ELSEVIER

International Journal of Pharmaceutics 164 (1998) 103–111

international journal of pharmaceutics

# Physical stability of size controlled small unilameller liposomes coated with a modified polyvinyl alcohol

Hirofumi Takeuchi *, Hiromitsu Yamamoto, Toshitada Toyoda, Hidekazu Toyobuku, Tomoaki Hino, Yoshiaki Kawashima

*Gifu Pharmaceutical University, Mitahora, Gifu 502, Japan*

Received 2 May 1997; received in revised form 25 August 1997; accepted 19 November 1997

**Abstract**

The purpose of this work is to evaluate the improvement in physical stability of polymer coated liposomes. Small unilamellar liposomes (dimilistoylphosphatidylcholine (DMPC): dicetyl phosphate (DCP): cholesterol (Chol) = 7:3:1) 100 nm in diameter were coated with polyvinyl alcohol (PVA) and a modified PVA, which bears a long alkyl chain at the end of molecule (PVA-R), by simply mixing the liposomal suspension with the polymer solutions. Changes in particle size and zeta potential for the polymer coated liposomes confirmed the existence of a thick polymer layer on the surface of liposomes, especially for the PVA-R coated liposomes. The amount of PVA-R coating increased with increasing polymer concentration, and the estimated molar ratio of PVA-R coating to phospholipids in the liposomes was 1/120 at the highest polymer concentration (2%). The physical stability of polymer coated liposomes was evaluated by measuring the change in particle size and the retention of entrapped contents after perturbing the system with freeze-drying and followed by rehydration (FD-RH). PVA-R coated liposomes showed almost complete retention of particle size and a larger entrapped content, while a dramatic increase in particle size and a reduction in the percentage entrapped, which suggested the occurrence of aggregation and fusion of liposome particles during FD-RH, were observed for non-coated and PVA-coated liposomes. Aggregation of liposomes in calf serum was effectively prevented by coating liposomes with PVA-R. 

*Keywords:* Liposome; Stability; Polymer coating; Surface modification; Freeze-drying; Serum

## 1. Introduction

Liposomes have been extensively studied for their potential use as drug carriers. For the successful introduction of liposomal formulations in drug therapy, much attention must be paid to

* Corresponding author. Tel: +81 58 2373931 ext. 245; fax: +81 58 2375979; e-mail: takeuchi@gifu-pu.ac.jp.

0378-5173/98/$19.00 
*PII* S0378-5173(97)00404-3

their stability, because liposomes tend to degrade or aggregate and fuse, which leads to leakage of entrapped drug during storage or after administration.

Many attempts have been made to enhance the stability of liposomes. Among them, surface modification of liposomes is an attractive method to improve liposomal stability both in vitro and in vivo. Sunamoto et al. (1983, 1984) have demonstrated improvement in chemical and physical stability of polymer coated liposomes prepared with polysaccharide derivatives such as mannan or amylopectin. So far several substances such as poloxamer (Jamshaid et al., 1988), polysorbate 80 (Kronberg et al., 1990), carboxymethyl chitin (Dong and Rogers, 1991), carboxymethyl chitosan (Alamelu and Rao, 1991) and dextran derivatives (Elferink et al., 1992) have been used for preparation of polymer-coated liposomes. While the possibility of coating liposomes with these polymers has been reported, few papers have dealt with the systematic evaluation of the physical stability of polymer coated liposomes. Moreover, contravening results have been reported, such as that polymer-coated liposomes showed little or less stability than non-coated liposomes (O'Connor et al., 1985; Jamshaid et al., 1988). To clarify the effectiveness of polymer coatings on the physical stability of liposomes, more information is required, especially with respect to the relationship between properties of coatings and improvement in stability of liposomes.

In this study, small unilamellar liposomes (SUVs) 100 nm in diameter were coated with polyvinyl alcohol (PVA) and a modified PVA, which bears a long alkyl chain at the end of molecule (PVA-R). Properties of coatings were evaluated by measuring the particle size and zeta potential. The physical stability of the polymer coated liposomes was investigated by evaluating the change in particle size and the retention of entrapped FlTC-dextran after inducing stress in the liposomes by freeze-drying followed by rehydration or incubation in serum. Effects of polymer coatings on liposome stability are discussed with consideration of the coating properties of these polymers.

## 2. Experimental

### 2.1. Materials

L-α-dimilistoylphosphatidylcholine (DMPC, Nippon Oil and Fats Co.), dicetyl phosphate (DCP, Sigma) and cholesterol (Chol, Sigma) were used as received. Fluorescein isothiocyanate dextran (FlTC-dex) of three different molecular weights (4400, 9300, 18 900) and 5(6)-carboxyfluorescein (CF) were purchased from Sigma. Polyvinyl alcohol (PVA) and polyvinyl alcohol having a hexyl group at the end of molecule (PVA-R) were a gift from Kuraray Co. The degree of polymerization of the polymers was 480. Calf serum (Life Technologies) was used as obtained. All other reagents were of analytical grade.

### 2.2. Preparation of liposomes and polymer-coated liposomes

Small unilamellar liposomes (SUVs) composed of DMPC, DCP and cholesterol (7:3:x in molar ratio, x = 1,2,3) were prepared by thin film hydration followed by extrusion with the Extruder (Lipex Biomembranes) equipped with polycarbonate membranes. In a typical procedure, 155.1 mg of DMPC, 53.6 mg of DCP and 12.6 mg of cholesterol were dissolved in a small amount of chloroform, and the solution was rotary evaporated at 50°C to obtain a thin lipid film. The thin lipid film was dried in a vacuum oven overnight to ensure complete removal of the solvent, and hydration was carried out with 4 ml of phosphate buffer solution (pH 7.4) using a vortex mixer. The resultant multilamellar liposomal suspension was extruded and then incubated at 10°C for 30 min.

Liposomes containing FlTC-dex or CF for stability tests were prepared in the same manner except a phosphate buffer solution containing an appropriate amount of FlTC-dex (2 mg/ml) or CF (20 μM) was used for hydration instead of phosphate buffer solution.

For preparation of polymer-coated liposomes, an appropriate amount of PVA or PVA-R was dissolved in phosphate buffer solution (pH 7.4). An aliquot of SUV liposome suspension was

mixed with the same volume of polymer solution at various concentrations (0–4%). The final polymer concentration in the liposome suspension was 0–2%. The mixed solution was incubated at 10°C for 60 min. To adjust the liposomal concentration of non-coated liposomes to coated ones, the same amount of phosphate buffer was added to the non-coated liposomes followed by incubation under the same conditions.

### 2.3. Properties of liposomes

The size of liposomes was measured by dynamic light-scattering (LPA-300, Otuka Electronics).

The zeta potential of the liposomes was measured with a zeta meter (Laser Zee Meter 501, Penkem, Inc.). To measure the zeta potential, an aliquot of liposomal suspensions was diluted with a large amount of phosphate buffer solution at pH 7.4.

To calculate the amount of polymeric coating, an aliquot of a liposomal suspension (0.3 ml) was ultra-centrifuged at 75 000 rpm for 120 min with CS-100 (Hitachi). After adding 3 ml of boric acid solution (4%) and 0.6 ml of 0.05 M iodine test solution, which was prepared by diluting the 0.5 M iodine test solution specified in JPXIII with distilled water, to 0.05 ml of the supernatant and diluting to 10 ml with distilled water, the polymer concentration was measured spectrophotometrically at a wavelength of 620 nm. The amount of coating was calculated from the reduced polymer concentration in the solution after coating.

### 2.4. Stability tests

The liposomal suspension was subjected to freeze-drying followed by rehydration (FD-RH) with water to assess the physical stability of the liposomes. One ml of liposomal suspension was preparatively frozen at −100°C and applied to the freeze-dryer (Neocool, Yamato Japan) at −100°C for 3 h. The freeze-dried cake was rehydrated with 1 ml of distilled water by vortex-mixing till the cake was optically degraded to form a slightly turbid solution. The particle size of the dispersed liposomes was measured with a LPA-300 (Otuka Electronics). For aggregated particles (larger than about 3 μm in size) Cis-1 (Gala) Inc.), which is a laser-based time of transition analysis system combined with an image analysis system, was used.

To evaluate the physical stability of liposomes with regard to the retention of entrapped content, the percent of entrapped FlTC-dex or CF was measured before and after FD-RH. Aliquots of the liposomal suspension (0.25 ml each) were taken before and after FD-RH. The samples were ultracentrifuged to form pellets at 75 000 rpm for 120 min with a CS-100 (Hitachi). The pellets resuspended into a constant volume of phosphate buffer solution (pH 7.4) were mixed with 10% Triton X-100 solution and heated at 60°C for a few minuets to collapse the liposomal structures. The concentration of FlTC-dex or CF in the solution was measured with a spectrofluorimeter (F3010, Hitachi). The percent of encapsulated substance was calculated by dividing the amount of substance in the pellets by the amount in the whole liposomal suspension, which was observed with spectrofluorimeter after the liposomal structure was destroyed in the same manner. The retention of content was calculated using these entrapped percentages.

In measuring the stability of liposomes in calf serum, an aliquot of liposomal suspension diluted 100-fold with the calf serum was incubated at 37°C for 60 min. The change in particle size was evaluated in the same manner as the physical stability test.

## 3. Results

### 3.1. Characterization of liposomes coated with PVA-R

The SUVs consisting of DMPC, DCP and Chol in a molar ratio of 7:3:x (x = 1,2,3) were coated with PVA-R or PVA at various concentrations in the solution. The particle size of the liposomes is listed in Table 1. The particle size of PVA-R-coated liposomes in each formulation was increased with the increasing concentration of polymer solution in the coating, suggesting the

Table 1
Particle size of liposomes before and after coating with PVA-R

| Lipid composition (DMPC/DCP/Chol) | Concentration of PVA-R (%) | Dw (nm) | | D (coated)/D (original) |
|---|---|---|---|---|
| | | Original | Coated | |
| 7:3:1 | 0.0 | 117.1 | 119.8[a] | 1.02 |
| | 0.5 | ↓ | 143.5 | 1.23 |
| | 1.0 | ↓ | 146.7 | 1.25 |
| 7:3:2 | 0.0 | 111.7 | 106.7[a] | 0.96 |
| | 0.5 | ↓ | 115.1 | 1.03 |
| | 1.0 | ↓ | 131.6 | 1.18 |
| 7:3:3 | 0.0 | 113.0 | 113.2[a] | 1.00 |
| | 0.5 | ↓ | 128.8 | 1.14 |
| | 1.0 | ↓ | 143.4 | 1.27 |

D (original) = Dw of non-coated liposomes; D (coated) = Dw of PVA-R coated liposomes.
[a]The values were observed for the non-coated liposomes after adding phosphate buffer solution instead of the polymer solution.

formation of a coating layer on the surface of the liposome particles and an increase in thickness of the coating layer with an increase in the concentration of the polymer solution.

The particle size ratio of a polymer coated liposome to a non-coated one was calculated for the liposomes DMPC/DCP/Chol = 7:3:1 with several runs and the values were plotted against the concentration of the polymer solution used in coating (Fig. 1). The result confirmed reproducibility of the increase in particle size by coating with the polymer. When PVA was used as the coating polymer, the extent of change in particle size was much smaller than when PVA-R was used.

The zeta potential of the SUV liposomes (DMPC/DCP/Chol = 7:3:1) coated with PVA-R or PVA was measured to elucidate the difference in the manner of coating by the polymers on the surface of liposomes. The zeta potential of PVA-R coated liposomes gradually reached zero with the increasing concentration of the polymer solution used in the coating (Fig. 2). The change in the zeta potential may be attributed to the increase in thickness of the polymer layer formed on the surface of liposomes that can move the

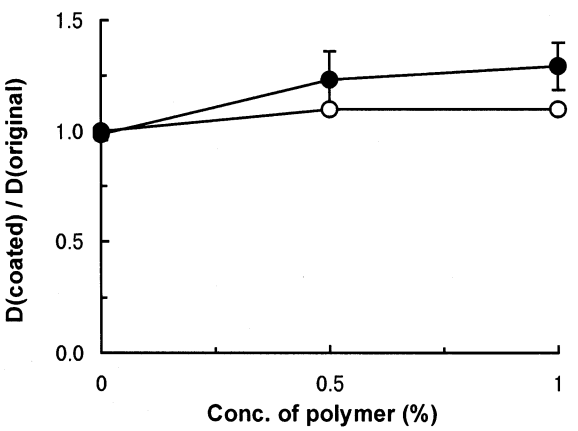


Fig. 1. Particle size change in liposomes by coating with PVA-R or PVA. Lipid composition of liposomes: DMPC/DCP/Chol = 7:3:1. ●: PVA-R, ○: PVA.

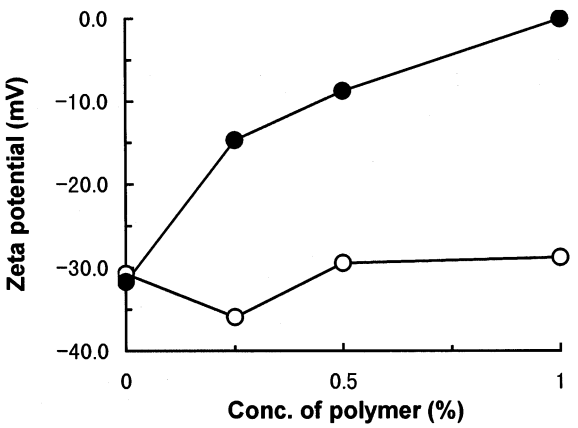


Fig. 2. Zeta potential of liposomes coated with PVA-R or PVA. Lipid composition of liposomes: DMPC/DCP/Chol = 7:3:1. ●: PVA-R, ○: PVA.

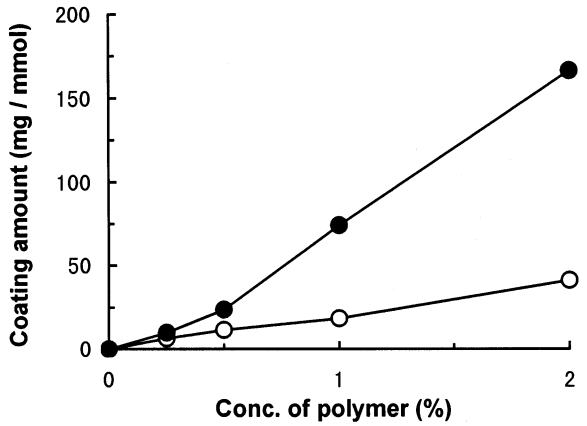


Fig. 3. Amount of PVA-R or PVA coating onto liposomes. Lipid composition of liposomes: DMPC/DCP/Chol = 7:3:1. ●: PVA-R, ○: PVA.

sheared plane into the bulk solution side. The zeta potential of PVA-coated liposomes remained almost constant regardless of the concentration of the polymer solution used in coating. This suggested formation of a thin coating layer of PVA, which corresponded to the results for particle size measurement of PVA-coated liposomes.

The amount of polymer coating the surface of the liposomes was estimated by measuring the polymer concentration of the supernatant in the centrifuged liposomal suspension after coating. The amount of PVA-R coating increased with increasing concentration of PVA-R used in coating (Fig. 3). When the amount of coating is calculated on a molar basis by considering the molecular weight of polymer to be about 20 000, the molar ratio of PVA-R to phospholipids is 1/120 at a polymer concentration of 2%. Although the mount of PVA coating also increased with the concentration of the polymer solution, the amount was much smaller than PVA-R when compared at the same polymer concentration.

### 3.2. Physical stability of polymer coated liposomes

The physical stability of the liposome (DMPC/DCP/Chol = 7:3:1) was evaluated by measuring the change in particle size and the retention of entrapped contents after perturbing the system by freeze-drying followed by rehydration (FD-RH). Table 2 lists the ratios of particle size and percent of entrapped FlTC-dexs (FITC-L, M, H) or CF retained for PVA-R coated or non-coated liposomes to original values after FD-RH. The higher values of particle size ratio for non-coated liposomes suggested aggregation and/or fusion of liposomal particles during FD-RH. Such perturbation in liposomal particles was also confirmed by reduction in the retention of entrapped FlTC-dex. The PVA-R coated liposomes showed smaller change in particle size and higher percent retention, suggesting their resistance to physical stresses. The values improved more by increasing the concentration of polymer, i.e. the amount of the coating. When the concentration of polymer solution was 1% in the coating, the particle size of the coated liposomes was almost retained after a physical stress was applied with FD-RH. The retention of FITC-H, having the highest molecular weight among the FlTC-dexs tested, by PVA-R coated liposomes was almost 100%. A similar correlation between coating efficiency and physical stability was observed for the liposomes having different amounts of cholesterol in their formulation (DMPC/DCP/Chol = 7:3:x, x = 2,3) (data is not shown).

When PVA was used for coating instead of PVA-R, the protective effect against the physical stress with FD-RH was much decreased (Fig. 4). The lower coating efficiency of PVA compared to PVA-R (Fig. 3) may be responsible for the differences in physical stability of the polymer-coated liposomes.

### 3.3. Stability of liposomes in serum

The effects of polymer coating on the stability of liposomes in calf serum was also evaluated by measuring the change in particle size. The particle size of noncoated liposomes (DMPC/DCP/Chol = 7:3:1) after incubation in calf serum increased approximately 10-fold, which suggested that aggregation and/or fusion of liposomes occurred in the serum. The aggregation and/or fusion was depressed by the PVA-R coating as shown in Fig. 5. The polymer layer on the surface of liposomes may effectively prevent the adsorp-

Table 2
Change in particle size and percent of entrapped-substance retained for PVA-R coated liposomes after freeze-drying and rehydration (FD-RH)

| Substance (MW) | Concentration of PVA-R (%) | Particle size ratio (−) after FD-R/before FD-R | | Remaining ratio of substance (%) after FD-R/before FD-R | |
|---|---|---|---|---|---|
| | | 1st | 2nd | 1st | 2nd |
| FITC-L (4400) | 0.00 | 33.1 | 39.5 | 30.7 | 10.9 |
| | 0.25 | 8.1 | 23.0 | 72.3 | 45.3 |
| | 0.50 | 1.9 | 4.2 | 64.8 | 54.9 |
| | 1.00 | 1.2 | 1.5 | 70.7 | 54.5 |
| FITC-M (9300) | 0.00 | 59.5 | 65.7 | 42.1 | 39.3 |
| | 0.25 | 14.1 | 21.4 | 68.6 | 67.0 |
| | 0.50 | 2.4 | 6.5 | 76.2 | 62.1 |
| | 1.00 | 1.0 | 1.4 | 69.3 | 65.8 |
| FITC-H (18 900) | 0.00 | 35.6 | 40.3 | 60.3 | 50.1 |
| | 0.25 | 22.4 | 23.8 | 100 | 79.0 |
| | 0.50 | 3.2 | 3.8 | 97.9 | 93.0 |
| | 1.00 | 1.0 | 1.2 | 99.7 | 95.9 |
| CF (376.3) | 0.00 | 63.7 | 72.5 | 61.6 | 48.0 |
| | 1.00 | 1.1 | 1.2 | 70.4 | 61.5 |

Lipid Composition = DMPC/DCP/Chol = 7:3:1; MW = molecular weight; 1st = after FD-RH; 2nd = after double FD-RH.

tion of aggregation promoting components such as proteins in serum. The effect of the PVA coating was not sufficient to prevent the destabilization of liposomes in the serum (Fig. 5).

When comparing the stability of non-coated liposomes containing different amounts of cholesterol (DMPC/DCP/Chol = 7:3:x, x = 1,2,3), it was found that stability of liposomes in serum can be improved by increasing the amount of cholesterol in the liposomal formulation. The rigid structure of liposomes containing larger amounts of cholesterol is responsible for the improved stability. However, these non-coated liposomes are still sus-

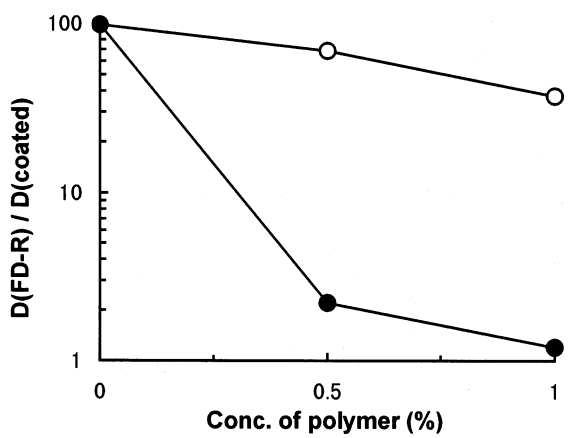


Fig. 4. Relative particle size of liposomes coated with PVA-R or PVA after freeze drying-rehydration (FD-RH) relative to that before FD-RH. Lipid composition of liposomes: DMPC/DCP/Chol = 7:3:1. ●: PVA-R, ○: PVA.

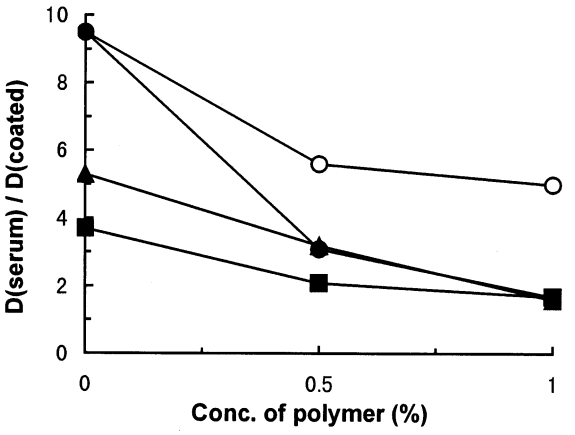


Fig. 5. Effect of polymer coating on changes in the particle size of liposomes after incubation in calf serum. Open symbol: PVA coated liposomes, closed symbol: PVA-R coated liposomes. Lipid composition of liposomes: ○,●: 7:3:1, ▲: 7:3:2, ■: 7:3:3.

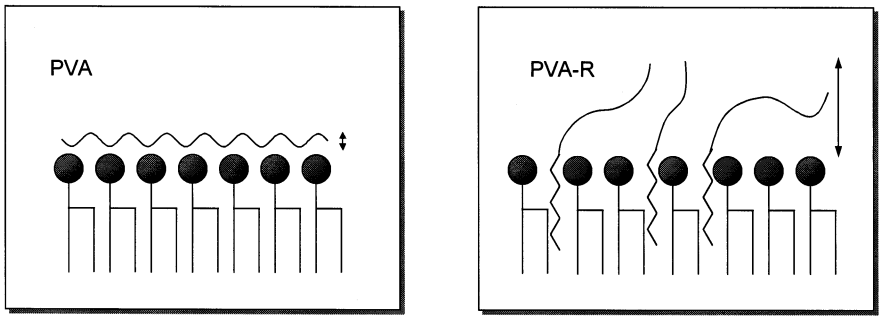


Fig. 6. Schematic drawing of different manners of coating of PVA and PVA-R on the surface of liposomes.

ceptible to destabilization, and an improvement in stability was conferred by PVA-R coating (Fig. 5).

## 4. Discussion

In considering the difference in coating properties of PVA and PVA-R, we note the differences in the manner by which these polymers coat. Hydrophillic polymers having a hydrophobic moiety in the molecule such as PVA-R coat the surface of liposomes by anchoring onto the phospholipid bilayer (Ringsdorf et al., 1991), while the possible coating mechanism of PVA is a simple physical adsorption. A different manner of coating is schematically drawn in Fig. 6. The difference in zeta potential of PVA or PVA-R coated liposomes can be explained well by the different coating manner.

The amount of PVA-R coating was much higher than that of PVA when compared at the same polymer concentration. This can also be attributed to the different manner of coating. The molar ratio of PVA-R to phospholipids in PVA-R coated liposomes with a 2% polymer solution is 1/120. Ringsdorf et al. (1991) have estimated the molar ratio of octadecyl-pyrene coupled with poly N-isopropylacrylamide to DMPC forming small unilamellar liposomes showing the upper level of inserted hydrophobic anchor groups to the phospholipid is 1/110 lipids. There is a good accordance between these two values.

Jamshaid et al. (1988) have estimated the apparent thickness of a polyoxyethylene-polyoxypropylene (POE-POP) block copolymer layer formed on the surface of polystyrene microspheres and SUVs composed of egg phosphatidylcholine as ca. 2–10 nm, depending on the type of POE-POP polymers and concentration of polymer. The estimated thickness of PVA-R layers on the surface of liposomes, ca. $30 \pm 10$ nm, is thicker than the observed value for the POE-POP layer. Considering the higher molecular weight of PVA-R and the coating mechanism described above, the thicker coating layer of PVA-R may be reasonable.

While there are some papers reporting the improved physical stability of polymer coated liposomes (Sunamoto et al., 1983; Dong and Rogers, 1991; Elferink et al., 1992), few systematic evaluations have been done to clarify the mechanism of the protective effects of the coating layer on the surface of the liposomes. Moreover, Jamshaid et al. (1988) has reported a contravening result, namely that the polymer coated liposomes were less stable than non-coated liposomes with respect to retention of entrapped contents.

The stability test with FD-RH reported in this paper revealed that the PVA-R coated liposomes are much more stable than non-coated liposomes. Retention of original particle size and percent entrapped FlTC-dex for PVA-R coated liposomes revealed the most complete protective effects of the polymer layer on the surface of liposomes against the physical stresses during FD-RH. It has been widely accepted that addition of cryoprotectants such as carbohydrates to liposomal suspensions leads to stabilization of the liposome particles during freeze-drying or freeze-thawing

(Crommelin and van Bommel, 1984; Crowe et al., 1987). The protective effects are ascribed to formation of amorphous glass states of the additives with water that prevent phase separation and fusion (Miyajima et al., 1986) and/or replacing water hydrogen bonds and forming a puseudohydration phase through their interaction with phospholipid head groups (Strauss et al., 1986; Crowe et al., 1988). PVA forms a glassy state at low temperature, and hydrogen bonds form between the polymer and water molecules. In considering these characteristics of the polymer, a similar protective mechanism for the aggregation and/or fusion of liposomes during freezing or dehydration as in the case of carbohydrates is expected for the polymer layer on the surface of liposomes.

However, PVA coated liposomes showed fewer protective effects than PVA-R coated liposomes, although PVA has a similar chemical structure and molecular weight to PVA-R. The difference in amount of coating (Fig. 3) partly explains the fewer protective effects of PVA-coated liposomes. The physical spacing effect (Strauss et al., 1986) is an alternative explanation for the excellent stability of PVA-R coated liposomes. The thick polymer layers of PVA-R on the surface of liposomes forms spaces between the liposome particles that may prevent the liposome particles from contacting.

From the practical point of views a dried form of liposomes is preferable for storage with respect not only to the retention of particle size and entrapped content but also in terms of the chemical stability of entrapped drugs. To achieve optimum conditions, critical parameters have been extensively studied. In cryoprotectant addition the effects of cryoprotectant depend on the freezing temperature and a higher freezing temperature ($-25$°C) is necessary to enable complete retention of entrapped content (Fransen et al., 1986). As the freezing temperature of $-100$°C in this study is more severe than the critical temperature, a grate protective effect of PVA-R coatings is expected. It is also preferable that the added amount of PVA-R is relatively small in comparison to cryoprotectants.

The PVA-R coated liposomes retained their original particle size after incubation in serum by protecting against the adsorption of various species in serum (Fig. 5). In considering i.v. injection of liposomes, an increase in particle size of liposomes in serum is not preferable for circulation because larger particles tend to be removed from the blood by the cells of the mononuclear phagocyte system. We have demonstrated the prolonged circulation time of PVA-R coated liposomes containing doxorubicine compared with non-coated and PVA coated liposomes after i.v. injection in rats (Takeuchi et al., 1996). The retention of particle size of PVA-R coated liposomes after incubation in serum (Fig. 5) is well correlated to the in vivo stability of PVA-R coated liposomes.

## 5. Conclusion

PVA-R can form a thick polymer layer on the surface of liposomes by the simple method of mixing a liposomal suspension with a polymer solution followed by incubation. The excellent physical stability of the resultant PVA-R-coated liposomes was confirmed by freeze-drying and rehydration tests. Aggregation of liposomes in serum was effectively prevented by the polymer coating. The physically adsorbed coating layer of PVA on the surface of liposomes was less effective for improving the physical stability of liposomes, although PVA used for coating has a molecular weight similar to PVA-R. Based on these results, a thicker polymer layer on the surface of liposomes is necessary to improve the physical stability of the liposomes, and a hydrophillic polymer having a hydrophobic moiety at the end of the molecule such as PVA-R is one of the most promising materials for the preparation of liposomes stabilized with coatings.

## Acknowledgements

Part of this work was supported by a Grant-in-Aid for Scientific Research (C, 07807197) from the Ministry of Education, Science, Sports and Culture, Japan.

## References


Alamelu, S., Rao, K.P., 1991. Studies on the carboxymethyl chitosan-containing liposomes for their stability and controlled release of dapson. J. Microencapsulation 8, 505–515.

Crommelin, D.J.A., van Bommel, E.M.G., 1984. Stability of liposomes on storage: freeze dried, frozen or as an aqueous dispersion. Pharm. Res. 159–163.

Crowe, J.H., Crowe, L.M., Carpenter, J.F., Aurell Wistrom, C., 1987. Stabilization of dry phospholipid bilayers and proteins by sugars. Biochem. J. 242, 1–10.

Crowe, J.H., Crowe, L.M., Carpenter, J.F., Rudolph, A.S., Aurell Wistrom, C., Spargo, B.J., Anchordoguy, T.J., 1988. Interactions of sugars with membranes. Biochim. Biophys. Acta 947, 367–384.

Dong, C., Rogers, J.A., 1991. Polymer-coated liposomes: stability and release of ASA from carboxymethyl chitin-coated liposomes. J. Contl. Rel. 17, 217–224.

Elferink, M.G.L., de Wit, J.G., Veld, G., In't, R.A., Driessen, A.J.M., Ringsdorf, H., Konings, W.N., 1992. The stability and functional properties of proteoliposomes mixed with dextran derivatives bearing hydrophobic anchor groups. Biochim. Biophys. Acta 1106, 23–30.

Fransen, G.J., Salemink, P.J.M., Crommelin, D.J.A., 1986. Critical parameters in freezing of liposomes. Int. J. Pharm. 33, 27–35.

Jamshaid, M., Farr, S.J., Kearney, P., Kellaway, I.W., 1988. Poloxamer sorption on liposomes: comparison with polystyrene latex and influence on solute efflux. Int. J. Pharm. 48, 125–131.

Kronberg, B., Dahiman, A., Carifors, J., Karlsson, J., Artursson, P., 1990. Preparation and evaluation of sterically stabilized liposomes: colloidal stability, serum stability, macrophage uptake, and toxicity. J. Pharm. Sci. 79, 667–671.

Miyajima, K., Tomita, K., Nakagaki, M., 1986. Effect of saccharides on the freezing and thawing of liposome dispersion. Chem. Pharm. Bull. 34, 2689–2697.

O'Connor, C.J., Wallace, R.G., Iwamoto, K., Taguchi, T., Sunamoto, J., 1985. Bile salt damage of egg phosphatidylcholine liposomes. Biochim. Biophys. Acta 817, 95–102.

Ringsdorf, H., Venzmer, J., Winnik, F.M., 1991. Interaction of hydrophobically-modified poly-N-isopropylacrylamides with model membranes – or playing a molecular accordion. Angew. Chem. Int. Ed. Engl. 30, 315–318.

Sunamoto, J., Iwamoto, K., Takada, M., Yuzuriha, T., Katayama, K., 1983. Improved drug delivery to target specific organs using liposomes coated with polysacchrides. Polymer Sci. Technol. 23, 157–168.

Sunamoto, J., Iwamoto, K., Takada, M., Yuzuriha, T., Katayama, K., 1984. Polymer coated liposomes for drug delivery to target specific organs. In: Anderson, J.M., Kim, S.W. (Eds.), Recent Advances in Drug Delivery Systems. pp. 153-162.

Strauss, G., Schurtenberger, P., Hauser, H., 1986. The interaction of saccharides with lipid bilayer vesicles: stabilization during freeze-thawing and freeze-drying. Biochim. Biophys. Acta 858, 169–180.

Takeuchi, H., Toyoda, T., Toyobuku, H., Yamamoto, H., Hino, T., Kawashima, Y., 1996. Improved stability of doxorubicin-loaded liposomes by polymer coating. Proc. of Control. Rel. Bioact. Mater. 23, 409–410.


.