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Research Paper

# Preparation and pharmacokinetics of docetaxel based on nanostructured lipid carriers

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## Abstract

**Objectives** This study describes the preparation and pharmacokinetics of docetaxel based on freeze-dried nanostructured lipid carriers (NLCs).

**Methods** The docetaxel-incorporated NLCs were developed using hot high-pressure homogenisation, and lyophilised to obtain freeze-dried docetaxel NLCs. The influences of different concentrations of lipid matrices, ratio of drug to lipid, and different cryoprotectants on the characteristics of the NLCs were investigated.

**Key findings** Freeze-dried docetaxel NLCs were spherical, with 5% (w/w) docetaxel loading efficiency and were stable for at least 6 months at 25ºC. X-ray powder diffraction and differential scanning calorimetry analysis suggested that docetaxel was distributed in a molecular or amorphous status. In-vitro release studies showed sustained drug release, with the cumulated release rate of 13% within 24 h without burst release. The freeze-dried docetaxel NLCs also showed sustained-release properties after intravenous injection into rats. The area under the plasma–concentration time curve and mean residence time were increased 4.90 and 2.82 times compared with docetaxel solution. The concentration of docetaxel in the lungs was significantly higher in rats treated with the NLCs than in those given docetaxel solution.

**Conclusions** Docetaxel NLCs have an organ-targeting effect and prolonged mean retention time and have potential for the treatment of lung cancer.

**Keywords** docetaxel; nanostructured lipid carriers; hot high-pressure homogenisation; X-ray powder diffraction; pharmacokinetics; tissue distribution

## Introduction

Docetaxel is an antineoplastic agent belonging to the taxoid family. It disrupts the microtubular network in cells, inhibiting mitosis. Docetaxel is an effective medicine not only for breast cancer, non-small cell lung cancer and prostate cancer, but also for ovarian cancer. However, to improve its poor water solubility, docetaxel injection contains Tween-80 and 13% solution of ethanol as solubilisers. It frequently causes severe hypersensitive responses. A suitable system of docetaxel is therefore needed for intravenous administration.[1,2]

Solid lipid nanoparticles (SLNs) represent a colloidal carrier system for controlled drug delivery, consisting of a biocompatible lipid as the core and amphiphilic surfactant as the outer shell, and have been found to be suitable for lipophilic drugs. SLNs have been attracting researchers all over the world since the beginning of the 1990s because of the favourable biocompatibility, site-specific targeting, excellent physical stability, protection of encapsulated labile drugs from degradation and controlled drug release.[3–5] However, certain disadvantages of SLNs that need to be taken into account are limited drug-loading capacity and drug expulsion during storage.[6] These limitations can be circumvented by using nanostructured lipid carriers (NLCs).

NLCs are developed by mixing solid lipids with certain spatially incompatible liquid lipids, leading to the formation of special structures of the lipid matrix of three types: the imperfect structured type, the structureless type and the multiple type. These features improve drug-loading capacity and avoid or minimise drug expulsion.[6,7]

High-pressure homogenisation (HPH) is the main method used to prepare NLCs at elevated temperature (hot HPH technique) or at or below room temperature (cold HPH technique), and is suitable for large-scale industrial production. The particle size is decreased by cavitation and turbulences. In this work, NLCs prepared by hot HPH were

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stored in a lyophilised state, which was expected to improve the stability and shelf-life of the drug carriers. The aim of this work was to evaluate the potential of NLCs as drug carriers for docetaxel, through the study of preparation procedure, characterisation and stability, as well as the pharmacokinetics and tissue distribution of freeze-dried docetaxel NLCs after intravenous injection into rats.

## Materials and Methods

### Materials

Docetaxel was provided by Ningbo Chemical Factory (Ningbo, China). Palmic acid was purchased from Shanghai Shun Qiang Biological Technology Company (Shanghai, China). Octadecanoic acid was purchased from the Shanghai Chemical Agent Company of the China Medicine Clique (Shanghai, China). Glycerol monostearate was purchased from the Changsha Organic Reagent Factory (Changsha, China). Compritol 888 (ATO) was supplied by Gattefossé (St-Priest, France). Poloxamer 188 was provided by BASF (Schwarzheide, Germany). Long-chain triglyceride and Miglyol 812N (MCT) were obtained from Sasol (Hamburg, Germany). Soybean lecithin was purchased from Shanghai Taiwei Pharmceutical Industry (Shanghai, China). Tween-80 was purchased from Lvshun Chemical Industry Works (Lvshun, China) and Brij 78 was from Sigma (St Louis, MO, USA). All other chemicals were of analytical grade.

### Animals

Male Wistar rats (200 ± 20 g) were from the Shenyang Pharmaceutical University Animal Institution. They were housed under standard conditions and were given a commercial diet and water *ad libitum*.

All animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the National Research Council. The protocol was approved by the local animal ethics committee.

### Preparation of docetaxel NLCs

NLCs were prepared by hot HPH. Briefly, a mixture of lipid (ATO : MCT, 5 : 4 w/w) and docetaxel were melted as the oil phase. Under mechanical stirring at 20 000 rpm, the aqueous surfactant solution consisting of soybean lecithin/Brij 78 (1 : 3, w/w) was quickly dispersed into the oil phase at the same temperature. The resultant pre-emulsion was homogenised at 800 rpm using an NS1001L (Niro-Soavi, Parma, Italy). After five homogenisation cycles the nanoemulsion obtained was cooled to room temperature and the lipid recrystallised, forming NLCs.

Appropriate amounts of cryoprotectants (5%, w/v) were dissolved in the NLC dispersion. The samples were frozen at −85ºC for 8 h before being lyophilised using an Eyela FDU-1100 (Prkakikai, Tokyo, Japan). The drying time was 36 h. The freeze-dried NLC powders were collected for later experiments after rehydration.

### Transmission electron microscopy (TEM)

NLCs were viewed under a JEM-1200 EX electron microscope (JEOL, Tokyo, Japan) by conventional negative staining methods using 0.3% phosphotungstic acid buffer (pH 6.0).

### Particle size distribution and zeta potential

An aliquot of freeze-dried docetaxel NLCs was reconstituted in deionised water. The particle size and zeta potential of docetaxel NLCs were measured by dynamic light scattering and electrophoretic light scattering, using an LS 230 Coulter counter (Beckmann Coulter, Krefeld, Germany) and a zeta potential analyser (Delsa 440 SX, Beckman Coulter), respectively.

### Entrapment efficiency

The drug entrapment efficiency (EE%) was determined by ultrafiltration as follows: 5 ml drug-loaded nanoparticles were placed in a stirred cell (Millipore 8010, Millipore Corporation, Bedford, OH, USA) which was fitted with a filter membrane (molecular weight cut-off: 50 000) under nitrogen. The ultrafiltrate was collected and the drug content in the ultrafiltrate determined by HPLC using a Diamonsil $C_{18}$ (5 $\mu$m, 250 mm × 4.6 mm) (Dikma, Tianjin, China) analytical column.[8] The injection volume was 20 $\mu$l and docetaxel was detected at 228 nm. The mobile phase consisted of acetonitrile and water (53 : 47 v/v) delivered at a flow rate of 1.0 ml/min. A 1 ml sample of nanoparticle suspension was diluted with absolute ethanol to determine the total docetaxel by HPLC. The drug loading capacity (L%) was determined from the equation: $((W_1 - W_2)/(W_1 - W_2 + W_3)) \times 100$ where $W_1$, $W_2$ and $W_3$ were the weight of docetaxel added to the system, weight of docetaxel in the ultrafiltrate and weight of lipid added to the system, respectively. EE% was calculated from the equation: $EE\% = ((W_1 - W_2)/W_1) \times 100$.

### X-ray powder diffraction and differential scanning calorimetry

Measurements were carried out on the following samples: docetaxel, a physical mixture of drug and lipids created by mechanical vorticisation, blank freeze-dried NLCs and freeze-dried docetaxel NLCs.

X-ray diffraction equipment (D/Max-2500Pc, Rigaku, Osaka, Japan) was used to investigate the crystal change at 50 kV, 300 mV for Cu K$\alpha$; the filter was a graphite monochromator.

Differential scanning calorimetry (DSC) was performed using a DSC-60 differential scanning calorimeter (Shimadzu, Kyoto, Japan). The scan was performed at 10ºC/min across 10–300ºC under nitrogen and cooled to 25ºC; an empty aluminium pan was used as reference.

### Stability studies

Freeze-dried docetaxel NLCs were stored at room temperature in a desiccator for 6 months. Changes in drug content, particle size, encapsulation efficiency[7] and cumulative release rate were measured at predetermined time intervals. Changes in the appearance of the dried powder were also included in stability studies.

### In-vitro release

The drug-release profile from NLCs was studied using an in-vitro dialysis method. Briefly, an aliquot of reconstituted freeze-dried docetaxel NLCs were diluted 50-fold with phosphate buffer (pH 7.4, 3% Tween 80, v/v) and incubated in buffer at 37ºC under magnetic stirring in a dialysis bag (molecular weight cut-off: 7000). Samples of 1 ml were withdrawn from the release medium and replaced with an equal volume of buffer at each sampling time. The amount of docetaxel was determined by HPLC. All the operations were carried out in triplicate.

### Pharmacokinetics and tissue distribution

For the in-vivo study, 12 male Wistar rats were fasted overnight and randomly divided into two groups of six. Docetaxel solution as reference and reconstituted freeze-dried docetaxel NLCs (10 mg/kg) were injected intravenously. Blood samples were collected from the orbital plexus at predetermined time points into heparinised tubes and then centrifuged at 5000 rpm for 5 min to separate the plasma.

To analyse plasma samples, paclitaxel (10 $\mu$l of a 10 $\mu$g/ml methanol solution) was added as internal standard to 100 $\mu$l plasma and the mixture extracted with ether before being centrifuged. Docetaxel in the supernatant was dried under nitrogen and redissolved in methanol before measurement by the LC-10AT HPLC (Shimadzu) as described above. The concentration of docetaxel in each sample was determined from a calibration curve.

For the tissue distribution studies, 18 male Wistar rats were fasted overnight and randomly divided into two groups of nine. Docetaxel solution for reference and reconstituted freeze-dried docetaxel NLCs (10 mg/kg) were injected intravenously. Another three animals were sacrificed without treatment and their tissues used as blank controls and for preparation of control spiked samples.

Animals were decapitated in groups of three at 7, 120 and 240 min and the heart, liver, spleen, lungs, kidneys and brain removed, washed of residual blood, air dried and weighed. Tissue samples were homogenised with saline to 0.2 mg/ml, then 10 $\mu$l methanol solution containing paclitaxel 10 $\mu$g/ml as an internal standard was added. Tissue samples were vortexed for 3 min and then centrifuged. The supernatants were collected and analysed by HPLC as described above.

### Statistical analysis

Statistical analyses of the effects of increasing concentrations of lipids (2.0, 3.0, 5.0, 8.0, 10.0% w/w), and drug : lipid ratio (1 : 21, 1 : 19, 1 : 17, 1 : 15, 1 : 13 w/w) on mean particle size and EE% of the various formulations were performed using the Kruskal–Wallis test. Storage stability of freeze-dried doctaxel NLCs at 25ºC (change in content, mean particle size, EE% and cumulative release rate each month) was also evaluated by the Kruskal–Wallis test. In all cases, post-hoc comparisons of the means of individual groups were performed using the least significant difference test.

Serum drug concentration versus time data for docetaxel in individual rats were analysed by non-compartmental estimations using 3p87 software (Chinese Society of Mathematical Pharmacology, Beijing, China). Serum and tissue concentrations of docetaxel obtained from rats were pooled to provide mean concentrations. Statistical analysis of the pharmacokinetic parameters (clearance, mean residence time from time 0 extrapolated to infinity (MRT) and area under the plasma–concentration time curve from time 0 extrapolated to infinity (AUC)) for docetaxel solution and freeze-dried docetaxel NLCs were carried out using the Mann–Whitney *U* test. Differences in tissue drug concentration at different time points were estimated by an independent-samples *t*-test. A significance level of $P < 0.05$ denoted significance in all cases. Statistical analysis was performed using SPSS (v. 17.0).

## Results

### Preparation of docetaxel NLCs

With hot HPH, the water phase containing emulsifiers was added dropwise to the melted lipid matrix at 80ºC and then cooled to room temperature to obtain the NLCs.

On the basis of preliminary experiments, the amount of lipid was the major factor affecting the EE% of NLCs prepared by hot HPH. The encapsulation efficiency of formulations with different lipid matrix contents (2.0, 3.0, 5.0, 8.0 and 10.0% w/v) are shown in Table 1. Aggregation and sedimentation occurred at a lipid content of 10.0%. The Kruskal–Wallis analysis indicated significant differences in both mean particle size and EE% with different lipid matrix contents. Post-hoc comparisons showed that there were no significant differences in mean particle size between the 2.0%, 3.0% and 5.0% lipid groups, while there was significant difference between the 5.0% and 10.0% groups. There were no significant differences in EE% between the 2.0%, 3.0% and 5.0% groups but there were significant differences between the 5.0%, 8% and 10.0% lipid groups.

Table 2 shows that the loading efficiency increased with the increasing ratio of drug to lipids; the optimal encapsulation efficiency reached was 98.1%, at the ratio of 1: 19, and fell when the ratio was higher than 1: 15. The Kruskal–Wallis analysis indicated significant differences in both mean particle size and EE% at the different drug : lipid ratios (1 : 21, 1 : 19, 1 : 17, 1 : 15 and 1 : 13 groups). Post-hoc comparisons showed no significant differences in mean particle size between 1 : 21, 1 : 19 and 1 : 17 groups but there were significant differences between 1 : 17, 1 : 15 and 1 : 13 groups. There were no significant differences in EE% between 1 : 21, 1 : 19 and

**Table 1** Effects of lipid concentration on mean particle size, drug entrapment efficiency and stability of nanostructured lipid carriers

| Lipid concentration (%) | Mean particle size (nm) | EE% | Stability[a] |
|---|---|---|---|
| 2.0 | 141.2 ± 12.7 | 85.1 ± 1.2 | – |
| 3.0 | 146.3 ± 19.4 | 92.5 ± 1.3 | ++ |
| 5.0 | 156.8 ± 9.7 | 98.3 ± 1.5 | ++ |
| 8.0 | 173.6 ± 11.2 | 99.0 ± 1.1 | + |
| 10.0 | 184.4 ± 16.7 | 98.9 ± 0.8 | – |

[a]Stability definitions: ++ unchanged within 30 days; + precipitation appeared within 10 days; – precipitation or gelatination appeared within 2 days. EE%, entrapment efficiency. Values are mean ± SD ($n$ = 3).

**Table 2** Effects of the concentration of docetaxel on mean particle size, drug entrapment efficiency and stability of nanostructured lipid carriers

| Drug : lipid ratio (w/w) | Mean particle size (nm) | EE% | Stability[a] |
|---|---|---|---|
| 1 : 21 | 145.6 ± 20.1 | 98.3 ± 1.2 | ++ |
| 1 : 19 | 155.2 ± 11.8 | 98.1 ± 1.3 | ++ |
| 1 : 17 | 163.9 ± 14.7 | 98.2 ± 0.9 | + |
| 1 : 15 | 193.9 ± 12.6 | 95.8 ± 1.5 | – |
| 1 : 13 | 214.3 ± 17.4 | 91.9 ± 1.6 | – |

[a]Stability definitions: ++ unchanged within 30 days; + precipitation appeared within 10 days; – precipitation or gelatination appeared within 2 days. EE%, entrapment efficiency. Values are mean ± SD ($n$ = 3).

1 : 17 groups but there was significant difference between the 1 : 17 and 1 : 13 groups.

## Investigation of cryoprotectants

The appearances, morphology and stability of the lyophilised nanoparticles were determined after rehydration with lactose, sucrose, trehalose, dextran and mannitol (5% w/v).

In terms of the protective effect of lyoprotectants (lactose, sucrose, trehalose and dextran), the size and encapsulation efficiency of freeze-dried docetaxel NLCs were similar to these of docetaxel NLCs. In particular, the dried product obtained with trehalose was satisfactory in appearance and was easy to reconstitute with unchanged and uniform size distribution. As far as mannitol was concerned, the lyophilised product was difficult to rehydrate but possessed smooth and bright appearance.

## Characterisation

### *Morphology analysis*

The morphological characterisation of docetaxel NLCs and freeze-dried docetaxel NLCs is shown in Figure 1. The nanoparticles had a spherical or ellipsoid shape.

### *Characteristics of NLCs before and after lyophilisation*

The EE% of docetaxel NLC suspension and lyophilised powder was 98.3% and 97.8%, respectively, while the zeta potential was −43.6 mV and −38.8 mV, respectively, which indicated that the encapsulation efficiency and zeta potential of docetaxel NLCs changed little after lyophilisation. However, the unimodal particle size was larger after the lyophilisation process, increasing from 156 nm to 245 nm.

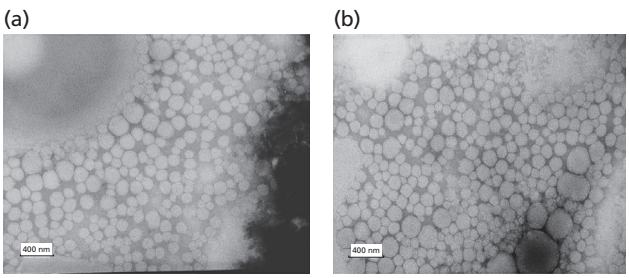


**Figure 1** Transmission electron microscopy images of nanostructured lipid carriers (a) before and (b) after lyophilisation

### *X-ray diffraction analysis*

X-ray diffraction was used to investigate the crystallisation forms. Typical diffractograms of docetaxel, physical mixture of drug and lipids, empty freeze-dried NLCs and freeze-dried docetaxel NLCs are shown in Figure 2. Docetaxel and lipid matrix had characteristic diffraction peaks. The physical mixture of docetaxel and lipid matrix possessed all the major diffraction peaks of docetaxel and Compritol 888 ATO. For the diffractograms of freeze-dried docetaxel NLCs, the diffraction peaks of docetaxel almost disappeared while the diffraction peaks of lipid matrix also decreased significantly.

### *Differential scanning calorimetry characterisation*

The DSC curves of docetaxel, physical mixture, empty freeze-dried NLCs and freeze-dried docetaxel NLCs illustrated that docetaxel exhibits a strong peak at 225ºC; the physical mixture showed three major peaks at 175, 225 and 251ºC; empty freeze-dried NLCs showed a blunted peak at 251ºC; freeze-dried docetaxel NLCs showed a decrease in the main peak at 251ºC compared with empty NLCs and no peaks around 225ºC.

### *Stability of freeze-dried docetaxel NLCs*

Because of the instability of the NLC dispersion, freeze-dried NLCs were used to improve the storage stability. No significant changes in drug content, particle size, EE% and culmulative release rate were observed for NLCs after rehydration during the long-term stability study. The dried products had a smooth, full and compact appearance and could be rapidly reconstituted (Table 3). The mean particle

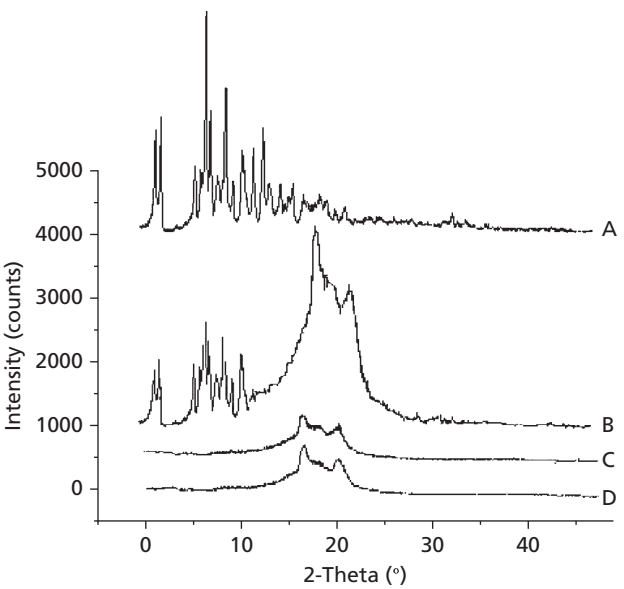


**Figure 2** X-ray diffraction analysis of docetaxel formulations. X-ray powder diffractograms of (A) docetaxel; (B) physical mixture of drug and lipids; (C) empty freeze-dried nanostructured lipid carriers (NLCs) and (D) freeze-dried docetaxel NLCs.

**Table 3** Storage stability of freeze-dried docetaxel nanostructured lipid carriers at 25°C

| Time (month) | Appearance | Content (%) | Mean particle size (nm) | EE% | Cumulative release rate (%) |
|---|---|---|---|---|---|
| 0 | smooth, compact, white | 100.1 ± 1.0 | 245.3 ± 13.6 | 97.8 ± 1.5 | 12.9 ± 1.3 |
| 1 | smooth, compact, white | 99.9 ± 1.1 | 253.7 ± 26.8 | 97.6 ± 1.4 | 12.0 ± 1.1 |
| 2 | smooth, compact, white | 99.8 ± 0.9 | 256.9 ± 12.2 | 97.7 ± 1.1 | 13.0 ± 1.1 |
| 3 | smooth, a little loose, white | 99.7 ± 1.2 | 261.6 ± 16.6 | 97.8 ± 1.6 | 13.1 ± 1.0 |
| 6 | smooth, a little loose, white | 99.7 ± 1.3 | 259.7 ± 19.4 | 97.6 ± 1.3 | 13.0 ± 0.9 |

Drug contents are given as a percentage of the initial concentration measured at time zero; values are means ± SD ($n$ = 3).

size of NLCs after 6 months' storage ranged from 245.3 ± 13.6 nm to 259.7 ± 19.4 nm. The percentage of remaining docetaxel after 6 months was 99.7%. The Kruskal–Wallis analysis showed no significant difference in content, mean particle size, EE% and cumulative release rate at 0, 1, 2, 3 and 6 months.

### *In-vitro release study*

The in-vitro release profile of freeze-dried docetaxel NLCs was investigated in phosphate buffer (pH 7.4). For up to 24 h, there was no obvious burst release of drug, and the prolonged release phenomenon was similar to that of docetaxel NLCs. In the hot HPH process, drug dissolved initially in the lipid matrix. The in-vitro release profile of docetaxel from NLCs suggested that about 13% of docetaxel was released within 24 h, exhibiting a good prolonged-release profile (Figure 3).

### *Pharmacokinetics and tissue distribution*

The effects of NLCs on the pharmacokinetic profile of docetaxel were studied. Figure 4 shows plasma concentration–time curves of docetaxel after intravenous administration of two formulations. At all time points docetaxel serum concentrations were higher for the NLCs than with the solution. After the injection of reconstituted freeze-dried docetaxel NLCs, docetaxel was still detectable after 12 h whereas with docetaxel solution the minimum concentration was reached after 4 h. The mean measured peak serum concentration achieved was 25.2 $\mu$g/ml for docetaxel solution and about 44.9 $\mu$g/ml for docetaxel NLCs. After intravenous administration of docetaxel solution, free docetaxel is rapidly distributed compared with distribution of docetaxel incorporated in NLCs. Table 4 lists the pharmacokinetic parameters of docetaxel after intravenous administration of solution and freeze-dried docetaxel NLCs to rats. The Mann–Whitney $U$ test indicated significant differences in clearance, MRT and AUC between the two formulations.

The NLCs significantly altered the tissue distribution pattern of docetaxel in rats compared with the commercial drug solution, as shown in Figure 5. The independent-samples $t$-test showed significant differences in docetaxel

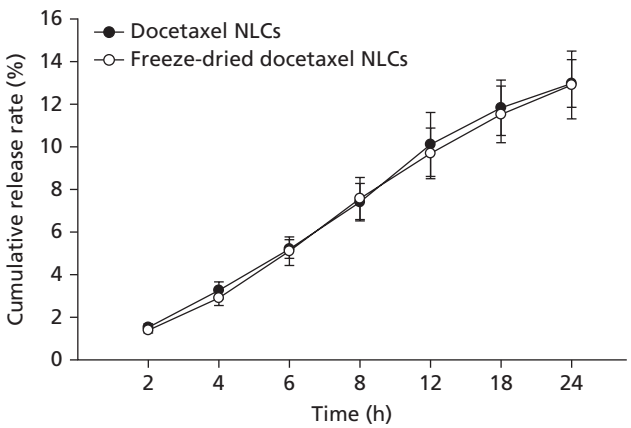


**Figure 3** In-vitro release study. Average cumulated dissolution rate of docetaxel from freeze-dried docetaxel nanostructured lipid carriers (NLCs) and docetaxel NLCs *in vitro*. Value are means ± SD, $n$ = 3.

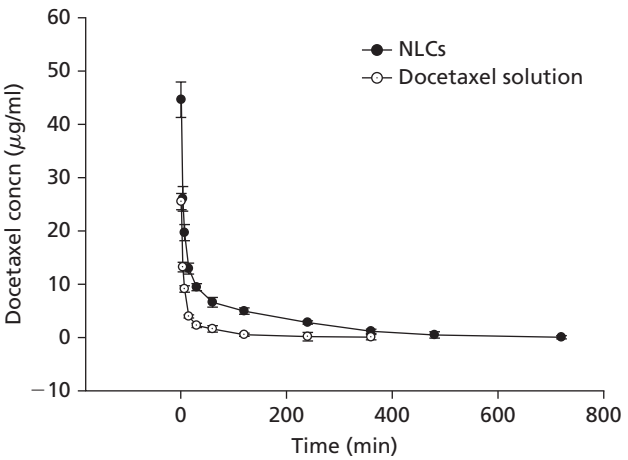


**Figure 4** Pharmacokinetic study of docetaxel formulations. Plasma concentrations of docetaxel after intravenous administration of docetaxel solution and freeze-dried docetaxel nanostructured lipid carriers (NLCs) to rats (10 mg/kg). Values are means ± SD, $n$ = 6.

**Table 4** Pharmacokinetic parameters of docetaxel after intravenous administration of docetaxel solution and freeze-dried docetaxel nanostructured lipid carriers to rats (10 mg/kg)

| Formulation | Clearance (ml/min) | MRT (min) | AUC (μg/ml·min) |
|---|---|---|---|
| Docetaxel solution | 6.19 ± 0.66 | 45.38 ± 4.56 | 399.14 ± 38.54 |
| NLCs | 1.25 ± 0.10 | 128.05 ± 7.22 | 1956.62 ± 142.61 |

AUC, area under the plasma–concentration time curve; MRT, mean residence time; both from time 0 extrapolated to infinity; NLCs, nanostructured lipid carriers. Values are means ± SD ($n$ = 6).

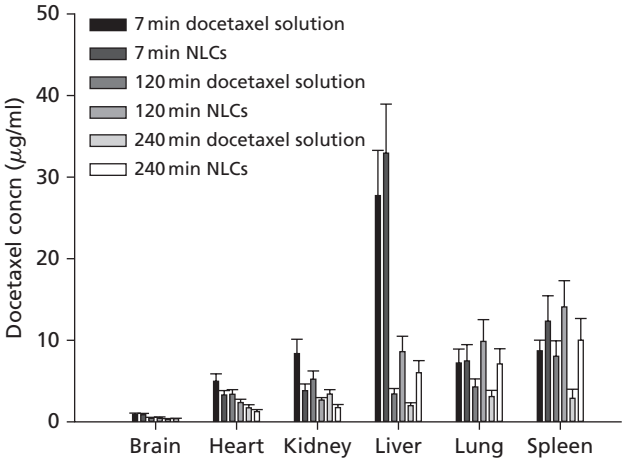


**Figure 5** Tissue distribution study of docetaxel formulations. Tissue distribution of docetaxel at different time points after intravenous administration of docetaxel solution and freeze-dried docetaxel nanostructured lipid carriers (NLCs) to rats (10 mg/kg). Value are means ± SD, $n = 3$.

concentration between docetaxel solution and freeze-dried docetaxel NLCs in heart at 7 min, kidney at 7, 120 and 240 min, and in liver, lungs and spleen at 120 and 240 min. There were no significant differences in docetaxel concentrations between docetaxel solution and freeze-dried docetaxel NLCs in brain any time point, in the heart at 120 and 240 min, and in the liver, lungs and spleen at 7 min.

## Discussion

### Factors influencing encapsulation efficiency

Docetaxel-loaded NLCs were successfully prepared by HPH at 80ºC. The process parameters, such as the amount of lipids and emulsifers, were crucial factors that affected the efficacy of drug incorporation. In the suspensions with 3–8% lipids, the EE% increased markedly to 99.0%. It was assumed that the incorporation of liquid lipid to solid lipid would lead to massive crystal order disturbance and the resulting matrix should offer enough space to accommodate drug molecules, thus contributing to the elevated drug entrapment efficiency and loading capacity.[9,10] Aggregation and sedimentation appeared at the highest lipid concentration. This might be due to the fact that the emulsifier forms a film around the lipid droplets, preventing nanoparticles from aggregating and improving the stability of the nanoparticle dispersion.[11] With a fixed concentration of soyabean lecithin : Brij 78 (1 : 3) as emulsifiers, when the concentration of the lipid exceeds a certain amount, the emulsifiers will not be able to coat the surface of all the lipid droplets, which leads to aggregation.

Interestingly, the soyabean lecithin/Brij 78 emulsifiers not only improved stability but also increased encapsulation efficiency of NLC. This is mainly because Brij 78 (polyoxyethylene (20) stearyl ether) consists of a hydrophilic polyethylene glycol (PEG) chain, which stabilises the nanoparticles, and a hydrophobic head, which has affinity for the lipid materials, leading to higher drug entrapment efficiency.[12]

### Investigation of cryoprotectors

Nanoparticles are usually prepared in an aqueous environment. A major drawback of these dispersions is their thermodynamic-driven tendency to lower their interfacial surface area with the environment and thus to aggregate. The addition of cryoprotectors is necessary to obtain a better redispersion of the dry product. Trehalose was found to be the most effective cryoprotector for preventing aggregation during lyophilisation and subsequent reconstitution of NLCs.

The stabilisation of materials in sugar glasses has been explained by the vitrification theory and particle isolation theory. The vitrification theory refers to the formation of a glassy sugar matrix in which diffusion on a relevant time scale is inhibited. The particle isolation hypothesis refers to the formation of a sugar matrix that acts as a physical barrier between the particles. Both the physical barrier and lack of translational movement prevent aggregation.[13–15]

### X-ray diffraction analysis and differential scanning calorimetry characterisation

X-ray diffraction analysis and DSC are widely used to investigate the status of lipids. X-ray diffraction analysis makes it possible to assess the length of the long and short spacings of the lipid lattice. For the diffractograms of freeze-dried docetaxel NLCs, the diffraction peaks of docetaxel almost disappeared while the diffraction peaks of lipid matrix also decreased significantly. This phenomenon should be attributed to a crystal change. Several factors could have led to the crystal change: for example, docetaxel dispersed in lipid matrix in the molecular state, or most of the lipid matrix could have been converted into the amorphous state during the preparation process.

DSC uses the fact that different lipid modifications possess different melting points and melting enthalpies.[16] The change in the calorimetric peak shape demonstrated the preferential dissolution of docetaxel in molecular form in the lipid matrix.

### Stability of NLCs

No significant changes in drug content, mean particle size and EE% were observed for NLCs after rehydration during the long-term stability study, and the NLCs had a smooth, full and compact appearance and could be rapidly reconstituted (Table 3). The data show that lyophilised NLCs had a similar particle size and drug content to the initial preparation, which could be ascribed to the following three facts. Firstly, it is well known that sugars can be applied to prevent aggregation of nanoparticles during drying and storage.[17,18] Secondly, the zeta potential of drug-loaded NLCs was about −40 mV, which was required for full electrostatic stabilisation. Thirdly, Brij, as an emulsifier, possesses a long PEG tail, which could also act as a steric stabiliser. The enlarged lyophilised particle size may be explained by an increased hydrodynamic diameter of the NLCs due to coating of the vesicle surface with the saccharides through hydrogen bonding with lipids.

#### *Pharmacokinetics and tissue distribution*

To assess the pharmacokinetic behaviour of the most efficacious docetaxel-loaded NLCs, two different

formulations of docetaxel (freeze-dried docetaxel NLCs and docetaxel solution) were given by intravenous injection to conscious male rats. In-vitro release studies indicated that docetaxel was released slowly from the NLCs for at least 24 h, whereas free docetaxel was not readily distributed, which explains the higher initial serum concentrations with the NLCs than the docetaxel solution. As shown in Table 4, AUC increased approximately 6-fold with the freeze-dried docetaxel NLCs compared with the solution, with a corresponding decrease in clearance. Docetaxel dissolved in molecular form in the lipid matrix and released slowly from the NLCs leads to slower elimination, which may explain the higher AUC and lower clearance with the NLCs. The MRT was increased about 3-fold with NLCs compared with docetaxel solution. This could be explained by two points. Firstly, the release profile of drug depends on the entrapped state of drug in the lipid matrix and the degradation of lipid. Docetaxel existed in the ATO/MCT matrix and the lipid matrix delayed degradation and slowed down the release of drug from nanoparticles compared with solution. Secondly, one of the emulsifiers, Brij, improved the stability of NLCs in plasma[12] and prolonged the circulating time. The steric barrier presented by PEG, together with a narrow size distribution of NLCs, results in a higher blood level of docetaxel following the administration of freeze-dried docetaxel NLCs.

The docetaxel concentration in different tissues was determined to assess the distribution of docetaxel when administered intravenously as freeze-dried docetaxel NLCs and injection solution to rats. NLCs accumulated primarily in liver, spleen and lung compared with the solution; in particular, the docetaxel concentration in the lungs was significantly higher in the NLC-treated rats than the solution-treated rats. The higher concentration of docetaxel found in liver, spleen and lung in NLC-treated rats could be related to the slow drug release from NLCs to the targeted organs. The clearance behaviour and tissue distribution of intravenously injected particulate drug carriers are greatly influenced by their size, surface features and opsonisation. Opsonins are adsorbed on the nanoparticle surface and promote particle recognition by the reticuloendothelial system (RES) organs.[19,20] Lipid emulsions are rapidly taken up by the RES in the liver and spleen after intravenous administration.[21] Various attempts have been made to achieve long circulation times by avoiding macrophage uptake, such as stealth SLNs. NLCs changed the biodistribution of docetaxel, especially the increased concentration of drug in the lungs. This may be due to the presence of a long hydrophilic chain of Brij around the shell of NLCs, with longer retention time in the circulation and accumulation in the lungs, which have a rich blood flow with high capillary permeability. The uptake of NLCs by the brain was not improved because of its hydrophilicity.

## Conclusions

We have described the characterisation and stability analysis of freeze-dried docetaxel NLCs. Docetaxel NLCs were prepared by HPH with 5% drug loading efficiency; after freeze-drying the encapsulation efficiency achieved was 97.8%, with unimodal size distribution. The lyophilised product was stable for at least 6 months.

The pharmacokinetics and tissue distribution studies in rats show that it is possible to improve the therapeutic efficacy of lipophilic drugs like docetaxel by incorporation into NLCs. When compared with docetaxel solution, freeze-dried docetaxel NLCs showed high uptake in RES organs after intravenous administration, and the docetaxel concentration in the lungs was significantly higher with NLCs than following injection of standard solution. The formulation approach could be used to improve the stability and tissue distribution of poorly soluble drugs.

## Declarations

### Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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