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A novel controlled release formulation for the anticancer drug paclitaxel (Taxol®): PLGA nanoparticles containing vitamin E TPGS

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Abstract

Paclitaxel (Taxol®) is one of the best antineoplastic drugs found from nature in the past decades. Like many other anticancer drugs, there are difficulties in its clinical administration due to its poor solubility. Therefore an adjuvant called Cremophor EL has to be employed, but this has been found to cause serious side-effects. However, nanoparticles of biodegradable polymers can provide an ideal solution to the adjuvant problem and realise a controlled and targeted delivery of the drug with better efficacy and fewer side-effects. The present research proposes a novel formulation for fabrication of nanoparticles of biodegradable polymers containing d-α-tocopheryl polyethylene glycol 1000 succinate (vitamin E TPGS or TPGS) to replace the current method of clinical administration and, with further modification, to provide an innovative solution for oral chemotherapy. In the modified solvent extraction/evaporation technique employed in this research, the emulsifier/stabiliser/additive and the matrix material can play a key role in determining the morphological, physicochemical and pharmaceutical properties of the produced nanoparticles. We found that vitamin E TPGS could be a novel surfactant as well as a matrix material when blended with other biodegradable polymers. The nanoparticles composed of various formulations and manufactured under various conditions were characterised by laser light scattering (LLS) for size and size distribution, scanning electron microscopy (SEM) and atomic force microscopy (AFM) for morphological properties, X-ray photoelectron spectroscopy (XPS) for surface chemistry and differential scanning calorimetry (DSC) for thermogram properties. The drug encapsulation efficiency (EE) and the drug release kinetics under in vitro conditions were measured by high performance liquid chromatography (HPLC). It was concluded that vitamin E TPGS has great advantages for the manufacture of polymeric nanoparticles for controlled release of paclitaxel and other anti-cancer drugs. Nanoparticles of nanometer size with narrow distribution can be obtained. A drug encapsulation efficiency as high as 100% can be achieved and the release kinetics can be controlled.

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

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1. Introduction

The application of biodegradable polymeric particles in the scale of micrometers and nanometers as a controlled release dosage form of anticancer drugs has generated immense interest. Due to their relatively large size, however, the microspheres were not appropriate to direct the drug to target tissues or cells via systemic circulation or across the mucous membrane [1–3]. Nanoparticles, instead, were successfully used for systemic, oral, pulmonary, transdermal and other administration routes for various purposes including drug targeting, enhancement of drug bioavailability and protection of drug bioactivity and stability [4–6]. Also, nanoparticles can improve the bioavailability of poorly absorbed drugs, thus enhancing oral delivery [3,4]. Moreover, the nanoparticles are able to permeate cells for cellular internalisation and connective tissue permeation and so deliver the drug efficiently to the targeted tissue without clogging capillaries [7,8]. The ability of nanoparticles to improve drug diffusion through biological barriers is a typical benefit for the delivery of anticancer agents. The enhanced endocytic activity and leaky vasculature in the tumor could result in accumulation of intravenously administered nanoparticles [9]. Some studies have indicated that nanoparticle-bound antitumour agents showed prolonged drug retention in tumours, reduction in tumour growth and prolonged survival of tumour-bearing animals [10–13].

The polymer matrix of the nanoparticles must meet several requirements such as biocompatibility, biodegradability, mechanical strength, and ease of processing. The best known class of biodegradable materials for controlled release are the poly (lactide-co-glycolide)s (PLGAs). In addition, a wide variety of biopolymers such as bovine serum albumin (BSA), human serum albumin (HSA), collagen, gelatine, and hemoglobin have been studied for their application to drug delivery systems [2].

Various drug release profiles can be achieved by controlling the molecular weight, the copolymer ratio, the drug loading, the microparticle size and porosity, and the fabrication conditions [14,15]. The fabrication technique for micro/nanoparticles should be chosen based on the nature of the polymer, the drug, the intended use, and the duration of the therapy [2,16,17]. The resulting pharmaceutical properties of the micro/nanoparticles may be determined by various factors which include the nature, solubility and loading of the drug; the polymer type, composition, and molecular weight; the property of organic solvent; the concentration and mixed ratio of the water and the oil phases; the nature and the concentration of the emulsifier; the mechanical strength of stirring/agitation; and other conditions such as temperature and pH, etc. Among them, the matrix material and the emulsifier/stabiliser/additive can play a key role.

The present research proposes d-α-tocopheryl polyethylene glycol 1000 succinate (vitamin E TPGS or TPGS) as a novel emulsifier as well as a novel matrix material when blended with other biodegradable polymers in the preparation of nanoparticles for controlled release of anticancer drugs with paclitaxel as a prototype, which is one of the best natural antineoplastic drugs of the past decades. The therapeutic efficacy of this drug has been limited due to its poor aqueous solubility. In current clinical administration of paclitaxel, an adjuvant called Cremophor EL is needed, which has been proved to be responsible for most serious side-effects of the dosage form such as hypersensitivity reaction, nephrotoxicity, neurotoxicity and cardiotoxicity [18–24]. The nanoparticles can provide a solution for the problems caused by Cremophor EL and with further modification, promote oral chemotherapy due to their extremely small size and adhesive properties.

A widely used emulsifier in the preparation of micro/nanoparticles is poly (vinyl alcohol) (PVA) [25–29]. To our knowledge, there have been no comprehensive studies on applying vitamin E TPGS as an emulsifier, let alone using it as matrix material. The only exception is a recent publication by the authors, in which they initiated and evaluated the feasibility and advantages of TPGS utilised as emulsifier in the solvent evaporation/extraction technique

[25]. The present work more intensively investigated TPGS used as emulsifier and further pursued the possibility of applying TPGS as a matrix material blended with PLGA for the manufacture of nanoparticles for clinical administration of paclitaxel. The preparation, characterisation and in vitro release kinetics of the nanoparticles of this novel formulation were studied. The different formulations with various ratios of the oil phase, the aqueous phase, the polymer material and the TPGS either as emulsifier or as a component of the matrix material were evaluated and optimised. The results demonstrated that vitamin E TPGS could be an excellent emulsifier for fabrication of polymeric nanoparticles, achieving very good emulsifying effects and high drug encapsulation efficiency. The desired size and size distribution, surface morphology, and in vitro release kinetics can be obtained. Moreover, this is the first time in the literature that TPGS has been used as a matrix material for nanoparticle preparation, having a self-emulsifying effect in the emulsification process.

2. Materials and methods

2.1. Materials

Poly(DL-lactide-co-glycolide) (PLGA; L/G=50/50, MW 40,000–75,000; L/G=75/25, MW 90,000–120,000; and L/G=85/15, MW 90,000–120,000), poly(DL-lactide) (PLA; MW 106,000), polyvinyl alcohol (PVA; MW 30,000–70,000; the viscosity of a 4% solution was 4–6 cp at 20 °C; the degree of hydrolysis was 87–90%) were purchased from Sigma (USA). Paclitaxel of 99.8% purity was purchased from Yunnan Hande Biotechnology, China. Vitamin E TPGS was a gift from Eastman Chemical, USA. Methylene chloride (dichloromethane, DCM, analytical grade) was purchased from Mallinckrodt (Mallinckrodt Laboratory Chemicals, Mallinckrodt Baker, USA). Acetonitrile used as mobile phase in high performance liquid chromatography (HPLC) was purchased from EM Science (ChromAR, HPLC grade, Mallinckrodt Baker, USA). Ultra-high pure water produced by UHQ Water Purification System (USF-ELGA lab water, Millipore, Singapore) was utilised for HPLC analysis. Deionised water was used throughout the experiment. The in vitro release measurement was carried out at pH 7.4 and 37 °C in phosphate-buffered saline (PBS), which was purchased from Sigma. All other chemicals used were of reagent grade.

2.2. Nanoparticle preparation

The paclitaxel loaded nanoparticles were fabricated by a modified oil-in-water single emulsion solvent evaporation/extraction technique [25]. Briefly, known amounts of mass of polymer and paclitaxel were added into DCM, which was suitably stirred to ensure that all material was dissolved. The solution of organic phase was slowly poured into the stirred aqueous solution with or without emulsifier and sonicated simultaneously at 50 W in pulse mode (Misonix, USA). The formed o/w emulsion was gently stirred at room temperature (22 °C) by a magnetic stirrer overnight to evaporate the organic solvent. The resulting sample was collected by centrifugation (10,000 rpm, 10 min, 16 °C; Eppendorf model 5810R, Eppendorf, Hamburg, Germany) and washed once or twice with deionised water for some samples. The produced suspension was freeze-dried (Alpha-2, Martin Christ Freeze Dryers, Germany) to obtain a fine powder of nanoparticles, which was placed and kept in a vacuum dessicator. The loading ratio of paclitaxel for the preparation was 1–10%.

2.3. Encapsulation efficiency

The drug entrapped in the nanoparticles was determined in triplicate by HPLC (Agilent LC1100, Agilent Technologies, Singapore). A reverse phase Inertsil® ODS-3 column (150×4.6 mm i.d., pore size 5 μm, GL Science, Tokyo, Japan) was used. A 3-mg sample of nanoparticle powder was dissolved in 1 ml of DCM and 5 ml of acetonitrile–water (50:50) was then added. A nitrogen stream was introduced to evaporate the DCM until a clear solution was obtained. The solution was filtered into a vial for HPLC detection of the paclitaxel concentration. The mobile phase consisted of a mixture of acetonitrile and water (50:50, v/v), and was delivered at a flow rate of 1.00 ml/min with a pump (HP 1100 high pressure gradient pump, Agilent Technologies, Sing-

apore). A 50-μl aliquot of the sample was injected with an autoinjector (HP 1100 Autosampler, Agilent Technologies, Singapore). The column effluent was detected at 227 nm with a variable wavelength detector (VWD). The calibration curve for the quantification of paclitaxel was linear over the range of standard concentration of paclitaxel at 10–60,000 ng/ml with a correlation coefficient of $R^2=1.000$. The solvent for calibration was the mixture of acetonitrile and water (50:50, v/v).

Correction of the calculated encapsulation efficiency may be needed in case of inefficient extraction [29]. To decide whether correction is needed, the recovery efficiency factor of the extraction procedure on encapsulation efficiency was determined as follows. The same amount of pure paclitaxel as that loaded in the nanoparticles and 3.0–5.0 mg of placebo nanoparticles or polymer were dissolved in 1 ml of DCM. Then 5 ml of acetonitrile–water (50:50) was added. The same procedure as described above was carried out. The resulting factor was 100%, which means that 100% of the original amount of the paclitaxel could be detected. No correction was needed. The encapsulation efficiency of paclitaxel was obtained as the mass ratio between the amount of paclitaxel incorporated in nanoparticles and that used in the nanoparticle preparation.

2.4. Nanoparticle characterisation

2.4.1. Size and size distribution

The particle size and size distribution of the prepared nanoparticles were measured by laser light scattering (LLS, 90 Plus Particle Sizer, Brookhaven Instruments, USA). The dried powder samples were suspended in deionised water and sonicated before measurement. The obtained homogeneous suspension was examined to determine the volume mean diameter, size distribution and polydispersity.

2.4.2. Morphology

Scanning electron microscopy (SEM, JSM-5600 LV, JEOL USA) and atomic force microscopy (AFM, Multimode™ Scanning Probe Microscope, Digital Instruments, USA) were employed to determine the shape and surface morphology of the produced nanoparticles. SEM requires coating of the sample with platinum, which was performed in an Auto Fine Coater (JFC-1300, JEOL USA). AFM was conducted with Nanoscope IIIa in the tapping mode. The nanoparticle sample was mounted on metal slabs using double-sided adhesive tapes and scanned by the AFM maintained in a constant-temperature and vibration-free environment.

2.4.3. DSC analysis

The thermogram characteristics of selected batches of nanoparticles were determined by differential scanning calorimetry thermogram analysis (DSC, 2920 Modulated, Universal V2.6D, TA Instruments, USA) on the glass transition temperatures (T_g) or melting point (T_m). The following steps were taken during the process. Samples (8 mg) were equilibrated at −10 °C and purged with pure dry nitrogen at a flow rate of 40 ml/min. The nitrogen was heated to 120 °C at 20 °C/min, after which it was held isothermally for 3 min. The samples were cooled back to −10 °C at the same rate. After 5 min of isothermal stage, the second heating cycle proceeded at 5 °C/min temperature ramp speed to 120 °C. The T_g of polymer was obtained by taking the mid-point of the slope during glass transition. In the present research, two heating cycles were conducted. Indium was used as the standard reference material to calibrate the temperature and energy scales of the DSC instrument. As a control, the pure material was analysed to observe the change of the T_g or T_m.

2.4.4. Surface analysis

X-ray photoelectron spectroscopy (XPS, Kratos Axis HSi, Kratos Analytical, Shimadzu, Japan) was utilised to analyse the surface chemistry of the nanoparticles. The angle of X-ray was 90°. The analyser was used in fixed transmission mode with pass energy of 80 eV for the survey spectrum covering a binding energy range from 0 to 1200 eV. Peak curve fitting of the C1s (atomic orbital 1s of carbon) envelope was performed using the software provided by the instrument manufacturer.

2.5. In vitro release study

The release rate of paclitaxel from the nanoparti-

cles was measured in PBS medium (pH 7.4) by HPLC in triplicate. Paclitaxel loaded nanoparticles (10 mg) were suspended in 10 ml of buffer solution in screw capped tubes and placed in an orbital shaker bath (GFL-1086, Lee Hung Technical, Bukit Batok Industrial Park A, Singapore), which was maintained at 37 °C and shaken horizontally at 120 min^{-1}. After a particular time interval, the tubes were taken out of the water bath and centrifuged at 11000 rpm for 15 min. The precipitated nanoparticles were resuspended in 10 ml of fresh buffer and then put back in the shaker bath. The supernatant was taken for analysis of paclitaxel concentration, extracted first with 1 ml of DCM, followed by adding 3 ml of the mixture of acetonitrile and water (50:50, v/v), and then evaporated under a stream of nitrogen until a clear solution was obtained. HPLC analysis was conducted as previously described. Similarly to the measurement of encapsulation efficiency, the extraction procedure needs to be analysed for the extraction recovery efficiency due to inefficient recovery. Known mass of pure paclitaxel was dealt with the same procedure as mentioned above. The determined factor was 77.5%, which meant that the obtained extraction solution contained 77.5% of the original amount of paclitaxel after the related process. The data obtained for analysis of the in vitro release were corrected accordingly.

3. Results and discussions

3.1. A novel formulation of nanoparticles for controlled release of paclitaxel

Structurally, vitamin E TPGS has a dual nature, similar to an amphiphile, with part of the molecule exhibiting lipophilicity and another part exhibiting hydrophilicity, which is necessary for use as a surface-active agent. Although the exact portion of the hydrophilic polar head and the lipophilic alkyl tail may not be elucidated obviously, it is accepted that the polyethylene glycol portion behaves as the polar head while the tocopherol succinate portion behaves as the lipophilic tail (Fig. 1). Moreover, not only does the TPGS molecule possess a bulky shape and large surface area, but it is also miscible with

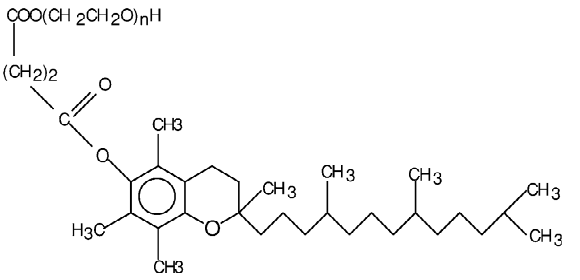

Fig. 1. Chemical structure of vitamin E TPGS.

water as well as being soluble in oil. The special structure–property relationship of TPGS suggests its potential use as an emulsifier for various oil–water immiscible systems. We further realised that, blended with hydrophobic polymers such as PLA and PLGA, TPGS could be used as a component of the matrix material to improve the controlled release property of nanoparticles. This is a unique characteristic of TPGS superior to many other emulsifiers such as PVA.

Our result showed that used as emulsifier added into the water phase in the microencapsulating process, TPGS was very effective at improving the emulsification process for microencapsulation. In fabrication of nanoparticles by the solvent extraction/evaporation technique, the concentration needed for the traditional emulsifier PVA was normally at least 1% (w/v) [25–28]. However, under the same fabrication conditions, the required amount of TPGS is only 0.015% (w/v), an amount 67 times less than the PVA but with the same emulsifying effects. Also, blended with biodegradable polymers such as PLGA, TPGS could be adopted as a matrix material of nanoparticles, which was added and dissolved in the oil phase. The mixture of PLGA and TPGS has a self-emulsifying effect, which can form nanoparticles with no need to add another surfactant stabiliser. In both cases, the resulting nanoparticles have the desired size with narrow size distribution and the desired release kinetics. The drug encapsulation efficiency could be as high as 100% by optimising the formulation. XPS investigation showed that the surface of the fabricated nanoparticles was dominated by the TPGS molecules, which demonstrated the emulsification role of TPGS. Our work indicated

the most significant advantage by employing vitamin E TPGS either as an efficient stabiliser or as a component of the matrix material. This achievement significantly improves the solvent extraction/evaporation technique for fabrication of nanoparticles.

3.2. Formulation optimisation

A number of nanoparticle samples were fabricated using different types of PLGA including PLA, PLGA (85:15), PLGA (75:25) and PLGA (50:50). Vitamin E TPGS of various concentrations was utilised as emulsifier. The nanoparticles with TPGS blended with PLGA as matrix material were also fabricated by changing the ratio of polymer and TPGS. The basic characteristics of the products involved in the formulation studies are presented in Table 1. Samples E1–E4 were prepared by using TPGS as emulsifier at high concentration (0.06%, w/v). Samples E5–E8 and E13–E16 were made with a medium concentration of TPGS (0.03%, w/v), while samples E9–E12 employed a low TPGS concentration (0.015%, w/v). Samples E7, E13, E14 and E8, E15, E16 were prepared by varying the polymer concentration and using a medium concentration of emulsifier. Moreover, samples M1–M3 were produced by applying a blended mixture of TPGS and PLGA as matrix material with no other emulsifier or surfactant stabiliser (self-emulsified).

3.2.1. Morphology of nanoparticles

Under SEM and AFM observation (Figs. 2 and 3), the nanoparticles all had a fine spherical shape with various degrees of smooth surface. The AFM technique was used to study the detailed morphology of nanoparticles, as the prepared nanoparticles are too small to be closely investigated by SEM due to its limited magnification. The AFM images reveal the fine structure of the nanoparticle surface as showed in Fig. 3. They gives clear 3D morphological images of spherical nanoparticles of sub-300 nm diameter, and confirmed that there was no aggregation or adhesion among the nanoparticles. Furthermore, the surface morphology of the nanoparticles could be seen closely from the AFM images. It was noticeable from the zoom-in picture that caves and/or cracks existed on the particle surfaces and single particles showed a certain roughness on their surface although multi-particle images gave relatively smoother surface morphology. Although the topography of the

Table 1
The nanoparticles products and their properties

Sample No.	Polymer	TPGS concentration (%, g/ml)	Polymer concentration (%, g/ml)	Paclitaxel loading (%, w/w)	Mean diameter (nm)±S.E.	Poly-dispersity	Recovery yield (%)	Encapsulation efficiency (%)
E1	PLA	0.06	0.125	–	979.0±257.7	0.005	38.5	–
E2	PLGA (85:15)	0.06	0.125	–	1764.1±141.1	0.184	38.9	–
E3	PLGA (75:25)	0.06	0.125	–	914.8±380.1	0.186	27.1	–
E4	PLGA (50:50)	0.06	0.125	–	686.1±251.7	0.373	55.9	–
E5	PLA	0.03	0.125	2.4	589.0±244.5	0.326	44.7	43.0
E6	PLGA (85:15)	0.03	0.125	–	1028.2±371.8	0.288	55.8	–
E7	PLGA (75:25)	0.03	0.125	2.4	272.5±169.5	0.245	41.7	50.4
E8	PLGA (50:50)	0.03	0.125	2.4	515.5±317.9	0.005	64.3	53.2
E9	PLA	0.015	0.125	–	846.7±348.7	0.243	25.9	–
E10	PLGA (85:15)	0.015	0.125	–	567.4±362.6	0.277	21.4	–
E11	PLGA (75:25)	0.015	0.125	–	699.3±286.9	0.005	46.9	–
E12	PLGA (50:50)	0.015	0.125	–	895.4±318.8	0.052	31.3	–
E13	PLGA (75:25)	0.03	0.188	0.83	444.5±70.8	0.005	40.5	49.0
E14	PLGA (75:25)	0.03	0.25	0.62	654.6±80.9	0.005	56.7	80.3
E15	PLGA (50:50)	0.03	0.188	0.83	636.4±90.6	0.088	47.4	54.3
E16	PLGA (50:50)	0.03	0.25	0.62	369.1±80.8	0.230	37.7	83.8
M1	PLGA (50:50)	PLGA/TPGS=2/1	9.9	552.3±81.4	0.005	46.7	~100	
M2	PLGA (50:50)	PLGA/TPGS=1/1	9.9	622.1±102.4	0.005	43.6	~100	
M3	PLGA (50:50)	PLGA/TPGS=1:2	9.9	844.3±115.6	0.005	29.7	~100	

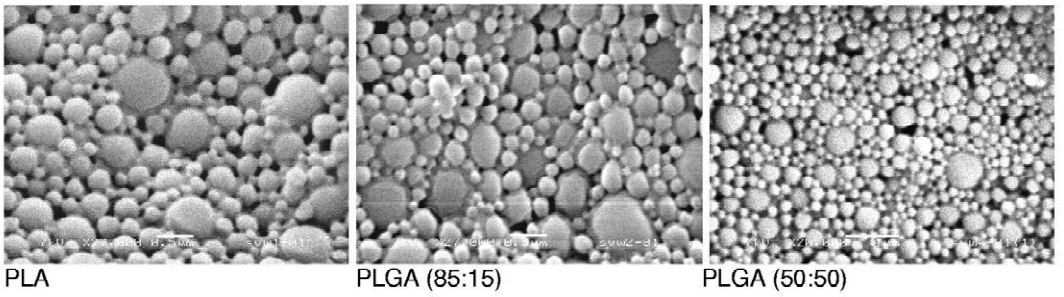

Fig. 2. SEM images of nanoparticles prepared by different molecular weight of polymer with different concentration of TPGS as emulsifier or by blended mixture of PLGA (50:50) and TPGS without using any other emulsifier.

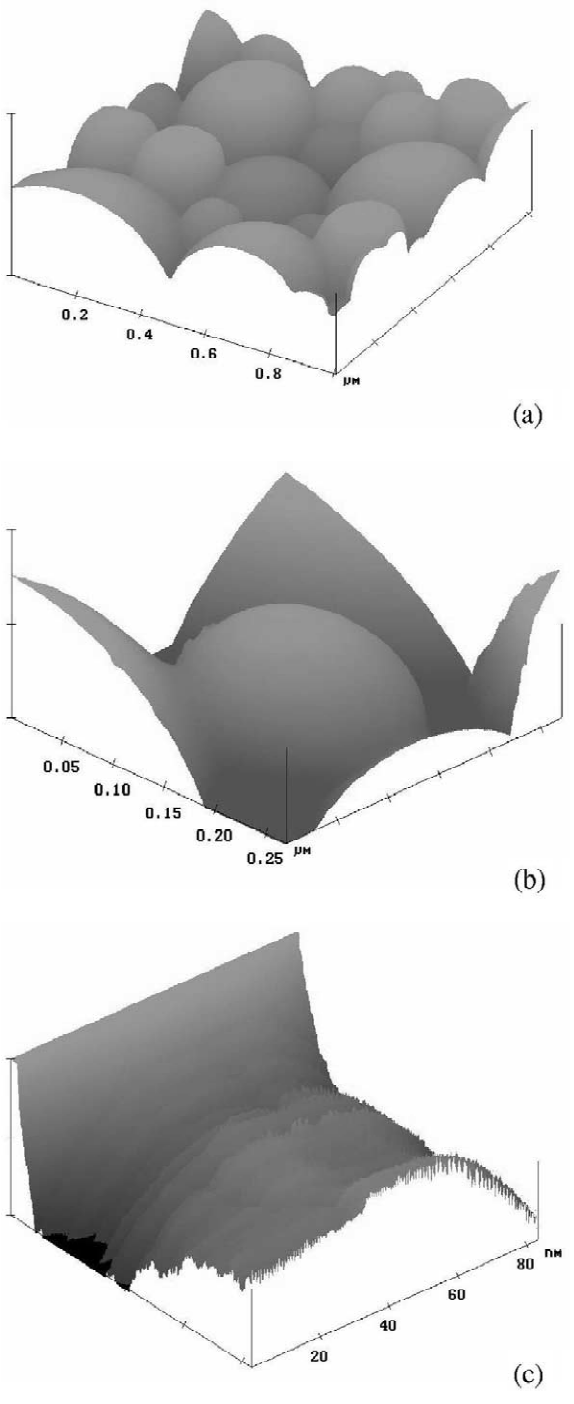

Fig. 3. AFM images of nanoparticles prepared with TPGS as emulsifier: (a) multi-particles; (b) single particle; (c) zoom-in of the nanoparticle surface.

nanoparticles may be complex, the roughness and the caves observed on the surface could provide physical evidence of diffusion release mechanism. The relatively smooth surface supported the assumption that the release of drug from nanoparticles might be caused by both diffusion and matrix erosion.

3.2.2. Particle size and size distribution

The mean size averaged by particle volume and polydispersity of all samples were determined and are listed in Table 1. Fig. 4 illustrates the particles size distribution. It can be observed that the size of particles was in the range 300–1000 nm. It was shown that, in general, the size of nanoparticles was smaller when fabricated with 0.03% (w/v) TPGS as emulsifier (samples E5–E8, E13–E16), and the polydispersity at this emulsifier concentration was narrower than those of the nanoparticles fabricated at higher concentration (0.06%, samples E1–E4) and lower concentration (0.015%, samples E9–E12) of TPGS. This is easy to understand: the emulsifier remains at the interface separating the oil and water phases. Smaller particles have larger total surface area and thus need more emulsifier. Too little emulsifier would result in no nanoparticles formed and too much emulsifier may result in particle aggregation.

As regards the composition of PLGA, when the L/G ratio decreased, smaller nanoparticles were obtained. The size distribution became narrower as well. This result was more notable for PLGA (50:50) (samples E8, E15, E16) and PLGA (75:25) (samples E7, E13, E14) polymers although other properties of the nanoparticles were quite similar. Further, in order to investigate the effects of polymer concentration on the formulation properties, different concentrations of polymer in the oil phase were used to fabricate the nanoparticles. For PLGA (75:25), the nanoparticle size increased slightly with increasing concentration of the polymer used, and the polydispersity decreased to a better value of 0.005. However, the trend was the opposite for PLGA (50:50). The particle size decreased with increasing polymer concentrations while the polydispersity worsened slightly. This may be attributed to the different natures of the two co-polymers used. As the ratio of L/G is decreased, the hydrophilicity of the polymer increases, and thus the interaction amongst the

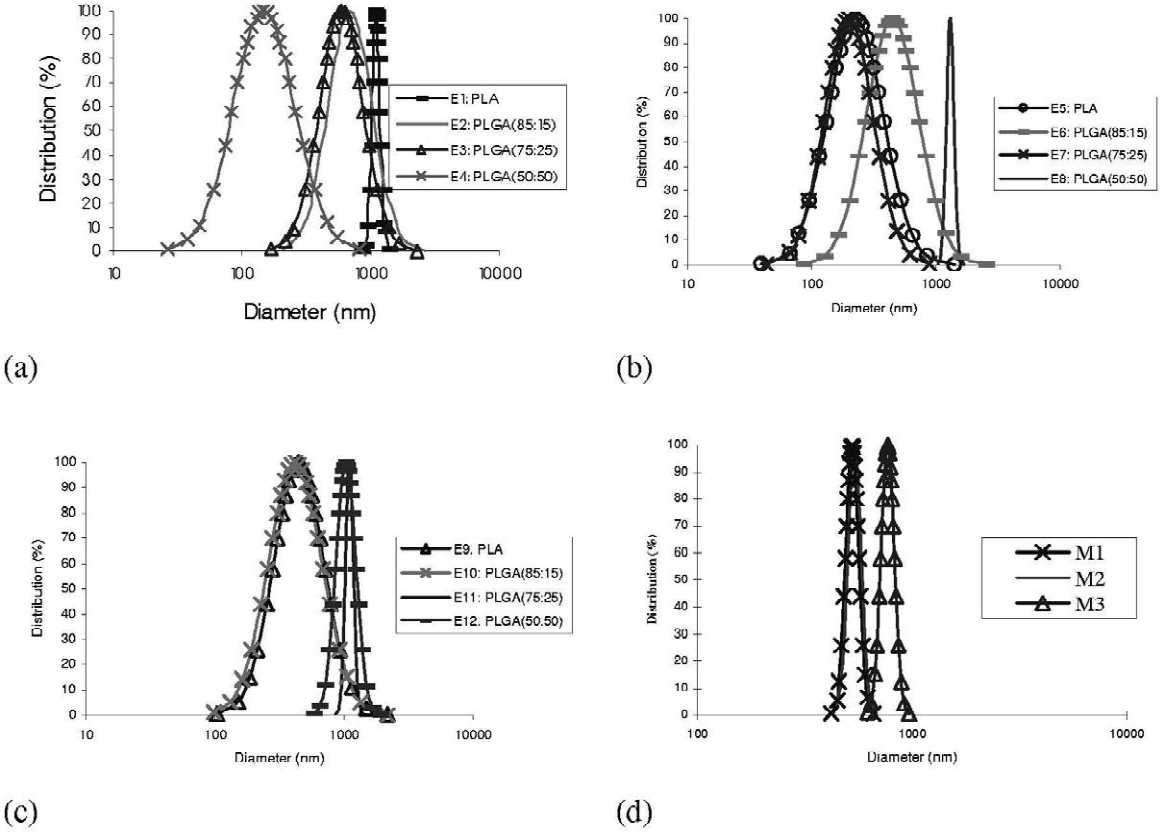

Fig. 4. Size distribution of produced nanoparticles under various experiment parameters: (a) TPGS as emulsifier with high concentration (0.06%); (b) TPGS as emulsifier with medium concentration (0.03%); (c) TPGS as emulsifier with low concentration (0.015%); (d) TPGS as matrix blended with PLGA with no other emulsifier (ratio for PLGA–TPGS: M1 (2:1), M2 (1:1), M3 (1:2)). The horizontal axis is plotted on log-normal scale.

polymer, oil phase and aqueous phase changes accordingly. In addition, the nanoparticles (samples M1–M3) prepared with the mixture of TPGS and PLGA had somewhat smaller size and quite narrow polydispersity. The polydispersity measurement seemed to contradict the SEM images in Fig. 2d, in which the large particles might result from aggregation of small sized particles during the freeze drying process. Generally, there was a tendency for small sized nanoparticles to aggregate during the lyophilisation process, which might be one disadvantage of the freeze drying technique [30]. The particle size was measured by laser light scattering after suspending the particle powder in deionised water by sonication to form a homogeneous dispersion. The surface property of nanoparticles, such as the distribution of surfactant stabiliser on the surface, was found to significantly influence the redispersion of the freeze-dried nanoparticles [31,32]. The aggregated nanoparticles may be easily and thoroughly redispersed to give nanoparticles of smaller and uniform size as displayed in Fig. 4. Further investigation is being carried out.

3.2.3. Yield and encapsulation efficiency

From the results listed in Table 1, it can be observed that the nanoparticle yield was higher for those emulsified by vitamin E TPGS at a medium concentration (0.03%); the yield was clearly low when using TPGS either at high concentration (0.06%) or at low concentration (0.015%). Of the various types of PLGA, it seemed that the yield

increases with decrease of the L/G ratio of PLGA copolymer. It was obvious that PLGA (50:50) consistently gave higher yield regardless of the emulsifier concentrations and other preparation conditions. One main reason might be that when the hydrophobic property increases, the material in the o/w emulsion system with bulky water phase was relatively easier to aggregate but did not form a stable emulsion system. Thus, the PLA frequently gave lower yield in comparison with PLGA. On other hand, however, there was no clear difference in the yield when the polymer concentration varied. Furthermore, as regards the various combinations of TPGS and PLGA as matrix material for samples M1–M3, the yield was decreased when the ratio of TPGS was increased. This should result from the high solubility of TPGS in water. When larger amounts of TPGS were added to the o/w emulsion system, more would be dissolved in the water.

Another aspect worth noticing is the encapsulation efficiency of drug entrapped in nanoparticles. This has been previously investigated by the authors [25]. The present study found that most of the experimental parameters influence the drug encapsulation efficiency in the nanoparticles, drug loading possibly being one of the most significant factors. It could be understood from the present work that, when the drug loading was low, the amount of drug entrapped in the nanoparticles would be decreased. In contrast, when the drug loading ratio was increased to 10%, the drug encapsulation efficiency could reach 100%.

Considering the particle size, size distribution and polydispersity, the nanoparticle yield and the drug encapsulation efficiency, it can be concluded that of the emulsifier concentrations investigated, 0.02–0.03% vitamin E TPGS concentration has the best nanoparticle yield, whereas higher or lower TPGS concentration resulted in low yield. Moreover, it could be observed that both high and low TPGS concentration could cause aggregation or adhesion of nanoparticles during the fabrication process. The shape of the nanoparticles also became less spherical and the particles were less homogeneous.

The result found for TPGS is different from that for other emulsifiers such as PVA [26–28]. This could be due to the unique physicochemical properties of vitamin E TPGS. TPGS is miscible with water and forms solutions with water at concentrations up to ~20% (w/w), beyond which liquid crystalline phases may form. The amphiphilic characteristic of the TPGS molecule leads to its self-association in water when concentration exceeds a threshold known as the critical micelle concentration (CMC), which is ~0.02 wt% in water. Above CMC, TPGS begins to form micelles and continues to form relatively low viscosity solutions with water until a concentration of ~20 wt% is obtained. When the TPGS concentration is above this value, higher viscosity liquid crystalline phases start to form. As its concentration increases, the structure of the TPGS/water liquid crystalline phase evolves gradually from isotropic globular micellar to isotropic cylindrical micellar, mixed isotropic cylindrical micellar and hexagonal, mixed hexagonal and reversed hexagonal, reversed globular micellar, and finally to the lamellar phase [33]. In fabrication of nanoparticles by the single emulsion solvent evaporation/extraction technique, the role of the surfactant stabiliser is to stabilise the dispersed-phase droplets and inhibit coalescence. The amphipathic surfactants align themselves at the droplet surface so as to promote stability by lowering the free energy at the interface between two phases and resisting coalescence and flocculation of the nanoparticles. However, at higher concentration, the state of TPGS in the aqueous dispersing phase has changed and it can not exert a stabilising effect on the formation of emulsion system, droplet separation and stabilisation, as well as nanoparticles hardening. In contrast, it was evident that when the concentration was too low, it did not act as a emulsifier. Therefore, TPGS would not be able to perform as a good surfactant at both higher and lower concentrations and could not produce nanoparticles with ideal properties. The adhesion, flocculation or aggregation of the nanoparticles could easily occur during the fabrication process. The optimal concentration, confirmed in the present research, was 0.02–0.03 wt%, which is very close to the critical micelle concentration (CMC). However, when TPGS was blended together with PLGA as the matrix material, none of abovementioned effects occurred. This is because all the materials were dissolved in the oil phase and there was no interaction between TPGS and water. During this process for making nanoparticles, no other emulsifier was needed; the material is self-emulsify-

ing. This is another significant property of TPGS in drug delivery systems, and deserves further research to probe its great potential.

3.3. DSC analysis

Fig. 5 depicts the DSC thermogram analysis on the phase transition temperatures (T_g or T_m) of the pure material and the drug loaded nanoparticles. Fig. 5a gives the glass transition temperature of PLGA (50:50) at ~45 °C in the second heating cycle. With reference to Fig. 5b, the pure vitamin E TPGS shows the endothermic peak of melting point at ~41 °C for the first heating cycle and at a lower value of ~38 °C for the second heating cycle. These results were in accordance with those found in the literature [33]. The higher melting point exhibited for the first heating cycle demonstrated a solid TPGS with relatively high crystallinity. This crystalline state requires more thermal energy for melting than the lower-energy amorphous states that form during rapid cooling of the sample. Fig. 5c shows thermograms of PLGA nanoparticles fabricated at various concentrations of TPSG as emulsifier. The graphs are very similar each to other and quite similar to that of pure PLGA, which indicates that the influence of TPGS as emulsifier was not significant. From Fig. 5d which shows the thermogram characteristic of the nanoparticles produced from the mixture of TPGS and PLGA, it can be seen that the range of phase

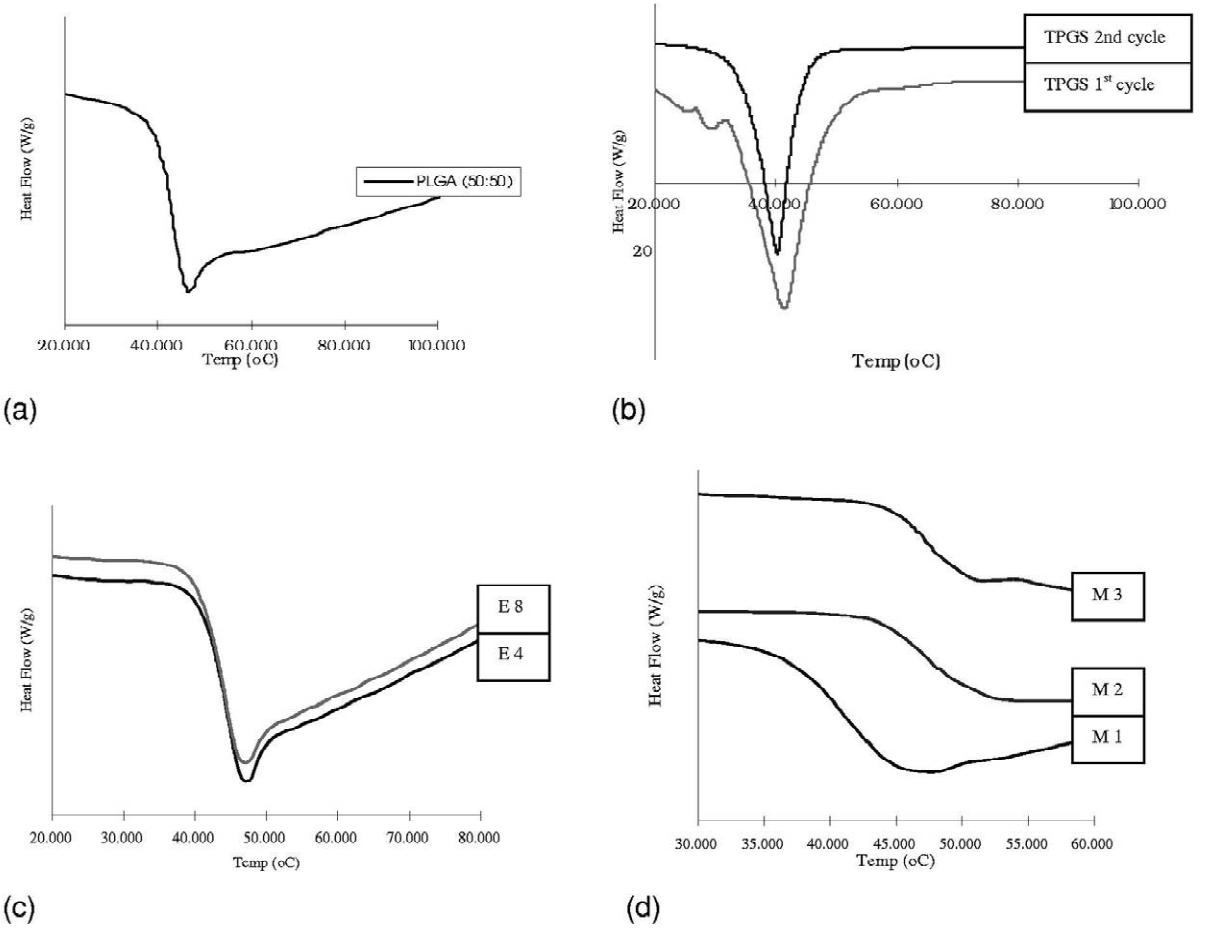

Fig. 5. DSC thermogram of second heating cycle of fabricated nanoparticles and related material: (a) pure PLGA (50:50); (b) pure TPGS; (c) PLGA nanoparticles with TPGS as emulsifier; (d) nanoparticles with blended mixture of PLGA and TPGS as matrix.

transition temperature appears broader and the data drop between glass transition of pure PLGA and the melting point of pure TPGS. The shape of the curves is very different from that of the melting peak. This may be due to the joint domination of both PLGA and TPGS of the thermal property of the nanoparticles. The two materials were blended together and made each other much more amorphous. Overall, it could be seen that the fabricated nanoparticles all displayed phase transition that corresponded to the amorphous solid material and did not show any melting peaks.

3.4. Surface analysis

Polymeric nanoparticles might be taken up by macrophages of the mononuclear phagocytes system, which may provide an opportunity to deliver drugs to certain cellular or tissue sites and may involve various complicated and simultaneous mechanisms [34,35]. Among them, interaction between nanoparticle and cell play an important role. Surface property analysis of drug-loaded nanoparticles should help elucidate the mechanism of particle–cell interactions. The XPS (ESCA) technique was adopted in the present study to investigate the surface chemistry of the nanoparticles. The results infer the type of chemical bonds at the surface of the nanoparticles and the fabricated nanoparticles and are shown in Table 2 and Fig. 6. Clearly, no matter which type of PLGA was used, the nanoparticles fabricated using TPGS as emulsifier all had almost similar relative ratios amongst the suggested chemical bonds and the ratios were in accordance with those found for pure vitamin E TPGS. Hence, it can be inferred that the TPGS was mainly distributed over the surface of the nanoparticles, which meant that the TPGS does act as emulsifier to form and stabilise the nanoparticles in the fabrication process. The result showed that the emulsifier could not be fully washed away when the formed nanoparticles were just washed once or twice during fabrication [25]. When using TPGS blended with PLGA as the matrix material, it was notable that as more TPGS was used, the proportion of C–C/C–H bonds increased while the proportion of O–C=O bonds decreased. Making a comparison with pure TPGS, the result may be attributed to the high proportion of C–C/C–H bonds in the structure of TPGS. This also reflected that in the process of forming nanoparticles, there were more TPGS molecules distributed on the nanoparticle surfaces. In essence, it can be seen that TPGS can act not only as emulsifier but also as a novel kind of matrix material which has self-emulsifying effects. Due to the amphiphilic nature of TPGS, repeated washing would be able to remove the TPGS from the nanoparticle surfaces.

3.5. In vitro release

The in vitro release behaviour of the various paclitaxel-loaded nanoparticles is summarised in the

Table 2
XPS (C1s) analysis of prepared nanoparticles and involved materials

Sample	XPS C1s envelope ratios (%)		
	C–C/C–H	C–O	O–C=O
PLGA (50:50)	49.5	26.9	23.6
PLGA (75:25)	62.4	20.4	17.2
PLA	50.7	26.7	22.5
TPGS	57.7	30.0	12.3
PLGA (50:50) nanoparticles with TPGS as emulsifier	54.5	28.0	17.5
PLGA (75:25) nanoparticles with TPGS as emulsifier	58.7	25.4	16.0
PLA nanoparticles with TPGS as emulsifier	58.1	24.4	17.5
PLGA-TPGS (2:1) nanoparticles	62.6	23.9	13.5
PLGA-TPGS (1:1) nanoparticles	74.4	10.2	15.4
PLGA-TPGS (1:2) nanoparticles	78.1	7.9	14.0

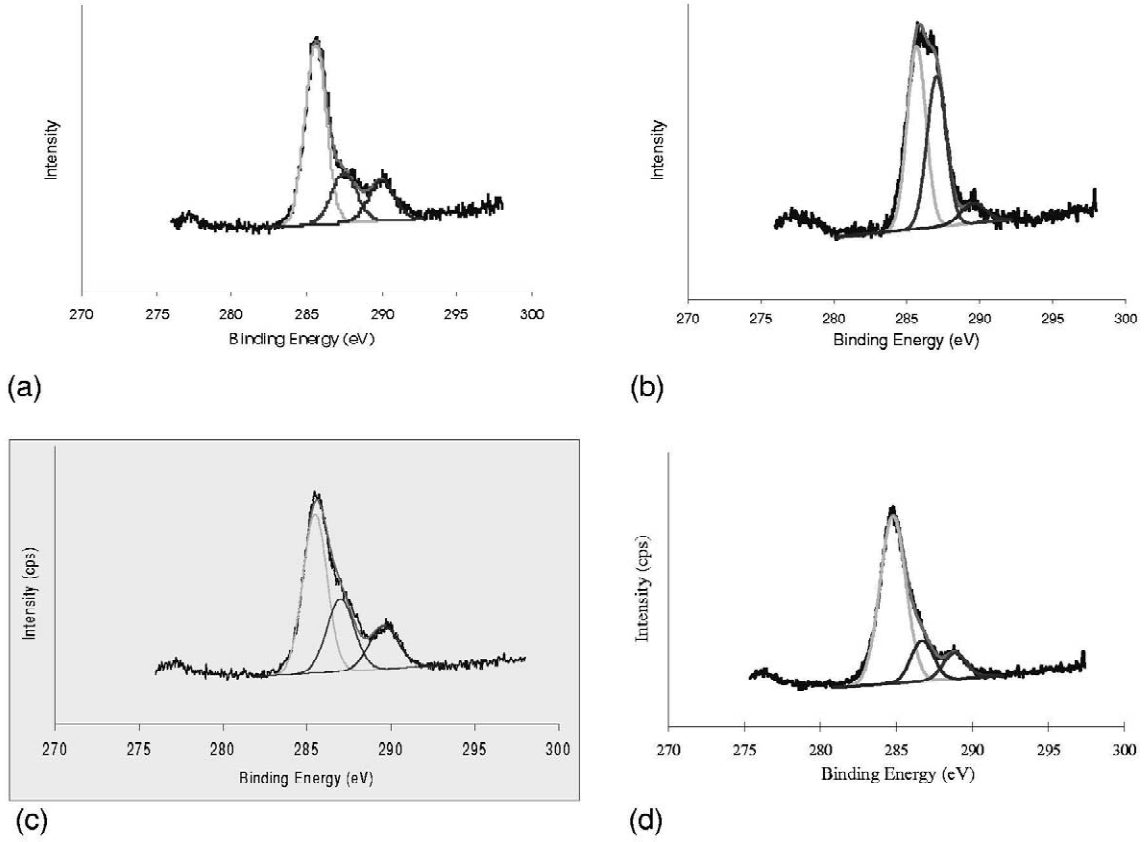

Fig. 6. Surface chemistry analysis of C1s with X-ray photoelectron spectroscopy: (a) PLGA (50:50); (b) vitamin E TPGS; (c) PLGA (50:50) nanoparticles using TPGS as emulsifier; and (d) PLGA-TPGS (1:1) nanoparticles.

cumulative percentage release shown in Fig. 7. Release over 1 month was measured. Fig. 7a shows the release profiles for nanoparticles fabricated from various types of PLGA using 0.03 wt% TPGS as emulsifier. The initial release burst was prominent for all three types of polymers during the first day of release, being greater than 15%. The release gradually decreased and remained constant even after 1 month. PLGA (75:25) and PLGA (50:50) had similar release properties, with slightly slower release as time progressed. PLA gave the slowest rate and extent. This was as expected because of its highly hydrophobic nature and molecular weight (MW), which prevented the drug from diffusing from the polymer matrix into the aqueous solution. Size was also a factor. Being the largest of the three batches of nanoparticles (Table 1), the rate and extent of release would be the slowest. Fig. 7b shows the release curves when TPGS was used as fabrication material blended with PLGA; as the ratio of TPGS increased, the relative release rate also increased. This is because as more TPGS was used, the nanoparticles became more hydrophilic. When exposed to an aqueous environment, the nanoparticles degraded more easily. The initial release burst, which was less than 10%, lasted for about a day, and the drug release rate then tapered gradually to slower rates. A further point is that the paclitaxel release from the M series with TPGS mixed with PLGA as matrix was less than that of E series with polymer only as matrix. One reason might be related to the physicochemical natures of the material, drug, and surfactant as well as their interactions within the nanoparticles. Both polymer material and the paclitaxel were quite

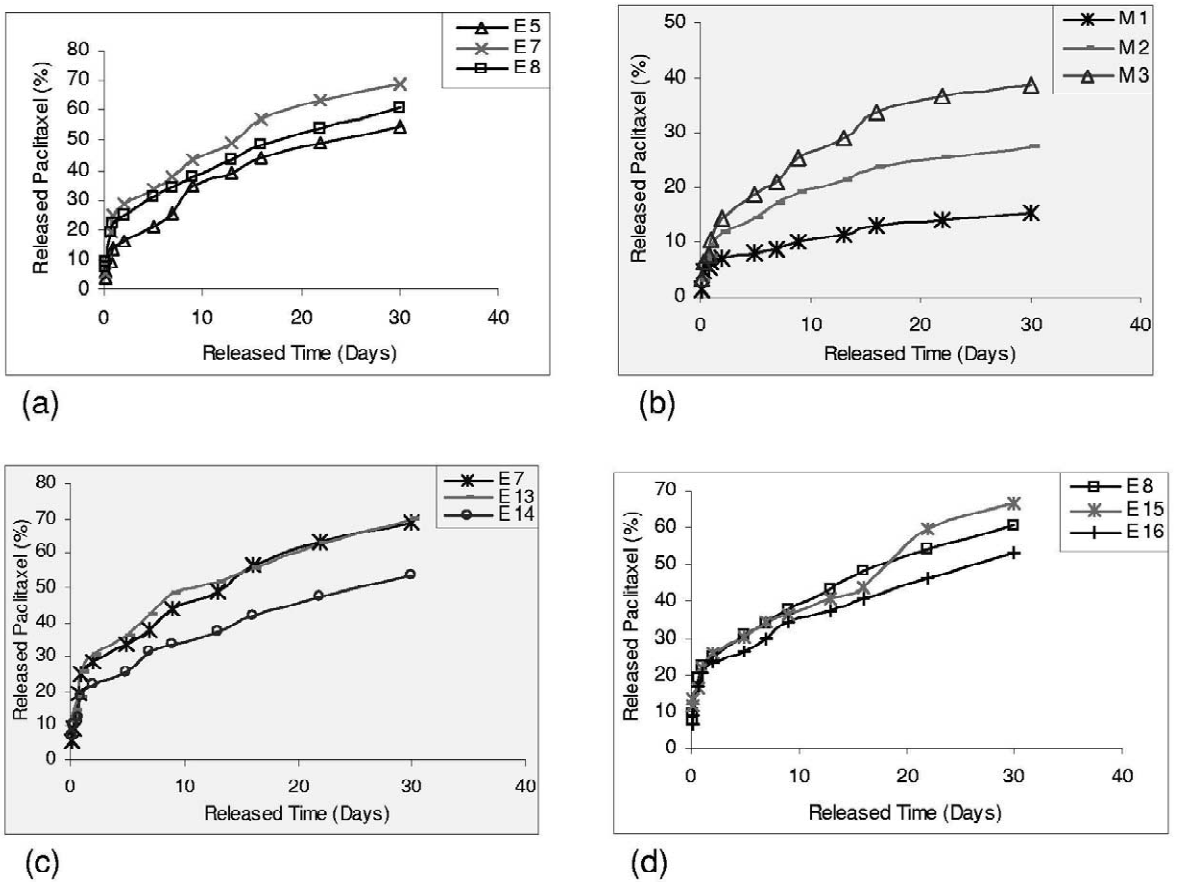

Fig. 7. In vitro release curves of paclitaxel loaded nanoparticles prepared under various experiment parameters (a) E5: PLA, E7: PLGA (75:25), E8: PLGA (50:50); (b) Ratio for PLGA-TPGS: M1 (2:1), M2 (1:1), M3 (1:2); (c) PLGA (75:25) concentration – E7: 0.125, E13: 0.188, E14: 0.25; (d) PLGA (50:50) concentration – E8: 0.125, E15: 0.188, E16: 0.25.

hydrophobic and could not dissolve in water. TPGS was amphiphilic but its hydrophobic ability was greater than its hydrophilic capability [33]. Therefore when TPGS was used as matrix material blended with PLGA and no other surfactant substance was added, the interaction or affinity between the polymer matrix and the drug might be enhanced and thus caused the slower drug release. The DSC study showed that TPGS and PLGA made each other much more amorphous when the two materials were blended together, which might also indicate that the interaction between them greatly increased.

Additionally, the differences in release rates for the particles prepared with various polymer concentrations displayed in Fig. 7c,d were as described before for size. The release of drug from smaller particles was clearly faster than that from larger ones. In accordance with the observations made in the SEM and AFM studies, the initial burst could be due to the diffusion release of paclitaxel distributed near the surface and in the outer portion of the nanoparticles. Afterwards, the release rates slowed as it would require time for the matrix material to erode in the aqueous environment. Thus the release mechanisms of diffusion, matrix swelling and polymer erosion, might be the main causes of the release behaviour [36].

4. Conclusions

The present research proposed a novel formulation by applying vitamin E TPGS either as emulsifier or as a component of the matrix material to fabricate

nanoparticles by freeze-dry solvent extraction/evaporation technique for controlled release of the antineoplastic drug paclitaxel. Investigation of the preparation, characterisation and in vitro release of the nanoparticles was carried out. The different formulations with various ratios of oil phase, aqueous phase, polymer material and surfactant were evaluated and optimised. Our results demonstrated that vitamin E TPGS could be an efficient emulsifier for fabrication of polymeric nanoparticles by the single emulsion technique, which can achieve excellent effects in drug encapsulation efficiency, size and size distribution, morphological and physicochemical properties, and in vitro release kinetics of the nanoparticles, and may have the potential to improve nanoparticle adhesion to cells and the hemodynamic properties of the nanoparticles in the blood flow. This is the first time in the literature that TPGS blended with other biodegradable polymers has been used as matrix material for nanoparticle fabrication, and has a self-emulsifying effect. In this research, a drug encapsulation efficiency as high as 100% has been achieved. AFM and SEM revealed the nanoparticles were fine spherical shapes. The particle size and size distribution strongly depended on the amount of TPGS added in the fabrication. DSC study concluded that when TPGS and PLGA were blended together to make the nanoparticles, they made each other much more amorphous. The fabricated nanoparticles all displayed phase transition behaviour that corresponded to that of the amorphous solid material and did not show any melting peaks. XPS investigation indicated that the surface of the fabricated nanoparticles was dominated by the TPGS molecules. We can conclude then that vitamin E TPGS has advantages either as emulsifier or as matrix material blended with PLGA for the manufacture of nanoparticles for controlled release of paclitaxel. The formulation can also be applied to make nanoparticles for clinical administration of other drugs and gene therapy.

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