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Research paper

# Effects of process and formulation parameters on characteristics and internal morphology of poly(D,L-lactide-co-glycolide) microspheres formed by the solvent evaporation method

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**Abstract**

Taking ABT627 as a hydrophobic model drug, poly-(lactic-co-glycolic acid) (PLGA) microspheres were prepared by an emulsion solvent evaporation method. Various process parameters, such as continuous phase/dispersed phase (CP/DP) ratio, polymer concentration, initial drug loading, polyvinyl alcohol concentration and pH, on the characteristics of microspheres and in vitro drug release pattern of ABT627 were investigated. Internal morphology of the microspheres was observed with scanning electron microscopy by stereological method. CP/DP is a critical factor in preparing microspheres and drug loading increased significantly with increasing CP/DP ratios accompanied by a remarkably decreased burst release. At CP/DP ratio 20, microspheres with a core-shell structure were formed and the internal porosity of the microspheres decreased with increasing CP/DP ratio. Increase in PLGA concentration led to increased particle sizes and decreased drug release rates. ABT627 release rate increased considerably with increasing PVA concentrations in the continuous phase from 0.1% to 0.5%. The maximum solubility of ABT627 in PLGA was approximately 30%, under which ABT627 was dispersed in PLGA matrix in a molecular state. Increase in initial drug loading had no significant influence on particle size, drug encapsulation efficiency, burst release and internal morphology. However, drug release rate decreased at higher drug loading. Independent of process parameters, ABT627 was slowly released from the PLGA microspheres over 30 days, by a combination of diffusion and polymer degradation. During the first 13 days, ABT627 was mainly released by the mechanism of diffusion demonstrated by the unchanged internal morphology. In contrast, a core-shell structure of the microspheres was observed after being incubated in the release medium for 17 days, independent of drug loading, implying that the ABT627/PLGA microspheres degraded by autocatalytic effect, starting from inside of the matrix. In conclusion, hydrophobic drug release from the PLGA microspheres is mainly dependent on the internal morphology and drug distribution state in the microspheres.


*Keywords:* PLGA; Microspheres; Internal morphology

## 1. Introduction

Poly-(lactic-co-glycolic acid) (PLGA), a biodegradable and biocompatible polymer, has received tremendous interest for the development of microparticulate formulations [1,2]. By encapsulating the drug in a PLGA matrix from which it is released at a relatively slow rate over a prolonged time, controlled release allows less frequent administrations, thereby increasing patient compliance and reducing discomfort [3]. Since microspheres can be administered by injection, one can also achieve localized drug delivery and high local concentrations [4], such as for vaccine delivery [5]. The delivery of chemotherapeutic agents

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using polymeric microspheres has become a popular research area [6,7]. For anticancer drugs, intratumoral injection would be preferred with reduced systemic toxicity, sustained drug concentration in the target site, prolonging its pharmacological activity.

So far, numerous investigations concentrated on the microencapsulation of hydrophilic drug substances, such as proteins and peptides, into PLGA matrices using the double emulsion method and the influence of process parameters on burst release and encapsulation efficiency were extensively studied [8,9]. However, only scant information is available regarding lipophilic drug substances [10–12], which were mainly prepared by the oil-in-water emulsion/solvent evaporation process [2]. To the best of our knowledge, no information is available regarding the influence of process parameters on the internal morphology of microspheres prepared by this method. Since morphology is a crucial factor influencing the drug release profiles from microspheres especially during the pore diffusion process [13], this study is absolutely essential. ABT627 is a newly synthesized chemotherapeutic agent for the treatment of prostate cancer. It is lipophilic with slight solubility in water. Therefore, taking ABT627 as a lipophilic model drug, effect of continuous phase/dispersed phase ratio (CP/DP), PLGA concentration, continuous phase pH, polyvinyl alcohol concentration and initial drug loading on the physicochemical characteristics of the developed microspheres was studied, and internal morphology of the microspheres was analyzed by stereological method to elucidate the distribution and the release mechanism of ABT627 from microspheres.

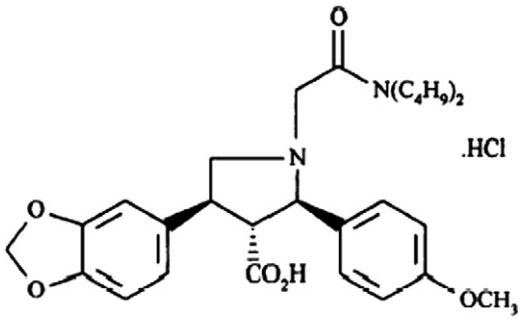
## 2. Materials and methods

### 2.1. Materials

PLGA (Resomer RG503 Mr 34,000) was purchased from Boehringer–Ingelheim (Ingelheim, Germany). Polyvinyl alcohol (PVA, 88 mol % hydrolyzed, MW 130 kDa) was purchased from Clariant GmbH (Frankfurt, Germany). ABT627 was provided by Abbott Laboratories (Lake Forest, USA); its structure is shown in Scheme 1. The partition coefficients of ABT627 between *n*-octanol and pH 5.1, 7.0 and 9.0 buffers were determined at ambient temperature. The partition coefficients were 2.74 (RSD = ±1.82%) at pH 5.1, 2.67 (RSD = ±1.03%) at pH 7.0 and 1.65 (RSD = ±1.46%) at pH 9.0. The compound has two ionizable moieties, the pyrrolidine nitrogen and the carboxylic acid. The p$K_a$ for the carboxylic acid is 6.53 ± 0.04 and the p$K_a$ for the nitrogen is 3.10 ± 0.07. All other chemicals used were of analytical grade.

### 2.2. Preparation of ABT627 loaded PLGA microspheres

ABT627 loaded PLGA microspheres were prepared by a conventional oil-in-water (O/W) microencapsulation method. Briefly, ABT627 was dissolved in methylene chloride (DCM) containing appropriately concentrated PLGA. The resulting organic phase was then injected into aqueous phase containing PVA as an emulsifier to form an o/w emulsion. A high-speed homogenizer Ultra Turrax (Janke-Kunkel, Staufen, Germany, type TP 25) was used for the emulsification operated at 8000 rpm for 30 s. The final emulsion was subsequently stirred at 200 rpm with a propeller mixer for 3 h at room temperature to evaporate methylene chloride. The microspheres were collected by filtration and washed three times with distilled water. The microspheres were then lyophilized. The relative yield was calculated based on the amount of lyophilized microspheres of each formulation obtained relative to the amount of solid materials used in the dispersed phase.




Scheme 1. Structure of ABT627.

### 2.3. Analytical method of ABT627

The content of ABT627 in the microspheres was measured using an HPLC assay with *p*-diethoxybenzene as an internal standard. HPLC assays were performed on an ODS-2 HPLC column (15 × 0.45 cm i.d. pore size Inertsil 5 μm, Meta Chem, Varian). The column temperature was maintained at 40 °C. The mobile phase was a mixture of acetonitrile:$H_2O$ (adjust the pH to 3.3 using orthophosphoric acid) 53:47 delivered at a flow rate of 1 ml/min. Samples of 20 μl were injected and detected at 234 nm with a variable wavelength detector (VWD). Keeping the internal standard concentration constant at 72 μg/ml, different concentrated ABT627 solutions were prepared and the PAR (peak area ratio) values were measured. In the concentration range of 23.96–119.8 μg/ml, a linear correlation was found between concentration and PAR with a correlation coefficient of $r = 0.999$ ($n = 5$). To study the in vitro release of ABT627 from the microspheres, a standard curve in a wide range of concentrations without internal standard was established. A linear correlation was found between concentration and peak area in the concentration range of 0.37–118 μg/ml ($r = 0.9999$, $n = 9$).

### 2.4. Determination of ABT627 content in the microspheres

ABT627 content in the microspheres was measured using an extraction method. Approximately 10 mg of the microspheres was incubated with 1 ml of acetonitrile. The

microspheres were not completely dissolved in the acetonitrile. After vortexing and 10 min incubation, 4 ml of the diluent (water:acetonitrile, 1:1) was added and mixed, whereupon the polymer precipitated. The suspension was centrifuged at 4000 rpm and the supernatant was collected for HPLC assay. One milliliter of the supernatant and 0.3 ml internal standard solution were added into a volumetric flask and added to volume 5 ml. The concentration of ABT627 was analyzed with the HPLC assay method described above. Triplicate samples were prepared for each of the formulations. The actual drug loading and encapsulation efficiency (EE) were calculated using the following equation:

$$\text{Theoretical drug loading} = \frac{\text{drug(tot.)}}{\text{drug(tot.)} + \text{polymer}}$$

$$\text{Actual drug loading} = \frac{\text{drug(enc.)}}{\text{drug(tot.)} + \text{polymer}}$$

$$\text{Encapsulation efficiency} = \frac{\text{actual drug loading}}{\text{theoretical drug loading}} \times 100\%$$

### 2.5. Particle size measurement

The particle size distribution of the prepared microspheres was measured using a MasterSizer X (Malvern Instruments) based on the laser light scattering. Weighed microsphere samples were suspended in 0.1% aqueous Tween 80 solution (1 ml) and vortexed before measurement. The homogeneous suspension was examined to determine the particle size distribution. Results are reported as volumetric mean diameter [$D(4,3)$] ($n = 3$).

### 2.6. Morphology

For the morphology studies, freeze-dried particles were visualized using scanning electron microscopy. Samples were sprinkled on a double-sided adhesive tape attached to an aluminum stub and fixed onto a graphite surface. Excess samples were removed and the stub sputter coated with gold. The coated samples were viewed under a scanning electron microscope at 25 kV to reveal the surface quality and porosity of microspheres (The CamScan Series 4 Scanning Electron Microscopes, Cambridge Scanning Company Limited, England). Cross-sectional morphology of microspheres was obtained by embedding the microspheres in an aqueous solution containing 30% gelatin and 5% glycerin, as described previously [13,14].

### 2.7. Differential scanning calorimetry (DSC)

Thermal characterization of microspheres was performed with a Perkin-Elmer DSC 7 (Perkin-Elmer, Wellesley, MA). Samples were weighed (approximately 5 mg) and placed in sealed aluminum pans. The equipment was calibrated with indium. The samples were scanned at 10 °C/min from −40 to 270 °C in nitrogen atmosphere (flow rate 61 ml/min). The sample was first heated from −40 to 270 °C. It was then quenched to −40 °C with liquid nitrogen, and heated again to 270 °C. The melting temperature ($T_m$) was determined from the endothermic peak of the DSC curve recorded in the first heating scan. The glass transition temperature ($T_g$) was determined from the DSC curve recorded in the second heating scan. Reported glass transition temperatures are midpoint values.

### 2.8. In vitro release

The release rate of ABT627 from microspheres was measured in phosphate buffer saline (PBS) medium (pH 7.4) containing 0.5% SDS by an HPLC assay. The release medium was selected based on solubility studies to maintain the sink condition. Approximately 10 mg ABT627 loaded microspheres was suspended in 10 ml PBS in screw-capped tubes and placed in an orbital shaker (Rotatherm, Liebisch, Germany) maintained at 37 °C and rotating at 30 rpm. At predetermined time intervals, the tubes were taken out of the shaker and centrifuged at 2000 rpm for 5 min. One milliliter of the supernatant was taken for analysis. Fresh medium of the equal volume was added in the meantime. The precipitated microspheres pellets were resuspended in the buffer and placed back in the shaker. The amount of drug released during the first 24 h was designated as "burst release".

## 3. Results and discussion

### 3.1. Effect of continuous phase/dispersed phase (CP/DP) ratio

Keeping drug loading (20%), PVA concentration (0.1%), organic phase volume (5 ml) and polymer concentration (10%) constant, the effect of CP/DP ratio on the properties of the microspheres was investigated, as shown in Fig. 1.

No significant difference in particle size was observed ($P > 0.05$). All microspheres have a spherical shape without pores on the surface, with size approximately 20 μm (Fig. 2). However, the drug loading and encapsulation efficiency increased remarkably with increasing CP/DP ratio ($P < 0.05$). Similar phenomena were reported for the encapsulation of progesterone [12]. Additionally, the surface of microspheres was smoother at higher CP/DP ratios, probably due to the faster solidification rate. It has been reported that the porosity in a system of microspheres is determined during microspheres hardening as the organic solvent evaporates during preparation [12]. CP containing a large amount of water resulted in faster polymer precipitation and therefore less porous spheres were formed [15]. Similarly, burst release depended on CP/DP ratio as well and $13.7 \pm 0.28\%$ burst release was found at CP/DP ratio 20 compared to that of $1.68 \pm 0.11\%$ at CP/DP ratio 90, despite the relatively low drug loading at CP/DP ratio 20. This can probably be explained by differing distributions of ABT627 in the microspheres due to differing evap-

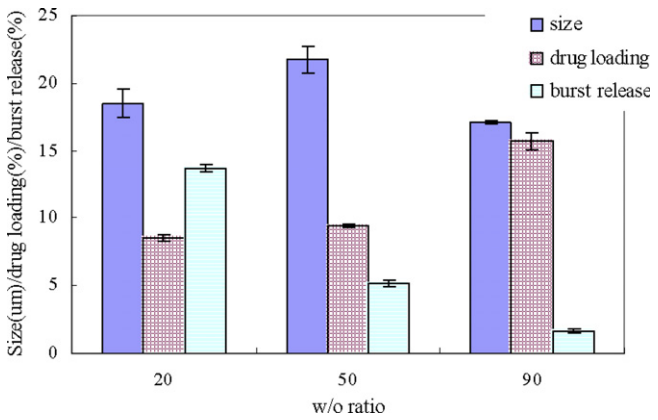



Fig. 1. Effect of aqueous/organic phase ratio on the properties of PLGA–ABT627 microspheres.

oration rates of DCM. The solubility of DCM in water is about 2% (v/v). When the aqueous volume is less, DCM will distribute into the aqueous phase slowly which is controlled by DCM evaporation rate, therefore, drug substance will migrate to the microsphere surface together with DCM due to its superior solubility in the solvent, leading to drug-enriched microsphere surface. In contrast, when the aqueous volume is large, DCM distributed into the water phase immediately and ABT627 was solidified in the matrix of the polymer without delay. It is well known that drug substance near the surface will diffuse out of the microspheres first, causing burst release. Moreover, it has been indicated previously that the morphology of microspheres depends on the rate of polymer precipitation and solvent removal at the interface [16]. From the cross-sectional images of the microspheres before release study (Fig. 2), it is clear that internal phase of the microspheres is CP/DP ratio-dependent and a core-shell structure was observed at CP/DP ratio 20 and the porosity decreased with increasing CP/DP ratio. Porosity has an important effect on drug release characteristics and is related to the initial burst effect [17,18].

The cumulative release profiles of ABT627 from microspheres prepared at different CP/DP ratios were significantly different, as shown in Fig. 3. Higher release rates were observed for the microspheres prepared at CP/DP 20 during the first 15 days despite lower drug loading. The internal pore structure of biodegradable polymeric delivery systems seems to play an important role in the release characteristics of the entrapped agents [19]. The core-shell structure of the microspheres prepared at CP/DP ratio 20 decreased the diffusion distance compared to the microspheres prepared at CP/DP 50 and 90, in which drug substance was distributed more homogeneously in the solid spherical polymer matrix, leading to faster release rates. In general, the drug release pattern from PLGA microspheres was found to be biphasic, as a combination of diffusion and polymer degradation. Initially, drug is released via diffusion through the polymer matrix as well as through the porous voids of the polymer structure, but degradation of PLGA continuously changes the drug release pattern. All ABT627 batches exhibited a typical S-shaped release pattern. Low release rate during the first 13 days can be attributed to pore diffusion of the drug substance from the polymer matrix. This is supported by the unchanged micro-morphology of the microspheres after 7 days release independent of CP/DP ratio (Fig. 2). An extremely fast drug release was found between 13 and 21 days. This can probably be explained by the fact that local pH dropped within microspheres due to the production of acidic groups as a result of the polymer hydrolysis. Fu et al., using a pH sensitive dye technique, have reported a pH drop to 1.5 within PLGA microspheres after 15 days in phosphate-buffered saline [20]. The solubility of ABT627 is pH-dependent and increased with decreasing pH, which probably contributed to the fast release after 13 days. In addition, as PLGA degradation is catalyzed by protons, this micro-pH decrease can lead to autocatalytic effects and, thus, accelerate polymer degradation and drug release [21]. The core-shell structure of the microspheres after 17 days release (Fig. 2) provided direct evidence for proton catalyzed PLGA degradation, starting from inside of the microsphere. At this stage all the microspheres attached to each other, losing their individual morphology. The release slowed down and leveled off on day 25, at which point more than 80% drug substance was released.

### 3.2. Effect of PLGA concentration in the organic phase

Keeping all other parameters constant, the effect of polymer concentration (5–30%) on the properties of the microspheres was investigated, as shown in Fig. 4a. Particle size increased with increasing polymer concentration and remarkable size increases occurred when the polymer concentration increased from 10% to 15%. Further increase in polymer concentration up to 30% increased particle size marginally. It was noticed that drug loading increased considerably when PLGA concentration increased from 5% to 10%, which can be explained by the increased viscosity of the organic phase and denser internal structure, therefore less drug loss during the evaporation process. However, no remarkable further increase was found when increasing PLGA concentration to 30%, with drug encapsulation efficiency approximately 91%. A low polymer concentration resulted in high internal porosity (data not shown) and thus, high internal burst at PLGA 5%. Burst release decreased remarkably when PLGA concentration increased from 5% to 10% and remained constant up to 20%. The burst release was less than 2% for all the microspheres prepared at PLGA concentration >10%.

The in vitro release profiles are shown in Fig. 4b. All the microspheres exhibited a typical S-shaped release pattern. ABT627 was released faster at a PLGA concentration of

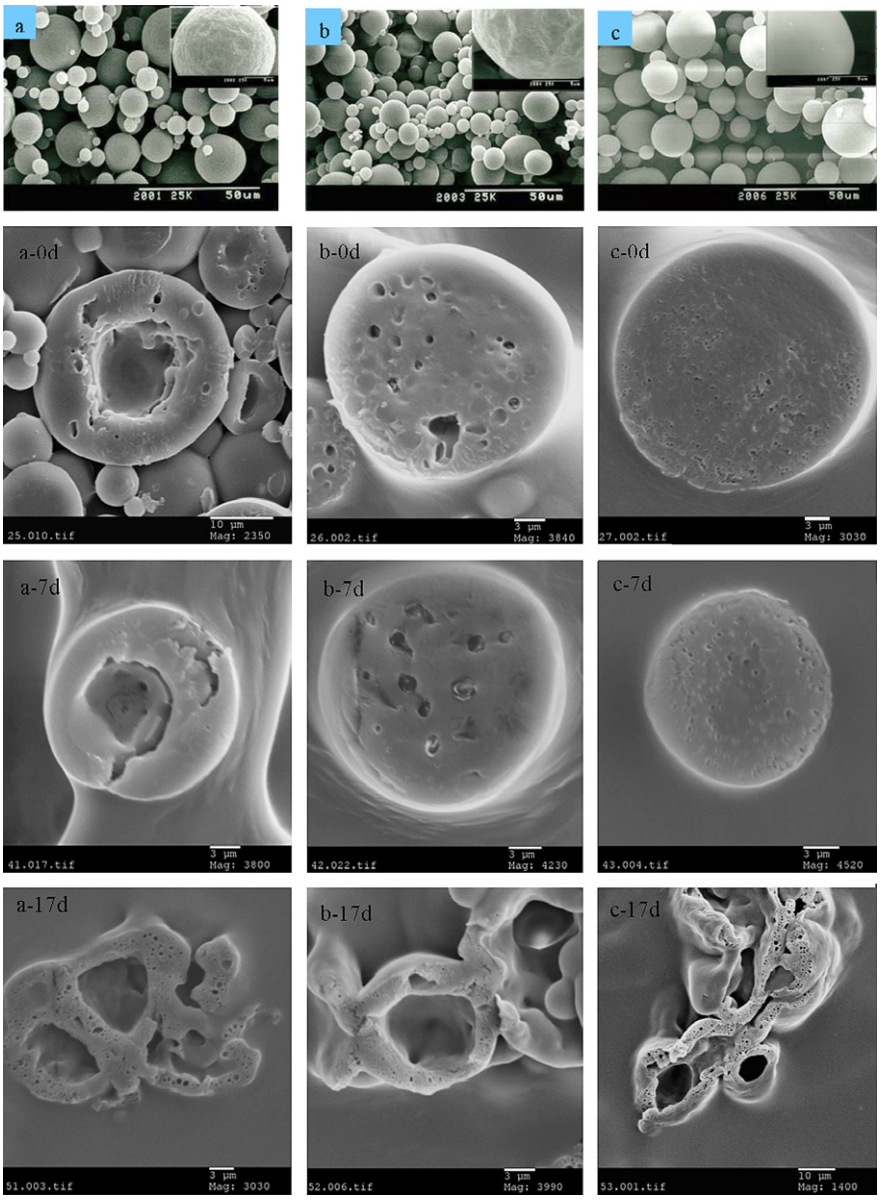



Fig. 2. Surface and internal morphology of the microspheres prepared at different CP/DP ratio and after 0, 7 and 17 days of release. Insets show the surface of microspheres at a high magnification. (a) CP/DP 20, (b) CP/DP 50, (c) CP/DP 90.

5%. No significant difference in relative release was found when PLGA concentration was in the range of 10–20% percent. However, considerably decreased release was observed when PLGA concentration was as high as 30%. Two reasons could be responsible for the lower burst with increasing polymer concentration. One is that larger microspheres have smaller surface area. The other is high viscosity restricted the diffusion of drug substance out of the matrix.

### 3.3. Effect of PVA solution pH

Solubility of ABT627 is pH dependent, and its minimum was found at pH 4–5. Keeping CP/DP ratio at 90, PLGA concentration at 10% and theoretical drug loading at 20%, the effect of PVA solution pH on the properties of microspheres was studied and the results are shown in Fig. 5a. A slightly increased particle size was observed at pH 4.0. No significant difference in particle size was found between pH 4.5 and 6.5 ($P > 0.05$). However, the drug loading increased with decreasing pH and a statistically increased drug loading was found at pH 4.0 and pH 4.5 compared with that of pH 5.67 ($P < 0.05$). Therefore, controlling the pH of the aqueous phase is essential to increase the drug loading in some cases. Changing the pH of the external water phase to modulate drug entrapment has been reported previously [22]. The release profiles are shown in Fig. 5b. No significant influence of PVA solution pH on the in vitro release of ABT627 was found in the range investigated.

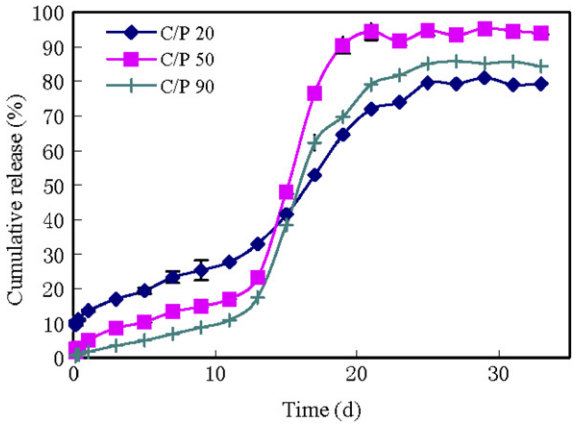
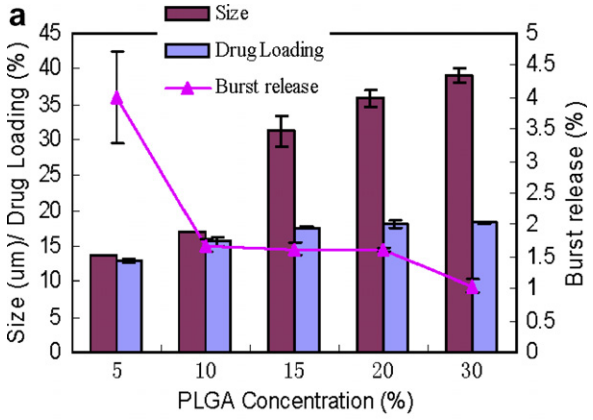



Fig. 3. Effect of aqueous/organic phase ratio on the in vitro release of ABT627 from the microspheres.



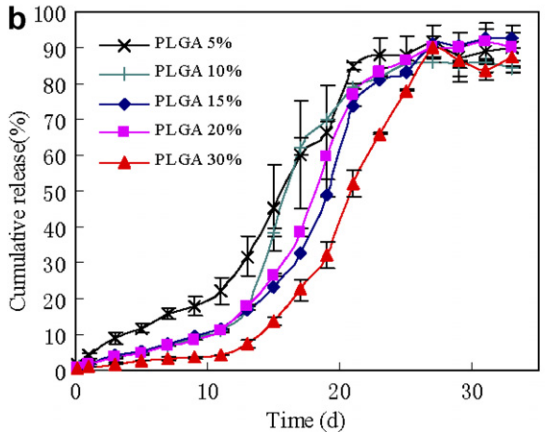
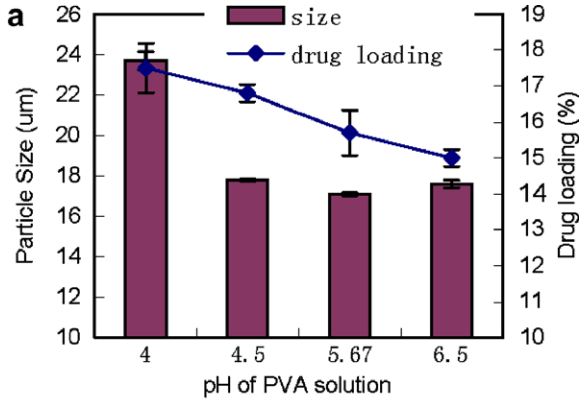




Fig. 4. (a) Particle size, drug loading and burst release versus PLGA concentration. (b) In vitro release profiles versus PLGA concentration.

### 3.4. Effect of PVA solution concentration

Keeping PVA solution pH at 4.5, the effect of PVA concentration on the properties of the microspheres was studied and the results are shown in Fig. 6a. PVA content in the water phase did not affect the particle morphology and size distribution significantly. However, when PVA concentration changed from 0.1% to 0.05%, drug encapsulation efficiency decreased from 82.3% to 72.0%. Normally, stability of o/w emulsion increased with increasing PVA concentration in the water phase against coalescence. It seems that 0.05% PVA is not sufficient to stabilize the emulsion droplets, leading to decreased encapsulation efficiency. However, a further increased PVA concentration (0.5%) did not lead to a higher drug loading, implying that 0.1% PVA might be a suitable concentration. Burst release amount decreased slightly with increasing PVA concentration. Similar phenomenon was reported previously [23].



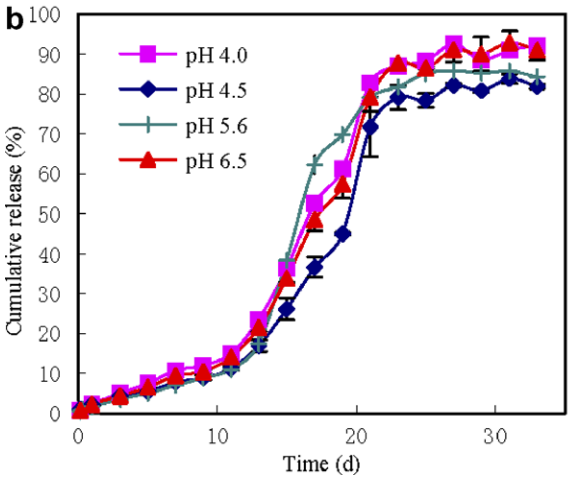




Fig. 5. (a) Particle size, drug loading and burst release versus PVA solution pH. (b) In vitro release profiles versus PVA solution pH.

The release profiles are shown in Fig. 6b. No significant difference in release percentage was found between microspheres prepared with 0.05% and 0.1% PVA. However, remarkably increased release was found during the first 20 days when PVA concentration increased to 0.5%, probably due to the surfactant effect of PVA remaining on the surface of the microspheres.

### 3.5. Solubility of ABT627 in PLGA matrix

The solubility of ABT627 in PLGA matrix was studied with DSC since DSC is a very useful tool in the investigation of thermal properties and can provide both qualita-

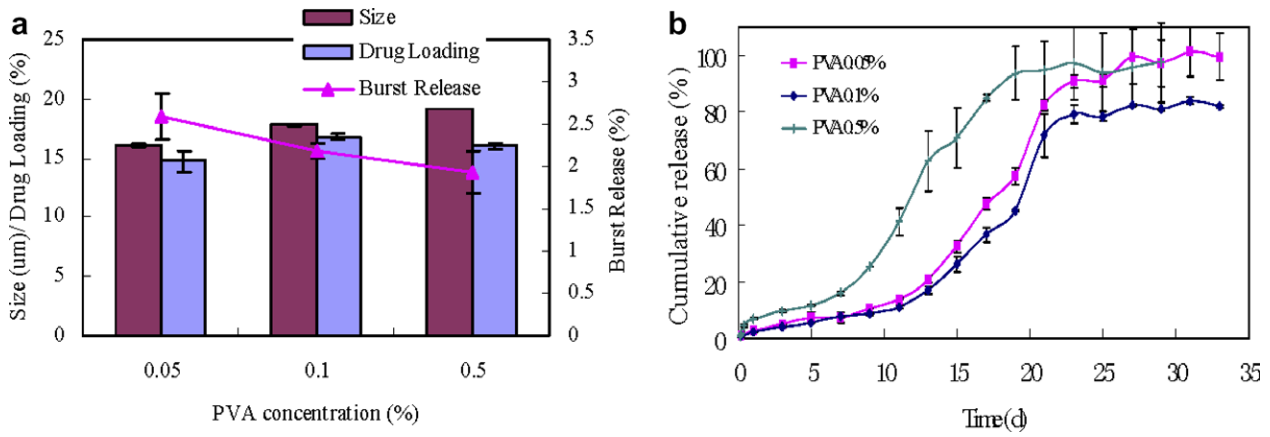



Fig. 6. (a) Particle size, drug loading and burst release versus PVA concentration. (b) In vitro release profiles versus PVA concentration.

tive and quantitative information about physicochemical state of the drug inside the matrix [24,25]. There is no detectable endotherm if the drug is present in a molecular dispersion or a solid solution state in the polymeric matrix. This amorphous nature of the drug may have pronounced pharmaceutical significance as it could lead to an improved biological activity. In order to investigate the dispersion state of the drug in the polymer matrix and determine the maximal drug embedding capacity in the polymer, first of all, the interaction between the polymer and drug substance ABT627 was investigated with different blend films. Melting endotherm of pure ABT627 was found to be 227 °C. The glass transition temperature and melting temperatures of different samples were investigated and influence of drug content on the $T_g$ of the polymer is shown in Fig. 7. $T_g$ decreased with drug content until drug loading 30%. A good correlation between $T_g$ and ABT627 concentration was established in ABT627 concentration range of 0–30% ($T_g = -0.8575C + 40.36$, $r = 0.995$, $n = 4$) and no melting temperature of the drug substance was observed, suggesting that the drug is molecularly dispersed in the matrix. However, when the drug loading is as high as 50%, $T_g$ started to increase, crystalline drug appeared on the film surface. These results implied that the maximum solubility of ABT627 in PLGA is approximately 30%.

Furthermore, since DCM was known to have a high affinity for PLGA, the glass transition temperature of the blank microspheres was investigated and compared with that of the pure polymer. No difference was found between each other (40.97 versus 40.96), indicating that the residual traces of DCM entrapped were too low to affect the $T_g$ of PLGA. Therefore, the $T_g$ shift recorded in DSC was solely due to the presence of ABT627.

### 3.6. Influence of initial drug loading

To determine an optimal drug loading, based on the data from DSC studies, keeping CP/DP ratio 90, polymer concentration 10%, 0.1% PVA solution pH 4.5, influence of

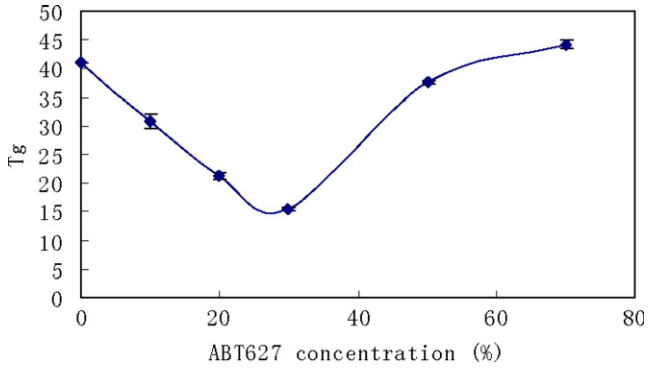



Fig. 7. The relationship between ABT627 concentration and glass transition temperature of PLGA RG503.

theoretical drug loading (20%, 30%, 35%, 40%, 45%) on the properties of the microspheres was investigated. The results are listed in Table 1.

The initial drug loading did not influence either the mean particle size or size distribution significantly, as demonstrated previously [25]. All the drug encapsulation efficiencies were higher than 90%. The high encapsulation efficiency of a hydrophobic drug like ABT627 is due to its high partition coefficient, and therefore, its retention in the organic phase as the microspheres solidify. However, the yield decreased slightly with increasing drug loading, probably due to increased viscosity of the dispersing phase. Concerning the burst release, approximately 1% of ABT627 was released from the microspheres after 24 h incubation irrespective of drug loading, implying that most of the drug substance was embedded into the internal of the microspheres instead of adsorbing on the surface. This point was further supported by the observation of the surface morphology. Fig. 8 shows that all the microspheres were spherical in shape with smooth surfaces. No crystalline drug was found on the surface of the microspheres even when the drug loading was as high as 45%. Internal morphology observation revealed that increasing drug loading did not change the micro-morphology of the

Table 1
Effect of drug loading on the properties of ABT627 microspheres

| Batch | Size $D[4,3]$ (μm) | Theo. drug loading (%) | Actual drug loading (%) | EE (%) | Yield (%) | Burst release (%) |
|---|---|---|---|---|---|---|
| ABT627-21 | 22.3 ± 0.2 | 30 | 28.5 ± 1.3 | 94.2 ± 4.3 | 83.9 | 0.90 ± 0.02 |
| ABT627-22 | 18.7 ± 0.2 | 35 | 33.5 ± 0.8 | 96.2 ± 2.5 | 82.6 | 1.28 ± 0.05 |
| ABT627-23 | 19.6 ± 0.1 | 40 | 37.2 ± 1.4 | 93.0 ± 3.4 | 75.6 | 1.12 ± 0.14 |
| ABT627-24 | 19.8 ± 0.1 | 45 | 42.9 ± 1.7 | 95.5 ± 3.8 | 76.4 | 1.04 ± 0.16 |

microspheres, which is mainly controlled by the process parameters (Fig. 8f).

In vitro release of different microspheres is shown in Fig. 9. ABT627 release from the microspheres is drug loading dependent, the higher the drug loading the lower the release rate. ABT627 was released faster at drug loading 20%, no remarkable difference was found between drug loading 30 and 40%. Release slowed down significantly at drug loading 45%. Similar phenomena were reported by several authors, that is, faster release at lower drug content and decreased release rates at a higher drug loading [26–28]. Hydrophobic drug was thought to crystallize inside the microspheres and phase separation occurred within microspheres at higher drug loadings, whereas a molecular dispersion was achieved at lower drug loading. As a result, crystallized or aggregated hydrophobic drug dissolved more slowly. Our results can probably be explained by the phase behavior of the drug substance in the polymer matrix. Therefore, DSC was employed to study the existing state of ABT627 in the microspheres at different drug loading. It showed that when the drug loading was 20%, all the drug substance was dispersed in the polymer matrix in an amorphous state. By contrast, a small part of ABT627 existed in a crystalline state within drug loading 30–40%, indicated by a small melting peak in the DSC curve in the first heating run, and the ratio increased at drug loading 45% (data not shown). This result is in good agreement with the decreased in vitro release rate at higher drug loading due to the low dissolution rate of drug substance at a crystalline state.

However, with another slight water-soluble crystalline drug substance, fentanyl, an opposite phenomenon was observed [16]. It was found that with the fentanyl-loaded PLGA microspheres, the release of microspheres with low drug loading was slower than that with high drug loading. It was explained that the crystals of fentanyl in low initial drug loading were finely dispersed in the PLGA matrix and the release pattern of fentanyl was not affected by the

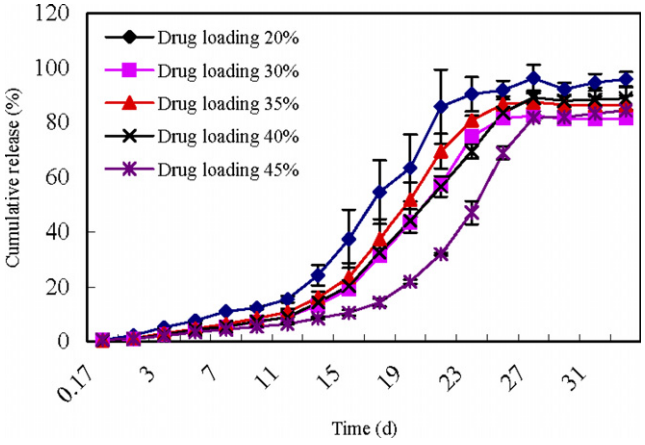



Fig. 9. Effect of drug loading on the in vitro release of ABT627 from PLGA microspheres.

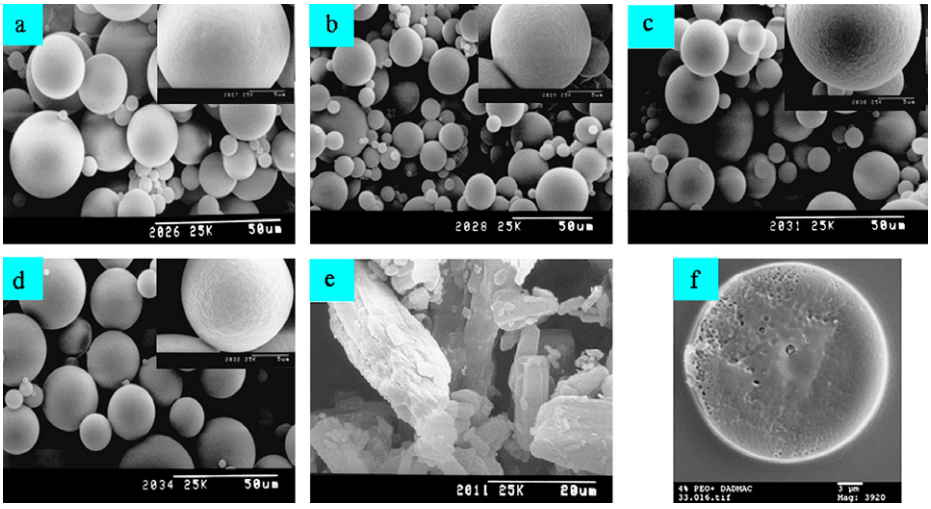



Fig. 8. Morphology of the microspheres prepared at different drug loading. Inserts show the surface of microspheres at a high magnification. (a) 30%, (b) 35%, (c) 40%, (d) 45%, (e) ABT627 pure drug, (f) internal morphology of microsphere with drug loading 45%.

biodegradation of PLGA. In contrast, at higher initial drug loading drugs were entrapped in polymer matrix like network and were released by simple dissolution and diffusion. X-ray analysis did show that part of the drug substance existed in a crystalline state even at drug loading 10%. Therefore, the release rate of drug substance from PLGA microspheres depends to some extent on the miscibility of the drug substance with the polymer.

## 4. Conclusions

For the microencapsulation of hydrophobic drug substance into PLGA matrix, continuous phase/dispersing phase ratio is a crucial factor. Drug loading increased significantly with increasing CP/DP ratio accompanied by remarkably decreased burst release. At CP/DP ratio 20, microspheres with a core-shell structure were observed and the internal porosity of the microspheres decreased with increasing CP/DP ratio. Increase in PLGA concentration increased particle size but decreased drug release rate. Increase PVA concentration in the continuous phase from 0.1% to 0.5% increased ABT627 release rate. The maximum solubility of ABT627 in PLGA microspheres is approximately 30%, under which ABT627 was dispersed in PLGA matrix in a molecular state. Its release rate decreased with increasing initial drug loading. ABT627 was slowly released from the PLGA microspheres over 30 days, by a combination of pore diffusion and polymer degradation. During the first 13 days, ABT627 was mainly released by the mechanism of diffusion, supported by the unchanged internal morphology of the microspheres after 7 days release. Internal morphology observation after incubating the microspheres for 17 days indicated that the ABT627/PLGA microspheres were mainly degraded by auto-catalyzation from the inside, as revealed by the core-shell structure of the microspheres at that release stage. In summary, hydrophobic drug release from PLGA microspheres is mainly dependent on the internal morphology and drug distribution state in the microspheres.

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