Pharmaceutical Properties of Nanoparticulate Formulation Composed of TPGS and PLGA for Controlled Delivery of Anticancer Drug

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Abstract — A suitable management of the pharmaceutical property is needed and helpful to design a desired nanoparticulate delivery system, which includes the carrier nature, particle size and size distribution, morphology, surfactant stabiliser according to the technique applied, drug-loading ratio and encapsulation efficiency, surface property, etc. All will influence the in vitro release, in vivo behaviour and tissue distribution of administered particulate drug loaded nanoparticles. The main purpose of the present work was to determine the effect of drug loading ratio when employing TPGS as surfactant stabiliser and/or matrix material to improve the nanoparticulate formulation. The model drug employed was paclitaxel.

Index Terms—Biomaterials; Drug delivery; Surfactant stabiliser; Paclitaxel; D-α-tocopheryl polyethylene glycol 1000 succinate.

I. INTRODUCTION

The major goal in designing polymeric nanoparticles for delivery of a specific drug includes realising the controlled and targeted release of the active agent to the specific site of action at the therapeutically optimal rate. One of the most important concerns 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 of the particles, etc. All may have significant influence on the *in vivo* behaviour and tissue distribution of the drug loaded in the nanoparticles. With regard to the manufacture of polymeric nanoparticles, the solvent extraction/evaporation method is one of the most widely employed techniques and poly (vinyl alcohol) (PVA) is the most commonly used emulsifier in the process. However, as a functional surfactant, PVA tends an interconnected network with the polymer at the surface and is difficult to be removed after emulsification despite repeated washing [1]. It has been found that nanoparticles with higher amount of residual PVA have relatively lower cellular uptake [2]. PVA emulsified nanoparticles are thus not satisfactorily biocompatible and may be toxic for human body. Recently, we have successfully used 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 stabiliser added in the water phase or as a matrix component material added in the oil phase in the process [3, 4]. The model drug adopted was paclitaxel, which is one of the best antineoplastic drugs found from the nature in the past decades. Paclitaxel has excellent therapeutic efficacy against a wide spectrum of cancers, especially for ovarian and breast cancer. The clinical application of this drug has been limited due to its poor aqueous solubility. In its current clinical administration, an adjuvant called Cremophor EL is needed, which has been found responsible for most of the serious side effects of the dosage form and these include hypersensitivity reaction, nephrotoxicity, neurotoxicity and cardiotoxicity [5,6]. 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.

There are various parameters in the manufacturing

process which determine the physicochemical and pharmaceutical properties of the paclitaxel loaded polymeric nanoparticles such as the polymer type, its molecular weight/co-polymer ratio, the emulsifier employed, the drug loading ratio, the oil to water phase ratio, the mechanical strength of mixing, the pH and the temperature, etc. The present work was focused on the influence of the emulsifier type/quantity and the drug loading ratio on the physicochemical and pharmaceutical properties of the paclitaxel loaded PLGA/TPGS nanoparticles, which were manufactured by a modified single emulsion solvent extraction/evaporation technique. TPGS was introduced first as an emulsifier added in the water phase and then as a matrix material component added in the oil phase at various mole ratios in the emulsification process. Paclitaxel was loaded at various levels. Various equipments were used to characterise and analyse 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 thermal properties, and the high performance liquid chromatography (HPLC) for drug encapsulation efficiency and the measurement of *in vitro* release kinetics. We found that the drug encapsulation efficiency and the *in vitro* release behaviour could be significantly influenced by the drug loading ratio and the type and quantity of the surfactant stabiliser used and that vitamin E TPGS could be a novel and effective emulsifier as well as a nanoparticle matrix component material. Compared with PVA, the TPGS emulsified nanoparticles can have more favourable properties such as higher encapsulation efficiency (up to 100%) and could be easier to be removed from particles surface after the nanoemulsification.

II. MATERIALS AND METHODS

A. Material

Poly (DL-lactide-co-glycolide) (PLGA, L/G=50/50, MW (40,000-75,000) and polyvinyl alcohol (PVA, MW 30,000-70,000, the degree of hydrolysis is 87 to 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 (HPLC grade) was used as the mobile phase in HPLC and was purchased from Mallinckrodt Baker Inc. USA. Ultra-high pure water produced by UHQ Water Purification System was utilised for HPLC analysis. Deionised 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 used were of reagent grade and were used as received without further purification.

B. Methods

Fabrication and Collection of Nanoparticles --- 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 dichloromethane (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 stabilisers and sonicated simultaneously with energy output of 12W in a pulse mode using a sonicator (Misonix Incorporated, USA). The formed o/w emulsion was stirred by magnetic stirrer continuously for at least six 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, 10 min, 16ºC. 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 3-4 times to remove the surfactant residue. The produced suspension was dried under lyophilisation (Alpha-2 Martin Christ Freeze Dryers, Germany) to obtain the fine powder of nanoparticles, which was placed and kept in vacuum dessicator.

Characterization of Nanoparticles --- 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. SEM required a coating of the sample with platinum, which was done in an Auto Fine Coater (JFC-1300, JEOL USA). 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 deionised water and was sonicated for a suitable time period before the measurement. The volume mean diameter, size distribution and polydispersity of the resulting homogeneous suspension was determined. The surface chemistry of the nanoparticles was analysed by X-ray Photoelectron Spectroscopy (XPS, SXIS His-165 Ultra, Kratos Axis HSi, Kratos Analytical, Shimadzu Corporation, Japan). Peak curve fitting of the C1s (atomic orbital 1s of carbon) envelope was performed using XPSPeak 4.1 software. The thermal characteristics of drug loaded nanoparticles were analysed by the differential scanning calorimetry (DSC 822e, Mettler Toledo, STARe

software), and the glass transition temperatures (Tg) or melting point (Tm) was measured. 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 analysed.

Incorporation Capability and In Vitro Release of Paclitaxel Loaded nanoparticles --- The amount of entrapped paclitaxel in nanoparticles was detected in triplicate by HPLC (Agilent LC1100). A reverse phase Inertsil® ODS-3 column (150 x 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). 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. The *in vitro* release of paclitaxel from nanoparticles was measured in triplicate in PBS at pH 7.4. Ten mg 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 $37^{o}C$ 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 for 12 minutes. 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 due to 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.

III. RESULTS AND DISCUSSION

Morphology, Size and Size Distribution of Prepared Nanoparticles --- AFM and SEM were utilised to study the morphological property of the nanoparticles. From the SEM images (Fig.1), all nanoparticles from all formulations
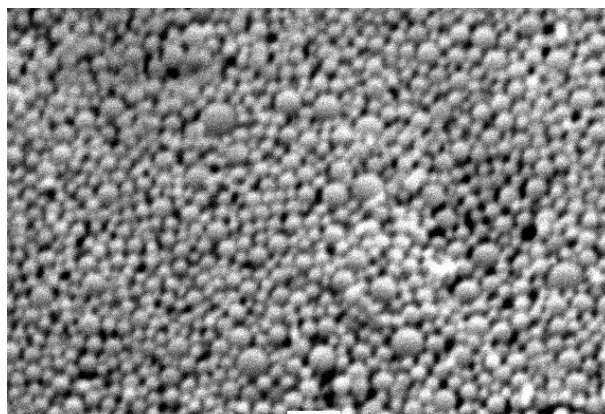

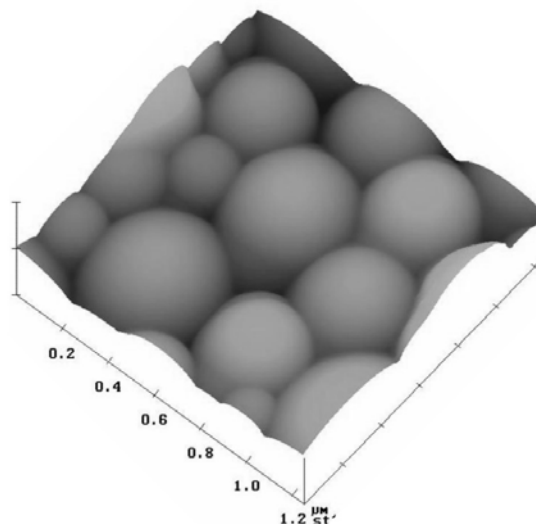Fig.1. SEM images of nanoparticles composed of PLGA/TPGS with TPGS as emulsifier (the bar was 1 μm).

Fig.2. AFM images of nanoparticles composed of PLGA/TPGS with TPGS as emulsifier.

displayed in spherical shapes and did not show aggregation although the particles might be too small for the resolving power of SEM. There was no obviously difference

amongst different formulations of nanoparticles. Under AFM with higher resolution (Fig.2), the distinct spherical nanoparticles could be observed, both single nanoparticle and multi-nanoparticles. The particles were closed and sorted out well from each other without adhesion or cohesion. The surface was relatively smooth. The result showed that the difference in drug loading-ratio had no significant influence on the nanoparticles morphology, no matter the particles were composed of polymer only or the
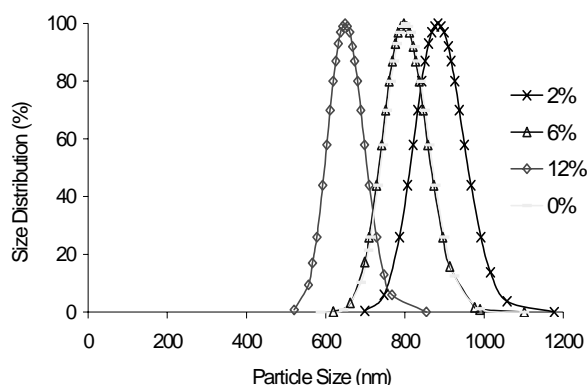
the polymer PLGA employed in the nanoparticles formulation was not influenced significantly by the procedure. But the melting peak of TPGS did not displayed, which meant that it was made amorphous when blended with polymer for fabrication of nanoparticles.

Surface Chemistry --- The XPS results were summarised in Table 1. For all samples, the elemental ratios for C and O were similar and did not seem to be affected by the difference in drug loading ratio or emulsifier. Some of the

Fig.3. Size distribution of nanoparticles composed of PLGA and TPGS with TPGS as emulsifier and with various drug-loading ratios.

Table 1. 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)	63.15	36.85	0.00	41.84	0.00	30.97	27.18
PVA	63.9	36.1	0.00	55.47	34.60		9.93
TPGS	69.10	30.9	0.00	46.9	50.7	0.00	2.4
Paclitaxel	78.40	19.55	2.05	68.62	15.15	8.90	7.33
p1	68.13	31.87	0.00	42.70	16.58	17.87	22.85
p2	66.79	32.73	0.48	45.73	15.56	17.02	21.68
p3	67.10	32.90	0.00	45.17	13.21	18.61	23.01
p4	69.60	30.40	0.00	48.90	14.72	15.72	20.66
t1	69.41	30.59	0.00	42.48	19.41	18.79	19.31
t2	70.94	29.06	0.00	46.35	17.90	17.17	18.58
t3	71.40	28.12	0.47	49.92	15.13	17.68	17.27
t4	70.50	29.50	0.00	44.72	18.14	17.18	19.96
tp1	66.41	33.01	0.58	37.79	17.55	20.53	24.13
tp2	68.14	31.74	0.12	45.95	15.43	17.90	20.72
tp3	67.00	33.00	0.00	42.03	15.66	19.51	22.80
tp4	68.09	31.91	0.00	48.90	16.73	15.98	18.39
tt1	72.75	27.25	0.00	46.27	18.88	16.24	18.60
tt2	74.24	25.76	0.00	50.59	17.74	15.64	16.02
tt3	75.33	24.53	0.14	51.65	18.69	15.15	14.51
tt4	71.34	28.66	0.00	46.29	19.11	16.12	18.48

mixture of polymer and TPGS, and emulsified by PVA or TPGS. Fig. 3 illustrated the representative size distribution of the nanoparticles prepared with various drug-loading ratio. Summarily, the noparticles reached the smallest when both TPGS and PVA were employed into the formulation. And PVA emulsified nanoparticles were relatively smaller than the TPGS emulsified nanoparticles.

Thermal Characteristic --- The pure paclitaxel showed an endothermic peak of melting at about 223.0 ℃ but no related peak displayed for all the prepared nanoparticles with or without drug entrapped. However, the physical mixture of pure paclitaxel and placebo nanoparticles gave a broadened peak shifted to a lower temperature at about 220 ℃. The content of paclitaxel in the physical mixture (the ratio of paclitaxel to placebo nanoparticles was 1:9) was even higher than that in the nanoparticles (12% drug loading ratio). The result concluded 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 mixed with TPGS after the fabrication and all related treatment, no matter the drug loading was low (2%) or high (12%) [7]. Meanwhile, the glass transition temperature of

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 the nanoparticles. This may also suggest that the nanoparticles produced were in the form of matrix system where the drug is distributed evenly or random. Therefore, it is possible to observe the presence of the drug near or at the surface even at a very low amount, most of which could not be detected by the XPS. Nevertheless, it was expected that the drug was more concentrated inside the nanoparticles because the drug paclitaxel is highly hydrophobic, it tends to stay away from aqueous environment. This could be confirmed by the elemental distribution of nanoparticles with high drug loading ratio as the distribution of N near or at the surface did not increased along with the increased drug loading ratio. The even and random distribution of paclitaxel was also agreed with the result of DSC analysis. Besides, the presence of paclitaxel near or at the surface may influence the in vitro release behaviour that may show a high burst release. As to the XPS curve fitting analysis, 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 that

corresponded to all the initial specific C1s environments. In comparison with the value from the 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 suggested the distribution or adsorption of the emulsifier PVA or TPGS on the surface during the nanoparticles formation to achieve stabilisation of the polymer/water interface. The retaining of C1s envelope corresponding to C-O-C=O indicated the existence of PLGA at the particles surface. The analysis revealed that the surface of the fabricated nanoparticles was composed of both the matrix material PLGA and the surfactant stabiliser PVA or TPGS. The distribution percentage of each substance was approximately close to 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 the nanoparticles surface to it at the pure material from PVA was higher than from TPGS, no matter if TPGS was used together with PLGA as matrix material. The result may suggest that the emulsifier molecules of PVA left on the particles surface was more than those of TPGS, meaning that the PVA was difficult to be removed away than TPGS after same cleaning procedure.

Paclitaxel Encapsulation Efficiency --- The encapsulation efficiencies of the drug in all the nanoparticulate formulations were measured. Overall, the nanoparticles prepared with TPGS as emulsifier demonstrated higher drug encapsulation efficiencies compared to the ones prepared with PVA as emulsifier. TPGS could effectively increase the encapsulation efficiency up to 100% (i.e. 97.5% in sample tt2, when TPGS as material matrix and as surfactant stabiliser), while PVA could only efficiently encapsulate until 60.5 when PLGA was used as material in sample p3 (PLGA as material matrix and PVA as surfactant stabiliser) and 65.5% in sample tp3 (when TPGS was mixed together with PLGA as matrix material while PVA as surfactant stabiliser). It could be concluded that TPGS was a more effective emulsifier than PVA since concentration of TPGS used was much lower than concentration of PVA (0.025% versus 1.0%). Meanwhile, there was a trend in the relation between the drug loading-ratio and the encapsulation efficiency. Generally, increase in drug loading-ratio from 2% to 12 % resulted in an increase in the encapsulation efficiency although an optimal drug loading ratio might be confirmed by determining more points of drug loading-ratios versus encapsulation efficiencies to probably obtain the correlation between them.

In Vitro Release of Paclitaxel from Various Nanoparticles --- Nanoparticles of various formulations were determined for their cumulative release of encapsulated paclitaxel under *in vitro* condition. The curves were showed in Fig. 4. Generally, the release profiles were biphasic with an initial burst attributed to the drug associated near particles surface, followed by a linear release phase. Clearly, both the emulsifier and the drug loading ratio could influence the in vitro release behaviour significantly. A general trend was that the release rate decreased with the increased drug loading ratio for all formulations (Fig. 4, a-d). As discussed previously, the drug loading ratio did not have significant effects on the nanoparticles size and morphology. Thus, the difference in the release behaviour could not be related to the particle size. 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 less drug diffusion for the release. PVA emulsified nanoparticles (Sample p and Sample tp) exhibited faster release than those emulsified by TPGS (Sample t and Sample tt). This may be because TPGS is more hydrophobic molecule, smaller but with bigger bulk area and could cause more compact matrix structure, resulting in a lower degradation rate of the polymer and/or slower diffusion of the encapsulated drug.

IV. CONCLUSION

The present work fabricated and characterised paclitaxel loaded PLGA and PLGA-TPGS nanoparticle formulations that utilised various paclitaxel loading ratio and emulsifier. The results demonstrated that the pharmaceutical property of nanoparticles could be influenced either by paclitaxel loading ratio or by surfactant stabiliser. In vitro release characteristics were closely related to the paclitaxel loading ratio which was also an important formulation parameter on the drug encapsulation efficiency. Generally, the release rate decreased with the increased drug loading-ratio for all formulations. TPGS-emulsified nanoparticles exhibited slower release compared to PVA-emulsified nanoparticles. Initial burst release was observed for all nanoparticles formulation. When incorporating TPGS as material matrix together with PLGA, the initial burst release reduced compared other formulations with PLGA only as material matrix at same drug-loading ratio. Also in that case, the release rate of PVA emulsified nanoparticles was distinctly faster than that of TPGS emulsified nanoparticles. The increment was even more when paclitaxel loading ratio increased. The surface chemistry of the nanoparticles in different formulation was similar, which suggested that drug-loading ratio had slightly influence on the particles surface property. Also, PVA was difficult to be removed away than TPGS after same cleaning procedure. To sum up, both paclitaxel loading ratio and the surfactant stabiliser were important formulation parameters that could
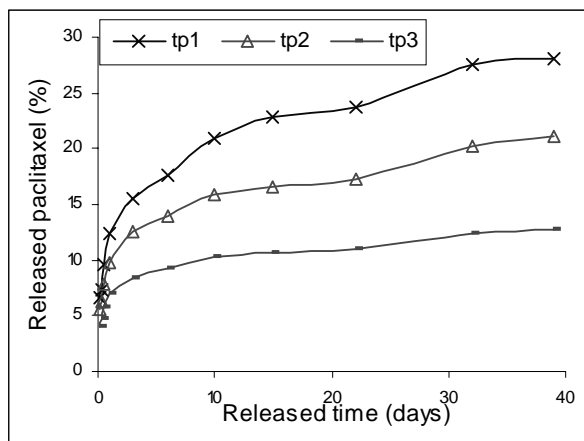
be applied to modify or alter the different pharmaceutical properties of nanoparticles for the controlled release of anticancer drug paclitaxel. As emulsifier, TPGS was comparable to PVA but possessed potential advantages to be applied.
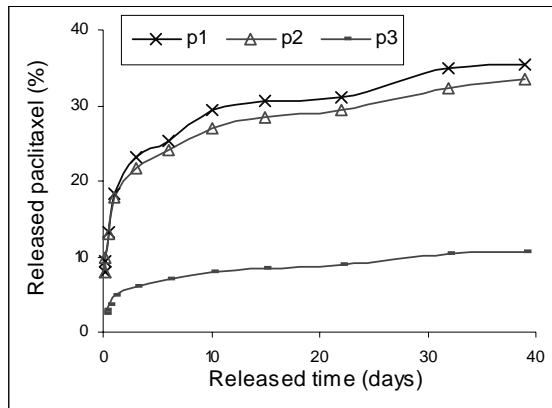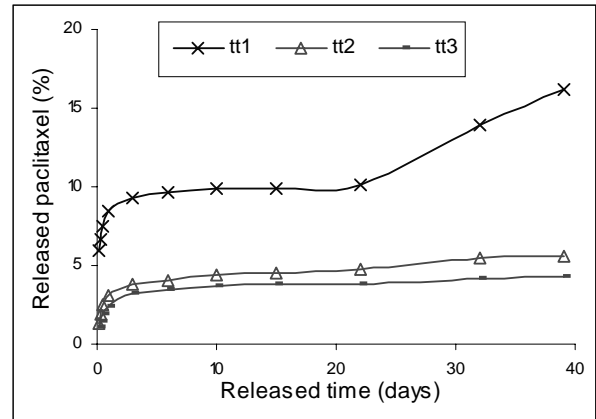

(a)
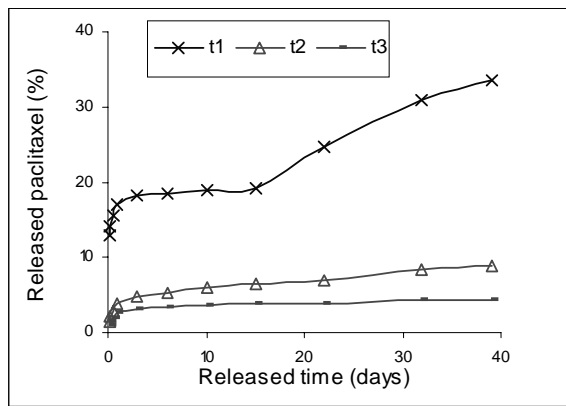
(b)

(c)

(d)

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).

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