1 Introduction

1.1 Context and Motivation

Nowadays, the use of nanoparticles (NPs) in biotechnology is of major interest due to the amazing potential displayed by mixed systems combining the properties of materials and the specific architectures and functions of the biological world. In the past few years, intensive research activities have been devoted to the characterization of the interaction between inorganic nanoparticles and biomolecules among which were proteins,[1] DNA molecules[2] and phospholipid molecules (typically in the form of bilayers).[3] This is an important issue with respect to using nanoparticles in nanomedicine but also in the related field of nanotoxicology.[4-7] In that context, mixed systems of phospholipid vesicles and inorganic nanoparticles exhibit a particularly strong potential owing to the specific properties of both species.

On one hand, nanoparticles are attractive as they exhibit strongly size-dependent properties,[8-10] which allows their use as functional system components, as for instance in sensor applications or as delivery vehicles.[11-13] But they are now also in abundant use as antimicrobial agents (e.g. Ag nanoparticles)[14, 15] or in the case of oxide nanoparticles for various purposes, such as biomarkers for controlled release.[16-18]

On the other hand, being rather versatile self-assembled systems with respect to size and detailed structure, vesicles, which for the case of phospholipids are often called liposomes, are frequently employed in pharmaceutical[19, 20] and cosmetic formulations or for drug delivery.[21-23] This is the case as they are able to transport hydrophobic molecules within their bilayer and/or hydrophilic molecules in their interior, thereby being very flexible carrier systems. Furthermore, phospholipids are the main component of natural membranes and thereby their vesicle bilayers can serve as good model systems to study interactions of particles or other colloidal systems with biological membranes.

Hence, combining the properties of phospholipid vesicles and nanoparticles as well as observing the influence of nanoparticles on biological membranes for which liposomes may serve as model system may lead to innovative approaches for novel medical applications. In addition, they are a relevant model system for investigations regarding nanotoxicity,[24] since nanoparticles have received increased scrutiny and attention with respect to their potential effects on health issues. Moreover, nanoparticles can serve also as formulating agents to

control the properties of membranes since they are frequently employed in vesicle formulations as applied in pharmacy or cosmetics.

Accordingly, fundamental studies in this field are necessary in order to gain a systematic understanding of the relevant aspects of the interactions between nanoparticles and lipid bilayers.

1.2 Silica Nanoparticles

Before being used for medical purposes, NPs have been subjected to numerous preventive studies about their cytotoxicity.[25-28] In this respect, silica particles appear to be a preferential choice since silica is considered as a biocompatible material due to its low cytotoxicity[25] and is furthermore required for the production of structural material of many living organisms.[29] But more than its low toxicity, the versatility of silica in synthesis as well as in surface functionalization is the main argument of the use of silica particles in biotechnology.[17] As a matter of fact, silica particles can be easily synthetized by various methods[16, 30-33] and the silanol groups on their surface can react with various compounds to form amine, carboxyl, thiol, or other groups, i.e., it exists a large variety of possible chemical surface modifications. However, silica surface modification can also be performed by passive adsorption of molecules such as proteins,[1, 17] granting silica particles an amazing potential as very versatile drug carriers. Finally, the hydrophilic nature of the bare silica particle surface allows for the appearance of specific structures when interacting with liposomes as discussed later in this chapter (see paragraph 1.4.3.1 and 1.4.3.2)

1.3 Liposomes: Definition and Properties

Phospholipids are amphiphilic molecules composed of a hydrophilic headgroup (phosphate) and two hydrocarbon chains thereby yielding to a packing parameter value p[34-36] between 0.5 and 1, granting them a truncated cone or cylindrical form (p being defined as: $p = v/(a.l)$; with v being the volume of the hydrophobic chains, a the head group area of the lipid and l its stretched length).[34] Thus, phospholipids have a tendency for the formation of bilayers,[37] also in the form of closed bilayers, i.e. vesicles.[38, 39]

Phospholipid vesicles, often called liposomes, are frequently used in cosmetic and pharmaceutical formulations. This is mostly due to their ability to solubilize hydrophobic compounds and to allow for rheological control of the formulations.[19, 23] Liposomes are also known to have a significant potential as drug carriers.[21, 22] This is the case as they are able to transport hydrophobic molecules in their bilayer and/or hydrophilic molecules in their

interior, thereby being very flexible carrier systems. In addition, having cell membrane mimetic surfaces, they facilitate the transport through membranes while being non cytotoxic and may serve as model systems for experimental and theoretical studies on cell membranes.

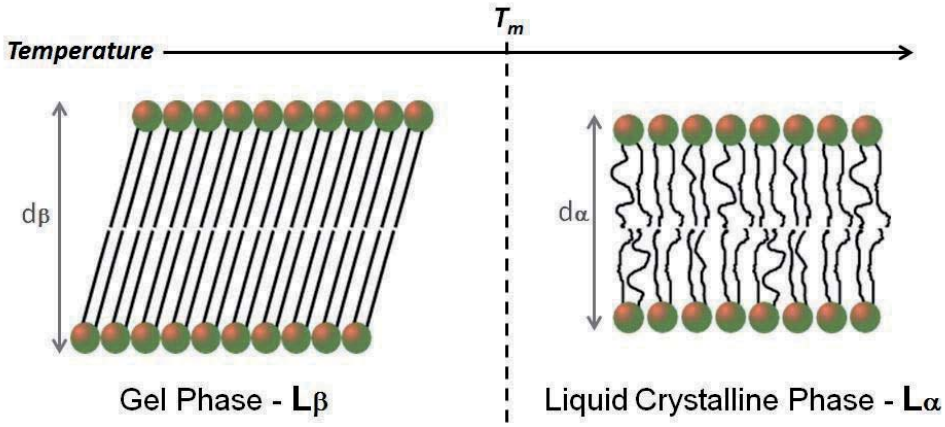

Figure 1-1: Structural models associated with thermal transition for phospholipid bilayers. Bilayer with crystalline ordering of the alkyl chains (L_β) with a thickness d_β and liquid crystalline disordered bilayer (L_α) of smaller thickness d_α.

Pure phospholipid bilayer membranes exhibit a sharp main phase transition[40] which is lipid specific and corresponds to the melting of their hydrocarbon chains. Below the chain melting temperature (T_m), the phospholipid molecules are locked in place,[41] bound tightly together by the van der Waals forces between their hydrocarbon chains, so that the bilayer is in a "solid" state (gel phase), leading to the appearance of characteristic angular shapes in the case of vesicles.[42] On the contrary, at temperatures above T_m, the lipids exhibit lateral as well as inter-layer mobility (flip-flop)[43] responsible for the fluidity of the membrane (see Figure 1-1). When passing through its phase transition, the bilayer vesicle membrane experiences a temporary permeability allowing the release of molecules from its interior.[44] This last property being one other reason why liposomes are used as nanocontainers in drug delivery technologies, since they allow achieving on-demand release of encapsulated molecules by passing through the phase transition temperature.

The formation of liposomes occurs naturally but in many circumstances is done using appropriate procedures. Phospholipids dispersed in water "spontaneously" form giant multilamellar vesicles (MLVs) which are huge enough to be assimilated to a lamellar phase (it should be noted that typically during dispersion shear is applied, which is mainly responsible for the formation of the vesicles from these lamellar dispersions). However, starting from an MLV suspension, the preparation of well-defined small unilamellar vesicle (SUV) dispersions of specific composition, size and polydispersity can be relatively easily

achieved using one or a combination of the different available techniques among which are sonication, rehydration of lipid film, or extrusion.[22, 23, 39, 45]

These preparation processes are required since the normal equilibrium state of phospholipids is a lamellar phase, whereas the vesicles are only metastable.[46] Hence, this imposes one limitation to the possible applications of phospholipid vesicles as they are intrinsically unstable, while it might be noted that in some surfactant systems spontaneous vesicle formation and thermodynamic stability are present, especially in the case of cationic surfactants or mixtures of surfactants.[47-50]

1.4 Interaction in Mixed Systems Liposome/Nanoparticle

1.4.1 Role of the Lipid Bilayer Phase Behavior

An important aspect for the understanding of the interactions between lipid bilayers and nanoparticles is the phase behavior of the lipids. In that context it is important to note that the properties of a given bilayer membrane in the gel phase (L_β) are extremely different from those it exhibits in the fluid phase (L_α).

Temperature/°C	***20***	***50***
V_L/Å^3	1144	1232
D/Å	63.5	67
A/Å^2	47.9	64
d_{GL}/Å	47.8	38.5
d_W/Å	15.7	28.5
d/Å	42.4	46.5
d_W'/Å	11.1	20.5

Table 1-1: Parameters that characterize the structure of DPPC (dipalmitoylphosphatidylcholine) bilayers in the liquid phase (50°C) and in the gel phase (20°C). V_L: lipid molecular volume. D: lamellar repeat spacing. A: average interfacial area per lipid. d_{GL}: Gibbs-Luzzati bilayer thickness. d_W: Gibbs-Luzzati Water thickness. d: steric bilayer thickness. d_W': steric water thickness. Figure reprinted and adapted with permission from referecence 52.

First of all, the structural arrangement of the phospholipid molecules is different. As described earlier, in the gel phase lipid-acyl chains are conformationally highly ordered while in the fluid phase the bilayer possesses both translational disorder and a high degree of lipid-acyl-chain disorder. This implies that as the bilayer is heated from the gel to the fluid phase, its thickness decreases substantially due to the conformational changes experienced by the acyl-chains.[41] This decrease was analyzed using both the Gibbs-Luzzati thickness (d_{GL}) model[51] (which partitions the overall bilayer thickness into a lipid region d_L and a water region d_W thereby ignoring details of the interfacial region) as well as with the

steric bilayer thickness (*d*) model (which takes into account the presence of water molecules mixed with the polar heads in the interfacial region) for the case of dipalmitoylphosphatidylcholine (DPPC) (Table 1-1). This work confirmed that this decrease is not model-dependent and typically in the range of 10 to 20%.[52] A concomitant increase in the bilayer area in the fluid state occurs, coming along with the corresponding decrease of the bilayer thickness, and that, of course, means one has then an enhanced hydration of the head group area. Similarly the state of the phospholipid chains has an important effect on the vesicle properties, in particular, when it comes to the deposition of supported lipid bilayers on nanoparticles where the surface ratio liposome/nanoparticle is of main interest. It can also be noted that the L_α-phase shows a much larger swelling, which can be attributed to the undulation forces that are much more pronounced compared to the L_β-phase (as the L_α-phase possesses much lower bending moduli, see Table 1-2), where the Helfrich undulation repulsion between two bilayers can be described by:[53]

$$E_{und}(D) = \frac{3\pi^2}{128}\frac{(kT)^2}{\kappa_b D^2} \qquad \textbf{1-1}$$

which gives the free energy of the steric interaction per unit area of membrane (κ_b: mean bending modulus of one bilayer, D: distance between two adjacent membranes).

Lipid	T_m/°C	T/°C	$(T - T_m)$/°C	κ_c/k_BT
14:0 PC	24	22	−2	100.0 ± 4.99
		24	0	20.9 ± 0.61
		28	+4	13.9 ± 0.24
		35	+11	15.3 ± 0.31
		45	+21	13.9 ± 0.44
		60	+36	8.2 ± 0.12
16:0 PC	41	30	−11	49.6 ± 2.78
		41	0	36.1 ± 1.49
		60	+19	9.5 ± 0.18
18:0 PC	54	40	−14	79.1 ± 3.23
		60	+6	13.6 ± 0.24

Table 1-2: Bending elasticity κ_c of saturated bilayers (PC: phophatidylcholine) in D_2O at different temperatures, measured with the neutron spin-echo (NSE) technique. Figure reprinted and adapted with permission from reference 59.

More importantly the fluidity of the membrane, as characterized by the bending elasticity, is dramatically altered by the phase transition. The bending elasticity (κ), described by the Helfrich theory[54] as the energy required to bend a bilayer away from its spontaneous curvature, is determining the balance between adhesive and elastic forces responsible for the bending (and potential wrapping) of the bilayer around a particle.[55, 56] Values of the

bending elasticity for a given bilayer have been found to decrease drastically when the bilayer is heated through the phase transition to its fluid phase[57-59] (Table 1-2). This observation is to be related to the inability of the gel membrane to bend around particles or to fuse on flat surfaces, as a result of its high rigidity[60].

Finally, in the case of zwitterionic phospholipids, the electrostatic properties of the bilayer are also altered by the conformational changes occurring during the main phase transition. The dielectric permittivity of the lipid bilayer as well as its conductivity in aqueous solution have been found to increase while passing from the gel to the fluid phase.[61, 62] These changes, resulting from the decrease of the dipolar correlation taking place between the headgroup dipoles in the hydrophilic part of the bilayer, have a non-negligible influence on the surface interactions taking place between lipid bilayers and nanoparticles.

1.4.2 Nature of the Interaction between Liposome and Nanoparticles

The interaction between lipid bilayers and nanoparticles is rather complex and includes van der Waals, double layer, hydration, hydrophobic, thermal undulation and protrusion forces.[63] The precise nature of the interaction, as well as the structure it creates, is in all cases related to the properties of the lipid and the solid surface used as well as the nature of the dispersing medium.

1.4.2.1 Van der Waals Interaction

Describing the strength of van der Waals forces in mixed liposome/nanoparticle systems in a quantitative manner is challenging due to the geometry and nature of the system components. In the ideal case of a sphere (medium 1: nanoparticle) interacting with a shell (medium3: liposome) in an aqueous medium (medium 2), the van der Waals energy is given by the equation:[64, 65]

$$V_{vdw} = -A_{123}\frac{R_1 R_2}{6(R_1+R_2)}\left(\frac{1}{D}-\frac{1}{(D+d)}\right)-\frac{A}{6}\ln\left(\frac{D}{D+d}\right) \qquad \textbf{1-2}$$

where R_1 is the radius of the sphere (particle), R_2 the outer radius of the shell, d the thickness of the shell, D the distance between the surfaces of the two objects and A_{123} the Hamaker constant of the system. An approximate value of A_{123} may be given in terms of the Hamaker constants of the individual media as follows:[64, 66]

$$A_{123} \approx (\sqrt{A_{11}}-\sqrt{A_{22}})(\sqrt{A_{33}}-\sqrt{A_{22}}) \qquad \textbf{1-3}$$

where A_{xx} is the Hamaker constant between two semi-infinite planes of medium x in vacuum.

For "symmetrical" systems, the Hamaker constant is always positive, leading to attractive forces, but between dissimilar surfaces, as in the case of mixed liposome/nanoparticle systems, A_{123} can be either positive or negative leading respectively to attractive or repulsive forces. For phospholipid bilayer interacting with oxide particles (SiO_2, TiO_2) the Hamaker constant is typically (3-4)×10^{-21} J ≈ 0.75 to 1 kT (for T = 25°C).[60, 67, 68]

1.4.2.2 Electrostatic Interaction

In general, the electrostatic double-layer interaction energy between two identical planar surfaces decreases exponentially with the distance. However, for two surfaces of different charge densities or potentials, which is the case in mixed liposome/nanoparticle systems, this interaction energy can exhibit a minimum or a maximum at some finite distance.[64] The study on those asymmetric systems has led to different approximate equations for the interaction energy of two surfaces of unequal but constant potentials.[69-71] The "Hogg – Healy – Fuerstenau" equation (HHF)[64, 69] for two different planar surfaces of low constant potentials ψ_1 and ψ_2 in 1:1 electrolyte is given by:

$$V_{edl}(D) = \frac{\varepsilon_0 \varepsilon \kappa [2\psi_1\psi_2 - (\psi_1^2 + \psi_2^2)e^{-\kappa D}]}{(e^{+\kappa D} - e^{-\kappa D})} \qquad \textbf{1-4}$$

where D is the distance between the surfaces and κ their Debye length. This equation has recently been successfully implemented to characterize the double layer interaction between lipid bilayer and silica substrates.[60]

Nevertheless, when working with nanoparticles and vesicles, one often has to take into account the curvature of the interacting surfaces. To this aim, applying the Derjaguin approximation on equation 1-4 leads to the following formula for the approximate interaction energy between dissimilar double layers on two spherical particles with radii R_1 and R_2:[69]

$$V_{edl}(D) = \frac{\varepsilon R_1 R_2 (\psi_1^2 + \psi_2^2)}{4(R_1 + R_2)} \left[\frac{2\psi_1\psi_2}{(\psi_1^2 + \psi_2^2)} \ln\left(\frac{1 + e^{-\kappa D}}{1 - e^{-\kappa D}}\right) + \ln(1 - e^{-2\kappa D}) \right] \qquad \textbf{1-5}$$

It is important to note that the HHF formula is based on the Debye-Hückel linear approximation. This approximation is thus applicable for sufficiently low potentials (commonly ψ < 25 mV). More recent work using the Poisson-Boltzmann expression for the potential has led to more accurate analytical approximations.[70]

Alternatively, The Gouy-Chapman Theory has also been used to describe the electrostatic interaction energy between a flat substrate with surface potential ψ_{sub} and a flat bilayer of surface potential ψ_{bil} in monovalent salt solution[72, 73] leading to the following formula:

$$V_{edk}(D) = 64kT\rho_{\infty} \tanh\left(\frac{e\psi_{sub}}{4kT}\right)\tanh\left(\frac{e\psi_{bil}}{4kT}\right)\frac{e^{-\kappa D}}{\kappa} \qquad \textbf{1-6}$$

where T is the temperature, k the Boltzmann constant and ρ_{∞} the ions concentration. This equation also assumes a constant surface potential at any distance D.

1.4.2.3 Hydration Forces

In systems containing phospholipid bilayers, van der Waals and double layer interactions, as jointly expressed by the DLVO theory, often fail to precisely describe the phenomena taking place at short distances due to the presence of repulsive hydration forces,[74, 75] as it has been observed experimentally by means of the surface force apparatus. These repulsive forces are believed to arise from the presence of a layer of water molecules, strongly bound to the hydrophilic surfaces and preventing them from approaching any closer than the thickness of 2 water molecules.[64, 74, 76]

The full nature of this interaction is still an active field of research. However, in the case of hydrophilic surfaces in aqueous solution, the hydration energy is attributed to stable structured water layers hydrogen bonded to the solid surfaces, while the hydration repulsion between two lipid membranes is believed to be dominated by entropic factors (microscopic thermal fluctuations).

Tero et al.[73] were the first to propose an expression for the calculation of the hydration energy between lipid bilayer and hydrophilic surfaces, by assuming that the hydration between lipid membrane and solid substrate (W_{hyd}) is the average of each hydration energy, giving:

$$W_{hyd} = \frac{W_{solid} + W_{lipid}}{2} \qquad \textbf{1-7}$$

where W_{solid} is the hydration force between hydrophilic substrates, depending on the nature of the substrate and its medium. Empirically, W_{solid} has been found to decay exponentially with the distance (D) between the surfaces:[64, 72, 73]

$$W_{solid} = W_0 e^{-D/\lambda_0} \qquad \textbf{1-8}$$

where λ_0 is on the order of 1 nm and W_0 depends on the hydration of the surfaces but is usually in the range of 3-30mJ.m^{-2} ≈ 0.7 to 7 kT.nm^{-2} (for T = 25°C).

On the other hand, W_{lipid} is believed to arise from thermal fluctuations[77] and thus depends on the three different types of thermal motion that a membrane can experience: protrusion, peristalsis and undulation. The energetic contribution of these different motions can be separately expressed as follows:[64, 73]

$$W_{protrusion} = 2.7\Gamma kTe^{-(\alpha_p D/kt)} \quad \textbf{1-9}$$

$$W_{peristalsis} = \frac{(kT)^2}{20k_a D^4} \quad \textbf{1-10}$$

$$W_{undulation} = \frac{3\pi^2 (kT)^2}{64k_b D^3} \quad \textbf{1-11}$$

where Γ is the surface density of protruding head groups, α_p the (hydrophobic) protrusion energy per unit length, k_a the area expansion modulus and k_b the bending modulus. Typical values for these parameters are: α_p = 2.5×10^{-11} J.m^{-1}, k_a = 0.15 J.m^{-2} and k_b = 10^{-19} J[64, 73] while Γ depends on the molecular occupying area of the chosen lipid.

It should be noted that undulation forces may be neglected in the case of membranes carrying unscreened surface charges or being under tension. Nevertheless, when considering every contribution, the hydration energy between a bilayer and a flat hydrophilic substrate is given by the expression:

$$W_{protrusion} = 2.7\Gamma kTe^{-(\alpha_p D/kt)} \quad \textbf{1-12}$$

Additionally, it should be mentioned that the geometry of curved surface tends to smother short range interaction (hydration) and emphasizes longer range van der Waals and electrostatic double layer forces.[74, 78, 79] This is of course of crucial importance when studying the balance of forces in mixed liposomes/nanoparticles systems where both species often exhibit a curved surface.

1.4.2.4 Hydrophobic Interaction

Hydrophobic interactions may arise when phospholipid bilayers, subjected to a stretching force or stress, expand laterally and expose areas of their hydrophobic interior to the aqueous solvent.

Hydrophobic attraction plays an important role in the mechanisms of vesicle fusion or particle embedding, when hydrophobic particles are internalized into the hydrophobic interior of the membrane. In the case of vesicle-vesicle interaction, the increase in stress experienced by the membrane would be directly proportional to the increased adhesion

force between two vesicles.[64] In the case of particle embedding, hydrophobic attraction can be expressed as the balance between the free energy change to move a hydrophobic sphere from pure water into a hydrophobic membrane (ΔG_{emb}) and the energy penalty to deform the bilayer (ΔG_{def}).[80] Where ΔG_{emb} is given by:[80, 81]

$$\Delta G_{emb} = \pi D^2 \gamma \qquad \textbf{1-13}$$

where D is the nanoparticle diameter and γ the liquid-vapor surface tension of water.

Ultimately, the interplay between Van der Waals, double layer, hydration and hydrophobic interactions can be tuned by playing on numerous parameters among which pH, ionic strength and temperature in order to obtain various structures such as supported lipid bilayers, internalized particles and decorated vesicles.

1.4.3 Resulting Structures

The interactions taking place in mixed liposome/nanoparticle systems lead to a variety of original structures which present a significant potential for different industrial applications. In the following, the different structures arising in systems containing liposomes and hydrophilic nanoparticles are described, as well as the balance of force contributions responsible for their formation. It is to be noted that phenomena such as particle embedding in bilayer membranes or adsorption of phospholipid monolayers on particle surfaces are not being discussed here as they occur in systems containing hydrophobic particles thereby having less relevance to the present work.

1.4.3.1 Supported Lipid Bilayer (SLB) Formation and Particle Internalization

Supported lipid bilayers (SLB) are continuous fluid lipid membranes ($T > T_m$) adsorbed on a solid substrate, and separated from this substrate by a thin water layer (1-3 nm).[82] It is important to note the necessity of having a bilayer in the fluid phase (to have a membrane of sufficiently low elasticity)[57-59] and a hydrophilic substrate (to bind the supporting water layer) to achieve the deposition of SLB.

SLB formation has been studied first for the case of planar substrates (glass, silica, mica) as a basis to produce innovative catalytic surfaces or immobilized protein arrays,[60, 67, 82-91] as well as to study bilayer-bilayer interactions, as they are important in biology, under well-defined conditions. Nowadays, this field of research has been extended to the case of nanoparticles, with the intention of designing nanovectors by rendering nanoparticles biocompatible through the deposition of a lipid bilayer onto their surface.[92-94] Such studies