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Double-walled microspheres for the sustained release of a highly water soluble drug: characterization and irradiation studies

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Abstract

Composite double-walled microspheres with biodegradable poly(L-lactic acid) (PLLA) shells and poly(D,L-lactic–co-glycolic acid) (PLGA) cores were fabricated with highly water-soluble etanidazole entrapped within the core as solid crystals. This paper discusses the characterization, in vitro release and the effects of irradiation on this class of microsphere. Through the variation of polymer mass ratios, predictable shell and core dimensions could be fabricated and used to regulate the release rates. A direct and simple method was devised to determine the composition of the shell and core polymer based on the different solubilities of the polymer pair in ethyl acetate. A distribution theory based on solubility parameter explains why highly hydrophilic etanidazole has the tendency to be distributed consistently to the more hydrophilic polymer. Release profiles for normal double-walled samples have about 80% of drug released over 10 days after the initial time lag, while for irradiated double-walled samples, the sustained release lasted for more than 3 weeks. Although sustained release was short of the desired 6–8 weeks required for therapy, a low initial burst of less than 5% and time lags that can be manipulated, allows for administration of these microspheres together with traditional ones to generate pulsatile or new type of releases. The effects of irradiation were also investigated to determine the suitability of these double-walled microspheres as delivery devices to be used in conjunction with radiotherapy. Typical therapeutic dosage of 50 Gy was found to be too mild to have noticeable effects on the polymer and its release profiles, while, sterilization dosages of 25 kGy, lowered the glass transition temperatures and crystalline melting point, indirectly indicating a decrease in molecular weight. This accelerated degradation of the polymer, hence releasing the drug.

Keywords: Double-walled reservoir microspheres; Etanidazole; Radiosensitizer; Poly(D,L-lactic–co-glycolic acid); Poly(L-lactic acid); Irradiation

1. Introduction

Drug discovery alone is insufficient in treating diseases; often correct dosing and targeting are equally important for clinical success. Researches in controlled drug release systems specifically look into

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these areas to enhance the efficacy of therapeutics for specific treatment regimens such as chemotherapy. Traditional microsphere drug delivery systems using a single polymer have several inherent flaws such as high initial burst [1], low encapsulation efficiency for highly water soluble drugs [2], inability to lend themselves to pulsatile or zero order release and lack of sustained release for periods suitable for periodic therapy [3].

Composite double-walled microspheres adapted for the encapsulation of a highly water-soluble radiosensitizers, etanidazole (SR-2508, NSC-301467, EF5; 2-nitroimidazole) have the ability to circumvent some of these limitations. They are also advantageous over many earlier attempts to simply coat existing microspheres using pan coating, fluidized beds or spray drying [3–5]. The effect of a shell and core structure, with drug encapsulated only in the core, presents additional barriers to diffusion, eliminating initial bursts, shielding the encapsulated drug from external mediums, and therefore reducing leaching. This lends itself to new types of release profiles.

Clinical trials carried out with the family of nitroimidazole hypoxic cell radiosensitizers, to which etanidazole belongs, have yielded disappointing results. This is partly due to systemic toxicities, which develop when the drug was administered systemically over a long treatment schedule [6,7]. It would appear that delivery of etanidazole by biodegradable polymers could be the appropriate vehicle for intratumoral delivery of this drug for prolonged periods and with minimal systemic side effects.

Therefore, it was the goal of this study to adapt methods of double-walled fabrication [8,9] with modifications, for the successful encapsulation of highly water-soluble etanidazole, resulting in reduced initial bursts as well as sustained release profiles suitable for the radiosensitizers. Simple characterization and identification methods developed to distinguish between the shell and core polymer compositions and the locality of the drug would further enable us to explain and predict expected release patterns. Moreover, since radiosensitizers are most commonly administered together with radiotherapy after tumor surgery to reduce the dosages applied to patients [10–13], the subjection to irradiation would be inevitable for the delivery system. Hence, the study also calls for the effects of γ-irradiation on release profiles, both in smaller clinical doses of 50 Gy [14], and industrial sterilization of 25 kGy, to be examined.

2. Materials and methods

2.1. Materials

Poly(L-lactic acid) (PLLA, mw 85 000–160 000), poly(D,L-lactic–co-glycolic acid 50:50) (PLGA, mw 40 000–75 000), etanidazole (mw 214) and poly-(vinyl alcohol) (PVA) (mw 31 000–50 000) were all purchased from Sigma–Aldrich, USA and used without modification. HPLC grade acetonitrile was purchased from Fisher (Fair Lawn, NJ, USA). Phosphate buffer saline (PBS) used for in vitro release study was obtained from Pierce (Rockford, USA) containing 0.1 M sodium phosphate, 0.15 M sodium chloride, pH 7.2. All other materials or solvents used were of analytical grade.

2.2. Preparation of double-walled microspheres

Fabrication of double-walled microspheres combines the phenomenon of phase separation of two polymers in organic solvent when critical concentrations are attained and the process of solvent evaporation [8]. Etanidazole loaded microspheres were prepared by this modified oil-in-oil-in-water (O/O/W) emulsion solvent evaporation technique, utilizing the polymer incompatibility between PLLA and PLGA which results in their complete phase separation [9].

Separate solutions of PLLA and PLGA in dichloromethane (DCM) (15–20%, w/v) were prepared. Typical DCM volumes used were between 335 and 1000 μl. The preparation of the PLGA polymeric solution slightly differs, in that the drug was added to DCM, sonicated using an ultrasonic probe (model XL2000, Misonix, NY, USA) at 2 W output for 30 s to break down the drug crystals into smaller filaments (<20 μm), prior to the addition of the polymer PLGA. The two polymeric solutions were then added together and sonicated at 2 W for 20 s to create an oil-in-oil (O/O) emulsion, evident with the originally clear polymeric solutions become

translucent with a milky look. Addition of the emulsion dropwise into 200 ml of nonsolvent of PVA aqueous solution (2.5%, w/v) creates an O/O/W emulsion. Stirring using a mechanical stirrer (IKA Eurostar Model TD, Germany) at the rate of 250 rpm for 4 h allows for the extraction and evaporation DCM as well as the hardening of the microspheres. Filtration, washing and freeze-drying under vacuum (Christ Alpha1-2 Freeze Dryer Model 100200, Germany) followed. Fabricated microspheres were stored in a desiccator to prevent hydrolytic degradation of the biodegradable polymer under humidity.

Microspheres having varying shell thickness and core diameter were prepared in the same manner by altering the polymer mass ratio (w/w) of PLLA and PLGA ranging from 3:1 to 1:1. Single polymer (PLLA and PLGA) microspheres intended for characterization and baseline comparison were also prepared using the well-established single emulsion method commonly found in the controlled release literature [1,2].

2.3. Characterization

The morphology of both unloaded and etanidazole loaded microspheres were studied with a scanning electron microscope (SEM, JSM-5600LV, Jeol, Tokyo, Japan), where the surface and cross-sectional morphology as well as degradation of the microspheres at various stages of in vitro release were investigated. The microspheres to be examined were first cross-sectioned using a microtome blade with a frozen holding media and mounted onto metal stubs with double-sided carbon tape. The samples were air-dried before being coated with a layer of platinum using an auto fine coater (JFC-1300, Jeol).

Observations using optical microscope (Olympus Vanox Model BX60, Japan) were carried out to identify different polymer layers in the double-walled microspheres based on the difference in crystalline structures as well as to identify the distribution of the drug within the loaded microspheres by noticing the distinct yellow crystalline drug filaments distribution. In preparation for optical microscopy, the microspheres were sectioned using a microtome blade and mounted onto glass slides for viewing under cross Polaroid.

Particle size distributions and mean particle sizes were determined using Coulter laser diffraction particle size analyzer (Beckman Coulter, Coulter LS 230, Small volume module, CA, USA). Microspheres were suspended in ultrapure water and allowed to flow through the analyzer.

A novel method for determining the composition of the core and shell polymer was devised especially for our study to be applied to the unique polymer pair. The method has its basis in the different solubility of the polymer pair PLLA and PLGA in ethyl acetate. PLGA is soluble but not PLLA. The double-walled microspheres were first cross-sectioned approximately at the centerline. Each half is then immersed individually into a small amount of ethyl acetate for dissolution for about 10 min with little or no agitation. The remnant of the cross-sectioned microsphere is then removed for optical observation. The solution is also examined to ensure that the core has not fallen out in any case. Hence, two possible scenarios of either a hollow core or the remnant of a core could result depending on whether the core or the shell dissolves. Optical microscopic observations of cross-sectional views would enable us to identify remaining PLLA polymer to be either that of the shell or the core and if they were completely phase separated.

This method would be employed together with IR study using Fourier transformed infra-red (FTIR) spectra obtained using FTIR microscope (Bio-Rad UMA 500) connected to FTIR spectrophotometer mainframe (Bio-Rad FTS-3500 ARX) and analyzed using Bio-Rad analysis software in the mid IR range (wave number 400–4000 cm^{-1}, resolution 2 cm^{-1}). Standard microspheres of single polymer and double-walled composite microspheres were cross-sectioned into halves and mounted on a gold slide for examination. Ten points were randomly selected in the core and shell using the software to obtain the transmission spectra. An average of these spectra were then obtained and compared with that of the single polymer microspheres, used as reference for analysis of the composition of respective zones.

Encapsulation efficiency is defined as the ratio of actual to theoretical loading of the drug within the microspheres as described in equation

$$\text{Efficiency}\ (\%) = \frac{C_{\text{actual}}}{C_{\text{theoretical}}} \times 100 \qquad (1)$$

where C_{actual} (mg) is the actual amount of drug contained in microspheres and $C_{theoretical}$ (mg) the theoretical loading that is equal to total amount of drug used initially. The actual amount of drug encapsulated within the microspheres was determined using an extraction method where 5 mg of microspheres were accurately weighed out in triplicate and dissolved in 2 ml of DCM, chloroform or dimethyl sulfoxide (DMSO) each.

Extraction of the drug was carried out with the use of 5 ml of deionised water where the water-soluble drug etanidazole will preferentially partition. The solution with two immiscible phases is then centrifuged at 90.6 *g* for 10 min before the top layer of water is extracted, filtered of any residual particles and analyzed for its drug concentration using high-performance liquid chromatography (HPLC) (Agilent HPLC series 1100, USA). The mobile phase selected for analyzing etanidazole was 5% acetonitrile in water, delivered at a flow-rate of 1 ml/min through a reverse phase Inertsil ODS-3 column (C_{18}, ∅ 5 μm, 4.6×200 mm, GL Science, Tokyo, Japan). From the glass sample bottle, 25 μl was injected using an autoinjector and detected at 324 nm. The amount of drug was calculated by integrating the peak area using the HP CHEMSTATION software (Hewlett-Packard REV A.07.01, USA). A standard curve was built up in the same process.

For release studies, etanidazole loaded microspheres (5±0.5 mg) were accurately weighed in triplicates and placed in vials containing 1.8 ml of PBS (pH 7.2, Pierce, Pockford, IL, USA). The vials were maintained at physiological temperature of 37 °C in a thermostat oscillating waterbath at 120 rpm (Cetomat WR, Braun Biotech, Germany). A 1.8 ml volume of the aliquots were collected at preselected times after centrifugation (Kubota Centrifuge Model 2100, Japan) at 90.6 *g* for 5 min and the vials replaced with the same amount of freshly prepared PBS. The etanidazole content in the supernatant was analyzed using the HPLC system as described above. The peak areas obtained were compared against calibration to determine the drug concentration and the fraction of drug released at each data point calculated. A fresh amount of PBS was added to the microspheres to replace the removed supernatant.

Irradiations of samples were carried out using a Gamma Chamber (^{60}Co. source, half life 5.27 years) with dosage of 50 Gy, 25 kGy applied to the samples at a dose rate of 2.5 Gy/h. Dry ice was added to the sample during the course of radiation to lower the local temperature of the sample and to prevent the sample from undergoing thermal degradation. This is a common practice when high irradiation doses are employed [15,16].

Thermal analysis of the microspheres was performed using a modulated differential scanning calorimeter (DSC, DSC 2920 modulated DSC, TA instruments) equipped with controller (TAC 7/DX, TA instruments) connected to a cooling system. The samples (about 6.5 mg) were placed in sealed aluminum pans and were subjected to heating from −20 °C to 200 °C for the first heating ramp, cooled to −10 °C and reheated on the second ramp to 200 °C all at a rate of 10 °C/min. Data obtained were processed on TA universal analyzer software and glass transition temperatures (T_g) and crystalline melting points (T_m) identified. For the purpose of degradation studies, data obtained from the second heating ramp were used, since we are interested in the equilibrium state of the polymer in that if changes have resulted due to hydrolytic cleavage and information on the polymer crystallized under the same conditions.

Degradation studies were carried out according to the following procedures: loaded microspheres and blank microspheres (20±5 mg) were each accurately weighed and placed in vials containing 10 ml of PBS buffer maintained at 37 °C in a thermostat oscillating waterbath at 120 rpm. The microspheres were removed at predesignated times for extensive study using SEM and DSC. SEM studies were carried out on the loaded microspheres to study the effect of polymer degradation on drug release and the relation between polymer physical properties and characteristics points in the release profile. While, blank microspheres were intended for thermal DSC study to characterize any change in polymer T_g and T_m under degradation.

3. Results

3.1. Preparation of double-walled microspheres

The combination PLLA and PLGA in DCM at

above their critical concentrations result in the exhibition of immiscibility [9]. Application of the ultrasonic probe dispersed the two polymeric mixtures into an oil-in-oil emulsion, which was evident from the change of the clear polymeric solutions to a translucent and milky solution. The dispersion of this O/O emulsion into an aqueous continuous phase of nonsolvent PVA with mechanical stirring eventually resulted in the formation of an oil-in-oil-in-water (O/O/W) emulsion. Extraction of DCM ensued by evaporation hardens the microspheres. The resulting microsphere (Fig. 1A) can be classified as a class of reservoir-dispersed matrix system where etanidazole was dispersed within the core, and the shell acts as a rate-limiting barrier to drug release.

By keeping the fabrication conditions constant it was shown that the particle size distribution between batches could be kept relatively constant. Different fabrications of PLLA/PLGA mass ratio 2:1 and 1:1, yielded volume mean diameter of 432.3 (±179.2) μm, and 422.3 (±174.5) μm, respectively. While altering the mass ratios of PLLA to PLGA, the thickness of the shell wall and the diameter of the core can be varied. This is shown in Fig. 1B–D, where double-walled microspheres having mass ratios of PLLA to PLGA ratio (w/w) from 1:1 to 2.5:1 were deliberately fabricated to be relatively large (~450 μm), to allow for easy cross-sectioning at the microspheres' centreline with reasonable accuracy with the microtome blade.

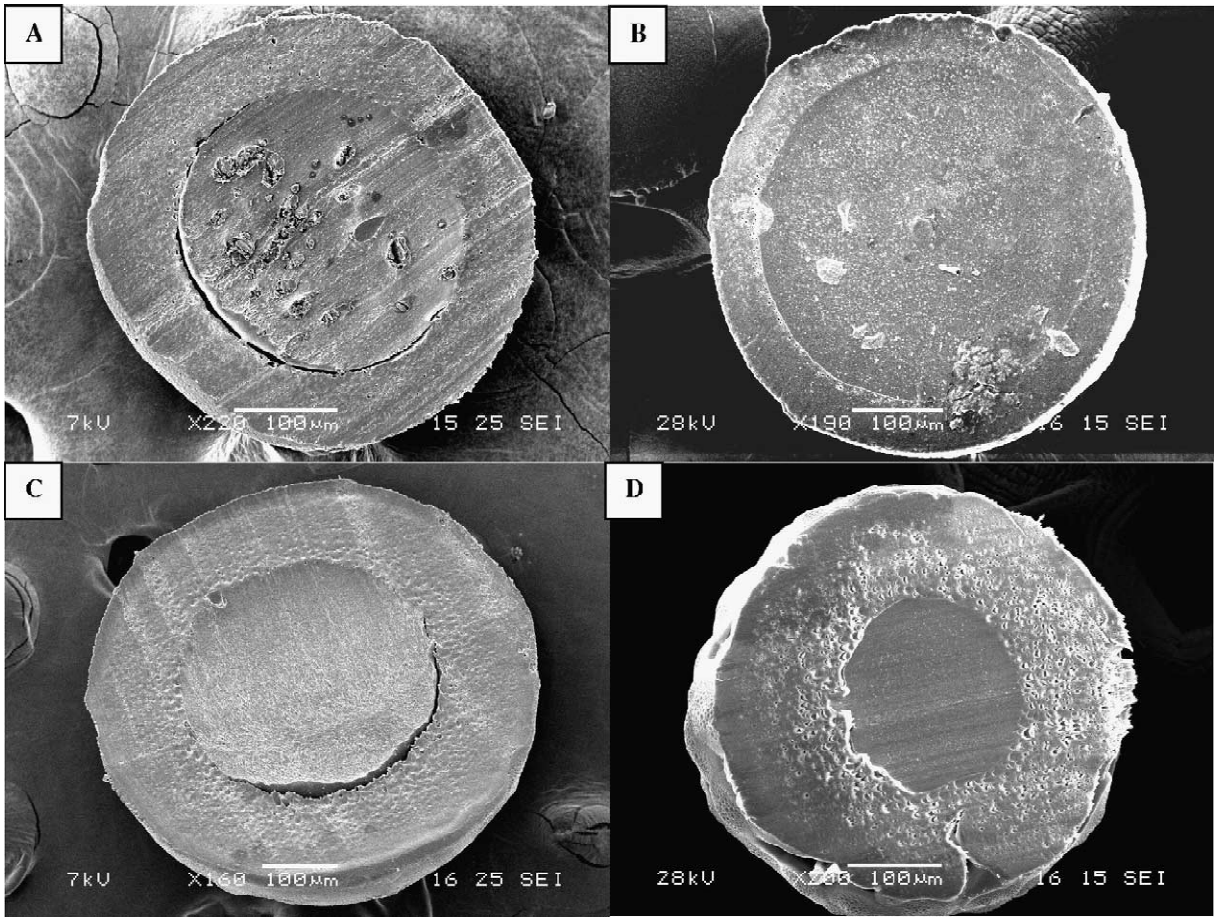

Fig. 1. SEM photographs of cross-sectional view of double-walled microspheres. (A) Etanidazole loaded microsphere, PLLA–PLGA (2:1); (B) unloaded microsphere, PLLA–PLGA (1:1); (C) unloaded microsphere, PLLA–PLGA (2:1); (D) unloaded microsphere, PLLA–PLGA (2.5:1).

3.2. Identification of shell and core polymer composition

Cross-sectional views of doubled-walled microspheres after dissolution in ethyl acetate was observed under SEM (Fig. 2). Optical observations showed the configuration of a hollow core with a surrounding solid shell that was insoluble in the solvent consistently for various batches of fabrications. No particles that are of the size of the core were observed to be residual in the small amount of ethyl acetate added. Close up observation of the remnant shell revealed it to be porous (Fig. 2C) while numerous small spheres can be found in the void vacated by the dissolved core (Fig. 2D). Therefore, based on known solubility of the PLGA and insolubility of PLLA in ethyl acetate, the shell and core composition can be positively identified as PLLA and PLGA, respectively. Although each is impregnated with small spheres of the other polymer, which results in pores in the shell, and remnant small spheres after PLGA has been dissolved.

FTIR spectra of PLLA and PLGA produced distinguishable spectra in the wave numbers region from 1300 to 1500 cm^{-1}. The cross-sections of the double-walled microspheres were analyzed using FTIR microscope, where the shell and core regions were scanned separately. Comparisons with the

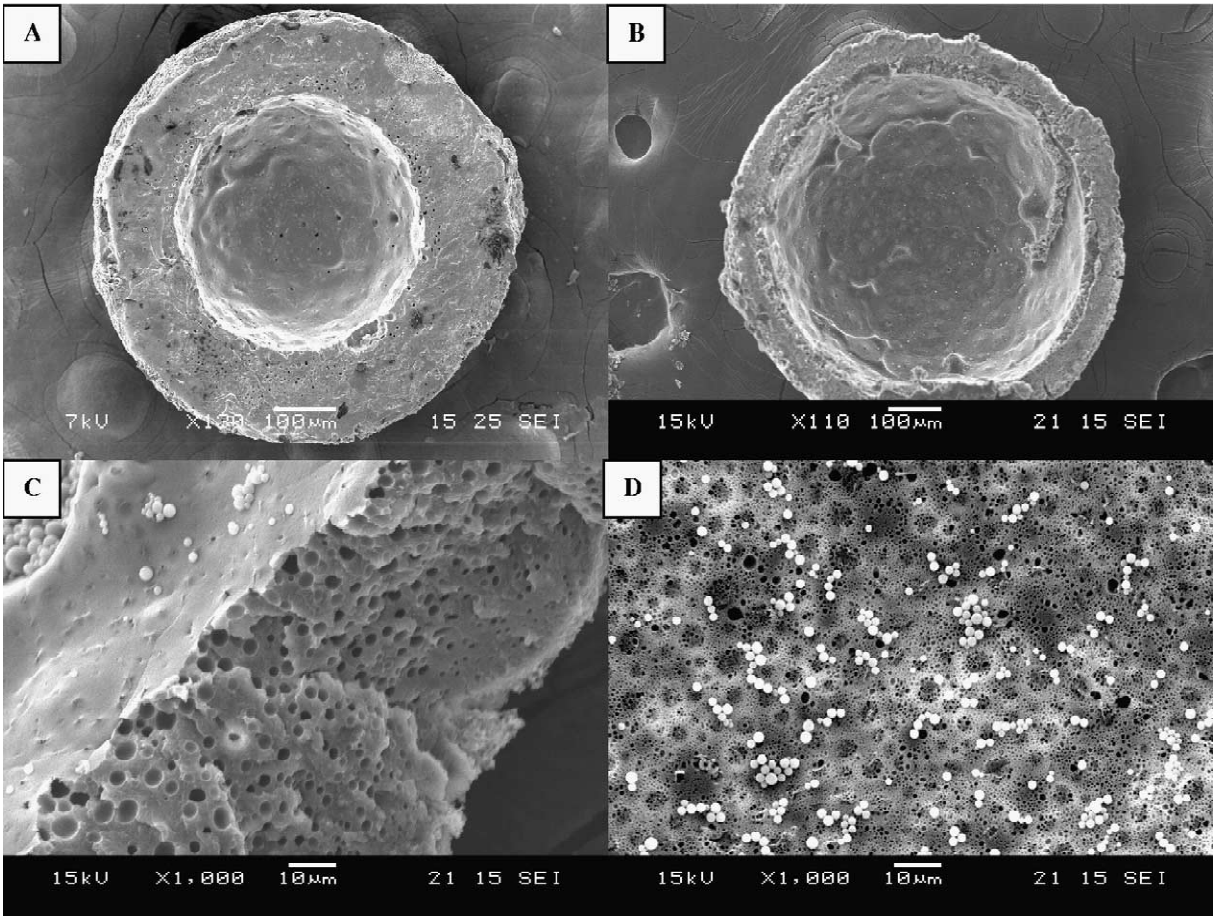

Fig. 2. SEM photographs of unloaded double-walled microspheres after dissolution with ethyl acetate. (A) PLLA–PLGA (2:1); (B) PLLA–PLGA (1:1); (C) close-up view of porous shell; (D) close-up of small sphere left in void vacated by dissolved core.

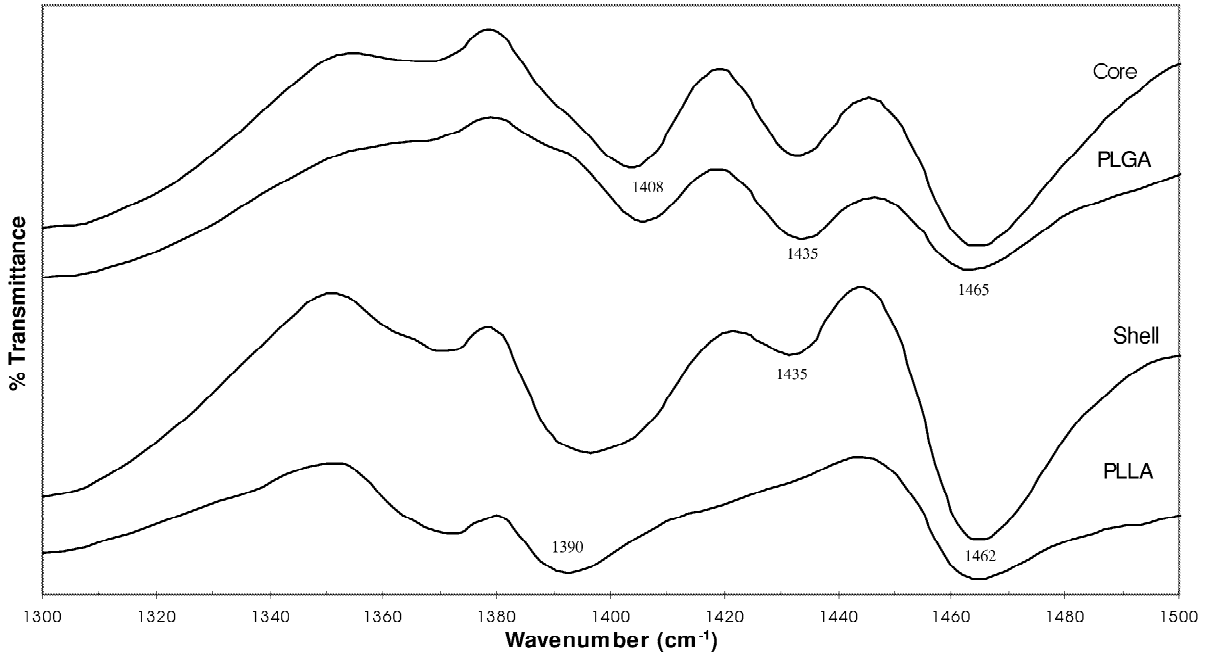

Fig. 3. FTIR spectra identifying the shell and core polymers of double-walled microsphere with polymer ratio, PLLA–PLGA (2:1).

spectra of pure polymers allowed for positive identification of the composition (Fig. 3) via known characteristic wave numbers of the polymers [9].

PLLA showed a C–H bending vibration of methyl group at 1390 and 1462 cm^{-1} while PLGA showed a C–H bending vibration of methyl group at 1408 and 1465 cm^{-1} and an additional C–H vibration of methylene group at 1435 cm^{-1}. The spectra of the core correspond closely to that of PLGA while the shell to PLLA except for an additional a peak at 1435 cm^{-1}. However, this peak is much smaller relative to that at the other two characteristic peaks and could possibly be due to small amount of PLGA entrapped within as shown by the porous structure of the shell after they have been dissolved.

DSC thermograms of double-walled microspheres studied showed two glass transition temperatures, one each for PLLA and PLGA, and a single crystalline melting point for PLLA, since PLGA is amorphous and has no crystalline melting point. The points are identified in DSC thermograms where they plotted together with the thermograms of the pure PLLA and PLGA polymers that were used as control. Their respective T_g and T_m values are shown in Table 1.

SEM and optical microscopy observed drug crystal distribution in the translucent polymeric microspheres to be within the core only as shown in Fig. 4. Etanidazole existed within the core layer as dispersed solid crystal filaments that are slightly yellowish. Microspheres of single polymer demonstrated a low

Table 1
Thermal properties of pure polymers and double-walled microspheres obtained from DSC thermograms

	Glass transition temperature, T_g (°C)	Crystalline melting point, T_m (°C)
PLGA 50:50	39.85	–[a]
PLLA	60.80	174.04
PLLA–PLGA (2:1)	47.72, 60.44[b]	173.54
PLLA–PLGA (1:1)	47.54, 59.88[b]	173.44

[a] PLGA 50:50 is amorphous and has no crystalline melting point.

[b] The two glass transition temperatures refer to those of PLGA and PLLA, respectively, in the composite.

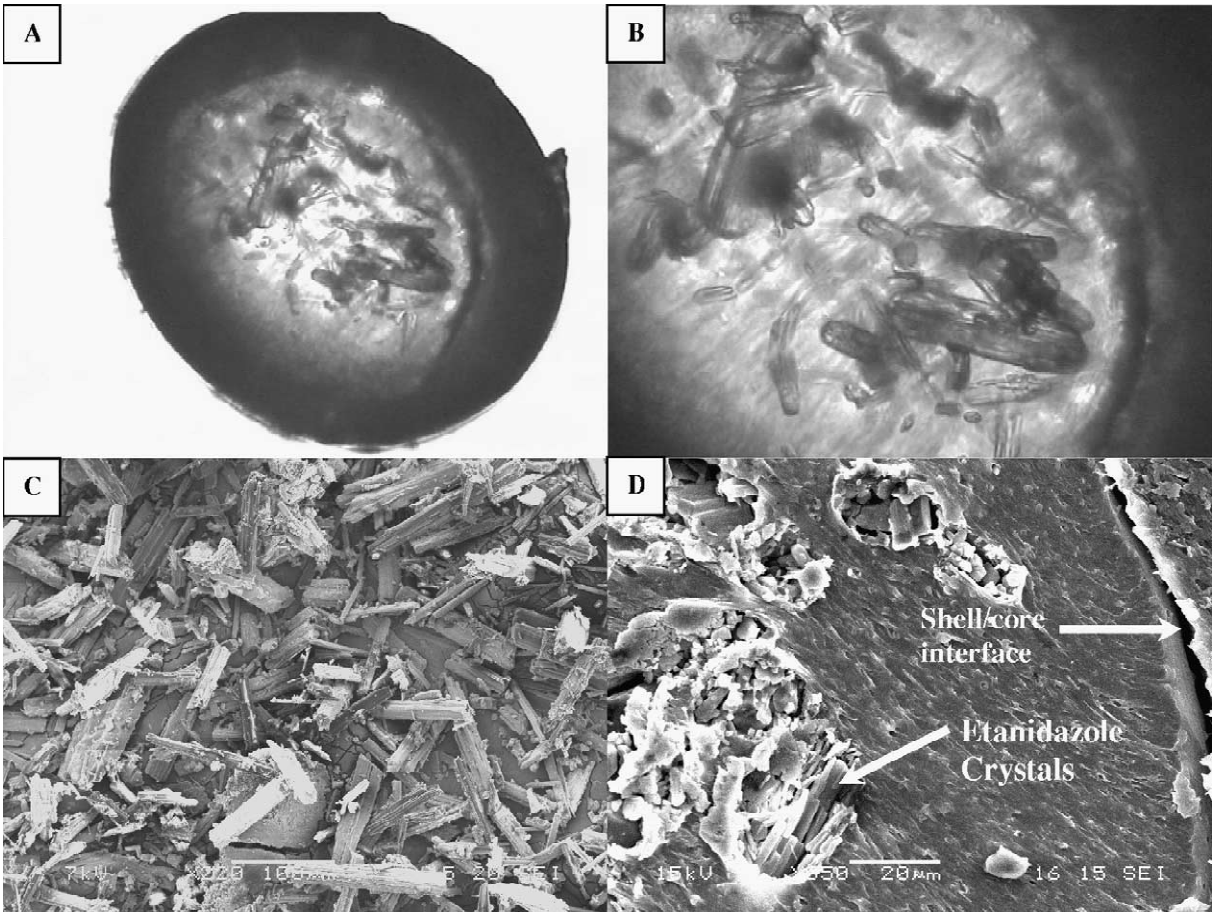

Fig. 4. (A) Optical photographs of double-walled microspheres with drug in the core, polymer ratio PLLA–PLGA, 2:1. (B) Close-up on drug crystals. (C) SEM of etanidazole crystals. (D) SEM close-up showing etanidazole in the core.

efficiency of entrapment below 50% for the highly water soluble etanidazole (100 mg/ml). The fabrication using a double-walled configuration, however, was able to produce encapsulation of 55% and higher. That is a consistent 5–10% improvement over each of the two single polymer systems as Table 2 illustrates.

3.3. In vitro release

The cumulative percentage of etanidazole release from the two formulations of PLLA–PLGA (2:1) and PLLA–PLGA (1:1), with 5% drug loading were examined in vitro in PBS buffer for a period of up to 2 months (Fig. 5A). The profiles exhibited a low

Table 2
Comparison of encapsulation efficiency of different fabrications

Sample	Encapsulation efficiency (%)
PLLA single polymer	49.38±1.77
PLGA single polymer	39.59±1.89
Double-walled PLLA–PLGA (2:1, w/w)	56.69±0.21
Double-walled PLLA–PLGA (1:1, w/w)	54.83±0.30

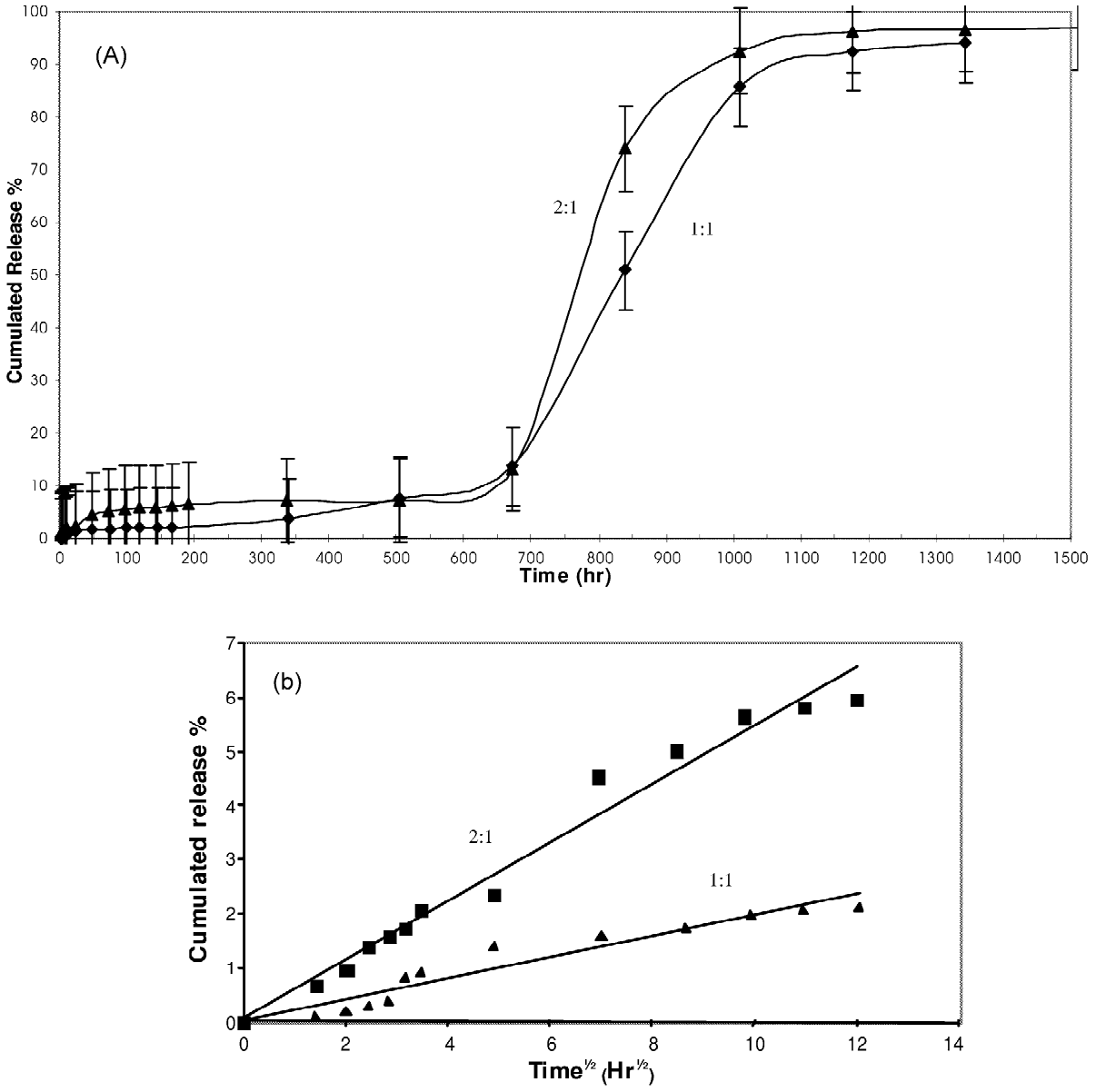

Fig. 5. (A) Cumulative release profile of etanidazole from double-walled microspheres. Mass ratios (2:1) (▲); 1:1 (♦). (B) Plot of cumulated release Q_t versus $t^{1/2}$ to show diffusion controlled drug release. (■) PLLA–PLGA (2:1, w/w); (▲) PLLA–PLGA (1:1, w/w).

initial burst followed by a characteristic lag phase and an almost linear sustained release for about 300 h leading up to 2 months where it plateaus off. The plot of the initial release data for percent cumulated release versus square root of time, yields a linear profile (Fig. 5B). This suggests that the initial release is diffusion controlled [17] while the subsequent rapid release is controlled by degradation where drug is flowing out through a rate-limiting shell layer that is undergoing degradation.

3.4. Gamma irradiation

Two different dosages of 50 Gy (representative of

the total dosage for the entire course of radiotherapy) and 25 kGy (commonly known as the industrial overkill) were used in the study. Impacts of 50 Gy on the physical properties of polymeric carrier were negligible hence its effect on the release profile was also insignificant. While doses greater than 25 kGy resulted in leftwards shifts in T_g and T_m (Fig. 6), which can be indirectly correlated to decrease in molecular weight, by using Flory Fox Eqs. (2) and (3) [18,19]

$$T_g = T_g^{\infty} - \frac{K}{\bar{M}_n} \tag{2}$$

$$\frac{1}{T_m} - \frac{1}{T_m^{\infty}} = \frac{2RM_o}{\Delta H_m \bar{M}_n} \tag{3}$$

where T_g^{∞} is the T_g at infinite mw and K is a constant representing the excess free volume of the end groups of polymer chain. T_m^{∞} is the T_m at the infinite T_m at the infinite mw, R is the gas constant, M_o is the mw of the repeat unit and ΔH_m is the heat of fusion per mole of repeat unit.

The molecular weight of polymers decreases due to radiolytic cleavage and scission of polymer chain [15,16], which is consistent with our understanding of the effect of gamma irradiation and supported by the data by Yip [20]. For PLGA 50:50, at 0 kGy dose, mw = 42 100, M_n = 23 600 and polydispersity = 1.79 while at >25 kGy dose, mw = 31 000, M_n = 18 000 and polydispersity = 1.73. Consequently, the release profile is preempted by a time period of 14 days and the main release phase where most of the drugs (~80%) are release, prolonged to about 500 from 300 h without irradiation (Fig. 7).

Degradation studies were conducted using DSC to determine the effects of polymer in vitro degradation on drug release profiles as well as the effects that gamma irradiation have on polymeric degradation rates. From Table 1, there exist two distinct T_g values for double-walled microspheres, one each for PLLA and PLGA and a single crystalline melting point for PLLA since PLGA is amorphous. All three points showed leftward shifts as degradation progresses in vitro. For nonirradiated samples, it was found that the most significant shifts in the T_m and T_g (Fig. 8A) were between 28 and 35 days. This corresponded to the onset of the bulk release of

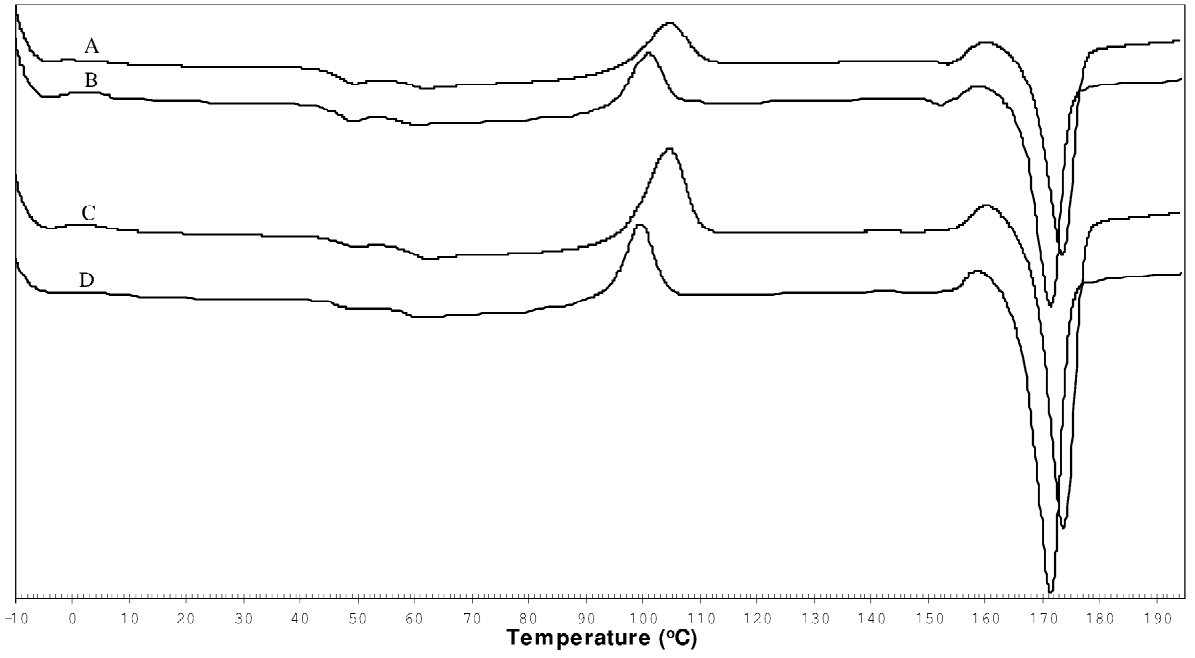

Fig. 6. DSC thermograms studying effects of gamma irradiation on double-walled microspheres. (A) PLLA–PLGA 1:1, 0 Gy (B) PLLA–PLGA 1:1, >25 kGy (C) PLLA–PLGA 2:1, 0 Gy (D) PLLA–PLGA 2:1, >25 kGy.

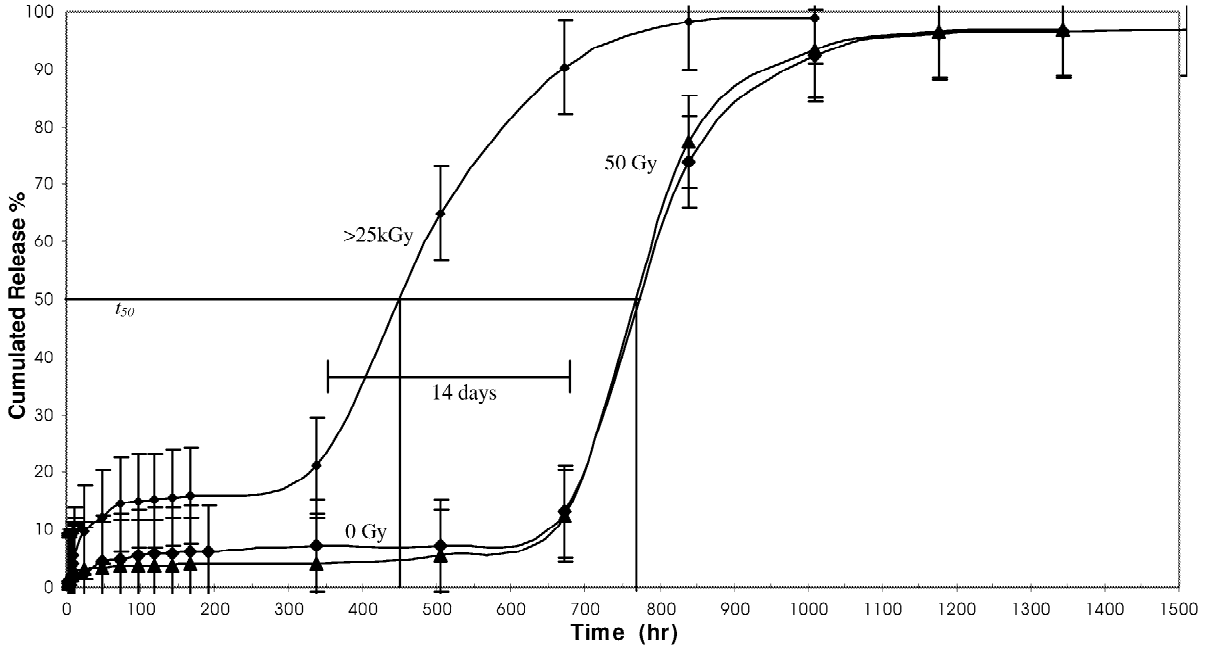

Fig. 7. Release profile of sample 2:1 (PLLA–PLGA, 2:1 w/w) with irradiation dose of 0 Gy (●), 50 Gy (▲) and >25 kGy (♦).

etanidazole around 700 h (Fig. 7). This trend was again observed for irradiated samples where the most drastic reductions in T_g and T_m (Fig. 8B) were between 14 and 21 days, corresponding to the onset of bulk etanidazole release around 350 h (Fig. 7). Meanwhile, SEM photographs have shown nonirradiated samples and irradiated samples having similar extent of degradation, but at earlier times for the latter (Fig. 9). The differences between the time periods have been consistently observed to be around 14 days for 25 kGy of irradiation administered.

4. Discussion

4.1. Double-walled microspheres

A combination of polymeric solutions with concentrations exceeding critical levels results in the exhibition of immiscibility. Solvent evaporation of PLLA and PLGA in DCM [9] concentrates and separates the polymers allowing for double-walled microspheres formation where one layer engulfs the other according to the theory of spreading coefficient [3,21]. The resultant double-walled microspheres have shells of uniform thickness (Fig. 1) versus those produced via traditional coating technology. This condition is critical for the present delivery system, which depended upon the release of drug as the polymer coating erodes acting as a rate-limiting barrier to release.

Proof of distinct polymeric layers and their compositions in double-walled microspheres was established using a novel solubility test using ethyl acetate applicable to this specific polymer pair and FTIR differentiation of the compositions of the shell and core. Cross-sectional views showing remnant shell with a dissolved core leaving a crater-like cavity indicated that the core consisted of mostly PLGA and the shell, PLLA. As discussed earlier, this cavity could not have been the result of the core dropping out. The reason being that dissolution was done on the cross-sectioned halves individually with little agitation and no residues beside the shell was observed to be remnant in the solvent.

Close-up views also revealed the presence of highly porous shells after dissolution and small spheres left in the core cavity. This suggests that while phase separation between the two polymers was complete, there were also kinetic factors govern-

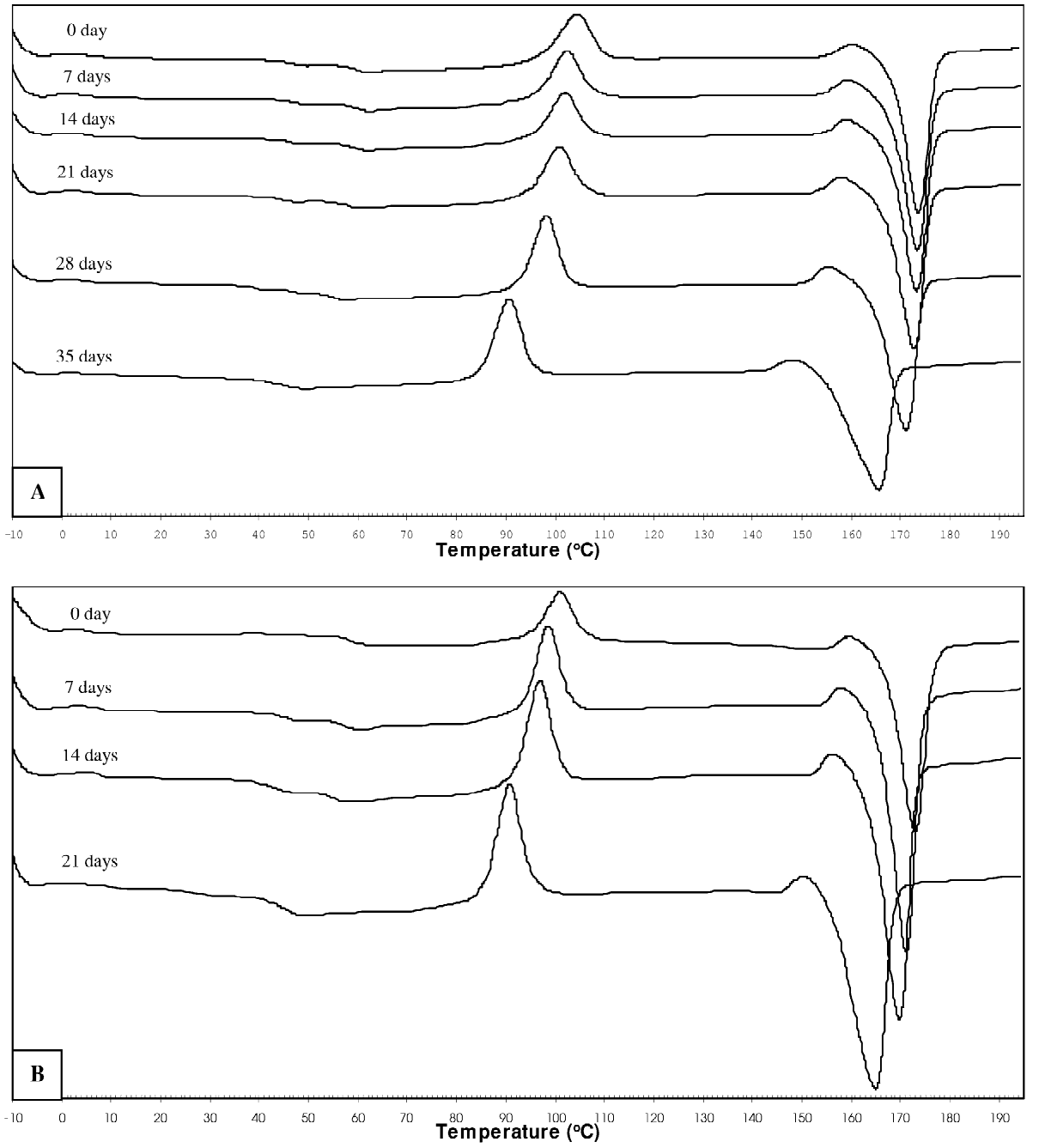

Fig. 8. DSC thermogram of double-walled microspheres, polymer ratio PLLA–PLGA (2:1), undergoing degradation. (A) Non-irradiated sample (B) irradiated sample.

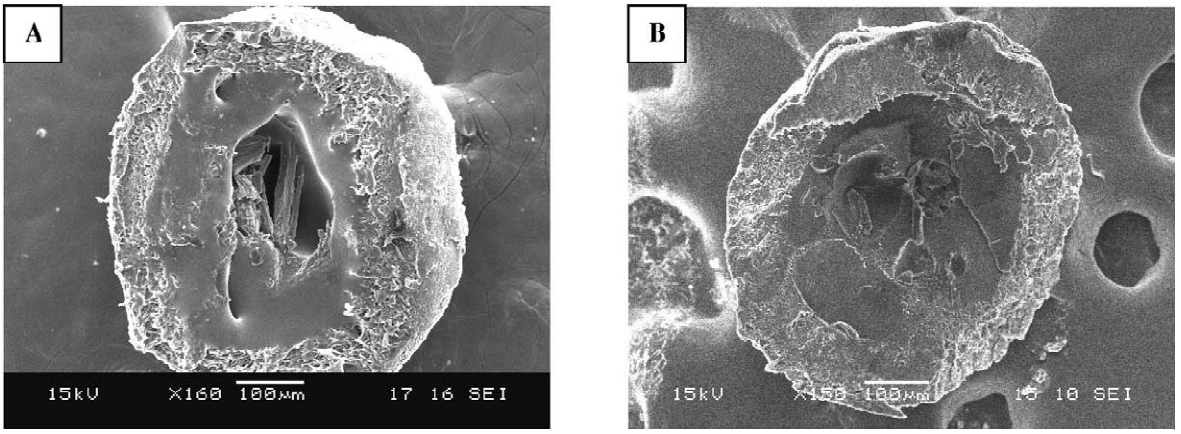

Fig. 9. SEM photographs of double-walled microspheres, polymer ratio PLLA–PLGA (2:1), undergoing in vitro degradation. (A) Non-irradiated sample after 28 days (B) irradiated sample after 14 days.

ing the solidification process at work. Due to insufficient time allowed for the polymers to move into and coalesce with their respective phases, small amount of each polymer can be found trapped in the phase of the other polymer. This can be anticipated as during solvent loss, polymeric solution becomes increasingly more concentrated, hence viscous, inhibiting movement. Therefore, small amount of PLLA remained entrapped within the PLGA and vice versa.

Nevertheless, small spheres of more hydrophilic and faster degrading PLGA entrapped within the stronger PLLA shell are useful for the formation of channels and pores as it degrades before PLLA (Fig. 2C). This increased the porosity of the shell, enhancing drug release through the otherwise low permeability PLLA shell. The porous shells in turn functioned as rate-limiting barriers similar to membranes.

FTIR spectra further substantiate our findings showing that the spectra of the core coincides with PLGA while the spectra of the shell coincides with PLLA, with characteristic wave numbers of the polymer pairs similar to those successfully employed in Matsumoto et al. [9]. The shell spectra while predominantly resembling PLLA contained a small peak typical of PLGA at 1435 cm^{-1}. This can be attributed to our observations that the shell has small amount of PLGA entrapped.

4.2. Effect of polymer mass ratios

Changes in mass ratios of PLLA to PLGA (w/w) have been observed to result in the variations of shell thickness and core diameter (Fig. 1) while mean particle size remained constant. This can be explained in the light that particle size is dependent only upon fabrication conditions such as stirring speed and surfactant concentrations [22]. Hence, if fabrication conditions were kept constant for the various batch processes, similar particle size distribution can be obtained. With varying mass ratio of polymers, the resultant dimension of the shell and core changes accordingly. Fabrication conditions for the study were deliberately chosen such that, double-walled microspheres fabricated would have sizes ~450 μm. This is to allow for cross-sectioning at the microspheres' centerline to be carried out with sufficient accuracy and ease.

Having identified that the core consists mostly of PLGA and the shell of PLLA, with lesser PLGA and more PLLA in each particle, a core of smaller diameter and shell of greater thickness can be expected. This was consistent with our observations (Fig. 1B–D). With changes to the thickness of the wall, which is the rate-limiting barrier to drug release, release profiles will then be different (Fig. 5A). Making the assumptions of conservation of mass, constant polymer density, volume additivity

and perfect dispersion, accurate predictions of shell thickness (d_{wt}) and core diameter of the fabricated microspheres (d_{core}) can be made using Eqs. (4)–(7) shown below

$$V_{total} = [(V_{PLLA}) + (V_{PLGA})] = \left[\left(\frac{M_{PLLA}}{\rho_{PLLA}} \right) + \left(\frac{M_{PLGA}}{\rho_{PLGA}} \right) \right] \quad (4)$$

$$N_p = \left(\frac{V_{total}}{(\pi/6) d_p^3} \right) \times \% \text{ yield} \quad (5)$$

$$d_{core} = \sqrt[3]{\frac{6 \times V_{PLGA} \times \% \text{ yield}}{\pi \times N_p}} \quad (6)$$

$$d_{wt} = \frac{d_p - d_{core}}{2} \quad (7)$$

where V_{total} is total particle volume, V_{PLLA} is volume of PLLA phase, V_{PLGA} is volume of PLGA phase, M_{PLLA} is mass of PLLA used, M_{PLGA} is mass of PLGA used, ρ_{PLLA} is density of PLLA and ρ_{PLGA} is density of PLGA. N_p is total number of microspheres produced and d_p is the mean volume diameter of microspheres obtained from size distribution data. Application of the method to predict resulting dimensions of the single microsphere shown in Fig. 1B has been carried out and shown in Table 3 to contain less than 2% error. This confirmed our earlier hypothesis and fulfilled the motivation of altering mass ratios, which was to allow for the engineering of shell thickness and core diameter.

Although etanidazole was initially added to PLGA solution, after the formation of the translucent O/O emulsion, it was impossible to say definitely in which phase was the drug now localised. However, both SEM and optical microscopy on the final double-walled microspheres found the phenomenon of etanidazole localising predominantly within the core (PLGA) polymer. This allowed for the formation of a dispersed drug matrix PLGA reservoir core, which was then engulfed by PLLA resulting in the formation double-walled composite type microsphere. This offered plausible explanations as to why better etanidazole encapsulation efficiency was achieved for double-walled relative to each of the single polymer microspheres (Table 2). Since the hydrophobic PLLA helped to shield highly water-soluble etanidazole dispersed into PLGA phase from the aqueous PVA media during fabrication. Thereby, reducing leaching and improving entrapment efficiency significantly by 5–10%. This improvement is significantly larger than the standard errors for the test and therefore could not have been a result of it.

Distribution theory [23], which states that the distribution of a drug substance varies according to the solubility parameter of the polymer–DCM solutions and that of the drug, may provide a plausible explanation for our observations, since it has been successfully applied to predict distribution of Cisplatin and amino acids within bisphasal polymeric solutions [9]. The theory is represented in Eq. (8)

$$\log \frac{X_{PLGA}}{X_{PLLA}} = V_{drugs} \frac{(\delta_{drug} - \delta_{PLLA-DCM})^2 - (\delta_{PLGA-DCM} - \delta_{drug})^2}{2.3RT} \quad (8)$$

where X_{PLGA} and X_{PLLA} are the concentrations of drug in the PLGA and PLLA phase, respectively; δ_{drug}, $\delta_{PLGA-DCM}$ and $\delta_{PLLA-DCM}$ are solubility parameters of drug, PLGA phase and PLLA phase, respectively. V_{drug} is the molecular volume of the drug; R is the gas constant and T is the absolute temperature.

Qualitatively, since PLGA and etanidazole are both more hydrophilic (i.e. more polar) than PLLA,

Table 3
Estimation of wall thickness and core diameter of double-walled microsphere from batch with polymer ratio PLLA–PLGA 1:1 using Eqs. (4)–(7). $\rho_{PLLA} = 1.34$ g/cm^3; $\rho_{PLGA} = 1.24$ g/cm^3, $M_{PLLA} = 100$ mg, $M_{PLGA} = 100$ mg, $d_p = 453 \times 10^{-6}$ m

$d_{core,\ calculated}$ (μm)	$d_{core,\ actual}$ (μm)	Error (%)	$d_{wt,\ calculated}$ (μm)	$d_{wt,\ actual}$ (μm)	Error (%)
354.84	360.00	1.43	49.08	50.00	1.84

we can deduce that their solubilities will be more alike than the pair of PLLA (i.e. less polar) and etanidazole. Therefore, the numerator of the expression on the right hand side would positive. Multiplying with a large value of V_{drug} (since it is solid), the concentration ratio of X_{PLGA}/X_{PLLA} would give a value very much greater than 1, clearly implying a preferential localization of etanidazole to the PLGA polymer core, which is the actual situation being observed. The solubility parameter of the drug is defined in solid state since it is suspended into the polymeric solution and not dissolved into it.

In vitro release revealed that double-walled microspheres provided effective suppression of the undesirable initial burst of drug release where less than 10% of etanidazole was released within the first 6 days. This worked out to less than 0.5 mg/week implying that the local drug concentration was unlikely to exceed toxicity level where detrimental effects will be felt. This fulfilled one of the key motivations for a double-walled system intended for application with highly water-soluble drug. A small initial release was followed by a time lag phase. The plot of cumulated release percentage versus square root of time yielded a linear profile indicating a diffusion controlled release mechanism [17].

This time lag period can be engineered through careful selection of the doses of irradiation to modify the release. This was clearly demonstrated where 25 kGy irradiated microspheres released 14 days earlier than a polymeric system without irradiation (Fig. 7). This may be due to diminished molecular weights as a result of irradiation. Such a degree of freedom existing in the release profile would be most versatile allowing for a combination of these new composite double-walled microspheres with traditional monolithic polymer drug delivery systems which will provide an earlier burst. Achieving pulsatile, double burst or near linear release has been suggested to be beneficial in treating growing tumors and for medical applications such as vaccines that may require a second and subsequent booster burst.

The intermediate release phase where the bulk of the drug was released spans about 300 h for nonirradiated microspheres and up to 500 h for 25 kGy-irradiated microspheres. Admittedly, this is still short of the desired 6–8 weeks of sustained release, but it does points to the potential of using irradiation to modify the release profile of double-walled microspheres to achieve this target. The sudden change in the gradient of release in this phase can be attributed to a change in release mechanism from diffusion controlled to degradation controlled where the formation of pores and channels influence release more substantially than diffusion.

The onset of sustained release phase has been positively correlated to the most significant decrease in T_g and T_m values suggesting that this phase is strongly influenced by the state of degradation of the microspheres. As earlier explained, the presence of small amount of PLGA within the shell after degradation would lead to pore and channel formation. Since PLGA degrades after about a month in vitro, faster than PLLA, this leaves a porous shell engulfing a core that is also highly degraded. Therefore, etanidazole will be released at an increased rate, leading to its rapid depletion.

5. Conclusions

The double-walled polymeric drug delivery system is able to circumvent most of the limitations of traditional monolithic polymer systems. Developments in this paper have allowed for accurate identification of the composition of the shell and core polymer, prediction of the shell and core dimensions and the preferential distribution of etanidazole to the core. The suitability of the system, which is intended for implantation and used concurrently with radiotherapy, was also evaluated in terms of the effects of gamma irradiation on polymeric carriers. It was found that clinical doses of irradiation failed to have any observable effects while typical sterilization doses cause radiolytic cleavage leading to earlier breakdown of polymer structure during degradation and modification of drug release profile. The delivery system designed for this work was able to achieve higher entrapment efficiency for a highly water-soluble drug along with release profiles that contained a time lag and sustained release phase that was modified advantageously by high irradiation doses. The time lag in turn allows for the combination of double-walled system with traditional microspheres for more general therapeutic applications.

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