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Liposomal dispersions and oil–water emulsions based on lipodipeptides

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A comparison of the properties of liposomal dispersions and oil–water emulsions based on lipodipeptides as delivery systems for biologically active substances has been carried out.

Liposomes and emulsions are the most promising classes of colloid systems used for drug delivery.[1–4] Liposomal forms allow one to prepare bioavailable therapeutic agents based on water-insoluble compounds. Active ingredients included in liposomes are protected from the destructive effects of blood serum components, thus allowing the administered dose of an agent to be reduced. Furthermore, transport across a cell plasma membrane can be simplified considerably.[5]

Oil–water emulsions are also widely used for the delivery of drug substances. In this case, the dispersed phase of the resulting colloid carrier serves as a container for lipophilic compounds. An active compound is distributed between dispersed phase and dispersion medium, and when the system contacts a plasma membrane, the drug is transported across the barrier.[6]

The delivery of bioactive compounds can also be performed based on cationic peptides.[7] They contain hydrocarbon chains that can undergo conformational rearrangements in acidic media, thus avoiding hydrolysis in the endosome.

Our laboratory has an extensive library of cationic amphiphiles based on lipodipeptides. The hydrophobic fragment is formed from mono- and diesters of L-glutamic acid or L-glutamine and saturated or unsaturated aliphatic alcohols (C_8, C_{14}, C_{16}, $C_{18:1}$).

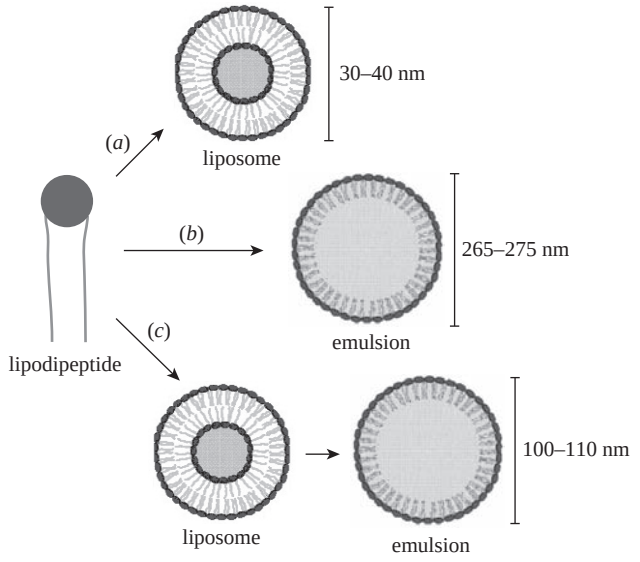

Figure 1 Preparation of liposomes and emulsions: (a) Liposomes were obtained by the hydration of a thin lipid film. The vesicle size was 30–40 nm. (*b*) Emulsions were obtained by the dropwise addition of an oil phase containing an emulsifier to water. The particle size was 265–275 nm. (*c*) A liposomal dispersion based on $OrnGlu(C16)_2$ was used as the initial aqueous component of an emulsion. The final emulsion was obtained after homogenisation of the resulting colloid solution and addition of residual water. The particle size was 100–110 nm.

Liposomes on this basis are promising transport systems due to easier interaction of the resulting species with epithelium, accelerated transport of conjugates in biological fluids, and high efficiency of penetration into the cell.[8]

Furthermore, owing to their amphiphilic structure, lipodipeptides are good emulsifiers that can stabilise an emulsion by decreasing the interfacial tension and by imposing electrical charges of the same sign on emulsion particles.

We performed a comparative study of the physicochemical properties of liposomes and submicron emulsions based on lipodipeptides.

Liposomes [Figure 1(*a*)] were obtained by the hydration of a thin lipid film followed by ultrasonic treatment and passing through a polycarbonate membrane extruder with a pore diameter of 100 nm. The main characteristics were determined for liposomes based on dihexadecyl *N*-(L-ornithyl)-L-glutamate [$OrnGln(C16)_2$], which was synthesized previously.[8] According to photon correlation spectroscopy, the vesicle size was 30–40 nm. The phase transition for bilayer aggregates was studied by the trapping of eosin dye with amphiphile molecules.[9] The phase transition temperature was 40 °C. The zeta potential was 50 mV; owing to this, the electrostatic repulsion of particles appeared in aqueous dispersions. Thus, the prepared colloid solutions stored at room temperature are stable for a week.

The pH-sensitive liposomes can be used for the controlled release of an agent directly in tumour cells.[10] The change in pH is the cause of restructuring the morphology of a lipid bilayer, which leads to destabilization of liposomes. Amphiphiles have a relatively small polar group, as compared with the hydrophobic part, and they can be changed from a lamellar phase to an inverted hexagonal phase. To study the pH sensitivity of liposomes, lipodipeptide dispersions in a prosphate buffer solution (pH 8.0) containing Uranin A (sodium fluorescein) were prepared. The dye not included in internal space was removed by gel filtration. The pH value was varied by the addition of 0.1 M hydrochloric acid;

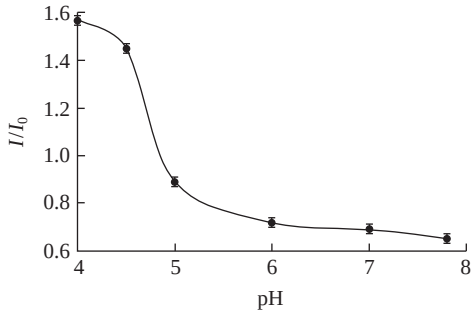

Figure 2 Effect of solution pH on the relative fluorescence intensity of Uranin A included in liposomes.

this was accompanied by an increase in the Uranin A fluorescence intensity. It was shown that the change was insignificant as pH decreased to 5.0, while the intensity increased abruptly below this pH value due to destabilisation of the bilayer and release of the dye from the liposome internal spaces (Figure 2).

We studied the properties of colloid solutions as a function of the emulsifier structure. Natural phospholipids [soybean lecithin Lipoid S100 (Lipoid KG)] and synthetic lipodipeptide OrnGln$(C16)_2$ were used as the emulsifiers. The emulsions obtained using a mixture of cationic lipodipeptides and phospholipids showed a higher stability than colloid solutions containing individual emulsifiers. The best results were obtained at a soybean lecithin/dihexadecyl *N*-(L-ornityl)-L-glutamate ratio of 5:1 (Figure 3).

Soybean oil (100%, Inax LLC) and squalene (98%, Sigma-Aldrich) were used as the oil phase. No considerable differences in the effects of these modifying agents on the stability of emulsions and particle size of the dispersed phase were found.

The concentrations of surfactants serving as stabilisers in the preparation of emulsions were varied. All the experiments were carried out at room temperature. The stability of the emulsions was determined visually based on the start of oil and water separation, as judged by the occurrence of a yellowish layer in the upper part of the emulsion. The emulsions containing 0.5–1.3% Tween 80 or Brij 78 in the aqueous phase were most stable.

The effects of Tween 80 (Fluka) and Brij 78 (Sigma-Aldrich) on the particle size and stability of colloid systems in time were studied. The emulsions containing Brij were stable even at 0.5% surfactant. Further addition of the compound did not affect considerably the emulsion stability. In the case of Tween 80, the dispersions were stable at 1.3% surfactant. Long-term stability could not be reached at lower concentrations, whereas at higher content of this component, the emulsions broke down within an hour.

The determination of aggregate sizes in the dispersed system by photon correlation spectroscopy has shown that emulsions containing a mixture of Lipoid S100 and OrnGln$(C16)_2$ have smaller dispersed phase particles (265–275 nm) and hence lower viscosity, which is a beneficial factor for the penetration of such systems into a cell, compared with emulsions containing only one emulsifier.

A process for inclusion of drugs into liposomal dispersions and emulsions based on lipodipeptides has been developed. Doxorubicin (Doxorubicin-Ferein, Bryntsalov A), a model anti-tumour agent, was included into liposomes by passive loading *via* trapping the aqueous phase fraction containing the drug in a closed bilayer structure spontaneously formed by amphiphiles in the presence of water, by freezing-thawing, as well as by active loading along an ammonium sulfate concentration gradient.

The efficiency of anti-tumour agent inclusion was determined using a calibration plot of absorption intensity (at 480 nm) *versus* the concentration of doxorubicin released from liposomes upon destruction of the latter with ethanol. As doxorubicin is transferred to a water-ethanol solution, a characteristic maximum appears in the spectrum.

The percentage of the agent included in liposomes was about 90% in the case of simple mixing but exceeded 95% for the other two methods. The percentage of doxorubicin inclusion in emulsions also amounted to 95%.

Hydrophobic α-tocopherol (96%, Sigma-Aldrich) was embedded to the liposomes and emulsion. The liposomal dispersion showed a small percentage of the embedded active compound (53%). In the case of emulsions, a necessary drug amount was dissolved in an oil phase, which was added dropwise to the aqueous phase. Hence, the advantage of cationic emulsions over liposomes is that relatively large amounts of lipophilic drugs can be solubilised in the hydrophobic environment of emulsion particles. Furthermore, unlike liposomes, cationic emulsions remain relatively stable at 25 °C for longer periods (about six months). However, the emulsion particles obtained are much larger than liposomes.

We succeeded in decreasing the particle size using a liposomal dispersion based on OrnGlu$(C16)_2$ as the initial aqueous component [Figure 1(*c*)]. The final emulsion was obtained after homogenisation of the resulting colloid solution and addition of residual water. As a result, the particle size of the dispersed phase decreased to 100–110 nm, while the emulsion remained stable for six months at room temperature. As for pH-sensitivity, a decrease in pH to 4.5 resulted in an abrupt change in the optical density of the solutions. This property is of importance in the design of drug delivery systems with pH-controlled release.

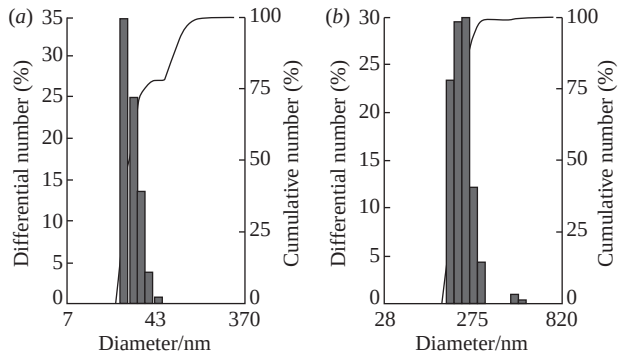

Figure 3 Particle sizes of (*a*) a liposome and (*b*) dispersed phase of soybean lecitin/dihexadecyl *N*-(L-ornityl)-L-glutamate ratio of 5:1.

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References

1 Sh. M. Shaheen, F. R. Shakil Ahmed, Md. N. Hossen, M. Ahmed, Md. Sh. Amran and Md. Anwar-Ul-Islam, *Pakistan J. Biol. Sci.*, 2006, 1181.
2 B. J. Boyd, *Drug Delivery Report*, 2005, 63.
3 M. J. Lawrence and G. D. Rees, *Adv. Drug Delivery Rev.*, 2000, **45**, 89.
4 U. A. Budanova, O. O. Koloskova and Yu. L. Sebyakin, *Mendeleev Commun.*, 2010, **20**, 326.
5 Y. Barenholz, *Curr. Opin. Colloid Interface Sci.*, 2001, **6**, 66.
6 C. Solans, P. Izquierdo, J. Nolla, N. Azemar and M. J. Garcia-Celma, *Curr. Opin. Colloid Interface Sci.*, 2005, **10**, 102.
7 L. P. Cavalcanti, O. Konovalov, I. L. Torriani and H. Haas, *Nucl. Instrum. Methods Phys. Res. B*, 2005, **238**, 290.
8 Yu. L. Sebyakin and U. A. Budanova, *Bioorg. Khim.*, 2006, **32**, 453 (*Russ. J. Bioorg. Chem.*, 2006, **32**, 407).
9 Yu. L. Sebyakin, U. A. Budanova and L. Yu. Guryeva, *Biol. Membrany*, 2007, **24**, 273 [*Biochemistry (Moscow), Ser. A: Membr. Cell Biol.*, 2007, **24**, 212].
10 D. C. Drummond, M. Zignani and J. Leroux, *Prog. Lipid Res.*, 2000, **39**, 409.

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