We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Colloid Stability Influences on the Biological Organization and Functions

Camillo La Mesa and Gianfranco Risuleo

Abstract

It is common to entities having sizes in the nano/micro-scale range be that, real or bio-intended systems, to undergo the action of many different forces, imparting them colloid stability. Ubiquitary electrostatic contributions, sometimes dominant, may overlap with steric stabilization ones; their combination effectively takes place in most cases. The two effects are jointly responsible, for instance, for the control of many phenomena such as: adhesion onto cells of alien agents, cellular separation during morpho-functional evolution, uptake of exogenous materials into cells and tissues. We evidence here, how the combination of these forces operates, and indicate the procedures leading to their effectiveness, when required for purposes inherent to biomimicry.

Keywords: biological systems, biomimetic systems, surface charge density, sterical stabilization, adhesion

1. Introduction

It took a long time before the characteristics peculiar to biological organisms, were considered on the more solid grounds dictated by Physics and Chemistry. It is enough to remind the violent criticism raised by many outstanding scientists against D'Arcy Thompson book "*On growth and form*", dating the second decade of the last century. The successful fate of the book urged biologists to account not only for the taxonomic rules inherent to biological organisms and to focus, much more reasonably, on the physical forces, geometrical, and morphological constraints responsible for biological organization, growth and functions. It is convincingly stated there that "In the growth of a shell, for instance, we can conceive no simpler law than this, that it shall widen and lengthen in the same unvarying proportions: and this simplest of laws is that which Nature tends to follow. The shell, like the creature within it, grows in size but does not change its shape; and the existence of this constant relativity of growth, or constant similarity of form, is of the essence, and may be made the basis of a definition, of the equiangular spiral." [1]. To the best of our knowledge this is the first effort to explain the growth of animal shapes in terms of geometry and topology, and to account for the modes in which organisms self-organize, following the rules dictated by their own genome. With respect to the gene/body shape one should consider the phenomenon of epistasis, i.e., the effect of one gene modulated by the genetic background [2]. Originally this definition meant that the phenotypic effect of one gene is affected by one or more different

genetic *loci*. Thus, epistatic mutations have different "combinatorial" rather than individual effects. This was originally a genetics concept but, nowadays, is of common use in biochemistry, computational biology and evolutionary biology. Epistasis stems from interactions between or, reciprocally, within genes and this leads to non-linear effects. Therefore, it has a dramatic influence on the shape of evolutionary landscapes, which leads to profound consequences for evolution and evolutionary potentials of the phenotypic traits [3].

The statements of D'Arcy Thompson's statement become convincing also if applied to the micro/nanoscale range. Here, the role played by physical forces at short distances comes in full evidence, that is: when the biological organization modes in their lower stage, as in dispersed cells, are considered (however, in a fully organized, functional organism, the role of physical forces may become extremely complex). The stability and, eventually, the organization of such objects is dictated by the overlapping, or dominance, of van der Waals, *vdW*, electrostatic, osmotic, elastic, steric and many other forces. Their combination with what is dictated by gene expression, leads to an optimal topology-ruled shape that a biological system, be it a cell or a tissue, not to speak of a whole organism, assumes. In what follows we put in evidence the role of major contributions when the stabilization in dispersed form is required, or naturally occurs.

To proceed along this line, we discuss separately the physical origins of both electrostatic and steric effects. Examples based on real biological systems, shall be given and the pathways inducing/reducing the onset, or disappearance, of these effects will be discussed. We must be aware that the action of many forces is required to attain a preferred organization mode in cells and tissues, where electrostatic interactions are prevalent, safe the enzyme/substrate where VdW forces are prevalent. We also know that the mentioned forces are dominant, in the terms dictated by energy costs, not considering their modulus (provided the sum of all ΔG terms is <0). This fact, combined with the genetically driven rules, fulfills requirements needed for optimal biological activity and functions. In what follows we describe, in sequence, the quintessential features of both electrostatic and steric forces, which are required to understand how important they are in living systems. In the final part of this chapter we mention some pertinent examples on the role that electrostatic and steric effects may jointly play.

2. Electrostatic aspects

Sufficiently high electrical potentials, ψ, are responsible for the kinetic stability of colloids. These facts avoid the onset of undesired effects, such as sedimentation [4], creaming [5], and/or clustering [6]. The foundations of colloid stability date to the 1940s of the last century and are formalized in DLVO theory, which applies to real dispersions. The original theory combines electrostatics and statistical thermodynamics, and is formalized in the well-known Poisson-Boltzmann, or P-B, equation. The effects considered in that theory are responsible for the stabilization of clays [7], inorganic colloids as $Al(OH)_3$ [8], latexes [9], cells, vesicles, viruses, etc. [10–13]. Note that counter-ion condensation onto DNA, and biopolymers in general, is expressed in terms of the same theory [14, 15].

The balance of attractive vs. repulsive electrostatic forces depends on the sign of their surface potentials, ψ's. On this line, Parsegian and Gingell showed that charged surfaces of the same sign, eventually differing in modulus, may never become attractive. Conversely, particles/surfaces bearing opposite sign attract or repel, depending on experimental conditions [16]. That statement was demonstrated for the surface charge densities of colloid A and B, termed σ_A and σ_B, (with $\sigma_A \neq \sigma_B$)

separated by a distance $0 < x < l$. If the upper and lower limits of the derivative, hereafter indicated as $[d(ze\psi/KT)/dx]_{|x=0}$ and $[d(ze\psi/KT)/dx]_{|x=1}$, respectively, differ in sign, the corresponding function must be null somewhere between the integration limits. As a consequence, like-charged surfaces always repel, whatever is x, σ or ψ. The question to be addressed is how to find rational procedures reducing that unescapable fate.

In what follows, we indicate how to face the problem and to control, or minimize, repulsions. Imagine having two large cells, each characterized by a given σ. We suppose that the respective surface charge densities are equal in modulus. The potential that one cell exerts on the other is ψ. In the P-B equation, the difference among the respective exponentials, that is, $\exp^{\pm(ze\psi/KT)}$, is considered. Proper transformation in hyperbolic form reduces the function to [17]

$$\exp^{+(ze\psi/KT)} - \exp^{-(ze\psi/KT)} = 2\sinh(ze\psi/KT) \tag{1}$$

which is easily linearized if $ze\psi << KT$. In words, the Euler-based approximation underlying Eq. (1) holds in linear perturbation regimes. The theory relies on the fact that the effect of ψ decreases with distance from the source (i.e., where charges are located), and fulfills the law stating

$$\psi = \psi^{o} exp^{-kx} \tag{2}$$

Implicit in the equation is the statement that, a distance x from a surface of well-defined potential, ψ decreases and its decay depends on the number of charges; more properly, on the concentration in excess of positive, or negative, ions. In words, it is as if we were in presence of a double layer, of length $1/k$, located a distance x apart from the source. To reduce the effect of ψ on its surroundings, thus,

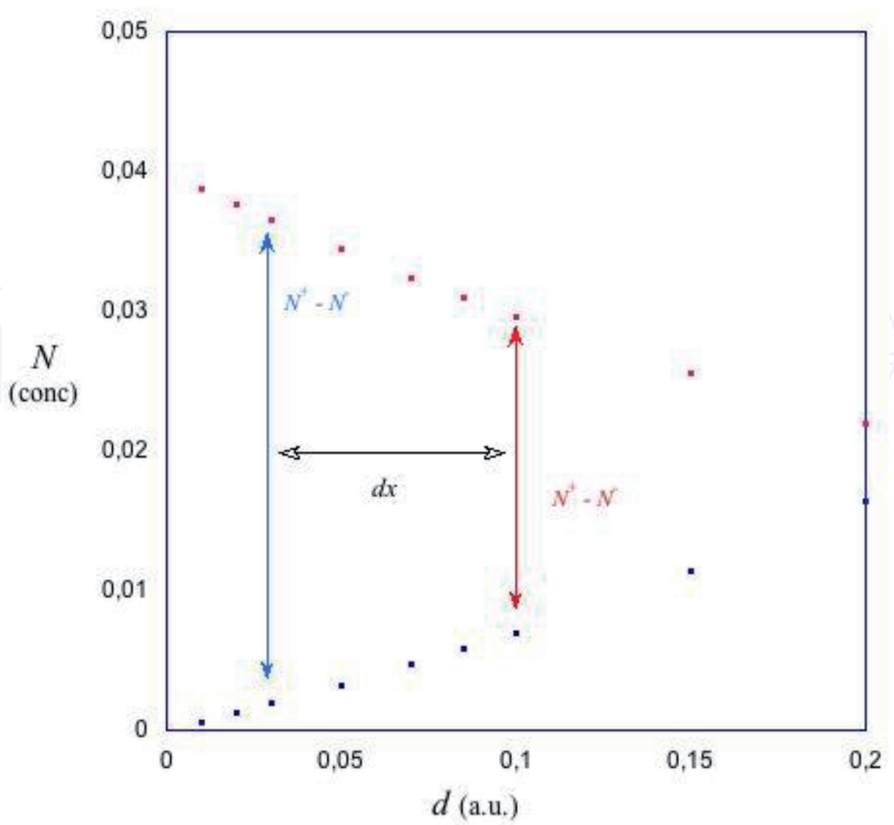

Figure 1.
Excess concentration of positive ions, (N+—N−), vs. distance, d. dx is the double layer thickness. The surfaces have different charge density. The difference among them, is dσ. The same holds in case of excess negative charges.

it is enough adding salt in excess. The potential is screened and repulsion between particles substantially minimized. In this way, colloids do not "feel" each other as it was before adding swamping electrolytes.

The problem can be properly addressed discussing the case of surface charge density, σ. As a consequence of Eq. (1), ψ decreases with distance. On moving from a reference point, $x°$, to x, σ decreases. The system behaves as a capacitor, and the local, punctual, concentration of ions, ρ, decreases with x. This is expressed by writing the integral of σ from $x°$ to the point of interest as

$$\sigma = -\int \rho dx \tag{3}$$

The meaning of Eqs. (2) and (3) is visualized in **Figure 1**. At long, on a molecular scale, distances the value of the integral in Eq. (3) approaches the equilibrium ionic concentration, and electro-neutrality is thus ensured. Therefore, (N^+—N^-) values in the above figure refer to the excess of counter-ions around a given charged body. Swamping electrolytes reduce the effect of potential at long distances and favor coalescence.

In many procedures intended for food chemistry, for instance, salting is by far the preferred route to reduce repulsive forces between entities in the given medium and to induce phase separation [18].

3. Sterical stabilization

The role that such effect plays in controlling colloid stability, with particular emphasis to biological systems is considered [19]. The concept was originally intended to latexes stabilization, and later extended to bio-systems. The term "*sterical stabilization*" indicates that macromolecules protect particles from flocculation, or coagulation. It applies to systems in which stabilizers are surface bound to the particles in question, which would flocculate if not protected. Sterical stabilization is intended to systems in which binding to a given surface is permanent. When binding is covalent, the drawbacks inherent to depletion [20, 21], which occurs when the stabilizer is weakly bound and may transfer toward the bulk, are missing. In that case, stabilizers partition between the particle surface and the bulk. This favors the onset of osmotic gradients, detaching stabilizers from the particle' surface with coagulation of no longer stabilized colloids. Predicting sterical stabilization is cumbersome if all these effects are not accounted for. As a matter of fact, polymer moieties protruding outward a given particle are solvated, sometimes charged; their state, conformation and degrees of freedom jointly depend on polymer-medium interactions. In other words, entropic contributions are not only due to changes in conformational degrees of freedom, as proposed by van der Waals's school [22, 23]. More precisely, the Gibbs energy, due to entropic and also enthalpy-based terms [24–26], is the result of different contributions.

We consider the following entropic terms

$$\Delta S_{tot} = \Delta S_{conform} + \Delta S_{solv} + other\ \Delta S\ terms \tag{4}$$

where the subscripts indicate conformational, solvation, and all other contributions, respectively. We do not consider, in a first stage, terms due to charging/discharging of polar moieties. Neither shall we consider osmotic repulsion, which is significant when the "coronas" surrounding the nanoparticles are compact. The requirements needed to have effective repulsion between polymer moieties, and in coronas stabilization too, crucially depend on the solvent. It is not casual, thus, that different stabilizers are needed to disperse colloids in polar, or non-polar, media.

The original hypothesis by van der Waals, which relies only on conformational entropy, is not convincing, since the contribution due to the solvent cannot be null. In fact, the solvent features are responsible for a number of formulation possibilities, including those intended to bio-systems.

To come in more details on the basics of steric stabilization, we report some details describing the first stages of surface anchoring, i.e., polymer wrapping. That process is responsible for macromolecule binding thereon, and not only. We describe below that effect, without entering in much details as to whether binding is covalent or not. Imagine a homo-polymer binding onto a solid surface; in consequence of that, units in the chain face outward, and may bind in a second place. The only physical restriction is that chains cannot enter the particle but may substantially adsorb thereon in many points. The process depends on polymer affinity toward the surface; in words, surface coverage is dictated by thermodynamics. We assume that:

a. polymers and colloidal nano-particles, *NP*s, are mono-disperse;

b. polymer size is << *NP*s radius;

c. wrapping units are much shorter than the fully extended polymer one;

d. different parts of the same polymer may wrap;

e. partition between bulk and surface-bound states may occur.

We impose X_f to be the overall mole fraction of polymer molecules in the medium; it is either free or interacting with particles. The latter is the sum of wrapping and protruding parts. Interacting polymers are divided in two classes, i.e., wrapped, α, and protruding, ε (=1 − α), states. The equilibrium between such states, $K_{\alpha,\varepsilon}$, is defined as

$$K_{\alpha,\varepsilon} = \alpha/\varepsilon = \alpha/(1 - \alpha) \tag{5}$$

As in most classical books on Colloid Chemistry ([27, 28], and references therein), Eq. (5), defines the ratio of wrapped to protruded states, and gives a binding probability, *P*, for adsorbed segments. For an *i*th state, it is defined as

$$P_i = k_i \exp^{-[(\pi+w)ali/KT]} \exp^{-[\eta/KT]} \tag{6}$$

where P_i refers to a state characterized by a length l_i, and width equal to a. We assume the latter to be the cross section of the main chain, **Figure 2**. Terms w and π are energies per unit volume. Their balance depends on the dominance of attractive/repulsive forces acting on the *NP* surface. η is a rotational energy, when the pre-exponential k_i is a proper weighing factor. P_i depends on the balance of all energy terms acting in the first layer around particles. The forces acting in the corona depend on the average local polymer concentration, calculated layer by layer. This statement is due to the fact that the content of polymer sub-units in a layer depends on surface curvature, and/or bulkiness, as well [29]. Expectedly, the solvation is not uniform along the protruding polymer chains. Similar cases occur when polymer coverage is close-grained and does not allow surface adsorption of other species. Thus, poly-oxy-ethylene glycols, PEO's, and structurally related polymers (mostly PEO-PPO-PEO block co-polymers) are widely used to avoid protein adsorption and find application if biomedical-intended NPs must not adsorb albumin or lysozyme, otherwise easily nucleating onto the mentioned NPs [30–32]. This common application is in use, others are mentioned below.

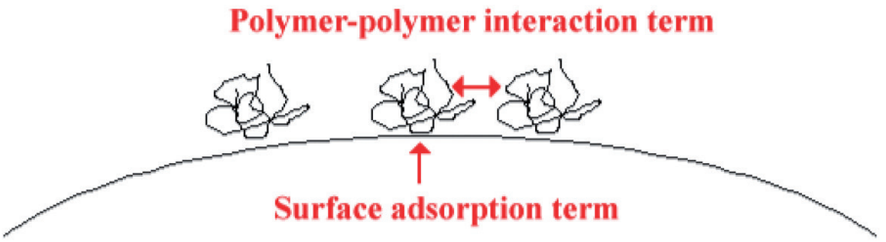

Figure 2.
Polymer adsorption for loops sizes << NP diameter. The surface adsorption term, has energy = w. Polymer-polymer interactions at the NP surface, π, are attractive or repulsive. At saturation wrapping has reached its maximum value. Above that threshold, w and π energies balance.

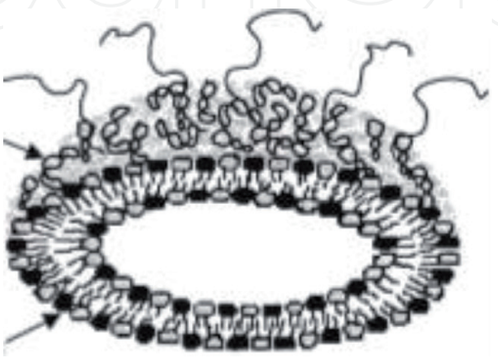

Figure 3.
Reconstructed liposomes with DNA compacted on the outer surface. The lower left arrow indicates lipid head groups; the upper one random coils represents compacted DNA. Protruding whips indicate PEO chains anchored to the bilayer. Such chains are hydrated, elastic, densely packed, and do not allow other species to come in contact with liposomes.

Figure 3 describes how to get the stabilization of synthetic liposomes against coalescence and is widely used in transfection technologies.

4. Biological implications

Physics and Chemistry allow to clarify some items of biology, despite substantial problems, due to a terrific increase in complexity inherent to living systems. As a matter of fact, these consist of many parts substantially differing from each other, in reciprocal relation. A first glance to a biological sample allows to observe some organization details. Understanding the hierarchy of active forces, packing modes, and processes taking place in bio-systems is discouraging, unless systematic efforts are stricken out. Therefore, most attempts to relate cell complexity to a number of forces acting therein are not trivial. Such attempts require going from simplicity to complexity, and to focus only on the essential modes of interaction. To focus only on the electrostatic and steric effects is a promising starting hypothesis. The first accounts for forces decaying with distance in a predictable way, irrespective of the medium [33]. The second conveys the impression that the geometry dictated by an arrangement of springs, or semi-rigid protrusions, facing outward the particle surface implies repulsion [34]. This fact is more convincing if we account for the additional role of osmotic forces [35], quite often associated to steric stabilization.

Cells always systematically trigger surface charges, acting as a physical barrier against the entry of exogenous material. Passive diffusion is not enough [36], unless it is substantially assisted by other transfer pathways. Imagine having the necessity to insert a nucleic acid in the cell. It is absolutely necessary to make use of chaperons, capable to overcome the protecting action of electric barriers [37]. This action relies on

transfection technologies capable to neutralize/invert the charge of hosts to be transferred through membranes, thus favoring uptake. For instance, DNA is neutralized, or adsorbed on oppositely charged vesicles, and transferred into the hosting cells [38–42]. Attraction between cells and transfectants is electrostatic in nature, and its action favors fusion with the cell membrane. It is a phenomenon comparable to the behavior met when viruses enter a cell [43–45]. This mimicry overlaps with other physical effects, due to membrane curvature elasticity [46, 47], its fluid state [48], and so forth [49].

It is known that steric stabilization has a large effect on macrophage uptake in vitro [50]. This effect is tuned by the fixed thickness of aqueous layers, at least in lipid-based chaperones. To ensure long-lasting circulation to immuno-liposomes (and avoid their biodegradation), combination of sterical stabilization with a superior targetability is attained by attaching monoclonal antibodies. These are formed directly on the distal ends of liposome-grafted PEO chains [51, 52]. Similar effects are attained when the more rigid poly-L-lysine is used as sterical stabilizer. In this case, electrostatic effects, due to pH-sensitive charging/discharging of the peptide moieties, are significant. The coupling of antibodies to membranes allow anchorage even in mild basic conditions without the need for antibody derivatization. On the same line, lipo-plexes sterically stabilized with PEO derivatives are used [53]. Relevant are some reports on doxorubicin-loaded liposomes, previously stabilized by adsorption of heparin; these systems show a marked antitumor activity. Heparin is also a coating material stabilizing and protecting liposomes against adverse immune reactions [54], or, in presence of adjuvants, induces drug accumulation at the tumor site [55]. The above list is far from being exhaustive and indicates that steric stabilization, in conjunction with other contributions, is responsible for advanced applications in most fields of bio-medicine and molecular medicine [56–60].

5. Final remarks

The "visions" of nature have been different throughout the development of natural sciences. Often physicists, chemists and biologists had a different, sometimes conflicting, way of looking at nature and investigating natural phenomena. One example for all: physical and chemical phenomena (reactions) were considered essentially irreversible in the "classical" history of natural sciences. On the contrary, biological phenomena were considered reversible, and fluidity, change of shape and behavior were dominant. It is not a coincidence that one of the masterpieces of theoretical biology written by J. Monod (1910–1976), one of the most authoritative biologists of last century is entitled "*Chance and necessity*" [61]. As the title suggests, chance seems to rule the biological phenomena: how life arose, the "plasticity" of many biological events. But, necessity is the driving force; a sort of constraint, impeding the same phenomena to escape the stringent laws permitting the completion of a fully functional living organism. Indeed, these strict laws are often represented by physico-chemical (essentially weak) interactions that give a determinant contribution to biological organization and functions. In other words, one could confidently conclude that Occam razor in its popular acceptation, once again, holds true: if there are competing ideas, the simplest one is possibly the correct one. This formulation is not actually the Occam's razor, but rather the law of maximum parsimony. Occam's razor states that in the case of competing hypotheses making the same predictions, the solution with the fewest assumptions is most likely to be true, provided that hypotheses predicting different solutions are not discarded by default. Ockham stated this principle in various ways, but the most popular version, "Entities are not to be multiplied without necessity" (originally "Non sunt multiplicanda entia sine necessitate.") was formulated by the Irish Franciscan philosopher John Punch.

Author details

Camillo La Mesa[1*] and Gianfranco Risuleo[2]

1 Department of Chemistry, Sapienza Università di Roma, Rome, Italy

2 Department of Biology and Biotechnologies, Sapienza Università di Roma, Rome, Italy

*Address all correspondence to: camillo.lamesa@uniroma1.it

IntechOpen

References

[1] Thompson D'AW. On Growth and Form. Cambridge University Press; 1917

[2] Gros PA, Le Nagard H, Tenaillon O. The evolution of epistasis and its links with genetic robustness, complexity and drift in a phenotypic model of adaptation. Genetics. 2009;**182**:277-293

[3] Rieger R, Michaelis A, Green MM. A Glossary of Genetics and Cytogenetics: Classical and Molecular. New York: Springer-Verlag; 1968. ISBN 9780387076683

[4] Karaiskakis G, Graff KA, Caldwell KD, Giddings C. Sedimentation field-flow fractionation of colloidal particles in river water. International Journal of Environmental Analytical Chemistry. 1982;**12**:1-15

[5] Robins MM. Emulsions—Creaming phenomena. Current Opinion in Colloid & Interface Science. 2000;**5**:265-272

[6] Yasrebi M, Shih WY, Aksay IA. Clustering of binary colloidal suspensions: Experiment. Journal of Colloid and Interface Science. 1991;**142**:357-368

[7] Bell FG. Lime stabilization of clay minerals and soil. Engineering Geology. 1996;**42**:223-237

[8] Luo Q, Campbell DR, Babu SV. Stabilization of alumina slurry for chemical-mechanical polishing of copper. Langmuir. 1996;**12**:3563-3566

[9] Thompson KL, Armes SP, York DW, Burdis JA. Synthesis of sterically-stabilized latexes using well-defined poly(glycerol monomethacrylate) macromonomers. Macromolecules. 2010;**43**:2169-2177

[10] Schill RO, Mali B, Dandekar T, Schnölzer M, Reuter D, Frohme M. Molecular mechanisms of tolerance in tardigrades: New perspectives for preservation and stabilization of biological material. Biotechnology Advances. 2009;**27**:348-352

[11] Crowe JH, Crowe LM, Carpenter JF, Wistrom CA. Stabilization of dry phospholipid bilayers and proteins by sugars. The Biochemical Journal. 1987;**242**:1-10

[12] Kostarelos K, Luckham PF, Tadros TF. Steric stabilization of phospholipid vesicles by block copolymers. Vesicle flocculation and osmotic swelling caused by monovalent and divalent cations. Journal of the Chemical Society, Faraday Transactions. 1998;**94**:2159-2168

[13] Jung M, Hubert DHW, Bomans PHH, Frederik PM, Meuldijk J, van Herk AM, et al. New vesicle-polymer hybrids: The parachute architecture. Langmuir. 1997;**13**:6877-6880

[14] Bloomfield VA. DNA condensation by multivalent cations. Biopolymers. 1997;**44**:269-282

[15] Bloomfield VA. DNA condensation. Current Opinion in Structural Biology. 1996;**6**:334-341

[16] Parsegian VA, Gingell D. On the electrostatic interaction across a salt solution between two bodies bearing unequal charges. Biophysical Journal. 1972;**12**:1192-1204

[17] Fogolari F, Brigo A, Molinari HJ. The Poisson-Boltzmann equation for biomolecular electrostatics: A tool for structural biology. Journal of Molecular Recognition. 2002;**15**:377-392

[18] Vauthier C, Bouchemal K. Methods for the preparation and manufacture of polymeric nanoparticles.

Pharmaceutical Research. 2009;**26**: 1025-1058

[19] Allen TM, Cullis PR. Liposomal drug delivery systems: From concept to clinical applications. Advanced Drug Delivery Reviews. 2013;**65**:36-48

[20] Tardani F, La Mesa C. Attempts to control depletion in the surfactant-assisted stabilization of single-walled carbon nanotubes. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2014;**443**:123-128

[21] De Gennes PG. Polymer solutions near an interface. Adsorption and depletion layers. Macromolecules. 1981;**14**:1637-1643

[22] Mackor EL. A theoretical approach of the colloid-chemical stability of dispersions in hydrocarbons. Journal of Colloid Science. 1951;**6**:492-495

[23] Mackor EL, van der Waals JH. The statistics of the adsorption of rod-shaped molecules in connection with the stability of certain colloidal dispersions. Journal of Colloid Science. 1952;**7**:535-550

[24] Napper DH. Flocculation studies of sterically stabilized dispersions. Journal of Colloid and Interface Science. 1970;**32**:106-114

[25] Napper DH, Netsehey A. Studies of the steric stabilization of colloidal particles. Journal of Colloid and Interface Science. 1971;**37**:528-535

[26] Evans R, Napper DH. Steric stabilization. Comparison of theories with experiment. Kolloid-Zeitschrift und Zeitschrift für Polymere. 1973;**251**:409-414

[27] Vold RD, Vold MJ. Colloid and Interface Chemistry. Reading, Mass: Addison-Wesley; 1983. pp. 134-148, and references therein

[28] Adamson AW. Physical Chemistry of Surfaces. Vth ed. New York: Wiley; 1990. pp. 421-449, and references therein

[29] Chithrani BD, Ghazani AA, Chan WCW. Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. Nano Letters. 2006;**6**:4662-4668

[30] Gref R, Luck M, Quellec P, Marchand M, Dellacherie E, Harnish S, et al. Stealth corona-core nanoparticles surface modified by polyethylene glycol (PEG): Influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. Colloids and Surfaces B: Biointerfaces. 2000;**18**:301-313

[31] Malmsten M, Linse P, Cosgrove T. Adsorption of PEO-PPO-PEO block copolymers at silica. Macromolecules. 1992;**25**:2474-2481

[32] Docter D, Westmeier D, Markiewicz M, Stolte S, Knauer SK, Stauber RH. The nanoparticle biomolecule corona: Lessons learned—Challenge accepted? Chemical Society Reviews. 2015;**44**:6094-6121

[33] More correctly, the whole process is controlled by the medium permittivity, ε

[34] Liufu S, Xiao H, Li Y. Adsorption of poly(acrylic acid) onto the surface of titanium dioxide and the colloidal stability of aqueous suspensions. Journal of Colloid and Interface Science. 2005;**281**:155-163

[35] Kim S-H, Park J-G, Choi TM, Manoharan VN, Weitz DA. Osmotic-pressure-controlled concentration of colloidal particles in thin-shelled capsules. Nature Communications. 2014;**5**:3068

[36] Di L, PerArtursson P, Avdeef A, Ecker GF, Faller B, Fischer H, et al.

Evidence-based approach to assess passive diffusion and carrier-mediated drug transport. Drug Discovery Today. 2012;**17**:905-912

[37] Valenzano KJ, Benjamin ER, René P, Bouvier M. Pharmacological chaperones: Potential for the treatment of hereditary diseases caused by mutations in G protein-coupled receptors. In: Gilchrist A, editor. CPCR Molecular Pharmacology and Drug Targeting: Shifting Paradigms and New Directions. Wiley; 2010. pp. 460-500

[38] Barbetta A, La Mesa C, Muzi L, Pucci C, Risuleo G, Tardani F. Chapt. VII: Cat-anionic vesicle-based systems as potential carriers in nanotechnologies. In: Ahmed W, Phoenix DA, editors. Nanobiotechnology. UK: One Central Press; 2014. pp. 152-179

[39] Rai R, Alwani S, Badea I. Polymeric nanoparticles in gene therapy: New avenues of design and optimization for delivery applications. Polymers. 2019;**11**:745-780

[40] Shende P, Ture N, Gaud RS, Trotta F. Lipid- and polymer-based plexes as therapeutic carriers for bioactive molecules. International Journal of Pharmaceutics. 2019;**558**:250-260

[41] Bonincontro A, La Mesa C, Proietti C, Risuleo G. A biophysical investigation on the binding and controlled DNA release in a cetyltrimethylammonium bromide-sodium octyl sulfate cat-anionic vesicle system. Biomacromolecules. 2007;**8**:1824-1829

[42] Bonincontro A, Falivene M, La Mesa C, Risuleo G, Ruiz Pena M. Dynamics of DNA adsorption on and release from SDS-DDAB cat-anionic vesicles: A multitechnique study. Langmuir. 2008;**24**:1973-1978

[43] Du X, Wang J, Zhou Q, Zhang L, Wang S, Zhang Z, et al. Advanced physical techniques for gene delivery based on membrane perforation. Drug Delivery. 2018;**25**:1516-1525

[44] Garcia-Guerra A, Dunwell TL, Trigueros S. Nano-scale gene delivery systems. Current technology, obstacles, and future directions. Current Medicinal Chemistry. 2018;**25**:2448-2464

[45] van der Schaar HH, Rust MJ, Chen Chen C, van der Ende-Metselaar H, Wilschut J, Xiaowei ZX, et al. Dissecting the cell entry pathway of dengue virus by single-particle tracking in living cells. PLoS Pathogens. 2008;**4**:e1000244

[46] Temin HM. On the origin of RNA tumor viruses. Annual Review of Genetics. 1974;**8**:155-177

[47] Deuling HJ, Helfrich W. The curvature elasticity of fluid membranes: A catalogue of vesicle shapes. Journal de Physique. 1976;**37**:1335-1345

[48] Winterhalter M, Helfrich W. Effect of surface charge on the curvature elasticity of membranes. The Journal of Physical Chemistry. 1988;**92**:6865-6867

[49] Losa DA, Murata N. Membrane fluidity and its roles in the perception of environmental signals. Biochimica et Biophysica Acta—Biomembranes. 2004;**1666**:142-157

[50] Barbetta A, Pucci C, Tardani F, Andreozzi P, La Mesa C. Size and charge modulation of surfactant-based vesicles. The Journal of Physical Chemistry. B. 2011;**115**:12751-12758

[51] Zeisig R, Shimada K, Hirota S, Arndt D. Effect of sterical stabilization on macrophage uptake in vitro and on thickness of the fixed aqueous layer of liposomes made from alkylphosphocholines. Biochimica et Biophysica Acta. 1996;**1285**:237-245

[52] Bendas G, Krause A, Bakowsky U, Vogel J, Rothe U. Targetability of novel immunoliposomes prepared by a new antibody conjugation technique. International Journal of Pharmaceutics. 1999;**181**:79-93

[53] Sanders NN, De Smedt SC, Cheng SH, Demeester J. Pegylated GL67 lipoplexes retain their gene transfection activity after exposure to components of CF mucus. Gene Therapy. 2002;**9**:363-371

[54] Duehrkop C, Leneweit G, Heyder C, Fromell K, Edwards K, Ekdahl KN, et al. Development and characterization of an innovative heparin coating to stabilize and protect liposomes against adverse immune reactions. Colloids and Surfaces B: Biointerfaces. 2016;**141**:576-583

[55] Park J, Hwang SR, Choi JU, Alam F, Byun Y. Self-assembled nanocomplex of PEGylated protamine and heparin-suramin conjugate for accumulation at the tumor site. International Journal of Pharmaceutics. 2018;**535**:38-46

[56] Merino M, Zalba S, Garrido MJ. Immunoliposomes in clinical oncology: State of the art and future perspectives. Journal of Controlled Release. 2018;**275**:162-176

[57] Rasenack N, Müller BW. Micron-size drug particles: Common and novel micronization techniques. Pharmaceutical Development and Technology. 2004;**9**:1-13

[58] Salem HF, Eid K, Sharaf MA. Formulation and evaluation of silver nanoparticles as antibacterial and antifungal agents with a minimal cytotoxic effect. International Journal of Drug Delivery. 2011;**3**:293-304

[59] Bakowsky H, Richter T, Kneuer C, Hoekstra D, Rothe U, Bendase G, et al. Adhesion characteristics and stability assessment of lectin-modified liposomes for site-specific drug delivery. Biochimica et Biophysica Acta. 2008;**1778**:242-249

[60] Xu X, Ho W, Zhang X, Bertrand N, Farokhzad O. Cancer nanomedicine: From targeted delivery to combination therapy. Trends in Molecular Medicine. 2015;**21**:223-232

[61] Monod J. Chance and Necessity: An Essay on the Natural Philosophy of Modern Biology. New York: Alfred A. Knopf; 1971. ISBN: 0-394-46615-2