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Preparation and characterization of modified lipid nanoparticles for doxorubicin controlled release

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Low drug encapsulation efficiency and burst release are the main drawbacks of lipid nanoparticles. To solve these problems, lipid nanoparticles containing a cross-linked lipid network were prepared in this study. Monostearin (MS) and octadecylamine (ODA) were used as the solid lipids, and oleic acid (OA) was used as liquid lipid. The use of a small amount of ODA was to form cross-linked network by glutaraldehyde in the presence of OA. Using doxorubicin (DOX) as a model drug, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and glutaraldehyde cross-linked NLC were prepared. The physicochemical properties of these lipid nanoparticles such as average diameter, drug loading, drug encapsulation efficiency and *in vitro* drug release were investigated. The drug loading capacities and encapsulation efficiency were improved by mixing OA with the solid lipids to produce the nanoparticle matrix, and were further increased by the cross-linking of glutaraldehyde. The faster drug release was obtained when the NLC with higher OA content. After cross-linking by glutaraldehyde, the burst release of DOX from NLC could be reduced, in comparison to both SLN and non cross-linked NLC. The results indicated the NLC with cross-linked network are a potential drug delivery system with improved drug encapsulation and controlled drug release.

1. Introduction

Nanostructured lipid carriers (NLC) based on mixture of solid lipids with spatially incompatible liquid lipids are a new colloidal carrier for controlled drug delivery (Müller et al. 2002a). As a type of lipid nanospheres, NLC combines the advantages of emulsions, liposomes, polymeric nanoparticles and solid lipid nanoparticles (SLN) (Yang et al. 1999a, 1999b). As a new generation of SLN, in contrast to the more or less highly ordered SLN being yielded from solid lipids or blends of solid lipids, the incorporation of liquid lipids to solid lipids leads to massive crystal order disturbance. The resulting matrix of lipid particles shows great imperfections in the crystal lattice and leaves enough space to accommodate drug molecules, thus, leading to improved drug loading capacity (Jenning et al. 2000; Jenning et al. 2001; Souto et al. 2004). In addition, by controlling the amount of liquid lipids added to the formulation, a controlled drug release can be achieved with NLC (Müller et al. 2002b). In our previous paper (Hu et al. 2005), we reported that the encapsulation efficiency of NLC prepared by the solvent diffusion method increased with increasing liquid lipid content, but on the other hand an enhanced drug release rate was observed.

Doxorubicin (DOX) is an anthracycline cytostatic antibiotic with activity against a variety of malignancies, especially in the treatment of soft tissue, and bone sarcoma (Minko et al. 2000). Due to its cumulative dose-related (Hoff et al. 1979) and irreversible cardiotoxicity (Coukell et al. 1997, Keizer et al. 1990), the clinical usefulness of this drug is limited. Up to now, various targeted drug delivery systems, such as liposomes (Gabizon et al. 1997), microspheres (Stolnik et al. 1995), polymeric micelles (Kwon et al. 1994), and chemical conjugates (Dvorak et al. 1999), have been developed to minimize the toxic side effects of the drug and enhance its therapeutic efficacy. The therapeutic effectiveness of these targeted doxorubicin delivery systems were improved for the treatment of some tumors by intravenous injection (Minko et al. 2000). However, few applications for oral administration were reported. Lipid nanoparticles have been mainly used through oral administration, which is aimed to increase drug bioavailability and work as target delivery. The bioavailability of poorly water-soluble drugs can be improved via the oral administration route, when the drug is encapsulated in lipid nanoparticles (Humberstone et al. 1997). The lower drug loading capacity and burst release behavior were the main drawbacks for the oral administration of lipid nanoparticles. The lower drug loading capacity means the use of higher lipid concentration, which may cause the higher viscosity of the formulation and instability of lipid nanoparticles. The burst release may lead to drug loss during *in vivo* transport of lipid nanoparticles, and hence cause side effects of the drug.

In order to obtain lipid nanoparticles with higher drug loading capacity and less burst release, lipid nanoparticles with cross-linked network were induced. Using DOX as a model hydrophobic drug, DOX-loaded SLN,

NLC and NLC cross-linked by glutaraldehyde were prepared by the solvent diffusion method, and the physicochemical properties of the lipid nanoparticles such as their average diameter, zeta potential, drug encapsulation efficiency, drug loading, and *in vitro* drug release were investigated.

2. Investigations, results and discussion

2.1. Function of the liquid lipid incorporated into solid lipid

The DOX loaded SLN and NLC with different OA content were prepared by the solvent diffusion method in an aqueous system. The properties of resulting NLC and SLN, such as volume average diameter, zeta potential and polydispersity indices are listed in Table 1. No obvious effect of OA content on particle size and zeta potential of the lipid nanoparticles was found. However, the EE and DL of lipid nanoparticles increased with increasing the content of OA in the lipid matrix. This was basically consistent with the results reported by Yuan et al. (2007). The liquid lipid can lead to massive crystal order disturbance, and the resulting matrix of lipid particles indicates great imperfections in the crystal lattice and leaves enough space to accommodate drug molecules, thus the EE and DL can be improved (Souto et al. 2004).

The *in vitro* drug release profiles from the DOX-loaded SLN and NLC with different amounts of OA content are shown in Fig. 1. The drug release rate of lipid nanoparticles was affected by the OA content. The drug release rate from lipid nanoparticles was enhanced by increasing OA content. The *in vitro* drug release rate of NLC was faster than that of SLN, which has been attributed to the presence of liquid lipid in the solid lipid matrix. Due to the presence of liquid lipid NLC became more imperfect, and faster drug diffusion rate was achieved via mixing the matrix of solid lipid and liquid lipid compared to that of through solid lipid matrix.

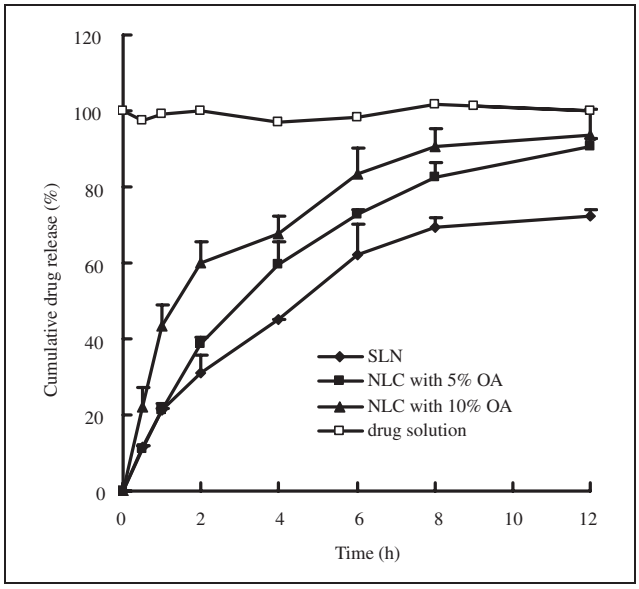

Fig. 1: *In vitro* DOX release profiles from DOX loaded SLN and NLC with different OA content (n = 3)

2.2. Effects of charged amounts of drug

To investigate their drug loading capabilities, NLC were prepared using different charged amounts of DOX. The OA content in NLC was fixed at 10 wt%. The results are shown in Figs. 2–4. As shown in Fig. 2, the volume average diameter of NLC increased, but the absolute values of zeta potential decreased as the charged amounts of drug increased. The increased particle size of NLC can be due to the decreased absolute values of zeta potential and the higher viscosity of molten oil phase in the preparation process, because the molten point of DOX is higher than that of lipid materials. The decrease of absolute values for zeta potential could be attributed to two factors: the increased drug content could reduce the charge density and absolute values of zeta potential; and the bigger particle size led to the lower charge density of particle surface. The effects of the charged amounts of drug on encapsulation efficiency (EE) and drug loading (DL) of NLC are shown in Fig. 3. It was found that the EE of NLC decreased with increasing amounts of charged drug, but DL could be highly improved. When 7.5 wt% drug was charged, DL could reach to as high as 4.25 wt%. Figure 4 shows the profiles of *in vitro* drug released from NLC with different DLs. The faster rate of drug release was observed in the NLC with higher DL, and the highest rate of the NLC was achieved when 7.5 wt% drug was added. This was probably related to the drug distribution nearby the surface of NLC, which increased as the charged amounts of drug increased. Thus the rate of drug release was enhanced, and the EE of NLC was reduced.

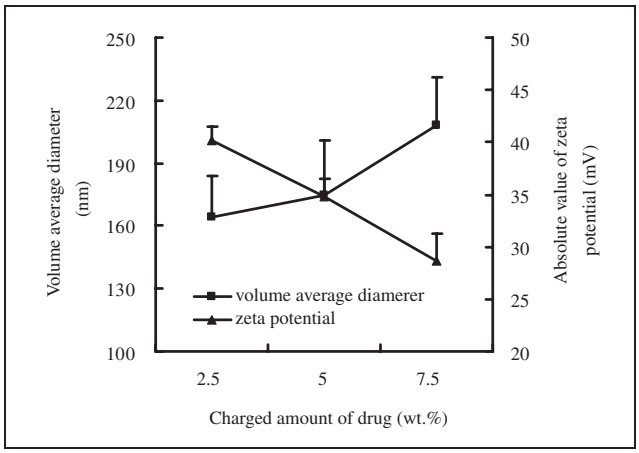

Fig. 2: Variations of volume average diameter and absolute value of zeta potential for NLC prepared by using different amount of charged drug (n = 3)

Table 1: Properties of DOX loaded SLN and NLC

Liquid lipid content (wt%)	d_v (nm)	PI	Zeta potential (mV)	EE (wt%)	Drug loading (wt%)
0	185 ± 38.7	0.195	−36.1 ± 2.7	57.3 ± 2.91	2.78
5	175 ± 25.8	0.219	−34.8 ± 1.7	66.72 ± 0.23	3.23
10	174 ± 38	0.213	−35.2 ± 1.2	69.90 ± 4.56	3.38

d_v, PI and EE indicate the volume average diameter, polydispersity index and drug encapsulation efficiency, respectively

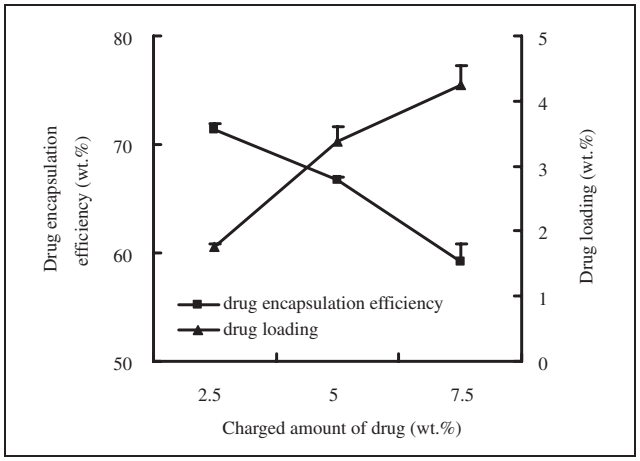

Fig. 3: Variations of drug encapsulation efficiency and drug loading for NLC prepared by using different amount of charged drug (n = 3)

2.3. *Effect of cross-linking by glutaraldehyde*

In order to further improve the EE of NLC, 1 wt% ODA was used instead of the same amount of MS, and the NLC with 10 wt% OA content was prepared by cross-linking using different amounts of glutaraldehyde. It is well known that a nucleophilic reaction between amino groups and aldehyde groups produces a Schiff's base, which is also the main product of the reaction between glutaraldehyde and ODA. The effects of reaction temperature and amount of glutaraldehyde on the particle size of glutaraldehyde cross-linked NLC were shown in Figs. 5 and 6. When the reaction temperature was lower (room temperature), cross-linking between NLC occurred and led to the obvious increase in particle size (Fig. 5). On the other hand, when the reaction temperature was higher (70 °C), no significant change of particle size was observed. This might be due to the molten oil phase at high temperature, and the cross-linking mainly occurred in NLC. However, the NLC was a solid state in room temperature, the cross-linking mainly occurred between NLC. Figure 6 shows the effect of the charged amount of glutaraldehyde on the particle size of glutaraldehyde cross-linked NLC. As shown in Fig. 6, when glutaraldehyde was added in an appropriate ratio ($n_{ODA}:n_{glu} = 1:5$, $1:9$), the particle sizes were almost constant for 6h. However, when the glutaraldehyde concentration reached a higher

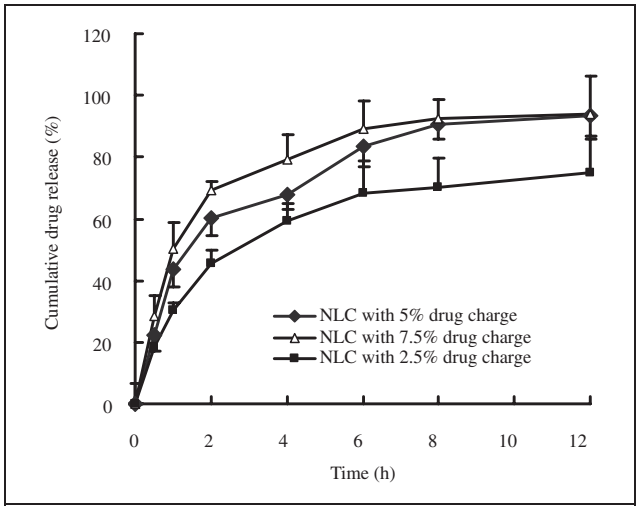

Fig. 4: *In vitro* DOX release profiles of NLC prepared by using different amount of charged drug (n = 3)

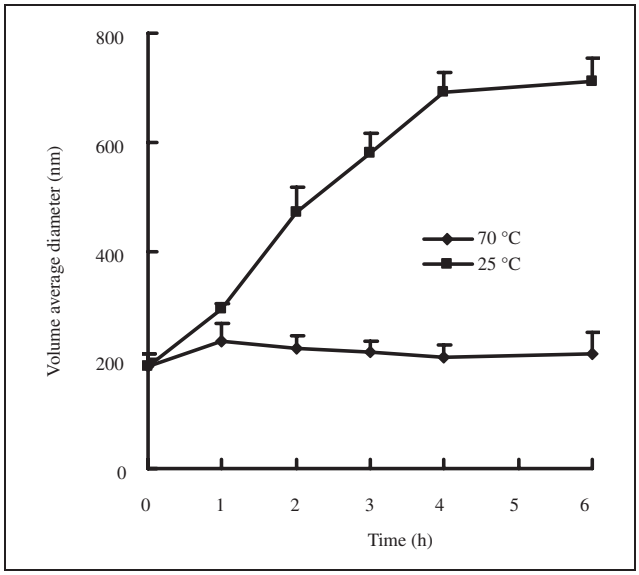

Fig. 5: Effects of reaction temperature and time on the particle size of NLC cross-linked by glutaraldehyde

ratio ($n_{ODA}:n_{glu} = 1:13$), the particle sizes increased significantly, and was 10 times the initial size after 6 h.

The effects of cross-linking of glutaraldehyde on the EE and DL of NLC were investigated. The changes of the EE and DL are given in Table 2. It is clear that the EE and DL of nanoparticles were increased from 66.72 to 87.2% and from 3.38 to 4.18%, respectively, when the NLC was cross-linked by glutaraldehyde ($n_{ODA}:n_{glu} = 1:9$) for 6 h.

In vitro release curve of glutaraldehyde cross-linked DOX-loaded NLC is shown in Fig. 7. In the case of NLC with 10 wt% OA and 5 wt% drug charged, complete drug release was observed within 12 h. However, only about 60 wt% drug was released from DOX loaded NLC cross-linked by glutaraldehyde ($n_{ODA}:n\text{glu} = 1:9$), at this time. The drug release rate in the initial stage was significantly reduced. This may be due to the formation of a cross-linking network between glutaraldehyde and ODA, which is considered to increase the resistance of the matrix material for the diffusion of the drug through the hardened matrix and consequently retard the release rate of the drug from the NLC (Warren et al. 1998; Domb et al. 1990).

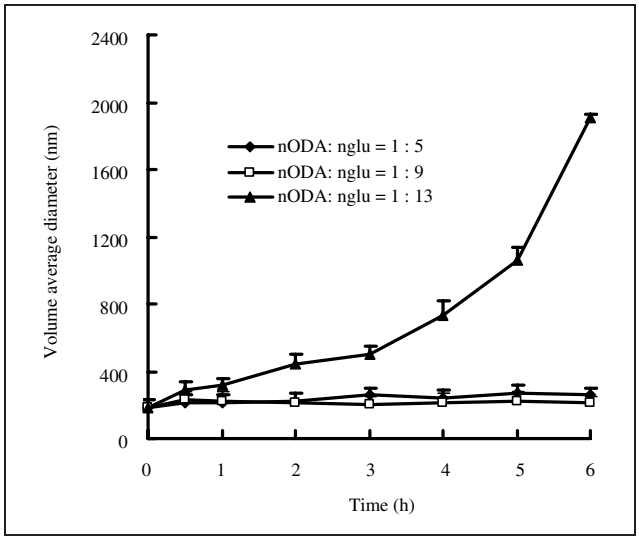

Fig. 6: Effect of charged amount of glutaraldehyde on the particle size of NLC cross-linked by glutaraldehyde

Table 2: Properties of NLC before and after cross-linking by glutaraldehyde

Sample	d_v (nm)	PI	Zeta potential (mV)	EE (wt%)	Drug loading (wt%)
NLC	174 ± 38	0.213	-35.2 ± 1.2	69.90 ± 4.56	3.38
Cross-linked NLC	213 ± 26.5	0.131	-40.8 ± 3.6	87.2 ± 0.94	4.18

d_v, PI and EE indicate the volume average diameter, polydispersity index and drug encapsulation efficiency, respectively

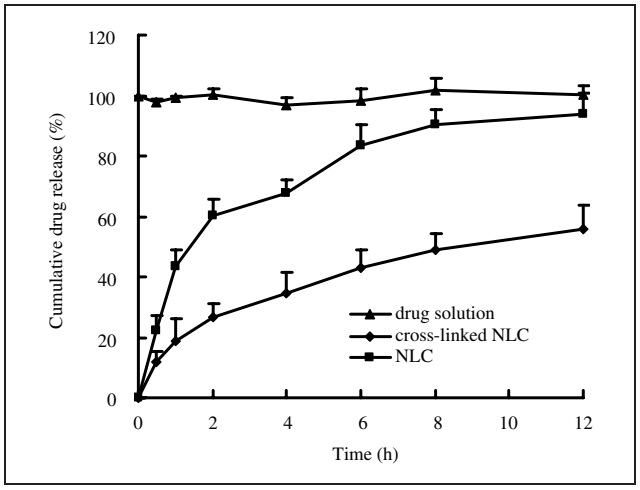

Fig. 7: *In vitro* release profiles of DOX from NLC cross-linked by glutaraldehyde

In conclusion, compared with SLN, NLC showed advantages such as higher EE, and higher DL. However, the drug release rate was increased with increasing content of liquid lipid (OA). When the NLC was cross-linked by glutaraldehyde, EE and DL were further improved, and the drug release rate in the initial stage could be reduced significantly. NLC preparated in this study could potentially be exploited as a carrier with improved DL and controlled drug release properties. In future experiments *in vivo*, we expect that DOX-loaded NLC can improve the drug bioavailability after oral administration.

3. Experimental

3.1. Materials

Monostearin was purchased from Shanghai Chemical Reagent Co. Ltd., (China). Oleic acid (Hangzhou Shuanglin Chemical Industry Co. Ltd., China) was chosen as liquid lipid material for NLC. Doxorubicin (DOX) was kindly donated from Zhejiang Haizheng Pharmaceutical Co. Ltd., China. Octadecylamine (ODA, 95%) was purchased from Fluka. Glutaraldehyde was purchased from Wulian Chemical plant, China. All other solvents were analytical or chromatographic grade.

3.2. Preparation of SLN and NLC

DOX loaded SLN and NLC were prepared by the solvent diffusion method in an aqueous system reported in our previous study (Hu et al. 2005). Briefly, 30 mg lipid materials (MS with 0, 5 or 10 wt% OA) and 2.5–7.5 wt% DOX relation to lipid materials were completely dissolved in a mixture of DMSO (1.5 ml) and ethanol (1.5 ml) in a water bath at 70 °C. The resultant organic solution was quickly dispersed into 27 ml distilled water under mechanical stirring (DC-40, Hangzhou Electrical Engineering Instruments, China) with 400 rpm in water bath at 70 °C for 5 min. The obtained pre-emulsion (melted lipid droplets stabilized in a hot surfactant solution) was the cooled at room temperature until drug-loaded SLN or NLC dispersion was obtained.

3.3. Preparation of glutaraldehyde cross-linked NLC

The glutaraldehyde cross-linked NLC with 10 wt% OA content were prepared by using a small amount of ODA as lipid material to react with glutaraldehyde. The weight ratio of ODA to total lipid was 1 wt%. NLC containing ODA was cross-linked by dropping glutaraldehyde aqueous solution into the NLC dispersion under mechanic stirring with 400 rpm at room temperature or at 70 °C. The molar ratio of ODA to glutaraldehyde was controlled at 1 : 5, 1 : 9, 1 : 13, respectively.

3.4. Determination of particle size and zeta potential

The volume average diameter and zeta potential of DOX-loaded lipid nanoparticles in dispersion were determined with a Zetasizer (Nano-zs90, Malvern Instruments, UK) after the nanoparticles dispersion diluted 20 times with distilled water.

3.5. Drug encapsulation efficiency and drug loading

The doxorubicin content was measured by a fluorescence spectrophotometer. The excitation wavelength, emission wavelength and slit openings were set at 505 nm, 565 nm and 5 nm, respectively. The calibration curve of fluorescence intensity against concentration of DOX (μg/ml) is shown in Eq. (1)

$$y = 539.3x + 19.058 \quad (1)$$

fitting the concentration range, 0.05–2μg/ml ($r = 0.9998$, where y = fluorescence intensity, and x = DOX concentration).

To determine the entrapment efficiency of DOX in the NLC and SLN, the pH of the prepared lipid nanoparticle dispersion was adjusted to 1.20 by adding 0.1M HCl, then was centrifuged (64R, Beckman, USA) at 25,000 rpm for 15 min, and then being the supernatant and precipitate of lipid nanoparticles collected. The solid residue was dispersed in 50 ml phosphate buffered saline (PBS) solution (pH 7.4), in order to dissolve the free drug absorbed on the surface of the nanoparticles, and then centrifuged separately. Both the drug content in supernatant (W_{s1}) and in PBS (W_{s2}) were determined by fluorescence spectrophotometer. The efficiency of drug encapsulation (EE) and drug loading (DL) of nanoparticles were calculated from Eqs. (2) and (3),

$$EE = \left(\frac{W_a - W_{s1} - W_{s2}}{Wa}\right) \times 100\% \quad (2)$$

$$DL = \left(\frac{W_a - W_{s1} - W_{s2}}{W_a - W_{s1} - W_{s2s} + W_L}\right) \times 100\% \quad (3)$$

where W_a and W_L are the weight of drug added in system, weight of lipid added in system, respectively.

3.6. In vitro release studies

The drug release profiles of DOX from lipid nanoparticles were assessed in vitro. After washed with PBS (pH 7.4) as described in section 3.5, the lipid nanoparticles were dispersed in 100 ml phosphate buffered saline (PBS) solution (pH 7.4) using 150 ml appropriate glass test-tubes. The resulting samples were shaken horizontally (SHELLAB1227–2E, SHELLAB, USA) at 60 strokes per min under 37 °C. Aliqnots of the dispersion (1 ml) was withdrawn from the system at definite time interval and centrifuged (64R, Beckman, USA) at 25,000 rpm for 15 min. The supernatant was determined by the fluorescence spectrophotometer method as described above. As a control, the DOX solution was prepared by dispersing 1 ml DOX DMSO solution (1 mg/ml) in 99 ml PBS. Each releasing experiment was performed in triplicate.

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