

Polyelectrolyte multilayer capsules as vehicles with tunable permeability

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Available online 7 October 2004

Abstract

This review is devoted to a novel type of polymer micro- and nanocapsules. The shell of the capsule is fabricated by alternate adsorption of oppositely charged polyelectrolytes (PEs) onto the surface of colloidal particles. Cores of different nature (organic or inorganic) with size varied from 0.1 to 10 μm can be used for templating such PE capsules. The shell thickness can be tuned in nanometer range by assembling of defined number of PE layers. The permeability of capsules depends on the pH, ionic strength, solvent, polymer composition, and shell thickness; it can be controlled and varied over wide range of substances regarding their molecular weight and charge. Including functional polymers into capsule wall, such as weak PEs or thermosensitive polymers, makes the capsule permeability sensitive to correspondent external stimuli. Permeability of the capsules is of essential interest in diverse areas related to exploitation of systems with controlled and sustained release properties. The envisaged applications of such capsules/vesicles cover biotechnology, medicine, catalysis, food industry, etc.

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Keywords: Colloid; Encapsulation; Layer-by-layer; Controlled release; Nanoengineering

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1. Introduction

Creation of novel materials is one of the driving forces for the evolution of fundamental knowledge and industrial

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capabilities. The whole range of composites are widely used nowadays: from macro-composites, employed for example in construction, to micro- and nano-engineered materials used in electronics, optical, textile, paper, pharmaceutical, etc., industries. Knowledge about colloids and their properties plays probably the main role in the development of nano-composites. Improvement of these materials lays either in variation of their chemical composition or in modification of their surface properties. The latter option provides higher flexibility and accurate tuning of the material properties. Various methods can be utilized to modify the colloidal surface: from nonspecific sorption and chemisorption to chemical grafting.

One of the major contributions to the field of surface modification dates back to the 1990s when Decher et al., developed the electrostatic self-assembly (ESA), first proposed by Iler in 1966 [1]. They used anionic and cationic bipolar amphiphiles, adsorption of which led to charge reversal [2]. The multilayers could be built just by alternative dipping of a substrate into solutions of the corresponding bolaamphiphiles of opposite charge. In the same year they extended the method to polyelectrolytes (PEs) [3,4]. The only crucial factor for successful deposition is the surface charge reversal upon deposition of layers, which can be achieved by choice of proper deposition conditions. Since then various polyelectrolyte species could be adsorbed onto the surface of solid or liquid materials by means of electrostatic adsorption, forming layered structures with unique properties. It has been established that films can contain more than 1000 polyelectrolyte multilayers (PEMs). The thickness of the adsorbed layers can be tuned precisely in the range of few Ångströms. The universal character of the method does not impose any restriction on the type of polyelectrolyte. To date, this method has been used for more than 50 different charged macromolecules including synthetic polyelectrolytes, conductive polymers and biopolymers (proteins and nucleic acids). The multilayers can be also given diverse shapes as soon as various materials beside polyelectrolytes, such as organic, inorganic particles and crystals, biomolecules, lipids and viruses allow com-

plicated laminated structures to be formed. The use of polymers as the coating component is often advantageous in comparison with employment of their low-molecular weight analogues. Mechanical strength, elasticity, electrical, optical and other properties make them unique “building” blocks for creation of composite materials. The incorporation of proteins and nucleic acids in multilayer films may lead to the application as biosensors and in biotechnology [5–8]. The latter may even provide the base for development of ultrathin multistep chemical catalysts or photosynthesis systems mimicking plants. Applications can be also found in fabrication of optical devices [9,10] and gas separation membranes [11]. The efficient use of the multilayer coating is interlinked with investigation of their properties. Although the methods of studying multilayer properties are straightforward, they are very often not applicable or are time consuming because of the low amount of material studied, and the possible influence of the surface used as a support for layer growth. To eliminate the first factor, one should increase drastically the total surface of the multilayers, which can be done by using colloidal particles as templates [12]. Dissolution of the colloidal core could avoid the second objection. The derived hollow capsules [13,14] allow the study of polyelectrolyte multilayers at the liquid–liquid interface, which seems to be very difficult while working on the solid support.

Besides the auxiliary hollow polyelectrolyte multilayer shells and empty capsules represent a substantial system carrying much potential. The investigation of this continuously growing topic was started in the late 1990s [13–17]. Different materials have been used as a templating support: from latexes and inorganic crystals to protein aggregates, cells and even oil droplets. Two ways of alternate adsorption have been suggested: (1) sequential addition of matching amounts of polyelectrolytes into solution; (2) intermediate removal by centrifugation or filtration of polyelectrolyte excess before addition of each next layer (Fig. 2.1). As well as for flat surfaces, different substances can be used for capsule fabrication, including polymers, small molecules and ions, and particles. The core material can be removed

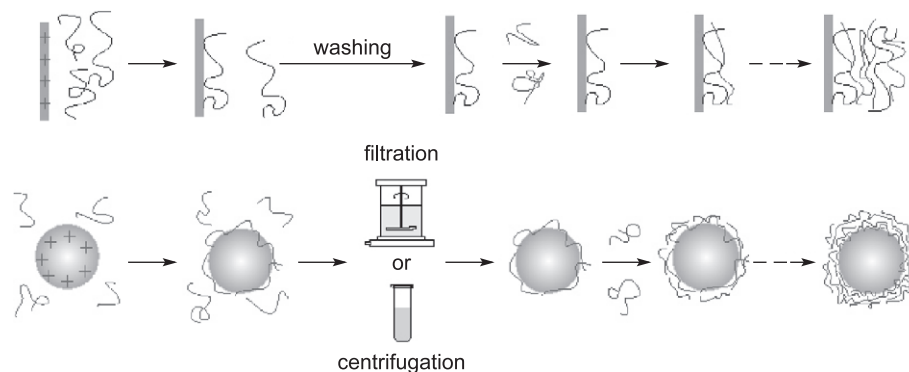


Fig. 2.1. Scheme of multilayer formation on flat substrates (top) and colloidal particles (bottom). Polyelectrolyte added into a system adsorbs onto the template leading to the charge reversion. After removal of PE excess (by washing of flat substrate or filtration or centrifugation of colloidal cores), oppositely charged PE is added. The circle is repeated to obtain a multilayer film or shell.

afterwards by dissolution [14] or calcination [18] to leave a hollow capsule. These capsules may have potential as drug delivery systems [19] capable of sustained release [20], microreactors [21–23], catalytic systems [21,22], etc.

Capsule-based materials are expected to have several advantages: (1) Very active and, probably, permselective materials can be obtained due to the specific morphology and high surface areas. (2) Formation of composite coverage of few different active substances would lead to several-in-one catalytic systems, capable of promoting a number of reactions simultaneously. The presence of a polymer matrix could lead to selectivity in catalysis due to the steric, enantiomeric, or electrostatic influence. (3) The polymeric matrix could serve as a protective envelope, shielding the active molecule from probable environmental hazards. (4) The solubility of these materials in different solvents can be easily tuned by changing the outer most layer: a hydrophobic layer would enhance oil solubility, a hydrophilic one would enhance the water solubility. Simultaneously it is easy to regulate the affinity of the capsule to a surface by attachment of specific chemical groups.

One of the most important peculiarities of these multilayers is the selective permeability for different species. The film permeability depends on layer thickness, porosity, structure of multilayers as well as on chemistry, charge and size of a permeable compound. The first investigations in this direction [24] showed that these films exclude macromolecules with large molecular size whilst they are readily permeable for small polar molecules.

The main target of this chapter is to show the current achievements in the field of regulation the permeability of polyelectrolyte multilayer capsules.

2. Core influence on capsule properties

The procedure of capsule formation is a multistep process. Though the further properties of capsules depend strongly on the choice of polyelectrolytes employed, adsorption conditions, such as ionic strength, temperature, solvent composition, number of layers, etc., an extremely important step, determining success of the experiment is the proper choice of the cores type. In this chapter we describe the main classes of the templates used for capsules fabrication.

In order to fabricate intact capsules consisting only of the material used during the coating, the process of core dissolution should result in 100% elimination of the core without affecting the PE multilayers. In fact, the molecular weight of core components is typically hundred times more than that of the polymers forming the shell, and the task of complete core removal seems to be complicated from chemical point of view. At present, a variety of cores have been exploited to template hollow capsules. Generally, they can be classified into three groups differed by dissolution

products and chemistry occurring with PE components of the shell.

2.1. Organic cores made of water-insoluble oligomers

We attribute to this class the widely studied melamine formaldehyde (MF)-cores dissolvable at low pH and in some organic solvents, polystyrene (PS) cores soluble in tetrahydrofuran (THF) and bio-friendly polylactic acid/poly(lactic-co-glycolic acid) (PLA/PLGA) soluble in acetone/*N*-methyl-2-pyrrolidinone mixture. In spite of a good monodispersity (with exception of PLA/PLGA) and technologically well-established process of synthesis, their drawback is sticking of the core material to the capsule wall. Naturally, the oligomers formed upon dissolution can easily get entangled with PE multilayer, making difficult their removal even after several washing cycles. In the paper of Gao et al. [25] the rest of MF-in hollow polyelectrolyte capsules was shown to reach 30% of the capsule weight. The osmotic stress occurring upon dissolution of MF-cores may cause rupture of PE multilayer shell. In some cases the capsule stays intact during the dissolution process only if it is assembled of not more than 8–10 polyelectrolyte layers [25,26]. The mentioned processes bring uncertainty in further study of permeability properties of the capsules fabricated on the polymeric templates.

2.2. Core dissolvable into small molecules and ions

These can be either ionic or molecular crystals soluble in acidic or basic conditions or in an organic solvent. At present, different carbonate particles (CaCO_3 , CdCO_3 and MnCO_3) [27,28] and SiO_2 particles [29] have been used for PE multilayer templating. Main advantage of exploitation of such inorganic particles is absence of the osmotic stress upon dissolution and complete elimination of the core. As shown in Ref [27], the possible rest of Mn-ions in the capsules does not exceed the ratio of 1:1000 to SO_3^- groups of polystyrenesulfonate PSS. As a consequence, these capsules can comprise high number of layers and have a permeability coefficient significantly lower than that of the latex-templated capsules, which is a proof of an intact capsule wall.

2.3. Core subjected to strong oxidation

A biological cell is a typical example of this kind of cores. Assembly of polyelectrolyte multilayers can be performed using fixed erythrocyte cells as templates [30–32]. The removal of the cell cores can be achieved by an oxidation with sodium hypochlorite solution. In result hollow capsules are produced. However, the treatment with NaOCl solution changes the chemical composition of the capsules drastically [31]. Oxidation of polyelectrolytes and appearance of covalent bonds lead to cross-linking of the polymer chains.

Actually, biological cells represent a monodisperse and naturally very cheap source of templates for capsules production. But their use for scientific purpose, such as permeability study, is limited by possible PE employed and undefined chemical modification of PE shells.

We also could address the attention of readers to templating exclusively inorganic hollow spheres by calcinations of organic components [18] and synthesized organic/inorganic shells at ambient conditions [33]. But these branches of the capsule research are far from purely polymeric vesicles considered here being off-topic of this review.

As one can see from mentioned above about core influence, employment of different core material results in different permeability properties. Ibarz et al. [34] proposed the approach to “heal” the capsules surface by post-preparation assembly of several additional polyelectrolyte layers. Permeation of small molecules such as dyes through the walls of the capsules templated on latex cores is limited by free diffusion through defects and is almost equal to the diffusion through water. Assembling of sequential layers on pre-formed hollow capsules can decrease permeability coefficient by several orders of magnitude due to the closing of the pores occurred during the dissolution process. The diffusion coefficient of such capsules is close to that of the capsules templated on molecular crystals [35,36] or on carbonate or silica particles (Fig. 2.1).

3. Permeability for low molecular weight compounds

A major challenge in the development of the carrier systems of submicron dimensions lays in the field of advanced drug formulations. It comprises the elaboration of delivering systems capable to provide sustained release of bioactive materials [37]. Mainly, these bioactive compounds are small molecules with different solubility and diffusion coefficients. If we consider only physical entrapment without any covalent binding in order to develop delivery system one has to encapsulate the active molecules within the shell proving certain release characteristics. One

possible way to form an essential barrier for small polar molecules would be the use of lipids as a layer constituents as has been proposed in Refs. [38,39]. The formation of thicker capsule walls might be another straightforward way to decrease permeation [40]. Thus, increasing the layer number is expected to decrease molecules penetration. The potential use of polyelectrolyte capsules for drug release, however, requires more quantitative data on the permeation of small molecules through polyelectrolyte walls. In this chapter, different aspects of the permeability of low molecular weight substances through the polyelectrolyte multilayers are discussed.

3.1. Permeability as a function of film thickness

To verify the influence of the number of layers on the permeation rate of low molecular weight ions, fluorescein was used as a model [20]. One advantage of using fluorescein consists of its self-quenching at high concentrations and in solid state enabling distinction between dilute and concentrated state. It should be also mentioned that the fluorescein structure and properties are quite similar to that of many drugs. Fluorescein microparticles were covered with a different number of polystyrenesulfonate/polyallylamine hydrochloride (PSS/PAH) layers at low pH where fluorescein is insoluble. Afterwards core dissolution was initiated by a pH change and monitored by the increasing fluorescence in the bulk. Fig. 3.1.1 provides the scheme of fluorescein particle encapsulation and release. After LbL adsorption (Fig. 3.1.1A–C) core dissolution is initiated by changing the pH from pH 2.0 to pH 8.0 (Fig. 3.1.1D–E) and completed after a certain period of time (Fig. 3.1.1F). Fluorescein particles rapidly dissolve at pH 8.0. The idea was thus to slow down the rate of core dissolution by covering the particles with polyelectrolyte multilayers. Shell walls consisting of a different number of layers were fabricated and examined in regard to their fluorescein permeability behavior.

In Fig. 3.1.2 typical time-dependent fluorescence curves, which were obtained by switching the pH to 8.0 are shown.

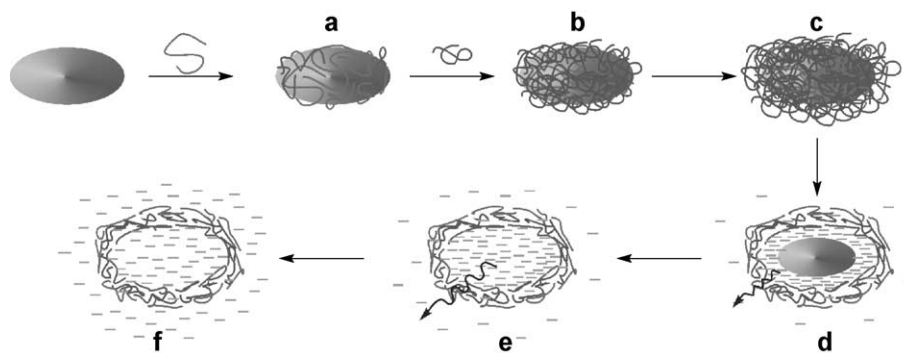


Fig. 3.1.1. Scheme of the polyelectrolyte multilayer deposition process and of the subsequent core dissolution. The initial steps (a–d) involve stepwise shell formation on a fluorescein core. After the desired number of polyelectrolyte layers is deposited the coated particles are exposed to pH8 (e) and core dissolution with fluorescein penetration into the bulk is initiated resulting finally in fully dissolved cores and remaining empty capsules (f).

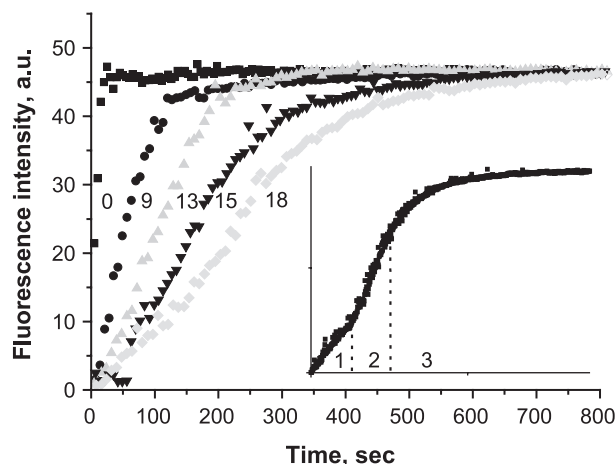


Fig. 3.1.2. Fluorescence increase upon time, obtained by dissolving fluorescein particles covered with shells of different thickness (9, 13, 15, and 18 PE layers), compared with bare (0 PE layers) fluorescein particles. Insert shows three stages of core material release.

Fluorescein particles covered by shell of different thickness (9, 13, 15, and 18 layers) are compared with the control showing the fluorescence increase upon dissolution of the bare fluorescein particles.

It can be seen (Fig. 3.1.2 insert) after a comparatively short induction period (1) (which varies and sometimes is negligible) the rate of dissolution becomes constant (2), meaning the constant concentration gradient before finally the fluorescence in the bulk levels off (3). Without going deep into discussions about different stages of core dissolution and mathematical interpretation of the dissolution curves described elsewhere [20,41], let us rather return to the behavior of the permeability coefficient as a function of the multilayers structure.

For 8–18 layers the permeability value varied from 7×10^{-9} to 2×10^{-9} m/s. Assuming a thickness of 3 nm for each individual polyelectrolyte layer, the permeability can be converted into a diffusion coefficient (D) by means of multiplying the permeability with the shell wall thickness. The calculated diffusion coefficients ranged from 1.7×10^{-16} to 1×10^{-16} m²/s.

If the permeability of the polyelectrolyte multilayer is provided by diffusion through the entangled polymer

network, it should scale with the inverse of the layer thickness. The behavior of the permeability as a function of the number of layers is shown in Fig. 3.1.3. As can be seen the permeability decreases with increasing layer number much faster than expected from a straightforward thickness increase. Only from approximately eight layers onwards the permeability multiplied by the shell thickness becomes constant indicating that the permeability is now controlled by the thickness increase, where, the diffusion-limiting region is the polyelectrolyte layer. This finding is consistent with the earlier observations [42] where it was shown that the conformation of the first eight layers differs from that of further assembled layers. These deeper layers are denser resulting in a fivefold reduction of the estimated diffusion coefficient as can be inferred in Fig. 3.1.3. Loose structure of the interfacial layers lead to the slow increase of the dissolution time, and only presence of the internal dense layers can strongly increase this time.

Let us discuss in more detail the permeation mechanism. One may distinguish between diffusion through water filled pores and a “bulk” diffusion mechanism through the homogeneous phase of the polyelectrolyte multilayer shell. For thinner walls the drastic dependence on shell thickness may be explained either by existence of pores that are successively closed by further layer deposition or by a thickness dependent diffusion coefficient. Diffusion dependent on layer depth was indeed observed when the diffusion of polar labels in planar polyelectrolyte films was studied. Diffusion coefficients ranging between 10^{-18} and 10^{-20} m²/s were obtained [42]. In order to compare our results with those of the previous study we should remark the following differences.

- In Ref. [42], the films were prepared by drying after each adsorption step which is not possible during particle coating and which is known to result in a denser film.
- The measurement in Ref. [42] consisted in depositing a dye probe in a defined depth into the film and observing time dependent fluorescence changes due to quencher diffusion. For sufficiently small pore concentration, which is surely the case here, this technique is only

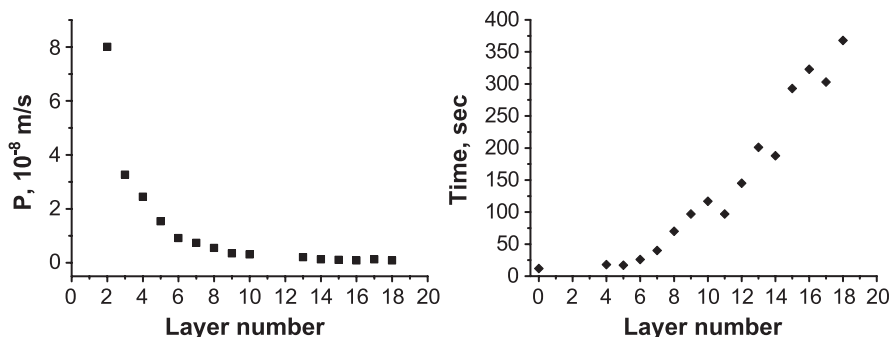


Fig. 3.1.3. Fluorescein permeability coefficient (left) and time of core dissolution (right) as a function of layer number.

sensitive to “bulk” diffusion and will not reflect the permeation through pores.

Thus one may expect that the diffusion coefficients derived from permeability data are larger than values measured in Ref. [42], and from this work we also know that they may vary by at least an order of magnitude with salt conditions.

Comparing the diffusion coefficient D obtained in this study with that in bulk water ($\sim 10^{-10}$ m²/s), which is an upper limit for the diffusion in the pore volume we may estimate the pore area in the shell wall. From this comparison, it can be concluded that the pore area constitutes not more than 10^{-6} of the total surface.

Next, we address a possible mechanism of pore formation. It is quite conceivable that the hydrostatic pressure difference arising as a result of the water flux driven by the difference in osmotic pressure appearing upon fluorescein core dissolution creates a tension in the wall, which may widen existing or create new pores. As seen in Fig. 3.1.2, especially for a larger numbers of layers, in the beginning of dissolution the release is sustained. The pores are formed as a result of a rearrangement of polyelectrolyte molecules, which facilitate the permeation.

3.2. Permeability as a function of ionic strength and charge of a permeating molecule

Another parameter that can influence the multilayers structure is the ionic strength. It has been shown that the multilayer structure is affected if the salt concentration is changed once the film is built up [43–46]. These structural rearrangements happen in both cases of increase or decrease of the ionic strength. A variety of multilayer properties may change on salt concentration shift—the layers thickness, surface morphology and permeability. The main reason for this is rearrangements in the PE complex. The similar experiment with dissolution of coated fluorescent particle at different ionic strengths has been employed to investigate the ionic strength influence. Fluorescein particles were coated with 19 PSS/PAH layers having a thickness of 29 nm. The shell thickness was kept constant in order to investigate the properties of the “bulk” layers; besides that, the permeability had to be limited by permeation through

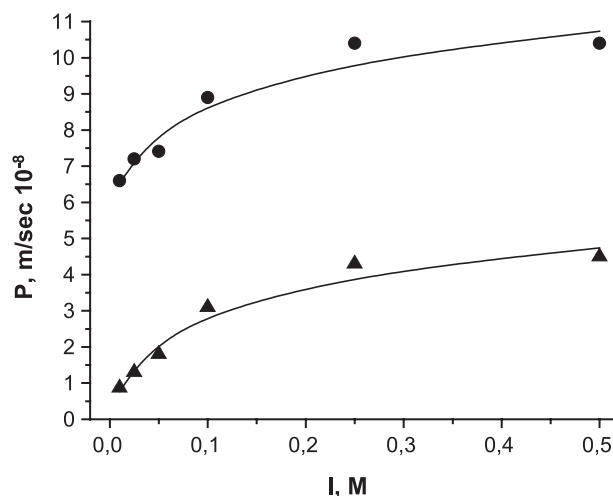


Fig. 3.2.2. Permeability of PEM capsules as a function of ionic strength at pH 7.0 (circles) and pH 8.0 (triangles). Lines represent the fitting of the data by Eq. (3) (see later).

these inner layers, in other words, influence of the outer layers (first and last four layers, which are more loose) had to be negligible. Core dissolution was conducted in pH 7 and 8 buffers with varying ionic strength. A typical set of curves obtained at different ionic strengths varying from 10 to 500 mM is depicted in Fig. 3.2.1.

As seen from the graph in Fig. 3.2.2, the permeability coefficient has a strong nonlinear dependence on the salt concentration. From a law, which describes the influence of the ionic strength on the permeability, one may conclude on the mechanism governing the permeation of the molecules through PEMs.

In the work of Farhat et al. [47] it was discussed that the diffusion of charged ions through the PEM occurs via hopping from site to site. So far, each dissociable PE group is a potential “vehicle” and acts only being in the dissociated state. Hence, the salt concentration is expected to increase the content of free sites in the PE complex. It was suggested that the permeability of PEM for ions of different charges depends dramatically on their charges [47,48]. This dependence obeys a power law $P \sim k \cdot [\text{salt}]^n$, where n is the ion charge [47], since the hopping theory describes the ion penetration. Although this approximation explains the growth of the flux at higher salt concentration, it, however,

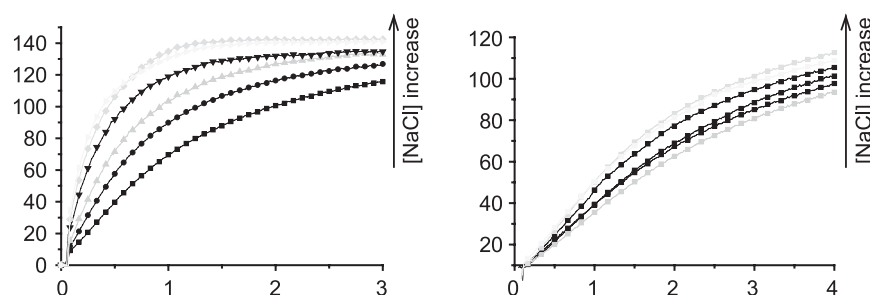


Fig. 3.2.1. Release curves at pH 8.0 (left) and pH 7.0 (right) at 10, 25, 50, 100, 250, and 500 mM NaCl (from bottom) at a starting phase of the dissolution.

cannot explain the obvious non-square dependency in our case. This mismatching is possible due to the fact that the pH of the system described in Ref. [47] was not altered, while in the given example the pH was shifted by 5 and 6 units. This pH shift changes the ratio between positive and negative charges within the multilayers and may loosen their structure. The theory mentioned implies the assumption that the mobility of ions cannot depend on the amount of additional water, which appears as a result of PEM swelling because the layers contain a high-enough amount of water in their unswollen state. Although the change of the PSS/PAH multilayer thickness was not registered [49], which can be coupled to the amount of “sucked” water upon immersion of PEM into salt solution, the morphology of PEMs changes drastically—in the work of Dubas et al. [49] an unexpected smoothing of the multilayer surface upon salt treatment was found. To describe the strength of electrostatic interactions, we can use the Debye length, which for 1:1 electrolytes (like NaCl) is $1/k = 0.304/\sqrt{C_{\text{NaCl}}}$ nm. This means that $1/k$ changes from 3.04 to 0.43 nm going from 10 to 500 mM NaCl solution. The ionic strength influences not only the range of the electrostatic forces, but also their amplitudes [50]. The interactions between two polyelectrolytes depend dramatically on the salt concentration and in many cases the increase of the ionic strength leads to the total destruction of the interpolyelectrolyte complex (PEC). Addition of salt to PEMs decreases the attractive forces between the oppositely charged polyelectrolytes on one hand, and the repulsive forces between the polyelectrolytes of the same charge. While the first phenomena leads to the swelling of the multilayers [49,51], the second may cause their shrinkage [52]. This explains a seeming contradiction between experimentally observed behaviors of PEMs prepared at different conditions.

The release of fluorescein, with two negative charges at our experimental conditions should be a function of C_{NaCl}^2 according to the mentioned theory [47], but, as we will see that is not a case here. Hence, there should be another mechanism governing the dependence of the release on the salt concentration. It is highly probable that the release takes place through water-filled cavities in the PEC. The fraction of water in the complex should be related to the intermolecular force between neighboring polyelectrolytes. The free energy of PE interaction F in the case of relatively high ionic strength can be described as dependence on the Debye length $1/k$ and the fraction of PE charged groups F , which is the part of the variable P_1 [53]. The constant P_2 depends on the nature of a polyelectrolyte and other charge independent parameters:

$$F \sim -P_1 \frac{1}{k} + P_2 \quad (1)$$

We must point out that this equation can be used within the approximation of the intermixed layer structure not to consider the repulsion forces between segments of the same charge, which was proved by neutron reflectivity [54]. The

equilibrium constant for the bond dissociation between PSS and PAH segments obeys the Vant Hoff exponential law $K \sim \exp(-F/kT)$. The fraction of water filled cavities inside the multilayer, h , must proportionally increase with a decrease of the amount of PSS/PAH bonds:

$$h \sim K \sim \exp\left(-\frac{F}{kT}\right) \sim \text{const} \exp\left(\frac{1}{\sqrt{C_{\text{NaCl}}}} \text{const}'\right) \quad (2)$$

So far, if this equation is valid, it should describe the release rate dependence on the ionic strength. The permeability coefficient P and the time of release should obey the following law:

$$\frac{1}{A^*} \sim P = \left(p_1 \exp\left(\frac{1}{\sqrt{C_{\text{NaCl}}}} p_2\right)\right)^{-1} + p_3 \quad (3)$$

Parameter p_3 depends on the non-interpolyelectrolyte interactions, such as interactions between a permeating molecule and multilayers. This relationship can be compared with the Arrhenius equation—the pre-exponential parameter p_1 is a frequency factor, p_2 can be compared with activation energy of pore formation and ionic strength with temperature.

Eq. (3) fits well the experimental data of the permeability coefficients vs. salt concentration (Fig. 3.2.2). The fitting equations for pH 7.0 and pH 8.0 were $P = (1.4 \times 10^7 \exp(0.34/\sqrt{C_{\text{NaCl}}}))^{-1} + 6.3 \times 10^{-8}$ and $P = (1.5 \times 10^7 \exp(0.32/\sqrt{C_{\text{NaCl}}}))^{-1} + 5 \times 10^{-9}$ respectively. This suggests that the permeation through the PEMs is a function of the free energy of the interpolyelectrolyte interaction and can be changed by parameters responsible for the polyelectrolyte complex (PEC) formation.

Acidity is another parameter, which influences the binding in PECs if at least one of the polyelectrolytes is a weak polyacid or polybase. As soon as the number of intrinsic binding sites can vary with changing the pH due to the protonation/deprotonation of the weak component, several processes may take place: (1) osmotic pressure increase because of incoming ions compensating an excessive charge, (2) breakage of the internal electrostatic bonds, (3) decrease of the free energy of polyelectrolyte interaction, because the coefficient P_1 in Eq. (1) is proportional to the charge densities of PE chains; (4) besides that, the appearance of the charge on the immobile surface can suppress its penetrability for the ions of the same charge. We showed above that the first two mechanisms influence the polymer permeability. The permeation of the small fluorescein molecule depends also on the pH value of the solution. The permeability values range from 1.0×10^{-7} to 6.6×10^{-8} m/s for pH 7.0 and from 4.5×10^{-8} to 8.7×10^{-9} at pH 8.0, while the salt concentration is varied from 500 to 10 mM. Deceleration of the fluorescein anion permeation by negatively charged walls leads to a 1.5- to 5-fold decrease of the permeability, depending on the ionic strength, with an increase of the alkalinity by one pH unit. As soon as the charge compensation between polyelectrolytes is maximal

at the conditions of assembly, at pH 2.0, any shift from this pH to alkaline region would lead to a charge increase due to deprotonation of the PAH, and the higher the deviation, the larger the charge induced. The negative charge of the capsule wall is higher at pH 8.0 than at pH 7.0, which increases the repulsion between the multilayers and fluorescein molecules, resulting in a permeability decrease. This phenomenon was not registered in previous works [55]. One of the explanations for this may lay in the mechanism of ion permeation. If permeation occurs via water-filled compartments inside the polyelectrolyte layers and if the ion is small enough not to feel the charge of the compartment walls, the pH influence can be negligibly small; a typical example is the permeation of metal ions. Oppositely, the interactions between ions and polyelectrolytes cannot be omitted if the ion size is comparable to the size of the cavities responsible for the permeation. In our case, two opposite processes are proceeding simultaneously. On one hand, the pH increase induces the in-layer repulsion between negatively charged PSS segments and increases the osmotic pressure, and on the other hand, the total negative charge should repel the fluorescein anions. These two phenomena work in opposite direction. The former increases the permeation, while the latter decreases it. In the case of fluorescein, which has a doubly negative charge, its repulsion from the multilayers determines the release rate. It is highly possible that for monovalent anions of the same size as fluorescein molecules, the first process would dominate, resulting in an increase in permeability with increasing pH. For triply charged anions, repulsion by the capsule wall may lead to a strong decrease of the permeability coefficients.

The permeability of variably charged ions through the multilayers was intensively studied on planar multilayers by the groups of Bruening and Schlenoff [47,48,56–59]. If the ions were bearing the same charge as the multilayer membrane, the permeability was reducing with the increase of the ion charge. It was proposed to employ this finding in creation of ion separating membranes. Ion-permeability study for capsule-forming multilayers is difficult due to methodological reasons. At present the only attempt to measure ion permeability on capsules was proposed in Ref. [60]. Dissolution of multilayer coated magnesium oxalate microcrystals was studied by conductivity measurements. For doubly charged ion the diffusion coefficient was estimated to be around 10^{-9} cm²/s, which four orders of magnitude lower than the coefficient of Mg²⁺ free diffusion in water.

3.3. Temperature annealing and resealing by additional layers

Polyelectrolyte multilayer vesicles can be subjected to temperature treatment, which significantly influences their properties. As shown by Leporatti et al. [61] at raising temperature to 95 °C the PE capsules keep their integrity but undergo irreversible structural changes. The resulted struc-

ture is characterised by decreasing the capsule diameter with simultaneous increasing of shell thickness as revealed by scanning force microscopy. From thermodynamic point of view this effect can be explained by approaching the equilibrium state of polyelectrolyte complexes. Indeed, the polyelectrolyte capsules represent PE complex topologically organized into hollow sphere, as formed by templating on spherical (or other shape) particles. However, the coiled structure would be an energetically favoured state for PE complexes. Applying energy to the polyelectrolyte chains, the electrostatic coupling between opposite charges is disturbed and PE chains reorganise to find counter charged groups at a more favourable conformation. Obviously, any new position for polymer chains results in their retraction and the whole PE capsule shrinks. This process is dependent on the average number of coupled charged per one polymer molecule. For instance, the capsules made of multivalent ions and oppositely charged PE may shrink to coil during several hours at a room temperature.

Widely used PE composition (PSS/PAH)₄ shrinks by the factor of 1.3–1.5 in diameter upon 30 min treatment at 90 °C. Since the volume occupied by the capsule wall is constant, an increase of the multilayer thickness during the shrinkage obeys the square law as a function of the capsule diameter. Thickness measurements [61] proved this square dependence and demonstrated the more coiled structure of folds PE shells form upon drying.

As described above, the thickness increase upon annealing results in reduction of the permeability. However, in reality the effect is much stronger than one would expect from just a thickness increase and a constant diffusion coefficient. As demonstrated by Ibarz et al. [62], the permeability coefficient can be changed by two orders of magnitude (Fig. 3.3.1) after heating at 80 °C. The PE chains rearrangement caused by temperature leads to annealing defects in PE multilayers.

Glinel et al. [63] exploited thermosensitive polymers as constituents of a capsule wall. Hollow capsules were

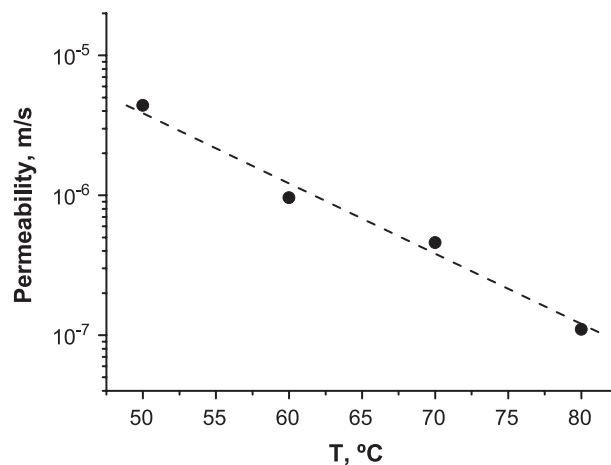


Fig. 3.3.1. Permeability coefficient changes of PAH/PSS capsules templated on MF-cores, 8 layers. Annealing time is 30 min.

fabricated by LbL deposition of oppositely charged diblock co-polymers each containing a poly(*N*-isopropylacrylamide) (PNIPAM) block (80 mol%) and a charged negative or positive block (20 mol%). PNIPAM is known to undergo a phase transition at a temperature of 32 °C (so called lower critical solution temperature), after which it is not soluble. This transition is rather sharp and completely reversible upon cooling. The obtained capsules exhibit changes in morphology and the permeability at elevated temperatures. However, this process is only partially reversible in the multilayers. Relaxation requires more than a month, which limits their thermosensitivity to one heating cycle.

3.4. Permeability regulation by chemical modification

Chemical modification can be another way to induce the excessive charge into the PE multilayers and change other characteristics of the film. One of the methods was proposed by Bruening [64], in which his group was using the polyacrylic acid, partially modified by Fisher esterification with different alcohols, as a multilayers constituent. Depending on the nature of the derivatizing alcohol, the hydrophobicity of the film could be tuned. The hydrophobicity of ester groups was shown to result in advancing water contact angles to more than 100°. Cross-linking of these hydrophobic films via heat-induced amidation stabilized coatings over a wide pH range but did not significantly influence their permeability. Base-promoted hydrolysis of the ester groups made the films extremely hydrophilic and selectively permeable to positively charged ions over negatively charged ones due to the high density of newly formed-COO⁻ groups.

It has to be mentioned here that cross-linking of the reactive groups in the multilayers is sometimes the only possibility of making films composed of weak polyelectrolytes, stable at wide range of pH and ionic strength. One of the examples is PAA/PAH multilayers, which readily decompose upon shift of pH or ionic strength, can be cross-linked in order to gain the stability [65,66]. Besides that, the properties of the film become completely different, so PAA/PAH film has a resistance of about 5 MΩ cm² after amidization, even when the film is only 10-nm thick.

Generally speaking, since the bulk of multilayers is not charged due to overcompensation of the polyelectrolytes, the charge of a film is determined by the outer most layer. It was shown that the charge of the capping polyelectrolyte influences strongly the permeation rate of ions. The permeation rate of the ions having the charge of the same sign as that of the film is much lower than the permeation of counterions [47].

Another possibility of increasing the internal film charge is to use the charged nonstoichiometric complex of a polyelectrolyte and a low-molecular weight ion as a constituent during the multilayer assembly [67]. So, alternating deposition of PAA partially complexed with Cu²⁺

and PAH followed by removal of copper ions and deprotonation resulted in fixed-COO⁻ sites in these films. The permselectivity of the multilayers was increased strongly in a direction of repulsion of multiply charged anions and for example for Cl⁻/SO₄²⁻ ions the separation constant of 610 could be achieved.

Another method of chemical modification of a multilayer capsule wall was suggested by Dähne et al. [68] who proposed synthesis of charged polymers in the shells. As a result of polymerisation, polyelectrolyte was formed in the capsule interior as well as in the wall leading to a strong permselectivity of capsules, which became very much selective to charge of a permeating molecule, excluding small molecules of the same charge as the polyelectrolyte.

4. Permeability for high molecular weight compounds

From previous work it is known that the capsule wall composed of PSS/PAH multilayers has semipermeable properties [38,69]. It is permeable for small molecules, such as dyes and ions while under most conditions the high molecular weight compounds are excluded. One of the explanations for this is the much smaller size of the pores in the multilayer film in comparison to the hydrodynamic radius of the permeating molecule. Electrostatic interactions should not be taken into account since the behavior is similar for all types of molecules investigated-positive, charged and neutral. High concentration of polyelectrolyte in the external solution leads to the osmotic stress and rupture of the capsule wall. The possibility to control and reversibly change the capsule permeability for low and high molecular weight molecules would be desirable for many applications, such as medicine, biotechnology, catalysis, cosmetics, nutrition, where selective encapsulation and release is required.

4.1. Permeability as a function of pH

Each polyelectrolyte pair that can be employed as layer component has unique properties that depend on the properties of each individual component and on their complex. The LbL assembly buildup leads to the formation of a stoichiometric interpolyelectrolyte complex, however this stoichiometry can be broken upon any shift from the adsorption conditions. The most important parameters that may lead to this shift are pH and the ionic strength. The change of the stoichiometry would necessarily lead to the occurrence of the surplus charge, and as a consequence, to the change of physico-chemical properties of multilayers.

Let us take an example of the classical PSS/PAH multilayers. At the pH of capsule formation, pH 7.0, the charge densities on both polyelectrolytes determine their stoichiometric ratio during adsorption. Since the polymers are irreversibly adsorbed in the shell wall a pH decrease does not induce polymer desorption. However, charging of

the PAH may occur in acidic conditions, which would induce positive charge into the shell wall. This may alter the shell wall morphology by enhancing the mutual repulsion, which could lead to defects in the polymer network. Moreover, if to estimate the steric energy of PAH at pH 10.0 (degree of protonation close to 0) and pH 3.0 (degree of protonation close to 1), stretching of the PAH will occur because of the growth of the steric energy when changing the pH value from 7.0 to 3.0 (Fig. 4.1.1) (the calculated value considers energies arising from bond stretching, angle bending, bond torsion, van der Waals and electrostatic interactions, and the cross-interactions, like bend-stretch term are added to the sum. Though the calculation may be not the most precise one, it illustrates the influence of the charge density on the macromolecule's conformation). The fully charged oligomer molecule has a contour length of 7% higher than the uncharged one. Besides that, the ions, which will appear upon charging, will enhance the osmotic pressure inside the layers. These three facts may cause swelling of the layers with ensuing loosening of the structure and subsequent enhanced capsule wall permeability.

The energy calculation shows that the energy change on charging is dominated by the Coulomb repulsion of charges on the PAH. A stronger effect is expected for Coulomb repulsion between PAH in adjacent layers leading to stronger swelling.

The permeability properties of hollow polyelectrolyte capsules templated on MF latex particles and carbonate crystals and consisting of four PSS-PAH bilayers have been investigated for diverse polymers, such as polyions PSS and PAH, neutral dextran and a wide range of proteins [22,23,28,70,71].

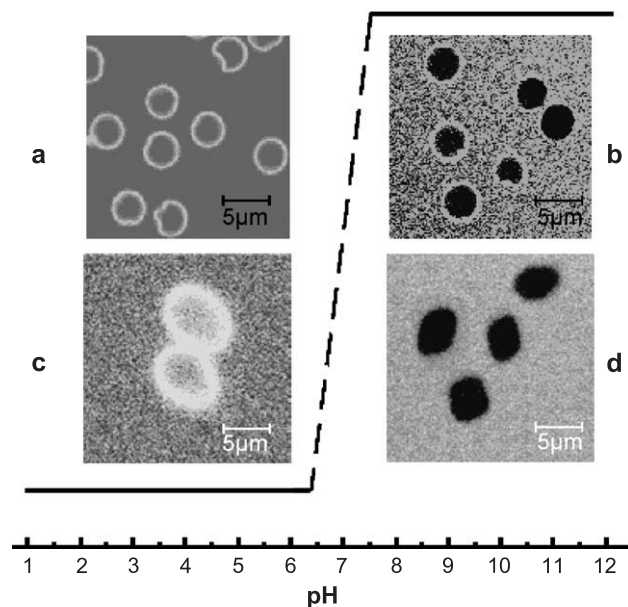


Fig. 4.1.2. Permeability of MF- and CdCO₃-derived capsules for FITC-dextran. Open (a, c) at pH 3.5 and closed (b, d) at pH 10.0 states of (PSS/PAH)₄ hollow capsules prepared on MF particles (a, b) and CdCO₃ crystals (c, d).

The permeability was monitored by means of confocal laser scanning fluorescent microscopy (CLSM). Fig. 4.1.2 (right) shows confocal images of capsules in the presence of fluorescently labeled (FITC-) dextrans at pH higher than 7. The exclusion of macromolecules by the capsule wall is clearly seen. However, at acidic conditions permeability increases drastically and the capsules interior gets filled with polymer. This permeability change at pH alternations is completely reversible process as shown on polymers

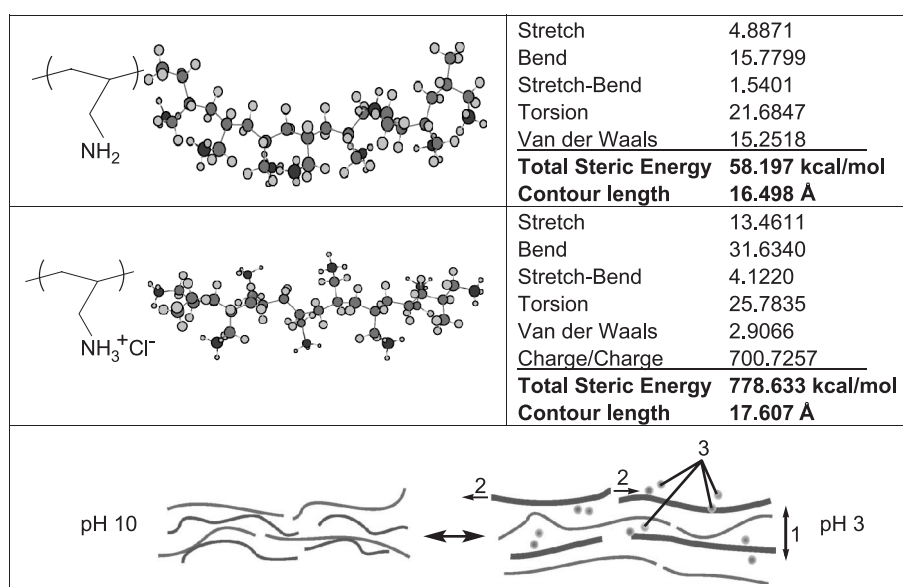


Fig. 4.1.1. Table-conformation of PAH at pH 7.0 and 3.0. The steric energies (in kcal/mol) are calculated for the seven units allylamine oligomer in CS Chem3D Pro (CambridgeSoft Corporation, USA). Bottom-scheme of layer swelling due to repulsion between adjacent layers (1), PAH segments (2) and nascent osmotic pressure from incoming ions (3) while changing pH from 10 to 3.

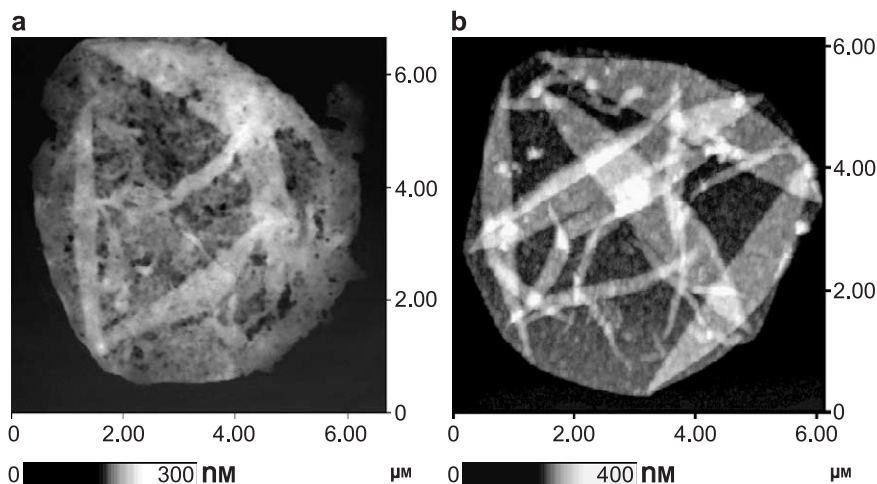


Fig. 4.1.3. SFM images of capsules treated with pH 3.5 (a) and pH 12 (b) buffers before drying.

income [28,70] as well as protein release from the capsules [72].

The structure of the capsules was studied by means of scanning force microscopy (SFM) as a function of pH (Fig. 4.1.3). Large holes up to 100 nm in diameter can be identified in the capsule walls, which had been exposed to acidic solution while no changes compared with the control occurred after incubation at pH 10 [28]. Most remarkable is the reversibility of these structural changes. Upon transfer of the capsules, exposed previously to pH 3.5, into an alkaline solution of pH 10 the holes could not be observed. Comparable observations were recently reported concerning the behavior of PAH-PSS multilayers on flat surfaces [73]—the presence of holes up to hundred nanometers in diameter was established after the multilayers composed of strong and weak polyelectrolytes were incubated at certain pH. In Ref. [74], PSS/PAH planar films were treated with pH 3.2 buffer, that lead to 40% swelling. The swelling corresponded to dramatic increase in film permeability.

4.2. Permeability as a function of solvent polarity

It is a known fact that many polyelectrolyte complexes can be dissolved by treatment with organic solvents. It happens because of changing a solvent quality—for instance

for a PSS/PAH pair, which forms an insoluble complex in water and a soluble one in ethanol, changing the ratio in water–ethanol mixture, one can pass through Θ -conditions. At this particular composition (around 20% of ethanol by volume [75]), the solvent changes its quality from poor to good, affecting the polyelectrolyte complex. This may lead to loosening of the film structure, or even to dissolution. At 20% of ethanol in water Tieke et al. [75] have registered a dramatic increase of the permeation rate through the PSS/PAH membrane and partial desorption of polyelectrolyte layers.

This phenomenon was utilized to change the capsule wall permeability. Experiments, similar to that described in a previous chapter, were conducted. Capsules suspension was mixed with ethanol in the presence of a labeled polymer. Ethanol containing capsules, contrary to that without ethanol, were permeable for the whole range of polymers studied. No difference in permeation of PSS, Dextrans (Mw 77 000 and 2 000 000), PAH, and various proteins was found pointing on a nonspecific mechanism of permeation. The similarity of ethanol treatment to the pH influence consists also in reversibility of the polymer network rearrangements. Washing of the ethanol treated capsules with water leads to self-healing of the multilayers and the wall morphology changes to that of native capsule.

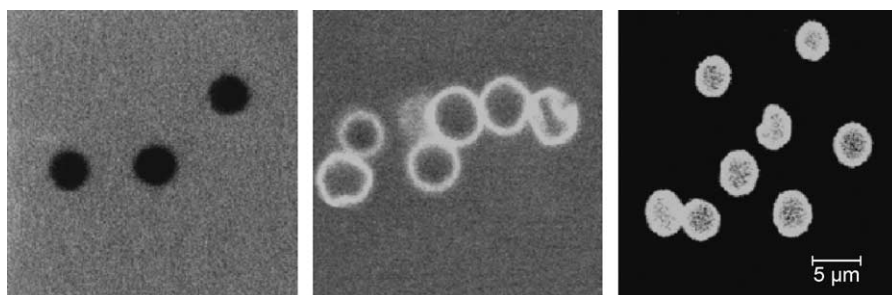


Fig. 4.3.1. Encapsulation of urease into capsules. Left: capsules in water; middle: in ethanol/water mixture; right: capsules with encapsulated urease again in water.

4.3. Encapsulation of polymers

The possibility to switch the permeability of the capsule wall for macromolecules between an open and a closed state provides an elegant means to load macromolecules into capsules and to release them afterwards. The capsules might be loaded at low pH or in the presence of ethanol, and after increasing the pH or washing with water, the material is captured inside. This possibility of encapsulation is demonstrated in Fig. 4.3.1.

Filled capsules can serve as microreactors. The advantage of encapsulated enzymes and proteins in general, is their inaccessibility for inhibitors, proteases, which are polymers and cannot permeate the capsule wall, and, of course, for bacteria. The activity of enzymes encapsulated is higher as compared with typical values of chemically immobilized enzymes preserving 50% of activity and less, so glucose oxidase loaded into the capsule preserves 60% of its activity [71,76]. The yield of encapsulation can reach 100% [23]. The native conformation of proteins does not change upon encapsulation process. The spectrum of capsules with hemoglobin encapsulated was recorded for verification of possible changes that could occur during loading or due to the contact of the protein with the capsule wall. There was no change registered in a position of a so called Soret band, which is very sensitive to the changes of hem conformation and the maximum of adsorption at 409 nm (Soret band) shifts by more than 2 nm if any changes occur [76].

5. Conclusions

PE multilayer capsules present a rather unique class of polymeric vesicles with wide possibilities for permeability variation. Originated on planar surfaces the multilayers topologically organized in the shape of hollow spheres became attractive systems. Intensive studies in last decade on flat multilayer PE films significantly contributed in estimation of properties of the hollow spheres towards their employment as vesicles and microcontainers for chemical reactions. Indeed, the selective permeability allows the performing the chemical reaction exclusively in capsule interior [77]. In this review we focused only on permeability properties of shells composed of PE multilayers. These multilayers provide a wide spectrum of permeability coefficients for different species ranging from ions to macromolecules depending to layer number, overall charge, pH, salt or solvent. Besides of obvious applied aspect of exploitation of such vesicles as carrier systems with defined permeability the fundamental research is still encouraged. So, diffusion coefficients for permeation of different low molecular weight compounds through the multilayers can be easily found if the film is fabricated in the capsule shape. Several different approaches can be used for that: release of a core-forming compound from the shell and measurement of the conductivity or fluorescence increase; or exchange of

the material between the capsule volume and the bulk. Diffusion coefficients for small ions and molecules were found to be 4–6 orders of magnitude lower, than those for their free diffusion in water.

Capsules and multilayers in general are very sensitive to the external conditions, so shift in pH, ionic strength or solvent leads to the change of their permeability properties. Besides that, this permeability change is reversible, which gives an opportunity to encapsulate various polymers. Encapsulation of polyelectrolytes leads to the local shift of pH inside the capsule, hydrophilic molecules—to the polarity shift; encapsulated enzymes can serve catalytic purposes. Capsules with all these polymers encapsulated could be successfully used as microreactors, or crystallization compartments.

Pure chemical ways of permeability regulation like cross-linking, hydrolysis, polymerization, etc., are based on creation of charged groups inside the film matrix. The ions of the same charge with the matrix repulse from the multilayers, while oppositely charged ions can use the excessive charged groups in the film as carriers, penetrating the film faster. Strong variation of the permeability of the ions of different valence was shown to have much potential for creation of ion separating membranes.

Exploration of the capsules' and multilayers' permeability properties is a rapidly developing topic, carrying much potential, which already gained much interest in industry. We believe, possibilities to control the permeability of the multilayered capsules will find its stable position on the market of drug carriers, biosensing, micro-reactors and catalysts, construction materials, and many others.

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