Association morphologies of amphiphilic polyelectrolyte diblock copolymers

Korobko, Alexander Viktorovitch

CHAPTER 1

Introduction

1.1. General Introduction

The purpose of this thesis is to investigate self-assembled structures of polyelectrolyte diblock copolymers. We will mainly focus on micelles and vesicles, which are ordered at a "meso" length scale: larger than the sizes of the individual constituents, but still microscopic in the nano- to micrometer range. The hierarchical structure from the single-molecule to the mesoscale is controlled by the chemical properties of the copolymer, the possible presence of salt, the solvent, and the preparation procedure [1]. Micelles and vesicles are made of the same building blocks, but they differ in molecular organization. Copolymer micelles and vesicles have many physical, chemical, biomedical, and biotechnological applications. Their structural, dimensional, and multi-functional features provide an opportunity for e.g., transport of medically active substances, mimic biological membrane processes, and control of gelation, lubrication, and flow behavior of complex fluids [2–7]. Nowadays, they also play a pivotal role in the markets for detergencies, catalysis, oil recovery, and separation (chromatography and electrophoresis) technology to name a few. They also play a major role in the rapid growth of nanotechnology. The development of nanotechnological applications is impossible however without fundamental knowledge of the interactions between the copolymer blocks and their effect on the (non)equilibrium properties of the self-assembled micellar and vesicular structures.

With respect to the considerable amount of previous work on polyelectrolyte copolymer micelles, here we will mainly focus on concentrated, crowded systems.

The micelles are surrounded by a coronal brush made of the polyelectrolyte attachments. One of the pressing questions is whether these coronal brushes shrink or interpenetrate when the micelles are accommodated in an increasingly crowded volume. This question becomes even more challenging if one considers the osmotic effects of the small counterions both trapped in the coronal layer and freely dispersed in the surrounding medium. A complete description of inter- and intramicellar structure on a variety of length scales is clearly needed in order to understand the functionality of this class of nano-structured materials. Polyelectrolyte diblock copolymers can also be used to produce vesicles. These copolymer vesicles are characterized by much higher mechanical and chemical stabilities compared to the conventional vesicles made of lipids. In order to optimize the vesicles for specific applications, the properties of the membrane can be delicately tuned by the way the vesicles are prepared and by the choice of material. In the second part of this thesis we will explore a new method to encapsulate DNA within these copolymer vesicles and we will show that this new class of carrier system can be used for reverse gene delivery.

1.2. Polyelectrolytes

1.2.1. Amphiphilic Diblock Copolymers and DNA

Amphiphilic diblock copolymers with a polyelectrolyte block comprise two linearly attached moieties: a polyelectrolyte and a hydrophobic chain part. The hydrophilic polyelectrolyte block bears acid or a base group which may dissociate in a polar solvent, ionize and release a counterion. In addition to the conventional factors, such as the presence of salt, the quality of solvent, and the chemical composition and symmetry of the respective blocks, the amphiphylic behavior has a profound effect on the complexity of the meso-scale structure.

The DNA molecule is a natural polyelectrolyte due to the negative charge of the phosphate groups making up the backbone. According to Watson and Crick [8], the double helical form of DNA is made of two anti-parallel polynucleotide chains, which are kept together by base pairing. The stability of the double helix is controlled by small ion screening of the electrostatic repulsive interaction between the negatively charged backbones [9]. Under biological conditions, plasmid DNA exists in a closed circular, supercoiled state in which the DNA duplex is wound around another part of the same molecule to form a higher order helix. When one strand of the duplex is broken (nicked), the superhelix unwinds and the DNA molecule takes the form of

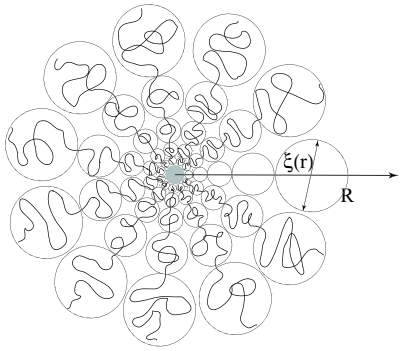

Figure 1.1: Neutral micelle in a good solvent.

an open circle (ocDNA). A break in both strands opens the circle and the molecule becomes linear.

1.3. Curved Polyelectrolyte Brushes

1.3.1. Theoretical Investigations

Neutral Brushes The structure of spherical polymer brushes has been a subject of intense theoretical study, mostly based on scaling concepts initially advanced by de Gennes [10]. It is convenient to begin the theoretical description of spherical micelles formed by self-assembly of copolymers without considering the effects of charge, Figure 1.1. The equilibrium size of such a micelle is driven by conformational free energy and the free energy of short-range interaction between monomers and scales as

$$R(f) \simeq \begin{cases} N^{1/2} f^{1/4} a, & \theta \text{ conditions}, \\ N^{3/5} \upsilon^{1/5} f^{1/5} a, & \text{good conditions}. \end{cases} \tag{1.1}$$

Here, f denotes the number of chains per micelle, each containing N monomer units of length a, and R is the end-to-end distance. The interaction between monomer units is described by the second virial coefficient υ, which reflects pair contacts between monomers and depends on temperature, $\upsilon = (T - \theta)/T$.

In order to estimate the radial monomer density profile, the polymer brush is

represented as a system of concentric spherical shells of blobs of size $\xi(r)$ [11]. Within such a blob, the chain is subjected to either unperturbed Gaussian or excluded volume statistics, while on larger length scales the brush forms a radial array of blobs. The correlation length, $\xi(r)$, is determined by the local concentration of monomers, and scales as $(ca^3)^{-1}a$ for θ solvent and $(ca^3)^{-3/4}\upsilon^{-1/4}a$ in the case of good solvent. Close packing of the blobs in the shell of radius r and thickness $\xi(r)$ implies the radial dependency of the blob size, $\xi(r) \cong rf^{-1/2}$ and monomer density profile $c(r) \sim f^{2/3}r^{-4/3}$ [11].

For micelle concentrations above the overlap concentration, the solution can be viewed as a dispersion of micelles immersed in a matrix of overlapping chains ends (sea of blobs). Within the domain of a micelle, the chain statistics is the same as for individual, diluted micelles. In the sea of blobs, however, the chain statistics is thought to be same as in a concentrated polymer solution.

Charged Brushes Polyelectrolyte brushes carry electrolyte groups which may dissociate and release counterions. Such systems have potentially much richer behavior then their neutral counterparts, because of the Coulomb interaction between charges, screening, and osmotic forces caused by ions confined in the interfacial layer. Two different classes exist. When the fraction of ionized groups is very small, the electrostatic screening length is much larger than the micelle size, and hence, inside the corona there is no screening of Coulomb interaction. With increasing charge fraction the majority of the counterions are trapped within the corona, and now, the concomitant osmotic pressure gives the main contribution to the corona stretching force. In the present thesis, all micelles are in this so-called osmotic regime and we merely summarize the theoretical results pertaining to the latter class of spherical brushes.

Quenched brush Strong polyelectrolytes with a fixed degree of dissociation and fixed distribution of charges along the chain constitute a quenched brush. The balance of the osmotic pressure of the retained counterions and configurational elasticity of the chains determines the size of the micelles

$$R(f) \simeq Na\alpha^{-1/2}, \tag{1.2}$$

which does not depend on the grafting density. For the osmotic quenched brush, the fraction of trapped counterions does not vary along the radius, chains are uniformly stretched, and the monomer density profile decays as r^{-2} [12], see Figure 1.2a. When salt is added, it penetrates into the polyelectrolyte brush and at the periphery of the micelle the screening is dominated by the salt ions. The local balance between the

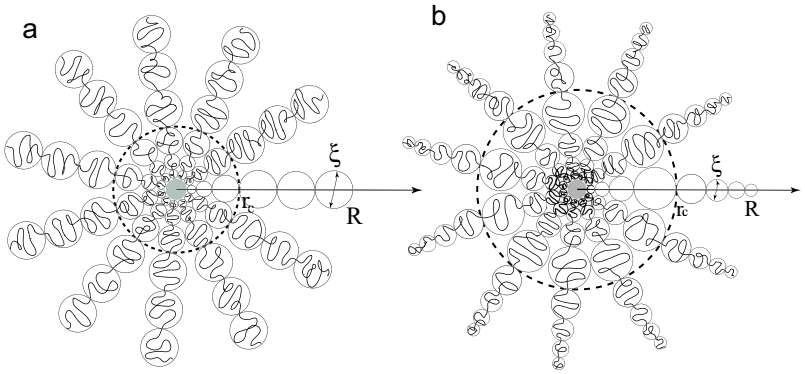

Figure 1.2: Polyelectrolyte micelle with quenched (a) and annealed (b) outer region, $r \geq r_c$. In the inner region, $r \leq r_c$, the statistics corresponds to the neutral curved brush in θ-solvent.

differential osmotic force and the local tension in the chain than results in a radial scaling similar to the neutral spherical brush $c(r) \cong r^{-4/3}$.

Annealed brush Weak polyelectrolytes with a small fraction of dissociating monomers, $\alpha < 0.1$, show the local dissociation-recombination balance determined by the mass action law, $\alpha(r) \simeq K/c_{H^+}(r)$. Here K is the ionization constant, and H^+ is the concentration of ions (in the case of polyacid). The phase diagram of the annealed brush is richer than the one for the quenched brush. Because of the dissociation and recombination balance, the charge fraction is now no longer constant and increases with increasing r. A remarkable result of this charge annealing effect is that the blob size decreases with increasing distance away from the core, Figure 1.2b, and the density scales as $c(r) \sim r^{-8/3}$ or $c(r) \sim r^{-5/3}$ without or with volume interactions, respectively [12].

Simulations The scaling results were intensively scrutinized by Self Consistent Field (SCF) [13, 14], Molecular Dynamics (MD) [15–17] and Monte Carlo (MC) [18, 19] computer simulations. In general, the studies confirm the scaling results in the limits of small and large number of chains and small/large concentrations of salt. However, the computer simulations often show a non-uniform distribution of the counterions and chain fluctuations, effects which are not captured by the scaling approaches.

Some of the computational results are particularly of importance in the context of the experiments reported in this thesis. The SCF calculations [13] shows contraction

of the polyelectrolyte star even before the overlap concentration $c^* = Nf/R^3$), in accordance with our observations. The MD simulations of polyelectrolyte stars [17] confirm that the solution structure is liquid-like. However, with increasing concentration above c^*, the interpenetration of the stars qualitatively modifies the structure factor: the height of the main peak decreases and that of the second peak increases with increasing density. This behavior was explained by the existence of two relevant length scales of the system: one is related to the inter-star distance, whereas the other corresponds with the size of the star itself [20].

1.3.2. Experimental Results

Polyelectrolyte diblock copolymer solutions exhibit structures at a variety of length scales: from the nano-sized structures in the core and corona of the micelles to micro-sized clusters of micelles. Small Angle Neutron and X-Ray Scattering (SANS and SAXS, respectively) have been used in a complementary way to probe these structures [21–29]. The size of the micelles and their polydispersity can also be estimated by Light Scattering (LS) [30–33], whereas the mechanical and the flow behavior at different time scales have been tested by rheometry [33–38].

Subjected to a beam of X-rays or neutrons, the scattered intensity profile $I(q)$ carries information about the structure of the micelles and inter-micelle organization. The momentum transfer, $q = 4\pi/\lambda \sin(\theta/2)$, is defined by the wave length λ of the radiation and scattering angle θ between the incident and scattered beams. For spherical micelles, this intensity can be factorized: $I(q) \sim P(q)S(q)$, where the form factor $P(q)$ describes the structure of micelles and $S(q)$ reflects the structural correlation among micelles.

If the structural arrangement of the micelles is liquid-like, the Percus-Yevick approximation for hard spheres can be employed to calculate the structure factor. However, the soft nature of the micelles calls for a long-range, Yukawa-like repulsive potential [20, 39]. In practice, the structure factor is evaluated with an effective hard sphere model, possibly supplemented with a short range attraction (Baxter sticky hard sphere model [40, 41]). The value of the derived effective diameter is than compared with the structural value and the difference is taken as an indication of the softness of the micelle.

For polyelectrolyte diblock copolymer micelles with relatively large aggregation number (~ 100), high charge and minimal screening conditions, SANS experiments with contrast variation [25, 26, 42] have shown that the coronal layers are fully stretched. The counterions are confined in the corona and their radial distribution

is very close to the one pertaining to the corona forming segments. With increasing concentration, inter micelle correlations become more pronounced and the structure factor now exhibits a strong correlation peak [29, 43–45]. The position of this peak scales with concentration as $c^{1/3}$. Besides the regular diffraction peak caused by inter-micelle interference, another diffuse scattering peak appears at higher angles. The latter correlation peak scales with concentration as $c^{1/2}$ [29, 46, 47] and has been associated with the correlation of monomers pertaining to chains of the same ($c < c^*$) and different micelles ($c > c^*$).

The addition of salt screens the electrostatic interactions, causes contraction of the brushes, and suppresses inter-micelle interference [25, 32, 48–50]. The density profile, as has been reported for osmotic micelles in the salt dominated regime [25], now exhibits two regions as predicted by scaling theory in Ref. [12]. In the inner region (close to the core) the brush is not affected by the salt and the density profile obeys r^{-2} scaling. In the outer region, the screened electrostatic excluded volume interaction rescales the density profile to $r^{-4/3}$. The overall radius scales with the salt concentration as $c_s^{-1/5}$ [48], if the concentration of salt exceeds the ionic strength of the counterions coming from dissociation of the polyelectrolyte. Remarkably, annealed brushes at low salt concentrations swell upon an increase of the salt concentration [32] due to the additional ionization of the chains [12].

1.4. Polyelectrolyte Vesicles

With the Human Genome Project [51] and the genetic basis of many diseases [52], a considerable amount of work has been devoted to the design and characterization of gene delivery systems. These systems are able to protect and transfer genes through cell membranes and have the potential to cure disease in situ at the genomic level. Phospholipid vesicles (liposomes) are made of lipid amphiphiles and can be considered as the predecessor of the polymer based formulations [53–56]. Liposomes are closed spherical membranes with a typical thickness in the range 3 to 5 nm and are capable to fuse with the cell membrane [53]. Although liposomes can easily be formed, they are rather unstable due to the small membrane thickness and large membrane fluctuations. Their limited stability and poor membrane permeability for polar molecules have stimulated research to more stable and advanced carrier systems based on polymers [5, 57–59].

The flexibility of polymer chemistry meets the needs for the design of an efficient and safe non-viral gene delivery system. The obvious requirements are protection

of the gene transfer vector against a hostile environment, controlled administration, stability, non-toxicity, and bio-compatibility [60, 61]. Polymeric vesicles and polymer/DNA complexes (polyplexes) have the additional advantage that they provide protection against nuclease degradation and controlled release [62–70]. Furthermore, the carrier is often coupled to a ligand which can bind to a specific receptor on the targeted cell. A general characteristic of these carrier systems is the large extent to which the DNA is compacted by polycations [71, 72], proteins [73], colloidal particles [74–77], or dendrimers [78, 79].

1.4.1. Polymersomes

Like phospholipids, amphiphilic block copolymers self assemble into vesicles by different pathways: hydration [80]; electro formation [81]; solvent evaporation [5]. Compared to liposomes, polymer vesicles (polymersomes) are different in respect of the considerably higher molecular weight of the building blocks [3, 82–84] (Mw $\gg$ 1 kD compared to $<$ 1 kD for lipids). The thick polymer bilayer results in a decreased fluidity and increased stability of the membrane. Polymersomes are easily formed if their bilayer bending elasticity is low and the surface tension is high. The formation of both phospholipids and polymersomes is a two-step process: the amphiphile forms a bilayer; this bilayer may close into a vesicle [85, 86]. The high bending modulus of a polymeric membrane indicates a higher energy required to form the vesicles, $E \sim k_c$, and leads to larger vesicle sizes [87]. This bending energy can be estimated from the surface tension and the size of a vesicle according to $E \simeq R\gamma$ [85].

Mechanical properties for liposomes [88] and polymersomes [83, 89, 90] have been studied with the micropipette aspiration technique [91]. The dilation (relative excess area) of the vesicle is measured in relation to the membrane tension and elastic deformation. Both liposomes and polymersomes are characterized by almost the same bending elastic modulus, k_c, of the order of 10 k_BT. The stretching of polymersomes is more pronounced at high tension, with the stretching modulus in the range 150 - 450 dyn/cm [83, 89, 90]. These values are on the same order or slightly higher than the values reported for liposomes (230 dyn/cm) [88]. Not surprisingly, polymersomes are more robust under the applied forces and the critical tension where they become unstable occurs at about a 20% dilation factor. For reference, liposomes rupture at 5% of the relative excess area [83].

Polymersomes are attractive for encapsulation and controlled release of drugs because of their increased stability and tunable properties. Encapsulation can be achieved during the vesicle preparation (electro formation, film rehydration) or by

control of the permeability of the membrane. Release can be achieved by hydrolysis-driven membrane degradation (for PEG-based polymers [70]), or adhesion of the polymersome to the cell and further phagocytic uptake (endocytosis, [92]).

1.4.2. Multilayer Capsules

Recently, polyelectrolyte multilayer microcapsules have attracted much attention. These systems have been designed for their enhanced stability and encapsulation capabilities. The principle is based on the layer-by-layer adsorption of oppositely charged polyelectrolytes [93] onto a template colloidal particle. The template can subsequently removed, and the compartment can be used to encapsulate a drug or substance [94, 95]. The number of bilayers in the shell can be varied up to ten, and the total thickness of the shell could be up to 20 nm. Encapsulation by the layer-by-layer thin film technology has been applied for uncharged small molecules [96], enzymes [97, 98], proteins [99], polyelectrolytes [100], DNA [101], polysaccharides [102], surfactants, phospholipids, nanoparticles [103], crystals [104], dyes [105, 106], and even single cells [107, 108].

1.5. Thesis Outline

The aim of the thesis is twofold. First, polyelectrolyte copolymer micelles will be studied to elucidate the role of the micelle concentration on micelle structure and inter micelle organization. Secondly, we will study giant vesicles as an example of a superstructure self-assembled by DNA and oppositely charged polyelectrolyte copolymer.

The structure of the thesis is as follows.

In Chapter 2 the structure of spherical micelles of the diblock copolymer poly (styrene-*block*-acrylic acid) [PS-*b*-PA] in water was investigated with small angle neutron scattering (SANS). The intermicelle correlation and the extension of the polyelectrolyte chains in the coronal layer have been investigated through the overlap concentration. With increasing packing fraction the corona shrinks and/or interpenetrate in order to accommodate the micelles in the increasingly crowded volume. At high charge and minimal screening conditions, the corona layers interpenetrate once the volume fraction exceeds the critical value 0.53.

In Chapter 3 a more detailed account is given of the experiments reported in Chapter 2. Furthermore, the counter-ion distribution, the structure of the micellar

solution and their effect on the flow properties and the visco-elastic behavior are discussed. The counterion structure factor was obtained with small angle X-ray scattering (SAXS). It is shown that interpenetration of the polyelectrolyte brushes controls the fluid rheology: the viscosity increases dramatically and the parallel frequency scaling behavior of the dynamic moduli shows the formation of a physical gel.

Chapter 4 describes the preparation and analysis of cationic diblock copolymer poly(butadiene-*b*-N-methyl 4-vinyl pyridinium) [PBd-*b*-P4VPQ] vesicles loaded with dsDNA fragments (contour length 54 nm). Encapsulation is achieved with a single emulsion technique. The PBd block forms an interfacial brush, whereas the cationic P4VPQ block complexes with DNA and enhances the stability of capsules. Under a change of the quality of the solvent, the PBd brush collapses and a capsule is formed. This process has been studied with phase contrast, polarized light, and fluorescence microscopy as well as scanning electron microscopy. The compaction of DNA is shown by the appearance of liquid crystalline textures under crossed polarizers and the increase in fluorescence intensity of labeled DNA. To form vesicles, the capsules are dispersed in aqueous medium supported by an osmotic agent. The universality of the method will be demonstrated by the encapsulation of pUC18 plasmid (further detailed in chapter 5) and the "charge inverse" system: cationic poly(ethylene imine) encapsulated by the anionic diblock poly(styrene-b-acrylic acid).

In Chapter 5 we further discuss the preparation and characterization of similar cationic vesicles, but loaded with cloning vector DNA (pUC18 or pEGFP-N1). The integrity of the DNA after encapsulation and subsequent release was confirmed by gel electrophoresis. We demonstrate "reverse" transfection of in vitro cultured HeLa cancer cells growing on plasmid-copolymer vesicles deposited on a glass substrate by the fluorescence of the expressed green fluorescent protein in cultured cells.

Bibliography

[1] Cameron, N.S., Corbierre, M.K., Eisenberg, A., Can. J. Chem. **77**, 1311, 1999.

[2] Savic, R., Luo, L., Eisenberg, A., Maysinger, D., Science, **300**, 615, 2003.

[3] Discher, D.E., Eisenberg, A., Science, **297**, 967, 2002.

[4] Langer, R., Science, **393**, 58, 2001.

[5] Zhang, L., Yu, K., Eisenberg, A., Science, **272**, 1777, 1996.

[6] De Smedt, S.C., Demeester, J., Hennink, W.E., Pharmaceutical Research, **17**, No 2, 113, 2000.

[7] Francis, M.F., Cristea, M., Winnik, F.M., Pure and Applied Chemistry, **76**, 1321, 2004.

[8] Watson, J.D., Crick, F.H., Nature, **171**, 737, 1952.

[9] Marko, J.F., Siggia, E.D. Phys. Rev. E, **52**, 2912, 1995.

[10] de Gennes, P.-G., Scaling Concepts in Polymer Physics (Cornell University Press, Ithaca, 1979)

[11] Daoud, M., and Cotton, J.P. J. Phys. (Paris), **43**, 531, 1982.

[12] Borisov, O.V., and Zhulina, E.B., Eur. Phys. J. B, **4**, 205, 1998.

[13] Klein Wolterink, J., Leermakers, F.A.M., Fleer, G.J., Koopal, L.K., Zhulina, E.B., and Borisov, O.V., Macromolecules, **32**, 2365, 1999.

[14] Klein Wolterink, J., van Male, J., Cohen Stuart, M.A., Koopal, L.K., Zhulina, E.B., Borisov, O.V., Macromolecules, **35**, 9176, 2002.

[15] Grest, G.S., Kremer, K., and Witten, T.A., Macromolecules, **20**, 1376, 1987.

[16] Jusufi, A., Likos, C.N., and Löwen, H., Phys. Rev. Lett., **88**, 8301, 2002.

[17] Jusufi, A., Likos, C.N., and Löwen, H., J. Chem. Phys., **116**, 11011, 2002.

[18] Batoulis, J., Kremer, K., Macromolecules, **22**, 4277, 1989.

[19] Roger, M., Guenoun, P., Muller, F., Belloni, L., Delsanti, M. Eur. Phys J. E, **9**, 313, 2002.

[20] Likos, C.N. Physics Reports, **348**, 267, 2001.

[21] Feigin, L.A., Svergun, D.I., Structure analysis by Small-Angle X-Ray and Neutron Scattering. Plenum Press, New York

[22] Cogan, K.A., Gast, A.P., Capel, M., Macromolecules, **24**, 6512, 1991.

[23] Förster, S., Wenz, E., and Lindner, P., Phys. Rev. Lett., **77**, 95, 1996.

[24] Guenoun, P., Muller, F., Delsanti, M., Auvray, L., Chen, Y.J., Mays, J.W., and Tirrell, M., Phys. Rev. Lett., **81**, 3872, 1998.

[25] van der Maarel, J.R.C., Groenewegen, W., Egelhaaf, S.U., and Lapp, A., Langmuir, **16**, 7510, 2000.

[26] Groenewegen, W., Egelhaaf, S.U., Lapp, A., and van der Maarel, J.R.C., Macromolecules **33**, 3283, 2000.

[27] Hickl, P., Ballauff, M., Lindner, P., Jada, A., Colloid Polym. Sci., **275**, 1027, 1997.

[28] Marques, C.M., Izzo, D., Charitat, T., Mendes, E., Eur. Phys. J. B, **3**, 353, 1998.

[29] Heinrich, M., Rawiso, M., Zilliox, J.G., Lesieur, P., Simon, J.P. Eur. Phys. J. E, **4**, 131, 2001.

[30] Vagberg, L.J.M., Cogan, K.A., Gast, A.P., Macromolecules, **24**, 1670, 1991.

[31] Lee, A.S., Butun, V., Vamvakaki, M., Armes, S.P., Pople, J.A., Gast, A.P., Macromolecules, **35**, 8540, 2002.

[32] Guo, X., Ballauff, M., Phys. Rev. E, **64**, 051406, 2001.

[33] Kapnistos, M., Vlassopoulos, D., Fytas, G., Mortensen, K., Fleischer, G., and Roovers, J., Phys. Rev. Lett. 85, 4072, 2000

[34] Hamley, I.W., Fairclough, J.P.A., Ryan, A.J., Ryu, C.Y., Lodge, T.P., Gleeson, A.J., and Pedersen, J.S., Macromolecules, **31**, 1188, 1998.

[35] Watanabe, H., Yao, M-L., Sato, T., and Osaki, K., Macromolecules, **30**, 5905, 1997.

[36] Stiakakis, E., Vlassopoulos, D., Loppinet, B., Roovers, J., and Meier, G. Phys. Rev. E, **66**, 051804, 2002.

[37] Buitenhuis, J., Förster, S., J. Chem. Phys., **107**, 262, 1997.

[38] Bhatia, S.R., and Mourchid, A., Langmuir, **18**, 6469, 2002.

[39] Brown, G.J., Richards, R.W., and Heenan, R.K., Polymer, **42**, 7663, 2001.

[40] Baxter, R.J., J. Chem. Phys., **49**, 2770, 1968.

[41] Liu, Y.C., Chen, S.H., Huang, J.S., Phys. Rev. E, **54**, 2, 1698, 1996.

[42] Groenewegen, W., Lapp, A., Egelhaaf, S.U., and van der Maarel, J.R.C., Macromolecules, **33**, 4080, 2000.

[43] Willner, L., Jucknischke, O., Richter, D., Farago, B., Fetters, L.J., Huang, J.S., Europhys. Lett., **19**, 297, 1992.

[44] Richter, D., Jucknischke, O., Willner, L., Fetters, L.J., Lin, M., Huang, J.S., Roovers, J., Toporovski, C., Zhou, L.L., J. Phys IV, Suppl., **3**, 3, 1993.

[45] Mendes, E., Lutz, P., Bastide, J., Boue, F., Macromolecules, **28**, 174, 1995.

[46] Muller, F., Delsanti, M., Auvray, L., Yang, J., Chen, Y.J., Mays, J.W., Deme, B., Tirrell, M., and Guenoun, P. Eur. Phys. J. E, **3**, 45, 2000.

[47] Guenoun, P., Delsanti, M., Gazeau, D., Mays, J.W., Cook, D.C., Tirrell, M., Auvray, L., Eur. Phys. J. B, **1**, 77, 1998.

[48] Muller, F., Guenoun, P., Delsanti, M., Deme, B., Auvray, L., Yang, J., and Mays, J.W., Eur. Phys. J. E, **15**, 465, 2004.

[49] Mei, Y., Ballauff, M., Eur. Phys. J. E, **16**, 341, 2005.

[50] Muller, F., Romet-Lemonne, G., Delsanti, M., Mays, J.W., Daillant, J., and Guenoun, P. J. Phys.: Condens. Matter, **17**, S3355, 2005.

[51] Lander, E.S., Linton, L.M., et al., Nature, **409**, 860, 2001.

[52] Verma, I.M., Somia, N., Nature, **389**, 239, 1997.

[53] Felgner, P.L., Gadek, T.R., Holm, M., Roman, R., Chan, H.W., Wenz, M., Northrop, J.P., Ringold, G.M., and Danielsen, M., Proc. Natl. Acad. Sci. USA, **84**, 7413, 1987.

[54] Rosoff, M., Vesicles, Marcell Dekker, 768, 1996.

[55] Cevc, G., Phospholipids Handbook, Marcel Dekker, 992, 1993.

[56] Akoh, C.C., Min, D.B., Food Lipids, Marcel Dekker, 1040, 1992.

[57] Wu, G.Y., Wu, C.H., J. Biol. Chem., **262**, 4429, 1987.

[58] Behr, J.-P., Demeneix, B., Loeffler, J.P., Perez-Mutul, J., Proc. Natl. Acad. Sci. USA, **86**, 6982, 1989.

[59] Kabanov, A.V., Kabanov, V.A., Bioconjugate Chem., **6**, 7, 1995.

[60] Pack, D.W., Hoffman, A.S., Pun, S., Stayton, P.S., Nature Reviews, **4**, 581, 2005.

[61] Pouton, C.W., Seymour, L.W., Advanced Drug Delivery Reviews, **34**, 3, 1998.

[62] Mathiowitz, E., Jacob, J.S., Jong, Y.S., Carino, G.P., Chickering, D.E., Chaturvedi, P., Santos, C.A., Vijayaraghavan, K., Montgomery, S., Bassett, M., Morrell, C., Nature, **386**, 410, 1997.

[63] Edlund, U., Albertsson, A-C., Advances in Polymer Science, **157**, 68, 2002.

[64] Jong, Y.S., Jacob, J.S., Yip, K.P., Gardner, G., Seitelman, E., Whitney, M., Montgomery, S., and Mathiowitz, E., Journal of Controlled Release, **47**, 123, 1997.

[65] Cohen, H.,Levy, R.J., Gao, J., Fishbein, J., Kousaev, V., Sosnowski, S., Slomkowski, S., Golomb, G., Gene Therapy, **7**, 1896, 2000.

[66] Hirosue, S., Muller, B.G., Mulligan, R.C., Langer, R., Journal of Controlled Release, **70**, 231, 2001.

[67] Prasmickaite, L., Hogset, A., Selbo, P.K., Engesaeter, B.O., Hellum, M., Berg, K., British Journal of Cancer, **86**, 652, 2002.

[68] Nardin, C, Bolikal, D., Kohn, J., Langmuir, **20**, 11721, 2004.

[69] Putnam, D., Gentry, C.A., Pack, D.W., Langer, R., Proc. Natl. Acad. Sci. USA, **98**, 1200, 2001.

[70] Ahmed, F., Discher, D.E., Journal of Controlled Release, **96**, 37, 2004.

[71] Dias, R.S., Pais, A.A.C.C., Miguel, M.G., and Lindman, B., J. Chem. Phys., **119**, 8150, 2003.

[72] Gebhart, C.L., Kabanov, A.V., J. Bio. Compat. Pol., **18**, 143, 2003.

[73] Schiessel, H., J. Phys.: Condens. Matter, **15**, R699, 2003.

[74] Dias, R.S., Lindman, B., Miguel, M.G., J. Phys. Chem. B, **106**, 12600, 2002.

[75] Thurmond II, K.B., Remsen, E.E., Kowalewski, T., Wooley, K.L., Nucleic Acids Research, **27**, 14, 2966, 1999.

[76] Koltover, I., Salditt, T., Radler, J., Safinya, C.R., Science, **281**, 78, 1998.

[77] Kneuer, C., Bioconjugate Chem., **11**, 926, 2000.

[78] Budker, V.G., Slattum, P.M., Monahan, S.D., Wolff, J.A., Biophysical Journal, **82**, 1570, 2002.

[79] Chen, W., Turro, N.J., Tomalia, D.A. Langmuir, **16**, 15, 2000.

[80] Walter, A., Vinson, P.K., Kaplun, A., Talmon, Y. Biophys. J., **60**, 1315, 1991.

[81] Angelova, M.I., Soleau, S., Meleard, P., Faucon, J.F., and Bothorel, P. Progr. Coll. Polm. Sci., **89**, 127, 1992.

[82] Nardin, C, Winterhalter, M., Meier, W., Langmuir, **16**, 7708, 2000.

[83] Discher, B.M., Won, Y.-Y., Ege, D.S., Lee, J.C-M., Bates, F.S., Discher D.E., Hammer, D.A., Science, **284**, 1143, 1999.

[84] Aranda-Espinoza, H., Bermudez, H., Bates, F.S., Discher, D.E., Phys. Rev. Lett., **87**, 208301, 2001.

[85] Antonietti, M., Förster, S., Adv. Mater., **15**, 1323, 2003.

[86] Sevink, G.J.A., Zvelindovsky, A.V., Macromolecules, **38**, 7502, 2005.

[87] Wang, W., McConaghy, A.M., Tetley L., and Uchegbu, I.F., Langmuir, **17**, 631, 2001.

[88] Rawicz, W., Olbrich K.C., McIntosh, T., Needham, D., and Evans, E., Biophysical Journal, **79**, 328, 2000.

[89] Bermudes, H., Brannan, A.K., Hammer, D.A., Bates, F.S., and Discher, D.E., Macromolecules, **35**, 8203, 2002.

[90] Dimova, R., Seifert, U., Pouligny, B., Forster, S., and Dobereiner, H.-G. Eur. Phys. J. E, **7**, 241, 2002.

[91] Evans, E., Rawicz, W., Phys. Rev. Lett., **64**, 2094, 1990.

[92] Photos, P.J., Bacakova, L., Discher, B., Bates, F.S., and Discher, D.E., Journal of Controlled Release, **90**, 323, 2003.

[93] Decher, G., Science, **277**, 1232, 1997.

[94] Donath, E., Sukhorukov, G.B., Caruso, F., Davis, S., Mohwald, H. Angew. Chem. Int. Ed., **37**, 2202, 1998.

[95] Sukhorukov, G.B., Donath, E., Lichtenfeld, H., Knippel, E., Knippel, M., Budde, A., and Mohwald, H., Colloids Surf. A, **137**, 353, 1998.

[96] Caruso, F., Yang, W., Trau, D., Renneberg, R., Langmuir, **16**, 8932, 2000.

[97] Onda, M., Lvov, Y., Ariga, K., Kunitake, T. Biotechnol. Bioeng., **51**, 163, 1996.

[98] Caruso, F., Trau, D., Mohwald, H., Renneberg, R., Langmuir, **16**, 1485, 2000.

[99] Lvov, Y., Ariga, K., Ichinose, I., Kunitake, T., J. Am. Chem. Soc., **117**, 6117, 1995.

[100] Radtchenko, I.L., Sukhorukov, G.B., and Mohwald, H., Colloids and Surfaces A, **202**, 127, 2002.

[101] Shchukin, D.G., Patel, A.A., Sukhorukov, G.B., Lvov, Y.M., J. Am. Chem. Soc., **126**, 3374, 2004.

[102] Qui, X.P., Leporatti, S., Donath, E., Mohwald, H., Langmuir, **17**, 5375, 2001.

[103] Shchukin, D.G., Radtchenko, I.L., Sukhorukov, G.B., J. Phys. Chem. B, **107**, 86, 2003.

[104] Pargaonkar, N., Lvov, Y.M., Li, N., Steenekamp, J.H., de Villiers, M.M., Pharm. Res., **22**, 826, 2005.

[105] Sukhorukov, G.B., Dähne, L., Hartmann, J., Donath, E., Mohwald, H., Adv. Mater., **12**, 112, 2000.

[106] Sukhorukov, G.B., Brumen, M., Donath, E., Mohwald, H., J. Phys. Chem. B, **103**, 6434, 1999.

[107] Moya, S., Dähne, L., Voigt, A., Leporatti, S., Donath, E., Mohwald, H., Colloids and Surfaces A, **183 – 185**, 27, 2001.

[108] Diaspro, A., Silvano, D., Krol, S., Cavalleri, O., and Gliozzi, A., Langmuir, **18**, 5047, 2002.