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Formulation of Anastrozole Microparticles as Biodegradable Anticancer Drug Carriers

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ABSTRACT

The purpose of this study was to develop poly(d,l-lactic-co-glycolic acid) (PLGA)–based anastrozole microparticles for treatment of breast cancer. An emulsion/extraction method was used to prepare anastrozole sustained-release PLGA-based biodegradable microspheres. Gas chromatography with mass spectroscopy detection was used for the quantitation of the drug throughout the studies. Microparticles were formulated and characterized in terms of encapsulation efficiency, particle size distribution, surface morphology, and drug release profile. Preparative variables such as concentrations of stabilizer, drug-polymer ratio, polymer viscosity, stirring rate, and ratio of internal to external phases were found to be important factors for the preparation of anastrozole-loaded PLGA microparticles. Fourier transform infrared with attenuated total reflectance (FTIR-ATR) analysis and differential scanning calorimetry (DSC) were employed to determine any interactions between drug and polymer. An attempt was made to fit the data to various dissolution kinetics models for multiparticulate systems, including the zero order, first order, square root of time kinetics, and biphasic models. The FTIR-ATR studies revealed no chemical interaction between the drug and the polymer. DSC results indicated that the anastrozole trapped in the microspheres existed in an amorphous or disordered-crystalline status in the polymer matrix. The highest correlation coefficients were obtained for the Higuchi model, suggesting a diffusion mechanism for the drug release. The results demonstrated that anastrozole microparticles with PLGA could be an alternative delivery method for the long-term treatment of breast cancer.

KEYWORDS: Breast cancer, microencapsulation, biodegradation, anastrozole, PLGA.

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INTRODUCTION

Many breast cancers have estrogen receptors, and growth of these tumors can be stimulated by estrogen. Treatment of breast cancer has included efforts to decrease estrogen levels by the use of antiestrogens and progestational agents.[1] Anastrozole is a nonsteroidal aromatase inhibitor. It is chemically described as 1,3-benzene diacetonitrile, a, a, a', a'-tetramethyl-5-(1H-1,2,4-triazol-1-ylmethyl). It is a potent and selective nonsteroidal aromatase inhibitor. The dose of anastrozole (Arimidex tablet) is 1 mg orally once a day. For patients with advanced breast cancer, the drug should be continued until tumor progression ends. For adjuvant treatment of early breast cancer in postmenopausal women, the median duration of therapy is 31 months. To increase patient compliance, a sustained delivery system of anastrozole could be used. One of the technological resources used to improve the permanence of drugs at the site of action is the use of therapeutic systems prepared using biodegradable polymers. Erodible matrices offer the advantage of biodegrading, disappearing gradually while releasing the drug from the site of action. Furthermore, the administration of these devices formulated as microparticles provides the advantage of facilitating their injection through standard infiltration needles.[2]

Poly(d,l-lactic-co-glycolic acid) (PLGA), a copolymer of poly(lactic acid) and poly(glycolic acid), has been studied extensively as a polymeric carrier for biodegradable microspheres. Because of these biodegradable polymers' long history of safe use in humans in the form of surgical sutures and their commercial availability in various monomer ratios and molecular weights, a wide variety of drugs ranging from small-molecular-weight therapeutic agents to peptide hormones, antibiotics, and chemotherapeutic drugs have been studied using these biodegradable polymers.[3] PLGA microparticles have proven to be successful drug delivery systems for different classes of drugs, such as nonsteroidal anti-inflammatory drugs, anticancer drugs like paclitaxel, peptides like luteinizing hormone–releasing hormone agonists, and steroid hormones. When the appropriate polymer composition with a known rate of degradation is selected, the polymers can be exploited to produce a drug delivery

system that releases the active agent at a predetermined rate. Several methods have been used in preparation of microspheres, for both natural and synthetic polymers. Emulsification by solvent evaporation is the most popular method for preparing PLGA microspheres, because of its reproducibility and the uniformity of particle size. The purpose of the present work was to develop a validated analytical method for the quantitation of anastrozole using gas chromatography/mass spectroscopy (GC/MS), to formulate anastrozole-loaded PLGA microspheres and to determine the physicochemical characteristics of the developed microspheres. In addition, the interaction of the drug with the employed polymer was also investigated.

Materials and Methods

Materials

PLGA (50:50) of different viscosity grades was purchased from Lactel International Absorbable Polymers (Pelham, AL). Anastrozole (purity 99.87%) (CAS No 120511-73-1) was purchased from Chemagis USA Inc (Mountain Lakes, NJ). Polyvinyl alcohol (PVA) (molecular weight 70000:100000 Da), clomipramine hydrochloride (purity 99.6%), dibasic potassium hydrogen phosphate (KH_2PO_4), sodium hydroxide, and hydrochloric acid (37%) were obtained from Sigma Chemical Co (St Louis, MO). Methylene chloride (high-performance liquid chromatography grade) was obtained from Fisher Scientific Co (Norcross, GA). All chemicals were used as received. Reagents used were of analytical grade, and preparation of buffer and its dilutions was done with Millie-Q demineralized double-distilled water.

Development of Anastrozole Analytical Method

Anastrozole detection by high-performance liquid chromatography was found to be inadequate because of low sensitivity and limits of quantitation.[4] Therefore, GC/MS was used to achieve adequate sensitivity and selectivity during method development. An internal standard (clomipramine hydrochloride) was used during this assay to correct for the extraction efficiency and variations in the MS response. A 5973N Agilent GC system with an MS detector (Agilent Technologies Co, Palo Alto, CA) and Chemstation analytical software was used for analysis. It was equipped with split/splitless capillary injectors, electronic pressure controllers, and direct injection liners. An HP-5MS column (J and W Scientific, Köln, Germany, 30 m × 0.25 mm × 0.25 μm film thickness) was used. The stationary phase was 100% dimethylpolysiloxane. High-purity helium (99.9%) was the carrier gas. Pressure was maintained at 15.2 psi, and makeup flow was 54.2 mL/min. The temperature was 100°C for sample intake, 280°C for detector, and 250°C for column. The split vent flow was 65 mL/min, the purge vent flow was 5 mL/min, and the splitless time was 1 minute.

Fifteen milligrams of anastrozole reference standard were dissolved in 10 mL methylene chloride to obtain a solution containing 1.5 mg anastrozole/mL. The solution was serially diluted with methylene chloride to obtain the following concentrations: 50, 100, 250, 500, 1000, and 1500 μg/mL. The solutions were spiked with 100 μL of 1 mg/mL of the internal standard in methylene chloride. The standard curves were constructed with the peak area ratio (anastrozole/internal standard) as a function of anastrozole concentration.

For the validation of the developed analytical method, percent recovery, precision, and accuracy were assessed. For recovery, 4 solutions of anastrozole were prepared in distilled water, with concentrations of 1.5, 1, 0.5, and 0.25 mg/mL. One milliliter of each solution was poured into a new tube and evaporated to dryness. For GC/MS analysis, the residues were dissolved in 1 mL of methylene chloride, spiked with 100 μL of 1 mg/mL of the internal standard solution, vortex-shaken, and transferred to microvials for analysis. Recovery was calculated by comparing the peak areas of the extracted samples to those of the analytical samples of identical theoretical concentrations. Interday and intraday statistics were generated for each sample. For accuracy, the arithmetic means ± standard deviations were determined for the 4 concentrations. The relative coefficients of variation of the measured concentrations were calculated and used to express precision.[5]

Thermal Analysis of Anastrozole

Thermal gravimetric analysis (TGA) was performed using a TA Thermogravimetrical Analyzer (SDT 2960 Simultaneous DSC-TGA, TA Instruments Co, New Castle, DE). The temperature ramp speed was set at 10°C/min, and the percentage weight loss of the samples was monitored from 25°C to 500°C. Volatiles were removed from samples by storing powders under vacuum with desiccants at 25°C for 72 hours prior to thermal studies.[6]

Preparation of Anastrozole Microparticles

PLGA microspheres loaded with anastrozole were prepared by an oil-in-water (O/W) emulsion/solvent evaporation technique as described by Sinha and Trehan.[7] The different formulations, conditions, and variables applied for the preparation of anastrozole microparticles are shown in Table 1. Typically, PVA solution was prepared in demineralized water at room temperature, heated to facilitate the dissolution of PVA, and then allowed to cool at room temperature. The oil phase consisted of the required amount of the drug dissolved in 10 mL of dichloromethane in which was dissolved the

Table 1. Composition and Encapsulation Parameters of Anastrozole-Loaded PLGA Microspheres*

Batch No	Formulation Variables						Encapsulation Parameters		
	Polymer %†	Polymer Inherent Viscosity dL/g	Drug %†	Stirring Rate	PVA %/100 mL	O/W Ratio	Targeted Drug Loading (%)	Actual Drug Loading (%)‡	EEF (%)‡
1	2.5	1.08	1.0	800	0.5	1:10	28.50	3.90	13.6
2	5.0	1.08	1.0	800	0.5	1:10	16.70	2.50	14.9
3	7.5	1.08	1.0	800	0.5	1:10	11.76	2.40	20.5
4	7.5	0.82	1.0	800	0.5	1:10	11.76	2.24	19.1
5	7.5	0.60	1.0	800	0.5	1:10	11.76	1.90	16.3
6	7.5	0.82	1.0	1200	0.5	1:10	11.76	2.05	17.4
7	7.5	0.82	1.0	1600	0.5	1:10	11.76	1.80	15.4
8	7.5	0.82	1.0	800	1.0	1:10	11.76	2.08	17.8
9	7.5	0.82	1.0	800	2.0	1:10	11.76	1.73	14.7
10	7.5	1.08	1.0	800	0.5	1:50	11.76	2.65	22.5
11	7.5	1.08	1.0	800	0.5	1:20	11.76	1.28	10.9
12	7.5	1.08	0.5	800	0.5	1:10	6.20	0.70	11.3
13	7.5	1.08	2.0	800	0.5	1:10	21.05	6.80	32.4
14	7.5	1.08	2.0	800	0.5	1:50	21.05	11.80	56.1

*PLGA indicates poly(d,l-lactic-co-glycolic acid); PVA, polyvinyl alcohol; O/W, oil-in-water; EEF, encapsulation efficiency.

†Polymer and drug concentration in methylene chloride (10 mL).

‡SDs did not exceed 2% of the reported value.

specified amount of PLGA. This oil phase was added to 50, 100, or 200 mL of PVA solution, which was stirred using a Lightnin Stirrer (General Signal Corp, Stamford, CT) at 800, 1200, or 1600 rpm for 4 hours to achieve emulsification followed by complete solvent evaporation. The resulting microspheres were harvested from the PVA solution by filtration under vacuum using Whatman No. 5 filter paper (Whatman, Inc, Florham Park, NJ). The microspheres were washed twice with distilled water and vacuum-dried in a Multiple Module Vacuum Oven (Vacuum Atmosphere Co, West Midland, UK) at room temperature for 2 days to remove any trace of solvents and extend the storage life.

Microsphere Image Analysis

The surface morphology and shape of microspheres was photographed and analyzed by scanning electron microscopy (SEM) (Nova 600 Nanolab, FEI Co, Hillsboro, OR). The samples were mounted onto aluminum specimen stubs using double-sided adhesive tape and fractured with a razor blade. The samples were sputter-coated with gold/palladium for 120 seconds at 14 mA under argon atmosphere for secondary electron emissive SEM.

Microsphere Size Distribution

The particle size distribution of the prepared microspheres was determined by mesh analysis using the ATM Model L3P Sonic Sifter Separator (John Morris Scientific, Palmerston North, New Zealand) equipped with a series of 6 screens and a pan. A ~10-g sample was tested with a pulse setting of 5, a sift setting of 5, and a total sifting time of 5 minutes.

Determination of Encapsulation Efficiency

The encapsulation efficiency (EEF) of anastrozole was determined by measuring the total amount of anastrozole present in each 10-mg sample (ie, experimental core loading) and comparing this value with the expected amount of anastrozole in each of the samples based on the drug loading during the preparation (ie, theoretical core loading). The EEF was calculated from Equation 1:

$$EEF = \frac{Dm \times 100}{Dt} \quad (1)$$

where Dt is the amount of anastrozole loaded to PLGA solution and Dm is the amount of anastrozole in the prepared microparticles.[8] Experiments were run in triplicate.

In Vitro Release of Anastrozole From Microparticles

Both the dissolution of anastrozole pure powder and its release from the prepared microparticles were performed using a modified dialysis method.[9] The release media were distilled water, 0.1N HCl (pH 1.2) or phosphate buffer (pH 7.4). An amount of microspheres equivalent to 10 mg anastrozole was suspended in 5 mL of the dissolution medium containing 0.02% sodium azide to prevent microbial growth in a 7 mL Tube-O-Dialyzer tube (1.5 cm internal diameter × 5.5 cm length). The dialysis tubes were capped with a Spectrapor dialysis membrane-windowed screw cap (molecular weight cutoff 10 000), placed in a dialysis tank filled with 25 mL of the same release medium and incubated

in a thermostatic shaking incubator (LabScientific, Inc, Livingston, NJ) adjusted at 37°C with continuous agitation at a rate of 120 rpm. At selected time intervals, during 35 days, 1 mL was withdrawn from the external release medium, and replaced by the same volume of fresh medium. The drug released in each sample was determined using a calibration curve; the reported values are averages of 3 replicates.

Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectroscopy was performed with Nicolet Impact 410 (Thermo Electron Co, Newington, NH) attached to an attenuated total reflectance (ATR) accessory. ATR was fitted with a single bounce diamond at 45° internally reflected incident light providing a sample area of 1 mm in diameter with a sampling depth of several microns. A small amount of the sample was directly placed on the diamond disk and scanned for absorbance over the range from 4000 to 500 cm^{-1} wave numbers at a resolution of 1 cm^{-1}.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) thermograms were taken using the above-mentioned TA Thermogravimetrical Analyzer in a standard aluminum pan. Nitrogen was the sweeping gas, and the heating rate was 10°C/min. Samples (5 mg) were loaded in a pan without further treatment. The initial and end temperatures were 25°C and 300°C, respectively. Indium was used as the standard reference material to calibrate the temperature and energy scales of the DSC instrument.

Results and Discussion

Anastrozole Analytical Method

TGA was done for the pure anastrozole powder (spectrum not shown) to confirm that the entire drug injected is detected by the GC/MS system at the selected detector temperature. TGA showed that 100% sample mass loss occurred at temperatures of 270ºC to 300ºC, which is in good agreement with the selected detector temperature. The retention times were 5.1 minutes for anastrozole and 5.32 minutes for the internal standard (Figure 1A). Figure 1 depicts the mass spectral profiles of the GC fractions corresponding to anastrozole and the internal standard. The quantitation of anastrozole and the internal standard is achieved via their molecular ion peaks monitoring at m/z 293 and 314, respectively. The limit of detection for this assay is ~50 ng/mL. The limit of quantitation was defined as 5 times background noise. A 0.15 μg/mL concentration was as low as this method could reproducibly quantitate anastrozole. The validated concentration range of this assay was 50 to 1500 μg/mL. Over the 3 days of validation, the mean R^2 value was 0.997, with a coefficient of variation (CV) of 0.13%; the mean slope value was 0.2282, with an SD of ±0.0134; and the mean intercept was 0.0072, with an SD of ±0.0003.

The interday and intraday precision and accuracy of the method were assessed by analyzing the samples at different concentrations, in triplicates, on the same day and on different days. The average recovery of anastrozole from aqueous solution was 98% (95.6%-102.1%) over the validated range of 0.25 to 1.5 mg/mL. For concentrations of calibration standards, the precision around the mean value ranged from 2.33% to 7.2%. Consequently, the present results indicate that this was a sufficiently accurate and precise assay method for carrying out the in vitro studies.

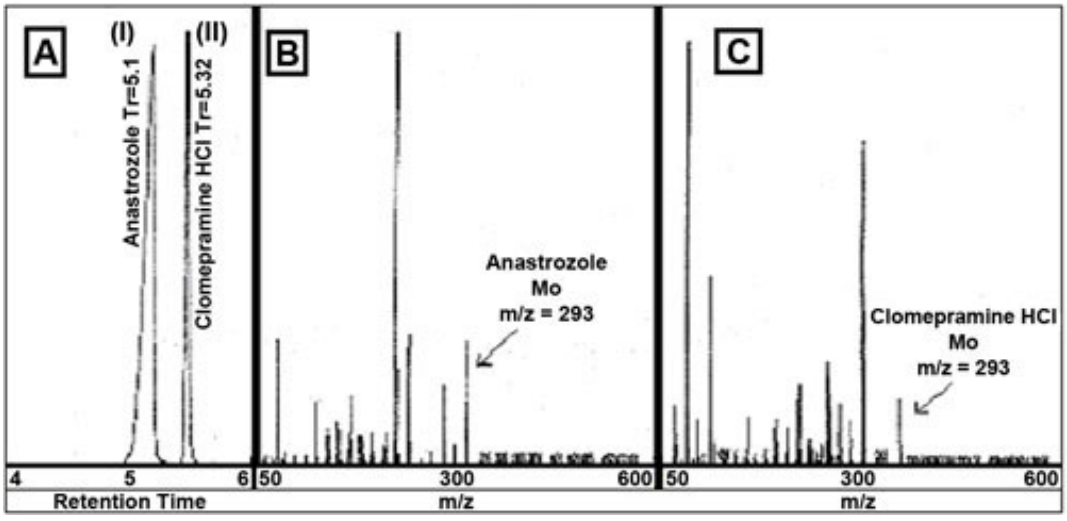

Figure 1. Typical gas chromatography/mass spectrometry reconstructed ion chromatograms of anastrozole, AI, and clomipramine hydrochloride, AII; mass spectral characteristics of anastrozole (B) and clomipramine hydrochloride (C).

Evaluation of Anastrozole Microparticles

Microsphere Morphology and EEF

The microspheres formulated were well formed, spherical, smooth, and nonporous. Moreover, anastrozole loading had no effect on the surface morphology of the prepared microparticles, compared with the blank microparticles. The EEF and the actual values of anastrozole loading within the prepared PLGA microspheres are shown in Table 1. Actual drug loadings were not close to the theoretical drug loadings because some drug partitioned into the aqueous medium during the emulsification and the evaporation of the organic solvent steps. In addition, some drug was lost in the repeated washings of the microspheres with water during the harvesting and extraction process. The results in Table 1 show that the EEF increased by increasing drug loadings, polymer loadings, polymer inherent viscosities, or the ratio of organic to aqueous phase volumes (O/W ratio). The decreased efficiency of the lower drug loadings compared with the higher loadings was assumed to be due to the higher relative percentage of the total drug lost at the steps of the fabrication process.[10] Wang et al[11] reported that the increase in the mass of the polymer results in a corresponding increase in viscosity that directly influences the stability of the emulsion and therefore the encapsulation of the hydrophilic molecules increases. The effect of increasing O/W ratio on the EEF was expected because as the volume of the aqueous phase decreases, less drug is lost to the external aqueous phase, leading to an increase in the EEF. On the other hand, a decrease in the EEF was detected by increasing both the percentage of the surfactant, namely PVA, in the aqueous phase and the stirring rate. This result could be attributed to the facilitated diffusion of the drug into the external aqueous media by increasing both the shearing force brought by the stirring paddle and the surfactant activity of PVA. It is important to note that the yield of microparticles would be drastically reduced by the absence of PVA; therefore, a small amount of PVA was needed as a stabilizer to prevent coalescence of the emulsified droplets. Consequently, to achieve the highest EEF, we prepared batch 14 using the optimal values for all the formulation variables studied. It provided an EEF of 56.1%, which was considered to be high enough for further studies.

Particle Size Distribution

The particle size distributions of the prepared microparticles are presented in Table 2. The microparticles formulated were found to be in the size range of 420 to 75 μm. Increases in the polymer loadings, drug loadings, polymer viscosities, or O/W ratios were accompanied by increases in the portion of prepared microparticles that had higher particle sizes. The increase in the particle size with increasing drug loadings or polymer concentrations and inherent viscosities has been observed by other authors for PLGA polymers.[12,13] This was probably caused by the increasing viscosity of the dispersed phase (polymer solution), resulting in poor dispersibility of the PLGA solution into the aqueous phase. There is likely to be higher viscous resistance against the shear forces during the emulsification. Coarse emulsions are obtained at higher polymer concentrations and viscosities, which led to the buildup of bigger particles during the diffusion process. This fact is explained by the greater probability that the desolvated macromolecules (or small aggregates formed from these molecules) coalesce in a more concentrated solution, thereby forming larger particles.[14] Increasing the O/W ratio led to a decrease in particle size distribution. The coalescence of droplets can be prevented by a large amount of aqueous phase available for diffusion in the O/W emulsion and hence smaller particles were produced.[15] An increase in the fraction of

Table 2. Particle Size Distribution of Anastrozole-Loaded PLGA Microspheres*

Batch No	420:300 μm	300:180 μm	180:106 μm	106:75 μm	<75 μm
1	0.6	34.3	58.0	5.9	1.2
2	3.1	60.5	33.3	1.9	1.2
3	19.0	65.6	12.3	2.3	0.9
4	7.0	30.6	56.2	5.7	0.5
5	6.7	28.0	55.5	8.1	1.7
6	5.4	20.8	58.8	12.7	2.3
7	3.2	16.2	60.8	18.5	1.3
8	3.0	30.4	60.0	5.5	1.1
9	1.0	23.3	63.8	10.2	1.6
10	9.8	74.3	10.2	5.1	0.6
11	12.7	42.0	41.8	2.9	0.6
12	5.3	50.1	39.2	4.6	0.8
13	6.0	72.8	7.1	11.3	2.8
14	38.5	36.4	18.6	3.6	2.9

*Values in the table are percentages of total weight, with SDs not exceeding 2% of the measured value. PLGA indicates poly(d,l-lactic-co-glycolic acid).

small particles was also observed by increasing either the stirring rate from 800 to 1600 rpm or the concentration of PVA in the external medium from 0.5% to 2% (wt/vol). To obtain emulsified systems, the addition of energy is a fundamental step. Increasing the stirring rate produces smaller emulsion droplets through stronger shear forces and increased turbulence.[16] On the other hand, a high concentration of emulsifier led to a narrower granulometric distribution. This phenomenon can be expected from the stabilizing function of an emulsifier. It is easy to understand that an insufficient amount of emulsifier would fail to stabilize all the microparticles, and thus some of them would tend to aggregate. As a result, larger microparticles might be produced.[17]

Release Studies

It is reasonable to conclude that the release profiles of anastrozole from the microparticles in all cases showed 2 distinct phases. An initial burst release phase occurs within the first week, followed by a gradual release phase. The release profiles of anastrozole from the microparticles could be explained by 2 diffusion processes, one occurring after the other. The diffusion of the aqueous dissolution medium into the matrix results in the dissolution of the drug, which is then followed by drug diffusion through the pores into the dissolution medium.[18] The release of anastrozole from batch 3 was performed in distilled water and at different pHs (results not shown). It was found that the pH change had a negligible effect on the release of the drug from the PLGA microspheres. Anastrozole does not contain any ionizable groups and therefore does not ionize at any pH. Its solubility, then, is practically unaffected by the pH change.[19] Consequently, all the release studies performed afterward were conducted in distilled water. The drug release rate increased with the increase in anastrozole loading or O/W ratio during fabrication of microparticles (Figures 2 and 3).

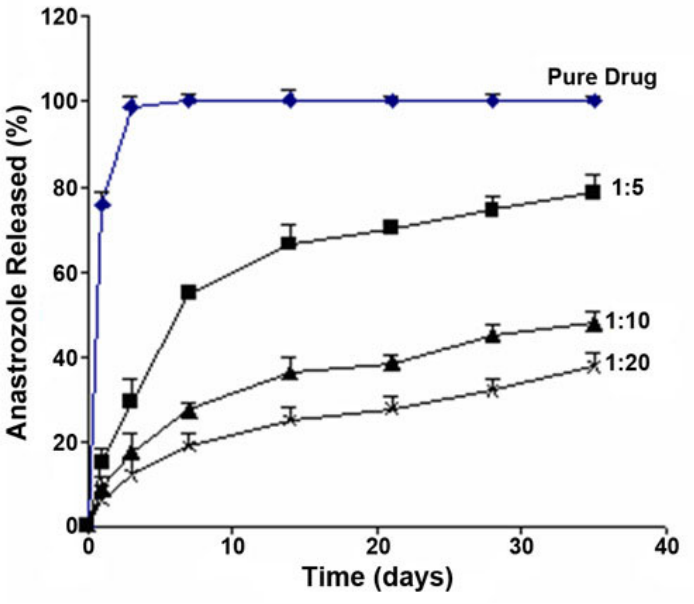

Figure 3. Release profile of anastrozole from poly(d,l-lactic-co-glycolic acid)–based microspheres as a function of ratio of organic phase to aqueous phase volumes during microparticle formulation (n = 3).

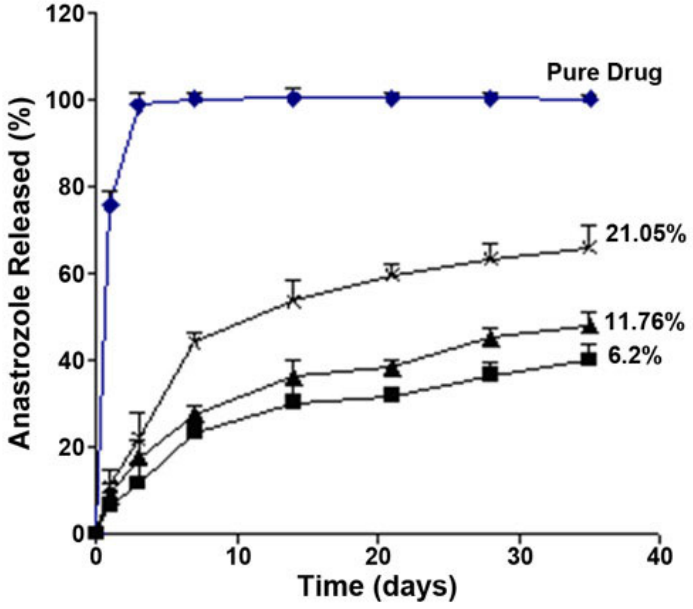

Figure 2. Release profile of anastrozole from poly(d,l-lactic-co-glycolic acid)–based microspheres as a function of drug loading (n = 3).

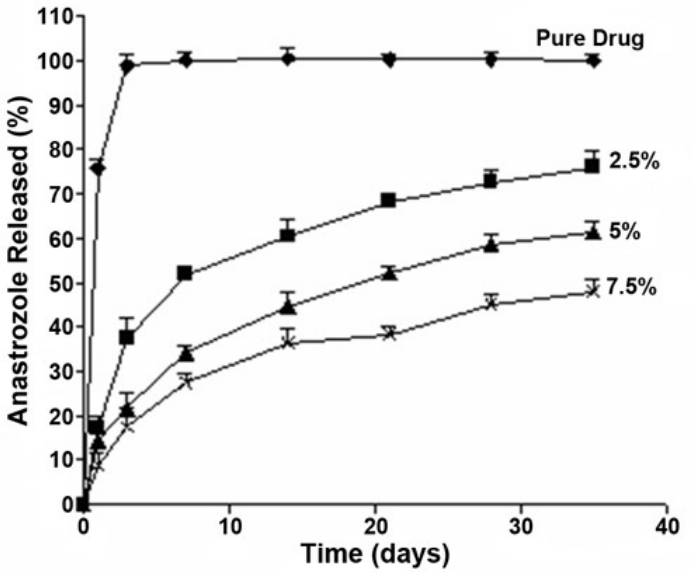

Figure 4. Release profile of anastrozole from poly(d,l-lactic-co-glycolic acid)–based microspheres as a function of polymer loading (n = 3).

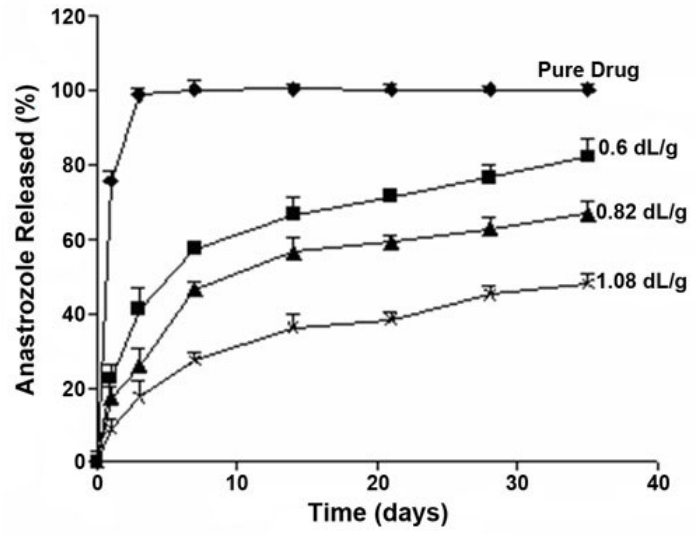

Figure 5. Release profile of anastrozole from poly(d,l-lactic-co-glycolic acid)–based microspheres as a function of polymer inherent viscosity (n = 3).

At higher drug loadings, the path length shortened and the drug closer to the surface leached out into the release medium. This created empty pores with a corresponding decrease in force, which enabled drugs in the inner portion to leach out at a faster rate. A similar case was reported by Chen and Singh[20] for the encapsulation of testosterone within a PLGA-based in situ gel-forming delivery system. The authors reported that increased drug loading provided simpler pathways, lower tortuosity, and greater porosity for diffusion and facilitation of the water movement into and out of the matrix. On the other hand, increasing the O/W ratio led to increasing the amount of the drug encapsulated, which in turn increased the drug release rate.

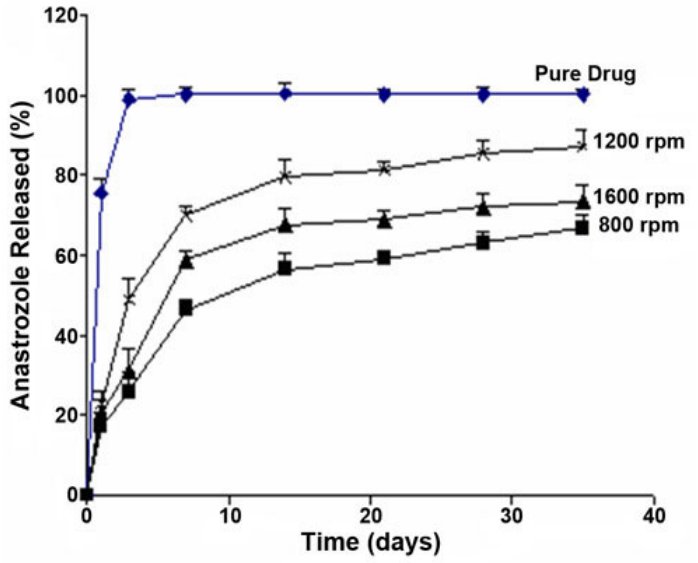

Figure 6. Release profile of anastrozole from poly(d,l-lactic-co-glycolic acid)–based microspheres as a function of the stirring rate applied during microparticle formulation (n = 3).

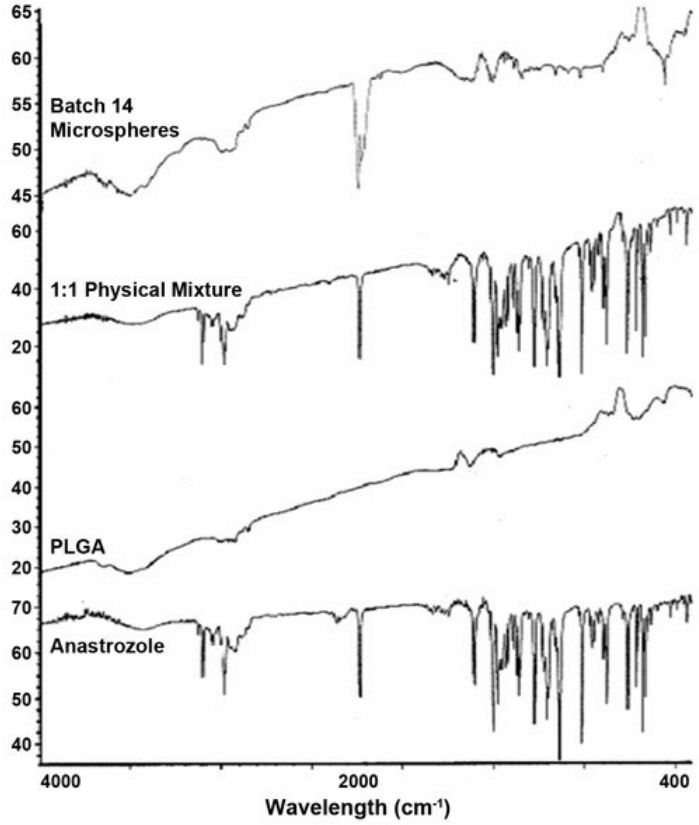

Figure 7. Fourier transform infrared spectra of pure anastrozole, blank PLGA 50/50 microspheres, 1:1 physical mixture of anastrozole and PLGA, and anastrozole-loaded PLGA microspheres (batch 14). PLGA indicates poly(d,l-lactic-co-glycolic acid).

The drug release rate decreased with the increase in polymer concentrations and viscosities or stirring rate (Figures 4, 5, and 6). The viscosity grade or the molecular weight of a polymer is known to influence the rate of drug release from microparticles. The permeability of the polymer decreases with increasing viscosity grade, which yields a decreased rate of drug release.[21] The PVA concentration in the external phase was inversely proportional to the amount of drug released (results not shown). Although this effect was not significant enough to influence the release patterns, it may have affected the amount of drug encapsulated within the prepared microspheres. Increasing the surfactant level in the aqueous phase led to a decrease in the EEF that was accompanied by a slow drug release rate. On the other hand, an increase in the stirring rate from 600 to 1600 rpm (batches 4, 6, and 7) during the microparticle fabrication was accompanied by an increase in both the initial burst of the drug and the extent of the drug released within 35 days. Changing the stirring rate may have affected the particle size distribution. Increasing the mixing speed generally results in decreased microsphere mean size.[22]

FTIR-ATR Studies

The FTIR spectra of anastrozole-loaded PLGA microspheres, the 1:1 physical mixture, and the individual components are depicted in Figure 7. No differences in the positions of the absorption bands were observed in spectra of the anastrozole physical mixture with PLGA, indicating that there are no chemical interactions in the solid state between the drug and the polymer. As shown in Figure 7, no difference was observed for the absorption of the cyanide groups of the drug in the spectra of its PLGA microspheres. Moreover, the ATR spectrum of the drug-loaded microspheres is dominated by the PLGA absorption, with only a minor contribution of the anastrozole bands. The ATR technique measures to a depth of only 1 to 2 μm, so only the drug at the surface of the microspheres is detected.[23] Because of the wavelength-dependent penetration depth of the IR radiation in the ATR technique, the spectrum is biased toward absorption bands at lower energy (smaller wavenumber). As a result, no chemical interaction between the drug and the investigated polymer was found.

DSC Analysis

Figure 8 shows the DSC thermograms of the same samples used for FTIR analysis. The DSC trace of anastrozole showed a sharp endothermic peak at 83.3°C, its melting point. The physical mixture of anastrozole and PLGA showed nearly the same thermal behavior as the individual components, indicating that there was no interaction between the drug and the polymer in the solid state. The absence of the endothermic peak of the drug at 83.3°C in the DSC of its PLGA-based microspheres suggests that the drug existed in an amorphous or disordered-crystalline phase as a molecular dispersion or a solid solution state in polymeric matrix.[24]

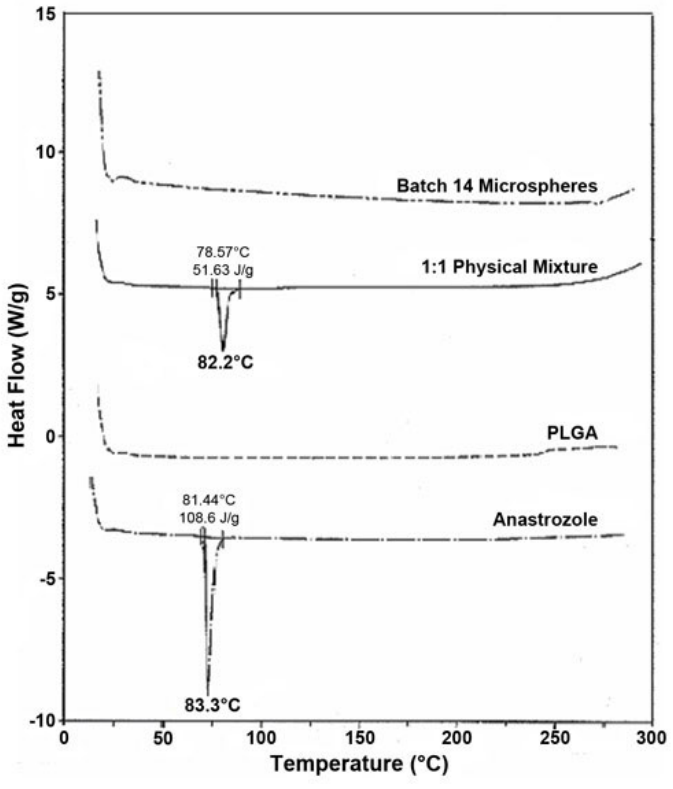

Figure 8. Differential scanning calorimetry thermograms of pure anastrozole, blank PLGA 50/50 microspheres, 1:1 physical mixture of anastrozole and PLGA, and anastrozole-loaded PLGA microspheres (batch 14). PLGA indicates poly(d,l-lactic-co-glycolic acid).

CONCLUSION

The highest entrapment of anastrozole within PLGA microspheres could be obtained by tailoring the formulation variables. DSC analysis and release kinetics showed that anastrozole was uniformly dispersed in an amorphous or solid solution state in the polymer matrix. A sustained and localized delivery system of anastrozole was formulated to provide anticancer therapy for at least 35 days. Studies on the animal experiments are in progress.

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