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Research article

Influence of processing on the stability and release properties of biodegradable microspheres containing thioridazine hydrochloride

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Abstract

Biodegradable microspheres of poly(DL-lactic-co-glycolic acid) (PLGA) containing thioridazine HCl were produced by four emulsion–solvent evaporation methods including an O/W emulsion method, an O/O emulsion method, a W/O/W multiple emulsion method, and a W/O/**O**/O multiple emulsion method. Gel permeation chromatography was used to determine the molecular weight of the polymer before and after processing. Resultant microspheres were either incubated in an oven at 40°C, or stored in a desiccated chamber at 20°C. Change in the molecular weight of the polymer was monitored as a function of time. Premature degradation of the polymer was evident in microspheres produced by the O/W conventional solvent evaporation method. Thioridazine HCl catalyzed hydrolysis of PLGA was evident in normalized molecular weight distribution plots of the O/W microspheres. The in vitro release of thioridazine HCl from multiphase microspheres produced by potentiometric dispersion was compared with the release of drug from conventional microspheres prepared from the same polymer. Release of thioridazine HCl from multiphase microspheres of the W/O/**O**/O type occurred by diffusion during initial stages of drug release. © 1997 Elsevier Science B.V.

Keywords: Multiphase microsphere; Stability; Thioridazine; Potentiometric dispersion; Poly(DL-lactic-co-glycolic acid)

1. Introduction

The encapsulation of amine based drugs in polyesters comprised of lactic and glycolic acid presents formidable challenges to the researcher due to the tendency of such drugs to promote hydrolytic degradation of these polymers. The successful encapsulation of these entities requires high drug loading in the microspheres, prevention of degradation during the encapsulation process, and predictable release of the drug compound from the microspheres. To achieve these goals, multiple emulsion techniques and other innovative modifications have been made to the conventional solvent evaporation process.

Common problems reported with W/O/W microspheres include the rapid initial release of drug, termed the burst effect, and instability of certain drugs after encapsulation due to contact with either the polymer or the solvent used in the system [1]. The burst effect has been shown to be related to a porous microstructure of the microspheres which allowed rapid hydration of the device [2,3]. For conventional O/W microspheres, the degradation rate of the polymer was accelerated by the encapsulation of tertiary amine based drugs [4–8]. Multiphase microspheres offer the possibility of protection of the encapsulated drug from degradation by the polymer, and can protect poly(DL-lactic-co-glycolic acid) from degradation induced by the encapsulated drug [9–11].

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The release of drug and the rate of degradation of polymeric drug delivery devices are both influenced by the molecular weight of the polymer, and degradation has been investigated by analysis of the loss of molecular weight by gel permeation chromatography [12]. Although many researchers have investigated the in vivo and in vitro degradation of biodegradable microspheres, few studies have addressed the effect of chemical interaction or hydrolytic degradation induced by encapsulated compounds, on the molecular weight of the biodegradable polymer.

2. Materials and methods

2.1. Materials

Poly(DL-lactic-co-glycolic acid) (PLGA), (M_w 100 000, and M_w 64 000, lactic acid/glycolic acid 75/25), were purchased from Birmingham Polymers Inc., Birmingham, AL. Purified gelatin was obtained from Fisher, Fair Lawn, NJ. Thioridazine HCl, and polyvinyl alcohol, (M_w 40 000) were obtained from Sigma, St. Louis, MO. Soybean oil USP, and aluminum monostearate USP/NF were purchased from Spectrum, Gardena, CA. Sorbitan mono-oleate (Span® 80), and polyoxyethylene sorbitan mono-oleate (Tween® 80), were obtained from ICI, Wilmington, DE.

2.2. Preparation of microspheres

Multiphase microspheres containing thioridazine HCl were made by emulsifying the aqueous phase (W), with a protective oil (O), to form a primary W/O emulsion. The primary emulsion was dispersed in a polymer solution (O), and the emulsion was then dispersed in a hardening solution (O). The W/O/**O** emulsion was dispersed in the hardening solution by forcing it at a constant rate of infusion through a narrow electrically conductive stainless steel tube 5 cm in length. A power source (Acopian, Model # PO5HP12, Easton, PA) supplied an electric potential of 1800 V to the tube and targeted a circular anode with a diameter of 28 mm, positioned in the hardening solution. The hardening solution was continuously agitated at 200 rpm by means of a magnetic stirrer for 24 h to allow for evaporation of the organic solvent from the microspheres. The resulting microspheres were collected by vacuum filtration and rinsed three times with hexane to remove any residual oil. The microspheres were then rinsed three times with a solution of purified water and Tween® 80. The microspheres were allowed to dry for 24 h in a vacuum dessicator and recovered as a free flowing powder.

For microspheres produced by a water-in oil-in water (W/O/W) method, the drug was dissolved in 0.5 ml of distilled water (W) and 0.8 g of PLGA was dissolved in 8 ml of methylene chloride (O). The two solutions were then combined and emulsified using a Polytron® homogenizer. The resultant primary emulsion was added to 80 ml of an aqueous 2% polyvinyl alcohol solution (W) and agitated with a lightin® mixer at 500 rpm for 3 h. The microspheres were recovered by filtration and stored in a dessicator for 24 h.

Microspheres of the O/W and O/O type were prepared by a solvent evaporation technique using mechanical agitation. In the case of the O/O type microspheres, thioridazine was dispersed in a solution of PLGA and acetonitrile. The polymer solution was then dispersed in a continuous phase of mineral oil containing 0.5% Span® 80. A three blade propeller was used, and the system was agitated at 500 rpm. For the O/W type microspheres, the solvent used was methylene chloride, and the continuous phase was comprised of purified water containing 0.5% Tween 80.

2.3. Dissolution studies

The duration of each in vitro dissolution experiment was three months, and all dissolution testing was performed in a Vanderkamp® sustained release apparatus (Vankel, Edison, NJ). This apparatus consisted of a 16 station rotating bottle apparatus, a 30 gallon water bath (O'Dell, San Antonio, TX) and a Tecam TE-7 Tempette combined water bath heater and circulator (Duxford, England).

Dissolution testing was performed in VanKel® 100 ml dissolution test bottles which were sealed with Teflon® caps. Dissolution media consisted of distilled water. Each dissolution bottle contained 75 ml of dissolution medium which ensured adequate head space in each vessel to facilitate uniform dispersion of the microspheres throughout the dissolution media for the duration of the experiment. To prevent unwanted leakage, the bottles were double sealed with Parafilm laboratory film. Bottles were rotated end-over-end at a fixed rate of 15 rpm. To prevent the removal of microspheres during sampling, the bottles were allowed to stand for 30 min prior to sample removal. The sample size was 100 μl for all dissolution experiments.

2.4. Scanning electron microscopy (SEM)

Scanning electron microscopy was used to examine the surface and internal morphology of the PLGA microspheres. Samples of microspheres were attached to brass SEM stages using 3M double sided tape and were then coated with gold palladium for 60 s under an argon atmosphere using a Pelco® Model 3 cold sputter module (TED Pella, Tusin, CA) in a high vacuum evaporator equipped with an omni-rotary stage. Samples were examined with a JEOL scanning electron

microscope (Model JSM-35C, Jeol USA, Peabody, MA) at 25 kV.

Micrographs were taken with a Polaroid 545 Land Camera and Polaroid 4 × 5 Type 53 film (Polaroid, Cambridge, MA). Transverse cross sections of the microspheres were prepared by embedding the microspheres in a slab of gelatin and sectioning the slab with a scalpel. The sections were then mounted on an SEM stage as described above.

2.5. Gel permeation chromatography

Gel permeation chromatography was used to determine the molecular weight of PLGA. Molecular weight averages were determined using a Waters® system (Waters Associates, Milford, MA) which included a WISP Model 710B Auto-injector, a Model 510 double piston HPLC pump, 3 Ultrastyragel® columns in series, a Temperature Control Module with column heater, and a Model 410 Differential Refractometer. The column sizes were 100, 10^3, 10^4 Å, and the pump flow rate was set a 1.0 ml/min. The columns were held at a constant temperature of 30°C.

Calibration of the unit was achieved using retention time peak analysis of monodispersed polystyrene molecular weight standards from 600 to 400 000 in molecular weight, and all molecular weights were determined relative to those standards. Tetrahydrofuran (THF) stabilized with 0.025% BHT was utilized as the solvent and mobile phase for all samples. An interface module connected the refractometer to an NEC Powermate SX Plus desktop computer. An injection volume of 100 μl was used for each sample. Samples were prepared by dissolving the microspheres in THF at a concentration of 0.001 g/ml and filtering into scintillation vials through a 0.2 micron nylon filter.

The retention time for PLGA was 23 min, with complete elution of all components within 40 min. To ensure that polymeric degradation was not occurring during the sample run, unprocessed PLGA was analyzed at the beginning and end of each analysis period.

2.6. Particle size distribution

The particle size distribution of the microspheres was determined using a Shimadzu SALD 1100 laser diffraction type particle size analyzer (Shimadzu, Columbia, MD). The laser operates at a wavelength of 780 nm with an output of 3 mW. Calibration of the particle size analyzer was performed using 0.5, 1, and 35 μm monodispersed polystyrene beads. Particle size distribution data consisted of volume percent distribution, light energy distribution, cumulative volume distribution, and differential distribution. Samples were read in triplicate, and the data were processed by a direct fit method. All samples were dispersed in filtered purified water containing 0.5% Tween® 80 as a dispersing agent immediately prior to analysis and agitated to ensure a uniform dispersion free of aggregates.

2.7. Drug loading

Drug loading of the microspheres was determined by first dissolving 50 mg of microspheres in 100 ml of chloroform. The UV absorbance was read at 267 nm using a Beckman Model DU-65 Spectrophotometer (Beckman, Fullerton, CA). Placebo microspheres were tested to ensure that other components of the microsphere formulations did not display UV absorbance at the scanning wavelength. A calibration curve was generated using thioridazine standards dissolved in chloroform.

2.8. Moisture content analysis

A Karl Fisher coulometric moisture analyzer Model Aquatest 8 (Photovolt, Indianapolis, IN) was used to determine the amount of encapsulated water in the microspheres. The Aquatest 8 Karl Fisher coulometric moisture analyzer was placed in a low humidity environment with restricted air flow. The reagents were utilized according to the manufacturer's specifications. The Karl Fisher coulometric moisture analyzer was calibrated and standardized with three injections of 4 μl of purified water. Each test sample was comprised of 50 mg of microspheres accurately weighed and rapidly introduced into the Karl Fisher reaction vessel. A 2 min delay was programmed into the unit to allow the microspheres to dissolve. Each sample was tested in triplicate and the average of the three determinations was reported.

3. Results and discussion

3.1. Microsphere size and surface morphology

Poly(DL-lactic-co-glycolic acid) microspheres containing thioridazine were prepared by four emulsion solvent evaporation methods; oil-in-water (O/W), oil-in-oil (O/O), water-in-oil-in-water (W/O/W) and water-in-oil-in-oil-in-oil (W/O/**O**/O), as described in the methods section. PLGA with molecular weights of either 64 000 or 100 000 was used to prepare the microspheres. The particle size and surface morphology of the microspheres were examined visually by scanning electron microscopy. Photomicrographs of microspheres of PLGA of molecular weight 64 000 produced by either conventional O/W solvent evaporation or an O/O solvent evaporation method are shown in Fig. 1a and b, respectively. The particle size distribution for the O/O type microspheres was more narrow than that of

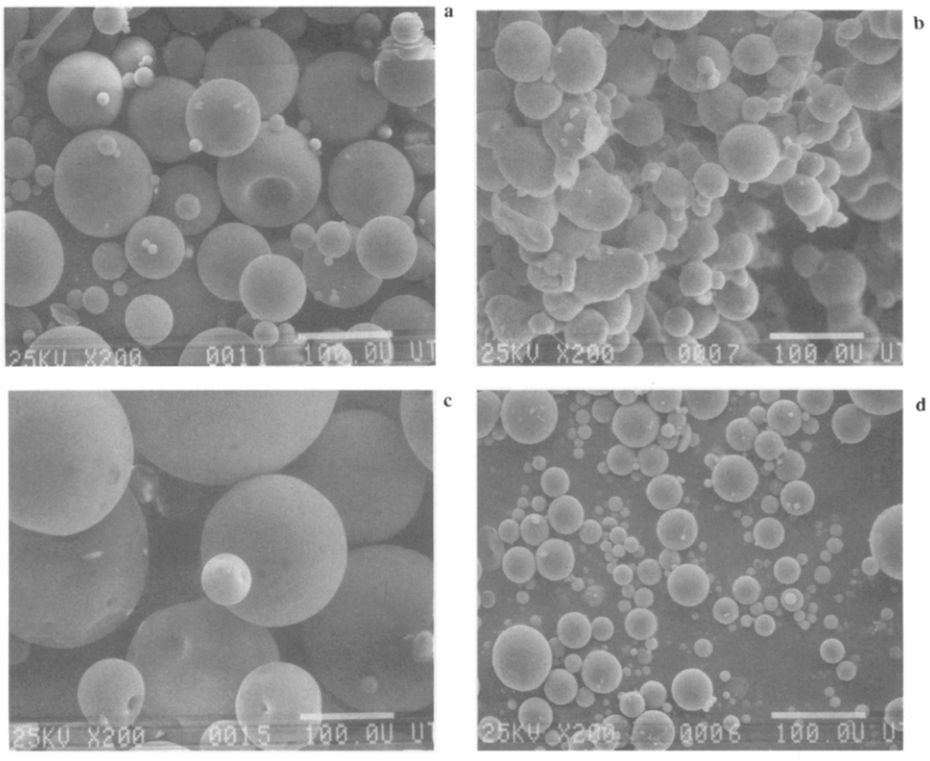

Fig. 1. Scanning electron micrographs of PLGA microspheres containing thioridazine prepared by an: (a) O/W emulsion method, (b) O/O emulsion method. (c) W/O/W emulsion method, and (d) W/O/**O**/O potentiometric dispersion method.

the O/W microspheres. The O/O microspheres displayed a greater tendency for aggregation, which was attributed to the slower diffusion rate of the solvent into the continuous oil phase. The microspheres remained in a softened state for longer periods of time, and through random collisions which occurred during the hardening period, the microspheres adhered to each other. In many cases, the aggregated microspheres were found to coalesce and larger spheres and agglomerates were formed. The effects of coalescence were particularly pronounced in the lower molecular weight PLGA, and examination of the surface morphology revealed larger microspheres which were formed from the coalescence of the smaller microspheres. This aggregation and resultant coalescence of the microspheres can clearly be seen in Fig. 1b.

Multiphase microspheres of the W/O/W type are shown in Fig. 1c for PLGA of molecular weight 64 000. The microspheres ranged in size from less than 10 μm to approximately 250 μm. The surface of the microspheres appeared to be smooth, but on close examination, small surface imperfections were observed. Surface impacts and imperfections on the microspheres were due to the high shear created by the propeller which was used for agitation.

The surface morphology of W/O/**O**/O microspheres produced by potentiometric dispersion is shown in Fig. 1d. The photomicrograph revealed a particle size distribution ranging from 10 to 160 μm and smooth microspheres which were free of defects. These microspheres displayed a more narrow particle size distribution than microspheres produced with the PLGA of molecular weight of 100 000. In both cases, the polymer was dispersed into the continuous phase with a 0.40 mm infusion tube at a voltage of 1800 vdc. The difference in particle size was attributed to the increased viscosity of the higher molecular weight polymer.

The particle size distributions determined by laser light diffraction studies confirmed the observed particle size distribution seen in the photomicrographs, and the results appear in Fig. 2. Microspheres produced by the W/O/**O**/O method had a mean particle size of 75 μm, with 75% of the microspheres under 100 μm in size. The microspheres produced by the W/O/W method had a mean particle size of 140 μm, with 75% of the microspheres below 190 μm in size. Microspheres of the O/W type had a mean particle size of 160 μm, and the microspheres of the O/O type had a mean particle size of 200 μm. Microspheres produced by the O/W emulsion solvent evaporation had the broadest particle size distribution which was considered normal for this type of microspheres. A wide particle size distribution was also seen with the O/O type microspheres. The percentage of large microspheres produced by this system were in fact aggregates of smaller microspheres. Partial or complete coalescence prevented the production of dis-

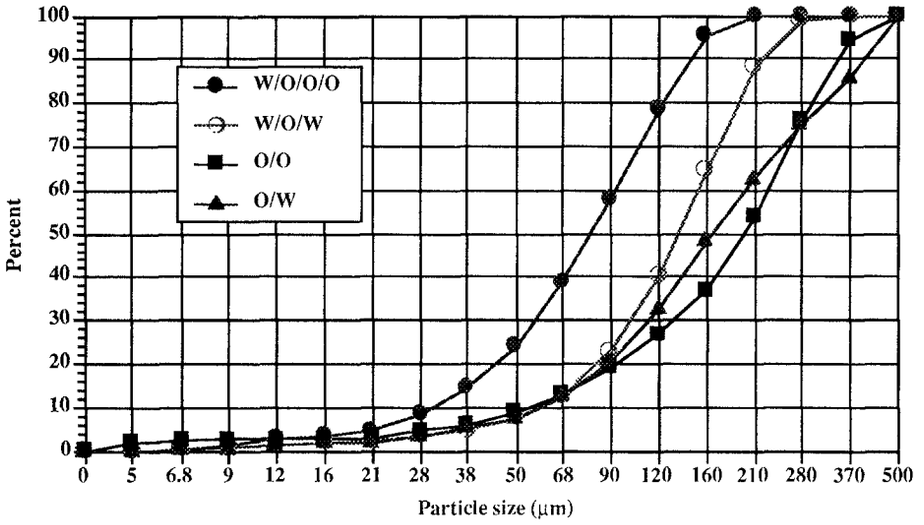

Fig. 2. Particle size distributions of poly(DL-lactic-co-glycolic acid) microspheres containing thioridazine HCl produced by O/O, O/W, W/O/W, or W/O/**O**/O solvent evaporation methods. (PLGA molecular weight 100 000).

crete microspheres, but instead, produced irregular shaped agglomerates. Microspheres produced with the lower molecular weight PLGA displayed similar distributions.

The encapsulation efficiency and drug content of the microspheres were determined and the results are presented in Table 1 for microspheres produced with PLGA 64 000. For both polymers, the thioridazine HCl loading was lowest for microspheres produced by the conventional O/W method. The encapsulation efficiency for PLGA of molecular weight of 100 000 and 64 000 was 18.3 and 23.8% respectively. Low drug loading efficiency for water soluble drugs such as thioridazine HCl is a common problem associated with microspheres produced by O/W solvent evaporation methods, due to the tendency of water soluble drugs to partition into the aqueous continuous phase. Microspheres of the O/O type displayed a higher drug loading. The encapsulation efficiency for PLGA 100 000 was 43.8% of theoretical, while the encapsulation efficiency for PLGA 64 000 was found to be 23.0%. Microspheres of the W/O/W type displayed much higher encapsulation efficiencies, with 69.0% of theoretical loading for microspheres produced with PLGA 64 000 and 99.5% for microspheres produced with PLGA 100 000. The improved encapsulation efficiency of the W/O/W type microsphere was attributed to the successful prevention of drug partitioning into the continuous phase due to the formation of a multiple emulsion. The higher drug loading for the PLGA of molecular weight of 100 000 was attributed to the higher viscosity of the polymer solution. Microspheres of the W/O/**O**/O type also displayed very high drug loading, with both molecular weight grades of PLGA achieving drug loadings of approximately 100%. High encapsulation efficiencies realized for the W/O/**O**/O type microspheres were attributed to the ability of the primary emulsion to prevent drug partitioning into the external oil continuous phase.

Table 1
Encapsulation efficiencies and drug content of poly(DL-lactic-co-glycolic acid) microspheres produced by O/W, O/O, W/O/W, or W/O/**O**/O emulsion solvent evaporation methods

Method	M_w	Encapsulation efficiency (%)	Drug content (mg/g)
O/W	64 000	23.75	17.81
O/W	100 000	18.25	13.69
O/O	64 000	23.0	17.25
O/O	100 000	43.75	21.87
W/O/W	64 000	69.0	13.8
W/O/W	100 000	99.5	19.9
W/O/**O**/O	64 000	99.97	19.99
W/O/**O**/O	100 000	100.1	20.02

3.2. Influence of processing and encapsulated drug on polymer stability

The molecular weight of the PLGA microspheres was determined using gel permeation chromatography. Samples were stored at 40°C and the molecular weight was determined at regular intervals over a 4 month period. The changes in the molecular weight as a function of time are shown in Figs. 3 and 4 for PLGA 64 000 and PLGA 100 000 respectively. For both polymers, microspheres produced by the anhydrous O/O system were found to be very stable, with only a slight loss in molecular weight over a 4 month period. However, microspheres produced by the O/W process experienced a significant reduction in molecular weight over the same time period. Microspheres produced with PLGA 100 000 experienced a 64% reduction in molecular weight while PLGA 64 000 expe-

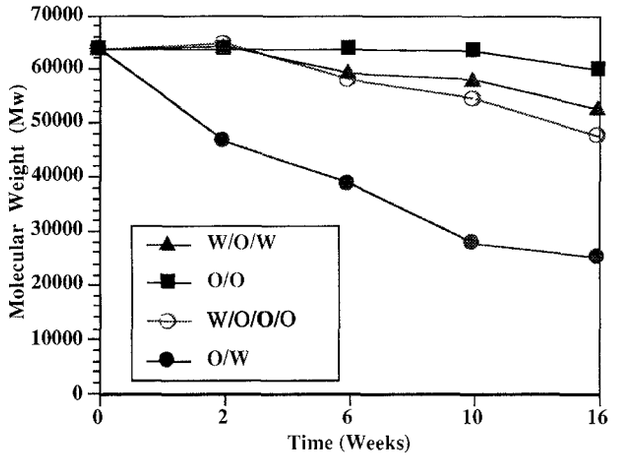

Fig 3. Change in the molecular weight of poly(DL-lactic-co-glycolic acid) microspheres containing thioridazine HCl vs. time, prepared by O/W, O/O, W/O/W, and W/O/**O**/O emulsion solvent evaporation methods: poly(DL-lactic-co-glycolic acid) $M_w = 64\,000$.

rienced a 61% reduction in molecular weight. The hydrolytic degradation of the PLGA was accelerated due to the presence of the thioridazine. Microspheres of either the W/O/W type or the W/O/**O**/O type exhibited only a slight loss of molecular weight over the period investigated. The stability of the O/O type microsphere system in this case was not surprising, in that the anhydrous system did not facilitate hydrolytic degradation.

Polymer hydrolysis in the presence of basic amine compounds was correlated to the decline in molecular weight of the polymer used in the W/O system. Similar results were also found by Cha and Pitt [13] who showed that the catalytic effect of four basic drugs increased as the basicity of the drugs increased. The rates of drug release and polymeric degradation were strongly affected by the encapsulated compound.

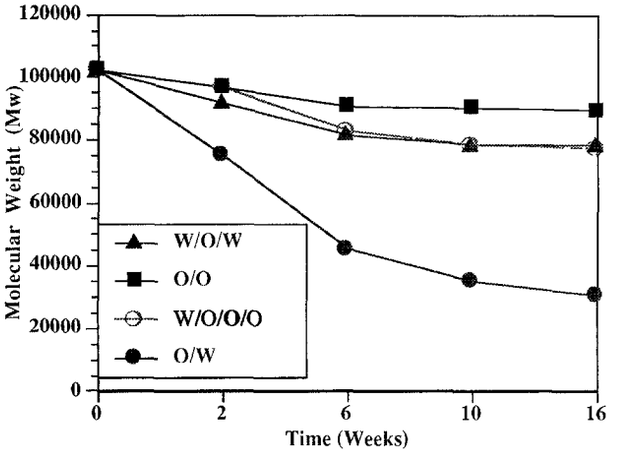

Fig. 4. Change in the molecular weight of poly(DL-lactic-co-glycolic acid) microspheres containing thioridazine HCl vs. time, prepared by O/W, O/O, W/O/W, and W/O/**O**/O emulsion solvent evaporation methods. poly(DL-lactic-co-glycolic acid) M_w-100 000.

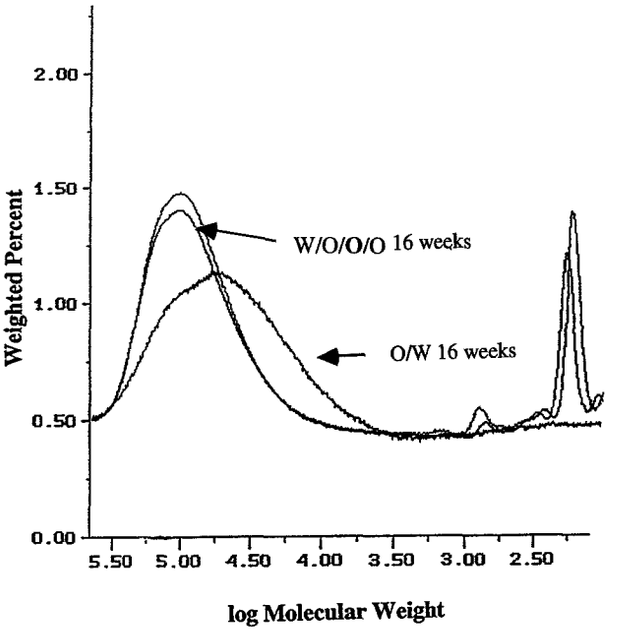

Fig. 5. Normalized distribution plot of un-processed poly(DL-lactic-co-glycolic acid) of molecular weight of 64 000 superimposed on distribution plots of the polymer after 16 weeks storage of microspheres at 40°C.

Maulding and co-workers [4] found that the degradation rate of poly(DL-lactic acid) (PLA) was accelerated in the presence of the tertiary amino compound, thioridazine. The PLA component of microcapsules containing thioridazine base showed a decrease in molecular weight during microcapsule fabrication and in the course of dissolution studies. This effect was evidenced by a rapid release of thioridazine as a function of time. Polymer hydrolysis did not occur in the placebo microspheres or in microspheres which contained thioridazine pamoate, a protonated form of thioridazine. This suggested that the nucleophilic nitrogen of thioridazine was required for reaction with the ester linkages of the polymer.

Degradation of PLGA microspheres produced by the O/W method was investigated by analysis of molecular weight normalized distribution plots from gel permeation chromatography studies as seen in Fig. 5. The normalized distribution plot of un-processed PLGA 64 000 and the polymer after 16 weeks storage of microspheres produced by either the O/W or the W/O/**O**/O method indicated that limited hydrolysis of the polymer occurred during storage of the W/O/**O**/O microspheres. The thioridazine HCl catalyzed hydrolysis of PLGA was evident in the normalized distribution plots of the O/W microspheres.

The change in molecular weight in PLGA versus time of placebo microspheres of the O/W and W/O/**O**/O and microspheres containing thioridazine HCl incubated for 16 weeks at 40°C is presented in Fig. 6. PLGA (M_w = 64 000) microspheres of the W/O/**O**/O type showed a

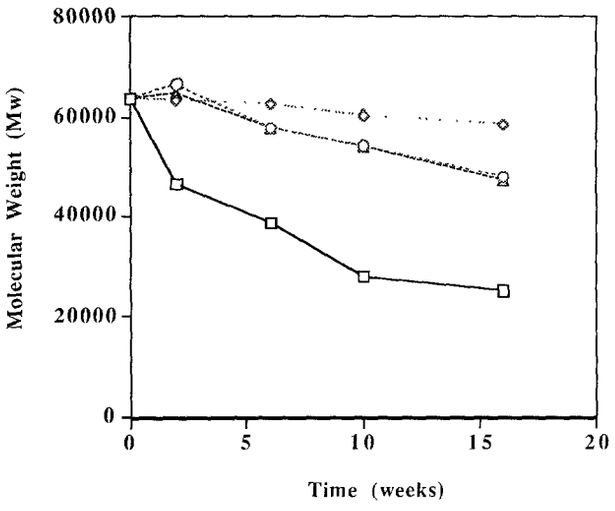

Fig 6. The change in molecular weight in poly(DL-lactic-co-glycolic acid) vs. time of placebo microspheres of the O/W and W/O/**O**/O type and microspheres containing thioridazine HCl incubated for 16 weeks at 40°C. Key (□), thioridazine HCl O/W; (◇), placebo O/W; (△), thioridazine HCl W/O/**O**/O; (○) placebo W/O/**O**/O.

reduction in molecular weight of 19% for both the placebo formulation and the formulation which contained the drug, whereas microspheres prepared by the O/W method displayed a substantial change in polymer molecular weight between the placebo and thioridazine HCl formulations. These findings demonstrated that the W/O/**O**/O type microspheres successfully prevented the encapsulated drug from direct contact with the polymer.

Similar results were obtained for microspheres of the W/O/W type. This was surprising, due to the process involved in the production of the W/O/W type microspheres. It was hypothesized that hydrolytic degradation was related to the entrapped water content of the different types of microspheres.

The accessibility of water to the ester bonds of poly(DL-lactic-co-glycolic acid) has been reported to be a significant factor in the degradation of PLGA [14,15]. In three of the four processes utilized to produce microcapsules, the polymer was in contact with substantial amounts of water. For microspheres prepared by the W/O/W and the W/O/**O**/O methods, a portion of water is encapsulated within the microsphere. For the W/O/W type microsphere, most of the entrapped water is removed by either vacuum evaporation or lyophilization. These procedures, however, will promote the development of pores or channels throughout the microsphere, and allow rapid hydration and release of the encapsulated drug during the initial stages of in vitro dissolution testing. The W/O/**O**/O type microsphere encapsulated a portion of water which comprised the inner phase of the multiple emulsion. This water however, was contained in the inner phase of a water-in-oil emulsion, so contact with the polymeric material was restricted.

The O/O system, which demonstrated the most stable formulation with regard to hydrolytic degradation of the polymer by an amine, contained very little water available to promote hydrolytic degradation. The O/W system, which demonstrated the greatest degree of degradation allowed the most contact between the water and polymer. Polymeric degradation in this system was detected within 2 weeks of manufacture.

Karl Fisher analysis was performed on microspheres prepared by the four processes previously described to determine the amount of encapsulated water in the microspheres, and the results are shown in Table 2. Microspheres of the O/W type contained 0.55% water. This encapsulated water, together with the encapsulated amine, promoted rapid hydrolytic degradation of the polymer in the O/W type microsphere. The O/O microsphere, on the other hand, was found to have only 0.12% water and no significant change in molecular weight of the polymer over a 4 month period was noted.

Microspheres of the W/O/W type were found to have 0.39% entrapped water. During the production of W/O/W type microspheres, water is removed through vacuum drying or lyophilization. The W/O/W type microspheres exhibited good polymer stability in the presence of the thioridazine HCl, which indicated that the water detected in the microspheres was not available to promote massive hydrolytic degradation. Pitt and co-workers [16] found that the rate of hydrolytic chain scission of PLGA was relatively insensitive to the water content of immiscible blends such as poly(vinyl alcohol) and PLGA, but increased exponentially after they became miscible. The immiscibility of the poly(vinyl alcohol) and PLGA which comprised the W/O/W microspheres enhanced the stability of these microspheres.

Microspheres of the W/O/**O**/O type exhibited a larger amount of internal moisture, 0.61%. This level of moisture was higher than that of the O/W type microsphere, yet the microspheres displayed excellent poly-

Table 2
Water content of PLGA microspheres containing thioridazine HCl as determined by Karl Fisher analysis

Microsphere type	PLGA (%) M_w = 64 000	PLGA (%) M_w = 100 000
O/W	0.55	0.51
O/O	0.12	0.09
W/O/W	0.39	0.29
W/O/**O**/O	0.61	0.60

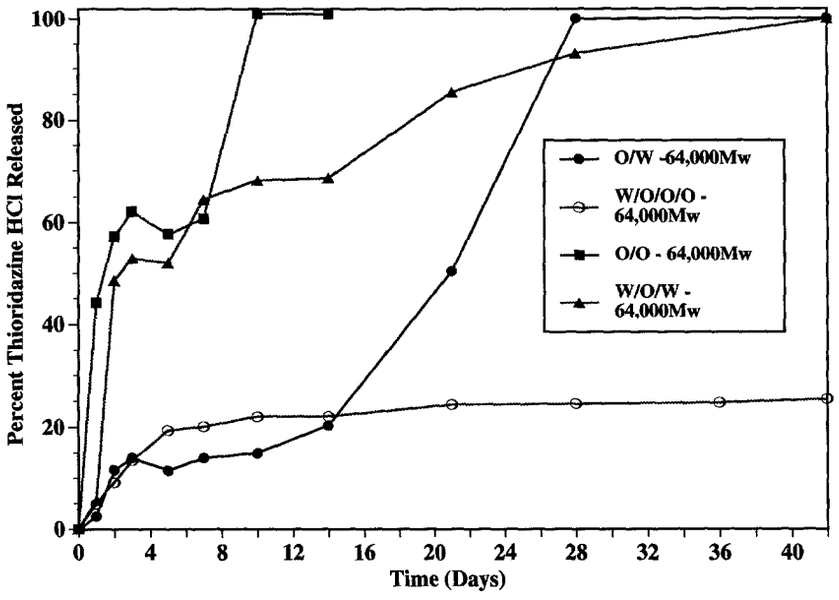

Fig. 7. Release of thioridazine HCl from poly(DL-lactic-co-glycolic acid) microspheres ($M_w = 64\,000$) in purified water at 37°C, performed in rotating bottle apparatus at 15 rpm.

mer stability. The internal water was incorporated in the primary emulsion together with the thioridazine HCl. The high water content, coupled with the polymeric stability indicated that the internal dispersed phase of the primary emulsion had very limited contact with the polymer.

3.3. Release properties of thioridazine HCl from PLGA microspheres

Dissolution studies were performed on microspheres prepared by the four methods, and the results are shown in Figs. 7 and 8 for microspheres of PLGA with molecular weights of 64 000 and 100 000 respectively. The W/O/W type microspheres of PLGA 64 000 showed a significant burst effect, with 43% of the drug released within the first 48 h of the test period. A burst of 10% thioridazine HCl was released within 24 h for W/O/W microspheres prepared with the higher molecular weight polymer. The burst effect had been well documented for microspheres of the W/O/W type [17–20] and is usually associated with the porosity of the microspheres. Several parameters which affect the porosity of W/O/W type microspheres include the rate of solvent removal, solvent type and the drug load [21,22]. The removal of encapsulated water by vacuum drying or lyophilizing may also produce channels which increase the porosity of the microspheres and promote a rapid burst release of the drug.

The O/O type microspheres also exhibited a substantial burst, with 58% of the drug released within the first 48 h of the test. The initial burst was followed by a lag period, and then a second burst began on day 6. The O/O type microspheres were determined to be a matrix type particle, with the thioridazine dispersed throughout the polymeric matrix. The initial burst was attributed to release of the drug from the surface regions of the microspheres, followed by a slower release of drug as water penetrated the polymeric matrix. Release profiles of drugs from matrix type microspheres have been previously reported [23–25].

The O/W emulsion solvent evaporation method used to produce microspheres also resulted in a matrix type microsphere. As with the case of the O/O type matrix, polymer degradation and the formation of pores in the matrix were necessary before drug release could be initiated. A lag period of 6 days was seen for microspheres comprised of PLGA molecular weight of 100 000, as compared to a lag period of 14 days for thioridazine HCl microspheres of PLGA with a molecular weight of 64 000. Both O/W formulations released thioridazine HCl after the lag period. The PLGA 100 000 microspheres dose dumped within 5 days after the lag period. Microspheres of PLGA 64 000 released thioridazine HCl over a 2 week period.

Microspheres of the W/O/O/O type released 12% of the drug load within 48 h. The remaining drug was released from the microspheres over a period of weeks, with drug release being attributed to both diffusion in the early stages of release and later a more rapid release, attributed to the bulk erosion of the microspheres. Release profiles for PLGA 100 000 were similar in appearance. A lengthy release of drug from the microspheres was not unusual in that PLGA with a compositional ratio of 75:25 lactide to glycolide was expected to have a biodegradation time of about 5 months [26].

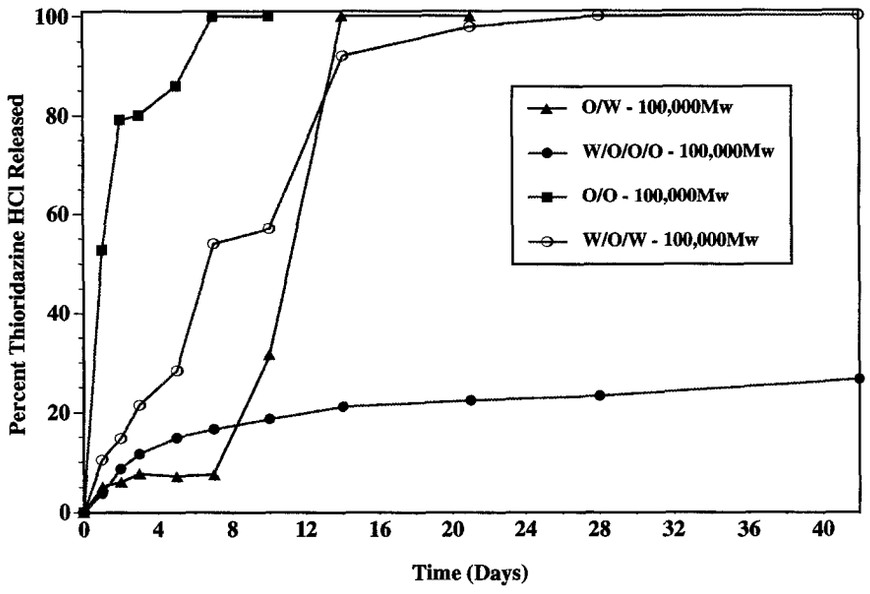

Fig. 8. Release of thioridazine HCl from multiphase microspheres of PLGA of molecular weight of 100 000 in purified water at 37°C, performed in rotating bottle apparatus at 15 rpm.

Examination of the dissolution profiles indicated different mechanisms of release of the drug due to the different processing mechanisms involved in the production of the microspheres. In the case of the O/W microspheres, release of thioridazine HCl was biphasic, and was preceded by a short lag time. Only 11% of the drug was released after 2 days. Another phase of slow drug release, often called the lag time, was observed for a 12 day period followed by rapid release until the drug was exhausted. Matrix type microspheres exhibit a lag time during which the dissolution medium penetrates the polymer matrix prior to drug release. The O/W microspheres of PLGA containing thioridazine deviated from this model, in that the initial release occurred prior to the lag time, and a bi-phasic release was observed.

Thioridazine HCl is soluble in methylene chloride, which was used in the O/W emulsion solvent evaporation process. During the solvent evaporation process, the thioridazine recrystallized as solvent was removed from the system. This produced drug rich and drug poor domains within the polymeric matrix. Thioridazine HCl particles that were located close to the surface of the microsphere diffused rapidly into the dissolution media. An ensuing lag time was required as water hydrated the deeper recesses of the microsphere. Examination of the microspheres after 21 days of dissolution testing revealed a very clear phase separation of polymer and drug within the microsphere. Phase separation occurred during the solvent evaporation process, and a well defined core of drug enriched polymer was surrounded by a polymer phase of reduced drug content. Bodmeier and McGinity [27,28] investigated solvent-non-solvent polymer interactions on the formation of PLA microspheres containing quinidine sulfate and found that the rate of polymer precipitation was strongly affected by the rate of diffusion of the organic solvent into the aqueous phase. Rapid diffusion of the solvent into the aqueous phase caused a precipitation of polymer on the outer regions of the microsphere. A slower diffusion of the drug from the inner core allowed the development of thioridazine HCl crystals to form inside the polymeric matrix, and resulted in the higher drug loading observed in the O/W type microspheres.

PLGA microspheres produced by an anhydrous O/O solvent evaporation system exhibited a large initial burst for both polymer molecular weights investigated, followed by a short lag time of approximately 2 days. The remaining drug was released within a 6 day period. The O/O microspheres utilized acetonitrile as the solvent for the polymer and mineral oil as the continuous phase. Since thioridazine HCl was insoluble in the acetonitrile/polymer solution, the drug was dispersed throughout the microcapsule. The drug did not form drug enriched regions in the microsphere, but rather remained dispersed throughout the matrix. During dissolution, thioridazine HCl close to the microsphere surface was released. Upon further hydration of the microsphere, the remaining drug was released through pores formed throughout the matrix.

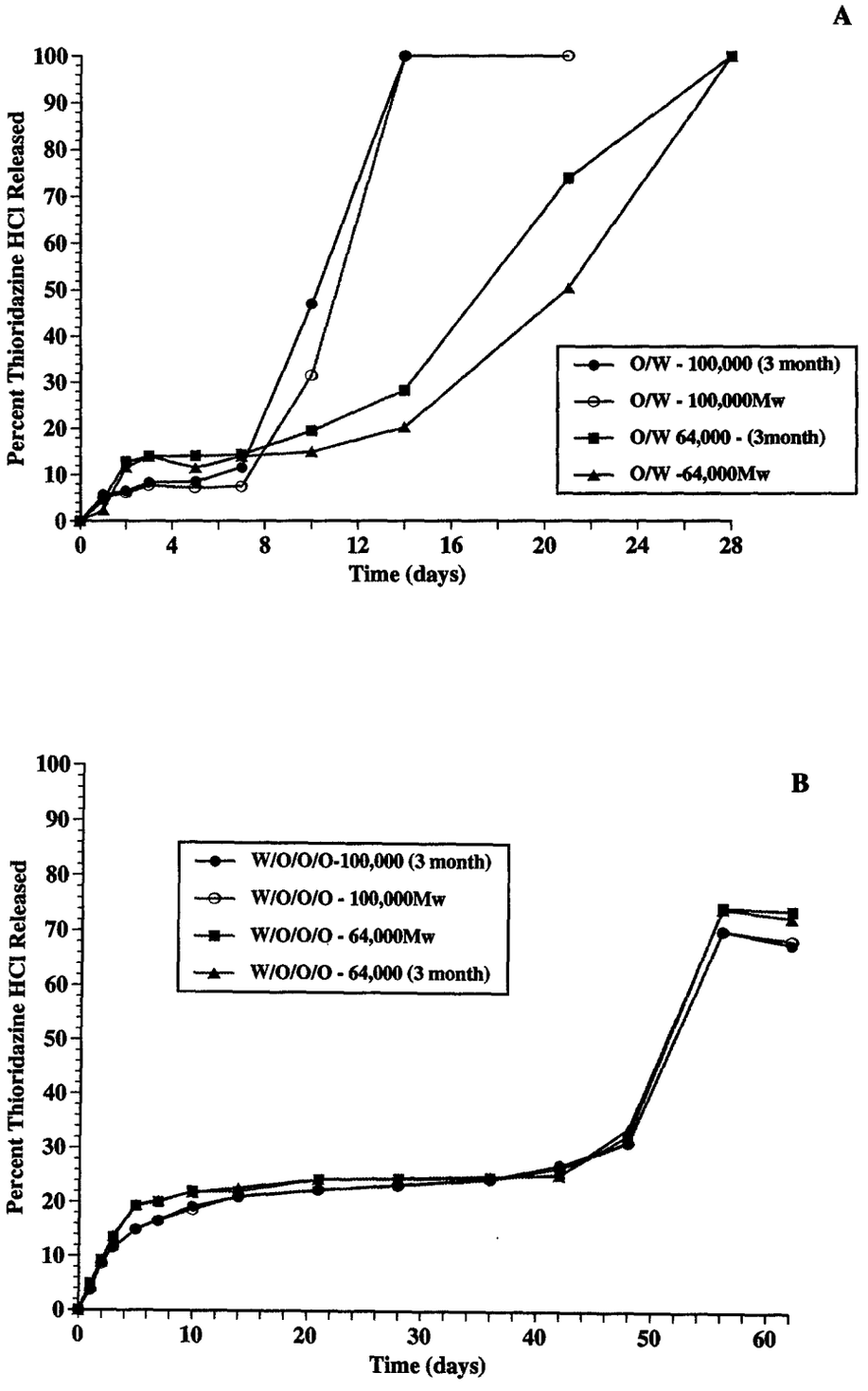

Fig. 9. Release of thioridazine HCl from O/W and W/O/O/O PLGA microspheres at initial and 3 month storage intervals. (a) $M_w = 64\,000$ (b) $M_w = 100\,000$.

In the case of the multiphase microspheres of the W/O/O/O produced by potentiometric dispersion, penetration of water into the polymeric matrix initiated an initial drug release, which was followed by an extended lag time. Examination of W/O/O/O microspheres after 21 days of dissolution revealed a smooth microsphere surface, with no evidence of rupture. Evidence of visible pores or other surface imperfections were not detected. After 90 days, however, fragments of microspheres were observed. Close examination of the fragments revealed circular indentations in which the drug containing emulsion resided, and indicated massive bulk erosion

of the polymer. The rapid onset of polymeric degradation coupled with the incomplete release (76%) of thioridazine HCl was investigated. During dissolution of the multiphase microspheres, a small initial amount of thioridazine was released as the microsphere began to hydrate. After a 40 day period, sufficient water permeated the microsphere and diffusion of the thioridazine HCl from the encapsulated emulsion commenced. The release of the drug, however, accelerated the degradation of the polymeric matrix, and promoted further drug release. Although rapid degradation occurred after the onset of drug release during the second pulse phase, the results of this research clearly indicated that multiphase microspheres of the W/O/**O**/O type can successfully prevent the degradation of PLGA during microsphere fabrication and storage, and can control the release of reactive compounds.

3.4. Influence of storage on release properties

Changes in the polymer molecular weight during storage will affect the physicochemical and release characteristics of PLGA microspheres. Multiphase microspheres of the W/O/**O**/O type and conventional microspheres of the O/W type comprised of PLGA containing thioridazine HCl were stored in sealed containers at 40°C for 3 months. The effect of change in dissolution rate as a function of storage was investigated. Dissolution tests were performed as previously described after a 3 month storage interval, and the results are shown in Fig. 9a and b.

Microspheres of the O/W type showed a substantial change in release of thioridazine after 3 months of storage. The change in release was more pronounced after the lag time. This was due to the lower concentration of drug in the outer regions of the microspheres. The lag time was attributed to the rate at which the dissolution media penetrated the polymeric matrix. The microspheres which were stored for 3 months released the drug from the drug enriched inner core at a faster rate when compared to the initial release profiles for these microspheres. Microspheres of the W/O/**O**/O type which were stored for 3 months at 40°C showed no significant change in release rates. This was attributed to the stability of the polymeric matrix which surrounded the W/O emulsion in the microsphere.

The change in the release characteristics of the O/W PLGA microspheres corresponds to the reduction in molecular weight of the polymer. The thioridazine catalyzed hydrolytic degradation produced oligomers of lower molecular weight. These lower molecular weight fractions increased the permeability of the polymeric matrix. Incorporation of lower molecular weight fractions in PLA and PLGA to modify release characteristics of polymeric devices has been reported by other investigators. Bodmeier et al. [29] prepared biodegradable films and microspheres from blends of high and low molecular weight PLA by solvent casting and an emulsification-solvent evaporation method respectively, and investigated the effects on release of salicylic acid, caffeine and quinidine sulfate. The addition of low molecular weight poly(DL-lactide) clearly accelerated drug release from both films and microspheres. Acceleration of drug release due to a change in polymer molecular weight has been reported by other researchers. [30–32].

4. Conclusions

Drug loading, encapsulation efficiency, and polymer stability were influenced by the method of microsphere preparation. Multiphase microspheres of the W/O/**O**/O type were shown to successfully prevent hydrolytic degradation of PLGA when a reactive material such as thioridazine HCl was encapsulated. Microspheres produced by the conventional O/W emulsion solvent evaporation method exhibited accelerated degradation of PLGA. Changes in the dissolution profiles of O/W type microspheres stored for a 3 month period were attributed to polymeric degradation. Multiphase microspheres of the W/O/**O**/O type containing thioridazine HCl were found to be stable, and no change in release characteristics during storage was found.

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