Available online at www.sciencedirect.com
SCIENCE DIRECT
International Journal of Pharmaceutics 313 (2006) 136–143
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Stability and pharmacokinetic studies of *O*-palmitoyl amylopectin anchored dipyridamole liposomes

Cheng Ji *, Zhu Jia-bi, Wen Na, Xiong Fei

School of Pharmacy, China Pharmaceutical University, Nanjing, Jiangsu 210009, PR China

Received 13 October 2005; received in revised form 15 January 2006; accepted 20 January 2006
Available online 15 March 2006

Abstract

Modified polysaccharides have been used widely to increase physico-chemical stability of liposomes. However, the stability and pharmacokinetic studies on the polysaccharides modified anchored liposomes containing hydrophobic drugs which exist in lipid bilayer membranes were insufficient as compared with the liposomes carrying hydrophilic or ionic drugs in inner aqueous phase. In the present study, a hydrophobic drug, dipyridamole (DIP), was entrapped into liposomes through film hydration. Amylopectin was palmitoylated and anchored on the surface of plain DIP liposomes. Subsequently, the stabilities of DIP ethanol solution, plain DIP liposomes (PDL) and anchored DIP liposomes (ODL) against irradiation, disperse medium, biofluid, long-term storage were determined and compared. The concentrations of DIP in plasma of rats and its pharmacokinetic behaviors after intravenous administration of DIP injection, PDL and ODL were studied by RP-HPLC. The pharmacokinetic parameters were computed by software 3p97 programme. The results showed that ODL could increase stabilities more of DIP in vitro as compared with PDL. The plasma concentration–time curves of DIP after intravenous administration of DIP injection, PDL and ODL were all in accordance with open two-compartment model. Pharmacokinetic parameters of DIP injection, PDL and ODL in rats were significantly different. The present findings suggest that anchored liposomes could increase stabilities of DIP in vitro as compared with plain liposomes. Furthermore, the difference of pharmacokinetic profiles was due to the targetability of anchored liposomes.

Keywords: *O*-Palmitoyl amylopectin; Liposomes; Dipyridamole; Stabilization; Pharmacokinetics

1. Introduction

Liposomes are widely investigated as potential drug carriers due to their ability to protect and carry drug molecules (Ranade, 1989; Allen and Moase, 1996). However, stability of the liposomal preparations remains to be the major problem for the controlled release and efficacious targeting of the active drugs. Thus, sterically stabilized liposomes have been designed which differ from conventional liposomes in alteration of their surface through the use of natural components such as glycolipids (Allen, 1994; Satish and Surolia, 2002) or synthetic polymers such as polyethylene glycol, polyvinyl alcohol or polyacrylamide (Sato and Sunamoto, 1992; Parr et al., 1994; Torchilin et al., 1994; Ishiwata et al., 1995; Takeuchi et al., 2000).

Polysaccharides are also attractive for liposomes coating because of their protein-rejecting ability, biodegradability, low toxicity and cell targetability through specific moieties (Sato and Sunamoto, 1992; Letourneur et al., 2000; Venkatesan and Vyas, 2000; Sihorkar and Vyas, 2001; Mehvar, 2003). Liposomes anchored with natural polysaccharides (mannan (Vyas et al., 2000, 2005), amylopectin (Takada et al., 1984; Miyazaki et al., 1992; Deol and Khuller, 1997; Poiani et al., 1997; Vyas et al., 2004) and pullulan (Takada et al., 1984; Sehgal and Rogers, 1995; Matsukawa et al., 2000; Vyas et al., 2005)) have been described. The polysaccharides were chemically modified with a hydrophobic anchor and subsequently integrated with the lipid bilayer membranes.

Increase of structural stability, long stability and membrane integrity was recorded in black lipid membranes anchored with hydrophobized polysaccharides by Moellerfeld et al. (1986). A series of investigations demonstrated the stabilities of polysaccharides anchored liposomes in buffer solutions, serum, plasma or simulated biological conditions in vitro (Sehgal and Rogers,

* Corresponding author. Tel.: +86 25 8533 8217.
E-mail address: chengji45@sohu.com (J. Cheng).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.01.031

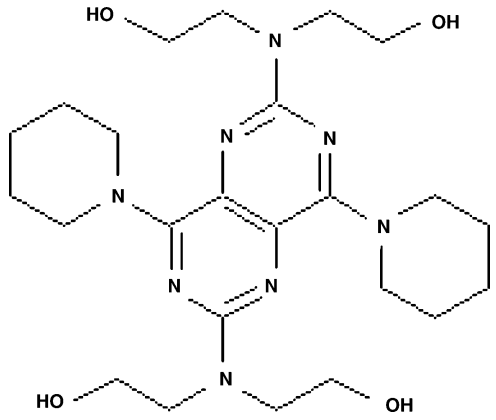

Fig. 1. The chemical structure of dipyridamole.

1995; Moreira and Almeida, 1996; Moreira et al., 1997; Deol and Khuller, 1997; Mumper and Hoffman, 2000; Venkatesan and Vyas, 2000; Vyas et al., 2000). Otherwise, the in vivo studies mainly focused on the tissue or cell targetabilities of polysaccharides anchored liposomes (Takada et al., 1984; Miyazaki et al., 1992; Deol and Khuller, 1997; Poiani et al., 1997; Ichinose et al., 1998; Grinsell et al., 2001; Mehvar, 2003; Vyas et al., 2004, 2005).

However, most of the studies to date cover in vitro stability aspects of polysaccharides anchored liposomes that carried hydrophilic or ionic drugs in inner aqueous phase and lack sufficient parallel studies for hydrophobic drugs existing in lipid bilayer membranes. Moreover, the in vivo stability studies for polysaccharides anchored liposomes were fairly limited without detailed evaluation through pharmacokinetic parameters. So, it is worth further dealing with in vitro and in vivo stabilities of polysaccharides anchored liposomes containing the hydrophobic drugs.

In this research, *O*-palmitoyl amylopectin (OPA), a modified polysaccharide, was synthesized and characterized. Then, the OPA was used for anchoring the liposomes containing the hydrophobic model drug, dipyridamole (DIP; Fig. 1), which is widely used as a coronary vasodilator and antiplatelet agent in medicine (FitzGerald, 1987), and has also been researched for treating lung diseases (De la Cruz et al., 1996; Curtin and Turner, 1999; Arnold et al., 2000; Pan et al., 2002). Subsequently, the characterization of OPA anchored DIP liposomes and comparative in vitro stability and in vivo pharmacokinetic profiles of OPA anchored DIP liposomes against plain DIP liposomes were studied.

2. Materials and methods

2.1. Chemicals

Egg yolk phosphatidylcholine (EPC) was obtained from Lipoid (Ludwigshafen, Germany). Cholesterol was purchased from Sigma (St. Louis, MO, USA). Amylopectin was obtained from MPbio (Irvine, CA, USA) and used without further purification. DIP was a gift sample from Yabao Pharm Inc. (Shanxi, China). DIP injection was purchased from Hefeng Pharm Inc. (Shanghai, China). All other reagents and solvents were of analytical grade or better.

2.2. Preparation of DIP liposomes (PDL and ODL)

Plain DIP liposomes (PDL) were prepared through film hydration as reported by Bangham et al. (1965) with slight modifications. Briefly, EPC, cholesterol and DIP (mass ratio = 100:12:2.25) were dissolved in chloroform. A thin film of dry lipid was deposited on the inner wall of the flask by evaporating the solvent under vacuum at 40 °C. The film was hydrated at 40 °C with pH 7.4 PBS.

Then, the OPA anchored DIP liposomes (ODL) were prepared by anchoring *O*-palmitoyl amylopectin on the surface of PDL. OPA was synthesized by esterification of amylopectin in DMSO under catalytic conditions following the procedure as described by Hammerling and Westphal (1967). Unanchored PDL were incubated with OPA for coating. Coating parameters were optimized by measuring the change in zeta potential of the liposomal suspension with zetasizer 3000 (Malvern, UK) (Vyas et al., 2004). Coating ratio of OPA with respect to total lipid was varied with the designated 2-h incubation time. Similarly, at an optimized lipid:OPA ratio the incubation time periods were varied. The superfluous OPA was removed through centrifugation (20,000 rpm, 4 °C, 30 min) using 3K30 refrigerated centrifuge (Sigma, Germany). Then, the ODL for following experiments were prepared according to the optimal coating parameters.

2.3. Characterization of the liposomes

The vesicle size and span were measured by Mastersizer 2000 (Malvern). The morphology was observed by H7000 transmission electron microscope (Hitachi, Japan). The entrapment efficiency (EE) was measured by passing a Sephadex G50 column and calculated following the equation: $EE\,(\%) = (D_1/D_2) \times 100$, where D_1 was the amount of DIP in liposomes after column chromatography and D_2 was the amount of DIP in liposomes before column chromatography. One hundred microliters of liposomal suspension was diluted to 10 ml by ethanol for HPLC assay. Twenty microliters of the samples was injected into a Lichrosphere C18 column, 4.6 mm × 15 cm, 5 μm (Hanbang Analytical Instrument Co. Ltd., China). The column was eluted with methanol–pH 4.6, 0.1% Na_2HPO_4 (3:1, v/v). Detection was made by UV adsorption measurement at 288 nm (flow rate 1 ml/min). Peak areas were recorded and drug concentration was calculated from standard curves. The assay was linear over the tested concentration range (3–15 μg/ml). The precision and accuracy of this method were also satisfying.

2.4. Stabilities of the DIP liposomes

2.4.1. Photostability

DIP ethanol solution, PDL and ODL were irradiated under aerobic conditions in a dark cabinet, with an array of six TLK40W/10R UVA lamps (Philips, Holland) whose dominant emission was at a wavelength of 365 nm. The distance from the light source to the test samples was 10 cm. The irradiation

was measured by a TN-2365 UVA radiometer (Taina, Taiwan, China) during the experiment. The irradiations were performed at 25 °C to avoid any possible effect of heat on the degradation. At scheduled intervals, 50 μl test samples were diluted to 10 ml by ethanol, the analytical samples for fluorescence spectra were prepared by diluting the ethanol dilutions 10-fold with water, then the fluorescence intensity was measured (λ_{ex} = 308 nm and λ_{em} = 494 nm, slit width = 3 nm) with a RF-5301 fluorescence spectrophotometer (Shimadzu, Japan).

2.4.2. Stability study in PBS and FCS

The similar 1 ml containing 1 mg DIP of PDL, ODL or DIP injection was diluted one-fold with pH 7.4 PBS or fetal calf serum (FCS), respectively; the 2.0 ml sample of the diluted liposomal suspension or DIP injection was placed in a cellulose membrane tubing (Shanghai Chemical Reagent Co. Ltd., China) with molecular weight cutoff at 8000–10,000, which was pretreated by soaking in water overnight and washed with deionized water. The two ends of the tubing were tightened and the bag soaked in 150 ml, pH 7.4 PBS used as release medium. Experiments were carried out at 37 °C for 24 h. At scheduled intervals, 200 μl of the release medium was collected for HPLC assay. The same volume of blank medium with the same temperature was added immediately. The DIP release percentage was obtained according to the equation: drug release (%) = $(D_t/D_0) \times 100$, where D_t and D_0 indicated the amount of drug released from liposomal suspension at certain intervals and the total amount of drug in liposomal suspension, respectively.

2.4.3. Long-term stability

The PDL and ODL were kept in amber colored glass ampoules flushed with nitrogen and stored at 4 ± 2 °C for a period of 12 weeks. At scheduled intervals, aliquots of liposomes were removed and the leakage profile was determined according to the measurement for entrapment efficiency. The DIP leakage percentage was calculated following the equation: drug leakage (%) = $(D_0 - D_t)/D_0 \times 100$, where D_0 and D_t were the entrapment efficiencies (measured using the same method as mentioned above) of the same formulation at the start of the experiment and scheduled intervals. The vesicle size and span were measured simultaneously. Their variance percentage (%) = $(D_t - D_0)/D_0 \times 100$, where D_0 and D_t were the measured size or span values at the start of the experiment and scheduled intervals, respectively.

2.5. Pharmacokinetics in rats

The PDL, ODL or DIP injection was injected into male S–D rats via tail vein at a dose of 1.25 mg DIP/kg body weight. Plasma samples were obtained at various intervals after administration (0.083 h, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h and 12 h). To each 200 μl of plasma sample, 200 μl of 0.1 M NaOH was added and mixed. Subsequently, 3 ml dichloromethane was added. Extraction was conducted by vortexing for 10 min and centrifugation (3000 rpm, 4 °C, 15 min). The organic phase was transferred to a glass tube and evaporated to dryness under a gentle stream of nitrogen at 40 °C, eventually the residue dissolved in 150 μl ethanol for HPLC assay. The standard curves ranging

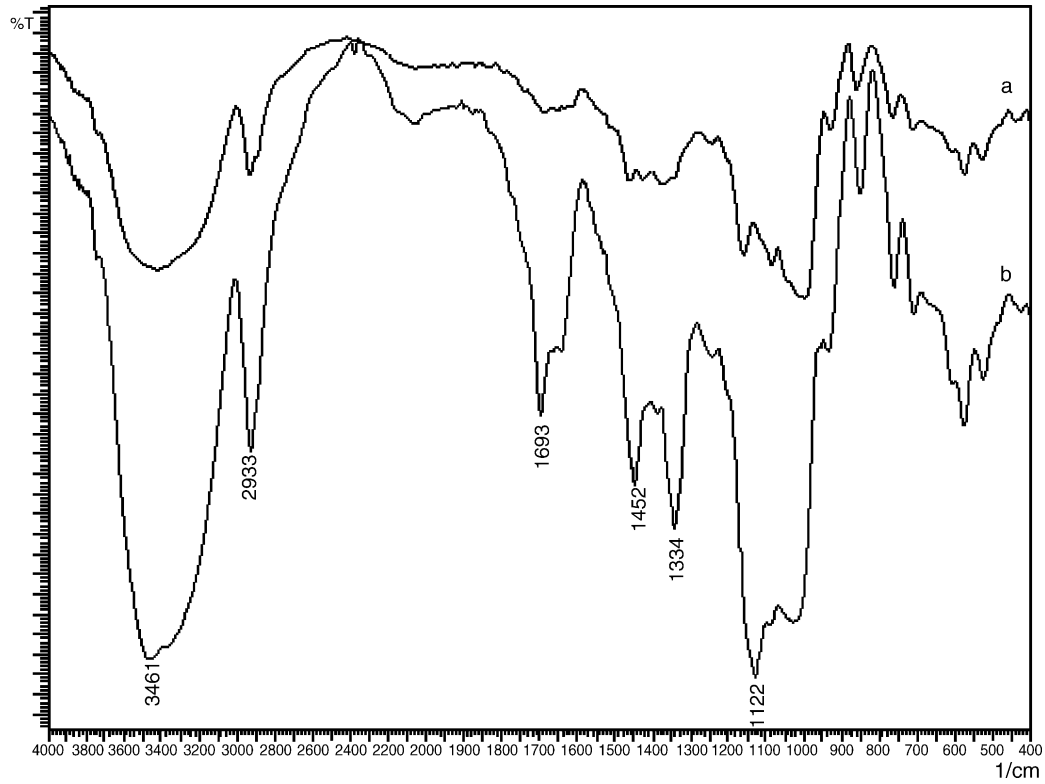

Fig. 2. The comparative IR spectra of amylopectin (a) and OPA (b).

from 0.05 to 5 μg/ml were linear ($r = 0.9996$). The precision and accuracy of this method were also satisfying.

The area under the plasma concentration–time profile (AUC) was calculated using a log-linear trapezoidal method (3P97 programme, Mathematic Pharmacological Committee, Chinese Pharmacological Society, China). Systemic plasma clearance (Cl) was calculated as dose/$AUC_{t0-tinf}$. The volume of distribution (V_d) was calculated as mean residence time (MRT) multiplied with Cl. MRT was derived from the equation $AUMC_{t0-tinf}/AUC_{t0-tinf}$. The data were analyzed for statistical significance by the *t*-test. All results were expressed as the mean ± standard deviation (S.D.).

3. Results and discussions

3.1. Preparation and characterization of DIP liposomes

OPA was characterized by IR analysis. Comparison of the IR spectra (Fig. 2) of OPA with amylopectin revealed that the peak corresponding to C=O stretching vibration expected at 1735 cm^{-1} (Pavia et al., 1979) appeared with a shift at 1693 cm^{-1}. It may be a credit to a consequence of intra-molecular hydrogen bonds between carbonyl and hydroxyl groups which suppress the stretching force vibration of C=O band. The presence of hydrogen bonds was further confirmed from the OH stretching vibration at 3461 cm^{-1}. This represents a polymeric band. However, there is a shift in band from 3600–3200 cm^{-1} to 3461 cm^{-1}, which may be attributed to the intra-molecular single bridge between hydrogen bonds. Furthermore, the C–O and C–H stretching vibrations appeared as the characteristic bands at 1122 cm^{-1} and 2933 cm^{-1}, respectively. From these observations it can be concluded that there exists an ester bond between amylopectin and *O*-palmitoyl anchor.

The following experiments were conducted to optimize the procedure of OPA coating. The parameters studied include lipid:OPA ratio and optimum incubation time. Through the similar incubation time, the value of zeta potential varied marginally on addition of OPA up to 20% OPA:lipid (w/w). On further addition of OPA, there is an obvious change in the zeta potential value (Fig. 3a). It indicated that at an optimal 20% weight ratio of OPA:lipid, the integration of OPA in the lipid bilayer membranes occurred at saturation level while further addition

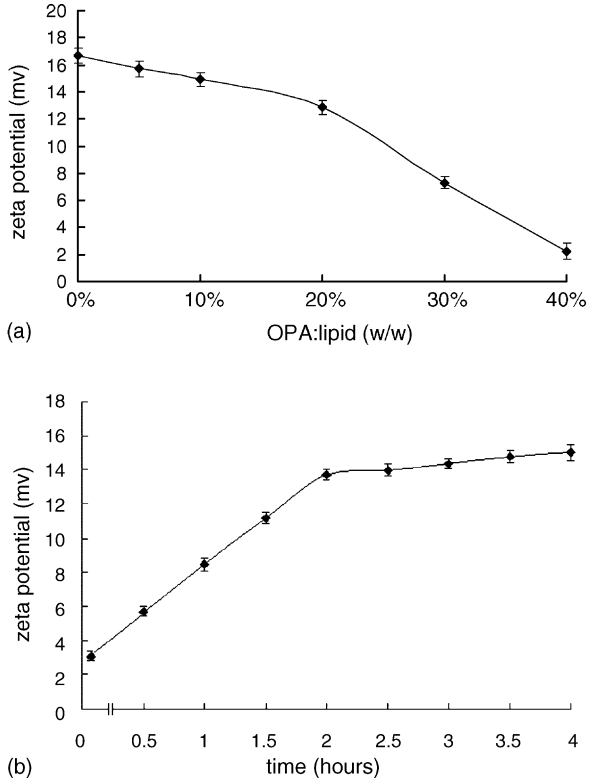

Fig. 3. Optimization of OPA anchoring process to DIP liposomes. Optimization of: (a) OPA:lipid ratio (w/w) and (b) incubation time. The first measuring point in (b) means the value of zeta potential measured as soon as possible after mixing PDL with OPA. Each point represents the mean ± S.D. ($n = 3$).

results in an increase in the surface potential values, which was attributable to the excess of free negatively charged OPA. The incubation time for coating was optimized by measuring the surface potential of the liposomes with the optimum OPA:lipid ratio at different incubation times. The surface potential value rose up steeply during the initial time. However, the change in surface potential after 2 h was negligible to nil (Fig. 3b). The increase in the surface potential values may be attributed to the charge quenching of anchored free OPA. With longer incubation time, the amount of free OPA decreased as measured in terms of surface potential. The results showed that at the end of 2 h, the interaction and integration of added OPA could have been completed. The 2 h was considered as the optimum coating incu-

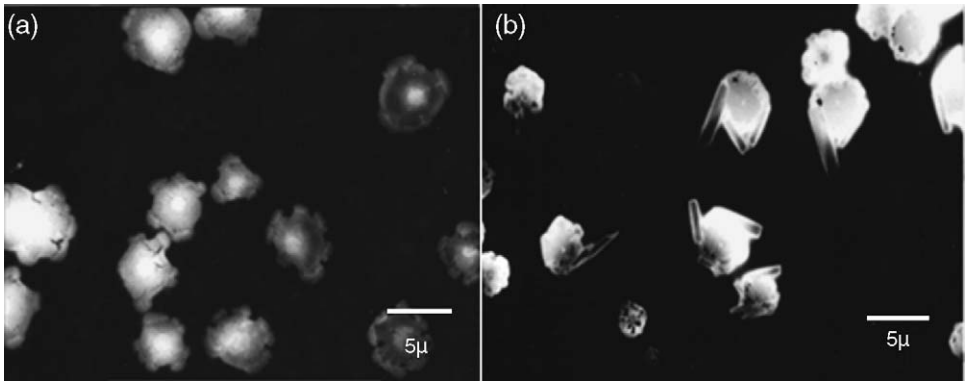

Fig. 4. Transmission electron microscope photographs of PDL (a) and ODL (b).

bation time. The ODL for following experiments were prepared by incubating OPA with PDL at 20% OPA:lipid (w/w) for 2 h.

The PDL and ODL prepared in this study visually appeared as green to yellowish suspensions. Morphologically, the PDL was spherical in shape and multilamellar in nature. However, the ODL was observed opaque probably due to the OPA coating (Fig. 4). The vesicle size of PDL compares well with ODL, i.e. 3.86 ± 0.10 μm and 3.75 ± 0.16 μm, respectively. The span of PDL and ODL was 1.17 ± 0.05 and 1.19 ± 0.02, respectively.

Relatively high entrapment efficiency ($88.08 \pm 0.84\%$) of PDL could be attributed to the lipophilic nature of the DIP, it tends to interact with the hydrophobic domain of lipid membranes (Betageri and Dipali, 1993; Patricia et al., 1997). An obvious decrease in the entrapment efficiency was measured ($83.25 \pm 0.76\%$) with OPA coating. According to the hydrophobicity of DIP, the presence of a hydrophobic and rigid molecule such as palmityl from OPA in the DIP liposomes had a negative effect on entrapment efficiency. Vyas et al. (2000, 2004) considered that *O*-steroyl amylopectin or *O*-palmitoylated mannan coated liposomes did not affect the entrapment efficiency of hydrophobic drugs such as rifampicin or amphotericin B, though the values of entrapment efficiency in the case of coated liposomes were actually lower than those of plain liposomes. In this research, the entrapment efficiency of DIP liposomes decreased significantly after the coating. It was supposed that these results may be attributed to the specific location of DIP in lipid membrane which was close to the bilayer surface in the border of hydrophobic–polar heads interface (Patricia et al., 1997). However, rifampicin was deeply buried inside the lipid bilayer (Rodrigues et al., 2003). Amphotericin B was able to take a vertical position with regard to the membrane surface. Such an orientation helped amphotericin B's polar head to interact with a polar head of the PC (Sternal et al., 2004). The palmityl group which intercalated in lipid bilayer may occupy competitively the specific position of DIP molecular, which differs from that of rifampicin or amphotericin B, set DIP molecules away from lipid bilayer and induce the decrease of entrapment efficiency.

3.2. Photostability of liposomes

The photostability study was carried out by fluorescence measurements at different time intervals. The irradiation on the samples was found to be 1.78–1.83 mW/cm^2 during the experiment. It was showed that DIP followed a first order kinetics and a good linearity was obtained by plotting the logarithm of intensities as a function of time, as in ethanol and in liposomes, in agreement with the following equation: $\log(\%I) = -kt + 2$, where $\%I$ was percent residual intensity, k the photodegradation rate constant, t the time (min) and 2 means the logarithm of initial percent intensity (100%).

The degradation was evaluated on the basis of k and $t_{1/2}$, with respect to the initial percent intensity. DIP degradation curves are plotted in Fig. 5, while Table 1 summarizes the mentioned degradation kinetic parameters based on data from three samples for each interval.

As is evident, the DIP entrapped into liposomes permitted a good photostability of the drug. Whereas in ethanol DIP degraded almost completely after 8 h, the PDL showed an increased stability if compared with the ethanol solution but a higher degradation rate than in the ODL.

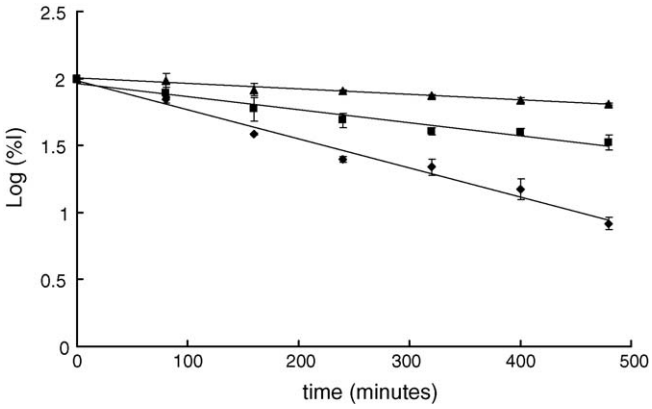

Fig. 5. Photodegradation plots of DIP, in ethanol solution (♦), PDL (■) and ODL (▲). Each point represents the mean ± S.D. ($n = 3$).

The entrapment of the drugs in liposomes represents one of the most frequently studied photoprotective carriers and successful results have been reported in many studies (Habib and Asker, 1991; Morgan et al., 1995; Bisby et al., 2000; Ragno et al., 2003; Ioele et al., 2005). The photodegradation process of DIP and its degradation product have been well described (Kigasawa et al., 1984; Vargas et al., 2001, 2002). Previous data indicated that the DIP is localized at the border of the polar and nonpolar regions, the pyrimido-pyrimidine nucleus is placed close to this border and the substituents are located in the nonpolar part (Borissevitch et al., 1995; Patricia et al., 1997). Moreover, the photodegradation of DIP takes place on the piperidine ring (Kigasawa et al., 1984; Vargas et al., 2002), so the results obtained from this photostability study led us to suppose that the photoprotection of PDL may rely on the position of the active group, which was located in the bilayer interface. The better photoprotection of ODL was probably the consequence of the shading effect from polysaccharides which intercalated in lipid bilayer membranes.

3.3. Stability study in PBS and FCS

Release properties of PDL and ODL were measured as the accumulative release percentages in outer medium during 24 h to compare the stability of them in PBS and FCS. In the experimental protocol, the DIP was released promptly from DIP injection either in PBS or in FCS. This showed that the DIP could run through the cellulose membrane tubing freely.

It could be seen that PDL was able to release $51.17 \pm 1.73\%$ of the drug, while ODL showed $35.55 \pm 0.79\%$ drug release at

Table 1
The kinetic parameters of photodegradation for DIP in ethanol solution and liposomes

Matrix	k	$t_{1/2}$ (min)	r^2
DIP ethanol solution	2.21×10^{-3}	136.20	0.9823
PDL	1.09×10^{-3}	275.81	0.9367
ODL	4.01×10^{-4}	749.84	0.9744

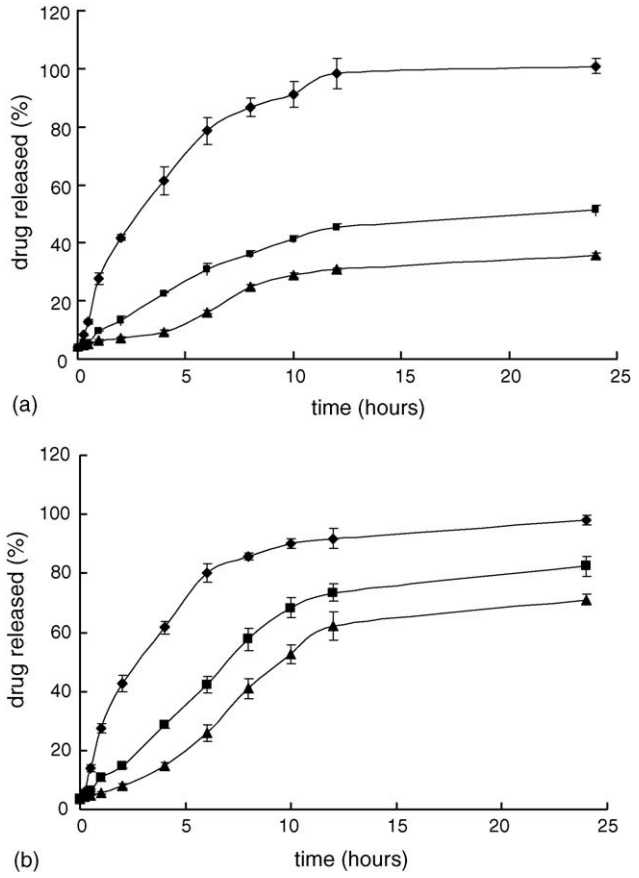

Fig. 6. Dynamic release profiles of DIP ethanol solution (♦), PDL (■) and ODL (▲) in pH 7.4 PBS (a) and FCS (b). Each point represents the mean ± S.D. ($n = 3$).

the end of the experiment (Fig. 6a). These data presented that ODL retarded the release of entrapped drug effectively in comparison to PDL in PBS. As the incubation periods increased the drug release also increased in the case of PDL for a longer time period and an almost sustained release profile was observed near the end of the experimental protocol. For the ODL, however, a biphasic release was observed. The drug release profiles followed the same trend in the FCS (Fig. 6b), as compared with PBS, the drug release was obviously higher in the case of liposomes ($82.46 \pm 3.35\%$ and $71.07 \pm 1.76\%$, respectively, from PDL and ODL) at the end of the experiment. The release record of ODL was significantly low as compared with PDL.

It was well known that the DIP has the pH-dependent solubility and insoluble in a charge neutrality condition. According to our experimental data, the solubility of DIP was 2.33×10^{-2} mg/ml and 2.51×10^{-2} mg/ml in pH 7.4 PBS and pH 7.4 FCS, respectively, at 37 °C. It was supposed that the release profiles of PDL and ODL in PBS or FCS related really to the solubility of DIP which is the rate limiting step of the release progress in pH 7.4 condition. Through the comparatively analysis of measured release profiles, the comparatively tardier and lower release in the case of ODL could be ascribed to the more stable outer surface of the vesicles. The more stable construction of ODL kept DIP in lipid bilayer more stably. On account of parallel solubility of DIP in PBS and FCS, the destruction of the vesicles and the modified polysaccharides by bioactive

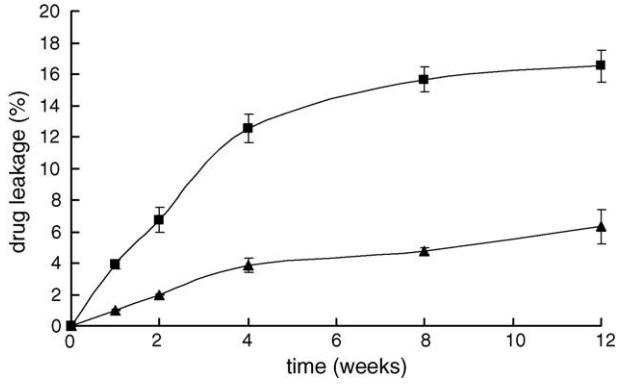

Fig. 7. Leakage profiles of PDL (■) and ODL (▲) during long-term storage. Each point represents the mean ± S.D. ($n = 3$).

substance in FCS could be a rational explanation for the phenomenon that the drug releases from both PDL and ODL in FCS were pronouncedly faster and higher than those in PBS.

3.4. Long-term stability

Long-term stability of the liposomes was examined by measuring the entrapment efficiency, vesicle size and span as a function of time at 4 ± 2 °C.

It could be seen that ODL recorded a $3.87 \pm 0.44\%$ and $6.30 \pm 1.12\%$ leakage, respectively, at the end of the 4th and 12th weeks (Fig. 7). Meanwhile, PDL showed $3.94 \pm 0.29\%$ leakage in the 1st week, and subsequently, further degradation leading to a $12.54 \pm 0.89\%$ leakage after 4 weeks. It was seen that vesicles lost their integrity and $16.52 \pm 1.03\%$ of leakage was recorded after 12 weeks of storage at 4 ± 2 °C.

During the long-term stability experiments, the vesicle size and span of PDL were found to have changed (Fig. 8). Similar trends were observed on ODL; however, the ODL showed an overall slower variation. The results were well anticipated and appreciated due to the stability of the lipid bilayer membranes interpolated with OPA. Thus, from these results, it was observed that ODL remained more stable during long-term cold storage.

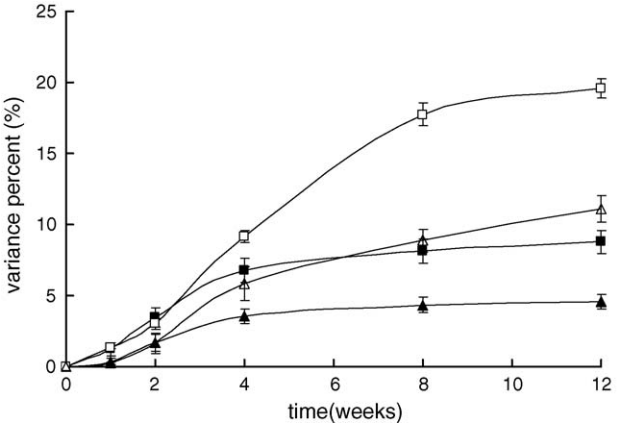

Fig. 8. Variance profiles of vesicle size ((■) PDL and (▲) ODL) and span ((□) PDL and (△) ODL). Each point represents the mean ± S.D. ($n = 3$).

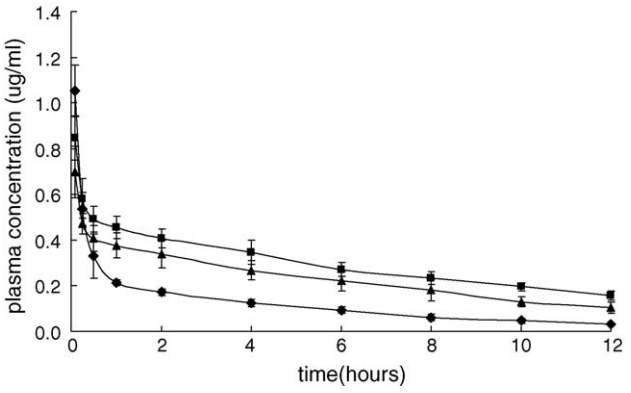

Fig. 9. Plasma concentration–time profiles in rats after intravenous administration of DIP injection (♦), PDL (■) and ODL (▲). Each point represents the mean ± S.D. ($n = 5$).

3.5. Pharmacokinetics in rats

The intravenous administration of DIP injection resulted in relatively higher plasma concentrations of DIP within half an hour after administration and then rapidly declined (Fig. 9). This could account for the rapid distribution and metabolism of the free DIP. Plasma concentration of DIP after the administrations of PDL was significantly higher than the injection after 30 min. ODL also exhibited steady higher plasma concentrations than injection after 30 min, but it was noticeably lower than that obtained after the administration of PDL. This decrease in plasma concentration in the case of ODL may be due to the enhanced affinity to macrophages and consequently hepatosplenic and lung clearance of modified amylopectin anchored DIP liposomes (Takada et al., 1984; Miyazaki et al., 1992; Deol and Khuller, 1997; Poiani et al., 1997; Sihorkar and Vyas, 2001; Vyas et al., 2004). The simulated results of pharmacokinetic data showed that open two-compartment model was fitted to all of DIP injection, PDL and ODL.

The main pharmacokinetic parameters of DIP after intravenous administration of the different formulations are illustrated in Table 2. Entrapment of drug in liposomes was applied as an approach to decrease the elimination clearance. In the present study, PDL resulted in a significant increase in the AUC, MRT and $t_{1/2\beta}$ ($p < 0.05$), which correlated with the significant decrease of the Cl ($p < 0.05$). These results were ascribed to the protection of the lipid bilayer membranes and slow drug release from liposomes. In addition, V_d of PDL was larger than those of free DIP, although statistically not significant ($p > 0.05$). The slight increase of V_d indicated the growth trend of the drug uptaken by MPS.

The declines of AUC, MRT and $t_{1/2\beta}$ could be obtained from ODL as compared with PDL ($p < 0.05$). The shortened circulation time was also correlated with a significant augmentation of Cl ($p < 0.05$). All these observations, together with the quiet increase of V_d ($p > 0.05$), were in agreement with the results from tissue distribution experiment (data not shown in this paper), which showed the MPS-rich tissues (such as liver, spleen, especially lung) took a greater uptake on ODL than on PDL. The elimination of ODL was faster, although ODL had the slower release trait in vitro studies.

Table 2
The main pharmacokinetics parameters of DIP after intravenous administration of DIP injection, PDL and ODL to rats (each value represents the mean ± S.D., $n = 5$)

Parameters	DIP injection	PDL	ODL
AUC ((μ_g/ml) h)	1.704 ± 0.121	5.326 ± 0.603*	3.722 ± 0.536*,#
MRT (h)	5.001 ± 0.380	10.319 ± 0.944*	8.245 ± 2.615*
V_d (l/kg)	0.807 ± 0.125	1.024 ± 0.251	1.288 ± 0.521
Cl (l/(kg h))	0.746 ± 0.067	0.238 ± 0.027*	0.332 ± 0.061*,#
$t_{1/2\alpha}$ (h)	0.122 ± 0.032	0.090 ± 0.036	0.083 ± 0.037
$t_{1/2\beta}$ (h)	3.955 ± 0.645	7.237 ± 0.421*	6.204 ± 0.558*,#

* Significantly different from DIP injection ($p < 0.05$).
\# Significantly different from PDL ($p < 0.05$).

4. Conclusions

In this research, OPA was synthesized and used to anchor DIP liposomes successfully. In vitro studies certified that the OPA anchored liposomes could increase stabilities of DIP against irradiation: disperse medium, biofluid and long-term storage as compared with plain liposomes. However, those results were inversely related with the values from the in vivo study, which showed the variances of pharmacokinetic parameters for ODL were a little bit lower than those for PDL. These results were correlated with the tissue targetability of OPA anchored liposomes. Modified amylopectin anchored liposomes were reported (Takada et al., 1984; Miyazaki et al., 1992; Deol and Khuller, 1997; Poiani et al., 1997; Sihorkar and Vyas, 2001; Vyas et al., 2004) to be identified and uptaken selectively by MPS, especially by the lung macrophages and monocytes; these reported were also confirmed by another tissue distribution experiment. Subsequent to these observations, investigations on OPA anchored liposomes are ongoing to elucidate the mechanism of stabilization and explore their potential as a delivery system for the treatment of lung diseases.

References

Allen, T.M., 1994. The use of glycolipids and hydrophilic polymers in avoiding rapid uptake of liposomes by the mononuclear phogocyte system. Adv. Drug Deliv. Rev. 13, 285–309.

Allen, T.M., Moase, E.H., 1996. Therapeutic opportunities for targeted liposomal drug delivery. Adv. Drug Deliv. Rev. 21, 117–133.

Arnold, P., Hayden, S.J., Haynes, S., Hasan, A., Hamilton, J.R., 2000. Use of dipyridamole for pulmonary hypertension following repair of total anomalous pulmonary venous drainage. Intensive Care Med. 26, 146.

Bangham, A.D., Standish, M.M., Watkins, J.C., 1965. Diffusion of univalent ions across the lamellae of swollen phospholipids. J. Mol. Biol. 13, 238–252.

Betageri, G.V., Dipali, S.R., 1993. Partitioning and thermodynamics of dipyridamole in the *n*-octanol/buffer and liposome systems. J. Pharm. Pharmacol. 45, 931–933.

Bisby, R.H., Mead, C., Morgan, C.G., 2000. Active uptake of drugs into photosensitive liposomes and rapid release on UV photolysis. Photochem. Photobiol. 72, 57–61.

Borissevitch, I.E., Borges, C.P., Yushmanov, V.E., Tabak, M., 1995. Localization of dipyridamole molecules in ionic micelles effect of micelle and drug charges. Biochim. Biophys. Acta 1238, 57–62.

Curtin, N.J., Turner, D.P., 1999. Dipyridamole-mediated reversal of multidrug resistance in MRP over-expressing human lung carcinoma cells in vitro. Eur. J. Cancer 35, 1020–1026.

De la Cruz, J.P., Olveira, C., Gonzalez-Correa, J.A., Benitez, A., Sanchez de la Cuesta, F., 1996. Inhibition of ferrous-induced lipid peroxidation by dipyridamole, RA-642 and mopidamol in human lung tissue. Gen. Pharmacol. 27, 855–859.

Deol, P., Khuller, G.K., 1997. Lung specific stealth liposomes: stability, biodistribution and toxicity of liposomal antitubercular drugs in mice. Biochim. Biophys. Acta 1334, 161–172.

FitzGerald, G.A., 1987. Dipyridamole. N. Engl. J. Med. 316, 1247–1257.

Grinsell, M., Weinhold, L.C., Cutler, J.E., Han, Y., Kozel, T.R., 2001. In vivo clearance of glucuronoxylomannan, the major capsular polysaccharide of *Cryptococcus neoformans*: a critical role for tissue macrophages. J. Infect. Dis. 184, 479–487.

Habib, M.J., Asker, A.F., 1991. Photostabilization of riboflavin by incorporation into liposomes. J. Parenter. Sci. Technol. 45, 124–127.

Hammerling, U., Westphal, O., 1967. Synthesis and use of *O*-stearoyl polysaccharides in passive hemagglutination and hemolysis. Eur. J. Biochem. 1, 46–50.

Ichinose, K., Yamamoto, M., Khoji, T., Ishii, N., Sunamoto, J., Kanematsu, T., 1998. Antitumor effect of polysaccharide coated liposomal adriamycin on AH66 hepatoma in nude mice. Anticancer Res. 18, 401–404.

Ioele, G., Cione, E., Risoli, A., Genchi, G., Ragno, G., 2005. Accelerated photostability study of tretinoin and isotretinoin in liposome formulations. Int. J. Pharm. 293, 251–260.

Ishiwata, H., Vertut-Doi, A., Hirose, T., Miyajima, K., 1995. Physical–chemistry characteristics and biodistribution of poly(ethylene glycol)-coated liposomes using poly(oxyetylene) cholestery ether. Chem. Pharm. Bull. 43, 1005–1011.

Kigasawa, K., Shimizu, H., Hayashida, S., Ohkubo, K., 1984. Decomposition and stabilization of drug XX. Photodecomposition and stabilization of dipyridamol. Yakugaku Zasshi 104, 1191–1197.

Letourneur, D., Parisel, C., Prigent-Richard, S., Cansell, M., 2000. Interactions of functionalized dextran-coated liposomes with vascular smooth muscle cells. J. Control. Release 65, 83–91.

Matsukawa, S., Yamamoto, M., Ichinose, K., Ohata, N., Ishii, N., Kohji, T., Akiyoshi, K., Sunamoto, J., Kanematsu, T., 2000. Selective uptake by cancer cells of liposomes coated with polysaccharides bearing 1-aminolactose. Anticancer Res. 20, 2339–2344.

Mehvar, R., 2003. Recent trends in the use of polysaccharides for improved delivery of therapeutic agents: pharmacokinetic and pharmacodynamic perspectives. Curr. Pharm. Biotechnol. 4, 283–302.

Miyazaki, T., Kohno, S., Sasayama, K., Inoue, Y., Hara, K., Ogasawara, M., Sato, T., Sunamoto, J., 1992. Polysaccharide-coated liposomal amphotericin B for the treatment of murine pulmonary candidiasis. Tohoku J. Exp. Med. 168, 483–490.

Moellerfeld, J., Prass, W., Ringsdorf, H., Hamazaki, H., Sunamoto, J., 1986. Improved stability of black lipid membranes by coating with polysaccharide derivatives bearing hydrophobic anchor groups. Biochim. Biophys. Acta 857, 265–270.

Moreira, J.N., Almeida, L.M., 1996. Evaluation of in vitro stability of large unilamellar liposomes anchored with a modified polysaccharide (*O*-palmitoyl pullulan). J. Mater. Sci. Mater. Med. 7, 301–303.

Moreira, J.N., Almeida, L.M., Geraldes, C.F., Madeira, V.M.C., Costa, M.L., 1997. Carboplatin liposomes anchored with *O*-palmitoyl pullulan: in vitro characterization. Int. J. Pharm. 147, 153–164.

Morgan, C.G., Yianni, Y.P., Sandhu, S.S., Mitchell, A.C., 1995. Liposome fusion and lipid exchange on ultraviolet irradiation of liposomes containing a photochromic phospholipid. Photochem. Photobiol. 62, 24–29.

Mumper, R.J., Hoffman, A.S., 2000. The stabilization and release of Hirudin from liposomes or lipid-assemblies coated with hydrophobically modified dextran. AAPS Pharm. Sci. Technol. 1 (article 3).

Pan, J., Lou, W., Chen, L., Liu, X., 2002. The effect of dipyridamole and adenosine on pulmonary fibrosis in mice. Zhonghua Jie He He Hu Xi Za Zhi 25, 273–275.

Parr, M.J., Ansell, S.M., Choi, L.S., Cullis, P.R., 1994. Factors influencing the retention and chemical stability of poly (ethylene glycol)-lipid conjugates incorporated into large unilamellar vesicles. Biochim. Biophys. Acta 1195, 21–30.

Patricia, M.N., Luis, E.A., Marcel, T., 1997. Binding of dipyridamole to phospholipid vesicles: a fluorescence study. Biochim. Biophys. Acta 1328, 140–150.

Pavia, D.L., Lampman, G.L., Kriz, G.S., 1979. Infrared absorption process. In: Hoit, G., Rinehart, K.L., Weinstein, I.B. (Eds.), Introduction to Spectroscopy: A Guide for Students of Organic Chemistry. WB Saunders, Philadelphia, PA, pp. 13–15.

Poiani, G.J., Kemnitzer, J.E., Fox, J.D., Tozzi, C.A., Kohn, J., Riley, D.J., 1997. Polymeric carrier of proline analogue with antifibrotic effect in pulmonary vascular remodeling. Am. J. Respir. Crit. Care Med. 155, 1384–1390.

Ragno, G., Cione, E., Garofalo, A., Genchi, G., Ioele, G., Risoli, A., Spagnoletta, A., 2003. Design and monitoring of photostability systems for amlodipine dosage forms. Int. J. Pharm. 265, 125–132.

Ranade, V.V., 1989. Drug delivery systems. 1. Site-specific drug delivery using liposomes as carriers. J. Clin. Pharmacol. 29, 685–694.

Rodrigues, C., Gameiro, P., Prieto, M., de Castro, B., 2003. Interaction of rifampicin and isoniazid with large unilamellar liposomes: spectroscopic location studies. Biochim. Biophys. Acta 1620, 151–159.

Satish, P.R., Surolia, A., 2002. Preparation and characterization of glycolipid-bearing multilamellar and unilamellar liposomes. In: Basu, S.C., Basu, M. (Eds.), Liposome Methods and Protocols, vol. 199. Humana Press, Totowa, NJ, pp. 193–202.

Sato, T., Sunamoto, J., 1992. Recent aspects in the use of liposomes in biotechnology and medicine. Prog. Lipid Res. 31, 345–372.

Sehgal, S., Rogers, J.A., 1995. Polymer-coated liposomes: improved liposome stability and release of cytosine arabinoside (Ara-C). J. Microencapsul. 12, 37–47.

Sihorkar, V., Vyas, S.P., 2001. Potential of polysaccharide anchored liposomes in drug delivery, targeting and immunization. J. Pharm. Pharm. Sci. 4, 138–158.

Sternal, K., Czub, J., Baginski, M., 2004. Molecular aspects of the interaction between amphotericin B and a phospholipid bilayer: molecular dynamics studies. J. Mol. Model 10, 223–232.

Takada, M., Yuzuriha, T., Katayama, K., Iwamoto, K., Sunamoto, J., 1984. Increased lung uptake of liposomes coated with polysaccharides. Biochim. Biophys. Acta 802, 237–244.

Takeuchi, H., Kojima, H., Yamamoto, H., Kawashima, Y., 2000. Polymer coating of liposomes with a modified polyvinyl alcohol and their systemic circulation and RES uptake in rats. J. Control. Release 68, 195–205.

Torchilin, V.P., Shtilman, M.I., Trubetskoy, V.S., Whiteman, K., Milstein, A.M., 1994. Amphiphilic vinyl polymers effectively prolong liposome circulation time in vivo. Biochim. Biophys. Acta 1195, 181–184.

Vargas, F., Cheng, A.T., Velutini, G., Marcano, E., Sanchez, Y., Fraile, G., Velasquez, M., 2001. In vitro antioxidant and photo-oxidant properties of dipyridamole. Int. J. Toxicol. 20, 363–368.

Vargas, F., Rivas, C., Fuentes, A., Cheng, A.T., Velutini, G., 2002. The photochemistry of dipyridamole. J. Photochem. Photobiol. A: Chem. 153, 237–243.

Venkatesan, N., Vyas, S.P., 2000. Polysaccharide coated liposomes for oral immunization—development and characterization. Int. J. Pharm. 203, 169–177.

Vyas, S.P., Katare, Y.K., Mishra, V., Sihorkar, V., 2000. Ligand directed macrophage targeting of amphotericin B loaded liposomes. Int. J. Pharm. 210, 1–14.

Vyas, S.P., Kannan, M.E., Jain, S., Mishra, V., Singh, P., 2004. Design of liposomal aerosols for improved delivery of rifampicin to alveolar macrophages. Int. J. Pharm. 269, 37–49.

Vyas, S.P., Quraishi, S., Gupta, S., Jaganathan, K.S., 2005. Aerosolized liposome-based delivery of amphotericin B to alveolar macrophages. Int. J. Pharm. 296, 12–25.