Lipid modified polyelectrolyte microcapsules with controlled diffusion†

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The lipid coating introduced directly on (polystyrene sulfonate/polyallylamine hydrochloride)$_5$ polyelectrolyte microcapsule surfaces significantly reduces the permeability of capsule walls estimated by fluorescence recovery after photobleaching (FRAP).

Layer-by-layer assemblies of amphiphilic molecules and polyelectrolytes have a major role as biomimetic systems for various applications.[1–3] The assembly of lipid bilayer membranes alternated with polyelectrolytes has been demonstrated on solid supports.[4,5] The polyelectrolyte/lipid architecture formed as microcapsules can have some advantages over liposomes due to better structural support and control over size of microcapsules.[5]

A lipid membrane on solid or polymer-precoated templates can be created by two major techniques, namely direct vesicle fusion[5,6] and the Langmuir–Blodgett (LB) technique.[4,6] In both cases, it was shown that electrostatic forces play an important role in film formation. In the case of vesicle (liposome) adsorption, a uniform lipid bilayer forms very quickly, within 5 min of the addition of the solution to a substrate, and covers more than 95% of the surface.[6] The almost perfect bilayer coverage was shown on silica for neutral zwitterionic lipids and their mixtures with low concentrations of negatively charged lipids. A uniform bilayer was also obtained for lipids on mica and polyethylenimine-supported surfaces.[6] In some cases, adsorption of more then one lipid bilayer on polycation-coated surfaces was assumed taking into account the thickness of the obtained films.[5]

Here, we report results on the direct coating of polyelectrolyte microcapsules with lipid bilayers of different composition, and the first quantification of its influence on capsule permeability using the fluorescence recovery after photobleaching (FRAP) technique. The polyelectrolyte/lipid microcapsules, with controllable permeability, are of interest for drug delivery applications.

We prepared unilamellar vesicles of 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) containing 5, 10, 17, 20% (w/w) of 1,2-dipalmitoyl-*sn*-glycero-3-phosphate (DPPA) and those of L-α-phosphatidylglycerol (PG) with 10, 20% (w/w) of L-α-phosphatidylcholine (PC) by mixing the reagents in chloroform followed by solvent evaporation and continuous sonication of the obtained mixtures in deionized water (pH 6.5). Negatively charged lipid bilayers were deposited from 1 mg/mL suspensions in alternation with polyethylenimine (PEI), polyallylamine (PAH), poly(diallyldimethylammonium chloride) (PDDA), and chitosan on silver Quartz Crystal Microbalance (QCM) resonators.[7] The resonators were first coated with three precursor polyallylamine/polystyrenesulfonate (PAH/PSS) layers.[8] Frequency changes of the resonators were measured only after deposition of polyelectrolyte layers to prevent rearrangement of lipid bilayers due to drying. The total thickness of the assembled films was less than 50 nm, which allows us to assume that the Sauerbrey equation is valid.[9]

Taking into account available data,[4–6] we believe that the prepared films consist of lipid bilayers sandwiched between two polycation layers. Deposition of multiple bilayers of a surfactant alternated with polyvinyl sulfate by the LB method was previously reported.[4] The mean thicknesses for (lipid bilayer/polyelectrolyte) layers for different compositions are shown in Table 1. The thickness of the layers is in the range 2–7 nm.

We found that a stable assembly can be obtained while the concentration of a charged lipid in the mixtures is 20%. The obtained (lipid bilayer/polyelectrolyte) layer thickness is in good agreement with the reported values for a DPPC bilayer.[5,6,10] The total thickness of a DPPC bilayer is 4.6–5.0 nm (including a 3.4–3.7 nm hydrophobic layer) in the gel state and 3.7–4.5 nm (2.6–3.0 nm for hydrophobic layer) in the fluid state. Admixture of another lipid (surfactant, hydrocarbon, *etc.*) into a lipid bilayer can dramatically change the main transition temperature of the mixture[11] and, therefore, influence the lipid bilayer thickness. In addition, oppositely charged substances can incorporate themselves between lipid heads changing the thicknesses of the hydrophilic and hydrophobic parts of the lipid bilayer; the effect is more pronounced for the mixtures with charged lipids.[12] However, in the case of the mixtures with low concentration of charged lipid (5–10% w/w DPPA), formation of incomplete lipid

Table 1 Thickness of (lipid bilayer/polyelectrolyte) bilayers

Bilayer composition[a]	Freq. shift[b]/Hz	Thickness[c]/nm
DPPA–DPPC 5% (w/w)/PEI	166 ± 51	2.65 ± 0.82
DPPA–DPPC 10% (w/w)/PEI	133 ± 47	2.12 ± 0.75
DPPA–DPPC 17% (w/w)/PEI	409 ± 23	6.54 ± 0.37
DPPA–DPPC 20% (w/w)/PEI	284 ± 27	4.55 ± 0.43
DPPA–DPPC 5% (w/w)/PAH	366 ± 39	5.86 ± 0.62
DPPA–DPPC 10% (w/w)/PAH	190 ± 40	3.05 ± 0.64
DPPA–DPPC 17% (w/w)/PAH	199 ± 39	3.18 ± 0.62
DPPA–DPPC 20% (w/w)/PAH	161 ± 18	2.58 ± 0.29
DPPA–DPPC 5% (w/w)/PDDA	111 ± 10	1.78 ± 0.16
DPPA–DPPC 10% (w/w)/PDDA	491 ± 10	7.85 ± 0.16
DPPA–DPPC 17% (w/w)/PDDA	327 ± 10	5.23 ± 0.16
DPPA–DPPC 20% (w/w)/PDDA	273 ± 42	4.37 ± 0.67
PG–PC 10% (w/w)/chitosan	339 ± 65	5.42 ± 1.05
PG–PC 20% (w/w)/chitosan	307 ± 25	4.91 ± 0.38

[a] The percentages indicate total weight percent of charged lipid components in a mixture. [b] The average of two independent experiments. [c] The frequency shift of the QCM resonators was converted into thickness using the experimental equation Δd (nm) = −0.016 ΔF (Hz).

† Electronic supplementary information (ESI) available: confocal images of the fluorescence recovery after photobleaching for capsules with different lipid coatings in an FITC-dextran solution. See http://www.rsc.org/suppdata/cc/b4/b415774e/

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bilayers (or polyelectrolyte layers on the top of lipid bilayers) because of the absence of electrostatic charge is possible. In the case of strong polycation PDDA, the formation of thicker layers can be explained by the deposition of more than one DPPA–DPPC bilayer on the top of the polycation layer in order to compensate for its high positive charge.

The assembly of PG–PC vesicles in alternation with cationic chitosan shows a bilayer thickness of ~5 nm. The last architecture is of considerable importance since chitosan is a non-toxic, natural, biocompatible polymer with antimicrobial properties, which makes this architecture suitable for *in-vivo* applications.[13]

For microparticle and microcapsule coatings we chose conditions giving a stable assembly and providing a 4–5 nm thick bilayer, which is consistent with the length of two lipid molecules.[4–6,10] The lipid mixtures with 20% w/w charged lipid were used hereafter. Lipid layers in alternation with polyelectrolytes were assembled on 3 μm polymethacrylate microspheres (PMA) precoated with two PAH/PSS bilayers, and changes in surface charge (ζ-potential) of the microparticles were measured after each adsorption step. The detailed procedure can be found elsewhere.[14] Latex particles were used in this series of experiments in order to avoid the interference of multivalent ions, which can precipitate lipids.[15] The ζ-potential values (Fig. 1) show complete charge reversal with synthetic polyelectrolytes such as PDDA, PEI, and PAH, and the assembly with PAH shows the most consistent alternation of surface charge. For assembly of chitosan and PG–PC, a shift in magnitude of positive charge was found, but not complete charge reversal, probably because of perturbation of lipid bilayers by chitosan.[16]

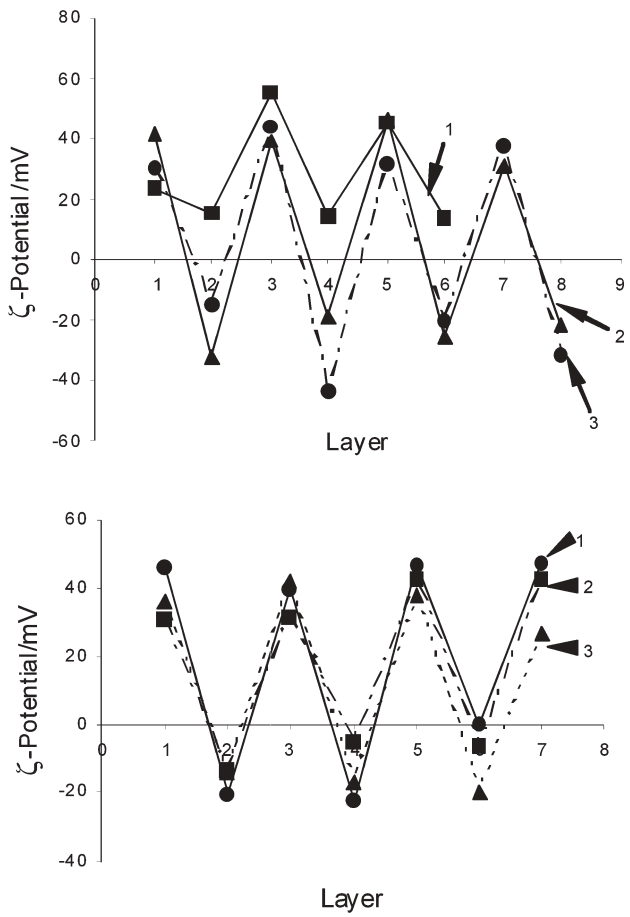

Fig. 1 ζ-Potential analysis of polyelectrolyte/lipid assemblies on PMA/$(PAH/PSS)_2$ microparticles, the first layer corresponding to the respective polycation. (Top): lipid – DPPA–DPPC 20% w/w; 1 – PDDA, 2 – PEI, 3 – PAH. (Bottom): lipid – PG–PC 20% w/w; 1 – chitosan, 2 – PEI, 3 – PAH.

The influence of a lipid coating on diffusion through polyelectrolyte layers was demonstrated with the example of PSS/PAH capsules with well-studied permeability.[17] The hollow 5 bilayer PSS/PAH capsules were fabricated using $MnCO_3$ cores.[18,19] The possibility of capsule coating with lipid bilayers was confirmed by deposition of a DPPC–17% w/w DPPA mixture with 3% admixture of a lipid labeled with fluorescein (FITC)[20] on the top of the hollow capsules (Fig. 2).

The operational sequence proposed by Moya[5] was employed for coating capsules with a lipid bilayer for FRAP experiments. The hollow $(PSS/PAH)_5$ capsules with an outermost PAH layer were suspended in a 10 μM solution of FITC-dextran of MW 4300 for 2 h to enable uniform distribution of the dyed polymer through the interior of the capsules and surrounding solution. This was followed by the addition of lipid vesicles to form the outermost layer on the polyelectrolyte capsules. Rinsing the capsules with the FITC-dextran solution followed in order to remove any unadsorbed liposomes from the mixture. The final sample for FRAP experiments was a suspension of capsules coated with a lipid bilayer in a solution of FITC-dextran. Single capsules were isolated under a Leica confocal laser scanning microscope, photobleached, and the fluorescence recovery after photobleaching was monitored.[17] As anticipated, the results indicate far slower fluorescence recovery for lipid-coated capsules than for uncoated ones as can be seen from Fig. 3. The diffusion properties are a representation of about 70% of the population.

The diffusion coefficient of FITC-dextran of MW 4300 into the polyelectrolyte microcapsules was evaluated on the basis of a fluorescence recovery model proposed by Axelrod and coworkers.[21] For uncoated $(PSS/PAH)_5$ capsules, the value is $(7.1 \pm 2.1) \times 10^{-12}$ cm^2/s. For lipid-coated capsules the value is $(1.4 \pm 0.3) \times 10^{-13}$ cm^2/s (DPPA/DPPC-coated), and $(1.5 \pm 0.4) \times 10^{-13}$ cm^2/s (PG–PC-coated). These results indicate nearly 2 orders of magnitude difference in the diffusion coefficient for FITC-dextran of MW 4300 for lipid-coated and uncoated microcapsules. It is worth mentioning that FITC fluorescence recovery in lipid-coated capsules was only slightly slower than that in uncoated

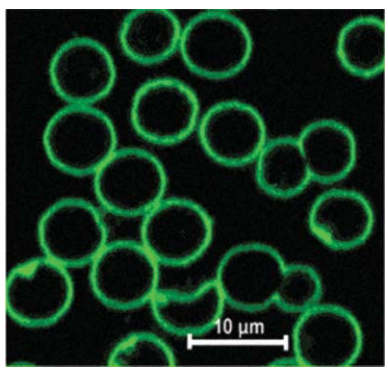

Fig. 2 Confocal image of $(PSS/PAH)_5$ capsules coated with a fluorescein-labeled lipid mixture.

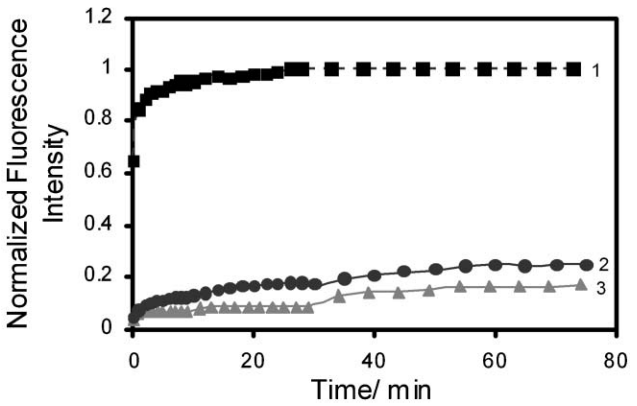

Fig. 3 Fluorescence recovery for FITC-dextran of MW 4300 in $(PSS/PAH)_5$ capsules coated with different lipid mixtures: (1) uncoated, (2) PG–PC 20% coated, (3) DPPA–DPPC 20% coated. The intensity of the capsule interior is shown relative to that of the surrounding solution.

ones, indicating that in spite of the deposited lipid bilayers a high diffusion coefficient through microcapsule walls remains for low molecular weight substances.

The diffusion coefficients of FITC-dextran of MW 4300 into uncoated and lipid-coated capsules, estimated by us, are higher than those which can be expected from previously reported data.[5,17,22] We speculate that another core type used for capsule preparation is the main reason for the higher diffusion coefficients. Previous studies on lipid-modified capsules were based on melamine formaldehyde microparticles and red blood cells as soluble cores; however, recent evidence indicates incomplete dissolution of such cores, which could lead to residues in the capsules and polyelectrolyte capsule swelling.[18] Various diffusion models used for the evaluation of diffusion coefficients can influence the results.

In summary, the multilayer architecture of lipid/polyelectrolyte capsules has been reported. The lipid coating introduced directly on polyelectrolyte microcapsule surfaces significantly reduces the permeability of capsule walls. The size, wall composition, and diffusion properties of the microcapsules are closer to biological cell membrane architecture than many other model systems. These lipid/polyelectrolyte microcapsules could be a further development of traditional liposome delivery system technologies.

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