Berberine hydrochloride is found in plant tissues. In the past, berberine hydrochloride as an intestinal antiseptic via oral administration has been used in Chinese medicine and in folk medicine in Japan. Recently, the anticancer activity, immunosuppression, and anti-inflammatory effect of berberine hydrochloride have been reported. But due to the poor absorption of berberine hydrochloride via oral administration and the strong side-effect via parenteral administration, the use of berberine hydrochloride was restricted. A new targeting drug delivery liposome of berberine hydrochloride can reduce the side-effect and improve the drug efficiency. The various influencing factors on preparation of the berberine hydrochloride liposome have important signification to exploit the new drug delivery liposome. The purpose of this present study is to find the effect of cholesterol concentration on the preparation of berberine hydrochloride liposomes. The experimental results indicated that (1) the encapsulation efficiency of berberine hydrochloride liposomes with various concentration lipids had a typical bell-shaped dependence on the cholesterol concentration with a maximum value of 3.0 mg/mL, and (2) the lower critical temperature of berberine hydrochloride liposomes can be reduced by increase of the cholesterol concentration. Acknowledgment. This work was supported by the National Natural Science Foundation of China (No. 20273032, 20573056) and the New Technique Foundation of Jiangsu Province, P. R. China (No. BG-2005041).