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Degradation of phosphatidylcholine in liposomes containing carboplatin in dependence on composition and storage conditions

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Abstract

In this study, the hydrolytic degradation of phosphatidylcholine in aqueous liposome-dispersions and the stability of the anti cancer drug carboplatin enclosed in the liposomes were investigated in dependence on liposome composition and storage conditions. Cholesterol containing liposomes show a high stability of the phosphatidylcholine and the encapsulated carboplatin during six months storage in refrigerator. The hydrolytic degradation of phosphatidylcholine is strongly increased by addition of the antioxidant ascorbyl palmitate, but despite the partial hydrolysis the advantages of the lipid membrane are retained — no degradation of the drug and no changes in the particle size were detected during six months storage in refrigerator, in contrast to storage at room temperature.

Keywords: Phosphatidylcholine liposomes; Lipids; HPLC; Particle size; Stability

Drug delivery systems like liposomes are gaining more and more importance in pharmaceutical research because of the enhancement of therapeutic efficiency of the enclosed drugs and the prolongation of the therapeutic effect. But the liposomal formulations have limited storage stability. Phosphatidylcholine is most commonly used in pharmaceutical liposome preparations. It is known for drug effluxing effects (Au et al., 1987). The use of hydrogenated phosphatidylcholine and the addition of cholesterol (Ch) increase the stability of the lipid membrane (Kibat and Stricker, 1985), and a former addition of an antioxidant such as a-tocepherol or butyl hydroxy toluene decreases the oxidative degradation (Grit and Crommelin, 1993).

Aqueous carboplatin (CP) containing liposomes are known for their hematopoiesis stimulating effects (Fichtner et al., 1993). In this study the degradations of phosphatidylcholine and carboplatin (CP) in multilaminar liposomes containing aqueous CP were investigated in dependence on composition and storage conditions. The liposomes consist of fully hydrogenated soy-bean-phosphatidylcholine (PC) with or without Ch and/or ascorbyl palmitate (AP). AP has antioxidative effects and stabilises the lipid membrane.

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The liposomes were prepared according to the 'film' method (mass composition PC/Ch/AP = 70/10/15) followed by filtration through polycarbonate filters near by transition state temperature and several steps of centrifugation, water-washing and redispersion to remove nonenclosed CP. The

Table 1
Contents of PC and degradations products after 6 months storage (in% with respect to the value observed after preparation)

Storage conditions	Content of PC, LPC and PE (in%) in liposomes consisting of			
	PC	PC + Ch	PC + AP[a,b]	PC + Ch + AP[a,b]
4°C and darkness	PC 94.6 LPC 5.4 PE 0.0	PC 100 LPC 0.0 PE 0.0	PC 60.0 LPC 11.0 PE 5.0	PC 65.3 LPC 12.0 PE 5.0
Room temperature and day light	PC 85.8 LPC 14.2 PE 0.0	PC 100 LPC 0.0 PE 0.0	PC 19.0 LPC 59.6 PE 5.0	PC 21.8 LPC 55.1 PE 5.0

[a] The difference to 100% consists of water soluble compounds which were separated during preparation of the samples for HPLC.
[b] pH of the liposome preparation: 3.5–4.0.

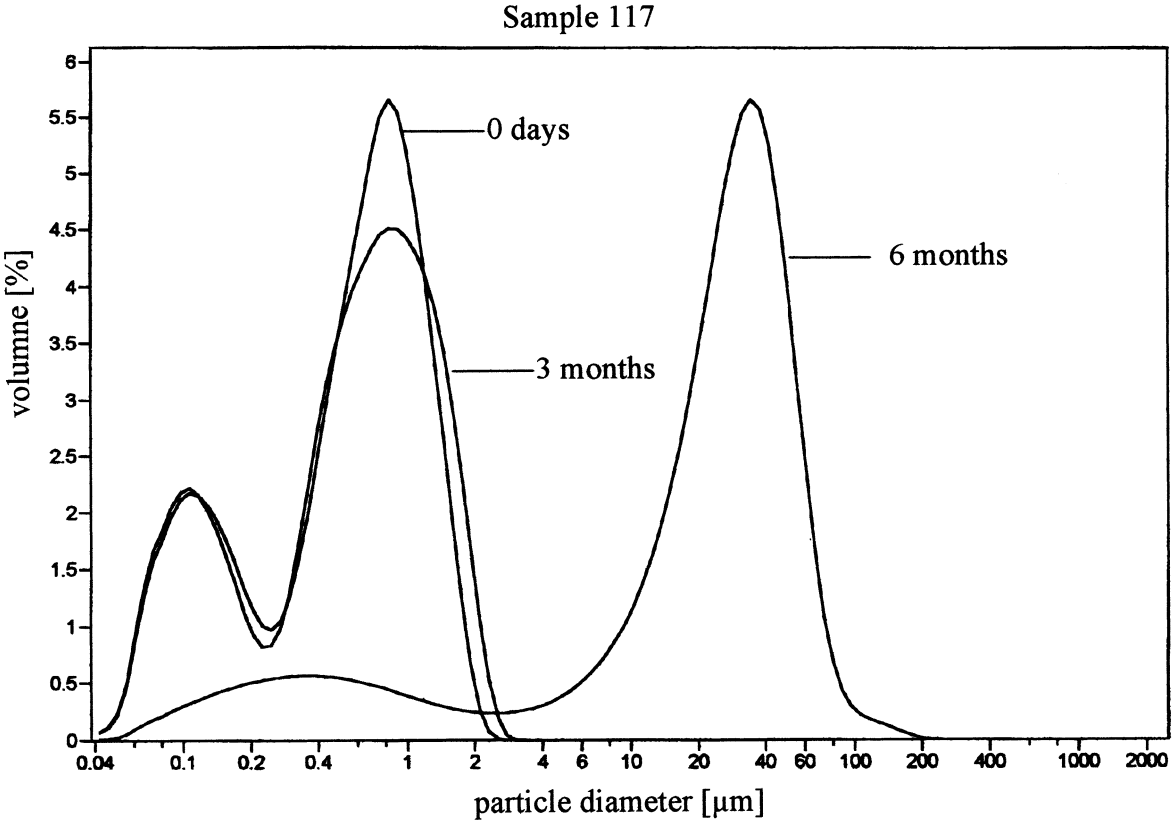

Fig. 1. Particle size distribution of sample 117 (stored at RT).

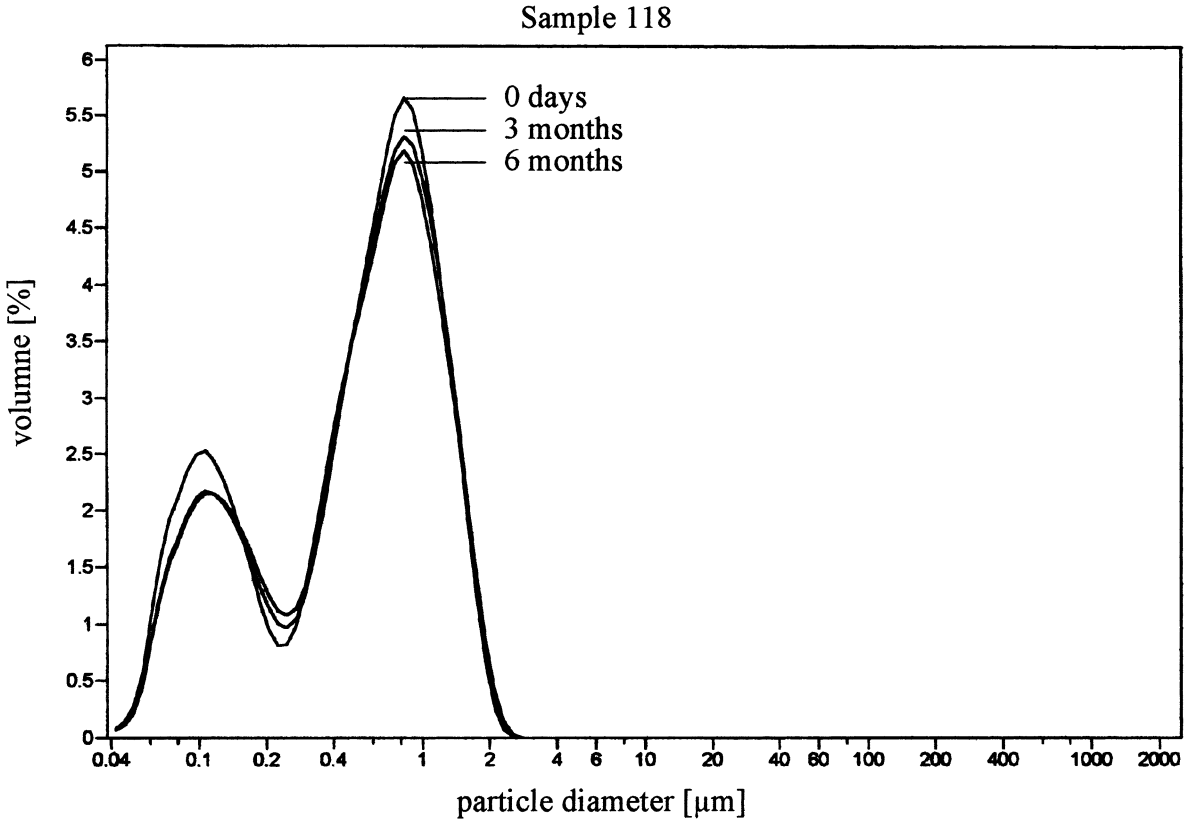

Fig. 2. Particle size distribution of sample 118 (stored in refrigerator).

Table 2
Content of CP in liposomal preparations after 6 months storage (in% with respect to the values observed after preparation)

Storage conditions	CP-content[a] (in%) in liposomes consisting of			
	PC	PC+Ch	PC+AP[b]	PC+Ch+AP[b]
4°C and darkness	100	100	92	92
Room temperature and day light	93	95	51	45

[a] HPLC on RP8 with phosphatbuffer/ion pair reagent, UV 210 nm.
[b] pH of the liposome preparation: 3.5–4.0.

preparations were stored in refrigerator in the darkness or at room temperature (RT) under day light.

In aqueous dispersion PC is hydrolysed to form free fatty acids and lyso-PC (LPC) as the main degradation products. Additionally the hydrolysis results in a little content of phosphatidylethanolamine, in which the nitrogen atom is demethylated. Theoretically, LPC and PE can further be hydrolysed to give glycerophospho compounds, but such substances were not observed in this study.

Table 1 shows the contents of PC, LPC and PE in the liposome preparations (in% with respect to the values observed after preparation) six months after storage at the given conditions. They were

detected using a HPLC-method with an NH_2-column, an eluent consisting of acetonitrile/methanol/water and a light scattering detector.

The data show that CP containing liposomes made from PC are sufficiently stable under refrigerator storage. The addition of Ch yields liposomes which are stable at RT too. CP-liposomes from PC + Ch show sedimentations which were excluded by adding the antioxidant AP. But the hydrolytic degradation of PC is strongly increased by this addition, possibly because of the low pH of the resulting liposome dispersion (3.5–4.0). According to the literature the lipid bilayer structure is less stable when the liposomes contain an elevated concentration of LPC, and the membrane becomes more sensitive for fusion (Weltzien, 1979). The comparison of the particle size distributions of the PC + Ch + AP-liposomes at RT (sample 117) or stored in refrigerator (sample 118) given in Figs. 1 and 2 shows dramatic changes in dependence on storage time and temperature.

Although the liposomes of sample 118 consist of 12% LPC no changes in particle size are detected after three or six months with respect to the curve observed after preparation. Also the curves of the particle size mesurements of PC- and PC/Ch-liposomes show the same behavior. On the contrary, in the liposomal-dispersion of sample 117 fusions and a strong growth of the particles was observed. Especially the curve after 6 months, which shows this undesirable effect, caused by the high amount of LPC (because the CP containig liposomes are to be intravenously applied the liposomes must have particle sizes of less than 2 μm. Therefore, liposome growth has to be avoided).

Despite the partial hydrolysis of PC the advantages of the lipid membrane were retained over the observed period by storing in refrigerator. As the data in Table 2 show, the stability of CP in the PC- and PC/Ch-liposomes is high.

This stability is comparable to the stability of CP in aqueous solution (by storage in refrigerator stable for at least 6 months, at RT only 93% of the initial content was detected after this time). CP has also in PC + Ch + AP-liposomes an acceptable stability after refrigerator storage. But AP has a negative effect on stability of CP too: especially at RT the degradation of CP is strongly accelerated.

Although PC-liposomes show greater drug relase values (Kibat and Stricker, 1985; Au et al., 1987) the efflux of CP out of PC + Ch + AP-liposomes is low. After six months storage in refrigerator less than 1% of the drug CP was released (PC- and PC/Ch-liposomes were not measured).

Summerizing, aqueous liposomes containing CP can be stored for at least 6 months in refrigerator without destruction of the liposomes. The hydrolytic degradation of PC is strongly increased by addition of AP, especially during storage at RT.

It seems to be possible to produce stable liposome without any antioxidant, if fully hydrogenated PC, which is less suseptible for oxidation degradation (Lang et al., 1990) is used: the Ch-oxidation can be avoided by fitting Ch in the lipid membrane and storage at low temperatures. Corresponding investigations are in progress.

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