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Characterization of biodegradable poly(D,L-lactide-co-glycolide) polymers and microspheres

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Abstract: Characterization of biodegradable polymers used for controlled drug delivery is essential to ensure reproducibility of *in vitro* and *in vivo* performance. Selected characterization techniques established for poly(D,L-lactide-co-glycolide) (PLGA) copolymers included DSC to analyse thermal behavior, ^{13}C-NMR to determine exact comonomer ratios and comonomer sequencing, cloud point titration to establish solubility, SEC to monitor molecular weight averages and polydispersity, SEM to observe surface morphology, BET gas adsorption to analyse surface area, tapped bulk density measurements to suggest internal pore structure and porosity and finally *in vitro* degradation to analyse degradation times and profiles. Comonomer ratios of 50:50 PLGAs were found to be closer to stated values for Boehringer Ingelheim polymers than for polymers from two other suppliers. Implementing such a characterization program for biodegradable polymers ensures the production of reproducible and reliable controlled drug delivery systems.

Keywords: *Biodegradable polymers, characterization methods, poly(D,L-lactide-co-glycolide).*

Introduction

When working with biodegradable polymer systems, the selection of appropriate characterization techniques is an important step toward assurance of a reproducible and effective product. Biodegradable polymers have numerous physical and chemical characteristics, such as molecular weight averages and distribution, glass transition and/or melting temperatures, comonomer ratios and sequencing for copolymers. All of these properties could influence the physical behaviour of raw polymers. When polymers are fabricated into controlled drug delivery systems, additional qualities such as surface area, bulk density, surface morphology and particle size are introduced, and may affect both degradation of and drug release from the polymeric system. This requires analysis of these properties for a complete understanding and eventual prediction of the system's behaviour. A wide variety of analytical techniques to characterize these different aspects of the polymers and polymer systems is available, ranging from end-group analysis, ultracentrifugation, dilute solution viscosity measurements and size exclusion chromatography (SEC) for molecular weight determination, and differential thermal analysis, thermogravimetric analysis and differential scanning calorimetry (DSC) for thermal analysis, to sieving, light diffraction and scanning electron microscopy (SEM) for particle size determination. Variations in results obtained from such a large collection of techniques make it difficult to compare values of a specific characteristic that were obtained by different methods. For example, end group analysis and ultracentrifugation offer absolute values for molecular weight, but dilute solution viscometry and SEC do not and thus require calibration standards. Separate values of molecular weight averages are achieved using each method that, although comparable to values obtained by the identical technique, cannot be directly compared with one another. Furthermore, varying materials or parameters used in a specific technique can often change results, thereby complicating reliable comparisons, as in using different mobile phases in SEC, or different scanning rates in DSC. For these reasons, a researcher working with polymer systems must carefully select and validate a collection of techniques that fully characterize the polymer, and maintain these same techniques throughout their research. This paper will discuss a characterization program consisting of a collection of techniques and

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methods chosen to comprehensively analyse important properties of a biodegradable polymer, poly(D,L-lactide-co-glycolide) (PLGA) and microspheres prepared from the polymer. The techniques include: DSC for thermal analysis; ^{13}C nuclear magnetic resonance spectroscopy (NMR) to determine the comonomer ratio and sequence distribution of copolymers; SEC for molecular weight; SEM for surface morphology; gas adsorption for determination of specific surface area; tapped bulk volume measurements to determine bulk density of the microspheres; and *in vitro* analysis of degradation behaviour. PLGA was selected for study because of its past and continuing importance as a suitable biodegradable and biocompatible polymer for use in drug delivery.

Differential Scanning Calorimetry

DSC provides accurate and precise quantitative data due to the separate treatment of the sample and the reference according to the power compensated "null balance" principle [1]. As the sample and reference are individually heated at the same constant rate, the energy which is absorbed or evolved by the sample during an endothermic or exothermic event, respectively, is electrically compensated for by the subsequent addition or subtraction of an equivalent amount of energy to the sample, thus maintaining isothermal conditions between the sample and reference holders. Figure 1 shows a DSC thermogram in which the heat flow rate, dH/dt, is plotted vs temperature or time. An endothermic transition is represented by a positive peak, and an exothermic transition by a negative peak. With DSC, thermochemical parameters such as heat capacity and heat of fusion can be determined, making this an excellent technique for thermal characterization of polymers.

PLGA 50:50 copolymers are amorphous and exhibit glass transition (approximately 45°C), but no melting points. DSC was used to determine and compare the glass transition temperatures, T_g, and changes in heat capacities, ΔC_p, of selected copolymer raw materials. The T_g is defined as the temperature at which a rigid or "glassy" polymer converts to a softer "rubbery" polymer upon heating due to an increase in the free volume of the polymer. The opposite transition from a rubber to a glass occurs upon cooling. In a thermogram shown in Fig. 2, the T_g is depicted by a relatively abrupt change in slope, which actually signifies a change in heat capacity according to the following equation

$$\Delta C_p = C_{p^2} - C_{p^1}, \qquad (1)$$

where C_{p^1} and C_{p^2} are the heat capacities immediately preceding and following the T_g, respectively. Individual C_p values are determined according to the equation

$$C_p = (dH/dT)_p, \qquad (2)$$

where H and T are the enthalpy and temperature, respectively. The actual T_g is calculated by extending the tangents of the curve immediately preceding and following the transition, then measuring the temperature at one-half the vertical distance between these two lines (Fig. 2).

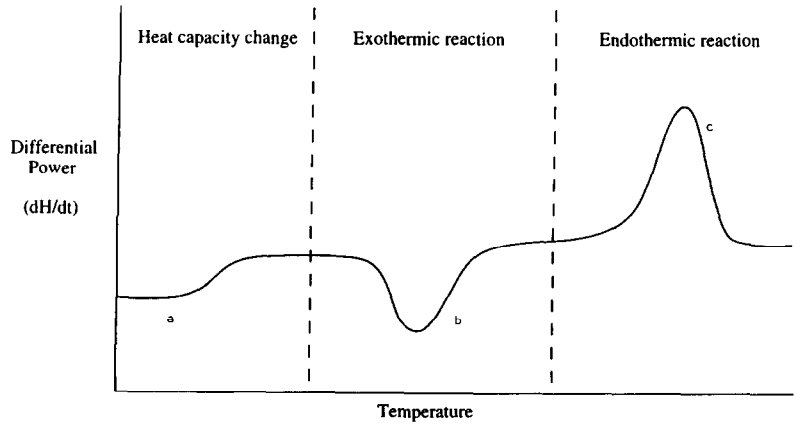

Figure 1
DSC Thermal Events: (a) a normal glass transition; (b) a crystallization exotherm and (c) a melting endotherm.

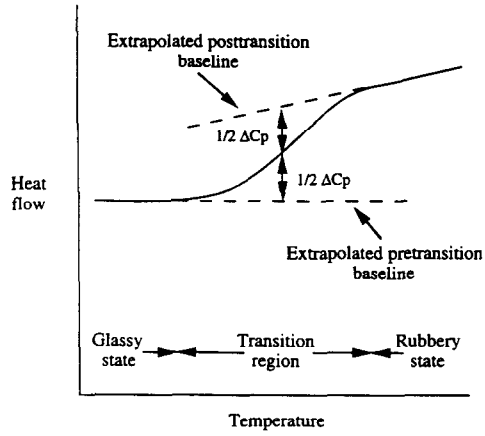

Figure 2
Heat capacity change in the glass transition region.

Materials and methods for DSC

The DSC technique was accomplished using a calorimeter connected to a refrigeration system (Perkin–Elmer DSC 7, Norwalk, CT, USA). Calibration of the system was performed by a temperature and area calibration of an indium standard. Sample analysis was accomplished by carefully weighing 5–10 mg of PLGA copolymer (Boehringer Ingelheim, Germany) and sealing it in an aluminum sample pan. An empty sealed aluminum pan was used as the reference. While under a nitrogen purge, the sample and reference were heated at a rate of 5°C min^{-1} from 25 to 80°C, cooled at a rate of 10°C min^{-1} to 25°C, then heated again at 5° min^{-1}, this time to 65°C. Two heating scans, as shown in Fig. 3, were performed because the first heating is affected by the thermal history of the polymer, and therefore may not reliably indicate the original polymeric properties [2]. An uncharacteristic peak at the glass transition, known as kinetic overshoot, occurred due to the structure introduced to the polymer in the past [3]. By heating the polymer once past the T_g, then cooling the polymer below the T_g at a faster rate than that used for heating, any "history" of the polymer can be eliminated. The correct T_g occurred at 43.8°C (Fig. 3b) and ΔC_p was calculated (0.42 J g^{-1} $°C^{-1}$) by the microprocessor.

^{13}C nuclear magnetic resonance spectroscopy (NMR) for comonomer ratio and sequence distribution determination of copolymers

^{13}C NMR has been a valuable technique for studying the microstructure of polymers [4, 5]. Pulsed Fourier transform (FT) ^{13}C NMR has

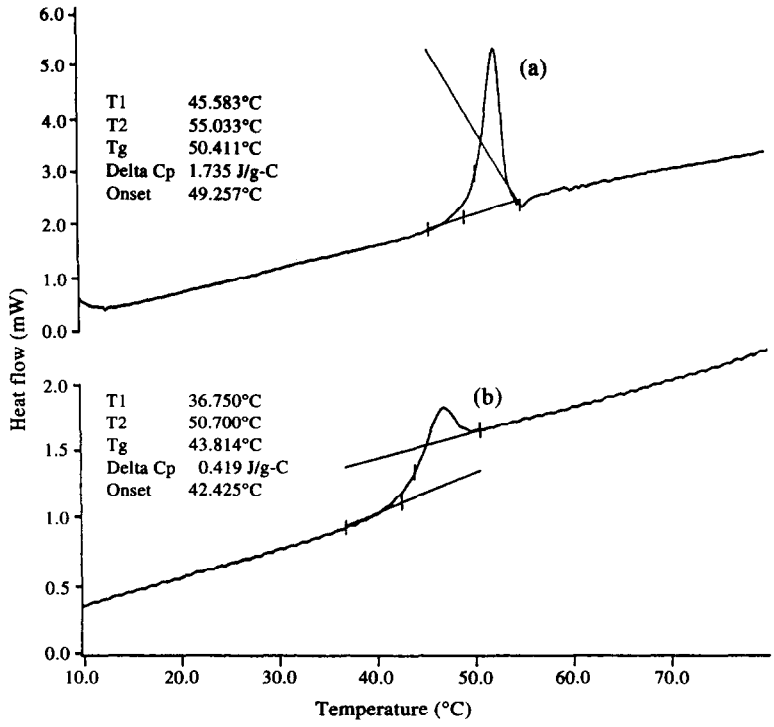

Figure 3
DSC thermograms for PLGA 50:50. (a) First heating showing kinetic overshoot and (b) second heating showing T_g at 43.8°C and ΔCp of 0.42 J g^{-1} $°C^{-1}$.

been used to specifically determine the sequence distribution of many types of copolymers, for instance poly(ethylene-1-butene) [6], poly(styrenebutadiene) and poly(ethylene-vinyl-acetate) [7]. The technique relies on separate chemical shifts produced by carbons in specific molecular environments. Quantitative analysis requires: (1) a sufficiently powerful radiofrequency (RF) pulse to irradiate all of the nuclei equally; (2) enough relaxation time for the nuclei of interest to completely return to equilibrium after the pulse and prior to the next pulse; and (3) elimination of differential nuclear Overhauser enhancement (NOE). As a spectrum is proton decoupled, signal enhancement of a multiplet is observed as the multiplet collapses into a single line. The extent is usually greater than would be expected due to simple collapse. This additional signal increase is termed the nuclear Overhauser enhancement and is created by ^{13}C-^{1}H dipole–dipole interactions [8].

For ^{13}C NMR, a sufficient delay occasionally approaches 100 s, which would substantially increase the minimum experiment acquisition time from under 1 h to 10 or more hours. Fortunately, large molecules such as polymers generally feature short relaxation times and require pulse delays of only a few seconds. Any relaxation and differential NOE problems can be overcome by the addition of a paramagnetic substance, also known as a relaxation agent, such as chromium (III) acetylacetonate ($Cr(acac)_3$) and iron chelates.

Since the microstructure of a polymer can affect the physical behaviour, i.e. solubility or degradation, of a polymer, it is a valuable property to analyze. A few studies examining the microstructure of PLGA copolymers using ^{13}C NMR have been reported. Avgoustakis and Nixon observed two resonances at 166.6 and 166.7 ppm representing carbonyls of the glycolic acid (GA) unit of the copolymer in specific environments of GA–GA–GA and GA–GA–LA (lactic acid unit) [9]. They concluded that the reported spectrum represents a blocky or heterogeneous copolymer and not a random or homogeneous copolymer (which should exhibit additional resonances near 166 ppm representing the LA–GA–LA and LA–GA–GA arrangements). Similarly, Bendix used ^{13}C NMR to compare PLGAs prepared by two methods; polycondensation of lactic and glycolic acids without catalyst, which is thought to produce totally random copolymers, and ring-opening melt condensation of lactide and glycolide, which produces more heterogeneous copolymers [10]. By comparing relative intensities of the previously-mentioned peaks, average poly(glycolide) block lengths ranging from 1 for the random polycondensation copolymers to about 4.6 for the heterogeneous ring-opening melt condensation copolymers were observed. These studies support the use of ^{13}C NMR as the chosen method to study comonomer ratios and sequence distributions of copolymers.

Materials and methods for ^{13}C NMR

The following materials were used in the validation of the NMR technique: 75MHz ^{13}C-NMR instrument (Varian, Palo Alto, CA, USA); PLGA 50:50, 75:25 and 85:15 (Boehringer Ingelheim, Germany); PLGA 50:50 (Birmingham Polymers Inc., Birmingham, AL, USA); PLGA 54:46 (Medisorb Technologies International LP, Cincinnati, OH, USA); deuterated dichloromethane (CH_2Cl_2) as the solvent; and glass NMR tubes with 5 mm o.d. $Cr(acac)_3$ was used as a relaxation agent.

The following parameters were selected: 90° pulse width = 8.7 ms, pulse delay = 2 s, spectral width = 13 000 Hz, decoupler mode on. Samples were prepared in deuterated CH_2Cl_2 at concentrations of 50 mg ml^{-1}. Total acquisition time was approximately 2 h to give a good signal-to-noise ratio.

Figure 4 shows the ^{13}C-NMR spectrum obtained for PLGA. Comonomer ratios were determined by integrating the methine group of the lactide unit (LA) at 69 ppm and for the methylene group of the glycolide unit (GA) at 61 ppm. These integral values, I_{LA} and I_{GA}, respectively, were converted into comonomer ratios, R_{LA} and R_{GA}, using the following equations

$$R_{LA} = \frac{I_{LA}}{I_{LA} + I_{GA}} \quad (3)$$

$$R_{GA} = \frac{I_{GA}}{I_{LA} + I_{GA}} \quad (4)$$

The comonomer sequence distributions for PLGA were obtained by comparing the relative intensities of the GA carbonyl resonances shown in Fig. 5. The peak at 166.8 ppm represents the GA carbonyl most directly connected to a LA unit (GA–LA), and the

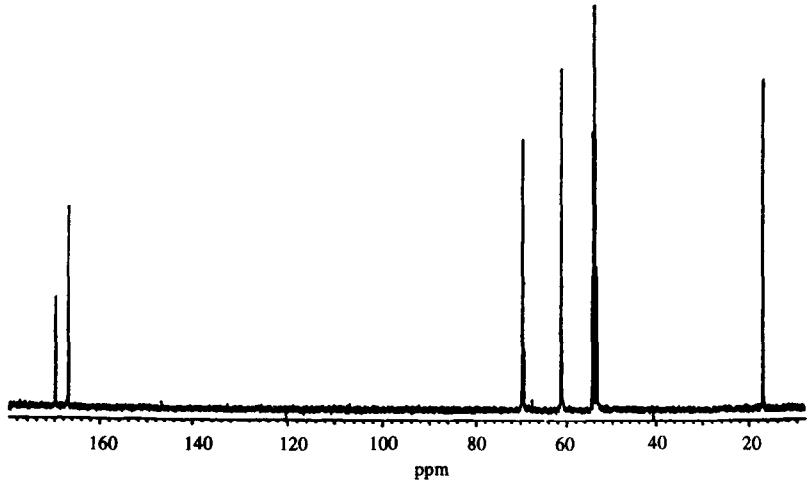

Figure 4
NMR spectra of PLGA 50:50.

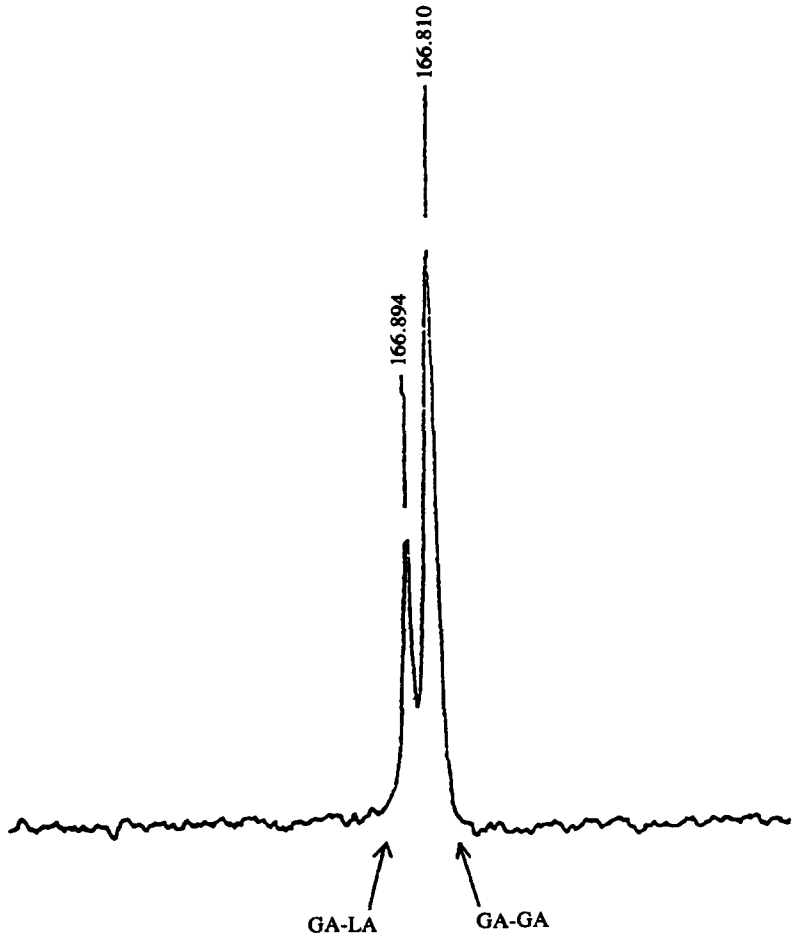

Figure 5
Assignment of the GA carbonyl resonances; GA–LA at 166.9 ppm and GA–GA at 166.8 ppm.

peak at 166.9 ppm represents the GA carbonyl most directly attached to another GA unit (GA–GA). Table 1 shows that the experimentally determined LA:GA comonomer ratios were in excellent agreement with the values reported by the individual manufacturers. Further, the peak intensity values for the GA carbonyl peaks, and the LA methine (69 ppm) and GA methylene (61 ppm) groups in the PLGA spectrum were compared with

Table 1
Comonomer ratios for PLGA as determined by ^{13}C NMR

Sample	LA integral (I_{LA})	GA integral (I_{GA})	Measured LA:GA ratio (R_{LA}:R_{GA})	Stated LA:GA ratio (R_{LA}:R_{GA})
Boehringer	43.33	43.49	50:50	
50:50	49.25	48.83	50:50	50:50
Lot 95034	41.45	40.83	50:50	
Boehringer	32.46	32.98	50:50	
50:50	31.86	30.99	51:49	50:50
Lot 15029	43.35	42.14	51:49	
Boehringer	46.12	45.73	50:50	
50:50	41.22	40.50	50:50	50:50
Lot 15096	56.13	55.15	50:50	
Boehringer	50.71	49.37	51:49	
50:50	28.12	26.93	51:49	51:49
Lot 211686	43.46	40.62	52:48	
Boheringer	44.13	43.58	50:50	
50:50	19.88	19.80	50:50	50:50
Lot 211688	19.84	19.57	50:50	
Birmingham	47.44	40.85	54:46	
50:50	17.62	15.52	53:47	Not stated
	48.71	40.26	55:45	
Medisorb	34.92	25.38	58:42	
54:46	47.67	36.43	57:43	54:46
	29.72	21.59	58:42	
Boehringer	64.14	20.90	75:25	75:25
75:25				
Boehringer	91.81	16.08	85:15	85:15
85:15				

and without the addition of the relaxation agent $Cr(acac)_3$, and were found to be equivalent.

The ratio, R_{cms}, was formed according to the following

$$R_{cms} = \frac{\text{intensity of GA–LA carbonyl peak}}{\text{intensity of GA–GA carbonyl peak}}. \quad (5)$$

The lower the R_{cms} value, the higher the degree of heterogeneity or blockiness of the copolymer. For various samples of commercial PLGA 50:50, R_{cms} values ranged from 0.42 to 0.57, indicating differing degree of blockiness [11].

Cloud point titration for solubility determination

Since polymers are large chain molecules, their dissolution in solvents requires a two stage process [12]. First, the solvent diffuses into the polymer matrix, causing swelling of the polymer and eventual gelation. Second, the gelled polymer fragments slowly untangle themselves from the intact matrix and move out into the solvent, effectively dissolving. Stirring facilitates the dissolution process.

The solubility of a polymer is important in many of the analytical methods, i.e. SEC and viscosity, and in the fabrication into various types of drug delivery devices, i.e. microspheres and membranes. Therefore, knowledge of the extent of a polymer's solubility in commonly used organic solvents is helpful. Additionally, small differences in the solubilities of a family of copolymers, such as the PLGA copolymers, can signal subtle variations in comonomer ratios and/or comonomer sequencing [13].

Cloud point titration offers a fast, simple and effective method for determining the solubility behavior of many polymers. It works on the principle of precipitating a dissolved polymer out of a good solvent by the addition of a non-solvent. Although an absolute measure of polymer solubility is not achieved by this method, the data can be compared for polymers in the same solvent, providing information about relative degrees of dissolution capacity.

Materials and methods for cloud point titration

The solubilities of several lots of PLGA 50:50 copolymers (mw 30 000 Da (Boehringer)) in HPLC grade CH_2Cl_2 and acetonitrile were determined by adapting the cloud point titration technique described by Dunn *et al.* [13]. Of the polymer 125 mg were trans-

ferred to a 20-ml glass test tube and dissolved in 5 ml of either CH_2Cl_2 or acetonitrile. The solutions were slowly titrated with HPLC grade methanol to the cloud point where the polymer began to precipitate, as noted by a faint but distinct cloudiness which remained upon shaking. The relative solubility was calculated as the volume of methanol added per mass of PLGA dissolved. The results from duplicate runs for various lots of PLGA 50:50 are shown in Table 2.

Size Exclusion Chromatography for Molecular Weight Determination

Although numerous techniques (ebulliometry, electrospray mass spectroscopy, light scattering and end-group analysis [14]) are available for molecular weight determination of polymers, the two most familiar methods are solution viscometry and SEC. Neither of these methods is a direct or absolute method of molecular weight determination, meaning that they rely not only on the individual polymer characteristics, but also on other factors such as solvent type and temperature for viscometry, and solvent type and calibration standards for SEC. Nevertheless, these methods give reliable relative results.

Viscometric determination of molecular weight relies on the intrinsic viscosity, which is the viscosity of the polymer at infinite dilution. Intrinsic viscosity, η, can be determined either by measuring the specific viscosity [15] or inherent viscosity [12] and then calculating the molecular weight from the Mark Houwink Sakurada equation [15].

$$\eta = KM^a. \tag{6}$$

K and a are constants that are unique for each polymer–solvent combination. Typical values for K fall around 0.2×10^{-4} dl g^{-1}, and those for a usually range from 0.5 to 0.8 for random coil polymers [12]. Kenley, *et al.*, determined these values for PLGA 50:50 in tetrahydrofuran (THF), specifically $K = 1.07 \times 10^{-4}$ and $a = 0.761$ [16].

SEC, commonly referred to as gel permeation chromatography (GPC), involves the separation of macromolecules according to their size. A polymer solution is passed through an appropriate porous column in which the smaller polymer molecules can access and enter the pores and the larger molecules are excluded [17]. As a result, the larger molecules elute from the column sooner than the smaller molecules, and molecular size differentiation is achieved. A typical chromatogram is shown in Fig. 6, where detector response is plotted versus retention time or volume. Using SEC, several different molecular weight averages can be calculated. These include number-average molecular weight, M_n, and weight-average molecular weight, M_w, as calculated by the following [18]

$$M_n = \frac{\Sigma n_i M_i}{\Sigma n_i} = \frac{\Sigma w_i}{\Sigma w_i / M_i} \tag{7}$$

$$M_w = \frac{\Sigma n_i M_i^2}{\Sigma n_i M_i} = \frac{\Sigma w_i M_i}{\Sigma w_i}, \tag{8}$$

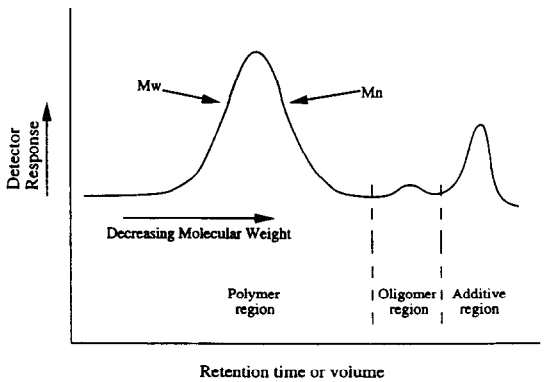

Figure 6
Hypothetical SEC chromatogram showing approximate locations for molecular weight averages M_n and M_w.

Table 2
PLGA 50:50 relative solubilities as determined by cloud point titration

Sample	CH_2Cl_2 solubility (ml CH_3OH/g PLGA)	CH_3CN solubility (ml CH_3OH/g PLGA)
15029	30	28
15096	28	28
211686	31	30
211688	29	28
Average ± SD	29.5 ± 1.3	28.5 ± 1.0

where n_i = number of moles of the ith component, M_i = the molecular weight of the ith component, w_i = weight of the ith component. The M_n value is mostly effected by smaller molecules, whereas M_w is more influenced by larger molecules. Consequently, $M_n < M_w$ for polydisperse polymers. Approximate positions of these values on an SEC curve are illustrated in Fig. 6. The SEC chromatogram gives valuable information about the actual molecular weight distribution, MWD, of the polymer. Skewing or tailing in the chromatogram reveals wide MWDs. The polydispersity, PD, of the polymer represents the breadth of the MWD and can be calculated as follows

$$PD = M_w/M_n. \quad (9)$$

The higher the PD, the wider the MWD.

Materials and methods for SEC

The SEC system consisted of a solvent delivery device, a photodiode array UV detector, a computer installed with software capable of directly analysing SEC molecular weight data, two Ultrastyragel THF columns — 500 Å for molecular weights of 100–10 000 Da and 104 Å for molecular weights of 5000–600 000 Da — connected in series (all from Waters Chromatography, Milford, MA, USA), and an auto sampler; HPLC grade THF as both the solvent and mobile phase; narrow polystyrene standards with molecular weights ranging from 500 to 170 000 Da for calibration; and PLGA 50:50.

System calibration was carried out using polystyrene standard solutions prepared in degassed, prefiltered THF at concentrations of 1 mg ml^{-1}. The system was allowed to equilibrate at a flow of 1 ml min^{-1} and, with a detector setting of 220 nm, 20 ml of the standard solutions were injected. Correlation coefficients of >0.999 were routinely achieved for standard curves of log (MW) vs retention time. Polymer samples were prepared in THF at concentrations of 10 mg ml^{-1}, stirred gently overnight, then filtered with a 0.2 PVDF syringe filter. Duplicate injections were made for all samples. Table 3 lists the M_w, M_n and PD values for three separate samples of a PLGA batch. Figure 7 shows a typical chromatogram for a 50:50 PLGA sample at zero time and after 50% mass loss.

Scanning Electron Microscopy for Surface Morphology

When working with microparticulates, it is often helpful to visualize particle shapes and surface characteristics in order to correlate other determined characteristics such as surface area and bulk density. Furthermore, if the microspheres are prepared from biodegradable polymers, it is interesting to note how surface morphology changes over time as the polymer erodes. SEM is an excellent tool for physical observation of morphological features of the microspheres, both initially and during the degradation process.

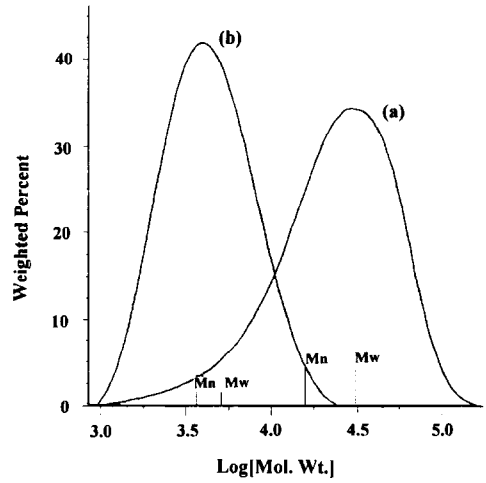

Figure 7
SEC chromatograms of PLGA microspheres before and after approximately 40% mass loss. (a) Before suspending in 0.1 M PBS, pH 7.4 at 37°C, M_w = 31007, M_n = 16075 and PD = 1.94; (b) after 6 weeks, M_w = 5121, M_n = 3653 and PD = 1.40.

Table 3
Reproducibility of SEC method using PLGA samples

Run	M_w	M_n	PD
1	29,464	16,052	1.84
2	29,848	16,927	1.76
3	29,480	17,420	1.69
Average ± SD	29,597 ± 217	16,800 ± 693	1.76 ± 0.08

Materials and methods for SEM

Blank microspheres were prepared by a phase separation technique from 30 000 Da MW PLGA 50:50 (Resomer® RG503, Boehringer Ingelheim, Germany). This process has been described previously [19]. The SEM microspheres were placed on an aluminum stud which was coated with an adhesive label. The mount was coated with conductive gold palladium and then examined at magnifications of 50×, 200× and 1500× using 2–5 kV power using a Model S-800, Hitachi SEM. The field at 200× usually contained 2–5 microspheres while that at 1500× showed only the surface of one microsphere. Figure 8 shows photomicrographs of PLGA microspheres at the three magnifications. These can be used to qualitatively assess morphology as well as particle size distribution and surface pore diameter. The 40× magnification was used to estimate size distribution and measure mean particle size. Large pores and mean pore size were measured from the 200× mag, while the 1500× mag was used to discern small pores and estimate their size. The measurements were made with a ruler with millimeter divisions and converted to micrometers using the appropriate correction factors. Figure 9 shows the morphology of these microspheres after biodegradation in phosphate buffered saline for a period of six weeks in which approximately 40% mass loss had occurred.

Gas Adsorption for Surface Area Measurements of Microspheres

Surface area of a microsphere delivery system may affect drug release and biodegradation [20–23]. Two methods for direct determination of surface area are air permeability and gas adsorption. Air permeability is based on the rate at which air is able to flow through the substance with permeability being inversely proportional to surface area. The gas adsorption method depends on the amount of gas adsorbed onto the surface of the sample, called the adsorbent. Both methods are applicable for non-porous polymeric materials, but air permeability cannot efficiently measure surface area created by small and tortuous surface pores like those present in many fabricated polymeric drug delivery systems, whereas gas adsorption can.

Materials and methods for gas adsorption

Surface area was measured by the Brunauer, Emmett and Teller (BET) technique [24] employing a Micromeritics ASAP 2000 instrument with krypton as the adsorption gas. Following calibration with alumina particles measurements were made by first weighing the empty sample holder, stopper and filling rod assembly, then adding the microspheres to the holder and reweighing the entire assembly. The sample was degassed under vacuum at 40°C for several hours and reweighed. Analysis was accomplished at approximately −195°C using liquid nitrogen and measuring the void space with a non-adsorbing gas, helium. The helium was then evacuated and research purity krypton gas added. Krypton was selected because it has a lower saturation pressure (2.7 mmHg) than nitrogen (760 mmHg), making it a more sensitive adsorbing gas [25]. This allows for using smaller sample sizes of microspheres with low surface areas. The amount of gas adsorbed onto the adsorbent was measured and the surface area determined by the microprocessor.

Sample sizes between 0.05 and 1.3 g from a batch of microspheres were analyzed and the results are shown in Table 4. Agreement of calculated surface area values for duplicate runs of the same mass of sample was very good

Table 4
Reproducibility of BET gas adsorption surface area analysis

Sample mass (g)	BET surface area ($m^2\ g^{-1}$)	Correlation coefficient
1.3120	0.2201 ± 0.0019	0.9998
1.3120	0.2209 ± 0.0018	0.9998
0.7088	0.2202 ± 0.0019	0.9998
0.7088	0.2175 ± 0.0017	0.9998
0.3013	0.2220 ± 0.0016	0.9998
0.3013	0.2189 ± 0.0018	0.9998
0.1657	0.2207 ± 0.0016	0.9998
0.1657	0.2108 ± 0.0022	0.9997
0.0504	0.2190 ± 0.0107	0.9931

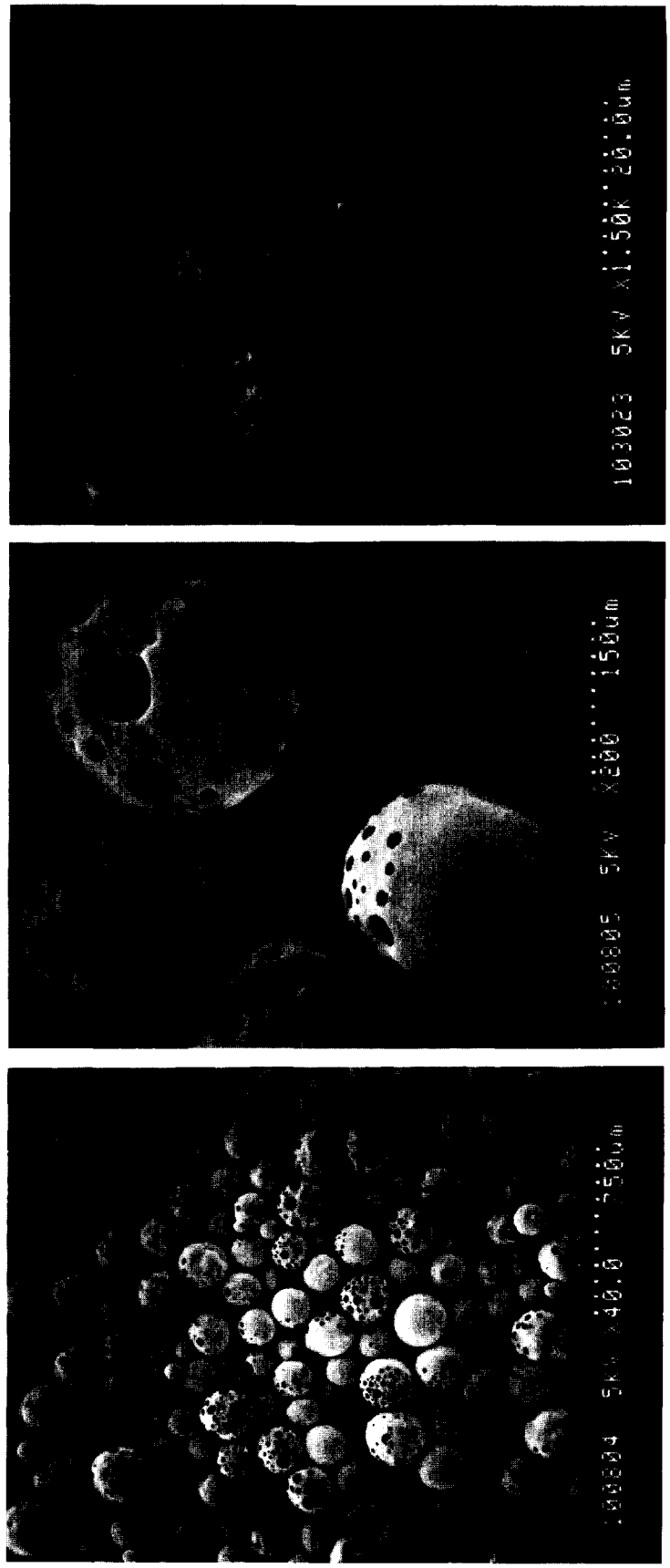

Figure 8
SEM photomicrographs of PLGA microspheres at 40×, 200× and 1500×.

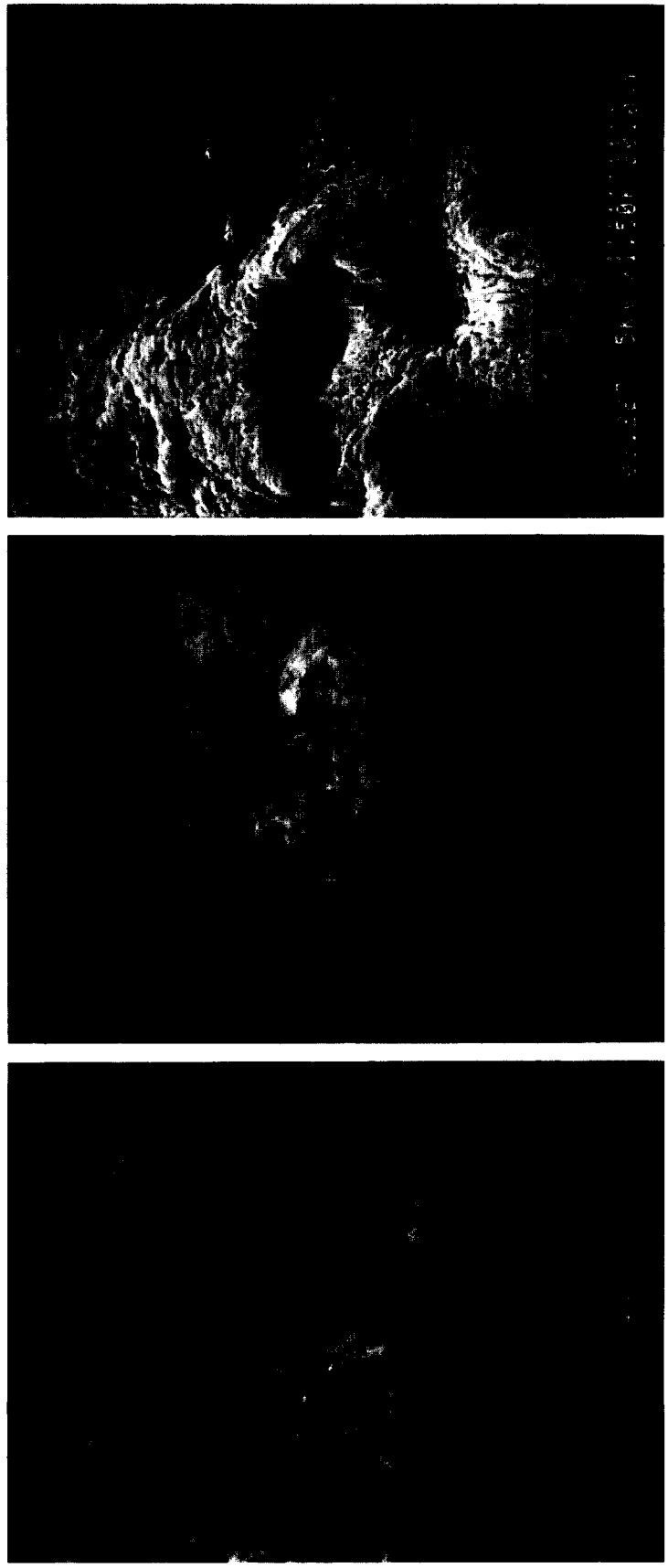

Figure 9
SEM photomicrographs of PLGA microspheres shown in Fig. 8 after *in vitro* degradation.

for sample sizes larger than 0.3 g. Similarly, surface area values were very consistent and correlation coefficients showed excellent linearity for sample sizes larger than 0.3 g. Therefore, to ensure accurate and reproducible results, a sample size of 0.5 g is recommended for surface areas less than 0.5 $m^2\ g^{-1}$.

Tap Method for Bulk Density Measurements

The density of a substance is simply expressed as the weight per unit volume. However, there are several types of densities that describe particles, two of which are true density and bulk density [12]. True density is the actual density of the material discounting all void spaces caused by packing arrangement and intraparticular pores. Bulk density, D_b, in g cc^{-1}, is the mass, m, of the packed particles divided by the bulk volume, V_b, which is the volume occupied by the dry particles in a graduated cylinder or test tube

$$D_b = \frac{m}{V_b}. \qquad (10)$$

Highly porous particles of the same size and shape as non-porous particles will have a lower D_b density than the non-porous particles. D_b is determined after tapping a particle-filled graduated cylinder until the particles tightly pack and the V_b remains constant.

Materials and methods for the tap measurements

Using the tap method, a D_b of 0.38 g cc^{-1} was determined for a standard substance, methylcellulose. This was within 2.6% of the reported value, indicating good accuracy. About 1.4 g of dry microspheres were quantitatively transferred to a 10 ml graduated cylinder and the volume noted. The cylinder was subsequently tapped on a padded flat surface from a vertical distance of approximately 0.5 inch, until the volume occupied by the particles remained unchanged. This value was then recorded as V_b, and the D_b calculated using equation 10. The reproducibility or precision of the method was determined by performing three runs on the same batch of PLGA microspheres. The results, listed in Table 5, reveal excellent agreement with the average $D_b = 0.265 \pm 0.002$ g cc^{-1} and a deviation of less than 1%.

In vitro Analysis of Degradation Behaviour

Biodegradable polymers can degrade by a heterogeneous or surface process where only the outside of the system exposed to the degradation media erodes while the inside remains intact, or by a homogeneous or bulk process as does PLGA, where the entire polymer matrix, inside and out, simultaneously degrades [26]. Figure 10 illustrates the two processes. The molecular weight and mass loss profiles are illustrated in Fig. 11. During the bulk degradation of a hydrolytically unstable polymer such as PLGA, an almost immediate and rapid decrease in molecular weight occurs as susceptible bonds in the polymer chains are cleaved. As a result, smaller polymer fragments are produced. This initial molecular weight decrease precedes any polymer mass loss. When the small fragments begin to obtain a critical molecular weight, they become soluble in the aqueous degradation media, causing mass loss.

Materials and methods for in vitro *degradation analysis*

In vitro *set up*. The *in vitro* degradation media was phosphate buffered saline (PBS) at a pH of 7.4, prepared with distilled water passed through a filtration system, and the following chemicals: sodium phosphate monobasic monohydrate, sodium phosphate dibasic anhydrous, sodium chloride and sodium azide, the latter to prevent bacterial growth. One

Table 5
Reproducibility of tapped bulk density measurements for a batch of PLGA microspheres

Trial	Mass (g)	Bulk volume (cc)	Number of taps	Bulk density (g cc^{-1})
1	1.30	4.84	80	0.267
2	1.36	5.15	60	0.263
3	1.45	5.50	60	0.263
Average ± SD				0.265 ± 0.002

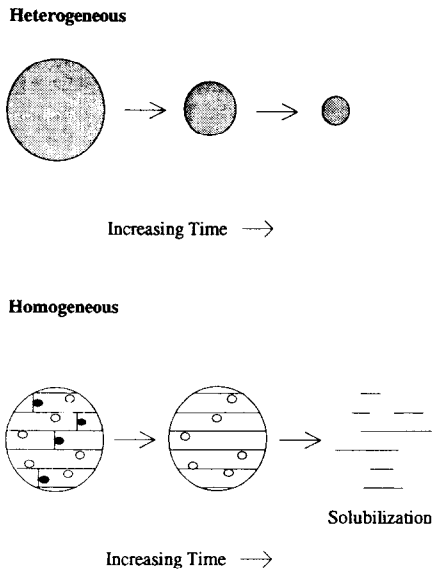

Figure 10
Illustrations of the two basic types of polymer degradation.

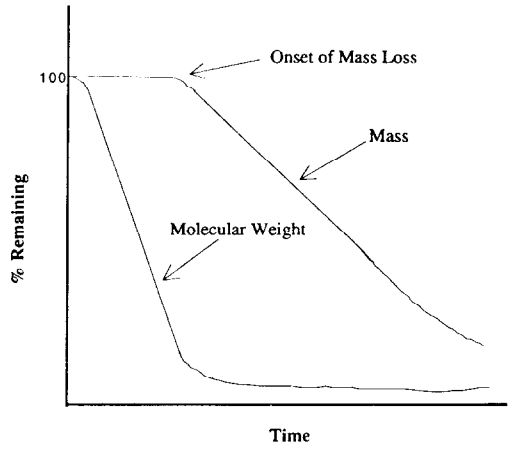

Figure 11
An example of molecular weight decrease and mass loss during *in vitro* degradation of PLGA.

hundred milligram portions of the PLGA 50:50 samples were individually weighed and transferred to 20 ml scintillation vials. Ten millilitres of PBS were introduced into each of the sample filled vials. The samples were then agitated in a shaker bath (37°C and 50 cycles min^{-1}). Duplicate samples were pulled at appropriate times and analysed for molecular weight by SEC and mass loss by gravimetry.

Mass loss analysis by gravimetry

Gravimetric analysis for polymer mass loss was accomplished on an electrobalance. One-hundred-milligram portions of the dry polymer samples were carefully weighed prior to introduction to the degradation media. This initial mass of microspheres were labelled m_i, and incubated in PBS for a designated time. The polymer samples were filtered, rinsed with distilled water, and dried for 24 h in a vacuum oven at room temperature. The dried samples were weighed to determine the mass after degradation, m_d, and the % mass remaining was calculated

$$\% \text{ mass remaining} = \frac{m_d}{m_i} \times 100\%. \quad (11)$$

For the microspheres shown in Fig. 9 a mass loss of 40% was determined after 6 weeks.

Summary and Conclusions

Thorough characterization of biodegradable polymer systems intended for use as controlled drug delivery devices requires a varied collection of carefully-selected techniques and methods that fully define the important physical and chemical aspects of the polymer itself, and of the polymer as fabricated into the desired drug delivery device. In selecting the appropriate techniques, one must consider which techniques measure the polymer characteristics that most affect the polymer's physical behaviour and the ease and availability of the technique. The eight techniques described provide a basic and thorough plan for characterizing PLGA polymers; a fundamental step toward understanding and predicting the behaviour of biodegradable drug delivery devices.

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