Chapter 5

The Versatile Dioctadecyldimethylammonium Bromide

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Additional information is available at the end of the chapter

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Abstract

Dioctadecyldimethylammonium bromide (DODAB) is a quaternary ammonium surfactant (Quat) with interesting properties and applications. In this chapter, DODAB characteristics as compared to other Quats emphasize its self-assembly in aqueous solutions and the novel applications involving this useful cationic lipid so easily combined with biomolecules and interfaces to yield a wide range of novel uses in many fields such as delivery of drugs, vaccines and genes, design of nanoparticles, modification of interfaces, and many others yet to come.

Keywords: quaternary ammonium surfactants, self-assembly in water, cationic lipid in novel applications

1. The quaternary ammonium surfactants (Quats)

The quaternary ammonium surfactants or "Quats" encompass many individual chemicals [1, 2]. They are present in thousands of end-use formulations, many of which are blends of various Quats [1]. Common uses include disinfection, detergency, fabric softening, antistatic, and wood preservation [2]. The chemical structure determines their chemical behavior and utility. Quats will be strongly cationic due to their quaternary and positively charged nitrogen able to attach to surfaces, both organic and inorganic [3]. With remarkable chemical stability, they can exhibit long-lasting biocidal effects [4]. They attract anions, for example, soaps, detergents and hard water constituents, for example, carbonate and sulfate [5]. They are attracted by negatively charged cells such as bacteria or fungus and become attached to them eventually causing their cytoplasmic membrane to leak with membrane damages leading to antimicrobial effects [6–9]. Certain Quats will biodegrade and the biodegradability decreases with increase in their alkyl chain length [10, 11]. The degradation takes place via partitioning to sludge and processing by biodegradation. The complex Quats biodegradation occurs in

several steps and depends on the Quat chemical structure, Quat interactions with the sludge determining adsorption and desorption, microorganisms present in the sludge and the presence or absence of anions; alkylammonium surfactants chemically modified with biological moieties such as carbohydrates, amide, aminoacids or peptides were better degraded [12]. From the point of view of Quats synthesis, compounds bearing more than one positive charge were readily obtained at economical cost from compounds with at least two tertiary amines that could be readily quaternized; some of these displayed potent antibacterial and antibiofilm activity and did not trigger bacterial resistance systems as those from methicillin-resistant Staphylococcus aureus (MRSA); mono-Quats and several bis-, tris- and tetra-Quats tested against bacteria within a few hundred generations yielded a lack of resistance for Quats of higher charge when compared to mono-Quats [13].

Quats chemical structure determines their self-assembly in water solution. The theory for the self-assembly of dilute surfactant solutions is well established and very successful [14, 15]. This theory applies also to Quats since their amphiphilic molecular nature includes polar and apolar regions in the same molecule. The theory relates the self-assembly in water solution with the geometric parameter v/al. The definition of v/al is given by v, the volume of the hydrocarbon region of the surfactant; a, the optimal head group area, and l, as the optimal hydrocarbon chain length related to its maximum extended length. One should notice that the nature and shape of the assemblies are intimately related to the v/al value. For instance, in the case of spherical micelles, $v/al < 1/3$ whereas for vesicles or bilayers, $½ < v/al \leq 1$. When bilayer vesicles are the desired structure, larger v is required as is the case of the double-chained surfactants. Single-chained surfactants and lower v are required for micellar structures. For example, a single-chained quaternary ammonium surfactant such as cetyltrimethylammonium bromide (CTAB) has a lower v than the corresponding double-chained quaternary ammonium surfactant. The self-assembly of CTAB and dioctadecyldimethylammonium bromide [DODAB] from calculations for their respective geometric parameters predicts, as indeed observed, CTAB molecules assembling as micelles and DODAB molecules assembling as bilayers in water solutions.

Not only the molecular geometry of the Quats determines their assembly in water solution: specific counterion effects also do [16]. Counterion adsorption and Stern layer effects change the optimal headgroup area a. In general, counterions will adsorb to some extent to the surfactant headgroups. Specific interactions of a nonelectrostatic origin like dehydration or hydration of the surfactants, conformational changes in the surfactant headgroup, size of the adsorbed counterion are important because they determine the thickness of the Stern layer and the actual surface potential. Specific counterions can change the lateral interactions between surfactants in a micelle, monolayer or bilayer. By means of the direct force measurement technique developed by Israelachvili [15] after depositing DODAB bilayers with the Langmuir-Blodgett technique on two molecularly smooth mica surfaces and bringing these surfaces together in an aqueous solution, the measurements of the interaction forces between the bilayers as a function of their separation a repulsive double-layer force are experienced. Fitting the measured double-layer force with theory allows the surface potential to be estimated, from which the binding affinity of the ions can be determined [15]. Apart from the

repulsive double-layer interaction, the van der Waals interaction and possibly the ion-ion correlation interaction, which are both attractive, must be taken into account [17]. The interactions between bilayers of dihexadecyldimethylammonium acetate and bromide surfactants, which are soluble in water and adsorbed from solution as a bilayer onto the mica surfaces, were determined by Pashley and coworkers [18]. Marra employed the Langmuir-Blodgett deposition technique for an insoluble surfactant like DODAB so that the solution did not contain any aggregates and the binding of anions to the quaternary ammonium headgroups would not depend sensitively on the precise length of the hydrocarbon tails [16]. The anions investigated bound to the headgroups following a lyotropic series where the least hydrated, smallest anions bound with highest affinity [16]. Lateral interactions between DODAB adjacent molecules in a monolayer at the air-water interface and interactions between bilayers of DODAB surfactants exhibited a pronounced ion specificity. Large hydrated counterions like the fluoride, hydroxide, and acetate ions gave expanded monolayer compression isotherms. Fluoride, hydroxide, and acetate counterions did not bind to DODAB headgroups. Following the lyotropic series for anion sizes F-> C1-> Br-, the smaller the (hydrated) anion, the more contracted the monolayer [16]. For dioctadecyldimethylammonium (DODA) acetate, chloride or bromide, vesicle size and zeta-potentials were inversely related; an increase in the zeta-potential was accompanied by a decrease in vesicle size, in accordance with the self-assembly theory; DODA acetate bilayer vesicles had the largest, less tightly bound and more hydrated counterion and exhibited the smallest size in comparison with those obtained from the other DODA salts [19].

2. DODAB hybrid assemblies

DODAB remarkable interactive capability with opposite charges of silica particles [20–23], silicon wafers [24], polymeric particles [25–31]; polymer films [32–34], drugs [35–45], nucleic acids [31, 46], oligonucleotides [47–49], proteins [30, 50–54], peptides [9, 55–57], polyelectrolytes [8, 9, 36, 58, 59] and many other important surfaces, biological cells, molecules and nanostructures [60–67] is at the root of DODAB popularity in the literature spanning a huge variety of subjects. Today (December 10th, 2016) a search in American Chemical Society, PubMed and Scopus databases retrieved 104, 140 and 1208 documents, respectively, quoting DODAB. Therefore, this review just gives an overview on DODAB recent possibilities, and many others have already appeared or are yet to come.

The interaction between DODAB and solid surfaces like silicon wafers depends on the charge density of the solid surface, which depends on the nature and concentration of bound counterions and DODAB ability to displace them; the cation more tightly bound to the negatively charged surface solid surface should be Li+ that would be difficult to displace by the DODAB cation, in contrast to the loosely bound Cs+ with its large ion radius and low charge density. In summary, DODAB adsorption proceeded in accordance with charge density on the solid surface thus depending on nature and concentration of counterions and DODAB ability to displace them; increasing the ionic strength increases silanol dissociation, surface charge density,

and DODAB adsorption [24]. The effect of monovalent salt nature and concentration over a range of low ionic strengths (0–10 mM LiCl, NaCl, KCl, or CsCl) and at two different pH values (6.3 and 10.0) on DODAB adsorption onto flat SiO_2 surfaces evaluated by in situ ellipsometry. This technique allowed precise evaluation of thin film thicknesses on very smooth solid surfaces such as those of silicon wafers. Thereby, DODAB adsorption isotherms of high affinity showed adsorption maxima consistent with bilayer deposition only around 10 mM monovalent salt at both pH values. In contrast, when pure water was the intervening medium, DODAB adsorption decreased substantially. The nature of counterion on the charged solid surface was also important to determine DODAB adsorption: at 10 mM CsCl or LiCl, the highest and the lowest affinity constants for DODAB adsorption onto SiO_2 were, respectively, obtained [24]. This was understandable from the fact that DODAB adsorption onto the solid surface required as a first step the displacement and cation exchange at the solid surface. DODAB adsorption consistently followed the expected facility of cation exchange at the surface required for DODAB adsorption. **Figure 1** illustrates the effect of counterion nature and

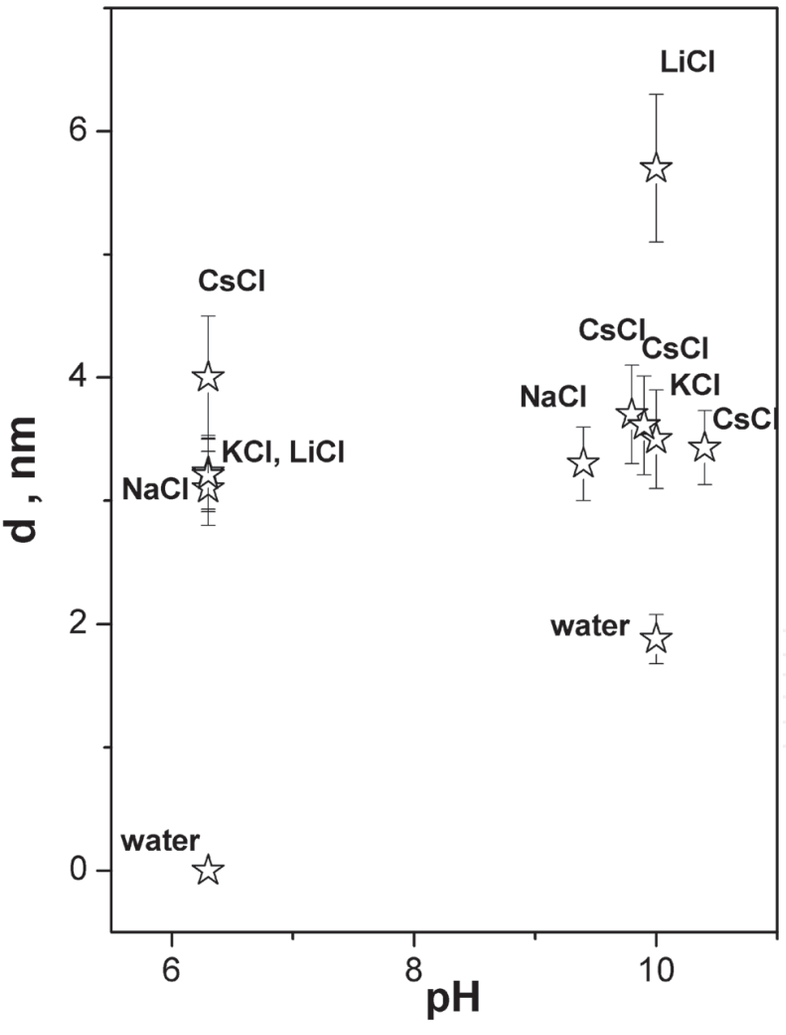

Figure 1. The effect of monovalent salt nature and concentration at 0 and 10 mM LiCl, NaCl, KCl, or CsCl and pH 6.3 or 10.0 on the thickness of the DODAB adsorbed layer deposited from bilayer fragments onto flat SiO_2 surfaces from in situ ellipsometry [24]. Reprinted with permission from Ref. [24]. Copyright (2006) American Chemical Society.

concentration on DODAB adsorption from bilayer fragments (BF) onto silicon wafers as determined from in situ ellipsometry measurements [24].

The changes of the electrostatic repulsion between adjacent DODAB molecules in a bilayer as the one due to interaction with counterions or oppositely charged inorganic or organic species can drastically change the nature of DODAB assemblies. For example, monovalent salt at a moderate concentration was reported to induce fusion of DODAB bilayer fragments [68–71] with induction of hydrophobic defects at the bilayer-water interface [72]. When the electrostatic repulsion is high as in pure water or in the presence of low concentrations of poorly bound counterions, interdigitation represents a way of relaxing the intermolecular repulsion in the bilayer; adhesion between DODAB bilayers due to interdigitation between DODAB molecules in the bilayer [26], molecular dynamic simulations [73], differential scanning calorimetry (DSC), and X-ray scattering in the subgel state [74] further supported DODAB tendency to display hydrophobic moieties in its assemblies for relaxation of the electrostatic repulsion.

Other interesting instances refer to the formation of catanionic bilayers from DODAB and anionic oleosiloxanes [75] or oleic acid [76]; DODAB membrane fragments and fatty-acid esters of cyclosiloxanes formed dense multibilayered vesicles; the transformation took place once the ester groups hydrolyzed to yield carboxyl groups yielding the anionic silicone surfactant in situ and the catanionic system with DODAB. The oleo-silica compound was obtained via hydrosilylation of methyl undec-10-enoate with 1,3,5,7-tetra-methylcyclotetrasiloxane (1). Flat DODAB/oleic acid bilayer sheets were obtained at about 1:1 molar ratios for DODAB/oleic acid binary dispersions; the relaxation of the electrostatic repulsion between DODAB molecules in the bilayer due to the incorporation of OA into DODAB bilayer decreased the membrane curvature and increased the aggregate size; introduction of the fatty acid around equimolar ratios led to flat DODAB/OA bilayer assemblies in the dispersions [76]. The electrostatic attraction between DODAB and anionic amphiphiles decreased the mean area per molecule, increased the geometric parameter v/al, and increased the aggregate size similarly to the fusogenic effects reported upon increasing counterion concentration [68–72, 75, 76].

Figure 2 shows cryo-transmission electron micrographs (cryo-TEM) of vitrified DODAB bilayer fragments obtained by sonication of DODAB in water [77], unilamellar vesicles of about 200–400 nm obtained by vaporization of a DODAB chloroform solution in water at 70 degrees centigrades (above the gel to liquid-crystalline phase transition temperature of the DODAB bilayer and above the chloroform boiling point) [78] and very large unilamellar DODAB vesicles from salt-induced fusion of DODAB bilayer fragments [68, 69].

Combinations of DODAB and dihexadecylphosphate (DHP) yielded miscible catanionic bilayers over a range of molar ratios, though DODAB and DHP miscibility in the bilayer domain was non-ideal [79]. For vesicles with DODAB as the predominant lipid, small sizes, high positive zeta potential, low main transition temperature, less angular structure, good stability, and high internal water compartment contrasted with similar properties determined for the DHP-rich vesicles; DODAB improved the bilayer fluidity of DHP vesicles both in the liquid-crystalline and in the rippled bilayer phases [79]. Interestingly, the reduction

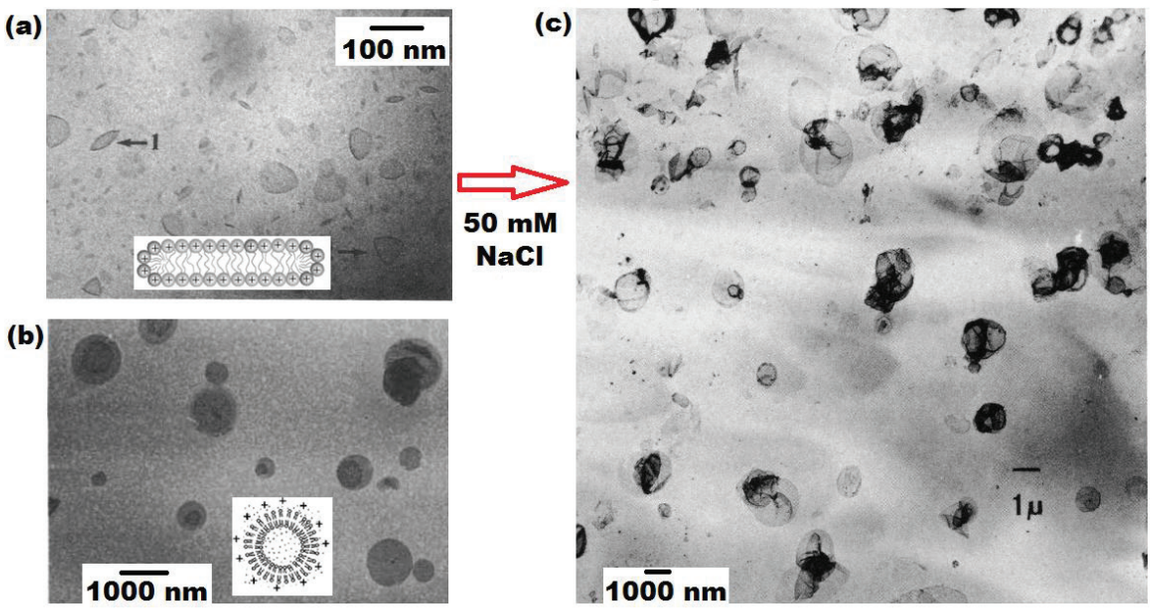

Figure 2. DODAB dispersions in water solutions obtained by different dispersion methods. (a) Cryo-transmission electron micrographs (cryo-TEM) of vitrified DODAB bilayer fragments obtained by sonication of DODAB in water [77]. Reprinted with permission from Ref. [77]. Copyright (1995) American Chemical Society. (b) Transmission electron microscopy of electronically stained large unilamellar DODAB vesicles (200–400 nm mean diameter) from vaporization of a DODAB chloroform solution in water at 70°C [78]. Reprinted from Ref. [78]. Copyright (1983) with permission of Elsevier. (c) Transmission electron microscopy of electronically stained and very large micrometric unilamellar DODAB vesicles obtained by NaCl-induced fusion of DODAB bilayer fragments [69]. Reprinted from Ref. [69]. Copyright (1986) with permission of Elsevier.

of positive charges on the DODAB/DHP vesicles improved also the survival of mammalian cells in culture [79]. These results might become important for future drug/gene delivery applications.

Cholesterol has been suggested to play a role in stable vesicle formation by adjusting the molecular packing of the vesicular bilayer. The Langmuir monolayer approach with infrared reflection-absorption spectroscopy (IRRAS) elucidated the effects of cholesterol on molecular packing of double-chained cationic surfactants [80]. Combining cholesterol with DXDAB monolayers at the air-water interface (X meaning the hydrocarbon chain length) reduced desorption of DXDAB with short alkyl chains, for example, ditetradecyldimethylammonium bromide or dihexadecyldimethylammonium bromide, into the water sub-phase and condensed the DXDAB monolayers [80]. For the DODAB monolayers, cholesterol had a dual effect inducing both order and disorder of the neighboring hydrocarbon chains; the flexible alkyl side-chain of cholesterol along with the corresponding portion of neighboring hydrocarbon chains formed a fluidic region, counteracting the conformational order induced by the sterol ring of cholesterol interacting with the alkyl chains [80].

The effect of varying the molar proportion of DODAB and neutral dipalmitoylphosphatidylcholine (DPPC) in DODAB/DPPC vesicles revealed a high bilayer and coloidal stability

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with good miscibility for the binary system and absence of phase separation at a molar proportion equal to 1 [81]. The demixing and crystallization of DODAB/DPPC binary lipid system were recently found to take place when DODAB or DPPC was dominant in the mixture (DPPC/DODAB = 1/2 or DPPC/DODAB = 2/1); when DODAB was no more than equimolar (e.g., DPPC/DODAB = 2/1 and 1/1), there was good miscibility in absence of DODAB crystallization [82]. At high or low DODAB, DPPC molar proportions, phase separation occurred upon cooling so that gel domains rich in DODAB phase-separated from DPPC-DODAB domains or DPPC domains. This phase separation for the gels would mean demixing and crystallization originating DODAB-rich and DPPC-rich tilted gel separated domains upon incubation at low temperatures [82].

Figure 3 illustrates the development of interdigitated regions in the DODAB bilayer as predicted from molecular dynamics simulation at two instants in time [73].

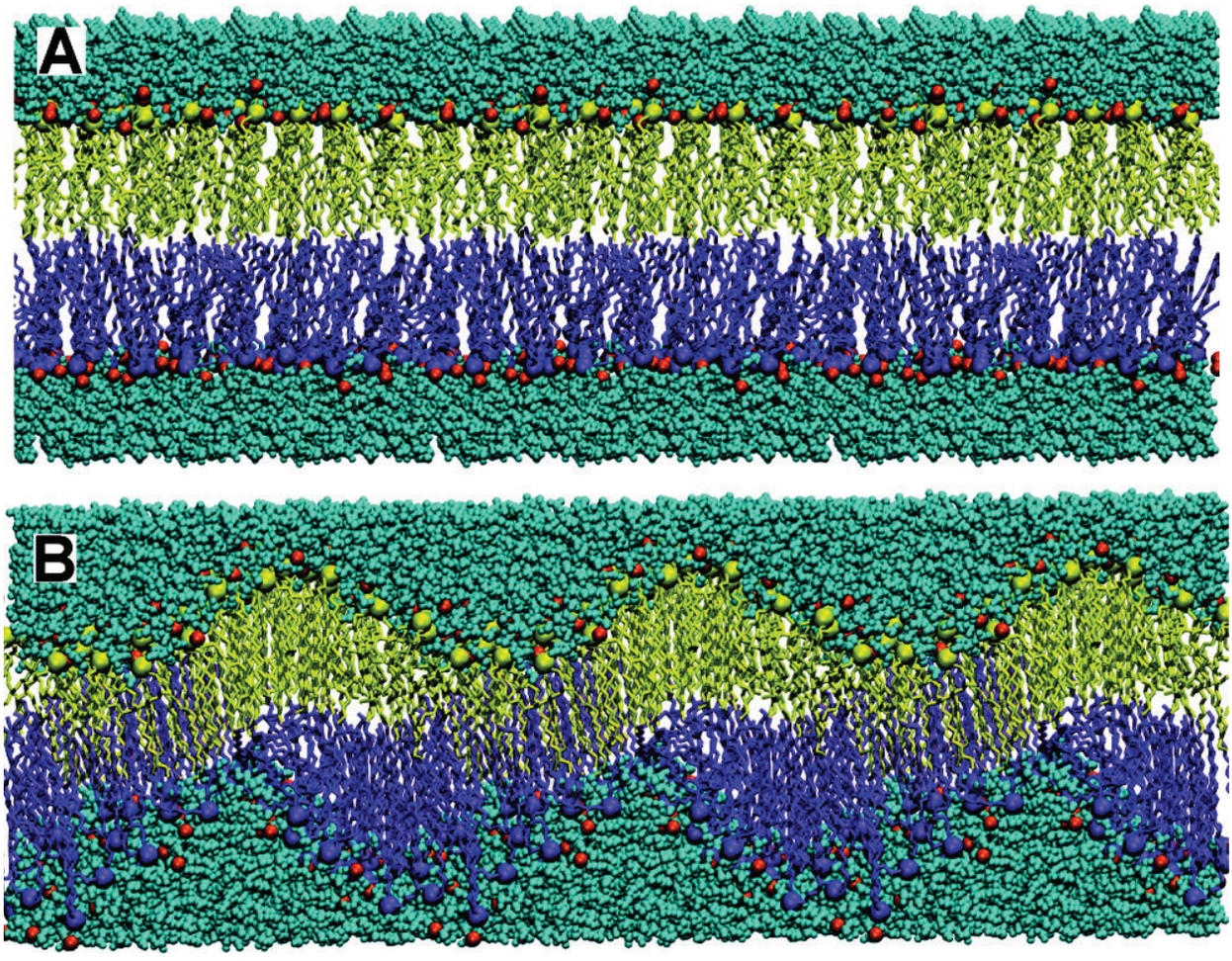

Figure 3. Molecular dynamics simulations of the DODAB bilayer at two different instants in time: 0 (A) and 90 ns (B); DODAB molecules assembled as a conventional (A) or as an interdigitated bilayer (B) where the hydrophilic quaternary ammonium heads were represented as spheres, similarly to the bromide ions; the water molecules are displayed as small spheres [73]. Reprinted with permission from Ref. [73]. Copyright (2010) American Chemical Society.

3. Novel applications for DODAB hybrid assemblies

Aqueous solubilization of water-insoluble materials is highly important for pharmaceuticals, detergency, emulsion polymerization, enhanced oil recovery, and textile dyeing. Among colloidal Self-assembled structures, micelles/vesicles are efficient solubilizers but the solubilization properties of bilayers of vesicles are superior [83, 84]. A series of double-chained surfactants, with increasing chain length (C12–18) mixed with single chained dodecylethyldimethylammonium bromide (DODABB) solubilized curcumin thanks to hydrophobic-hydrophobic and electrostatic interactions with preservation of curcumin antioxidant activity in food [85].

Aiming at the production of nanoparticles (NPs) for drug delivery, DODAB has been very useful to harmonize oppositely charged polysaccharides such as carboxymethylcellulose [58] or hyaluronic acid [86] with hydrophobic drugs such as amphotericin B [36], indomethacin [45], and tocoferol (vitamin E) [86]. Carboxymethylcellulose/DODAB/indomethacin NPs were prepared by direct injection of DODAB/indomethacin ethanol solution into a carboxymethylcellulose water solution [45]. Similarly, hyaluronate/soybean lecithin/DODAB/vitamin E NPs were prepared by direct injection of vitamin E/soybean lecithin/DODAB ethanol solution into hyaluronic acid water solution; further incorporation of these NPs in polymeric, bioadhesive films containing Aloe vera extract, hyaluronic acid, sodium alginate, polyethyleneoxide (PEO) and polyvinylalcohol (PVA) represented an innovative treatment for skin wounds [86].

A three-dimensional layer-by-layer (LbL) structure composed by xanthan and galactomannan biopolymers on DODAB liposome template created a LbL structure up to eight layers, evaluated using quartz crystal microbalance (QCM) and zeta potential analysis; these bilayer-coated NPs increased up to five times the sustained release of epidermal growth factor (EGF) and could be useful for improving the release profile of low-stability drugs like EGF [87].

The approach of combining important biomolecules such as proteins or nucleic acids with DODAB and further stabilizing the hybrids with hydrophilic polymers has been very useful for several biomedical and biotechnological applications. For instance, the delivery of DNA plasmids or small interference RNA (siRNA) to cells requires nanocarrier stability after in vivo administration though too strong stabilization can decrease the carrier efficiency; after characterizing DODAB/monoolein/pDNA or siRNA lipoplexes [88, 89], the nanocarriers were pegylated and tested for stability in serum and gene silencing in cultured cancer cells with promising results: pegylation avoided siRNA dissociation from the nanocarriers in human serum and improved transfection efficiency [90]. Stable lipoplexes of small size (100–160 nm) with a positive surface charge (>+45 mV) were readily internalized by human non-small cell lung carcinoma (H1299) cells and were efficient in promoting gene silencing. Monolein had a similar gene silencing ability as the commonly used helper lipid 1,2-dioleyl-3-phosphatidylethanolamine (DOPE), but with much lower cytotoxicity [91]. More recently, the same DODAB/monolein system was used to incorporate cell wall surface proteins (CWSP) from *Candida albicans* aiming at the production of an antigen delivery system (ADS) for a potential vaccine against candidiasis; the system facilitated antigen uptake by dendritic cells in vitro

and induced higher levels of pro-inflammatory cytokines and opsonizing specific IgG antibodies in serum of mice immunized subcutaneously [92].

DODAB was also used to treat spores of *Bacillus subtilis* aiming at gene gun delivery of DNA plasmids in mice; DODAB treated spores allowed efficient plasmid adsorption and could be loaded into biolistic cartridges and efficiently delivered into mice for induction of specific cellular and antibody responses required for DNA vaccines in vivo [93].

For textile materials, sometimes modification of the wettability of hydrophobic surfaces is essential. For instance, DODAB adsorption to hydrophobic polypropylene (PP) thin films dramatically enhanced surface adsorption of different proteins from soybeans and represented a facile treatment to obtain PP-modified surfaces that were completely hydrophilic [94].

DODAB combinations with graphene enhanced adsorption of hydrophobic analytes and improved the design of novel sensors for phenolic compounds; graphene/DODAB films exhibited remarkable synergistic effects toward the oxidation of tetrabromobisphenol TBBPA, due to the greatly increased TBBPA accumulation in the film and magnitude of the peak currents detected by chronocoulometry [95]. In another interesting instance, immobilization of urease for urea biosensing was achieved employing a DODAB monolayer at the air-water interface and natural exopolysaccharides from microalgae in the aqueous subphase; both DODAB and polysaccharide provided an appropriate microenvironment for the enzyme, enhanced its adsorption in the monolayer and could be used for the production of films supported on solid substrates [96].

Interestingly, the anisotropic polymerization of DNA adsorbed to a DODAB monolayer at the air-water interface yielded a one-dimensionally assembled belt-shaped structure and a unimolecular thickness for the polymerized DNA; thereby, the polymerization could be regulated in the two-dimensionally confined medium of the Langmuir-Blodgett film [97].

In another instance, DODAB monolayers allowed to ascertain the nanostructure of assembled oligonucleotides; two oligonucleotides, a 19-mer bearing thrombin binding aptamer sequence and a 21-mer with human telomeric sequence were end-labeled with fluorescent groups and their fluorescence spectra and G-quadruplex folding at DODAB monolayer interface were reported for the first time. Thanks to film balance measurements (pressure-area isotherms), the fluorescence spectra recording using a fiber optic accessory interfaced with a spectrofluorimeter and the DODAB monolayer, the fluorescence energy transfer efficiency of monolayer adsorbed probes increased significantly in the presence of sodium or potassium ion in subphase, which indicated that the probes retained their cation binding properties when adsorbed at the DODAB monolayer interface [98].

In the fields of antimicrobials and adjuvants for vaccines, DODAB has also been playing important roles. Biocompatible NPs of poly (methylmethacrylate) (PMMA) were synthesized in the presence of DODAB and characterized by dynamic light scattering for sizing, polydispersity and zeta potential analysis, scanning electron microscopy (SEM) for morphology visualization, and plating plus colony-forming unities (CFU) counting for

the determination of antimicrobial activity; there was a high permanent load of DODAB in the NPs, and a remarkable antimicrobial activity of PMMA/DODAB NPs, which was much higher than the one determined for DODAB itself [61]. PMMA particles loaded with DODAB were thus obtained from particle synthesis by emulsion polymerization in the presence of DODAB, a facile, fast, low-cost approach to obtaining highly efficient antimicrobial nanoparticles with a permanent DODAB load. Other hybrid DODAB assemblies with the antimicrobial peptide gramicidin (Gr) reunited the complementary antimicrobial properties of DODAB with those of the peptide [56]. DODAB dispersed as large closed bilayer vesicles (LV) or bilayer disks (BF) was added of gramicidin (Gr), which is an antimicrobial peptide assembling as channels in membranes, increasing their permeability toward cations and displaying high toxicity against mammalian cells; DODAB/Gr bilayers exhibited microbicidal action and reduced cytotoxicity against eukaryotic cells [56]. The novel formulations were characterized by dynamic light scattering for sizes an zeta-potentials, leakage from large vesicles induced by transmembrane gramicidin pores with dissipation of osmotic gradients, determination of lytic effects on bacteria and plating plus viable bacteria counting over a range of DODAB and/or Gr concentrations [56]. Gr dimers reconstituted functional channels in LV and the insertion of these channels in DODAB bilayer increased the charge density for LV but did not affect charge density of BF, with Gr at the BF borders. DODAB/Gr combinations diminished the high peptide toxicity against *Saccharomyces cerevisae* and had the advantage of broadening the spectrum of antimicrobial activity for the combination by inducing *Escherichia coli* and *Staphylococcus aureus* lysis and bacterial death. Thereby, the cytotoxicity of the peptide against eukariotic cells was reduced, and the spectrum of antimicrobial activity was broadened since DODAB and Gr displayed complementary activities [56]. More recently, the PMMA/DODAB and DODAB/Gr antimicrobial systems revealed potential uses in food microbiology for killing important food-borne pathogens such as *Escherichia coli, Salmonella enterica, Staphylococcus aureus* and *Listeria monocytogenes* [9].Nowadays, a large family of bacterial genes (generally termed quaternary ammonium genes) encode efflux pumps capable of expelling many Quat structures from bacterial cells, leading to a decrease in susceptibility to Quats [99]. Since bacteria will inevitably find ways of resisting the existing antibiotics and Quats, maybe hybrid assemblies of antimicrobials will prove strategical to overcome resistance. **Table 1** shows some schematic representations of DODAB combinations with gramicidin [56] or biocompatible PMMA polymer in PMMA/DODAB nanoparticles [61]. Their antimicrobial effects against food-borne bacteria were summarized on **Table 2** [9].

In vaccine development, adjuvants and immunostimulants have the important task of presenting antigens to the immune system eliciting an amplified and antigen-specific immune response. Among the adjuvants, DODAB is especially important due to its biomimetic hybrid nanostructures with an outer DODAB coating or an inner DODAB core, which join the advantages of particles and lipids and permit a robust control over size-dependent immune responses in vivo. Recently, hybrid nanomaterials based on DODAB with potential for combination with antigens and immunostimulants for vaccine development were reviewed [100]. For instance, in compositions with derivatives of the myco-bacterial cell wall component, the cord factor trehalose dimycolate (TDM), which is the most abundant glycolipid in the mycobacterial cell wall, DODAB yielded highly efficacious immunoadjuvant formulations

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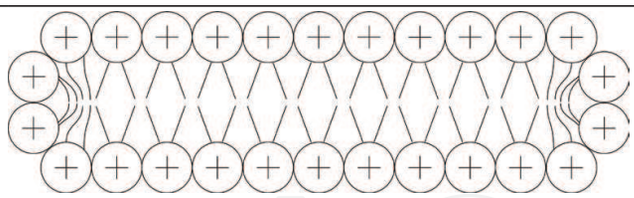
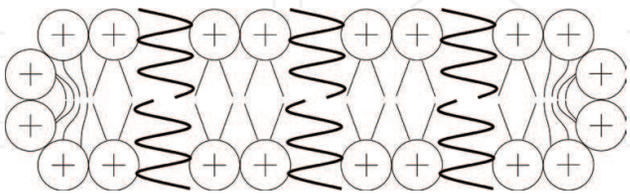
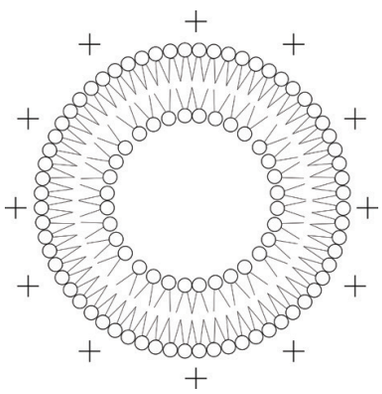
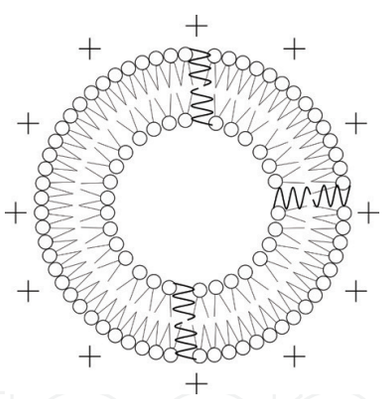
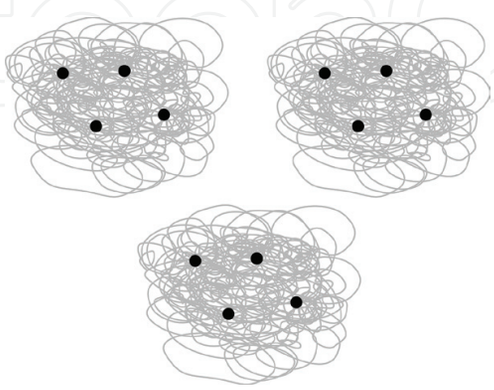
Assemblies	Schematic representation	References
DODAB BF		[71, 77]
DODAB BF/Gr		[9]
DODAB LV		[71, 78]
DODAB LV/Gr		[55, 56]
PMMA/DODAB		[33, 61]

Table 1. Some DODAB supramolecular assemblies: DODAB bilayer fragments (BF) or large closed vesicles (LV), antimicrobial peptide gramicidin D (Gr) and its assemblies with DODAB BF or DODAB LV and DODAB molecules in PMMA biocompatible polymer.

Assembly	MBC in mM; mg/mL/reduction in log(CFU/mL)			
	E. coli	*S. enterica*	*S. aureus*	*L. monocytogenes*
Gr	0.010; 0.019/0.3	0.010; 0.019/0.5	0.010; 0.019/2.1	0.005; 0.009/7.6
DODAB BF	0.063; 0.039/7.6	0.500; 0.316/1.3	0.063; 0.039/3.4	0.125; 0.079/7.8
DODAB BF/Gr	0.031; 0.019/7.5	0.250; 0.158/0.9	0.015; 0.010/3.8	0.125; 0.079/8.0
DODAB LV	0.015; 0.010/4.5	0.500; 0.316/0.7	0.015; 0.010/2.9	0.250; 0.158/5.7
DODAB LV/Gr	0.015; 0.010/4.6	0.500; 0.316/0.4	0.031; 0.019/2.7	0.063; 0.039/6.0
PMMA/DODAB NPs	–; 2.500/2.2	–; 1.250/0.1	–; 5.000/3.1	–; 5.000/1.5

Minimal bactericidal concentrations (MBC) (in mM; mg/mL) and log of viability reduction at MBC for the cationic assemblies were determined against important food-borne pathogens. For DODAB/Gr combinations, the molar ratio was [Gr] = 0.1 [DODAB]. Adapted from Ref. [9].

Table 2. Antimicrobial activity of DODAB and some of its hybrid assemblies with the antimicrobial peptide gramicidin (Gr) or the biocompatible polymer PMMA.

for tuberculosis vaccines able to induce cell-mediated immunoresponses against intracelular bacteria [101, 102]. In general, DODAB has been combined not only with antigens of interest but also with important immunostimulants such as oligonucleotides, glycolipids or lipopeptides [100].

DODAB-covered particles and DODAB bilayer fragments were often used as immunoadjuvants since DODAB can both adsorb onto several hydrophobic or hydrophilic particles and present antigens (Ag) to elicit amplified immunoresponses [65]. The electrostatic attraction drives the adsorption of a cationic DODAB bilayer onto oppositely charged polystyrene sulfate (PSS) nanoparticles (NPs) over a range of particle sizes [25, 27]. Adsorption isotherms and electrokinetic properties of the covered particles show the deposition of DODAB onto silica or PSS particles at maximal adsorption [21, 22, 25, 27, 28]. At maximal adsorption, the area per DODAB molecule adsorbed onto PSS particles is 0.286 nm^2, which is half of the usual area per monomer in DODAB monolayers at the air-water interface and suggests bilayer deposition onto the polystyrene surface; electrokinetic properties of the covered particles are very similar to those of DODAB vesicles [25]. The hydrodynamic diameter of particles in the particles/DODAB mixtures increases 9–10 nm. A tiny concentration of 10-micromolar is required for bilayer coverage of 10^9 particles (300 nm diameter) per mL at sub-toxic DODAB concentrations. DODAB toxicity against fibroblasts in cell culture becomes significant above 0.1 mM DODAB; there is 50% of cell death at 0.5 mM DODAB [103]. Lipid-covered NPs are useful for antigen presentation [30].

The mean molecular area of DODAB in a monolayer at the air-water interface is 0.6 nm^2 [70]. For particles with 300 nm of mean diameter, the bilayer coverage of total surface area on 5×10^9 particles/mL requires 10 μM DODAB only [30]. At this minute amount, the usual DODAB toxicity is not relevant. In contrast, DODAB vesicles used as immunoadjuvants over the millimolar range of DODAB concentrations may be toxic in vivo [52]. Antigen (Ag) adsorption to the PSS/DODAB assembly does not disturb the order of the particulate over a

range of Ag concentrations; the PSS/DODAB system at 5×10^9 particles/mL accommodates well up to 25 μg/mL Ag with narrow size distributions for PSS/DODAB/Ag NPs over this range of Ag concentrations [30]. This homogeneity for the particle size in the dispersions yields low polydispersities determined by dynamic light scattering, inside the 0.05–0.10 range [30].

DODAB molecules ultrasonically dispersed in aqueous solution are nano-sized bilayer disks or bilayer fragments (BF); the electrostatic repulsion at low ionic strength keeps the BF stable in aqueous dispersions [39, 64]. DODAB BFs are antimicrobial agents [39, 43], carriers for hydrophobic drugs [104] and useful for the production of lipid-covered particles such as bilayer-coated sílica [22] or PSS [28]. DODAB BFs also present antigens to the immune system inducing cellular immune responses [54]. These open bilayers differ from their mother vesicles by especial features. They do not respond to osmotic gradients because they do not have an inner aqueous compartment. They have a discoidal shape with disks exhibiting one bilayer thickness and both faces available to display antigens [54]. They have domains of fluid and gel lipid phases [105]. They solubilize hydrophobic molecules sometimes in contrast to their mother vesicles that do not do so as in the case of amphotericin B [104]. DODAB BF interact with proteins, oligonucleotides or DNA via both the hydrophobic effect and the electrostatic attraction at low ionic strength. Bovine serum albumin (BSA) purified 18/14 kDa antigens from *Taenia crassiceps* cysticerci (18/14-Tcra) or a recombinant heat-shock protein (hsp-18 kDa) from *Mycobacterium leprae* adsorb on DODAB BF [54]. DODAB BF/Ag NPs are stable over a range of DODAB and Ag concentrations; these ranges vary with the Ag nature and are different for different antigens [54]. The production of cytokines by lymph nodes (LN) cells of immunized mice in culture is important to determine the nature of immune response induced by PSS/DODAB/Ag or DODAB BF/Ag. The mice immunized with antigen alone, adjuvant/antigen or adjuvant alone provide LN cells in culture that produce different cytokines depending on Ag and adjuvant nature [54]. A sandwich kit enzyme-linked immunosorbent assay (ELISA) determines the analytical concentrations of the cytokines produced after reestimulating the cells in culture. The cytokines profile is rather different from immunization with the parasite and the bacterium antigens [54]. The high levels of IL-12 and IFN-gamma induced by PSS/DODAB/Ag and DODAB BF/Ag when Ag is hsp-18kDa shows that these adjuvants are useful for the design of subunit vaccines against intracelular bacteria. IL-12 and IFN-gamma are the most important cytokines in innate responses to intracellular bacteria such as M. leprae or tuberculosis; when Ag is 18/14-Tcra, there is an enhancement in production of IL-10 and Il-13 by LN cells elicited by DODAB BF/Ag. These cytokines are typically associated with responses to allergens and parasites such as helminths and mediate differentiation of CD4+-T cells into Th2 cells [106]. On the other hand, the *Mycobacterium leprae* antigen carried by DODAB BF or PSS/DODAB adjuvants elicits low levels of these cytokines. Responses are indeed different for the helminthes and the bacteria antigens and antigen-specific as they should be [54, 106].

IL-10 exerts an inhibitory effect on macrophages and dendritic cells by decreasing the production of IL-12 and the expression of class II major histocompatibility complex (MHC) [106]. Macrophages and DCs also secrete IL-12 that induces T cells differentiation into Th1 and natural killer (NK) cells with increased IFN-gamma synthesis and cytotoxic activity. The adaptive immunity against intracellular bacteria is principally cell mediated and consists of

activation of macrophages by CD4+T cells as well as killing of infected cells by CD8+ cytotoxic T lymphocytes (CTL). Naïve CD4+ T cells may differentiate into distinct subsets, such as Th1 and Th2 cells in response to different antigens.

Due to its chemical stability and low cost when compared to other natural or synthetic lipids, DODAB has been intensively investigated aiming at subunit vaccine design. Major problems of liposomal formulations based on DODAB are the high DODAB concentration (1–10 mM DODAB) and the large liposomes size [52, 106, 107]. Minimization of DODAB dose is required for administration in vivo. DODAB BF effectively present antigens at 0.1 mM DODAB only; supported DODAB bilayers on PSS or silica require even lower DODAB concentrations [22, 25, 30, 54]. The total surface area on the BF dispersion available for antigen association are much larger than the one for closed, large and sometimes multibilayered liposomes. Thus, the first advantage of DODAB BF, PSS/DODAB or silica/DODAB as adjuvants would be the low DODAB concentration required for Ag presentation. The second advantage of BF is the nanosize. Depending on sonication power and time plus composition of the dispersing medium that determine colloidal stability, DODAB BF/Ag complexes have a few tenths of nanometers in size (40–80 nm). This size is effective for antigen delivery to antigen-presenting cells (APCs), generating potent and combined humoral and CD8+ T cell immunity [109–111]. Over a range of low DODAB and antigen concentrations ([DODAB] ≤0.1 mM; 0.001–0.05 mg/mL antigen), adjuvant/antigen combinations were cationic, stable, homodisperse and immunogenic at low DODAB dose, low cost, low sizes for improved dendritic cells uptake, high chemical stability, prone to present several different antigens and displaying low or even absent cytotoxicity. They were remarkably superior to alum due to their ability to elicit the cellular Th1 immune response. Contrary to alum or DODAB LV (1–10 mM DODAB), local or systemic adverse effects in mice were completely absent over the 0.1–0.01 mM DODAB range. Silica/DODAB, PSS/DODAB, and DODAB BF are available over the sub-200 nm range of sizes thus presenting potential also for design of mucosal vaccines. The third advantage of BF is the absence of depots at the site of injection, an inflammatory reaction that is not always desirable [54]. These depots occur for DODAB large vesicles (LV) and appear due to inflammatory responses at the site of injection [107, 108]. Similar sizes for adjuvant and adjuvant-antigen complexes evidenced that the antigens readily adsorbed and stabilized the adjuvant; conversely, the adjuvant also stabilized the antigens preventing antigen-antigen aggregation as often observed for protein-protein interactions [30, 54].

An important component of the early innate immune response to viruses and bacteria is IL-12 that enhances the IFN-gamma production and the development of Th1 cells; IL-12 is involved in the combat of infections by cell-mediated immunity, for example, leishmaniasis [106]. Subunit vaccines against protozoa that survive within macrophages require as principal defense mechanism the cell-mediated immunity, particularly directed to macrophage activation by Th1 cell-derived cytokines. Immune responses to leishmaniasis against the parasite *Leishmania donovani* involve cell-mediated immune response of the Th1 type and CD4+ Th1 cells activation for killing phagocytosed parasites. Leishmania-specific Th1 CD4+ T cells produce IFN-gamma, that activates macrophages to kill intracellular parasites. On the other hand, the parasite activates Th2 cells increasing their production of Th2 cytokines that suppress the activity of macrophages and increase parasite survival

[106]. Similarly, during the liver stages of malaria, CD8+ T cells kill infected hepatocytes and induce the secretion of IFN-gamma activating the production of nitric oxide and other agents by the hepatocytes for killing the parasites. IL-12 stimulates IFN-gamma production inducing resistance to sporozoite challenge in rodents and non-human primates [106]. Il-12 also increases the cytotoxic activity of natural killer (NK) cells after viral infections thereby mediating the NK cell killing of virus-infected cells for combating the infection. Recombinant DNA vaccines expressing membrane and envelope of viral proteins may benefit from the DODAB BF or PSS/DODAB adjuvants, which can also carry DNA [31] or oligonucleotides [49].

DNA sequences containing unmethylated CpG dinucleotide generate danger signals that are recognized by the immune system; they are typical of bacteria and viruses but rare in vertebrates activating cells that express Toll-like receptor 9 and induce an innate immune response characterized by the production of Th1 cytokines [112]. Both CpG and DODAB improve Th1 responses against antigens when used separately. DODAB BF/CpG presenting ovalbumin (OVA) also enhanced Th1 immune responses [50]. DODAB BF/CpG/OVA also did not result in any observable depot effect at the site of prime suggesting their direct action on the antigen presenting cells (APC) of the draining LN. Only NPs can specifically target LN-resident cells [113]. The interstitial flow convects sub-100 nm NPs into the draining lymphatic vessels; NPs are not trapped in the tissue interstitium. Nano-sizes allow direct LN targeting without the use of specific ligands. In the LN, antigen-presenting cells (APCs) rapidly capture the NPs. A few reviews are available on DODAB applications for the development of novel hybrid assemblies useful as immunoadjuvants, gene or RNA carriers [114–118].

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References

[1] Block SS. Disinfection, Sterilization and Preservation (5th edition). Block SS (Ed.), Lippincott, Williams & Wilkins, Philadelphia, 2001. 1481 p.

[2] Attwood D, Florence AT, editors. Surfactant Systems: Their Chemistry, Pharmacy and Biology. 1st ed. London EC4P 4EE: Chapman & Hall, 1983. 794 p. Available from http://www.springer.com/br/book/9789400957770

[3] Carmona-Ribeiro AM. Lipid-based biomimetics in drug and vaccine delivery, In: Mukherjee A, editor. Biomimetics Learning from Nature. Olajinica: InTech: 2010. pp. 507-534, doi:10.5772/8792. Available from: http://www.intechopen.com/books/biomimetics-learning-from-nature/lipid-based-biomimetics-in-drug-and-vaccine-delivery

[4] Carmona-Ribeiro AM, Barbassa B, Melo LD. Antimicrobial Biomimetics. In Cavrak M, editor., Biomimetic Based Applications, Olajinica: InTech: 2011. pp. 227-284. doi:10.5772/14400. Available from: http://www.intechopen.com/books/biomimetic-based-applications/antimicrobial-biomimetics

[5] Dai C, Li W, Cui Y, Sun Y, Wu W, Xu Z, Liu Y, Yang Z, Wu V. The effect of functional groups on the sphere-to-wormlike micellar transition in quaternary ammonium surfactant solutions, Colloids Surf A: Physicochem Eng Aspects. 2016;**500**:32-39. doi:10.1016/j.colsurfa.2016.04.024. (http://www.sciencedirect.com/science/article/pii/S0927775716302564)

[6] Carmona-Ribeiro AM, Vieira DB, Lincopan N. Cationic surfactants and lipids as anti-infective agents. Anti-Infect Agents Med Chem 2006;**5**:33-54. doi:10.2174/187152106774755572

[7] Vieira DB, Carmona-Ribeiro AM. Cationic lipids and surfactants as antifungal agents: mode of action. J Antimicrob Chemother 2006;**58**:760-767. doi:10.1093/jac/dkl312

[8] De Melo Carrasco LD, Sampaio JL, Carmona-Ribeiro AM. Supramolecular cationic assemblies against multidrug-resistant microorganisms: activity and mechanism of action. Int J Mol Sci. 2015;**16**(3):6337-6352. doi:10.3390/ijms16036337

[9] Carrasco LD, Bertolucci R Jr, Ribeiro RT, Sampaio JL, Carmona-Ribeiro AM. Cationic nanostructures against foodborne pathogens. Front Microbiol. 2016;**7**:1804. doi:10.3389/fmicb.2016.01804

[10] Zhang C, Cui F, Zeng GM, Jiang M, Yang ZZ, Yu ZG, Zhu MY, Shen LQ. Quaternary ammonium compounds (QACs): a review on occurrence, fate and toxicity in the environment. Sci Total Environ. 2015;**518-519**:352-362. doi:10.1016/j.scitotenv.2015.03.007

[11] Garcia MT, Kaczerewska O, Ribosa I, Brycki B, Materna P, Drgas M. Biodegradability and aquatic toxicity of quaternary ammonium-based gemini surfactants: effect of the spacer on their ecological properties. Chemosphere. 2016;**154**:155-60. doi:10.1016/j.chemosphere.2016.03.109

[12] Brycki B, Waligórska M, Szulc A. The biodegradation of monomeric and dimeric alkylammonium surfactants. J Hazard Mater. 2014;**280**:797-815. doi:10.1016/j.jhazmat.2014.08.021

[13] Minbiole KPC, Jennings MC, Ator LE, Black JW, Grenier MC, LaDow JE, Caran KL, Seifert K, Wuest WM. From antimicrobial activity to mechanism of resistance: the

multifaceted role of simple quaternary ammonium compounds in bacterial eradication. Tetrahedron. 2016;72:3559-3566. doi:10.1016/j.tet.2016.01.014

[14] Israelachvili JN, Mitchell DJ, Ninham BW. Theory of self-assembly of lipid bilayers and vesicles. Biochim Biophys Acta. 1977;**470**(2):185-201. doi:10.1016/0005-2736(77)90099-2

[15] Israelachvili JN. Intermolecular and Surface Forces. 3rd ed. San Diego: Academic Press; 2011. 674 p. doi:10.1016/B978-0-12-375182-9.10025-9

[16] Marra J. Effects of counterion specificity on the interactions between quaternary ammonium surfactants in monolayers and bilayers. J Phys Chem. 1986;**90**(10):2145-2150. doi:10.1021/j100401a031

[17] Guldbrand L, Jonsson B, Wennerstrom H, Linse P. Electrical double layer forces. A Monte Carlo study. J Chem Phys. 1984;**80**:2221-2228. doi:10.1063/1.446912

[18] Pashley RM, McGuiggan PM, Ninham BW, Brady J, Evans DF. Direct measurements of surface forces between bilayers of double-chained quaternary ammonium acetate and bromide surfactants. J Phys Chem. 1986;**90**(8):1637-1642. doi:10.1021/j100399a037

[19] Nascimento DB, Rapuano R, Lessa MM, Carmona-Ribeiro AM. Counterion effects on properties of cationic vesicles. Langmuir 1998;**14**(26):7387-7391. doi:10.1021/la980845c

[20] Rapuano R, Carmona-Ribeiro AM. Physical adsorption of bilayer membranes on silica. J Colloid Interface Sci. 1997;**193**(1):104-111. doi:10.1006/jcis.1997.5060

[21] Rapuano R, Carmona-Ribeiro AM. Supported bilayers on silica. J Colloid Interface Sci. 2000;**226**:299-307. doi:10.1006/jcis.2000.6824

[22] Moura SP, Carmona-Ribeiro AM. Cationic bilayer fragments on silica at low ionic strength: competitive adsorption and colloid stability. Langmuir. 2003;**19**(17):6664-6667. doi:10.1021/la034334o

[23] Moura SP, Carmona-Ribeiro AM. Adsorption behavior of DODAB/DPPC vesicles on silica. J Colloid Interface Sci. 2007;**313**(2):519-26. doi:10.1016/j.jcis.2007.04.061

[24] Pereira EM, Petri DF, Carmona-Ribeiro AM. Adsorption of cationic lipid bilayer onto flat silicon wafers: effect of ion nature and concentration. J Phys Chem B. 2006;**110**(20):10070-10074. doi: 10.1021/jp060737w

[25] Carmona-Ribeiro AM, Midmore BR. Synthetic bilayer adsorption onto polystyrene microspheres. Langmuir. 1992;**8**(3):801-806. doi:10.1021/la00039a013

[26] Tsuruta LR, Lessa MM, Carmona-Ribeiro AM. Interactions between dioctadecyldimethylammonium chloride or bromide bilayers in water. Langmuir. 1995;**11**(8):2938-2943. doi:10.1021/la00008a016

[27] Tsuruta LR, Lessa MM, Carmona-Ribeiro AM. Effect of particle size on colloid stability of bilayer-covered polystyrene microspheres. J Colloid Interface Sci. 1995;**175**(2):470-475. doi:10.1006/jcis.1995.1477

[28] Carmona-Ribeiro AM, De Moraes Lessa M. Interactions between bilayer membranes and látex. Colloids Surf. A: Physicochem. Eng. Aspects. 1999;**153**(1-3):355-361. doi:10.1016/S0927-7757(98)00532-9

[29] Pereira EMA, Vieira DB, Carmona-Ribeiro AM. Cationic bilayers on polymeric particles: effect of low NaCl concentration on surface coverage. J Phys Chem B. 2004;**108**(31):11490-11495. doi:10.1021/jp048555u

[30] Lincopan N, Espíndola NM, Vaz AJ, Carmona-Ribeiro AM. Cationic supported lipid bilayers for antigen presentation. Int J Pharm. 2007;**340**(1-2):216-22. doi:10.1016/j.ijpharm.2007.03.014

[31] Rosa H, Petri DF, Carmona-Ribeiro AM. Interactions between bacteriophage DNA and cationic biomimetic particles. J Phys Chem B. 2008;**112**(51):16422-16430. doi:10.1021/jp806992f

[32] Pereira EMA, Petri DF, Carmona-Ribeiro AM. Synthetic vesicles at hydrophobic surfaces. J Phys Chem B 2002;**106**(34):8762-8767. doi:10.1021/jp020735l

[33] Pereira EM, Kosaka PM, Rosa H, Vieira DB, Kawano Y, Petri DF, Carmona-Ribeiro AM. Hybrid materials from intermolecular associations between cationic lipid and polymers. J Phys Chem B. 2008;**112**(31):9301-9310. doi:10.1021/jp801297t

[34] Melo LD, Palombo RR, Petri DF, Bruns M, Pereira EM, Carmona-Ribeiro AM. Structure-activity relationship for quaternary ammonium compounds hybridized with poly(methyl methacrylate). ACS Appl Mater Interfaces. 2011;**3**(6):1933-1939. doi:10.1021/am200150t

[35] Vieira DB, Pacheco LF, Carmona-Ribeiro AM. Assembly of a model hydrophobic drug into cationic bilayer fragments. J Colloid Interface Sci. 2006;**293**(1):240-247. doi:10.1016/j.jcis.2005.06.046

[36] Vieira DB, Carmona-Ribeiro AM. Cationic nanoparticles for delivery of amphotericin B: preparation, characterization and activity in vitro. J Nanobiotechnol. 2008;6:6. doi:10.1186/1477-3155-6-6

[37] Pacheco LF, Carmona-Ribeiro AM. Effects of synthetic lipids on solubilization and colloid stability of hydrophobic drugs. J Colloid Interface Sci. 2003;**258**(1):146-154. doi:10.1016/S0021-9797(02)00103-0

[38] Lincopan N, Mamizuka EM, Carmona-Ribeiro AM. In vivo activity of a novel amphotericin B formulation with synthetic cationic bilayer fragments. J Antimicrob Chemother. 2003;**52**(3):412-418. doi:10.1093/jac/dkg383

[39] Carmona-Ribeiro AM. Bilayer-forming synthetic lipids: drugs or carriers? Curr Med Chem. 2003;**10**(22):2425-1446. doi:10.2174/0929867033456611

[40] Lincopan N, Mamizuka EM, Carmona-Ribeiro AM. Low nephrotoxicity of an effective amphotericin B formulation with cationic bilayer fragments. J Antimicrob Chemother. 2005;**55**(5):727-734. doi:10.1093/jac/dki064

[41] Lincopan N, Carmona-Ribeiro AM. Lipid-covered drug particles: combined action of dioctadecyldimethylammonium bromide and amphotericin B or miconazole. J Antimicrob Chemother. 2006;**58**(1):66-75. doi:10.1093/jac/dkl153

[42] Lincopan N, Santana MR, Faquim-Mauro E, da Costa MH, Carmona-Ribeiro AM. Silica-based cationic bilayers as immunoadjuvants. BMC Biotechnol. 2009;**9**:5. doi:10.1186/1472-6750-9-5

[43] Carmona-Ribeiro AM. Lipid bilayer fragments and disks in drug delivery. Curr Med Chem. 2006;**13**(12):1359-70. doi:10.2174/092986706776872925

[44] Barbassa L, Mamizuka EM, Carmona-Ribeiro AM. Supramolecular assemblies of rifampicin and cationic bilayers: preparation, characterization and micobactericidal activity. BMC Biotechnol. 2011;**11**:40. doi:10.1186/1472-6750-11-40

[45] Lima EG, Gomes LR, Carmona-Ribeiro AM. Stable indomethacin dispersions in water from drug, ethanol, cationic lipid and carboxymethyl-cellulose. Pharm Nanotechnol. 2016;**4**(2):126-135. doi:10.2174/2211738504666160304195436. (Available from http://www.eurekaselect.com/node/140208/article)

[46] Kikuchi IS, Carmona-Ribeiro AM. Interactions between DNA and synthetic cationic liposomes. J Phys Chem B. 2000;**104**(13):2829-2835. doi:10.1021/jp9935891

[47] Kikuchi, IS, Viviani W, Carmona-Ribeiro AM. Nucleotide insertion in cationic bilayers. J Phys Chem A. 1999;**103**:8050-8055. doi:10.1021/jp9911090

[48] Nantes IL, Correia FM, Faljoni-Alario A, Kawanami AE, Ishiki HM, Amaral AT, Carmona-Ribeiro AM. Nucleotide conformational change induced by cationic bilayers. Arch Biochem Biophys. 2003;**416**(1):25-30. doi:10.1016/S0003-9861(03)00280-7

[49] Rozenfeld JH, Oliveira TR, Lamy MT, Carmona-Ribeiro AM. Interaction of cationic bilayer fragments with a model oligonucleotide. Biochim Biophys Acta. 2011;**1808**(3):649-655. doi:10.1016/j.bbamem.2010.11.036

[50] Rozenfeld JH, Silva SR, Ranéia PA, Faquim-Mauro E, Carmona-Ribeiro AM. Stable assemblies of cationic bilayer fragments and CpG oligonucleotide with enhanced immunoadjuvant activity in vivo. J Control Release. 2012;**160**(2):367-373. doi:10.1016/j.jconrel.2011.10.017

[51] Carvalho LA, Carmona-Ribeiro AM. Interactions between cationic vesicles and serum proteins Langmuir. 1998;**14**(21):6077-6081. doi:10.1021/la980345j

[52] Tsuruta LR, Quintilio W, Costa MH, Carmona-Ribeiro AM. Interactions between cationic liposomes and an antigenic protein: the physical chemistry of the immunoadjuvant action. J Lipid Res. 1997;**38**(10):2003-2011.

[53] Lincopan N, Carmona-Ribeiro AM. Protein assembly onto cationic supported bilayers. J Nanosci Nanotechnol. 2009;**9**(6):3578-3586. doi:10.1166/jnn.2008.003

[54] Lincopan N, Espíndola NM, Vaz AJ, da Costa MH, Faquim-Mauro E, Carmona-Ribeiro AM. Novel immunoadjuvants based on cationic lipid: preparation, characterization and activity in vivo. Vaccine. 2009;**27**(42):5760-71. doi:10.1016/j.vaccine.2009.07.066

[55] Carvalho CA, Olivares-Ortega C, Soto-Arriaza MA, Carmona-Ribeiro AM. Interaction of gramicidin with DPPC/DODAB bilayer fragments. Biochim Biophys Acta. 2012;**1818**(12):3064-3071. doi:10.1016/j.bbamem.2012.08.008

[56] Ragioto DA, Carrasco LD, Carmona-Ribeiro AM. Novel gramicidin formulations in cationic lipid as broad-spectrum microbicidal agents. Int J Nanomed. 2014;**9**:3183-3192. doi:10.2147/IJN.S65289

[57] Carmona-Ribeiro AM, de Melo Carrasco LD. Novel formulations for antimicrobial peptides. Int J Mol Sci. 2014;**15**(10):18040-18083. doi:10.3390/ijms151018040

[58] Correia FM, Petri DF, Carmona-Ribeiro AM. Colloid stability of lipid/polyelectrolyte decorated latex. Langmuir. 2004;**20**(22):9535-9540. doi:10.1021/la048938j

[59] Melo LD, Mamizuka EM, Carmona-Ribeiro AM. Antimicrobial particles from cationic lipid and polyelectrolytes. Langmuir. 2010;**26**(14):12300-12306. doi:10.1021/la101500s

[60] Leão-Silva AC, Naves AF, Pereira EM, Petri DF, Carmona-Ribeiro AM. Assembly of horseradish peroxidase within supported cationic bilayers. Biotechnol Prog. 2011;**27**(5):1433-41. doi:10.1002/btpr.640

[61] Naves AF, Palombo RR, Carrasco LD, Carmona-Ribeiro AM. Antimicrobial particles from emulsion polymerization of methyl methacrylate in the presence of quaternary ammonium surfactants. Langmuir. 2013;**29**(31):9677-9684. doi:10.1021/la401527j

[62] Carmona-Ribeiro AM. Preparation and characterization of biomimetic nanoparticles for drug delivery. Methods Mol Biol. 2012;**906**:283-294. doi:10.1007/978-1-61779-953-2_22

[63] Carmona-Ribeiro AM. Biomimetic particles in drug and vaccine delivery. J Liposome Res. 2007;**17**(3-4):165-72.

[64] Carmona-Ribeiro AM. Biomimetic nanoparticles: preparation, characterization and biomedical applications. Int J Nanomed. 2010;**5**:249-59.

[65] Carmona-Ribeiro AM. Biomimetic systems in nanomedicine. In: Torchilin, V. (Ed,) Handbook of Nanobiomedical Research. Singapore: World Scientific; 2014. Available from: http://www.worldscientific.com/doi/abs/10.1142/97898 14520652_0063.

[66] Carmona-Ribeiro AM. Chapter 4—Interactions between bilayer vesicles, biomolecules and interfaces. In Handbook of Surfaces and Interfaces of Materials, edited by Hari Singh Nalwa, Burlington: Academic Press, 2001, pp. 129-165. doi:10.1016/B978-01251 3910-6/50061-X

[67] Mamizuka EM, Carmona-Ribeiro AM. Cationic liposomes as antimicrobial agents. In: Antonio Méndez Vilas. (Org.). Communicating Current Research and Educational Topics and Trends in Applied Microbiology. 1ed. Badajoz: Formatex, 2007, v. 2, pp. 636-647. ISBN-13: 978-84-611-9423-0. Available from http://www.formatex.org/microbio/pdf/pages636-647.pdf

[68] Carmona-Ribeiro AM, Yoshida LS, Chaimovich H. Salt effects on the stability of dioctadecyldimethylammonium chloride and sodium dihexadecyl phosphate vesicles. J Phys Chem. 1985;**89**(13):2928-2933. doi:10.1021/j100259a045

[69] Carmona-Ribeiro AM, Chaimovich H. Salt-induced aggregation and fusion of dioctadecyldimethylammonium chloride and sodium dihexadecylphosphate vesicles. Biophys J. 1986;**50**(4):621-628. doi:10.1016/S0006-3495(86)83501-9

[70] Claesson PM, Carmona-Ribeiro AM, Kurihara K. Dihexadecyl phosphate monolayers: intralayer and interlayer interactions. J Phys Chem. 1989;**93**(2):917-922. doi:10.1021/j100339a071

[71] Carmona-Ribeiro AM. Synthetic amphiphile vesicles. Chem Soc Rev. 1992;**21**:209-214. doi:10.1039/CS9922100209

[72] Carmona-Ribeiro AM. Interactions between charged spheric vesicles. J Phys Chem. 1993;**97**(45):11843-11846. doi:10.1021/j100147a043

[73] Jamróz D, Kepczynski M, Nowakowska M. Molecular structure of the dioctadecyldimethylammonium bromide (DODAB) bilayer. Langmuir. 2010;**26**(19):15076-15079. doi:10.1021/la102324p

[74] Feitosa E, Adati RD, Hansson P, Malmsten M. Thermal and structural behavior of dioctadecyldimethylammonium bromide dispersions studied by differential scanning calorimetry and X-ray scattering. Langowski J, ed. PLoS One. 2012;**7**(9):e44702. doi:10.1371/journal.pone.0044702.

[75] Kepczynski M, Bednar J, Kuźmicz D, Wydro P, Nowakowska M. Spontaneous formation of densely stacked multilamellar vesicles in dioctadecyldimethylammonium bromide/oleosiloxane mixtures. Langmuir. 2010;**26**(3):1551-1556. doi:10.1021/la904094e

[76] Kepczynski M, Lewandowska J, Witkowska K, Kędracka-Krok S, Mistrikova V, Bednar J, Wydro P, Nowakowska M. Bilayer structures in dioctadecyldimethylammonium bromide/oleic acid dispersions. Chem Phys Lipids. 2011;**164**(5):359-367. doi:10.1016/j.chemphyslip.2011.04.007

[77] Andersson M, Hammarstroem L, Edwards K. Effect of bilayer phase transitions on vesicle structure and its influence on the kinetics of viologen reduction. J Phys Chem. 1995;**99**:14531-14538. **doi:**10.1021/j100039a047

[78] Carmona-Ribeiro AM, Chaimovich H. Preparation and characterization of large dioctadecyldimethylammonium chloride liposomes and comparison with small sonicated vesicles. Biomembranes. 1983;**733**(1):172-179. doi:10.1016/0005-2736(83)90103-7

[79] Chou TH, Liang CH, Lee YC, Yeh LH. Effects of lipid composition on physicochemical characteristics and cytotoxicity of vesicles composed of cationic and anionic dialkyl lipids. Phys Chem Chem Phys. 2014;**16**(4):1545-53. doi:10.1039/c3cp54176b

[80] Kuo AT, Chang CH. Elucidating the effects of cholesterol on the molecular packing of double-chained cationic lipid langmuir monolayers by infrared reflection-absorption spectroscopy. J Oleo Sci. 2015;**64**(4):455-465. doi:10.5650/jos.ess14266

[81] Sobral CN, Soto MA, Carmona-Ribeiro AM. Characterization of DODAB/DPPC vesicles. Chem Phys Lipids. 2008;**152**(1):38-45. doi:10.1016/j.chemphyslip.2007.12.004

[82] Wu FG, Wu RG, Sun HY, Zheng YZ, Yu ZW. Demixing and crystallization of DODAB in DPPC-DODAB binary mixtures. Phys Chem Chem Phys. 2014;**16**(29):15307-15318. doi:10.1039/c4cp01707b

[83] Lawrence MJ. Surfactant systems: their use in drug delivery. Chem Soc Rev. 1994;**23**:417-424. doi:10.1039/CS9942300417

[84] Maswal M, Pandith AH, Islam N, Dar AA. Co-solubilization of the hydrophobic drugs carbamazepine and nifedipine in aqueous nonionic surfactant media. J Solut. Chem. 2013;**42**:1374-1392. doi:10.1007/s10953-013-0036-4

[85] Kumar A, Kaur G, Kansal SK, Chaudhary GR, Mehta SK. Enhanced solubilization of curcumin in mixed surfactant vesicles. Food Chem. 2016;**199**:660-666. doi:10.1016/j.foodchem.2015.12.077

[86] Pereira GG, Detoni CB, Balducci AG, Rondelli V, Colombo P,Guterres SS, Sonvico F. Hyaluronate nanoparticles included in polymer films for the prolonged release of vitamin E for the management of skin wounds. Eur J Pharm Sci. 2016;**83**:203-211. doi:10.1016/j.ejps.2016.01.002

[87] Kaminski GA, Sierakowski MR, Pontarolo R, Santos LA, de Freitas RA. Layer-by-layer polysaccharide-coated liposomes for sustained delivery of epidermal growth factor. Carbohydr Polym. 2016;**140**:129-135. doi:10.1016/j.carbpol.2015.12.014

[88] Silva JP, Oliveira IM, Oliveira AC, Lúcio M, Gomes AC, Coutinho PJ, Oliveira ME. Structural dynamics and physicochemical properties of pDNA/DODAB:MO lipoplexes:effect of pH and anionic lipids in inverted non-lamellar phases versus lamellar phases. Biochim Biophys Acta. 2014;**1838**(10):2555-2567. doi:10.1016/j.bbamem.2014.06.014

[89] Silva JP, Oliveira AC, Lúcio M, Gomes AC, Coutinho PJ, Oliveira ME. Tunable pDNA/DODAB:MO lipoplexes: the effect of incubation temperature on pDNA/DODAB:MO lipoplexes structure and transfection efficiency. Colloids Surf B: Biointerfaces. 2014;**121**:371-379. doi:10.1016/j.colsurfb.2014.06.019

[90] Oliveira AC, Raemdonck K, Martens T, Rombouts K, Simón-Vázquez R, Botelho C, Lopes I, Lúcio M, González-Fernández Á, Real Oliveira ME, Gomes AC, Braeckmans K. Stealth monoolein-based nanocarriers for delivery of siRNA to cancer cells. Acta Biomater. 2015;**25**:216-29. doi:10.1016/j.actbio.2015.07.032

[91] Oliveira AC, Martens TF, Raemdonck K, Adati RD, Feitosa E, Botelho C, Gomes AC, Braeckmans K, Real Oliveira ME. Dioctadecyldimethylammonium:monoolein nanocarriers for efficient in vitro gene silencing. ACS Appl Mater Interfaces. 2014;**6**(9):6977-6989. doi:10.1021/am500793y

[92] Carneiro C, Correia A, Lima T, Vilanova M, Pais C, Gomes AC, Real Oliveira ME, Sampaio P. Protective effect of antigen delivery using monoolein-based liposomes in experimental hematogenously disseminated candidiasis. Acta Biomater. 2016;**39**:133-145. doi:10.1016/j.actbio.2016.05.001

[93] Aps LR, Tavares MB, Rozenfeld JH, Lamy MT, Ferreira LC, Diniz MO. Bacterial spores as particulate carriers for gene gun delivery of plasmid DNA. J Biotechnol. 2016;**228**:58-66. doi:10.1016/j.jbiotec.2016.04.027

[94] Salas C, Genzer J, Lucia LA, Hubbe MA, Rojas OJ. Water-wettable polypropylene fibers by facile surface treatment based on soy proteins. ACS Appl Mater Interfaces. 2013;**5**(14):6541-6548. doi:10.1021/am401065t

[95] Chen X, Wang Y, Tong J, Xia S, Zhou Y, Wu K Electrochemical sensing platform for tetrabromobisphenol A at pM level based on the synergetic enhancement effects of graphene and dioctadecyldimethylammonium bromide. Anal Chim Acta. 2016;**935**:90-96. doi:10.1016/j.aca.2016.06.052

[96] de Brito AK, Nordi CS, Caseli L. Algal polysaccharides as matrices for the immobilization of urease in lipid ultrathin films studied with tensiometry and vibrational spectroscopy: physical-chemical properties and implications in the enzyme activity. Colloids Surf B Biointerfaces. 2015;**135**:639-645.

[97] Yonamine Y, Cervantes-Salguero K, Minami K, Kawamata I, Nakanishi W, Hill JP, Murata S, Ariga K. Supramolecular 1-D polymerization of DNA origami through a dynamic process at the 2-dimensionally confined air-water interface. Phys Chem Chem Phys. 2016;**18**(18):12576-81. doi:10.1039/c6cp01586g

[98] Swiatkowska A, Kosman J, Juskowiak B. FRET study of G-quadruplex forming fluorescent oligonucleotide probes at the lipid monolayer interface. Spectrochim Acta A Mol Biomol Spectrosc. 2016;**152**:614-21. doi:10.1016/j.saa.2015.01.102

[99] Jennings MC, Minbiole KPC, Wuest WM. Quaternary ammonium compounds: an antimicrobial mainstay and platform for innovation to address bacterial resistance. ACS Infect Dis. 2015:**1**:288−303. doi:10.1021/acsinfecdis.5b00047

[100] Carmona-Ribeiro AM. Chapter Thirteen—Nanomaterials based on lipids for vaccine development, In Micro and Nano Technologies, edited by Mariusz Skwarczynski and Istvan Toth, William Andrew Publishing, 2017, pp. 241-257, Micro and Nanotechnology in Vaccine Development, ISBN 9780323399814, doi:10.1016/B978-0-323-39981-4.00013-0. (http://www.sciencedirect.com/science/article/pii/B9780323399814000130)

[101] Holten-Andersen L, Doherty TM, Korsholm KS, Andersen P. Combination of the cationic surfactant dimethyl dioctadecyl ammonium bromide and synthetic mycobacterial cord factor as an efficient adjuvant for tuberculosis subunit vaccines. Infect Immun. 2004;**72**(3):1608-1617.

[102] Agger EM. Novel adjuvant formulations for delivery of anti-tuberculosis vaccine candidates. Adv Drug Deliv Rev. 2016;**102**:73-82. doi:10.1016/j.addr.2015.11.012

[103] Carmona-Ribeiro AM, Ortis F, Schumacher RI, Armelin MCS. Interactions between cationic vesicles and cultured mammalian cells. Langmuir. 1997;**13**:2215-2218. doi:10.1021/la960759h

[104] Vieira DB, Carmona-Ribeiro AM. Synthetic bilayer fragments for solubilization of amphotericin B. J Colloid Interface Sci. 2001;**244**:427-431. doi:10.1006/jcis.2001.7975

[105] Cocquyt J, Olsson U, Olofsson G, Van der Meeren P. Temperature quenched DODAB dispersions: fluid and solid state coexistence and complex formation with oppositely charged surfactante. Langmuir. 2004;**20**(10):3906-3912. doi:10.1021/la036080c

[106] Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology. 6th ed. Philadelphia: Saunders Elsevier; 2006. 566 p.

[107] Davidsen J, Rosenkrands I, Christensen D, Vangala A, Kirby D, Perrie Y, Agger EM, Andersen P. Characterization of cationic liposomes based on dimethyldioctadecylammonium and synthetic cord factor from M. tuberculosis (trehalose 6,6'-dibehenate)—a novel adjuvant inducing both strong CMI and antibody responses. Biochim Biophys Acta. 2005;**1718**(1-2):22-31. doi:10.1016/j.bbamem.2005.10.011

[108] Smith Korsholm K, Agger EM, Foged C, Christensen D, Dietrich J, Andersen C S, Geisler C, Andersen P. The adjuvant mechanism of cationic dimethyldioctadecylammonium liposomes. Immunology. 2007;**121**:216-226. doi:10.1111/j.1365-2567.2007.02560.x

[109] Fifis T, Gamvrellis A, Crimeen-Irwin B, Pietersz GA, Li J, Mottram PL, McKenzie IF, Plebanski M. Size-dependent immunogenicity: therapeutic and protective properties of nano-vaccines against tumors. J Immunol. 2004;**173**(5):3148-3154. doi:10.4049/jimmunol.173.5.3148

[110] Foged C, Brodin B, Frokjaer S, Sundblad A. Particle size and surface charge affect particle uptake by human dendritic cells in an in vitro model. Int J Pharm. 2005;**298**(2):315-322. doi:10.1016/j.ijpharm.2005.03.035

[111] Xiang SD, Scholzen A, Minigo G, David C, Apostolopoulos V, Mottram PL, Plebanski M. Pathogen recognition and development of particulate vaccines: does size matter? Methods. 2006;**40**(1):1-9. doi:10.1016/j.ymeth.2006.05.016

[112] Vollmer J, Krieg AM. Immunotherapeutic applications of CpG oligodeoxynucleotide TLR9 agonists. Adv Drug Deliv Rev. 2009;**61**:195-204.

[113] Manolova V, Flace A, Bauer M, Schwarz K, Saudan P, Bachmann MF. Nanoparticles target distinct dendritic cell populations according to their size. Eur J Immunol. 2008;38:1404-1413.

[114] Katz D, Lehrer S, Galan O, Lachmi B, Cohen S, Inbar I, Samina I, Peleg B, Heller D, Yadin H, Chai D, Freeman E, Schupper H, Fuchs P. Unique immunomodulating properties of dimethyl dioctadecyl ammonium bromide (DDA) in experimental viral vaccines. Adv Exp Med Biol. 1996;**397**:115-125.

[115] Perrie Y, Kastner E, Kaur R, Wilkinson A, Ingham AJ. A case-study investigating the physicochemical characteristics that dictate the function of a liposomal adjuvant. Hum Vaccin Immunother. 2013;**9**(6):1374-1381. doi:10.4161/hv.24694

[116] Carmona-Ribeiro AM (2014). Cationic nanostructures for vaccines. In Ht Duc (editor) Immune Response Activation, InTech, doi:10.5772/57543. Available from: http://www.intechopen.com/books/immune-response-activation/cationic-nanostructures-for-vaccines

[117] Hafner AM, Corthésy B, Merkle HP. Particulate formulations for the delivery of poly(I:C) as vaccine adjuvant. Adv Drug Deliv Rev. 2013;**65**(10):1386-1399. doi:10.1016/j.addr.2013.05.013

[118] Zhang Y, Wang Z, Gemeinhart RA. Progress in microRNA delivery. J Control Release. 2013;**172**(3):962-974. doi:10.1016/j.jconrel.2013.09.015

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