in the polymeric wall of the microspheres after two weeks, forming porous structures. These pores enlarged because of erosion and connected with each other to form aqueous channels through which drug could diffuse. The emulsion globules that were removed by vacuum during the SEM sample preparation are clearly demonstrated as small craters in the cross sections of these particles. The dark color of the dye can be seen on the surface of the microspheres in the optical photographs in Fig. 5, where multiphase microspheres containing brilliant blue have been exposed to the dissolution medium for 1 week. The clear regions surrounding the microspheres were gelatin fragments from the mounting procedure. The arrows in the photograph indicate fissures or areas of erosion caused by hydration and swelling of the polymer through which the emulsion diffused.

The surface morphological features of the multiphase microspheres prior to dissolution testing are shown in Fig. 6. The depressions found in the surface of many of the microspheres were created following their manufacture, during the vacuum drying of the microspheres to remove the residual solvents. The particle size of the microspheres varied from 50 to 500 μm, with a mean diameter of approximately 275 μm. Since the emulsion droplets were randomly distributed throughout the matrix of the multiphase microspheres, the

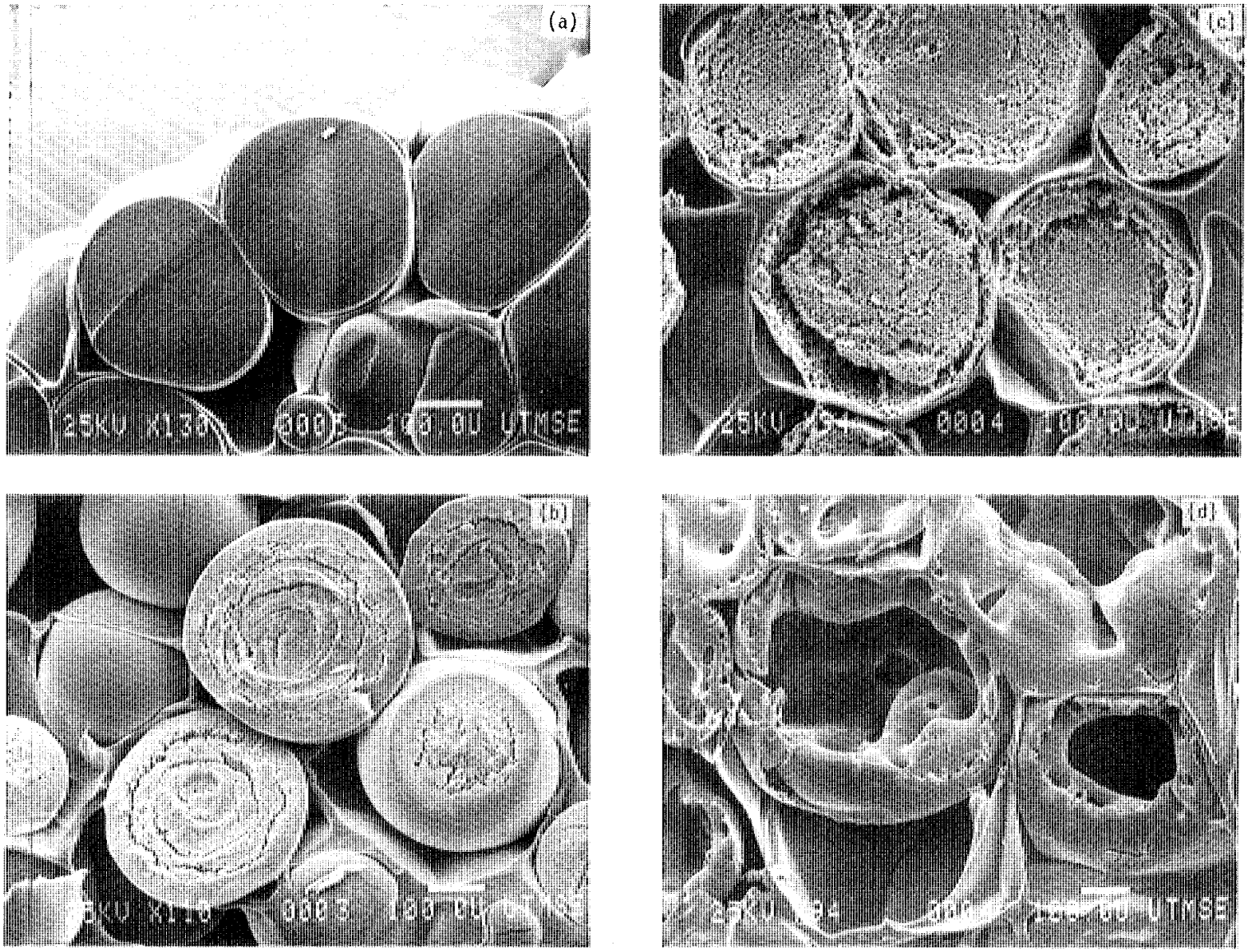

Fig. 3. Morphological properties of cross sections of conventional PLGA microspheres containing brilliant blue during an *in vitro* dissolution test. The samples [initial (a)] were observed after 1 week (b), 2 weeks (c), and 4 weeks (d) by scanning electron microscopy.

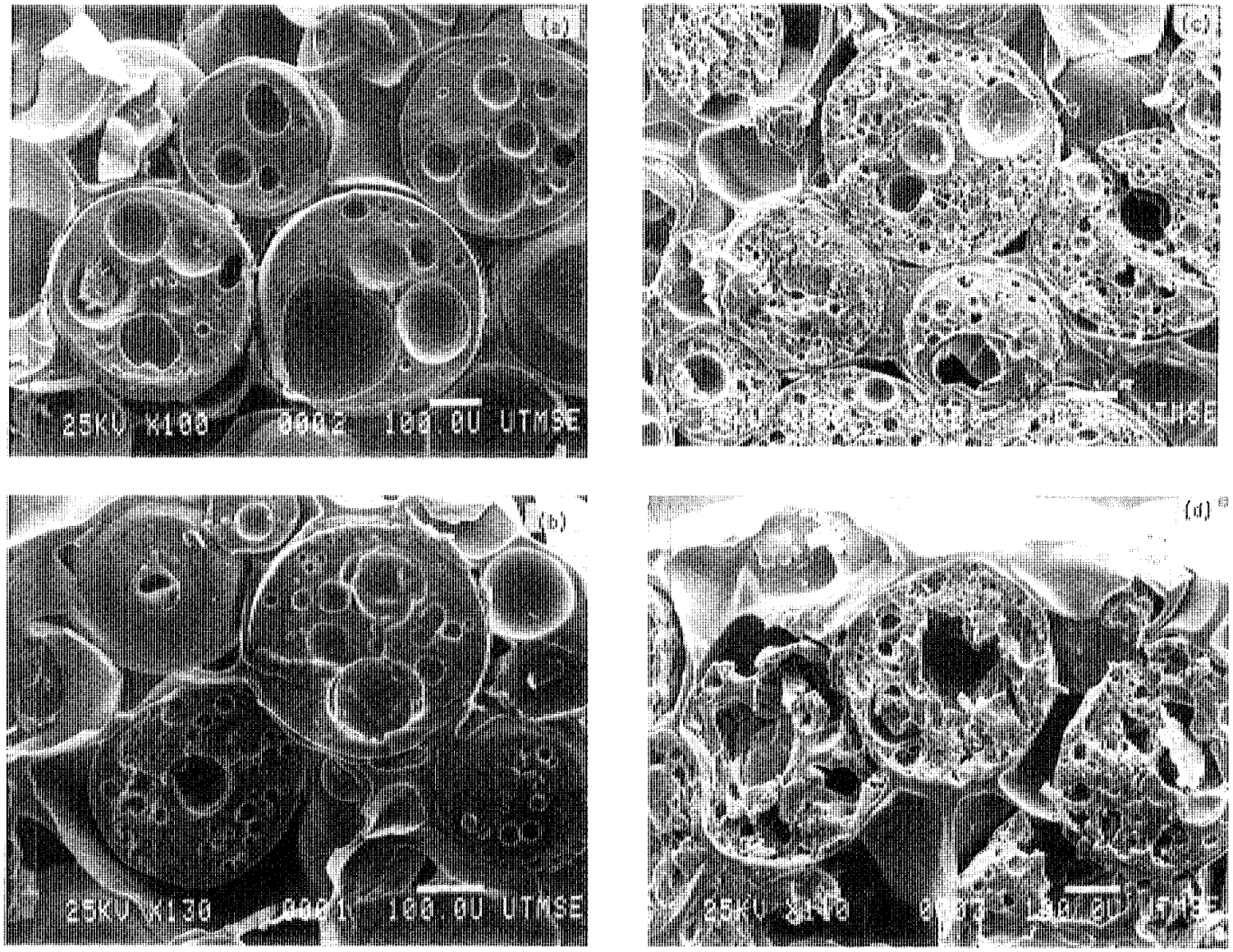

Fig. 4. Morphological properties of cross sections of multiphase PLGA microspheres containing brilliant blue during an *in vitro* dissolution test. The samples [initial (a)] were observed after 1 week (b), 2 weeks (c), and 4 weeks (d) by scanning electron microscopy.

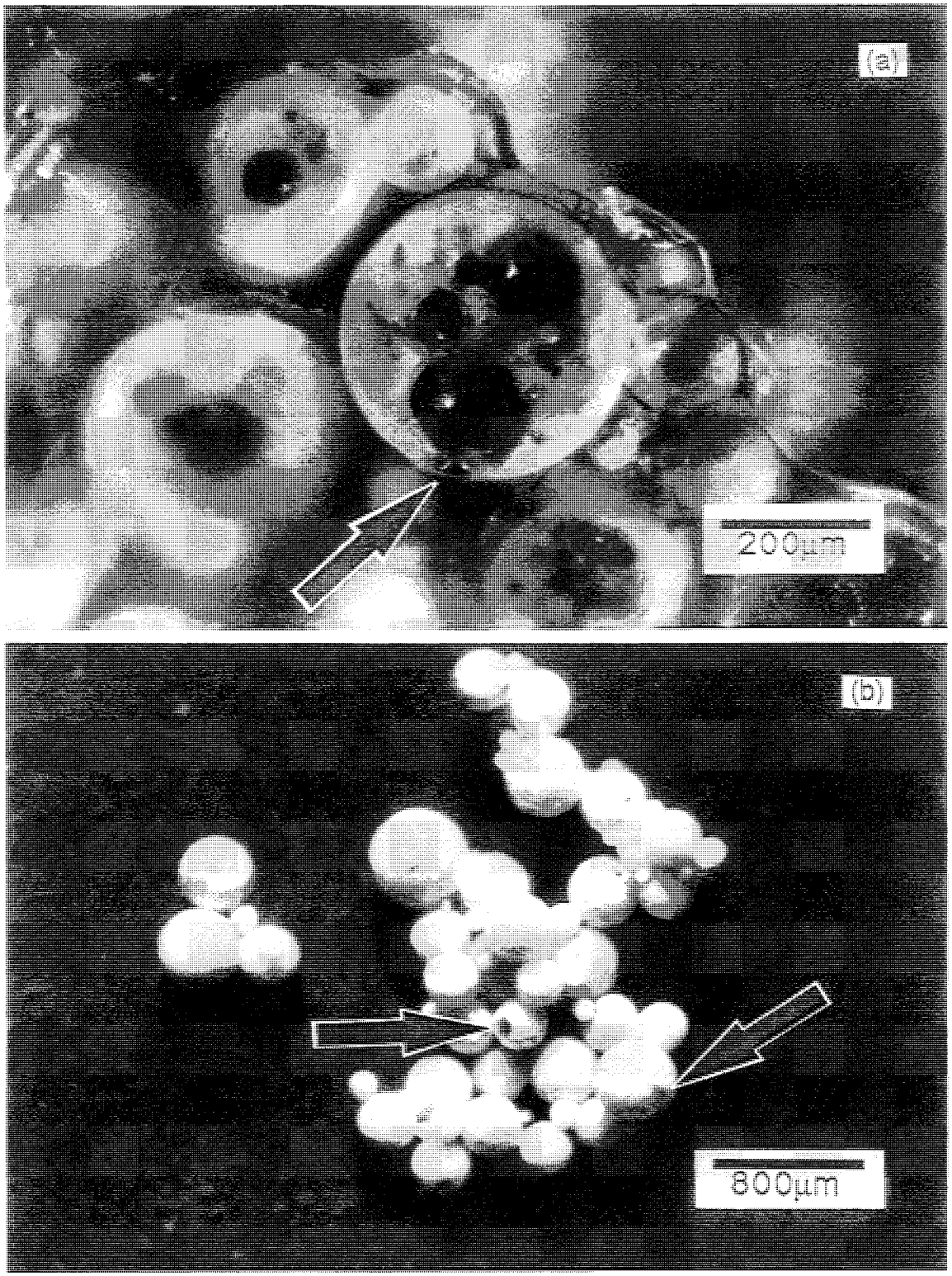

Fig. 5. Optical micrographs of cross sections (a) and complete microspheres (b) of PLGA multiphase microspheres containing brilliant blue after an *in vitro* dissolution test in buffered saline medium (pH 7.4) for 1 week.

polymer walls of the multiphase microspheres that were in contact with the emulsion droplets would be relatively thin compared to other portions of the microsphere. Although significant erosion of the internal structure occurred with both microspheres during the latter stages of the dissolution process, the surface of the beads remained relatively smooth throughout the duration of the dissolution test. The Span 80 present in the soybean oil and mineral oil used in the man-

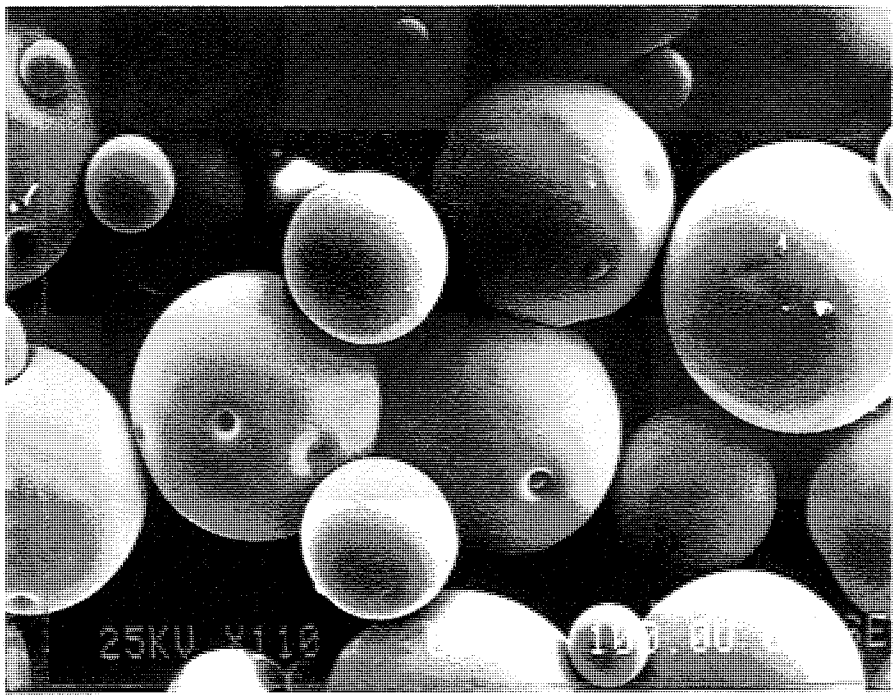

Fig. 6. Scanning electron micrograph of the surface morphology of multiphase microspheres.

ufacture of the microspheres may have been adsorbed onto the surface of the polymeric beads, thus protecting the surface from extensive hydrolytic breakdown.

Since the release profiles of both the dye and the chlorpheniramine maleate from the conventional and multiphase microspheres were distinctly different and the internal structures of the particles prepared by the two processes were also dissimilar, the influence of the emulsion on the stability of the polymer during the dissolution test was investigated. The hydrolytic degradation of the PLGA during the dissolution process from both the multiphase and the conventional microspheres was studied by following the changes in the molecular weight of the polymer by GPC. These results are shown in Fig. 7. A comparison of these results with the lag time in the dissolution profiles in Figs. 1a and 2a indicated that for the CPM and the brilliant blue, approximately 60 and 50%, respectively, of the polymer had degraded before the drug release process had been initiated. Since there was no

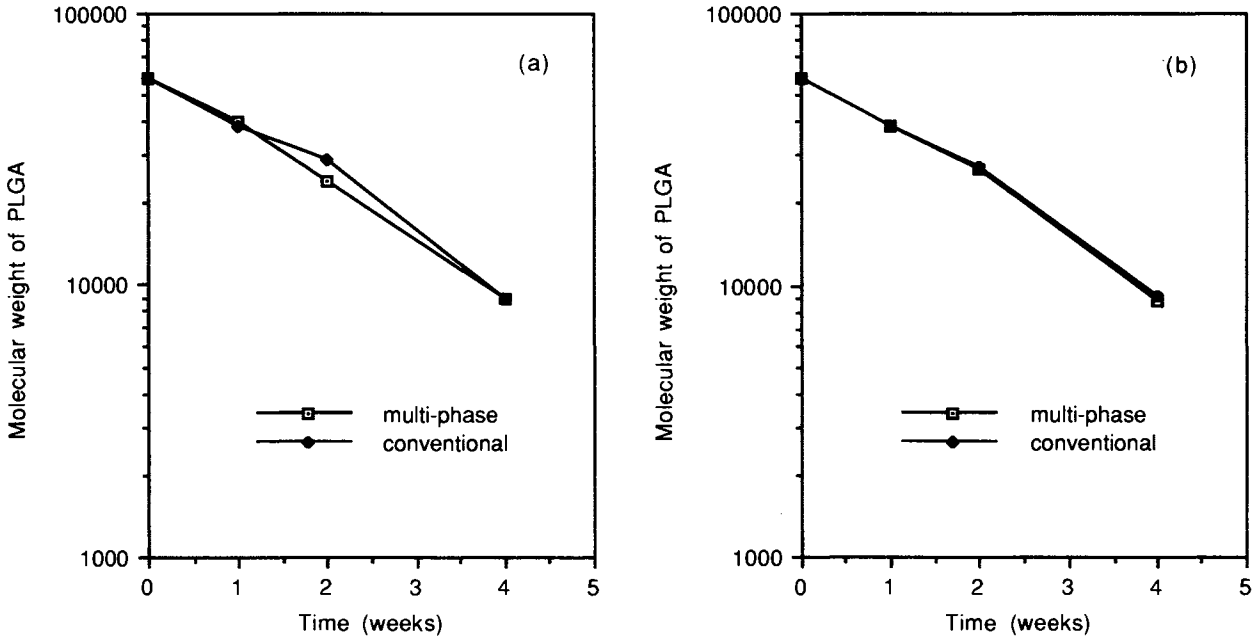

Fig. 7. Influence of drugs [BB (a) and CPM (b)] and the method of preparation of microspheres on the stability of PLGA during an *in vitro* dissolution test in phosphate-buffered saline (pH 7.4).

significant difference during the dissolution test in the rate of decrease in M_w between the conventional and the multiphase microspheres, it was concluded that the presence of the emulsion in the multiphase microspheres displayed no significant influence on decreasing or increasing the degradation rate of the polymer.

In conclusion, the results of this study have demonstrated that the release of water-soluble model compounds from multiphase microspheres was independent of the drug loading levels in the microspheres. A lag time for drug release was shown with the conventional microspheres of 10 and 15 days for the chlorpheniramine maleate and the dye, respectively. The dissolution rate of both compounds from the conventional microspheres was dependent upon the loading levels in the respective formulations. Scanning electron microscopy demonstrated polymeric erosion occurred in the microspheres during the dissolution process. Results from gel permeation chromatography demonstrated that the relative rate of decrease in molecular weight of the polymer was the same during the dissolution testing for microspheres prepared by both processes. Future studies will focus on methods to reduce the mean particle size of the multiphase microspheres and to develop techniques to obtain a more linear release of the soluble compounds from the microspheres.

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