Designing drug-loaded multi-layered polymeric microparticles

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Abstract	<p>This work reports how novel multi-layered (from double-layered to quadruple-layered) microparticles comprising immiscible polymers can be fabricated through a simple, economical, reliable and versatile one-step solvent evaporation method. These multi-layered microparticles would be excellent candidates to overcome problems inherent in single-layered microparticles for drug delivery. Particle morphologies, layer configurations, and drug distribution were determined by scanning electron microscopy and Raman mapping. Key process parameters achieving the formation of the multi-layered structure were identified. Encapsulation of multiple drugs and layer localization of these drugs within these multi-layered microparticles has also shown to be possible, which were driven by drug-polymer affinity. This one-step fabrication technique can therefore be used for tailoring particle designs, thus facilitating the development of multiparticulate drug delivery devices.</p>	

3 Designing drug-loaded multi-layered polymeric microparticles

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7 **Abstract** This work reports how novel multi-layered (from
8 double-layered to quadruple-layered) microparticles com-
9 prising immiscible polymers can be fabricated through a
10 simple, economical, reliable and versatile one-step solvent
11 evaporation method. These multi-layered microparticles
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20 possible, which were driven by drug-polymer affinity. This
21 one-step fabrication technique can therefore be used for tai-
22 loring particle designs, thus facilitating the development of
23 multiparticulate drug delivery devices.
24

25 1 Introduction

26 Polymer particles have been extensively used in the med-
27 ical industries to deliver drugs [1, 2]. Considered for par-
28 enteral, oral, nasal or pulmonary administration, they have
29 the potential to achieve sustained and controlled release of

the encapsulated drugs, while the drugs can be protected 30
from degradation and physiological clearance before they 31
reach the release site [3]. However, monolithic or single- 32
layered polymeric microparticles may suffer from several 33
inherent limitations, including initial burst release of drugs 34
[4], a lack of time-delayed or pulsatile release of drugs and 35
the inability to achieve constant drug release [5–7]. 36

The fabrication of multi-layered microparticles is an 37
important step towards an attractive and robust approach to 38
provide a variety of drug delivery kinetics and profiles, 39
where some layers contain drug substances; others are rate- 40
limiting layers [8–11]. For example, multi-layered micro- 41
particles could provide pulsatile drug release kinetic, in 42
which the drug-loaded core is surrounded by non-drug- 43
holding layers with an outermost layer containing the initial 44
bolus of drugs. Through the layer localization of drugs in 45
different layers, two or more different drugs could be 46
released at different doses with time. Furthermore, two or 47
more particle populations can be readily combined into the 48
multi-layered microparticles as a dosage form, allowing for 49
easy adjustment of pharmacokinetic profile and/or creation 50
of synergistic and multiple strengths of a single micropar- 51
ticle. The unique hydrolytic degradation characteristics and 52
structural attributes of multi-layered microparticles would 53
also enable the distinctive drug release profiles [12, 13]. 54

Several processes have been developed to fabricate 55
double-layered microparticles, including hot melt tech- 56
nique, pan- or dip-coating [14], fluidized beds [15], spray- 57
drying [16, 17], a precision particle fabrication technology 58
[18, 19], and emulsion solvent evaporation [20, 21]. Unlike 59
other fabrication process where pre-fabricated microparti- 60
cles were further coated, the solvent evaporation technique 61
is a one-step process used to fabricate double-layered 62
microparticles with higher yields, highly uniform coating, 63
and a controllable particle size ranging from 20 to 64

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1,000 μm [22–24]. The solvent evaporation technique is also more economical since expensive and complex fabrication equipment is not required. In addition, poorly soluble drugs can be directly loaded into the coating layer in the solvent evaporation technique, in comparison to the fluidized bed method [15, 21]. On another note, the solvent evaporation technique has been widely utilized to fabricate the microparticles in a small scale for research purpose. While the development of multi-layered (more than two layers) remains a major challenge in the one-step fabrication, composite microparticles so far obtained from the solvent evaporation method are only double-layered, although the third or more layers can be added in separate steps [21, 25]. These additional steps would result in quality-control problems and a decrease in yield [5, 22]. A fabrication technique that requires only single step and provides easy formation of multi-layered (more than two layers) particles with layer localization of drugs would therefore facilitate the development of different ingenious designs of the particulate drug-delivery system.

In the present work, the fabrication of novel multi-layered (from double-layered to quadruple-layered, and potentially multilayered) microparticles using a one-step solvent evaporation technique is reported. These multi-layered microparticles were composed of poly(D,L-lactide-co-glycolide, 50:50) (PLGA), poly(L-lactide) (PLLA), poly(ethylene-co-vinyl acetate, 40 wt% vinyl acetate) (EVA) and polystyrene (PS). The polymers were selected based on the mutual immiscibility. Developing double-layered microparticles is challenging, and the complexity of the process dramatically increases with each additional polymer. Key process parameters achieving the formation of the multi-layered structure were identified. Ibuprofen, lidocaine base and metoclopramide monohydrochloride monohydrate (henceforth referred to as metoclopramide HCl) were used as model drugs with different hydrophobicities to understand how multiple drug loading and localization of drugs in different layers of the microparticles can be realized through this one-step fabrication technique. We believe that our work suitably illustrates the potential of a novel fabrication technique for the multi-layered microparticles (more than two layers), in which a wide range of multi-layered particulate systems can be used to realize low-cost and efficient process schemes in different drug-delivery applications.

2 Materials and methods

2.1 Materials

Poly(L-lactic acid) (PLLA, intrinsic viscosity/IV: 2.38, Bio Invigor), poly(DL-lactic-co-glycolic acid, 50:50) (PLGA,

IV: 1.18, Bio Invigor), poly(ethylene-co-vinyl acetate, 40 wt% vinyl acetate) (EVA, molecular weight/MW 42 kDa, Aldrich), poly(styrene) (PS, monocarboxy terminated, MW 50 kDa, Aldrich) and poly(vinyl alcohol) (PVA, MW 30–70 kDa, Sigma-Aldrich) were used without further purification. The drugs, ibuprofen, lidocaine base, metoclopramide HCl, were purchased from Sigma-Aldrich and used as received. Dichloromethane (DCM) from Tedia Company Inc. was of high-performance liquid chromatography (HPLC) grade and used as received.

2.2 Determination of polymer cloud point

The four polymers (PLGA, PLLA, PS and EVA) were selected on the basis of mutual immiscibility. To determine the polymer cloud point, a homogenous polymer solution (2% (w/v)) was prepared in which polymers were dissolved in DCM. The polymer solution was then transferred to a graduated cylinder and allowed to sit undisturbed in a fume hood at room temperature. When cloudy phases became apparent due to polymer phase separation, the volume of the solution was recorded [26, 27].

2.3 Fabrication of microparticles

Single-layered and multi-layered microparticles were prepared using an emulsion solvent evaporation method [5, 20]. The polymers were first dissolved in DCM. After which, the resultant polymer solution was first ultra-sonicated for 1 min and was subsequently added to a PVA aqueous solution (0.5% (w/v)) with oil-to-water ratio of 0.013 and emulsified at 400 rpm using an overhead stirrer (Calframo BDC1850-220) at room temperature (25°C). The evaporation of DCM results in the phase separation of the polymers, yielding multi-phase microparticles. Finally, the microparticles were centrifuged, rinsed with de-ionized water, lyophilized, and stored in a desiccator for further characterization. Table 1 details the processing parameters investigated in this study. For the fabrication of multiple drugs-loaded quadruple-layered microparticles, ibuprofen (5% w/w), lidocaine base (10% w/w) and metoclopramide HCl (20% w/w) were first added to the polymer solution and the resulting solution was then ultrasonicated for 1 min to achieve complete homogenization. The same process parameters for fabricating particle S4 were employed to produce multiple drugs-loaded quadruple-layered microparticles.

2.4 Characterization

2.4.1 Scanning electron microscope (SEM)

The internal morphologies of microparticles were observed using a JEOL JSM-6360A SEM at an accelerating voltage

Table 1 Parameters used to fabricate the composite microparticles

Sample	Polymer solution concentration (% w/v)	Polymer mass ratios			
S1	10	PLGA			
		0.3 g			
S2	7.5	PLGA	PLLA		
		0.2 g	0.1 g		
		2	1		
S3	7.5	PLGA	PLLA	EVA	
		0.2 g	0.1 g	0.05 g	
		4	2	1	
S4	13	PLGA	PLLA	PS	EVA
		0.4 g	0.2 g	0.1 g	0.04 g
		10	5	2.5	1
S5	13	PLGA	PLLA	PS	EVA
		0.4 g	0.2 g	0.05 g	0.05 g
		8	4	1	1
S6	7.5	PLGA	PLLA	PS	EVA
		0.4 g	0.2 g	0.1 g	0.04 g
		10	5	2.5	1
S7	5	PLGA	PLLA		
		0.2 g	0.1 g		
		2	1		

785 nm near infrared diode laser, and a 10× objective lens was used to collect the backscattered light. Scans were performed in a spectral window from 300 to 1,900 cm⁻¹ and acquisition time for each Raman spectrum was around 35 s. Spectral pre-processing that included spike removal due to cosmic rays was first carried out before the Raman mapping data was further analyzed using the band-target entropy minimization (BTEM) algorithm. The BTEM algorithm was developed to reconstruct the pure component spectral estimates [28, 29]. When all normalized pure component spectra of all of the underlying constituents were reconstructed, the relative contributions of each measured point of these signals could be calculated by projecting them back onto the baseline-corrected and normalized data set. The spatial distribution of each of the underlying constituents was then generated.

3 Results and discussion

3.1 Formation of multi-layered microparticles

The fabrication of multi-layered microparticles using a one-step solvent evaporation is based on the inherent phase separation of immiscible polymers in the emulsion droplets during solvent extraction and evaporation [5]. As the concentration of polymers in the emulsion droplets increases and reaches the cloud point as a result of solvent extraction and evaporation, polymers phase separate to yield composite multi-layered microparticles. Cloud point is a polymer solution concentration at which one polymer becomes immiscible with the other polymers and they begin to phase separate, which can be determined experimentally [26, 27].

With this solvent evaporation technique, we demonstrated an evolution from single-layered to quadruple-layered (S1–S4) and potentially multi-layered, microparticles. The process parameters used to fabricate these microparticles are listed in Table 1. Figure 1 shows the SEM images for these microparticles and their corresponding pure-component Raman spectra estimates and associated score images. The particles were spherical in shape. Particle sizes of S1–S4 measured by SEM were 316.2 ± 59.8, 258.4 ± 60.3, 275.9 ± 70.9 and 438.2 ± 77.1 μm, respectively, in diameter. In this study, larger microparticles were deliberately fabricated to allow for accurate Raman mapping of the polymer layer configuration. The Raman mapping results (Fig. 1) show the double-layered PLGA(shell)/PLLA(core), triple-layered PLGA(shell)/PLLA(middle-layer)/EVA(core) and quadruple-layered PLGA/PLLA/PS/EVA (shell to core) microparticles. A multi-layered morphology can be formed only when the polymer solution was prepared above the cloud point at which a homogeneous

of 5 kV. Before analysis, cross-sectioned microparticles mounted on metal stubs were prepared with a razor blade. Samples were then coated with gold using a sputter coater model SPI-module. Ten microparticles from every sample batch were randomly chosen to be viewed under the SEM. Since particle configurations were found to be consistent within each sample batch, only one representative SEM micrograph was shown. The particle size (in terms of diameter) was measured from the SEM images using the ImageJ software.

2.4.2 Raman mapping

The final particle configuration and drug distribution were verified by Raman mapping measurements. Multi-layered microparticles that had been cross-sectioned were placed under the microscope objective with laser power up to approximately 20 mW. Raman point-by-point mapping measurements on Particle S4 (for example) were then performed on the area of 640 × 600 μm² with a step size of 10 μm in both the X and Y directions using a Raman microscope (InVia Reflex, Renishaw) equipped with a near infrared enhanced deep-depleted thermoelectrically Peltier cooled CCD array detector (576 × 384 pixels) and a high grade Leica microscope. The sample was irradiated with a

Fig. 1 SEM images of cross-sectioned microparticles and their corresponding pure-component Raman spectra estimates and associated score images obtained via BTEM. **a** Particle S1. **b**, **c** particle S2. **d**, **e** particle S3. **f**, **g** particle S4

Fig. 2 Scheme of the mechanism involved in the formation of the multi-layered microparticles

multi-phase polymer solution was first created and a well-controlled polymer precipitation rate was subsequently achieved. The scheme of the internal phase separation of polymers in the emulsion droplet is shown in Fig. 2. When this multi-phase polymer solution was initially poured into the surfactant-containing aqueous solution, the immiscible polymers then immediately phase separated as small droplets (the coacervate phase) containing organic volatile solvent (e.g. DCM) and polymer within the emulsion droplets [27]. During the solvent removal process, the volatile solvent must first diffuse into the aqueous phase and then evaporate at the water/air interface [30]. The solubility of DCM in water is about 2% (v/v) [31]. Therefore, the oil-to-water ratio of 0.013 was used in order for DCM diffusing rapidly into the water without any delay. The time available for solvent partitioning between each polymer coacervate phase was thus reduced at a considerably higher precipitation rate. The surface tension of each of polymer coacervate phases was about the same due to similar DCM content. As such, the polymers in the system have no preferential distribution to form an interface with the surrounding aqueous phase or with other polymers. Eventually, the multi-layered structure was kinetically trapped as a transient intermediate, where a higher mass polymer formed the outer layer, engulfing the polymer phase with lower mass [23, 32]. In contrast, with the polymer mass ratios of PLGA/PLLA/PS/EVA 8:4:1:1, a triple-layered structure with two separate polymer cores of EVA and PS was observed for particle S5 (Fig. 3a, b), where the same masses of PS and EVA were used for the fabrication.

The starting polymer solution concentration above the cloud point of all polymers is one of the key process parameter in achieving the multi-layered structure. When the starting polymer solution concentration was at 7.5% (w/v) (S6), only PLGA was phase separated from the PLLA/PS/EVA solution, as evident from a distinctive yellowish liquid phase observed in the clout point test [20]; PLLA, PS and EVA remained miscible until solvent extraction caused the concentration to increase to the cloud point of these four polymers, which is at 12% (w/v). A quadruple-layered structure was not observed with this starting concentration of 7.5% (w/v), as shown in Fig. 4a. The mobility of the polymer chains in the system decreases

continuously with increasing of the polymer solution concentration during the extraction and evaporation of the solvent. This would then hinder the formation of PLLA, EVA and PS coacervate droplets when the concentration increased to 12% (w/v) before the migration of these coacervate phases to their respective layers can take place. Hence, a core comprising the coalescence of three phase-separated polymers (PLLA, EVA and PS) encapsulated with a PLGA shell was observed (Fig. 4b). This core-shell configuration was again determined by the polymer mass

Fig. 3 a SEM images of cross-sectioned microparticles (particle S5) and b their corresponding pure-component Raman spectra estimates and associated score images obtained via BTEM

Fig. 4 **a** SEM images of cross-sectioned microparticles (particle S6) and **b** their corresponding pure-component Raman spectra estimates and associated score images obtained via BTEM

Fig. 5 **a** SEM images of cross-sectioned microparticles (particle S7) and **b** their corresponding pure-component Raman spectra estimates and associated score images obtained via BTEM

ratios, where PLGA with higher mass engulfed the polymers (PLLA, EVA and PS) with lower masses.

The final polymer layer configuration is determined from the polymer mass ratios only when the precipitation rate is high enough to kinetically trap the non-equilibrium configuration. For the binary-phase microparticles fabricated at PLLA/PLGA mass ratio of 1:2, different core-shell configuration was observed for different initial polymer solution concentrations of 5 and 7.5% (w/v), both were above the cloud point of 4% (w/v). The solution concentration of 5% (w/v) resulted in a PLLA shell and PLGA core structure (S7) (Fig. 5a b), whereas a layer inversion was observed for the solution concentration of 7.5% (w/v) (Fig. 1b, c). It is shown that the considerable lower starting polymer concentration of 5% (w/v) would have provided sufficient time (require more time for the solvent to be extracted) and enough mobility (less viscous system) for the polymers to reconfigure themselves accordingly to thermodynamic equilibrium, as determined by the changes in interfacial energies of the components

during solvent extraction. During solvent extraction, a greater amount of DCM was partitioned into the PLGA coacervate phases, due to a higher degree of interaction between the DCM and PLGA [20]. DCM being hydrophobic caused the DCM-rich PLGA coacervate droplets to migrate towards the inner core, away from the aqueous solution. PLLA coacervate droplets, on the other hand, had a greater affinity for the continuous aqueous phase [20]. Therefore, for this set of parameters, the thermodynamic factor prevailed over kinetics. On the other hand, the solution concentration of 7.5% (w/v) sufficiently provided faster precipitation rate (thus reducing the time for solvent partitioning between each polymer coacervate phase) to allow kinetic factors to dominate over the thermodynamic factor. Hence, this caused PLGA to engulf PLLA, according to the polymer mass ratios.

The spreading coefficient, which can be theoretically predicted from the surface tensions of the solid and melted polymers and its outer aqueous phase, is able to indirectly estimate the configuration (i.e. complete engulfing, partial engulfing or no engulfing) of the two immiscible polymer phases in the emulsion droplet [5, 27, 33]. However, the spreading coefficient changes continually as

solvent is extracted since the change in polymer solution concentration will alter the effective interfacial energies [26]. In addition, the process of forming the particle's internal structure is dynamic, as the mobility of the polymer phases in the system decreases steadily during solvent extraction. When the solvent content decreases to a certain level, the polymer phases within the emulsion droplets cannot move at all to reach the thermodynamic equilibrium configuration dictated by the spreading coefficient. Furthermore, partial encapsulation at which one polymer does not completely engulf the other polymer is often observed if the polymer precipitation rate is slow enough to facilitate the formation of thermodynamic equilibrium configuration [26]. Therefore, it is preferred to adjust the solvent extraction rates (or polymer precipitation rate) by optimizing the process parameters to kinetically trap the multi-layered structure in a non-equilibrium configuration, which has been shown in this paper. The polymers in the multi-layered microparticles are thus selected on the basis of mutual immiscibility only; on the other hand, the surface tension of each of the polymer is not a key criterion as a result of kinetics factors governing the phase separation and solidification of polymers.

3.2 Multiple drug localization in quadruple-layered microparticles

Multiple drugs were encapsulated in the microparticles through this one-step solvent evaporation method. Drug distribution within the microparticles was verified using Raman mapping (Fig. 6). Ibuprofen and metoclopramide HCl were found to localize in the EVA core and outermost PLGA layer, respectively, whereas most lidocaine base was predominantly dispersed in the PLLA layer, however, some lidocaine base was located in PLGA. The pure component Raman spectrum of ibuprofen could not be separated from that of EVA using BTEM analysis due to homogenous (molecular) dispersion of ibuprofen within the EVA matrix. Strong affinity between hydrophobic long ethylene chains of EVA and hydrophobic ibuprofen drove the drug to be dispersed in the EVA. Likewise, highly hydrophilic metoclopramide HCl had a strong affinity with the relatively more hydrophilic PLGA. The solubility parameter of lidocaine base ($20.15 \text{ MPa}^{0.5}$) was found to be close to that of PLLA ($20.6 \text{ MPa}^{0.5}$), as compared to PLGA ($22.4 \text{ MPa}^{0.5}$), EVA ($18.1 \text{ MPa}^{0.5}$) and PS ($18.6 \text{ MPa}^{0.5}$). These solubility parameters were calculated using group contribution methods based on the chemical structure [10, 26, 34]. We can deduce that the alike solubilities resulted in more lidocaine base localized in the PLLA layer.

Fig. 6 **a** SEM image of a cross-sectioned ibuprofen + lidocaine base + metoclopramide HCl-loaded quadruple-layered PLGA/PLLA/PS/EVA microparticle and **b** its corresponding pure-component Raman spectra estimates and associated score images obtained via BTEM

4 Conclusions

This work highlighted the fabrication of multi-layered (from double-layered to quadruple-layered, and potentially multilayered) microparticles comprising immiscible polymers through a one-step solvent evaporation method. Encapsulation of multiple drugs and layer localization of drugs in a single multi-layered microparticle were also possible. This fabrication technique, by virtue of its simplicity and reliability, can facilitate the design of multi-layered microparticles as a drug carrier.

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References

- Freiberg S, Zhu X. Polymer microspheres for controlled drug release. *Int J Pharm.* 2004;282(1–2):1–18.

2. Brannon-Peppas L. Recent advances on the use of biodegradable microparticles and nanoparticles in controlled drug-delivery. *Int J Pharm.* 1995;116(1):1–9.
3. Freitas S, Merkle HP, Gander B. Microencapsulation by solvent extraction/evaporation: reviewing the state of the art of microsphere preparation process technology. *J Control Release.* 2005; 102(2):313–32.
4. Jalil R, Nixon JR. Microencapsulation using poly (L-lactic acid) IV: release properties of microcapsules containing phenobarbitone. *J Microencapsul.* 1990;7(1):53–66.
5. Pekarek KJ, Jacob JS, Mathiowitz E. Double-walled polymer microspheres for controlled drug-release. *Nature.* 1994;367(6460): 258–60.
6. Sanchez A, Gupta RK, Alonso MJ, Siber GR, Langer R. Pulsed controlled-release system for potential use in vaccine delivery. *J Pharm Sci.* 1996;85(6):547–52.
7. Rodriguez M, Vila-Jato JL, Torres D. Design of a new multiarticulate system for potential site-specific and controlled drug delivery to the colonic region. *J Control Release.* 1998;55(1): 67–77.
8. Lee TH, Wang JJ, Wang CH. Double-walled microspheres for the sustained release of a highly water soluble drug: characterization and irradiation studies. *J Control Release.* 2002;83(3):437–52.
9. Shi M, Yang YY, Chaw CS, Goh SH, Mochhala SM, Ng S, et al. Double walled POE/PLGA microspheres: encapsulation of water-soluble and water-insoluble proteins and their release properties. *J Control Release.* 2003;89(2):167–77.
10. Rahman NA, Mathiowitz E. Localization of bovine serum albumin in double-walled microspheres. *J Control Release.* 2004; 94(1):163–75.
11. Berklund C, Cox A, Kim K, Pack DW. Three-month, zero-order piroxicam release from monodispersed double-walled microspheres of controlled shell thickness. *J Biomed Mater Res.* 2004;70A(4):576–84.
12. Pekarek KJ, Dyrud MJ, Ferrer K, Jong YS, Mathiowitz E. In vitro and in vivo degradation of double-walled polymer microspheres. *J Control Release.* 1996;40(3):169–78.
13. Lee WL, Loei C, Widjaja E, Loo SCJ. Altering the drug release profiles of double-layered ternary-phase microparticles. *J Control Release.* 2011;151:229–38.
14. Gopferich A, Alonso MJ, Langer R. Development and characterization of microencapsulated microspheres. *Pharm Res.* 1994; 11(11):1568–74.
15. Watano S, Nakamura H, Hamada K, Wakamatsu Y, Tanabe Y, Dave RN, et al. Fine particle coating by a novel rotating fluidized bed coater. *Powder Technol.* 2004;141(3):172–6.
16. Wang FJ, Wang CH. Sustained release of etanidazole from spray dried microspheres prepared by non-halogenated solvents. *J Control Release.* 2002;81(3):263–80.
17. Lee HK, Park JH, Kwon KC. Double-walled microparticles for single shot vaccine. *J Control Release.* 1997;44(2–3):283–93.
18. Pollauf EJ, Kim KK, Pack DW. Small-molecule release from poly (D,L-lactide)/poly (D,L-lactide-co-glycolide) composite microparticles. *J Pharm Sci.* 2005;94(9):2013–22.
19. Berklund C, Pollauf E, Pack DW, Kim K. Uniform double-walled polymer microspheres of controllable shell thickness. *J Control Release.* 2004;96(1):101–11.
20. Lee WL, Foo WL, Widjaja E, Loo SCJ. Manipulation of process parameters to achieve different ternary-phase microparticle configurations. *Acta Biomater.* 2010;6:1342–52.
21. Mathiowitz E, Langer RS. Preparation of multiwall polymeric microcapsules. US Patent No. 4861627; 1989.
22. Pekarek KJ, Jacob JS, Mathiowitz E. One-step preparation of double-walled microspheres. *Adv Mater.* 1994;6(9):684–7.
23. Tan EC, Lin RY, Wang CH. Fabrication of double-walled microspheres for the sustained release of doxorubicin. *J Colloid Interface Sci.* 2005;291(1):135–43.
24. Leach K, Noh K, Mathiowitz E. Effect of manufacturing conditions on the formation of double-walled polymer microspheres. *J Microencapsul.* 1999;16(2):153–67.
25. Mathiowitz E, Jacob JS, Chickering Iii DE, Pekarek KJ. Preparation of multiwall polymeric microcapsules from hydrophilic polymers. US Patent No. 6511749; 2003.
26. Pollauf EJ, Pack DW. Use of thermodynamic parameters for design of double-walled microsphere fabrication methods. *Biomaterials.* 2006;27(14):2898–906.
27. Loxley A, Vincent B. Preparation of poly(methylmethacrylate) microcapsules with liquid cores. *J Colloid Interface Sci.* 1998; 208(1):49–62.
28. Widjaja E, Lee WL, Loo SCJ. Application of Raman microscopy to biodegradable double-walled microspheres. *Anal Chem.* 2010; 82:1277–82.
29. Widjaja E, Li CZ, Chew W, Garland M. A general and robust algorithm for pure component spectral recovery. Band target entropy minimization. *Anal Chem.* 2003;75:4499–507.
30. O'Donnell PB, McGinity JW. Preparation of microspheres by the solvent evaporation technique. *Adv Drug Deliv Rev.* 1997;28(1): 25–42.
31. Mao SR, Shi Y, Li L, Xu J, Schaper A, Kissel T. 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. *Eur J Pharm Biopharm.* 2008;68(2):214–23.
32. Matsumoto A, Matsukawa Y, Suzuki T, Yoshino H, Kobayashi M. The polymer-alloys method as a new preparation method of biodegradable microspheres: principle and application to cisplatin-loaded microspheres. *J Control Release.* 1997;48(1):19–27.
33. Wang Y, Guo BH, Wan X, Xu J, Wang X, Zhang YP. Janus-like polymer particles prepared via internal phase separation from emulsified polymer/oil droplets. *Polymer.* 2009;50(14):3361–9.
34. Krevelen DW, Hoftyzer PJ. Properties of polymers their estimation and correlation with chemical structure. Amsterdam: Elsevier; 1976.