

Nanospheres of Biodegradable Polymers: A System for Clinical Administration of an Anticancer Drug Paclitaxel (Taxol®)

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Abstract

Introduction: Paclitaxel is a natural antineoplastic drug. It has been approved by the Food and Drug Administration (FDA) for the treatment of various cancers, especially ovarian and breast cancers with the best effect over other anticancer agents. Due to its high hydrophobicity however, an excipient such as Cremophor EL has to be used, which causes additional serious side effects. This study was intended to investigate the feasibility to apply nanospheres of biodegradable polymers as an alternative system to achieve best results with least side effects. **Materials and Methods:** FDA-approved biodegradable polymers such as poly(D, L-lactic-co-glycolic acid) (PLGA) and poly(D, L-lactic acid) (PLA) were used to fabricate paclitaxel-loaded nanospheres by the solvent extraction/evaporation technique. The nanospheres fabricated in various conditions were characterised by laser light scattering for size and size distribution, scanning electron microscopy (SEM) and atomic force microscopy (AFM) for surface morphology, X-ray photoelectron spectroscopy (XPS) for the chemical structure of the surface. The drug encapsulation efficiency (EE) and the in vitro release kinetics were measured by HPLC. **Results:** The products ranged from 300 nm to 700 nm with low polydispersity. The in vitro drug release can last more than 6 months at an approximately constant rate after an initial burst. The release kinetics can be fully controlled by the material used and the fabrication conditions. **Conclusions:** Nanospheres of biodegradable polymers can be an ideal carrier for paclitaxel to eliminate the side effects caused by the excipient currently used in clinical administration. The technique developed can also be applied to other anticancer drugs.

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Key words: Atomic force microscopy, Chemotherapy, Controlled release, Encapsulation efficiency, Surface property

Introduction

Paclitaxel (Taxol®) is a diterpenoid extracted from the bark of a rare, slow growing Pacific yew or Western yew tree (*Taxus brevifolia*). Its antitumour activity was detected in 1967 in the US NCI (National Cancer Institute) screening of cytotoxic agents from natural products and was then found later to be a novel, promising antineoplastic drug of special effect in clinical application for breast and ovarian cancers.¹ Its therapeutic efficacy also includes head and neck cancer, small cell lung cancer, colon cancer, multiple myeloma, melanoma, and Kaposi's sarcoma. It was approved by the US FDA (Food and Drug Administration) for ovarian cancer in 1992, for advanced breast cancer in 1994 and for early stage breast cancer in October 1999. The action mechanism of paclitaxel has been intensively

investigated and the results suggested that paclitaxel acts to inhibit mitosis in tumour cells by binding to microtubules. Microtubules are made up of tubulins and involved in various cellular functions, e.g. movement, nutrition ingestion, cell shape control, sensory transduction and spindle formation during cell division. Paclitaxel aids polymerisation of tubulin dimers to form microtubules and thus stabilises the microtubules. The microtubules formed due to paclitaxel action are stable and thus dysfunctional, leading to cell death.²⁻⁴

There are two limitations for clinical application of paclitaxel. One is its availability as a natural product. Although extraction of paclitaxel in large scale has increased yields to 0.04%, four trees have to be sacrificed to produce 2 g of the drug for the chemotherapy of 1 patient. This is not

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affordable from an environmental protection viewpoint. Although having been achieved, full synthesis is not practical, as more than 200 steps are needed and the price is thus too high. Although under continuous improvement in the past two decades, the full synthesis is still not commercially practical. An appropriate solution seems to be a semisynthesis, which extracts paclitaxel from needles and twigs of the more abundant English yew trees. Another limitation is its difficulty in clinical administration. Paclitaxel is highly hydrophobic (water insoluble, with water solubility $\leq 0.5 \text{ mg/L}$). An excipient consisting of Cremophor EL (polyoxyethylated castor oil) and dehydrated alcohol has to be used in current clinical administration of paclitaxel,^{5,6} which causes additional side effects. Although still under investigation as to which side-effects are bound to paclitaxel itself and which are caused by the excipient, it is believed that side-effects caused by Cremophor EL include hypersensitivity reactions, nephrotoxicity and neurotoxicity. It was also reported that Cremophor EL has influence on the functions of endothelial and vascular muscle, such as vasodilation, laboured breathing, lethargy, and hypotension. It is thus toxic to myocardium. In application, it causes leaching of diethylhexylphthalate (DEHP) from plasticised polyvinyl chloride (PVC) containers and intravenous infusion line.⁷⁻¹⁰

Due to the limitation of paclitaxel in its clinical administration, its dosage and infusion period have thus been limited.¹¹ The general dose of paclitaxel is 135 or 175 mg/m^2 as a 3- or 24-h infusion every 3 weeks.¹² Best clinical effects have not been achieved yet. Alternative dosage forms are thus necessary to overcome the problem caused by Cremophor EL. Such alternative dosages may include liposomes,¹³⁻¹⁵ mixed micelles,^{16,17} parenteral emulsions,^{18,19} cyclodextrin complexes,²⁰ and nanoparticles.²¹ Among them, some dosage forms can dissolve sufficient quantities of paclitaxel and have shown improved antitumour effects in animal models. However, problems such as the *in vitro* stability of the liposomes and dose-limiting toxicity due to the vehicles have also been noticed.^{18,20}

The present paper investigated the feasibility to apply nanospheres of biodegradable polymers as an alternative administration system of paclitaxel. The use of biodegradable polymeric micro/nanospheres for controlled delivery of anticancer agents has advantages in enhancing therapeutic efficacy and reduces systemic side effects. Certain satisfactory results in clinical trials have been obtained.^{22,23} In addition, it has been reported that nanospheres show significant advantages over microspheres.²⁴⁻²⁶ Microspheres of size less than 1 μm enable intravenous injection (the dimension of capillaries is about 4 μm) as well as intramuscular and subcutaneous administration by minimising possible irritant reactions.^{27,28}

The biodegradable polymers used in this research are poly(D,L-lactic-co-glycolic acid) (PLGA) (lactic/glycolic ratio 50/50 or 75/25) and poly(D,L-lactic acid) (PLA), all FDA approved.^{29,30} The solvent evaporation technique, or the single emulsion technique, which is one of the widely used methods to prepare nanospheres with encapsulation of lipophilic drugs such as paclitaxel, is employed.³¹ The first step in this method is to formulate an emulsion. The oil phase that is formed by dissolving the drug and polymer in an organic solvent is dispersed in the aqueous phase to extract/evaporate the organic solvent. Since the oil phase is easy to aggregate in the aqueous phase, macromolecular emulsifiers, such as gelatin and PVA (polyvinyl alcohol), have to be used due to their high viscosity in the aqueous solution and stronger adsorption around the emulsion drops.^{32,33} To evaluate the quality, structure and properties of the paclitaxel-loaded polymeric nanospheres fabricated under various conditions, characterisation is conducted by various means such as the laser light scattering for size and size distribution, scanning electron microscopy (SEM) and atomic force microscopy (AFM) for surface morphology, X-ray photoelectron spectroscopy (XPS) for the chemical structure of the surface. The drug encapsulation efficiency and the *in vitro* release kinetics are measured by high performance liquid chromatography (HPLC). A 3-month release measurement shows that the drug release can last up to 6 months and even longer period at an approximately constant release rate after an initial burst of 20% to 40% release in the first day. The release kinetics can be fully controlled by the design of polymeric nanospheres.

Application of nanospheres of biodegradable polymers is not confined to clinical administration of paclitaxel. The technique can also be applied for a better administration of other anticancer drugs such as adriamycin,^{32,33} camptothecin,^{34,35} fluorouracil,³⁶ irinotecan,³⁷ and anti-malarial drugs such as halofantrine and lumefantrine.³⁸ Controlled release can then be realised to achieve better clinical effects with less side effects and to simplify the administration.

Materials and Methods

Materials

Paclitaxel (Taxol®) is the product of Dabur India Limited Corporation, India. Polyvinyl alcohol (PVA) (MW = 30,000 to 70,000) was purchased from Sigma Chemical Company (St. Louis, MO). Poly(D, L-lactic-co-glycolic acid) with a molar ratio of 75:25 or 50:50 and poly (lactic acid) were also the products of Sigma Chemical Company.

Methods

(a) *Preparation of Nanospheres:* Certain amount of paclitaxel and 200 mg of PLGA was dissolved in 16 mL of methylene chloride (DCM). The formed solution was

added to 250 mL of distilled water containing the emulsifier. The resulting emulsion was sonicated with an energy output of 50W in a pulsed mode for 90 seconds. The oil-in-water emulsion was stirred overnight at room temperature with the magnetic stirrer to evaporate methylene chloride. The produced nanospheres were collected by centrifugation (12,000 rpm, 30 min) and washed with water 4 times to remove excessive emulsifier. The product was freeze-dried to get fine powder.

(b) Characterisation of Nanospheres: The laser light scattering was done with particle size analyser (90 Plus), which is the product of Brookhaven Instruments Company. Dried nanospheres were coated with gold for 3 minutes before SEM was done with Hitachi S-4100. Atomic force microscopy (AFM) images were obtained from Nanoscope IIIa, Digital Instrument, Santa Barbara, CA, USA. X-ray photoelectron spectroscopy (XPS) was obtained by Axis His (a product from Kratos Analytical Ltd) with magnesium as the node. For all samples, the survey spectrum recorded covered a binding energy range of 0 to 1200 eV using the pass energy of 80 eV. Curve fitting was performed using the software supplied by the manufacturer.

(c) In Vitro Release Test: Typically, 10 mg of nanospheres was dispersed in phosphorus buffer solution (PBS), in which the pH was maintained at 7.4. The buffer solution was kept in an orbital shaker that was vibrated at a constant rate of 166 rpm and the temperature was kept constant at 37.2°C. At given time intervals, 3 tubes of each formula of nanospheres were withdrawn and centrifuged at 12,000 rpm for 5 minutes. The precipitated nanospheres were taken and re-suspended in 10 mL of fresh release medium and placed back in the shaker, while the supernatant solution was kept for HPLC analysis. In order to examine the paclitaxel content in the supernatant solution, the following procedures were adopted. Paclitaxel in the release medium firstly extracted with 1 mL of DCM was reconstituted in 1 mL of mobile phase (acetonitrile: water = 1:1); thereafter, DCM was evaporated under a stream of nitrogen. For HPLC analysis, the C-18 column was used and the mobile phase was delivered at a rate of 1 mL/min. 100 µL of sample was injected with an auto-sampler and the column effluent was detected at 227 nm with an ultraviolet detector.

(d) Encapsulation Efficiency Measurement: About 3 mg of paclitaxel-loaded nanospheres was dissolved in 1 mL DCM and 9 mL of mobile phase was added to the solution subsequently. A nitrogen stream was introduced to evaporate DCM at room temperature until a clear solution was obtained. The resulted solution was analysed by HPLC.

(e) Extraction Factor and Recovery Efficiency Measurement: Due to inefficient extraction, the extraction factor had to be analysed for calibration. The procedure

was as follows:³⁹ paclitaxel of known concentration in aqueous solutions (varying from 0.05 to 0.5 mg/L) was extracted and its concentration was investigated by HPLC. The results showed that the extracted solution contained 16% of the original amount of the paclitaxel. Therefore, the *in vitro* release result determined by this method was corrected by 16% due to inefficient extraction.

Similarly, known amount of paclitaxel in DCM was subjected to the procedure mentioned before. The result showed that 78% paclitaxel remained after these procedures. Consequently, 78% was used to correct the encapsulation efficiency got from the method mentioned above.

Results and Discussion

(a) Surface Morphology of Nanospheres: The morphology of nanospheres of various polymers fabricated under various conditions was examined by SEM and the result is shown in Figure 1. From this image, the nanospheres appeared to be round in shape and the surface was smooth. The size of nanospheres ranged from 300 nm to 1 µm, while the most frequent size of nanospheres was about 700 nm. Nevertheless, the prepared nanospheres were too small for SEM to investigate closely due to its limited magnification. Therefore, atomic force microscopy (AFM) was employed in our research to study the detailed morphology of nanospheres. The advantage of this approach is that it can reveal the structure with very high resolution, as shown in Figure 2. Figure 2a gives an overview of a typical nanosphere with the diameter of circa 500 nm and its surface seemed to be rather smooth. However, this was to some extent misleading because a certain degree of roughness of the superficies was noticeable in a closer investigation of its coronal as shown in Figure 2b. Further investigation by AFM shows the existence of complex topography of nanospheres, which contain some micro-caves on the surface rather than simple smooth morphology (Fig. 3). These are the evidence of diffusion release mechanism.

(b) Surface Chemistry of Nanospheres: X-ray photoelectron spectroscopy (XPS) was employed in order to analyse the surface chemistry of nanospheres. The details have to be neglected due to the space limitation. Interested readers can refer to our other publications.^{40,41}

(c) Effect of the Emulsifier Concentration on the Size and Encapsulation Efficiency: Two batches of nanospheres with different amount of PVA were prepared. Their size distribution characterised by the laser light scattering and encapsulation efficiencies (EE) measured by HPLC were shown in Table I and Figure 4.

Obviously, both Table II and Figure 4 illustrate that higher concentration of emulsifier leads to a reduced size of nanospheres. This can be expected from the function of emulsifier as stabiliser of the emulsion to form nanospheres.

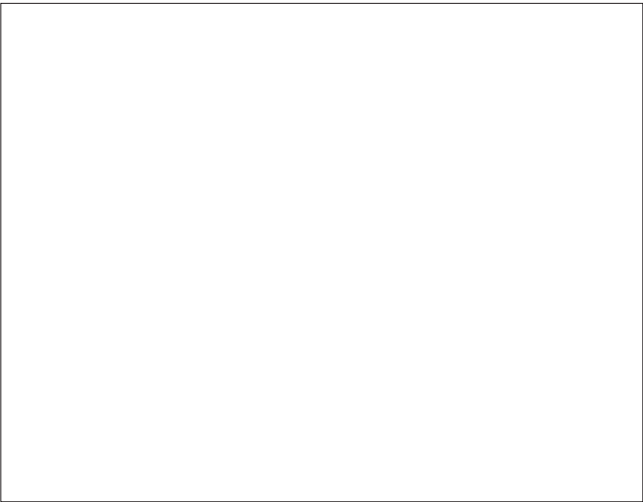


Fig. 1. Morphology of poly (lactic-co-glycolic acid) (PLGA)/paclitaxel nanospheres characterised by scanning electron microscopy.



Fig. 3. A high resolution AFM image (250 nm x 250 nm) shows a porous structure on the nanosphere's surface, which could be an evidence for the diffusion mechanism of the paclitaxel release from the nanosphere (the unit is μm).

It is easy to understand that insufficient emulsifier will fail to stabilise all the nanospheres and thus some nanospheres tend to aggregate with each other. As a result, nanospheres with larger sizes are produced.

The amount of emulsifier plays a fundamental role not only in the size of fabricated nanospheres, but also in the encapsulation efficiency as well. The outcome of this study indicated that higher amount of PVA will result in reduced encapsulation efficiency of nanospheres, which can possibly be explained by two facts.

First, excessive emulsifier may cause the loss of drugs in the fabrication process. The reason goes as follows: if the amount of emulsifier used is more than the critical level to coat all the surfaces, the excessive emulsifier might interact with the available free drugs during nanosphere formation. Consequently, some drugs will leak into the excessive PVA rather than PLGA phase.



Fig. 2. Topography (height analysis) of poly (lactic-co-glycolic) (PLGA)/paclitaxel nanospheres characterised by atomic force microscopy.

TABLE I: THE ENCAPSULATION EFFICIENCY AND SIZE OF NANOSPHERES PREPARED BY DIFFERENT EMULSIFIERS

Sample code	Polymer	Amount of PVA (wt%)	Effective diameter (nm)	EE (%)
A	PLGA (50/50)	4	1298.1 $\pm$ 61.4	19.0
B	PLGA (75/25)	4	801.0 $\pm$ 3 8.0	22.9
C	PLA	4	631.1 $\pm$ 4.6	16.0
D	PLGA (75/25)	2	973.5 $\pm$ 41.0	40.2

EE: encapsulation efficiency

Second, larger size will yield higher encapsulation efficiency. It has been reported that the larger the diameter of the nanospheres prepared, the higher their encapsulation efficiency.^{28,42} Such a phenomenon can be explained by the fact that the increase of diameter causes the decrease of the total surface area per unit volume, which consequently reduces the possibility of drug loss by diffusion towards the suspending medium during the fabrication procedure. This explanation can also be crucial to understand that encapsulation efficiency of nanospheres is usually lower than that of microspheres.⁴³

It can thus be concluded that the presence of an optimum value of emulsifier for encapsulation efficiency is justifiable. This finding is not only applicable to nanospheres, but also

TABLE II: SELECTED PROPERTIES OF THE THREE AMORPHOUS PLA/PLGA POLYMERS

Polymer	MW (g/mol)	Tg (°C)	Estimated degradation time (months)
Poly (DL-Lactide)	90,000 ~ 120,000	55 to 60	12 ~ 16
Poly 75:25 (DL-lactide-co-glycolide)	90,000 ~ 126,000	50 to 55	4 ~ 5
Poly 50:50 (DL-lactide-co-glycolide)	40,000 ~ 75,000	45 to 50	1 ~ 2

MW: molecular weight

Tg: glass transition temperature

Fig. 4. Effect of emulsifier concentration on log-normalised size distribution of nanospheres (sample B and D).

Fig. 5 Effect of polymer property on log-normalised size distribution of nanospheres (sample A, B and C).

and their effects on the size and encapsulation efficiency of the fabricated nanospheres are summarised in Table I and Figure 5. It happened that although the molar ratio and molecular weight of polymers have certain effects on the encapsulation efficiency, the effects, however, were not significant among the polymers used in this research. The explanation will be given below. On the other hand, the results suggested that higher content of lactic acid would lead to a reduced effective size of nanospheres, which might be related with the viscosity of the polymer.

Figure 6 shows the *in vitro* release profiles of nanospheres made up of different polymers. Since the degradation time of polymers is usually more than 2 months, its effect on the release in the first 20 days is less significant in comparison with other factors. Therefore, the dominant release mechanism during this period would be diffusion. By juxtaposing the release profiles of PLGA (75/25) and PLA, it is not surprising to find that increase of lactic acid component tends to accelerate the release rate of nanospheres. This suggests that increment of L/G ratio will lead to a higher diffusion rate of paclitaxel within the polymeric matrix. This may be explained by the increased hydrophilicity⁴⁶ and free volume^{47,48} caused by the side methyl chain, which incline to produce accelerated diffusivity and mobility.⁴⁷

Fig. 6. In vitro release of poly (lactic-co-glycolic acid) (PLGA) and poly (lactic acid) (PLA) nanospheres.

to microspheres.^{44,45}

(d) *Effects of Polymer Composition on the Size, Encapsulation Efficiency and In Vitro Release:* In general, the type and composition of biodegradable polymers play a significant role in determining the properties and thus the release mechanism and kinetics of the fabricated nanospheres. One of the objectives of this research is to identify and evaluate the effects of polymer composition on the resulting nanospheres. The properties of different polymers investigated in this study are listed in Table II,

In addition, it is believed that the molecular weight of polymers also plays a fundamental role in the resulted release profile. In general, low molecular weight leads to a high diffusion coefficient of the drug molecule within the polymeric matrix. Therefore, although PLGA (50/50) was expected to have a lower release rate because of the lower ratio of L/G than PLGA (75/25), its lower molecular weight tends to produce a quicker release. Consequently, these two effects counteract each other, and the resulted release rate of PLGA (50/50) was almost equal to that of PLA in the first 20 days of release.

Besides, a greater release profile of PLGA (50/50) was observed 20 days later since its degradation began to become dominant and its degradation rate is larger than that of PLA.

Conclusions

PLGA nanospheres loaded with paclitaxel were firstly prepared with solvent extraction/evaporation technique. Their size and size distribution and topography have been characterised by the laser light scattering, SEM as well as AFM for the first time. Also, the effects of emulsifier (PVA) on the size and encapsulation efficiency have been investigated and the results indicate the existence of an optimum amount regarding to the encapsulation efficiency. Effects of L/G ratio and molecular weight of PLGA on the release profile have been studied, which suggest that high content of lactic acid and low molecular weight can result in an increased release rate of paclitaxel. Therefore, the release kinetics of fabricated nanospheres can be fully controlled.

It should be pointed out that to evaluate the clinical effects of biodegradable polymeric nanospheres in administration of paclitaxel, further *in vivo* studies would be crucial and the relation between the *in vitro* and *in vivo* release has been a problem in the controlled release area.

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