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Fabrication, characterization and in vitro release of paclitaxel (Taxol®) loaded poly (lactic-co-glycolic acid) microspheres prepared by spray drying technique with lipid/cholesterol emulsifiers

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Abstract

Spray dry technique was applied to produce paclitaxel loaded microspheres of biodegradable poly (lactic-co-glycolic acid) (PLGA) as an alternative delivery system. Various emulsifiers such as L-α-dipalmitoyl-phosphatidylcholine (DPPC), cholesterol, polyvinyl alcohol (PVA), gelatin were incorporated in order to achieve high encapsulating efficiency of paclitaxel in the microspheres and desired properties for a sustained release. Atomic force microscopy (AFM) and scanning electron microscopy (SEM) showed that the surface of the microspheres with high ratio of lipid was spherical and smooth. Those made with other emulsifiers had rougher surface with pores. Incorporation of lipid, cholesterol or gelatin can significantly increase the drug content in the microspheres. The differential scanning calorimetry (DSC) result indicated that the paclitaxel trapped in the microspheres existed in an amorphous or disordered-crystalline status in the polymer matrix. The zeta potential of the microspheres was negative in general and was strongly influenced by the type of the emulsifiers used in fabrication. The system formulated with cholesterol was most stable. The release profiles of various formulations with PVA, gelatin as well as low ratio of DPPC showed almost zero-order release kinetics in the first 3 weeks after an initial burst less than 5% in the first day. The release rate then gradually decreased. The microspheres fabricated with high ratio of DPPC exhibited large initial burst. When cholesterol was combined together with DPPC as an emulsifier, the release became faster.

Keywords: Paclitaxel; Microspheres; Spray dry; Emulsifiers; Anticancer agent

Abbreviations: PLGA, poly (lactic-co-glycolic acid); DPPC, dipalmitoyl-phosphatidylcholine; PVA, polyvinyl alcohol; DSC, differential scanning calorimetry; SEM, scanning electron microscopy; AFM, atomic force microscopy; NCI, US National Cancer Institute; FDA, The US Food and Drug Administration; MDR, multidrug resistance; PBS, Phosphate buffered saline; DCM, dichloromethane

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1. Introduction

Paclitaxel (Taxol®) is one of the best antineoplastic drugs found from nature in the past decades. It has been widely used in the clinical treatment of various cancers especially breast cancer and ovarian cancer. It was discovered, isolated and its antitumor activity was identified in the 60's of the twentieth century [1]. It has a unique mechanism of action to inhibit the growth and separation of cancer cells. It blocks cells in the late 62-mitotic phase of the cell cycle by stabilizing the microtubule cytoskeleton [2]. Due to its novel antineoplastic activity, paclitaxel has been approved by the US Food and Drug Administration (FDA) to treat various types of cancers [3,4]. There are two main difficulties, however, which confine its more effective clinical application. One is the resource of the drug. The most concentrated natural source of the compound is the bark of the Pacific yew tree, *Taxus brevifolia*. It takes all of the bark of six large trees, each 100 years old, to produce enough paclitaxel to treat just one patient. This is not affordable from the nature. The total chemical synthesis of paclitaxel has been achieved. But it may not be commercially practical because of the complex and unusual chemistry. The possible solution is the semisynthesis of the drug from other more abundant source such as English yew trees and Chinese red bean trees. Another limitation of paclitaxel is its high insolubility in water and most pharmaceutical solvents [5]. Adjuvant such as Cremophor EL has to be used in its current clinical administration. Cremophor EL is a polyethoxylated castor oil derivative commonly used as a drug solubilizer, which, by itself, causes serious side effects such as hypersensitivity reactions, nephrotoxicity, neurotoxicity and cardiotoxicity [2,6–12]. To eliminate the toxicity of the adjuvant, improve efficacy and eliminate premedication, the effective therapy with paclitaxel is thus mainly focused on developing new drug delivery systems to circumvent the problem associated with Cremophor EL. A successful administration of paclitaxel requires a formulation, which does not employ toxic adjuvant, releases the paclitaxel over an extended period of time and stabilizes the compound during long term storage of the formulation. Meanwhile, it should be practical to produce on a large scale. There have been attempts to deliver paclitaxel devoid of Cremophor EL, which include nanocapsules [13], liposomes [14], water-soluble prodrugs [15], enzyme-activatable prodrugs in conjugation with antibodies [16], albumin conjugates [17], complexes with cyclodextrins [18], parenteral emulsion [19], all with limited success. None of them has reached the stage of replacing Cremophor EL based vehicle. Applying microspheres of biodegradable polymers for chemoembolization to enhance therapeutic efficacy of anticancer agents while reducing the systemic side effects has been pursued to achieve satisfactory result [20]. The formulations are usually prepared by the solvent evaporation technique, either freeze dry or spray dry [21–25].

The spray dry technique has been an important and widely applied technique in the pharmaceutical and biochemical fields [24,25]. For fabricating microparticulate systems, the spray dry can be applicable to both heat resistant and heat sensitive drugs, both water soluble and water insoluble drugs. This technique is also applicable to both hydrophilic and hydrophobic polymers. It is a one stage continuous process, which produces dry powders, granules or agglomerates from drug-excipient solutions and/or suspensions. This technique can be adaptable in an industrial scale, which is superior to most of other fabrication procedures being only good for laboratory-scale operation. Furthermore, the polymeric microsphere drug delivery systems produced by this technology have a potential to provide new types of administered routes, such as oral dosage forms, targeting systems to organs and tissues and long-acting parenteral biodegradable systems [26,27].

In the development of biodegradable polymeric micro/nanoparticles as drug delivery devices, the type of surface active substances or stabilizers involved in the fabrication procedure has an important effect on drug loading, physical and pharmaceutical properties, in vitro release property as well as in vivo character of the product. The present work employed the spray dry technique to produce paclitaxel loaded microspheres of biodegradable poly (lactic-co-glycolic acid) (PLGA). Various emulsifiers were applied in their fabrication to achieve high encapsulate efficiency of paclitaxel and desired properties for sustained release of paclitaxel with less side effects. The fabrication, characterization and in

vitro release of paclitaxel loaded PLGA microspheres were investigated. The influence of different kinds of emulsifiers on the encapsulation efficiency, size and size distribution, surface structure and morphology, physicochemical properties, and more importantly, the release kinetics of the drug were discussed.

2. Materials and methods

2.1. Materials

Paclitaxel was purchased from Dabur India Limited, India and Hande Biotechnology, China. Poly (DL-lactide-co-glycolide) (PLGA, 75:25, MW 90000–126000), polyvinyl alcohol (PVA, MW 30000–70000), gelatin (Type B: From Bovine Skin), L-α-dipalmitoylphosphatidylcholine (DPPC, $C_{40}H_{80}NO_8P$, FW 734.0), cholesterol (5-cholesten-3β-ol, $C_{27}H_{46}O$, FW 386.7, grade: 99+%) were purchased from Sigma Chemical Co., USA. The organic solvent methylene chloride/dichloromethane (DCM) was 'Baker Analyzed' HPLC solvent. Acetonitrile used as mobile phase in high performance liquid chromatography (HPLC) was Mallinckrodt chrom AR HPLC grade. Phosphate buffered saline (PBS) which was also purchased from Sigma Chemical Co. was used as buffer solution for the in vitro release measurement. Distilled water produced by Millipore (Millipore Corporation, Bedford, MX 01730, USA) was used throughout.

A laboratory scale spray-drying was carried out by using the Büchi mini spray dryer B-191 (Buchi Laboratory-Techniques, Switzerland) with a standard nozzle (0.7 mm diameter) to prepare various types of paclitaxel-loaded PLGA microspheres. The operating conditions were set as follows: inlet air temperature (50±2)°C, outlet temperature (42±2°C), spray flow control (700 NL/h), pump setting at feed spray rate (4.5–5.0 ml/min), aspirator setting level (100%), atomization pressure (6 bar). Additives such as PVA, gelatin, DPPC and cholesterol were applied to investigate their effects on the encapsulation efficiency, size and size distribution, morphology, physicochemical properties and release kinetics. Polymeric material, paclitaxel, and the additive were dissolved in an appropriate volume of dichloromethane (DCM), then stirred at room temperature using a magnetic stirrer (EYELA Magnetic stirrer RC-2) until all components were dissolved or suspended homogeneously. The solution or suspension system was spray dried till no more production can be sprayed out. The dried product was then collected. The obtained microspheres were stored in a vacuum dessicator at room temperature. Placebo microspheres in which paclitaxel was absent was prepared with the same procedure.

2.2. Microspheres characterization

The size and size distribution of the prepared microspheres were measured by the dynamic laser light scattering (Brookhaven Instruments Corporation 90 Plus Particle Sizer). The dried powder samples were suspended in deionized water. After slight sonication, the obtained homogeneous suspension was determined for the volume mean diameter and polydispersity. The shape and surface morphology of the fabricated microspheres were examined with the scanning electron microscopy (SEM, Hitachi 5-4100, or Philips XL 30) after gold palladium coating of the microspheres using an ion-coater. The results of size and size distribution were further confirmed by SEM as well. Higher resolution images for the morphology of microspheres were obtained microspheres using an ion-coater. The results of size and size distribution were further confirmed by SEM as well. Higher resolution images for the morphology of microspheres were obtained from atomic force microscopy (AFM, Multimode Scanning Probe Microscope, Digital Instruments). The zeta potential of all types of microspheres was measured by laser doppler anemometry (Zeta Plus, Zeta Potential Analyzer, Brookhaven Instruments Corporation). Standard buffer solution of 0.05 M potassium hydrogen phthalate aqueous solution at pH 4.0, 0.025 M potassium dihydrogen phosphate aqueous solution at pH 7.0 and 0.01 M tetra-sodium boric acid aqueous solution at pH 9.0 were employed. Differential scanning calorimetry (DSC) was performed (Netzsch DSC 200 instrument). The samples were purged with nitrogen. The heat flow rate was recorded from 20 to 300°C at a rate of 10°C/min. Indium was used as the standard reference material

to calibrate the temperature and energy scales of the DSC instrument.

2.3. Encapsulation efficiency

The measurement of paclitaxel content in the microspheres was carried out in triplicate using HPLC according to the method given in references [21] and [22] with certain modification. 3.0 mg of paclitaxel-loaded microspheres was dissolved in 1 ml of DCM. Then 3 ml of acetonitrile–water (50:50, v/v) was added and mixed. A nitrogen stream was introduced to evaporate DCM at room temperature until a clear solution was obtained. The solution was put into vial for HPLC detection. A reverse phase Inertsil® ODS-3 column (150×4.6 mm i.d., pore size 5 μm, GL Science, Tokyo, Japan) was used. The mobile phase consisted of a mixture of acetonitrile–water (50:50, v/v), and was delivered at a flow rate of 1.00 ml/min with a pump (Perkin Elmer-S200). A 100 μl aliquot of the samples was injected with an autoinjector (Perkin Elmer-ISS200). The column effluent was detected at 227 nm with an UV detector. The calibration curve used for the quantification of paclitaxel in the microspheres was linear over the range of 200–6000 ng/ml (standard concentration of paclitaxel) with a correlation coefficient of $r^2 = 0.9991$. The solvent for calibration is a mixture of acetonitrile–water (50:50, v/v).

A correction of the calculated encapsulation efficiency is needed since inefficient extraction may exist [22,28]. To determine the recovery efficiency coefficient of the extraction procedure, known weights of pure paclitaxel from 100 to 1000 μg and 3.0 mg of placebo microspheres or polymer were dissolved in 1 ml of DCM, and then subjected to the same extraction procedure in triplicate as described above. The resulted factor was 79.0%, which meant that about 80% of the original amount of the paclitaxel were detected. The encapsulation efficiency of paclitaxel in the microspheres, after corrected accordingly, was calculated as the weight ratio of the drug entrapped in the microspheres to that used for the preparation.

2.4. In vitro release study

The in vitro release of paclitaxel loaded microspheres was measured in PBS in triplicate at temperature of 37°C. 10 mg of paclitaxel loaded microspheres were suspended in 10 ml of PBS containing 0.1% (w/v) Tween 80 in a screw capped tubes, which were placed in an orbital shaker bath (GFL-1086, Lee Hung Technical Company, Bukit Batok Industrial Park A, Singapore) maintained at 37°C and shaken horizontally at 130 min^{-1}. At given time intervals, the tubes were centrifuged at 10,000 to 12,000 r.p.m. for 10 min. The precipitated microspheres pellets were resuspended in 10 ml of fresh buffer and placed back in the shaker bath. The supernatants were taken for analysis. Paclitaxel concentration in the release medium was determined by adding 1 ml of DCM for extraction which was followed by adding 3 ml of the mixture of acetonitrile–water (50:50, v/v). Evaporation until a clear solution was carried out under a stream of nitrogen. HPLC analysis can then be done as previously described.

Similar to the investigation of encapsulation efficiency, the extraction procedure had to be analyzed to obtain the extraction factor. For this purpose, a known weight of pure paclitaxel was subjected to the same procedure as the method mentioned above. The determined factor was 46.0%, which meant that the obtained extraction solution only contained 46% of the original paclitaxel after all the related process. The data obtained for analysis of the in vitro release should be corrected accordingly.

3. Results and discussion

3.1. Microsphere preparation and encapsulation efficiency

Six types of paclitaxel loaded PLGA microspheres along with placebo PLGA microspheres were fabricated by applying several types of emulsifiers. The concentration of PLGA in organic solvent was 2.5% (w/v). The initial loading level of paclitaxel in all materials was 2.0% (w/w). The yield of preparation and the encapsulation efficiency of paclitaxel in various microspheres are listed in Table 1. The production yield was not significantly affected by adding PVA, gelatin or low ratio of DPPC, but might be lowered by application of high ratio of DPPC.

Table 1
Preparation and properties of all types of PLGA microspheres

Samples	Yield (%)	EE (%)	Mean diameter (nm)±S.E.	Polydispersity
1. PLGA only	23.3		1109.6±84.0	0.181
2. PLGA+Paclitaxel (2.5%)	47.4	61.1	1953.4±112.5	0.041
3. PLGA+Paclitaxel (2.0%)+PVA (1.9%)	47.9	63.1	1696.0±85.9	0.158
4. PLGA+Paclitaxel (2.1%)+DPPC (2.1%)	46.9	84.1	1718.7±101.6	0.033
5. PLGA+Paclitaxel (1.8%)+Gelatin (2.1%)	47.1	70.1	2914.2±179.8	0.164
6. PLGA+Paclitaxel (2.3%) +DPPC (20%)+cholesterol (20%)	54.9	79.1	853.3±39.3	0.340
7. PLGA+Paclitaxel (2.1%)+DPPC (40%)	11.9	81.8	991.9±68.2	0.248

However, it was greatly increased when DPPC and cholesterol were introduced together. The encapsulation efficiency of paclitaxel in the microspheres was only 61.1% without any additives. It was increased to 63.1% or 70.1% when PVA or gelatin was incorporated, respectively. And it was greatly increased to 84.1% for low ratio DPPC, 79.1% for DPPC/cholesterol and 81.8% for high ratio DPPC. These results may be explained as hydrophobic surfactants contribute to the corporation of the high hydrophobic agent paclitaxel into the related formulation system.

Obviously, there is advantage for the DPPC and/or cholesterol as additives to increase the encapsulation efficiency of paclitaxel entrapped into the microspheres.

3.2. Size and morphology characteristics of the microspheres

The particle size and size distribution were summarized in Table 1 and diagramed in Fig. 1. The mean size of the fabricated microspheres laid within a narrow range of 0.9–3.0 μm, which was slightly influenced by incorporating different additives in the formulations. The microspheres produced by adding gelatin possessed the largest mean size, which was much larger than that of the microspheres prepared by using the high ratio of DPPC as emulsifier, which had the smallest mean size. As shown in Fig. 1, the size of all the seven types of microspheres in the small-to-large order was, PLGA+Paclitaxel+DPPC (high ratio), PLGA+Paclitaxel+DPPC+Cholesterol, PLGA only, PLGA+Paclitaxel+PVA, PLGA+Paclitaxel, PLGA+Paclitaxel+DPPC (low ratio), PLGA+Paclitaxel+Gelatin. The SEM images (Fig. 2) showed that the microparticles were roughly spherical but somewhat irregular with minor or grass distortions due to the formation of holes or shrivellings. The microspheres containing both DPPC and cholesterol or high ratio of DPPC only were of relatively regular shape and smooth nonporous surface. Those with other additives were of porous or dimple surface. All batches showed no aggregation.

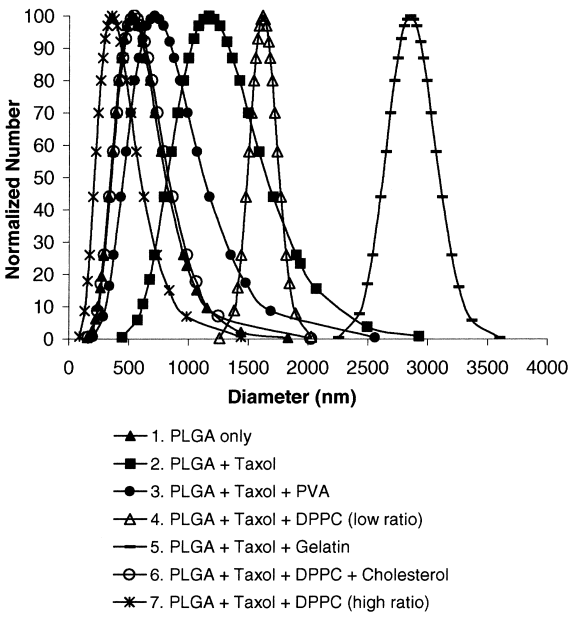

Fig. 1. Normalized particle size distribution of various types of PLGA microspheres loaded with paclitaxel.

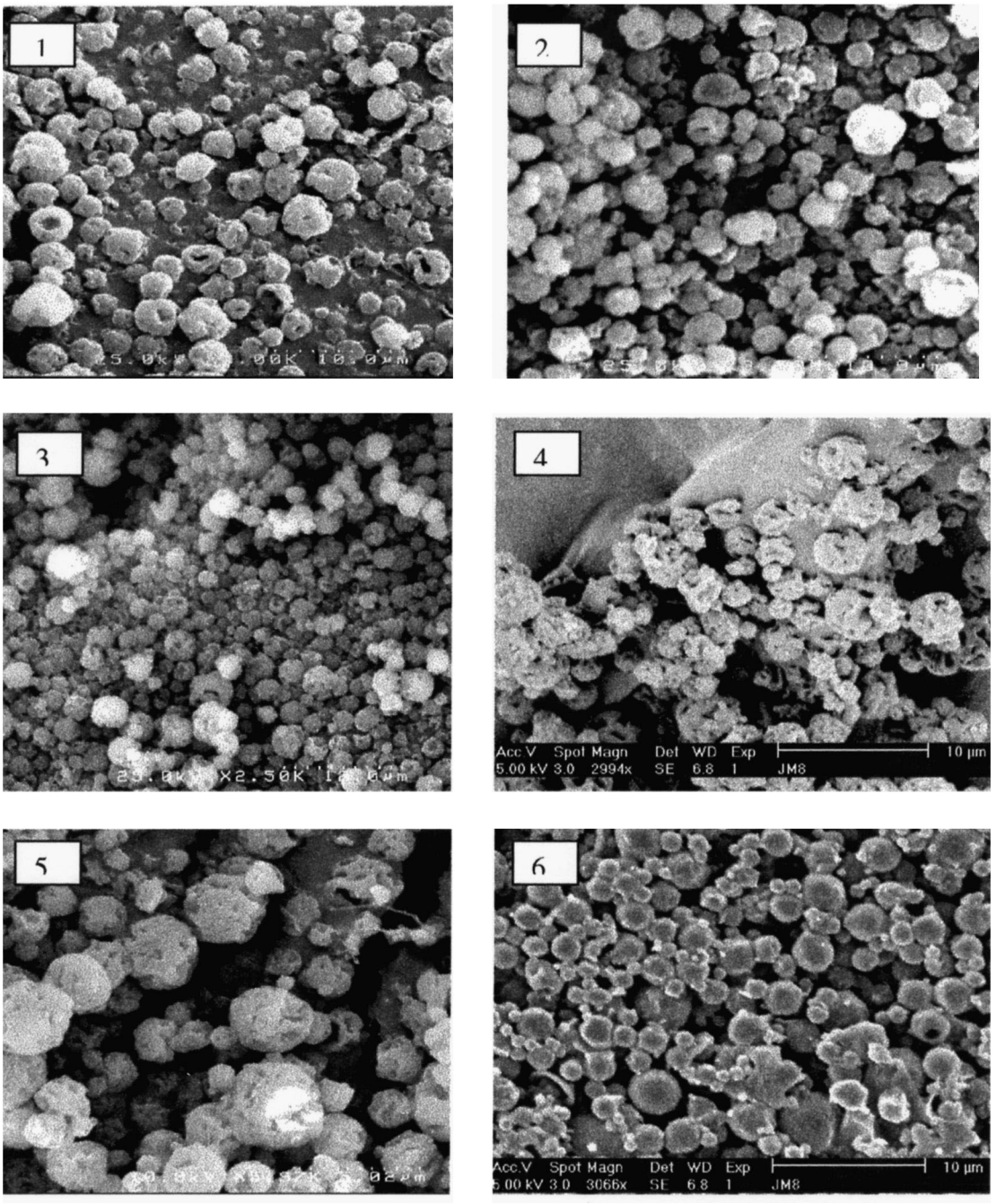

Fig. 2. Scanning electron micrographs (SEM) showing the surface morphology of the various types of paclitaxel loaded PLGA microspheres prepared. (1) PLGA only; (2) PLGA+Paclitaxel; (3) PLGA+Paclitaxel+PVA; (4) PLGA+Paclitaxel +DPPC (low ratio); (5) PLGA+Paclitaxel±Gelatin; (6) PLGA+Paclitaxel+DPPC+Cholesterol.

In preparation of microparticles by the spray drying technique, the achievement of smooth spherical products was found to be difficult because the formation of fibres due to insufficient forces to break up the liquid into droplets encountered in the spray drying of polymeric solution [29]. The influence of process parameters, polymer concentration and the nature of organic solvent on the microparticle characteristics had been described previously. However, it seemed that these parameters had no noticeable effect on the particle size in a certain range [30–32].

Comparatively, less demonstration was available on the effect of the additives on the microsphere characteristics and the drug release from the delivery system. The present work aimed to investigate the effects of the additives or emulsifiers on the fabrication and property of the microspheres of PLGA and paclitaxel. As appeared in the size distribution graphs and SEM images, the relatively larger microspheres may result from the high viscosity of the original suspension or solution before spraying and infusion of the particles formed in the process due to the increased frequency of collisions. The irregular crumpled surface could be due to the rapid drying from the surface to the interior. In addition, the presence of some additive such as gelatin may exert a plasticizing effect on the microspheres before the drying. In contrast, DPPC and cholesterol are lipids, which are amongst the natural emulsifiers and possess the advantage of amphiphiles. They could be dissolved in DCM completely and form subsequently a more homogeneous and stable emulsion system, which had an appropriate viscosity. Moreover, the surface tension of the solvent/air interface of the adsorbed emulsifiers was reduced effectively. Therefore, after spray drying, the microspheres of high ratio lipid demonstrated small particle size, regular shape and smooth surface.

One of the features in characterization in this paper is the application of the atomic force microscopy (AFM) to achieve images of the microspheres in high resolution. AFM technique has been widely developed to provide surface-dependent information in three dimensions on a nanometer scale. It is capable of resolving surface details down to the atomic level depending on operating conditions. It is difficult to get optimal image for solid micro/nanoparticle samples in general. The images of shape and surface property of the microspheres formulated with various emulsifiers were obtained successfully by applying tapping mode AFM and those made with high ratio of DPPC as emulsifier were shown in Fig. 3. The smooth surface of the microsphere can be observed clearly from Figs. 3a,b and the pore on the sphere surface can be seen from Fig. 3c.

3.3. DSC analysis

The DSC technique can provide qualitative and quantitative information about the physicochemical status of the drug in the microspheres, which was reported to be involved in the endothermic or exothermic process [33]. The related thermal transitions include melting, recrystallization, decomposition, out-gassing, or a change in heat capacity. DSC is useful to monitor different samples of the same material to assess their similarities or differences or the effects of additives on the thermal properties of a material. Using the DSC analysis of drug, polymer materials and produced microspheres, the nature of the drug inside the polymer matrix can be assessed, which may emerge in solid solution, metastable molecular dispersion or crystallization [33] and may display relevant properties during in vitro release. There is no detectable melting endotherm if the drug presents in a molecular dispersion or a solid solution state in the polymeric microspheres loaded with enough amount of drug. The spray dry may result in the formation of an amorphous or disordered-crystalline drug phase due to rapid drying of the slurry droplets [34]. A higher thermodynamic activity of the amorphous drug phases is related to a more common crystalline form and has particular pharmaceutical significance. The increased solubility can result in improved biological activity. In the present work, the DSC thermograms of pure paclitaxel, pure PLGA materials, placebo microsphere, physical mixture of paclitaxel and PLGA with the same ratio as that of the paclitaxel loaded microspheres were detected and shown in Figs. 4–6. Since the melting endotherm peak of paclitaxel could not be detected in the physical mixture of paclitaxel and polymer with the same ratio as that in the microspheres loaded with 2.5% of paclitaxel because of a lack of sensitivity of the apparatus, microspheres with 10%

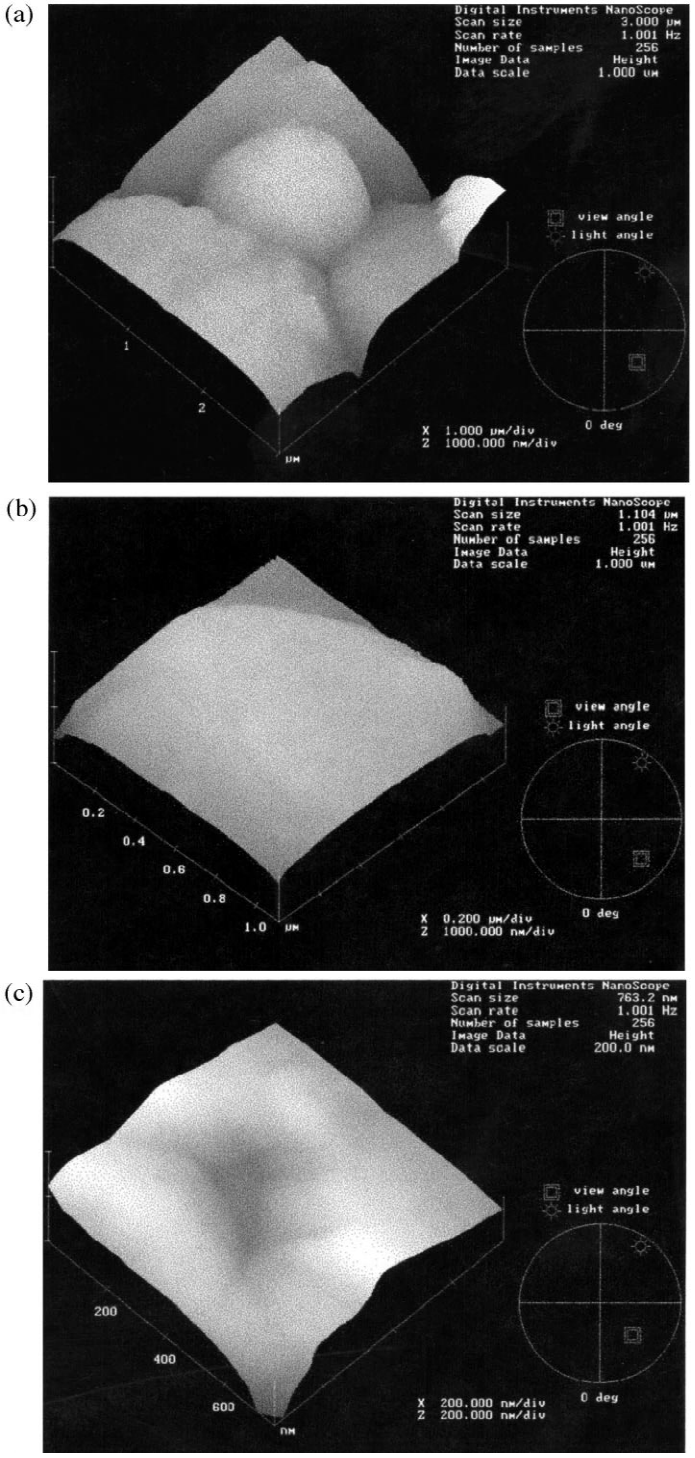

Fig. 3. Atomic force microscopy (AFM) images showing the shape and surface of paclitaxel loaded PLGA (75:25) microspheres with lipid (DPPC) as emusifier.

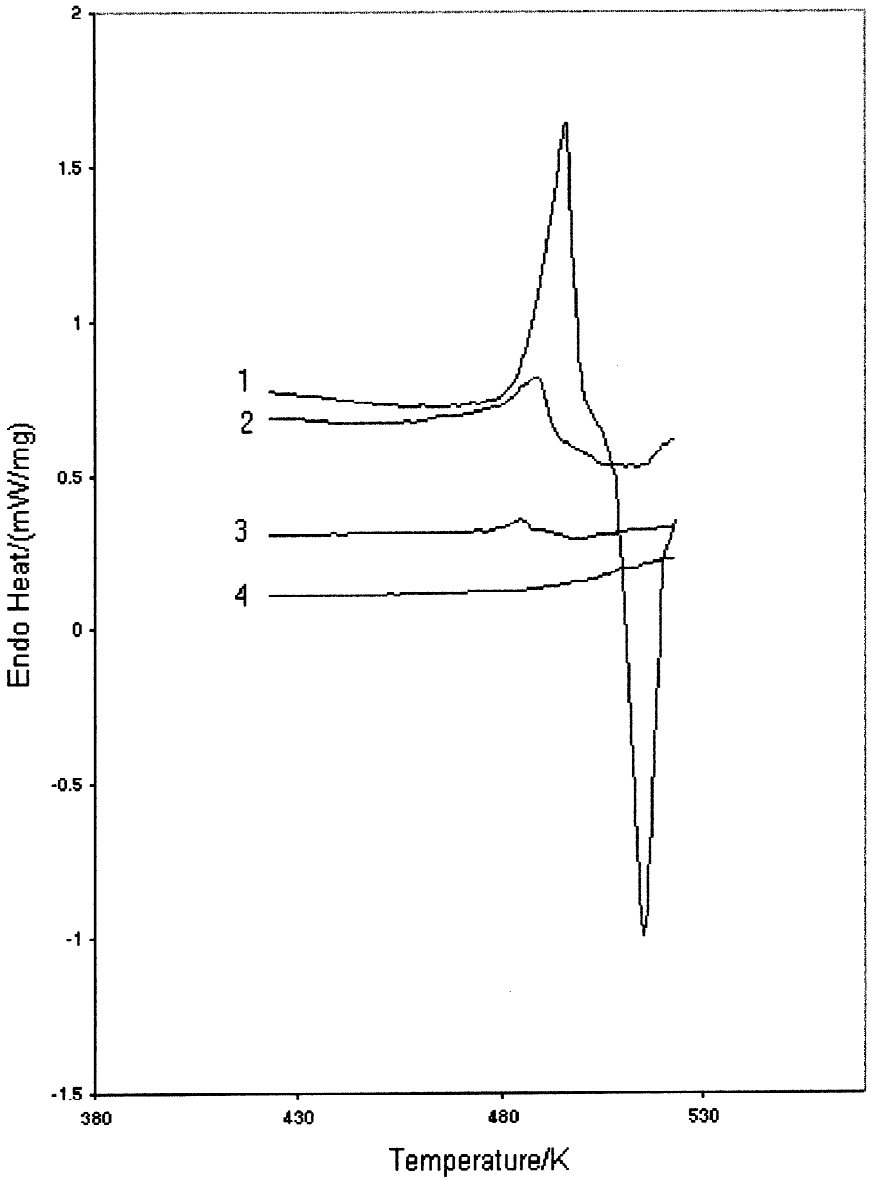

Fig. 4. DSC thermograms of (1) 100% Paclitaxel; (2) Physical mixture of 30% Paclitaxel and 70% PLGA; (3) Physical mixture of 10% Paclitaxel and 90% PLGA; (4) Physical mixture of 5% Paclitaxel and 95% PLGA.

of paclitaxel were prepared for the DSC measurement. As control, the physical mixtures of paclitaxel and PLGA with 10% and higher ratio of paclitaxel were proceeded (Fig. 4). Under the experimental condition, an endothermic peak of melting of pure paclitaxel at 223.0°C (496.0 °K) was broadened and shifted to a lower temperature for the paclitaxel-PLGA physical mixture. It can be seen from Fig. 4 that the peak was at 216.0°C (489.0 °K) for the 30% sample, and was shifted to about 209.0°C (482.0 °K) for the 10% sample. There was no peak observed at the temperature range of 150–250°C (430–530 °K) for pure PLGA material and the placebo microspheres (Fig. 5). There was also no peak appeared in

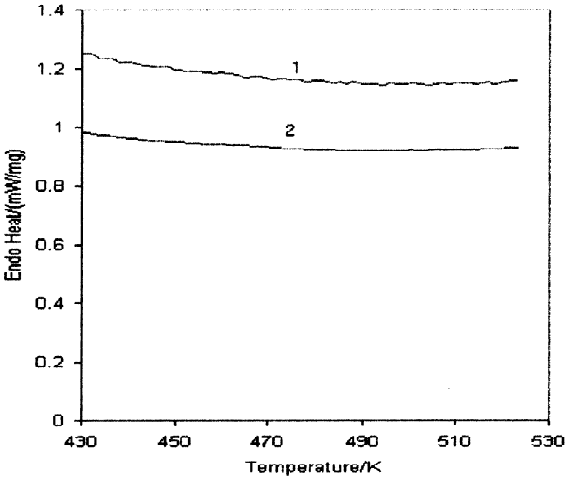

Fig. 5. DSC thermograms of (1) Placebo microsphere; (2) Pure PLGA.

this temperature range for all the drug loaded microspheres produced with the different kinds of emulsifiers (Fig. 6). The experimental outcome confirms the general results of spray drying [34]. It can thus be concluded that the paclitaxel formulated in all types of microspheres existed in an amorphous or disordered-crystalline drug phase of a molecular dispersion or a solid solution state in the polymer matrix during short period after fabrication. In other words, the fabrication process and the various emulsifiers applied in the present work did not change the physical state of the drug formulated in the microspheres.

3.4. Zeta potential analysis

Almost all particulate or microscopic materials in contact with a liquid acquire an electronic charge on their surfaces. Zeta potential is an important and useful indicator of this charge, which can be used to predict and control the stability of colloidal suspensions or emulsions [35]. Zeta potential is the difference in electrical potential between a tightly bound layer of ions on particle surfaces and the bulk liquid in which the particles are suspended. It can be quantified by tracking the charged particles when they migrate in a voltage field, as is done in a zeta potential analyzer.

The greater the zeta potential, the more likely the suspension is to be stable because the charged particles repel one another and thus overcome the natural tendency to aggregate. As a result, higher value of zeta potential implies more stable suspension, and lower value indicates colloid instability, which could lead to aggregation. Zeta potential

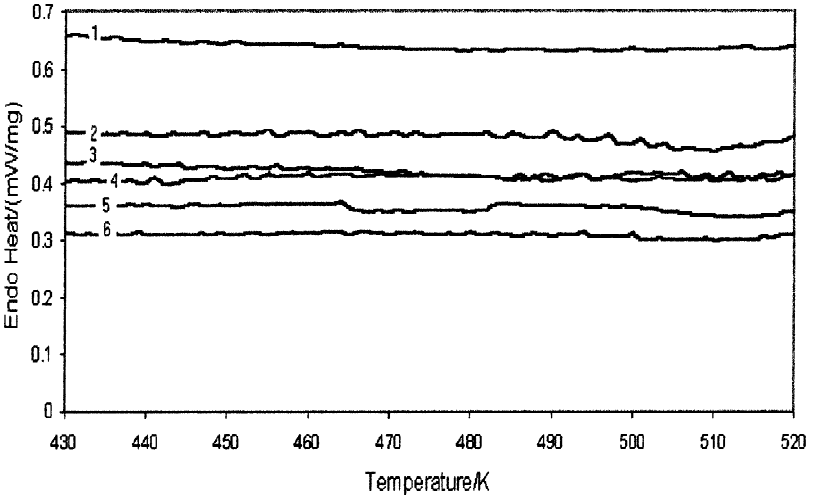

Fig. 6. DSC thermograms of (1) Paclitaxel loaded microspheres with high ratio of DPPC; (2) Paclitaxel loaded microspheres with Gelatin; (3) Paclitaxel loaded microspheres with PVA; (4) Paclitaxel loaded microspheres without any emulsifier; (5) Paclitaxel loaded microspheres with high ratio of cholesterol; (6) Paclitaxel loaded microspheres with low ratio of cholesterol (For all samples, the paclitaxel loading ratio is 10%).

changes with salt concentration, pH, surfactant concentration, etc. The measurement of zeta potential is often the key to understanding dispersion and aggregation processes such as diverse water purification, ceramic slip casting, as well as the formulation of paints, inks, cosmetics and a drug dosage form in pharmaceutics. A successful pharmaceutical suspension whose physical properties affect the patient's response to the product will not cake and will enjoy a long shell life. In the area of drug delivery, zeta potential study can be applied to investigate various systems such as microparticles, nanoparticles, liposomes, micelles, etc. In the present study, the zeta potential values were used as an indication of the stability of the various types of paclitaxel loaded PLGA microspheres. The larger the absolute value of its zeta potential, the more stable the microsphere system should be. The zeta potential of all types of microspheres measured in different pH systems were reported in Table 2 and diagramed in Fig. 7. In most cases the zeta potential was negative, implying negative charge on the surfaces. This may be attributed to the presence of ionized carboxyl groups on the microspheres surface [36]. The absolute value of the zeta potential increases with increasing pH value, and it was largest in the environment of pH 9.18, leading to the greatest stability. This result could be verified by the fact that in the zeta potential measurement it was in this circumstance that a stable value of zeta potential could be reached within the shortest time. Slightly positive values for Sample 3 (paclitaxel loaded microspheres made with PVA) and Sample 7 (paclitaxel loaded microspheres made with high ratio DPPC) were observed at pH 4.00 and pH 6.86. This inversion of zeta potential has been reported for

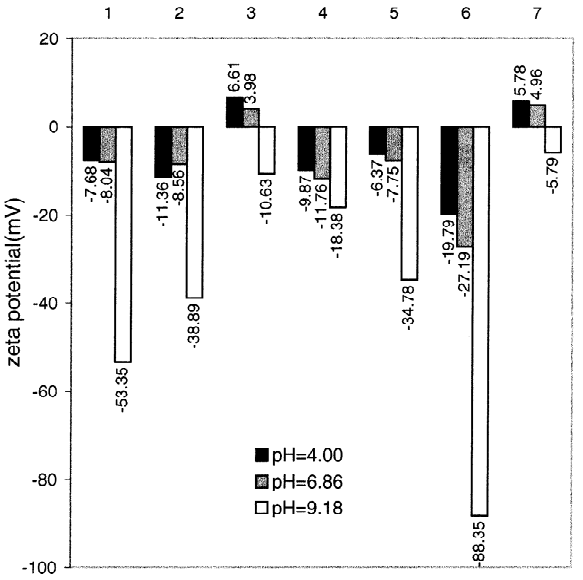

Fig. 7. Zeta potential of all types of PLGA microspheres loaded with paclitaxel. (Sap1) Placebo PLGA microspheres; (Sap2) Paclitaxel loaded microspheres without any excipient; (Sap 3) Pacitaxel loaded microspheres with PVA; (Sap4) Paclitaxel loaded microspheres with low ratio DPPC; (Sap5) Paclitaxel loaded microspheres with gelatin; (Sap6) Paclitaxel loaded microspheres by combining DPPC and cholesterol; (Sap7) Paclitaxel loaded microspheres with high ratio DPPC.

carboxylated polystyrene latex and attributed to a positive charge acquired by hydrogen bonding of hydronium ions to the carboxyl group [36]. By comparing the zeta potential of different types of microspheres systems, it could be seen that Sample 6 (paclitaxel loaded microspheres made with a combination of DPPC and cholesterol) was the most stable system in acid, neutral and basic conditions. In

Table 2
Zeta potential of microspheres with various emulsifiers measured at different pH

Samples	Zeta potential (mV)		
	PH 4.0	PH 7.0	PH 9.0
1. PLGA only	−7.68	−8.04	−53.35
2. PLGA+Paclitaxel (2.5%)	−11.36	−8.56	−38.89
3. PLGA+Paclitaxel (2.0%)+PVA (1.9%)	6.61	3.98	−10.63
4. PLGA+Paclitaxel (2.1%)+DPPC (2.1%)	−9.87	−11.76	−18.38
5. PLGA+Paclitaxel (1.8%)+Gelatin (2.1%)	−6.37	−7.75	−34.78
6. PLGA+Paclitaxel (2.3%) +DPPC (20%)+cholesterol (20%)	−19.79	−27.19	−88.35
7. PLGA+Paclitaxel (2.1%)+DPPC (40%)	5.78	4.96	−5.79

contrast, Sample 3 (paclitaxel loaded microspheres made with PVA) and Sample 7 (paclitaxel loaded microspheres with high ratio DPPC) were most unstable in all three circumstances. On the other hand, with respect to pH dependence, the stability of the microsphere system increased when the pH of environment became higher. Overall, the zeta potential was strongly influenced by the emulsifiers used in the formulations.

3.5. In vitro release

The in vitro release curves of paclitaxel from all types of microspheres were shown in Fig. 8. The drug release from the microspheres formulated with PVA (Sample 3), gelatin (Sample 5), low ratio DPPC (Sample 4), or without any additives (Sample 2) was nearly linear, indicating almost zero-order release kinetics in the first 3 weeks after an initial burst less than 5% in the first day. The release rate then decreased afterwards. This phenomenon reached a consensus with the normal in vitro drug release behaviour of microspheres prepared by spray drying method [25]. The paclitaxel released most rapidly from the microspheres formulated without any additives (sample 2) but the release curve was not steady.

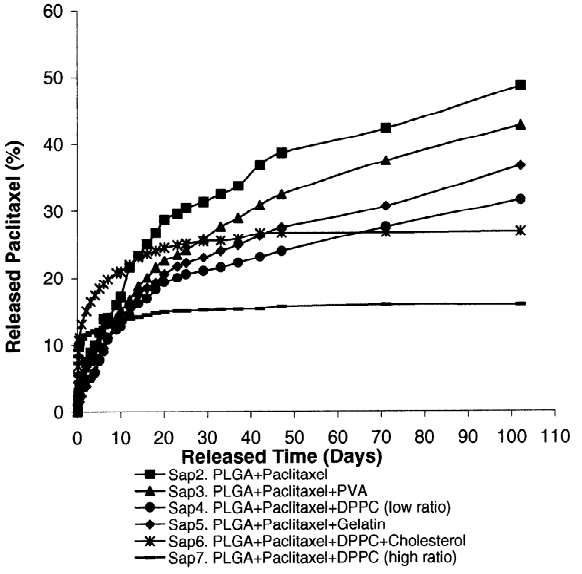

Fig. 8. In vitro release of paclitaxel from all types of microspheres.

The accumulative amount of paclitaxel released in 110 days was about 50% for the microspheres formulated without any additives (sample 2). The second fastest release occurred with the microspheres formulated with PVA (sample 3). The accumulative amount of paclitaxel released in 110 days was about 41%. The third fastest release was from the microspheres formulated with gelatin (sample 5). The accumulative amount of paclitaxel released in 110 days was about 35%. A sustained release came from the microspheres formulated with low ratio DPPC (sample 4). The accumulative amount of paclitaxel released in 110 days was about 30%. The microspheres fabricated with high ratio of DPPC (sample 7) had low release rate and exhibited a large initial burst release. Approximately 12% of paclitaxel was released within 3 days and thereafter the release became very slow. When cholesterol was combined with DPPC as emulsifier (sample 6), the microspheres had a better release curve but still displayed an initial burst. Roughly 25% of the paclitaxel loaded was released within 2 weeks and then the release became slow.

There are three primary mechanisms for the loaded drug to be released from PLGA microspheres: swelling, diffusion and degradation [37]. The release routes of drug are either by diffusion through tortuous paths in the polymer matrix, or by matrix erosion [38]. In the course of release, water is taken up by DL–PLGA microspheres immediately after being exposed to aqueous media [39]. The rate of water uptake depends on the hydrophilicity caused by glycolic acid of the polymer. The microspheres start swelling and allow the encapsulated drug to be released by diffusion through aqueous pores. This release mechanism is much faster than drug diffusion through the intact polymer barrier. The porosity of the spray-dried particles can promote the water penetration into the microsphere so as to increase the release. As shown in SEM of the microspheres after 1 and 2 weeks of in vitro release (Fig. 9), the PLGA microspheres lost their regular surface due to bulk hydrolysis. The initially spherical microspheres became deformed particles with deep surface folds. In contrast to these results, the presence of high ratio lipid in the formulation sensibly slowed down the drug release. This phenomenon should depend on the strong reduction of particle porosity. The release of

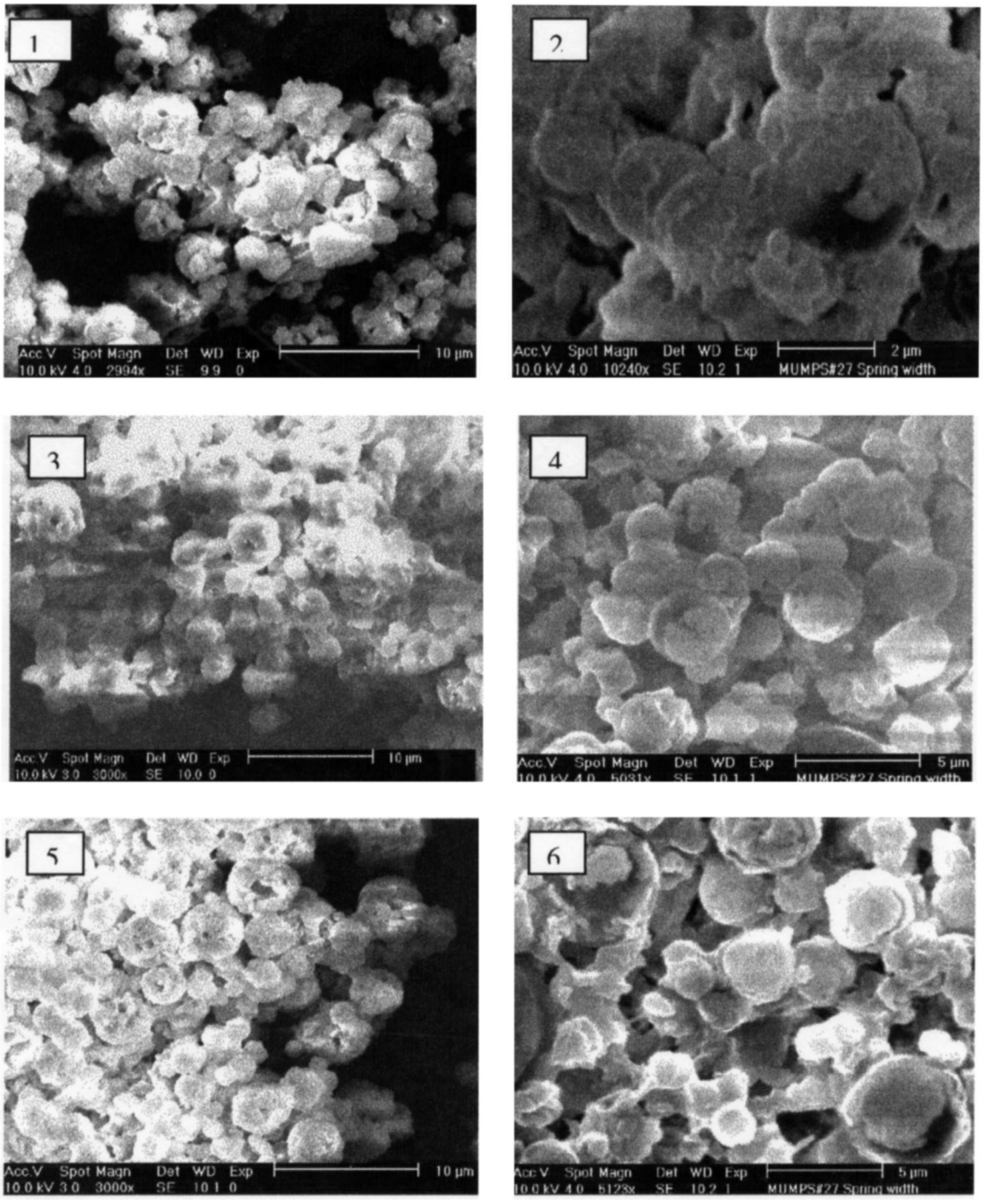

Fig. 9. Scanning electron micrographs (SEM) showing the morphology of the paclitaxel loaded PLGA microspheres during release stage. (1) Sap.3 after 1 week; (2) Sap.3 after 2 weeks; (3) Sap.4 after 1 week; (4) Sap.4 after 2 weeks; (5) Sap.5 after 1 week; (6) Sap.5 after 2 weeks.

the entrapped drug from less-porous biodegradable microparticles was preceded by hydrolysis of the particle matrix and diffusion of degraded chain fragments into the external medium. This evidence also suggested a strong interaction between lipid and drug for both of them were highly hydrophobic. The initial burst may be attributed to the immediate dissolution and release of the portion of the drug located on and near the surface of the microspheres [40].

The main consideration of the controlled release is to achieve a delivery profile that would release a constant amount of the drug over a desired period of time, which can be from several hours to several months, even more than one year, and thus raise the therapeutic index and to achieve convenience in administration. A profound design of biodegradable polymeric microspheres will include choosing an optimal drug loading, an ideal carrier materials and suitable additive as surfactant agent or stabilizer. Furthermore, the controlled release systems should both respond to changes in the biological environment. Moreover, a targeted delivery system should be developed in which the drug is to be directed to the specific cell, tissue, or site. The present study shows that application of an appropriate additive as emulsifier or stabilizer can also be a control factor in the controlled drug delivery formulations.

4. Conclusions

This study confirms that the spray drying technique can be well-adapted to produce fine biodegradable polymeric microspheres to deliver anticancer drug paclitaxel. The technique is easy to scale up, simpler and faster than the solvent evaporation/extraction technique (freeze dry) applied in microsphere fabrication. Application of an appropriate emulsifier or additive in the formulation could contribute to increase the encapsulation efficiency, to control the particle size and size distribution, to preserve the stability of the microsphere system, to keep the physicochemical properties of the drug, to improve its biological activity, and also to make the drug release more constant, sustained and effective. The result shows that low ratio DPPC is a suitable emulsifier to achieve these goals over the high ratio DPPC, cholesterol, PVA, and gelatin. We have investigated the effects of the chain length and chain unsaturation of DPPC on the morphology and release kinetics of paclitaxel loaded nanospheres fabricated by the single emulsion freeze dry technique [41]. It has been shown that lipids with saturated shorter chains have excellent effects. Obviously, with profound investigations including rational fabrication condition, optimal drug loading level, suitable additive of appropriate amount as emulsifier or stabilizer, the innovative drug delivery formulation of paclitaxel loaded microspheres can be achieved to be a effective delivery device to increase the therapeutic benefits and minimize the side effects for chemotherapy of various cancers.

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