PLGA/TPGS Nanoparticles for Controlled Release of Paclitaxel: Effects of the Emulsifier and Drug Loading Ratio

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Purpose. We successfully manufactured nanoparticles of biodegradable polymers for controlled release of paclitaxel. TPGS (d-α-tocopheryl polyethylene glycol 1000 succinate) could be a novel material to make nanoparticles of high drug encapsulation efficiency (EE) and desired physicochemical and pharmaceutical properties of the drug loaded nanoparticles. Among various controlling parameters in the process, the present work is to elucidate the effects of the surfactant stabilizer and the drug loading ratio.

Methods. Paclitaxel loaded PLGA nanoparticles were formulated at various drug-loading ratios by a modified single emulsion solvent extraction/evaporation technique. TPGS was introduced either as the emulsifier or as a matrix material component by using different technique. Polyvinyl alcohol (PVA) was also used for a comparison. The nanoparticles of various recipes were characterized by various state-of-the-art instrument technology for their properties.

Results. The EE and the *in vitro* release behavior were found significantly influenced by the drug loading ratio and the surfactant stabilizer encountered. TPGS involved nanoparticles can have high EE and other favorable properties.

Conclusions. TPGS could be a novel and effective emulsifier, which can result in high EE and desired properties of paclitaxel-loaded polymeric nanoparticles.

KEY WORDS: anti-cancer agent; biodegradable polymer; drug delivery; emulsifier; D-α-tocopheryl polyethylene glycol 1000 succinate; taxol.

INTRODUCTION

Polymeric nanoparticles offer a suitable means for delivering various therapeutic agents by either localized or targeted delivery to the tissue of interest (1–7). In such devices, the nanoparticles are formulated from biodegradable polymer in which the active agent is dissolved, entrapped, encapsulated, adsorbed, attached or chemically coupled in the matrices depending on the fabrication method (1,5). The major goal in designing polymeric nanoparticles for delivering a specific drug includes realizing the controlled and targeted release to the specific site of action at the therapeutically optimal rate. An important concern is a clear understanding of the pharmaceutical property of the prepared nanoparticles, including carrier nature, particle size and size distribution, surface and bulk morphology, surface chemistry, surface charge, thermogram property, drug encapsulation efficiency (EE), and drug release kinetics, etc. All may have significant influence on the *in vivo* behavior and tissue distribution of the drug loaded in the nanoparticles (2,8,9). With regard to the manufacture of polymeric nanoparticles, the solvent extraction or evaporation method is a widely used technique and poly(vinyl alcohol) (PVA) is the most commonly used emulsifiers in the process. The main advantage of PVA as emulsifier is that it results in particles of relatively small size and uniform size distribution (10,11). However, PVA tends to be associated with nanoparticles surface by forming an interconnected network with the polymer at the interface and difficult to be removed after emulsification (12,13). It has been found that PVA was non-biodegradable and potentially carcinogenic; it could modify the carrier's surface properties that determine the *in vivo* fate (14–17). Moreover, the nanoparticles with higher amount of residual PVA have relatively lower cellular uptake (18). PVA emulsified nanoparticles are thus not satisfactorily biocompatible and may be toxic. One purpose of our Chemotherapeutic Engineering Laboratory in the Bioengineering Corridor of The National University of Singapore is to explore and develop novel polymeric nanoparticle delivery systems for alternative administration of anticancer agents and with further development to promote oral chemotherapy. Recently, we successfully applied d-α-tocopheryl polyethylene glycol 1000 succinate (vitamin E TPGS or TPGS) in the formulation of poly(DL-lactide-co-glycolide) (PLGA) nanoparticles, which can be treated either as the surfactant stabilizer or as a matrix component material by varying the technique in the process. The model drug adopted was paclitaxel, which is one excellent natural antineoplastic drug against a wide spectrum of cancers, especially for ovarian and breast cancer. Its clinical application has been restricted because of its poor aqueous solubility and limited source. An adjuvant called Cremophor EL is needed for its current administration, which could cause serious side effects such as hypersensitivity reaction, nephrotoxicity, neurotoxicity, and cardiotoxicity (19–22). The polymeric nanoparticles have promising potential to solve the problems caused by Cremophor EL and could provide an alternative dosage form for clinical administration of paclitaxel. Our previous work studied various types of PLGA with different L/G ratio and molecular weight with TPGS as emulsifier in the nanoparticle formulation of paclitaxel (23). We demonstrated that TPGS could be used either as a very effective emulsifier or as a component of the matrix material when blended with PLGA. TPGS can achieve high emulsifying effects, desired particle size and size distribution, surface morphology, and *in vitro* release kinetics.

Various parameters in the manufacture process determine the physiochemical and pharmaceutical properties of the drug loaded nanoparticles such as the polymer type, its molecular weight or co-polymer ratio, the emulsifier used, the

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ABBREVIATIONS: PLGA, poly (lactic-co-glycolic acid); vitamin E TPGS, or TPGS, d-α-tocopheryl polyethylene glycol 1000 succinate; PVA, polyvinyl alcohol; HPLC, high performance liquid chromatography; LLS, laser light scattering; DSC, differential scanning calorimetry; SEM, scanning electron microscopy; AFM, atomic force microscopy; XPS, X-ray photoelectron spectroscopy; FDA, the US Food and Drug Administration; PBS, phosphate buffered saline; DCM, dichloromethane.

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drug loading ratio, the oil to water phase ratio, the mechanical strength of mixing, the pH, and the temperature and so forth. The present focuses on the influence of the emulsifier type/quantity and the drug loading ratio on the nature of the paclitaxel loaded PLGA/TPGS nanoparticles, which were manufactured by a modified single emulsion solvent extraction/evaporation technique. PVA emulsified PLGA nanoparticles were prepared for a comparison. Various state-of-the-art equipment were applied to characterize and analyze the produced nanoparticles such as the laser light scattering (LLS) for size and size distribution, the atomic force microscopy (AFM) and scanning electron microscopy (SEM) for surface morphology, the X-ray photoelectron spectroscopy (XPS) for surface chemistry, the different scanning calorimetry (DSC) for thermogram properties and the high performance liquid chromatography (HPLC) for drug encapsulation efficiency, and the measurement of *in vitro* release kinetics. Optimal design was pursued. We found that the drug encapsulation efficiency and the *in vitro* release behavior could be significantly influenced by the drug loading ratio and the type and quantity of the surfactant stabilizer. TPGS could be a novel and effective emulsifier and a nanoparticle matrices component.

MATERIALS AND METHODS

Material

Poly(DL-lactide-co-glycolide) (PLGA, L/G = 50/50, Av. Mol. Wt. 40,000–75,000) and polyvinyl alcohol (PVA, Av. Mol. Wt. 30,000–70,000, the degree of hydrolysis is 87–90%) were purchased from Sigma Chemical Co., USA. Paclitaxel was purchased from Dabur India Limited, India. TPGS (d-alpha tocopheryl polyethylene glycol 1000 succinate) was purchased from Eastman Chemical Company, USA. Acetonitrile with HPLC grade used as mobile phase in HPLC was purchased from Mallinckrodt Baker Inc. USA. Ultro-high pure water produced by UHQ Water Purification System was utilized for HPLC analysis. Deionized water was used throughout the experiment. The measurement of *in vitro* release was carried out in phosphate buffered saline (PBS), which was purchased from Sigma Diagnostics. All other chemicals encountered were of reagent grade.

Nanoparticles Formation

The nanoparticles were fabricated by a modified oil-in-water (o/w) single-emulsion solvent evaporation/extraction technique. Typically, known amounts of the polymer, TPGS and paclitaxel at a certain ratio were dissolved in DCM, which was stirred using a magnetic stirrer until all materials were dissolved. The organic phase was poured into the stirred aqueous solution containing one of the two surfactant stabilizers and sonicated simultaneously with energy output of 12 w in a pulse mode (Misonix Incorporated, USA). The formed o/w emulsion was stirred by a magnetic stirrer continuously for at least 6 hours to evaporate the organic solvent off. During the process, the micro/nano- droplets were solidified in the aqueous system. The resultant sample was separated and collected by centrifugation (11000 rpm [*g* is 11.5 regarding the centrifugation], 10 min, 16°C. 5810R, Eppendorf AG, Germany). The supernatant was decanted and pure deionised water was poured into the centrifuge tube that was well shaken to wash the collected nanoparticles three to four times to remove the surfactant residue. The produced suspension was dried under lyophilization (Alpha-2 Martin Christ Freeze Dryers, Germany) to obtain the fine powder of nanoparticles, which was placed and kept in vacuum dessicator. Drug loading ratio was set to be 2, 6 and12% respectively.

Morphology Study

The atomic force microscopy (AFM, Multimode™ Scanning Probe Microscope, Digital Instruments, USA) and the scanning electron microscopy (SEM, JSM-5600 LV, JEOL USA, Inc.) were conducted to observe the shape and surface morphology of the nanoparticles. AFM was performed by the tapping mode. Before operation, a small amount of nanoparticles was stuck on a double-sided tape attached on a metallic sample stand. SEM required a previous coating of the sample with platinum, which was done in an Auto Fine Coater (JFC-1300, JEOL USA).

Particle Size Analysis

The particle size and size distribution of the nanoparticles were measured by the laser light scattering (LLS, 90 Plus Particle Sizer, Brookhaven Instruments Co. USA). Suitable amount of the dried nanoparticles from each formulation was suspended in deionized water and was sonicated for a suitable period before the measurement. The resulted homogeneous suspension was determined for the volume mean diameter, size distribution and polydispersity. Each sample was run for 5 times with 1 min duration.

Surface Chemistry

The surface chemistry of the nanoparticles was analyzed by X-ray Photoelectron Spectroscopy (XPS, SXIS His-165 Ultra, Kratos Axis HSi, Kratos Analytical, Shimadzu Corporation, Japan). The angle of X-ray used in XPS was 90°. The analyzer was used in fixed transmission mode with pass energy of 40 eV for the survey spectrum covering a binding energy range from 0 to 1200 eV. Peak curve fitting of the C1s (atomic orbital 1 s of carbon) envelope was performed using XPSPeak 4.1 software.

Thermal Characterization

The thermogram characters of drug loaded nanoparticles were analyzed by the differential scanning calorimetry (DSC 822e, Mettler Toledo, STARe software) on the glass transition temperatures (Tg) or melting point (Tm). As a control, the pure material of paclitaxel, PLGA, TPGS, PVA and the physical mixture of paclitaxel with placebo nanoparticles (paclitaxel: placebo nanoparticles = 1:9) was also analyzed. Approximately 4–5 mg of each sample was sealed in a standard aluminum pan (40 μl) with lid. The temperature range of measurement was 20–250°C. The heat flow rate was set to 10°C per minute. After a measurement, the temperature was decreased back to starting temperature by liquid nitrogen. Indium was used as the standard reference material to calibrate the temperature and energy scales of the DSC instrument.

Table I. Nanoparticles Formulation and Related Properties

Samples no.	Materials	Emulsifier	Drug loading ratio (%)	Recovery yield (%)	EE (%)	Mean diameter ± S.E. (nm)	Polydispersity[a]
p1	PLGA	PVA	2	73.2	42.4	430.1 ± 66.6	0.005
p2	PLGA	PVA	6	76.8	43.1	421.6 ± 80.5	0.005
p3	PLGA	PVA	12	82.4	60.1	384.3 ± 68.4	0.005
p4	PLGA	PVA	0	41.9	—	471.7 ± 74.8	0.005
t1	PLGA	TPGS	2	62.3	38.9	605.0 ± 102.9	0.005
t2	PLGA	TPGS	6	80.0	85.3	618.4 ± 199.7	0.005
t3	PLGA	TPGS	12	75.4	88.3	707.5 ± 46.1	0.043
t4	PLGA	TPGS	0	31.6	—	899.8 ± 209.7	0.005
tp1	PLGA + TPGS	PVA	2	71.3	57.2	435.5 ± 72.3	0.005
tp2	PLGA + TPGS	PVA	6	78.7	63.9	472.4 ± 113.5	0.005
tp3	PLGA + TPGS	PVA	12	76.2	65.5	299.8 ± 80.4	0.005
tp4	PLGA + TPGS	PVA	0	62.0	—	659.3 ± 64.7	0.005
tt1	PLGA + TPGS	TPGS	2	47.9	59.2	883.4 ± 119.3	0.005
tt2	PLGA + TPGS	TPGS	6	74.6	97.5	761.6 ± 178.9	0.005
tt3	PLGA + TPGS	TPGS	12	68.4	94.4	648.9 ± 125.5	0.005
tt4	PLGA + TPGS	TPGS	0	39.0	—	801.6 ± 25.9	0.005

[a] The polydispersity was referred to the log normal distribution width of the particle diameter.

Drug Encapsulation Efficiency

The amount of entrapped paclitaxel in nanoparticles was detected in triplicate by HPLC (Agilent LC1100). A reverse phase Inertsil® ODS-3 column (150 × 4.6 mm ID, pore size 5 μm, GL Science, Tokyo, Japan) was used. The mobile phase consisted of a mixture of acetonitrile and water (50/50 v/v) and was delivered at a flow rate of 1 ml/min with a pump (HP 1100 High Pressure Gradient Pump). The column effluent was detected at 227 nm with a variable wavelength detector (HP 1100 VWD). A calibration curve of standard paclitaxel solution was used to obtain the paclitaxel concentration, which was linear over the range of 50–50,000 ng/ml with a correlation coefficient of $R^2 = 0.9999$. Known mass of nanoparticles was dissolved in a suitable amount of pure acetonitrile by vortex agitator. The clear solution was then put into HPLC vial to detect the paclitaxel concentration, which was injected in with an auto-injector (HP 1100 Autosampler). The encapsulation efficiency of paclitaxel in nanoparticles was determined as the mass ratio of the entrapped paclitaxel in nanoparticles to the theoretical amount of paclitaxel used in the preparation. Meanwhile, the recovery efficiency factor on encapsulation efficiency was determined as the ratio of the paclitaxel concentration obtained from HPLC to the theoretical concentration of the prepared solution, which was obtained by dissolving the physical mixture of pure paclitaxel and placebo nanoparticles with relevant ratio in acetonitrile. The resultant factor was 100%, which means that 100% of originally loaded amount of paclitaxel could be detected. No correction was needed.

In Vitro Paclitaxel Release

The *in vitro* release of paclitaxel from nanoparticles was measured in triplicate in PBS at pH 7.4. Ten milligrams of paclitaxel-loaded nanoparticles were suspended in 10 ml of PBS in a screw-capped tube and the tube was placed in an orbital shaker water bath (GFL-1086, Lee Hung Technical Company, Bukit Batok Industrial Park A, Singapore). The water bath was maintained at 37°C and shaken horizontally at 120 min^{-1}. At particular time intervals, the tubes were taken out from the water bath and were centrifuged at 12000 rpm (*g* is 11.5 regarding the centrifugation) for 12 min. The supernatant solution was collected from each tube for HPLC analysis and the precipitated nanoparticles were resuspended in 10 ml of fresh PBS and then put back into the water bath to continuous release measurement. The collected supernatant solution was extracted with 1 ml of DCM. A mixture of acetonitrile and water (50:50 v/v) was added to the extracted paclitaxel after the DCM had evaporated. The resultant solution was put into HPLC vial for HPLC analysis by the same procedure previously described. Similarly, the extraction recovery efficiency was measured because of inefficient extraction. A known mass of pure paclitaxel was treated with the same extraction procedure described above. The determined factor was 37%. That means the extracted solution contained 37% of the original paclitaxel after all the related processes. The data obtained from the detection were corrected accordingly.

RESULTS AND DISCUSSION

The pharmaceutical characteristics of the nanoparticles could be determined and influenced by many factors in the manufacturing process, including the formulation materials, the concentration of material, the ratio of oil to water phase, the type and concentration of emulsifiers, the drug loading ratio, the strength of mixing energy such as stirring, sonication, homogenizing, and the treatments after the nanoparticles formation such as centrifugation, washing, lyophilization, sterilization and pH and temperature (1,2). Amongst them, the emulsifier plays a key role in separation of the oil and the water phase to form the emulsion and in stabilization of the dispersed-phase droplets formed during emulsification. It inhibits coalescence of droplets and determines the particle size, size distribution, morphologic properties, and internal structure of the nanoparticles and thus, the release kinetics. Our lab has successfully identified the TPGS for manufacture of palclitaxel loaded nanopartilces, which can be added either in the water phase as a novel and effective emulsifier or in the oil phase as a matrix component material. Our aim of the present study is to find an optimal drug loading ratio with the

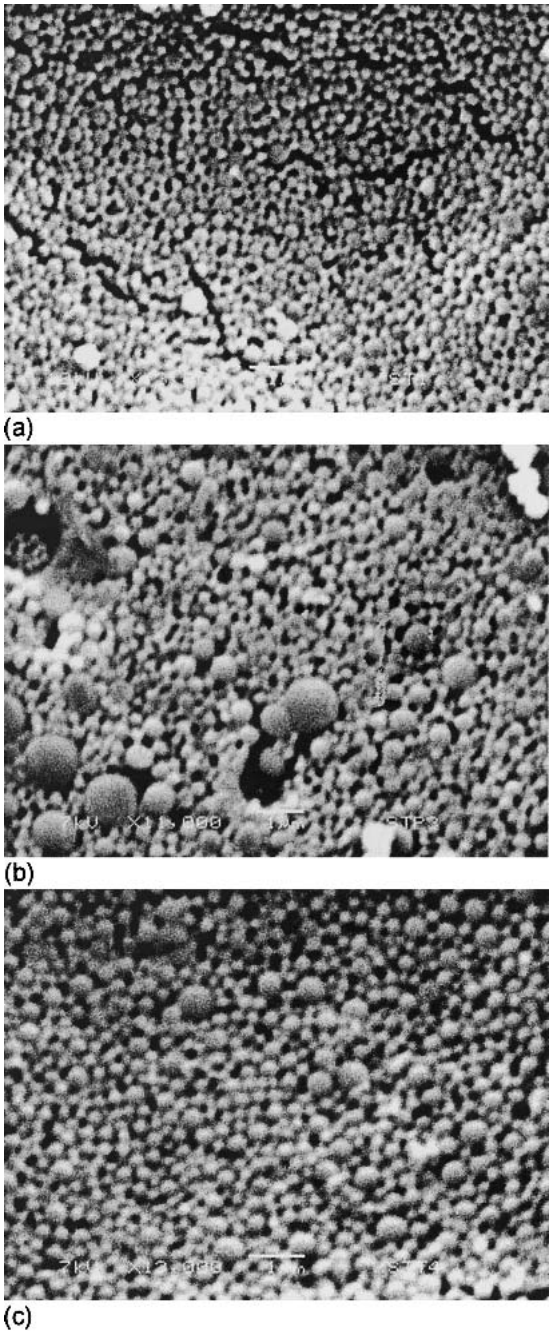

Fig. 1. SEM images of nanoparticles composed of PLGA/TPGS with TPGS/PVA as emulsifier (a) PLGA as material matrix with TPGS as emulsifier, (b) PLGA and TPGS as material matrix with PVA as emulsifier, (c) PLGA, and TPGS as material matrix with TPGS as emulsifier.

new surfactant stabilizer for the formulation of PLGA nanoparticles for an alternative clinical administration of paclitaxel to overcome the problem caused by the adjuvant Cremophor EL of Taxol®, which is the only dosage form available so far for clinical administration of paclitaxel. The investigation was focused on the influence of drug loading ratio and emulsifier on the fabrication, characterization and *in vitro* release behavior of the paclitaxel loaded PLGA/TPGS nanoparticles, which were fabricated in different formulations and displayed in Table I. The PLGA to TPGS ratio in Column 2 was 10:1. The concentration of emulsifiers was 1% for PVA and 0.025% for TPGS.

Morphology, Size and Size Distribution

AFM and SEM were utilized to investigate the morphologic property of the nanoparticles, which are shown in Figs. 1 and 2. From the SEM images (see Fig. 1), nanoparticles of all formulations displayed in spherical shapes and did not show aggregation although the particles might be too small for the limited resolution of the used SEM. There was no obvious difference in shape and surface morphology amongst the samples of various formulations. AFM has higher resolution (see Fig. 2), under which the distinct spherical nanoparticle could be observed for the single nanoparticle and for the nanoparticles gathered. The surface was relatively smooth in the scale of observation.

The size and size distribution of the nanoparticles were measured by LLS and the data was tabulated in Table I. The size distribution was specified in the intensity of the light scattering and the polydispersity was referred to the log normal distribution width of the particle diameter. All nanoparticle samples had a quite narrow polydispersity from 0.005 to 0.043 and the mean diameter ranged from 299.8 nm to 899.8 nm. We can see from Table I, a higher drug loading ratio may result in a smaller particle size in general although the influence of the drug loading ratio on the particle size and size distribution was not significant. The nanoparticles reached the smallest when both TPGS (as one component of material matrix) and PVA (as emulsifier) were used into the formulation and the PVA emulsified nanoparticles were relatively smaller than the TPGS emulsified nanoparticles. This is because there is a tendency for small size nanoparticle to aggregate during freeze-drying process that seemed to generate a variety of freezing and drying stresses, which may induce particle surface modification resulting in the formation of aggregates and it might be one disadvantage of freeze-drying method (24). Furthermore, there may be a limited amount of emulsifier on the surface of nanoparticle that may be favorable for nanoparticle formation. The emulsifier presents on the interface to separate the oil and the water phases to prevent the aggregation during the emulsification process. As nanoparticles have hardened, the emulsifier is not needed and should have been completely washed away. PVA as a macromolecular emulsifier was not easily washed away from particle surface. The PVA emulsified nanoparticles may thus have more residues left on the surface after washing process and hence, the nanoparticles were easily dispersed in water during the LLS measurement, which thus may result in smaller size (25,26). Unfortunately, the residue of PVA on nanoparticle surface is not favorable due to a few reasons for consideration of biocompatibility of nanoparticles (14–18). Unlike PVA, TPGS is a smaller molecular surfactant and easier to remove from nanoparticle surface. Our previous study has found by analyzing the surface chemistry of the nanoparticles that, when acting as surfactant stabilizer, TPGS would distribute mainly on the particles surface. If washing only 2 times or no washing after preparation, the existence of TPGS on the surface was significant; while, if washing up more than 4 times, the remaining of TPGS on the surface could not be detected by XPS analysis (23). TPGS emulsified nanoparticles thus exhibited mild aggregation by freeze-

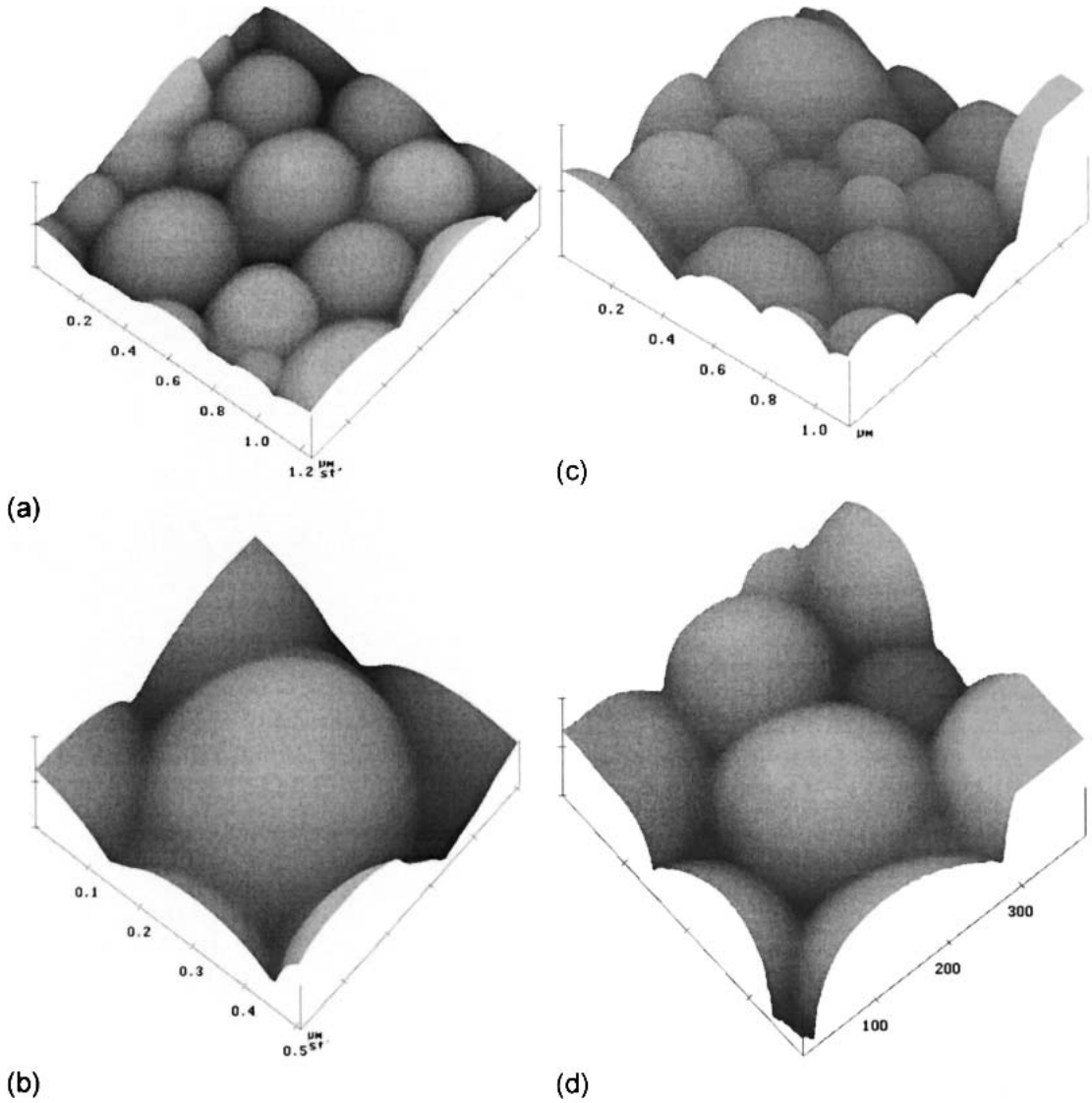

Fig. 2. AFM images of nanoparticles gather and single particle composed of PLGA/TPGS with TPGS as emulsifier (a) and (b) PLGA as material matrix and TPGS as emulsifier, (c) and (d) PLGA, and TPGS as material matrix and TPGS as emulsifier.

drying process. Several alternative solutions may be adopted, such as the use of lyoprotectant in preventing aggregation of nanoparticles (24).

Thermal Characteristics

To determine the physical status of paclitaxel inside nanoparticles and the thermal property of material matrix, DSC analysis was conducted. The results were showed in Fig. 3. It can be seen that the pure paclitaxel showed an endothermic peak of melting at about 223.0°C but no related peak displayed for all prepared nanoparticles with or without drug entrapped. However, the physical mixture of paclitaxel and placebo nanoparticles gave a broadened peak shifted to a lower temperature at about 220°C, although the content of paclitaxel in the physical mixture was even lower than that in the nanoparticles of 12% drug loading ratio. This means that the paclitaxel entrapped in the nanoparticles was in an amorphous or disordered-crystalline phase of a molecular dispersion or a solid solution state in the matrix of polymer or polymer/TPGS mixture (27). The glass transition temperature of PLGA was not influenced significantly by the procedure. But the melting peak of TPGS did not display, which meant that it was in amorphous state when blended with the polymer.

Surface Chemistry

XPS technique was adopted in the present study to analyze the chemical structure of nanoparticles surface. The investigation was done by inferring the relative percentage of elements C, O, and N presented in XPS spectra with C1s spectra in terms of peak assignments and relative percentage of each carbon environment from curve fitting over a binding energy range of 280 to 300 eV. The comparison between pure powder material and nanoparticles was made. The envelope fit for C1s regions was expected using four main peaks corresponding to C–C/C–H (at about 283–284 eV), C-OH(R) (1.4–2.1 eV shift), C-O-C=O (1.4–2.1 eV shift) and O-C=O (4.4 ± 0.1 eV shift) environments respectively (28,29). During

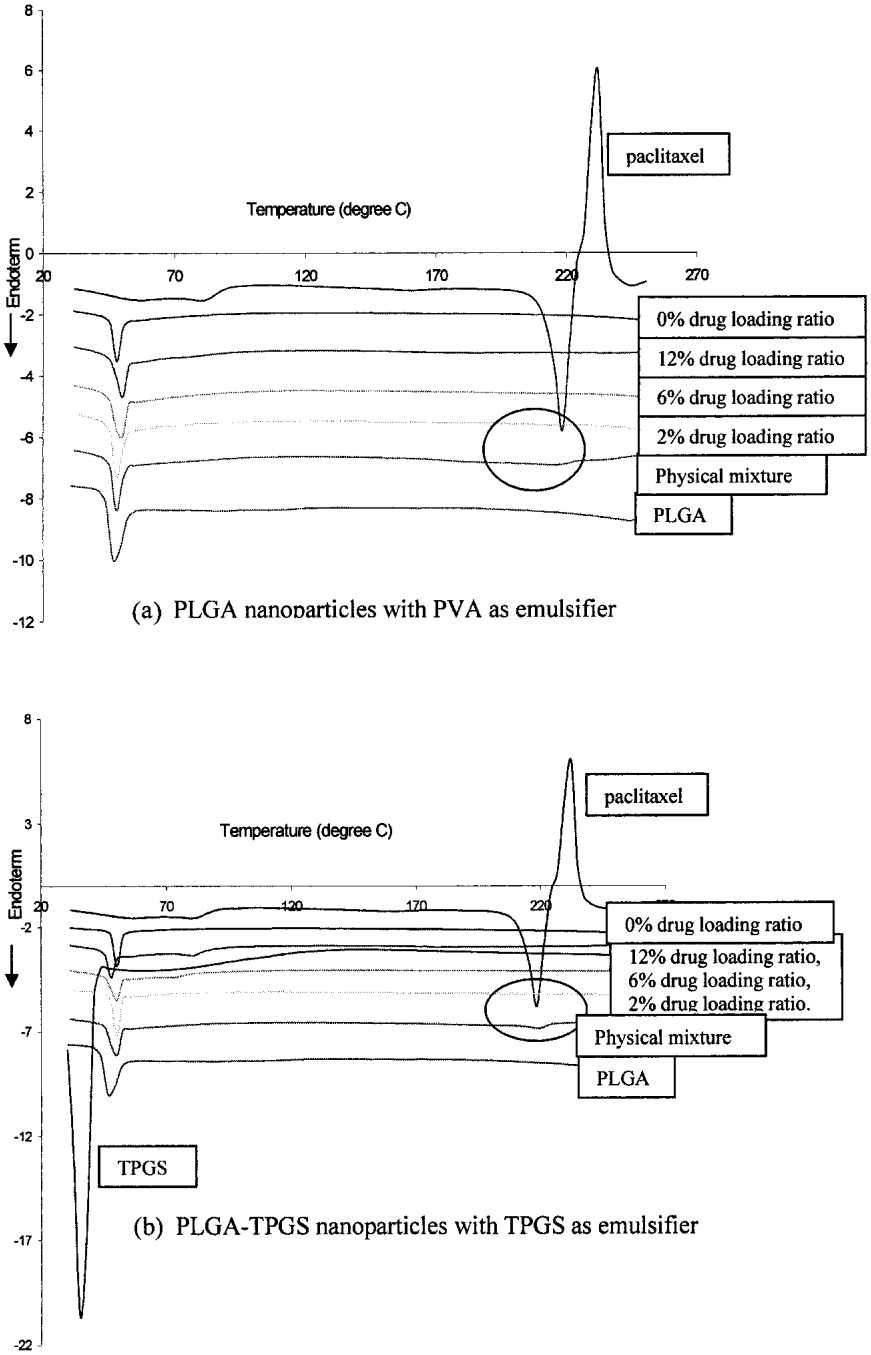

Fig. 3. DSC thermograms of nanoparticles prepared with different emulsifiers.

peak fitting, the full width at half maximum values (FHWM) was fixed to be same for all peaks to ensure the ionization cross section for an element was same for all component bonds. The obtained results were summarized in Table II.

The elemental ratio was the percentage of atomic concentration in each sample. For all samples, the elemental ratios for C and O were similar and did not seem to be affected by the drug loading ratio and the emulsifier used. Some of the samples had non-zero percentages for element N although the percentage was low, which may indicate the presence of N near or at the surface of nanoparticles. This suggests that the drug is distributed evenly or randomly in the nanoparticle matrix. Nevertheless, the drug should be more concentrated inside nanoparticles due to its high hydrophobicity. This is confirmed by the fact that the distribution of N on the surface did not increase significantly with the increase of drug loading ratio. This result agreed with the DSC analysis. From the XPS curve fitting analysis, the basic substance PLGA gave the expected three peaks corresponding to O=C-O, C-O-C=O and C-C/C-H, whilst PVA and TPGS gave O=C-O, C-OH(R) and C-C/C-H respectively. After fabrication procedure, all nanoparticles gave four peaks corresponded to all the initial specific C1s environments. In comparison with the value from basic material PLGA, the data from all nanoparticles displayed a significant increase in the region of C-OH(R) and decrease in the region of C-O-C=O. This sug-

Table II. Surface Chemistry of Produced Nanoparticles Analysed by XPS

Sample code	XPS elemental ratio (%)			XPS C1s envelope ratio (%)			
	C	O	N	C-C/C-H	C-OH (R)	C-O-C=O	O-C=O
PLGA (50/50)	63.1	36.8	0.0	41.8	0.0	30.9	27.2
PVA	63.9	36.1	0.0	55.5	34.6	0.0	9.9
TPGS	69.1	30.9	0.0	46.9	50.7	0.0	2.4
Paclitaxel	78.4	19.6	2.0	68.6	15.2	8.9	7.3
p1	68.1	31.9	0.0	42.7	16.6	17.9	22.8
p2	66.8	32.7	0.5	45.7	15.6	17.0	21.7
p3	67.1	32.9	0.0	45.2	13.2	18.6	23.0
p4	69.6	30.4	0.0	48.9	14.7	15.7	20.7
t1	69.4	30.6	0.0	42.5	19.4	18.8	19.3
t2	70.9	29.1	0.0	46.3	17.9	17.2	18.6
t3	71.4	28.1	0.5	49.9	15.1	17.7	17.3
t4	70.5	29.5	0.0	44.7	18.1	17.2	19.9
tp1	66.4	33.0	0.6	37.8	17.6	20.5	24.1
tp2	68.1	31.7	0.1	45.9	15.4	17.9	20.7
tp3	67.0	33.0	0.0	42.0	15.7	19.5	22.8
tp4	68.1	31.9	0.0	48.9	16.7	16.0	18.4
tt1	72.7	27.3	0.0	46.3	18.9	16.2	18.6
tt2	74.2	25.8	0.0	50.6	17.7	15.6	16.0
tt3	75.3	24.5	0.1	51.6	18.7	15.2	14.5
tt4	71.3	28.6	0.0	46.3	19.1	16.1	18.5

gested the distribution or adsorption of the emulsifier PVA or TPGS on particle surface during its formation. The distribution percentage of each substance was approximately 50% by comparing the envelope ratio of C-OH(R) and C-O-C=O. Another point to be highlighted is that, regarding the C-OH(R) coming from the emulsifier, the percentage of this carbon environment distributed on nanoparticles surface related to the pure material was higher from PVA than from TPGS, suggesting that the emulsifier residue was more for PVA emulsified nanoparticles than for TPGS emulsified samples.

Drug Encapsulation Efficiency

The encapsulation efficiencies of the drug in all nanoparticle formulations were measured. The data was tabulated in Table I. It could be seen that nanoparticles prepared with TPGS as emulsifier demonstrated higher drug encapsulation efficiency compared with those prepared with PVA as emulsifier. TPGS could effectively increase the encapsulation efficiency up to 100% (ie, 97.5% in Sample tt2), whereas PVA could only efficiently encapsulate at most 60.5% of the drug in Sample p3 when PLGA was used as the matrix material and 65.5% in Sample tp3 when TPGS was mixed together with PLGA as matrix material. Moreover, TPGS was a more effective emulsifier than traditional PVA because the concentration of TPGS used was much lower than that of PVA (0.025% vs. 1.0%), 40 times more effective. Meanwhile, increase in the drug loading ratio could result in an increase in the encapsulation efficiency. There may be a minimum as well as a maximum amount of the drug loading that can be encapsulated efficiently. All of the organic phase (ie, DCM), polymer material and drug molecules may partition or diffuse across the interface from oil phase to water phase, which contributes to a substantial lowering of the recovery yield as well as the drug encapsulation efficiency. There should be an equilibrium concentration. The correct choice of emulsifier thus has a significant effect on localization of drug molecules and reducing the drug molecule leakage from the oil droplets, and hence improving the drug encapsulation efficiency in the nanoparticles. The drug encapsulation efficiency can be achieved by TPGS emulsified nanoparticles as high as 100%, which may have clinical and economical significance especially for such an effective and expensive drug as paclitaxel.

In Vitro Release

Nanoparticles of various formulations were determined for their cumulative release of encapsulated paclitaxel under *in vitro* condition. The curves were shown in Fig. 4. The release of paclitaxel from the nanoparticles involved an initial rapid release phase, which was followed by a phase of relatively slow release. The fraction of drug released in the initial burst depended on nanoparticles composition or formulation. Generally, the mechanisms by which active agents can be released from a delivery system are the combination of diffusion of the active agent passes through the polymer that forms the controlled-release device, polymeric erosion, swelling, and degradation. Any or all of these mechanisms may occur in a given release system. The degradation of PLGA is slow, therefore the release mechanism of paclitaxel from nanoparticles may depend on the drug diffusion and the PLGA surface and bulk erosions or swelling. In the present typical matrix drug delivery system, the polymer, drug and additive had been mixed to form a homogeneous system, in which the diffusion occurred when the drug passes from the polymer matrix into the external environment, which referred to both on a macroscopic scale—as through pores in the polymer matrix—or on a molecular level, by passing between polymer chains. As the release continued, its rate normally decreased with this type of system, because the drug had a progressively longer distance to travel and thus required a longer diffusion time to release. Clearly, both of the surfactant and the drug loading ratio could influence the *in vitro*

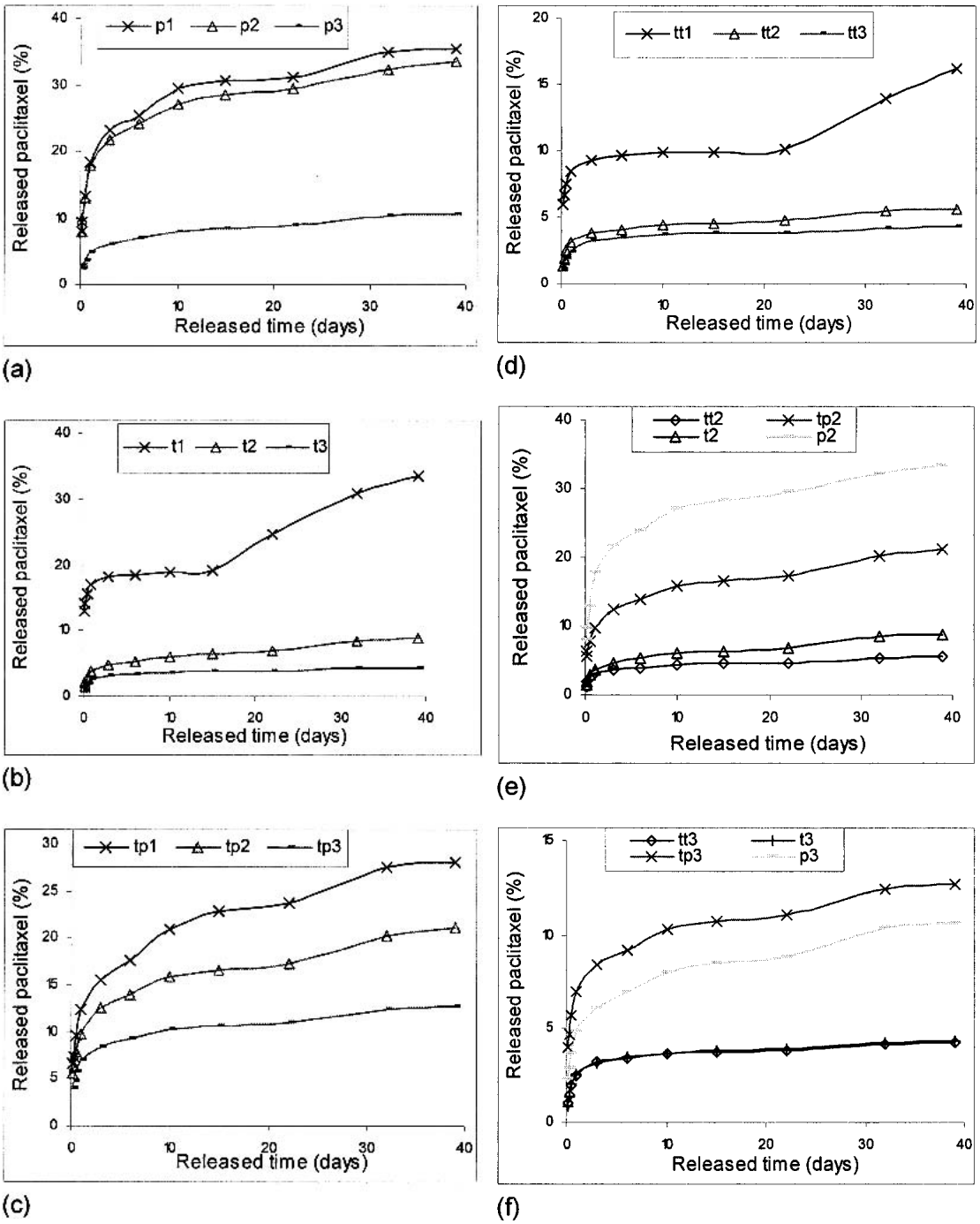

Fig. 4. Release curves of paclitaxel from different nanoparticle formulations under *in vitro* condition (a) PLGA nanoparticles with PVA as emulsifier and different drug loading ratio, (b) PLGA nanoparticles with TPGS as emulsifier and different drug loading ratio, (c) PLGA-TPGS nanoparticles with PVA as emulsifier and different drug loading ratio, (d) PLGA-TPGS nanoparticles with TPGS as emulsifier and different drug loading ratio, (e) Nanoparticles with 6% drug loading ratio and different emulsifier, (f) Nanoparticles with 10% drug loading ratio and different emulsifier.

release behavior significantly. A general trend was that the release rate decreased with the increased drug-loading ratio for all formulations (see Fig. 4, a–d). After 1 month, the accumulative amount of paclitaxel released was about 30%, 8%, and 5% for nanoparticles with PLGA as matrix and TPGS as emulsifier regarding the drug loading 2%, 6%, and 12%, respectively. Similarly, when PLGA together with TPGS as matrix and PVA as emulsifier, the amount was about 27%, 20%, and 12.5% with regard to the three kinds of drug loading respectively. As discussed previously, the drug-loading ratio did not have significant effects on the nanoparticles size and morphology. Thus, the difference in the release behavior could not be related to the particle size. A concern for the behavior in the studied case may be due to the presence of a

compact domain in the nanoparticles. For nanoparticles of a same size, increase in the drug loading ratio causes their internal structure more compact, hindering the water penetration into the particles and hence there was less drug diffusion for the release. Some studies reasoned this behavior by suggesting a formation of a homogeneous matrix with the drug randomly distributed throughout the polymer particle at low loading and a heterogeneous matrix at high drug loading [30]. PVA emulsified nanoparticles (Sample p and Sample tp) exhibited faster release than those emulsified by TPGS (Sample t and Sample tt). For example, when drug loading was 2%, the accumulative amount of paclitaxel released was about 27% after 1 month for the PVA emulsified nanoparticles using PLGA and TPGS together as matrix. However, the released amount was about 13% for the TPGS emulsified nanoparticles when also using PLGA and TPGS as matrix. This may be because TPGS is both hydrophobic and hydrophilic, smaller but with bigger bulk area. It might reach and stay in the domains of both the pores in the polymer matrix and the polymer chains, causing more compact matrix structure thus resulting in a lower erosion, swelling as well as degradation rate of the polymer or slower diffusion of the encapsulated drug through the matrix. One more interesting point was that when TPGS was applied as material matrix together with PLGA, the release rate of PVA emulsified nanoparticles was distinctly faster than that of TPGS emulsified nanoparticles (see Fig. 4, e and f, Sample tp and tt). The increment of the released paclitaxel was even more when drug loading ratio was increased.

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